"Variability mapping of beneficial microbes in different soils of Maharashtra"

A Thesis submitted to

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST. AHMEDNAGAR,
MAHARASHTRA, INDIA

In partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

AGRICULTURAL MICROBIOLOGY

by

MURUMKAR DATTATRAYA RANGNATH
(Reg. No. 08/55)

DEPARTMENT OF PLANT PATHOLOGY AND AGRICULTURAL MICROBIOLOGY,

POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
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MAHARASHTRA, INDIA
2011

CANDIDATE'S DECLARATION

I hereby declare that this thesis or a part thereof has not been submitted by me or any other person to any other University or Institute for a Degree or Diploma.

Place : MPKV, Rahuri (D. R. Murumkar)

Date : / / 2011

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CERTIFICATE

This is to certify that the thesis entitled, "**VARIABILITY MAPPING OF BENEFICIAL MICROBES IN DIFFERENT SOILS OF MAHARASHTRA**" submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State, India in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in **AGRICULTURAL MICROBIOLOGY**, embodies the results of a piece of bonafide research work carried out by **Mr. MURUMKAR DATATRAYA RANGNATH** under my guidance and supervision and that no part of the thesis has been submitted to any other university for degree or diploma or publication in other form. The assistance and help received during the course of this investigation and source of reference have been duly acknowledged.

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Maharashtra State (India)

CERTIFICATE

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Place :MPKV, Rahuri
Date : / /2011 *(D. R. Murumkar)*

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LIST OF ABBREVIATIONS

ARA	:	Acetylene reduction assay
Asp	:	*Azospirillum*
Asp-BNF	:	*Azospirillum lipoferum* (MPKV strain)
Azt	:	*Azotobacter*
Azt-BNF	:	*Azotobacter chroococcum* (MPKV strain)
bp	:	Base pair
C.D.	:	Critical difference
CFU	:	Colony forming unit
CRD	:	Completely randomized design
CTAB	:	N-cetyl-N,N,N-trimethyl ammonium bromide
C.V.	:	Coefficient of variation
DAI	:	Days after inoculation
DNA	:	Deoxyribonucleic acid
dNTP	:	Deoxynucleotide triphosphate
EDTA	:	Ethylene diamine tetraacetic acid
EI	:	Evenness index
E long.	:	East longitude
et al.	:	Etalia (and other)
etc.	:	Et cetera (and so forth)
FID	:	Flame ionization detector
GC	:	Gas chromatograph
GPS	:	Global positioning system
g	:	gram
g^{-1}	:	Per gram
hr^{-1}	:	Per hour
i.e.	:	That is
in vitro	:	In laboratory

kb	:	Kilobase pair
LB broth	:	Luria bertani broth
MPN	:	Most probable number
mg	:	Milligram
mg protein^{-1}	:	Per milligram protein
ml	:	Milliliter
mM	:	Millimolar
mm	:	Millimeter
µl	:	Micro liter
ng	:	Nanogram
µm	:	Micrometer
nmol	:	Nano mole
nm	:	Nanometer
N lat.	:	North latitude
No.	:	Number (s)
PCR	:	Polymerase chain reaction
2D PCO	:	Two dimensional principal coordinate scatter plot analysis
3D PCO	:	Three dimensional principal coordinate scatter plot analysis
pH	:	Potential of hydrogen ion concentration
pp.	:	Page (s)
Pi	:	Inorganic phosphorus
PSB	:	Phosphate solubilizing bacteria
PSB-BNF	:	*Bacillus megaterium* (MPKV strain)
PSF	:	Phosphate solubilizing fungi
PSF-BNF(A)	:	*Aspergillus awamori* (MPKV strain)
PSF-BNF(P)	:	*Penicillium digitatum* (MPKV strain)
RAPD	:	Random amplified polymorphic DNA
RBA	:	Random bacterial primer

RFU	:	Random fungal primer
rRNA	:	Ribosomal ribonucleic acid
Rh	:	*Rhizobium*
Rh-BNF	:	*Rhizobium* spp. (MPKV strain)
RI	:	Richness index
RNase	:	Ribonuclease
rpm	:	revolutions per minute
S	:	Swedberg unit
S.E.±	:	Standard error
SI	:	Shannon's index
spp.	:	Species
TBE buffer	:	Tris boric acid EDTA buffer
TCP	:	Tricalcium phosphate
TE	:	Tris-EDTA (buffer)
Tris	:	Tris (hydroxymethyl) amino-methane
UPGMA	:	Unweighted pair group method and arithmetic average
UV	:	Ultra violet
viz.,	:	Videlicet (namely)
%	:	Per cent

ABSTRACT

Variability mapping of beneficial microbes in different soils of Maharashtra

by

Murumkar Dattatraya Rangnath

A candidate for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

AGRICULTURAL MICROBIOLOGY

Mahatma Phule Krishi Vidyapeeth,

Rahuri – 413 722

2011

Research Guide : **Dr. S. G. Borkar**

Department : Plant Pathology and Agricultural Microbiology

Microorganisms present in the soil play an important role in nutrient availability to crops by solubilization, mobilization and recycling of nutrients in soil. The soil microorganisms that enhance nutrient availability to crop plants include nitrogen fixers and phosphate solubilizers which have been used to develop biofertilizers. Therefore, attempts were made to study the variability among the nitrogen fixing and phosphate solubilizing isolates recovered from the rhizosphere soils of different physiographic regions of Maharashtra with respect to morphological, cultural, biochemical and physiological characterization as well as their beneficial functions and genetic variation.

A total of 263 nitrogen fixing and 93 phosphate solubilizing isolates were obtained during the year 2009 from the rhizosphere soils of different physiographic regions of Maharashtra *viz.*, Western

Konkan Coast, Western Ghats (*Sahyadris*), Western Maharashtra, North Maharashtra, Marathwada and Vidarbha. Among the nitrogen fixers, 94 *Azotobacter*, 76 *Rhizobium* and 93 *Azospirillum* isolates were obtained. Among the phosphate solubilizers, 47 phosphate solubilizing bacteria and 46 phosphate solubilizing fungi were isolated.

The present investigation revealed the distinct variation in cell morphology among the different isolates of *Azotobacter*. The cells of all *Azotobacter* isolates were motile and gram negative in reaction. Out of 94 *Azotobacter* isolates, cells of 48 isolates were rod shaped, whereas 46 isolates were oval in shape. The cell size varied among the isolates and was in the range of 1.1 to 4.4 x 2.0 to 11.8 µm. On the basis of cell arrangement, four distinct groups of isolates were formed and these were: 50 of the *Azotobacter* isolates were single celled, 26 with cells in pairs, 14 with cells in chain and 4 with irregular clumps. Further, the cells of all the *Rhizobium* isolates were rod shaped, motile and gram negative in reaction. A wide variation was observed in cell size varying from 0.4 to 2.8 x 0.7 to 10.0 µm among the *Rhizobium* isolates. In addition, the distinct variation was noticed in cell morphology among the different *Azospirillum* isolates. The cells of 83 *Azospirillum* isolates were non-motile, whereas 10 isolates had motile cells. The cell shape varied among the various *Azospirillum* isolates in that cells of 44 isolates were helicle/S-shape, 35 with vibrioid shape and 14 as curved rods. The distinct variation in cell size among the isolates was recorded which was in the range of 1.0 to 2.8 x 3.3 to 24.8 µm. Further, the cells of all phosphate solubilizing bacterial isolates were rod shaped, motile and gram positive in reaction. A wide variation was observed in cell size varying from 0.4 to 3.1x1.8 to 11 µm among the various PSB isolates.

The distinct variation was observed in colonial morphology, spore characteristics and microscopic appearance among the phosphate solubilizing *Aspergillus* and *Penicillium* isolates. The colonial

morphology of *Aspergillus* isolates revealed that the white colonies turned black or brown as culture matures. On the basis of colonial morphology, four distinct groups of *Aspergillus* isolates were formed. The first group comprised of eight *Aspergillus* isolates producing white floccose mycelium spreading rapidly and formed black coloured spores, second group of seven isolates producing velvety green mycelium which became almost black in due course, five isolates included in third group produced white cottony, loose woven thread like mycelium forming brown-black spores in due course and nine isolates involved in fourth group produced white colony, thread like mycelium forming brown-black spores in due course. On the basis of microscopic appearance, three distinct groups of *Aspergillus* isolates were formed. The first group comprised of 11 *Aspergillus* isolates consisting of hyphae-aseptate, conidiophore-septate, red coloured, globose vesicle, conidia-red and round, second group of 10 isolates having hyphae-colourless, conidiophores branching, septate, globose vesicle, conidia-small, red and round and 8 isolates included in third group comprised of hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black and round.

The colonial morphology of *Penicillium* isolates revealed that the mature cultures usually turned greenish or blue-green. On the basis of colonial morphology, six distinct groups of *Penicillium* isolates were formed. Out of 18 *Penicillium* isolates, three isolates from each group produced the different mycelial forms *viz.*, velvet, velutinous, fasciculate and cotton-wool followed by spore colour varied from dark-green, yellow, orange and blue-green. Based on the microscopic examination, three distinct groups of *Penicillium* isolates were formed. The first group comprised of 8 *Penicillium* isolates consisting of branched conidiophores that are borne on a single rope of fertile hyphae, second group of 4 isolates having branching conidiophores

that are borne on aerial mycelia and 5 isolates included in third group comprised of branching conidiophores arise from a septate mycelium.

Based on colonial morphology, spore characteristics and microscopic examination, out of 46 PSF isolates, 29 were tentatively identified as *Aspergillus awamori* and 17 as *Penicillium digitatum*.

The colonies of *Azotobacter* isolates appeared to be slimy, milky, viscid, mucoid, glistening and opaque with aged cultures producing light brown to dark brown pigmentation. Further, the colonies of *Rhizobium* isolates appeared white-opaque, mucilaginous, semi-translucent, translucent and milky to watery translucent, whereas the colonies of *Azospirillum* isolates formed subsurface pellicles on semi-solid N-free malate medium and the pink pigmentation was produced on BMS agar medium. Furthermore, the colonies of PSB isolates appeared to be creamy as well as whitish on Pikovskaya's agar medium. Among the PSB isolates, the zone of phosphate solubilization ranged from 5 to 14 mm, whereas in case of PSF isolates, the diameter of zone of P-solubilization ranged from 11 to 30 mm in *Aspergillus* isolates and 9-24 mm in *Penicillium* isolates.

As regards biochemical characters, all the 94 *Azotobacter* and 76 *Rhizobium* isolates were positive for starch hydrolysis, H_2S production, catalase test and oxidase test, but were negative for gelatin liquefaction. Regarding utilization of different carbon sources; mannitol, glucose, sucrose and fructose were used as a sole carbon source for growth by all the *Azotobacter* isolates, whereas mannitol, glucose, sucrose and arabinose were used as a sole carbon source for growth by all the *Rhizobium* isolates, while malate and citrate showed negative result. Further, all the 93 *Azospirillum* isolates were positive for catalase test and oxidase test, but were negative for starch hydrolysis, H_2S production and gelatin liquefaction. Out of 93 *Azospirillum* isolates, 83 isolates utilized glucose and malic acid as a sole carbon source and positive for biotin requirement, whereas

10 isolates utilized only malic acid as a sole carbon source for growth and showed negative result for glucose and biotin requirement.

Based on the morphological, cultural, biochemical and physiological characterization, out of 263 nitrogen fixing isolates, 94 isolates were tentatively identified as *Azotobacter chroococcum*, 76 isolates as *Rhizobium* spp., 83 isolates as *Azospirillum lipoferum* and 10 isolates as *Azospirillum brasilense*.

All the 47 PSB isolates were positive for starch hydrolysis, gelatin liquefaction and catalase test, but were negative for H_2S production, KOH and oxidase test. Fructose, glucose and maltose were used as a sole carbon source by all the isolates, whereas mannitol and citrate showed negative result. Based on the morphological, cultural, biochemical and physiological characterization, 47 PSB isolates were tentatively identified as *Bacillus megaterium*.

A significant variation in nitrogenase activity (5.3 to 291.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}) was recorded among the 94 different *Azotobacter* isolates tested. The isolate Azt-21 recorded significantly highest nitrogenase activity (291.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}) than the other isolates tested. Out of 94 *Azotobacter* isolates, 11 isolates *viz.*, Azt-21, 25, 50, 64, 70, 82, 97, 129, 135, 142 and 148 recorded significantly higher nitrogenase activity than the standard MPKV strain Azt-BNF. Further, the nitrogenase activity among the 76 *Rhizobium* isolates ranged from 1.9 (Rh-65) to 119.7 (Rh-69) nmol C_2H_4.mg protein^{-1}.hr^{-1}. Out of 76 *Rhizobium* isolates, 6 isolates *viz.*, Rh-64, 69, 72, 109, 113 and 132 recorded significantly higher nitrogenase activities than the standard MPKV strain Rh-BNF. Moreover, among the 93 *Azospirillum* isolates, the nitrogenase activity varied significantly (7.5 to 394.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}). The isolate Asp-97 recorded significantly highest nitrogenase activity (394.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}) than the other isolates tested. Out of 93 *Azospirillum* isolates, 6 isolates *viz.*, Asp-28, 50, 97, 124, 132 and

150 recorded significantly higher nitrogenase activities than the standard MPKV strain Asp-BNF.

Based on the nitrogenase activity, the highly efficient nitrogen fixing isolates *viz.*, 12 *Azotobacter*, 7 *Rhizobium* and 7 *Azospirillum* isolates alongwith standard MPKV strain were further studied for their genetic variability.

All the 47 PSB and 46 PSF isolates were able to solubilize inorganic insoluble phosphate. The amount of Pi released by the PSB isolates ranged from 2.06 (PSB-59) to 52.38 (PSB-15) per cent at 10 days after inoculation. Out of 47 PSB isolates, 6 isolates *viz.*, PSB-15, 33, 41, 72, 100 and 119 recorded significantly higher P-solubilizing ability than the standard MPKV strain PSB-BNF. The decrease in pH of the TCP broth was found to have highly significant positive correlation (r = +0.781**) with the amount of Pi released at 10 DAI. Further, the amount of Pi released by the *Aspergillus* isolates ranged from 45.80 (PSF-4) to 87.17 (PSF-71) per cent at 10 days after inoculation. Out of 29 *Aspergillus* isolates, 5 isolates *viz.*, PSF-28, 55, 64, 71 and 100 recorded significantly higher P-solubilizing ability than the standard MPKV strain PSF-BNF(A). The decrease in pH of the TCP broth was found to have highly significant positive correlation (r = +0.905**) with the amount of Pi released at 10 DAI. Moreover, among the 17 *Penicillium* isolates, the amount of Pi released varied significantly (26.30 to 69.77 per cent) at 10 days after inoculation. The isolate PSF-61 recorded significantly highest P-solubilization (69.77%) followed by PSF-77 (53.44%) than the standard MPKV strain PSF-BNF(P) and other isolates tested. The decrease in pH of the TCP broth was found to have highly significant positive correlation (r = +0.833**) with the amount of Pi released at 10 DAI.

Based on the phosphate solubilizing ability, the highly efficient phosphate solubilizing isolates *viz.*, 8 PSB isolates, 8 *Aspergillus*

isolates and 5 *Penicillium* isolates alongwith standard MPKV strain were further studied for their genetic variability.

Impact analysis of weather parameters and cropping system on distribution of efficient N_2 fixing and P solubilizing isolates across the physiographic regions of Maharashtra indicated that both, weather parameters and cropping system had no singnificant influence on distribution of efficient N_2 fixing and P solubilizing isolates. However, the population of these isolates varied significantly with cropping system across the regions. The maximum population of *Azotobacter, Rhizobium* and *Azospirillum* isolates (10.17, 8.33 and 5.40 $x10^4$ CFU g^{-1} soil, respectively) was recorded in paddy-wheat, pigeonpea-wheat/pigeonpea-chickpea and paddy/nachni cropping system in Kolhapur and Latur district of Western Ghats and Marathwada, respectively. Similarly, the maximum population of PSB and PSF isolates (2.83 and 2.33 $x10^4$ CFU g^{-1} soil) was recorded in pigeonpea-wheat/pigeonpea-chickpea, pigeonpea-safflower and green gram-sorghum/green gram-safflower cropping systems of Latur, Beed and Solapur district of Marathwada and Western Maharashtra, respectively. The overall results revealed that crop related factors have more critical influence on the abundance of native microbial populations than soil or climatic factors.

The genetic variability within each group of the selected nitrogen fixing and phosphate solubilizing isolates along with the standard MPKV strain was analyzed by employing random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. The genomic DNA of 12 *Azotobacter chroococcum*, 7 *Rhizobium* spp. and 7 *Azospirillum lipoferum* isolates alongwith standard MPKV strains *viz.*, Azt-BNF, Rh-BNF and Asp-BNF, respectively were subjected to PCR amplification using twenty five random oligonucleotide primers. Moreover, the genomic DNA of 8 PSB isolates and 13 PSF isolates *viz.*, 8 *Aspergillus awamori* and 5 *Penicillium digitatum* isolates alongwith

standard MPKV strains *viz.*, PSB-BNF, PSF-BNF(A) and PSF-BNF(P), respectively were subjected to PCR amplification using twenty five random oligonucleotide primers.

The PCR amplified products of the 12 selected isolates of *Azotobacter* along with standard MPKV strain Azt-BNF with respect to each of the 25 random primers showed 1123 polymorphic bands. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.40 and minimum genetic similarity of 0.15. The highest genetic similarity of 0.40 could be noted between the isolates Azt-08 and Azt-25. The primer RBA-24 was found to be the best primer for determination of variability among the *Azotobacter chroococcum* isolates. This primer generated 54 bands of different molecular weight of which 16 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Azotobacter* isolates for use as passport data.

The amplification profiles of the 7 selected isolates of *Rhizobium* along with standard MPKV strain Rh-BNF with respect to each of the 25 random primers showed 905 polymorphic bands. The dendrogram constructed showed three clusters with the maximum genetic similarity of 0.27 and minimum genetic similarity of 0.22. The highest genetic similarity of 0.27 could be noted between the isolates Rh-64 and Rh-69. The primer RBA-7 was found to be the best primer for determination of variability among the *Rhizobium* spp. isolates. This primer generated 66 bands of different molecular weight of which 28 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Rhizobium* isolates for use as passport data.

The amplified products of the 7 selected isolates of *Azospirillum* along with standard MPKV strain Asp-BNF with respect to each of the 25 random primers showed 820 polymorphic bands. The dendrogram constructed showed four clusters with the maximum genetic similarity

of 0.53 and minimum genetic similarity of 0.23. The highest genetic similarity of 0.53 could be noted between the isolates Asp-127 and Asp-132. The primer RBA-4 was found to be the best primer for determination of variability among the *Azospirillum lipoferum* isolates. This primer generated 63 bands of different molecular weight of which 30 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Azospirillum* isolates for use as passport data.

The PCR amplified products of the 8 selected isolates of PSB along with standard MPKV strain PSB-BNF with respect to each of the 25 random primers showed 600 polymorphic bands. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.87 and minimum genetic similarity of 0.29. The highest genetic similarity of 0.87 could be noted between the isolates PSB-33 and PSB-39. The primer RBA-21 was found to be the best primer for determination of variability among the *Bacillus megaterium* isolates. This primer generated 41 bands of different molecular weight of which 10 were unique and isolate specific in the present study. This primer can be used for fingerprinting of PSB isolates for use as passport data.

The amplification profiles of the 8 selected isolates of *Aspergillus* along with standard MPKV strain PSF-BNF(A) with respect to each of the 25 random primers showed 656 polymorphic bands. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.59 and minimum genetic similarity of 0.16. The highest genetic similarity of 0.59 could be noted between PSF-115 isolate and PSF-BNF strain. The primer RFU-23 was found to be the best primer for determination of variability among the *Aspergillus awamori* isolates. This primer generated 33 bands of different molecular weight of which 9 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Aspergillus* isolates for use as passport data.

The amplified products of the 5 selected isolates of *Penicillium* along with standard MPKV strain PSF-BNF(P) with respect to each of the 25 random primers showed 453 polymorphic bands. The dendrogram constructed showed three clusters with the maximum genetic similarity of 0.83 and minimum genetic similarity of 0.40. The highest genetic similarity of 0.83 could be noted between the isolates PSF-97 and PSF-101-1. The primer RFU-2 was found to be the best primer for determination of variability among the *Penicillium digitatum* isolates. This primer generated 37 bands of different molecular weight of which 8 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Penicillium* isolates for use as passport data.

The diversity analysis using different diversity indices *viz.*, Shannon's index (SI), Evenness index (EI) and Richness index (RI) was carried out for 263 nitrogen fixing and 93 phosphate solubilizing isolates. The Shannon's index of the pooled data for nitrogen fixing and phosphate solubilizing isolates was 1.09 and 1.02 respectively, suggesting high diversity of these isolates in six different physiographic regions of Maharashtra. The diversity of tentatively identified 263 nitrogen fixing and 93 phosphate solubilizing isolates in terms of Shannon's index ranged from 0.37 to 1.10 and 0.33 to 1.05, respectively. The maximum diversity of nitrogen fixing isolates was found in Marathwada region (SI of 1.10), whereas phosphate solubilizing isolates in Vidarbha region (SI of 1.05).

The evenness index of the pooled data was 0.79 and 0.93 for nitrogen fixing and phosphate solubilizing isolates respectively, indicating the dominance of one species over the others. Among the different physiographic regions, Marathwada showed maximum evenness index (1.00) for nitrogen fixers indicating more even distribution of nitrogen fixing isolates in this region, whereas Vidarbha showed maximum evenness index (0.96) for phosphate solubilizers

indicating more even distribution of phosphate solubilizing isolates in this region.

The Richness index for the individual physiographic regions ranged from 0.46 to 0.85 for nitrogen fixing isolates and 0.58 to 1.06 for phosphate solubilizing isolates. Among the different physiographic regions, Western Maharashtra showed maximum richness index (0.85) for nitrogen fixers indicating richness of nitrogen fixing isolates in this region, whereas Marathwada showed maximum richness index (1.06) for phosphate solubilizers indicating richness of phosphate solubilizing isolates in this region.

Thus, the present investigation revealed distinct variability among the nitrogen fixing and phosphate solubilizing isolates recovered from the rhizosphere soils of various physiographic regions of Maharashtra with respect to morphological, cultural, biochemical and physiological characterization as well as beneficial functions and genetic makeup. The population dynamics study revealed that crop related factors have more critical influence on the abundance of native microbial populations than soil or climatic factors. Furthermore, diversity analysis study indicated the richness of nitrogen fixing isolates in Sangli, Kolhapur, Pune, Solapur and Ahmednagar district of Western Maharashtra and phosphate solubilizing isolates in Latur, Beed and Parbhani district of Marathwada, whereas the maximum diversity of nitrogen fixing and phosphate solubilizing isolates was found in Marathwada and Vidarbha region, respectively.

1. INTRODUCTION

Microbial diversity studies are important in understanding the microbial ecology in soil and functioning of ecosystems. Microorganisms are the most numerous and ancient biotic communities which have successfully colonized every possible ecological niche on this planet. The microorganisms recycle important nutrients and thereby improve the health and functioning of soil ecosystem and the whole environment. They play a vital role in maintaining soil fertility and productivity, pest and disease control and detoxification of natural and man-made pollutants. The extraordinary activity of microorganisms is based on their remarkable metabolic diversity and genetic adaptability.

The structure and diversity of bacterial communities can be analyzed by several methods. The cultural methods are insufficient for estimating biodiversity as the standard laboratory media used support the growth of less than one per cent of the total bacterial population. Thus, the microbes that are studied and exploited by humans constitute only a minute fraction of earth's microbial diversity because many species yet await recognition and realize their genetic potential. The information relating taxonomic diversity to functioning of the ecosystem is hardly available. To gain thorough knowledge of the microbial processes within an ecosystem, the study of functional diversity coupled with taxonomic diversity is essential. Meager information is available on genetic diversity and the influence of genetic and taxonomic diversities on the functional diversity and ecosystem properties.

The microbial resources have contributed significantly to improvement of agriculture due to the latest advances in recombinant DNA technology. Microbial populations and microbial

processes in soil can be better understood through comparison of phenotypic and DNA-based analysis of bacterial diversity. The comparative analyses of the small subunit rRNA (l6S and 18S) and other gene sequences have shown that the greatest extent of earth's biodiversity is among microorganisms (Hugenholtz *et al.*, 1998). Results from the application of recombinant DNA technology and molecular phylogenetic methods to a number of diverse environments confirm that our view of microbial diversity was limited and further it points to a wealth of novel and environmentally important diversity yet to be explored (Pace, 1997).

Microorganisms present in the soil play an important role in nutrient availability to crops by solubilization, mobilization and recycling of nutrients in soil. The soil microorganisms that enhance nutrient availability to crop plants include nitrogen fixers and phosphate solubilizers which have been used to develop biofertilizers.

The biological nitrogen fixation is one of the most important microbial activity as it makes the recycling of atmospheric nitrogen on earth possible and gives a fundamental contribution to nitrogen homeostasis in the biosphere (Aquilantia *et al.*, 2004). Among the various diazotrophs, the ecological distribution of *Azotobacter* spp. is related to diverse factors determining the presence or absence of this bacterium in specific soil. It has been shown that the soil characteristics and climatic conditions affect the distribution of this microorganism (Dobereiner and Pedrosa, 1988). The free living rhizobial strains isolated from rhizosphere soils of legume crops are able to fix nitrogen as scored by acetylene reduction while growing in the absence of a plant host (Bedmar and Olivares, 1979). *Azospirillum* is considered to be the most important rhizobacterial genus for improving plant growth under a variety of environmental

and soil conditions (Bashan *et al.*, 2004). *Azospirillum* exhibit acetylene reduction assay (ARA) under micro-aerophilic condition (Kim *et al.*, 2005).

Soil microbes play a key role for solubilization of inorganic phosphate to release phosphorus which is available for crop growth and development (Rodriguez and Fraga, 1999). High proportion of these phosphate solubilizing microorganisms (PSM) are concentrated in the rhizosphere of plants (Vazquez *et al.*, 2000). Many soil fungi and bacteria are known to solubilize inorganic phosphates (Illmer and Schinner, 1992).

The study of molecular diversity by PCR based RAPD analysis facilitates placement of the strains in genetically distinct and related groups (Lee and Henry, 2001and Megha *et al.*, 2007).

Keeping in view these facts, the present investigation was planned to study the diversity of nitrogen fixing and phosphate solubilizing microorganisms in different soils of Western Konkan Coast, Western Ghats (*Sahyadris*) and North Deccan Plateau of Maharashtra with the following objectives.

1. To isolate, enumerate and characterize the beneficial microorganisms *viz.*, nitrogen fixers (*Azotobacter, Rhizobium* and *Azospirillum*) and phosphate solubilizers (PSB and PSF) from the rhizosphere soils of different physiographic regions of Maharashtra.
2. To study the functional diversity of nitrogen fixing and phosphate solubilizing microorganisms with special reference to beneficial activities for agriculture.
3. To analyze the molecular diversity within each group of nitrogen fixing and phosphate solubilizing microorganisms by PCR based RAPD analysis.
4. To prepare the map of beneficial microbes of the soils of Maharashtra state.

2. REVIEW OF LITERATURE

The present investigation was carried out to isolate and characterize nitrogen fixing and phosphate solubilizing microorganisms from rhizosphere soils of various physiographic regions of Maharashtra and study their functional diversity with respect to their role for soil functioning and also the molecular diversity of some selected isolates with beneficial traits. The literature reviewed for discussing the results of this investigation are presented below under the appropriate heads, which will provide an overview of the current status of the research work on the concerned aspects.

2.1 Microbial diversity:

The microbes play immense role in agriculture, industry, medicine and environment because of their remarkable inherent physiological and functional diversity. Much better known and exploited microbial activities are augmentation, supplementation and recycling of plant nutrients, so vital to sustainable agriculture (Johri *et al.,* 2005).

Biological diversity is the variability among the living organisms and the ecological complexes in which they occur. It also includes the diversity found at the genetic, species and ecosystem levels (Nei and Li, 1979). Comparative analyses of small subunit rRNA (16S or 18S rRNA) and other gene sequences show that life falls into three primary domains *viz.,* bacteria, eukarya and archea. Based on studies using rRNA trees, the main extent of earth's biodiversity was found to be amongst microbes (Hugenholtz *et al.*, 1998).

Measuring biodiversity of a habitat or community has prime importance in ecology and conservation biology, because of its academic necessity and utility in devising conservation strategies. A variety of indices are available to quantify the diversity of biological communities. The most popular and widely used measures are Shannon's, Simpson's, Fischer's alpha-log series (Magurron, 1988) and Avalanche index (Ganeshaiah *et al.,* 1997). Majority of these techniques are used to study the diversity of plants and animals, but they have also been used for studying the microbial diversity. Such type of diversity analysis using diversity indices was carried out for mycorrhizae (Beena *et al.,* 2000) and for bacteria from soils (Torsvik *et al.,* 1990).

Rao and Venkateswarlu (1983) carried out a comprehensive study on the microbial ecology of Indian desert soils. The different groups of microorganisms *viz.*, fungi, actinomycetes, bacteria, *Azotobacter* and nitrifying bacteria were present, but in relatively low numbers, there was considerable variation in numbers from one site to another within the desert as the physicochemical characters of the soils varied. In general the low organic matter content and poor moisture availability of desert soils were the major factors limiting optimum microbial activity.

Price (1988) stated that the existence of each living organism depends upon microorganisms since they are the decomposers in the food chain. Various potentials of microorganisms *viz.*, nitrogen fixation, solubilization of inorganic insoluble phosphate, production of plant growth promoting substances, biological control of insect pests, diseases and weeds of crops etc. have been realized and exploited for today's agriculture. The knowledge accumulated on the importance of microorganisms has compelled the scientists

think about the microbial diversity. Thus, the study of microbial diversity is gaining increasing interest.

Hill *et al.* (2000) stated that the traditional analysis of soil microbial communities on culturing techniques using a variety of culture media designed to maximize the recovery of diverse microbial populations. However, only a small fraction (<0.1%) of the soil microbial community has been accessible with this approach. To overcome these problems, in recent years, molecular methods for soil microbial community analysis have provided phylogenetic diversity of microbial communities in soil. Among the most useful of these methods are those in which small subunit rRNA genes are amplified from soil-extracted nucleic acids. Microbial rRNA genes can be detected directly from soil samples and sequenced. The use of these techniques provides new ways of assessing soil microbial diversity.

Theron and Cloete (2000) suggested the use of molecular techniques for determining microbial diversity and community structure in natural environments. They proposed the recombinant DNA techniques, 16S rRNA sequence analysis and use of fluorescently labelled population of specific rRNA probes for studying diversity and phylogeny of microorganisms.

Kari and James (2001) characterized the rhizosphere and root interior microbial communities through fatty acid methyl ester (FAME) analysis and community level physiological profiles and reported that the plant variety has notable influence on microbial composition of rhizosphere.

Girvan *et al.* (2003) studied the soil bacterial communities in three separate arable farms in eastern England using a polyphasic approach combining traditional soil analysis, analysis of the physiological properties of the isolates as well as nucleic acid

profiling and reported that soil factor was the key determinant of the bacterial diversity in arable soils.

The influence of soil amendments on microbial properties of the acidic, heavy metal contaminated soil from Cu-Ni smelter-damaged landscape of Canada was studied by Okonski *et al.* (2003) who reported that the abundance of bacteria, actinomycetes and microfungi was found to be extremely low in these soils. They counted and identified representatives of 14 species of soil micro-fungi. The highest biodiversity of microfungal community and actinomycetes was recorded in soil treated with zeolite (Shannon's index 2.6 and 1.8 respectively) whereas limed soil had the least diversity of both the groups (Shannon's index value of 0.9 and 0.7 respectively).

2.2 Isolation of beneficial microorganisms from soil:

2.2.1 Isolation of nitrogen fixing microorganisms from soil

Subramaney and Abraham (1962) isolated *Azotobacter chroococcum* strains from red loamy soils of India. Rangaswamy and Sadasivam (1964) studied the distribution of *Azotobacter* around Chidambaram, Madras State and found them to be wide spread. Two most commonly occurring species, *Azotobacter beijerinckii* appeared in acidic soils and *Azotobacter chroococcum* in neutral to alkaline soils. The number of *Azotobacter chroococcum* in Indian soils rarely exceeds from 10^4 to 10^9/g of soil (Subba Rao *et al.*, 1993).

Sadowsky *et al.* (1983) isolated fast and slow growing *Rhizobium* strains from soils collected in the provinces of Shansi, Honan, Shandong, Shanghai and Beijing, Peoples' Republic of China using cango-red yeast extract mannitol agar (CRYEMA) medium. Soberon-Chavez and Najera (1989) isolated *Rhizobium*

strains from soil of two different geographical origins in Mexico. Naz *et al.* (2009) isolated *Rhizobium* sp. from the rhizosphere soils of four different localities of Khewra (Pakistan).

Han and New (1998) isolated 285 *Azospirillum* strains from soils from seven geographic regions of New South Wales, Australia by using nitrogen-free semisolid malate (NFB) medium. Tejera *et al.* (2005) isolated *Azotobacter chroococcum* and *Azospirillum brasilense* from the rhizosphere soils of sugarcane collected from four different agricultural locations in Granada (Spain). Kim *et al.* (2005) isolated *Azospirillum lipoferum* and *Azospirillum brasilense* strains from the rhizosphere soils of different cereals and grasses using semi-solid malate medium (NFB) supplemented with 50 $mg.L^{-1}$ yeast extract.

Suliasih and Widawati (2005) has studied the occurrence of nitrogen fixing bacteria *viz.*, *Azotobacter chroococcum, Rhizobium* sp. and *Azospirillum* sp. from the soil samples of Wamena Biological Garden which is one of the mountain range-biota ex-citu conservation at Eastern part of Indonesia.

Doroshenko *et al.* (2007) isolated three *Azospirillum lipoferum* strains from the soil of a *Sphagnum* peat bog (Moscow). Muthukumarasamy *et al.* (2007) isolated *Azospirillum* strains from rhizosphere soils of rice grown in Yeongnam (South Korea).

Ilyas *et al.* (2008) isolated *Azospirillum lipoferum* strains from the roots and rhizosphere soils of maize grown under arid, semiarid and irrigated areas of Pakistan. He also isolated *Rhizobium* strains from the rhizosphere soils of same agro-ecological regions using cango-red yeast extract mannitol agar (CRYEMA) medium. *Rhizobium* and *Azospirillum* isolated from irrigated areas showed higher colony count as compared to those from semi-arid areas particularly at reproductive stage. Tulajappa *et al.* (2008) isolated ten *Azotobacter chroococcum* from different agro-climatic zones of

Karnataka, India by Waksman No.77 N-free agar by a serial dilution plate technique.

Bhatia *et al.* (2009) isolated seventy six *Azotobacter* strains from the rhizosphere of cotton plants from farmers' fields from four regions of India following cotton-wheat rotation cropping, *viz.*, Haryana, Punjab, Rajasthan and Maharashtra by enrichment in Jensen's medium. Twenty *Azotobacter chroococcum* isolates were finally selected on the basis of efficient plant growth-promoting properties.

Ilyas and Bano (2010) isolated *Azospirillum brasilense* strains from the roots and rhizosphere soils of wheat grown under different agro-climatic regions of Attok, Kallar and Islamabad (Pakistan). The survival of *Azospirillum* isolates from plants of irrigated field and those from well-watered pots was higher than that of *Azospirillum* strains isolated from roots and rhizosphere soils of plants grown under arid and semiarid (14–8% soil moisture) field conditions and under water-stressed (8% soil moisture) conditions in pots.

2.2.2 Isolation of phosphate solubilizing microorganisms from soil

Nair and Subba Rao (1977) isolated two efficient phosphate solubilizing microorganisms *viz.*, *Pseudomonas* sp. and *Aspergillus niger* and reported that they were capable of solubilizing 49 % and 49.7 % of the supplied tricalcium phosphate.

Chhonkar and Tarafdar (1984) studied the abundance of phosphate solubilizing microorganisms in different soils. The bacterial and fungal population was higher in both slightly acidic and alkaline soils, whereas, actinomycetes were higher in acidic soils.

Venkateshwarlu *et al.* (1984) observed that the population of phosphate solubilizing microorganisms was generally low in

desertic soils (Aridsols) possibly due to low level of organic matter and high temperature regimes. *Bacillus cereus, Pseudomonas fluorescens, Aspergillus niger* were some of the predominant species of phosphorus solubilizers found in majority of the soils.

Rokade and Patil (1992) observed that the phosphate solubilizing fungal population generally occurred more in acidic to neutral soils, while the bacterial population was found more in alkaline soils. The phosphate solubilizing microorganisms generally occurred large in population in the rhizosphere than non-rhizosphere soils of different crops.

Nahas *et al.* (1994) isolated 31 phosphate solubilizing bacteria and 11 phosphate solubilizing fungi from 13 different soil types from various regions of the Sao Paulo State, Brazil. Total bacterial and fungal population ranged from 2.7 to 142.6 x 10^5 and 2.3 to 57.7 x10^3 CFU g^{-1} of soil, respectively.

Vazquez *et al.* (2000) isolated 13 phosphate solubilizing bacterial strains from the rhizosphere of black and white species of mangroves in a semiarid coastal lagoon of Mexico and one phosphate solubilizing fungal strain of *Aspergillus niger* from rhizosphere of black mangroves. Spectrophotometric quantification of phosphate solubilization showed that *Vibrio proteolyticus* was the most active solubilizing species among the bacteria.

Kundu *et al.* (2002) isolated about 73 isolates of phosphate solubilizing bacteria (PSB) from the rhizosphere of cowpea, maize, sorghum, cotton and pearlmillet. Eleven promising isolates were selected based on their rate of phosphate solubilization for subsequent studies. Sorghum and pearlmillet showed more PSB followed by maize, cowpea and cotton. The isolates showed variable halozones on medium containing tricalcium phosphate. A decrease in pH of medium was noticed in almost all the isolates.

Suliasih and Widawati (2005) isolated 12 efficient phosphate solubilizing bacteria from the soil samples of Wamena Biological Garden which is one of the mountain range-biota ex-citu conservation at Eastern part of Indonesia.

Pradhan and Sukla (2005) isolated phosphate solubilizing fungi *viz.*, *Aspergillus fumigatus* and *Penicillium* sp. from the rice field soil of Bhubaneswar, Orissa, India. Both the strains showed diverse levels of phosphate solubilizing activity in liquid broth culture in presence of various carbon and nitrogen sources.

Chen *et al.* (2006) isolated 36 strains of phosphate solubilizing bacterial isolates from the subtropical soils of Central Taiwan. Castagno *et al.* (2008) isolated 17 phosphate solubilizing bacterial isolates from the samples of saline-alkaline soils collected from the rhizosphere of *Lotus tenuis* plants grown Chascomus, Argentina.

Hariprasad and Niranjana (2009) isolated 43 phosphate solubilizing rhizobacterial (PSRB) isolates from 37 rhizospheric soil samples from tomato growing regions of Karnataka. Among the 43 isolates, 33 were found to be positive for solubilizing both inorganic and organic forms of phosphorous.

Chakraborty *et al.* (2010) isolated 400 phosphate solubilizing fungal strains from soil samples collected from forest, river basin, agricultural fields and rhizosphere of plantation crops of North Bengal, India. Among the screened isolates, ninety showed phosphate solubilizing activity. Out of these, ten isolates belongs to *Aspergillus niger, A. melleus and A. clavatus* which showed maximum phosphate solubilizing activities and were further tested for their efficiencies in liquid medium using two types of inorganic phosphates.

Deepa *et al*. (2010) isolated 42 phosphate solubilizing fungal strains from paddy soils of southern peninsular region of Tamil Nadu, India. Out of 42 fungal strains, 12 phosphate solubilizing fungal strains isolated from rice rhizosphere soil showed higher solubilization of phosphate and release more amount of phosphorus and phosphatase when compared with other fungal strains isolated from different field soils.

Shiva Reddy *et al*. (2010) isolated phosphate solubilizing bacterial strains of *Bacillus megaterium* from soils of different agro-climatic zones of Karnataka by the enrichment culture technique in Spreber's medium. Characteristically all the isolates were gram positive and rod shape. All isolates showed the phosphate solubilization zone around the colony.

2.3 Population dynamics of microorganisms:

2.3.1 Population dynamics of nitrogen fixing microorganisms

Bashan and Levanony (1985) have developed the improved selection technique for isolation and enumeration of *Azospirillum beasilense*. The technique was based on successive liquid enrichments in nitrogen-free semisolid medium supplemented with streptomycin, followed by the most probable number counting method and verification on a selective medium.

Rao and Venkateswarlu (1985) have applied the most probable number (MPN) method for enumerating the *Azospirillum* population in the rhizosphere of pearl millet. The most probable number of *Azospirillum* varied among different root zones of pearl millet with maximum in the rhizosphere (3.46×10^4 g^{-1} soil).

Venkateswarlu *et al*. (1997) correlated native rhizobial populations (*Bradyrhizobium* sp.) in 88 soil samples from 13 important legume growing locations with mean annual rainfall, soil

pH, organic carbon, clay content and crop history. The populations showed significant relationship with organic carbon and previous crop history but clay content, mean annual rainfall and soil pH had no influence. The population, however, increased significantly following crop cultivation. The increase was more with pigeonpea and sorghum than with sunflower. When pigeonpea, groundnut and sorghum were grown as preceding crops, the population remained high (> log10 3.0 MPN/g soil). The overall results revealed that crop related factors have more critical influence on the abundance of native rhizobial populations than soil or climatic factors.

Suliasih and Widawati (2005) studied the occurrence of nitrogen fixing bacteria *viz.*, *Azotobacter chroococcum, Rhizobium* sp. and *Azospirillum* sp. from the soil samples of Wamena Biological Garden which is one of the mountain range-biota ex-citu conservation at Eastern part of Indonesia. Microbial population was estimated by plate count method. The results showed that the microbial population ranged from $5.0x10^3$ – $1.5x10^7$ CFU of bacteria g^{-1} soil.

Narayan and Kehri (2008) studied the population dynamics of *Azotobacter chroococcum* in wheat and paddy fields with different degrees of agricultural intensification in plains of Northern India. The results showed that irrespective of the degree of intensification, the highest population of *A. chroococcum* (1.52 to 3.15 x 10^4 g^{-1} oven dry soil) was recorded in the wheat fields with lowest degrees of intensification and the lowest in the rice fields (0.15 to 0.64 x 10^4 g^{-1} oven dry soil) with the highest degrees of intensification. This may be attributed to the changes in the chemical properties of the soil with reference to the land use practices and prevailing water logged condition during the rice crop season.

Naz *et al.* (2009) measured the survival efficiency of *Rhizobium* isolates in culture (log cfu g^{-1} soil). The difference in the survival efficiency (as measured by the colony count) among the isolates of Khewra Salt range may be attributed to the difference in the root exudates of different plants and their relative tolerance to saline condition.

Ilyas and Bano (2010) studied the survival efficiency (Log cfu/g) of *Azospirillum* isolates. Survival efficiency (measured as cfu/g soil) of isolates from irrigated field of NARC, Islamabad (soil moisture 25%) revealed higher values than those of isolates from rhizosphere soil of plants grown in arid (soil moisture 8%) and semiarid field (soil moisture 14%) areas.

2.3.2 Population dynamics of phosphate solubilizing microorganisms

Nahas *et al.* (1994) reported the occurrence of phosphate solubilizing bacteria and fungi from 13 different soil types. Total bacterial and fungal population ranged from 2.7 to 142.6 x 10^5 and 2.3 to 57.7 x10^3 CFU g^{-1} of soil, respectively.

Suliasih and Widawati (2005) isolated 12 efficient phosphate solubilizing bacteria from the soil samples of Wamena Biological Garden which is one of the mountain range-biota ex-citu conservation at Eastern part of Indonesia. Microbial population was estimated by plate count method. The results showed that the microbial population ranged from 5.0x10^3 – 7.5x10^6 CFU of bacteria g^{-1} soil.

Fallah (2006) studied the abundance and distribution of phosphate solubilizing bacteria and fungi in 50 soil samples collected from North of Iran. The PSB populations ranged from 0 to 10^7 cells g^{-1} soil with 94% of the soil samples containing PSB microorganisms. The soil sample from Berry cultivated region

contained the greatest PSB population equaling $3.85x10^6$ cells g^{-1} soil. Eighty six percent of the soil samples contained PSF ranging up to 10^6 cells g^{-1} soil. The greatest PSF population of $1.8x10^5$ cells g^{-1} soil was found in the fermenting residues of the tea processing plant. Bacteria comprised the greatest percentage of phosphate solubilizing microorganisms in the soil samples that were examined.

Seshadri and Lakshminarasimhan (2007) studied the population dynamics of P-solubilizers in the rhizosphere of major weed species from a tropical delta soil of India. The population ranged from 0 to $74x10^3$ CFU g^{-1} soil.

2.4 Morphological and cultural characterization of microorganisms

2.4.1 Cell morphology

Several research workers (Beijerinck, 1901; Jensen and Petersen, 1954; Johnstone, 1974; Apte and Shende, 1981 and Tchan and New, 1986) reported that the cells of *Azotobacter chroococcum* were oval to rod shaped measuring 1.5 to 3 x 0.5 to 6 µm and they occurred singly, or in pairs of irregular clumps and rarely in chains of more than 4 cells, motile with peritrichous or polar flagella or non-motile with abundant cyst formation.

The *Rhizobium* cells were reported as rod shaped measuring 0.5 to 0.9 x 1.2 to 3.0 µm, motile by one polar flagella (Jordan, 1986).

In semi-solid nitrogen-free malate (Nfb) medium, *Azospirillum lipoferum* develops predominantly into pleomorphic cells (elongated S-shaped forms and enlarged ovoid forms) at 37°C within 48 hrs, in contrast to *Azospirillum brasilense* which retain mainly the enlarged vibriod forms and several refractile, ovoid C forms and motile cells (Tarrand *et al.*, 1978). *Azospirillum lipoferum* grows as elongated

cells (1.4-1.7 µm x 5 to over 30 µm long) which are non-motile and have an S shape or helical shape. The *Azospirillum* cells were reported as plump, slightly-curved and straight rods, about 1.0 µm in diameter and 2.1 to 3.8 µm in length, often with pointed ends, motile in liquid medium by a single polar flagellum; on solid medium at 30°C numerous lateral flagella of shorter wavelength are also formed (Tarrand *et al.*, 1978).

Bacillus was reported as rods having round or squared ends and may be small 0.5 x 12 µm or large 2.5 x 10 µm, motile by peritrichous flagella (Claus and Berkeley, 1986).

Mittal *et al.* (2008) characterized PSF strains up to species level based on colonial morphology, spore characteristics and microscopic examination and identified as *Aspergillus awamori* and *Penicillium digitatum*. He reported that *Aspergillus awamori* strains showed hyphae-aseptate, conidiophores thick walled, smooth, septate, red coloured, globose vesicle, conidial heads globose, conidia-red and round, sterigma in two series and *Penicillium digitatum* strains showed conidiophores with smooth walled, branching conidiophores arise from a septate mycelium.

2.4.2 Staining

The cells of *Azotobacter chroococcum* were gram negative in reaction and non-spore forming (Johnstone, 1974; Becking, 1981; Apte and Shende, 1981 and Tchan and New, 1986). The strains of *Azotobacter chroococcum* may produce moderate to copious amount of capsular slime (Johnstone, 1974; Mulder and Brotonegoro, 1974). However, Apte and Shende (1981) observed that all the strains of *Azotobacter chroococcum* are heavily capsulated.

The cells of *Azospirillum* were gram negative in reaction (Tarrand *et al.*, 1978). The cells of *Rhizobium* were gram negative

and non-spore forming bacteria (Vincent *et al.*, 1979 and Jordon, 1986).

Bacillus is gram positive in reaction and endospore forming bacteria (Claus and Berkeley, 1986).

Halebian *et al.* (1981) used a simple, rapid method utilizing a 3% solution of potassium hydroxide (KOH) to distinguish between gram-positive and gram-negative bacterial strains and tested on 213 strains of anaerobic bacteria representing 19 genera.

2.4.3 Colony characters

Grey (1953) reported that the pure cultures of *Azotobacter* produced small (0.5 to 1.0 mm diameter) cream opaque colonies on milk agar medium. Mishustin and Shilinikova (1972) reported that 3 to 4 days old *Azotobacter* colonies appear as flat, soft, milky and mucoid on agar plates.

Tchan and New (1986) reported that the colonies of *Azotobacter chroococcum* were generally 2 to 6 mm in diameter, opaque, entire, low convex, viscid, glistening and smooth. Variant colony forms may arise through desiccation in the quantity of extra cellular polysaccharides produced.

Cheruku (2004) observed that the pure cultures of *Rhizobium* produced circular, convex, entire colonies which had semi translucent mucilagenous appearance and had slow growing rate on YEMA medium.

Adiguzel *et al.* (2010) reported that the pure cultures of *Rhizobium* produced circular colonies with regular borders, raised in elevation, creamy in color, showing intermediate to high production of mucus on YEMA medium.

On BMS agar after 1-2 weeks of incubation at 33-35°C, colonies of *Azospirillum* were pink, opaque, irregular or round, often

wrinkled, and typically have umbonate elevations (Tarrand *et al.*, 1978).

Tejera *et al.* (2005) reported that the colonies of *Azospirillum brasilense* on malate medium were opaque and non slimy, whereas the colonies of *Azotobacter chroococcum* on N-free medium were slightly viscous, semi-translucent during early growth and later dark brown.

Claus and Berkeley (1986) observed that the colonies of pure cultures of *Bacillus* were whitish or creamy coloured.

Mittal *et al.* (2008) reported that strains of *Aspergillus* awamori showed rapidly growing brownish black colonies with slightly radially furrowed usually white sometimes yellow basal mycelium and strains of *Penicillium digitatum* showed colonies with restricted growth, blue-green, reverse bright yellow with the same pigmentation diffusing into agar.

2.4.4 Pigmentation

The aged cultures of *Azotobacter chroococcum* formed an insoluble black-brown pigment on Jensen's or Ashby's medium (Beijerinck, 1901; Jensen and Petersen, 1954; Norris and Chapman, 1968 and Johnstone, 1974), commonly attributed to the presence of melanin. This melanin was formed as a result of tyrosinase, a copper containing enzyme.

Sundara Rao and Iswaran (1959) observed heavy turbidity and scum at the surface in solution containing sucrose and lactose by *Azotobacter chroococcum*, but no growth in peptone broth.

Pigmentation is best on BMS agar incubated in the light. The strains of *Azospirillum lipoferum* and *Azospirillum brasilense* form colonies that have a very deep pink colour (Tarrand *et al.*, 1978 and Tejera *et al.*, 2005).

On nutrient agar, the strains of *Bacillus megaterium* showed growth heaped and nonspreading, glossy or moderately dull, sometimes slightly rugose; on aging some shade of yellow to pink; on long incubation, growth and medium may become brown or black (Claus and Berkeley, 1986).

2.5 Biochemical test:

2.5.1 Starch hydrolysis

Azotobacter, Rhizobium and *Bacillus* were found to have the ability to hydrolyze starch as reported by Tchan and New (1986); Tejera *et* al. (2005); Singh *et al.* (2008); Bhatia *et al.* (2009); Claus and Berkeley (1986) and Gaind and Gaur (1991) whereas *Azospirillum* was found negative towards starch hydrolysis (Tarrand *et al.*, 1978 and Tejera *et* al., 2005).

2.5.2 H_2S production

Azotobacter and *Rhizobium* showed positive reaction towards H_2S production (Norris and Chapman, 1968; Tchan and New, 1986; Ilyas *et al.*, 2008 and Naz *et al.*, 2009). Species belonging to *Bacillus* genus were found to have 90% negative reaction for H_2S production (Claus and Berkeley, 1986 and Gaind and Gaur, 1991). *Azospirillum* was found negative towards H_2S production (Ilyas *et al.*, 2008 and Ilyas and Bano, 2010).

2.5.3 Gelatin liquefaction

Azotobacter had both positive and negative reaction towards gelatin liquefaction, showing variation possessed by different sp. (Tchan and New, 1986). *Rhizobium* and *Azospirillum* showed negative reaction towards gelatin liquefaction (Ilyas *et al.*, 2008; Naz *et al.*, 2009; Sadowsky *et al.*, 1983 and Tarrand *et al.*, 1978).

Bacillus has ability to liquefy gelatin (Claus and Berkeley, 1986 and Gaind and Gaur, 1991).

2.5.4 Catalase and Oxidase test

Catalase and oxidase reactions of bacteria were reported by several research workers. *Azotobacter* was oxidase and catalase positive (Johnstone, 1974; Tchan and New, 1986 and Bhatia *et al.*, 2009). *Rhizobium* and *Azospirillum* showed catalase and oxidase positive reaction (Naz *et al.*, 2009; Tarrand *et al.*, 1978; Tejera *et* al., 2005; Kim *et* al., 2005; Ilyas *et al.*, 2008 and Ilyas and Bano, 2010). Bacilli are either oxidase positive or catalase positive (Claus and Berkeley, 1986 and Gaind and Gaur, 1991).

2.6 Functional diversity of microorganisms:

2.6.1 Nitrogen fixing ability of microorganisms

Hardy *et al.* (1968) demonstrated the methodology, characteristics and application of the sensitive acetylene-reduction assay for N_2 fixation by nitrogenase preparations and bacterial cultures in the laboratory and by legumes and free-living bacteria *in situ*. The validity of measuring N_2 fixation in terms of reduction of acetylene (C_2H_2) to ethylene (C_2H_4) was established through extensive comparisons of these activities using defined systems, including purified N_2ase preparations and pure cultures of N_2 fixing bacteria.

Pagan *et al.* (1975) reported the solid defined CS7 medium for the induction of nitrogenase in free living rhizobia. Turner and Gibson (1980) reported the acetylene reduction assay for nitrogen fixation in *Rhizobium*.

Kaneshiro *et al.* (1978) isolated 272 free living rhizobial strains from soils of Peoria, Illinois and reported nitrogenase

activity of 69 rhizobial strains grown on a defined CS7 medium by acetylene reduction assay (ARA). Out of 69 strains, 32 *Rhizobium japonicum* strains expressed the nitrogenase activity in the range of 2 to 45 nmol C_2H_4.mg protein^{-1}.hr^{-1} and 37 unclassified strains showed the nitrogenase activity in the range of 1 to 40 nmol C_2H_4.mg protein^{-1}. hr^{-1}.

Bedmar and Olivares (1979) reported nitrogen fixation (as scored by acetylene reduction) by free-living *Rhizobium meliloti* strain while growing in a defined medium in the absence of a plant host. The highest nitrogenase activity (96.7 nmol C_2H_4.mg protein^{-1}. hr^{-1}) was recorded by *R. meliloti* strain growing in defined liquid medium under normal atmosphere in the sealed tubes.

Kaneshiro *et al.* (1983) showed the positive correlation between increase in number of pleomorphic cells with their acetylene reducing activity in free-living cowpea-type *Rhizobium* sp. strain 32Hi (3200 nmol C_2H_4.mg protein^{-1}. hr^{-1}) followed by *Rhizobium japonicum* strains USDA 26 and USDA 110 (2600 and 65 nmol C_2H_4.mg protein^{-1}.hr^{-1}, respectively) grown on a GMG differential agar medium.

Li and Hung (1987) measured the nitrogenase activity as 16 to 22 nmol C_2H_4.mg protein^{-1}.hr^{-1} by *Azospirillum* strains isolated from western forest research centre, Lebanon, Oregon by acetylene reduction assay.

Ramaswamy and Bal (1987) reported asymbiotic nitrogen fixation by *Rhizobium* sp. 127E15 grown in six different induction media and acetylene reduction activity was detected in all six different induction media of which the medium CS7, LNB5 and MW giving significantly higher nitrogenase activity of 9.0, 18.3 and 12.1 nmol C_2H_4.mg protein^{-1}. hr^{-1}, respectively. In the induced cultures, large pleomorphic forms of bacteroids were produced. Considerable

acetylene reduction activity (7.5 nmol C_2H_4.mg protein^{-1}. hr^{-1}) was recorded in the rhizosphere of the lima bean plants that were inoculated with rhizobia and grown in pot cultures. Trace amounts of activity (0.02 nmol C_2H_4.mg protein^{-1}. hr^{-1}) could also be detected in the rhizobia adhering to the rhizoplane.

Gajendiran and Mahadevan (1989) measured N_2 fixation (acetylene reduction assay) in free living bacteria *viz.*, *Azotobacter chroococcum* and *Azospirillum lipoferum* isolated from rhizosphere soils with nitrogenase activity of 290 and 1332 nmol C_2H_4.mg protein^{-1}.hr^{-1}, respectively.

Murkute *et al.* (1990) tested the efficacy of 32 strains of *Azotobacter chroococcum* isolated from different soil zones of Maharashtra and found that ten strains of *Azotobacter* fixed nitrogen 15.4 to 20.38 mg/g of sucrose; twelve strains fixed 10.30 to 13.59 mg/g of sucrose while remaining ten strains fixed minimum (6.44 to 9.5 mg/g of sucrose) nitrogen.

Malik *et al.* (1997) determined the nitrogenase activity of *Azospirillum lipoferum* and *Azospirillum brasilense* strains by acetylene reduction assay (ARA) and reported highest nitrogenase activity of *A. lipoferum* strains (686 nmol C_2H_4.mg protein^{-1}. hr^{-1}) followed by *A. brasilense* strains (215 nmol C_2H_4.mg protein^{-1}. hr^{-1}).

Han and New (1998) tested 285 *Azospirillum* strains isolated from soils from seven geographic regions of New South Wales, Australia for nitrogenase activity by acetylene reduction assay (ARA). There was wide variation in nitrogenase (acetylene reduction) activity among the 285 different isolates grown in nitrogen-free malate semisolid medium (0.0-154.9 nmol C_2H_4.mg protein^{-1}.hr^{-1}), with differences between regions in average activities of isolates (12.5-79.9 nmol C_2H_4.mg protein^{-1}.hr^{-1}). The isolates of *Azospirillum lipoferum* exhibited a higher average nitrogenase activity compared

to *Azospirillum brasilense*, both in Nfb medium (67.6 compared with 39.2 nmol C_2H_4.mg protein^{-1}.hr^{-1}) and in association with wheat roots (4.3 compared with 2.0 nmol C_2H_4.mg dry root^{-1}. day^{-1}). A high nitrogen fixation rate in pure culture (>110 nmol C_2H_4.mg protein^{-1}. hr^{-1}) was achieved by 24% of identified isolates of *A. lipoferum*, but not by any identified *A. brasilense*. Therefore majority of the most active nitrogen fixers were *Azospirillum lipoferum*.

Kim *et al.* (2005) determined the nitrogenase activity of thirteen strains of *Azospirillum brasilense* and two strains of *Azospirillum lipoferum* isolated from the rhizosphere of different crops of Korea by acetylene reduction assay (ARA). There was wide variation in nitrogenase activity among the different isolates selected, ranging from 187 to 387 nmol C_2H_4.mg protein^{-1}.hr^{-1}.

Tejera *et al.* (2005) determined the nitrogenase activity of *Azotobacter chroococcum* and *Azospirillum brasilense* strains isolated from the sugarcane rhizosphere by acetylene reduction assay (ARA). The amount of acetylene reduced by *A. chroococcum* isolates was in the range of 79.6 to 329.5 nmol C_2H_4.mg protein^{-1}.hr^{-1} whereas in case of *Azospirillum brasilense* isolates, acetylene reduction was in the range of 91.5 to 135.1 nmol C_2H_4.mg protein^{-1}.hr^{-1}.

Muthukumarasamy *et al.* (2007) reported the nitrogenase activity of different diazotrophic bacterial isolates of each group in the range of 3 to 44 nmol C_2H_4.mg protein^{-1}.hr^{-1}.

Bhatia *et al.* (2009) estimated nitrogenase activity of twenty *Azotobacter chroococcum* strains isolated from four cotton growing regions of India, *viz.*, Haryana, Punjab, Rajasthan and Maharashtra by acetylene reduction assay (ARA). The ARA of these isolates varied from 17.4 to 385.0 nmol C_2H_4.mg protein^{-1}.hr^{-1}. The highest nitrogenase activity recorded by Punjab isolates (52.8 to 385.0 nmol C_2H_4.mg protein^{-1}.hr^{-1}) followed by Haryana, Rajasthan and

Maharashtra isolates (17.4 to 209.2, 50.0 to 105.1 and 18.6 to 46.0 nmol C_2H_4.mg protein^{-1}.hr^{-1}, respectively).

2.6.2 Phosphate solubilizing ability of microorganisms

Mehta and Bhide (1970) isolated 42 efficient phosphate solubilizing fungal cultures from different soils of Maharashtra and showed that there was no correlation between pH of soil and degree of phosphate solubilization.

Bardiya and Gaur (1972) studied the isolates such as *Bacillus megaterium, Bacillus polymyxa, Bacillus pulvifaciens, Bacillus subtilis, Bacillus circulans* which solubilized tricalcium phosphate. The highest amount i.e. 72.25 mg P_2O_5 out of 100 mg added phosphorus was solubilized by *Bacillus pulvifaciens*.

Nair and Subba Rao (1977) isolated two efficient phosphate solubilizing microorganisms *viz.*, *Pseudomonas* sp. and *Aspergillus niger* and reported that they were capable of solubilizing 49 % and 49.7 % of the supplied tricalcium phosphate.

Arora and Gaur (1979) carried out screening of phosphate solubilizing microorganisms on the basis of zone of clearance of tricalcium phosphate in Pikovskaya's solid medium around the colony growth. Further, they reported the ability of *Bacillus megaterium, Bacillus polymyxa, Pseudomonas striata, Aspergillus awamori* and *Penicillium digitatum* to solubilize tricalcium phosphate in Pikovskaya's liquid medium. *Aspergillus awamori* was the most effective solubilizer recording highest phosphate solubilizing ability (75 mg P_2O_5/ml) followed by *Penicillium digitatum* (45 mg P_2O_5/ml). Among the bacteria, *Pseudomonas striata* recorded maximum phosphate solubilization (35 mg P_2O_5/ml) followed by *Bacillus megaterium* (27 mg P_2O_5/ml).

Wani *et al*. (1979) reported the phosphate solubilizing activity of four phosphomicrobials *viz*., *Bacillus polymyxa, Pseudomonas striata, Aspergillus awamori* and *Penicillium digitatum*. All these isolates more efficiently solubilized tricalcium phosphate than rock phosphate in the liquid medium.

Venkateswarlu *et al*. (1984) isolated two phosphate solubilizing bacteria (*Bacillus cereus* and *Pseudomonas flourescens*) and two phosphate solubilizing fungi (*Aspergillus niger* and *Penicillium pinophillum*) from different parts of Western Rajasthan. *In vitro* evaluation of these cultures indicated that fungi were more efficient than bacteria in phosphorus solubilization. Phosphorus release by all the organisms was associated with the production of organic acids like lactic, gluconic and succinic in the medium.

Haque (1986) isolated twenty four bacteria and two fungi from the rhizosphere soil of rice, jute and dhaincha growing in four important soil tracts of Bangladesh and tested for the solubilization of rock phosphate in liquid medium. Variations were observed among the cultures, fungi being the better, in respect of rock phosphate dissolution. In almost all the cultures, pH was decreased in the medium. Positive relationship was found between the amount of rock phosphate solubilized and decrease in pH of the medium at seven days' incubation.

Yin (1988) reported that microorganisms like *Bacillus, Flavobacter, Pseudomonas* sp., *Bacillus polymyxa* and *Bacillus subtilis* have phosphate solubilizing ability of 20 to 30 mg/g of tricalcium phosphate.

Yadav and Singh (1991) reported that the efficiency of microorganisms in phosphate solubilization varied widely, *Bacillus megaterium* being superior to other microorganisms. *B. megaterium* showed the maximum effect by releasing 41 per cent of the total P

added in the medium while *Bacillus cereus, Penicillium digitatum* and *Aspergillus niger* showed 22, 28 and 35 per cent release, respectively. The maximum decrease in pH was recorded with *Bacillus megaterium* (from 6.0 to 4.2) and *Bacillus cereus* (from 6.6 to 5.6). A significant negative correlation (r= -0.973) was observed between pH of the broth and amount of P released by *Bacillus megaterium*.

Nahas (1996) isolated forty two soil isolates (31 bacteria and 11 fungi) which were studied for their ability to solubilize rock phosphate and calcium phosphate in culture medium. Eight bacteria and 8 fungi possessed solubilizing ability. *Pseudotnonus cepacia* and *Penicillium putpurogenum* showed the highest activity. A high correlation was observed between final pH and soluble phosphate only for the rock phosphates inoculated with the highest concentration of solubilizing bacteria (r = - 0.73 to - 0.98).

Whitelaw *et al.* (1999) isolated *Penicillium radicum*, a phosphate solubilizing fungus from the rhizosphere of wheat roots. He reported that the phosphate solubilization was highest from $CaHPO_4$ (475 mg P l^{-1}), $Ca_3(PO_4)_2$ (360 mg P l^{-1}) and colloidal aluminium phosphate (207 mg P l^{-1}). The main mechanism for phosphate solubilization was acid production leading to a decrease in pH.

Vazquez *et al.* (2000) demonstrated the phosphate solubilizing potential of the rhizosphere microbial community in mangroves when culture media supplemented with insoluble tribasic calcium phosphate. He reported that the four *Bacillus* strains have phosphate solubilizing ability of 20 to 110 mg/L. Further, qualitative determination of phosphate solubilizing capacity of *Aspergillus niger* on solid medium showed 5mm halos around the periphery of each fungal colony after 24 hrs. The entire dish turned

clear within 48 hrs of incubation. Quantitative analysis showed that the conc. of soluble phosphate in liquid culture increased from 50 to 430 mg/L and the pH in the culture showed a drastic drop (from 6.5 to 3.0).

Alam *et al.* (2002) isolated ten most efficient phosphate solubilizing bacteria and three fungi (PSM) from maize rhizosphere and grown *in vitro* for seven days on Pikovskaya's medium following analyses were carried out i.e. solubilization index, pH change, phosphorus (P) solubilized, P immobilized and organic acids produced under *in vitro* conditions. P solubilization index of these isolates ranged from 1.63-3.29. Drop in pH of the medium ranged from 7 to 3.2 with the continuous growth of these isolates for seven days. Study showed that more P was immobilized (0.2-0.46%) than solubilized (0.09-0.22%). Bacteria were found to be more active than fungi in conversion of insoluble P to soluble P.

Pradhan and Sukla (2005) tested two fungal isolates (*Aspergillus* sp. and *Penicillium* sp.) for their tricalcium phosphate (TCP) solubilization efficiency in both solid and liquid medium. Phosphate solubilization was related to pH decrease caused by growth of fungus in medium containing glucose as carbon source. *Aspergillus* sp. solubilized 480 µg/ml of phosphorus, while *Penicillium* sp. solubilized 275 µg/ml of phosphorus from 0.5% tricalcium phosphate.

Chen *et al.* (2006) isolated 36 strains of phosphate solubilizing bacteria (PSB) from Central Taiwan. The solubilization of TCP in the liquid medium by different strains was accompanied by a significant drop in pH to 4.9 and 6.0 from an initial pH of 6.8–7.0 after 72 hrs. The soluble-P concentration in the medium ranged between 31.5 and 519.7 mg/L with variations among different isolates. Among ten *Bacillus megaterium* isolates, drop in pH

was in the range of 5.1 to 6.0 from an initial pH 7.0 of the medium and the soluble-P concentration in the medium ranged between 72.2 and 270.2 mg/L.

Rajankar *et al.* (2007) isolated thirty three phosphate solubilizing fungi and bacteria from saline belt of Purna river basin. On the basis of phosphate solubilizing ability, it was observed that the fungi *viz.*, *Aspergillius* spp., *Penicillium* spp. and *Fusarium* spp. have the more solubilizing ability of inorganic insoluble phosphate than bacteria, *viz.*, *B.subtilis*, and *B.megatherium*. Hence the application of biofertilizer prepared by above mentioned fungi should be helpful to reduce the salinity of soil by neutralization phenomenon, because these microorganisms release the acid in very minute quantity in phosphate solubilization.

Mittal *et al.* (2008) reported the phosphate solubilizing activity of six phosphate-solubilizing fungi (two strains of *Aspergillus awamori*, and four of *Penicillium citrinum*) isolated from rhizosphere of various crops located around Chandigarh, India in Pikovskaya's liquid medium varied from 38 to 760 μg ml^{-1} for tricalcium phosphate (TCP) and 28–248 μg ml^{-1} for mussoorie rock phosphate (MRP).

Oliveira *et al.* (2008) reported phosphate solubilizing efficiency of forty-five strains isolated from rhizosphere soil of maize grown in an oxisol of the Brazilian Cerrado Biome in a modified Pikovskaya's liquid medium containing tricalcium phosphate ($Ca_3(PO_4)_2$). Strains B17 and B5, identified as *Bacillus* sp. and *Burkholderia* sp., respectively, were the most effective, solubilizing 67% and 58.5% of the total P ($Ca_3(PO_4)_2$) after 10 days. Among the microorganisms showing ability to solubilize tricalcium phosphate, *Penicillium citrinum* (F95) and *Aspergillus terreus* (F93) were the most efficient solubilizing 44% and 42% of total P, respectively.

Tao *et al*. (2008) isolated inorganic P-solubilizing bacteria (IPSB) and organic P-mineralizing bacteria (OPMB) from subtropical flooded and temperate non-flooded soils of Beijing, China. Ten OPMB strains were isolated and identified as *Bacillus cereus* and *Bacillus megaterium,* and five IPSB strains as *B. megaterium, Burkholderia caryophylli, Pseudomonas cichorii,* and *Pseudomonas syringae*. The IPSB strains exhibited inorganic P-solubilizing abilities ranging between 25.4–41.7 µg P mL^{-1} and organic P-mineralizing abilities between 8.2–17.8 µg P mL^{-1}. Each of the OPMB strains also exhibited both solubilizing and mineralizing abilities varying from 4.4 to 26.5 µg P mL^{-1} and from 13.8 to 62.8 µg P mL^{-1}, respectively.

Hariprasad and Niranjana (2009) isolated 43 phosphate solubilizing rhizobacteria (PSRB) from rhizospheric soil samples of tomato collected from tomato growing regions of Karnataka and estimated phosphate solubilizing ability under *in-vitro* condition. The isolates, PSRB 7 and PSRB 19 showed phosphate solubilization of 143 and 141 µg/ml respectively in Pikovskaya's broth, which were significantly higher over other PSRB isolates. Almost all selected PSRB isolates lowered the pH of culture.

Panhwar *et al*. (2009) reported that P solubilization by the different bacterial strains was significantly influenced by the sources of P used in the broths. Inoculation of NBRIP broth containing TCP with the strain PSB-10 solubilized the highest P (40.56%), while comparatively lower P was solubilized by the bacteria in CIRP broth containing rock phosphate as P source. The positive correlation was noticed between considerable amount of P solubilized with decrease in pH of the medium.

Chakraborty *et al*. (2010) obtained ninety P-solubilizing isolates from soil samples collected from forest, river basin, agricultural fields and rhizosphere of plantation crops of North

Bengal and tested their phosphate solubilizing activity on Pikovskaya's agar medium by the appearance of halos around the inoculums. Ten isolates which showed maximum phosphate solubilizing activities in Pikovskaya's agar medium were further tested for their activities in liquid medium using two types of inorganic phosphates, tricalcium and rock phosphate. *Aspergillus niger* isolate showed maximum solubilization of phosphorous (856 mg/L) whereas *Aspergillus clavatus* isolate showed minimum (799 mg/L) phosphorous solubilization when the media were supplemented with tricalcium phosphate. When the medium was supplemented with rock phosphate, *Aspergillus melleus* isolate showed maximum of 385 mg/L phosphorous solubilization and *Aspergillus clavatus* isolate showed minimum of 288 mg/L phosphorous solubilization.

Chaudhari *et al.* (2010) reported that three fungal isolates (*Aspergillus awamori, A. niger* and *A. fumigatus*) and two bacterial isolates (*Bacillus* strain-1 and *Bacillus* strain-2) solubilized P in the range of 47.4 to 61.0 % using tricalcium phosphate and 9.0 to 18.2 % using rock phosphate as P source. The fungal isolates solubilize more phosphate as compared to bacterial isolates.

Deepa *et al.* (2010) isolated twelve efficient strains of phosphate solubilizing fungi belongs to *Aspergillus, Alternaria, Cladosporium, Fusarium, Penicillium* and *Rhizopus* from paddy soils of southern peninsular region of Tamil Nadu, India. The mineral phosphate solubilizing (MPS) activities of twelve isolates were tested in tricalcium phosphate medium. The maximum phosphate solubilizing activity was recorded on 9th day after incubation for all fungal strains ranging between 393.9 to 4701.8 µg/ml. The positive correlation was noticed between considerable amounts of phosphorus released with decrease in pH of the culture filtrate.

Qian *et al.* (2010) isolated twelve phosphate solubilizing bacteria, including eight organic P-solubilizing bacteria (OPBs) and four inorganic P-solubilizing bacteria (IPBs). Laboratory tests on P release ability revealed that IPBs were more effective at releasing P than OPBs. The most efficient IPB strain could accumulate over 170 $mg \cdot L^{-1}$ orthophosphate, while the equivalent OPB strain only liberated less than 4 $mg \cdot L^{-1}$ orthophosphate in liquid culture.

Yadav *et al.* (2011) tested phosphate solubilizing ability of *Aspergillus niger* strain BHUAS01, a plant growth promoting fungus (PGPF) isolated from rhizosphere of chickpea at different sources of carbon. *Aspergillus niger* was found to solubilize maximum tricalcium phosphate (512 $\mu g\ mL^{-1}$) at glucose as carbon source and minimum activity (348 $\mu g\ mL^{-1}$) of phosphate solubilization at sucrose as carbon. Further the effect of different salinity (1% NaCl, 1% KCl and 1% CaCl2) was tested at different pH (6.0, 7.0 and 8.0) under *in vitro* condition. *A. niger* strain BHUAS01 showed maximum significant solubilization of tricalcium phosphate (495 $\mu g\ mL^{-1}$) in presence of 1% $CaCl_2$ in modified Pikovskaya's broth at pH 8.0 than other salt concentration.

2.7 Molecular diversity of microorganisms:

The polymerase chain reaction (PCR) is the *in-vitro* amplification of particular DNA sequences using random or specific primers and a thermostable DNA polymerase (Joshi *et al.,* 1999). The diversity analysis as well as finger printing of individuals can be performed with the help of PCR based RAPD markers.

The random primers are used in PCR reactions for randomly amplified polymorphic DNA (RAPD) studies (Williams *et al.,* 1990).

Theron and Cloete (2000) suggested the use of molecular techniques for determining microbial diversity and community

structure in natural environments. They proposed the methods of recombinant DNA techniques, 16S rRNA sequence analysis and the use of fluorescently labelled population specific rRNA probes in the studies of diversity and phylogeny of microorganisms.

RAPD has been used as a tool to study the diversity of microorganisms. Rangarajan *et al.* (2001) analysed populations of *Pseudomonas* for their biochemical characters and genetic diversity using molecular tools including RAPD and PCR-RFLP and found that increased salinity caused selection of *Pseudomonas pseudoalcaligens* and *Pseudomonas alcaligens,* irrespective of the host rhizosphere.

2.7.1 Molecular diversity of nitrogen fixing microorganisms

Young and Cheng (1998) determined the genetic relationships among six strains of rhizobia, including three strains of *Rhizobium fredii* and three strains of *Bradyrhizobium japonicum*, using random amplified polymorphic DNA (RAPD) technique. In this study, 46 arbitrary 10mer primers were employed for RAPD, generating a total of 251 informative fragments. A dendrogram of phylogenetic relationships among the six strains was constructed. The results indicated that geographical distribution may affect phylogeny, as there were closer relationships among the four Taiwanese strains, SB138, SB562, SB368 and SB651, than between these strains and USDA192, which originated from mainland China. The strain USDA110, obtained from the United States, was used in the parsimony analysis. The greatest similarity (55.6%) existed between two strains of *B. japonicum*, SB562 and SB138 and the lowest in *R. fredii* (44.4%) between two strains of *R. fredii*, SB368 and USDA192. They also found a RAPD marker specific to the four Taiwanese SB strains used in the study.

Sikora and Redzepovic (2003) characterized eighteen *Bradyrhizobium japonicum* isolates as well as reference strains genotypically by RAPD fingerprinting. Total genomic DNAs were amplified with six oligonucleotide primers. The amplification patterns revealed a high level of polymorphism. All primers produced multiple DNA products ranging in size from 0.15 to 2.8 kb. The primers produced up to 14 products per strain. The dendrogram derived from RAPD profiles showed that all *B. japonicum* strains could be divided into two major clusters.

Pinto *et al*. (2004) determined the genetic relationship of 85 *Arachis pinto* nodulating *Rhizobium* strains using the RAPD methods. The analysis included 75 strains isolated from Cerrado soils and 10 other ones of different origins. The results indicated that there is a high level of similarity between these strains and that geographic distribution may affect their phylogenetic relationship. In addition, the results allowed the selection of the most suitable primers for characterization of these *Rhizobium* strains which will be useful for implementation of competitiveness studies in Cerrado soils.

El-Fiki (2006) used RAPD fingerprinting for strain identification and the assessment of genetic diversity within a field population of *Rhizobium* (*Bradyrhizobium archus*, *Bradyrhizobium japonicum* and *Rhizobium leguminosarum* bv. *Trifolii*). Total genomic DNAs from different field isolates were amplified using two different arbitrary primers. Different banding patterns were obtained for all strains. Cluster analysis showed the ralationship of *R. leguminosarum* bv. *Trifolii* with *B. archus* (69%) and *B. japonicum* (63%). The results indicated that RAPD is a very discriminative and efficient method for differentiating and studying genetic diversity of *Rhizobium* strains.

Bhatia *et al.* (2008) used genetic fingerprinting to study the diversity among *Azotobacter* spp. isolated from four different cotton-wheat cropping regions of India. On the basis of acetylene reduction, out of 76 free-living diazotrophs isolated from the rhizospheric soil of cotton, 20 efficient *Azotobacter* spp were selected for further studies. BIOLOG cataloguing divided them into two main groups, but amplified ribosomal DNA restriction analysis clustered the isolates from the four regions having different soil types into four separate sub-clusters. Variations observed could be due to domestication of the isolates in different agro-ecological niches.

Ilyas *et al.* (2008) assessed the genetic diversity among the *Rhizobium leguminosarum* and *Azospirillum lipoferum* isolates by RAPD analysis. All the isolates showed reproducible DNA banding pattern. Diversity among the isolates was assessed on the basis of variation of size, number and intensity of bands. DNA was amplified by using RAPD-PCR revealed banding patterns depending on the number and size of amplified products was observed for *Rhizobium* and *Azospirillum* isolates. All the *Rhizobium* and *Azospirillum* isolates shared different DNA banding pattern. On the basis of carbon/nitrogen utilization pattern, three groups of *Rhizobium* isolates were identified that fell into two groups on the basis of banding pattern of RAPD. Similar grouping were recognized for *Azospirillum* on the basis of carbon/nitrogen utilization pattern and RAPD analysis.

Shamseldin *et al.* (2008) isolated thirty four Egyptian free living rhizobial strains directly from soils without the use of a trap host and species status was determined by using partial sequencing of 16S rDNA.

Tulajappa *et al.* (2008) estimated molecular diversity of ten *Azotobacter chroococcum* strains isolated from different agro-climatic zones of Karnataka, India by using ten selected RAPD primers. A total of 103 bands were scored out of which 87 were found to be polymorphic (84.97%). A dendrogram divided the isolates into two groups separated by 37 linkage distances and the dissimilarity matrix showed a maximum difference of 64% between the isolates of the north eastern transition zone and the central dry zone and a minimum difference of 18% between the isolates of the eastern dry zone and the hilly zone. The isolate from zone 4 was clustered separately from the group. Thus, RAPD markers analysis proved to be a quick, simple and significant testing method to assess genetic diversity among *Azotobacter chroococcum* isolates.

Bhatia *et al.* (2009) determined the genetic diversity of twenty *Azotobacter chroococcum* isolates by RFLP (restriction fragment length polymorphism) analysis of the functional gene *nif*H. Genetic analysis of these isolates depicted a similarity coefficient of $\geq$80% among them, suggesting that though the isolates were obtained from different cotton soils of India, still they have large commonality in the *nif*H gene and constituted a homogeneous *nif*H population.

Naz *et al.* (2009) assessed the genetic diversity among the five isolates of *Rhizobium* sp. by RAPD-DNA finger printing and PCR was done for the presence of 16S-rRNA gene. On the basis of carbon/nitrogen source utilization patterns, *Rhizobium* isolates placed in five different groups and were designated as Rkh1, Rkh2, Rkh3, Rkh4 and Rak5 but RAPD tests categorized the isolates into two clusters. The RAPD results were further analyzed by MVSP software; similarity matrix was measured and converted into dendrogram using UPGMA clustering method.

Rajeswari and Mangai (2009) determined the *nifH* gene sequence of the nitrogen-fixing bacterium *Azotobacter* spp. with the use of polymerase chain reaction (PCR). The phylogenetic tree revealed that isolated *Azotobacter* spp. was distantly related to uncultivated and uncultured organisms. They did not form any branch with other *Azotobacter* spp. in the data base.

Rajasundari *et al*. (2009) isolated ten soil rhizobia from different field locations and further subjected to RAPD analysis to study the genetic diversity. It was found that *Rhizobium* isolates ALN 7, SOB 1, EB 36 and MBS 19 were clustered together to form cluster 1 followed by *Rhizobium* isolates MBS 9, TNAU 14, COG 15 were belong to cluster 2 and CBS 9 and ALN 2 formed the fourth cluster. Isolate CBS 12 was found to have more divergence (>90%) to make the separate cluster. COG 15 with TNAU 14 and SOB 1 with *Rhizobium* ALN 7 were clustered together with more than 50% similarity. The results indicated that RAPD provided a high degree of discrimination between the strains.

Ilyas and Bano (2010) determined the genetic biodiversity and polymorphism among the isolated *Azospirillum* strains by the random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. On the basis of unweighted pair group method with arithmetic means (UPGMA) cluster analysis of *Azospirillum* strains isolated from rhizosphere soil and roots of wheat plants using OP-01 and OP-06 primer, isolates were grouped into two clusters. Isolates within each cluster were closely related to each other, whereas isolates in different clusters had greater genetic distance from each other.

Kanimozhi and Panneerselvam (2010) assessed the genetic diversity among the ten *Azospirillum* isolates by RAPD analysis. RAPD fingerprinting analysis was made using the primer OP07. All

the ten isolates was shared different DNA banding pattern in RAPD finger printing analysis. Most of the amplified fragments were between 234 and 2027 bp. RAPD profiles of *Azospirillum* isolates were greatly diversified based on their banding pattern.

2.7.2 Molecular diversity of phosphate solubilizing microorganisms

Chen *et al*. (2006) used the RAPD technique to elucidate the polymorphism among the 36 strains of phosphate solubilizing bacteria (PSB) isolated from Central Taiwan and ascertained their possible relationship with mineral phosphate solubilizing (MPS) activities. Out of the 80 random primers used, only 20 primers were effective in resolving the difference. The similarity coefficient was calculated by NTSYS software based on the RAPD binary data and showed that similarity among 36 isolates ranged between 0.15 and 0.82 and formed four distinct clusters.

Mittal *et al*. (2008) assessed the genetic diversity among six phosphate-solubilizing fungi (two strains of *Aspergillus awamori*, and four of *Penicillium citrinum*) isolated from rhizosphere of various crops located around Chandigarh, India by RAPD technique. The RAPD analysis of the genomic DNA of six PSF revealed that all the isolates were different from one another. Out of eight different primers used, T2 and T5 primers-based RAPD banding pattern could distinguish all the six PSF. Percentage of similarity was estimated by simple matching.

Chakraborty *et al*. (2010) analyzed molecular diversity of ten P-solubilizing fungi isolated from soil samples collected from forest, river basin, agricultural fields and rhizosphere of plantation crops of North Bengal by RAPD analysis. In the present investigation, four random decamer primers *viz*., OPD-5, OPB-2, OPB-3 and OPB-6 gave sufficient polymorphism among the isolates of *A. clavatus*, *A.*

niger and *A. melleus*. The amplified fragments ranged from 1100 to 600 bp in size. A total of 127 polymorphic bands were obtained with an average of 31.75 bands/ primer. UPGMA cluster analysis divided the ten isolates into two groups with the genetic similarity ranging from 0.35- 0.61. One group consisted of four isolates of *A. niger* and five isolates of *A. melleus,* while the other group had one isolate of *A. clavatus*.

Shiva Reddy *et al*. (2010) carried out the molecular characterization of ten phosphate solubilizing bacterial strains of *Bacillus megaterium* isolated from soils of different agro-climatic zones of Karnataka by the random amplification of polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique. A total of twenty RAPD bands produced from the selected two primers were used for fingerprinting and for estimation of genetic diversity among ten isolates of *B. megaterium.* The cluster analysis based on twenty RAPD bands revealed that all the ten *B. megaterium* isolates formed two major clusters. To visualize the genetic relatedness among the *B. megaterium* isolates in detail, the principal component analysis (PCA) was done for 81 RAPD bands generated by 10 decamer random primers.

3. MATERIAL AND METHODS

The present investigation entitled, "Variability mapping of beneficial microbes in different soils of Maharashtra" was carried out at the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri during the period from June, 2009 to February, 2011. The aim of the study was to isolate and characterize nitrogen fixing and phosphate solubilizing microorganisms from the rhizosphere soils of various physiographic regions of Maharashtra *viz.*, Western Konkan Coast, Western Ghats (*Sahyadris*) and North Deccan Plateau (Upper, Lower and Metamorphic) and study their functional diversity with respect to their role for soil functioning. It was also intended to study the molecular diversity of some selected nitrogen fixing and phosphate solubilizing isolates with beneficial traits and prepare the map of beneficial microbes for soils of Maharashtra state. The material used and the methods followed for the present investigations are outlined below.

3.1 Survey for collection of rhizosphere soil samples

A total of 150 rhizosphere soil samples (0-15 cm) were collected by using GPS technique as per the soil series prescribed by Challa *et al.* (1999) from different physiographic regions of Maharashtra *viz.*, Western Konkan Coast, Western Ghats (*Sahyadris*) and North Deccan Plateau (Upper, Lower and Metamorphic) so as to study the variability of beneficial microorganisms specially of nitrogen fixing microorganisms (*Azotobacter, Rhizobium* and *Azospirillum*) and phosphate solubilizing microorganisms (PSB and PSF) to ascertain most efficient strains for their functionality in soil. The abstract of soil samples collected from various physiographic regions of Maharashtra for studying the microbial diversity is presented in Table-1.

Table 1 Abstract of soil samples collected from different physiographic regions of Maharashtra for studying the microbial diversity

Sr. No.	Name of the District	* No. of soil samples collected for studying the microbial diversity					Total number of soil samples collected
		Western Konkan Coast	Western Ghat	North Deccan Maharashtra Plateau			
				Upper	Lower	Lower (M)	
1.	Sindhudurg	7	5	-	-	-	12
2.	Ratnagiri	7	2	-	-	-	9
3.	Raigad	4	-	-	-	-	4
4.	Thane	4	4	-	-	-	8
5.	Kolhapur	-	6	5	-	-	11
6.	Satara	-	1	5	-	-	6
7.	Sangli	-	-	5	2	-	7
8.	Pune	-	2	6	-	-	8
9.	Solapur	-	-	3	5	-	8
10.	Ahmednagar	-	-	8	1	-	9
11.	Nasik	1	4	4	6	-	15
12.	Dhule	-	1	1	2	-	4
13.	Nandurbar	-	2	-	-	-	2
14.	Jalgaon	-	-	-	2	-	2
15.	Aurangabad	-	-	-	1	-	1
16.	Beed	-	-	1	2	-	3
17.	Latur	-	-	3	-	-	3
18.	Osmanabad	-	-	1	-	-	1
19.	Parbhani	-	-	-	1	-	1
20.	Hingoli	-	-	-	1	-	1
21.	Nanded	-	-	-	1	-	1
22.	Jalna	-	-	-	1	-	1
23.	Buldhana	-	-	-	1	-	1
24.	Washim	-	-	-	1	-	1
25.	Akola	-	-	-	1	-	1
26.	Amravati	-	-	-	1	-	1
27.	Yeotmal	-	-	-	1	-	1
28.	Wardha	-	-	-	4	-	4
29.	Nagpur	-	-	-	2	11	13
30.	Chandrapur	-	-	-	1	3	4
31.	Bhandara	-	-	-	-	1	1
32.	Gondia	-	-	-	-	1	1
33.	Gadchiroli	-	-	-	-	5	5
	Total	**23**	**27**	**42**	**37**	**21**	**150**

* As per the soil series prescribed by Challa *et al.* (1999).

3.2 Isolation of beneficial microorganisms from soil

The isolation of nitrogen fixing and phosphate solubilizing microorganisms on selective media *viz.*, *Azotobacter* on Jensen's agar medium; *Azospirillum* on nitrogen-free malate semi-solid medium; *Rhizobium* on congo red yeast extract mannitol agar (CRYEMA) medium and phosphate solubilizing microorganisms on Pikovskaya's medium (Appendix-III) was carried out by serial dilution of soil and agar plating method (Aneja, 2003).

Ten gram composite rhizosphere soil samples from each soil series was suspended in 90 ml of sterilized water blanks. Serial dilutions were made from 10^{-1} to 10^{-6}. One ml aliquot of dilutions from 10^{-3} to 10^{-5} was transferred to sterilized petriplates separately. The sterilized selective medium before solidification (45°C temperature) was poured in each petriplate and mixed the contents in plates by rotating the plates gently taking care that medium should not touch the lid. After solidification, plates were kept at 28$\pm$2°C in BOD incubator for 4-5 days. All the plates were observed for the appearance of bacterial/fungal colony.

3.2.1 Selection of *Azotobacter* colony

Azotobacter cells grown as raised, slimy colonies on Jensen's agar medium and aged cultures showed yellowish brown/black colouration due to pigment production. A loopful of *Azotobacter* colony was purified by streak plate method on fresh Jensen's agar plate for single colony. These purified cultures of *Azotobacter* were maintained on the slants of Jensen's agar medium.

3.2.2 Selection of *Rhizobium* colony

Rhizobium cells formed white, translucent, glistening, elevated and comparatively small colonies on the CRYEMA medium. Moreover, the *Agrobacterium* colonies, the most common

contaminant picked up the colour of congo red dye added in the medium. After careful selection, a loopful of *Rhizobium* colony was purified by streak plate method on fresh YEM agar plate for single colony. These purified cultures of *Rhizobium* were maintained on the slants of YEMA medium.

3.2.3 Selection of *Azospirillum* colony

The isolates showing characteristic subsurface pellicle formation were selected from nitrogen-free malate semi-solid medium in test tubes. The isolates were purified by repeated subculturing. A loopful of culture was streaked on malate agar plates containing 1 per cent NH_4Cl. After a week of incubation, typical small, white dense single colonies were picked and transferred to semisolid N-free malate medium in culture tubes. The isolates that formed characteristic subsurface white pellicle in this medium were tentatively considered as *Azospirillum*.

The final purification was done by streaking on potato infusion agar (BMS agar medium). Typical pink often wrinkled colonies on potato infusion agar were transferred to semi-solid medium for storage and characterization.

3.2.4 Selection of phosphate solubilizing bacterial/fungal colony

The formation of clear zone of P-solubilization around the colonies grown on Pikovskaya's medium were selected, purified, subcultured and maintained on the slants of Pikovskaya's agar for further use.

3.2.5 Outline of beneficial microbes isolated from soils of Maharashtra

The details of nitrogen fixing and phosphate solubilizing microorganisms isolated from different soils of Maharashtra in accordance with GPS locations and further coding of these

microorganisms are summarized in Appendix-I and Appendix-II, respectively.

3.3 Population dynamics study

The population of nitrogen fixing (*Azotobacter* and *Rhizobium*) and phosphate solubilizing microorganisms was estimated by counting the number of colonies of the respective organisms per plate by a colony counter and computing the average number of particular group of microborganisms per gram of soil by using the following formula adapted by James (1978):

$$\text{Viable cell count (CFU/g soil)} = \frac{\text{Average plate count x Dilution factor}}{\text{Dry weight of soil}}$$

The population of *Azospirillum* was enumerated from the soils employing the most probable number (MPN) method (Cochran, 1950). This method relies upon the pattern of positive and negative growth of *Azospirillum* in the tubes inoculated with a consecutive series of dilutions (10^{-3}, 10^{-4} and 10^{-5}) of the soil. The positive tubes were observed for the development of subsurface pellicle in the nitrogen-free malate semi-solid medium. Based on number of positive tubes, the population is estimated from the appropriate MPN index numbers of the MPN table by using the following formula:

$$\text{Population of } \textit{Azospirillum}\text{/g of dry soil} = \frac{\text{MPN index X Dilution factor (middle dilution)}}{\text{Dry weight of the sample}}$$

3.4 Morphological and cultural characterization

All the isolates were examined for their cell morphology, colony morphology and gram reaction as per the standard procedures given by Cappuccino and Sherman (1987).

3.4.1. Cell morphology

All the nitrogen fixing and phosphate solubilizing bacterial isolates were examined for their cell morphology (cell shape, cell size and cell arrangement) using the trinocular microscope along with image analysis software DigiPro™ version 4.0. The cell motility test was performed using cavity slide by hanging drop technique.

3.4.2 Gram staining

A thin bacterial smear of one day old culture was made on a clean slide, fixed by gentle heating and Gram stained was performed for all the nitrogen fixing and phosphate solubilizing bacterial isolates by standard procedures given by Cappuccino and Sherman (1987). The isolates which retained dark blue or violet colour of primary stain (crystal violet) were gram-positive, whereas those that lose the crystal violet and counter stained by safranin (appeared red) were referred as gram-negative.

3.4.3. Colony morphology

The colonial morphology (colony growth, form, margin, elevation, appearance and pigmentation) of all the nitrogen fixing and phosphate solubilizing bacterial isolates was examined by culturing on variety of media.

3.4.4. Morphological and cultural study of fungal isolates

The phosphate solubilizing fungal isolates were identified on the basis of cultural and microscopic features followed by the method of Subramanian (Subramanian, 1971; Barnet and Hunter, 1972; Aneja, 2003). The colonial morphology of fungal isolates was examined on Pikovskaya's agar medium and microscopic appearance by lactophenol cotton blue staining technique which determined the type of reproductive mycelium i.e. conidiophores.

3.5 Biochemical characterization

The isolates were subjected to biochemical characterization by employing the standard procedures given by Cappuccino and Sherman (1987). Different biochemical tests performed are briefly outlined below.

3.5.1 Starch hydrolysis

Starch agar medium (Appendix-III) was poured in petriplates. A single streak inoculation of organism was done into the centre of plate and incubated at 28±2°C for 24-48 hrs. Upon incubation, the plates were flooded with 10 ml of iodine solution (Appendix-I). Hydrolyzed starch appeared as clear zone around microbial colony because of β-amylase activity. Reddish brown zone around the colony indicated partial hydrolysis of starch as a result of α-amylase activity. The unhydrolyzed starch formed a blue colour with iodine.

3.5.2 H_2S production

Large test tubes having SIM agar (Appendix-III), containing $Fe(NH_4)SO_4$ that behave as the H_2S indicator adjusted to pH 7.3 were used and inoculated with each isolate. Ferrous ammonium sulphate in the medium serves as an indicator by combining with the gas forming an insoluble black ferrous sulphide precipitate that was seen along the line of stab inoculation and is indicative of H_2S production. Observation for blackening in tubes was taken after three days of incubation.

3.5.3 Gelatin liquefaction

The medium consisting of nutrient broth supplemented with 12 per cent gelatin (Appendix-III) was used. The test tubes containing medium was inoculated with the bacterial isolates and

incubated for 24 hrs at 28±2°C. Following incubation, the cultures were placed in a refrigerator at 4°C for 30 minutes. The cultures that remain liquefied indicated gelatinase and demonstrate gelatin hydrolysis, whereas the cultures those solidify on refrigeration lack gelatinase activity and gave negative reaction.

3.5.4 Catalase test

A loopful of growth was placed on a glass slide and emulsified with drops of H_2O_2 10 per cent (v/v). The effervescence caused by liberation of free gas bubbles was considered for the presence of catalse in culture (MacFaddin, 1980).

3.5.5 Oxidase test

Oxidase test was performed according to Steel (1961). Tetramethyl-para-phenylenediamine dihydrochloride (Appendix-III) reagent dissolved in warm water and stored in dark bottle. A strip of filter paper was dipped in this reagent and air dried. With the help of sterile wire loop, one day old colonies of bacterial cultures from trypticase soy agar (Appendix-III) plates were transferred on this filter paper strip. The observations for presence or absence of colony colour changes from pink to maroon and finally to black was recorded. The change of colour indicated a positive test.

3.6 Physiological characterization

3.6.1 Utilization of different carbon sources

The culture medium was amended with different carbon sources and autoclaved. Sterilized petriplates were poured with 15-20 ml medium with different carbon sources and allowed to solidify. After solidification, 10 µl of 24 hrs old cultures of the test

organisms were spotted on plates with each carbon source. The plates were incubated for 48 hrs at 28 ±2°C and the ability of the isolates to grow on different carbon sources was noted.

Sr. No.	Microorganism	Culture medium	Carbon source utilization
1.	*Azotobacter*	Jensen's agar medium	Mannitol, glucose, sucrose, fructose, malate and citrate
2.	*Rhizobium*	CRYEMA medium	Mannitol, glucose, sucrose, arabinose, malate and citrate
3.	*Azospirillum*	N-free malate semi-solid medium	Glucose and malic acid
4.	PSB	Pikovskaya's medium	Fructose, glucose, maltose, mannitol and citrate

3.7 Functional diversity of nitrogen fixing microorganisms

3.7.1 Nitrogen fixing ability of free-living bacteria

Nitrogenase enzyme catalyses the reduction of atmospheric nitrogen in biological systems and is also capable of reducing several other substrates like acetylene (C_2H_2). The acetylene is an inhibitor of N_2-reduction and is converted by nitrogenase to ethylene (C_2H_4) led to development of a simple method for determining N_2-fixation.

3.7.1.1 Assay of nitrogenase activity by acetylene reduction method

The acetylene reduction assay (ARA) is an indirect method for measuring N_2-fixation at a time point (Hardy *et al.*, 1968). It is a highly sensitive and non-destructive method. The technique involves incubation of nitrogenase containing system (free-living bacteria) in a known atmosphere of acetylene (10% C_2H_2) in the gas phase, and after an optimum time of incubation (24 hrs), ethylene

(C_2H_4) produced is measured by a gas chromatograph using flame ionization detector (FID).

Equipment and glasswares:

1. Assay vials/containers with rubber serum stoppers.
2. Compressed acetylene (C_2H_2) gas cylinder
3. Compressed ethylene (C_2H_4) standard gas cylinder
4. Compressed N_2 and H_2 gas cylinder
5. Gas tight syringes (1, 2, 5 and 10 ml)
6. BOD incubator
7. Gas chromatograph (Model: Varian GC-3700) with Porapak T column and FID detector

3.7.1.2 Procedure for acetylene reduction assay (ARA) for free-living bacteria

Free living bacteria like *Azotobacter* and *Azospirillum* expressed nitrogenase activity on Jensen's agar and semisolid nitrogen free malate medium, respectively, but a few bacteria like rhizobia required stringent conditions of growth for the expression of acetylene reduction activity. The specific defined medium used for the induction of nitrogenase in free-living *Rhizobium* was solid CS 7 medium (Appendix-III) (Pagan *et al.*, 1975).

The following steps were followed for the assay:

1. The slants were prepared by taking 5 ml CS 7 medium in each of 15 ml test tubes for *Rhizobium*. The Jensen's agar medium was used for preparing slants for *Azotobacter* and semisolid nitrogen-free malate medium for *Azospirillum*.
2. A loopful culture of each isolate of respective organism in three replicates was streaked on the slants and incubated at 28±2°C for 8 days.
3. The cotton plugs were replaced with air tight rubber serum stoppers.

4. 10% of air in test tube was removed and replaced with equal volume of acetylene gas using gas-tight syringe.
5. The tubes were incubated at 28±2°C for 24 hrs.
6. After completion of incubation period, 1 ml of gas sample from test tube was injected into the pre-conditioned gas chromatograph for ethylene estimation and recorded the output data as peaks using a strip-chart recorder and noted the attenuation.
7. The volume of assay tube gas phase was recorded.
8. Similarly recorded values of 1 ml standard ethylene gas.
9. Total protein of each sample tube was estimated by collecting bacterial cells in 2 ml of 2N NaOH. Keep the cell suspension in boiling water for 10 min. Cool it, neutralize with 2 ml of 2N HCl and protein was estimated by Lowry's method using Folin-Phenol reagent (Lowry *et al.*, 1951).
10. The nitrogenase activity was calculated by using following formula.

$$\text{nmole } C_2H_4 \text{ produced.mg protein}^{-1}.\text{hr}^{-1} = \frac{Ce \times Ps \times Va \times As \times 60}{Pstd \times Vs \times Astd \times T \times P}$$

Where,

Ce = Concentration of ethylene in standard (in nmoles)

Ps = Peak height for sample (in cm)

Va = Volume of assay tube gas phage (in ml)

As = Attenuation used for sample

Pstd = Peak height for standard ethylene (in cm)

Vs = Volume of gas sample injected for analysis (in ml)

Astd = Attenuation used for standard ethylene

T = Time for incubation (in min).

P = Protein content of bacterial growth on slant (in mg)

3.8 Functional diversity of phosphate solubilizing microorganisms

3.8.1 Phosphate solubilizing ability of the bacterial isolates

The ability of the bacterial isolates to solubilize insoluble inorganic phosphate was tested by spotting 10 µl overnight cultures on Pikovskaya's agar (Appendix III) plates and incubating at 28-30°C for 2-3 days. The isolates which showed clear zone of solubilization of tricalcium phosphate (TCP) around the colony were noted as phosphate solubilizers. The diameter of the zone of TCP solubilization was measured and expressed in millimeters.

3.8.2. Quantitative estimation of Pi released from tricalcium phosphate for bacterial isolates

The bacterial isolates positive for P solubilization on Pikovskaya's agar medium were subjected to quantification of Pi released from TCP in broth medium. The Erlenmeyer flasks containing 50 ml Pikovskaya's broth (Pikovskaya, 1948) were inoculated with 500 µl overnight culture of each isolate in two replicates and incubated for 10 days at 28 ± 2°C. The amount of Pi released in the broth was estimated at 10 days of incubation in comparison with the uninoculated control. The reduction in pH of the broth from the initially adjusted pH of 7.0 was also noted after 10 days of incubation so as to monitor the amount of acidity produced and study its correlation with the Pi released. The TCP broth cultures were spun at 10,000 rpm for 10 minutes to separate the cells and insoluble phosphate and the available P content of the supernatant was estimated by using phosphomolybdic blue colour method (Jackson, 1973).

3.8.3 Phosphate solubilizing ability of the fungal isolates

The ability of the fungal isolates to solubilize insoluble inorganic phosphate was carried out by allowing the fungi to grow on selective media, i.e. Pikovskaya's agar plates and incubating at

28-30°C for 7-10 days. The appearance of a transparent halo zone around the fungal colony indicated the phosphate solubilizing activity of the fungus. The diameter of the zone of TCP solubilization was measured and expressed in millimeters.

3.8.4. Quantitative estimation of Pi released from tricalcium phosphate for fungal isolates

The fungal isolates positive for P solubilization on Pikovskaya's agar medium were subjected to quantification of Pi released from TCP in broth medium. The Erlenmeyer flasks containing 50 ml Pikovskaya's broth (Pikovskaya, 1948) were inoculated with 5 mm mycelial discs from 7 day old culture of each fungal isolate grown on PDA in two replicates and incubated for 10 days at 28±2°C. The pH of the medium was adjusted to 7.0. The mycelia were harvested after 10 days of incubation by filtering and the change in the pH of the culture filtrate was recorded after centrifuging the medium at 10000 rpm for 5 min. The available P content of the supernatant was estimated by using phosphomolybdic blue colour method (Jackson, 1973) described below.

Reagents used

Chloromolybdic acid

For preparing this reagent, 7.5 g of ammonium molybdate was dissolved in 150 ml distilled water followed by addition of 162 ml conc. HCl. The total volume was made to one liter with distilled water and the reagent was stored in amber coloured bottle at 4°C.

Chlorostannous acid

This reagent was prepared by dissolving 25 g of $SnCl_2.2H_2O$ in 100 ml conc. HCl and making the total volume to one liter with distilled water. It was also stored in amber colored bottle at 4°C.

Procedure

One ml of the TCP broth culture supernatant was dispensed in 50 ml volumetric flask. To this, 10 ml of chloromolybdic acid was added, mixed thoroughly and the volume was made to approximately 3/4th with distilled water. Chlorostannous acid (0.25 ml) was added and the final volume was made to 50 ml with distilled water and mixed thoroughly. The flasks were kept 15 min for colour development and the blue colour developed was read in a spectrophotometer at 610 nm using a reagent blank.

Preparation of standard curve

Potassium dihydrogen phosphate (0.2195 g KH_2PO_4) dried at 40°C for 3-4 hrs was dissolved in 400 ml distilled water. After addition of 25 ml of 7N H_2SO_4 to it, the volume was made to one liter with distilled water and mixed thoroughly. Twenty ml of this was diluted further to 500 ml with distilled water to obtain 2 ppm stock P solution.

The standards of P (0 to 2 ppm P) were prepared by using this stock 2 ppm P solution. These standards were also subjected to colour development as above and read in a spectrophotometer at 610 nm. The standard curve of P was plotted and the amount of P solubilized (per cent Pi released) by the isolates from TCP was calculated by using following formula:

$$\% \text{ Pi released} = \frac{R \times \text{Volume of chlomolybdic acid} \times \text{Final volume made}}{\text{Volume of chlorostannous acid} \times \text{Aliquot taken}} \times \frac{1}{100}$$

Where,

R = O.D. x slope factor

O.D. = Optical density (absorbance at 610 nm)

$$\text{Slope factor} = \frac{\text{Sum of standards of P conc.}}{\text{Sum of O.D.}}$$

Volume of chlomolybdic acid used = 10 ml

Volume of chlorostannous acid used = 0.25 ml

Final volume made = 50 ml

Aliquot taken = 1 ml of TCP broth culture supernatant/ fungal filtrate

3.9 Molecular diversity of selected nitrogen fixing and phosphate solubilizing microorganisms

The molecular diversity within each group of the selected nitrogen fixing and phosphate solubilizing isolates along with the standard MPKV strains was analyzed by employing random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique as described below.

Sr. No.	Microorganism	Number of efficient strains used for diversity analysis	Standard MPKV strains
1.	*Azotobacter chroococcum*	12	Azt-BNF
2.	*Rhizobium* spp.	7	Rh-BNF
3.	*Azospirillum lipoferum*	7	Asp-BNF
4.	PSB- *Bacillus megaterium*	8	PSB-BNF
5.	PSF		
i.	*Aspergillus awamori*	8	PSF-BNF(A)
ii.	*Penicillium digitatum*	5	PSF-BNF(P)

3.9.1 Isolation of genomic DNA of bacteria

The isolation of genomic DNA of nitrogen fixing (*Azotobacter, Rhizobium* and *Azospirillum*) and phosphate solubilizing (*Bacillus*) isolates and MPKV standard strains [Azt-BNF, Rh-BNF, Asp-BNF and PSB-BNF] was carried out by following method, modified that of Shiva Reddy *et al.* (2010). The chemicals and reagents used for DNA isolation are listed in Appendix-IV.

Procedure for isolation of genomic DNA of bacteria:

1. The bacterial isolates were grown in LB broth for 24-48 hrs.
2. About 1.5 ml of bacterial culture was taken in micro centrifuge tube, centrifuged at 12000 rpm for 2 min. This procedure was repeated for six times.

3. The supernatant was discarded and the cell pellets dissolved in 500 µl of extraction buffer (2% sarcosyl in T_{50} E_{20}). After that 10 µl of RNase A and 5 µl of Protease K were added per sample.
4. The samples were incubated in water bath at 50°C for 60 min.
5. Each sample was vortex for 1-2 min.
6. After that equal volume of Tris-HCl saturated phenol (pH 8.0) was added and centrifuged at 12000 rpm for 10 min.
7. The upper face was collected in new micro centrifuge tube. To that tube equal volume of chloroform was added and centrifuged at 12000 rpm for 10 min. and the upper face was collected.
8. To the upper face 0.7 volume of chilled isopropanol was added and mix thoroughly and kept in -20°C for overnight.
9. Again centrifuged at 12000 rpm for 15 min. at 4°C.
10. The supernatant was discarded and 250 µl 70% chilled ethanol was added and centrifuged at 12000 rpm for 5 min.
11. The supernatant was discarded.
12. The DNA pellet was dried and resuspended in 100 µl of TE buffer.

3.9.2 Isolation of genomic DNA of fungi

The isolation of genomic DNA of phosphate solubilizing fungal isolates (*Aspergillus* and *Penicillium*) and MPKV standard strains [PSF-BNF(A) and PSF-BNF(P)] was carried out by following method, modified that of Mittal *et al.* (2008). The chemicals and reagents used for DNA isolation are listed in Appendix-IV.

Procedure for isolation of genomic DNA of fungi:

The genomic DNA was extracted from the mycelial mat of each fungal isolate grown on PDA for 7 days at 28±2°C. For this, aerial mycelia were collected by scrapping it from the agar surface with sterile scalpel and the mycelial mat was suspended in sterile

distilled water for washing. These mats were further taken out and placed in aluminum foil for drying at 60°C in hot air oven for half an hour. After drying the mycelial mats were collected in another aluminum foil and kept in freeze at 4°C. Further the genomic DNA was isolated by applying following procedure.

1. 100mg of dried fungal mat was ground to a fine powder in liquid nitrogen using a mortar and pestle without allowing it to thaw.
2. The contents in the morter was transferred to the preheated micro centrifuge tubes and mixed with 1ml of CTAB extraction buffer. After that 5µl of β-mercaptoethanol and 10 µl of RNase A were added per sample.
3. The tubes were inverted for several times and incubated for 1 hr. at 60°C with intermittent shaking at 10 min. interval.
4. The tubes were cooled to room temperature and each sample was vortex for 1-2min.
5. After that equal volume of Tris-HCl saturated phenol (pH 8.0) was added and centrifuged at 12000 rpm for 10 min.
6. The upper face was collected in new micro centrifuge tube. To that tube equal volume of chloroform was added and centrifuged at 12000 rpm for 10 min. and the upper face was collected.
7. To the upper face 0.7 volume of chilled isopropanol was added and mix thoroughly and kept in -20°C for overnight.
8. Again centrifuged at 12000 rpm for 15 min. at 4°C.
9. The supernatant was discarded and 250 µl 70% chilled ethanol was added and centrifuged at 12000 rpm for 5 min.
10. The supernatant was discarded.
11. The DNA pellet was dried and resuspended in 100 µl of TE buffer.

3.9.3 Quantification of genomic DNA

The quantification of genomic DNA was done both by gel electrophoresis and NanoDrop ND-1000 USA, UV visible spectrophotometer. The 2 µl DNA samples were subjected to gel electrophoresis using 0.8% agarose along with molecular weight marker DNA (2µl λ DNA having concentration of 20 ng/µl). The gel was observed under UV transilluminator and the size of DNA bands was compared with λ DNA marker and concentration of DNA was expressed as ng/µL.

In NanoDrop ND-1000 USA, UV visible spectrophotometer, ultra violet light of specific wavelength (260 nm for DNA and 280 nm for protein) was passed through sample. Biomolecules present in the sample were responsible for the absorbance of the light and absorbed light was directly proportional to quantity of biomolecules in the sample. A part of light get absorbed in the sample and measured as OD. The DNA obtained in sample having ratio of ODs (260/280nm) near 1.8 showed good quality of DNA. The concentration of DNA was expressed as ng/µL.

3.9.4 Agarose gel electrophoresis

The DNA samples were subjected to gel electrophoresis using 0.8 per cent agarose along with molecular weight marker DNA (λ DNA Hind III digest). The gel was observed under UV transilluminator and documented.

3.9.5 RAPD-PCR

The molecular diversity and polymorphism within each group of the selected nitrogen fixing and phosphate solubilizing isolates along with the standard MPKV strains were determined by the random amplification of polymorphic DNA-polymerase chain reaction (RAPD-

PCR) technique adapted by Teaumroong and Boonkerd (1998). The details are given below.

3.9.5.1 Template DNA

The purified genomic DNA samples from the individual isolates were diluted to working concentration of 20 ng/µl and used as template DNA.

3.9.5.2 Random primers

The following commercial kits of random decamer DNA primers were obtained from M/S. Bangalore GeNei Pvt. Ltd., Bangalore, India. A total of fifty random decamer DNA primers were used. Each of these primers (3 O.D. = 99 µg = 30000 picomoles]) were dissolved in 1500 µl sterilized milli Q water to get final concentration of 20 picomoles/µl and incubated at room temperature for 3-4 hrs. by vortexing frequently in between. The DNA sequences (5' – 3') of twenty five each of bacterial and fungal RAPD primers used for DNA amplification are given in Table 2 and Table 3, respectively.

3.9.5.3 Taq DNA polymerase

Taq DNA polymerase and 10X Taq buffer A and Taq buffer B obtained from M/S. Bangalore Genei Pvt. Ltd., Bangalore were used.

3.9.5.4 Deoxynucleotide triphosphates

dNTP mix obtained from M/S. Bangalore Genei Pvt. Ltd., Bangalore was used.

3.9.5.5 Thermocycler

Eppendorf Mastercycler, Germany was used for the cyclic amplification of DNA.

Table 2 Bacterial RAPD primers and their sequences

Sr. No.	Primer Name	Primer Sequences (5'- 3')
1	RBA 1	5' AAA ACC GGG C 3'
2	RBA 2	5' ACA GGG CTC T 3'
3	RBA 3	5' ACA GGG GTG T 3'
4	RBA 4	5' ACC GGG TTT C 3'
5	RBA 5	5' AGG GGC GGC A 3'
6	RBA 6	5' ATC CTG CCT G 3'
7	RBA 7	5' ATC GGG TCC T 3'
8	RBA 8	5' ATC GGG TCG A 3'
9	RBA 9	5' ATC TGC GAG C 3'
10	RBA 10	5' CCC GCC TTC C 3'
11	RBA 11	5' CCG GCC CCA A 3'
12	RBA 12	5' CCG GCC TTA A 3'
13	RBA 13	5' CCG GCC ATA C 3'
14	RBA 14	5' CCG GCC TTC C 3'
15	RBA 15	5' CCG GCT GGA A 3'
16	RBA 16	5' CCG GGG AAA C 3'
17	RBA 17	5' CCG GGG TTA T 3'
18	RBA 18	5' CCG GGG TTT G 3'
19	RBA 19	5' CCT GCG CTT A 3'
20	RBA 20	5' CCT GGC GGT G 3'
21	RBA 21	5' CCT GGG CCA G 3'
22	RBA 22	5' CCT GGG CTG G 3'
23	RBA 23	5' CCT GGG CTT G 3'
24	RBA 24	5' CCT GGG CTT A 3'
25	RBA 25	5' CCT GGG GGA T 3'

Table 3 Fungal RAPD primers and their sequences

Sr. No.	Primer Name	Primer Sequences (5'- 3')
1	RFU 1	5' CCT GGG CCA G 3'
2	RFU 2	5' CCT GGG CGA G 3'
3	RFU 3	5' CCT GGG CTG G 3'
4	RFU 4	5' CCT GGG CTA T 3'
5	RFU 5	5' CCT GGG CTT G 3'
6	RFU 6	5' CCT GGG CTA C 3'
7	RFU 7	5' CCT GGG CTT A 3'
8	RFU 8	5' CCT GGG TCG A 3'
9	RFU 9	5' CCT GGG TGC A 3'
10	RFU 10	5' CCT GGG TGA C 3'
11	RFU 11	5' CCT GGC TTA C 3'
12	RFU 12	5' CCT GGG TTA C 3'
13	RFU 13	5' CGG GGG ATG G 3'
14	RFU 14	5' CTC CCT GAC C 3'
15	RFU 15	5' GAG CAC CTG T 3'
16	RFU 16	5' GAG CAC GTC A 3'
17	RFU 17	5' GAG CAC GGC A 3'
18	RFU 18	5' GAG CAC GGA G 3'
19	RFU 19	5' GAG CTC GCA T 3'
20	RFU 20	5' GAG GGC ATG T 3'
21	RFU 21	5' CCG GCC CCA A 3'
22	RFU 22	5' CCG GCC TTA A 3'
23	RFU 23	5' CCG GCC ATA C 3'
24	RFU 24	5' CCG GCC TTC C 3'
25	RFU 25	5' CCG GCT GGA A 3'

3.9.5.6 Reaction mix

One primer was used at a time to study the polymorphism within each group of the selected nitrogen fixing and phosphate solubilizing isolates along with the standard MPKV strains by RAPD-PCR assay with genomic DNA extracts from all the strains as template DNA. The master mix (Appendix-IV) required for each isolate was freshly prepared to avoid handling errors. The master mix was distributed as 19 µl per tube and 1 µl of template DNA from the respective isolates was added to make the total reaction volume to 20 µl.

3.9.5.7 PCR amplification

The DNA amplification was done by following the PCR method proposed by Castrillo and Brooks (1998) with certain modifications as shown below:

Cycle	Stage	Temperature (°C)	Duration (min.)	No. of cycles
I	Initial denaturation	94	5	1
II	Denaturation Annealing Extension	94 36 72	1 1 2	40
III	Final Extension Hold	72 4	10 30	1 -

After completing specified cycles of amplification, the samples were stored at 4°C in a refrigerator and later the contents were subjected to agarose gel electrophoresis.

3.9.5.8 Separation of amplified products by agarose gel electrophoresis

The reagents required for agarose gel electrophoresis are listed in Appendix IV. Twenty µl of the amplified products from each tube along with 4 µl of loading dye (Appendix IV) were separated on 1.2 per cent agarose gel (Appendix IV) using 1X TBE buffer of pH 8.0

(Appendix IV) along with 7 µl 1kb gene ruler (Fermentas) DNA ladder. Electrophoresis was performed for 3 hrs at 80 volts in a submarine electrophoresis apparatus (Bio Rad No. 96). The gel was stained with Ethidium bromide (0.1%) and photographed using gel documentation system (using FluorChem software).

3.9.5.9 Analysis of RAPD profiles

The amplification profiles for all the primers were compared with each other and the bands of DNA fragments were scored as present (1) or absent (0) generating the binary (0/1) matrices in MS Excel 1997-2003 format.

The binary data were analyzed under the SIMQUAL module of NTSYS PC-2.0 (Rohlf, 1998) using Dice Coefficient (Nei and Li, 1979). SAHN module based on UPGMA clustering method (Sneith and Sokal, 1973) was used to generate a tree (dendrogram).

Principal coordinate analysis (PCO) was performed to estimate the genetic distance between each group of the isolates by using NTSYS software. It involves first transforming similarity value to scalar products by DCENTER. Then analyzing the product matrix by EIGEN to get Eigenvectors (which is PCO) and Eigenvalues, and finally getting its 2D/3D scatter plot graph.

The per cent polymorphism was computed as,

$$\text{Per cent polymorphism} = \frac{\text{Total number of polymorphic bands}}{\text{Total number of bands}} \times 100$$

3.10 Assessment of bacterial diversity

Different indices of diversity *viz.*, Shannon's index, evenness index and richness index were computed for all the nitrogen fixing and phosphate solubilizing isolates as per the procedure elaborated by Ludwig and Reynolds (1988).

3.10.1 Shannon's index

It determines the average degree of uncertainty in predicting to what species individuals chosen at random from a collection of 'S' species and 'N' individuals will belong. This average uncertainty increases as the number of species becomes even. Thus $H^1 = 0$ if and only if there is one species in the sample and it is maximum only when all species are represented by the same number of individuals. It was estimated by using the formula,

$$H^1 = - \sum_{i=1}^{S} [(ni/N) \text{ In } (ni/N)]$$

Where, ni = Number of individuals belonging to the i^{th} species.
N = Total number of individuals in the study area.

3.10.2 Evenness index

The evenness index was calculated using the formula,

$$E = \frac{H^1}{\ln(S)}$$

Where, H^1 = Shannon's index
S = Number of species

Usually E approaches to zero as a single species become more and more dominant in a community. The value of E increases with evenness of the species.

3.10.3 Richness index

Richness index was calculated as follows

$$R = \frac{S}{N\ ½}$$

Where, S = Number of species
N = Number of isolates

3.11 Statistical analysis

The statistical analysis of the data was carried out by employing completely randomized design (CRD). The critical differences were calculated at P=0.01 level of significance for the *in-vitro* experiments wherever F tests were significant and interpretation of the results was carried out in accordance with Panse and Sukhatme (1985).

4. EXPERIMENTAL RESULTS

The present study covered the isolation and characterization of nitrogen fixing and phosphate solubilizing microorganisms from the rhizosphere soils of various physiographic regions of Maharashtra *viz.*, Western Konkan Coast, Western Ghats (*Sahyadris*), Western Maharashtra, North Maharashtra, Marathwada and Vidarbha. The isolates were tested for their beneficial traits like N_2-fixation and P-solubilizaiton. Based on the *in vitro* analysis, the efficient strains were further analyzed for molecular diversity.

4.1 Isolation of beneficial microorganisms from soil

One hundred fifty rhizosphere soil samples collected from various physiographic regions of Maharashtra were screened for the presence of nitrogen fixing and phosphate solubilizing microorganisms on a variety of culture media. A total of 263 nitrogen fixing and 93 phosphate solubilizing microorganisms were isolated and presented in Table 4. Among the nitrogen fixers, 94 *Azotobacter*, 76 *Rhizobium* and 93 *Azospirillum* isolates were obtained. Among the phosphate solubilizers, 47 phosphate solubilizing bacteria and 46 phosphate solubilizing fungi were isolated (Plate 1 to 8).

The details of N_2-fixing and P-solubilizing isolates obtained from various physiographic regions of Maharashtra in accordance with GPS locations and further coding of these microorganisms are summarized in Appendix-I and Appendix-II, respectively.

Table 4 Abstract of nitrogen fixing and phosphate solubilizing microorganisms isolated from different physiographic regions of Maharashtra

a) Nitrogen fixing microorganisms:

Sr. No.	Physiographic Regions	Species of nitrogen fixing microorganisms				Total isolates
		Azotobacter chroococcum	*Rhizobium* spp.	*Azospirillum lipoferum*	*Azospirillum brasilense*	
1	Western Konkan Coast	8	3	11	2	24
2	Western Ghats	19	9	22	1	51
3	Western Maharashtra	28	24	18	4	74
4	North Maharashtra	9	9	3	1	22
5	Marathwada	9	10	11	-	30
6	Vidarbha	21	21	18	2	62
	Total	**94**	**76**	**83**	**10**	**263**

b) Phosphate solubilizing microorganisms:

Sr. No.	Physiographic Regions	Species of phosphate solubilizing microorganisms			Total isolates
		Bacillus megaterium	*Aspergillus awamori*	*Penicillium digitatum*	
1	Western Konkan Coast	6	5	-	11
2	Western Ghats	7	5	1	13
3	Western Maharashtra	14	4	7	25
4	North Maharashtra	3	4	1	8
5	Marathwada	6	3	3	12
6	Vidarbha	11	8	5	24
	Total	**47**	**29**	**17**	**93**

4.2 Population dynamics study

4.2.1 *Azotobacter* isolates

A wide variation in microbial population among the different isolates of *Azotobacter* was observed and presented in Table 5. The *Azotobacter* population ranged from $1.03x10^4$ to $10.17x10^4$ CFU g^{-1} soil. The significantly highest *Azotobacter* population ($10.17x10^4$ CFU g^{-1} soil) was recorded by Azt-70 isolate obtained from Kolhapur district of Western Maharashtra (16° 16'5.24" North latitude and 74° 14'17.9" East longitude) followed by Azt-148 and Azt-113 isolate ($9.67x10^4$ CFU g^{-1} soil) from Bhandara and Solapur district of Vidarbha and Western Maharashtra (20°51'3"N lat. 80°13'9"E long. and 17°51'30"N lat. 75°45'30"E long.), respectively.

4.2.2 *Rhizobium* isolates

The colony counts of free living rhizobial strains isolated from rhizosphere soils of different regions of Maharashtra revealed significant variations ($1.07x10^4$ to $8.33x10^4$ CFU g^{-1} soil) (Table 6). Among the different *Rhizobium* isolates, maximum values of colony count ($8.33x10^4$) was obtained for Rh-72 isolate recovered from Latur district of Marathwada region (18°28'0"N lat. 76°41'00"E long.) whereas the lowest microbial count ($1.07x10^4$) coming from Rh-98 isolate of Nasik district of North Maharashtra (19°54'55"N lat. 73°42'00"E long.).

4.2.3 *Azospirillum* isolates

The microbial population of *Azospirillum* isolates varied among the different regions of Maharashtra (Table 7). The significantly highest *Azospirillum* count ($5.40x10^4$ MPN g^{-1} soil) was recorded by Asp-28 isolate obtained from the rhizosphere soils of Kolhapur district of Western Maharashtra (17°01'52"N lat. 73°52'06"E long.) followed by Asp-72 ($4.50x10^4$ MPN g^{-1} soil) and

Table 5 Population dynamics of *Azotobacter* isolates by serial dilution of soil and agar-plating method.

Sr. No	Isolate	*Azotobacter* count (x 10^4 CFU g^{-1} soil)	Sr. No	Isolate	*Azotobacter* count (x 10^4 CFU g^{-1} soil)
1	Azt-4	3.45 (4.53)	27	Azt-50	8.33 (4.92)
2	Azt-8	3.67 (4.56)	28	Azt-52	4.33 (4.62)
3	Azt-9	1.17 (4.07)	29	Azt-53	4.50 (4.64)
4	Azt-10	3.83 (4.57)	30	Azt-54	3.50 (4.53)
5	Azt-12	1.33 (4.12)	31	Azt-55	3.17 (4.47)
6	Azt-18	1.11 (4.04)	32	Azt-56	5.50 (4.73)
7	Azt-21	9.33 (4.97)	33	Azt-57	2.67 (4.43)
8	Azt-23	4.83 (4.68)	34	Azt-58	3.67 (4.54)
9	Azt-25	5.50 (4.74)	35	Azt-60	3.16 (4.49)
10	Azt-26	4.17 (4.61)	36	Azt-61	3.50 (4.53)
11	Azt-27	7.67 (4.88)	37	Azt-63	2.33 (4.35)
12	Azt-28	3.37 (4.51)	38	Azt-64	6.67 (4.81)
13	Azt-29	5.17 (4.71)	39	Azt-65	1.67 (4.20)
14	Azt-32	3.87 (4.57)	40	Azt-66	5.00 (4.69)
15	Azt-33	5.33 (4.72)	41	Azt-67	3.83 (4.57)
16	Azt-34	2.83 (4.44)	42	Azt-68	5.16 (4.70)
17	Azt-36	1.50 (4.16)	43	Azt-69	6.50 (4.80)
18	Azt-37	4.67 (4.66)	44	Azt-70	10.17 (5.00)
19	Azt-39	2.00 (4.26)	45	Azt-72	6.33 (4.79)
20	Azt-40	4.67 (4.66)	46	Azt-73	4.17 (4.62)
21	Azt-42	4.83 (4.68)	47	Azt-75	5.33 (4.72)
22	Azt-43	3.33 (4.52)	48	Azt-77	6.00 (4.77)
23	Azt-44	6.17 (4.79)	49	Azt-78	4.00 (4.59)
24	Azt-45	1.07 (4.03)	50	Azt-79	5.17 (4.70)
25	Azt-48	4.00 (4.59)	51	Azt-81	3.67 (4.54)
26	Azt-49	4.50 (4.65)	52	Azt-82	8.10 (4.90)

contd…

Table 5 contd…

Sr. No	Isolate	*Azotobacter* count (x 10^4 CFU g^{-1} soil)	Sr. No	Isolate	*Azotobacter* count (x 10^4 CFU g^{-1} soil)
53	Azt-83	6.11 (4.78)	74	Azt-114	4.67 (4.65)
54	Azt-84	4.87 (4.68)	75	Azt-118	5.33 (4.72)
55	Azt-86	3.67 (4.55)	76	Azt-120	3.50 (4.53)
56	Azt-88	1.03 (4.01)	77	Azt-122	2.00 (4.29)
57	Azt-89	2.87 (4.44)	78	Azt-125	2.83 (4.44)
58	Azt-91	1.67 (4.19)	79	Azt-126	1.17 (4.06)
59	Azt-93	1.33 (4.11)	80	Azt-129	6.33 (4.79)
60	Azt-94	1.11 (4.04)	81	Azt-130	4.50 (4.65)
61	Azt-97	3.67 (4.55)	82	Azt-131	4.00 (4.59)
62	Azt-99	2.33 (4.35)	83	Azt-132	5.33 (4.72)
63	Azt-101	4.67 (4.66)	84	Azt-133	3.17 (4.49)
64	Azt-102	3.17 (4.49)	85	Azt-135	4.50 (4.65)
65	Azt-104	1.21 (4.08)	86	Azt-136	2.33 (4.35)
66	Azt-105	2.00 (4.28)	87	Azt-137	2.67 (4.41)
67	Azt-106	4.67 (4.65)	88	Azt-138	3.50 (4.54)
68	Azt-107	4.83 (4.67)	89	Azt-141	4.83 (4.67)
69	Azt-108	3.33 (4.51)	90	Azt-142	4.17 (4.62)
70	Azt-109	5.17 (4.71)	91	Azt-144	2.83 (4.43)
71	Azt-110	5.67 (4.74)	92	Azt-147	5.17 (4.71)
72	Azt-112	3.33 (4.51)	93	Azt-148	9.67 (4.98)
73	Azt-113	9.67 (4.98)	94	Azt-149	6.33 (4.79)

	S.E. $\pm$	0.10
	C.D. at 1%	0.36
	C.V.%	2.98

Figures in parentheses represent the logarithmic transformation values for statistical analysis

Table 6 Population dynamics of *Rhizobium* isolates by serial dilution of soil and agar-plating method

Sr. No	Isolate	*Rhizobium* count (x 10^4 CFU g^{-1} soil)	Sr. No	Isolate	*Rhizobium* count (x 10^4 CFU g^{-1} soil)
1	Rh-13	4.17 (4.60)	39	Rh-85	3.83 (4.58)
2	Rh-21	5.50 (4.73)	40	Rh-86	4.33 (4.62)
3	Rh-22	3.67 (4.55)	41	Rh-93	1.33 (4.11)
4	Rh-28	3.83 (4.58)	42	Rh-95	1.67 (4.20)
5	Rh-29	3.33 (4.51)	43	Rh-97	3.50 (4.52)
6	Rh-37	4.50 (4.64)	44	Rh-98	1.07 (4.03)
7	Rh-39	2.17 (4.33)	45	Rh-100	3.67 (4.54)
8	Rh-40	3.67 (4.55)	46	Rh-101	4.17 (4.61)
9	Rh-42	1.83 (4.23)	47	Rh-102	3.83 (4.58)
10	Rh-43	1.17 (4.06)	48	Rh-105	6.17 (4.78)
11	Rh-44	5.50 (4.73)	49	Rh-106	5.50 (4.73)
12	Rh-50	4.50 (4.64)	50	Rh-107	4.67 (4.66)
13	Rh-51	3.00 (4.47)	51	Rh-108	4.17 (4.61)
14	Rh-53	3.50 (4.53)	52	Rh-109	5.17 (4.70)
15	Rh-54	4.67 (4.67)	53	Rh-112	2.17 (4.32)
16	Rh-55	2.50 (4.38)	54	Rh-113	5.23 (4.71)
17	Rh-56	3.83 (4.57)	55	Rh-114	3.87 (4.58)
18	Rh-58	3.50 (4.54)	56	Rh-115	4.17 (4.61)
19	Rh-60	4.67 (4.65)	57	Rh-117	4.68 (4.66)
20	Rh-61	2.33 (4.35)	58	Rh-118	3.17 (4.49)
21	Rh-62	6.33 (4.79)	59	Rh-120	4.33 (4.63)
22	Rh-64	4.83 (4.68)	60	Rh-123	2.67 (4.42)
23	Rh-65	3.00 (4.47)	61	Rh-124	1.33 (4.11)
24	Rh-66	4.17 (4.62)	62	Rh-125	1.23 (4.09)
25	Rh-67	3.83 (4.58)	63	Rh-126	1.07 (4.03)
26	Rh-68	3.33 (4.51)	64	Rh-129	3.17 (4.49)
27	Rh-69	8.17 (4.91)	65	Rh-130	3.83 (4.57)
28	Rh-70	7.67 (4.88)	66	Rh-132	5.67 (4.74)
29	Rh-71	5.33 (4.72)	67	Rh-133	3.67 (4.55)
30	Rh-72	8.33 (4.92)	68	Rh-136	3.33 (4.51)
31	Rh-73	6.50 (4.81)	69	Rh-138	4.33 (4.63)
32	Rh-74	2.67 (4.42)	70	Rh-139	4.17 (4.62)
33	Rh-76	2.33 (4.35)	71	Rh-140	4.33 (4.62)
34	Rh-78	7.17 (4.85)	72	Rh-143	4.67 (4.66)
35	Rh-80	5.33 (4.72)	73	Rh-144	3.50 (4.53)
36	Rh-81	5.83 (4.76)	74	Rh-146	5.17 (4.70)
37	Rh-82	7.16 (4.85)	75	Rh-147	4.67 (4.66)
38	Rh-84	5.17 (4.71)	76	Rh-148	3.83 (4.57)
				S.E. $\pm$	0.09
				C.D. at 1%	0.34
				C.V.%	2.82

Figures in parentheses represent the logarithmic transformation values for statistical analysis

Table 7 Population dynamics of *Azospirillum* isolates by Most Probable Number (MPN) method.

Sr. No	Isolate	MPN of *Azospirillum* (x 10^4 g^{-1} soil)	Sr. No	Isolate	MPN of *Azospirillum* (x 10^4 g^{-1} soil)
1	Asp-1	0.12 (3.07)	27	Asp-40	3.50 (4.53)
2	Asp-2	0.17 (3.21)	28	Asp-41	1.40 (4.14)
3	Asp-3	0.26 (3.41)	29	Asp-42	0.24 (3.37)
4	Asp-4	0.14 (3.13)	30	Asp-43	0.64 (3.77)
5	Asp-5	2.20 (4.33)	31	Asp-44	1.50 (4.16)
6	Asp-8	0.33 (3.50)	32	Asp-46	3.50 (4.53)
7	Asp-10	0.17 (3.20)	33	Asp-47	0.45 (3.64)
8	Asp-11	0.26 (3.39)	34	Asp-48	0.33 (3.48)
9	Asp-14	0.24 (3.35)	35	Asp-49	3.50 (4.54)
10	Asp-15	1.40 (4.14)	36	Asp-50	1.40 (4.11)
11	Asp-16	0.17 (3.19)	37	Asp-51	0.21 (3.29)
12	Asp-21	0.32 (3.47)	38	Asp-55	0.54 (3.72)
13	Asp-23	0.20 (3.26)	39	Asp-56	0.45 (3.62)
14	Asp-24	0.39 (3.55)	40	Asp-57	1.20 (4.06)
15	Asp-25	0.12 (3.07)	41	Asp-59	0.17 (3.21)
16	Asp-27	0.20 (3.33)	42	Asp-60	0.26 (3.39)
17	Asp-28	5.40 (4.73)	43	Asp-62	1.80 (4.25)
18	Asp-29	3.50 (4.53)	44	Asp-63	2.80 (4.43)
19	Asp-31	0.39 (3.59)	45	Asp-64	0.17 (3.20)
20	Asp-33	0.41 (3.60)	46	Asp-67	0.36 (3.53)
21	Asp-34	0.38 (3.56)	47	Asp-68	0.72 (3.83)
22	Asp-35	0.21 (3.30)	48	Asp-69	1.40 (4.12)
23	Asp-36	2.80 (4.43)	49	Asp-70	2.80 (4.44)
24	Asp-37	2.10 (4.31)	50	Asp-72	4.50 (4.63)
25	Asp-38	0.12 (3.07)	51	Asp-73	2.80 (4.43)
26	Asp-39	0.45 (3.63)	52	Asp-75	0.21 (3.31)

contd…

Table 7 contd…

Sr. No	Isolate	MPN of *Azospirillum* (x 10^4 g^{-1} soil)	Sr. No	Isolate	MPN of *Azospirillum* (x 10^4 g^{-1} soil)
53	Asp-78	1.40 (4.12)	74	Asp-116	1.40 (4.12)
54	Asp-82	2.10 (4.30)	75	Asp-117	1.80 (4.22)
55	Asp-83	3.50 (4.53)	76	Asp-119	0.20 (3.28)
56	Asp-85	2.80 (4.44)	77	Asp-120	0.39 (3.57)
57	Asp-86	2.10 (4.31)	78	Asp-121	0.26 (3.34)
58	Asp-87	0.12 (3.07)	79	Asp-123	0.45 (3.63)
59	Asp-88	0.17 (3.20)	80	Asp-124	0.41 (3.59)
60	Asp-90	0.39 (3.58)	81	Asp-127	0.17 (3.20)
61	Asp-92	0.20 (3.27)	82	Asp-129	1.20 (4.06)
62	Asp-95	0.26 (3.39)	83	Asp-130	2.80 (4.43)
63	Asp-96	0.41 (3.59)	84	Asp-132	1.40 (4.12)
64	Asp-97	3.80 (4.55)	85	Asp-134	0.39 (3.56)
65	Asp-99	0.21 (3.29)	86	Asp-136	0.45 (3.62)
66	Asp-101	0.39 (3.55)	87	Asp-140	0.12 (3.08)
67	Asp-102	0.41 (3.57)	88	Asp-142	0.17 (3.21)
68	Asp-103	0.26 (3.40)	89	Asp-145	3.50 (4.53)
69	Asp-106	1.20 (4.06)	90	Asp-147	0.72 (3.86)
70	Asp-107	1.80 (4.23)	91	Asp-148	0.54 (3.71)
71	Asp-108	2.80 (4.44)	92	Asp-149	1.80 (4.24)
72	Asp-112	0.54 (3.72)	93	Asp-150	1.20 (4.07)
73	Asp-113	0.20 (3.25)			

	S.E. $\pm$	0.14
	C.D. at 1%	0.51
	C.V.%	5.18

Figures in parentheses represent the logarithmic transformation values for statistical analysis

Asp-97 ($3.80x10^4$ MPN g^{-1} soil) isolates obtained from Latur and Parbhani district of Marathwada, respectively. The significantly lowest colony count ($0.12x10^4$) was recorded by Asp-1, 25, 38, 87 and 140 isolates obtained from rhizopshere soils of Sindhudurg, Ratnagiri, Nasik, Ahmednagar and Nagpur district, respectively.

4.2.4 PSB isolates

The phosphate solubilizing bacterial population varied significantly ($0.16x10^4$ to $2.83x10^4$ CFU g^{-1} soil) among the soil samples collected from different physiographic regions of Maharashtra (Table 8). The significantly highest PSB population ($2.83x10^4$) was recorded by PSB-72 isolate obtained from rhizopshere soils of Latur district of Marathwada region, whereas minimum population ($0.16x10^4$) from PSB-11, 69, 95 and 125 isolate was noticed in rhizosphere soils of Ratnagiri, Sangli, Beed and Wardha district, respectively.

4.2.5 PSF isolates

The phosphate solubilizing fungal population varied significantly ($0.16x10^4$ to $2.33x10^4$ and $0.33x10^4$ to $1.83x10^4$ CFU g^{-1} soil) among the *Aspergillus* and *Penicillium* isolates obtained from rhizosphere soils of different regions of Maharashtra (Table 9 a and b), respectively. As regards *Aspergillus* isolates, the significantly highest population ($2.33x10^4$) was recorded by PSF-82 isolate recovered from rhizopshere soils of Beed district of Marathwada region (18°44'00"N lat. 75°50'30"E long.), whereas least population ($0.16x10^4$) coming from PSF100 and PSF-128 isolates obtained from rhizopshere soils of Solapur and Dhule district, respectively. In case of *Penicillium* isolates, the significantly highest population ($2.33x10^4$) was recorded by PSF-61 isolate obtained from rhizopshere soils of Solapur district of Western Maharashtra (17°48'35"N lat. 75°33'00"E long.).

Table 8 Population dynamics of phosphate solubilizing bacteria (PSB isolates) by serial dilution of soil and agar-plating method.

Sr. No	Isolate	PSB count (x 10^4 CFU g^{-1} soil)	Sr. No	Isolate	PSB count (x 10^4 CFU g^{-1} soil)
1	PSB-6	0.33 (3.49)	25	PSB-79	0.67 (3.78)
2	PSB-10	1.67 (4.20)	26	PSB-81	0.33 (3.49)
3	PSB-11	0.16 (3.18)	27	PSB-83	0.83 (3.90)
4	PSB-15	1.50 (4.16)	28	PSB-93	0.33 (3.50)
5	PSB-17	0.33 (3.48)	29	PSB-95	0.16 (3.20)
6	PSB-20	0.83 (3.91)	30	PSB-100	2.25 (3.91)
7	PSB-33	0.50 (3.68)	31	PSB-102	1.17 (4.05)
8	PSB-39	1.33 (4.11)	32	PSB-106	1.50 (4.16)
9	PSB-41	1.17 (4.05)	33	PSB-107	1.00 (3.97)
10	PSB-42	1.00 (3.99)	34	PSB-109	0.50 (3.68)
11	PSB-46	1.17 (4.05)	35	PSB-111	1.33 (4.10)
12	PSB-48	0.50 (3.67)	36	PSB-113	0.50 (3.61)
13	PSB-50	1.00 (3.93)	37	PSB-119	1.17 (4.04)
14	PSB-56	0.83 (3.90)	38	PSB-125	0.16 (3.19)
15	PSB-59	0.33 (3.48)	39	PSB-129	0.83 (3.90)
16	PSB-60	1.00 (3.96)	40	PSB-130	0.67 (3.80)
17	PSB-61	0.83 (3.91)	41	PSB-132	0.33 (3.48)
18	PSB-64	1.17 (4.01)	42	PSB-136	0.50 (3.67)
19	PSB-68	0.67 (3.79)	43	PSB-140	2.00 (4.30)
20	PSB-69	0.16 (3.19)	44	PSB-142	0.50 (3.67)
21	PSB-72	2.83 (4.45)	45	PSB-143	0.67 (3.80)
22	PSB-73	1.17 (4.04)	46	PSB-144	1.00 (3.99)
23	PSB-75	1.33 (4.08)	47	PSB-148	1.33 (4.08)
24	PSB-78	1.67 (4.21)			

	S.E. $\pm$	0.16
	C.D. at 1%	0.61
	C.V.%	5.92

Figures in parentheses represent the logarithmic transformation values for statistical analysis

Table 9 Population dynamics of phosphate solubilizing fungi (PSF isolates) by serial dilution of soil and agar-plating method.

a) *Aspergillus* isolates

Sr. No	Isolate	PSF count (x 10^4 CFU g^{-1} soil)	Sr. No	Isolate	PSF count (x 10^4 CFU g^{-1} soil)
1	PSF-4	1.67 (4.20)	16	PSF-100	0.16 (3.17)
2	PSF-7	0.67 (3.80)	17	PSF-101	1.87 (3.50)
3	PSF-8	0.83 (3.90)	18	PSF-115	1.17 (4.02)
4	PSF-11	0.33 (3.48)	19	PSF-119	1.50 (4.14)
5	PSF-19	0.50 (3.69)	20	PSF-120	0.67 (3.80)
6	PSF-28	1.17 (4.05)	21	PSF-122	0.50 (3.79)
7	PSF-32	1.83 (4.24)	22	PSF-124	0.50 (3.66)
8	PSF-40	1.17 (4.03)	23	PSF-127	0.33 (3.48)
9	PSF-41	1.00 (3.95)	24	PSF-128	0.16 (3.19)
10	PSF-44	0.67 (3.81)	25	PSF-129	1.17 (4.03)
11	PSF-55	0.50 (3.66)	26	PSF-132	0.83 (3.89)
12	PSF-57	1.00 (3.97)	27	PSF-135	0.50 (3.65)
13	PSF-64	1.33 (4.09)	28	PSF-143	0.83 (4.05)
14	PSF-71	2.00 (4.30)	29	PSF-148	1.83 (4.25)
15	PSF-82	2.33 (4.34)			

	S.E. ±	0.17
	C.D. at 1%	0.67
	C.V.%	6.31

b) *Penicillium* isolates

Sr. No	Isolate	PSF count (x 10^4 CFU g^{-1} soil)	Sr. No	Isolate	PSF count (x 10^4 CFU g^{-1} soil)
1	PSF-30	0.33 (3.51)	10	PSF-97	0.83 (3.90)
2	PSF-61	1.83 (4.25)	11	PSF-101-1	0.50 (3.68)
3	PSF-65	0.67 (3.81)	12	PSF-102	0.67 (3.82)
4	PSF-77	1.00 (3.99)	13	PSF-118	2.33 (4.36)
5	PSF-79	0.67 (3.81)	14	PSF-120-1	0.83 (3.91)
6	PSF-80	1.17 (4.06)	15	PSF-136	1.33 (4.12)
7	PSF-82-1	1.33 (4.09)	16	PSF-138	1.67 (4.21)
8	PSF-85	1.50 (4.17)	17	PSF-141	0.50 (3.68)
9	PSF-92	1.13 (4.05)			

	S.E. ±	0.11
	C.D. at 1%	0.44
	C.V.%	3.83

Figures in parentheses represent the logarithmic transformation values for statistical analysis

4.3 Morphological characterization

4.3.1 *Azotobacter* isolates

The distinct variation in cell morphology among the different isolates of *Azotobacter* was observed (Table 10, Plate 9). The cells of all *Azotobacter* isolates were motile and gram negative in reaction. Out of 94 *Azotobacter* isolates, cells of 48 isolates were rod shape and 46 isolates having oval in shape. The cell size varied among the isolates and it was in the range of 1.1 to 4.4 x 2.0 to 11.8 µm. On the basis of cell arrangement, four distinct groups of isolates were formed. Fifty *Azotobacter* isolates were single celled, 26 isolates having cells in pairs, 14 isolates having cell in chain and cells of 4 isolates formed irregular clumps. The cell morphology of MPKV *Azotobacter* strain (Azt-BNF) revealed that cells were rod shaped measuring 3.3-3.5 x 5.8-9.3 µm and they occurred in pairs, chain and forms irregular clumps.

4.3.2 *Rhizobium* isolates

The cells of all *Rhizobium* isolates were rod shaped, motile and gram negative in reaction (Table 11, Plate 10). A wide variation was noticed in cell size among the different *Rhizobium* isolates and it was in the range of 0.4 to 2.8 x 0.7 to 10.0 µm. The cells of MPKV strain were motile, rod shaped measuring 2.3-2.8 x 5.1-7.3 µm.

4.3.3 *Azospirillum* isolates

The cells of all isolates were gram negative in reaction. The distinct variation in cell morphology among the different *Azospirillum* isolates was noticed (Table 12, Plate 11). On the basis of cell motility, two distinct groups of isolates were formed. The cells of 83 *Azospirillum* isolates were non-motile whereas 10 isolate having motile cells. The cell shape differed among the various *Azospirillum* isolates. Out of 93 *Azospirillum* isolates,

Table 10 Morphological characteristics of *Azotobacter* isolates

Sr. No.	Isolate	Gram reaction	Cell morphology			
			Cell shape	Cell size (µm)	Cell arrangement	Cell motility
1	Azt-4	Negative	Rods	1.6-2.0 x 4.0-7.5	Single cells	Motile
2	Azt-8	Negative	Rods	2.6-3.2 x 5.2-11.8	Cells are single as well as in pairs	Motile
3	Azt-9	Negative	Rods	1.3-1.5 x 3.9-6.5	Cells are in pairs	Motile
4	Azt-10	Negative	Oval	1.6-1.8 x 3.5-3.9	Single cells	Motile
5	Azt-12	Negative	Rods	2.1-2.2 x 4.9-6.1	Cells are in chain	Motile
6	Azt-18	Negative	Rods	1.5-1.7 x 3.3-4.8	Single cells	Motile
7	Azt-21	Negative	Rods	4.1-4.4 x 7.3-9.9	Cells are in chain & form irregular clumps	Motile
8	Azt-23	Negative	Rods	1.6-1.9 x 4.1-7.3	Single cells	Motile
9	Azt-25	Negative	Rods	3.7-3.9 x 6.8-9.3	Cells are in chain	Motile
10	Azt-26	Negative	Rods	1.2-1.6 x 3.7-5.1	Cells are in pairs	Motile
11	Azt-27	Negative	Oval	1.2-1.5 x 2.3-3.1	Single cells	Motile
12	Azt-28	Negative	Oval	1.7-2.1 x 3.3-4.4	Cells are in pairs	Motile
13	Azt-29	Negative	Rods	2.1-2.5 x 4.8-7.2	Cells form irregular clumps	Motile
14	Azt-32	Negative	Rods	2.0-2.2 x 5.1-8.9	Single cells	Motile
15	Azt-33	Negative	Oval	1.5-1.7 x 3.1-3.7	Single cells	Motile
16	Azt-34	Negative	Oval	1.3-1.8 x 2.5-3.3	Cells are in pairs	Motile
17	Azt-36	Negative	Oval	1.6-1.8 x 3.3-3.8	Single cells	Motile
18	Azt-37	Negative	Rods	1.8-2.2 x 4.4-7.9	Single cells	Motile
19	Azt-39	Negative	Rods	2.1-2.3 x 5.3-8.4	Cells are in pairs	Motile
20	Azt-40	Negative	Rods	1.9-2.4 x 4.3-7.7	Cells are in pairs	Motile
21	Azt-42	Negative	Rods	1.6-1.8 x 4.1-9.7	Cells are in chain	Motile
22	Azt-43	Negative	Oval	1.5-1.9 x 2.7-4.1	Single cells	Motile
23	Azt-44	Negative	Rods	1.8-2.1 x 3.3-4.0	Single cells	Motile
24	Azt-45	Negative	Oval	1.4-1.9 x 2.5-4.1	Cells are in pairs	Motile
25	Azt-48	Negative	Oval	1.3-1.5 x 2.3-3.2	Single cells	Motile
26	Azt-49	Negative	Rods	2.1-2.4 x 4.9-7.1	Cells are in chain	Motile
27	Azt-50	Negative	Rods	1.7-2.0 x 4.3-8.0	Cells are in pairs	Motile
28	Azt-52	Negative	Oval	1.4-1.8 x 2.5-3.7	Single cells	Motile
29	Azt-53	Negative	Rods	1.9-2.2 x 4.4-7.3	Cells are in chain	Motile
30	Azt-54	Negative	Oval	1.2-1.6 x 2.3-3.7	Single cells	Motile
31	Azt-55	Negative	Oval	1.7-2.1 x 3.1-4.8	Cells are in pairs	Motile
32	Azt-56	Negative	Oval	1.3-1.5 x 2.3-2.9	Cells are in pairs	Motile

contd…

Table 10 contd…

Sr. No.	Isolate	Gram reaction	Cell morphology			
			Cell shape	Cell size (µm)	Cell arrangement	Cell motility
33	Azt-57	Negative	Rods	1.6-1.8 x 4.1-5.9	Single cells	Motile
34	Azt-58	Negative	Oval	1.5-1.7 x 3.1-3.6	Single cells	Motile
35	Azt-60	Negative	Oval	1.8-1.9 x 3.3-3.5	Single cells	Motile
36	Azt-61	Negative	Rods	2.1-2.4 x 4.7-7.9	Cells are in chain	Motile
37	Azt-63	Negative	Oval	1.4-1.6 x 2.5-3.4	Single cells	Motile
38	Azt-64	Negative	Rods	2.0-2.4 x 4.8-10.9	Single cells	Motile
39	Azt-65	Negative	Rods	2.3-2.5 x 5.6-8.8	Cells are in clumps	Motile
40	Azt-66	Negative	Rods	2.0-2.1 x 5.1-6.3	Cells are in chain	Motile
41	Azt-67	Negative	Rods	1.5-1.7 x 4.1-6.0	Cells are in pairs	Motile
42	Azt-68	Negative	Rods	1.9-2.2 x 4.4-5.8	Cells are in pairs	Motile
43	Azt-69	Negative	Oval	1.4-1.7 x 2.6-3.3	Single cells	Motile
44	Azt-70	Negative	Oval	1.3-1.7 x 2.7-3.6	Single cells	Motile
45	Azt-72	Negative	Oval	1.2-1.4 x 2.3-2.5	Cells are in pairs	Motile
46	Azt-73	Negative	Rods	2.1-2.4 x 5.1-6.3	Cells are in chain	Motile
47	Azt-75	Negative	Oval	1.3-1.5 x 2.3-2.9	Single cells	Motile
48	Azt-77	Negative	Oval	1.4-1.7 x 2.6-3.3	Single cells	Motile
49	Azt-78	Negative	Rods	2.2-2.3 x 5.1-7.2	Cells are in pairs	Motile
50	Azt-79	Negative	Rods	1.8-2.1 x 4.4-5.9	Cells are in pairs	Motile
51	Azt-81	Negative	Oval	1.1-1.3 x 2.3-2.8	Cells are in pairs	Motile
52	Azt-82	Negative	Oval	1.3-1.7 x 2.4-3.5	Single cells	Motile
53	Azt-83	Negative	Oval	1.7-2.1 x 3.3-3.8	Single cells	Motile
54	Azt-84	Negative	Oval	1.4-1.6 x 2.5-3.0	Cells are in pairs	Motile
55	Azt-86	Negative	Rods	2.0-2.3 x 4.9-7.1	Cells are in chain	Motile
56	Azt-88	Negative	Rods	1.6-1.9 x 4.1-6.8	Cells are in pairs	Motile
57	Azt-89	Negative	Oval	1.3-1.5 x 2.4-2.9	Single cells	Motile
58	Azt-91	Negative	Oval	1.4-1.7 x 2.5-3.0	Single cells	Motile
59	Azt-93	Negative	Oval	1.5-1.6 x 3.1-3.7	Cells are in pairs	Motile
60	Azt-94	Negative	Rods	2.3-2.7 x 5.2-8.1	Single cells	Motile
61	Azt-97	Negative	Oval	1.5-1.9 x 2.8-3.5	Single cells	Motile
62	Azt-99	Negative	Oval	1.3-1.7 x 2.4-3.2	Cells are in pairs	Motile
63	Azt-101	Negative	Rods	2.1-2.4 x 5.2-7.7	Single cells	Motile
64	Azt-102	Negative	Rods	1.4-1.9 x 4.2-6.1	Cells are in pairs	Motile
65	Azt-104	Negative	Rods	1.3-1.7 x 2.9-4.8	Single cells	Motile

contd…

Table 10 contd…

Sr. No.	Isolate	Gram reaction	Cell morphology			
			Cell shape	Cell size (µm)	Cell arrangement	Cell motility
66	Azt-105	Negative	Oval	1.5-1.8 x 2.7-3.1	Single cells	Motile
67	Azt-106	Negative	Oval	1.3-1.7 x 2.4-3.0	Cells are in pairs	Motile
68	Azt-107	Negative	Oval	1.2-1.4 x 2.1-2.4	Single cells	Motile
69	Azt-108	Negative	Rods	2.0-2.4 x 4.1-5.8	Cells are in chain	Motile
70	Azt-109	Negative	Rods	2.3-1.7 x 5.9-8.7	Irregular clumps	Motile
71	Azt-110	Negative	Oval	1.3-1.8 x 2.2-3.0	Single cells	Motile
72	Azt-112	Negative	Oval	1.2-1.5 x 2.0-8.0	Single cells	Motile
73	Azt-113	Negative	Rods	1.9-2.3 x 4.3-6.8	Single cells	Motile
74	Azt-114	Negative	Rods	2.0-2.2 x 4.9-6.7	Cells are in pairs	Motile
75	Azt-118	Negative	Oval	1.4-1.7 x 2.4-3.0	Cells are in chain	Motile
76	Azt-120	Negative	Rods	1.2-1.6 x 3.9-5.8	Single cells	Motile
77	Azt-122	Negative	Rods	1.7-1.9 x 4.4-6.0	Cells are in chain	Motile
78	Azt-125	Negative	Rods	1.3-1.7 x 3.7-5.1	Cells are in chain	Motile
79	Azt-126	Negative	Oval	1.4-1.6 x 2.9-3.0	Single cells	Motile
80	Azt-129	Negative	Rods	2.2-2.5 x 5.1-6.9	Cells are in chain	Motile
81	Azt-130	Negative	Oval	1.5-3.6 x 3.8-6.7	Single cells	Motile
82	Azt-131	Negative	Rods	3.1-3.3 x 3.6-8.7	Single cells	Motile
83	Azt-132	Negative	Oval	1.3-1.5 x 2.7-2.9	Single cells	Motile
84	Azt-133	Negative	Oval	1.7-1.8 x 3.2-3.7	Cells are in pairs	Motile
85	Azt-135	Negative	Oval	1.5-3.6 x 3.8-6.7	Single cells	Motile
86	Azt-136	Negative	Rods	1.2-1.7 x 2.3-4.5	Single cells	Motile
87	Azt-137	Negative	Rods	1.4-1.6 x 2.3-7.4	Single cells	Motile
88	Azt-138	Negative	Rods	1.9-2.3 x 4.7-8.4	Cells are in chain	Motile
89	Azt-141	Negative	Rods	1.8-2.1 x 4.6-8.1	Single cells	Motile
90	Azt-142	Negative	Oval	1.6-1.9 x 2.8-4.1	Single cells	Motile
91	Azt-144	Negative	Oval	1.7-1.9 x 3.6-3.9	Single cells	Motile
92	Azt-147	Negative	Rods	2.3-2.7 x 5.2-6.9	Cells are in pairs	Motile
93	Azt-148	Negative	Oval	1.3-1.7 x 2.3-3.0	Single cells	Motile
94	Azt-149	Negative	Oval	1.2-1.4 x 2.0-2.5	Cells are in pairs	Motile
95	Azt-BNF	Negative	Rods	3.3-3.5 x 5.8-9.3	Cells are in chain & form irregular clumps	Motile

Azt-BNF = MPKV strain (*Azotobacter chroococcum*)

Table 11 Morphological characteristics of *Rhizobium* isolates

Sr. No.	Isolate	Gram reaction	Cell morphology		
			Cell shape	Cell size (µm)	Cell motility
1	Rh-13	Negative	Rods	0.9-1.1 x 2.2-3.1	Motile
2	Rh-21	Negative	Rods	1.3-1.4 x 2.1-3.9	Motile
3	Rh-22	Negative	Rods	1.5-1.8 x 2.9-5.5	Motile
4	Rh-28	Negative	Rods	0.8-1.0 x 2.0-3.8	Motile
5	Rh-29	Negative	Rods	0.9-1.3 x 2.4-3.7	Motile
6	Rh-37	Negative	Rods	0.7-1.0 x 2.3-3.5	Motile
7	Rh-39	Negative	Rods	1.4-1.7 x 3.2-4.1	Motile
8	Rh-40	Negative	Rods	0.6-0.8 x 1.9-2.8	Motile
9	Rh-42	Negative	Rods	1.0-1.4 x 2.6-4.1	Motile
10	Rh-43	Negative	Rods	0.9-1.2 x 2.4-4.2	Motile
11	Rh-44	Negative	Rods	1.5-1.7 x 3.1-4.7	Motile
12	Rh-50	Negative	Rods	1.1-1.4 x 2.9-3.8	Motile
13	Rh-51	Negative	Rods	0.6-0.8 x 1.7-2.5	Motile
14	Rh-53	Negative	Rods	0.7-1.0 x 2.0-3.1	Motile
15	Rh-54	Negative	Rods	0.5-0.9 x 1.2-3.0	Motile
16	Rh-55	Negative	Rods	0.8-1.1 x 2.1-3.6	Motile
17	Rh-56	Negative	Rods	0.5-0.7 x 1.1-2.6	Motile
18	Rh-58	Negative	Rods	1.2-1.5 x 2.7-5.0	Motile
19	Rh-60	Negative	Rods	0.4-0.6 x 0.7-1.3	Motile
20	Rh-61	Negative	Rods	0.9-1.3 x 2.0-3.4	Motile
21	Rh-62	Negative	Rods	0.7-1.0 x 2.2-3.7	Motile
22	Rh-64	Negative	Rods	2.8-3.1 x 5.9-7.3	Motile
23	Rh-65	Negative	Rods	1.1-1.5 x 2.7-4.3	Motile
24	Rh-66	Negative	Rods	0.9-1.1 x 2.3-3.9	Motile
25	Rh-67	Negative	Rods	1.3-1.7 x 3.1-4.0	Motile
26	Rh-68	Negative	Rods	1.2-1.4 x 2.6-3.7	Motile
27	Rh-69	Negative	Rods	1.0-1.3 x 2.3-3.0	Motile
28	Rh-70	Negative	Rods	0.7-1.1 x 2.2-3.5	Motile
29	Rh-71	Negative	Rods	0.5-0.8 x 2.1-3.0	Motile
30	Rh-72	Negative	Rods	2.4-2.7 x 5.4-7.9	Motile
31	Rh-73	Negative	Rods	1.0-1.3 x 2.4-3.9	Motile
32	Rh-74	Negative	Rods	1.6-1.8 x 3.5-4.9	Motile
33	Rh-76	Negative	Rods	0.9-1.1 x 2.2-3.7	Motile
34	Rh-78	Negative	Rods	1.0-1.4 x 2.1-3.5	Motile
35	Rh-80	Negative	Rods	1.7-1.9 x 3.0-4.8	Motile
36	Rh-81	Negative	Rods	0.7-1.0 x 2.0-3.7	Motile
37	Rh-82	Negative	Rods	2.1-2.3 x 3.9-8.2	Motile
38	Rh-84	Negative	Rods	1.2-1.5 x 2.9-4.0	Motile
39	Rh-85	Negative	Rods	0.5-0.9 x 1.9-3.1	Motile

contd…

Table 11 contd…

Sr. No.	Isolate	Gram reaction	Cell morphology		
			Cell shape	Cell size (µm)	Cell motility
40	Rh-86	Negative	Rods	0.6-0.8 x 1.8-3.3	Motile
41	Rh-93	Negative	Rods	1.2-1.4 x 2.1-3.7	Motile
42	Rh-95	Negative	Rods	1.5-1.8 x 2.9-5.6	Motile
43	Rh-97	Negative	Rods	0.8-1.1 x 2.0-3.9	Motile
44	Rh-98	Negative	Rods	0.7-0.9 x 2.3-4.2	Motile
45	Rh-100	Negative	Rods	1.1-1.4 x 2.8-4.7	Motile
46	Rh-101	Negative	Rods	2.2-2.4 x 5.1-7.5	Motile
47	Rh-102	Negative	Rods	1.3-1.5 x 2.9-4.1	Motile
48	Rh-105	Negative	Rods	1.0-1.2 x 2.3-3.5	Motile
49	Rh-106	Negative	Rods	0.6-0.8 x 2.7-3.1	Motile
50	Rh-107	Negative	Rods	0.9-1.1 x 2.6-4.2	Motile
51	Rh-108	Negative	Rods	0.5-0.8 x 1.9-3.0	Motile
52	Rh-109	Negative	Rods	0.9-1.4 x 2.1-3.5	Motile
53	Rh-112	Negative	Rods	0.7-0.9 x 2.0-3.1	Motile
54	Rh-113	Negative	Rods	3.3-3.7 x 5.2-11.8	Motile
55	Rh-114	Negative	Rods	0.6-0.9 x 2.0-3.6	Motile
56	Rh-115	Negative	Rods	0.8-1.0 x 2.0-4.1	Motile
57	Rh-117	Negative	Rods	1.3-1.7 x 2.8-5.3	Motile
58	Rh-118	Negative	Rods	1.0-1.3 x 2.3-4.6	Motile
59	Rh-120	Negative	Rods	0.9-1.1 x 2.4-3.9	Motile
60	Rh-123	Negative	Rods	0.6-0.8 x 2.0-4.7	Motile
61	Rh-124	Negative	Rods	0.7-1.1 x 2.3-4.4	Motile
62	Rh-125	Negative	Rods	0.8-1.1 x 2.1-3.7	Motile
63	Rh-126	Negative	Rods	1.4-1.7 x 3.0-5.7	Motile
64	Rh-129	Negative	Rods	0.6-0.8 x 2.0-3.1	Motile
65	Rh-130	Negative	Rods	0.5-0.9 x 2.1-3.7	Motile
66	Rh-132	Negative	Rods	1.7-1.9 x 2.3-4.1	Motile
67	Rh-133	Negative	Rods	1.4-1.7 x 2.6-5.5	Motile
68	Rh-136	Negative	Rods	1.0-1.2 x 2.2-4.0	Motile
69	Rh-138	Negative	Rods	0.7-0.9 x 2.0-3.5	Motile
70	Rh-139	Negative	Rods	1.0-1.3 x 2.1-3.9	Motile
71	Rh-140	Negative	Rods	0.6-0.9 x 2.4-4.1	Motile
72	Rh-143	Negative	Rods	0.8-1.0 x 3.0-5.7	Motile
73	Rh-144	Negative	Rods	1.1-1.4 x 2.2-3.4	Motile
74	Rh-146	Negative	Rods	1.0-1.3 x 2.4-4.1	Motile
75	Rh-147	Negative	Rods	0.7-1.1 x 2.0-3.6	Motile
76	Rh-148	Negative	Rods	0.8-1.0 x 2.1-3.9	Motile
77	Rh-BNF	Negative	Rods	2.3-2.8 x 5.1-7.3	Motile

Table 12 Morphological characteristics of *Azospirillum* isolates

Sr. No.	Isolate	Gram reaction	Cell morphology		
			Cell shape	Cell size (µm)	Cell motility
1	Asp-1	Negative	Vibrioid	1.7-1.9 x 6.1-7.3	Motile
2	Asp-2	Negative	Helical	1.1-1.4 x 3.4-7.0	Non-motile
3	Asp-3	Negative	Helical	1.5-1.8 x 4.9-5.1	Non-motile
4	Asp-4	Negative	'S' shape	1.3-1.5 x 4.6-10.2	Non-motile
5	Asp-5	Negative	Vibrioid	1.6-1.9 x 5.1-9.8	Non-motile
6	Asp-8	Negative	Vibrioid	1.8-2.2 x 5.3-9.0	Non-motile
7	Asp-10	Negative	Helical	1.3-1.7 x 4.1-7.9	Non-motile
8	Asp-11	Negative	'S' shape	1.2-1.7 x 4.0-9.3	Non-motile
9	Asp-14	Negative	Curved rods	1.2-1.4 x 3.7-7.8	Motile
10	Asp-15	Negative	Vibrioid	1.6-1.8 x 4.7-9.8	Non-motile
11	Asp-16	Negative	Curved rods	1.4-1.6 x 5.0-9.1	Non-motile
12	Asp-21	Negative	Vibrioid	1.9-2.0 x 5.6-9.0	Non-motile
13	Asp-23	Negative	Helical	1.1-1.4 x 4.9-8.6	Non-motile
14	Asp-24	Negative	'S' shape	1.2-1.5 x 5.1-11.0	Non-motile
15	Asp-25	Negative	Vibrioid	1.3-1.6 x 5.3-8.7	Non-motile
16	Asp-27	Negative	Vibrioid	1.4-1.6 x 5.0-8.9	Non-motile
17	Asp-28	Negative	Spiral shape	1.6-2.1 x 10.7-17.8	Non-motile
18	Asp-29	Negative	Vibrioid	1.1-1.5 x 4.6-7.7	Non-motile
19	Asp-31	Negative	Vibrioid	1.2-1.8 x 5.1-9.3	Non-motile
20	Asp-33	Negative	Helical	1.6-1.9 x 5.7-11.3	Non-motile
21	Asp-34	Negative	'S' shape	1.1-1.3 x 4.9-8.1	Non-motile
22	Asp-35	Negative	Vibrioid	1.0-1.2 x 4.1-7.6	Motile
23	Asp-36	Negative	Curved rods	1.3-1.6 x 4.4-11.9	Non-motile
24	Asp-37	Negative	Vibrioid	1.9-2.3 x 4.9-8.8	Non-motile
25	Asp-38	Negative	Helical	1.6-2.0 x 5.1-9.5	Non-motile
26	Asp-39	Negative	Helical	1.2-1.8 x 5.0-11.1	Non-motile
27	Asp-40	Negative	'S' shape	1.7-2.1 x 4.1-9.7	Non-motile
28	Asp-41	Negative	Curved rods	1.6-2.0 x 4.7-11.0	Non-motile
29	Asp-42	Negative	Vibrioid	1.3-1.5 x 4.0-8.1	Non-motile
30	Asp-43	Negative	Vibrioid	1.9-2.4 x 5.3-8.6	Non-motile
31	Asp-44	Negative	Helical	1.4-1.9 x 4.3-7.9	Non-motile
32	Asp-46	Negative	Helical	1.1-1.5 x 4.1-8.7	Non-motile

contd…

Table 12 contd…

Sr. No.	Isolate	Gram reaction	Cell morphology		
			Cell shape	Cell size (µm)	Cell motility
33	Asp-47	Negative	Vibrioid	1.6-1.8 x 4.0-8.5	Non-motile
34	Asp-48	Negative	Long helical cells	1.5-1.9 x 5.3-18.4	Non-motile
35	Asp-49	Negative	Vibrioid	1.2-1.5 x 5.0-11.1	Non-motile
36	Asp-50	Negative	Elongated helical shape	2.6-2.8 x 20.8-24.8	Non-motile
37	Asp-51	Negative	'S' shape	1.3-1.7 x 5.3-16.1	Non-motile
38	Asp-55	Negative	Helical	1.1-1.6 x 4.4-8.9	Non-motile
39	Asp-56	Negative	Helical	1.8-2.3 x 5.6-11.7	Non-motile
40	Asp-57	Negative	'S' shape	1.6-1.8 x 4.1-7.9	Non-motile
41	Asp-59	Negative	Vibrioid	1.1-1.4 x 3.8-6.1	Motile
42	Asp-60	Negative	Helical	1.2-1.4 x 5.0-9.5	Non-motile
43	Asp-62	Negative	Vibrioid	1.1-1.5 x 4.1-9.0	Non-motile
44	Asp-63	Negative	Vibrioid	1.2-1.4 x 4.0-7.1	Non-motile
45	Asp-64	Negative	Curved rods	1.1-1.4 x 4.3-10.1	Non-motile
46	Asp-67	Negative	Helical	1.4-1.7 x 4.1-11.3	Non-motile
47	Asp-68	Negative	Vibrioid	1.6-1.8 x 4.0-9.2	Non-motile
48	Asp-69	Negative	Vibrioid	1.5-1.9 x 5.1-9.7	Non-motile
49	Asp-70	Negative	Helical	1.0-1.3 x 4.1-8.9	Non-motile
50	Asp-72	Negative	Helical	1.4-1.7 x 4.0-9.6	Non-motile
51	Asp-73	Negative	Curved rods	1.3-1.6 x 4.6-11.0	Non-motile
52	Asp-75	Negative	Vibrioid	1.2-1.7 x 4.0-6.6	Motile
53	Asp-78	Negative	Curved rods	1.1-1.3 x 4.1-9.7	Non-motile
54	Asp-82	Negative	Vibrioid	1.4-2.1 x 3.3-7.1	Non-motile
55	Asp-83	Negative	Helical	1.2-1.5 x 5.1-13.5	Non-motile
56	Asp-85	Negative	'S' shape	1.7-2.0 x 5.3-17.1	Non-motile
57	Asp-86	Negative	Vibrioid	1.3-1.9 x 4.4-9.4	Non-motile
58	Asp-87	Negative	Vibrioid	1.2-1.6 x 4.1-7.2	Non-motile
59	Asp-88	Negative	Curved rods	1.4-1.8 x 3.9-5.8	Motile
60	Asp-90	Negative	Helical	1.3-1.6 x 5.0-10.4	Non-motile
61	Asp-92	Negative	'S' shape	1.4-1.7 x 5.1-12.5	Non-motile
62	Asp-95	Negative	Vibrioid	1.1-1.3 x 4.3-8.0	Non-motile
63	Asp-96	Negative	Vibrioid	1.2-1.4 x 4.0-7.7	Non-motile

contd…

Table 12 contd…

Sr. No.	Isolate	Gram reaction	Cell morphology		
			Cell shape	Cell size (µm)	Cell motility
64	Asp-97	Negative	Elongated 'S' shape	1.7-1.9 x 5.5-16.4	Non-motile
65	Asp-99	Negative	Curved rods	1.4-1.8 x 4.9-8.8	Motile
66	Asp-101	Negative	Helical	1.6-1.9 x 5.0-13.8	Non-motile
67	Asp-102	Negative	Vibrioid	1.1-1.4 x 4.1-7.0	Non-motile
68	Asp-103	Negative	Vibrioid	1.3-1.5 x 4.3-8.1	Non-motile
69	Asp-106	Negative	Vibrioid	1.0-1.6 x 4.0-7.2	Non-motile
70	Asp-107	Negative	Helical	1.1-1.4 x 4.9-11.0	Non-motile
71	Asp-108	Negative	Helical	1.5-1.7 x 5.3-12.6	Non-motile
72	Asp-112	Negative	Vibrioid	1.0-1.6 x 4.0-5.7	Motile
73	Asp-113	Negative	Elongated 'S' shape	1.4-1.8 x 5.7-14.1	Non-motile
74	Asp-116	Negative	Vibrioid	1.2-1.4 x 4.1-7.7	Non-motile
75	Asp-117	Negative	Vibrioid	1.3-1.7 x 4.4-8.1	Non-motile
76	Asp-119	Negative	Vibrioid	1.0-1.3 x 4.0-7.1	Non-motile
77	Asp-120	Negative	'S' shape	1.6-1.9 x 4.9-10.7	Non-motile
78	Asp-121	Negative	Vibrioid/curved rods	1.4-1.7 x 4.0-5.9	Motile
79	Asp-123	Negative	Helical	1.6-2.0 x 5.1-14.3	Non-motile
80	Asp-124	Negative	Helical	1.8-2.1 x 4.2-12.6	Non-motile
81	Asp-127	Negative	Curved rods	1.5-1.7 x 4.7-8.9	Non-motile
82	Asp-129	Negative	Helical	1.5-2.0 x 5.7-18.6	Non-motile
83	Asp-130	Negative	Curved rods	1.4-1.6 x 4.4-8.7	Non-motile
84	Asp-132	Negative	Helical/spiral	1.9-2.4 x 4.4-11.5	Non-motile
85	Asp-134	Negative	Helical	1.4-1.7 x 5.1-10.2	Non-motile
86	Asp-136	Negative	Vibrioid	1.7-1.9 x 4.5-9.3	Non-motile
87	Asp-140	Negative	Curved rods	1.3-1.5 x 5.3-9.1	Non-motile
88	Asp-142	Negative	Helical	1.6-1.9 x 5.0-12.6	Non-motile
89	Asp-145	Negative	Curved rods	1.4-1.7 x 4.1-6.2	Motile
90	Asp-147	Negative	Helical	1.3-1.9 x 5.6-10.1	Non-motile
91	Asp-148	Negative	Vibrioid	1.2-1.5 x 4.1-8.1	Non-motile
92	Asp-149	Negative	'S' shape	1.4-1.7 x 4.9-10.3	Non-motile
93	Asp-150	Negative	Helical	1.6-1.9 x 5.8-17.3	Non-motile
94	Asp-BNF	Negative	Helical, 'S' shape	1.9-2.4 x 5.8-10.7	Non-motile

cells of 44 isolates were helicle/S-shape, 35 isolates having vibrioid shape and 14 isolates were curved rods. The great variation in cell size among the isolates was recorded and it was in the range of 1.0 to 2.8 x 3.3 to 24.8 µm. The cells of MPKV strain (Asp-BNF) were non-motile, helicle/S-shaped measuring 1.9-2.4 x 5.8-10.7 µm.

4.3.4 PSB (*Bacillus* isolates)

The cells of all phosphate solubilizing bacterial isolates were rod shaped, motile and gram positive in reaction (Table 13, Plate 12). A wide variation was noticed in cell size among the different PSB isolates and it was in the range of 0.4 to 3.1 x 1.8 to 11.0 µm. On the basis of cell arrangement, three distinct groups of PSB isolates were formed. Twenty eight PSB isolates were single celled, 10 isolates having cells in chains and cells of 9 isolates were single as well as in pairs. The cells of MPKV strain (PSB-BNF) were motile, rods measuring 2.6-3.1 x 1.8-11.0 µm and gram positive in reaction.

4.3.5 PSF isolates

The distinct variation in colonial morphology, spore characteristics and microscopic appearance among the different phosphate solubilizing fungal isolates *viz.*, *Aspergillus* and *Penicillium* isolates was observed (Table 14 and 15, Plate 13, 14 and 15).

4.3.5.1 *Aspergillus* isolates

On the basis of colonial morphology, four distinct groups of *Aspergillus* isolates were formed. The first group comprised of eight *Aspergillus* isolates producing white floccose mycelium spreading rapidly and formed black coloured spores, second group of seven isolates producing velvety green mycelium becoming almost black, five isolates included in third group produced white cottony, loose

woven thread like mycelium forming brown-black spores in due course and nine isolates involved in fourth group produced white colony, thread like mycelium forming brown-black spores in due course.

On the basis of microscopic appearance, three distinct groups of *Aspergillus* isolates were formed. The first group comprised of 11 *Aspergillus* isolates consisting of hyphae-aseptate, conidiophore-septate, red coloured, globose vesicle, conidia-red and round, second group of 10 isolates having hyphae-colorless, conidiophores branching, septate, globose vesicle, conidia-small, red and round and 8 isolates included in third group comprised of hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black and round. The cells of MPKV strain [PSF-BNF(A)] consisting of hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red and round.

4.3.5.2 *Penicillium* isolates

On the basis of colonial morphology, six distinct groups of *Penicillium* isolates were formed. Out of 18 *Penicillium* isolates, three isolates from each group produced the different mycelial forms *viz.*, velvet, velutinous, fasciculate and cotton-wool followed by spore colour varied from dark-green, yellow, orange and blue-green.

Based on the microscopic examination, three distinct groups of *Penicillium* isolates were formed. The first group comprised of 8 *Penicillium* isolates consisting of branched conidiophores that are borne on a single rope of fertile hyphae, second group of 4 isolates having branching conidiophores that are borne on aerial mycelia and 5 isolates included in third group comprised of branching conidiophores arise from a septate mycelium. The cells of MPKV strain [PSF-BNF(P)] consisting of branched conidiophores that are borne on a single rope of fertile hyphae.

Table 13 Morphological characteristics of phosphate solubilizing bacteria

Sr. No.	Isolate	Gram reaction	Cell morphology			
			Cell shape	Cell size (µm)	Cell arrangement	Cell motility
1	PSB-6	Positive	Rods	1.3-1.7 x 4.9-7.1	Single cells	Motile
2	PSB-10	Positive	Rods	1.0-1.4 x 3.7-7.5	Single cells	Motile
3	PSB-11	Positive	Rods	2.0-2.3 x 5.1-8.7	Single cells	Motile
4	PSB-15	Positive	Rods	1.5-1.8 x 4.9-9.3	Cells in chains	Motile
5	PSB-17	Positive	Rods	1.1-1.3 x 3.9-7.0	Single cells	Motile
6	PSB-20	Positive	Rods	0.7-1.0 x 2.8-5.1	Single cells	Motile
7	PSB-33	Positive	Rods	1.8-2.2 x 5.1-9.9	Cells in chains	Motile
8	PSB-39	Positive	Rods	1.2-1.5 x 4.0-10.6	Cells in chains	Motile
9	PSB-41	Positive	Rods	0.6-1.0 x 3.7-6.9	Single cells	Motile
10	PSB-42	Positive	Rods	0.8-1.0 x 3.5-7.7	Single cells	Motile
11	PSB-46	Positive	Rods	0.9-1.3 x 3.9-9.5	Single cells	Motile
12	PSB-48	Positive	Rods	0.7-1.0 x 3.1-6.8	Single cells	Motile
13	PSB-50	Positive	Rods	1.4-1.9 x 5.8-6.9	Cells in chains	Motile
14	PSB-56	Positive	Rods	1.7-2.2 x 5.7-10.9	Single cells	Motile
15	PSB-59	Positive	Rods	2.1-2.3 x 5.1-8.9	Single cells	Motile
16	PSB-60	Positive	Rods	0.7-1.0 x 3.1-5.7	Single cells	Motile
17	PSB-61	Positive	Rods	1.0-1.2 x 3.3-6.0	Single cells	Motile
18	PSB-64	Positive	Rods	1.2-1.3 x 4.9-10.1	Cells are in pairs	Motile
19	PSB-68	Positive	Rods	1.7-2.2 x 6.1-10.0	Cells in chains	Motile
20	PSB-69	Positive	Rods	0.9-1.3 x 3.5-10.0	Single cells	Motile
21	PSB-72	Positive	Rods	0.7-1.1 x 3.0-7.7	Single cells	Motile
22	PSB-73	Positive	Rods	1.3-1.5 x 4.4-8.1	Single cells	Motile
23	PSB-75	Positive	Rods	0.7-1.0 x 3.0-6.0	Single cells	Motile
24	PSB-78	Positive	Rods	1.0-1.2 x 3.6-7.9	Cells in chains	Motile
25	PSB-79	Positive	Rods	1.1-1.3 x 3.9-8.1	Cells are single	Motile
26	PSB-81	Positive	Rods	0.4-0.6 x 1.8-2.0	Single cells	Motile
27	PSB-83	Positive	Rods	0.9-1.3 x 3.3-7.1	Cells in chains	Motile
28	PSB-93	Positive	Rods	2.0-2.3 x 4.0-6.1	Single cells	Motile
29	PSB-95	Positive	Rods	1.9-2.1 x 5.1-9.6	Single cells	Motile
30	PSB-100	Positive	Rods	1.5-1.9 x 4.4-10.0	Cells are single & in chain	Motile
31	PSB-102	Positive	Rods	1.1-1.4 x 3.9-8.8	Cells in chains	Motile
32	PSB-106	Positive	Rods	1.3-1.5 x 4.1-9.5	Cells in chains	Motile
33	PSB-107	Positive	Rods	0.8-1.1 x 3.0-6.1	Single cells	Motile
34	PSB-109	Positive	Rods	0.6-0.9 x 2.9-4.7	Single cells	Motile
35	PSB-111	Positive	Rods	2.2-2.7 x 5.1-8.8	Cells are in chain	Motile
36	PSB-113	Positive	Rods	1.0-1.2 x 3.9-6.7	Single cells	Motile
37	PSB-119	Positive	Rods	1.9-2.5 x 7.1-10.0	Cells are in chain	Motile
38	PSB-125	Positive	Rods	1.5-1.7 x 4.1-8.9	Single cells	Motile
39	PSB-129	Positive	Rods	1.2-1.6 x 3.3-7.7	Cells in chains	Motile
40	PSB-130	Positive	Rods	2.3-2.9 x 3.1-4.5	Single cells	Motile
41	PSB-132	Positive	Rods	0.9-1.3 x 3.3-7.4	Single cells	Motile
42	PSB-136	Positive	Rods	0.7-1.0 x 2.8-4.9	Cells in chains	Motile
43	PSB-140	Positive	Rods	1.2-1.4 x 3.5-5.3	Single cells	Motile
44	PSB-142	Positive	Rods	2.2-2.4 x 3.4-4.9	Single cells	Motile
45	PSB-143	Positive	Rods	1.6-1.9 x 5.8-11.0	Cells are single & in pairs	Motile
46	PSB-144	Positive	Rods	1.3-1.5 x 5.0-9.3	Cells are single & in pairs	Motile
47	PSB-148	Positive	Rods	1.0-1.4 x 3.6-8.1	Single cells	Motile
48	PSB-BNF	Positive	Rods	2.6-3.1 x 4.9-8.3	Cells are single	Motile

Table 14 Colony morphology and microscopic appearance of PSF (*Aspergillus* isolates)

Sr. No.	Isolate	Colony morphology	Microscopic appearance
1	PSF-4	White floccose mycelium spreading rapidly, quickly, producing spores-black coloured	Hyphae-colorless, branching, conidiophores branching, septate, globose vesicle, conidia-small, red, round
2	PSF-7	Velvety, green-dark, green becoming almost black, spreading, reverse colourless	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
3	PSF-8	White cottony, loose woven thread like, forming brown-black spores in due course	Hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
4	PSF-11	White colony, loose woven thread like, forming brown-black spores in due course	Hyphae-colorless, branching, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
5	PSF-19	White floccose mycelium spreading rapidly, quickly, producing spores-black coloured	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
6	PSF-28	White cottony, loose woven thread like, forming brown-black spores in due course	Hyphae-colorless, branching, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
7	PSF-32	Velvety, green-dark, green becoming almost black, spreading	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
8	PSF-40	White colony, loose woven thread like, forming brown-black spores in due course	Hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
9	PSF-41	White floccose mycelium spreading rapidly, quickly, producing spores-black coloured	Hyphae-colorless, branching, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
10	PSF-44	White cottony, loose woven thread like, forming brown-black spores in due course	Hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
11	PSF-55	White cottony, loose woven thread like, forming brown-black spores in due course	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
12	PSF-57	White colony, loose woven thread like, forming brown-black spores in due course	Hyphae-colorless, branching, conidiophores branching, septate, globose vesicle, conidia-small, red, round
13	PSF-64	White floccose mycelium spreading rapidly, quickly, producing spores-black coloured	Hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
14	PSF-71	White floccose mycelium spreading rapidly, quickly, producing spores-black coloured	Hyphae-colorless, branching, conidiophores branching, septate, globose vesicle, conidia-small, red, round
15	PSF-82	White colony, loose woven thread like, forming brown-black spores in due course	Hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round

Table 14 contd…

Sr. No.	Isolate	Colony morphology	Microscopic appearance
16	PSF-100	White floccose mycelium spreading rapidly, quickly, producing spores-black coloured	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
17	PSF-101	White cottony, loose woven thread like, forming brown-black spores in due course	Hyphae-colorless, branching, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
18	PSF-115	Velvety, green-dark, green becoming almost black, spreading	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
19	PSF-119	White colony, loose woven thread like, forming brown-black spores in due course	Hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
20	PSF-120	Velvety, green-dark, green becoming almost black, spreading	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
21	PSF-122	White floccose mycelium spreading rapidly, quickly, producing spores-black coloured	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
22	PSF-124	White colony, loose woven thread like, forming brown-black spores in due course	Hyphae-colorless, branching, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
23	PSF-127	White floccose mycelium spreading rapidly, quickly, producing spores-black coloured	Hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
24	PSF-128	White colony, loose woven thread like, forming brown-black spores in due course	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
25	PSF-129	Velvety, green-dark, green becoming almost black, spreading, reverse colourless	Hyphae-colorless, branching, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
26	PSF-132	Velvety, green-dark, green becoming almost black, spreading	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
27	PSF-135	White cottony, loose woven thread like, forming brown-black spores in due course	Hyphae-colorless, branching, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
28	PSF-143	White colony, loose woven thread like, forming brown-black spores in due course	Hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black, round
29	PSF-148	Velvety, green-dark, green becoming almost black, spreading, reverse colourless	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round
30	PSF-BNF(A)	Velvety, green-dark, green becoming almost black, spreading	Hyphae-aseptate, colorless, conidiophore-septate, red coloured, globose vesicle, conidia-red, round

Table 15 Colony morphology and microscopic appearance of PSF (*Penicillium* isolates)

Sr. No.	Isolate	Colony morphology	Microscopic appearance
1	PSF-30	Velvet, dark-green spreading mycelium, aged cultures forming almost dark grey spores	Branching conidiophores that are borne on aerial mycelia, spheroidal conidia
2	PSF-61	Dark green to turquoise colony, forming blue-green spores in due course	Conidiophores-branched, that are borne on a single rope of fertile hyphae, subspheroidal conidia
3	PSF-65	Velutinous mycelium spreading rapidly, producing blue-green spores	Branching conidiophores occurs in bundles and produced in tufts, ellipsoidal conidia
4	PSF-77	Cotton-wool colony, forming clear to orange spores	Conidiophores produced along the whole length of synnemata and bundled at the top of synnemata, cylindrical conidia
5	PSF-79	Fasciculate mycelium loose woven thread like, later turn pale yellow to green	Striate conidia in chains develop at the end of the sterigmata arising from the metula of the conidiophores, rough walled conidia
6	PSF-80	Dark green to turquoise colony, forming blue-green spores in due course	Branching conidiophores arise from a septate mycelium, spiny conidia
7	PSF-82-1	Velvet, green-dark spreading mycelium, aged cultures forming almost dark grey spores	Conidiophores produced along the whole length of synnemata and bundled at the top of synnemata, cylindrical conidia
8	PSF-85	Yellow mycelial colony, forming yellow-green spores in due course	Branching conidiophores that are borne on aerial mycelia, spheroidal conidia
9	PSF-92	Yellow mycelial colony, forming yellow-green spores in due course	Striate conidia in chains develop at the end of the sterigmata arising from the metula of the conidiophore
10	PSF-97	Velutinous mycelium spreading rapidly, producing dark-green spores	Conidiophores-branched, that are borne on a single rope of fertile hyphae, echinulate conidia
11	PSF-101-1	Velutinous mycelium spreading rapidly, producing dark-green spores	Branching conidiophores that are borne on aerial mycelia, smooth walled conidia
12	PSF-102	Dark green to turquoise colony, forming blue-green spores in due course	Conidiophores-branched, that are borne on a single rope of fertile hyphae
13	PSF-118	Velvet, green-dark spreading mycelium, aged cultures forming almost dark grey spores	Striate conidia in chains develop at the end of the sterigmata arising from the metula of the conidiophores, smooth walled conidia
14	PSF-120-1	Fasciculate mycelium loose woven thread like, later turn yellow to green	Branching conidiophores that are borne on aerial mycelia, spheroidal conidia
15	PSF-136	Yellow mycelial colony, forming yellow-green spores in due course	Conidiophores-branched, that are borne on a single rope of fertile hyphae
16	PSF-138	Cotton-wool colony, forming clear to orange spores	Branching conidiophores arise from a septate mycelium, spheroidal conidia
17	PSF-141	Fasciculate mycelium loose woven thread like, later turn pale yellow to green	Conidiophores-branched, that are borne on a single rope of fertile hyphae
18	PSF-BNF(P)	Velutinous mycelium spreading rapidly, producing yellow-green spores	Conidiophores-branched, that are borne on a single rope of fertile hyphae

Based on colonial morphology, spore characteristics and microscopic examination, out of 46 PSF isolates, 29 were tentatively identified as *Aspergillus awamori* and 17 as *Penicillium digitatum*.

4.4 Cultural characterization

4.4.1 *Azotobacter* isolates

A wide variation in colony characters of *Azotobacter* isolates was observed on Jensen's agar medium (Table 16, Plate 16). Eighty four *Azotobacter* isolates were found to be having circular colonies with entire margin whereas ten isolates having irregular colonies with lobate margin. The elevation of the colonies varied from flat, raised to convex and the colonies appeared to be slimy, milky, viscid, mucoid, glistening and opaque and later aged cultures produced light brown to dark brown pigmentation. The growth of colonies varied from slight, moderate to abundant.

4.4.2 *Rhizobium* isolates

The colonies of all *Rhizobium* isolates were circular with entire margin (Table 17, Plate 17). The elevation of the colonies varied among the isolates. The colonies of forty four *Rhizobium* isolates were having raised elevation whereas thirty two isolates having convex elevation. The colonies appeared to be white-opaque, mucilaginous, semi-translucent, translucent and milky to watery translucent. The colonial growth varied from slight, moderate to abundant.

4.4.3 *Azospirillum* isolates

The colonies of all *Azospirillum* isolates formed subsurface pellicles on semi-solid N-free malate medium (Table 18, Plate 18). The pellicle size varied from 1 to 4 mm. The colonies of 50 *Azospirillum* isolates formed 1-2 mm sized thin pellicles, whereas

Table 16 Cultural characteristics of *Azotobacter* isolates on Jensen's agar medium

Sr. No.	Isolate	Colony characters on Jensen's agar medium					
		Growth	**Form**	**Margin**	**Elevation**	**Appearance**	**Pigmentation**
1	Azt-4	Slight	Circular	Entire	Raised	Opaque	Light brown
2	Azt-8	Moderate	Circular	Entire	Flat	Mucoid	Grey brown
3	Azt-9	Abundant	Circular	Entire	Convex	Slimy	Brown black
4	Azt-10	Abundant	Circular	Entire	Flat	Milky	Dark brown
5	Azt-12	Slight	Circular	Entire	Raised	Milky	Pale brown
6	Azt-18	Moderate	Irregular	Lobate	Flat	Slimy	Grey brown
7	Azt-21	Abundant	Circular	Entire	Raised	Milky	Dark brown
8	Azt-23	Abundant	Circular	Entire	Raised	Opaque	Pale brown
9	Azt-25	Abundant	Circular	Entire	Flat	Viscid	Light brown
10	Azt-26	Moderate	Circular	Entire	Convex	Viscid	Brown black
11	Azt-27	Abundant	Irregular	Lobate	Flat	Opaque	Brown black
12	Azt-28	Slight	Circular	Entire	Flat	Milky	Dark brown
13	Azt-29	Slight	Circular	Entire	Raised	Milky	Grey brown
14	Azt-32	Moderate	Circular	Entire	Raised	Slimy	Grey brown
15	Azt-33	Moderate	Circular	Entire	Convex	Slimy	Pale brown
16	Azt-34	Slight	Circular	Entire	Convex	Slimy	Light brown
17	Azt-36	Abundant	Circular	Entire	Convex	Mucoid	Brown black
18	Azt-37	Abundant	Circular	Entire	Flat	Opaque	Brown black
19	Azt-39	Slight	Circular	Entire	Raised	Opaque	Dark brown
20	Azt-40	Slight	Circular	Entire	Convex	Slimy	Pale brown
21	Azt-42	Moderate	Irregular	Lobate	Raised	Viscid	Grey brown
22	Azt-43	Abundant	Circular	Entire	Raised	Opaque	Dark brown
23	Azt-44	Abundant	Circular	Entire	Raised	Milky	Grey brown
24	Azt-45	Slight	Circular	Entire	Convex	Milky	Light brown
25	Azt-48	Moderate	Irregular	Lobate	Flat	Mucoid	Grey brown
26	Azt-49	Moderate	Circular	Entire	Raised	Opaque	Brown black
27	Azt-50	Slight	Circular	Entire	Raised	Opaque	Light brown
28	Azt-52	Abundant	Circular	Entire	Convex	Milky	Dark brown
29	Azt-53	Slight	Circular	Entire	Raised	Milky	Grey brown
30	Azt-54	Slight	Circular	Entire	Raised	Viscid	Grey brown
31	Azt-55	Moderate	Circular	Entire	Flat	Viscid	Pale brown
32	Azt-56	Moderate	Circular	Entire	Raised	Opaque	Light brown

contd…

Table 16 contd…

Sr. No.	Isolate	Colony characters on Jensen's agar medium					
		Growth	**Form**	**Margin**	**Elevation**	**Appearance**	**Pigmentation**
33	Azt-57	Moderate	Circular	Entire	Raised	Slimy	Brown black
34	Azt-58	Moderate	Circular	Entire	Raised	Opaque	Brown black
35	Azt-60	Slight	Circular	Entire	Convex	Opaque	Dark brown
36	Azt-61	Abundant	Circular	Entire	Flat	Viscid	Pale brown
37	Azt-63	Abundant	Circular	Entire	Convex	Viscid	Grey brown
38	Azt-64	Moderate	Circular	Entire	Raised	Mucoid	Dark brown
39	Azt-65	Moderate	Circular	Entire	Raised	Mucoid	Grey brown
40	Azt-66	Slight	Circular	Entire	Convex	Mucoid	Light brown
41	Azt-67	Abundant	Irregular	Lobate	Raised	Milky	Grey brown
42	Azt-68	Abundant	Circular	Entire	Raised	Milky	Brown black
43	Azt-69	Slight	Irregular	Lobate	Flat	Viscid	Light brown
44	Azt-70	Moderate	Circular	Entire	Raised	Opaque	Light brown
45	Azt-72	Abundant	Irregular	Lobate	Convex	Slimy	Grey brown
46	Azt-73	Abundant	Circular	Entire	Flat	Milky	Brown black
47	Azt-75	Moderate	Circular	Entire	Raised	Opaque	Dark brown
48	Azt-77	Moderate	Circular	Entire	Raised	Opaque	Pale brown
49	Azt-78	Slight	Circular	Entire	Convex	Slimy	Grey brown
50	Azt-79	Abundant	Circular	Entire	Convex	Slimy	Light brown
51	Azt-81	Abundant	Circular	Entire	Raised	Mucoid	Pale brown
52	Azt-82	Abundant	Circular	Entire	Raised	Glistening	Light brown
53	Azt-83	Moderate	Circular	Entire	Flat	Opaque	Brown black
54	Azt-84	Slight	Circular	Entire	Flat	Viscid	Brown black
55	Azt-86	Slight	Circular	Entire	Raised	Slimy	Pale brown
56	Azt-88	Abundant	Circular	Entire	Raised	Glistening	Light brown
57	Azt-89	Abundant	Circular	Entire	Flat	Milky	Dark brown
58	Azt-91	Moderate	Circular	Entire	Convex	Milky	Grey brown
59	Azt-93	Slight	Circular	Entire	Raised	Viscid	Light brown
60	Azt-94	Moderate	Irregular	Lobate	Convex	Slimy	Grey brown
61	Azt-97	Abundant	Circular	Entire	Raised	Milky	Brown black
62	Azt-99	Abundant	Circular	Entire	Raised	Viscid	Light brown
63	Azt-101	Moderate	Circular	Entire	Convex	Milky	Dark brown
64	Azt-102	Abundant	Circular	Entire	Flat	Opaque	Grey brown

contd…

Table 16 contd…

Sr. No.	Isolate	Colony characters on Jensen's agar medium					
		Growth	**Form**	**Margin**	**Elevation**	**Appearance**	**Pigmentation**
65	Azt-104	Slight	Circular	Entire	Raised	Opaque	Brown black
66	Azt-105	Slight	Circular	Entire	Raised	Viscid	Grey brown
67	Azt-106	Slight	Circular	Entire	Raised	Mucoid	Grey brown
68	Azt-107	Abundant	Circular	Entire	Convex	Mucoid	Dark brown
69	Azt-108	Moderate	Circular	Entire	Raised	Milky	Pale brown
70	Azt-109	Moderate	Irregular	Lobate	Raised	Opaque	Grey brown
71	Azt-110	Moderate	Circular	Entire	Convex	Mucoid	Pale brown
72	Azt-112	Slight	Circular	Entire	Raised	Viscid	Light brown
73	Azt-113	Abundant	Circular	Entire	Flat	Opaque	Brown black
74	Azt-114	Slight	Circular	Entire	Convex	Opaque	Brown black
75	Azt-118	Abundant	Circular	Entire	Flat	Opaque	Dark brown
76	Azt-120	Moderate	Circular	Entire	Raised	Milky	Pale brown
77	Azt-122	Moderate	Circular	Entire	Raised	Milky	Grey brown
78	Azt-125	Slight	Circular	Entire	Raised	Viscid	Dark brown
79	Azt-126	Slight	Circular	Entire	Convex	Slimy	Grey brown
80	Azt-129	Abundant	Circular	Entire	Convex	Slimy	Light brown
81	Azt-130	Slight	Circular	Entire	Raised	Mucoid	Grey brown
82	Azt-131	Slight	Circular	Entire	Convex	Mucoid	Dark brown
83	Azt-132	Moderate	Circular	Entire	Raised	Milky	Grey brown
84	Azt-133	Moderate	Circular	Entire	Raised	Viscid	Grey brown
85	Azt-135	Moderate	Circular	Entire	Raised	Milky	Pale brown
86	Azt-136	Abundant	Circular	Entire	Convex	Viscid	Light brown
87	Azt-137	Abundant	Circular	Entire	Flat	Opaque	Brown black
88	Azt-138	Slight	Circular	Entire	Raised	Slimy	Brown black
89	Azt-141	Moderate	Irregular	Lobate	Flat	Opaque	Dark brown
90	Azt-142	Moderate	Circular	Entire	Raised	Slimy	Pale brown
91	Azt-144	Abundant	Circular	Entire	Raised	Milky	Grey brown
92	Azt-147	Slight	Circular	Entire	Convex	Mucoid	Dark brown
93	Azt-148	Slight	Circular	Entire	Convex	Viscid	Grey brown
94	Azt-149	Moderate	Circular	Entire	Raised	Opaque	Light brown
95	Azt-BNF	Abundant	Circular	Entire	Convex	Viscid	Dark brown

Table 17 Cultural characteristics of *Rhizobium* isolates on yeast extract mannitol agar

Sr. No.	Isolate	Colony characters on Yeast Extract Mannitol Agar Medium				
		Growth	**Form**	**Margin**	**Elevation**	**Appearance**
1	Rh-13	Moderate	Circular	Entire	Convex	White-opaque
2	Rh-21	Slight	Circular	Entire	Convex	Semi-translucent
3	Rh-22	Slight	Circular	Entire	Raised	Semi-translucent
4	Rh-28	Abundant	Circular	Entire	Raised	Translucent
5	Rh-29	Abundant	Circular	Entire	Raised	White-opaque
6	Rh-37	Moderate	Circular	Entire	Convex	White-opaque
7	Rh-39	Moderate	Circular	Entire	Raised	Mucilagenous
8	Rh-40	Slight	Circular	Entire	Convex	Mucilagenous
9	Rh-42	Slight	Circular	Entire	Convex	Milky to watery translucent
10	Rh-43	Moderate	Circular	Entire	Convex	Translucent
11	Rh-44	Abundant	Circular	Entire	Convex	White opaque
12	Rh-50	Abundant	Circular	Entire	Raised	White opaque
13	Rh-51	Slight	Circular	Entire	Raised	Semi-translucent
14	Rh-53	Slight	Circular	Entire	Raised	Semi-translucent
15	Rh-54	Moderate	Circular	Entire	Convex	Translucent
16	Rh-55	Abundant	Circular	Entire	Convex	Mucilagenous
17	Rh-56	Abundant	Circular	Entire	Convex	Mucilagenous
18	Rh-58	Slight	Circular	Entire	Raised	Semi-translucent
19	Rh-60	Abundant	Circular	Entire	Convex	White-opaque
20	Rh-61	Moderate	Circular	Entire	Raised	Transluent
21	Rh-62	Abundant	Circular	Entire	Raised	Transluent
22	Rh-64	Slight	Circular	Entire	Raised	Semi-translucent
23	Rh-65	Abundant	Circular	Entire	Raised	Mucilagenous
24	Rh-66	Slight	Circular	Entire	Raised	Mucilagenous
25	Rh-67	Slight	Circular	Entire	Convex	Transluent
26	Rh-68	Moderate	Circular	Entire	Raised	White-opaque
27	Rh-69	Moderate	Circular	Entire	Raised	Translucent
28	Rh-70	Abundant	Circular	Entire	Raised	White-opaque
29	Rh-71	Moderate	Circular	Entire	Raised	White-opaque
30	Rh-72	Moderate	Circular	Entire	Convex	Translucent
31	Rh-73	Moderate	Circular	Entire	Raised	White-opaque
32	Rh-74	Abundant	Circular	Entire	Raised	White-opaque
33	Rh-76	Slight	Circular	Entire	Raised	Mucilagenous
34	Rh-78	Slight	Circular	Entire	Convex	Mucilagenous
35	Rh-80	Moderate	Circular	Entire	Convex	White-opaque
36	Rh-81	Abundant	Circular	Entire	Raised	White-opaque
37	Rh-82	Moderate	Circular	Entire	Raised	Translucent
38	Rh-84	Slight	Circular	Entire	Convex	Semi-translucent
39	Rh-85	Slight	Circular	Entire	Convex	Milky to watery translucent

contd…

Table 17 contd…

Sr. No.	Isolate	Colony characters Yeast Extract Mannitol Agar Medium				
		Growth	**Form**	**Margin**	**Elevation**	**Appearance**
40	Rh-86	Abundant	Circular	Entire	Raised	White-opaque
41	Rh-93	Abundant	Circular	Entire	Convex	Semi-translucent
42	Rh-95	Moderate	Circular	Entire	Convex	Translucent
43	Rh-97	Abundant	Circular	Entire	Convex	Translucent
44	Rh-98	Slight	Circular	Entire	Raised	White-opaque
45	Rh-100	Moderate	Circular	Entire	Raised	Milky to watery translucent
46	Rh-101	Abundant	Circular	Entire	Raised	Semi-translucent
47	Rh-102	Abundant	Circular	Entire	Convex	White-opaque
48	Rh-105	Moderate	Circular	Entire	Raised	White-opaque
49	Rh-106	Moderate	Circular	Entire	Raised	Mucilagenous
50	Rh-107	Moderate	Circular	Entire	Raised	Mucilagenous
51	Rh-108	Slight	Circular	Entire	Raised	Transluent
52	Rh-109	Slight	Circular	Entire	Convex	Semi-transluent
53	Rh-112	Abundant	Circular	Entire	Convex	Mucilagenous
54	Rh-113	Moderate	Circular	Entire	Raised	White-opaque
55	Rh-114	Slight	Circular	Entire	Convex	White-opaque
56	Rh-115	Slight	Circular	Entire	Raised	White-opaque
57	Rh-117	Moderate	Circular	Entire	Raised	Semi-translucent
58	Rh-118	Abundant	Circular	Entire	Raised	Mucilagenous
59	Rh-120	Moderate	Circular	Entire	Convex	Translucent
60	Rh-123	Slight	Circular	Entire	Raised	Translucent
61	Rh-124	Slight	Circular	Entire	Convex	Mucilagenous
62	Rh-125	Slight	Circular	Entire	Convex	White-opaque
63	Rh-126	Moderate	Circular	Entire	Convex	Milky to watery translucent
64	Rh-129	Abundant	Circular	Entire	Raised	Translucent
65	Rh-130	Abundant	Circular	Entire	Raised	White-opaque
66	Rh-132	Moderate	Circular	Entire	Raised	White-opaque
67	Rh-133	Moderate	Circular	Entire	Raised	Semi-translucent
68	Rh-136	Moderate	Circular	Entire	Convex	Semi-translucent
69	Rh-138	Slight	Circular	Entire	Convex	Translucent
70	Rh-139	Abundant	Circular	Entire	Convex	Translucent
71	Rh-140	Abundant	Circular	Entire	Raised	Mucilagenous
72	Rh-143	Slight	Circular	Entire	Raised	White-opaque
73	Rh-144	Slight	Circular	Entire	Convex	White-opaque
74	Rh-146	Moderate	Circular	Entire	Raised	Milky to watery translucent
75	Rh-147	Moderate	Circular	Entire	Raised	Translucent
76	Rh-148	Moderate	Circular	Entire	Raised	Semi-translucent
77	Rh-BNF	Abundant	Circular	Entire	Convex	White-opaque

Table 18 Cultural characteristics of *Azospirillum* isolates on semi-solid N free malate medium

Sr. No.	Isolate	Colony characters on semi-solid N free malate medium		
		Form	Size (mm)	Appearance
1	Asp-1	Pellicle	1	White dense subsurface thin pellicle
2	Asp-2	Pellicle	3	White dense thick pellicle
3	Asp-3	Pellicle	2	White globular thin pellicle
4	Asp-4	Pellicle	2	White dense thin pellicle
5	Asp-5	Pellicle	1	White dense thin pellicle
6	Asp-8	Pellicle	4	Dull white thick pellicle
7	Asp-10	Pellicle	3	White dense thick pellicle
8	Asp-11	Pellicle	3	White dense thick pellicle
9	Asp-14	Pellicle	2	White dense thin pellicle
10	Asp-15	Pellicle	4	White globular thick pellicle
11	Asp-16	Pellicle	2	White globular thin pellicle
12	Asp-21	Pellicle	1	White dense thin pellicle
13	Asp-23	Pellicle	2	White dense thin pellicle
14	Asp-24	Pellicle	4	White dense thick pellicle
15	Asp-25	Pellicle	3	White globular thick pellicle
16	Asp-27	Pellicle	3	White globular thick pellicle
17	Asp-28	Pellicle	1	White dense thin pellicle
18	Asp-29	Pellicle	2	White globular thin pellicle
19	Asp-31	Pellicle	4	White dense thick pellicle
20	Asp-33	Pellicle	3	White globular thick pellicle
21	Asp-34	Pellicle	2	White dense thin pellicle
22	Asp-35	Pellicle	3	White globular thick pellicle
23	Asp-36	Pellicle	1	White dense thin pellicle
24	Asp-37	Pellicle	3	White globular thick pellicle
25	Asp-38	Pellicle	2	White dense thin pellicle
26	Asp-39	Pellicle	1	White dense thin pellicle
27	Asp-40	Pellicle	3	Dull white dense thick pellicle
28	Asp-41	Pellicle	2	White globular thin pellicle
29	Asp-42	Pellicle	1	White globular thin pellicle
30	Asp-43	Pellicle	2	White dense thin pellicle
31	Asp-44	Pellicle	3	White dense thick pellicle
32	Asp-46	Pellicle	2	White globular thin pellicle
33	Asp-47	Pellicle	4	White dense thick pellicle
34	Asp-48	Pellicle	2	White dense thin pellicle
35	Asp-49	Pellicle	1	White dense thin pellicle
36	Asp-50	Pellicle	1	White globular subsurface thin pellicle
37	Asp-51	Pellicle	3	White dense thick pellicle
38	Asp-55	Pellicle	2	White globular thin pellicle
39	Asp-56	Pellicle	2	White globular thin pellicle

contd…

Table 18 contd…

Sr. No.	Isolate	Colony characters on semi-solid N free malate medium		
		Form	Size (mm)	Appearance
40	Asp-57	Pellicle	1	White dense thin pellicle
41	Asp-59	Pellicle	2	White dense thin pellicle
42	Asp-60	Pellicle	2	White globular subsurface thin pellicle
43	Asp-62	Pellicle	2	White dense thin pellicle
44	Asp-63	Pellicle	1	White dense thin pellicle
45	Asp-64	Pellicle	3	Dull white thin pellicle
46	Asp-67	Pellicle	2	White dense thin pellicle
47	Asp-68	Pellicle	3	Dull white thick pellicle
48	Asp-69	Pellicle	2	White dense thin pellicle
49	Asp-70	Pellicle	3	White globular thin pellicle
50	Asp-72	Pellicle	2	White dense thin pellicle
51	Asp-73	Pellicle	1	Dull white thick pellicle
52	Asp-75	Pellicle	1	Dull white thick pellicle
53	Asp-78	Pellicle	1	White dense thick pellicle
54	Asp-82	Pellicle	2	White globular subsurface thin pellicle
55	Asp-83	Pellicle	4	White globular thick pellicle
56	Asp-85	Pellicle	3	White dense thick pellicle
57	Asp-86	Pellicle	3	White dense thick pellicle
58	Asp-87	Pellicle	2	White dense thin pellicle
59	Asp-88	Pellicle	2	White dense thin pellicle
60	Asp-90	Pellicle	4	White dense thick pellicle
61	Asp-92	Pellicle	3	Dull white thick pellicle
62	Asp-95	Pellicle	3	White globular thick pellicle
63	Asp-96	Pellicle	3	White globular thick pellicle
64	Asp-97	Pellicle	1	White dense subsurface thin pellicle
65	Asp-99	Pellicle	3	White globular thick pellicle
66	Asp-101	Pellicle	2	White dense thin pellicle
67	Asp-102	Pellicle	3	White dense thick pellicle
68	Asp-103	Pellicle	3	White dense thick pellicle
69	Asp-106	Pellicle	4	White globular thick pellicle
70	Asp-107	Pellicle	3	White globular thick pellicle
71	Asp-108	Pellicle	2	White dense thin pellicle
72	Asp-112	Pellicle	4	White globular thick pellicle
73	Asp-113	Pellicle	4	Dull white thick pellicle
74	Asp-116	Pellicle	3	White dense thick pellicle
75	Asp-117	Pellicle	3	White globular thick pellicle
76	Asp-119	Pellicle	1	White dense thin pellicle
77	Asp-120	Pellicle	2	White dense thin pellicle
78	Asp-121	Pellicle	3	White globular thick pellicle

contd…

Table 18 contd…

Sr. No.	Isolate	Colony characters on semi-solid N free malate medium		
		Form	Size (mm)	Appearance
79	Asp-123	Pellicle	3	White globular thick pellicle
80	Asp-124	Pellicle	1	White globular thin pellicle
81	Asp-127	Pellicle	2	White dense thin pellicle
82	Asp-129	Pellicle	4	Dull white thick pellicle
83	Asp-130	Pellicle	1	White globular thin pellicle
84	Asp-132	Pellicle	1	White dense thin pellicle
85	Asp-134	Pellicle	2	White dense thin pellicle
86	Asp-136	Pellicle	3	White globular thick pellicle
87	Asp-140	Pellicle	2	White dense thin pellicle
88	Asp-142	Pellicle	1	White dense thin pellicle
89	Asp-145	Pellicle	2	White dense thin pellicle
90	Asp-147	Pellicle	4	White globular thick pellicle
91	Asp-148	Pellicle	3	White globular thick pellicle
92	Asp-149	Pellicle	4	Dull white thick pellicle
93	Asp-150	Pellicle	2	White globular thin pellicle
94	Asp-BNF	Pellicle	1	White dense subsurface thin pellicle

Table 19 Cultural characteristics of *Azospirillum* isolates on BMS agar medium

Sr. No.	Isolate	Colony characters on BMS agar medium				
		Form	Margin	Elevation	Pigmentation	Optical characters
1	Asp-1	Irregular	Serrate	Umbonate	Pink	Opaque
2	Asp-2	Round	Entire	Raised	Pink	Opaque
3	Asp-3	Round	Entire	Convex	Pink	Opaque
4	Asp-4	Irregular	Serrate	Umbonate	Pink	Opaque
5	Asp-5	Round	Entire	Raised	Pink	Opaque
6	Asp-8	Round	Entire	Raised	Pink	Opaque
7	Asp-10	Round	Entire	Convex	Pink	Opaque
8	Asp-11	Irregular	Lobate	Umbonate	Pink	Opaque
9	Asp-14	Round	Entire	Raised	Pink	Opaque
10	Asp-15	Irregular	Undulate	Raised	Pink	Opaque
11	Asp-16	Irregular	Lobate	Umbonate	Pink	Opaque
12	Asp-21	Round	Entire	Convex	Pink	Opaque
13	Asp-23	Round	Entire	Convex	Pink	Opaque
14	Asp-24	Round	Entire	Raised	Pink	Opaque
15	Asp-25	Irregular	Serrate	Convex	Pink	Opaque
16	Asp-27	Irregular	Serrate	Umbonate	Pink	Opaque
17	Asp-28	Round	Lobate	Convex	Pink	Opaque
18	Asp-29	Round	Entire	Raised	Pink	Opaque

contd…

Table 19 contd…

Sr. No.	Isolate	Colony characters on BMS agar medium				
		Form	**Margin**	**Elevation**	**Pigmentation**	**Optical characters**
19	Asp-31	Irregular	Undulate	Umbonate	Pink	Opaque
20	Asp-33	Irregular	Serrate	Umbonate	Pink	Opaque
21	Asp-34	Irregular	Serrate	Raised	Pink	Opaque
22	Asp-35	Round	Entire	Convex	Pink	Opaque
23	Asp-36	Irregular	Lobate	Convex	Pink	Opaque
24	Asp-37	Round	Entire	Raised	Pink	Opaque
25	Asp-38	Round	Entire	Raised	Pink	Opaque
26	Asp-39	Irregular	Serrate	Umbonate	Pink	Opaque
27	Asp-40	Round	Entire	Raised	Pink	Opaque
28	Asp-41	Round	Entire	Raised	Pink	Opaque
29	Asp-42	Irregular	Serrate	Umbonate	Pink	Opaque
30	Asp-43	Irregular	Serrate	Convex	Pink	Opaque
31	Asp-44	Irregular	Lobate	Convex	Pink	Opaque
32	Asp-46	Round	Entire	Raised	Pink	Opaque
33	Asp-47	Round	Entire	Umbonate	Pink	Opaque
34	Asp-48	Irregular	Undulate	Raised	Pink	Opaque
35	Asp-49	Round	Entire	Convex	Pink	Opaque
36	Asp-50	Irregular	Serrate	Raised	Pink	Opaque
37	Asp-51	Irregular	Lobate	Raised	Pink	Opaque
38	Asp-55	Irregular	Undulate	Convex	Pink	Opaque
39	Asp-56	Round	Entire	Raised	Pink	Opaque
40	Asp-57	Round	Entire	Raised	Pink	Opaque
41	Asp-59	Irregular	Undulate	Umbonate	Pink	Opaque
42	Asp-60	Round	Entire	Raised	Pink	Opaque
43	Asp-62	Round	Entire	Convex	Pink	Opaque
44	Asp-63	Irregular	Lobate	Umbonate	Pink	Opaque
45	Asp-64	Round	Entire	Umbonate	Pink	Opaque
46	Asp-67	Round	Entire	Convex	Pink	Opaque
47	Asp-68	Round	Entire	Raised	Pink	Opaque
48	Asp-69	Irregular	Lobate	Raised	Pink	Opaque
49	Asp-70	Round	Entire	Convex	Pink	Opaque
50	Asp-72	Round	Entire	Raised	Pink	Opaque
51	Asp-73	Irregular	Lobate	Convex	Pink	Opaque
52	Asp-75	Round	Entire	Umbonate	Pink	Opaque
53	Asp-78	Irregular	Serrate	Umbonate	Pink	Opaque
54	Asp-82	Irregular	Undulate	Raised	Pink	Opaque
55	Asp-83	Round	Entire	Raised	Pink	Opaque
56	Asp-85	Round	Entire	Convex	Pink	Opaque
57	Asp-86	Round	Entire	Convex	Pink	Opaque

contd…

Table 19 contd…

Sr. No.	Isolate	Colony characters on BMS agar medium				
		Form	**Margin**	**Elevation**	**Pigmentation**	**Optical characters**
58	Asp-87	Irregular	Lobate	Raised	Pink	Opaque
59	Asp-88	Round	Entire	Raised	Pink	Opaque
60	Asp-90	Round	Entire	Convex	Pink	Opaque
61	Asp-92	Irregular	Serrate	Umbonate	Pink	Opaque
62	Asp-95	Irregular	Serrate	Umbonate	Pink	Opaque
63	Asp-96	Round	Entire	Raised	Pink	Opaque
64	Asp-97	Irregular	Serrate	Umbonate	Pink	Opaque
65	Asp-99	Round	Entire	Raised	Pink	Opaque
66	Asp-101	Irregular	Undulate	Umbonate	Pink	Opaque
67	Asp-102	Round	Entire	Raised	Pink	Opaque
68	Asp-103	Round	Entire	Raised	Pink	Opaque
69	Asp-106	Irregular	Undulate	Raised	Pink	Opaque
70	Asp-107	Round	Entire	Convex	Pink	Opaque
71	Asp-108	Round	Entire	Raised	Pink	Opaque
72	Asp-112	Round	Entire	Convex	Pink	Opaque
73	Asp-113	Irregular	Serrate	Umbonate	Pink	Opaque
74	Asp-116	Round	Entire	Convex	Pink	Opaque
75	Asp-117	Round	Entire	Convex	Pink	Opaque
76	Asp-119	Irregular	Lobate	Raised	Pink	Opaque
77	Asp-120	Irregular	Undulate	Raised	Pink	Opaque
78	Asp-121	Round	Entire	Convex	Pink	Opaque
79	Asp-123	Round	Entire	Raised	Pink	Opaque
80	Asp-124	Irregular	Serrate	Umbonate	Pink	Opaque
81	Asp-127	Irregular	Undulate	Raised	Pink	Opaque
82	Asp-129	Round	Entire	Convex	Pink	Opaque
83	Asp-130	Irregular	Lobate	Raised	Pink	Opaque
84	Asp-132	Irregular	Undulate	Convex	Pink	Opaque
85	Asp-134	Round	Entire	Convex	Pink	Opaque
86	Asp-136	Irregular	Serrate	Umbonate	Pink	Opaque
87	Asp-140	Irregular	Serrate	Raised	Pink	Opaque
88	Asp-142	Round	Entire	Raised	Pink	Opaque
89	Asp-145	Round	Entire	Raised	Pink	Opaque
90	Asp-147	Round	Entire	Umbonate	Pink	Opaque
91	Asp-148	Irregular	Serrate	Raised	Pink	Opaque
92	Asp-149	Irregular	Undulate	Convex	Pink	Opaque
93	Asp-150	Irregular	Serrate	Umbonate	Pink	Opaque
94	Asp-BNF	Irregular	Lobate	Umbonate	Pink	Opaque

43 isolates formed 3-4 mm sized thick pellicles. The colonies of 83 isolates appeared to be white dense with white globular subsurface thin as well as thick pellicles whereas the dull white thick pellicles were shown by 10 isolates. The distinct variation in colony characters of *Azospirillum* isolates was noticed on BMS agar medium (Table 19). The round as well as irregular colonies with entire, serrate, lobate and undulate margins and raised, convex and umbonate elevations were observed among the different *Azospirillum* isolates. All isolates produced pink pigmentation on BMS agar medium having opaque in their optical characteristics.

4.4.4 PSB isolates

All PSB isolates formed circular colonies with entire as well as lobate margins, the elevation varied from flat, raised to convex and the colonies appeared to be creamy as well as whitish on Pikovskaya's agar medium (Table 20). The zone of phosphate solubilization varied from 5 to 14 mm among the different PSB isolates. The highest zone of 14 mm was produced by PSB-15 isolate (Plate 19).

On nutrient agar medium, all PSB isolates formed circular colonies with raised as well as convex elevations and colonies appeared to be moderately dull, glossy or slightly rugose on the medium and later aged cultures produced pink, yellow to brown pigmentation (Table 21, Plate 20).

Table 20 Cultural characteristics of phosphate solubilizing bacteria on Pikovskaya's medium

Sr. No.	Isolate	Colony characters on Pikovskaya's Medium				
		Zone of 'P' solubilization on TCP (mm)	Form	Margin	Elevation	Appearance
1	PSB-6	7.0	Circular	Entire	Flat	Creamy
2	PSB-10	9.0	Circular	Entire	Raised	Whitish
3	PSB-11	8.0	Circular	Lobate	Raised	Whitish
4	PSB-15	14.0	Circular	Entire	Convex	Whitish
5	PSB-17	10.0	Circular	Entire	Flat	Creamy
6	PSB-20	7.0	Circular	Entire	Raised	Whitish
7	PSB-33	9.0	Circular	Lobate	Flat	Creamy
8	PSB-39	11.0	Circular	Lobate	Raised	Creamy
9	PSB-41	10.0	Circular	Entire	Raised	Whitish
10	PSB-42	7.0	Circular	Entire	Convex	Whitish
11	PSB-46	9.0	Circular	Entire	Convex	Creamy
12	PSB-48	7.0	Circular	Lobate	Raised	Creamy
13	PSB-50	6.0	Circular	Lobate	Raised	Creamy
14	PSB-56	8.0	Circular	Entire	Raised	Whitish
15	PSB-59	5.0	Circular	Entire	Flat	Creamy
16	PSB-60	9.0	Circular	Entire	Convex	Creamy
17	PSB-61	7.0	Circular	Lobate	Convex	Creamy
18	PSB-64	8.0	Circular	Entire	Raised	Whitish
19	PSB-68	6.0	Circular	Entire	Raised	Whitish
20	PSB-69	6.0	Circular	Lobate	Convex	Creamy
21	PSB-72	10.0	Circular	Entire	Flat	Creamy
22	PSB-73	9.0	Circular	Lobate	Raised	Whitish
23	PSB-75	9.0	Circular	Entire	Convex	Whitish
24	PSB-78	8.0	Circular	Entire	Convex	Creamy
25	PSB-79	7.0	Circular	Lobate	Raised	Creamy
26	PSB-81	6.0	Circular	Lobate	Raised	Whitish
27	PSB-83	7.0	Circular	Entire	Raised	Creamy
28	PSB-93	5.0	Circular	Entire	Flat	Whitish
29	PSB-95	8.0	Circular	Entire	Raised	Whitish
30	PSB-100	12.0	Circular	Lobate	Flat	Creamy
31	PSB-102	9.0	Circular	Entire	Convex	Creamy
32	PSB-106	7.0	Circular	Lobate	Convex	Creamy
33	PSB-107	6.0	Circular	Entire	Raised	Whitish
34	PSB-109	10.0	Circular	Lobate	Convex	Whitish

contd…

Table 20 contd…

Sr. No.	Isolate	Colony characters on Pikovskaya's Medium				
		Zone of 'P' solubilization on TCP (mm)	Form	Margin	Elevation	Appearance
35	PSB-111	11.0	Circular	Entire	Flat	Creamy
36	PSB-113	10.0	Circular	Entire	Convex	Creamy
37	PSB-119	9.0	Circular	Entire	Raised	Whitish
38	PSB-125	8.0	Circular	Lobate	Flat	Whitish
39	PSB-129	9.0	Circular	Lobate	Convex	Whitish
40	PSB-130	6.0	Circular	Entire	Convex	Creamy
41	PSB-132	11.0	Circular	Entire	Raised	Creamy
42	PSB-136	7.0	Circular	Entire	Flat	Whitish
43	PSB-140	9.0	Circular	Lobate	Flat	Creamy
44	PSB-142	9.0	Circular	Entire	Raised	Creamy
45	PSB-143	6.0	Circular	Entire	Convex	Whitish
46	PSB-144	10.0	Circular	Entire	Flat	Whitish
47	PSB-148	9.0	Circular	Lobate	Convex	Creamy
48	PSB-BNF	9.0	Circular	Entire	Raised	Whitish

Table 21 Cultural characteristics of phosphate solubilizing bacteria on nutrient agar medium

Sr. No.	Isolate	Colony characters on Nutrient Agar Medium			
		Form	Elevation	Appearance	Pigmentation
1	PSB-6	Circular	Convex	Moderately dull	Yellow
2	PSB-10	Circular	Raised	Moderately dull	Brown
3	PSB-11	Circular	Convex	Glossy	Yellow
4	PSB-15	Circular	Raised	White	Pink
5	PSB-17	Circular	Raised	Moderately dull	Pink
6	PSB-20	Circular	Raised	Slightly rugose	Yellow
7	PSB-33	Circular	Flat	Moderately dull	Yellow
8	PSB-39	Circular	Convex	Creamy	Brown
9	PSB-41	Circular	Convex	Slightly rugose	Brown
10	PSB-42	Circular	Convex	Glossy	Pink
11	PSB-46	Circular	Raised	Glossy	Yellow
12	PSB-48	Circular	Raised	Glossy	Pink
13	PSB-50	Circular	Convex	Moderately dull	Yellow
14	PSB-56	Circular	Raised	Glossy	Brown

contd…

Table 21 contd…

Sr. No.	Isolate	Colony characters on Nutrient Agar Medium			
		Form	**Elevation**	**Appearance**	**Pigmentation**
15	PSB-59	Circular	Raised	Moderately dull	Yellow
16	PSB-60	Circular	Convex	Moderately dull	Brown
17	PSB-61	Circular	Raised	Glossy	Pink
18	PSB-64	Circular	Raised	Glossy	Brown
19	PSB-68	Circular	Convex	Slightly rugose	Yellow
20	PSB-69	Circular	Convex	Slightly rugose	Yellow
21	PSB-72	Circular	Raised	Glossy	Brown
22	PSB-73	Circular	Raised	Moderately dull	Pink
23	PSB-75	Circular	Convex	Glossy	Brown
24	PSB-78	Circular	Convex	Glossy	Brown
25	PSB-79	Circular	Raised	Moderately dull	Yellow
26	PSB-81	Circular	Convex	Moderately dull	Yellow
27	PSB-83	Circular	Convex	Moderately dull	Yellow
28	PSB-93	Circular	Raised	Glossy	Yellow
29	PSB-95	Circular	Raised	Glossy	Yellow
30	PSB-100	Circular	Convex	Moderately dull	Brown
31	PSB-102	Circular	Raised	Moderately dull	Pink
32	PSB-106	Circular	Convex	Glossy	Brown
33	PSB-107	Circular	Convex	Slightly rugose	Brown
34	PSB-109	Circular	Convex	Slightly rugose	Brown
35	PSB-111	Circular	Raised	Moderately dull	Yellow
36	PSB-113	Circular	Raised	Glossy	Yellow
37	PSB-119	Circular	Convex	Moderately dull	Pink
38	PSB-125	Circular	Raised	Glossy	Brown
39	PSB-129	Circular	Raised	Glossy	Yellow
40	PSB-130	Circular	Raised	Slightly rugose	Brown
41	PSB-132	Circular	Convex	Moderately dull	Brown
42	PSB-136	Circular	Convex	Glossy	Brown
43	PSB-140	Circular	Raised	Glossy	Yellow
44	PSB-142	Circular	Raised	Glossy	Pink
45	PSB-143	Circular	Raised	Moderately dull	Brown
46	PSB-144	Circular	Raised	Slightly rugose	Yellow
47	PSB-148	Circular	Convex	Creamy	Yellow
48	PSB-BNF	Circular	Convex	Glossy	Brown

4.5 Biochemical and physiological characterization

4.5.1 *Azotobacter* isolates

All the 94 isolates alongwith MPKV strain (Azt-BNF) were tested for their biochemical characters *viz.*, starch hydrolysis, H_2S production, gelatin liquefaction, catalase test, oxidase test and physiological characters like utilization of different carbon sources. All the 94 *Azotobacter* isolates were positive for starch hydrolysis, H_2S production, catalase test and oxidase test, but were negative for gelatin liquefaction (Table 22, Plate 21). Mannitol, glucose, sucrose and fructose were used as a sole carbon source for growth by all the isolates, while malate and citrate showed negative result (Table 23).

Based on the morphological, cultural, biochemical and physiological characterization, 94 isolates were tentatively identified as *Azotobacter chroococcum*.

4.5.2 *Rhizobium* isolates

All the 76 isolates alongwith MPKV strain (Rh-BNF) were tested for their biochemical characters *viz.*, starch hydrolysis, H_2S production, gelatin liquefaction, catalase test, oxidase test and physiological traits like utilization of different carbon sources. All the 76 *Rhizobium* isolates were positive for starch hydrolysis, H_2S production, catalase test and oxidase test, but were negative for gelatin liquefaction (Table 24, Plate 22). Mannitol, glucose, sucrose and arabinose were used as a sole carbon source for growth by all the isolates, while malate and citrate showed negative result (Table 25).

Based on the morphological, cultural, biochemical and physiological characterization, 76 isolates were tentatively identified as *Rhizobium* spp.

Table 22 Biochemical characteristics of *Azotobacter* isolates

Sr. No.	Isolate	Biochemical Tests				
		Starch hydrolysis	H_2S production	Gelatin liquefaction	Catalase test	Oxidase test
1	Azt-4	+	+	-	+	+
2	Azt-8	+	+	-	+	+
3	Azt-9	+	+	-	+	+
4	Azt-10	+	+	-	+	+
5	Azt-12	+	+	-	+	+
6	Azt-18	+	+	-	+	+
7	Azt-21	+	+	-	+	+
8	Azt-23	+	+	-	+	+
9	Azt-25	+	+	-	+	+
10	Azt-26	+	+	-	+	+
11	Azt-27	+	+	-	+	+
12	Azt-28	+	+	-	+	+
13	Azt-29	+	+	-	+	+
14	Azt-32	+	+	-	+	+
15	Azt-33	+	+	-	+	+
16	Azt-34	+	+	-	+	+
17	Azt-36	+	+	-	+	+
18	Azt-37	+	+	-	+	+
19	Azt-39	+	+	-	+	+
20	Azt-40	+	+	-	+	+
21	Azt-42	+	+	-	+	+
22	Azt-43	+	+	-	+	+
23	Azt-44	+	+	-	+	+
24	Azt-45	+	+	-	+	+
25	Azt-48	+	+	-	+	+
26	Azt-49	+	+	-	+	+
27	Azt-50	+	+	-	+	+
28	Azt-52	+	+	-	+	+
29	Azt-53	+	+	-	+	+
30	Azt-54	+	+	-	+	+
31	Azt-55	+	+	-	+	+
32	Azt-56	+	+	-	+	+
33	Azt-57	+	+	-	+	+
34	Azt-58	+	+	-	+	+
35	Azt-60	+	+	-	+	+
36	Azt-61	+	+	-	+	+
37	Azt-63	+	+	-	+	+
38	Azt-64	+	+	-	+	+
39	Azt-65	+	+	-	+	+
40	Azt-66	+	+	-	+	+
41	Azt-67	+	+	-	+	+

contd...

Table 22 contd...

Sr. No.	Isolate	Biochemical Tests				
		Starch hydrolysis	H_2S production	Gelatin liquefaction	Catalase test	Oxidase test
42	Azt-68	+	+	-	+	+
43	Azt-69	+	+	-	+	+
44	Azt-70	+	+	-	+	+
45	Azt-72	+	+	-	+	+
46	Azt-73	+	+	-	+	+
47	Azt-75	+	+	-	+	+
48	Azt-77	+	+	-	+	+
49	Azt-78	+	+	-	+	+
50	Azt-79	+	+	-	+	+
51	Azt-81	+	+	-	+	+
52	Azt-82	+	+	-	+	+
53	Azt-83	+	+	-	+	+
54	Azt-84	+	+	-	+	+
55	Azt-86	+	+	-	+	+
56	Azt-88	+	+	-	+	+
57	Azt-89	+	+	-	+	+
58	Azt-91	+	+	-	+	+
59	Azt-93	+	+	-	+	+
60	Azt-94	+	+	-	+	+
61	Azt-97	+	+	-	+	+
62	Azt-99	+	+	-	+	+
63	Azt-101	+	+	-	+	+
64	Azt-102	+	+	-	+	+
65	Azt-104	+	+	-	+	+
66	Azt-105	+	+	-	+	+
67	Azt-106	+	+	-	+	+
68	Azt-107	+	+	-	+	+
69	Azt-108	+	+	-	+	+
70	Azt-109	+	+	-	+	+
71	Azt-110	+	+	-	+	+
72	Azt-112	+	+	-	+	+
73	Azt-113	+	+	-	+	+
74	Azt-114	+	+	-	+	+
75	Azt-118	+	+	-	+	+
76	Azt-120	+	+	-	+	+
77	Azt-122	+	+	-	+	+
78	Azt-125	+	+	-	+	+
79	Azt-126	+	+	-	+	+
80	Azt-129	+	+	-	+	+
81	Azt-130	+	+	-	+	+
82	Azt-131	+	+	-	+	+

contd...

Table 22 contd...

Sr. No.	Isolate	Biochemical Tests				
		Starch hydrolysis	H_2S production	Gelatin liquefaction	Catalase test	Oxidase test
83	Azt-132	+	+	-	+	+
84	Azt-133	+	+	-	+	+
85	Azt-135	+	+	-	+	+
86	Azt-136	+	+	-	+	+
87	Azt-137	+	+	-	+	+
88	Azt-138	+	+	-	+	+
89	Azt-141	+	+	-	+	+
90	Azt-142	+	+	-	+	+
91	Azt-144	+	+	-	+	+
92	Azt-147	+	+	-	+	+
93	Azt-148	+	+	-	+	+
94	Azt-149	+	+	-	+	+
95	Azt-BNF	+	+	-	+	+

+ : Positive - : Negative

Table 23 Physiological characteristics of *Azotobacter* isolates

Sr. No.	Isolate	Utilization of different carbon sources					
		Mannitol	Glucose	Sucrose	Fructose	Malate	Citrate
1	Azt-4	++++	++++	++	++	-	-
2	Azt-8	++++	++++	++	++	-	-
3	Azt-9	++++	++++	++	++	-	-
4	Azt-10	++++	++++	++	++	-	-
5	Azt-12	++++	++++	++	++	-	-
6	Azt-18	++++	++++	++	++	-	-
7	Azt-21	++++	++++	+++	++	-	-
8	Azt-23	+++	++	++	++	-	-
9	Azt-25	+++	+++	++	++	-	-
10	Azt-26	++++	+++	++	++	-	-
11	Azt-27	++++	+++	++	++	-	-
12	Azt-28	++++	+++	++	++	-	-
13	Azt-29	++++	++	++	++	-	-
14	Azt-32	++++	++	++	++	-	-
15	Azt-33	++++	+++	++	++	-	-
16	Azt-34	++++	+++	++	++	-	-
17	Azt-36	+++	+++	++	++	-	-
18	Azt-37	+++	+++	++	++	-	-
19	Azt-39	+++	+++	++	++	-	-
20	Azt-40	+++	+++	++	++	-	-

contd...

Table 23 contd...

Sr. No.	Isolate	Utilization of different carbon sources					
		Mannitol	Glucose	Sucrose	Fructose	Malate	Citrate
21	Azt-42	+++	+++	++	++	-	-
22	Azt-43	+++	+++	++	++	-	-
23	Azt-44	++++	+++	++	++	-	-
24	Azt-45	++++	+++	++	++	-	-
25	Azt-48	++++	+++	++	++	-	-
26	Azt-49	++++	++	++	++	-	-
27	Azt-50	++++	++	+++	++	-	-
28	Azt-52	++++	++	++	++	-	-
29	Azt-53	+++	+++	++	++	-	-
30	Azt-54	+++	+++	++	++	-	-
31	Azt-55	+++	+++	++	+++	-	-
32	Azt-56	++++	+++	++	++	-	-
33	Azt-57	++++	+++	++	++	-	-
34	Azt-58	++++	+++	++	++	-	-
35	Azt-60	++++	+++	++	++	-	-
36	Azt-61	++++	+++	++	++	-	-
37	Azt-63	++++	++	++	++	-	-
38	Azt-64	++++	+++	+++	+	-	-
39	Azt-65	+++	+++	++	++	-	-
40	Azt-66	+++	+++	++	++	-	-
41	Azt-67	+++	+++	++	++	-	-
42	Azt-68	++++	+++	++	++	-	-
43	Azt-69	++++	+++	++	++	-	-
44	Azt-70	++++	+++	++	++	-	-
45	Azt-72	++++	++++	+++	++	-	-
46	Azt-73	+++	++	++	++	-	-
47	Azt-75	+++	+++	++	++	-	-
48	Azt-77	++++	+++	++	++	-	-
49	Azt-78	++++	+++	++	++	-	-
50	Azt-79	++++	+++	++	++	-	-
51	Azt-81	++++	++	++	++	-	-
52	Azt-82	++++	+++	+++	++	-	-
53	Azt-83	++++	+++	++	++	-	-
54	Azt-84	++++	+++	++	++	-	-
55	Azt-86	+++	+++	++	++	-	-
56	Azt-88	+++	+++	+++	++	-	-
57	Azt-89	+++	+++	++	++	-	-
58	Azt-91	+++	+++	++	++	-	-
59	Azt-93	+++	+++	++	++	-	-
60	Azt-94	+++	+++	++	++	-	-
61	Azt-97	++++	+++	++	++	-	-

contd...

Table 23 contd...

Sr. No.	Isolate	Utilization of different carbon sources					
		Mannitol	Glucose	Sucrose	Fructose	Malate	Citrate
62	Azt-99	++++	+++	++	++	-	-
63	Azt-101	++++	+++	++	++	-	-
64	Azt-102	++++	++	++	++	-	-
65	Azt-104	++++	++	+++	++	-	-
66	Azt-105	++++	++	++	++	-	-
67	Azt-106	+++	+++	++	++	-	-
68	Azt-107	+++	+++	++	++	-	-
69	Azt-108	+++	+++	++	+++	-	-
70	Azt-109	++++	+++	++	++	-	-
71	Azt-110	++++	+++	++	++	-	-
72	Azt-112	++++	+++	++	++	-	-
73	Azt-113	++++	+++	++	++	-	-
74	Azt-114	++++	+++	++	++	-	-
75	Azt-118	++++	++	++	++	-	-
76	Azt-120	++++	+++	+++	+	-	-
77	Azt-122	+++	+++	++	++	-	-
78	Azt-125	+++	+++	++	++	-	-
79	Azt-126	+++	+++	++	++	-	-
80	Azt-129	++++	+++	++	++	-	-
81	Azt-130	++++	+++	++	++	-	-
82	Azt-131	++++	+++	++	++	-	-
83	Azt-132	++++	++++	+++	++	-	-
84	Azt-133	+++	++	+++	++	-	-
85	Azt-135	+++	+++	++	++	-	-
86	Azt-136	++++	+++	++	++	-	-
87	Azt-137	++++	+++	++	++	-	-
88	Azt-138	++++	+++	++	++	-	-
89	Azt-141	++++	++	++	++	-	-
90	Azt-142	++++	+++	++	++	-	-
91	Azt-144	++++	+++	++	++	-	-
92	Azt-147	++++	+++	++	++	-	-
93	Azt-148	+++	+++	++	++	-	-
94	Azt-149	+++	+++	++	++	-	-
95	Azt-BNF	++++	+++	++	++	-	-

++++ : Maximum growth +++ : Good growth ++ : Moderate growth

+ : Less growth - : No growth

Table 24 Biochemical characteristics of *Rhizobium* isolates

Sr. No.	Isolate	Biochemical Tests				
		Starch hydrolysis	H_2S production	Gelatin liquefaction	Catalase test	Oxidase test
1	Rh-13	+	+	-	+	+
2	Rh-21	+	+	-	+	+
3	Rh-22	+	+	-	+	+
4	Rh-28	+	+	-	+	+
5	Rh-29	+	+	-	+	+
6	Rh-37	+	+	-	+	+
7	Rh-39	+	+	-	+	+
8	Rh-40	+	+	-	+	+
9	Rh-42	+	+	-	+	+
10	Rh-43	+	+	-	+	+
11	Rh-44	+	+	-	+	+
12	Rh-50	+	+	-	+	+
13	Rh-51	+	+	-	+	+
14	Rh-53	+	+	-	+	+
15	Rh-54	+	+	-	+	+
16	Rh-55	+	+	-	+	+
17	Rh-56	+	+	-	+	+
18	Rh-58	+	+	-	+	+
19	Rh-60	+	+	-	+	+
20	Rh-61	+	+	-	+	+
21	Rh-62	+	+	-	+	+
22	Rh-64	+	+	-	+	+
23	Rh-65	+	+	-	+	+
24	Rh-66	+	+	-	+	+
25	Rh-67	+	+	-	+	+
26	Rh-68	+	+	-	+	+
27	Rh-69	+	+	-	+	+
28	Rh-70	+	+	-	+	+
29	Rh-71	+	+	-	+	+
30	Rh-72	+	+	-	+	+
31	Rh-73	+	+	-	+	+
32	Rh-74	+	+	-	+	+
33	Rh-76	+	+	-	+	+
34	Rh-78	+	+	-	+	+
35	Rh-80	+	+	-	+	+
36	Rh-81	+	+	-	+	+
37	Rh-82	+	+	-	+	+
38	Rh-84	+	+	-	+	+
39	Rh-85	+	+	-	+	+
40	Rh-86	+	+	-	+	+

contd...

Table 24 contd...

Sr. No.	Isolate	Biochemical Tests				
		Starch hydrolysis	H_2S production	Gelatin liquefaction	Catalase test	Oxidase test
41	Rh-93	+	+	-	+	+
42	Rh-95	+	+	-	+	+
43	Rh-97	+	+	-	+	+
44	Rh-98	+	+	-	+	+
45	Rh-100	+	+	-	+	+
46	Rh-101	+	+	-	+	+
47	Rh-102	+	+	-	+	+
48	Rh-105	+	+	-	+	+
49	Rh-106	+	+	-	+	+
50	Rh-107	+	+	-	+	+
51	Rh-108	+	+	-	+	+
52	Rh-109	+	+	-	+	+
53	Rh-112	+	+	-	+	+
54	Rh-113	+	+	-	+	+
55	Rh-114	+	+	-	+	+
56	Rh-115	+	+	-	+	+
57	Rh-117	+	+	-	+	+
58	Rh-118	+	+	-	+	+
59	Rh-120	+	+	-	+	+
60	Rh-123	+	+	-	+	+
61	Rh-124	+	+	-	+	+
62	Rh-125	+	+	-	+	+
63	Rh-126	+	+	-	+	+
64	Rh-129	+	+	-	+	+
65	Rh-130	+	+	-	+	+
66	Rh-132	+	+	-	+	+
67	Rh-133	+	+	-	+	+
68	Rh-136	+	+	-	+	+
69	Rh-138	+	+	-	+	+
70	Rh-139	+	+	-	+	+
71	Rh-140	+	+	-	+	+
72	Rh-143	+	+	-	+	+
73	Rh-144	+	+	-	+	+
74	Rh-146	+	+	-	+	+
75	Rh-147	+	+	-	+	+
76	Rh-148	+	+	-	+	+
77	Rh-BNF	+	+	-	+	+

+ : Positive - : Negative

Table 25 Physiological characteristics of *Rhizobium* isolates

Sr. No.	Isolate	Utilization of different carbon sources					
		Mannitol	Glucose	Sucrose	Arabinose	Malate	Citrate
1	Rh-13	+++	+++	++	++	-	-
2	Rh-21	+++	+++	++	++	-	-
3	Rh-22	++++	+++	++	++	-	-
4	Rh-28	++++	+++	++	++	-	-
5	Rh-29	++++	+++	++	++	-	-
6	Rh-37	++++	++	++	++	-	-
7	Rh-39	++++	++	+++	++	-	-
8	Rh-40	++++	++	++	++	-	-
9	Rh-42	+++	+++	++	++	-	-
10	Rh-43	+++	+++	++	++	-	-
11	Rh-44	+++	+++	++	+++	-	-
12	Rh-50	++++	+++	++	++	-	-
13	Rh-51	++++	+++	++	++	-	-
14	Rh-53	++++	+++	++	++	-	-
15	Rh-54	++++	+++	++	++	-	-
16	Rh-55	++++	+++	++	++	-	-
17	Rh-56	++++	++	++	++	-	-
18	Rh-58	++++	+++	+++	+	-	-
19	Rh-60	+++	+++	++	+++	-	-
20	Rh-61	++++	+++	++	++	-	-
21	Rh-62	++++	++++	++	++	-	-
22	Rh-64	++++	++++	++	++	-	-
23	Rh-65	++++	++++	++	++	-	-
24	Rh-66	++++	++++	++	++	-	-
25	Rh-67	++++	++++	++	++	-	-
26	Rh-68	++++	++++	++	++	-	-
27	Rh-69	++++	++++	+++	++	-	-
28	Rh-70	+++	++	++	++	-	-
29	Rh-71	+++	+++	++	++	-	-
30	Rh-72	++++	+++	++	++	-	-
31	Rh-73	++++	+++	++	++	-	-
32	Rh-74	++++	+++	++	++	-	-
33	Rh-76	++++	++	++	++	-	-
34	Rh-78	++++	++	++	++	-	-
35	Rh-80	++++	+++	++	++	-	-
36	Rh-81	++++	+++	++	++	-	-
37	Rh-82	+++	+++	++	++	-	-
38	Rh-84	+++	+++	++	++	-	-
39	Rh-85	+++	+++	++	++	-	-
40	Rh-86	+++	+++	++	++	-	-

contd...

Table 25 contd...

Sr. No.	Isolate	Utilization of different carbon sources					
		Mannitol	Glucose	Sucrose	Arabinose	Malate	Citrate
41	Rh-93	+++	+++	++	++	-	-
42	Rh-95	+++	+++	+++	++	-	-
43	Rh-97	+++	+++	++	++	-	-
44	Rh-98	+++	+++	++	++	-	-
45	Rh-100	+++	+++	++	++	-	-
46	Rh-101	+++	+++	++	++	-	-
47	Rh-102	++++	+++	++	++	-	-
48	Rh-105	++++	+++	++	++	-	-
49	Rh-106	++++	+++	++	++	-	-
50	Rh-107	++++	++	++	++	-	-
51	Rh-108	++++	++	+++	++	-	-
52	Rh-109	++++	++	++	++	-	-
53	Rh-112	+++	+++	++	++	-	-
54	Rh-113	+++	+++	++	++	-	-
55	Rh-114	+++	+++	++	+++	-	-
56	Rh-115	++++	+++	++	++	-	-
57	Rh-117	++++	+++	++	++	-	-
58	Rh-118	++++	+++	++	++	-	-
59	Rh-120	++++	+++	++	++	-	-
60	Rh-123	++++	+++	++	++	-	-
61	Rh-124	++++	++	++	++	-	-
62	Rh-125	+++	+++	++	++	-	-
63	Rh-126	+++	+++	++	++	-	-
64	Rh-129	+++	+++	++	++	-	-
65	Rh-130	++++	+++	++	++	-	-
66	Rh-132	++++	+++	++	++	-	-
67	Rh-133	++++	+++	++	++	-	-
68	Rh-136	++++	++++	+++	++	-	-
69	Rh-138	+++	++	++	++	-	-
70	Rh-139	+++	+++	++	++	-	-
71	Rh-140	++++	+++	++	++	-	-
72	Rh-143	++++	+++	++	++	-	-
73	Rh-144	++++	+++	++	++	-	-
74	Rh-146	++++	++	++	++	-	-
75	Rh-147	++++	+++	+++	++	-	-
76	Rh-148	++++	+++	++	++	-	-
77	Rh-BNF	++++	+++	++	++	-	-

++++ : Maximum growth +++ : Good growth ++ : Moderate growth

+ : Less growth - : No growth

4.5.3 *Azospirillum* isolates

All the 93 isolates alongwith MPKV strain (Asp-BNF) were tested for their biochemical characters *viz.*, starch hydrolysis, H_2S production, gelatin liquefaction, catalase test, oxidase test and physiological characters like utilization of different carbon sources. The results are presented in Table 26. All the 93 *Azospirillum* isolates were positive for catalase test and oxidase test, but were negative for starch hydrolysis, H_2S production and gelatin liquefaction (Plate 23). Out of 93 *Azospirillum* isolates, 83 isolates utilized glucose and malic acid as a sole carbon source and were positive for biotin requirement, whereas 10 isolates utilized only malic acid as a sole carbon source for growth and showed negative result for glucose and biotin requirement. Based on the morphological, cultural, biochemical and physiological characterization, 83 isolates were tentatively identified as *Azospirillum lipoferum* and 10 isolates as *Azospirillum brasilense*.

4.5.4 PSB isolates

All the 47 isolates alongwith MPKV strain (PSB-BNF) were tested for their biochemical characters *viz.*, starch hydrolysis, H_2S production, gelatin liquefaction, catalase test, oxidase test and physiological characters like utilization of different carbon sources. All the 47 PSB isolates were positive for starch hydrolysis, gelatin liquefaction and catalase test, but were negative for H_2S production, KOH and oxidase test (Table 27, Plate 24). Fructose, glucose and maltose were used as a sole carbon source for growth by all the isolates, while mannitol and citrate which showed negative result (Table 28). Based on the morphological, cultural, biochemical and physiological characterization, 47 PSB isolates were tentatively identified as *Bacillus megaterium*.

Table 26 Biochemical and physiological characteristics of *Azospirillum* isolates

Sr. No	Isolate	Biochemical Tests					Carbon source utilization		Biotin requirement
		Starch hydrolysis	H_2S production	Catalase test	Oxidase test	Gelatin liquefaction	Glucose	Malic acid	
1	Asp-1	-	-	+	+	-	-	+	-
2	Asp-2	-	-	+	+	-	+	+	+
3	Asp-3	-	-	+	+	-	+	+	+
4	Asp-4	-	-	+	+	-	+	+	+
5	Asp-5	-	-	+	+	-	+	+	+
6	Asp-8	-	-	+	+	-	+	+	+
7	Asp-10	-	-	+	+	-	+	+	+
8	Asp-11	-	-	+	+	-	+	+	+
9	Asp-14	-	-	+	+	-	-	+	-
10	Asp-15	-	-	+	+	-	+	+	+
11	Asp-16	-	-	+	+	-	+	+	+
12	Asp-21	-	-	+	+	-	+	+	+
13	Asp-23	-	-	+	+	-	+	+	+
14	Asp-24	-	-	+	+	-	+	+	+
15	Asp-25	-	-	+	+	-	+	+	+
16	Asp-27	-	-	+	+	-	+	+	+
17	Asp-28	-	-	+	+	-	+	+	+
18	Asp-29	-	-	+	+	-	+	+	+
19	Asp-31	-	-	+	+	-	+	+	+
20	Asp-33	-	-	+	+	-	+	+	+
21	Asp-34	-	-	+	+	-	+	+	+
22	Asp-35	-	-	+	+	-	-	+	-
23	Asp-36	-	-	+	+	-	+	+	+
24	Asp-37	-	-	+	+	-	+	+	+
25	Asp-38	-	-	+	+	-	+	+	+
26	Asp-39	-	-	+	+	-	+	+	+
27	Asp-40	-	-	+	+	-	+	+	+
28	Asp-41	-	-	+	+	-	+	+	+
29	Asp-42	-	-	+	+	-	+	+	+
30	Asp-43	-	-	+	+	-	+	+	+
31	Asp-44	-	-	+	+	-	+	+	+
32	Asp-46	-	-	+	+	-	+	+	+
33	Asp-47	-	-	+	+	-	+	+	+

contd…

Table 26 contd...

Sr. No	Isolate	Biochemical Tests					Carbon source utilization		Biotin requirement
		Starch hydrolysis	H_2S production	Catalase test	Oxidase test	Gelatin liquefaction	Glucose	Malic acid	
34	Asp-48	-	-	+	+	-	+	+	+
35	Asp-49	-	-	+	+	-	+	+	+
36	Asp-50	-	-	+	+	-	+	+	+
37	Asp-51	-	-	+	+	-	+	+	+
38	Asp-55	-	-	+	+	-	+	+	+
39	Asp-56	-	-	+	+	-	+	+	+
40	Asp-57	-	-	+	+	-	+	+	+
41	Asp-59	-	-	+	+	-	-	+	-
42	Asp-60	-	-	+	+	-	+	+	+
43	Asp-62	-	-	+	+	-	+	+	+
44	Asp-63	-	-	+	+	-	+	+	+
45	Asp-64	-	-	+	+	-	+	+	+
46	Asp-67	-	-	+	+	-	+	+	+
47	Asp-68	-	-	+	+	-	+	+	+
48	Asp-69	-	-	+	+	-	+	+	+
49	Asp-70	-	-	+	+	-	+	+	+
50	Asp-72	-	-	+	+	-	+	+	+
51	Asp-73	-	-	+	+	-	+	+	+
52	Asp-75	-	-	+	+	-	-	+	-
53	Asp-78	-	-	+	+	-	+	+	+
54	Asp-82	-	-	+	+	-	+	+	+
55	Asp-83	-	-	+	+	-	+	+	+
56	Asp-85	-	-	+	+	-	+	+	+
57	Asp-86	-	-	+	+	-	+	+	+
58	Asp-87	-	-	+	+	-	+	+	+
59	Asp-88	-	-	+	+	-	-	+	-
60	Asp-90	-	-	+	+	-	+	+	+
61	Asp-92	-	-	+	+	-	+	+	+
62	Asp-95	-	-	+	+	-	+	+	+
63	Asp-96	-	-	+	+	-	+	+	+
64	Asp-97	-	-	+	+	-	+	+	+
65	Asp-99	-	-	+	+	-	-	+	-
66	Asp-101	-	-	+	+	-	+	+	+

contd…

Table 26 contd...

Sr. No	Isolate	Biochemical Tests					Carbon source utilization		Biotin requirement
		Starch hydrolysis	H_2S production	Catalase test	Oxidase test	Gelatin liquefaction	Glucose	Malic acid	
67	Asp-102	-	-	+	+	-	+	+	+
68	Asp-103	-	-	+	+	-	+	+	+
69	Asp-106	-	-	+	+	-	+	+	+
70	Asp-107	-	-	+	+	-	+	+	+
71	Asp-108	-	-	+	+	-	+	+	+
72	Asp-112	-	-	+	+	-	-	+	-
73	Asp-113	-	-	+	+	-	+	+	+
74	Asp-116	-	-	+	+	-	+	+	+
75	Asp-117	-	-	+	+	-	+	+	+
76	Asp-119	-	-	+	+	-	+	+	+
77	Asp-120	-	-	+	+	-	+	+	+
78	Asp-121	-	-	+	+	-	-	+	-
79	Asp-123	-	-	+	+	-	+	+	+
80	Asp-124	-	-	+	+	-	+	+	+
81	Asp-127	-	-	+	+	-	+	+	+
82	Asp-129	-	-	+	+	-	+	+	+
83	Asp-130	-	-	+	+	-	+	+	+
84	Asp-132	-	-	+	+	-	+	+	+
85	Asp-134	-	-	+	+	-	+	+	+
86	Asp-136	-	-	+	+	-	+	+	+
87	Asp-140	-	-	+	+	-	+	+	+
88	Asp-142	-	-	+	+	-	+	+	+
89	Asp-145	-	-	+	+	-	-	+	-
90	Asp-147	-	-	+	+	-	+	+	+
91	Asp-148	-	-	+	+	-	+	+	+
92	Asp-149	-	-	+	+	-	+	+	+
93	Asp-150	-	-	+	+	-	+	+	+
94	Asp-BNF	-	-	+	+	-	+	+	+

Table 27 Biochemical characteristics of PSB isolates

Sr. No.	Isolate	Biochemical Tests					
		Starch hydrolysis	H_2S production	Gelatin liquefaction	KOH	Catalase test	Oxidase test
1	PSB-6	+	-	+	-	+	-
2	PSB-10	+	-	+	-	+	-
3	PSB-11	+	-	+	-	+	-
4	PSB-15	+	-	+	-	+	-
5	PSB-17	+	-	+	-	+	-
6	PSB-20	+	-	+	-	+	-
7	PSB-33	+	-	+	-	+	-
8	PSB-39	+	-	+	-	+	-
9	PSB-41	+	-	+	-	+	-
10	PSB-42	+	-	+	-	+	-
11	PSB-46	+	-	+	-	+	-
12	PSB-48	+	-	+	-	+	-
13	PSB-50	+	-	+	-	+	-
14	PSB-56	+	-	+	-	+	-
15	PSB-59	+	-	+	-	+	-
16	PSB-60	+	-	+	-	+	-
17	PSB-61	+	-	+	-	+	-
18	PSB-64	+	-	+	-	+	-
19	PSB-68	+	-	+	-	+	-
20	PSB-69	+	-	+	-	+	-
21	PSB-72	+	-	+	-	+	-
22	PSB-73	+	-	+	-	+	-
23	PSB-75	+	-	+	-	+	-
24	PSB-78	+	-	+	-	+	-
25	PSB-79	+	-	+	-	+	-
26	PSB-81	+	-	+	-	+	-
27	PSB-83	+	-	+	-	+	-
28	PSB-93	+	-	+	-	+	-
29	PSB-95	+	-	+	-	+	-
30	PSB-100	+	-	+	-	+	-
31	PSB-102	+	-	+	-	+	-
32	PSB-106	+	-	+	-	+	-
33	PSB-107	+	-	+	-	+	-
34	PSB-109	+	-	+	-	+	-
35	PSB-111	+	-	+	-	+	-

contd…

Table 27 contd....

Sr. No.	Isolate	Biochemical Tests					
		Starch hydrolysis	H_2S production	Gelatin liquefaction	KOH	Catalase test	Oxidase test
36	PSB-113	+	-	+	-	+	-
37	PSB-119	+	-	+	-	+	-
38	PSB-125	+	-	+	-	+	-
39	PSB-129	+	-	+	-	+	-
40	PSB-130	+	-	+	-	+	-
41	PSB-132	+	-	+	-	+	-
42	PSB-136	+	-	+	-	+	-
43	PSB-140	+	-	+	-	+	-
44	PSB-142	+	-	+	-	+	-
45	PSB-143	+	-	+	-	+	-
46	PSB-144	+	-	+	-	+	-
47	PSB-148	+	-	+	-	+	-
48	PSB-BNF	+	-	+	-	+	-

+ : Positive - : Negative

Table 28 Physiological characteristics of PSB isolates

Sr. No.	Isolate	Utilization of different carbon sources				
		Fructose	Glucose	Maltose	Mannitol	Citrate
1	PSB-6	+++	++	++	-	-
2	PSB-10	+++	++	++	-	-
3	PSB-11	+++	++	++	-	-
4	PSB-15	+++	++	++	-	-
5	PSB-17	+++	++	++	-	-
6	PSB-20	++	++	++	-	-
7	PSB-33	++	+++	++	-	-
8	PSB-39	++	++	++	-	-
9	PSB-41	+++	++	++	-	-
10	PSB-42	+++	++	++	-	-
11	PSB-46	+++	++	+++	-	-
12	PSB-48	+++	++	++	-	-
13	PSB-50	+++	++	++	-	-
14	PSB-56	+++	++	++	-	-
15	PSB-59	+++	++	++	-	-

contd...

Table 28 contd...

Sr. No.	Isolate	Utilization of different carbon sources				
		Fructose	Glucose	Maltose	Mannitol	Citrate
16	PSB-60	+++	++	++	-	-
17	PSB-61	++	++	++	-	-
18	PSB-64	+++	+++	+	-	-
19	PSB-68	+++	++	+++	-	-
20	PSB-69	+++	++	++	-	-
21	PSB-72	++++	++	++	-	-
22	PSB-73	++++	++	++	-	-
23	PSB-75	++++	++	++	-	-
24	PSB-78	++++	++	++	-	-
25	PSB-79	++++	++	++	-	-
26	PSB-81	++++	++	++	-	-
27	PSB-83	++++	+++	++	-	-
28	PSB-93	++	++	++	-	-
29	PSB-95	+++	++	++	-	-
30	PSB-100	+++	++	++	-	-
31	PSB-102	+++	++	++	-	-
32	PSB-106	+++	++	++	-	-
33	PSB-107	+++	++	++	-	-
34	PSB-109	+++	++	++	-	-
35	PSB-111	++	++	++	-	-
36	PSB-113	++	+++	++	-	-
37	PSB-119	++	++	++	-	-
38	PSB-125	+++	++	++	-	-
39	PSB-129	+++	++	++	-	-
40	PSB-130	+++	++	+++	-	-
41	PSB-132	+++	++	++	-	-
42	PSB-136	+++	++	++	-	-
43	PSB-140	+++	++	++	-	-
44	PSB-142	+++	++	++	-	-
45	PSB-143	+++	++	++	-	-
46	PSB-144	++	++	++	-	-
47	PSB-148	+++	+++	+	-	-
48	PSB-BNF	+++	++	+++	-	-

++++ : Maximum growth +++ : Good growth ++ : Moderate growth

+ : Less growth - : No growth

4.6 Functional diversity of nitrogen fixing microorganisms

4.6.1 Nitrogenase activity of the *Azotobacter* isolates

The nitrogenase activity of the *Azotobacter* isolates was determined by acetylene reduction assay and the results are presented in Table 29. There was a wide range of variation in nitrogenase activity among the 94 different *Azotobacter* isolates tested (5.3 to 291.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}). The nitrogenase activity of all isolates was compared with standard MPKV strain. The isolate Azt-21 recorded significantly highest nitrogenase activity (291.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}) than the other isolates tested. The MPKV strain Azt-BNF had the nitrogenase activity of 149.4 nmol C_2H_4.mg protein^{-1}.hr^{-1}. Out of 94 *Azotobacter* isolates, 11 isolates *viz.*, Azt-21, 25, 50, 64, 70, 82, 97, 129, 135, 142 and 148 recorded significantly higher nitrogenase activity than the standard MPKV strain Azt-BNF. The isolate Azt-8 had the nitrogenase activity of 151.1 nmol C_2H_4.mg protein^{-1}.hr^{-1} which was statistically at par with MPKV strain. The nitrogenase activity of selected *Azotobacter chroococcum* isolates in comparison with standard MPKV strain Azt-BNF is shown in Figure 1.

The distribution of efficient N_2 fixing microbes (*Azotobacter chroococcum* isolates) across the physiographic regions of Maharashtra is given in Table 30. Impact analysis of weather parameters and cropping system on distribution of efficient N_2 fixers (*Azotobacter chroococcum* isolates) across the physiographic regions of Maharashtra is given in Table 31. The results showed that weather parameters and cropping system had no singnificant influence on distribution of efficient *Azotobacter chroococcum* isolates. However, the population of *A. chroococcum* isolates varied significantly with rainfall pattern and cropping system across the regions. The maximum population of *A. chroococcum* (10.17x10^4 CFU g^{-1} soil) was recorded in paddy-wheat cropping system in Kolhapur district of Western Maharashtra with

Table 29 Nitrogenase activity of *Azotobacter* isolates by Acetylene Reduction Assay (ARA)

Sr. No	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})	Sr. No	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})
1	Azt-4	27.6	27	Azt-50	194.2
2	Azt-8	151.1	28	Azt-52	19.3
3	Azt-9	34.1	29	Azt-53	25.1
4	Azt-10	69.4	30	Azt-54	54.7
5	Azt-12	19.5	31	Azt-55	81.3
6	Azt-18	11.3	32	Azt-56	39.8
7	Azt-21	291.5	33	Azt-57	93.4
8	Azt-23	81.1	34	Azt-58	17.8
9	Azt-25	158.4	35	Azt-60	20.6
10	Azt-26	17.9	36	Azt-61	15.4
11	Azt-27	13.3	37	Azt-63	23.9
12	Azt-28	91.6	38	Azt-64	236.7
13	Azt-29	87.9	39	Azt-65	8.4
14	Azt-32	101.7	40	Azt-66	57.8
15	Azt-33	73.1	41	Azt-67	75.1
16	Azt-34	19.4	42	Azt-68	91.6
17	Azt-36	46.5	43	Azt-69	104.3
18	Azt-37	29.7	44	Azt-70	259.4
19	Azt-39	16.2	45	Azt-72	111.5
20	Azt-40	23.4	46	Azt-73	81.6
21	Azt-42	41.1	47	Azt-75	16.2
22	Azt-43	13.7	48	Azt-77	36.3
23	Azt-44	78.5	49	Azt-78	78.1
24	Azt-45	11.6	50	Azt-79	38.7
25	Azt-48	13.8	51	Azt-81	61.2
26	Azt-49	53.9	52	Azt-82	205.6

contd…

Table 29 contd…

Sr. No	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})	Sr. No	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})
53	Azt-83	93.1	75	Azt-118	58.8
54	Azt-84	78.4	76	Azt-120	84.9
55	Azt-86	81.7	77	Azt-122	27.6
56	Azt-88	15.3	78	Azt-125	47.1
57	Azt-89	29.8	79	Azt-126	5.3
58	Azt-91	12.4	80	Azt-129	163.3
59	Azt-93	25.3	81	Azt-130	31.8
60	Azt-94	19.6	82	Azt-131	88.3
61	Azt-97	185.1	83	Azt-132	67.5
62	Azt-99	77.4	84	Azt-133	92.7
63	Azt-101	45.9	85	Azt-135	155.9
64	Azt-102	68.3	86	Azt-136	77.2
65	Azt-104	41.2	87	Azt-137	41.1
66	Azt-105	93.7	88	Azt-138	56.7
67	Azt-106	84.6	89	Azt-141	105.6
68	Azt-107	98.1	90	Azt-142	170.8
69	Azt-108	86.3	91	Azt-144	97.5
70	Azt-109	97.5	92	Azt-147	88.7
71	Azt-110	38.4	93	Azt-148	217.3
72	Azt-112	44.7	94	Azt-149	93.8
73	Azt-113	103.8	95	Azt-BNF	149.4
74	Azt-114	69.3			

	S.E. $\pm$	1.64
	C.D. at 1%	6.02
	C.V.%	3.82

Table 30 Distribution of efficient N_2 fixing microbes (*Azotobacter chroococcum* isolates) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Distribution of efficient N_2 fixing microbes (*A. chroococcum* isolates)
I	Western Konkan Coast	Sindhudurg	I, III
		Ratnagiri	-
		Thane	III
		Raigad	I, II
II	Western Ghats	Sindhudurg	II, III
		Ratnagiri	I
		Thane	III
		Kolhapur	II, III
		Satara	III
		Pune	III
		Nasik	III
		Dhule	III
		Nandurbar	I, III
III	Western Maharashtra	Pune	II, III
		Kolhapur	I, II
		Satara	II, III
		Sangli	II, III
		Solapur	II, III
		Ahmednagar	I, II, III
IV	North Maharashtra	Nasik	II, III
		Dhule	III
		Jalgaon	II
		Nandurbar	-
V	Marathwada	Latur	II, III
		Osmanabad	III
		Beed	I
		Nanded	III
		Parbhani	I, II
		Hingoli	-
		Jalna	-
		Aurangabad	III
		Buldhana	-
VI	Vidarbha	Washim	-
		Akola	III
		Amravati	III
		Yavatmal	I, III
		Wardha	II, III
		Nagpur	II, III
		Bhandara	I
		Chandrapur	I, II, III
		Gondia	-
		Gadchiroli	I, II, III

Categories of efficiency of *Azotobacter chroococcum* isolates:

I – Highly efficient (149.4 to 291.5 nmol C_2H_4.mg protein^{-1}.hr^{-1})

II – Moderately efficient (75.1 to 149.3 nmol C_2H_4.mg protein^{-1}.hr^{-1})

III – Less efficient (< 75 nmol C_2H_4.mg protein^{-1}.hr^{-1})

Table 31. Impact analysis of weather parameters and cropping system on distribution of efficient N_2 fixers (*Azotobacter chroococcum* isolates) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient N_2 fixer
					Max.	Min.	Kharif	Rabi	Summer	
I	Western Konkan Coast	Sindhudurg	Moderately shallow to very deep	3287	32.7	18.7	Paddy/ Nachni/ Varai	-	-	I (3.67) III (2.79)
		Ratnagiri	Shallow	3188	30.5	23.0	Paddy/ Nachni	-	-	-
		Thane	Moderately shallow to deep	2477	34.4	17.5	Paddy/ Nachni	-	-	III (2.28)
		Raigad	Moderately deep	1804	31.2	20.3	Paddy/ Nachni	-	-	I (9.33) II (4.83)
II	Western Ghats	Sindhudurg	Shallow	2245	32.7	18.7	Paddy	-	-	II (3.33) III (4.24)
		Ratnagiri	Shallow	2617	30.5	23.0	Paddy/ Nachni	-	-	I (5.50)
		Thane	Shallow	2477	34.4	17.5	Paddy	-	-	III (4.59)
		Kolhapur	Moderately deep	1904	31.8	18.8	Paddy/ Nachni	-	-	II (4.90) III (4.07)
		Satara	Very shallow	710	30.3	19.0	Grasses	-	-	III (4.67)
		Pune	Very shallow	1150	31.8	18.3	Grasses	-	-	III (2.00)
		Nasik	Shallow	1035	31.1	16.9	Paddy/ Nagli	-	-	III (2.95)
		Dhule	Moderately shallow	1035	31.8	18.9	Forest	-	-	III (4.00)
		Nandurbar	Moderately shallow	674	29.6	18.8	Sorghum	Wheat	-	I (8.33) III (4.50)

contd...

Table 31 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient N_2 fixer
					Max.	Min.	Kharif	Rabi	Summer/ Annual	
III	Western Maharashtra	Pune	Shallow to deep	700	31.8	18.3	Sorghum/ Pearlmillet	Wheat	Sugarcane	II (5.16) III (4.33)
		Kolhapur	Deep	1904	31.8	18.8	Paddy	Wheat	Sugarcane	I (10.17) II (3.74)
		Satara	Moderately deep	710	30.3	19.0	Sorghum	Chickpea /Wheat	Sugarcane	II (5.01) III (6.00)
		Sangli	Shallow to moderately deep	685	31.9	19.1	Groundnut /Sorghum	Wheat	Sugarcane	II (4.25) III (2.96)
		Solapur	Shallow to very deep	580	34.1	21.7	Green gram	Sorghum/ Safflower	Sugarcane	II (2.33) III (2.38)
		Ahmednagar	Very shallow to deep	550	31.5	17.8	Pearlmillet /Pigeaon-pea	Chickpea /Wheat	Sugarcane	I (6.67) II (4.17) III (2.63)
IV	North Maharashtra	Nasik	Moderately shallow to deep	1035	31.1	16.9	Pearlmillet /Onion	Wheat	Sugarcane	II (5.17) III (4.06)
		Dhule	Shallow	670	31.8	18.9	Pearlmillet	-	-	III (2.34)
		Jalgaon	Deep	710	32.3	18.8	Banana/ Cotton	-	-	II (3.50)
		Nandurbar	Modeately deep	740	29.6	18.8	Pigeaon-pea	Wheat/ Chickpea	-	-
V	Marathwada	Latur	Shallow to deep	880	32.0	18.8	Pigeaon-pea	Wheat/ Chickpea	Sugarcane	II (4.44) III (5.50)
		Osmanabad	Shallow to deep	880	31.9	17.3	Pigeaon-pea	Wheat/ Chickpea	-	III (4.71)
		Beed	Deep	700	31.4	18.2	Pigeaon-pea	Wheat/ Safflower	Sugarcane	I (8.10)
		Nanded	Shallow	890	32.1	19.8	Cotton/ Pigeaonpea	-	-	III (1.33)

contd...

Table 31 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient N_2 fixer
					Max.	Min.	Kharif	Rabi	Summer	
		Parbhani	Shallow to deep	840	31.9	18.9	Pigeaonpea	Safflower	-	I (3.67) II (4.67)
		Hingoli	Shallow to deep	840	31.7	18.6	Pigeaonpea	Chickpea	-	-
		Jalna	Shallow to deep	840	31.6	19.3	Pigeaonpea	Chickpea	-	-
		Aurangabad	Shallow to deep	750	31.8	18.8	Pearlmillet/ Sorghum	Safflower	-	III (3.17)
VI	Vidarbha	Buldhana	Deep	750	32.1	19.9	Cotton	-	-	-
		Washim	Shallow to deep	840	33.1	20.7	Sorghum	Safflower	-	-
		Akola	Shallow to deep	840	34.1	19.9	Sorghum	Safflower	-	III (4.67)
		Amravati	Shallow to deep	840	33.3	21.5	Sorghum	Safflower /Chickpea	-	-
		Yavatmal	Shallow to deep	990	31.9	20.1	Cotton	-	-	I (6.33) III (2.00)
		Wardha	Shallow to deep	1090	33.3	21.5	Cotton	-	-	II (4.83) III (4.08)
		Nagpur	Deep	1660	34.7	22.9	Cotton/ Paddy	-	-	II (3.83) III (3.59)
		Bhandara	Deep	1400	33.9	21.6	Cotton/Paddy	-	-	I (9.67)
		Chandrapur	Deep	1420	29.6	14.6	Paddy	-	-	I (4.50) II (2.83) III (5.33)
		Gondia	Deep	1420	30.1	18.6	Paddy	-	-	-
		Gadchiroli	Deep	1550	29.9	18.8	Paddy	-	-	I (4.17) II (5.00) III (3.50)

Figures in parentheses represent the population dynamics values (x10^4 CFU g^{-1} soil) of *Azotobacter chroococcum* isolates

annual rainfall 1904 mm followed by cotton/paddy fields of Bhandara district of Vidarbha ($9.67x10^4$ CFU g^{-1} soil) and paddy/nachni fields of Raigad district of Western Konkan Coast ($9.33x10^4$ CFU g^{-1} soil) with annual rainfall 1400 and 1804 mm, respectively.

4.6.2 Nitrogenase activity of the *Rhizobium* isolates

There was significant variation in nitrogenase activity among the 76 *Rhizobium* isolates tested (1.9 to 119.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}) (Table 32). The nitrogenase activity of all isolates was compared with standard MPKV strain. The isolate Rh-69 recorded significantly highest nitrogenase activity (119.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}) than the other isolates tested. The MPKV strain Rh-BNF had the nitrogenase activity of 71.3 nmol C_2H_4.mg protein^{-1}.hr^{-1}. Out of 76 *Rhizobium* isolates, 6 isolates *viz*., Rh-64, 69, 72, 109, 113 and 132 recorded significantly higher nitrogenase activities than the standard MPKV strain Rh-BNF. The isolate Rh-101 had the nitrogenase activity of 73.1 nmol C_2H_4.mg protein^{-1}.hr^{-1} which was statistically at par with MPKV strain. The nitrogenase activity of selected *Rhizobium* spp. isolates in comparison with standard MPKV strain Rh-BNF is shown in Figure 2.

The distribution of efficient N_2 fixing microbes (*Rhizobium* spp. isolates) across the physiographic regions of Maharashtra is given in Table 33. Impact analysis of weather parameters and cropping system on distribution of efficient N_2 fixers (*Rhizobium* spp. isolates) across the physiographic regions of Maharashtra is given in Table 34. The results showed that weather parameters and cropping system had no singnificant influence on distribution of efficient *Rhizobium* spp. isolates. However, the population of *Rhizobium* spp. isolates varied significantly with cropping system across the regions. The maximum population of *Rhizobium* spp.

Table 32 Nitrogenase activity of *Rhizobium* isolates by Acetylene Reduction Assay (ARA)

Sr. No	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})	Sr. No	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})
1	Rh-13	17.1	40	Rh-86	19.7
2	Rh-21	4.3	41	Rh-93	6.1
3	Rh-22	6.7	42	Rh-95	9.8
4	Rh-28	49.4	43	Rh-97	4.3
5	Rh-29	31.7	44	Rh-98	7.4
6	Rh-37	8.1	45	Rh-100	13.2
7	Rh-39	3.7	46	Rh-101	73.1
8	Rh-40	21.8	47	Rh-102	24.8
9	Rh-42	13.6	48	Rh-105	21.1
10	Rh-43	2.1	49	Rh-106	32.7
11	Rh-44	29.3	50	Rh-107	29.3
12	Rh-50	40.7	51	Rh-108	39.2
13	Rh-51	2.3	52	Rh-109	80.6
14	Rh-53	4.9	53	Rh-112	18.7
15	Rh-54	33.5	54	Rh-113	98.3
16	Rh-55	41.2	55	Rh-114	34.7
17	Rh-56	22.4	56	Rh-115	25.6
18	Rh-58	7.5	57	Rh-117	19.4
19	Rh-60	13.9	58	Rh-118	27.3
20	Rh-61	8.2	59	Rh-120	31.7
21	Rh-62	2.9	60	Rh-123	6.4
22	Rh-64	74.5	61	Rh-124	3.8
23	Rh-65	1.9	62	Rh-125	5.9
24	Rh-66	16.8	63	Rh-126	9.2
25	Rh-67	29.7	64	Rh-129	32.4
26	Rh-68	37.1	65	Rh-130	9.2
27	Rh-69	119.7	66	Rh-132	83.1
28	Rh-70	33.1	67	Rh-133	27.4
29	Rh-71	22.4	68	Rh-136	19.3
30	Rh-72	91.8	69	Rh-138	11.7
31	Rh-73	39.7	70	Rh-139	32.9
32	Rh-74	5.1	71	Rh-140	6.8
33	Rh-76	2.7	72	Rh-143	30.1
34	Rh-78	43.8	73	Rh-144	18.6
35	Rh-80	30.6	74	Rh-146	23.4
36	Rh-81	47.8	75	Rh-147	16.3
37	Rh-82	42.1	76	Rh-148	22.5
38	Rh-84	29.6	77	Rh-BNF	71.3
39	Rh-85	38.3			

	S.E. $\pm$	0.74
	C.D. at 1%	2.74
	C.V.%	4.67

Table 33. Distribution of efficient N_2 fixing microbes (*Rhizobium* spp.) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Distribution of efficient N_2 fixing microbes (*Rhizobium* spp. isolates)
I	Western Konkan Coast	Sindhudurg	-
		Ratnagiri	III
		Thane	-
		Raigad	III
II	Western Ghats	Sindhudurg	III
		Ratnagiri	-
		Thane	-
		Kolhapur	II, III
		Satara	III
		Pune	III
		Nasik	III
		Dhule	-
		Nandurbar	II
III	Western Maharashtra	Pune	II, III
		Kolhapur	III
		Satara	II
		Sangli	I, II, III
		Solapur	I, III
		Ahmednagar	I, II, III
IV	North Maharashtra	Nasik	I, III
		Dhule	III
		Jalgaon	III
		Nandurbar	-
V	Marathwada	Latur	I, II, III
		Osmanabad	
		Beed	II, III
		Nanded	III
		Parbhani	III
		Hingoli	III
		Jalna	
		Aurangabad	III
		Buldhana	-
VI	Vidarbha	Washim	-
		Akola	I
		Amravati	-
		Yavatmal	III
		Wardha	III
		Nagpur	II, III
		Bhandara	III
		Chandrapur	I, III
		Gondia	-
		Gadchiroli	III

Categories of efficiency of *Rhizobium* spp.:

I – Highly efficient (71.3 to 119.7 nmol C_2H_4.mg protein^{-1}.hr^{-1})
II – Moderately efficient (36.1 to 71.2 nmol C_2H_4.mg protein^{-1}.hr^{-1})
III – Less efficient (< 36 nmol C_2H_4.mg protein^{-1}.hr^{-1})

Table 34. Impact analysis of weather parameters and cropping system on distribution of efficient N_2 fixers (*Rhizobium* spp.) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient N_2 fixer
					Max.	Min.	Kharif	Rabi	Summer	
I	Western Konkan Coast	Sindhudurg	Moderately shallow to very deep	3287	32.7	18.7	Paddy/ Nachni/ Varai	-	-	-
		Ratnagiri	Shallow	3188	30.5	23.0	Paddy/ Nachni	-	-	III (3.92)
		Thane	Moderately shallow to deep	2477	34.4	17.5	Paddy/ Nachni	-	-	-
		Raigad	Moderately deep	1804	31.2	20.3	Paddy/ Nachni	-	-	III (5.50)
II	Western Ghats	Sindhudurg	Shallow	2245	32.7	18.7	Paddy	-	-	III (2.84)
		Ratnagiri	Shallow	2617	30.5	23.0	Paddy/ Nachni	-	-	-
		Thane	Shallow	2477	34.4	17.5	Paddy	-	-	-
		Kolhapur	Moderately deep	1904	31.8	18.8	Paddy/ Nachni	-	-	II (3.83) III (4.42)
		Satara	Very shallow	710	30.3	19.0	Grasses	-	-	III (3.67)
		Pune	Very shallow	1150	31.8	18.3	Grasses	-	-	III (2.17)
		Nasik	Shallow	1035	31.1	16.9	Paddy/ Nagli	-	-	III (1.83)
		Dhule	Moderately shallow	1035	31.8	18.9	Forest	-	-	-
		Nandurbar	Moderately shallow	674	29.6	18.8	Sorghum	Wheat	-	II (4.50)

contd...

Table 34 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient N_2 fixer
					Max.	Min.	Kharif	Rabi	Summer/ Annual	
III	Western Maharashtra	Pune	Shallow to moderately deep	700	31.8	18.3	Sorghum/ Pearlmillet	Wheat	Sugarcane	II (3.33) III (3.61)
		Kolhapur	Deep	1904	31.8	18.8	Paddy	Wheat	Sugarcane	III (5.72)
		Satara	Moderately deep	710	30.3	19.0	Sorghum	Chickpea /Wheat	Sugarcane	II (5.50)
		Sangli	Shallow to moderately deep	685	31.9	19.1	Groundnut /Sorghum	Wheat	Sugarcane	I (8.17) II (5.83) III (4.25)
		Solapur	Shallow to very deep	580	34.1	21.7	Green gram	Sorghum/ Safflower	Sugarcane	I (5.23) III (2.79)
		Ahmednagar	Very shallow to deep	550	31.5	17.8	Pearlmillet /Pigeaon-pea	Chickpea /Wheat	Sugarcane	I (4.83) II (6.50) III (5.28)
IV	North Maharashtra	Nasik	Moderately shallow to deep	1035	31.1	16.9	Pearlmillet /Onion	Wheat	Sugarcane	I (5.17) III (3.12)
		Dhule	Shallow	670	31.8	18.9	Pearlmillet	-	-	III (2.87)
		Jalgaon	Deep	710	32.3	18.8	Banana/ Cotton	-	-	III (4.33)
		Nandurbar	Modeately deep	740	29.6	18.8	Pigeaon-pea	Wheat/ Chickpea	-	-
V	Marathwada	Latur	Shallow to deep	880	32.0	18.8	Pigeaon-pea	Wheat/ Chickpea	Sugarcane	I (8.33) II (2.50) III (3.83)
		Osmanabad	Shallow to deep	880	31.9	17.3	Pigeaon-pea	Wheat/ Chickpea	-	-
		Beed	Deep	700	31.4	18.2	Pigeaon-pea	Wheat/ Safflower	Sugarcane	II (7.16) III (1.67)

contd...

Table 34 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient N_2 fixer
					Max.	Min.	Kharif	Rabi	Summer	
		Nanded	Shallow	890	32.1	19.8	Cotton/ Pigeaonpea	-	-	III (1.93)
		Parbhani	Shallow to deep	840	31.9	18.9	Pigeaonpea	Safflower	-	III (5.50)
		Hingoli	Shallow to deep	840	31.7	18.6	Pigeaonpea	Chickpea	-	III (3.50)
		Jalna	Shallow to deep	840	31.6	19.3	Pigeaonpea	Chickpea	-	-
		Aurangabad	Shallow to deep	750	31.8	18.8	Pearlmillet/Sorghum	Safflower	-	III (3.83)
VI	Vidarbha	Buldhana	Deep	750	32.1	19.9	Cotton	-	-	-
		Washim	Shallow to deep	840	33.1	20.7	Sorghum	Safflower	-	-
		Akola	Shallow to deep	840	34.1	19.9	Sorghum	Safflower	-	I (4.17)
		Amravati	Shallow to deep	840	33.3	21.5	Sorghum	Safflower /Chickpea	-	-
		Yavatmal	Shallow to deep	990	31.9	20.1	Cotton	-	-	III (2.92)
		Wardha	Shallow to deep	1090	33.3	21.5	Cotton	-	-	III (2.60)
		Nagpur	Deep	1660	34.7	22.9	Cotton/ Paddy	-	-	II (4.17) III (4.14)
		Bhandara	Deep	1400	33.9	21.6	Cotton/ Paddy	-	-	III (3.83)
		Chandrapur	Deep	1420	29.6	14.6	Paddy	-	-	I (7.67) III (3.50)
		Gondia	Deep	1420	30.1	18.6	Paddy	-	-	-
		Gadchiroli	Deep	1550	29.9	18.8	Paddy	-	-	III (4.46)

Figures in parentheses represent the population dynamics values (x 10^4 CFU g^{-1} soil) of *Rhizobium* spp. isolates

($8.33x10^4$ CFU g^{-1} soil) was recorded in the pigeonpea-wheat/ pigeonpea-chickpea cropping system in Latur district of Marathwada followed by groundnut-wheat/sorghum-wheat cropping pattern of Sangli district of Western Maharashtra ($8.17x10^4$ CFU g^{-1} soil) and pigeonpea-safflower/ pigeonpea-wheat of Beed district of Marathwada ($7.16x10^4$ CFU g^{-1} soil).

4.6.3 Nitrogenase activity of the *Azospirillum* isolates

A distinct variation in nitrogenase activity among the 93 *Azospirillum* isolates tested (7.5 to 394.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}) was observed (Table 35). The nitrogenase activity of all isolates was compared with standard MPKV strain. Among 83 *Azospirillum lipoferum* isolates, Asp-97 recorded significantly highest nitrogenase activity (394.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}) followed by Asp-28, 50, 150, 132 and 124 isolates with nitrogenase activity of 311.2, 287.1, 269.8, 256.4 and 248.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}, respectively than the standard MPKV strain Asp-BNF (239.6 nmol C_2H_4.mg protein^{-1}.hr^{-1}). The isolate Asp-127 had the nitrogenase activity of 241.3 nmol C_2H_4.mg protein^{-1}.hr^{-1} which was statistically at par with MPKV strain. The nitrogenase activity of selected *Azospirillum lipoferum* isolates in comparison with standard MPKV strain Asp-BNF is shown in Figure 3.

Among 10 *Azospirillum brasilense* isolates, Asp-75 recorded highest nitrogenase activity (39.3 nmol C_2H_4.mg protein^{-1}.hr^{-1}). The *Azospirillum lipoferum* isolates exhibited a higher average nitrogenase activity compared to *Azospirillum brasilense* isolates (83.2 compared with 20.1 nmol C_2H_4.mg protein^{-1}. hr^{-1}).

Based on the nitrogenase activity, the highly efficient nitrogen fixing isolates *viz.*, 12 *Azotobacter* isolates, 7 *Rhizobium* isolates and 7 *Azospirillum* isolates alongwith standard MPKV strain were further analyzed for their molecular diversity.

Table 35 Nitrogenase activity of *Azospirillum* isolates by Acetylene Reduction Assay (ARA)

Sr. No.	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})	Sr. No	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})
1	Asp-1	7.6	37	Asp-51	41.8
2	Asp-2	18.1	38	Asp-55	116.7
3	Asp-3	26.4	39	Asp-56	68.2
4	Asp-4	33.7	40	Asp-57	130.2
5	Asp-5	19.2	41	Asp-59	34.8
6	Asp-8	13.5	42	Asp-60	16.3
7	Asp-10	49.3	43	Asp-62	33.7
8	Asp-11	15.2	44	Asp-63	41.7
9	Asp-14	9.4	45	Asp-64	67.3
10	Asp-15	11.7	46	Asp-67	103.8
11	Asp-16	16.4	47	Asp-68	112.7
12	Asp-21	51.3	48	Asp-69	134.3
13	Asp-23	34.1	49	Asp-70	101.2
14	Asp-24	26.9	50	Asp-72	124.1
15	Asp-25	13.3	51	Asp-73	87.5
16	Asp-27	27.5	52	Asp-75	39.3
17	Asp-28	311.2	53	Asp-78	138.6
18	Asp-29	134.7	54	Asp-82	117.7
19	Asp-31	79.3	55	Asp-83	114.3
20	Asp-33	119.4	56	Asp-85	109.6
21	Asp-34	59.1	57	Asp-86	88.4
22	Asp-35	14.7	58	Asp-87	11.3
23	Asp-36	24.3	59	Asp-88	26.9
24	Asp-37	33.7	60	Asp-90	17.2
25	Asp-38	7.5	61	Asp-92	97.6
26	Asp-39	41.7	62	Asp-95	81.4
27	Asp-40	53.4	63	Asp-96	11.7
28	Asp-41	25.9	64	Asp-97	394.7
29	Asp-42	19.3	65	Asp-99	23.9
30	Asp-43	14.2	66	Asp-101	67.4
31	Asp-44	93.6	67	Asp-102	89.9
32	Asp-46	17.9	68	Asp-103	113.2
33	Asp-47	56.1	69	Asp-106	102.7
34	Asp-48	78.4	70	Asp-107	91.3
35	Asp-49	83.8	71	Asp-108	81.3
36	Asp-50	287.1	72	Asp-112	19.2

contd…

Table 35 contd…

Sr. No.	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})	Sr. No	Isolate	Nitrogenase activity (nmol C_2H_4.mg protein^{-1}.hr^{-1})
73	Asp-113	117.4	84	Asp-132	256.4
74	Asp-116	101.9	85	Asp-134	115.3
75	Asp-117	94.7	86	Asp-136	53.9
76	Asp-119	138.4	87	Asp-140	74.1
77	Asp-120	16.1	88	Asp-142	55.8
78	Asp-121	13.7	89	Asp-145	11.4
79	Asp-123	69.4	90	Asp-147	107.2
80	Asp-124	248.5	91	Asp-148	113.6
81	Asp-127	241.3	92	Asp-149	142.3
82	Asp-129	130.3	93	Asp-150	269.8
83	Asp-130	121.7	94	Asp-BNF	239.6
			S.E. ±		1.60
			C.D. at 1%		5.90
			C.V.%		3.38

The distribution of efficient N_2 fixing microbes (*Azospirillum lipoferum* isolates) across the physiographic regions of Maharashtra is given in Table 36. Impact analysis of weather parameters and cropping system on distribution of efficient N_2 fixers (*Azospirillum lipoferum* isolates) across the physiographic regions of Maharashtra is given in Table 37. The results showed that weather parameters and cropping system had no singnificant influence on distribution of efficient *Azospirillum lipoferum* isolates. However, the population of *A. lipoferum* isolates varied significantly with rainfall pattern and cropping system across the regions. The maximum population of *A. lipoferum* ($5.40x10^4$ MPN g^{-1} soil) was recorded in the paddy/nachni fields in Kolhapur district of Western Ghats with annual rainfall 1904 mm followed by pigeonpea-wheat cropping system of Latur district ($4.50x10^4$ MPN g^{-1} soil) and pigeonpea-safflower of Parbhani district of Marathwada ($3.80x10^4$ MPN g^{-1} soil) with annual rainfall 880 and 840 mm, respectively.

Table 36. Distribution of efficient N_2 fixing microbes (*Azospirillum lipoferum* isolates) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Distribution of efficient N_2 fixing microbes (*A. lipoferum* isolates)
I	Western Konkan Coast	Sindhudurg	III
		Ratnagiri	III
		Thane	III
		Raigad	III
II	Western Ghats	Sindhudurg	III
		Ratnagiri	III
		Thane	III
		Kolhapur	I, II, III
		Satara	III
		Pune	III
		Nasik	III
		Dhule	III
		Nandurbar	I, III
III	Western Maharashtra	Pune	III
		Kolhapur	II, III
		Satara	II, III
		Sangli	II, III
		Solapur	III
		Ahmednagar	III
IV	North Maharashtra	Nasik	III
		Dhule	-
		Jalgaon	III
		Nandurbar	-
V	Marathwada	Latur	II, III
		Osmanabad	-
		Beed	III
		Nanded	I
		Parbhani	I
		Hingoli	-
		Jalna	III
		Aurangabad	III
		Buldhana	III
VI	Vidarbha	Washim	-
		Akola	III
		Amravati	-
		Yavatmal	II, III
		Wardha	I, III
		Nagpur	I, II, III
		Bhandara	III
		Chandrapur	I, III
		Gondia	III
		Gadchiroli	III

Categories of efficiency of *Azospirillum lipoferum* isolates:

I – Highly efficient (239.6 to 394.7 nmol C_2H_4.mg protein^{-1}.hr^{-1})

II – Moderately efficient (120.1 to 239.5 nmol C_2H_4.mg protein^{-1}.hr^{-1})

III – Less efficient (< 120 nmol C_2H_4.mg protein^{-1}.hr^{-1})

Table 37. Impact analysis of weather parameters and cropping system on distribution of efficient N_2 fixers (*Azospirillum lipoferum* isolates) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient N_2 fixer
					Max.	Min.	Kharif	Rabi	Summer	
I	Western Konkan Coast	Sindhudurg	Moderately shallow to very deep	3287	32.7	18.7	Paddy/ Nachni/ Varai	-	-	III (0.22)
		Ratnagiri	Shallow	3188	30.5	23.0	Paddy/ Nachni	-	-	III (0.88)
		Thane	Moderately shallow to deep	2477	34.4	17.5	Paddy/ Nachni	-	-	III (0.19)
		Raigad	Moderately deep	1804	31.2	20.3	Paddy/ Nachni	-	-	III (0.26)
II	Western Ghats	Sindhudurg	Shallow	2245	32.7	18.7	Paddy	-	-	III (1.04)
		Ratnagiri	Shallow	2617	30.5	23.0	Paddy/ Nachni	-	-	III (0.26)
		Thane	Shallow	2477	34.4	17.5	Paddy	-	-	III (1.15)
		Kolhapur	Moderately deep	1904	31.8	18.8	Paddy/ Nachni	-	-	I (5.40) II (3.50) III (0.31)
		Satara	Very shallow	710	30.3	19.0	Grasses	-	-	III (3.50)
		Pune	Very shallow	1150	31.8	18.3	Grasses	-	-	III (1.98)
		Nasik	Shallow	1035	31.1	16.9	Paddy/ Nagli	-	-	III (0.55)
		Dhule	Moderately shallow	1035	31.8	18.9	Forest	-	-	III (0.33)
		Nandurbar	Moderately shallow	674	29.6	18.8	Sorghum	Wheat	-	I (1.40) III (3.50)

Table 37 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient N_2 fixer
					Max.	Min.	Kharif	Rabi	Summer/ Annual	
III	Western Maharashtra	Pune	Shallow to moderately deep	700	31.8	18.3	Sorghum/ Pearlmillet	Wheat	Sugarcane	III (0.40)
		Kolhapur	Deep	1904	31.8	18.8	Paddy	Wheat	Sugarcane	II (1.20) III (2.45)
		Satara	Moderately deep	710	30.3	19.0	Sorghum	Chickpea /Wheat	Sugarcane	II (1.40) III (2.23)
		Sangli	Shallow to moderately deep	685	31.9	19.1	Groundnut /Sorghum	Wheat	Sugarcane	II (1.40) III (2.10)
		Solapur	Shallow to very deep	580	34.1	21.7	Green gram	Sorghum/ Safflower	Sugarcane	III (0.28)
		Ahmednagar	Very shallow to deep	550	31.5	17.8	Pearlmillet /Pigeaon-pea	Chickpea /Wheat	Sugarcane	III (1.02)
IV	North Maharashtra	Nasik	Moderately shallow to deep	1035	31.1	16.9	Pearlmillet /Onion	Wheat	Sugarcane	III (0.19)
		Dhule	Shallow	670	31.8	18.9	Pearlmillet	-	-	-
		Jalgaon	Deep	710	32.3	18.8	Banana/ Cotton	-	-	III (0.30)
		Nandurbar	Modeately deep	740	29.6	18.8	Pigeaon-pea	Wheat/ Chickpea	-	-
V	Marathwada	Latur	Shallow to deep	880	32.0	18.8	Pigeaon-pea	Wheat/ Chickpea	Sugarcane	II (4.50) III (0.45)
		Osmanabad	Shallow to deep	880	31.9	17.3	Pigeaon-pea	Wheat/ Chickpea	-	-
		Beed	Deep	700	31.4	18.2	Pigeaon-pea	Wheat/ Safflower	Sugarcane	III (1.18)

Table 37 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient N_2 fixer
					Max.	Min.	Kharif	Rabi	Summer	
		Nanded	Shallow	890	32.1	19.8	Cotton	-	-	I (0.17)
		Parbhani	Shallow to deep	840	31.9	18.9	Pigeaonpea	Safflower	-	I (3.80)
		Hingoli	Shallow to deep	840	31.7	18.6	Pigeaonpea	Chickpea	-	-
		Jalna	Shallow to deep	840	31.6	19.3	Pigeaonpea	Chickpea	-	III (0.13)
		Aurangabad	Shallow to deep	750	31.8	18.8	Pearlmillet /Sorghum	Safflower	-	III (0.41)
VI	Vidarbha	Buldhana	Deep	750	32.1	19.9	Cotton	-	-	III (0.12)
		Washim	Shallow to deep	840	33.1	20.7	Sorghum	Safflower	-	-
		Akola	Shallow to deep	840	34.1	19.9	Sorghum	Safflower	-	III (0.39)
		Amravati	Shallow to deep	840	33.3	21.5	Sorghum	Safflower /Chickpea	-	-
		Yavatmal	Shallow to deep	990	31.9	20.1	Cotton	-	-	II (0.45) III (1.20)
		Wardha	Shallow to deep	1090	33.3	21.5	Cotton	-	-	I (0.41) III (1.03)
		Nagpur	Deep	1660	34.7	22.9	Cotton/ Paddy	-	-	I (1.20) II (2.30) III (1.41)
		Bhandara	Deep	1400	33.9	21.6	Cotton/ Paddy	-	-	III (0.54)
		Chandrapur	Deep	1420	29.6	14.6	Paddy	-	-	I (1.40) III (0.26)
		Gondia	Deep	1420	30.1	18.6	Paddy	-	-	III (0.17)
		Gadchiroli	Deep	1550	29.9	18.8	Paddy	-	-	III (0.72)

Figures in parentheses represent the population dynamics values (x 10^4 MPN g^{-1} soil) of *Azospirillum lipoferum* isolates

4.7 Functional diversity of phosphate solubilizing microorganisms

4.7.1 Phosphate solubilizing ability of the PSB isolates

All the 47 PSB isolates were tested for their ability to solubilize inorganic phosphate both qualitatively and quantitatively and their results are presented in Table 38. Quick analysis of P-solubilization was carried out on Pikovskaya's agar medium. All the 48 isolates were able to form zone of P-solubilization on the medium. The diameter of the zone of P-solubilization ranged from 5-14 mm in different isolates.

4.7.1.1 Quantitative estimation of Pi released from TCP

The amount of Pi released from tri-calcium phosphate by the PSB isolates in Pikovskaya's broth was estimated at 10 days after inoculation (Plate 25). The amount of Pi released from TCP by the isolates at 10 DAI ranged from 2.06 to 52.38 per cent (Table 38). The P-solubilizing ability of all isolates was compared with standard MPKV strain. The isolate PSB-15 recorded significantly highest P-solubilization (52.38%) than the other isolates tested. The MPKV strain PSB-BNF had the P-solubilizing ability of 19.29%. Out of 47 PSB isolates, 6 isolates *viz.*, PSB-15, 33, 41, 72, 100 and 119 recorded significantly higher P-solubilizing ability than the standard MPKV strain PSB-BNF. The isolate PSB-39 and PSB-140 had the P-solubilizing ability of 22.03% and 20.24% which was statistically at par with MPKV strain. The amount of Pi released from TCP by selected PSB (*Bacillus megaterium*) isolates in comparison with standard MPKV strain PSB-BNF is shown in Figure 4.

4.7.1.2 Decrease in pH of medium during phosphate solubilization

The decrease in pH of TCP broth from initially adjusted pH of 7.0 was also noted at 10 days after inoculation. The significant reduction

Table 38 Zone of P solubilization on Pikovskaya's agar and per cent Pi released from TCP broth by the PSB isolates

Sr. No.	Isolate	Zone of P solubilization on TCP (mm)	% Pi released from TCP after 10 days	Decrease in pH of medium (from initial pH 7.0) after 10 days
1	PSB-6	7.0	7.64	5.09
2	PSB-10	9.0	13.23	4.42
3	PSB-11	8.0	9.43	4.11
4	PSB-15	14.0	52.38	3.48
5	PSB-17	10.0	16.81	4.66
6	PSB-20	7.0	6.43	5.07
7	PSB-33	9.0	23.40	4.47
8	PSB-39	11.0	22.03	4.41
9	PSB-41	10.0	39.74	4.14
10	PSB-42	7.0	4.43	5.05
11	PSB-46	9.0	17.60	4.61
12	PSB-48	7.0	6.75	5.08
13	PSB-50	6.0	2.79	5.02
14	PSB-56	8.0	3.85	5.04
15	PSB-59	5.0	2.06	5.01
16	PSB-60	9.0	4.22	5.05
17	PSB-61	7.0	5.59	5.07
18	PSB-64	8.0	6.17	5.08
19	PSB-68	6.0	8.54	5.09
20	PSB-69	6.0	6.53	5.06
21	PSB-72	10.0	26.14	4.66
22	PSB-73	9.0	18.23	4.94
23	PSB-75	9.0	10.96	4.54
24	PSB-78	8.0	12.23	4.12
25	PSB-79	7.0	12.60	4.09

contd…

Table 38 contd…

Sr. No.	Isolate	Zone of P solubilization on TCP (mm)	% Pi released from TCP after 10 days	Decrease in pH of medium (from initial pH 7.0) after 10 days
26	PSB-81	6.0	8.96	5.17
27	PSB-83	7.0	15.44	4.70
28	PSB-93	5.0	11.01	4.88
29	PSB-95	8.0	3.58	5.20
30	PSB-100	12.0	29.72	4.10
31	PSB-102	9.0	16.13	4.65
32	PSB-106	7.0	11.07	4.51
33	PSB-107	6.0	5.32	5.11
34	PSB-109	10.0	13.02	4.88
35	PSB-111	11.0	16.55	4.61
36	PSB-113	10.0	16.86	4.77
37	PSB-119	9.0	22.56	4.38
38	PSB-125	8.0	13.91	4.66
39	PSB-129	9.0	11.38	4.58
40	PSB-130	6.0	7.80	5.01
41	PSB-132	11.0	13.60	4.52
42	PSB-136	7.0	6.85	5.09
43	PSB-140	9.0	20.24	4.11
44	PSB-142	9.0	10.12	4.79
45	PSB-143	6.0	4.80	5.11
46	PSB-144	10.0	12.86	4.67
47	PSB-148	9.0	10.17	4.71
48	PSB-BNF	9.0	19.29	4.26
		S.E.±	0.84	0.20
		C.D. at 1%	3.19	0.75
		C.V.%	8.90	5.93
	Correlation (r) of Pi released with decrease in pH			0.781**

in pH of the medium i.e. pH 3.48 was recorded by PSB-15 isolate followed by PSB-79, PSB-100 and PSB-11 isolates which reduced the pH of the medium to 4.09, 4.10 and 4.11, respectively (Table 38). The correlation of decrease in pH of the medium with the amount of Pi released was also worked out. At 10 days after incubation, they had highly significant positive correlation (r = + 0.781**).

The distribution of efficient phosphate solubilizing bacteria (*Bacillus megaterium* isolates) across the physiographic regions of Maharashtra is given in Table 39. Impact analysis of weather parameters and cropping system on distribution of efficient phosphate solubilizing bacteria (*Bacillus megaterium* isolates) across the physiographic regions of Maharashtra is given in Table 40. The results showed that weather parameters and cropping system had no singnificant influence on distribution of efficient *Bacillus megaterium* isolates. However, the population of *B. megaterium* isolates varied significantly with cropping system across the regions. The maximum population of *B. megaterium* ($2.83x10^4$ CFU g^{-1} soil) was recorded in the pigeonpea-wheat cropping system in Latur district of Marathwada followed by green gram-sorghum/green gram-safflower cropping system of Solapur district ($2.25x10^4$ CFU g^{-1} soil) of Western Maharashtra and cotton/paddy fields of Nagpur district of Vidarbha ($2.00x10^4$ CFU g^{-1} soil).

4.7.2 Phosphate solubilizing ability of the PSF (*Aspergillus* isolates)

All the 29 *Aspergillus* isolates were tested for their ability to solubilize inorganic phosphate both qualitatively and quantitatively. The results are presented in Table 41 and Plate 26. Quick analysis of P-solubilization was carried out on Pikovskaya's agar medium. All the 29 isolates were able to form zone of P-solubilization on the medium. The diameter of the zone of P-solubilization ranged from 11-30 mm in different isolates.

Table 39 Distribution of efficient phosphate solubilizing bacteria (*Bacillus megaterium* isolates) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Distribution of efficient PSB (*Bacillus megaterium* isolates)
I	Western Konkan Coast	Sindhudurg	II
		Ratnagiri	III
		Thane	-
		Raigad	III
II	Western Ghats	Sindhudurg	-
		Ratnagiri	-
		Thane	I
		Kolhapur	I
		Satara	-
		Pune	I, II
		Nasik	I, III
		Dhule	III
		Nandurbar	III
III	Western Maharashtra	Pune	II, III
		Kolhapur	-
		Satara	II
		Sangli	III
		Solapur	I, II, III
		Ahmednagar	II, III
IV	North Maharashtra	Nasik	II, III
		Dhule	-
		Jalgaon	I
		Nandurbar	-
V	Marathwada	Latur	I, III
		Osmanabad	-
		Beed	III
		Nanded	II
		Parbhani	II
		Hingoli	-
		Jalna	-
		Aurangabad	II
		Buldhana	
VI	Vidarbha	Washim	III
		Akola	-
		Amravati	-
		Yavatmal	II
		Wardha	II, III
		Nagpur	I, III
		Bhandara	II
		Chandrapur	II
		Gondia	-
		Gadchiroli	II, III

Categories of efficiency of *Bacillus megaterium* isolates:

I – Highly efficient (19.29 to 52.38 % Pi released from TCP at 10 DAI)
II – Moderately efficient (10.1 to 19.28 % Pi released from TCP at 10 DAI)
III – Less efficient (<10 % Pi released from TCP at 10 DAI)

Table 40. Impact analysis of weather parameters and cropping system on distribution of efficient PSB (*Bacillus megaterium* isolates) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient P solubilizers
					Max.	Min.	Kharif	Rabi	Summer	
I	Western Konkan Coast	Sindhudurg	Moderately shallow to very deep	3287	32.7	18.7	Paddy/ Nachni/ Varai	-	-	II (1.00)
		Ratnagiri	Shallow	3188	30.5	23.0	Paddy/ Nachni	-	-	III (0.25)
		Thane	Moderately shallow to deep	2477	34.4	17.5	Paddy/ Nachni	-	-	-
		Raigad	Moderately deep	1804	31.2	20.3	Paddy/ Nachni	-	-	III (0.83)
II	Western Ghats	Sindhudurg	Shallow	2245	32.7	18.7	Paddy	-	-	-
		Ratnagiri	Shallow	2617	30.5	23.0	Paddy/ Nachni	-	-	-
		Thane	Shallow	2477	34.4	17.5	Paddy	-	-	I (1.17)
		Kolhapur	Moderately deep	1904	31.8	18.8	Paddy/ Nachni	-	-	I (0.50)
		Satara	Very shallow	710	30.3	19.0	Grasses	-	-	-
		Pune	Very shallow	1150	31.8	18.3	Grasses	-	-	I (1.33) II (1.17)
		Nasik	Shallow	1035	31.1	16.9	Paddy/ Nagli	-	-	I (1.50) III (1.00)
		Dhule	Moderately shallow	1035	31.8	18.9	Forest	-	-	III (0.50)
		Nandurbar	Moderately shallow	674	29.6	18.8	Sorghum	Wheat	-	III (1.00)

contd...

Table 40 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient P solubilizers
					Max.	Min.	Kharif	Rabi	Summer/ Annual	
III	Western Maharashtra	Pune	Shallow to moderately deep	700	31.8	18.3	Sorghum/ Pearlmillet	Wheat	Sugarcane	II (1.00) III (0.84)
		Kolhapur	Deep	1904	31.8	18.8	Paddy	Wheat	Sugarcane	-
		Satara	Moderately deep	710	30.3	19.0	Sorghum	Chickpea /Wheat	Sugarcane	II (1.25)
		Sangli	Shallow to moderately deep	685	31.9	19.1	Groundnut /Sorghum	Wheat	Sugarcane	III (0.25)
		Solapur	Shallow to very deep	580	34.1	21.7	Green gram	Sorghum/ Safflower	Sugarcane	I (2.25) II (0.92) III (0.83)
		Ahmednagar	Very shallow to deep	550	31.5	17.8	Pearlmillet /Pigeaon-pea	Chickpea /Wheat	Sugarcane	II (1.17) III (1.17)
IV	North Maharashtra	Nasik	Moderately shallow to deep	1035	31.1	16.9	Pearlmillet /Onion	Wheat	Sugarcane	II (0.50) III (0.33)
		Dhule	Shallow	670	31.8	18.9	Pearlmillet	-	-	-
		Jalgaon	Deep	710	32.3	18.8	Banana/ Cotton	-	-	I (1.17)
		Nandurbar	Modeately deep	740	29.6	18.8	Pigeaon-pea	Wheat/ Chickpea	-	-
V	Marathwada	Latur	Shallow to deep	880	32.0	18.8	Pigeaon-pea	Wheat/ Chickpea	Sugarcane	I (2.83) III (0.83)
		Osmanabad	Shallow to deep	880	31.9	17.3	Pigeaon-pea	Wheat/ Chickpea	-	-
		Beed	Deep	700	31.4	18.2	Pigeaon-pea	Wheat/ Safflower	Sugarcane	III (0.16)

contd...

Table 40 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient P solubilizers
					Max.	Min.	Kharif	Rabi	Summer	
		Nanded	Shallow	890	32.1	19.8	Cotton/ Pigeaonpea	-	-	II (0.33)
		Parbhani	Shallow to deep	840	31.9	18.9	Pigeaonpea	Safflower	-	II (1.50)
		Hingoli	Shallow to deep	840	31.7	18.6	Pigeaonpea	Chickpea	-	-
		Jalna	Shallow to deep	840	31.6	19.3	Pigeaonpea	Chickpea	-	-
		Aurangabad	Shallow to deep	750	31.8	18.8	Pearlmillet/ Sorghum	Safflower	-	II (1.17)
VI	Vidarbha	Buldhana	Deep	750	32.1	19.9	Cotton	-	-	-
		Washim	Shallow to deep	840	33.1	20.7	Sorghum	Safflower	-	III (0.25)
		Akola	Shallow to deep	840	34.1	19.9	Sorghum	Safflower	-	-
		Amravati	Shallow to deep	840	33.3	21.5	Sorghum	Safflower /Chickpea	-	-
		Yavatmal	Shallow to deep	990	31.9	20.1	Cotton	-	-	II (0.83)
		Wardha	Shallow to deep	1090	33.3	21.5	Cotton	-	-	II (0.13) III (1.00)
		Nagpur	Deep	1660	34.7	22.9	Cotton/ Paddy	-	-	I (2.00) III (0.59)
		Bhandara	Deep	1400	33.9	21.6	Cotton/Paddy	-	-	II (1.33)
		Chandrapur	Deep	1420	29.6	14.6	Paddy	-	-	II (0.67)
		Gondia	Deep	1420	30.1	18.6	Paddy	-	-	-
		Gadchiroli	Deep	1550	29.9	18.8	Paddy	-	-	II (0.50) III (0.67)

Figures in parentheses represent the population dynamics values (x 10^4 CFU g^{-1} soil) of *Bacillus megaterium* isolates

4.7.2.1 Quantitative estimation of Pi released from TCP

The amount of Pi released from tri-calcium phosphate by the *Aspergillus* isolates in Pikovskaya's broth was estimated at 10 days after inoculation. The amount of Pi released from TCP by the isolates at 10 DAI ranged from 45.80 to 87.17 per cent (Table 41). The P-solubilizing ability of all isolates was compared with standard MPKV strain. The isolate PSF-71 recorded significantly highest P-solubilization (87.17%) than the other isolates tested. The MPKV strain PSF-BNF(A) had the P-solubilizing ability of 60.82%. Out of 29 *Aspergillus* isolates, 5 isolates *viz.*, PSF-28, 55, 64, 71 and 100 recorded significantly higher P-solubilizing ability than the standard MPKV strain PSF-BNF(A). The isolates PSF-8, 115 and 132 had the P-solubilizing ability of 65.03%, 63.98% and 61.87%, respectively which was statistically at par with MPKV strain. The amount of Pi released from TCP by selected PSF (*Aspergillus awamori*) isolates in comparison with standard MPKV strain PSF-BNF(A) is shown in Figure 5.

4.7.2.2 Decrease in pH of medium during phosphate solubilization

The decrease in pH of TCP broth from initially adjusted pH of 7.0 was also noted at 10 days after inoculation. The significant reduction in pH of the medium i.e. pH 3.01 was recorded by PSF-71 isolate followed by PSF-28, PSF-55 and PSF-64 isolates which reduced the pH of the medium to 3.27, 3.40 and 3.48, respectively (Table 41). The correlation of decrease in pH of the medium with the amount of Pi released was also worked out. At 10 days after incubation, they had highly significant positive correlation (r = + 0.905**).

The distribution of efficient phosphate solubilizing fungi (*Aspergillus awamori* isolates) across the physiographic regions of Maharashtra is given in Table 42.

Table 41 Zone of P solubilization on Pikovskaya's agar and per cent Pi released from TCP broth by the *Aspergillus* isolates

Sr. No.	Isolate	Zone of P solubilization on TCP (mm)	% Pi released from TCP after 10 days	Decrease in pH of medium (from initial pH 7.0) after 10 days
1	PSF-4	11.0	45.80	4.90
2	PSF-7	16.0	56.07	4.71
3	PSF-8	21.0	65.03	3.61
4	PSF-11	13.0	50.01	4.77
5	PSF-19	12.0	47.64	4.70
6	PSF-28	25.0	75.04	3.27
7	PSF-32	12.0	46.32	4.40
8	PSF-40	15.0	47.53	4.31
9	PSF-41	18.0	57.65	4.11
10	PSF-44	19.0	59.76	4.24
11	PSF-55	23.0	73.99	3.40
12	PSF-57	16.0	50.54	4.39
13	PSF-64	22.0	66.09	3.48
14	PSF-71	30.0	87.17	3.01
15	PSF-82	13.0	50.28	4.33
16	PSF-100	22.0	69.77	3.57
17	PSF-101	14.0	54.49	4.13
18	PSF-115	21.0	63.98	3.63
19	PSF-119	13.0	51.86	4.65
20	PSF-120	12.0	49.75	4.90
21	PSF-122	17.0	54.49	4.36
22	PSF-124	18.0	58.18	4.59
23	PSF-127	16.0	56.86	4.14
24	PSF-128	13.0	50.39	4.37
25	PSF-129	15.0	56.07	4.40
26	PSF-132	21.0	61.87	3.77
27	PSF-135	17.0	57.65	4.11
28	PSF-143	16.0	53.44	4.31
29	PSF-148	12.0	46.85	4.77
30	PSF-BNF(A)	20.0	60.82	3.98
		S.E.+	1.32	0.20
		C.D. at 1%	5.13	0.77
		C.V.%	3.24	6.69
	Correlation (r) of Pi released with decrease in pH			0.905**

Table 42. Distribution of efficient phosphate solubilizing fungi (*Aspergillus awamori* isolates) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Distribution of efficient PSF (*Aspergillus awamori* isolates)
I	Western Konkan Coast	Sindhudurg	I
		Ratnagiri	II
		Thane	II
		Raigad	II
II	Western Ghats	Sindhudurg	II
		Ratnagiri	-
		Thane	II
		Kolhapur	I, II
		Satara	II
		Pune	-
		Nasik	-
		Dhule	-
		Nandurbar	-
III	Western Maharashtra	Pune	-
		Kolhapur	I, II
		Satara	-
		Sangli	-
		Solapur	I
		Ahmednagar	I
IV	North Maharashtra	Nasik	I
		Dhule	II
		Jalgaon	II
		Nandurbar	-
V	Marathwada	Latur	I
		Osmanabad	-
		Beed	II
		Nanded	II
		Parbhani	-
		Hingoli	-
		Jalna	-
		Aurangabad	-
		Buldhana	-
VI	Vidarbha	Washim	-
		Akola	II
		Amravati	III
		Yavatmal	II
		Wardha	II
		Nagpur	II
		Bhandara	II
		Chandrapur	I, II
		Gondia	-
		Gadchiroli	II

Categories of efficiency of *Aspergillus awamori* isolates:

I – Highly efficient (60.82 to 87.17 % Pi released from TCP at 10 DAI)
II – Moderately efficient (31.1 to 60.81 % Pi released from TCP at 10 DAI)
III – Less efficient (<31 % Pi released from TCP at 10 DAI)

4.7.3 Phosphate solubilizing ability of the PSF (*Penicillium* isolates)

All the 17 *Penicillium* isolates were tested for their ability to solubilize inorganic phosphate both qualitatively and quantitatively. The results are presented in Table 43. Quick analysis of P-solubilization was carried out on Pikovskaya's agar medium. All the 17 isolates were able to form zone of P-solubilization on the medium. The diameter of the zone of P-solubilization ranged from 9-24 mm in different isolates.

4.7.3.1 Quantitative estimation of Pi released from TCP

The amount of Pi released from tri-calcium phosphate by the *Penicillium* isolates in Pikovskaya's broth was estimated at 10 days after inoculation. The amount of Pi released from TCP by the isolates at 10 DAI ranged from 26.30 to 69.77 per cent (Table 43). The P-solubilizing ability of all isolates was compared with standard MPKV strain. The isolate PSF-61 recorded significantly highest P-solubilization (69.77%) followed by PSF-77 (53.44%) than the other isolates tested. The MPKV strain PSF-BNF(P) had the P-solubilizing ability of 48.17%. Out of 17 *Penicillium* isolates, 3 isolates *viz.*, PSF-80, 97 and 101-1 recorded the P-solubilizing ability of 50.28%, 50.54% and 49.22%, respectively which was statistically at par with MPKV strain. The Amount of Pi released from TCP by selected PSF (*Penicillium digitatum*) isolates in comparison with standard MPKV strain PSF-BNF(P) is shown in Figure 6.

4.7.3.2 Decrease in pH of medium during phosphate solubilization

The decrease in pH of TCP broth from initially adjusted pH of 7.0 was also noted at 10 days after inoculation. The significant reduction in pH of the medium i.e. pH 3.13 was recorded by PSF-61 isolate followed by PSF-101-1, PSF-97 and PSF-80 isolates which reduced the pH of the medium to 3.70, 3.72 and 3.81, respectively (Table 43).

Table 43 Zone of P solubilization on Pikovskaya's agar and per cent Pi released from TCP broth by the *Penicillium* isolates

Sr. No.	Isolate	Zone of P solubilization on TCP (mm)	% Pi released from TCP after 10 days	Decrease in pH of medium (from initial pH 7.0) after 10 days
1	PSF-30	12.0	44.53	4.92
2	PSF-61	24.0	69.77	3.13
3	PSF-65	13.0	46.06	4.81
4	PSF-77	21.0	53.44	3.88
5	PSF-79	12.0	43.95	4.80
6	PSF-80	19.0	50.28	3.81
7	PSF-82-1	9.0	26.30	5.01
8	PSF-85	14.0	45.80	4.83
9	PSF-92	10.0	33.94	5.13
10	PSF-97	20.0	50.54	3.72
11	PSF-101-1	18.0	49.22	3.70
12	PSF-102	10.0	36.31	4.93
13	PSF-118	11.0	39.74	4.90
14	PSF-120-1	13.0	45.01	4.31
15	PSF-136	12.0	44.48	4.40
16	PSF-138	10.0	34.99	4.72
17	PSF-141	11.0	37.89	4.49
18	PSF-BNF(P)	15.0	48.17	3.84
		S.E.+	1.19	0.18
		C.D. at 1%	4.85	0.75
		C.V.%	3.79	5.91
	Correlation (r) of Pi released with decrease in pH			0.833**

The correlation of decrease in pH of the medium with the amount of Pi released was also worked out. At 10 days after incubation, they had highly significant positive correlation (r = + 0.833**).

Based on the phosphate solubilizing ability, the highly efficient phosphate solubilizing isolates *viz.*, 8 PSB isolates, 8 *Aspergillus* isolates and 5 *Penicillium* isolates alongwith standard MPKV strain were further analyzed for their molecular diversity.

The distribution of efficient phosphate solubilizing fungi (*Penicillium digitatum* isolates) across the physiographic regions of Maharashtra is given in Table 44.

Impact analysis of weather parameters and cropping system on distribution of efficient phosphate solubilizing fungi (*Aspergillus awamori* and *Penicillium digitatum* isolates) across the physiographic regions of Maharashtra is given in Table 45. The results showed that weather parameters and cropping system had no singnificant influence on distribution of efficient *Aspergillus awamori* and *Penicillium digitatum* isolates. However, population of PSF (*A. awamori* and *P. digitatum*) isolates varied significantly with cropping system across the regions. The maximum population of *A. awamori* ($2.33x10^4$ CFU g^{-1} soil) was recorded in the pigeonpea-wheat/pieonpea-safflower cropping system in Beed district of Marathwada followed by paddy-wheat cropping system of Kolhapur district ($2.00x10^4$ CFU g^{-1} soil) and sorghum-safflower of Akola district of Vidarbha ($1.87x10^4$ CFU g^{-1} soil). Similarly green gram-sorghum/ green gram-safflower cropping system resulted in maximum population of *P. digitatum* ($2.33x10^4$ CFU g^{-1} soil) followed by sorghum-chickpea/sorghum-wheat cropping system ($1.83x10^4$ CFU g^{-1} soil) of Solapur and Satara district of Western Maharashtra, respectively.

Table 44 Distribution of efficient phosphate solubilizing fungi (*Penicillium digitatum* isolates) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Distribution of efficient PSF (*Penicillium digitatum* isolates)
I	Western Konkan Coast	Sindhudurg	-
		Ratnagiri	-
		Thane	-
		Raigad	-
II	Western Ghats	Sindhudurg	-
		Ratnagiri	-
		Thane	-
		Kolhapur	II
		Satara	-
		Pune	-
		Nasik	-
		Dhule	-
		Nandurbar	-
III	Western Maharashtra	Pune	II
		Kolhapur	-
		Satara	I, II
		Sangli	-
		Solapur	I, II
		Ahmednagar	I, II
IV	North Maharashtra	Nasik	-
		Dhule	-
		Jalgaon	II
		Nandurbar	-
V	Marathwada	Latur	-
		Osmanabad	-
		Beed	II
		Nanded	-
		Parbhani	I
		Hingoli	-
		Jalna	-
		Aurangabad	II
		Buldhana	-
VI	Vidarbha	Washim	-
		Akola	I
		Amravati	-
		Yavatmal	-
		Wardha	II
		Nagpur	II
		Bhandara	-
		Chandrapur	-
		Gondia	-
		Gadchiroli	II

Categories of efficiency of *Penicillium digitatum* isolates:

I – Highly efficient (48.17 to 69.77 % Pi released from TCP at 10 DAI)
II – Moderately efficient (24.1 to 48.16 % Pi released from TCP at 10 DAI)
III – Less efficient (<24 % Pi released from TCP at 10 DAI)

Table 45. Impact analysis of weather parameters and cropping system on distribution of efficient PSF (*Aspergillus awamori* and *Penicillium digitatum* isolates) across the physiographic regions of Maharashtra

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient P solubilizers	
					Max.	Min.	Kharif	Rabi	Summer	*Aspergillus awamori*	*Penicillium digitatum*
I	Western Konkan Coast	Sindhudurg	Moderately shallow to very deep	3287	32.7	18.7	Paddy/ Nachni/ Varai	-	-	I (0.83)	-
		Ratnagiri	Shallow	3188	30.5	23.0	Paddy/ Nachni	-	-	II (0.50)	-
		Thane	Moderately shallow to deep	2477	34.4	17.5	Paddy/ Nachni	-	-	II (1.67)	-
		Raigad	Moderately deep	1804	31.2	20.3	Paddy/ Nachni	-	-	II (0.50)	-
II	Western Ghats	Sindhudurg	Shallow	2245	32.7	18.7	Paddy	-	-	II (1.83)	-
		Ratnagiri	Shallow	2617	30.5	23.0	Paddy/ Nachni	-	-	-	-
		Thane	Shallow	2477	34.4	17.5	Paddy	-	-	II (1.00)	-
		Kolhapur	Moderately deep	1904	31.8	18.8	Paddy/ Nachni	-	-	I (1.17) II (0.67)	II (0.33)
		Satara	Very shallow	710	30.3	19.0	Grasses	-	-	II (1.17)	-
		Pune	Very shallow	1150	31.8	18.3	Grasses	-	-	-	-
		Nasik	Shallow	1035	31.1	16.9	Paddy/ Nagli	-	-	-	-
		Dhule	Moderately shallow	1035	31.8	18.9	Forest	-	-	-	-
		Nandurbar	Moderately shallow	674	29.6	18.8	Sorghum	Wheat	-	-	-

contd...

Table 45 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient P solubilizers	
					Max.	Min.	Kharif	Rabi	Summer /Annual	*Aspergillus awamori*	*Penicillium digitatum*
III	Western Maharashtra	Pune	Shallow to moderately deep	700	31.8	18.3	Sorghum/ Pearlmillet	Wheat	Sugarcane	-	II (0.67)
		Kolhapur	Deep	1904	31.8	18.8	Paddy	Wheat	Sugarcane	I (2.00) II (1.00)	-
		Satara	Moderately deep	710	30.3	19.0	Sorghum	Chickpea /Wheat	Sugarcane	-	I (1.83) II (1.00)
		Sangli	Shallow to moderately deep	685	31.9	19.1	Groundnut /Sorghum	Wheat	Sugarcane	-	-
		Solapur	Shallow to very deep	580	34.1	21.7	Green gram	Sorghum/ Safflower	Sugarcane	I (0.19)	I (2.33) II (0.67)
		Ahmednagar	Very shallow to deep	550	31.5	17.8	Pearlmillet /Pigeaon-pea	Chickpea /Wheat	Sugarcane	I (1.33)	I (1.17) II (1.13)
IV	North Maharashtra	Nasik	Moderately shallow to deep	1035	31.1	16.9	Pearlmillet /Onion	Wheat	Sugarcane	I (1.17)	-
		Dhule	Shallow	670	31.8	18.9	Pearlmillet	-	-	II (0.16)	
		Jalgaon	Deep	710	32.3	18.8	Banana/ Cotton	-	-	II (1.09)	II (0.83)
		Nandurbar	Modeately deep	740	29.6	18.8	Pigeaon-pea	Wheat/ Chickpea	-	-	-
V	Marathwada	Latur	Shallow to deep	880	32.0	18.8	Pigeaon-pea	Wheat/ Chickpea	Sugarcane	I (0.50)	-
		Osmanabad	Shallow to deep	880	31.9	17.3	Pigeaon-pea	Wheat/ Chickpea	-	-	-
		Beed	Deep	700	31.4	18.2	Pigeaon-pea	Wheat/ Safflower	Sugarcane	II (2.33)	II (1.33)

contd...

Table 45 contd...

Sr. No.	Physiographic region	District	Soil type	Rainfall pattern (mm)	Temperature range (°C)		Cropping system			Distribution of efficient P solubilizers	
					Max.	Min.	Kharif	Rabi	Summer	*Aspergillus awamori*	*Penicillium digitatum*
		Nanded	Shallow	890	32.1	19.8	Cotton/ Pigeaonpea	-	-	II (0.33)	-
		Parbhani	Shallow to deep	840	31.9	18.9	Pigeaonpea	Safflower	-	-	I (0.83)
		Hingoli	Shallow to deep	840	31.7	18.6	Pigeaonpea	Chickpea	-	-	-
		Jalna	Shallow to deep	840	31.6	19.3	Pigeaonpea	Chickpea	-	-	-
		Aurangabad	Shallow to deep	750	31.8	18.8	Pearlmillet /Sorghum	Safflower	-	-	II (0.67)
VI	Vidarbha	Buldhana	Deep	750	32.1	19.9	Cotton	-	-	-	-
		Washim	Shallow to deep	840	33.1	20.7	Sorghum	Safflower	-	-	-
		Akola	Shallow to deep	840	34.1	19.9	Sorghum	Safflower	-	II (1.87)	I (0.50)
		Amravati	Shallow to deep	840	33.3	21.5	Sorghum	Safflower /Chickpea	-	III (0.50)	-
		Yavatmal	Shallow to deep	990	31.9	20.1	Cotton	-	-	II (0.83)	-
		Wardha	Shallow to deep	1090	33.3	21.5	Cotton	-	-	II (0.50)	II (1.50)
		Nagpur	Deep	1660	34.7	22.9	Cotton/ Paddy	-	-	II (0.50)	II (1.33)
		Bhandara	Deep	1400	33.9	21.6	Cotton/ Paddy	-	-	II (1.83)	-
		Chandrapur	Deep	1420	29.6	14.6	Paddy	-	-	I (0.83) II (0.50)	-
		Gondia	Deep	1420	30.1	18.6	Paddy	-	-	-	-
		Gadchiroli	Deep	1550	29.9	18.8	Paddy	-	-	II (0.83)	II (1.09)

Figures in parentheses represent the population dynamics values (x 10^4 CFU g^{-1} soil) of P solubilizing isolates

4.8 Molecular diversity of nitrogen fixing microorganisms

The molecular diversity within each group of the selected nitrogen fixing isolates along with the standard MPKV strain was analyzed by employing random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique.

4.8.1 RAPD-PCR analysis of genomic DNA of different *Azotobacter* isolates

The genomic DNA of twelve selected isolates of *Azotobacter chroococcum* alongwith MPKV strain, Azt-BNF were subjected to PCR amplification using twenty five random oligonucleotide primers (Table 46). The amplification profiles of 12 different isolates + 1 MPKV strain with 25 random bacterial primers are summarized in Table 47. It was observed that 1495 fragments were generated in all the 12 different isolates + 1 MPKV strain with 25 RAPD primers, out of which 1123 fragments were polymorphic. Amongst all the primers the maximum amplification was observed with RBA-20, RBA-16 and RBA-11 (62, 57 and 54 polymorphic bands, respectively) primers, whereas least banding pattern was generated by RBA-2 and RBA-4 (27 and 30 polymorphic bands respectively). The primer RBA-20 showed maximum per cent polymorphism of 87.32%. These 25 primers produced isolate specific 372 unique bands. The fingerprint data of 25 random primers were converted into a binary matrix based on the presence or absence of individual amplified bands for each isolate. The RAPD bands of particular molecular weight present in an isolate were marked as “1” and absence was marked as “0”. The cluster analysis was performed by using unweighted pair group method and arithmetic average (UPGMA) to distinguish *Azotobacter chroococcum* isolates. The computer software NTSYS–PC was used for data analysis.

Table 46 RAPD analysis of genomic DNA of twelve different isolates of *Azotobacter chroococcum*

Sr. No.	Details	
1	Total No. of primers used	25
2	No. of polymorphic primers	25
3	Total No. of bands	1495
4	Total No. of polymorphic bands	1123
5	Total No. of unique bands	372
6	Percentage polymorphic	75.12

Table 47 Total number of RAPD markers and polymorphic bands produced by random primers in twelve different isolates of *Azotobacter chroococcum*

Sr. No.	Random primers	No. of bands generated	Polymorphic bands	Monomorphic bands	Unique bands	% Polymorphic bands
1	RBA-1	66	46	-	20	69.69
2	RBA-2	51	27	-	24	52.94
3	RBA-3	62	41	-	21	66.12
4	RBA-4	43	30	-	13	69.76
5	RBA-5	59	49	-	10	83.05
6	RBA-6	58	44	-	14	75.86
7	RBA-7	62	44	-	18	70.96
8	RBA-8	64	55	-	09	85.93
9	RBA-9	60	46	-	14	76.66
10	RBA-10	66	51	-	15	77.27
11	RBA-11	74	54	-	20	72.97
12	RBA-12	46	37	-	09	80.43
13	RBA-13	62	44	-	18	70.96
14	RBA-14	61	47	-	14	77.04
15	RBA-15	64	49	-	15	76.56
16	RBA-16	75	57	-	18	76.00
17	RBA-17	43	30	-	13	69.76
18	RBA-18	58	49	-	09	84.48
19	RBA-19	45	30	-	15	66.66
20	RBA-20	71	62	-	09	87.32
21	RBA-21	62	46	-	16	74.19
22	RBA-22	63	51	-	12	80.95
23	RBA-23	61	46	-	15	75.40
24	RBA-24	54	38	-	16	70.37
25	RBA-25	65	50	-	15	76.92

All the 25 primers used generated scorable polymorphic bands for the isolates of *Azotobacter chroococcum* (Plate 27 and 28). All the primers showed the genetic polymorphism between the *Azotobacter chroococcum* isolates tested. However, RBA-24 was found to be the best primer for determination of variability among the *A. chroococcum* isolates. This primer generated 54 bands of different molecular weight (Table 48). Though some of the isolates share common bands most of the isolates differed in their banding pattern. Band 11, 13, 17, 36 and 51 were only present in Azt-21isolate, band 37 and 54 were only present in Azt-70 isolate, band 22 and 53 in Azt-50, band 25 and 50 in Azt-135, band 5 in Azt-BNF, band 14 in Azt-142, band 16 in Azt-8, band 26 in Azt-129 and band 40 in Azt-148 isolate, thereby indicating these are the genetically different isolates. None of the *Azotobacter chroococcum* isolate share all the bands and are therefore genetically variable. Though all the isolates of *A. chroococcum* were genetically variable, they share some common bands indicating phylogenetic relationship among them.

Dendrogram representing the genetic relationship among the isolates based on RAPD analysis was developed and presented in Figure 7, 8 and 9. On the basis of RAPD analysis, *Azotobacter chroococcum* isolates were classified into four broad groups. The group I further classified into two subgroups. The subgroup Ia consisted two isolates i.e. Azt-8 and Azt-25 having genetic similarity coefficient 0.40. The subgroup Ib consisted of single Azt-21 isolate having the genetic similarity coefficient 0.24. The group II consisted of two isolates i.e. Azt-50 and Azt-64 having genetic similarity coefficient 0.25. Likewise group III was further classified into three subgroups on the basis of genetic similarity coefficient. The subgroup IIIa consisted of single Azt-70 isolate having the genetic similarity coefficient 0.21, whereas subgroup IIIb consisted of Azt-82 and Azt-97 isolate having similarity coefficient 0.35. Similarly subgroup IIIc consisted of single Azt-129

Table 48 Determination of genetic variability in different isolates of *A. chroococcum* by DNA amplification profiles by RBA-24 primer

Band No.	Mol. wt. (bp)	***Azotobacter chroococcum* isolates**												
		Azt 8	Azt 21	Azt 25	Azt 50	Azt 64	Azt 70	Azt 82	Azt 97	Azt 129	Azt 135	Azt 142	Azt 148	Azt BNF
Isolate No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
1	5835	1	1											
2	4820	1	1	1	1	1		1	1	1				1
3	3824							1	1			1		
4	3786	1	1	1				1	1			1		
5	3355													1
6	3127				1	1	1							
7	3064			1						1			1	
8	3034							1	1					
9	2914									1			1	
10	2856	1	1		1									
11	2335		1											
12	2266	1		1				1	1					1
13	2133		1											
14	2029											1		
15	1853		1		1									
16	1780	1												
17	1710		1											
18	1546	1		1	1									
19	1500								1	1				
20	1455							1			1			
21	1370		1							1		1		
22	1329				1									
23	1277			1										1
24	1166				1			1				1		
25	1109										1			
26	1087									1				
27	1065		1										1	
28	1013				1	1								

contd…

Table 48 contd…

Band No.	Mol. wt. (bp)	***Azotobacter chroococcum* isolates**												
		Azt 8	Azt 21	Azt 25	Azt 50	Azt 64	Azt 70	Azt 82	Azt 97	Azt 129	Azt 135	Azt 142	Azt 148	Azt BNF
Isolate No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
29	973						1					1		1
30	907	1		1										
31	871		1									1		
32	828							1		1				1
33	764		1		1									
34	734					1	1							
35	727							1	1					
36	691		1											
37	637						1							
38	625	1	1	1						1		1		
39	619				1	1		1	1					
40	600												1	
41	554						1					1		
42	476				1	1								
43	471	1	1	1										
44	467							1	1	1	1	1	1	
45	409						1			1				
46	378		1	1										
47	359						1		1					1
48	276									1				1
49	226										1		1	
50	202										1			
51	188		1											
52	160						1				1			
53	148				1									
54	132						1							

1 = band present

isolate with similarity coefficient 0.18. The group IV again was classified into three subgroups. The subgroup IVa consisted of single Azt-135 isolate with similarity coefficient 0.17, whereas subgroup IVb consisted of Azt-142 and Azt-148 isolate having equal genetic similarity coefficient 0.24. Similarly subgroup IVc consisted of single Azt-BNF strain having distinct genetic similarity coefficient 0.15.

3D PCO scatter plot clearly represents the phylogenetic relationship among different isolates of *Azotobacter chroococcum*. The height of the points differentiates the distinctness or genetic similarity among the isolates. For example Azt-82 isolate showed highest pick which represent distinctness of this isolate from other isolates, whereas Azt-142 and Azt-148 isolates pointing on a similar height showed genetic similarity among them. Similarly 2D PCO scatter plot was also helpful to make up the group of the isolates on the basis of presence of points on particular axis.

4.8.2 RAPD-PCR analysis of genomic DNA of different *Rhizobium* isolates

The genomic DNA of seven selected isolates of *Rhizobium* spp. alongwith MPKV strain, Rh-BNF were subjected to PCR amplification using twenty five random oligonucleotide primers (Table 49). The amplification profiles of 7 different isolates + 1 MPKV strain with 25 random bacterial primers are summarized in Table 50. It was observed that 1589 fragments were generated in all the 7 different isolates + 1 MPKV strain with 25 RAPD primers, out of which 905 fragments were polymorphic. Amongst all the primers the maximum amplification was observed with RBA-4, RBA-14 and RBA-20 (47, 45 and 43 polymorphic bands, respectively) primers, whereas least banding pattern was generated by RBA-9, RBA-19 and RBA-17 (19, 24 and 27 polymorphic bands respectively). The primer RBA-4 showed maximum per cent polymorphism of 74.60%. These 25 primers produced isolate specific 683 unique bands.

Table 49 RAPD analysis of genomic DNA of seven different isolates of *Rhizobium* spp.

Sr. No.	Details	
1	Total No. of primers used	25
2	No. of polymorphic primers	25
3	Total No. of bands	1589
4	Total No. of polymorphic bands	905
5	Total No. of unique bands	683
6	Percentage polymorphic	56.95

Table 50 Total number of RAPD markers and polymorphic bands produced by random primers in seven different isolates of *Rhizobium* spp.

Sr. No.	Random primers	No. of bands generated	Polymorphic bands	Monomorphic bands	Unique bands	% Polymorphic bands
1	RBA-1	58	32	-	26	55.17
2	RBA-2	55	28	-	27	50.90
3	RBA-3	53	34	-	19	64.15
4	RBA-4	63	47	-	16	74.60
5	RBA-5	65	41	-	24	63.07
6	RBA-6	66	35	-	31	53.03
7	RBA-7	66	38	-	28	57.57
8	RBA-8	84	37	-	47	44.04
9	RBA-9	33	19	-	14	57.57
10	RBA-10	66	35	-	31	53.03
11	RBA-11	64	42	-	22	65.62
12	RBA-12	66	38	-	28	57.57
13	RBA-13	63	36	-	27	57.14
14	RBA-14	69	45	-	24	65.21
15	RBA-15	68	38	-	30	55.88
16	RBA-16	84	36	-	48	42.85
17	RBA-17	64	27	-	37	42.18
18	RBA-18	58	34	-	24	58.62
19	RBA-19	57	24	-	33	42.10
20	RBA-20	65	43	01	21	66.15
21	RBA-21	52	35	-	17	67.30
22	RBA-22	68	42	-	26	61.76
23	RBA-23	65	39	-	26	60.00
24	RBA-24	72	39	-	33	54.16
25	RBA-25	65	41	-	24	63.07

All the 25 primers used generated scorable polymorphic bands for the isolates of *Rhizobium* spp. (Plate 29). All the primers showed the genetic polymorphism between the *Rhizobium* spp. isolates tested. However, RBA-7 was found to be the best primer for determination of variability among the *Rhizobium* spp. isolates. This primer generated 66 bands of different molecular weight (Table 51). Though some of the isolates share common bands most of the isolates differed in their banding pattern. None of the *Rhizobium* spp. isolate share all the bands and are therefore genetically variable. Though all the isolates of *Rhizobium* spp. were genetically variable, they share some common bands indicating phylogenetic relationship among them.

Dendrogram representing the genetic relationship among the isolates based on RAPD analysis was developed and presented in Figure 10, 11 and 12. On the basis of RAPD analysis, *Rhizobium* spp. isolates were classified into three broad groups. The group I further classified into two subgroups. The subgroup Ia consisted two isolates i.e. Rh-64 and Rh-69 having genetic similarity coefficient 0.27. The subgroup Ib consisted of single Rh-72 isolate having the genetic similarity coefficient 0.24. Likewise group II was further classified into two subgroups on the basis of genetic similarity coefficient. The subgroup IIa consisted of Rh-101 and Rh-109 isolate having the genetic similarity coefficient 0.26, whereas subgroup IIb consisted of single Rh-113 isolate having similarity coefficient 0.25. The group III consisted of Rh-132 isolate and Rh-BNF strain having genetic similarity coefficient 0.22.

3D PCO scatter plot clearly represents the phylogenetic relationship among different isolates of *Rhizobium* spp. The height of the points differentiates the distinctness or genetic similarity among the isolates. For example Rh-109 isolate showed highest pick which represent distinctness of this isolate from other isolates, whereas the isolate Rh-132 and Rh-BNF strain pointing on a similar height showed

Table 51 Determination of genetic variability in different isolates of *Rhizobium* spp. by DNA amplification profiles by RBA-7 primer

Band No.	Molecular weight (bp)	***Rhizobium* spp. isolates**							
		Rh 64	Rh 69	Rh 72	Rh 101	Rh 109	Rh 113	Rh 132	Rh BNF
Isolate No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	5361					1	1		
2	4161	1							
3	3949				1				
4	3780	1							
5	3650			1		1			
6	3525	1							
7	3174			1				1	1
8	3012				1	1			
9	2986	1							
10	2784								1
11	2712				1				
12	2642	1							
13	2529		1		1			1	
14	2464	1				1			
15	2379			1	1				
16	2219	1							
17	2069				1	1			
18	1980	1							
19	1831							1	1
20	1799	1							
21	1707				1	1	1		
22	1634		1	1					1
23	1620							1	
24	1421	1		1					
25	1360				1				1
26	1302					1		1	
27	1268				1		1		1
28	1257	1	1						
29	1193			1		1			
30	1152							1	
31	1113	1				1			
32	1074			1					1

contd…

Table 51 contd…

Band No.	Molecular weight (bp)	***Rhizobium* spp. isolates**							
		Rh 64	Rh 69	Rh 72	Rh 101	Rh 109	Rh 113	Rh 132	Rh BNF
Isolate No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
33	1028	1	1		1		1		
34	951		1						
35	886	1	1						
36	827						1	1	
37	812	1							
38	798			1	1	1	1		
39	764		1					1	1
40	706					1	1		
41	659			1	1				
42	619	1							
43	603							1	1
44	588				1	1	1		
45	568		1						
46	511	1	1	1	1				
47	464		1				1		1
48	448	1				1			
49	407	1							
50	367		1		1	1			
51	351						1		
52	345	1							
53	339			1				1	
54	300	1	1						1
55	297				1	1			
56	282						1		
57	263	1							
58	258							1	
59	241			1					
60	233		1						
61	223	1							
62	197		1	1					
63	187					1			
64	171	1			1		1		
65	162		1						
66	136	1			1	1			

1 = band present

genetic similarity among them. Similarly 2D PCO scatter plot was also helpful to make up the group of the isolates on the basis of presence of points on particular axis.

4.8.3 RAPD-PCR analysis of genomic DNA of different *Azospirillum* isolates

The genomic DNA of seven selected isolates of *Azospirillum lipoferum* alongwith MPKV strain, Asp-BNF were subjected to PCR amplification using twenty five random oligonucleotide primers (Table 52). The amplification profiles of 7 different isolates + 1 MPKV strain with 25 random bacterial primers are summarized in Table 53. It was observed that 1397 fragments were generated in all the 7 different isolates + 1 MPKV strain with 25 RAPD primers, out of which 820 fragments were polymorphic.

Amongst all the primers the maximum amplification was observed with RBA-15/20 and RBA-22 (42 and 41 polymorphic bands, respectively) primers, whereas least banding pattern was generated by RBA-9, RBA-2 and RBA-5 (21, 25 and 26 polymorphic bands, respectively). The primer RBA-20 showed maximum per cent polymorphism of 70%. These 25 primers produced isolate specific 577 unique bands.

All the 25 primers used generated scorable polymorphic bands for the isolates of *Azospirillum lipoferum* (Plate 30). All the primers showed the genetic polymorphism between the *A. lipoferum* isolates tested. However, RBA-4 was found to be the best primer for determination of variability among the *A. lipoferum* isolates. This primer generated 63 bands of different molecular weight (Table 54). Though some of the isolates share common bands most of the isolates differed in their banding pattern. None of the *A. lipoferum* isolate share all the bands and are therefore genetically variable. Though all the isolates of *A. lipoferum* were genetically variable, they share some common bands indicating phylogenetic relationship among them.

Table 52 RAPD analysis of genomic DNA of seven different isolates of *Azospirillum lipoferum*

Sr. No.	Details	
1	Total No. of primers used	25
2	No. of polymorphic primers	25
3	Total No. of bands	1397
4	Total No. of polymorphic bands	820
5	Total No. of unique bands	577
6	Percentage polymorphic	58.70

Table 53 Total number of RAPD markers and polymorphic bands produced by random primers in seven different isolates of *Azospirillum lipoferum*

Sr. No.	Random primers	No. of bands generated	Polymorphic bands	Monomorphic bands	Unique bands	% Polymorphic bands
1	RBA-1	50	30	-	20	60.00
2	RBA-2	38	25	-	13	65.78
3	RBA-3	49	33	-	16	67.34
4	RBA-4	63	33	-	30	52.38
5	RBA-5	40	26	-	14	65.00
6	RBA-6	49	33	-	16	67.34
7	RBA-7	52	31	-	21	59.61
8	RBA-8	57	33	-	24	57.89
9	RBA-9	40	21	-	19	52.50
10	RBA-10	60	35	-	25	58.33
11	RBA-11	61	38	-	23	62.29
12	RBA-12	52	31	-	21	59.61
13	RBA-13	67	34	-	33	50.74
14	RBA-14	75	40	-	35	53.33
15	RBA-15	63	42	-	21	66.66
16	RBA-16	65	34	-	31	52.30
17	RBA-17	57	30	-	27	52.63
18	RBA-18	57	33	-	24	57.89
19	RBA-19	54	29	-	25	53.70
20	RBA-20	60	42	-	18	70.00
21	RBA-21	64	36	-	28	56.25
22	RBA-22	64	41	-	23	64.06
23	RBA-23	51	32	-	19	62.74
24	RBA-24	52	28	-	24	53.84
25	RBA-25	57	30	-	27	52.63

Table 54 Determination of genetic variability in different isolates of *Azospirillum lipoferum* by DNA amplification profiles by RBA-4 primer

Band No.	Molecular weight (bp)	***Azospirillum lipoferum* isolates**							
		Asp 28	Asp 50	Asp 97	Asp 124	Asp 127	Asp 132	Asp 150	Asp BNF
Isolate No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
1	5586		1			1	1	1	1
2	5033	1							
3	4778			1					
4	4679							1	1
5	4583				1				
6	4086				1			1	
7	3961					1			
8	3606				1				1
9	3532					1			
10	3495						1	1	
11	3388		1						
12	3353			1					
13	3117				1				
14	2808	1							
15	2694				1	1	1		
16	2504				1				
17	2427		1	1		1	1	1	1
18	2304				1				
19	2210								1
20	2164					1	1		
21	2142		1						
22	2120							1	
23	2033		1	1	1				
24	1851				1		1	1	
25	1832			1					
26	1813		1						1
27	1757				1	1	1	1	
28	1703		1						
29	1633			1					
30	1600								1
31	1567	1			1			1	
32	1534		1			1	1		

contd…

Table 54 contd…

Band No.	Molecular weight (bp)	*Azospirillum lipoferum* isolates							
		Asp 28	Asp 50	Asp 97	Asp 124	Asp 127	Asp 132	Asp 150	Asp BNF
Isolate No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
33	1441		1			1	1	1	1
34	1397	1							
35	1354			1					
36	1326							1	1
37	1272				1				
38	1220				1			1	
39	1146					1			
40	1122				1				1
41	1111					1			
42	1033						1	1	
43	1011		1						
44	960			1					
45	940				1				
46	902	1							
47	892				1	1	1		
48	830				1				
49	821		1	1		1	1	1	1
50	779				1				
51	755								1
52	666					1	1		
53	646		1						
54	570							1	
55	552		1	1	1				
56	472				1		1	1	
57	430			1					
58	380		1						1
59	360				1	1	1	1	
60	338		1						
61	311			1					
62	269								1
63	203	1			1			1	

1 = band present

Dendrogram representing the genetic relationship among the isolates based on RAPD analysis was developed and presented in Figure 13, 14 and 15. On the basis of RAPD analysis, *Azospirillum lipoferum* isolates were classified into four broad clusters. The cluster I consisted two isolates i.e. Asp-28 and Asp-50 having genetic similarity coefficient 0.40. Likewise cluster II consisted of Asp-97 and Asp-124 isolate having the genetic similarity coefficient 0.23. The cluster III consisted of Asp-127 and Asp-132 isolate with similarity coefficient 0.53. The cluster IV consisted of Asp-150 isolate and Asp-BNF strain having genetic similarity coefficient 0.50.

3D PCO scatter plot clearly represents the phylogenetic relationship among different isolates of *Azospirillum lipoferum*. The height of the points differentiates the distinctness or genetic similarity among the isolates. For example Asp-28 isolate showed highest pick which represent distinctness of this isolate from other isolates, whereas Asp-127 and Asp-132 isolates pointing on a similar height showed genetic similarity among them. Similarly 2D PCO scatter plot was also helpful to make up the group of the isolates on the basis of presence of points on particular axis.

4.9 Molecular diversity of phosphate solubilizing microorganisms

The molecular diversity within each group of the selected phosphate solubilizing isolates along with the standard MPKV strain was analyzed by employing random amplified polymorphic DNA-polymerase chain reaction (RAPD-PCR) technique.

4.9.1 RAPD-PCR analysis of genomic DNA of different PSB (*Bacillus* isolates)

The genomic DNA of eight selected isolates of *Bacillus megaterium* alongwith MPKV strain, PSB-BNF were subjected to PCR amplification using twenty five random oligonucleotide primers (Table 55). The amplification profiles of 8 different isolates + 1 MPKV strain with 25 random bacterial primers are summarized in Table 56. It was

observed that 798 fragments were generated in all the 8 different isolates + 1 MPKV strain with 25 RAPD primers, out of which 600 fragments were polymorphic. Amongst all the primers the maximum amplification was observed with RBA-20, RBA-13 and RBA-25 (38, 34 and 33 polymorphic bands, respectively) primers whereas least banding pattern was generated by RBA-7 and RBA-4 (13 and 15 polymorphic bands respectively). The primer RBA-1 showed maximum per cent polymorphism of 100%. These 25 primers produced isolate specific 198 unique bands.

The fingerprint data of 25 random primers were converted into a binary matrix based on the presence or absence of individual amplified bands for each isolate. The RAPD bands of particular molecular weight present in an isolate were marked as “1” and absence was marked as “0”. The cluster analysis was performed by using unweighted pair group method and arithmetic average (UPGMA) to distinguish *Bacillus megaterium* isolates. The computer software NTSYS–PC was used for data analysis.

All the 25 primers used generated scorable polymorphic bands for the isolates of *Bacillus megaterium* (Plate 31). All the primers showed the genetic polymorphism between the *Bacillus megaterium* isolates tested. However, RBA-21 was found to be the best primer for determination of variability among the *B. megaterium* isolates. This primer generated 41 bands of different molecular weight (Table 57). Though some of the isolates share common bands most of the isolates differed in their banding pattern. Band 21, 23, 31 and 37 were only present in PSB-140 isolate, band 1 and 38 were only present in PSB-72 isolate, band 3 and 41 in PSB-41, band 4 in PSB-100 and band 29 in PSB-15 isolate, thereby indicating these are the genetically different isolates. None of the *Bacillus megaterium* isolate share all the bands and are therefore genetically variable. Though all the isolates of *B. megaterium* were genetically variable, they share some common bands indicating phylogenetic relationship among them.

Table 55 RAPD analysis of genomic DNA of eight different isolates of phosphate solubilizing bacteria (*Bacillus megaterium*)

Sr. No.	Details	
1	Total No. of primers used	25
2	No. of polymorphic primers	25
3	Total No. of bands	798
4	Total No. of polymorphic bands	600
5	Total No. of unique bands	198
6	Percentage polymorphic	75.19

Table 56 Total number of RAPD markers and polymorphic bands produced by random primers in eight different isolates of phosphate solubilizing bacteria (*Bacillus megaterium*)

Sr. No.	Random primers	No. of bands generated	Polymorphic bands	Monomorphic bands	Unique bands	% Polymorphic bands
1	RBA-1	18	18	-	-	100.00
2	RBA-2	21	18	-	03	85.71
3	RBA-3	20	17	-	03	85.00
4	RBA-4	19	15	-	04	78.94
5	RBA-5	27	23	-	04	85.18
6	RBA-6	28	23	-	05	82.14
7	RBA-7	23	13	-	10	56.52
8	RBA-8	32	28	-	04	87.50
9	RBA-9	40	32	-	08	80.00
10	RBA-10	26	23	-	03	88.46
11	RBA-11	29	22	-	07	75.86
12	RBA-12	24	16	-	08	66.66
13	RBA-13	44	34	-	10	77.27
14	RBA-14	40	26	-	14	65.00
15	RBA-15	33	23	-	10	69.69
16	RBA-16	36	27	-	09	75.00
17	RBA-17	32	23	-	09	71.87
18	RBA-18	37	25	-	12	67.56
19	RBA-19	46	30	-	16	65.21
20	RBA-20	52	38	-	14	73.07
21	RBA-21	41	31	-	10	75.60
22	RBA-22	19	17	-	02	89.47
23	RBA-23	33	25	-	08	75.75
24	RBA-24	34	20	-	14	58.82
25	RBA-25	44	33	-	11	75.00

Table 57 Determination of genetic variability in different isolates of *Bacillus megaterium* by DNA amplification profiles by RBA-21 primer

Band No.	Molecular weight (bp)	***Bacillus megaterium* isolates**								
		PSB 15	PSB 33	PSB 39	PSB 41	PSB 72	PSB 100	PSB 119	PSB 140	PSB BNF
Isolate No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	5698					1				
2	5053	1	1	1			1	1		
3	4481				1					
4	4219						1			
5	4348								1	1
6	3855								1	1
7	3973		1	1	1					
8	3630						1	1		
9	3419	1	1	1						
10	3031				1					1
11	2770						1	1	1	
12	2688	1	1	1		1				
13	2557						1	1		
14	2383	1	1	1						
15	2290					1	1	1	1	
16	2113	1	1	1						1
17	2051					1	1			
18	1800					1		1		
19	1612						1	1		
20	1549	1	1	1						
21	1346								1	
22	1206				1	1				
23	1113								1	
24	948						1	1		1
25	893	1	1	1						
26	884						1	1		1
27	824	1	1	1						
28	580				1	1				
29	768	1								
30	610								1	1
31	479								1	
32	400					1			1	
33	377						1	1		1
34	355	1	1	1		1				
35	338						1	1		
36	318	1	1	1						
37	290								1	
38	279					1				
39	250	1	1	1			1	1		1
40	228	1	1	1			1	1		1
41	190				1					

1 = band present

Dendrogram representing the genetic relationship among the isolates based on RAPD analysis was developed and presented in Figure 16, 17 and 18. On the basis of RAPD analysis, *Bacillus megaterium* isolates were classified into four broad groups. The group I further classified into two subgroups. The subgroup Ia consisted of single PSB-15 isolate having the genetic similarity coefficient 0.82.

The subgroup Ib consisted two isolates i.e. PSB-33 and PSB-39 having genetic similarity coefficient 0.87. The group II consisted of single PSB-140 isolate having distinct genetic similarity coefficient 0.29. Likewise group III was further classified into two subgroups on the basis of genetic similarity coefficient. The subgroup IIIa consisted of PSB-100 and PSB-119 isolate having similarity coefficient 0.76, whereas subgroup IIIb consisted of single PSB-BNF strain having the genetic similarity coefficient 0.65. The group IV consisted of PSB-41 and PSB-72 isolate having equal genetic similarity coefficient 0.59.

3D PCO scatter plot clearly represents the phylogenetic relationship among different isolates of *Bacillus megaterium*. The height of the points differentiates the distinctness or genetic similarity among the isolates. For example PSB-140 isolate showed highest pick which represent distinctness of this isolate from other isolates, whereas PSB-41 and PSB-72 isolates pointing on a similar height showed genetic similarity among them. Similarly 2D PCO scatter plot was also helpful to make up the group of the isolates on the basis of presence of points on particular axis.

4.9.2 RAPD-PCR analysis of genomic DNA of different PSF (*Aspergillus* isolates)

The genomic DNA of eight selected isolates of *Aspergillus awamori* alongwith MPKV strain, PSF-BNF were subjected to PCR amplification using twenty five random oligonucleotide primers (Table 58). The amplification profiles of 8 different isolates + 1 MPKV strain with 25 random fungal primers are summarized in Table 59. It was observed that 905 fragments were generated in all the 8 different isolates + 1 MPKV strain with 25 RAPD primers, out of which 656 fragments were polymorphic.

Table 58 RAPD analysis of genomic DNA of eight different isolates of phosphate solubilizing fungi (*Aspergillus awamori*)

Sr. No.	Details	
1	Total No. of primers used	25
2	No. of polymorphic primers	25
3	Total No. of bands	905
4	Total No. of polymorphic bands	656
5	Total No. of unique bands	249
6	Percentage polymorphic	72.49

Table 59 Total number of RAPD markers and polymorphic bands produced by random primers in eight different isolates of phosphate solubilizing fungi (*Aspergillus awamori*)

Sr. No.	Random primers	No. of bands generated	Polymorphic bands	Monomorphic bands	Unique bands	% Polymorphic bands
1	RFU-1	43	29	-	14	67.44
2	RFU-2	46	38	-	08	82.60
3	RFU-3	63	47	-	16	74.60
4	RFU-4	37	27	-	10	72.97
5	RFU-5	36	25	-	11	69.44
6	RFU-6	26	19	-	07	73.07
7	RFU-7	30	18	-	12	60.00
8	RFU-8	30	21	-	09	70.00
9	RFU-9	32	24	-	08	75.00
10	RFU-10	37	27	-	10	72.97
11	RFU-11	25	16	-	09	64.00
12	RFU-12	29	15	-	14	51.72
13	RFU-13	41	31	-	10	75.60
14	RFU-14	36	27	-	09	75.00
15	RFU-15	29	19	-	10	65.51
16	RFU-16	32	23	-	09	71.87
17	RFU-17	41	28	-	13	68.29
18	RFU-18	35	27	-	08	77.14
19	RFU-19	37	21	-	16	56.75
20	RFU-20	38	32	-	06	84.21
21	RFU-21	49	37	-	12	75.51
22	RFU-22	32	27	-	05	84.37
23	RFU-23	33	24	-	09	72.72
24	RFU-24	31	22	-	09	70.96
25	RFU-25	37	32	-	05	86.48

Amongst all the primers the maximum amplification was observed with RFU-3, RFU-2 and RFU-21 (47, 38 and 37 polymorphic bands, respectively) primers, whereas least banding pattern was generated by RFU-12 and RFU-11 (15 and 16 polymorphic bands respectively). The primer RFU-25 showed maximum per cent polymorphism of 86.48%. These 25 primers produced isolate specific 249 unique bands.

All the 25 primers used generated scorable polymorphic bands for the isolates of *Aspergillus awamori* (Plate 32). All the primers showed the genetic polymorphism between the *A. awamori* isolates tested. However, RFU-23 was found to be the best primer for determination of variability among the *A. awamori* isolates. This primer generated 33 bands of different molecular weight (Table 60). Though some of the isolates share common bands most of the isolates differed in their banding pattern. Band 10, 17, 23 and 28 were only present in PSF-71 isolate, band 3 and 25 were only present in PSF-28 isolate, band 8 and 20 in PSF-132 and band 26 in PSF-8 isolate, thereby indicating these are the genetically different isolates. None of the *A. awamori* isolate share all the bands and are therefore genetically variable. Though all the isolates of *A. awamori* were genetically variable, they share some common bands indicating phylogenetic relationship among them.

Dendrogram representing the genetic relationship among the isolates based on RAPD analysis was developed and presented in Figure 19, 20 and 21. On the basis of RAPD analysis, *Aspergillus awamori* isolates were classified into four broad groups. The group I consisted of two isolates i.e. PSF-8 and PSF-28 having genetic similarity coefficient 0.38. The group II further classified into three subgroups. The subgroup IIa consisted of PSF-55 and PSF-100 isolate having the genetic similarity coefficient 0.52. The subgroup IIb consisted of single PSF-71 isolates having genetic similarity coefficient 0.36.

Table 60 Determination of genetic variability in different isolates of *Aspergillus awamori* by DNA amplification profiles by RFU-23 primer

Band No.	Molecular weight (bp)	*Aspergillus awamori* isolates								
		PSF 8	PSF 28	PSF 55	PSF 64	PSF 71	PSF 100	PSF 115	PSF 132	PSF BNF
Isolate No.		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1	3132		1							1
2	2699		1							1
3	2005		1							
4	1619				1		1			
5	1574	1	1	1						
6	1560							1		1
7	1489				1		1			
8	1448								1	
9	1395		1	1						
10	1307					1				
11	1295							1		1
12	1259	1	1	1	1		1			
13	1191							1		1
14	1106				1		1		1	
15	1055	1	1	1						
16	998							1	1	
17	953					1				
18	910				1		1			
19	901	1	1	1					1	1
20	814								1	
21	755	1	1	1	1		1			
22	741							1		1
23	688					1				
24	651			1	1		1	1	1	1
25	627		1							
26	604	1								
27	346	1						1	1	1
28	306					1				
29	279	1							1	
30	247		1	1	1	1		1		1
31	200	1				1				
32	174				1		1	1		
33	166			1					1	1

1 = band present

The subgroup IIc consisted of single PSF-132 isolates having genetic similarity coefficient 0.30. The group III consisted of PSF-115 isolate and PSF-BNF strain having genetic similarity coefficient 0.59. The group IV consisted of single PSF-64 isolate having distinct genetic similarity coefficient 0.16.

3D PCO scatter plot clearly represents the phylogenetic relationship among different isolates of *Aspergillus awamori*. The height of the points differentiates the distinctness or genetic similarity among the isolates. For example PSF-8 isolate showed highest pick which represent distinctness of this isolate from other isolates, whereas PSF-BNF strain and PSF-115 isolate pointing on a similar height showed genetic similarity among them. Similarly 2D PCO scatter plot was also helpful to make up the group of the isolates on the basis of presence of points on particular axis.

4.9.3 RAPD-PCR analysis of genomic DNA of different PSF (*Penicillium* isolates)

The genomic DNA of five selected isolates of *Penicillium digitatum* alongwith MPKV strain, PSF-BNF were subjected to PCR amplification using twenty five random oligonucleotide primers (Table 61). The amplification profiles of 5 different isolates + 1 MPKV strain with 25 random fungal primers are summarized in Table 62. It was observed that 691 fragments were generated in all the 5 different isolates + 1 MPKV strain with 25 RAPD primers, out of which 453 fragments were polymorphic. Amongst all the primers the maximum amplification was observed with RFU-2, RFU-3 and RFU-8 (29, 28 and 26 polymorphic bands, respectively) primers, whereas least banding pattern was generated by RFU-12 and RFU-19 (6 and 7 polymorphic bands respectively). The primer RFU-15 showed maximum per cent polymorphism of 91.66%. These 25 primers produced isolate specific 236 unique bands.

Table 61 RAPD analysis of genomic DNA of five different isolates of phosphate solubilizing fungi (*Penicillium digitatum*)

Sr. No.	Details	
1	Total No. of primers used	25
2	No. of polymorphic primers	25
3	Total No. of bands	691
4	Total No. of polymorphic bands	453
5	Total No. of unique bands	236
6	Percentage polymorphic	65.56

Table 62 Total number of RAPD markers and polymorphic bands produced by random primers in five different isolates of phosphate solubilizing fungi (*Penicillium digitatum*)

Sr. No.	Random primers	No. of bands generated	Polymorphic bands	Monomorphic bands	Unique bands	% Polymorphic bands
1	RFU-1	32	19	-	13	59.37
2	RFU-2	37	29	-	08	78.38
3	RFU-3	38	28	-	10	73.68
4	RFU-4	20	13	-	07	65.00
5	RFU-5	42	22	-	20	52.38
6	RFU-6	36	18	-	18	50.00
7	RFU-7	22	18	01	03	81.81
8	RFU-8	33	26	-	07	78.78
9	RFU-9	33	21	-	12	63.63
10	RFU-10	23	15	-	08	65.21
11	RFU-11	26	16	-	10	61.53
12	RFU-12	14	6	-	08	42.85
13	RFU-13	35	24	-	11	68.57
14	RFU-14	29	16	-	13	55.17
15	RFU-15	12	11	-	01	91.66
16	RFU-16	29	22	-	07	75.86
17	RFU-17	27	18	01	08	66.66
18	RFU-18	31	13	-	18	41.93
19	RFU-19	23	7	-	16	30.43
20	RFU-20	29	20	-	09	68.96
21	RFU-21	25	22	-	03	88.00
22	RFU-22	23	16	-	07	69.56
23	RFU-23	31	22	-	09	70.96
24	RFU-24	19	12	-	07	63.15
25	RFU-25	22	19	-	03	86.36

All the 25 primers used generated scorable polymorphic bands for the isolates of *Penicillium digitatum* (Plate 33). All the primers showed the genetic polymorphism between the *P. digitatum* isolates tested. However, RFU-2 was found to be the best primer for determination of variability among the *P. digitatum* isolates. This primer generated 37 bands of different molecular weight (Table 63). Though some of the isolates share common bands most of the isolates differed in their banding pattern. Band 8, 13, 15, 19, 27 and 31 were only present in PSF-77 isolate whereas band 3 and 20 were only present in PSF-80 isolate, thereby indicating these are the genetically different isolates. None of the *P. digitatum* isolate share all the bands and are therefore genetically variable. Though all the isolates of *P. digitatum* were genetically variable, they share some common bands indicating phylogenetic relationship among them.

Dendrogram representing the genetic relationship among the isolates based on RAPD analysis was developed and presented in Figure 22, 23 and 24. On the basis of RAPD analysis, *Penicillium digitatum* isolates were classified into three broad groups. The group I consisted of two isolates i.e. PSF-61 and PSF-80 having genetic similarity coefficient 0.64. The group II consisted of single PSF-77 isolate having distinct genetic similarity coefficient 0.40. The group III further classified into two subgroups. The subgroup IIIa consisted of PSF-97 and PSF-101-1 isolate having the genetic similarity coefficient 0.83. The subgroup IIIb consisted of single PSF-BNF strain having genetic similarity coefficient 0.61.

3D PCO scatter plot clearly represents the phylogenetic relationship among different isolates of *Penicillium digitatum*. The height of the points differentiates the distinctness or genetic similarity among the isolates. For example PSF-BNF strain showed shortest pick which represent distinctness of this isolate from other isolates, whereas PSF-61 and PSF-80 isolates pointing on a similar height showed genetic similarity among them. Similarly 2D PCO scatter plot was also helpful to make up the group of the isolates on the basis of presence of points on particular axis.

Table 63 Determination of genetic variability in different isolates of *Penicillium digitatum* by DNA amplification profiles by RFU-2 primer

Band No.	Molecular weight (bp)	***Penicillium digitatum* isolates**					
		PSF 61	PSF 77	PSF 80	PSF 97	PSF 101-1	PSF BNF
Isolate No.		**1**	**2**	**3**	**4**	**5**	**6**
1	2350		1		1	1	
2	2191		1		1	1	
3	1938			1			
4	1807				1	1	1
5	1775		1	1			
6	1570		1		1	1	
7	1464				1	1	
8	1239		1				
9	1261			1	1	1	1
10	1145			1			1
11	1106		1		1	1	
12	1013				1	1	1
13	978		1				
14	936	1		1			
15	873		1				
16	858				1	1	1
17	821	1		1			
18	814				1	1	1
19	793		1				
20	739			1			
21	671	1		1	1	1	1
22	631		1		1	1	1
23	578	1		1			
24	539	1		1			
25	511		1		1	1	1
26	460				1	1	1
27	390		1				
28	370	1		1			
29	379				1	1	1
30	316	1		1			
31	307		1				
32	294				1	1	1
33	260	1		1			
34	232	1		1	1	1	1
35	185				1	1	1
36	177	1		1			
37	160	1		1			

1 = band present

4.10. Assessment of microbial diversity using diversity indices

The diversity analysis using different diversity indices *viz.*, Shannon's index, Evenness index and Richness index was carried out for 263 nitrogen fixing and 93 phosphate solubilizing isolates recovered from the rhizosphere soils of six different physiographic regions of Maharashtra.

4.10.1 Assessment of diversity of nitrogen fixing microorganisms

A bulk of 263 nitrogen fixers isolated from the rhizosphere soils of six different physiographic regions of Maharashtra consisted of four species. The frequency of occurrence of these four species *viz.*, *Azotobacter chroococcum, Rhizobium* spp., *Azospirillum lipoferum* and *Azospirillum brasilense* in different physiographic regions of Maharashtra is presented in Table 64. The data on the relative occurrence of different species in these physiographic regions indicated that the rhizosphere soils of different physiographic regions harboured all the four species of nitrogen fixers except Marathwada region which harboured only three species. *Azotobacter chroococcum* was the dominant species distributed in the rhizosphere soils of all the six physiographic regions followed by *Azospirillum lipoferum* and *Rhizobium* spp. whereas *Azospirillum brasilense* was observed in the rhizosphere soils of five physiographic regions.

The values of the Shannon's index (SI), Evenness index (EI) and Richness index (RI) of the nitrogen fixing isolates from different physiographic regions of Maharashtra are presented in Table 65. The diversity of 263 tentatively identified nitrogen fixing isolates in terms of Shannon's index ranged from 0.37 to 1.10. The maximum diversity of nitrogen fixing isolates was found in Marathwada region (SI of 1.10) followed by Vidarbha (1.09), Western Maharashtra (1.08), Western Ghats (1.04) and North Maharashtra (1.00) whereas it was the least in Western Konkan Coast (SI 0.37).

Table 64 Occurrence of different species and isolates of nitrogen fixers in different physiographic regions of Maharashtra

Sr. No	Physiographic regions	Species of nitrogen fixing microorganisms				Total species	Total isolates
		Azotobacter chroococcum	*Rhizobium* spp.	*Azospirillum lipoferum*	*Azospirillum brasilense*		
1	Western Konkan Coast	8	3	11	2	4	24
2	Western Ghats	19	9	22	1	4	51
3	Western Maharashtra	28	24	18	4	4	74
4	North Maharashtra	9	9	3	1	4	22
5	Marathwada	9	10	11	-	3	30
6	Vidarbha	21	21	18	2	4	62
	Total	**94**	**76**	**83**	**10**	**4**	**263**

Table 65 Shannon's index, evenness and richness index of the nitrogen fixing isolates in different physiographic regions of Maharashtra

Sr. No.	Physiographic regions	Shannon's index	Evenness index	Richness index
1	Western Konkan Coast	0.37	0.27	0.82
2	Western Ghats	1.04	0.75	0.56
3	Western Maharashtra	1.08	0.78	0.85
4	North Maharashtra	1.00	0.72	0.46
5	Marathwada	1.10	1.00	0.55
6	Vidarbha	1.09	0.79	0.51

Table 66 Shannon's index, richness index and evenness index of the pooled data of all the physiographic regions of Maharashtra for nitrogen fixing isolates

Sr. No.	Species and diversity indices	Values
1	Total species	4
2	Shannon's index	1.09
3	Richness index	0.25
4	Evenness index	0.79

The evenness index for the 263 nitrogen fixing isolates ranged from 0.27 to 1.00. The highest evenness index of 1.00 was observed for Marathwada region followed by Vidarbha (0.79), Western Maharashtra (0.78), Western Ghats (0.75) and North Maharashtra (0.72), while the lowest evenness index of 0.27 was recorded for the Western Konkan Coast. The Richness index for the individual physiographic regions ranged from 0.46 to 0.85 for nitrogen fixing isolates. Among the different physiographic regions, Western Maharashtra showed maximum richness index (0.85), while North Maharashtra showed least richness index (0.46). The Shannon's index had a very strong positive correlation (r = +0.867) with the evenness index, while it had a strong negative correlation with the richness index (r = -0.553). The evenness index and the richness index in turn, also showed a strong negative correlation with each other.

The Shannon's index, richness index and evenness index were also calculated for the pooled data. The values of Shannon's index, richness index and evenness index of the pooled data of all the physiographic regions of Maharashtra obtained for the nitrogen fixing isolates were 1.09, 0.25 and 0.79 respectively (Table 66).

4.10.2 Assessment of diversity of Phosphate solubilizing microorganisms

A bulk of 93 phosphate solubilizers isolated from the rhizosphere soils of six different physiographic regions of Maharashtra consisted of three species. The frequency of occurrence of these three species *viz.*, *Bacillus megaterium, Aspergillus awamori* and *Penicillium digitatum* in different physiographic regions of Maharashtra is presented in Table 67. The data on the relative occurrence of different species in these physiographic regions indicated that the rhizosphere soils of different physiographic regions harboured all the three species of phosphate solubilizers except Western Konkan Coast region which harboured only two species. *Bacillus megaterium* was the dominant species distributed in the rhizosphere soils of all the six physiographic regions followed by *Aspergillus awamori* whereas *Penicillium digitatum* was observed in the rhizosphere soils of five physiographic regions.

Table 67 Occurrence of different species and isolates of phosphate solubilizers in different physiographic regions of Maharashtra

Sr. No	Physiographic regions	Species of phosphate solubilizing microorganisms			Total species	Total isolates
		Bacillus megaterium	*Aspergillus awamori*	*Penicillium digitatum*		
1	Western Konkan Coast	6	5	-	2	11
2	Western Ghats	7	5	1	3	13
3	Western Maharashtra	14	4	7	3	25
4	North Maharashtra	3	4	1	3	8
5	Marathwada	6	3	3	3	12
6	Vidarbha	11	8	5	3	24
	Total	**47**	**29**	**17**	**3**	**93**

Table 68 Shannon's index, evenness and richness index of the phosphate solubilizing isolates in different physiographic regions of Maharashtra

Sr. No.	Physiographic regions	Shannon's index	Evenness index	Richness index
1	Western Konkan Coast	0.33	0.48	0.58
2	Western Ghats	0.90	0.82	0.83
3	Western Maharashtra	0.97	0.88	0.60
4	North Maharashtra	0.97	0.88	0.87
5	Marathwada	1.04	0.95	1.06
6	Vidarbha	1.05	0.96	0.61

Table 69 Shannon's index, richness index and evenness index of the pooled data of all the physiographic regions of Maharashtra for phosphate solubilizing isolates

Sr. No.	Species and diversity indices	Values
1	Total species	3
2	Shannon's index	1.02
3	Richness index	0.31
4	Evenness index	0.93

The values of the Shannon's index (SI), Evenness index (EI) and Richness index (RI) of the phosphate solubilizing isolates from different physiographic regions of Maharashtra are presented in Table 68. The diversity of 93 tentatively identified phosphate solubilizing isolates in terms of Shannon's index ranged from 0.33 to 1.05. The maximum diversity of phosphate solubilizing isolates was found in Vidarbha region (SI of 1.05) followed by Marathwada (1.04), Western Maharashtra (0.97), North Maharashtra (0.97) and Western Ghats (0.90), whereas it was the least in Western Konkan Coast (SI 0.33).

The evenness index for the 93 phosphate solubilizing isolates ranged from 0.48 to 0.96. The highest evenness index of 0.96 was observed for Vidarbha region followed by Marathwada (0.95), Western Maharashtra (0.88), North Maharashtra (0.88) and Western Ghats (0.82), while the lowest evenness index of 0.48 could be recorded for the Western Konkan Coast. The Richness index for the individual physiographic regions ranged from 0.58 to 1.06 for phosphate solubilizing isolates. Among the different physiographic regions, Marathwada showed maximum richness index (1.06), while Western Konkan Coast showed least richness index (0.58). The Shannon's index had a very strong positive correlation (r = +0.746) with the evenness index, while it had a strong negative correlation with the richness index (r = -0.419). The evenness index and the richness index in turn, also showed a strong negative correlation with each other.

The Shannon's index, richness index and evenness index were also calculated for the pooled data. The values of Shannon's index, richness index and evenness index of the pooled data of all the physiographic regions of Maharashtra obtained for the phosphate solubilizing isolates were 1.02, 0.31 and 0.93 respectively (Table 69).

On the basis of diversity analysis, the map of beneficial microbes of the soils of Maharashtra State has been prepared representing the districtwise distribution of efficient nitrogen fixing and phosphate solubilizing isolates and presented in Figure 25.

5. DISCUSSION

Microbial diversity studies are important in understanding the microbial ecology in soil and functioning of ecosystems. Microorganisms present in the soil play an important role in nutrient solubilization, mobilization and recycling. The soil microorganisms that enhance nutrient availability to plants include nitrogen fixers and phosphate solubilizers which have been used to develop biofertilizers.

The biological nitrogen fixation is one of the most important microbial activity as it makes the recycling of atmospheric nitrogen on earth possible and gives a fundamental contribution to nitrogen homeostasis in the biosphere (Aquilanti *et al.*, 2004). Among the various diazotrophs, the ecological distribution of *Azotobacter* spp. is related to diverse factors determining the presence or absence of this bacterium in specific soil. It has been shown that the soil characteristics, land use and climatic conditions affect the distribution of this microorganism (Dobereiner and Pedrosa, 1988). The free living rhizobial strains isolated from rhizosphere soils of legume crops are able to fix atmospheric nitrogen as scored by acetylene reduction while growing in the absence of a plant host (Bedmar and Olivares, 1979). *Azospirillum* is also considered to be the most important rhizobacterial genus for improving plant growth under a variety of environmental and soil conditions (Bashan *et al.*, 2004). *Azospirillum* exhibit acetylene reduction assay (ARA) under micro-aerophilic condition (Kim *et al.*, 2005).

Soil microbes play a key role for solubilization of inorganic phosphate to release phosphorus which is available for plant growth and development (Rodriguez and Fraga, 1999). High proportion of these phosphate solubilizing microorganisms (PSM)

are concentrated in the rhizosphere of plants (Vesquez *et al.*, 2000). Many soil fungi and bacteria are known to solubilize inorganic phosphates (Illmer and Schinner, 1992).

The study of molecular diversity by PCR based RAPD analysis facilitates identification and placement of the strains in genetically distinct and related groups (Lee and Henry, 2001).

With this background the efforts were made to study the variability among the nitrogen fixing and phosphate solubilizing isolates recovered from the rhizosphere soils of different physiographic regions of Maharashtra regarding morphological, cultural, biochemical and physiological characterization as well as beneficial functions and genetic variation. The results obtained are discussed in length hereunder.

5.1 Isolation of beneficial microorganisms from soil

One hundred fifty rhizosphere soil samples collected from different physiographic regions of Maharashtra were screened for the presence of nitrogen fixing and phosphate solubilizing microorganisms on a variety of culture media. A total of 263 nitrogen fixing and 93 phosphate solubilizing microorganisms were isolated. Among the nitrogen fixers, 94 *Azotobacter* isolates, 76 *Rhizobium* isolates and 93 *Azospirillum* isolates were obtained. Among the phosphate solubilizers, 47 phosphate solubilizing bacteria and 46 phosphate solubilizing fungi were isolated.

Several research workers obtained these beneficial microbes from rhizosphere soils of different geographic regions. Subramaney and Abraham (1962) isolated *Azotobacter chroococcum* strains from red loamy soils of India. Soberon-Chavez and Najera (1989) isolated *Rhizobium* strains from soil of two different geographical origins in Mexico. Naz *et al.* (2009) isolated *Rhizobium* sp. from the rhizosphere

soils of four different localities of Khewra (Pakistan). Han and New (1998) isolated 285 *Azospirillum* strains from soils from seven geographic regions of New South Wales, Australia by using nitrogen-free semisolid malate (NFB) medium. Ilyas *et al.* (2008) isolated *Azospirillum lipoferum* strains from the rhizosphere soils of maize grown under arid, semiarid and irrigated areas of Pakistan. He also isolated *Rhizobium* strains from the rhizosphere soils of same agro-ecological regions using cango-red yeast extract mannitol agar (CRYEMA) medium. Ilyas and Bano (2010) isolated *Azospirillum brasilense* strains from the rhizosphere soils of wheat grown under different physiographic regions of Attok, Kallar and Islamabad (Pakistan).

Nahas *et al.* (1994) isolated 31 phosphate solubilizing bacteria and 11 phosphate solubilizing fungi from 13 different soil types from various regions of the Sao Paulo State, Brazil. Kundu *et al.* (2002) isolated about 73 isolates of PSB from the rhizosphere of cowpea, maize, sorghum, cotton and pearlmillet from farms of CCSHAU, Hisar. Chen *et al.* (2006) isolated 36 strains of PSB isolates from the subtropical soils of Central Taiwan. Deepa *et al.* (2010) isolated 42 PSF strains from paddy soils of southern peninsular region of Tamil Nadu, India. Shiva Reddy *et al.* (2010) isolated phosphate solubilizing bacterial strains of *Bacillus megaterium* from soils of different physiographic zones of Karnataka.

5.2 Population dynamics study of microorganisms

A wide variation in microbial population among the different nitrogen fixing isolates was observed. Among the nitrogen fixers, highest microbial population (10.17×10^4 CFU g^{-1} soil) was recorded by of *Azotobacter* isolate obtained from Kolhapur district of Western Maharashtra followed by *Rhzobium* isolate (8.33×10^4) from Latur

district of Marathwada region and *Azospirillum* isolate ($5.40x10^4$ MPN g^{-1} soil) from Kolhapur district of Western Maharashtra.

The microbial population varied significantly among the different phosphate solubilizing isolates. The highest microbial population ($2.83x10^4$ CFU g^{-1} soil) was recorded by PSB isolates obtained from rhizosphere soils of Latur district of Marathwada region followed by PSF isolates ($2.33x10^4$) obtained from Beed and Solapur district of Marathwada and Western Maharashtra region, respectively.

Suliasih and Widawati (2005) isolated nitrogen fixing bacteria *viz.*, *Azotobacter chroococcum, Rhizobium* sp. and *Azospirillum* sp. from the soil samples of Wamena Biological Garden which is one of the mountain range-biota ex-citu conservation at Eastern part of Indonesia and estimated microbial population which were ranged from $5.0x10^3$ to $1.5x10^7$ CFU g^{-1} soil. Rao and Venkateswarlu (1985) reported that the most probable number of *Azospirillum* varied among different root zones of pearl millet with maximum in the rhizosphere ($3.46x10^4$ g^{-1} soil).

The highest microbial population of PSB ($7.5x10^6$ CFU g^{-1} soil) was reported by Suliasih and Widawati (2005) obtained from the soil samples of Wamena Biological Garden. Seshadri and Lakshminarasimhan (2007) studied the population dynamics of P-solubilizers in the rhizosphere of major weed species from a tropical delta soil of India. The population ranged from 0 to $74x10^3$ CFU g^{-1} soil.

5.3 Morphological characterization

A total of 263 nitrogen fixing and 93 phosphate solubilizing isolates were studied for their morphological traits. There were considerable variations among the isolates in cell morphology. The

cells of all *Azotobacter* isolates were motile and gram negative in reaction. Out of 94 *Azotobacter* isolates, cells of 48 isolates were rod shape and 46 isolates having oval in shape. The cell size varied among the isolates and it was in the range of 1.1 to 4.4 x 2.0 to 11.8 µm. On the basis of cell arrangement, four distinct groups of isolates were formed. Fifty *Azotobacter* isolates were single celled, 26 isolates having cells in pairs, 14 isolates having cell in chain and cells of 4 isolates formed irregular clumps.

The cells of all *Rhizobium* isolates were rod shaped, motile and gram negative in reaction. A wide variation was noticed in cell size among the different *Rhizobium* isolates and it was in the range of 0.4 to 2.8 x 0.7 to 10.0 µm.

The cells of all *Azospirillum* isolates were gram negative in reaction. On the basis of cell motility, two distinct groups of isolates were formed. The cells of 83 *Azospirillum* isolates were non-motile whereas 10 isolate having motile cells. The cell shape differed among the various *Azospirillum* isolates. Out of 93 *Azospirillum* isolates, cells of 44 isolates were helicle/S-shape, 35 isolates having vibrioid shape and 14 isolates were curved rods. The great variation in cell size among the isolates was recorded and it was in the range of 1.0 to 2.8 x 3.3 to 24.8 µm.

The cells of all phosphate solubilizing bacterial isolates were rod shaped, motile and gram positive in reaction. A wide variation was noticed in cell size among the different PSB isolates and it was in the range of 0.4 to 3.1 x 1.8 to 11.0 µm. On the basis of cell arrangement, three distinct groups of PSB isolates were formed. Twenty eight PSB isolates were single celled, 10 isolates having cells in chains and cells of 9 isolates were single as well as in pairs.

On the basis of microscopic appearance, three distinct groups of phosphate solubilizing fungal (*Aspergillus*) isolates were formed. The first group comprised of 11 *Aspergillus* isolates consisting of hyphae-aseptate, conidiophore-septate, red coloured, globose vesicle, conidia-red and round, second group of 10 isolates having hyphae-colorless, conidiophores branching, septate, globose vesicle, conidia-small, red and round and 8 isolates included in third group comprised of hyphae-septate, colorless, conidiophore-septate, elliptical vesicle, conidia-small, dark brown-black and round.

Based on the microscopic examination, three distinct groups of phosphate solubilizing fungal (*Penicillium*) isolates were formed. The first group comprised of 8 *Penicillium* isolates consisting of branched conidiophores that are borne on a single rope of fertile hyphae, second group of 4 isolates having branching conidiophores that are borne on aerial mycelia and 5 isolates included in third group comprised of branching conidiophores arise from a septate mycelium.

Several research workers (Beijerinck, 1901; Jensen and Petersen, 1954; Johnstone, 1974; Apte and Shende, 1981 and Tchan and New, 1986) reported that the cells of *Azotobacter chroococcum* were oval to rod shaped measuring 1.5 to 3 x 0.5 to 6 µm and they occurred singly, or in pairs of irregular clumps and rarely in chains of more than 4 cells, motile with peritrichous or polar flagella. Further, the *Rhizobium* cells were reported as rod shaped measuring 0.5 to 0.9 x 1.2 to 3.0 µm, motile by one polar flagella (Jordan, 1986).

Tarrand *et al.* (1978) reported that in semi-solid nitrogen-free malate (Nfb) medium, *Azospirillum lipoferum* grows as elongated cells (1.4-1.7 µm x 5 to over 30 µm long) which are non-motile and have an S-shaped or helical form, in contrast to *Azospirillum*

brasilense which retain mainly the enlarged vibriod forms and motile cells.

Bacillus was reported as rods having round or squared ends and may be small 0.5 x 12 µm or large 2.5 x 10 µm, motile by peritrichous flagella (Claus and Berkeley, 1986). Furthermore, Mittal *et al.* (2008) characterized PSF strains up to species level based on colonial morphology, spore characteristics and microscopic examination and identified as *Aspergillus awamori* and *Penicillium digitatum*.

5.4 Cultural characterization

A total of 263 nitrogen fixing and 93 phosphate solubilizing isolates were further studied for their colony characters. A wide variation in colony characters of *Azotobacter* isolates was observed on Jensen's agar medium. Out of 94 *Azotobacter* isolates, 84 isolates were found to be having circular colonies with entire margin, whereas 10 isolates having irregular colonies with lobate margin. The elevation of the colonies varied from flat, raised to convex and the colonies appeared to be slimy, milky, viscid, mucoid, glistening and opaque and later aged cultures produced light brown to dark brown pigmentation.

The colonies of all *Rhizobium* isolates were circular with entire margin. The elevation of the colonies varied among the isolates. Out of 76 *Rhizobium* isolates, the colonies of 44 isolate having raised elevation whereas 32 isolate having convex elevation. The colonies appeared to be white-opaque, mucilaginous, semi-translucent, translucent and milky to watery translucent.

The colonies of all *Azospirillum* isolates formed subsurface pellicles on semi-solid N-free malate medium. Out of 93 *Azospirillum* isolates, the colonies of 50 isolates formed 1-2 mm

sized thin pellicles whereas 43 isolates formed 3-4 mm sized thick pellicles. On BMS agar medium, the round as well as irregular colonies with entire, serrate, lobate and undulate margins and raised, convex and umbonate elevations were observed among the different *Azospirillum* isolates. All isolates produced pink pigmentation on BMS agar medium with opaque in their optical characteristics.

Fourty seven PSB isolates formed circular colonies with entire as well as lobate margins, the elevation varied from flat, raised to convex and the colonies appeared to be creamy as well as whitish on Pikovskaya's agar. On nutrient agar medium, all PSB isolates formed circular colonies with raised as well as convex elevations and colonies appeared to be moderately dull, glossy or slightly rugose on the medium and later aged cultures produced pink, yellow to brown pigmentation.

On the basis of colonial morphology, four distinct groups of *Aspergillus* isolates were formed. The first group comprised of eight *Aspergillus* isolates producing white floccose mycelium spreading rapidly and formed black coloured spores, second group of seven isolates producing velvety green mycelium becoming almost black, five isolates included in third group produced white cottony, loose woven thread like mycelium forming brown-black spores in due course and nine isolates involved in fourth group produced white colony, thread like mycelium forming brown-black spores in due course.

Based on colonial morphology, six distinct groups of *Penicillium* isolates were formed. Out of 18 *Penicillium* isolates, three isolates from each group produced the different mycelial forms *viz.*, velvet, velutinous, fasciculate and cotton-wool followed by spore colour varied from dark-green, yellow, orange and blue-green.

Similar colony characters were observed by several research workers which are narrated as under.

Mishustin and Shilnikova (1972) reported that 3 to 4 days old *Azotobacter* colonies appear as flat, soft, milky and mucoid on agar plates. Tchan and New (1986) observed that colonies of *Azotobacter chroococcum* were opaque, entire, low convex, viscid, glistening and smooth on nitrogen free glucose medium. The aged cultures of *Azotobacter chroococcum* formed an insoluble black-brown pigment on Jensen's or Ashby's medium (Beijerinck, 1901; Jensen and Petersen, 1954; Norris and Chapman, 1968 and Johnstone, 1974), commonly attributed to the presence of melanin.

Cheruku (2004) and Adiguzel *et al.* (2010) reported that the pure cultures of *Rhizobium* produced circular, convex, entire colonies which had semi translucent mucilagenous appearance and had slow growing rate on YEMA medium.

On BMS agar after 1-2 weeks of incubation at 33-35^0 C, colonies of *Azospirillum* were pink, opaque, irregular or round, often wrinkled, and typically have umbonate elevations (Tarrand *et al.*, 1978 and Tejera *et al.*, 2005).

On nutrient agar, the strains of *Bacillus megaterium* showed growth heaped and nonspreading, glossy or moderately dull, sometimes slightly rugose; on aging some shade of yellow to pink; on long incubation, growth and medium may become brown or black (Claus and Berkeley, 1986).

Mittal *et al.* (2008) reported that strains of *Aspergillus* awamori showed rapidly growing brownish black colonies with slightly radially furrowed usually white sometimes yellow basal mycelium and strains of *Penicillium digitatum* showed colonies with restricted growth, blue-green, reverse bright yellow with the same pigmentation diffusing into agar.

5.5 Biochemical and physiological characterization

The biochemical characterization indicated that all the *Azotobacter* isolates were positive for starch hydrolysis, H_2S production, catalase test and oxidase test, but were negative for gelatin liquefaction. In the physiological characterization, it was noticed that all the isolates were able to utilize mannitol, glucose, sucrose and fructose as sole carbon source for growth except malate and citrate which showed negative result.

All the *Rhizobium* isolates were positive for starch hydrolysis, H_2S production, catalase test and oxidase test, but were negative for gelatin liquefaction. Mannitol, glucose, sucrose and arabinose were used as a sole carbon source for growth by all the isolates, whereas malate and citrate showed negative result.

All the *Azospirillum* isolates were positive for catalase test and oxidase test, but were negative for starch hydrolysis, H_2S production and gelatin liquefaction. Out of 93 *Azospirillum* isolates, 83 isolates utilized glucose and malic acid as a sole carbon source and positive for biotin requirement whereas 10 isolates utilized only malic acid as a sole carbon source and showed negative result for glucose and biotin requirement.

Taking into account the morphological, cultural and biochemical characteristics, ability of the isolates to utilize various carbon sources for their growth (Tchan and New, 1986; Jordan, 1986 and Tarrand *et al.*, 1978), out of 263 nitrogen fixing isolates, 94 isolates were tentatively identified as *Azotobacter chroococcum*, 76 isolates as *Rhizobium* spp., 83 isolates as *Azospirillum lipoferum* and 10 isolates as *Azospirillum brasilense*.

All the 47 PSB isolates were positive for starch hydrolysis, gelatin liquefaction and catalase test, but were negative for H_2S production, KOH and oxidase test. Fructose, glucose and maltose

were used as a sole carbon source for growth by all the isolates whereas mannitol and citrate showed negative result. Based on the morphological, cultural, biochemical and physiological characterization (Claus and Berkeley, 1986), 47 PSB isolates were tentatively identified as *Bacillus megaterium*.

Based on colonial morphology, spore characteristics and microscopic examination (Mittal *et al.*, 2008), out of 46 PSF isolates, 29 PSF isolates were tentatively identified as *Aspergillus awamori* and 17 PSF isolates were tentatively identified as *Penicillium digitatum*.

5.6 Functional diversity of microorganisms

The functional diversity of the isolates with respect to beneficial functions like nitrogenase activity and phosphate solubilizing ability was studied and the results are discussed hereunder.

5.6.1 Nitrogenase activity of the isolates

The significant variation in nitrogenase activity among the 94 *Azotobacter* isolates tested (5.3 to 291.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}) was noticed. The nitrogenase activity of all isolates was compared with standard MPKV strain. The isolate Azt-21 recorded highest nitrogenase activity (291.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}) followed by Azt-70, 64, 148, 82, 50, 97, 142, 129, 25, 135 and 08 isolates with nitrogenase activity of 259.4, 236.7, 217.3, 205.6, 194.2, 185.1, 170.8, 163.3, 158.4, 155.9 and 151.1 nmol C_2H_4.mg protein^{-1}.hr^{-1}, respectively. The MPKV strain Azt-BNF had the nitrogenase activity of 149.4 nmol C_2H_4.mg protein^{-1}.hr^{-1}.

These results are in agreement with the results of Tejera *et al.* (2005) who determined the nitrogenase activity of *Azotobacter chroococcum* isolated from the sugarcane rhizosphere which was in

the range of 79.6 to 329.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}. The present results are in conformity with the reports of earlier workers (Gajendiran and Mahadevan, 1989 and Bhatia *et al.*, 2009).

There was a wide variation in nitrogenase activity among the 76 free living *Rhizobium* isolates tested (1.9 to 119.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}). The nitrogenase activity of all isolates was compared with standard MPKV strain. The isolate Rh-69 recorded highest nitrogenase activity (119.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}) followed by Rh-113, 72, 132, 109, 64 and 101 isolates with nitrogenase activity of 98.3, 91.8, 83.1, 80.6, 74.5 and 73.1 nmol C_2H_4.mg protein^{-1}.hr^{-1}, respectively. The MPKV strain Rh-BNF had the nitrogenase activity of 71.3 nmol C_2H_4.mg protein^{-1}.hr^{-1}.

Similar results were reported by Kaneshiro *et al.* (1978) who determined nitrogenase activity among 69 free living rhizobial strains grown on a defined CS7 medium by acetylene reduction assay (ARA). Out of 69 strains, 32 *Rhizobium japonicum* strains expressed nitrogenase activity in the range of 2 to 45 nmol C_2H_4.mg protein^{-1}.hr^{-1} and 37 unclassified strains showed the nitrogenase activity in the range of 1 to 40 nmol C_2H_4.mg protein^{-1}.hr^{-1}. Several other research workers (Pagan *et al.*, 1975; Bedmar and Olivares, 1979; Kaneshiro *et al.*, 1983 and Ramaswamy and Bal, 1987) have also reported the nitrogenase activity of free living *Rhizobium* isolates by ARA.

A distinct variation in nitrogenase activity among the 93 *Azospirillum* isolates tested (7.5 to 394.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}) was observed. The nitrogenase activity of all isolates was compared with standard MPKV strain. Among 83 *Azospirillum lipoferum* isolates, Asp-97 recorded highest nitrogenase activity (394.7 nmol C_2H_4.mg protein^{-1}. hr^{-1}) followed by Asp-28, 50, 150, 132, 124 and 127 isolates with nitrogenase activity of 311.2, 287.1, 269.8, 256.4, 248.5 and

241.3 nmol C_2H_4.mg protein^{-1}.hr^{-1}, respectively. The standard MPKV strain Asp-BNF had the nitrogenase activity of 239.6 nmol C_2H_4.mg protein^{-1}.hr^{-1}. Among 10 *Azospirillum brasilense* isolates, Asp-75 recorded highest nitrogenase activity (39.3 nmol C_2H_4.mg protein^{-1} .hr^{-1}). The isolates of *Azospirillum lipoferum* exhibited a higher average nitrogenase activity compared to *Azospirillum brasilense* (83.2 compared with 20.1 nmol C_2H_4.mg protein^{-1}. hr^{-1}).

Similar results were reported by Malik *et al.* (1997) who determined the nitrogenase activity of *Azospirillum lipoferum* and *Azospirillum brasilense* strains by acetylene reduction assay (ARA) and reported highest nitrogenase activity of *A. lipoferum* strains (686 nmol C_2H_4.mg protein^{-1}.hr^{-1}) followed by *A. brasilense* strains (215 nmol C_2H_4.mg protein^{-1}. hr^{-1}). Han and New (1998) also reported nitrogenase activity among 285 *Azospirillum* strains isolated from soils from seven geographic regions of New South Wales, Australia. There was wide variation in nitrogenase (acetylene reduction) activity among the 285 different isolates (0.0-154.9 nmol C_2H_4.mg protein^{-1}.hr^{-1}), with differences between regions in average activities of isolates (12.5-79.9 nmol C_2H_4.mg protein^{-1} .hr^{-1}). The isolates of *Azospirillum lipoferum* exhibited a higher average nitrogenase activity compared to *Azospirillum brasilense* (67.6 compared with 39.2 nmol C_2H_4.mg protein^{-1}. hr^{-1}). The results obtained in the present investigation are comparable to those of earlier workers (Li and Hung, 1987; Gajendiran and Mahadevan, 1989; Kim *et al.*, 2005 and Tejera *et al.*, 2005).

5.6.2 Phosphate solubilizing ability of the isolates

All the 47 *Bacillus* isolates were able to solubilize tricalcium phosphate (TCP) on Pikovskaya's agar medium and the isolates displayed wide variations in the diameter of the zone of TCP solubilization (5-14 mm). Similar observations have been made

earlier with *Bacillus* strains by Chaudhari *et al.* (2010) and Qian *et al.* (2010).

These 47 isolates were further subjected to quantification of the Pi released in Pikovskaya's broth containing TCP. All the isolates showed TCP solubilization but differed significantly with respect to the amount of Pi released (2.06 to 52.38%) at 10 days after inoculation. The P-solubilizing ability of all isolates was compared with standard MPKV strain. The isolate PSB-15 recorded highest P-solubilization (52.38%) followed by PSB-41, 100, 72, 33, 119, 39 and 140 isolates with the release of 39.74, 29.72, 26.14, 23.40, 22.56, 22.03 and 20.24 % Pi, respectively. The MPKV strain PSB-BNF had the P-solubilizing ability of 19.29%.

Similar results were reported by Oliveira *et al.* (2008) who determined phosphate solubilizing efficiency of 45 strains isolated from rhizosphere soil of maize grown in Brazil in a modified Pikovskaya's liquid medium containing TCP. Strains B17 and B5, identified as *Bacillus* sp. and *Burkholderia* sp., respectively, were the most effective, solubilizing 67% and 58.5% of the total P ($Ca_3(PO_4)_2$) after 10 days. The present results are comparable to those of earlier workers (Bardiya and Gaur, 1972; Arora and Gaur, 1979; Wani *et al.*, 1979; Yin, 1988; Chen *et al.*, 2006; and Tao *et al.*, 2008).

In addition to the quantitative estimation of Pi released from TCP, the decrease in pH of the broth was also monitored so as to correlate the amount of the acidity produced with the amount of Pi released. At 10 days of incubation, the isolate PSB-15 brought down pH of the medium to 3.48 from the initially adjusted pH of 7.0. Many other isolates also recorded reduced pH of the medium indicating acid production. The decrease in pH of the medium had highly significant positive correlation ($r = + 0.781^{**}$) with the amount

of Pi released indicating that the production of acidity by the isolates favoured the process of P solubilization. Alam *et al.* (2002) also reported decrease in pH of the medium ranged from 7 to 3.2 during TCP solubilization by 10 phosphate solubilizing bacterial isolates due to the production of organic acids. Chen *et al.* (2006) reported that among ten *Bacillus megaterium* isolates, drop in pH was in the range of 5.1 to 6.0 from an initial pH 7.0 of the medium. Panhwar *et al.* (2009) reported positive correlation between considerable amount of P solubilized with decrease in pH of the medium.

Out of 46 phosphate solubilizing fungal isolates, 29 *Aspergillus* isolates and 17 *Penicillium* isolates were able to solubilize tricalcium phosphate (TCP) on Pikovskaya's agar medium and the isolates displayed wide variations in the diameter of the zone of TCP solubilization (11-30 mm and 9-24 mm, respectively). Similar observations have been recorded by earlier workers with *Aspergillus* and *Penicillium* strains (Vazquez *et al.*, 2000; Pradhan and Sukla, 2005; Rajankar *et al.*, 2007; Mittal *et al.*, 2008 and Chaudhari *et al.*, 2010).

Twenty nine *Aspergillus* isolates were further subjected to quantification of the Pi released in Pikovskaya's broth containing TCP. All the isolates showed TCP solubilization but differed significantly with respect to the amount of Pi released (45.80 to 87.17%) at 10 days after inoculation. The P-solubilizing ability of all isolates was compared with standard MPKV strain. The isolate PSF-71 recorded highest P-solubilization (87.17%) followed by PSF-28, 55, 100, 64, 08, 115 and 132 isolates with the release of 75.04, 73.99, 69.77, 66.09, 65.03, 63.98 and 61.87 % Pi, respectively. The MPKV strain PSF-BNF(A) had the P-solubilizing ability of 60.82%.

Similarly 17 *Penicillium* isolates were further subjected to quantification of the Pi released in Pikovskaya's broth containing

TCP. All the isolates showed TCP solubilization but differed significantly with respect to the amount of Pi released (26.30 to 69.77%) at 10 days after inoculation. The P-solubilizing ability of all isolates was compared with standard MPKV strain. The isolate PSF-61 recorded highest P-solubilization (69.77%) followed by PSF-77, 97, 80 and 101-1 isolates with the release of 53.44, 50.54, 50.28 and 49.22 % Pi, respectively. The MPKV strain PSF-BNF(P) had the P-solubilizing ability of 48.17%.

The isolates of *Aspergillus awamori* and *Penicillium digitatum* exhibited a higher average P-solubilizing ability compared to *Bacillus megaterium* (57.40 and 44.25 compared with 13.23 % Pi released) in TCP broth.

Rajankar *et al.* (2007) also reported that *Aspergillius* spp., and *Penicillium* spp. has the more solubilizing ability of inorganic insoluble phosphate than bacteria, *viz.*, *B.subtilis*, and *B.megatherium*. Chaudhari *et al.* (2010) reported that fungal isolates (*Aspergillus awamori, A. niger* and *A. fumigatus*) solubilize more phosphate (47.4 to 61.0 %) as compared to bacterial isolates (*Bacillus* strain-1 and *Bacillus* strain-2) (9.0 to 18.2 %).

The present results are in agreement with the reports of Oliveira *et al.* (2008) who reported phosphate solubilizing efficiency of forty-five strains isolated from rhizosphere soil of maize grown in Brazil. Among the 45 strains, *Penicillium citrinum* and *Aspergillus terreus* were the most efficient solubilizing 44% and 42% of total P in TCP broth, respectively. Similar results were reported by earlier workers (Arora and Gaur, 1979; Wani *et al.*, 1979; Venkateswarlu *et al.*, 1984; Yadav and Singh, 1991; Whitelaw *et al.*, 1999; Vazquez *et al.*, 2000; Mittal *et al.*, 2008; Chakraborty *et al.*, 2010 and Yadav *et al.*, 2011).

In addition to the quantitative estimation of Pi released from TCP, the decrease in pH of the broth was also monitored so as to correlate the amount of the acidity produced with the amount of Pi released. At 10 days of incubation, the *Aspergillus* isolate PSF-71 brought down pH of the medium to 3.01 from the initially adjusted pH of 7.0. Many other isolates also recorded reduced pH of the medium indicating acid production. The decrease in pH of the medium had highly significant positive correlation (r = + 0.905**) with the amount of Pi released indicating that the production of acidity by the isolates favoured the process of P solubilization. Similarly, the *Penicillium* isolate PSF-61 brought down pH of the medium to 3.13 from the initially adjusted pH of 7.0. Many other isolates also recorded reduced pH of the medium indicating acid production. The decrease in pH of the medium had highly significant positive correlation (r = + 0.833**) with the amount of Pi released indicating that the production of acidity by the isolates favoured the process of P solubilization. Vazquez *et al.* (2000) also reported decrease in pH of the medium ranged from 6.5 to 3.0 during TCP solubilization by phosphate solubilizing fungal isolates due to the production of organic acids. Similar results were reported by earlier workers (Yadav and Singh, 1991; Nahas, 1996; Whitelaw *et al.*, 1999; Alam *et al.*, 2002 and Pradhan and Sukla, 2005).

The studies also indicated lot of variation amongst the nitrogen fixing and phosphate solubilizing isolates with respect to their morphological, cultural, biochemical and physiological characteristics as well as functional properties. Some selected isolates were further subjected to molecular diversity analysis.

5.7 Distribution of efficient N_2 fixers and P solubilizers across the regions

The weather parameters and cropping system had no singnificant influence on distribution of efficient N_2 fixing and P solubilizing isolates across the physiographic regions of Maharashtra. However, the population of these isolates varied significantly with cropping system across the regions. The maximum population of *Azotobacter, Rhizobium* and *Azospirillum* isolates (10.17, 8.33 and 5.40 $x10^4$ CFU g^{-1} soil, respectively) was recorded in paddy-wheat, pigeonpea-wheat/pigeonpea-chickpea and paddy/nachni cropping system in Kolhapur and Latur district of Western Ghats and Marathwada, respectively. Similarly, the maximum population of PSB and PSF isolates (2.83 and 2.33 $x10^4$ CFU g^{-1} soil) was recorded in pigeonpea-wheat/pigeonpea-chickpea, pigeonpea-safflower and green gram-sorghum/green gram-safflower cropping systems of Latur, Beed and Solapur district of Marathwada and Western Maharashtra, respectively.

Similar results were reported by Venkateswarlu *et al.* (1997) who correlated native rhizobial populations (*Bradyrhizobium* sp.) in 88 soil samples from 13 important legume growing locations with mean annual rainfall, soil pH, organic carbon, clay content and crop history. The populations showed significant relationship with organic carbon and previous crop history but clay content, mean annual rainfall and soil pH had no influence. The population, however, increased significantly following crop cultivation. The increase was more with pigeonpea and sorghum than with sunflower. When pigeonpea, groundnut and sorghum were grown as preceding crops, the population remained high (> log10 3.0 MPN/g soil). The overall results revealed that crop related factors have more critical influence on the abundance of native rhizobial populations than soil or climatic factors.

Narayan and Kehri (2008) also studied the population dynamics of *Azotobacter chroococcum* in wheat and paddy fields with different degrees of agricultural intensification in plains of Northern India. The results showed that irrespective of the degree of intensification, the highest population of *A. chroococcum* (1.52 to 3.15 x 10^4 g^{-1} oven dry soil) was recorded in the wheat fields with lowest degrees of intensification.

5.8 Molecular diversity of nitrogen fixing microorganisms

The diversity analysis as well as fingerprinting of an individual can be achieved by using PCR based RAPD markers. Ilyas *et al.* (2008) assessed the genetic diversity among the *Rhizobium leguminosarum* and *Azospirillum lipoferum* isolates by RAPD-PCR analysis. Tulajappa *et al.* (2008) estimated molecular diversity of ten *Azotobacter chroococcum* strains isolated from different physiographic zones of Karnataka, India by using RAPD-PCR technique. On the basis of this information, the highly efficient nitrogen fixing isolates *viz.*, 12 *Azotobacter* isolates, 7 *Rhizobium* isolates and 7 *Azospirillum* isolates alongwith standard MPKV strain were analyzed for their RAPD profiles using twenty five random primers obtained from M/s. Bangalore GeNei Pvt. Ltd., Bangalore, India.

5.8.1 RAPD-PCR analysis of genomic DNA of different *Azotobacter* isolates

The PCR amplified products of the 12 selected isolates of *Azotobacter* along with standard MPKV strain Azt-BNF with respect to each of the 25 random primers showed 1123 polymorphic bands. The number of bands varied from 43 (primer RBA-17) to 75 (primer RBA-16). The average percent polymorphism was 75.12% per primer. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.40 and minimum genetic similarity of 0.15. The highest genetic similarity of 0.40 could be noted between the isolates Azt-08 and Azt-25. The primer RBA-24 was found to be the best

primer for determination of variability among the *Azotobacter chroococcum* isolates. This primer generated 54 bands of different molecular weight of which 16 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Azotobacter* isolates for use as passport data.

On the basis of UPGMA clustering analysis, *Azotobacter chroococcum* isolates were grouped into four clusters. The first cluster consisted of three isolates *viz.*, Azt-8, Azt-21 and Azt-25 which were recovered from Sindhudurg, Raigad and Ratnagiri district of Konkan region. The cluster I further classified into two sub-clusters. The first sub-cluster was formed by two isolates i.e. Azt-8 and Azt-25 having genetic similarity coefficient 0.40. The second sub-cluster consisted of single Azt-21 isolate having the genetic similarity coefficient 0.24. The isolate Azt-21 exhibited significantly highest nitrogenase activity than the other isolates tested. The cluster II consisted of two isolates i.e. Azt-50 and Azt-64 which were obtained from Nandurbar and Ahmednagar district of North Maharashtra region. Both the isolates having genetic similarity coefficient 0.25 and showed higher nitrogenase activity than the isolates from Konkan region except Azt-21. Likewise cluster III was further classified into three sub-clusters on the basis of genetic similarity coefficient. The sub-cluster IIIa consisted of single Azt-70 isolate recovered from Kolhapur district having the genetic similarity coefficient 0.21. The sub-cluster IIIb consisted of Azt-82 and Azt-97 isolate obtained from Beed and Parbhani district of Marathwada region having similarity coefficient 0.35. Similarly sub-cluster IIIc consisted of single Azt-129 isolate obtained from Yeotmal district with similarity coefficient 0.18. The isolate Azt-129 showed minimum nitrogenase activity as compared to isolates from Marathwada region. The cluster IV consisted of three isolates *viz.*, Azt-135, Azt-142 and Azt-148 and one standard MPKV strain Azt-BNF of which three isolates were recovered from

Chandrapur, Gadchiroli and Bhandara districts of Vidarbha region. The cluster IV further classified into three sub-clusters. The sub-cluster IVa consisted of single Azt-135 isolate with similarity coefficient 0.17, whereas sub-cluster IVb consisted of Azt-142 and Azt-148 isolate having equal genetic similarity coefficient 0.24. The isolates, Azt-135, Azt-142 and Azt-148 exhibited moderate nitrogenase activity. Similarly sub-cluster IVc consisted of single Azt-BNF strain having distinct genetic similarity coefficient 0.15.

2D PCO scatter plot analysis represents two distinct groups of *Azotobacter* isolates on the basis of presence of points on particular axis which were correlated with the grouping done on the basis of cell shape. The cells of seven isolates *viz.*, Azt-8, 21, 25, 50, 64, 129 and Azt-BNF were rod shaped, whereas six isolates *viz.*, Azt-70, 82, 97, 135, 142 and 148 were oval in shape.

The RAPD-PCR based molecular diversity analysis revealed that twelve *Azotobacter chroococcum* isolates with higher nitrogenase activity than the standard MPKV strain Azt-BNF were clustered into four separate clusters. Variations observed could be due to domestication of the isolates in different agro-ecological niches as well as their nitrogenase activity.

Similar results were reported by Tulajappa *et al.* (2008) who estimated molecular diversity of ten *Azotobacter chroococcum* strains isolated from different agro-climatic zones of Karnataka, India by using ten selected RAPD primers and observed them to be distributed in two clusters. Bhatia *et al.* (2008) also used genetic fingerprinting to study the diversity among *Azotobacter* spp. isolated from four different cotton-wheat cropping regions of India. On the basis of acetylene reduction, out of 76 isolates, 20 efficient *Azotobacter* spp were further selected for molecular studies and based on RAPD-PCR clustered into four separate clusters.

5.8.2 RAPD-PCR analysis of genomic DNA of different *Rhizobium* isolates

The amplification profiles of the 7 selected isolates of *Rhizobium* along with standard MPKV strain Rh-BNF with respect to each of the 25 random primers showed 905 polymorphic bands. The number of bands varied from 33 (primer RBA-9) to 84 (primer RBA-16). The average percent polymorphism was 56.95% per primer. The dendrogram constructed showed three clusters with the maximum genetic similarity of 0.27 and minimum genetic similarity of 0.22. The highest genetic similarity of 0.27 could be noted between the isolates Rh-64 and Rh-69. The primer RBA-7 was found to be the best primer for determination of variability among the *Rhizobium* spp. isolates. This primer generated 66 bands of different molecular weight of which 28 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Rhizobium* isolates for use as passport data.

On the basis of UPGMA clustering analysis, *Rhizobium* isolates were classified into three broad clusters. The cluster I further classified into two sub-clusters. The sub-cluster Ia consisted two isolates i.e. Rh-64 and Rh-69 which were recovered from Ahmednagar and Sangli district of Western Maharashtra region having genetic similarity coefficient 0.27. Both of the isolates showed maximum growth on glucose as a sole carbon source. The isolate, Rh-69 exhibited significantly highest nitrogenase activity than the other isolates tested. The sub-cluster Ib consisted of single Rh-72 isolate obtained from Latur district of Marathwada region having the genetic similarity coefficient 0.24. Likewise cluster II was further classified into two sub-clusters on the basis of genetic similarity coefficient. The sub-cluster IIa consisted of Rh-101 and Rh-109 isolates recovered from Akola and Nasik district having the genetic similarity coefficient 0.26, whereas sub-cluster IIb consisted of single Rh-113 isolate obtained from Solapur district having similarity coefficient 0.25. The

cluster III consisted of Rh-132 isolate recovered from Chandrapur district and Rh-BNF strain having genetic similarity coefficient 0.22.

2D PCO scatter plot analysis represents three distinct groups of *Rhizobium* isolates on the basis of presence of points on particular axis which were correlated with the grouping done on the basis of colony characteristics. The colonies of three isolates *viz.*, Rh-64, 101 and 109 appeared to be semi-translucent, whereas the colonies of two isolates (Rh-113 and Rh-132) and MPKV strain Rh-BNF were white opaque in appearance and the two isolates *viz.*, Rh-69 and Rh-72 were translucent in their colony appearance.

Sikora and Redzepovic (2003) characterized eighteen *Bradyrhizobium japonicum* isolates as well as reference strains genotypically by RAPD fingerprinting. The dendrogram derived from RAPD profiles showed that all *B. japonicum* strains could be divided into two major clusters. Similar results were reported by earlier workers with *Rhizobium* strains (Young and Cheng, 1998; Pinto *et al.*, 2004; El-Fiki, 2006; Ilyas *et al.*, 2008; Naz *et al.*, 2009 and Rajasundari *et al.*, 2009).

5.8.3 RAPD-PCR analysis of genomic DNA of different *Azospirillum* isolate

The amplified products of the 7 selected isolates of *Azospirillum* along with standard MPKV strain Asp-BNF with respect to each of the 25 random primers showed 820 polymorphic bands. The number of bands varied from 38 (primer RBA-2) to 75 (primer RBA-14). The average percent polymorphism was 58.70% per primer. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.53 and minimum genetic similarity of 0.23. The highest genetic similarity of 0.53 could be noted between the isolates Asp-127 and Asp-132. The primer RBA-4 was found to be the best primer for determination of variability among the *Azospirillum lipoferum* isolates. This primer generated 63 bands of different molecular weight of which 30 were unique and isolate specific in the

present study. This primer can be used for fingerprinting of *Azospirillum* isolates for use as passport data.

On the basis of UPGMA clustering analysis, *Azospirillum lipoferum* isolates were classified into four broad clusters. The cluster I consisted two isolates i.e. Asp-28 and Asp-50 which were recovered from Kolhapur and Nandurbar district having genetic similarity coefficient 0.40. Likewise cluster II consisted of Asp-97 and Asp-124 isolate obtained from Parbhani and Wardha district having the genetic similarity coefficient 0.23. The isolate, Asp-97 exhibited significantly highest nitrogenase activity than the other isolates tested. The cluster III consisted of Asp-127 and Asp-132 isolate recovered from Nanded and Chandrapur district with similarity coefficient 0.53. The cluster IV consisted of Asp-150 isolate obtained from Nagpur district and Asp-BNF strain having genetic similarity coefficient 0.50. The results indicated that the isolates within each cluster were closely related to each other, whereas isolates in different clusters had greater genetic distance from each other.

2D PCO scatter plot analysis represents four distinct groups of *Azospirillum* isolates on the basis of presence of points on particular axis which were correlated with the grouping done on the basis of cell shape. The cells of four isolates *viz.*, Asp-50, 124, 132, 150 and Asp-BNF strain were helical shaped, whereas cells of Asp-28, 97 and 127 isolates were spiral, elongated 'S' shaped and curved rods, respectively. Rest of the cell morphology and colony characters differed among the isolates.

Ilyas and Bano (2010) determined the genetic biodiversity and polymorphism among the isolated *Azospirillum* strains by the RAPD-PCR technique. On the basis of UPGMA cluster analysis of *Azospirillum* strains isolated from rhizosphere soil of wheat plants, the isolates were grouped into two clusters. Similar observations have

been recorded by earlier workers with *Azospirillum* strains (Ilyas *et al.*, 2008 and Kanimozhi and Panneerselvam, 2010).

5.9 Molecular diversity of phosphate solubilizing microorganisms

The diversity analysis as well as fingerprinting of an individual can be achieved by using PCR based RAPD markers. Mittal *et al.* (2008) assessed the genetic diversity among six phosphate-solubilizing fungi (two strains of *Aspergillus awamori*, and four of *Penicillium citrinum*) isolated from rhizosphere of various crops located around Chandigarh, India by RAPD technique. Shiva Reddy *et al.* (2010) carried out the molecular characterization of ten phosphate solubilizing bacterial strains of *Bacillus megaterium* isolated from soils of different physiographic zones of Karnataka by the RAPD-PCR technique. On the basis of this information, the highly efficient phosphate solubilizing isolates *viz.*, eight *Bacillus* isolates, eight *Aspergillus* isolates and five *Penicillium* isolates alongwith standard MPKV strain were further analyzed for their RAPD profiles using twenty five random primers obtained from M/s. Bangalore GeNei Pvt. Ltd., Bangalore, India.

5.9.1 RAPD-PCR analysis of genomic DNA of different PSB (*Bacillus* isolates)

The PCR amplified products of the 8 selected isolates of *Bacillus* along with standard MPKV strain PSB-BNF with respect to each of the 25 random primers showed 600 polymorphic bands. The number of bands varied from 18 (primer RBA-1) to 52 (primer RBA-20). The average percent polymorphism was 75.19% per primer. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.87 and minimum genetic similarity of 0.29. The highest genetic similarity of 0.87 could be noted between the isolates PSB-33 and PSB-39. The primer RBA-21 was found to be the best primer for determination of variability among the *Bacillus megaterium*

isolates. This primer generated 41 bands of different molecular weight of which 10 were unique and isolate specific in the present study. This primer can be used for fingerprinting of PSB isolates for use as passport data.

On the basis of UPGMA clustering analysis, *Bacillus megaterium* isolates were classified into four clusters. The first cluster consisted of three isolates *viz.*, PSB-15, PSB-33 and PSB-39 which were recovered from Western Ghat region of Nasik, Kolhapur and Pune district. The cluster I further classified into two sub-clusters. The sub-cluster Ia consisted of single PSB-15 isolate having the genetic similarity coefficient 0.82. The isolate, PSB-15 exhibited significantly highest P-solubilization than the other isolates tested. The sub-cluster Ib consisted two isolates i.e. PSB-33 and PSB-39 having genetic similarity coefficient 0.87. The cluster II consisted of single PSB-140 isolate obtained from Nagpur district of Vidarbha region having distinct genetic similarity coefficient 0.29. Likewise cluster III was further classified into two sub-clusters on the basis of genetic similarity coefficient. The sub-cluster IIIa consisted of PSB-100 and PSB-119 isolates recovered from Solapur and Jalgaon district having genetic similarity coefficient 0.76. The sub-cluster IIIb consisted of single PSB-BNF strain having the dice coefficient 0.65. The cluster IV consisted of PSB-41 and PSB-72 isolates recovered from Thane and Latur district having equal genetic similarity coefficient 0.59.

2D PCO scatter plot analysis represents three distinct groups of *Bacillus megaterium* isolates on the basis of presence of points on particular axis which were correlated with the grouping done on the basis of cell arrangement. The cells of three isolates *viz.*, PSB-15, 33 and 39 were in chains, whereas two isolates (PSB-41 and 72) were single celled and the cells of three isolates (PSB- 100, 119 and 140) alongwith PSB_BNF strain were single as well as in chains.

The RAPD-PCR based molecular diversity analysis revealed that eight *Bacillus megaterium* isolates with higher P solubilizing ability than the standard MPKV strain PSB-BNF were clustered into four separate clusters. Variations observed could be due to domestication of the isolates in different agro-ecological niches as well as their P solubilizing ability.

Similar results were reported by Chen *et al*. (2006) who used the RAPD technique to elucidate the polymorphism among the 36 strains of phosphate solubilizing bacteria (PSB) isolated from Central Taiwan and formed four distinct clusters. Shiva Reddy *et al*. (2010) also carried out the molecular characterization of ten PSB strains of *Bacillus megaterium* isolated from soils of different physiographic zones of Karnataka by the RAPD-PCR technique. The dendrogram has clearly depicted that all the ten *B. megaterium* isolates formed two major clusters.

5.9.2 RAPD-PCR analysis of genomic DNA of different PSF (*Aspergillus* isolates)

The amplification profiles of the 8 selected isolates of *Aspergillus* along with standard MPKV strain PSF-BNF(A) with respect to each of the 25 random primers showed 656 polymorphic bands. The number of bands varied from 25 (primer RFU-11) to 63 (primer RFU-3). The average percent polymorphism was 72.49% per primer. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.59 and minimum genetic similarity of 0.16. The highest genetic similarity of 0.59 could be noted between PSF-115 isolate and PSF-BNF strain. The primer RFU-23 was found to be the best primer for determination of variability among the *Aspergillus awamori* isolates. This primer generated 33 bands of different molecular weight of which 9 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Aspergillus* isolates for use as passport data.

On the basis of UPGMA clustering analysis, *Aspergillus awamori* isolates were classified into four broad clusters. The cluster I consisted of two isolates i.e. PSF-8 and PSF-28 which were recovered from Western Ghat region of Sindhudurg and Kolhapur district having genetic similarity coefficient 0.38. The cluster II further classified into three sub-clusters. The sub-cluster IIa consisted of PSF-55 and PSF-100 isolate obtained from scarcity zone of Latur and Solapur district having the genetic similarity coefficient 0.52. The sub-cluster IIb consisted of single PSF-71 isolates recovered from Kolhapur district of Western Maharashtra having dice coefficient 0.36. The isolate, PSF-71 exhibited significantly highest P solubilization than the other isolates tested. The sub-cluster IIc consisted of single PSF-132 isolates obtained from Chandrapur district of Vidarbha region having dice coefficient 0.30. The cluster III consisted of PSF-115 isolate recovered from Nasik district and PSF-BNF strain having genetic similarity coefficient 0.59. The cluster IV consisted of single PSF-64 isolate obtained from Ahmednagar district having distinct genetic similarity coefficient 0.16.

2D PCO scatter plot analysis represents three distinct groups of *Aspergillus awamori* isolates on the basis of presence of points on particular axis which were correlated with the grouping done on the basis of colony morphology. The colonies of three isolates *viz.*, PSF-8, 28 and 55 were white cottony, loose woven thread like, forming brown-black spores in due course, whereas the isolates, PSF-64, 71 and 100 formed white floccose mycelium spreading rapidly, quickly and producing black coloured spores. The velvety, dark green colonies fromed by the isolates, PSF-115, 132 and PSF-BNF(A) strain.

The RAPD-PCR based molecular diversity analysis revealed that eight *Aspergillus awamori* isolates with higher P solubilizing ability than the standard MPKV strain PSF-BNF(A) were clustered into four separate clusters. Variations observed could be due to domestication

of the isolates in different agro-ecological niches as well as their P solubilizing ability.

5.9.3 RAPD-PCR analysis of genomic DNA of different PSF (*Penicillium* isolates)

The PCR amplified products the 5 selected isolates of *Penicillium* along with standard MPKV strain PSF-BNF(P) with respect to each of the 25 random primers showed 453 polymorphic bands. The number of bands varied from 12 (primer RFU-15) to 42 (primer RFU-5). The average percent polymorphism was 65.56% per primer. The dendrogram constructed showed three clusters with the maximum genetic similarity of 0.83 and minimum genetic similarity of 0.40. The highest genetic similarity of 0.83 could be noted between the isolates PSF-97 and PSF-101-1. The primer RFU-2 was found to be the best primer for determination of variability among the *Penicillium digitatum* isolates. This primer generated 37 bands of different molecular weight of which 8 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Penicillium* isolates for use as passport data.

On the basis of UPGMA clustering analysis, *Penicillium digitatum* isolates were classified into three broad clusters. The cluster I consisted of two isolates i.e. PSF-61 and PSF-80 which were recovered from Solapur and Ahmednagar district of Western Maharashtra having genetic similarity coefficient 0.64. The isolate PSF-61 exhibited significantly highest P solubilization than the other isolates tested. The cluster II consisted of single PSF-77 isolate obtained from Satara district having distinct dice coefficient 0.40. The cluster III further classified into two sub-clusters. The sub-cluster IIIa consisted of PSF-97 and PSF-101-1 isolates recovered from Parbhani and Akola district having the genetic similarity coefficient 0.83. The sub-cluster IIIb consisted of single PSF-BNF strain having dice coefficient 0.61.

2D PCO scatter plot analysis represents three distinct groups of *Penicillium digitatum* isolates on the basis of presence of points on particular axis which were correlated with the grouping done on the basis of colony morphology. The colonies of two isolates *viz.*, PSF-61 and 80 were dark green, forming blue-green spores in due course, whereas the isolate, PSF-77 formed cotton-wool colony, forming clear to orange spores. The velutinous mycelium spreading rapidly and producing yellow-green spores formed by the isolates, PSF-97, 101-1 and PSF-BNF(P) strain.

The RAPD-PCR based molecular diversity analysis revealed that five *Penicillium digitatum* isolates with higher P solubilizing ability than the standard MPKV strain PSF-BNF(P) were clustered into three separate clusters. Variations observed could be due to domestication of the isolates in different agro-ecological niches as well as their P solubilizing ability.

Similar results were reported by Mittal *et al.* (2008) who assessed the genetic diversity among six phosphate-solubilizing fungi (two strains of *Aspergillus awamori*, and four of *Penicillium citrinum*) isolated from rhizosphere of various crops located around Chandigarh, India by RAPD technique. The RAPD analysis of the genomic DNA of six PSF revealed that all the isolates were different from one another. Chakraborty *et al.* (2010) also analyzed molecular diversity of ten P-solubilizing fungi isolated from soil samples collected from forest, river basin, agricultural fields and rhizosphere of plantation crops of North Bengal by RAPD analysis. UPGMA cluster analysis divided the ten isolates into two groups with the genetic similarity ranging from 0.35- 0.61.

5.10 Assessment of microbial diversity using diversity indices

Measuring biodiversity of a habitat or community has prime importance in ecology and conservation biology, because of its academic necessity and utility in devising conservation strategies. A variety of indices are available to quantify the diversity of biological communities. The most popular and widely used measures are Shannon's, Simpson's, Fischer's alpha-log series (Magurron, 1988), and Avalanche index (Ganeshaiah *et al.,* 1997). Majority of these techniques are used to study the diversity of plants and animals, but they have also been used for studying the microbial diversity. Such type of diversity analysis using diversity indices was carried out for microfungal populations, actinomycets (Okonski *et al.,* 2003), mycorrhizae (Beena *et al.,* 2000) and for bacteria from soils (Torsvik *et al.,* 1990).

Similar attempts were made in the present study to analyze diversity of 263 nitrogen fixing isolates and 93 phosphate solubilizing isolates recovered from the rhizosphere soils of six different physiographic regions of Maharashtra.

5.10.1 Assessment of diversity of nitrogen fixing microorganisms

The **Shannon's index** (SI) of the pooled data was 1.09 suggesting high diversity of nitrogen fixing isolates in six different physiographic regions of Maharashtra. The maximum diversity of nitrogen fixing isolates was found in Marathwada region (SI of 1.10) followed by Vidarbha (1.09), Western Maharashtra (1.08), Western Ghats (1.04) and North Maharashtra (1.00) whereas it was the least in Western Konkan Coast (SI 0.37). This indicated that the individual physiographic regions are quite variable with respect to nitrogen fixing isolates. Such variations could be credited to the differences in the soil types, plant species, root exudates, etc. in

different physiographic regions, which influence differentially the microflora under the region floor (Virginia, 1986). It is reported that the organic carbon derived from root exudates of different plant species exerts differential influence on soil microflora (Somani and Bhandari, 1989).

Richness index of the pooled data was 0.25 indicating that the nitrogen fixing isolates are not distributed equally in the soils of different physiographic regions of Maharashtra. The richness index for the individual physiographic regions ranged from 0.46 to 0.85. Among the different physiographic regions, Western Maharashtra showed maximum richness index (0.85) for nitrogen fixers indicating richness of nitrogen fixing isolates in this region. **An evenness index** of 0.79 for pooled data indicated the dominance of one species over the others. Among the different physiographic regions, Marathwada showed maximum evenness index (1.00) indicating more even distribution of nitrogen fixing isolates in this region. However, the Western Konkan Coast recorded an evenness index of 0.27 indicating dominance of a single species over others. Only *Azospirillum lipoferum* was the dominant species distributed among the nitrogen fixers in western konkan coast.

5.10.2 Assessment of diversity of phosphate solubilizing microorganisms

The **Shannon's index** (SI) of the pooled data was 1.02 suggesting high diversity of phosphate solubilizing isolates in six different physiographic regions of Maharashtra. The maximum diversity of phosphate solubilizing isolates was found in Vidarbha region (SI of 1.05) followed by Marathwada (1.04), Western Maharashtra (0.97), North Maharashtra (0.97) and Western Ghats (0.90) whereas it was the least in Western Konkan Coast (SI 0.33).

This indicated that the individual physiographic regions are quite variable with respect to phosphate solubilizing isolates. Such variations could be credited to the differences in the soil types, plant species, root exudates, etc. in different physiographic regions, which influence differentially the microflora under the region floor (Virginia, 1986). It is reported that the organic carbon derived from root exudates of different crop species exerts differential influence on soil microflora (Somani and Bhandari, 1989).

Richness index of the pooled data was 0.31 indicating that the phosphate solubilizing isolates are not distributed equally in the soils of different physiographic regions of Maharashtra. The richness index for the individual physiographic regions ranged from 0.58 to 1.06. Among the different physiographic regions, Marathwada showed maximum richness index (1.06) for phosphate solubilizers indicating richness of phosphate solubilizing isolates in this region. **An evenness index** of 0.93 for pooled data indicates the dominance of one species over the others. Among the physiographic regions, Vidarbha showed maximum evenness index (0.96) indicating more even distribution of phosphate solubilizing isolates in this region. However, the Western Konkan Coast recorded least evenness index of 0.48 indicating dominance of a single species over others. Only *Bacillus megaterium* was the dominant species distributed among the phosphate solubilizers in western konkan coast.

The results obtained on diversity of 263 nitrogen fixing and 93 phosphate solubilizing isolates recovered from six different physiographic regions of Maharashtra were comparable to those of Megha *et al.* (2007) who analyzed the diversity of 52 fluorescent pseudomonads from different forest soils of Western Ghats and

reported the existence of substantial diversity among the isolates in different forest soils.

Thus, it was evident from the study that a great diversity exists among the nitrogen fixing and phosphate solubilizing isolates recovered from the rhizosphere soils of various physiographic regions of Maharashtra with respect to morphological, cultural, biochemical and physiological characterization as well as beneficial functions and genetic makeup. The population dynamics study revealed that crop related factors have more critical influence on the abundance of native microbial populations than soil or climatic factors. Furthermore, diversity analysis study indicated the richness of nitrogen fixing isolates in Sangli, Kolhapur, Pune, Solapur and Ahmednagar district of Western Maharashtra and phosphate solubilizing isolates in Latur, Beed and Parbhani district of Marathwada, whereas the maximum diversity of nitrogen fixing and phosphate solubilizing isolates was found in Marathwada and Vidarbha region, respectively. The highly efficient nitrogen fixing and phosphate solubilizing isolates compared to standard MPKV strains however, need to be exposed to field conditions to study their performance. Therefore, there is a need to conserve this treasure of diversity for the future exploitation.

6. SUMMARY AND CONCLUSION

A total of 263 nitrogen fixing and 93 phosphate solubilizing isolates were recovered during the year 2009 from the rhizosphere soils of various physiographic regions of Maharashtra *viz.*, Western Konkan Coast, Western Ghats (*Sahyadris*), Western Maharashtra, North Maharashtra, Marathwada and Vidarbha. These isolates were further characterized and tentatively identified up to species level based on morphological, cultural, biochemical and physiological characterization. Their functional diversity with respect to beneficial traits like nitrogen fixation and phosphate solubilization was also analyzed. Based on the *in vitro* analysis, the molecular diversity of selected isolates within each group was also studied. The results obtained are summarized below.

- A total of 263 nitrogen fixing and 93 phosphate solubilizing microorganisms were isolated from the rhizosphere soils of different physiographic regions of Maharashtra. Among the nitrogen fixers, 94 *Azotobacter*, 76 *Rhizobium* and 93 *Azospirillum* isolates were obtained. Among the phosphate solubilizers, 47 phosphate solubilizing bacteria and 46 phosphate solubilizing fungi were isolated.
- A wide variation in microbial population among the different isolates of *Azotobacter* was observed. The significantly highest *Azotobacter* population (10.17×10^4 CFU g^{-1} soil) was recorded by Azt-70 isolate obtained from Kolhapur district of Western Maharashtra distributed at 16°16'5.24" North latitude and 74°14'17.9" East longitude followed by Azt-148 and Azt-113 isolates (9.67×10^4 CFU g^{-1} soil) from Bhandara and Solapur district of Vidarbha and Western Maharashtra which were

distributed at 20°51'3"N lat. 80°13'9"E long. and 17°51'30"N lat. 75°45'30"E long., respectively.

- The free living rhizobia isolated from rhizosphere soils of different regions of Maharashtra revealed significant variation ($1.07x10^4$ to $8.33x10^4$ CFU g^{-1} soil) in microbial population among the different *Rhizobium* isolates. The maximum values of population ($8.33x10^4$) recorded by Rh-72 isolate obtained from Latur district of Marathwada region (18°28'0"N lat. 76°41'00"E long.), whereas the lowest population ($1.07x10^4$) coming from Rh-98 isolate of Nasik district of North Maharashtra which were distributed at 19°54'55"N lat. 73°42'00"E long.

- The population of *Azospirillum* isolates varied among the different regions of Maharashtra. The significantly highest microbial count ($5.40x10^4$ MPN g^{-1} soil) was recorded by Asp-28 isolate obtained from the rhizosphere soils of Kolhapur district of Western Maharashtra (17°01'52"N lat. 73°52'06"E long.) followed by Asp-72 ($4.50x10^4$ MPN g^{-1} soil) and Asp-97 ($3.80x10^4$ MPN g^{-1} soil) isolates obtained from Latur and Parbhani district of Marathwada, respectively.

- The phosphate solubilizing bacterial population varied significantly ($0.16x10^4$ to $2.83x10^4$ CFU g^{-1} soil) among the soil samples collected from different physiographic regions of Maharashtra. The significantly highest PSB population ($2.83x10^4$) was recorded by PSB-72 isolate obtained from rhizosphere soils of Latur district of Marathwada region, whereas minimum population ($0.16x10^4$) coming from PSB-11, 69, 95 and 125 isolates of Ratnagiri, Sangli, Beed and Wardha district, respectively.

- The phosphate solubilizing fungal population varied significantly ($0.16x10^4$ to $2.33x10^4$ and $0.33x10^4$ to $1.83x10^4$ CFU g^{-1} soil)

among the *Aspergillus* and *Penicillium* isolates obtained from rhizosphere soils of different regions of Maharashtra, respectively. As regards *Aspergillus* isolates, the significantly highest population ($2.33x10^4$) was recorded by PSF-82 isolate recovered from Beed district of Marathwada region (18°44'00"N lat. 75°50'30"E long.). In case of *Penicillium* isolates, the significantly highest population ($2.33x10^4$) was recorded by PSF-61 isolate obtained from Solapur district of Western Maharashtra (17°48'35"N lat. 75°33'00"E long.).

- The distinct variation in cell morphology among the different isolates of *Azotobacter* was observed. The cells of all *Azotobacter* isolates were motile and gram negative in reaction. Out of 94 *Azotobacter* isolates, cells of 48 isolates were rod shape and 46 isolates having oval in shape. The cell size varied among the isolates and it was in the range of 1.1 to 4.4 x 2.0 to 11.8 µm. On the basis of cell arrangement, four distinct groups of isolates were formed.

- The cells of all *Rhizobium* isolates were rod shaped, motile and gram negative in reaction. A wide variation in cell size (0.4 to 2.8 x 0.7 to 10.0 µm) among the different *Rhizobium* isolates was noticed.

- The distinct variation in cell morphology among the different *Azospirillum* isolates was noticed. The cells of 83 *Azospirillum* isolates were non-motile, whereas 10 isolate having motile cells. The cell shape differed among the various *Azospirillum* isolates. Out of 93 *Azospirillum* isolates, cells of 44 isolates were helicle/S-shape, 35 isolates having vibrioid shape and 14 isolates were curved rods. The great variation in cell size among the isolates was recorded and it was in the range of 1.0 to 2.8 x 3.3 to 24.8 µm.

- The cells of all phosphate solubilizing bacterial isolates were rod shaped, non-motile and gram positive in reaction. A wide variation was noticed in cell size (0.4 to 3.1 x 1.8 to 11.0 μm) among the different PSB isolates.
- The distinct variation in colonial morphology, spore characteristics and microscopic appearance among the phosphate solubilizing *Aspergillus* and *Penicillium* isolates was observed. The colonial morphology of *Aspergillus* isolates revealed that white colonies become black or brown as culture matures, whereas in case of *Penicillium* isolates the mature cultures usually turn greenish or blue-green. On the basis of colonial morphology, four distinct groups of *Aspergillus* and six of *Penicillium* isolates were formed and regarding the microscopic appearance, three distinct groups of *Aspergillus* and six of *Penicillium* isolates were formed.
- Based on colonial morphology, spore characteristics and microscopic examination, out of 46 PSF isolates, 29 were tentatively identified as *Aspergillus awamori* and 17 were tentatively identified as *Penicillium digitatum*.
- The colonies of *Azotobacter* isolates appeared to be slimy, milky, viscid, mucoid, glistening and opaque and later aged cultures produced light brown to dark brown pigmentation whereas the colonies of *Rhizobium* isolates were white-opaque, mucilaginous, semi-translucent, translucent and milky to watery translucent. The colonies of *Azospirillum* isolates formed subsurface pellicles on semi-solid N-free malate medium and the pink pigmentation produced on BMS agar medium.
- The colonies of PSB appeared to be creamy as well as whitish on Pikovskaya's agar medium. The zone of phosphate solubilization

varied from 5 mm to 14 mm among the different PSB isolates. Among the PSF isolates, the diameter of zone of P-solubilization ranged from 11-30 mm in different *Aspergillus* isolates whereas 9-24 mm zone among the different *Penicillium* isolates.

- The biochemical characterization indicated all the 94 *Azotobacter* and 76 *Rhizobium* isolates were positive for starch hydrolysis, H_2S production, catalase test and oxidase test, but were negative for gelatin liquefaction. Regarding utilization of different carbon sources; mannitol, glucose, sucrose and fructose were used as a sole carbon source for growth by all the *Azotobacter* isolates, whereas mannitol, glucose, sucrose and arabinose were used as a sole carbon source for growth by all the *Rhizobium* isolates, while malate and citrate showed negative result. Further, all the 93 *Azospirillum* isolates were positive for catalase test and oxidase test, but were negative for starch hydrolysis, H_2S production and gelatin liquefaction. Out of 93 *Azospirillum* isolates, 83 isolates utilized glucose and malic acid as a sole carbon source and positive for biotin requirement, whereas 10 isolates utilized only malic acid as a sole carbon source for growth and showed negative result for glucose and biotin requirement.

- Based on the morphological, cultural, biochemical and physiological characterization, out of 263 nitrogen fixing isolates, 94 isolates were tentatively identified as *Azotobacter chroococcum*, 76 isolates as *Rhizobium* spp., 83 isolates as *Azospirillum lipoferum* and 10 isolates as *Azospirillum brasilense*.

- All the 47 PSB isolates were positive for starch hydrolysis, gelatin liquefaction and catalase test, but were negative for H_2S production, KOH and oxidase test. Fructose, glucose and

maltose were used as a sole carbon source for growth by all the isolates while mannitol and citrate showed negative result.

- Based on the morphological, cultural, biochemical and physiological characterization, 47 PSB isolates were tentatively identified as *Bacillus megaterium*.

- A significant variation in nitrogenase activity (5.3 to 291.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}) was observed among the 94 different *Azotobacter* isolates tested. The isolate Azt-21 recorded significantly highest nitrogenase activity (291.5 nmol C_2H_4.mg protein^{-1}.hr^{-1}) than the other isolates tested. Out of 94 *Azotobacter* isolates, 11 isolates *viz.*, Azt-21, 25, 50, 64, 70, 82, 97, 129, 135, 142 and 148 recorded significantly higher nitrogenase activity than the standard MPKV strain Azt-BNF.

- The nitrogenase activity among the 76 *Rhizobium* isolates ranged from 1.9 (Rh-65) to 119.7 (Rh-69) nmol C_2H_4.mg protein^{-1}.hr^{-1}. Out of 76 *Rhizobium* isolates, 6 isolates *viz.*, Rh-64, 69, 72, 109, 113 and 132 recorded significantly higher nitrogenase activities than the standard MPKV strain Rh-BNF.

- Among the 93 *Azospirillum* isolates, the nitrogenase activity varied significantly (7.5 to 394.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}). The isolate Asp-97 recorded significantly highest nitrogenase activity (394.7 nmol C_2H_4.mg protein^{-1}.hr^{-1}) than the other isolates tested. Out of 93 *Azospirillum* isolates, 6 isolates *viz.*, Asp-28, 50, 97, 124, 132 and 150 recorded significantly higher nitrogenase activities than the standard MPKV strain Asp-BNF.

- Based on the nitrogenase activity, the highly efficient nitrogen fixing isolates *viz.*, 12 *Azotobacter* isolates, 7 *Rhizobium* isolates and 7 *Azospirillum* isolates alongwith standard MPKV strain were further studied for their genetic variability.

- ❖ All the 47 PSB and 46 PSF isolates were able to solubilize inorganic insoluble phosphate. The amount of Pi released by the PSB isolates ranged from 2.06 (PSB-59) to 52.38 (PSB-15) per cent at 10 days after inoculation. Out of 47 PSB isolates, 6 isolates *viz.*, PSB-15, 33, 41, 72, 100 and 119 recorded significantly higher P-solubilizing ability than the standard MPKV strain PSB-BNF. The decrease in pH of the TCP broth was found to have highly significant positive correlation (r=+0.781**) with the amount of Pi released at 10 days.

- ❖ The amount of Pi released by the *Aspergillus* isolates ranged from 45.80 (PSF-4) to 87.17 (PSF-71) per cent at 10 days after inoculation. Out of 29 *Aspergillus* isolates, 5 isolates *viz.*, PSF-28, 55, 64, 71 and 100 recorded significantly higher P-solubilizing ability than the standard MPKV strain PSF-BNF(A). The decrease in pH of the TCP broth was found to have highly significant positive correlation (r = +0.905**) with the amount of Pi released at 10 days.

- ❖ Among the 17 *Penicillium* isolates, the amount of Pi released varied significantly (26.30 to 69.77 per cent) at 10 days after inoculation. The isolate PSF-61 recorded significantly highest P-solubilization (69.77%) followed by PSF-77 (53.44%) than the standard MPKV strain PSF-BNF(P) and other isolates tested. The decrease in pH of the TCP broth was found to have highly significant positive correlation (r = +0.833**) with the amount of Pi released at 10 days.

- ❖ Based on the phosphate solubilizing ability, the highly efficient phosphate solubilizing isolates *viz.*, 8 PSB isolates, 8 *Aspergillus* isolates and 5 *Penicillium* isolates alongwith standard MPKV strain were further studied for their genetic variability.

- Impact analysis of weather parameters and cropping system on distribution of efficient N_2 fixing and P solubilizing isolates across the physiographic regions of Maharashtra indicated that both, weather parameters and cropping system had no singnificant influence on distribution of efficient N_2 fixing and P solubilizing isolates. However, the population of these isolates varied significantly with cropping system across the regions. The maximum population of *Azotobacter, Rhizobium* and *Azospirillum* isolates (10.17, 8.33 and 5.40 $x10^4$ CFU g^{-1} soil, respectively) was recorded in paddy-wheat, pigeonpea-wheat/pigeonpea-chickpea and paddy/nachni cropping system in Kolhapur and Latur district of Western Ghats and Marathwada, respectively. Similarly, the maximum population of PSB and PSF isolates (2.83 and 2.33 $x10^4$ CFU g^{-1} soil) was recorded in pigeonpea-wheat/pigeonpea-chickpea, pigeonpea-safflower and green gram-sorghum/green gram-safflower cropping systems of Latur, Beed and Solapur district of Marathwada and Western Maharashtra, respectively. The overall results revealed that crop related factors have more critical influence on the abundance of native microbial populations than soil or climatic factors.

- The PCR amplified products of the 12 selected isolates of *Azotobacter* along with standard MPKV strain Azt-BNF with respect to each of the 25 random primers showed 1123 polymorphic bands. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.40 and minimum genetic similarity of 0.15. The highest genetic similarity of 0.40 could be noted between the isolates Azt-08 and Azt-25. The primer RBA-24 was found to be the best primer for determination of variability among the *Azotobacter chroococcum* isolates. This primer generated 54 bands of different molecular weight of which 16 were unique and isolate specific in the

present study. This primer can be used for fingerprinting of *Azotobacter* isolates for use as passport data.

- The amplification profiles of the 7 selected isolates of *Rhizobium* along with standard MPKV strain Rh-BNF with respect to each of the 25 random primers showed 905 polymorphic bands. The dendrogram constructed showed three clusters with the maximum genetic similarity of 0.27 and minimum genetic similarity of 0.22. The highest genetic similarity of 0.27 could be noted between the isolates Rh-64 and Rh-69. The primer RBA-7 was found to be the best primer for determination of variability among the *Rhizobium* spp. isolates. This primer generated 66 bands of different molecular weight of which 28 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Rhizobium* isolates for use as passport data.

- The amplified products of the 7 selected isolates of *Azospirillum* along with standard MPKV strain Asp-BNF with respect to each of the 25 random primers showed 820 polymorphic bands. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.53 and minimum genetic similarity of 0.23. The highest genetic similarity of 0.53 could be noted between the isolates Asp-127 and Asp-132. The primer RBA-4 was found to be the best primer for determination of variability among the *Azospirillum lipoferum* isolates. This primer generated 63 bands of different molecular weight of which 30 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Azospirillum* isolates for use as passport data.

- The PCR amplified products of the 8 selected isolates of PSB along with standard MPKV strain PSB-BNF with respect to each of the 25 random primers showed 600 polymorphic bands. The

dendrogram constructed showed four clusters with the maximum genetic similarity of 0.87 and minimum genetic similarity of 0.29. The highest genetic similarity of 0.87 could be noted between the isolates PSB-33 and PSB-39. The primer RBA-21 was found to be the best primer for determination of variability among the *Bacillus megaterium* isolates. This primer generated 41 bands of different molecular weight of which 10 were unique and isolate specific in the present study. This primer can be used for fingerprinting of PSB isolates for use as passport data.

- The amplification profiles of the 8 selected isolates of *Aspergillus* along with standard MPKV strain PSF-BNF(A) with respect to each of the 25 random primers showed 656 polymorphic bands. The dendrogram constructed showed four clusters with the maximum genetic similarity of 0.59 and minimum genetic similarity of 0.16. The highest genetic similarity of 0.59 could be noted between PSF-115 isolate and PSF-BNF strain. The primer RFU-23 was found to be the best primer for determination of variability among the *Aspergillus awamori* isolates. This primer generated 33 bands of different molecular weight of which 9 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Aspergillus* isolates for use as passport data.

- The amplified products of the 5 selected isolates of *Penicillium* along with standard MPKV strain PSF-BNF(P) with respect to each of the 25 random primers showed 453 polymorphic bands. The dendrogram constructed showed three clusters with the maximum genetic similarity of 0.83 and minimum genetic similarity of 0.40. The highest genetic similarity of 0.83 could be noted between the isolates PSF-97 and PSF-101-1. The primer

RFU-2 was found to be the best primer for determination of variability among the *Penicillium digitatum* isolates. This primer generated 37 bands of different molecular weight of which 8 were unique and isolate specific in the present study. This primer can be used for fingerprinting of *Penicillium* isolates for use as passport data.

- The diversity analysis using different diversity indices *viz.*, Shannon's index (SI), Evenness index (EI) and Richness index (RI) was carried out for 263 nitrogen fixing and 93 phosphate solubilizing isolates. The Shannon's index of the pooled data for nitrogen fixing and phosphate solubilizing isolates was 1.09 and 1.02 respectively, suggesting high diversity of these isolates in six different physiographic regions of Maharashtra. The diversity of tentatively identified 263 nitrogen fixing and 93 phosphate solubilizing isolates in terms of Shannon's index ranged from 0.37 to 1.10 and 0.33 to 1.05, respectively. The maximum diversity of nitrogen fixing isolates was found in Marathwada region (SI of 1.10), whereas phosphate solubilizing isolates in Vidarbha region (SI of 1.05).

- The evenness index of the pooled data was 0.79 and 0.93 for nitrogen fixing and phosphate solubilizing isolates respectively, indicating the dominance of one species over the others. Among the different physiographic regions, Marathwada showed maximum evenness index (1.00) for nitrogen fixers indicating more even distribution of nitrogen fixing isolates in this region, whereas Vidarbha showed maximum evenness index (0.96) for phosphate solubilizers indicating more even distribution of phosphate solubilizing isolates in this region.

- The Richness index for the individual physiographic regions ranged from 0.46 to 0.85 for nitrogen fixing isolates and 0.58 to 1.06 for phosphate solubilizing isolates. Among the different

physiographic regions, Western Maharashtra showed maximum richness index (0.85) for nitrogen fixers indicating richness of nitrogen fixing isolates in this region, whereas Marathwada showed maximum richness index (1.06) for phosphate solubilizers indicating richness of phosphate solubilizing isolates in this region.

On the basis of morphological, cultural, biochemical and physiological characterization as well as functional and molecular diversity studies it is concluded that the distinct variability existed among the nitrogen fixing and phosphate solubilizing isolates recovered from the rhizosphere soils of various physiographic regions of Maharashtra ranging from Western Konkan Coast, Western Ghats (*Sahyadris*), Western Maharashtra, North Maharashtra, Marathwada to Vidarbha regions. The population dynamics study revealed that crop related factors have more critical influence on the abundance of native microbial populations than soil or climatic factors. Furthermore, diversity analysis study indicated the richness of nitrogen fixing isolates in Sangli, Kolhapur, Pune, Solapur and Ahmednagar district of Western Maharashtra and phosphate solubilizing isolates in Latur, Beed and Parbhani district of Marathwada, whereas the maximum diversity of nitrogen fixing and phosphate solubilizing isolates was found in Marathwada and Vidarbha region, respectively.

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8. APPENDIX

Appendix-I

8.1 Details of nitrogen fixing and phosphate solubilizing microorganisms isolated from soil samples collected from different physiographic regions of Maharashtra for studying microbial diversity

I) Western Konkan Coast (23)

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
1	*Azospirillum*	Village: Pural Tahsil: Devgarh Dist: Sindhudurg	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	4 cm	Yellowish brown	16°55'20"N lat. 73°20'30"E long.
2	*Azospirillum*	Village: Ratnagiri Tahsil: Ratnagiri Dist: Ratnagiri	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	8 cm	Reddish brown	17°00'00"N lat. 73°16'19"E long.
3	*Azospirillum*	Village: Bhurapada Tahsil: Palghar Dist: Thane	Fine, mont. (cal), isohyperthermic, Vertic Halaquepts	Moderately shallow	53 cm	Dark grayish brown	19°32'00"N lat. 72°45'30"E long.
4	*Azotobacter, Azospirillum,* PSF(A)	Village: Virthan Tahsil: Palghar Dist: Thane	Fine,mont. (cal), isohyperthermic, Entic Haplusterts	Moderately deep	>85cm	Brown & dark gray brown	19°32'20"N lat. 72°44'30"E long.
5	*Azospirillum*	Village: Nivli Tahsil: Chiplun Dist: Ratnagiti	Fine, loamy, mixed, isohyperthermic, Udic Rhodustalfs	Moderately shallow	65 cm	Reddish brown	17°22'00"N lat. 73°27'30"E long.
6	PSB	Village: Harchil Tahsil: Ratnagiti Dist: Ratnagiti	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	18 cm	Yellowish red	16°55'30"N lat. 70°26'20"E long.
7	PSF(A)	Village: Nandgaon Tahsil: Chiplun Dist: Ratnagiti	Clayey-skeletal mixisohyperthermi c Typic Haplustepts	Moderately shallow	56 cm	Dark reddish brown	17°21'00"N lat. 73°33'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
8	*Azotobacter, Azospirillum,* PSF(A)	Village: Janvali Tahsil: Kankauli Dist: Sindhudurg	Fine, loamy, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	50 cm	Dark reddish brown	16°16'21"N lat. 73°43'53"E long.
9	*Azotobacter*	Village: Adeli Tahsil: Vengurla Dist: Sindhudurg	Fine, mixed, isohyperthermic, Ultic Haplustalfs	Moderately shallow	55 cm	Dark reddish brown	15°35'13.6"N lat. 73°41'29.6"E long.
10	*Azotobacter, Azospirillum,* PSB	Village: Vatora Tahsil: Vengurla Dist: Sindhudurg	Fine, mixed, isohyperthermic, Ultic Haplustalfs	Very deep	140 cm	Yellowish red	15°54'40"N lat. 73°39'6"E long.
11	*Azospirillum,* PSB, PSF(A)	Village: Tikali Tahsil: Ratnagiti Dist: Ratnagiti	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	15 cm	Reddish brown	16°55'30"N lat. 73°20'40"E long.
12	*Azotobacter*	Village: Achra Tahsil: Malvan Dist: Sindhudurg	Loamy, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	65 cm	Brown	16°13'3"N lat. 73°1'3"E long.
13	*Rhizobium*	Village: Gavalwadi Tahsil: Chiplun Dist: Ratnagiti	Fine-loamy, mixed, isohyperthermic, Udic Rhodustaf	Deep	>100 cm	Reddish brown	17°25'00"N lat. 73°28'30"E long.
14	*Azospirillum*	Village: Naringre Tahsil: Devgarh Dist: Sindhudurg	Fine, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	75 cm	Reddish brown	16°17'16"N lat. 73°27'20"E long.
15	*Azospirillum,* PSB	Village: Rajurbahula Tahsil: Nasik Dist: Nasik	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	7 cm	Brown	19°53'0"N lat. 73°41'30"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
16	*Azospirillum*	Village: Gunde Tahsil: Shahapur Dist: Thane	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	23 cm	Dark brown	19°26'10"N lat. 73°38'10"E long.
17	PSB	Village:Brahamanwadi Tahsil: Kankauli Dist: Sindhudurg	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	12 cm	Strong brown	16°17'10"N lat. 73°46'50"E long.
18	*Azotobacter*	Village: Nane Tahsil: Wada Dist: Thane	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	56 cm	Dark brown	19°38'30"N lat. 72°58'30"E long.
19	PSF(A)	Village: Ambet Tahsil: Man Dist: Raigad	Clay-skeletal, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	70 cm	Brown	18°5'00"N lat. 73°16'00"E long.
20	PSB	Village: Tamhane Tahsil: Mahad Dist: Raigad	Clay-skeletal, mixed, isohyperthermic, Typic Rhodustalfs	Moderately shallow	70 cm	Yellowish red	17°55'00"N lat. 73°10'00"E long.
21	*Azotobacter, Rhizobium, Azospirillum*	Village: Sakhar Tahsil: Poladpur Dist: Raigad	Fine, mixed, isohyperthermic, Udic Haplustalfs	Moderately deep	90 cm	Reddish brown	18°00'00"N lat. 73°34'00"E long.
22	*Rhizobium*	Village: Kenjule Tahsil: Khed Dist: Ratnagiri	Loamy-skeletal, mixed, isohyperthermic, Typic Haplustepts	Moderately deep	76 cm	Dark red	17°48'00"N lat. 73°34'00"E long.
23	*Azotobacter, Azospirillum*	Village: Bhorghar Tahsil: Mangaon Dist: Raigad	Clay-skeletal, mixed, isohyperthermic, Typic Haplustepts	Moderately shallow	56 cm	Dark reddish brown	17°14'40"N lat. 73°12'10"E long.

Appendix-I contd...

II) Western Ghats (27)

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
24	*Azospirillum*	Village: Ojahar Budrukh Tahsil: Sangameshwar Dist: Ratnagiri	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Shallow	40 cm	Dark reddish brown	17°01'44"N lat. 73°47'19"E long.
25	*Azotobacter, Azospirillum*	Village: Ojhar Tahsil: Rajapur Dist: Ratnagiri	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	16 cm	Yellowish red	16°44'24"N lat. 73°37'40"E long.
26	*Azotobacter*	Village: Harkul Tahsil: Kankauli Dist: Sindhudurg	Loamy-skeletal, mixed, isohyperthermic, Typic Haplustepts	Shallow	45 cm	Reddish brown	16°19'12"N lat. 73°50'11"E long.
27	*Azotobacter, Azospirillum*	Village: Manivili Tahsil: Murbad Dist: Thane	Fine-loamy, mixed isohyperthermic, Typic Haplustepts	Shallow	33 cm	Dark reddish brown	19°17'00"N lat. 73°18'00"E long.
28	*Azotobacter, Rhizobium, Azospirillum,* PSF(A)	Village: Golevane Tahsil: Sahuwadi Dist: Kolhapur	Fine-loamy, mixed isohyperthermic, Udic Rhodustalfs	Deep	100 cm	Reddish brown	17°01'52"N lat. 73°52'06"E long.
29	*Azotobacter, Rhizobium, Azospirillum*	Village: Ambarde Tahsil: Ajra Dist: Kolhapur	Fine, mixed isohyperthermic, Udic Rhodustalfs	Deep	125 cm	Dark reddish brown	16°5'59"N lat. 70°01'56"E long.
30	PSF(P)	Village: Manjra Tahsil: Sahuwadi Dist: Kolhapur	Loamy-skeletal, mixed, isohyperthermic, Udic Haplustalfs	Shallow	40 cm	Yellowish red	16°49'57"N lat. 73°49'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
31	*Azospirillum*	Village: Amboli Tahsil: Sawantwadi Dist: Sindhudurg	Fine-loamy mixed, isohyperthermic, Typic Haplustepts	Moderately deep	90 cm	Red & dark red	15°58'00"N lat. 73°59'00"E long.
32	*Azotobacter,* PSF(A)	Village: Amba Tahsil: Sawantwadi Dist: Sindhudurg	Fine-loamy mixed, isohyperthermic, Udic Rhodustalfs	Deep	125 cm	Reddish brown	17°0'48"N lat. 73°50'43"E long.
33	*Azotobacter, Azospirillum,* PSB	Village: Balkawadi Tahsil: Gargoti Dist: Kolhapur	Fine, mixed, isohyperthermic, Udic Rhodustalfs	Moderately deep	95 cm	Dark reddish brown	16°11.43'48"N lat 74°01'57.7"E long.
34	*Azotobacter, Azospirillum*	Village: Kodekhurd Tahsil: Gaganbavda Dist: Kolhapur	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Shallow	27 cm	Reddish brown	16°38'40"N lat. 73°54'30"E long.
35	*Azospirillum*	Village: Dahisar Tahsil: Palghar Dist: Thane	Clayey-skeletal, mixed, isohyperthermic, Typic Ustorthents	Very shallow	13 cm	Black gravely clay	19°38'30"N lat. 72°53'15"E long.
36	*Azotobacter, Azospirillum*	Village: Haloli Tahsil: Palghar Dist: Thane	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately deep	76 cm	Dark brown	19°39'15"N lat. 72°53'00"E long.
37	*Azotobacter, Rhizobium, Azospirillum*	Village: Verla Tahsil: Sawantwadi Dist: Sindhudurg	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Shallow	36 cm	Reddish yellow	15°59'04"N lat. 73°58'52"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
38	*Azospirillum*	Village: Nirguda Tahsil: Peint Dist: Nasik	Clayey-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	8 cm	Yellowish red & dark reddish brown	20°10'00"N lat. 73°25'00"E long.
39	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Mahakoshi Tahsil: Bhor Dist: Pune	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	13 cm	Brown	18°03'30"N lat. 73°02'05"E long.
40	*Azotobacter, Rhizobium, Azospirillum*, PSF(A)	Village: Barsewadi Tahsil: Wai Dist: Satara	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	12 cm	Very dark grayish brown	18°00'30"N lat. 73°56'15"E long.
41	*Azospirillum,* PSB, PSF(A)	Village: Mhakoshi Tahsil: Jawahar Dist: Thane	Clayey, mixed, isohyperthermic, Typic Ustorthents	Very shallow	16 cm	Yellowish brown & dark yellowish brown	19°59'00"N lat. 73°10'30"E long.
42	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Chinchoda Tahsil: Peint Dist: Nasik	Clayey-skeletal, mixed, isohyperthermic, Typic Ustorthents	Shallow	30 cm	Reddish brown & dark reddish brown	27°10'30"N lat. 73°26'30"E long.
43	*Azotobacter, Rhizobium, Azospirillum*	Village: Shrirange Tahsil: Sawantwadi Dist: Sindhudurg	Loamy, mixed, isohyperthermic, Lithic Haplustepts	Shallow	42 cm	Dark red	15°44'35"N lat. 74°06'18.5"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
44	*Azotobacter, Rhizobium, Azospirillum*, PSF(A)	Village: Ramghat Tahsil: Chandgad Dist: Kolhapur	Fine-loamy, mixed, isohyperthermic, Typic Haplustepts	Moderately deep	90 cm	Reddish brown & dark reddish brown	15°50'3"N lat. 74°06'18"E long.
45	*Azotobacter*	Village: Khaprale Tahsil: Sinnar Dist: Nasik	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	13 cm	Brown	19°48'15"N lat. 73°54'30"E long.
46	*Azospirillum*, PSB	Village: Indalkarwadi Tahsil: Purandar Dist: Pune	Clayey-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	8 cm	Dark reddish brown	18°25'45"N lat. 73°13'24"E long.
47	*Azospirillum*	Village: Sonmbre Tahsil: Sinnar Dist: Nasik	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	18 cm	Brown	19°48'00"N lat. 73°55'00"E long.
48	*Azotobacter, Azospirillum*, PSB	Village: Tarwade Tahsil: Dhule Dist: Dhule	Clayey-skeletal, mixed, isohyperthermic, Typic Ustorthents	Very shallow	12 cm	Brown & dark grayish brown	20°24'40"N lat. 73°38'30"E long.
49	*Azotobacter, Azospirillum*	Village: Chimanipada Tahsil: Navapur Dist: Nandurbar	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	55 cm	Very dark grey & very dark grayish brown	21°31'36"N lat. 74°30'20"E long.
50	*Azotobacter, Rhizobium, Azospirillum*, PSB	Village: Kolegaon Tahsil: Navapur Dist: Nandurbar	Clay, mixed, isohyperthermic, Typic Haplustepts	Shallow	36 cm	Very dark grayish brown	21°08'30"N lat. 73°53'30"E long.

Appendix-I contd...

III) North Deccan: Maharashtra Plateau

A) North Deccan Maharashtra Upper Plateau (42)

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
51	*Rhizobium*, *Azospirillum*	Village: Girnare Tahsil: Malegaon Dist: Nasik	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	8-15 cm	Brown	20°23'15"N lat. 74°20'15"E long.
52	*Azotobacter*	Village: Girnar Tahsil: Malegaon Dist: Nasik	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	18-22 cm	Brown & dark brown	20°22'15"N lat. 74°20'00"E long.
53	*Azotobacter*, *Rhizobium*	Village: Pimple Tahsil: Kalvan Dist: Nasik	Clayey-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	10-15 cm	Dark reddish brown	20°21'20"N lat. 73°56'30"E long.
54	*Azotobacter*, *Rhizobium*	Village: Mohabari Tahsil: Sakri Dist: Dhule	Clayey-skeletal, mixed, isohyperthermic, Typic Ustorthents	Shallow	41 cm	Dark brown	20°34'00"N lat. 73°55'20"E long.
55	*Azotobacter*, *Rhizobium*, *Azospirillum*, PSF(A)	Village: Karkata Tahsil: Ausa Dist: Latur	Clayey, montmorillonitic, isohyperthermic, Typic Haplustepts	Shallow	43 cm	Dark brown	18°23'00"N lat. 76°17'15"E long.
56	*Azotobacter*, *Rhizobium*, *Azospirillum*, PSB	Village: Laman Tanda Tahsil: Ahmadpur Dist: Latur	Clayey, montmorillonitic, isohyperthermic, Lithic Ustorthents	Very Shallow	15-22 cm	Dark brown	18°26'00"N lat. 76°46'15"E long.
57	*Azotobacter*, *Azospirillum*, PSF(A)	Village: Wadkudi Tahsil: Chandgad Dist: Kolhapur	Fine, mixed isohyperthermic, Udic Rhodustalfs	Deep	135 cm	Reddish brown & dark reddish brown	15°56'04"N lat. 74°14'26"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
58	*Azotobacter, Rhizobium*	Village: Khodal Tahsil: Junnar Dist: Pune	Loamy-skeletal, mixed (cal), isohyperthermic, Lithic Ustorthents	Very shallow	17 cm	Dark reddish brown	19°5'00"N lat. 74°41'00"E long.
59	*Azospirillum*, PSB	Village: Devargaon Tahsil: Niphad Dist: Nasik	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	5-10 cm	Brown	20°12'30"N lat. 74°9'30"E long.
60	*Azotobacter, Rhizobium, Azospirillum*, PSB	Village: Rokdwadi Tahsil: Ambegaon Dist: Pune	Loamy, mixed (cal), isohyperthermic, Lithic Ustorthents	Very shallow	9-21 cm	Dark yellowish brown	18°56'00"N lat. 74°8'00"E long.
61	*Azotobacter, Rhizobium,* PSB, PSF(P)	Village: Sarole Tahsil: Mohol Dist: Solapur	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	6-18 cm	Yellowish brown	17°48'35"N lat. 75°33'00"E long.
62	*Rhizobium, Azospirillum*	Village: Nandgaon Tahsil: Karjat Dist: Ahmednagar	Clayey, montmorillonitic, isohyperthermic, Lithic Ustorthents	Very shallow	7-18 cm	Brown & dark reddish brown	18°35'00"N lat. 74°55'30"E long.
63	*Azotobacter, Azospirillum*	Village: Ambawadi Tahsil: Shirala Dist: Sangli	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	12-20 cm	Dark reddish brown	17°3'20"N lat. 74°4'50"E long.
64	*Azotobacter, Rhizobium, Azospirillum,* PSB, PSF(A)	Village: Nimbhore Tahsil: Rahuri Dist: Ahmednagar	Loamy-skeletal, mixed (cal), isohyperthermic, Typic Ustorthents	Very shallow	10-22 cm	Brown	19°28'00"N lat. 74°29'00"E long.
65	*Azotobacter, Rhizobium,* PSF(P)	Village: Karvali Tahsil: Mohol Dist: Solapur	Clayey-skeletal, montmorillonitic (cal) isohyperthermic, Typic Ustorthents	Extremely shallow	9 cm	Brown	17°35'40"N lat. 75°42'30"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
66	*Azotobacter, Rhizobium*	Village: Jhikri Tahsil: Jamkhed Dist: Ahmednagar	Clayey, montmorillonitic, isohyperthermic, Lithic Haplusteps	Very shallow to Shallow	12-36 cm	Very dark grayish brown	18°39'30"N lat. 74°19'00"E long.
67	*Azotobacter, Rhizobium, Azospirillum*	Village: Ambulga Tahsil: Osmanabad Dist: Osmanabad	Clayey, montmorillonitic, isohyperthermic, Typic Haplustepts	Shallow	42 cm	Very dark grayish brown	18°27'15"N lat. 76°46'30"E long.
68	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Karlewadi Tahsil: Ambegaon Dist: Pune	Fine, mont (cal), isohyperthermic, Typic Haplustepts	Moderately deep	76-90 cm	Dark brown	18°57'49"N lat. 73°52'00"E long.
69	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Kameri Tahsil: Valva Dist: Sangli	Fine, mont (cal), isohyperthermic, Typic Haplustepts	Deep	105-150 cm	Very dark grayish brown	17°17'70"N lat. 74°56'00"E long.
70	*Azotobacter, Rhizobium, Azospirillum*	Village: Bumne Tahsil: Gargoti Dist: Kolhapur	Fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	160 cm	Browm & dark brown	16°16'5.24"N lat. 74°14'17.9"E long.
71	*Rhizobium,* PSF(A)	Village: Parpoli Tahsil: Ajra Dist: Kolhapur	Fine, mixed, isohyperthermic, Udic Haplustalfs	Moderately deep	80 cm	Browm & reddish brown	16°6'8"N lat. 74°5'11"E long.
72	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Bamni Tahsil: Latur Dist: Latur	Very fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	110-150 cm	Very dark grayish brown	18°28'0"N lat. 76°41'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
73	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Deothan Tahsil: Akole Dist: Ahmednagar	Fine-loamy, mixed, calcareous, isohyperthermic, Typic Ustifluvents	Deep	150 cm	Brown	19°38'00"N lat. 74°4'00"E long.
74	*Rhizobium*	Village: Ranjani Tahsil: Ambegaon Dist: Pune	Loamy, mixed (cal), isohyperthermic, Lithic Ustorthents	Very shallow	13-23 cm	Brown & dark brown	17°0'58"N lat. 74°30'00"E long.
75	*Azotobacter, Azospirillum,* PSB	Village: Ambade Tahsil: Bhor Dist: Pune	Loamy, mixed isohyperthermic, Lithic Ustorthents	Very shallow	12 cm	Strong Brown	18°3'00"N lat. 73°51'30"E long.
76	*Rhizobium*	Village: Patharwadi Tahsil: Atpadi Dist: Sangli	Clayey, mixed isohyperthermic, Lithic Ustorthents	Extremely shallow	7-14 cm	Dark reddish brown	17°17'00"N lat. 74°54'45"E long.
77	*Azotobacter*, PSF(P)	Village: Dudhebari Tahsil: Phaltan Dist: Satara	Loamy-skeletal, mixed (cal), isohyperthermic, Typic Ustorthents	Very shallow	18-20 cm	Very dark grayish brown	17°56'00"N lat. 74°30'00"E long.
78	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Sonawadi Tahsil: Phaltan Dist: Satara	Fine, montmorillonitic (cal), isohyperthermic, Vertic Haplustepts	Moderately deep	73-86 cm	Dark brown	17°57'00"N lat. 74°29'00"E long.
79	*Azotobacter*, PSB, PSF(P)	Village: Penjalwadi Tahsil: Bhor Dist: Pune	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	55-65 cm	Dark brown	18°11'00"N lat. 74°01'00"E long.
80	*Rhizobium*, PSF(P)	Village: Kolyachiwadi Tahsil: Rahuri Dist: Ahmednagar	Fine, mixed (cal), isohyperthermic, Fluventic Haplustepts	Moderately Deep	83 cm	Brown & dark brown	19°23'00"N lat. 74°27'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
81	*Azotobacter, Rhizobium,* PSB	Village: Kurli Tahsil: Vite Dist: Sangli	Fine, mont. (cal), isohyperthermic, Typic Calciustepts	Moderately deep	75 cm	Brown & dark brown	17°14'00"N lat. 74°33'45"E long.
82	*Azotobacter, Rhizobium, Azospirillum,* PSF(A), PSF(P)	Village: Wagha Tahsil: Kaij Dist: Beed	Fine, mont. (cal), isohyperthermic, Typic Haplustepts	Deep	88-150 cm	Dark brown	18°44'00"N lat. 75°50'30"E long.
83	*Azotobacter, Azospirillum,* PSB	Village: Targaon Tahsil: Koregaon Dist: Satara	Fine, mont. (cal), isohyperthermic, Typic Haplustepts	Deep	165 cm	Brown to dark brown	17°31'00"N lat. 74°09'00"E long.
84	*Azotobacter, Rhizobium*	Village: Shiware Tahsil: Shahuwadi Dist: Kolhapur	Very fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	165 cm	Dark brown	16°56'52"N lat. 74°01'12"E long.
85	*Rhizobium, Azospirillum,* PSF(P)	Village: Wathar Tahsil: Koregaon Dist: Satara	Fine, mont. (cal), isohyperthermic, Typic Haplustepts	Deep	150 cm	Very dark grayish brown	17°32'00"N lat. 74°11'00"E long.
86	*Azotobacter, Rhizobium, Azospirillum*	Village: Kondvan Tahsil: Shahuwadi Dist: Kolhapur	Fine, mixed, isohyperthermic, Udic Rhodustalfs	Moderately Deep	98 cm	Reddish brown	17°02'30"N lat. 73°54'40"E long.
87	*Azospirillum*	Village: Ghospuri Tahsil:Ahmednagar Dist: Ahmednagar	Clayey-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	5-15 cm	Dark reddish brown	18°54'45"N lat. 74°40'20"E long.
88	*Azotobacter, Azospirillum*	Village: Nizampur Tahsil: Sangola Dist: Solapur	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	5-20 cm	Yellowish brown & brown	17°19'00"N lat. 75°8'20"E long.
89	*Azotobacter*	Village: Sulaiwadi Tahsil: Vite Dist: Sangli	Clayey, mon., isohyperthermic, Lithic Ustorthents	Very shallow	12-24 cm	Dark reddish brown	17°14'30"N lat. 74°34'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
90	*Azospirillum*	Village: Davri Tahsil: Patan Dist: Satara	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	12-28 cm	Dark reddish brown	17°16'5"N lat. 73°55'59"E long.
91	*Azotobacter*	Village: Virgaon Tahsil: Akole Dist: Ahmednagar	Loamy-skeletal, mixed (cal), isohyperthermic, Typic Ustorthents	Very shallow	14 cm	Dark yellowish brown	19°37'00"N lat. 74°3'00"E long.
92	*Azospirillum,* PSF(P)	Village: Bhoirepathar Tahsil: Ahmednagar Dist: Ahmednagar	Fine, mont. (cal), isohyperthermic, Vertic Haplustepts	Moderately shallow	60-75 cm	Dark brown/ Very dark brown	19°36'00"N lat. 74°2'00"E long.

III) B) North Deccan Maharashtra Lower Plateau (37)

93	*Azotobacter, Rhizobium,* PSB	Village: Ancholi Tahsil: Biloli Dist: Nanded	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	5-20 cm	Brown dark brown	18°53'10"N lat. 77°27'00"E long.
94	*Azotobacter*	Village: Gulvanch Tahsil: Sinnar Dist: Nasik	Loamy, mixed, calcareous, isohyperthermic, Lithic Ustorthents	Very shallow	19 cm	Brown & dark brown	19°52'00"N lat. 74°7'00"E long.
95	*Rhizobium, Azospirillum,* PSB	Village: Beed Tahsil: Beed Dist: Beed	Clayey, mont. (cal), isohyperthermic, Typic Haplustepts	Shallow	34 cm	Dark brown	18°58'15"N lat. 75°48'00"E long.
96	*Azospirillum*	Village: Kavthe Mahankal Tahsil: Kavthe Mahankal Dist: Sangli	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	5 cm	Reddish brown	16°59'55"N lat. 74°55'30"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
97	*Azotobacter, Rhizobium, Azospirillum,* PSF(P)	Village: Jamthi Babala Tahsil: Hingoli Dist: Hingoli	Loamy-skeletal, mixed (cal), isohyperthermic, Lithic Ustorthents	Very shallow	14-20 cm	Very dark grayish brown	19°49'07"N lat. 77°5'4"E long.
98	*Rhizobium*	Village: Rajur Babala Tahsil: Nasik Dist: Nasik	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	10-22 cm	Strong Brown	19°54'55"N lat. 73°42'00"E long.
99	*Azotobacter, Azospirillum*	Village: Bhalvani Tahsil: Malsirus Dist: Solapur	Clayey, montmorillonitic, isohyperthermic, Lithic Ustorthents	Very shallow	8-20 cm	Dark brown	17°42'00"N lat. 75°7'30"E long.
100	*Rhizobium,* PSB, PSF(A)	Village: Kumbhoj Tahsil: Karmala Dist: Solapur	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	8-22 cm	Dark brown	18°17'30"N lat. 75°11'3"E long.
101	*Azotobacter, Rhizobium, Azospirillum,* PSF(A), PSF(P)	Village: Chandhai Tahsil: Mangrulpir Dist: Akola	Clayey, montmorillonitic, isohyperthermic, Lithic Haplustepts	Shallow	27-36 cm	Very dark grayish Brown	20°17.8'00"N lat. 73°24'00"E long.
102	*Azotobacter, Rhizobium, Azospirillum,* PSB, PSF(P)	Village: Satana Tahsil: Vaijapur Dist: Aurangabad	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	49-73 cm	Very dark grayish Brown	19°52'00"N lat. 74°47'00"E long.
103	*Azospirillum*	Village: Nandgaon Tahsil: Gondpipri Dist: Chandrapur	Fine-loamy, mixed, isohyperthermic, Typic Haplustepts	Shallow	40 cm	Brown & dark brown	19°51'30"N lat. 79°44'15"E long.
104	*Azotobacter*	Village: Ghospuri Tahsil: Ahmednagar Dist: Ahmednagar	Clayey, mont. (cal.), isohyperthermic, Typic Haplustepts	Shallow	28-45 cm	Very dark grayish brown	18°55'25"N lat. 74°40'50"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
105	*Azotobacter, Rhizobium*	Village: Dhulgaon Tahsil: Kavthe Mahankal Dist: Sangli	Fine, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Moderately deep	35-100 cm	Dark yellowish brown	16°57'30"N lat. 74°52'35"E long.
106	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Wadachiwadi Tahsil: Jintur Dist: Parbhani	Fine, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Deep	100-150 cm	Dark grayish brown & dark brown	19°34'00"N lat. 76°44'30"E long.
107	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Sewagram Tahsil: Wardha Dist: Wardha	Fine, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Deep	140-150 cm	Dark grayish brown & dark brown	20°44'00"N lat. 78°39'30"E long.
108	*Azotobacter, Rhizobium, Azospirillum*	Village: Umrer Tahsil: Umrer Dist: Nagpur	Fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	99-150 cm	Dark grayish brown & very dark grayish brown	20°52'00"N lat. 79°22'00"E long.
109	*Azotobacter, Rhizobium,* PSB	Village: Ranvad Tahsil: Niphad Dist: Nasik	Clayey, montmorillonitic, isohyperthermic, Lithic Haplustepts	Shallow	30-42 cm	Dark brown	20°09'15"N lat. 74°07'00"E long.
110	*Azotobacter*	Village: Nandurdi Tahsil: Niphad Dist: Nasik	Clayey, montmorillonitic (calcareous), isohyperthermic, Lithic Haplustepts	Shallow	44 cm	Dark brown	20°08'00"N lat. 74°05'30"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
111	PSB	Village: Purandarwada Tahsil: Malshirus Dist: Solapur	Fine, montmorillonitic (calcareous), isohyperthermic, Vertic Haplustepts	Moderately deep	80-110 cm	Very dark grayish brown	17°51'45"N lat. 74°51'00"E long.
112	*Azotobacter, Rhizobium, Azospirillum*	Village: Bandhalwadi Tahsil: Malshirus Dist: Solapur	Clayey, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Shallow	25-50 cm	Dark grayish brown & dark brown	17°44'00"N lat. 75°06'00"E long.
113	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Masala Tahsil: North Solapur Dist: Solapur	Fine, montmorillonitic (calcareous), isohyperthermic, Vertic Haplustepts	Moderately deep	100 cm	Dark grayish brown	17°51'30"N lat. 75°45'30"E long.
114	*Azotobacter, Rhizobium*	Village: Thejoda Tahsil: Satana Dist: Nasik	Fine, montmorillonitic (calcareous), isohyperthermic, Typic Haplustepts	Moderately shallow to deep	50-125 cm	Brown & dark brown	20°00'32"N lat. 74°14'00"E long.
115	*Rhizobium,* PSF(A)	Village: Belbare Tahsil: Kalwan Dist: Nasik	Fine, montmorillonitic, isohyperthermic, Udic Haplustepts	Deep	105 cm	Very dark grayish brown	20°21'30"N lat. 73°56'30"E long.
116	*Azospirillum*	Village: Dhondrai Tahsil: Gevarai Dist: Beed	Fine, montmorillonitic, (calcareous), isohyperthermic, Entic Haplustepts	Deep	80-160 cm	Brown	19°18'00"N lat. 75°41'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
117	*Rhizobium, Azospirillum*	Village: Mendki Tahsil: Katol Dist: Nagpur	Fine, montmorillonitic, (calcareous), hyperthermic, Typic Haplustepts	Deep	125 cm	Very dark grayish brown	21°21'00"N lat. 78°38'00"E long.
118	*Azotobacter, Rhizobium,* PSF(P)	Village: Sirpur Tahsil: Deoli Dist: Washim	Fine, montmorillonitic, (calcareous), hyperthermic, Vertic Haplustepts	Deep	90-150 cm	Dark brown	20°52'00"N lat. 78°23'00"E long.
119	*Azospirillum,* PSB, PSF(A)	Village: Mehunbara Tahsil: Jalgaon Dist: Jalgaon	Fine, montmorillonitic, isohyperthermic, Typic Haplustepts	Deep	150 cm	Dark brown & very dark grayish brown	20°35'10"N lat. 73°55'20"E long.
120	*Azotobacter, Rhizobium, Azospirillum,* PSF(A), PSF(P)	Village: Dahiwade Tahsil: Chopda Dist: Jalgaon	Loamy-skeletal, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	10-15 cm	Brown & dark brown	20°31'10"N lat. 73°55'00"E long.
121	*Azospirillum*	Village: Sewagram Tahsil: Wardha Dist: Wardha	Loamy, mixed, hyperthermic, Lithic Ustorthents	Extremely shallow	8 cm	Yellowish brown & brown	19°44'30"N lat. 78°40'00"E long.
122	*Azotobacter,* PSF(A)	Village: Koregaon Tahsil: Wani Dist: Amravati	Clayey, montmorillonitic, isohyperthermic, Lithic Haplustepts	Shallow	25 cm	Very dark grayish brown	19°56'00"N lat. 78°36'00"E long.
123	*Rhizobium, Azospirillum*	Village: Wanjri Tahsil: Kelapur Dist: Buldhana	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately shallow	56 cm	Dark grayish Brown & dark brown	19°57'00"N lat. 78°34'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
124	*Rhizobium, Azospirillum,* PSF(A)	Village: Garpit RF Tahsil: Karanja Dist: Wardha	Loamy-skeletal, mixed, hyperthermic, Lithic Ustorthents	Very shallow	18 cm	Very dark grayish brown	21°4'00"N lat. 78°28'00"E long.
125	*Azotobacter, Rhizobium,* PSB	Village: Talegaon Tahsil: Karanja Dist: Wardha	Clayey, montmorillonitic, hyperthermic, Lithic Ustorthents	Shallow	28 cm	Very dark grayish brown	21°08'00"N lat. 78°28'00"E long.
126	*Azotobacter, Rhizobium*	Village: Mulgi Tahsil: Akkalkuwa Dist: Dhule	Loamy, mixed, isohyperthermic, Lithic Ustorthents	Very shallow	10-15 cm	Reddish brown	21°34'30"N lat. 73°55'10"E long.
127	*Azospirillum,* PSF(A)	Village: Phulwari Tahsil: Kinwat Dist: Jalna	Clayey, mixed, isohyperthermic, Lithic Ustorthents	Extremely shallow	<10 cm	Dark brown	19°43'00"N lat. 78°7'00"E long.
128	PSF(A)	Village: Bodla Tahsil: Dhudgaon Dist: Dhule	Loamy-skeletal, mixed, isohyperthermic, Typic Ustorthents	Extremely shallow	7 cm	Brown & dark brown	21°34'30"N lat. 73°55'10"E long.
129	*Azotobacter, Rhizobium, Azospirillum,* PSB, PSF(A)	Village: Umri Tahsil: Yeotmal Dist: Yeotmal	Fine, montmorillonitic, isohyperthermic, Vertic Haplustepts	Moderately deep	64-82 cm	Dark grayish brown	20°00'00"N lat. 79°00'00"E long.

III) C) North Deccan Maharashtra Lower Plateau (Metamorphic) (21)

130	*Azotobacter, Rhizobium, Azospirillum,* PSB	Village: Manori Tahsil: Umrer Dist: Nagpur	Fine-loamy, mixed, hyperthermic, Typic Ustorthents	Shallow	28-50 cm	Dark grayish brown & Very dark grayish brown	20°42'00"N lat. 79°5.5'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
131	*Azotobacter*	Village: Abola Tahsil: Ramtek Dist: Nagpur	Fine-loamy, mixed, hyperthermic, Typic Haplustepts	Moderately deep	80 cm	Dark brown	21°23'20"N lat. 79°20'45"E long.
132	*Azotobacter, Rhizobium, Azospirillum,* PSB, PSF(A)	Village: Bamhni Tahsil: Sindewahi Dist: Chandrapur	Fine, montmorillonitic, hyperthermic, Entic Haplustepts	Deep	140 cm	Yellowish brown	20°45'15"N lat. 79°59'30"E long.
133	*Azotobacter, Rhizobium*	Village: Kinhala Tahsil: Umrer Dist: Nagpur	Fine, mont. hyperthermic, Typic Haplustepts	Deep	125-150 cm	Dark grayish brown	20°44'0"N lat. 79°9'00"E long.
134	*Azospirillum*	Village: Hatodi Tahsil: Ramtek Dist: Nagpur	Coarse-loamy, mixed, hyperthermic, Typic Haplustepts	Shallow	26 cm	Dark brown	21°20'30"N lat. 79°22'00"E long.
135	*Azotobacter,* PSF(A)	Village: Phutki Tahsil: Sindewahi Dist: Chandrapur	Fine-loamy, mixed, hyperthermic, Typic Haplustepts	Deep	>100 cm	Yellowish brown	20°14'00"N lat. 79°42'00"E long.
136	*Azotobacter, Rhizobium, Azospirillum,* PSB, PSF(P)	Village: Takali Tahsil: Kuhi Dist: Nagpur	Fine, montmorillonitic, hyperthermic, Typic Haplustepts	Deep	> 125-150 cm	Brown	21°2'00"N lat. 79°29'55"E long.
137	*Azotobacter*	Village: Sawarkhanda Tahsil: Kuhi Dist: Nagpur	Fine, mixed, hyperthermic, Typic Haplustepts	Moderately shallow	60 cm	Brown and dark brown	21°3'20"N lat. 79°29'30"E long.
138	*Azotobacter, Rhizobium,* PSF(P)	Village: Elgartola RF Tahsil: Ahiri Dist: Gadchiroli	Fine, mixed, hyperthermic, Typic Ustorthents	Shallow	35 cm	Brown and dark brown	19°31'00"N lat. 80°7'00"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
139	*Rhizobium*	Village: Nalikasa (Dalli) Tahsil: Kurkheda Dist: Gondia	Fine, mixed, hyperthermic, Vertic Haplustepts	Deep	100-125 cm	Brown and dark brown	20°32'5"N lat. 80°21'46"E long.
140	*Rhizobium, Azospirillum,* PSB	Village: Sitagondi Tahsil: Parseoni Dist: Nagpur	Coarse-loamy, mixed, hyperthermic, Typic Haplustepts	Shallow	50 cm	Very dark brown	21°32'00"N lat. 79°03'00"E long.
141	*Azotobacter,* PSF(P)	Village: Tekala Tahsil: Etapalli Dist: Gadchiroli	Fine-loamy, mixed, hyperthermic, Typic Haplustepts	Deep	100-110 cm	Brown & dark brown	19°28'30"N lat. 80°33'30"E long.
142	*Azotobacter, Azospirillum,* PSB	Village: Kasansur Tahsil: Etapalli Dist: Gadchiroli	Fine, mixed, hyperthermic, Typic Haplustepts	Moderately shallow	57 cm	Grayish brown & very dark grayish brown	19°28'00"N lat. 80°09'00"E long.
143	*Rhizobium,* PSB, PSF(A)	Village: Pediguradom RF Tahsil: Ahiri Dist: Gadchiroli	Fine-loamy, mixed, hyperthermic, Typic Haplustoll	Deep	125 cm	Dark reddish grey & dark reddish brown	19°28'30"N lat. 80°9'00"E long.
144	*Azotobacter, Rhizobium,* PSB	Village: Khatera Tahsil: Sindewahi Dist: Chandrapur	Fine, montmorillonitic, hyperthermic, Typic Haplustepts	Deep	125 cm	Brown & dark brown	20°44'30"N lat. 79°54'45"E long.
145	*Azospirillum*	Village: Bhiwkund Tahsil: Kuhi Dist: Nagpur	Loamy-skeletal, mixed, hyperthermic, Lithic Ustorthents	Extremely shallow	9 cm	Brown and dark brown	20°14'00"N lat. 79°28'15"E long.

Appendix-I contd...

Sample No.	Type of pure cultures isolated	Location	Soil classification	Soil type	Soil depth (cm)	Soil colour	GPS location
146	*Rhizobium*	Village: Palora Tahsil: Ramtek Dist: Nagpur	Fine-loamy, mixed hyperthermic, Typic Haplustepts	Deep	100 cm	Brown	21°22'0"N lat. 79°10'00"E long.
147	*Azotobacter, Rhizobium, Azospirillum*	Village: Andhali Tahsil: Kurkheda Dist: Gadchiroli	Fine, montmorillonitic, hyperthermic, Vertic Haplustepts	Deep	125 cm	Grayish brown & dark grayish brown	20°33'52"N lat. 80°16'45"E long.
148	*Azotobacter, Rhizobium, Azospirillum,* PSB, PSF(A)	Village: Palandur Tahsil: Deori Dist: Bhandara	Fine-loamy, mixed hyperthermic, Typic Haplustepts	Deep	145 cm	Brownish yellow & yellowish brown	20°51'3"N lat. 80°13'9"E long.
149	*Azotobacter, Azospirillum*	Village: Bhugaon Tahsil: Nagpur Dist: Nagpur	Fine, montmorillonitic, hyperthermic, Typic Haplustepts	Deep	125 cm	Very dark grayish brown	20°6'00"N lat. 79°22'00"E long.
150	*Azospirillum*	Village: Bahadura Tahsil: Nagpur Dist: Nagpur	Fine, montmorillonitic, hyperthermic (cal), Vertic Haplustepts	Moderately deep	90 cm	Dark brown	21°05'55"N lat. 79°10'09"E long.

Figures in parentheses of different physiographic regions of Maharashtra indicates number of soil samples collected for studying the microbial diversity

PSB = Phosphate Solubilizing Bacteria
PSF(A)= Phosphate Solubilizing Fungi (*Aspergillus*)
PSF(P)= Phosphate Solubilizing Fungi (*Penicillium*)

Appendix-II

8.2 Coding of nitrogen fixing and phosphate solubilizing cultures isolated from different soils of Maharashtra

Sample No.	*Azotobacter*	*Rhizobium*	*Azospirillum*	PSB	PSF	
					A	P
1	-	-	Asp-1	-	-	-
2	-	-	Asp-2	-	-	-
3	-	-	Asp-3	-	-	-
4	Azt-4	-	Asp-4	-	PSF-4	-
5	-	-	Asp-5	-	-	-
6	-	-	-	PSB-6	-	-
7	-	-	-	-	PSF-7	-
8	Azt-8	-	Asp-8	-	PSF-8	-
9	Azt-9	-	-	-	-	-
10	Azt-10	-	Asp-10	PSB-10	-	-
11	-	-	Asp-11	PSB-11	PSF-11	-
12	Azt-12	-	-	-	-	-
13	-	Rh-13	-	-	-	-
14	-	-	Asp-14	-	-	-
15	-	-	Asp-15	PSB-15	-	-
16	-	-	Asp-16	-	-	-
17	-	-	-	PSB-17	-	-
18	Azt-18	-	-	-	-	-
19	-	-	-	-	PSF-19	-
20	-	-	-	PSB-20	-	-
21	Azt-21	Rh-21	Asp-21	-	-	-
22	-	Rh-22	-	-	-	-
23	Azt-23	-	Asp-23	-	-	-
24	-	-	Asp-24	-	-	-
25	Azt-25	-	Asp-25	-	-	-
26	Azt-26	-	-	-	-	-
27	Azt-27	-	Asp-27	-	-	-
28	Azt-28	Rh-28	Asp-28	-	PSF-28	-
29	Azt-29	Rh-29	Asp-29	-	-	-
30	-	-	-	-	-	PSF-30
31	-	-	Asp-31	-	-	-
32	Azt-32	-	-	-	PSF-32	-
33	Azt-33	-	Asp-33	PSB-33	-	-
34	Azt-34	-	Asp-34	-	-	-
35	-	-	Asp-35	-	-	-
36	Azt-36	-	Asp-36	-	-	-
37	Azt-37	Rh-37	Asp-37	-	-	-
38	-	-	Asp-38	-	-	-
39	Azt-39	Rh-39	Asp-39	PSB-39	-	-

contd...

Appendix-II contd...

Sample No.	*Azotobacter*	*Rhizobium*	*Azospirillum*	PSB	PSF	
					A	P
40	Azt-40	Rh-40	Asp-40	-	PSF-40	-
41	-	-	Asp-41	PSB-41	PSF-41	-
42	Azt-42	Rh-42	Asp-42	PSB-42	-	-
43	Azt-43	Rh-43	Asp-43	-	-	-
44	Azt-44	Rh-44	Asp-44	-	PSF-44	-
45	Azt-45	-	-	-	-	-
46	-	-	Asp-46	PSB-46	-	-
47	-	-	Asp-47	-	-	-
48	Azt-48	-	Asp-48	PSB-48	-	-
49	Azt-49	-	Asp-49	-	-	-
50	Azt-50	Rh-50	Asp-50	PSB-50	-	-
51	-	Rh-51	Asp-51	-	-	-
52	Azt-52	-	-	-	-	-
53	Azt-53	Rh-53	-	-	-	-
54	Azt-54	Rh-54	-	-	-	-
55	Azt-55	Rh-55	Asp-55	-	PSF-55	-
56	Azt-56	Rh-56	Asp-56	PSB-56	-	-
57	Azt-57	-	Asp-57	-	PSF-57	-
58	Azt-58	Rh-58	-	-	-	-
59	-	-	Asp-59	PSB-59	-	-
60	Azt-60	Rh-60	Asp-60	PSB-60	-	-
61	Azt-61	Rh-61	-	PSB-61	-	PSF-61
62	-	Rh-62	Asp-62	-	-	-
63	Azt-63	-	Asp-63	-	-	-
64	Azt-64	Rh-64	Asp-64	PSB-64	PSF-64	-
65	Azt-65	Rh-65	-	-	-	PSF-65
66	Azt-66	Rh-66	-	-	-	-
67	Azt-67	Rh-67	Asp-67	-	-	-
68	Azt-68	Rh-68	Asp-68	PSB-68	-	-
69	Azt-69	Rh-69	Asp-69	PSB-69	-	-
70	Azt-70	Rh-70	Asp-70	-	-	-
71	-	Rh-71	-	-	PSF-71	-
72	Azt-72	Rh-72	Asp-72	PSB-72	-	-
73	Azt-73	Rh-73	Asp-73	PSB-73	-	-
74	-	Rh-74	-	-	-	-
75	Azt-75	-	Asp-75	PSB-75	-	-
76	-	Rh-76	-	-	-	-
77	Azt-77	-	-	-	-	PSF-77
78	Azt-78	Rh-78	Asp-78	PSB-78	-	-
79	Azt-79	-	-	PSB-79	-	PSF-79
80	-	Rh-80	-	-	-	PSF-80
81	Azt-81	Rh-81	-	PSB-81	-	-

contd...

Appendix-II contd...

Sample No.	*Azotobacter*	*Rhizobium*	*Azospirillum*	PSB	PSF	
					A	P
82	Azt-82	Rh-82	Asp-82	-	PSF-82	PSF-82-1
83	Azt-83	-	Asp-83	PSB-83	-	-
84	Azt-84	Rh-84	-	-	-	-
85	-	Rh-85	Asp-85	-	-	PSF-85
86	Azt-86	Rh-86	Asp-86	-	-	-
87	-	-	Asp-87	-	-	-
88	Azt-88	-	Asp-88	-	-	-
89	Azt-89	-	-	-	-	-
90	-	-	Asp-90	-	-	-
91	Azt-91	-	-	-	-	-
92	-	-	Asp-92	-	-	PSF-92
93	Azt-93	Rh-93	-	PSB-93	-	-
94	Azt-94	-	-	-	-	-
95	-	Rh-95	Asp-95	PSB-95	-	-
96	-	-	Asp-96	-	-	-
97	Azt-97	Rh-97	Asp-97	-	-	PSF-97
98	-	Rh-98	-	-	-	-
99	Azt-99	-	Asp-99	-	-	-
100	-	Rh-100	-	PSB-100	PSF-100	-
101	Azt-101	Rh-101	Asp-101	-	PSF-101	PSF-101-1
102	Azt-102	Rh-102	Asp-102	PSB-102	-	PSF-102
103	-	-	Asp-103	-	-	-
104	Azt-104	-	-	-	-	-
105	Azt-105	Rh-105	-	-	-	-
106	Azt-106	Rh-106	Asp-106	PSB-106	-	-
107	Azt-107	Rh-107	Asp-107	PSB-107	-	-
108	Azt-108	Rh-108	Asp-108	-	-	-
109	Azt-109	Rh-109	-	PSB-109	-	-
110	Azt-110	-	-	-	-	-
111	-	-	-	PSB-111	-	-
112	Azt-112	Rh-112	Asp-112	-	-	-
113	Azt-113	Rh-113	Asp-113	PSB-113	-	-
114	Azt-114	Rh-114	-	-	-	-
115	-	Rh-115	-	-	PSF-115	-
116	-	-	Asp-116	-	-	-
117	-	Rh-117	Asp-117	-	-	-
118	Azt-118	Rh-118	-	-	-	PSF-118
119	-	-	Asp-119	PSB-119	PSF-119	-
120	Azt-120	Rh-120	Asp-120	-	PSF-120	PSF-120-1
121	-	-	Asp-121	-	-	-
122	Azt-122	-	-	-	PSF-122	-
123	-	Rh-123	Asp-123	-	-	-

contd...

Appendix-II contd...

Sample No.	*Azotobacter*	*Rhizobium*	*Azospirillum*	PSB	PSF	
					A	P
124	-	Rh-124	Asp-124	-	PSF-124	-
125	Azt-125	Rh-125	-	PSB-125	-	-
126	Azt-126	Rh-126	-	-	-	-
127	-	-	Asp-127	-	PSF-127	-
128	-	-	-	-	PSF-128	-
129	Azt-129	Rh-129	Asp-129	PSB-129	PSF-129	-
130	Azt-130	Rh-130	Asp-130	PSB-130	-	-
131	Azt-131	-	-	-	-	-
132	Azt-132	Rh-132	Asp-132	PSB-132	PSF-132	-
133	Azt-133	Rh-133	-	-	-	-
134	-	-	Asp-134	-	-	-
135	Azt-135	-	-	-	PSF-135	-
136	Azt-136	Rh-136	Asp-136	PSB-136	-	PSF-136
137	Azt-137	-	-	-	-	-
138	Azt-138	Rh-138	-	-	-	PSF-138
139	-	Rh-139	-	-	-	-
140	-	Rh-140	Asp-140	PSB-140	-	-
141	Azt-141	-	-	-	-	PSF-141
142	Azt-142	-	Asp-142	PSB-142	-	-
143	-	Rh-143	-	PSB-143	PSF-143	-
144	Azt-144	Rh-144	-	PSB-144	-	-
145	-	-	Asp-145	-	-	-
146	-	Rh-146	-	-	-	-
147	Azt-147	Rh-147	Asp-147	-	-	-
148	Azt-148	Rh-148	Asp-148	PSB-148	PSF-148	-
149	Azt-149	-	Asp-149	-	-	-
150	-	-	Asp-150	-	-	-
Total	**94**	**76**	**93**	**47**	**29**	**17**

A= *Aspergillus* **P**= *Penicillium*

Appendix–III

8.3 Composition of different growth media/reagents/indicators used

A) BMS agar medium (Krieg and Dobereiner, 1984)	
Peeled potato	200.00 g
Malic acid	2.50 g
KOH	2.00 g
Sucrose	2.50 g
Biotin	0.10 g
Bromothymol blue (0.5% alcoholic solution)	1.00 ml
Agar	15 g
Distilled water	1000 ml
pH	7.0
B) Cango red yeast extract mannitol agar medium (Hofer, 1935)	
Mannitol	10.00 g
K_2HPO_4	0.50 g
$MgSO_4.7H_2O$	0.20 g
NaCl	0.10 g
Yeast extract	1.00 g
Cango Red (1%)	2.50 ml
Agar	18.00 g
Distilled water	1000 ml
pH	7.0
C) Iodine solution (for starch hydrolysis)	
Iodine	0.50 g
Potassium iodide	1.00 g
Distilled water	1000 ml
D) Jensen's agar medium (Norris and Chapman, 1968)	
Sucrose	20.00 g
K_2HPO_4	1.00 g
$MgSO_4$	0.50 g
Na_2MoO_4	0.001 g
$FeSO_4.7H_2O$	0.01 g
$CaCO_3$	2.00g
Yeast Extract	0.50 g
Agar	18.00 g
Distilled water	1000 ml
pH	7.0-7.2

contd...

Appendix-III contd...

E) Luria Bertani agar (LB agar) (Miller, 1987)	
Tryptone	10.00 g
Yeast extract	5.00 g
NaCl	5.00 g
Agar	15.00 g
Distilled water	1000 ml
pH	7.2
F) Nutrient agar (Anon, 1957)	
Peptone	5.00 g
Beef extract	3.00 g
Sodium chloride	5.00 g
Agar	18.00 g
Distilled water	1000 ml
pH	6.8-7.2
G) Nitrogen-free malate semisolid medium (Okon *et al.*, 1977)	
Malic acid	5.00 g
K_2HPO_4	0.50 g
KOH	4.00 g
$MgSO_4$	0.10 g
NaCl	0.02 g
$CaCl_2$	0.01 g
$FeSO_4$	0.05 g
Na_2MoO_4	0.002 g
$MnSO_4$	0.01 g
Bromothymol Blue (0.5% alcoholic solution)	2.00 ml
Yeast Extract	0.50 g
Agar	1.80 g
Distilled water	1000 ml
pH	6.9-7.3
H) Nutrient gelatin (Cappuccino and Sherman, 1987)	
Peptone	5.00 g
Beef extract	3.00 g
Gelatin	120.00 g
Distilled water	1000 ml
pH	6.8-7.0

contd...

Appendix-III contd...

I) Potato dextrose agar (Okon et al., 1977)	
Peeled potato	200.00 g
Dextrose	20.00 g
Agar	18.00 g
Distilled water	1000 ml
J) Pikovskaya's medium modified by Sundara Rao and Sinha (1963)	
Glucose	10.00 g
Tricalcium phosphate	5.00 g
$(NH_4)_2\ SO_4$	0.50 g
KCl	0.20 g
$MgSO_4.7H_2O$	0.10 g
$MnSO_4$	0.0001 g
$FeSO_4$	0.0001 g
Yeast extract	0.50 g
Agar	18.00 g
Distilled water	1000 ml
pH	7.0-7.2
K) SIM agar (Cappuccino and Sherman, 1987)	
Peptone	30.00 g
Beef extract	3.00 g
Ferrous ammonium sulphate	0.20 g
Sodium thisoulphate	0.025 g
Agar	3.00 g
Distilled water	1000 ml
pH	7.3
L) Starch agar (Bailey and Schott, 1970)	
Peptone	5.00 g
Beef extract	3.00 g
Starch solution	10.00 ml
Agar	15.00 g
Distilled water	1000 ml
pH	7.2

contd...

Appendix-III contd...

M) Solid CS 7 medium (Pagan *et al.*, 1975)	
Na-succinate	25 mM
L-Arabinose	25 mM
Myo-inositol	5.6 mM
Glutamine	2.0 mM
$MgSO_4.7H_2O$	0.14 mM
KCl	0.9 mM
$CaCl_2.2H_2O$	0.7 mM
$MnSO_4.7H_2O$	3.5 mM
KH_2PO_4	2.2 mM
H_3BO_3	82 mM
$ZnSO_4.7H_2O$	3.5 mM
KI	6.0 mM
$CuSO_4.5H_2O$	0.8 mM
$Na_2MoO_4.2H_2O$	0.4 mM
$CoCl_2.6H_2O$	0.4 mM
$FeSO_4.7H_2O$	54 mM
Na_2 EDTA	54 mM
Thiamine.HCl	15 mM
Nicotinic acid	41 mM
Pyridoxine-HCl	2.4 mM
Agar (Noble Difco)	1% w/v
pH	5.9
N) Tetramethyl-para-phenylenediamine dihydrochloride (for oxidase acitivity)	
Tetramethyl-para-phenylenediamine dihydrochloride	5.00 g
Distilled water	50.00 ml
O) Trypticase soy agar (Frazier *et al.*, 1967)	
Trypticase (animal peptone)	15.00 g
Phytone (soy peptone)	5.00 g
Sodium chloride	5.00 g
Agar	15.00 g
Distilled water	1000 ml
pH	7.3

Appendix–IV

8.4 Reagents used for molecular characterization

Reagents for genomic DNA isolation:

Stock solutions:

1M Tris (pH 8.0)

Tris	:	121.1 g
Water	:	800 ml
pH (adjusted with concentrated HCl)	:	8.0
Total volume	:	1000 ml

EDTA 0.5 M (pH 8.0)

EDTA	:	18.6 g
Water	:	80 ml
pH (adjusted with NaOH)	:	8.0
Total volume	:	100 ml

Proteinase K: 100 mg/ml

Dissolved 100 mg of proteinase K in 1 ml of sterilized milli Q water and stored at –20°C and used @ 5 µl/sample.

DNase free RNase A: 10 mg/ml

Dissolved 10 mg of DNAase free RNAase A in 1 ml of sterilized milli Q water and heated in thermal cycler at 95°C for 5 min. and stored at –20°C and used @ 10 µl/sample.

Extraction Buffer (2% Sarcosyl in $T_{50}E_{20}$): 50 ml

Stock conc.	Chemical		Quantity	Final conc.
1 M	Tris	:	2.5 ml	50 mM
0.5 M	EDTA	:	2.0 ml	20 mM
20%	Sarcosyl	:	5.0 ml	2%
	Sterilized milli Q water	:	40.5 ml	
	Total	:	50.0 ml	

The extraction buffer used @ 500 µl /sample

Appendix-IV contd...

CTAB Extraction Buffer: 20 ml

Stock conc.	Chemical		Quantity	Final conc.
1 M	Tris pH 7.5	:	2.0 ml	100 mM
5 M	NaCl	:	2.8 ml	700 mM
0.5 M	EDTA pH 8.0	:	2.0 ml	50 mM
20%	CTAB	:	1.0 ml	1%
	Sterilized milli Q water	:	12.2 ml	
	Total	:	20.0 ml	

The CTAB extraction buffer used @ 1 ml /sample

Use freshly made CTAB extraction buffer; warm buffer to 60-65°C before use.

Loading dye composition

Loading dye (6x) : 0.25% bromophenol blue
store at 4°C in 40% glycerol

Recipe for 1.2 per cent agarose gel (100 ml)

Agarose : 1.2 g
1X TBE Buffer : 100 ml
Ethidium Bromide (10 mg/ml) : 5 µl

10X TBE buffer composition

Tris base : 108 g
Boric acid : 55 g
0.5 M EDTA (pH 8.0) : 40 ml
Make total volume 1000 ml with sterilized milli Q water

Buffer	Concentration	10x Buffer Composition
Taq Buffer A (with $MgCl_2$)	10X	100 mM Tris (pH 9.0)
		500 mM KCl
		15 mM $MgCl_2$
		0.1% Gelatin

Buffer	Concentration	10x Buffer Composition
Taq Buffer B (without $MgCl_2$)	10X	100 mM Tris (pH 9.0)
		500 mM KCl
		0.1% Gelatin

Appendix-IV contd...

PCR reaction mixture (20 µl /tube) for RAPD analysis of nitrogen fixing (*Azotobacter*, *Rhizobium* and *Azospirillum*) isolates:

Stock conc.	Chemical		Quantity	Working conc.
10X with 15 mM $MgCl_2$	Taq Buffer A	:	2.0 µl	1X with 1.5 mM $MgCl_2$
25 mM (Extra)	$MgCl_2$	:	0.4 µl	0.5 mM
2.5 mM	dNTP mix	:	1.6 µl	0.2 mM
3U/µl	Taq DNA polymerase	:	0.33 µl	1 Unit
20 picomoles/µl	Primer	:	1.0 µl	1 picomoles/µl
20 ng/µl	Template DNA	:	1.0 µl	
	Sterile milli Q water	:	13.67 µl	
	Total volume	:	20 µl	

PCR reaction mixture (20µl/tube) for RAPD analysis of PSB (*Bacillus*) isolates:

Stock conc.	Chemical		Quantity	Working conc.
10X (without $MgCl_2$)	Taq Buffer B	:	2.0 µl	1X (without $MgCl_2$)
25 mM	$MgCl_2$	:	1.6 µl	2.0 mM
2.5 mM	dNTP mix	:	1.6 µl	0.2 mM
3U/µl	Taq DNA polymerase	:	0.33 µl	1 Unit
20 picomoles/µl	Primer	:	1.0 µl	1 picomoles/µl
20 ng/µl	Template DNA	:	1.0 µl	
	Sterile milli Q water	:	12.47 µl	
	Total volume	:	20 µl	

PCR reaction mixture (20µl/tube) for RAPD analysis of PSF (*Aspergillus* and *Penicillium*) isolates:

Stock conc.	Chemical		Quantity	Working conc.
10X with 15 mM $MgCl_2$	Taq Buffer A	:	2.0 µl	1X with 1.5 mM $MgCl_2$
25 mM (Extra)	$MgCl_2$	:	0.4 µl	0.5 mM
2.5 mM	dNTP mix	:	1.6 µl	0.2 mM
3U/µl	Taq DNA polymerase	:	0.33 µl	1 Unit
20 picomoles/µl	Primer	:	1.0 µl	1 picomoles/µl
20 ng/µl	Template DNA	:	1.0 µl	
	Sterile milli Q water	:	13.67 µl	
	Total volume	:	20 µl	

9. VITA

Mr. MURUMKAR DATTATRAYA RANGNATH

A candidate for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

AGRICULTURAL MICROBIOLOGY

2011

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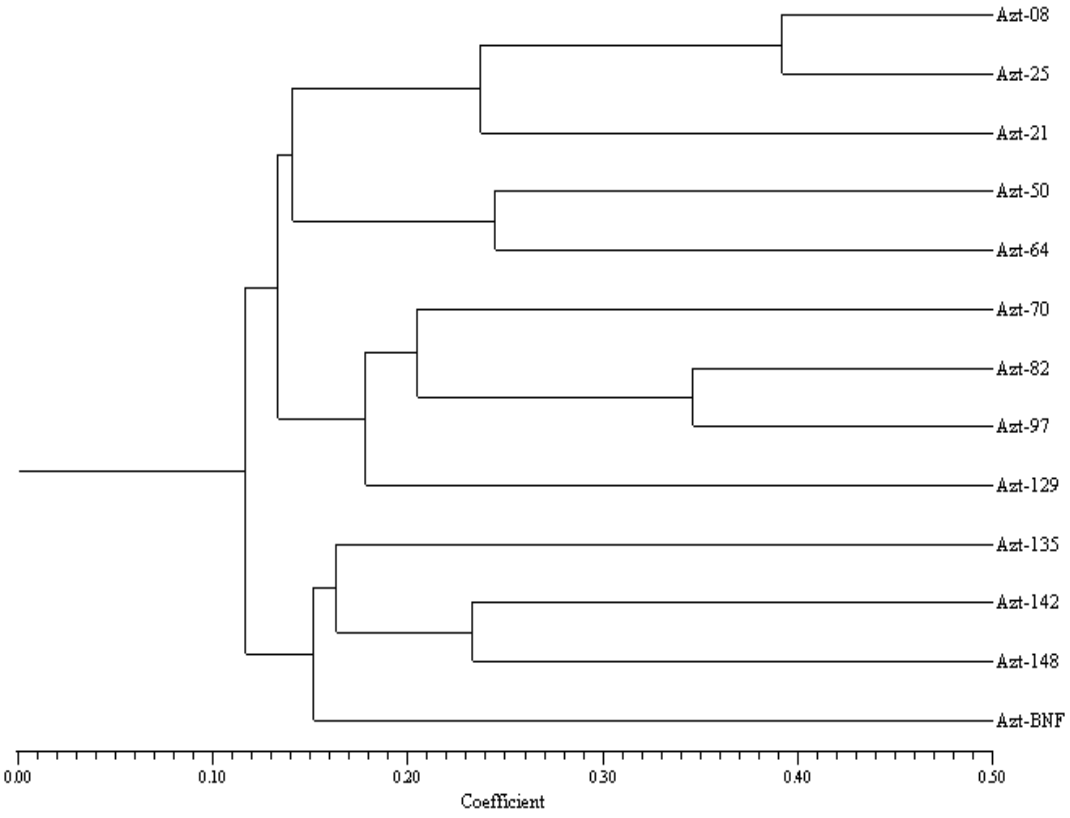


Figure 7. Dendrogram representing the clustering among different isolates of *Azotobacter chroococcum*

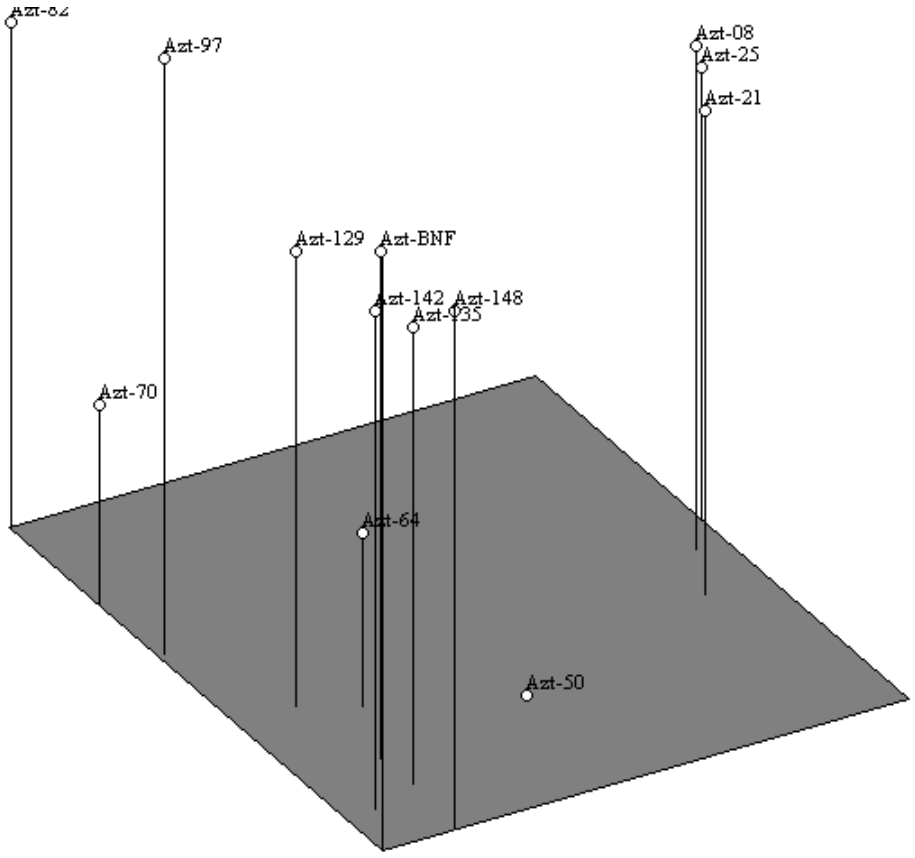


Figure 8. RAPD 3D PCO scatter plot representing the phylogenetic relationship among different isolates of *A. chroococcum*

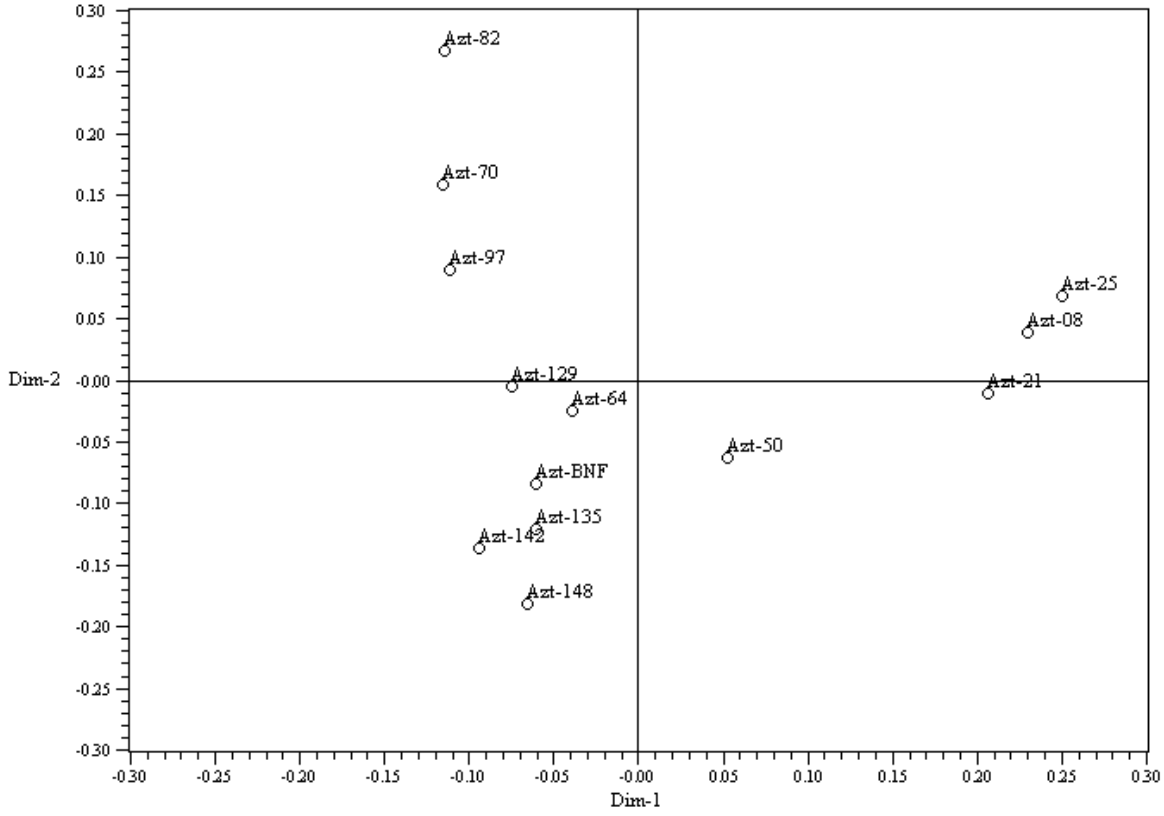


Figure 9. RAPD 2D PCO scatter plot representing the phylogenetic relationship among different isolates of *A. chroococcum*

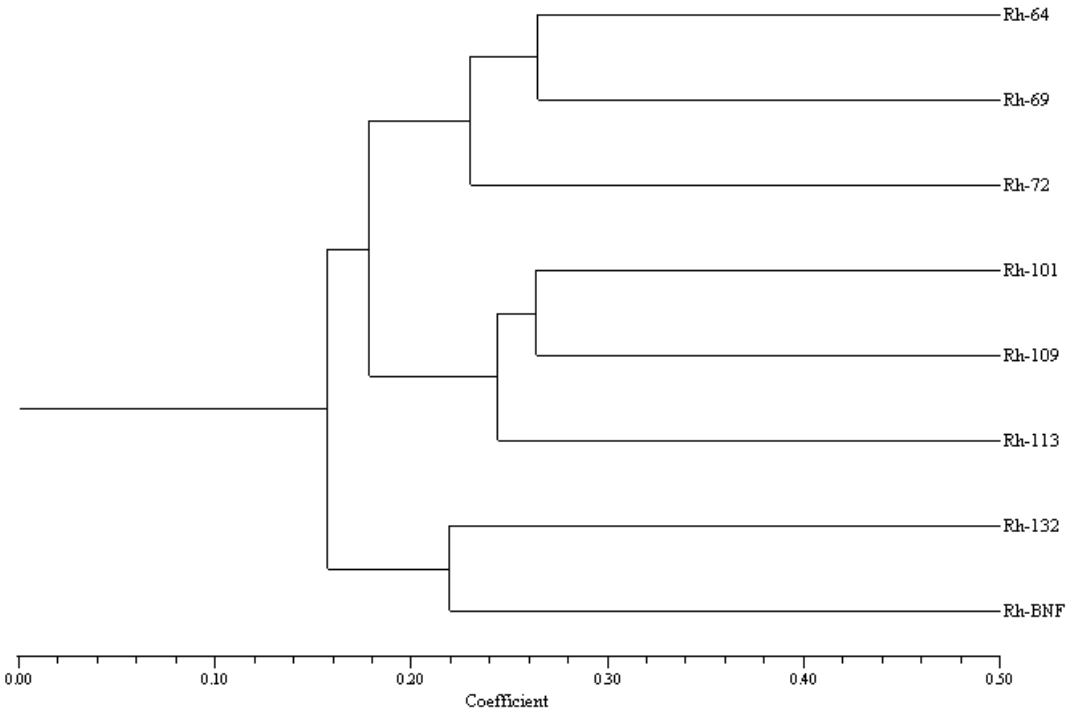


Figure 10. Dendrogram representing the clustering among different isolates of *Rhizobium* spp

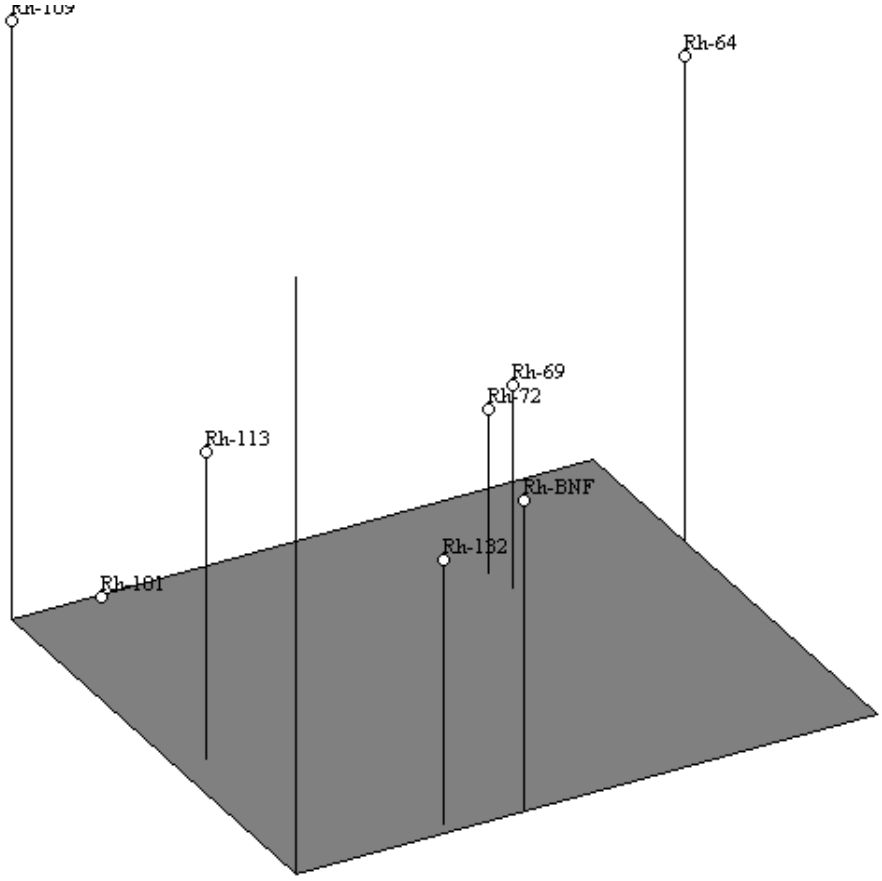


Figure 11. RAPD 3D PCO scatter plot representing the phylogenetic relationship among different isolates of *Rhizobium* spp

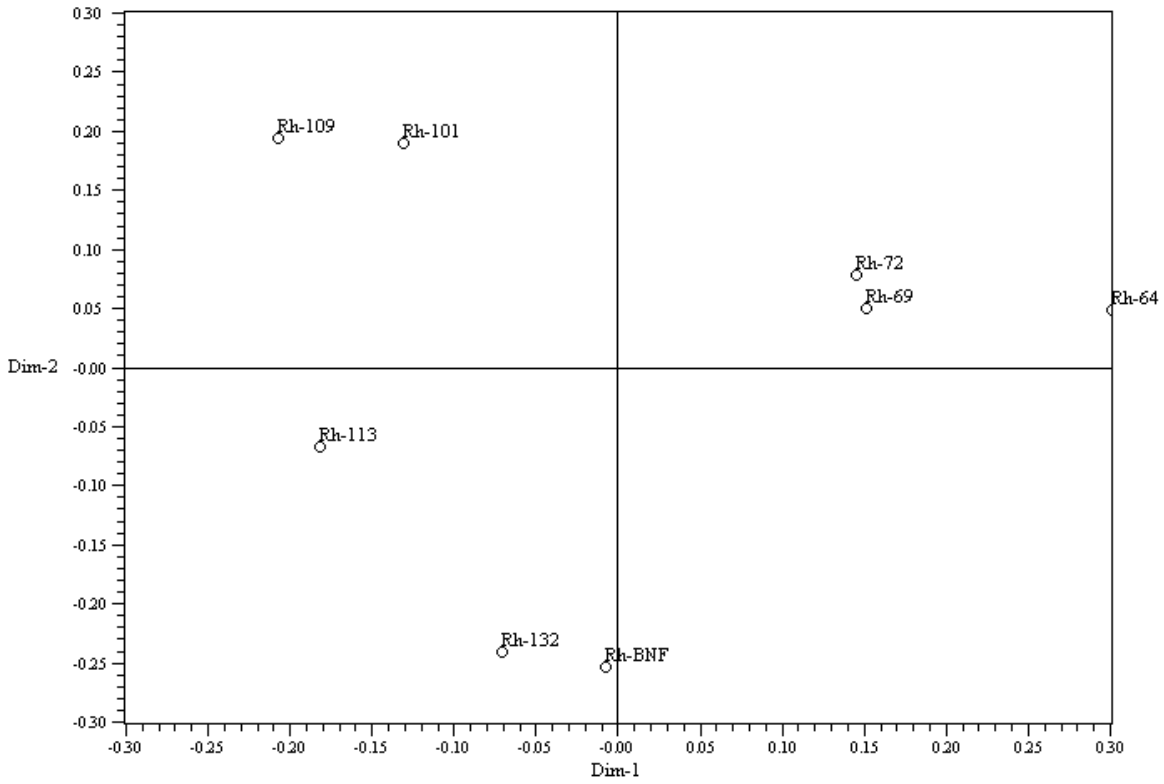


Figure 12. RAPD 2D PCO scatter plot representing the phylogenetic relationship among different isolates of *Rhizobium* spp

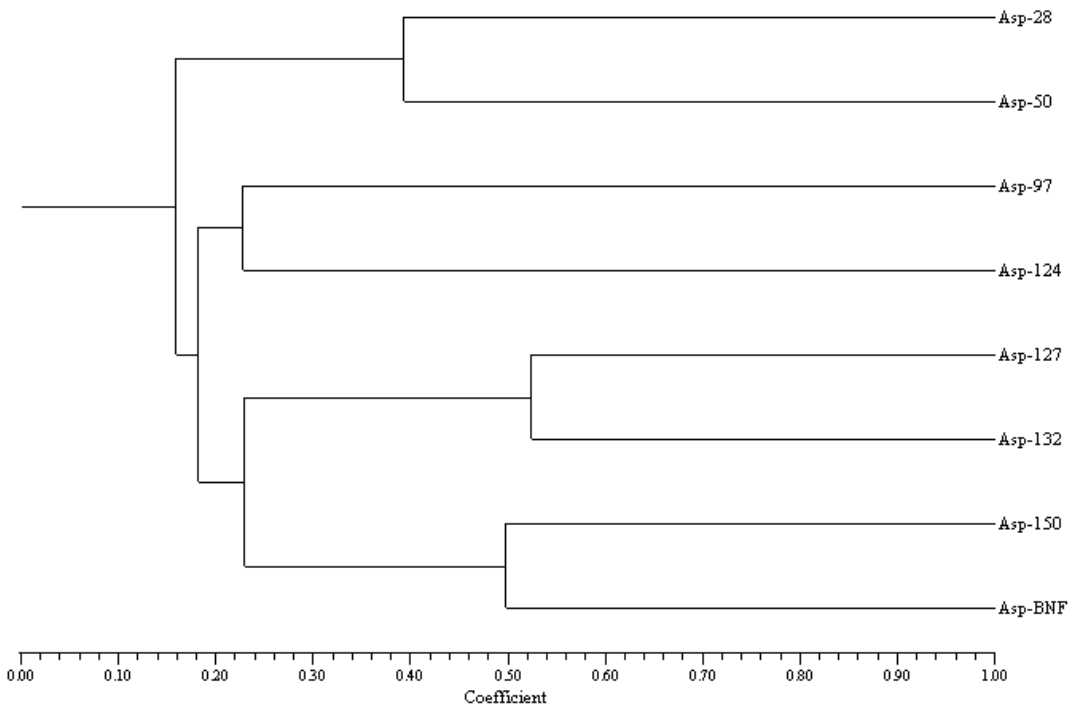


Figure 13. Dendrogram representing the clustering among different isolates of *Azospirillum lipoferum*

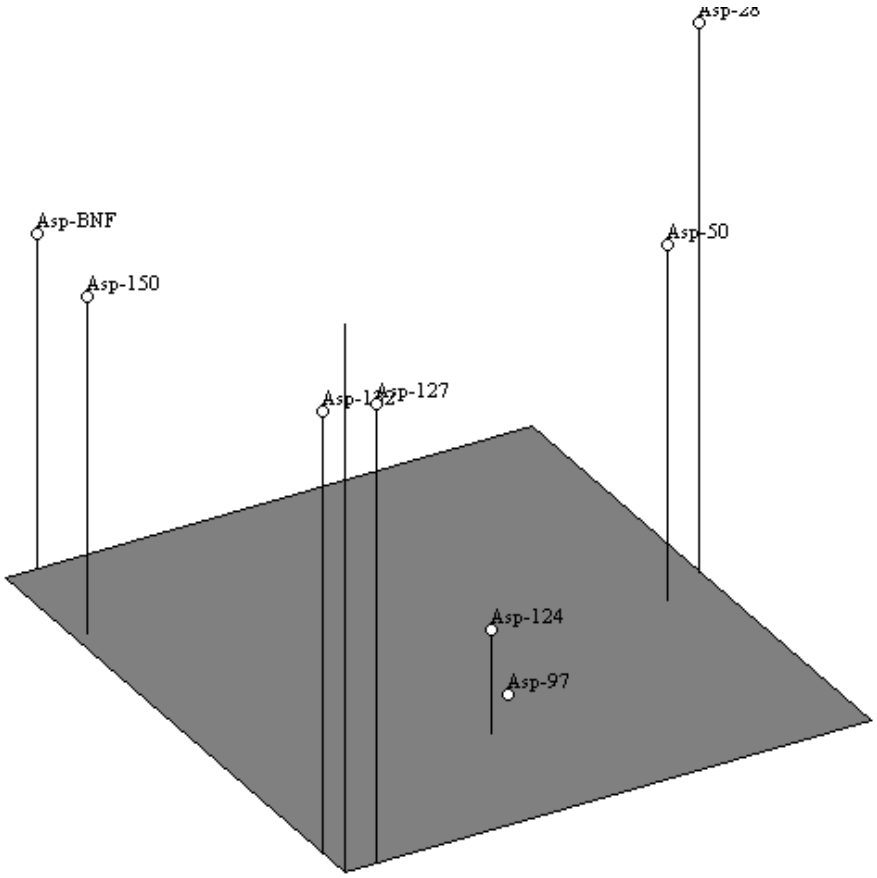


Figure 14. RAPD 3D PCO scatter plot representing the phylogenetic relationship among different isolates of *Azospirillum lipoferum*

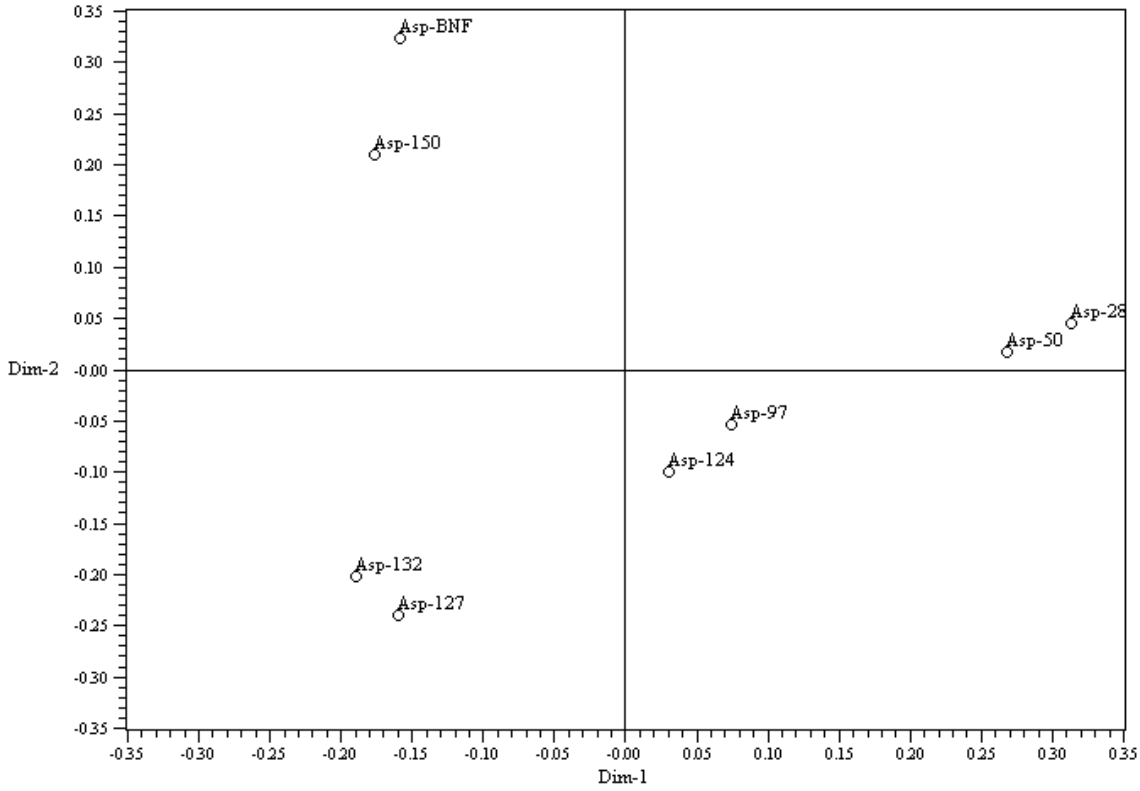


Figure 15. RAPD 2D PCO scatter plot representing the phylogenetic relationship among different isolates of *Azospirillum lipoferum*

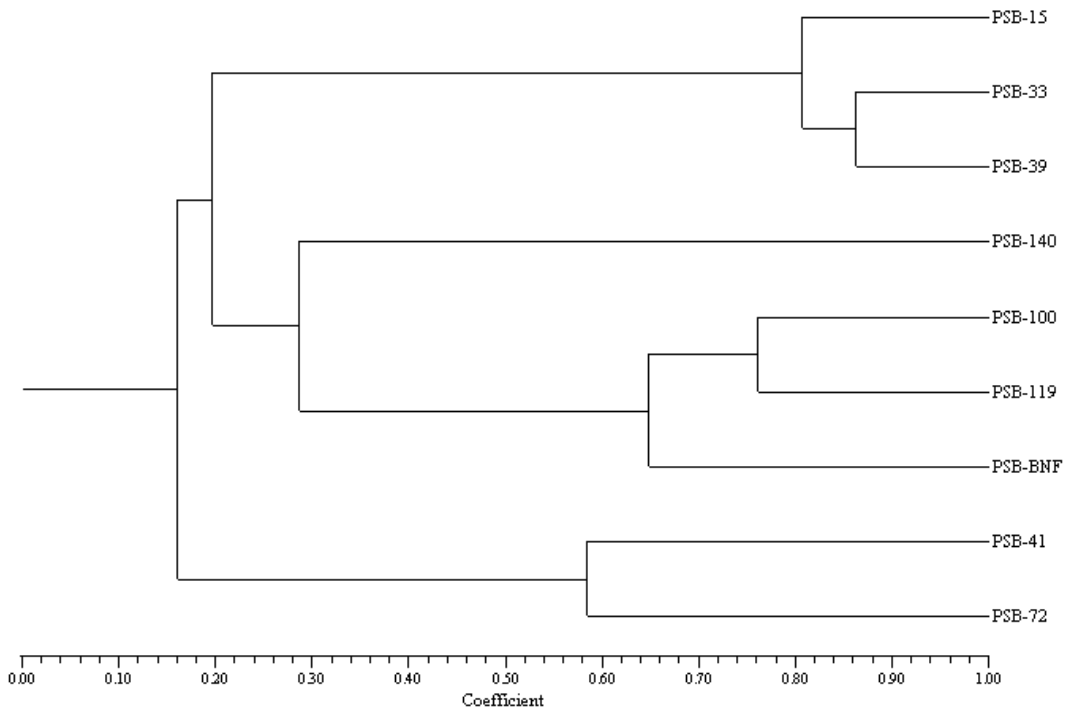


Figure 16. Dendrogram representing the clustering among different isolates of *Bacillus megaterium*

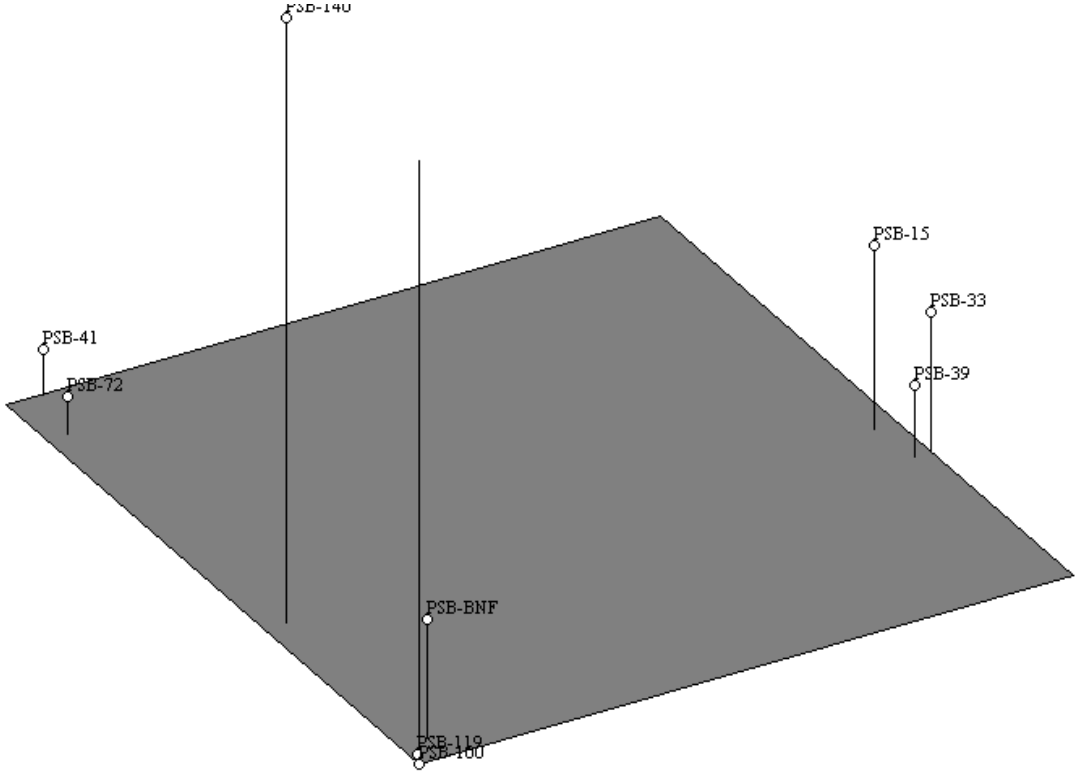


Figure 17. RAPD 3D PCO scatter plot representing the phylogenetic relationship among different isolates of *Bacillus megaterium*

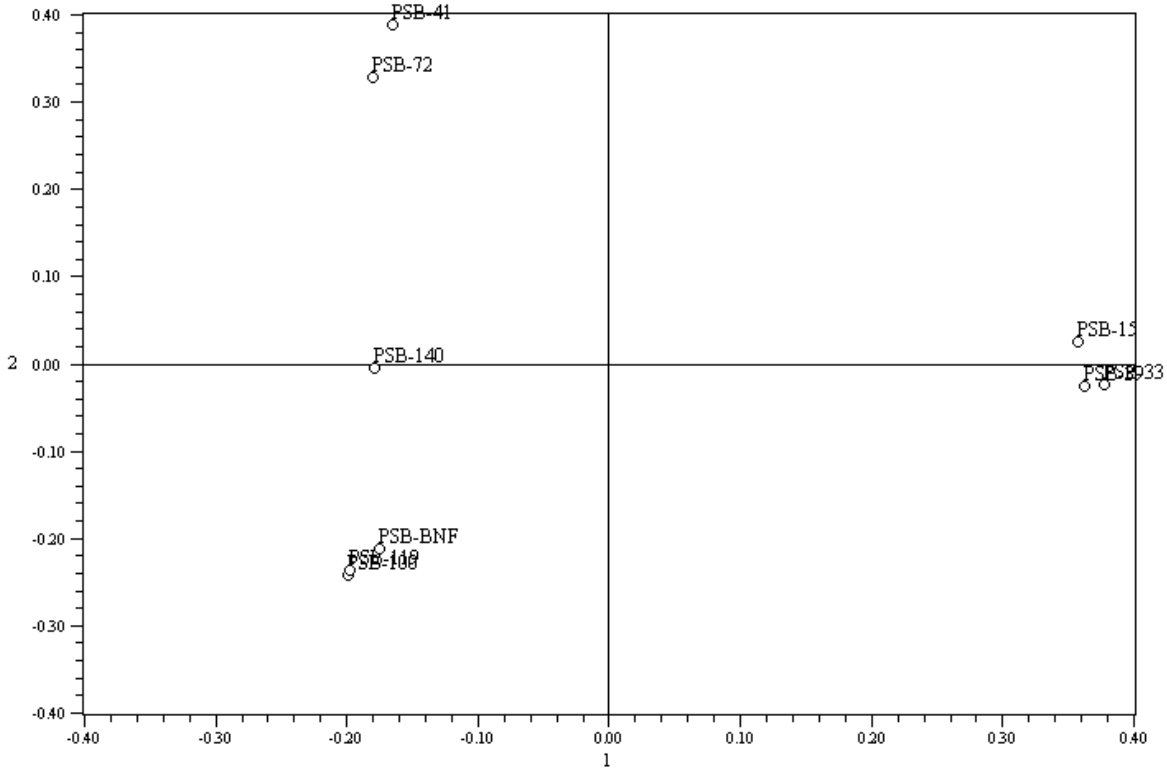


Figure 18. RAPD 2D PCO scatter plot representing the phylogenetic relationship among different isolates of *Bacillus megaterium*

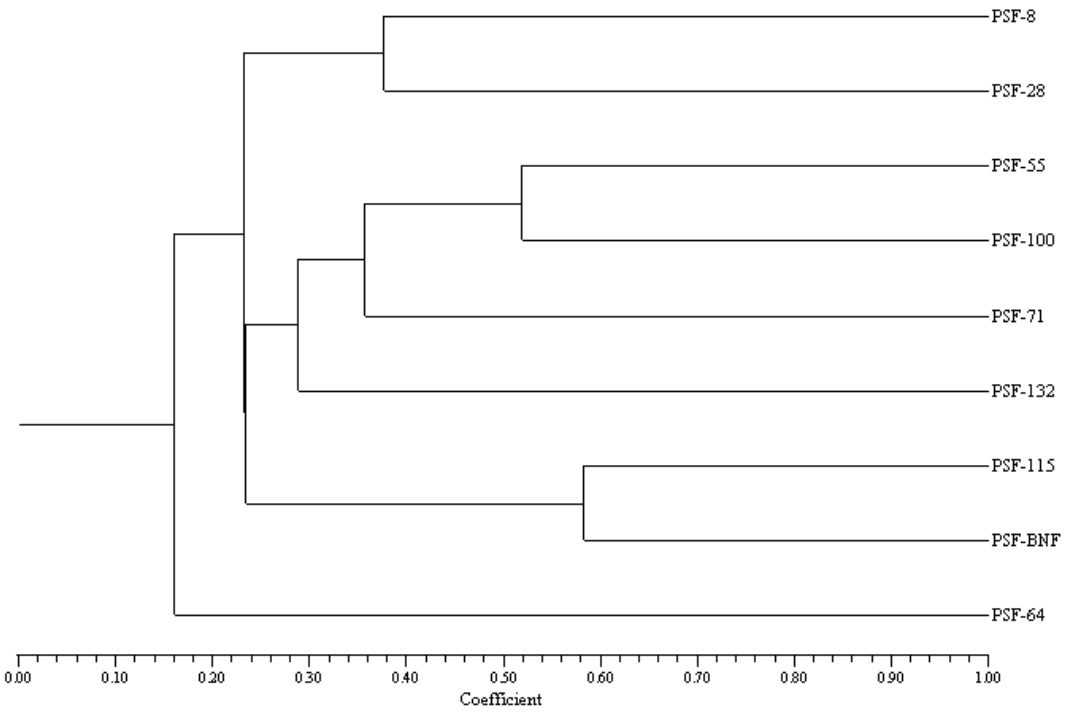


Figure 19. Dendrogram representing the clustering among different isolates of *Aspergillus awamori*

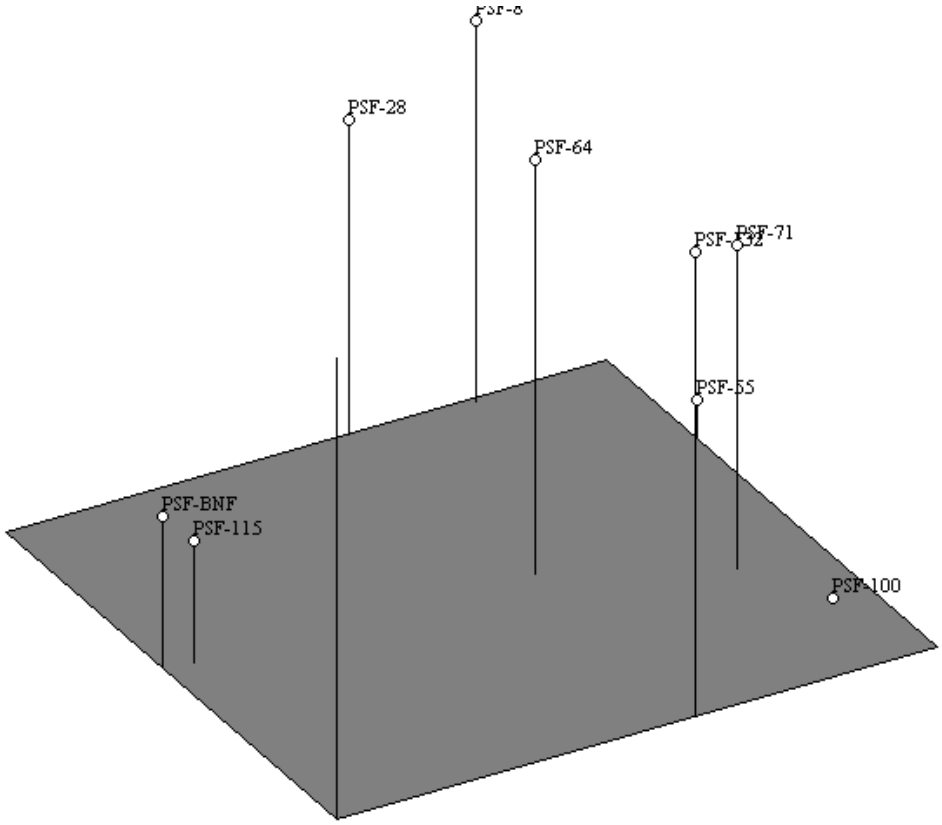


Figure 20. RAPD 3D PCO scatter plot representing the phylogenetic relationship among different isolates of *Aspergillus awamori*

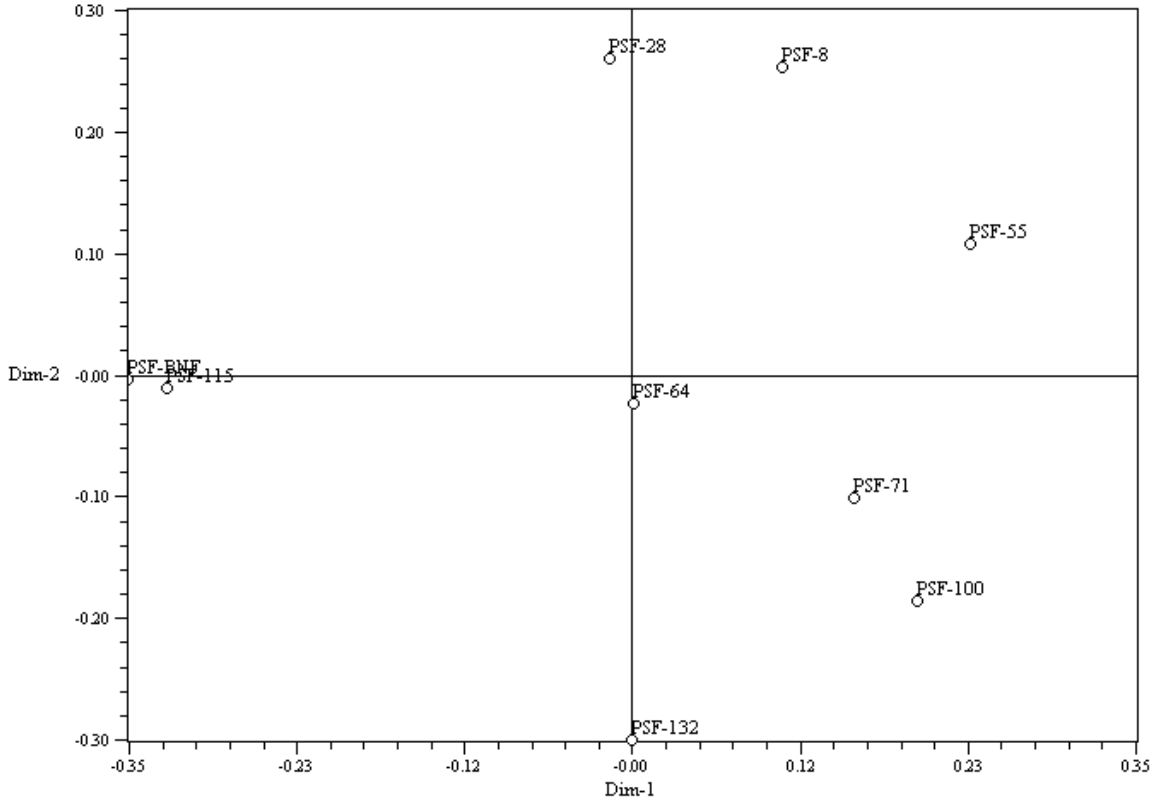


Figure 21. RAPD 2D PCO scatter plot representing the phylogenetic relationship among different isolates of *Aspergillus awamori*

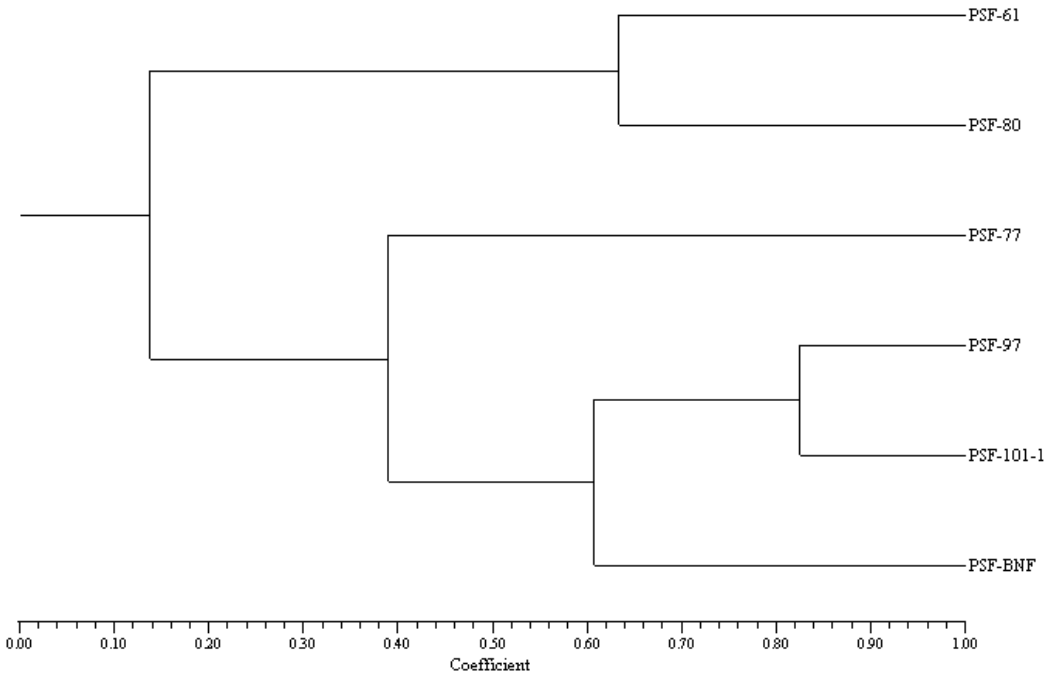


Figure 22. Dendrogram representing the clustering among different isolates of *Penicillium digitatum*

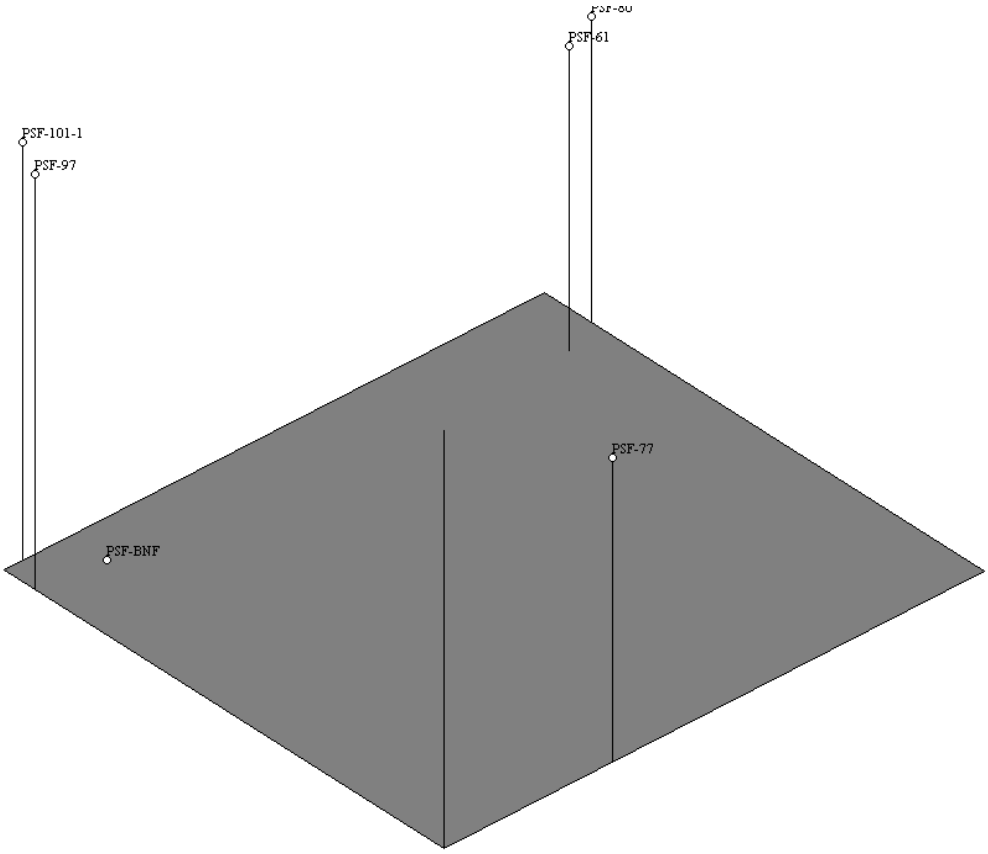


Figure 23. RAPD 3D PCO scatter plot representing the phylogenetic relationship among different isolates of *Penicillium digitatum*

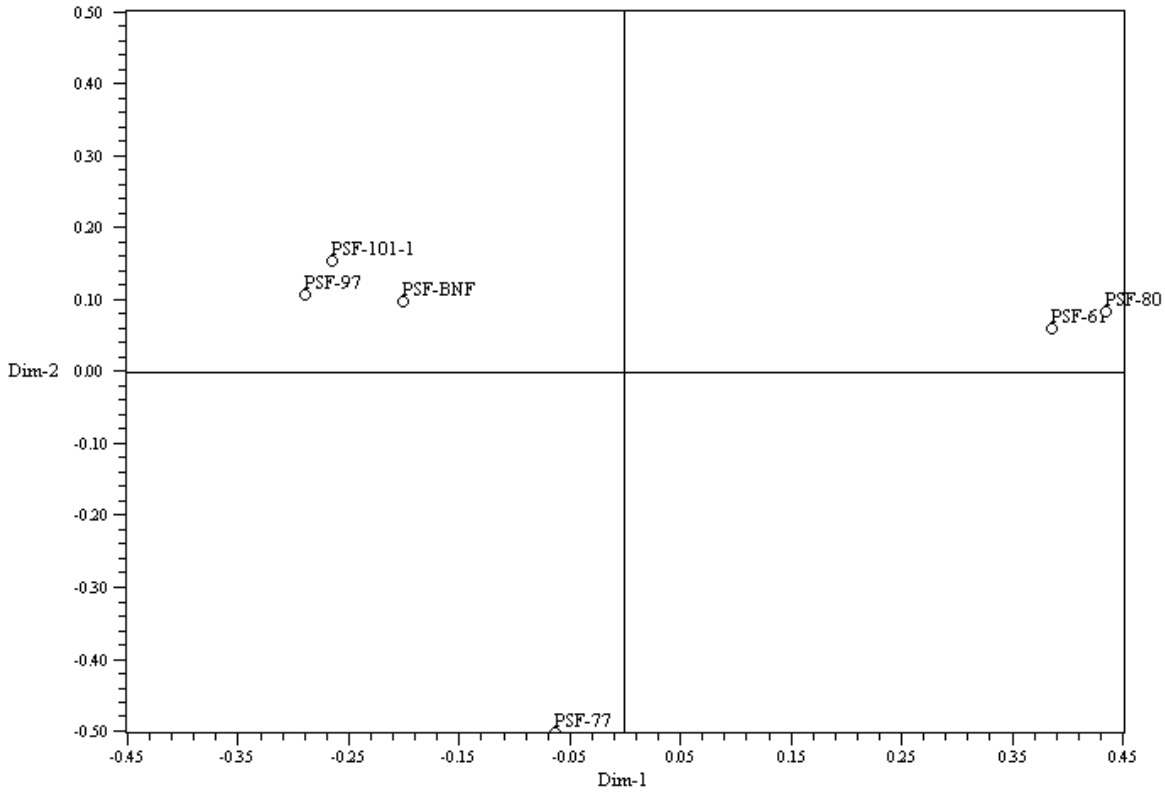


Figure 24. RAPD 2D PCO scatter plot representing the phylogenetic relationship among different isolates of *Penicillium digitatum*

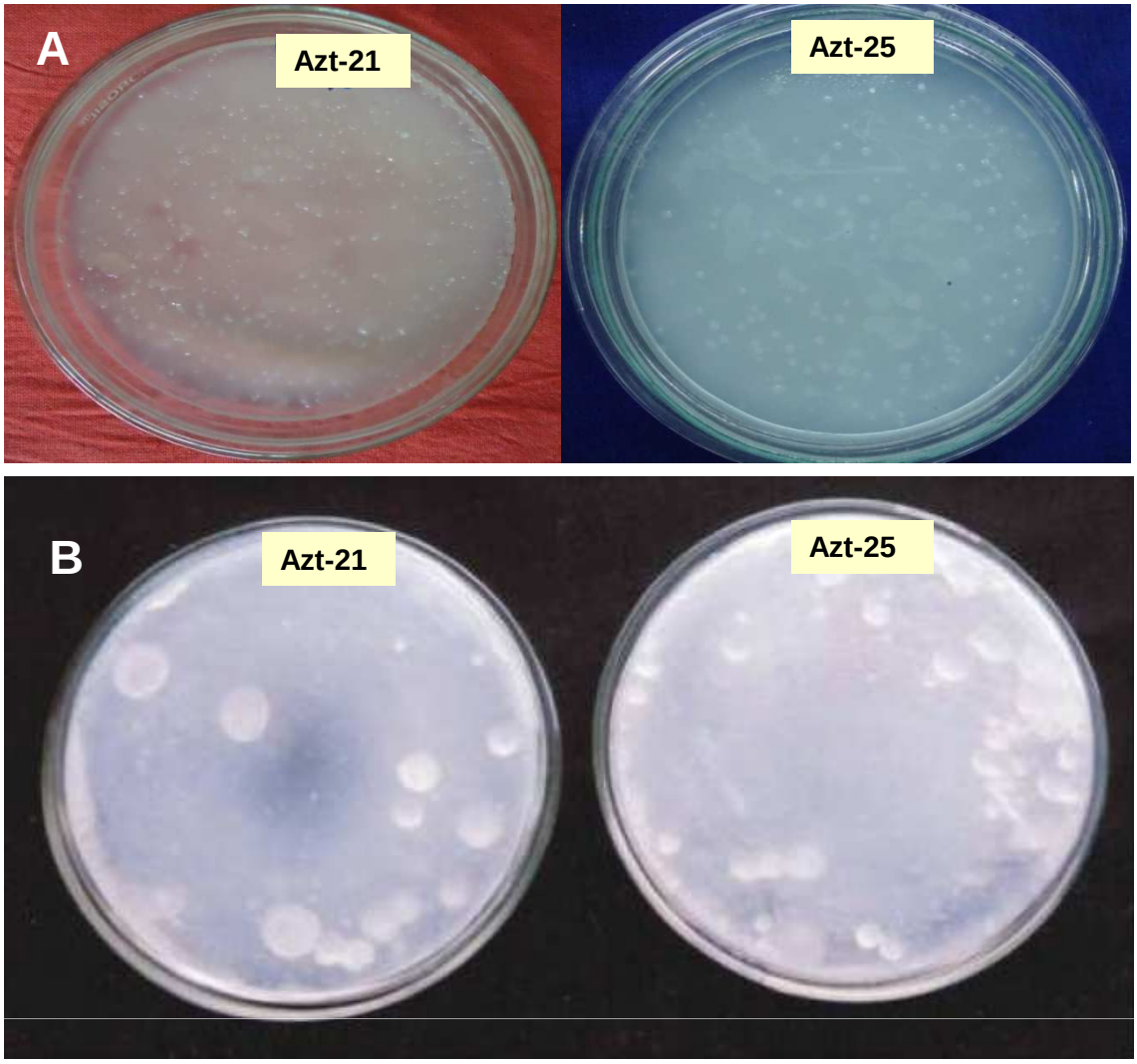


Plate 1. Growth of *Azotobacter* on Jensen's agar medium
A – After 2 days of incubation B – After 8 days of incubation

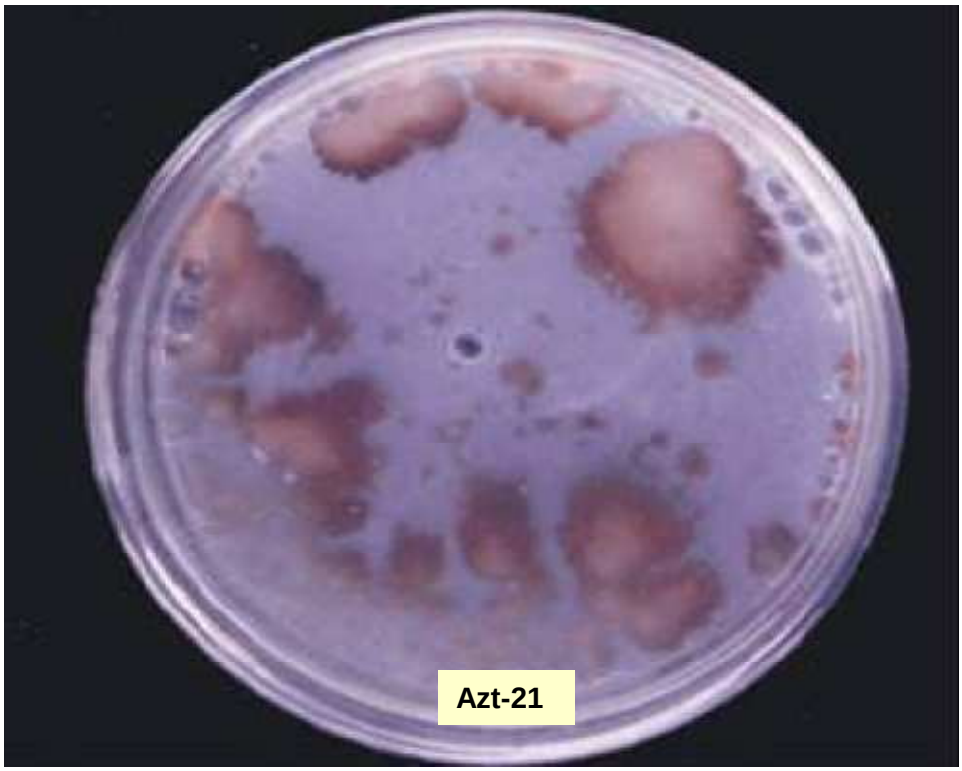


Plate 2. Brown pigment production from *Azotobacter* on Jensen's agar medium after 15 days of incubation

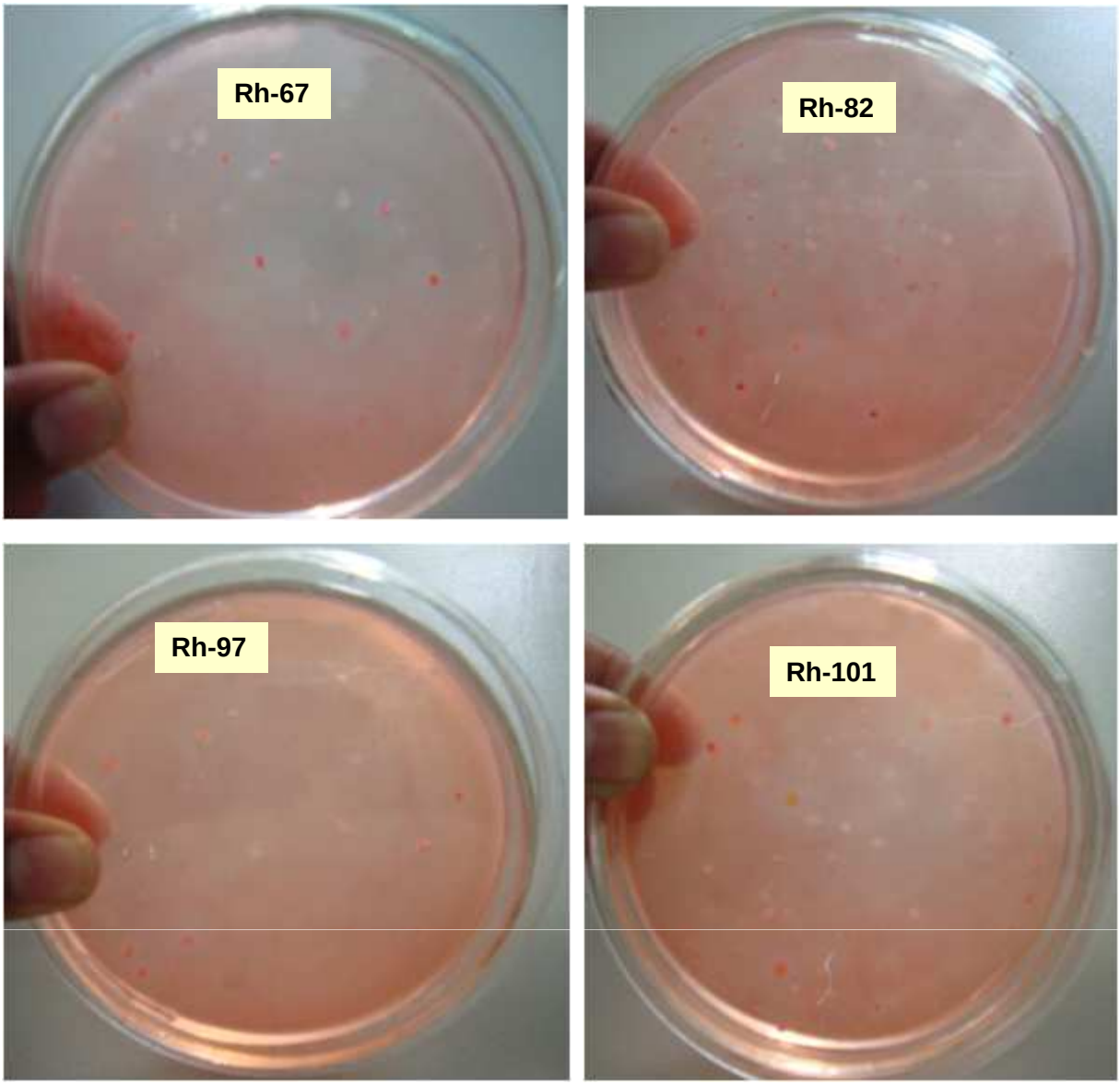


Plate 3. Growth of *Rhizobium* on CRYEMA medium
White colony - *Rhizobium* Red colony - *Agrobacterium*

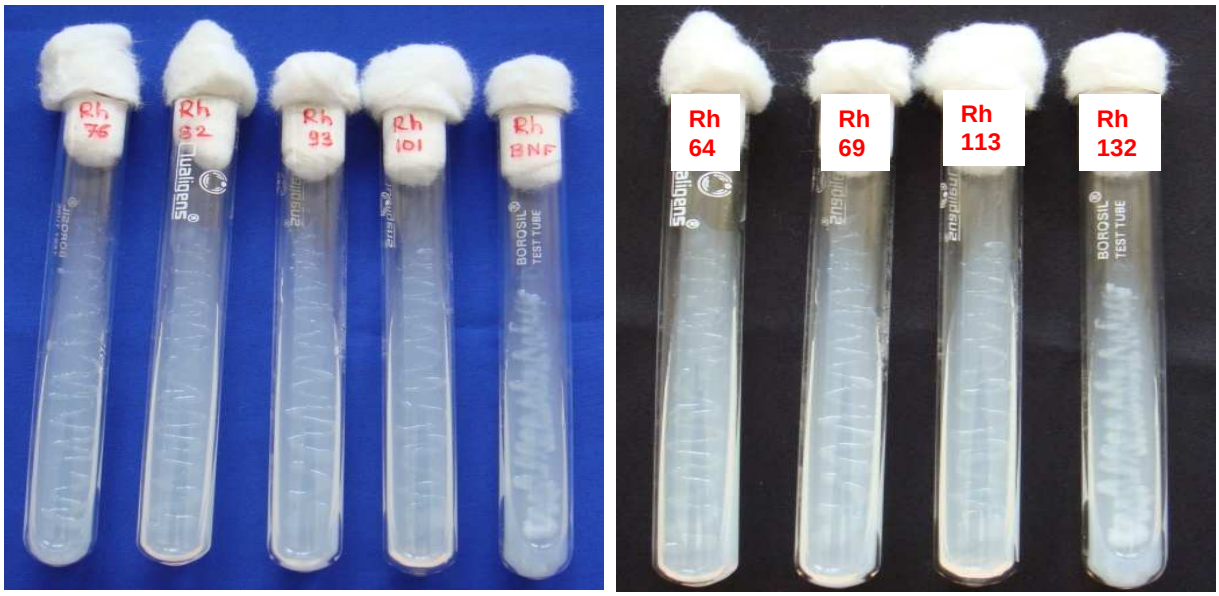


Plate 4. Pure cultures of *Rhizobium* isolates on YEMA medium

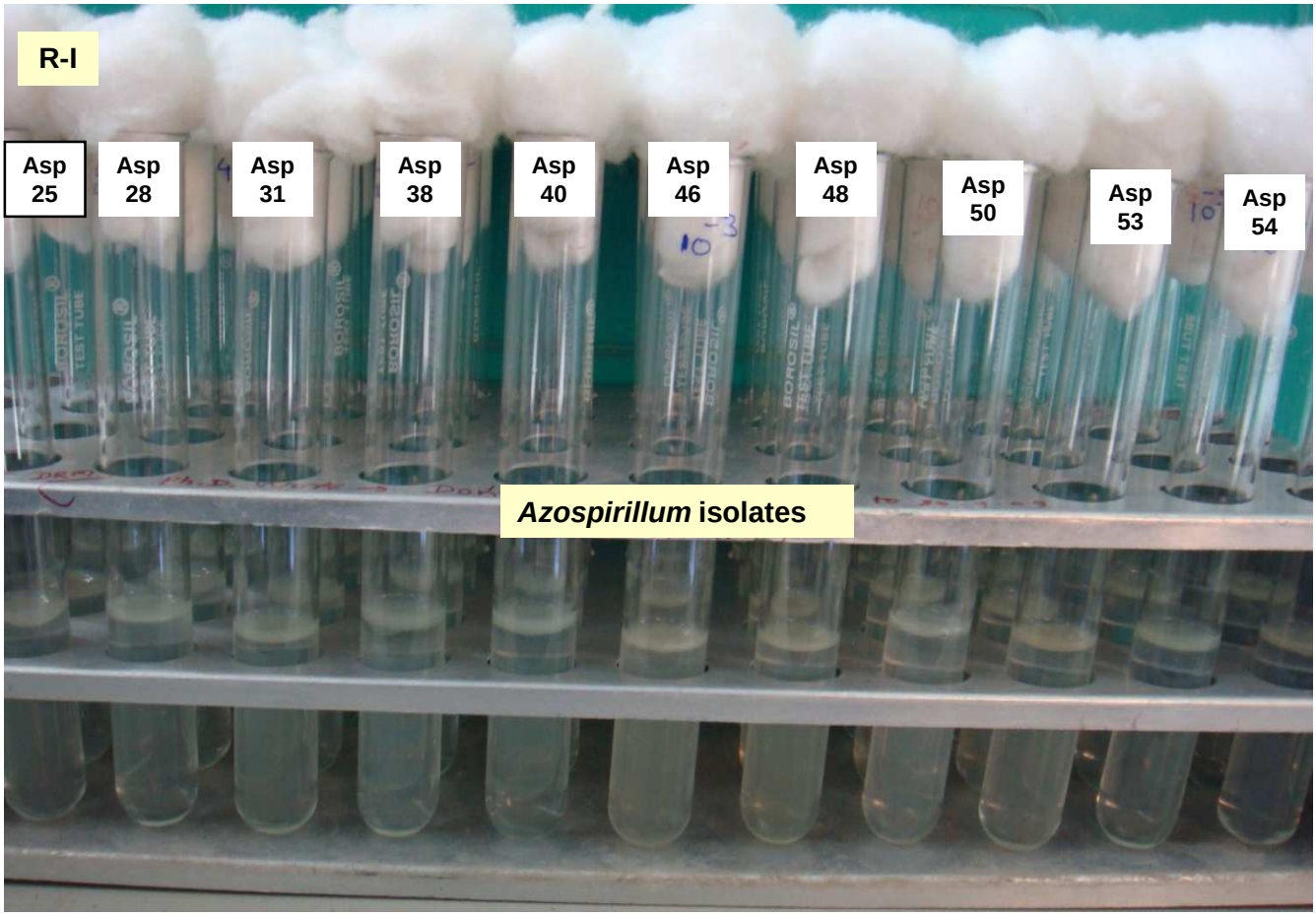


Plate 5. Subsurface pellicle formation by *Azospirillum* isolates on N-free malate semisolid medium

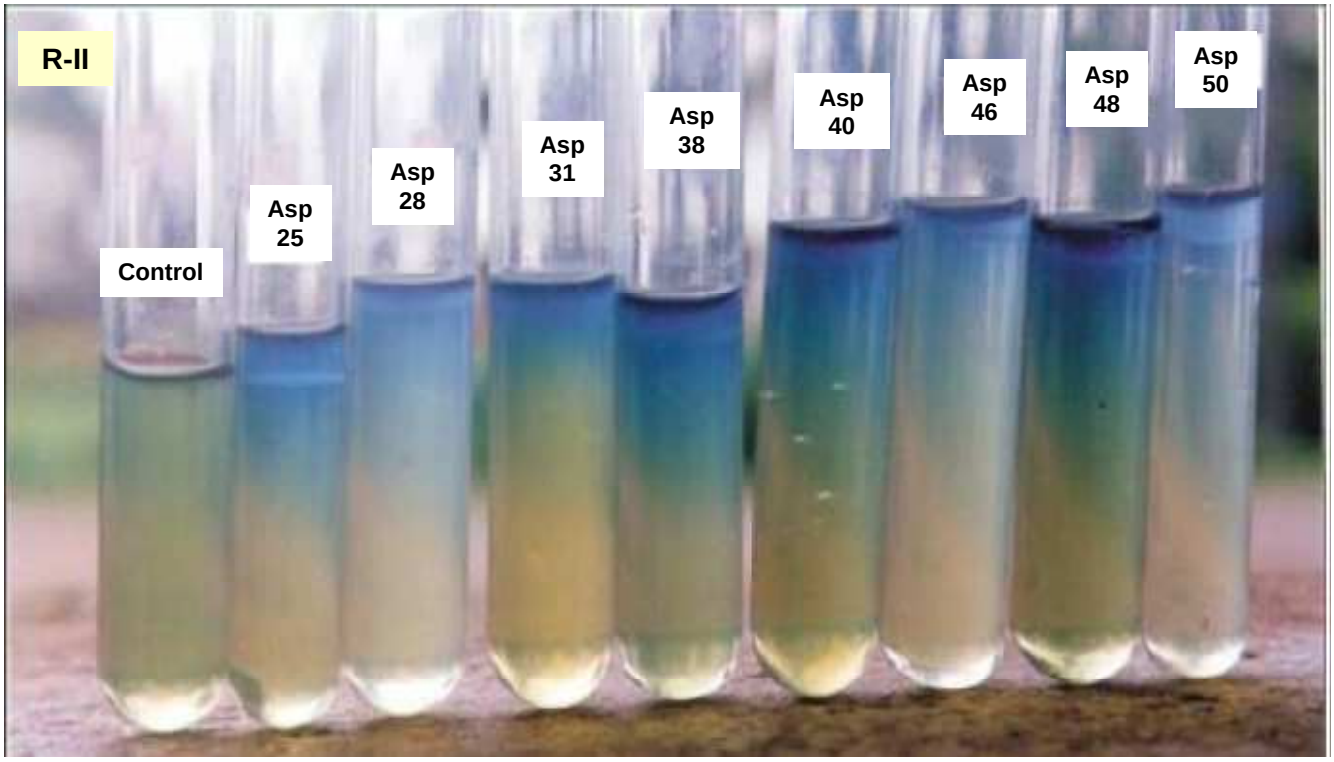


Plate 6. Change in colour of the medium from grass green to blue after incubation of a week and white pellicle formation indicate the *Azospirillum* isolate

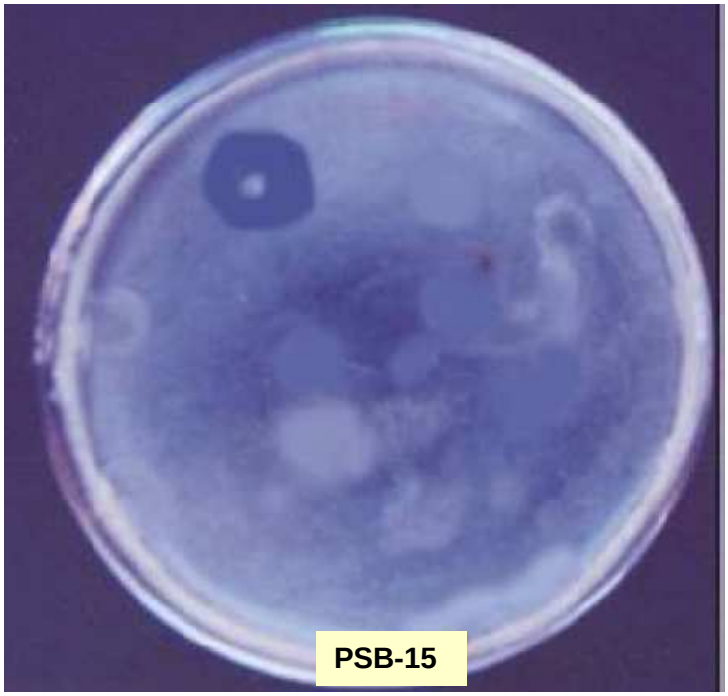
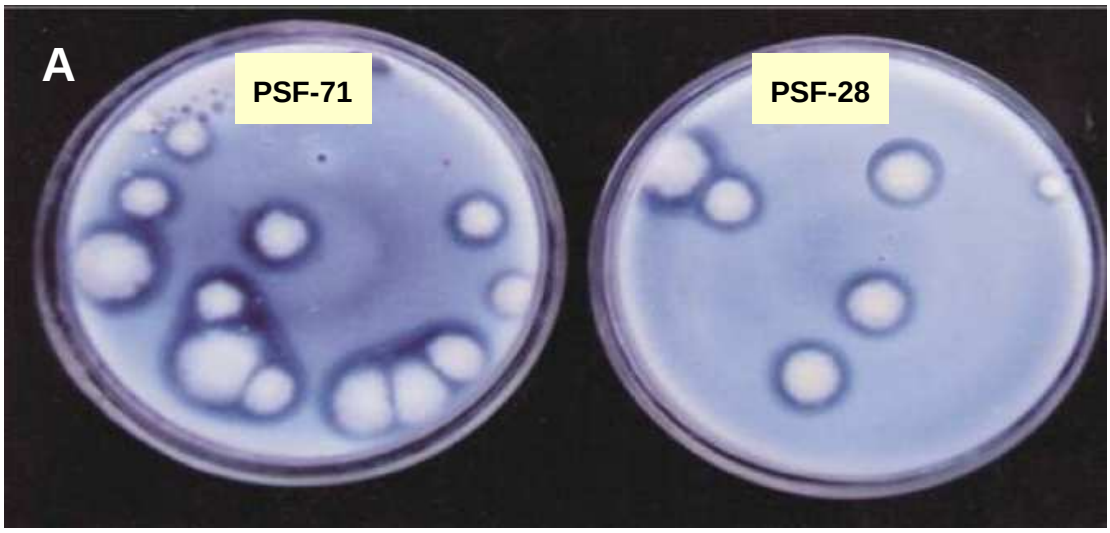


Plate 7. PSB isolate showing hallo zone of solubilization of tricalcium phosphate on Pikovskaya's agar medium



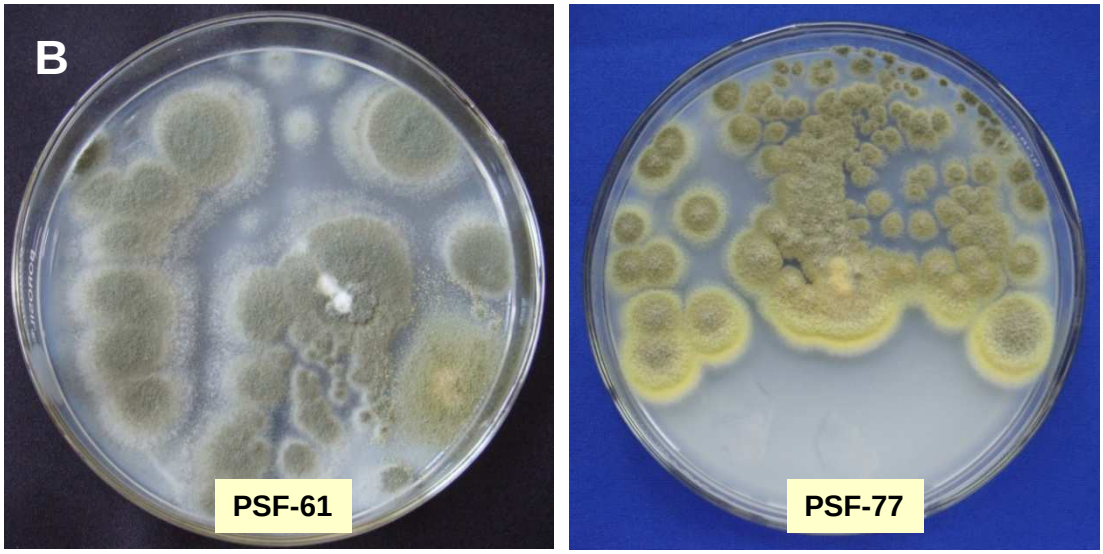


Plate 8. PSF isolates showing hallo zone of solubilization of tricalcium phosphate on Pikovskaya's agar medium
A) *Aspergillus* isolates B) *Penicillium* isolates

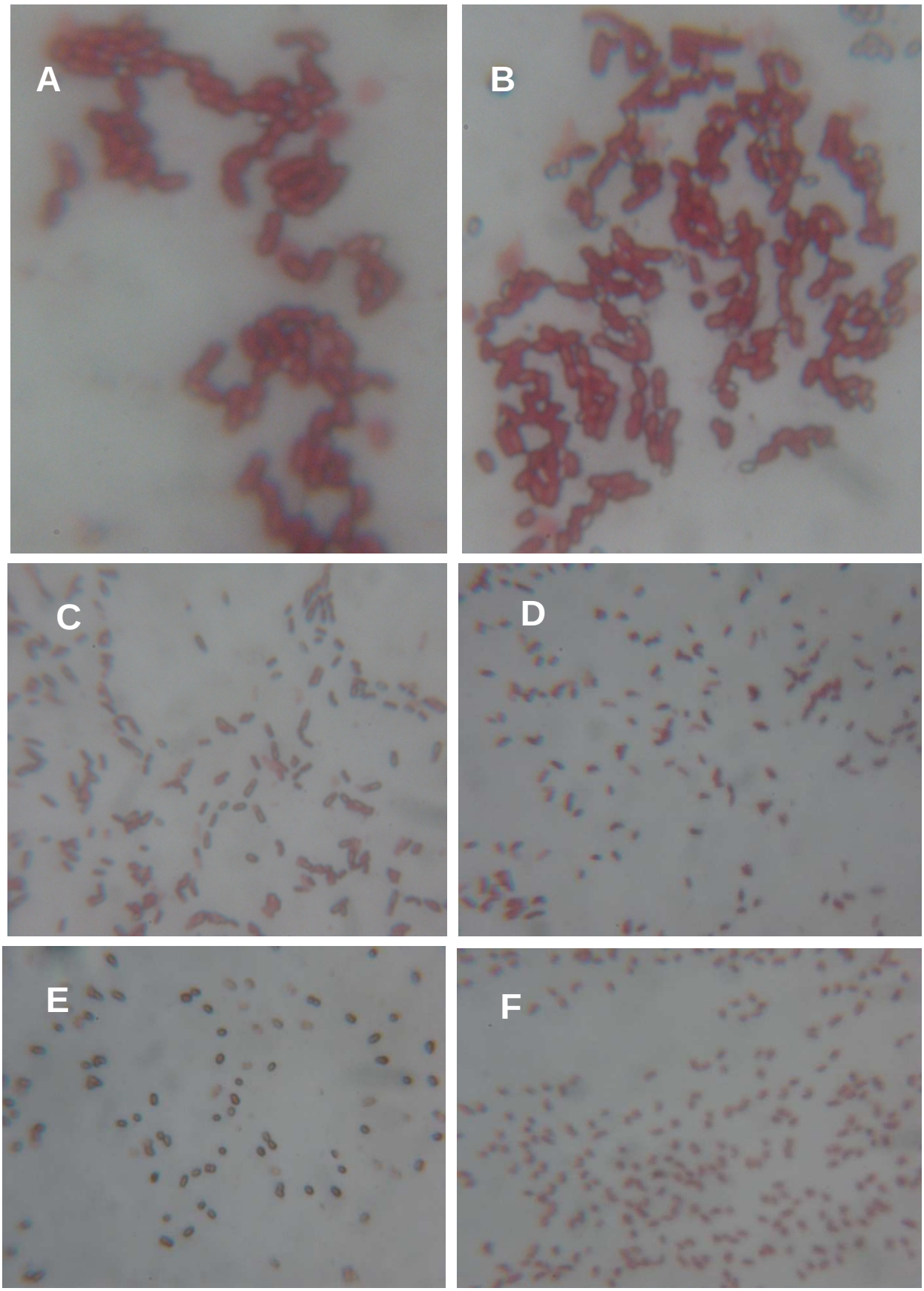


Plate 9. Cell morphology of *Azotobacter* isolates (A to F)
A) Azt-21 – Cells are rod shape, in chain & form irregular clumps B) Azt-25 – Cells are rod shape & in chain
C) Azt-08 – Cells are rod shape, single cells as well as in pairs D) Azt-64 – Single cells having rod shape
E) Azt-130 – Single cells having oval shape F) Azt-131–Single cells having oval shape

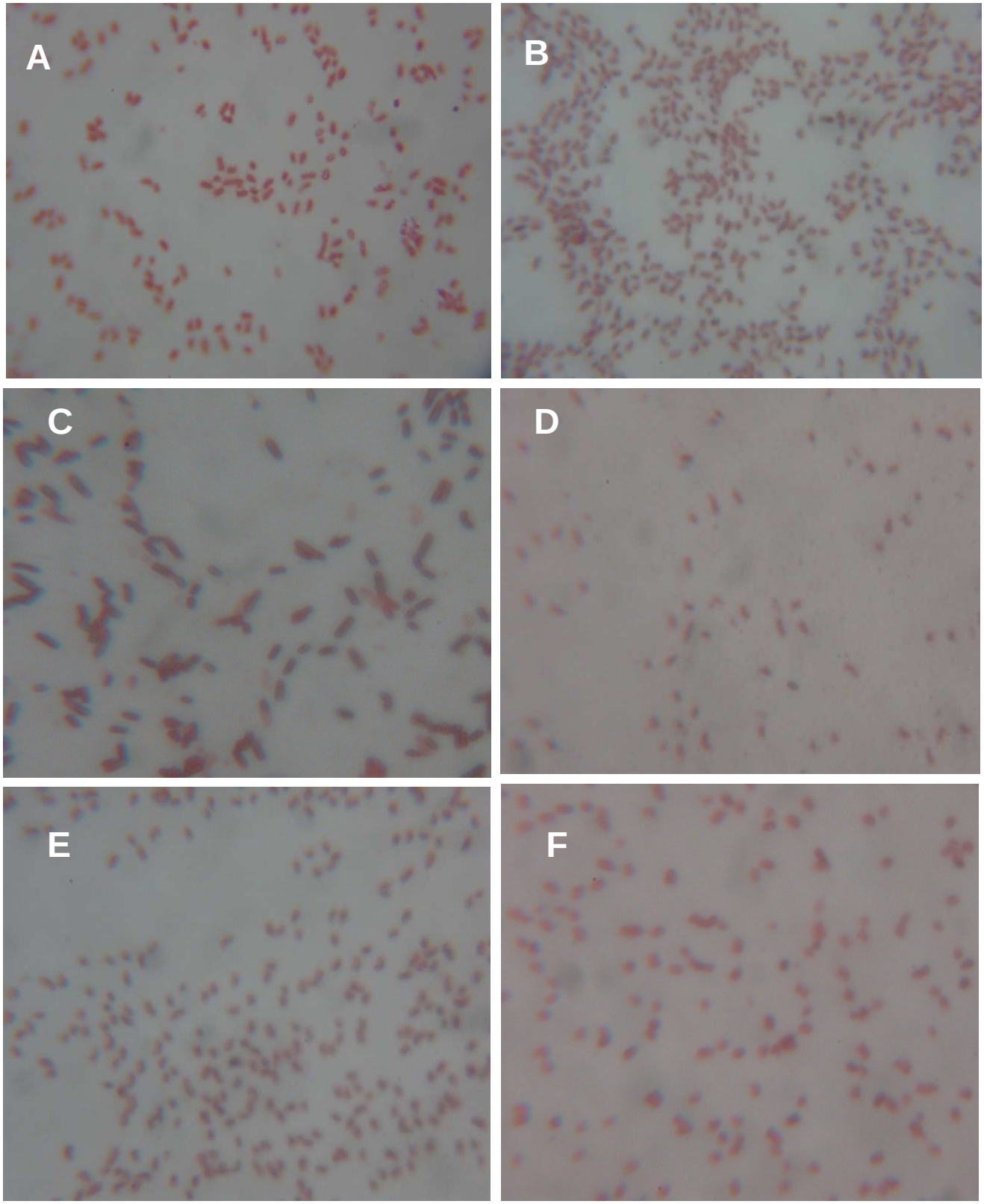


Plate 10. Cell morphology of *Rhizobium* isolates (A to F)-Cells are rod shape but varying in cell size
A) Rh-101 – Cell size: 2.2-2.4 x 5.1-7.5 µm **B) Rh-72 – Cell size: 2.4-2.7 x 5.4-7.9 µm**
C) Rh-113 – Cell size: 3.3-3.7 x 5.2-11.8 µm **D) Rh-82 – Cell size: 2.1-2.3 x 3.9-8.2 µm**
E) Rh-132 – Cell size: 1.7-1.9 x 2.3-4.1 µm **F) Rh-64 – Cell size: 2.8-3.1 x 5.9-7.3 µm**

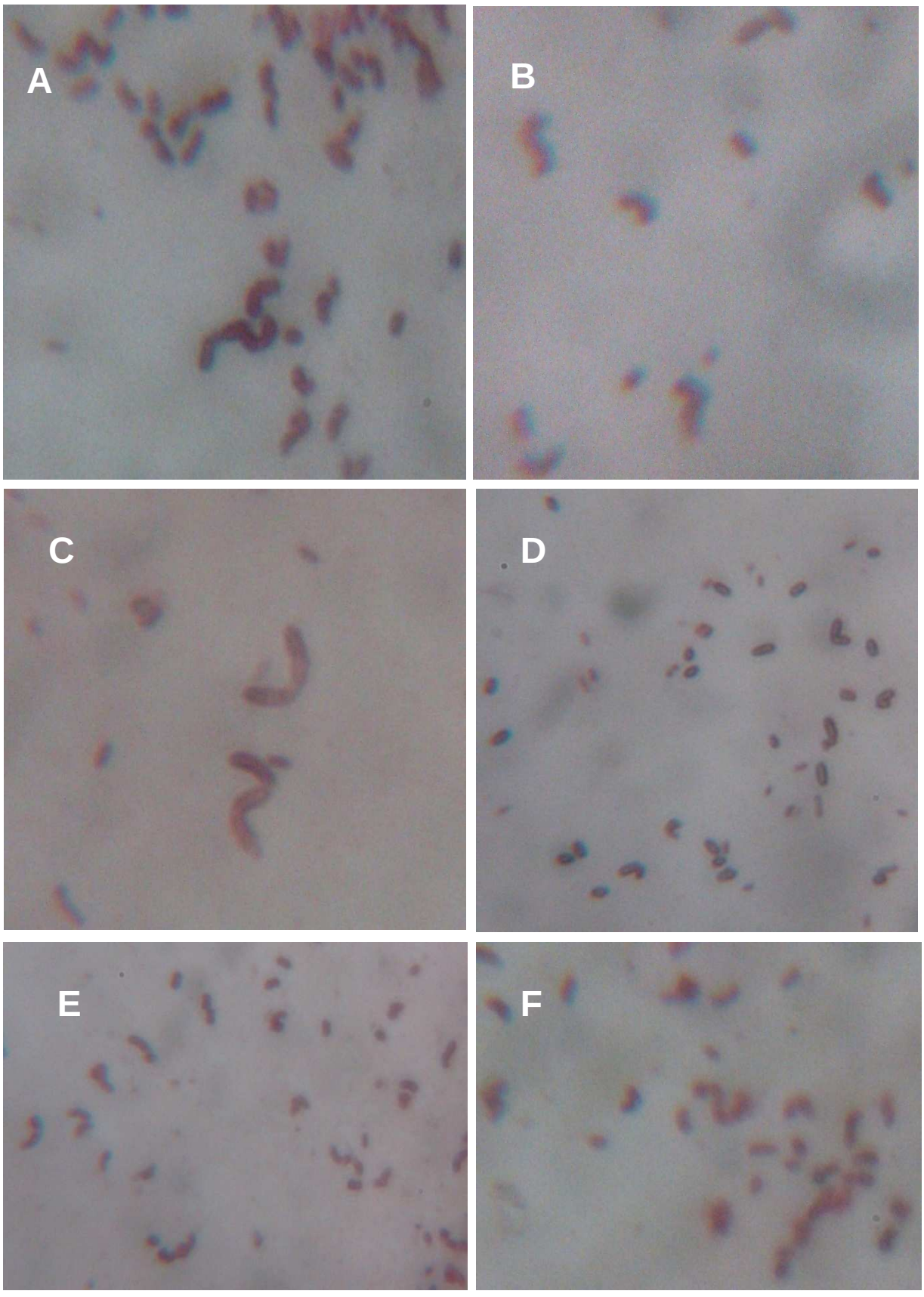


Plate 11. Cell morphology of *Azospirillum* isolates (A to F)

A) Asp-97 – Cells are elongated 'S' shape
B) Asp-28 – Cells are spiral shape
C) Asp-50 – Cells are elongated helical shape
D) Asp-121–Cells are vibrioid as well as curved rods
E) Asp-150 – Cells are helical shape
F) Asp-132 – Cells are helical/spiral shape

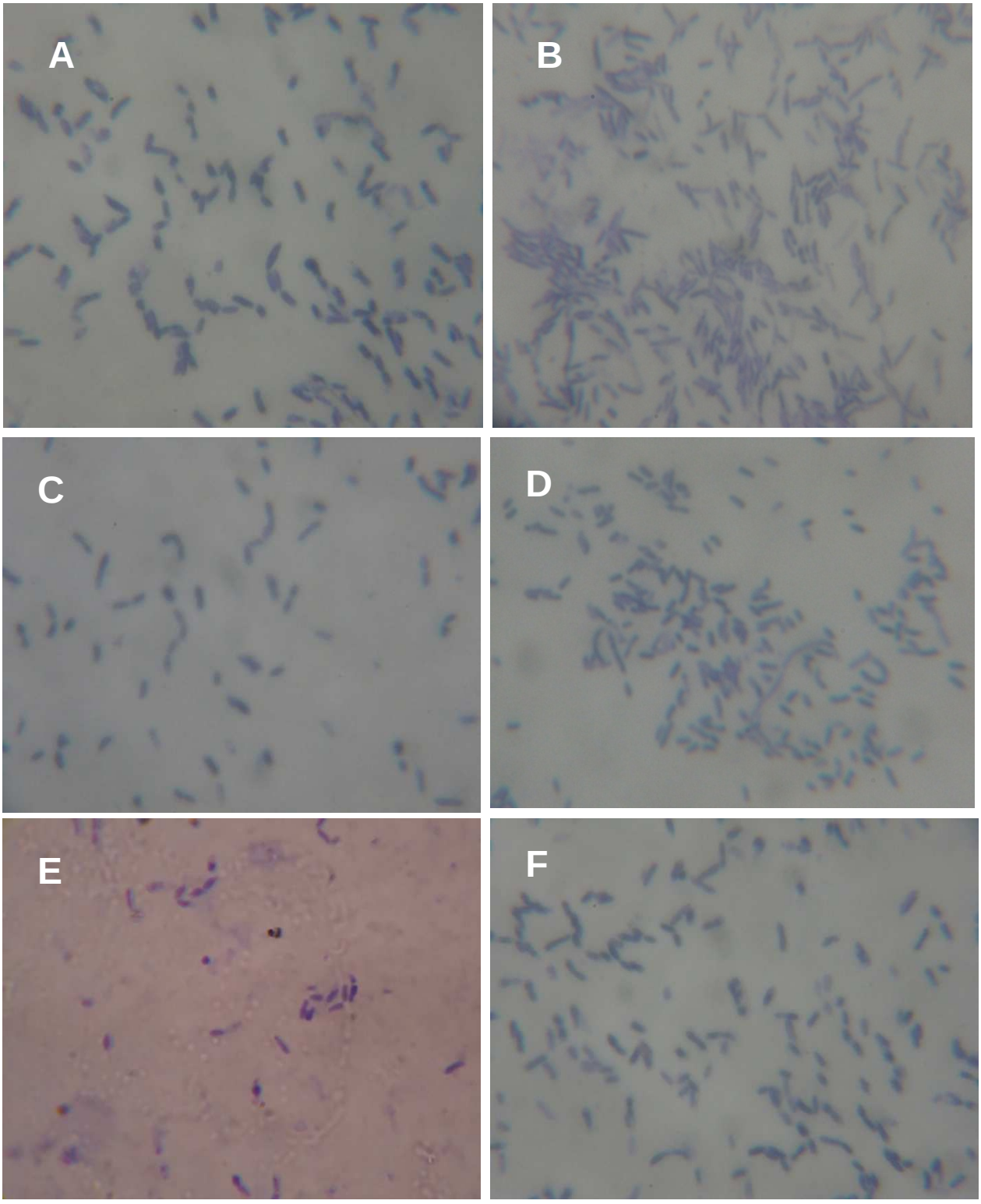


Plate 12. Cell morphology of PSB isolates (A to F) All cells are rod shaped.

A) PSB-15 – Cells in chain
B) PSB-33 – Cells in pairs
C) PSB-39 – Cells are single and in chains
D) PSB-41 – Single cells
E) PSB-72 – Cells are single and in pairs
F) PSB-100 – Cells are single and in chains

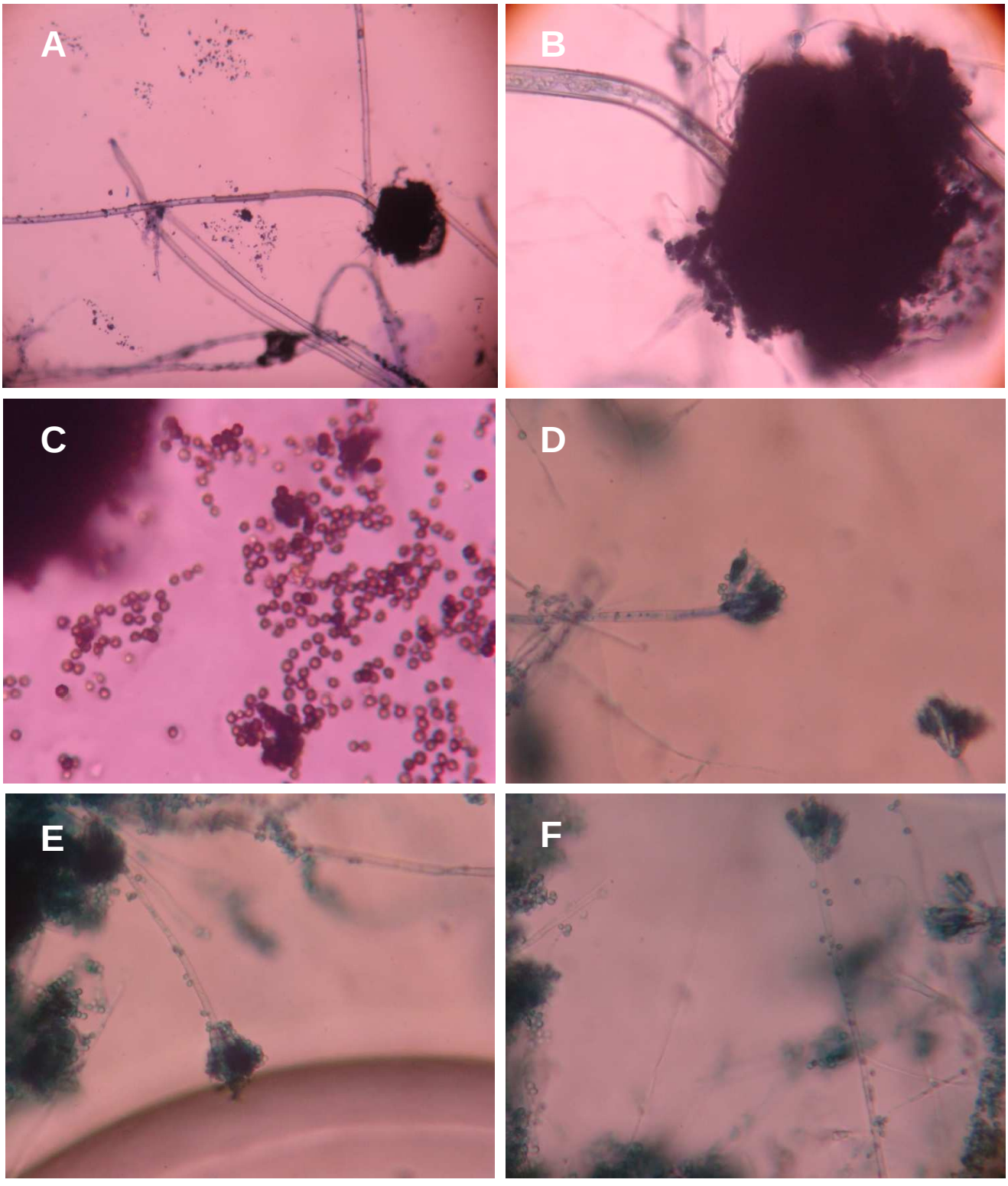


Plate 13. Microscopic appearance of PSF isolates

A to C – *Aspergillus* isolates

A) PSF-71 –Long conidiophores arise from a septate mycelium

B) PSF-28 – Conidiophore-septate, elliptical vesicle

C) PSF-55–Conidia-small, dark brown-black and round

D to F – *Penicillium* isolates

D) PSF-61 – Conidiophores-branched, that are borne on a single rope of fertile hyphae

E) PSF-77 – Conidiophores bundled at the top of synnemata

F) PSF-80 – Branching conidiophores arise from a septate mycelium, spiny conidia

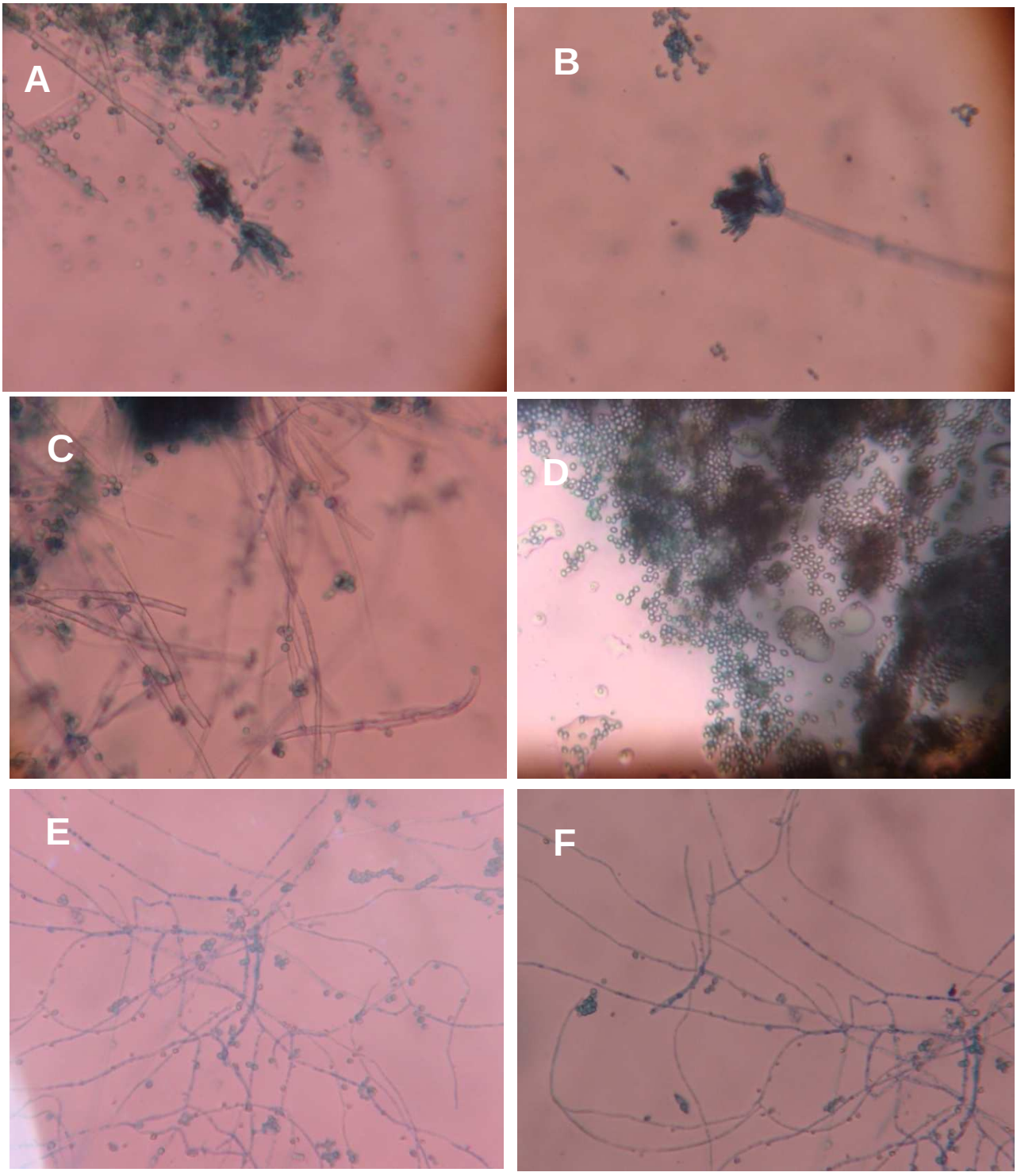


Plate 14. Microscopic appearance of PSF (*Penicillium* isolates) - A to F
(A) PSF-97 – Conidiophores-branched, that are borne on a single rope of fertile hyphae
(B) PSF-101-1 – Branching conidiophores that are borne on aerial mycelia
(C) PSF-138 – Branching conidiophores arise from a septate mycelium
(D) Conidia in chains develop at the end of the sterigmata (E) and (F) A septate mycelium

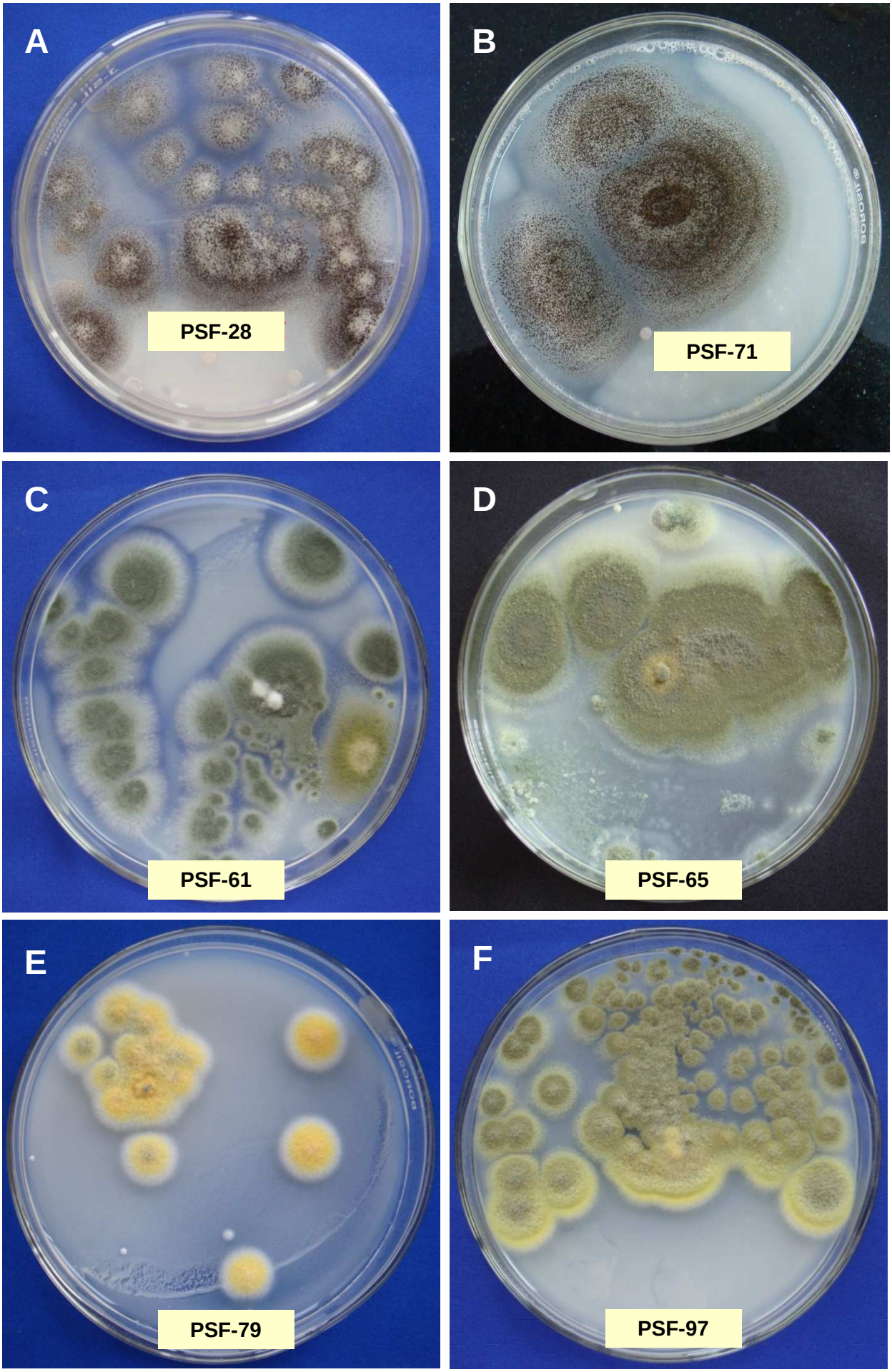


Plate 15. Colony morphology of PSF isolates on Pikovskaya's medium
(A) and (B) – *Aspergillus* isolates showing hallo zone of solubilization of TCP
(C) to (F) – *Penicillium* isolates showing hallo zone of solubilization of TCP

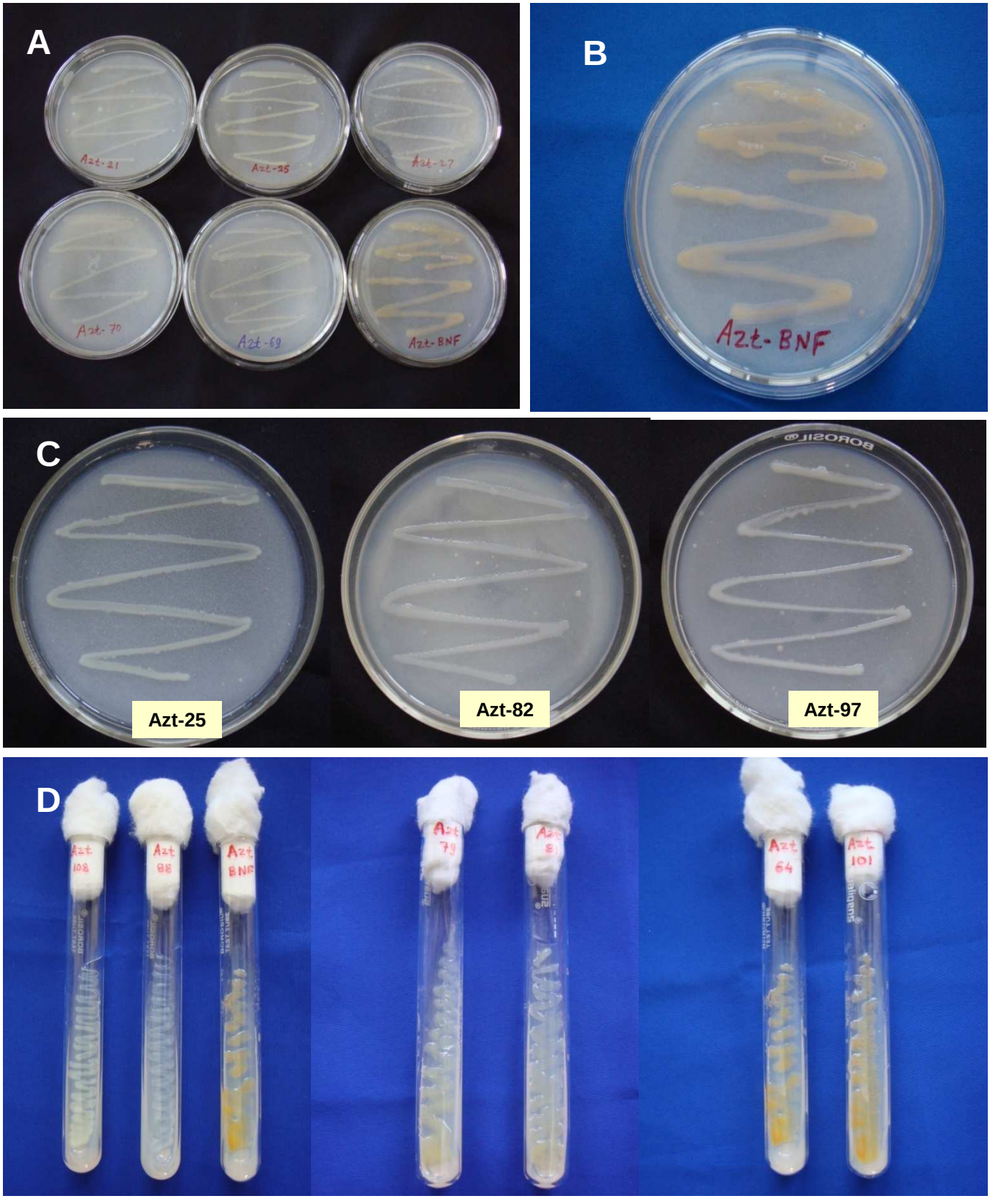


Plate 16. Cultural characteristics of *Azotobacter* isolates on Jensen's agar medium
(A) and (C) – Colony characters of *Azotobacter* isolates
(B) and (D) – Pigment production by aged cultures.

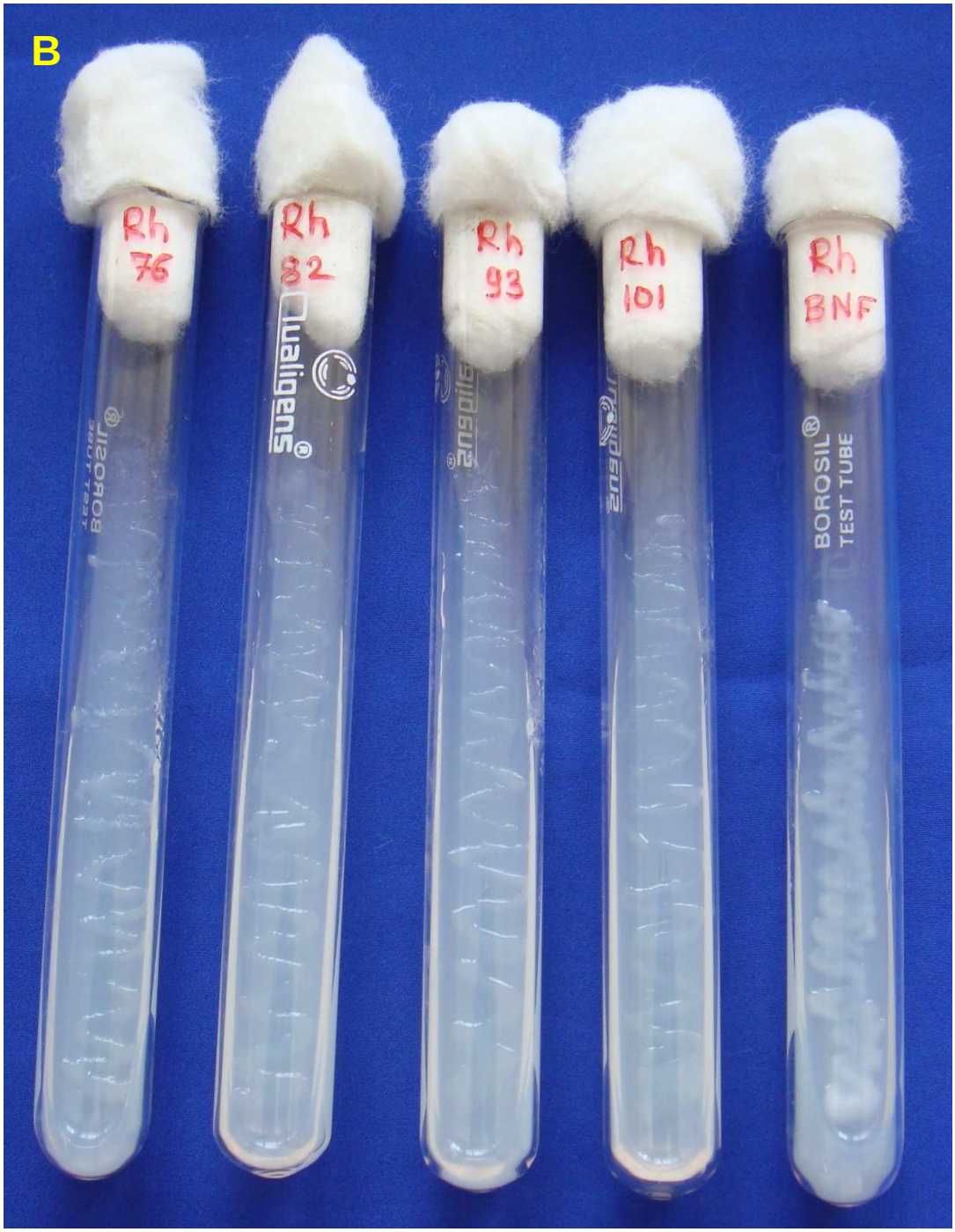


Plate 17. Cultural characteristics of *Rhizobium* isolates on YEMA medium
(A) and (B) – Colony characters of *Rhizobium* isolates

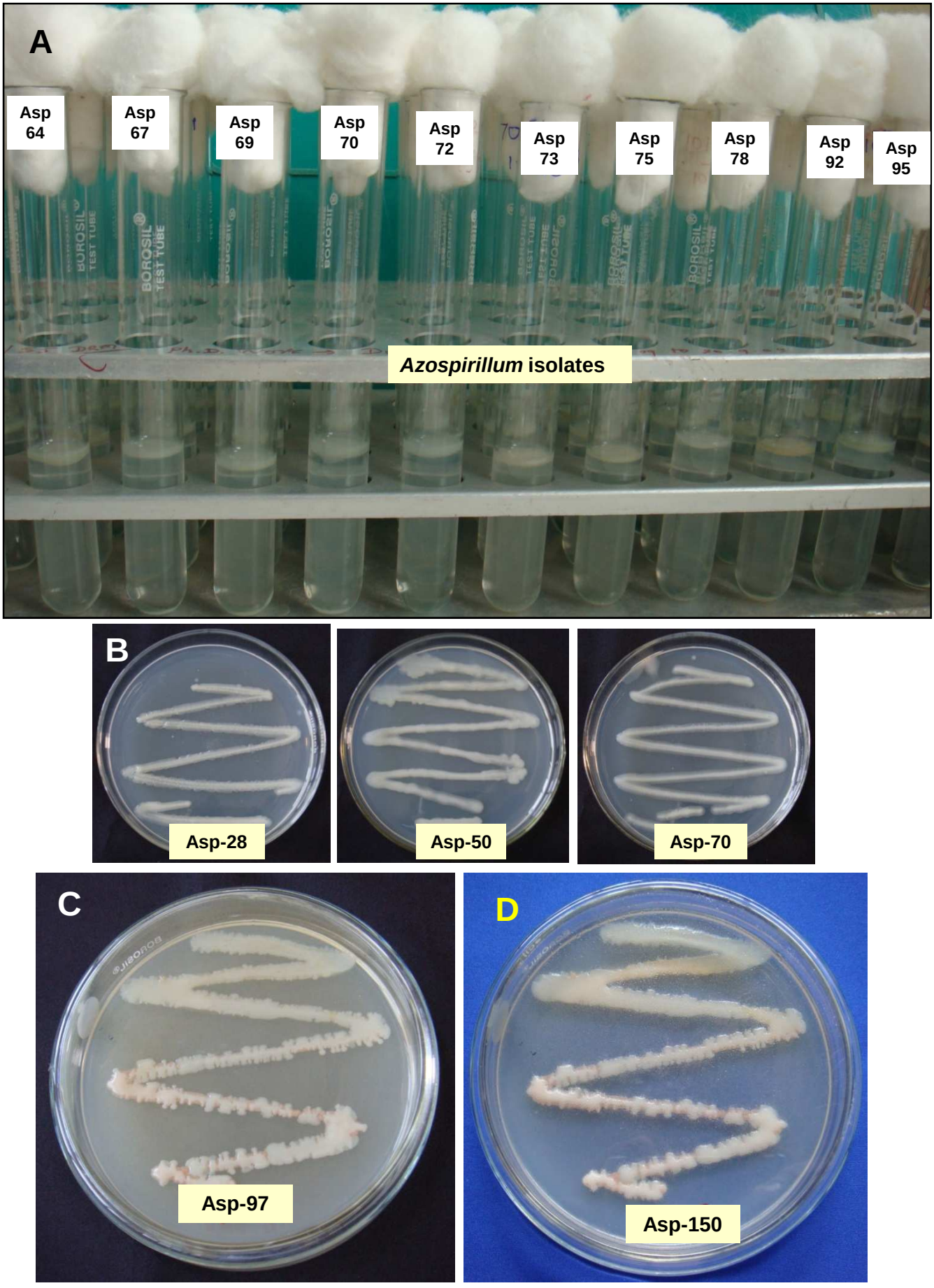
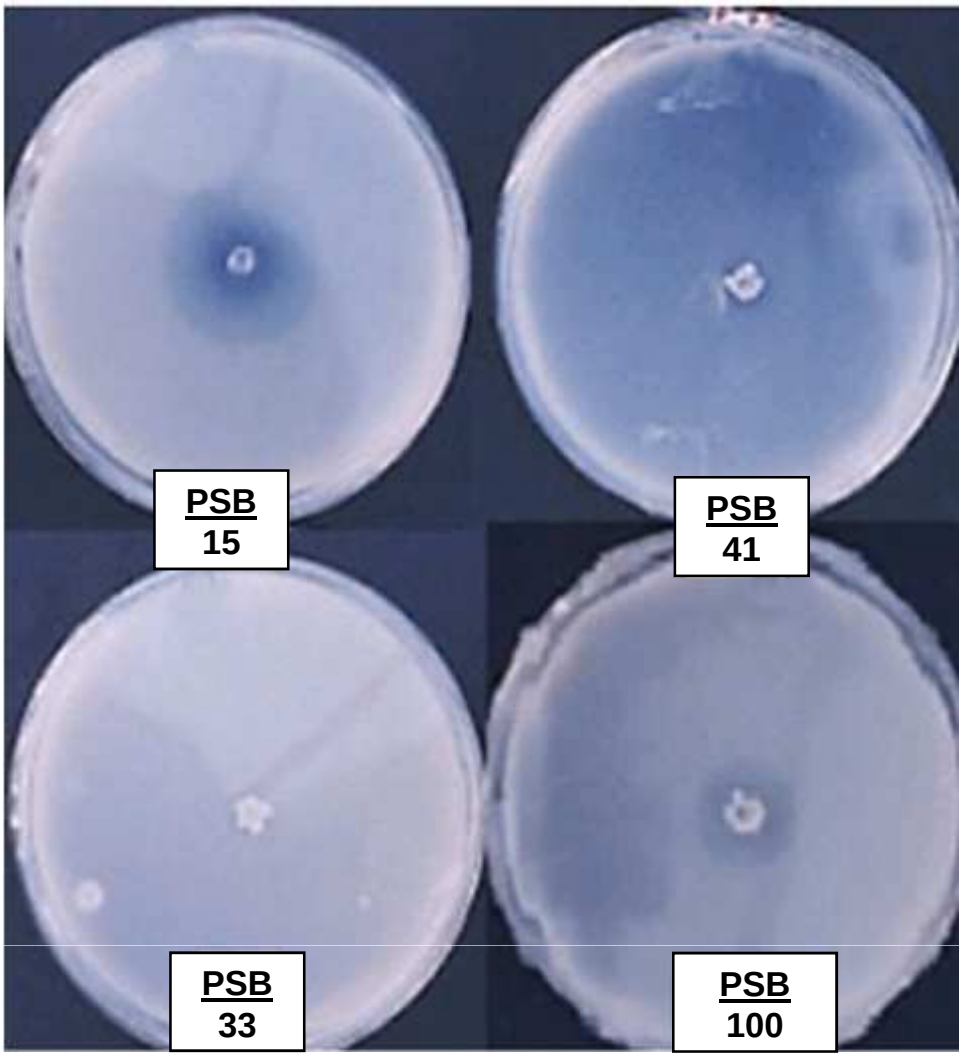


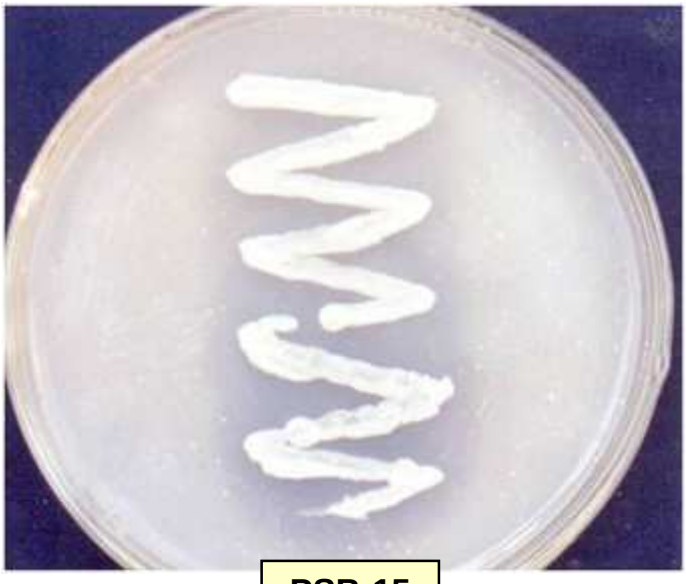
Plate 18. Cultural characteristics of *Azospirillum* isolates

(A) – Subsurface pellicle formation on N-free malate semisolid medium

(B) – Colony characters on BMS agar medium after one week of incubation

(C) and (D) – On BMS agar after two weeks of incubation colonies are pink, opaque, irregular, wrinkled and have umbonate elevation





PSB-15

Plate 19. PSB isolates showing hallo zone of solubilization of tricalcium phosphate on Pikovskaya's agar medium

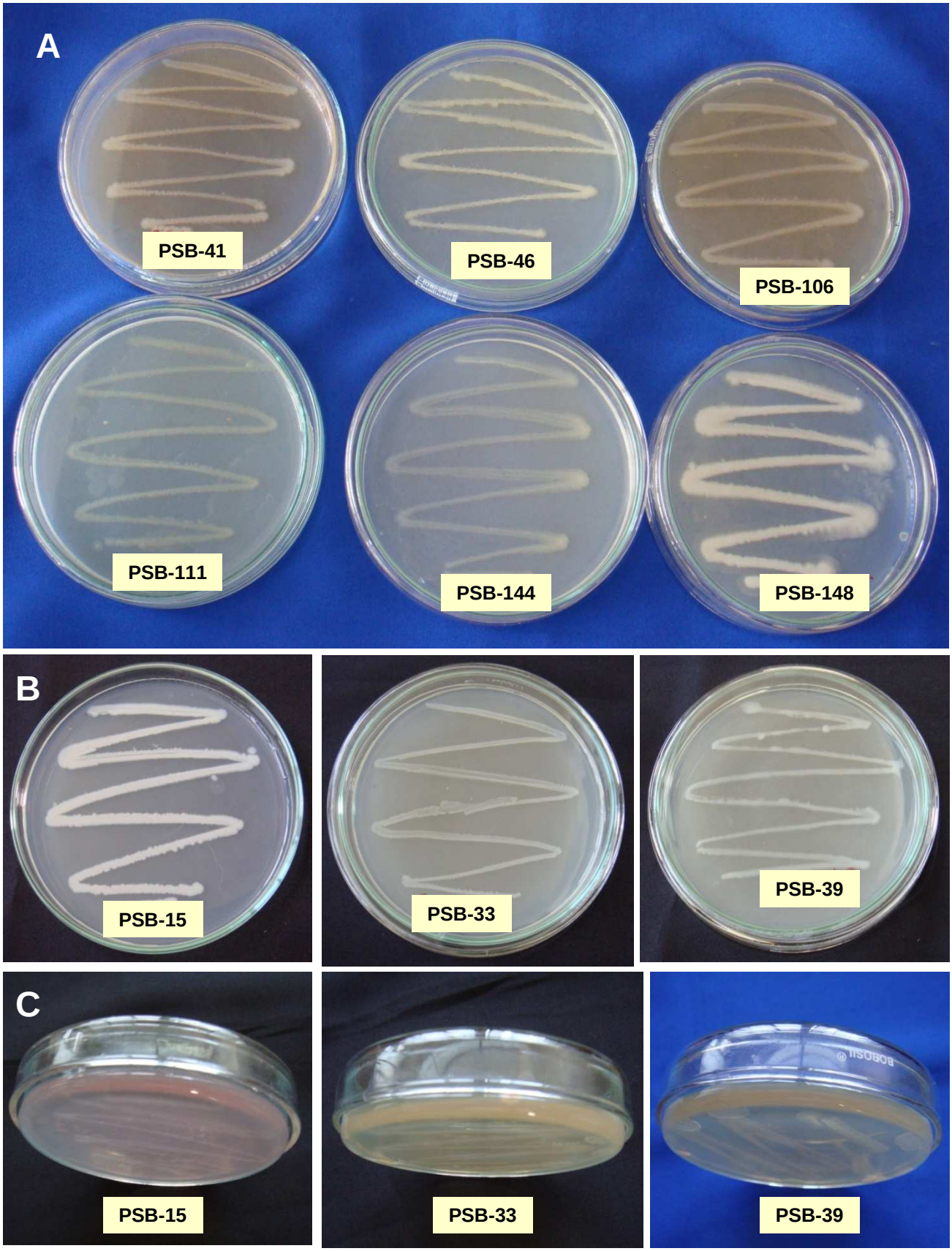


Plate 20. Cultural characteristics of PSB isolates

(A) and (B) – Colony characters on nutrient agar medium after four days of incubation

(C) – Pink, yellow to brown pigmentation on NA medium after two weeks of incubation

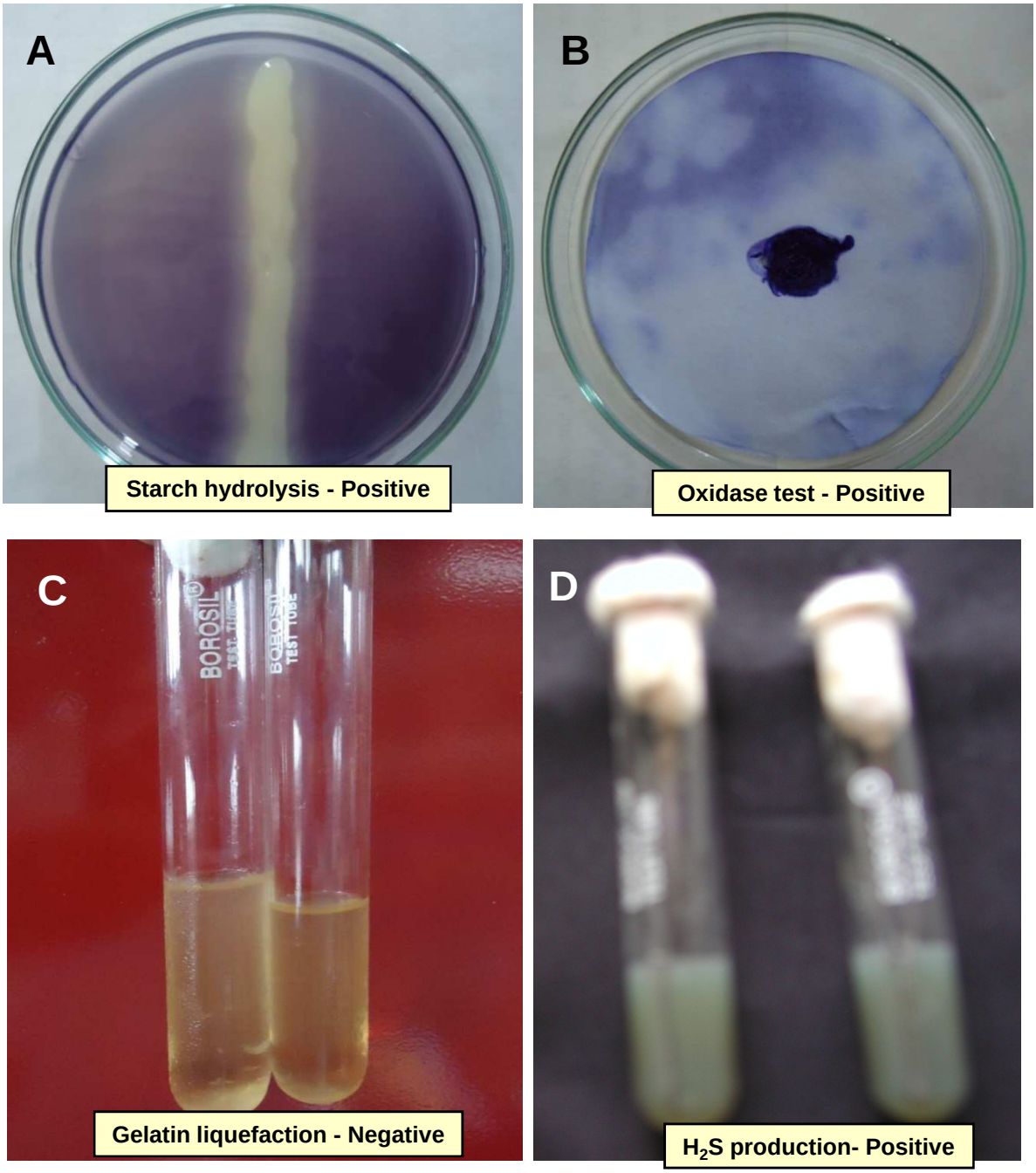
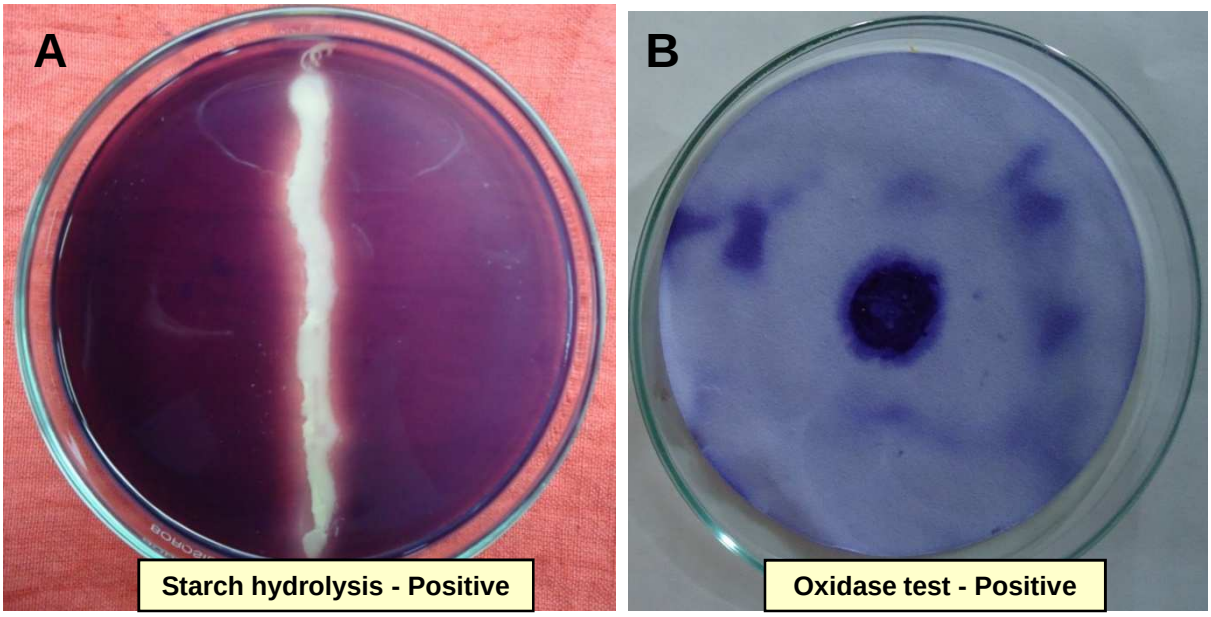
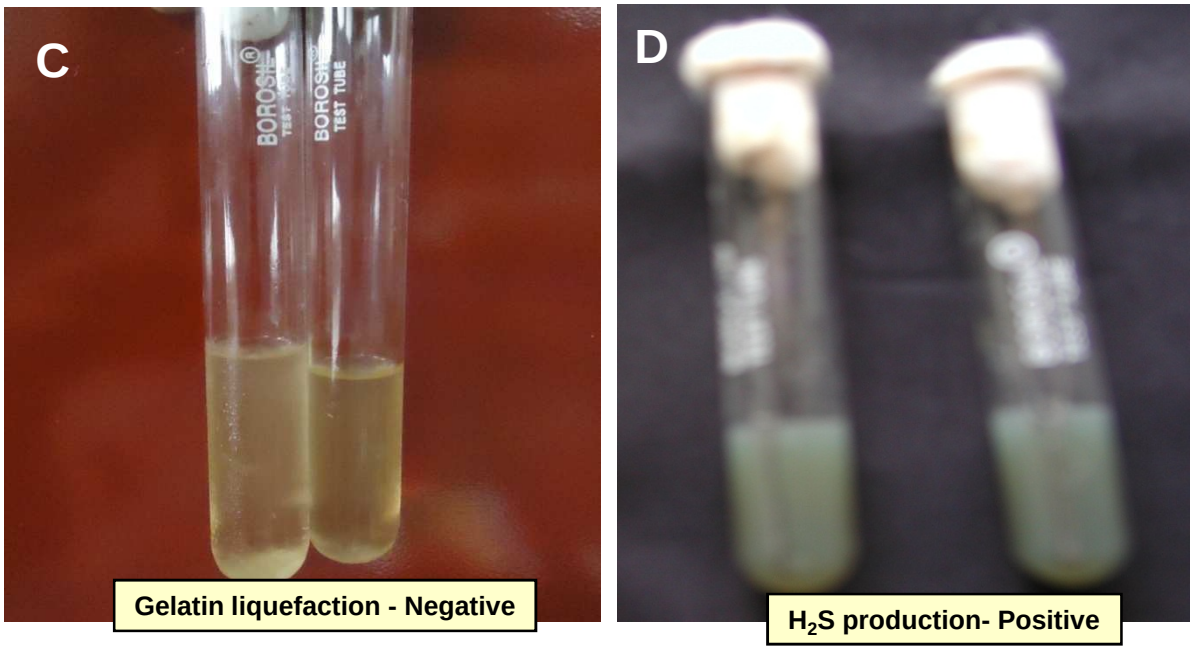


Plate 21. Different tests carried out for biochemical characterization of *Azotobacter* isolates (A to E)





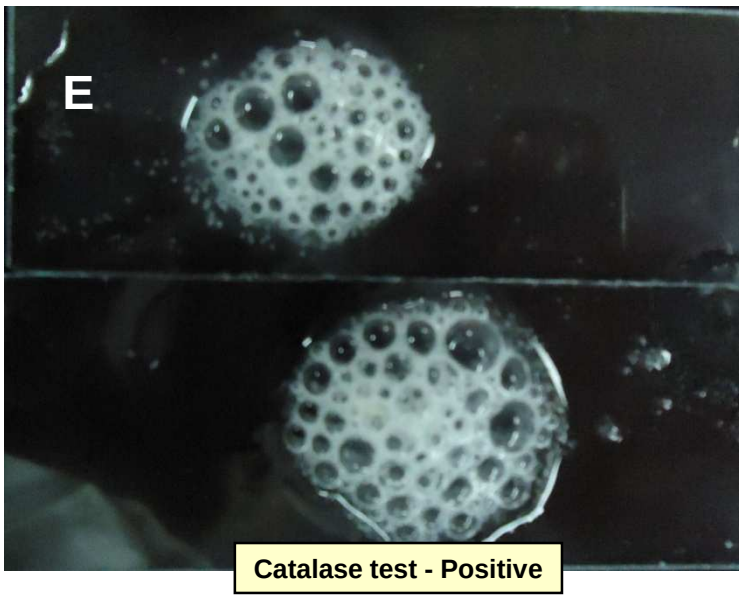


Plate 22. Different tests carried out for biochemical characterization of *Rhizobium* isolates (A to E)

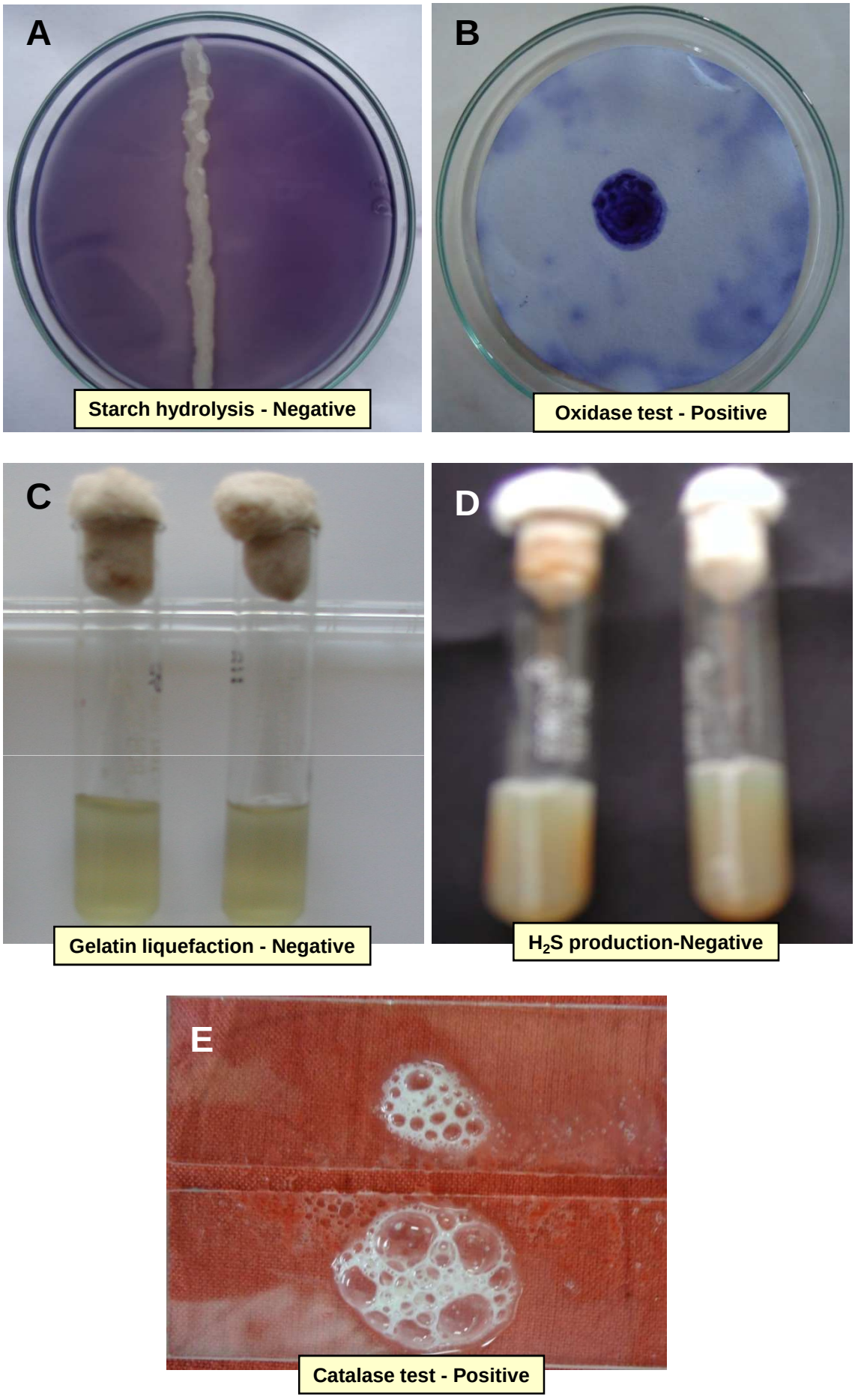


Plate 23. Different tests carried out for biochemical characterization of *Azospirillum* isolates (A to E)

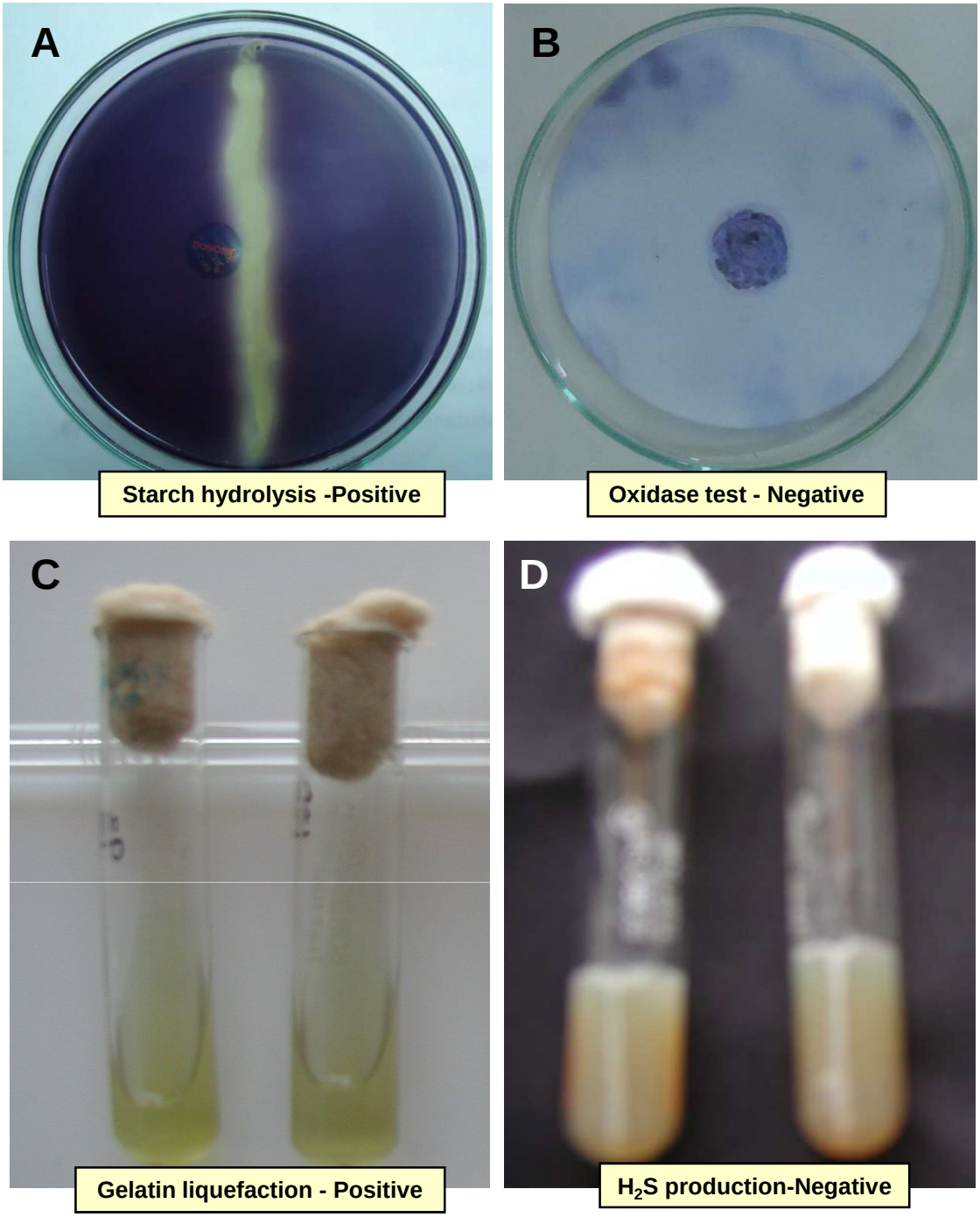


Plate 24. Different tests carried out for biochemical characterization of PSB isolates (A to E)

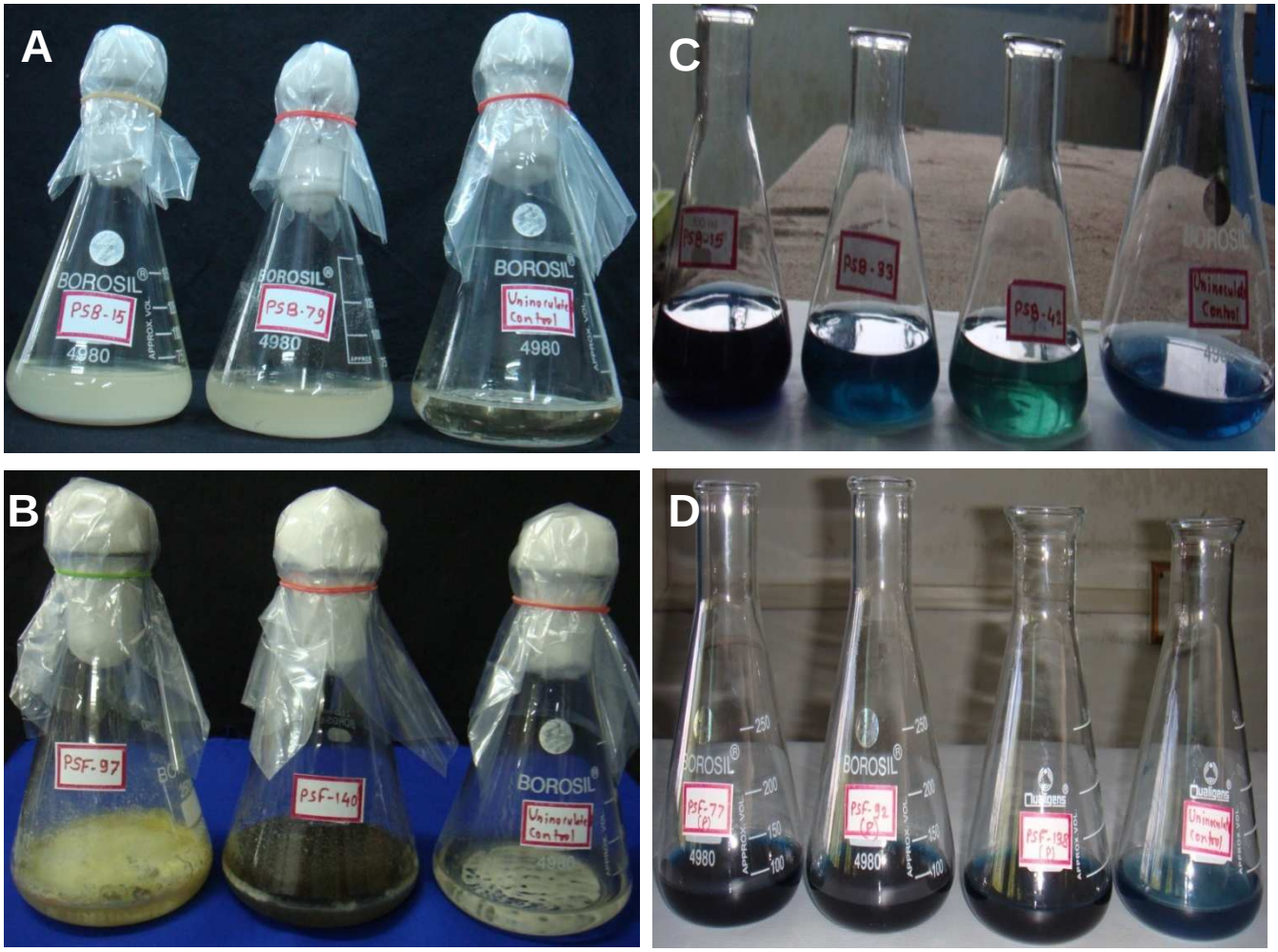


Plate 25. Quantitative estimation of Pi released from TCP by PSB and PSF isolates (A to D)
(A) Turbidity produced by PSB isolates on Pikovskaya's broth at 10 DAI
(B) Mycelial mat developed by PSF isolates on Pikovskaya's broth at 10 DAI
(C) and (D) The intensity of blue colour developed by PSB and PSF isolates was measured at 610 nm

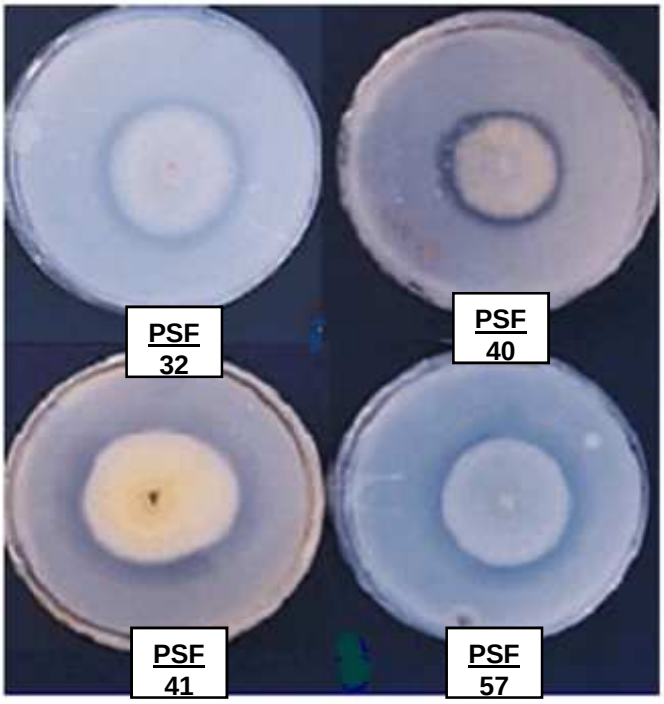
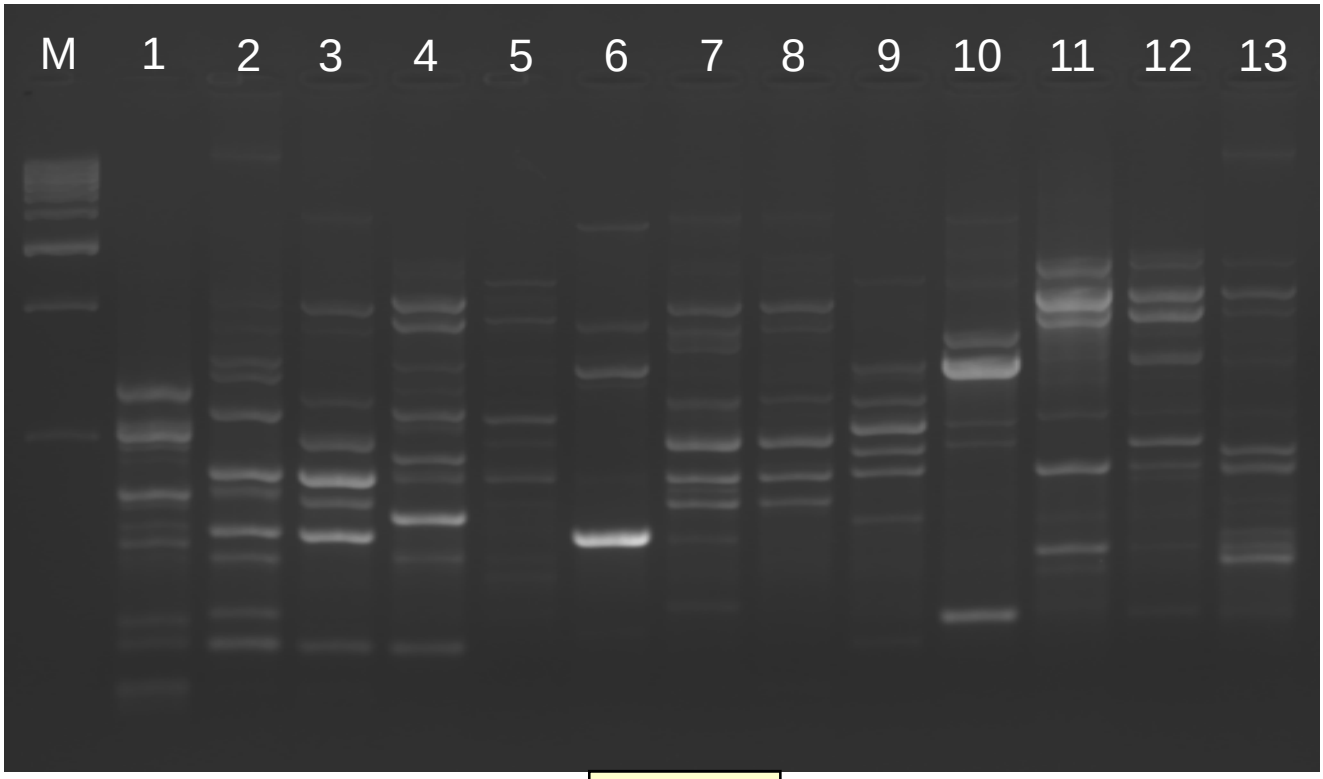
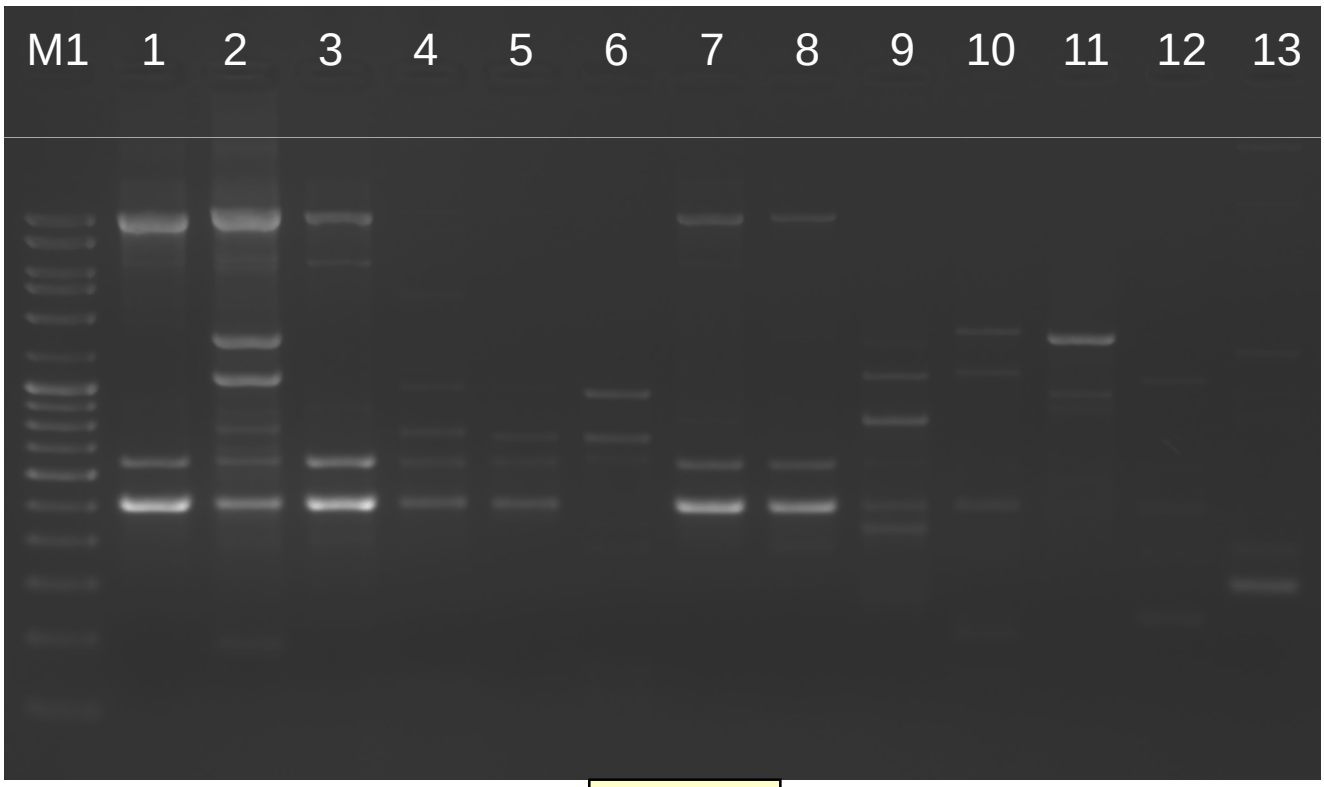


Plate 26. PSF isolates showing hallo zone of solubilization of TCP on Pikovskaya's agar medium



RBA-15



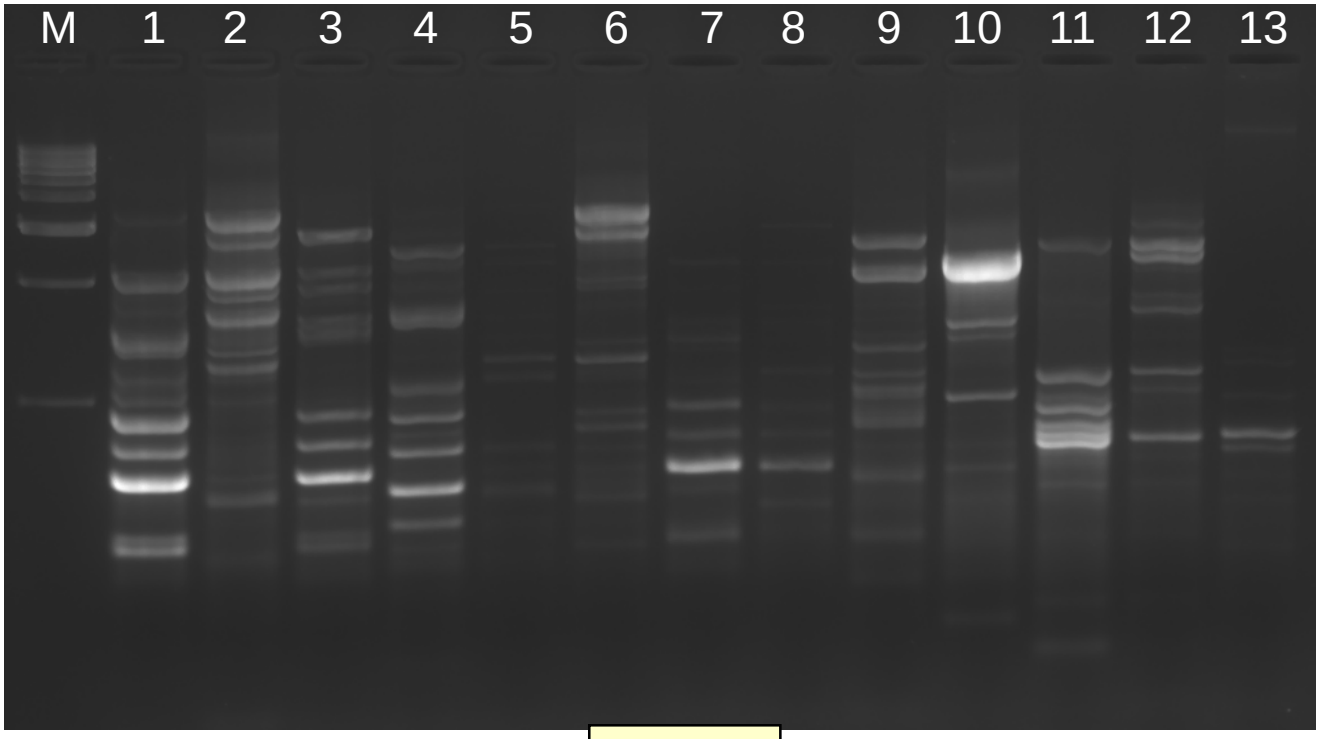
RBA-24

Plate 27. Amplification profiles of different isolates of *Azotobacter chroococcum* by RBA-15 and RBA-24 primer

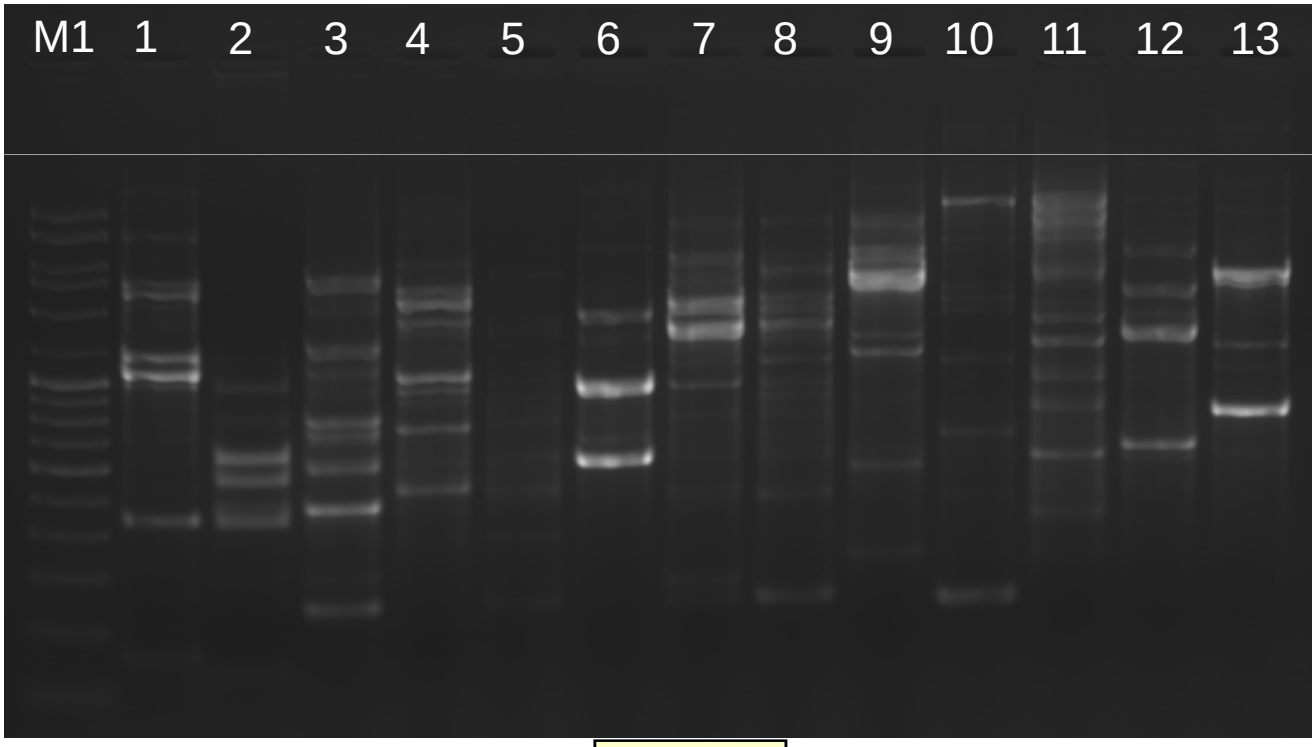
Lane M : Marker 1 kb (GeNei™), M1 : Marker 100 bp (GeNei™ Low Range DNA Ruler Plus)

***Azotobacter chroococcum* isolates:**

1 – Azt-08	**2 – Azt-21**	**3 – Azt-25**	**4 – Azt-50**	**5 – Azt-64**	**6 – Azt-70**	**7 – Azt-82**
8 – Azt-97	**9 – Azt-129**	**10 –Azt-135**	**11–Azt-142**	**12–Azt-148**	**13–Azt-BNF (MPKV strain)**	



RBA-10



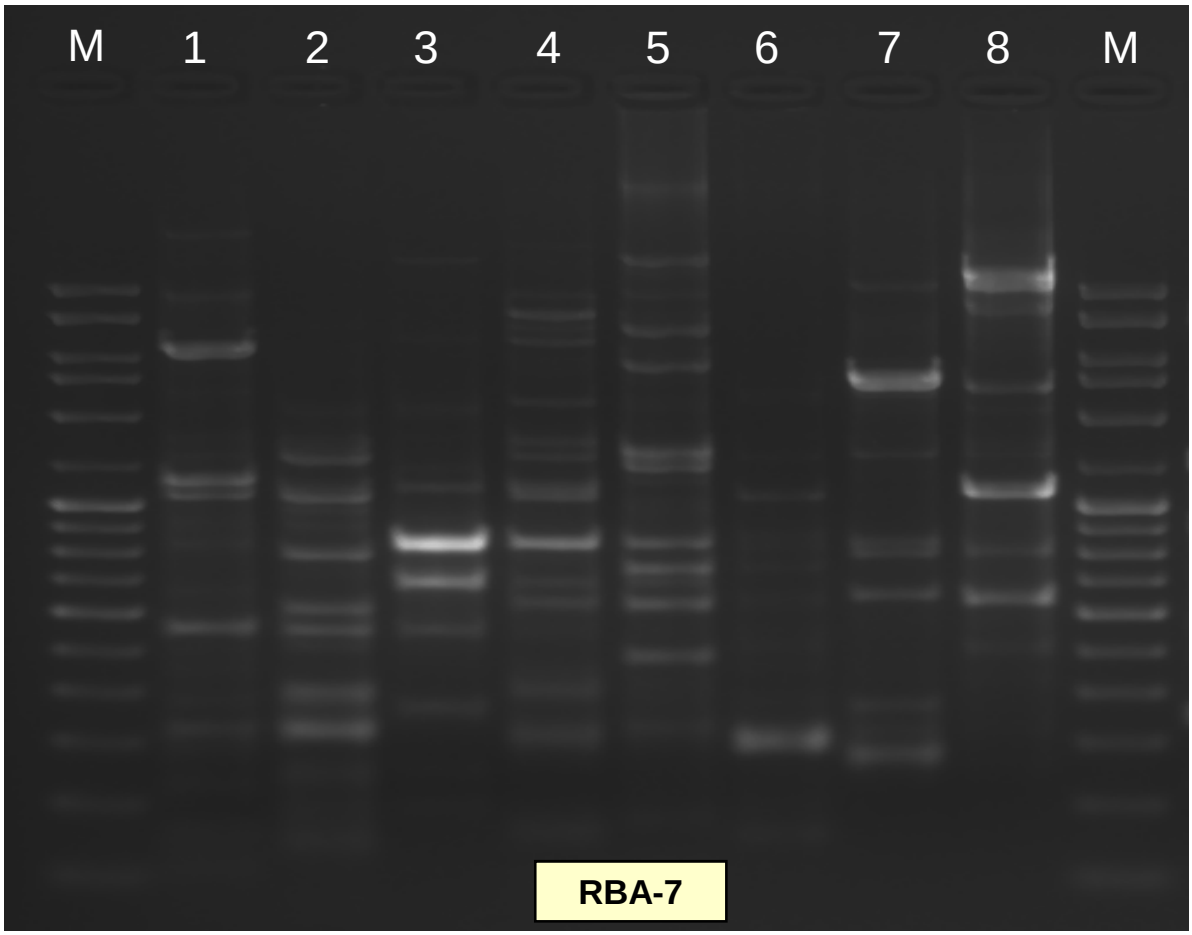
RBA-22

Plate 28. Amplification profiles of different isolates of *Azotobacter chroococcum* by RBA-10 and RBA-22 primer

Lane M : Marker 1 kb (GeNei™), M1 : Marker 100 bp (GeNei™ Low Range DNA Ruler Plus)

***Azotobacter chroococcum* isolates:**

1 – Azt-08	2 – Azt-21	3 – Azt-25	4 – Azt-50	5 – Azt-64	6 – Azt-70	7 – Azt-82
8 – Azt-97	9 – Azt-129	10 –Azt-135	11–Azt-142	12–Azt-148	13–Azt-BNF (MPKV strain)	



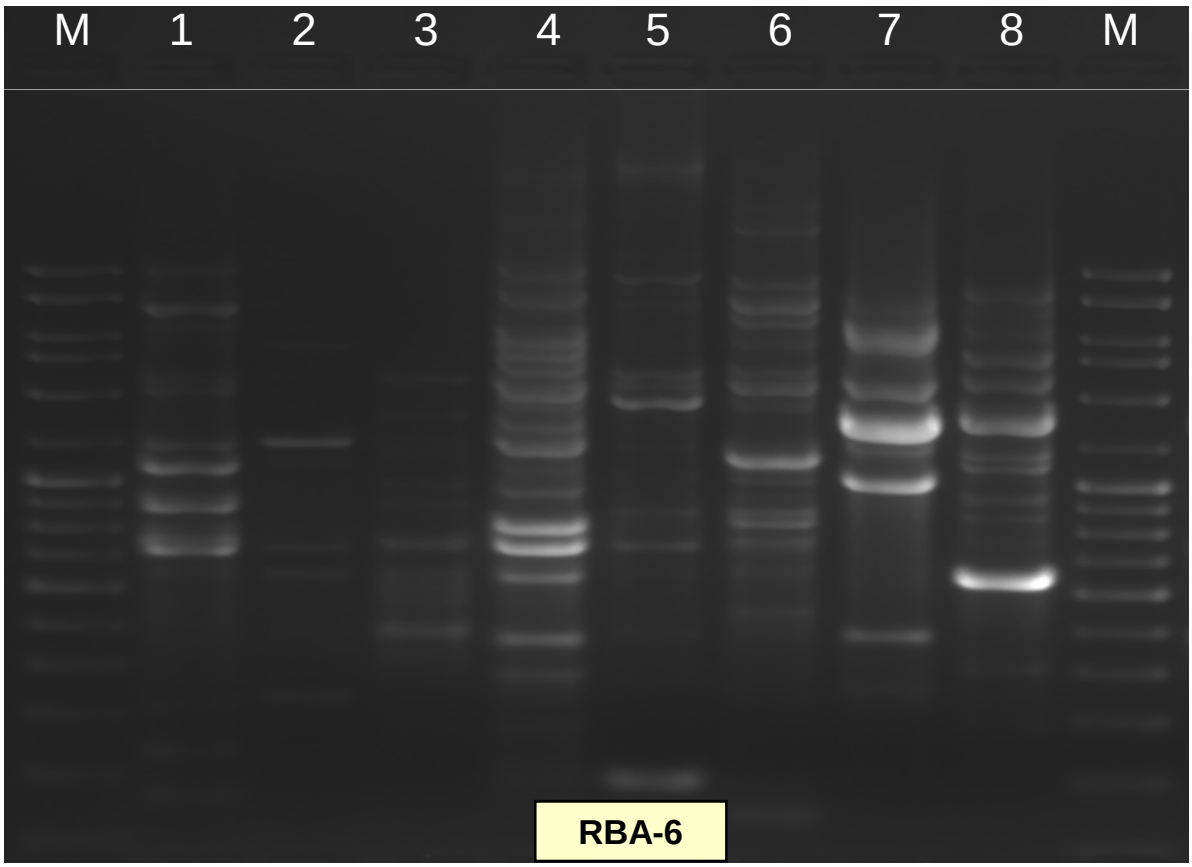


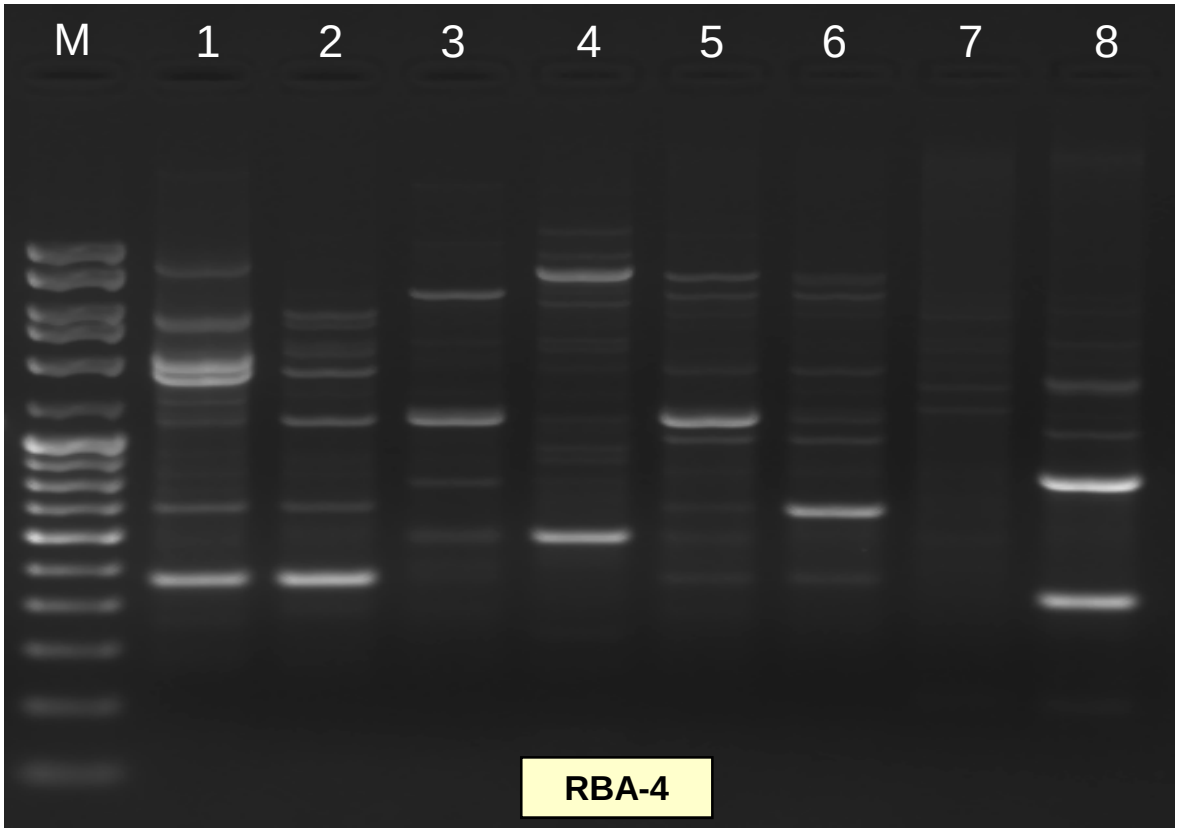
Plate 29. Amplification profiles of different isolates of *Rhizobium* spp. by RBA-7 and RBA-6 primer

Lane M : Marker 100 bp (GeNei™ Low Range DNA Ruler Plus)

***Rhizobium* spp. isolates:**

1 – Rh-64 2 – Rh-69 3 – Rh-72 4 – Rh-101 5 – Rh-109 6 – Rh-113

7 – Rh-132 8 – Rh-BNF (MPKV strain)



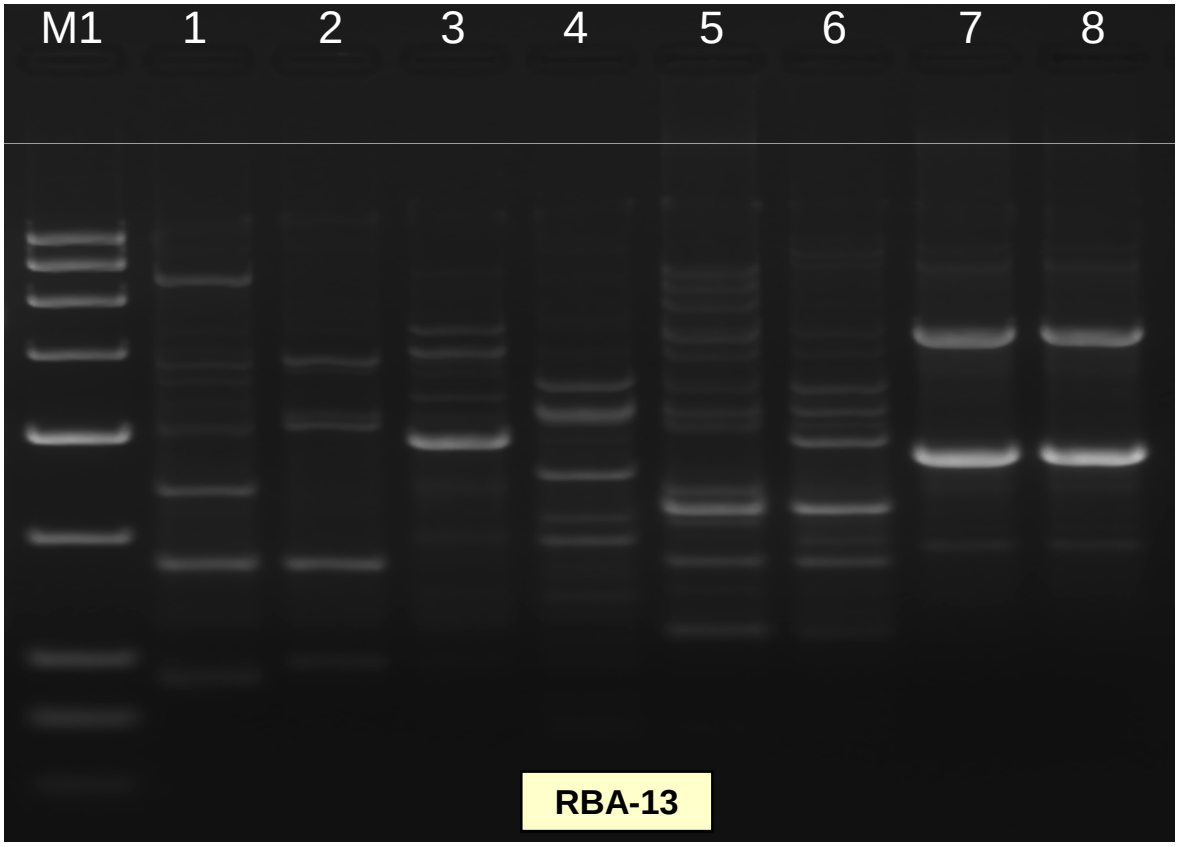
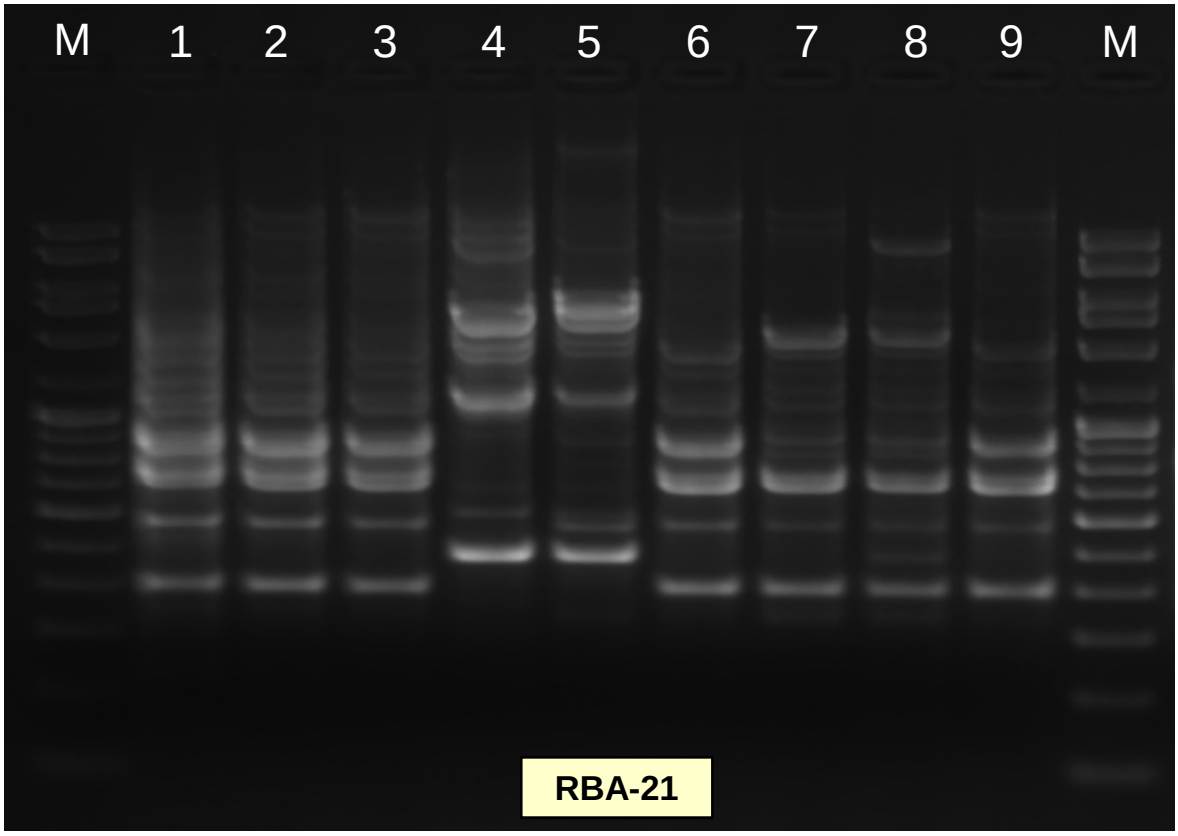


Plate 30. Amplification profiles of different isolates of *Azospirillum lipoferum* by RBA-4 and RBA-13 primer

Lane M : Marker 100 bp (GeNei™ Low Range DNA Ruler Plus)
Lane M1 : Marker 100 bp (GeNei™ Low Range DNA Ruler)

***Azospirillum lipoferum* isolates:**
1 – Asp-28 2 – Asp-50 3 – Asp-97 4 – Asp-124 5 – Asp-127 6 – Asp-132
7 – Asp-150 8 – Asp-BNF (MPKV strain)



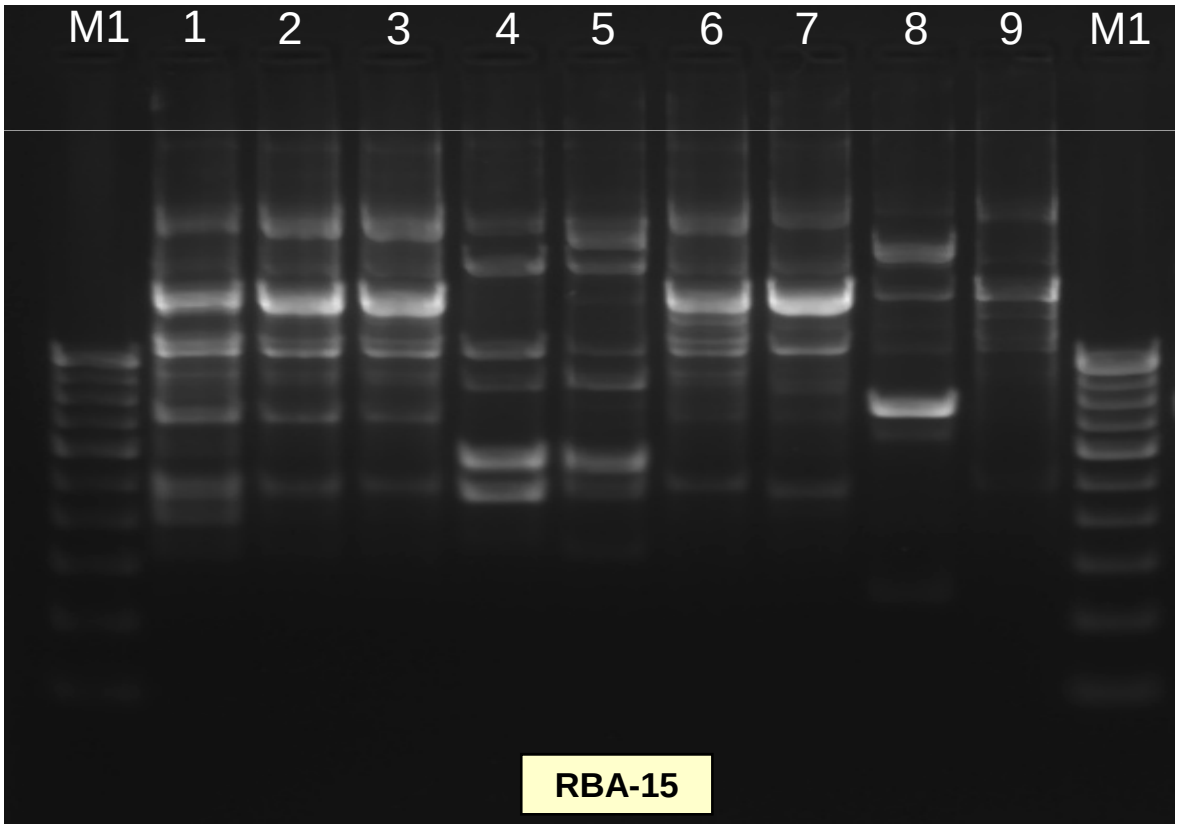
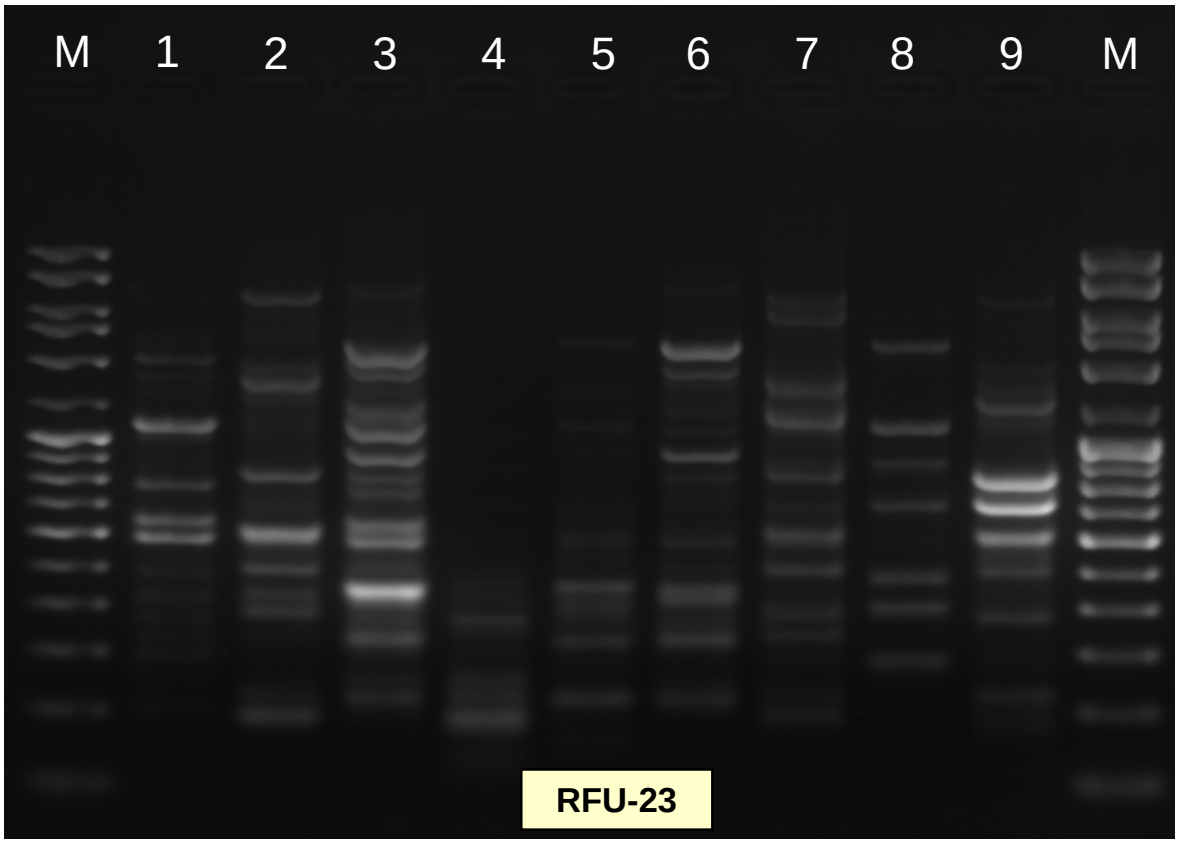


Plate 31. Amplification profiles of different isolates of *Bacillus megaterium* by RBA-21 and RBA-15 primer

Lane M : Marker 100 bp (GeNei™ Low Range DNA Ruler Plus)
Lane M1 : Marker 100 bp (GeNei™)

***Bacillus megaterium* isolates:**
1 – PSB-15 2 – PSB-33 3 – PSB-39 4 – PSB-41 5 – PSB-72 6 – PSB-100
7 – PSB-119 8 – PSB-140 9 – PSB-BNF (MPKV strain)



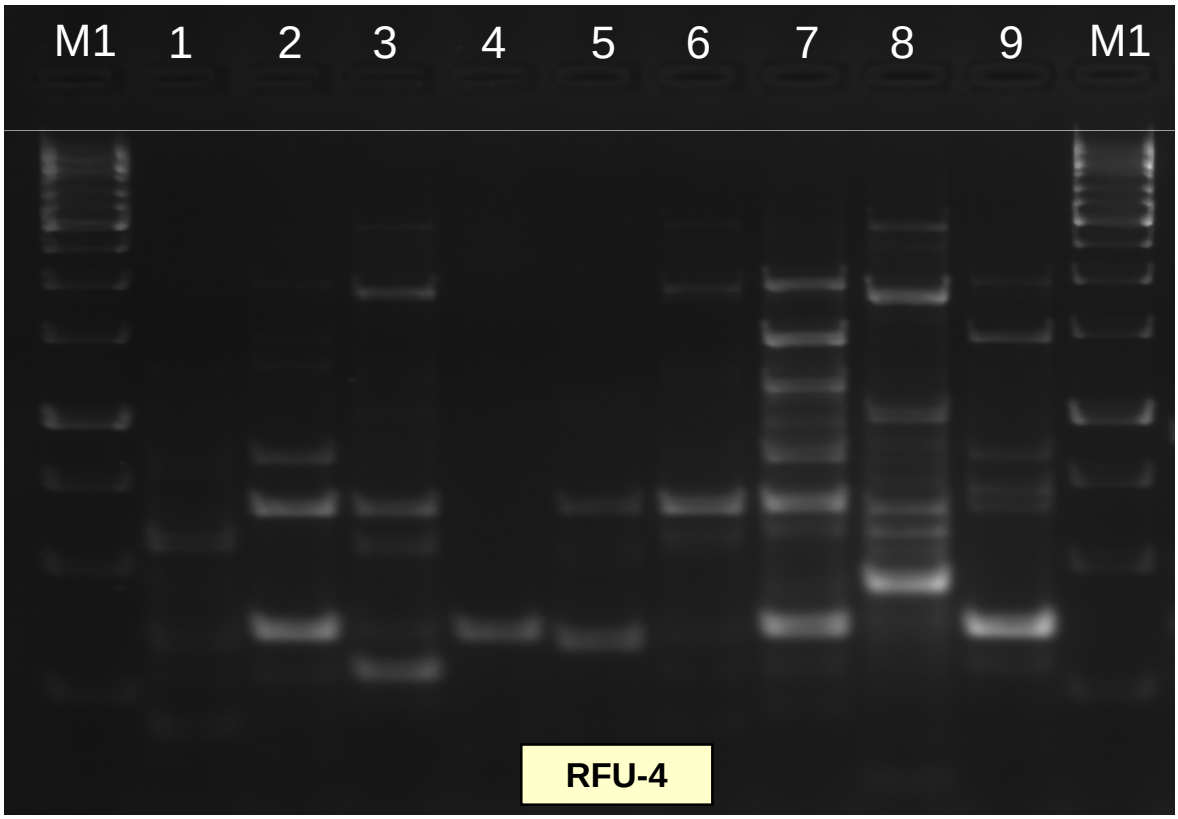


Plate 32. Amplification profiles of different isolates of *Aspergillus awamori* by RFU-23 and RFU-4 primer

Lane M : Marker 100 bp (GeNei™ Low Range DNA Ruler Plus)
Lane M1 : Marker 1 kb (Gene Ruler™)

***Aspergillus awamori* isolates:**
1 – PSF-08 2 – PSF-28 3 – PSF-55 4 – PSF-64 5 – PSF-71 6 – PSF-100
7 – PSF-115 8 – PSF-132 9 – PSF-BNF(A) (MPKV strain)

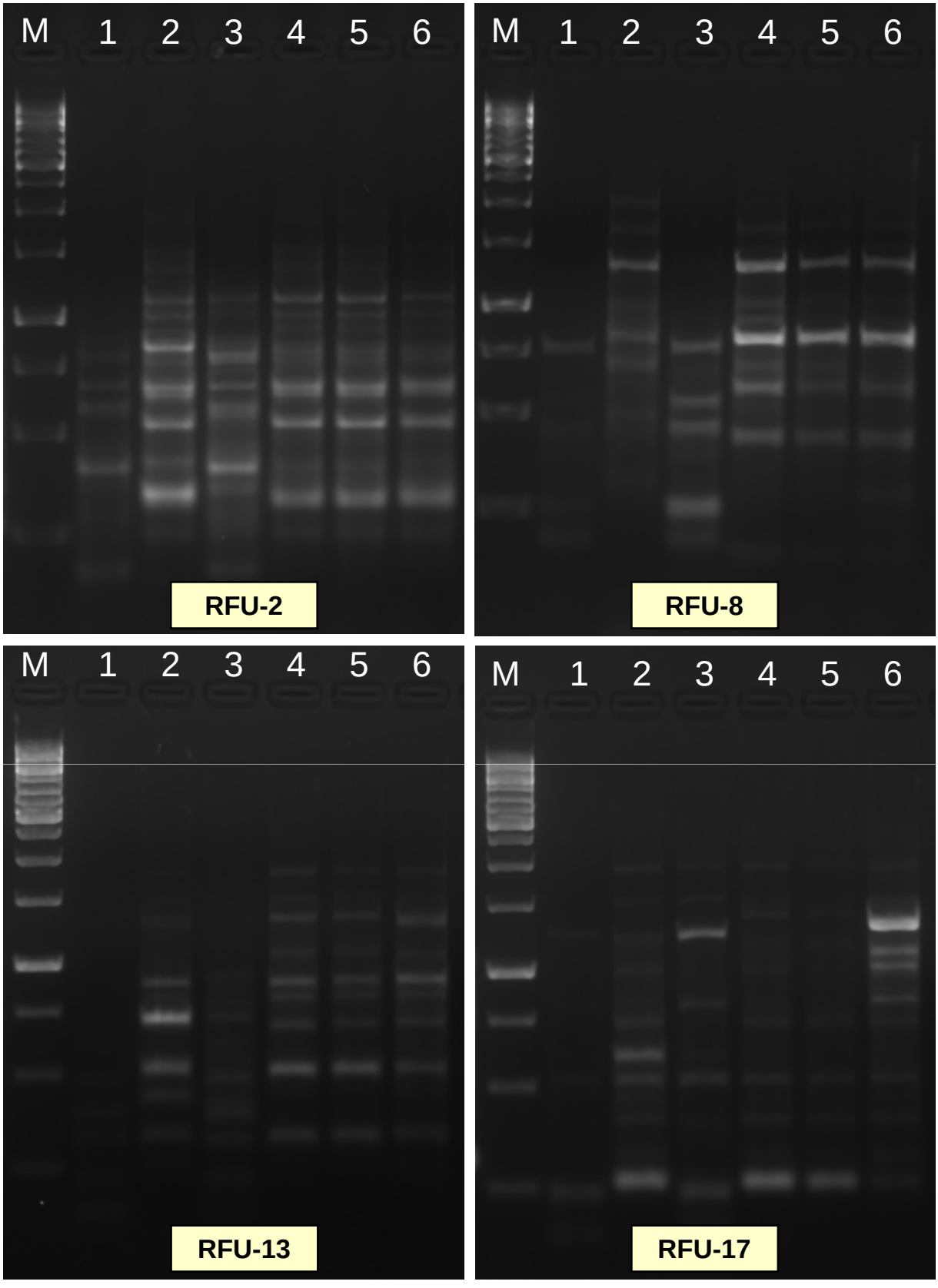


Plate 33. Amplification profiles of different isolates of *Penicillium digitatum* by RFU-2, RFU-8, RFU-13 and RFU-17 primer

Lane M : Marker 1 kb (Gene Ruler^{TM})

***Penicillium digitatum* isolates:**

1 – PSF-61 2 – PSF-77 3 – PSF-80 4 – PSF-97 5 – PSF-101-1

6 – PSF-BNF(P) (MPKV strain)

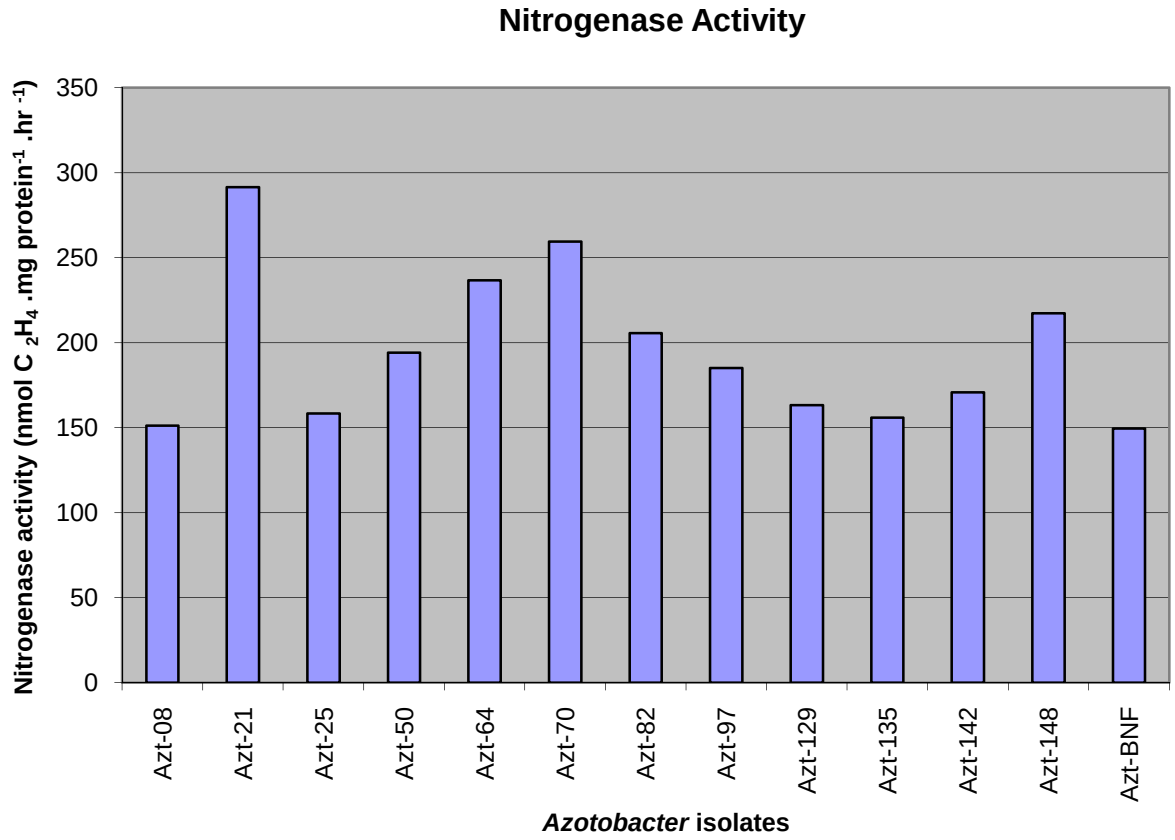


Figure 1. Nitrogenase activity of selected *Azotobacter chroococcum* isolates in comparison with standard MPKV strain Azt-BNF

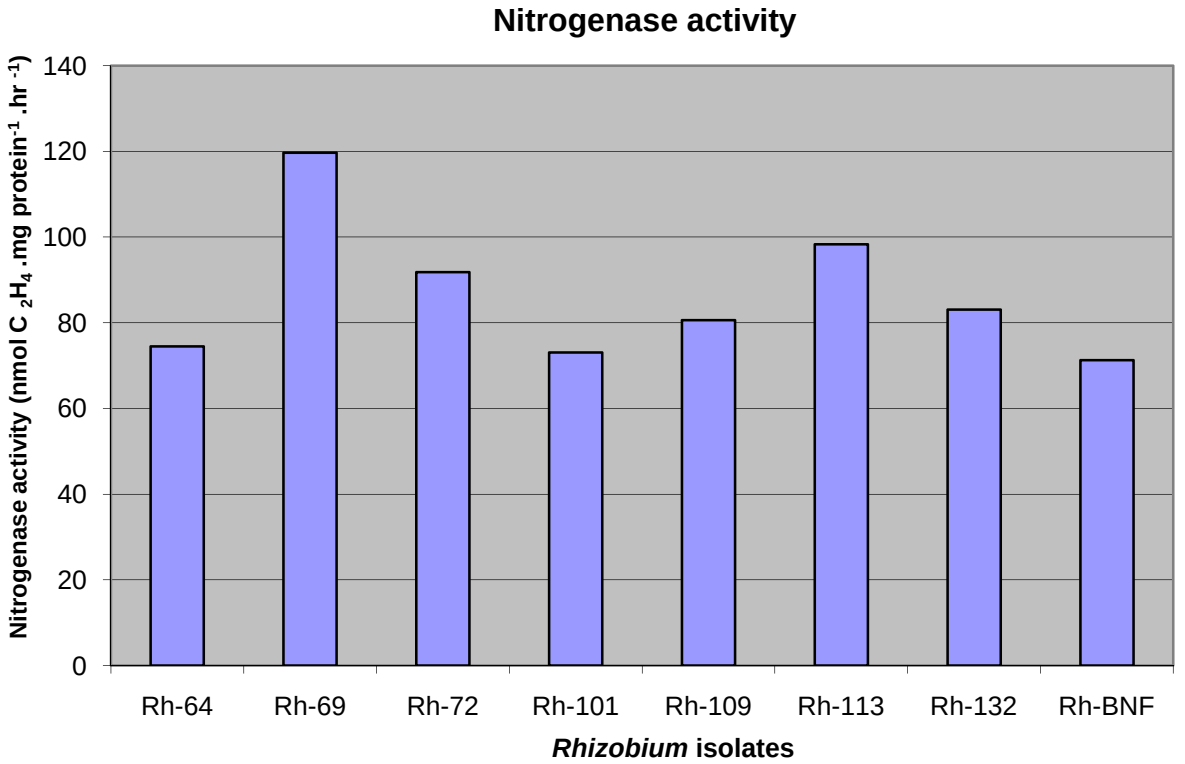


Figure 2. Nitrogenase activity of selected *Rhizobium* spp. isolates in comparison with standard MPKV strain Rh-BNF

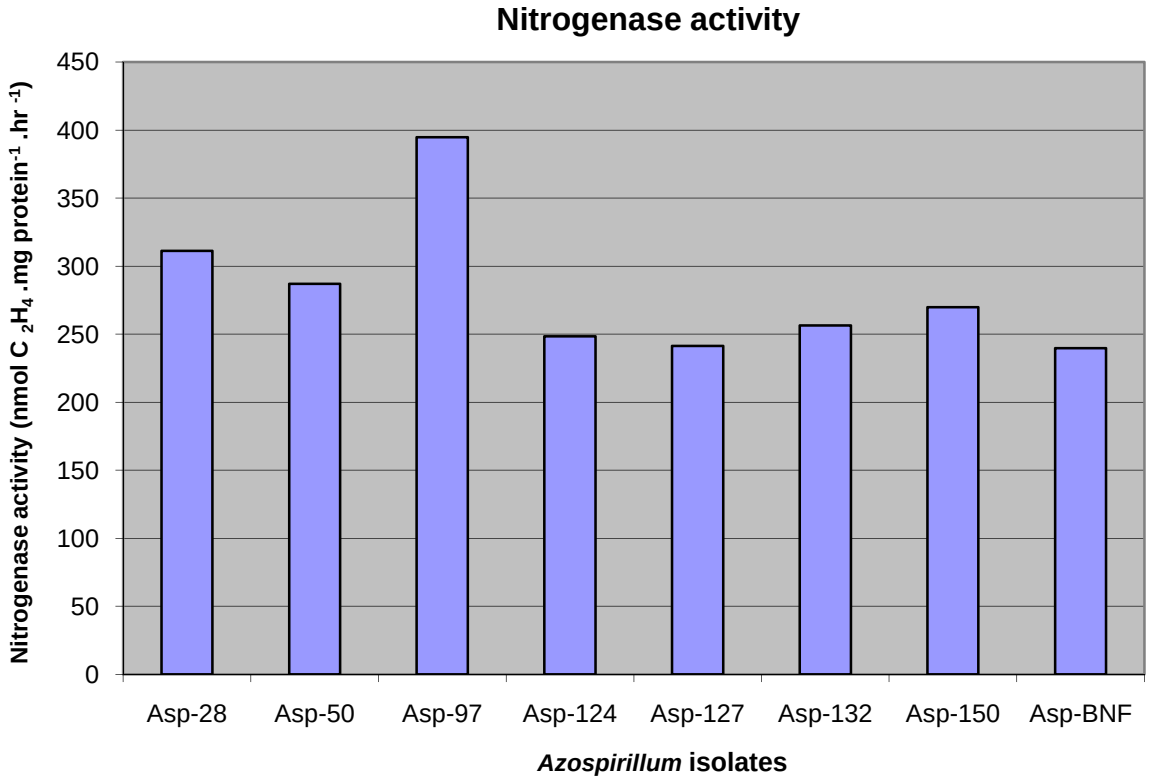


Figure 3. Nitrogenase activity of selected *Azospirillum lipoferum* isolates in comparison with standard MPKV strain Asp-BNF

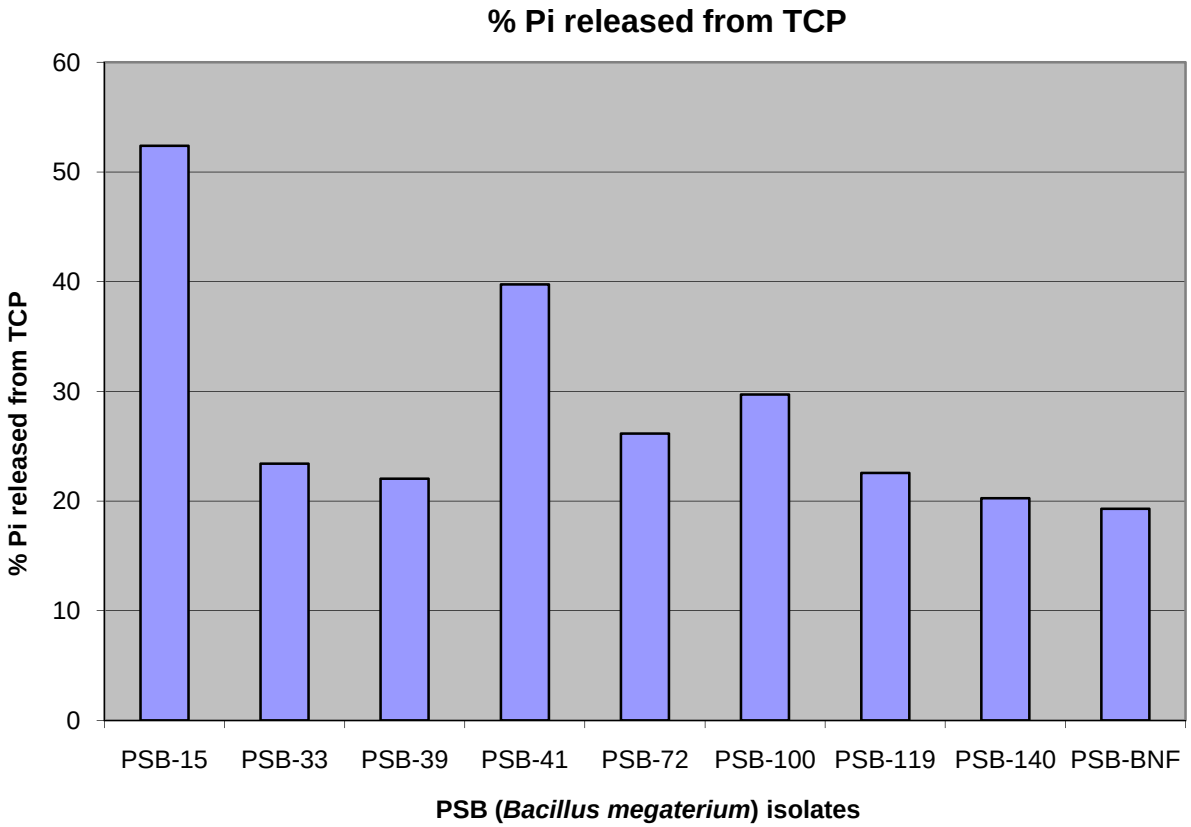


Figure 4. Amount of Pi released from TCP by selected PSB (*Bacillus megaterium*) isolates in comparison with standard MPKV strain PSB-BNF

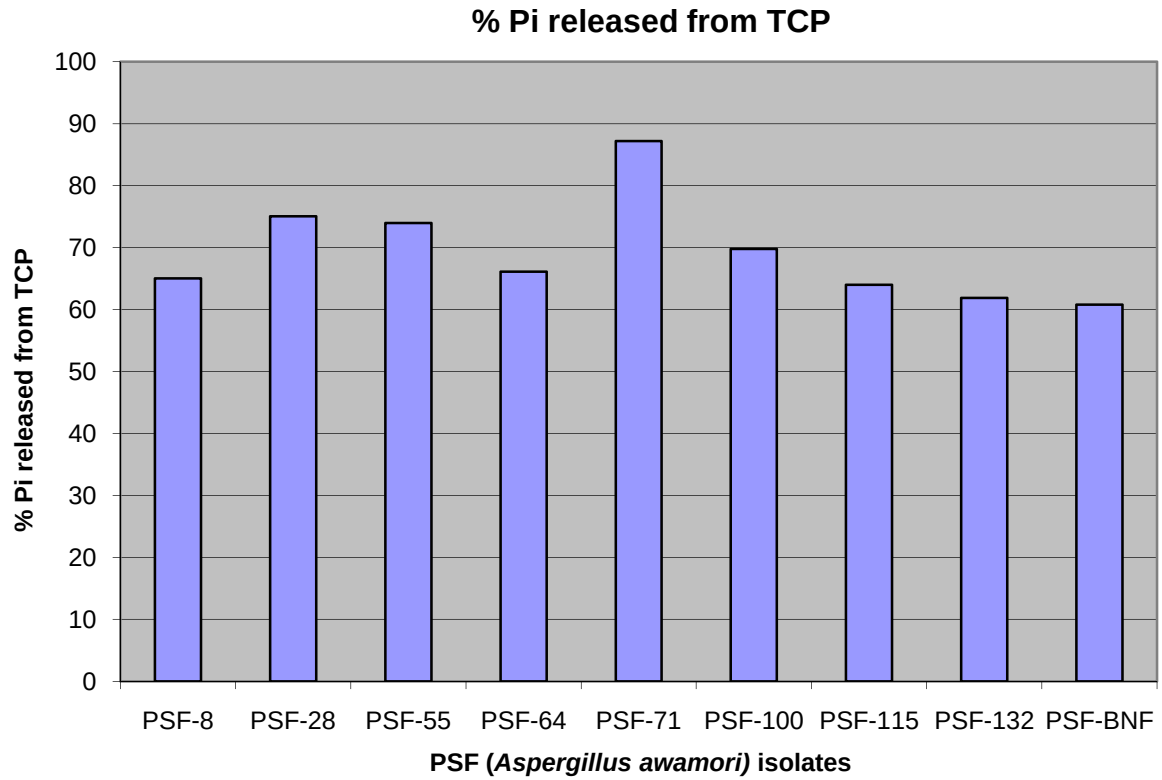


Figure 5. Amount of Pi released from TCP by selected PSF (*Aspergillus awamori*) isolates in comparison with standard MPKV strain PSF-BNF(A)

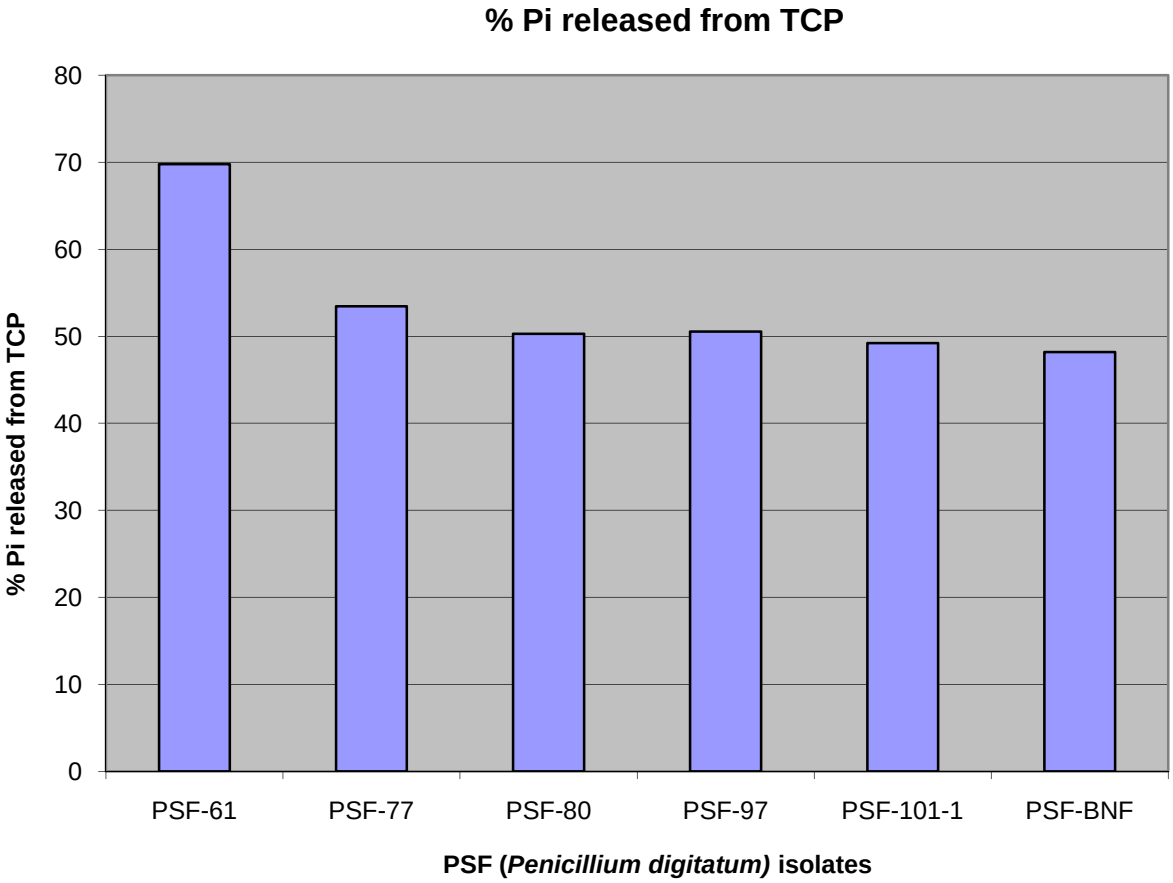


Figure 6. Amount of Pi released from TCP by selected PSF (*Penicillium digitatum*) isolates in comparison with standard MPKV strain PSF-BNF(P)

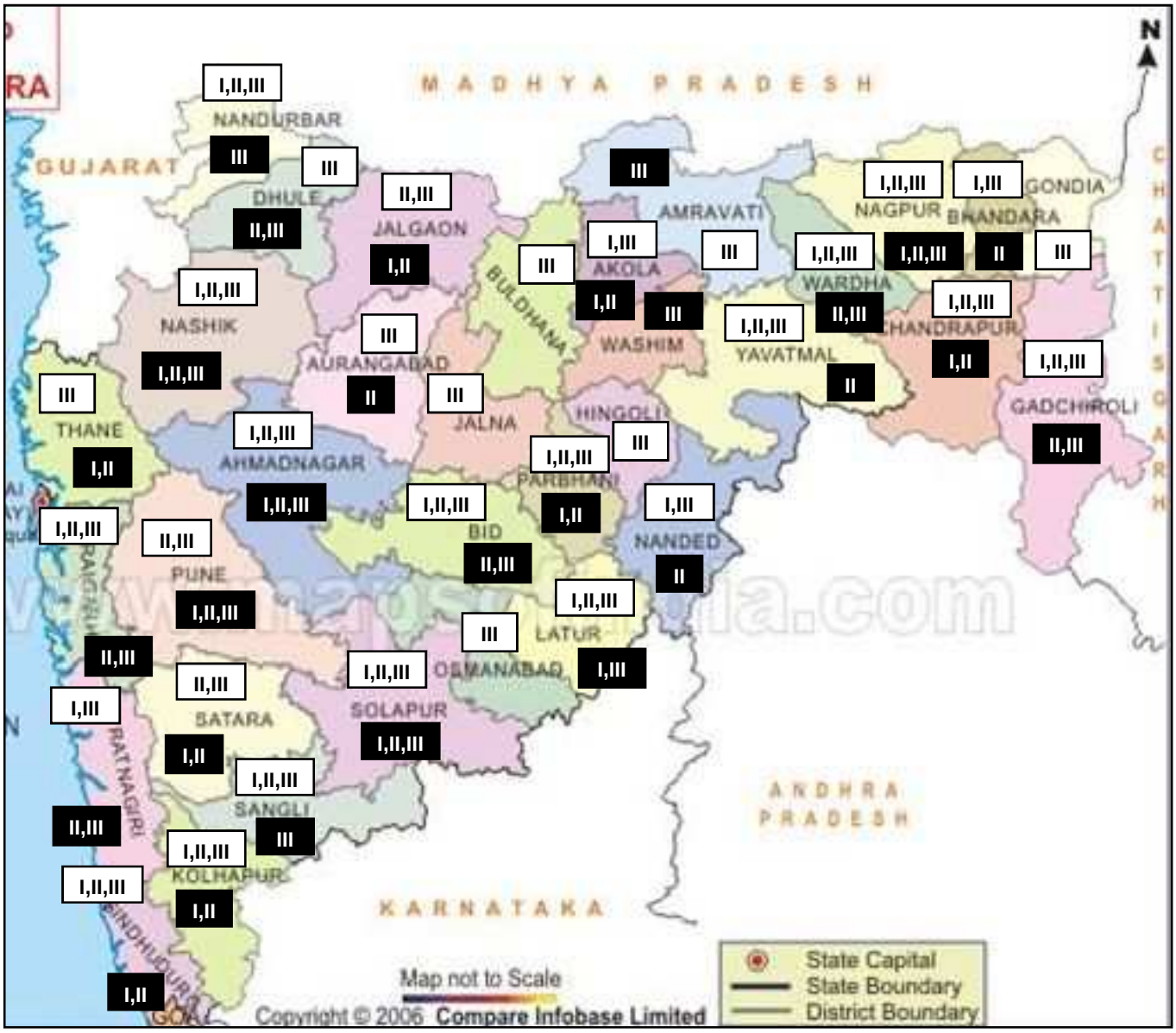


Figure 25. District wise distribution of efficient nitrogen fixing and phosphate solubilizing isolates in the soils of Maharashtra State

White Box – Efficient nitrogen fixing isolates

Black box – Efficient phosphate solubilizing isolates

Categories of efficiency of isolates: I = Highly efficient II= Moderately efficient III= Less efficient