

**INFLUENCE OF PLANT GROWTH
PROMOTING RHIZOBACTERIA (PGPR)
ON GROWTH PROMOTION OF RICE
[*Oryza sativa* L.]**

काशी हिन्दू
विश्वविद्यालय



BANARAS HINDU
UNIVERSITY

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Supervisor

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Submitted by

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Dear Sir,

I have great pleasure in forwarding the thesis entitled “**Influence of Plant Growth Promoting Rhizobacteria (PGPR) on Growth Promotion in Rice (*Oryza sativa* L.)**” submitted by **Mr. Shouvik Gorai, I.D. No. 16412GPB014, Enrollment No. 389677** in partial fulfillment of the requirements for the degree of **Master of Science (Agriculture)** in Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (U.P.) and placing on record that he has completed the requisite residential requirements as contained in the statutes of the university.

I certify that the entire scheme of investigation presented herein was planned and carried out solely by the candidate under my guidance and supervision. The data presented in thesis, to the best of my knowledge and belief, are genuine and original.

Thanking you,

Yours faithfully,

FORWARDED

(Prof. R. K. Singh)
Supervisor

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Dedicated to my beloved **parents**
and **Indian farmers**

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Date:

Place: Varanasi

(Shouvik Gorai)

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ABBREVIATIONS

% – Percentage

°C – Degree Centigrade

BNF – Biological nitrogen fixation

cm – Centimetre

Conc. – Concentrated

DW – Dry weight

FW – Fresh weight

g – Gram

h – Hour

IAA – Indole-3-acetic acid

kg – Kilogram

l – Litre

M – Molar

mg – Milligram

min. – Minute

ml – Millilitre

mM – Millimolar

mol. – Mole

N – Normal

NA- Nutrient Agar

nm – Nanometre

PGPR – Plant growth promoting rhizobacteria

rpm – Revolutions per minute

SOD – Superoxide dismutase

spp. – Species

UV/VIS – Ultraviolet/Visible

V – Volume

W – Weight

µmole – Micromole

YEM- Yeast Extract Mannitol



Introduction

“Start by doing what is necessary, then what is possible and suddenly you are doing the impossible.”

-St. Francis of Assisi

INTRODUCTION

Rice (*Oryza sativa*, L.), a member of Gramineae family is the leading food grain crop which has been reported to be used as a human food for the last almost 5000 years. Worldwide, more than 3.5 billion people depend on rice for more than 20% of their daily calorie intake (IRRI, 1997). More than 90% of world's rice is produced and consumed in Asia where 60% of world's population live (Khush, 2004; Zeigler and Barclay, 2008). Rice, the most important food crop of India, contributes nearly 45% to the total food grain production. It ranks first in the use of land (> 44 million ha), inputs (38-40% of fertilizers and 17 – 18% of pesticides) and water resources (> 50% irrigation water) though the use efficiency is significantly low (Tiwari, 2007). Given the increasing competition by urban and industrial water users, and the predicted adverse impacts of climate change on water availability, the present practice of rice production under flood-irrigated conditions has to cope with future water scarcity. Tuong and Bouman (2003) reported that by 2025, about 15 out of 75 million ha of Asia's flood-irrigated rice area alone will experience water shortage.

Rice plants require large amounts of mineral nutrients including N for their growth, development and grain production. Rice crops remove around 16–17 kg N for the production of each ton of rough rice including straw (De Datta 1981; Sahrawat 2000; Javaid and Bajwa 2010). However, most of the rice soils of the world are deficient in N, so N fertilizer application is required to meet a rice crop's N demand. Generally, urea is applied as the N source for rice production. But the efficiency of added urea-N utilization is very low, often only 30–40%, in some cases even lowers (Choudhury 2008). This low N-use efficiency is mainly due to denitrification, NH_3 volatilization and leaching losses (De Datta and Buresh 1989). NH_3 volatilization and denitrification cause atmospheric pollution through the production of greenhouse gases like N_2O and NH_3 . NO_3^- leaching causes groundwater toxicity. In addition to these environmental problems, the long-term use of urea depletes the soil organic matter. These problems are of great concern to soil and environmental scientists around the world. Alternate sources of N should be applied to minimize these problems if possible.

How can the current level of annual rice production of around 545 million tons be increased to about 700 million tons to feed an additional 650 million rice eaters by 2025 without irreversibly degrading the land resources, even with high levels of management inputs is indeed the great challenge in Asia. In order to ensure food security for growing population agricultural productivity must be increased proportionately. The other alternative method of providing nutrients for plant growth and yield production is use of soil microbes (Adesemoye *et al.*, 2008, Berg, 2009). Soil is a natural mix of weathered rock and organic matters and is the foundation of all crop production. Soil is biologically active and home to a wide range of living organisms, including soil microbes, earthworms and growing plant roots. Microorganisms present in soil include bacteria, actinomycetes, protozoa and fungi that affect soil structure and nutrient cycling. Generally, soil contains 10^7 to 10^9 of bacteria; 10^7 to 10^8 of actinomycetes; 10^5 to 10^6 of fungi per gram of soil (Coyne 1999). These microorganisms live in rhizosphere or root zone and play a significant role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients such as Nitrogen (N), Phosphorus (P) and Sulphur (Kunda and Gaur 1984). There is lot of diversity of microbes in the soil. Some bacteria living in soil freely while some are associated with the Rhizosphere soil of the plants because they depend on plant root exudates for their survival. Bacteria living in the soil are called free-living as they do not depend on root exudates for their survival. Bacteria associated with plants can be harmful and/or beneficial (Tilak *et al.*, 2005). Plants play an important role in selecting and enriching the types of bacteria by the constituents of their root exudates. Thus, depending on the nature and concentrations of organic constituents of exudates and the corresponding ability of the bacteria to utilize these as sources of energy, the bacterial community develops in the rhizosphere (Curl and Truelove, 1986). Rhizospheric bacterial communities, however, have efficient systems for uptake and catabolism of organic compounds present in root exudates (Barraquio *et al.*, 2000).

Plant growth promoting rhizobacteria (PGPR) are the soil bacteria inhabiting around/on the root surface and are directly or indirectly involved in promoting plant growth and development via production and secretion of various regulatory chemicals in the vicinity of rhizosphere. These PGPR promote plant growth in two different ways: (1) By direct mechanism which includes their capability of fixing atmospheric nitrogen, solubilizing phosphorus and iron, and of producing plant hormones, such as auxins, gibberelins, cytokinins, and ethylene (Kloepper, 1997). Changes in the plant hormone level also changes the root growth and shape by increasing root mass, root length,

and/or the branching of root hairs. Indole acetic acid (IAA) is one of the most physiologically active auxins. IAA is a common product of L-tryptophan metabolism by several microorganisms including PGPR (Lynch, 1985). Indirect mechanisms which involves the biological control of plant pathogens and deleterious microbes (bacteria, fungi, and viruses), through the production of antibiotics, lytic enzymes, hydrogen cyanide, catalase and limiting available Fe supply through producing siderophores or through competition for nutrients and space can improve significantly plant health and promote growth, as evidenced by increases in seedling emergence, vigor, and yield (Kumar *et al.*, 2012).

Various studies have documented the increased health and productivity of different plant species by the application of plant growth promoting rhizobacteria under both normal and stressed conditions (Ahemad and Kibret 2014). Strains with PGPR activity, mostly belonging to genera *Azospirillum*, *Azotobacter*, *Arthrobacter*, *Bacillus*, *Clostridium*, *Enterobacter*, *Gluconacetobacter*, *Pseudomonas* and *Serratia* have been reported (Hurek and Reinhold- Hurek 2003). Among these, species of *Pseudomonas* and *Bacillus* are the most extensively studied (Kumar *et al* 2011). These bacteria competitively colonize the roots of plants and can act as biofertilizers and/or antagonists (biopesticides) or simultaneously both. These characteristics have highlighted the use of efficient microorganisms to improve plant growth and manage soil and plant health with the aim to achieve sustainability in agriculture, which amalgamates environmental health, economic feasibility and social equity ensuring long-term productivity of natural resources and improved livelihood.

A variety of beneficial bacteria colonize the roots and other parts of rice. Interest in beneficial rhizobacteria associated with rice has increased recently due to their potential use as biofertilizer (Bashan *et al*, 1995). The beneficial effects of plant growth promoting rhizobacteria (PGPR) have been attributed to biological nitrogen fixation and production of phytohormones that promote root development and proliferation resulting in more efficient uptake of water and nutrients. Inoculation of indigenous strain of PGPR often showed better crop yield than inoculation with type strains (Murthy and Ladha,1988; Fulchieri and Frioni,1994). Over the last decade, asymbiotic nitrogen fixation has received much attention. Various associations between rice roots and nitrogen fixing bacteria have been described eg. rice with *Azospirillum brasilense*, *Enterobacter* spp. and *Pseudomonas* spp.

Recently, plant growth-promoting rhizobacteria (PGPR)-mediated Induced Systemic Resistance (ISR) has received considerable attention which provides a highly effective defense response that

is able to resist potential attack by microbial pathogens. Several mechanisms involved in the induction of systemic resistance include production of siderophores, salicylic acid, antibiotics and induction of pathogenesis related proteins (PR's). The production of enzymes related to pathogenesis (PR-proteins) such as chitinases and glucanases by strains of Rhizobacteria can lyse the fungal cell wall and are considered an important trait for antagonism (Saikia *et al* 2004). Apart from PR-proteins, the plants also produce other defense enzymes, including peroxidases. Metabolic changes involved in the defense mechanism of plants are correlated with changes in activity of key enzymes in primary and secondary metabolism. Peroxidase catalyses the formation of lignin (Figueiredo *et al* 2010). In addition to the pathogenesis-related (PR-proteins), the plants produce other enzymes of the defense, including peroxidases, phenylalanine ammonia-lyase (PAL), and polyphenoloxidase (PPO).

Recent studies indicate that microorganisms can also help crops to cope up with abiotic stresses. A variety of mechanisms have been proposed behind microbial elicited stress tolerance in plants. Production of indole acetic acid, gibberellins and some unknown determinants by PGPR, result in increased root length, root surface area and number of root tips, leading to enhanced uptake of nutrients thereby improving plant health under stress conditions (Egamberdieva and Kucharova, 2009). Recent study reports the potential of PGPR strains in alleviating drought stress and salinity stress by lowering the activity of antioxidant enzymes in plants as compared to uninoculated plants (Sandhya *et al.*, 2010; Omar *et al.*, 2009). In the last decade, bacteria belonging to different genera including *Rhizobium*, *Bacillus*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Microbacterium*, *Methylobacterium*, *Variovorax*, *Enterobacter*, etc. have been reported to provide tolerance to host plants under different abiotic stress environments. Use of these microorganisms per se can alleviate stresses in agriculture thus opening a new and emerging application of microorganisms has been reviewed (Venkateswarlu *et al.*, 2008; Yang *et al.*, 2009).

Thus, information on effect of bacterial PGPR on agronomic and plant physiological attributes of rice is generally lacking. With such research gaps existing and Keeping all these points in view present study “Influence of Plant Growth Promoting Rhizobacteria (PGPR) on Growth Promotion in Rice (*Oryza sativa* L.) was carried out with the following objectives:

- To characterize selected plant growth promoting endophytic rhizobacteria physiologically (temperature, pH, salt tolerance, antibiotic resistance).
- To characterize selected plant growth promoting endophytic rhizobacteria biochemically (IAA, siderophore production, solubilization of P, Zn, K).
- To study the effect of endophytic bacterial inoculation on germination of rice under laboratory condition.
- To study the impact of inoculation of endophytic bacterial strains alone as well as in combination on different plant growth parameter in rice.



Review of Literature

*“If you donot know history, you don't know anything.
You are a leaf that doesn't know its part of the tree.”*

-Michael Crichton

REVIEW OF LITERATURE

Plant growth is influenced by many abiotic and biotic factors. The thin layer of soil immediately surrounding plant roots extremely important and active area for root activity and metabolism. The rhizosphere concept was first introduced by Hiltner in the year 1904 to describe the narrow zone of soil surrounding the roots where microbe populations are stimulated by root activities (**Hiltner, 1904**). A large number of microorganisms such as bacteria, fungi, protozoa and algae coexist in the rhizosphere. Bacteria are the most abundant microorganisms in the rhizosphere, it is highly possible that they influence the plants physiology to a greater extent, especially considering their competitiveness in root colonization. Plants select those bacteria contributing most to their fitness by releasing organic compounds through exudates creating a very selective environment where diversity is low (**Garcia et al. 2001**).

Microorganisms that colonize the rhizosphere can be classified according to their effects on plants and the way they interact with roots, some being pathogens whereas other cause beneficial effects. The bacteria inhabiting the rhizosphere and have beneficial effect to plant via direct or in direct mechanism are termed Plant growth-promoting rhizobacteria (**Kloepper et al. 1980**). Various bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacte*, *Burkholderia*, *Bacillus* and *Serratia* have been reported to enhance the plant growth (**Glick, 1995**). There are several PGPR inoculants currently commercialized that seem to promote growth through at least one mechanism either by suppression of plant disease (Bioprotectants), improved nutrient acquisition (Biofertilizers) and phytohormone production (Biostimulants). The use of PGPR offers an attractive way to supliment chemical fertilizer and pesticides.

The pertinent literature available is reviewed under the following heads:

2.1 Plant growth promoting rhizobacteria (PGPR):

The rhizosphere is the volume of soil surrounding and under the influence of plant roots, and the rhizoplane is the plant root surfaces and strongly adhering soil particles (**Kennedy, 2005**).

Pinton *et al.*, (2001) reviewed that often, studies of the microbial ecology of the rhizosphere also include the rhizoplane. In the rhizosphere, very important and intensive biochemical interactions and exchanges of signal molecules takes place between the plant, soil, microorganisms and soil micro fauna.

Antoun and Klopper, (2001) reported that rhizobacteria are rhizosphere competent bacteria that aggressively colonize plant roots; they are able to multiply and colonize all the ecological niches found on the roots at all stages of plant growth, in the presence of a competing microflora. About 2 to 5% of rhizobacteria, when reintroduced by plant inoculation in a soil containing competitive microflora, exert a beneficial effect on plant growth and are termed plant growth promoting rhizobacteria (PGPR). PGPR are free-living bacteria and some of them invade the tissues of living plants and cause unapparent and asymptomatic infections.

Klopper *et al.*, (1989); Glick, (1995) reported various species of bacteria like *Pseudomonas*, *Azospirillum*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligenes*, *Arthrobacter*, *Burkholderia*, *Bacillus* and *Serratia* that enhances the plant growth. In their association with their host plant PGPR may differently colonize their host plant. They may colonize the rhizosphere, the root surface or the intercellular spaces of the host plant.

Martinez-Viveros *et al.*, (2010) classified PGPRs based on their degree of association with the plant root cells. PGPRs can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR). iPGPRs can enter plant cells and are able to produce specialized structures, the so-called nodules. ePGPRs, on the other hand, are found in the rhizosphere, or within the apoplast of the root cortex, but not inside the cells. According to their proximity to the plant root, ePGPRs can be divided into three classes: those living near, but not in contact with the roots; those colonizing the root surface; and those living in the spaces between cells of the root cortex (*Herbaspirillum seropedicae* colonize root intercellular space, aerenchyma and cortical cells, with a few penetrating stele to enter the vascular tissue).

Holguin *et al.*, (1999) described that PGPR may induce plant growth promotion by direct or indirect modes of action. Direct mechanisms includes i) nitrogen fixation ii) solubilization of phosphorus iii) sequestering of iron by production of siderophores iv) production of phytohormones such as auxins, cytokinins, gibberellins and v) lowering of ethylene concentration,

thus improve the plant nutrient status and stimulate the disease-resistance mechanisms. Indirect effects originate for example when PGPR act like biocontrol agents reducing diseases, when they stimulate other beneficial symbioses, or when they protect the plant by degrading xenobiotics in inhibitory contaminated soils.

Somers *et al.*, (2004) classified PGPR as biofertilizers (increasing the availability of nutrients to plant), phyto stimulators (plant growth promoting, usually by the production of phytohormones), rhizoremediators (degrading organic pollutants) and biopesticides (controlling diseases, mainly by the production of antibiotics and antifungal metabolites).

2.2 PGPR as endophytes

The plant roots are the interface between a multicellular eukaryote, soil and one of the richest microbial ecosystems on earth. Soil bacteria are able to multiply inside roots as endophytes and modulate plant growth and development, with implications ranging from enhanced crop productivity to phytoremediation.

Researcher have found bacterial endophytes in potato (Garbeva *et al.* 2001), maize (Fisher *et al.* 1992), rice (Stoltzfus *et al.* 1997), cotton (McInroy & Kloepper, 1995) and cucumber (Mahafee & Kloepper, 1997).

Bell *et al.*, (1995) studied that endophytes entered the plant tissue primarily through the roots, however, aerial portions of plants such as flowers, stems, and cotyledons, are also used for their entry.

Aravind *et al.* (2009) reported Indigenous endophytic bacteria from black pepper (*Piper nigrum* L.) belonging to genera like *Pseudomonas*, *Bacillus*, *Herbaspirillum*, *Serratia*, *Streptomyces* etc. Their use for biocontrol purpose would eliminate the need to select bacterial types with high rhizosphere competence that are often considered necessary for successful seed or root bacterization before or at planting.

Sun *et al.*, (2008) analyzed endophytic bacterial diversity in the roots of rice (*Oryza sativa* L.) by 16S rDNA cloning, amplified ribosomal DNA restriction analysis (ARDRA) and sequence homology comparison. Sequence analysis revealed diverse phyla of bacteria which consisted of alpha (*Methylobacterium* spp., [*Rhizobium*](#) spp.), beta (*Burkholderia* spp., *Nitrosomonas* spp.,

Azoarcus spp.), gamma (*Pseudomonas* spp.), delta (*Desulfobacter* spp.), and epsilon (*Campylobacter* spp.) subclasses of the Proteobacteria.

Procopio et al., (2009) isolated Seventy six endophytic bacterial strains from *Eucalyptus* sp. and were characterized by RAPD and amplified rDNA restriction analysis (ARDRA). The isolates were identified by 16S rDNA sequencing as *Pantoea* (45%), *Agrobacterium* spp. (21%), *Curtobacterium* spp. (9%), *Brevibacillus* spp. (8%), *Pseudomonas* spp. (8%), *Acinetobacter* spp. (4%), *Burkholderia cepacia* (2.6%), and *Lactococcus lactis* (2.6%).

Luo et al. (2011) isolated *Serratia* spp. an endophytic bacterium of *Solanum nigrum* L. showed cadmium and zinc hyper accumulation *in vitro* as well as *in vivo*.

2.3 The mechanisms PGPRs use to promote plant growth

2.3.1 Biological Nitrogen Fixation

Nitrogen (N) is one of the major plant nutrients, hence the ability of some microorganisms to make atmospheric nitrogen available for plants by biological nitrogen fixation is of great importance. Advances in agricultural sustainability will require an increase in the utilization of BNF as a major source of nitrogen for plants. 80% of total biological nitrogen fixation comes from symbiotic associations of *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium*, the obligate symbionts of leguminous plants. Non-symbiotic BNF carried out by free living diazotrophic bacteria belonging to genera *Azoarcus*, *Azospirillum*, *Burkholderias*, *Gluconacetobacter* and *Pseudomonas* that can stimulate non-legume plants growth (Antoun et al 1998).

Antoun et al. (1998) studied the effect *Bradyrhizobium* and *Rhizobium* as plant growth promoting rhizobacteria in non-leguminous crop (*Raphanus sativus* L.). *Bradyrhizobium japonicum* enhanced the N-availability and responsible for about 60% increase in radish dry matter yield.

Kanimozhi et al. (2010) reported application of root associated N₂ fixing (diazotrophic) bacteria from rice leads to significant increases in the shoot length (up to 60%), shoot dry weight (up to 33%) and the grain yield (up to 26%) of rice. Similarly, a positive co-relation of N₂ fixing efficiency of *Azospirillum* strain and the grain yield of paddy was also there.

Mehnaz et al. (2001) estimated Nitrogen-fixing ability of *Enterobacter* and *Azospirillum brasilense* isolated from rice by ARA-based MPN (acetylene reduction assay-based most probable number) in roots and shoots. Nitrogen fixation with the inoculants *Azospirillum* and *Enterobacter* was nearly 46% and 41% of the nitrogen derived from atmosphere respectively.

Mia et al. (2007) studied the effect of combined application of PGPR strain and N-fertilizers on growth of banana plantlets. They demonstrated that PGPR inoculation with 20 mg/kg minimal N-fertilizer (13% of total plant N) produced a synergistic effect on N₂ fixation with higher amounts of N₂ fixed (11.0 mg/plant; 12.5 % Ndfa), while higher doses of N-fertilizers inhibits the N₂ fixation.

2.3.2 Phosphate Solubilization

Phosphorus (P) is an essential plant nutrient with low availability in many agricultural soils. much of this P is in mineral forms and is only slowly available to plants. The ability of rhizospheric and endophytic bacteria to solubilize insoluble P minerals has been attributed to their capacity to reduce pH by the excretion of organic acids (e.g., gluconate, citrate, lactate and succionate) and protons (during the assimilation of NH₄⁺) (**Gyaneshwar et al., 1999**). These bacteria have been characterized as members of the *Bacillus*, *Burkholderia*, *Enterobacter*, *Klebsiella*, *Kluyvera*, *Streptomyces*, *Pantoea* and *Pseudomonas* genera.

Dave and Patel (1999) reported *in vitro* P-solubilization by psuedomonads isolates in different media namely bone meal, rock phosphate, diacalcium phosphate, TCP, ferrous phosphate and aluminum phosphate. The highest P solubilizatoin for TCP (163mg P₂O₅), rock phosphate (21mg P₂O₅), diacalcium phosphate (99mg P₂O₅) ferrous phosphate (24.8mg P₂O₅) and aluminum phosphate (5.3mg P₂O₅) was recorded. Bone meal was considered as most readily soluble form of phosphate.

Afzal and Asghari (2008) studied the effect of a Rhizobial strain and a P-solubilizer strain in single and in dual combination with and without P₂O₅ on wheat. It was concluded that single and dual inoculation along with P fertilizer is 30-40% better than only P fertilizer for improving grain

yield of wheat and dual inoculation without fertilizer (P) improved grain yield up to 20% as compared to P application.

Kumar *et al.* (2012) analysed P-solubilizers associated with French bean rhizosphere. Quantitative estimation of tri-calcium phosphate solubilization was found between 15 to 60 ng/ml. The phosphate solubilization zones was determined ranging from 4 mm to 20 mm on solid media.

Parani and Saha (2012) reported a *Pseudomonas fluorescens* strain solubilized tricalcium phosphate to substantial amount (968.5 mg l⁻¹) in Pikovskaya's (PVK) broth.

Srivastava *et al.* (2007) showed that out of 30 culture of bacteria and fungi, 26 isolates solubilized eight varying amount of phosphorus, some of the isolates showed positive antifungal activities as well and were identified as *Pseudomonas* spp., *Pseudomonas striata* continue to perform best in phosphate solubilization even under field conditions.

2.3.3 Indole-3-Acetic Acid (IAA) Production

The production of phytohormones by PGPR is now considered to be one of the most important mechanisms by which many rhizobacteria promote plant growth. Phytohormones are signal molecules acting as chemical messengers and play a fundamental role as growth and development regulators in the plants. The PGPR can stimulate plant growth through the production of auxins (indole acetic acid), gibberellines, and cytokinins or by regulating the high levels of endogenous ethylene in the plant. At present, auxin synthesizing rhizobacteria are the most well-studied phytohormone producers (**Spaepen *et al.*, 2007**). However, the effect of IAA on plants depends on the plant sensitivity to IAA and the amount of IAA produced from plant associated bacteria and induction of other phytohormones.

Joseph *et al.* (2007) reported 150 PGPR isolates belonging to *Bacillus*, *Pseudomonas*, *Azotobacter* and *Rhizobium* isolated from different rhizospheric soil of chick pea for production of indoleacetic acid (IAA). All the isolates of *Bacillus*, *Pseudomonas* and *Azotobacter* produced IAA, whereas only 85.7% of *Rhizobium* was able to produce IAA.

Ashrafuzzaman et al. (2009) isolated and characterized six indole acetic acid (IAA) producing PGPR isolates from the rhizosphere soil of rice. Significant increase in plant height, root length, and dry matter production of shoot and root of rice seedlings observed after application of selected isolates.

Ashraf et al. (2011) isolated ten bacterial strains from roots and rhizosphere of sugarcane showed IAA production in growth medium containing tryptophane. IAA production ranged from 1.1-4.49mg/L. Inoculation with selected isolates lead to maximum root area (37.40 mm²) and root length (288.47 mm).

Wahyudi et al. (2011) recorded a significant increase in the root length, shoot length and number of lateral root of soybean treated with IAA producing *Bacillus* species.

Rani et al. (2012) characterized sixty isolates for the indole acetic acid (IAA) production and other PGPR traits. PGPR isolates remarkably enhanced seed germination, shoot length, root length, dry matter production of shoot, nodule number and nodule mass of pigeon pea.

Etesami et al. (2015) isolated ten isolates from berseem clover plants which had been screened to detect isolates with good potential as rice growth-promoting agents indicated that seven out of ten endophytes, inoculated on five rice cultivars, were able to colonize within roots and promote plant growth. According to the results obtained, 7 isolates tested behaved as potentially good PGP and colonizing agents. Seedlings inoculated with IAA producing isolates yielded more shoot biomass (stem plus leaves), root length and colonization than the control plants inoculated with IAA non-producing strains and plants inoculated with PGPR producing other PGP traits.

2.3.4 Siderophore production

Siderophores are low molecular weight, extracellular compounds with a high affinity for ferric iron, that are secreted by microorganisms to take up iron from the environment (**Hofte, 1993**). These compounds are produced by various types of bacteria in response to iron deficiency which normally occurs in neutral to alkaline pH soils, due to low iron solubility at elevated pH (**Sharma and Johri, 2003**). Fe acquisition through siderophore production plays an essential role in

determining the competitive fitness of bacteria to colonize plant roots and to compete for iron with other microorganisms in the rhizosphere (**Crowley and Gries, 1994; Crowley, 2006**).

Arora et al. (2001) isolated root-nodulating bacterial strains of *Rhizobium meliloti* from the medicinal plant *Mucuna pruriens* capable to produce siderophores. Application of selected strains resulted in marked enhancement in percentage seed germination, seedling biomass, nodule number and nodule fresh weight of *M. phaseolina* compared to uninoculated and uninfected controls.

Meera and Balabaskar, (2012) reported *Pseudomonas fluorescens* isolates from rice rhizosphere. Significant diseases reduction and other prominent PGPR traits were recorded for all isolates.

Mishra and Kumar (2012) *in vitro* studied production of siderophore on different carbon sources by two PGPR strains. Application of selected isolates, significantly increased the growth of rice (*Pusa sugandha III*). Strains were identified with the help of 16S rRNA gene sequencing as *Bacillus amyloliquefaciens* and *Bacillus subtilis*. *Bacillus subtilis* was found to be more efficient as compared to *Bacillus amyloliquefaciens* in terms of its capability to produce siderophore.

Shrivastava and Kumar (2011) isolated 74 rhizobacterial strains from rice rhizosphere. Out of 74 isolates, only 29 (39.2%) diazotrophic rhizobacterial isolates showed siderophore production. The amount of siderophore produced by these isolates ranged between 1.45 to 22.22 µg/mg dry weights. 15 (51.7%) isolates were catechol type and 14 (48.3%) were hydroxamate type. TLC analysis of catechol type siderophores revealed that only 13.8% was exactly similar to 2, 3-dihydroxybenzoic acid (DHBA) whereas 86.2 % showed more than one type of siderophores or other catechol type siderophore with varying molecular weights.

2.4 Rice-microbes interaction

Dewan and Subba Rao (1979) have found that root biomass of rice seedlings increased when inoculated with *Azospirillum brasilense* and *Azotobacter chroococcum* singly or in combination. They also observed that this increase in the biomass of roots was more in unsterilized soil than in sterilized one with or without inorganic nitrogen in the form of urea.

Stoltzfus et al. (1997) indicated in their study that a large diversity of apparently diazotrophic and non-diazotrophic endophytic bacteria can be isolated from rice tissues, some of which are capable of re-colonizing their host when re-inoculated onto sterile rice seedlings.

Yanni et al. (1997) have examined that the clover symbiont, *Rhizobium leguminosarum* bv. *trifolii* colonizes rice roots endophytically in fields where these crops were rotated. Their result indicated that *Rhizobium leguminosarum* bv. *trifolii* significantly increased shoot and root growth of rice in growth chamber experiments, and grain yield plus agronomic fertilizer N-use efficiency of hybrid rice in a field inoculation experiment.

Ladha et al. (1997) investigated that nitrogen-fixing putative endophytic populations in the root and culm on field-grown rice plants grown in the absence of nitrogen fertilizer were significantly higher near heading stage.

El-Khawas and Adachi (1999) have observed that *Azospirillum brasilense* and *Klebsiella pneumoniae* in culture media supplemented with tryptophan produced high quantities of extracellular IAA. They investigated the effect of different concentrations of filter-sterilized culture supernatants of both strains on the development of rice roots grown in hydroponic culture medium and observed increased root elongation, root surface area, root dry matter and development of lateral roots and root hairs under optimum concentrations (6–8%) of bacterial supernatants compared to untreated roots. They also observed that high concentrations of the supernatant (more than 10%) strongly inhibited root elongation.

Biswas et al. (2000) have inoculated rice seedlings with different rhizobia in soil supplemented with varied amounts of mineral nitrogen. The results indicated that *Rhizobium leguminosarum* bv. *Trifolii*, *Rhizobium* sp. and *Bradyrhizobium* sp. increased rice grain and straw yields differently at different nitrogen rates.

Nandakumar et al., (2001) have shown in their results that PGPR treatment significantly increase plant growth as measured by the number of tillers, plant height and yield both in glasshouse and in the field experiments. They observed enhanced plant growth in treatments, which received a mixture of strains rather than a single strain. The increased plant growth ultimately resulted in significant increase in yield.

Mehnaz et al., (2001) have isolated PGPR from rice and observed their beneficial effect on two Basmati rice varieties. Their result showed that *Enterobacter* S1 and *Azospirillum* maximally increase root area and plant biomass in rice variety Basmati 385, whereas in the rice variety Super Basmati, *Enterobacter* R3 maximally increase of root area and plant biomass as well as observed that these bacteria also increase the nitrogen content in plants.

Verma et al. (2001) studied on the diversity of endophytic bacteria present in seeds of a deepwater rice variety on the basis of the presence of seven types of BOX-PCR fingerprints. For studying the endophytic colonization *P. agglomerans* strain was genetically tagged with the reporter gene, *gusA*. Histochemical analysis of the seedling grown in hydroponics showed that the tagged strain colonized the root surface, root hairs, root cap, points of lateral root emergence, root cortex and the stelar region and found that short thickened lateral roots showed better colonization by *P. agglomerans*.

Rediers et al., (2003) have isolated *Pseudomonas stutzeri* a nitrogen-fixing bacterium from rice plants and found that it colonize and infect rice roots and thus provide rice plants fixed nitrogen and enhance plants growth. They also focused their study on the identification of *P. stutzeri* genes that are switched on during rice root colonization and are switched off during free-living growth on synthetic medium.

Minamisawa et al. (2004) reported the existence of anaerobic nitrogen-fixing consortia (ANFICOs) consisting of N₂-fixing clostridia and diverse non-diazotrophic bacteria in non leguminous plants (gramineous plants) and found that these bacteria are widespread in wild rice. Their results indicated that clostridia are naturally occurring endophytes in gramineous plants and that clostridial N₂ fixation arises in association with non diazotrophic endophytes.

Mirza et al. (2006) have treated two rice varieties Super Basmati and Basmati 385 with nitrogen fixing PGPR (*Azospirillum brasilense*, *Azospirillum lipoferum*, *Zoogloea* and *Pseudomonas* sp.) as single-strain inoculants and observed increased shoot biomass and/or grain yield over that of non inoculated control plants in each inoculated treatment.

Ahanthem and Jha (2007) has observed that *Glomus* sp. and *Azotobacter chroococcum*, either alone or in combination with different levels of soil nitrogen application, positively affected crop growth over the treatments that received nitrogen only. This indicates that inoculation of rice plants with *Glomus* sp. and, the dual inoculation of *Glomus* sp. and *Azotobacter chroococcum* enhanced the plant growth when urea was incorporated up to the recommended dose.

Peng et al. (2009) have isolated twelve facultative anaerobic, endophytic diazotrophs from surface-sterilized roots of the wild rice species *Oryza latifolia* and characterized by phenotypic and molecular methods. The study indicated that *Enterobacter oryzae* isolated as a novel nitrogen fixing strain.

Prakamhang et al., (2009) explored the community of diazotrophic bacteria from the leaf, stem, and root parts of the rice. They observed that the expression level of the *nifH* gene in all roots from plants grown in N-fertilized soil was the lowest among the treatments studied. Their results confirmed the complexity of the endophytic diazotrophic bacterial community, and indicate that the type of plant tissue seems to influence the community structure.

Jha et al., (2009) have isolated a diversity of N₂-fixing (diazotrophic) bacteria from two traditional rice cultivars of North Bihar, India, where low levels of nitrogen fertilizers were applied. *Burkholderia cenocepacia*, *Pseudomonas* sp. and three diazotrophic PGPR reference strains were used as inoculation for growth promoting potential of rice. Significant increases in the shoot length, shoot dry weight and the grain yield per plant were observed in non-axenic pot and field trials with sustainable colonization of inoculated bacteria.

Panhwar et al., (2011) have shown in their study that Phosphate solubilizing *Bacillus* strains exhibited capability of producing organic acids from soil and plant roots and increased yield of aerobic rice under glasshouse conditions. They also observed high leaf chlorophyll content, photosynthesis rate and root development

Bhattacharjee et al., (2011), have focused their study on chemotaxis, colonization and rice growth promoting ability of indole acetic acid (IAA) and 1-aminocyclopropane-1-carboxylic acid (ACC)

deaminase-producing rhizobacteria *Rhizobium leguminosarum* bv. *trifolii*, previously isolated from root nodules of *Trifolium alexandrinum* L. and found that this bacteria promote the growth of four different rice varieties grown in India in terms of biomass, root branching and N content as well as successfully colonize the root surface of the rice variety.

Salamone et al. (2012) have reported that *Pseudomonas fluorescens* and *Azospirillum brasilense* has significant potential when applied to rice as a commercial formulation under field condition.

Hasan et al. (2014) isolated PGPR from sterilized roots of rice (*Oryza sativa* L.) Two of the PGPR isolates were found belonging to *Enterobacter spp.* while the other two isolates were *Azospirillum spp.* The two rice cultivars Basmati and Super Basmati were inoculated with bacterial isolates alone and in combination with *Rhizobium* and *Azospirillum* respectively and their growth responses were examined. Their results confirmed the beneficial effects of co-inoculation with sufficient increase in root length, shoot length and fresh and dry weight of plants, when compared to single inoculation or uninoculation.



Materials and Methods

“Creativity requires input, and that's what research is. You're gathering material with which to build.”

-Gene Luen Yang

MATERIALS AND METHODS

The present study was carried out at the PGPR Laboratory Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi (UP).

3.1 Bacterial strains used and their cultivation

Four plant growth promoting rhizobacterial endophytic strains used in this study were obtained from Prof. R. K. Singh (Sr. Microbiologist) Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi, India. These isolates were routinely maintained in Yeast Extract Mannitol (YEM) medium (Vincent, 1970) (Appendix-I) at $28 \pm 2^{\circ}\text{C}$. These strains were designated as RRE3, RRE6, BHU3, BHU8.

Table 3.1: Details of endophytic bacteria used in inoculation test

Strains	Details
RRE3	<i>Burkholderia cepacia</i>
RRE6	<i>Rhizobium leguminosarum</i> bv. <i>phaseoli</i>
BHU3	<i>Pseudomonas aeruginosa</i>
BHU8	<i>Pseudomonas saponiphila</i>

3.2 Plant material used

Seeds of the rice cultivar HUR 105 used in this study were provided by Prof. R. P. Singh (Plant breeder), Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi.

3.3 Growth Media

3.3.1 Media used for cultivation of bacteria

Strains of α -rhizobial strains were grown and maintained in Yeast Extract Mannitol (YEM) medium and β -rhizobial strains were maintained in BAz and BAc medium (Perin *et al.* 2006a) [Appendix II]. 1.5% (w/v) agar-agar was used to solidify media when required. Bacterial strains were incubated at $28\pm 2^\circ\text{C}$.

3.3.2 Media used for plant growth study

For plant growth study on rice, nitrogen free Thornton's medium (Thornton, 1930) with 1.5% agar (appendix-III) was used.

3.4 Glassware

Glassware used in this study were manufactured by Borosil (India) and Schott Duran (Germany). Thorough washing of glassware was done by several times with tap water followed by rinsing with double distilled water. Finally, the glasswares were dried in hot-air oven before use. Petridishes, clean culture tubes and Erlenmeyer flasks were used for culturing purpose. Gibson's tubes were used for invitro plant growth experiment. Culture tubes and flasks were plugged with non-absorbent cotton wool.

3.5 Chemicals

Most of the chemicals were obtained from E. Merck (Germany), Sigma-Aldrich (St. Louis, Missouri, USA), and Hi-Media (India). Double distilled water was used throughout the experiment for preparation of stock solution, media and reagents etc.

3.6 Sterilization

Sterilization of different growth media and glasswares were done by steam sterilisation in an autoclave at 15psi and 121°C for 15 min. The heat labile chemicals (aminoacids) were sterilized by filter sterilization using $0.22\mu\text{m}$ size Millipore filters. Seed material used for this study was

sterilized by using chemicals mercuric chloride and alcohol. All the inoculations and bacterial culture transfers were performed under sterile conditions of laminar air flow hood.

3.7 Physiological and Biochemical characterization

3.7.1 Detection of temperature tolerance

To determine the bacterial growth at different temperature, YEM agar plates were inoculated with 10 μ l of culture ($\pm 10^9$ CFU ml⁻¹) and incubated at four temperatures 15^oC, 25^oC, 35^oC, 45^oC. The same procedure was performed in YEM broth and optical density was recorded at 420 nm after two and four days of incubation.

3.7.2 Detection of pH tolerance

The ability of endophytic bacterial strains to tolerate a high pH was tested on YEM solid medium. YEM agar plate with six different level of pH 5, 6, 7, 8, 9, 10, 11 and 12 were maintained by using sodium hydroxide (NaOH). 10 μ l of culture ($\pm 10^9$ CFU ml⁻¹) of each bacterial strain were dropped onto the plates of varying degree of pH. These were incubated at 28-30^oC for 5 days. The same experiment was performed in YEM broth and optical density was measured at 420 nm after two and four days of incubation.

3.7.3 Detection of salt tolerance

The tolerance towards higher concentration of salt (Sodium chloride) of the plant endophytic bacterial strains was tested on YEM agar solid medium. YEM agar medium was prepared supplemented with serial increase of 0.1, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 % of NaCl for salt tolerance. 10 μ l of culture ($\pm 10^9$ CFU ml⁻¹) of each bacterial strain were dropped onto the plates of varying degree of salt concentration. The endophytic bacteria culture were incubated at 28-30^oC for 5 days. The same experiment was performed in YEM broth and optical density was measured at 420 nm after two and four days of incubation.

3.7.4 Phosphate solubilization

Pikovskaya's agar plates (Appendix IV) were spot inoculated with overnight grown bacterial cultures and incubated at 30 °C for 48 h. Formation of clear zone around the colonies indicate a positive test of phosphate solubilization (Pikovskaya, 1948). The radial area of clear

zone including colony was measured and phosphate solubilization activity was expressed in mm radial area solubilized per hour. The solubilization index was determined by following formula.

$$\text{Solubilization Index}(SI) = \frac{\text{colony diameter}(cm) + \text{halo zone diameter}(cm)}{\text{colony diameter}(cm)}$$

3.7.5 Zinc solubilisation

PGPR isolates were screened for their Zinc solubilizing activity on plate assay containing ZnO as an insoluble Zinc source. 10 µl of exponentially grown bacterial culture of each PGPR strain were spotted onto the plates (Appendix V). These plates were incubated at 28°C for 7 days. The radial clear zone surrounding the bacterial colony indicates a positive test for Zinc solubilisation. The solubilized clear halo zone including bacterial colony was measured and Zinc solubilization activity was expressed in millimeter radial area solubilized.

3.7.6 Pottasium solubilisation

Potash solubilization by the endophytic bacteria was screened over the Aleksandrov's agar medium plates (Appendix-VI). Each plate was inoculated with a spot of 10µl bacterial suspension and spotted on the medium. The plates were incubated at 28°C for 48-96 hours. The zone showing clearance around the colony indicated potash solubilizing ability of bacteria.

3.7.7 Indole acetic acid (IAA) production

IAA was determined by the method of Gorden and Webber (1951) [Appendix VII]. Tubes having 10 ml nutrient broth with 100µg/ml tryptophan and without tryptophan were inoculated with actively growing bacterial cultures individually and incubated under shaking conditions at 28±2 °C for 48 h. After incubation, the cultures were centrifuged at 10,000 rpm for 10 min. Two ml of Salkovsky reagent was added to 1.0 ml of culture supernatant and the resulting mixture was incubated at 30 °C for 25 min. Development of pink colour indicates IAA production by the test bacteria. Absorbance was recorded at 530 nm using UV/VIS spectrophotometer.

3.7.7.1 Quantification of IAA

Reagents

1. Stock solution of indole acetic acid (100 µg/ml)
2. Salkovsky reagent: 1.0 ml FeCl₃ (0.5 M) mixed with 50 ml HClO₄ (35%).

3.7.7.2 Standard curve

Different volumes (50-500µl) from the stock solution of IAA were taken in the tubes in duplicate. Final volume was made to 1.0 ml with distilled water (DW), to which 2 ml Salkovsky reagent was added and the mixture was incubated at 30⁰C for 25 min. Distilled water was taken as control. Absorbance was recorded at 530 nm. Graph was plotted using IAA concentrations verses absorbance.

3.7.8 Siderophore Production

Overnight grown bacterial cultures were spot inoculated on CAS (Chrome Azurol S) plates (Schwyn and Neilands, 1987) [Appendix IX]. Plates were incubated at 30⁰C for 48 hours. Appearance of orange halo around the bacterial colonies against a dark blue background indicates siderophore production.

3.7.9 Intrinsic Antibiotic tolerance

The intrinsic level of resistance for the bacterial isolates was tested with nine antibiotics (Tetracycline, Rifampicin, Streptomycin, Neomycin, Nalidixic acid, Kanamycin, Chloramphenicol, Ampicillin, Ciprofloxacin) disc which showed different concentration using NA media. Each bacterial isolate was mixed with NA media and pour plated. Antibiotics disc were placed on each plate and incubated at 30⁰C for 48 h and checked for the inhibition zones.

3.8 Plant growth experiment under laboratory condition

To study the effect of inoculation with different endophytic rhizobacterial strains on germination and other plant growth parameter of rice plant, plant infection test was carried out in Gibson's tubes (38 X 200 mm) having agar slants.

3.8.1 Collection of seeds

For conducting the experiment seeds of rice cultivar HUR 105 was obtained from Prof. R. P. Singh (Rice breeder), Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi.

3.8.2 Treatment details

The four PGPR strains, i.e., *Burkholderia cepacia* RRE3, *Rhizobium leguminosarum* bv. *phaseoli* RRE6, *Pseudomonas aeruginosa* BHU3 and *Pseudomonas saponiphila* BHU8 were selected for this study based on the earlier reports of these strain's plant growth promoting characteristics. Rhizobial strains were used alone as well as in co-inoculation with each other in all combinations separately. Four different consortium of rhizobacterial strains i.e., *Burkholderia cepacia* RRE3 and *Pseudomonas aeruginosa* BHU3 (a 1:1 ratio by volume of RRE3 and BHU3); *Burkholderia cepacia* RRE3 and *Pseudomonas saponiphila* BHU8 (a 1:1 ratio by volume of RRE3 and BHU8); *Rhizobium leguminosarum* bv. *phaseoli* RRE6 and *Pseudomonas aeruginosa* BHU3 (a 1:1 ratio by volume of RRE6 and BHU3); *Rhizobium leguminosarum* bv. *phaseoli* RRE6 and *Pseudomonas saponiphila* BHU8 (a 1:1 ratio by volume of RRE6 and BHU8) were used for comparing the effect of growth promotion by rhizobacterial co-inoculation. Along with these uninoculated control treatment consisted of water-treated seeds (without bacterial inoculation) was included for comparing the effectiveness of the treatments.

3.8.3 Experiment details

Experiment was laid out in a completely randomized design (CRD) with three replications of each nine treatments including control for the rice variety.

3.8.4 Preparation of bacterial inoculum

Each bacterial strain was grown in 150 ml Erlenmeyer flask containing 50 ml of Nutrient broth, incubated in incubator shaker (120 rpm) at 30°C. Bacterial cultures were grown up to a period to obtain 10^8 to 10^9 CFU ml⁻¹.

Table 3.2: Bacterial strains and their combinations used for inoculation

	Bacterial Strain	Designation
1	<i>Burkholderia cepacia</i>	RRE3
2	<i>Rhizobium leguminosarum</i> bv. <i>phaseoli</i>	RRE6
3	<i>Pseudomonas aeruginosa</i>	BHU3
4	<i>Pseudomonas saponiphila</i>	BHU8
5	<i>Burkholderia cepacia</i> and <i>Pseudomonas aeruginosa</i>	RRE3 + BHU3
6	<i>Burkholderia cepacia</i> and <i>Pseudomonas saponiphila</i>	RRE3 + BHU8
7	<i>Rhizobium leguminosarum</i> bv. <i>phaseoli</i> and <i>Pseudomonas aeruginosa</i>	RRE6 + BHU3
8	<i>Rhizobium leguminosarum</i> bv. <i>phaseoli</i> and <i>Pseudomonas saponiphila</i>	RRE6 + BHU8

3.8.5 Seed sterilization

Dehulled healthy and bold rice seeds were surface sterilized by immersion in 95% ethanol (for 5 minutes) and 0.1% acidified HgCl₂ (for 2 minutes). Treated seeds were washed five times with sterile distilled water.

3.8.6 Seed inoculation and Sowing

Just after sterilization seeds were soaked in exponentially grown bacterial cultures for one hour. The inoculated seeds were transferred to culture tubes having agar slants. One seed was planted in each tube and 1 ml of bacterial suspension was used for inoculation of each tube. Un-inoculated seeds (soaked in sterile water only) were served as control.

3.8.7 Growth conditions

Inoculated seeds were transferred in plant growth chamber programmed with 14 h light and 10 h dark period, 28⁰C/23⁰C day/night cycle of temperature and 70% relative humidity.

3.8.8 Harvesting

Plants were grown for 15 days. Watering was done regularly with distilled water. Plants were harvested 15 days after inoculation and the roots were carefully rinsed with water to remove agar adhering to it.

Data were recorded on individual plant growing in each plant infection tube and average value was taken for each treatment.

3.8.9 Plant Parameter

3.8.9.1 Germination

Rate of germination, time taken to germinate after inoculation was recorded.

3.8.9.2 Shoot length

Shoot length was measured from stem base of the emerging root to the tip of fully expanded leaf and values were recorded in cm.

3.8.9.3 Root length

Root length was measured from the base of the stem to the end of root tip.

3.9 Screening of selected PGPR strains for growth promotion of rice under poly house condition

A pot experiment was conducted in the poly house of Institute of Agricultural Sciences, Banaras Hindu University, Varanasi to evaluate the effects of rhizobacterial inoculation on different growth parameters of rice plants.

Collection of seeds, preparation of bacterial inoculum and treatment details followed same process as mentioned in plant growth experiment under laboratory condition.

3.9.1 Experiment details

The experiment was conducted in completely randomized design fashion with three replications of each nine treatments for rice variety HUR 105.

3.9.2 Soil collection and preparation of pot

Soil was collected from the upper 0-15 cm soil layer from agriculture farm of Institute of Agricultural Sciences, BHU, Varanasi. Soil was air dried and sieved with <5 mm mesh. Soil was steam sterilized in autoclave. After sterilization, soil was filled into the pots of 5 kg capacity. The pots were irrigated with sterilized water and left for 1-2 days for equilibration.

3.9.6 Seed sterilization

Same process was followed as mentioned in section 3.8.5

3.9.7 Seed inoculation and Sowing

Sterilized seeds were soaked in exponentially grown bacterial cultures for one hour. Five seeds for each treatment were taken for sowing in single pot. One ml of prepared bacterial culture was given to each pot by making hole in soil and seed was placed in the hole. Seeds were placed at the same depth (approximately 2 cm below the soil surface) in all inoculated pots. Un-inoculated seeds (soaked in sterile water only) were served as control.

3.9.8 Growth conditions

Experiment was carried out in a poly house under following growth conditions: Temperature: $28 \pm 2^{\circ}\text{C}$, Photo period: 16/8 hour day/night cycle, Light intensity: $400 \text{ Em}^{-2} \text{ s}^{-1}$ (400-700 nm), Relative humidity: 60%. After two weeks three equally germinated seedlings were maintained in each pot. All the pots were irrigated with sterilized water. After three weeks of sowing 20 ml of nitrogen free nutrient solution Thornton's medium (Thornton, 1930) [Appendix III] were given weekly to the each pot.

3.9.9 Sample collection and analysis

Plants were uprooted from the soil very carefully without disturbing the root system after 60 days of sowing and washed thoroughly with tap water to remove the soil particle adhering to the root system. After initial air drying, root/shoot fresh weight, height were recorded.

3.9.10 Plant growth parameter

3.9.10.1 Leaf Chlorophyll content

Chlorophyll content (Chlorophyll Content Index reading) of the upper two fully expanded leaves was measured by a CCI meter.

3.9.10.2 Shoot length

Plant height was measured from the soil base to the tip of fully expanded leaf and values were recorded in cm.

3.9.10.3 Root length

Root length was measured from the base of the stem to the end of root tip.

3.9.10.4 Root and shoot fresh weight

Immediately after harvesting plants were cut from stem base. Shoot and the root of the plant was weighed separately using weighing balance $\pm .001$ gm.

3.9.10.5 Root and shoot dry weight

Plant samples were dried in an oven at 60 °C for two to three days to get a constant weight. Dry matter of root/shoot of the plants was recorded and values were expressed in grams.

3.10 Data analysis

Complete Randomized Design (CRD) was used for laboratory and poly house plant growth experiments. Data were analyzed by using analysis of variance (ANOVA) with SPSS (Version 10.0) software. Treatment means were compared at 95% and 99% probability level (P=0.05 and 0.01) and the same set of data was further analyzed to calculate the least significant difference (LSD) at P=0.05 and 0.01 respectively.



Experimental Findings

*“Research is to see what everybody else has seen, and
to think what nobody else has thought.”*

-Albert Szent-Gyorgyi

EXPERIMENTAL FINDINGS

The objective of present study is to find out the growth promotion activity of potent plant growth promoting rhizobacteria on rice. Rice variety HUR105 (Malviya Sugandhit Dhan 105) used in this study is a new, high-yielding, scented, drought tolerant rice variety recommended for cultivation in eastern India.

4.1 Physiological characters:

4.1.1 Temperature tolerance

The growth of all the four endophytic bacterial colonies at different temperature was studied. Among these best growth of all these strains were found at 25°C whereas above (35°C) and below this temperature (15°C) retardation in colony growth was observed. However, the bacterial colony can also thrive at 45°C (Fig. 4.1).

4.1.2 pH tolerance

The bacterial growth was studied at a varying range of pH from 5 to 12. At pH 7 the best growth of colonies was observed. By increasing acidity or alkalinity of medium, a decrease in the growth of colonies was recorded (Fig. 4.2).

4.1.3 Salt tolerance

At different levels of NaCl concentration ranging from 0.1% to 10%, the growth of endophytic bacterial colonies was studied. It was observed that the best growth of colonies developed at 0.1% NaCl. With the increase in salt concentration in media a gradual decrease in the growth rate was observed (Fig. 4.3).

4.1.4 Intrinsic antibiotic resistance

It was evident that all the endophytic bacteria have a very high level of (600µg/ml) intrinsic antibiotic resistance (IAR). Among the antibiotic tested ciprofloxacin and kanamycin

have an inhibitory effect on RRE3 and caused maximum lethality. However, streptomycin has a more toxic effect on the growth of BHU3, BHU8 and RRE6 (Fig 4.4 to 4.12).

4.2 Biochemical characters

4.2.1 Indole 3-Acetic Acid (IAA) production

All endophytic rhizobacteria were grown in YEM broth supplemented with 100 µg/ml of tryptophan for IAA production. Variable response was shown by isolates in terms of IAA production. (Table 4.1). After 3 DAI, IAA production ranged from 4.58 to 14.15 µg/ml. Highest concentration was recorded in BHU8 (14.15 µg/ml) followed by RRE3 (12.36 µg/ml). Minimum IAA production was recorded in RRE6 (4.58 µg/ml) [Plate 4.3].

Table 4.1: Biochemical test of endophytic bacteria

Bacterial endophyte	IAA production (µg/ml) 3 DAI	Siderophore production
Control	ND	-
RRE3	12.36	++
RRE6	4.58	+
BHU3	9.67	+++
BHU8	14.15	++

Note: DAI= Days after inoculation

+ = low, ++ = medium, +++ = good

4.2.2 Siderophore production

All endophytic rhizobacterial strains used in this experiment were capable of producing siderophores. All isolates produced yellow-orange color zone on CAS agar plate hence considered as siderophore producers. A qualitative assessment of siderophore production test was carried out that is described in table. Based on the size of yellow colour zone they were categorized as low (+), medium (++) and good (+++) siderophore producer (Table 4.1). Maximum siderophore production was observed in BHU3 followed by RRE3 and BHU8 (Plate 4.4).

4.2.3 Phosphate solubilisation by endophytes under invitro condition

To determine the phosphate solubilisation activity, all the bacterial strains were grown in Pikovskaya's agar media. 6 days after inoculation diameter of bacterial colonies and solubilized zone (hallow area) was measured in millimeter. Phosphate solubilisation ability was indicated in terms of mm solubilized area per hour. All the four bacterial strains showed phosphate solubilisation ability (Plate 4.5). Maximum phosphate solubilisation was observed in BHU8 (0.37 mm/hr) followed by RRE6 (0.29 mm/hr), BHU3 (0.24 mm/hr) and RRE3 (0.16 mm/hr) [Table 4.2].

Table 4.2: Solubilization test of endophytic bacteria

Bacterial endophyte	Phosphate solubilisation (mm hr ⁻¹)	Zinc solubilisation (mm hr ⁻¹)	Pottasium solubilisation (mm hr ⁻¹)
Control	ND	ND	ND
RRE3	0.16	0.18	0.13
RRE6	0.29	0.07	ND
BHU3	0.24	0.11	0.07
BHU8	0.37	0.08	0.06

Note: ND- Not Detected

4.2.4 Zinc solubilisation by endophytes under invitro condition

To assess the potential zinc solubilizers ZnO used in media as source of insoluble zinc. Hallow zone surrounding the Bacterial colony was measured 6 days after inoculation (Plate 4.6). Highest zinc solubilisation activity was shown by RRE3 (0.18 mm/hr) and RRE6 (0.07 mm/hr) was recorded as minimum [Table 4.2].

4.2.5 Pottasium solubilisation by endophytes under invitro condition

All the bacterial strains were grown in Alexandrov's agar medium to screen the pottasium solubilisation ability, where mica was used as insoluble pottasium. Except RRE6 all the three bacterial strains showed clear zone of solubilisation (Plate 4.7). Maximum ability to solubilize pottasium was shown by RRE3 [Table 4.2].

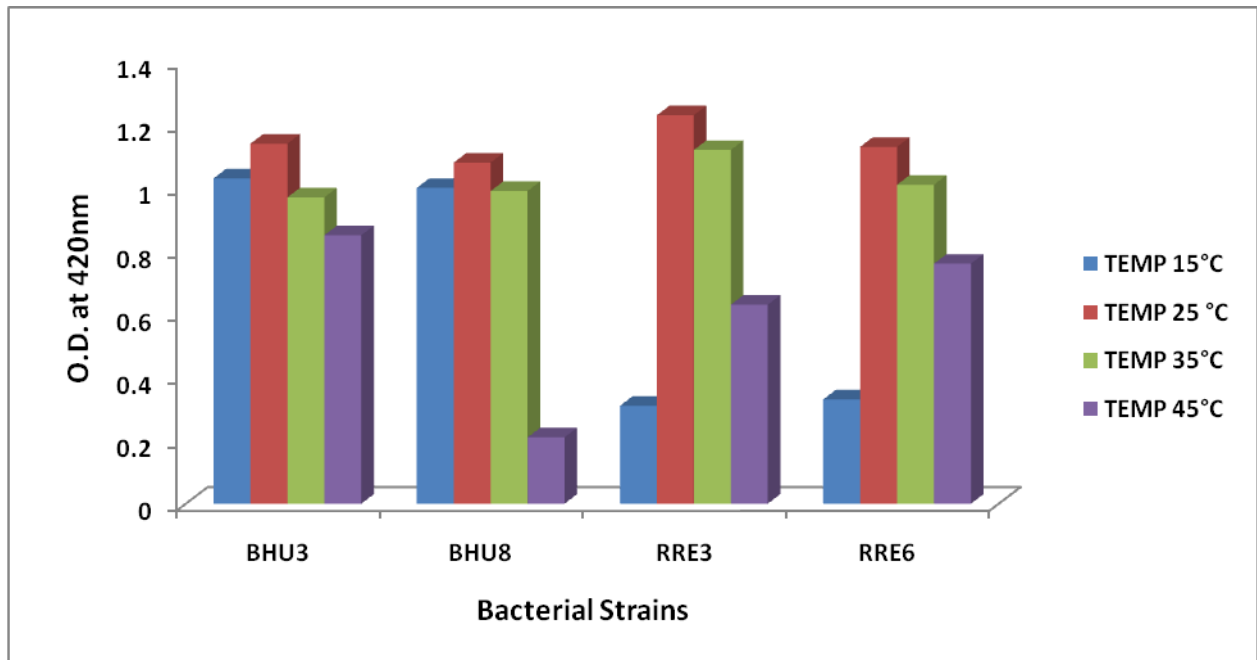


Fig 4.1: Growth of endophytic bacteria at different temperature in YEM medium

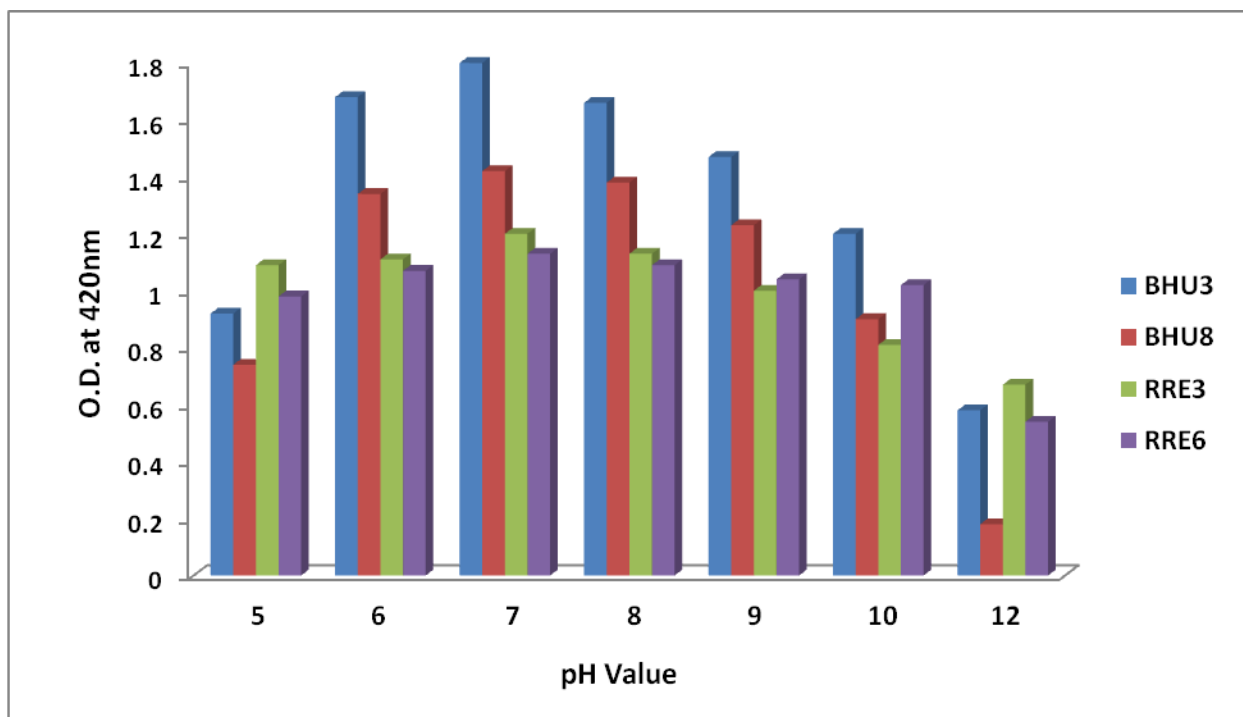


Fig 4.2: Growth of endophytic bacteria at different pH in YEM medium

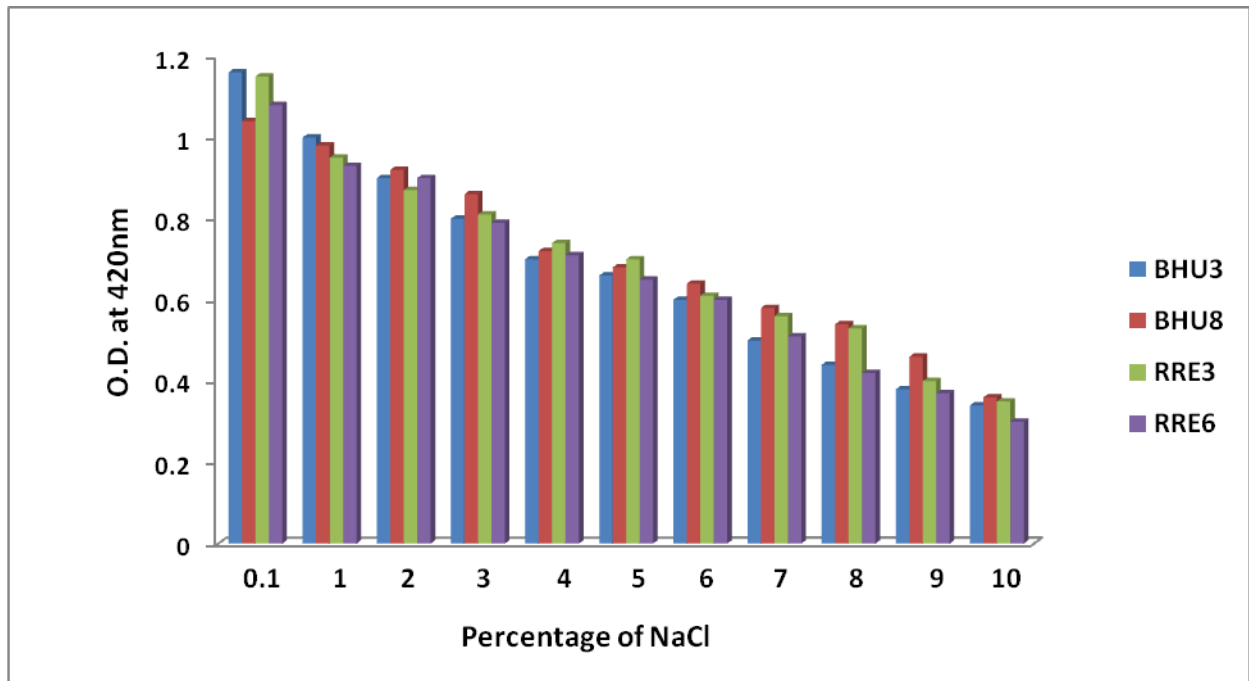


Fig 4.3: Growth of endophytic bacteria at different percentage of NaCl in YEM medium

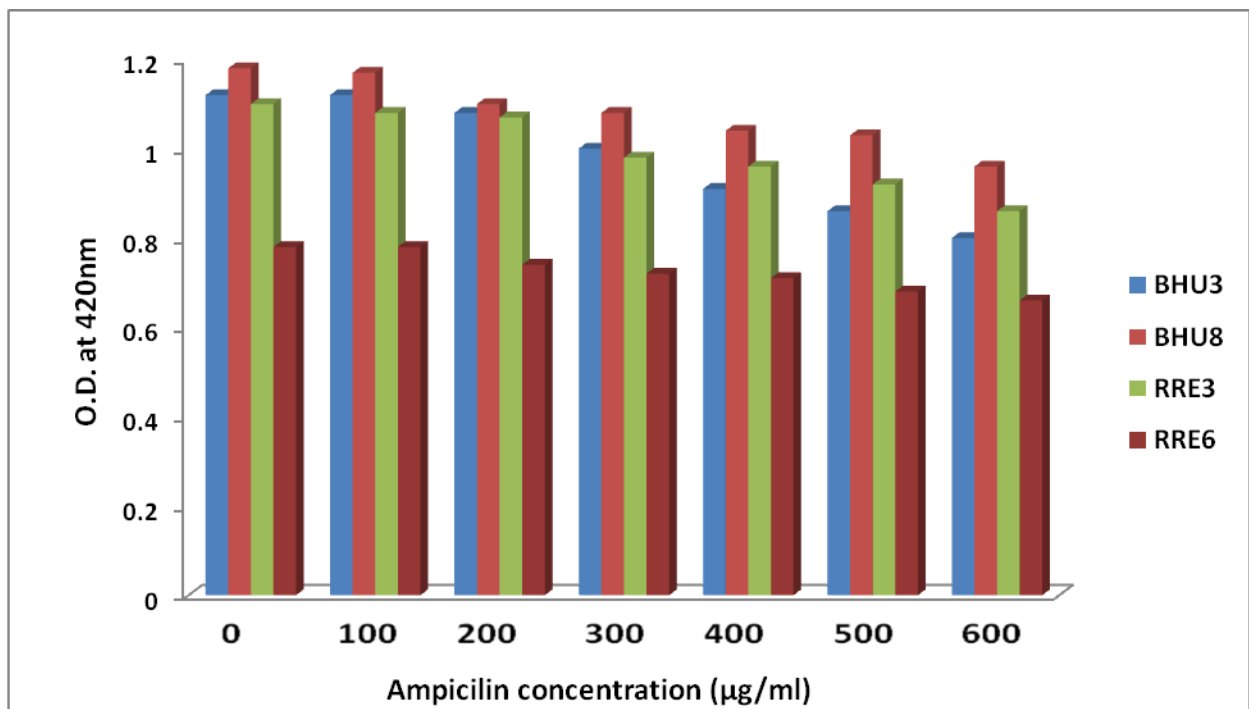


Fig 4.4: Growth of endophytic bacteria at different Ampicillin concentration.

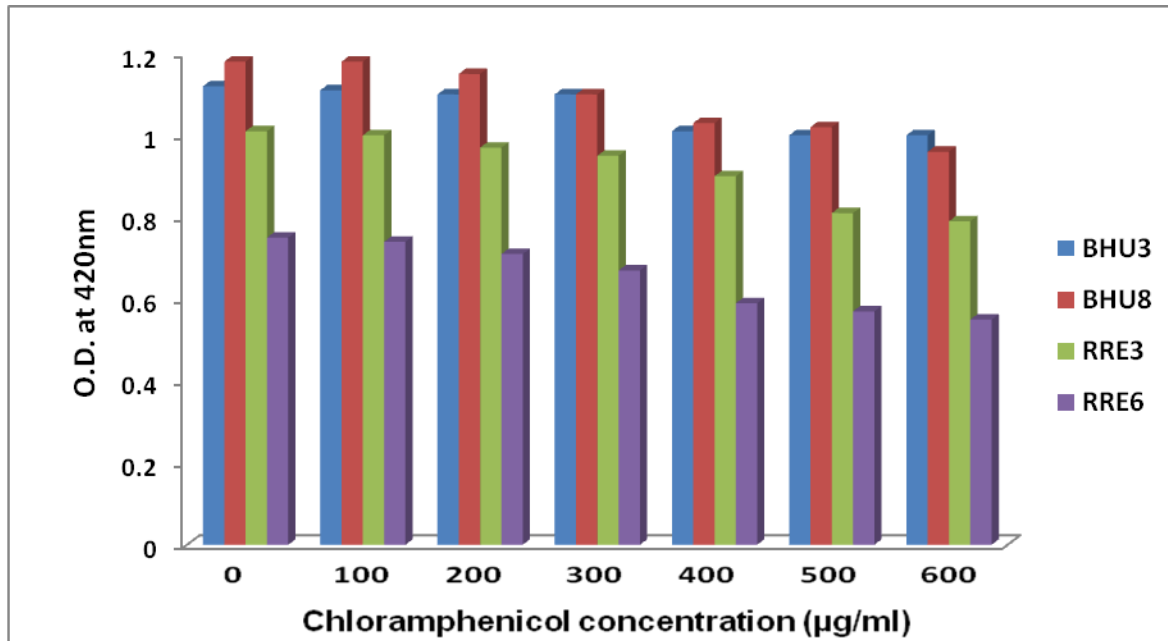


Fig 4.5: Growth of endophytic bacteria at different Chloramphenicol concentration.

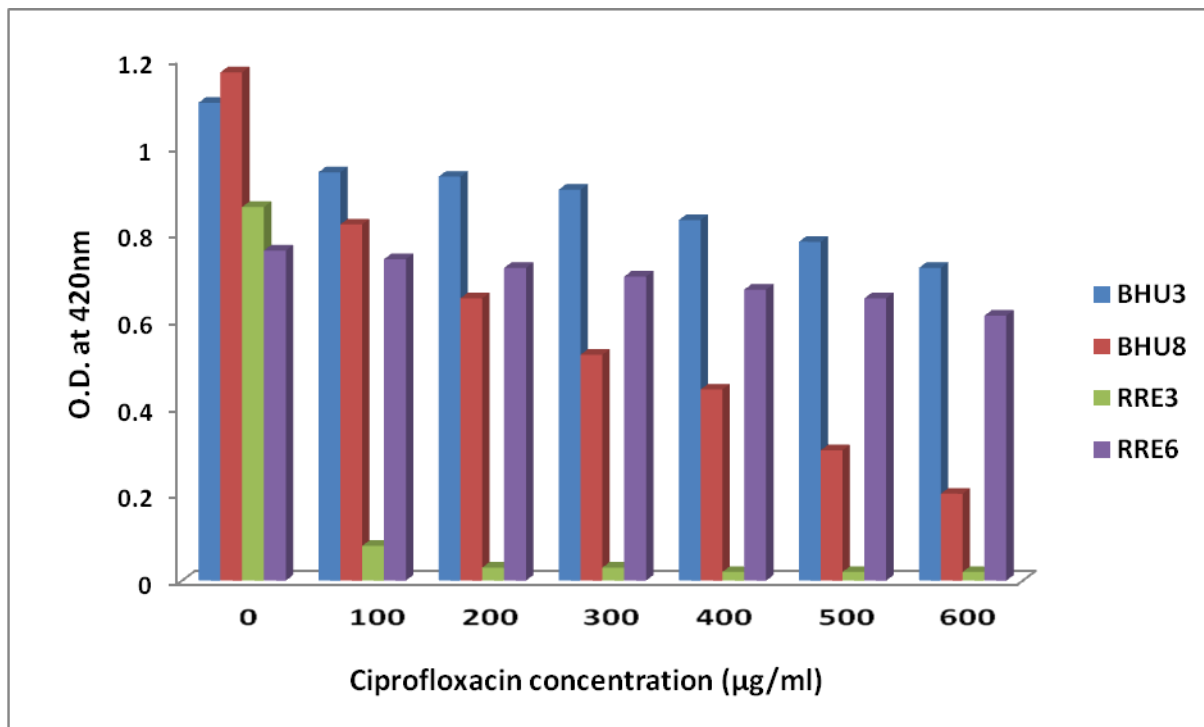


Fig 4.6: Growth of endophytic bacteria at different Ciprofloxacin concentration.

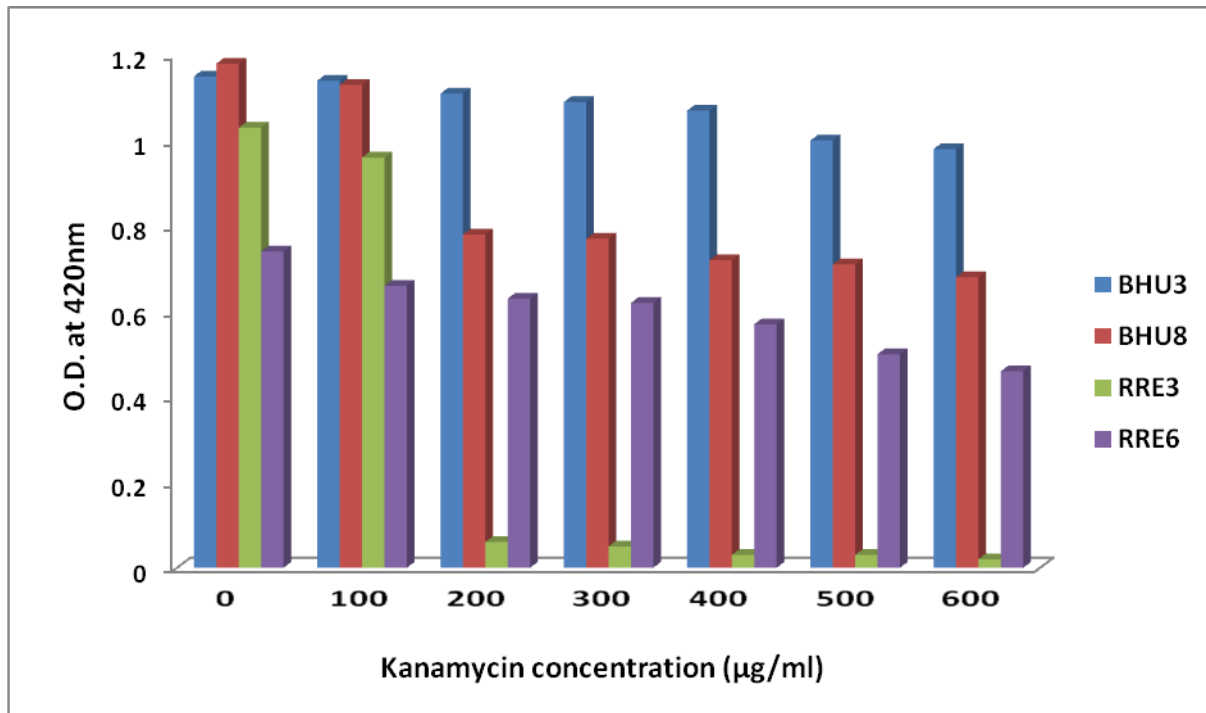


Fig 4.7: Growth of endophytic bacteria at different Kanamycin concentration.

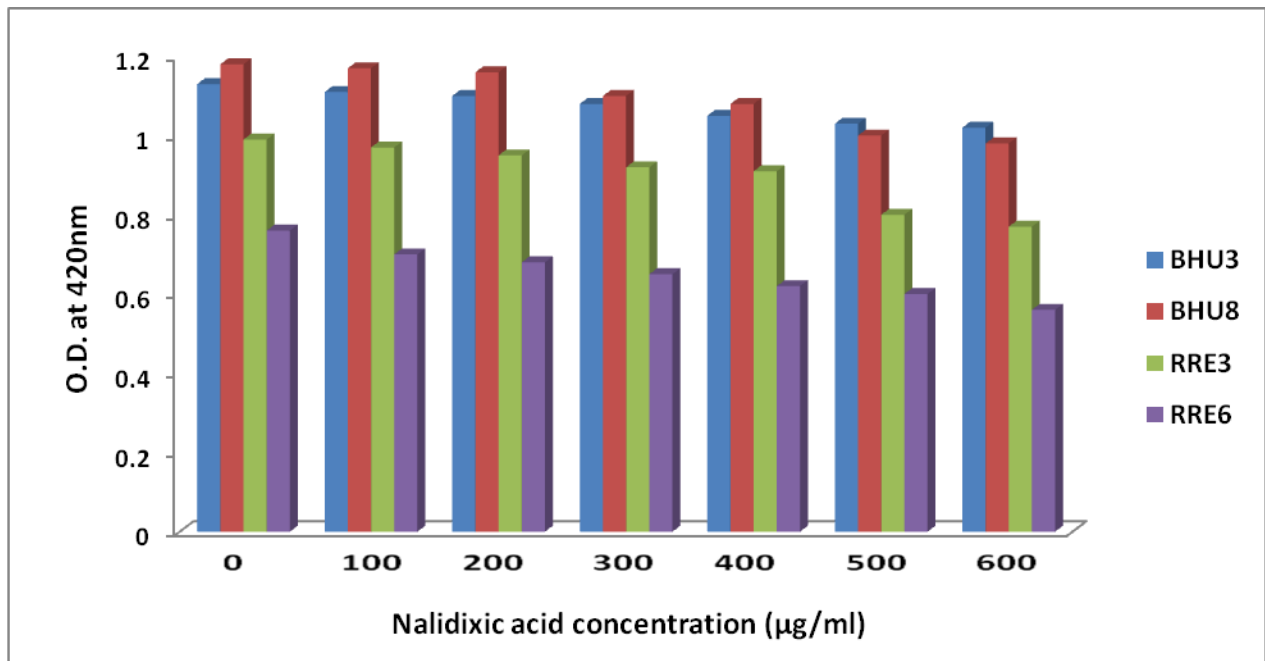


Fig 4.8: Growth of endophytic bacteria at different Nalidixic acid concentration.

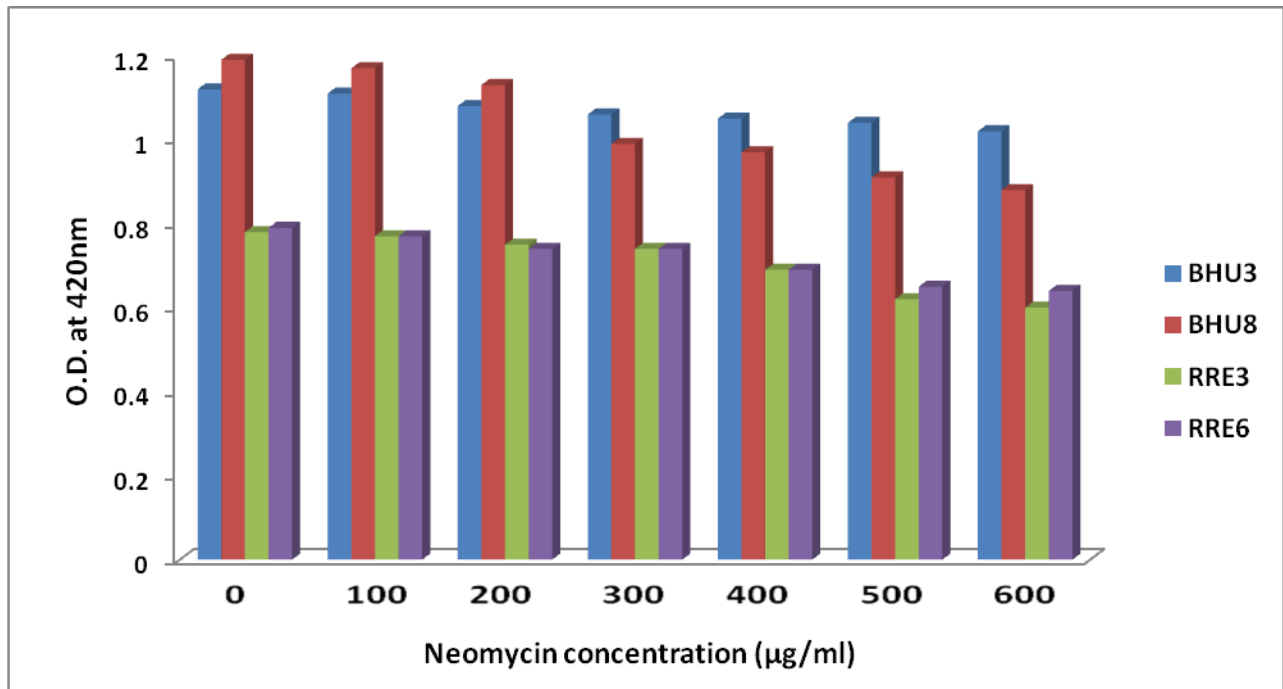


Fig 4.9: Growth of endophytic bacteria at different Neomycin concentration.

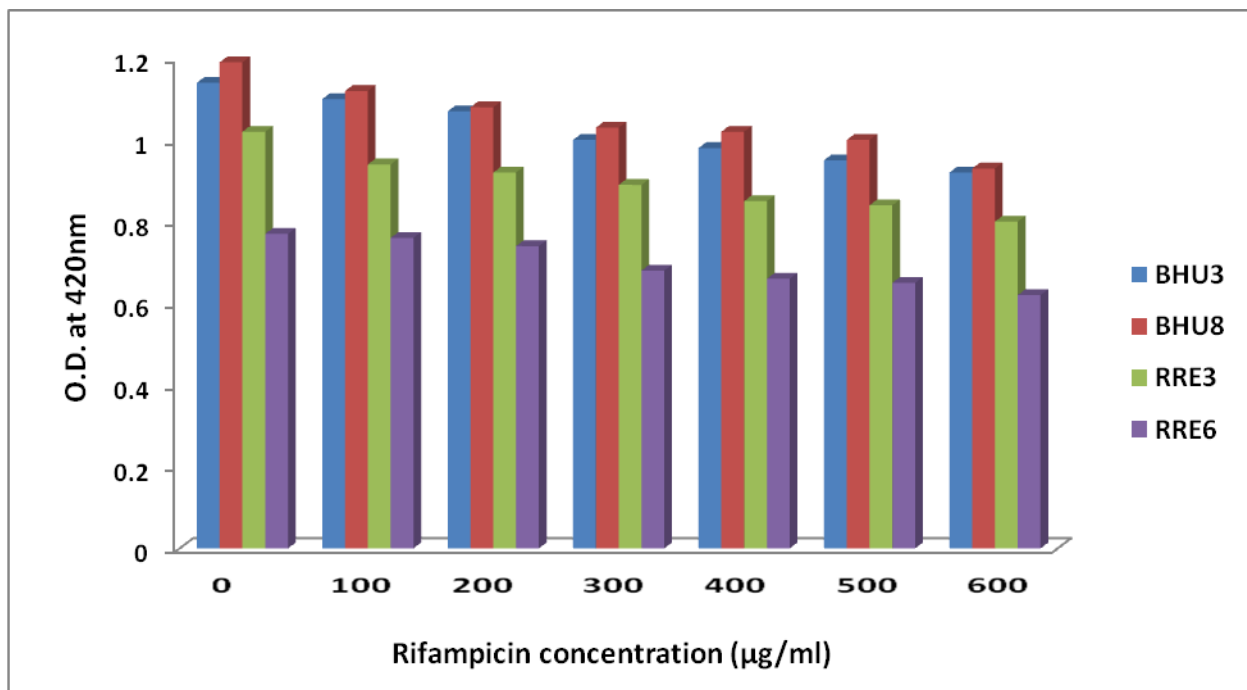


Fig 4.10: Growth of endophytic bacteria at different Rifampicin concentration.

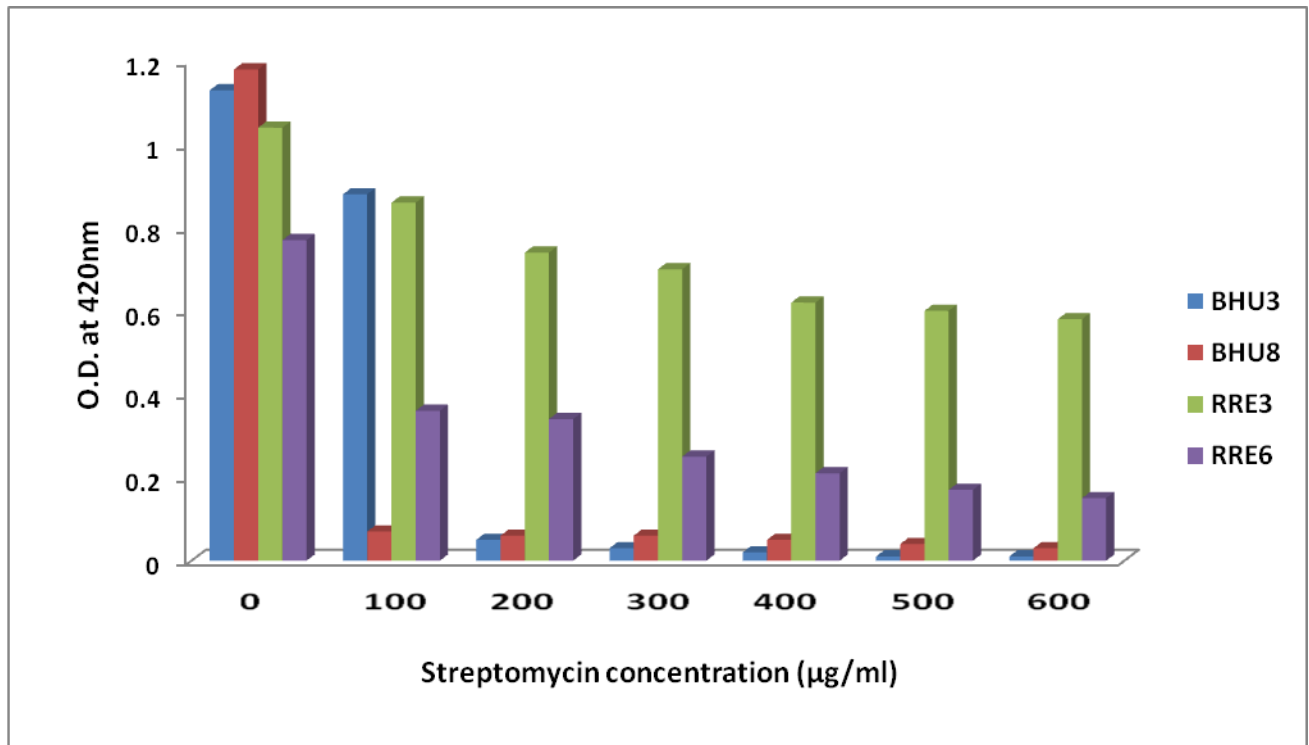


Fig 4.11: Growth of endophytic bacteria at different Streptomycin concentration.

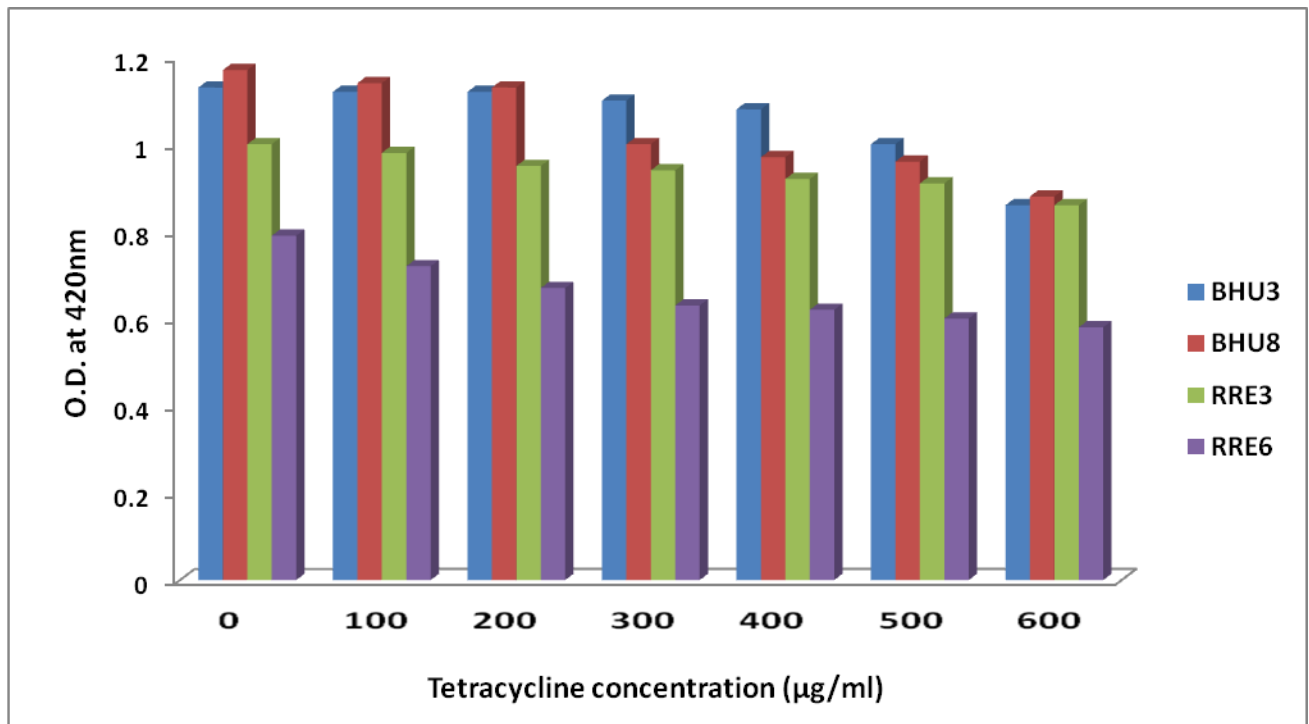


Fig 4.12: Growth of endophytic bacteria at different Tetracycline concentration.

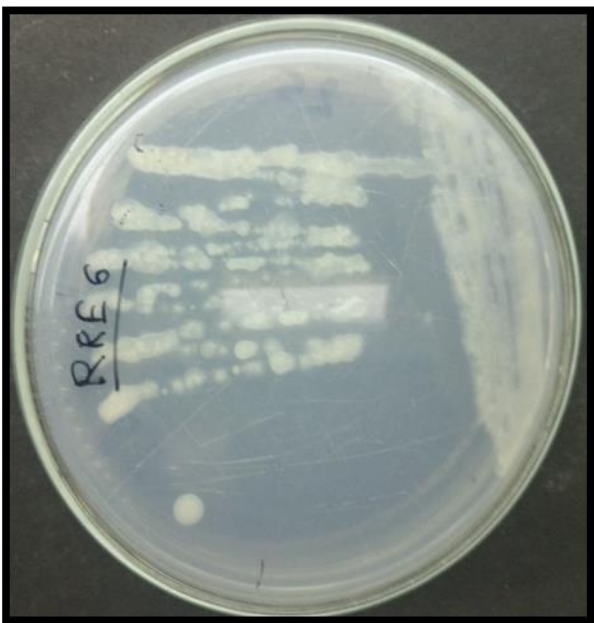
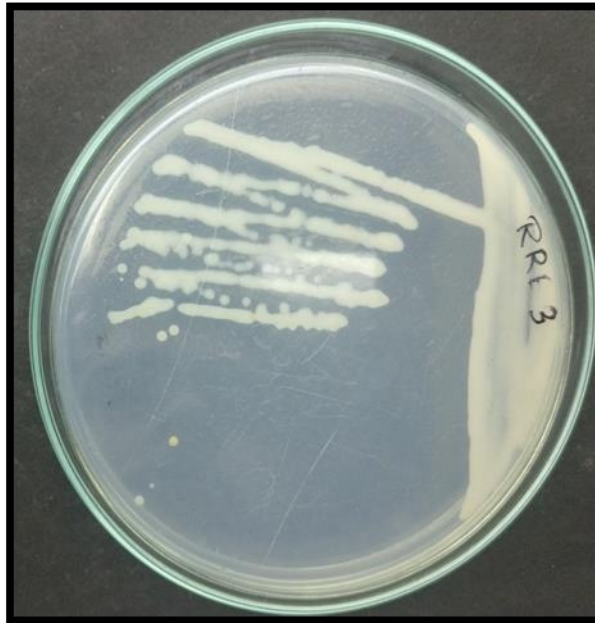
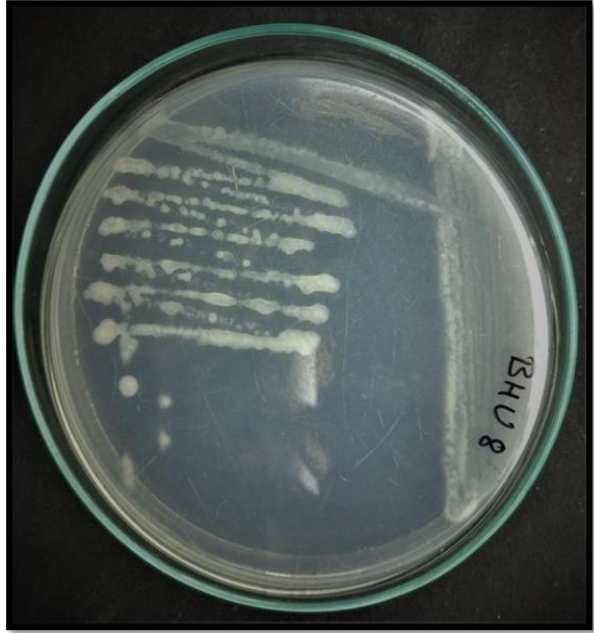


Plate 4.1: Growth of endophytic bacterial colony in YEM medium.

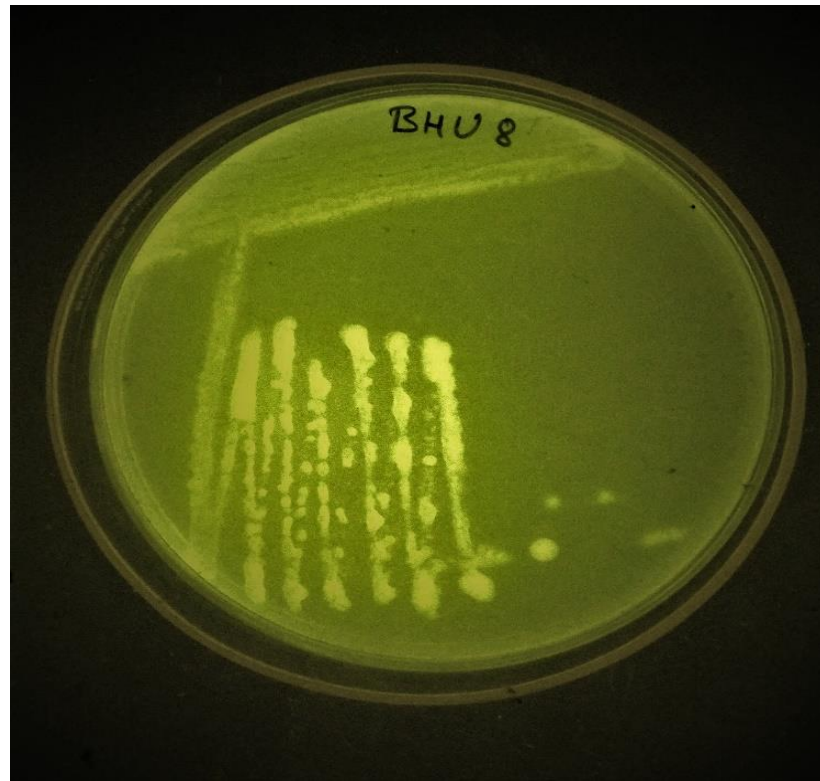


Plate 4.2: Strain BHU3 and BHU8 showing fluroscence under UV light

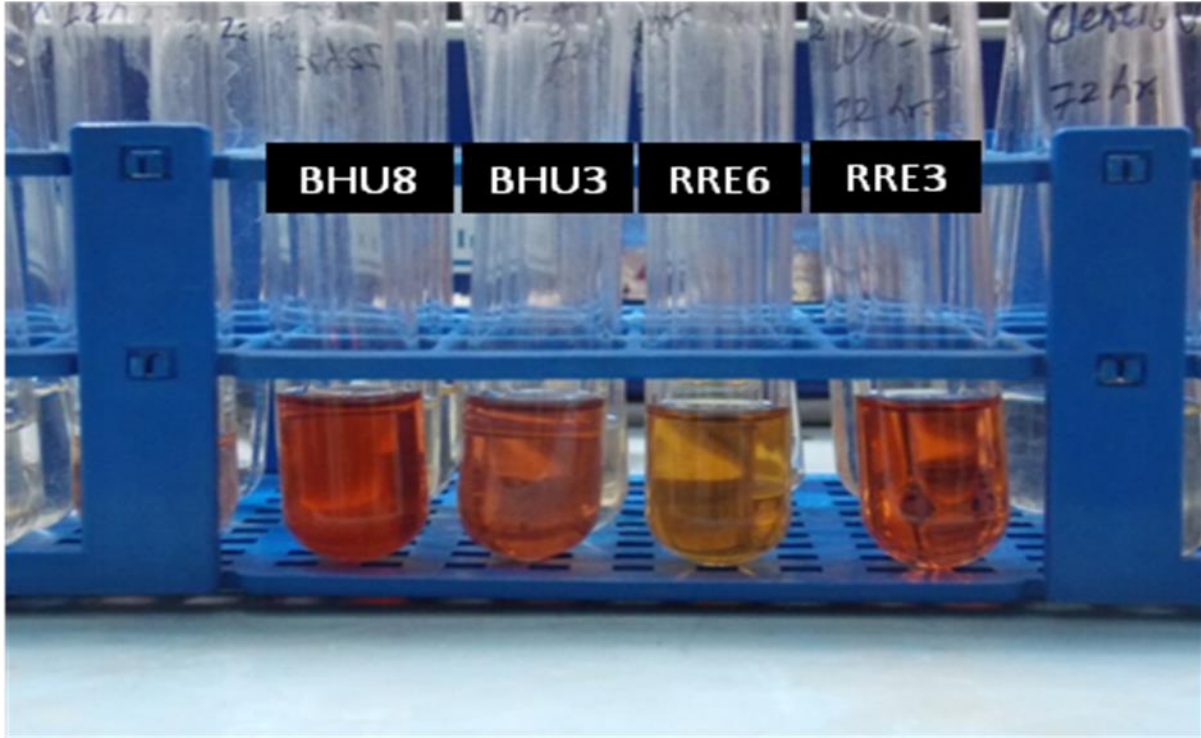


Plate 4.3: IAA production (pink colour development) by endophytic bacteria in test tube.

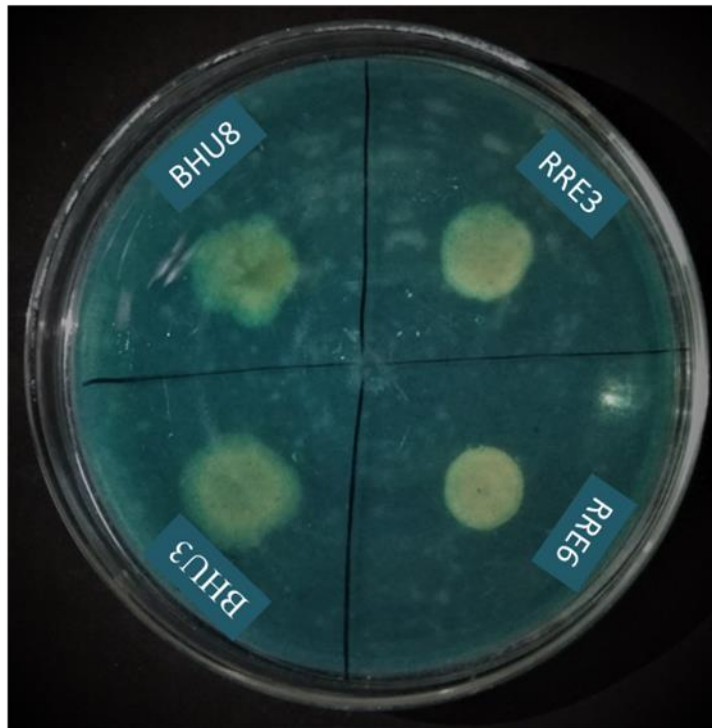


Plate 4.4: Siderophore production by endophytic bacteria on CAS medium.

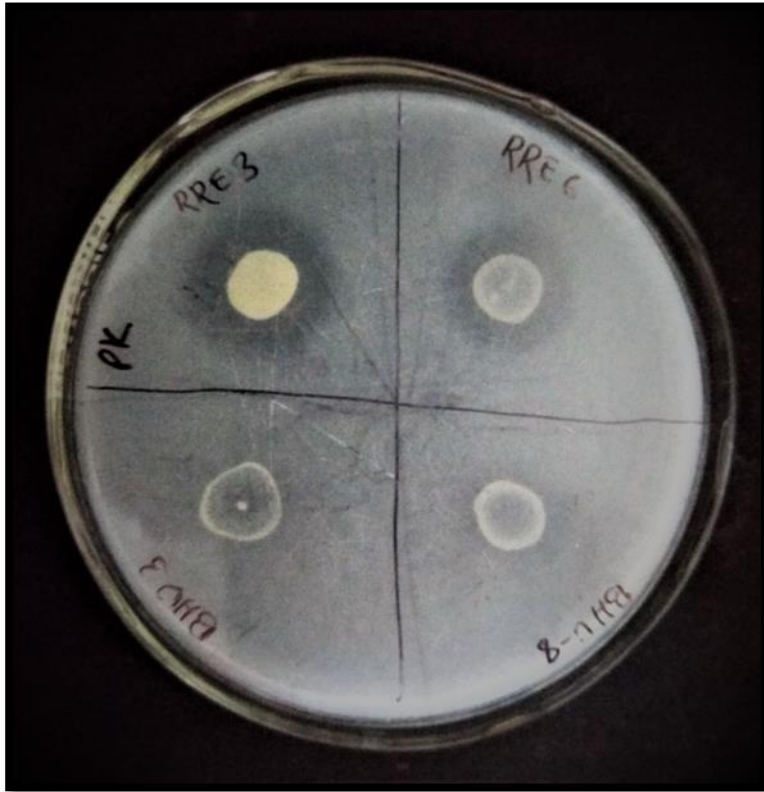


Plate 4.5: Phosphate solubilization (clear zone) of endophytic bacteria.



Plate 4.6: Zinc solubilization

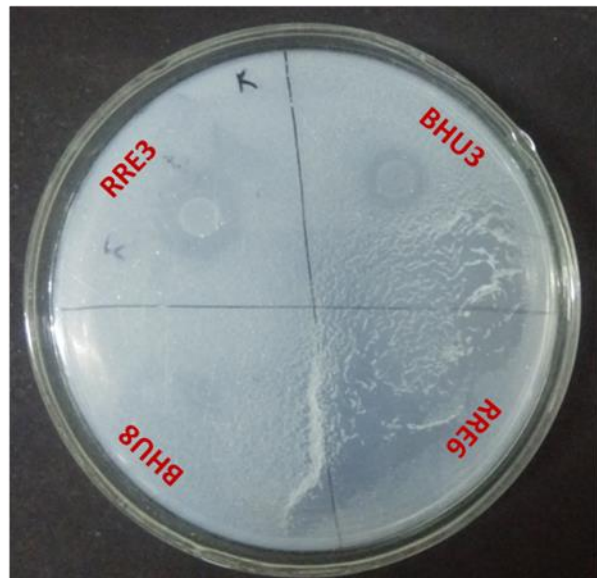


Plate 4.7: Potash solubilization

4.3 Effect of selected bacterial strains on the growth promotion of rice under laboratory condition

4.3.1 Germination

To study the effect of endophytic bacteria on germination of rice regular observation was taken. It showed that seeds inoculated with RRE6 took minimum time to germinate whereas BHU8 inoculated seeds took maximum time to germinate. The growth of root and shoot was also observed daily after germination. Regular observation revealed maximum growth rate in shoot elongation was with RRE3+BHU3 inoculated seedlings whereas that of minimum was recorded in seedlings inoculated with BHU8 (after control). Similarly for growth rate of root, maximum effect was shown by seedlings inoculated with RRE3+BHU8 and Minimum was recorded for RRE6 (Table 4.3).

4.3.2 Shoot length

Significant increase in average shoot length of the plants inoculated with endophytic bacterial strains was observed as compared to control (uninoculated). Maximum increase was recorded in coinoculation treatment of RRE3+BHU3 (58.77% increase over control) whereas RRE3 (35.09% increase) showed the best result among the treatments inoculated with single strain. Minimum increment was recorded in BHU8 (21.93% increase over control) [Table 4.3] (Plate 4.8).

4.3.3 Root length

Just like shoot length, average root length also showed significant improvement over control under laboratory condition. RRE3+BHU8 showed maximum improvement (77.18% increase over control) whereas minimum improvement was observed in case of BHU8 (35.56% increase) [Table 4.3] (Plate 4.8).

Table 4.3: Effect of inoculation of endophytic bacteria on plant growth promotion of rice (cv. HUR 105) under laboratory condition

Bacterial Strains	Shoot length (cm)	Root length (cm)
Control (Uninoculated)	14.33	7.77
RRE3	20.53 (35.09)*	11.93 (53.58)*
RRE6	18.77 (23.46)*	10.80 (39)*
BHU3	20.17 (32.68)*	11.47 (47.58)*
BHU8	18.53 (21.93)*	10.53 (35.56)*
RRE3+BHU3	24.13 (58.77)*	13.53 (74.17)*
RRE3+BHU8	23.60 (55.26)*	13.77 (77.18)*
RRE6+BHU3	21.90 (44.08)	12.50 (60.88)*
RRE6+BHU8	22.47 (47.81)*	11.63 (49.72)*
CD at 1%	3.16	2.02
CD at 5%	2.76	1.76
SEM±	0.78	0.50

Parenthesis – Percent increase over control

* Significant at P= 0.01

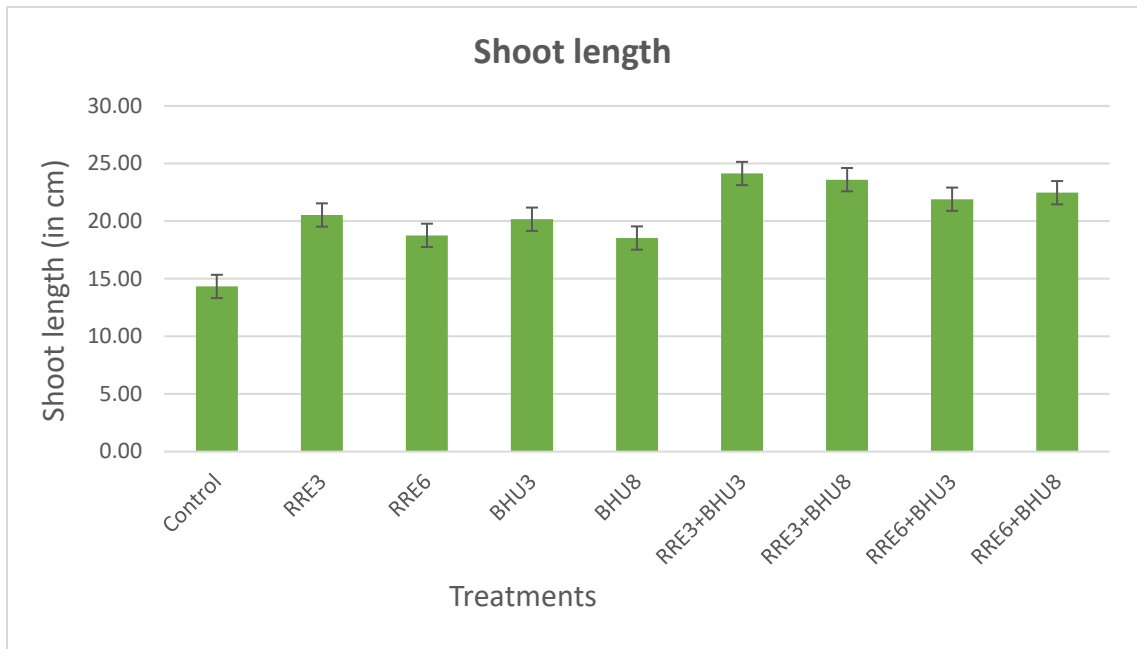


Fig 4.13: Effect of different endophytic bacterial strains on shoot length of rice under laboratory condition

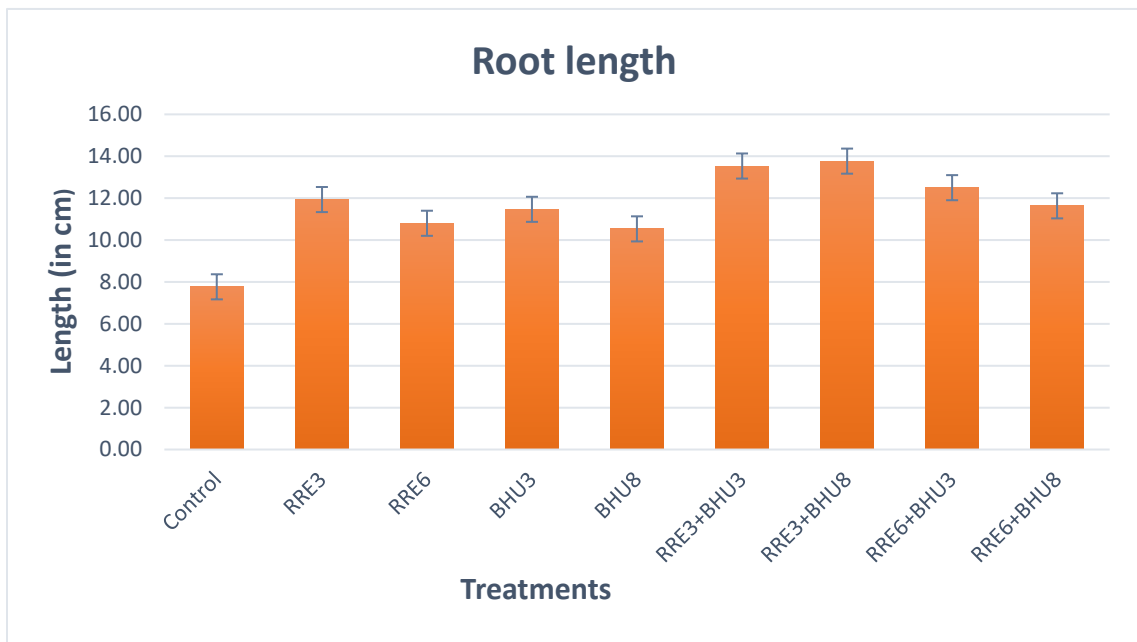


Fig 4.14: Effect of different endophytic bacterial strains on root length of rice under laboratory condition

4.4 Effect of selected bacterial isolates on the growth promotion of rice under poly-house condition

4.4.1 Effect of bacterial inoculation on leaf chlorophyll content

Effect of bacterial inoculation on plant leaf chlorophyll content was studied using Chlorophyll Content Index (CCI) meter. CCI value was recorded 35 days after sowing. Chlorophyll content has been increased in all the treatments when compared with the control plant. Average chlorophyll content for plant inoculated with RRE3 was of CCI value 4.80 (106.70% higher over control) whereas in control treatment it was 2.32. The CCI values of the other treatments are as follows- RRE6- 5.40 (132.54% higher over control), BHU3- 4.67 (100.96% higher over control), BHU8- 4.10 (76.56% higher over control), RRE3+BHU3- 6.09 (162.20%), RRE3+BHU8- 5.88 (153.11%), RRE6+BHU3- 6.49 (179.43%), RRE6+BHU8- 5.79 (149.28%). So best response was found in coinoculation of RRE6+BHU3 (179.43%) whereas minimum increase over control was seen in BHU8 (76.56% higher over control) [Table 4.4] (Plate 4.14).

4.4.2 Effect on number of functional leaves

Number of functional leaves that was counted on 35 DAS showed significant difference in all the rhizobial treated treatments over the control. Maximum increase in functional leaf no was seen in RRE6+BHU8- 21.78 that is 67.52% higher than control (13.00) followed by RRE6- 19.89 (52.99% higher). Least increase over control was shown by BHU8- 16.67 (28.21% higher) [Table 4.4].

4.4.3 Effect on Shoot length

Significant increase in shoot length was observed in all rhizobial treatments individually as well as in co-inoculation. However, RRE3+BHU8 (59.39 % increase over control) gave the best inoculation response in interaction with rice cultivar HUR105 followed by the RRE3+BHU3 (51.29 %), RRE6+BHU3 (48.13 %), RRE6+BHU8 (44.31 %), RRE6 (42.71 %), RRE3 (42.13 %),

Table 4.4: Effect of inoculation of endophytic bacteria on plant growth promotion of rice (cv. HUR 105) under poly house condition

Bacterial Strains	Chlorophyll Content Index (CCI)	No of functional leaves plant ⁻¹	Shoot length (cm)	Root length (cm)	Fresh Weight plant ⁻¹ (gm)		Dry weight plant ⁻¹ (gm)	
					Shoot	Root	Shoot	Root
Control (Uninoculated)	2.32	13.00	27.03	22.10	0.98	0.77	0.48	0.34
RRE3	4.80 (106.70)*	17.22 (32.48)	38.42 (42.13)	30.21 (36.70)	1.45 (48.38)	1.15 (49.55)	0.65 (37.08)	0.50 (47.59)
RRE6	5.40 (132.54)	19.89 (52.99)	38.58 (42.71)	32.50 (47.07)	1.33 (36.06)	1.19 (54.59)	0.61 (28.09)	0.53 (56.79)
BHU3	4.67 (100.96)	19.11 (47.01)	37.28 (37.90)	31.75 (43.68)	1.34 (37.28)	1.21 (57.18)	0.62 (29.20)	0.54 (60.23)
BHU8	4.10 (76.56)	16.67 (28.21)	38.22 (41.39)	29.02 (31.31)	1.42 (44.56)	1.17 (51.02)	0.63 (31.95)	0.50 (49.61)
RRE3+BHU3	6.09 (162.20)	19.67 (51.28)	40.90 (51.29)	35.57 (60.94)	1.67 (70.82)	1.41 (82.15)	0.74 (54.42)	0.62 (84.86)
RRE3+BHU8	5.88 (153.11)	18.67 (43.59)	43.09 (59.39)	37.46 (69.49)	1.80 (84.06)	1.59 (106.61)	0.82 (71.64)	0.70 (109.36)
RRE6+BHU3	6.49 (179.43)	18.33 (41.03)	40.04 (48.13)	35.10 (58.84)	1.66 (69.77)	1.43 (85.64)	0.73 (52.39)	0.67 (98.31)
RRE6+BHU8	5.79 (149.28)	21.78 (67.52)	39.01 (44.31)	33.26 (50.48)	1.54 (57.60)	1.35 (75.60)	0.70 (47.82)	0.60 (77.94)
CD at 5%	1.02	1.62	6.77	5.64	0.29	0.27	0.11	0.09
SEM±	0.34	0.54	2.26	1.88	0.10	0.09	0.04	0.03

* Parenthesis – Percent increase over control

BHU8 (41.39 %), BHU3 (37.90 %). BHU8 gave minimum increase over control [Table 4.4] (Plate 4.13).

4.4.4 Effect on root length

Root length of rice plants were also increased significantly when inoculated with endophytic rhizobacterial strains and almost same trend of different treatments were found in increasing root length. Effect of RRE3+BHU8 (37.46 cm) was most effective on HUR105 whereas minimum increment over control was recorded in BHU8 (29.02 cm) [Table 4.4] (Plate 4.12).

4.4.5 Effect on shoot fresh weight

Average shoot fresh weight of all the treatment over the replications was compared with control. Inoculation with bacterial strains was found effective in increasing shoot fresh weight over control. Average shoot fresh weight observed for plants inoculated with RRE3+BHU8 was 1.80 gm which was 84.06 % higher over control and it was recorded as maximum improvement in shoot fresh weight over control. RRE6 inoculated plants with average fresh weight 1.33gm (36.06 % higher over control) was minimum among all the inoculation treatments [Table 4.4].

4.4.6 Effect on shoot dry weight

A significant increase in shoot dry weight was observed in HUR105 with all four endophytic bacterial isolates and in co-inoculation. Highest increase in shoot dry weight was observed in plants inoculated with RRE3+BHU8 (0.82 gm) whereas minimum increment over control was recorded in RRE6 (0.61 gm) [Table 4.4].

4.4.7 Effect on root fresh weight

There was significant effect of inoculation of rhizobial strains on rice cultivar over control with respect to root fresh weight. Highest increase in root fresh weight was recorded in plants inoculated

with RRE3+BHU8 (106.61 % increase over control) followed by RRE6+RRE3 (85.64 %) and RRE3+RRE3 (82.15%). Minimum increase was with RRE3 (49.55%) [Table 4.4].

4.4.8 Effect on root dry weight

Rhizobial inoculation elicited a same trend of response on root dry weight of inoculated plant. Inoculation with coinoculation of two rhizobacterial strains RRE6+RRE3 exhibited highest root dry weight (109.36 % increase over control). Whereas, minimum increase was recorded in RRE3 (47.59 %) [Table 4.4].

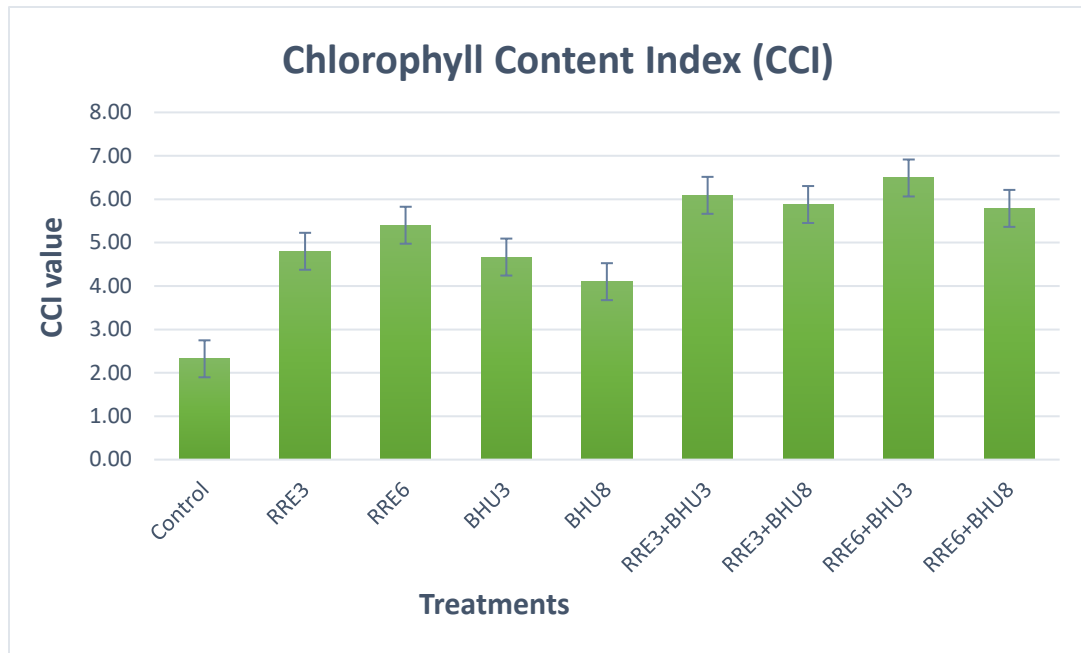


Fig 4.15: Effect of different endophytic bacterial strains on chlorophyll content of rice under poly house condition.

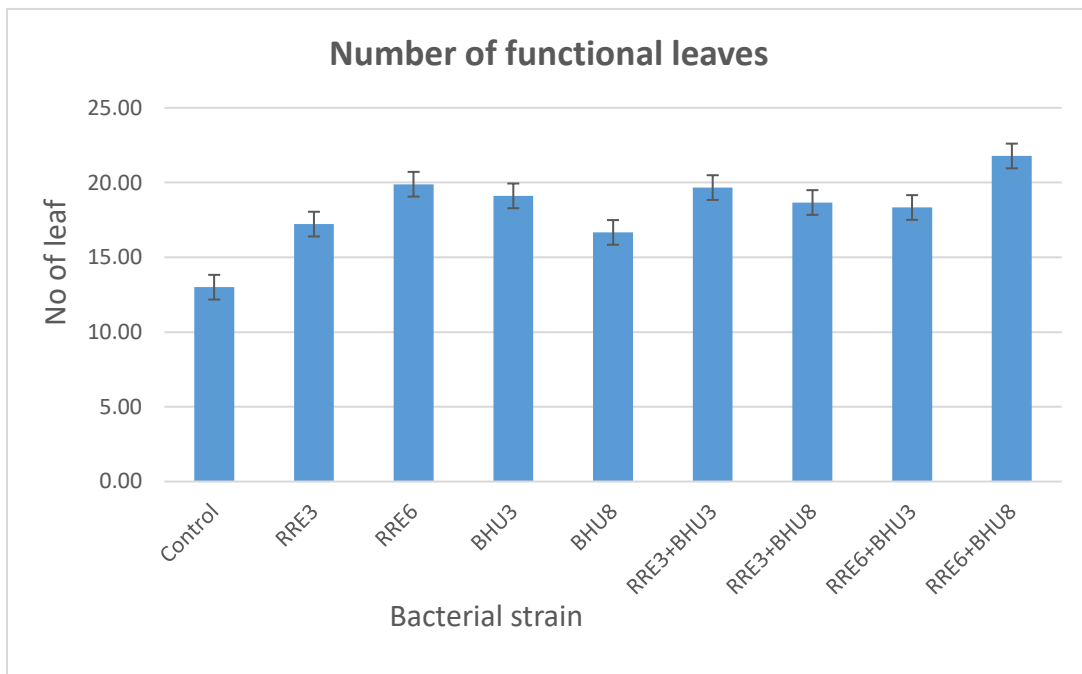


Fig 4.16: Effect of different endophytic bacterial strains on number of functional leaves of rice under poly house condition

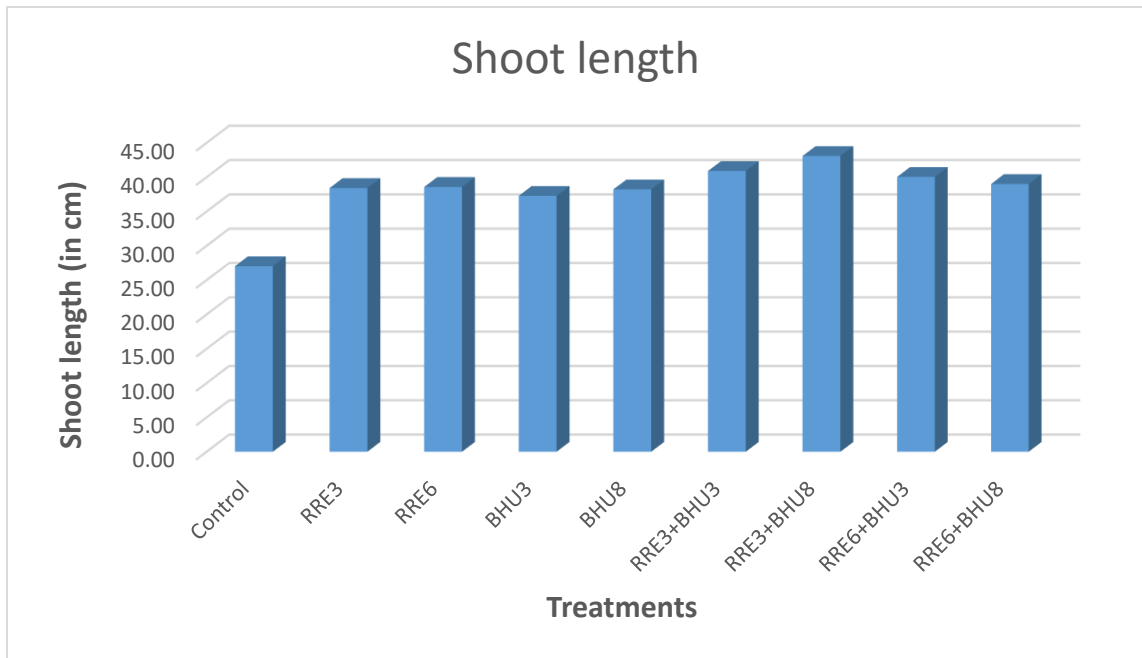


Fig 4.17: Effect of different endophytic bacterial strains on shoot length of rice under poly house condition

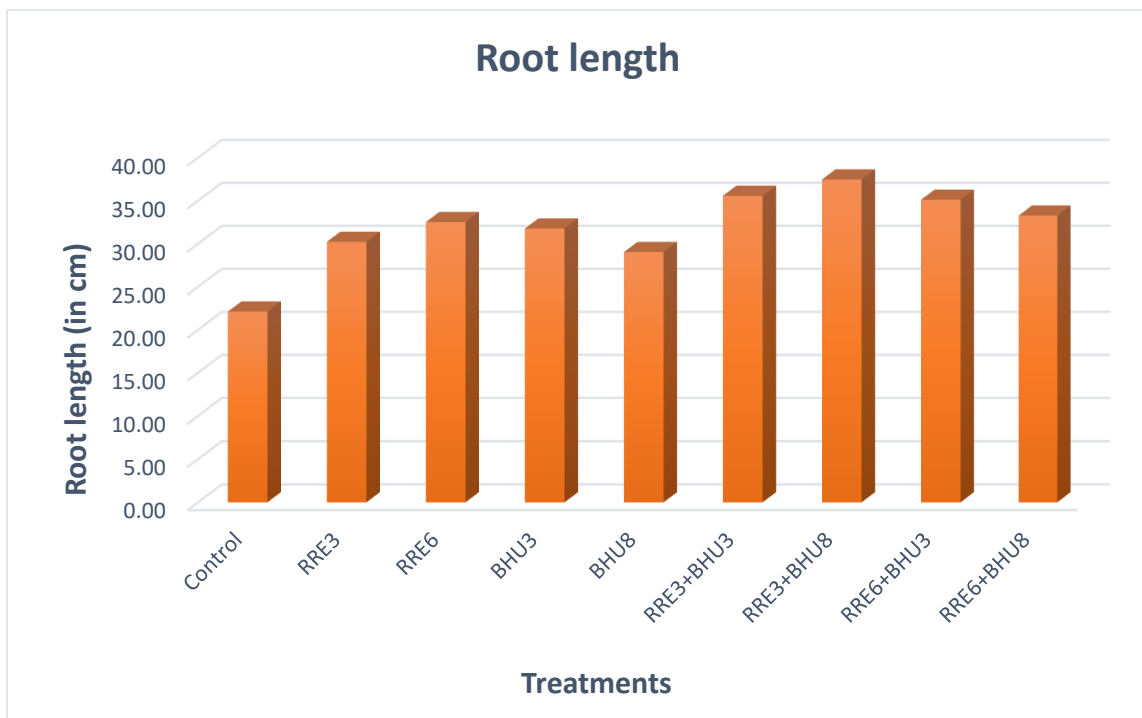


Fig 4.18: Effect of different endophytic bacterial strains on root length of rice under poly house condition

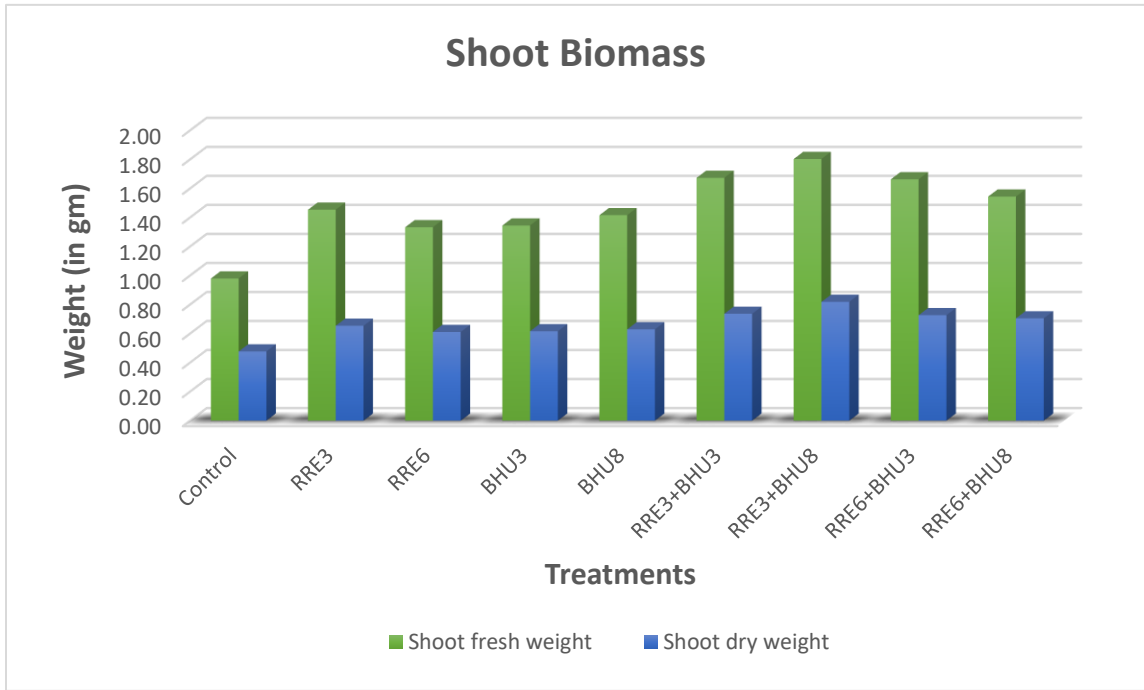


Fig 4.19: Effect of different endophytic bacterial strains on shoot biomass of rice under poly house condition

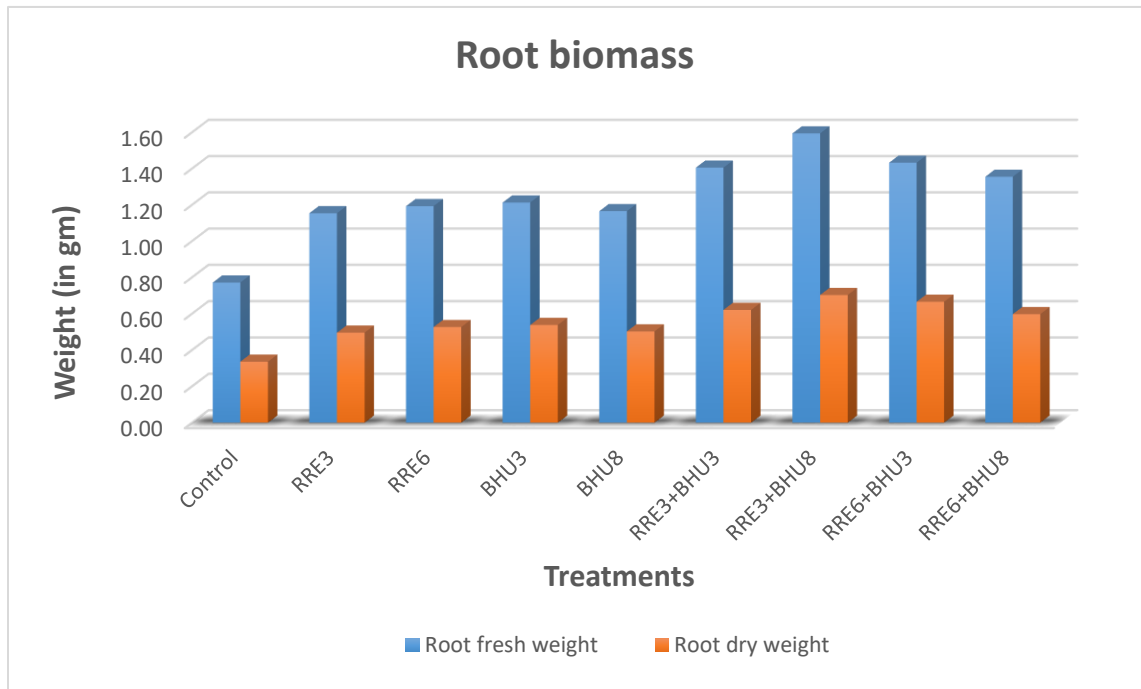


Fig 4.20: Effect of different endophytic bacterial strains on root biomass of rice under poly house condition

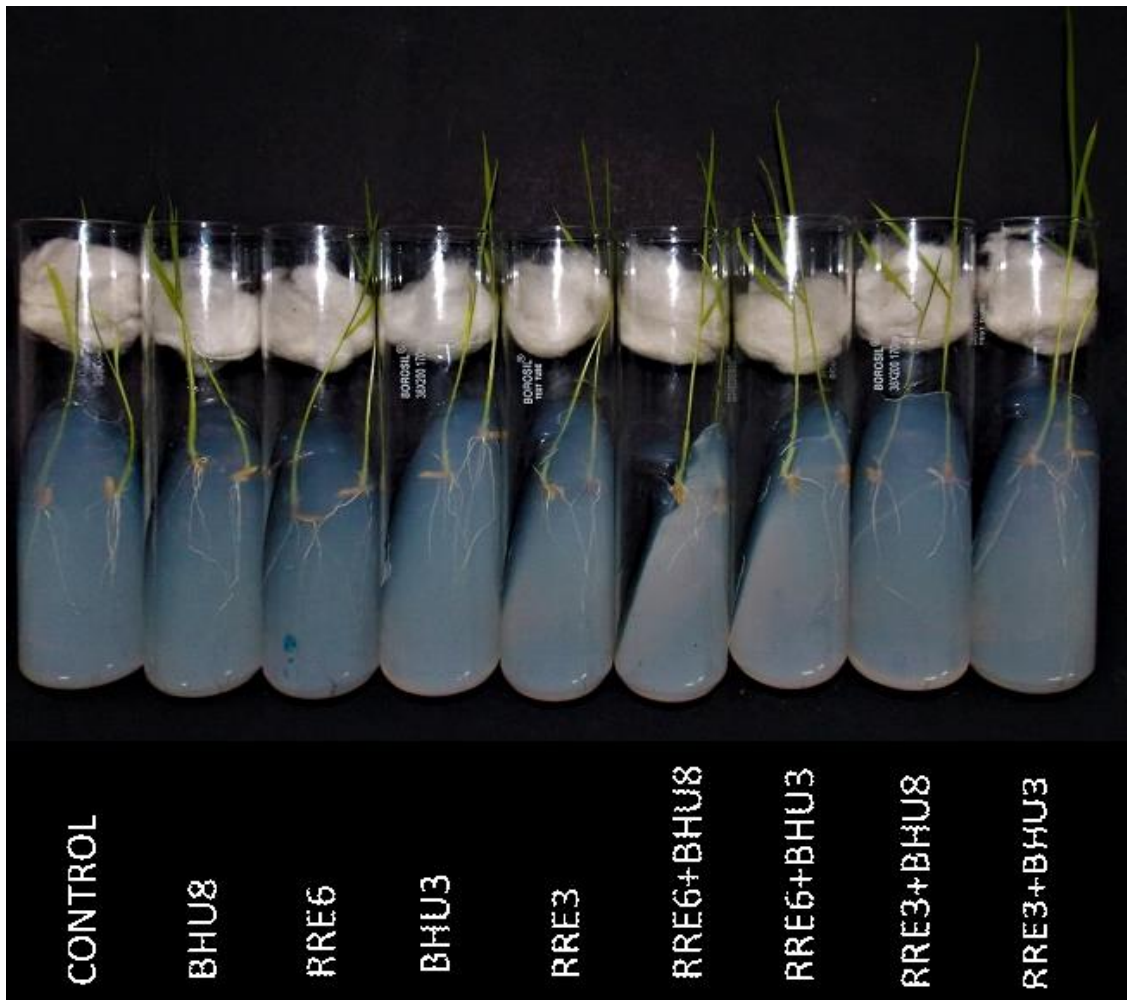


Plate 4.8: Effect of endophytic bacteria on rice seedlings growth after 15 days of inoculation under laboratory condition.



Plate 4.9: Rice plant grown under poly-house condition

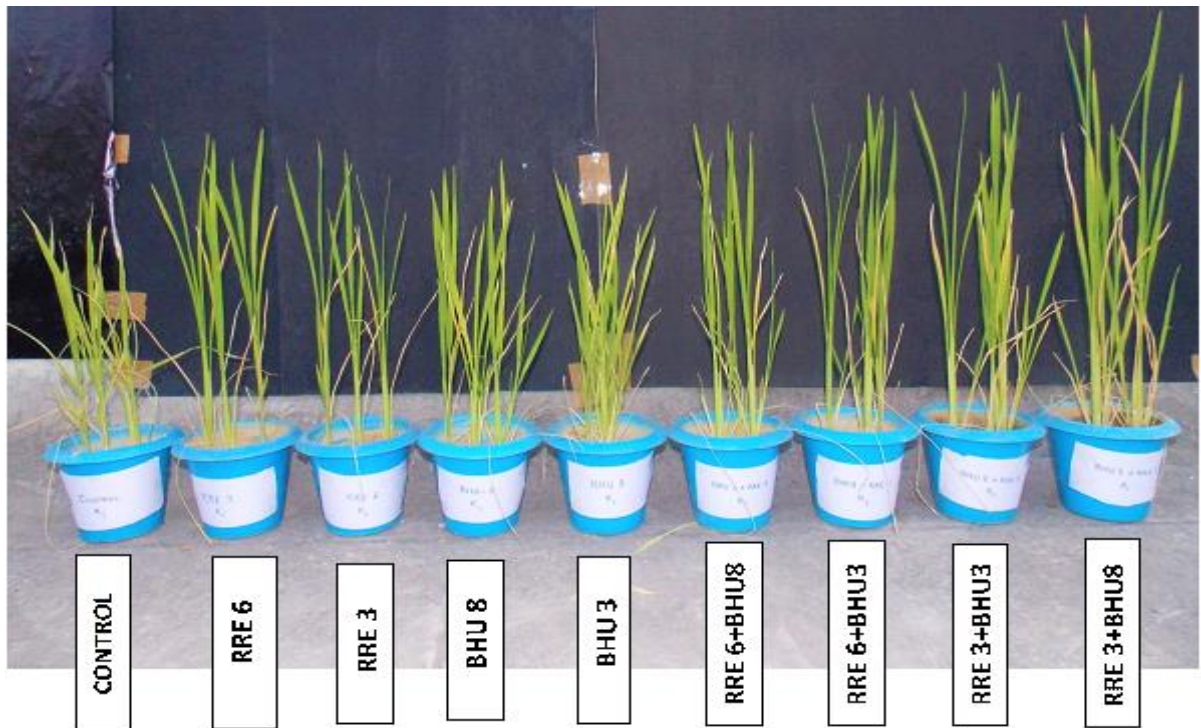


Plate 4.10: Effect of endophytic bacteria on growth of rice plant



Serial No	1	2	3	4	5	6	7	8	9
Treatment	CONTROL	BHU3	BHU8	RRE3	RRE6	RRE6+ BHU8	RRE6+ BHU3	RRE3+ BHU3	RRE3+ BHU8

Plate 4.11: Effect of endophytic bacteria on growth of rice 30 days after sowing.



Plate 4.12: Effect of different endophytic bacteria on root growth of rice



Plate 4.13: Effect of endophytic bacteria on growth of rice 60 days after sowing.



Plate 4.14: Chlorophyll content measurement of rice plant using CCI meter



Discussion

“The aim of argument, or of discussion should not be victory, but progress.”

-Joseph Joubert

DISCUSSION

Plant rhizosphere has been shown to be a preferred ecological niche for different types of soil microorganisms due to rich nutrient availability and is thus considered as a dynamic and versatile ecological environment of distinct microbes–plant interactions for harnessing essential micro- and macro-nutrients from a limited nutrient pool. To be an efficient PGPR, bacteria must be able to colonize roots because bacteria need to establish itself in the rhizosphere at population densities sufficient to produce beneficial effects. The exact mechanism by which PGPR stimulate plant growth is not clearly established, although several hypotheses such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of mineral nutrient uptake are believed to be involved.

In the present investigation, some endophytic PGPR viz. *Pseudomonas spp.*, *Burkholderia spp.*, and *Rhizobium spp.* were enumerated under different temperature treatments. Maximum growth was observed at 25°C. Kachhap et al., (2015) have reported greater numbers of PGPR in elevated temperature than ambient temperature. Their results attributed to the fact that with an increase in temperature, as has been predicted under future climate conditions, root exudation of organic C also increases. Zogg et al. (1997) found that microbial respiration increased dramatically with soil warming due to an apparent increase in the pool size of C metabolized by soil microbes at higher temperatures.

All the PGPRs in this experiment were screened in seven different pH. All these strains showed better growth between pH 6 to 8 and maximum growth was recorded at pH 7. The results are also in line with experimental studies of Sadi & Masoud (2012). They recorded most effective seed invigoration and improved seedling growth of sunflower inoculated with *Pseudomonas fluorescens* UTPF61 at pH 7.

Effect of different salt concentration on growth of PGPR strains was also studied in our experiment. The best growth of PGPR was observed at 0.1% salt concentration. With the increasing concentration of NaCl, a gradual decrease in growth rate was observed but they showed growth upto 10% salt concentration. In higher salt concentration *Pseudomonas saponiphila* BHU8

exhibited better growth than other PGPR strain. The result obtained from the study of Deshwal and Kumar (2013) indicated that all *Pseudomonas aeruginosa* and *P. fluorescens* strains showed growth in medium containing 1.5% NaCl. Above 1.75% NaCl concentration in medium, the survival number of *Pseudomonas* gradually reduced. No strains of *P. cepaciacan* tolerate 2.5% NaCl. All *Pseudomonas* strains produced normal PGPR activity of IAA, HCN, siderophore and P-solubilization up to 0.75, 1, and 1.25% NaCl respectively.

The solubilization of P in the rhizosphere is the most common mode of action implicated in PGPR to increase nutrient availability to host plants. In present study all four bacterial strains (BHU3, BHU8, RRE3, RRE6) showed clear phosphate solubilisation zones 0.24, 0.37, 0.16, 0.29 mm/h respectively. Singh et al., (2014) screened rhizobial isolates from root nodules of chickpea, pigeon pea, sweet pea, masoor and lobia. Out of 100 rhizobial isolates 70% showed phosphate solubilizing index in a range of 2.2 to 4.1 on Pikovskaya's agar media. Isolate RASH6 showed maximum solubilization index.

Similar to phosphate solubilisation, in the present investigation all the bacterial strains showed a fair amount of zinc solubilisation. *Burkholderia cepacia* RRE3 showed maximum Zn solubilisation (0.18 mm/h) whereas *Rhizobium leguminosarum* bv. *phaseoli* RRE6 solubilized minimum (0.07 mm/h). In case of potassium solubilisation except RRE6 all other endophytic bacteria solubilized potassium in Alexandrov's agar medium plate assay. *Burkholderia cepacia* RRE3 solubilized maximum K (0.13 mm/h) than others. Saravanan et al., (2004) assessed Zinc solubilizing ability of *Bacillus* sp. and *Pseudomonas* sp. using zinc oxide, zinc sulphide (sphalerite) and zinc carbonate in both plate and broth assays. ZSB-O-1 (*Bacillus* sp.) showed highest dissolution in the zinc sulphide (Sphalerite ore), with 2.80 cm of dissolution zone and 14.50 cm² of area in the plate assay. The ZSB-S-2 (*Pseudomonas* sp.) showed more solubilizing ability in the zinc oxide, with 3.30 cm clearing zone and 20.43 cm² area in the plate assay. The isolate ZSB-S-4 (*Pseudomonas* sp.) has highest solubilizing potential in zinc carbonate with 6.20 cm of dissolution zone and 13.40 cm² area in the plate assay. Thus, the solubilization potential varies among different cultures. The solubilization might be due to production of acids by the culture using zinc oxide, zinc sulphide (sphalerite) and zinc carbonate in both plate and broth assays. ZSB-O-1 (*Bacillus* sp.) showed highest dissolution in the zinc sulphide (Sphalerite ore), with 2.80 cm of dissolution zone and 14.50 cm² of area in the plate assay. The ZSB-S-2

(*Pseudomonas* sp.) showed more solubilizing ability in the zinc oxide, with 3.30 cm clearing zone and 20.43 cm² area in the plate assay. The isolate ZSB-S-4 (*Pseudomonas* sp.) has highest solubilizing potential in zinc carbonate with 6.20 cm of dissolution zone and 13.40 cm² area in the plate assay. Thus, the solubilization potential varies among different cultures. The solubilization might be due to production of acids by the culture, since the pH of the culture broth has been shifted from 7.0-7.3 to 4.8-6.5 after 15 days of inoculation.

PGPRs promote plant growth by secreting phytohormones. IAA is a phytohormone which is considered to be the most important native auxin, important signal molecule in regulation of plant development. All the four endophytic strains produced indole-3-acetic acid, the amount ranged from 4.58 µg/ml to 14.15 µg/ml after 72 h of incubation. *Pseudomonas saponiphila* BHU8 produced maximum amount of IAA (14.15 µg/ml). The rhizospheric bacterial isolates, in general, produced more amount of auxin than endophytes. Further, the results are corroborated with the findings of Sharma *et al.* (2012) where the production of IAA ranges from 10.0 to 30.0 mg/l.

The selected bacterial strains used in the present experiment were able to grow on CAS medium, showing their ability to produce siderophore. The maximum bright zone with yellowish colour was observed in case of *Pseudomonas aeruginosa* BHU3. The findings are in line with those of El-Azeem *et al.* (2007), who assayed siderophore production by rhizospheric microorganism using chrome azurol S (CAS) agar medium. Out of the 81 bacterial isolates, 73 strains gave positive reactions with the CAS plate assay and produced low molecular weight high affinity iron (III) chelating substances that transplant iron into bacterial cell called siderophores. Noori and Saud (2012) reported twenty rhizospheric isolates screened positive on chrome azurol S (CAS) agar medium.

In present study all bacterial endophytes showed high level (600 µg/ml) of intrinsic antibiotic resistance (IAR). Streptomycin was more toxic to RRE6, BHU3 and BHU8 as compared to other antibiotics whereas RRE6 showed sensitivity to kanamycin and ciprofloxacin. This finding is not in line with the results obtained by Mehnaz and Lazarovits (2006). They studied IAR pattern of *Pseudomonas putida* using antibiotics ampicillin, cyclohexamide, kanamycin, rifampicin, gentamycin, tetracycline streptomycin, spectinomycin. *Pseudomonas* showed sensitivity to tetracycline and kanamycin at 25 µg/ml and resistance to ampicillin, cycloheximide, spectinomycin upto 100 µg/ml.

The present study showed the effect of selected bacterial endophytes in single and co-inoculation on germination, shoot and root length, biomass production of rice cv. HUR105 when applied through seed inoculation.

Plant infection test carried out under controlled laboratory condition showed maximum improvement in rate of germination over control in case of seeds inoculated with RRE6. Seeds inoculated with RRE3+BHU3 exhibited higher shoot growth rate and RRE3+BHU8 exhibited higher root growth rate over other treatments. RRE3 in combination with BHU3 gave the best interaction response for shoot length of seedling (58.77% increase over control) whereas RRE3+BHU8 gave best result for root length (77.18% increase over control) over uninoculated control. The results are supported by de Souza et al., (2013) studied the bioinoculant potential on rice. They found that Rice inoculated with the bacterial isolates AC32 (*Herbaspirillum sp.*), CA1 (*Burkholderia sp.*), CA6 (*Burkholderia sp.*) and CA56 (*Rhizobium sp.*) presented significantly higher results for shoot length, dry shoot biomass, and dry root weight when compared with the uninoculated control plants under gnotobiotic conditions. These isolates also significantly enhanced the nutrient uptake of the rice plants.

Effect of endophytic PGPR strains individually and in coinoculation on plant growth parameters like shoot length, root length, chlorophyll content, shoot and root fresh weight, dry weight was tested under poly house condition also. Results from this study showed significant increase of these parameters over control. Maximum increase in chlorophyll content of rice was recorded in plants treated with consortium of RRE6+BHU3 (179.43% increase over control) over control. For number of functional leaves, plants coinoculated with RRE6+BHU8 (67.52%) gave maximum improvement. RRE3+BHU8 combination showed significantly increased value over uninoculated control for parameters shoot length (59.39%), root length (69.49%), shoot fresh weight (84.06%), root fresh weight (106.61%), shoot dry weight (71.64%), root dry weight (109.36%). Chen et al., (2017) obtained similar results in *Sedum alfredii* inoculated with endophytic bacterium *Pseudomonas fluorescens* Sasm05. They found Sasm05 had a positive effect on the content of chlorophyll which was increased by 24–44% with Sasm05 treatment. Hasan et al., (2014) studied the effects of PGPR isolates *Azospirillum* and *Enterobacter* with rhizobial co-inoculation on growth of rice plants. They found both Basmati and Superbasmati rice varieties inoculated with PGPR had greater plant height, stem width, root length and interestingly, the magnitude of

stimulation for root length, shoot length, biomass was greater in co-inoculated treatments was increased more in double inoculation as compared to single treatment.

From the study of Çakmakçı et al., (2007) it has been shown that inoculation of wheat plants with rhizobia resulted in increase in root elongation up to 20%, root dry weight up to 13%, shoot elongation 38% and SDW 36%. Similar rate of increase in wheat due to the application of PGPR is also reported. Therefore, our results are in agreement with these results and it can be concluded that the effect of PGPR alone or co-inoculation is not limited to rice plant but it shows their influence on other crops.



Summary and Conclusion

“Reasoning draws a conclusion, but does not make the conclusion certain, unless the mind discovers it by the path of experience.”

- Roger Bacon

SUMMARY AND CONCLUSION

Rice (*Oryza sativa* L.) is the main staple food in Asia, and the lowland rice makes a major contribution to global rice supply and rice-rice and rice-wheat system are often cited as examples of sustainable production system. The main reason for sustainable maintenance of soil fertility in the lowland rice paddies is the conservation and accumulation of organic matter (organic carbon and nitrogen) under prolonged submerged soil conditions. On the other hand, the rapid decomposition of organic matter in the presence of oxygen (O₂) under upland crop or lowland rice upland cropping systems, leads to a significant decline in the soil organic carbon. Native and applied plant growth promoting rhizobacteria (PGPR) play an important role in better crop growth, yield, nutrient uptake and maintenance of soil chemical and biological parameters in irrigated rice system.

With this background, Present study was planned to screen and characterize the potent plant growth promoting bacteria and to access their potential for growth promotion in rice. The finding of the study are summarized as followed.

- Four endophytic bacterial strains *Burkholderia cepacia* RRE3, *Rhizobium leguminosarum* *bv. phaseoli* RRE6, *Pseudomonas aeruginosa* BHU3, *Pseudomonas saponiphila* BHU8 were obtained from PGPR laboratory of Prof. R.K. Singh, Dept of Genetics and Plant Breeding, IAS, BHU. These bacterial strains were characterized for their plant growth promoting and biochemical properties.
- The bacterial strains were initially characterized for physiological characters like temperature tolerance, pH tolerance, salt tolerance and antibiotic resistance. All the bacterial strains showed luxuriant growth at 25⁰C and in a pH range of 6 to 8. Though they survived in a wide range of temperature and pH, above 35⁰C and below 15⁰C there was decline in bacterial growth. For the growth test with different concentration of salt, the best growth was obtained at 0.1% NaCl. With gradual increase in salt concentration there was a sharp retardation in colony growth. All the four PGPR strains exhibited a high range of

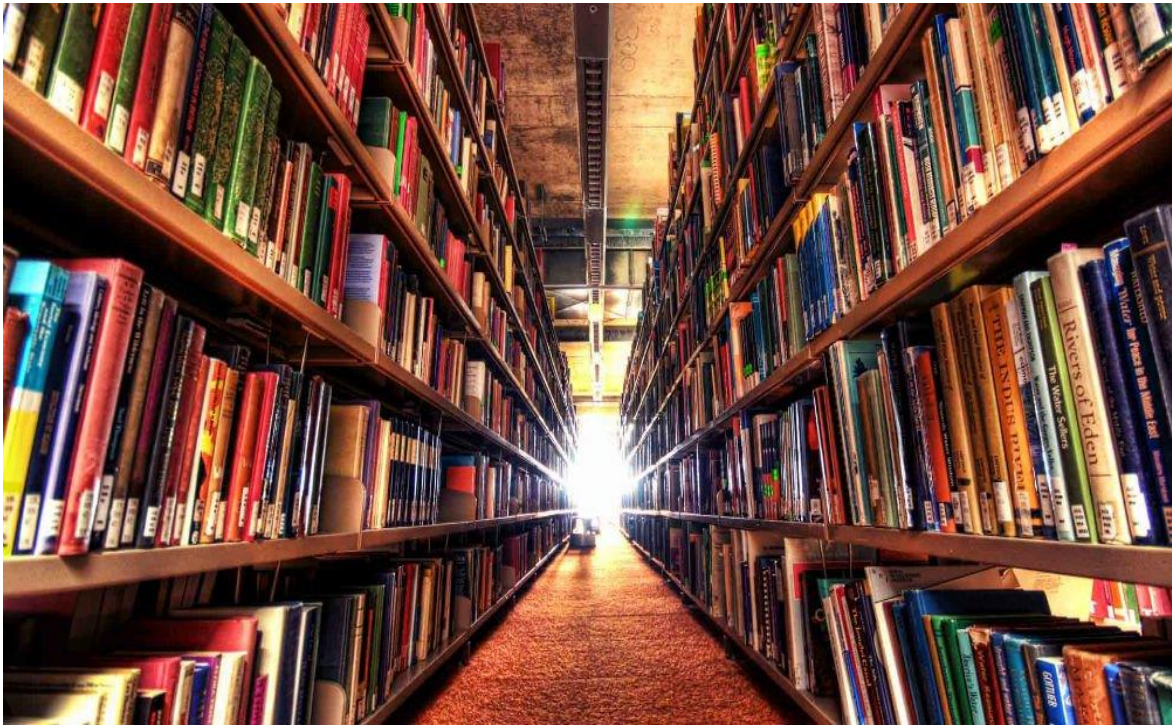
intrinsic antibiotic resistance to ampicillin, streptomycin, neomycin, kanamycin, ciprofloxacin, chloramphenicol, nalidixic acid, tetracyclin and rifampicin.

- The bacterial strains were also screened for biochemical characters. Maximum IAA production was recorded in RRE3 whereas BHU3 is the highest siderophore producer followed by RRE3 and BHU8 respectively.
- The endophytes gave positive results for P, Zn and K solubilisation. Highest phosphate solubilisation activity was found in BHU8 followed by RRE6, BHU3 and RRE3. RRE3 was highest zinc solubilizer. Except RRE6, all other strains solubilized potash.
- Four PGPRs, *Burkholderia* strain RRE3, *Rhizobium* strain RRE6, *Pseudomonas* strain BHU3 and BHU8 and combination of these strains with each other were used for germination and plant growth promotion test in rice cv. HUR105 under laboratory condition. Seeds inoculated with RRE6 took minimum time to germinate. A combination of RRE3 strain with BHU3 resulted maximum shoot growth rate and seeds inoculated with combination of strains RRE3 and BHU8 resulted maximum root growth rate. Maximum seedling shoot length was observed in RRE3+BHU3 (58.77% increased over control) and that of root length was recorded in RRE3+BHU8 (77.18% increased over control) over untreated control. Minimum improvement over control was shown by BHU8 for both shoot and root length.
- A similar plant growth promotion test using these four isolates and their combinations was conducted on rice under polyhouse condition. All the four PGPR strains and their combinations when applied through seed inoculation along with soil application were capable of significantly increasing root and shoot length, fresh and dry biomass of rice plants. Maximum chlorophyll content was recorded in RRE6+BHU3 (179.43%) treated plants whereas minimum improvement over control was recorded in BHU8 (76.56%). RRE6+BHU8 (67.52%) gave best result in improving number of functional leaves over control. In case of both shoot length and root length RRE3+BHU8 (59.39% and 69.49% increase over control respectively) showed maximum improvement over control. BHU3 (37.90%) and BHU8 (31.31%) showed minimum improvement over control for shoot and root length respectively. Best result for shoot and root fresh weight was exhibited by RRE3+BHU8 (84.06% and 106.61% increase over control respectively). A similar trend was followed for shoot and root dry weight also. Maximum biomass for shoot and root was

recorded in RRE6+BHU3 (71.64% and 109.36% increase over control respectively) treated plants over uninoculated control. In terms of shoot biomass RRE6 (28.09%) and root biomass BHU8 (49.61%) resulted minimum increment though they are significantly higher than control.

It is quite clear from the present investigations that plant growth promoting endophytic bacteria either alone or in combination resulted significant improvement over control. This increase in plant growth following inoculation is possibly attributed to the stimulatory effect of microbes that produce or induced plant growth regulators. Further results indicate that coinoculation of rice gave better result than plants inoculated with single strain. So in consortium they must exhibit synergistic, more stimulating effects when compared to growth responses following inoculation with either of the strain alone. The best combination of PGPR isolates among those tested was identified as *Burkholderia cepacia* strain RRE3 (β proteobacteria) plus *Pseudomonas saponiphila* strain BHU8 (γ proteobacteria). These results indicate that the strains isolated from rice field appear more promising for potential biofertilizers in crop fields and can be used for enhanced growth of plants in future.

However, the findings of present investigations i.e. inoculation of rice with endophytic bacterial strains needs to be re-examined with different cultivars of rice as many bacterial strains are host genotype specific and in different agro-climatic zones before arriving to a concrete conclusion.



Bibliography

“Literacy could be the ladder out of poverty.”

— Morgan Freeman

BIBLIOGRAPHY

- Adesemoye, A. O., Torbert, H. A., & Kloepper, J. W. (2008). Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. *Canadian journal of microbiology*, **54**(10), 876-886.
- Afzal, A., & Bano, A. (2008). Rhizobium and phosphate solubilizing bacteria improve the yield and phosphorus uptake in wheat (*Triticum aestivum*). *International Journal of Agricultural Biology*, **10**(1), 85-88.
- Ahanthem, S., & Jha, D. K. (2007). Response of rice crop inoculated with arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria to different soil nitrogen concentrations. *Mycorrhiza News*, **18**(4), 15-20.
- Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King Saud University-Science*, **26**(1), 1-20.
- Antoun, H., & Kloepper, J. (2001). Plant growth promoting rhizobacteria (PGPR).
- Antoun, H., Beauchamp, C. J., Goussard, N., Chabot, R., & Lalande, R. (1998). Potential of Rhizobium and Bradyrhizobium species as plant growth promoting rhizobacteria on non-legumes: effect on radishes (*Raphanus sativus* L.). In *Molecular microbial ecology of the soil* (pp. 57-67). Springer, Dordrecht.
- Aravind, R., Dinu, A., Eapen, S. J., Kumar, A., & Ramana, K. V. (2009). Isolation and evaluation of endophytic bacteria against plant parasitic nematodes infesting black pepper (*Piper nigrum* L.). *Indian Journal of Nematology*, **39**(2), 211-217.
- Arora, N. K., Kang, S. C., & Maheshwari, D. K. (2001). Isolation of siderophore-producing strains of Rhizobium meliloti and their biocontrol potential against Macrophomina phaseolina that causes charcoal rot of groundnut. *Current Science*, 673-677.

- Arslan, A. M., Mahmood, R., & Sajjad, M. M., (2011). Nitrogen Fixation and Indole Acetic Acid Production Potential of Bacteria Isolated from Rhizosphere of Sugarcane (*Saccharum officinarum* L.). *Advances in Biological Research* 5(6): 348-355
- Ashrafuzzaman, M., Hossen, F. A., Ismail, M. R., Hoque, A., Islam, M. Z., Shahidullah, S. M., & Meon, S. (2009). Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. *African Journal of Biotechnology*, 8(7).
- Barraquio, W. L., Segubre, E. M., Gonzalez, M. A. S., Verma, S. C., James, E. K., Ladha, J. K., & Tripathi, A. K. (2000). Diazotrophic enterobacteria: What is their role in the rhizosphere of rice. *The Quest for Nitrogen Fixation in Rice*. Eds. JK Ladha and PM Reddy, 93-118.
- Bashan, Y., Puente, M. E., Rodriguez-Mendoza, M. N., Toledo, G., Holguin, G., Ferrera-Cerrato, R., & Pedrin, S. (1995). Survival of Azospirillum brasilense in the Bulk Soil and Rhizosphere of 23 Soil Types. *Applied and Environmental Microbiology*, 61(5), 1938-1945.
- Bell, C. R., Dickie, G. A., Harvey, W. L. G., & Chan, J. W. Y. F. (1995). Endophytic bacteria in grapevine. *Canadian journal of Microbiology*, 41(1), 46-53.
- Berg, G. (2009). Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. *Applied microbiology and biotechnology*, 84(1), 11-18.
- Bhattacharjee, R. B., Jourand, P., Chaintreuil, C., Dreyfus, B., Singh, A., & Mukhopadhyay, S. N. (2012). Indole acetic acid and ACC deaminase-producing *Rhizobium leguminosarum* bv. trifolii SN10 promote rice growth, and in the process undergo colonization and chemotaxis. *Biology and fertility of soils*, 48(2), 173-182.

- Biswas, J. C., Ladha, J. K., Dazzo, F. B., Yanni, Y. G., & Rolfe, B. G. (2000). Rhizobial inoculation influences seedling vigor and yield of rice. *Agronomy Journal*, **92**(5), 880-886.
- Çakmakçı, R., Erat, M., Erdoğan, Ü., & Dönmez, M. F. (2007). The influence of plant growth-promoting rhizobacteria on growth and enzyme activities in wheat and spinach plants. *Journal of Plant Nutrition and Soil Science*, **170**(2), 288-295.
- Chen, B., Luo, S., Wu, Y., Ye, J., Wang, Q., Xu, X., ... & Yang, X. (2017). The Effects of the Endophytic Bacterium *Pseudomonas fluorescens* Sasm05 and IAA on the Plant Growth and Cadmium Uptake of *Sedum alfredii* Hance. *Frontiers in microbiology*, **8**.
- Choudhary, R. L. (2008). *performance of rice hybrids grown by system of rice intensification (SRI) with plant growth promoting rhizobacteria* (PGPR Doctoral dissertation).
- Coyne, M. S., & Coyne, M. S. (1999). *Soil microbiology: an exploratory approach*. New York: Delmar.
- Crowley, D. E. (2006). Microbial siderophores in the plant rhizosphere. In *Iron nutrition in plants and rhizospheric microorganisms* (pp. 169-198). Springer, Dordrecht.
- Curl, E. A., & Truelove, B. (1986). The rhizosphere. Springer-Verlag, Berlin. *The rhizosphere. Springer-Verlag, Berlin*.
- Dave, A., & Patel, H. H. (1999). Inorganic phosphate solubilizing soil Pseudomonads. *Indian Journal of Microbiology*, **39**(3), 161-164.
- De Datta, S. K. (1981). *Principles and practices of rice production*. International Rice Research Institute.

- De Datta, S. K., & Buresh, R. J. (1989). Integrated nitrogen management in irrigated rice. In *Advances in Soil Science* (pp. 143-169). Springer, New York, NY.
- de Salamone, I. E. G., Funes, J. M., Di Salvo, L. P., Escobar-Ortega, J. S., D'Auria, F., Ferrando, L., & Fernandez-Scavino, A. (2012). Inoculation of paddy rice with *Azospirillum brasilense* and *Pseudomonas fluorescens*: impact of plant genotypes on rhizosphere microbial communities and field crop production. *Applied soil ecology*, **61**, 196-204.
- de Souza, R., Beneduzi, A., Ambrosini, A., Da Costa, P. B., Meyer, J., Vargas, L. K., ... & Passaglia, L. M. (2013). The effect of plant growth-promoting rhizobacteria on the growth of rice (*Oryza sativa* L.) cropped in southern Brazilian fields. *Plant and soil*, **366**(1-2), 585-603.
- Deshwal, V. K., & Kumar, P. (2013). Effect of salinity on growth and PGPR activity of Pseudomonads. *Journal of Academia and Industrial Research*, **2**(6), 353-356.
- Dewan, G. I., & Rao, N. S. (1979). Seed inoculation with *Azospirillum brasilense* and *Azotobacter chroococcum* and the root biomass of rice (*Oryza sativa* L.). *Plant and Soil*, **53**(3), 295-302.
- Egamberdieva, D. (2009). Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiologiae Plantarum*, **31**(4), 861-864.
- El-Azeem, S. A. M. A., Mehana, T. A., & Shabayek, A. A. (2007). Some plant growth promoting traits of rhizobacteria isolated from Suez Canal region, Egypt. In *African Crop Science Conference Proceedings* (Vol. 8, pp. 1517-1525).
- El-Khawas, H., & Adachi, K. (1999). Identification and quantification of auxins in culture media of *Azospirillum* and *Klebsiella* and their effect on rice roots. *Biology and Fertility of Soils*, **28**(4), 377-381.

- Etesami, H., Alikhani, H. A., & Hosseini, H. M. (2015). Indole-3-acetic acid (IAA) production trait, a useful screening to select endophytic and rhizosphere competent bacteria for rice growth promoting agents. *MethodsX*, **2**, 72-78.
- Fåhraeus, G. (1957). The infection of clover root hairs by nodule bacteria studied by a simple glass slide technique. *Microbiology*, **16**(2), 374-381.
- Figueiredo, M. D. V. B., Seldin, L., de Araujo, F. F., & Mariano, R. D. L. R. (2010). Plant growth promoting rhizobacteria: fundamentals and applications. In *Plant growth and health promoting bacteria* (pp. 21-43). Springer, Berlin, Heidelberg.
- Fisher, P. J., Petrini, O., & Scott, H. L. (1992). The distribution of some fungal and bacterial endophytes in maize (*Zea mays* L.). *New Phytologist*, **122**(2), 299-305.
- Fulchieri, M., & Frioni, L. (1994). Azospirillum inoculation on maize (*Zea mays*): effect on yield in a field experiment in central Argentina. *Soil Biology and Biochemistry*, **26**(7), 921-923.
- Garbeva, P., Van Overbeek, L. S., Van Vuurde, J. W. L., & Van Elsas, J. D. (2001). Analysis of endophytic bacterial communities of potato by plating and denaturing gradient gel electrophoresis (DGGE) of 16S rDNA based PCR fragments. *Microbial ecology*, **41**(4), 369-383.
- Garcia, J. L., Probanza, A., Ramos, B., and Manero, F. J. G., (2001). Ecology, genetic diversity and screening strategies of plant growth promoting rhizobacteria. *Journal of Plant Nutrition and Soil Sciences* **164**: 1-7
- Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. *Canadian journal of microbiology*, **41**(2), 109-117.
- Gordon, S. A., & Weber, R. P. (1951). Colorimetric estimation of indoleacetic acid. *Plant physiology*, **26**(1), 192.

- Gyaneshwar, P., Parekh, L. J., Archana, G., Poole, P. S., Collins, M. D., Hutson, R. A., & Kumar, G. N. (1999). Involvement of a phosphate starvation inducible glucose dehydrogenase in soil phosphate solubilization by *Enterobacter asburiae*. *FEMS microbiology letters*, *171*(2), 223-229.
- Hasan, M., Bano, A., Hassan, S. G., Iqbal, J., Awan, U., Rong-ji, D., & Khan, K. A. (2014). Enhancement of rice growth and production of growth-promoting phytohormones by inoculation with *Rhizobium* and other rhizobacteria. *World Applied Sciences Journal*, *31*(10), 1734-1743.
- Hiltner, L. T. (1904). U neuere Erfahrungen und Probleme auf dem Gebiet der Bodenbakteriologie und unter besonderer Berücksichtigung der Grundung und Brache. *Arb. Dtsch. Landwirt. Ges.*, *98*, 59-78.
- Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil. *Circular. California agricultural experiment station*, *347*(2nd edit).
- Höfte, M. (1993). Classes of microbial siderophores. *Iron chelation in plants and soil microorganisms*, 3-26.
- Holguin, G., & Patten, C. L. (1999). *Biochemical and genetic mechanisms used by plant growth promoting bacteria*. World Scientific.
- Hurek, T., & Reinhold-Hurek, B. (2003). *Azoarcus* sp. strain BH72 as a model for nitrogen-fixing grass endophytes. *Journal of Biotechnology*, *106*(2-3), 169-178.
- IRRI, (1997). Almanac, 2nd edition. Manila, Philippines: International Rice Research Institute, Los banos, Laguna, Philippines.

- Javaid, A., & Bajwa, R. (2011). Field evaluation of effective microorganisms (EM) application for growth, nodulation, and nutrition of mung bean. *Turkish Journal of Agriculture and Forestry*, **35**(4), 443-452.
- Jha, B., Thakur, M. C., Gontia, I., Albrecht, V., Stoffels, M., Schmid, M., & Hartmann, A. (2009). Isolation, partial identification and application of diazotrophic rhizobacteria from traditional Indian rice cultivars. *European journal of soil biology*, **45**(1), 62-72.
- Joseph, B., Ranjan Patra, R., & Lawrence, R. (2012). Characterization of plant growth promoting rhizobacteria associated with chickpea (*Cicer arietinum* L.). *International Journal of Plant Production*, **1**(2), 141-152.
- Kachhap, S., Chaudhary, A., & Singh, S. D. (2015). Response of plant growth promoting rhizobacteria (pgpr) in relation to elevated temperature conditions in groundnut (*Arachis hypogaea* L.). *The Ecoscan*, **9**(3and4), 771-778.
- Kanimozhi, K., & Panneerselvam, A. (2010). Studies on isolation and nitrogen fixation ability of *Azospirillum* spp. isolated from Thanjavur district. *Der Chemica Sinica*, **1**(3), 138-145.
- Khush, G. S. (2004, February). Harnessing science and technology for sustainable rice-based production systems. In *FAO rice conference* (Vol. 4).
- Kloepper, J. W. (1994). Plant growth-promoting rhizobacteria (other systems) *Azospirillum/plant associations*, 137-166.
- Kloepper, J. W., Leong, J., Teintze, M., & Schroth, M. N. (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature*, **286**(5776), 885.
- Kloepper, J. W., Lifshitz, R., & Zablutowicz, R. M. (1989). Free-living bacterial inocula for enhancing crop productivity. *Trends in biotechnology*, **7**(2), 39-44.

- Kumar, A., Kumar, A., Devi, S., Patil, S., Payal, C., & Negi, S. (2012). Isolation, screening and characterization of bacteria from Rhizospheric soils for different plant growth promotion (PGP) activities: an in vitro study. *Recent research in science and technology*, **4**(1).
- Kumar, R. R., Karajol, K., & Naik, G. R. (2011). Effect of polyethylene glycol induced water stress on physiological and biochemical responses in pigeonpea (*Cajanus cajan* L. Millsp.). *Recent Research in Science and Technology*, **3**(1).
- Kundu, B. S., & Gaur, A. C. (1984). Rice response to inoculation with N₂-fixing and P-solubilizing microorganisms. *Plant and Soil*, **79**(2), 227-234.
- Ladha, J. K., Barraquio, W. L., & Revilla, L. (1997). Isolation of endophytic diazotrophic bacteria from wetland rice. In *Opportunities for Biological Nitrogen Fixation in Rice and Other Non-Legumes* (pp. 15-24). Springer, Dordrecht.
- Luo, S., Wan, Y., Xiao, X., Guo, H., Chen, L., Xi, Q., ... & Chen, J. (2011). Isolation and characterization of endophytic bacterium LRE07 from cadmium hyperaccumulator *Solanum nigrum* L. and its potential for remediation. *Applied microbiology and biotechnology*, **89**(5), 1637-1644.
- Lynch, J. M. (1985). Origin, nature and biological activity of aliphatic substances and growth hormones found in soil. In *Soil organic matter and biological activity* (pp. 151-174). Springer, Dordrecht.
- Mahaffee, W. F., & Kloepper, J. W. (1997). Bacterial communities of the rhizosphere and endorhiza associated with field-grown cucumber plants inoculated with a plant growth-promoting rhizobacterium or its genetically modified derivative. *Canadian journal of microbiology*, **43**(4), 344-353.

- Martínez-Viveros, O., Jorquera, M. A., Crowley, D. E., Gajardo, G. M. L. M., & Mora, M. L. (2010). Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *Journal of soil science and plant nutrition*, **10**(3), 293-319.
- McInroy, J. A., & Kloepper, J. W. (1995). Survey of indigenous bacterial endophytes from cotton and sweet corn. *Plant and soil*, **173**(2), 337-342.
- Meera, T., & Balabaskar, P. (2012). Isolation and characterization of *Pseudomonas fluorescens* from rice fields. *International Journal of Food, Agriculture and Veterinary Science*, **2**(1), 113-120.
- Mehnaz, S., Mirza, M. S., Haurat, J., Bally, R., Normand, P., Bano, A., & Malik, K. A. (2001). Isolation and 16S rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. *Canadian journal of microbiology*, **47**(2), 110-117.
- Minamisawa, K., Nishioka, K., Miyaki, T., Ye, B., Miyamoto, T., You, M., ... & Sein, T. (2004). Anaerobic nitrogen-fixing consortia consisting of clostridia isolated from gramineous plants. *Applied and environmental microbiology*, **70**(5), 3096-3102.
- Mirza, M. S., Mehnaz, S., Normand, P., Prigent-Combaret, C., Moëgne-Loccoz, Y., Bally, R., & Malik, K. A. (2006). Molecular characterization and PCR detection of a nitrogen-fixing *Pseudomonas* strain promoting rice growth. *Biology and Fertility of Soils*, **43**(2), 163-170.
- Mishra, V. K., & Kumar, A. Plant growth promoting and phytostimulatory potential of *Bacillus Subtilis* and *Bacillus Amyloliquefaciens*. *ARPJN Journal of Agricultural and Biological Science* **7**:7-12
- Murty, M. G., & Ladha, J. K. (1988). Influence of *Azospirillum* inoculation on the mineral uptake and growth of rice under hydroponic conditions. *Plant and soil*, **108**(2), 281-285.

- Nandakumar, R., Babu, S., Viswanathan, R., Sheela, J., Raguchander, T., & Samiyappan, R. (2001). A new bio-formulation containing plant growth promoting rhizobacterial mixture for the management of sheath blight and enhanced grain yield in rice. *Biocontrol*, **46**(4), 493-510.
- Noori, M. S. S., & Saud, H. M. (2012). Potential plant growth-promoting activity of *Pseudomonas* sp. isolated from paddy soil in Malaysia as biocontrol agent. *Journal of Plant Pathology and Microbiology*, **3**(2), 1-4.
- Panhwar, Q. A., Radziah, O., Rahman, A. Z., Sariah, M., Razi, I. M., & Naher, U. A. (2011). Contribution of phosphate-solubilizing bacteria in phosphorus bioavailability and growth enhancement of aerobic rice. *Spanish Journal of Agricultural Research*, **9**(3), 810-820.
- Parani, K., & Saha, B. K. (2012). Prospects of using phosphate solubilizing *Pseudomonas* as bio fertilizer. *European Journal of Biological Sciences*, **4**, 40-44.
- Peng, G., Zhang, W., Luo, H., Xie, H., Lai, W., & Tan, Z. (2009). *Enterobacter oryzae* sp. nov., a nitrogen-fixing bacterium isolated from the wild rice species *Oryza latifolia*. *International journal of systematic and evolutionary microbiology*, **59**(7), 1650-1655.
- Perin, L., Martínez-Aguilar, L., Castro-González, R., Estrada-De Los Santos, P., Cabellos-Avelar, T., Guedes, H. V., ... & Caballero-Mellado, J. (2006). Diazotrophic Burkholderia species associated with field-grown maize and sugarcane. *Applied and environmental microbiology*, **72**(5), 3103-3110.
- Pikovskaya, R. I. (1948). Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, **17**, 362-370.
- Prakamhang, J., Minamisawa, K., Teamtaisong, K., Boonkerd, N., & Teaumroong, N. (2009). The communities of endophytic diazotrophic bacteria in cultivated rice (*Oryza sativa* L.). *Applied soil ecology*, **42**(2), 141-149.

- Procópio, R. E. L., Araújo, W. L., Maccheroni Jr, W., & Azevedo, J. L. (2009). Characterization of an endophytic bacterial community associated with Eucalyptus spp. *Genetics and Molecular Research*, **8**(4), 1408-1422.
- Rani, M. U., Arundhathi, A., & Reddy, G. (2012). Screening of rhizobacteria containing plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing growth of pigeon pea. *African Journal of Biotechnology*, **11**(32), 8085-8091.
- Rediers, H., Bonnacarrere, V., Rainey, P. B., Hamonts, K., Vanderleyden, J., & De Mot, R. (2003). Development and application of a dapB-based in vivo expression technology system to study colonization of rice by the endophytic nitrogen-fixing bacterium *Pseudomonas stutzeri* A15. *Applied and environmental microbiology*, **69**(11), 6864-6874.
- Sadi, M. S., & Masoud, A. (2012). Effect of pH on stability, sunflower growth promotion and biocontrol potential of a talc-based formulation of 'Pseudomonas fluorescens' UTPF61. *Australian Journal of Crop Science*, **6**(3), 463.
- Sahrawat, K. L. (2000). Macro-and micronutrients removed by upland and lowland rice cultivars in West Africa. *Communications in Soil Science and Plant Analysis*, **31**(5-6), 717-723.
- Saikia, R., Kumar, R., Singh, T., Srivastava, A. K., Arora, D. K., & Lee, M. W. (2004). Induction of defense related enzymes and pathogenesis related proteins in *Pseudomonas fluorescens*-treated chickpea in response to infection by *Fusarium oxysporum* f.sp. *ciceri*. *Mycobiology*, **32**(1), 47-53.
- Sandhya, V. S. K. Z., Ali, S. Z., Grover, M., Reddy, G., & Venkateswarlu, B. (2010). Effect of plant growth promoting *Pseudomonas* spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. *Plant Growth Regulation*, **62**(1), 21-30.

- Saravanan, V. S., Subramoniam, S. R., & Raj, S. A. (2004). Assessing in vitro solubilization potential of different zinc solubilizing bacterial (ZSB) isolates. *Brazilian Journal of Microbiology*, **35**(1-2), 121-125.
- Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. *Analytical biochemistry*, **160**(1), 47-56.
- Sharma, A., & Johri, B. N. (2003). Growth promoting influence of siderophore-producing Pseudomonas strains GRP3A and PRS9 in maize (*Zea mays* L.) under iron limiting conditions. *Microbiological research*, **158**(3), 243-248.
- Sharma, B. C., Subba, R., & Saha, A. (2012). Kurthia sp, a novel member of phosphate solubilising bacteria from rhizospheric tea soil of Darjeeling Hills, India. *Journal of Pharmacy and Biological Sciences*, **2**(3), 36-39.
- Shrivastava, U. P., & Kumar, A. (2011). Biochemical characterization of siderophore producing plant growth promoting rhizobacteria of rice rhizosphere. *Nepal Journal of Integrated Sciences*, **1**, 31-37.
- Singh, S., Gupta, G., Khare, E., Behal, K. K., & Arora, N. K. (2014). Phosphate solubilizing rhizobia promote the growth of chickpea under buffering conditions. *International Journal of Pure and Applied Bioscience*, **2**, 97-106.
- Somers, E., Vanderleyden, J., & Srinivasan, M. (2004). Rhizosphere bacterial signalling: a love parade beneath our feet. *Critical reviews in microbiology*, **30**(4), 205-240.
- Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS microbiology reviews*, **31**(4), 425-448.

- Srivastava, J., Chandra, H., & Singh, N. (2007). Allelopathic response of *Vetiveria zizanioides* (L.) Nash on members of the family Enterobacteriaceae and *Pseudomonas* spp. *The Environmentalist*, **27**(2), 253-260.
- Stoltzfus, J. R., So, R. M. P. P., Malarvithi, P. P., Ladha, J. K., & De Bruijn, F. J. (1997). Isolation of endophytic bacteria from rice and assessment of their potential for supplying rice with biologically fixed nitrogen. *Plant and Soil*, **194**(1-2), 25-36.
- Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X., & Song, W. (2008). Endophytic bacterial diversity in rice (*Oryza sativa* L.) roots estimated by 16S rDNA sequence analysis. *Microbial ecology*, **55**(3), 415-424.
- Thornton, H. G. (1930). The influence of the host plant in inducing parasitism in lucerne and clover nodules. *Proceedings of Royal Society. London*, **106**(742), 110-122.
- Tilak, K. V. B. R., Ranganayaki, N., Pal, K. K., De, R., Saxena, A. K., Nautiyal, C. S., ... & Johri, B. N. (2005). Diversity of plant growth and soil health supporting bacteria. *Current science*, 136-150.
- Tiwari, K. N. (2007). Site-Specific Integrated Nutrient Management for Sustainable Rice Production and Growth. *Journal of the Indian Society of Soil Science* **55**(4): 444-454.
- Tuong, T. P., & Bouman, B. A. M. (2003). Rice production in water-scarce environments. *Water productivity in agriculture: Limits and opportunities for improvement*, **1**, 13-42.
- Venkateswarlu, B., Desai, S., & Prasad, Y. G. (2008). Agriculturally important microorganisms for stressed ecosystems: challenges in technology development and application. *Agriculturally important microorganisms*, **1**, 225-246.

- Verma, S. C., Ladha, J. K., & Tripathi, A. K. (2001). Evaluation of plant growth promoting and colonization ability of endophytic diazotrophs from deep water rice. *Journal of Biotechnology*, **91**(2-3), 127-141.
- Vincent, J. M. (1970). A manual for the practical study of the root-nodule bacteria. *A manual for the practical study of the root-nodule bacteria*.
- Wahyudi, A. T., Astuti, R. P., Widyawati, A., Mery, A., & Nawangsih, A. A. (2011). Characterization of *Bacillus* sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting rhizobacteria. *Journal of Microbiology and Antimicrobials*, **3**(2), 34-40.
- Yang, J., Kloepper, J. W., & Ryu, C. M. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in plant science*, **14**(1), 1-4.
- Yanni, Y. G., Rizk, R. Y., Corich, V., Squartini, A., Ninke, K., Philip-Hollingsworth, S., ... & Schmidt, T. M. (1997). Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. In *Opportunities for Biological Nitrogen Fixation in Rice and Other Non-Legumes* (pp. 99-114). Springer Netherlands.
- Zeigler, R. S., & Barclay, A. (2008). The relevance of rice.
- Zogg, G. P., Zak, D. R., Ringelberg, D. B., MacDonald, N. W., Pregitzer, K. S. and White, D. C. 1997. Compositional and functional shifts in microbial communities due to soil warming. *Soil Science Society of America. Journal*. **23**: 123-134.

Appendices

Appendix-I

Yeast Extract Mannitol (YEM) Media (Vincent 1970)

Sl.No	Constituent	g/l
1.	Yeast Extract	1.0
2.	Mannitol	10.0
3.	K ₂ HPO ₄	0.5
4.	MgSO ₄ .7H ₂ O	0.2
5.	NaCl	0.1
6.	CaSO ₄	3.0
7.	pH	6.8

❖ 15gm/L agar was added to make plate

Appendix-II

BAz media

Constituents	gL ⁻¹
Azelaic acid	2.0
K ₂ HPO ₄	0.4
KH ₂ PO ₄	0.4
MgSO ₄ .7H ₂ O	0.2
CaCl ₂ .2H ₂ O	0.02
Na ₂ MoO ₄ .H ₂ O	0.002
FeCl ₃	0.01
Bromothymol blue	0.075
Agar -agar	2.3
pH	5.7

BAc media

Constituents	gL ⁻¹
Azelaic acid	2.0
L-citrulline	0.2
K ₂ HPO ₄	0.4
KH ₂ PO ₄	0.4
MgSO ₄ .7H ₂ O	0.2
pH	5.7

Appendix-III

Thornton's Plant Growth Medium (Thornton, 1930)

Constituents	gL ⁻¹
Ca ₃ (PO ₄) ₂	2.0
K ₂ HPO ₄	0.5
MgSO ₄	0.2
NaCl	0.1
FePO ₄	1.0
FeCl ₃	0.01
Micronutrients	1 ml/L of stock solution
pH	6.6

Micronutrient stock solution

Constituents	gL ⁻¹
H ₃ BO ₃	2.86
MnCl ₂	2.08
ZnSO ₄	0.22
Na ₂ MoO ₄	0.11
CuSO ₄	0.08

Appendix-IV

Pikovskaya's Medium (Pikovskaya, 1948)

Sl.No.	Constituent	g/l
1.	Yeast Extract	0.50
2.	Dextrose	10.0
3.	Calcium Phosphate	5.0
4.	Ammonium sulphate	0.50
5.	Potassium chloride	0.20
6.	Magnesium sulphate	0.10
7.	Manganese Sulphate	0.0001
8.	Ferrous sulphate	0.0001
9.	Agar-Agar	20

Appendix-V

Media for zinc solubilization

Sl.No	Constituent	g/l
1.	Dextrose	1.0
2.	Ammonium sulphate	0.1
3.	Potassium chloride	0.02
4.	Di-potassium phosphate	0.01
5.	Magnesium sulphate	0.02
6.	Zinc oxide	0.1
7.	pH	6.8

Appendix-VI

Aleksandrov's agar medium

Sl.No.	Constituent	g/l
1.	Magnesium sulphate	0.50
2.	Calcium carbonate	0.10
3.	Potassium alumino silicate	2.00
4.	Glucose	5.00
5.	Ferric chloride	0.005
6.	Calcium phosphate	2.00
7.	Agar-Agar	20.00
8.	pH	7.2

Appendix-VII

IAA production medium

Sl.No.	Constituent	Amount
1.	Tryptophane	100 $\mu\text{g mL}^{-1}$
2.	FeCl ₃	1.0mL
3.	Perchloric acid	50.0mL
4.	YEM Broth	25.0mL
5.	pH	6.8

Appendix-VIII

King's B agar media

Sl.No.	Constituent	Amount
1.	Proteose peptone No.3	20.0
2.	Dipotassium hydrogen phosphate	1.50
3.	Magnesium sulphate heptahydrate 1	1.50
4.	Agar	20.0
5.	pH	7.2±0.2

Appendix-IX

CAS agar media

Sl.No.	Constituent	g/l
1.	Chrome Azurol S	60.5mg/50ml d.w.
2.	Hexadecyltrimethyl ammonium bromide	72.9mg/40ml d.w.
3.	Kings Medium B base	42.23
4.	Distilled water	900ml
5.	pH	6.8±0.2

*Mix Chrome Azurol S solution and Hexadecyltrimethyl ammonium bromide solution and then add it to 10 ml of 1mM FeCl₃.6H₂O solution prepared in 10 mM HCl. Add the final solution to 900 ml of Kings Medium B base.