CHARACTERIZATION OF GROWTH PROMOTING BACTERIA FROM SPENT MUSHROOM COMPOST AND THEIR EFFICACY ON YIELD AND QUALITY OF WHITE BUTTON MUSHROOM

Thesis

by

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CERTIFICATE - I

This is to certify that the thesis titled **" Characterization of growth promoting bacteria from spent mushroom compost and their efficacy on yield and quality of white button mushroom"** submitted in partial fulfillment of the requirements for the award of degree of **MASTER OF SCIENCE** in the discipline of **MICROBIOLOGY** of Dr. Yashwant Singh Parmar University of Horticulture & Forestry, (Nauni) Solan (HP) - 173230 is a bonafide research work carried out by **Ms. Manjula Bhardwaj (F-2020-46-M)** D/o Shri Ram pal under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigations have been fully acknowledged.

Place: Nauni, Solan
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CERTIFICATE-II

" **Characterization of growth promoting bacteria from spent mushroom compost and their efficacy on yield and quality of white button mushroom"**submitted by **Ms. Manjula Bhardwaj (F-2020-46-M)** D/o Shri Ram pal to the Dr. Yashwant Singh Parmar University of Horticulture & Forestry, (Nauni) Solan (HP)- 173230 India in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in the discipline of **MICROBIOLOGY** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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Date
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CONTENTS

ABBREVIATIONS USED

%	:	Per cent
$^{\circ}C$	:	Degree centigrade
µg	:	Microgram
µl	:	Microlitre
BLAST	:	Basic Local Alignment Tool
BNF	:	Biological Nitrogen Fixation
bp	:	Base pair
CAS	:	Chrome-azurol-S
Ca	:	Calcium
CD	:	Critical Difference
CRD	:	Completely Randomized Design
Cfu	:	Colony forming units
Cm	:	Centimetre
Conc.	:	Concentrated
Df	:	Degree of freedom
DNA	:	Deoxyribose nucleic acid
dNTP	:	Deoxyribo nucleotide triphosphate
dSm^{-1}	:	Deci Siemens per meter
EC		Electrical conductivity
EDTA	:	Ethylene Diamine Tetraacetic Acid
Fe	:	Iron
g	:	Gram
h	:	Hour
Ha	:	Hectare
HCN	:	Hydrogen cyanide
IAA	:	Indole-3-acetic acid
K	:	Potassium
kg	:	Kilogram
min	:	Minute
ml	:	Millilitre
Mm	:	Millimetre
MSS	:	Mean Sum of Square
N	:	Nitrogen
NA	:	Nutrient agar
NC	:	Nutrient content

NFB	:	Nitrogen fixing bacteria
ng	:	Nanogram
NH_3	:	Ammonia
nm	:	Nanometre
NU	:	Nutrient uptake
OC	:	Organic Carbon
OD	:	Optical Density
P	:	Phosphorus
PCR	:	Polymerase Chain Reaction
PGPR	:	Plant Growth Promoting Rhizobacteria
ppm	:	Parts per million
PSB	:	Phosphate solubilizing bacteria
PSM	:	Phosphate solubilising microorganism
PVK	:	Pikovskaya's medium
RE	:	Restriction Endonuclease
RNA	:	Ribose nucleic acid
rRNA	:	Ribosomal ribose nucleic acid
rpm	:	Rotations per minute
sp.	:	Species
sec	:	Second
SMC	:	Spent mushroom compost
UPGMA		Unweighted Pair Group Method with Arithmetic Mean
UV	:	Ultra violet
Vol.	:	Volume
Var.	:	Variety
TCP	:	Tricalcium phosphate

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Chapter - 1

INTRODUCTION

The spent mushroom compost (SMC) is a valuable by-product of mushroom cultivation. It consists of partially degraded paddy or wheat straw, coconut husk, bagasse, or other agricultural waste. Compost is formed of recyclable agro-waste such as wheat straw, reed plant wastes, waste paper, oat straw, waste tea leaves, and certain water plants. After a few cycles of mushroom cultivation, it is biochemically modified by fungal enzymes into a simpler form and is enriched with protein. Due to different characteristics, the possible applications of SMC were reported as enzyme source, animal nutrition source, and its utilization in energy production, or even bioremediation and in agriculture as bio fertilizer. It is a rich source of carbon, nitrogen, and other elements. The aged SMC are a rich source of micro-organisms. The microbial diversity in SMC is greatly affected by the initial material used and its thermal pasteurization treatment. The bacteria within SMC are adapted to the harsh conditions of mushroom cultivation, they play a beneficial role in recycling compost nutrients. These adapted bacteria from spent mushroom compost need to be isolated and optimized based on growth- promoting traits for better mushroom yield.

The isolation and characterization of microbial diversity with potential growth promoting traits in SMC are of prime importance for researchers and farmers. These beneficial microbes of spent mushroom compost evaluated for plant growth-promoting activities *viz.*, phosphate solubilization, nitrogen fixation, siderophore, IAA, HCN production, and biocontrol agent. Such beneficial microbes can be utilized as efficient biofertilizers and are verified as secure and proficient methods of increasing crop yields and production, andalso help to improve the soil health. (Premachandra *et al*., 2016; Vejan *et al*., 2016). Recently numerous PGP bacterial genera such as *Azotobacter, Arthrobacter, Bacillus, Burkholderia, Klebsiella, Enterobacter*, *Pseudomonas, Serratia,* etc. have been used as biofertilizers due to their growth promoting traits, availability and safe handling as reported by various authors. (Di Benedetto *et al.,* 2017). The use of mushroom growth promoting bacteria as bioinoculant within substrate media create a beneficial metabolic and physical interdependent interaction among fungi and bacteria. Association of bacteria with mycelia of fungi increases hyphae extension, stimulate primordia formation, growth and fruiting of *Pleurotus* and *Agaricus* mushroom. Bacteria promotes the formation of beneficial bacterial

communities that produce growth-stimulating chemicals, which are thought to be implicated in the start of pin heads (Kumari and Naraian, 2021). Several studies have been published on the positive effects of casing soil microorganisms on *A. bisporus*, particularly *Pseudomonas putida* and *Alcaligenes faecalis*, *Pseudomonas putida*, initiate basidiome formation and stimulates fungal growth.

Mushrooms have been favored as food by mankind for a long time. Mushrooms supply a rich addition to the diet in the form of protein, carbohydrates, valuable salts, minerals, and vitamins. There are 2000 edible types of mushrooms, but the white button mushroom is the one that's most commonly eaten worldwide. White button mushroom (*Agaricus bisporus*) is the most popular species, widely cultivated in temperate countries and cooler regions of the tropics. It is the most frequently cultivated edible mushroom species in the world, as well as the most popular cultivar among artificially grown fungi, accounting for 31.8 percent of worldwide mushroom cultivation and 85 percent of total yield in India.

To form the fruit bodies, *A. bisporus* requires two different substrates: compost for nutrition, on which it grows vegetatively, and nutrient-deficient casing soil, in which the appropriate physicochemical/biological conditions stimulate the initiation of the pin head formation process for fruit body production. The white button mushroom (*Agaricus bisporus*) is a significant nutritional and medicinal species.It contains combination of vital amino acids, fatty acids, carbs, low calories, crude fibres, trace minerals, and vitamins which has role in human nutritional and medicinal sectors. (Owaid *et al*., 2017)White button mushrooms are the rich source of vitamin D and antioxidants. They contain certain compound which have anti-inflammatory and anti-cancerous compounds that can help to fight against chronic diseases and keep us healthy.

The cultivation of *Agaricus bisporus* (Lange) Imbach, a button mushroom, is a biotechnological technique that recycles ligninocellulosic wastes (spent substrates), which may be utilized in a variety of ways. Scientific knowledge and practical expertise are used to consistently produce good mushroom crops. The casing layer is an essential growth characteristic and a cause of variance in commercial crop output, quality, and uniformity. Farm yard manure (FYM) has been in favor in the Indian subcontinent as a casing medium for mushroom production due to its simple availability and non-availability of peat moss, which is commonly used for casing in Europe and the United States. Recent publications have emphasized the advantages of employing FYM as a casing material over other agro

industrial wastes. The casing layer must be strong and deep enough to support the growth of the mushrooms, in addition to presenting a high water absorption capacity. Additionally, the casing layer must be able to withstand frequent irrigation without losing its structure and be able to maintain good permeability to water and gases (O_2 and CO_2) the casing layer also needs to have a low salt concentration, and low nutritional value, and neutral or slightly alkaline pH. Thus, the casing layer must contain calcium carbonate and have a high cation exchange capacity. (Ghasemi *et al*., 2020)

In mushroom cultivation, the microorganisms present in the casing play a crucial role in the initiation and development of primordial and uniform distribution of sporophores, higher mushroom yield, and early cropping. There is a succession of microbial populations in the stages of mushroom cropping which is influenced by casing type affecting mushroom yield. However, isolates from SMC have not been reported and tested for its effect on mushroom yield which may have better potential.

Keeping in view the present status of knowledge, there is a need to screen bacterial diversity associated with SMS and to study their application on the growth and yield of white button mushrooms. Hence, the present investigation will be carried out with the following objectives.

OBJECTIVES

i) Isolation and screening of growth promoting bacteria from spent mushroom compost.
ii) Morphological, biochemical and physiological characterization of selected bacterial isolates.
iii) To study the effect of growth promoting bacteria on casing and substrate for yield and quality of white button mushroom.

Chapter - 2

REVIEW OF LITERATURE

The microorganisms are well recognized for its vital role in soil fertility and plant health. They provide large amount of nutrients to the plants. These microorganisms also decompose organic wastes and residues, detoxify pesticides, suppress plant pathogens, enhance nutrient acquisition and produce bioactive compounds such as hormones and enzymes (Koskey *et al*., 2017). Mushroom compost may contain beneficial microorganisms that grow in residues of straw, poultry litter and benefit mushroom growth. These microorganisms in casing play a crucial role in initiation and development of primordial, higher mushroom yield, and early completion of cropping throughout their entire life cycle. The uncultivated and farmed mushrooms interact with other microbes. These interactions necessitate unrelated combinations of physical contact (adhesion) and molecular communication via signalling molecules, chemotaxis, metabolite exchange, and conversion, all of which have an impact on the interacting organisms and their surroundings. The role of bacteria are involved in several stages of mushroom development, including substrate conversion and adaption, mycelial hyphal elongation during substrate mycelial colonisation, and stimulation of fruiting body production. Bacterial-fungal interactions have primarily been studied in the context of ectomycorrhizal fungi. (Suarez *et al*., 2020).

The current status of knowledge on the topic has been reviewed under the following heads:

2.1 Isolation of bacteria from spent mushroom compost.

Spent mushroom compost is the remaining by product of *Agaricus bisporus* and is mainly consist of a thermally treated cereal straw/animal manure mixture colonized by the fungal biomass.

Omokaro *et al.* (2013) reported species of fungal genera such as *Aspergillus* (40.9%), *Fusarium* (22.4%), *Mucor* (5.6%), *Rhizopu*s (11.6%) and *Trichoderma* (2.3%) which were associated with the substrate of mushroom compost. The bacterial species reported by them were *Bacillus* (36.67%), *Clostridium* (16.67%), *Enterobacter* (18.33%), *Escherchia* (15%) and *pseudomonas* (13.33%).

Young *et al*. (2013) studied the effect of cultivable mushroom growth promoting bacteria on *Agaricus blazei* productivity. They isolated 56 bacterial isolates from casing soil associated with *A. blazei* and reported bacterial count in *A blazei* from triplicates at 1.3X 10^6 cfu/g.

Sharma *et al*. (2015) conducted a study on isolation of compost associated microbes from rural composts and two fungal cultures were isolated in respective media and selected on the basis of qualitative and quantitative test. Qualitative screening was done on the basis of clear zone formation by growing the culture in their respective media.

Sung *et al*. (2016) isolated and characterized growth promoting rhizobacteria from button mushroom compost. They isolated an auxin-producing bacterial strain (strain 5-1) from button mushroom compost and classified as a novel strain of *Enterobacter aerogenes* based on chemotaxonomic and phylogenetic analyses. This isolate *E. aerogenes* 5-1 was confirmed to produce indole-3-acetic acid (IAA), precursor of auxin hormones, using TLC and HPLC analyses and maximum (109.9 mgL^{-1}) concentration of IAA was detected in culture broth. They further investigated the growth-promoting effects of isolate on crops and concluded that inoculated treatment had more adventitious root induction and root growth of mung bean and lettuce than those of the control.

Premalatha *et al*. (2017) isolated cellulolytic bacteria from microbial decomposed leaf litter compost and farm yard manure and these isolates were reported as high cellulase and xylanase activities. They showed that strain ACCA2 was useful for large-scale cellulase production.

Mathipriya *et al.* (2020) reported bacterial diversity in spent mushroom compost by using molecular techniques. They reported 50 bacterial isolates associated with genera *Bacillus, Paeribacillus, Exiguobacterium, Staphylococcus, Desemzia, Carnobacterium, Aethrobacter* and *Microbacterium* of bacterial division *Firmicutes* and *Actinobacteria.*

Istifadah *et al*. (2021) isolated bacteria and fungi from waste substrate of shiitake mushrooms (*Lentinula edodes*) and oyster mushrooms (*Pleurotus sp*.) Seven bacterial isolates and four fungal isolates were obtained from SMS of *L. edodes*, whereas five bacterial isolates and two fungal isolates were obtained from SMS of *Pleurotus sp.* Two isolates SB4 and SB7 found to be best and showed disease reduction.

2.2 Growth promoting traits of bacteria

Several mechanisms have been proposed for growth promoting bacteria to promote growth in plants. It includes, phosphate solubilzation, nitrogen fixation, siderophore production and biological control.

2.2.1 GPB as Phosphate solubilizer

Phosphorus (P) is the second important plant growth-limiting nutrient after nitrogen and abundantly available in soils in both organic and inorganic forms. Ironically, soils may have large reserves of total P, but the amounts available to plants is usually a very small proportion of this total (Kalayu *et al*., 2019). The low availability of P to plants is because the vast majority of soil P is found in insoluble forms, and plants can only absorb P in two soluble forms, the monobasic and dibasic (H_2PO^{4-} and $HPO4^{2-}$).The phosphate solubilizing microorganisms (PSM) make this inaccessible form of P available to plants.

Fatima *et al*. (2009) isolated seven plant growth-promoting rhizobacterial (PGPR) strains from the rhizoplane and rhizosphere of wheat. Out of 4 isolates, namely *Azospirillum* (WPR-42, WP-3), *Pseudomonas* (WPR-61) and *Azotobacter* (WPR-51) had the potential to convert insoluble P fractions into soluble form.

Mohammadi (2011) studied different phosphate solubilizing strains such as *Bacillus sp.*, *Pseudomonas sp., Burkholderia sp., Pseudomonas sp. and Flavobacterium sp*. In comparison to the uninoculated control, these bacterial strains showed substantial increases in phosphatase activity, organic phosphorus mineralization, and accessible P in soil.

Young *et al*. (2013) studied the effect of cultivable mushroom growth promoting bacteria on *Agaricus blazei* productivity. They reported that out of 56 isolates ten isolates had phosphate solubilisation ability.

Hussain *et al*. (2013) studied the five promising strains i.e, *Burkholderia sp., Bacillus sp., Pseudomonas sp., Flavobacterium sp. and pseudomonas sp.* with capability of solubilizing both organic and inorganic phosphorus. These bacterial strains showed significant increases in plant height, root length, shoot dry weight, root dry weight and grain yield over uninoculated control. Similarly, they showed significant increases in the rhizosphere phosphate activity, mineralization of organic P.

Oteino *et al.* (2015) studied the ability of endophytic bacterial isolates to produce gluconic acid (GA), solubilize insoluble phosphate and stimulate the growth of *Pisum sativum* L. plants. They reported that PGPR bacteria releasing organic acids into the soil which solubilize the phosphate complexes converting them its ortho-phosphate which is available for plant uptake and utilization.

Elias *et al.* (2016) isolated and characterized phosphate solubilizing fungi from different rhizospheres. They reported that 359 fungal isolates were obtained from haricot bean, faba bean, cabbage, tomato, and sugarcane and out of these isolates, 167 (46.52%) were solubilized inorganic phosphate.

Sharma *et al*. (2017) isolated 15 phosphate-solubilizing bacteria from the rhizospheric soil of an apple tree. Isolate An-15-Mg (*Pseudomonas aeruginosa*) showed maximum in-vitro phosphate solubilization index of 46 mm on Pikovskaya's agar plates and 76 µg/ml present in liquid assay.

Zhang *et al*. (2017) used waste mushroom leftovers residues (cotton seed hulls, corn cob, and biogas) for isolation of bacteria. They found that all the bacterial strains were phosphate solubilizing and ranged from 17.31–60.87 µg/l.

Saleemi *et al*. (2017) conducted experiment on integrated effect of plant growth promoting rhizobacteria and phosphate-solubilizing microorganisms on the growth of wheat (*Triticum aestivum L*.) under rainfed condition. They isolated 63 bacterial strains as PGPR and seven as phosphate-solubilizing microbes (PSM). The P-solubilization was found to be in range of 25 to 130.1 µg/ml.

Kour *et al*. (2019) isolated 86 rhizobacterial isolates from various cereal crops. They found 48 isolates as drought adaptive and P-solubilizers. The EULWNA-33 strain of *Pseudomonas libanensis* showed potential plant growth-promoting abilities and was able to solubilize a significant amount of P in water-deficient conditions.

Elhaissoufi *et al*. (2020) studied phosphate solubilizing rhizobacteria had a stronger influence on wheat root traits. They reported that 5 contrasting PSB (*Pseudomonas sp.*) isolates increased wheat root traits, particularly PSB5 which increased root biomass and PSB3 that had greater effect on root diameter.

Dewi *et al*. (2020) isolated and characterized PGPB from coconut coir dust, which includes phosphate solubilization, IAA generation, protease enzyme and siderophore production. They reported that out of 40 isolates only 5 isolates have shown positive result of phosphate-solubilizing activity.

Lellapalli *et al.* (2021) isolated phosphate-solubilizing rhizobacteria from rice (*Oryza sativa*). They reported that 16 strains out of 52 had phosphate solubilizing activity of which 4 potential isolates were evaluated for morphological and biochemical analysis. Out of 4 isolates PCPSMR15 isolate showed promising plant growth-promoting traits.

2.2.2 GPB as nitrogen fixers

Nitrogen (N) is one of the main plant nutrients, becoming a limiting factor in agricultural ecosystems due to heavy losses by rainfall or mineral leaching. Number of PGPR Strains such as *Azoarcus* sp*., Beijerinckia* sp*., Klebsiella pneumoniae, Pantoeaag glomerans* and *Rhizobium* sp. reported to fix atmospheric nitrogen in soil and make it available to plants (Bashan and De-Bashan, 2010).

Jha *et al.* (2013) studied the role of *Bacillus pumilus* and *Pseudomonas pseudoalcaligenes* on paddy rice (*Oryza sativa L.*) under greenhouse conditions and reported that the PGPR inoculated plants were increase the concentrations of N (26%), P (16%), K (31%), and reduced concentrations of Na (71%) and Ca (36%) as compared to non-inoculated control plants under saline conditions. It also showed the significant increases in the shoot length (up to 60%), shoot dry weight (up to 33%) and the grain yield (up to 26%) of rice.

Gupta *et al*. (2015) studied the non-symbiotic nitrogen fixation by free living diazotrophs and can stimulate the growth of radish and rice plants. They reported that genera *Acetobacter, Azotobacter, Azospirillum, Burkholderia, Enterobacter, Gluconacetobacter, Pseudomonas, Anabaena, and Nostoc* include non-symbiotic nitrogen-fixing bacteria. A comprehensive approach to disease management is provided by the inoculation of crops with non-symbiotic bacteria, which also help to keep agricultural nitrogen levels stable.

Gopalkrishan *et al.* (2017) isolated 11 rhizobia from the nodules of chickpea and characterized for nitrogen fixation potential and growth promoting ability. Only 4 isolates (ICKM-9, ICKM-15, ICS-31 and ICS-32) were found to fix nitrogen more than 4.0 n moles.

Korir *et al.* (2017) studied the effect of *Paenibacillus polymyxa, Bacillus megaterium*), IITA-PAU 987 and IITA-PAU 983. They reported that co-inoculation of isolated PGPR and reference strains rhizobia (CIAT 899) have a synergistic effect on bean growth and improved effectiveness of *Rhizobium* biofertilizers for common bean production.

Singh *et al*. (2020) studied the diversity of nitrogen fixing rhizobacteria associated with sugarcane. They reported that 350 different bacterial strains isolated from rhizospheric soil of sugarcane and 22 isolates were selected based on their plant growth promotion traits, biocontrol, and nitrogenase activity. They further concluded that *Bacillus* isolates have N-fixation and biocontrol property against two sugarcane pathogens *Sporisorium scitaamineum* and *Ceratocystis paradoxa.*

2.3 GPB as siderophore producers

Iron is an essential growth element for all living organisms. Iron is a cofactor for large number of enzymes and iron chelating proteins; therefore it is the most important micronutrient used by bacteria and is essential for their metabolism. PGPR produce low molecular weight compounds called siderophores to reduce iron deficiency. Plants assimilate iron from bacterial siderophores by means of different mechanisms, for instance, chelate and release of iron, the direct uptake of siderophore-Fe complexes or by a ligand exchange reaction. Plant growth promotion and siderophore mediated Fe-uptake as a result of siderophore producing rhizobacterial inoculations have been reported (Rajkumar *et al*., 2010).

Duhan (2013) studied the role of siderophore production in nitrogen fixation and iron uptake in pigeon pea (*Cajanus cajan*). He reported that 9 rhizobial mutants overproduced siderophores (0.68 to 8.05µg of hydroxamate N/ mg protein) as compared to wild type (2.2 µg of hydroxamate N/mg of protein.

Chakraborty and Parmar (2016) reported that siderophore producing bacterial and fungal strains had a potential in growth promotion due to low solubility of iron in the soil. The siderophore producers, chelate iron and make it available to the plant. They reported that *Pseudomonas fluorescens* was able to produce water soluble yellow green siderophore in succinate medium. Siderophore production was also proved to be useful for plant growth promotion due to increase in root length, shoot length and number of leaves of leguminous plants like *Lens Culinaris* and *Phaseoluslunatus* when grown under iron limiting conditions with siderophore supplements.

Zhang *et al*. (2017) isolated 13 bacteria from waste mushroom leftovers residues and found 6 isolates to be siderophore producers. The three strains M05, M06, M08 being highest siderophore producers.

Batool and Iqbal (2018) isolated phosphate solubilizing bacteria, a total of 30 isolates obtained from various plants rhizosphere out of which 10 best phosphate solubilizing strains were selected on the basis of their high P solubilization and good plant growth promoting traits (auxin, siderophore production, HCN production and Nitrogen fixation). The phosphate solubilization ability of selected bacterial strains was in the range of 30–246 mg/ml in liquid broth and 4–7 solubilization index on agar plate.

Bagmare *et al*. (2019) tested laboratory stock cultures of plant growth promoting bacteria for siderophore production. They reported that 8 PGPR isolates and out of these 7 were showed positive result for siderophore production. However, they reported that *Pseudomonas florescence* (75%) and *Azospirullum lipoferum* (67%)being highest siderophore producers.

Subramanium *et al.* (2020) isolated *Pseudomonas aeruginosa* PSA01 and *Pseusomonas fluorescence* PSF02 from rhizosphere of agricultural soil in Salem district of Tamil Nadu. Both isolates proved to produce siderophore and solubilized phosphate. They showed that the isolates *P. fluorescens* (62.8 percent) and *P. aeruginosa* (60.5percent) are powerful producers with limited iron.

Dewi *et al*. (2020) isolated and characterized PGPB from coconut coir dust, which includes phosphate solubilization, IAA generation, protease enzyme and siderophore production. They reported that out of 40 isolates only 8 isolates have shown positive result of siderophore production.

Kumari *et al.* (2021) isolated siderophore producing bacteria from the rhizospheric soil of *Eragrostic cynosuroides* by CAS agar screening and CAS shuttle assay method. Among 5 isolates, DR2 produced a high level of siderophore (69.81 SU%) and identified as *Bacillus subtitlis* DR2 (KP455653) based on 16S rRNA gene sequencing and phylogenetic analysis. They reported that the strain *B.subtilis* DR2 showed more siderophore yield (80.60 SU %) under optimized condition.

2.4 GPB as bio control agents

Plant growth promoting bacteria (PGPB) offers promise for establishing environment friendly sustainable agriculture systems and as a notable alternative to these harmful chemicals due to their wide range of direct and indirect mechanisms of plant growth promotion. Indirect mechanisms, which involves plant growth promotion through disease suppression. Disease suppression mechanisms include antibiosis, Induced Systemic Resistance (ISR), high affinity siderophore production, and production of lytic enzymes.

Harish *et al.* (2009) performed an experiment in which plant growth-promoting rhizospheric and endophytic bacterial strains were used to induce systemic resistance against BBTV in tissue-cultured banana plantlets. Co-inoculation of *Pseudomonas fluorescens* strain Pf1 and strain CHA0 (rhizobacteria) and *Bacillus subtilis* strain EPB22 (endophyte) showed reduction of infection by 80 per cent over control plants.

Sharma and Kaur (2010) isolated *Pseudomonas* and *Bacillus* rhizobacterial species from carnation to evaluate their growth promoting effects on carnation as to select and developed more efficient indigenous plant growth promoting and disease suppressing bioagents. They reported that inoculation with best selected isolates resulted in 40 per cent reduction in diseases incidence as compared to control.

Sundaramoorthy and Balabaskar (2012) isolated the strains of *Bacillus subtilis* (EPCO16 and EPC5) and *Pseudomonas fluo*rescens (Pf, Py15 and Fp7) individually and in combination for their effectiveness against early blight of tomato incited by *Alternaria solani* under controlled conditions. The results revealed that the strains of *Bacillus subtilis* and *Pseudomonas fluorescens* were compatible. Their application was found to effectively inhibit the mycelial growth of the pathogen and promote the growth of tomato seedlings in comparisons to individual strains application.

Tan *et al.* (2013) isolated two tomato root colonizing strains, *Bacillus amyloliquefaciens* CM-2 and T-5, and evaluated for their antagonistic activities against pathogenic *Ralstonia solanacearum* (RS) in roots of tomato seedlings. They showed favorable responses for the formation of ammonia, indole acetic acid, siderophores, and phosphate solubilizing activity. The data demonstrated the isolated strains' potential for biocontrol of tomato bacterial wilt.

Kumar *et al.* (2014) screened *Pseudomonas* and *Bacillus* sp. i*n-vitro* for abiotic stress tolerance, plant growth promoting and biocontrol activity. They found P59 strain possessed promising antagonistic activity and drought tolerance. The magnitude of antagonism shown by *Bacillus* isolates was also higher when compared to *Pseudomonas* strains.

Dinesh *et al*. (2015) isolated 100 PGPR strains from rhizospheric soil of ginger (*Zingiber officinale* Rosc.). They examined PGPR strains for inhibition in vitro for *Pythium myriotylum*, which causes soft rot in ginger. They reported that 5 PGPR demonstrated a suppression of *P. myriotylum* that was greater than 70percent, according to the findings. These 5 PGPR were employed for further growth promotion and biocontrol investigations in the greenhouse and field and were identified as *Burkholderia cepacia, Bacillus amyloliquefaciens, Serratia marcescens, S. marcescens*, and *Pseudomonas aeruginosa.* Overall, they concluded that *B. amyloliquefaciens* and *S. marcescens* might serve as effective substitutes for chemical treatments in the growth promotion and management of soft rot disease in ginger.

Laid *et al*. (2016) isolated 93 isolates to evaluate their biocontrol properties against *Fusarium culmorum*. They selected 4 isolates (D2, D5, D8, and AST1) through positive effect of PGPR traits and biocontrol characters in bread wheat (*Triticum aestivum* L.) such as inhibition of pathogenic fungi when treated with selected isolates during seed germination.

Islam *et al.* (2016) studied the ability of PGPR to suppress *Phytophthora* crown rot in cucumber. They isolated 66 bacterial isolates, and 10 were chosen because they had characteristics that favoured plant development and were antagonistic to several phytopathogens. The *Phytophthora* crown rot caused by *Phytophthora capsici* was reduced in cucumber seeds treated with these bacterial isolates.

Paramanandham *et al.* (2017) isolated *Pseudomonas aeruginosa* isolates *viz*., JO and JO7 from the rhizosphere soil and evaluated for the growth promotion traits, germination percentage, shoot and root length and disease resistance in tomato (*Solanum lycopersicum* L.). They found that isolates were capable of providing significant improvement in growth of plants and successfully suppressed disease severity of *Fusarium oxysporum* and *Alternaria solani* in pot experiments.

Sharma *et al.* (2017) isolated fifteen *Pseudomonas* sp. strains capable of phosphate-solubilizing bacteria from the rhizospheric soil of an apple tree. Twelve isolates showed

positive results for HCN production and reported maximum HCN production in isolate named An-15-mg of these fifteen isolates.

Zulphikar *et al.* (2018) isolated antagonistic bacteria from spent mushroom compost and evaluated its effect for damping off disease in tomato. They reported that *Bacillus subtillus* from compost control the disease by 75 percent.

Soufi *et al*. (2020) selected effective *Bacillus amyloliquefaciens* Bc2 and *Trichoderma harzianum* against anthracnose disease in strawberry cultures (*Fragaria ananassa Duch*.).They reported that strains *B. amyloliquefaciens* Bc2 and *Trichoderma harzianum TR* are also efficient biological control agents for grey mould, and powdery mildew on strawberries cultivated in the field. Moreover, they have also been found to boost the growth of the strawberry plants and the amount of fruits produced.

Kanjanasopa *et al*. (2021) carried out research to assess the PGPR as biocontrol agents in Rice. They investigated the use of PGPB (*Parabur kholderia sp*.strain) SOSO3 to provide disease protection in rice (*Oryza sativa L.)*. They showed that SOSO3 inhibits the growth of *Curvularia lunata, Rhizobacteria solani, Pyricularia oryzae, Helminthosporium oryzae,* and *Fusarium moniliforme* by 17.2%, 1.1%, 8.3%, 32.5%, and 35.4%, on plate assay respectively.

2.5 Growth promoting bacteria as IAA production

Indole-3-acetic acid (IAA) is the main member of auxins family produced by plants as it plays an important role in a number of plant activities such as leaf formation, embryo development, root initiation and development, abscission (falling of leaves), phototropism, geotropism, fruit development, etc. IAA helps in the enhancement of root length with increase in number of root branches, root hairs and root laterals that aid in uptake of nutrients from surrounding. Since IAA has been found to be very important for plant growth and development, extensive studies have been performed on IAA after its discovery as a plant hormone. It has been found that different bacteria, fungi and algae are capable of producing physiologically active amounts of IAA. (Chandra *et al*., 2018).

Ashraf *et al*. (2011) isolated twelve bacterial strains from root of sugarcane growing in different areas. They identified 10 isolates as *Pseudomonas* and 2 isolates as *Azotobacte*r. The *Pseudomonas* strain A17 was highest IAA (4.49 mg/l) producer followed by strains A4

and A11, also the *Azotobacte*r strains Azoto1 and Azoto2 were capable of producing IAA of amount 0.2 and 0.1 mg/l respectively.

Lenin and Jayanthi (2012) isolated 20 PGPR strains with 5 strains from each species i.e., *Azospirillum lipoferum, Azotobacter chroococcum, Pseudomonas fluorescens,* and *Bacillus megaterium* from the rhizosphere of *Catharanthus roseus.* All the isolates were IAA producers with *Azospirillum lipoferum* strain CRAS-2 (74.2 µg 25 ml^{-1} of broth) being highest IAA producer.

Goswami *et al*. (2013) reported *Pseudomonas* spp. olive green (OG) as a plant growth-promoting bacteria (PGPB). The isolate was evaluated for multiple plant growth-promoting traits and IAA production of 29 mg ml^{-1}.

Dasgupta *et al*. (2015) isolated and screened 12 rhizobacterial strains i.e., DD1 to DD12 from the rhizosphere soil of Dhaincha (*Sesbania bispinosa)*. After screening they observed 7 isolates as positive Indole-3-acetic acid (IAA) producers.

Baggam *et al*. (2017) studied the agricultural soil and forest soil for isolation and screening of Indole Acetic Acid producing bacteria. 6 bacterial isolates namely I1, I2, I3, I4, I5, I6 were tested for IAA for 1, 4, 6, 8 days. They reported that all the isolates showed IAA production ranging between 0 to 33µg/ml. They further reported that isolate I4 (27.1µg/ml) and I6 (33.5µg/ml) being highest producers.

Zhang *et al*. (2017) isolated 13 bacterial isolates from mushroom residues and found 8 isolates to be indole acetic acid producers ranging between 8.06–62.43 mg/l and strain M07 was highest IAA producer.

Chandra *et al*. (2018) isolated 20 rhizobacterial isolates from *Stevia rebaudiana* rhizosphere and screened 3 isolates for higher IAA production. The isolate CA1001 produced maximum IAA of 91.7µg ml^{-1} at optimised temperature of 37ºC and also achieved higher IAA production of 104µg ml^{-1} with dextrose (1%) as carbon source, followed by isolate CA2004 which showed higher yield of IAA in ammonium nitrate supplemented media, and beef extract as supplements followed by isolate CA2003.

Mike-Anosike *et al*. (2018) investigated the production of indole acetic acid by soil bacteria and screened six species of bacteria and identified as *Staphylococcus* sp (RSS1),

Micrococcus sp. I (RSS2), *Micrococcus* sp. II (RSS3), *Bacillus* sp I (RSS4), *Bacillus* sp II (RSS5) and *Pseudomonas* sp. (RSS6).They reported that the concentrations of IAA produced by the bacterial isolates ranges from 4.0mg/L by *Pseudomonas* sp. to 10.0mg/L by *Micrococcus* sp. I (RSS2).

Albdaiwi *et al*. (2019) isolated 74 halotolerant bacterial isolates from rhizosphere and endorhizosphere of durum wheat (*Triticum turgidum* subsp. durum). Out of 35 bacterial strains, Only 10 strains were able to produce levels of IAA above 10 µg/ml, while 15 produced levels less than 5 µg/ml.

Dewi *et al*. (2020) isolated and characterized PGPB from coconut coir dust, which includes phosphate solubilization, IAA generation, protease enzyme and siderophore production. They reported that out of 40 isolates only 4 isolates have shown positive result of IAA production of 28 ppm by High Performance Liquid Chromatography analysis

Suliasih *et al*. (2021) isolated and screened IAA producing bacteria from peat land area in West Kalimantan and optimized some culture conditions for maximum IAA production. They isolated 19 isolates for IAA producing bacteria with the amount ranging between 2.88-5.14 µg/ml. They further found that maximum IAA producing bacteria was *Bacillus siamensis*.

Yeom et al., (2021) studied Plant growth promoting traits of bacterial strains solated from white button mushroom (*Agaricus bisporus).* they reported that out of twenty one bacterial strains, all strains produced IAA ranging from 20µg/ml to 250µg/ml.

2.6 OPTIMIZATION OF CULTURAL CONDITIONS FOR BACTERIAL ISOLATES

Growth of bacteria influenced by the component of medium and physical facto such as pH, temperature, agitation, dissolved oxygen and inoculum density. The activity and stability of an organism is influenced by the type of strain, cultivation conditions and growth medium composition. Temperature is a vital environmental factor which controls the growth and production of metabolites by microorganism and usually varies from one organism to another (Kumar *et al*., 2017). The pH of the medium is influenced by the growth of microorganism and plays an important role in terms of inducing enzyme production and morphological changes in the microbes (Kathiresan and Manivannam, 2006). Numerous

factors such as dry season, corrosiveness, alkalinity, saltiness can influence the microbial growth.

Rasul *et al*. (2012) reported high phenotypic and genetic diversity among rhizhobial symbiotic with *Millettia pinnata*. They isolated 29 isolates from *P. pinnata* nodules, and showed that they can grow in a pH range of 4.0-10.0 and a temperature range of 35°C to 45°C.

Pawar *et al*. (2014) studied the temperature and pH tolerance of root nodulating bacteria isolated from Soybean (Glycine max. L.) root nodules. They reported bacterial isolates were grown best at 36°C, while rhizobia grown best at pH 6.5 to 7.5.

Chandra *et al*. (2018) isolated 20 isolates from rhizosphere of *Stevia rebaudiana* and screened them for IAA production. Selected 3 isolates with higher IAA production were further optimized for pH and showed maximum IAA production at pH 5, temperature 37ºc and growth in specific medium supplemented with carbon and nitrogen sources. Isolate CA1001 showed best result under these optimized conditions with overall higher IAA yield.

Rana *et al.* (2017) isolated and screened the amylase producing bacteria from spent mushroom compost. There result showed that high amylase production was obtained with apple pomace after 72 h of incubation, at pH 9.0 and temperature of 45°C.

Kumari *et al.,* (2021) studied the importance of pH for solubility of iron and efficient production of siderophore. There result showed that maximum siderophore production (75.80 %) was obtained at pH 8.0.

2.7 MOLECULAR CHARACTERIZATION OF GROWTH PROMOTING BACTERIA

Zarenejad *et al*. (2011) studied evaluation of indigenous potent mushroom growth promoting bacteria (MGPB) on *Agaricus bisporus* production. Out of 23 bacterial strains,2 bacterial strains were selected (Bt4 and Ps7) and were identified by 16s rRNA gene sequencing as *Pseudomonas putida.*

Young *et al*. (2013) studied the effect of cultivable mushroom growth promoting bacteria on *Agaricus blazei* productivity. A total of 56 *A. blazei* -associated bacterial isolates were obtained from casing soil and identified by 16S rRNA gene sequencing. Bacterial isolates were identified as *actinobacteria* (60%), *firmicutes* (20%), and *proteobacteria* (20%).

Kuan *et al*. (2015) isolated PGPR strains from maize roots under greenhouse conditions. These strains were identified as *Klebsiella* sp. Br1, *Klebsiella pneumonia* Fr1, *Bacillus pumilus* S1r1 and *Acinetobacter* sp. S3r2 and a reference strain used was *Bacillus subtilis* UPMB10.

Dasgupta *et al*. (2015) isolated Indole-3-acetic acid producing, phosphate solubilising bacteria from the rhizospheric soil of Dhaincha *(Sesbania bipinosa*). They identified best 3 isolates by 16s rRNA partial gene sequencing as *Escherrchia coli, Psedomonas fluorescens, Burkholderia sp*

Dinesh *et al*. (2015) isolated a total of 100 PGPR strains from rhizospheric soil of ginger (*Zingiber officinale* Rosc.). They identified these strains with 16S rDNA sequencing, all the strains were identified as *Burkholderia cepacia, Bacillus amyloliquefaciens, Serratia marcescens, S. marcescens*, and *Pseudomonas aeruginosa* as inhibitor of *Pythium myriotylum.*

Habib *et al.* (2016) studied molecular characterization of stress tolerant plant growth promoting rhizobacteria (PGPR) for growth enhancement of rice. They identified two isolates UPMR7 and UPMR17 based on 16s rRNA gene sequences as *Bacillus* sp and *Citrobacter* sp.

Stanojevic *et al*. (2016) isolated 108 isolates from samples of straw and chicken manure compost at various stages of the composting process and casing soil used for growing button mushrooms. All 23 bacterial isolates were characterized as Gram-positive and catalase-positive. They identified that bacterial strains belong to *Bacillus subtilis*, *B. amyloliquefaciens, B. licheniformis and B. pumilus species*.

Singh *et al.* (2017) isolated growth promoting bacteria from the Rhizosphere of Vegetable Crop *Momordica charantia*. They identified bacterial strains as *Bacillus, Azotobacter, Pseudomonas and Acinetobacter*. Nearly 50% isolates of Pseudomonas and Acinetobacter.

Santosa *et al*. (2018) studied Molecular characterization of Plant Growth Promoting Rhizobacteria using 16S rRNA sequences in the organic rice field. They identity that the nine isolates were of *Pseudomonas aeruginosastrain* RI-98-1, *Stenotrophomonas maltophilia* strain S431, *Bacillus subtilis* strain CEB2, *Bacillus cereus* strain ATCC 14579 clone EA195, S. *maltophilia* strain 5517, *Exiguobacterium acetylicu*m strain

SSA-3 , *Serratia nematodiphilastrain* HC4, Bacillus cereus strain ANP221, and *Acinetobacter junii.*

Kumari and Naraian (2020) isolated rhizospheric soil bacteria from different crops and scrutinized 2165 bacterial isolates. They obtained 26 isolates that showed growth-promoting attributes with oyster mushroom of which strain identified as *Glutamicibacter arilaitensis* MRC119 with 16S rRNA was most efficient.

Lellapalli *et al*. (2021) isolated phosphate solubilisng rhizobacteria from rice (*Oryza sativa)* .They identified the isolate on the basis of 16S rRNA gene sequencing as genus *Lysinibacllus pakistanensis* PCPMR15.

Kumari *et al.* (2021) isolated siderophore producing bacteria from the rhizospheric soil of *Eragrostic cynosuroides.* They identified the isolate as *Bacillus subtitlis* DR2 based on 16S rRNA gene sequencing and phylogenetic analysis.They also found that isolate was positive for oxidase, citrate, ammonia, Voges-Proskauer reaction and starch hydrolysis.

3. Effect of growth promoting bacteria on the growth and yield characteristics of white button mushroom

Kim *et al.* (2008) reported that Pseudomonas sp. P7014 enhanced the growth of edible oyster mushroom *P.eryngii*.

Riahi *et al*. (2011) studied effect of bacterial and cyanobacterial culture on growth, quality and yield of *Agaricus bisporus*. They inoculated *pseudomonas spp* and cyanobacteria into the casing soil. They found was a significant difference of mushroom yield as compared to the control. They further reported that Inoculation of cyanobacteria into the casing soil significantly increased mushroom yield and quality.

Ebadi *et al* (2012) studied the effect of plant growth promoting bacteria on morpho physiological properties of button mushroom. They reported 12.7 per cent high yield of mushrooms with PGPR in comparison to uninoculated control.

Zarenejad *et al.* (2012) studied the evaluation of indigenous growth promoting bacteria (MGPB) on white button mushroom. They studied 274 isolates and out of them 23 strains were selected as potent mushroom growth promoting bacteria (GPB) for inoculation of casing layer. They reported that best three bacterial strains Bt4, Fd8, and Ps7 increases the mushroom production with 14%, 13.4% and 12% increase, repectively.

Mollayi and Besharati (2012) studied effects of plant growth promoting rhizobacteria (PGPR) on some quality and quantity traits of button mushroom *(Aaricus bisporus)* in different industrial and agricultural wastes as growth beds. They inoculated the beds with mixture of *Bacillus* and *pseudomonas,* except the uninoculated control. They further reported that the highest fresh weight (751.5 gr $plot^{-1}$) were measured in the bed with PGPR inoculation whereas lowest (592.6 gr $plot^{-1}$) was found in the bed without inoculation.

Young *et al*. (2013) studied the effect of cultivable mushroom growth promoting bacteria on *Agaricus blazei* productivity. A total of 56 *A. blazei* -associated bacterial isolates were obtained from casing soil and identified by 16S rRNA gene sequencing. Bacterial isolates were identified as *actinobacteria* (60%), *firmicutes* (20%), and *proteobacteria* (20%). They reported that the ten isolates had phosphate-solubilisation ability, eight showed nitrogen-fixation capability, and 12 isolates promoted. *A. blazei* mycelium growth. They further reported that bacterial inoculation increases fresh mushroom yield up to 215 per cent and increases total polysaccharide protein content two-fold in comparison to uninoculated control.

Khalili *et al.* (2013) studied the effect of plant growth promoting rhizobacteria on yield and quality of button mushroom. They inoculated casing with suspensions of the PGPR *Bradyrhizobium japonicum, Rhizobium leguminosrum*, and *pseudomonas putida* and compared to a non-inoculation control. They reported that *Pseudomonas putida* inoculation in casing and substrate increased button mushroom yield and quality.

Prabhat *et al*. (2013) reported application of *Pseudomonas putida* (1x 10^8 cfu/ml) in casing was found to be high yielding with 28 percent biological efficiency of white button mushrooms.

Ahlawat and Manikandan (2015) studied evaluation of bacterial innoculants for yield enhancement of white button mushroom. They reported that mixing of *A.faecalis* and B subtilis in FYM + SMS based casing materials increases the fruit body yield of *Agaricus bisporus* compared to uninnoculated casing soil treatments.

Mohammad and Sabba (2015) reported 26 percent increase in mushroom yield when inoculated with *Pseudomonas putida* in comparison with non innoculated sets.

Febriyansyah *et al*. (2018) studied about the potency of growth promoting bacteria on mycelial growth of edible mushroom *Pleurotus ostreatus.* They scrutinized 19 bacterial isolates from *P. ostreatus* and found 3 isolates to be effective in increasing the mycelial growth when treated with the *Pleurotus ostreatus* and identified them as strains *Bacillus cereus* strain ATCC 14579, *Bacillus aryabhattai* strain B8W22 and *Acinetobacter pittii* strain ATCC 19004 with 16S rDNA sequencing.

Helay *et al.* (2020) studied effect of PGPB strains i.e., *Serratia marcescens* (AP-4), *Pseudomonas poae* (AP-19), *Plantibacter flavus* (AP-21), and *Bacillus amyloliquefaciens* subsp. *plantarum* (AP-303) on collard plant (*Brassica oleracea* var. *acephala*). The treatment with strain AP-303 revealed highest improved vegetative growth, pigment contents, nutritional values over control.

Kumari and Naraian (2020) isolated 2165 bacterial isolates .Among them 26 isolates found exhibiting positive traits of mushroom growth promotion. They reported 58 percent increase in yield of oyster mushroom (*P. florida*) when treated with growth promoting bacteria *Glutamibacter arilaitensis* MRC119.

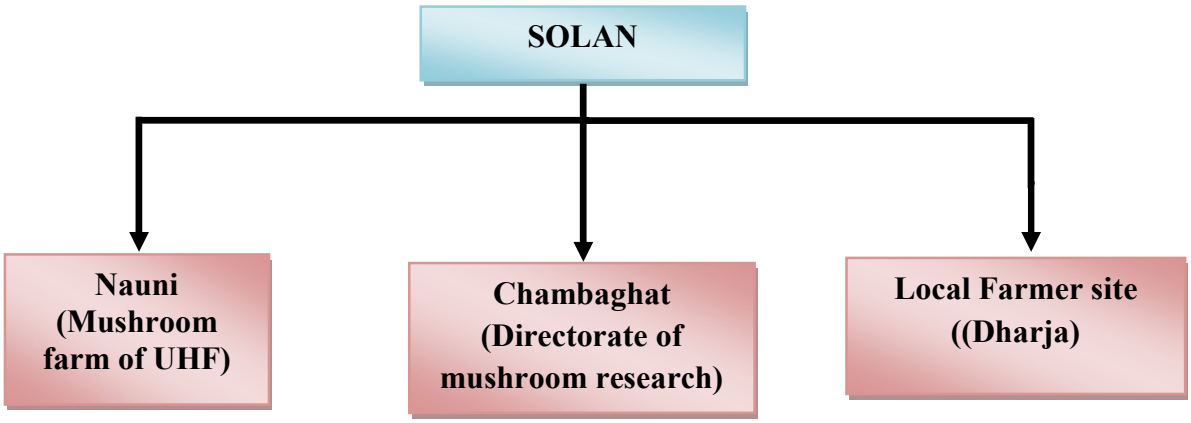
Chapter - 3

MATERIALS AND METHODS

The present study entitled **'Characterization of Growth promoting bacteria from spent mushroom compost and their efficacy on yield and quality of white button mushroom'** will be carried out in Laboratory of Basic sciences and also in Mushroom farm under Lab conditions of Dr YSP university of Horticulture and Forestry, Nauni Solan, HP During 2020-2022. The studies were conducted to select best plant growth promoting rhizobacteria from spent mushroom compost and to study their response in the improvement of growth and yield of white button mushroom.

3.1 COLLECTION OF SAMPLE

The samples of spent mushroom compost were collected from different locations/sites of distt Solan (HIMACHAL PRADESH) .the three different sites are Chambaghat (1502 amsl), Nauni (1250 amsl), and from local farmer of district Solan. The mushroom compost were produced after 3-4 mushroom harvest. Samples were placed in plastic sbags and stored in laboratory of Department of Basic Sciences for further isolation and for carrying on the analytical work.



Number of sites = 3

Replications = 3

Statistical design = RBD

3.2 MEDIA USED FOR ISOLATION OF BACTERIAL COUNT

Composition of the media (Atlas, 1995) used for the study are as follows:

3.2.1 Nutrient Agar (NA) Medium

Constituents	**:**	**quantity/litre**
Beef extract	:	3g
Peptone	:	5g
NaCl	:	5g
Agar	:	20g
pH	:	6.8± 0.01

3.2.2 Pikovskaya's (PVK) Broth

Glucose	:	10g
$Ca_3 (PO_4)_2$	:	5g
$(NH_4)_2SO_4$	:	0.5g
KCl	:	0.2g
$MnSO_4.7H_2O$	:	0.1g
$MnSO_4$	:	Trace
$FeSO_4$	:	Trace
Yeast Extract	:	0.5g
Bromocresol Purple	:	0.01%
pH	:	6.8±0.1

3.2.3 Pikovskaya's (PVK) Agar

Pikovskaya's Broth + 20g Agar/Litre

3.2.4 Jensen's medium (N-Free medium)

K_2HPO_4 anhydrous	:	1g
$MgSO_4.7H_2O$	:	1g
NaCl	:	0.5g
$FeSO_4$	:	0.1g
Sucrose	:	20g
$Ca(CO_3)_2$	:	2g
Agar	:	20g
pH	:	6.8±0.1

3.2.5. Chrome-azurol-S agar

CAS	:	0.06g
HDTMA	:	0.07g
HCl	:	0.02g
FeCl3	:	0.2g
Agar	:	20g
pH	:	6.8±0.1

3.2.6. Soil Extract Medium

Glucose	:	1.5g
K_2HPO_4	:	0.5g
Agar	:	20g
Soil extract	:	100ml
pH	:	6.8 ± 0.01

3.3. CHEMICALS AND REAGENTS

Analytical grade (AR) chemicals and reagents procured from standard company were used for different experiments under present investigation.

3.4. MICROBIOLOGICAL METHODS

3.4.1 Sterilisation

Glasswares used during the work were washed thoroughly in detergent water, running tap water followed by rinsing in distilled water. Glasswares were sterilized in hot air over at 180ºC temperature for 20 min. All the media , water blanks etc. were sterilised in autoclave at 15 pounds per square inch pressure of pure steam for 20 min. Laminar air flow chamber was sterilised by disinfectant followed by ultra violet (UV) radiation for 30 min before start of the work.

3.4.2 Isolation and enumeration of bacteria from organic sources of nutrients

3.4.2. Isolation of bacteria

One gram of sample (spent mushroom compost) were dissolved in 9 ml of sterilized distilled water and serial dilutions were made up to 10^{-8} under aseptic conditions. The serially

diluted suspensions of samples were spread on pre-poured nutrient agar medium and also on the selective media: Nitrogen free medium (Jensen, 1987) for nitrogen fixing activity, Pikovskaya medium (Pikovskaya, 1948) for phosphate solubilizing 37 ability as described by Subba Rao (1999). The petri plates were incubated for 24 - 48 h at 35 ± 2˚C temperature in incubator. The microbial count appeared on the plate was expressed as colony forming unit (cfu) per gram/ml of sample (spent mushroom compost)

3.5 MAINTENANCE OF THE CULTURES

The isolated cultures were purified by streak plate method and maintained on the slants of respective medium at 4°C in refrigerator. Bacterial cultures were maintained on nutrient agar at 4°C. Sub-culturing of bacterial cultures was done once in fortnight on respective medium at incubation temperature of 28 ± 1°C.

3.5.4 Measurement of growth

3.5.4.1 Preparation of inoculum

A bacterial cell suspension (OD 1 at 540 nm) of 48 h old culture grown on nutrient broth at the rate of 10 per cent was used as inoculum in all experiments, unless mentioned otherwise.

3.5.4.2 Turbidity

Growth was monitored by measuring the change in absorbance of the inoculated broth at 540 nm using un-inoculated broth as blank.

3.5.4.3 Viable counts

Appropriate dilutions of bacterial cell suspension were used to seed NA plates. The number of viable cells in the initial population was obtained by counting the number of colonies that developed after incubating the plates and multiplying this figure by dilution factor.

$$\text{Colony forming unit (cfu/g)} = \frac{-\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of culture plate}}$$

3.6 SCREENING OF BACTERIAL ISOLATES ON THE BASIS OF PLANT GROWTH PROMOTING TRAITS

The screening of the bacterial isolates for various plant growth promoting activities like P-solubilization, growth on N-free medium, siderophore production, HCN, and auxin

production(IAA) were performed by adopting the standard methods. The brief descriptions of these methods are as follows:

3.6.1 Phosphate solubilizing activity

Each of purified isolate were streaked in a straight line on PVK medium as described by Pikovskaya (1948) and was incubated for 72 h at 30 ± 2°C. Colonies showing solubilization halos (>0.1mm diameter) were selected.

3.6.2 Nitrogen fixing activity

Each pure isolate was streaked in a straight line on Jensen's medium and cultured for 72 to 120 hours, with the plates that showed bacterial colony growth being chosen.

3.6.3 Siderophore production

The CAS plate assay method was used to measure siderophore production (Schwyn and Neilands, 1987). CAS (60.5 mg/50 mL distilled water) was mixed with 10 mL iron solution to make sterilised blue agar (1 mM $FeCl_3.6H_2O$ in 10 mM HCl). The hexadecyltrimethyl ammonium bromide (HDTMA) solution was produced by dissolving 72.9 mg HDTMA in 40 mL distilled water. As a result, 100 mL of CAS dye were made. 750 mL of nutritional agar was combined with 30.24 g of 1, 4 piperazine diethane sulphonic acid, and the pH was adjusted to 6.8 with 0.1N NaOH. It was autoclaved separately and then mixed with Chrome azurol- S (100 ml) under aseptic conditions before being used in subsequent tests.

A bit of 72 h old culture of each test bacterium was placed on prepoured blue coloured chrome-azurol-S agar (CAS) plates. Plates were incubated at 30 ± 2°C for 24 h and observed for production of orange halo around the bit.

$$\text{Percent siderophore efficiency} = \frac{Z\text{-}C \quad X\ 100}{C}$$

Where,

Z= size of Halozone

C= colony size

The halozone diameter around the colony was calculated by subtracting colony size from total size. Phosphate solubilization index (PSI) was measured using the formula (Edi-Premono *et al.*, 1996).

3.6.4 HCN Production

Bakker and Schippers (1987) approach was used to screen bacterial isolates for the generation of hydrogen cyanide (HCN) . Bacterial cultures were streaked on King's B medium modified with 1.4 g/l glycine on prepoured plates. Whatman No.1 filter paper strips were soaked in 0.5 percent picric acid in 2% sodium carbonate, then inserted in the lid of each petriplate, sealed with parafilm, and incubated for 1 to 4 days at 35°C. The findings of the uninoculated control were compared. The colour of the filter paper on the plates was changed from yellow to orange brown.

3.6.5 Quantitative estimation of indole-3-acid

Bacterial cultures were grown in modified Luria Bertani broth amended with 5 mM L-tryptophan, 0.065% sodium dodecyl sulphate and 1% glycerol 72 h at 35˚C under shaking conditions. The cultures were centrifuged at 15,000 rpm for 20 minutes and supernatants were collected and stored at 4°C.

The method described by Gorden and Palleg (1957) was used to determine the IAA equivalents i.e. 3 ml of supernatant was pipette out into test tube and 2 ml of Salkowski reagent (2 ml 0.5 M $FeCl_3$ + 98 ml 35% $HCLO_4$) was added to it. The tubes containing the mixture were left for 30 minutes (in dark) for colour development. Intensity of colour was measured spectrophotometrically at 535 nm. Similarly, colour was also developed in standard solution of IAA (10-100 µg/ml) and a standard curve was established by measuring the intensity of this colour.

3.7 MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF SELECTED ISOLATES.

The most efficient bacterial isolates selected on the basis of plant growth promoting traits and *in vitro* antagonistic activity were subjected to morphological, physiological and biochemical characterization as per the criteria of Bergey's Manual of Systematic Bacteriology (Sherman and Cappuccino, 2005).

3.7.1 Morphological characterization

Morphological characteristics of isolates including colony morphology, Gram's reaction and cell shape were investigated.

3.7.2 Physiological characterization

Separate experiments were performed for optimization of conditions for growth of selected bacterial isolates.

3.7.2.1 Effect of pH on the growth of bacterial isolates

5 ml of nutrient broth was taken in test tubes. The medium was adjusted to various pH (3, 5, 7, 9 and 11) using 0.1 N NaOH or 0.1 N HCl as the case may be. Each tube was inoculated with 0.1 ml of 48 h old bacterial cell suspension (OD 1.0 at 540 nm) of selected isolates. The experiment was carried out in triplicates. The pH suited for maximum growth was selected on the basis of turbidity caused by the bacterial growth in test tube.

3.7.2.2 Effect of temperature on growth of bacterial isolates

5 ml of nutrient broth was taken in test tubes and inoculated with 0.1 ml of 48 h old bacterial cell suspension (OD 1.0 at 540 nm). The optimum temperature for growth was selected on the basis of turbidity caused by the bacterial growth in test tube. Growth curves were drawn by growing the culture at various temperatures.

3.7.2.3 Effect of incubation period on growth of bacterial isolates

5ml of nutrient broth was taken in test tubes and inoculated with 0.1 ml of 48 hr old bacterial cell suspension (O.D. 1.0 at 540 nm). Each test tube was incubated for different time period (24 h, 48 h, 72 h, 96 h and 120 h) and observed for turbidity. The optimum incubation period for growth was maintained for further experimentation.

3.7.3 Biochemical characterization

The selected bacterial isolates were tested using following biochemical tests:

1. Indole Test

Interpretation

- **Positive**: Pink coloured ring after addition of Kovac's reagent.
- **Negative**: No colour change even after the addition of Kovac's reagent.

2. Methyl red Test

Interpretation

- **Positive**: Development of red colour after adding methyl red reagent
- **Negative**: No colour change after adding methyl red reagent

3 Citrate utilization

Interpretation

- **Positive:** Medium turns royal blue.
- **Negative**: Medium remains green

4. Starch Hydrolysis

Interpretation

Iodine has been added to this starch agar plate.

- **Positive**: The zone of clearing surrounding *bacterial isolate* indicate that isolate wereable to hydrolyze starch.
- **Negative**: The absence of any clearing indicates that isolate were able to hydrolyze starch.

5. Casein Hydrolysis

Interpretation

- **Positive**: Clearing is observed around or beneath colony growth (hydrolysis).
- **Negative**: No clearing is observed around and beneath the inoculum.

6. Hydrogen Sulphide production

Interpretation

- **Positive**: Blackening of medium
- **Negative**: Non blackening of medium

7. Catalase test

Interpretation

- **Positive**: Effervescence (bubble formation)
- **Negative**: No bubble formation

8. Voges proskauer test

Interpretation

- **Positive**: Development of a red colour 15 minutes after the addition of the reagents
- **Negative**: No colour change

9. Carbohydrates fermentation

Interpretation

- **Positive**: Change in colour to bright red to pale yellow indicating acid production
- **Negative**: No colour change of broth

3.8 MOLECULAR CHARACTERIZATION OF SELECTED RHIZOBIAL ISOLATES BY 16S rRNA SEQUENCING

3.8.1 Genomic DNA extraction by conventional method (Sambrook *et al*., 1989)

Bacterial isolates were incubated overnight at 28°C in YEM broth at 200 rpm. The cells were harvested and processed for DNA isolation.

Requirements:

96-100% Ethanol

Sterile, DNase- free pipette tips and microcentrifuge tubes

RNase A (50 mg/ml)

10% SDS

Phenol: Chloroform: Isoamyl Alcohol (25:24:1)

Extraction buffer (20 mg/ml lysozyme, 100 mM Tris HCl, 50 mM EDTA, 500 mM NaCl)

TE buffer (10 mM Tris HCl, 1 mM EDTA)

Procedure

1 ml of overnight grown culture was transferred to a microcentrifuge tube and centrifuged at 13,000 rpm for 1 min and supernatant was discarded.

2 Bacterial pellet was suspended in 500 µl of extraction buffer and 50 µl of 10 % SDS. Cell pellet was resuspended by vortexing or pipetting.

3 Incubation at 65°C water bath was done for 30 min until the sample lysate becomes clear. During incubation, tube was inverted at every 3 min.

4 After 65°C incubation, 2 µl of RNase A (50 mg/ml) was added to sample lysate and mixed by vortexing. Then incubated at room temperature for 5 min.

5 To the lysate, equal volume of Phenol:Chloroform:Isoamyl Alcohol (25:24:1) was added and mixed well.

6 The above mixture was centrifuged at 10,000 rpm for 5 min at room temperature. Two layers were formed. Upper aqueous layer was collected in new eppendorf tube with the help of pipette.

7 The Phenol:Chloroform:Isoamyl Alcohol extraction step was repeated.

8 The above mixture was centrifuged at 10,000 rpm for 5 min at room temperature. Two layers were formed. Upper aqueous layer was collected in another new eppendorf tube with the help of pipette.

9 1/10 volume of 5M NaCl and 2.5 volume of absolute ethanol was added to aqueous phase collected in eppendorf tube.

10 Incubation was done at -20°C overnight.

11 The above mixture was centrifuged at 12,000 rpm for 20 min at room temperature and supernatant was discarded.

12 The DNA pellet was washed with 1 ml of 70% ethanol.

13 The above mixture was centrifuged at 12,000 rpm for 5 min at room temperature and supernatant was discarded.

14 DNA pellet was air dried for about 15 min until all the residual ethanol got evaporated.

15 Finally, the DNA pellet was suspended in appropriate amount of TE and was quantified.

3.8.2 Gel electrophoresis

The isolated DNA was finally suspended in 100 µl of elution buffer and quantified on 1% (w/v) agarose gel.

3.8.2.1 Preparation of agarose gel

The agarose (as required) was added in 100 ml of 1X TAE buffer and mixed by heating until the agarose solubilized completely. The slurry was cooled and ethidium bromide was added in concentration of 0.5 µg/ml, mixed thoroughly and poured onto gel casting tray and comb was placed in gel.

3.8.2.2 Sample preparation of agarose gel electrophoresis

The sample of DNA was mixed with the gel loading dye (5:1 ratio) and slowly added into the gel submerged in 1xTAE gel running buffer.

3.8.2.3 Electrophoresis

The electrophoresis was performed at 1-5 V/cm^2 voltage until two dyes resolve and migrated the appropriate distance through the gel. After electrophoresis, the gel was observed under UV light and documented using gel documentation system (Bio-rad).

3.8.2.4 Primers used for sequencing of 16S rDNA

Universal primers for 16S rDNA amplification of bacterial isolates used were pA (5'-AGAGTTTGATCCTGGCTCAG-3') and pH (5'-AAGGAGGTGATCCAG CCGCA-3') designed by Edwards *et al*. (1989).

3.8.2.5 PCR amplification of 16S rDNA

PCR reaction was carried out in 20 µl reaction containing ~50 ng of template DNA, 20 p moles of each primers, 0.2 mM dNTPs and 1 U Taq polymerase (Genei, Banglore) in 1xPCR buffer. Amplification was performed using thermo-cycler (multigene PCR system, Labnet). Reactions were cycled 35 times at 94°C for 3 min, 50°C for 30 sec, 72°C for 1.5 min followed by final extension at 72°C for 10 min. The PCR products were analyzed on 1 per cent agarose gel in 1xTAE buffer, run at 100V for 1 h and the size was determined by using 1 kb DNA ladder (Thermo Scientific).

3.8.2.6 Gel elution was done by Hi Yield Gel/PCR DNA Extraction Kit

3.8.2.7 Gel dissociation

Agarose gel slice containing relevant DNA fragments was excised and extra agarose was removed to minimize the size of the gel slice. 300 mg of the gel slice was transferred into a microcentrifuge tube. 500 µl of DF buffer was added to the sample and mixed by vortexing. Incubation was done at 55°C for 10-15 min until the gel slice gets completely dissolved. During incubation, the tubes were inverted at every 2-3 min and dissolved sample mixture was cooled at room temperature.

3.8.2.8 DNA binding

A DF column was placed in 2 ml collection tube. 800 µl of sample mixture (from above step) was applied into the DF column and centrifuged at 13,000 rpm for 30 seconds. Flow through was discarded and DF column was placed back in 2 ml collection tube.

3.8.2.9 Wash

600 µl of wash buffer (ethanol added) was added into DF column and centrifuged at 13,000 rpm for 30 sec. Flow through was discarded and DF column was placed back in 2 ml collection tube. It was again centrifuged for 3 min at 13,000 rpm to dry the column matrix

3.8.2.10 DNA elution

Dried column was transferred into a new micro centrifuge tube. 15-30 µl of elution buffer or distilled water was added into the centre of column matrix. Stand for 2 min until elution buffer or distilled water was absorbed by the matrix. Centrifugation was done for 2 min at 13,000 rpm to elute purified DNA.

3.8.2.11Sequence and phylogenetic analysis

Representative bacterial isolates of each genotypic profile were chosen for 16S rDNA partial sequencing. The sequencing was performed from Eurofins lab, Bangalore, India, using both forward and reverse primers as mention in section 3.9.3. The sequence alignment was done using the Clustal W (Thompson *et al*., 1994) and manually edited using the Bioedit package (Hall, 1999). The cladograms were constructed by neighbor-joining method (Saitou and Nei, 1987) with the Kimura-2-parameter model (Kimura, 1980) and were bootstrapped using the software programs in the MEGA 6.0 package (Tamura *et al*., 2013).

3.8 TO STUDY THE EFFICACY OF SELECTED GROWTH PROMOTING BACTERIAL ISOLATES ON GROWTH AND YIELD OF WHITE BUTTON MUSHROOM.

The efficacy of selected bacterial isolates on white button mushroom will be carried out.

T_{O_1} = Uninnoculated casing

T_{O_2} = Uninnoculated substrate

T_1 = GPB_1 on casing

T_2 = GPB_1 on substrate

T_3 = GPB_2 on casing

T_4 = GPB_2 on substrate

T_5 = $GPB_1 + GPB_2$ on casing

T_6 = $GPB_1 + GPB_2$ on substrate

T_7 = GPB1 + GPB2 on casing and substrate

Where,

GPB1 is growth promoting bacteria isolated from Nauni , Solan

GPB2 is growth promoting bacteria isolated from Local site of Solan (Dharja)**.**

Bacterial isolates = 2

Total treatments = 9

Replications = 3

Experimental Design = RBD

3.9 PHYSICO-CHEMICAL PROPERTIES, AVAILABLE NUTRIENTS AND MICROBIOLOGICAL STATUS OF CASING MIXTURE AND SPENT MUSHROOM COMPOST.

Potting mixture was analyzed before the application of treatments and after the termination of experiment for important physico-chemical properties, available nutrient and microbiological status by adopting the following standard procedures:

3.9.1 pH and electrical conductivity

The soil pH was determined in 1:2.5 (soil: water suspension) and the electrical conductivity of the supernatant liquid was recorded and expressed in dSm^{-1} (Jackson, 1973).

3.9.2 Organic carbon

Organic carbon was determined by Chromic acid titration method of Walkley and Black (1934).

3.9.3 Available nitrogen

Available nitrogen was determined by alkaline permagnate method of Subbiah and Asija (1956).

3.9.4 Available phosphorous

0.5 N $NaHCO_3$ at 8.5pH was used to extract available phosphorus (Olsen's *et al*., 1954) and determined spectrophotometrically.

3.19.5 Available potassium

Available potassium was extracted by normal neutral ammonium acetate (Merwin and Peech, 1951) and determined on flame photometer.

3.9.6 Total microbial count

The compost mixture was also analysed for total bacterial counts. 1g of compost mixturewas taken in 9 ml of sterilized water blank and the soil suspension was diluted in 10 fold series, then microbial counts was determined by standard pour plate technique on different media as described by (Subba Rao, 1999). The population was expressed as colony forming units per gram of soil (cfu/g soil).

3.10 OBSERVATIONS RECORDED FOR MUSHROOM PARAMETERS

3.10.1 Sporophore parameters

a) Days taken for completion of case run

b) Days taken for first harvest post casing

After first case run, days taken for first harvesting of mushrooms was recorded.

c) Total length of mushroom

Length of mushroom will be measure with ruler scale in centimeters (cm) from the bottom of mushroom to cap of mushroom.

d) Individual weight of fruit

The fresh weight of individual fruit bodies from each treatment was measured by cutting the above ground portion of mushroom with the help of knife and weighed with the help of weighing balance and the fresh weight was expressed in grams.

e) Length of stalk

Length of stalk of each mushroom was measured with the help of scale and it was measure in centimeters (cm)

f) **Width of stalk**

Width of stalk of each fruit wa measured with the help of ruler scale in centimeters (cm)

g) Average diameter of mushroom cap (cm)

h) Yield (kg/100kg of compost)

i) Disease and pest if any.

3.10.2 Yield parameter

$$\text{Biological efficiency (B. E) \%} = \frac{\text{Total wt. of mushroom}}{\text{Weight of compost}} \text{ X } 100$$

3.10.3 Mushroom biochemical parameters

3.10.3.1Antioxidant enzyme assay

The mushroom extracts were mixed with methanol (96 %) and 63 µmol/L solution of DPPH. After 30 min. at room temperature, the absorbance was measured at 517 nm and converted into percentage of radical scavenging activity (Zeković *et al*., 2010). The comparative analysis of samples was made by calculating DPPH scavenging activity which stands for the relative decrease of absorbance in the samples analysed. DPPH scavenging activity was calculated by using the following equation:

DPPH scavenging activity = $100 \times (Ac - As)/Ac$ where:

Ac - absorbance of the control

As- absorbance of the sample

3.10.3.2 Total phenolic content

Total phenols (TP) were determined spectrophotometrically with *Folin-Ciocalteu* reagent (Waterhouse, 2002). The sample (2 mL) was dissolved in ethanol and mixed with 10 mL Folin-Ciocalteau's reagent diluted 1/10 with distilled water. After few minutes sodium carbonate (8 mL) was added to this solution. This solution was stored in dark place for two hours and after that, the absorbance was measured at 765 nm. A standard curve was prepared using gallic acid as standard with a concentration range from 100 to 500 µg/mL. Results are expressed in mg of gallic acid equivalents per gram (mg GAE g-1) of mushrooms.

3.10.3.3 Total ash content

This method was described by (Raghuramulu *et al*., 2003).In a crucible, one gram of the sample was precisely weighed. The crucible was set on a clay pipe triangle and heated over a low flame until thoroughly charred, then cooked in a muffle furnace for around 56 hours at 600° C. It was then weighed after cooling in a dessicator. The crucible was then heated in the muffle furnace for 1 hour, cooled, and weighed to assure complete ashing. This was done until the ash was virtually white or greyish white in hue and two successive weights were the same.

Total ash was calculated as:

$$\text{Ash content (g/100 g sample)} = \frac{\text{weight of ash}}{\text{Weight of sample taken}} \text{ X } 100$$

3.10.3.4 Total protein content

A mushroom sample of 0.1 gm was placed in 10 ml of cell lysis solution and allowed to sit at room temperature for two days. The samples were then centrifuged for 10 minutes at 7000 rpm, and the preparation was used to estimate protein using Lowry's technique. (Lowry *et al*.,1951)

3.10.3.5 Total carbohydrates content

The total carbohydrate content of wild edible mushrooms is estimated by the anthrone method (Hedge and Hofreiter, 1962).

3.10.3.6 Total crude fibre content

This method was described by (Raghuramulu *et al*., 2003).Ten grams of moisture and fat-free sample was taken in a beaker and 200 *ml* of boiling 0.255 N H_2SO_4 was added. The mixture was boiled for 30 minutes keeping the volume constant by the addition of water at frequent intervals. The mixture was then filtered through a muslin cloth and the residue washed with hot water till free from acid. The material was then transferred to the same beaker, and 200 *ml* of boiling 0.313 N NaOH added. After boiling for 30 minutes (keeping the volume constant as before) the mixture was filtered through a muslin cloth and the residue washed with hot water till free from alkali, followed by washing with some alcohol and ether. It was then transferred to a crucible, dried overnight at 80~100℃ and weighed (We) in an electric balance .The crucible was heated in a muffle furnace (at 600℃)for 5~6 hours, cooled and weighed again (Wa). The difference in the weights (We-Wa) represents the weight of crude fiber.

$$\text{Crude fiber (g/100 g sample)} = \frac{\text{100 - (moisture + fat)}}{\text{(We-Wa)/Wt of sample}}$$

3.11 ANALYSIS OF PLANT AND SOIL NITROGEN, PHOSPHORUS AND POTASSIUM (NPK)

3.11.1 Sample collection and preparation

The mushroom and compost samples were collected treatment wise and immediately weighed and brought to the laboratory in paper bags. All the samples were washed in series,

first with tap water then 0.1 N HCl followed by distilled water. The washed samples were allowed to dry in air subsequently in hot air oven at 60°C till constant weight was observed. Oven dried samples of were ground and sieved (40 meshes). Thereafter, they were stored in butter paper bags for chemical estimation.

3.11.2 Digestion of samples

Total N

The digestion of 0.50g samples for estimating nitrogen was carried out in the concentrated H_2SO_4 in presence of digestion mixture having following composition:

Potassium sulphate (K_2SO_4)	=	400 parts
Mercuric oxide (HgO)	=	3 parts
Copper sulphate ($CuSO_4.5H_2O$)	=	20 parts
Selenium powder (Se powder)	=	1 part

Total P and K

For the estimation of P and K 0.5 g plant sample was digested in diacid mixture prepared by mixing nitric acid and perchloric acid (4:1) taking all relevant precautions as suggested by Piper (1966).

3.11.3 Estimation of nutrient elements (NPK)

The nitrogen was estimated in Kjaldhel apparatus. The phosphorus content was determined by Vanado molybdo-phosphoric yellow colour method. However, potassium was determined by flame-photometer (Jackson, 1973).

3.12 STATISTICAL ANALYSIS

The data recorded on plant, soil and microbiological properties will be statistically analyzed by using MS-Excel and OPSTAT packages (Sheoran *et al*., 1998). The mean values of data will be used for the analysis of variance (ANOVA) as described by Panse and Sukhatme (2000) by using Completely Randomized Design as given in Table 1.

Analysis of variance (ANOVA) by Randomized Block Design (RBD)

Sources of Variation	Degree of Freedom	Sum of squares	Mean sum of squares	F cal
Replication	(r-1)	Sr	Sr/(t-1)=Mr	Mr/Me
Treatment	(t-1)	St	Se/(N-t)=Mt	Mt/Me
Error	(r-1)(t-1)	Se	Se/(N-t)=Me	
Total	(N-1)	ST	St	

Where,

R = Number of replications

T = Number of treatments

S_r = Sum of squares due to replications

S_t = Sum of squares due to treatments

S_e = Sum of squares due to errors

S_T = Total sum of squares

M_r = Mean sum of squares due to replications

M_t = Mean sum of squares due to treatments

M_e = Mean sum of squares due to error

The "F" calculated value will be compared with "F" table value at 5% level of significance. If "F" calculated value is higher than, F" table value, the treatment effect was considered significant i.e. one of the treatment pair differ significantly and critical difference (CD) will be calculated.

The standard error of mean SE(m) and critical difference (CD) for comparing the means of any two treatments will be calculated as below:

$SE(m) = \pm (Me / r)^{1/2}$ $SE(d) = \pm (2Me / r)^{1/2}$

CD = SE(d) x t (5%) value at error degrees of freedom.

Where,

SE(m) = Standard error of mean SE(d) = Standard error of difference

$CD_{(0.05)}$ = Critical difference at 5% level of significance

Chapter - 4

RESULTS AND DISCUSSION

The present investigation entitled "**Characterization of growth promoting bacteria from spent mushroom compost and their efficacy on quality and yield of white button mushroom"** was conducted at the Department of Basic Sciences, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Nauni-Solan during the year 2020-22. The studies were conducted to select best growth promoting bacteria from spent mushroom substrate and to study their response in the improvement of growth and yield of white button mushroom.

The findings obtained from the present study are presented and discussed in this chapter:

4.1 ISOLATION AND ENUMERATION OF BACTERIA ASSOCIATED WITH SPENT MUSHROOM COMPOST

The data pertaining to bacterial count from spent mushroom compost (SMC) in nutrient agar medium are presented in Table 1, Fig 1 and Plate 1. It is evident from the data that mushroom compost selected from different locations had significant effects on the total bacterial count. The maximum bacterial count of 6.78 $\times 10^6$ cfu/g was recorded from DMR Chambaghat of district Solan. However, the minimum bacterial count 5.43 $\times 10^6$ cfu/g was recorded from Local farmer site (Dharja) of district Solan. On the basis of predominant growth of bacterial isolates on nutrient agar by calculating 10^6cfu/g, total 25 bacterial isolates were selected. Among 25 isolates 14 isolates from Chambaghat, Solan represented as CSI-14, 7 isolates from Nauni named as NSI-7 and 4 isolates from Local Farm, Dharja, Solan represented LSI-4 were selected for further studies. (Table 2)

Yohalem *et al*. (1996) reported that the media of button mushroom are habitat for useful microorganisms. They collected different samples from different sites of mushroom compost and found variation of bacterial population in all the collected samples. This variation may be due to composition of compost used for mushroom cultivation. The microbial communities also depends upon, time of sampling and environmental conditions such as temperature during and cultivation of mushroom. Similar results were reported for

white button mushroom compost by Siyoum *et al.*, 2016 reported similar results with 7.6×10 log cfu/g bacteria in SMC of white button mushroom. Rossouw and Korsten (2017) reported high cfu/g (5.2 to 2.4 log cfu/g) form spent mushroom compost.

Table 1: Isolation and enumeration of total bacterial population

Sites	**Bacterial count log (×10^6 cfu/g)**
Chambaghat (directorate of mushroom research)	6.78
Nauni (Mushroom farm of UHF)	5.47
Local farmer (Dharja)	5.43
$CD_{0.05}$	0.05

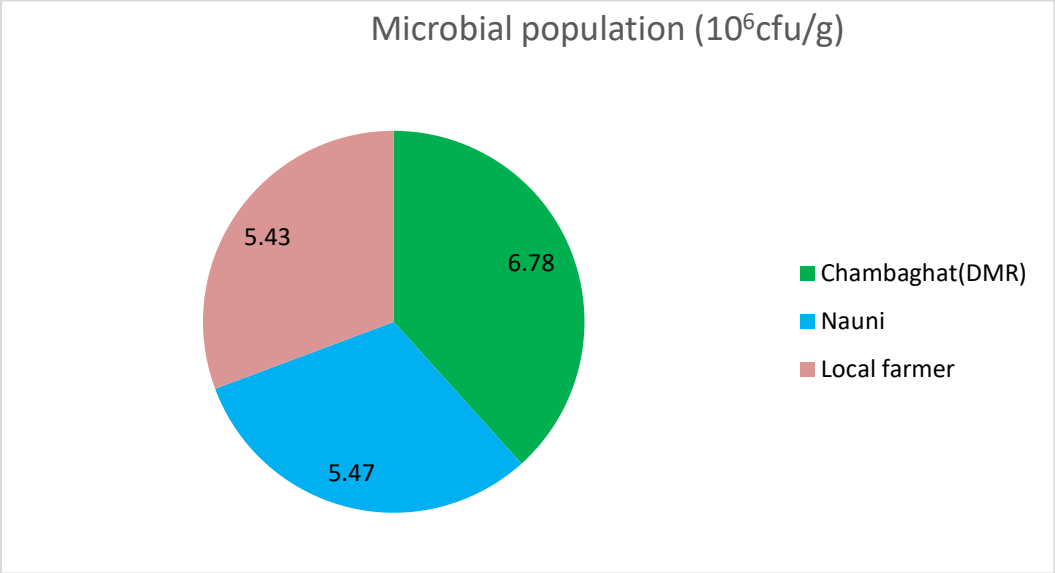


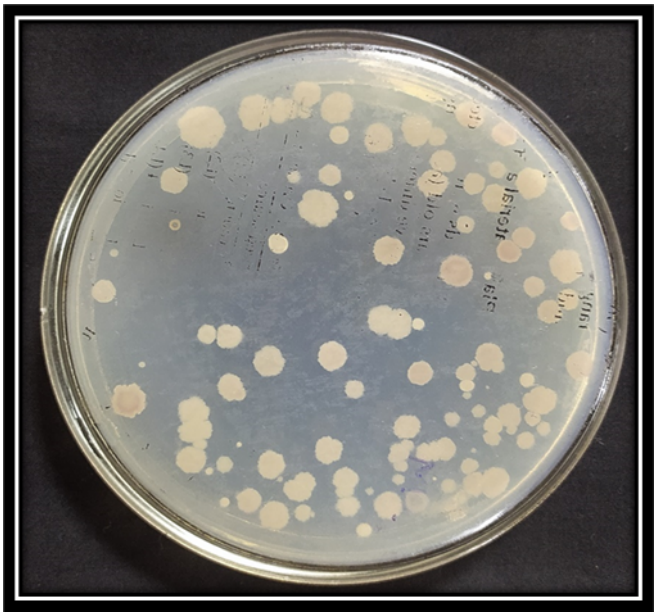
Fig 1: Comparative microbial population isolated from spent mushroom compost of different sites.

Table 2: Total number of isolates selected from spent mushroom compost

District	**Sites**	**Isolates name**	**No. of isolates**
Solan	Chambaghat	CSI (Chambaghat solan isolate)	14
Solan	Nauni	NSI (Nauni Solan isolate)	7
Solan	Local famer	LSI (Local Solan isolate)	4

4.2. SCREENING OF BACTERIAL ISOLATES FOR MULTIFARIOUS GROWTH PROMOTING TRAITS

The twenty five selected isolates, which represents 14 isolates from DMR Chambaghat, 7 from UHF Nauni mushroom farm and 4 from Local farmer of Dharja Solan



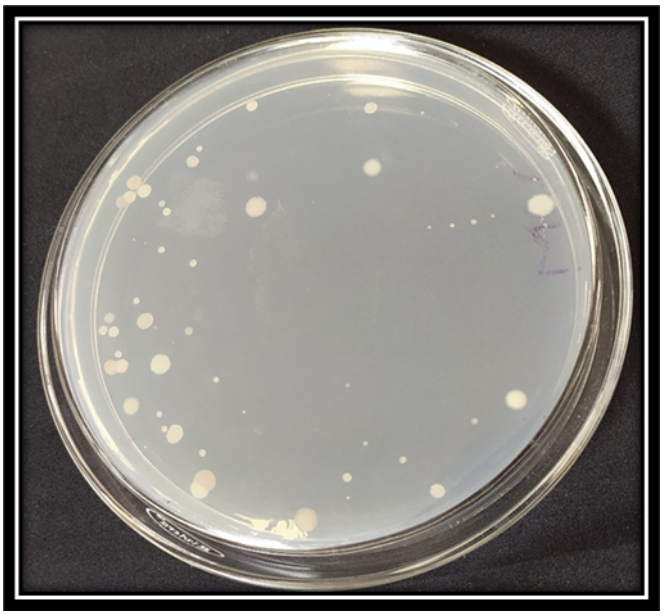


Plate 1: Enumeration of bacteria from spent mushroom compost

were screened for multifarious growth promoting traits such as IAA production, phosphate solubilization, Nitrogen fixing ability, HCN production and siderophore production.

Table 3: Screening of bacterial isolates for multifarious growth promoting traits isolated from spent mushroom compost

Bacteria Isolates	IAA Production	P-Solublization	N-Free medium	Siderophore production	HCN Production
CS1	++	++	+	-	++
CS2	+	+	++	++	++
CS3	-	-	-	-	-
CS4	-	-	-	+++	-
CS5	-	-	+	+	-
CS6	-	+++	+	+	+++
CS7	-	-	++	-	-
CS8	-	-	-	-	-
CS9	-	-	+	-	-
CS10	-	-	-	+	-
CS11	++	++	+++	++	-
CS12	++	++	+	-	-
CS13	-	+	-	+	+
CS14	-	-	+	-	++
NS1	-	-	-	+	-
NS2	+++	+++	+	++	+++
NS3	-	-	+	+	-
NS4	++	++	++	-	-
NS5	-	+	++	-	+++
NS6	-	-	-	-	+
NS7	-	-	-	++	-
LS1	-	++	++	-	-
LS2	++	+	-	+	-
LS3	-	-	+	++	-
LS4	+++	+++	+++	++	++

IAA Production: no IAA production (-), 50-100% (++), ≤ 50% (+), ≥ 100 % (+ + +)
P-solubilization: no P-solubilization (-), 50-100 % (+ +), ≤ 50% (+), ≥ 100 % (+ + +)
Siderophore units: no activity (-), 50-75 (+ +), ≤ 50 (+), ≥ 75 (+ + +)
Growth on N-free medium: 30-60 %(+ +) and 60-100% (+ + +).

The data presented in Table 4 and Fig 2 for various growth promoting traits showed that out of 25 bacterial isolates only 10 bacterial isolates were found as IAA producer (40%), 15 isolates were found as N fixer(60%), 12 were phosphate solubilizers (48%), 13 were siderophore producer (52%)and only 9 isolates were HCN producer (36%). The isolate NS2 showed the maximum growth promoting traits followed by LS4. However, bacterial isolates CS3, CS8 and CS9 have not shown any growth promotion activities (Table 3). It is evident from Fig 2 that bacterial isolates from compost of local farm of Dharja and mushroom farm of Nauni had maximum growth promoting traits, whereas spent mushroom compost of

Chambaghat was recorded with bacterial isolates having low growth promoting traits. The variation of traits from different sites may be due to composition of compost of different sites and the environmental conditions during mushroom cultivation and time of sampling of compost.

Carrasco and Preston (2020) and Chin *et al*. (2022) have also reported that composition and conditions of mushroom cultivation affect the microbial communities. In addition to the cultivation environment, biological factors can also play a pivotal part of production cycle of edible fungi & the microbial communities of compost.

Table 4: Percentage of isolates for Growth promoting traits of selected isolates from different sites.

Sites	Nitrogen fixers	IAA production (%)	P-solubilisation (%)	Siderophore production (%)	HCN production (%)
Chambaghat (Directorate of mushroom research)	57.1% (8/14)	35.7 % (5/14)	42.8 % (6/14)	50% (7/14)	35.7% (5/14)
Nauni(Mushroom farm of UHF)	57.1% (4/7)	33.3 % (2/7)	42 % (3/7)	57.1% (4/7)	42.8 % (3/7)
Local farmer (Dharja)	75% (3/4)	75 % (3/4)	75 % (3/4)	50% (2/4)	25% (1/4)
Total	60% (15/25)	40 % (10/25)	48% (12/25)	52% (13/25)	36% (9/25)

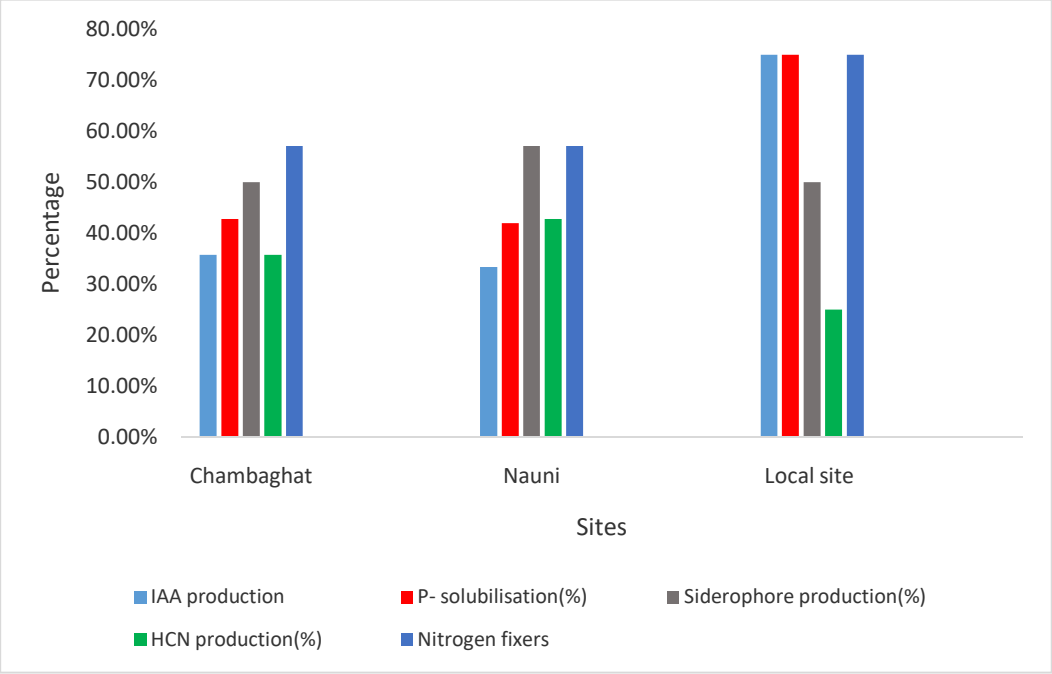


Fig 2: Percent of isolates exhibiting growth promoting traits

4.2 QUANTITATIVE ESTIMATION OF SELECTED BACTERIAL ISOLATES FOR GROWTH PROMOTING TRAITS

The selected 25 bacterial isolates were further screened for quantitative estimation of Phosphate solubilization, siderophore production and IAA production.

4.2.1 Quantitative screening of Phosphate solubilizing bacterial isolates

The data presented in Table 5 and Plate 2 revealed that among 25 selected bacterial isolates maximum phosphate solubilization index was observed with bacterial isolate NS2 (2.81) whereas minimum was recorded with isolate CS1(2.23). All the selected bacterial isolates registered a significant variation for solubilization of tricalcium phosphatein liquid medium. The maximum (214.7 µg/ml) P-solubilization was recorded with NS2 isolate with the decrease in final pH 7.00 to 5.67. The bacterial isolates NS2 showed maximum (93.73%) phosphate solubilization efficiency, However minimum efficiency was recorded for bacterial isolate CS1.(Fig 3)

Table 5: Solubilization of tri calcium phosphate (TCP) by selected bacterial isolates insolid and liquid Pikovskaya's medium.

Bacterial Isolates	P- solubilization in solid medium		P- solubilization in liquid medium (µg/ml)****	Final pH of supernatant
	Phosphate solubilization index (PSI)**	% P-solubilization efficiency (%SE)***		
CS1	2.23	22.03	64.50	4.24
CS2	2.38	29.29	65.50	5.31
CS6	2.77	79.46	93.73	4.81
CS10	2.69	50.53	80.63	5.32
CS11	2.64	59.37	84.93	3.99
CS12	2.38	49.11	73.33	3.41
NS2	2.81	93.73	214.76	5.67
NS4	2.65	74.51	91.33	4.34
NS5	2.76	62.61	102.76	5.34
LS1	2.45	72.06	110.16	5.21
LS2	2.81	77.84	103.86	4.98
LS4	2.92	86.70	127.53	3.56
$CD_{0.05}$	0.13	4.21	4.15	0.02

*Figures in parentheses are arc sin transformed values

PSI = zone size + colony size/colony size

***Per cent solubilisation efficiency (% S.E.)** = $\frac{Z-C}{}\times 100$; where C = colony diameter, Z = halozone Diameter C

**** **P-solubilized** = T-C; where, T=P-solubilized in test, C=P-solubilized in control

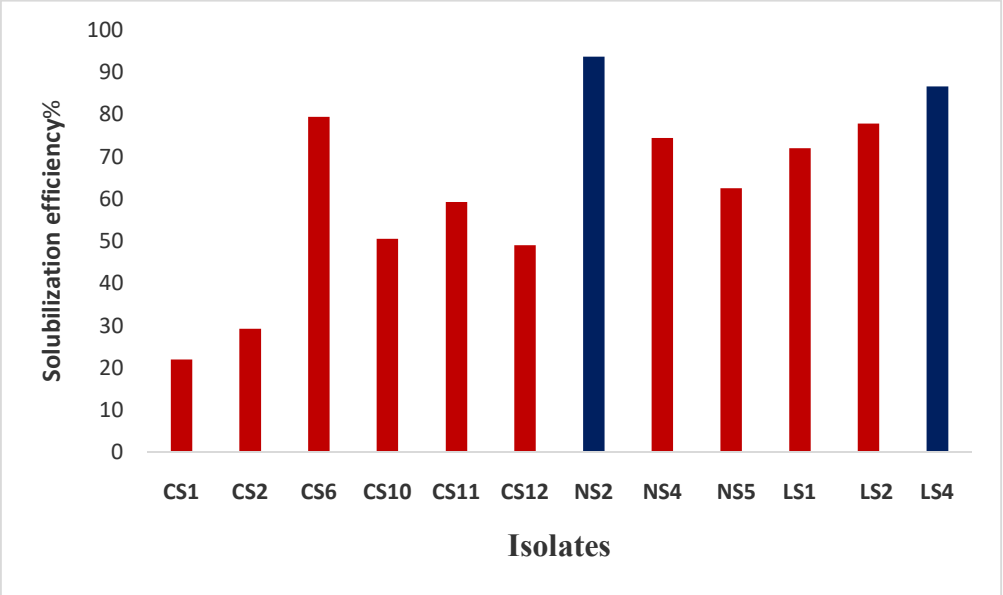
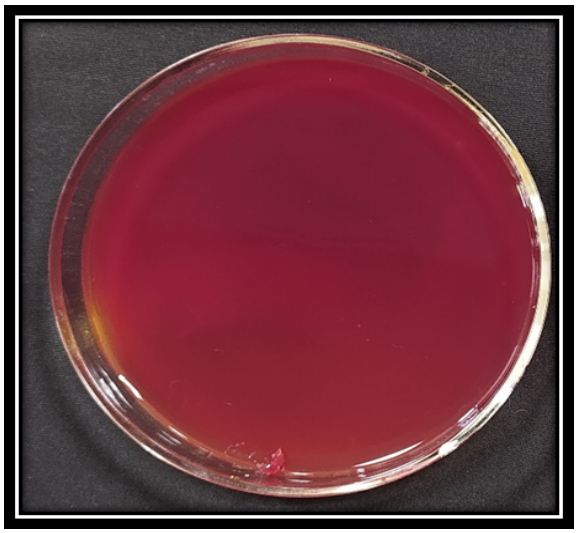


Fig 3: P-Solubilization efficiency of selected bacterial isolates from spent mushroom compost

Phosphorus is major essential macronutrients for normal plant growth and development. Microorganisms provide a biological rescue system capable of solubilizing the insoluble inorganic P of soil and make it available to the plants. Some microorganisms has ability to convert insoluble Phosphorus (P) to an accessible form such as Orthophosphate, is an important PGP traits for increasing the plant yield of the crops. Exploitation of P-Solubilising Bacteria (PSB) have enormous potential in increasing the availability of accumulated phosphate by lowering soil pH through production of organic acid, chelation, ion exchange reaction (Delvasto *et al*.2006). The rhizospheric phosphate utilizing bacteria is a promising source for plant growth promotion in agriculture soil. The phosphate solubilizing bacteria used as inoculants to increase the P uptake in soil by the crops.

The P-solubilization by bacteria has also been reported by other workers. Paul and Sinha (2017) found Solubilization Index of 2.85 for *Pseudomonas aeroginosa* KUPSB12, using Pikovskaya agar plates. A clear zones around colonies are due to the solubilization of phosphate found in Pikovskaya medium. Maheswar and Sathiyavani, (2012) reported *B. subtilis* and *B. cereus* were formed halozones around the bacterial colony on pikoviskaya agar medium. Mohammad *et al*., (2018) reported that phosphate solubiisation index for bacterial isolates varies in the range between 2.5 to 3.2 on pikovskaya agar medium. However, Batool and Iqbal, (2019) who also reported the phosphate solubilization index in



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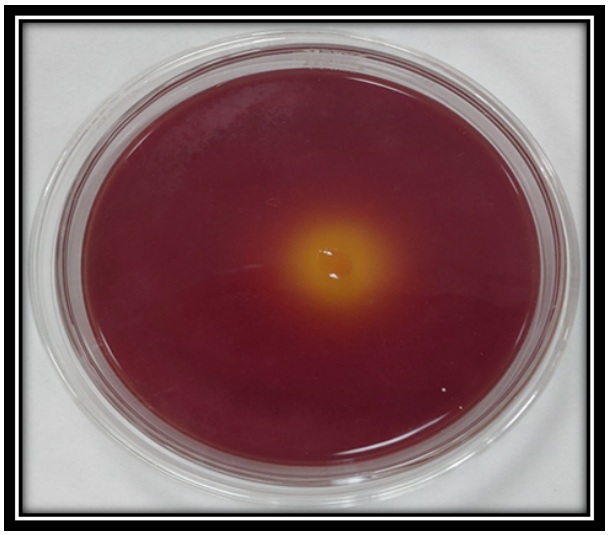
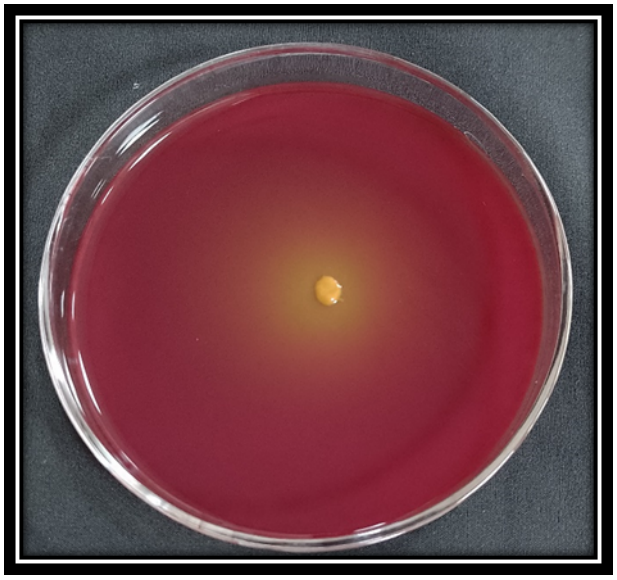
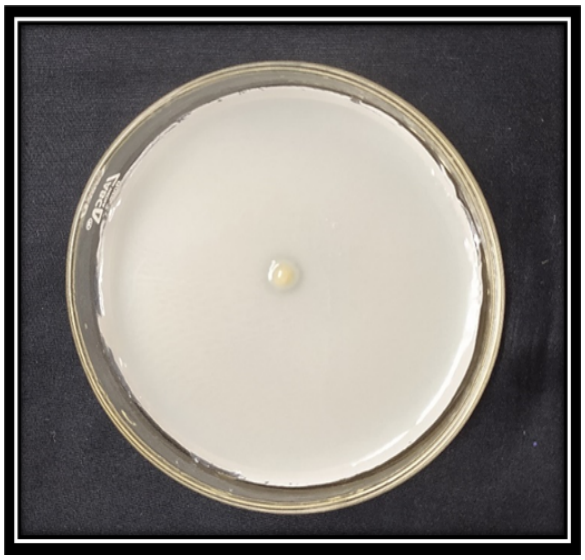


GPB1



GPB2

Plate 2 : P solubilization by isolate NS2 and LS4 on Pikovskaya's medium plates



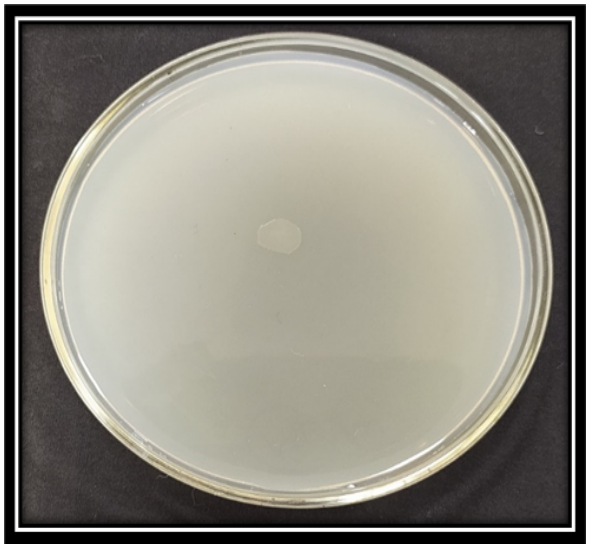


Plate 3: Growth of isolate NS_2 and LS_4 on N free Jensen media

the range of 4–7 on agar plate and 30–246. 9 µg/ml in liquid broth by the selected bacterial isolates.

4.2.2 Quantitative screening of siderophore producing bacterial isolates.

Siderophores are low molecular weight compounds that are formed when the iron is present in limited amount. They have a high specific activity to chelate the ferric ion (Fe III) and act as carriers for the transfer of Fe (III) into microbial cells. The siderophore production efficiency of selected bacterial isolates were measured qualitatively on the basis of bright zone on CAS medium. The data presented in Table 6 revealed that all the twenty five selected bacterial isolates from spent mushroom compost showed a significant variation for percent siderophore efficiency and siderophore production in liquid medium. The maximum (96.37%) siderophore unit (SU) was observed in NS2 bacterial isolate followed by LS4. The minimum (17.46 %) siderophore unit (SU) was observed in bacterial isolate NS4 on solid CAS media. Quantitative estimation of siderophore using CAS liquid assay of selected bacterial isolates revealed that maximum (286.21 µg/ml) siderophore production was recorded with bacterial isolates NS2 with decrease in pH (7.00 to 4.18). The minimum siderophore production (14.31 µg/ml) was recorded with bacterial isolate NS4 with decrease in final pH of supernatant (7.00 to 3.12).It is clearly observed from the Fig 4 that bacterial isolates NS2 and LS4 have shown high percentage of siderophore among the 25 tested bacterial isolates.

Table 6: Siderophore production efficiency (%) on CAS medium by selected bacterial isolates

Bacterial Isolates	% Siderophore production efficiency (%SE) on solid medium	Siderophore production in liquid medium (µg/ml)	Final pH of supernatant**
CS1	74.06	105.60	5.31
CS2	61.79	165.18	4.98
CS6	50.30	43.167	4.54
CS10	75.90	117.73	4.60
CS11	85.53	124.76	3.41
CS12	56.52	82.88	4.71
NS2	96.37	286.21	4.18
NS4	17.69	14.31	3.12
NS5	38.01	112.54	3.51
LS1	78.82	170.02	3.92
LS2	54.44	285.82	3.12
LS4	87.61	253.85	3.67
$CD_{0.05}$	**1.37**	**1.47**	**0.06**

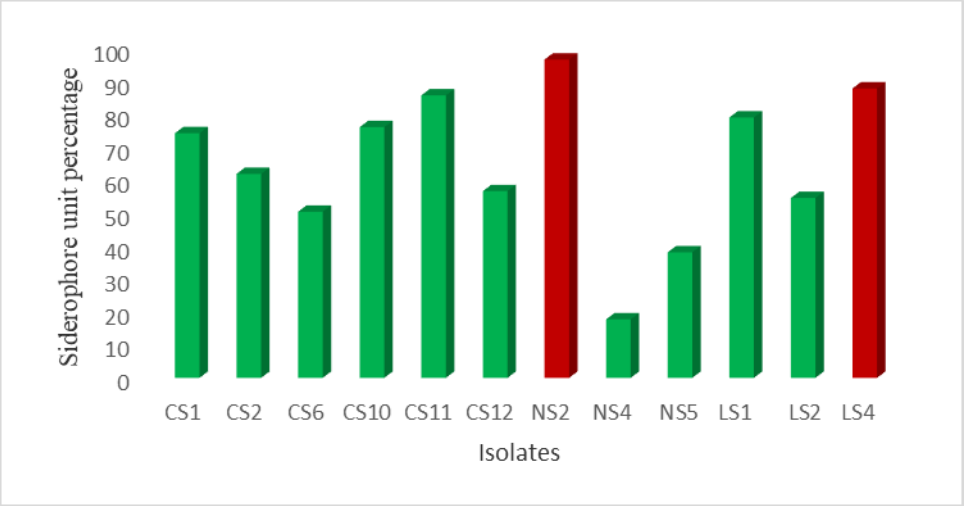


Fig 4: Percentage of siderophore production efficiency by selected bacterial isolates from spent mushroom compost.

The present results are in line with earlier workers who reported that bacterial isolates as siderophore producers. Gupta and Gopal (2008) isolated 10 PGPR isolates out of which only 6 were found to produce siderophore. They concluded that siderophore producing bacteria protect plants at two levels i.e, by limiting growth of plant pathogen and triggering plants defensive mechanisms (Ramos *et al.,*2010). Bagmare *et al*. (2019) reported 75 per cent siderophore production in *Pseudomonas florescence* and 67 per cent production in *Azospirullum lipoferum*. Dewi *et al*. (2020) reported 8 bacterial strains were positive for siderophore production. Subramanium *et al.* (2020) reported 60.51 per cent siderophore production in *P. fluorescens* and 62.48 percent in *P. aeruginosa* with zone diameter 6.53 mm and 9.4mm respectively.

4.2.3 Indole-3-acetic acid (IAA) production by selected bacterial isolates.

The data presented in Table 7 represents IAA production by selected bacterial isolates from spent mushroom compost. The IAA production by the bacterial isolates significantly varied from 17.19-79.60 µg/ml with the variation at final pH from 4.2 to 7.1. The bacterial isolate NS2 produce maximum (79.60 µg/ml) amount of IAA followed by bacterial isolate LS4 with decrease in pH from 7.00 to 5.70 and 4.83 respectively. However, bacterial isolate NS5 showed minimum (17.19 µg/ml) IAA production with reduction in pH from 7.00 to 5.80 after 48h of incubation at 37^{o}C. Similar results were observed by Baggam *et al*. (2017) for 2 strains with highest IAA production of 27.1µg/ml and 33.5µg/ml respectively. Yeom *et al*., (2021) also reported six bacterial strains producing IAA ranging between 20µg/ml to 250µg/ml.

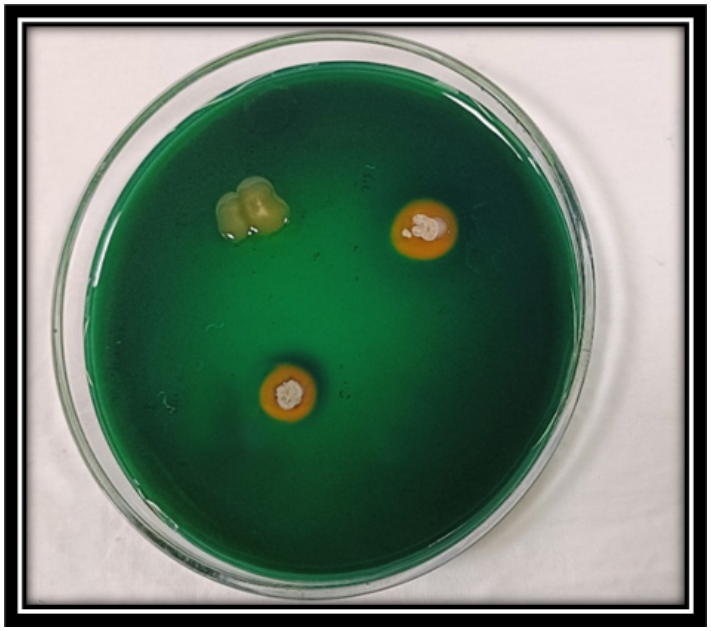


Plate 4: Siderophore production by two selected isolates

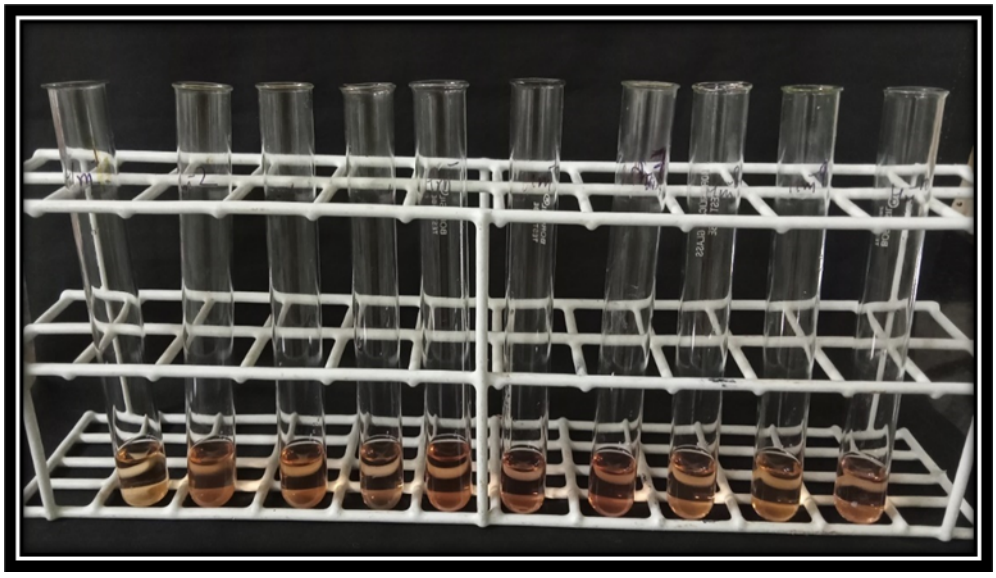
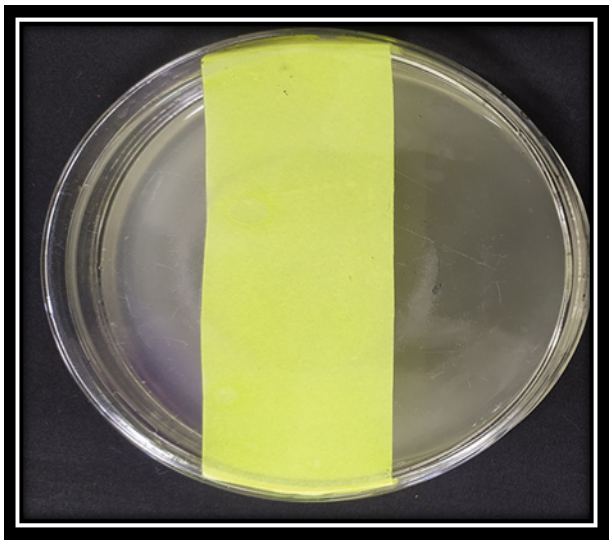


Plate 5: IAA production by isolate NS2 and LS4



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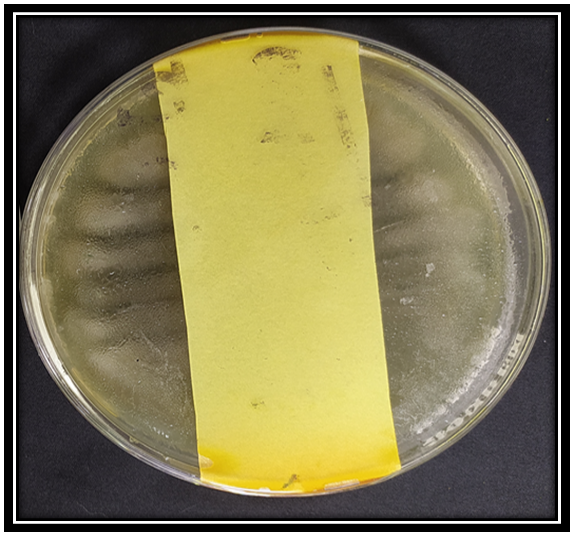
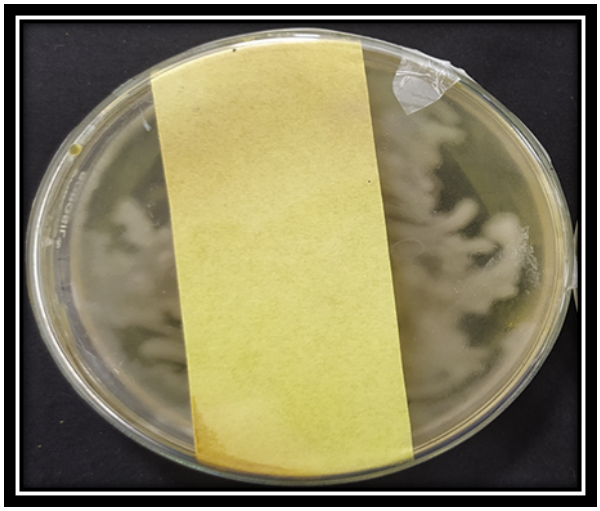


GPB1



GPB2

Plate 6: HCN Production by selected bacterial isolate

Table 7: IAA production by selected bacterial isolates

Bacteria Isolates	IAA production by bacterial isolates (µg/ml)	Final pH of supernatant
CS1	22.42	4.93
CS2	20.22	5.76
CS6	24.29	4.20
NS5	17.19	5.80
CS11	66.11	6.13
CS12	21.73	7.10
NS2	79.60	5.70
LS1	17.77	7.03
LS2	32.51	4.43
LS4	25.68	4.83
$CD_{0.05}$	**1.35**	**1.14**

Likewise Dewi *et al*. (2020) reported 4 isolates showing positive result for IAA production. Joseph *et al.* (2007) showed the highest IAA production in all isolates of *Bacillus, Pseudomonas and Azotobacter* (100%) followed by *Rhizobium* (85.7%). Similar variation in IAA production by *Bacillus* and *Pseudomonas sps*. was also reported by Minaxi *et al*. (2011), Kumar *et al*. (2012) and Jangu and Sindhu (2011). The IAA production was generally affected by the culture conditions, growth stage and substrate availability for bacteria (Mohite *et al*., 2013). The results of present investigations are in conformity with Sarayloo *et al*. (2014) who have reported Indole acetic acid (IAA) production in the range of 63.42 to 93.62 µg/ml by *Rhizobium rhizogenes,* isolated from soybean.

4.3. MORPHOLOGICAL, PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF SELECTED BACTERIAL ISOLATES

Two isolates namely NS2 and LS4 were selected on the basis of maximum PGP traits. The selected 2 bacterial isolates, NS2 (Mushroom farm UHF Nauni) and LS4 (Local farmer, Dharja) characterized for morphological, physiological and biochemical characterization by using the standard procedures.

4.3.1 Morphological and Biochemical characterization of selected bacterial isolates.

The data presented in Table 8 showed the morphological attributes of isolates NS2 and LS4. Gram staining was performed to check the shape and gram reaction of the selected bacterial isolates by the light microscope. The results revealed that bacterial isolate NS2 was gram positive and the isolate LS4 was found as gram negative. The bacterial isolates, NS2

was rod shaped, white, irregular, convex elevation, entire margin, granular surface, slimy texture However bacterial isolate LS4 was rod shaped, circular, raise elevation, entire margin, smooth surface and slimy texture.

The results are supported by Tsegaye *et al*., (2019) reported that 84.5per cent isolates were Gram-negative and 15.5 percent were Gram-positive, and among bacterial genus *Pseudomonas Bacillus, Enterobacter, Serratia, Chryseobacter, Citrobacter, Flavobacter and Klebsiella.* Khan *et al*. (2018) also characterized the form, elevation, margin, opacity and colour of the selected bacterial isolates. They selected a total of 14 bacterial strains for gram staining and out of these selected isolates only 11 bacterial strains were Gram negative while the 3 strains were Gram positive. Vinayarani and Prakash (2018) who reported that out of 50 isolates, 43 were Gram-negative while 7 were Gram-positive

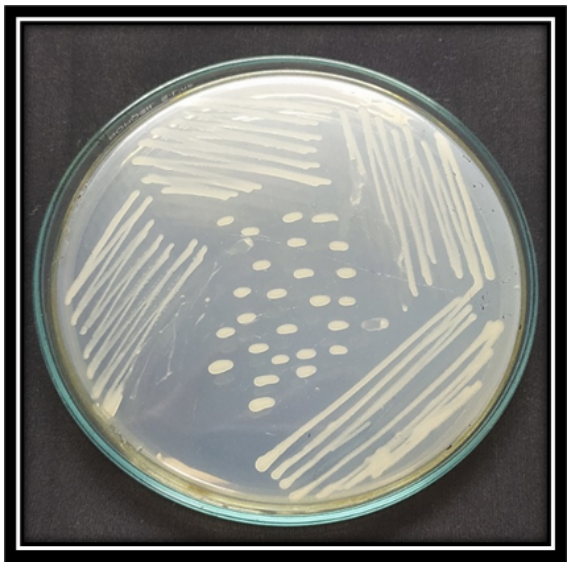
Table 8: Morphological characterization of selected bacterial isolates

Bacteria Isolates	Colony color	Form	Elevation	Margin	Surface	Texture	Shape	Arrangement	Colour	Gram's reaction
NS2	White	irregular	convex	Entire	Granular	Slimy	Rods	Cluster	Pink	+
LS4	White	Circular	Raised	Entire	Smooth	Slimy	Rods	Cluster	Pink	-

Table 9: Biochemical characterization of selected bacterial isolates.

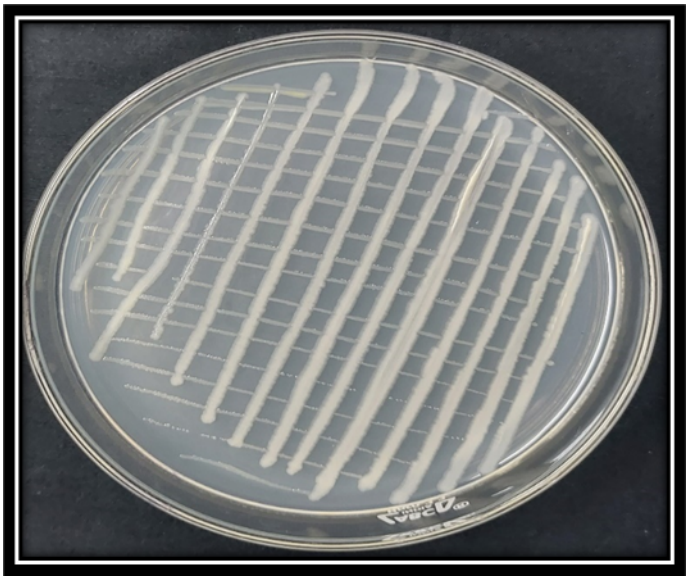
Bacterial isolates	Indole test	Methyl-Red test	Voges Proskauer test	Hydrogen sulphide production	Catalase test	Citrate utilisation	Starch hydrolysis	Casein hydrolysis
NS2	-	+	+	-	+	+	-	-
LS4	-	+	+	-	+	-	-	-
Carbohydrate test	Xylulose	Fructose	Sucrose	Galactose	Maltose	Mannitol	Lactose	Xylulose
NS2	+	+	+	+	+	+	-	+
LS4	+	+	+	+	+	+	-	+

Two selected bacterial isolates namely NS2 from Mushroom farm and LS4 from local farmer were characterized by a series of biochemical test and results are presented in Table 9 Results of present study revealed that all bacterial isolates were negative for indole test and positive for methyl red test. Among the selected bacteria isolates all were positive for citrate utilization. In this experiment, all selected bacterial isolates show halos around the colonies indicating positive results. All the bacterial isolates were positive for catalase test as well as able to ferment carbohydrates. All the selected bacterial isolates were positive for Voges



GPB1 (NS2)

GPB2 (LS4)



Consortia (GPB1 + GPB2)

Plate 7: Selected Bacterial isolates (GPB 1 + GPB 2)

proskauer test and unable to hydrolyze Casein. In case of carbon utilization, both the isolates were able to ferment xylulose, sucrose, galactose, maltose, mannitol fructose except lactose.

According to the Bergey's manual of systematic bacteriology the results on morphological and biochemical tests of selected bacterial isolates in the present study revealed that bacterial isolates were gram positive and negative in reaction and rods and coccus in shape so these selected bacterial isolates may belongs to the *Bacillus* and *Pseudomonas* sp.The present results are in conformity with those Rathaur *et al*. (2012) who also observed similar morphological characteristics for *Withania somnifera*. Similarly, Prabhukarthikeyan *et al*. (2017) isolated a total of 60 bacterial isolates from the turmeric rhizosphere and out of which 15 bacterial isolates characterize biochemically. They reported that all the *Pseudomonas* isolates shows positive reaction for the following test *viz*., Glucose utilization, Nitrate utilization, Citrate utilization and Catalase activity.

4.3.2 PHYSIOLOGICAL CHARACTERIZATION OF SELECTED BACTERIAL ISOLATES

4.3.2.1 Effect of incubation period on the growth of selected bacterial isolates.

The results presented in Table 10 and fig 5 represents the growth of isolates NS2 and LS4 at incubation period of 0-72 hrs. The results showed that maximum growth of selected bacterial isolate NS2 and LS4 was obtained at incubation period of 48 hrs with the variable count of 41×10^6 cfu/g and 51×10^6 cfu/g respectively. Both the isolates showed no growth in the period of 0 to12 h. However, significant decrease in the growth of selected bacterial isolates was observed after 48 hrs of incubation. It is clearly evident from fig that both the isolates have been shown increased growth after 12 hrs of incubation and decrease growth after 48 hrs of incubation. Our results are in line with Singh (2013) and Pahari and Mishra (2017) who also reported the best growth of *Bacillus* sp. at 48 hours of incubation

Table 10: Effect of different incubation period on the growth of selected bacterial isolates

Incubation period (h)	NS2	LS4	NS2	LS4
0	-	-	0	0
12	-	-	0	0
24	+	+	26	32
36	+++	+++	34	40
48	+++	+++	41	51
60	+	+	33	46
72	+	+	24	43
$CD_{0.05}$			**1.79**	**3.11**

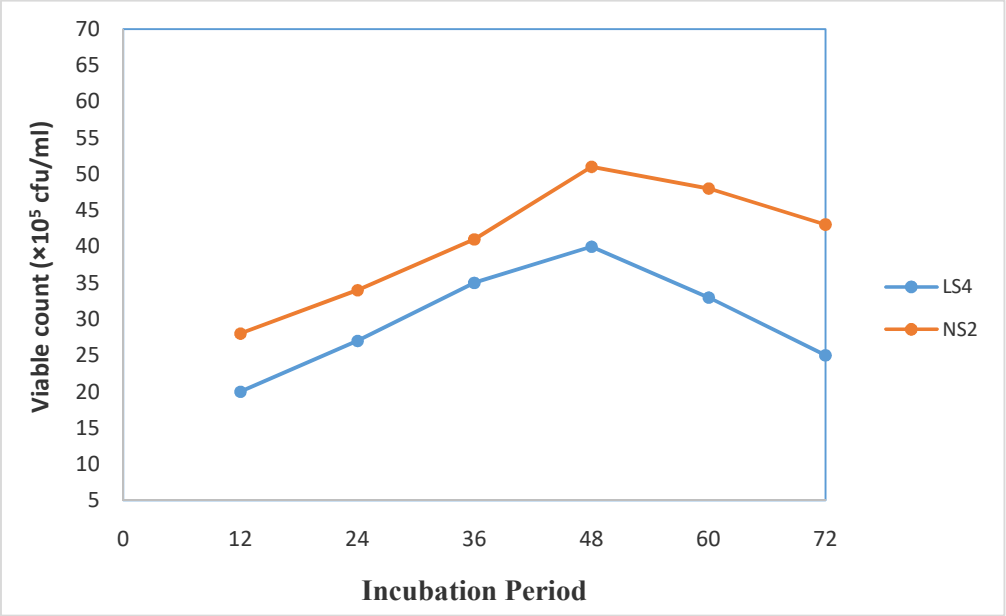


Fig 5: Effect of incubation period on growth of selected bacterial isolates from spent mushroom compost

4.3.2.2 Effect of temperature on the growth of selected bacterial isolates

The data presented in Table 11 revealed the bacterial growth at different temperature from 25-45°C. The maximum growth of selected bacterial isolates was recorded at the temperature of 35°C with viable count of 41 × 10^6 cfu/ml for NS2 isolate and 36 × 10^6 cfu/ml for LS4 isolate. As the temperature increased above 35°C well marked decrease in growth of bacterial isolates was observed (Fig 6). Both the isolates were able to grow at the temperature range 20-35º C. It was found that none of the tested bacteria were able to grow after 45 ºC.

Table 11: Effect of different temperature on the growth of bacterial isolates.

	Bacterial isolates		Viable count (×10^6 cfu/ml)	
Temperature (°C)	**NS2**	**LS4**	**NS2**	**LS4**
25	+	+	31	27
30	+	+	39	37
35	+++	+++	41	36
40	++	++	27	26
45	+	+	21	21
$CD_{0.05}$			1.84	1.84

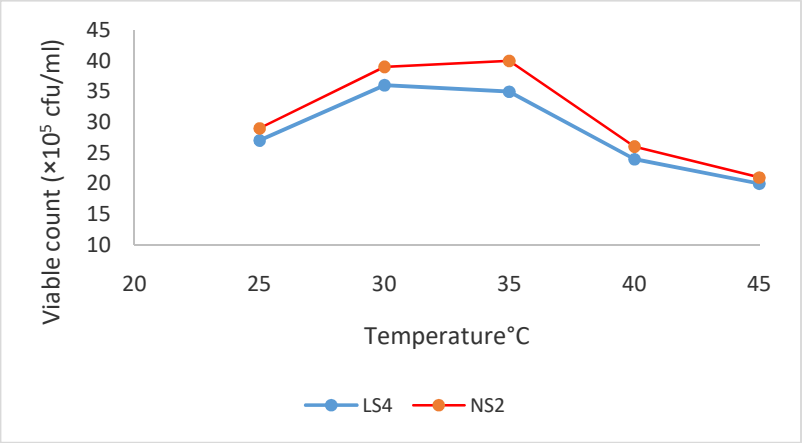


Fig 6: Effect of temperature on growth of selected bacterial isolates from spent mushroom compost

Temperature is the factor that affects the growth of bacteria. Zahid *et al*. (2015) reported that all isolates were able to grow in temperatures ranging between 25 and 40◦ C, and optimum temperature of 35°C was best for the growth of bacterial isolates. However, Rathaur *et al*., (2012) reported the growth of all isolates was good in the temperature range of 20ºC to 28ºC.

4.3.2.3 Effect of pH on the growth of selected bacterial isolates

Data presented in Table 12 represented the effect of different pH on the growth of selected bacterial isolates. The results showed that both the selected bacterial isolates were able to grow at pH range of 5-9.0 pH 7.0.However, the selected bacterial isolates showed very poor growth below pH 5 and after 9.0 pH. It was observed that optimum growth of bacterial isolates was recorded at pH of 7.0 and decreases of growth below and above optimum level (Fig 7).

Table 12: Effect of different pH on the growth of selected bacterial isolates

	Bacterial isolates		**Viable count (×10^6 cfu/ml)**	
pH	**NS2**	**LS4**	**NS2**	**LS4**
5.0	+	+	22	20
6.0	+	+	29	28
7.0	+++	+++	39	35
8.0	++	++	35	31
9.0	++	++	12	12.3
$CD_{0.05}$			**2.73**	**1.71**

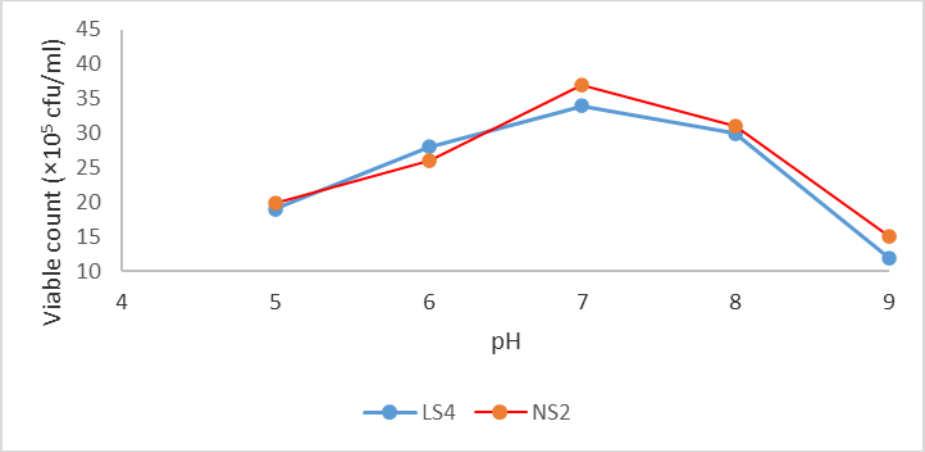


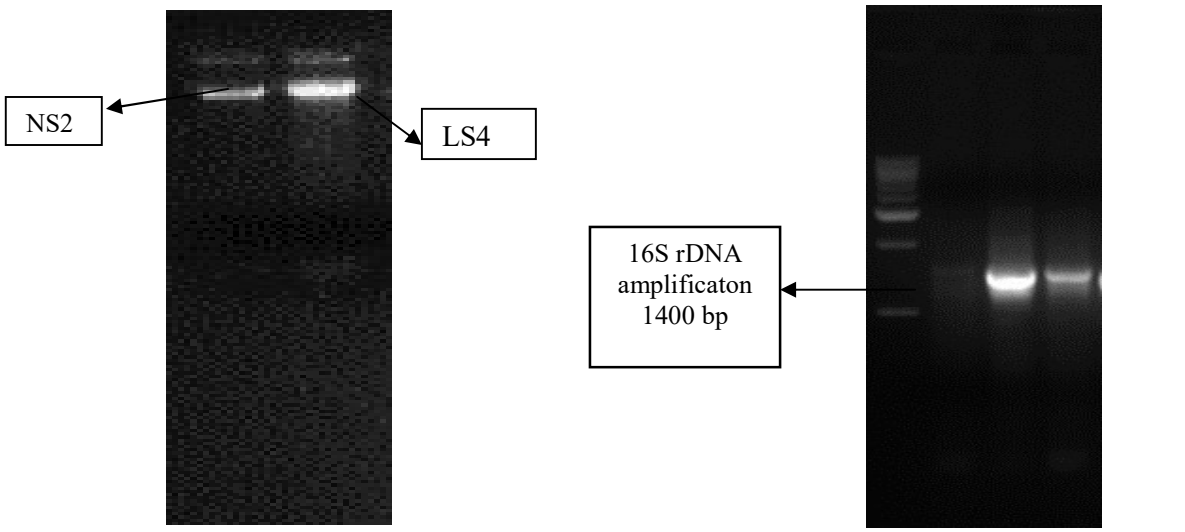
Fig 7: Effect of pH on growth of bacterial isolates from spent mushroom compost

The maximum bacterial growth was observed at pH 7.0 with viable count 39× 10^6 cfu/ml for NS2 and 35× 10^6 cfu/ml for LS4. The findings of current study are similar with the study conducted by Dinesh *et al*. (2015) who also reported that pH 7.0 is optimum for the growth of *Bacillus* and *Pseudomonas* sp. Similar results were reported by Rasul *et al*. (2012) for 29 isolates which could grow in a pH range of 4.0-10.0. Similar morphological, biochemical and physiological characteristics of *Bacillus* sp. and *Serratia sp*. have already been reported by Allaf (2010); Kaushal (2011), Ghani *et al*. (2013).

4.4 MOLECULAR CHARACTERISATION

The most efficient growth promoting two bacterial isolates viz., NS2 (GBP1) and LS4 (GPB2) were selected. These bacterial isolates exhibited all mushroom growth promoting traits and were selected for identification up to species level by molecular technique based on 16S rRNA sequencing. The DNA was extracted by conventional method (Sambrook *et al*., 1989) and purified DNA was amplified using Universal bacterial primers. The amplicon of expected size i.e. (1400 bp) was obtained (plate).

Fig 8. Neighbour joining tree based on relationship of bacterial isolates GPB1 with the analyzed sequences. (Accession number are mentioned isolate)



Genomic DNA of bacterial isolates | **Amplification of DNA**

Plate 8: Molecular identification of bacterial isolates based on 16 S rRNA amplification.

Corrected sequence of strain GPB1 (*Serratia fonticola*) Accession No. OP743914

TGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTCGAGCGGTAGCAC
AGGGAGCTTGCTCCTGGGTGACGAGCGGCGGACGGGTGAGTAATGTCTGGGAAACT
GCCCGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCTTC
GGACCAAAGTGGGGGACCTTCGGGCCTCACACCATCGGATGTGCCCAGATGGGATT
AGCTAGTAGGTGAGGTAATGGCTCACCTAGGCGACGATCCCTAGCTGGTCTGAGAG
GATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG
TGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTGTGAAG
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CACCGTTCATTGACGTTACTCGCAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCC
GCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCACGC
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CGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGAACCCTGGTAGTCCACGC
TGTAAACGATGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACG
CGTTAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGA
CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAAC
CTTACCTACTCTTGACATCCACAGAACTTTCCAGAGATGGATTGGTGCCTTCGGGAA
CTGTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTTAA
GTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGCGTAATGGCGGGAACTC
AAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCAT
GGCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCATATACAAAGAGAAGCGA
ACTCGCGAGAGCAAGCGGACCTCATAAAGTATGTCGTAGTCCGGATCGGAGTCTGC
AACTCGACTCCGTGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGA
ATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAA
GAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGG
GTGAAGTCGTACA

Corrected sequence of strain GPB2 (*Priestia megaterium*) Accession No. OP743915

GACGTTAGCGGCGGACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGA
TAACTTCGGGAAACCGAAGCTAATACCGGATAGGATCTTCTCCTTCATGGGAGATGA
TTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGTGCATTAGCTAGTTG
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CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATC
TTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTC
GGGTCGTAAAACTCTGTTGTTAGGGAAGAACAAGTACAAGAGTAACTGCTTGTACCT
TGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATA
CGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTTTC
TTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTGGAAACTGGGG
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TGACCTGGGCTACACACGTGCTACAATGGATGGTACAAAGGGCTGCAAGACCGCGA
GGTCAAGCCAATCCCATAAAACCATTCTCAGTTCG

Plate 9. Molecular identification of bacterial isolates GPB1, GPB2 based on 16S rRNA amplification

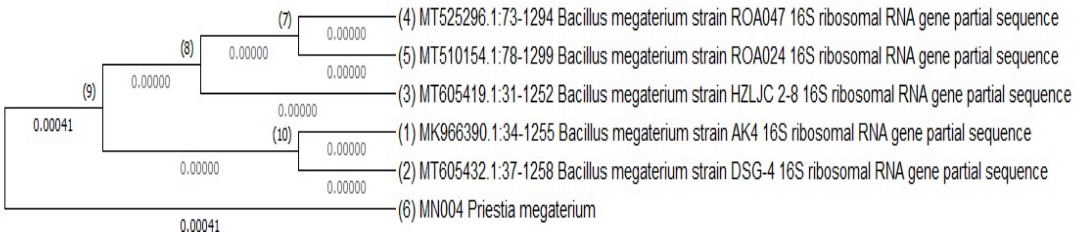

Fig 9. Neighbour joining tree based on relationship of bacterial isolates GPB2 with the analyzed sequences. (Accession number are mentioned isolate)

The sequence data of the 16S rRNA of selected isolates were subjected to BLAST analysis. As 16S rRNA gene sequence provide accurate grouping of organism even at subspecies level it is considered as a powerful tool for the rapid identification of bacterial species (Jill and Clarridge, 2004). The sequence analysis of 16S rRNA revealed that strain GPB1 (Accession No. OP743914) showed maximum similarity of 99.00 per cent with *Serratia fonticola* and strain GPB2 (Accession No. OP743915) showed maximum similarity of 99.00 per cent with *Priestia megaterium***.** The phylogenetic analysis of 16S rRNA sequence of the isolates along with the sequence retrieved from the NCBI was carried out with MEGA X using the neighbor joining method with 1,000 bootstrap replicates.

4.5 EFFECT OF SELECTED GROWTH PROMOTING BACTERIA ISOLATES ON GROWTH AND YIELD OF WHITE BUTTON MUSHROOM

4.5.1 Compatibility of selected bacterial isolates

The compatibility of selected bacterial isolates viz., GPB1, GPB2 were tested and showed in Plate5. It was found that the two selected bacterial isolates were able to grow on the nutrient agar plate without inhibiting the growth of the other isolate. Thus, the two growth promoting isolates GPB1 and GPB2 are compatible to each otherand thus used for the further experiment under net house conditions for cultivation of white button mushroom.

4.5.2 Effect of selected bacterial isolates on the growth parameters of White button mushrooms.

A persual of data presented in Table 13 revealed the effect of bacterial inoculation on the yield of white button mushroom and mushroom growth parameters. The application of selected bacterial isolates showed a significant variation for all the growth parameters of mushroom viz. total length of mushroom, length and width of stalk, diameter of cap, individual weight of mushroom, and yield of mushroom. It was found from the present

investigation that all growth parameters and yield showed significant increase over uninoculated control. Plate (9a and 9b) represents the growth characters of mushroom with different treatments. Overall a total of 20-24 days were taken for case run after spawning and 19-20 days taken for first harvest after casing for all the treatments.

Table 13: Effect of selected bacterial isolate on mushroom growth parameters.

Treatments	Total length of mushroom (cm)	Individual weight of mushroom(g)	Length of stalk (cm)	Width of stalk (cm)	Average diameter of cap (cm)	Yield (kg/100kg compost)
To1(uninnoculated control in casing)	1.80	12.50	0.70	1.06	2.33	10.63
To2(uninnoculated control in substrate)	1.93	12.63	0.80	1.36	2.36	10.63
T1(GPB1 on casing)	2.56	14.86	1.53	2.20	3.30	11.53
T2(GPB1 on substrate)	3.10	13.20	2.06	2.26	3.40	12.56
T3 (GPB2 on casing)	3.36	14.36	1.60	2.36	4.16	13.16
T4(GPB2 on substrate)	3.13	16.36	2.00	1.80	4.43	14.60
T5(GPB1 + GPB2 on casing)	4.30	18.20	2.40	2.26	5.46	15.66
T6(GPB1+GPB2 on substrate)	4.13	19.20	2.33	2.50	5.16	15.60
T7(GPB1+GPB2 on casing and substrate)	4.33	21.10	2.70	2.66	6.26	16.53
CD $_{0.05}$	**0.31**	**0.51**	**0.21**	**0.30**	**0.48**	**0.67**

The data presented in table 13 showed that the maximum total length of mushroom (4.33cm), individual mushroom weight (21.10g), width of stalk (2.66cm), length of stalk(2.70cm), average diameter of cap of mushroom (6.26cm) were recorded by Treatment T7 (GPB1+ GPB2) on casing and substrate whereas the minimum values total length of mushroom (1.80cm), individual weight of mushroom (12.50g) , width of stalk (1.06cm) , length of stalk (0.70cm), average diameter of cap of mushroom (2.33 cm)were recorded by Treatment TO1 which was uninnoculated control. The highest yield of mushroom (16.53kg) was also recorded with Treatment T7 which was statistically superior to all the treatments. It was found that with application of consortia of bacterial isolates NS2 and LS4 on casing as well as on substrate (T7) yield of mushroom increased with 55 percent over uninnoculated

Uninnoculated control TO1

Uninnoculated control TO2

Treatment T1

Treatment T2

Treatment T3

Treatment T4

Plate 10a : Effect of selected bacterial isolates on quality of mushroom

control (Fig 10).The results showed that inoculation of mushroom growth promoting bacteria treatment had more stimulating effect on growth and yield of mushrooms. The increase in growth parameters with biofertilizers may be attributed to the improved nutrient availability specially N (nitrogen fixation), P (P- solubilization) and Fe (siderophore) to the mushroom by direct mechanism.

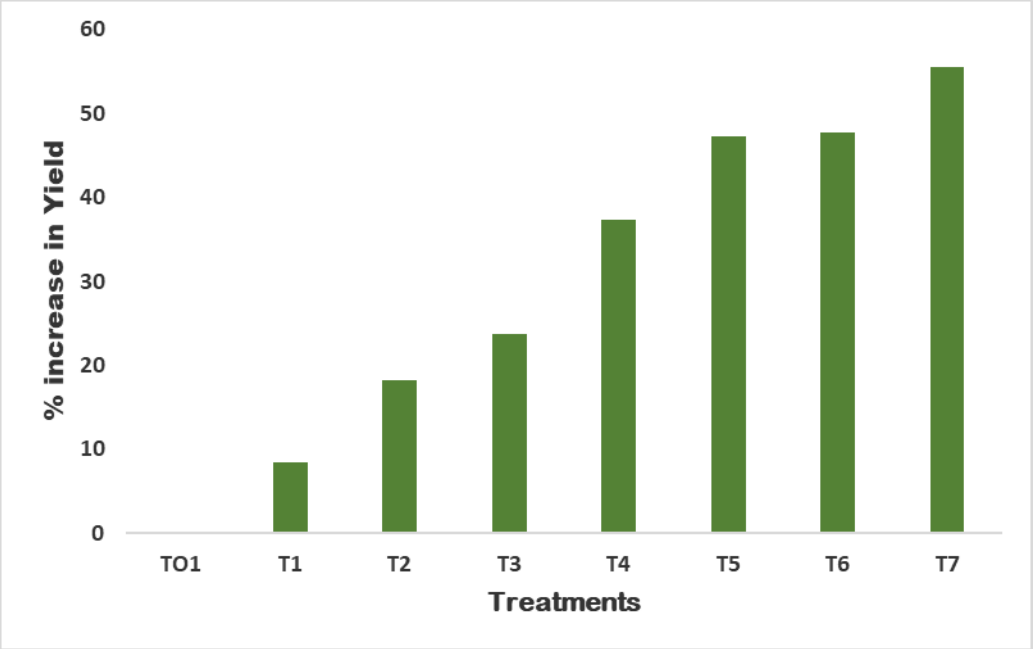

Fig 10: Percent increase in yield of white button mushroom.

Similar results were reported by Ahlawat (1998) who reported that more than 40 per cent isolates were found to increase the growth of strains S-11, S-310, and U-3. Kim *et al* (2008) reported that *Pseudomonas sp*.P7014 enhanced the growth of edible oyster mushroom *P.eryngii* in bottle cultures. Young *et al*. (2013) reported a significant increase of 64 percent in yield of *Agaricus blezzi* inoculated with two bacterial isolates *Exignobacterium* sp. (JN03) and *Arthobacter* sp. (JN12) over uninoculated cultures. Mohammad and Sabaa (2015) also recorded a 26.6 percent increase in mushroom yield when inoculated with *Pseudomonas putida* in comparison with uninnoculated control. Cetin *et al*. (2016) also recorded the positive effect of bacterial isolates, which provided 8–40 percent enhancement in the total yield of *A. bisporus* mushroom.

4.5.3. BIOLOGICAL EFFICIENCY

A perusal data in Table 14 showed that the biological efficiency for all the three flushes ranged between 15.32- 24.83%, 14.22- 22.76%, and 10.23- 15.56% respectively. A

significant increase of biological efficiency was recorded for all the treatment in all the three flushes of mushroom in comparison to uninoculated control. The highest biological efficiency was observed by treatment T7 (24.83 percent) (GPB1+ GPB2) with inoculation of on both casing and substrate) followed by T6. The results of present investigation indicate that inoculation of GPB into casing and substrate has great impact on yield and quality of white button mushroom. It was clearly observed from fig 10 that biological efficiency increased in all the treatments over uninnoculated control. It was found that treatment T7 showed 63.1 percent increase in biological efficiency in first flush, 60.2 percent increase in second flush, and 53.1 percent increase in third flush over uninoculated control.

Table14: Effect of selected bacterial isolates on biological efficiency of mushroom.

Treatments	**First flush (%)**	**Second flush (%)**	**Third flush (%)**
TO1(Uninnoculated casing)	15.32	14.22	10.23
TO2 (Uninnoculated substrate)	15.21	14.22	10.16
T1 (GPB1 on casing)	18.39	16.25	11.86
T2 (GPB1 on substrate)	17.90	17.38	10.93
T3 (GPB2 on casing)	19.52	16.44	12.60
T4 (GPB2 on substrate)	20.83	18.81	11.40
T5 (GPB1 + GPB2 on casing)	22.19	19.24	13.26
T6 (GPB1+ GPB2 on substrate)	22.47	21.34	13.40
T7(GPB1+ GPB2 on both Casing and substrate)	24.83	22.76	15.56
$CD_{0.05}$	0.68	0.24	0.57

The mushroom substrate and casing soil inoculated with MGP improved the mushroom yield and Biological efficiency significantly. This increase in growth and yield parameters may be due to availability of nutrients and metabolism and enzyme activities in the compost with the application of (MGPB) mushroom growth promoting bacteria. Bacteria were closely attached to mycelia surface and enhanced the growth. The findings of current study are similar with the study conducted by Khalili *et al*. (2015) who reported that biological efficiency were higher 12.1 percent and 20.64 percent and 13.43 percent and 21.08 percent of the control, respectively when substrate or both substrate and casing were inoculated with *P. putida*. This is in agreement with the findings of Iqbal *et al*., (2005), where they clarified that Oyster mushroom gave the maximum flushes on sawdust substrate.

Treatment T5

Treatment T6

Uninoculated control

Treatment T7

Plate 10 b : Effect of selected bacterial isolates on quality of mushroom

P.fluorescens strains reported to promote the formation of the primordium growth of fruiting body of *Pleurotus* (Kim *et al*.2008). Zarenejad *et al* (2012) also reported that among 23 listed bacterial strains *P. putida* was best for improving yield and fruiting bodies of *Agaricus bisporus*. Silva *et al.* (2020) reported BE ranged from 92.7 to 148.8 percent and yield from 349.3 to 560.5 g kg^{-1} in *Pleurotus ostreatus*. Liang *et al.* (2019) reported the biological efficiencies of *A. polytricha* cultivated on the experimental substrates were between 124.70 percent and 148.12 percent.

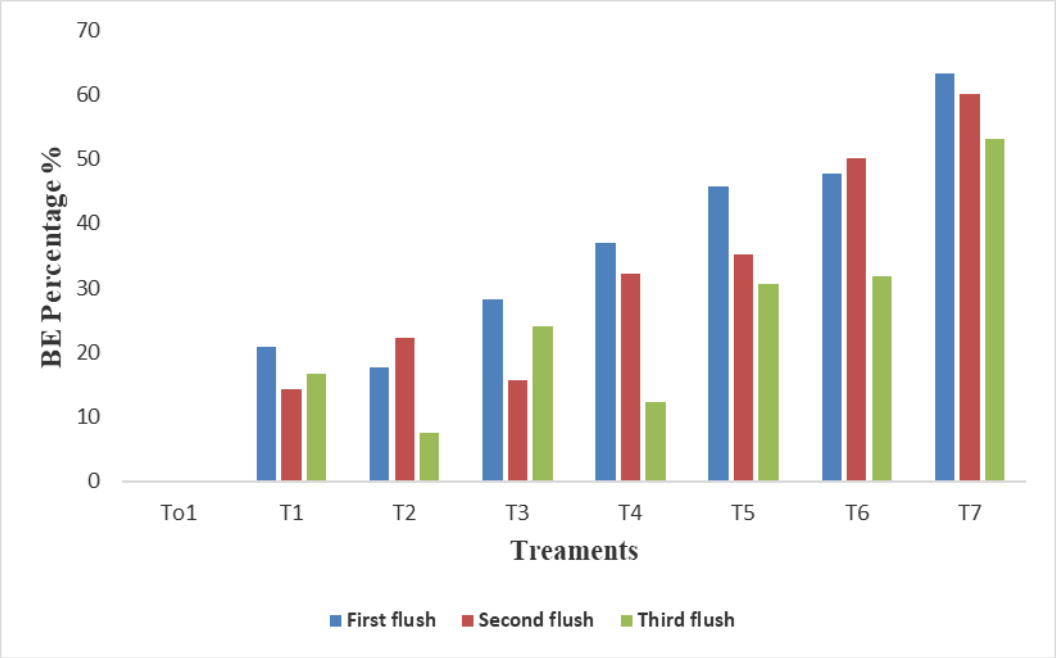

Fig 11: Effect of selected bacterial isolates on biological efficiencies of mushroom

4.5.4. Effect of selected bacterial cultures on quality parameters of white button mushrooms.

The data on quality parameters (Antioxidant enzyme assay, total phenolic content, total ash content, total protein content, total carbohydrate content and total crude fibre content) were presented in Table 15. A significant increase was found in all the treatments over uninnoculated control. The data revealed that The maximum antioxidant activity (32.56) , phenol content(12.40mg GAE/g), ash content (1.54 g/100g),protein content (4.10 g/100g), Crude fibre content (3.57g/100g) and carbohydrate content (61.60 g/100g) were recorded with treatment T7(GPB1 + GPB2 on casing and substrate) which was statistically superior to all the treatments .However the minimum antioxidant activity (21.51%) , phenol content(7.13 mg GAE/g), ash content (0.82g/100g),protein content (2.44g/100g),

carbohydrate content (35.36g/100g) and Crude fibre content (1.10g/100g) were recorded with uninoculated control.

Table 15: Effect of bacterial isolates on mushroom quality parameters.

Treatments	Quality Parameters (%)					
	Antioxidant enzyme assay (DPPH)(%)	Phenolic content	Ash content (g/100g sample)	Protein content (g/100g)	Carbohydrate content g/100g	Crude fibre content
T_{O1}	21.51	7.13	0.82	2.44	35.36	1.10
T_{O2}	21.54	7.26	0.82	2.35	35.53	1.92
T_1	24.78	8.20	1.28	3.05	42.36	2.17
T_2	27.31	9.33	1.26	3.11	51.90	2.11
T3	28.67	8.46	1.15	3.43	48.93	2.54
T4	26.44	10.46	1.30	3.34	57.03	3.08
T5	28.14	11.43	1.42	3.89	59.16	3.46
T6	27.35	10.73	1.45	3.70	59.50	3.37
T7	32.56	12.40	1.54	4.10	61.60	3.57
$CD_{(0.05)}$	**0.96**	**0.38**	**0.04**	**0.13**	**0.80**	**0.09**

It is evident from Fig (12) that all the quality parameters are correlated with each other and showed more than 50 percent increase for quality parameters with T7 treatments over uninnoculated control. The increase of antioxidant activity may be due to increase of phenol contents. The increase of quality parameter in the treatments with inoculation of growth promoting bacteria may be due to enhanced availability of essential nutrients in the compost, responsible for synthesis of biochemical constituents of white button mushroom. The consortia of both the bacterial isolates had more impact (T7) than the single bacterium. The other possible reason of increase may be attributed to increase of carbohydrates in all treatment which directly affect the other quality attributes of mushroom.

Similar results were reported by Prabhu *et al*. (2016) maximum DPPH radical scavenging activity of 37.04±0.15 and 28.04±0.41 % in *Pleurotus florida* and *Calocybe indica*. The results of phenol content are in accordance with Alispahić *et al* .(2015) who reported total phenolic content in white button mushroom ranged from 4.94 mg GAE/g to 7.66 mg GAE/g. The phenolic content in a study by Giannenas *et al*. (2011) was 8.85 mg GAE/g of dry weight, and Dubost *et al*. (2007) reported 8.00-10.65mg GAE /g in button mushroom. Maknali *et al. (*2021) reported that inoculated treatment with Pseudomonas had

8.46 percent higher ash content than the non-inoculated treatment. The earlier reports showed varied amount of ash content in white button mushroom which may be due to variation of compost of nutrient availability. The total ash content reported by Alam *et al*. (2008) in *Pleurotus ostreatus, P. sajor-caju, P. florida* and *C. indica* were 1.1~1.3 g, 1~1.2 g, 1.1~1.2 g and 1.2~1.4 respectively. The ash content reported by Okechukwu *et al*., (2011) showed a range of 3.20 - 25.10 percent in *Agaricus bisporus*. Sinha *et al*. (2021) reported the ash content in the fresh white button mushroom was found to be 0.93±0.01 g/100g.

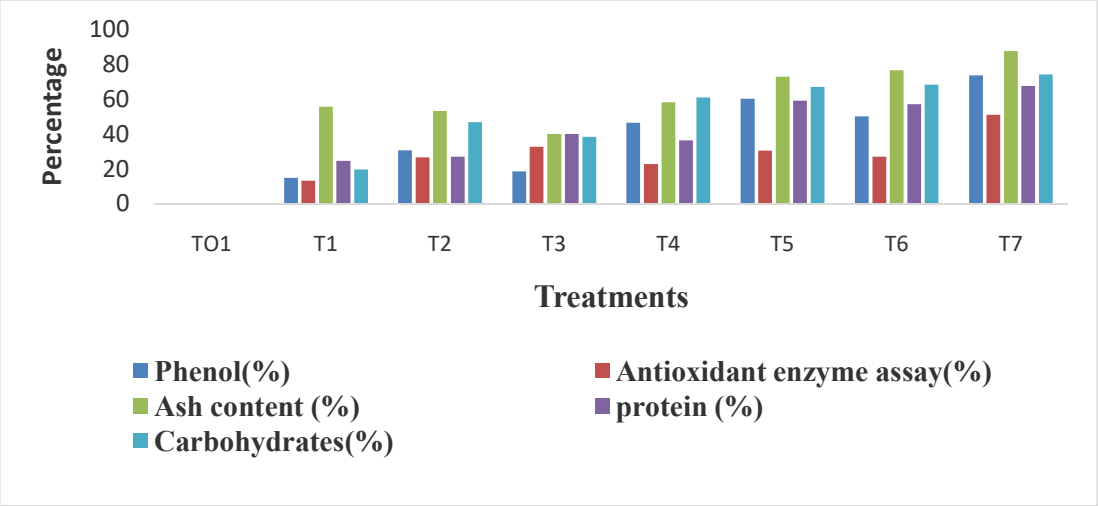

Fig 12: Percent increase in mushroom quality parameter.

Sinha *et al*. (2021) reported the protein value as 3.27±0.12/100g in fresh white button mushroom**.** These high carbohydrate contents are in accordance with Shin *et al*.,(2007), Hong *et al*.,(2007),Dhundar *et al* ., 2008). Kumari and Srivastava (2020) who also reported highest carbohydrate content in *Astraeus hygrometricus* (53.2g percent), followed by *Volvariella volvacea* (48.8 percent), *Pleurotus ostreatus* (42.7 percent), *Termitomyces clypeatus* (36.5 percent) respectively on dry weight basis. However Sinha et al. (2021) found the mean quantum of carbohydrate in the fresh white button mushroom was 2.66±0.61 %. A lot of variation was reported for crude fibre content of mushroom. Masamba and Mawale (2010) reported crude fibre content within the range of 0.2 – 0.5(g/100g) in *T. letetsui, C. cibarius and A. bisporous.* Zahid *et al.,* (2010) reported fibre content content of mushrooms to be in a range of 0.58-1.11g% in oyster mushroom and milky white mushrooms (*Calocybe indica*).

4.5.5. Effect of selected bacterial isolates on nutrient uptake

The tried treatments registered a significant increase in NPK content of mushroom (table 16).The maximum Nitrogen content (6.30 %), Phosphorus content (0.81%), and

potassium content (5.00%) were recorded by Treatment T7 followed by Treatment T5 .However, the minimum was recorded by treatment TO1 and TO2 which were uninnoculated control. Fig 13 indicates that with application of consortium of selected bacterial isolates T7 nitrogen increased upto 85.2 percent, P upto 72 percent and K values increased upto 80 percent over uninnoculated control. The NPK content in mushroom may be due to mineralization of compost via application of biofertilizers due to phosphate solubilization, Siderophore production, and nitrogen fixation. Similar results were reported by Khalili *et al*. (2015) nitrogen content within range of 4.5– 5.3 % and potassium within range of 4.3 – 5.1%).

Table 16: Effect of bacterial isolates on N P K of white button mushroom.

Treatments	Nutrient uptake (%)		
	N (%)	P (%)	K (%)
TO1(Uninnoculated casing)	3.46	0.47	2.66
TO2 (Uninnoculated substrate)	3.53	0.48	2.73
T1 (GPB1 on casing)	4.20	0.57	3.63
T2 (GPB1 on substrate)	4.63	0.63	3.13
T3 (GPB2 on casing)	4.80	0.65	3.70
T4 (GPB2 on substrate)	5.33	0.69	4.13
T5 (GPB1 + GPB2 on casing)	5.96	0.76	4.43
T6 (GPB1+ GPB2 on substrate)	5.93	0.78	4.16
T7(GPB1+ GPB2 on both Casing and substrate)	6.30	0.81	5.00
$CD_{0.05}$	**0.43**	**0.03**	**0.20**

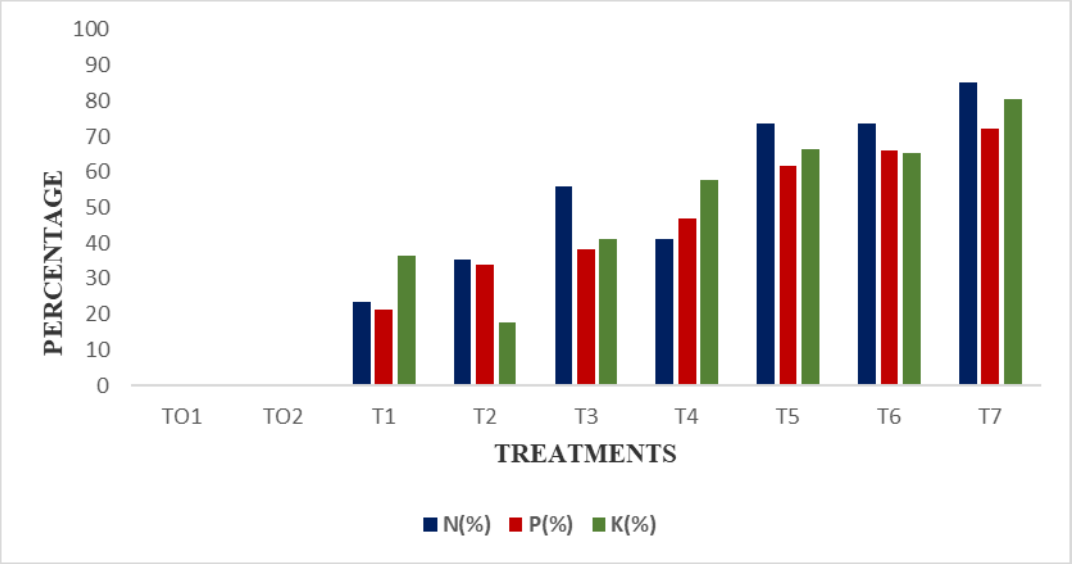

Fig 13: Percent increase of nutrient content N P K of mushroom

4.6 Effect of selected bacterial isolates on physico chemical properties, nutrient availability and microbial count of compost.

The data on the physico-chemical properties of soil were recorded at the start and termination of the experiment.

4.6.1 Physico-chemical properties, nutritional status and total microbial count of compost (initial status)

The data pertaining to initial physico-chemical status of the soil are presented in the Table 17 A perusal of data showed that the compost was nearly neutral (pH 6.22), EC (1.56dSm^{-1}) and organic carbon was 12.30 per cent. The available N (0.59 percent) and P (0.36 percent) contents were in medium range. However, available K (0.90 percent) was in high range. The total bacterial count was 6.12×10^{6} cfu/g soil on nutrient agar medium.

4.6.2 At the end of experiment

The data pertaining to effect of different treatments on the physico-chemical and biological properties of compost are presented in Table 17. Data revealed that none of the treatment influenced compost pH and electrical conductivity over control. However, organic carbon significantly increased over control. The corresponding values recorded were 6.14-6.88 for pH, 1.5 to 1.74 (dSm^{-1}) for electrical conductivity and 20.18 to 26.15 % organic carbon. All the treatments registered significant increase in available NPK content. The highest available N content (1.07 %) and available P content (0.79 %) of compost was recorded with treatment T_7 (GPB1 + GPB2) However, the minimum (0.62%) available N content and available P content (0.42%) of compost were recorded with treatment T_{O1} (Uninoculated control).The maximum available K content (1.70%) of soil was recorded with treatment T_7(GPB1 + GPB2).The minimum (0.97%) available K content of compost was recorded with T_{O1} (Uninoculated) control. The figure 13 showed per cent increase of nutrient uptake after the completion of experiment over control under net house conditions. The treatment T7 registered significant per cent increase 72.5% in nitrogen, 88 % increase in phosphorus and 75.2 % increase in potassium over uninoculated control (T1).

The solubilization of P in the rhizosphere is the most common mode of action implicated in biofertilizer that increases nutrient availability to host plants (Bhattacharyya and Jha, 2012). The application of biofertilizer have enhanced the solubilization, mobilization, availability and uptake of N, P and K by the mushroom (Zahir *et al*., 2004).The

results obtained in the present study with respect to N, P and K are in agreement with the results reported by Gupta et al. (2015) and Gopalkrishan *et al.*,(2017). Jha and Subramaniam, (2013) reported that the PGPR inoculated plants were increase the concentrations of N (26 per cent), P (16 per cent), K (31 per cent. Similar results has been reported by Duhan 2013, Zhang *et al*., 2017, Sharma *et al*., 2017).

Table 17. Physico-chemical and biological properties at the end of the experiment

Treatments	pH	EC (dSm-1)	OC (%)	Available N (%)	Available P (%)	Available K (%)	Microbial count ($\times 10^6$ cfu/g soil
Initial status (compost mixture)	6.22	1.54	12.30	0.59	0.36	0.90	6.12
TO1	6.53	1.43	20.18	0.62	0.42	0.97	6.23
TO2	6.14	1.47	21.31	0.62	0.43	0.98	6.25
T1	6.45	1.49	22.30	0.85	0.52	1.42	8.10
T2	6.40	1.56	23.12	0.73	0.56	1.33	8.30
T3	6.88	1.64	21.62	0.86	0.60	1.52	7.30
T4	6.61	1.73	23.25	0.81	0.56	1.61	8.70
T5	6.65	1.74	25.44	0.94	0.64	1.60	9.16
T6	6.55	1.58	24.55	0.95	0.72	1.67	10.56
T7	6.36	1.55	26.15	1.07	0.79	1.70	10.83
$CD_{0.05}$	N/S	N/S	0.86	0.04	0.06	0.08	0.54

4.6.3 Microbial count (at the termination of experiment)

The data presented in Table 17 revealed the significant variation in the microbial count over uninoculated control. The total microbial count was varied from (6.23 to 10.83 × 10^6 cfu/g soil) NA medium. The maximum (10.83 × 10^6 cfu/g soil) microbial counts on NA medium was observed in spent mushroom compost whose substrate were treated with consortia of bacterial isolates (GPB1 +GPB2)(T7). Whereas, the minimum count (6.23× 10^6 cfu/g soil) was recorded with uninoculated control (TO2). Ahlawat and Manikandan (2015) reported that after inoculation of *Bacillus subtilis* casing treatments enhanced 1.72 and 2.73 folds higher bacterial count than control FYM + SMS based casing. Similarly, Riahi *et al*.,(2011) concluded that the inoculation of native *P. putida* isolated from casing soil at the primordia formation stage would be very efficient for increasing mushroom yield and quality.

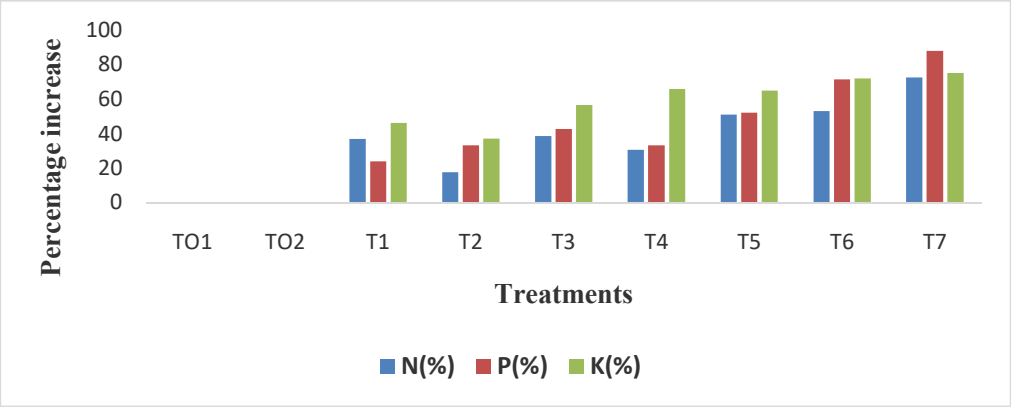

Fig 14: Percent increase in NPK.

Chapter - 5

SUMMARY AND CONCLUSION

❖ The present study entitled **'Characterization of Growth promoting bacteria from spent mushroom compost and their efficacy on yield and quality of white button mushroom'** will be carried out in Laboratory of Basic sciences and also in Mushroom farm under Lab conditions of Dr YSP university of Horticulture and Forestry , Nauni Solan, HP During 202--2022. The studies were conducted to select best plant growth promoting rhizobacteria from spent mushroom compost and to study their response in the improvement of growth and yield of white button mushroom.

❖ The salient observations recorded from the present investigation are summarized here:

1. A variation was observed for bacterial count isolated from spent mushroom compost from different locations of distt Solan. The maximum bacterial population (6.73×10^{6}cfu/g) was recorded from compost collected from DMR Chambaghat Solan.
2. A total of 25 bacterial isolates 14 from Chambaghat,7 from Nauni and 4 from Local site were selected on the basis of predominant growth 48 percent were P-solubilizer,36 percent were HCN producers, 52 percent were siderophore producers and 40 isolates were IAA producers and 60 percent were nitrogen fixers.
3. Among the selected bacterial isolates from the maximum P-solubilization (93.73%) was recorded with bacterial isolate NS2 on solid PVK medium. In the liquid medium, maximum P-solubilization (214.76 µg/ml) was recorded with NS2 bacterial with the decrease in final pH from 7.00 to 5.67, whereas minimum was recorded with bacterial isolate CS1 (22.03 %).
4. Among the selected twenty five bacterial isolates spent mushroom compost maximum (96.37%) siderophore unit (SU) was observed in NS2 bacterial isolate The minimum (17.69%) siderophore production efficiency was recorded with isolate NS4.On liquid assay maximum (286.21µg/ml) siderophore production was recorded with bacterial isolates NS2 with decrease in pH (7.00 to 4.18), whereas minimum was recorded with bacterial isolate NS4 (14.31µg/ml.)

5. Bacterial isolate NS2 from compost of Nauni produced a significantly higher amount of IAA (79.60 µg/ml) with decrease in pH from 7.00 to 5.70, whereas, minimum IAA production was recorded with isolate NS5 (17.19 µg/ml) after 48 hrs of incubation at 37°C.
6. On the basis of growth promoting traits viz., P-solubilization, siderophore production, IAA production and HCN production two isolates NS2 & LS4 were selected for further study.
7. The selected isolates (NS2 and LS4) were white in color, cluster in arrangement, entire in margin, and slimy colonies with raised elevation in LS4 and convex elevation of NS2. In Gram', NS2 bacterial isolate was gram positive rods and LS4 was negative for gram staining. On the basis molecular identification GPB1 was identified as *Serratia fonticola* and GPB2 as *Priestia megaterium.*
8. Among the selected bacterial isolates both the bacterial isolates were negative for indole test, Starch hydrolysis, casein hydrolysis and positive for methyl red test, Voges Prokauer test, catalase test, and citrate utilization test. In case of carbon utilization these isolates were able to ferment xylulose, fructose, sucrose, galactose, maltose, manitol and glucose except lactose The biochemical analysis of the selected bacterial isolates indicated that most of these as *Bacillus* and *Pseudomonas*.
9. The selected bacterial isolates showed maximum growth at 35°C after an incubation period of 48 h. Both the selected isolates were (NS2 and LS4) able to grow at pH ranges from 5.0 to 8.0. However, none of them was found to grow at pH below 4.0 and above pH 9.0.
10. The application of selected bacterial isolates significantly increased the growth and yield parameters of mushroom over uninoculated control. the maximum total length of mushroom (4.33cm), individual mushroom weight (21.10g), width of stalk (2.66cm), length of stalk(2.70cm), Average diameter of cap of mushroom (6.26cm) were recorded by Treatment T7 (GPB1+ GPB2 on casing and substrate whereas the minimum values total length of mushroom (1.80cm), Individual weight of mushroom (12.50g) , width of stalk (1.06cm) , length of stalk (0.70cm), Average diameter of cap of mushroom (2.33 cm)were recorded by Treatment TO1 which was uninnoculated control. The highest yield of mushroom (16.53 kg) was also recorded with Treatment T7 which was statistically superior to all the treatments.
11. The treatment T7 (GPB1+ GPB2 on casing and substrate) registered 55 percent increase in yield of white button mushroom over uninnoculated control.

12. The highest biological efficiency was observed by treatment T7 which showed 63.1 percent increase in biological efficiency in first flush, 60.2 percent increase in second flush, and 53.1 percent increase in third flush over uninoculated control.
13. The maximum antioxidant activity (32.56) , phenol content(12.40mg GAE/g), ash content (1.54 g/100g),protein content (4.10 g/100g), Crude fibre content (3.57g/100g) and carbohydrate content (61.60 g/100g) were recorded with treatment T7(GPB1 + GPB2 on casing and substrate) which was statistically superior to all the treatments .However the minimum antioxidant activity (21.51) , phenol content(7.13 mg GAE/g), ash content (0.82g/100g),protein content (2.44g/100g), carbohydrate content (35.36g/100g) and Crude fibre content (1.10g/100g) were recorded with uninoculated control.
14. A significant increase in quality parameters were recorded with combined application of bacterial isolate over control. The treatment T_7 (GPB1+ GPB2 with inoculation of on both casing and substrate) registered 51.3 per cent increase in antioxidant enzyme assay content,67.83 per cent increase in protein content , 74.5 percent in carbohydrate content , 87.8 per cent increase in ash content content over uninoculated control (T_1).
15. The highest available N content (6.30 %), available P content (0.81) and available K content (5.00%) of soil was recorded with treatment T_7 (GPB1+ GPB2 with inoculation of on both casing and substrate). The minimum (3.46%) available N content, available P content (0.47%) and available K content (2.73%) of white button mushroom were recorded with treatment T_1 (Uninoculated control).
16. The treatment T7 registered 85.2 % percent increase in nitrogen content ,72.3 percent increase in phosphorus content and 80.4 % increase in potassium content of white button mushroom over uninoculated control

17. All the treatments registered significant increase in available NPK content of compost. The highest available N content (1.07 %) and available P content (0.79 %) of compost was recorded with treatment T_7 (GPB1 + GPB2 on casing and substrate) However, the minimum (0.62 per cent) available N content and available P content (0.42 per cent) of compost were recorded with treatment T_{O1} (Uninoculated control).The maximum available K content (1.70 %) of soil was recorded with treatment T_7(GPB1 + GPB2 on casing and substrate).The minimum (0.97 per cent) available K content of soil was recorded with T_{O1} (Uninoculated) control.

18. The total microbial count was varied from (6.2 to 10.8 × 10^6 cfu/g soil) NA medium. The maximum (10.8 × 10^6 cfu/g soil) microbial counts on NA medium was observed in spent mushroom compost whose substrate were treated with consortia of bacterial isolates (GPB1 + GPB2) (T7). Whereas, the minimum count (6.2× 10^6 cfu/g soil) was recorded with uninoculated control (TO1).

From the present investigation, it was concluded that spent mushroom compost can be a good source of growth promoting bacterial for mushroom cultivation. On the basis of efficiency selected isolates, the consortia of two isolates NS2 and LS4 have significantly increased yield and quality characteristics of white button mushroom.. The consortia of both the isolates increase available NPK content of compost which also increased the nutritional status of mushrooms. Hence, these isolates have enormous potential to be used as multifunctional biofertilizer for enhanced productivity of white button mushrooms. The developed technology may be recommended to the farmers after mutilocational trials.

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APPENDIX-I

1.1 Preparation of the standard curve for P- estimation

i) 50 ppm standard solution of P was prepared by dissolving 0.2195 g KH_2PO_4 in distilled water and made final volume to 1000 ml, the working standard solution was prepared by following procedures:

Volume of standard solution (ml)	Concentration (ppm)			
0.5	1	+ 5ml Ammonium molybdate	+1 ml. working solution $SnCl_2$	Final volume 25ml.
1.0	2			
1.5	3			
2.0	4			
2.5	5			
3.0	6			

ii) Estimation of Phosphorous in sample

a) 50 ml PVK broth + 10 per cent inoculum (1 O.D at 540nm)

b) Incubation under shaking condition

c) Centrifugation

d) 1ml Culture fitrate + distilled water = 25 ml (dilution 25 times)

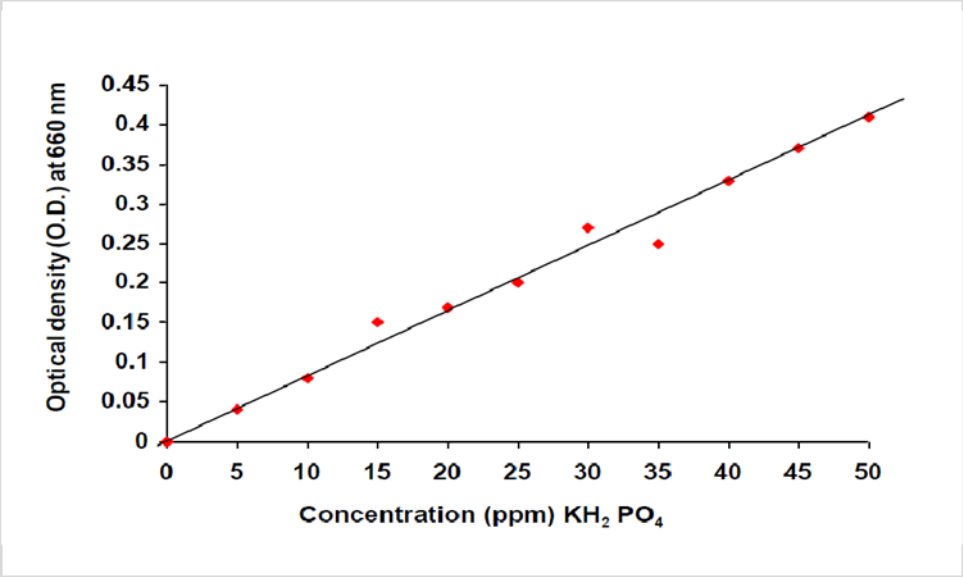
e) 5ml of above CF (step d) + 5ml. Ammonium molybdate + 1ml working solution of $SnCl_2$ + d. w = final volume to 25ml (dilution 5 times) O.D at 660 nm.

Total dilution 25 x 5 = 125 times

iii) Calculations:

Concentration (ppm) from standard curve x 125 (dilution factor) = P-solubilized (ppm)

Final concentration calculated after deducting from P-solubilized in control



Standard curve for Phosphorous

1.2 Composition of CAS assay solution

1. **2 mM CAS (stock solution):** 0.121 g CAS in 100 ml distilled H_2O

2. **1 mM Fe (stock solution):** 1 mM $FeCl_3.6H_2O$ in 10 mM HCl

3. **Piperazine buffer:** Dissolved 4.307 g piperazine in 30 ml distilled water. Added 6.75 ml concentration HCl to bring the pH to 5.6

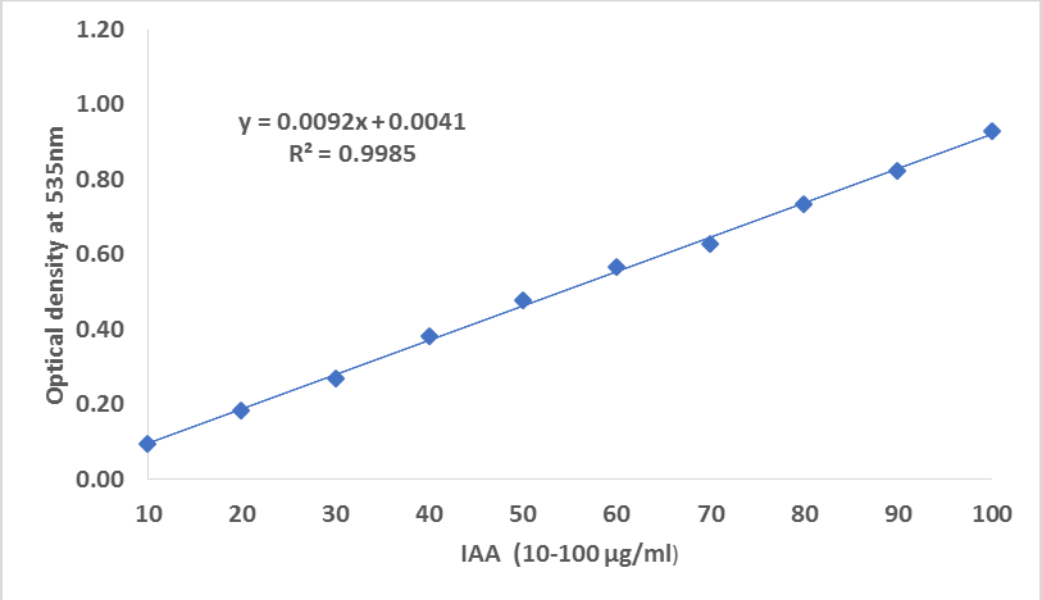
4. **Hexadecyl trimethyl ammonium bromide (HDTMA):** Dissolved 0.0219g HDTMA in 50 ml distilled water in a 100 ml mixing cylinder.

Procedure: Mixed 1.5 ml Fe solution with 7.5 ml CAS solution and added to the HDTMA in the mixing cylinder. Added piperazine solution to the mixing cylinder and brought volume up to 100 ml with water.

1.3 Preparation of standard curve (100 ppm) for IAA:

10 mg of IAA (99.00 %) pure was dissolved in 50 ml distilled water and the final volume was made to 100 ml in a volumetric flask. This is 100 ppm standard solution prepared by methods as described in following table.

IAA (ml)	Distilled water (ml)	Final volume (ml)	Salkowski regent (ml)	ppm (ml)	Optical density (O.D.) at 535 nm
0.3	2.7	3	2	10	0.929
0.6	2.4	3	2	20	0.824
0.9	2.1	3	2	30	0.734
1.2	1.8	3	2	40	0.628
1.5	1.5	3	2	50	0.567
1.8	1.2	3	2	60	0.476
2.1	0.9	3	2	70	0.383
2.4	0.6	3	2	80	0.268
2.7	0.3	3	2	90	0.184
3.0	0.0	3	2	100	0.095



Standard curve of indole-3-acetic acid

APPENDIX-II

A) Effect of bacterial inoculation on per cent increase average yield of mushroom.

Treatments	Per cent increase on yield Over T1(Control)
TO1(Control)	0
TO2 (control)	0
T1 (GPB1 on casing)	8.46
T2 (GPB1 on substrate)	18.15
T3 (GPB2 on casing)	23.8
T4 (GPB2 on substrate)	37.34
T5 (GPB1 + GPB2 on casing)	47.31
T6 (GPB1+ GPB2 on substrate)	47.75
T7(GPB1+ GPB2 on both Casing and substrate)	55.5

B) Effect of rhizobial inoculation on per cent increase on available NPK

Treatments	**Per cent increase on root length**		
	N	**P**	**K**
TO1(Control)	0	0	0
TO2 (control)	0	0	0
T1 (GPB1 on casing)	23.5	21.2	36.4
T2 (GPB1 on substrate)	35.2	34	17.6
T3 (GPB2 on casing)	55.8	38.2	41.3
T4 (GPB2 on substrate)	41.1	46.8	57.8
T5 (GPB1 + GPB2 on casing)	73.5	61.7	66.5
T6 (GPB1+ GPB2 on substrate)	73.7	65.9	65.4
T7(GPB1+ GPB2 on both Casing and substrate)	85.2	72.3	80.4

C) Effect of rhizobial inoculation on per cent increase on available NPK

Treatments	**Per cent increase in BE**		
	1st flush	**2nd flush**	**3rd flush**
TO1(Control)	0	0	0
TO2 (control)	0	0	0
T1 (GPB1 on casing)	20.9	14.27	16.73
T2 (GPB1 on substrate)	17.6	22.2	7.5
T3 (GPB2 on casing)	28.3	15.6	24.01
T4 (GPB2 on substrate)	36.9	32.2	12.2
T5 (GPB1 + GPB2 on casing)	45.8	35.15	30.5
T6 (GPB1+ GPB2 on substrate)	47.7	50.07	31.8
T7(GPB1+ GPB2 on both Casing and substrate)	63.24	60	53.1

APPENDIX-III

A) ANNOVA OF BACTERIAL COUNT

Source of Variation	DF	Mean Squares
		Bacterial count
Treatment	2	1.760
Error	6	0.001
Total	8	

B) ANOVA OF P-SOLUBILIZATION

Source of Variation	DF	Mean Squares	
		Qualitative	Quantitative
Treatment	11	1469.388	4887.085
Error	24	6.138	6.001
Total	35		

C) ANNOVA OF SIDEROPHORE PRODUCTION

Source of Variation	DF	Mean Squares	
		Qualitative	Quantitative
Treatment	11	1,550.331	27,419.982
Error	24	0.659	13.223
Total	35		

D) ANNOVA OF IAA PRODUCTION

Source of Variation	DF	Mean Squares	
		Qualitative	pH
Treatment	11	1,427.035	3.007
Error	24	0.624	0.449
Total	35		

E) ANNOVA OF MUSHROOM YIELD PARAMETERS

Source of Variation	Df	Mean Sum of Square				
		Total length of mushroom (cm)	Individual weight of mushroom(g)	Length of stalk(cm)	Width of stalk(cm)	Average diameter of cap(cm)
Treatment	9	2.764	28.623	1.457	0.861	5.652
Error	17	0.032	0.086	0.016	0.030	0.077
Total	26					

F) ANNOVA TABLE OF MUSHROOM QUALITY PARAMETERS

Source of Variation	Df	Mean Sum of Square					
		Antioxidant enzyme assay (DPPH) (%)	Phenolic content	Ash content (g/100g sample)	Protein content (g/100g)	Carbohydrate content g/100g	Crude fibre content
Treatment	9	36.768	10.491	0.198	1.089	316.695	2.118
Error	17	0.303	0.049	0.001	0.006	0.213	0.003
Total	26						

G) ANNOVA OF NPK OF WHITE BUTTON MUSHROOM

Source of Variation	Df	Mean Sum of Square		
		N(%)	P(%)	K(%)
Treatment	9	3.316	0.047	1.798
Error	17	0.062	0.000	0.016
Total	26			

H) ANOVA OF NPK SOIL PHYSICO-CHEMICAL PROPERTIES

Source of Variation	Df	Mean Sum of Square		
		N(%)	P(%)	K(%)
Treatment	9	0.080	0.055	0.290
Error	17	0.001	0.001	0.002
Total	26			

DEPARTMENT OF BASIC SCIENCES
DR Y S PARMAR UNIVERSITY OF HORTICULTURE AND FORESTRY
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Title of Thesis	**:**	**"Characterization of growth promoting bacteria from spent mushroom compost and their efficacy on quality and yield of white button mushroom"**
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Major Discipline	**:**	Microbiology
Minor Discipline	**:**	i) Biochemistry ii) Soil Science and Water Management
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Total Pages of the Thesis	**:**	81+vi
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ABSTRACT

Microrganisms in mushroom casing and substrate play a crucial role in initiation and development of primordial. The spent mushroom compost can be a good source of growth promoting bacterial for mushroom cultivation. The present investigation entitled "Characterization of growth promoting bacteria from spent mushroom compost and their efficacy on quality and yield of white button mushroom" was carried out in the Microbiology Laboratory of Department of Basic Sciences during the years 2020-2022.The samples of spent mushroom compost (SMC) were collected from different location of Solan (Chambaghat (DMR), Nauni (UHF), Local farmer (Dharja) The isolation of bacterial isolates were carried out by using Nutrient agar medium. A total of 25 bacterial isolates from SMC were selected and purified from spent mushroom compost. Out of 25 bacterial isolates (14 from Chambaghat,7 from Nauni, 4 from Local farmer site) 12 were P-solubilizer, 15 were nitrogen fixers, 9 were HCN producers, 13 were siderophore producers and 10 were IAA producers. Only 2 bacterial isolates (GPB1 and GPB2) out of 25 isolates were selected on the basis of various PGPR traits .Bacterial isolate NS2 showed maximum P-solubilization qualitative (93.3%) and quantitative (214.76 µg/ml), siderophore production (96.37%) on solid CAS medium and (286.21µg/ml) in liquid assay, IAA production (79.60 µg/ml), HCN .These two bacterial isolates (GPB1, GPB2) were tested for growth of white button mushroom in field experiment. Mushrooms innoculated with Treatment 7(GPB1+ GPB2 on casing and substrate) showed significant increase in yield (75 %) and biological efficiency in all three flushes over Uninoculated control. The treatment T7 (GPB1+ GPB2) registered 51.3 per cent increase in antioxidant enzyme assay content,67.83 per cent increase in protein content , 74.5 percent in carbohydrate content , 87.8 per cent increase in ash content over uninoculated control (T1).This treatment also increased NPK content of compost over uninoculated control. Hence these bacterial isolates have enormous potential to be used as biofertilizers for enhanced growth and quality of white button mushroom.

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Date:

Signature of the Major Advisor
Name: Dr (Mrs) Neerja Rana
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Fellowship/ Scholarship/ Gold Medals/Awards/ Any other Distinction : Yes

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(Manjula Bhardwaj)