Microbial Augmentation of Low Productive Soil Towards Increased Productivity in Organic Farming of Soybean Plants

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By

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This thesis is dedicated to my late parents,
whose visions and motivations are my strength, both in their presence and absence.

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List of Abbreviations

- + : Positive
- - : Negative
- 16S rRNA: 16 Svedberg unit ribosomal ribonucleic acid
- A: Absorbance
- ABA: abscissic acid
- ACC: Aminocyclopropane 1-carboxylic acid
- Al: Aluminum
- AMF: Arbuscular Mycorrhizal Fungi
- ANOVA: Analysis of variance
- B: Boron
- BHI: Brain Heart Infusion
- BOD: Biological oxygen demand
- BSM
- BNF: biological nitrogen fixation
- C: Carbon
- Ca: Calcium
- CAS: Chrome Azurol S
- Cfu: Colony forming unit
- Chl: Chlorophyll
- Cl: Chlorine
- cm: Centimetre
- Co: Cobalt
- CO_2: Carbon dioxide

- CFS: cell free supernatant
- CRA: Congo Red Agar
- Cu: Copper
- DF media: Dworkin-Foster media
- DNA: Deoxy ribonucleic acid
- EBI: European Bioinformatics
- FAO: Food and Agricultural Organisation
- Fe: Iron
- $FeCl_3$: Ferric chloride
- FW: Fresh weight
- *g*: acceleration due to gravity, 9.8 m/s^2
- GA: Gibberelic acid
- H_2O: Water
- H_2O_2: Hydrogen peroxide
- HDTMA: hexadecyltrimethylammonium bromide
- HCl: Hydrochloric acid
- Hydrogen (H)
- I: Iodine
- IAA: Indole acetic acid
- ICAR: Indian Council of Agriculture Research
- ISR: Induced Systemic Resistance
- I.U.: International Unit
- JA: Jasmonic acid
- K: Potassium
- KNO_3 : Potassium nitrate

- KOH: Potassium hydroxode
- KSB: Potassium solubilizing bacteria
- L.: Linnaeus
- LB broth: Luria Bartani broth
- LCO: Lipo-chito oligosaccharides
- LDL: low-density lipoprotein
- LPS: Lipo-polysaccharides
- MCL: Maximum Composite Likelihood
- MDA: Malonic aldehyde
- Mg: Manganese
- Mn: Manganese
- Mo: Molybdenum
- µg: Microgram
- $\mu g L^{-1}$: Microgram per litre
- $\mu M\ gm^{-1}$: Micromolar per gram
- $\mu g\ ml^{-1}$: Microgram per millilitre
- MEGA: Molecular Evolutionary Genetics Analysis
- $mg\ kg^{-1}$: Milligram per kilogram
- $Mg\ L^{-1}$: Milligram per litre
- mM: Millimolar
- mm: Millimetre
- Mn: Manganese
- MR-VP: Methyl-Red-Voges Proskauer
- N: Nitrogen
- NA: Nutrient agar

- Na: sodium
- NaCl: Sodium chloride
- NB: Nutrient broth
- NBS: nature-based solutions
- NGS: Next generation sequencing
- Ni: Nickel
- nBLAST: Nucleotide BLAST
- NCBI: National Center for Biotechnology Information
- Ni: Nickel
- nm: Nanometer
- NO_3^-: nitrate
- NH_4^+ : ammonium
- Nr: reactive nitrogen
- O: Oxygen
- O_2: Molecular Oxygen
- $O_{2^o}^{\ -}$: Superoxide radical
- OD: Optical Density
- OTU: Operational taxonomic unit
- P: Phosphorus
- PCR: Polymerase Chain Reaction
- PDA: potato dextrose agar
- PGP: plant growth promoting
- PGPB: Plant Growth Promoting Bacteria
- PGPM: Plant growth-promoting microorganisms
- PGPR: Plant Growth Promoting Rhizobacteria

- pH: Potential of Hydrogen (negative base 10 logarithm of H^+ ion activity)
- PO_4^{3-}: Phosphate ion
- PSB: Phosphate solubilizing bacteria
- ROS: reactive oxygen species
- S: Sulfur
- SA: Salicylic acid
- Se: Selenium
- SHC: Soil Health Card
- Si: Silicon
- SMA: Skimmed milk agar
- SOC: Soil organic carbon
- SMC: Soil microbial community
- SOM: Soil organic matter
- TOC: Total organic carbon
- USDA: United States Department of Agriculture
- VOC: Volatile Organic Compound
- W.B.: West Benga
- W.H.O.: World Health Organization
- Zn: Zinc

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CHAPTER-1

Introduction

Soils are living ecosystems that provide the substratum and proper environment for supporting various life forms. According to FAO, (2019), Soil fertility is defined as the capabilities of soils to support the growth and development of plants by supplying essential nutrients and a habitat with suitable chemical, physical and biological traits congenial for sustaining plant life, whereas, 'soil productivity is the resultant effect of several factors influencing crop yield' (FAO, 2020). Healthy soil is an integral part of soil fertility and is essential for agricultural sustainability.

1.1. Depletion of fertility and productivity of arable land

According to (FAO, 2020), soil degradation is considered as an alteration in the soil health status including physical, chemical and biological health of soil, which subsequently diminishes the ability of the ecosystem to supply 'goods and services' to the beneficiaries. Various natural and anthropogenic interventions such as excessive use of agrochemicals, inadequate return of organic matter to cultivated land, monoculture, soil erosion, and deforestation are the significant driver of soil degradation. Loss of mineral nutrient from arable lands, is one of the most common form of soil health degradation. Intensification of cropping practice through extensive use of agrochemicals has been the major drivers for a quantum leap in crop production after the green revolution. Over-exploitation of agricultural lands to feed the rapidly growing world population has negatively impacted the structure and function of soil by depleting its nutrient levels, lowering microbiological diversity, and crop productivity (Huang 2019) posing a serious threat to global food security. The damaged soils fail to regain their fertility satisfactorily and are also unable to regenerate naturally (Goenster et al. 2017). Thus, sustainability of agricultural systems has become a major challenge across the world as well as, in India where 54.6% of the total workforce depends on agricultural sector for their livelihood (Census 2011). According to FAO, about 33 percent of the soil of our planet is degraded (FAO, 2022).

1.2. Microbial augmentation of low productive soil

Research works spanning over the last few decades have established the importance of beneficial soil microbes (BSM) in upgradation of of soil fertility and productivity. Among the BSMs, plant growth promoting bacteria (PGPB) can directly influence plant structure and performance through mobilization of soil nutrients, secretion of plant beneficial secondary metabolites (such as phytohormones, siderophore) as well as

indirectly protect plants from biotic and abiotic stresses (Gouda et al. 2018). In the rapidly growing sectors of sustainable agriculture, BSMs are assumed to steer the bio-based revolution in the near future as a potential alternative to complement or replace chemical fertilizers and pesticides (Manfredini *et al*. 2021; Malusà *et al.* 2021).

1.2.1. Utilization of native/resident plant growth promoting (PGP) bacterial consortia

Most of the studies on agricultural sustainability are related to the soil amendment practices with single strain microbial inoculant having multifarious PGP activity which, in some instances, encountered inconsistencies in field condition (Manfredini *et al*. 2021). Sometimes their establishment in the targeted soil environment become problematic, because most of the beneficial plant-microbe interactions are specific to plant genotypes and the introduced inoculants have to interact with different plant genotypes in the new environment (Mawarda *et al.* 2020). To overcome these limitations, some attempts were initiated to apply consortia of native soil microorganisms as biofertilizer, biocontrol and bio-stimulant farm inputs (FAO, 2020). Thakur *et al.* (2019) observed that on-farm utilization of the locally adapted and biologically diverse resident microbial members – in contrast to introduced microbes - may also be adopted as an effective method to increase biotic resistance against the invading foreign phytopathogens. Hu. *et al.* (2021) suggested a unique strategy of designing multi-strain bacterial inoculants to enhance and rejuvenate the resident BSMs, already present in the rhizosphere. Combined application of beneficial and environment-friendly microbial inoculants (e. g. Phosphate-solubilizers and Nitrogen-fixers) in agricultural field together with inorganic fertilizers are now gaining popularity (FAO, 2022). Previous report indicates that only a single poly-microbial inoculation can exert remarkable impact on agricultural productivity and soil fertility (Bargaz *et al.* 2018).

1.2.3. Microbiome based approach

Microbiome-based research has revolutionized our perception of indigenous microbiome-inoculant interactions as well as co-evolution of plants as holobionts. Novel studies are revealing that soil biological diversity and functioning can be effectively restored through integrated manipulation and control of whole soil microbiome (FAO, 2020). A plant-favourable microbiome can be established in the crop field through the introduction of

PGP microbes, which exert their plant-favourable activity after introduction, building up a critical amount of biomass into the soil (Vassileva *et al.* 2020). The effect of the introduced microbial inoculants may be transient or prolonged in the soil and it is dependent on the diversity of autochthonous soil microbial communities (Mawarda *et al.* 2020; Mallon et al. 2015). They may exert a consistent impact in field conditions on plant productivity (Vassileva *et al*. 2020) either due to the complementation effects of plant-favourable functions at the consortium level or because of imminent diversity effects in the plant-associated microbiome (Hussani 2018).These modulations may alter plant performance and soil health, and thereby, inducing unpredictable feedback reactions (Berg *et al.* 2021). The potential of residual multi-strain PGP bacterial consortia as effective microbial inoculant to rejuvenate damaged arable land and to improve plant vigour, is yet to be properly explored, especially, in the perspective of West Bengal, India, very few studies have been conducted so far.

1.3. Organic farming towards agricultural sustainability

Organic farming is "a system which avoids and largely excludes the use of artificial inputs" (USDA). It is based on ecological processes, biological diversity and nutrient cycles that operate in local conditions. It protects and preserves soil health through sustainable and eco-friendly crop management practice, conservation and restoration activities. (Gamage *et al.*, 2023). A combination of novel technologies such as biofertilizer and vermicompost is an essential part of this system. Biofertilizers contain one/more species of microorganisms involved in the mobilization of nutritionally important elements through biological processes, such as biodegradation of complex organic molecules, compost, N-fixation, P-solubilization, and secretion of PGP metabolites in soil. Additionally, biofertilizer application improves biological health of soil by directly suppressing pathogens through antagonistic interactions via modification of the indigenous microbiota (Zhang *et al.* 2020). Furthermore the natural products (like vermicomposts, agro-industrial wastes, etc.) promote the growth of indigenous soil microbes within the soil-plant system (Strachel *et al*. 2017). Globally, at least 72.3 million hectares of farmland belonging to 3.1 million farmers of 187 countries are operating organically (Willer *et al.* 2021). In the perspective of West Bengal, India, adequate initiatives has not yet been undertaken to popularize organic farming for restoration and utilization of nutrient depleted agricultural soil.

The investigation pertaining to the thesis is an endeavour to explore the implications of soil amendment with residual PGP bacteria (*Bacillus.* spp., *Pseudomonas* sp.) isolated from the resident microbial flora of two types of over-exploited agricultural soil (alluvial soil and lateritic soil) of West Bengal, India. Two novel consortia combinations were designed using residual PGP bacteria. Their impact on growth promotion of *Gylcine max* (L) Meril. (soybean) plants, influence on the resident soil bacterial community structure, and soil nutrient level composition, were investigated in pot trial condition. This unique soil amendment strategy can be applied to enhance plant productivity of long-term cultivated soil towards agricultural sustainability. The resilient crop, Soybean (*Glycine max* L. Meril), can be grown in the transformed soil, specially of fallow and marginal lands, during the rain-fed kharif season. Introduction of this underutilized crop can help to improve the socioeconomic status of many small and marginal farmers in rural West Bengal.

In this context, this investigation was undertaken with the following objectives:

The objectives of the current investigation

1. Collection and characterization of low productive agricultural soil from different localities
2. Isolation and screening of bacteria from the selected soil samples for their plant growth promoting (PGP) traits
3. Utilization of the selected resident PGP bacterial isolates to design novel multi-strain bio-inoculant for soil upgradation and growth promotion of *Glycine max* (L.) Merill. (soybean) plant
4. Evaluation of the impacts of the novel multi-strain bacterial inoculants on plant growth, soil nutrient status, and resident soil bacterial community structure.

CHAPTER-2

Literature Review

2.1. Low productive Soil: Nutrient Deficiency

Soils have the inherent ability to supply nutrients for supporting plant life. Nearly 95% of our food materials are of soil origin (FAO, 2020).

2.1.1. Soil: The Nutrient base for plants

Soils are the primary source of nutrients on which the living world thrives. It is a complex ecosystem which supports a bewildering array of functions for originating, capturing, transforming, and recycling the essential elements through biogeochemical cycles. Microbiota of healthy soils can transform inaccessible sources of soil nutrients into plant-available forms. Furthermore, they provide proper chemical and biological environment that regulates the nutrient uptake by the plant roots and nutrient leakage to the surroundings (Peoples et al., 2014). It is currently established that plants need 17 essential nutrients for completing their life cycle (Hodges, 2010). Except carbon (C), hydrogen (H), and oxygen (O), plants acquire all the 14 elements through their roots from this soil or from fertilizers and manures (Parikh & James 2012). The macronutrients e.g., nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), and magnesium (Mg) are needed in huge quantities. The micronutrients like iron (Fe), manganese (Mn), boron (B), zinc (Zn), copper (Cu), molybdenum (Mo), nickel (Ni), and chlorine (Cl) are required in relatively small amounts by plants (Table 1). Plants also require some essential elements, e.g. sodium (Na), silicon (Si), cobalt (Co), iodine (I), selenium (Se), and aluminum (Al) which enhance their growth and productivity conferring resistance against various biotic and abiotic stresses (Singh & Schulze 2015)**.** The soil minerals, reserved in solid soil phase, serve as the main source and sink for essential plant nutrients, which are made accessible to plants through a series of chemical, physical, biological processes, and mineralization of soil organic matter (SOM). In fact, the availability and/or deficiency of macro- and micronutrients are regulated by various of complex processes and interactions related to the physical, chemical and biological properties of soil (Jones and Darrah, 1994). Soil organic matter (SOM) plays an important role has a leading role in this regard. A balanced and continuous availability of macro- and micronutrients is essential for proper metabolic activities of crops.

Among all the essential macronutrients, nitrogen (N) is needed by plants in the highest quantity. It is considered as one of the most common limiting factors in crop

productivity and most frequently lost from the soil reserve. The nitrate (NO_3^-) and ammonium (NH_4^+) are the plant-available forms of soil nitrogen. The atmosphere is the largest reservoir of the Earth's N. Nearly, 99% of this nitrogen are present in a highly stable non-reactive form and are non-available to 99% of life forms of the planet**.** It is transformed into bio-available, reactive N (Nr) form only through the natural phenomena lightning and microbial N-fixation (Galloway et al., 2003)**.** Most of the soil has limited anion exchange capacity, thus, leaching of N in the form of NO_3^- ions, as well as their removal and in percolation water is a common problem in many agricultural lands. To compensate for the leaching, excessive amounts of N fertilizers are used (Weil and Brady, 2017, Singh & Schulze 2015). A substantial amount of N is also lost from soil due to the de-nitrification process which is regulated by soil microbes.

Phosphorus is one of the scarcest and limiting plant nutrients found in the Earth's crust being rare in occurrence in high concentration (Weil and Brady, 2017). This element is made bio-available mainly through the process weathering of primary soil minerals and requires to be supplemented as mineral fertilizer or biofertilizer to some agricultural lands. According to Weil and Brady (2017), soil P mostly occur in phosphate forms such as PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, and $H_3PO_4^-$, primarily as PO_4^- either in its inorganic, organic, dissolved or particulate form. Thus, the transformation of the immobilized mineral P to its bio-available forms is a great challenge towards agronomic management of crop lands. Generally, roots absorb P in the form of ortho-phosphate and as certain forms of organic P. As the concentrations of dissolved P in the soil solution is very low, it moves to the root surface through diffusion.

Out of the essential nutrients, **Potassium (**K) is the most abundant one, accounting for about 0.5-2.5% of the soil mass. K-feldspar and mica are the most common K, existing in primary mineral form, about 98% of which is unavailable for life forms. Usually, less than 1% fraction are bio-available. They are released from the soil reservoir following weathering or dissolution of K minerals and meet up the K requirements for many of the global crops (Brouder *et al.* 2021).

Element		Symbol	Primary forms used by plants	Plant content (range/ per cent)
Macronutrients	Nitrogen	N	NH_4+, NO_3^-	0.5-5
	Phosphorus	P	HPO_4^{2-}, $H_2PO_4^-$	0.5-5
	Potassium	K	K^+	0.1-5
	Calcium	Ca	Ca^{2+}	0.5-5
	Magnesium	Mg	Mg^{2-}	0.05-5
	Sulphur	S	$SO4^{2-}$	0.05-0.5
Micronutrients	Iron	Fe	Fe^{3+}, Fe^{2+}	50-1000
	Manganese	Mn	Mn, Mn^{2+}	20-200
	Zinc	Zn	Zn2+	10-100
	Copper	Cu	Cu^{2+}	2-20
	Boron	B	H_3BO_3	2-100
	Molybdenum	Mo	Mo, MoO_4^{2-}	0.1-10
	Chlorine	Cl	Cl^-	100-1000

Table 2.1.1. Essential mineral nutrients taken up by plants, and their concentrations in plant tissue **(Source:** FAO, 2022. https://doi.org/10.4060/cc0900en).

Nutrient	Form of absorption	Metabolic form	Mobility in the plants	Mobilization soil to roots
N	NO_3^-	NH_4^+	++	Mass flow
	NH_4^+	NH_3		
	Urea	NH_2OH^-		
	Amides			
	Amino acids			
P	$H_2PO_4^-$	$H_2PO_4^-$	+	Diffusion
	HPO_4^{-2}	HPO_4^{-2}		
		PO_4^{-3}		
K	K^+	K^+	++	Diffusion
Ca	Ca^{++}	Ca^{++}		Interception
				Mass flow
Mg	Mg^{++}	Mg^{++}		Interception
				Mass flow
S	SO_4^{-2}	S-H/S-S	±	Mass flow
Mn	Mn^{++}	Mn^{++}	±	Mass flow
				Chelates
				Interception
Zn	Zn^{++}	Zn^{++}	±	Mass flow
				Chelates
				Interception
Cu	CuOH	Cu^{++}	-	Mass flow
	CuCl			
	Chelates			
Fe	Fe^{++}	Fe++	-	Mass flow
	Fe^{+++}			
	Chelates			
B	H_3BO_3		Mass flow	
	H_2BO_3			
	HBO_3^{-2}			
	BO_3^{-3}			
	$B(OH)_4$			
	$B_4O_7^{-2}$			
Mo	MoO_4^{-2}		+	Mass flow
	$HMoO_4^-$			
Cl	Cl^-		+	Mass flow

'+' indicates mobile in plant;' -' indicates non-mobile in plants

Table 2.1.2. Soil nutrients, their adsorption form, metabolic form, mobility in plant and mechanism of mobilization from soils to roots. (Source: FAO, 2022. https://doi.org/10.4060/cc0900en)

The essential nutrients which are needed by plant in relatively small amounts are categorized into the group of **Micronutrients.** They include boron (B), chloride (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni) and zinc (Zn). A proper balance of all the essential elements (both macro and micronutrients) are needed for normal growth of plants and optimum crop yield. These nutrients are conserved in soil as inorganic state (silicates, oxides, $HMoO_4^-$). The micronutrients such as Fe, Mn, Cu, Zn, and Ni are absorbed by plant roots as cations, whereas B, Mo, and Cl are absorbed as anionic forms (e.g. H_3BO_3 and MoO_4^{2-}). Fe and Mn are generally present in large amount in most soils. Besides the concentration of soil micronutrients, their chemical forms also have a role on the bio-availability, deficiency, and toxicity of the macronutrients.

2.1.2. Degradation of soil health: Depletion in soil fertility and productivity

"Soil health is the capacity of soil to function as a living system, with ecosystem and land use boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and promote plant and animal health. Healthy soils maintain a diverse community of soil organisms that help to control plant disease, insect and weed pests, form beneficial symbiotic associations with plant roots; recycle essential plant nutrients; improve soil structure with positive repercussions for soil water and nutrient holding capacity, and ultimately improve crop production" (FAO, 2008).According to FAO, (2019), Soil fertility is defined as the capabilities of soils to support the growth and development of plants by supplying essential nutrients and a habitat with suitable chemical, physical and biological traits congenial for sustaining plant life, whereas, ‘soil productivity is the resultant effect of several factors influencing crop yield’ (FAO, 2020). Deterioration in the soil health results in a ‘diminished capacity of the ecosystem’ to supply ‘goods and services’ to the ‘beneficiaries’ leading to soil degradation (FAO, 2020).

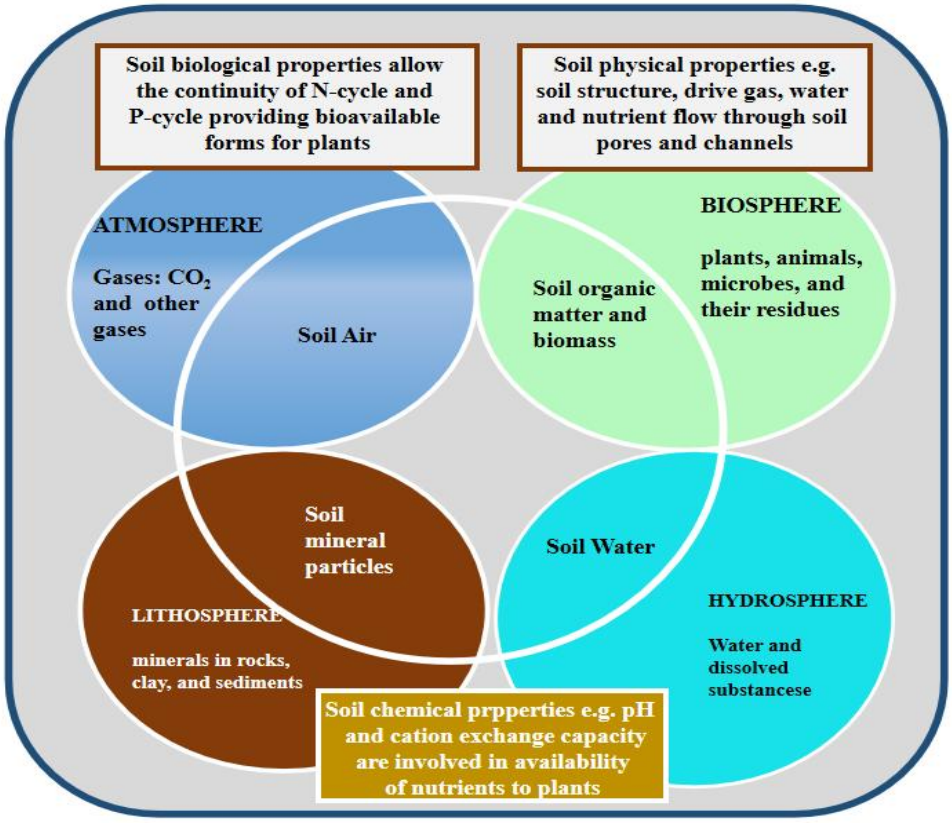


Fig 2.1.1. Conception of soil fertility encompassing integral physical, chemical, and biological parameters and some natural processes showing the convergence abiotic and biotic components of soil. (Self-developed) Source: FAO, 2022. https://doi.org/10.4060/cc0900en

In the present-day-world, productivity of soil has become a major concern due to degradation of physical, chemical, and biological health of soil. According to FAO, about 33 percent of the soil of our planet is degraded. The contributing factors for nutrient-depletion are both natural and anthropogenic interventions such as excessive use of chemical fertilizers, pesticides, inadequate return of organic matter to cultivated land, monoculture, soil erosion, and deforestation. All soils do not possess the same level of plant-available nutrients. In many areas, soils are non-fertile in nature having very low agricultural potential, whereas in some regionss, soil fertility has been depleted due to some degradative processes. In both the instances crop productivity is significantly poor due to soil nutrient depletion. Furthermore, in some regions soil fertility has been adversely affected due to improper management practices with excessive application of nutrients leading to serious environmental hazards. A

'contrasting scenario of nutrient imbalances' in agricultural lands, is presently being experienced in different regions of the world (FAO, 2022).

Soil degradation due to nutrient imbalance

Soils are essential for sustenance of life in our planet and it contributes around 95% of our food nutrients, as a whole. It is the reservoir of essential plant nutrients, although their availability may be restricted due to some factors like natural deficiencies, immobilization in solid phase, or due to soil degradation related to inappropriate agricultural management practices. A substantial amount of mineral nutrients is lost from cultivated lands after every harvest. The fertility of the arable lands will be progressively lost, if adequate amounts of fertilizers are not applied. According to FAO (2015) documents, imbalanced fertilizer application is a common cause for soil fertility depletion in intensive farming system. Soil nutrients e.g., N and P are crucial for plant growth, and if escape from the soil-plant systems, they generally act as pollutants that are quite troublesome as well as expensive to retrieve (Sutton *et al.* 2011). Many global reports indicate serious environmental disturbances in biogeochemical cycles of N and P, mainly due to excessive use of chemical fertilizers**.** During the period of 1961–2013, an increase in nitrogen/phosphate fertilizer ratio by 0.8 g N/g P decade^{-1} was recorded which may have a profound impact on global agro-ecosystem functions in near future (Lu and Tian, 2017).

Worldwide, especially in the developing countries, soil in several areas is becoming low productive, consequently producing nutrient-deficient crops. It is a major threat towards global food security. Nutrient imbalances are observed in an agro-ecosystem, when the nutrient input (atmospheric deposition, fertilizer) exceeds/decreases the output (leaching, crop harvesting). It is one of the most striking reasons for soil degradation. Nutrient mining either induces nutrient depletion or make the soils toxic (due to high concentration) to plants, ultimately the soil fails to support crops.

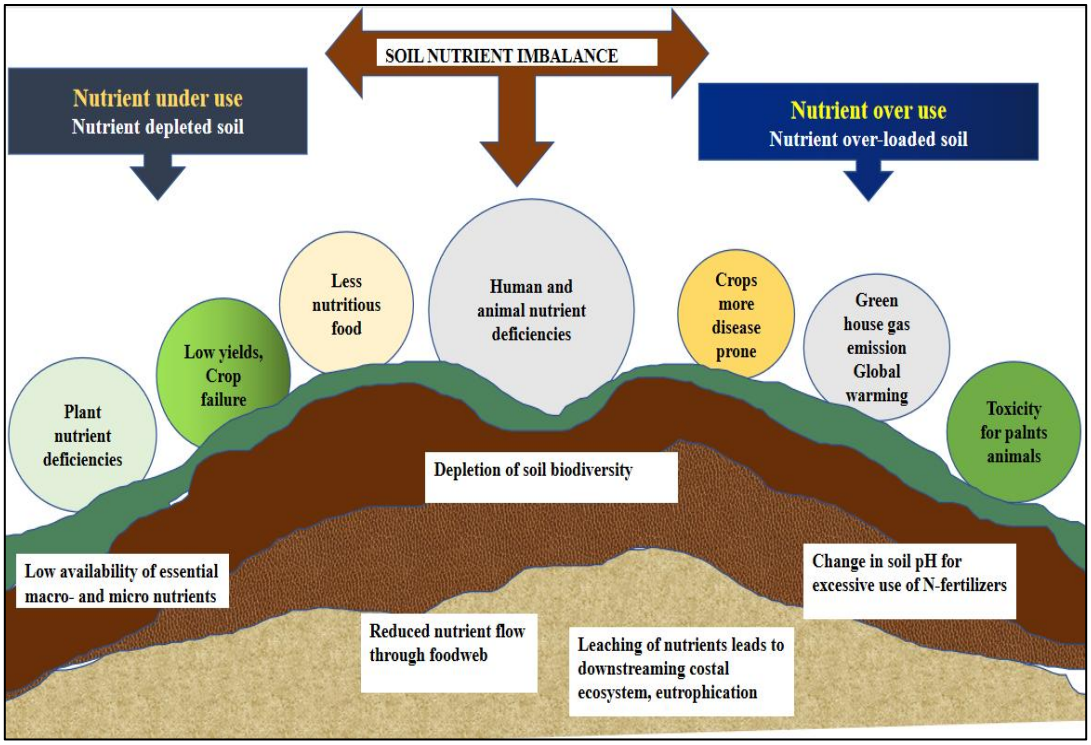


Fig 2.1.2. Effects of Soil nutrient imbalances on crops, animals, and the environment (Self- developed. Source: FAO, 2022. https://doi.org/10.4060/cc0900en)

Soil degradation due to nutrient Mining

Agriculturally productive soils have an inherent potential of supplying a substantial amount of its cumulative nutrients to plant roots through soil solutions during a crop cycle, thereby promoting plant growth. In intensive cropping practice, a substantial quantity of essential nutrients is removed from soil during continued crop production round the year. A grave situation, 'Nutrient mining' occurs, when the amount of soil nutrients taken up by a crop is not recycled back and/ or replenished to the depleted nutrient pool of the field (Sanyal 2014). It is emerging as a major challenge in the intensively cultivated areas of many countries like India where arable lands are under tremendous pressure to meet up the expanding demand for food. Around the world, decades of cultivation practices have depleted the pool of sol nutrients due to loss of both macro- and micronutrients through the process of nutrient mining. The currently adopted nutrient management strategies by majority of the farmers is aggravating the problem due to insufficient or imbalanced nutrient applications (Majumder *et al.* 2016). Nutrient mining is also reported to occur through some natural processes such as leaching and volatilization. The depletion in the inherent fertility of arable lands may seriously jeopardize crop productivity and food security of a country in near future. In

the context of Indian soils, there is a urgent need to integrate the concept of nutrient mining with the proper crop production strategies (Majumder et al. 2016).

Soil nutrient	**Nutritional status**				
	Very low	**Low**	**Medium**	**High**	**Very high**
Soil organic carbon (SOC) per cent	< 0.25	0.25–0.50	0.5–0.75	0.75–1.0	> 1.0
Nitrogen (kg/ha)	140	140–280	280–560	560–700	> 700
Phosphorus (kg/ha)	< 5	5–10	10–25	25–40	> 40
Potassium (kg/ha)	< 60				
Boron (ppm)	> 0.5				
Copper (ppm)	> 2.0				
Zinc (ppm)	> 0.6				
Iron (ppm)	> 4.5				
Manganese (ppm)	>2.0				

Table 2.1.3. Criteria for deficiency or sufficiency of nutrients in different types of soil. Source: Indian Council of Agriculture Research, Ministry of Agriculture and Farmers' Welfare. (Khurana and Kumar, 2022).

Note: According to the Soil Health Card scheme, (SHC), Govt. Of India, soil containing "very low", "low" or "medium" amount of macronutrients e.g. N, SOC, P and K are treated as nutrient deficient. Soils having "high" or "very high" macronutrient nutrient levels, are considered as 'nutrient sufficient'. Soils containing below the prescribed content of a micronutrient such as, B, Cu, Fe, Mn, S and Zn, etc. are treated as deficient. Soils having the micronutrients as same or above the prescribed levels, are micronutrient sufficient (Khurana and Kumar, 2022).

2.1.3. Nutritional Status of Indian Soil

The Union Ministry of Agriculture and Farmers' Welfare, India, introduced the Soil Health Card (SHC) scheme in 2014–15, as part of the National Mission of Sustainable Agriculture. Under this scheme, a large no. of soil testing are conducted across the country and based on

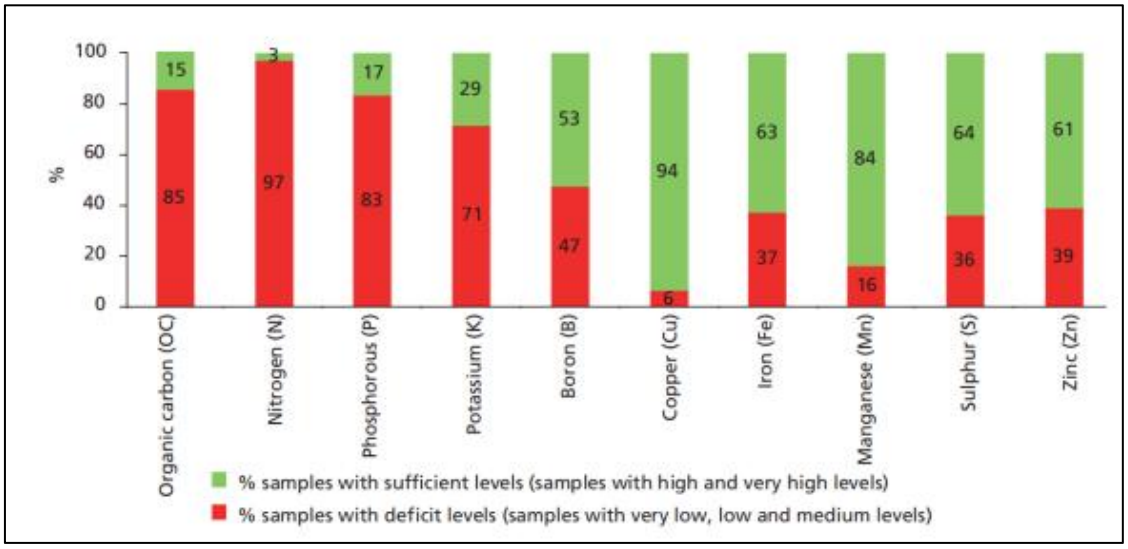


Fig 2.1.3. Status of organic carbon, macronutrients, and micronutrient content in Indian soil (Khurana and Kumar, 2022)

Note: Based on the available data (https://www.soilhealth.dac.gov.in.) the Figure was drawn showing the cumulative results of cycle 1 and cycle 2 testing under SHC scheme are furnished. Source: Khurana and Kumar (2022).

the reports, soil health cards are issued to the farmers. In Cycle-1(from 2015–16 to 2016–17) and Cycle-2 (from 2017–18 to 2018–19) nearly, 5.27 crore soil samples from different areas have been tested. Furthermore, nearly 19.64 lakh soil samples were tested under the Model Villages Programme. In government-approved laboratories, from across the country, over five crore soil samples were checked for their nutritional status were checked for their nutritional status during the period of 2015–16 to 2018–19.

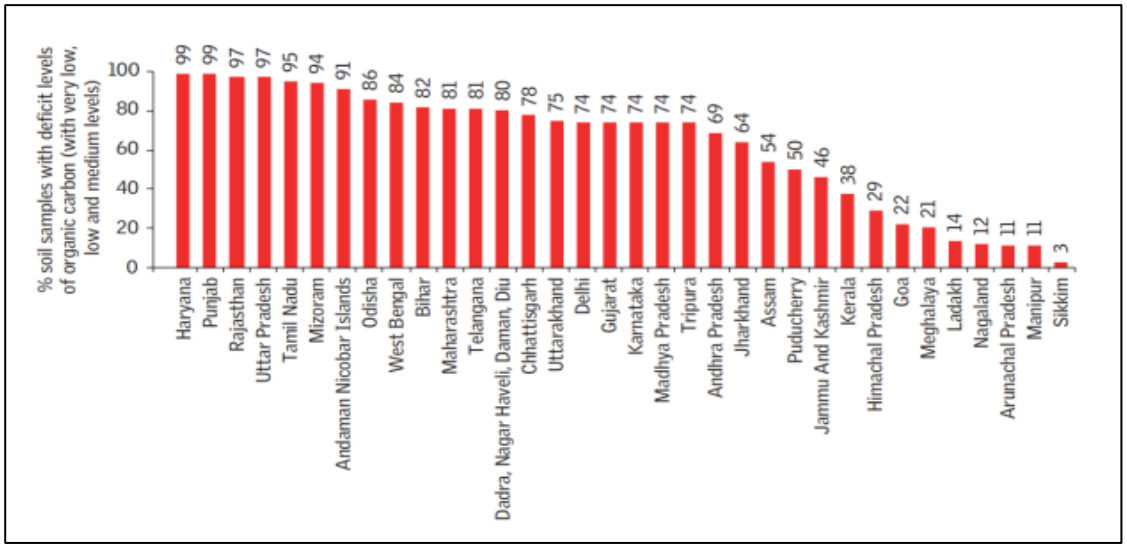


Figure 2.1.4. Status of SOC deficiency in soil of Indian States and Union territories (Khurana and Kumar, 2022)

Note: Based on the available data (https://www.soilhealth.dac.gov.in.) the Figure was drawn showing the cumulative results of cycle 1 and cycle 2 testing under SHC scheme are furnished. Source: Khurana and Kumar (2022).

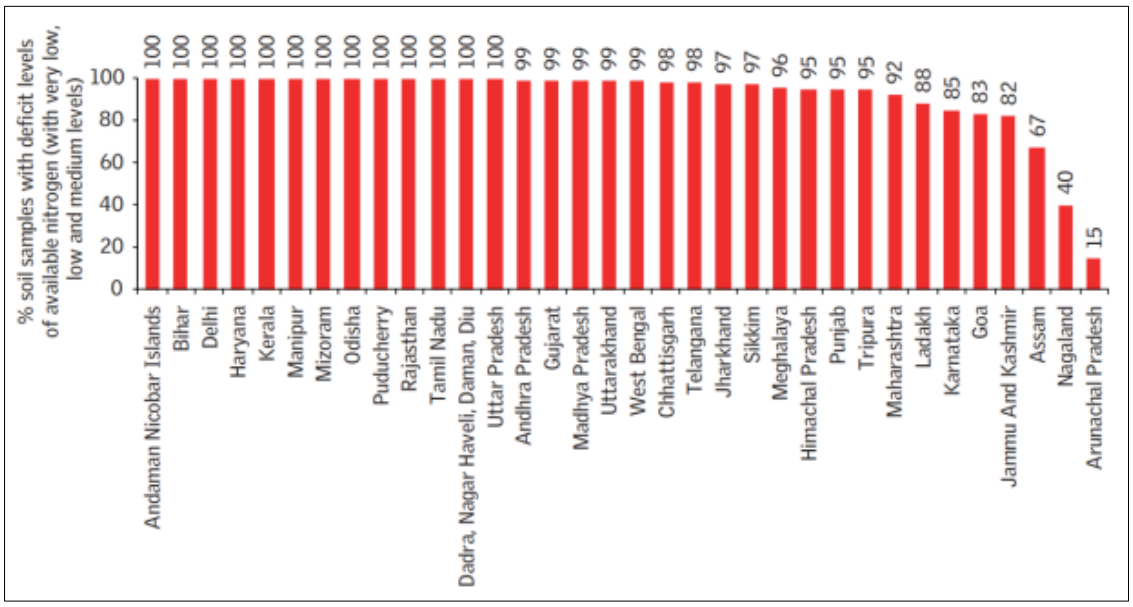


Fig 2.1.5. Available Nitrogen (N) deficiency in soils of Indian States and Union territories (Khurana and Kumar, 2022)

Note: Figures calculated based on the data available on the website https://www.soilhealth.dac.gov.in. The combined results of cycle 1 and cycle 2 testing under SHC scheme are furnished. **Source**: Soil Health Card (SHC) scheme, Union Ministry of Agriculture and Farmers' Welfare, India

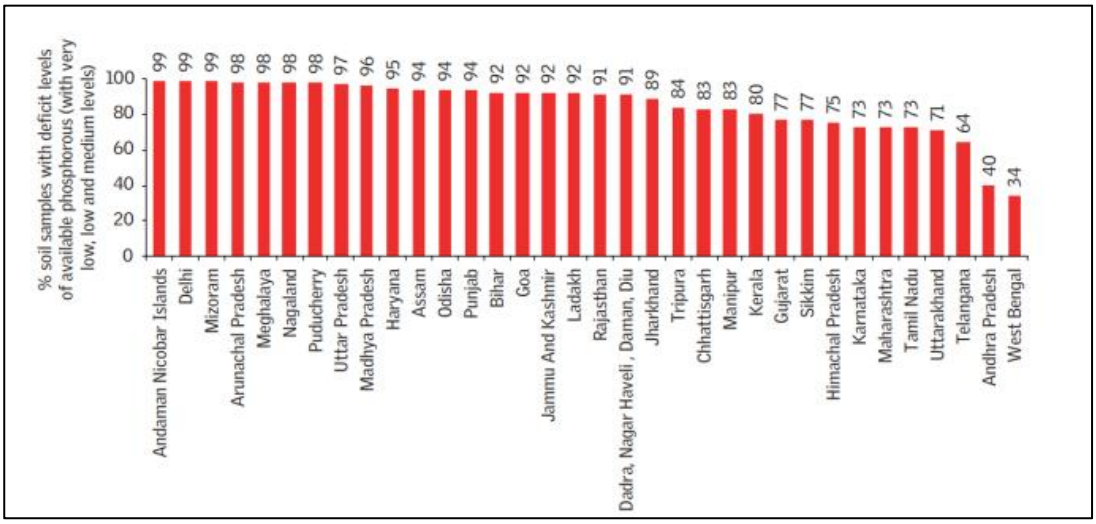


Fig 2.1.6. Available P deficiency in soils of Indian States and Union territories (Khurana and Kumar, 2022)

Note: Based on the available data (https://www.soilhealth.dac.gov.in.) the Figure was drawn showing the cumulative results of cycle 1 and cycle 2 testing under SHC scheme are furnished. Source: Khurana and Kumar (2022).

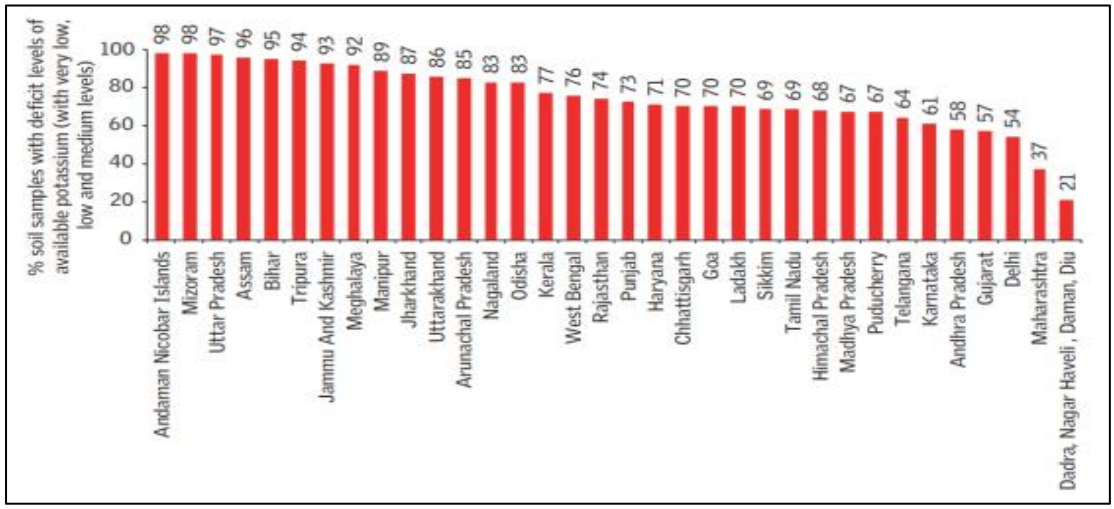


Fig 2.1.7. Available Potassium (K) deficiency in soils of Indian States and Union territories (Khurana and Kumar, 2022).

Note: Based on the available data (https://www.soilhealth.dac.gov.in.) the Figure was drawn showing the cumulative results of cycle 1 and cycle 2 testing under SHC scheme are furnished. Source: Khurana and Kumar (2022).

The results indicate acute and widespread depletion of organic carbon (SOC) and essential macronutrient content in Indian soils. About 50% of the tested soil samples from 24 states and Union territories (UTs), were observed to have low TOC. Out of

them, over 90% from 7 states were deficient in TOC, Haryana showing the poorest condition**.** Depletion of N level in Indian soil is very alarming. About 50% of the tested soil samples from 32 states and UTs are N-deficient, among which more than 90% samples were from 27 states and UTs. Severe N-depleted condition was recorded in 15 states and Union territories (Andaman and Nicobar Islands, Bihar, Dadar and Nagar Haveli and Daman and Diu, Delhi, Haryana, Kerala, Madhya Pradesh, Manipur, Mizoram, Odisha, Puducherry, Rajasthan, Tamil Nadu, Uttarakhand and Uttar Pradesh) where 99-100% of the tested samples have very poor N-level. Depletion in P content is also widespread in soils across the Indian subcontinent where 32 states and UTs have nearly 50% of tested soil samples deficient in P content. Out of them, more than 90% samples from 19 states and UTs are P-deficient. K deficiency is also prevalent in Indian soils. About 32 Indian states and UTs were observed to have more than 50% of their soil samples K deficient, out of which 8 states and UTs detected to have above 90% K-deficient samples**.** In addition to the collected data related to the SHC scheme, many other sources also indicated a deteriorating level of mineral nutrient content, SOC and humus in Indian soil (Trivedi *et al.* 2010).

2.2. Soil Microbial Inoculants: Upgradation of Soil Health for Agricultural Sustainability

Soil harbours millions of microorganisms which are intricately related to each other forming a complex and dynamic network essential for the efficient functioning of ecosystems. However, in soils, degraded by intensive cropping practice, depletion of microbial diversity leads to suppression of many plant-beneficial functions and destabilize their expression by the respective microbes (Hu *et al.* 2021). In the context of current agricultural scenario, especially in tropical areas, utilization of beneficial soil microorganisms as bio-inoculants has emerged as a promising strategy to modulate degraded soil health towards agricultural sustainability. It is now established that plant-associated soil microbiota play an important role in agriculture by stimulating growth and development of plants and protecting them from environmental extremities related to various biotic and abiotic stresses (Hardoim et al. 2015).

2.2.1. Microbial inoculants as a nature-based solution to improve soil nutrient level

The Innovative strategies for modulating *in situ* microbiome for a sustainable agriculture, involve application of beneficial microorganisms as single strain bacterial inoculant or as multi-strain consortium. The potential utilization of inoculants can exert beneficial effects e.g. mobilization and transfer of nutrient to plants, upgradation of soil structural composition and water dynamics, and resistance towards soil-borne phytopathogens. Additionally, it helps to mitigate the consequent challenges of soil degradation through biological N fixation (BNF) and facilitating bio-availability of some essential mineral nutrients. Till now, most of the inoculants are based on a single strain, which in some instances, encountered inconsistencies in field. Sometimes their establishment in the targeted soil environment become problematic, because most of the beneficial plant-microbe interactions are specific to plant genotypes and the introduced inoculants must interact with different plant genotypes in the new environment. Furthermore, the inoculant strain(s) must compete with the resident microbiota of the soil and plant niche. To overcome this problem, multi-strain consortia have been formulated using different beneficial microbes. According to FAO **(2020),** utilization of native consortia of resident soil microbes as biofertilizer, biocontrol agent and bio-stimulant, is a promising approach in farming practice**.** The sourcing of native

consortia of soil microbes will enable farmers utilize locally adapted and biologically diverse inoculants. Thakur and his associates (2019) observed that the on-firm application of resident microbial species instead of alien microorganisms, may be used as a unique method to increase biotic resistance against intruding foreign pathogens.

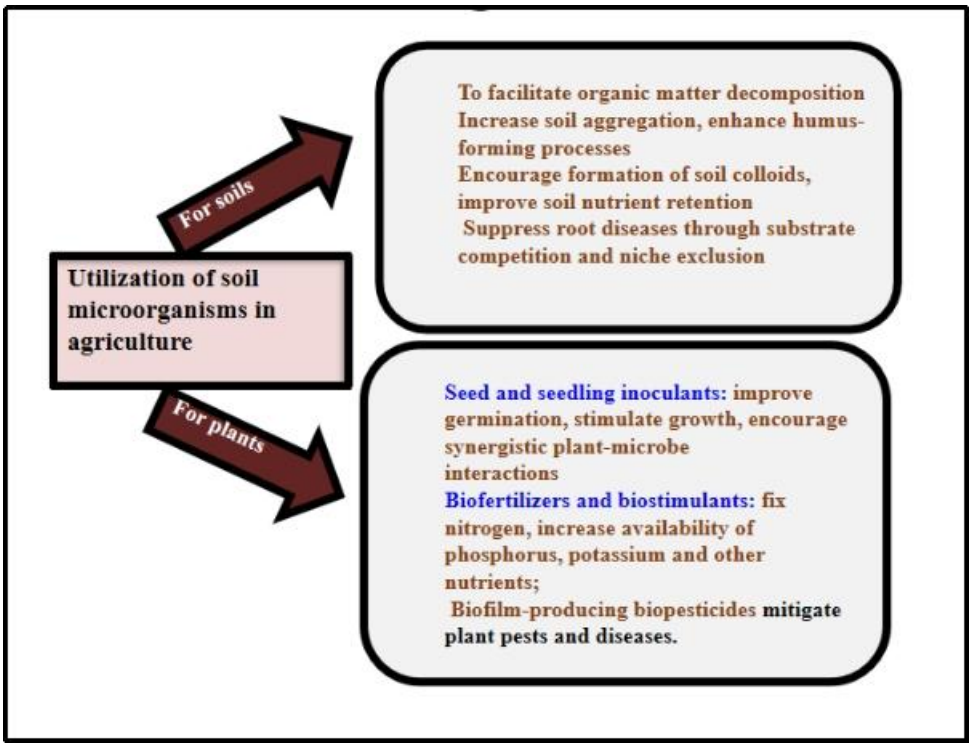


Fig 2.2.1. Multifarious benefits through utilization of soil microorganisms in agriculture (Self-developed)

Utilization of beneficial soil microorganisms for quality enhancement of soil, where native microbiological diversity has decreased due to various anthropogenic activities, is considered as one of the most effective nature-based solutions (NBS) for ensuring agricultural and environmental security as they imitate the natural processes which are involved in ecosystem functioning Arnés-García and Santivañez (2021). The NBS activities are related related 'to protection, sustainable management, and restoration natural or altered ecosystem and 'that address societal challenges effectively, simultaneously providing benefits for human well-being and biodiversity' (FAO, 2022). These methods can be effectively utilized for upgradation of soil fertility and soil nutrient level using microbial inoculants as biofertilizers.

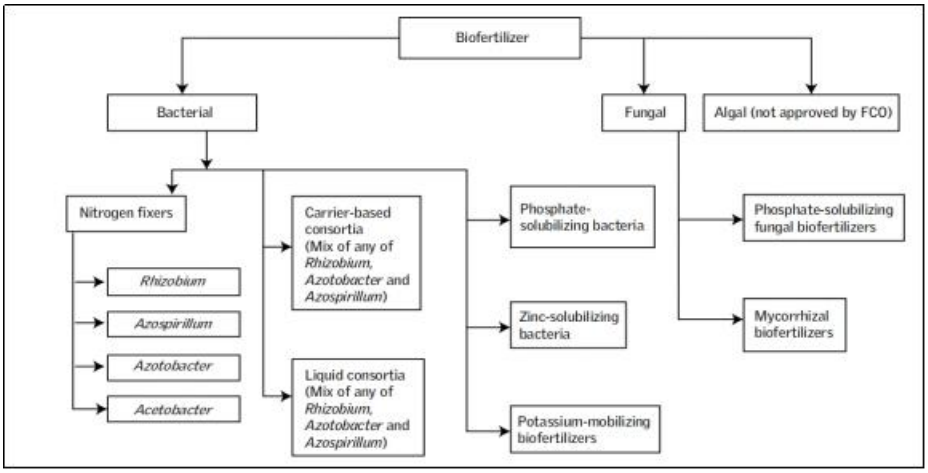


Fig 2.2.2. Biofertilizers approved under Fertilizer control order, India. Source: Fertilizer- Inorganic, organic and mixed (Control, Order, 1985, amended in July, 2021 (Khurana A. and Kumar, 2022).

A biofertilizer is an umbrella term applied for products containing alive or dormant microbes such as bacteria, fungi, actinomycetes and algae, singly or as a composite inoculant which, after introduction into the soil, facilitate biological N-fixation or solubilization/ mobilization of soil nutrients (FAO, 2019). For improving soil nutrient content, beneficial microorganisms are deliberately introduced especially to the soils, where microbial activities have been depleted due to various anthropogenic interventions or some other causes. Improvement in soil NPK content can be achieved through various approaches such as, fixation of atmospheric N utilizing symbiotic and free-living diazotrophs, introduction of P-solubilising (PSB) and K-solubilsing/ mobilising (KSB) bacteria into the soil. Biological N fixation (BNF) is a promising alternative to reduce the overuse of chemical N fertilizers. It is established that BNF contribute about 60% of the N fixed in the soil (FAO, 2022). Application of PSB increases the availability of immobilized soil P. Schlitz and collaborators (2018) found improved crop yield in P-deficient soil following the application of Arbuscular Mycorrhizal Fungi (AMF), P-solubilizers, and N fixers as biofertlizers. The biofertilizers not only enrich soil content but also effectively improve soil health by directly suppressing phytopathogens or antagonistic interactions through modification of the resident microbial community (Zhang et al., 2020). Furthermore, effective utilization of microorganisms help to remediate contaminated soils and waters. The established importance of soil microorganisms in sustainable agriculture, has triggered a great commercial interest across the world toward the development of microbiome-

based solutions for protecting crops and supplying nutrients to them (Sessitsch et al., 2018).

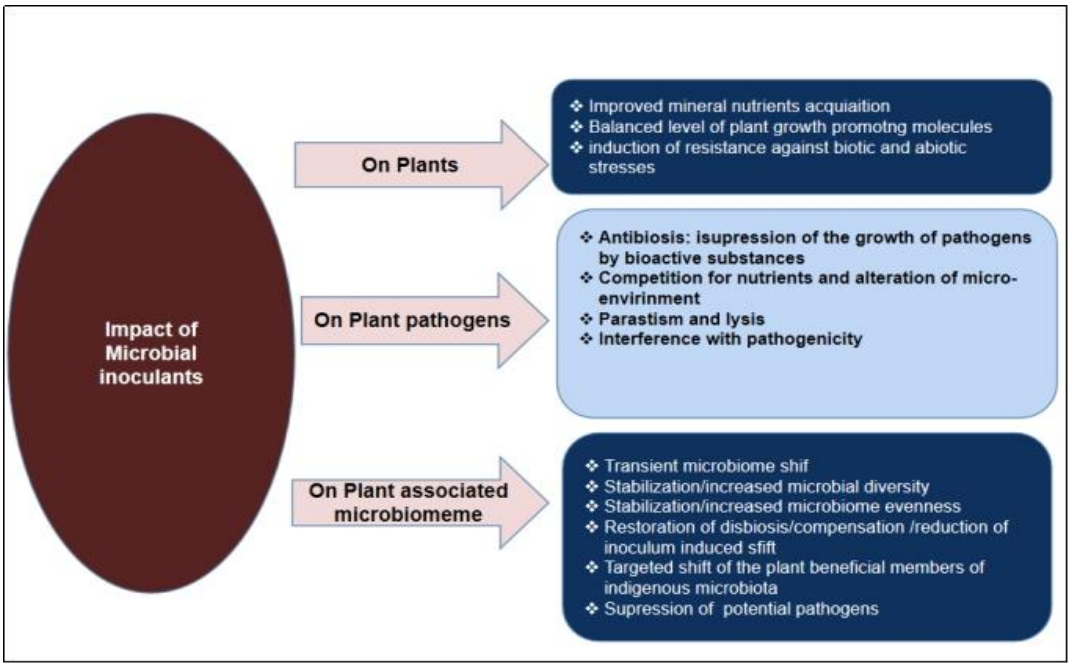


Fig 2.2.3. Mode of action and consequent effects of introduced -microbial inoculant on plant, plant pathogens, and plant associated Microbiome (Self-developed)

There is still scarcity of good products in the market, and usually many laboratory-tested products fail to exert promising results under field trial conditions (Compant *et al.*, 2019). The ability of the inoculant strain(s) to overcome the tough competition they face in the soil and plant root-microbiome and to interact synergistically with other microbes, is significant constrain. Here, a better perception is required regarding the core microbiota, microbiome networks, and the regulatory mechanisms operating in field condition (Tohu *et al.* 2018). Despite the challenges, commercialization of biofertilizers has remarkably expanded. In global perspective, the demand for biofertilizers is expected to escalate about 13 percent from 2017 to 2025 (FAO, 2022). Government of India has introduced several schemes and programmes such as Paramparagat Krishi Vikas Yojana, Mission Organic Value Chain Development for North Eastern Region, National Food Security Mission for promoting production and utilization of biofertilizers and organic fertilizers Khurana and Kumar (2022). Data procured from diffident locations of our country, have revealed that several poor quality biofertilizers are widespread in Indian market. A multi-prong collaborative effort between the Centre and states is urgently needed for ensuring the production,

quality control, and availability of biofertilizers in Indian market Khurana and Kumar (2022).

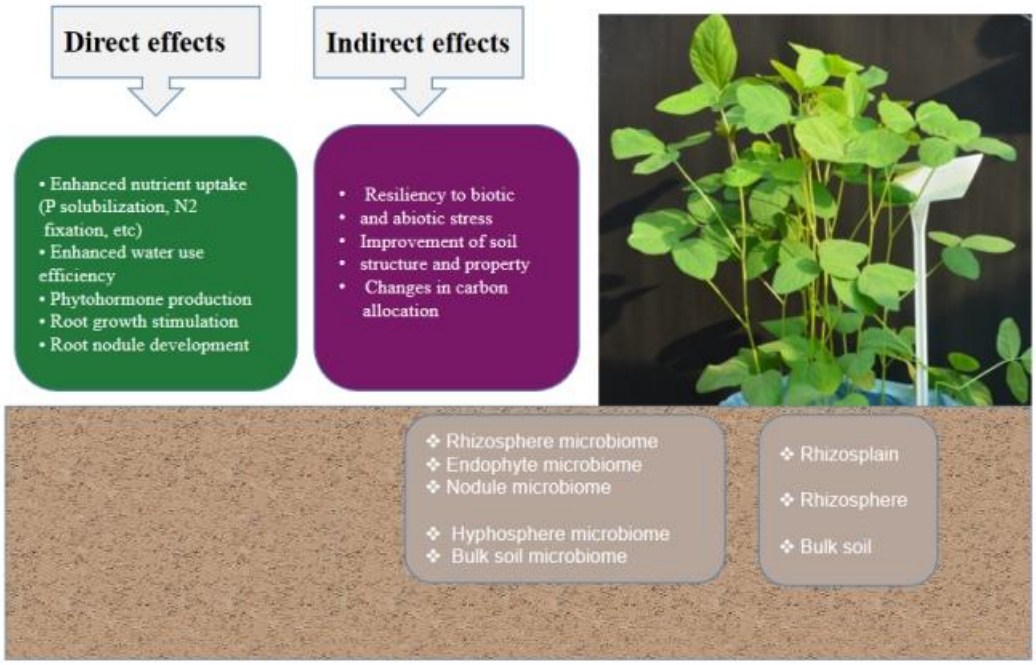


Fig 2.2.4. Overview of the functions of single strain/multi-strain soil microbial inoculants involved in the main elements and processes of plant growth and yield promotion (Self developed). Source: Bargaz (2018).

2.2.2. Impact of bacterial inoculants in plant growth promotion

Plant growth-promoting microorganisms (PGPMs) benefit plants mainly through three different ways: (i) As biofertilizer: Nutrient acquisition and mobilization (e.g., N-fixation, P-solubilization, K-mobilization), (ii) As Phytostimulator (phytohormone production), directly promoting the plant growth, (iii) As biocontrol agents: protecting plants against phytopathogens (Hardoim *et al.* 2015). Currently, most of the inoculant strains are chosen relied on their activities. Co-inoculation of PGPB strains as consortia enhances plant growth due to the combined multifarious effects of different agriculturally beneficial mechanisms exerted by the microbes, as well as increase the efficiency of the inoculant. Introduction of microbial inoculants in soil most often results in modulation of resident microbiome which in turn, affects plant performance (Cornell, 2021). Effects of some microbial inoculants on plant growth and defence are furnished in Table 2.2.1.

Microbial inoculant	Plant Species	Impact	References
Pseudomonas reactans EDP28, *Pantoea alli* ZS 3-6, *Rhizoglomus irregulare*	*Zea mays*	K+ content increased resulting an effective reduction in Na+ level in plant	Moreira *et al.* 2020
Rhizophagus irregularis, *Pseudomonas jessenii*, *P. synxantha*	*Triticum aestivum*	The ability of PGPR to colonize roots increased; dehydrogenase and alkaline phosphatase activity in soil increased	Singh *et al.* 2020
Thervelics R: a mixture of cells of *Bacillus subtilis* C-3102 and carrier materials	*Oryza sativa* and *Hordeum vulgare*	Increased production of siderophore, IAA, and protease; higher amount of dry matter production	Jamily et al., 2019
Bacillus subtilis, B, megatorium	*Cuminum cyminum*	Seed yield and essential oil content improved in plants	Mishra *et al.* 2019
Bradyrhizobium sp.	*Glycine max*	Phosphorus utilization efficiency and uptake of N and P by soybean plants increased	Fituma *et al.* 2018
Pseudomonas syringae pv. *syringae* Pss20 and *Pseudomonas tolaasii* Pt18	*Dacus carota*	In carrot root formation and biocontrol activity improved	Etminani and Harighi, 2018

Table 2.2.1. Application of some microbial inoculants as single strain or consortia and their effect on plant growth and defence.

2.2.3. Bacterial inoculants in modulating soil microbiome *in situ*

Microbiome-based researches have drastically changed our perception of indigenous microbiota-inoculant interactions as well as co-evolution of plants as holobionts. Previous studies revealed tha**t** the fate of the introduced microbial inoculant varies depending on the . According to Berg *et al.* (2021) six types of modulation in plant microbiota may occur: (i) transient shift in microbiome, (ii) microbial diversity may stabilize or increase, (iii) plant microbiome evenness may be stabilized or increased, (iv) a dysbiosis/compensation may be restored or a pathogen-induced shift reduced, (v) targeted shifts may occur towards plant friendly microbes of the native microbiome, and (vi) potential phytopathogens may be suppressed. In sustainable cropping systems, PGP microbial inoculants are purposefully introduced in soil for plant performance enhancement and soil upgradation. Several studies confirmed that release of microbial inoculants into soil, sometimes have negligible effects or a transitory or a long-term

effect on resident microbiome. Such modulations may alter plant performance and soil performance and soil health and thereby inducing unpredictable

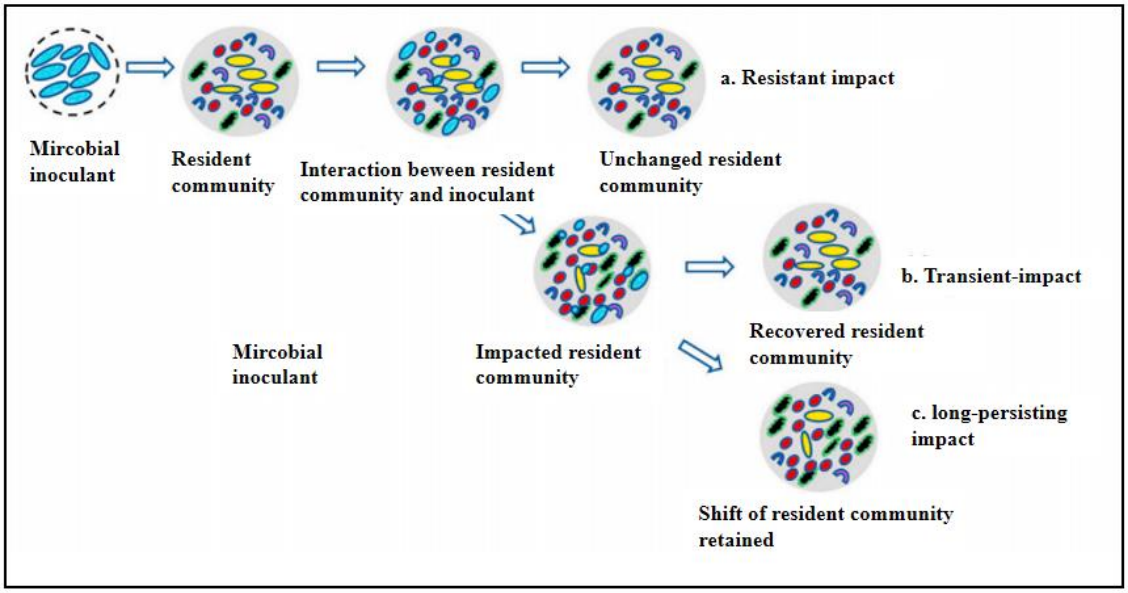


Fig. 2.2.5. Impact of microbial inoculants on soil community structure. Adopted from: Mawarda *et al.* (2020). a. Restant effect with no change in native bacterial community; b. Transient effect with recovered resident bacterial community; c. a long persistant shift in in native bacterial community. Feedback reactions (Berg *et al.* 2021). Microbial inoculation may lead to tremendous change in the number and composition of the taxonomic groups.

Although, the observed post-inoculation effects depend mainly on the inoculum type and methods used to address the dynamics of soil microbiome composition and disturbance regime (Cornell, 2021). Due to paucity of adequate information, the mode of action and the the consequent implications introduced-inoculum on soil microbiota is poorly understood. Literature mining pointed out multiple underlying mechanisms explaining the post-inoculation changes in the resident soil microbial community**.** So far, the mode of action and the the consequent implications introduced-inoculum on soil microbiota are poorly perceived. Literature mining pointed out four different underlying mechanisms explaining the driving post-inoculation changes of the resident soil microbial community (SMC). The mechanisms include: **(a)** resource competition, (b) antagonism, (c) synergism, and (d) an indirect influence related to plant root exudates (Mawarda *et al*. 2020).

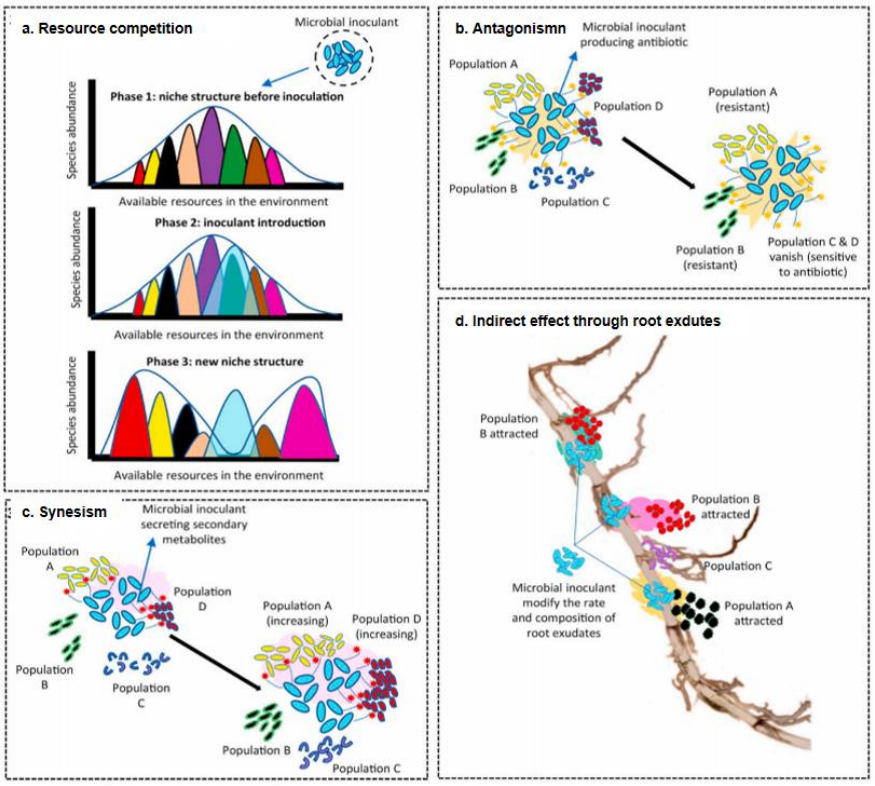


Fig 2.2.6. Four predicted mechanisms for alteration of resident microbiome community composition following introduction of microbial inoculants (a). Resource competition at the initial stage, (b). Antagonistic interaction between the introduced microbes and resident microbial community, (c). Synergism, (d). Indirect mechanism: Modification of the resident microbiome through root exudates. Adopted from: Mawarda *et al.*(2020).

Establishment of a correlation between the impacts on soil microbial community structure and the functional metabolic pathways of the soil microbial communities, is still a cereous concern to the scientific communities. The mechanisms of these driving factors in the context of the crop plant, the transient, short-term and long-term effects, and site variations are yet to be properly investigated in the perspective of agricultural sustainability. For better understanding, exploration of a broad array of omic approaches such as metagenomics, metatranscriptomics, metabonomics, and metaproteomics, are essential (Berg *et al.* 2021).

Regarding resource competition, the limiting resources left unconsumed by the native microbes and the consumption rate by the resident as well as, the introduced species determine the fate of the intruding species (Yang *et al.* 2017). As a consequence of post-inoculation impact, soil microbial diversity may also decrease due to dominant

and suppressive effects exerted by the native taxa. (Janouskova *et al.* 2017). Interestingly, even the species which fails to establish itself, it may increase 'the abundances of rare or subordinate taxa due to competitive release' modulating the resident microbial community structure (Mawarda *et al.* 2020). The introduced species may adversely affect the the growth and functionality of the native communities in their vicinity, through antibiosis. In agricultural sector, several biocontrol agents such as *Bacillus, Pseudomonas, Streptomyces, Burkholderia* possess this ability. Synergism between some resident microbes and the bio-inoculants, can influence native community structure by fostering other soil microbes, where the microbes are dependent on each other from marginal mutual support to absolute dependence. Plant root exudates contain diverse array of biochemicals which help to flourish the specific microorganisms capable of metabolizing these compounds (Mawarda *et al.* 2020).

2.3. Soybean plant: Enhancement of its performance through microbial inoculants under adverse abiotic conditions

Across the world, several studies are being conducted to explore the potential of microbial inoculants to enhance quality and productivity of soybean plants.

2.3.1. Soybean (*Glycine max* (L). Merrill) : the'miracle crop'

The multidimensional crop**,** Soybean, is an erect bushy slow-growing annual herb belonging to the legume family, Fabaceae. It is well adapted to a wide range of soil and climatic conditions and takes about 3 to 5 months to attain maturity. Fully mature seeds are generally harvested and dried up to 13-14% moisture content. The 'golden bean' seeds are the most important commercially exploited plant part containing about 40 protein and 21% oil. Globally, soybean is widely used as oil seeds. This strategic crop has multiple uses as human food, animal feed and industrial raw material (Khojely *et a*l. 2018).

Food use

Soybean has high nutritional value. The soya protein is comparable to cow's milk and animal proteins as it contains all the essential amino acids such as, glycine, tryptophan, and lysine. Besides containing a substantial amount of nutrients and proteins, the seed are rich in carbohydrate (about 33% dry weight of seeds) of which 16.6% is soluble sugar contributing to digestibility and nutritional value of soybean-based food products. The seeds are also rich in vitamin (vit)-A, vit-B, vit-D and vit-E; minerals e.g., Ca, Fe,

and K and phospholipid like lecithin (Hasanuzzaman 2022). They are considered as a promising supplement for essential constituents in human diet (Liu *et al.* 2020**).** Soya seeds can be used as a good natural source of isoflavones**.** Both non-fermented and fermented soya-based food products are consumed worldwide. The common fermented food materials include soy sauce, miso, and natto whereas, bean curd (tofu), yuba (soy milk-protein extract), kinako (roasted soybean powder), nimane (cooked pods), sprouted beans, and soy-milk are popular non-fermented food items**.** Green and immature pods are consumed as a green vegetable (edamame) (Hasanuzaman 2022).

Soybean oil is widely used in world market in preparation of various food and non-food products. In the food industry, it is used to fry or bake foods, and as a cooking oil and applied in salad and margarin (Sudarić, 2020). Soybean germ oil, a byproduct of protein preparation,

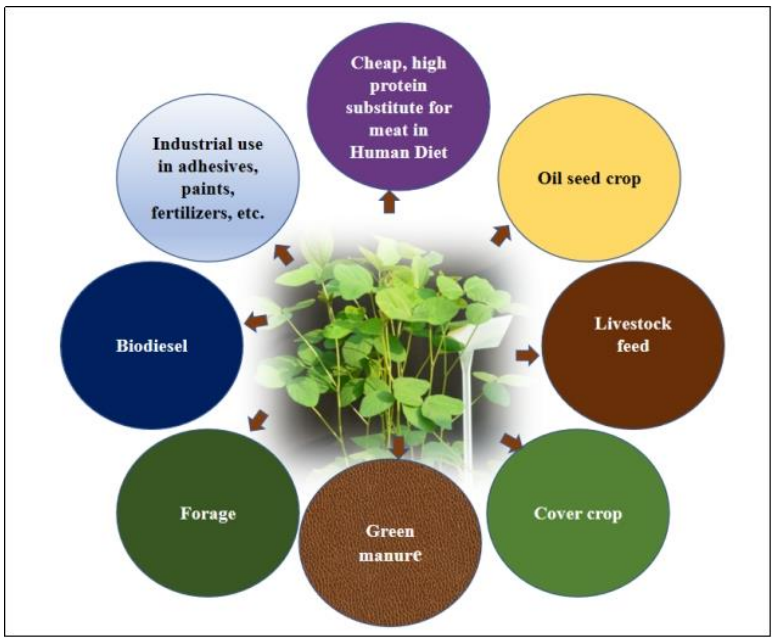


Fig. 2.3.1. Multidimensional uses of *Glycine max* (L) (Self-developed).

can reduce plasma cholesterol and is used to treat hyper cholesterolemia (Zhu *et al.* 2019). It is now established that soybean has anti-hypertensive, antimicrobial, antioxidant, anti-diabetic, and anticancer properties due to its high calorific value, high concentrations of β-carotene, vitamin-C, essential amino acids. (Sanjukta and Rai, 2016**).** Soybean is also used for making meat analogue, which has lipid and blood pressure lowering as well as, low-density lipoprotein (LDL), cholesterol oxidation, and digestibility increasing ability (Kalenik *et al.* 2017). Soybean meal is adored as a good quality animal feed because it contains a substantial amount of easily digestable protein with perfect amino acid profiling, small quantity of crude fibre, good amount of P.

Furthermore, the raw seeds and whole plant can be used as cattle feed (Biketi 2020). Soybean oil is also popularly used as an affordable aquafeed across the world, because of its good fatty acid profile (Biketi 2020).

Industrial use

Nowadays, this plant is gaining popularity as a potent biodiesel crop (Zhang et al. 2022). Solid waste of soybean is widely used to prepare "methyl soyate", a renewable substitute for petroleum diesel, which is now being used for various industrial purpose. Globally, soybean is emerging as a promising substitute to petrochemical raw materials for a wide variety of industrial product e.g., rubbers, fibers, plastics, coatings, solvents, lubricants, adhesives, and thousands of consumer goods (Sudarić, 2020). Soybean meal along with bark, is a raw material to prepare a cost-effective, formaldehyde-free, and eco-friendly, water-resistant wood adhesive with better mechanical strength, and thermal stability for plywood industry (Luo 2020).

Role in improving Agricultural sustainability of soil

Apart from the multifarious uses of soybean in human food and animal feed, this versatile plant plays a very significant role in agricultural sustainability through biological N-fixation (BNF) due to its partnership with the indigenous N-fixing symbionts, *Bradyrhizobium* spp**.** In addition to that, the AMF-induced synergism with soybean roots improves bio-avaiability and uptake of P by plants, proliferating P-solubilizing fungal genera in soil (Meena et al. 2018). It can be easily grown as an inter-crop for improving soil fertility. Therefore, crop rotation of soybean with cereal crops is now highly recommended (Adamič, 2021).

2.3.2. Microbial amendment of soil under adverse environmental conditions for enhancing performance of soybean plants

In general, rhizospheric soils of soybean grown crop lands usually contain a diverse group of native N-fixing symbionts, *Bradyrhizobium,* the characteristic soybean-rhizobia bacterial members and AMF. Strains of *Bradyrhizobium* induces rapid roots-nodulation of soybean seedlings, AMF-induced synergism improves bio-avaiability and uptake of P by plants, proliferating P-solubilizing fungal memebers (Meena *et al.* 2018). This partnership, helps soybean plants to thrive across various soil-types in P-limiting conditions. Furthermore, the plant root exudates exert a great impact on the rhizospheric microbiome (Perez Jaramillo *et al.* 2016). The combined regulatory processes and interactions between *Bradyrhizobium,* AMF-induced synergism and other beneficial soil microbes are the key players for smooth running of various soil processes operating in soybean rhizosphere. In environment-friendly low-budget

cropping practice, such interactions are essential for soil sustenance and better crop yield. In this field, extensive studies are being conducted across the world for effective utilization of co-inoculants of Rhizobial strains with other beneficial soil-microorganisms-PSB-PGPR, and AMF (Meena *et al.* 2018).

Despite the wide adaptability of *Glycine max* (L). to various soil and climatic conditions, this plant is sensitive to various abiotic and biotic stressors i.e., high salinity, drought, extreme temperature, toxic metals/metalloids, and phytopathogenic attack. Adverse biotic and abiotic factors cast a negative impact on the structure and performance of soybean plants accounting for about 26.3% and 69.3% loss of crop productivity, respectively (Hasanuzaman 2022). To mitigate the issue, application of native plant growth promoting bacteria (PGPB) is emerging as a promising eco-friendly strategy. It has been well established that the mutualistic interactions with appropriate PGPB strains can help the plants to tolerate various abiotic stressors through their multifarious directly and indirectly mechanisms such as nutrient sequestration, phytohormone, and ACC deaminase production, which are beneficial to plant growth and productivity (Agha, 2023). ACC deaminase enzyme keeps the plants active longer, even under abiotic stressors, by lowering the level of the senescence hormone, ethylene. Badawy et al. (2022) have shown that potent PGPB strains play a dual role in making plants tolerant to abiotic and biotic stresses, and thereby, promoting plant growth and performance, though the immense potential of PGPB are yet to be properly explored.

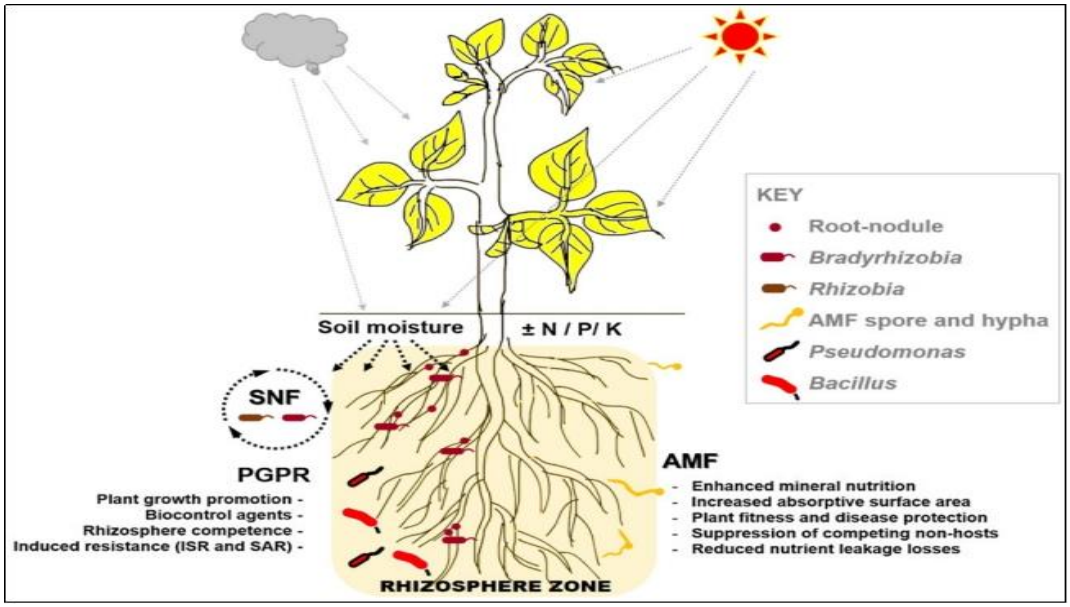


Fig. 2.3.2. Soybean rhizosphere, rhizospheric interactions and processes. Adopted from: (Meena et al. 2018) DOI 10.1007/s10725-017-0334-8

Water deficit condition

Globally, draught is one of the most destructive abiotic stresses, adversely affecting crop yield and food security (Seleiman et al., 2021). Soybean plants are also vulnerable to water stress condition and the visible effects can be detected at different growth phases, starting from seedling to seed maturity stage of the plant (Han et al. 2022, Igiehon et al. 2021). Short dry spell of of tropical summer cropping period along with the high temperature and elevated evapo-transpiration rate, adversely impacts the critical developmental activities i.e., flowering and grain filling of soybean (Basu et al., 2016). At the cellular level, water scarcity increases generation of ROS which in turn, causes impairment of various cellular processes including shrinkage of plant cell protoplast and increase in membrane permeability ultimately, leading to cell death (Rashid et al. 2022).

Across the world extensive works are on progress to unfold the potential of PGPB strains in increasing the productivity of soybean grown under various stresses. A plethora of PGPB and their metabolites including phytohormones, lipo-chitooligosaccharides (LCOs) and lipopolysaccharides (LPS) may help to alleviate ROS-induced damage through activation of appropriate physiological and metabolic pathways (Fukami et al. 2018)**.** Moretti et al. 2021) demonstrated that in tropical region the bacterial consortium containing *Bradyrhizobium* spp., *Azospirillum brasilense* strains and rhizobial metabolites application can increase chl a, chl b, total chl , and carotenoid content of soybean leaves. Furthermore, Moretti *et al.* (2021) opined that the application helped the plants to combat water deficiency through osmolyte accumulation, antioxidant metabolism, and upregulation of stress-responsive gene. Beneficial effects were also visible in nodule formation, root and shoot growth, improved photosynthetic efficiency, production of photo-assimilates which were finally reflected to grain filling.

Salt stress condition

Soybean crop is moderately sensitive to salt stress and prolonged exposure to salinity is detrimental to plant health (Arora et al. 2016). Overall growth and developmental processes are negatively affected under saline conditions due to excess production of ROS, resulting in osmotic stresses. It causes ionic imbalance and toxicity impairing normal cellular metabolic processes, Detrimental effects are reflected on seedling establishment, physiological and metabolic activities, ultimately limiting soybean yield and crops quality (Hasanuzzaman 2022). Several studies unravelled the potential of PGPB to improve plant performance by mitigating salt stress.

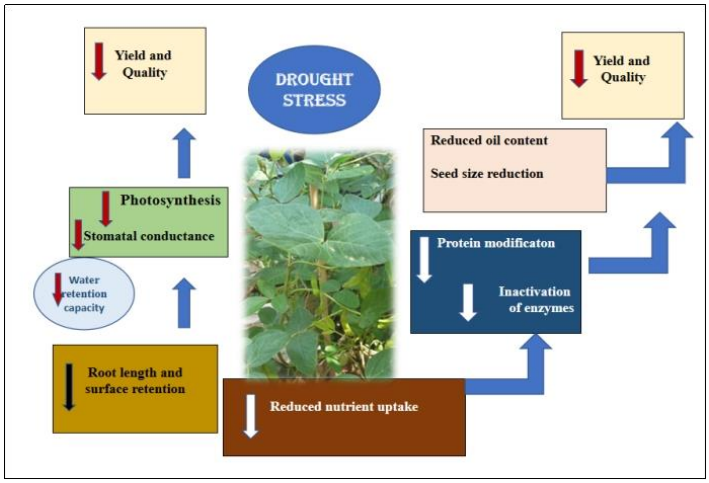


Fig. 2.3.3. Drought stress leading to various deleterious impacts on *Glycine max* (L) crops. (Self-developed, Reference: Rasheed et al. 2022)

Level of Salinity	Microbial inoculum	Salinity tolerant responses	References
100 micro molar sodium chloride 22 days	*Bradyrhizobium japonicum*	Promoted root nodule formation and growth of seedlings; elevated chlorophyll and carotenoid; improvement of photochemical potential of PSIIL; maintenance of thylakoid ultra structure and chloroplast; increased root isoflavone content	Kim *et al.* 2016
120 micro molar sodium chloride 22 days	*Bacillus amyloliquefaciens* H-2-5	Plant growth promotion and GA_4 content; reduced ABA, SA, JA, and proline level in plant	Kim *et al.* 2017
100 sodium chloride 7 days	*Pseudomonas* sp. strain AK- 1, and *Bacillus* sp. strain SJ-5	Increase in fresh weight of plant and photosynthesis activity, elevated chlorophyll, and water content, reduced osmotic injury	Kumari *et al.* 2015
70 and 140 mM NaCl, 7 d	*Porostereum spadiceum* GH786	Promoted growth of seedling, GA and isoflavone content; decreased Abscissic acid and Jasmonic acid production; reduced the rate of transpiration	Humaun *et al.* 2017

Table 2.3.1. Beneficial microbes-mediated mechanism to increase salt tolerance of soybean. Source: Hasanuzzaman *et al.* 2022.

DOI: http://dx.doi.org/10.5772/intechopen.102835 2.4.

Studies conducted by Khan (2019) showed that the PGPR strains such as *Arthrobacter woluwensis* AK1, *Microbacterium oxydans, A. aurescens, B. megaterium, and B. aryabhattai* can remarkably improve salt tolerence potential of soybean plants. Accorging to Agha (2023) a combined treatment of *Enterobacter* Delta PSK and the natural symbiotic partner *B. japonicum* improve soybean plant growth under salinity stressors confirming the potential of *Enterobacter* Delta PSK to combat osmotic stress (Agha et al. 2023). Khan et al. (2019) reported phytohormones, antioxidants, and ACC deaminase production by *Curtobacterium* sp. SAK1 to combat salt-stress and promote growth of soybean plants. Similar findings were reported by Sofy et al. (2021) where *Bacillus subtilis* and *Pseudomonas fuorescens* enhanced the synthesis of photosynthetic pigment in pea plants under high salinity condition. Zhao et al. (2018) opined that enhanced absorption of P, N, and K might be the underlying cause of PGPB-stimulated pigment production in treated *Glycine max* (L.) plants. These findings suggest for utilization of PGPB as a promising solution to overcome salinity problems (Dawood et al. 2022).

2.4.Application of Metagenomics in Agricultural Sustainability

During the last decade, several studies have been conducted on soil and plant-associated microbiomes. Furthermore, it has been recognised that plants along with their associated microbes act jointly in a concerted manner constitute the 'holobiome', which greatly influences plant performance (Sanchez-Cañizares et al., 2017). In traditional laboratory culture-based techniques, only about 0.1% of soil microbes are culturable and the vast microbial world is remaining unexplored (Rashid M, Stingl, 2015). However, novel molecular technique of next-generation metagenomic sequencing facilitates an in-depth evaluation of soil microbial diversity and subsequently, help to identify the functional groups of microbial members. This highly efficient and cost-*effective method can be applied to know the in situ assessment of agricultural soil health.* This technique, coupled with computational biology and conventional culture-based methods, help the agricultural biologists to have a comprehensive knowledge about the soil microbes and their impact on associated cropping patterns. The innovative approach of microbiome-based technologies help us to identify functional soil microbes, which are the key players for ecosystem functioning and regulating plant growth.

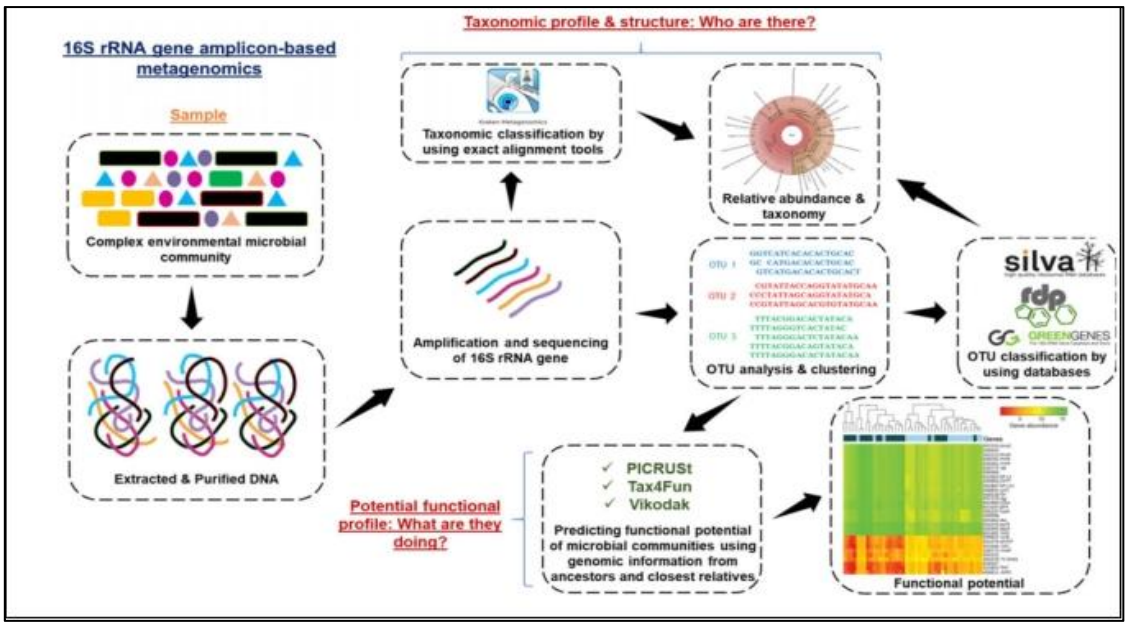


Fig 2.4.1. General pipeline and bioinformatic tools used to analyse metagenomic 16s rRNA data sets for obtaining taxonomic and functional profiles of any microbial communities. (Adopted from Morgan and Huttenhower, 2012)

2.4.1. Taxonomic profiling of soil microbial community

Soil is the reservoir of myriad of microorganisms which are beneficial for agriculture. Traditional laboratory culture technique cannot explore the potential of most soil microorganisms which are not culturable (Bevivino and Dalmastri, 2017). The emerging area of metagenomics, has unraveled the mystery of soil microbiome, offering a scope to understand the composition of the microbial community structure, and of a micro-niche of special interest. Thus, it helps us to understand the structural compositions of bacteria, archaea, and fungal members of a soil microbiome (Martínez-Porchas and Vargas-Albores, 2017). It reveals the identity of the existing taxa, as well as their relative abundance in the sample under study (Philippot et al., 2013). This technique helps us to have a comprehensive idea about the total soil microbiome by sequencing and analysing the extracted DNA. It is a non-culturable approach that enables us to study the genomes of whole microbial community in an environmental sample, in a greater scale. This technique unravels the vast microbial diversity existing in a soil environment and thereby, facilitates the utilization of novel microbial members in agricultural sectors for sustainable crop production (Garrido-Oter et al., 2018).

2.4.2. Prediction of functional metabolic pathway and soil microbial network

Soil of agricultural lands represents a challenging and diversified ecosystem, presenting unlimited resources. Beneficial soil microbes are essential proper ecosystem functioning, majority of them being non-culturable in laboratory condition, their functional potential is still untapped. Metagenomic analysis of a microbiome enables us to study the predictable functional contributions of the microbial community by identifying and quantifying the gene composition (Philippot et al., 2013). All productions in agricultural sectors are intricately related to the biotic organisms that are co-inhabiting and interacting with each other and with their surrounding environments (Martínez-Porchas and Vargas-Albores, 2017). Furthermore, plant and associated microbial communities interact with each other and with the plant. For sustainable agricultural production, some of these interactions are essential (Philippot et al., 2013). The biochemical substances produced in the above-ground parts of plants may either positively or negatively influence the rhizospheric microbial community composition

and vice versa. These interactions can even improve crop yield in the above-ground plant parts (Igiehon and Babalola, 2018). Metagenomic tools have facilitated the identification of agriculturally beneficial unique enzymes from soil which, may be aptly used to reduce our dependency on chemical fertilizers and pesticides promoting a sustainable environment (Nwachukwu and Babalola, 2022). Some of the enzymes in this category are: sulfatases, dehydrogenase, phosphatases, amylases, xylanases, lipases, cellulases, proteases (Peng et al., 2018; Salem et al., 2020). Application of such untapped soil resources in commercial scale, is increasing rapidly and metagenomic tools can be successfully implemented to meet the massive demand of these biocatalyst molecules (Ghosh et al., 2018). Utilization of enzymes in agriculture will improve soil health and ensure sustainable crop production (Ahmed et al., 2018).

2.4.3. Combined Traditional methods and Metagenomics strategies for Precision Agriculture

Literature search reveals that application of microorganisms with PGP traits can increase crop productivity by 50–60% (Abram, 2015). The importance of microorganisms to enhance plant growth and performance is well-established, but till now, the vast majority of the rhizospheric microbiota is yet to be explored. A combination of the traditional methods with metagenomic approach can be effectively used to assess the functional and structural characteristics of the soil microbial community (Batista and Singh, 2021).

Additionally, prediction-based analysis of the complex of soil microbial community network, including the interactions among these communities with crop plants and their surrounding soil environments, have been possible through the application computational biological methods (Guerrero- Ramirez *et al*., 2018). These methods also have a degree of predictive power to understand the responses of a soil system to various changes in climatic conditions, new cropping systems, and soil management practices. The metagenomic tools also benefit the field practitioners to choose the potent agriculturally beneficial soil microbes for utilization as symbiotic/free-living nitrogen-fixers and the associated crop plants. Such inoculants can be used as seed coats or in powdered form or as liquid biofertilizers, applied to soil during planting of seeds or seedlings (Rocha et al., 2018). In microbiome-based precision agriculture, it may be predicted which functions are needed to provide externally into the soil in the

form of organic amendment e.g., organic compound, microorganisms, and plant extract. Such predictive models will enable us to forecast proper crops/agronomic practices suitable for a particular type of soil/microbiome (FAO 2020).

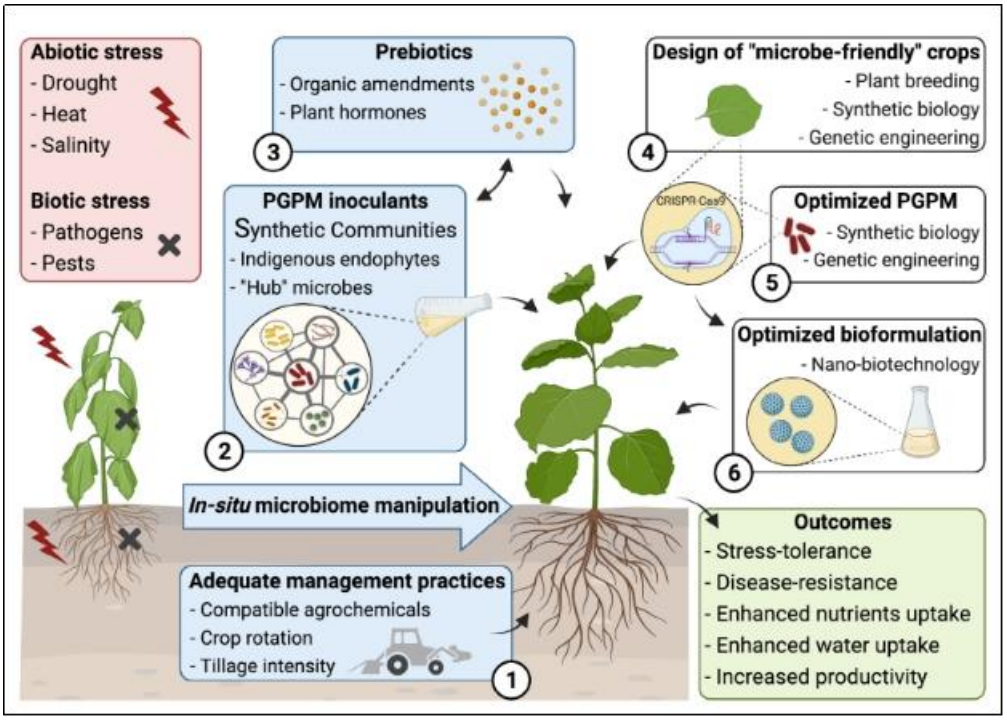


Fig 4.2.2. Traditional and metagenomics-based strategies for promoting the establishment of plant-microbiome association for improving crop yield. After application of microbial inoculants in the crop land multiple biotic and abiotic factors (red box) may influence the success of the inoculum. To overcome the issue, the traditional method of (blue box) *in situ* manipulation of the plant-associated microbiota can be induced for facilitating the establishment of plant-beneficial microorganisms. Traditional strategies are: management practices, (1) (2) introduction of PGP microbes and (3) application of plant probiotics. Emerging strategies (white boxes) are: (4) designing microbe-friendly crop; (5) optimization of PGP strains, (6) optimization of bio-inoculant formulations. (Adopted from Batista and Singh, 2021).

CHAPTER-3

Materials and Methods

List of chemicals and reagents used

A list of all the chemicals and reagents used in this investigation is furnished in **Table 3.1.** The names of the manufacturers are also included.

Name of the Chemical/Reagent/Medium	**Manufacturer**
Absolute Ethanol	HiMedia
Acetic acid	Merck
Acetone	Merck
Agar	HiMedia
Aleksandrow medium	HiMedia
Aminocyclopropane 1-carboxylic acid (ACC)	Merck
Ammonium molybdate	Merck
Ammonium meta vanadate	Merck
Antibiotic paper discs	HiMedia
Barritt's reagent	HiMedia
Beef extract	HiMedia
Brain Heart Infusion agar (BHI agar)	HiMedia
Bromo Thymol Blue	Loba Chemie
Boric acid	Merck
Casamino acid	Sigma-Aldrich
Chitin	Sigma-Aldrich
Christensen's Urea Agar medium	Himedia
Chrome Azurol S (CAS)	HiMedia
Citric acid	Merck
Congo red dye	Merck
Copper sulphate	Merck
Crystal Violet	Merck

Name of the Chemical/Reagent/Medium	Manufacturer
Dimethyl sulfoxide (DMSO)	Merck
Diphenyl-1 picrylhydrazyl	HiMedia
Ethanol (E.M. Grade)	Merck
Ethylene diamine tetraacetic acid (EDTA)	HiMedia
Para-dimethyl amino-benzaldehyde	Merck
Ferric chloride	Merck
Ferrous sulphate ($FeSO_4.7H_2O$)	Merck
Gluconic acid	Merck
Gibberelic acid (GA_3)	Sigma-Aldrich
Gilatin	HiMedia
Guaiacol	Sigma-Aldrich
Hexadecyl trimethylammonium bromide (HDTMA)	Merck
Hydrochloric acid	Merck
Hydrogen peroxide	Merck
Indole acetic acid	HiMedia
Jensen's medium	HiMedia
Kovac's reagent	Loba Chemial
Kraft lignin (MSM-KL)	Sigma-Aldrich
Laminarin	Sigma-Aldrich
Luria Bertini broth	HiMedia
Magnesium sulphate	Merck
Manganese sulphate	Merck
Methyl red	Loba Chmie
MOBIO PowerSoilTM DNA isolation kit	Qiagen
Molybdenum oxide (MoO3)	
α napthylamine	HiMedia
Nessler's reagent	Loba Chemie
Nitric acid	Merck
Nitrous acid	Merck
Nutrient agar	HiMedia
Nutrient broth	HiMedia

Name of the Chemical/Reagent/Medium	Manufacturer
Perchloric acid	Merck
Peptone	HiMedia
Picric acid	Loba Chemie
Piperazine-1, 4-bis (2-ethane sulfonic acid) (PIPES)	HiMedia
Pikovskaya broth	HiMedia
Potassium nitrate	Merck
Potassium hydrogen phosphate	Merck
Potassium ferro cyanide	Merck
Potato dextrose agar	HiMedia
Pyrogallol	Sigma- Aldrich
Seeds of *Glycine max* (L) Merill variety JS-0335	ICAR-Indian Institute of Soybean Research, Indore
Soluble Starch	Loba Chemie
Simmon's citrate agar	HiMedia
Sodiun nitrite	Merck
Sulfanilic acid	Merck
Tetrazolium salt	Sigma-Aldrich
Tryptone broth	HiMedia
Tryptophan	Sigma-Aldrich
Tetramethyl para phenylene diamine dihydrochloride	Merck
Tween-80	Merck
Yeast extract	HiMedia
Zinc acetate	Merck
Zinc sulphate	Merck

Table 3.1. List of chemicals and reagents used

Instruments and Tools Used

- Agilent Bioanalyzer (DNA 1000 chip)
- Aligner software (for consensus DNA sequence/contigs generation): BioEdit
- Bright-field compound light microscope: Dewinter DIG1510, 5.1 MP 1/ 2.5'' CMOS sensor
- BOD incubator: Bio Techno Lab BTL-6
- DNA sequencer: Thermo Fisher ABI 3730xl Genetic Analyzer, BDT v3.1 Cycle sequencing kit
- Eluting PCR amplicons: Qiagen- QIAquick gel extraction kit
- FASTQC pipeline
- Flame photometer: Systonic
- Freezer (-20 ^{0}C): Blue Star CHFSD150DHSW/DHPW
- Illumina MiSeq
- KAPA HiFi HotStart ReadyMix PCR Kit (KAPA BIOSYSTEMS).
- Kjeldahl apparatus: Dolphin Labware KDU
- Illumina MiSeq
- MOBIO PowerSoil™ DNA isolation kit (Qiagen, United States)
- Multiple alignment software: Clustal W
- NanoDrop™ 2000 Spectrophotometer (ND2000, Thermo Scientific)
- PCR system: ProFlex, Life Technologies
- Phylogenetic tree construction: MEGA 7
- Python software version 3.11+
- Qubit™ dsDNA BR (Broad Range) Assay Kit (ThermoFisher Scientific)
- SILVAngs (1.3) pipeline
- Spectrophotometer: BIO-RAD T-100
- UV-Visible spectrophotometer: Optizen
- Venny 2.1.0 version

MATERIALS AND METHODS

3.1. Collection and characterization of soil sample

The soil samples were collected from five different long-term used and abandoned agricultural fields located in different districts of South Bengal such as, South 24 parganas (Bahadurpur, Masat), Birbhum (Sadaipur), Nadia (Majdia, Hanskhali), abiding by the protocol of (TNAU-2013). For microbiological study, soil samples were taken in sterile zip locked bags and stored in -20 ^{0}C refrigerator till further use. Collected soils were sun dried and homogenized with morter and pestle, prior to analysis for their physico-chemical parameters. Freshly collected soil from sampling field was used for conducting pot experiments. The pH level, water holding capacity, and soil particle size (sand, silt, clay) were determined (Jackson, M.L, 1967; Gavlak et al. 2005). Soil nutrient status was analysed. The organic carbon content of soil (SOC), available nitrogen (N), phosphorus (P), potassium (K), Zinc (Zn) and iron (Fe) level were estimated using standard methods as followed in Mandal (2020). Details of the sampling site is furnished in Table 3.2.

Soil Sample No.	**Soil Sample code**	**Sampling site**		**Location**
		Village	**District**	
1	A	Bahadurpur	South 24 parganas	22.2038° N, 88.1777° E
2	B	Masat	South 24 parganas	22.2615°N 88.2454°E
3	C	Sadaipur	Birbhum	23.8242° N, 87.4542° E
4	D	Majdia	Nadia	23.401893°N 88.394087°E
5	E	Hanskhali	Nadia	23.367747°N 88.601146°

Table 3.2. Soil sampling sites and their location

3.2. Isolation, preliminary morphological, and biochemical characterization

The serial dilution-agar plate method was used to isolate bacteria from the selected soil samples. In a conical flask 90 ml sterile distilled water was taken, to it 10 g of soil sample was added and placed it on a rotatory shaker for 5 min to get a homogeneous mixture (Kumar et al 2012). It was then serially diluted using sterile distilled water. From appropriate dilutions, 200 µl aliquot was taken out, plated aseptically on sterilized Nutrient agar (NA) medium (HiMedia), incubated at 37ºC for 24 hours (h). The colonies showing predominant growth (1-3mm in diameter) on NA medium culture plates were chosen and re-streaked using quadrate streaking method on sterilized NA plates to obtain pure cultures.

3.2.1. Morphological characterization

Preliminary morphological characteristics of the isolates were studied following the protocols described in Cappuchino and Sherman (2017). The colony characteristics such as colour, texture, elevation and margin were observed. For studying the morphological features of the bacterial cells such as shape and Gram nature, Dewinter DIG1510, 5.1 MP 1/ 2.5'' CMOS sensor bright-field optical microscope was used.

3.2.2. Biochemical characterization

The biochemical characteristics e.g. indole production, MR-VP test, citrate utilization, oxidase, catalase, urease, nitrate reductase production tests were performed according to the protocols described in Cappuchino and Sherman, (2017).

Indole production test

Tryptophan broth medium (peptone 10 g L^{-1}, NaCl 5g L^{-1}, pH 7.4) was prepared. For preparation of Kovac's reagent, 10 g of p-dimethyl amino-benzaldehyde was dissolved in 150 ml Amyl alcohol, then 50ml concentrated Hydrochloric acid (HCl) was added to it slowly, and stored in refrigerator. The tryptophan broth medium was inoculated with 1 % (v/v) inoculum of each of the bacterial isolates and kept under incubation at 37º C

for 48 hrs. Then 0.5 mL of the Kovac's reagent was poured to the culture tubes carefully along the walls, without shaking. The tubes were gently agitated after completion of reagent addition. Development of a reddish ring on the top of the reaction mixture indicated the ability of bacteria to hydrolyze tryptophan.

Methyl red-Voges Proskauer (MR-VP) tests

For the tests, two sets of dextrose phosphate broth were prepared (peptone- 5 g L^{-1}, K_2HPO_4 5 g L^{-1}, dextrose- 5 g L^{-1}, pH 7.6). Both of the sets were inoculated with 1 % (v/v) of the respective bacterial isolates and incubated at 37º C for 48 h. After incubation, one set of bacterial cultures were treated with 5-6 drops of methyl red indicator (0.1 g methyl red in 200 ml 95 % ethanol) and to the other one, 1-2 mL of Barritt's reagent (5 % α napthol in 100 % ethanol and 40 % KOH) was added. After the addition of reagents, culture tubes were shaken gently. Development of red colour was considered as an MR positive result whereas, in Barritt's reagent treated cultures, appearance of copperish red colour indicates a VP positive result.

Nitrate reduction test

Nitrate broth media (beef extract- 3 g L^{-1}, KNO_3 1 g L^{-1}, peptone- 5 g L^{-1}, pH 7), reagent A (0.25 g α napthylamine in 50 mL 30 % acetic acid) and reagent B (0.15 g sulfanilic acid in 50 mL 30 % acetic acid) were prepared. The sterilized media were were inoculated with 1 % (v/v) inoculum of respective bacterial cultures kept in incubation at 37 ^{0}C for 24 h. Then the bacterial cultures were treated with 1 ml each of reagent A and reagent B, respectively. Development of red colour indicates positive result for nitrate reduction test. If no colour change is observed after the addition of the reagents, zinc dust was added to the tube for reducing the nitrates (if any) to nitrites. Development of red colour after addition of zinc dust, it was taken as a true negative result.

Citrate utilization test

Sterilized slants were prepared using Simmon's citrate agar media (HiMedia), pH 6.9 and were inoculated with respective bacteria isolates. The inoculated slants were incubated for 24 h at 37ºC. A change of media colour from green to deep blue

following incubation, was considered as a positive reaction regarding citrate utilization. No change in media colour (remained green), indicated a negative result.

Oxidase test

A filter paper was soaked in tetramethyl para phenylene diamine dihydrochloride solution (1.5 %) and moistened with sterile distilled water. The colony of each of the test bacterial isolates was pricked to it. A change in colour change of the filter paper to deep blue/purple within 10-30 seconds indicated a positive result.

Catalase test

The bacterial isolates were streaked on NA medium and kept in incubation for 24 h at 28 ± 2^0C. A small amount of bacterial colony was taken on a clean, dry glass slide using a sterile loop, and 4-5 drops of 3% hydrogen peroxide were added to it. Production of catalase was detected by the rapid evolution of gas bubbles within a few seconds**.**

3.3. Screening of soil bacterial isolates for their *in vitro* NPK acquisition ability

The mineral nutrient harnessing potential of the selected bacterial colonies were examined on selective media.

3.3.1. Determination of N-fixing ability

The bacterial isolates from the selected colonies were grown separately on nitrogen-free Jensen's medium (M-710, HiMedia) for isolation of *in vitro* N-fixers **Ahmad et al., 2008).** The medium was supplemented with the acid-base colour indicator Bromo Thymol Blue (BTB). The N-fixing bacterial colonies were detected by their ability to change the colour of modified Jensen's medium within 5-8 days of inoculation from greenish blue (initial colour) to dark-blue (intermediate stage) and finally to yellow indicating different steps from N-fixation, nitrification to ammonification **(Sulistiyani, and Meliah., 2017)**. Only the isolates having *in vitro* N-fixing ability were chosen and purified by repeated sub-culturing, and maintained for further screening.

3.3.2. Qualitative and quantitative estimation of phosphate solubilization

For the detection of inorganic phosphate solubilization ability, isolates were spot inoculated on Pikovskaya agar (PKV) medium (HiMedia) (Pikovskaya, 1948). Phosphate solubilizing bacteria (PSB) formed a zone of clearance around the bacterial colonies. The colonies with halo-zones were selected to calculate their solubilization index (SI) using the formula (Edi-Premono *et, al.* (1996): Phosphate SI = (colony diameter + halo zone diameter)/colony diameter . *In vitro,* phosphate solubilization ability of the bacterial isolates was assessed quantitatively by the Vanado molybdate phosphate yellow colour method using Barton's reagent in accordance with the protocol of Pande *et al.*, 2019. Preparation of Barton's reagent: Soution-A. 25 g of ammonium molybdate was dissolved in 400 mL of water. Solution-B. 25 g of ammonium meta vanadate was added to 300 mL of boiling water (after cooling), then 250 mL of concentrated HNO_3 was poured to it. Finally solution-A and solution-B were mixed and the volume was made up to 1 L adding distilled water.] Freshly grown bacterial culture (10 µl) was inoculated in Pikovskaya broth medium and was incubated at 28 ± 1°C for 8 days. Phosphate solubilization potential was tested at 2^{nd}, 4^{th}, 6^{th}, and 8^{th} day, respectively. On each day, 1 mL of the inoculated culture was centrifuged for 20 min at 10,000 rpm and subsequently filtered using 0.45 µM milipore filter. To 0.1 mL of culture filtrate, 0.25 mL of Barton's reagent was mixed and the final volume was adjusted up to 5mL by adding double distilled water. The reaction mixture was then incubated for 10 min till appearance of yellow colour and intensity of yellow colour as measured at 430 nm. The amount of soluble phosphorus present in the supernatant was was measured based on a standard curve prepared form known concentration of KH_2PO_4 (Pande *et al.,*2019). Laboratory strain *Bacillus* sp. was kept as control.

3.3.3 Qualitative and quantitative estimation of potassium solubilizing efficiency

Potassium (K) solubilizing bacterial (KSB) colonies was detected on modified Aleksandrow medium (Himedia) containing mica as source of mineral potash, using acid-base indicator dye, BTB, prepared in 70% (weight/volume) ethanol (Rajawat *et al.*, 2016; Etesami *et.al.*, 2017). The bacterial isolates were streaked on the media and kept

at 37 °C incubator for 72 h. The isolates capable of changing the colour of the inoculated media to yellow, due to organic acid formation within 72 h of incubation, were considered as potassium solubilizing bacteria (PSB) and taken for quantification of K solubilization in accordance with the method followed by Saiyad *et.al.* (2015). Hundred ml of sterile Aleksandrow medium was taken in a 250 ml of Erlenmeyer flask and 100 µL of bacterial inoculum (10^8 cfu mL^{-1}) was added to it and incubated at 30 ± 2 ^{0}C for 10 days in a rotary shaker at 150 rpm speed. The culture suspension of each of the isolates was spinned for 10 min at 10,000 rpm and 1 mL of the supernatant was transferred to a 50 mL volumetric flask. The volume was adjusted to 50 mL using distilled water and mixed properly. Then the reaction mixture was fed to a flame photometer for measuring the amount of K released in the medium from mineral mica due to solubilization by the test bacterium. Using flame photometer, the amount of K released in the medium was checked at 3rd, 5th, 7th, and 10th day of incubation.

All the experiments were run in triplicates to minimize the error. Only the bacterial isolates showing positive results for i*n vitro* mineral harnessing potential were selected, sub-cultured on NA slants, and maintained at 4 ^{0}C for further studies.

3.4. Compatibility study among promising isolates and designing multi-strain bacterial consortia

This study was performed by T-streaking method on NA plates (Fukui *et al*., 1994). Based on the nutrient harnessing ability (NPK) and compatibility study, 5 isolates (S1, S3, S5, S7, R1, an R2) from soil sample-A, and isolate L3 from soil sample-C were chosen for utilization as bacterial inoculants in plant growth promotion study.

3.6. Preliminary pot trial experiment for selecting most potent multi-strain bacterial consortia growing *Glycine max* L. as test plant

The preliminary yield trial was conducted using the field soil sample-A (silty clay soil of Bahadurpur). *Glycine max* (L) Merill. variety JS-0335 procured from the ICAR Indian Institute of Soybean Research, Indore, was used as test plant. Before sowing, seeds were surface sterilized using sodium hypochlorite solution (0.1%) for 5 min and

then rinsed thoroughly with sterile distilled water 5 times. The non-sterilized, freshly collected soil from the field was used for this experiment. For the experiment earthen pots of 28 cm diameter were used, each pot contained 5 kg of non-sterilized experimental soil. The pots of each treated and untreated set-up were maintained in four replicates. Six seeds were planted per pot. Four replicates were maintained for each set-up. The inoculant formulation contained 20% of bacterial of consortium in LB Broth culture (48 hours old) with bacterial concentration of 4.5 X 10^8, 30 % sunflower oil, 20 % Tween-80, and 30 % water. Each consortium contained three different bacterial isolates at 1:1:1 ratio. The first dose of inoculant formulation (20 mL pot^{-1}) was applied in soil after 15 days of the seedling emergence stage, followed two successive doses on 35 days and 55 days stages, respectively. An equal amount of water was applied to each pot on every two days. Once in every week, de-weeding was practiced.

The experimental design is presented in Table 3.3.

Experimental Set-up	**Consortia Combination used**	**Bacterial Isolate Code used**
Control (non-sterilized field soil)	Untreated soil	No bacterial inoculum used
Treatment-1 (non-sterilized field soil + consortium-1)	Combination-I	S3+S7+R1
Treatment-2 (non-sterilized field soil + consortium-II)	Combination-II	S1+S7+R2
Treatment-3 (non-sterilized field soil + consortium-III)	Combination-III	S3+S5+R1

Table 3.3. Preliminary pot trial experiment design

In vivo plant growth promotion study

The efficacy of the consortia was tested based on their impact on selected vegetative and reproductive growth parameters of the potted plants. Data were recorded every 4, 8, and, 12 weeks after the seedling emergence (WAE) stage for analysing vegetative parameters of plants like the total no. of leaves, leaf area, plant height, and the no. of root nodules plant^{-1}. Yield related traits such as the total no. of pods node^{-1}, total no. of pods plant^{-1}, and pod size, and pod weight were recorded.

Estimation of chlorophyll content of leaves

Chlorophyll (chl) content of leaves such as chl-a, chl-b and total chl(a+b) were measured spectrophotometrically at 4, 8, and 12 WAE respectively. To extract chlorophyll pigment, 80% acetone was used. Freshly collected mature leaves (2nd and 3rd leaves) from the pot-grown soybean plants were washed properly in running water. Then 0.5 g of the leaf segments (0.5 X 0.5 cm^2 dimension) were placed in 10 mL of 80% acetone solution for extraction of chlorophyll and incubated at room temperature for 24 h. Then the leaf pieces were taken out and the chlorophyll extracts were spinned for 5 min at 15,000. The absorbance of the supernatants for each of the experimental set up, was observed at wavelengths of 645 nm and 663 nm in a UV vis spectrophotometer (Liang, 2017). Samples showing absorbance >1, were diluted by half using 80% acetone solution and re-examined. The chl-a, chl-b and chl(a+b) content were estimated according to the equation of Arnon *et.al* (1949):

$$\text{Chlorophyll a } (\mu g\ mL^{-1}) = 12.7\ (A_{663}) - 2.69\ (A_{645})$$

$$\text{Chlorophyll b } (\mu g/mL) = 22.9\ (A_{645}) - 4.68\ (A_{663})$$

$$\text{Total chlorophyll (a+b) } (\mu g/mL) = 20.2\ (A_{645}) + 8.02\ (A_{663})$$

3.6. Characterization of the promising bacterial isolates (from efficient consortia combinations) for their additional agriculturally beneficial traits

The bacterial isolates from the consortia combinations showing better performance with respect to vegetative and reproductive parameters, were chosen for additional PGP characterization.

3.6.1. Quantitative measurement of Indole-3-acetic acid (IAA) production

The experiment was conducted using Salkowski reagent following the protocol as described in Sarker and Rashid (2013) with slight modification. [Salkowski reagent preparation: 2 mL of 0.5 M $FeCl_3$ was mixed with 49 mL 70% Perchloric acid and 49 mL of water. It was stored in an amber bottle at room temperature.] The selected bacterial isolated were grown in LB broth in two separate set ups, in up one set 0.1% tryptophan was added. They were kept under incubation at 28 ^oC for 48 h. After incubation, the culture broth was spinned at 1000 rpm for 10 min. Two mL of the aliquot was taken in a test tube, 2 mL of freshly prepared Salkowski reagent was poured into it, and then, kept in incubation for 25 to 30 min. The development of pink color in the reaction mixture confirmed IAA production, and its intensity was measured at 540 nm in a UV-VIS spectrophotometer. The amount of auxin produced, was detected from the standard curve prepared from known concentrations (10 to100 µg mL^{-1} of IAA). This experiment was performed with three replicates and the data on estimated IAA concentration were presented as the mean values of repetitions ± standard deviation. The standard laboratory strain *Bacillus* sp. Was used as control.

3.6.2. Quantitative estimation of Gibberellic acid (GA_3)

GA_3 producing potential of the bacterial isolates were estimated using the standard colorimetric method of Hollbrook *et al.* (1961) with few modifications as followed by Sharma *et al*. (2017). Two different set ups were maintained where bacterial cultures were grown for 5 days and 7 days in 50 ml flasks. In 15 mL of bacterial culture supernatant, two ml of Zinc acetate reagent was added and kept for 2 min. [Zinc acetate

reagent preparation: 21.9 g zinc acetate was added to 1 mL of glacial acetic acid. The volume was adjusted to 100 ml adding distilled water]. Two mL of potassium ferrocyanide (10.6% in distilled water) was mixed to it and spinned at 2000 rpm for 15 min. Then 5 ml of supernatant was taken and 5 mL of 30 % HCI was poured into it. After 75 min of incubation of the reaction mixture at 20ºC, the amount of GA_3 production was detected measuring the absorbance at 254 nm and estimated from the standard curve, prepared from known concentration of Gibberellic acid (GA_3, Hi-media). Five ml of 5 % HCL solution was used as blank. The laboratory strain *Bacillus* sp. was used as control.

3.6.3. Detection and quantitative estimation of ACC deaminase production

To detect ACC deaminase production by the selected isolates, minimal Dworkin-Foster (DF) media was used. [Minimal DF media composition: KH_2PO_4 4 g L^{-1}, Na_2HPO_4 6 g L^{-1}, $MgSO_4.7H_2O$ 0.2 g L^{-1}, Glucose 2 g L^{-1}, Gluconic acid 2 g L^{-1}, Citric acid 2 g L^{-1}, Trace elements: $FeSO_4.7H_2O$ 1 mg L^{-1}, H_3BO_3 10 mg L^{-1}, $MnSO_4.H_2O$ 11.19 mg L^{-1}, $ZnSO_4.7H_2O$ 124.6 mg L^{-1}, $CuSO_4.5H_2O$ 78.22 mg L^{-1}, MoO_3 10 mg L^{-1}]. Log phase culture of each isolates were taken and centrifuged at 10,000 rpm. The cell pellets were collected and washed twice using saline water. The pellets were again suspended and spotted on the minimal DF media plates (in triplicate) amended with 3mM ACC as the only source of nitrogen. The inoculated plates were incubated at 28ºC for 72 h. Minimal DF media amended with ammonium sulphate as positive control and minimal DF media without any nitrogen source was kept as negative control (Kumar *et. al.* 2012).

The ACC deaminase enzyme was then quantified colorimetrically by ninhydrin-ACC assay method, in accordance with the protocol of Li Z., *et al.*, 2011. A single colony was picked up fresh culture on LB agar medium, inoculated in a tube containing 5 ml of LB broth media and incubated over night at 28ºC on a shaker at 200 rpm. From each culture, 2 mL of suspension was harvested in a 2 mL micro-centrifuge tube and spinned at 8000 g for 5 min. The cell pellets were collected, washed twice using 1 mL of liquid DF medium, taken in a 12 mL culture tube, suspended in 2 mL DF-ACC medium, and were kept under incubation for 24 h on a shaker at 200 rpm. Parallely, an un-inoculated DF-ACC medium sample (2 mL) was incubated. From each culture tube 1 ml of suspension was taken in a 1.5 ml centrifuge tube and spinned at 8000 g for 5

min. Then 100 µL volume of each supernatant was placed in a 1.5 mL centrifuge tube and diluted to 1 mL with liquid DF medium. From each of the 10 times diluted supernatant, 60 µL was used for 96-well PCR-plate ninhydrin-ACC assay. Sixty mL of working solution and 120 mL of ninhydrin reagent were dispensed by pipetting into the wells of the PCR plate and was kept in floating condition on a boiling water bath for 30 mins. As blank un-inoculated DF medium was used. The experiment was run in triplicate to minimize the error. The intensity of the resultant Ruhemann's Purple colour variation was measured spectrophotometrically at 570 nm. The bacterial isolate having visibly lower colour depth and absorbance compared to that of the uninoculated DF-ACC medium, was considered as ACC deaminase enzyme producing isolate. The enzyme activity was measured from the prepared standard curve of ACC concentration ranging from 0.05 mmole L^{-1} to 0.35 mmole L^{-1}. The amount of ACC utilized in the medium indicated the activity of ACC deaminase enzyme (Li Z., *et al.*, 2011). The standard laboratory strain *Bacillus* sp. was used as control.

3.6.4. Siderophore production

For detection of siderophore production by the bacterial isolates, O-CAS assay method was followed (Pérez-Miranda et al., 2007). Chrome azurol S (CAS) medium (blue agar medium) was following the method of Schwyn and Neilands (1987), without the addition of nutrients. For preparation of 1 L of CAS agar, 60.5 mg CAS was dissolved in 50 ml water, to it 10 ml Ferric chloride solution (1 mM FeCl, $6H_20$, 10 mM HCl) was added and mixed properly. To 72.9 mg HDTMA (dissolved in 40 mL water) solution, this solution was slowly added and stirred continuously. A dark blue liquid was obtained. This solution was autoclaved along with a mixture of 750 ml H_20, 100 mL 10 X MM9 salts, 15 g agar, 30.24 g Pipes, and 12.00 g of a 50 % (w/w) NaOH solution. The NaOH solution was required for raising the pH of Pipes (6.8). The mixture solution was cooled down to 50°C and to it 30 mL casamino acids (l0 %) was added as the C-source. The blue dye solution was carefully added with enough agitation for mixing to avoid foam generation. A freshly prepared medium was applied uniformly as an overlay over the agar plates containing 24 h grown bacterial cultures. After 15 mins, the overlaid regions surrounding the bacterial colonies were carefully observed to detect any change in color in the overlaid medium. The development of an orange, yellow or purple halo zone around the bacterial colony confirmed siderophore

production (Pérez-Miranda et al., 2007). The basic principle of the experiment is that the brightly coloured Fe+3- dye complex, binds with the strong ligand siderophore, resulting in the formation of an iron-ligand complex. This iron-ligand complex releases free dye which changes the colour of the blue medium to yellow (Sasirekha and Srividya, 2016). Plates containing the overlay without any bacterial colony showed no change in the coloration and were considered as the control set. The experiment was repeated thrice with three replicates each.

Siderophore typing

This experiment was performed based on the method followed by Radhakrishnan *et al.* (2014). Sterilized Fiss glucose minimal medium (supplemented with 300µg $FeSO_4$) was inoculated with fresh bacterial cells and kept on a rotary shaker at 35ºC for 24 hours for incubation. It was then centrifuged at 5000 rpm for 10 min. In sterilized screw capped tube, the cell free supernatant (CFS) of each of the isolates were collected. The hydroxamate, catecholate siderophore and carboxylate siderophore producing ability was checked by tetrazolium test, Arnow's test and spectrophotometric method, respectively (Radhakrishnan *et al.* 2014). On addition of tetrazolium salt and NaOH to the CFS a deep red colour develops in the presence of hydroxamate siderophore. Catecholate siderophores are detected by the appearance of a pink chromogen that show maximum absorbption at 515 nm following addition of nitrous acid, molybdate and alkali to the CFS. For carboxylate siderophores formation of a copper complex was detected that showed maximum absorption between 190-280 nm.

3.6.5. Biofilm production

Biofilm producing ability was detected on Congo Red Agar (CRA) medium following a modified method of (Roy *et al.* 2020). To prepare the CRA medium 0.8g of the CRA (Merck) was added to 1L of Brain Heart Infusion Agar (BHI, Himedia), and then 3% sucrose was mixed to it as supplement. The test bacteria were streaked onto CRA plates and incubated at 37 °C for 24-48 hrs. Production of black coloured colonies with a dry crystal-like consistency on Congo red plates indicated biofilm production. Congo red acts as an indicator binding to biofilm around the colonies.

3.6.6. Cellulase producing ability

Cellulose degradation potential of bacterial isolates was detected by growing them on the cellulose Congo-Red agar medium [composition: KH_2PO_4 0.5 g, $MgSO_4$ 0.25 g, cellulose 2 g, agar 15 g, Congo-Red 0.2 g, and gelatin 2.0 g; distilled water 1L; pH 6.8–7.2]. Congo-Red acted as an indicator for the detection of cellulase activity in the medium. Colonies showing a discoloration of Congo-Red after 24-72 h of incubation at 37^{o}C were considered to have cellulolytic activity **(**Gupta et al. 2012).

3.6.7. Amylase producing ability

Starch hydrolyzing efficacy was determined by inoculating the bacteria on pre-sterilized starch agar [Beef extract 3.0 g L^{-1}, Peptone 5 g L^{-1}, Starch 2 g L^{-1}, Agar 2 %, pH 7.2 ± 1] plates. The inoculated plates were incubated at 28 ± 2^{o}C for 48 h. Then, iodine solution was added to the bacterial colonies. The appearance of a halo zone of hydrolysis surrounding the colony confirmed amylase producing ability of the isolates (Deb *et al.,* 2013).

3.6.8. Protease producing ability

Each bacterial isolate was grown on skim milk agar plate (SMA), for detection of the proteolytic activity. Composition of SMA: Skimmed milk powder 28.0 g L^{-1}, Tryptone 5 g L^{-1}, Yeast extract 2.5 g L^{-1}, Dextrose - glucose 1.0 g L^{-1}, agar 15.0 g L^{-1}, final pH-7.0 ± 0.2. Each of them was streaked on SMA plates and incubated at 28 ± 2^{o}C for 72 h. The appearance of a zone of clearance around the colonies indicated proteolysis in the inoculated media (Rahman et al., 2018).

3.6.9. Laccase producing potential

To detect the laccase enzyme producing potential, bacterial isolates were grown on to nutrient agar medium supplemented with 0.5 mM Guaiacol. The plates were incubated for 96 h at 25 to 33^{o}C, formation of brown colouration around the bacterial colonies were considered as positive result. (Sheikhi., *et al.,*2017).

3.6.10. Urease producing ability

Christensen's Urea Agar medium was used for qualitative estimation of urease enzyme. The bacterial isolates were grown on the sterilized slants and incubated for 7 days at 35°C ± 2°C. A change in colour of bacterial colony from orange (initial) to red colour, was considered as positive test for urease producing ability of the isolates (Vashishta, et al., 2017).

3.6.11. Pectinase producing ability

The bacterial isolates were spot inoculated on Pectin agar media [composition: $NaNO_3$ 1.0 g L^{-1}, KCl 1.0 g L^{-1}, K_2HPO_4 1.0 g L^{-1}, $MgSO_4$ 0.5 g L^{-1}, Yeast Extract 0.5 g L^{-1}, Pectin 10.0 g L^{-1}, Agar 20.0 g L^{-1}]. The inoculated plates were incubated for 48 h at 37°C. After incubation, Gram's iodine solution was added to the culture plates, after 5 mins, the colonies showing clearing zone were marked positive. (Priya and Sashi, 2014).

3.6.12. Chitinase producing ability

Chitinase producing potential of the bacterial isolates was checked by plate assay method using colloidal chitin agar medium (medium composition: Na_2HPO_4 6 g, KH_2PO_4 3 g, NH4Cl 1g, yeast extract 0.05 g, agar15 g, colloidal chitin 1% (w/v) in 1L water). Colloidal chitin was made from the commercial chitin (Hi Media) (Wiwat, 1999). The chitin powder (20 g) was mixed slowly to conc. HCl (350 ml) and kept at 4°C overnight under vigorously stirring condition. In 2 liters of ice-cold 95% ethanol, the mixture was added stirring quickly, and kept overnight at 235°C. The solution was then centrifuged at 5000 g for 20 min at 4°C. The precipitate was collected and washed with sterile distilled water until the colloidal chitin reached at neutral pH (7.0). Then neutral colloidal chitin is lyophilized to dry. After the above treatment, the loose colloidal chitin was used as a substrate. The isolates were streaked on sterilized colloidal chitin agar medium and incubated at 37°C for 96 h. The colonies with a distinct hydrolysis area on the streak line were considered to be positive for chitinase activity (Saima et al. 2013).

3.6.13. β 1,3 glucanase producing ability

For detecting β 1,3 glucanase producing potential, the bacterial isolates were grown on solid minimal medium supplemented with 1gL^{-1} laminarin. After inoculation, the bacterial isolates were incubated for 2-3 days at 37^{o}C. Degradation of laminarin was detected under UV illumination after calcofluor white staining (Blättel *et al.* 2011).

3.6.14. Peroxidase producing ability

The plate assay method was used to determine peroxidase producing ability of the isolates. They were inoculated on NA plates and incubated at 37^{o}C for 48 h of incubation. Thirty µL of 0.4% H_2O_2 (v/v) and 1% pyrogallol prepared in distilled water, was poured on each bacterial colony. A change in colour of the colonies from brown to yellow within a few minutes indicated a positive reaction (Falade., *et al.* 2017**)**.

3.6.15. Lignin peroxidase producing ability

Minimal Salt Media which contain 0.5 g L^{-1} of kraft lignin (MSM-KL) as sole carbon source. The composition of MSM-KL medium: KL (0.5 g L^{-1}); K_2HPO_4 (4.55 g L^{-1}); KH_2PO_4 (0.53 g L^{-1}); $CaCl_2$ (0.5 g L^{-1}); $MgSO_4$ (0.5 g L^{-1}); NH_4NO_3 (5 g L^{-1}) with trace elements CuSO4 (0.2 mg L^{-1}), $FeSO_4$ (0.01 g L^{-1}); $MnSO_4$ (0.1 mg L^{-1}) and $ZnSO_4$ (0.1 mg L^{-1}). The plates were incubated at 50^{o}C for 7 days observed till the colony formation. Decolouration of media around the bacterial colony due to Guaiacol oxidation, indicated lignin peroxidase activity **(Lai *et al.* 2017).**

3.6.16. Hydrogen cyanide producing ability

Hydrogen cyanide (HCN) production capability of the bacterial isolates was tested on NA medium amended with glycine (4.4g L^{-1}). Exponentially growing culture (10^8/mL) of each isolate was streaked on plates containing pre-sterilized medium and incubated at 28 ± 2^{o}C for 48 h. A sterile filter paper saturated with 0.5% picric acid in 1% Na_2CO_3 solution was kept on the upper lids of each Petri plate and then sealed air-tight with parafilm. Production of HCN by the positive strains, was indicated by the colour change of picric acid-soaked yellow filter which turned to reddish brown colour (Bakker and Schipperes, 1987). An un-inoculated control was maintained.

3.6.17. Ammonia (NH_3) production

Ammonia production ability of the bacterial isolates was detected in peptone broth (peptone 10 g L^{-1}, NaCl 5 g L^{-1}). The freshly grown culture of each of the bacterial isolates was inoculated separately in test tubes containing 10 mL of peptone broth and incubated at 37°C. One mL of Nessler's reagent was added to 72 hrs old broth cultures (10 mL) each. The formation of deep yellow to brown colour confirmed the ammonia producing potential of the bacterial isolates (Cappuccino and Sherman, 2017).

All the tests were conducted in triplicates and repeated thrice to minimize the experimental errors.

3.6.18. Antagonistic activity against fungal pathogen

Antagonistic activity of the bacterial isolates, S3, S5, S7 and R1 against fungal pathogen isolated from infected soybean plants during the investigation.

Isolation and identification of the fungal pathogen

The infected leaf samples were washed properly with tap water. The leaf segments with infective lesions were surface sterilized with 1% sodium hypochlorite solution for 2 min, ringed in sterilized distilled water for 3-4 times and then, surface water was blot off with sterile filter paper. The leaf pieces were inoculated on Potato Dextrose Agar (PDA) medium to which rifampicin (10 mg l^{-1}) and ampicillin (200 mg l^{-1}) were added as a precautionary measure. The inoculated plates were incubated at 30°C for 7 to 10 days and was observed daily.

Dual culture plate assay method was used for studying anti-fungal potential of the bacterial isolates. All the selected bacterial isolates were tested for detecting their anti-fungal activity against the pathogen on dual culture plate containing potato dextrose agar (PDA) and NA at equal ratio (1:1) (Kumar *et al.* 2012). At the centre of the sterilized agar plate a well of 3 mm diameter was made using a cork borer. A fungal disc was placed within the well and each of the isolates was inoculated on a sterilized agar plate 2 cm apart from fungal disc and incubated 28 $\pm 1^0$C for 7 days. Formation of inhibition zone indicated anti-fungal activity. The diameter of inhibition zone was

recorded and antagonistic activity was calculated by the formula 100 × (C − T)/C [T' treated; 'C' control]. An uninoculated control was maintained.

3.6.19. Antibiotic sensitivity test

Agar disk-diffusion method (CLSI, 2012**)** was followed for testing antibiotic sensitivity of the bacterial isolates. Six hours old bacterial inoculum from broth culture was uniformly spread over the surface of NA plates using a sterile spreader. Then, commercially available antibiotic containing paper discs (5 mm in diameter), were placed on the agar surface and slightly pressed with the help of a a sterilized inoculating loop. The Petri dishes were incubated for 24 h at 35 ºC ± 2. The antimicrobial agents diffuse into the agar and influence growth of the test bacteria. After the incubation period, confluent bacterial growth was observed. The diameters of inhibition zones were measured in mm. Findings of antibiotic susceptibility assay of isolates were interpreted into 3 different categories, such as, susceptible (S), intermediate (I) or resistant (R) according to the (CLSI, 2012). The antibiotic discs used were: chloramphenicol 30 mg, tetracycline 30 µg, Vancomycin 30 µg, Rifampicin 30 µg, Ciprofloxacin 10 µg, Co-trimoxazole 25 µg, and Polymixin 300 µg. The Inhibition zone was measured and classified as resistant, sensitive or intermediate based on comparison with standard reference table. Tests were performed in triplicate.

3.7. Molecular identification of the promising isolates

Potential PGP bacterial isolates from soil sample. A and soil sample.C, were identified using the 16S rRNA Sanger dideoxy method. DNA was isolated from fresh pure culture of each of the bacterial isolates. The quality of DNA from each of the samples was checked on 1.0% Agarose Gel, on which for each of the samples a discrete PCR amplicon band of 1500 bp was detected. The 16S rDNA gene fragment of a isolate was amplified by 27F and 1492R primers.

Primers used:

27F- AGAGTTTGATCCTGGCTCAG

1492R- TACGGTTACCTTGTTACGACTT

Electrophoresis method was used to separate the amplified DNA on 0.8% agarose gel, run in 1× TAE buffer at 50V for 30 to 45 minute, till the proper migration of the DNA fragments. DNA quantification of every bacterial specimen was performed using Nanodrop (Biotech instruments, USA) and then DNA was preserved at−80^0C for future use. The ratio of absorbance at 260 nm and 280 nm (~1.8 to 2.0) was observed for assessing the purity of DNA. PCR amplification was carried out with a total amount of 25 µl solution containing 10 pmol each of forward and reverse primers, 2.5 mM of $MgCl_2$, 200 µM each of the four deoxy ribonucleotide triphosphates (dNTPs), 0.5 Unit of Taq DNA polymerase, 1 x concentration of PCR buffer (Invitrogen, Life Technologies, Brazil) and 50 to 100 ng of isolated bacterial genomic DNA. The template was denatured by heat treatment at pre-denaturation of 95 °C for 5 minutes, followed by 39 cycles of denaturation of 30 seconds duration at 95 °C, 45 seconds of annealing and 1 min for elongation at 72°C, with a final extension of 7 min at 72°C. The amplicons were resolved in 1.5% agarose gel using 0.5x tris-acetate-EDTA (TAE) buffer.

The PCR amplicon of each of the bacterial samples was purified for eliminating contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon were performed with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The next step was generation of Consensus sequence of 16S rDNA gene from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the NCBI genbank database. Based on maximum identity score first ten sequences were chosen and aligned using multiple alignment software program Clustal W at the European Bioinformatics (EBI) site http://www.ebi.eic.uk/clustalw). The final sequences of five bacterial isolates were submitted to NCBI GenBank.

For construction of plylogenetic tree and the evolutionary history were inferred using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). From 1000 replicates, the bootstrap consensus tree was predicted and considered for representing the evolutionary history of the taxa analyzed (Felsenstein, 1985). The branches which corresponded to partitions reproduced in less than 50% bootstrap replicates, were collapsed. The percentage of replicate trees clustering the associated

taxa together in the bootstrap test (1000 replicates), are furnished next to the branches (Felsenstein,1985). Initial tree(s) for the heuristic search obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances, estimated using the Maximum Composite Likelihood (MCL) approach, and the topology with superior log likelihood value were then selected. The analysis involved 11 nucleotide sequences. The 1st+2nd+3rd+Non-coding codon positions were included. All positions containing gaps and missing data were eliminated. Evolutionary analyses were constructed in MEGA7 (Kumar *et al.* 2015).

3.8. Selection of most potent PGPB isolates and formulation of multi-strain bio-inoculants

Based on the PGP potential of the bacterial isolates, the interaction study among themselves, and antibiotic sensitivity assay, Bacterial Combination-I, (S3+S5+R1) and Combination-II (S3+S5+R1+L3) were finally chosen for utilization as the novel multi-strain bioinoculant for application in soil sample.A and sample.C, respectively. From freshly grown NA plate cultures, each of the selected isolates were separately inoculated in Luria Bartani (LB) (HiMedia) media and incubated for 48 h at 35 ± 2^{o}C. The microbial consortium suspension in LB Broth culture with bacterial concentration of 4.5 X 10^{8}, containing each of the individual cultures at 1:1:1 ratio for combination-I and 1:1:1:1 ratio for combination-II, respectively, were prepared as inoculum. The bioinoculant formulation contained contained 20 % of bacterial of consortium in LB Broth culture (48 h old) with bacterial concentration of 4.5 X 10^{8}, 30 % sunflower oil, 20 % Tween-80 and 30 % water.

3.9. Survival assay of the bioinoculants

To find out the survival period of the bioinoculant in soil, twenty gm of sterilized soil sample-A and soil sample-C were taken separately in sterile 100 mL Erlenmeyer flask, inoculated with the bacterial inoculant formulation, mixed thoroughly using a sterilized glass rod, and stored in a BOD incubator for three months. Bacterial population was measured at every 15 days interval up to 90 days after storage, using standard plate count method. Bacterial density was expressed as CFU mL^{-1} of inoculant. The experiment was conducted in thrice and the findings are expressed as Mean ± standard deviation.

3.11. Evaluation of the impacts of the novel multi-strain bio-inoculants on plant growth, soil nutrient status, and resident soil bacterial community composition

The impact of soil amendment following application of the composite bio-inoculants, were evaluated through a three pronged approach:

- Improvement of plant growth and yield related characteristics
- Upgradation of soil nutritional status, and
- Modulation of resident soil bacterial community towards improvement in biological health of soil.

3.11.1. Evaluation of *in vivo* growth-promotion efficacy of the multi-strain bacterial inoculants

The pot trial experiment was designed to assess *in vivo* plant growth promotion ability of the amendments in open air conditions. *Glycine max* (L) Meril. var JS-0335 was used as the test plant. The seeds were procured from the ICAR-Indian Institute of Soybean Research, Indore. The non-sterilized, freshly collected soil from the field was used for this work. Before sowing, seeds were surface sterilized using sodium hypochlorite solution (0.1%) for 5 min and then washed thoroughly with sterile distilled water 5 times.

Experimental design

The pot trial experiment was carried out in polythene bags of 28 cm X 28 cm X 26 cm dimension, each containing 5 kg of non-sterilized experimental soil. The bags of each treated and untreated set-up were maintained in four replicates. In the amendment, vermicompost procured from Nimpith Krishi Vigyan Kendra (West Bengal), was applied @ 100gm kg^{-1} of soil in the bag. Details of the experimental design are furnished below in Table 3.4.

Experimental set up		Bacterial isolates used in bioinoculant formulations
Code	**Composition**	
Control [SU]	Untreated field soil	
Treatment-1 [SV]	Field soil + Vermicompost	Combination-I. For Soil Sample.A. Bacterial isolates [S3+S5+R1]
Treatment-2 [SBC]	Field soi + Consortium	Combination-II. For Soil Sample.C Bacterial isolates [S3+S5+R1+ L3]
Treatment-3 [SVBC]	Field soil + Vermicompost + Consortium	

Table 3.4. Experimental design showing different treatments and consortia combination

The topsoil was covered (1.5cm layer) with coco peat procured from local nursery, and six seeds were sown randomly in each pot. Twenty ml of the first dose of inoculant formulation was applied to the pots of the respectively treated set-up, near the rhizospheric region of the plants 15 days after the seedling emergence stage followed by two successive doses at 35- and 55-day stages, respectively. An equal amount of water was applied to each pot on every two days. De-weeding was practiced once in every week.

In vivo plant growth promotion study

The efficacy of the amendments was tested based on their impact on selected vegetative and reproductive growth characteristics of the potted plants. Data were recorded every 4, 8, and, 12 weeks after the seedling emergence (WAE) stage for analysing vegetative parameters of plants like the total number of leaves, leaf area, plant height, and the number of root nodules plant^{-1}. The first onset of flowering (days) and the total number of pods node^{-1}, total number of pods plant^{-1}, and pod size, were recorded. After harvesting, the no. of seeds pod^{-1}, and the dry weight of 100 seeds were kept in record.

Chlorophyll-a, chlorophyll-b, and total chlorophyll content of freshly collected mature leaves were measured at 4, 8, and 12 WAE stages respectively. Chlorophyll pigments of the leaves were extracted in accordance with the protocol of Liang, (2017) and estimated following the equation of Arnon et.al (1949). The crude protein and fat content of soybean seeds were estimated by the Kjeldahl method following the Indian Standard protocol for food and feed (IS-7219:1973) and using the method as described in Sadasivam and Manickam (2008), respectively.

Statistical analyses

Python soft ware version 3.11+ and its modules along with scientific computation libraries were used for plotting, analysing, and visualizing the data obtained during the investigative procedure. We performed one-way ANOVA on the vegetative and reproductive parameters, and subsequently conducted Tukey's Post Hoc test to investigate the significant differences of the means of these characteristics. Both the ANOVA and Tukey's test were conducted at a standard significance level of 5% ($p>0.05$). Normality test was performed to determine, if the concerned variables follow normal distribution pattern or not. Based on it, paired sample t-test was performed to detect, whether the effect of combined treatment of vermicompost and bacterial consortia on different plant parameters were significant or not. To study the effect of the combined treatment of vermicompost and bacterial consortia on vegetative growth and yield related characteristics of soybean plants, the observed

plant parameters in SVBC condition were compared with those of SU condition by applying paired t-test. Finally Logistic Regression mode was followed to find out if the improvement in vegetative parameters of plants such as, total no. of leaves plant-1 (X_1), leaf area (X_2), total chlorophyll content of leaves (X_3) and total no. of root nodules (X_4) , is reflected on the yield characteristics, which is captured best by total no of pods plant-1. Total no. of pods plant-1 (Yi) is the dependent binary variable. The empirical specification is:

$Yi = \alpha + \beta_1 X_1 + \beta 2\ X_2 + \beta 3\ X_3 + \beta 4\ X_4 +$ error term

Where,

Yi = 0, at vegetative state,

Yi = 1, at harvesting state,

3.11.2. Analysis of soil nutrient status in different experimental set-up across different stages of plant growth

For ascertaining the effect of soil amendment, through out the pot trial experimental stage soil nutrient status such as, available nitrogen, phosphorus and potassium levels were checked at 8, 12, and 16weeks stages after seedling emergence in accordance with the standard protocols as followed by Mandal (2020). The findings are graphically represented as Mean with error bars.

3.11.3. Metagenomic analysis of amended and non-amended soil to assess the impact of the amendment on soil bacterial community composition

For metagenomic analysis, field soil sample was sampled abiding by the protocol of TNAU-2013 https://agritech.tnau.ac.in/agriculture/agri_soil_sampling.html/TNAU-2013). Soil samples from pots of each experimental set-up, were collected from the rhizospheric region, at fruit harvesting stage of soybean plants and pooled separately. Collected field and pot soil samples were stored separately at -20^{0}C refrigerator until DNA extraction.

DNA extraction

Next generation sequencing method targeting the 16S rRNA metagenomic amplicons was used to analyze soil microbiomes pre- and post-amendment. For extraction of DNA, water samples were spun at 7000 x g for 15 mins to get cell pellet and that was considered as starting material for DNA extraction. The MO BIO PowerSoil™ DNA isolation kit was utilized for isolation and extraction of bacterial DNA from sieved soil samples for execution of downstream metagenomic analyses (Bag et al., 2016).

MiSeq Library Preparation and Sequencing

20 ng of DNA from each sample was used for amplification of V3 and V4 hyper variable regions of 16S gene with the help of KAPA HiFi HotStart ReadyMix PCR Kit (KAPA BIOSYSTEMS). In this protocol, the gene-specific sequences targeted only to the 16S V3 and V4 region. Then the library concentration was determined in a Qubit.3 Fluorometer (Life technologies using The Qubit™ dsDNA BR (Broad Range) Assay Kit (ThermoFisher Scientific).The DNA thus obtained was sequenced on Illumina MiSeq using reagent kit V3 in accordance with the manufacturer's protocol to generate 2×300 bp paired-end reads.

Raw Data Quality Check

The quality check of sequenced raw reads is essential for assessing data quality. It was performed to study some of the relevant characteristics of the ensemble of next generation sequencing reads like length, quality scores and base distribution. Data with low quality reads were discarded. The quality of raw reads of Illumina sequencing was checked to find the ambiguous bases, Phred score & gt; Q30, read length, nucleotide base content and some other characteristics using FASTQC. Then, quality assessment was carried out using NanoDrop™ 2000 Spectrophotometer followed by semi-quantitative estimation of DNA via agarose gel electrophoresis. QUBIT assay was performed to obtain the precise concentration of the extracted DNA.

Assembly and Gene Finding

Gene library preparation was carried out by amplifying the standardized V3-V4 region of 16SrRNA as per Illuimina gene library construction protocol [Primer Details: 16S Amplicon PCR Forward Primer = 5′TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG; 16S Amplicon PCR Reverse Primer = 5′GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC and adaptor sequences: Forward overhang: 5′ TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG (locus specific sequence) Reverse overhang: 5′ GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG (locus specific sequence) 341F = CCTACGGGNGGCWGCAG and 805R = GACTACHVGGGTATCTAATCC].

The library thus generated was quantified with qPCR and Agilent Bioanalyzer (DNA 1000 chip). The sequenced raw reads were processed through the FASTQC pipeline for quality checking followed by which the screened sequences surpassing the quality threshold were finally assembled via homopolymer elimination and minimization of artefactual noise and probable contamination using SYLVA–NGS (1.3) pipeline (Lepinay et al., 2018).

OTU Clustering

The processed reads were clustered into OTUs with the help of QIIME (Quantitative Insights into Microbial Ecology,) software or SYLVA–NGS to identify the microbial community. These OTUs were then utilized for taxonomic assignment, analysis of phylogenetic diversity and estimation of abundance (en.wikipedia.org). In accordance with the bioinformatics pipeline described by Ganguli et al. (2017), Operational Taxonomic Units (OTUs) were clustered using QIIME2, and microbial abundances were analysed using KRONA charts (Estaki et al. 2020) **f**or analyzing microbial communities**.**

Bioinformatic analysis

Thereby, the individual microbes present in the field soil under controlled and varying experimental conditions were identified and their differential abundances were obtained. The user-end reads yielded from Illumina sequencing were used as query sequences;

subjected to the LAST algorithm for matching against the RDP_16S_18S database allowing for the analyses of archaeal, bacterial, or eukaryotic matches, at different taxonomic levels, using an alignment score cut-off of 0.8, subsequent to the elimination of reads having very high e-values. The data obtained herein was used for downstream analyses. Starting from the widest taxonomic level, it assigns a taxonomic label to each read. The taxon that receives the most hits is used for this. The analysis continues until a confidence level is breached or numerous taxa are supported by the same quantity of high-quality hits. The confidence threshold gives the most numerous taxon a statistical measure of support while also reflecting the quality of the underlying alignments. Reads that do not conform to the requirements for the assignment are put in the unmatched class. All unmatched or unclassified reads were removed from the data for downstream analyses. Using Krona Tools, representative taxon is displayed in interactive graphs (Ondov et al, 2011). Species-level predictions for bacteria and archaea are made as a result of enhanced database naming consistency. Identification of common elements between the field soil microbial consortia under differential treatment conditions was done using Venny 2.1.0 to generate Venn Diagrams **(**Oliveros, J.C., 2007- 2015).

CHAPTER-4

RESULTS

OBJECTIVE-I: Collection and Characterization of Low Productive Soil

4.1. Collection and characterization of soil sample

Soil samples were collected from 5 different sites located in three districts of the southern part of West Bengal, as shown in Table 3.1. of Materials and Methods section. The physico-chemical characteristics of the collected soil samples are furnished in Table 4.1.

Parameters	Soil sample and soil type	Sample-A Silty clay	Sample-B Silty clay	Sample-C Red lateritic	Sample-D Clay loam	Sample-E Clay loam
Physical	pH at 25 ^{0}C	6.83 ± 0.01	7.11 ± 10	5.99± 0.06	7.95 ± 12	6.74 ± 06
	Sand (%)	37.0 ± 0.05	40.0 ± 0.35	61.4± 0.03	38.4 ± 0.08	35.2 ± 0.24
	Silt (%)	30.7 ± 0.06	31.5 ± 0.86	29.7± 0.06	20.1 ± 0.03	24.0 ± 0.05
	Clay (%)	32.3 ± 0.03	28.5 ± 0.07	16.6± 0.04	41.5 ± 0.05	40.8 ± 0.03
	Water holding capacity (%)	44.7 ± 0.20	46.5 ± 0.06	31.4± 0.11	58.6 ± 0.06	61.3 ± 0.02
Chemical	Total organic carbon (%)	0.34 ± 0.42	0.86 ± 0.03	0.57 ± 0.01	0.86 ± 0.05	1.51 ± 0.04
	Available N (mg kg^{-1})	48.7 ± 0.16	75.7 ± 0.06	81.91 ± 0.02	67.1 ± 0.05	74.6 ± 0.03
	Available P (mg kg^{-1})	27.25 ± 0.057	82.3 ± 0.02	6.90 ± 0.01	152.4 ± 0.05	38.23 ± 0.05
	Available K (mg kg^{-1})	136.60 ± 0.22	147.2 ± 0.03	126.57 ± 0.02	142.1 ± 0.09	153.1 ± 0.06
	Available Fe (mg kg^{-1})	24897.74± 0.08	21664.33± 0.03	95780.11± 0.05	18130± 0.11	15921.53± 0.05
	Available Zn (mg kg^{-1})	52.53± 0.15	70.14± 0.07	29.60± 0.02	88.03± 0.04	74.38± 0.15

Table 4. 1. Physico-chemical characteristics of selected soil samples

- The findings of Table 4.1. indicates that among the 5 collected soil samples, the sample-A (Bahadurpur, South 24 Pgs) was observed to contain low levels of available N (48.7 mg kg^{-1}), available P (27.25 mg kg^{-1}), and SOC (0.34%) contents. Thus, appeared to be depleted in these three essential soil nutrients.
- Soil sample-C (Sadaipur, Birbhum) was found to be deficient in available phosphorus (6.09 mg kg^{-1}) content and had a relatively low level of SOC (57 %). This sample, red lateritic in nature, was highly rich in iron content (95780.11± 0.05 (mg kg^{-1}). The water holding capacity of this sample was relatively poor (31.4 %) compared to that of the other samples.

- With respect to the nutrient status, soil sample-A and sample-C appeared to be over-exploited and poor quality (Table 4.1) soil and were chosen for the current investigation.

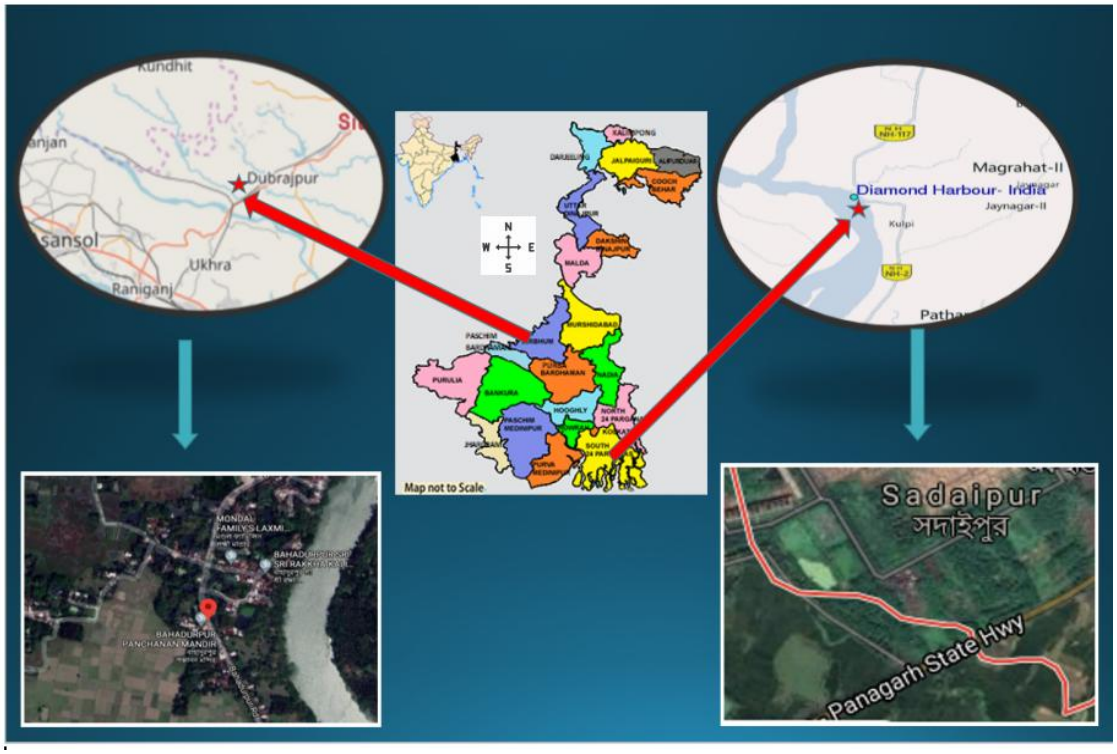


Fig 4.1. Soil Sampling sites selected for the investigation (Source: Google Map.)

OBJECTIVE-II: Isolation, Characterization and Screening of Bacteria with Plant Growth Promoting Traits from the Selected Soil Samples

4.2. Isolation, preliminary morphological, and biochemical characterization

Following serial dilution-agar plate method, bacteria were isolated from the two selected soil samples (sample-A and sample-C). Altogether 16 bacterial colonies showing prominent growth on NA plates, ware chosen from them. Ten isolates were selected from soil sample-A and were labeled as: S1, S2, S3, S4, S5, S6, S7, R1, R2, and R3, respectively. From soil sample-C, 6 bacterial isolated were picked up and labelled as: L1, L2, L3, L4, and L5, respectively.

Isolate code	Colour of colony	Margin	Elevation	Gram Nature and shape
S1	White, translucent	Entire	Flat	Gram-negative, rod
S2	Greyish White, opaque	Entire	Convex	Gram-negative, rod
S3	Greyish White, translucent	Entire	Convex	Gram-positive, short rod
S4	White, translucent	Entire	Flat	Gram-negative, rod
S5	Greyish white, translucent	Wavy	Elevated	Gram-positive, rod
S6	White, opaque	Entire	Flat	Gram-negative, rod
S7	Off white, opaque	Circular	Convex	Gram-positive, rod
R1	Creamy white, opaque	Irregular	Elevated	Gram-positive, rod
R2	Fuzzy white, opaque	Entire	Flat	Gram-positive, short rod
R3	Creamy white, translucent	circular	Elevated	Gram-negative, short rod
L1	White, translucent	Entire	Flat	Gram-positive, short rod
L2	Yellowish, opaque	Circular	Flat	Gram-positive, cocci
L3	Creamy white, opaque	Circular	Elevated	Gram-negative, rod
L4	Creamy white, translucent	Entire	Flat	Gram-negative, rod
L5	Greyish white, opaque	irregular	Convex	Gram-negative, rod

Table 4.2. Colony morphology, shape, and Gram nature of Bacterial Isolates

4.2.1. Morphological characterization

Morphological characteristics such as colour, elevation, and margin of the prominent bacterial colonies on NA plates, were studied.

The shape and Gram nature of the bacterial cells from the respective colonies were observed under the microscope. The observations are presented in Table 4.2. The pure culture of the isolated bacterial colonies on NA plates is shown in Fig 4.2.

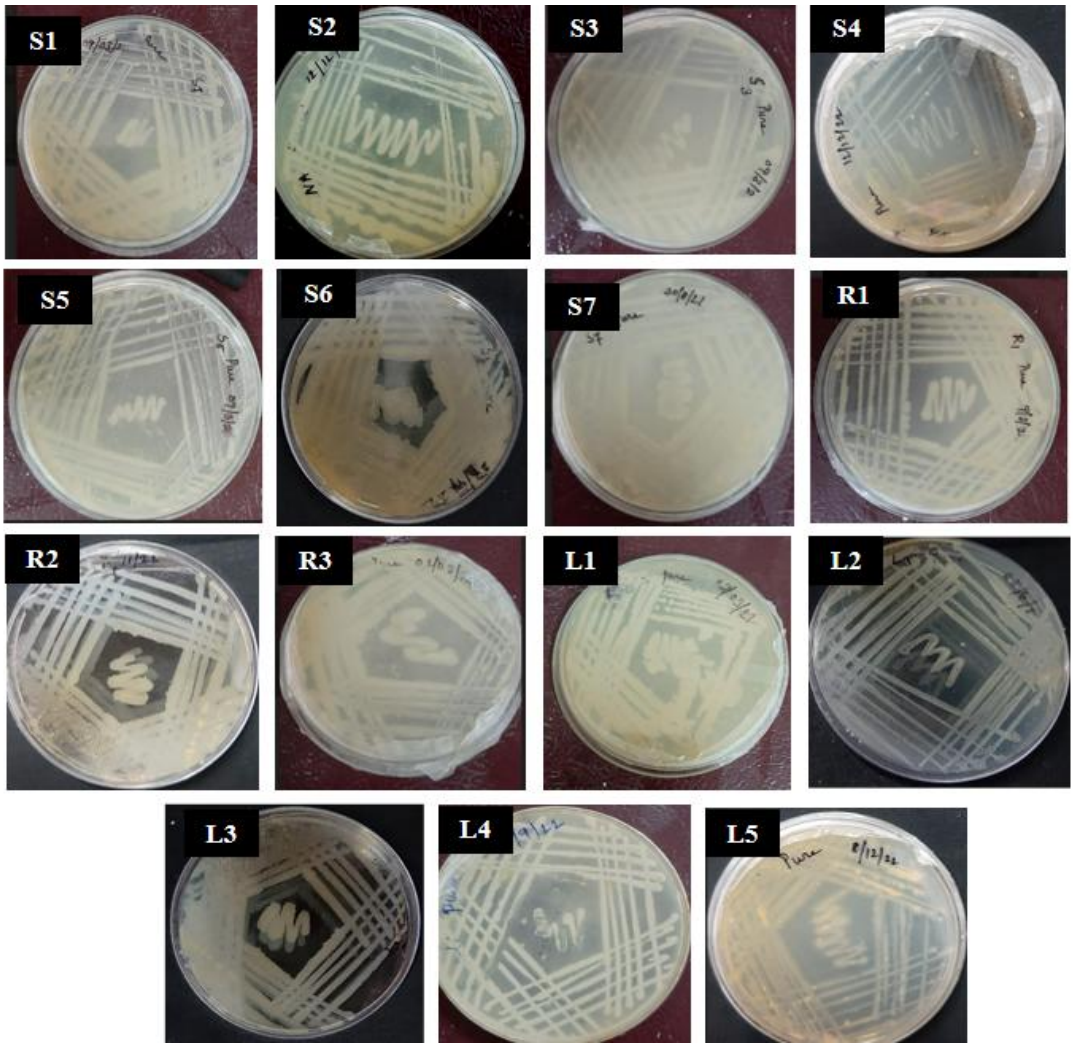


Fig 4.2. Colonies of bacterial isolates S1, S2, S3, S4, S5, S6, S7, R1, R2, R3, L1, L2, L3, L4, L5 respectively, on NA plates.

The major findings indicate that, out of the 16 bacterial isolates, only one was coccus and the others were bacilli in shape. Regarding Gram nature, 7 isolates were detected as Gram -ve and 9 were Gram +ve in nature.

4.2.2. Biochemical characterization

In order to have a preliminary idea about the biochemical characteristics, some tests were performed as enlisted in section 3.2.2. The results are furnished in Table 4.3.

Isolate code	Producing ability				Methyl Red test	Citrate utilization	Voges Proskeur test
	Catalase	Oxidase	Nitrate Reductase	Indole			
S1	+	+	-	-	-	+	+
S2	+	+	-	-	-	+	-
S3	+	+	-	-	-	+	-
S4	+	-	+	+	+	-	-
S5	+	-	-	-	-	+	+
S6	+	+	-	-	-	+	+
S7	+	variable	+	-	-	+	-
R1	+	-	+	-	-	+	-
R2	+	+	-	-	-	+	-
R3	+	-	+	+	+	-	-
L1	+	-	+	-	-	-	+
L2	-	-	-	-	-	-	+
L3	+	+	+	-	-	+	-
L4	+	variable	+	-	-	+	+
L5	+		+	-	-	+	

'+' = Positive '-'= Negative

Table 4.3. Biochemical Characterization of Bacterial Isolates

The Table 4.3. indicates the normal activity of different enzymes along with IMViC test. Furthermore, it is apparent from the table that none of the bacterial isolates are common coliform bacteria.

4.1. Screening of soil bacterial isolates for their *in vitro* NPK acquisition ability

The mineral nutrient (NPK) acquisition potential of the selected bacterial colonies were examined on selective media.

4.3.1. Determination of N-fixing ability

In vitro N-fixing capability of the bacterial isolates was checked by growing them separately on nitrogen-free Jensen's medium (M-710, HiMedia) the results are presented in Fig 4.3.

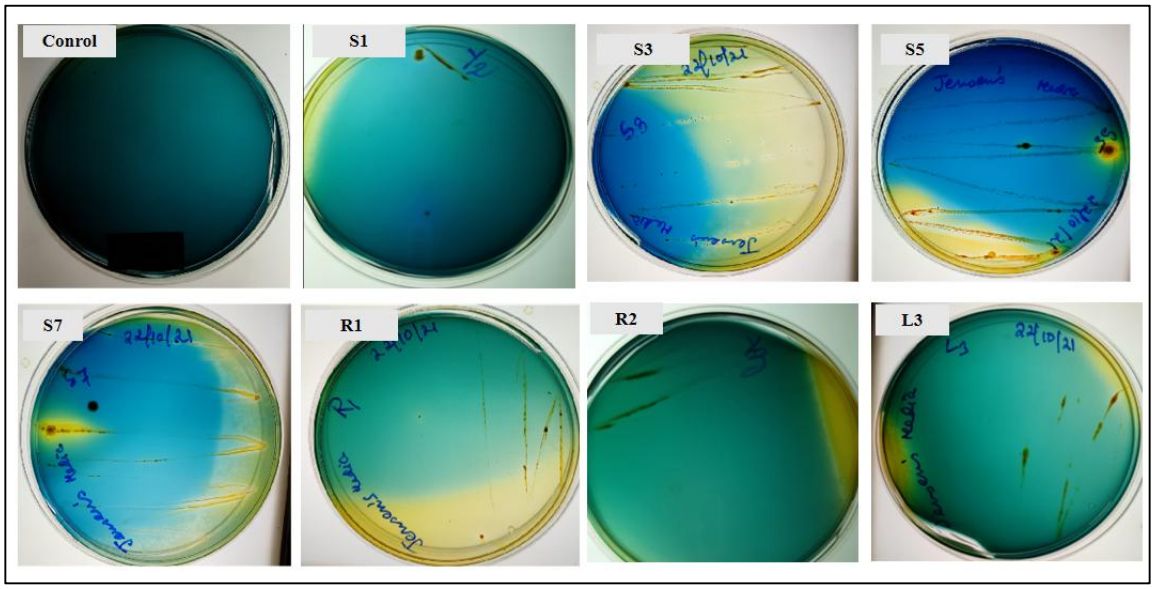


Fig 4.3. Plate assay method showing *in vitro* N-acquisition ability of the bacterial isolates S1, S3, S5, S7, R1, R2, and L3 on N-free Jensen's media.

- The observations from the Fig 4.3 shows that the bacterial isolates S1, S3, S5, S7, R1, R2, and L3 were able to change the greenish blue colour of modified Jensen's medium within 5-8 days of inoculation to blue (intermediate stage) and finally to yellow colour, indicating different steps of N-fixation, to nitrification and ammonification.
- Among total 15 bacterial isolates, 6 of the bacterial isolates S1, S3, S5, S7, R1, R2 were from soil sample-A, and the only isolate L3 from soil sample-C, were found to have N-fixing ability.
- Only these 7 N-fixing bacterial isolates were chosen for the screening of their phosphate and potassium solubilization potential.

4.3.2. Qualitative and quantitative estimation of phosphate solubilization

The P-solubilizing bacterial (PSB) isolates are able to form a clear solubilization zone around the colonies on the Pivovskaya agar medium, as shown in Fig 4.4.a. The experiments were done in triplicate and showed similar result, photographs represent one of three sets. For the quantitative estimation of phosphate solubilization, the standard curve for phosphate and solubilization potential by the selected bacterial isolates, are furnished in Fig 4. b. and Fig 4.c. respectively.

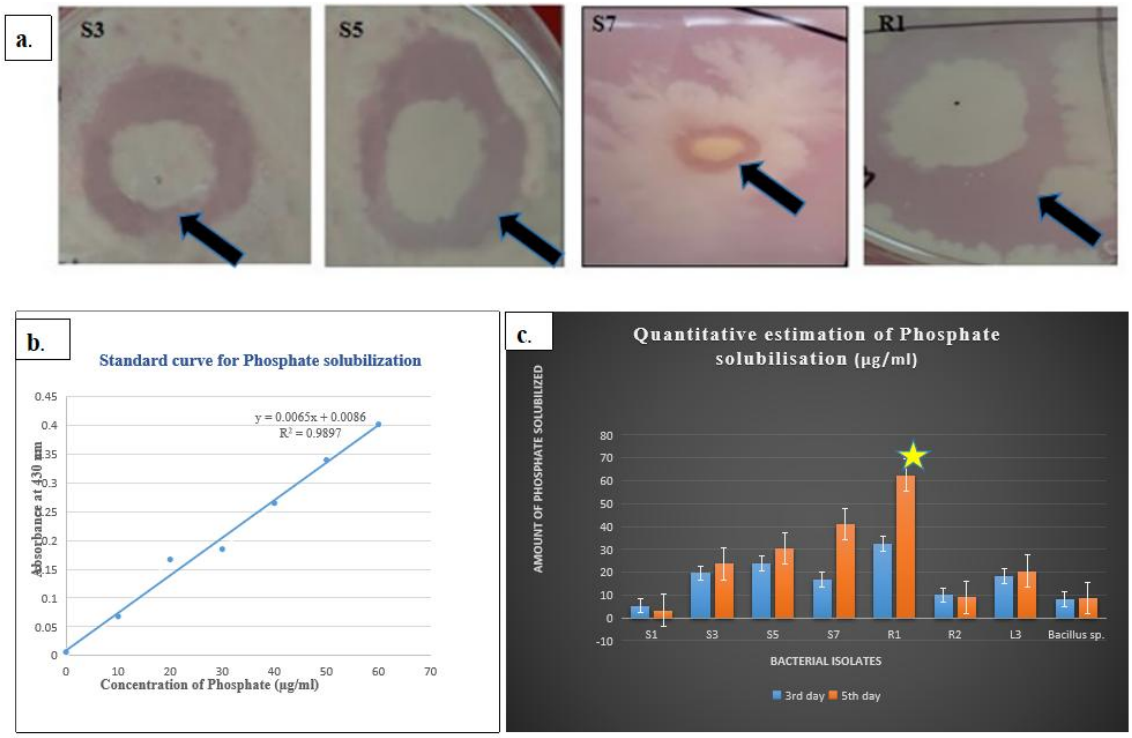


Fig 4.4. Phosphate solubilization potential of selected bacterial isolates. a. Plate assay method showing solubilization zone around the bacterial colonies on the Pivovskaya agar medium, the pointers highlighting solubilization zones; b. Standard curve for phosphate; c. Phosphate solubilization by the bacterial isolates on the 3rd and 5th day of incubation on the Pikovskaya broth medium.

- The major findings of Fig 4.4. indicate that, among the 7 isolated bacterial colonies, only 4 (S3, S5, S7 and R1) were able to form a distinct zone of clearance on Pikovskaya agar plate. These 4 isolates were considered as potent PSB isolates.

- Phosphate solubilization (SI) index of the isolates S3, S5, S7, and R1 were observed to have 1.4 ± 0.03 cm, 1.1 ± 0.05 cm, 0.8 ± 0.06 cm, 1.9 ± 0.07 cm, respectively. The isolate R1 appeared to be maximum based on S.I. value.

- The phosphate solubilizing ability of the bacterial isolates was checked from 3rd days and 5th days old culture. All the isolates solubilized a higher amount of phosphate on the 5th day.

- The bacterial isolate R1 has the highest phosphate solubilizing potential (62.5 µg/ml) followed by S7, S5, S3, and L3 in 5-day-old culture filtrate.

3.3.2. Qualitative and quantitative estimation of potassium solubilization

Qualitative estimation of the K-mobilizing potential of the bacterial isolates were studied on Aleksandrow medium. The isolates S1, S3, S5, S7, R1, R2, and L3 were capable of changing the colour of the modified Aleksandrow media to yellow, due to organic acid formation, within 72 h of incubation and were considered as KSB (Fig 4.5. a). The experiments done in triplicate and showed similar result, photographs represent one of three sets.

Quantification of potassium mobilization capability of the bacterial isolates were estimated. The results are graphically represented in Fig 4.5.b.

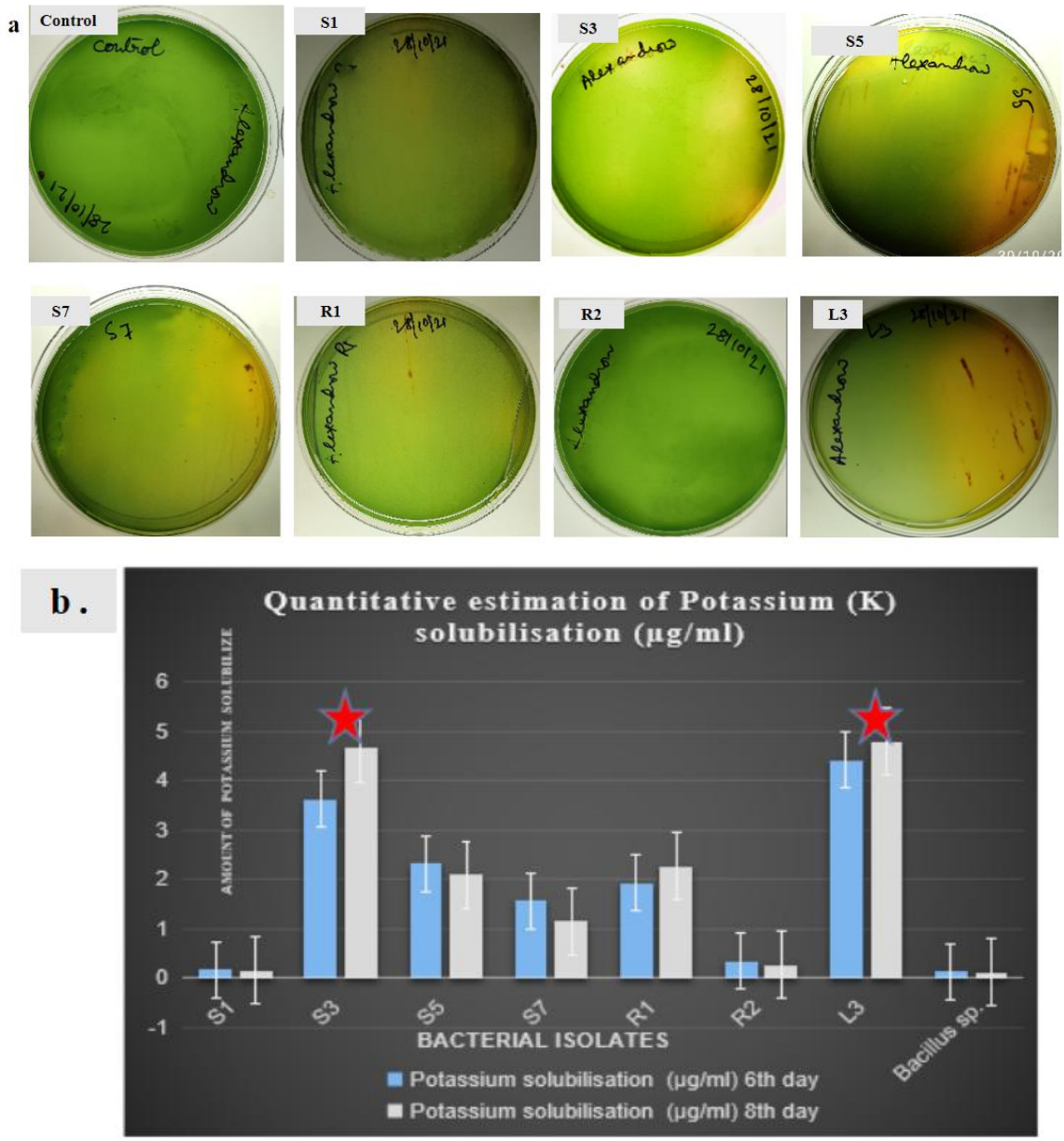


Fig 4.5. Potassium solubilization potential of selected bacterial isolates.a. Plate assay method showing potassium solubilization potential of selected bacterial isolates. The experiments were done in triplicate and showed similar results, photographs represent one of three sets; b**.** Quantitative estimation of Potassium solubilization ability of the bacterial isolates S1, S3, S5, S7, R1,R2, and L3. A laboratory strain of *Bacillus* was kept as control.

- **The results of Fig 4.5. shows that t**he bacterial isolates S3, S5, S7, R1, and L3 were capable of changing the colour of the modified Aleksandrow media to yellow, due to solubilization of potassium to organic acid, within 72 h of incubation. These 5 bacterial isolates were considered as KSB.

- In both 6th and 8th-day-old cultures, L3 solubilized the maximum amount of Potassium (4.71 µg/ml), closely followed by S3, R1. The isolate S7 was detected to have the lowest K-solubilizing potential.

- These seven isolates (S1, S3, S5, S7, R1, R2 ,and L3) were selected for further study and maintained by repeated subculturing on NA plates.

OBJECTIVE -III: Utilization of the Selected Resident Bacterial Isolates to design Novel Multi-strain Bioinoculant for Plant Growth Promotion and Soil upgradation

3.4. Compatibility study among promising isolates and designing multi-strain bacterial consortia

The results of the interaction study among the bacterial isolates are presented in Table 4.4. and Fig 4.6.

Bacterial Isolate code	S1	S3	S5	S7	R1	R2	L3
S1	NA	+	+	+	+	+	+
S3	+	NA	+	+	+	+	+
S5	+	+	NA	-	+	+	+
S7	+	+	-	NA	+	+	+
R1	+	+	+	+	NA	+	+
R2	+	+	+	+	+	NA	+
L3	+	+	+	+	+	+	NA

'+' = Positive interaction, '-'= Negative interaction, NA =Not applicable

Table 4.4. Compatibility study among bacterial isolates

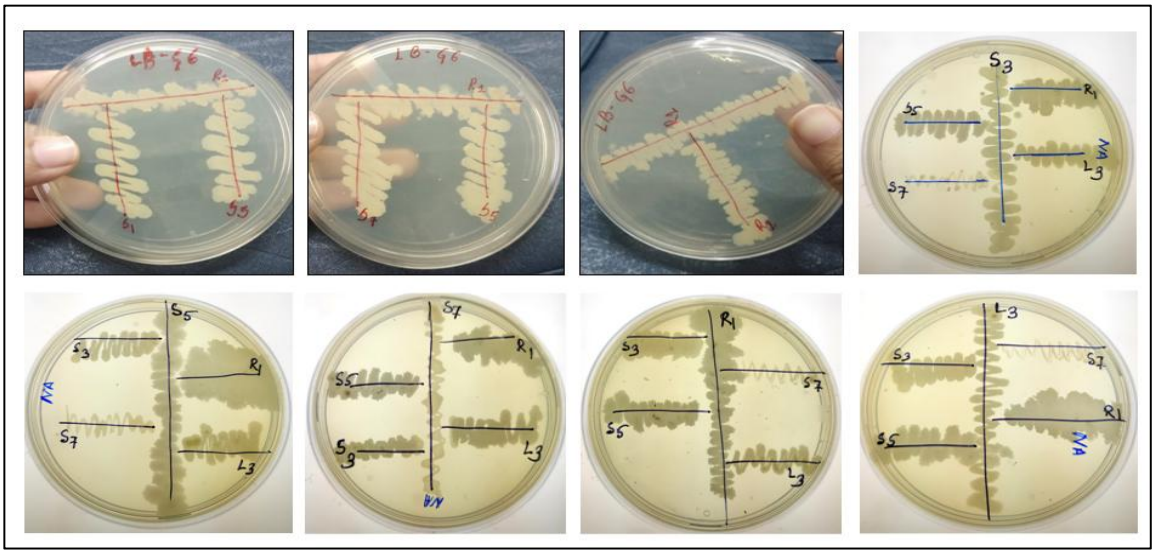


Fig 4.6. Compatibility study showing interaction among the bacterial isolates

- The results furnished in Table 4.4. and Fig 4.6. confirms that no inhibitory interaction was present among the isolates S3, S5, R1, and L3. The bacterial isolate S7 showed a slightly inhibitory effect against S5 and therefore, these two isolates were not considered for using together in the formulation of multi-strain consortia.
- Three different consortia combinations were designed. Combination-1: S3+S7+R1; Combination-2: S1+S7+R2; Combination-3: S3+S5+R1

4.5. Preliminary pot trial experiment for selecting the most potent multi-strain bacterial consortia growing *Glycine max* L. as a test plant

The preliminary yield trial was conducted using soil sample-A (soil from Bahadurpur, South 24 arganas) in pot trial condition. The findings are furnished below in Fig 4.7. and Fig 4.8.

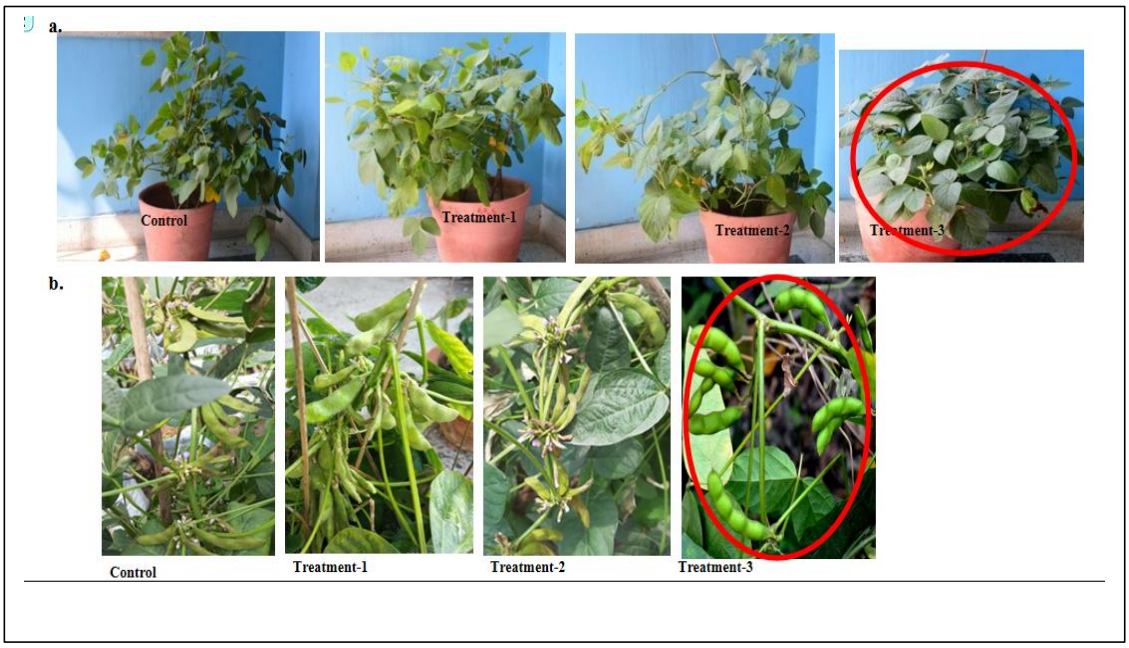


Fig 4.7. Preliminary pot-trial experiment. a. Treated and untreated plants at 12-week stage, marked area indicating luxuriant growth with maximum leaf density; b. Treated and untreated plants at the fruiting stage, marked area shows bigger fruit and seed size. Control (untreated), Treatment-1 (S3+S7+R1), Treatment-2 (S1+S7+R2), Treatment-3 (S3+S5+R1).

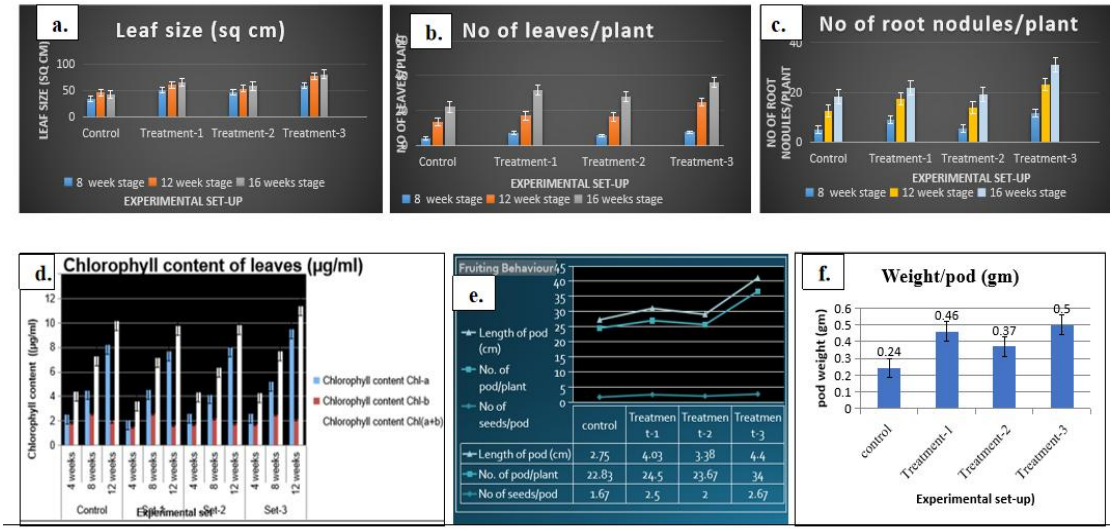


Fig 4.8. Effect of different consortia on vegetative characteristics and fruiting behaviour of of *Glycine max* Merill. plants in preliminary pot trial experiment. Fig a. leaf size; b. No. of leaves plant^{-1}; c. no. of root nodules plant^{-1}; d. chlorophyll content of leaves; e. fruiting behaviour; f. pod weight. Columns represent mean values of the data for each characteristic and the error bars represent the standard deviation.

- The observations of *in vivo* plant growth promotion study show significant variations in vegetative and fruiting-related traits of the test plants in the different experimental setup, following treatment with three different consortia, over to that of the control setup.
- The treatment-3 set-up (S3+S5+R1) exhibited a maximum increase in leaf density, and leaf area followed by the treatment-1 set-up (S3+S7+R1). The control set-up (untreated) was the poorest with respect to the observed vegetative parameters.
- Maximum improvement in the total number of fruits and fruit size was observed in the T-3 set-up (S3+S5+R1) followed by the T-1 set-up (S3+S7+R1) and T-2 set-up ((S1+S7+R2).
- The Fig 4.8. indicates significant improvements in vegetative, and yield related traits of the test were detected following inoculant application. The highest improvement with respect to all the observed parameters, was recorded in T-3 (S3+S5+R1) set-up followed by T-1 (S3+S7+R1) and T-2 (S1+S7+R2) set-up indicates a strong positive influence of consortium amendment.
- A remarkable increase in leaf size in T-3 (81 cm^2) followed by T-1 (62 cm^2) and T2 (54 cm^2) compared to untreated (42 cm^2) set-up, at 12week stage compared to the untreated one. A striking improvement in leaf no. T-3 (36), T-1 (35) over control (22) condition.
- About 78% increase in root nodule no. $plant^{-1}$ was observed in the T-3 set-up over to that of the untreated one. The total no.of pods $plant^{-1}$ increased from 22.8 in the untreated condition to 34 in the T-3 condition. About 50%, 35% and
- Consortium combimation-3 (S3+S5+R1) appeared to be the best with respect to vegetative and reproductive parameters, followed by combination-1 (S3+S7+R1) and combination-2 ((S1+S7+R2). Therefore, the isolates S3, S5, S7, and R1 (from soil sample-A) were selected for further PGP characterization and utilization in the plant growth promotion study.

4.6. Characterization of the promising bacterial isolates (from efficient consortia combinations) for their additional agriculturally beneficial traits

The bacterial isolates S3, S5, S7, and RI (from soil sample-A) and the only PGP isolate, L3 (from soil sample-C) were further tested for additional agriculturally beneficial characteristics.

4.6.1. Quantitative estimation of Indole acetic acid (IAA)production

Indile-3-acetic acid producing capability of the bacterial isolates was quantitatively estimated and the amount of IAA produced *in vitro* condition is presented in Fig 4.11.

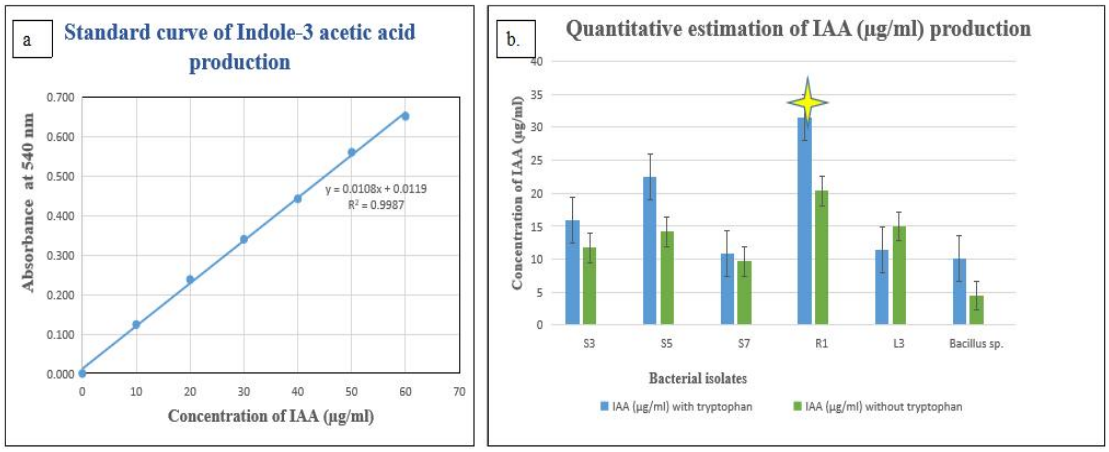


Fig 4.9. Quantitative estimation of IAA production by the selected isolates S3, S5, S7, R1 and L3. a. Standard curve for IAA; b. IAA in the LB broth, the presence and absence of tryptophan.

- The observations in Fig 4.9. indicate that the isolate R1 is the highest producer of IAA, both in the presence (31.43 µg mL^{-1}) and absence (20.12 µg mL^{-1}) of tryptophan.
- In the absence of precursor tryptophan, L3 can produce a higher amount of IAA than that of S5 and S3.
- S7 Isolate R1 appeared to be the most potent IAA producer followed by L3, S5, S3, and produced the least amount of IAA both in the presence and absence of tryptophan.

4.6.2. Quantitative estimation of Gibberellic acid (GA_3)

In vitro GA_3 producing potential of the selected bacterial isolates were checked and the results are furnished in Fig 4.10.

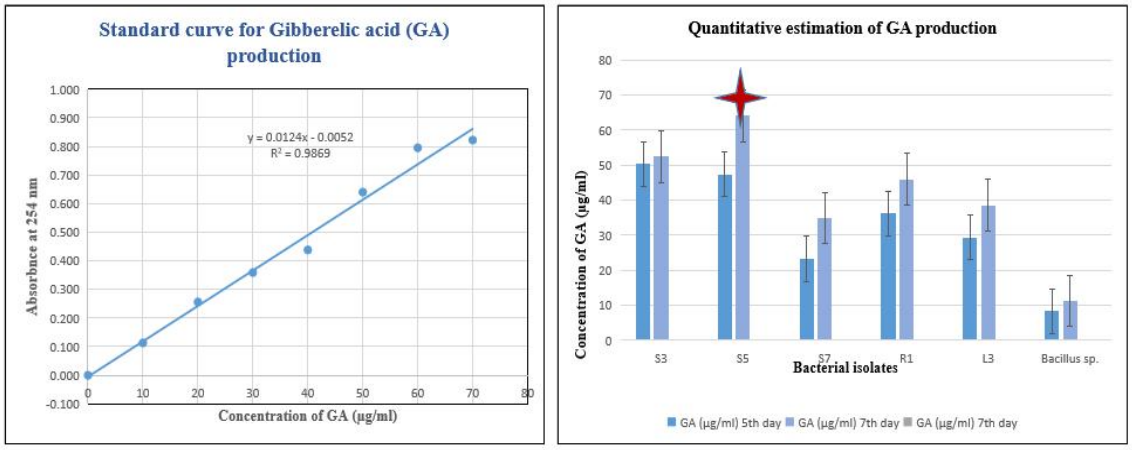


Fig 4.10. Quantitative estimation of GA_3 production by the selected bacterial isolates. a. Standard curve for GA_3; b. Concentration of GA_3 produced by the bacterial isolates at 5^{th} and 7^{th} day old bacterial culture filtrate.

- The major findings from Fig 4.10. are that, the isolate S5 produced highest amount (66.4 µg mL^{-1}) of GA_3 at 7^{th} day of incubation, followed by S3, R1, L3 and S7. At 5^{th} day stage, the maximum quantity (49.7 µg mL^{-1}) of GA_3 was produced by the bacterial isolate S3 followed by S5, R1, L3 and S7.
- In both 5^{th} and 7th-day culture filtrate, the concentration of GA_3 was produced in the least amount in S7.
- Isolate S5 appeared to be the most potent GA_3 producer followed by S3, R1, and L3.

4.6.3. Detection and Quantitative Estimation of ACC deaminase Production

ACC deaminase enzyme is a stress metabolite that makes the plants tolerant to adverse abiotic conditions. The selected PGP bacterial isolates were tested for their ACC deaminase enzyme production ability.

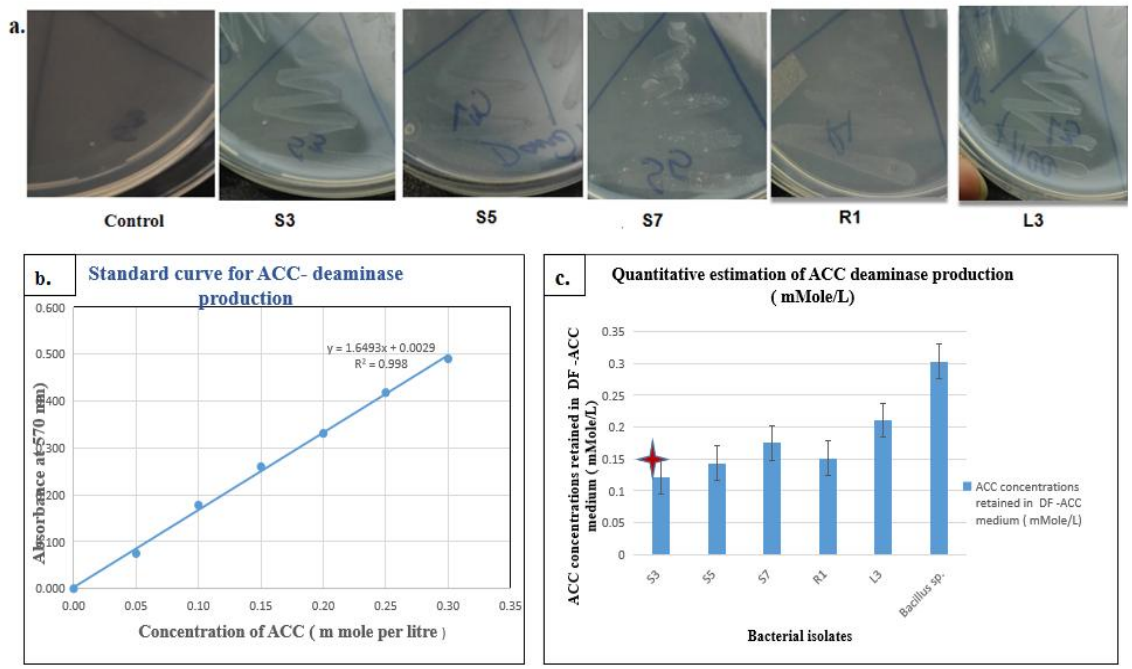


Fig 4.11. ACC deaminase production by the bacterial isolates. a. Growth of S3, S5, S7, R1 and and L3 on DF medium; b. Standard curve for ACC; c. ACC deaminase activity indicated by the concentration of ACC remaining in the medium containing 3.0 mmol/L of ACC, after 16 hours of incubation of each of the bacterial isolate.

- The results in Fig 4.11. indicate that all the isolates showed growth on DF media indicating their ACC deaminase-producing ability.
- Quantification of the enzyme activity was determined colorimetrically by the ninhydrin-ACC assay method.
- The highest ACC deaminase activity was noted for the strain L3, followed by S3, R1, S5, and S7. The standard laboratory strain *Bacillus* sp. strain has negligible activity with around 0.296 mmol/L of ACC retained in the medium.

4.6.4. Siderophore production and Siderophore typing

The findings of O-CAS assay method for the detection of siderophore producing capability of the bacterial isolates are presented in Fig 4.12.a. The observation of the Hydroximate type of siderophore typing is shown in Fig 4.12.b.

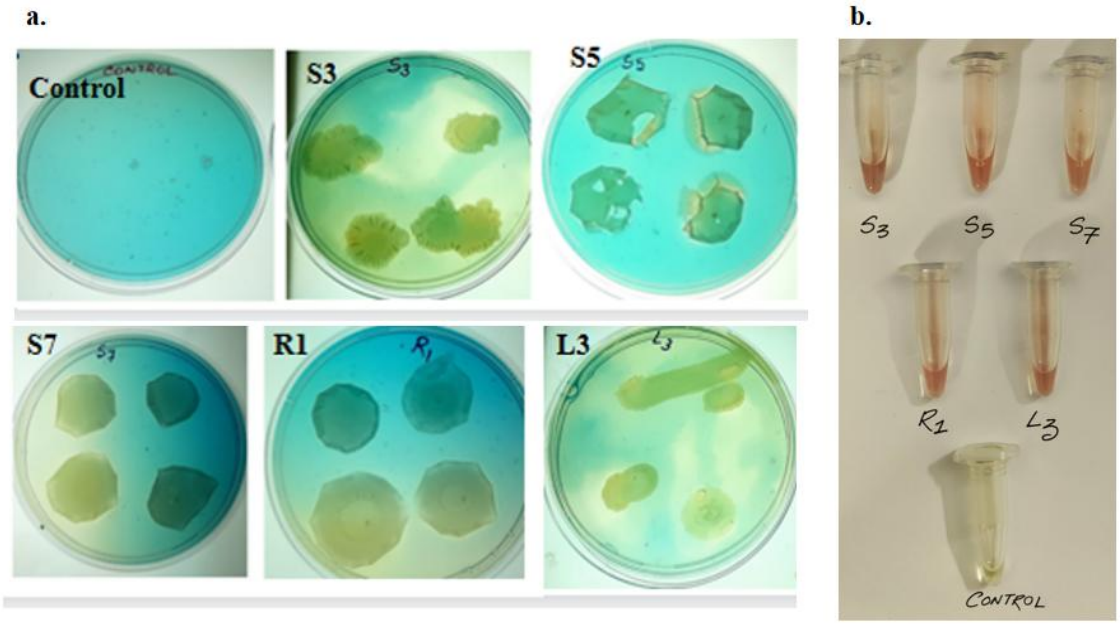


Fig 4.12. Siderophore production by bacterial isolates S3, S5, S7, R1, and L3. a. O-CAS assay culture plates showing siderophore production, change of colour of the blue medium to yellow; b. Hydroximate type of siderophore production as indicated by the development of red colour on addition of tetrazolium salt and NaOH to the CFS.

- The results shown in Fig 4.12. indicate the appearance of a yellow halo zone around the bacterial colonies confirming siderophore production by all the five bacterial isolates S3, S5, S7, R1 and L3 in O-CAS assay method. The brightly coloured Fe+3- dye complex of the blue agar medium, binds with the strong ligand siderophore, resulting in the formation of an iron-ligand complex. This iron-ligand complex releases free dye which changes the colour of the blue medium to yellow.
- All the strains produced Hydroximate type of siderophore as indicated by the development of red colour on addition of tetrazolium salt and NaOH to the CFS; S5 was able to form both catecholate and hydroximate types of siderophore

4.6.5. Biofilm producing ability of the bacterial isolates

The observations of Congo red agar plate assay method of biofilm production by the bacterial isolates are furnished in Fig 4.13.

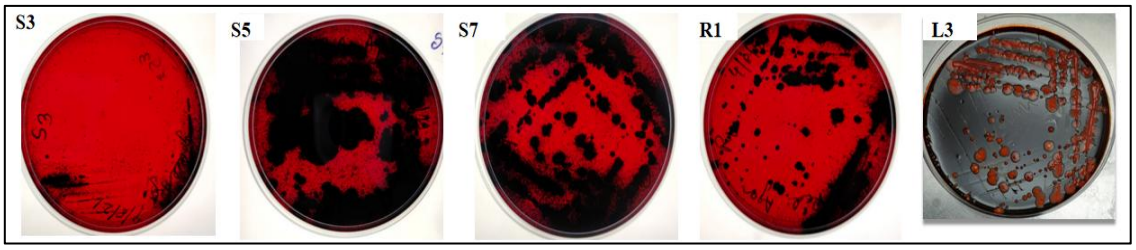


Fig 4.13. Congo red agar plate assay method of biofilm production by the bacterial isolates S3, S5, S7, R1 and L3

The results in Fig 4.13. shows that:

- Isolates S3, S5, S7, R1, and L3 produced black-coloured colonies with a dry crystal like consistency on Congo red plates indicating biofilm production. It is evident that these isolates were capable of producing biofilm but in varying amounts.
- Isolate S5 has the highest producing ability, followed by S7, R1, L3, and S3.

4.6.6. Cellulase production by bacterial isolates

Cellulase production ability of the bacterial isolates was detected by plate assay method. Colonies showing a discoloration of the medium after 24-72 h of incubation at 37^{o}C were considered to have cellulolytic activity. The findings are displayed in Fig 4.14.a.

4.6.7. Amylase production by bacterial isolates

Starch hydrolyzing potential through production amylase, was checked by plate assay method. The appearance of a clear zone of hydrolysis around the colony following addition of Iodine, indicated amylase producing ability of the isolates. The results are furnished in Fig 4.14.b.

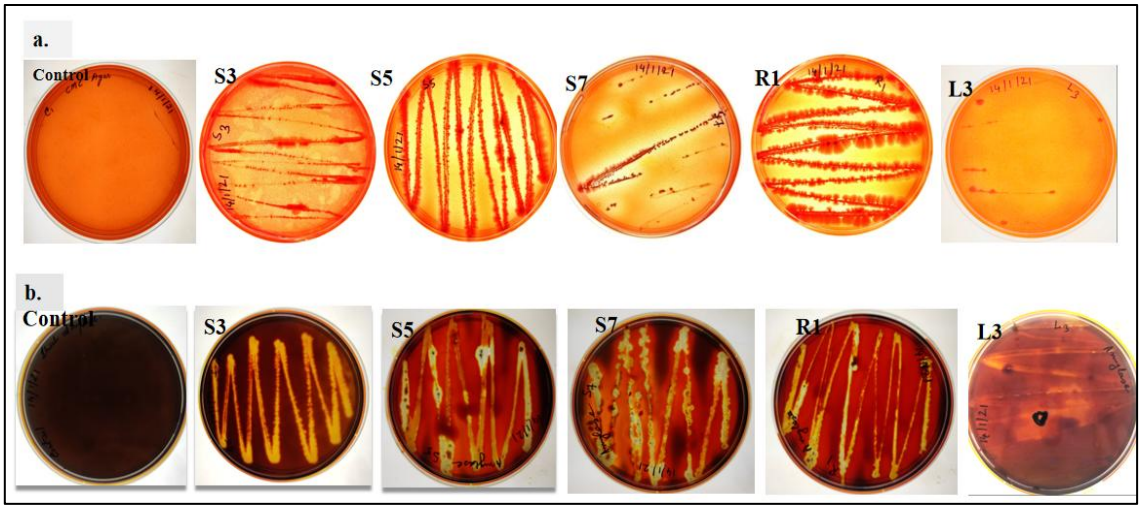


Fig 4.14. *In vitro* production of cellulase and amylase enzymes by the bacterial isolates S3, S5, S7, R1 and L3 on selective media. a Cellulase production; b. Amylase production.

- The result shown in Fig 4.14.a. and 4.14.b. indicate that except L3, other four isolates (S3, S5, S7, and R1) have amylase and cellulase enzyme production ability.

4.6.8. Protease production

The proteolytic activity of isolates S3, S5, S7, R1, and L3 were tested on skim milk agar plates (SMA). The results are presented in Table 4.5.

4.6.9. Laccase production

To detect the laccase enzyme-producing potential, bacterial isolates were grown on to NA medium supplemented with 0.5 mM Guaiacol (an inducer of laccase). Formation of brown colouration around the bacterial colonies was considered as positive result as shown in Fig 4.15.a.

4.6.10. Urease producing potential

Christensen's Urea Agar medium was used for detection of urease enzyme producing bacterial isolates. A changes in colour of bacterial colony from orange (initial) to red,

was considered as positive test for urease producing ability of the isolates. The results are presented in Fig 15.b. and Table 4.5.

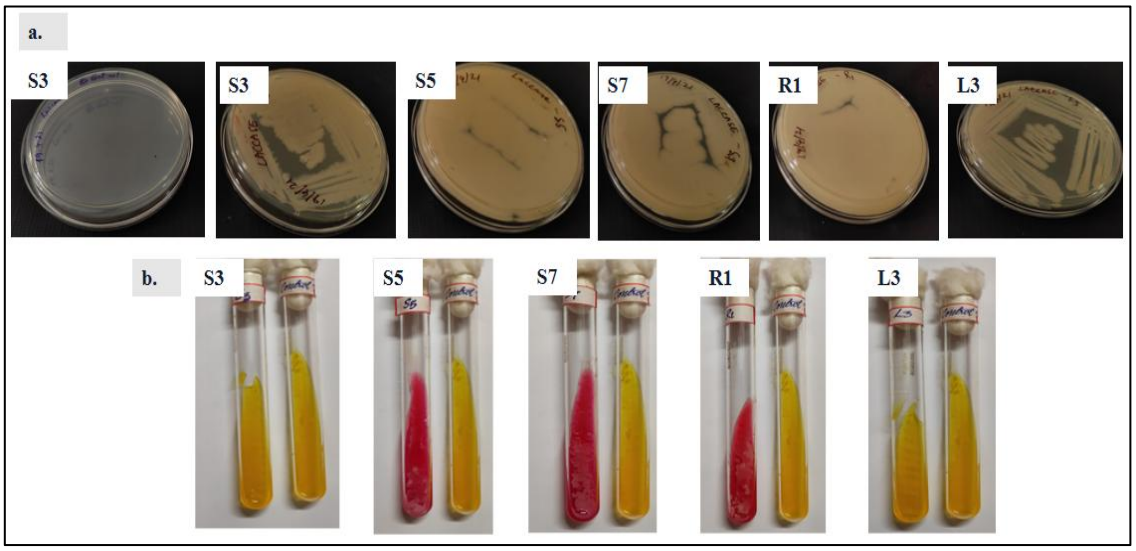


Fig 4.15. *In vitro* production of Laccase and Urease on selective media. a. Laccase; b. Urease by the bacterial isolates S3, S5, S7, R1,, and L3.

- The results furnished in Fig 4.15.a. indicate that all five isolates were laccase producers.
- Fig 4.15.b. shows that the isolates S5, S7, and R1 have urease-producing potential, whereas S3 and L3 were non-producers of urease.

4.6.11. Pectinase producing potential

Bacterial isolates were spot inoculated on Pectin agar media plates for detection of pectinase enzyme-producing bacterial isolates. The results are presented in Table 4.5.

4.6.12. Chitinase producing ability

Chitinase producing potential of the bacterial isolates was checked by plate assay method using colloidal chitin agar medium. The results are presented in Table 4.5. and Fig 16.a.

4.6.13. β 1,3 glucanase Chitinase producing ability

For detecting β 1,3 glucanase producing potential, the bacterial isolates were grown on solid minimal medium supplemented with $1gL^{-1}$ laminarin. The results are presented in Table 4.5. and Fig 16.b.

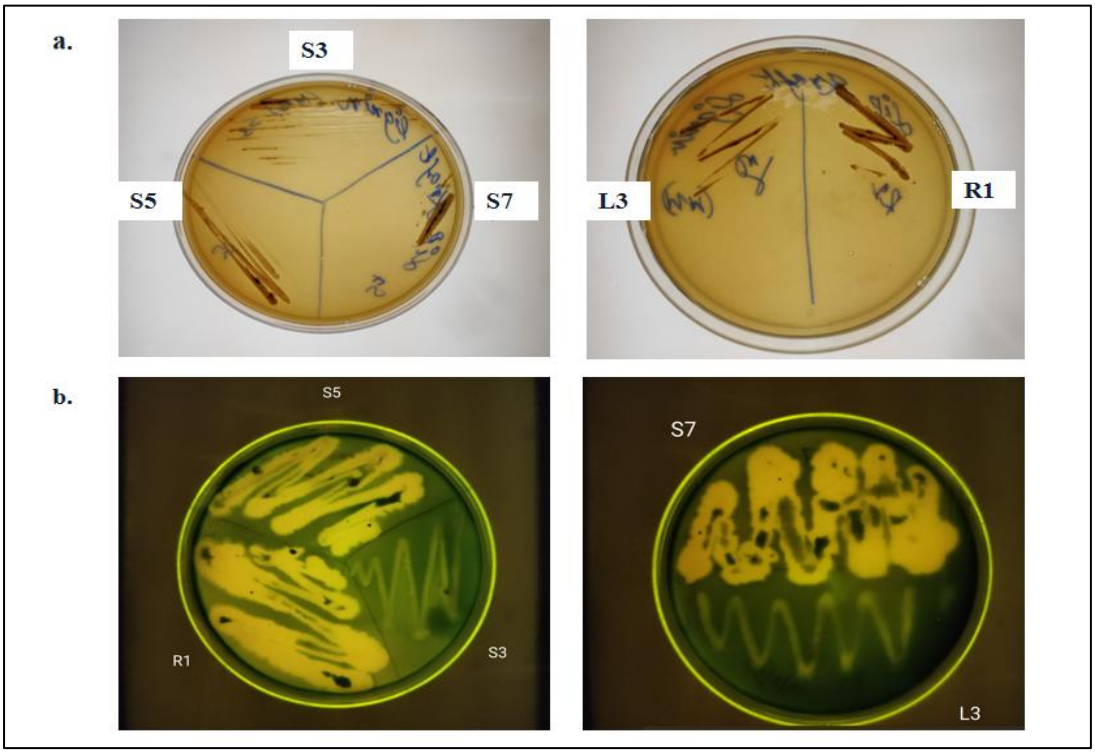


Fig 4.16. Culture plates showing β 1,3 glucanase enzyme production by the isolates S3, S5, S7, R1, and L3.. a. Chitinase; b. β 1,3 glucanase.

- The results show that all the five isolates produced chitinase enzyme.
- Bacterial isolates S5, S7, and R1 have β 1,3 glucanase-producing potential, whereas S3 and L3 lacked this ability.

5. 4.6.14. Peroxidase production

The plate assay method was used to determine peroxidase peroxidase-producing ability of the isolates. The results are presented in Fig 4.17. and Table 4.5.

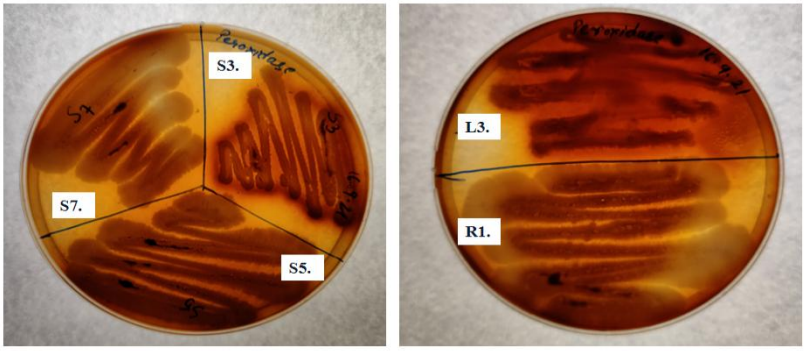


Fig 4.17. The plate assay showing peroxidase production by the isolates

Fig 4.17 indicates that except S5, all other isolates were positive for peroxidase enzyme production.

4.6.15. Lignin peroxidase

Minimal Salt Media which contained 0.5 g L^{-1} of kraft lignin as only source of C, was used to detect Lignin peroxidise producing potential of the bacterial isolates. The results are presented in Table 4.5.

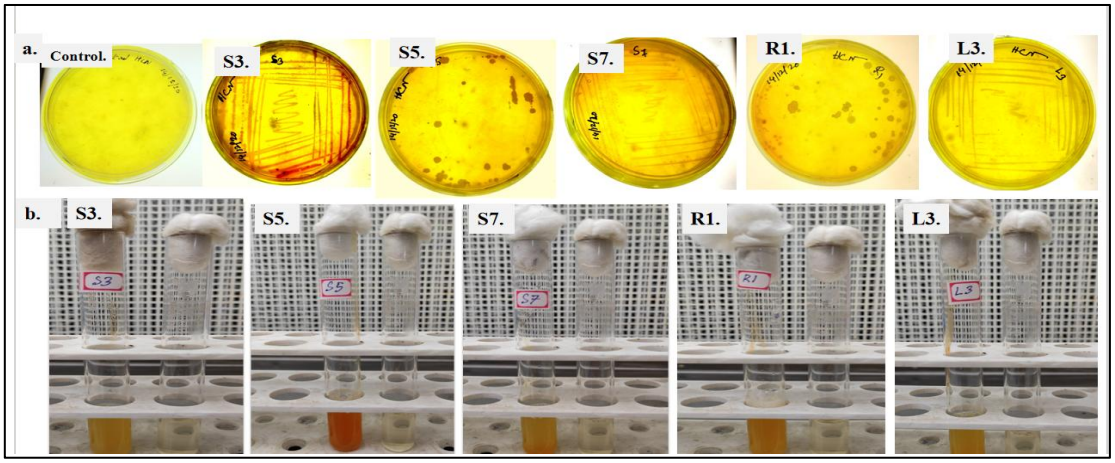


Fig 4.18. Production of antimicrobial metabolite. a. HCN production; b. NH_3 production

4.6.16. Hydrogen cyanide (HCN)

The production capability of HCN, a strong antimicrobial metabolite, of the bacterial isolates was tested. The results are presented in Table 4.5 and Fig 4.18.

4.6.17. Ammonia (NH_3) production

HCN is a volatile secondary metabolite useful for biocontrol of phytopathogens. The production potential of HCN by the bacterial isolates was tested. The results are presented in Table 4.5.and Fig 4.18.

Serial No	Production of agriculturally beneficial metabolites	Bacterial isolates				
		S3	S5	S7	R1	L3
1	**IAA**	+ ve	+ ve	+ ve	+ ve	+ ve
2	**GA**	+ ve	+ ve	+ ve	+ ve	+ ve
3	**ACC deaminase**	+ ve	+ ve	-ve	+ ve	+ ve
4	**Siderophore**	+ ve	+ ve	+ ve	+ ve	+ ve
	Siderophore type	Hydoximate	Hydroximate, catecholate	Hydroximate, catecholate	Hydroximate	Hydroximate
5	**Biofilm**	+ ve	+ ve	+ ve	+ ve	+ ve
6	**Amylase**	+ ve	+ ve	+ ve	+ ve	-ve
7	**Cellulase**	+ ve	+ ve	+ ve	+ ve	-ve
8	**Protease**	+ ve	+ ve	-ve	+ ve	+ ve
9	**Pectinase**	+ ve	+ ve	-ve	+ ve	- ve
10	**Laccase**	+ ve	+ ve	+ ve	+ ve	+ ve
11	**Urease**	-ve	+ ve	+ ve	+ ve	-ve
12	**Lignin peroxidase**	-ve	-ve	-ve	+ ve	-ve
13	**Chitinase**	+ ve	+ ve	-ve	+ ve	+ ve
14	**β 1, 3 Glucanase**	-ve	+ ve	+ ve	+ ve	-ve
15	**Peroxidase**	+ ve	-ve	+ ve	+ ve	+ ve
16	**HCN**	+ ve	+ ve	+ ve	+ ve	-ve
17	**NH_3**	+ ve	+ ve	+ ve	+ ve	+ ve

'+' = Production '-'= No production

Table 4.5. Production of agriculturally beneficial metabolites by the bacterial isolates

The major findings of the characterization of the selected bacterial isolates are:

- **Direct plant growth promoting metabolite production:** All the isolates can produce IAA and GA_3. Except S7, other isolates have ACC deaminase enzyme production potential.
- **Direct plant growth promoting metabolite production:** Except L3, other isolates are potent amylase and cellulase enzyme producers. All five of the bacterial isolates (S3, S5, S7, R1, and L3) were detected to have siderophore and biofilm-producing potential. Only isolate R1 possesses lignin peroxidase-producing ability. The isolate S7 was not able to produce protease, pectinase, and chitinase enzymes in *in vitro* conditions. Pectinase, beta 1,3 glucanase production ability was lacking in L3.
- **Antimicrobial compounds production:** All the isolates were the potent producers of NH_3 in *in vitro* conditions, S3 having a weak producing ability; except L3, others can produce HCN.

4.6.18. Antagonistic activity against fungal pathogen

Antagonistic activity against fungal pathogen isolated from infected leaves of soybean plants.

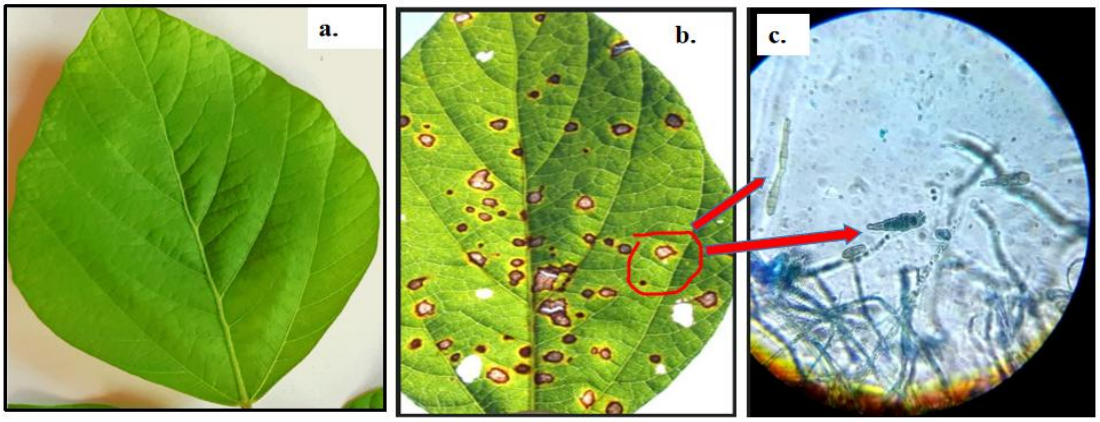


Fig 4.19. Isolation of fungal pathogen from diseased leaves. a. a healthy leaf; b. a diseased leaf showing fungal lesion; c. microscopic field showing conidia of the fungal pathogen. The isolated fungal pathogen was identified on basis of its vegetative and reproductive characteristics as *Alternaria* sp. Antagonistic activity the bacterial isolates were tested against fungal pathogen *Alternaria* sp. The result of the study are presented in Fig 4.20. and Table 4.6.

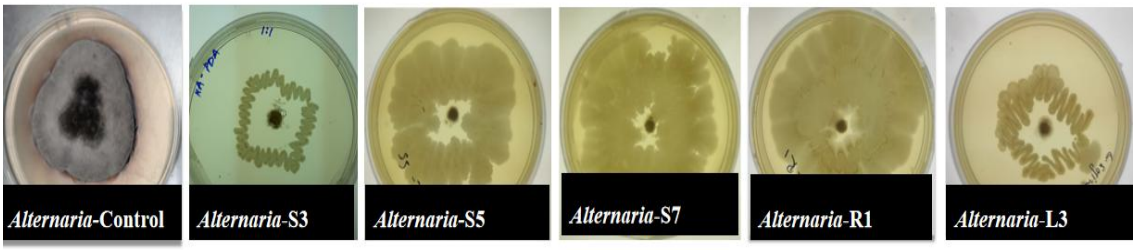


Fig 4. 20. Dual culture plate assay showing Anti-fungal activity of the bacterial isolates

Anti fungal activity against *Alternaria alternata*	**Bacterial isolates**				
	S3	**S5**	**S7**	**R1**	**L3**
Zone of inhibition (cm)	1.1 ± 0.02	0.2 ± 0.12	0.4 ± 0.25	0.4 ± 0.06	0.8 ± 0.08
% of inhibition	73.8 ± 0.24	95.2 ± 0.03	90.5 ± 0.17	90.5 ± 0.10	80.9 ± 0.13

Table 4.6. Anti-fungal activity of the bacterial isolates

Results of Fig 4.21. and Table 4.6. indicate that all the 5 tested bacterial isolates were able to inhibit the growth of the pathogenic fungus, *Alternaria* sp., although in varying degrees.

4.7. Antibiotic sensitivity assay of the promising isolates

The results of the antibiotic sensitivity assay are presented in Table 4.7. and Fig 4.20. The test was run in triplicates. the data is represented as mean with the standard error of the mean. For categorizing the response as ‘sensitive’, ‘intermediate’ or ‘resistant’, the zone of inhibition of bacterial growth in the presence of each of the respective antibiotics, was compared to the standard values (CLSI, 2012).

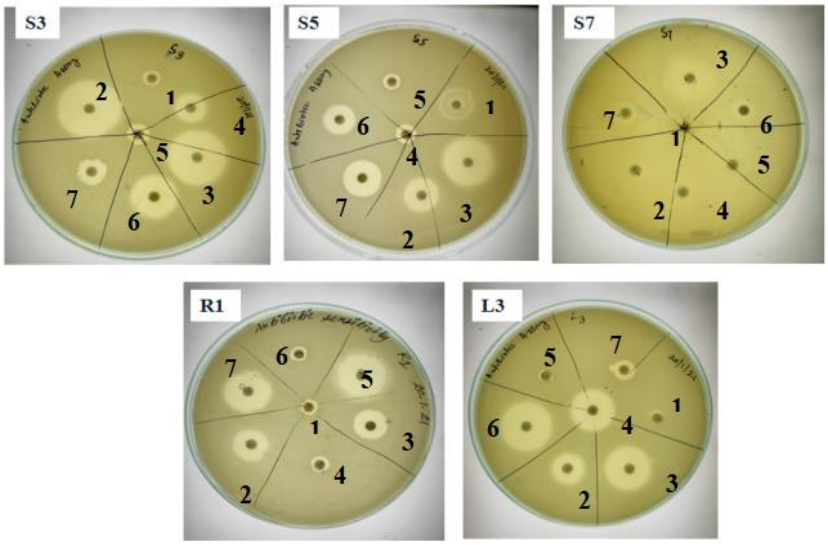


Fig 4.21. Antibiotic sensitivity assay of isolates S3, S5, S7, R1, and L3 showing the inhibition zone.

S=sensitive, I= intermediate, R= resistant; Dia= diameter, '-' no inhibition zone

Antibiotic	**Bacterial isolate**									
	S3		**S5**		**S7**		**R1**		**L3**	
	Zone Diameter	S/I /R	Zone Diameter	S/I/ R	Zone Diameter	S/I/ R	Zone Diameter	S/I /R	Zone Diameter	S/I/ R
1. Ampilox (10 µg)	03±0.01		12±0.09	I	-	R	02±0.10	R	-	R
2.Chloramphenicol (20 µg)	29±0.03	S	26±0.09	S	-	R	24±0.09	S	16±0.07	I
3.Ciprofloxacin 10 (µg)	35±0.04	S	33±0.13	S	24±0.00	S	35±0.23	S	26±0.04	S
4.Rifampicin (10 µg)	14±0.11	I	06±0.01	R	-	R	07±0.01	R	24±0.05	S
5.Streptomycin 30 (µg)	10±0.05	R	04±0.09	R	-	R	35±0.06	S	-	R
6.Tetracyclin 30 (µg)	31±0.21	S	25±0.12	S	02±0.03	R	10±0.02	I	30±0.01	S
7.Vancomycin 30 (µg)	12±0.06	S	25±0.05	S	03±0.04	R	31±0.13	S	12±0.06	I

Table 4. 7. Antibiotic sensitivity assay showing the interpretive categories (CLSI, 2012).

Based on the antibiotic sensitivity tests as shown in Fig 4.21 and Table 4.7., it is concluded that the isolates have varied responses to each of the antibiotics tested, and the

zone of inhibition also showed variations. Significantly, the isolate S7 was a multi-strain resistant isolate, only sensitive to Ciprofloxacin and therefore, was not considered for utilization in multi-strain bioinoculant designing.

4.8. Molecular identification of the promising isolates

The 7 efficient PGP bacterial isolates S3, S5, S7, R1, and L3 were identified based on their 16S rRNA gene sequence. For molecular identification, the unique 16S rDNA regions of the 5 isolates were amplified. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out. The 16S rDNA gene sequence was used to carry out BLAST with the NCBI GenBank database. The strains were identified based on nucleotide homology and phylogenetic analysis. For strain S3, 99% query cover and 99.88% identification score was observed with *Bacilus zhanzhouensis* strain MCCC 1A08372. For the strain S5 and R1, 100% query cover and 100% identification score was observed with *Bacillus cereus* strain ATCC 14579 and *Bacillus subtilis* strain S16, respectively. Strain S7 showed high similarity with *Bacillus subtilis* strain BJ3-2 having 100% query cover and 99.93% identification score. For strain L3, 99.56% query cover and 99.58% identification score were observed with *Pseudomonas gessardii* strain ST3SE. The sequences were submitted to the NCBI GenBank Database and their accession numbers were obtained, which are furnished as in Table 4.8.

Isolate code	**Submitted in NCBI as**	**NCBI Accession NO.**
S3	*Bacillus zhanzhouensis* MMAM	NCBI Acc: MT185655
S5	*Bacillus cereus* strain MMAM3	NCBI Acc: MT30003
S7	*Bacillus subtilis* strain MMAM4	NCBI Acc: MT30004.1
R1	*Bacillus subtilis* strain MMAM2	NCBI Acc: MT725461.1
L3	*Pseudomonas* sp. strain MMAM5	NCBI Acc: ON237480

Table 4.8. NCBI submission details of Bacterial isolates

Description	Max score	Total score	Query cover	E-value	Per. Ident
***Bacillus zhanzhouensis* strain MCCC 1A8372 16S ribosomal RNA, partial sequence**	**1552**	**1552**	**99%**	**0**	**99.98%**
Bacillus safensis strain NRBC 100820 16S ribosomal RNA gene, partial sequence	1552	1552	99%	0	99.98%
Bacillus pumilus strain NBRC 1209216S ribosomal RNA gene, partial sequence	1552	1552	99%	0	99.98%
Bacillus pumilus strain CIP 52.67 16S ribosomal RNA, partial sequence	1552	1552	99%	0	99.98%
Bacillus australimaris strain MCCC 1A05787 16S ribosomal RNA gene, partial sequence	1546	1546	99%	0	99.76%
Bacillus aerius strain 2K 6S ribosomal RNA gene, partial sequence	1530	1530	99%	0	99.41%
Bacillus stratosphericus strain 41KF2a 16S ribosomal RNA, partial sequence	1530	1530	99%	0	99.41%
Bacillus altitudinis strain 41KF2b 16S ribosomal RNA gene, partial sequence	1530	1530	99%	0	99.41%
Bacillus safensis strain FO-36b16S ribosomal RNA gene, partial sequence	1528	1528	98%	0	99.76%
Bacillus pumilus strain ATCC 7061 16S ribosomal RNA gene, partial sequence	1528	1528	98%	0	99.76%

Table 4.9. Sequences producing significant alignments for bacterial isolate S3

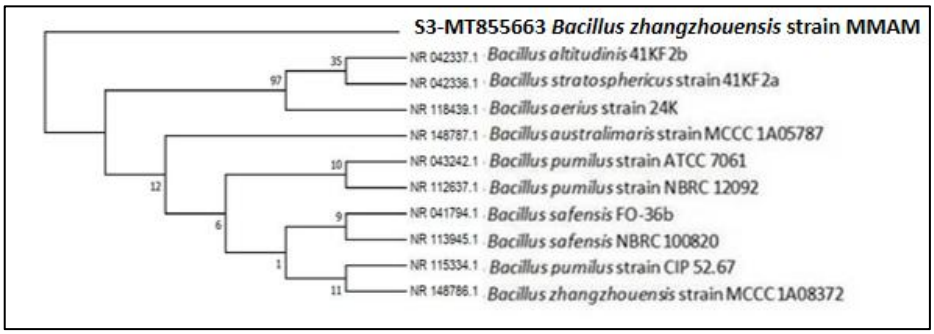


Fig 4.22. Phylogenetic tree of isolate S3.

Description	Max score	Total score	Query cover	E-value	Per. Ident
***Bacillus cereus* strain ATCC 14579 complete genome**	**1434**	**18638**	**100%**	**0**	**100%**
Bacillus anthracis strain ATCC 17OD930 complete genome	1434	14271	100%	0	100%
Bacillus cereus strain LUPA60-166 16s ribosomal RNA gene, partial sequence	1434	1434	100%	0	100%
Bacillus pseudomycoides strain SCSB.1 16S ribosomal RNA gene, partial sequence	1434	1434	100%	0	100%
Bacillus pseudomycoides strain SCSB.8 16S ribosomal RNA gene, partial sequence	1434	1434	100%	0	100%
Bacillus cereus strain BT-RVCE02 16S ribosomal RNA gene, partial sequence	1434	1434	100%	0	100%
Bacillus paranthracis strain PR1 chromosomal complete genome	1434	20049	100%	0	100%
Bacillus cereus strain FORC chromosomal complete genome	1434	20001	100%	0	100%
Bacillus cereus strain BTCB 16S ribosomal RNA gene, partial sequence	1434	1434	100%	0	100%
Bacillus cereus strain S12 i6S ribosomal RNA gene, partial sequence	1434	4345	100%	0	100%

Table 4.10. Sequences producing significant alignments for bacterial isolate S5

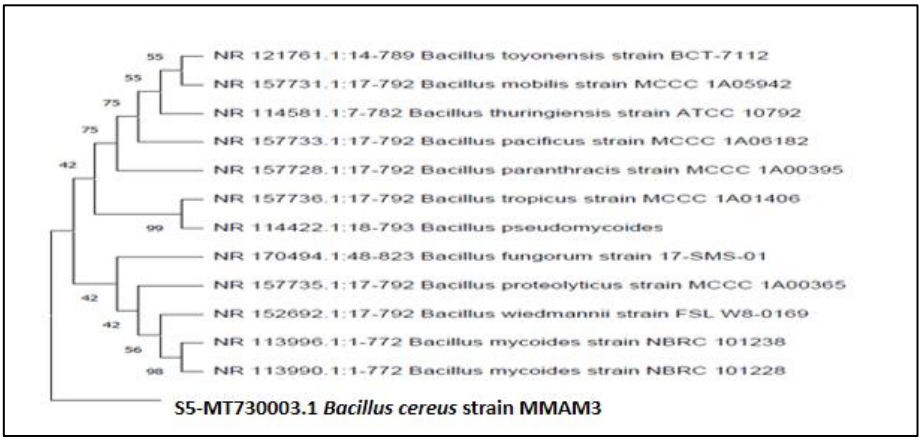


Fig 4.23. Phylogenetic tree of isolate S5

Description	Max score	Total score	Query cover	E-value	Per. Ident
***Bacillus subtilis* strain BJ3-2 complete genome sequence**	**2560**	**25528**	**100%**	**0**	**99.93%**
Bacillus tequilensis strain BK206 16S ribosomal RNA gene, partial sequence	2560	2560	100%	0	99.93%
Bacillus sp. strain MD-5 complete genome sequence	2560	30726	100%	0	99.93%
Bacillus subtilis strain MJ01 complete genome sequence	2560	25589	100%	0	99.93%
Bacillus sp. strain HNINUP40 16S ribosomal RNA gene, partial sequence	2560	2560	100%	0	99.93%
Bacillus subtilis strain *AER314.2* 16S ribosomal RNA gene, partial sequence	2560	2560	100%	0	99.93%
Bacillus sp. strain S20605 16S ribosomal RNA, partial sequence	2560	2560	100%	0	99.93%
Bacillus subtilis strain OH2377A 16S ribosomal RNA gene, partial sequence	2560	2560	100%	0	99.93%
Bacillus subtilis strain GZD-23 16S ribosomal RNA gene, partial sequence	2560	2560	100%	0	99.93%
Bacillus sp strain *3-10(2012)* 16S ribosomal RNA gene, partial sequence	2560	2560	100%	0	99.93%

Table 4.11. Sequences producing significant alignments for bacterial isolate S7

Fig 4.24. Phylogenetic tree of isolate S7

Description	Max score	Total score	Query cover	E-value	Per. Ident
***Bacillus subtilis* strain S16 16S ribosomal RNA, partial sequence**	**1430**	**1430**	**100%**	**0**	**100%**
Bacillus subtilis strain S15 16S ribosomal RNA gene, partial sequence	1430	1430	100%	0	100%
Bacillus tequililensis strain MMFG37 16S ribosomal RNA gene, partial sequence	1430	1430	100%	0	100%
Bacillus subtilis strain A1 16S ribosomal RNA gene, partial sequence	1430	1430	100%	0	100%
Bacillus halotolerance 16S ribosomal RNA gene, partial sequence	1430	1430	100%	0	100%
Bacillus subtilis strain JP2 16S ribosomal RNA gene, partial sequence	1430	1430	100%	0	100%
Bacillus sp. strain (in. Bacteria) NRC2 2017 16S ribosomal RNA, partial sequence	1430	1430	100%	0	100%
Bacillus sp. strain (in. Bacteria) NRC1 2017 16S ribosomal RNA, partial sequence	1430	1430	100%	0	100%
Bacillus subtilis strain BJ3-2 complete genome sequence	1430	1430	100%	0	100%
Bacillus subtilis subsp. strain *inquosorum* 16S ribosomal RNA gene, partial sequence	1430	1430	100%	0	100%

Table 4.12. Sequences producing significant alignments for bacterial isolate R1

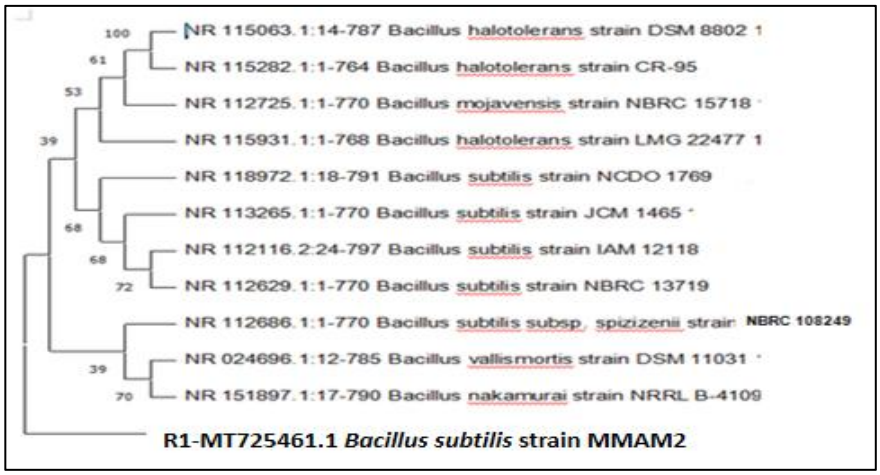


Fig 4.25. Phylogenetic tree of isolate R1

Description	Max score	Total score	Query cover	E-value	Per. Ident
Pseudimonas gessardii strain ST3SE 16S ribosomal RNA, partial sequence	**2612**	**2612**	**100%**	**0**	**99.58%**
Pseudomonas sp strain CFSAN08495 complete genome sequence	2612	15657	100%	0	99.58%
Pseudomonas paralactis strain CFH1-0116S ribosomal RNA gene, partial sequence	2612	2612	100%	0	99.58%
Pseudomonas fluorescens strain 4f 16S ribosomal RNA gene, partial sequence	2612	2612	100%	0	99.58%
Pseudomonas azotoformans strain BG4 16S ribosomal RNA gene, partial sequence	2612	2612	100%	0	99.58%
Pseudomonas fluorescens strain YPS3 16S ribosomal RNA gene, partial sequence	2612	2612	100%	0	99.58%
Pseudomonas sp. strain FDAARGOS38 chromosome, complete genome sequence	2612	15634	100%	0	99.58%
Pseudomonas sp. strain CF10.6 16S ribosoma RNA, partial sequence	2612	2612	100%	0	99.58%
Pseudomonas azotoformans strain F7 chromosome, complete genome sequence	2612	12967	100%	0	99.58%
Pseudimonas gessardii strain PBCUUn10 16S ribosomal RNA gene, partial sequence	2612	2612	100%	0	99.58%

Table 4.13. Sequences producing significant alignments for bacterial isolate L3

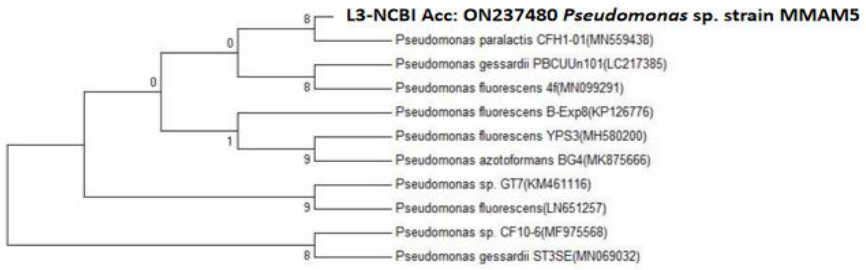


Fig 4.26. Phylogenetic tree of isolate L3.

4.9. Selection of most potent PGPB isolates and formulation of multi-strain bio-inoculants

Based on the PGP potential of the bacterial isolates, the interaction study among themselves, and the antibiotic sensitivity assay, bacterial isolates were finally chosen for

utilization as PGP bacterial inoculants in a plant growth promotion study. With respect to PGP characteristics, among the 5 isolates, S7 appeared to be comparatively inferior (Table 4.5.). Furthermore, the antibiotic sensitivity test reveals that S7 was resistant to most of the antibiotics tested Table 4.7. and Fig 4.20.) , and interaction study shows that S7 was slightly inhibitory to S5 (Table 4.4). Considering these points, S3, S5, R1 and L3 were selected for designing multi-strain bacterial consortia. Bacterial Combination-I, (S3+S5+R1) and Combination-II (S3+S5+R1+L3) were finally designed as novel multi-strain bioinoculants for application in soil sample-A and sample-C, respectively.

4.10. Survival assay of the bioinoculants

The survival period of the two bioinoculants Combination-I and Combination-II in soil was checked at a fifteen-day interval period up to ninety days and the findings are displayed in Table 4.14.

Consortium combination	**Population density (X 10^7CFU mL^{-1})**						
	Days after storage						
	0	15	30	45	60	75	90
Combination.1. S3+S5+R1	TNTC	TNTC	15.08	0.82	0.69	0.072	0.008
Combination.2. S3+S5+R1+L3	TNTC	28.85	8.60	0.17	0.001	0.0004	0.00

Table 4.14. Survival assay of bioinoculants

Results in Table 4.14. indicates that the Consortium Combination.1. and Combination.2. were viable for 60 days and 45 days, respectively.

OBJECTIVE IV: Evaluation of the Efficacy of the Novel Multi-strain Bioinoculant in Pot Trial Condition Growing *Glycine max* L.Merill (soybean) the Test Plant

4.11. Evaluation of the impacts of the novel multi-strain bio-inoculants on plant growth, resident soil bacterial community structure, and soil nutrient status.

The impact of soil amendment following application of the composite bio-inoculants, were evaluated through a three pronged approach:

- Improvement of plant growth and yield related characteristics
- Upgradation of soil nutritional status, and
- To detect any shift of resident soil bacterial community composition towards improvement in biological health of soil.

4.11.1. Evaluation of *in vivo* growth-promotion efficacy of the multi-strain bacterial inoculants

The implications of microbial amendment of soil on the vegetative growth and reproductive performance of soybean plants were assessed through the pot trial experiment.

4.11.1.1. Pot trial experiment in soil sample-A

The effect of soil amendment on the vegetative and reproductive growth of soybean plants in soil sample-A are furnished below in Fig 4.27. , Fig 4.28. and Fig 4.29.

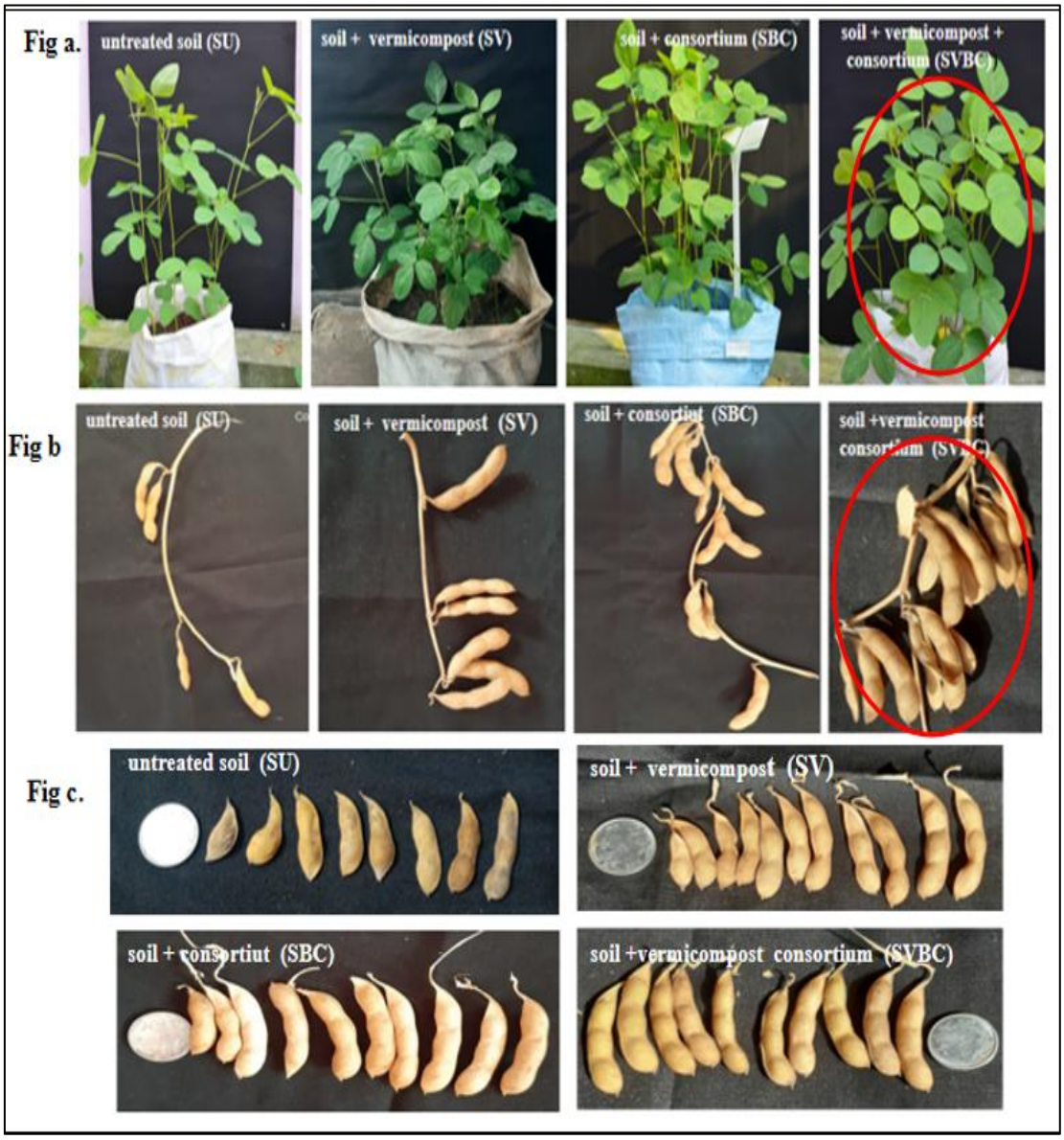


Fig 4.27. Pot trial Experiment in soil sample-A, showing vegetative and yield related traits of *Gycine max* L. (Merill) plants in soil sample A. a. Vegetative growth pattern of plants at 8 weeks after seedling emergence stage in treated and untreated set-ups; b. A portion of twigs of untreated and treated plants show fruiting; c. Mature fruits. SU = untreated soil, SV = soil amended with vermicompost, SBC = treated with bacterial consortium, and SVBC = soil amended with vermicompost and bacterial consortium

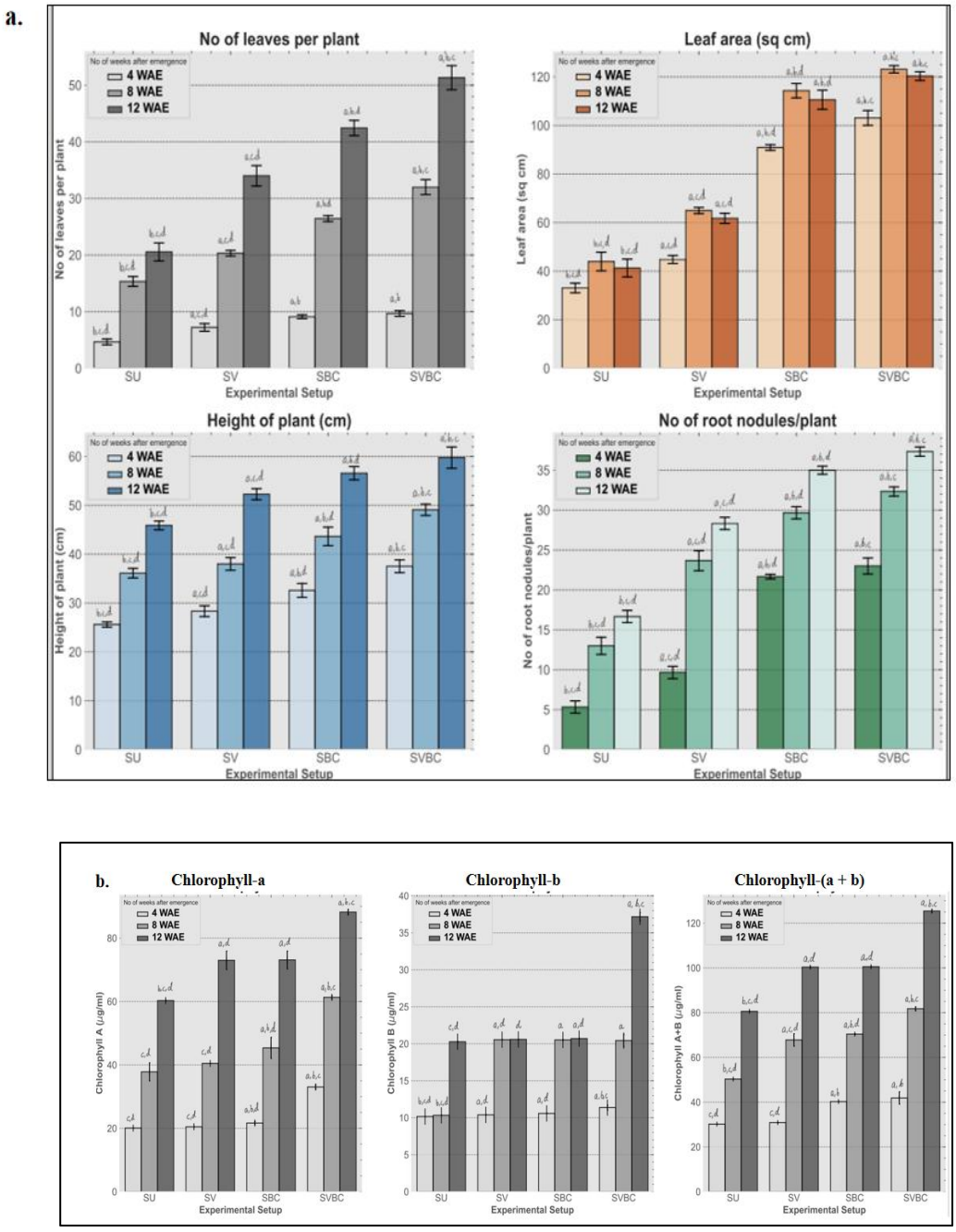


Figure 4.28. Effect of different treatments on vegetative, characteristics of *Glycine max* Merill. Plants in soil sample A. a. vegetative parameters; b. chlorophyll content of leaves; Columns represent mean values of the data for each characteristic and the error bars represent the standard deviation. Different letters on columns imply the significant difference between the means of the data ($p<0.05$) as evaluated by the Tukey's HSD test after a one-way ANOVA test. SU= untreated soil, SV= soil amended with vermicompost, SBC= treated with bacterial consortium, and SVBC= soil amended with vermicompost and bacterial consortium.

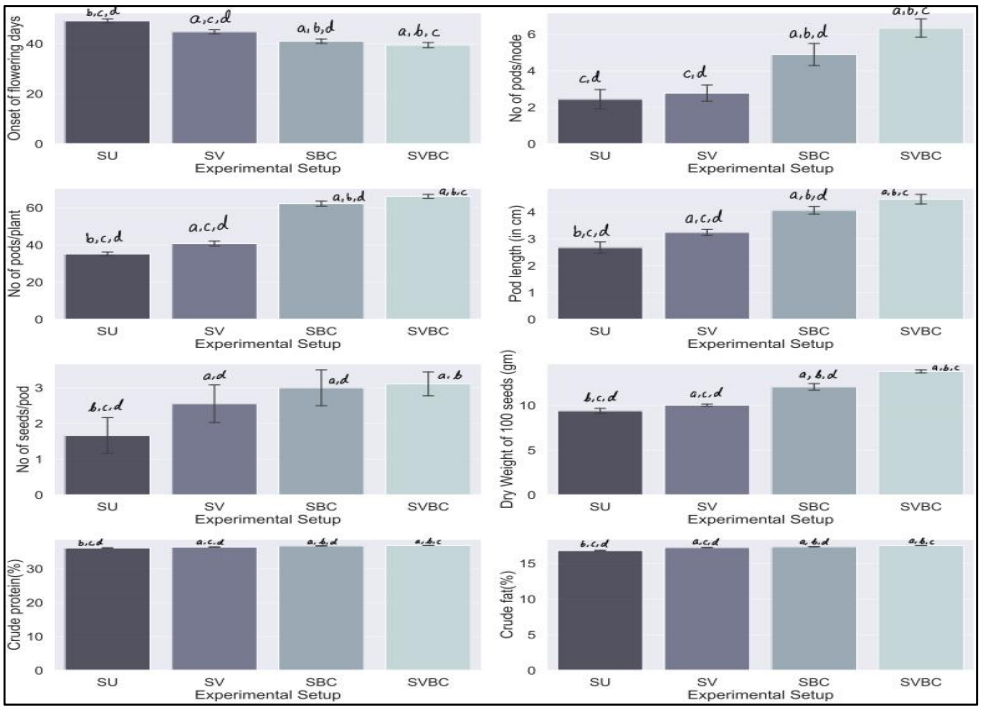


Fig 4.29. Pot trial experiment in soil sample-A, showing the effects of different treatments on reproductive and yield attributes of *Glycine max* Merill. Columns represent the mean values of the data for each characteristic and the error bars represent the standard deviation. Different letters on columns imply the significant difference between the means of the data ($p<0.05$) as evaluated by Tukey's HSD test after a one-way ANOVA test. SU = untreated soil, SV = soil amended with vermicompost, SBC = treated with bacterial consortium, and SVBC = soil amended with vermicompost and bacterial consortium

The observations in Fig 4.27, Fig 4.28, and Fig 4.29. indicate:

- Over all improvement in soybean plant growth and performance was observed following amendment practices in all the experimental set-up (SV, SBC, and SVBC) in varying degrees; maximum improvement recorded in SVBC condition.
- Plant height increased by 14.13%, 21.73%, and 32.6% in the SV, SBC, and SVBC set-ups with respect to the SU condition at 12 WAE; the total number (mean) of leaves plant^{-1} observed, at 12 WAE stages in SU, SV, SBC, and SBVC set-ups were

24, 33, 40, and 52, respectively; the similar trend of improvement was recorded in leaf area, and nodule numbers at 4, 8, and 12 WAE stages over to that of the SU. The total chlorophyll content of the leaves of potted plants showed a significant increase by 24.6%. 25.6%, and 55.4%, respectively under SV, SBC, and, SVBC condition, at 12 WAE stage over to the SU set-up.

- Soil augmentation with the joint-treatment of vermicompost and the novel consortium, exerted a positive effect on the initial blossoming stage as evidenced by 8.1%, 16%, and 20.4% decrease in the first onset of flowering days in SV, SBC, and SVBC in comparison to that of SU set-up. The application consortium incredibly increased the dry weight of seeds by 7.4%, 24.46%, and 45.7% in SV, SBC, and SBVC set-ups, respectively. Total no.of pods plant^{-1}, crude protein, and fat content of seeds improved remarkably in SBC and SBVC conditions.
- The findings indicate a strong positive influence of combined vermicompost-consortium amendment of most of the observed plant parameters under pot trial conditions.

4.11.1.2. Pot trial experiment in soil sample-C

The effect of soil amendment on the vegetative and reproductive growth of soybean plants in soil sample-C is furnished below in Fig 4.30. and Fig 4.31 and Fig 4.32.

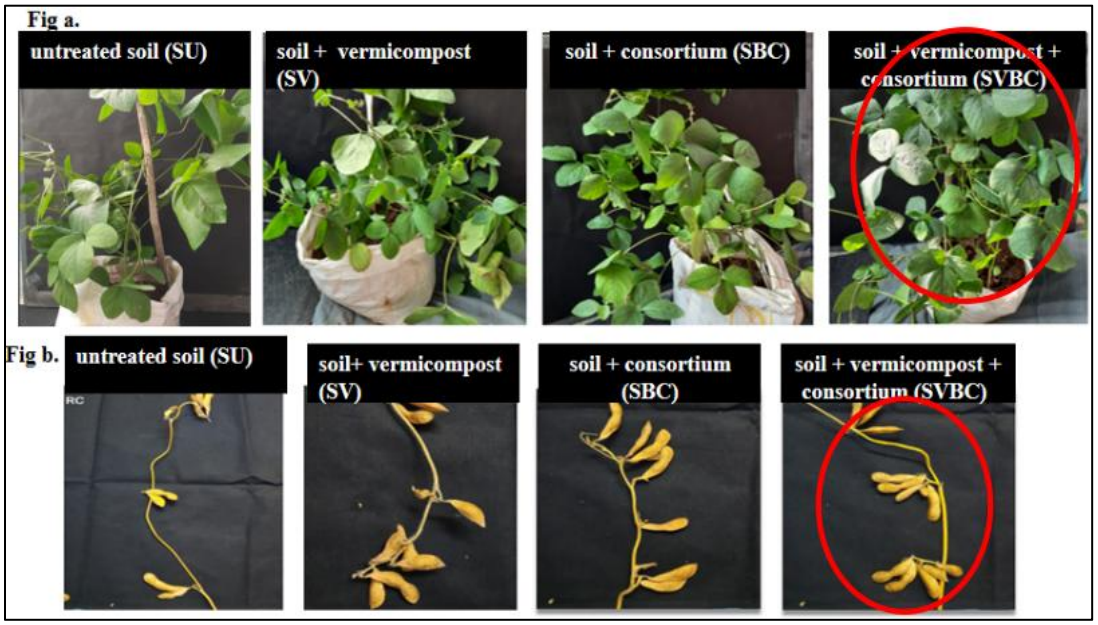


Fig 4.30. Pot trial Experiment showing vegetative and yield related traits of *Gycine max* L. (Merill) plants in soil sample C**.** a. Vegetative growth pattern of plants at 8 weeks

after seedling emergence stage in treated and untreated set-ups; b. A portion of twigs of untreated and treated plants show fruiting; c. Mature fruits.

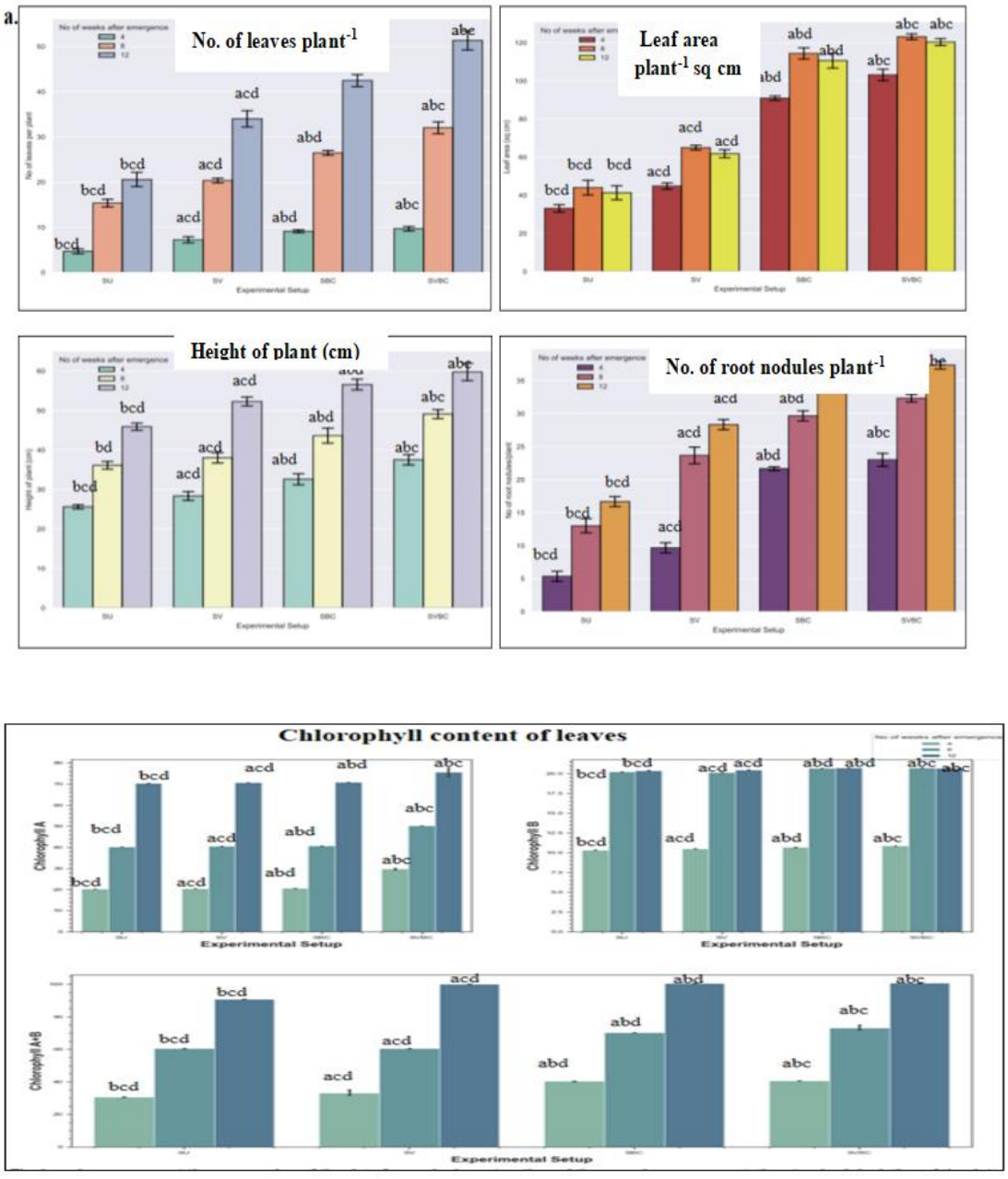


Fig 4.31. Effect of different treatments on vegetative, characteristics of *Glycine max* Merill. Plants in soil sample-C. a. vegetative parameters; b. chlorophyll content of leaves; Columns represent mean values of the data for each characteristic and the error bars represent the standard deviation. Different letters on columns imply the significant difference between the means of the data ($p<0.05$) as evaluated by Tukey's HSD test after

a one-way ANOVA test. SU= untreated soil, SV= soil amended with vermicompost, SBC= treated with bacterial consortium, and SVBC= soil amended with vermicompost and bacterial consortium.

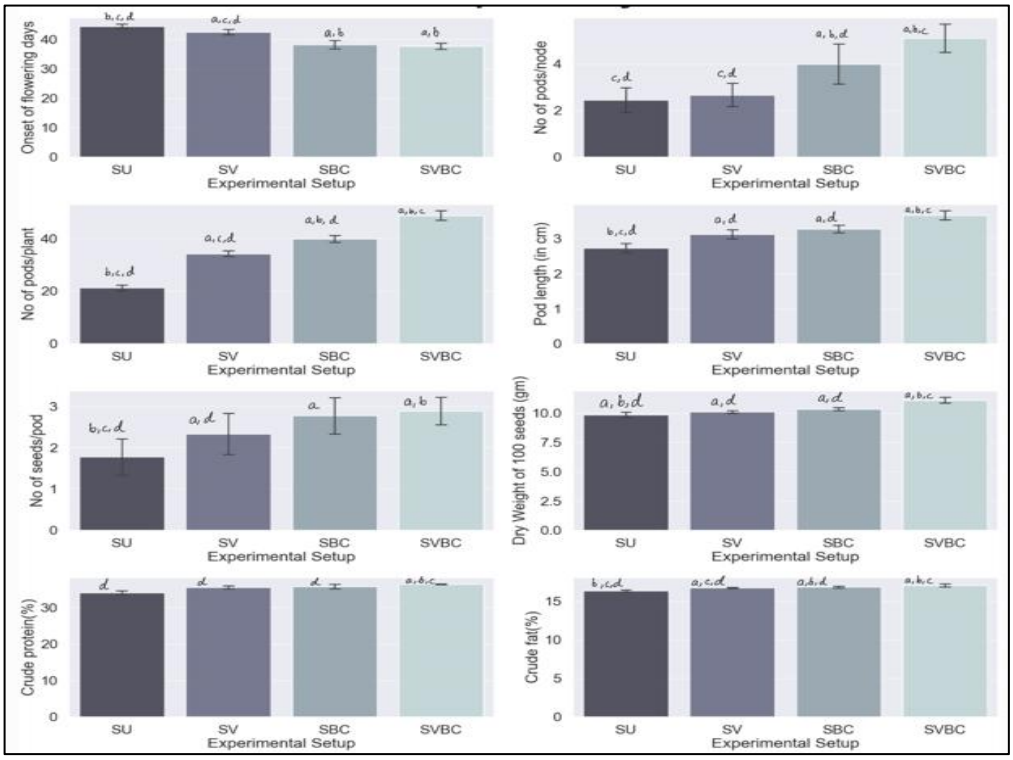


Fig 4.32. Effect of different treatments on vegetative and yield attributes of *Glycine max* Merill. Plants in soil sample-A SU= untreated soil, SV= soil amended with vermicompost, SBC = treated with bacterial consortium, and SVBC= soil amended with vermicompost and bacterial consortium. Columns represent the mean values of the data for each characteristic and the error bars represent the standard deviation. Different letters on columns imply the significant difference between the means of the data ($p<0.05$) as evaluated by Tukey's HSD test after a one-way ANOVA test.

The results in Fig 4.30., Fig 4.31, and Fig 4.32. indicate:

- Plant height increased by 15.4%, 30.1%, and 41.2% in the SV, SBC, and SVBC set-ups with respect to the SU condition at 12 WAE; leaf area (mean) of plants at 12 WAE stages increased in SV, SBC, and SBVC set-ups by 14.6%, 43.2%, and 67.3%, respectively compared to SU. A similar trend was recorded in the total no. of leaves and nodules in plants. The chlorophyll content of leaves marginally increased, as

indicated by 2.2%, 4.05%, and 5.8% increases in the (a+b) content in SV, SBC, and SVBC conditions, respectively.

- Soil augmentation with the joint treatment of vermicompost and the novel consortium, exerted a positive effect on the first onset of flowering days, as evidenced by 6.6%, 11.1%, and 16.7% decrease in SV, SBC, and SVBC compared to that of SU set-up. The application consortium improved the dry weight of seeds by 2.04%, 3.9%, and 5.6% in SV, SBC, and SBVC set-ups, respectively. Total no.of pods plant^{-1}, crude protein, and fat content of seeds improved marginally in SBC and SBVC conditions.
- The findings indicate a positive influence of different treatments on most of the observed parameters under the pot trial condition, the highest improvement being recorded in the SVBC condition.

Statistical analysis of plant performance

Tests of Normality showed that all the concerned variables related to plant growth and performance followed normal distribution as confirmed by the Shapiro-Wilk test for both soil samples (Table 4.16., 4.17.). The test statistics were not significant at the 5% level**.** Hence, we proceeded to t-Test which is appropriate for this study.

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Total_leaves_SU	.138	12	.200*	.975	12	.958
Total_chlorophyll_SU	.202	12	.192	.882	12	.093
root_nod_SU	.226	12	.091	.859	12	.048
pods_plant_SU	.230	12	.080	.900	12	.160
dry_wt_100_seeds_SU	.287	12	.007	.783	12	.006
Protein_content_seeds__SU	.159	12	.200*	.928	12	.355
Total_leaves_SVBC	.180	12	.200*	.957	12	.748
Total_chlorophyll_SVBC	.197	12	.200*	.911	12	.219
root_nod_SVBC	.145	12	.200*	.948	12	.615
pods_plant_SVBC	.205	12	.176	.890	12	.118
dry_wt_100_seeds_SVBC	.156	12	.200*	.965	12	.849
Protein_content_seeds_SVBC	.145	12	.200*	.932	12	.400

a. Lilliefors Significance Correction,

*. This is a lower bound of the true significance.

Table 4.15. Tests of Normality: Soil sample-A

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
Total_leaves_SU	.144	12	.196	.885	12	.134
Total_chlorophyll_SU	.198	12	.184	.873	12	.003
root_nod_SU	.204	12	.060	.838	12	.048
pods_plant_SU	.221	12	.019	.891	12	.095
dry_wt_100_seeds_SU	.274	12	.005	.804	12	.008
Protein_content_seeds__SU	.135	12	.073	.916	12	.066
Total_leaves_SVBC	.176	12	.198	.960	12	.162
Total_chlorophyll_SVBC	.167	12	.200*	.897	12	.084
root_nod_SVBC	.148	12	.194	.951	12	.168
pods_plant_SVBC	.201	12	.167	.830	12	.201

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistic	df	Sig.	Statistic	df	Sig.
dry_wt_100_seeds_SVBC	.138	12	.097	.952	12	.641
Protein_content_seeds_SVBC	.135	12	.200*	.836	12	.422

Table 4.16. Tests of Normality: Soil sample-C

Pair No.	Variables	t-value	df	p-value
1	Leaves_SU_SVBC	27.567	11	.000
2	Chl (a+b)_SU_SVBC	-90.312	11	.000
3	Root_nodule_SU_SBVC	34.413	11	.000
4	pods_plant_SU_SVBC	86.216	11	.000
5	Dry_wt_100_seeds_SU_SVBC	-49.542	11	.000
6	Protein_content_seeds_SU_SVBC	30.488	11	.000

Table 4.17. Paired sample t-Test for Soil sample-A

Pair No.	Variables	t-value	df	P-value
1	Leaves_SU_SVBC	16.619	11	.000
2	Chl (a+b)_SU_SVBC	4.354	11	.001
3	Root_nodule_SU_SBVC	28.447	11	.000
4	pods_plant_SU_SVBC	72.508	11	.000
5	Dry_wt_100_seeds_SU_SVBC	-53.512	11	.000
6	Protein_content_seeds_SU_SVBC	22.045	11	.000

Table 4.18. Paired sample t-Test for Soil sample-C

			Score	df	Sig.
Step 0	Variables	No_leaves	17.913	1	.000
		Leaf_area	17.869	1	.000
		Chl_content	17.984	1	.000
		root_nodules	17.631	1	.000
	Overall Statistics		17.988	4	.001

Table 4.19. Logistic regression for Soil sample-A

			Score	df	Sig.
Step 0	Variables	No_leaves	17.724	1	.000
		Leaf_area	17.989	1	.003
		Chl_content	17.807	1	.000
		root_nodules	17.751	1	.000
	Overall Statistics		17.980	4	.001

Table 4.20. Logistic regression for Soil sample-C

The statistical analyses in Table 4.15 to Table 4.20 indicate:

- For both, soil sample-A and soil sample-B, it was observed that t-statistic was highly significant at 5% level which confirmed that there has been significant improvement in the total no. of leaves plant^{-1}, total no. of root nodules plant^{-1}, total no. of pods plant^{-1}, and dry weight of 100 seeds in SVBC condition over to that of SU condition. These findings confirm the efficacy of the amendment.

- Finally, Logistic regression model indicates that, for plants grown in soil sample-A, if there is per unit rise in the no. of leaves plant^{-1} (X_1), leaf area (X_2), Chl (a+b) content of leaves (X_3) and total no. of root nodules, then there is likelihood that the no. of pod plant^{-1} will increase by 17.91, 17.86, 17.98,17.63 and 17.99 units, respectively (Table 4.19). Similarly, for plants grown in soil sample-C, the Logistic regression model indicates that, if there is per unit rise in the no. of leaves plant^{-1} (X_1), leaf area (X_2), chl (a+b) content of leaves (X_3) and total no. of root nodules, then there is a likelihood that the no. of pod plant^{-1} will increase by 17.72, 17.98, 17.80, and 17.85 units, respectively (Table 4.20).

4.11.2. Analysis of soil nutrient status in different experimental set-up across different stages of plant growth

To ascertain the effect of soil amendment, carried out in the pot trial experimental stage, the soil nutrient status such as, available nitrogen, phosphorus and potassium levels were checked at 8, 12, and 16 weeks stages after seedling emergence. The results are furnished below.

4.11.2.1. Soil sample-A: Analysis of soil nutrient status

The level of soil nutrient like available NPK and SOC content were checked at regular intervals and the results are furnished below:

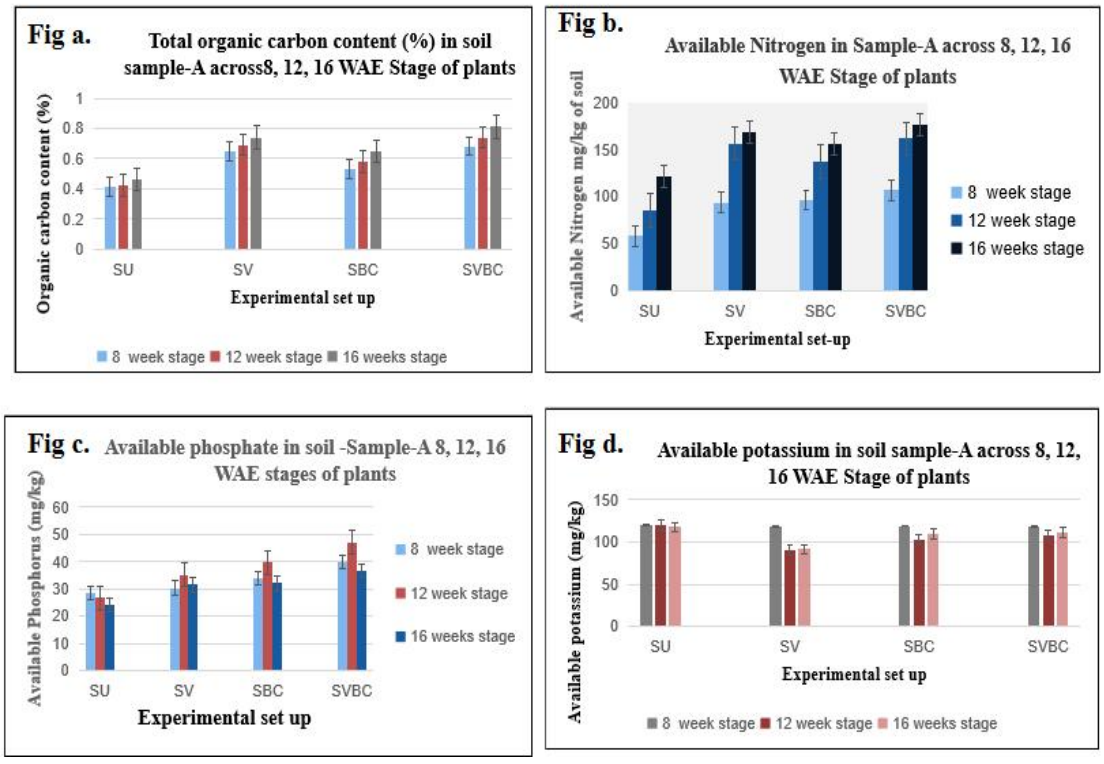


Fig 4.33. Soil sample-A: Analysis of soil nutrient status across 4, 8, and 12 WAE stages of plants. a. SOC content; b. Available N content; c. Available P content; d. Available K content.

The results in Fig 4.33. indicate that in Soil sample-A:

- A steady increase in SOC in all the experimental set-ups, across the observed period; 60%, 41%, and 76% in SV, SBC, and SVBC conditions compared to the untreated.
- Available N: A steady increase in all the experimental set-ups, across the observed period; 19%, 37%, and 68.6% in SV, SBC, and SVBC conditions compared to the untreated
- Available Phosphate: The highest level at 16 WAE except in SU. Increased about 6.5%,60.6%, and 90.75% in SV, SBC, and SVBC set-up. Steady mobilization and acquisition
- Available Potassium: Sharp fall in SV (22.49%), a gradual decrease in SBC, SVBC, 7.21%, 5.35% decrease over to that of untreated condition. Rapid acquisition in SV condition; consortia facilitated utilization of soil potassium by the plants but poor mobilization in all the treatments.

4.11.2.2. . Soil sample-C: Analysis of soil nutrient status

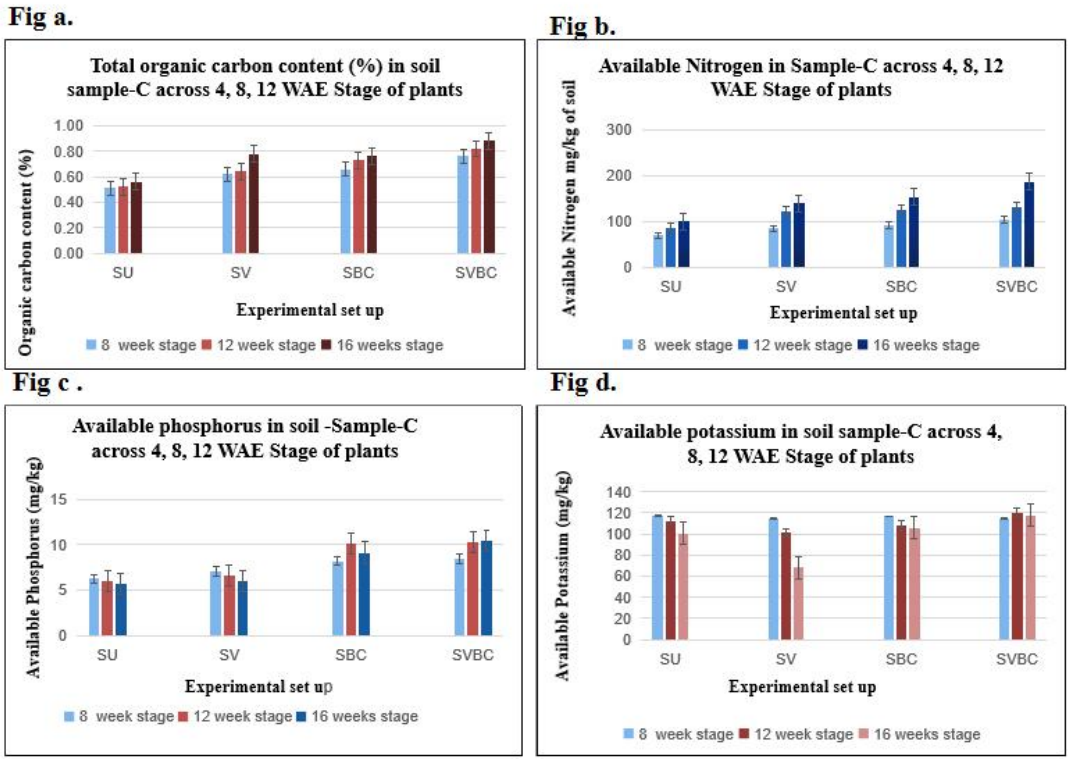


Fig 4.34. Soil sample-C: Analysis of soil nutrient status across 4, 8, and 12 WAE stage of plants. a. SOC content; b. Available N content; c. Available P content; d. Available K content.

The results in Fig 4.34. indicate:

- A steady increase in SOC in all the experimental set-ups, across the observed period; 37%, 35.7%, and 56% in SV, SBC, and SVBC conditions compared to the untreated set-up.
- Available N: A steady increase in all the experimental set-ups, across the observed period; 39.4%, 54.5%, and 87.8% in SV, SBC, and SVBC conditions compared to the untreated
- Available Phosphate: The highest level at 16 WAE except in SU. Increased about 5.09%, 60.6%, and 83.1% in SV, SBC, and SVBC set-up. Steady mobilization and acquisition in the SBC and SVBC condition

4.11.3. Insights into bacterial community composition in treated and untreated soil

Next-generation sequencing and Metagenomic analysis of soil bacterial community in amended and non-amended soil were carried out to any shift in bacterial community structure towards improvement in soil health.

4.11.3.1. Soil Sample A: Comparative Metagenomic analysis of soil bacterial community structure in amended and non-amended soil

Next-generation metagenomic sequencing and bioinformatics analysis of five different Datasets of untreated field soil and the soil, under four different experimental conditions, were carried out. The results are furnished below.

Dataset	Sample Code	Experimental Condition	Analysis Code	NCBI SRA Project: PRJNA689214
				SRA Accession Numbe
1	S	Field Soil	SAM3	SRX 9768638
2	SU	Field Soil+Soybean Plant	SAM2	SRX 9815238
3	SV	Field Soil+Vermicompost+ Soybean Plant	DHB3	SRX 19133782

Dataset	Sample Code	Experimental Condition	Analysis Code	NCBI SRA Project: PRJNA689214
				SRA Accession Numbe
4	SBC	Field Soil+Bacterial Consortium+Soybean Plant	DHB1	SRX 19133762
5	SVBC	Consortium+Soybean Plant	DHB2	SRX 19133763

***Direct URL to data:** https://www.ncbi.nlm.nih.gov/bioproject/PRJNA689214

Table 4.21. Analysed Datasets of uninoculated and inoculated soil sample-A

The metagenomic data set of the 5 soil samples was submitted to NCBI SRA.

DATASET	Sample Code	Alpha diversity	Shanon Diversity index
1	S	228	2.418
2	SU	368	3.142
3	SV	281	3.439
4	SBC	377	3.426
5	SVBC	328	3.496

Table 4.22. Alpha and Shannon Diversity Indices of the analysed Datasets of soil sample-A

The findings in Table 4.22. indicate:

- Datasets 1, 2, 3, 4 and 5 show alpha diversities of 228, 368, 281, 377 and 328 respectively and Shannon diversity indices of 2.418, 3.142, 3.439, 3.426 and 3.496 respectively.
- The diversity profiles indicate that although the 5th dataset has a relatively lower alpha diversity compared to the 4th dataset, it has a higher index of Shannon diversity, thereby establishing a higher richness and uniformity in distribution of the total number of genera in the SBVC sample

Bacterial abundance in five different datasets in Soil sample-A

Abundance in five different datasets (S,SU, SBC and SVBC), at phyla and genera level are presented in Fig 4.36, 4.37, 4.38,4.39, and 4.40

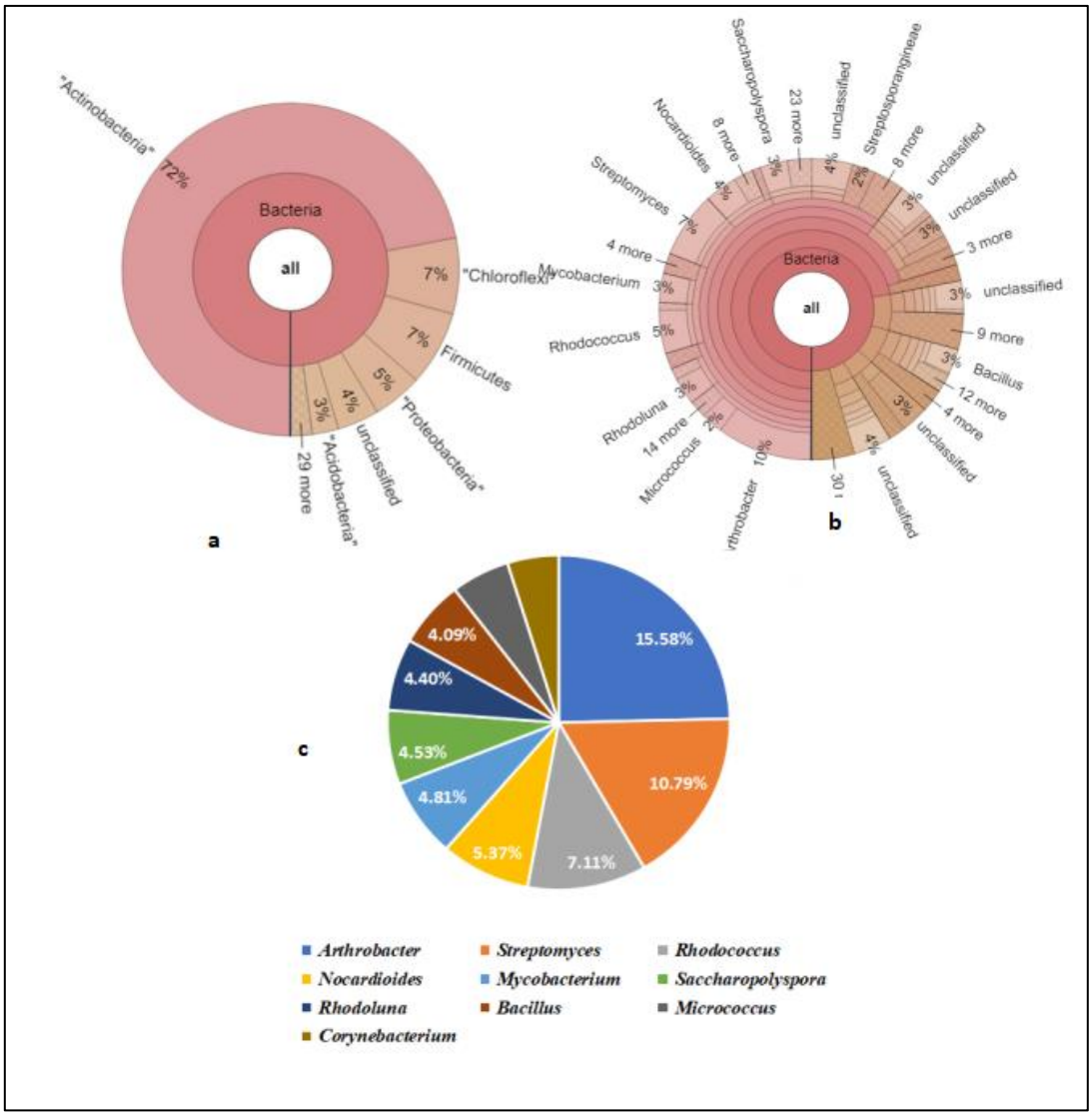


Fig 4.35. Bacterial abundance in S (untreated field soil) condition in soil sample-A. a. Krona chart representation of Phyla level abundance of prevalent bacterial assemblage; b. Krona chart representation of Genera level abundance of prevalent bacterial assemblage; c. Pie chart representing the top 10 scoring genera

The Fig 4.35. indicates that:

The Krona map yielded by running the raw sequence reads through a suitable pipeline revealed that Actinobacteria was the most abundant phylum, followed by Chloroflexi, Firmicutes, Proteobacteria, and Acidobacteria in soil sample (S). Upon further screening

of the putative top 10 genera that were most abundant in the given sample, *Arthrobacter* and *Streptomyces* had relative abundances of 15.58% and 10.79%.

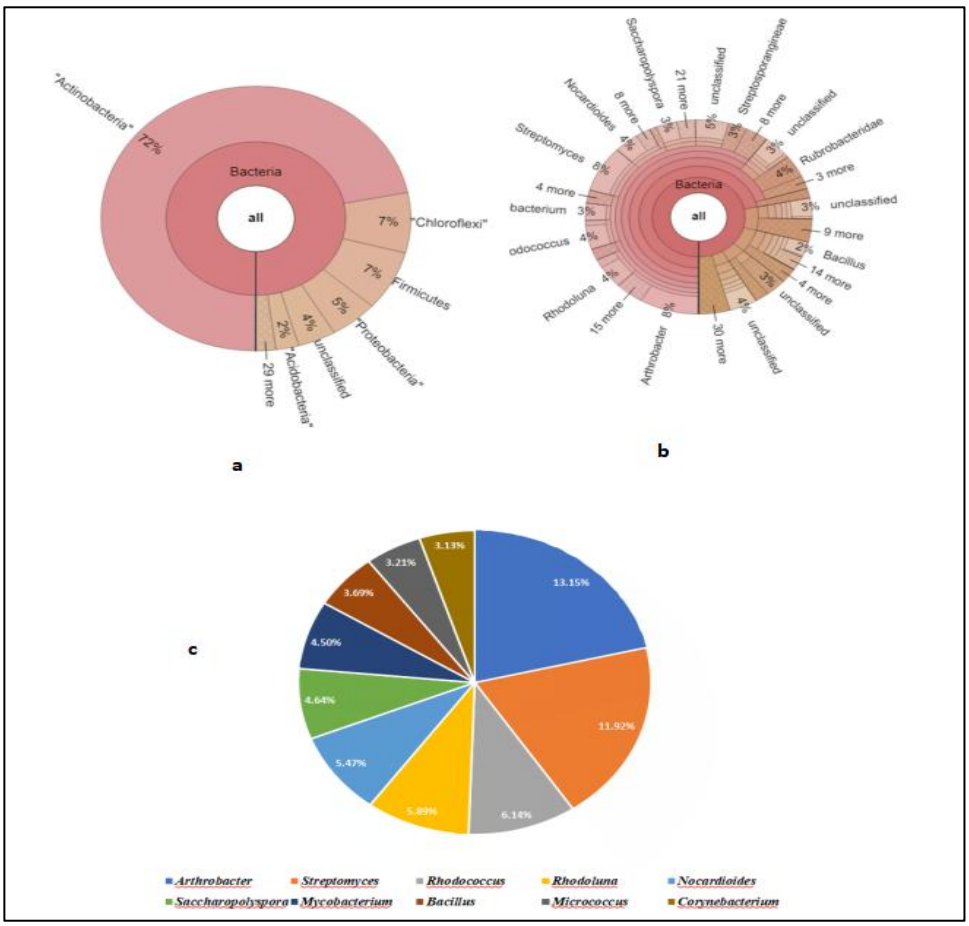


Fig.4.36. Bacterial abundance in SU (Untreated field soil with soybean plants) condition in soil sample-A. a. Krona chart representation of Phyla level abundance of prevalent bacterial assemblage, b. Krona chart representation of Genera level abundance of prevalent bacterial assemblage, c. Pie chart representing the top 10 scoring genera.

The Fig 4.36. indicates that:

It was found that Actinobacteria was the most abundant phylum, followed by Chloroflexi, Firmicutes, Proteobacteria, and Acidobacteria. The untreated field soil sample in the presence of soybean plants (SU) contained the following top 4 genera: *Arthrobacter* and *Streptomyces* (8%), *Rhodococcus* (4%), *Mycobacterium* (3%), and *Bacillus* (2%). Upon

further screening of the putative top 10 genera that were most abundant in the given sample, were Arthrobacter and Streptomyces had relative abundances of 13.15% and 11.92%

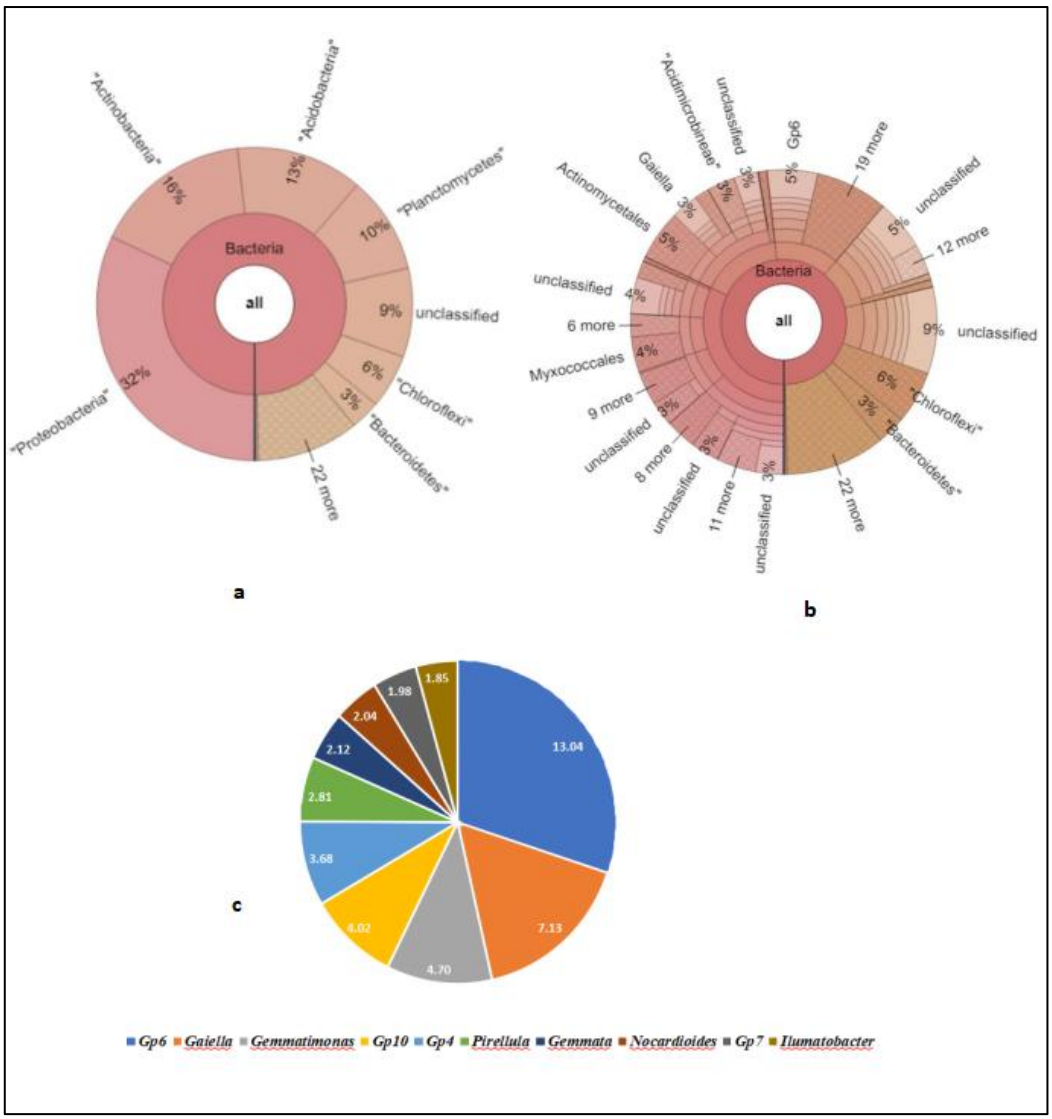


Fig. 4.37. Bacterial abundance in SV (Field soil treated with vermicompost + soybean plants) condition condition in soil sample-C. a. Krona chart representation of Phyla level abundance of prevalent bacterial assemblage, b. Krona chart representation of Genera level abundance of prevalent bacterial assemblage, c. Pie chart representing the top 10 scoring genera.

The Fig 4.37. reveals that:

The Krona map revealed that Actinobacteria appeared to be the most abundant phylum, followed by Acidobacteria, Planctomycetes, Chloroflexi, Bacteroidetes, and

Proteobacteria in the soil sample SU. It included the following top 4 genera: Gp6 (5%), *Gaiella* (3%), *Gemmatimonas* (2%), and Gp10 (2%)

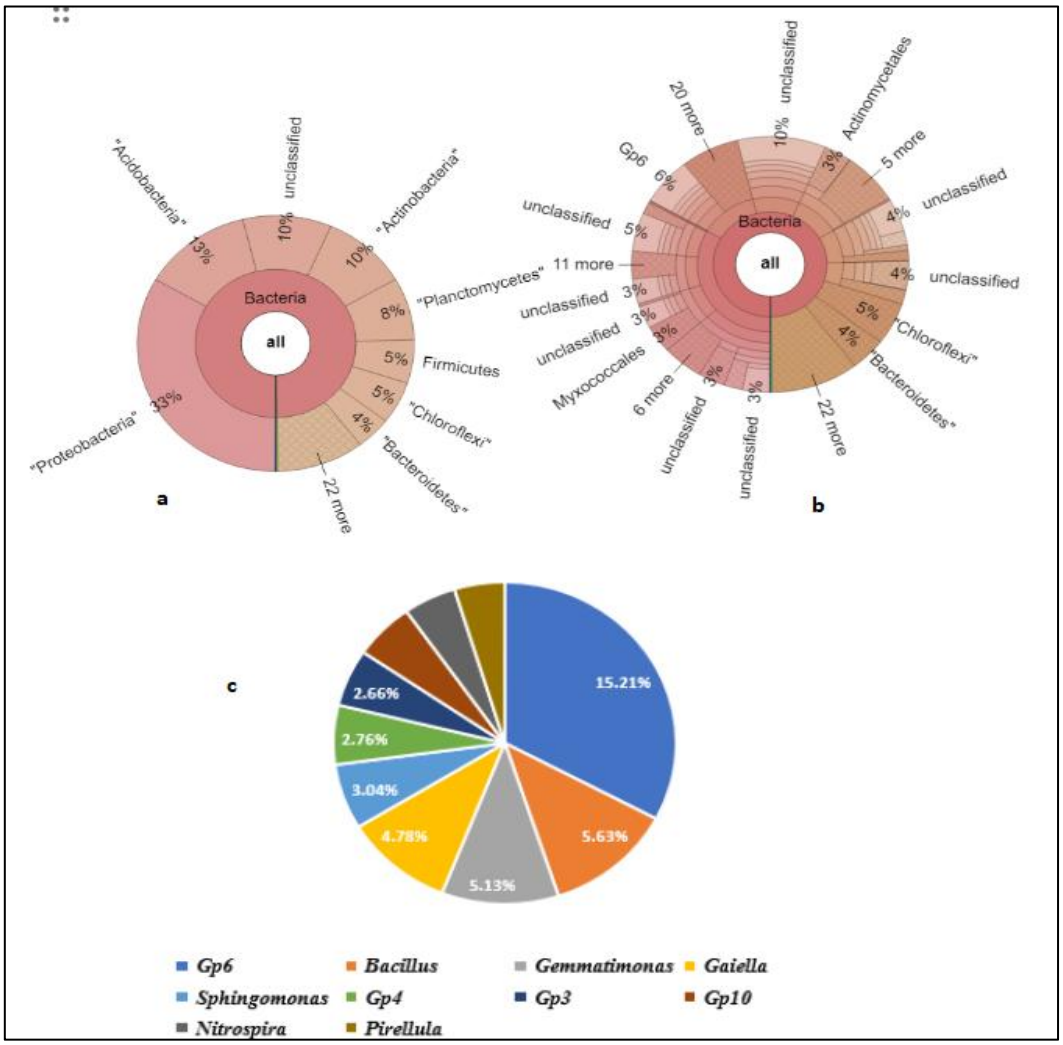


Fig. 4.38. Bacterial abundance in SBC (Field soil treated bacterial consortium+soybean plant) condition in soil sample-A. a. Krona chart representation of Phyla level abundance of prevalent bacterial assemblage, b. Krona chart representation of Genera level abundance of prevalent bacterial assemblage, c. Pie chart representing the top 10 scoring genera.

The Fig 4.38. reveals that:

Proteobacteria was observed to be the most abundant phylum, followed by Acidobacteria, Actinobacteria, Planctomycetes, Firmicutes, Chloroflexi, and Bacteroidetes in soil samlpe SV. It contained of the following top 4 genera: Gp6 (6%), *Bacillus* (3%), *Gemmatimonas*

(2%), and *Gaiella* (2%). Upon further screening of the putative top 10 genera recorded to be the most abundant in the given sample, *Gp6* and *Bacillus* have relative abundances of 15.21% and 5.63%.

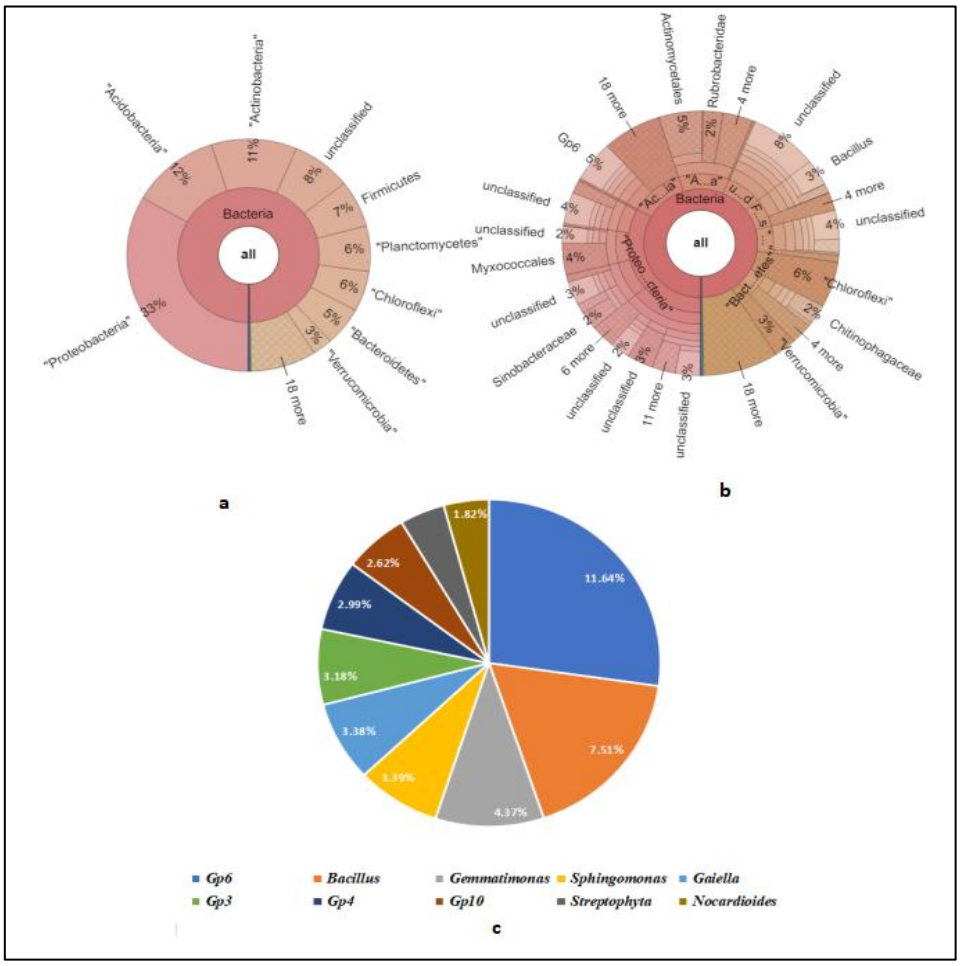


Fig. 4.39. Bacterial abundance in SVBC (Field soil treated with vermicompost +bacterial consortia+soybean plants) condition in soil sample-C. . a. Krona chart representation of Phyla level abundance of prevalent bacterial assemblage, b. Krona chart representation of Genera level abundance of prevalent bacterial assemblage, c. Pie chart representing the top 10 scoring genera.

The Fig 4.39. reveals that:

The Krona map revealed that Proteobacteria was the most abundant phylum, followed by Acidobacteria, Actinobacteria, Firmicutes, Planctomycetes Chloroflexi, Bacteroidetes, and Verrucomicrobia. The soil sample SBC contained the following top 4 genera: Gp6 (6%), *Bacillus* (3%), *Gemmatimonas* (2%), and *Sphingomonas* (2%). Upon further

screening of the putative top 10 genera that were most abundant in the given sample, Gp6 and *Bacillus* have relative abundances of 11.64% and 7.51%

Analysis of datasets of untreated and treated datasets as presented in the Krona charts (Fig 4.36. to 4.40.) indicate that:

- Actinobacteria was found to be the most prevalent phylum in the first two datasets i.e., untreated field soil (S) and (SU). However, a pronounced shift of phyla was observed in all the remaining experimental datasets toward Proteobacteria.
- Among the most abundant putative top 10 genera in the S and SU condition, *Arthrobacter* and *Streptomyces* have high relative abundances of 15.58% and 10.79%, respectively.
- Among the most abundant putative top 10 genera in the Consortium treated sample (SBC), (SVBC): The relative abundance of *Bacillus* sp. was high: 5.63% (SBC) and 7.51% (SBVC)
- The bacterial genera unique to the untreated field soil sample (S) were *Neptuniibacter, Lysinimonas, Alcanivorax, Campylobacter, Neisseria, Methylococcu*s, and *Oceanobacillus.*
- The set of bacteria that were found to be unique to the soil sample SU, includes *Anaerotruncus, Dialister, Rhodoferax, Parvimonas, Negativicoccus, Hoeflea,* and *Ruegeria.*
- The soil sample in SV condition, revealed a unique set of bacterial genera which include *Rhodoplanes, Neochlamydia, Byssovorax, Thermogutta, Verrucomicrobium, Luedemannella,* and *Tahibacter*.
- When exposed to the treatment with the defined bacterial consortium, the field soil sample in SBC condition exhibited a unique bacterial profile consisting of *Thauera, Ignavibacterium, Thermoactinomyces, Solitalea, Syntrophobacter, Fluviicola,* and *Solimonas.*
- Under the concerted application of vermicompost and the bacterial consortium (SVBC), a unique bacterial profile was isolated from the experimental soil which included *Rhodanobacter, GpV, Clostridium, Okibacterium, Dokdonella, Phycicoccus,* and *Pedobacter.*

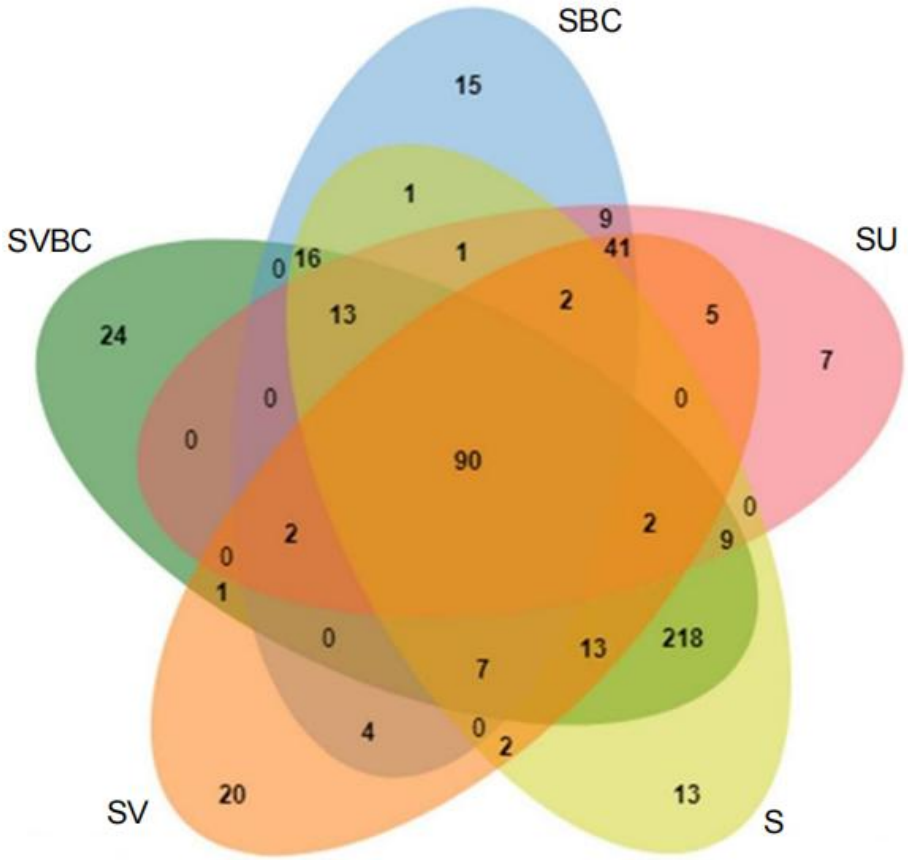


Fig. 4.40. Comparative Venn diagram depicting the common and unique bacterial members among the five samples under study in soil sample-A.

The results obtained in Fig 4.40. indicate that:

- The Venn diagram shows common and unique members at OTU level in the bacterial community of untreated and treated setup.
- All five datasets shared 90 OTU of bacterial genera. The enriched soil of SBVC shows the highest number of unique bacterial members (24 OTU) followed by SV (20 OTU), SBC (15), S(13 OTU), and SU (7 OTU) respectively. A higher abundance of unique members in the SVBC condition indicates the positive impact of combined vermicompost and consortium treatment on soil microbial health.

- SVBC set up shared maximum OTU (218) with untreated field soil (S), indicating an improvement in overall soil microbial health thus promoting more and more associative beneficial bacterial assemblages.

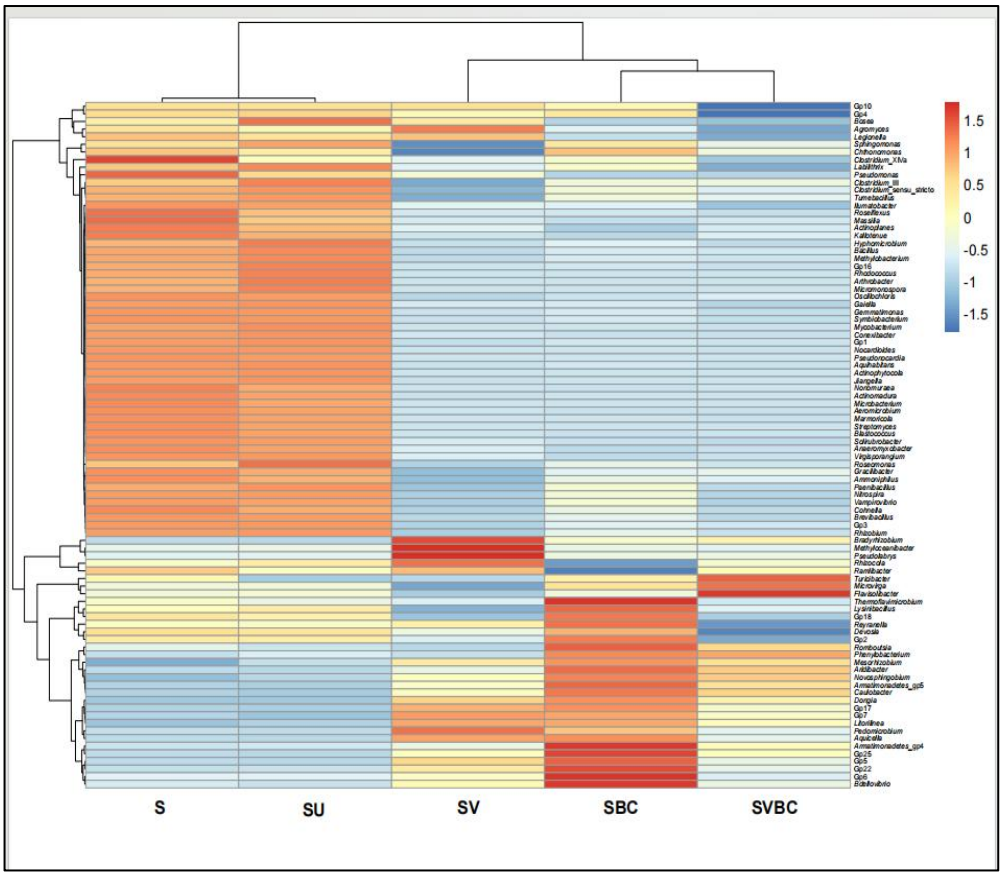


Fig. 4.41. Heatmap depicting the abundance of important growth-promoting bacterial members in the five samples under study in soil sample-A.

The heat map presentation in Fig. 4.41. indicates that:

- The bacterial assemblages found to be common between the consortium-treated datasets were *Rhizobium, Bradyrhizobium, Mesorhizobium, Microbacterium, Paenibacillus, Bacillus, and Pseudomonas.*
- *The functional genera in the SBVC soil sample show the highest level of enrichment in functional genera.*
- The functional genera show an intermediate level of abundance in the case of the soil samples SBC and SV set up.

- The untreated field soil (S) and soil sample SU exhibited the lowest level of abundance in the functional genera as presented in the Heat map.

4.11.3.2. Soil Sample C: Comparative Metagenomic analysis of soil bacterial community structure in amended and non-amended soil

Next generation metagenomic sequencing and bioinformatics analysis of the datasets of untreated field soil and the treated field soil samples, under four different experimental conditions, were carried out. The results furnished below.

Dataset	Sample Code	Experimental Condition	Analysis Code	NCBI SRA Project: PRJNA689214
				SRA Accession Numbe
1	S	Field Soil	SAM3	SRX 9815210
2	SV	Field Soil+Vermicompost+ Soybean Plant	DHB3	SRX 2193273
3	SBC	Field Soil + Bacterial Consortium+Soybean Plant	DHB1	SRX 2193274
4	SVBC	Consortium+Soybean Plant	DHB2	SRX 2193275

***Direct URL to data:** https://www.ncbi.nlm.nih.gov/bioproject/PRJNA689214

Table 4.23 Analysed Datasets of uninoculated and inoculated soil sample-C

DATASET	Sample Code	Alpha diversity	Shanon Diversity index
1	S	185	3.41
2	SV	304	5.17
3	SBC	208	4.67
4	SVBC	200	4.78

Table 4.24. Alpha and Shannon Diversity Indices of the analysed Datasets of soil sample-C

The findings in Table 4.24. indicate:

- Datasets 1, 2, 3, and 4 show alpha diversities of 185, 304, 208, and 200 respectively and Shannon diversity indices of 3.41, 5.17, 4.67, and 4.78 respectively.

- The diversity profiles indicate that the 4th dataset (SVBC) has a relatively lower alpha (208) diversity compared to the 2nd (SV) and 3rd (SBC) datasets. The Shannon diversity index of SV (5.17) was highest followed by SVBC (4.78) and SBC (4.67), thereby establishing a higher richness and uniformity in distribution of the total number of genera in the treated samples, whereas untreated field soil (S) showed lowest value (3.41).

Bacterial abundance in five different datasets of untreated and treated Soil sample-C

Abundance in five different datasets (S,SU, SBC and SVBC), at phyla and genera level are presented below:

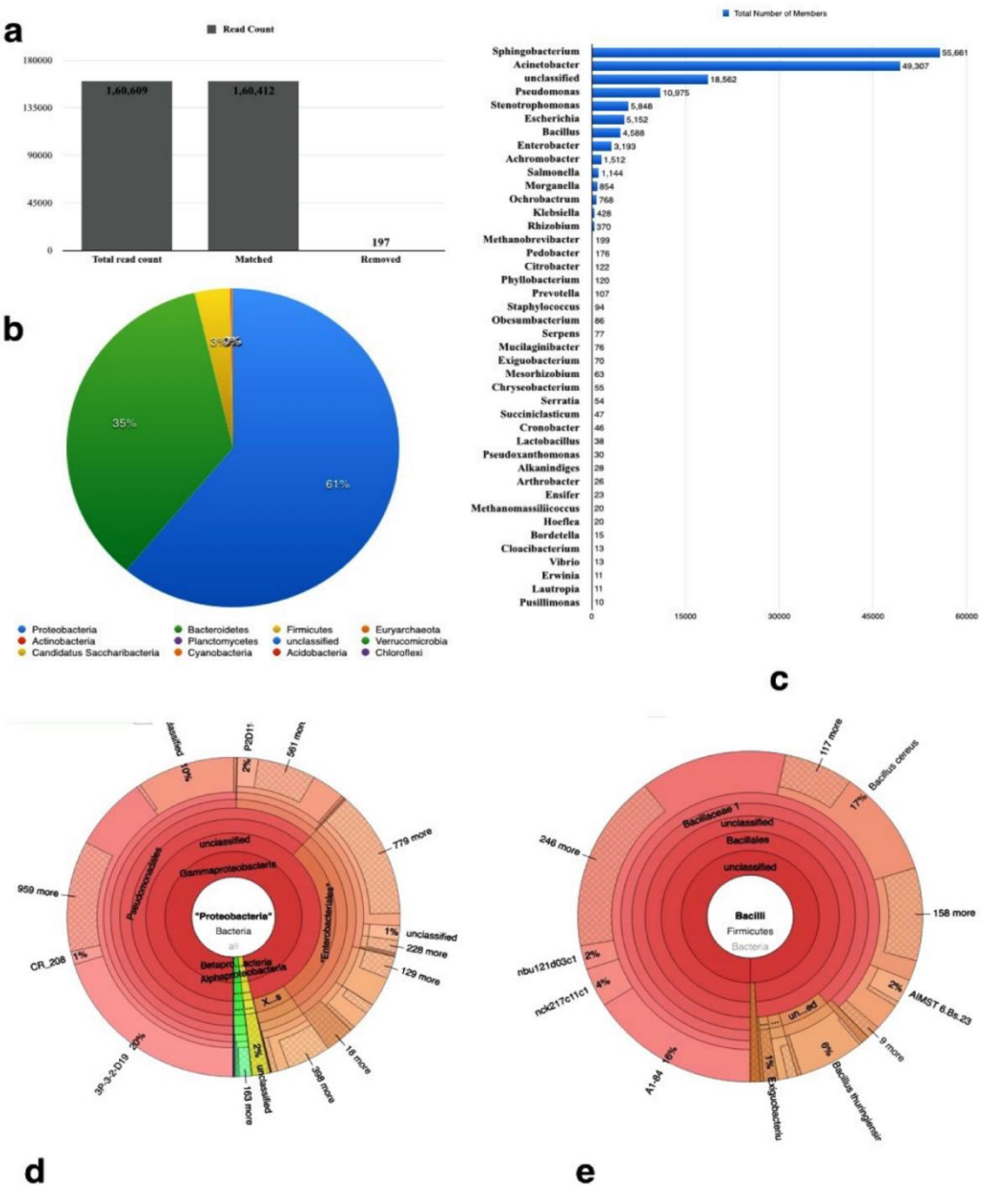


Fig 4.42 Untreated field soil) (S). of soil sample-C. Data Profile obtained through sequencing. a) Read Counts as obtained after initial quality check where 197 reads were discarded; b) Phylum abundances c) Genus level abundances exhibit the predominance of. Crona chart to depict the distribution of Proteobacterial members; (e) Phylogenetic representation using Krona chart to depict the distribution of the *Bacillus* clade at the genus level (Source: Mukhopadhyay *et al*. 2020).

The Fig 4.42. indicates

- Proteobacteria was the most dominant phylum (68%), followed by Bacteroidota and Firmicutes.

- *Sphingobacterium (65%)*, *Acinetobacter* (18%), *Pseudomonas* (10%), Bacillus (7%), and *Flavobacterium* (1%), were predominant at genus level.

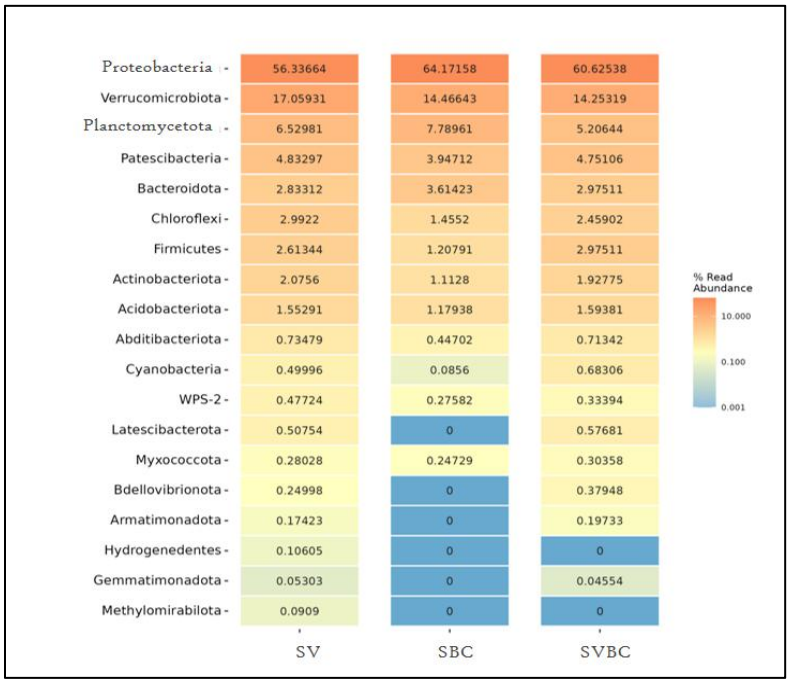


Fig 4.43. Heat map showing dominant bacterial phyla in the treated soil samples in SV, SBC, and SVBC conditions in soil sample-C.

Fig 4.43. indicates that:

- Proteobacterial abundance at Phylum level were detected in the soil at SV, SBC, and SVBC conditions, followed by Verrucomicrobiota, Planctomycetota, Patescibacteria, Bacteroidota, Chloroflexi, Firmicutes and Actinobacteria.
- The observed Proteobacterial abundance were 56.33%, 64.17%, and 60.62%, respectively in SV, SBC, and SVBC setup, showing a maximum decline in Proteobacteial abundance after vermicompost treatment. Proteobacterial abundance also showed a trend of decline following introduction of bacterial consortium in soil, both in the presence and absence of vermicompost.

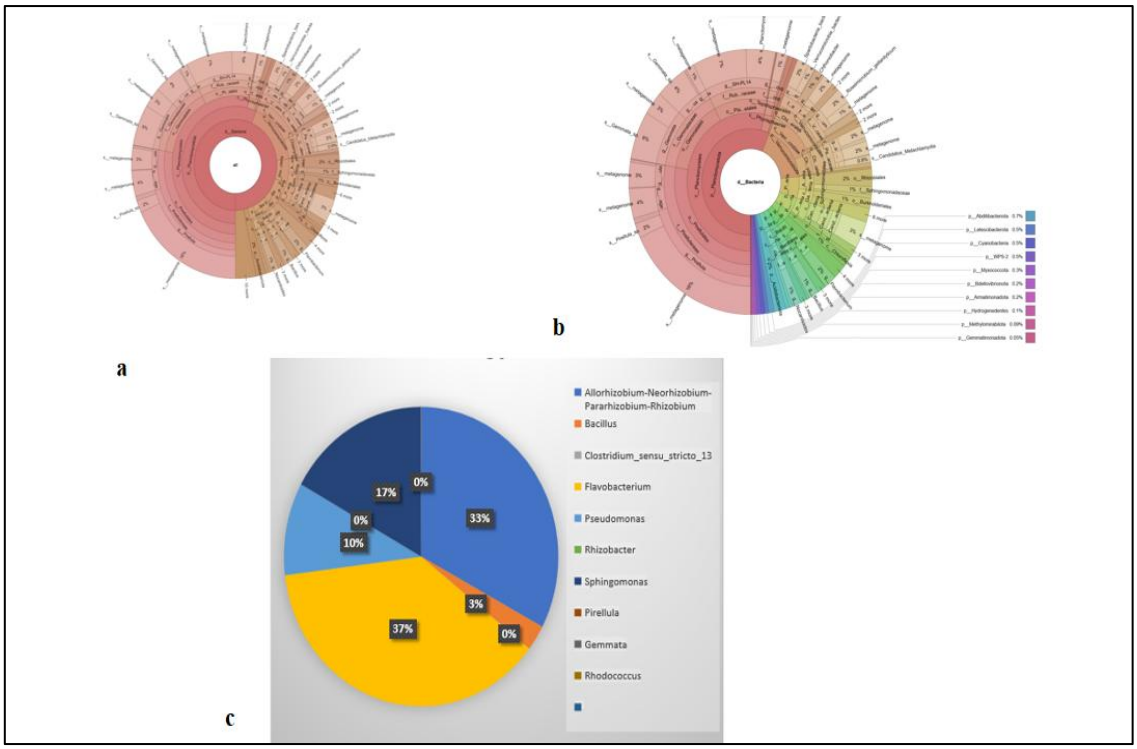


Fig. 4.44. Bacterial abundance in SV (Field soil treated with vermicompost+bacterial concortia+soybean plants) condition in soil sample-C. a. Krona chart representation of Phyla level abundance of prevalent bacterial assemblage, b. Krona chart representation of Genera level abundance of prevalent bacterial assemblage, c. Pie chart representing the top 10 scoring genera.

Fig 4.44. reveals that:

- Proteobacteria (56.33%) was observed to be the most abundant phylum, followed by Verrucomicrobiota, Planctomycetota, Patescibacteria, Bacteroidota, Chloroflexi, Firmicutes and Actinobacteria in soil sample SV. It contained of the following top putive genera: *Allorhizobium-Mesorrhizobium-Rhizobium* (33%), *Flavobacterium* (37%),*Sphingomonas* (17%), *Bacillus* (3%) and *Pueudomonas* (10%).

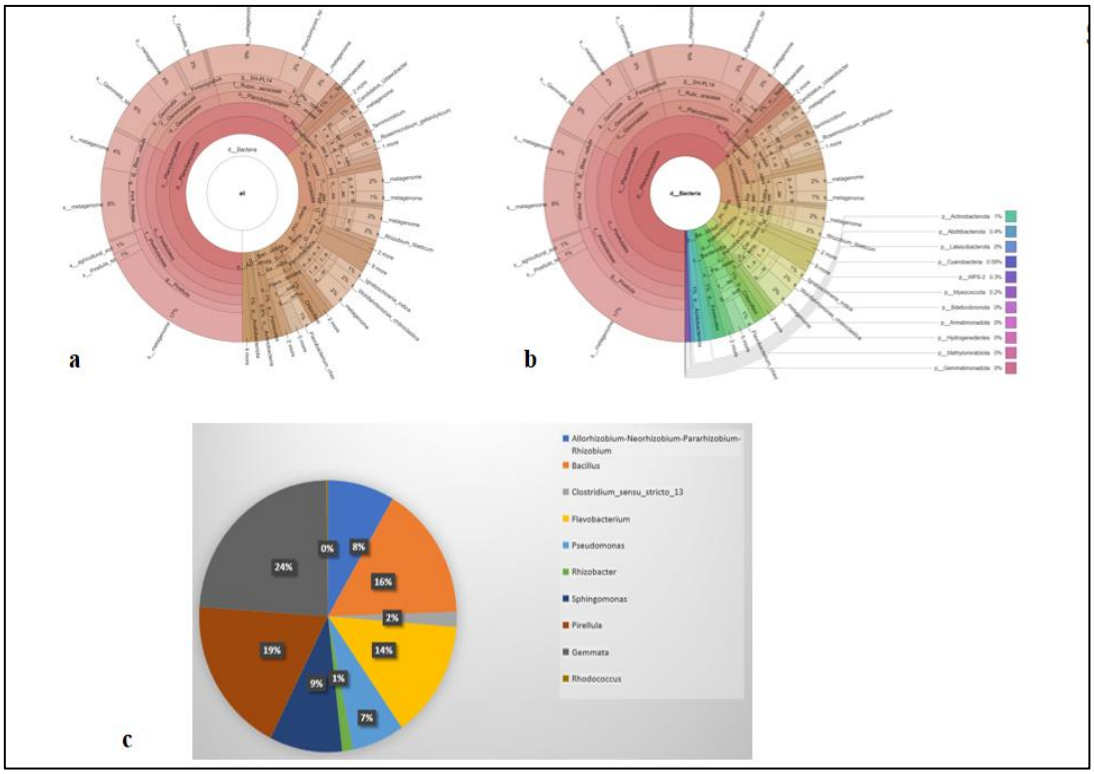


Fig. 4.45. Bacterial abundance in SBC (Field soil treated with vermicompost+bacterial consortia+soybean plants) condition. a. Krona chart representation of Phyla level abundance of prevalent bacterial assemblage, b. Krona chart representation of Genera level abundance of prevalent bacterial assemblage, c. Pie chart representing the top 10 scoring genera.

Fig 4.45. Indicates that:

- Proteobacteria was observed to be the most abundant phylum, followed by Verrucomicrobiota, Planctomycetota, Bacteroidota, Chloroflexi, Firmicutes, and Actinobacteria in soil sample SBC. It contained of the following top genera: *Gemmata* (24%), *Flavobacterium* (14%), *Sphingomonas* (9%), *Bacillus* (16%) *Allorhizobium-Mesorrhizobium-Rhizobium* (8%), and *Pseudomonas* (7%).

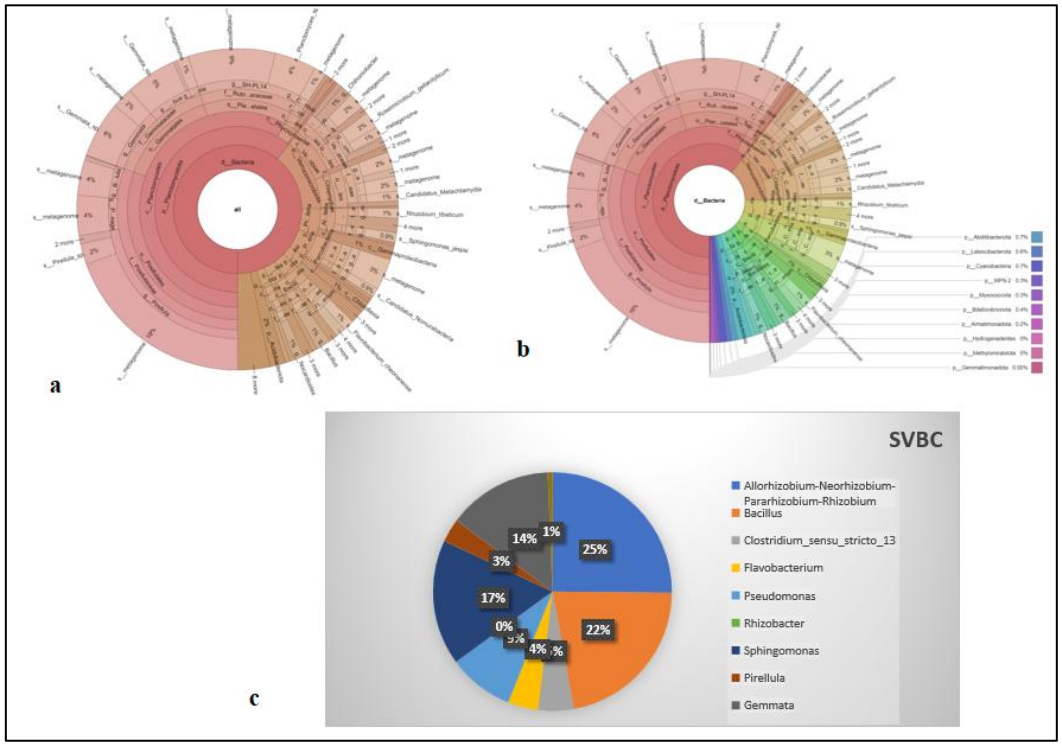


Fig. 4.46. Bacterial abundance in SVBC (Field soil treated with vermicompost+bacterial consortia+soybean plants) condition in soil sample-C. a. Krona chart representation of Phyla level abundance of prevalent bacterial assemblage, b. Krona chart representation of Genera level abundance of prevalent bacterial assemblage, c. Pie chart representing the top 10 scoring genera.

Fig 4.46. Indicates that:

- Proteobacteria was observed to be the most abundant phylum, followed by Verrucomicrobiota, Planctomycetota, Bacteroidota, Chloroflexi, Firmicutes, and Actinobacteria in soil sample SVBC. It contained of the following top putitive genera: *Allorhizobium-Mesorrhizobium-Rhizobium* (24%), *Bacillus* (22%), *Sphingomonas* (17%), , *Gemmata* (14%), *Pueudomonas* (9%) and *Flavobacterium* (4%).

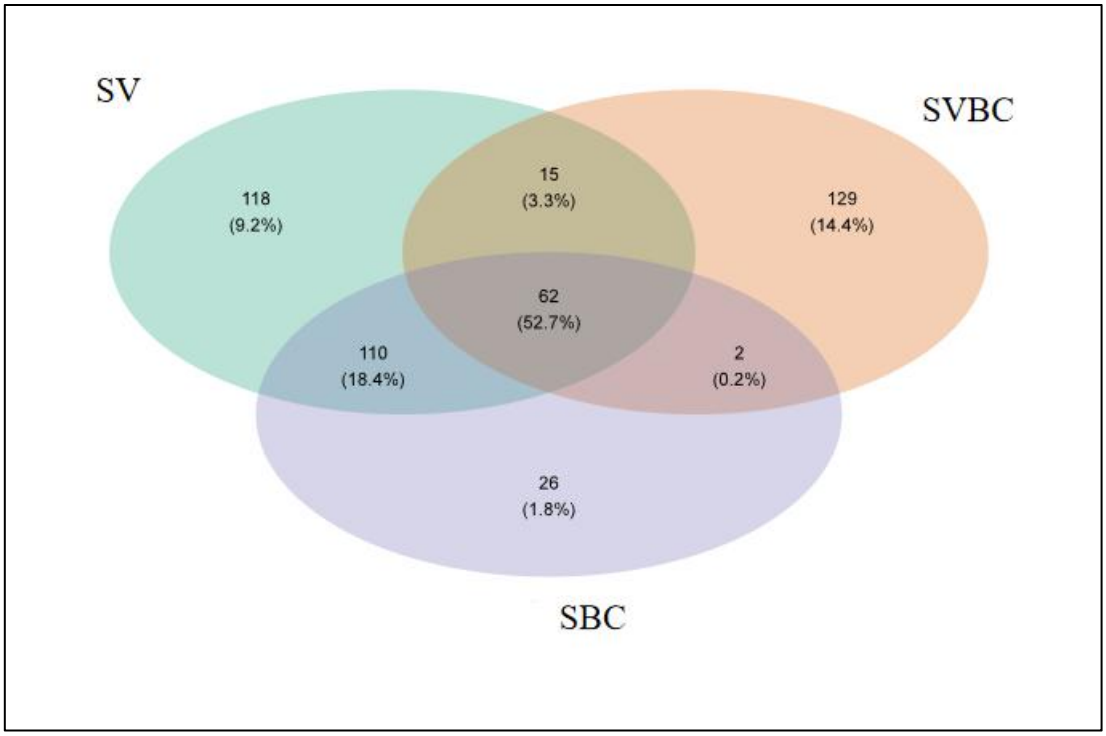


Fig. 4.47. Comparative Venn diagram depicting the common and unique bacterial members among the five samples under study in soil sample-C.

The results obtained in Fig 4.47. indicate that:

- The Venn diagram shows common and unique members at OTU level in the bacterial community of untreated and treated setup. All three datasets shared 52 OTU of bacterial genera. The enriched soil of SBVC shows the highest number of unique bacterial members (129 OTU) followed by SV (118 OTU), SBC (26 OTU) respectively. A higher abundance of unique members in the SV and SVBC conditions indicate the positive impact of vermicompost, as well as, combined vermicompost and consortium treatment on soil microbial health. Decrease in the unique members in SBC condition indicates that the introduced inoculant suppressed some genera.

CHAPTER-IV

Discussions

Soil is the natural medium for the sustenance of plant life in our planet. It is the reservoir of essential plant nutrients, although their availability may be restricted due to some factors like natural deficiencies, immobilization in solid phase, or due to soil degradation related to inappropriate agricultural management practices. A fertile soil is able to supply essential plant nutrients and favourable habitat with a suitable chemical, physical and biological environment, for sustaining plant growth (FAO, 2019). The resultant effect of several factors influencing crop yield, is manifested as soil productivity. In healthy soil, a diverse community of soil-inhabiting organisms helps to protect plants from several biotic stresses such as pathogenic fungi, bacteria, insects, and pests, as profitable symbionts in plant roots; recycling the nutrients essential for plants; improving soil structure, and thereby, improve crop production (FAO, 2008). Globally, the productivity of soil has become a major concern due to the degradation of the physical, chemical, and biological health of soil. According to Huang *et al.* (2019), over-exploitation of agricultural soil and persistent application of agro-chemicals has been adversely impacting soil structural and functional properties resulting in depletion of nutrients, soil microbiological diversity, fertility, and crop productivity. About 33 percent of the soil of our planet is degraded (FAO, 2022). The contributing factors for nutrient-depletion are both natural and anthropogenic interventions such as excessive use of chemical fertilizers, pesticides, inadequate return of organic matter to cultivated land, monoculture, soil erosion, and deforestation. In intensive cropping practice, a huge quantity of essential nutrients are removed from soil during continued crop production round the year.When the amount of soil nutrients taken up by a crop is not recycled back and/or replenished to the depleted nutrient pool of the field (Sanyal 2014), 'Nutrient mining occurs, causing progressive loss of fertility of the arable lands. The currently adopted nutrient management strategies by majority of the farmers is aggravating the problem due to insufficient or imbalanced nutrient applications (Majumder *et al.* 2016). It is a major challenge in the intensively cultivated areas of many countries like India, where arable lands are under tremendous pressure to meet up the expanding demand for food. According to FAO (2015) document, imbalanced fertilizer application is a common cause for soil fertility depletion in intensive farming system. During the period of 1961–2013, a rise in Nitrogen/Phosphate fertilizer ratio by 0.8 g Nitrogen/g Phosphate per decade was recorded, which may have a profound impact on global agro-ecosystem functions in near future (Lu and Tian, 2017). Worldwide, especially in the developing countries, the low productive soil is producing nutrient-deficient crops, seriously jeopardizing food security of a country in near future.

Literature mining indicates that the damaged agro-ecosystem of such soil can be reclaimed by restoration of microbial diversity which helps to replenish many plant-favourable services at community-level, and consequently, improve plant health (Delgado-Baquerizo 2016). In this perspective, for the current investigation, from five low productive abandoned agricultural fields, two different types of over-exploited lands were chosen, alluvial soil of Bahadurpur of South 24 Parganas (soil sample-A) and lateritic soil of Sadaipur, Birbhum (soil sample-C). The soil sample-A was observed to contain low level available N (48.7 mg kg^{-1}), available P (27.25 mg kg^{-1}) and SOC (0.34%). Soil sample-C was found to be deficient in available phosphorus (6.09 mg kg^{-1}) content along with relatively low level of SOC (57 %), and poor water holding capacity (Table 4.1). Being red lateritic in nature, Soil sample-C was observed to be highly rich in iron content (95780.11 mg kg^{-1}). From these two different types of long-term used arable soils (soil sample-A and soil sample-C), five promising bacteria strains with multifarious PGP activity, were isolated. The novel PGP bacterial isolates are: Isolate S3. *Bacillus zhanzhouensis* MMAM (NCBI Acc: MT185655), Isolate S5. *Bacillus cereus* strain MMAM3 (NCBI Acc: MT30003), Isolate S7. *Bacillus subtilis* strain MMAM4 (NCBI Acc: MT30004.1), Isolate R1. *Bacillus subtilis* strain MMAM2 (NCBI Acc: MT725461.1), and Isolate L3. *Pseudomonas* sp. strain MMAM5 (NCBI Acc: ON237480) [Table 4.8]. They possess mineral nutrient (NPK) harnessing ability and were able to produce IAA, GA, ACC deaminase, siderophore, and biofilm, several lytic enzymes, pectinase, beta 1,3 glucanase peroxidase, HCN, and NH_3 in i*n vitro* condition (Mukhopadhyay et al. 2022). In the present work, four potent resident PGPB isolates, *Bacillus subtilis* strain MMAM2, *Bacillus zhanzhouensis* strain MMAM, *Bacillus cereu*s strain MMAM3 and *Pseudomonas* sp. strain MMAM5 were used in designing two different multi-strain bio-inoculants (consortium combination-I and consortium combination-II) for plant-growth promotion study in the same soil samples (soil sample-A and soil sample-C), as mentioned in section 4.9. The effects of the inoculants on growth promotion of soybean plants in vermicompost-treated and vermicompost-untreated pot soil were evaluated for both soil sample-A (Fig 4.27. to Fig 4.29) and soil sample-C (Fig 4.30. to Fig 4.32). Furthermore, soil nutrients such as, NPK and SOC levels, spanning around different stages of plant growth at 4 different experimental conditions, were also studied for soil sample-A (Fig 4.33) and soil sample-C (Fig 4.34.). Finally, the efficacy of the inoculant on the community structure of resident soil microbiome, was analysed in order to find out any shift in the microbial flora in pot soil, following application of bacterial inoculum (Section 4.11.3).

In the present study, over all improvement in soybean plant growth and performance was observed in all the experimental set-up (SV, SBC, and SVBC) in varying degrees, following soil amendment. A strong positive influence of combined vermicompost-consortium amendment, was detected regarding of most of the observed plant parameters under pot trial condition, in soil sample-A. The highest improvement regarding most of the observed plant parameters, was recorded in SVBC followed by SBC condition (Fig 4.27. to Fig 4.29). Total no. of leaves plant^{-1}, leaf size, chlorophyll content of the leaves, plant height, of potted soybean plants showed a significant increase under SV, SBC, and, SVBC condition, at 12 WAE stage over to that of SU set-up. Total no.of pods plant^{-1}, dry weight of seeds, crude protein and fat content of seeds improved remarkably in inoculum treated condition (SBC and SBVC). In soil sample-C, the experimental set-up SV, SBC, and SVBC exhibited varying effects on leaf density, leaf area, plant height and nodule numbers at 8, and 12 WAE stages of the plants over that of the SU, better performance exhibited by consortium treated set-ups (Fig 4.31.a). But no significant increase in chlorophyll content of leaves was detected (Fig 4.31.b)The maximum improvement with respect to yield related traits like, total no.of pods plant^{-1}, dry weight of seeds and crude protein content was recorded in SVBC followed by SBC and SV condition (Fig 4.32). These observations indicate a strong positive influence of combined vermicompost-consortium amendment on most of the observed parameters under pot trial condition. Our findings corroborate with the observation of previous researchers that several PGPB positively influence plant growth and performance. They benefit plants mainly through three different ways: (i) As biofertilizer: Nutrient acquisition and mobilization (e.g. N-fixation, P-solubilization, K-mobilization), (ii) As Phytostimulator (phytohormone production), directly promoting the plant growth, (iii) As biocontrol agents: protecting plants against phytopathogens (Hardoim *et al.* 2015). It is now well established that improvement in crop yield can be achieved through indigenous or inoculated PGPB via enhanced nutrient availability or phytohormones production (Backer *et al.* 2018; Bechtaoui *et al.* 2020). The *de novo* biosynthesis of plant growth enhancers (such as cytokinins and IAA) synergistically reinforces the phytohormone signaling cascades thereby, augmenting host tolerance to various biotic and abiotic stresses from the environment that they are constantly subjected to (Naveed *et al*. 2015). According to Zhang *et al.* (2015) different plant beneficial activities like IAA, siderophore, ammonia production, and P-solubilization potential are significantly high in biofilm forming PGPR which show strong antimicrobial activity, and suppress many plant pathogenic microbes

(Pandin *et al.,* 2017). *Bacillus subtilis* Rhizo SF48, producing considerable amounts of ACC deaminase, enhanced growth and prevented drought stress-induced damage in the inoculates plants (Gowtham, H.G., 2020). Many soil bacteria are able to produce a plethora of hydrolytic enzymes, which are directly associated with the mineralization of organic materials thus, facilitating the nutrient mineralization and carbon cycling process (Sinsabaugh, 2008). Positive impacts on plant growth due to *Bacillus*-induced enhanced nutrient acquisition and hormonal modulations following treatment with *Bacillus*-based formulations have been observed in recent studies (Tsotetsi, 2022). Furthermore, Hu *et al.* (2021) reported that application of multi-strain microbial consortia inoculants (*Pseudomonas* spp.) is capable of enhancing plant growth more effectively compared to that of single-strain inoculants. This report supports our findings where, better growth of the consortium treated soybean plants in soil sample-C, was observed . Co-inoculation of *Glycine max* L. plants with *Bradyrhizobium japonicum* and *Azospirillum brasilense* inoculants showed outstanding results for improving grain yield and nodulation over that of the non-inoculated control (Hungria *et al.* 2013). A recent study reported that a multi-strain bacterial inoculant of *Pseudomonas chlororaphis* H1 and *Bacillus altitudinis* Y1 remarkably enhanced soybean plant growth, yield performance, enrich the beneficial bacterial composition around root and rhizospheric region with a positive effect on soil improvement (Zhang *et al.* 2023). Thus, our findings are in line with the previous studies in this arena.

For ascertaining the effect of soil amendment on soil nutrient status, the level of available NPK and SOC content were checked at 8, 12, and 16 WAE, during the pot trial experiment. In soil sample-A, a steady increase in SOC in all the experimental set-up, across the observed period was recorded. Available N level steadily increased in all the experimental set-up, across the observed period, 19%, 37%, and 68.6% in SV, SBC and SVBC condition respectively, compared to the untreated one. Available soil P content increased about 6.5%,60.6%, 90.75% in SV, SBC, SVBC set-up at 16 WAE indicating a steady mobilization and acquisition of P. Regarding available K, a sharp fall in SV (22.49%), gradual decrease in SBC, SVBC, 7.21%, 5.35% decrease over to that of untreated condition indicating a rapid acquisition in SV condition due to consortia facilitated utilization of soil potassium by the plants but poor mobilization in all the treatments. The potential utilization of inoculants can exert several beneficial effects such as, mobilization and transfer of nutrient to plants, upgradation of soil structural composition and water dynamics, and resistance towards soil-borne phytopathogens (WHO, 2022). Additionally, PGP inoculants

help to mitigate the consequent challenges of soil degradation through biological N fixation (BNF) and facilitating bio-availability of some essential mineral nutrients (WHO, 2022. According to FAO (2020), utilization of native consortia of resident soil microbes as biofertilizer, biocontrol agent and bio-stimulant, is a promising approach in farming practice. These methods can be effectively utilized for upgradation of soil fertility and soil nutrient level applying microbial inoculants as biofertilizers. It is established that BNF contribute about 60% of the N fixed in the soil (FAO, 2022). Application of PSB increases the availability of immobilized soil phosphate found an improved crop yield in P-deficient soil, following the application of AMF, P-solubilizers, and N fixers as biofertlizer Shi *et al*. (2022.a). Shi *et al*. (2022.b) showed that introduction of microbial inoculants in soil can increase SOM and available nutrient level. The similar trends were detected in our study. The growth promotion of inoculated soybean plants may be correlated to the improvement in soil nutrient status which is in consistent with the previous findings of Shi *et al*. (2022.b).

In the recent years, the implications of introduced bioinoculants on soil microbial community composition are extensively investigated. Xing *et al*. (2022) explored the effect of co-inoculation with three beneficial bacteria (*Bradyrhizobium japonicum* 5038 (R5038), *Bacillus aryabhattai* MB35-5 (BA) and *Paenibacillus mucilaginosus* 3016 PM), individually and as a combination, on soybean rhizosphere bacterial community composition and soil properties. Their findings confirms that several PGPB with multifaceted functions could effectively be used together as a composite bacterial inoculum, which coordinately shift the rhizospheric bacterial community composition and enhance plant performance. In our study, the analysis of metagenomic data sets of treated and untreated soil of two different types (soil sample-A, soil sample-C), indicated a modulation of soil bacterial community composition following soil augmentation. According to Willis (2019), analysis of the alpha diversity in amplicon sequencing data appears to be a common first approach to measuring variations between environments in terms of microbial ecology to summarize an ecological community structure according to its richness (number of taxonomic groups), evenness (distribution of abundances of the groups) or both. The set of metagenomic analyses of soil sample-A, conducted during this study, demonstrated that in the inoculum-treated soil samples α-diversity was high over to that of the untreated field soil as observed in SU (228), SBC (377) and SVBC (328), respectively. The diversity profiles of soil sample-A reveals that although the 5th dataset (SVBC) i.e., field soil treated with both vermicompost and bacterial consortia in the presence of soybean plant, had a higher index of Shannon diversity, thereby,

establishing a higher richness and uniformity in distribution of the total number of genera in the given sample. The findings of our investigation are in line with the observations of Shi et al. (2022b), indicating an improved α-diversity level after bacterial inoculation, compared to the control condition in the same time period.

The established importance of soil microorganisms in sustainable agriculture, has triggered a great commercial interest across the world toward the development of microbiome-based solutions for protecting crops and supplying nutrients to them (Sessitsch *et al.* 2018). A striking constrain is the ability of the inoculant strain(s) to overcome the tough competition they face in the soil and plant root-microbiome and to interact synergistically with other microbes (Tohu *et al*. 2018). Thakur *et al.* (2019) observed that on-farm utilization of the locally adapted and biologically diverse resident microbial members – in contrast to introduced microbes - may also be adopted as an effective method to increase biotic resistance against the invading foreign phytopathogens. Utilization of beneficial soil microorganisms for quality enhancement of soil, where native microbiological diversity has decreased due to various anthropogenic activities, is considered as one of the most effective nature-based solutions (NBS) for ensuring agricultural and environmental security; these microbes imitate the natural processes which are involved in ecosystem functioning Arnés-García and Santivañez (2021). In our investigation three native PGP bacterial isolates (*Bacillus* sp.), were introduced into soil as multi-stain bacterial inoculant in soil sample-A, intending to restore the diversity of agriculturally beneficial bacterial assemblages in the over-exploited soil. In soil sample-C, one PGP isolate (*Pseudomonas* sp.) along with 3 potent isolates having multifarious PGP traits (*Bacillus* sp.), were introduced in soil.

To elucidate the impact of the multi-strain bacterial inoculants on soil bacterial community composition, metagenomic analyses of the non-amended and amended soil samples were carried out for both the soil types (soil sample-A and soil sample-C). The present investigation revealed that Actinobacteria was the most prevalent phylum in the untreated field soil and in the soil, treated with vermicompost in presence of soybean plant In soil sample-A. However, a pronounced shift of bacterial phyla was observed in all the bacterial consortia treated experimental datasets, toward Proteobacteria indicating a significant impact of the inoculant on soil bacterial community. Usually, soil Proteobacteria appear to be the dominant phylum showing highest diversity at genetic and metabolic level (Shi et al. 2022a.). The shift in bacterial abundance towards Proteobacteria in our study, could be a

positive indication of soil health upgradation which might have exerted a multifaceted role, contributing to plant growth and development, promoting nutrient balance and increasing N-acquisition via N-fixation (Miliute and Buzaite 2015). About 80% of BNF are contributed by leguminous plant-microbe associations between *Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium,* and *Allorhizobium* and their abundances are dictated by ecological, edaphic, genetic and agronomic parameters (Sindhu et al. 2019). Literature sources confirm Actinobacteria as one of the common abundant phyla, suggesting their involvement in nutrient cycling, soil quality improvement, and crop yield enhancement along with maintenance of plant health thus, being a reliable contender as a biofertilizer alternative to conventional inorganic supplements in agricultural (Boubekri et al. 2022). In the current study, some of the PGPB bacterial assemblages, found to be common between the consortium-treated datasets were, *Rhizobium, Bradyrhizobium, Mesorhizobium, Microbacterium, Paenibacillus, Bacillus,* and *Pseudomonas.* Literature sources reveal that selected strains of *Pseudomonas* can substantiate the productivity of the soybean-wheat cropping system in regions of Central India with an enhanced content of clayey minerals in the soil, whereby they were found to boost soil enzyme activities, total system productivity and nutrient uptake in field trial (Sharma et al. 2011). Compatible strains of *Pseudomonas* were reported to contribute elevated solubilization of inorganic phosphate, production of IAA, ACC deaminase, and biofilm biosynthesis along with improved grain yield and soil quality parameters, synergistically, compared to independent inoculation with single strains (Kumawat et al. 2019). The bacteria belonging to the genera of *Bacillus* and *Paenibacillus* have been evidenced to be mobilizing host plant nutrition thereby supporting their growth, along with antagonizing pathogenic infestations of insect pests, bacteria, fungi, and nematodes by modulation of host defense cascades and triggering induced systemic resistance (ISR) thus making them suitable contenders for application in sustainable agricultural practices (Govindasamy et al. 2010). Furthermore, in our work, the analyses revealed that among the most abundant putative top10 genera in the Consortium treated sample (SBC), (SVBC): The relative abundance of *Bacillus* sp.was high: 5.63% (SBC) and 7.51% (SBVC) indicating a significant change in abundance of *Bacillus* sp. after bacterial inoculation in soil. In soil sample-C, the common bacterial assemblage *Allorhizobium-Mesorrhizobium-Rhizobium* (33%), *Flavobacterium* (37%), *Sphingomonas* (17%), *Bacillus* (3%) and *Pseudomonas* (10%), was predominant in amended condition.

The Venn diagram of soil sample-A (Fig 4.40) shows common and unique members at OTU level in the bacterial community of untreated and treated set up. All the five datasets shared 90 OTU of bacterial genera. The enriched soil of SBVC shows the highest number of unique bacterial members (24 OTU) followed by SV (20 OTU), SBC (15), S(13 OTU) and SU (7 OTU) respectively. Higher abundance of unique members in SVBC condition indicates the positive impact of combined vermicompost and consortium treatment on soil microbial health. SVBC set up shared maximum OTU (218) with untreated field soil (S), indicating an overall improvement in native soil bacterial genera thus, promoting more and more associative beneficial bacterial assemblages. In soil sample-C (Fig 4.47), a higher abundance of unique members in the SV and SVBC conditions indicate the positive impact of vermicompost, as well as, combined vermicompost and consortium treatment on soil microbial health. Decrease in the unique members in SBC condition indicates that the introduced inoculant suppressed some genera. After growing soybean plants, no significant change in bacterial composition was found in untreated field soil.

The results of the current study further predicted that, among the analysed soil datasets of both types of experimental soil, the combined treatment of vermicompost with the selected bacterial consortium exhibited a significantly higher magnitude of activation of the most prevalent functional pathways as compared to the remaining datasets. The functional pathways related to carbon fixation, oxidative phosphorylation, pentose phosphate pathway, and quorum sensing were also elevated. The selectively enriched pathways of terpenoid backbone synthesis in almost all the datasets can be correlated with existing literature sources which substantiate this functionality in *Salvia miltiorrhiza* seeds from seven different geographic origins whereby, it has shown to provide important precursors for terpenoid biosynthesis thus, indicating a significant level of secondary metabolism for enhancing biotic and abiotic stress resistance (Chen et al. 2018). A significant down-regulation of some selective metabolic pathways might have occurred in the untreated field soil, which might be suggestive of the adaptive trait of specialized and dynamic carbon utilization from sources like α-pinene, naphthalene secreted in the root exudates as a part of the unique microenvironment utilized by bacteria like *Pseudomonas, Burkholderia, Mycobacterium, Streptomyces, Sphingomonas, Pseudomonas, Ralstonia,* etc. in the rhizospheric bacterial consortium in soybean thus, leading to a decrease in common carbon metabolism pathways (Liu et al. 2019).

A report of Ma *et al*. (2018), revealed a significant over-representation of several bacterial classes and genera, which were observed to be involved in symbiotic N-fixation, plant health promotion, biol-control and soil catalase activity promotion, following bacterial inoculant treatment. Furthermore, a decrease in some taxa with negative impacts on soil quality, was noticed in the study of Ma et al. (2018). Application of the microbial consortium might have resulted in an elevated crosstalk among the microbial members of the niche in the treated soil samples. Along with an increase in crosstalk, elevated expression of metabolic pathways might be present, indicating a modulation of resident bacterial assemblage at the community level towards the improvement in soil biological health.

Finally, it can be concluded that, the application of the resident PGPB strains to the soil, in combination with vermicompost, might have enriched the plant-beneficial soil microbes already present in the microbiome resulting plant growth promotion in the soil of Bahadurpur (soil sample-A). The composite PGP bacterial inoculant probably improved the soil health facilitating nutrient mobilization and uptake by the soybean plants in the red lateritic soil (soil sample-C).Vermicompost might have an added advantage for plant growth promotion, as a soil prebiotic increasing the population of resident associative beneficial bacteria and also as a nutrient source for the bacterial strains already existing within the soil–plant system (Strachel et al., 2017, Vassileva et al., 2020).

The unique soil amendment strategy, based on utilization of residual soil BSM, can be adopted to enhance plant productivity in long-term cultivated soil and for agricultural sustainability.

CHAPTER-5

Summary of major findings

OBECTIVE-I

Collection and Characterization of Low Productive Soil

- Among the 5 collected soil samples, sample-A (Bahadurpur, South 24 Pgs) was observed to contain low levels of available N (48.7 mg kg^{-1}), available P (27.25 mg kg^{-1}), and SOC (0.34%) contents. Thus, appeared to be depleted in these three essential soil nutrients.

- Soil sample-C (Sadaipur, Birbhum) was found to be deficient in available phosphorus (6.09 mg kg^{-1}) content and had a relatively low level of SOC (57 %) contents. This sample being red lateritic in nature, was highly rich in iron content. The water holding capacity of this sample was relatively poor (31.4 %) compared to that of the other samples.

- With respect to the nutrient status, soil sample-A, and sample-C appeared to be nutrient depleted over-exploited (Table 4.1) soil and were chosen for the current investigation

OBJECTIVE-II

Isolation, Characterization, and Screening of Bacteria with Plant Growth Promoting Traits from the Selected Soil Samples

- Altogether 15 bacterial colonies showing prominent growth on NA media, were selected, 6 of the bacterial isolates S1, S3, S5, S7, R1, and R2 were from soil sample-A, and the only isolate L3 from soil sample-C were found to have N-fixing ability.

- Only these 7 N-fixing bacterial isolates were chosen for the screening of their phosphate and Potassium solubilization potential.

- Among the 7 isolated bacterial colonies, only 4 (S3, S5, S7, and R1) were able to form a distinct zone of clearance on the Pikovskaya agar plate. The isolates S3, S5, S7, and R1 were observed to have the (SI) index value of 1.4 ± 0.03 cm, 1.1 ± 0.05 cm, 0.8 ± 0.06 cm, 1.9 ± 0.07 cm, respectively and were considered as potent PSB candidate. The phosphate solubilizing ability of the bacterial isolates was measured

from 3rd days and 5th days old cultures. All the isolates solubilized a higher amount of phosphate on the 5th day. The bacterial isolate R1 has the highest phosphate solubilizing potential (62.5 µg/ml) followed by S7, S5, S3, and L3 in 5-day-old culture filtrate.

- The bacterial isolates S3, S5, S7, R1, and L3 were capable of changing the colour of the modified Aleksandrow media to yellow, due to the solubilization of potassium to organic acid, within 72 h of incubation. These 5 bacterial isolates were considered as KSB. In both 6th and 8th-day-old cultures, L3 solubilized the maximum amount of Potassium (4.71 µg/ml), closely followed by S3, R1. The isolate S7 was detected to have the lowest K-solubilizing potential.
- Thus, from soil sample-A, four of the bacterial isolates S3, S5, S7, and R1 were detected to have NPK acquisition potential whereas, S1 and R2 have only N-fixing ability. From soil sample-C, only the bacterial isolate L3 was a potent N-fixer, K-solubilizer, and P-solubilizer.
- These seven isolates (S1, S3, S5, S7, R1, R2, and L3) were selected for further study and maintained by repeated subculturing on NA plates.

OBJECTIVE -III

Utilization of the Selected Resident Bacterial Isolates to Design Novel Multi-strain Bioinoculant for Plant Growth Promotion and Soil Upgradation

- Compatibility study among these bacterial isolates detected no inhibitory interaction among the isolates S3, S5, R1, and L3. The bacterial isolate S7 showed a slightly inhibitory effect against S5 and therefore, these two isolates were not considered for using together in the formulation of multi-strain consortia.

- Three different consortia combinations were designed for testing their plant growth promotion potential. Consortia combination-1: S3+S7+R1, combination-2: S1+S7+R2; and combination-3: S3+S5+R1.

- Preliminary pot-trial experiment for selecting the most potent consortia using *Glycine max* L. as test plant, showed significant improvement in vegetative, and yield-related traits, in consortia-treated plants.
- Significant improvements in vegetative, and yield-related traits of the test were detected following inoculant application. The highest improvement with respect to all the observed parameters was recorded in T-3 (S3+S5+R1) set-up followed by T-1 (S3+S7+R1) and T-2 (S1+S7+R2) set-up indicating a strong positive influence of consortium amendment.
- A remarkable increase in leaf size in T-3 (81 cm^2) followed by T-1 (62 cm^2) and T2 (54 cm^2) compared to the untreated (42 cm^2) set-up, at 12-week stage compared to the untreated one. A striking improvement in leaf no. T-3 (36), T-1 (35) over control (22) condition.
- About 78% increase in root nodule no. $plant^{-1}$ was observed in T-3 set-up over to that of the untreated one. The total no. of pods $plant^{-1}$ increased from 22.8 in the untreated condition to 34 in the T-3 condition. About 50%, 35%, and 16% increase in dry weight of pod compared to that of untreated condition were also recorded in T-3, T-1, and T-2 condition.
- Consortium combination-3 (S3+S5+R1) appeared to be the best combination with respect to vegetative and reproductive parameters, followed by combination-1 (S3+S7+R1) and combination-2 (S1+S7+R2). Improvement in plant performance after application of these 2 inoculants in soil, indicated a strong positive influence of consortia on most of the observed plant parameters under pot trial conditions.
- Therefore, the isolates S3, S5, S7, and R1 (from soil sample-A) were chosen for further PGP characterization and utilization in the plant growth promotion study.
- Among the 5 selected isolates, isolate R1 was the highest producer of IAA, both in the presence (31.43 µg mL^{-1}) and absence (20.12 µg mL^{-1}) of tryptophan. In the absence of a precursor, L3 can produce a higher amount than that of S5 and S3. S7 Isolate R1 appeared to be the most potent IAA producer followed by L3, S5, and S3, and produced the least amount of IAA both in the presence and absence of tryptophan.
- Quantitative estimation of GA_3 production by the bacterial isolates indicated that S5 produced highest amount (66.4 µg mL^{-1}) of GA_3 at 7^{th} day of incubation,

followed by S3, R1, L3 and S7. At 5th day stage, the maximum quantity (49.7 µg mL^{-1}) of GA_3 was produced by the bacterial isolate S3 followed by S5, R1, L3 and S7. In both 5th and 7th day culture filtrate, the concentration of GA_3 were produced in least amount in S7. Isolate S5 appeared to be the most potent GA_3 producer followed by S3, and L3.

- ACC deaminase is an abiotic stress tolerant metabolite improving plant growth and development. All the isolates showed growth on DF media indicating their ACC deaminase producing ability. Quantification of the enzyme activity was determined colorimetrically by the ninhydrin-ACC assay method. The highest ACC deaminase activity was noted for the strain L3, followed by S3, R1, S5, and S7.
- The findings of O-CAS assay method indicated that all the five bacterial isolates S3, S5, S7, R1 and L3 have siderophore producing capability. All the strains produced Hydroximate type of siderophore; S5 is able to form both catecholate and hydroximate types of siderophore.
- Indirect plant growth promoting metabolite production: Except L3, other isolates are potent amylase and cellulase enzyme producer. All the five of the bacterial isolates (S3, S5, S7, R1, and L3) were detected to have siderophore and biofilm producing potential but in varying amounts. Isolate S5 has the highest biofilm producing ability, followed by S7, R1, L3 and S3. Only the isolate R1 possess lignin peroxidase producing ability. Bacterial isolates S5, S7 and R1 have urease producing potential, whereas S3 and L3 were non producer of urease. Pectinase, beta 1,3 glucanase production ability was lacking only in L3. All the five isolates were positive for peroxidase and laccase enzyme production. The isolate S7 was not able to produce protease, pectinase, and chitinase enzyme in *in vitro* condition.
- Antimicrobial compounds production: All the isolates were potent producer of NH3 in in vitro condition, S3 having a weak producing ability; except L3, others can produce HCN. Bacterial isolates S5, S7 and R1 have β 1,3 glucanase producing potential, whereas S3 and L3 lacked this ability. All the five isolates produced chitinase enzyme.
- All the 5 tested bacterial isolates were able to inhibit the growth of the pathogenic fungus, *Alternaria*, isolated from the diseased plants.

- Isolates S3, S5, R1 and, L3 appeared to be more potent PGPB. The isolate S7 was rather a poor performer regarding PGP ability.
- The antibiotic sensitivity assay revealed that the tested bacterial isolates have varied response to each of the antibiotics tested, and the zone of inhibition also showed variations. Significantly, the isolate S7 was resistant to all the antibiotics tested, except Ciprofloxacin and therefore, was not considered for utilization in multi-strain bioinoculant designing.
- Molecular identification of the promising isolates based on 16s rRNA sequencing:

Isolate code	**Submitted in NCBI as**	**NCBI Accession NO.**
S3	*Bacillus zhanzhouensis* MMAM	NCBI Acc: MT185655
S5	*Bacillus cereus* strain MMAM3	NCBI Acc: MT30003
S7	*Bacillus subtilis* strain MMAM4	NCBI Acc: MT30004.1
R1	*Bacillus subtilis* strain MMAM2	NCBI Acc: MT725461.1
L3	*Pseudomonas* sp. strain MMAM5	NCBI Acc: ON237480

- Based on the PGP potential of the bacterial isolates, the interaction study among themselves, and antibiotic sensitivity assay, bacterial isolates were finally chosen for utilization as multi-strain PGPB inoculant in plant growth promotion study. With respect to PGP characteristics, among the 5 isolates, S7 appeared to be comparatively inferior. Furthermore, antibiotic sensitivity test reveals that S7 was was resistant to most of the antibiotics tested and interaction study showed that S7 was slightly inhibitory to S5. Considering these points, S3, S5, R1 and L3 were finally selected for designing multi-strain bacterial consortia. Bacterial Combination-I, (S3+S5+R1) and Combination-II (S3+S5+R1+L3) were designed as novel multi-strain bioinoculants for application in soil sample-A and sample-C, respectively.
- Survival assay of the multi-strain bio-inoculants in soil, showed that consortium combination.1. andcombination.2. were viable for 60 days and 45 days, respectively.

OBJECTIVE IV

Evaluation of the Efficacy of the Novel Multi-strain Bioinoculant in Pot Trial Condition Growing *Glycine max* L.Merill (soybean) the Test Plant

The impact of the soil augmentation method was evaluated in a tripartite way for both of the soil sample-A and soil sample-B:

Assessment of *in vivo* growth-promotion efficacy of the PGPB consortium in Soil Sample-A.

- Application of bacterial inoculant, both with and without vermicompost, had strong positive influence on the plant growth and productivity. Overall improvement in soybean plant growth and performance was observed following amendment practices in all the experimental set-up (SV, SBC, and SVBC) in varying degrees. A strong positive influence of combined vermicompost-consortium amendment (SVBC), was detected regarding of most of the observed parameters under pot trial condition.
- Plant height increased by 14.13%, 21.73%, 32.6% in the SV, SBC, SVBC set-ups with respect to the SU condition at 12 WAE; similar trend was recorded in leaf density, leaf area, and nodule numbers at 4, 8, and 12 WAE stages over to that of the SU. The highest improvement was recorded in SVBC followed by SBC condition. Total chlorophyll content of the leaves of potted plants showed a significant increase by 24.6%. 25.6%, and 55.4%, respectively under SV, SBC, and, SVBC condition, at 12 WAE stage over to the SU set-up.
- Total no.of pods plant^{-1}, dry weight of seeds, crude protein and fat content of seeds improved remarkably in consortium treated (SBC and SBVC) conditions.

Assessment of *in vivo* growth-promotion efficacy of the PGPB consortium in Soil Sample-C.

- Plant height increased by 15.4%, 30.1%, 41.2% in the SV, SBC, SVBC set-ups with respect to the SU condition at 12 WAE; leaf area (mean) of plants at 12 WAE stages increased in SV, SBC, and SBVC set-ups by 14.6%, 43.2%, and 67.3%,

respectively compared to SU. A similar trend was recorded in total no. of leaf and nodules in plants. Chlorophyll content of leaves marginally increased, as indicated by 2.2%, 4.05% and 5.8% increase in chl(a+b) content in SV, SBC, and SVBC conditions, respectively.

- Soil augmentation with the joint-treatment of vermicompost and the novel consortium, exerted a positive effect on the first onset of flowering days, as evidenced by 6.6%, 11.1%, and 16.7% decrease in SV, SBC, and SVBC compared to that of SU set-up. Application consortium improved the dry weight of seeds by 2.04%, 3.9% and 5.6% in SV, SBC and SBVC set-ups, respectively. Consortium inoculated plants showed improvement in yield parameters, (both SBC and SVBC). Crude protein content increased marginally in treated conditions, 2.2% in SBC and 3.5% in SVBC condition.

- The findings indicate a positive influence of different treatments on most of the observed parameters under pot trial condition, highest improvement being recorded in SVBC condition.

- During statistical analysis of observed plant parameters, in both, soil sample-A and soil sample-C, it was found that t-statistic was highly significant at 5% level which confirmed that there has been significant improvement in the total no. of leaves plant^{-1}, total no. of root nodules plant^{-1}, total no. of pods plant^{-1}, and dry weight of 100 seeds in SVBC condition, over to those of SU condition.

- Finally, Logistic regression model indicates that, for plants grown in soil **sample-A,** if there is per unit rise in the no. of leaves plant^{-1} (X_1), leaf area (X_2), total chl (a+b) content of leaves (X_3) and total no. of root nodules, then there is likelihood that the no. of pod plant^{-1} will increase by 17.91, 17.86, 17.98,17.63 and 17.99 units, respectively (Table 4.19). Similarly, for plants grown in soil sample-C, Logistic regression model indicates that, if there is per unit rise in the no. of leaves plant^{-1} (X_1), leaf area (X_2), total chl (a+b) content of leaves (X_3) and total no. of root nodules, then there is likelihood that the no. of pod plant^{-1} will increase by 17.72, 17.98, 17.80, and 17.85 units, respectively. These findings indicated that improvement in vegetative growth of plants was also reflected in enhancement of yield performance.

- Overall vegetative and reproductive growth pattern of the bacterial consortia treated plants revealed that plants grown in loamy soil (soil sample-A), performed better in comparison with the plants grown in red lateritic soil (soil sample-C). This might have occurred due the presence very high level of iron content in the red lateritic soil.

Analysis of soil nutrient status in different experimental set-up across different stages of plant growth Soil sample-A.

- A steady increase in TOC in all the experimental set-ups, across the observed period; 60%, 41%, and 76% in SV, SBC, and SVBC conditions compared to the untreated.
- Available N: A steady increase in all the experimental set-ups, across the observed period; 19%, 37%, and 68.6% in SV, SBC, and SVBC conditions compared to the untreated, probably due to the combined influence of the introduced consortium along with the symbiotic N-fixing bacteria of soybean plants.
- Available Phosphate: Highest level at 16 WAE except in SU. Increased about 6.5%,60.6%, and 90.75% in SV, SBC, and SVBC set-up. Steady mobilization and acquisition is indicated in amended condition.
- Available Potassium: Sharp fall in SV (22.49%), a gradual decrease in SBC, SVBC, 7.21%, 5.35% decrease over to that of untreated condition. Rapid acquisition in SV condition; consortia facilitated utilization of soil potassium by the plants but poor mobilization in all the treatments.

Analysis of soil nutrient status in different experimental set-up across different stages of plant growth: Soil sample-C.

- A steady increase in TOC in all the experimental set-ups, across the observed period; 37%, 35.7%, and 56% in SV, SBC, and SVBC conditions compared to the untreated set-up.

- Available N: A steady increase in all the experimental set-ups, across the observed period; 39.4%, 54.5%, and 87.8% in SV, SBC, and SVBC conditions compared to the untreated one, probably due to the combined influence of the introduced consortium along with the symbiotic N-fixing bacteria.

- Available Phosphate: Highest level at 16 WAE except in SU. Increased about 5.09%, 60.6%, and 83.1% in SV, SBC, and SVBC set-up. Steady accumulation and acquisition in the SBC and SVBC condition.

- Available Potassium: Sharp fall in SV (31.7) %, but a slow increase in SBC (5.6%), and SVBC (17.05%) treatments compared to that of untreated condition indicating both acquisition and mobilization of soil K in treated condition, more effectively in SBVC.

Comparative Metagenomic analysis of soil bacterial community in amended and non-amended Soil Sample-A

- The diversity profiles indicate that although the 5th data set i.e., SVBC condition, has a relatively lower alpha diversity compared to the 4th one, it has a higher Shannon diversity index, thereby establishing a higher richness and uniformity in the distribution of the total number of genera in the given sample.

- The set of metagenomic analyses demonstrated that Actinobacteria was the most prevalent phylum in the first two datasets i.e., untreated field soil (S) and (SU). However, a pronounced shift of phyla was observed in all the remaining experimental datasets towards Proteobacteria.

- Among the most abundant putative top 10 genera in the S and SU condition, *Arthrobacter* and *Streptomyces* have high relative abundances of 15.58% and 10.79%, respectively. Among the most abundant putative top 10 genera in the Consortium treated sample (SBC), (SVBC): The relative abundance of *Bacillus* sp. was high: 5.63% (SBC) and 7.51% (SBVC). The bacterial genera unique to the untreated field soil sample were *Neptuniibacter, Lysinimonas, Alcanivorax, Campylobacter, Neisseria, Methylococcu*s, and *Oceanobacillus.* The set of bacteria that were found to be unique to the soil sample SU, includes *Anaerotruncus, Dialister, Rhodoferax, Parvimonas, Negativicoccus, Hoeflea,* and *Ruegeria.* The soil sample in SV condition, revealed a unique set of bacterial genera which include

Rhodoplanes, Neochlamydia, Byssovorax, Thermogutta, Verrucomicrobium, Luedemannella, and *Tahibacter*. When exposed to the treatment with the defined bacterial consortium, the field soil sample in SBC condition exhibited a unique bacterial profile consisting of *Thauera, Ignavibacterium, Thermoactinomyces, Solitalea, Syntrophobacter, Fluviicola,* and *Solimonas.* Under the concerted application of vermicompost and the bacterial consortium (SVBC), a unique bacterial profile was isolated from the experimental soil which included *Rhodanobacter, GpV, Clostridium, Okibacterium, Dokdonella, Phycicoccus,* and *Pedobacter.*

- The Venn diagram shows common and unique members at the OTU level in the bacterial community of untreated and treated setups. All five datasets shared 90 OTU of bacterial genera. The enriched soil of SBVC shows the highest number of unique bacterial members (24 OTU) followed by SV (20 OTU), SBC (15), S (13 OTU), and SU (7 OTU) respectively. A higher abundance of unique members in the SVBC condition indicates the positive impact of combined vermicompost and consortium treatment on soil microbial health. SVBC set up shared maximum OTU (218) with untreated field soil (S), indicating an improvement in overall soil microbial health thus promoting more and more associative bacterial assemblages. The enriched soil of SVBC set up shared maximum OTU (218) with untreated field soil (S), indicating an improvement in overall soil microbial health thus promoting more and more plant-beneficial associative bacterial assemblages.

- Some of the bacterial assemblages of PGPB, common between the consortium-treated datasets, were *Rhizobium, Bradyrhizobium, Mesorhizobium, Microbacterium, Paenibacillus, Bacillus, and Pseudomonas.* The bacterial assemblages found to be common between the consortium-treated datasets were *Rhizobium, Bradyrhizobium, Mesorhizobium, Microbacterium, Paenibacillus, Bacillus, and Pseudomonas.* The functional genera in the SBVC soil sample show the highest level of enrichment. The functional genera show an intermediate level of abundance in the case of the soil samples SBC and SV setup. The untreated field soil (S) and soil sample SU exhibited the lowest level of abundance in the functional genera.

Comparative Metagenomic analysis of soil bacterial community in amended and non-amended Soil Sample-C

- The diversity profiles indicate that the 4th dataset (SVBC) has a relatively lower alpha (208) diversity compared to the 2nd (SV) and 3rd (SBC) datasets. The Shannon diversity index of SV (5.17) was highest followed by SVBC (4.78) and SBC (4.67), thereby establishing a higher richness and uniformity in distribution of the total number of genera in the treated samples, whereas untreated field soil (S) showed lowest value (3.41).

- The set of metagenomic analyses demonstrated that Proteobacteria was the most prevalent phylum in all the experimental datasets; however, a decline in their dominance at phyla level was observed in all the amended datasets compared to that of the untreated one (S). In the 1st dataset Bacteroidota and Firmicutes were among other predominant phyla, whereas in the treated datasets Verrucomicrobiota, Planctomycetota, Patescibacteria, Bacteroidota, Chloroflexi, Firmicutes and Actinobacteria were found to be abundant.

- The observed Proteobacterial abundance were 56.33%, 64.17%, and 60.62%, respectively in SV, SBC, and SVBC setup, respectively, showing a decline in Proteobacteial abundance after vermicompost treatment.

- In the untreated field soil (S) *Sphingobacterium (65%)*, *Acinetobacter* (18%), *Pseudomonas* (10%), *Bacillus* (7%), and *Flavobacterium* (1%), were predominant at genus level. Soil sample-SV contained of the following top genera: *Allorhizobium-Mesorrhizobium-Rhizobium* (33%), *Flavobacterium* (37%), *Sphingomonas* (17%), *Bacillus* (3%) and *Pseudomonas* (10%). Soil sample SBC contained of the following top genera: *Gemmata* (24%), *Flavobacterium* (14%), *Sphingomonas* (9%), *Bacillus* (16%) *Allorhizobium-Mesorrhizobium-Rhizobium* (8%), and *Pseudomonas* (7%). Soil sample SVBC contained of the following top putitive genera: *Allorhizobium-Mesorrhizobium-Rhizobium* (24%), *Bacillus* (22%), *Sphingomonas* (17%), *Gemmata* (14%), *Pueudomonas* (9%) and *Flavobacterium* (4%).

- Bacterial diversity at genus level indicates a shift in soil bacterial community composition. The abundance of some agriculturally beneficial genera such as, *Bacillus, Allorhizobium-Mesorrhizobium-Rhizobium, Flavobacterium* has

increased in consortium treated soil, both in the presence or presence of vermicompost. However, SVBC condition showed maximum improvement probably due to the combined effect of vermicompost-consortium amendment.

- The Venn diagram shows common and unique members at OTU level in the bacterial community of untreated and treated setup. All three datasets shared 52 OTU of bacterial genera. The enriched soil of SBVC shows the highest number of unique bacterial members (129 OTU) followed by SV (118 OTU), SBC (26 OTU) respectively. A higher abundance of unique members in the SV and SVBC conditions indicate the positive impact of vermicompost, as well as, combined vermicompost and consortium treatment on soil microbial health. Decrease in the unique members in SBC condition indicates that the introduced inoculant suppressed some genera.

- The functional genera in the SBVC soil sample show the highest level of enrichment. The functional genera show an intermediate level of abundance in the case of the soil samples SBC and SV setup. The untreated field soil (S) and soil sample SU exhibited the lowest level of abundance in the functional genera.

Finally, the results of the present investigation indicated that application of the composite inoculants of PGPB strains to the soil, in combination with vermicompost, might have resulted *in situ* modification of resident microbiome promoting an increased number of agriculturally beneficial soil microbial assemblages and the resultant effects have been reflected in the promotion in plant growth and performance.

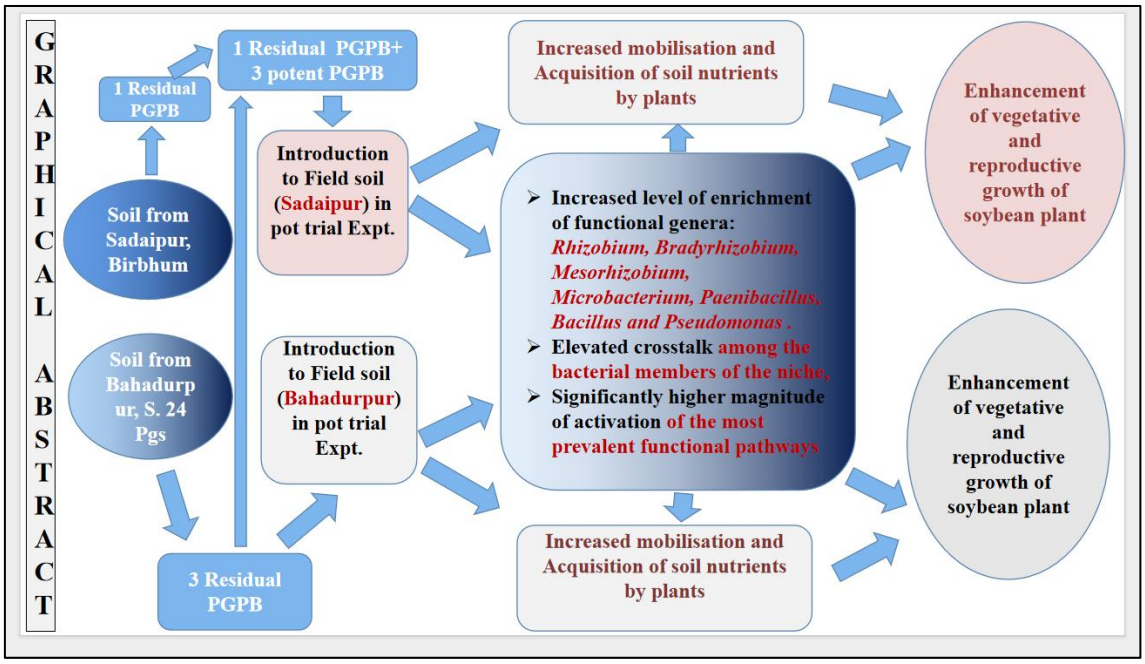
Conclusion

As the world grapples with burgeoning populations and the concomitant challenges of ensuring food security, microbial innovations could be the linchpin. The United Nations' Sustainable Development Goals, predict a mounting global prospect of microbial inoculants in ushering a sustainable agriculture. There is still a scarcity of microbial inoculants-based good products in the market, and usually, many laboratory-tested products fail to exert promising results under field trial conditions (Compant *et al.*, 2019). Despite the challenges, the commercialization of biofertilizers has remarkably expanded. In the global perspective, demand for biofertilizers is expected to escalate by about 13 percent from 2017 to 2025 (FAO, 2022). In this context, the utilization of residual BSM to enhance plant productivity in over-exploited nutrient-depleted soil can emerge as a promising strategy for futuristic agriculture.

Future Prospects

- In future, this novel technology can be implemented to enhance soil fertility and crop productivity by organic farming in nutrient-depleted soil, at a minimum cost.
- In the perspective of West Bengal, the resilient crop, Soybean (*Glycine max* L. Merril), can be grown in the transformed soil, especially in fallow and marginal lands, during the rain-fed kharif season.
- The introduction of this underutilized crop can help to improve the socioeconomic status of many small and marginal farmers in rural West Bengal.
- Extensive field trials are needed to validate the field efficacy of this strategy for large scale implementation.

Schematic Representation of The Work



Publications

- **Meenakshi Mukhopadhyay,** Arup Kumar Mitra, Debapriya Maitra, Bedaprana Roy, Archisman Chakraborty, Sudeshna Shyam Choudhury. (2022). Development of a Novel Consortium Using Bacteria With Multiple Plant Beneficial Traits From Over-Exploited Agricultural Soil. Journal of Environment and Sociobiology, (S.l.), p. 245-256, dec. 2022. ISSN 2454-2601. https://www.i-scholar.in/index.php/JESEBA/article/view/221422 **(UGC CARE enlisted journal).**

- Arup Kumar Mitra, **Meenakshi Mukhopadhyay**, Sampurna Mondal, Pallab Ghosh, Sohini Chattopadhyay, Ritushree Ganguly, Pritam Kanjilal, Sharanya Kundu, Bedaprana Roy, Debapriya Maitra and Sucharita Roy. (2022). Statistical Analysis of the Effect of Bacterial Consortia in Soybean Production. Acta Scientific Microbiology 5.5 (2022):p. 31-43. DOI: 10.31080/ASMI.2022.05.1056

- .**Meenakshi Mukhopadhyay,** Arup Kumar Mitra, Sudeshna Shyam Choudhury, Sayak Ganguli (2021). Metagenome Dataset of Lateritic Soil Microbiota from Sadaipur, Birbhum, West Bengal, India. Data in Brief 36 (2021), **ELSEVIER**. https://doi.org/10.1016/j.dib.2021.107041

- **Meenakshi Mukhopadhyay,** M Ghosal, S. Biswas, S. Basu, S.K Ghosh, S. S. Choudhury, A. K. Mitra. (2020). Improvement of Soybean Production in Low Productive Soil by the Utilization of Native Microbial Flora; Precision Agriculture and Sustainable Crop Production; Today & Tomorrow's Printers and Publishers, New Delhi-110002.,pp. 155-171. ISBN 9788170196679 (INDIA), ISBN 1-55528-1 (USA).

- **Meenakshi Mukhopadhyay,** Ashutosh Mukherjee, Sayak Ganguli, Archisman Chakraborti, Samrat Roy, Sudeshna Shyam Choudhury, Arup Kumar Mitra *et al.* (2023). Microbial Marvels in Soil Amendment: Plant-Growth Promotion, Bacterial Community Modulation, Sustainable Development and Futuristic Socioeconomic Implications. Under review in Frontiers in Microbiology.

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Data Article

Metagenome dataset of lateritic soil microbiota from Sadaipur, Birbhum, West Bengal, India

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ABSTRACT

The data represents the bacterial community profile obtained through metagenomic sequencing of soil sample, collected from the 'Rarh' region of West Bengal, which is characterized by the lateritic badlands dating back to the late Pleistocene. Taxonomic binning and operational taxonomic unit (OTU) prediction of the Illumina sequencing data indicated the abundance Proteobacteria (61%) followed closely by Bacterioidetes (35%). The top two most abundant genera identified, were Sphingobacterium and Acinetobacter respectively. Chemical properties of soil, such as pH, organic carbon content, available nitrogen, phosphorus, and potassium were also analyzed for enabling future researchers to correlate the abundance of microbial taxa with the prevalent conditions. These findings can be effectively used to formulate strategic microbiome engineering through bioaugmentation for a sustainable agricultural system.

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Research Article

Statistical Analysis of the Effect of Bacterial Consortia in Soybean Production

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Abstract

Soybean (*Glycine max*) is known as one of the most important legume plants, making a huge commercial contribution to vegetable oil production, meat production, even human nutrition. For marginalized farmers whose livelihood is dependent on soy, organic farming can be a great solution to improve its production. The use of organic farming essentially replaces the use of chemical fertilizers/pesticides and promotes the growth of Plant Growth Promoting Rhizobacteria (PGPR) which are eco-friendly and beneficial for plants in many ways. Different strains of bacteria M3, M7, M1 of *Bacillus sp.* can act as potential PGPRs. As part of the experiment, soil was collected and characterized by biochemical analysis in order to get an idea of how to improve soil quality. The CFU count of the soil was determined through serial dilutions and standard plate count technique followed by proper incubation. Two types of soil were considered: Garden soil (only) as normal control and garden soil plus vermicompost as the positive control. The treatments/inoculum included solid media, LB Broth, and water suspension which were applied separately. The pots were then prepared accordingly as positive and normal control setups and three different treatments had been applied. The growth was closely monitored for several weeks. The changes in the morphological and reproductive parameters for different treatments were quantified by Causal Impact Analysis for vegetative characteristics. For Reproductive Characteristics, ANOVA and LSD are performed. The results of this study indicated that LB treatments in normal, as well as, in the positive control, showed an overall better growth than the rest. Based on the above-mentioned tests, it is evident that the overall performance was best in the consortia when applied to LB broth. Additionally, the correlation coefficient shows that the vegetative and reproductive characteristics are highly correlated.

Keywords: Inoculum; Normal Control; Positive Control; Consortia; Strain; Vermicompost; Serial Dilution

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DEVELOPMENT OF A NOVEL CONSORTIUM USING BACTERIA WITH MULTIPLE PLANT BENEFICIAL TRAITS FROM OVER-EXPLOITED AGRICULTURAL SOIL

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ABSTRACT

Soil is a treasure trove of myriad microbial communities that encompass a bewildering array of physiological, metabolic, and genomic diversity essential for sustenance of soil fertility. Over-exploitation of arable lands with extensive use of agrochemicals has negatively impacted soil structure and function by lowering crop productivity. Such damaged agro-ecosystem can be recovered through restoration of microbial richness to replenish various plant-beneficial services at community level. Incorporation of potential single or multiple plant-growth promoting bacteria (PGPB) in soil is a unique strategy in modern sustainable agriculture. In this study, PGPB strains were isolated from a long-term used agricultural soil of Bahadurpur, West Bengal. Only the bacterial isolates having nitrogen, phosphorus and potassium acquisition ability were further screened for multiple plant growth promoting (PGP) traits. Isolates S3, S5, and R1 showed nitrogen-fixing, phosphate and potassium solubilizing ability. They were tested for production of IAA, GA, ACC deaminase, siderophore, biofilm, lytic enzymes, and volatile biochemical compounds under *in vitro* condition. Three most potent isolates (S3, S5 and R1) were selected for development of a multi-strain consortium to be utilized as bio-inoculant. Based on 16s rRNA gene sequence they were identified as *Bacillus zhangzhouensis* strain MMAM, *B. cereus* strain MMAM3, and *B. subtilis* strain MMAM2.

Key words: *Long-term used agricultural soil, Plan-growth promoting bacteria, Multi-strain consortium, Bio-inoculant.*

Seminar Presentations and Achievements

Oral presentation

- Paper entitled 'Futuristic Microbial Solution Upgradation of Over-exploited Arable Land' in **Billateral International Conference** on Exotoxicology and Environmental Sciences (ICEES-2022) held during 19th-20th October, 2022 at Khulna University, Khulna, Bangladesh.

- Paper entitled **'**Improvement of Soybean Production in Low Productive Soil by The Utilization Microbial Flora'. **International Conference** (February 25-27, 2020) on Climate Cha nge,Precision Agriculture&Innovative Disease Control Strategies For Sustainable Agriculture, University Department of Botany. T,M, Bhagalpur University,Bagalpur-812007, Bihar, India.

- Paper entitled 'Isolation of potential micro-organisms from degraded soil and their utilization in improving the soil productivity'.Two Days **National Seminar** on "Waste Management for Greener and Cleaner Environment" © 2019 (25-26 March,2019) , organized by Department of Ecological Studies and International Centre for Ecological Engineering in collaboration with ENVIS Resource Partner, University of Kalyani.

Poster presentation

- Poster entitled 'Positive Complementation of Residual Bacterial Community towards Plant Growth Promotion in Long-term Used Agricultural Soil' in 1st **Botanical Congress** organised (**An International Meet)** organised by Botanical Society of Bengal in Collaboration with Dept. Of Botany, University of Calcutta, held on 23-25th March, 2023.

- Poster entitled 'Development of a novel Consortium with Multiple Plant Beneficial Traits using Bacteria fron an Over-exploited Agricultural land' in **International Conference** on Climate Change: Global Co-operation organised by St. Xavier's College (Autonomous), Kolkata held on the 26th and 27th August, 2022.

- Poster entitled Microbial Augmentation of Low Productive Soil Towards better Productivity of Disease free Glycine max L. Meril. **International conference.** January 24-25, 2020) on Algae, Fungi and Plants: Systematic to Application, Organised by CAS, Department of Botany, Calcutta University in Collaboration with Botanical Survey of India.

- Poster entitled 'Phyllosphere Actinomycetes in Control of Frog Eye Leaf Spot of *Glycine max* (Soybean plant;). **107th Indian Science Congress** (3-7 January, 2020) in Section of Environmental Sciences.

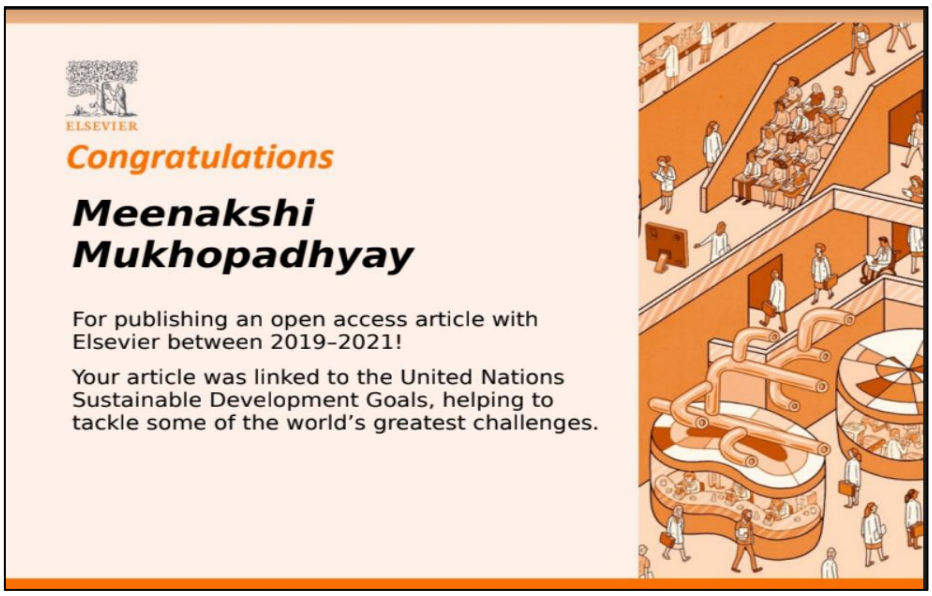
- Poster entitled 'In situ Improvement of Low Productive Soil Using Resident Microbes'. **National Seminar** in Frontiers of Biological Sciences(21-22

September, 2019),St Xavier's College, Kolkata, Presidency College and CSIR-Indian Institute of Chemical Biology.

- Poster entitled Characterization of Potential Microorganisms from Low Productive Soil for Their Subsequent utilization in Improving Productivity. Agriculture and Environmental Sciences Section; **National Conference** on " Future India: Science and Technology"(27-28 February, 2019, jointly organized by City College and Indian Science Congress Association, Kolkata Chapter.

Achievement

- Applauded by ELSEVIER Group of journals for the article, 'Metagenome Dataset of Lateritic Soil Microbiota from Sadaipur, Birbhum, West Bengal, India', published in 'Data in Brief' which was linked to the United Nation's SDGs helping to tackle some of the world's greatest challenges.



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References

Abram F. (2015). Systems-based approaches to unravel multi-species microbial community functioning. Comput. Struct. Biotechnol. J. 13, 24–32. doi: 10.1016/j.csbj.2014.11.009

Adamič S, Leskovšek R. Soybean (*Glycine max* (L.) Merr.) Growth, Yield, and Nodulation in the Early Transition Period from Conventional Tillage to Conservation and No-Tillage Systems. Agronomy. 2021; 11(12):2477. https://doi.org/10.3390/agronomy11122477

Agha MS, Haroun SA, Abbas MA, Sofy MR, Mowafy AM. 2023. Growth and Metabolic Response of Glycine max to the Plant Growth‐Promoting *Enterobacter* Delta PSK and *Bradyrhizobium japonicum* Under Salinity StressJournal of Plant Growth Regulation. https://doi.org/10.1007/s00344-023-10967-4

Ahmad, F, Ahmad I, and Khan M.S. 2008. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities. Microbiol. Res. 163: 173-181. https://doi.org/10.1016/j.micres.2006.04.001

Ahmed V Verma, MK Gupta, S Mandhan V, and Chauhan NS. (2018). Metagenomic profiling of soil microbes to mine salt stress tolerance genes. Front. Microbiol. 9, 159. doi: 10.3389/fmicb.2018.00159

Arnés-García M and Santivañez T. 2021. Hand in hand with nature – Nature-based Solutions for transformative agriculture: A revision of Nature-based Solutions for the Europe and Central Asia region, supported by Globally Important Agricultural Heritage Systems (GIAHS) examples. https://doi.org/10.4060/cb4934en

Arnon DI.1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology. Volume 24, pp. 1-15.

Arora N, Miransari M. 2016. Abiotic and Biotic Stresses in Soybean Production. 2016. http://dx.doi.org/10.1016/B978-0-12-801536-0.00006-2

Badawy IH, Hmed AA, Sofy MR, Al-Mokadem AZ. 2022. Alleviation of Cadmium and Nickel Toxicity and Phyto-Stimulation of Tomato Plant L. by Endophytic *Micrococcus luteus* and *Entero bacter cloacae.* Plants 11(15):2018.

Bag S, Saha B, Mehta O, Anbumani D, Kumar N, Dayal M, Pant A, Kumar P, Saxena S, Allin KH, Hansen T, Arumugam M, Vestergaard H, Pedersen O, Pereira V, Abraham P, Tripathi R, Wadhwa N Bhatnagar S Prakash VG, and Das B. 2016. An Improved Method for High Quality Metagenomics DNA Extraction from Human and Environmental Samples. Scientific reports, 6, 26775. https://doi.org/10.1038/srep26775

Bakker AW and Schippers B. 1987. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas* spp. mediated plant growth-stimulation. Soil Biol. Biochem. 19: 451-457. https://doi.org/10.1016/0038-0717(87)90037-X

Bargaz A, Lyamlouli K, Chtouki M, Zeroual Y and Dhiba D. 2018. Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. Frontiers in Microbiology, 9: 1606. https://doi.org/10.3389/fmicb.2018.01606

Batista BD and Singh BK. 2021. Realities and hopes in the application of microbial tools in agriculture. Microbial Biotechnology, John Wiley & Sons Ltd and Society for Applied Microbiology., Microbial Biotechnology, 14, 1258–1268.

Basu S, Ramegowda V, Kumar A, Pereira A. 2016. Plant adaptation to drought stress. F1000Research 5, 1–10. https://doi.org/10.12688/F1000RESEARCH.7678.1

Bechtaoui N, Raklami A, Benidire L, Tahiri A, Göttfert M, and Oufdou K (2020) Effffects of PGPR co-inoculation on growth, phosphorus nutrition and phosphatase/phytase activities of faba bean under difffferent phosphorus availability condition. Polish J. Environ. Stud. 29, 1557–1565. doi: 10.15244/pjoes/110345

Becker M, Patz S, Becker Y, Berger B, Drungowski M, Bunk B, et al. (2018) Comparative genomics reveal a flagellar system, a type VI secretion system and

plant growth-promoting gene clusters unique to the endophytic bacterium Kosakonia radicincitans. Front. Microbiol. 9:1997. https://doi: 10.3389/fmicb.2018.01997

Berg G, Kusstatscher P, Abdelfattah A, Cernava T, and Smalla K. (2021). Microbiome Modulation-Toward a Better Understanding of Plant Microbiome Response to Microbial Inoculants. Front Microbiol.12:650610. doi: 10.3389/fmicb.2021.650610. PMID: 33897663; PMCID: PMC8060476

Bevivino, A., and Dalmastri, C. (2017). "Impact of agricultural land management on soil bacterial community: A case study in the mediterranean area," in Soil biological Communities and Ecosystem Resilience, eds P. G. Lukax and M. Gamboni (New York, NY: Springer), 77–95.

Biketi A. 2020. Suitability of soybean-blood meal mixture as replacement of fish meal in nile tilapia (Oreochromis niloticus) rations. African Environmental Review Journal. 2020; 4:103-110

Blättel V, Larisika M, Pfeiffer P, Nowak C, Eich A, Eckelt J, König H.2011. Beta-1,3-glucanase from *Delftia tsuruhatensis* strain MV01 and its potential application in vinification. Appl Environ Microbiol. Feb;77(3):983-90. doi: 10.1128/AEM.01943-10

Brady NC, and Weil RR. 2008. The Nature and Properties of Soil, 14th ed. Upper Saddle River, NJ: Prentice Hall.

Brouder SM, Volenec JJ, and Murrell, TS. 2021. The potassium cycle and its relationship to recommendation development. In TS Murrell, RL Mikkelsen, G. Sulewski, R. Norton and M.L. Thompson, eds. Improving Potassium Recommendations for Agricultural Crops. pp. 1–46. Paper presented at, 2021, Cham. https://doi.org/10.1007/978-3-030-59197-7_1

Boubekri, K, Soumare A, Mardad I, Lyamlouli K, Ouhdouch Y, Hafidi M, Kouisni L (2022) Multifunctional role of Actinobacteria in Agricultural Production Sustainability: A Review. Microbiological Research, 261, 127059

Cappuccino JG., Sherman N. 2017. Microbiology: A Laboratory Manual. 11th Edn, Global edition. ISBN-13 978-0321840226. Pearson Education Limited.

Census 2011. Annual Report 2020-21. Department of Agriculture, Cooperation & Farmers' Welfare. Ministry of Agriculture & Farmers' Welfare Government of India. www.agricoop.nic.in

Chang C, Nasir F, Ma L et al. 2017. Molecular communication and nutrient transfer of arbuscular mycorrhizal fungi, symbiotic nitrogen-fixing bacteria, and host plant in tripartite symbiosis. In: Sulieman S, Tran LS (eds) Legume nitrogen fixation in soils with low phosphorus availability Springer, Cham. https://doi.org/10.3390/plant s11152018

Chen H, Wu H, Yan B, Zhao H, Liu F, Zhang H, Sheng Q, Miao F, Liang Z (2018) Core Microbiome of Medicinal Plant Salvia miltiorrhiza Seed: A Rich Reservoir of Beneficial Microbes for Secondary Metabolism? International Journal of Molecular Sciences. 19(3):672

CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. CLSI Guideline. Vol. 34. M100-S24 ed. Committee for Clinical Laboratory Standards; 2012:50-98

Compant S, Samad A, Fais H. and Sessitsch A. 2019. A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. Journal of Advanced Research, 19: 29-37.

Cornell C, Kokkoris V, Richards A, Horst C, Rosa D, Bennett JA and Hart MM (2021) Do Bioinoculants Affect Resident Microbial Communities? A Meta-Analysis. Front. Agron. 3:753474. doi: 10.3389/fagro.2021.753474

Dawood MF, Sofy MR, Mohamed HI, Sofy AR, Abdel-kader HA. (2022). Hydrogen Sulfde Modulates Salinity Stress in Common Bean Plants by Maintaining Osmolytes and Regulating Nitric Oxide Levels and Antioxidant Enzyme Expression. J Soil Sci Plant Nut. https://doi.org/10.1007/s42729-022-00921-w

Deb P, Talukdar SA, Mohsina K, Sarker PK, and Sayem SM. (2013). Production and partial characterization of extracellular amylase enzyme from *Bacillus*

amyloliquefaciens P-001. Springer Plus 2:1-12. https://doi.org/10.1186/2193-1801-2-154

Edi-Premono, M, Moawad A.M. and Vleck, P.L.G. (1996). Effect of Phosphate Solubilizing *Pseudomonas putida* on the Growth of Maize and Its Survival in the Rhizosphere; Indonesian Journal of Crop Science, 11, 13

Estaki M, Jiang L, Bokulich NA, McDonald D, González A, Kosciolek T, Martino C, Zhu Q, Birmingham A, Vázquez-Baeza Y, Dillon MR, Bolyen E, Caporaso JG, and Knight R. (2020). QIIME 2 Enables Comprehensive End-to-End Analysis of Diverse Microbiome Data and Comparative Studies with Publicly Available Data. Current protocols in bioinformatics, 70(1), e100. https://doi.org/10.1002/cpbi.100.

Etminani F and Harighi B. (2018). Isolation and Identification of Endophytic Bacteria with Plant Growth Promoting Activity and Biocontrol Potential from Wild Pistachio Trees. Plant Pathol J. Jun;34(3):208-217. doi: 10.5423/PPJ.OA.07.2017.0158

Etesami H, Emami S, Alikhani HA. (2017). Potassium solubilizing bacteria (KSB): Mechanisms, promotion of plant growth, and future prospects-A review. Journal of Soil Science and Plant Nutrition, 17 (4), 897-911

Falade A, Eyisia O, Mabinyaa L, Nwodoa U, Okoh A. (2017). Peroxidase production and ligninolytic potentials of fresh water bacteria *Raoultella ornithinolytica* and *Ensifer adhaerens.* Biotechnology Reports. http://dx.doi.org/10.1016/j.btre.2017.10.001

Felsenstein J. (1985). Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39:783-791.

Food and Agriculture Organization of the United Nations, (FAO). Rome . (2008). An international technical workshop Investing in sustainable crop intensification The case for improving soil health. Integrated Crop Management Vol.6-2008.

Food and Agriculture Organization of the United Nations, (FAO). Rome.(2015). Agroecology for food Security And nutrition Proceedings of the FAO international symposium18-19 September 2014,Rome, Italy. Soil health and

agricultural sustainability: the role of soil biota. Available from: https://www.researchgate.net/publication/284640031_Soil_health_and_agricultural_sustainability_the_role_of_soil_biota.

Food and Agriculture Organization of the United Nations, (FAO). Rome. (2019). The international Code of Conduct for the sustainable use and management of fertilizers: -. Rome, Italy, FAO. 56 pp. https://doi.org/10.4060/CA5253EN

Food and Agriculture Organization of the United Nations, Rome (FAO). 2020. Towards a definition of soil health. Intergovernmental Technical Panel on Soils. Letter no. 1. Rome, Italy, FAO.

Food and Agriculture Organization of the United Nations, (FAO). Rome. (2022). Soils for nutrition: state of the art. Rome. https://doi.org/10.4060/cc0900en

Fituma T, Tana T, Alemneh AA. (2018). Response of soybean to Bradyrhizobium japonicum inoculation and phosphorus application under intercropping in the Central Rift Valley of Ethiopia, South African Journal of Plant and Soil, 35:1, 33-40, DOI: 10.1080/02571862.2017.1331382

Fukui R, Schroth, MN, Hendson M, and Hancock JG. (1994). Interaction between strains of pseudomonads in sugar beet spermospheres and their relationship to pericarp colonization by Pythium ultimum in soil. Phytopathology 84: 1322-1330.

Fukami J, DeLa Osa, C, Ollero FJ, Megías M, Hungria M. (2018). Co-inoculation of maize with *Azospirillum brasilense* and *Rhizobium tropici* as a strategy to mitigate salinity stress. Funct. Plant Biol. 45, 328–339. https://doi.org/10.1071/FP17167.

Galloway, JN, Aber JD, Erisman JW, Seitzinger SP, Howarth RW, Cowling EB, and Cosby BJ. (2003). The nitrogen cascade. Bioscience, 53(4): 341–356.

Gamage A, Gangahagedara R, Gamage J, Jayasinghe N, Kodikara N, Suraweera P, Merah O. (2023). Role of organic farming for achieving sustainability in agriculture. Farming System 1 (2023) 100005. https://doi.org/10.1016/j.farsys.2023.100005

Ganguli S, Rahaman S, Bera AR., Vishal V, Malik S, Roopalakshmi K, and Singh PK (2017). Rhizospheric metagenome of the terrestrial mangrove fern Acrostichum from Indian Sunderbans. Genomics data, 14, 53–55. https://doi.org/10.1016/j.gdata.2017.09.001

Garrido-Oter, R., Nakano, R. T., Dombrowski, N., Ma, K. W., Team, T. A., Mchardy, A. C., et al. (2018). Modular traits of the rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. Cell Host Microbe 24, 155–167.e5. doi: 10.1016/j.chom.2018.06.006

Gavlak R., Horneck D, and Miller R. (2005). Plant, soil and water reference methods for the Western Region. Western Regional Extension Publication (WREP) 125, WERA-103 Technical Committee, http://www.naptprogram.org/files/napt/western-states-method-manual-2005.

Ghosh S., Ahmad, R., Gautam, V. K., and Khare, S. K. (2018). Cholesterol-oxidase-magnetic nanobioconjugates for the production of 4-cholesten-3-one and 4-cholesten-3, 7-dione. Biores. Technol. 254, 91–96. doi: 10.1016/j.biortech.2018.01.030

Goenster S, Gründler C, Buerkert A, Joergensen RG. (2017) Soil microbial indicators across land use types in the river oasis Bulgan sum center, Western Mongolia. Ecol Indic 76:111–118

Gouda S, Kerry RG, Das G, Paramithiotis S, Shin HS, and Patra JK. (2018) Revitalization of plant growth promoting rhizobacteria for sustainable development in agriculture. Microbiol. Res. 206, 131–140. doi: 10.1016/j.micres.2017.08.016

Gowtham HG, Duraivadivel P, Hariprasad P, Niranjana SR. (2017). A novel split-pot bioassay to screen indole acetic acid producing rhizobacteria for the improvement of plant growth in tomato (Solanum lycopersicum L.). Sci. Hortic. 224, 351–357. https:// doi.org/10.1016/j.scienta.2017.06.017

Govindasamy V et al. (2010) *Bacillus* and *Paenibacillus* spp.: Potential PGPR for Sustainable Agriculture. In: Maheshwari D. (eds) Plant Growth and Health

Promoting Bacteria. Microbiology Monographs, vol 18. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-13612-2_15

Guerrero-Ramirez, G, Valdez-Cordoba C., Islas-Cisneros J, and Trevino, V. (2018). Computational approaches for predicting key transcription factors in targeted cell reprogramming (Review). Molecular Medicine Reports, 18, 1225-1237. https://doi.org/10.3892/mmr.2018.9092

Gupta P, Samant K, and Sahu A. (2012),Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential, International Journal of Microbiology, doi:10.1155/2012/578925

Hamayun M, Hussain A, Khan SA, Kim HY, Khan AL, Waqas M, et al. 2017. Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. Frontiers in Microbiology.8:686. DOI: 10.3389/fmicb.2017.00686

Han ML, Lv QY, Zhang J, Wang T, Zhang C.-X, Tan R-J, Wang Y-L, Zhong L- Y. Gao Y-Q, Chao Z-F, Li Q-Q. Chen G-Y, Shi Z, Lin H-X., Chao D-Y. (2022). Decreasing nitrogen assimilation under drought stress by suppressing DSTmediated activation of Nitrate Reductase 1.2 in rice. Mol. Plant 15 (1), 167–178.

Hardoim PR, van Overbeek L, Berg G, Pirttilä AM, Compant S, Campisano A, Döring M, and Sessitsch A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. Microbiology and Molecular Biology Review. 79: 293-320

Hashem A, Abd_Allah EF, Alqarawi AA, Wirth S, Egamberdieva D. (2019). Comparing symbiotic performance and physiological responses of two soybean cultivars to arbuscular mycorrhizal fungi under salt stress. Saudi Journal of Biological Sciences. 26:38-48

Hassani MA, Durán P, Hacquard S. (2018). Microbial interactions within the plant holobiont. Microbiome 6, 58. doi:10.1186/s40168-018-0445-0

Hassanuzzaman M, Parvin K, Anee TI, Chowdhury Masud AA and Nowroz F. (2022). Salt Stress Responses and Tolerance in Soybean. Plant Stress Physiology - Perspectives in agriculture. In: Salt Stress Responses and Tolerance in Soybean. DOI: http://dx.doi.org/10.5772/intechopen.102835

Hasanuzzaman M, Nahar K, Rahman A, Anee TI, Alam MU, Bhuiyan TF, *et al*. (2017). Approaches to enhance salt stress tolerance in wheat. In: Wanyera R, editor. Wheat Improvement, Management and Utilization. Rijeka: Intech. pp. 151-187

Heritage Systems (GIAHS) examples. Budapest, FAO. Food & Agriculture Org. https://doi. org/10.4060/cb4934en

Holbrook AA, Edge WJW, Bailey and F. (1961). Spectrophotometric method for determination of gibberellic acid. Adv. Chem. 28: 159-167. https://doi.org/10.1021/ba-1961-0028.ch018

Havlin JL. *et al. S*oil Fertility and Fertilizers: An Introduction to Nutrient Management, 7th ed. Upper Saddle River, NJ: Prentice Hall, 2005.

Hodges, S.C. (2010). Soil fertility Basics. NC Certified Crop Advisor Training . Chapter 1. Basic Concepts. Soil Science Extension. North Carolina State University. http://www2.mans. edu.eg/projects/heepf/ilppp/cources

Hu J, Yang T, Friman V, Kowalchuk GA, Hautier Y, Li M,Wei Z, Xu Y, Shen Q, and Jousset A. (2021). Introduction of probiotic bacterial consortia promotes plant growth via impacts on the resident rhizosphere microbiome. Proc. R. Soc. B 288: 20211396. https://doi.org/10.1098/rspb.2021.1396.

Huang R, Mcgrath S, Hirsch P, Clark, I, Storkey J, Wu, L et al. (2019). Plant–microbe networks in soil are weakened by century-long use of inorganic fertilizers. Microb. Biotechnol. 12, 1464–1475. doi: 10.1111/1751-7915.13487

Igiehon, N. O., and Babalola, O. O. (2018). Below-ground-above-ground plant microbial interactions: focusing on soybean, rhizobacteria and mycorrhizal fungi. Open Microbiol. J. 12, 261. doi: 10.2174/1874285801812010261

Igiehon NO, Babalola OO, Cheseto X, Torto B. (2021). Effects of rhizobia and arbuscular mycorrhizal fungi on yield, size distribution and fatty acid of soybean seeds grown under drought stress. Microbiol. Res. 242.

Ilangumaran G, Schwinghamer TD, Smith DL. Rhizobacteria from root nodules of an indigenous legume enhance salinity stress tolerance in soybean. (2020). Frontiers in Sustainable Food Front. Sustain. Food Syst.Sec. Crop Biology and Sustainability Volume 4.| https://doi.org/10.3389/fsufs.2020.617978

IS:7219-1973 (Reaffirmed 2005). Indian Standard protocol for food and feed (IS-7219:1973).

Jackson ML. (1967). Soil Chemical Analysis. J. Plant Nutr. Soil Sci. 85, 251-252. https://doi.org/10.1002/jpln.19590850311

Jamily AS, Koyama Y, Win TA, Toyota K, Chikamatsu S, Shirai T, Ya-suhara T (2019). Effects of inoculation with a commercial micro-bial inoculant Bacillus subtilis C-3102 mixture on rice and barleygrowth and its possible mechanism in the plant growth stimulatoryeffect. J Plant Prot Res 59(2):193–205. DOI: 10.24425/jppr.2019.129284

Janoušková M, Krak K, Wagg C, Štorchová H, Caklová P, and Vosátka M. (2013). Effects of inoculum additions in the presence of a pre-established arbuscular mycorrhizal fungal community. Appl. Environ. Microbiol. 79, 6507–6515. doi: 10.1128/AEM.02135-13

Jones DL, Darrah PR. (1994). Role of root derived organic-acids in the mobilization of nutrients from the rhizosphere. Plant and Soil, 166(2): 247-257.

Jones Jr., J.B. (2012). Plant nutrition and soil fertility manual. Second edition. New York, CRC Press. 230 pp. https://doi.org/10.1201/b1157

Joo GJ, Kim YM, Ki JT, Rhee IK, Kim JH, and Lee IJ.. (2005),Gibberellins-producing rhizobacteria increase endogenous gibberellins content and promote growth of red peppers Journal of Microbiology; 43 pp. 510-515

Kalenik TK, Costa R, Motkina EV, Kosenko TA, Skripko OV, Kadnikova IA. (2017). Technology development of protein rich concentrates for nutrition in extreme conditions using soybean and meat by-products. Acta Sci Pol Technol Aliment. 2017 Jul-Sep;16(3):255-268. doi: 10.17306/J.AFS.0501. PMID: 29055974.

Khan MA, Asaf S, Khan AL, Ullah I, Ali S, Kang S-M, Lee I-J (2019). Alleviation of salt stress response in soybean plants with the endophytic bacterial isolate Curtobacterium sp. SAK1. Ann Microbiol 69(8):797–808. https://doi.org/10.1007/s13213-019-01470-x

Khojely DM, Ibrahim SE, Sapey E, Han T. (2018). History, current status, and prospects of soybean production and research in sub Saharan Africa. The Crop J 6(3):226–235. https://doi.org/10. 1016/j.cj.2018.03.006

Khurana A. and Kumar V. (2022). State of Biofertilizers and Organic Fertilizers in India, Centre for Science and Environment, New Delhi Published by Centre for Science and Environment. www.cseindia.org

Kim MJ, Radhakrishnan R, Kang SM, You YH, Jeong EJ, Kim JG, et al. (2017). Plant growth promoting effect of Bacillus amyloliquefaciens H-2-5 on crop plants and influence on physiological changes in soybean under soil salinity. Physiology and Molecular Biology of Plants. 23:571-580

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16:111-120. https://doi.org/10.1007/BF01731581

Kumar P, Dubey RC, and Maheshwari DK. (2012). Bacillus strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. Microbiol. Res. 167: 493-499. https://doi.org/10.1016/j.micres.2012.05.002 .

Kumar S, Stecher G, Tamura K. (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Boil. Evol. 33: 1870-1874. https://doi.org/10.1093/molbev/msw054

Kumari S, Vaishnav A, Jain S, Varma A, Choudhary DK. (2021). Bacterialmediated induction of systemic tolerance to salinity with expression of stress alleviating enzymes in soybean (*Glycine max* L. Merrill). Journal of Plant Growth Regulation. 2015;34:558-573 Systems. 4:308. DOI: 10.3389/fsufs.2020.617978

Kumawat KC, Sharma P, Sirari A, Singh I, Gill BS, Singh U, Saharan K. Synergism of Pseudomonas aeruginosa (LSE-2) nodule endophyte with Bradyrhizobium sp. (LSBR-3) for improving plant growth, nutrient acquisition and soil health in soybean. World J Microbiol Biotechnol. 2019 Mar 5; 35(3):47. doi: 10.1007/s11274-019-2622-0. PMID: 30834977

Lai CMT, Chua HB, Danquah MK and Saptoro A. (2017) . Isolation of Thermophilic Lignin Degrading Bacteria from Oil-Palm Empty Fruit Bunch (EFB) Compost. IOP Conf. Ser.: Mater. Sci. Eng. 206 012016DOI 10.1088/1757-899X/206/1/012016

Langille MGI. (2018). Exploring linkages between taxonomic and functional profiles of the human microbiome. MSystems 3(2), e00163–e00117.

Lepinay C, Mihajlovski A, Touron S, Seyer D, Bousta F, & Martino PD. (2018). Bacterial diversity associated with saline efflorescences damaging the walls of a French decorated prehistoric cave registered as a World Cultural Heritage Site. International Biodeterioration & Biodegradation, 130, 55-64. https://doi.org/10.1016/j.ibiod.2018.03.016.

Liang Y, Urano D, Liao KL et al. (2017). A nondestructive method to estimate the chlorophyll content of Arabidopsis seedlings. Plant Methods 13, 26. https://doi.org/10.1186/s13007-017-0174-6

Liu F, Hewezi T, Lebeis SL et al. (2019) Soil indigenous microbiome and plant genotypes cooperatively modify soybean rhizosphere microbiome assembly. BMC Microbiol 19, 20.

Liu Z, Li H, Gou Z, Zhang Y, Wang X, Ren H, Wen Z, Kang B-K, Li Y, Yu L, Gao H, Wang D, Qi X, Qiu L. (2020). Genome-wide association study of soybean seed germination under drought stress. Mol. Genet. Gen. 295 (3), 661–673.

Li Z., Chang S, Lin L, Li Y, and An Q., (2011). A colorimetric assay of 1-aminocyclopropane-1-carboxylate (ACC) based on ninhydrin reaction for rapid screening of bacteria containing ACC deaminase, Letters in Applied Microbiology ISSN 0266-8254, doi:10.1111/j.1472-765X.2011.03088.x

Lu, C. & Tian, H. (2017). Global nitrogen and phosphorus fertilizer use for agriculture production in the past half century: shifted hot spots and nutrient imbalance. Earth System Science Data, 9(1): 181–192. https://doi.org/10.5194/essd-9-181-2017

Luo J, Zhou Y, Gao Q, Li J, Yan N. (2020). From wastes to functions: A new soybean meal and bark-based adhesive. ACS Sustainable Chemistry & Engineering. 29:10767-10773

Ma M, Jiang X, Wang Q, Guan D Li, L Ongena, M, and Li J. (2018). Isolation and Identification of PGPR Strain and its Effect on Soybean Growth and Soil Bacterial Community Composition. International Journal of Agriculture and Biology, 20, 1289–1297. doi:10.17957/IJAB/15.0627

Majumder K., Sanyal SK, Dutta . S.K., Satyanarayana T. Singh VK. (2016). Nutrient Mining: Addressing the Challenges to Soil Resources and Food Security. # Springer India 2016 .U. Singh et al. (eds.), Biofortifification of Food Crops, DOI 10.1007/978-81-322-2716-8_14

Malusà E, Berg G, Biere A, Bohr A, Canfora L, Jungblut AD, Kepka W, Kienzle J, Kusstatscher P, Masquelier S, Pugliese M, Razinger J, Tommasini MG, Vassilev N, Meyling NV, Xu X, Mocali S. (2021). A Holistic Approach for Enhancing the Efficacy of Soil Microbial Inoculants in Agriculture: From Lab to Field Scale. Glob. J. Agric. Innov. Res. Dev [Internet]. 15 Special issue: Biofumigation 7 Symposium. https://doi.org/10.15377/2409-9813.2021.08.14.

Manfredini A, Malusà E, Costa C, Pallottino F, Mocali S, Pinzari F, Canfora L. (2021). Current methods, common practices and perspectives in tracking and monitoring bioinoculants in soil. Front. Microbiol.. Volume 12, 31. https://doi.org/10.3389/fmicb.2021.698491

Mandal S, Karmakar M and Rahman F. H. (2020). Study on Soil Nutrient Status According to Global Positioning System in Different Blocks of Birbhum District of West Bengal Current Journal of Applied Science and Technology, 39(21)2020: 64.

Maron PA, Sarr A, Kaisermann A, Lévêque J, Mathieu O, Guigue J., et al. (2018). High microbial diversity promotes soil ecosystem functioning. Appl. Environ. Microbiol. 84, 02738–02717. doi: 10.1128/AEM.02738-17.

Martínez-Porchas, M., and Vargas-Albores, F. (2017). Microbial metagenomics in aquaculture: a potential tool for a deeper insight into the activity. Rev. Aquacult. 9, 42–56. doi: 10.1111/raq.12102

Mawarda PC, Le Roux X, Dirk van E, J and Salles JF. (2020). Deliberate introduction of invisible invaders: a critical appraisal of the impact of microbial inoculants on soil microbial communities. Soil Biol. Biochem. 148, 107874.

Mawarda CP , Roux XL, Elsas JDV , Salles JF. (2020). Deliberate introduction of invisible invaders: A critical appraisal of the impact of microbial inoculants on soil microbial communities. Soil Biology and Biochemistry 148 (2020) 107874 . https://doi.org/10.1016/j.soilbio.2020.107874

Meena RS., Vijayakumar V., Yadav GS., Mitran T. 2018. Response and interaction of Bradyrhizobium japonicum and arbuscular mycorrhizal fungi in the soybean rhizosphere. Plant Growth Regul (2018) 84:207–223 DOI 10.1007/s10725-017-0334-8

Miliute I, Buzaite O & Baniulis, Danas & Stanys, Vidmantas (2015). Bacterial endophytes in agricultural crops and their role in stress tolerance: A review. Zemdirbyste-Agriculture. 102. 465-478. 10.13080/z-a.2015.102.060

Mishra BK, G Lal, YK Sharma, Krishna Kant, Saxena SN, and Dubey PN. (2019). Effect of microbial inoculants on cumin (Cuminum cyminum Linn.) growth and yield. International J. Seed Spices 9(1), January 2019:53-56.

Morgan XC, Huttenhower C. (2012). Chapter 12: Human microbiome analysis. PLoS Comput Biol. 8(12):e1002808. doi: 10.1371/journal.pcbi.1002808.

Moretti LG, Crusciol CAC, Bossolani JW, Calonego JC et al. (2021). Beneficial microbial species and metabolites alleviate soybean oxidative damage and increase grain yield during short dry spells. European Journal of Agronomy 127 (2021) 126293. https://doi.org/10.1016/j.eja.2021.126293

Moreira H, Pereira SIA, Vega A, Castro P M.L., Marques APGC. (2020). Synergistic effects of arbuscular mycorrhizal fungi and plant growth-promoting bacteria benefit maize growth under increasing soil salinity, Journal of Environmental Management,Volume 257, 109982, ISSN 0301-4797, https://doi.org/10.1016/j.jenvman.2019.109982

Mukhopadhyay M, Mitra AK, Choudhury SS, and Ganguli S. (2021). Metagenome dataset of lateritic soil microbiota from Sadaipur, Birbhum, West Bengal, India. Data Brief. 36:107041. doi: 10.1016/j.dib.2021.107041

Mukhopadhyay M, Arup Kumar Mitra, Debapriya Maitra, Bedaprana Roy, Archisman Chakraborty, Sudeshna Shyam Choudhury. (2022). Development of a Novel Consortium Using Bacteria With Multiple Plant Beneficial Traits From Over-Exploited Agricultural Soil. Journal of Environment and Sociobiology, (S.l.), p. 245-256, dec. 2022. ISSN 2454-2601. https://www.i-scholar.in/index.php/JESEBA/article/view/221422

Naveed M, Qureshi MA, Zahir ZA, Hussain MB, Sessitsch A, Mitter B (2015) L-Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by Burkholderia phytofirmans PsJN. Annals of Microbiology, 65: 1391–1389.

Nwachukwu BC. and Babalola OO. 2022. Metagenomics: A Tool for Exploring Key Microbiome With the Potentials for Improving Sustainable Agriculture.Front. Sustain.Food Sys. 2022, 6, 886987. doi: 10.3389/fsufs.2022.

O'Callaghan M, Ballard RA, Wright D. (2022). Soil microbial inoculants for sustainable agriculture: Limitations and opportunities. Soil use and Management. Volume38, Issue 3, July 2022.https://doi.org/10.1111/sum.12811

Oliveros JC (2007-2015) Venny. An Interactive Tool for Comparing Lists with Venn's Diagrams. https://bioinfogp.cnb.csic.es/tools/venny/index.html

Ondov BD, Bergman NH, & Phillippy AM. (2011) Interactive metagenomic visualization in a Web browser. BMC bioinformatics, 12, 385. https://doi.org/10.1186/1471-2105-12-385

Pande A, Kaushik S, Pandey P, and Negi A. (2020). Isolation, characterization, and identification of phosphate-solubilizing *Burkholderia cepacia* from the sweet corn cv. Golden Bantam rhizosphere soil and effect on growth-promoting activities. Int. J. Veg. Sci. 26: 591-607. https://doi.org/10.1080/19315260.2019.1692121

Pandin C, Le Coq D, Canette A, Aymerich S, Briandet R. 2017. Should the biofilm mode of life be taken into consideration for microbial biocontrol agents? Microb. Biotechnol. 10, 719-734. https://doi.org/10.1111/1751-7915.12693

Parikh SJ and James BR. (2012). Soil: The foundation of agriculture. *Nature Education Knowledge* 3(10), 2.

Peng, W., Li, X., Song, J., Jiang, W., Liu, Y., and Fan, W. (2018). Bioremediation of cadmium-and zinc-contaminated soil using Rhodobacter sphaeroides. Chemosphere 197, 33–41. doi: 10.1016/j.chemosphere.2018.01.017

Peoples M, Richardson AE, Simpson R and Fillery IRP. (2014). Soil: Nutrient Cycling. In: Encyclopedia of Agriculture and Food Systems. pp. 197–210. https://doi.org/10.1016/B978-0-444- 52512-3.00094-2

Perez-Jaramillo JE, Mendes R, Raaijmakers JM. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. Plant Mol Biol. 90: 635 – 44

Pérez-Miranda S, Cabirol N, George-Téllez R, Zamudio-Rivera LS, Fernández FJ. (2007). O-CAS, a fast and universal method for siderophore detection. J Microbiol Methods. 2007 Jul;70(1):127-31. doi: 10.1016/j.mimet.2007.03.023.

Philippot, L., Raaijmakers, J. M., Lemanceau, P., and Van Der Putten, W. H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. Nat. Rev. Microbiol. 11, 789–799. doi: 10.1038/nrmicro3109

Pikovskaya RI. (1948). Mobilization of Phosphorus in Soil Connection with the Vital Activity of Some Microbial Species. Microbiology, 17, 362-370.

Priya V and Sashi V. (2014), Pectinase enzyme producing Microorganisms, International Journal of Scientific and Research Publications, Volume 4, Issue 3, March 2014 1, ISSN 2250-3153

Radhakrishnan M, Samshath KJ, and Balagurunathan R. (2014) Hydroxamate siderophore from Bacillus spSD12 isolated from iron factory soil. Curr World Environ.9(3), 990-993.

Rahman M.S, Islam, MR, Mondol OK, Rahman MS, Sabrin F, Zohora, US. (2018). Screening of protease producing bacteria from tannery wastes of leather processing industries at Hazaribag, Bangladesh. Jahangirnagar Univ. J. Biol. Sci. 7:23-34. https://doi.org/10.3329/jujbs.v7i1.37970

Rajawat MVS, Singh S, Tyagi SP, Saxena AK. (2016). A modified plate assay for rapid screening of potassium-solubilizing bacteria. Pedosphere 26: 768-773. https://doi.org/10.1016/S1002-0160(15)60080-7.

Rashid A , Mahmood A , Maqbool R et al. 2022. Key insights to develop drought-resilient soybean: A review. Journal of King Saud University – Science 34 (2022) 102089. https://doi.org/10.1016/j.jksus.2022.102089

Rashid M, Stingl U. (2015). Contemporary molecular tools in microbial ecology and their application to advancing biotechnology. Biotechnol Adv. Dec; 33(8):1755-73. doi: 10.1016/j.biotechadv.2015.09.005

Rocha I, Ma Y, Carvalho MF, Magalhaes C, Janouskova M, Vosatka M, Freitas H. and Oliveira RS. (2018). Seed coating with inocula of arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria for nutritional enhancement of maize under different fertilization regimes. Archives of Agronomy and Soil Science, 65: 31-43

Roy B, Maitra D, Mitra A K. (2020). "Chapter 2 Methods of Sample Preparation and Assay of Bacterial Biofilms with Special Reference to Their Significance in Agriculture and Extreme Environments",Springer Science and Business Media LLC, 2020.

Sadasivam S. and Manickam. (2008). A Biochemical Method. 3rd Edition, New Age International Publishers, New Delhi.

Saima KM, Roohi AIZ. (2013) Isolation of novel chitinolytic bacteria and production optimization of extracellular chitinase. Journal of Genetic Engineering and Biotechnology 11:39–46

Salem, M. A., Perez De Souza, L., Serag, A., Fernie, A. R., Farag, M. A., Ezzat, S. M., et al. (2020). Metabolomics in the context of plant natural products research: from sample preparation to metabolite analysis. Metabolites 10, 37. doi: 10.3390/metabo10010037

Saiyad SA, Jhala YK and Vyas RV. (2015). Comparative efficiency of five potash and phosphate solubilizing bacteria and their key enzymes useful for enhancing and improvement of soil fertility. Int. J. Sci. Res. 5(2): 2250- 3153

Sanjukta S, Rai AK. (2016). Production of bioactive peptides during soybean fermentation and their potential health benefits. Trends in Food Science and Technology. 2016;50:1-10

Sanyal SK. (2014). Nutrient mining in soil-an issue of concern. Newsletter Indian Society of Soil Science, New Delhi. No. 36: 1-3.

Sarker A and Al-Rashid Jubair. (2013). Analytical protocol for determination of Indole 3- Acetic Acid (IAA) production by Plant Growth Promoting Bacteria (PGPB).

Sasirekha B. and Srividya S. (2016) Siderophore production by Pseudomonas aeruginosa FP6, a biocontrol strain for *Rhizoctonia solani* and *Colletotrichum gloeosporioides* causing diseases in chilli. Agri Nat Res. 50, 250 -256.

Schütz L, Gattinger A, Meier M, Müller A, Boller T, Mäder P, and Mathimaran N. (2018). Improving Crop Yield and Nutrient Use Efficiency via Biofertilization—

A Global Meta-analysis. Frontiers in Plant Science, 8. https://www.frontiersin.org/article/10.3389/fpls.2017.02204

Schwyn B, Neilands JB. (1987). Universal chemical assay for the detection and determination of siderophores. Anal Biochem. Jan;160(1):47-56. doi: 10.1016/0003-2697(87)90612-9. PMID: 2952030.

Shahbandeh, M. Soybean Production Worldwide 2012/13–2022/23, by Country. https://www.statista.com/statistics/263926/soybean-production-in-selected-countries-since-1980.

Seleiman MF, Al-Suhaibani N, Ali N, Akmal M. Alotaibi M, Refay Y. Dindaroglu T, Abdul-Wajid HH, Battaglia ML. (2021). Drought stress impacts on plants and different approaches to alleviate its adverse effects. Plants 10, 259.

Sharma, SK. (2011). Selection of Plant Growth-Promoting Pseudomonas spp. That Enhanced Productivity of Soybean-Wheat Cropping System in Central India. Journal of Microbiology and Biotechnology. Journal of Microbiology and Biotechnology. https://doi.org/10.4014/jmb.1012.12018.

Sheikhi F, Ardakani M, Enayatizamir N, Rodriguez-Couto S. (2012). The Determination of Assay for Laccase of Bacillus subtilis WPI with Two Classes of Chemical Compounds as Substrates,Indian J Microbiol, Doi 10.1007/s12088-012-0298-3

Sindhu SS, Sharma R, Sindhu S, Sehrawat A (2019). Soil Fertility Improvement by Symbiotic Rhizobia for Sustainable Agriculture. In: Panpatte, D., Jhala, Y. (eds) Soil Fertility Management for Sustainable Development. Springer, Singapore. https://doi.org/10.1007/978-981-13-5904-0_7

Singh A. (2022). Soil salinity: a global threat to sustainable development. Soil Use Manag 38(1):39–67. https://doi.org/10.1111/sum. 12772

Singh V, Sharma S, Kunal S, Gosal, SK, Choudhary R, et al. (2020). Synergistic Use of Plant Growth-Promoting Rhizobacteria, Arbuscular Mycorrhizal Fungi, and Spectral Properties for Improving Nutrient Use Efficiencies in Wheat (Triticum

aestivum L.), Communications in Soil Science and Plant Analysis, 51:1, 14-27, DOI: 10.1080/00103624.2019.1689259

Sofy MR, Aboseidah AA, Heneidak SA, Ahmed HR (2021).. ACC deaminase containing endophytic bacteria ameliorate salt stress in Pisum sativum through reduced oxidative damage and induction of antioxidative defense systems. Environ Sci Poll Res 28(30):40971–40991. https://doi.org/10.1007/ s11356-021-13585-3

Sofy MR, Mancy AG, Alnaggar AEAM, Refaey EE, Mohamed HI, Elnosary ME. (2022). A polishing the harmful efects of Broad Bean Mottle Virus infecting broad bean plants by enhancing the immunity using diferent potassium concentrations. Not Bot Horti Agro Cluj-Nap 50(1):12654. https://doi.org/10.15835/nbha50112654

Serna-Chavez HM, Fierer N and, van Bodegom, P.M. (2013). Global drivers and patterns of microbial abundance in soil: Global patterns of soil microbial biomass. Global Ecology and Biogeography, 22: 1162–1172. https://doi.org/10.1111/geb.12070

Sessitsch A, Brader G, Pfaffenbichler N, Gusenbauer D. and Mitter B. (2018). The contribution of plant microbiota to economy growth. Microbiology and Biotechnology, 11(5): doi:10.1111/1751-7915.13290

Sharma,S, Sharma A, Kaur M.(2018). Extraction and evaluation of gibberellic acid from *Pseudomonas* sp.: Plant growth promoting rhizobacteria. J. Pharmacogn. Phytochem.7:2790-2795.

Shi, J.W.; Lu, L.X.; Shi, H.M.; Ye, J.R. (2022a.). Effects of plant growth-promoting rhizobacteria on the growth and soil microbial community of Carya illinoinensis. Curr. Microbiol. 79, 352.

Shi H, Lu L, Ye J, Shi L. (2022b.)Effects of Two Bacillus velezensis Microbial Inoculants on the Growth and Rhizosphere Soil Environment of Prunus davidiana. International Journal of Molecular Sciences. 23(21):13639. https://doi.org/10.3390/ijms232113639

Singh B, and Schulze D.G. (2015). Soil minerals and plant nutrition. Nature Education Knowledge, 6(1): 1.

Sinsabaugh RL, Lauber CL, Weintraub MN, Ahmed B, Allison, SD, Crenshaw C, Contosta, A.R., Cusack D, Frey S, Gallo, ME, and Gartner TB. (2008). Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11: 1252-1264. https://doi.org/10.1111/j.1461-0248.2008.01245.x

Strachel R, Wyszkowska J, and Bacmaga M. (2017). The Role of Compost in Stabilizing the Microbiological and Biochemical Properties of Zinc-Stressed Soil. Water Air Soil Pollut. 228, 349. doi: 10.1007/s11270-017-3539-6

Sudarić A. (2020). 'Introductory Chapter: Soybean - Quality and Utilization'. Soybean for Human Consumption and Animal Feed. IntechOpen. doi:10.5772/intechopen.93942.

Sulistiyani TR, and Meliah S. (2017). Isolation and Characterization of Nitrogen Fixing Endophytic Bacteria Associated with Sweet Sorghum (*Sorghum bicolor*), in: Proceedings The SATREPS Conference 1: 110-117

Sutton MA, Oenema O, Erisman JW, Leip A, van Grinsven H, and Winiwarter W. (2011). Too much of a good thing. Nature, 472(7342): 159–161

Tamil Nadu Agricultural University, India, TANU Agritech Portal, Resource Management: Soil: Soil Sampling Procedure. https://agritech.tnau.ac.in/agriculture/agri _ soil _ sampling.html/TNAU-2013.

Tewari S, Arora NK, Miransari M. (2016). Plant growth promoting rhizobacteria to alleviate soybean growth under abiotic and biotic stresses. In: Abiotic and Biotic Stresses in Soybean Production: Volume 12016, Pages 131-155 https://doi.org/10.1016/B978-0-12-801536-0.00006-2

Thakur MP, Phillips HRP, Brose U, de Vries F. Lavelle P, Loreau M, Mathieu J, et al. (2019). Toward an integrative understanding of soil biodiversity. Biological Reviews, 95(2): 350-364.

Tohu H, Peay KG, Yamamichi M, Narisawa K, Hiruma K, Naito, K, Fukuda S, et al. (2018). Core microbiomes for sustainable agroecosystems. Nature Plants, 4: 247-257. doi10.1038/s41477-018-0139-4

Trivedi TP. et al 2010. Degraded and Wastelands of India Status and Spatial Distribution, Indian Council of Agricultural Research New Delhi. Available at https://icar.org.in/files/Degraded-and-Wastelands.

Tsotetsi T, Nephali L, Malebe M, Tugizimana F. Bacillus for Plant Growth Promotion and Stress Resilience: What Have We Learned? Plants (Basel). 2022 Sep 22;11(19):2482. doi: 10.3390/plants11192482. PMID: 36235347; PMCID: PMC9571655

Vashishta R, Attri S, Sharma D, Shuklaa A, Goel G. (2017),Monitoring biocalcifification potential of Lysinibacillus sp. isolated from alluvial soils for improved compressive strength of concrete. Microbiological Research, doi.org/10.1016/j.micres.2017.12.010

Vassileva M, Flor-Peregrin E, Malusa´ E and Vassilev N. (2020). Towards Better Understanding of the Interactions and Effificient Application of Plant Beneficial Prebiotics, Probiotics, Postbiotics and Synbiotics. Front. Plant Sci. 11:1068. doi: 10.3389/fpls.2020.01068.

Wang Z, Chen Z, Kowalchuk GA, Xu Z, Fu X, Kuramae EE. Succession of the Resident Soil Microbial Community in Response to Periodic Inoculations. Appl Environ Microbiol. (2021). 13;87(9):e00046-21. doi: 10.1128/AEM.00046-21. PMID: 33637572; PMCID: PMC8091015

Weil RR. and Brady NC. (2017). The nature and properties of soils (global edition). Harlow: Pearson.

Willer H, Travnícek J, Meier C, Schlatter B. (2021). The World of Organic Agriculture. Statistics and Emerging Treands 2021. Research Institute of Organic Agriculture FiBL, and IFOAM-Organic International, Bonn, v20210301

Willis Rarefaction AD (2019). Alpha Diversity, and Statistics. Frontiers in microbiology, 10, 2407

Wiwat C, Siwayaprahm P, Bhumiratana A. (1999). Purification and characterization of chitinase from *Bacillus circulan*s No.4.1. Curr Microbiol.39(3):134-40. doi: 10.1007/s002849900434. PMID: 10441726

Xing P, Zhao Y, Guan D, Li L, Zhao B, Ma M, Jiang X, Tian C, Cao F, Li J. Effects of Bradyrhizobium Co-Inoculated with Bacillus and Paenibacillus on the Structure and Functional Genes of Soybean Rhizobacteria Community. Genes (Basel). 2022 Oct 22; 13(11):1922.

Yang T, Wei,Z, Friman, VP Xu, Y Shen, Q Kowalchuk, GA Jousset A. (2017). Resource availability modulates biodiversity-invasion relationships by altering competitive interactions. Environmental Microbiology 19 (8), 2984–2991. https:// doi.org/10.1111/1462-2920.13708

Zhang L, Shi N, Fan J, Wang F, George TS, and Feng G. ()2018. Arbuscular Mycorrhizal Fungi Stimulate Organic Phosphate Mobilization Associated With Changing Bacterial Community Structure Under Field Conditions. Environmental Microbiology, 20 (7):2639-2651

Zhang M, Liu S, Wang Z, Yuan Y, Zhang Z, Liang Q, Yang X, Duan Z, Liu Y, Kong F. (2022) Progress in soybean functional genomics over the past decade. Plant Biotechnol J20(2):256. https://doi.org/10.1111/pbi.13682

Zhang N, Yang D, Wang D, Miao, Y, Shao, J, Zhou X, Xu Z, Li Q, Feng, H, Li S, Shen Q. (2015). Whole transcriptomic analysis of the plant-beneficial rhizobacterium *Bacillus amyloliquefaciens* SQR9 during enhanced biofilm formation regulated by maize root exudates. BMC Genom. 16, 1-20. https://doi.org/10.1186/s12864-015-1825-5

Zhang W, Mao G, Zhuang J, Yang H. The co-inoculation of Pseudomonas chlororaphis H1 and Bacillus altitudinis Y1 promoted soybean [Glycine max (L.) Merrill] growth and increased the relative abundance of beneficial microorganisms in rhizosphere and root. Front Microbiol. 2023 Jan 9;13:1079348

Zhang X, Davidson EA, Zou T, Lassaletta L, Quan Z, Li T, and Zhang, W. (2020). Quantifying nutrient budgets for sustainable nutrient management. *Global Biogeochemical Cycles*, 34(3).

Zhou C, Zhu L, Ma Z, Wang J. (2018). Improved iron acquisition of *Astragalus sinicus* under low iron-availability conditions by soilborne bacteria *Burkholderia cepacia*. J Plant Int 13(1):9–20. https://doi.org/10.1080/17429145.2017.1407000

Zhu H, Chen J, He Z, Hao W, Liu J, Kwek E, et al. (2019). Soybean germ oil reduces blood cholesterol by inhibiting cholesterol absorption and enhancing bile acid excretion. Food and Function. 10:1836-1845

http://www.ncbi.nlm.nih.gov

http://www.ebi.eic.uk/clustalw