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REVIEW

Plant–Microbiome driven sustainable crop protection demonstrated through native root bacterial consortia

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Plant- microbiome play pivotal role in shaping crop health, productivity, and resilience against biotic and abiotic stresses. Currently agricultural production globally is highly impacted by climate change with a strong correlation with pest and diseases incidence impacting agriculture productivity. Climate-smart and resilient agricultural practices are inevitable to combat the adverse impacts of climate change, and manage pest and diseases.Indiscriminate use of chemical fertilizers and pesticide risks agriculture by deteriorating the soil and water resources, and also developing resistance to pesticide, with residual impact in crops. Hence, there is increasing demand for adopting alternative strategies for production of safe crop with significant reduction of synthetic chemical fertilisers and pesticides. Root associated microbiome with beneficial Plant Growth Promoting (PGP) traits offers an eco-friendly approach to improve plant nutrition absorption, provides defense against biotic and abiotic stress and reduces the use of chemical inputs. In our lab a large number of PGPRS with biocontrol efficiency have been isolated, characterised and researched for their PGPR traits both in *in vivo* and *in vitro* conditions. For eg., 2,4-DAPG producing *P. fluorescens* MSSRF 51, *P. brassicacearum* MSSRF 56, *P. putida* MSSRF D41, *P. corrugata* MSSRF 39 and phenazine producing *P. aeruginosa* MSSRF C20, *P. chlororaphis* MSSRF C15, and *Bacillus*sp MSSRF T15 isolated from agricultural soil exhibited antagonistic activity against broad spectrum fungal pathogens namely *Pyricularia grisea* TN508, *Gaeumannomyces graminis* DSM1463, *Fusarium oxysporum* DSM 62297, *F. graminearum* MML 4005, and *F. solani* MML 4002. The antibiotic coding genes involved in the synthesis of antibiotic 2,4-DAPG in *Pseudomonas* sp. MSSRF D41 and phenazine-1-carboxamide (PCN) in *P. chlororaphis* MSSRF C15 was identified. The antagonistic isolates also exhibited plant growth promoting traits such as production of siderophores, hydrolytic enzymes, Indole Acetic Acid, and phosphate solubilisation etc. Application of beneficial PGPR either as individual or as consortium including arbuscular mycorrhizal fungi showed increased grain yields in millets and pulses by 30 % compared to single inoculation under intercropping systems. Thus the plant-associated microbiomes confer fitness advantages to the plant host, including growth promotion, nutrient uptake, stress tolerance and resistance to pathogens.

Keywords : Actinomycetes, biocontrol, *Fusarium oxysporum*, *Pyricularia oryzae*, Rhizobacteria,

INTRODUCTION

Estimates of 2050 food demand exhibit a projection of 50 to 60 % increase between 2019 and 2050 (Falcon *et al.* 2022) but at a slower pace of growth than the previous decade due to demographic trends.

Globally agricultural productivity is severely impacted due to the current climate change vagaries that cause huge economic losses to the tune of 12 billion USD annually, which keeps rising steadily (FAO. 2025. The State of Food and Agriculture 2025 – Addressing land degradation across landholding scales. Rome. https://doi.org/10.4060/cd7067en). Though conventional agricultural practices have been instrumental in meeting the food demands and managing pest and disease over decades, however intensive intensification of chemical pesticide use has resulted in adverse impacts thus limiting its use (Massawe *et al.* 2018; Zubair *et al.* 2021). Greenhouse gas emissions from agriculture are expected to increase by 7.5% in the next decade just less than half the projected output growth

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indicating a significant fall in the carbon intensity of agricultural production. The global use of inorganic N fertilizers increased from 12.0 Tg (1012 g) year^{-1} in 1961 to 108 Tg year^{-1} in 2019 (IFA, 2022). Excess application of nitrogen inputs significantly affects the soil microbes and below-ground activities, which has huge negative environmental impacts by influencing the quality of water and air, which in turn influences human health (Andersen *et al.* 2014) (Zubair *et al.* 2021). Excessive reliance on synthetic fertilizers and pesticides, while offering short-term gains, has led to soil nutrient depletion, and development of pesticide-resistant pathogens, environmental pollution, thereby limiting their use in the agricultural sector (Gao *et al.* 2016, Massawe *et al.* 2018) and toxic residues in the food, thus challenging the supply of safe food to humans. Plant diseases exert a heavy toll on global food production (Savary *et al*. 2019). In 2019, >2 million tons of pesticides were used (Sharma *et al*. 2019), with potentially deleterious impacts on ecosystems, including the soil biome (Gandara *et al*. 2024). Although Pesticides are necessary for crop protection and food security, inappropriate use can lead to serious consequences (Beyuo *et al.* 2024).

Sustainable alternatives to chemical inputs

Current Sustainable intensification of agriculture necessitates the adoption of climate-smart and environmentally resilient practices. Apart from climate change vagarias phytopathogens pose a serious threat to crop productivity worldwide, to provide adequate food for the growing population efficient management strategies need to be adopted (Ayaz *et al.* 2021, Farzand *et al.* 2021).Growing evidence is establishing connections between microorganisms and core elements of host biology, ecology, and evolution e.g, (Hacquard *et al.* 2015;Haney *et al.* 2015; Martinez-Medina *et al.* 2016; Davenport *et al.* 2017; Sherwin *et al.* 2019). Alternatively, the phytomicrobiome is emerging as an critical determinant of plant health, growth and productivity; an important component of sustainable agriculture. Efficient Plant Growth-Prompting Rhizobacteria (PGPR) have emerged as beneficial alternative for mitigation of both biotic and abiotic stress and reduced dependency on synthetic agrochemicals. Currently there is intensive focus by researchers exploring the use of beneficial microorganisms as an eco-friendly strategy for plant growth and protection and to maximise yield potential (Gao *et al.* 2016, Zubair *et al.* 2021). Diverse bacterial genera havedemonstrated great potential as biostimulants and biocontrol agents and also play a significant role in preventing important diseases in major crops, including fungi (Schoina *et al.*2011) eg.nitrogen-fixing rhizobia, arbuscular mycorrhizal fungi, and biocontrol *Pseudomonas, Bacillus* agents etc. Although several PGPR based formulations are commercially available, which have been proven efficient in both lab and field conditions, their inconsistent performance due to pathogen pressure and plant-microbe interaction has not been studied intensively. Understanding the molecular and ecological basis of these interactions is essential to develop robust bioformulations of biocontrol agents for specific pathogens. Among the culturable groups, the rhizosphere associated microbial communities have gained greater attention for stress tolerance and disease suppression. Research in the last decade has expanded the knowledge of microbiome diversity, functional traits and plant microbe interaction,regulating green house gas emissions, which paves opportunities to develop advance microbial applications tailored to address contemporary agricultural challenges, including climate change, biotic and abiotic stress, and declining soil health (Hacquard *et al*. 2015; Haney *et al*. 2015; Pieterse *et al*. 2016; Davenport *et al*. 2017; Sherwin *et al*. 2019).

Role of the plant microbiome as an ecological partner

The plant microbiota harbors enormous microbial communities that defy analytical methodologies to study dynamics underlying plant microbiome interactions (Suman *et al.* 2021). The research gaps to fully leverage microbiome functions for sustainable plant production needs greater attention (Compant *et al.* 2025).Plant–microbiome interactions are significant determinant for plant growth, fitness and productivity (Trivedi *et al.* 2020).The advent of next- generation sequencing (NGS) and high throughput molecular techniques have revolutionised microbiome research,

Fig 1: *In vitro* antagonistic activities of pepper rhizospheric bacteria against *Fusarium* spp.

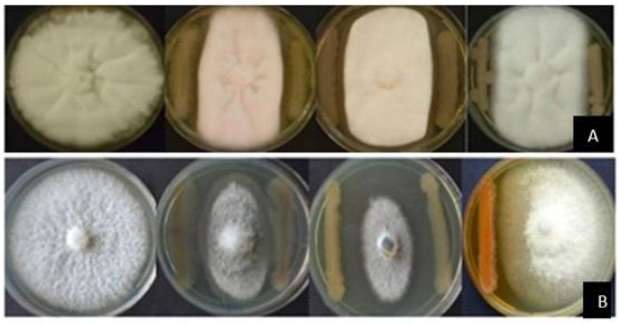

Fig. 2 : *In vitro* Antagonistic activities of tomato rhizosphericof bacteria against (A) *Fusarium oxysporum*and (B) *Pyricularia oryzae*

enabling comprehensive profiling of microbial communities and their functions. The next generation tools revealed that microbial community composition is shaped by host genotypes, development stage, and environmental conditions, underscoring the complexity of plant- microbiome interactions (Lundberg *et al.* 2012).There is a necessity to decipher the complex structural and functional diversity within plant microbiomes to reveal its immense potential in agriculture, while the conventional approaches have ignored many beneficial microbial strains, thus creating a huge gap in understanding the

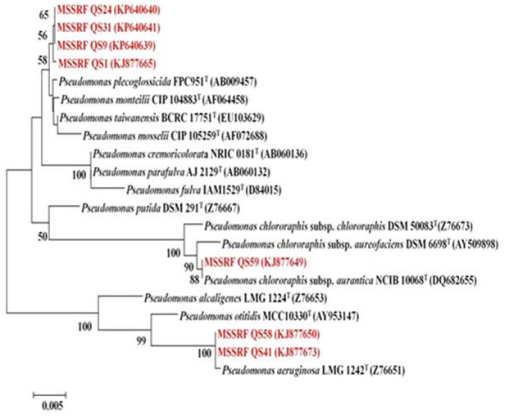

Fig. 3: 16S rDNA based phylogenetic relationship of isolated antagonistic bacteria with *Pseudomonas* spp.

microbial communications along with the genetic adaptations. Emerging biocontrol strategies employ antagonistic microorganisms, utilizing phyllosphere microecology and systemic resistance to combat this disease. Furthermore, there is a growing public concern over pesticide/ fungicide use for environmental reasons, and biocontrol agents have proven to be a reliable alternative to overcome these challenges. Several studies have reported the beneficial role of rhizobacteria in promoting plant growth and disease suppression in food crops. PGPR can modulate plant growth and development by producing/modulating phytohormone pathways such as auxin, cytokinins, abscisic acid, ethylene, and gibberellin (Orozco-Mosqueda *et al*. 2023).

The Diversity and functional traits of rhizosphere, endophytic and phyllosphere-microbiome

Plant microbial communities depending upon on their habitat are classified asrhizosphere, phyllosphere, and endophytic microbiome based on their habitat and functional role (Gupta *et al*.2021). Thus understanding the plant-microbiome interactions specific to their functional properties will provide opportunities to develop strategies for sustainable agricultural practices (Barea, 2015). The plant microbiome interactions modulate host beneficial properties particularly nutrient acquisition and defense, along with future agricultural applications (Gupta *et al.* 2021). Despite these advancements conventional methods have historically overlooked many

beneficial microbial taxa, limiting our understanding of the microbial communication and genetic adaptation. The plant microbiota harbors enormous microbial communities that defy analytical methodologies to study dynamics underlying plant microbiome interactions (Suman *et al.* 2021). Plant microbiome interactions modulate host beneficial properties particularly nutrient acquisition and defense, along with future agricultural applications (Gupta *et al.*2021). Therefore increasing interest in "green pesticides" i.e., the use of native soil microbes as natural antagonists of plant pathogens is becoming more and more popular. The plant growth promoting rhizobacteria (PGPR) are major drivers in the biocontrol of phytopathogens and are common inhabitants of the rhizospheres and phyllos-pheres. They can survive in a broad range of environmental conditions and also have a high influence on the soil and plant's health(Costa *et al*. 2006 b; Ayyadurai *et al*. 2007).

Biocontrol efficiency of root-associated bacteria

Various species of rhizobacteria belonging to the genera *Alcaligenes, Arthrobacter, Azospirillum, Azotobacter, Bacillus, Bradyrhizobium, Burkholderia, Enterobacter, Flavobacterium, Klebsiella, Mesorhizobium, Pseudomonas, Rhodococcus, Streptomyces, Serratia* etc. have been reported to promote plant growth and antagonise plant pathogens (Ahmad *et al.* 2008; Tariq *et al.* 2014; Gupta *et al.* 2017, Tariq *et al.*2017). Antagonistic rhizobacteria can inhibit the growth of several phytopathogens through various mechanisms ie., competing for space and nutrients, producing bacteriocins, lytic enzymes, antibiotics and siderophores (Jing *et al.* 2007).*In vitro* assessment of antagonism by rhizospheric bacteria of pepper (Fig.1) and tomato (Fig.2) against fungal pathogens have been illustrated Eubacterial genera, including *Bacillus, Burkholderia, Enterobacter, Herbaspirillum, Ochrobactrum, Pseudomonas, Serratia, Staphylococcus and Stenotrophomonas* are a few of the well-known antagonistic bacteria. These bacteria have been broadly described for a wide range of antagonistic activities to combat phytopathogens (Soylu *et al.* 2005; Berg, 2009; Tariq *et al.* 2010).

Symbiotic relationships and nutrient cycling

Microbial communities associated with their hosts have been shown to promote plant growth, nutrient uptake, and resistance to pathogens (Boukhalfa and Crumbliss, 2002; Crosa and Walsh, 2002; Andrews *et al.* 2003; Crowley, 2006; Zhou *et al.*2016). Although individual members of plant-associated microbial communities can possess certain beneficial traits (Crosa and Walsh, 2002; Crowley, 2006) the manifestation of a trait in the community is an emergent property that cannot be predicted by the individual members.Thus harnessing plant microbiome to mitigate challenges influenced by biotic and abiotic stress, climate change, declining soil health etc. that impact in crop production has been addressed through the microbial application (Compant *et al.* 2025). The plant microbiome comprises a plethora of beneficial, commensal, and pathogenic microor-ganisms that play important roles in plant growth and health (Raaijmakers and Weller, 2001; Vorholt, 2012, Philippot *et al.* 2013, Lemanceau *et al.* 2017, Mendes *et al.* 2018).Microorganisms, particularly nitrogen-fixing rhizobia as well as mycorrhizae and biocontrol agents, have been applied for decades to improve plant nutrition and health. Still, there are limitations regarding efficacy and consistency under field conditions (Suman *et al.* 2021). Also, the wealth of expanding knowledge on microbiome diversity, functions and interactions represents a huge source of information to exploit for new types of application. Finally, we discuss research gaps to fully leverage microbiome functions for sustainable plant production (Compant *et al.* 2025).Plant–microbiome interactions are significant determinant for plant growth, fitness and productivity (Trivedi *et al.* 2020).

Plant–Microbe Interactions in the Rhizosphere

The rhizosphere represents a highly dynamic ecological niche where complex interactions occur between plants and microorganisms. Both pathogenic and non-pathogenic microorganisms coexist in the rhizosphere region and often compete for space and limited nutrient resources, influencing plant health and productivity (Smith and Jones, 2015; Zhao *et al*. 2020). However, the

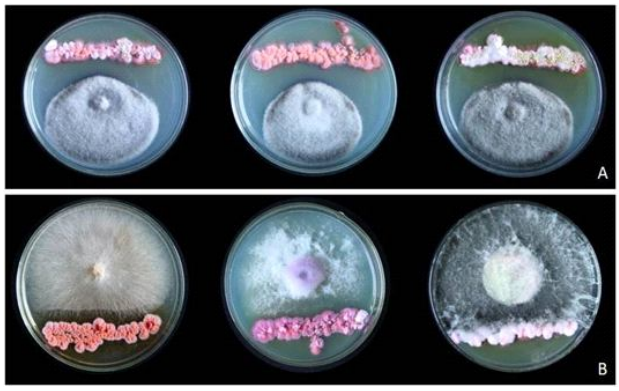

Fig. 4 : Antagonistic activities of *Streptomyces* sp. PM5 against (A) three *P. oryzae* isolates; (B) *R. solani*and *F. oxysporum in vitro*

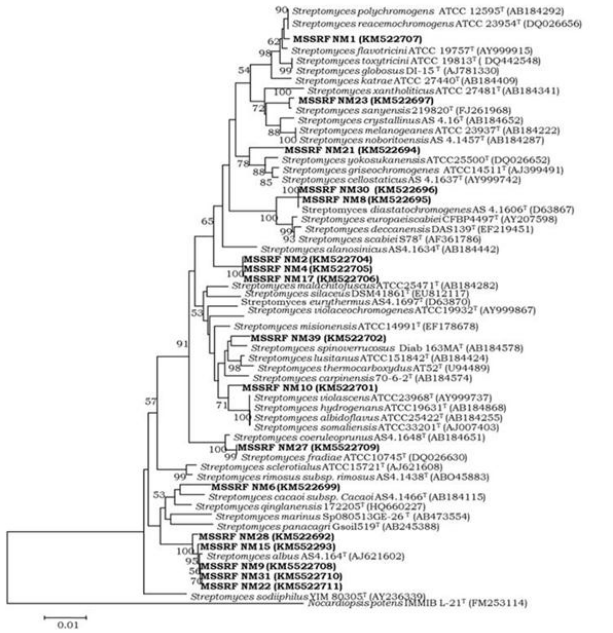

Fig 5 : 16 S rDNA based phylogenetic relationship of the actinomycetes isolates with *Streptomyces* species

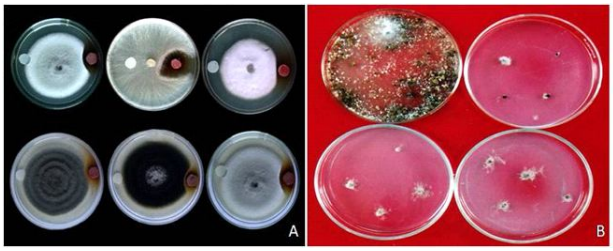

Fig. 6 : (A & B) Fungicidal activity of bacterial culture filtrates against broad spectrum of phytopathogens

beneficial plant–microbe associations in the rhizosphere have been shown to promote plant growth and alleviate both biotic and abiotic stresses (Kumar *et al*. 2018; Hernández and Singh, 2021).

Plants actively shape their rhizosphere microbiome by releasing root exudates, which act as both chemical signals and nutrient sources that selectively attract beneficial micro-organisms (Badri and Vivanco, 2009; Lundberg *et al.*2012). For instance, *Arabidopsis thaliana* secretes malic acid that facilitates colonization by *Bacillus subtilis*, thereby strengthening plant defense responses against *Pseudomonas syringae (*Rudrappa *et al.*2008*)*. Such exudation processes are crucial in enabling plants to nourish and recruit microbial partners that provide protection against pathogens. Beneficial bacteria synthesize a wide range of natural products that are crucial for managing plant diseases. Across microbial sources several antimicrobial metabolites have been identified, approximately 2,900 antibiotics from bacteria, 4,900 from fungi, and nearly 8,700 from actinomycetes, many of which include lipopeptides such as iturin, surfactin, and fengycin (Berdy, 2005; Raaijmakers *et al.*2006).Several prominent root-colonising bacteria have been reported, the most common being *Pseudomonas* and *Bacillus,* widely associated with various crops. For example, *Pseudomonas jessenii* RU47 has been reported to efficiently colonize lettuce roots, suppress *Rhizoctonia solani*, reduce disease severity, and enhance plant growth (Farrag and Fotouh, 2010).Successful colonization of roots by beneficial micro-organisms depends not only on the traits of the microbial species but also on host plant characteristics and the composition of surrounding microbial communities (Berendsen *et al.* 2012).

Biocontrol Agents

Biological control assumes special significance because it is eco-friendly and cost effective control strategy, and can be integrated with other disease management practices such as cultural and even partial chemical control to achieve higher levels of crop protection for sustaining the crop yields (Mathivanan *et al.* 2005). Further, it is increasingly becoming more popular as it is a potential non-chemical tool for crop protection against plant pathogens (O'Brien, 2017; Tariq *et al.* 2020; Collinge *et al.* 2022; Shen *et al.* 2023). Many bacteria, fungi and actinomycetes are playing a vital role in biological control of plant

diseases. Several beneficial fungal and bacterial species have been reported for the control of both root borne as well as aerial disease management by adopting different mechanisms. The common groups are *Trichoderma* spp. *Bacillus* spp. and *Pseudomonas* spp. which have been reported for the suppression of soil borne pathogens such as *Fusarium oxysporum* and *Rhizoctonia solani.*

Bioinoculants and its types for sustainable crop production

Bacteria as Biocontrol agents

Pseudomonas species, in particular, are notable producers of antimicrobial natural products, including extracellular enzymes such as cellulase, chitinase, proteases, and â-glucanase, as well as compounds like phenazines, siderophores, hydrogen cyanide, and 2,4-diacetylphloroglucinol, which are widely reported to suppress diverse phytopathogens (Haas & Défago, 2005).Among bacterial lipopeptides, fengycins and iturins are especially effective against fungal pathogens, damaging hyphae and conidia of *Fusarium graminearum* and *Monilinia fructicola* (Ongen-aasnd Jacques, 2008; Romero *et al*. 2007). Although primarily reported as antifungal, compounds have also demonstrated antibacterial activity against *Xanthomonas campestris* and *Pectobacteriumcarotovorum* (Bashan *et al.* 2004) *X.axonopodis*pv. *vesicatoria* (Arguelles-Arias *et al*. 2009), and *Ralstonia solanac-earum* (Velivelli*et al*. 2014). There has been consistent effort to use pseudomonads for the control of number of plant disease and for plant growth promotion in different crops. (Raaij makers and Weller, 2001; Kwak *et al*. 2009; Raudales *et al*. 2009; Immanuel *et al*. 2012; Lanteigne *et al*.2012; Mishra and Arora, 2012; Santoyo *et al*. 2012; Weller *et al*. 2012; Wu *et al*. 2012; Zhou *et al*. 2012b; Asadhi*et al*. 2013; Shi *et al*. 2013; Yin *et al*. 2013). *Pseudomonas* spp. are dominant plant-associated group of bacteria known for their beneficial interaction with the host plants (Haas &Défago, 2005). 16S rDNA based phylogenetic relationship of isolated antagonistic bacteria with *Pseudomonas* species have been presented in Fig 3.Most of them exhibit a wide range of beneficial properties such as salt tolerance, production of antibiotics and enzymes, solubilisation of phosphate, production of indoleacetic acid (IAA); gibberellic acid; cytokinins; enzyme 1-aminocyclopropane-1-carboxylic acid deaminase and N-acyl homoserine lactone for signal communications. They provide effective protection against bacterial and fungal pathogens, parasites and certain nematodes (Weller *et al.*, 2002). Some isolates of Pseudomonads tolerate salinity upto 1.5 M concentration (Rangarajan *et al.* 2002). High salinity tolerant pseudomonads may also tolerate other stresses like UV or drought, due to changes in the ecophysiology and membrane structure of pseudomonads. In addition, *Bacillus* spp. produce a diverse array of metabolites such as mycosubtilin, subtilisin, sublancin, bacilysin, chlorotetain, mycobacillin, rhizocticins, bacillaene, and difficidin, which have demonstrated strong potential in biocontrol (Stein, 2005; Chen *et al*. 2009).

Actinomycetes as biocontrol agents

Actinomycetes, especially *Streptomyces* spp., are widely recognized as biocontrol agents due to their antagonistic activity against diverse plant pathogens (Fig.4) such as *Fusarium oxysporum*, *Rhizoctonia solani*, and *Phytop-hthora capsici*, often mediated by antibiotic production or extracellular enzymes (Lee and Hwang, 2002, Sujina *et al.* 2017). 16S rDNA based phylogenetic relationship of the actinomycetes isolates with *Streptomyces* species have been presented in Fig 5. Endophytic isolates from banana roots inhibited *F. oxysporum* f. sp. *cubense* (Cao *et al.* 2023), while strains like *S. pilicatus*, *Frankia sp.* and *S. halstedi* showed broad-spectrum antifungal activity (Zaitlin *et al.* 2003, Aghighi *et al.*2004). Commercial formulations such as Mycostop (*S. griseoviridis*) and Actinovate (*S. lydicus*) are effectively used in vegetables, ornamentals, and turf ((Mathivanan and Manibhushanrao, 2004, Minuto *et al.* 2006), and novel applications like chitosan–polyphosphate beads with *S. melanosporofaciens* protected potato tubers from scab (Jobin *et al*. 2005). Similarly, *S. padanus* and *S. xantholiticus* reduced damping-off in Chinese cabbage when applied alone or with biofungicide formulations (Chung *et al*. 2005). Beyond their biocontrol role, actinomycetes are prolific producers of bioactive metabolites with

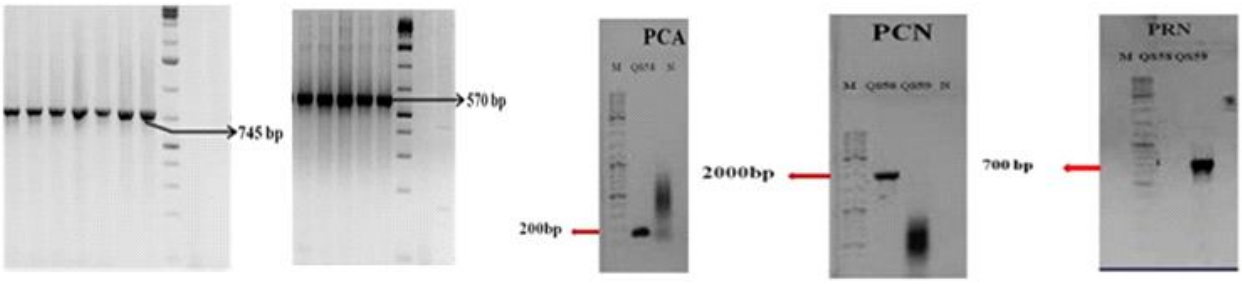

Fig 7 : Antibiotic coding genes (DAPG coding genes, hcnAB coding genes and phenazine coding genes) in *Pseudomonas chlororaphis* MSSRF QS59

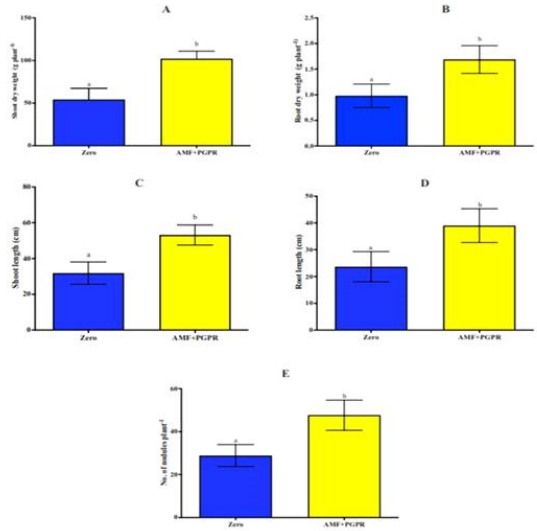

Fig. 8: Biomass of pigeon pea seedlings raised in the nursery. (A) Shoot dry weight (B) root dry weight (C) shoot length (D) root length and (E) Number of nodules

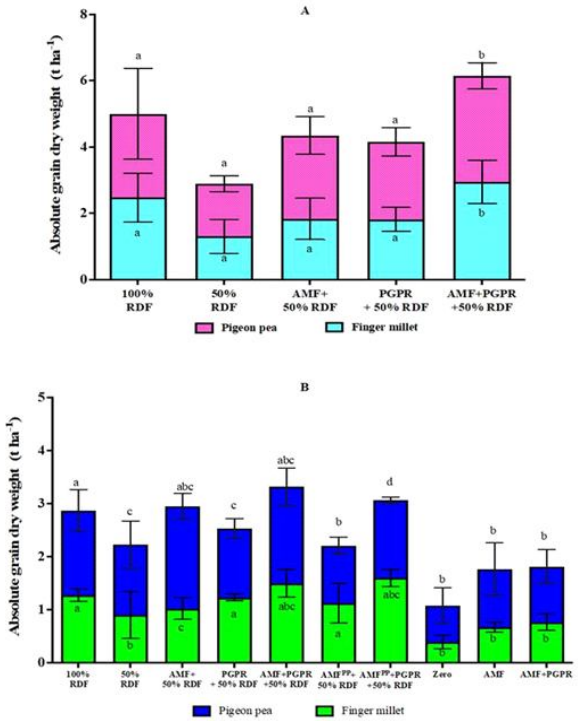

Fig 9: Yield parameters of finger millet and pigeon pea under different microbial treatments

antibacterial, antifungal, antiparasitic, herbicidal, and immunosuppressive activities, making them valuable as eco-friendly alternatives to chemical pesticides in crop protection. Efficient control of *solani* by *Streptomyces* sp. PM5 has been reported. Two antifungal aliphatic compounds, SPM5C-1 and SPM5C-2 with a lactone and ketone carbonyl unit, respectively obtained from *Streptomyces* sp. PM5 were evaluated under *in vitro* and *in vivo* conditions against major rice pathogens, *Pyricularia oryzae* and *R. solani* (Prabavathy *et al.* 2006). *Streptomyces* is the dominant genera among all actinomycetes that are studied extensively for the production of antimicrobial metabolites against plant pathogens (Fig.6 A &B)

Mechanisms of pathogen suppression

The role of rhizosphere associated microbiomes are very much critical for plant health and disease and pest control. The role of efficient biocontrol agents is increasingly being recognised as suitable alternatives to chemical fungicides (Zhou *et al.* 2021)). Phytopathogens pose a severe threat to crop productivity worldwide, to meet the food demand of the growing population and to provide safer food, an efficient, eco-friendly disease management is required to control pests and diseases of various crops. Therefore, biological control assumes special significance because it is eco-friendly and cost effective control strategy. Further, biological control or biocontrol can be integrated with other disease management practices such as cultural and even partial chemical control to achieve higher levels of crop protection for sustaining the crop yields (Mathivanan *et al*. 2000 a; 2004; 2005). Further, it

is increasingly becoming more popular as it is a potential non- chemical tool for crop protection against plant pathogens (O'Brien, 2017; Tariq *et al*.2020; He *et al*. 2021; Collinge *et al*. 2022). Many bacteria, fungi and actinomycetes are playing a vital role in biological control of plant diseases. Various microbial antagonists including the some species of bacterial genera viz., *Bacillus*, *Pseudomonas*, *Burkholderia*, *Agrobacterium* and *Enterobacter*, *Streptomyces* and fungi, *Trichoderma*, *Gliocladium* and *Coniothyrium* have shown enormous biocontrol potential against various foliar and root diseases in several crop plants (Prabavathy, 2005; Prabavathy *et al*. 2006; Mathivanan *et al*. 2005; Srinivasan and Mathivanan, 2009; 2011; Babbal *et al*. 2017; Chenniappan *et al*. 2019; Poveda *et al*. 2021; Bonaterra *et al*. 2022; Boro *et al*. 2022; Gusella *et al*. 2022) and several commercial bioproducts have already been developed using bacterial and fungal biocontrol agents, evaluated against various plant diseases and currently available in the global market for farm use.

There has been consistent effort to use pseudomonads for the control of number of plant disease and for plant growth promotion in different crops (Asadhi *et al.* 2013; Shi *et al.* 2014; Weller, 2014, Wu *et al;* 2021). *Pseudomonas* spp. are dominant plant-associated group of bacteria known for their beneficial interaction with the host plants (Haas and Defago, 2005). Most of them exhibit a wide range of beneficial properties such as salt tolerance, production of antibiotics and enzymes, solubilisation of phosphate, production of indoleacetic acid (IAA); gibberellic acid; cytokinins; enzyme 1-aminocyclopropane-1-carboxylic acid (Baldi *et al.* 2021) deaminase and N-acyl homoserine lactone for signal communications. They provide effective protection against bacterial and fungal pathogens, parasites and certain nematodes (Weller *et al.* 2002). Some isolates of Pseudomonads tolerate salinity upto 1.5 M concentration (Rangarajan *et al.* 2003). High salinity tolerant pseudomonads may also tolerate other stresses like UV or drought, due to changes in the ecophysiology and membrane structure of pseudomonads.

Secondary metabolites for the control of phytopathogens

The biocontrol ability of pseudomonads is directly correlated with production of various potential antibiotics such as 2,4-diacetylphloroglucinol (2,4-DAPG), phenazines, pyoluteorin (PLT), pyrrolnitrin (PRN), hydrogen cyanide (HCN) and cell wall lytic enzymes. There is intensified evidence that 2,4-diacetylphloroglucinol (DAPG) plays an vital role in the biological control of plant diseases as it exhibits broad antiviral, antibacterial, antifungal and antihelminthic, properties (Yang *et al.* 2014) and induce systemic resistance and systemic tolerance in the host plant (Iavicoli *et al.* 2003). The antifungal metabolite DAPG is synthesized through the expression of the genes *phlFACBDE* which forms the *phl* operon. The gene cluster responsible for the biosynthesis of DAPG was first isolated by from *P. fluorescens* Q2-87 and about six genes viz., *phlFACBDE* involved in the synthesis of 2,4-DAPG was reported. (Bhetwal *et al.*2021) predicted the presence of eight ORFs in *phl* gene cluster (*phlHGFACBDE*). ORFs *phlG, phlA, phlC, phlB, phlD*, and *phlE* had a common 3´- 5' orientation, while *phlH*and*phlF*were oppositely oriented. The different functions of these genes have been well characterized: *phlD* plays an important role in the biosynthesis of the precursor molecule for DAPG production, *phlE* acts as an exporter of DAPG metabolic (toxic) intermediates, *phlG* is involved in the degradation of DAPG, *phlF* is a pathway-specific repressor and *phlH* acts as a regulator. Among these genes *phlD* is a key gene involved in the synthesis of monoacetylphloroglucinol (MAPG), the precursor of DAPG from acetoacetyl-CoA which encodes for a polyketide synthetase (Bangera and Thomashow, 1999). 2,4-DAPG producing fluorescent pseudomonads have been identified in several distinct groups of pseudomonads (Mazzola, 2004; Naik *et al.* 2008; Berg, 2024). Promising biocontrol pseudomonads have been identified based on their ability to produce 2,4-DAPG, amplified ribosomal DNA restriction analysis (ARDRA) fingerprints, BOX and ERIC-PCR and RAPD fingerprinting. (Frapolli *et al.* 2007) used multilocus sequence typing (MLST) approach to group the worldwide collection of Phl-producing pseudomonads. This led to the identiûcation of six genotypes (Saati-Santamaría *et al.* 2021) of Phl-producing pseudomonads and proved to be as discriminative as ERIC-PCR. The production of DAPG in *in vitro* condition was media dependent. The expression of *phlD* gene and the production of 2,4-DAPG significantly changed

over time, which was also influenced by the presence of the pathogen, growth medium and was a time-dependent response ((Notz, 2005, Terhonen *et al.* 2013, Paulin *et al.* 2017). Similarly, (Notz, 2005) studied how biotic factors affecting expression of the 2,4-Diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* CHA0. Furthermore, DAPG expression is regulated by the two main component systems *GacA/GacS* which globally regulates the production of extracellular enzymes and secondary metabolite. Like in other prokaryotic two-component systems, the membrane bound sensor kinase (*GacS*) functions as an environmental sensor and activates the cognate response regulator (*GacA*) by phosphorylation. The response regulator undergoes a conformational change, which is thought to lead to transcriptional regulation of target genes.

Antibiotic coding genes in biocontrol strains

Biosynthetic loci involved in the production of antibiotics phenazines and pyrrolnitrin were detected by PCR. Antibiotic coding genes in *Pseudomonas chlororaphis* MSSRF QS59 have been presented in Fig. 7. The *phzC*gene encodes for 2-keto-3-deoxyarabinoheptulosonate-7-phosphate (DAHP) synthase and *phzD* for isochorismatase, involved in the phenazine biosynthetic pathway. The *phzH* gene encodes for an asparagine synthetase-like enzyme that converts phenazine-1-carboxylate to phenazine-1-carboxamide.

Application strategies in experimental field trials

Three methods namely, foliar spray, soil inoculation, and seed coating are commonly used to deliver microbes to plants (Rocha *et al*. 2019; Sivaram *et al*. 2023). Even upon inoculation with a high pathogen inoculum, the negative impact of pathogens in the suppressive soil on the plant host tends to diminish over time (Weller *et al*. 2002; Spooren*et al*. 2024). There is increasing evidence that the use of beneficial bioinoculants, particularly the combined inoculation of AMF and PGPB has the potential to substantially reduce mineral fertilizer input (Nath Bhowmik and Das, 2018). Intercropping of pulses with cereals is common in rainfed areas worldwide and is also known as the sole cropping system in agriculture (Layek *et al.* 2014; Baweja *et al.* 2020). The association of bacteria PGPRs with AM is highly specific and influenced by various factors, e.g., the production of extracellular polysaccharides by two PGPRs, *Azospirillum* and *Rhizobium*, significantly enhanced the attachment of bacterial strains to mycorrhizal root and AM fungal structures. This can significantly influence the movement of bacterial strains rhizospheres and also contributes to developing effective formulations of microbial inoculums (Bianciotto *et al.* 2001). Experimental trials conducted in Kolli hills using combined inoculation of the AM fungi and PGPR significantly influenced the percentage of root colonization by AM fungi as well as plant uptake of nutrients. Certain bacterial strains such as *Pseudomonas* spp. can colonize both the plant roots and AM hyphae, can influence the AMF spore germination by affecting the spore wall (Boer *et al.*2005), producing stimulants such as CO_2, or by influencing the AM fungal P absorption (Ruiz-Lozano and Bonfante, 2000).

Consortia Biofertilizer application in Nursery

The biofertilizers were applied as consortia (i) PGPRs - *Pseudomonas* sp. MSSRFD41 and Rhizobium were applied in the concentration of 10^8 CFU/ml as seed coating at the rate of 5 ml per Kg seed. Additionally, a band application (along the planting rows) was applied at the rate of 49.5 litre (consisting 1 x 10^8 CFU per ml) together with the 7.5 t FYM per ha. AMF - *Glomus leptoticum* for pigeon pea (AMFpp = 5 g/ plant) and *Glomus fasciculatum* for finger millet (AMFfm = 1 g/ plant hill) were used. The other comparative treatments were 50% RDF combined with biofertilizers either AMF or Pseudomonas or both and compared to only Biofertilizers, No input and 100% RDF. The pigeon pea seedlings raised in the nursery were maintained for 30-35 days before transplantation after the first shower of rain. The biometric data showed that the seedlings that received dual inoculation of AMF + PGPR were significantly taller, had longer roots, and had more no of nodules compared to the other treatments. The combination of AMF and PGPR appears to be the most effective treatment and indicates that dual inoculation of AMF and PGPR is best suitable for improving the growth of pigeon pea seedlings (Fig.8).

Biofertilizer consortia application on Grain Yield in Kolli hills

The total grain and straw yield of both finger millet and pigeon pea was significantly higher than the uninoculated control yield. Application of AMF+PGPR increased grain yield in finger millet by 94% as compared to uninoculated control and the absolute straw yield in pigeon pea was 54% higher compared to zero input and yield from AMF+PGPR with 50% RDF grain yield of finger millet was 17% and pigeon pea was 14% higher compared to 100 % RDF. Similarly, the treatments with 50% RDF with dual inoculations of AMF+PGPR, showed an overall trend of increased absolute grain and yield in both finger millet and pigeon pea respectively compared to single inoculation of either AMF or PGPR (Fig.9).

Consortium application outperformed single inoculation, indicating functional complementarity among strains and potential resilience to pathogen interference. Integration with arbuscular mycorrhizal fungi further enhanced nutrient uptake and plant vigor, suggesting that multi-functional microbial consortia may provide a sustainable alternative to chemical pesticides and fertilizers.

Mass production of bioinoculants

The augmentation of bioinoculants in plant defense against pests and diseases, increasing agricultural yield while reducing reliance on chemical fertilizers has been well studied. With global concerns of soil degradation, environmental and water pollution and climate change there is increasing interest among researchers, farmers and policy makers and industries to promote environment friendly biologicals for the control of pest and disease and minimise the use of harmful insecticides and fungicides (Barbosa *et al.* 2025; Ehis-Eriakha *et al.* 2025; Kumar *et al.* 2025). Technological advancement in mass production through fermentation technologies, improved strain selection, use of microbial consortia, immobilization and encapsulation techniques, liquid formulations, and carrier systems can help in scale up production. Strengthening regulatory frameworks to ensure quality, safety, and efficacy and supporting patenting of the efficient strains will improve adoption of this technology. Microbiome based approaches to strengthen the native microbial communities will yield better results.

The key constraint impeding the utilization of bioinoculants is their restricted shelf life, and availability of quality products for the end users. Researchers have investigated several formulation processes and carrier materials to improve the survival rate of these bacteria. The longevity of bioinoculants can be extended by enhancing formulation techniques, selecting suitable carrier materials, and maintaining ideal storage conditions. Smart delivery technologies, such as nanobioformultion, freeze-drying technology, capsule formulations, rhizosphere engineered bioinoculants and direct seed injection techniques can be adopted to increase shelf life and effectiveness (Monica *et al.*2025).

However, to harness the potential of the plant microbiome in agroecological environments, we still have to overcome multiple major challenges: i) the identification of the right microbe/microbial consortium for inoculation; ii) the delivery of focal microbes that can invade and persist in the native microbiome; and iii) regulatory hurdles, including public acceptance and regulatory approval. Rigorous quality control measures during the manufacturing process are critical to ensure consistency and reliability in microbial formulations. To ensure quality, standards should be established for parameters like viable cell counts, purity, and effectiveness, and this should be implemented throughout the production process, from the initial culture selection to the final formulation and packaging (Mishra *et al.* 2023).

Challenges -Limited Number of Registered Biopesticides and Lack of Awareness

Several research studies have demonstrated strong efficacy of biocontrol agents (BCAs), but currently represent less than 5% of the global crop protection market (Galli *et al.*2024). The major constraint is lengthy and complex process of identifying, characterizing, and registering potential microbial candidates, which often demands strong collaboration between academia and industry (Chandler *et al.*2011, Glare *et al.*

2012). In addition, the use of naturally occurring resources for disease management raises ecological, ethical, and legal concerns, as the introduction of novel microbial strains may affect local biodiversity (Glare *et al.* 2012; Kumar *et al.* 2021). The deployment (PGPR) in controlled environments such as greenhouses has proven more feasible. These systems allow for better management of microbial introductions while minimizing ecological risks (Berg, 2009). Still, the expansion of PGPR-based biocontrol has been hampered by regulatory challenges, since approval procedures differ greatly across countries. For instance, in Australia, the high cost of developing and registering new BCAs has been identified as a major barrier to commercialization. Effective registration therefore requires coordinated action among government agencies, universities, and private companies, supported by policies that recognize both ecological and financial benefits (Glare *et al.* 2012). At the global level, organizations such as the International Organization for Biological Control (IOBC) work toward harmonizing standards and providing recommendations to ease these bottlenecks (Eilenberg *et al.* 2001, Hokkanen, 2015). From the farmers' perspective, biopesticides are often perceived as less reliable and less profitable compared to chemical pesticides, which deliver faster and more predictable results (Compant *et al.* 2010). This perception, combined with limited technical knowledge and lack of training, reduces adoption rates. Strengthening farmer awareness through community-based programs, training sessions, and extension services could enhance confidence in BCAs (Berg, 2009, Pretty and Pervez Bharucha, 2015). Importantly, PGPR-based biocontrol reduces dependence on chemical inputs, thereby lowering production costs while indirectly improving biodiversity and environmental sustainability. However, the limited awareness and accessibility of commercial products remain significant obstacles. Building trust and increasing knowledge within the farming community are therefore essential to promote large-scale adoption of biopesticides. Our findings reaffirm the potential of PGPR to serve as biofertilizers and biocontrol agents under climate-challenged agricultural systems.

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DECLARATION

Conflict of Interest. Author has no conflict of interest.

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