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Vermicompost Derived *Pseudomonas Plecoglossicida* Strain Efficient of Growth Promoting and Managing of Root Rot Disease in Pea Plants (*Pisum Sativum*)

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The present evaluation is to characterise and assess the selected novel vermicompost-based Plant Growth–Promoting (PGP) bacteria on pea crops. Vermicompost tea was prepared using the production assembly of two plastic sieve containers and a storage bin. Among the five bacterial isolates from vermicompost tea, a single prominent isolate was found optimistic for many of the PGPR traits like nitrogen fixation, indole acetic acid production, phosphate solubilization, plant defence enzymes, anti–fungal activity and siderophore secretion. The bacterization of pea seeds with vermicompost tea and N3 bacterial culture indicated an increase in germination, vigour index and fresh seed weight, when compared to the control. The isolate showed significant growth and exhibited a variety of plant growth- promoting traits on pea crops, making it a suitable inoculant. The selected bacterium showed antifungal activity against *Fusarium oxysporium* and prominent expression of β–1,3glucanase, phenylalanine ammonia–lyase, peroxidase and chitinase defense proteins. The pot study of the pea plants treated with the vermicompost and N3 isolate on the 5th, 10th, and 15th day showed the optimum root length, shoot length, and dry biomass weight with reference to the control. The isolate was identified as *Pseudomonas plecoglossicida* [Yank1] derived from the phenotypic and genotypic characteristics. The Yank1 strain received accession number MF153408 to deposition in GenBank. Thus, the current study proposes the beneficial effects of *Pseudomonas plecoglossicida* isolated from vermicompost tea for the first time as PGPR on pea plant through collective action mode.

Keywords: Biocontrol activity, Nitrogen fixing, Phosphate solubilizer, Plant defense enzymes, Vermicompost tea

Introduction

Worldwide soil–borne fungal diseases of green peas unfavourably affect crop growth and pod yield. Among these diseases, powdery mildew and root rot caused by *Erysiphe pisi* and *Fusarium oxysporum*, respectively, are considerably important to the farming community. The pea plant's susceptibility to diseases has been due to poor plant health and oxidative stress factors.[1] Successful supervision of seed–borne root and foot rot diseases of pea plants is by crop rotation and seed treatment with pesticides and fungicides. These practices caused the ecological deterioration; pathogen resistance to fungicides increases the cost of production and ultimately affects human health.

Biocontrol agents have been the most effective alternatives for controlling diseases and reducing losses in many pulses and horticultural crops.[2] Usage of eco-friendly vermi-composting tea spray on beans, radish, tobacco, rice and tomato has been beneficial as vermicompost harbors nitrogen–fixing and several other decomposing microorganisms in its gut, and the excreta enhances the nutrient content of the soil.[3] Thus, employing microorganisms that supplement or boost nutrient availability provides sustainable solutions for problems in agriculture.[4]

Application of the Plant Growth–Promoting Rhizobacteria (PGPR) formulations on peanut[5], tomato[6] and chickpea[7] has proved economical and sustainable. In contrast to mutually symbiotic species, PGPR interrelates with a huge collection of plant species and encompasses a wide taxonomic variety. PGPR increase the nourishment of plants by using the associative nitrogen fixed and by phosphate solubilization or by the production of phytosiderophores. They facilitate shoot and root escalation through phytohormone production and elicit plant defense mainly by the induced systemic resistance.[8] During the increased plant–pathogen interactions, mechanisms at the molecular level point to the fast production of a huge quantity of oxygen-

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free radicals, and antimicrobial and antifungal lytic defence proteins.[9] In this context, an aggressive bacterium, *Bacillus subtilis* strain AR12, was shown to manage bacterial wilt of tomato grown in the greenhouses. *Bacillus subtilis* and *B. cereus* have promoted plant growth,[10] and an increase in water stress resistance in green gram plants by PGPR.[11] *Pseudomonas spp*. Strains on pigeon pea plants have specifically established the significant management of soil–borne diseases.

Plant Growth-Promoting Bacteria (PGPB) likely boost plant growth and crop yields by releasing helpful substances.[12] These substances encourage plants to produce hormones like IAA, cytokinins, and gibberellins, and they also help with nitrogen fixation and dissolving phosphates in the soil. China has witnessed success using PGPB on growing crops like rice, soybeans, and corn.[13] However, there isn't much research on how PGPB affect pea plants.[14] This means there's a great opportunity to find useful PGPB for farming and gardening. That's why this study aims to find a PGPB from homemade vermicompost tea, its features, and its affect on plant growth and other beneficial traits. This investigation is sharing findings about a specific bacterium, *Pseudomonas plecoglossicida*, and how it helps pea plants grow.

Materials and Methods

Preparation of Vermicompost Tea

The earthworms (*Esenia foetida)* were collected from soil ploughed at the University of Agricultural Sciences, GKVK campus, Bengaluru, India (13.0846 N and 77.572 E). An assembly of two sieved plastic containers and a storage bin is used to produce vermicompost. The earthworms were maintained in fertilised soil before applying them to the compost. Soil (700 g) and crushed leaves (300 g) collected from the campus of Dayananda Sagar College of Engineering, Bengaluru, were added to the upper container of an assembly of two sieved plastic containers with a storage bin. Earthworms (30–40) were spread on the mixture, wetted daily and left for 45 days for vermicomposting. Moisture content was maintained at the compost bed by sprinkling water at regular intervals, and after 45 days of composting, vermicompost tea was prepared by mixing 1 kg of vermicompost in 1L of aerated water for 22 hours and filtered using a clean muslin cloth before draining it off and placing it in plastic bottles for further usage in experiments.

Screening of Nitrogen–Fixing and PGP Bacteria from Vermicompost Tea

The bacterial colonies inoculated using vermicompost tea inoculums of 10^{-5} to 10^{-7} dilutions separately on selective nitrogen–free liquid medium, and growth was monitored at 37 ± 2°C for a maximum period of 6–7 days.[13] The growth of N_2 – fixing bacteria was monitored by the formation of turbidity in the flasks. Each culture (250 µl) was spread uniformly on an LB plate using a spreader and incubated at 37°C for 16–24 hours. Single pure colonies (~15) with pigmentation and a translucent look were selected and aseptically transferred onto N_2–free Ashby's agar and incubated at RT 27 ± 2°C for 6–7 days according to the procedure.[15] The fifteen bacterial colonies checked from vermicompost tea, merely five colonies formed a drastically higher scale of turbidity in the nitrogen–free liquid medium. The bacterial colony-forming units (CFU) were determined on every 15th day using a typical colony count method. Five bacterial isolates named N1, N2, N3, N4 and N5 from the entire colony–forming units showed major growth and were restreaked onto Ashby agar medium to gain pure isolates and further molecular characterisation and growth studies were conducted.

Determination of PGP traits of vermicompost isolates

The five bacterial isolates are characterised by their expression of growth–promoting qualities. Estimation of Auxin (Indole–3–acetic acid, IAA) was carried out by using the fast microplate technique by using Salkowski reagent.[16] The five vermicompost isolates were characterised for expressing plant growth–promoting traits. The estimation of siderophore was determined by the Blue Agar CAS.[17] *In vitro* antifungal activity of bacterial isolates was determined by placing a fungal disk of *Fusarium oxysporum* of 8 mm size on the periphery of ~1.0 cm of the Petri plate containing glucose, casamino acid, and yeast extract.[18] N1 to N5 bacterial isolates were streaked on the other periphery of the plate and incubated at 28 ± 2°C for five days till the complete coverage of the fungus in the control was seen.

Molecular and Bioinformatic Analysis

Genomic DNA from the N3 isolate, grown for 12 hr in LB media, was isolated using PureLink™ Genomic DNA Mini Kit. Amplification of the 16S rRNA gene fragment was carried out using universal

27F and 1492R primers. The Polymerase chain reaction mixture (25 µL) was made up of N3 genomic DNA (100 ηg), Phusion high fidelity Master mixture (14 µL) and forward and reverse primers (2µM) respectively. The conditions were of an initial denaturation step of 98°C for 1 min followed by denaturation step of 35 cycles. The 16SrRNA gene sequence of N3 isolate was used to carry out 98°C for 30 sec, annealing at 58°C for 60 sec, extension at 72°C for 30 sec and a final extension at 72°C for 15 min. DNA sequencing of the amplicon was done at Eurofins Genomics, Bengaluru, India. The consensus sequence of the 16S rRNA gene of the N3 isolate was obtained from sequence BLAST with the NCBI GenBank database. The maximum identity score of the first fifteen sequences was selected and aligned using the ClustalW software program. The phylogenetic tree and the distance matrix were constructed using MEGA11.0 and analyzed using the Kimura 2 model.[19] The evolutionary relationship between the sequences was studied using the bootstrap values. The RNA structure prediction was carried out using UNAFold software 4.0.

Seed Bacterization and Germination Assay of Pea Seeds

The Arka Chaitra pea seeds from the Indian Institute of Horticulture Research, Bengaluru were soaked in a 0.03% $HgCl_2$ solution for 6 minutes and then rinsed them three times with sterile water.[20] After that, they took 80 of these seeds and soaked them in sterile water, vermicompost tea, or an N3 bacterial culture (10^7 cells/ml) for about 10 to 12 hours, giving them a gentle shake now and then. Once they were done soaking, they let the seeds dry in the shade. For the seeds treated with vermicompost tea and the bacterial culture, the bacterial count was about 10^2 colony-forming units (CFU) per seed before planting. They then planted the treated seeds and some untreated control seeds in sterile Petri plates lined with sterile water-soaked Whatman No.1 filter paper. These plates were placed in an incubator set at the ideal temperature of 27 ± 1°C and 85%–90% relative humidity. They checked the seedlings at days 0, 3, 6, 9, and 12 to do biochemical tests. They also kept an eye on the plates all week to check on their growth. To measure shoot and root growth, they picked out 10 seedlings from each of the two groups. They figured out the germination percentage and vigor index on the 7th day, using the method described by Govindappa *et al*.[21]

Statistical Analysis

Different treatments yielded data and were analyzed using ANOVA software and for mean values comparison, Duncan's Multiple Range Test was applied. Important differences were measured at the 0.05 level using Origin software (version 8.5.0) 2010.

Determination of Activities of Defense–Related Enzymes

Control, vermicompost and N3 treated seedlings (2g) grown for 4 days were washed using sterilized water and homogenized. The extract was taken in pre–chilled 5 ml of 50 mM phosphate buffer solution containing 1 mM EDTA and Phenyl methane sulphonyl fluoride (0.5 mM) was added. The homogenate was centrifuged at 13000 rpm for 30 min at 4°C. The supernatant was taken as defense enzyme source and stored at −20°C until further use. The protein content was estimated using the Bradford method.[22] β–1,3 glucanase activity was determined by the DNS method.[23] Phenylalanine ammonia–lyase (PAL) activity was determined at 290 nm by estimating the concentration of T–cinnamic acid.[24] Peroxidase assay was determined by using pyrogallol. Chitinase activity was determined for the supernatant as described by T Shimahara and Takiguchi's method.[25]

SDS–PAGE of Induced Defense Proteins

The extract of germinated seedlings of control and treated seeds were the source of induced proteins estimated by Bradford[22] and analyzed by SDS–PAGE.[26] BL Ultra pre–stained protein ladder from Gene Direx was loaded and the SDS–PAGE gel was visualized by staining using Coomassie blue G–250 solution.

Plant Growth Measurements and Pot Trials

Earthen pots of 2 kg capacity diameter of 17 cm were packed with 1.5 kg of sterile non–manure soil. Three replications of four bacterisized seeds were sowed in each earthen pot and a control was maintained. Pots were placed in a glasshouse with a optimum temperature 26 ± 2°C and 65% RH and reduced to two plants per pot after a week. On alternate days, the earthen pots were sprinkled with autoclaved water for 30 day period. In the set of experiments, vermi teas were prepared with a dilution range of 5–10%. Fresh teas as foliar spray were applied to pots at transplanting, and then 4 times

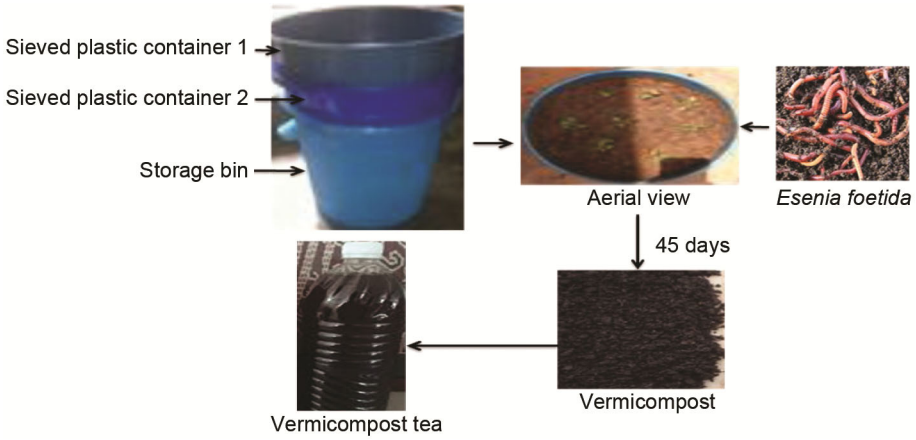

Fig. 1 — Vermicompost production assembly

weekly for 6 weeks. Three replicates and proper control were maintained in the experiment. The inoculation was done with the fungi *Fusarium oxysporum* to the 7 days seedlings of one set of treatment. After 25 days of the period, length of roots and shoots and total chlorophyll content[27] were recorded as indicative parameters of pea plant growth.

Results

Preparation of Vermicompost Tea

Earthworms (*Esenia foetida),* collected from soil ploughed at the GKVK campus, University of Agriculture Sciences, Bengaluru, India (13.0846 N and 77.572 E). An assembly of two sieved plastic containers with a storage container, as shown in Fig. 1, was used to produce vermicompost, and after 45 days, composted vermicompost tea was prepared. The vermicompost tea of 1 litre is obtained from every 1.0kg of vermicompost. Pant *et al.*,[(28)] reported that vermicompost boosted the concentration of vital plant nutrients such as carbon, nitrogen, potassium and phosphorus, as compared to control soil. The significant improvement of the soil in terms of physicochemical and biological properties validated by chemical analysis, and hence vermicompost is considered to be the paramount source for PGPB.

Isolation and Screening of Nitrogen–Fixing Bacteria

Overall, fifteen vermicompost-based bacterial colonies were isolated. The colony morphology was whitish, creamy, smooth, and irregular, with a 3mm–6mm diameter, transparent and glistening appearance. Out of the 15 colonies grown on LB plates, only five had higher nitrogen–fixing capacity as tested on Ashby Agar medium. Gram staining technique showed 20% of them to be gram–positive cocci

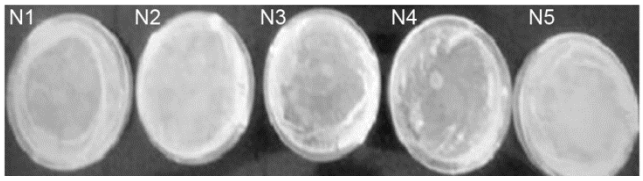

Fig. 2 — Five unique isolates from vermicompost tea on Nitrogen–free Ashby media

forms, 25% of gram–positive long rods, and 55% of gram–negative rods. Further, a single soil grain technique also produced N_2-fixing bacteria from vermicompost on Ashby agar media. The CFU/ml for vermicompost tea was 2.66×10^6 when compared to 1.63×10^6 of the soil sample and twice the concentration compared to the soil at a dilution of 10^{-4} to 10^{-7}. It is suggested that vermicompost consortia contain more bacteria than the soil. Some colonies showed a dark brownish pigmentation, and a few had a greenish–yellow pigmentation. This study focuses on nitrogen–fixing bacteria, as vermicompost is known to contain a consortium of such beneficial bacteria (Fig. 2). The five unique colonies named N1, N2, N3, N4, and N5 were isolated based on the methodology of Capowiez *et al.*[29] and the characteristics of the isolates were observed.

Five isolates are unique and categorised into three morphologies depending on the cell type they were classified as bacilli, gram–negative, short and large. There were also gram–negative bacilli, short and small, and cysts (Fig. 2). A few exhibited brown and green pigmentation. The vermicompost isolate has more rod–shaped bacterial species, compared to cocci–shaped. The bacterial species in vermicompost preferred to be in a chain pattern, while in the usual soil, they were found in bunches. In vermicompost, most bacteria are gram-positive and grow much

Table 1 — Comparison of plant growth–promoting parameters exhibited by the vermicompost isolates

Isolate	Nitrogen fixation	Phosphate solubilization	HCN production	IAA production	Siderophore production	Antifungal activity (mm)
N1	++	–	++	++	+	+
N2	+	–	+	++	–	+
N3	++++	++	++++	++++	++	+++
N4	++	+	++	+	+	+
N5	+++	–	+	+	–	+

++++ indicates very good , +++ good, ++ moderate activity, + low activity and – indicates no activity.

quicker than those found in regular soil. It is noticeable that they have a wider range of colours and colony patterns. The production of phytohormone IAA by the isolates was in the range of 2.85 µg/ml – 15.88 µg/ml without the presence of tryptophan, which was beneficial unlike the auxin production in response to the tryptophan supplemented medium or as in the exogenous application of tryptophan.[30]

The growth inhibition of pea pathogen *Fusarium oxysporum* by the isolated bacterial colonies N1, N2, N3, N4, and N5 was observed, and the percentage inhibition varied with each isolate, with the N3 isolate showing maximum inhibition of 50% compared to the others, which showed 30%–42%. The N3 isolate showed a decrease in fungal growth and the development of a zone of inhibition by the secretion of antifungal metabolites that lyse the pathogen cell wall. This category of antifungal activity was also seen against *Rhizoctonia solani*, *Sclerotium rolfsii and S. sclerotiorum* along with HCN production, solubilization and formation of phosphate and siderophore respectively (Table 1).

Molecular Identification of N3 Isolate

The 16S rRNA gene was amplified using the genomic DNA of the N3 isolate as a template. As shown in Fig. 3, the consensus sequence length was determined to be 1380 base pairs.

The BLASTn analysis showed that the N3 isolate was 99% homologous with bacterial strains *Pseudomonas plecoglossicida, P. guariconensis* strain C–416S, *Pseudomonas putida strain EH63 16S, and P. plecoglossicida* strain SR4. The BLAST was performed against the non–redundant databases, and the BLOSUM 62 software was used to identify the strain of *Pseudomonas guariconensis* employing the maximum likelihood method. The method provided probabilities of the sequences to a model of their evolution on a particular tree and helped to conclude that isolate N3 is a unique and novel strain of *Pseudomonas plecoglossicida*, which was named

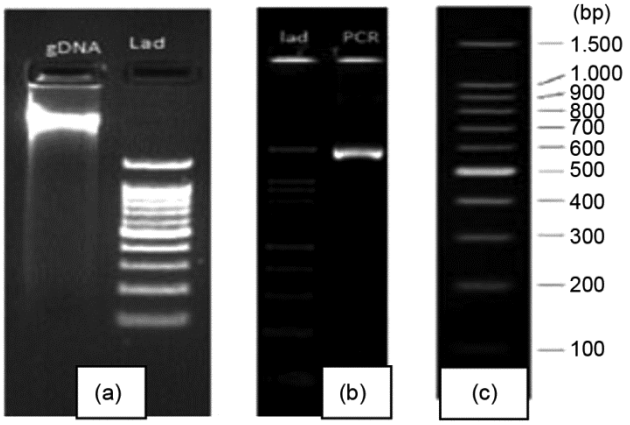

Fig. 3 — Agarose gel electrophoresis: (a) Genomic DNA of N3 isolate, (b) PCR amplicon of 16S rRNA of N3 bacterial isolate (c) 100 bp DNA ladder

Yank1, as it was isolated from the vermicompost. With accession number MF153408, the Yank1 strain was added to GenBank (NCBI). The bacterium *P. plecoglossicida* is an efficient phosphate solubiliser. The Yank1 isolate was found to have high similarity with *Pseudomonas plecoglossicida* by nucleotide homology and phylogenetic analysis. The phylogenetic analysis of 16S rRNA by the Neighbour–joining method revealed that the isolate N3 matched *Pseudomonas plecoglossicida* and is close to *Pseudomonas guariconensis* strain C, whose accession number is KT748640.

The Neighbor–Joining technique was used to determine the phylogenetic relationship (Fig. 4) between nitrogen–fixing bacteria (N3 isolate) and specific bacteria based on the 16S rRNA gene sequences of closely related species. MEGA version 11.0 was used to calculate the evolutionary distances using the Maximum Composite Likelihood approach.

This evolutionary analysis involved eleven nucleotide sequences of 16S rRNA from bacteria that were similar both morphologically and metabolically. From the closest bacteria in the distance to the farthest bacteria, as calculated by the Maximum Composite

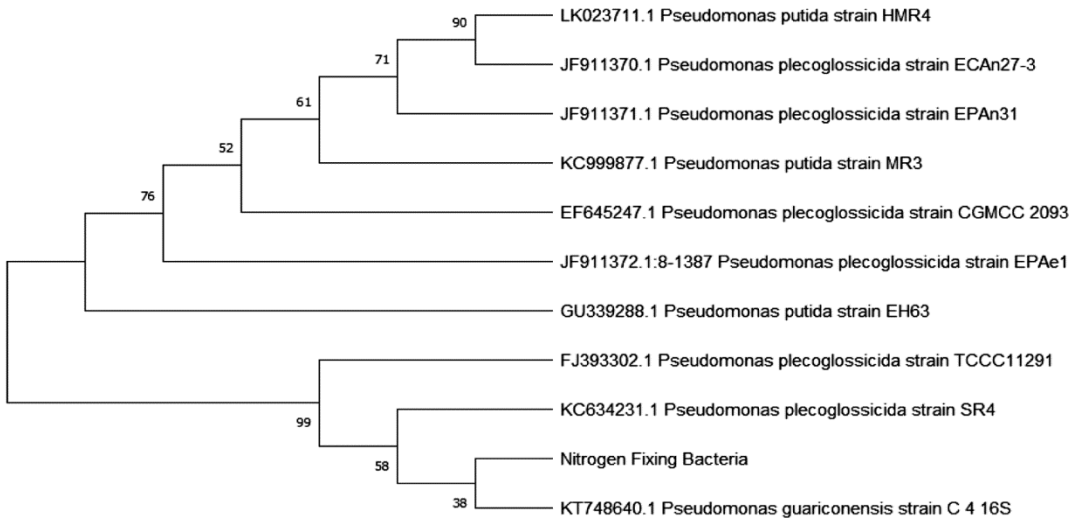

Fig. 4 — The phylogenetic relationship between nitrogen–fixing bacteria (N3 isolate) and specific bacteria based on the 16S rRNA gene sequences of closely related species

Likelihood method by using MEGA11.0 software, the N3 isolate showed a 99% identity with *Pseudomonas plecoglossicida strain* with accession number (FJ3933021) and a total score of 2532 obtained from the BLAST studies. *In* comparison to *Pseudomonas guariconensis* C_416S ribosomal RNA, the total score is 2527. Here in the phylogenetic tree, the distant bacteria are *Pseudomonas plecoglossicida* ECAn27–3.

The RNA structure of the 16S rRNA sequences was predicted using UNAFold software to understand the stability of the gene sequences, usually calculated and expressed in terms of Gibbs free energy. As UNAFold.4 uses the nearest–neighbour energy rule to gauge the energy of the structure, Fig. 5 shows the RNA secondary structure of *Pseudomonas plecoglossicida* strain TCCC119291 with free energy observed to be $\Delta G = -514.79$ kcal/mol. Initially, found to be −570.2 kcal/mol. The RNA secondary structure of our isolate and the value obtained for ΔG is −468.28 kcal/mol and the initial ΔG was −525.3 kcal/mol, respectively. Gibbs free energy of both structures specifies the stability of the fold and provides the minimised energy structure, but sometimes can deviate from the natural complexities of the system. The calculation is proof of the stability of nucleotides in the novel organisms. Thus, the RNA fold structure explains the closeness of the two strains.[31]

Germination Assay of Pea Seedlings

N3–coated pea seeds reduced the incidence of the disease by 50%–60% in pea seedlings on germination when compared to the control (Fig. 6).

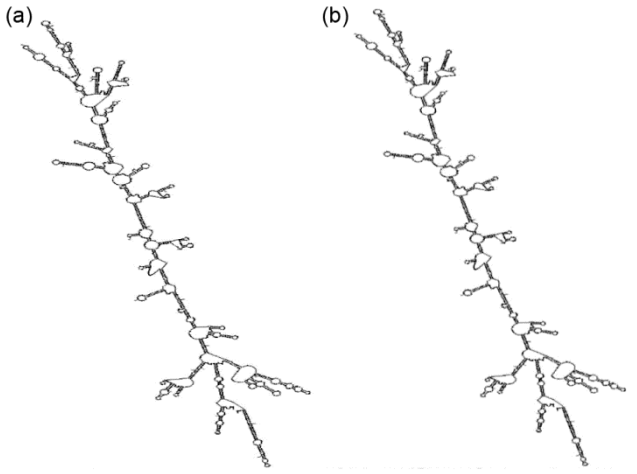

Fig. 5 — RNA structure prediction of *Pseudomonas plecoglossicida*: (a) strain TCCC119291 (b) and N3 isolate

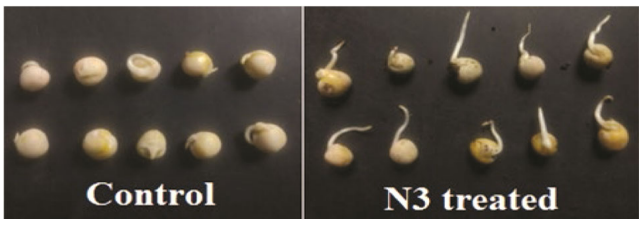

Fig. 6 — Seed germination assay of control, N3 isolate treated *Pea* seedlings

The N3 and vermicompost tea treatments of the pea seedlings showed a high vigour index, high percentage of germination, and high seed fresh weight on comparison to the control seedlings. PGPR inoculation of pea seeds showed increased root and shoot length owing to the increase in the production of gibberellins

Table 2 — Effect of N3 isolate and vermicompost tea treatments challenged with *F. oxysporum* on the vigour index, disease incidence, germination percentage and fresh weight of pea seedlings; (A batch of forty germinated pea seedlings was taken, and the mean standard error of three replicates represented the data).

Treatment	Germination (percentage)	Incidence of disease (percentage)	Vigor index	Fresh Seed weight (g)
Control	55.40 ± 1.09	12.90 ± 0.27	1444 ± 66	1.09 ± 0.011
Vermicompost tea	79.08 ± 1.29	2.19 ± 0.12	1776 ± 72	1.55 ± 0.022
N3 isolate	73.98 ± 1.09	2.29 ± 0.41	1744 ± 26	1.57 ± 0.065

Defence Enzyme Induction in Pea Seedlings

The activities of peroxidase, *β*–1,3 glucanase, Phenylalanine lyase and chitinase were monitored in various treatments at every stage of pea seedlings' growth, showing high variation between the control and treated plants. Consistently, PGPR inoculated pea plants with N3 isolate possessed nearly 1.5–2.0 times higher activity of the defense enzymes compared to control plants. Among the three PGPR (N2, N3, and N5) tested, the N3 isolate was adequate in inducing elevated activities of these enzymes. When three treatments were administered to investigate the induction of systemic resistance in pea plants, the activity was not further increased.

Vermicompost tea and N3 treatments were found to incite Phenylalanine Ammonia–Lyase (PAL) activity significantly in pea plants (Fig. 7c). Peroxidase activity was also shown to be induced notably in both the treated pea plants after seven days (Fig. 7a). While pea plants challenged with the pathogen *Fusarium oxysporum*, the activities of PAL and peroxidase improved after 7 days. The prompt establishment of these defence parameters in plants treated with N3 and vermicompost indicated the presence of Induced Systemic Resistance (ISR). Elevated levels of both β–1,3–glucanase and chitinase were observed within a week in the plants treated with vermicompost and N3, distinguishably from the control group and pea plants infected with *Fusarium oxysporum* after just 5–6 days. The overall defence enzyme activity profile was higher in treated plants. According to the findings mentioned, the activities of peroxidase, chitinase, β–(1, 3) glucanase, and PAL demonstrated a significant increase as a result of the treatment with the PGPR strain. This could help us understand how microorganisms that encourage plant development affect plants that produce defensive enzymes. Therefore, it can be said that one of the primary causes of the increased growth of pea plants may be the N3 isolate identified as *P plecoglossicida.*

The protein expression analysis (Fig. 8) done by SDS–PAGE in control N3 and vermicompost-induced

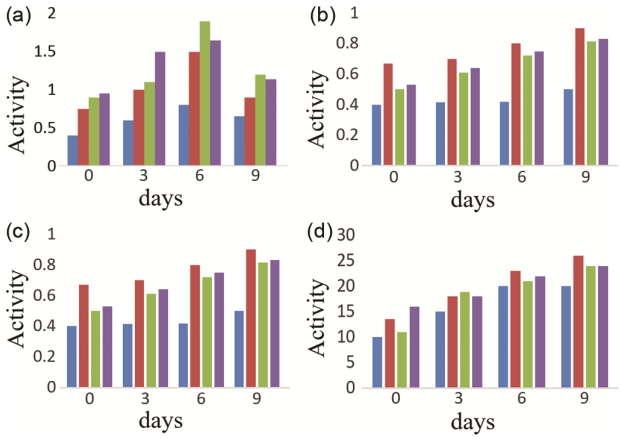

Fig. 7 — Plant defense enzyme activities observed in the pea seedlings over nine days (a) Peroxidase (ΔA unit/min/mg of protein) (b) β–1, 3 Glucanase (µmol of glucose /min/mg of protein) (c) Phenylalanine ammonia lyase(µmol T–cinnamic acid/min/mg of protein) (d) Chitinase (µg of N-acetyl glucoseamine formed /min/mg of protein) (Blue: Control seedlings, Red: N3 treated, Green: N5 treated, and Purple: N2 treated, Enzyme activity values were the mean of three replicates).

seedlings for a growth period of 3 days and 6 days showed high protein expression in the bandwidth of 10 kDa, 14 kDa, 24 kDa, 33 kDa, 44 kDa, 47 kDa, 50 kDa, and 95 kDa as compared to the control extract. These PGPR–induced protein expression results observed were similar to and comparable to studies reported in rice cultivars infected with the sheath blight pathogen[30] and in *Erwinia caratovara* subsp. *carotovora–challenged* tomato plants.[31] The results were also comparable to nematode *Globodera rostochiensis* challenged potato plants[32] and groundnut plants treated with *Pseudomonas guariconensis* challenged with *A. niger*. The protein expression profile correlates to Thaumatin type protein of 24 kDa, β–1,3glucanase of 33 kDa and chitinase of 47 kDa, 50 kDa. This preliminary analysis needs to be validated through the characterisation of the induced proteins by activity staining of the defence enzymes. This validation study would enable us to understand the molecular mechanism of resistance and gain a better insight into the molecular mechanism of PGPR–induced resistance in plants.

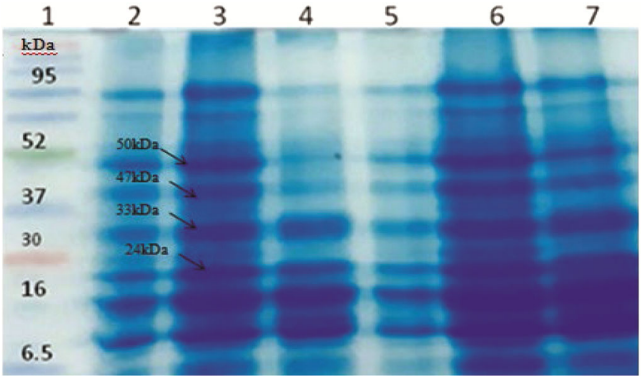

Fig. 8 — SDS Polyacrylamide gel electrophoresis of expressed proteins of untreated control, vermicompost and N3-treated pea seedlings; Lane1: BLUltra Prestained protein ladder, Lane 2: protein extract of control seedlings for 3 days, Lane 3: Protein extract from N3 treated seedlings that have matured for 3 days–4 days, Lane 4: Protein extract from seedlings treated with vermicompost grew to full size in 3–4 days, Lane 5: Protein extract of control for 6 days, Lane 6: protein extract of N3 treated seedlings grown for 6 days, Lane7: Protein extract of vermicompost treated seedlings for 6 days.

Discussion

Fusarium oxysporum is extremely infectious and leads to *Fusarium* wilt in pea plants. The pathogen endures on agricultural residues and within the soil as chlamydospores for 5 – 6 years, even without any vulnerable host, and reproduces using contaminated soil and seeds. Previous management of *Fusarium* wilt in chickpeas has been challenging since no single direct approach is fully effective. While employing a resistant legume variety is the most economical and effective control measure, the effectiveness of Fusarium wilt resistance is restricted by the presence of nine evolutionarily diverse pathogenic species in FO[33], in contrast to the strains of *Bacillus spp*., *Trichoderma spp*., and *Pseudomonas spp*., which were isolated from the rhizosphere of crops. Non–pathogenic isolates from compost effectively manage plant pathogens and help vegetation in mobilising and acquiring nutrients.[34] This new group of microorganisms with plant growth–promoting and biocontrol properties is typically more prevalent in forest pasture soils and herbal composts than in arable soils.[34] Thus, herbal or vermicompost may be the optimal option for sourcing PGPR.

Understanding PGPR mechanisms is a key to the development of sustainable agricultural practices. Germination delay, slow emergence and delayed plant growth characteristics induced by biotic stress factors that lead to an increased risk of root rot infection, primarily caused by *Botrytis, Pythium, Fusarium, and Rhizoctonia solani*, causing yield loss in various crops such as maize, oats, canola, soy, potatoes and lentils.[35] The previous studies indicate *Bacillus and Pseudomonas spp* to be the predominant PGPR among various genera, whereas not much was reported for pea plants. This is the motivation to explore the isolation, identification, and characterisation of a potent strain of PGPB from vermicompost tea. It might protect the pea plants from the *Fusarium oxysporum* pathogen that leads to root rot disease and encourage plant growth. PGPRs from the *Pseudomonas* genus, acting as biocontrol agents, are shown to be resistant to bacterial or fungal pathogens. The transcriptome analysis of *P. fluorescences* WcS417r exhibited substantial gene expression changes in roots. Detailed analysis showed that among five isolates of active PGP, the N3 isolate emerged as a promising PGPR with a better nitrogen–fixing ability and better plant growth–promoting molecular profile.

Germination assay results show that N3 and vermicompost tea–treated pea seeds were vigorously germinating compared to control seeds, and disease resistance was reduced by 40%. One of the reasons would be cytokinin, e.g. Zeatin production by N3 isolate and vermicompost, and it is documented especially in *Pseudomonas fluorescence.*[36] Gibberellins and cytokinins production has been demonstrated in several PGPR noted to *Bacillus spp.* and *Azobacter spp.*[37] Under the investigational conditions, seed bacterization also showed the same results. As the mechanisms of plant growth promotion by these bacteria are uncovered, the potential for developing new applications through additional plant–PGPB pairings will be explored. This study demonstrates the extraction of plant growth–promoting bacteria from vermicompost tea and evaluates various PGPR characteristics through *in vitro* screening in controlled pot experiments.

Plants have multiple robust defence strategies that react to biotic stress, with the interplay of induced systemic resistance and systemic acquired resistance enhancing protection against pathogens.[38] The activation of defences in peas through N3 isolate and various vermicompost treatments primarily involves defence enzymes, including peroxidase, phenylalanine ammonia–lyase, chitinase, and β–1, 3 glucanase.[39] Activities of peroxidase and chitinase in mature plants are likewise stimulated by fungi functioning as PGPR.[40] The SDS –PAGE profile of

induced proteins in N3 and vermicompost–treated pea plants indicated that the expression is high in the case of treated compared to control, and the results were supported by the activity profile of the defence enzymes. Further identification and overexpression would pave the way to understand the induced resistance by PGPR. The results add to the hypothesis that *Ps. plecoglossicida* functions as a mycorrhiza helper bacterium.[41] In conclusion, the N3 isolate was proven to possess PGP properties and lessen the incidence of disease by 55%–70%. These region–specific PGPRs can save the farmers from the problems of costly chemical fertilisers by contributing comparatively to low–cost alternate bio–fertilizers while reducing soil degradation and in that way making agriculture and horticulture sustainable.

Conclusions

The present study demonstrates that vermicompost tea is a rich source of beneficial plant growth–promoting bacteria (PGPB). Fifteen nitrogen-fixing bacterial colonies were isolated from vermicompost tea, among which five isolates (N1–N5) showed promising plant growth–promoting traits. Among them, the N3 isolate exhibited the highest nitrogen fixation, phosphate solubilization, siderophore production, IAA production, and strong antifungal activity against plant pathogens such as *Fusarium oxysporum*. Molecular characterisation using 16S rRNA gene sequencing identified the N3 isolate as a strain closely related to *Pseudomonas plecoglossicida*, which was designated as strain Yank1. The isolate significantly improved pea seed germination, vigour index, and fresh seed weight while reducing disease incidence. Additionally, N3 treatment enhanced defence enzyme activities, including peroxidase, phenylalanine ammonia-lyase, β-1,3-glucanase, and chitinase, indicating the induction of systemic resistance in plants. Pot experiments further confirmed enhanced plant growth and reduced disease severity. Overall, the study highlights the potential of vermicompost-derived *Pseudomonas plecoglossicida* strain Yank1 as an effective biofertilizer and biocontrol agent on pea plants for sustainable agriculture.

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