

***Providencia vermicola* AAU PR1- A New Bioinoculant for Agriculture with Multiple Utility**

Panpatte Deepak G.,* Shelat Harsh N., Jhala Yogeshvari K. and Vyas Rajababu V.

Department of Agricultural Microbiology, B. A. College of Agriculture,
Anand Agricultural University, Anand-388110, Gujarat, India

*Corresponding Author E-mail: dgpanpatte@gmail.com

Received: 22.06.2020 | Revised: 30.07.2020 | Accepted: 8.08.2020

ABSTRACT

In present investigation isolation and characterization of native Providencia vermicola was attempted from rhizospheric soil of sickplot, Model farm, AAU, Vadodara, Gujarat. From the overall morphological, biochemical and 16S rDNA sequencings, the isolate was identified and designated as Pr. vermicola AAU PR1. The isolate showed positive PGPR traits viz. phosphate solubilisation, potash solubilisation, IAA production and ACC deaminase production. Moreover, the isolate also showed inhibition of hatching of M. incognita eggs after 24, 48 and 72 hrs. of exposure. The isolate was also showed inhibition of multiple fungal pathogens viz. A. alternate (ITCC-5503), A. alternate (ITCC-6134), A. alternate (ITCC-7067), A. niger (ITCC-6202), A. niger (ITCC-6738), M. phaseolina (ITCC-6734), M. phaseolina (ITCC-6749), P. aphanidermatum (ITCC-5488), F. solani (ITCC-6846), F. udum, F. oxysporum. The mechanism behind the biocontrol potential of the strain includes its ability to produce siderophores, cell wall degrading enzymes viz. and cellulose, chitinase and lipase as well as volatile compound hydrogen cyanide. To the best of our knowledge this is the first time report wherein both biocontrol and plant growth promoting traits were found positive a strain of Pr. Vermicola.

Keywords: *Providencia vermicola, PGPR, Meloidogyne incognita, biocontrol, chitinase, hydrogen cyanide.*

INTRODUCTION

In present era of organic farming peoples are becoming well aware about the negative effects of agro-chemicals on soil and human health. In recent years, scientists have diverted their attention towards exploring the potential of beneficial microbes, for nutrient management and plant protection. Bio-inoculants are easy to deliver, improve plant

growth, activate resistance mechanism in the host, increase biomass production and yield. Searching out the newer strains with multiple utility as biofertilizers and biocontrol can widen the scope of the microbial inoculant. Since registration of biocontrol agents for commercial availability is very slow, exploring the PGPR strains with biocontrol and biofertilizer is an acceptable green approach.

Cite this article: Panpatte, D.G., Shelat, H.N., Yogeshvari, J.K., & Vyas, R.V. (2020). *Providencia vermicola* AAU PR1- A new bioinoculant for agriculture with multiple utility, *Ind. J. Pure App. Biosci.* 8(5), 185-194. doi: <http://dx.doi.org/10.18782/2582-2845.8160>

In addition, the present day bio-products can be further improved to obtain greater levels of nutrient management and disease reduction. These antagonists act through antibiosis, secretion of volatile toxic metabolites, mycolytic enzymes, parasitism and through competition for space and nutrients.

Bacterial genera *Pseudomonas*, *Bacillus*, *Streptomyces* and *Agrobacterium* are bio control agents, which has been commercialized and marketed. Till date none of the product available in market based on *Providencia* genus. Some of the researchers have explored the potential of *Pr. Vermicola* as plant growth promoter in various crops viz. Cauliflower (*Brassica oleracea* var. *botrytis* L.) and Cabbage (*Brassica oleracea* var. *capitata* L.) (Gowtham et al., 2015), Rapeseed (Hussain et al., 2015), Rubber (Hidayati et al., 2014). Only one report is available representing the potential of *Pr. vermicola* to inhibiting egg hatching of *M. incognita* in tomato (Ammar et al., 2015), whereas none of the report is there showing inhibition of phytopathogenic fungi by *Pr. vermicola*.

In present study, we have isolated and characterized new strain of *Pr. vermicola* with both PGPR and biocontrol traits which is the first time report wherein single strain of *Pr. vermicola* was sowed positive PGRP traits as well as biocontrol potential against phytopathogenic nematodes and soil borne fungi.

MATERIALS AND METHODS

Collection of soil samples

For isolation, suppressive soil samples (rhizospheric and non-rhizosphere soil) were collected from the Model Farm and from Diploma school, AAU, Vadodara.

Isolation of *Pr. vermicola*

Isolation of *Pr. vermicola* strains was carried out on Nutrient agar media by serial dilution technique. The dominant colonies on the nutrient agar plates were further purified and characterized on the basis of morphological, cultural and biochemical characteristics. For identification and phylogenetic relationship of the potential isolates, 16S *rDNA* sequencing

was carried out as described by Waturangi et al. (2011).

Genomic DNA extraction: The isolate was grown in Luria broth for 24 hrs, and genomic DNA was extracted by C-TAB method. The integrity and concentration of purified DNA was determined by agarose gel electrophoresis. The total genomic DNA extracted was dissolved in sterile distilled water and stored at 4°C.

DNA Sequencing Analysis: DNA fragment from five isolates were chosen to be sequenced based on the dominant isolates that isolated from soil samples. PCR product was purified using DNA Gel Extraction Kit (Banglore Genei, India). Purified products were sequenced using big dye terminator with ABI PRISM™ model 3130 Genetic Analyzer. The sequence data of bacterial isolate was compared to sequence from GenBank in National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov) using BLASTN program for identification and phylogenetic analysis of the isolates.

Plant growth promoting traits of isolate Phosphate solubilizing activity

The isolate was spot inoculated on Sperber's media supplemented with Tri-calcium Phosphat (TCP) as insoluble form of phosphorous to evaluate its phosphate solubilisation potential. Furthermore, erlenmeyer flasks (250 ml) containing 100 ml of the liquid Sperber's medium was inoculated with 100 µl of bacterial suspension (approx. 10⁷ cfu/ml). The assay was performed in triplicate keeping one uninoculated control. The flasks were incubated on rotary shaker (150 rpm) at 30± 2°C. After 7 days, measurement of liberated P following Vanado-molybdate method was carried out. The graph of OD versus concentration of phosphate in µg was plotted for the standard and samples were compared to calculate P concentration.

Potash solubilisation activity

Bacterial strain was spot inoculated on Alendreskov's media containing mica as a raw insoluble potash substrate to check their potash solubilization activity (Hu et al., 2006). Plates were incubated at 28±2°C and examined for

development of clear halo of potash solubilization around colonies.

Fifty (50) ml of Aleksandrov media in 100 ml Erlenmeyer flask was taken with mica. Flasks were autoclaved at 121°C for 15 min and medium was allowed to cool and inoculated with 1ml of bacterial suspension (10^8 cells/ml) and incubated at $30\pm 2^\circ\text{C}$ for 10 days. After 5 days each 50 ml flask was harvested and checked for potassium release by centrifuging at 10,000 rpm for 10 min and supernatant was retained. 1ml of supernatant was taken in 50 ml volumetric flask and volume was made to 50 ml with distilled water and mixed thoroughly. After that the solution was fed to flame photometer for K released from the mineral feldspar and mica individually by bacterial suspension.

Indole acetic acid (IAA) production

In vitro IAA production by selected isolates was determined using the protocol described by Khalid et al. (2004). For this purpose, 10 ml Glucose Phosphate Broth (GPB) medium was prepared in 100 ml Erlenmeyer flasks, autoclaved and cooled. L-Tryptophan was filter sterilized passing through 0.2 μm membrane filter and added at desired concentration (15 $\mu\text{g/ml}$) to the liquid medium. One flask without tryptophan was also kept for comparison. The flasks were inoculated with 1.0 ml of 3-days old bacterial broth (10^7 CFU/ml) and incubated at $30\pm 2^\circ\text{C}$ for 48 h. Un-inoculated control was kept for comparison. After incubation, the contents were filtered through Whatman filter paper No. 2. For measuring IAA, 3.0 ml of filtrate was taken in test tube and 2.0 ml of Salkowski reagent was added. The contents in the test tubes were allowed to stand for $\frac{1}{2}$ h for color development. Similarly, color was also developed in standard solutions of IAA. The intensity of color was measured at 535 nm by spectrophotometer. Standard curve was prepared and used to calculate IAA produced by isolate.

ACC-deaminase activity

Qualitative screening of bacterial isolate for ACC deaminase enzyme production was carried out based on their ability to use ACC

(1-Aminocyclopropane-1-Carboxylate) as a sole nitrogen source in the sugar free minimal salt medium. Cultures were spot inoculated on petri plates containing DF salt minimal medium supplemented with 3 mM ACC substrate. Plates containing DF minimal medium without ACC served as negative control and with $(\text{NH}_4)_2\text{SO}_4$ (2.0 gm/l) as a nitrogen source serve as positive control. The plates were incubated for 3-4 days at $30\pm 2^\circ\text{C}$. Growth of isolates on ACC supplemented plates was compared with positive and negative control plates. Isolates grown well on ACC plates were considered as ACC deaminase enzyme producers (Daun et al., 2009).

Determination of nematicidal potential

In vitro study was carried out to test efficacy of isolate on egg hatching of *M. incognita* J2 under laboratory conditions with treatment comprising of sterile distilled water as control. For this experiment, 259 freshly hatched *M. incognita* J2 (Average of 3 egg mass) were transferred to 2.5cm diameter petri dishes containing 1 ml of bacterial broth (10^8 cells/ml) separately. Petri dishes were maintained at $28 \pm 2^\circ\text{C}$ in an incubator. Immobilized J2 were counted under stereoscopic microscope at 60X magnification after 24, 48 and 72 h of incubation. Each treatment was replicated 3 times.

Determination of fungistatic activity

Phytopathogenic fungal cultures *viz.* *A. alternata* (ITCC-5503) from chilli, *A. alternata* (ITCC-6134) from tomato, *A. alternata* (ITCC-7067) from potato, *A. niger* (ITCC-6202) from Citrus reticulate, *A. niger* (ITCC-6738) from sorghum seeds, *M. phaseolina* (ITCC-6734) from tomato seeds, *M. phaseolina* (ITCC-6749) from soybean seeds, *P. aphanidermatum* (ITCC-5488) from ginger rhizome, *F. solani* (ITCC-6846) from chilli roots were procured from Indian Type Culture Collection, IARI, New Delhi and revived on PDA media. Isolate was tested *in vitro* for their biocontrol potential by dual inoculation technique (Foldes et al., 2000). Inhibition of fungal growth was recorded at 5th and 7th days after co-incubation and compared

with normal fungal growth. The radial growth of mycelium was measured and per cent

inhibition (PI) was calculated as follows.

$$\text{Per cent inhibition (PI)} = \frac{(C - T)}{C} \times 100$$

Where, C is the growth of test pathogen (mm) in the absence of the antagonistic isolate.

T is the growth of test pathogen (mm) in the presence of the antagonistic isolate.

Biocontrol traits

Siderophore production

The production of siderophore by isolate was assessed through plate assay. Chrome Azurol S blue agar medium (CAS) was used to detect siderophore production by the isolates (Alexander & Zuberer, 1991). The overnight grown test bacterial cultures were spot inoculated on individual CAS plates and incubated at 30±2°C for 24 h. The cultures showing yellow to orange colored ring around the colonies were considered as siderophore production positive.

Production of cell wall degrading enzymes

The lipolytic activity was determined by streaking isolates on Tributyrin agar plates (Lawrence et al., 1967). The chitinolytic activity was determined by streaking bacterial isolates on Nutrient agar medium supplemented with 0.2 % colloidal chitin (Sessitsch et al., 2004). Zone of chitin digestion was considered as positive result. The cellulase activity was determined by streaking bacterial strain on cellulose agar plate (Ibrahim & El- Diwany, 2007).

RESULTS AND DISCUSSION

Isolation and characterization of *Pr. vermicola* AAU PR1

Colonial characteristics of the isolate are noted in Table 1. The isolate was Gram -ve, short rod occurring singly. Isolate was found to be positive for ONPG, Lysine utilization, Ornithine utilization, Urease, Phenyl alanine deamination, Nitrate reductase, Citrate utilization, Methyl red, Indole as well as production of amylase, oxidase and gelatinase enzymes. Among multicarbon compounds tested isolate was found to utilize Maltose,

Arabinose, Xylose, Rhamnose, Cellobiose, Melibiose, Sachharose, Raffinose, Trehalose, Glucose, Fructose, Galactose, Mannose, Sodium gluconate, Glycerol, Dulcitol, Inositol, Mannitol, Adonitol, Arabitol, α-methyl D-gloside and Melezitol. From 16S *rRNA* partial gene sequence isolate PR1 was identified as *Pr. vermicola* with 99 % identity and 100 % query coverage to *Providencia vermicola* strain OP1 (Accession No. NR_042415.1) (Figure 1). The sequence has been deposited to NCBI, USA vide accession number KJ161325.

As a source of isolation rhizospheric soils from sick plots were selected as the chances of getting efficient biocontrol bacterial strains are maximum under naturally suppressive soil because natural suppressive soils contain mixture of microbial communities with variable modes of actions to inhibit the plant pathogens. Till date efforts were made to isolate the *Providencia* spp. from animal or human wastes, entomopathogenic nematodes as well as rhizospheric soils of various crops. In present study we have isolated *Pr. vermicola* strain from naturally suppressive soil. Further it is important to identify the isolate through biochemical and molecular characterization. Besides biochemical characterization, the sequencing of the 16S ribosomal RNA (rRNA) gene is another identification technique used for phylogenetic placement, identification and diversity analysis of bacteria. Based on the results obtained from overall biochemical and molecular studies the isolate was identified to be the new strain of *Pr. vermicola*. Somvanshi et al. (2006) *Pr. vermicola* sp. nov., isolated from infective juveniles of the entomopathogenic nematode *Steinernema thermophilum*. All strains were positive for tryptophan deaminase, indole production, acetoin production, fermentation/oxidation, catalase positive as well as positive for

utilization of ribose, glucose, fructose, mannose, N-acetylglucosamine and gluconate.

Plant growth promoting traits of isolate

The isolate showed 2.8 mm zone of P solubilisation on Sperber's medium and showed 168.58 µg/ml P release from TCP in liquid medium at 7 DAI. Similarly isolate showed 2.0 mm zone of K solubilisation on Aleksandrov's medium and release of 2.45 µg/ml K from mica in liquid media at 5 DAI. Moreover, both the isolates produced considerable amounts of IAA in tryptophan supplemented medium (Table 2). Isolate also showed production of growth hormone Indole 3 acetic acid 4.05 µg/ml in without tryptophan and 17.50 µg/ml in presence of 15 ppm tryptophan in media. Isolate was found to grow luxuriously on control plates containing (NH₄)₂SO₄ as nitrogen source, whereas, grew poorly on plates containing nitrogen free media. Moreover, the strain showed luxurious growth on plates having ACC as sole source of nitrogen showing their ability to produce enzyme ACC deaminase (Table 2).

The isolate was found positive for plant growth promoting traits *viz.* phosphate solubilisation, potash solubilisation, IAA production as well as ACC deaminase production. It is known that Phosphorus and potash are found to be the major limiting factors for crop productivity. Conversely, a huge amount of fixed P is present in acidic and alkaline soils in the form of Ca-phosphates, and Fe and Al-phosphates, respectively (Ahemad & Kibert, 2014) which is to be made available to plant through microorganisms. Similarly IAA plays a key role in cell division and growth in addition to its significance root proliferation (Vassilev et al., 2006; & Seo & Park, 2009). Results indicate that that the isolate may reduce ethylene concentration in the plants during various biotic and abiotic. As it possess enzyme ACC deaminase which enables the plant growth and development under stress condition by decreasing plant ethylene level and thereby protecting plants from stress. So this phenomenon may be a key to reduce the post infection stress in the plant after pathogen infection which may increase

the capacity of the plants to withstand biotic stress.

Nematicidal potential against *M. incognita*

Pr. vermicola AAU PR1 showed reduction in egg hatching 670, 658 and 652 at 24, 48 and 72 hrs post inoculation as compared to water control showing 172, 165 and 157 at 24, 48 and 72 hrs post inoculation respectively (Table 3). Results revealed that there was a pronoun effect of the bacteria on egg-masses hatching under the light microscope. It was observed that the gelatinous matrix turned dark brown to black color. Further examination under the light microscope showed that there were less numbers of hatching juveniles either motile or immotile. Similarly, Ammar et al. (2015) in pots experiment under the greenhouse conditions, tomato seedlings inoculated by the dark brown egg-masses failed to exhibit any root galls. The inhibitory effect of bacteria *P. vermicola* against the plant pathogenic nematode *M. javanica* was examined under the greenhouse condition. The obtained results showed that there were no any developed symptoms on tomato roots. As strains was isolated from rhizospheric region it colonize the rhizosphere aggressively wherein competition with root pathogens for nutrients and root surface colonization has been proposed as an important trait for biological control, although in recent years its significance has been debated (Haas & Defago, 2005). Competition may concern the aquisition of organic substrates released by seeds and roots (Kamilova et al., 2005), as well as micronutrients such as soluble iron, which is often in limiting amounts in soil. Iron acquisition entails the production of iron transporters (siderophores). Once complexed to ferric iron in soil or the root zone, the siderophores are then taken up using outer membrane receptors. In a context of biological control, competition for iron involves the synthesis of siderophores of higher affinity compared with siderophores used by phytopathogens. Similarly another biocontrol trait responsible for observed nematicidal and antifungal activity may be production of cell wall degrading enzymes such as cellulase,

chitinase and lipase which can degrade important cell wall components of phyto-pathogens.

Fungistatic activity against phyto-pathogenic fungi

Fungal growth was measured at 3 DAI and 5 DAI, wherein *Pr. vermicola* AAU PR1 showed a wide range of inhibitory effect against tested fungal pathogens (Table 1). In particular, *Pr. vermicola* AAU PR1 (KJ161325) exhibited high (100 %) inhibitory effect on *A. niger* (ITCC-6738) and *P. aphanidermatum* (ITCC-5488), whereas failed to inhibit *A. alternate* (ITCC-5503), *A. alternate* (ITCC-7067) and *M. phaseolina* (ITCC-6734) (Table 4). In present study to the best of our knowledge and based on the literature surveyed this is the first time report wherein *Pr. vermicola* found as fungistatic or inhibitory to phyto-pathogenic fungi.

Biocontrol traits of isolate

The isolate produced yellow-orange color zone (3.2 mm) on CAS agar plate hence considered as siderophore producers. Siderophore bind most of the available iron (Fe^{+3}) in rhizosphere

thereby preventing any fungal pathogen in immediate vicinity from proliferation due to lack of iron (Glick & Basan, 1997). The isolate was also found positive for production of cell wall degrading enzymes viz. lipase, chitinase and cellulase. Diby et al. (2005) showed that biocontrol bacterial strains found efficient in suppressing root rot of black pepper (*Piper nigrum* L.), caused by *Phytophthora capsici*, were tested *in vitro* for their efficacy in lysing the cell wall of the pathogen, *P. capsici*. The antagonists produced mycolytic enzymes viz. β -1,3-glucanases, β -1,4-glucanases and lipases. Similarly biocontrol bacterial strains isolated from the rhizosphere of chickpea (*Cicer arietinum* L.) and green gram (*Vigna radiata* L.) produce appreciable amounts of cellulose and chitinase enzyme in culture-free supernatants and showed growth inhibition of the two fungi *Pythium aphanidermatum* (Oomycete) and *Rhizoctonia solani* (Basidiomycete) in plates on potato dextrose agar medium which was correlated with cell wall degrading enzyme production (Sindhu & Dadarwal, 2001).

Table 1: Characterization of *Pr. vermicola* AAU PR1

Tests	<i>Pr. vermicola</i> AAU PR1
Colonial characteristics	
Shape	Round
Margin	Entire
Elevation	Slightly raised
Texture	Smooth
Opacity	Opaque
Pigmentation	Off white
Morphological characteristics	
Shape	Short Rod
Arrangement	Single
Gram's reaction	G -ve
Biochemical characteristics	
ONPG	+
Lysine utilization	+
Ornithine utilization	+
Urease	+
Phenyl alanine deamination	+
Nitrate reductase	+
H ₂ S production	-
Citrate utilization	+
Voges proskauer's	-
Methyl red	+

Indole	+
Malonate utilization	-
Esculine hydrolysis	-
Maltose	+
Arabinose	+
Xylose	+
Adonitol	-
Rhamnose	+
Cellobiose	+
Melibiose	+
Sachharose	+
Raffinose	+
Trehalose	+
Glucose	+
Fructose	+
Galactose	+
Sucrose	-
Mannose	+
Inulin	-
Sodium gluconate	+
Glycerol	+
Salicin	-
Dulcitol	+
Inositol	+
Sorbitol	-
Mannitol	+
Adonitol	+
Arabitol	+
Ethythretol	-
α -methyl D-gloside	+
Melezitol	+
Gelatinase production	+
Amylase production	+
Oxidase production	+

Keys: +: positive test, -: negative reaction

Table 2: Plant Growth Promoting traits of *Pr. vermicola* AAU PR1

Name of culture	Plant Growth Promoting traits						
	Nutrient solubilisation				Growth Hormone production		Stress relieving enzyme production
	Phosphate solubilisation		Potash Solubilisation		Concentration of IAA ($\mu\text{g/ml}$)		ACC deaminase Production
	Zone on Sperber's media with TCP as P source at 7 DAI (mm)	Release of P from TCP at 7 DAI ($\mu\text{g/ml}$)	Zone on Aleksandrov's media with mica as K source at 5 DAI (mm)	Release of K from Mica at 5 DAI ($\mu\text{g/ml}$)	Without tryptophan	With 15 ppm tryptophan	
<i>Pr. vermicola</i> AAU PR1	2.8	168.58	2.0	2.45	4.05	17.50	+

Table 3: Nematicidal activity of *Pr. vermicola* AAU PR1 against *M. incognita* after 24, 48, 72 h. of exposure

Tr. No.	Treatment details	No. of J ₂ after exposure					
		24 h.		48 h.		72 h.	
		Hatched	Unhatched	Hatched	Unhatched	Hatched	Unhatched
T ₁	<i>Pr. vermicola</i> AAU PR1	102 (10.07)	670 (25.87)	116 (10.73)	658 (25.66)	120 (10.91)	652 cd (25.54)
T ₂	Sterile Distilled water	598 (24.44)	172 (13.11)	603 (24.56)	165 (12.83)	609 (24.67)	157 (12.51)

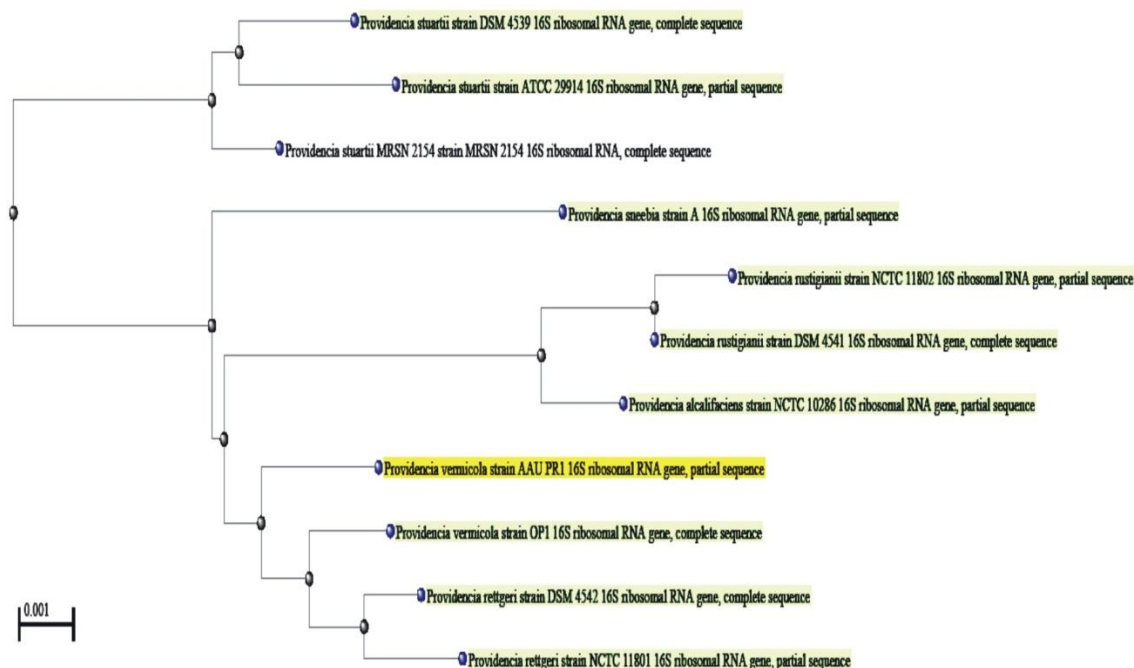
Note: Initial count 259 eggs/egg mass (Average of 3 egg mass)

Table 4: *In vitro* antagonistic activity of native biocontrol bacteria against phyto- pathogenic fungi

Fungal Pathogen	Inhibition of fungal pathogen (%)	
	3 DAI	5 DAI
<i>A. alternate</i> (ITCC-5503)	0.00	0.00
<i>A. alternate</i> (ITCC-6134)	0.00	73.33
<i>A. alternate</i> (ITCC-7067)	0.00	0.00
<i>A. niger</i> (ITCC-6202)	11.11	40.00
<i>A. niger</i> (ITCC-6738)	84.62	100.00
<i>M. phaseolina</i> (ITCC-6734)	0.00	1.64
<i>M. phaseolina</i> (ITCC-6749)	9.52	11.29
<i>P. aphanidermatum</i> (ITCC-5488)	100.00	100.00
<i>F. solani</i> (ITCC-6846)	9.68	10.77
<i>F. udum</i>	2.35	17.63
<i>F. oxysporum</i>	10.35	24.17

Table 5: Biocontrol traits of *Pr. vermicola* AAU PR1

Name of culture	Siderophore production	Production of cell wall degrading enzymes			HCN production
	Zone diameter (mm)	Lipase	Cellulase	Chitinase	
<i>Pr. vermicola</i> AAU PR1	3.2	+	+	+	+

**Fig. 1: Phylogenetic tree based on 16S rDNA sequence of isolate AAU PR1**

CONCLUSION

The results of present study clearly brought out that, the *Pr. vermicola* isolate having plant growth promoting ability as well as biocontrol activity against phyto-pathogenic nematodes and fungi. These novel indigenous isolate indicated wide scope and prospects as agriculturally beneficial bioinput to reduce chemical inputs for sustainable agriculture in a long run.

REFERENCES

- Ahemad, M., & Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *Journal of King Saudi University of Sciences*, 26, 1–20.
- Alexander, D. B., & Zuberer, D. A. (1991). Use of chrome-azurol-S reagents to evaluate siderophore production by rhizosphere bacteria. *Biology and Fertility of Soils*, 12, 39–45.
- Ammar, A., Youssef, S. A., & Masoud, S. I. (2015). A potential biocontrol and PGPR activities of bacteria *Providencia vermicola* against root knot nematode *Meloidogyne javancia*. *Journal of Nematology*, 47(3), 218–281.
- Daun, J., Muller, K. M., Charles, T. C., Vesely, S., & Glick, B. R. (2009). 1-Aminocyclopropane-1-Carboxylate (ACC) Deaminase genes in Rhizobia from sourthen Saskatchewan. *Microbial Ecology*, 57, 423-436.
- Diby, P., Saju, K. A., Jisha, P. J., Sarma, Y. R., Kumar, A., & Anandaraj, M. (2005). Mycolytic enzymes produced by *Pseudomonas fluorescens* and *Trichoderma* spp. against *Phytophthora capsici*, the foot rot pathogen of black pepper (*Piper nigrum* L.). *Annals of Microbiology*, 55, 129–133.
- Foldes, T., Banhegyi, I., Herpai, Z., Varga, L., & Szigeti, J. (2000). Isolation of *Bacillus* strains from the rhizosphere of cereals and in vitro screening for antagonism against phytopathogenic, food-borne pathogenic and spoilage micro-organisms. *Journal of Applied Microbiology*, 89, 840–846.
- Glick, B. R., & Bashan, Y. (1997). Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of phytopathogens. *Biotechnological Advances*, 15, 353–378.
- Gowtham, H. G., Singh, S. B., & Niranjana, S. R. (2015). Evaluation of plant growth promoting ability of *Providencia* spp. collected from north eastern region of India in crucifers. *IJASR*, 5(3), 321-328.
- Haas, D., & Défago, G. (2005). Biological control of soil-borne pathogens by fluorescent pseudomonads. *National Review of Microbiology*, 3, 307–319.
- Halt, J. G., Peter, N. R., Sneath, H. A., Staley, J. T., & William, S. T. (1994). *Burgeys Manual of Detrimintave Bacteriology* 9th Edition. Pub. William and Wilkins, Baltimore pp: 559.
- Hidayati, U., Chaniago, I. A., Munif, A., & Santosa, D.A. (2014). Potency of plant growth promoting endophytic bacteria from rubber plants (*Hevea brasiliensis* Miill. Arg.). *Journal of Agronomy*, 13(3), 147-152.
- Hu, X. F., Chen, J., & Guo, J. F. (2006). Two phosphate- and potassium-solubilizing bacteria isolated from Tianmu Mountain, Zhejiang, China. *World Journal of Microbiology and Biotechnology*, 22, 983-990.
- Hussain, K., Hameed, S., Shahid, M., Ali, A., Iqbal, J., & Hahn, D. (2015). First Report of *Providencia vermicola* strains characterized for enhanced rapeseed growth attributing parameters. *International Journal of Agriculture and Biology*, 17, 1110–1116.
- Ibrahim, A. S. S., & El-diwany, A. I. (2007). Isolation and identification of new cellulases producing thermophilic bacteria from an egyptian hot spring

- and some properties of the crude enzyme. *Australian Journal of Basic and Applied Sciences*, 1(4), 473-478.
- Kamilova, F., Validov, S., Azarova, T., Mulders, I., & Lugtenberg, B. (2005). Enrichment for enhanced competitive plant root tip colonizers selects for a new class of biocontrol bacteria. *Environmental Microbiology*, 7, 1809–1817.
- Khalid, A., Arshad, M., & Zahir, Z. A. (2004). Screening plant growth-promoting rhizobacteria for improving growth and yield of wheat. *Journal of Applied Microbiology*, 96, 473-480.
- Lawrence, R. C., Fryer, T. F., & Reiter, B. (1967). Rapid method for the quantitative estimation of microbial lipases. *Nature*, 213, 1264-1265.
- Seo, P. J., & Park, C. M. (2009). Auxin homeostasis during lateral root development under drought condition. *Plant Signal Behavior*, 4, 1002–1004.
- Sessitsch, A., Reiter, B., & Berg, G. (2004). Endophytic bacterial communities of field grown potato plants and their plant growth promoting and antagonistic abilities. *Canadian Journal of Microbiology*, 50, 239–249.
- Sindhu, S. S., & Dadarwal, K. R. (2001). Chitinolytic and cellulolytic *Pseudomonas* sp. antagonistic to fungal pathogens enhances nodulation by *Mesorhizobium* sp. *Cicer* in chickpea. *Microbiology Research*, 156, 353–358.
- Somvanshi, V. S., Lang, E., Straubler, B., Sproer, C., Schumann, P., Ganguly, S., Saxena, A. K., & Stackebrandt, E. (2006). *Providencia vermicola* sp. nov., isolated from infective juveniles of the entomopathogenic nematode *Steinernema thermophilum*. *International Journal of Systemic & Evolutionary Microbiology*, 56, 629–633.
- Vassilev, N., Vassileva, M., & Nikolaeva, I. (2006). Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. *Applied Microbiology and Biotechnology*, 71, 137–144.
- Waturangi, D. E., Francisca, I., & Susanto, C. O. (2011). Genetic diversity of methylotrophic bacteria from human mouth based on Amplified Ribosomal DNA Restriction Analysis (ARDRA). *Hayatian Journal of Biosciences*, 18(2), 77-81.