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# Report of indole acetic acid (IAA) producing endophytic bacteria from the edible fern *Ampelopteris prolifera* (Retz.) copel

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## Abstract

Endophytic bacteria are the plant beneficial bacteria that thrive inside plant tissue and can improve plant growth without causing any negative impact. In the present study endophytic bacteria were isolated from leaf, rachis, rhizome and root of an edible fern *Ampelopteris prolifera* (Retz.) Copel were screen for Indole Acetic Acid (IAA) production. Three isolates RART4, WARH3 and WART2 were identified as *Bacillus megaterium* (MZ310576), *Lynsinibacillus varians* (MZ310567) and *Paenibacillus sp.* (MZ310568) respectively based on 16s rDNA sequencing were produced significant amount of IAA in the presence of 0.1% tryptophan. We have observed RART4 produced highest amount of IAA among the isolates. The enzymatic activities, sugar fermentation and antimicrobial agent resistance abilities of the isolates were recorded. In vitro treatment of the isolate RART4 for plant growth promotion resulted in substantial increase in root, shoot, primary and secondary leaf length, biomass, chlorophyll, and protein content as compared to the control.

**Keywords:** endophytic bacteria, *Ampelopteris prolifera* (Retz.) copel, indole acetic acid (IAA), *Bacillus megaterium, lynsinibacillus varians*, *Paenibacillus sp.*, plant growth promotion

## Introduction

Endophytic bacteria are the plant beneficial bacteria that can thrive within the plant tissue without imposing any negative impact of their host. They are ubiquitously associated with almost every plant studied and may create a variety of symbiotic, mutualistic, commensal, and trophobiotic interactions [1]. In general, the density of endophytic bacteria is higher in the roots and subsequently declines in the stems and leaves [2]. Many endophytic bacteria have been isolated from the roots, stems, leaves, flowers, fruits, and seeds of many monocot and dicot plants, according to a literature review [3] but exploration of endophytes among pteridophytes are very countable [4-6]. Endophytic bacteria have already been reported to play a key role in plant growth, development, and yield by generating wide range of phytohormones such as auxins, cytokinins, and gibberellic acids [7]. The principle and first auxin sequestered from plants is indole -3-acetic acid (IAA) [8], which is considered the most important signalling molecule among the various plant growth regulators that regulates plant growth and development, including organogenesis, tropic responses, cell division and differentiation, and gene regulation [9]. Several types of endophytic bacteria have the potential to create IAA, which is beneficial to plant development. The bacterial IAA increases root surface area and length, which enhances nutrient absorption by plants [10], as well as loosening plant cell walls, which allows for more root exudates, which boosts microbial activity [11]. Namwongsa *et al*., 2019 [12], isolated indole-3-acetic acid (IAA) generating *Micrococcus luteus* from *Helianthus tuberosus* L. and found that it boosted the plant's height, shoot and root weight, root length, root diameter, root volume, root area, and root surface. Mukherjee *et al*., 2017 [4], isolated an IAA-producing endophytic bacteria Bacillus from the pteridophytes *Ophioglossum reticulatum*. A diazotrophic endophytic bacterium, *Burkholderia vietnamiensis*, that has been isolated from wild cottonwood (*Populus trichocarpa*), produced plant growth promoting hormone IAA [8].

*Ampelopteris prolifera* (Retz.) Copel is a terrestrial scrambling fern with stems, short creeping rhizome. The plant produces proliferous buds scattered along the rachis of the fronds which can grow into new plants. Young fronds of this fern are sold as Dheki sak at the local market and consumed as fresh or cooked vegetable. It is used in ethnomedicine to treat headaches. This fern is occasionally planted as a decorative plant [13]. In the present study, endophytic bacteria from the surface sterilized leaf, rachis, rhizome and root of an edible fern *A. prolifera* were isolated, characterized and evaluated their potentiality for the production of IAA.

## Materials and Methods

### *Collection of plant materials*

The plant (Figure-1) materials were collected from Shibpur (23.6421363 N, 87.6837508 E), Bolpur Road, Birbhum, West Bengal, The healthy plants along with soil were collected in a zipper pack, brought to the laboratory and the isolation process was proceeds within two hours. Till then the plant materials were stored at $4^0$C temperature in refrigerator.

Herbarium specimens were prepared in triplicate following the standard protocol [14] and submitted in the departmental herbarium (VBBOTSS 0109F).

**Isolation of endophytic bacteria**

Bacterial endophytes were isolated from 1gm of leaf, rachis, rhizome and root of healthy *A prolifera* separately. The collected parts of the plant were washed thoroughly under running tap water and then distilled water and transferred to the sterile glass vial for surface sterilization. The samples were surface sterilized by performing a consecutive immersion in 70% ethanol for 2min, sodium hypochloride (4% w/v available chlorine) for 4min, and 70% ethanol for 30sec followed by five rinses of sterile distilled water. For the confirmation of the effective decontamination, 100 µl aliquots of the sterile distilled water that was used in the final rinse was plated on to tryptic soya agar (TSA), (Sigma - Aldrich) and incubated at 28-30$^0$C for 24-96 hours and observed the presence or absence of the growth of microorganisms. For bacterial isolation, the plant samples were fully pestle with 10 ml of phosphate buffer saline (PBS) in a mortar and kept it for 60 minutes for natural precipitation. Then the supernatant was taken and serially diluted up to $10^{-2}$ dilution with PBS and checked for bacterial count. All the samples were observed to have a countable number of bacteria at $10^0$ dilutions. So this dilution was used to isolate the endophytic bacteria. 100 µl of aliquots of the serially diluted samples were plated on TSA medium in triplicate. Plates were then incubated at 28-30$^0$ C for 24 to 96 hours and observed for the growth of bacterial colonies. By the process of diluting streaking method, morphologically distinguishable bacterial colonies were isolated in pure form and maintained by regular sub-culturing on the same media.

**Screening for Indole-3-acetic acid producing endophytic bacteria**

Production of indole-3-acetic acid (IAA) by the endophytic bacteria was determined following Salkowski colorimetric assay. Endophytic bacterial isolates were grown in tryptophan broth at 28-30 °C for 96 hours on a rotary shaker. The broth culture was centrifuged at 10,000 rpm for 10 min. After that 1ml of supernatant was collected and to this 2 ml of salkowski reagent (2ml 0.5M $FeCl_3$, 49 ml distilled water and 70% perchloric acid) and 3 ml of distilled water was added. The absorbance of the samples was read at 530 nm using UV Vis Spectrophotometer after incubation for 30 min in dark at room temperature for the development of pink colour. The quantitative estimation of IAA was done from the standard curve prepared in the same way with authentic IAA from Sigma Aldrich (USA)

**Identification of selected endophytic bacterial isolates**

For phenotypical characterization colonies of endophytic bacterial isolates were characterized after 24- 96hrs postinoculation for the following traits: colony size, colour, form, elevation, margin, transparency, cell shape, cell size, gram nature, endospore and motility following the standard laboratory method [5-7]. For morphological characterization, light microscopic (zeiss trinocular microscope and Cells were measured using the software Axion Vision LE, version 4.8.2.0.) as well as scanning electron microscopic (SEM) (Gemini SEM450-8216010130) studies were carried out (Figure-3). For SEM studies bacterial cells were prepared by following the method of Mandal *et al*., 2013 [15] with slight modification and were sprayed on cover glasses. All the cover glasses were coated with gold using an ion sputter (Coater IB-2, Gike Engineering, Japan) and observed under SEM. Determination to the ability of production of hydrolytic enzymes by the isolates was done by performing a screening test for catalase, oxidase, protease, amylase, and cellulase [16-17]. Fermentation of carbohydrates was carried out in a fermentation tube containing Durham tubes and phenol red is used as $p^H$ indicator. Positive fermentation reaction changed the phenol red to yellow due to the production of acid or produce gas which is trapped in the Durham tube along with the production of acid.

**Molecular identification was conducted using 16s rDNa gene sequencing**

DNA was isolated from the pure culture and its quality was evaluated on 1.0% Agarose Gel, a single band of high-molecular weight DNA has been observed. 16S rDNA gene fragment was amplified by 27F and 1492R primers. One discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose gel. The PCR amplicon was purified to get rid of contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was administrated with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. The consensus sequence of 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST (Basic Local Alignment Search Tool) with the database of NCBI (National Centre for Biotechnology Information) genbank database. Supported maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated and the phylogenetic tree was constructed following Kimura's two parameter model [18] using MEGA 7 using neighbor joining method with 1000 bootstrap values [19].

**Antibiotic Sensitivity test**

Antimicrobial agent resistance of the endophytes were done individually using the recommended working concentrations *i.e.* 100 µg/ml for ampicillin and streptomycin, 50 µg/ml for kanamycin and rifampin, 25 µg/ml for chloramphenicol and 15 µg/ml for tetracycline. Endophytes were considered sensitive to an antibiotic if no visible growth were observed on plates containing antibiotic when there was visible growth on control plates after incubation [20].

**Effect of IAA producing Endophytic bacteria on plant growth**

*Vigna unguiculata* (L.) Walp. Seeds were taken and washed with running tap water and surface sterilized with 70% alcohol for 2-3 minutes, 0.2% Mercuric chloride ($HgCl_2$) for 5 mins respectively. After that surface-sterilized seeds were rinsed thoroughly with sterile distilled water for several times to remove the traces of $HgCl_2$. Seeds were allowed to germinate in aseptic conditions using paper towel method with slight modification [21]. The germinated roots were treated with bacterial culture ($1.3x\ 10^7$) for 24 hours at 30-30$^0$ C and sowed in a plastic pot containing a mixture of sterilized soils and vermicomposts (3:1 v/v) and allowed to grow in a natural environment. After 10 days of the seedling plantation again soil was treated with bacteria culture. The plants were assed for shoot length, root length; primary-secondary leaves count, Biomass, Chlorophyll, Protein content at the age of 30 days and compared to the untreated control plant.

***Shoot length Measurement***

The shoots of all the plantlets were excised and measured manually using scales. The average shoot measurement for each pot was calculated.

**Root length Measurement**

All the plantlets were removed gently from pots and every root of every plantlet was measured manually using scale. The average root measurement for every pot was calculated.

***Biomass Estimation***

Plants were blotted dry and weighed after the harvesting period and biomass was calculated on dry weight basis (g). For the determinations of dry weight fresh plant tissues were kept separately in hot plate at $100^0$ C till a constant weight is obtained.

**Chlorophyll estimation**

For the total chlorophyll estimation, the Arnon's (1949) [22] method was employed. About 100 mg of fresh debris free leaves were taken and crushed with 10 ml of methanol and centrifuged at 5000 rpm for 10 minutes. Take the supernatant and the volume was made up to 10 ml by adding methanol to the supernatant. The residual part was kept apart for protein estimation. Development of the colour was measured at 650 nm. Then chlorophyll content was calculated by using Arnon's formula. The concentration of chlorophyll was expressed as mg/gm tissue.

**Protein estimation**

Total estimation of protein was determined by the Lowry's (1951) [23] method. The hot air-oven dried pellet (residual part from chlorophyll estimation) was digested in 2 ml of 1 (N) NaOH solution for one hour at $80^0$ C in a hot water bath. Then centrifuged at 5000 rpm by addition of 2 ml of distilled to each. Take 0.2 ml supernatant of plant extract and volume was made up to 1 ml by adding distilled water. After that 1 ml of reagent A (mixture of 10% Na2Co3 in 0.5 (N) of NaOH solution, 1% cupric sulphate (CuSO4, 5H2O) solution and 2% sodium – potassium tartarate solution in the ratio of 20: 1: 1) was added to the diluted extract. About 5 minutes later 0.5 ml of reagent B (1: 2 Folin – Ciocalteau's phenol reagent from the original stock solution with distilled water) was added to the reaction mixture. Then the final reaction mixture was shaken vigorously and left for 20 minutes at room temperature. A blue colour appeared which indicated the presence of protein in the reaction mixture. Then 2.5 ml of distilled water was added to the extract mixture and the absorbance was read at 650 nm. The total content of protein in plant extract was determined by comparing with a standard curve prepared from Bovine Serum Albumin (BSA). The concentration of proteins was expressed as mg/gm tissue.

**Statistical analysis**

All experimental data were entered in a Microsoft® Excel 2010 spread sheet and mean was calculated and the standard deviation value was given in graph.

**Results**

Endophytic bacterial colonies were observed on tryptic soya agar plates after 48-96 hours of incubation. In this present study, a total of 32 morphologically distinguishable endophytic bacteria from different parts of *A. prolifera* were isolated.

Nine of the 32 endophytic bacterial isolates produced IAA, as evidenced by the formation of a pink colour after treatment with Salkowski's reagent. IAA production concentrations varied from 2.95 µg/ml to 15.89 µg/ml. The isolates RART4 was emerged as the best producer (15.89 µg/ml) followed by WARH3 (7.60 µg/ ml) and WART2 (6.07 µg/ml). RART4, WARH3 and WART2 isolates were chosen based on their ability to produce IAA. The phenotypic (Table-1) and biochemical profiles (Table-2) of these three isolates were used to further characterise them. It was observed that RART4, WARH3, and WART2 are motile gram positive rods in nature. In RART4 and WART2, endospore development was found. WARH3 can generate catalase, oxidase, protease, amylase, and cellulase, according to an enzyme screen of these endophytic bacterial isolates. Except for oxidase, all of the enzymes produced by RART4 were shown to be positive. WART2 is also capable of producing catalase and oxidase (Table-2). The sugar fermentation profiles of these three endophytes (Table 3) revealed that they could all ferment glucose, lactose, and sucrose.

It has been observed that RART4 was sensitive to all antibiotics tested and WARH3 showed resistance to streptomycin, tetracycline and kanamycin while WART2 showed resistance to kanamycin and chloramphenicol (Table-4). DNA extraction, 16s rDNA gene amplification, and sequencing were performed on the endophytic bacterial isolates. RART4 was found to be identical to *Bacillus megaterium*, WARH3 to *Lynsinibacillus varians*, and WART2 to *Paenibacillus* sp., according to BLAST analysis. RART4, WARH3, and WART2 16S rDNA gene sequences were submitted to GenBank under accession numbers MZ310576, MZ310567, and MZ310568, respectively. Based on nucleotide homology and phylogenetic analysis, the isolates RART4, WARH3, and WART2 exhibited remarkable resemblance with *Bacillus megaterium*, *Lynsinibacillus varians*, and *Paenibacillus* sp., respectively. Using the neighbour-joining method and 1000 bootstrap values, a phylogenetic tree of closely related sequences acquired from NCBI was constructed. (Figure 3).

RART4 (*Bacillus megaterium*), one of the three endophytic bacterial isolates, was administered to the plant to monitor its growth activity.

After 30 days, all of the plantlets cultivated in pots containing endophytic bacterial cultures (RART4) had different growth patterns (Figure-4). In comparison to the control, seeds treated with endophytic isolates had increased shoot and root length, primary and secondary leaf count, biomass, chlorophyll, and protein content (Table- 5).

**Fig 1:** *Ampelopteris prolifera* (Retz.) Copel

**Table 1:** Micromorphological characteristics of endophytic bacterial isolates

| | Colony Morphology | | | | | | Cell shape | Cell size (µm) | Gram nature | Endospore | Motility |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Isolates | colony size | colour | form | elevation | margin | tramsparent | | | | | |
| *RART4* | Large | white | circular | convex | entire | opaque | rod | 2.88- 5.63 x 0.91- 1.03 | +ve | + | + |
| *WARH3* | medium | white | circular | convex | entire | opaque | rod | 1.92- 2.4 x 0.61- 0.65 | +ve | - | + |
| *WART2* | small | white | circular | convex | entire | opaque | rod | 2.03- 5.07 x 0. 64 -1.03 | +ve | + | + |

**Table 2:** Biochemical Characterization of the endophytic bacterial isolates

| Isolates | Production of enzymes | | | | |
|---|---|---|---|---|---|
| | Catalase | Oxidase | Protease | Amylase | Cellulase |
| *RART4* | + | - | + | + | + |
| *WARH3* | + | + | + | + | + |
| *WART2* | + | - | - | - | + |

'+'= positive, '-' = negative

**Table 3:** Carbohydrate fermentation

| Isolates | Carbohydrates | | |
|---|---|---|---|
| | Glucose | Lactose | Mannitol |
| *RART4* | AG | A | A |
| *WARH3* | AG | A | A |
| *WART2* | A | A | A |

A-Acid, AG- Acid & Gas

**Table 4:** Antibiotic resistance tests of the isolates

| Isolates | Antibiotics | | | | | |
|---|---|---|---|---|---|---|
| | Ampicillin (100 µg/ml) | Streptomycin (100 µg/ml) | Tetracycline (15 µg/ml) | Kanamycin (50 µg/ml) | Chloramphenicol (25 µg/ml) | Rifampicin (50 µg/ml) |
| *RART4* | S | S | S | S | S | S |
| *WARH3* | S | R | R | S | S | S |
| *WART2* | S | S | S | R | R | S |

S- Sensitive, R – Resistance

**Table 5:** Growth assessment of pot experiment by IAA producing endophytic bacteria on seed germination.

| Sl. No. | Growth Assessment | Treatment | | One-way ANOVA α < 0.05 * = significant difference with control |
|---|---|---|---|---|
| | | Control | RART4 | |
| 1 | Root Length(cm) | 7.83 | 9.3 | F-value: 3.56 |
| 2 | Shoot length(cm) | 17.33 | 24 (*) | F-value: 60.78 |
| 3 | Primary leaves(cm) | 5 | 5.96 | F-value: 12.509 |
| 4 | Secondary leaves (cm) | 2.6 | 5.43 (*) | F-value: 11.15 |
| 5 | Biomass(gm) | 0.73 | 1.41 (*) | F-value: 111.78 |
| 6 | Chlorophyll(mg/gm) | 5.2 | 6.4 (*) | F-value: 55.25 |
| 7 | Protein (mg/gm) | 58.35667 | 65.93 (*) | F-value: 387.24 |

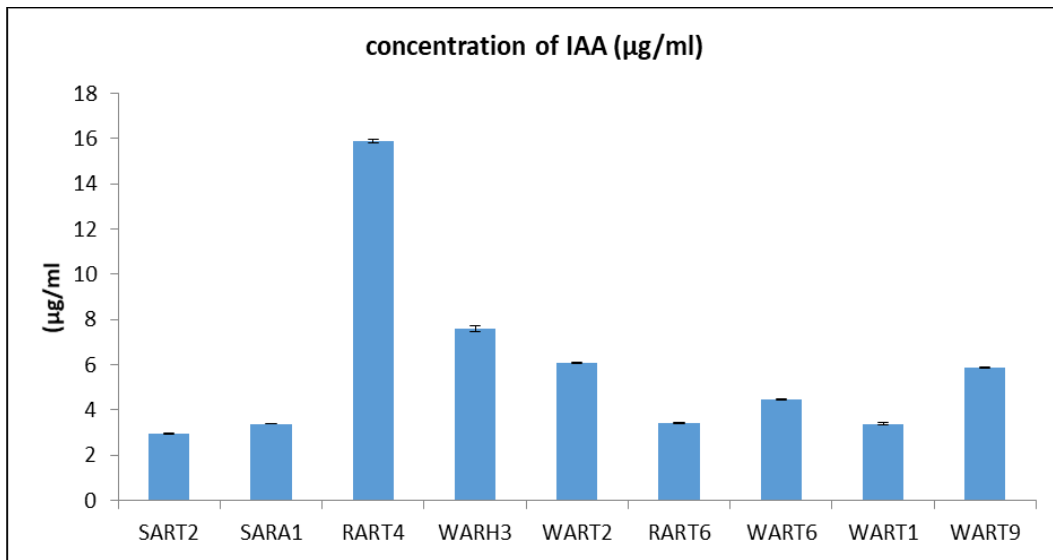



**Fig 2:** IAA production by the endophytic bacteria isolated *A. prolifera*.

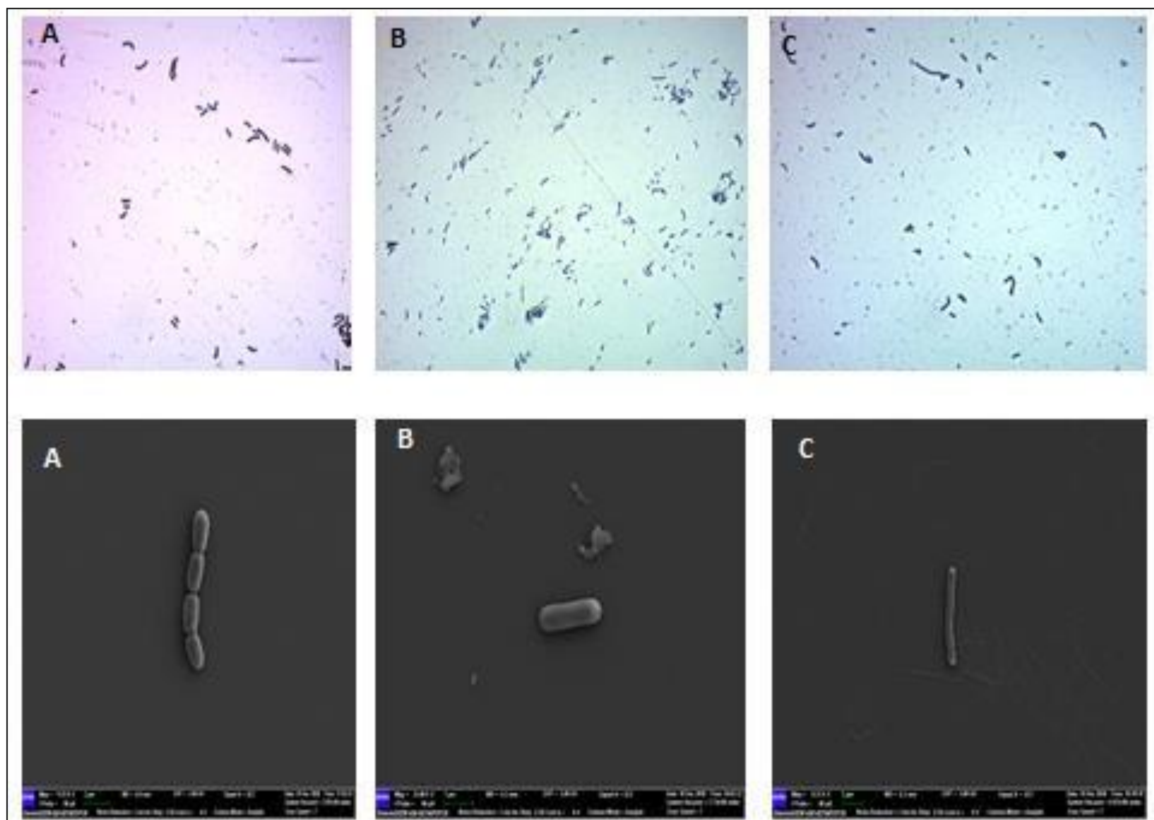
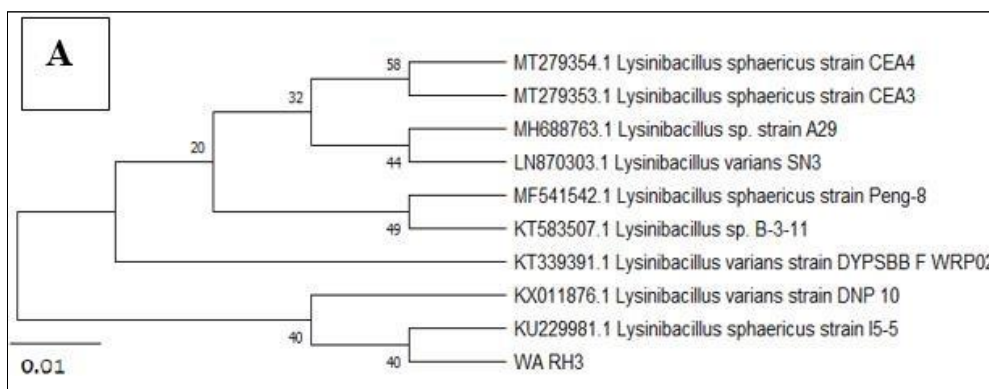



**Fig 3:** Gram staining and Scanning electron micrograph of isolated endophytic bacterial strains: A- *Bacillus megaterium,* B-*Lynsinibacillus varians*, C- *Paenibacillus sp.*



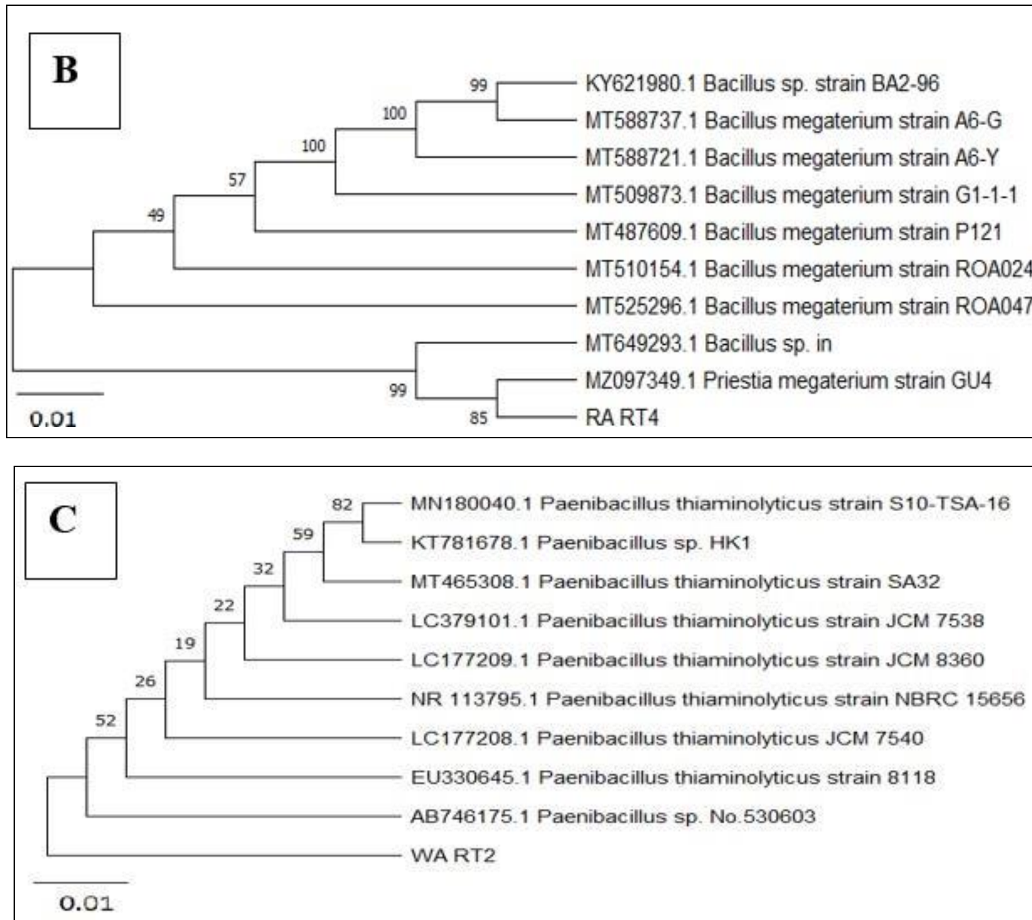



**Fig 4:** Phylogenetic tree of 16s rRNA gene sequences of isolates with closest related sequences obtained from NCBI using MEGA7. A-*Bacillus megaterium,* B-*Lynsinibacillus varians*, C- *Paenibacillus sp.*

**Fig 5:** Growth assessment of pot experiment by IAA producing endophytic bacteria on seed germination

## Discussions

Indole acetic acid (IAA) is an essential plant growth hormone that regulates plant growth and development. Some endophytic bacteria produce it as well, and it plays an important role in plant-bacteria interactions. A total of 32 morphologically identifiable endophytic bacteria from various parts of *A. prolifera* were identified in this investigation. In the presence of tryptophan, nine isolates produced IAA (Figure-2), with RART4 being the most prolific generator, followed by WARH3 and WART2.

These three isolates are gram-positive motile rods that generate hydrolytic enzymes such as Catalase, protease,

amylase, and cellulase, which are necessary for colonisation of the plant's root. IAA synthesis by Gram positive bacteria has been described by several authors [24-26]. Quantification of enzymes produced by plant-associated endophytic bacteria is commonly used in biotechnological and pharmaceutical procedures since they are a prospective source of enzymes. All three endophytic isolates could ferment glucose, lactose, and mannitol (Table 3) and were resistant to streptomycin, tetracyclin, kanamycin, and chloramphenicol (Table 4).

The 16S rDNA gene was used to identify endophytic bacterial genera quickly and efficiently. RART4 was shown to be identical to *Bacillus megaterium*, WARH3 to *Lynsinibacillus varians*, and WART2 to *Paenibacillus* sp., according to BLAST analysis of sequences. RART4 was shown to be clustered with *Bacillus megaterium*, WARH3 with *Lynsinibacillus varians*, and WART2 with *Paenibacillus* sp. in phylogenetic analysis using the MEGA7 neighbour joining technique.

These endophytic bacterial strains have been reported as IAA producing endophytes from various plants. The genus *Bacillus megaterium*, *Paenibacillus sp*. reported as endophytic bacteria from *Dicksonia sellowiana* HOOK (Dicksoniaceae) [27]. J. Wu *et al*., 2019 [28] isolated two *Bacillus megaterium* strains from hybrid *Pennisetum* which can produce 3.00 and 2.76 mg.L$^{-1}$ IAA respectively. The genus *Lynsibacillus* isolated from corn root can produce 10.2 mg.L$^{-1}$ IAA [29]. Two endophytic bacteria *Paenibacillus* strains from the medicinal plant *Lonicera japonica* were screened and characterised by Zhao *et al*. 2014 [30] can produce 18.3 and 29.3 mg.L$^{-1}$ IAA respectively.

IAA assists the plant in boosting nutrient intake by expanding root area and length. Inoculation with IAA-producing bacteria resulted in the development of lateral roots, according to a literature review [31, 32]. Through an in vitro root growth promotion experiment, considerable root growth, shoot growth, primary and secondary leaf length, biomass, chlorophyll, and protein content by the endophytic bacterial isolates RART4 were detected (Figure-5)

All of these characteristics, as demonstrated by the isolate RART4, suggest that it could be a useful bio-inoculant that could be used in organic farming to mitigate the environmental impact of chemical fertilizers while also ensuring the quality of agricultural products.

## Conclusions

The endophytic bacterial isolates from A. prolifera generated many hydrolytic enzymes and had the potential to create a substantial amount of IAA, which supports plant growth, according to the findings. On the basis of 16s rDNA gene sequencing, these three effective IAA producing endophytes were identified as *Bacillus megaterium* (MZ310576), *Lynsinibacillus varians* (MZ310567), and *Paenibacillus* sp. (MZ310568), respectively. According to the literature, this is the first report of endophytic bacteria and IAA synthesis from *A. prolifera*. These isolates might be highly beneficial in the future for the synthesis of economically relevant hydrolytic enzymes and as a biofertilizer for the encouragement of agricultural crop plant growth and development.

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## References

1. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. Bacterial endophytes: recent developments and applications. *FEMS microbiology letters,*2008:278(1):1-9.
2. Lamb TG, Tonkyn DW, Kluepfel DA. Movement of Pseudomonas aureofaciens from the rhizosphere to aerial plant tissue. *Canadian journal of microbiology,*1996:42(11):1112-1120.
3. Rosenblueth M, Martínez-Romero E. Bacterial endophytes and their interactions with hosts. *Molecular plant-microbe interactions*,2006:19(8):827-837.
4. Mukherjee A, Bhattacharjee P, Das R, Pal A, Paul AK. Endophytic bacteria with plant growth promoting abilities from Ophioglossum reticulatum L. *AIMS microbiology*,2017:3(3):596.
5. Das G, Park S, Choi J, Baek KH. Anticandidal potential of endophytic bacteria isolated from Dryopteris uniformis (Makino). *Jundishapur Journal of Microbiology*, 2019, 12(1).
6. Munir E. Mamangkey J, Lutfia A. Antibacterial and phosphate solubilization activity of endophytic bacteria isolated from Pterydophyta (Tectaria barberi). In *IOP Conference Series: Earth and Environmental Science*, IOP Publishing,2019:305(1):012019.
7. Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology*,2012:28(4):1327-1350.
8. Aziz K, Nawaz M, Nazir J, Anjum A A, Yaqub T, Ahmad MUD, Rehman MU, Aziz G and Khan M. 2015. Isolation, characterization and effect of auxin producing bacteria on growth of Triticum aestivum. *J. Anim. Plant Sci*,2018:25*:*1003-1007.
9. Ryu RJ, Patten CL. Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by TyrR in Enterobacter cloacae UW5. *Journal of bacteriology*,2008:190(21):7200-7208.
10. Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. *Plant and soil*,2003:255(2):571-586.
11. Glick BR. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 2012.
12. Namwongsa J, Jogloy S, Vorasoot N, Boonlue S, Riddech N, Mongkolthanaruk W. Endophytic Bacteria Improve Root Traits, Biomass and Yield of Helianthus tuberosus L. under Normal and Deficit Water Conditi, 2019, 1777-1789.
13. Mondal S, Sukul S. Diversity. *Ethnobotany and Phytochemistry of Pteridophytes from Birbhum District, West Bengal. Ph.D. Thesis*, Department of Botany, Visva-Bharati (A Central University), 2016.
14. Woodland DW. Contemporary plant systematics. 2nd Ed. *Andrews University Press. Berrien Spring, Michgian, United States of America,* 1997. ISBN 1-883925014-2. 619.
15. Mandal V, Sen SK, Mandal NC. Production and partial characterisation of an inducer-dependent novel

antifungal compound (s) by Pediococcus acidilactici LAB 5. *Journal of the Science of Food and Agriculture*,2013:93(10):2445-2453.
16. Shields P, Cathcart L. Oxidase test protocol. *American Society for Microbiplogy,* 2010.
17. Aneja KR. Experiments in microbiology, plant pathology and biotechnology, 2007. *New age* International. ISBN: 81-224-1494-x.
18. Kimura M. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution,*1980:16:111-120.
19. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets.Molecular Biology and Evolution (submitted), 2015.
20. Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P *et al*. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Applied and environmental microbiology*,2002:68(5):2198-2208.
21. Gholami A, Shahsavani S, Nezarat S. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *World Academy of Science, Engineering and Technology*,2009:49:19-24.
22. Arnon DI. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*, Plant Physiol,1949:24:1-15.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *J Biological Chemistry,*1951:193:265-75.
24. Mohite B. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. *Journal of soil science and plant nutrition*,2013:13(3):638-649.
25. Ozdal M, Ozdal OG, Sezen A, Algur of and Kurbanoglu, EB. 2017. Continuous production of indole-3-acetic acid by immobilized cells of Arthrobacter agilis. *3 Biotech*,2017:7(1):23.
26. Bhutani N, Maheshwari R, Negi M, Suneja P. Optimization of IAA production by endophytic Bacillus spp. from Vigna radiata for their potential use as plant growth promoters. *Israel Journal of Plant Sciences*,2018:65(1-2):83-96.
27. Barros IDA, Araújo WL, Azevedo JL. The effect of different growth regimes on the endophytic bacterial communities of the fern, Dicksonia sellowiana hook (Dicksoniaceae). *Brazilian Journal of Microbiology*,2010:41:956-965.
28. Wu J, Kamal N, Hao H, Qian C, Liu Z, Shao Y *et al*. Endophytic Bacillus megaterium BM18-2 mutated for cadmium accumulation and improving plant growth in Hybrid Pennisetum. *Biotechnology Reports*,2019:24:e00374.
29. Yu J, Guan X, Liu C, Xiang W, Yu Z, Liu X *et al*. Lysinibacillus endophyticus sp. nov., an indole-3-acetic acid producing endophytic bacterium isolated from corn root (Zea mays cv. Xinken-5). *Antonie van Leeuwenhoek*,2016:109(10):1337-1344.
30. Zhao L, Xu Y, Lai XH, Shan C, Deng Z, Ji Y. Screening and characterization of endophytic Bacillus and Paenibacillus strains from medicinal plant Lonicera japonica for use as potential plant growth promoters. *Brazilian Journal of Microbiology*,2015:46:977-989.
31. Teale W D, Paponov IA and Palme K. 2006. Auxin in action: signalling, transport and the control of plant growth and development. *Nature reviews Molecular cell biology*, *7*(11), 847-859.
32. Chaiharn M and Lumyong S. 2011. Screening and optimization of indole-3-acetic acid production and phosphate solubilization from rhizobacteria aimed at improving plant growth. *Current microbiology*, *62*(1), 173-181.
33. Kachhawa G, Charan SK, Choudhary R. Diversity and pollination probability of insect pollinators of Tagetes Erecta L. in the Chomu Tehsil, Rajasthan, India. International Journal of Entomology Research. 2020;5(6):106-10.