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Amit Rakshit and Soma Sukul

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Studies on Two Indole Acetic Acid (IAA) Producing Endophytic Bacteria from *Thelypteris interrupta* (Wild.) K. Iwats and their Effect on Seed Germination

Amit Rakshit[1] and Soma Sukul*[2]

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A B S T R A C T

Endophytic bacteria isolated from the various parts such as leaf, rachis, rhizome and root of *Thelypteris interrupta* (Wild.) K. Iwats were screen for Indole Acetic Acid (IAA) production. Two isolates STL1 and RT5 were identified as *Kocuria flava* (MZ310566) and *Bacillus* sp. (MZ310573) respectively based on 16s rDNA sequencing were produce significantly high amount of IAA (≥60µg/ml) in the presence of tryptophan. The enzymatic activities, sugar fermentation and antimicrobial agent resistance abilities of the isolates were recorded. In vitro application of those isolates for growth promotion resulted to increase in the root, shoot length, primary and secondary leaf length, biomass, chlorophyll and protein content as compared to the control.

***Key words*:** Endophytic bacteria, Indole Acetic Acid (IAA), *Kocuria flava*, *Bacillus* sp., Enzymatic activity

Endophytic bacteria are those bacteria that live within the plant tissue without causing any apparent disease symptoms or gaining benefit other than residency. Plants constitute large and diverse niches for endophytic organisms and are ubiquitously associated with almost every plant studied. Endophytic bacteria can form a range of different relationships such as symbiotic, mutualistic, commensal and trophobiotic [1]. The population of endophytic bacteria is larger in roots and gradually decreases in stems and leaves [2]. Literature survey revealed that many endophytic bacteria were isolated from roots, stems, leaves, flowers, fruits and seeds of various monocot and dicot plants [3] but very few were reported from pteridophytes [4-6]. It is evident that endophytic bacteria have played a crucial role in plant growth, development and yield by producing a wide range of phytohormones, like auxins, cytokinins, and gibberellic acids [7]. Indole -3- Acetic Acid (IAA) is the principle and the first auxin sequestered from plants [8] and it is considered as the most important signaling molecule among the different plant growth regulators that regulates plant growth and development including organogenesis, tropic responses, cell division and differentiation, and gene regulation [9]. Various endophytic bacterial strains possess the ability to produce IAA and help the plant growth promotion significantly. It has been shown that the bacterial IAA increases the root surface area and length and thereby increasing nutrient uptake by plants [10] and also loosen the plant cell walls facilitating an increased amount of root exudates which increases microbial activity [11]. In recent years, isolated indole-3-acetic acid (IAA) producing *Micrococcus luteus* from *Helianthus tuberosus* L. that increased height, shoot and root weight, root length, root diameter, root volume, root area and root surface of the plant [12]. Mukherjee *et al*. [4], isolated an endophytic bacterium *Bacillus* from the pteridophytes *Ophioglossum reticulatum* having IAA producing ability. *T. interrupta* is semi-aquatic or terrestrial, herb, lower pinnae not or little reduced, rhizome long, creeping, scales flat, thin, scales without superficial hairs. The immature leaf crosier has been sold in the local market of Bolpur as well as in the local market of Durgapur, Asansol etc. as "Dhekhi Sak". This plant is also used as fodder. In ethno medicine it is used in stomach problems, diarrhoea, as anticancerous, antiviral and antibacterial agents [13]. In the present study, endophytic bacteria from the surface sterilized leaf, rachis, rhizome and root of *T. interrupta* were isolated, characterized and evaluated their potentiality for the production of IAA.

* **Soma Sukul**
✉ soma.chunari@visva-bharati.ac.in

[1-2] Department of Botany (UGC- DRS & DST- FIST Sponsored), Visva- Bharati (A Central University), Santiniketan - 731 235, Birbhum, West Bengal, India

MATERIALS AND METHODS

Collection of plant materials

The plant (Fig 1) materials were collected from *khoai* (23°41′515″ N, 87°40′309″ E and Elevation: 200ft) Santiniketan, 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°C temperature in refrigerator. Herbarium specimens were prepared in triplicate following the standard protocol [14] and submitted in the departmental herbarium (VBBOTSS 0110F).

Fig 1 *Thelypteris interrupta* (Wild.) K. Iwats

Isolation of endophytic bacteria

Bacterial endophytes were isolated from 1gm of leaf, rachis, rhizome and root of healthy *T. interrupta.* 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 distlled 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° 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% perchholic 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).

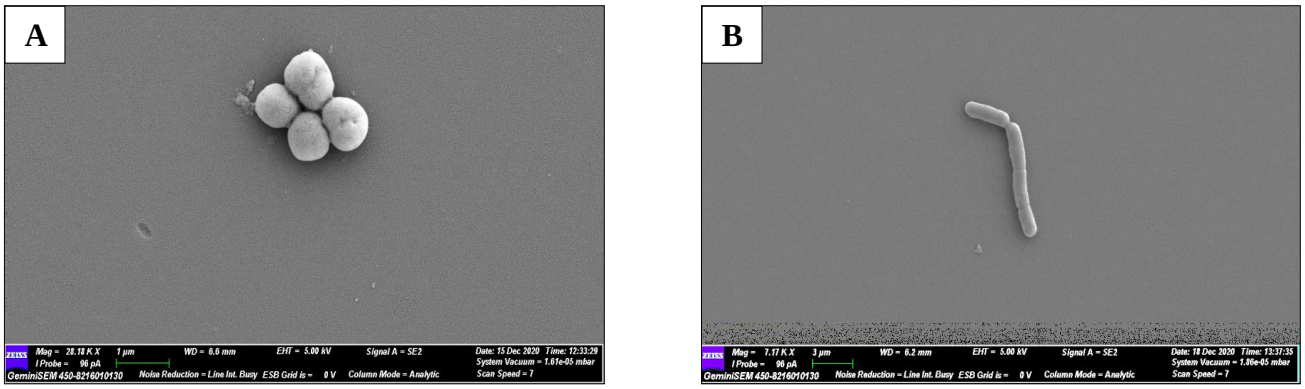

Fig 3 Scanning electron micrograph of isolated bacterial strains: A-*Kocuria flava*, B- *Bacillus* sp.

Identification of selected endophytic bacterial isolates

For phenotypical characterization colonies of endophytic bacterial isolates were characterized after 24-96 hours 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 (Fig 3). For SEM studies bacterial cells were prepared by following the method of Mandal *et al.* [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 respective bacterial culture ($1.3x\ 10^7$) for 24 hours at 30-30°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°C till a constant weight is obtained.

Chlorophyll estimation

For the total chlorophyll estimation, the Arnon's [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 [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°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 AND DISCUSSION

Endophytic bacterial colonies were observed on tryptic soya agar plates after 48-96 hours of incubation. In this present study, a total of 31 morphologically distinguishable endophytic bacteria from different parts of *T. interrupta* were isolated. Most of the isolates showed the production of IAA in the presence of tryptophan but the only two isolates STL1 and RT5 produce greater than 60µg / ml of IAA (Fig 2). Preliminary characterizations of these two

isolates were done on the basis of phenotypical (Table 1) and biochemical characteristics (Table 2). It was observed that. STL1 is non-motile gram-positive coccus in nature and RT5 is motile gram-positive rod.

Table 1 Micromorphological characteristics of bacterial isolates

Isolates	Colony morphology						Cell shape	Cell size (µm)	Gram nature	Endospore	Motility
	Colony size	Colour	Form	Elevation	Margin	Transparent					
Kocuria flava	Small	Yellow	Circular	Convex	Entire	Opaque	Coccus	0.59-1.08 dia	+ve	-	-
Bacillus sp	Large	White	Circular	Ubonate	Entire	Opaque	Rod	2.68 – 5.29 x 0.81 – 2.11	+ve	-	+

Table 2 Biochemical characterization of the endophytic isolates

Isolates	Production of enzymes				
	Catalase	Oxidase	Protease	Amylase	Cellulase
Kocuria flava	+	-	-	+	+
Bacillus sp	+	+	+	+	+

Table 3 Carbohydrate fermentation

Isolates	Carbohydrates		
	Glucose	Lactose	Mannitol
Kocuria flava	-	-	-
Bacillus sp	AG	A	A

A-Acid, AG- Acid and Gas

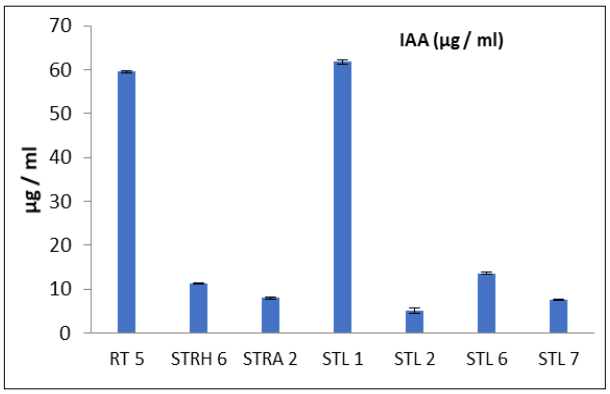

Fig 2 IAA production by the endophytic bacteria isolated *T. interrupta*

Enzymatic screening of these endophytic bacterial isolates showed that STL1 can produce catalase and cellulose while RT5 can produce catalase, oxidase, amylase, protease, cellulase (Table 2). Sugar fermentation profile (Table 3) of these two endophytes showed that only RT5 could ferment glucose, lactose and sucrose. It has been observed that STL1is sensitive to all antibiotics tested and RT5 shows resistance to streptomycin, kanamycin and chloramphenicol (Table 4).

The endophytic bacterial isolates were further directed to DNA extraction, 16s rDNA gene amplification and sequencing. BLAST results showed the identity of isolates STL1 towards *Kocuria flava and* TRT5 towards *Bacillus* sp. The 16S rDNA gene sequences of STL1 and RT5 were submitted to GenBank under accession numbers MZ310566 and MZ310573respectively.respectively. The isolates STL1 and RT5 showed high similarity with *Kocuria flava* and *Bacillus sp.* respectively based on nucleotide homology and phylogenetic analysis. A phylogenetic tree of closest related sequences obtained from NCBI was constructed by using neighbour-joining method with 1000 bootstrap values (Fig 4).

All the plantlets grown in pots supplied with respective endophytic bacterial cultures showed varied growth patterns after 30 days of the seedling (Fig 5). It showed that the seeds were treated with the isolates STL1 and RT5 increase of shoot and root length, primary and secondary leaf count (Table 5), biomass, chlorophyll and protein content (Fig 6-8) as compared to the control.

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)
Kocuria flava	S	S	S	S	S	S
Bacillus sp	S	R	R	S	R	S

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

Growth assessment	One-way ANOVA α = 0.05 *Significant difference with control			
	Control	*Kocuria flava*	*Bacillus* sp.	
Root Length	7.83	9.63 (*)	8.93	F-value: 3.56 *P*-value: 0.03
Shoot length	17.33	22.7 (*)	25.26 (*)	F-value: 60.78 *P*-value: 0.004
Primary leaves	5	6.96 (*)	6.53 (*)	F-value: 12.509 *P*-value: 0.002
Secondary leaves	2.6	5.26 (*)	5.6 (*)	F-value: 11.15 *P*-value: 0.003

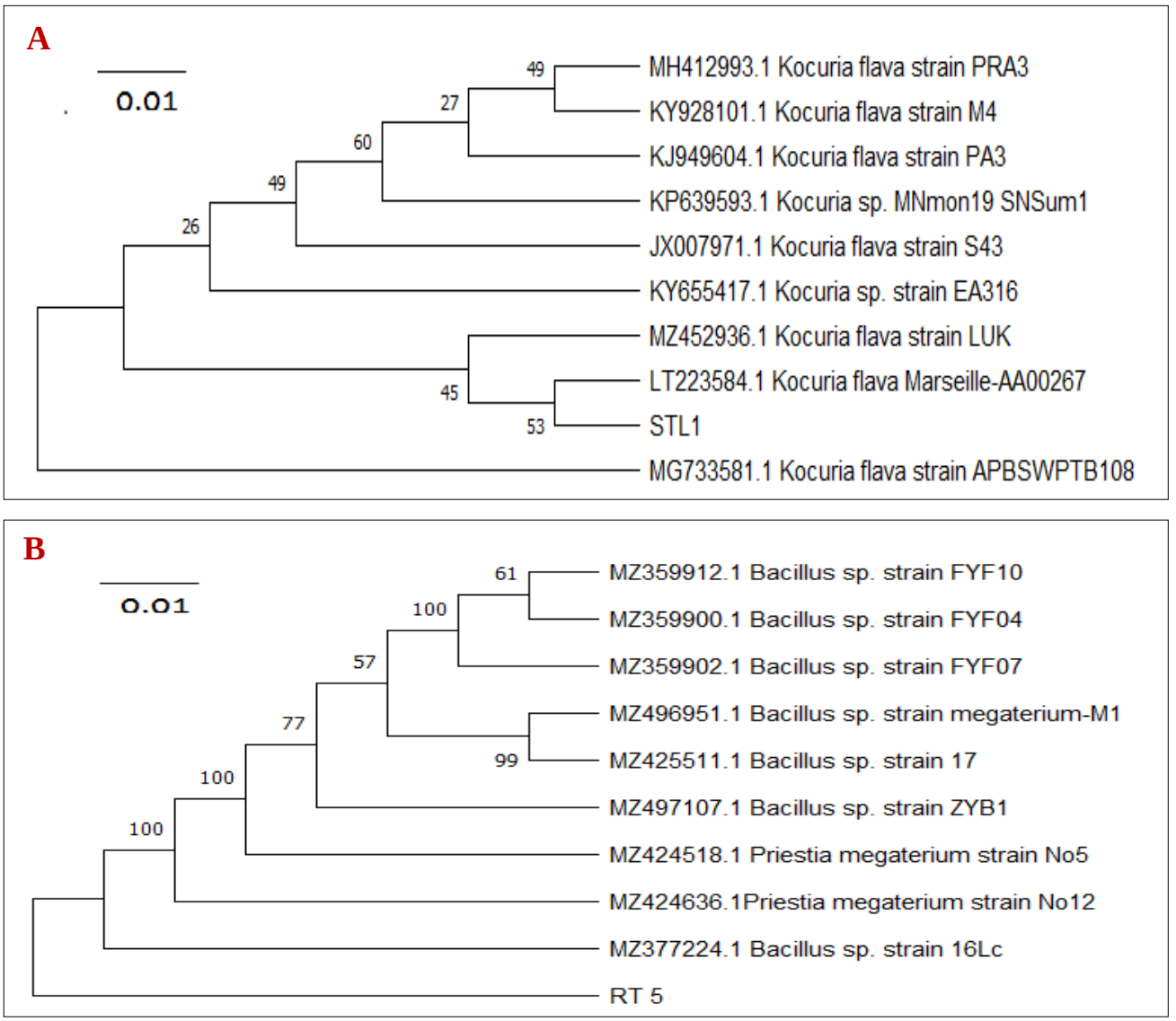

Fig 4 Phylogenetic tree of 16s rRNA gene sequences of isolates with closest related sequences obtained from NCBI using MEGA7. A- *Kocuria flava*, B- *Bacillus* sp.

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

Indole acetic acid (IAA) is considered as the most important plant hormone, also synthesized by endophytic bacteria plays a spectacular role in plant-bacteria interaction. In this present study, a total of 31 morphologically distinguishable endophytic bacteria from different parts of *Thelypteris interrupta* were isolated. Among these seven isolates produce Indole acetic acid (IAA) in the presence of tryptophan (Fig 2) and the isolate STL 1 being the maximum producer of IAA followed by RT5. Both isolates were gram positive in nature and produce hydrolytic enzymes Catalase, amylase, cellulase that are important to colonize to the root of the plant. Several authors reported the production of Indole acetic acid (IAA) by Gram positive bacteria [24-26]. As plant-associated endophytic bacteria are a possible source of enzymes, quantification of those enzymes are often utilized in biotechnological and pharmaceutical processes. The endophytic isolates RT5 could also ferment glucose, lactose and mannitol (Table 3) and were resistant to streptomycin, tetracyclin, chloramphenicol (Table 4).

The identification of endophytic bacterial genera using 16S rDNA gene is a rapid and efficient tool. BLAST analysis of sequences showed the identity of isolates STL1 towards *Kocuria flava* and RT5 towards *Bacillus* sp. Phylogenetic analysis using MEGA7 neighbour joining method also resulted in clustering of STL1 with *Kocuria flava* and RT5 with *Bacillus sp*. The genus *Bacillus* have been reported as Indole acetic acid (IAA) producing endophytes from different plants and also from the pteridophytes *Ophioglossum reticulatum* [4]. Kocuria flava reported as endophytic bacterial strains isolated from the shoot-tips of banana [27] but not from pteridophytes.

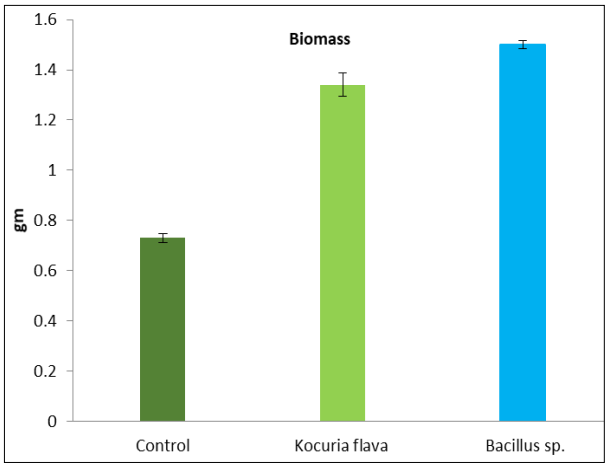

Fig 6 Effect of IAA producing endophytic bacteria on biomass

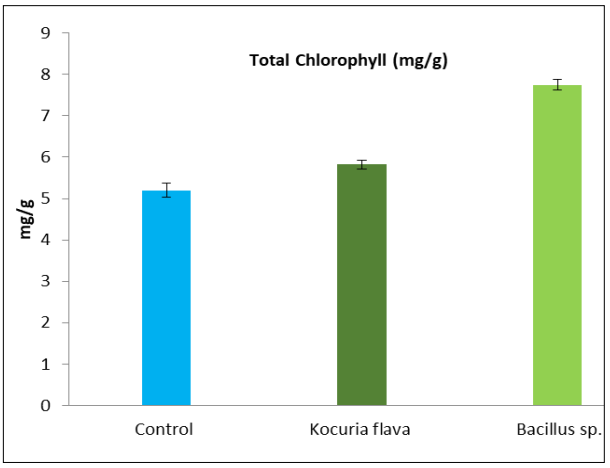

Fig 7 Effect of IAA producing endophytic bacteria on total chlorophyll content

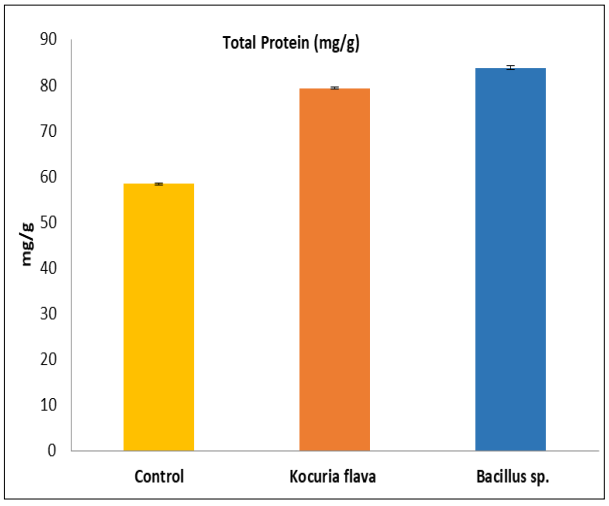

Fig 8 Effect of IAA producing endophytic bacteria on total protein content

IAA helps the plant to increase nutrient uptake by increasing root surface and length. Literature survey indicated that inoculation with IAA producing bacteria resulted in the proliferation of lateral roots [28-29]. In this study significant root growth, shoot growth, the length of primary and secondary leaves as well as biomass, chlorophyll and protein content by these two endophytic bacterial isolates were observed *in vitro* root growth promotion assay. All these characteristics as exhibited by the isolates STL1 and RT5 could be an effective bio-inoculant and be used in organic farming to mitigate the impact of chemical fertilizer on the environment, but also ensures the quality of agricultural products.

CONCLUSION

From the overall study, we can say that two endophytic bacterial isolates from *Thelypteris interrupta* produced several hydrolytic enzymes and have the ability to produce a sufficient amount of IAA which promotes the plant growth. These two efficient IAA producing endophytes were identified on the basis of 16s rDNA gene sequencing as *Kocuria flava* (MZ310566) and Bacillus sp. (MZ310573) respectively. These isolates can be very much useful for future applications for the production of commercially important hydrolytic enzymes and as biofertilizer for the promotion of growth and development of agricultural crop plants.

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LITERATURE CITED

1. Ryan RP, Germaine K, Franks A, Ryan DJ, Dowling DN. 2008. Bacterial endophytes: recent developments and applications. *FEMS Microbiology Letters* 278(1): 1-9.
2. Lamb TG, Tonkyn DW, Kluepfel DA. 1996. Movement of Pseudomonas aureofaciens from the rhizosphere to aerial plant tissue. *Canadian Journal of Microbiology* 42(11): 1112-1120.
3. Rosenblueth M, Martínez-Romero E. 2006. Bacterial endophytes and their interactions with hosts. *Molecular Plant-Microbe Interactions* 19(8): 827-837.
4. Mukherjee A, Bhattacharjee P, Das R, Pal A, Paul AK. 2017. Endophytic bacteria with plant growth promoting abilities from *Ophioglossum reticulatum* L. *AIMS Microbiology* 3(3): 596.
5. Das G, Park S, Choi J, Baek KH. 2019. Anticandidal potential of endophytic bacteria isolated from *Dryopteris uniformis* (Makino). *Jundishapur Journal of Microbiology* 12(1).
6. Munir E. Mamangkey J, Lutfia A. 2019. Antibacterial and phosphate solubilization activity of endophytic bacteria isolated from Pterydophyta (*Tectaria barberi*). *In*: *IOP Conference Series: Earth and Environmental Science*, IOP Publishing. 305(1): 012019.
7. Bhattacharyya PN, Jha DK. 2012. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. *World Journal of Microbiology and Biotechnology* 28(4): 1327-1350.
8. Aziz K, Nawaz M, Nazir J, Anjum AA, Yaqub T, Ahmad MUD, Rehman MU, Aziz G, Khan M. 2015. Isolation, characterization and effect of auxin producing bacteria on growth of *Triticum aestivum*. *Jr. Anim. Plant Sciences* 25: 1003-1007.
9. Ryu RJ, Patten CL. 2008. Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by TyrR in Enterobacter cloacae UW5. *Journal of Bacteriology* 190(21): 7200-7208.
10. Vessey JK. 2003. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil* 255(2): 571-586.
11. Glick BR. 2012. Plant growth-promoting bacteria: mechanisms and applications. *Scientifica*, 2012.
12. Namwongsa J, Jogloy S, Vorasoot N, Boonlue S, Riddech N, Mongkolthanaruk W. 2019. Endophytic bacteria improve root traits, biomass and yield of *Helianthus tuberosus* L. under normal and deficit water conditions. pp 1777-1789.
13. Mondal S, Sukul S. Diversity. 2016. *Ethnobotany and Phytochemistry of Pteridophytes from Birbhum District, West Bengal*. *Ph. D. Thesis*, Department of Botany, Visva-Bharati (A Central University).
14. Woodland DW. 1997. Contemporary plant systematics. 2nd Ed. *Andrews University Press*. *Berrien Spring, Michgian, United States of America*. ISBN 1-883925014-2. 619.
15. Mandal V, Sen SK, Mandal NC. 2013. Production and partial characterization of an inducer-dependent novel antifungal compound (s) by *Pediococcus acidilactici* LAB 5. *Journal of the Science of Food and Agriculture* 93(10): 2445-2453.
16. Shields P, Cathcart L. 2010. Oxidase test protocol. *American Society for Microbiology*.
17. Aneja KR. 2007. *Experiments in Microbiology, Plant Pathology and Biotechnology*. New Age International. ISBN: 81-224-1494-x.
18. Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16: 111-120.
19. Kumar S, Stecher G, Tamura K. 2015. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33(7): 1870-1874.
20. Zinniel DK, Lambrecht P, Harris NB, Feng Z, Kuczmarski D, Higley P, Ishimaru CA, Arunakumari A, Barletta RG, Vidaver AK. 2002. Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Applied and Environmental Microbiology* 68(5): 2198-2208.
21. Gholami A, Shahsavani S, Nezarat S. 2009. The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize. *World Academy of Science, Engineering and Technology* 49: 19-24.
22. Arnon DI, 1949. Copper enzymes in isolated chloroplasts polyphenol oxidase in *Beta vulgaris*. *Plant Physiology* 24: 1-15.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ, 1951. Protein measurement with the folin phenol reagent. *Jr. Biological Chemistry* 193: 265-275.
24. Mohite B. 2013. 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* 13(3): 638-649.
25. Ozdal M, Ozdal OG, Sezen A, Algur OF, Kurbanoglu EB. 2017. Continuous production of indole-3-acetic acid by immobilized cells of *Arthrobacter agilis*. *3 Biotech* 7(1): 23.
26. Bhutani N, Maheshwari R, Negi M, Suneja P. 2018. Optimization of IAA production by endophytic *Bacillus spp*. from Vigna radiata for their potential use as plant growth promoters. *Israel Journal of Plant Sciences* 65(1/2): 83-96.
27. Thomas P, Sekhar AC. 2017. Cultivation versus molecular analysis of banana (*Musa sp*.) shoot-tip tissue reveals enormous diversity of normally uncultivable endophytic bacteria. *Microbial Ecology* 73(4): 885-899.
28. Teale W D, Paponov IA, 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.
29. Chaiharn M, 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.