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Leclercia adecarboxylata - A Putative PGP Phytohormone Producing Endophytic Bacterial Isolate Molecular characterization of *Leclercia adecarboxylata*

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ABSTRACT: Endophytic bacteria that promote the plant growth and development have been isolated from rice apoplastic fluid. The rice leaf apoplast is an intracellular compartment that exists outside the plasma membrane and contains the plant's cell wall. Nutrient transport, cell signalling, water absorption, export, and plant endophytic interactions are all the significant functions of the apoplast. Endophytic bacteria have the capacity to improve the plant development and nutrient acquisition by fixing atmospheric nitrogen, solubilizing phosphorus, potassium, zinc, and producing phytohormones such as IAA, gibberellic acid, and siderophore in subsistent quantities. Nine distinct endophytic bacterial isolates were isolated from varied selected locations, and examined based on morphological and biochemical methods. Among the nine isolates, RAF-2 is a distinct endophytic bacteria has been characterized at molecular level sequencing and identified as *Leclercia adecarboxylata* (OM169350), and the pioneering work reported on rice. It has been found as the significant phytohormone producer includes IAA (29.77 µg 25 mL^{-1}), GA$_3$ (6.02 µg 25 mL^{-1}), and has an excellent ACC deaminase activity (19.33 µmol α-Kβ mg^{-1} protein^{-1} h^{-1}) respectively. RAF-2 (*Leclercia adecarboxylata*) (OM169350) isolated from rice apoplastic fluid was performed as the best in producing phytohormones such as IAA, GA$_3$, and has an excellent ACC deaminase activity, and this could be recommended as an effective phytohormone producer for the sustainable production of crops.

Keywords: Apoplastic fluid, Endophytic bacteria, Plant growth promoting endophytes, *Leclercia adecarboxylata*, ACC deaminase activity.

INTRODUCTION

Rice (*Oryza sativa* L.) is the world's major cereal crop, feeding more than 60% of the world's population. India is the world's second largest rice producer and consumer. Rice production is diminishing in conventional farming, while input and agrochemical prices are mounting. Farmers often abandon this profession due to the low cost advantages and the introduction of numerous seed variants as they are less informed of the new technologies. Agriculture contributes significantly to the gross domestic product of low-income developing nations. Above 50 per cent of Indians rely on agricultural practices. The use of agrochemicals in increasing doses typically degrades soil and food quality, resulting in lower crop yields.

Rice is composed of 80 per cent carbohydrates, 7 to 8 per cent protein, 3 per cent fat, 3 per cent fibre and very low amounts of lipid. Knowing its significance, United Nations designated the year 2004 as the "International Year of Rice". According to the 'Ministry of Agriculture and farmers' welfare, rice production in India remains at 118.43 million tonnes (Directorate of Economics and Statistics, 2020). Rice production in the financial year 2021/2022 remains at 124 million metric tonnes, the volume that includes 106 million metric tonnes of *Kharif* rice and 18 million metric tonnes of *rabi* rice. In Tamil Nadu, rice is grown on 2.2 million hectares, mostly irrigated and partly under rainfed conditions. The average production in the state is around 2.8 tonnes per hectare. In rice production, Tamil Nadu holds the fourth position in our country (2020-2021). In Tamil Nadu, rice has been cultivated in an area of 1.83 million hectares with a production of 5.84 million tonnes (Amrutha and Santhy 2018).

Plant growth promoting bacteria is the free-living microorganism that colonizes plant roots and various aerial parts of the plant and has favourable impacts on plant development. PGPB enhances the growth of plants using their own metabolism, initiates root development, and stimulates the enzymatic activity of the plant or "helping" other beneficial microorganisms to enhance their ability towards the growth and development of the crop. Plant growth enhancing bacteria are employed to increase growth characteristics by direct (or) indirect methods such as phytohormone, siderophore, nutrient solubilization, nitrogen fixation, and phytopathogen antagonism. PGPRs have been shown to improve plant growth development under adverse climatic conditions.

In the present study, plant growth promoting endophytic bacteria has been explored *via,* apoplastic fluids that present inside the rice leaves. In plant tissues, apoplast is a distinctive extracellular compartment that exists outside the plasma membrane, including the cell wall, and allows materials to freely diffuse. The apoplast channel in rice leaves allows "the transfer of water and solutes across tissues and organ". The phenomenon is named as an apoplastic transport". Its capability of establishing a symbiotic relationship with the host can be more beneficial. Several important biological processes take place in the apoplastic compartment of plant leaves, including cell wall formation, cellular water and nutrient uptake and export, plant endophytic interactions and pathogen defence response (Mousa *et al.,* 2015).

Furthermore, RAF-2 was identified by 16S rRNA sequencing. An NCBI BLAST search of the 16S rRNA sequence revealed that RAF-2 is closely related to the genus *Leclercia*. To further confirm this finding, a detailed phylogenetic analysis (MEGA 7.0) was performed, closely related sequences were aligned, and a neighbor-joining tree was constructed using MEGA 7.0. The RAF-2 showed high sequence homology and formed a subclade with *Leclercia adecarboxylata*. Based on these results, RAF-2 was identified as *L. adecarboxylata*, and its 16S rRNA sequence was submitted to the NCBI gene bank under the accession number OM169350.

Leclercia adecarboxylata, a member of the Enterobacteriaceae family, is a Gram-negative bacterium that is motile, aerobic, and omnipresent. Leclerc (1962) identified and called this strain as *Escherichia adecarboxylata*, and Tamura *et al*. (1986) gave it the generic name *Leclercia*. In the soil, plants and microbes naturally communicate, establishing a limited and intricate communication network. This network is based on biochemical to molecular signals that may be modified depending on the type of interaction (Souza *et al*., 2015). Direct growth is aided by the availability of nutrients like as nitrogen and phosphate, as well as the generation of plant regulators such as auxins, cytokinins, and amino acids. These regulators primarily stimulate central and lateral root development, increasing the absorption surface and thereby the root's nutrition and water intake. In the present study, the PGP strain *Enterobacter* sp. / *L. adecarboxylata* (RAF-2) was isolated from apoplastic fluid of rice, and characterized its ability to produce multiple bioactive metabolites including IAA, GA_3, and ACC deaminase activity.

MATERIALS AND METHODS

Apoplastic fluid extraction from rice leaves. The extraction method for apoplastic fluids was carried out by the process of infiltration-centrifugation (Klement, 1965). Infiltration duration varies depending on the size of the leaf and the type of syringe used. 3 to 4 cm long leaf blades from rice crop were clipped off. Clipping was done at the tillering stage, which ranges from 35 to 40 DAT. Fresh leaf fragments were collected, 2 to 3 cm in size, after surface sterilization with Mercuric chloride (0.01 per cent) for one minute, rinsed with distilled water, then 90 per cent Ethyl alcohol, and finally washed with sterile distilled water. The pressurization and depressurization process used in this technique because they are inserted in a syringe filled with 40 mL of sterile distilled water (syringe method). After infiltration, the leaf tips were centrifuged at 4°C for 10 minutes at speeds up to 2500*g*. The apoplastic fluids and solids (bacteria) collected from the leaves were gently resuspended, transferred to a 1.5 mL microcentrifuge tube, and centrifuged at 2,320*g* for 5 minutes at 4°C. 1.5 mL Eppendorf tube was filled halfway with the recovered apoplastic washing fluid. Apoplastic fluid was preserved in glycerol stock at 4°C for further studies. The apoplastic fluid was obtained from the rice leaves of different rice varieties including ADT-45, ADT-36 and CO-43 from Cuddalore, Villupuram and Thiruvannamalai districts of Tamil Nadu. The fresh leaf weight (*i.e*, before infiltration), Infiltrated leaf weight, and after centrifugation leaf weight, were weighed using weigh balance and the results were expressed in grams (Fig. 1a and b).

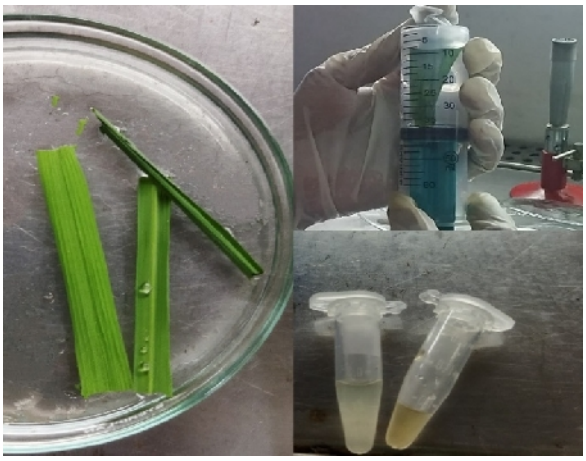

Fig. 1a. Leaf infiltration by centrifugation method.

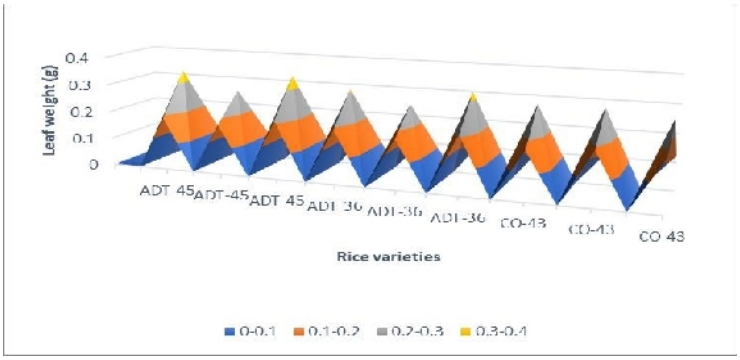

Fig. 1b. Comparison of leaf weight before and after infiltration by centrifugation method.

Enumeration of plant growth promoting endophytic microbial population (El-Deeb *et al.*, 2013). PGP endophytic microbes were isolated from apoplastic fluid of rice. To assess the existence of endophytic bacterial, fungal, and actinomycetes population, the apoplastic fluid collected from the rice leaf was serially diluted up to 10^6 dilutions. The 10^5 dilutions were plated on sterile Petri plates containing Luria Bertani agar (LB) medium for the growth of bacterial colonies at 37°C for two days, 10^3 dilutions were plated on sterile Petri plates containing Martin's Rose Bengal Agar medium (RBA) for the growth of fungal colonies at 28°C for three days, and 10^4 dilutions were plated on sterile Petri plates containing Kenknight's agar medium (KKA) for the growth of actinomycetes colonies at were 30±2ºC for five to seven days. In each plate, the number of bacterial, fungal, and actinomycetes colonies were counted and enumerated using the given formula,

Colony forming unit/mL =

$$\frac{(\text{Number of colonies} \times \text{Total dilution factor})}{\text{The volume of culture plated in mL}}$$

Designation of different endophytic bacterial isolates. The different endophytic bacteria isolated from apoplastic fluid of rice were designated as RAF-1, RAF-2, RAF-3, RAF-4, RAF-5, RAF-6, RAF-7, RAF-8, RAF-9 sequentially.

Characterization of plant growth promoting endophytic bacteria. As part of this procedure, all of the isolates were tested for colony morphology, including colour, shape, ability to form endospores, and Gram reaction.

Cell shape. The morphology of the endophytic bacterial isolates was studied using a simple staining technique. A loop of culture was placed on a clean slide, the bacterial culture was thinly smeared on the slide, crystal violet was used to stain the smear, the smear was rinsed with water, and the slide was examined under a microscope.

Gram staining Technique (Hucker and Conn 1923). Huckers modified technique was used to stain the inoculants using Gram staining. Under oil immersion, the slides were examined using a light microscope. Gram-positive bacteria have a violet appearance, whereas gram-negative bacteria have a pinkish red appearance.

Motility test (Aneja, 2006). Endophytic cultures were inspected microscopically using a hollow slide after being cultured for 72 hours to observe bacterial movement.

Endospore staining (Hussey and Zayaitz 2007). The Schaeffer-Fulton method (or a modification) is the most common method used to perform endospore staining technique.

Biochemical characterization of endophytic bacterial isolates

Catalase test (Aneja, 2006). A loop holding a 24-hour old culture of endophytic bacterial isolate grown on nutritional agar slants was transferred to a glass tube containing 0.5 ml distilled water and well mixed with 0.5 ml of 3% hydrogen peroxide solution, and the presence of effervescence was noted.

Oxidative fermentation test (Aneja, 2006). Fermentation media was prepared and sterilized in autoclave at 15lb pressure for 15 minutes. Each specified fermentation tubes of media inoculated with the organisms and labelled. A layer of oil was added on the top of the media present in test tube and incubated at 35°C for 24-48 hours. Results were observed and noted.

IAA test (Aneja, 2006). Test cultures were inoculated into test tubes containing 1 per cent tryptone broth and incubated for 48 hours at 35°C. Following the 48-hour incubation period, 1 mL of Kovac's reagent was added to each tube containing tryptone broth, and the tubes were shaken for 10 to 15 minutes. The tubes were then left to stand, allowing the reagent to rise to the top. The reddening of the alcohol layer in the tubes within a few minutes showed indole synthesis.

Methyl Red test (Aneja, 2006). The endophytic bacterial isolates were inoculated with Methyl Red test broth made in set of test tubes and incubated for 48 hours at 30°C. A few drops of methyl red alcoholic solution were added to the set of test tubes. The appearance of a distinct red hue was prediction of a positive MR test result.

Voges-proskauer test (Aneja, 2006). The endophytic bacterial isolates were inoculated in the set of tubes, a naphthol solution (5 per cent solution in 70% ethyl alcohol) was added and gently shaken for 15 minutes. The emergence of red hue suggested a favourable response of acetyl methyl carbinol synthesis. This showed a positive VP test result.

Citrate utilization test (Aneja, 2006). Endophytic bacterial isolates were inoculated into Simmons citrate agar medium and cultured for 48 hours at 37°C. A change in pH causes the medium to discolour from green to blue, indicating a favourable response. The carbon and energy sources of citrate, which was found in the Simmons medium.

Starch hydrolysis test (Aneja, 2006). Make a single line streak across the plate with the unknown microorganism. Incubate at either 25 or 37°C. Flood the plate with iodine after incubation and development.

Urease test (Aneja, 2006). The endophytic bacterial isolates were inoculated to urea agar plates and incubated for 24 to 48 hours. The medium's hue shifts from blue to red.

Hydrogen sulphide production (Aneja, 2006). The production of hydrogen sulphide was studied using sulphide indole motility (SIM) medium. SIM agar stabs were made, and endophytic bacterial isolates were inoculated into them. Stabs were inoculated and incubated at 30°C for 48 hours after inoculation. H_2S generation was demonstrated by black colouration along the line of stab injection after 48 hours of incubation.

Triple sugar Iron test (Aneja, 2006). Touch the top of a well-isolated colony with a straight inoculation needle. Inoculate TSI by stabbing through the middle of the medium to the tube's bottom, then streaking the agar slant's surface. Incubate the tube at 35° to 37°C in ambient air for 18 to 24 hours with the cap loosely on. Examine the medium's reaction. The indication of colour changes in the medium from orange-red to deep red.

Carbon utilization of endophytic bacterial isolates. Five different endophytic bacterial isolates were tested for their capacity to use various carbon sources. Glucose, Sucrose and Lactose were added at a one percent concentration. The medium was sterilized and adjusted to a pH of 7.0. The medium was sterilized before being inoculated with 0.1 mL of 48-hour old isolates. The growth of the tubes was measured after two to three days of incubation at room temperature. Examine the tube to determine if it contains gas and acid production.

Molecular characterization. The chosen isolate was identified *via* phylogenetic analysis of the 16S rRNA gene. Sequence Scanner Software v1 was used to check the sequence quality (Applied Biosystems). MEGA 7.0 was used to perform sequence alignment and any necessary modification of the acquired sequences. The amplified sequences were validated to be 16S rRNA by using the NCBI's BLAST program's similarity index (http://www.ncbi.nlm.nih.gov/). The species used in this study were assigned to the following species based on their higher percentage resemblance to the reference species.

Phytohormone production by *Leclercia adecarboxylata.* Among all the selected endophytic bacterial isolates, *Leclercia adecarboxylata* produces phytohormones such as IAA, GA_3 and ACC deaminase activity in enormous amount.

Quantification of Indole-3-acetic Acid (IAA). The production of indole-3-acetic acid (IAA) was measured in Luria–Bertani (LB) broth supplemented with 5 mM L-tryptophan. Bacterial cultures (10^7 CFU mL^{-1}) were injected into LB broth and shaken at 30°C for 36 hours at 120 rpm. Following the incubation time, the culture was centrifuged at 10,000 rpm for 15 minutes at room temperature. One millilitre of supernatant was treated with 2 mL of Salkowski reagent (2% 0.5 M $FeCl_3$ in 35% perchloric acid). Two drops of orthophosphoric acid were also added, and the mixture was kept in the dark to allow the colour to develop. The optical density at 530 nm was measured after 2 hours.

Gibberellic acid production (GA_3). The gibberellic acid production was determined by the following method of Borrow *et al.* (1955). 100 mL Luria Bertani broth was made and sterilized. The selected bacterial isolates were introduced to the medium separately and cultured for seven days at 37°C. The cultures were centrifuged at 8000*g* for 10 minutes after seven days of incubation to eliminate the bacterial cells. Two ml of zinc acetate solution and fifteen ml of culture were pipetted individually into the test tubes. Two mL potassium ferrocyanide solution was added after two minutes and centrifuged at 8000g for ten minutes. Five millilitres of supernatant were added to five millilitres of 30% hydrochloric acid, and the combination was incubated for 75 minutes at 27°C. Five per cent hydrochloric acid was used to make the blank. A UV-VIS spectrophotometer was used to detect absorbance at 254 nm. The amount of GA_3 produced by the culture was determined and represented as µg 25 ml^{-1} broth using a standard graph created with gibberellic acid solutions of known proportions.

ACC deaminase activity. The 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity was determined by culturing bacterial isolates on nitrogen-free medium supplemented with 3 mM ACC as a nitrogen source. The activity of ACC deaminase in bacterial isolates was measured UV spectrophotometrically in terms of α-ketobutyrate synthesis at 540 nm and compared to a standard curve of α-ketobutyrate.

RESULTS AND DISCUSSION

The apoplastic fluid obtained from rice leaves (Three different varieties *viz.,* ADT-45, ADT-36, and CO-43) were collected from nine different locations of three districts in Tamil Nadu,

India. From the collected samples, the fresh leaf weight (*i.e*, before infiltration), Infiltrated leaf weight, and after centrifugation leaf weight were also taken for comparison. The maximum leaf weight taken from the ADT-45 rice variety had recorded 0.210g as fresh leaf weight, 0.345g as infiltrated leaf weight, and 0.135g as after centrifugation leaf weight. Followed by ADT-36 rice variety had recorded 0.210g as fresh leaf weight, 0.325g as infiltrated leaf weight, and 0.129g as after centrifugation leaf weight (Fig. 1a and b).

The relative occurrence of plant growth promoting microbial population belonging to the various genera was determined from the apoplastic fluid of rice leaves

collected from nine different locations in Tamil Nadu. The endophytic bacterial population was expressed as CFU mL^{-1} of sample. The endophytic bacterial population in rice ranged from 12×10^4 to 32×10^4 CFU mL^{-1}.

The maximum bacterial population was 32×10^4 CFU mL^{-1} recorded from Chengam of Thiruvannamalai district, and the minimum population of 12×10^4 CFU mL^{-1} was recorded from Vanur, Villupuram district. The maximum fungal population was 72×10^3 CFU mL^{-1} from Chengam of Thiruvannamalai district, the minimum population of 50×10^3 CFU mL^{-1} was recorded from Annamalai Nagar, Cuddalore district. There is no actinomycetes growth in apoplastic fluid obtained from rice leaves (Fig. 2).

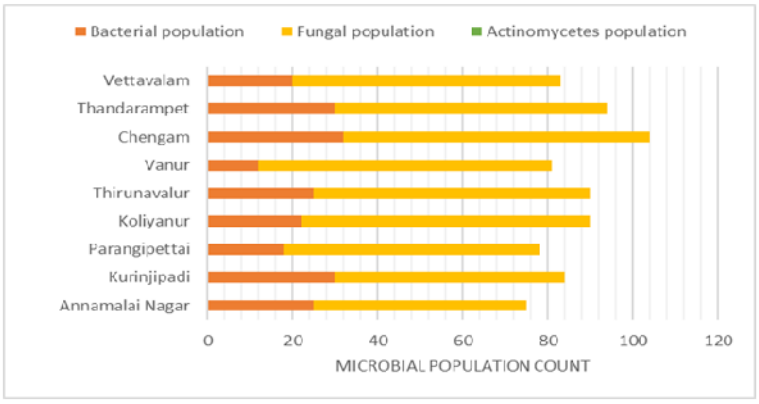

Fig. 2. Analysis of microbial population obtained from apoplastic fluid of rice leaves.

Nine Plant growth promoting isolates were studied and based on the results of morphological and biochemical tests, the species of the genus was ascertained. The morphological characterization of the bacteria was determined using bergey's manual of systematic bacteriology. Endophytic bacterial isolates from various areas of the rice plant were tentatively identified as *Azospirillum*, *Bacillus*, *Pseudomonas*, *Paenibacillus*, and *Methylobacterium* sp. by biochemical analysis, and the details are included in (Table 1 and 2). The nine different PGP isolates were further subjected to utilization of different carbon sources such as glucose, lactose and sucrose. It has been reported as most of all the isolates of endophytic bacterial strains can utilize the carbon sources and involved in gas production (Table 3). *In vitro* plant growth promoting activity of different endophytic bacterial isolates were analysed for its efficiency to produce IAA, GA_3 and ACC deaminase activity. Based on the efficiency of the endophytic bacterial isolates in IAA production the isolate RAF 2 (29.77 µg 25 mL^{-1}) recorded the highest, and this was followed by (28.50 µg 25 mL^{-1}) RAF-3 and the least was recorded by RAF-9 (17.47 µg 25 mL^{-1}). Highest amount of GA_3 was produced by the isolate RAF 2 (6.02 µg 25 mL^{-1}) and the least GA_3 was produced by RAF-9 (4.90 µg 25 mL^{-1}). ACC deaminase activity was recorded maximum for the isolate RAF 2 (9.33 µ mol α-Kβ mg^{-1} protein h^{-1}) and the minimum was produced by RAF-9 (1.00 µ mol α-Kβ mg^{-1} protein h^{-1}). The results for *invitro* plant growth promoting activity of nine different endophytic bacterial isolates were tabulated in Fig. 3. The best and effective strain RAF-2 which performed better in maximum IAA, GA_3, and has an effective ACC deaminase activity.

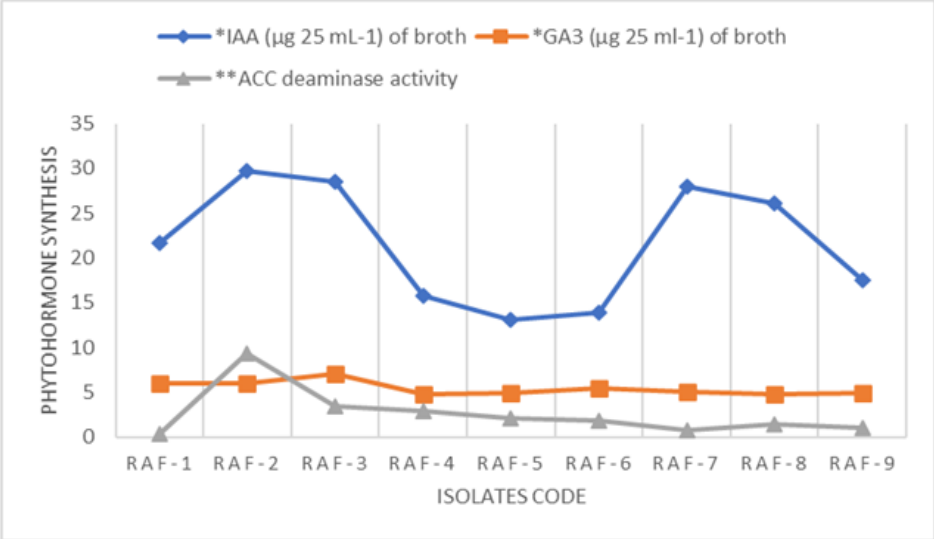

Fig. 3. Phytohormone production by the selected endophytic bacterial isolates.

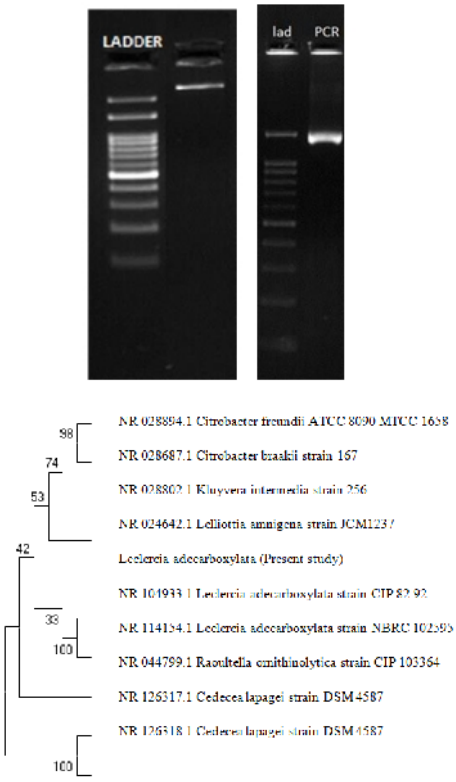

Fig. 4. gDNA and 16S Amplicon QC data: gDNA 16SrRNA amplicon.

Evolutionary relationships of taxa using 16S rRNA gene sequences

Leclercia adecarboxylata

TTGCTCTCGGGTGACGAGTGGCGGACGGGTGA
GTAATGTCTGGGAAACTGCCTGATGGAGGGGG
ATAACTACTGGAAACGGTAGCTAATACCGCAT
AATGTCGCAAGACCAAAGAGGGGGACCTTCGG
GCCTCTTGCCATCGGATGTGCCCAGATGGGATT
AGCTAGTAGGTGGGGTAATGGCTCACCTAGGC
GACGATCCCTAGCTGGTCTGAGAGGATGACCA
GCCACACTGGAACTGAGACACGGTCCAGACTC
CTACGGGAGGCAGCAGTGGGGAATATTGCACA
ATGGGCGCAAGCCTGATGCAGCCATGCCGCGT
GTATGAAGAAGGCCTTCGGGTTGTAAAGTACTT
TCAGCGAGGAGGAAGGCATTGTGGCTAATAAC
CGCAGTGATTGACGTTACTCGCAGAAGAAGCA
CCGGCTAACTCCGTGCCAACAGCCGCGGTAAT
ACGGAGGGTGCAAGCGTTAATCGGAATTACTG
GGCGTAAAGCGCACGCAGGCGGTCTGTTAAGT
CAGATGTGAAATCCCCGGGCTCAACCTGGGAA
CTGCATTTGAAACTGGCAGGCTTGAGTCTTGTA
GAGGGGGGTAGAATTCCAGGTGTAGCGGTGAA
ATGCGTAGAGATCTGGAGGAATACCGGTGGCG
AACGCGGCCCCCTGGACAAAGACTGACGCTCA
GGTGCGAAAGCGTGGGGAGCAAACAGGATTAG
ATACCCTGGTAGTCCACGCCGTAAACGATGTC
GACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTC
CGGAGCTAACGCGTTAAGTCGACCGCCTGGGG
AGTACGGCCGCAAGGTTAAAACTCAAATGAAT
TGACGGGGGCCCGCACAAGCGGTGGAGCATGT
GGTTTAATTCGATGCAACGCGAAGAACCTTACC
TACTCTTGACATCCAGAGAACTTGCCAGAGATG
GCTTGGTGCCTTCGGGAACTCTGAGACAGGTGC
TGCATGGCTGTCGTCAGCTCGTGTTGTGAAATG
TTGGGTTAAGTCCCGCAACGAGCGCAACCCTT
ATCCTTTGTTGCCAGCGGTTAGGCCGGGAACTC
AAAGGAGACTGCCAGTGATAAACTGGAGGAAG
GTGGGGATGACGTCAAGTCATCATGGCCCTTAC
GAGTAGGGCTACACACGTGCTACAATGGCGCA
TACAAAGAGAAGCGACCTCGCGAGAGCAAGCG
GACCTCATAAAGTGCGTCGTAGTCCGGATTGG
AGTCTGCAACTCGACTCCATGAAGTCGGAATC
GCTAGTAATCGTAGATCAGAATGCTACGGTGA
ATACGTTCCCGGGCCTTGTACACACCGCCCGTC
ACACCA.

Kang *et al*. (2019) successfully isolated, *Leclercia adecarboxylata* Mo1 among 36 isolates, and selected based on its IAA producing capability gene, based on the salkowski test and PCR results. The IAA quantification results was revealed that Mo1 produced significant amount of IAA (9.815 ± 0.62 µg mL^{-1}), Po4 solubilization (68.3 µg of P released from 100 mg of tricalcium phosphate), ACC deaminase activity (29.3 µ mol α-KB/mg Protein/h) respectively. Snak *et al*. (2021) reported, the characterization of PGP capacity of *Leclercia adecarboxylata* strain palotina, formerly isolated in corn, and their growth promoting traits such as IAA production and P solubilization were examined and recorded. Scagliola *et al.* (2016) affirmed the potential PGPB candidates have the ability of solubilizing phosphate and Indole acetic acid production.

Table 1: General morphological characters of PGP endophytic bacterial isolates obtained from apoplastic fluids of rice.

Isolate code	Cell shape	Texture	Gram reaction	Motility test	Endospore staining
RAF-1	Rod	Slimy	Negative	Motile	Negative
RAF-2	Rod	Slimy	Positive	Non-Motile	Negative
RAF-3	Rod	Creamy	Positive	Non-Motile	Negative
RAF-4	Rod	Hard and sticky	Positive	Motile	Negative
RAF-5	Rod	Creamy	Negative	Non-Motile	Negative
RAF-6	Rod	Smooth and shiny	Positive	Motile	Negative
RAF-7	Rod	Creamy	Positive	Motile	Negative
RAF-8	Rod	Slimy	Positive	Motile	Negative
RAF-9	Coccid rod	Slimy	Positive	Motile	Negative

Note: Tentative identification according to Bergey's Manual of Determinative Bacteriology

Table 2: Biochemical characterization of PGP endophytic bacterial isolates obtained from the apoplastic fluid of rice.

Sr. No.	Isolate code	Catalase test	Oxidative fermentation test	IAA	IMvic test		Citrate Utilization test	Starch hydrolysis	Urease test	Hydrogen Sulphide production	TSI	Tentatively Identified Species
					Methyl Red test	Voges Proskauer test						
1.	RAF-1	+++	+	-	-	+	-	-	+	-	-	*Pseudomonas fluorescens*
2.	RAF-2	+++	+	+	-	+	+	-	+	+	+	*Leclercia adecarboxylata*
3.	RAF-3	+++	+	-	-	+	+	+++	+++	-	-	*Azospirillum lipoferum*
4.	RAF-4	+++	-	+	+	-	+	+	-	+	+	*Paenibacillus* sp.,
5.	RAF-5	+++	+	-	-	+	+	++	++	-	-	*Azospirillum brasilense*
6.	RAF-6	+++	+	-	-	-	+	+	-	+	-	*Bacillus megaterium*
7.	RAF-7	+++	+	+	-	-	+	-	-	-	-	*Bacillus pumilus*
8.	RAF-8	+++	+	-	-	-	+	+	-	-	-	*Bacillus cereus*
9.	RAF-9	+++	+	-	-	-	-	-	+	-	-	*Methylobacterium* sp.,

Note: Tentative identification according to Bergey's Manual of Determinative Bacteriology
+: Positive result -: Negative result +++: High Production ++: Moderate Production +: Slight Production

Table 3: Carbon utilization test of PGP endophytic bacterial isolates obtained from the apoplastic fluid of rice.

Isolate code	Glucose	Lactose	Sucrose
RAF-1	+++	-	+
RAF-2	++	+	+
RAF-3	+++	+	-
RAF-4	++	-	+++
RAF-5	+	+	-
RAF-6	+	-	-
RAF-7	-	+	-
RAF-8	-	-	-
RAF-9	+	-	+

Note: Tested for acid production after 24h of incubation at 30^{o}C;
+: Positive result -: Negative result +++: High Production ++: Moderate Production
+: Slight Production.

CONCLUSION AND FUTURE SCOPE

Plant growth promoting endophytic bacterial isolates *viz., Azospirillium, Bacillus, Pseudomonas, Leclercia, Paenibacillus, Methylobacterium* were isolated from rice leaves and examined through Morphological and biochemical characterizing methods. *in vitro* plant growth promoting activity were assessed for its efficiency to produce IAA, GA_3, and analysis of ACC deaminase activity, From the following studies, it could be concluded that among the different bacterial endophytes, RAF-2 (*Leclercia adecarboxylata*) (OM169350) isolated from rice apoplastic fluid was performed as the best in producing phytohormones such as IAA, GA_3, and has an excellent ACC deaminase activity, and this could be recommended as an effective phytohormone producer for the sustainable production of crops.

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Conflict of Interest. None.

REFERENCES

Amrutha, K. K., and Santhy, P. (2018). Influence of different management practices and fertilizer levels on the growth and yield of rice in sodic soil. *Madras Agricultural Journal*, *105*(march (1-3), 1.

Aneja, K. R. (2006). Experiments in microbiology, plant pathology and biotechnology. *New Age International*.

Borrow, A., Brian, P. W., Chester, V. E., Curtis, P. J., Hemming, H. G., Henehan, C., and Radley, M. (1955). Gibberellic acid, a metabolic product of the fungus

Gibberella fujikuroi: some observations on its production and isolation. *Journal of the Science of Food and Agriculture*, *6*(6), 340-348.

Directorate of Economics and Statistics, 2020. (https://eands.dacnet.nic.in/)

El-Deeb, B., Fayez, K., and Gherbawy, Y. 2013. Isolation and characterization of endophytic bacteria from *Plectranthus tenuiflorus* medicinal plant in Saudi Arabia desert and their antimicrobial activities. *Journal of plant interactions*, *8*(1), 56-64.

Hussey, M. A., & Zayaitz, A. (2007). Endospore stain protocol. *Am Soc Microbiol*, *8*, 1-11.

Kang, S. M., Shahzad, R., Bilal, S., Khan, A. L., Park, Y. G., Lee, K. E. and Lee, I. J. (2019). Indole-3-acetic-acid and ACC deaminase producing *Leclercia adecarboxylata* MO1 improves *Solanum lycopersicum* L. growth and salinity stress tolerance by endogenous secondary metabolites regulation. *BMC Microbiology*, *19*(1), 1-14.

Mousa, W. K., Shearer, C. R., Limay-Rios, V., Zhou, T. and Raizada, M. N. (2015). Bacterial endophytes from wild maize suppress *Fusarium graminearum* in modern maize and inhibit mycotoxin accumulation. *Frontiers in plant science*, *6*, 805.

Hucker, G. J. and Conn, H. J. (1923). Methods of Gram staining.

Klement Z. (1965). Method of obtaining fluid from the intercellular spaces of foliage and the fluid's merit as substrate for phytobacterial pathogens. *Phytopathology*, *55*, 1033–1034.

Leclerc H.(1962). Biochemical study of pigmented Enterobacteriaceae. *Ann Inst Pasteur* (Paris) *102*, 726–741.

Scagliola, M., Pii, Y., Mimmo, T., Cesco, S., Ricciuti, P. and Crecchio, C. (2016). Characterization of plant growth promoting traits of bacterial isolates from the rhizosphere of barley (*Hordeum vulgare* L.) and tomato (*Solanum lycopersicon* L.) grown under Fe sufficiency and deficiency. *Plant physiology and biochemistry*, *107*, 187-196.

Snak, A., Vendruscolo, E. C. G., Santos, M. F. D., Fiorini, A. and Mesa, D. (2021). Genome sequencing and analysis of plant growth-promoting attributes from *Leclercia adecarboxylata*. *Genetics and Molecular Biology*, *44*.

Souza, R., de, Ambrosini, A. and Passaglia, L. M. P. (2015). Plant growth promoting bacteria as inoculants in agricultural soils. *Genet Mol Biol.*, *38*, 401–419.

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