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Ignatzschineria cameli Strain KAUPDF7-A First Report on Plant Growth Promoting Rhizobacteria (PGPR) from the Post-flood Affected Soils of Kerala

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ABSTRACT

Background: Kerala flood in 2018 and 2019 had reduced the yield in many agricultural plots of Attapadi, Kerala, India. The scope of the study was to identify the potential native plant growth promoting rhizobacteria from the post flood-affected sites to rejuvenate the nutrient depleted soils.

Methods: The present study was carried out in the department of agricultural microbiology, College of Agriculture, Kerala Agricultural University, Vellanikara, Kerala during 2020-2022. Bacteria were isolated from post flood-affected agricultural soils of Attapadi in Palakkad district of Kerala, India. Three morphologically distinct isolates were screened for cellulase, laccase and dehydrogenase to select the best bacterial isolate that could produce the multifunctional enzymes for rejuvenation of flood-affected soils. The isolate were also screened for plant growth promotion traits such as; indole acetic acid (IAA), phosphate solubilizing ability, nitrogen fixing ability and potassium solubilizing ability.

Result: The most promising isolate was identified as *Ignatzschineria cameli*, which was found to be a high indole acetic acid producer along with phosphate and potassium solubilizing ability and revealed as the first report of PGPR from post flood-affected soils of Kerala.

Key words: Cellulase, Dehydrogenase, IAA, *Ignatzschineria cameli,* Laccase, PGPR.

INTRODUCTION

The 2018 flood crisis in Kerala was one of the most disastrous natural calamities to hit the region. Known for its agricultural products, the high range region of Attapadi in the Palakkad district with hectares of agricultural land had severely affected crops (rice, pepper, cardamom, tea, coffee, coconut, arecanut, rubber, coconut and coffee) by flood. Because of the lack of nutrients and microorganisms in the afflicted areas of Attapadi, the yield of crops decreased in many of the flooded soils. At higher elevations, the severely flood-affected soils revealed a decline in microbial biomass (Mace *et al*., 2016).

Plant growth regulators (PGRs) mediate interactions between plant growth promoting microorganisms and the rhizosphere region of soil, which benefits the plants and might result in the geochemical cycling of soil nutrients (Rana *et al*., 2011). The decline of production in the impacted agricultural fields may be related to the loss of these potential microbial biomass which resulted in the depletion of soil nutrients. In order to improve the growth and yield of agricultural crops and maintain the sustainability of agro-ecosystems, it is crucial to screen and select effective PGPRs and use them in the integrated nutrient management strategies. However, more research are required because the long-term implications of are still unknown (Mace *et al*., 2016).

To identify potential PGPRs with soil rejuvenating ability, screening for their enzyme producing ability is relevant. The soil benefits from cellulose, which is the most prevalent polysaccharide in plant cell walls (Richards, 1988). Exo, endo and β-glucosidase, which make up the important enzyme cellulase, cooperate to break down the substrates of cellulose polymers. After cellulose, lignin is the most prevalent source of soil-based carbon (Kubicek, 2012). According to Datta *et al*. (2017), laccases and peroxidase enzymes can depolymerize phenolic and non-phenolic polymer lignin as well as the insoluble lignin. They can also cause lignin degradation by low molecular weight free radicals like OH. Dehydrogenases are only found within cells and they play a key role in the early stages of soil oxidation (Januszek *et al*., 2014) by transferring hydrogen from organic substrates to an inorganic acceptor (Zhang *et al*., 2010).

In the present studies, the test location was a two-acre tract in Thazhesambarcode, Attapadi (11.071808,

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76.571502), interplanted with banana, arecanut, coconut and pepper. According to the farmer, the region's overall crop yield was 50% lower than in previous years. The property experienced flooding in 2018 and 2019 due to its proximity to the river. The isolates TSFAB1, TSFAB2 and TSFAB3 (three morphologically different bacterial isolates obtained from the flood-affected locations of Thazhesambarcode, Attapadi) were screened for the enzymes, cellulase, laccase and dehydrogenase.

The primary objective of the study was to identify a potentially beneficial multifunctional isolate that can promote plant growth in the flood-affected areas. The found isolate may also be used to revitalize nutrient-depleted soils, perhaps increasing the reduced crop output in the afflicted areas.

MATERIALS AND METHODS

The test site was a 2-acre post flood-affected soils in Thazhesambarkode, Attapadi (11.071808, 76.571502), Kerala, India, interplanted with banana, arecanut, coconut and pepper. The field experienced flooding in 2018 and 2019, since it was near a river. The experiment was carried out in the department of agricultural microbiology, College of Agriculture, Kerala Agricultural University, Vellanikara, Kerala during 2020-2022. The region was affected as a result of the river's increased water level. The crop's output decreased by approximately fifty percent from prior years. Soil samples were collected by quadrant sampling method.

The bacterial isolates were isolated on Soil Extract Agar by serial dilution and plate count method (Aneja, 2003). The population was recorded and the isolates with varying colony morphology were purified and preserved for further work. The three selected isolates were screened for 3 enzymes, *i.e*. dehydrogenase (TTC method) (Thalmann, 1968), Laccase (Bavendamm, 1928) and Cellulase (Sazci and Erenler, 1986) by agar plate based screening method. Based on the maximum multi-functional enzyme activities, one isolate (TSFAB1) was further screened for plant growth promoting traits. Indole Acetic Acid (IAA) was quantified for the selected microbial isolate, TSFAB1 using Salkowski's reagent method (Gordon and Weber, 1951). Nitrogen was quantified by Kjeldhal method (Kjeldahl, 1883), phosphorous with ANSA reagent (Olsen, 1982) and potassium quantified using flame photometer (Knudsen *et al*., 1982).

Colony morphology of the selected isolate was analyzed in nutrient agar. Microscopic analysis by Gram's staining and spore staining was performed. The motility of the isolate was monitored by hanging drop method and in SIM medium with triphenyl-tetrazolium chloride (*TTC*). Biochemical characteristics were analyzed for indole utilization (I), methyl red (MR), Voges-Proskauer (VP), fermentation of sugars (glucose, fructose, lactose, sucrose, maltose and D-mannitol), catalase, oxidase and citrate utilization (Basak and Shetty, 2021).

DNA of the selected bacteria was isolated using NucleoSpin Tissue Kit following manufacturer's instructions. The isolated DNA of bacteria was amplified using 16S rRNA primers (16S-RS-F, Forward primer CAGGCCTA ACA CATGCAAGTC and 16S-RS-R Reverse primer GGGC GGWGTGTACAAGGC). The PCR amplification was carried out in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). ExoSAP-IT treated PCR products were sequenced in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, USA) following manufactures protocol. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al*., 2010).

RESULTS AND DISCUSSION

The bacterial population and soil parameters analyzed are presented in Table1. The bacterial population was recorded as 9×10^4 cfu/g of soil. Three morphologically different isolates (TSFAB1, TSFAB2 and TSFAB3) were selected for enzymatic screening. One isolate (TSFAB1) which exhibited maximum multifunctional enzyme producing ability was selected (Table 1 and Fig 1).

Table 1: Values of the parameters analyzed in the soil sample collected from Thazhesambarcode, Attapadi of Kerala India.

Sample	Bacterial count	Moisture content	pH
Thazhesamabarcode soil sample	9×10^4cfu/g	12.40%	6.82 (Neutral)
	Morphologically different isolates selected : 3 isolates		
Enzyme screening of isolates	Dehydrogenase	Laccase	Cellulase
TSFAB1	++++	+++	++++
TSFAB2	+++	+ +	++
TSFAB3	+	-	+
	Maximum Multifunctional Isolate selected: TSFAB1		
	PGPR activities quantified for TSFAB1		
Phosphate (µg/ml)	IAA (µg/ml)	Potassium (µg/ml)	Nitrogen (µg/ml)
8.8	92.75	4.42	0.91

++++: Maximum enzyme producing isolate, +++: Medium enzyme producing isolate, ++: Low enzyme producing isolate, +: Very low enzyme producing isolate, -: Negative enzyme producer.

Colony characteristics of the isolate showed that the colonies were small, off-white in color with a raised elevation with an excavation in the centre, irregular form and with undulate margin (Fig 2). The cells were gram negative short rods (Fig 3). They were non-sporulating, highly motile and oxidase positive but could not utilize citrate. Voges-Proskauer (VP) tests showed positive reaction, whereas indole (I) production and methyl red (MR) test were negative. The isolate could utilize glucose, fructose and sucrose, partial fermentation of mannitol and maltose recorded. Lactose was not utilized by the isolate (Fig 4).

The potential multifunctional enzymatic ability revealed that TSFAB1 was a maximum dehydrogenase and cellulase producer with efficient laccase production. Based on the maximum multifunctional enzyme producing ability, the isolate selected (TSFAB1) were further screened for the four plant growth promoting parameters; IAA, nitrogen, phosphorus and potassium. Plant growth promoting rhizobacteria (PGPR) fixes atmospheric nitrogen, dissolves soil-insoluble phosphates and stimulates the release of various phytohormones that promote plant development (Mahanty *et al*., 2017). The plant growth promoting traits (phosphate solubilization, indole acetic acid production (IAA), potassium solubilization and nitrogen utilization) analyzed showed that the TSFAB1 produce 92.75 µg/ml/ hour indole acetic acid in Luria Bertani broth containing 0.05% L-Tryptophan at room temperature. TSFAB1 also produced phosphate (8.8 µg/ml), potassium (4.42 µg/ml)

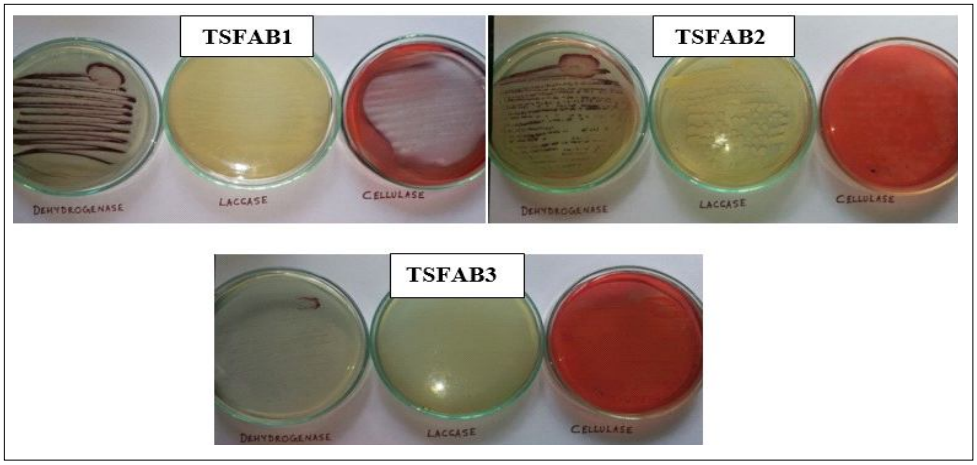


Fig 1: Enzyme screening (dehydrogenase, laccase and cellulase) of the three isolates (TSFAB1, TSFAB2 and TSFAB3).

Fig 2: Colony characteristics of *Ignatzschineria cameli.*

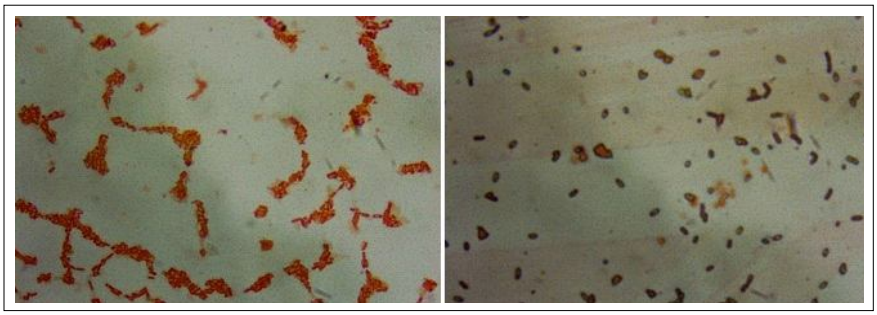
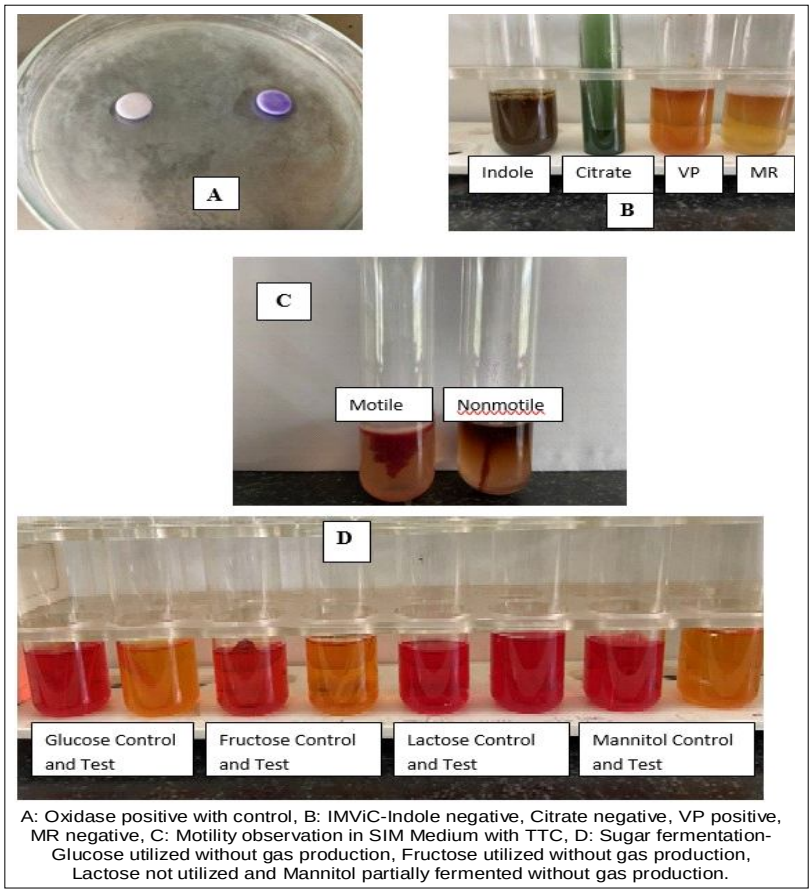


Fig 3: Gram stained picture of *Ignatzschineria cameli.*



A: Oxidase positive with control, B: IMViC-Indole negative, Citrate negative, VP positive, MR negative, C: Motility observation in SIM Medium with TTC, D: Sugar fermentation- Glucose utilized without gas production, Fructose utilized without gas production, Lactose not utilized and Mannitol partially fermented without gas production.

Fig 4: Biochemical tests.

and nitrogen (0.91µg/ml) (Table 1). IAA enhances plant development and yield. The generation of the trait in the medium also depends on tryptophan concentration, bacterial stage, incubation and media conditions (Bessai *et al*., 2022). *Rhizobium* species (142 µg IAA/ml) (Kumar *et al*., 2012) *Pseudomonas aeuruginoasa* (116±0.13 and 108±0.26 ìg IAA/ml) (Uzma *et al*., 2022) and *Bacillus safesnsis* (85.70±3.55 µg IAA/ml) (Lakshmanaan *et al*., 2022) are the common PGPR species which are reported with higher quantities of indole acetic acid (IAA).

The isolate (TSFAB1) were identified using morphological and biochemical characters and confirmed by 16SrRNA sequencing as *Ignatzschineria cameli.* The species was frequently described as being linked to infectious illnesses in humans and animals (Barker *et al*., 2014; Le Brun *et al*., 2015; Heddema *et al*., 2016). *Ignatzschineria cameli* was confirmed as the isolate by molecular (16S rRNA) sequencing. The 16SrRNA gene partial sequence obtained was searched using National Centre for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) and found 100% similarity with *Ignatzschineria sp.* and *Ignatzschineria cameli*. The sequence was 763 bases in length. The obtained partial sequence was submitted to National Centre for Biotechnology Information (NCBI) Gen Bank (Accession number: OP435588). According to Park *et al.* (2021), an *Ignatzschineria* sp. identified was a highly auxin (IAA) producing bacterial strain found in Korea and had an impact on plant growth. However, the species was not specified. According to our research, *Ignatzschineria cameli*, a potentially multifunctional isolate with PGPR activities, was found in flood affected soil samples from Thazhesambarcode, Attapadi Kerala. The isolate has reportedly been linked to necrotic foot tissue in camels and related maggots in the United Arab Emirates (Tsang *et al*., 2018). The isolate is a first report as PGPR from flood affected areas of Kerala.

CONCLUSION

An efficient and potential PGPR isolate (*Ignatzschineria cameli)* was identified from the flood affected agricultural soils of Attapadi, Kerala, India.

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Conflict of interest: None.

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