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Isolation and Characterization of Halophilic Plant Growth Promoting *Rhizobacteria* from Marine Sediment, Water and Coastal Sanddune Plant and It's Screening for Plant Growth Regulators

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ABSTRACT

Background: Plant growth promoting rhizobacteria (PGPR) is beneficial bacteria that are colonizing in the plant root and enhance into the plant growth. The use of PGPR is steadily increasing in agriculture and offers an attractive way to replace chemical fertilizers and pesticides. In this present study, PGPR were isolated from marine sediment, marine water and coastal sand dune plants. From that, 480 different bacterial strains were isolated. Antimicrobial activity and screening of enzyme producing bacteria from the aquatic and terrestrial environments acted as plant growth inducer. Among 480 strains, the 14 strains of *Pseudomonas* spp exhibited the production of indole acetic acid (IAA), extracellular *polysaccharides* (EPS) and antimicrobial activity. This study concluded that PGPR from marine environment can be used as plant growth promoters in agricultural crops.

Methods: Twenty two marine sediments, marine water, coastal sandune plant samples were collected from coastal area of Marakkanam slattern, (12.1899°N, 79.9249°E) Tamil Nadu, India at the depth of 10 cm.

Result: These 14 selected strains from marine environment can be potentially used in therapeutic and plant growth applications.

Key words: Antimicrobial activity, Extracellular *polysaccharides* (EPS), Indole Acetic Acid (IAA), Marine bacterium, Plant growth promoting rhizobacteria.

INTRODUCTION

Soils, marine water and marine sediments harbor high levels of biodiversity and support biogeochemical processes that are relating to life on earth. The soil and sediment function within food webs. The structure of these soil and sedimentary food webs, as well as their role in transforming carbon and nutrients, are often studied separately because of the habitat-centered organization of the research (Ward *et al*., 2017). *Ipomoea* is the largest genus in the flowering plant family convol vulaceae, converting over 600 species. It is a large and diverse group, with common names morning glory, water convolvulus, sweet potato, bindweed, moonflower, *etc*. The soil is in contact with the plant's roots, which may include microorganisms and their activities change its composition. Gram-negative Rhizobium fixes nitrogen in soil. In these bacteria, plant cells infiltrate root nodules; they transform ambient nitrogen into ammonia using nitrogenase and supply glutamine to the plant. Halotolerant and halophilic bacteria live in salty settings, while moderately halotolerant germs are more common (Srivastava and Rauniyar, 2020; Shidhi *et al*., 2021). Competence of the bacteria to respond and sustain in fluctuations of external osmolarity is note worthy function for endurance and propagation in diverse ecological niches acquired during evolution through expression of salt stress and large scale tolerant genes from the bacteria (Das *et al*., 2019). Isolation and classification of salt stress tolerant bacteria from the ocean environment has biotechnological significance through high production of functional bio molecules such as exopolysaccharides, hydrolytic enzymes, osmolytes, *etc*. Diverse halophilic and halotolerant bacteria have been found in aquatic environments and salt stress tolerant genes are being extracted. Isolation, functional screening, characterisation and molecular classification of marine salt-tolerant

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microorganisms are employed for agriculture (Niu *et al*., 2018). The food containing unabsorbed antimicrobials and secreted antimicrobial metabolites in the water and environmental sediment of salmon farming sites often retain their antimicrobial activity and can remain in the aquatic environment for variable period of time depending on their initial concentrations, biodegradability, physical and chemical characteristics (Watts *et al*., 2017). Studies of halo tolerant microorganisms can tolerate salinity stress and even survive in its absence. The study aimed to collect and select the most salt-tolerant microorganisms from sediment and water samples of marine environments for probing osmotic stress in the halo tolerance of crop plants. Coastal sand dunes plants are narrow and mounds of sand with clear bounds determined by the sea and landward sand movement restrictions. Globally, sand dunes encompass 6×10^6 km^2 of land. This ecology is different from inland dunes and sea. These are natural barriers against sea waves; the seashore protects inland natural habitats or developed places. Sand dunes plants are crucial to the richness of our seashore ecosystems. These researches examine halo-tolerant rhizosphere bacteria from marine water, marine sediment and coastal sand dune ipomoea plants: PGPR, IAA, antibacterial activity and EPS (Chu *et al*., 2019) were isolated and characterized. Then the effects of all the strain on the above plants with respect to their protein and chlorophyll content were performed (Alejandro *et al*., 2020).

Materials and Methods

This experiment was conducted 2021 at the Department of Biotechnology, Dr. M.G.R. Education and Research Institute (Deemed to be University), Chennai, Tamil Nadu, India.

Procurement of samples

Twenty two marine sediment, marine water, coastal sandune plant samples were collected from coastal area of Marakkanam slattern, (12.1899°N, 79.9249°E) Tamil Nadu, INDIA at the depth of 10 cm. The samples were collected in sterile air tight bottles and labeled with date of collection and transported to the laboratory for further investigation.

Media ingredients

King's B Medium: It is used for the production of pigments especially non-fluorescent pigment. Serial dilution 10 gm of soil sample diluted in 90 ml of distilled water and kept in shaker for 60 mins. Further serial dilution methodology was carried out while plating for 10^{-3} diluted sample and 10^{-5} diluted sample was carried using kings B medium (Simon *et al*., 2011).

Plant sample

Plant samples were taken from Marakkanam (12.2158°N, 79.9831°E). The plant sample was crushed and 95 ml of distilled water was added to 5 gm of the sample. Further serial dilutions were done and King's B medium was used to spread and pour plates for the 10^{-3} diluted sample and the 10^{-5} diluted sample (Giuliano *et al*., 2019; Gupta and Pandey, 2019).

Water sample

Marakkanam slattern, (12.1899°N, 79.9249°E) a different place was taken and serial dilution of 1gm of soil sample in 9 ml of distilled water and kept in the shaker for 60 mins. Further serial dilution methodology was carried out and spread method plating and pour method plating for 10^{-3} and 10^{-5} was carried out on Kings B medium (Giuliano *et al*., 2019; Gupta and Pandey, 2019).

Isolation of rhizobacteria

The plates were incubated at 37°C for 24 h. Obtained colonies were further sub cultured to get pure colonies. Secondary screening of fluorescent pseudomonades producing bacteria Kings B production medium was again prepared and poured into Petri plates and colonies were streaked and incubated for two days (Giuliano *et al*., 2019; Gupta and Pandey, 2019).

Screening of bacterial strains for the production of iaa

The selected bacterial cultures were inoculated in to nutrient broth medium with 1% of tryptone and incubated at room temperature for 2 days. The role of tryptone acts as precursor for Indole acetic acid biosynthesis. After 2 days of incubation, the grown culture was centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected to which salkowski reagent was added. IAA positive culture supernatants should give dark pink colour (Niu *et al*., 2018). Salkowski reagent is a mixture of 0.5 M ferric chloride ($FeCl_3$) and 35% perchloric acid ($HClO_4$) which upon reaction with IAA yields pink color, due to IAA complex formation with and reduction of Fe^{3+}. After addition of reagent, the optical densities of 409 samples were recorded at 530 nm using UV visible Spectrophotometer. Blank was also maintained as the negative control (Kumar *et al*., 2021).

Extracellular polysaccharides

Out of 480 strains, 79 strains were selected on the basis of their mucoid or sticky appearance (Surya *et al*., 2019). These 79 strains were then inoculated into the labelled test tubes respectively that consist of Yeast and Malt Extract with Glucose (YMG) broth. After inoculation, test tubes were kept for 4 days incubation at room temperature (Ragavan and Das, 2019).

Antimicrobial activity

480 samples were sub cultured in King's B media and was kept for overnight incubation. *Vibrio* culture was swabbed on 120 Nutrient agar plates in sterile environment using cotton swab (Bailey *et al*., 2013). Then the sub cultured strains were inoculated on nutrient agar plates. (4 strains were inoculated on one nutrient agar plate) $4 \times 120 = 480$. After inoculating strains, Ampicillin disc was placed at the centre on each agar plates. Allow it for incubation (37°C

for 24 hrs). On overnight incubation, measure the zone of inhibition of all strains against *Vibrio* pathogen (Giuliano *et al*., 2019).

Sample collection

Samples were collected from Marakkanam (12.1899°N, 79.9831°E) Tamil Nadu, India at depth of 10 cm (Fig 1).

Isolation of Rhizobium bacteria

Colony characteristics

Bacteria show characteristic growth on solid media under appropriate cultural condition. The colonies may be varying in diameter, in outline (circular, wavy, rhizoid, *etc*.) as shown in Fig 2; elevation (flat, raised, convex, *etc*.) and translucency (transparent, translucent and opaque). The colony color may be varying (yellow, brown, white, *etc*.). In some bacteria the background (medium) may get a characteristics color (Srivastava and Rauniyar, 2020).

Secondary screening of rhizobium producing bacteria

Among the 530 isolated screened 480 isolated showed prominent pseudomonas productions in primary screening. They were subjected to secondary screening on same enrichment agar media for the consistence of the isolate rhizobium producing ability. Based on the secondary screening of the rhizobium production isolated of 480 showed prominent activities in both of the screening (Srivastava and Rauniyar, 2020). Hence the isolate 480 was taken for further analysis (Fig 3).

Screening of bacterial strains for the production of IAA

The selected bacterial cultures were injected into 1% tryptone nutrition broth for 2 days (Chu *et al*., 2019). Tryptone

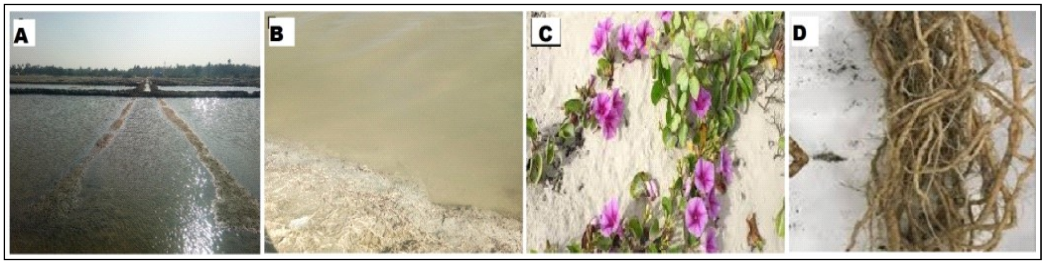

Fig 1: Sample collections.

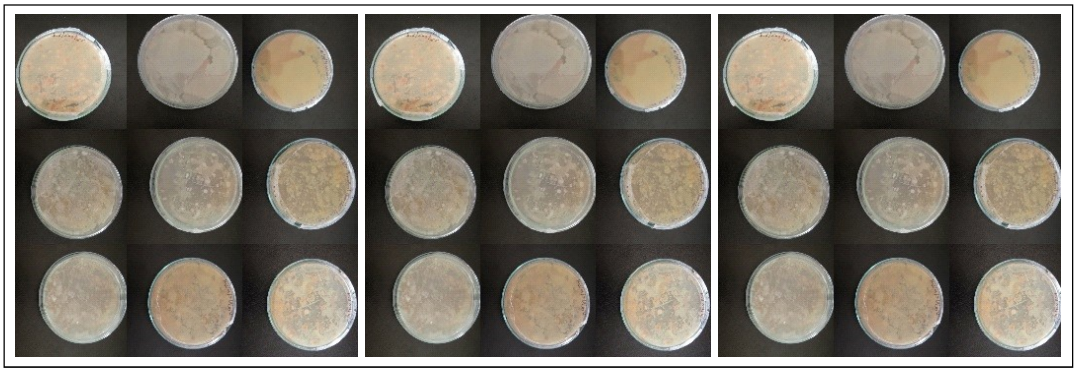

Fig 2: Isolated of *Rhizobium* bacteria culture.



Fig 3: Secondary screening of rhizobium producing bacteria.

helps biosynthesize Indole acetic acid. The 2-day-old culture was centrifuged at 10,000 rpm for 10 minutes. Salkowski reagent was added to supernatant (Surya *et al*., 2019). 530 nm UV visible Spectrophotometer optical density of 409 samples (Chu *et al*., 2019). Blank was the negative control (Fig 4).

Extracellular *polysaccharides*

79 strains out of 480 were chosen for their mucoid or sticky look. 79 strains were put into YMG broth test tubes (Chu *et al*., 2019). Test tubes were incubated at room temperature for 4 days after inoculation. Capsular EPS protects bacterial cells from unfavourable circumstances. EPS-producing bacteria hold and circulate free phosphorus to the plant. EPS-producing bacteria buffer from desiccation, adhere to surfaces and trigger plant defence in plant-microbe interactions (Simon *et al*., 2011).

In 480 (Fig 5) strains we get 14 potential strains which have IAA (Chu *et al*., 2019). EPS (Myo *et al*., 2019) and antimicrobial activity (Kose *et al*., 2019).

The potential strains have IAA, EPS and antimicrobial activity

The following conclusions have been drawn on the basis of present piece of work. Most of studies of pseudomonas are form bacterial but plant also found to be good source for the isolation of rhizobium. The bacteria will have better growth agent than bacterial of the pseudomonas since it has many side effects (Chu *et al*., 2019; Myo *et al*., 2019; Kose *et al*., 2019).

All 1-14 strains incubated IAA production, EPS production and antibacterial activity (Table 1).

Results and Discussion

The selected bacterial cultures were inoculated to nutrient broth medium with 1% of tryptone and incubated at room temperature for 2 days. The role of tryptone acts as precursor for Indole acetic acid biosynthesis. After 2 days of incubation, the grown culture was centrifuged at 10,000 rpm for 10 minutes. Supernatant was added to Salkowski reagent. Positive IAA culture supernatants should be pink. Salkowski Reagent: Dissolve 2.03 g ferric chloride in 500 mL distilled water and add 300 mL H_2So_4. In 480 strains, 95 are pink. After adding reagent, 480 samples' optical density was measured at 530 nm with a UV-Spectrophotometer. Blank was a negative control. 80% of Rhizosphere bacteria make IAA, a plant growth regulator. Tryptophan is IAA's precursor. Based on extracellular Polymeric substance (EPS), 79 strains were selected based on their mucoid or sticky appearance. These 79 strains were then inoculated into the labeled test tubes respectively that consist of YMG broth. After inoculation, test tubes were kept for 4 days incubation at room temperature.

A total of 480 samples were sub cultured in King's B media and was kept for overnight incubation. *Vibrio* culture was swabbed on 120 nutrient agar plates in sterile environment using cotton swab. Then the sub cultured strains were streaked on nutrient agar plates $4 \times 120 = 480$ each plate we cultured 4 strains (Table 2).

After putting the strains on the agar plates, an ampicillin disc was put in the middle of each one. Allow it to sit for 24 hours at 37°C. Measure the zone of inhibition of all the Strains against the *Vibrio* pathogen after letting them sit for a day.

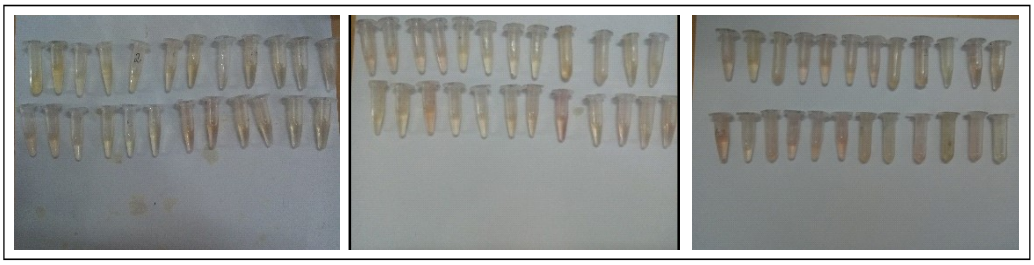

Fig 4: Bacterial strains for the production of IAA.

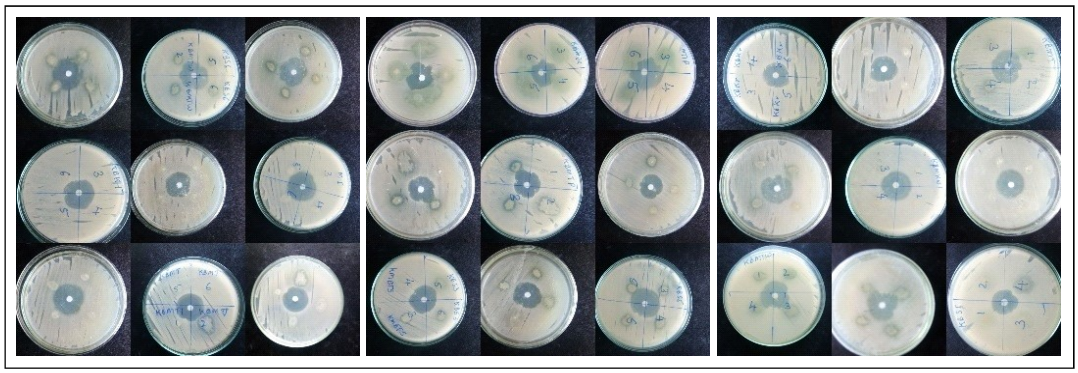

Fig 5: Antimicrobial activity.

The present study is based on the rice rhizosphere population in the marine sediment, water, coastal sand dune plant (*Ipomoea* spp) in Kings B medium. We tested 480 *pseudomonades* spp strains from rice rhizosphere soils for IAA. IAA is a phytohormone, a form of auxins, vital for plant growth and development (Ward *et al*., 2019).

Bacterial cells produce capsular EPS to protect themselves against unfavorable environmental conditions. The EPS producing bacteria helps to hold the free phosphorous and circulating essential nutrient to the plant. EPS-producing bacteria protect plants from desiccation, plant invasion and plant defence in plant-microbe interactions. Phosphorus is a plant macronutrient. PSB solubilize inorganic phosphorus from insoluble materials. PSB are phosphate biofertilizers (Chu *et al*., 2019).

For determining the phosphate solubilizing ability, the isolated bacterial strains were spot inoculated in Pikovskaya's medium. These cultures were incubated for 5 days at room temperature. Antibacterial Isolating and screening enzyme-producing bacteria from aquatic and terrestrial environments may yield plant growth promotion samples. Antimicrobial action kills disease-causing microorganisms. Antimicrobials are utilised. Antibacterial, antifungal, or antiviral are antimicrobials. They all suppress infection in distinct ways. A zone of inhibition test, also termed a Kirby-Bauer Test, measures antibiotic resistance and the ability of solids and fabrics to prevent microbial growth. This test measures and compares antimicrobial activity in fabrics, surfaces and liquids. Using a sterile swab, one million single-strain cells are distributed on an agar plate and incubated with the antimicrobial item (ex: Ampicillin disk). If the bacterial or fungal strain is susceptible to the antimicrobial agent, then a zone of inhibition appears on the agar plate. If it is resistant to the antimicrobial agent, then no zone is evident (Kose *et al*., 2019).

Table 1: The potential strains have IAA, EPS and antimicrobial activity.

Strains	IAA production	EPS production	Anti-bacterial activity
KBMT (Soil)- 1	Yes	Yes	Yes
KBMT (Water)- 2	Yes	Yes	Yes
KBMT (Soil)- 30	Yes	Yes	Yes
KBMT (Soil)- 5	Yes	Yes	Yes
KBMDK (Soil)- 1	Yes	Yes	Yes
KBMT (Root)- 1	Yes	Yes	Yes
KBMT (Root)- 2	Yes	Yes	No
KBMT (Root)- 3	Yes	Yes	Yes
KBMDK (Water)- 3	Yes	Yes	Yes
KBMDK (Water)- 4	Yes	Yes	Yes
KBMDK (Water)- 6	Yes	Yes	Yes
KBMT (Root)- 2	Yes	Yes	No
KBK (Plant)- 1	Yes	Yes	No
KBMT (Root)- 3	Yes	Yes	Yes

Table 2: Strains no 1to 14 strains incubated IAA production, EPS production and antibacterial activity.

Sample code	Sample name	IAA/ optical density at 530 nm	EPS production/ quantification	Anti-bacterial activity/ zone of inhibition (mm)
KBMT	(Soil)- 1	1.122	1.090	25 mm
KBMT	(Water)- 2	0.608	1.120	25 mm
KBMT	(Soil)- 3	1.153	1.120	14 mm
KBMT	(Soil)- 5	1.113	1.100	15 mm
KBMDK	(Soil)- 1	1.383	1.070	20 mm
KBMT	(Root)- 1	1.156	1.130	19 mm
KBMT	(Root)- 2	0.754	1.070	17 mm
KBMT	(Root)- 3	1.153	1.110	21 mm
KBMDK	(Water)- 3	1.119	1.160	13 mm
KBMDK	(Water)- 4	0.712	1.110	14 mm
KBMDK	(Water)- 6	1.364	1.070	14 mm
KBMT	(Root)- 2	1.153	1.101	16 mm
KBK	(Plant)- 1	1.218	1.100	20 mm
KBMTR	(Root)- 3	1.447	1.080	23 mm

Finally we concluded that the potential isolation is from the rice rhizosphere soil. This study indicate that the potential of the bacteria to produce antimicrobial compounds which can be useful for many therapeutically applications.

CONCLUSION

Thus the study concludes in the present study, out of 480 strains isolated from marine sediments, marine water and coastal sand dune plants only 14 strains showed resistance in IAA, EPS and antimicrobial activity. The selected (KBMT (Soil)-1, KBMT (Water)-2, KBMT (Soil)-3, KBMT (Soil)-5, KBMDK (Soil)-1, KBMT (Root)-1, KBMT (Root)-2, KBMT (Root)-3, KBMDK (Water)-3, KBMDK (Water)-4, KBMDK (Water)-6, KBMT (Root)-2, KBK (Plant)-1, KBMTR (Root)-3. All these strains were isolated from the marine sediments, marine water and coastal sand dune plants, Marakkanam Chennai. These 14 selected strains from marine environment can be potentially used in therapeutic and plant growth applications.

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Conflict of interest

All authors declare that they have no conflict of interest.

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