ISOLATION AND CHARACTERIZATION OF PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) FROM SALT TOLERANT RICE (ORYZA SATIVA L. VAR. KORGUT)

Dissertation submitted to the Goa University in partial fulfilment of the requirement for

DEGREE OF

MASTER OF SCIENCE

IN BOTANY

By

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UNDER THE GUIDANCE OF

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DECLARATION

I undersigned hereby declare that the dissertation work embodied in this thesis entitled "**Isolation and Characterization of Plant Growth-Promoting Rhizobacteria (PGPR) from Salt Tolerant Rice (*Oryza sativa* L. var Korgut)**" has been carried out by me, under the guidance of **Dr. Siddhi K. Jalmi,** Assistant Professor, Goa University, Taleigao Plateau, Goa. This research work is original and in partial fulfilment of the requirement for the award of the degree of Masters in Science in Botany, Department of Botany, Goa University. The work presented in the dissertation has not been submitted for the award of any other degree or diploma to any other University or Institution.

Date: Ms. Ashweta Ashok Gaude

CERTIFICATE

The dissertation work embodied in this thesis entitled "**Isolation and Characterization of Plant Growth-Promoting Rhizobacteria (PGPR) from Salt Tolerant Rice (*Oryza sativa* L. var Korgut)**" has been carried out by **Ms. Ashweta Ashok Gaude** under the guidance of **Dr. Siddhi K. Jalmi**, Assistant Professor, in the Department of Botany, Goa University. This work is original and has not been submitted so far in part or in full, for the award of any degree or diploma by any University or Institute.

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1. INTRODUCTION

Rice (*Oryza sativa* L.), belonging to the family Gramineae, is the predominant staple food providing over 20% of calorie needs of nearly two-thirds of the world's population (Liu *et al*., 2019). One-fifth of the world's inhabitants depend upon rice cultivation for livelihoods. According to FAO statistics, China is the largest producer of rice with about 204.23 Million tons (mt), followed by India and Indonesia (FAO, 2012). In Goa, it is cultivated over an area of around 42, 973 ha with a production of 1, 55,818 mt. annually. Average productivity of the crop is about 3, 399 Kg/ha (Guha, 2021). The crop is cultivated in three different topographical situations i.e. rainfed uplands (Morod) and the rainfed lowlands with (Kher) and coastal saline lands (Khazans).

The coastal saline soils in Goa, India are locally known as *Khazan* lands. Khazan ecosystems are reclaimed wetlands and mangrove areas, where tidal influence is regulated by the construction of embankments and sluice gates (Teri, 2018). A created network of bunds protects the agricultural fields and adjoining villages from tidal flows. Khazan lands have three main features: sluice gate, poim and two types of bunds.

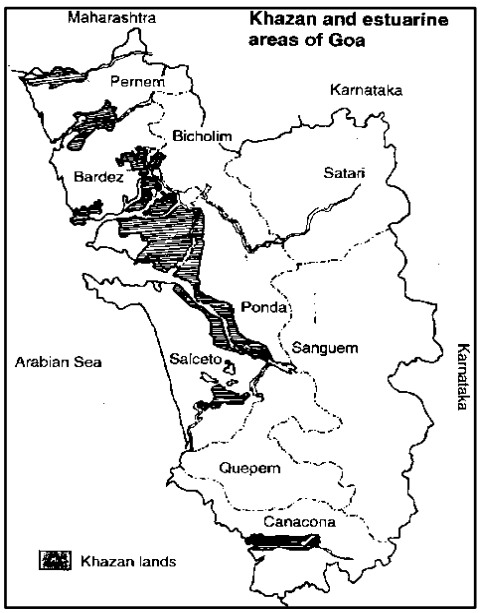


Fig 1: Map showing Khazan land

The dykes prevent saline water from coming onto the lands, the sluice gates regulate the flow of saline water, and the canals help in the drainage and circulation of water (Sonak *et al*.,2012).'True'-managed Khazan lands are located in the estuarine basins of Tiracol, Chapora, baga, Mandovi-Zuari complex, along Cumbarjua canal, Sal, Talpona and Galjibaga rivers. They are predominantly rice and fish fields. Crops grown in khazan fields currently are mainly salt tolerant varieties of rice.

Salt tolerance is an important constrain for rice, which is generally categorized as a typical glycophyte. Soil salinity is one of the major constraints affecting rice production worldwide, especially in the coastal areas (Bheema *et al*., 2017). Among different abiotic stresses to plants, soil salinity is one of the most important factors for reduced crop production. Causes of soil salinity are different in different parts of the world. Accumulation of salts under irrigated agriculture in arid and semi-arid regions is normally observed due to excess evapotranspiration, less precipitation and irrigation. Such kind of salinity is normally characterized by the alkaline soil reaction (high soil pH) (Mahajan *et al*., 2016). Sea water intrusion is one of the most common causes of salinity in coastal areas of India. It is estimated that ~10% of the world's croplands are affected by salinity (Li *et al*., 2007).

Soil salinity has major impacts on growth and productivity of plants, and represents a significant threat to the sustainable agricultural systems. High concentrations of salt stress adversely affect the physiological processes in plants, such as ion homeostasis, lipid metabolism, photosynthesis, and protein synthesis. Soil salinity is one of the important factors affecting soil microbial activities and crop productivity in most of the humid and sub-humid conventional rice growing areas of coastal Asia.

Plant growth-promoting rhizobacteria (PGPR) are free-living soil bacteria thriving in the plant rhizosphere. They have been studied as plant growth promoters and used for increasing agricultural productivity. These bacteria actively colonize the rhizospheric region of root and positively influence plant growth by facilitating the absorption of various nutrients such as nitrogen and phosphate and providing hormones to the plant. These also act antagonistically to bacterial and fungal pathogens thus protecting the plants from pathogens. Hence, PGPR can either directly or indirectly facilitate growth of plants. Indirect stimulation of plant growth includes mechanisms by which the bacteria prevent phytopathogens from inhibiting plant growth and development. Bacteria showing indirect effect on growth promotion are use as biocontrol agents. While direct stimulation may include providing plants with fixed nitrogen, phytohormones, iron that has been sequestered by bacterial siderophores, and soluble phosphate. Presence of 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity in several rhizospheric bacteria and regulation of ACC, a precursor to plant ethylene levels, is one of the principal mechanisms by which bacteria exert beneficial effects on plants under abiotic stress (Barnawal *et al.* 2017; Bharti *et al.* 2016; Kang *et al.* 2014). Looking at the beneficial properties of these bacteria, they are widely used as biofertilizers, bioprotectants in place of the harmful chemical fertilizers and pesticides, for improving the agricultural crop yield and reducing the crop loss due to environmental stresses.

The PGPR are known in alleviating plant environmental stress by several mechanisms, however these are not studied extensively. The microbiota associated with each plant varies among different plants depending on the type of root exudates secreted by the plant. The PGPR associated with rhizosphere of salt tolerant rice will possibly imbibe the property of alleviating salt stress in this cultivar in addition to imparting beneficial properties to the plant. The current study aims to isolate the plant growth promoting

bacteria from the rhizosphere of salt tolerant rice growing in coastal saline fields. Accessing its beneficial properties under salt stress will provide us with one of the mechanism how these rice plants can tolerate high level of salt in fields. The isolated halotolerant plant growth-promoting rhizobacteria (PGPR) could be used as alternative strategy other than breeding to alleviate salinity problems in rice plants grown in the coastal areas. It can stimulate not only plant growth and development, but also alleviate the negative effects on plants caused by salinity and other abiotic stresses (Hardoim *et al*., 2015).

1.1 OBJECTIVES

Considering this background, the objectives for the current study were formulated.

1. Isolation of Rhizospheric and Endophytic microbes from salt tolerant Korgut rice.
2. Characterization of isolates for plant growth-promoting activity.
3. Determining the growth promotion activity of bacterial isolates in seed germination.

2. REVIEW OF LITERATURE

The growth promotion effect on plant by plant growth promoting bacteria is vastly studied in various research laboratories across the globe. One of the study showing the effects of PGPB on the growth of Arabidopsis seedlings is depicted by (Gao *et al*., 2019), which reports the inhibitory effect on the elongation of primary roots and promotion of the lateral root formation and increasing the fresh weight of the shoot. Since there was an increase in the surface area of root system after the inoculation of PGPB, the nutrient and water uptake in soil by the plants was also improved; thus, the growth of plants was also promoted. The isolated strains i.e. DC-12, EC-3, DR-3, HR-4 and IR-10 showed significant effects on the growth and root structure of *Arabidopsis* seedling. It was seen that the plant growth promotion traits were greater in the rhizospheric soil than the bulk soil.

Another study reported plant growth promoting bacterial isolates PGB4, PGT1, PGT2, PGT3, PGG1 and PGG2 induced the production of Indole Acetic Acid (IAA), whereas PGT3 isolate was able to solubilize phosphorus. They found that most of the isolates resulted in the significant increase in the plant height, root length and dry matter production of shoot and root of rice seedlings. It also increased the seed germination of rice (Ashrafuzzaman, *et al*., 2009).

The effectiveness of rhizobacteria containing ACC deaminase activity was demonstrated for inducing salt tolerance and consequently improving the growth of rice plants under salt stress conditions. They assessed the plant growth promoting capabilities of the isolates based on the enhanced plant parameters of 15 day old plants grown under gnotobiotic conditions from rice seeds inoculated with the eleven strains of isolates without stress. They observed that all the plant growth parameters were significantly higher in bacteria treated plants as compared to the control ones (Bal *et al*., 2012).

In study performed by Damodaran, sixteen rhizobacteria were isolated through natural selection from saline sodic soils and characterized using morphological and biochemical parameters. The bacterial isolates were assessed for their plant growth-promoting rhizobacteria traits such as IAA production, ammonia and HCN production, phosphate solubilisation, etc. Among the sixteen isolates screened, two (B-1 and B-3) of them exhibited positive response to all the *in-vitro* PGPR traits studied. These isolates were identified as belonging to genus *Bacillus*. These were also screened them for *in-vitro* salt tolerance and Na^+ uptake pattern where two salt tolerant rhizobacteria B-1 and B-2 showed all PGPR traits with tolerance to salinity. Furthermore, they carried out the pot culture experiment to check the vigour index where these two isolates B-1 and B-2 showed the higher vigour index in the tomato seedlings (Damodaran *et al*., 2013).

Similarly, 74 isolates were isolated from rhizosphere and endorhizosphere of durum wheat plants cultivated in saline environments in the Ghor region near the east of the dead sea. Effect of selected PGPR strains on growth of salt sensitive and salt tolerant durum wheat genotypes under high salt stress conditions was studied. Their studies demonstrated that there were six halotolerant PGPR strains which improved the survival in inoculated plants under high salt stress which was observed by the high germination percentages and seedling root growth as comparison to the non-inoculated plants. Three strains improved durum wheat tolerance to water deficit stress and four strains showed antagonistic effect against *Fusarium culmorum* that causes crown rot disease. They observed that halotolerant PGPR strains improve the productivity of durum wheat under different stress conditions (Albdaiwi *et al*., 2019).

The effect of salt tolerant microbes and organic matter supplementation on rice plant growth and soil chemical and biological properties in coastal saline soils under pot conditions were studied. They compared three microbial inoculants namely; *Pseudomonas*

multiresinivorans, *Microbacterium esteraromaticum*, and *Bacillus subtilis* individually and their consortium along with amended farmyard manure (FYM) against untreated control and FYM without the microbial inoculant. The treatments with combined application of *B. subtilis* with FYM gave the best results. They concluded that the combined application of salt tolerant microorganisms and organic amendment helps rice plants alleviate salt stress and improves the plant growth (Bhambure *et al*., 2017).

Assessment of the isolates for their functional quantitative activities and their PGP properties based on germination and IAA production was carried out.Molecular and biochemical properties of the isolates were used to identify the strains which were isolated on the respective media for colonial growth. They identified 15 bacterial isolates from which a third belonged to the genus *Burkholderia* and a fifth belonged to the genus *Stenotrophomonas* sp. On inoculating on mung bean seeds, the bacterial isolates showed increase in root and shoot length as well as high seedling vigour along with significant IAA production depending upon their high functional activities. They then suggested that these functional bacterial strains could potentially be included in bio-fertilizer formulations for crop growth on acid soils (Tang *et al*., 2020).

Four low temperature surviving PGPR strains were isolated from root nodules of pea *(Pisum sativum* L.*)* plant growing in different agro-climatic regions. All the isolated strains showed growth at up to 5℃ in yeast extract-mannitol-mineral salts broth. Among these strains, PR-12-12 and PR-12-15 showed higher cell growth. They then studies these strains for their in vitro characteristics where all the PGPR strains showed Phosphate solubilisation activity ranging from 16-25 mm (7 DAI), produced phytohormone indole-3-acetic acid (IAA) in the range of 62.7-198.1 ug/ml (Meena *et al*., 2015)

A novel strain of halotolerant bacteria; *Glutamicibacter sp.* (YD01) was isolated and identified using 16 rDNA analysis. It was observed that the tolerance level of the strain to be 10% NaCl and besides that it showed other growth promoting traits such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme activity and IAA production. They stated that the inoculation of this strain could enhance the tolerance of rice (*Oryza sativa* L.) plants to salt stress by regulation of plant ethylene production, ACC content, ACC oxidase activity and improving K^+ acquisition (Yuan *et al*., 2020)

The effect of PGPR on lowering down the salt stress was investigated in many studies, of which one of the studies is listed here. In this study, different treatments were used as follows; Control(T1), Salt tolerant isolate KH-1(T2), Salt tolerant isolate KH-2(T3), Salt tolerant isolate KH-3 (T4), PGPR-I (*Pseudomonas*) (T5) and PGPR-II (*Azotobacter*) (T6). They sowed the rice under saline conditions with the inoculation of salt tolerant PGPR. The results were that the growth and yield of the plants were improved. It showed significant increase in plant height, biomass and yield over control. The inoculation of PGPR isolate KH-2 produced maximum grain yield in rice followed by PGPR-II as compared to other treatments including control. This concluded that with the application of salt tolerant isolate KH-2 there is a significant increase in rice production (Javed, *et al*., 2020).

From the rhizosphere region of mung bean plants; isolated 39 bacterial isolates on King's B and nutrient agar media. They further identified four isolates as *Pseudomonas* spp., *Bacillus* sp., *Acinetobacter* sp. using biochemical and 16S rDNA gene sequencing analysis. They screened the isolates for *in vitro* plant growth promoting attributes such as IAA production, phosphate solubilisation, ammonia production, catalase production, siderophore production and antagonistic activity against phytopathogenic *Rhizoctonia solani*. All the bacterial strains showed significant PGPR attributes and were able to

produce indole-3-acetic acid. The result suggests that the isolates possess, multiple plant growth-promoting traits and can be used as a potential candidate on the soil-plant system to increase their growth as well as productivity (Upadhyay *et al*., 2018).

Seventy bacteria were isolated from the rhizosphere of potato cv. Hartapel that grew at an altitude of 700 m above sea level. From these; 36 isolates were capable of producing IAA, GA, Siderophore and solubilizing phosphate. Isolate HB8 produced highest amount of IAA, while isolate HB32 produced highest amount of GA. Isolate HB18 produced salicylate type siderophore and isolate HB3 showed highest phosphate solubilisation. Three isolates namely; HB3, HB8 and HB31 positively produced HCN. This suggested the use of these isolates for biostimulant, biofertilizer and bioprotectant against soil borne pathogens (Kesaulya, *et al*., 2015).

Salinity stress is a barrier to crop production, quality yield, and sustainable agriculture. It affects seed germination, plant growth, and development, resulting in major agricultural yield losses worldwide. All growth phases undergo several morphological changes due to salinity stress. It also causes various biochemical changes which include antioxidant enzyme activation, modulation of phytohormones, changes in ion uptake, generation of reactive oxygen species (ROS), and disruption of photosynthetic pathways. Plant growth-promoting bacteria (PGPB) elicit salinity tolerance and positively affect plant life cycles, promoting plant growth by direct and indirect mechanisms.

Several studies have reported various mechanisms by beneficial microbes which induce stress tolerance in plants; the mechanisms such as producing gibberellins, indole acetic acid and some unidentified elements which results in increased root surface, root length area, root tips and also it increases the nutrient content leading to better health of the plant under stress conditions (Shahid *et al*., 2018). PGPR can produce different growth

hormones such as cytokines, accumulate abscisic acid (ABA) as well as antioxidants which detoxifies ROS (Barassi *et al*., 2006). It also produce ethylene which are important for several post- transcriptional modifications, also controls plant homoeostasis (Tiwari *et al*., 2014).

With these reports it is noted that PGPB isolated from rhizospheric and endophytic regions of various plants exerts positive and beneficial effects on the growth and development of plant. Many reports suggest that this beneficial effect and tolerance against various biotic and abiotic environmental stresses is imparted by PGPB by providing the unavailable nutrients, phytohormones and also by regulating several stress responsive genes. Hence, the study of isolating rhizospheric and endophytic beneficial bacteria from the roots of salt tolerant rice cultivar growing in khazan field will be crucial in identifying the beneficial bacteria associated with this cultivar. Further, study of the beneficial properties like nutrient acquisition, phytohormone production and stress alleviation imposed by PGPR on local rice cultivar will provide us with possible role of this PGPR in salt stress resistance exhibited by this important local rice cultivar.

3. MATERIALS AND METHODS

3.1 Collection site

The samples were collected in the month of August from the Khazan land cultivated with Korgut rice which is located at Raia, South Goa. The location of the collection site (15º19’18.3” N, 73º59’41.2” E) was recorded by Google maps (Fig. 2). This is one of the living khazan situated in the Salcette Taluka, South Goa. It lies along the Zuari estuaries which were reclaimed by the earlier communities. Korgut are mainly cultivated in the kharif season in this area. The sample was collected in a sterile plastic bag before they were transferred to the Laboratory at Department of Botany, Goa University.

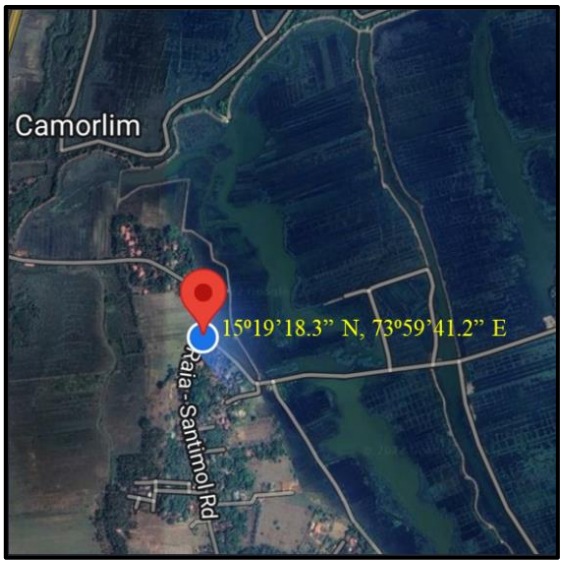


Fig 2: Collection site

3.2 Isolation of Rhizospheric and Endophytic Bacteria

To isolate the rhizospheric bacteria, the roots were gently shaken by immersing them in the sterile distilled water to remove the loosely bound soil clumps, leaving behind the soil firmly adhering to the roots (Rhizoshperic soil). This was further used for the serial dilution. The roots were cut into small pieces using sterile blade; suspended and vortexed

in 10ml of the 0.9% NaCl solution i.e. saline solutiuon. Further, 6 fold serial dilutions were prepared. 100 µl aliquote from 3^{rd} to 6^{th} fold of rhizospheric soil were spread plated on the Nutrient Agar Medium. The plates were incubated at 27℃ and monitored for two weeks for colony formation.

For the isolation of endophytic bacteria, the roots of the collected sample were rinsed in the sterile distilled water until the rhizospheric soil was removed. Thereafter, the roots were sterilized by soaking them into 70% ethanol for 30 seconds then rinsed with sterile distilled water thrice; followed by surface sterilization by soaking the roots in 1% Sodium hypochlorite for 1 minute. The roots were rinsed again with sterile distilled water 4-5 times. Using sterile mortar pestle; the roots were macerated using 1ml of 0.9% NaCl, and serial dilutions were prepared using 1 ml of tissue extract up to 6 folds. 100µl of aliquote were spread plated from 3^{rd} to 6^{th} dilution on the plated containing Nutrient Agar medium and then the plates were incubated at 37℃ for two weeks monitoring for colony formation.

The colonies of rhizospheric and endophytic bacterial isolates were examined for its morphological characters which included; shape, colour, texture, margin, appearance, pigmentation. The colonies with distinct morphological features were further sub-cultured on Nutrient Agar medium supplemented with 5% NaCl for isolating halotolerant bacteria. The plates were then stored at 4℃ for further use.

3.3 Halotolerance Assay

Bacterial isolates were screened for halotolerance by inoculating them on the NA plates supplemented with different concentrations of NaCl (5%, 10% and 20%). The inoculated plates were incubated at 37℃ for 7 days. On Day 7 the plates were observed for the bacterial growth.

3.4 Screening for Plant Growth Promoting Traits

3.4.1 Nitrogen production/fixation Assay

The isolates were spot inoculated on the plates with Jensen's medium. Jensen's media was prepared by mixing 24.1 g of Jensen's Broth and 7.5g of Bacteriological Agar in 1000 ml of distilled water, autoclaved at 121℃, 15 psi pressure for 15-20 minutes. The plates were prepared in sterile conditions under laminar air flow. The plates were then incubated at room temperature for 4 days for colony formation. The growth of the bacterial isolates on the medium was observed and colony diameter was measured using ruler, the growth indicated the nitrogen producing ability of the isolates.

3.4.2 Phosphate solubilization Assay

The Phosphate solubilisation ability of the bacterial isolates was studied by spot inoculating the isolates on the modified Pikovskaya agar plates. The media was prepared by mixing 31.3g of Pikovskaya media powder in 1000 ml of Distilled water and autoclaved at 121℃ at 15 psi pressure for 15-20 minutes. The media as poured in plated under laminar air flow. The plated were sealed using paraffin after inoculation. The isolates having the phosphate solubilisation ability was confirmed when they form a transparent holo zones around the bacterial colonies after 4 days of incubation at room temperature.

3.4.3 Cellulose Degradation Assay

The isolates were spot inoculated on the plates containing 0.02% Congo red supplemented with 2% of Cellulose. The media was prepared by adding 0.5 g KH_2PO_4, 0.25 g $MgSO_4$, 2g of Cellulose powder, 0.02% Congo red, 5g Agar and 2g of Gelatin in 1000 ml of distilled water; autoclaved at 121℃ , 15 psi pressure for 15-20 minutes. The plates were

inoculated under laminar air flow. The plates were incubated at room temperature for 4 days until the zone of clearance was observed. The isolates which degraded cellulose formed a clearance zone around the bacterial strain.

3.5 Qualitative Assays:

3.5.1 Ammonia Production Assay

Bacterial strains were grown in 10 ml of Peptone broth supplemented with 2% sucrose. The media constituents used for preparation were 5g Peptone water, 0.5g Ferrous sulphate, 2g Disodium hydrogen phosphate, 0.5g Calcium nitrate and 20g Sucrose in 1000 ml of distilled water. The media was sterilized using autoclave at 121℃, 15 psi pressure for 15-20 minutes.10 ml of LB media was transferred in each tube under laminar air flow maintaining all the sterile conditions. Loop-full of bacterial culture was inoculated in the media using sterile nichrome loop. Inoculated test tubes were incubated at 36℃ for 48-72h on a shaker at 150 rpm. After the growth of bacterial culture, 0.5 ml of Nessler's reagent was added to the suspension culture which resulted in the formation of brown-yellow colour compound. Formation of brown to yellow colour compound suggests the production of Ammonia.

3.5.2 IAA production Assay

The bacterial isolates were cultured by inoculation of loop-full of bacterial isolate in 5ml of Nutrient broth and incubating it at 37℃ in dark for 24 h in a shaker at the speed of 150 rpm and then, 100µl of bacterial culture from the suspension culture was transferred to 5 ml of NB medium supplemented with 0.15% of L-tryptophan. After the incubation in the dark at 30℃ with continuous shaking at 150 rpm for 4 days, 2 ml of bacterial culture was centrifuged at 8000 rpm and 4℃ for 10 minutes and 1 ml of supernatant was transferred to

the new test tube. After that, 1 ml of Salkowski reagent(10 ml of 35% $HCLO_4$ + 1 ml of 0.5 M $FeCl_3$) was added to the supernatant and the mixture was incubated at 30°C dark for 30 minutes. It formed pale pink coloured compound. The development of pink colour in the test tubes indicated the production of IAA.

3.6 Evaluation of Growth Promoting Ability of the Isolates

3.6.1 Germination Test

De-husked rice grains (*Oryza sativa* L. var. Jaya) were soaked in 70% ethanol for 5 minutes in a sterile beaker. The ethanol was discarded and the grains were surface sterilized with 0.5% Mercuric Chloride for 30 seconds and washed 5 times with sterile distilled water. Bacterial suspension culture prepared in a NB medium were taken and the rice seeds were soaked in a bacterial culture suspension along with 0.06% gum acacia solution and incubated overnight at room temperature. Each seed was then placed in the test tube containing 1% agar and incubated at room temperature for 7 days. Numbers of seeds germinated were observed regularly for the 7 days.

4. RESULTS

4.1 Isolation of Rhizospheric and Endophytic Bacteria

Out of 49 isolates that grew on the NA media, 32 were rhizospheric and 17 were endophytic. The majority of the colonies had creamy colour and smooth texture. For the halotolerance assay, out of 49 isolates; 45 isolates tolerated salinity level of 10% NaCl of which 31 were rhizospheric and 14 were endophytic. Further, 32 isolates were able to tolerate salinity level up to 20% NaCl of which 22 were rhizospheric and 10 were endophytic (Plate 1).

4.2 Screening for Plant Growth Promoting Traits

4.2.1 Nitrogen production/fixation assay

All the selected isolates based on the halotolerance assay were screened for different plant growth promotion traits. In this study, 36 out of 49 bacterial isolates showed growth on Jensen media. Jensen media being the nitrogen free media; is recommended for detection and cultivation of nitrogen fixing bacteria. The growth of the bacteria on Jensen media suggests that the isolates can produce/fix nitrogen. Sucrose in the media acts as the energy source. Sodium molybdate increases the fixation of nitrogen. Of these 25 were Rhizospheric isolates (Table 1) and 11 were Endophytic isolates (Table 2) that grew on the Jensen's medium after incubating plates for 7 days at 37℃ (Plate 2). The colony diameter was measured of all the isolates grown on the medium. The colony diameter ranged from 0.1 cm to 1.3 cm in the Rhizospheric isolates and 0.2 cm to 1.1 cm in the Endophytic isolates (Table 1 and Table 2).

4.2.2 Phosphate solubilisation assay

In this assay, 42 out of 49 bacterial isolates grew on Pikovskaya medium and of which 30 Rhizospheric and 11 were were Endophytic isolates (Plate 3). The bacterial colonies

showed the zone of clearance after incubation period of 7 days on Pikovskaya agar plates. Pikovskaya media is recommended for detection of phosphate solubilisation bacteria. Yeast extract in the medium provides nitrogen and other nutrients necessary to support bacterial growth. Dextrose acts as an energy source. Different salts and yeast extract supports the growth of organisms. Phosphate-solubilizing bacteria will grow on this medium and form a clear zone around the colony, formed due to phosphate solubilization in the vicinity of the colony. The clearance zone was measured in centimetres. From 30 Rhizospheric isolates; isolate R-6 showed the maximum clearance zone i.e. 0.5cm followed by R-29(0.45 cm), R-11(0.41 cm) and R-8 and R-10 (0.38 cm) (Table 3). Among 11 Endophytic; isolate E-2 and E-5 showed the maximum clearance zone i.e. (0.75 cm) followed by E-3 showing 0.61 cm of clearance zone (Table 4) (Fig 3).

4.2.3 Cellulose degradation assay

For Cellulose degradation 30 out of 49 isolates; 16 Rhizopsheric and 14 Endophytic showed the clearance zone after 7 days of inoculation at 37℃ on the plates containing 0.02% Congo red supplemented with 2% of Cellulose (Plate 4). From the Rhizospheric isolates maximum clearance zone was exhibited by R-27 (4.4 cm) followed by R-18 (3.0 cm) and R-17 (2.3 cm) (Table 5). From the Endophytic isolates maximum activity was shown by E-15 (4.2 cm) followed by E-6 (2.35 cm) (Table 6).

4.2.4 Ammonium Production Assay

In the qualitative assay for determining production of ammonia through nitrogen fixation, 24 out of 49 Ammonium producing isolates of which 13 Rhizospheric and 11 Endophytic formed the yellow to brown colour compound Peptone broth supplemented with 2% sucrose. R-1 showed the crimson yellow colour compound, R-10 formed a canary yellow compound and rest formed yellow compound. From the Endophytic; majority of the

isolates formed yellow-orange compound which included E-8, E-12, E-13, E-14 and E-3 showed the formation of Crimson yellow compound (Plate 5).

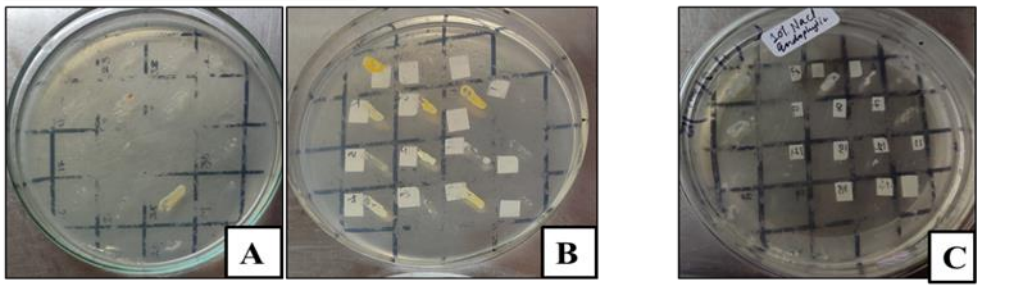
4.2.5 IAA Production Assay

In this assay, 34 out of 49 IAA producing isolates; 20 Rhizospheric and 14 Endophytic isolates were showing positive results to the IAA production assay by forming pink coloured compound. Among the Rhizospheric, isolates R-2, R-6, R-7, R-12, R-13, R-19, R-21 and R-29 formed the pale pink coloured compound. From the Endophytic, majority of the isolates formed lighter shades of pink except for the isolates E-2, E-4, E-9, E-12, E-13 and E-14 which showed clear pink coloured compound (Plate 6).

4.2.6 Germination assay

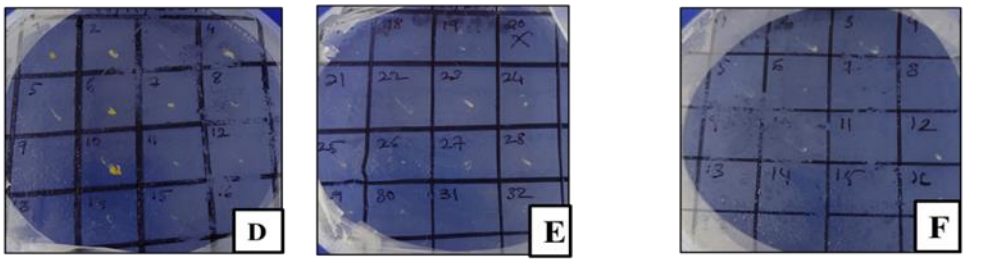
The growth promotion effect of isolated bacterial isolates was tested by performing germination assay on de-husked seeds of Rice (*Oryza sativa* L. var. Jaya). Most of the Rhizospheric isolates except R-2, R-11 and R-29 promoted the germination within three days of inoculation. Similarly, many of the endophytic isolates except E-2, E-4, E-10 and E-12 promoted germination within three days of inoculation. Some isolates; R-31, R-14, R-23, E-7 and E-8 promoted shoot growth on Day 7.

Halotolerance Assay



Rhizospheric isolates on 10% NaCl

Endophytic isolates on 10% NaCl

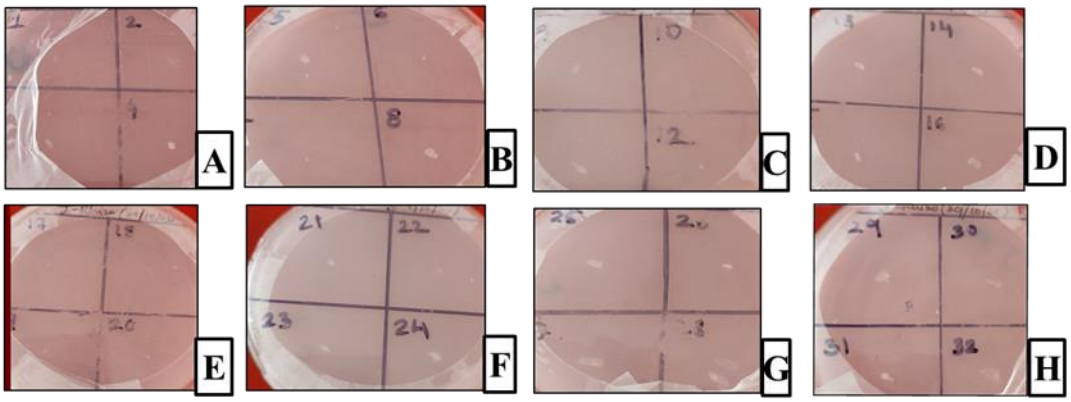


Rhizospheric isolates on 20% NaCl

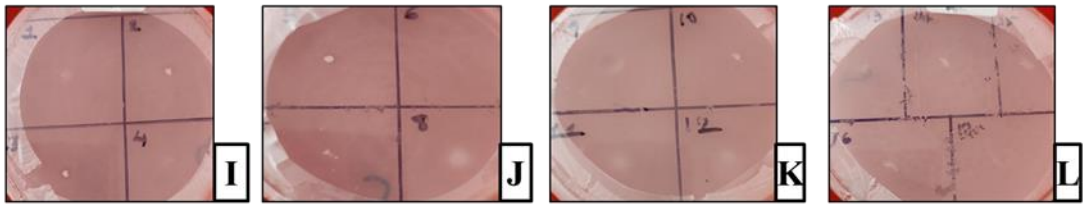
Endophytic isolates on 20% NaCl

Plate 1:Rhizospheric and Endophytic halotolerant isolates A-B. 10% NaCl Rhizospheric;C.10% NaCl Endophytic;D-E.20% NaCl Rhizospheric;F.20% NaCl Endophytic

Nitrogen Production Assay



Rhizospheric isolates grown on Jensen's Medium



Endophytic isolates gron on Jensen's Medium

Plate 2:Plates containing isolates showing Nitrogen production ability when grown on Jensen's medium, A-H: Rhizospheric isolates (24/32) and I-L: Endophytic isolates(11/17).

Table 1: Nitrogen Production and fixation by Halo-tolerant Rhizospheric bacterial isolates

Sr. No.	Isolate Code	Growth on Jensen's Media
1	R-1	+
2	R-2	++
3	R-3	++
4	R-4	+
5	R-5	++
6	R-6	-
7	R-7	-
8	R-8	-
9	R-9	++
10	R-10	-
11	R-11	++
12	R-12	-
13	R-13	++
14	R-14	++
15	R-15	++
16	R-16	++
17	R-17	+++
18	R-18	-
19	R-19	-
20	R-20	-
21	R-21	+
22	R-22	++
23	R-23	++
24	R-24	+++
25	R-25	++
26	R-26	++
27	R-27	+++
28	R-28	+++
29	R-29	++
30	R-30	++
31	R-31	++
32	R-32	+++

Table 2: Nitrogen Production and fixation by Halo-tolerant Endophytic bacterial isolates

Sr. No.	Isolate Code	Growth on Jensen's Media
1	E-1	+++
2	E-2	++
3	E-3	+
4	E-4	+++
5	E-5	++
6	E-6	-
7	E-7	+
8	E-8	+++
9	E-9	+++
10	E-10	++
11	E-11	+++
12	E-12	+++
13	E-13	-
14	E-14	-
15	E-15	-
16	E-16	-
17	E-17	-

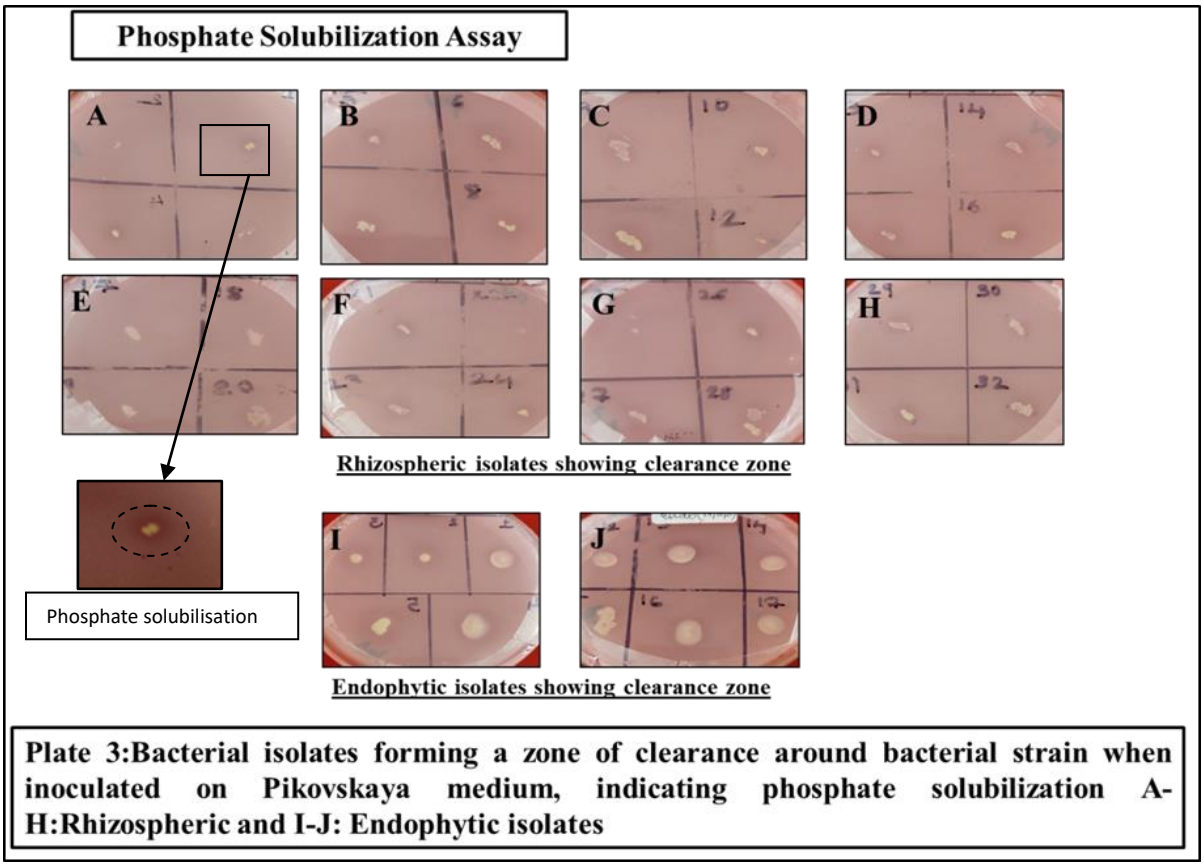


Plate 3:Bacterial isolates forming a zone of clearance around bacterial strain when inoculated on Pikovskaya medium, indicating phosphate solubilization A-H:Rhizospheric and I-J: Endophytic isolates

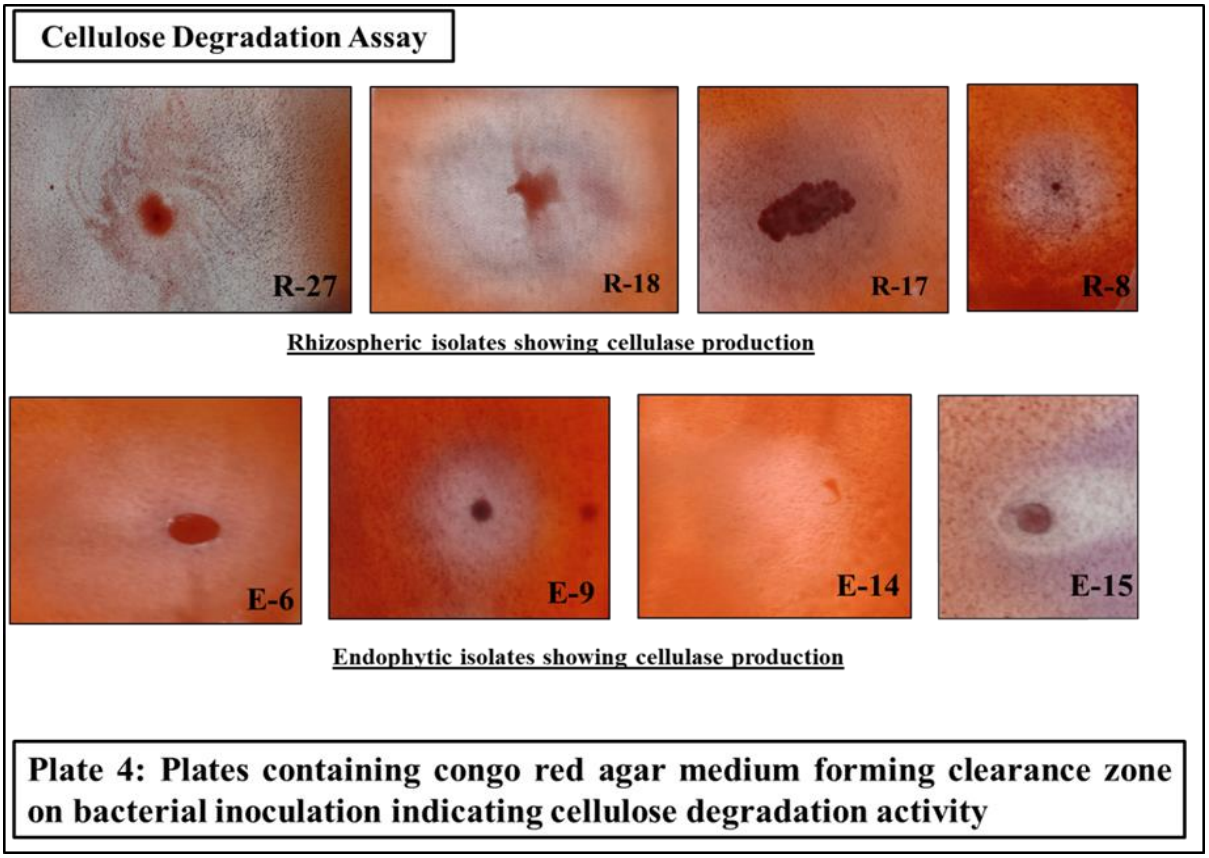


Plate 4: Plates containing congo red agar medium forming clearance zone on bacterial inoculation indicating cellulose degradation activity

Table 3: Phosphate solubilisation activity by the isolated Rhizospheric bacteria

Sr. No.	Isolates Code	Colony Diameter (cm) (C.D)	Clearance Zone Diameter (cm) with colony (C.Z)	Clearance Zone (cm) =(C.Z – C.D)
1	R-1	0.29	0.4	0.11
2	R-2	0.2	0.3	0.1
3	R-3	0.5	0.6	0.1
4	R-4	0.25	0.45	0.2
5	R-5	0.34	0.65	0.31
6	R-6	0.7	1.2	0.5
7	R-7	0.51	0.85	0.34
8	R-8	0.52	0.9	0.38
9	R-9	0.8	1.15	0.35
10	R-10	0.4	0.78	0.38
11	R-11	0.89	1.3	0.41
12	R-12	0.3	0.5	0.2
13	R-13	0.3	0.52	0.22
14	R-14	0.6	0.9	0.3
15	R-15	0.5	0.65	0.15
16	R-16	0.4	0.68	0.28
17	R-17	0.5	0.6	0.1
18	R-18	0.58	0.62	0.04
19	R-19	0.4	0.48	0.08
20	R-20	0.65	0.8	0.15
21	R-21	0.48	0.75	0.27
22	R-22	0.31	-	-
23	R-23	0.61	0.7	0.09
24	R-24	0.32	0.6	0.28
25	R-25	0.15	-	-
26	R-26	0.5	0.7	0.2
27	R-27	0.6	0.95	0.35
28	R-28	0.55	0.7	0.15
29	R-29	0.7	1.15	0.45
30	R-30	0.75	1.12	0.37
31	R-31	0.46	0.8	0.34
32	R-32	0.7	0.92	0.22

Table 4: Phosphate Solubilization activity by Endophytic bacterial isolates

Sr. No.	Isolates Code	Colony Diameter (cm) (C.D)	Clearance Zone Diameter (cm) with colony (C.Z)	Clearance Zone (cm) =(C.Z – C.D)
1	E-1	0.9	1.2	0.3
2	E-2	0.4	1.15	0.75
3	E-3	0.49	1.1	0.61
4	E-4	1.2	1.4	0.2
5	E-5	0.8	1.55	0.75
6	E-6	-	-	-
7	E-7	-	-	-
8	E-8	-	-	-
9	E-9	-	-	-
10	E-10	-	-	-
11	E-11	-	-	-
12	E-12	0.7	0.85	0.15
13	E-13	0.8	1.1	0.3
14	E-14	0.8	0.95	0.15
15	E-15	1.1	1.2	0.1
16	E-16	1.0	1.15	0.15
17	E-17	0.8	0.9	0.1

Table 5: Cellulose Degradation activity on Congo-Red by Rhizospheric isolates

Sr. No.	Isolates Code	Colony Diameter (cm) (C.D)	Clearance Zone Diameter (cm) with colony (C.Z)	Clearance Zone (cm) =(C.Z – C.D)
1	R-1	0.2	1.2	1
2	R-2	0.1	-	-
3	R-3	0.3	1.85	1.55
4	R-4	0.2	1.45	1.25
5	R-5	0.6	2.2	1.6
6	R-6	0.2	-	-
7	R-7	0.1	-	-
8	R-8	0.2	2.3	2.1
9	R-9	0.5	-	-
10	R-10	0.3	-	-
11	R-11	0.2	1.8	1.6
12	R-12	0.6	-	-
13	R-13	0.6	1.9	1.3
14	R-14	0.4	-	-
15	R-15	0.3	-	-
16	R-16	0.2	-	-
17	R-17	0.5	2.8	2.3
18	R-18	0.7	3.7	3.0
19	R-19	0.1	-	-
20	R-20	0.1	-	-
21	R-21	0.4	0.65	0.25
22	R-22	0.6	1.8	1.2
23	R-23	0.1	1.8	1.7
24	R-24	0.8	-	-
25	R-25	0.1	-	-
26	R-26	0.3	0.6	0.3
27	R-27	0.2	4.6	4.4
28	R-28	0.5	0.9	0.4
29	R-29	0.1	-	-
30	R-30	0.6	1.2	0.6
31	R-31	0.2	-	-
32	R-32	0.6	1.3	0.7

Table 6: Cellulose Degradation activity on Congo-Red by Endophytic isolates

Sr. No.	Isolates Code	Colony Diameter (cm) (C.D)	Clearance Zone Diameter (cm) with colony (C.Z)	Clearance Zone (cm) =(C.Z – C.D)
1	E-1	0.3	-	-
2	E-2	0.6	1.1	0.5
3	E-3	0.4	0.7	0.3
4	E-4	0.5	1.5	1
5	E-5	0.9	-	-
6	E-6	0.4	2.75	2.35
7	E-7	0.2	0.8	0.6
8	E-8	0.6	1.35	0.75
9	E-9	0.5	1.35	0.85
10	E-10	0.6	1.6	1
11	E-11	0.3	-	-
12	E-12	0.4	1.7	1.3
13	E-13	0.4	0.9	0.5
14	E-14	0.4	1.8	1.4
15	E-15	0.3	4.5	4.2
16	E-16	0.9	1.2	0.3
17	E-17	0.7	1.8	1.1

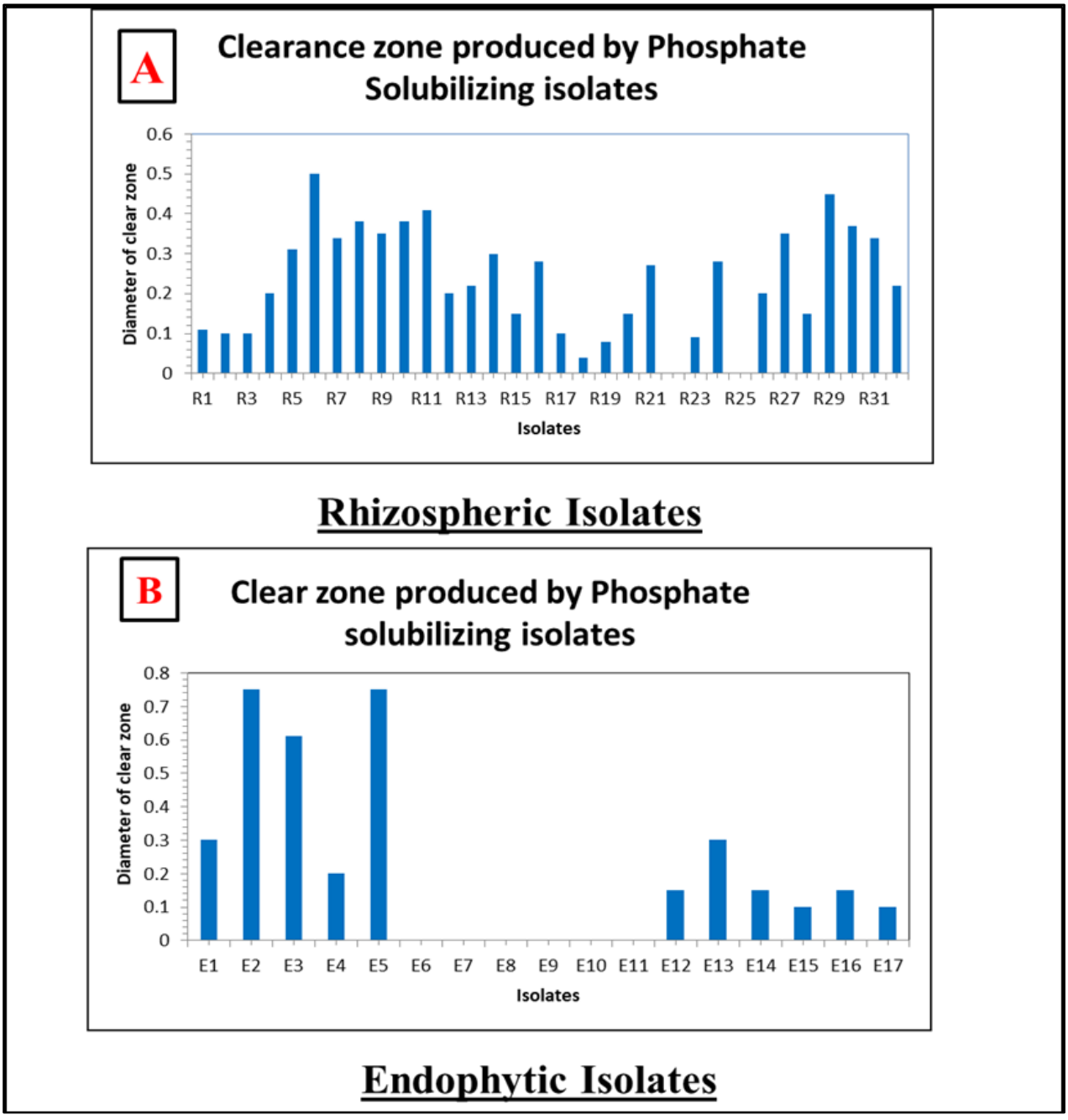


Fig 3: Shows the graph representing the Phosphate solubilizing activity of isolated bacterial strains. The clear zone obtained is shown in centimetre unit. Graph A:Rhizospheric Isolates and Graph B: Endophytic Isolates

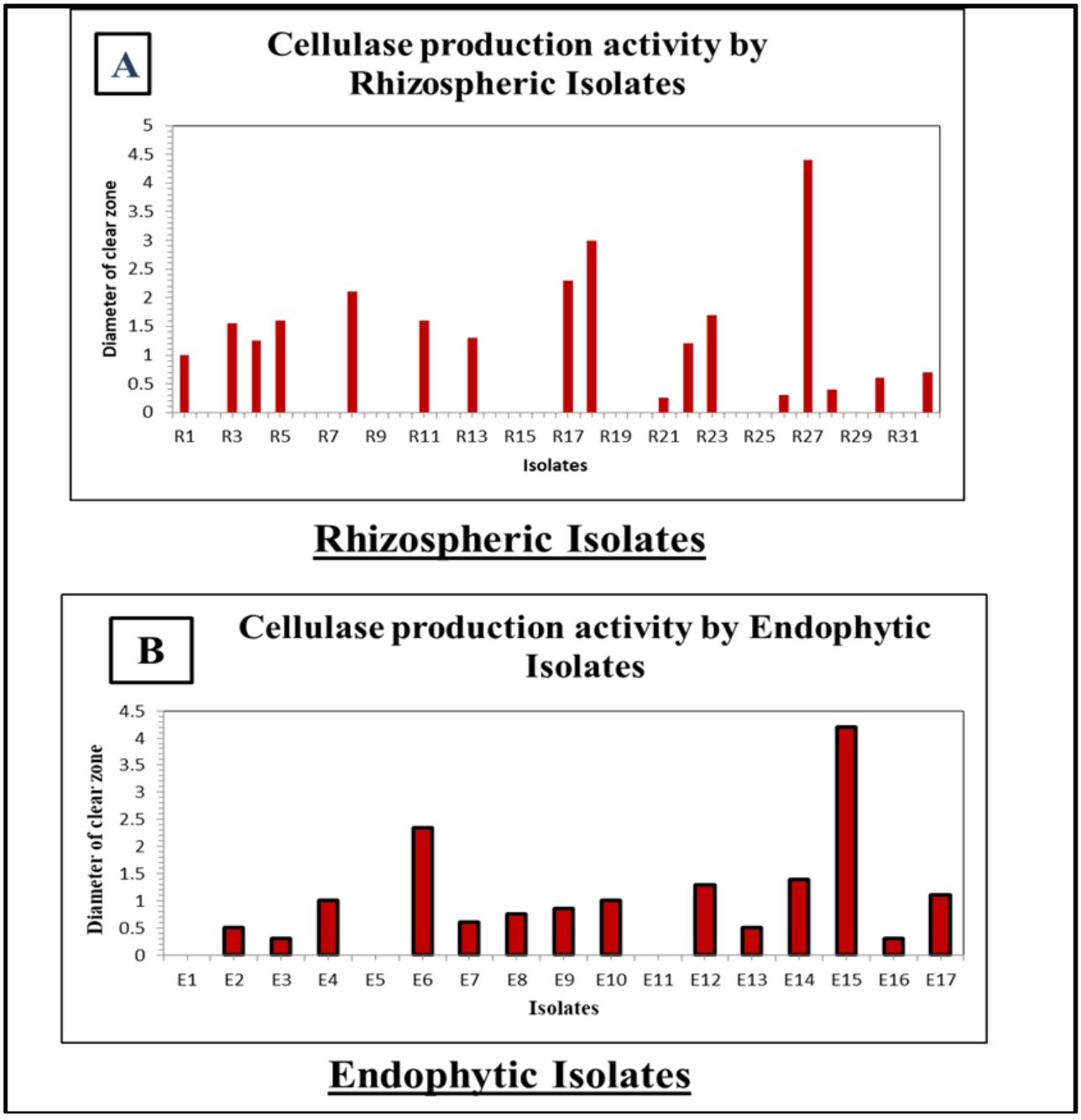


Fig 4:Shows the graph representing the Cellulose degradation activity of isolated bacterial strains, the clear zone is measured in centimetres. Graph A:Rhizospheric Isolates and Graph B: Endophytic Isolates

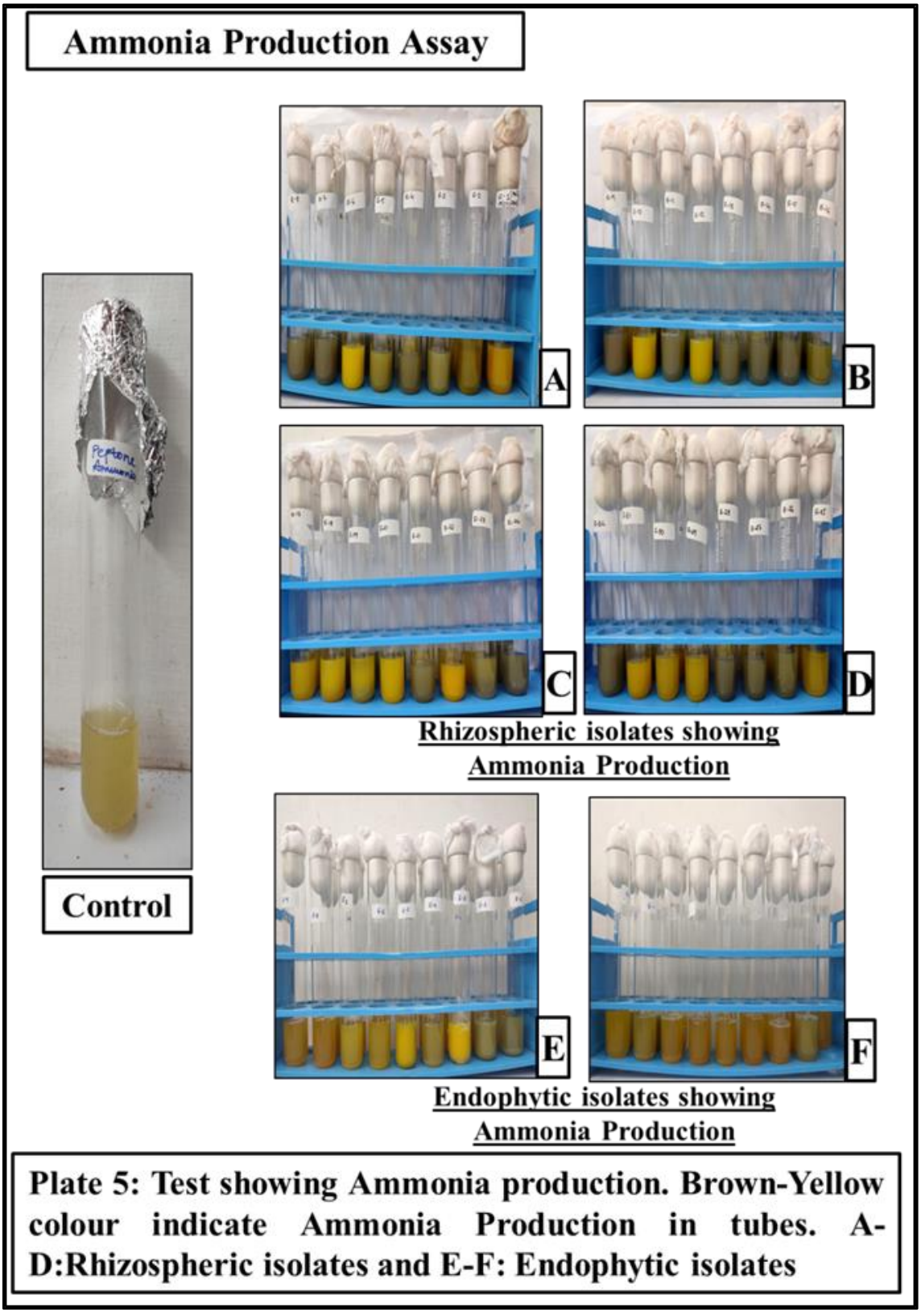
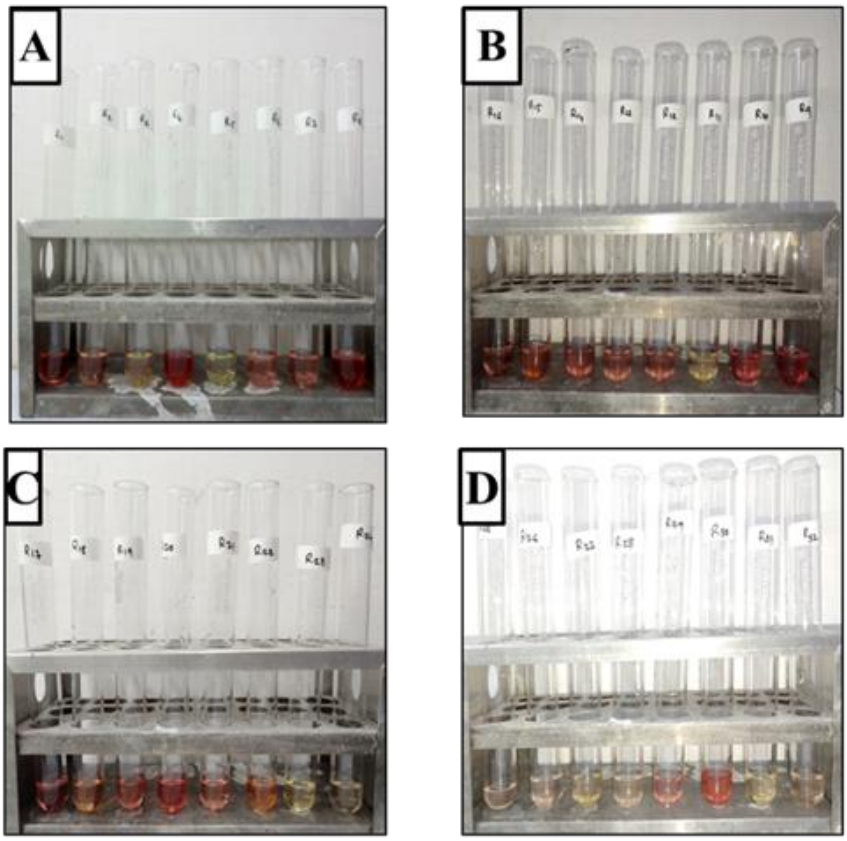
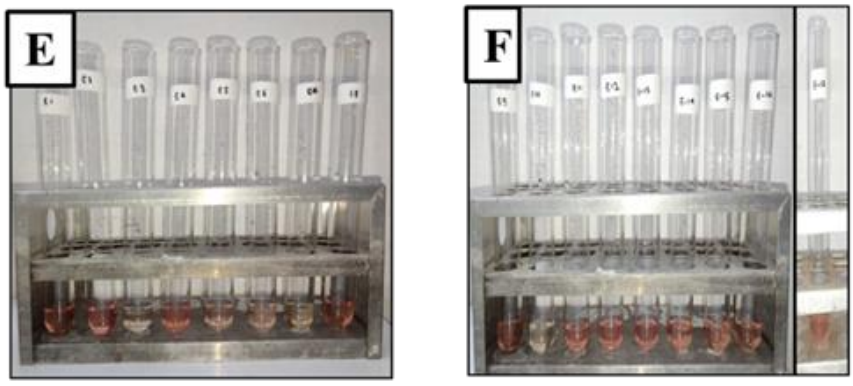


Plate 5: Test showing Ammonia production. Brown-Yellow colour indicate Ammonia Production in tubes. A-D:Rhizospheric isolates and E-F: Endophytic isolates

IAA Production Assay



Rhizospheric isolates showing IAA production



Endophytic isolates showing IAA production

Plate 6: Qualitative estimation of IAA production. Pink colour indicate production of IAA. A-D:Rhizospheric isolates and E-F: Endophytic isolates

Table 7: Numbers of seeds germinated on inoculation of Rhizospheric isolates

Sr. No.	Isolates Code	Germinated seeds (Day 3)	Germinated seeds (Day 5)	Germinated seeds (Day 7)
1	R-1	+	+	+
2	R-2	-	-	-
3	R-3	+	+	+
4	R-4	-	+	+
5	R-5	+	+	+
6	R-6	+	+	+
7	R-7	+	+	+
8	R-8	+	+	+
9	R-9	+	+	+
10	R-10	+	+	+
11	R-11	-	-	-
12	R-12	+	+	+
13	R-13	+	+	+
14	R-14	+	+	+
15	R-15	+	+	+
16	R-16	-	+	+
17	R-17	+	+	+
18	R-18	-	+	+
19	R-19	+	+	+
20	R-20	+	+	+
21	R-21	+	+	+
22	R-22	-	+	+
23	R-23	-	+	+
24	R-24	+	+	+
25	R-25	-	+	+
26	R-26	+	+	+
27	R-27	+	+	+
28	R-28	+	+	+
29	R-29	-	-	-
30	R-30	-	+	+
31	R-31	+	+	+
32	R-32	+	+	+

Table 8): Numbers of seeds germinated on inoculation of Endophytic isolates

Sr. No.	Isolates Code	Germinated seeds (Day 3)	Germinated seeds (Day 5)	Germinated seeds (Day 7)
1	E-1	+	+	+
2	E-2	-	-	-
3	E-3	-	+	+
4	E-4	-	-	-
5	E-5	+	+	+
6	E-6	+	+	+
7	E-7	+	+	+
8	E-8	+	+	+
9	E-9	+	+	+
10	E-10	-	-	-
11	E-11	+	+	+
12	E-12	-	-	-
13	E-13	-	+	+
14	E-14	+	+	+
15	E-15	+	+	+
16	E-16	-	-	+
17	E-17	+	+	+

5. DISCUSSION

Soil salinity is a major abiotic stress which has affected about 50% production of the rice; globally (Manohara *et al*., 2021). At seedling and reproductive stages, rice plants are commonly salt sensitive (Munns & Tester, 2008). The higher concentration of salt in the soil induces different stress to the rice plants which in turn affects the growth, photosynthetic rate and ultimately leads to death of the plant (Rahman *et al*., 2016). Salinity is a common problem in the coastal areas and is often characterized by seawater ingression during tides resulting in salt accumulation over the surface (Vinod *et al*., 2013). Another feature of the khazan lands are continuous waterlogging, flooding and poor drainage (Amanullah *et al*., 2007).

Apart from all these factors farmers are traditionally growing rice in these Khazan lands which may have been lead to inherent salt-tolerant trait in certain rice varieties such as the indigenous salt-tolerant rice variety from Goa; Korgut. One of the reasons for Korgut being a salt-tolerant variety may be due to its association with the Plant growth- promoting microbes. These are the beneficiary microbes that colonize the roots of the plant. Many of the reports suggest the role of PGPR in promoting the growth and development of plant as well as in helping in alleviating the environmental stress (Javed *et al.*, 2020). Studies identifying and characterizing the associated microbiota of the korgut variety has not been carried out and remains to be a promising area to be explored in order to determine the effect of the rhizospheric and endophytic microflora in salt stress tolerance of korgut rice variety.

In this study, we isolated 49 halo-tolerant microbes; 32 Rhizospheric and 17 Endophytic. These isolates were screened further for different plant growth promotion traits from which R-1, R-17 and R-30 showed positive results for all the PGP traits. R-17 had very high

activity of Nitrogen and Cellulase production but it was moderately halo-tolerant up to 10% NaCl as compared to other two isolates R-1 and R-30 which were tolerant to 20% NaCl. Other isolates such as R-4, R-13 and R-21 showed results for all the screened PGP traits except for production of ammonia. It has been reported earlier by (da Silva *et al*., 2011) that on successive culturing the microbes lose their characteristic pellelic growth which makes the nitrogenase activity assessment difficult. Isolates R-9 and R-22 showed better PGP traits except for Cellulase production and Phosphate solubilization respectively.

Among the Endophytic isolates screened for PGP traits, there was no isolate showing result for all the PGP traits but about 82% isolates showed better cellulose degrading and IAA production activity. In isolates E-2 and E-12 it was seen that there was a production of IAA but seeds were not germinated when treated with the same isolates. One of the reasons for this may be the quality of the seeds used and the handling of seeds during whole procedure. In a study carried out by (Tabatabaei *et al*., 2016), similar results have been obtained. In their studies they stated that this might be due to the elevated levels of IAA or some other metabolite production by the isolates. However in a report by (Banowetz *et al*., 2008) a strain of *Pseudomonas* suppressed germination of *Poaannua* seeds and some other graminaceous species, which he assumed due to the production of 4-formylaminooxyvinyl glycine. Although there were about 80% isolates which showed the results for seed germination. E-5 showed high result for phosphate solubilisation but it lacked the Cellulose degrading activity.

6. CONCLUSION

This work led to the isolation of the bacterial species thriving the rhizospheric and endophytic region of the salt tolerant korgut rice variety growing in the saline khazan land.

It was noted that the isolates were halotolerant and 49 halo-tolerant bacterial isolates were successfully isolated from rice (*Oryza sativa* L. var. Korgut) collected from Raia, South Goa.

32 isolates were from the rhizosphere of the roots and 17 were isolated from the Endophytic region.

Further these isolates were screened for plant growth-promoting traits wherein isolates exhibited different PGP traits such as IAA production, phosphate solubilisation, cellulose degradation, nitrogen production and ammonia production.

All the isolates were further tested for seed germination of salt sensitive variety of rice i.e. Jaya where isolates were successfully enhanced the germination percentagee of the seeds.

This isolates could be studies further for identifying the species and characterizing other beneficial properties exhibited by them on plants, which could give us potential candidates for the use as biofertilizers for the fields having high salinity. Also these could be used to alleviate the salt stress in salt susceptible cultivars.

Further, identification of the isolated bacterial strains should be carried out using 16S rDNA gene sequencing. Using these isolates for studying their effect on plant growth parameters would help in understanding their role in salt tolerant crops cultivated in khazan lands.

7. SUMMARY

The PGPR are known in alleviating plant environmental stress by several mechanisms, however these are not studied extensively. The micro-biota associated with each plant varies among different plants depending on the type of root exudates secreted by the plant.

This study is an attempt to isolate the PGPR strains from the rhizopheric and endophytic region of salt tolerant rice variety korgut. The results of this study are presented below:

A total of 49 bacterial cultures were isolated from the sample; 32 from rhizosphere and 17 from endophytic region.

All the isolates were screened for PGP traits like nitrogen fixation, phosphate solubilisation, cellulose degradation, IAA production and ammonia production.

Isolates from rhizospheric region; R-1, R-17 and R-30 showed positive results for all the PGP traits. Among endophytic isolates; E-2 and E-12 showed positive results for all PGP traits.

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