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Isolation, Screening, and Characterization of PGPR from Halophytic Rhizosphere

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Abstract
Climate change affects agricultural fields by facing many threats. Among them, salt stress is the major abiotic threat. To address this issue, salt-tolerant PGPRs have been used. They can create a symbiotic relationship with their host to increase salt tolerance capacity. A study evaluated the ability of salt-tolerant PGPR isolated from the 07 different halophyte rhizospheres from the Adri region of Junagadh district. A total of 198 bacterial isolates were isolated, with most being gram-positive, capsule-forming, non-spore-forming, and existing in single or chain cell arrangements. A salt tolerance test was carried out for screening up to 30% of NaCl concentrations. A total of 08 isolates could tolerate 30% NaCl concentration. These were selected for further studies. During pH tolerance, all 08 could tolerate pH up to 11, but none isolates could tolerate < 7 pH. Temperature tolerance record: 04 isolates were psychrophiles and 02 were thermotolerant. Further, 06 isolates produced IAA and 03 solubilized P, but none isolates solubilized Zn. Production of NH_3, catalase activity, and HCN production was done by 05 isolates. AEND5 was identified as a potent isolate, demonstrating the potential of salt-tolerant PGPRs in reducing synthetic pesticide and fertilizer use in agriculture.

Keywords: Halophytes, ST-PGPR, Salt tolerance, IAA production, P solubilization, Zn solubilization, NH_3 production, Catalase activity, and HCN production.

1. Introduction

Crop production is facing challenges due to global climate change. This has led to various environmental stresses such as salinity, rainfall, drought, extreme temperatures, and floods, which pose a threat to agricultural sustainability. With the increasing global population, higher food demand, and depletion of availability of cultivable land, these challenges are becoming more severe. Amongst these environmental stresses, soil salinity affects cultivable land areas the most, leading to reduced crop productivity and quality [1]. It is estimated that currently, about 62 million hectares, or 20 percent of the world's irrigated land, is affected by salinity [2]. India has a coastline of 7516.6 km [6100 km of mainland coastline + coastline of 1197 Indian islands] touching 13 States and Union Territories (UTs) [3]. Where the state of Gujarat has the longest coastline of 1600 km [4,1], the Kutch and Saurashtra regions of Gujarat span roughly 1125 km. Every year, the area within 1 km of the coastline experiences elevated salinity due to

the mixing of groundwater and oceanic saline water. So far, a strip of land approximately 5 to 7.5 km wide has been affected by salinity [1].

The excessive deposition of soluble salt in cultivable land has adversely affected important soil processes such as respiration, residue decomposition, nitrification, denitrification, microbial biodiversity [7,2], as well as its direct impact on crop yields. When a plant is under salt stress, it triggers several signalling pathways. This includes the production of stress tolerance hormones, the activation and synthesis of antioxidant enzymes, the production of Osmo protectants, and the production of polyamines. All of these processes help the plant cells to survive [5,6,1]. It also inhibits diverse physiological and metabolic processes of plants, even impacting their survival. The conventional methods of reclamation of saline soil, which involve scraping, flushing, leaching, or adding an amendment (such as gypsum, CaCl2, etc.), are limited in their success and also adversely affect the agroecosystems [2]. To improve the productivity of saline soil without harming the environment, sustainable methods need to be developed. Breeding salt-tolerant plants and developing salt-resistant crop varieties have not been effective. However, Microbial versatility in harsh environments like arid regions, saline regions, thermophilic regions, and acidic regions is more important because microbes present over there have unique genetic and physiological characteristics to survive and grow in those harsh conditions [8,9,10].

The plant microbiome, including plant growth-promoting rhizobacteria (PGPR), ectomycorrhizal fungi (AMF), and pathogenic microbes, affects the morphology and metabolism of the halophytes [8,11,12]. Halophytes – plants that can tolerate high levels of salt in their environment are a useful reservoir of halotolerant bacteria with plant growth-promoting abilities [7]. Microbes exhibit salt tolerance and dependence, including various genera of salt-tolerant plant growth promoting rhizobacteria (ST-PGPR) found in extremely alkaline, saline, and sodic soils that can mitigate various biotic and abiotic stresses in plants and enhance the productivity of plants those facing salt stress by increasing the availability and uptake of carbon, nitrogen, and minerals from soil [8,13,14] and make them useful for reclaiming saline agroecosystems [2].

PGPRs such as *Rhizobium, Klebsiella, Pseudomonas,* and *Enterobacter* are rhizospheric as well as facultative endophytes, and they reside in host tissues' intercellular spaces and establish a symbiotic association [8, 15, 16]. The identification of halophilic PGPRs will enable agriculture on saline soil [7]. The utilization of ST-PGPR in saline agriculture can enhance productivity and improve soil fertility. The ability to produce Osmo protectants, compatible solutes, and specialized transporters is key to adaptive responses toward salt stress. ST-PGPR are now utilized as bioinoculants to enhance crop yields, protect from phytopathogens, and improve soil health [2].

Previous studies on halophytes have focused on the physiological and genetic regulation of salinity resistance. However, it is important to note that salinity tolerance in halophytes is also related to complex microbial ecological processes within their rhizosphere. Despite this, plant-microbe interactions in saline habitats have not been extensively studied. Therefore, the present study will mainly focus on halophyte microbiome diversity and functions [8].

1. Materials and Methods

1.1 Sample Collection

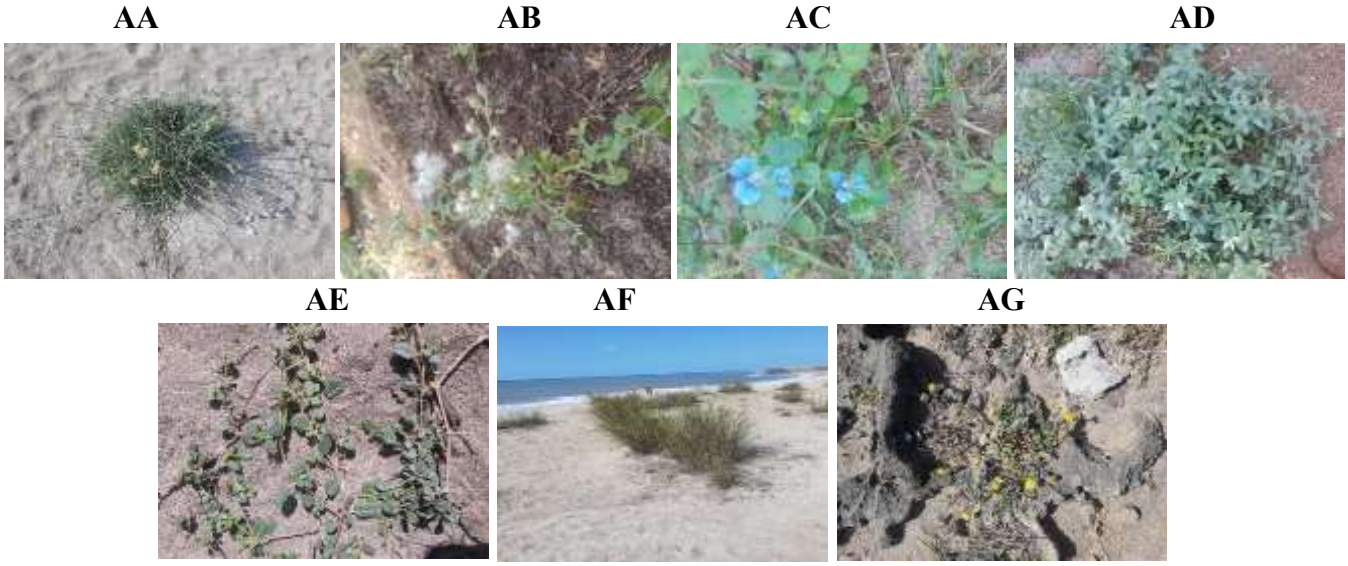
The present study was conducted with rhizospheric culturable bacteria isolated from the saline barren soil samples collected from the rhizospheric region of halophytes named AA, AB, AC, AD, AE, AF, and AG mentioned in Table -1 of Adri (20.96^{O}N, 70.29^{O}E) region from Gujarat, India. The halophytes were

uprooted from the soil, and the soil surrounding and attached to the roots was put in autoclaved bags and brought to the laboratory, and stored at 4 °C.

Table 1 – Halophyte samples from the Adri site

Sample code	Common name of a halophyte	Scientific name of a halophyte
AA	Button sedge	*Fimbristylis cymose*
AB	Little ironweed	*Vernonia cinerea*
AC	Benghal dayflower	*Commelina benghalensis*
AD	Seaside heliotrope	*Helitropium curassavicum*
AE	Khakhi weed	*Alternanthera pungens*
AF	Marram grass	*Ammophila arenaria*
AG	Fleabane	*Pulicaria dysenterica*

Figure 1 – Halophyte samples from the Adri site



1.2 Sample Processing and Isolation of Rhizobacteria

The 10 g rhizospheric soil of each halophyte sample was aseptically introduced into 100 mL of sterile 0.8gm% n-saline and prepared soil suspension. Soil suspensions were kept on a shaker at 200 RPM for 1 hour. Afterward, a series of decimal dilutions up to 10^{-2} were carried out in test tubes using sterile distilled water. To isolate potential rhizospheric bacteria soil suspensions, 10^{-1} and 10^{-2} dilutions of each halophyte sample were inoculated on three different sterile agar mediums NA - Nutrient Agar, YEMA – Yeast Extract Mannitol Agar with 0.01gm% congo red, and KB - King's B medium with extra 5gm% NaCl salt by four flame method. All plates were incubated at 37^0C for 24-48hr. After incubation, the bacteria with distinct colony morphology were selected and again sub-cultured to get purified isolates [17].

1.3 Microscopic observation

Based on various staining traits, bacteria can be roughly identified. Gram's staining, spore staining, and capsule staining were performed according to Experimental Microbiology by Rakesh J. Patel.

1.4 Physicochemical Analysis of Soil Sample

The collected soil samples were analysed for physicochemical parameters like Soil particle size, Soil pH, Soil salinity, and Soil water holding capacity.

To determine particle size, 100 g of each soil sample was dried in an oven to remove moisture content. Use a set of sieves arranged in an order of fineness (0.5cm → 0.4cm → 0.3cm → 0.2cm → 0.1cm). Place 100 g of the dried sample on top and shake the entire stack of the sieve manually. Weigh and record retained material on each sieve to calculate the percentage of material retained and passed through each sieve [22]. To measure the pH and salinity of the soil, a pH meter and a conductivity meter were used, respectively. Firstly, 10 grams of each soil sample was added to 100 ml of distilled water and mixed thoroughly. After allowing the soil particles to settle down, a pH electrode and conductivity cell were introduced to each sample, and the pH value as well as the conductivity value were recorded. To determine the water holding capacity of the soil, filter paper, a funnel, and 20 grams of pre-weighed soil sample are used. Next, lay the soil sample and funnel with filter paper on a conical flask, then gradually add 100 ml of distilled water. Measure the post-weight of moist soil and calculate the water-holding capacity after all the water has been filtered out.

1.5 Screening of Salt Tolerance

The isolated bacteria were spotted (0.1ml) in triplicate on sterile NA enriched with 05gm%, 10gm%, 15gm%, 20gm%, 25gm%, and 30gm% NaCl, to determine their salt tolerance at (±2) 37ºC temperature. After incubation, colony formation was noted in colony diameter, and as on that, the isolates were classified as halotolerant, moderate halophiles, and halophiles based on how well they could grow in various NaCl concentrations [18].

For further study, only those 08 isolates were chosen that could tolerate a 30 gm% concentration of NaCl.

1.6 Screening of pH Tolerance

By spotting the same volume of all bacterial suspensions (0.1 ml) in triplicate on a sterile NA solid medium with pH values of 3, 5, 7, 9, and 11, the pH tolerance of selected isolates was assessed. After that, the plates were incubated at 37 °C for 48–72 h., and the colony diameter was used to quantify the growth [19]. Based on colony diameter, isolates were classified as acidophiles, neutrophiles, and alkaliphiles.

1.7 Screening of Temperature Tolerance

To evaluate the best growth temperature range, the same amounts of bacterial suspension (0.1 ml) were spotted, in triplicate, on a sterile NA solid medium, and plates were incubated at 5º C, RT, 37 º C, 45º C, and 65º C. Observation of bacterial growth noted as temperature range that isolates can tolerate. Bacterial growth was evaluated qualitatively on bacterial spots and quantitatively by measuring colony diameter and noted as psychrophiles, mesophiles, and thermophiles [19].

1.8 Screening of Salt-Tolerant Rhizobacteria for PGP Traits

1.8.1 Indole-3-Acetic Acid (IAA) Production

Salkowski's technique was used to estimate indole acetic-3-acid (IAA) in selected salt-tolerant isolates. The isolates were aliquoted (1 ml at 0.1 OD at 600nm) and inoculated in 9.0 ml of PCA liquid medium (PCA - 5.0 gm/l Tryptone, 1.0 gm/l Glucose, 2.5 gm/l yeast extract, and 5% NaCl) and incubated for 24 hours under shaking conditions at 135 RPM. The 1 ml of supernatant (collected by centrifuging bacterial

growth solution at 8000 RPM for 20 min) was combined with 2 ml of Salkowski's reagent (49 ml of 35% $HClO_4$ + 1.0 ml of a 0.5 M $FeCl_3$) and kept in the dark at 27°C for 2 hours. The absorbances were measured at 530nm and compared with an IAA standard curve to determine the IAA production [17].

1.8.2 Phosphate Solubilization

The phosphate solubilization assay involved spot inoculation of each bacterial isolate on Pikovskaya's agar (Pikovskaya agar – 10gm/l Glucose, 5 gm/l Tricalcium Phosphate, 0.5 gm/l Ammonium sulphate, 0.2 gm/l KCl, 0.1 gm/l $MgSO_4$, Trace $MnSO_4$ and $FeSO_4$, 0.5 gm/l Yeast extract, 15 gm/l agar powder, 5gm% NaCl and 7 pH). The plates were incubated at 28 ± 1°C for 48 hours in a BOD incubator. After incubation, a distinct halo zone close to the bacterial growth was measured and, by using the following equation calculated the phosphate solubilization index [20] was calculated.

$$\text{Phosphate solubilization index (SI)} = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}}$$

1.8.3 Zinc Solubilization

The bacterial capacity to solubilize Zinc was assessed utilizing Tris-mineral agar medium (10gm/l D-glucose, 1.0 gm/l $(NH_4)_2SO_4$, 0.2 gm/l KCl, 0.1gm/l K_2HPO_4, 0.2gm/l $MgSO_4$, 5g% NaCl, and pH = 6.75 ± 0.25) revised with three distinctive sources of insoluble zinc compounds; zinc oxide (ZnO) (15.23 mM), zinc phosphate ($Zn_3(PO_4)_2$) (5.0 mM), and zinc carbonate ($ZnCO_3$) (5.2 mM) at a 0.1% Zn last concentration. The selected bacterium was spotted on each Tris-mineral medium, and plates were incubated at 30°C for 10 days. Zinc solubilizers showed a clear halo zone around the colony. If the zone was not observed, then the plate was flooded with 0.1% Congo red solution. The experiment was performed in triplicate, and the calculated zinc solubilization index was calculated by following the equation [20, 21].

$$\text{Zinc solubilization index (SI)} = \frac{\text{Colony diameter} + \text{Halo zone diameter}}{\text{Colony diameter}}$$

1.8.4 NH_3 Production

The ammonia production of isolates was estimated using Nessler's technique. 9.0 ml of peptone water (peptone water - 10 gm/l peptone, 50 gm/l NaCl) was used to inoculate aliquots (1.0 ml at 0.1 OD at 600 nm) of the selected salt-tolerant isolates and incubated for 24 hours at 27 °C with stirring at 135 RPM. The development of brown to yellow color was quantified using a spectrophotometer. The 1 ml of supernatant (collected by centrifuge the bacterial growth solution at 8000 RPM for 20 min) was combined with 1.0 ml of Nessler's reagent and diluted to 10.0 ml of distilled water for the final step. Ultimately, the concentration of ammonia in the medium was measured at 450 nm. Compare each absorbance value to an ammonium-sulfate ($(NH_4)_2SO_4$) standard curve [19].

1.8.5 Catalase Activity

Place a drop of selected isolates from a 30% NaCl concentration on a clean glass slide and add one drop of 3% H_2O_2. Observe effervations and record the result [37].

1.8.9 HCN Production

Using Lorck's approach (1948), HCN production was discovered. 4.4 g glycine/l was added to the nutrition broth before bacteria were streaked on modified agar plates. 2 gm% sodium carbonate and 0.5 gm% picric acid solution-soaked Whatman filter paper No. 1 was positioned on top of the plate. After sealing the plates with parafilm, they were incubated for 72-96 hrs at 36 ± 2°C. The generation of HCN was detected by the color changing from orange to red [18].

1.9 Trials' Design and Statistical Analysis

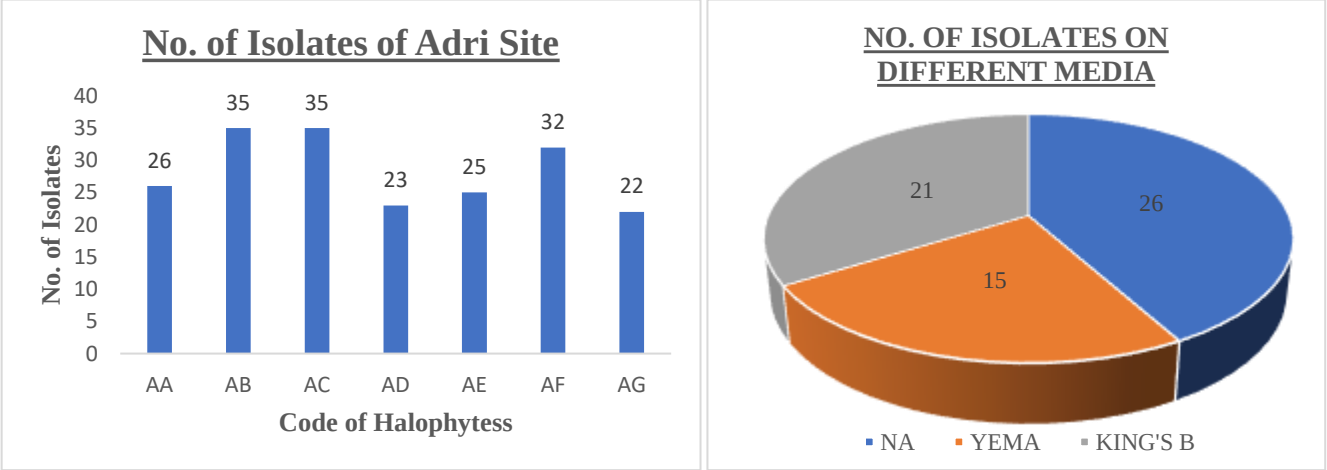
All trials were carried out by applying a randomized block design during the experiments. All experiment was performed individually in triplicate. The results are expressed as mean value (n = 3). One-way analysis of variance (ANOVA) was used to compare the bacterial isolates for in vitro IAA, P-solubilization, Zn-solubilization, NH_3 production, catalase activity, and HCN Production.

2 Observation and Results

2.1 Isolation of Rhizobacteria and Microscopic observation:

Soil is a reservoir of a variety of microbial flora that includes beneficial as well as harmful microorganisms. From different halophytes total of 198 rhizospheric bacterial isolates were isolated from the Adri region of Junagadh district. No. Of isolates per halophyte from different media is mentioned in Figure 2.

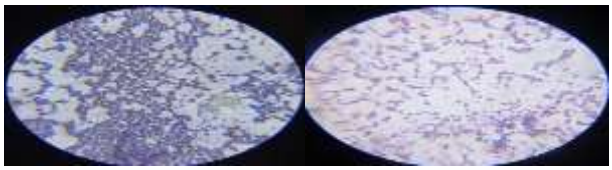
Figure 2 – No. Of Isolates from each halophyte and the media



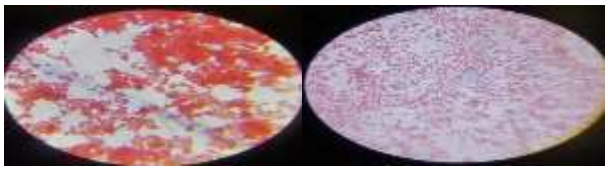
In the present study, rhizobacterial species were identified based on microscopic and morphological structures. Each isolate has different colony characteristics, including size, shape, margin, elevation, appearance, pigment, and opacity were observed mentioned in Figure 3. All 198 isolates were microscopically observed under a 100X oil emulsion lens, and their staining ability was checked by Gram, capsule, and spore staining mentioned in Figure 4 and Table 2.

Figure 3 – Isolates from each halophyte

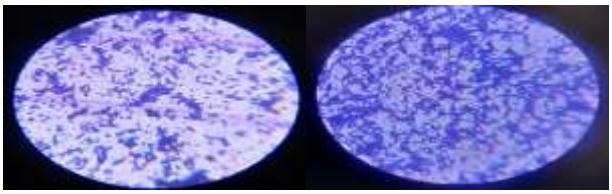
Figure 4 –Gram's, Capsule, and Spore staining



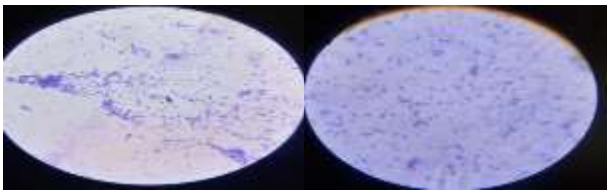
Gram's Positive



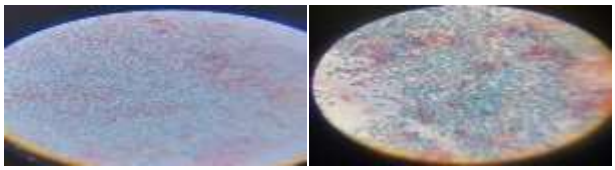
Gram's Negative



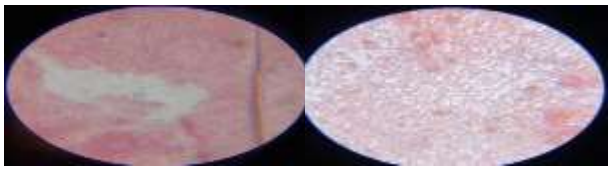
Capsule Positive



Capsule Negative



Spore Positive



Spore Negative

Table 2 – Microscopic observation of all isolates

Plant Code	Total isolates	Gram's Staining		Spore Staining		Capsule Staining		Shape of isolates		
		Positive	Negative	Positive	Negative	Positive	Negative	Bacilli	Cocci	Coccobacilli
AA	26	16	10	10	16	13	13	10	13	03
AB	35	23	12	15	20	14	21	11	14	10
AC	35	24	11	10	25	13	22	08	18	09
AD	23	12	11	09	14	08	15	12	10	01
AE	25	21	04	13	12	18	07	05	17	03
AF	32	26	06	09	23	19	13	11	15	06
AG	22	17	05	10	12	12	10	12	08	02
Total	**198**	**139**	**59**	**76**	**122**	**97**	**101**	**69**	**95**	**34**

2.2 Physicochemical Analysis of Soil Sample

The physicochemical assessment of soil samples, including particle size, pH, salinity, and water holding capacity, was performed from the Adri region of Junagadh. Table 3 shows the result of soil particle size and type of soil. Table 4 shows the results of soil pH, soil salinity, and water-holding capacity of soil. The

type of soil was determined by the sieve analysis [22]. All soil samples have ~8 pH, the salinity range was measured between 1.75 to 3.25 mʊ/cm, and the water holding capacity range was determined between 10.51% to 18.98%.

Table 3 –Soil Particle Size

Plant code	pH	Salinity by EC(mʊ/cm)	Water Holding Capacity (%)
AA	8.02	2.4	10.51
AB	8.44	2.8	11.97
AC	8.37	1.84	12.61
AD	8.43	2.0	18.98
AE	8.20	1.75	15.46
AF	8.53	1.94	13.55
AG	8.38	3.25	17.70

Table 4 –Soil pH, Soil Salinity, and Soil Water Holding Capacity

Plant code	pH	Salinity by EC(mʊ/cm)	Water Holding Capacity (%)
AA	8.02	2.4	10.51
AB	8.44	2.8	11.97
AC	8.37	1.84	12.61
AD	8.43	2.0	18.98
AE	8.20	1.75	15.46
AF	8.53	1.94	13.55
AG	8.38	3.25	17.70

Figure 5 –Soil pH, Soil Salinity, and Soil Water Holding Capacity

2.3 Screening of Salt Tolerance:

All isolates were screened by inoculating them with additional concentrations of NaCl salt at 5%, 10%, 15%, 20%, 25%, and 30% in a sterile NA medium. The number of isolates for each salt concentration is shown in Figure 6. The spotted plates containing bacterial isolates at varying salt concentrations are

displayed in Figure 7. AAK14, ABYD2, ABK22, ACY11, ADYD2, AEND5, AFY13, and AGY11 are particular isolates that can withstand a 30% concentration of NaCl salt.

Figure 6 –Salt Tolerant Assay

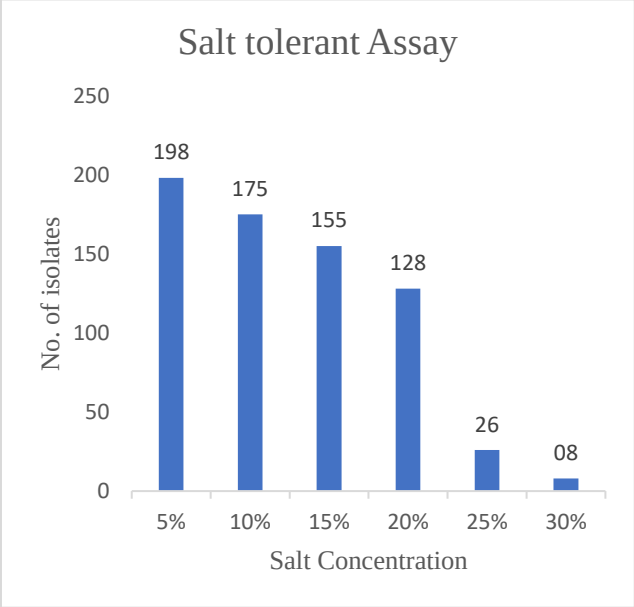
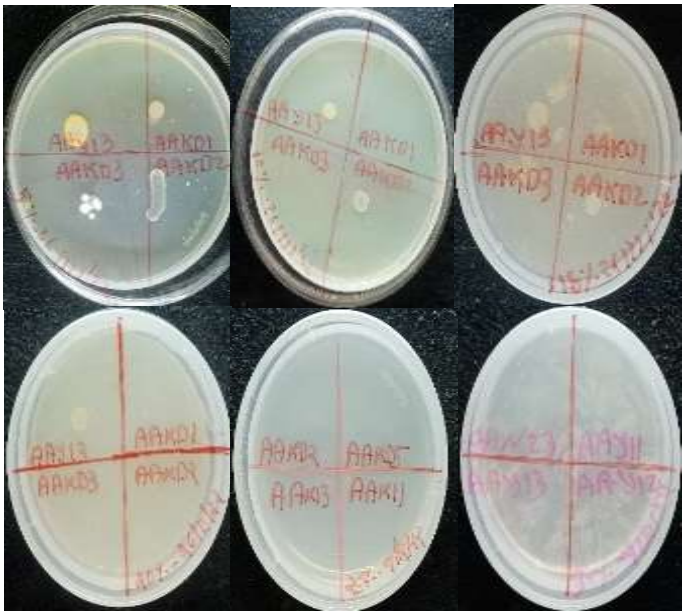


Figure 7 – Salt tolerant Assay- spotted plates

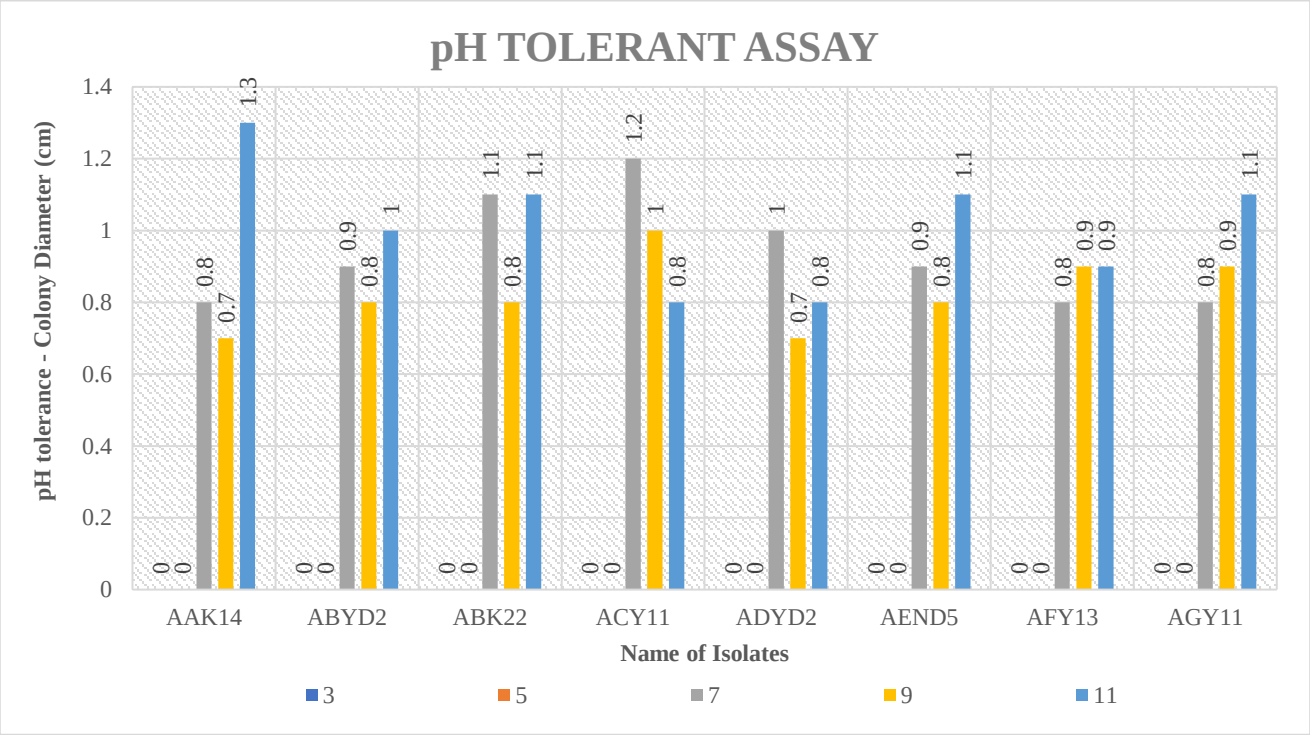


2.4 Screening of pH Tolerance:

A broad pH range, including 7, 9, and 11, is tolerated by all of the selected isolates. Three and five pH are intolerable to all isolates. No isolate was able to grow its pH below 7. Therefore, every isolate was an alkaliphile. The growth of isolates on plates with varying pH values is shown in Figure 8. Additionally, figure 9 illustrates the measured colony diameter (cm) as a proxy for the pH tolerance of a subset of isolates.

Figure 8 - pH Tolerant Assay- Spotted Plates

Figure 9 – pH Tolerant Assay

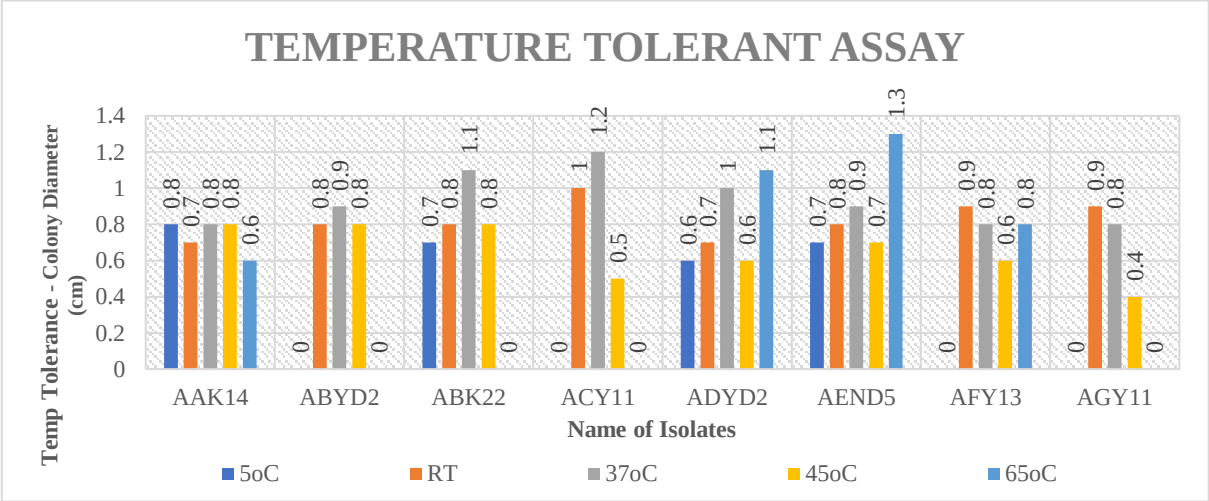


2.5 Screening of Temperature Tolerance:

A wide temperature range, including 5ºC, RT, 37 °C, 45 °C, and 65 °C, is tolerated by the selected isolates. Room temperatures=, 37 °C and 45 °C temperatures were tolerated by all the selected isolates. Half of the isolates could grow under 5ºC and 65 °C temperatures. The growth of isolates on plates with varying temperature values is shown in Figure 10. Additionally, Figure 11 illustrates the measured colony diameter (cm) as a proxy for the temperature tolerance of a subset of isolates.

Figure 10 - Temperature-tolerant Assay- spotted plates

Figure 11 – Temperature Tolerant Assay



2.6 Screening of Salt-Tolerant Rhizobacteria for PGP Traits:

Natural and agricultural saline soils are an important reservoir of halotolerant/halophilic microorganisms. The rhizospheric bacteria of halophytes are well adapted to saline stress due to low water potential in a dry or saline climate to which they are exposed [23]. These plant growth promoters follow certain mechanisms during the entire sequential process for nutrient mobilization, phytohormones for growth and development, and chemical agents for defense-related issues of the crops [24]. The mechanisms behind the scenes could mainly be divided into two types, direct mechanisms and indirect mechanisms. The present study characterizes direct mechanisms, including plant growth-promoting traits (PGP traits) viz. production of IAA; solubilization of P and Zn; production of NH_3, catalase enzyme, and HCN.

2.6.1 Indole-3-Acetic Acid (IAA) Production:

Phytohormones regulate the protective response of plants under biotic and abiotic stresses. Indole acetic acid (IAA) is one of the most physiologically active auxins that stimulate cell elongation by modifying certain conditions like, 1) an increase in osmotic contents of the cell, 2) an increase in permeability of water into the cell, 3) a decrease in wall pressure, and 4) an increase in cell wall synthesis [25]. IAA is a metabolite derived from Trp by many Trp-dependent and Trp-independent pathways in plants and bacteria. In the Trp tryptophan-dependent pathway, tryptophan is converted to indole-3-acetamide (IAM) by tryptophan-2-monooxigenase, and IAM is metabolized to IAA by IAM-hydrolase [26]. Trp-independent pathway might contribute significantly to the newly synthesized IAA; however, extensive Trp-to-IAA conversion also occurs [27].

Figure 12 – IAA Production

Blank AAK14 ABYD2 ACY11 ABK22 AEND5

IAA production was quantified with the use of Salkowski's reagent by the spectrophotometric method. Color development was visible at the highest IAA concentration within a minute and continued to increase in dark conditions for 30 minutes. Therefore, the optical density was measured after 30 minutes. The intensity of the dark reddish pink color development in the tube, checked spectrophotometrically at 530 nm, indicated the strong IAA synthesis by the selected isolates. IAA production in PCA broth ranged from 24.09 – 48.73 µg/ ml. Amongst all the isolates, IAA production was higher in the isolate ABK22 and lower in the isolate AAK14. Table 5 describes the data on IAA production, and Figure 12 shows the color changes in test tubes. A similar finding was reported previously from *Pseudomonas knackmussii* isolated from the rhizosphere of *Salsola tetrandra* [38].

2.6.2 Phosphate Solubilization

P is an essential macronutrient, particularly second to N_2, is available in both organic and inorganic forms that limit plant growth [30,31]. In saline soil, the essential plant nutrients remain in insoluble form. Many soil microorganisms solubilize unavailable forms of bound phosphorus - $Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$ into the plant available forms, HPO_4^{-2} or $H_2PO_4^{-2}$ for improved plant growth and sustenance under the higher salinity [32, 33].

P-solubilizing bacteria (PSB), belonging to the genera *Bacillus, Pseudomonas, Achromobacter, Alcaligenes, Brevibacterium, Serratia, Xanthomonas,* and *Rhizobium,* hydrolyse inaccessible P forms and convert them into the absorbable forms [34]. The solubilization of P by PGPR is achieved through acidification, chelation, ion exchange reactions, and production of low molecular weight organic acids such as acetic, lactic, malic, succinic, tartaric, gluconic, 2-ketogluconic, oxalic, and citric acids [35, 36].

The efficacies of P solubilization are shown in Table 5 and Figure 13. When the zone of solubilization was detected surrounding the isolated colony on modified Pikovskaya agar, then calculate the solubilization index (SI) was calculated with the equation mentioned in the protocol by using the diameter of the colony and zone. The estimated range of P-solubilizing isolates from halophytes was 2.25. Amongst the studied rhizospheric isolates, three isolates solubilized the same amount of P – ABYD2, AEND5, and AGY11, as well as five isolates did not solubilize P - AAK14, ABK22, ACY11, ADYD2, and AFY13. Similar data were reported from *Klebsiella* sp. and *Vibrio* sp. isolated from the rhizosphere of *Kochia indica* [39].

Figure 13 –Phosphate solubilization

2.6.3 Zinc Solubilization

Zn is one of the essential trace elements or micronutrients required for the normal growth of plants [40]. The majority of the soils possess high Zn content that exists in the unavailable forms: smithsonite ($ZnCO_3$), sphalerite (ZnS), zincite (ZnO), franklinite ($ZnFe_2O_4$), wellemite (Zn_2SiO_4), and hopeite ($Zn_3(PO_4)_2 \cdot 4H_2O$) [41]. Plants absorb Zn mainly in the form of Zn^{2+}, zinc hydrate, and organic zeolite [34].

Microbes can increase the solubility of low soluble Zn compounds and make their Zn accessible to other organisms, through various strategies, such as reducing soil pH, chelation, and altering root system [43]. Zn solubilizing bacterial genera viz., *Thiobacillus thioxidans, Thiobacillus ferroxidans, Acinetobacter, Bacillus, Gluconacetobacter, Pseudomonas* and facultative thermophilic iron oxidizers are reported [44]. Zn solubilization was estimated by plate assay using modified Pikovskaya's agar medium supplemented with 0.1% zinc carbonate. Halo zones were not observed around the colonies in a single isolate, after flooding the plates with a 0.1% Congo red-containing solution. The solubilization index of the isolates to solubilize Zn was quantitatively determined based on the results of the plate assay. Zn solubilization index was tested by using the equation mentioned in the protocol. The quantitative data of Zn solubilization of rhizospheric halophytes isolated from the selected halophytes are shown in Table 5 and Figure 14. Comparable research reported earlier from *Pseudomonas chlororaphis* and *P. aurantiaca* isolated from the rhizosphere of *Brachiaria mutica* [45].

Figure 14 – Zinc Solubilization

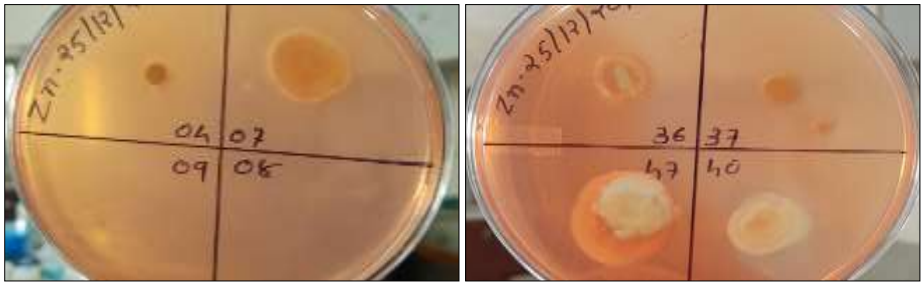
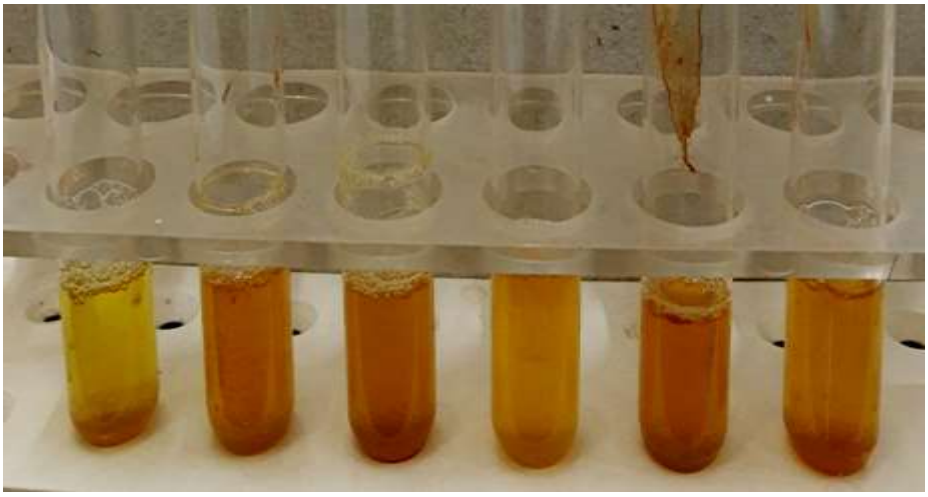


Figure 15 – NH_3 Production



Code: 04-AAK14, 07-ABYD2, 08-ABK22, 09-ACY11, 36-ADYD2, 37-AEND5, 40-AFY13, 47-AGY11

2.6.4 NH_3 Production:

NH_3 production plays an important role in plant growth increasing root and shoot growth and hence total plant biomass [28]. Ammonia is released during organic matter decomposition, improving soil structure and plant nutrition, resulting in higher productivity of the crop and enhanced tolerance against phyto parasites [29].

NH_3 production was spectrophotometrically quantified by Nessler's reagent at 450nm. A change in color development from yellow to brown or orange suggested the highest NH_3 concentration within 2-3 minutes. All isolates produced ammonia in varying concentrations of 8.86 to 16.87 µg/ml (Table 5 and Figure 15).

2.6.5 Catalase Activity

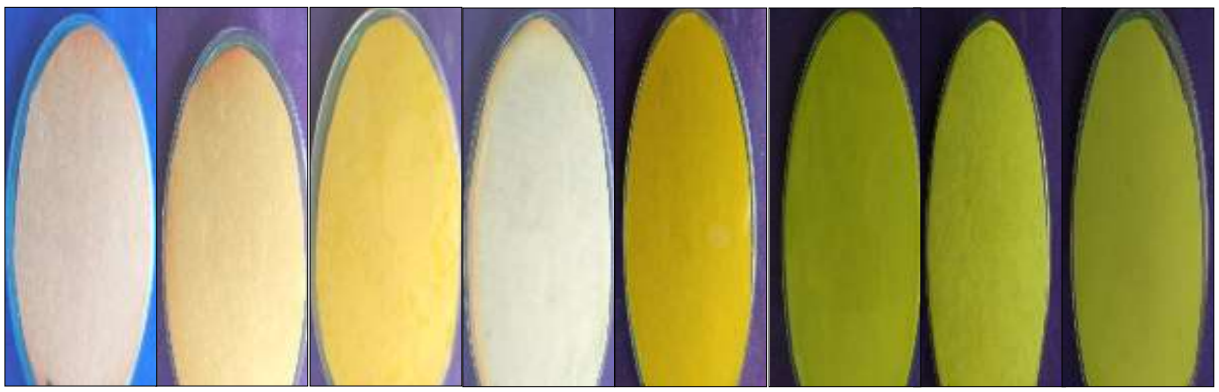
Many rhizospheric bacteria benefit plant growth indirectly through biocontrol mechanisms and the production of hydrolytic enzymes. Among the various ways, due to the production of lytic enzymes, the isolates degrade the membrane constituents of the phytopathogens [46]. The extracellular hydrolytic enzyme production of all the selected isolates is represented in Table 5 and Figure 16. Five isolates showed catalyic activity.

Figure 16 – Catalase Production

2.6.6 HCN Production

Cyanide is a dreaded chemical produced by some deleterious rhizobacteria. It has toxic properties and acts as a general metabolic inhibitor. It is synthesized, excreted, and metabolized by bacteria, algae, fungi, plants, and insects as part of their strategies to fight against predation or establish competition. The host plants are generally not negatively affected by inoculation with cyanide-producing bacteria [47]. Cyanide production was detected after 72h of incubation. A color change of Whatman filter paper from yellow to orange, red, brown, or reddish brown was an indication of the weak, moderate, or strongly cyanogenic potential, respectively (Table 5, Figure 17). Of the Out of eight halophytes, AAK14 was a strong HCN producer, ACY11 and ADYD2 were moderate HCN producers, AEND5 and AGY11 were weak HCN producers, whereas ABYD2, ABK22, and AFY13 were non-HCN producers. Similar findings were reported earlier from *Pseudomonas* sp. isolated from *Atriplex halimus* [42].

Figure 17 – HCN Production



AAK14 ACY11 ADYD2 AEND5 AGY11 ABYD2 ABK22 AFY13

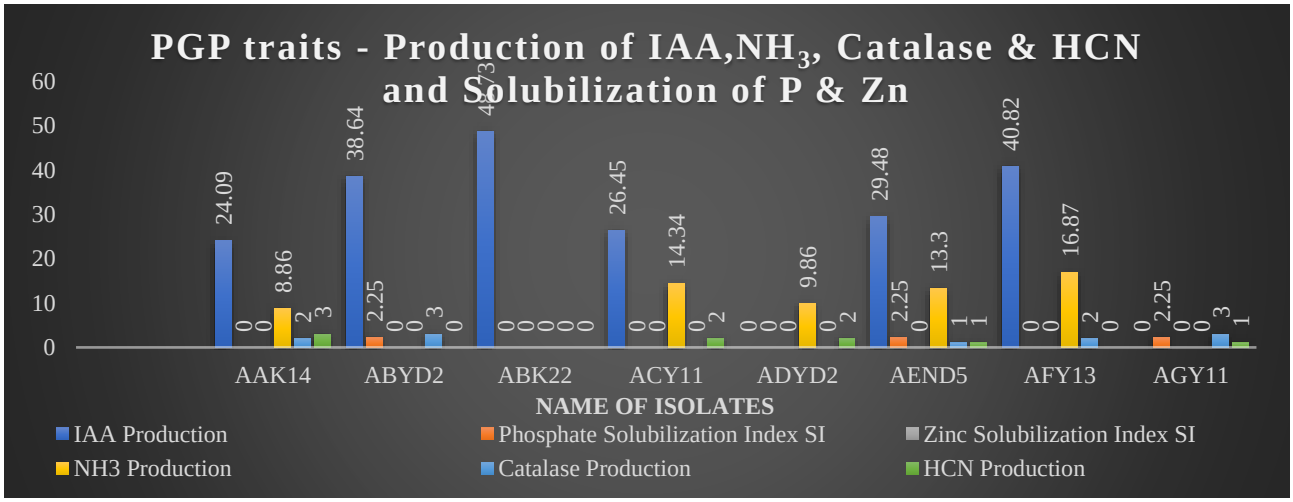


Figure 18 – PGP Traits

Conclusion

A total of 198 bacterial species were identified in the current study from the soil sample collected in the Junagadh district's Adri area. All isolates were identified morphologically and microscopically using accepted techniques. Out of the 198 isolates, 8 isolates (AAK14, ABYD2, ABK22, ACY11, ADYD2, AEND5, AFY13, and AGY11) have demonstrated greater salt tolerance efficiency, according to the data. The finest PGP features include the capacity to withstand a variety of pH ranges (3, 5, 7, 9, and 11) and temperature ranges (5ºC, RT, 37ºC, 45ºC, and 65ºC), as well as the ability to produce IAA, ammonia, and HCN, solubilize phosphate, and exhibit catalase activity. Every test result is positive in the AEND5 isolate. Thus, we believe that this particular species of bacteria can be used as a biofertilizer.

Data Availability Statement

The raw datasets supporting the conclusions of this article will be made available by the authors, without undue reservation. Raw data can be found in online repositories. The names of the repository/repositories are cited in the article.

References

1. Shweta B, Ragini R, Neepa P. Alleviation of impacts of salt stress on the growth parameters of groundnut (Arachis hypogaea L..) Employing halotolerant PGPR. Int. J. of Adv. Res. 2019 Nov 7; 331-338. (ISSN 2320-5407).
2. Egamberdieva D, Wirth S, Bellingrath-Kimura SD, Mishra J, Arora NK. Salt-tolerant plant growth-promoting rhizobacteria for enhancing crop productivity of saline soils. Frontiers in microbiology. 2019 Dec 18;10:2791.
3. https://www.pmfias.com/coastline-of-india-indian-coastline-coastal-plains-of-india/
4. Garg P. Energy scenario and Vision 2020 in India. Journal of Sustainable Energy & Environment. 2012 Aug;3(1):7-17.
5. Groß F, Durner J, Gaupels F. Nitric oxide, antioxidants and prooxidants in plant defence responses. Frontiers in Plant Science. 2013 Oct 29;4:419.
6. Schirawski J, Perlin MH. Plant–microbe interaction 2017—the good, the bad, and the diverse. International Journal of Molecular Sciences. 2018 May 5;19(5):1374.

7. Etesami H, Beattie GA. Mining halophytes for plant growth-promoting halotolerant bacteria to enhance the salinity tolerance of non-halophytic crops. Frontiers in microbiology. 2018 Feb 8;9:148.
8. Mukhtar S, Malik KA, Mehnaz S. Microbiome of halophytes: Diversity and importance for plant health and productivity. Microbiology and Biotechnology Letters. 2019;47(1):1-0.
9. Olsen GJ, Woese CR, Overbeek R. The winds of (evolutionary) change: breathing new life into microbiology. Journal of Bacteriology. 1994 Jan;176(1):1-6.
10. Podell S, Ugalde JA, Narasingarao P, Banfield JF, Heidelberg KB, Allen EE. Assembly-driven community genomics of a hypersaline microbial ecosystem. PLoS One. 2013 Apr 18;8(4):e61692.
11. Smith DL, Subramanian S, Lamont JR, Bywater-Ekegärd M. Signaling in the phytomicrobiome: breadth and potential. Frontiers in Plant Science. 2015 Sep 9;6:709.
12. Upadhyay SK, Singh DP. Effect of salt-tolerant plant growth-promoting rhizobacteria on wheat plants and soil health in a saline environment. Plant Biology. 2015 Jan;17(1):288-93.
13. Ruppel S, Franken P, Witzel K. Properties of the halophyte microbiome and their implications for plant salt tolerance. Functional Plant Biology. 2013 Apr 9;40(9):940-51.
14. Mukhtar S, Mirza MS, Awan HA, Maqbool A, Mehnaz S, Malik KA. Microbial diversity and metagenomic analysis of the rhizosphere of para grass (Urochloa mutica) growing under saline conditions. Pak J Bot. 2016 Apr 1;48(2):779-91.
15. Rodríguez H, Fraga R. Phosphate-solubilizing bacteria and their role in plant growth promotion. Biotechnology advances. 1999 Oct 1;17(4-5):319-39.
16. Singh RP, Jha PN. Alleviation of salinity-induced damage on the wheat plant by an ACC deaminase-producing halophilic bacterium Serratia sp. SL-12 isolated from a salt lake. Symbiosis. 2016 Jun;69:101-11.
17. Sorty AM, Meena KK, Choudhary K, Bitla UM, Minhas PS, Krishnani KK. Effect of plant growth-promoting bacteria associated with halophytic weed (Psoralea corylifolia L) on germination and seedling growth of wheat under saline conditions. Applied biochemistry and biotechnology. 2016 Nov;180:872-82.
18. Damodaran T, Sharma DK, Mishra VK, Jha SK, Kannan R, Sah V, Rai RB. Isolation of Salt Tolerant Endophytic and Rhizospheric Bacteria by Natural Selection and screening for promising plant growth promoting Rhizobacteria [PGPR] and growth vigor in Tomato under sodic soil. African Journal of Microbiology Research. 2013 Nov 7; Vol. 7(44), pp. 5082-5089.
19. Castiglione S, Oliva G, Vigliotta G, Novello G, Gamalero E, Lingua G, Cicatelli A, Guarino F. Effects of compost amendment on glycophyte and halophyte crops grown on saline soils: Isolation and characterization of rhizobacteria with plant growth-promoting features and high salt resistance. Applied Sciences. 2021 Feb 27;11(5):2125.
20. Karnwal A. Screening, isolation, and characterization of culturable stress-tolerant bacterial endophytes associated with Salicornia brachiata and their effect on wheat (Triticum aestivum L.) and maize (Zea mays) growth. Journal of Plant Protection Research. 2019;59(3).
21. Mahdi I, Allaoui A, Fahsi N, Biskri L. Bacillus velezensis QA2 potentially induced salt stress tolerance and enhanced phosphate uptake in quinoa plants. Microorganisms. 2022 Sep 14;10(9):1836.
22. Carter MR, Gregorich EG, editors. Soil sampling and methods of analysis. CRC Press; 2007 Aug 3.
23. Khan LA, Halo AB, Elyassi A, Ali S, Al-Hosni K, Hussain J, Al-Harrasi A, Lee IJ (2016). Indole acetic acid and ACC deaminase from endophytic bacteria improve the growth of *Solarium lycopersicum*. Electronic Journal of Biotechnology, 19:58-64.

24. Yadav AN (2020). Plant Microbiomes for Sustainable Agriculture: Current Research and Future Challenges. InPlant Microbiomes for Sustainable Agriculture, 25:475-482.
25. Zhao Y (2010). Auxin biosynthesis and its role in plant development. Annual review of plant biology, 61:49-64.
26. Matsukawa E, Nakagawa Y, Iimura Y, Hayakawa M (2007). Stimulatory effect of indole-3-acetic acid on aerial mycelium formation and antibiotic production in *Streptomyces* sp. Actinomycetologica, 21:32-39.
27. Mohite B (2013). Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. Journal of soil science and plant nutrition, 13:638-649.
28. Marques AP, Pires C, Moreira H, Rangel AO, Castro PM (2010). Assessment of the plant growth promotion abilities of six bacterial isolates using *Zea mays* as an indicator plant. Soil Biology and Biochemistry, 42:1229-1235.
29. Mota MS, Gomes CB, Souza Júnior IT, Moura AB (2017). Bacterial selection for biological control of plant disease: criterion determination and validation. Brazilian journal of microbiology, 48:62-70.
30. Khan MS, Zaidi A, Wani PA (2007) Role of phosphate-solubilizing microorganisms in sustainable agriculture review. Agron Sustain Dev 27: 29-43.
31. Vessey JK (2003) Plant growth-promoting rhizobacteria as biofertilizers. Plant & Soil 255: 571-586.
32. Sáenz-Mata J, Palacio-Rodríguez R, Sánchez-Galván H, Balagurusamy N (2016). Plant growth-promoting rhizobacteria associated with halophytes: potential applications in agriculture. Sabkha Ecosystems, 5:411-425.
33. Kapri A, Tewari L (2010). Phosphate solubilization potential and phosphatase activity of rhizospheric *Trichoderma* sp. Brazilian Journal of Microbiology, 41:787-795.
34. Saghafi D, Delangiz N, Lajayer BA, Ghorbanpour M (2019). An overview of the improvement of crop productivity in saline soils by halotolerant and halophilic PGPRs. 3 Biotech, 9: 261.
35. Sáenz-Mata J, Palacio-Rodríguez R, Sánchez-Galván H, Balagurusamy N (2016). Plant growth-promoting rhizobacteria associated with halophytes: potential applications in agriculture. Sabkha Ecosystems, 5:411-425.
36. Jha CK, Saraf M (2015). Plant growth-promoting rhizobacteria (PGPR): a review. Journal of Agricultural Research and Development, 5:108-119.
37. Marakana T, Sharma M, Sangani K (2018). Isolation and characterization of halotolerant bacteria and it's effects on wheat plant as PGPR, The Pharma Innovation Journal, 7: 102-110.
38. Rabhi NE, Silini A, Cherif Silini H, Yahiaoui B, Lekired A, Robineau M, Esmaeel Q, Jacquard C, Vaillant Gaveau N, Clément C, Aït Barka E (2018). *Pseudomonas knackmussii* MLR6, a rhizospheric strain isolated from a halophyte, enhances salt tolerance in *Arabidopsis thaliana*. Journal of Applied Microbiology, 125:1836-1851.
39. Yaqoob C, Awan HA, Maqbool A, Malik KA (2013). Microbial diversity of the rhizosphere of Kochia (*Kochia indica*) growing under saline conditions. Pakistan journal of botany, 45:59-65.
40. Iqbal U, Jamil N, Ali I, Hasnain S (2010). Effect of zinc-phosphate-solubilizing bacterial isolates on the growth of *Vigna radiata*. Annals of microbiology, 60:243-248.
41. Jagana CS, Baba ZA, Krishnanand SI, Zargar MY, Badri Z, Khan IJ (2019). Isolation and Characterization of Zinc Solubilizing Bacteria from Kashmir Himalayas, India.International Journal of Current Microbiology and Applied Sciences, 8:1248-1258.

42. Arif F, Ghoul M (2018). Halotolerance of Indigenous Fluorescent *Pseudomonads* in the Presence of Natural Osmoprotectants. Annual Research & Review in Biology, 24:1-11.
43. Vaishnav A, Varma A, Tuteja N, Choudhary DK (2016). PGPR-mediated amelioration of crops under salt stress. Plant-Microbe Interaction: An Approach to Sustainable Agriculture, 205-226.
44. Dhaked BS, Triveni S, Reddy RS, Padmaja G (2017). Isolation and screening of potassium and zinc solubilizing bacteria from different rhizosphere soil. International Journal of Current Microbiology and Applied Sciences, 6: 1271-1281.
45. Izzah S, Rizwan M, Baig DN, Saleem RS, Malik KA, Mehnaz S (2017). Secondary Metabolites production and plant growth promotion by *Pseudomonas chlororaphis* and *P. aurantiaca* strains isolated from cactus, cotton, and para grass. Journal of Microbiology and Biotechnology, 27:480-491.
46. Subrahmanyam G, Kumar A, Sandilya SP, Chutia M, Yadav AN (2020). Diversity, Plant Growth Promoting Attributes, and Agricultural Applications of Rhizospheric Microbes. Plant Microbiomes for Sustainable Agriculture, 1: 1-52.
47. Saharan BS, Nehra V (2011). Plant growth-promoting rhizobacteria: a critical review. Life Sciences and Medical Research, 21:30.