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Isolation and characterization of halotolerant bacteria and it's effects on wheat plant as PGPR

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Abstract
Salinization is recognized as the main threats to environmental resources and human health in many countries, affecting almost 1 billion worldwide/ globally representing about 7% of earth's continental extent. It has been estimated that an approximate area of 7 million hectares of land is covered by saline soil in India. Agricultural crops exhibit a spectrum of responses under salt stress. The impacts of salinity include low agricultural productivity, low economic returns and soil erosions. Soil salinity imposes ion toxicity, osmotic stress, nutrient (N, Ca, K, P, Fe, Zn) deficiency and oxidative stress on phosphorous uptake from soil. In order to access the tolerance of plants to salinity stress, growth or survival of the plants, alleviation of abiotic stress is can be done by rhizosphere bacteria. Studies on these shown that inoculations with selected PGPR and other microbes could serve as the potential tool for alleviating salinity stress in salt sensitive crops. It can be useful in developing strategies to facilitate sustainable agriculture in saline soils.

Keywords: Salinization, saline soil, salt stress, ion toxicity, osmotic stress, phosphorous, rhizosphere, alleviation of abiotic stress, PGPR

1. Introduction
Salinity is one major limiting factor to plant growth and crop productivity [1, 2]. Salinity is one of the most critical constraints which hamper agricultural productions in many areas worldwide [3]. Currently 800 million hectares of land throughout the world are affected by levels of salt that could substantially reduce crop productivity [2]. The adverse effects of salinity on plant growth includes the implementation of salt-tolerant bacteria with natural growth promoting ability in such conditions [3]. Certain soil bacteria can help the plants to avoid or partially overcome a variety of environmental stresses [3]. Little Rann of Kutch is nominated to be a "biosphere reserve" which is defined by the areas of terrestrial and coastal ecosystems internationally recognized within the framework of UNESCO's Man and Biosphere (MAB) program [4, 5]. Inspite of such ecosystem uniqueness possessed by little Rann of Kutch, It is least explored for study rhizobacterial diversity harbored in the rhizosphere of plants adapted to this ecosystem4. Rhizobacteria is the group of bacteria residing in the rhizosphere of plant, and they are widely studied for plant growth promoting (PGP) traits they possess [6]. Soil salinity in agriculture soils refers to the presence of high concentration of soluble salts in the soil moisture of the root zone. These concentrations of soluble salts through their high osmotic pressures affect plant growth by restricting the uptake of water by the roots [7]. Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on germination, plant vigour and crop yield [8]. Strategies for alleviation of salt stress involve developing salt-resistant cultivars, leaching excess soluble salts from upper to lower soil depths, flushing soils that contain soil crusts at the surface, reducing salt by harvesting salt-accumulating aerial plant parts in areas with negligible irrigation water or rainfall for leaching, and amelioration of saline soils under cropping and leaching [9]. Plants vary greatly in their tolerance to salts. However, the performance of crops under saline conditions depends on seed germination, plantlet appearance, establishment and also tolerance at later stages of growth [10]. When plant is exposure to salinity by NaCl, water and ion transport processes may be affected and disturb plant nutrient situation and ionic balance or disordered physiological process. Salinity decrease water availability for the seed by taking down osmotic potential and in second stage cause to toxicity and change in enzyme activity. Salt stress affects germination percentage, germination rate and seedling growth in different ways depending on plant species [10, 11]. Plants growing in saline soils experience osmotic stress due to increases in the concentration of Na+ and Cl-, leading to ionic imbalance in

tissues and resulting inhibition of nutrient uptake [12, 13]. Antioxidant enzymes are involved in resistance to various abiotic stresses, including salinity [14, 15]. Inter cultivars variation for salt tolerance at different levels of NaCl salinity at the germination or seedling stage has been assessed in wheat [16, 17]. Slight halophiles grow best in 0.3 to 0.8 M NaCl (1.8 to 4.7%), moderate halophiles 0.8 to 3.4 m (4.7 to 20%), and extreme halophiles 3.4 to 5.1 M (20 to 30%) NaCl [18] High soil salinity adversely affects the physical and chemical properties of soil, thereby directly affecting the growth and diversity of organisms that live in or on soil such as plants, microbes, protozoa and nematodes. In plants long-term high soil salinity conditions cause ionic and osmotic stress that adversely affects the functioning of various biochemical process [19, 20]. Plant growth promoting rhizobacteria (PGPR) could play a significant role in the development of sustainable agriculture [21]. Interestingly, as an alternative to breeding and genetic manipulation, plant salt tolerance can also be improved by the application of salt tolerant microorganisms, and beneficial microorganism, such as plant growth promoting bacteria (PGPR), that inhibit the rhizosphere have been investigated for their potential to alleviate salt stress [22]. Phosphorus (P) is the second most important plant nutrient after nitrogen [23]. Phosphate-solubilizing microbes play essential parts in biogeochemical phosphorus cycling in usual and agricultural environments. Phosphate-solubilizing microbes can convert the insoluble phosphorus to soluble forms $HPO^{2}_{4}{}^{-}$ and $H2PO^{-}_{4}$ by acidification, chelation, exchange reactions and polymeric substances formation [24, 25]. Therefore, the use of phosphate-solubilizing microbes in agricultural practice would not only offset the high cost of manufacturing phosphate fertilizers but would also mobilize insoluble phosphorus in the fertilizers and soils to which they are applied [24] Indole acetic acid (IAA) is one of the most physiologically active auxin [26]. IAA is a common product of L- tryptophan metabolism produced by several microorganisms including Plant Growth-Promoting Rhizobacteria (PGPR) [26, 27]. Bacteria that colonize the rhizosphere and plant roots, and enhance plant growth by any mechanism are referred to as PGPR. PGPR can exhibit a variety of characteristics responsible for influencing plant growth. The common traits include production of plant growth regulators (like auxin, gibberellin, and ethylene), siderophores, HCN and antibiotics [28, 29]. Indole acetic acid helps in the production of longer roots with increased number of root hairs [30]. The present study aims to isolate salt-tolerant bacteria from the rhizosphere of a facultative halophyte, characterize them by morphological, biochemical and molecular means; and to define whether any of the isolated bacteria possess PGPR actions by their tendency to fix atmospheric nitrogen, solubilization of mineral like phosphorus, production of IAA [7].

2. Materials and Methods

2.1 Rhizospheric Soil Sampling and Soil Analysis

The rhizosphere, indicates the thin deposit of soil adjoining plant roots and the soil engaged by the roots, supports large active groups of bacteria (PGPR) [31, 32]. Soil Sample was collected on 28th December of 2017 from the Kutch region of Gujarat state. The sample was collected from two villages of Kutch one is Koday which is situated in Mandvi and another is Mathal which is situated in Nakhatrana. Total of three samples were collected out of which 2 from Koday village and 1 from Mathal village. The first soil sample was collected from the roots of the wheat plant from Koday village. The second soil sample was also collected from the roots of the wheat plant from Mathal village. The third soil sample was collected from the wheat field from Koday village. These regions are near to seashore so they are rich in salts. 5g of soil from the roots of the wheat plant was taken as sample. The soil was scraped using forcep. The samples were investigated for microorganisms within 5 days of gathering samples. The collected soil sample was examined for chemical properties like pH and conductivity.

2.2 Isolation

For isolation, 1gm soil sample was serially diluted upto 10-4. From the last two dilution 0.1ml was inoculated on nutrient agar plates. Media used was Nutrient agar medium. This media was obtained from Hi Media Laboratories Pvt. Ltd. In Nutrient agar medium, additionally added sodium chloride (NaCl) 1M. In 200ml of distilled water, added 6gm of agar agar powder, 5.6gm of nutrient agar and 11.6gm of NaCl (1M). Then Autoclave it and poured them in sterile petri plates in laminar air flow. Agar plate using spread plate technique for isolation of desired bacteria. Plates inoculated with suspensions were incubated at 28o to 30o C for 48h for growth of halotolerant bacteria. Numbers of colonies are obtained in all the nutrient agar plates. Again Nutrient agar medium with additionally added NaCl. In 200ml of distilled water added 6gm of agar powder, 5.6gm of nutrient agar and 11.6gm of NaCl (1M). Then Autoclave it and poured them in sterile petri plates. Resulted colonies from the plates were selected with the help of sterile wire loop and using streaking plate technique streaked the colony on the nutrient agar plate and then incubated at 28o to 30o C for 48h. Same preparation was done repeatedly to obtain the pure line of isolates under 1M NaCl (5.8%) stress conditions. At last the pure lines of colonies obtained needed to be characterize and named that isolates as K1BI, K2B2, K1B2, K2C1, K1F1, K1E1, KD1, K2D1, and MB2. Same procedure was followed using Nutrient Agar medium with 2M NaCl (11.6%). Numbers of colonies obtained were selected and cultured again to obtain pure lines for the same and their colony characteristics were recorded.

Fig 2.2: Geographical location for sample collection a) Koday, Mandvi b) Mathal, Nakhatrana

2.3 Identification of halotolerant bacteria

2.3.1 Gram's staining

Gram staining is used to distinguish the bacteria as gram positive and gram negative [18]. For this cleaned the slide with detergent. Then marked all the slide by names of obtained isolates. With the help of sterile wire loop put the drop of distilled water on the slide. Single colony with the help of sterile wire loop was picked and made the heat fix smear on the slide. Next put the 1 drop of crystal violet on the smear for 1 min. After 1 min slide was washed by tap water drop wise. 1-2 drops of iodine were added on the smear for 30 sec. After 30 sec slide was washed with alcohol. After the slide was dried 1 drop of saffranine was added on the smear and kept for 1 min and then washed by tap water drop wise. Then 1 drop of oil was put on the slide to observe the slide under 100 X which is oil immersion lens using compound microscope.

2.3.2 Biochemical Test

2.3.2.1 Methyl Red (M-R) Test

Principle: As the medium (GPB) used is firmly buffered and methyl red is pH indicator having a range between 6.2 to 4.4, so pH at which methyl red identifies acid in considerable lower than the pH for other indicators used in bacterial media.

Procedure: Glucose phosphate broth 50ml was prepared. Then dissolved it by heating and poured it in the test tubes. Then sterilize all the test tubes in autoclave. After sterilization GPB containing tubes were inoculated with test culture and incubated all the test tubes in incubator at 28º- 30 ºC for 48 hours. 5 drops of methyl red indicator added in all the test tubes and observed the color change in all the test tubes.

2.3.2.2 Voges Proskauer (V-P) Test

Principle: In the existence of alkali and air acetone is reacted to diacetyl, which responds with the guanidine nucleus of arginine existence in protein of peptones to produce pink color. Test is made delicate by adding α- naphthol, which act as catalyst.

Procedure: First of all, prepared a glucose phosphate broth 50ml. Then dissolved it by heating and poured it in all the test tubes. Then sterilized all the test tubes in autoclave. Next inoculated GPB with test culture and incubated all the test tubes in incubator at 28º-30 ºC for 48 hours. Next added 0.6ml of α- naphthol and 0.2ml of KOH solution in all the test tubes. Then shake all the test tubes well. Then observed the color change in all the test tubes.

2.3.2.3 Citrate Utilization Test

Principle: This test define the capacity of bacteria to use citrate as a sole of carbon and energy. There is release of carbon di oxide and nitrogen which later form carbonate and hydroxide respectively. All this increase the pH and blue color gives positive test.

Procedure: For this test, Simmon's citrate agar was prepared (50ml). In the medium additionally added agar agar powder 1.5 gm. Then dissolved it by heating and poured it in all the test tubes. Then sterilized all the test tubes in autoclave. The test tubes were kept in the slant position. Next day streaked the test culture on that slants and kept in the incubator at 37oC for 48 hours. The color change in all the test tubes was observed.

2.3.2.4 Indole Production Test

Principle: Indole is one of the metabolic degradation products of the amino acid tryptophan. Bacteria that own the enzyme tryptophanase are proficient of hydrolyzing and deaminating tryptophan with the production of indole, pyruvate and ammonia.

Procedure: 50 ml of tryptone broth was prepared. Then dissolved it by heating and poured it in the test tubes. Then sterilized all the test tubes using autoclave. Test tubes containing tryptone broth were inoculated with test culture and kept for incubation in incubator at 37 ºC for 48 hours. Next added 1ml Kovac's reagent in all the test tubes. Then observed the formation of the pink color ring which if present indicates positive test.

2.3.2.5 Urea Hydrolysis Test

Principle: Stuart's urea broth is a medium in which urea is the only nitrogen source. Urease is an enzyme that can hydrolyze urea. The ammonia so produced reacts in solution to form ammonium carbonate, resulting in alkalization and an increase in the pH of the medium.

Procedure: First of all, prepared Stuart's Urea broth 50ml. Then dissolved it by heating and poured it in all the test tubes. Then sterilized all the test tubes in autoclave. Next inoculated Stuart's Urea broth with test culture and incubated all the test tubes in incubator at 28o-30 ºC for 48 hours. Then observed the color change in all the test tubes.

2.3.2.6 Nitrate Reduction Test

Principle: Bacteria having nitrate reductase when grown in a medium comprising nitrate as only source of nitrogen will convert nitrate to nitrite. This formation can be observed by adding sulphanilic acid, which forms a diazonium salts, which in turn respond with α- naphthylamine, result in formation of red azo dye.

Procedure: First of all, prepared a peptone nitrate broth 50ml.Then dissolved it by heating and poured it in all the test tubes. Then sterilized all the test tubes in autoclave. Next inoculated PNB with test culture and incubated all the test tubes in incubator at 28º-30ºC for 48 hours. Next added 0.5ml of reagent A that is α- naphthylamine and 0.5ml of reagent B that is sulphanilic acid. Then observed the development of red color within 30 seconds. But the colored was not developed so added pinch of zinc dust and mixed all the test tubes well. Then observed the color change in all the test tubes.

2.3.2.7 Starch Hydrolysis Test:

Principle: It is centered on color reaction of non-hydrolyzed starch with iodine.it provides deep-blue color but its breakdown products progressively becomes violet, brownish red and lastly colorless.

Procedure: First of all, prepared a starch agar 250ml in a flask. Then sterilized the flask in autoclave. Next poured the plate with starch agar. Next day streaked the plate with test culture and incubated all the plates in incubator at 28o-30 ºC for 48 hours. After incubation the plates were flooded with iodine and observed the zone surrounding the colonies. Appearence of the clear zone indicate the positivity of the test.

2.3.2.8 Catalase Test

Procedure: First of all, washed the slide with detergent. Then marked the obtained isolates on the slide. Then put the drop of test culture on the slide. Next put the 4 to 5 drops of hydrogen peroxide on the slide. Then observed the gas bubble formation on the slide. Formation of gas bubbles confirm that the test is positive.

2.3.3 Assessment of PGP Traits

2.3.3.1 Indole-3-acetic acid (IAA) Production [36, 37].

For IAA production first of all, prepared salkowski reagent 50ml, 35% perchloric acid and 1ml 0.5M FeCl3 solution. Then mixed it well. Then prepared the tryptophan broth 55ml. Then dissolved it by heating and poured in all the test tubes. Then sterilized the same in autoclave. Then inoculated the tryptophan broth with obtained isolates. Incubated all the test tubes in incubator at 28o-30 ºC for 48 hours. After incubation added the 2ml of salkowski reagent in all the test tubes. Observed the color change as the indicator of IAA production. Phosphate Solubilization [31].

2.3.3.2 Phosphate Solubilization [31]

Phosphate solubilization of the isolates was checked using Pikovskaya's agar medium (Hi Media, Mumbai). In these medium added 0.5gm tricalcium phosphate, 0.12gm yeast extract, 2.5gm dextrose, 0.125gm ammonium sulphate, 0.05gm potassium chloride, 0.025gm magnesium sulphate, 0.000025gm manganese sulphate, 0.000025gm ferrous sulphate and 3.75gm agar agar powder. Then sterilized the medium in autoclave. Then poured the medium in all the sterile petri plates. Next day put the 2µl of drop in all the plates. Incubated all the plates in incubator at 28o-30 ºC for 6-7 days. Then observed the zone of phosphate solubilization.

2.3.4.3 Hydrogen cyanide (HCN) Production [31, 38, 39, 40]

Production was done by streaking the bacterial isolates on King's B agar medium. For these test prepared King's B medium 250ml. In these medium mixed 10.55gm of King's B and 4.4gm% glycine in 250ml of distilled water. Then sterilized the medium in autoclave. Then poured the medium in all the sterile petri plates. Next day picked the colony from our obtained isolates and streaked on the plates of King's B medium. Then placed the Whatmann Filter Paper No.1 (soaked in picric acid) between the lid and the base of petri plates. Then sealed the plates with para film and put in the incubator at 28o-30 ºC for 5-6 days. Then observed the color change of the Whatmann filter paper No.1 from deep yellow to reddish brown was considered the HCN production.

2.3.3.4 Nitrogen Fixation Test

For these test prepared Jensen's medium 250 ml. In these medium mixed 9.77gm of Jensen's broth and 3.75gm of agar powder in 250ml of distilled water. Then sterilized the medium in autoclave. Then transfer the medium in all the sterile petri plates. Next day picked the colony from our obtained isolates and streaked on the plate of Jensen's medium. Incubated all the plates in incubator at 28o-30 ºC for 5-6 days. Then observed the growth of rhizospheric bacteria.

3. Result

3.1 Isolation and Morphology characteristics

A total of 11 isolates obtained as a result of spreading technique followed by streaking. The isolates were coded as K1BI, K2B2, K1B2, K2C1, K1F1, K1E1, KD1, K2D1, MB2, 2M1and 2M3. All the isolates colonies have round, rarely large in size, some are translucent while others transparent, regular in margin, flat in elevation. The texture of most isolates colonies was smooth, some are of rough also. The pigmentation ranged from cream-white, yellow and orange. Maximum growth occurred at 1M and 2M NaCl (w/v) at 270-300C, and pH ranged from 7.5-8.0.

Table 3.1: Colony characteristics of isolated halotolerant bacteria

Sample	Shape	Size	Opacity	Margin	Elevation	Texture	Pigmentation
K1B1	Round	Small	Transparent	Irregular	Flat	Rough	Yellow
K2B1	Round	Small	Translucent	Regular	Raised	Smooth	Cream-white
K2B2	Round	Large	Translucent	Irregular	Raised	Smooth	Orange
K2C1	Round	Small	Transparent	Regular	Flat	Smooth	Cream-white
K1F1	Round	Small	Translucent	Irregular	Raised	Rough	Cream-white
K1E1	Round	Large	Translucent	Regular	Flat	Smooth	Cream-white
MB2	Round	Large	Translucent	Irregular	Raised	Rough	Cream-white

3.2 Biochemical test

3.2.1 Methyl-Red test

As the medium (GPB) used is firmly buffered and methyl red is pH indicator having a range between 6.2 to 4.4, so pH at which methyl red identifies acid in considerable lower than the pH for other indicators used in bacterial media.

All isolates K1B1, K2B2, K1B2, K2C1, K1F1, K1E1, KD1, K2D1, and MB2 showed Methyl-red positive test.

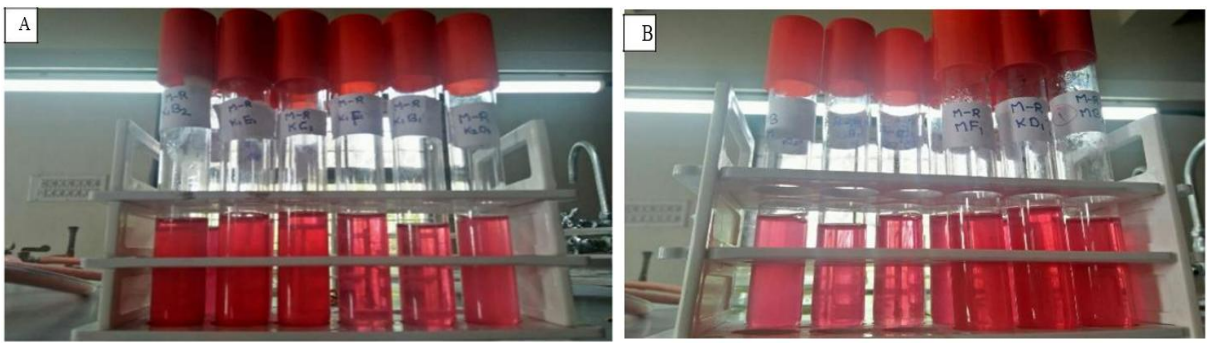

Fig 3.2.1: Methyl red test A) K1B2, K1E1, KC1, K1B1, K2D2, K1F1; B) MF1, KD1, MB2

3.2.2 Citrate Utilization Test

This test define the capacity of bacteria to use citrate as a sole of carbon and energy. There is release of carbon di oxide and nitrogen which later form carbonate and hydroxide respectively. All this increase the pH and blue color gives positive test. Three strains (K1B1, K2B1, and K2C1) showed Citrate Utilization positive test.

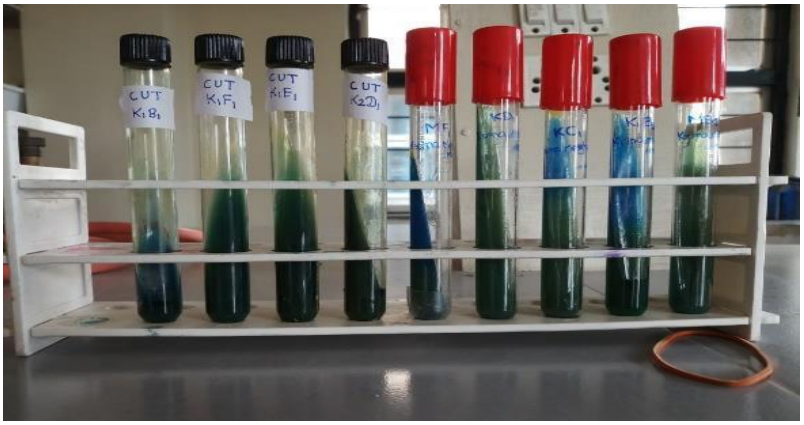

Fig 3.2.2: Citrate utilization test

3.2.3 Indole Production Test

Indole is one of the metabolic degradation products of the amino acid tryptophan. Bacteria that own the enzyme tryptophanase are proficient of hydrolyzing and deaminating tryptophan with the production of indole, pyruvate and ammonia. None isolates showed Indole Production positive test.

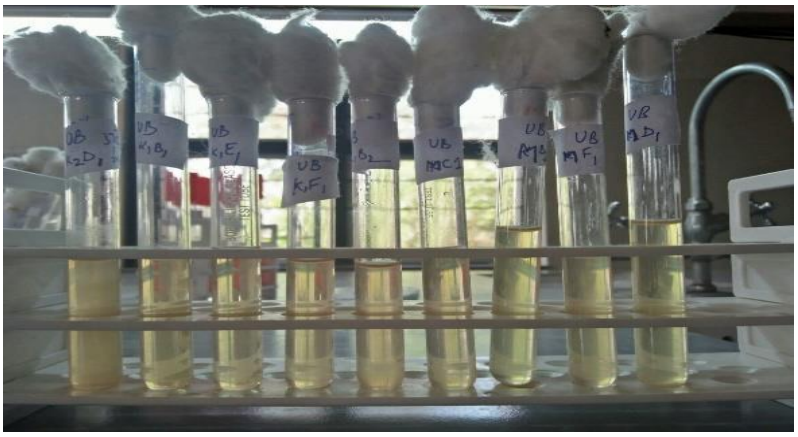

Fig 3.2.3: Indole acetic test

3.2.4 Nitrate reduction Test

Bacteria having nitrate reductase when grown in a medium comprising nitrate as only source of nitrogen will convert nitrate to nitrite. This formation can be observed by adding sulphanilic acid, which forms a diazonium salts, which in turn respond with α-naphthylamine, result in formation of red azo dye.

No isolates showed Nitrate reduction positive test.

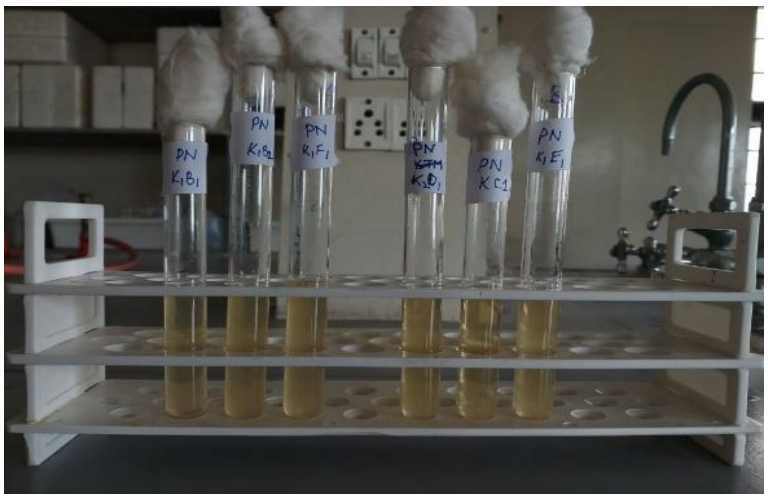

Fig 3.2.4: Nitrate reduction test

3.2.5 Starch Hydrolysis Test

It is centered on color reaction of non-hydrolyzed starch with iodine.it provides deep-blue color but its breakdown products progressively becomes violet, brownish red and lastly colorless.

All isolates showed Starch Hydrolysis positive test.

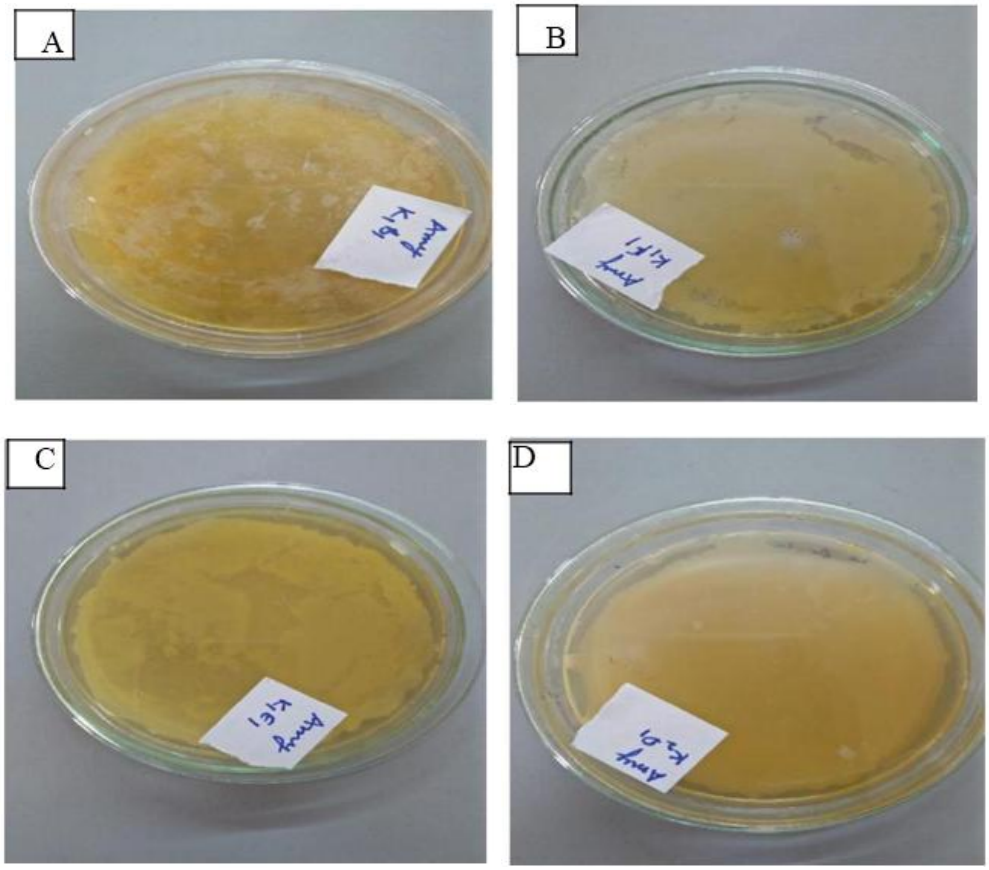

Fig 3.2.5: (A-D) Starch hydrolysis test

3.2.6 Catalase Test

None of the isolates showed Catalase positive test.

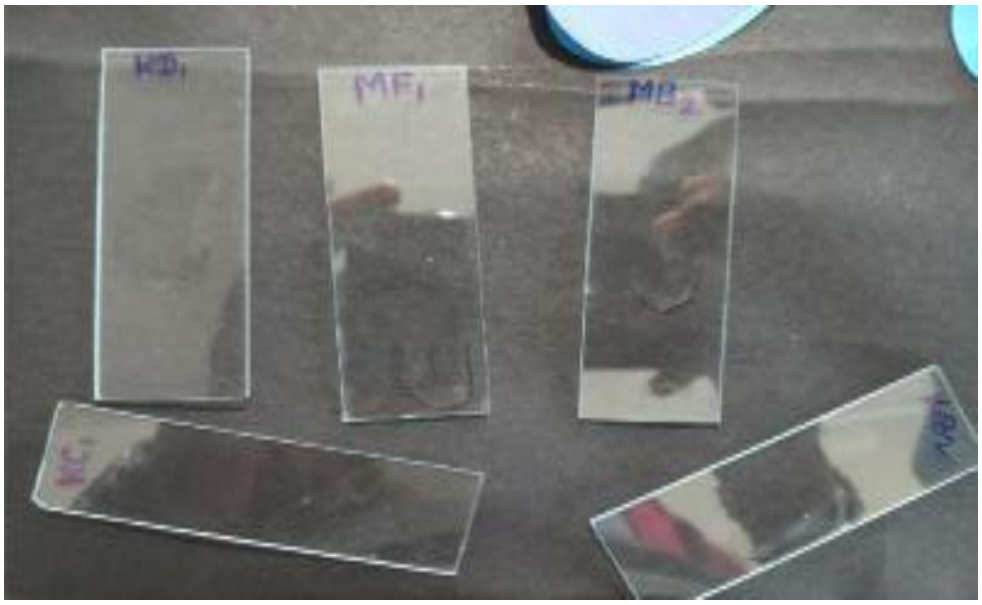

Fig 3.2.6: Catalase test

3.2.7 Urea Hydrolysis Test

Stuart's urea broth is a medium in which urea is the only nitrogen source. Urease is an enzyme that can hydrolyze urea. The ammonia so produced reacts in solution to form ammonium carbonate, resulting in alkalization and an increase in the pH of the medium. No isolates showed Urea Hydrolysis positive test.

Table 3.2: Biochemical test result of halotolerant isolates

Sample	Methyl Red (M-R) Test	Voges Proskauer (V-P) Test	Citrate Utilization Test	Indole Production Test	Nitrate reduction Test	Starch Hydrolysis Test	Catalase Test	Urea Hydrolysis Test
K1B1	+	-	+	-	-	+	-	-
K2B1	+	-	+	-	-	+	-	-
K2B2	+	-	-	-	-	+	-	-
K1F1	+	-	-	-	-	+	-	-
K1E1	+	-	-	-	-	+	-	-
K2C1	+	-	+	-	-	+	-	-
MB2	+	-	-	-	-	+	-	-
K2D1	+	-	-	-	-	+	-	-
KD1	+	-	-	-	-	+	-	-

3.3 Assessment of PGP traits

3.3.1 Production of Indole acetic acid (IAA)

Out of 11 isolates 9 strains (K1B1, K2B2, KD1, K2D1, K1F1, K1E1, MB2, 2M(1), 2M(3))gives production of IAA and 2 strains (K1B2 and K2C1) not shown production of IAA.

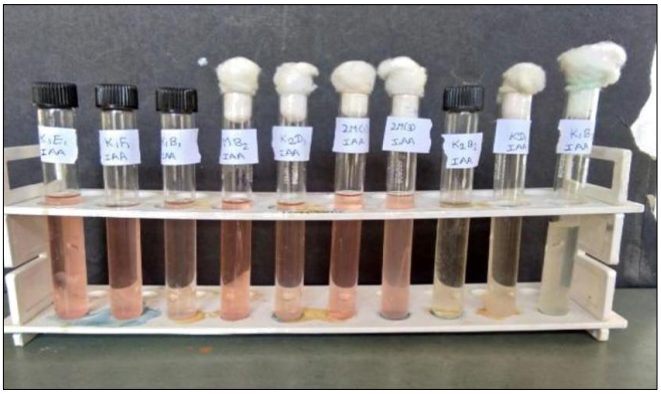

Fig 3.3.1: Salkowski's reagent test

3.3.2 Production of hydrogen cyanide (HCN)

Out of 11 isolates 10 strains (K1B1, K1B2, K2B2, KD1, K2D1, K2C1, K1F1, K1E1, MB2 AND 2M 1) gives production of HCN and 1 strain 2M3 not gives production of HCN.

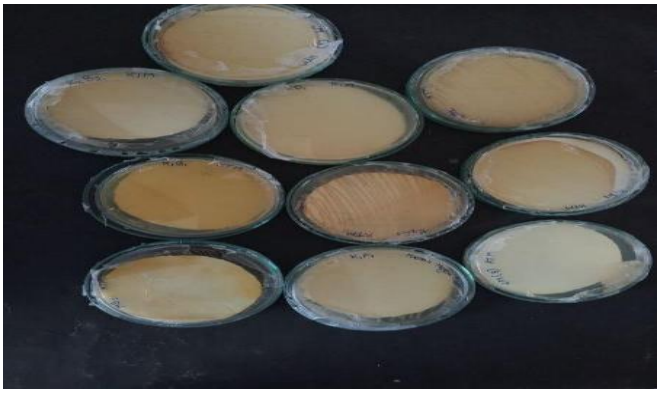

Fig 3.3.2: HCN Production test

3.3.3 Phosphate Solubilization (PS)

Out of 11 isolates 4 strains K2B2, K2D1, K1F1 and K1E1have ability to solubilize inorganic phosphate rest of 7 strains K1B1, K1B2, KD1, K2C1, MB2, 2M1 and 2M3have not ability to solubilize inorganic phosphate.

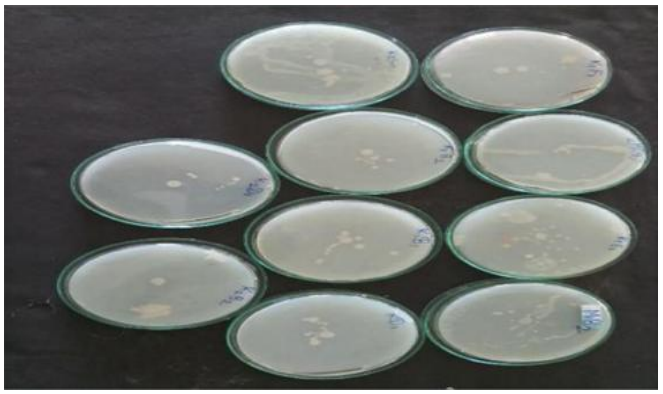

Fig 3.3.3: Phosphate solubilizing test

3.3.4 Determination of Nitrogen Fixation

Out of 11 isolates 7 strains K1B1, KD1, K2C1, K1F1, K1E1, MB2 and 2M3gives positive result for Nitrogen fixation and 4 strains K1B2, K2B2, K2D1 and 2M1gives negative result for Nitrogen fixation.

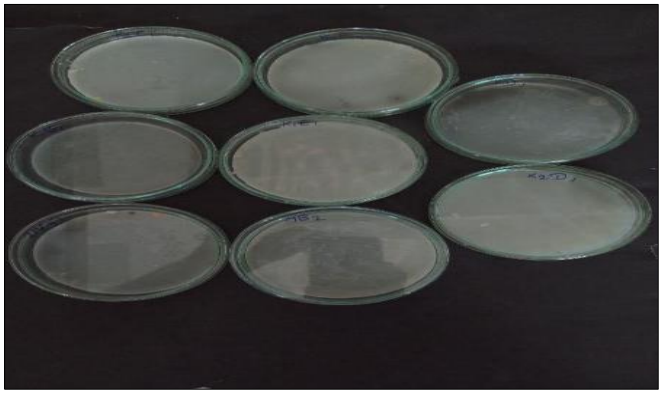

Fig 3.3.4: Nitrogen fixation test

Table 3.3: Assessment of halotolerant isolates for PGPR

Sample	IAA	HCN	Phosphate Solubilization	Nitrogen fixation
K1B1	+	+	-	+
K1B2	-	+	-	-
K2B2	+	+	+	-
KD1	+	+	-	+
K2D1	+	+	+	-
K2C1	-	+	-	+
K1F1	+	+	+	+
K1E1	+	+	+	+
MB2	+	+	-	+
2M (1)	+	+	-	-
2M (3)	+	-	-	+

4. Conclusions

From the present study it is clear that the area selected for sampling is having diversity of microorganism which are moderate halo tolerant bacteria. They are responding to IAA production in considerable amount in tryptophan broth. Over all isolates are showing positive response for IAA. Beside this isolates have shown significant role in phosphate solubilization, HCN production and nitrogen fixing capacity. Maximum response is for IAA and HCN production.

So, finally it is clear that rhizospheric soil of koday and mathal (Kutch region, Gujarat) was having not only halotolerant but also had plant growth promoting traits. These can be introduced against abiotic stress as biofertilizers and act as a boon for sustainable agriculture under stress condition.

5. Discussion

Bacteria encourage plant development by both primary as well as secondary mechanisms with various results depending on a number of factors. High salinity is one of the most common environmental worried factor that unfriendly upset plant output by concerning the plant growth as well as progress (Ramadoss *et al.*). To build up plant growth under saline condition, direct use of salt-accepting bacteria has drawn significant study both in industry and in research. In the current study, an enormous number of halo tolerant bacteria were isolated and characterized for their tolerance levels of NaCl. In this consideration all the isolates at higher NaCl concentrations grew with long stationary. Isolation of PGPR from saline soil was the main approach of this study. So, total of 11 isolates were selected from the sample. Out of that 9 isolates were available in 1M NaCl and rest two from 2M.Results shown that 9 isolates belong to gram positive and 2 isolates belong to gram negative. Various biochemical test has shown positive response for some strains. These are in agreement with the previous study reported by Halophilic bacteria grew better at temperature of 28-370 C and at pH7.0-8.0 on medium supplemented with 5-20% NaCl concentration (Roohi *et al*). The bacteria separated in this study grew best at 28-300C and pH 7.5-8.0 supplemented with 5-12% NaCl.These strains with high salt tolerance were further characterized for the PGP activities including IAA production, P- Solubilization and HCN production. IAA making by bacteria existing in the rhizosphere is very crucial property giving to plant growth. IAA, a member of the club of phytohormones, is considered to be the most crucial native auxin (Damam *et al*). All 7 isolates from 9 are positive for IAA production.

After several dilutions and sub culturing in the liquid as well as solid medium, colonies were isolated in the meliorate medium containing 1% and 2% NaCl. A total of 9 from 1M and 2 from 2M of strains were isolated underneath aerobic conditions from the sample. These strains were characterized morphologically as well as biochemically. Most of the isolates have IAA and HCN activity.10 out of 11 have shown positive result for HCN, which is related with the study done by (Reetha *et al.*). As IAA is produced by 9 strains, they may promote root growth directly, by stimulating cell elongation or cell division response for phosphate solubilization and nitrogen fixation are showing less positive response as compared to previously discussed ones. 4 Isolates (K2B2, K2D1, K1F1, and K1E1) out of 11 isolates shown positive activity for phosphate solubilization. The results for HCN producing bacteria are very effective as only one isolate (2M3) did not respond although there is variation in the intensity in the color change. Nitrogen is another important element required by plants.K1B1, KD1, K2C1, K1E1, MB2, 2M3 show positive response as role in nitrogen fixation bacteria.

The use of such microorganisms can induce tolerance to abiotic stresses in the host as biofertilizers may be a boon to agriculture since urbanization and industrialization are fast depleting our cultivable lands. Under such conditions, agriculture will gradually shift towards hitherto uncultivable areas such as coastal areas and waste lands and these microorganisms can contribute to sustainable agriculture under adverse conditions.

Further studies can be done using these isolates for detection of IAA and other PGP traits. Also *in vitro* pot trials can be done on different plants and their dry weight, root and shoot length etc. can be compared, so that these isolates can preferably play an important role as bio fertilizer.

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7. References

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