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Research Article

ASSESSMENT OF THE EFFECT OF HALO–TOLERANT BACTERIUM ON PLANT GROWTH PROMOTION

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ABSTRACT

A total of 12 halobacterial strains were isolated from soil samples of various salterns in Tamilnadu, India and screened their plant growth promoting activity. All the isolates were produce Indole Acetic Acid (IAA), Ammonia and 7isolates produced Hydrogen Cyanide (HCN). Siderophore production and phosphate utilization were negative. In this present study only one isolate (M5) showed good growth in halophilic medium and plant growth promoting activities such as IAA, HCN and Ammonia production. The plant growth promotion effects was tested in pot trials with various crops like Cotton, Jatropa, Lady's finger and Sorghum. Selected isolate significantly increased the plant shoot length and seed germination percentage in pot conditions compared to control. Further this isolate was identified as *Chromohalobactersalexigens*based on 16s rRNA sequencing.

Keywords: Halobacteria, PGPR**,** *Chromohalobactersalexigens,* IAA, HCN.

INTRODUCTION

Bioprospecting may be defined as the search for new or better bioproducts from biological sources. In particular, bioprospecting relies on provision of a bioresource, a supply of novel biodiversity. Halophiles are basically salt-loving organisms that inhabit hypersaline environments and it can be classified as slight halophiles showing optimum growth at 2–5% NaCl, moderate halophiles at 5–20% NaCl, and extreme halophiles show 20–30% NaCl.

Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that can be found in the rhizosphere, at root surfaces and in association with roots, which can improve the extent or quality of plant growth directly and/or indirectly. The use of microorganisms with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture (Freitas*et al.* 2007). Direct promotion of growth by PGPR occurs when the rhizobacteria produce metabolites that promote plant growth such as auxins (Asghar*et al*., 2002), cytokinins (Arkhipova*et al*., 2005) and gibberellins (Joo*et al*., 2004) as well as through the solubilization of phosphate minerals (Freitas*et al*., 2007). Indirect growth promotion occurs through the elimination of pathogens by the production of cyanide and siderophores. PGPR beneficial effects have been exploited in many areas including biofertilizers, microbial rhizoremediation and biopesticides (Adesemoye*et al*., 2006).

Salinity is one major limiting factor to plant growth and crop productivity (Allakhverdiev*et al*., 2000).Soil salinity is the major cause limiting plant productivity worldwide.Salinity stress adversely affects total dry matter and plant growth, as most of the energy available is used in to make osmotic adjustments by the plant (MunnsandTermaat (1986). A new biological approach 'plant-microbe interaction' to conquer salinity troubles has recently gained a great

interest from many workers throughout the world (Adesemoye*et al*., 2006).The screening of salt tolerant lines/cultivars has been attempted by many researchers on various species at seedling growth stage (Ashraf, 1999). Both water availability and salt stress can impair coleoptiles growth, thus affecting seedling establishment in the field (Schachtmanand Munns, 1992). The use of rhizobacteria is one of the most acceptable approaches to reduce the effect of salt-stress on plants as they are endowed with mechanisms which either modulate or ameliorate the salt stress (Dodd and Perez-Alfocea, 2012).

To overcome this problem, application of plant growth promoting halophilic bacteria (PGPB) in salt-affected soil, bioinoculant with salt-tolerant property is required for better survival and perform well in the field.Hence, the present study was conducted in an attempt to isolate and characterize halotolerant bacteria from saline habitats and evaluate their ability of plant growth promoting activity under saline stress conditions.

MATERIALS AND METHODS

Isolation and screening of microorganisms

Sediment and water sample from salterns situated all over Tamil Nadu, India were collected in sterile plastic containers, brought to the laboratory under low temperature. Halophilic microorganisms were isolated from serially diluted samples by plating on halophilic medium. All the isolated bacterial strains were further stored for screening of plant growth promoting activity.

Phenotypic and Biochemical characterization

The selected bacterial strain was characterized by subjecting to routine microbiological and biochemical tests (Cappuccino and Sherman, 1992) and identified using the key provided by Bergey'sManual of Determinative Bacteriology (1994).

Screening of phosphate solubilizing Bacteria

Modified Pikovskaya agar plates were prepared and test isolates were streaked on plates, then the plates were the incubated at 37°C and observed for 2-7 days. The strains forming zone of clearance were maintained by streaking on nutrient agar slants and stored at 4°C.

Siderophore production

Production of siderophore was detected by standard method (Schwyn andNeiland, 1987) using chrome azurol S (CAS) as indicator. The isolates were spot inoculated at the center of the plate and incubated for 7 days. The change in the colour of the medium around the bacterial spot was an indication of siderophore production.

IAA production

Indole acetic acid (IAA) production was detected as described by Brick *et al*. (1991). Bacterial cultures were grown for 7 days in halophilic medium containing supplement of 20g NaCl at 37°C. Fully grown cultures were centrifuged at 3,000 rpm for 30 min. The supernatant (2 mL) was mixed with 2 drops of orthophosphoric acid and 4 mL of Salkowski reagent (50 mL, 35% of perchloric acid and 1 mL 0.5 M $FeCl_3$solution). Development of pink colour indicated IAA production.

Hydrogen cyanide (HCN) production

HCN production was determined by color change of filter paper (Alstrom and Burns, 1989). Loop-full of bacterial suspension was inoculated on nutrient agar medium (Merck, Germany) containing 4.4 g L^{-1} glycine. Filter papers were soaked in a reagent solution (sodium carbonate 2% and picric acid 0.5%) and placed in the upper lid of Petri dishes. To prevent volatilization, the plates were sealed with parafilm and incubated at 37°C for 7 days. One plate without inoculation of bacterium was considered as control. If HCN was produced, yellow filter papers changed to cream, light brown, dark brown and eventually turn into reddish-brown.

Production of Ammonia

Bacterial isolates were tested for the production of ammonia in peptone water. Freshly grown cultures were inoculated in 10 ml peptone water in each tube and incubated for 7 days at 37°C. Nessler's reagent (0.5 ml) was added in each tube. Development of brown to yellow colour was a positive test for ammonia production (Cappuccino and Sherman,1992).

Seed germination test

Cotton, Jatropa, lady's finger and sorghumseeds collected from Tiruchengode, were soaked in culture for30 min. Ten seeds were sowedin pots and germination of seeds was observed and shoot length and number of leaves was recorded every 5^{th} day.

RESULTS

Isolation and screening of PGPR

Twelve bacterial strains were selected based on distinct morphology on nutrient agar (NA) (20% NaCl) plates. Colonies were selected based on colour, shape, size and abundance. These were screened for salt tolerance and growth in nutrient broth amended with various concentrations of NaCl. Growth was measured after every 24 hours till seventh day. All grew well in 20% NaCl. All the 12 isolates designated as M1 to M12 were screened for the plant growth promoting factors.

Phenotypic and Biochemical characterization of halophilic PGPR

Morphological and physiological characteristics and the responses of the isolates were presented in Table 1**.**

Based on morphological, biochemical tests and 16S rRNA gene homology the test strain was identified as a strain of *Chromohalobactersalexigens*.

Siderophore production

All the halophilic isolates showed maximum growth on CAS medium but none of the changed the colour of CAS agar. This result indicates none of the isolates producedsiderophore on the salinity condition. (Table-2)

IAA production

When the culture supernatant of the halophilic isolates were mixed with 2 ml of Salkowski's reagent and incubated at room temperature in dark 28±2°C for 30 min, it was observed that all the isolates produced IAA but in varying quantities. Development of pink colour in the test was compared with control.(Table-2)

Hydrogen cyanide (HCN) production

HCN production was indicated by change in color of the filter paper to red. It is reported that HCN indirectly influences plant growth promotion. Out of 12 isolates 7 isolates produced the hydrogen cyanide.(Table-2)

Production of Ammonia

Development of yellow-brown color after addition of Nessler's reagent indicates a positive test for ammonia production. All the isolates changed the color after addition of reagent, indicating all the isolates to be positive for ammonia production (Table-2).

Phosphate solubilization

Pikovaskaya's agar with NaCl, when inoculated with the halophilic isolates did not show any clear zone of hydrolysis around the bacterial growth, which indicated their inability to solubilize phosphates (Table-2).

Seed germination

The PGP effect of halophilic isolates on seed germination was evaluated by pre – treating Cotton, Jatropa, lady's finger and sorghumseeds with the isolates. It was noted that halophilic PGPR isolate increased seed germination by 10 to 20% over control and the M5 isolate remarkably influenced the germination of seeds.

Shoot length and number of leaves

The PGPR isolate (M5) significantly induced the height of seedlings (Fig-1 and 2). This result revealed that plant height increased in PGPR treated plants over uninoculated control. The plant shoot length was recorded at 3 different intervals 5th, 10th and 15th day. This result indicates the test isolates have the plant growth promoting activity. The lowest shoot length and number of leaves was noted in uninoculated control.

DISCUSSION

PGPRs are commonly used as inoculants for improving the growth and yield of agricultural crops, however screening for the selection of effective PGPR strains seems to be very critical. High salinity is one of the most common environmental stress factor that adversely affect plant productivity by retarding the plant growth and development. To promote plant growth under saline condition, direct use of salt-tolerant bacteria has drawn considerable research interest both in industry and in academics. In the present study, a large number of halotolerant bacteria were isolated, and screened for their tolerance levels of NaCl. Finkel and Kolter, (1999) reported that all the isolates at higher NaCl concentrations grew with long stationary phase. This could be due to the synthesis of protective factors and adaptation of current environmental conditions.

IAA, a member of the group of phytohormones, is generally considered to be the most important native auxin. IAA may function as important signal molecule in the regulation of plant development. Of twelve isolates, all isolates showed positive for IAA production. Our results were supported by Upadhyay*et al*. (2009)they found that only 18% (24 out of 130) of strains isolated from wheat

rhizosphere in soils of Varanasi, were found tolerant to 8% of NaCl, while maintaining PGP activities. It has been reported that IAA production by PGPR can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mirza*et al*., 2001). Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1992).

Phosphorus is one of the major nutrients, second only to nitrogen in requirement for plants. Most of phosphorus in soil is present in the form of insoluble phosphates and cannot be utilized by the plants (Pradhan and Sukla, 2006). The ability of bacteria to solubilize mineral phosphates has been of interest to agricultural microbiologists as it can enhance the availability of phosphorus and iron for plant growth. Ashrafuzzaman*et al*. (2009) showed that phosphate were not solubilized by all the seven *Bacillus* isolates, isolated from rice rhizosphere and Wahyudi*et al*. (2011) showed that one out of twelve isolates of *Bacillus* from soybean rhizosphere were not able to solubilize the phosphorus.

Another important trait of PGPR, that may indirectly influence the plant growth, is the production of ammonia. Our results showed all the isolates were produce ammonia. Ammonia production by the PGPB helps influence plant growth indirectly.

Siderophore production of a particular organism has been studied with CAS medium supplemented with amino acids, which have suggested that siderophore production is substrate dependent. From our results, none of the isolates showed positive for siderophore production and phosphate solubilization. Incontrast Ramados*set al*.(2013) reported five high salt tolerating bacteria with PGP activities. SL3 and J8W had multiple PGP activities (IAA and siderophore positive), however, SL32 and PU62 showed only siderophore and P-solubilization activities respectively.

Hydrogen cyanide (HCN) is an important attribute of PGPR which influences plant growth indirectly and strengthen the host disease resistance mechanism (Schipper*set al*., 1990). In the present study, seven isolates were found to produce HCN.Other report suggests that HCN has antimicrobial activity and effectively control the growth of plant pathogenic fungus.

Shoot length and number of leaves of culture inoculated plants possessed higher number of leaves on comparison to control in the present study.Our study was highly supported by Hala*et al* 2011, they reported increasing concentration of bacteria supernatant gave significant production in number of leaves/plant as the highest production was obtained by using 30mg/l which gave 3.4 and 3.7leaves/plant in the 1 and 2 seasons, respectively.

Several studies have been performed with most focusing on the effect of different types of bacteria on the growth parameters. Sheng (2005) on stated that *Bacillus edaphicus* NBT strain increased cotton plant height, Medina *et al.* (2007) on *Alpiniapurpurata*and Mafia *et al.* (2009) on *Eucalyptus globules*. They found that plant height and number of leaves were increased with *Azospirillumbrasilense*, *Azotobacterchroococcum*and *Bacillus megaterium*and *subtilis*, recently Sandeep*et al.* (2011) on *Amaranthusgangetcus*, proved that *Azotobacterchroococcum*raised the plant height, root length and leaves number.

Table 1: Biochemical characters of the test isolates

Test/ Isolate No	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
Gram reaction	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve	+ ve
Shape	Cocci	Rods	Cocci	Rods	Rods	Rods	Rods	Cocci	Cocci	Rods	Cocci	Rods
Spore appreance	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab
Oxidase	+ ve	+ ve	+ ve	-ve	+ ve	-ve	-ve	-ve	-ve	+ ve	-ve	-ve
Catalase	-ve	+ ve	+ ve	-ve	+ ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Glucose	-ve	-ve	-ve	+ ve	+ ve	-ve	+ ve	-ve	+ ve	-ve	+ ve	+ ve
Arabinose	-ve	-ve	-ve	-ve	+ ve	-ve	-ve	-ve	-ve	-ve	+ ve	+ ve

+ve Positive reaction
-ve Negative reaction
Ab absent

Table 2: Growth hormone studies of the test isolates

Isolates no/Test	IAA	Siderophore	HCN	Ammonia	Phosphate solubilization
M1	+ve	-ve	+ve	+ve	-ve
M2	+ve	-ve	+ve	+ve	-ve
M3	+ve	-ve	-ve	+ve	-ve
M4	+ve	-ve	+ve	+ve	-ve
M5	+ve	-ve	+ve	+ve	-ve
M6	+ve	-ve	+ve	+ve	-ve
M7	+ve	-ve	+ve	+ve	-ve
M8	+ve	-ve	-ve	+ve	-ve
M9	+ve	-ve	-ve	+ve	-ve
M10	+ve	-ve	-ve	+ve	-ve
M11	+ve	-ve	-ve	+ve	-ve
M12	+ve	-ve	+ve	+ve	-ve

+ve Positive
-ve Negative

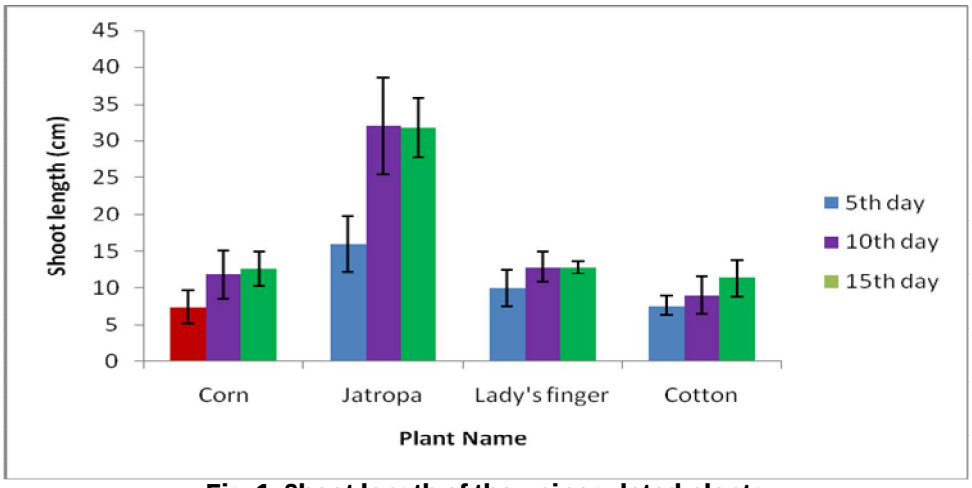

Fig. 1: Shoot length of the uninoculated plants

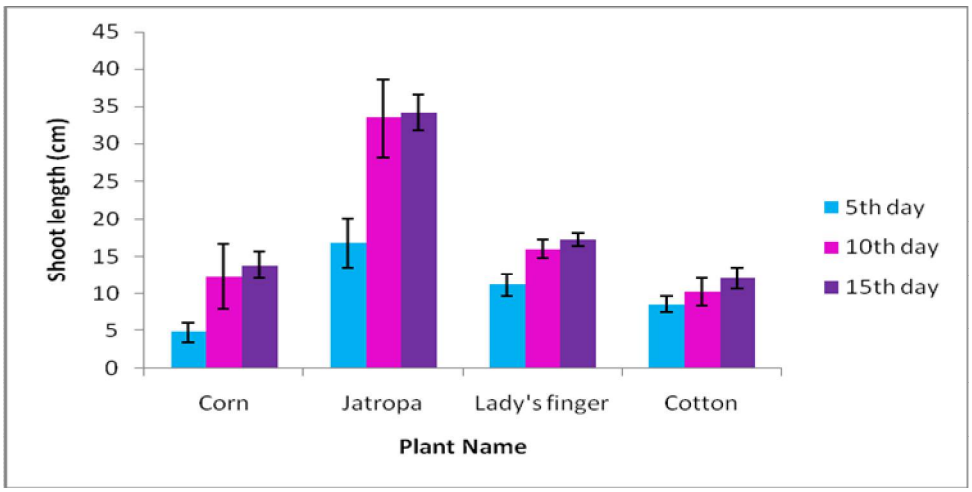

Fig. 2: Shoot length of the inoculated plants

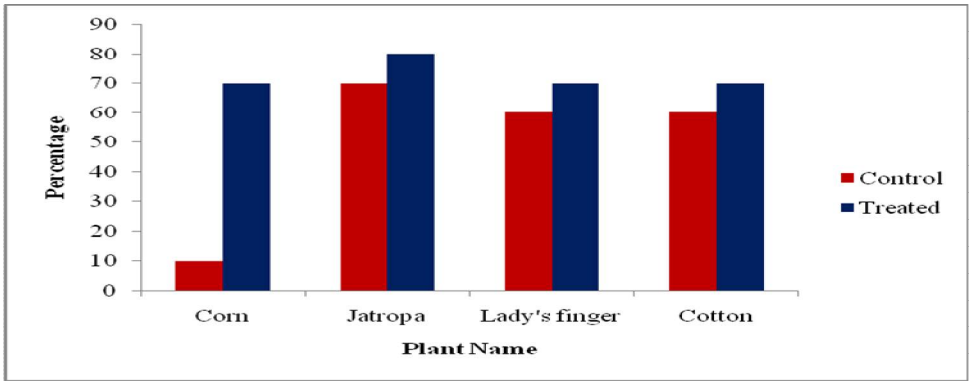

Fig. 3: Seed germination percentage of control and treated seeds

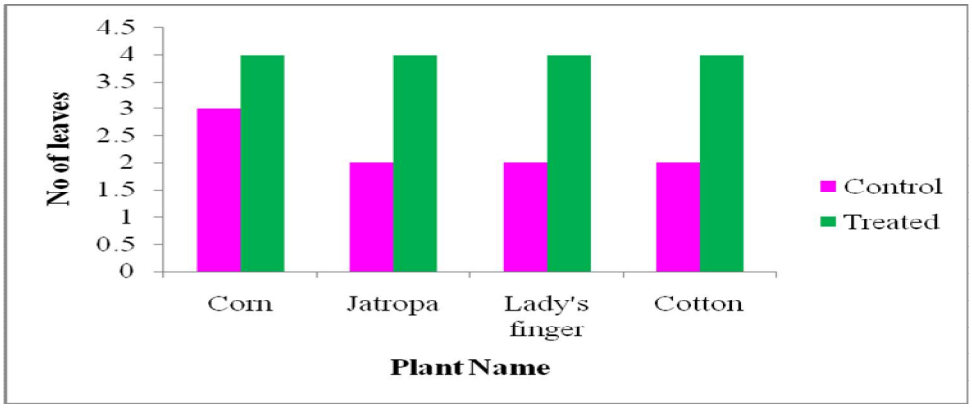

Fig. 4 : Number of leaves in control and treated plants

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