International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Effect of Halobacterium in Promoting the Plant Growth

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Abstract: ***Fifteen Halobacterial strains were isolated from soil samples of various salterns in Tamilnadu, India, and identified an isolate which produced the plant growth promoting hormone Indole Acetic Acid (IAA), and in addition tested the ability of the isolate whether it could solubilize phosphate. The effectiveness of the isolate in plants seed germination and growth also analyzed crops like Cotton, Tomato, Lady's finger and Maize etc were used. The isolate was identified as Chromohalobacter salexigens based on morphological, biochemical reactions and 16s rRNA sequence. Further the optimal growth conditions such as pH, NaCl concentrations and temperature were studied for the effective isolation of the isolate.***

Keywords: Halobacteria, Plant Growth Promoting Rhizosphere Bacteria, IAA, Chromohalobacter salexigens, Optimization

1. Introduction

Many microorganisms possess the ability to survive under harsh conditions and is being intensively studied for better understanding their various applications. 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. Salinity is one major limiting factor to plant growth and crop productivity [1]. High salinity affects plant growth through osmotic effects, toxicity of salt ions, changes in physical and chemical properties of soil, etc. The screening of salt tolerant lines/cultivars has been attempted by many researchers on various plant species at seedling growth stage [2]. Water availability and salt stress are the predominating that impair coleoptiles growth, affecting seedling establishment in the field [3].

Microorganisms that colonize the rhizosphere, inhabit plant roots and exert a positive effect to plants are termed Plant Growth Promoting Rhizobacteria (PGPR). The use of microorganisms with the aim of improving nutrients availability for plants is an important practice and necessary for agriculture [4] Direct promotion of growth by PGPR occurs when the rhizobacteria produce metabolites that promote plant growth such as auxins, cytokinins and gibberellins [5,6,7] as well as through the solubilization of phosphate minerals. 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 [8].

Application of plant growth promoting halophilic bacteria (PGPB) in salt-affected soil is being developed for better survival and performance in the field. Hence, the present study was conducted in an attempt to isolate and characterize halotolerant bacteria from saline habitats, optimize their growth characters and evaluate their ability of plant growth promoting activity under saline stress conditions.

2. Materials and Methods

Enrichment and Isolation of microorganisms

Soil sample from salterns situated of ECR, Chennai and Tuticorin, Tamil Nadu, India were collected in sterile plastic containers. 10g of the collected soil sample was inoculated in 100ml of Halophilic broth medium and incubated at room temperature for 5 – 7 days in shaker for enrichment. The enriched soil samples were inoculated on halophilic agar plates and incubated at room temperature for 3 – 5 days for development of isolated colonies. All the isolated bacterial strains were screened for production of plant growth promoting substance like Indole acetic acid.

Screening of production of plant growth promoting substances by Halobacterium Indole Acetic Acid (IAA) production [9]

Bacterial cultures were incubated in dark on orbital shaker at 200 rpm for 72 h in halophilic broth medium supplemented with 50 mg/l of L-Tryptophan. The broth cultures were centrifuged at 3,000 rpm for 30 mins. 2 ml of the individual supernatant was mixed with 2 drops of orthophosphoric acid and 4 ml of Salkowski's reagent (50 ml 35% of perchloric acid and 1 ml 0.5 M $FeCl_3$ solution) and incubated in dark for 30mins. Development of pink colour indicated IAA production.

Phosphate solubilization

The isolates were streaked on Modified Pikovskaya agar plates and incubated at 37°C for 2-7 days. The Phosphate Solubilising Bacteria (PSB) show clear zones around the bacterial colony. The strains forming zone of clearance were maintained by streaking on nutrient agar slants and stored at 4°C.

Morphological and Biochemical identification

The selected bacterial strain was characterized by subjecting to routine morphological and biochemical tests and identified [10,11].

Molecular characterization
Bacterial Genomic DNA was isolated by using the InstaGeneTM Matrix Genomic DNA isolation kit. The gene fragment was amplified with 27F/1492R primer. The amplified PCR product was purified and sequenced using 518F/800R primer.

Seed germination test
Cotton, tomato, lady's finger and corn seeds collected from local agricultural centres. Ten seeds were surface sterilized with hydrogen peroxide solution for 1 – 2 mins and then the treated seeds were soaked in culture (10^8 cfu/ml) of the selected isolate for 30 min. The seeds were placed in sterile petri dishes that had filter paper moistened with 10 – 50mM concentration of NaCl. Germination of seeds was observed and shoot length and root length was recorded every 5^{th}, 10^{th} and 15^{th} day.

Optimization of growth parameters (pH, Temperature, NaCl Concentration)
The isolate SSB 12 was inoculated in Halophilic agar medium with various NaCl concentrations of 10, 15, 20 and 25% and incubated at room temperature for 7 days and observed for growth. The optimal pH for growth of the isolate was studied by inoculating the strain in the medium with pH variation (5, 9, and 11) and incubated at room temperature for 7 days and observed for growth. The effective growth temperature was estimated by inoculating the effective strain in halophilic medium and incubating the inoculated plates at 25°C, 37°C and 50°C and observing for growth.

3. Results

Enrichment and Isolation of microorganisms
Fifteen Halobacterial strains (SSB1 – SSB15) were isolated from soil samples of various salterns in Tamilnadu, India after enrichment and isolation in Halophilic medium on 5 – 7 days incubation.

IAA production and phosphate solubilization
When the culture supernatant of all 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, only one isolate – SSB12, was found to produce IAA. Development of pink colour in the test was compared with control. All the isolates had no ability to solubilize phosphates (Table-2)

Phenotypic, Biochemical and Molecular characterization of halophilic PGPR
Morphology and physiological characteristics of SSB – 12 is presented in Table 1**.** The isolate is a Gram negative rod, non – sporing and non – motile. Molecular analysis based on 16S rRNA gene homology of 1429bp partial sequence, the test strain was identified as a strain of *Chromohalobacter salexigens*

Seed germination
The treated seeds were allowed to germinate in Petri plates at room temperature. The growth was measured as shoot length and seed germination percentage at 10^{th}, 15^{th}, 20^{th} and 25^{th} day and recorded (Fig 1, 2, 3, 4, and 5). The shoot length of treated plants were found to be longer than un-inoculated with corn showing maximum growth and cotton with minimum growth. The root length of treated plants was more or less equal to that of control but yet a bit longer. The overall growth percentage was more than 5% on comparing to control.

Optimization of growth parameters
Optimization conditions like pH, temperature and NaCl were tested for effective strain. The strain was found to resist upto 25% NaCl with optimal growth at 20%. It was able to grow at all the pH range with 9 being the optimal and room temperature being the effective and optimal growth temperature.

4. Discussion

Plant Growth Promoting Rhizosphere bacteria is group of non – pathogenic soil rhizosphere bacteria that play a beneficial role in plant health and nutrition. PGPRs are commonly used as inoculants for improving the growth and yield of agricultural crops. The benefit may be in plant growth, supplementing plant nutrition or providing plant growth regulators. However screening for the selection of effective PGPR strains seems to be very critical [12].
High salinity is one of the most common environmental stress factors 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, 15 halotolerant bacteria were isolated, and screened for their tolerance levels of NaCl. 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 [13].

Present investigation was supported by the result that found 18% (24 out of 130) of strains isolated from wheat rhizosphere soils of Varanasi, were found tolerant to 8% of NaCl, while maintaining PGP activities like IAA production and phosphate solubilisation [14]. 5 root tip colonizer bacteria were isolated from rhizosphere of wheat grown in saline soils. They were tested and found positive for salt tolerance at 5% NaCl and IAA production under saline conditions [15].

Eighty four halotolerant bacterial strains were isolated from saline habitat and characterized the growth at different NaCl concentrations (5 – 25%). They reported that 5 strains were able to grow effectively at 20% NaCl. They inoculated wheat seedling under slat stress with the isolated strains and observed increased root length upto 71.7% when compared with control [16].

Several studies have been performed with most focusing on the effect of different types of bacteria on the growth parameters. *Bacillus edaphicus* NBT strain increased cotton plant height,[17] *Alpinia purpurata* [18] and on *Eucalyptus globules* [19]. They found that plant height and number of leaves were increased with *Azospirillum brasilense, Azotobacter chroococcum* and *Bacillus megaterium* and *subtilis*, recently. *Azotobacter chroococcum* raised the plant

height, root length and leaves number on *Amaranthus gangetcus* [20].

5. Conclusion

The overall improvement in seedling vigour through a significant increase in various physiological parameters suggests that these strains have a plant-growth promoting ability on tested seedlings under saline conditions and hence could be used for seed inoculation for better establishment of seedlings in such environment. The plants with enhanced seedling vigour can help in better establishment of plant growth in saline soils. This study show that these isolates having best characteristics of plant growth promoting potential under soil condition that help in the seed germination, root and shoot length promotion and also increase the biomass of the tested plants.

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Table 1: Morphology and physiological characteristics of SSB – 12

Test/ Isolate No	*SSB12*
Gram reaction	- ve
Shape	Rods
Spore appreance	Ab
Oxidase	-ve
Catalase	+ve
Glucose	+ ve
Arabinose	+ ve
Motility	-ve

+ve Positive reaction
-ve Negative reaction
Ab absent

Table 2: Growth hormone studies of the test isolates

Isolates no/Test	IAA	Phosphate solubilization
SSB1	-ve	-ve
SSB2	-ve	-ve
SSB3	-ve	-ve
SSB4	-ve	-ve
SSB5	-ve	-ve
SSB6	-ve	-ve
SSB7	-ve	-ve
SSB8	-ve	-ve
SSB9	-ve	-ve
SSB10	-ve	-ve
SSB11	-ve	-ve
SSB12	**+ve**	**-ve**
SSB13	-ve	-ve
SSB14	-ve	-ve
SSB15	-ve	-ve

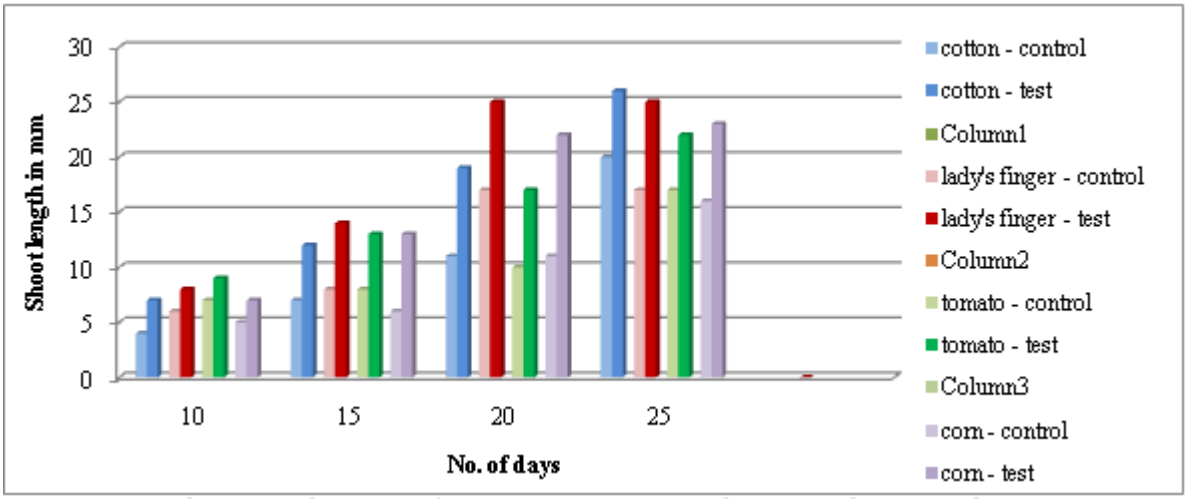


Figure 1: Shoot length of the uninoculated and inoculated plants

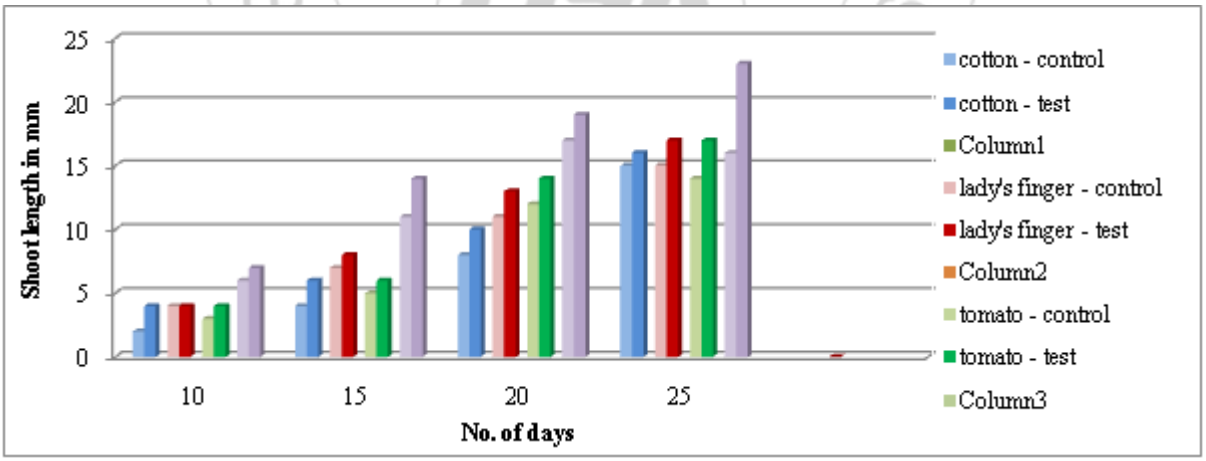


Figure 2: Root length of the uninoculated and inoculated plants

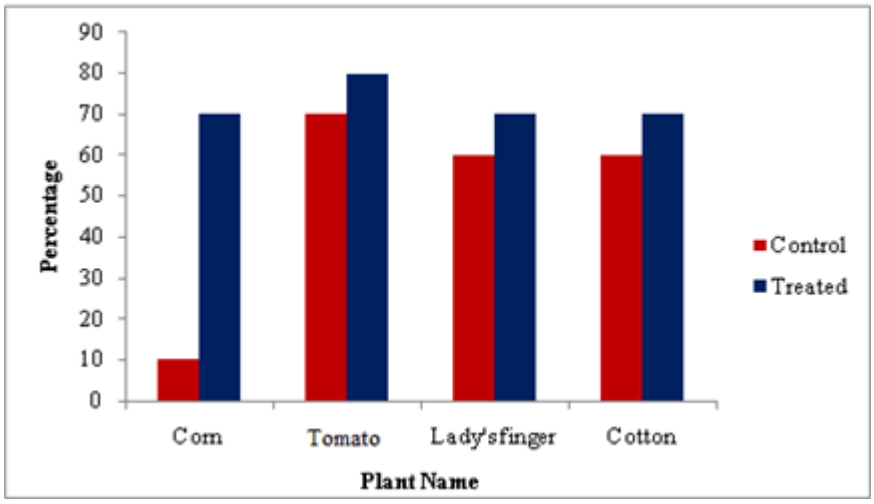


Figure 3: Seed germination percentage of control and treated seeds

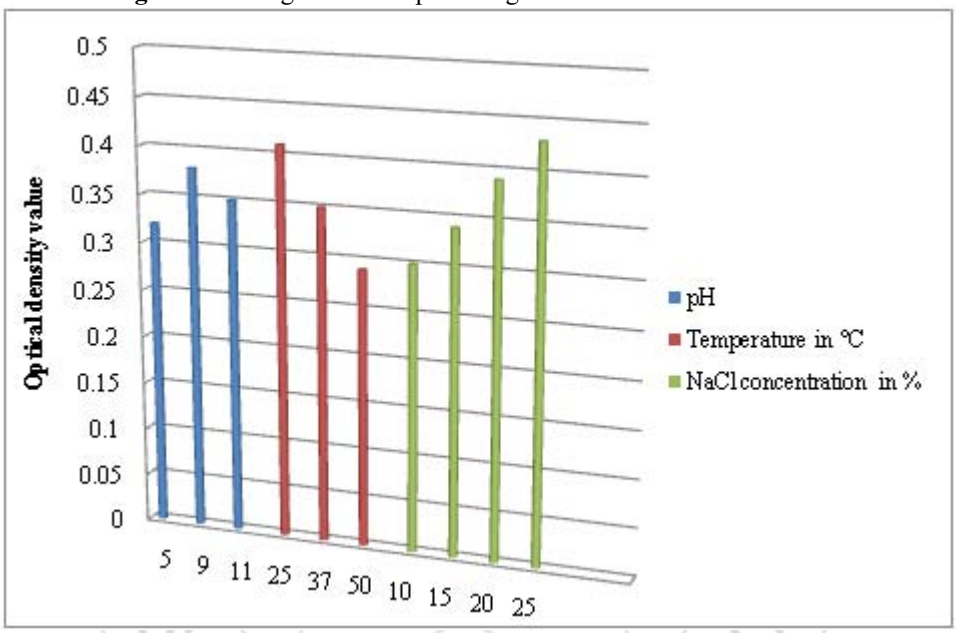
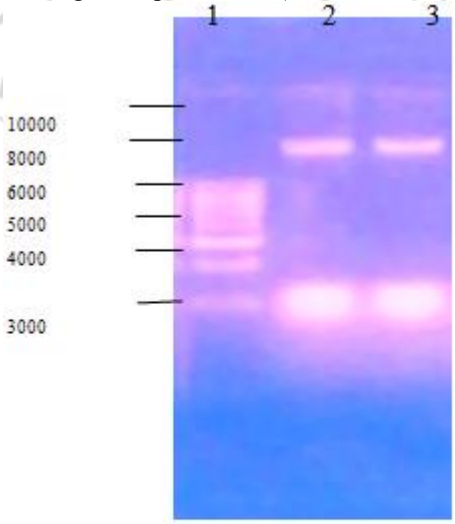


Figure 4: Optimization of growth parameters (NaCl concentration, pH and temperature)



+ve Positive,
-ve Negative

0.8% Agarose gel
Lane-1: 1Kbs lader – DNA marker
Lane-2 & 3: Sample DNA Extracted from *Chromohalobacterium* sp.

Plate 1: Molecular characterization of *Chromohalobacter salexigens*