Screening and biochemical characterisation of salt tolerant guard cell and rhizosphere bacteria from rice

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Abstract

One of the main abiotic stresses that have a significant impact on plant growth is soil salinity. High salinity has an impact on the agricultural productivity of more than 20% of cultivated land worldwide. Rice growth and productivity are significantly impacted by soil salinity. The negative effects of salinity are thought to be mitigated by specific bacteria that thrive in salt-affected soils, and these bacteria are also thought to be able to promote plant growth. Nevertheless, it is believed that some bacteria living in salt-affected soils might decrease the negative effects of salinity and can aid in the growth and development of plants. Ninety-one bacteria isolated, sixty-three Rhizosphere bacteria and twenty-eight guard cell endophytes were subjected to different NaCl concentrations and the bacterial isolates which were able to survive at 10% NaCl concentrations were selected for further study. These halotolerant bacterial isolates were subjected to biochemical characterisation. These halotolerant bacterial isolates can be exploited for agricultural improvement in saline areas and further studied for plant growth promotion.

Keywords: Salinity, Bacteria, Rhizosphere, Guard cell

1. Introduction

One significant barrier to plant growth and crop productivity is salinity [1]. Because of insufficient irrigation water, excessive fertilisation, and desertification processes, cultivated soils around the world are becoming progressively salinized. More than 800 million hectares of land worldwide are affected by levels of salt that could significantly lower crop output [2]. Cultivating salt-resistant cultivars, draining excess soluble salts from upper to lower soil levels, flushing soils with soil crusts at the surface, and harvesting aerial plants that can accumulate salt are all methods for minimising salt stress [3]. An alternative to reducing salt stress is to inoculate plant growth-promoting bacteria (PGPB) into crop seeds and seedlings. When considering crop production losses resulting from the severity of abiotic stresses, particularly salt, the stress tolerance offered by microbial inoculants becomes increasingly significant. The positive effects of PGPB under salinity have been linked to enhanced stomatal conductance, hydraulic conductance, osmolyte accumulation, sequestering harmful Na^+ ions, and photosynthetic activity [4]. To balance the osmotic pressure inside the cytoplasm with the surrounding medium, halotolerant bacteria keep their ionic concentrations at a low level by synthesising compatible solutes. They can adjust to changes in the saline environment, such as saline soils, owing to their internal environment maintenance mechanisms and the characteristics of the cytoplasmic membrane [5]. These halotolerant bacteria which can promote plant growth can be isolated from saline zones and used for agricultural purposes.

2. Materials and methods

2.1 Screening of bacterial isolates against NaCl concentration

Rhizosphere bacteria and guard cell endophytes were isolated from three different rice ecosystems *i.e.,* Conventional, SRI and Aerobic planting systems cultivated in three different districts of salt-affected areas of Tamil Nadu, India. Tryptic soy broth was prepared with different NaCl concentrations (5, 10, 15, 20 and 25% w/v). Isolated twenty-eight guard cell bacteria and sixty-three rhizosphere bacteria were inoculated in Tryptic soy broth containing different salt concentrations and incubated at 28ºC for 48 h. The growth was measured in terms of optical density at 600 nm in a spectrophotometer (M/s. Shimadzu, Japan) at 24 h and 48 h. The colonies which were able to proliferate under varying concentrations of NaCl in the liquid tryptic soy medium were considered as halotolerant bacteria. Fourteen halotolerant bacteria able to survive in 10% NaCl concentration were subjected to biochemical characterization.

2.2 Biochemical characterization of bacterial isolates

2.2.1 Gram staining

For Gram staining, 24 h old culture was used. 1-2 drops of halotolerant bacterial suspension were placed on the grease-free glass slides, air dried and heat fixed by passing over the flame. Initially, the heat-fixed smear was stained by adding crystal violet dye as the primary stain over the smear and allowed to stain for 60 s and rinsed with running tap water. Smear was then flooded with Lugol's Iodine solution for 30 s and washed under running tap water. Then the smear was decolourized with 70% ethanol for 15 s and rinsed with water. To counterstain, safranin was added and allowed to stain for 30 s and finally rinsed with water, air dried and observed under the light microscope. Gram-positive cells appeared purple whereas gram-negative bacteria appeared pink or red [6].

2.2.2 Indole production test

The halotolerant bacterial isolates were inoculated in peptone water broth [7] and incubated at 28±2°C for 48-96 h. After incubation 0.5 ml of Kovac’s reagent was added and shaken. The development of pink or red colour in the alcohol layer indicated a positive reaction [8].

2.2.3 Methyl red test

The methyl red test was performed by inoculating the isolates in 5 ml glucose phosphate broth [7] and incubating them at 28±2°C for 2 to 5 days. As growth occurs, five drops of 0.04 % solution of alcoholic methyl red was added and observed for bright red colour formation which indicated a positive result or yellow colour indicated a negative result [9].

2.2.4 Voges- Proskauer test

The test bacteria were inoculated in 5 ml glucose phosphate broth [7] and incubated at 28±2°C for 48 h. As growth occurred, 1ml of potassium hydroxide containing 0.3 % creatine and 3ml of α-naphthol solution were added. The positive reaction was indicated by the development of pink colour within 2-5 min [9].

2.2.5 Citrate utilization test

The citrate utilization test was performed by streaking the halotolerant bacterial isolates on Simmon’s citrate agar slants [7]. The slants were incubated at 28±2°C for 48 h and observed for the colour change in medium from green to blue which indicated a positive result [10].

2.2.6 Urease test

Halotolerant bacterial isolates were streaked on urea agar slants. Slants were incubated at 28±2°C overnight and observed for the development of purple pink colour which indicated a positive urease test [11].

2.2.7 Nitrate reduction test

The nitrate reductase activity of the isolates was tested by inoculating the organism in 5 ml nitrate broth. Broth incubated at 28±2°C for 96 h and after incubation one ml of α-napthylamine reagent and one ml of sulphanilamide reagent were added. Red colour development within a few minutes indicated the presence of nitrate, a positive reaction [8].

2.2.8 Catalase test

The halotolerant bacterial isolates were streaked on the nutrient agar plates and a drop of three percent H_2O_2 was placed on colonies of nutrient agar. Effervescence indicated the catalase-positive reaction [9].

3. Results and Discussion

3.1 Growth of bacterial isolates against NaCl concentration

Growing salinization of soil and groundwater resources is a result of cultivation practices, particularly poor irrigation management [12]. Crop production and yield are impacted by salinity because salt stress has a negative impact on plant growth [13]. However, the amount of growth suppression was reduced when the plants were inoculated with plant growth-promoting bacteria, and the treated plants responded to abiotic stress conditions with greater growth and dry weight than non-inoculated control plants; this is supported by Li, et al. [8] and Kohler, et al. [14].

Leaf samples and rhizosphere soil samples were collected from conventional, SRI and aerobic rice planting systems in salt-affected areas. A total of twenty-eight and sixty-three bacterial isolates were selected based on colour, shape, size and abundance from guard cell protoplast and rhizosphere soil, respectively. These ninety-one bacterial isolates were subjected to growth with different salt concentrations (5, 10, 15, 20 and 25% w/v NaCl) in tryptic soy broth. From the isolated guard cell bacteria, sixteen and five isolates were able to survive in 5% and 10% NaCl concentrations, respectively. From the isolated rhizosphere bacteria forty-one, nine and five isolates were able to survive in 5%, 10% and 15% NaCl concentrations, respectively. None of the isolates was able to survive in >15% NaCl concentrations (Fig. 1). Fourteen bacterial isolates (five guard cell bacteria and nine rhizosphere bacteria) were selected for further experiments. Only 18% (24 out of 130) of strains isolated from wheat rhizosphere in Varanasi soils were found to be tolerant to 8% of NaCl while sustaining Plant Growth Promoting (PGP) activities [15]. Siddikee, et al. [16] observed that several halotolerant bacteria could resist high salt concentrations (1.75 M NaCl) and were able to promote plant development in the presence of salt concentrations that inhibited plant growth. Jha and Subramanian [17] reported that halotolerant bacteria isolated from the rhizosphere of rice were able to promote the growth of rice even under saline conditions.

3.2 Biochemical characterization of halotolerant bacteria

Among the isolates RSH7, RSH8 and RSH10 were gram-positive and the remaining isolates were gram-negative. Six isolates were positive for the indole production test and the remaining were negative. All the isolates showed negative results for the methyl red test except RSH6 and vice versa for the Voges Proskauer test. All the isolates tested positive for the urease and nitrate reduction tests except RSH14 and RSH9, respectively. Among the fourteen isolates, ten isolates showed positive result for the catalase test. Nine isolates were able to change the colour of the medium from blue to green which indicated positive for the citrate utilization test (Table 1).

4. Conclusion

In conclusion, this work offers a futuristic perspective on soil microorganisms and plant guard cell endophytes on adaption and use as a natural fertiliser, where soil salinity is a significant problem. The bacterial isolates discussed above can live in high-salt environments and should be further examined for activities that promote plant growth. These halotolerant isolates could be the possible factor to revive the fertility of agricultural soil affected by salinity.

CONFLICT OF INTEREST

The authors declare that no conflict of interest, financial or otherwise.

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Table 1. Biochemical characteristics of selected halotolerant bacterial isolates

Sl. No	Isolate No.	Gram staining	Indole test	Methyl red test	Voges Proskauer test	Urease test	Nitrate reduction test	Catalase test	Citrate utilization test
1	GCH1	-	-	-	+	+	+	+	+
2	GCH2	-	+	-	+	+	+	-	+
3	GCH3	-	+	-	+	+	+	-	+
4	GCH4	-	+	-	+	+	+	+	+
5	GCH5	-	+	-	+	+	+	+	+
6	RSH6	-	+	+	-	+	+	+	+
7	RSH7	+	-	-	+	+	+	-	-
8	RSH8	+	+	-	+	+	+	+	-
9	RSH9	-	-	-	+	+	-	+	-
10	RSH10	+	-	-	+	+	+	+	+
11	RSH11	-	-	-	+	+	+	+	-
12	RSH12	-	-	-	+	+	+	+	+
13	RSH13	-	-	-	+	+	+	+	-
14	RSH14	-	-	-	+	-	+	-	+

‘+’ – Positive, ‘-’ – Negative.

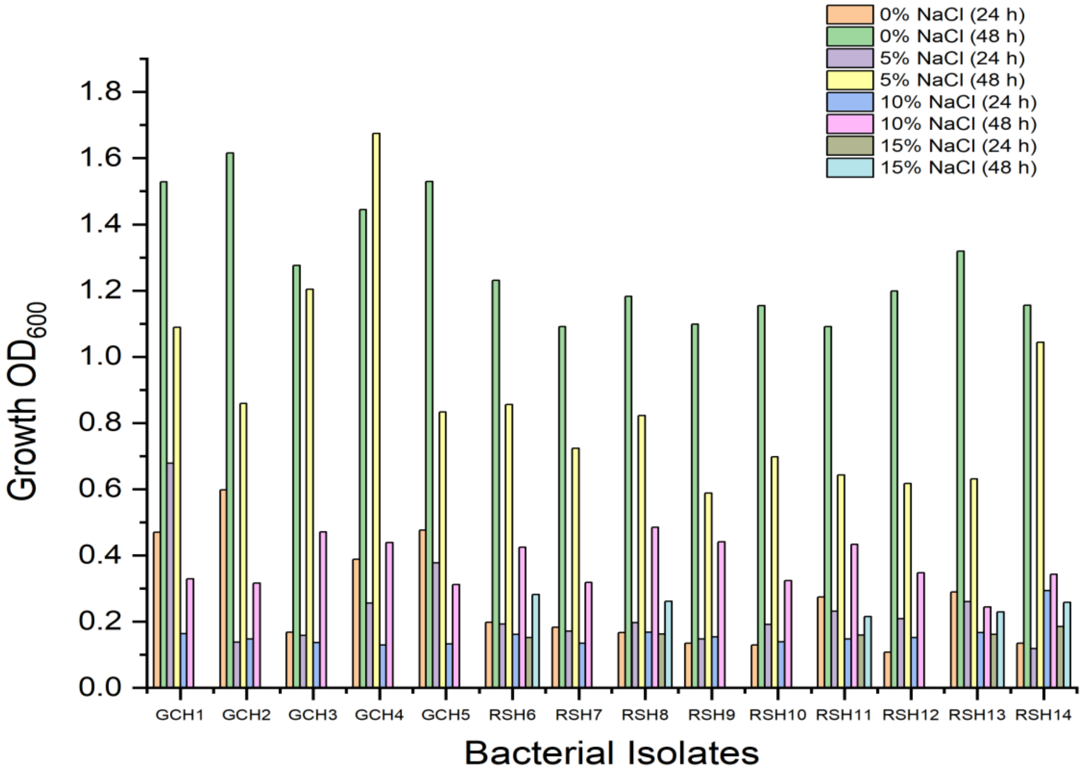

Figure 1. Growth of bacteria isolates at different NaCl concentrations (5%, 10% and 15% NaCl)