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Efficient role of rice associated Plant growth promoting bacteria

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ABSTRACT

Rice-associated plant growth-promoting bacteria (PGPB) have a major role in promoting growth and development using a variety of mechanisms. In this study, we isolated bacteria from drought stressed fields to check their effects on rice plants. Bacterial strains were selectively isolated based on their growth on selective media and different biochemical tests. These bacteria were identified by partial sequencing of 16s rDNA. We have found that two isolates, *Brevibacterium* sp. (B4) and *Priestia aryabhattai* (B16) (MZ081422.1), have a magnificent role in the growth of rice plants compared to mock-treated plants after drought stress treatment.

Key words: *PGPB, 16s rDNA, Brevibacterium sp., Priestia aryabhattai*

Introduction

Rice is the staple food of more than 90% of the Asian population, and it is the most cultivated cereal all over the world. Rice contributes around 50% of the calorific value of food consumed in Asian countries, owing to rice production and demand increasing by 1.6–1.8% per year. High-yielding varieties necessitated massive amounts of chemically synthesised fertilizer, resulting in the addition of hazardous elements to the food chain and environment. These effects increase the numbers of diseases in humans and animals as well. So, it is important to know the possible role of bacteria in growth and development of plant. In many studies, the role of plant growth promotion is studied in terms of nitrogen fixation, phosphorus solubilization, and defence promotion (Numan *et al.*, 2018).

In our study, we have isolated bacteria from rice fields in order to study their beneficial effects on rice plants after drought stress treatment. We found that two isolates, *Brevibacterium* sp. (B4) and *Priestia aryabhattai* (B16) (MZ081422.1), have a magnificent role in the growth of rice plants compared to mock-treated plants after drought stress treatment.

Materials and Methods

To isolate the bacteria, the soil sample was collected

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from a drought-stressed field of rice from a nearby root zone from the Bihar Agriculture University soil farm. Soil samples were collected from five different points at a depth of 5–10 cm, all soil samples were then pooled into one composite soil sample. This pooled sample is further used for the isolation of beneficial bacteria. To isolate the sample, 0.05 g of soil was mixed with 100 ml of sterile water for proper mixing vertex for 1 min, and the supernatant was collected after centrifugation at 5000×g for 10 min. The supernatant was re-dissolved in 100 ml of sterile water and serially diluted to 10^{-3}, 10^{-4}, and 10^{-5}. The serially diluted sample was spread with the help of an L-shaped sterile in Luria Bertani (LB)-agar media and incubated at 30 ^{o}C for 18 h in the incubator. Further single colonies from each plate were streaked in fresh LB-agar media for different assays and stored at 4 °C in a refrigerator.

The isolated bacteria were grown in 5 ml of Luria Bertani (LB) broth for overnight at 30 °C and DNA isolation was done by following the protocols of Carpenter *et al.,* 2018. For the identification of bacteria, the extraction of bacterial genomic DNA and 16S rDNA gene PCR amplification process have been taken from Kumari *et al.* (2020).

For morphological identification and characterization, different biochemical tests have been performed, such as Gram staining, Simmons citrate agar test, carbohydrate test, mobility assay, IAA production assay, H_2S production assay using SIM media (SRL), and gelatine hydrolysis test. Some specific tests were checked, such as the Methyl-Red test and the Voges-Proskauer test for species level characterization of bacteria (Brown, 2009).

To test the effect of bacteria on plant growth, Brevibacterium sp. (B4) and Priestia aryabhattai (B16) (MZ081422.1) were inoculated in 100 ml of LB-broth for 18 hours. To check the response of bacteria as plant growth promoting activity, the culture of selected isolates was given to 21-day old rice seedlings planted in pots along with a mock supplemented with LB-broth. In ten rice pots for each treatment, five seedlings were planted, supplemented with each full-grown culture along with a mock to check the beneficial effect of bacteria. Drought stress treatment given to above treated and mock plants by decreasing the water content of pot by 5% (Zu *et al.*, 2017). For testing of critical effects on plants, pot soil was not supplemented with any minerals or fertilisers.

Results and Discussion

To know the bacterial status of a drought-stressed rice field, we isolated 10 bacteria from soil samples. Among eighteen isolates of bacteria, two of them are positive for many biochemical assays, so these two were taken for further study for beneficial bacterial effects on rice plants. To confirm the real identity of bacteria, the 16S rDNA gene, a molecular marker for identification of bacterial species, was amplified from total genomic DNA using 16s rDNA universal primers (Fig. 1). The PCR product was sequenced by outsourcing to Xcelris Genomics Services, Gujarat. The BLASTN (https://blast.ncbi.nlm.nih.gov/Blast) searches showed 96-97% sequence similarity in the cases of bacterial isolate 4 (B4) with *Brevibacterium frigoritolerans* and bacterial isolate 16 (B16) with *Priestia aryabhattai.* The bacterial isolates B4 and B16 were Gram positive, chain cocci and cylindrical-rod shaped, Gram negative, respectively (Fig. 2). Bacterial isolates were non-motile, aerobic, and formed

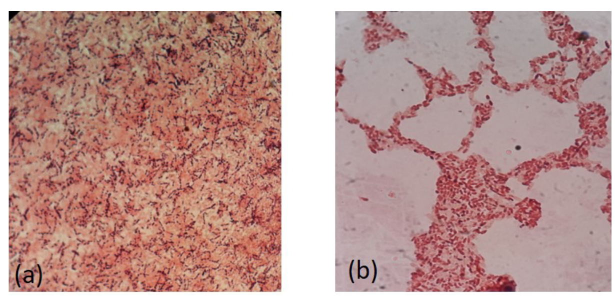

Fig. 2. Gram staining of bacteria (a). *Brevibacterium* sp.(B4) Gram +Ve, Chain cocci (b). *Priestia aryabhattai* (B16), Bacillus, Gram -Ve

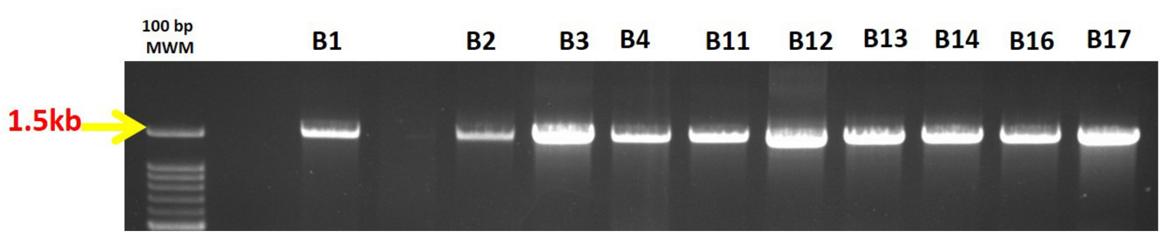

Fig. 1. 16S rDNA PCR of bacterial isolates

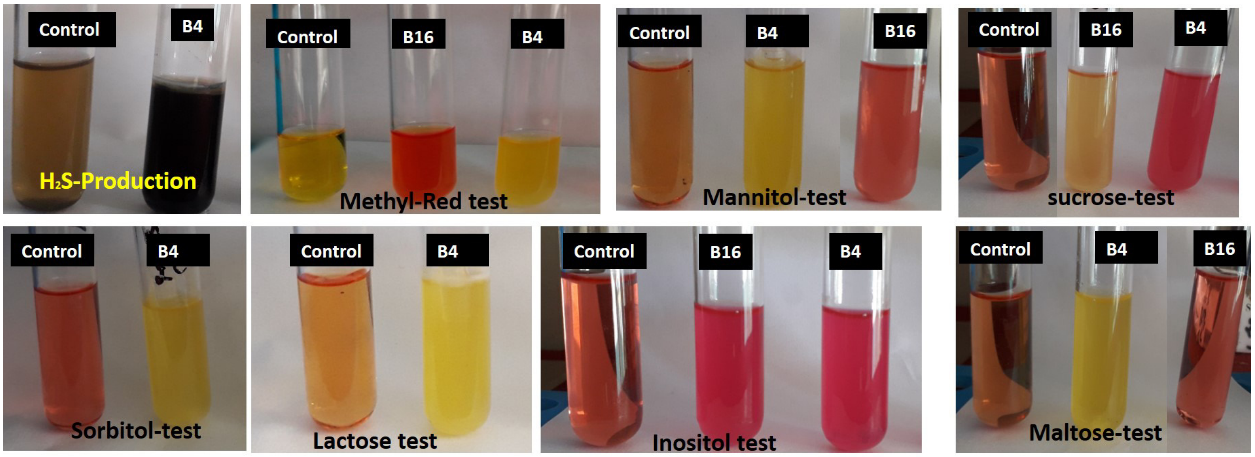

Fig. 3. Different biochemical test. H_2S production, MR-test, Carbohydrate test (Mannitol, sucrose, sorbitol, lactose, inositol, maltose test) was performed to characterise the bacteria

Table 1. List of biochemical tests of bacterial isolates

Biochemical test	*Brevibacterium* sp.	*Priestia aryabhattai* (MZ081422.1)
Gram staining	Gram positive	Gram negative
Shape	Chain cocci	Bacillus
Catalase test	Positive	Positive
Gelatine-hydrolysis test	Negative	Negative
Methyl-Red (MR) test	Negative	Positive
Voges-Proskauer (VP) test	Negative	Negative
Citrate agar test	Negative	Negative
H_2S Production	Positive	Negative
IAA production	Negative	Negative
Mobility	Non-motile	Non-motile
Lactose-test	Positive	Negative
Galactose-test	Negative	Negative
Inositol-test	Strongly inhibited	Strongly inhibited
Sorbitol-test	Positive	Negative
Maltose-test	Positive	Strongly inhibited
Sucrose-test	Strongly inhibited	Positive
Mannitol	Positive	Strongly inhibited

the creamiest, off-white colonies. They have the ability to degrade H_2O_2 to water with the help of the catalase enzyme. Both the isolates were positive and negative for various biochemical assays like gelatine-hydrolysis test, Methyl-Red (MR) test, Voges-Proskaure (VP) test, Citrate agar test, H_2S production, and different carbohydrate assays (Fig. 3, Table 1).

Brevibacterium sp. (B4) and *Priestia aryabhattai* (B16) (MZ081422.1) have more effects on plant growth compared to mock-treated plants after drought stress treatment. *Brevibacterium* sp. (B4) has a greater effect than *Priestia aryabhattai* (B16) among these two isolates. *Brevibacterium* sp. (B4) may solubilise basic nutrients like phosphate and may have the ability to provide nitrogen and potassium, which are needed for proper plant growth. Plant growth promoting bacteria form symbiotic associations between plants and bacteria in which bacteria colonise the internal tissue of plants to provide nutritious effects (Basu *et al.*, 2017). PGPBs are therefore inoculants that promote plant growth, inhibit environmental stress, and can be used for bio-stimulation, biocontrol, and biofertilization. (Numan *et al.*, 2018). *Brevibacterium* sp. has a plant growth promoting effect on wheat and salt and drought stress responses in maize (Meena and Saharan, 2017; Zhang *et al.*, 2019). So, the solubilisation effect of nutrients needs to be critically examined for plant growth promoting characteristics of bacteria.

References

Basu, S., Rabara, R. and Negi, S. 2017. Towards a better greener future - an alternative strategy using biofertilizers. I: Plant growth promoting bacteria. *Plant Gene.* 12 : 43–49.

Brown, A. E. 2009. *Benson's Microbiological Applications Laboratory Manual in General Microbiology.* McGraw-Hill Higher Education, New York.

Carpenter, S, C, D., Mishra, P., Ghoshal, C., Dash, P. and Wang, L. 2018. A strain of an emerging Indian pathotype of *Xanthomonas oryzae* pv. oryzae defeats the rice bacterial blight resistance gene xa13 without inducing a clade III SWEET gene and is nearly identical to a recent Thai isolate. *Front Microb*. 9: 2703.

Kumari, D., Parasad, B, D., Sahni, S. and Ghatak, A. 2020. Identification and Functional Characterization of *Xanthomonas oryzae* pv. oryzae Isolates. *Curr J App Sci Technol*. 39(4): 78-84.

Meena, T.N., Saharan, B.S. 2017. Plant growth promoting traits shown by bacteria Brevibacterium frigrotolerans SMA23 isolated from *Aloe vera* rhizosphere. *Agr Sci Digest*. 37 : 226–231.

Numan, M., Bashir, S., Khan, Y., Mumtaz, R. and Shinwari, Z. 2018. Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. *Microbiol Res*. 209: 21–32.

Zhang, C., Li, X., Yin, L., Liu, C. and Zou, H. 2019. Analysis of the complete genome sequence of Brevibacterium frigoritolerans ZB201705 isolated from drought-and salt-stressed rhizosphere soil of maize. *Ann Microbiol*. 69(13) : 1489-96.

Zu, X., Lu, Y., Wang, Q., Chu, P. and Miao W. 2017. A new method for evaluating the drought tolerance of upland rice cultivars. *The Crop Journal*. 5(6): 488-98.