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Role of Rhizosphere Bacteria Isolated from Wild-type Varieties of Sundarbans to Endure Salt Tolerance

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Abstract

Bangladesh is a low-lying riverine country where a large number of populations live in the saline zone. Salinization is a burning issue around the world that affect the plant growth as well as crop production. Due to soil salinization, a fifth of the world's agricultural land has been spoiled. From the geographical setting, Bangladesh is one of the most vulnerable countries to the impact of various natural hazards and disasters. Sundarbans also experienced such natural hazards and disasters, e.g., tsunamis, floods, and cyclones every year for being its location. Moreover, the study area's salinization level is increasing daily. As a result, the plants in the study area are facing a critical situation due to the rapidly changing saline levels. The study attempted to analyze rhizosphere bacteria isolated from wild-type varieties of Sundarbans to endure salt tolerance. This study was conducted using primary and secondary data. Wild-type variety and soil samples were collected from the Sundarbans. To isolate salt-tolerant bacteria, different concentrations of salt were used. The salt tolerance isolates were used to observe the role of seed germination and plant growth. The isolated salt-tolerant bacteria showed their effects on plant growth and seed germination in saline soil. *Bacillus* and *Acinetobacter* bacterial species were identified using 16S rRNA gene sequencing, which promoted plant growth and seed germination at a high salt level. Since rhizosphere bacteria play an essential role in enduring salt tolerance, it can be a straightforward and eco-friendly strategy to reduce plant salt stress.

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Introduction

The world population is increasing at an alarming rate, but agricultural productivity is not growing at the required rate because the plant faces various stresses (Raza *et al.,* 2019). One fifth of the world's agricultural land has been rendered unusable as a result of soil salinization. An increase in the amount of salt in the ground is caused by flooding, rising sea levels, and a lack of fresh water (Haque, 2006).

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In particular, when farmers irrigate their farms with marginally saline surface water towards the start of the low flow period, salt entry increases soil salinity. In 1997, the Soil Resources Development Institute (SRDI) reported that during the low flow season, soil salt levels varied from 8 to 15 dS/m south of Khulna and Bagerhat cities. Additionally, it is reported that during the low flow seasons in the 1980s, a number of sub-districts south of the Sundarbans—including Kachua, Mollahat, and Fultali—which "were known to be non-saline in the pre-Farakka period"—began to acquire soil salinity (Pooja Shrivastava & Rajesh Kumar, 2015). Therefore, crops cannot grow in the saline zone. According to research by the UN (United Nations), salinization causes the loss of an additional 100 acres of soil every day (Shrivastava and Kumar, 2015). The rising global need for food is threatened by this. Generally, plants face biotic and abiotic stresses consisting of different types of phytopathogen, drought, salt, and cold negatively affect plants and lousy weather (Suzuki, Rivero, Shulaev, Blumwald, & Mittler, 2014). Among these stresses, increasing soil salinity seriously affects plant health and growth, which causes the reduction of agricultural productivity to reduce the saline zone (Machado & Serralheiro, 2017). Scientists reported that salinity decreased the germination rate of some plants (Mbarki *et al.,* 2020). Because it induces water deficit, osmotic stress, stomatal closer, reduction of leaf expansion, and reduced photosynthetic activity because high salt concentrations make it harder for roots to absorb water. Soil salinity increases the reactive oxygen species (ROS) and causes an increase in leaf malondialdehyde (MDA) which is well-recognized for membrane lipid peroxidation (Sakuma *et al.,* 2006). The plant used different strategies to take out the excess salt. The prominent one is the Salt overly sensitive (SOS) pathway (Yamaguchi-Shinozaki & Shinozaki, 1994). Therefore, to cope with soil salinity in agriculture, many strategies have been introduced consisting of salt resistance varieties of different cultivars. One promising way is to useplant growth-promoting rhizobacteria (PGPR) (Shi, Quintero, Pardo, & Zhu, 2002). Reducing soil salinity or enduring the plant at high salt levels can be the solution to salt stress. In this study many wild type plants growing in farmland of low saline zone and not halophyte were found survive in high salt level in Sundarbans. The wild-type plant may have been endured by environmental factors or biological agents. Rhizosphere bacteria can play a role in plant growth (Saeed *et al.,* 2021). If rhizosphere bacteria have the capacity to endure salt tolerance it will help farmers raise crop production very effectively. Inoculation with salt- tolerant plant growth promoting bacteria are now being used for enhancing crop yields, protection and improving soil fertility (Egamberdieva, Wirth, Bellingrath-Kimura, Mishra, & Arora, 2019). This research work was aiming to find bacteria that endure salt tolerance to the plant. Rhizosphere bacteria isolated from wild-type variety of saline-prone regions were assayed for salt tolerance and plant growth promotion. This study could discover an environmental resource to solve the salt stress obstacle and develop crop production.

Objectives

The main objectives of this research were to collect rhizosphere soil from wild-type variety of saline-prone areas and isolate salt-tolerant bacteria. The isolated salt-tolerant bacteria would be used for bio-inoculation with plants in high salt-level soil. The isolated rhizosphere bacteria would be determined using 16S rRNA gene sequencing.

Materials and Methods

Sample collection

Rhizosphere soil was collected from wild-type plant varieties of saline-prone areas of Sundarbans in a

sterilized jar. pH, moisture, and salt level of the surroundings were also recorded. Various parts of the plants were also collected for taxonomical analysis.

Culture of rhizosphere bacteria

Rhizosphere soil was diluted in autoclaved distilled water. And cultured in TSB (Tryptic soy broth) media to obtain rhizosphere bacteria (Nordstedt & Jones, 2020).

Isolation and screening of bacteria for salt-tolerant rhizosphere bacteria

The inoculum of isolated rhizosphere bacteria was cultured on TSA (Tryptic soy agar) media supplemented with 10% NaCl. One gram of soil sample was dissolved in 100 mL of autoclaved distilled water, diluted 10–10,000 fold, and plated on TSA plates supplemented with 10% NaCl and followed the standard methods. To isolate salt-tolerant bacteria, different concentrations of salt were used, and for isolating salt-absorbing bacteria, salt concentration was measured using a TDS (Total dissolved solids) meter. The bacterial culture was isolated and preserved for further investigation. Salt-tolerant bacterial load was recorded as CFU/ml.

Seed germination stimulation trials with salt-tolerant rhizosphere bacteria

The individual bacterial colony was used to evaluate the ability to induce seed germination at a high salt level. Autoclaved compost fertilizer, soil, and coconut peat were utilized because the absence of other microorganisms was ensured before the seed germination trial was started. Wheat seed was used for this experiment. The seed was surface sterilized with ethanol and $HgCl_2$. Seed germination was observed in a sterilized closed seedling tray (Kearl *et al.,* 2019).

Plant growth stimulation trials with salt-tolerant rhizosphere bacteria

After seed germination, the plantlets were transferred to the sterilized jar containing autoclaved soil, compost, and cocopeat. The transfer process was done in an aseptic condition to prevent other microorganisms. Three jars were maintained without salt, with high salt but without bacteria, and high salt media with bacterial culture.

Molecular identification of isolated bacteria

Isolation of genomic DNA

Pure culture of isolated bacteria was used for genomic DNA isolation. A single colony was taken in lysis buffer and incubated in a sonication machine at 4°C for half an hour and centrifuged to discard the cell debris. Genomic DNA was precipitated by Ethanol (Zhang *et al.,* 2005)

PCR, Agarose gel electrophoresis, PCR product purification, and 16S rRNA gene sequencing

Genomic DNA was used for PCR using universal Primer 27F and 1492R. Initial denaturation was done at 96° C for 30 seconds, the annealing temperature was 52°C, and the extension was run at 72°C. The number of the cycle was 35 (Frank *et al.,* 2008). The PCR product was run in Agarose gel using 1% Agarose for 40 minutes. And the expected band around 1.5K was sliced and kept in a fresh tube for DNA extraction from the gel. With the help of the Quick Gel Extraction and PCR Purification Combo, amplified DNA was extracted from the gel. With the help of the Quick Gel Extraction and PCR Purification Combo

kit, amplified DNA was extracted from the gel (Catalog number: K220001, Thermo Fisher Scientific). The purified PCR product was used for 16S rRNA gene sequencing following Sanger methods (Johnson *et al.,* 2019). The sequence was aligned by Codon code aligner software and compared with NCBI (National Center for Biotechnology Information) database.

Phylogenetic analysis

The identified bacteria and other soil bacteria were analyzed for a phylogenetic relationship. The phylogenetic tree was constructed by Phylogeny.fr (http://www.phylogeny.fr) (Dereeper *et al.,* 2008). It is an online tool for phylogenetic analysis that may help users analyze their data in a quick and reliable manner while still adhering to acknowledged standards.

Results and Discussion

Rhizosphere bacteria were isolated from wild-type variety of saline-prone areas of Sundarbans and farmland of Kushtia

In this study, 12 varieties were selected, and rhizosphere soil was collected in sterilized jar. Different parts of the plants were collected for taxonomical analysis. The collected plants for rhizosphere bacteria were identical in both study areas (Fig. 1).

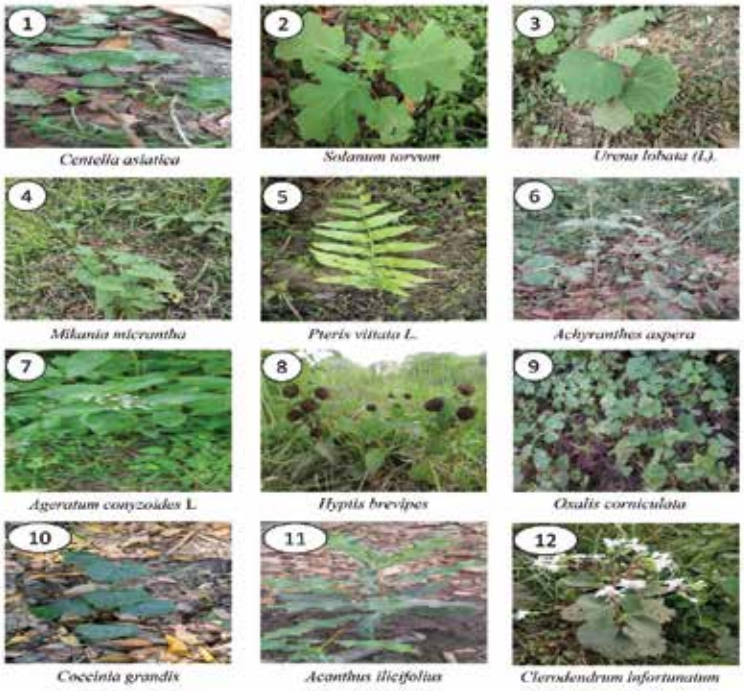

Fig. 1. Wild-type varieties from which rhizosphere bacteria were collected

When rhizosphere soil was collected, the pH, moisture level, and salt level (TDS) were recorded (Table 1). pH levels varied at different places as well as moisture and salt level were also different. But the data did not indicate pH and moisture have any impact on salt levels.

Though the variation in pH level has an effect on some environmental factors, this study did not show a direct relation to the difference in salt level (Fig. 2). Soil pH is equally essential as soil salinity. Salt content and pH were the two important factors in controlling the community composition, but the direct relationship between pH and salt level was not described (Zhao *et al.,* 2018).

Moisture levels were also recorded, and in this study, no relation was found with the salt level of the soil (Fig. 3). Moisture levels can influence salt levels by means of solubilizing salt. Moisture has the ability to solubilize organic matter (Zhu *et al.,* 2020). In this study, no relation was found between moisture level and salt salinity.

Salt-tolerant rhizosphere bacteria were found in abundance in the Sundarbans region

Table 1. pH, moisture, and salt level of the soil from where plant samples were collected

	Sundarbans			Farmland of Kushtia		
Sample number	pH	Moisture (%)	Salt level (ppm)	pH	Moisture (%)	Salt level (ppm)
1	6	40	88.1	5.9	43.75	38
2	4.4	77.5	125.4	6	31.25	54
3	4.5	75	129.3	5.4	40	23
4	4.4	82.5	82	5.2	61.25	43
5	5.3	61.25	183	5.9	37.5	46
6	4.2	75	105.8	5.8	41.25	35
7	4.6	70	131	6	27.5	57
8	6.7	50	157.1	5.5	56.25	22
9	4.6	75	160	5.5	53.75	25
10	5.2	62.5	100	5.3	43.75	31
11	4.6	62.5	134	5.9	40	62
12	3.8	85	147	5.8	37.5	32

The inoculum of rhizosphere bacteria was incubated on TSA media supplemented with 10% NaCl. After 24 hours of incubation, only salt-tolerant bacteria were grown (Fig. 4). In this study, 10% NaCl was used to screen high salt-tolerant rhizosphere bacteria. A higher salt level in the media showed low bacterial growth (Emese *et al.,* 2010).

The microbial load of salt-tolerant bacteria was significantly higher than the bacteria isolated from the rhizosphere soil of the Kushtia region (Fig. 5). After 24 hours of incubation, total colonies were counted, and pure culture was preserved for further study. Due to the high salt concentration in soil, halophile and halotolerant bacteria were present at significant density in rhizosphere soil of wild-type varieties of Sundarbans. These types of bacteria can mitigate the effects of salinity stress (Khan *et al.,* 2021).

Seed germination was stimulated with salt-tolerant rhizosphere bacteria

Seed germination was observed in three groups. Group A was induced with 10% NaCl and incubated with salt-tolerant bacteria, Group B was induced with 10% NaCl but no incubation with salt-tolerant bacteria,

and Group C was inoculated for germination without salt and bacteria (Figure 6). Isolated rhizosphere bacteria of sample 7 and sample 9 showed a positive response to seed germination. At high salt levals,

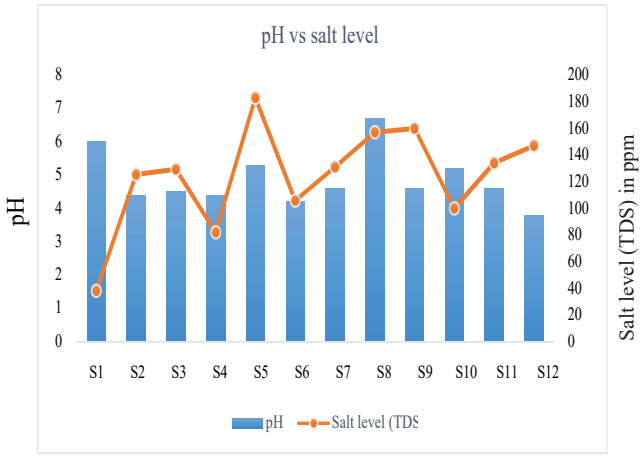

Fig. 2. pH and salt level of the soil from where plant samples were collected in Sundarbans

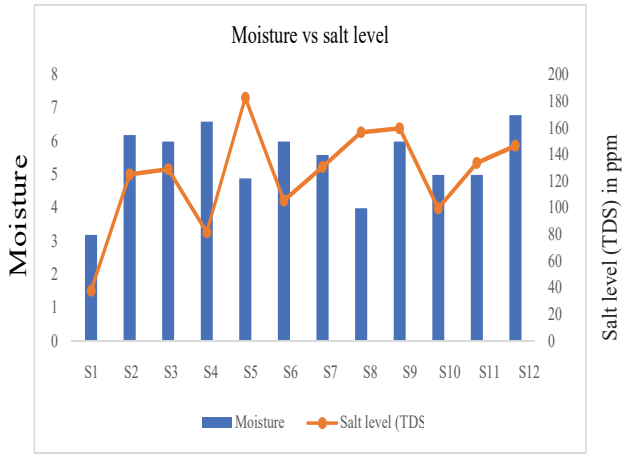

Fig. 3. Moisture and salt level of the soil from where plant samples were collected in Sundarbans

bacteria from sample 7 stimulated seed germination at 70%, whereas without bacterial inoculation, it was only 10% as well as sample 9 stimulation of seed germination at 75%, whereas without bacterial inoculation, that was only 5%. A single colony was isolated to make pure culture from samples 7 and 9, and inoculation was repeated, and the pure culture showed a similar influence on seed germination.

Plant growth was stimulated with salt-tolerant rhizosphere bacteria

The germinated seeds were transferred to a sterilized seedling tray and measured the length after 7 days. Two isolates induced plant growth at high salt levels (Fig. 7). The length of plants incubated with bacteria was increased significantly than the plant growth in high salt levels without inoculation.

Molecular identification of rhizosphere bacteria showing stimulation ability on seed germination and plant growth at a high salt level

Halotolerant bacteria having an influence on seed germination and plant growth were cultured and used for Genomic DNA isolation. The genomic DNA was used for PCR and 16S rRNAgene sequencing (Fig. 8). Bacteria from sample 7 was identified as *Metabacillus indicus*, and sample 9 was identified as *Bacillus amyloliquefaciens.*

The phylogenetic relationship was analyzed with soil bacteria and represented in fig 9. These bacteria showed closely relationship with soil bacteria.

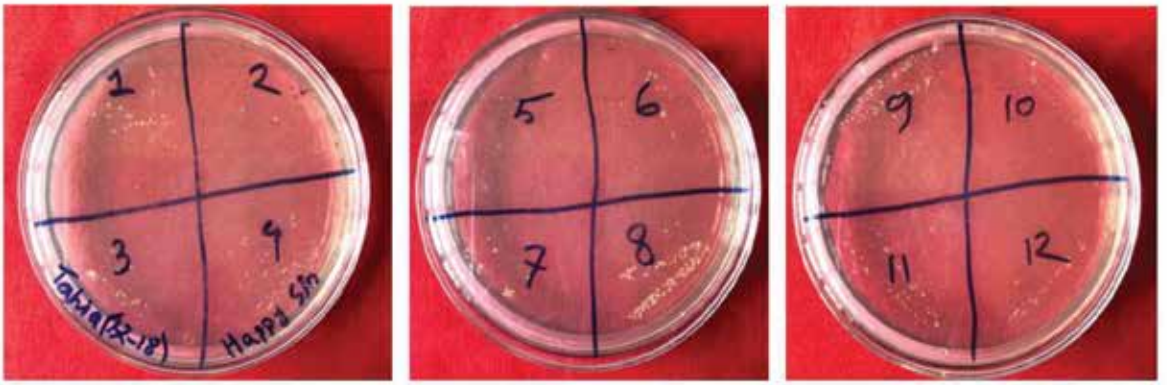

Fig. 4. Screening of high salt-tolerant bacteria using TSA media supplemented with 10% NaCl and rhizosphere soil were diluted to 1µg/ml.

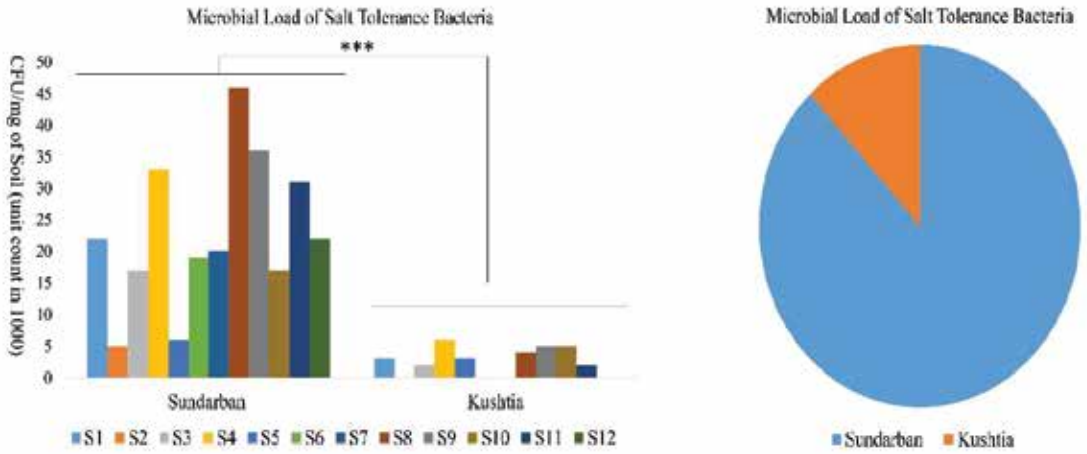

Fig. 5. Microbial load of high salt-tolerant bacteria

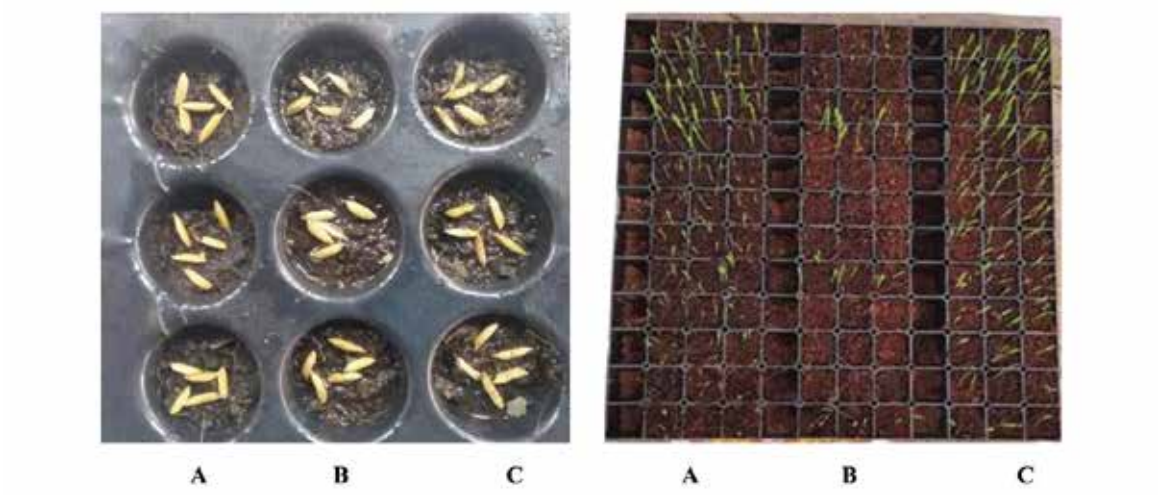

Fig. 6. The seed germination stimulation trials with salt-tolerante rhizosphere bacteria

Table 2. The seed germination rate of stimulation trials with salt-tolerant rhizosphere bacteria

Sample	Group A	Group B	Group C
1	0%	0%	90%
2	5%	5%	95%
3	15%	15%	100%
4	15%	10%	100%
5	10%	5%	95%
6	10%	10%	90%
7	70%	10%	95%
8	15%	15%	100%
9	75%	5%	90%
10	10%	5%	80%
11	0%	0%	90%
12	15%	10%	100%
	Second trial with pure culture		
7	75%	10%	95%
9	75%	10%	90%

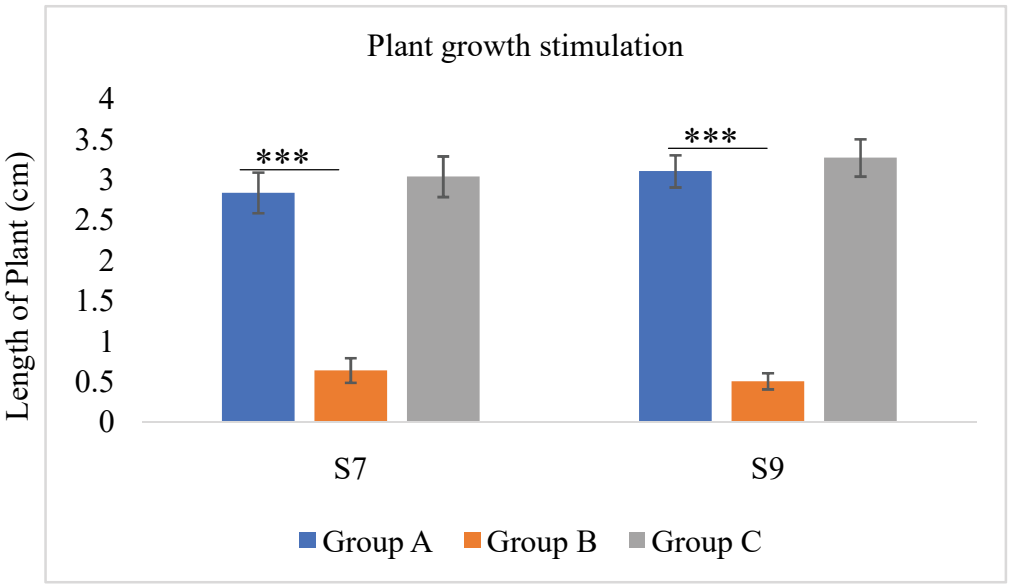

Fig. 7. Plant growth was stimulated with salt-tolerant rhizosphere bacteria. Group A contains salt and bacteria, Group B contains only salt, and Group C has no salt and no bacteria.

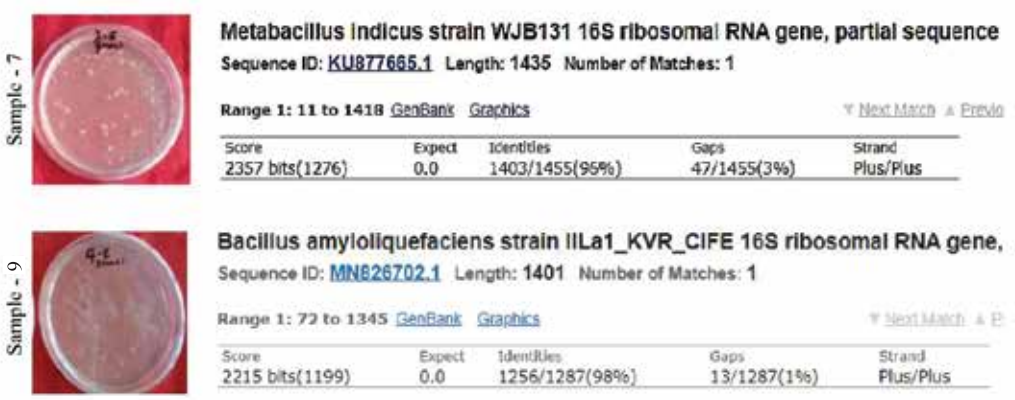

Fig. 8. 16S rRNA gene sequencing of 2 selected salt-tolerant identified bacteria

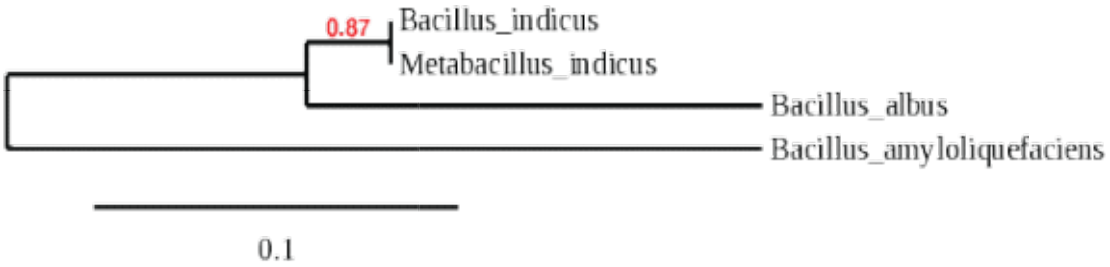

Fig. 9. Phylogenetic relationship of rhizosphere bacteria

Conclusions

Wild-type varieties of saline-prone areas like Sundarbans contain high salt-tolerant rhizosphere bacteria. The microbial load of salt tolerance bacteria was significantly higher in the rhizosphere soil of Sundarbans than those of farmland. Some of the bacteria showed an influence on seed germination and plant growth. The bacteria were identified using 16S rRNA gene sequencing. The isolates were identified as *Metabacillus indicus* and *Bacillus amyloliquefaciens*. Farmers can use these bacteria during the cultivation of crops in the soil.

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Conflict of interest

Authors declared no conflict of interest

Statement of author's credit

MAHMJ has role in supervision, writing-draft manuscript, review and editing, MAK has role in writing-

draft manuscript and methodology, SA, FNT, NN has role in methodology and data collection, AR and ACP has role in data analysis and methodology.

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