Региональные проблемы. 2018. Т. 21, № 3(1). С. 58–61. DOI: 10.31433/1605-220X-2018-21-3(1)-58-61.

CLIMATE CHANGE: CHALLENGES FOR AGRICULTURAL ENVIRONMENT

UDK 631.95(549.3)

SALINITY INTRUSION AND COASTAL AGRICULTURE: ADAPTATION STRATEGIES USING SALT-TOLERANT PLANT-GROWTH PROMOTING RHIZOBACTERIA FOR SUSTAINABLE FOOD SECURITY

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The salinity level in the coastal ecosystem and agricultural lands is being increased gradually due to the climate change effect, and Bangladesh is no exception to suffer salinity intrusion threatening its food security. In order to bring the salinity-affected lands under agriculture, the application of salt-tolerant, plant growth-promoting rhizobacteria (PGPR) as biofertilizer could be a method of choice. The current research reports the isolation of a salt-tolerant PGPR, identified as Bacillus aryabhattai MS3 from a coastal rice field of Bangladesh. Under laboratory condition, the strain showed profound plant growth-promoting activities: nitrogen fixation, production of indole acetic acid (IAA), phosphorus solubilization and siderophore production under 200 mM salinity. While in soil, rice growth under non-saline condition was comparable in between biofertilizer-added and control pots, the scenario was statistically significant when challenged with salts, 46% and 8% survival were recorded respectively. The PGPR supported the plants under salinity by increasing the availability of nutrients, accelerating IAA and chlorophyll production, enhancing proline accumulation, and decreasing malondialdehyde formation. The semi-quantitative reverse transcription-PCR demonstrated that the bacterium selectively up-regulated the plant expression of NHX1 gene under salinity, thereby conferring tolerance to salt stress. Overall, the application of salt-tolerant biofertilizer could be a non-transgenic innovation to support plant growth for coastal lands under changing climate conditions.

Keywords: *Bangladesh, plant growth-promoting rhizobacteria, salinity, salt-tolerance.*

Introduction

Under the ongoing changing climate conditions, saltwater intrusion is expected to worsen the fertility of coastal agriculture around the world, and Bangladesh being a flat and low-lying country is no exception. Once crossed the threshold limit, salinity conditions may decrease seed germination either by creating osmotic potential or by toxic effects, leading to retardation in plant development. Genetic engineering and molecular marker assisted breeding technologies are being used to develop saline tolerant crops, while the use of alternate technologies through non-transgenic approaches, like application of plant growth-promoting rhizobacteria (PGPR) for ameliorating abiotic stress is gaining importance and momentum for consideration [12]. The isolation of a salt-tolerant PGPR, identified as *Bacillus aryabhattai* MS3 from a coastal rice field of Bangladesh and its effect on growth of rice plants in the form of biofertilizer under regular and salt-stressed condition, are presented in current work.

Methods

Soil, water and sediment samples collected from the rice fields of salinity-affected coastal region of Bangladesh were subjected for bacterial growth on Jensen agar media after processing. As the media was devoid of nitrogen source, it only enabled selection of the nitrogen fixing bacteria, and the count was 53. The isolates were subsequently tested for other plant growth promoting attributes under a stress of 200 mM salinity in laboratory conditions that include (i) N_2 fixation by Kjeldahl method, (ii) phosphate solubilization by Molybdenum blue method [5] in NBRIP (National Botanical Research Institute's Phosphate) broth, (iii) Indole acetic acid (IAA) production [4] in Yeast extract mannitol media, and (iv) Siderophore producing ability by universal chrome azurol S (CAS) method [11].

The hemolytic activity of the selected PGPR on sheep blood agar was performed to ascertain it's safe use if entered in the human food chain [2]. To observe the growth pattern of the isolates under both normal

and saline conditions, culture was grown in batch system employing varied salinity under control (0%) and the test conditions (0.63%, 1.25%, 2.5%, 5%, 7.5%, 10% and 15%) in nutrient broth with the initial inoculum of 10^9 CFU ml^{-1}. The absorbance of the growing culture was measured spectrophotometrically at 600 nm in every two-hour intervals.

To understand the ability of the selected PGPR as biofertilizer to support the growth of a salt-sensitive rice cultivar, *Oryza sativa* BR 28 under normal and stress conditions; pot experiments were conducted under controlled environment. Mass production of the PGPR, and a bacterial strain, *E. coli* DH5α to be used as control was performed in Yeast extract mannitol broth (YEM). Biofertilizer was formulated aseptically according to the Bureau of Indian Standards (BIS) guidelines [3]. Prepared biofertilizer was uniformly mixed with soil and watered. Duplicate earthen pots for each test condition (PGPR, control- *E. coli* DH5α and uninoculated) were employed. The seeds were placed on filter paper, incubated at 56°C for 24 hours followed by soaking with tap water for 2 to 3 days. Then around 2L hydroponic solution was taken in the plastic tray and germinating seeds were left floating on the solution via cock sheet as a supporting material. After 12 days of cultivation in hydroponic solution, plants were transferred into earthen pot. Length of root, stem, and leaves were measured for each plant. After 45 days of rice cultivation under regular condition, a stress of 200 mM salinity was applied to plants where applicable [8]. Then after 25 days of salt application, length of stem and leaf were determined, and the pool of mRNA was extracted from plant tissues (root and stem).

For estimating IAA, total carbohydrate, chlorophyll, protein, proline and malondialdehyde (MDA) in the cultivated rice plants, 0.1 g leaf $stem^{-1}$ plant tissue sample was taken each time to follow the method described in [4, 7, 10, 6, 1, 9], respectively.

Transcriptomic analysis of plants' salt responsive genes namely, *NHX1*, *GIG* and *BZ8* [8] was performed to observe their changes in the modulation of expession upon addition of PGPR. 30 mg of plant tissue (root and stem) was processed for mRNA extraction using GSure™ Ultra Nucleic Acid Isolation Kit (GCC biotech, India), which were eventually reverse transcribed into cDNA by using ImProm-II™ Reverse Transcription System (Promega, USA). cDNAs were then used as templates to compare the expression of genes in biofertilizer-applied and control plants. The amplified products were electrophoresed on 1% agarose gel and the images were captured using gel Doc (AlphaImager, USA).

Results

Among the 53 bacterial isolates, the isolate that exhibited profound plant growth-promoting activities under 200 mM salinity, was identified as *Bacillus aryabhattai*, hereafter *B. aryabhattai* MS3. Identification was based on its biochemical characterization, sequence analysis of 16S rDNA followed by its phylogenetic and molecular evolutionary analyses by blasting the sequence on NCBI. Sequence data was deposited to GenBank, and the assigned accession number was MG209571.

The dynamic growth pattern of MS3 in batch culture in presence of 200 mM salt revealed that growth curve of MS3 exhibited longer lag phage under stress when compared to normal state (no added salt); however, it rapidly matched with the later with similar growth rate indicating higher tolerance against salt stress.

The plant growth promoting (PGP) properties of MS3 under *in vitro* laboratory condition showed comparable PGP attributes when grown under normal and saline conditions. The abilities were recorded for nitrogen fixation (11 and 7%), IAA production (35 and 25 μg mL^{-1}), phosphate solubilization (3.0 and 2.4 μg μL^{-1}) and siderophore release (70% and 20% unit) respectively. The isolate added in soil in the form of biofertilizer to support growth of a salt-sensitive rice cultivar under non-saline condition demonstrated no significant difference in between biofertilizer-added and control pots. The scenario, however was statistically significant when plants were challenged with 200 mM salts added after 45 days of cultivation that produced 46% and 8% survival rates respectively, estimated after 25 days of salt application.

Plants fundamentally cope with the unfavorable effects of elevated salinity stress by different metabolic changes. Plant hormones control plant growth and play role in adaptation to various stresses. The endogenous content of plant hormones in leaf and stem tissues of MS3 applied rice plants was found higher compared to uninoculated plant tissues. The IAA in stem and leaf tissues of inoculated rice was 5.3 and 7.8 μg g^{-1} as compared with control (uninoculated) plants (3.7 and 4.5 μg g^{-1}) under 200 mM NaCl. Similar trend was also reflected in chlorophyll content in leaf and stem tissues of MS3 inoculated rice (1.3 and 1.9 μg g^{-1}) versus uninoculated (0.1 and 0.4 μg g^{-1}). Proline, a compatible solute thought to provide salt-tolerance gives a positive correlation for tolerance. Its content was found higher (189 μM g^{-1}) in MS3-inoculated compared to uninoculated control rice plants (170 μM g^{-1}) under salinity stress condition. Conversely, Lipid peroxidation of cell mem-

brane is increased under prolong exposure to stress. The amount of MDA produced because of lipid peroxidation in rice was lowest (5 μM g^{-1}) in MS3-inoculated plants as opposed to uninoculated plants (12 μM g^{-1}) under salinity condition.

To address the molecular nature of acquisition of salt tolerance in plants, the semi-quantitative reverse transcription-PCR (SQRT-PCR) was conducted extracting total mRNAs of plants followed by converting them into cDNA, to be used as templates only to analyze transcriptomic distribution of three salt-tolerant genes in plants: *NHX1, GIG* and *BZ8*. While under normal condition, the expression of all three genes were evident, the bacterium selectively up-regulated the expression of *NHX1* gene only under salinity, a situation not evident in controls (Figure), therefore is thought to confer salt-tolerance to plants.

Discussion

We are moving to a post-carbon era, where climate change mitigation and adaptation are combined with other goals to build a sustainable future. This ties in closely with the United Nations' Sustainable Development Goals. The strain *B. aryabhattai* MS3 demonstrated phenomenal adaptation at elevated salt concentrations, still delivering plant-growth promoting attributes through plant-microbe interaction. As the study suggests, they aided plants by increasing availability of nutrients (P, Fe), accelerating IAA and chlorophyll production, enhancing proline accumulation, and decreasing malondialdehyde formation; the cumulation of which resulted in survivability of plants under salinity stress compared to the control condition. Further, the upregulation of sodium proton antiporter (*NHX1)* gene, which express a plant vacuole membrane protein responsible for catalyzing uptakes of Na^+ from cytosol into the plant vacuole in exchange of H^+ under higher salt concentration could advocate for tolerance. Entrepreneurship for constructing bio-bank composed of such potential PGPR could be a way forward for climate-change preparedness program for its effective application as biofertilizer for sustainable agriculture and food security under changing climate conditions.

Conclusion

The novel salt-tolerant strain, *Bacillus aryabhattai* MS3 has the potential to support plant survivability under salinity stress in the form of bio-fertilizer by modulating salt-tolerant gene expression, and providing vital plant-growth promoting activities to plants.

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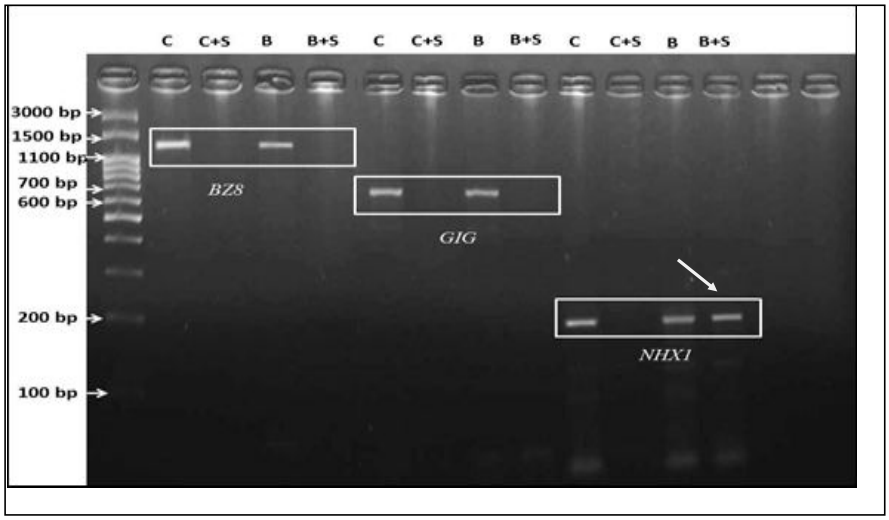

Figure. SQRT-PCR of NHX1, GIG and BZ8 plant genes, electrophoresed on 1% agarose gel. Plants either bio-fertilized (B) with MS3, or not under normal (C), or salt-stressed (S) conditions were treated for analyses. Specific expression of NHX1 is indicated by arrowhead in bio-fertilized plants under salinity. Marker used was 100 bp-3 kb DNA ladder

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