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ScienceDirect

Rice Science, 2017, 24(5): 264−273

Enhancement of Growth and Grain Yield of Rice in Nutrient Deficient Soils by Rice Probiotic Bacteria

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Abstract: Plant associated bacteria are promising alternatives to chemical fertilizers for plant growth and yield improvement in an eco-friendly manner. In this study, rice associated bacteria were isolated and assessed for mineral phosphate solubilization and indole-3-acetic acid (IAA) production activity *in vitro*. Six promising strains, which were tentatively identified as phylotaxon *Pseudochrobactrum* sp. (BRRh-1), *Burkholderia* sp. (BRRh-2), *Burkholderia* sp. (BRRh-3), *Burkholderia* sp. (BRRh-4), *Pseudomonas aeruginosa* (BRRh-5 and BRRh-6) based on their 16S rRNA gene phylogeny, exhibited significant phosphate solubilizing activity in National Botanical Research Institute phosphate growth medium, and BRRh-4 displayed the highest phosphate solubilizing activity, followed by BRRh-5. The pH of the culture broth declined, resulting in increase of growth rate of bacteria at pH 7, which might be due to organic acid secretion by the strains. In presence of *L*-tryptophan, five isolates synthesized IAA and the maximum IAA was produced by BRRh-2, followed by BRRh-1. Application of two most efficient phosphate solubilizing isolates BRRh-4 and BRRh-5 by root dipping (colonization) of seedling and spraying at the flowering stage significantly enhanced the growth and grain yield of rice variety BRRI dhan-29. Interestingly, application of both strains with 50% of recommended nitrogen, phosphorus and potassium fertilizers produced equivalent or higher grain yield of rice compared to the control grown with full recommended fertilizer doses, which suggests that these strains may have the potential to be used as bioinoculants for sustainable rice production.

Key words: indole-3-acetic acid; mineral phosphate solubilization; rice; plant growth promotion; plant associated bacterium; grain yield; fertilizer

Rice (*Oryza sativa* L.) is the staple food for more than half of the world's population, making it the most important cereal crop. With the increase of world's population, the global rise in rice consumption portends an increased pressure on our dwindling agricultural land. It has been reported that to feed the fast growing world's population, annual cereal production will need to rise to about 3.0 billion tons by 2050 (FAO, 2009) from around 2.6 billion tons today (FAO, 2017). To meet this demand, high-yielding varieties are being developed, which require extensive application of fertilizers such as nitrogen (N) and phosphorus (P) (Hazell, 2010). As currently practiced, an additional 40 and 20 million metric tons of chemical N and P fertilizers, respectively, will be required for food production by 2040 (Gregory et al, 2010). The alarming increase in synthetic chemical fertilizer has led to degradation in soil, deterioration in

Received: 29 October 2016; **Accepted:** 24 February 2017

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Peer review under responsibility of China National Rice Research Institute

http://dx.doi.org/10.1016/j.rsci.2017.02.002

air and water quality, which threatens the environmental sustainability (Tilman et al, 2001). Clearly, there is an urgent need to develop efficient, sustainable and green crop production systems for future use.

Phosphorus is an essential macro-nutrient for plant growth and development. Despite a high level of total P content in most agricultural soils, only 0.1% of it exists in soluble form for plant uptake (Richardson and Simpson, 2011). Therefore, costly and environment-degrading P fertilizers are applied in modern cropping systems to maintain P balance in soil for plant nutrition. However, almost 75%–90% of applied chemical P fertilizers are rapidly immobilized by forming complex with Al^{3+} or Fe^{3+} in acidic soils or with Ca^{2+} in calcareous soils (Stevenson, 1999; Islam and Hossain, 2012), resulting in shortage of available P for plant nutrition (Merbach et al, 2010). Farmers, therefore, apply several folds excess of P fertilizer. However, rapid depletion of global sources of rock P (Steen, 1998), rapidly increasing costs of inorganic P fertilizers and growing demands for organic foods have led to search for alternatives to environment-degrading synthetic P fertilizers.

Free-living plant-associated bacteria that may directly or indirectly exert beneficial effect on plant growth and development are generally referred as plant probiotic bacteria (Glick, 2012; Islam and Hossain, 2012). They are well-known to enhance plant growth and improve yield by increasing plant nutrient use efficiency by solubilization and mineralization of nutrient components particularly, mineral P (Sarkar et al, 2012), N-fixation (Islam et al, 2016), and synthesis of phytohormones such as indole-3-acetic acid (IAA) (Islam et al, 2016; Khan et al, 2016). Thus, a significant decrease in the use of chemical fertilizers could be achieved by applying plant-associated probiotic bacteria as bio-inoculants, which is an eco-friendly promising alternative to costly and environment-degrading industrial fertilizers (Rodríguez and Fraga, 1999; Islam and Hossain, 2012; Khan et al, 2016). Therefore, the objective of current study was to assess the role of native rice probiotic bacteria in order to reduce the use of chemical fertilizers without compromising with the growth and yield of rice under nutrient poor soil conditions.

MATERIALS AND METHODS

Experimental sites

The plant and soil samples were collected for isolation of bacteria from the experimental farm of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh (24.75º N and 90.50º E), Bangladesh and the field laboratory of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur (24.09º N and 90.25º E), Bangladesh.

Collection of plant materials and isolation of bacteria

Seed samples of rice varieties BINA dhan-4, BINA dhan-5, BINA dhan-6, BINA dhan-7, BINA shail, Iratom-24, Kalijira and root samples along with rhizosphere soils of BINA dhan-7 and Paijam were collected from the experimental sites for bacterial isolation. However, seeds of rice variety BRRI dhan-29 were collected from the field laboratory of BSMRAU to use in pot experiment.

To isolate endophytic bacteria [*Pseudochrobactrum* sp. (BRRh-1), *Burkholderia* sp. (BRRh-2), *Burkholderia* sp. (BRRh-3), *Burkholderia* sp. (BRRh-4), *Pseudomonas aeruginosa* (BRRh-5 and BRRh-6)], 2–5 g seed samples were washed thoroughly with distilled water and rinsed in 70% ethanol for 5 min, followed by 5 times washing with distilled water, and then surface sterilized with 1% NaClO for 1 min followed by 5 times washing with sterilized distilled water, and finally the tissue was rinsed in 100% ethanol for 1 min followed by 5 times washing with sterilized distilled water. The tissue was then aseptically macerated with homogenizers. For rhizoplane bacteria isolation, root samples were washed thoroughly with sterilized distilled water and then homogenized by a vortex mixture for 1 min in 20 mL of sterile distilled water in a sterile test tube. To isolate rhizobacteria, rhizosphere soil samples (1 g) were added to sterile distilled water (10 mL). Serial dilutions (up to 10^{-9}) of each resulting suspension were made, and exactly 100 µL aliquot from each dilution was spread on nutrient agar plates and incubated at 25 ºC for 48 h (Sarkar et al, 2012). Morphologically distinct colonies were purified by repeated streak culture on the same medium. The purified bacterial isolates were then stored in 20% glycerol solution at -20 ºC for further study.

Biochemical characterization of isolated bacteria

The gram reaction was conducted as described by Vincent and Humphrey (1970). A number of biochemical tests were performed to characterize the isolated bacteria following the criteria of Bergey et al (1994). For testing KOH solubility, bacterial isolates