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Phosphate and potassium-solubilizing bacteria effect on the growth of rice

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ABSTRACT

The objective of this study was to investigate the ability of three phosphate-solubilizing bacteria (PSB), including *Pantoea ananatis* (KM977993), *Rahnella aquatilis* (KM977991) and *Enterobacter* sp. (KM977992), to release potassium (K) from mica and also to evaluate their effect in promoting the growth of rice (cv. 'Tarom Hashemi') plants at an early stage of development. These isolates significantly solubilized K from mica in both solid and liquid medium *in vitro*. After 25 days of growth in liquid AM medium, K-solubilization (KS) for *P. ananatis*, *Enterobacter* sp. and *R. aqautillis* was 38.9, 33.6 and 15.5 $\mu g\,ml^{-1}$, respectively. KS of the isolates increased as pH of the culture medium declined ($r = -0.83$, $P < 0.0053$), as a result of organic acid production. Single KSB inoculations increased plant height (PlHe), stem diameter (SD), root length (RL), leaf area (LA) and biomass dry weight (BDW) by 4.09–10.8%, 4.07–10.4%, 8.0–13.1%, 19.8–21.4% and 7.53–15.7%, respectively, in a pot experiment while PlHe, BDW, SPAD value, K uptake in the leaves, stem, and root of rice seedling also increased by 10.8–15.1%, 27.4–65.3%, 8.64–12.0%, 38.5–76.9%, 17.6–52.9% and 25.0–75.0%, respectively, in a field experiment, when compared to the control. The results of both experiments indicate that the values of all measured parameters were higher when rice seedlings were inoculated with *P. ananatis* than with*Enterobacter* sp. and *R.aquatilis*. Based on our results, these isolates can be used as both PSB and KSB to enhance rice growth and also can be worthy of commercial development.

1. Introduction

Potassium (K) is one of the most important macronutrients that requested for plant growth. It is necessary for plant metabolisms such as synthesis of cells, the activity of enzymes, protein and vitamin production. Moreover, K increase plant resistance to abiotic and biotic stresses and also regulation of metabolic pathways (Maqsood et al., 2013; Epstein and Bloom, 2005). Deficiency of K, however, resulted in a slow grow in the plants, produce smaller seeds and finally lower yields (Gupta et al., 2015). Among of all macronutrients, K is taken up in the maximum value than others in rice which resulted in the better rice growth and development (Fageria, 2015). The amount of soluble K in soil are usually very low (K^+, 2% of total K in the soil which can be directly take-up by plants) and more than 90–98% of K in the soil exists as insoluble mineral K, exchangeable and non-exchangeable forms (Shanware et al., 2014). In intensive agriculture production, farmers usually using large amounts of chemical fertilizers, which can cause adverse effects on the environment. Therefore, introduce alternative sources of fertilizer such as microbial activation can be an effective way to reach a sustainable agriculture and to decline the use of chemical fertilizers (Bakhshandeh et al., 2015). To date, numerous studies focused on evaluating plant–microbe interactions with some rhizobacteria which were belonging to the *Azospirillum*, *Agrobacterium*, *Bacillus*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Micrococcus*, *Pseudomonas*, *Rhizobium* and *Serratia* genera (Bakhshandeh et al., 2014). These bacteria influence on the plant growth using both direct and indirect mechanisms that fully presented in Gupta et al. (2015). In addition, many studies recently indicate that some rhizobacteria such as *Pseudomonas*, *Bacillus*, *Klebsiella* and *Pantoea* can release K from insoluble minerals such as mica, illite and others by various mechanisms like organic acid production (Shanware et al., 2014; Meena et al., 2015). Generally, K-solubilizing bacteria (KSB) release K using production of organic acids like citric, oxalic, tartaric, succinic and α-ketogluconic acids and also enhance

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the dissolution of K compounds by supplying protons and by complexing of metal ions like Fe^{2+}, Al^{3+} and Ca^{2+}. Similarly, phosphate solubilization also occurs by organic acid production and proton extrusion by phosphate-solubilizing rhizobacteria (PSB) (Meena et al., 2016). Consequently, these bacteria use a common mechanism to solubilize insoluble phosphorus (P) and K in the soil. On the other hand, PSB can increase the availability of P in the soil or the P concentration in plant tissues, and also enhances the availability and uptake of K in the soil and plant tissues (Meena et al., 2016). Saha et al. (2016) indicate that both *Bacillus licheniformis* and *Pseudomonas azotoformans* had higher K solubilizing (7.22 and 6.03 $\mu g\,ml^{-1}$ at optimal conditions, respectively) among of rhizobacteria which were isolated from rice rhizosphere soil. In tobacco (*Nicotiana tabacum* L.) Subhashini (2015) reported that seedling roots inoculated with *Frateuria aurantia* as an effective KSB enhanced biomass dry weight (BDW) and uptake of K and nitrogen in a field experiment and also in a pot experiment (Zhang and Kong, 2014). Similarly, Sugumaran and Janarthanam (2007) in groundnut (*Arachis hypogaea* L.) indicated that BDW and oil content increased by 1.25 and 35.41%, respectively, when the seeds inoculated with *Bacillus mucilaginosus* (the ability of K-solubilization (KS) was 4.29 $mg\,l^{-1}$ in muscovite mica after 4 days at 28 °C) in comparison to the control. *Enterobacter hormoechei* remarkably increased root length (RL), fruit maturity, K and chlorophyll content in cucumber (*Cucumis sativus* L.) (Prajapati and Modi, 2016). Sheng (2005) reported that *Bacillus edaphicus* NBT, significantly increased BDW and uptake of K in cotton (*Gossypium hirsutum* L.) and rape (*Brassica napus* L.) when illite was applied in the soil. Therefore, the objective of this study was to investigate the efficiency of three PSB proposed by Bakhshandeh et al. (2014) including *Pantoea ananatis*, *Rahnella aquatilis* and *Enterobacter* sp. to release K from mica and also to evaluate their efficiency as KSB on rice growth (cv. 'Tarom Hashemi') at an early stage of development. Based on our knowledge, this is first study about *R. aquatilis* as a KSB.

2. Material and methods

2.1. Laboratory test

A laboratory experiment was conducted to evaluate the ability of KS of three effective PSB including *P. ananatis*, *R. aquatilis* and *Enterobacter* sp. with GenBank accession numbers KM977993, KM977991, and KM977992, respectively. These strains were isolated from rice paddy soil in northern Iran. The soil samples collection, isolation and identification methods are fully described in Bakhshandeh et al. (2014).

2.2. Qualitative analysis of KS

All isolates were tested for their KS ability on a modified Aleksandrov medium (AM) containing in 1 l: 5 g, glucose; 0.5 g, $MgSO_4{\cdot}7H_2O$; 0.005 g, $FeCl_3$; 0.1 g, $CaCO_3$; 2 g, $CaPO_4$; 1 g, mica as a K mineral and 15 g; agar, pH 7.0–7.5. The bacteria were cultured on solid AM (5 μl of a bacterial suspension containing about 10^6 CFU ml^{-1}) and incubated for 12 days at 30 ± 1 °C, with three replications. A clear zone around the bacterial colony on solid AM medium considered as a positive KSB. The clear zone was measured twice, at 6 and 12 days after incubation, by a digital caliper (mm) to determine the potassium solubilization efficiency (PSE) of the isolates, using Khandeparkar's method that was proposed by Shanware et al. (2014) [PSE = (solubilization diameter/growth diameter) × 100].

2.3. Quantitative analysis of KS

Three replicate flasks containing liquid AM were inoculated with each isolate, using 10 μl of a bacterial suspension containing about 10^6 CFU ml^{-1}, into 40 ml AM medium supplemented with mica (without agar). Non-inoculated liquid AM was used as a control. At the end of experiment, 10 ml cultures were sampled to determine pH (measured by a pH meter) and available K concentration (after 25 days inoculation at 30 ± 1 °C). The flame photometric method suggested by Sugumaran and Janarthanam (2007) was used to measure the amount of available K into the medium. Briefly, cultures were centrifuged (10,000 × *g*, 10 min, 20 °C) and then solubilized K value was estimated by subtracting the soluble K of the inoculated sample from that of the corresponding sample of non-inoculated control where K was partially released by the autoclaving.

2.4. Pot experiment

The plant growth promoting activity of these isolates was tested in a pot experiment. The experiment was conducted in a randomized complete block design (RCBD) with four times replicates, in rice (cv. 'Tarom Hashemi' a local and low-yielding rice cultivar with a high grain quality and appropriate for cultivation in the most parts of northern Iran). Rice seeds were germinated at room temperature (RT, 25 °C) for 5 days, and then inoculated with each bacterial suspension as well as a control (non-inoculated), for 5 h at RT before planting in pots. The isolates were grown in nutrient broth medium (NB, Merck, Germany, 8 $g\,l^{-1}$), as followed Bakhshandeh et al. (2015). Control rice seedlings were treated in the same manner with non-inoculated NB medium. After inoculation, two healthy rice seedlings (with a RL about 3 mm) were randomly selected and planted in each pot. Generally, we used 32 rice seedlings in the pot experiment (4 treatments × 4 replications × 2 seedling per pot). The pots were filled with 700 g of soil (containing 1 kg: 333 g, rotted sawdust; 67 g, perlite; 33 g, peat moss and 567 g, sand) which was sterilized using an autoclave (high pressure at 15 psi, 121 °C for 60 min^{-1}) twice before use. Pots were irrigated daily with water (at a depth of 1 cm above soil surface) during the experiment. Thirty-two days after planting, rice plants were harvested to measure, plant height (PlHe), BDW, number of leaves in the plant (NoL), stem diameter (SD), RL and plant leaf area (PLA). A ruler (cm) was used to determine PlHe and RL, and also SD measured with a digital caliper (mm). The BDW was determined by oven-dried at 70 °C to a constant weight. The PLA was estimated by the sum of the individual leaf area (LA) in plant. Individual LA was calculated using proposed method by Palaniswamy and Gomez (1974) [LA (cm^2) = 0.75 × *LW*; *L*, leaf length, and *W*, the maximum width of the leaf].

2.5. Field experiment

A field experiment was conducted in a paddy field of Mazandaran province (Babol City, located at 36°46′ N, 52°56′ E, and 25 m asl) as a split plot arrangement in a RCBD with three replications in 2016. The experiment was performed at an early stage of rice growth in a seedbed. Two methods, namely rice seedling and soil inoculations by each bacterial suspension were used as the main plot and three single inoculations with the KSBs as well as a control (non-inoculated), served as the subplots. Totally, we had 24 plots in the field experiment (2 methods × 4 treatments × 3 replications). The rice cultivar was the same in the pot experiment (cv. 'Tarom Hashemi'). At first method, germinated seeds of rice (5 days old with a RL about 3 mm) inoculated with each bacterial suspension which was prepared as in Bakhshandeh et al. (2015) (700 g germinated seeds into 4 l water contained 400 ml of bacterial suspension with a final density of 10^7 CFU ml^{-1}) for 5 h before planting in the seedbed. In the second method, the soil of each plot was treated with 400 ml of each bacterial suspension for 1 h before planting by hand (at a soil depth of 0–7 cm). Control rice seedlings were also treated in the same manner with non-inoculated NB medium