Research Article

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Enhancing the ability of rice to adapt and grow under saline stress using selected halotolerant rhizobacterial nitrogen fixer

https://doi.org/10.1515/opag-2022-0195
received February 8, 2021; accepted April 1, 2023

Abstract: Salinity stress has become the major devastating constraint for rice growth. Halotolerant rhizobacterial nitrogen fixer (HRNF) was investigated for increasing the nitrogenase activity (NA), organic acid (OA), gibberellic acid (GA), and indole 3-acetic acid (IAA) productions, seedling growth, and rice yield. Six N fixers were isolated using Ashby's (Ab_1, Ab_2, and Ab_3) and Okon's media (Az_1, Az_2, and Az_3). Furthermore, bioassay was carried out using rice seedling grown on nitrogen-free medium. The Ab_3 and Az_2 isolates were selected and biomolecularly identified as *Pseudomonas stutzeri* and *Klebsiella pneumoniae*, respectively. These selected bacteria were used as active ingredients for Halotolerant rhizobacterial inoculant (HRI) dosage trials (0, 500, 1,000, and 1,500 g ha^{-1}) on simple pot experiments. The Az group isolates had 3–5 times higher ability in fixing N and producing OA, GA, and IAA than the Ab group isolates. Furthermore, N-uptake, number of panicles, filled grain, and the rice yield of HRI treated pots were significantly increased. Application of 1,000–1,500 g HRI ha^{-1} had resulted in a significant increase in the yield of rice grain (26.10–28.27 g $plant^{-1}$ or 15.4–25.09%) which was higher than the control. This result concludes that HRI could contribute in enhancing the ability of rice to adapt and grow under saline stress.

Keywords: halotolerant N fixer, rhizobacteria, rice yield, *Pseudomonas stutzeri*, *Klebsiella pneumoniae*

1 Introduction

Salinity stress is one of the most devastating problems for rice cultivation in coastal areas and causes major significant reduction in their growth and yield along with decreased crop yield and soil quality [1–3]. The tolerance of rice variety to salt stress differs; however, most of the high yielding ones are generally intolerant to saline conditions [4,5]. Rice fields in Indonesia that are affected by salinity stress are increasing continuously and to some extent have become unproductive soils, particularly in dry season [6]. Furthermore, the increasing salinity level could inhibit plant photosynthesis, protein synthesis, and lipid metabolism which leads to a reduction in crop yield [7–9]. High salt content in the soil produces a negative effect on the nutrient balance and uptake by plants which causes osmotic stress [10–12]. Therefore, agricultural practices are forced to be more intensive in producing enough food for the rapidly growing population by combating soil degradation, salinization, and desertification of agricultural soils. Such circumstances require suitable biotechnology not only to improve crop yield but also soil health through interactions between plant roots and soil microorganisms [13,14].

New strategies have been developed to alleviate the salinity and other toxic effects on crop growth and yield, including the application of environmentally friendly fertilizers, known as beneficial microorganism [8,15,16]. Recent studies have revealed promising results on the application of the plant growth promoting rhizobacteria

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(PGPR) for alleviating both biotic and abiotic stress [17–20]. Moreover, the PGPR is one of the best alternatives in reducing salinity stress on crop growth [21–23]. PGPR is a group of free living saprophytic bacterial microorganisms that live in the plant rhizosphere and colonize their root system. Furthermore, these microbes colonize the seeds or roots and gain support from the root exudates that are rich in carbohydrates, amino acids, and other growth substances. The PGPR generally provides the plant with a compound that is synthesized by the bacterium for facilitating the uptake of nutrients from the environment [24–26]. In addition, it increases germination rates, root length, growth characteristic (leaf area, chlorophyll content), nutrient uptake, and yield. Protein content and the tolerance to drought and salt stress were also increased significantly. Furthermore, the presence of this PGPR that has tolerance to the saline ecosystem and improves the growth of the crop also belongs to different genera bacteria (*Rhizobium, Bacillus, Pseudomonas, Pantoea, Paenibacillus, Burkholderia, Achromobacter, Azospirillum, Microbacterium, Methylobacterium, Variovorax, Enterobacter*) [8,27,28]. The use of these microorganisms could alleviate saline stress and open different new and emerging applications.

Nitrogen is one of the most important elements for rice farming, and its application is directly proportional to the yield of this crop. However, intensive use of inorganic N fertilizer would accelerate the decomposition of soil organic matter. In addition, this crop is less responsive to the increased use of fertilizers which is also known as levelling off [29,30].

The aim of this study is to find the superior potential halotolerant N fixer PGPR that has the ability to fix nitrogen, produce organic acid (OA) and phytohormone (gibberellic acid; GA and indole 3-acetic acid; IAA), and also to increase the growth and yield of rice. The selected superior potential isolates of Halotolerant rhizobacterial nitrogen fixer (HRFN) were meant to be used as consortia of halotolerant rhizobacteria inoculant (HRI), and as bioagent (BA) or microbial fertilizers (MFs) for increasing the growth and yield of rice under salinity stress.

2 Materials and methods

2.1 Isolates of HRNF PGPR

The composite soil samples were taken for the isolation of HRNF from paddy rhizosphere at different salinity levels in Muara Baru Village, Karawang District, West Java Province, Indonesia. Furthermore, the isolation of HRNF was carried out using a series of plate dilution and salinized Ashby's nitrogen free media at 6 dS m^{-1} (*moderately saline*) which was modified from that reported by Rao [31]. It consists of 20 g mannitol, 0.2 g K_2HPO_4, 0.2 g NaCl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.1 g K_2SO_4, and 5.0 g $CaCO_3$ [32]. Afterwards, it was diluted in 1 L distilled water to obtain HRNF isolates of Az_1, Az_2, and Az_3 (Table 1).

Subsequently, the biochemical activity of all isolates was analyzed to measure the nitrogenase activity (NA), the production growth substance IAA, GA, and OA. The highest NA of isolate from each group was chosen for biomolecular identification in the Laboratory of Biotechnology Research Center in Bogor. Furthermore, the selected isolates were used as active ingredient for the formulation of HRI in the form of powder with the base ingredients being 50% peat + 17.5% compost + 17.5% biochar + 5% dolomite + 5% guano + 5% nutrition.

2.2 Activity of enzyme nitrogenase

The acetylene reduction assay method was used to measure the NA [33] and all isolates were cultured in a nutrient broth. 0.5 ml culture of each isolate were added to vials individually and incubated for 48 h at 22°C in a thermostatic incubator. A gastight syringe was used to exchange 10% of the air volume from the respective assay vessel with acetylene. Five minutes after the addition of acetylene, subsequently 500 µL of gas sample was taken and injected into the gas chromatography (Model DS 6200). Furthermore, the determination of the calibration curve was carried out by injecting 100, 200, 300, 400, and 500 µL of ethylene standard into the gas chromatography. The concentration of ethylene was measured with a calibration curve.

2.3 Production of OA, GA, and IAA

OA, GA, and IAA production were measured by high performance liquid chromatography (HPLC) [34]. OA determination was analyzed by reversed phase chromatography

Table 1: HRNFs isolated from different salinity levels

Isolates	Electrical conductivity (dS m^{-1})	Soil salinity
Ab_1 and Az_1	<2 dS m^{-1}	Low
Ab_2 and Az_2	4–6 dS m^{-1}	Medium
Ab_3 and Az_3	>6–8 dS m^{-1}	High

using a GraceSmart RP 18.5 μ column, and was read at λ = 210 nm. Quantification was done by comparing the peak heights of the standard compound of lactate, pyruvate, succinate, and malate [35]. GA and IAA analysis used a nutrient broth for culturing the *isolates* which was then incubated at 28°C for 3 days. Subsequently, isolate suspension (20 mL) was taken and centrifuged at 8,000 × *g* for 30 min at 4°C and about 1 L of supernatant was extracted with 100 μL of ether for three times. Finally, the extracted samples were added with 60% methanol and 10 μL of extract solution was taken and injected into a column chromatograph that is equipped with a different ultraviolet detector (280 nm). Furthermore, the quantification of the measured parameter was calculated by comparing peak heights.

2.4 Rice seedling growth

The bioassay on the growth of the rice seedling of Mekongga variety was conducted from 30th May to 15th June 2017 in the biofertilizers production unit, Arthauly in Bandung. The experiment was arranged as a completely randomized design consisting of six N-fixer bacterial isolates (Ab_1, Ab_2, Ab_3, Az_1, Az_2, and Az_3) and replicated three times. Furthermore, the Yoshida-free N medium was used for the experiment [36]. About 5 mL of Yoshida medium that contains 10 ppm of phosphorus (P), 40 ppm of potassium (K), 40 ppm of calcium (Ca), 40 ppm of magnesium (Mg), and enriched with micronutrient (B, Fe, Mn, Cu) were filled in a tube (100 mL vol) and sterilized at 121°C and 15 Psi in autoclave. Subsequently, the medium was inoculated with 1 mL of isolate suspension (10^9 CFU). The rice seeds were sterilized with 0.02% $HgCl_2$ for 1 min and then washed with aquadest and germinated in a Petri dish for 5 days. Furthermore, they were then planted in the Yoshida medium in the tube. The observed responses consist of the shoot height which was measured from the base of the stem to the tip of the leaf, the root length which was measured from the base to the tip of the root, and the plant dry weight which was measured on the 14th day after sowing.

2.5 Bioassay of HRI on N uptake and rice grain yield

Simple bioassay consisting of four dosages of inoculant consortia of Ab_3 and Az_2 (0, 500, 1,000 and 1,500 g ha^{-1}) such as MF or BA with three replications was carried out to investigate the contribution of selected HRI in improving the growth and yield of rice at the Agricultural Extension Station in Cilamaya Wetan in Karawang District (6°.15′44″, 107°34′24″, located about 0.5 m above sea level). The selected isolates were active ingredient of HRI and were identified biomolecularly. The HRI was prepared by 35 mL of the inoculant suspension containing about 10^9 CFU mL^{-1} of HRI into an aluminum sachet containing 65 g of powder organic based carrier (45% peat soil, 25% biochar, 25% compost, 5% additive). In addition, the final weight of the inoculant was 100 g $sachet^{-1}$. The experimental pots (10 L) were filled with 8 kg of soil media and the water content was maintained at a saturated condition. The treatments (100 g of inoculant) which were diluted in 10 kg of fine compost and mixed homogeneously were placed at the planting area. Subsequently, two rice seedlings (21 days old) of Inpari 34 variety were planted and the water levels were maintained at 2 cm high. Furthermore, all the experimental pots were placed under field condition. 500 kg ha^{-1} of compost was applied together with bacterial inoculant, while the mixed inorganic fertilizer (200 kg urea, 100 kg SP-36, and 100 kg of KCl ha^{-1}) applications were placed 3–5 cm into the soil with about 10 cm from the rice plant on the 15th and 42nd days after planting. The water level of the pots was maintained at 2 cm high, and the factors that were observed include nitrogen uptake [37], percentage of filled grain, and grain yield pot^{-1}.

2.6 Statistical analysis

The completely randomized design or simple bioassay of the pot experiment was arranged for the observed data and statistically analyzed using the Statistical Analysis System (SAS; SAS Institute, North Carolina State University). Furthermore, the F-Test was carried out to show the significant effects on the tested variables. Finally, the Duncan Multiple Range Test (DMRT) or least significant difference (LSD) at $P < 0.05$ were also carried out [38].

3 Results and discussion

3.1 Biochemical activity and selected isolates molecular identification

The results showed that the selected indigenous N-fixer isolates had the ability to fix N_2 (Table 2). Furthermore, the relatively high NA (117–121 μM mL^{-1} h^{-1}) was recorded from the isolates of Az_1, Az_2, and Az_3. The NA ranged from 24 to 29 μM mL^{-1} ha^{-1} in the isolates of Ab_1, Ab_2, and Ab_3,

Table 2: NA, OA, GA, and IAA of HRNF isolates

Isolates	NA ($\mu M\ mL^{-1}\ ha^{-1}$)	OA ($\mu M\ mL^{-1}\ ha^{-1}$)				GA (ppm)	IAA (ppm)	Method
		Lactate	Pyruvate	Succinate	Malate			
Ab_1	24	0	1	2	4	1	10	HPLC
Ab_2	26	0	2	4	2	1	12	HPLC
Ab_3	29	0	1	5	2	2	11	HPLC
Az_1	117	11	17	11	24	16	96	HPLC
Az_2	119	21	56	70	96	28	112	HPLC
Az_3	121	34	24	21	19	11	75	HPLC

which was significantly lower. Moreover, Table 2 showed that the Az isolates had the ability to produce relatively high OA (lactate, pyruvate, succinate, and malate), GA, and IAA compared to the Ab isolates group.

The results showed that the biochemical activity of Az isolates was higher and more superior than the Ab isolates. Therefore, for further research based on the ability to fix nitrogen (NA), the Ab_3 and Az_2 isolates were chosen as representative ingredient for each group to formulate HRI or PGPR bioagent for alleviating the salinity stress. Subsequently, isolates of Ab_3 and Az_2 have been identified biomolecularly as *Pseudomonas stutzeri* (98.88% similarity) and *Klebsiella pneumoniae* (99.63% similarity), respectively (Figure 1).

3.2 Salinity effect on the growth of rice seedlings

The growth of rice seedling (shoot height, root length, and biomass) of the Mekongga variety was decreased due to the increase in the salinity (Tables 3–5).

The shoot height of the rice seedling was not significantly different due to the inoculation of N-fixer at 2–4 $dS\ m^{-1}$ of electrical conductivity (ECe), while the lowest shoot height at 6 ECe was recorded due to the inoculation of Az_3 (9.67 cm). Furthermore, the high shoot height of the rice seedling occurred due to the inoculation of Ab_1, Az_1, and Az_2 at higher salinity (ECe = 6 $dS\ m^{-1}$). Furthermore, the root length was affected differently by the inoculation of

Nr.	Isolate code	Molecular Identification Results
		Nucleotide Sequences and Isolate Name (% similaritas)
1	Ab_3	CACTAAGATCTCAAGGATCCCAACGGCTAGTCGACNNNGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGGTTGCTCCC CACGCTTTCGCACCTCAGTGTCAGTATTAGCCCAGGTGGTCGCCTTCGCCACTGGTGTTCCTTCCTATATCTACGCATTTCACC GCTACACAGGAAATTCCACCACCCTCTGCCATACTCTAGCTCGCCAGTTTTGGATGCAGTTCCCAGGTTGAGCCCGGGGCTTT CACATCCAACTTAACGAACCACCTACGCGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTTCGTATTACCGCGGC TGCTGGCACGAAGTTAGCCGGTGCTTATTCTGTTGGTAACGTCAAAACAGCAAGGTATTAACTTACTGCCCTTCCTCCCAACTT AAAGTGCTTTACAATCCGAAGACCTTCTTCACACACGCGGCATGGCTGGATCAGGCTTTCGCCCATTGTCCAATATTCCCCAC TGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGACTGATCATCCTCTCAGACCAGTTACGGATCGTCGCCT TGGTGAGCCTTTACCTCACCAACTAGCTAATCCGACCTAGGCTCATCTGATAGCGCGAGGTCCGAAGATCCCCCGCTTTCTCC CGTAGGACGTATGCGGTATTAGCGTTCCTTTCGAAACGTTGTCCCCCACTATCAGGCAGATTCCTAAGCATTACTCACCCGTC CGCCGCTGAATCATGGAGCAAGCTCCAATCATCCGCTCGACTTGCATGTGTTAGGACTG ***Pseudomonas stutzeri* (98.88% similarity)**
2	Az_2	TCAAGGNGCACAACCTCCAAATCGACATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCG CACCTCGAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATCTCTACGCATTTCACCGCTACACC TGGAATTCTACCCCCCTCTACAAGACTCTAGCCTGCCAGTTTCGAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCACATCCG ACTTGACAGACCGCCTGCGTGCGCTTTACGCCCAGTAATTCCGATTAACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCA CGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATCGACAAGGTTATTAACCTTATCGCCTTCCTCCCCGCTGAAAGTAC TTTACAACCCGAAGGCCTTCTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCC TCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTAGGTGA GCCGTTACCCCACCTACTAGCTAATCCCATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCACTTTGGTCTTGCG ACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATCCCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCG CTCGTCACCCGAGAGCAAGCTCTCTGTGCTACCGCTCGACTTGCATGTGTTAAGGCCTG ***Klebsiella pneumoniae* (99.63% similarity)**

Figure 1: Molecular identification and nucleotide sequences of Halotolerant NFB isolates (Ab_3 – *Pseudomonas stutzeri*) and Az_2 – (*Klebsiella pneumonia*).

Table 3: Effect of HRNF isolates on shoot height (cm) of Mekongga rice seedlings under different salinity stress

Isolates of HRNF	ECe		
	$s_1 \leq$ 2 dS m^{-1}	s_2 = 4 to <6 dS m^{-1}	$s_3 \geq$ 6–8 dS m^{-1}
Ab_1	29.27 a	29.27 a	15.73 b
Ab_2	30.40 a	25.70 a	12.53 ab
Ab_3	31.90 a	28.73 a	11.73 ab
Az_1	31.33 a	28.10 a	16.50 b
Az_2	31.77 a	26.30 a	15.76 b
Az_3	30.77 a	27.83 a	9.67 a

Mean values followed by the same letter are not significantly different at DMRT of 5% (p = 0.05). ECe – electrical conductivity.

Table 5: Effect HRNF isolates on the dry weight (biomass) of Mekongga rice seedlings under different saline stress at 14 days after sowing

Isolates of HRNF	ECe		
	$s_1 \leq$2 dS m^{-1}	s_2 = 4–6 dS m^{-1}	$s_3 \geq$6–8 dS m^{-1}
Ab_1	92.33 a	83.33 b	60.00 b
Ab_2	110.00 a	126.67 b	36.67 ab
Ab_3	126.67 a	160.00 c	83.33 b
Az_1	106.67 a	136.67 b	60.00 b
Az_2	130.00 a	63.33 a	53.33 b
Az_3	103.33 a	110.00 b	20.00 a

Mean values followed by the same letter are not significantly different at DMRT of 5% (p = 0.05). ECe – electrical conductivity.

N-fixers. From low to medium salinity levels (2–4 dS m^{-1}), the lowest root length was recorded in Az_1 and the resulted highest root length was about 4.37 cm at the high salinity levels (ECe = 6 dS m^{-1}) but not significantly different with Ab_1.

Either Ab (Ab_1, Ab_2, and Ab_3) or Az isolates (Az_1, Az_2, and Az_3) resulted in indifferent biomass (dry weight) during the 14 days seedling period at lower salinity level (ECe = 2 dS m^{-1}). Furthermore, the different effects of N-fixer inoculation were found at the higher salinity level (4–6 dS m^{-1}). The highest biomass was recorded by inoculation with the isolates of Ab_3 and the lowest was found with Az_2 at the medium salinity level (ECe = 4–6 dS m^{-1}). At the higher salinity level (ECe ≥ 6–8 dS m^{-1}), the high biomass was obtained by the inoculation of the isolates of Ab_1, Ab_3, and Az_1 and the lowest was obtained by Az_3. This result indicated that the presence of N-fixers as PGPR could enhance the ability of rice seedling to adapt and grow under salinity stress.

The ability of HRNF to fix nitrogen from the atmosphere is seen in the NA and the production of OA, IAA, and GA. However, this activity is affected by the increasing level of salinity which is seen in the reduction in rice development.

The presence of PGPR isolates in the growth medium alleviates the effects of salinity on Mekongga by producing enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Furthermore, ACC deaminase-containing plant growth-promoting bacteria have been documented to facilitate the growth of a variety of plants under high salinity conditions.

Table 4: Effect of HRNF isolates on root length (cm) of Mekongga rice seedlings under different salinity stress

Isolates of HRNF	ECe		
	s_1 ≤2 dS m^{-1}	s_2 = 4 to <6 dS m^{-1}	s_3 ≥6–8 dS m^{-1}
Ab_1	5.90 b	5.17 ab	3.03 ab
Ab_2	4.33 ab	5.00 ab	2.80 a
Ab_3	5.33 ab	5.93 b	2.73 a
Az_1	3.07 a	4.40 ab	4.37 b
Az_2	4.00 ab	4.03 a	2.13 a
Az_3	4.60 ab	3.47 ab	1.83 a

Mean values followed by the same letter are not significantly different at DMRT of 5% (p = 0.05). ECe – electrical conductivity.

3.3 Bioassay of HRI on rice grain yield

Consortia of *Klebsiella pneumoniae* and *Pseudomonas stutzeri as* HRI had a significant effect on the increasing N uptake (content and total uptake, number of panicles, filled grain, and rice grain yield) (Table 6). The performance of rice growth was better visually than control (the leaf was greener and the performance was more healthy or robust). Furthermore, the increasing dosage of HRI had enhanced all the observed responses significantly. The highest results were obtained by the application of 1,000–1,500 g inoculant of HRI which resulted in the rice grain yield of about 26.10–28.27 g plant^{-1} (15.4–25.09% higher than control). This result was supported by the N uptake (4.75–5.26 g plant^{-1}), number of panicles (22.92–26.92 plant^{-1}), and filled grain (65.68–69.75%) or about 37.8–52.5%, 15.8–41.1%, and 15.1–39.3% higher than control, respectively. The soil physical-chemical properties were soil texture, namely, silty clay, acid soil (pH = 5.04), medium organic carbon content (2.44% Org-C), medium total nitrogen (0.25% N), high content of exchangeable Na (2.01 cmol kg^{-1}), high salinity (ECe = 6.64 dS m^{-1}), very low base saturation (14.24%).

Table 6: Effective dosage of HRI on N uptake (50 days old), number of panicles, filled grain and rice grain yield on saline soils in Cilamaya Wetan

Dosage of HRI (ha^{-1})	N-uptake		Panicle Nr. ($panicle\ plant^{-1}$)	Filled g rain (%)	Rice grain yield	
	(%)	$g\ plant^{-1}$			$g\ plant^{-1}$	Increment (%)
D_0 = 0 g	2.77 a (±0.07)	3.45 a (± 0.48)	19.08 a (±2.47)	57.08 a (±5.8)	22.60 a (±0.38)	—
D_1 = 500 g	2.82 a (±0.11)	4.44 b (± 0.53)	21.58 a (±2.04)	61.66 a (±6.21)	23.63 a (±0.34)	4.5
D_2 = 1,000 g	2.95 a (±0.11)	4.75 b (±0.62)	22.92 b (±1.63)	65.68 b (±2.71)	26.10 b (±0.93)	15.4
D_3 = 1,500 g	3.01 b (±0.08)	5.26 b (±0.75)	26.92 b (±1.01)	69.75 b (±1.22)	28.27 b (±1.00)	25.09

Mean values followed by the same letter are not significantly different at LSD of 5% (p = 0.05).

4 Discussion

4.1 Biochemical activity

The presence of bacteria isolated in rice rhizosphere could contribute to the availability of nitrogen, production growth substances (GA and IAA), and OA that are important for the increase in plant growth and to improve the health of rhizobiome [13,14,19,39]. Furthermore, the capacity to fix N is highly dependent on the NA [30]. The biological nitrogen fixation by bacteria are dominant in the rice rhizosphere particularly in flooded soil ecosystem [40]. The amount of soil microbes in rhizosphere are usually affected by the root exudates (sugars and amino acids), and this leads to the reduction in N_2 to NH_3 catalyzed by nitrogenase enzyme. Consequently, it is highly affected by the O_2 concentration. The high respiration activity in rhizosphere and the protection of nitrogenase enzyme in the cells might possibly lead to the higher activity of nitrogenase enzyme [41]. In addition, the six isolates had the ability to synthesize enzyme nitrogenase, OA, GA, and IAA (Table 2). Therefore, this N-fixer bacterial isolates could increase plant growth, thereby, promoting rhizobacteria (PGPR) [28,42].

The presence of IAA, gibberellins, and other growth factors produced by the PGPR increases the root length and tip numbers which contribute to the increase in the nutrients uptake and the health of the plant [7,26]. Furthermore, the PGPR contributed in improving the growth of various crops (tomato, pepper, canola, bean, lettuce, and other food crops) under saline ecosystem [43–45]. *Pseudomonas stutzeri* and *Klebsiella pneumoniae* are well known as N-fixer rhizobacteria or PGPR [46–48].

4.2 Salinity effect on the growth of rice seedling

The existence of PGPR of HRNF as functional microbes are undoubtedly placed under saline soil stress. Generally, most rice seeds, such as Mekongga, are usually salt sensitive especially in their early growth stage [49].

Salinity increases the osmotic potential of growth medium and as a result, seeds require more energy to absorb water, resulting in decreased germination [2,9,50,51]. However, when the seeds were inoculated with the PGPR suspension, their growth continued to increase even at a lower rate. Nia et al. [52] studied the effect of inoculation of PGPR isolated from saline or non-saline soil on yield components of wheat and they observed that inoculation with the two isolates increased salinity tolerance of wheat plants. Furthermore, the saline-adapted isolate significantly increased the shoot dry weight under severe water salinity. Plant homoeostasis was regulated by ethylene phytohormone endogenously and caused the reduction in root and shoot growth under high salinity.

The plant ACC deaminase was sequestered and degraded by bacterial cells to supply nitrogen. Moreover, removing ACC deaminase is good for plants because it reduces the harmful effects from ethylene, alleviating stress and improving the crops' growth [22,25]. Higher K^+/Na^+ ratio also has an effect on the reduction in electrolyte leakage and a decreased uptake of Na^+ in order to prevent plants from being exposed to high salt salinity [42,53].

4.3 Effect of HRI on rice grain yield

Pseudomonas stutzeri and *Klebsiella pneumoniae* played a significant role in alleviating salinity stress, growth, and crop yield [46–48,54,55]. In addition, the application of ameliorant combined with HRI as a BA could increase the effectiveness of the inoculant and the growth of rice significantly [14].

5 Conclusion

The isolates of Az_1, Az_2, and Az_3 have a high ability to fix N, produce OA and phytohormone (IAA and GA)

compared to the isolates of Ab group (Ab_1, Ab_2, and Ab_3). The highest biomass of rice seedling with about 160 and 83.3 g tube^{-1} was obtained from treated seeds with the inoculation of Ab_3 at a medium–high salinity level, respectively. The consortia application of HRI (*Pseudomonas stutzeri* and *Klebsiella pneumoniae*) was able to increase the N-uptake, number of panicles, filled gain, and rice grain yield significantly. Furthermore, the application of 1,000–1,500 g ha^{-1} of HRI was able to produce rice grain yield of about 26.10–28.27 g plant^{-1} (15.4–25.09% higher than control). This result concludes that the presence of HRI as BA or MFs could enhance the ability of rice to adapt and grow under saline field conditions. HRI could contribute to enhance the rice ability to adapt and grow on saline soils.

Acknowledgments: We are thankful for financial support of Academic Leadership Grant (ALG) program of Padjadjaran University and to late Mr. Sudjana Brilyan for his excellent work and contribution for this research.

Funding information: The study was financed with sources from Academic Leadership Grant (ALG) program of Padjadjaran University.

Conflict of interest: The authors state no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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