Research Article

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The use of endophytic growth-promoting bacteria to alleviate salinity impact and enhance the chlorophyll, N uptake, and growth of rice seedling

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Abstract: Soil salinity is a major limiting factor for crop productivity, which increases continuously due to climate change. This barrier can possibly be overcome with the occurrence of halotolerant endophytic bacteria which reportedly plays an important role in protecting plants against various environmental stresses. Therefore, plant growth-promoting microbes are used in agriculture as an inexpensive and eco-friendly technology to enhance crop productivity in saline areas. In this study, the three isolates with nitrogen fixation ability were applied for mitigation of salt stress. The isolates were coded as C3A1, C8D2, and K10P4 and applied to rice plants by seed priming method. Furthermore, they were given as single innoculant or combined as a consortium compared to control, which was without the addition of endophytic bacteria, while the inoculated seed was planted on saline semisolid Fahraeus media at 4 dS m^{-1}. The results showed that the single isolate of K10P4 endophytic bacteria increased the dry weight of rice plants, N uptake, and chlorophyll of plants in saline conditions. The combination of K10P4 isolate with C8D2 was synergistic and increased the population of endophytic bacteria in root tissue and chlorophyll content compared to the combination of C3A1 or three isolates. Meanwhile, the use of the 16S ribosomal RNA method on C3A1, C8D2, and K10P4 indentified the isolates as *Ochrobactrum tritici* (C3A1), *Pseudomonas stutzeri* (C8D2), and *Pseudomonas stutzeri* (K10P4).

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1 Introduction

Agricultural land close to the coastal area is vulnerable to salinity stress [1] because salts from the sea easily enter the ground through tides and seawater intrusion. In the long term, constant tidal wave will lead to salt saturation in soil and ground water sources [2]. Salt buildup in upper soil layer may also occur as the water used for irrigation often contains high levels of salt. Similarly, climate change, massive groundwater use, increased use of poor quality water in irrigation, and the introduction of large-scale irrigation associated with intensive agriculture and poor drainage accelerates the emergence of various salt-related environmental stress [3]. Land irrigation that uses salt-contaminated water for a long period can accumulate NaCl in the soil and damage crops [4], which affects crop growth and productivity worldwide [5]. Based on the Natural Resources Conservation Service criteria, one indicator to determine land salinity threat is the value of electric conductivity (EC), where the soil is considered to be high in salinity when the EC value is >4 dS m^{-1} [6,7]. In Indonesia, the main rice-producing areas are along the northern coast of the island of Java. The provinces of East, Central, West Java and Banten are the four provinces in north coast of Java with the coastal length of 1,316 km. These areas usually experience decline in rice production during the dry season. In 2017, seawater intrusion hit around 540,000 ha of rice fields during the dry season and the rice yields decreased by an average of 0.65 t ha^{-1} compared to the rainy season [8].

Salinity affects agricultural production [9], reduces the productivity of many crops [3], and causes a complexly

adverse effect between morphology, physiology, and biochemical processes such as seed germination, plant growth, and the absorption of nutrients and water in plants [10]. Therefore, plants that grow on saline soils experience high osmotic stress, poisoning, and crop nutrient disorders, or equilibrium [11]. Soil salinity is caused by the accumulation of salt in the soil and causes decrease in fertility. Its effect on plants includes osmotic stress and ionic toxicity, which affect all the major plant processes, such as photosynthesis, cellular metabolism, and nutrition [12].

The use of saline soil for agriculture requires handling salinity issues; meanwhile, using an ecofriendly and sustainable approach will be the microbe-based strategy of reducing salt stress [5]. This includes the use of endophytic bacteria living in saline soils in rice plants, which colonize plant tissue without causing harm to the host and can be beneficial to plants [13]. However, the bacteria can be detrimental when their presence is pathogenic in plants, and neutral when they do not affect plants.

Saline soil habitats are generally low in nitrogen (N) [14], therefore N inputs from N_2-fixing bacteria are very important in this environment. Salt effect in plant habitat can interfere with water and nutrients (especially N) absorption from the soil and are toxic to most organisms [15]. Potential of indigenous rice microbiomes, which mostly are endophytic, epiphytic, and rhizoplane diazotrophs, should be explored in order to obtain efficient N-fixing microorganims that can be directly or genetically improved and sustain crop production under the N-deficient condition and also decrease the application of chemical fertilizer [16]. The N_2-fixing bacteria that live in saline soils colonize the rhizosphere and salt-tolerant plants because of the presence of root exudate. The rhizosphere of the plant is very active and abundant in microbial habitat due to the secretion of plant root exudates of various nutritive substances and the microbial community in the surroundings. Therefore, the existence of nutritional substances in the rhizosphere plays an important role in the development and activity of the microbial community [17]. These microbes are associated with plant roots or live inside the host plant tissue.

Endophytic bacteria improve plant nutrition by fixing nitrogen from the air. Its contribution as plant nitrogen supplier is greater than that of free-living bacteria because fixed nitrogen is distributed and can be utilized immediately and loss from leaching is close to negligible. Furthermore, endophytes are better than the other rhizospheric and rhizoplane bacteria by providing fixed nitrogen directly to their host [18]. This is because nitrogen-fixing endosymbiont supplies nitrogen that is biologically fixed directly to plants [19]. Study on the application of superior stain of nitrogen-fixing endophytes in rice plant can aid the nitrogen uptake in plants, especially in the saline land ecosystem. This study observes the effect of a single and consortium of endophytic bacterial isolate addition towards the total population, N uptake, chlorophyll content, and rice plant dry weight in saline conditions.

2 Materials and methods

Endophytic bacterial isolates used were isolated from the tissues of rice plants grown in saline soils of Cirebon and Karawang district in West Java. These isolates were selected by acetylene reduction assay method in order to their ability to fixate nitrogen. The three isolates of N-fixing endophytic bacteria were from the paddy plants in saline soils of two locations; two isolates were obtained from the root and leaf tissues of paddy plants from Cirebon (codes C3A1 and C8D2), while that from leaf tissue of paddy plants were from Karawang (code K10P4). Meanwhile, the three isolates from paddy plants were inoculated in rice seeds planted on Fahraeus semisolid saline medium with EC value of 4 dS m^{-1} with the addition of single and a combination of the three isolates. The rice seeds also were grown on sterile straw paper under saline conditions. Straw paper was immersed in 4 dS m^{-1} sodium chloride solution based on the equation $y = 0.653 + 8.19x$ obtained from the standard NaCl and EC curves and sterilized using an autoclave at 121°C for 15 min.

The semisolid Fahraeus media used consisted of NaCl, $MgSO_4$, $CaCl_2$, KH_2PO_4, $2H_2O$, ferric citrate, yeast extract, and microelement ingredients, which were dissolved in distilled water of 1 L. Sodium chloride was added into semisolid Fahraeus medium and salinity level was adjusted to 4 dS m^{-1} based on the equation $y = 1.064 + 7.649x$ which was obtained from the the standard NaCl and EC curves. One agar slant of endophytic bacteria isolate was suspended into 200 mL of sterile nutrient broth and incubated at room temperature (±25°C) on a 120 rpm rotary shaker for 24 h. At the end of incubation, the bacterial density of the suspension was 10^8 CFU mL^{-1}.

The samples were completely randomized and consisted of eight treatments. Each treatment was replicated three times. The treatments tested were control (untreated), isolate C3A1, isolate C8D2, isolate K10P4, bacterium consortium of isolates C3A1 and C8D2, bacterium consortium of isolates C3A1 and K10P4, bacterium consortium of isolates

C8D2 and K10P4, and bacterium consortium of isolates C3A1, C8D2, and K10P4.

The data variance was analyzed by IBM SPSS version 17. If variance was detected, data were subjected to Duncan's Multiple Range Test with 5% confidence level. Observations were made on several parameters such as the population of endophytic bacteria, chlorophyll content, N uptake of rice plants, and dry weight of plants. Furthermore, the population of endophytic bacteria was analyzed by the pour plate count method. For measuring dry weight, plants were dehydrated in a 80°C oven for 48 h and placed inside a desiccator for 2 h until a constant weight was achieved. The analysis of chlorophyll content was performed with a chlorophyll meter soil plant analysis development (SPAD). In the measurement of chlorophyll, the sample used consisted of two plants with each comprising three points: upper, middle, and lower leaves. The equation for conversion from SPAD (chorophyll content index, CCI) to μmol m^{-2} for rice plants is: $-64 + 57^{*}(CCI)^{0.68}$ based on ref. [20], while the N content of rice plants was analyzed using the Kjeldahl method.

The DNA from bacterial isolates of rice plants was extracted using the Wizard DNA genomic extraction kit (Promega), and the 16S rRNA gene was amplified with primers 27F (5′ AGA GTT TGA TCC TGG CTC AG 3′) and 1492R (5′ GTT TAC CTT GTT ACG ACT T 3′) [21]. The amplification process was carried out by mixing 2 μL of printed DNA, 1.0 μL of each primer, 12.5 μL of GoTaq Green Master Mix 2×, and nuclease-free water to a volume of 25 μL. This was conducted under these conditions: initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1.5 min, primer attachment at 55°C for 45 s, polymerization for 1 min at 72°C, and polymerization time extension for 1 min in the last cycle. The reaction was stopped when temperature declined to 15°C and the purification of PCR products was carried out by the ethanol precipitation method [22].

3 Results and discussion

3.1 Population of endophytic bacteria in roots and shoot of rice plant

The inoculant density or inoculated population at the time of seed treatment was 1×10^7 CFU mL^{-1} and when it was isolated from rice plants, the density ranged from 1.0×10^4 to 1.0×10^5 CFU mL^{-1} (Table 1). The population density was applied based on a study [23] recommendation of 1.0×10^7 CFU mL^{-1}. Application with this population density has succeeded in roots and leaves colonization of cotton plants with the population of endophytes being 1.1×10^3 to 8.7×10^5 CFU g^{-1} in root tissue and 1.6×10^4 CFU g^{-1} in leaf weight. Application of 5×10^8 CFU mL^{-1} of endophytic bacteria in canola plantations successfully colonized 10^4–10^7 CFU g^{-1} of bacteria per plant [24].

Based on the observations, the highest population of endophytic bacteria was in the roots. This is in line with a previous study that identified the plant roots with a higher population density of endophytic bacteria compared to other parts above the ground [25]. The highest population of the bacteria in roots of rice plants was in the C8D2 single isolate and C8D2-K10P4 consortium. In the shoot, only C3A1 was detected. Synergism between two or more types of microbes occurs when microorganisms do not compete in the same nutrient source. Similarly, mixed microbial cultures also allowed their components to interact with each other synergistically via physical or biochemical activities, thereby simultaneously improving viability [26]. Soaking seeds with high bacterial density gives bacteria a higher chance of invading due to the differences in suspension pressure, which pressed the cell into the seeds through the pedicel. Therefore, when the seeds are removed from the suspension, the bacteria remain in the embryo [27].

Table 1: Endophytic bacteria population ($\times 10^4$) in root and shoot of rice plant in saline media

Treatments	Root	SD	Shoot	SD
Control (NB)	00.00	0.00^{a}	00.00	0.00^{a}
C3A1 (isolates from rice plant roots from Cirebon)	10.05	3.24^{ab}	31.80	8.36^{c}
C8D2 (isolates from rice plant leaves from Cirebon)	59.83	28.20^{c}	16.32	17.40^{abc}
K10P4 (isolates from rice plant leaves from Karawang)	10.14	12.55^{ab}	13.57	21.59^{abc}
C3A1 and C8D2	33.87	4.26^{bc}	08.98	6.52^{ab}
C3A1 and K10P4	40.77	33.39^{bc}	25.40	12.61^{bc}
C8D2 and K10P4	13.37	3.67^{ab}	01.10	0.00^{a}
C3A1, C8D2, and K10P4	27.50	9.92^{ab}	04.40	5.72^{ab}

Numbers followed with the same letter in superscript are not significantly different (Duncan Multiple Range Test, $\alpha = 5\%$).

3.2 Plant N uptake in saline media

The observations showed that the highest plant N uptake was obtained through the addition of K10P4 isolates in the form of a single bacterial isolate treatment (Table 2). This occurred because, in the previous stages of the study, K10P4 isolate produced the highest phytohormone on GA3 growth hormone, which stimulated the nutrient uptake of N, P, and K.

Furthermore, the highest plant N content was in the K10P4 treatment of a single endophytic bacterial isolate because the synergistic effect was not in a consortium. Meanwhile, bacteria nitrogen fixation is a natural process of changing atmospheric nitrogen (N_2) into a simple soluble nontoxic form (NH_4^+ primarily) which is used by plant cells for the synthesis of various biomolecules. It is one of the major sources of nitrogen for plants and an important step of distribution in the ecosystem [28].

Synergistic bacterial consortium isolates showed better results compared to the isolates in a single culture. It also stimulated physical and biochemical activities by increasing several beneficial aspects of plant physiology [29]. Meanwhile, synergism between two or more types of microbes occurs when there is no competition in the process of getting the nutrients needed by each microbe. Therefore, both microbes provide a beneficial effect on the host plant through a greater supply of nitrogen when one releases the metabolites needed by other microbes in fulfilling nutrients. A study showed that the use of a single inoculant *Acinetobacter* sp. which has the highest nitrogenase activity leads to a lower N supply when compared to the use of *Pseudomonas* sp. and *Acinetobacter* sp. mixture at each level of N fertilizer dosage [26].

Table 2: Effect of single and consortium of endophytic bacteria on N uptake of rice plants in saline media

Treatments	N (%)	SD
Control (NB)	1.52	0.19^{a}
C3A1 (isolates from rice plant roots from Cirebon)	2.72	0.15^{c}
C8D2 (isolates from rice plant leaves from Cirebon)	2.44	0.35^{bc}
K10P4 (isolates from rice plant leaves from Karawang)	3.52	0.46^{d}
C3A1 and C8D2	2.35	0.26^{bc}
C3A1 and K10P4	2.27	0.26^{bc}
C8D2 and K10P4	2.24	0.33^{bc}
C3A1, C8D2, and K10P4	1.95	0.28^{ab}

Numbers followed with the same letter in superscript are not significantly different (Duncan Multiple Range Test, $\alpha = 5\%$).

Nitrogen is part of the chlorophyll molecule that controls the ability of plants to carry out photosynthesis. It acts as a constituent of chlorophyll, where a sufficient amount of chlorophyll gives greener and more durable leaves. Meanwhile, nitrogen-deficient plants can be detected through the yellowish to dark greenish leaves, which is closely related to photosynthesis rate and grain yield in rice. It is also a sensitive indicator for the dynamic changes in plants, therefore, its status monitoring during the growing period is essential to achieve efficient fertilizer management and higher grain yield in rice production [30].

3.3 Chlorophyll content of rice plants

The application of endophytic bacterial consortium increases the chlorophyll content of leaves of rice plants (Table 3). The highest chlorophyll content was discovered in the application of K10P4 and C8D2 endophytic bacterial isolates, and their combination.

During photosynthesis, the function of chlorophyll is the formation of plant biomass and the capacity of photosynthesis. According to Moharana and Dutta [31], the total chlorophyll content in rice plants ranges from 1.13 mg g^{-1} to 7.26 mg g^{-1} in the reproductive phase of rice cultivation. Dobermann and Fairhurst [32] also reported a SPAD value of 35 for the most developed upper leaves used as a value of the N deficiency limit in the superior varieties of indica rice that were transplanted. Moreover, the limit for direct planting is SPAD 32–33 and was lower in the observed values due to the

Table 3: Chlorophyll content of rice plants due to endophytic bacteria application in saline media

Treatments	Chlorophyll		
	CCI	µmol m^{-2}	SD
Control (NB)	12.06	21.01	3.59^{a}
C3A1 (isolates from rice plant roots from Cirebon)	19.85	55.28	3.69^{abc}
C8D2 (isolates from rice plant leaves from Cirebon)	21.95	63.70	1.61^{c}
K10P4 (isolates from rice plant leaves from Karawang)	22.92	67.51	4.97^{c}
C3A1 and C8D2	15.43	36.49	3.95^{abc}
C3A1 and K10P4	12.31	22.17	7.55^{ab}
C8D2 and K10P4	20.13	56.40	2.96^{bc}
C3A1, C8D2, and K10P4	13.85	29.39	1.07^{ab}

Conversion equation for CCI to µmol m^{-2} ($-64 + 57 * [CCI]^{0.68}$) based on ref. [20]. Numbers followed with the same letter in superscript are not significantly different (Duncan Multiple Range Test, $\alpha = 5\%$).

growth phase of plants aged 21 days after plantation. Chlorophyll synthesis such as Chlorophyll a ($C_{55}H_{72}O_5N_4Mg$) and Chlorophyll b ($C_{55}H_{70}O_6N_4Mg$) requires certain factors such as light intensity, nitrogen, magnesium, iron, temperature, water, and trace elements (Mn, Cu, and Zn). If any of the factor is incomplete, the synthesis of chlorophyll will be affected [33].

The rice seedlings introduced with isolates K10P4 and C8D2 endophytic bacteria which can fix nitrogen also have a higher chlorophyll compared to control seedlings. The high chlorophyll value measured on the inoculated oil palm seedlings shows a good sign of plant health and it is closely related to photosynthesis activity and N status of the leaves. Lack of N in leaves can be identified through loss of green pigment as observed in control seedlings which reduced their photosynthesis ability [34]. Nitrogen is a macronutrient needed by plants in large quantities and is a major factor in the formation and constituent of chlorophyll in the form of protein. Furthermore, its influence on photosynthesis and other pathways is dependent on the genotype and the leaf region [35]. The formation of chlorophyll begins with the fixation of N_2 gas present in the soil and air by bacteria and converted to ammonia, which is transported through the xylem to the leaves. Therefore, the larger the quantity of ammonia in the leaves, the more chlorophyll will be formed [36].

3.4 Dry weight of rice plants

The results of this study showed that endophytic bacteria consortium did not increase the percentage of germination compared to a single isolate. Significant increase in plant's dry weight was detected in plants treated with K10P4 isolate (Table 4). Furthermore, both the single inoculate and consortium treatments showed a greater dry plant weight compared to control. This indicated that carrying a consortium and one endophytic bacterial isolate is good at increasing plant dry weight. Treatment with all endophytic bacterial isolates also increased the root's fresh and dry weights of inoculated rice plants. Therefore, rice seedlings' growth promotion can be attributed to the multiple plant growth-promoting properties of inoculated bacteria such as indole-3-acetic acid production, nitrogen fixation, and phosphate solubilization [37].

An increase in rice plant growth is caused by the ability of endophytic bacteria to produce growth hormones. Meanwhile, plant growth hormone regulates several aspects of growth and development such as the formation and maintenance of meristem tissue [38]. A previous study has shown that rice plants inoculated with antioxidant-producing bacteria have high dry weight compared to controls [39]. The application of endophytic bacteria also increases the dry weight of plants because the production of antioxidant enzymes by bacteria reduces the chloroplast damage for photosynthesis to proceed more optimally.

The dry plant weight is strongly influenced by the availability of endophytic diazotroph bacteria. According to Ji et al. [40], plants treated with CB-R05 endophytic isolate were taller with a significantly enhanced dry weight of leaf and root compared to the control. The five endophytic isolates (KW7-S22, KW7-S06, SW521-L21, CB-R05, and HS-R01), particularly CB-R05, can be used to facilitate an effective growth promotion in rice plants. Furthermore, the CB-R05 isolate showed a more significant growth effect than the type from the rhizosphere. Dongjin plants treated with CB-R05 isolate also showed a significant growth-promoting effect than the untreated control.

3.5 Identification of endophytic bacteria

The results of molecular identification of bacteria types against three isolates with codes C3A1 (origin from rice

Table 4: Effect of endophytic bacteria on dry weight of rice plant in saline media

Treatments	Dry plant weight (mg)	SD
Control (NB)	13.87	0.21^{a}
C3A1 (isolates from rice plant roots from Cirebon)	18.69	0.44^{b}
C8D2 (isolates from rice plant leaves from Cirebon)	18.97	0.37^{bc}
K10P4 (isolates from rice plant leaves from Karawang)	20.93	0.60^{d}
C3A1 and C8D2	18.69	0.44^{b}
C3A1 and K10P4	18.69	0.44^{b}
C8D2 and K10P4	20.41	2.20^{cd}
C3A1, C8D2, and K10P4	19.24	0.44^{bc}

Numbers followed with the same letter in superscript are not significantly different (Duncan Multiple Range Test, $\alpha = 5\%$).

plant roots from Cirebon), C8D2 (origin from rice plant leaves from Cirebon), and K10P4 (origin from rice plant leaves from Karawang) are shown in Table 5.

Endophytic N-fixing bacteria identified by C8D2 and K10P4 are *Pseudomonas stutzeri*. Meanwhile, previous studies showed that the ability of nitrogen fixation is determined by the characteristics of nif genes and nitrogenase enzymes in *Pseudomonas stutzeri* [41], which increases the growth and fixation of N_2 in upland rice [42,43]. Inoculation with N_2-fixing *Pseudomonas stutzeri* A1501 significantly altered the diazotrophic communities of the rhizosphere and root surface composition, where it became dominant in the rhizosphere, increased the indigenous diazotrophs' population and ammonia oxidizers [44], and was used as seed treatment [45]. According to Hakeem and Akhtar [46], *Pseudomonas mendocina* Khrs2, *Pseudomonas stutzeri* Khsr2, and *Pseudomonas putida* Khsr4 can produce indole-3-acetic acid, gibberellic acid, trans-zeatin riboside, and

Table 5: Molecular endophytic bacteria identification

No.	Isolate code	Results of molecular identification Nucleotide order and type (% similarity)
1	C3A1	GCGCACGTAGGCGGACTTTTAAGTCAGGGGTGAAATCCCGGGGCTCAACCCCGGAACT GCCTTTGATACTGGAAGTCTTGAGTATGGTAGAGGTGAGTGGAATTCCGAGTGTAGAGGT GAAATTCGTAGATATTCGGAGGAACACCAGTGGCGAAGGCGGCTCACTGGACCATTACTG ACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGT AAACGATGAATGTTAGCCGTTGGGGAGTTTACTCTTCGGTGGCGCAGCTAACGCATTAAAC ATTCCGCCTGGGGAGTACGGTCGCAAGATTAAAACTCAAAGGAATTGACGGGGGCCCGCA CAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCCCTTGACAT ACCGGTCGCGGACACAGAGATGTGTCTTTCAGTTTGGCTGGACCGGATACAGGTGCTGCATG GCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCC CTTAGTTGCCAGCATTTAGTTGGGCACTCTAAGGGGACTGCCGGTGATAAGCCGAGAGGAA GGTGGGGATGACGTCAAGTCCTCATGGCCCTTACGGGCTGGGCTACACACGTGCTACAATGG TGGTGACAGTGGGCAGCGAGCACGCGAGTGTGAGCTAATCTCCAAAAGCCATCTCAGTTCGG ATTGCACTCTGCAACTCGAGTGCATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGC CGCGGTGAATACGTTCCCGGGCCTTGTACAC ***Ochrobactrum tritici*** 16S ribosomal RNA gene, partial sequence (100%)
2	C8D2	CGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCCTCCA AAACTGGCGAGCTAGAGTATGGCAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCG TAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGGCTAATACTGACACTGAGGTG CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGAC TAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAG TACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATG GATTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGA TGTTGGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTAAGGTGGGC ACTCTAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCC TTACGGCCTGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAATCCGCGAGGTGGA GCTAATCCCATAAAACCGATCGTAG ***Pseudomonas stutzeri*** 16S ribosomal RNA gene, partial sequence (99%)
3	K10P4	CGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAAGCCCCGGGCTCAACCTGGGAACTGCATC CAAAACTGGCGAGCTAGAGTATGGCAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGCG TAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTGGGCTAATACTGACACTGAGGTG CGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGAC TAGCCGTTGGGATCCTTGAGATCTTAGTGGCGCAGCTAACGCATTAAGTCGACCGCCTGGGGAGT ACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGT TTAATTCGAAGCAACGCGAAGAACCTTACCAGGCCTTGACATGCAGAGAACTTTCCAGAGATGGA TTGGTGCCTTCGGGAACTCTGACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTT GGGTTAAGTCCCGTAACGAGCGCAACCCTTGTCCTTAGTTACCAGCACGTTAAGGTGGGCACTC TAAGGAGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTT ACGGCCTGGGCTACACACGTGCTACAATGGTCGGTACAAAGGGTTGCCAAGCCGCGAGGTGGAG CTAATCCCATAAAACCGATCGTAGTCCGGATCGCAGTCTGCAACTCGACTGCGTGAAGTCGGA ATCGCTAGTAATCGTGAATCATAATGTCACGGTGAATACGTTCCCGGGCCTTGTCGA ***Pseudomonas stutzeri*** 16S ribosomal RNA gene, partial sequence (99%)

abscisic acid. In the rhizosphere colonization of rice plants by *Pseudomonas putida* A15, Rediers et al. [47] discovered an expression of miaA involved in the production of trans-zeatin cytokinins and associated with plant growth hormones.

Endophytic N_2-fixing bacteria identified from C3A1 are *Ochrobactrum tritici*, which was originally isolated from wheat rhizoplane [48] and recently discovered from wastewater [49]. A previous study has shown that endophytic bacteria in rice seeds consisted of *Ochrobactrum* spp., namely R3–*O. tritici* and R12–*O. grignonense* [50]. *Anthropactic anthropic* isolated from *Helianthus tuberosus* plants has the effect of stimulating plant growth in the form of N fixation symbiosis, optimizing the formation of root morphology and increasing plant absorption [51]. *Ochrobactrum intermedium* L115 can increase the dry weight of the upper parts and roots of plants, indicating that the bacteria have the ability as plant growth-promoting rhizobacteria, which is capable of producing indole acetic acid, siderophore, and ACC deaminase [52]. Furthermore, the bacteria also have a high tolerance to high temperature and 300 mM NaCl.

4 Conclusion

The isolate of K10P4 endophytic *Pseudomonas stutzeri* bacteria from Karawang increases the dry weight, N uptake, and chlorophyll by 20.93 mg, 3.5%, and 67.51 μmol m^{-2}, respectively, of rice plants in saline conditions. Furthermore, its combination with C8D2 synergistically increased the population of endophytic bacteria in root tissue and chlorophyll content compared to the combination with C3A1 isolate or the combination of the three isolates. Meanwhile, the isolates were identified as follows: C3A1 endophytic bacteria from rice plant roots of Cirebon (*Ochrobactrum tritici*), C8D2 from rice plant leaves of Cirebon (*Pseudomonas stutzeri*), and K10P4 from rice plant leaves of Karawang (*Pseudomonas stutzeri*).

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