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ENDOPHYTIC BACTERIA FROM BANANA PLANT IMPROVES THE GROWTH AND YIELD OF BLACK RICE PLANT

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SUMMARY

Three bacterial isolates selected as plant growth-promoting endophytic bacteria (PGPEB) from "Klutuk" banana (*Musa balbisiana* L.) gained scrutiny for first-time testing on black rice. The presented study pursued an investigation on the impact of endophytic bacteria inoculation obtained from "Klutuk" banana plants on the growth and production traits of black rice. For this research, the three bacterial isolates (K10, K324, and K111) served as inoculums for black rice seeds, testing for synergism. The three isolates inoculation into black rice plants used both single and consortium inoculation methods. With agar media, black rice growth observation began 14 days after plantation (DAP) while on soil, at 30 and 140 DAP with inoculation. Three isolates did not show any antagonistic reactions. Overall, isolate K10 showed less significant improvement in growth and yield traits of black rice compared with two other isolates, i.e., K324 and K111. With agar media, the endophytic bacteria inoculations did not show a significant effect on the growth of black rice, and even isolate K324 was inhibiting in action. However, on soil media, isolate K324 significantly enhanced the number of roots and shoot length in black rice compared with the control at 30 DAP. The single inoculation with isolate K111 has caused increased productivity based on the weight of the root, dry grain weight per clum, plant height, root length, leaf length, and panicle length in black rice. Isolate K324 promotes the growth of black rice on the soil media at 30 DAP, while isolate K111 improves the production traits on the soil media at 140 DAP. The study findings provide a significant basis for the positive impact of endophytic bacterial inoculation on black rice growth and yield traits.

Keywords: Black rice (*Oryza sativa* L.), endophytic bacteria, bacterial isolates, "Klutuk" banana, agar and soil media, growth and yield traits

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Key findings: Endophytic bacteria isolates from "Klutuk" bananas have been illustrative of plant growth-promoting bacteria. Bacterial inoculation significantly improved black rice growth and yield traits. Isolate K324 enhanced the growth on soil media at 30 DAP, while isolate K111 enriched black rice growth and yield traits on soil media at 140 DAP.

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INTRODUCTION

The "Klutuk" banana (*Musa balbisiana colla*) plant with the BB genome (2n = 2x = 22) is a wild type of cultivar localized in the Java area, Indonesia (Sunandar, 2017). It is known as a relatively resistant cultivar due to its natural biotic and abiotic stress resistance (Tripathi *et al.*, 2008; Ravi *et al.*, 2013; Sutanto *et al*., 2014). Other reports showed that banana cultivars with the B genome have a relatively higher expression of resistance genes than bananas with the A genome (Daniells *et al*., 2001; Santos *et al*., 2018). "Klutuk" banana being a drought-stress-tolerant cultivar (Ravi *et al.,* 2013; Kurniawan *et al.,* 2017**),** also has resistance against *Xanthomonas* **(**Tripathi *et al.,* 2008), *Fusarium* **(**Sutanto *et al.,* 2014; Warman and Aitken, 2018), and banana bunchy top virus (Ngatat *et al.,* 2022).

Besides the influence of genetic resistance, the banana plant is also a recipient of impacts from bacteriome factors as the second associated genome (Berendsen *et al.,* 2012). In this case, a group of endophytic bacteria with plant growth-promoting (PGP) properties influences the resistance. Based on a metagenomic analysis, a report also indicated the "Klutuk" cultivar has a specific core microbiome from groups of *Bacillus* and *Pseudomonas* that supports the cultivar's resistance (Rahayu *et al.,* 2021a). Although, with the core microbiome's identification, limited confirmation about the effects of these bacterial inoculations proliferated.

In a previous study, endophytic bacteria isolation from the "Klutuk" banana cultivar and its characterization for PGP properties ensued in various crop plants (Rahayu *et al.,* 2021b; Abed *et al*., 2022; Al-Kurtany *et al*., 2023). The PGP characteristics included the ability to fix N_2, produce indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, dissolve Fe and PO_4, and inhibit the development of fungus *Fusarium oxysporum f.sp.* Cubense (Rahayu *et al.,* 2021b). Bacterial isolates K10, K111, and K324 possessed superior PGP characteristics compared with other strains and combined to form a consortium with complementary characters (Rahayu *et al.,* 2021b). These three bacterial isolates acquired from the "Klutuk" banana plant exhibit resistance to abiotic and biotic stresses and possess a multitrait PGPEB.

This characteristic will probably elicit a positive growth response in black rice plants since microbes associated with plants, including the endosphere, can stimulate the plant growth by producing phytohormones and providing nutrients (Saad *et al.,* 2020). Furthermore, several endophytic bacteria strains with a broad host range have existed, making them suitable for various crop plants' use (Afzal *et al.,* 2019). However, all these isolates require in-planta evaluations to discover more characters and effects on diverse crop plants, especially black rice.

Black rice is a good source of protein, vitamins, fiber, iron, and mineral contents and is remarkably high in several antioxidants compared with white rice. Therefore, its demand in foreign countries increases despite the relatively expensive price (Kristamtini *et al.,* 2018). Black rice is a local genetic resource-rich in anthocyanins as antioxidants, which possess numerous benefits, including anti-inflammatory and anticancer activities, preventing premature aging, protecting the brain and stomach from damage, inhibiting tumor cells, and preventing obesity and diabetes (Kristamtini and Wiranti, 2017; Kristamtini *et al.,* 2018; Suryanti *et al.,* 2020; Sari and Wahyuni, 2017). Also, it is beneficial for recovering from neurological diseases, increasing the brain's memory capacity, and neutralizing free radicals in the body. However, several production barriers are present, negatively affecting its grain yield, including long harvest time, inadequate cultivation, and fluctuating productivity (Suryanti *et al.,* 2020).

Black rice seedling quality improvement through inoculation with endophytic bacteria in single and consortium forms is one way to overcome these challenges (Vanegas and Uribe-Vélez, 2014; Chandra and Sharma, 2021). Current wide use of endophytic bacteria-based biological fertilizers continues for non-black rice (Gangmei and George, 2017; Sulandjari and Yunindanova, 2018; Herliana *et al.,* 2019; Maharani *et al.,* 2020; Susanto *et al*., 2020; Hasanah and Pratiwi, 2022). Research has shown that the endophytic bacterium *Ochrobactrum intermedium*, associated with medicinal plants, significantly enhances seed germination, seedling growth, and yield production of black rice plants in net house conditions (Singh *et al*., 2018). Similarly, applying *Streptomyces sp.* isolated from Indian black rice has revealed considerable enhancement in black rice seedlings' growth (Ningthoujam *et al*., 2016). Moreover, inoculating endophytic bacteria in black rice is a common practice to enhance its resistance to biotic and abiotic stresses (Rahma *et al*., 2022; Xue *et al*., 2020).

Previous efforts of bacterial inoculation to enhance the quality of black rice concentrated on a single inoculation method, and none of these studies have explored employing endophytic bacteria from "Klutuk" banana plants. The concerned work assessed the beneficial effects of three endophytic bacteria from banana plants on the growth and yield of black rice when applied individually and as a consortium. These three isolates have previously demonstrated potential PGP characteristics in a separate study (Rahayu *et al*., 2021b), although in-planta testing is yet to proceed. The single and consortium inoculations occurred on both agar and soil media. This paper discusses the different effects on black rice growth with bacterial inoculation on the agar and soil media. The study findings hope to contribute to developing a biological fertilizer formulation for black rice cultivation.

MATERIALS AND METHODS

Black rice and endophytic bacteria

The novel study used the black rice cultivar *'Sembada Hitam,'* a local variety of the District Sleman registered as a promising cultivar through a decree issued by the Ministry of Agriculture, Indonesia. The three isolates, selected out of 93 endophytic bacterial isolates, stood out for their PGP characteristics, previously isolated from the "Klutuk" banana plant. Based on an earlier evaluation, the characteristics of these three bacterial isolates appear in Table 1 (Rahayu *et al.*, 2021b).

Table 1. The characteristics of three PGP endophytic bacterial isolates, IAA: Indole Acetic Acid, ACC: 1-aminocyclopropane-1-carboxylate, PSI: phosphate solubility index, FoC: *Fusarium oxysporum* f. sp. Cubense.

Isolates Code	Ammonia production (mM)	IAA production (ppm)	ACC deaminase activity	Siderophore production (%)	PSI	Antagonism vs. FoC (%)
K10	0.61	73.45	-	34.32	-	-
K324	2.28	-	+	44.44	-	49
K111	-	72.6	-	41.67	2.8	-

Plus value (+) indicates the presence; the negative value (-) indicates the absence of the parameter.

Synergism evaluation of three isolates

A synergistic test used the cross-streak method following Ethica *et al*. (2019) to determine the presence and absence of an inhibitory reaction among isolates. For this evaluation, streaking all isolates on the Nutrient Agar (NA) medium crossly to each other was at a 90° angle, then incubated at 28 °C for 24 h. Evaluating the presence of synergism or antagonism among the isolates was qualitative. In cases where no growth inhibitions or clear areas showed around the contacted isolates, one can infer that no antagonistic activity occurred. Conversely, if growth inhibition arose among the isolates, it indicates the presence of antagonistic action.

Preparation of bacterial suspension

The in-planta evaluation proceeded by growing the consortium members individually in Nutrient Broth (NB) medium for 24 h. After incubation, cell harvesting by centrifugation continued, followed by washing with phosphate buffer and making them into a suspension with OD 600 = 0.8^{-1} (Ríos-Ruiz *et al.,* 2020).

Inoculation of black rice seeds with endophytic bacteria

Preparing the black rice seeds comprised sterilizing with 70% ethanol for 1 min, then with 1.2% (w/v) NaClO solution for 15 min, and rinsing four times with sterile distilled for 5 min. Meanwhile, mixing the consortium members was in the same ratio (Table 2). The in-planta test included soaking the seeds in a suspension of endophytic bacteria for 24 h with a ratio of 10 mL suspension per 20 black rice seeds (Ji *et al.*, 2014). Some sown seeds in water agar media, while some in pots with soil media remained in the greenhouse. The greenhouse experiment plan used a randomized complete design with four replications. Subsequently, measuring and recording the plants' height, leaf length/width, panicle number per clum, filled grain per panicle, panicle length, 1000-grain weight, the weight of fresh roots/clum, the weight of dry roots/clum, the weight of fresh leaves and stem/clum, the weight of dry leaves and stem/clum, and grain yield per clum progressed.

Molecular identification of bacterial isolates K324 and K111

The bacterial gDNA extraction followed the recommended procedure, using the Presto™ Mini gDNA Bacteria Kit, Geneaids, USA. Subsequently, the bacterial gDNA amplification by the 16S rDNA gene used universal primers 1492r and 27f and sequencing employing two-way primers (Rahayu *et al.,* 2021a). With the sequencing results formed contiguous, identification continued using the BLAST from National Center for Biotechnology Information (NCBI).

Statistical analysis

Analyzing data on growth and yield traits of black rice used one-way analysis of variance, then subjected to further scrutiny in cases where a significant difference occurs (Rahayu *et al.*, 2021a). Also, the statistical analysis of the data used the SAS ver. 9.2.

Table 2. Bacterial treatments used in the experiment.

Treatment code	Combination of bacterial strains
BE1	K10 + K111 + K324
BE2	K324 + K111
BE3	K10 + K111
BE4	K10 + K324
BE5	K10
BE6	K111
BE7	K324
BE8	Control/ uninoculated/no bacteria

RESULTS

Synergism test of consortium members

Since this experiment used consortium inoculation beside the single one, the presence or absence of resistant action needs checking to ensure the growth of all isolates. The synergism test among the three bacterial isolates (K10, K111, and K324) showed no inhibition even though isolate K324 growth was more dominant, and its colony showed a red-blood color (Figure 1). The absence of growth inhibition indicates no antagonistic reaction among the isolates; thus, consortium injection is possible for execution.

Growth of black rice in agar media

In this study, the vegetative growth of black rice in agar media emerged at 14 days after planting (DAP), with no significant effect of endophytic bacteria inoculation observed (Figure 2), even in the BE2, BE6, BE7 treatments, where a significant inhibition in the black rice plants' growth showed compared with other regimens.

Growth and productivity of black rice in the pot experiment

The bacterial inoculation on soil media toward black rice showed varied values compared with the water agar media. Inoculation with isolate K324 gave a significant increase in growth compared with control (uninoculated) plants, especially in shoot length and number of roots after 30 DAP (Figure 3). In addition, a significant increase in grain yield of black rice occurred at 140 DAP after isolate injection compared with the control. Inoculating with single and consortium bacterial isolates on the soil media also indicated significant effects compared with the control. The specific single inoculation with K111 significantly improved grain yield (Figure 4b) and plant height (Figure 4c).

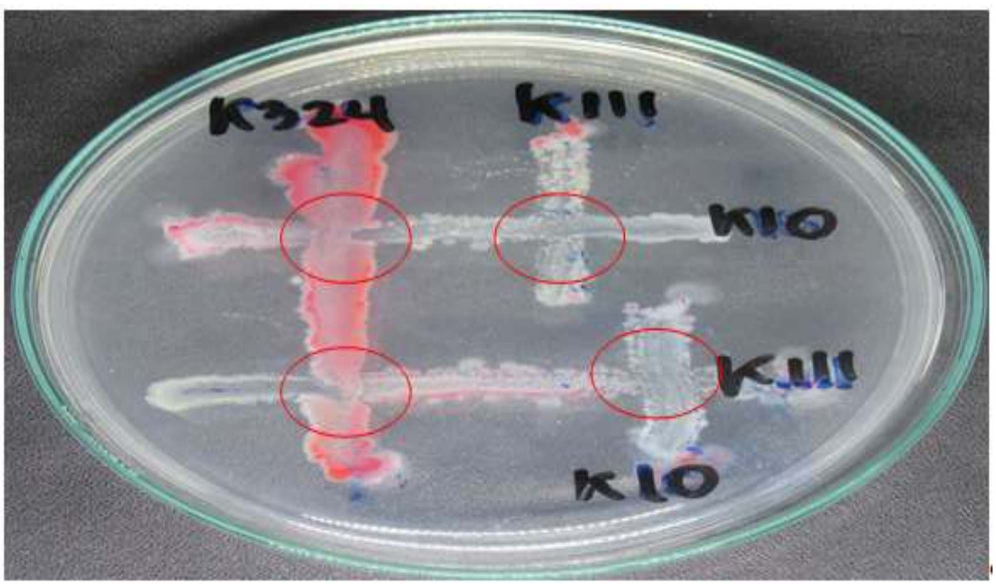

Figure 1. Synergism test among K10, K111, and K324 isolates showed no inhibition zone. Three isolates were crossly streaked on the NA medium and incubated for 24 h. Black ink markers indicate the isolates while the red circles denote the contacted area between two different isolates. This experiment had three repetitions (n=3).

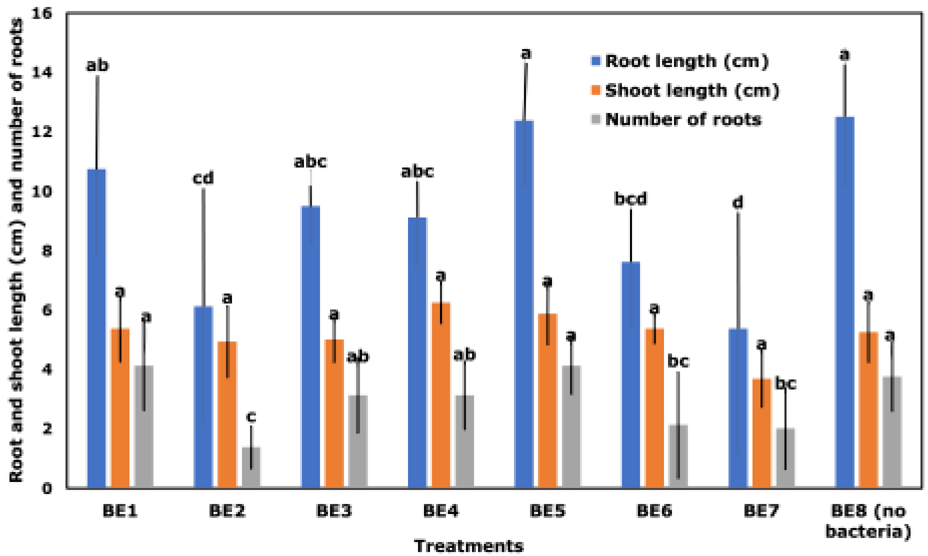

Figure 2. The growth of black rice plant on agar water media at 14 DAP on Duncan Multiple Range Test. Different letter indicates significant difference ($P \leq 0.05$) with eight replicates on each experimental sample (n=8). Bars represent the standard deviation.

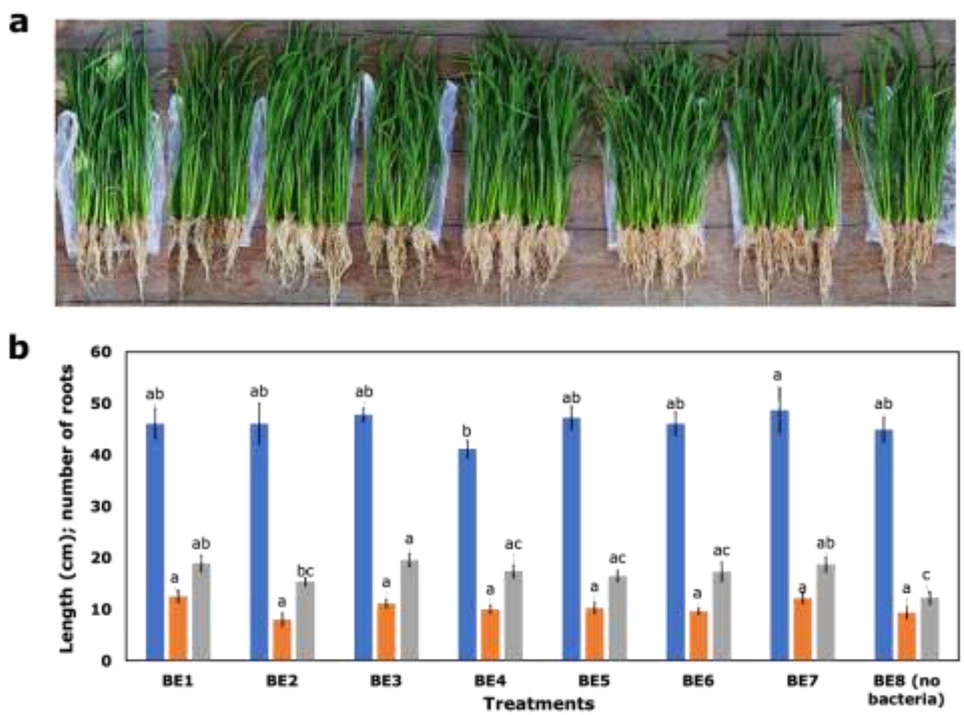

Figure 3. The growth of black rice plant under greenhouse conditions at 30 DAP. **(a)** The photographs of black rice plants. **(b)** Statistical analysis of growth of black rice plants on Duncan Multiple Range Test. Blue box: shoot length, orange box: root length, grey box: number of roots. Different letter indicates significant difference ($P \leq 0.05$) with eight replicates on each experimental sample (n=8); bars indicate the standard deviation.

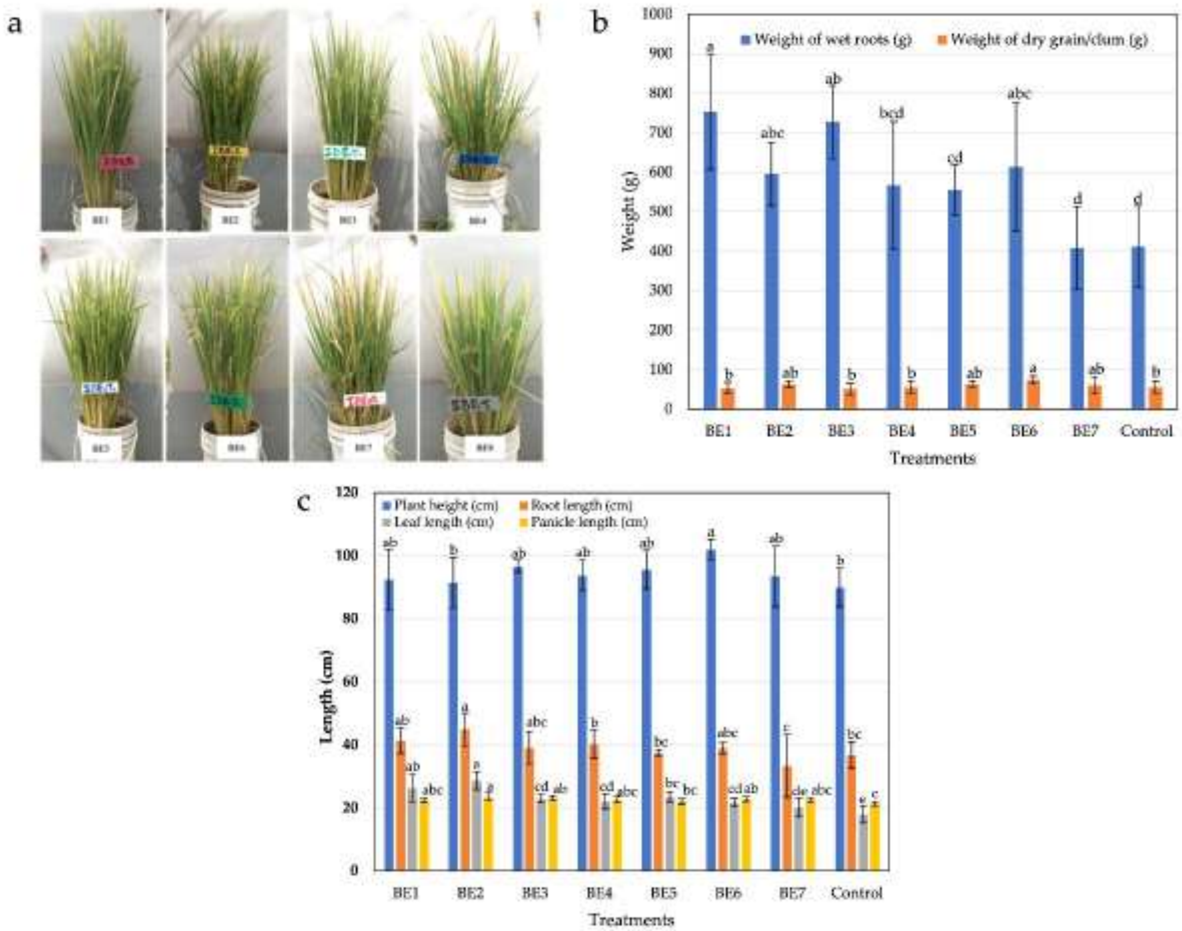

Figure 4. Evaluation of growth of black rice plants at 140 DAP. **(a)** The photograph of black rice plants inoculated by bacteria, (**b**) Statistical analysis of weights of root and dry grain/clum of black rice plants on Duncan Multiple Range Test. (**c**) Statistical analysis of plant height and root, leaf, and panicle lengths at 140 DAP after inoculation on Duncan Multiple Range Test. Different letter denotes significant difference at p ≤ 0.05. The data were analyzed based on eight replicates on each experimental sample (n=8); bars show the standard deviation. Tables 3 and 4 show all the data of black rice productivity.

All the inoculants except isolate K324 improved the growth of roots. Besides, the weight of fresh roots significantly increased at 140 DAP using BE1, BE2, BE3, BE4, BE5, and BE6 treatments. Remarkably, single inoculation with K111 isolate substantially improved the black rice's grain yield. The dry grain/clum weight considerably increased by isolate K111 inoculation at 140 DAP (Figure 4b). These improvements correspond to enhanced plant height and root, leaf, and panicle lengths. These four parameters' notable improvement by single inoculation of isolate K111 is in Figure 4c. The data of black rice at 140 DAP for growth and productivity traits are available in Tables 3 and 4.

Table 3. The effect of endophytic bacteria inoculation on growth parameters under greenhouse conditions (140 DAP). Different letter indicates significant difference ($P \leq 0.05$) with four replicates on each experimental sample (n=4).

Treatments	Plant height (cm)	Number of roots	Root length (cm)	Leaf length (cm)	Width length (cm)	Weight of dry roots (g)	Weight of wet roots (g)
BE1: K10+K324+K111	92.50 ± 9.70 ab	41.37 ± 4.33 a	41.38 ± 4.03 ab	26.30 ± 4.26 ab	1.08 ± 0.06 bc	271.00 ± 118.56 ab	753.25 ± 147.25 a
BE2: K324+K111	91.50 ± 7.95 b	42.00 ± 0.41 a	44.88 ± 5.19 a	28.75 ± 2.78 a	1.05 ± 0.04 c	223.38 ± 38.20 ab	595.50 ± 80.27 abc
BE3: K10+K111	96.62 ± 1.97ab	39.62 ± 6.88 a	39.13 ± 5.07 abc	22.84 ± 1.25 cd	1.15 ± 0.07 ab	298.00 ± 53.46 a	727.75 ± 91.59 ab
BE4: K10+K324	93.75 ± 4.86 ab	45.12 ± 6.54 a	40.38 ± 4.52 b	22.07 ± 2.46 cd	1.18 ± 0.10 a	224.63 ± 65.49 ab	566.38 ± 161.43 bcd
BE5: K10	95.62 ± 6.05 ab	43.00 ± 6.19 a	37.38 ± 1.03 bc	23.53 ± 1.66 bc	1.01 ± 0.06 c	282.88 ± 119.12 ab	555.25 ± 63.18 cd
BE6: K111	102.00 ± 3.10 a	46.62 ± 1.02 a	39.00 ± 1.84 abc	21.74 ± 1.34 cd	1.18 ± 0.06 a	262.25 ± 29.26 ab	613.50 ± 162.38 abc
BE7: K324	93.50 ± 9.65 ab	39.62 ± 10.38 a	33.38 ± 9.93 c	20.10 ± 3.03 de	1.08 ± 0.07 bc	219.63 ± 48.52 ab	407.63 ± 105.32 d
BE8: No bacteria	90.00 ± 6.12 b	42.75 ± 7.29 a	36.75 ± 4.13 bc	17.91 ± 2.73 e	1.10 ± 0.04 abc	190.13 ± 40.66 b	411.38 ± 103.71 d

Table 4. Endophytic bacteria inoculation on the yield and yield component of black rice at 140 DAP. n = 4. Averages followed by the same letter in the column do not show significant differences (DMRT, $p \leq 0.05$).

Treatments	Panicle length (cm)	Number of grain content per panicle	Weight of 1000 grain (g)	Weight of dry grain/clum (g)	Weight of wet leaves and stem (g)	Weight of dry leaves and stem (g)
BE1: K10+K324+K111	22.49 ± 0.77 abc	90.95 ± 13.43 a	2.00 ± 0.00 b	53.38 ± 14.63 b	331.13 ± 49.55 a	166.63 ± 15.47 a
BE2: K324+K111	23.51 ± 1.24 a	88.83 ± 10.15 a	2.00 ± 0.00 b	62.75 ± 8.19 ab	326.75 ± 68.62 a	169.75 ± 36.70 a
BE3: K10+K111	23.06 ± 0.87 ab	84.29 ± 11.80 a	2.13 ± 0.25 ab	51.25 ± 14.17 b	372.38 ± 32.67 a	164.75 ± 16.58 a
BE4: K10+K324	22.64 ± 0.91 abc	78.33 ± 13.35 a	2.00 ± 0.41 b	56.00 ± 15.22 b	364.13 ± 42.20 a	176.38 ± 29.79 a
BE5: K10	22.02 ± 0.91 bc	84.17 ± 7.27 a	2.13 ± 0.25 ab	63.88 ± 7.45 ab	377.88 ± 14.30 a	167.25 ± 11.96 a
BE6: K111	22.87 ± 0.70 ab	89.66 ± 5.38 a	2.50 ± 0.35 a	74.12 ± 9.38 a	375.88 ± 16.25 a	181.50 ± 22.34 a
BE7: K324	22.61 ± 0.70 abc	85.50 ± 6.97 a	2.25 ± 0.50 ab	60.25 ± 21.28 ab	344.13 ± 67.08 a	159.88 ± 29.36 a
BE8: No bacteria	2 1.39 ± 0.57 c	76.25 ± 10.67 a	2.00 ± 0.00 b	56.12 ± 15.04 b	321.13 ± 70.47 a	144.50 ± 40.83 a

Genetic similarity in isolates K324 and K111 within NCBI database

The shared results showed that isolate K324 significantly increased the shoot length and the number of roots on the soil media at 30 DAP. Also, isolate K111 revealed a significant improvement in the grain yield of black rice at 140 DAP. Therefore, the genetic similarity assessment of these two isolates ran through the NCBI database. The result showed the highest identity of isolate K324 corresponds to *Serratia marcescens* subsp. sakuensis strain WTB52, while isolates K111 corresponds to *Variovorax gossypii* strain HNG12 (Table 5). Subsequently, depositing the isolates K324 and K111 in the gene bank received the codes MW0799909 and MW079900, respectively. The high similarity between isolate K324 and *Serratia marcescens* species was supported by the presence of red color pigment of the colony, as shown in Figure 5. Genus *Serratia* was known to produce red-color pigments, namely, prodigiosin (Venil and Lakshmanaperumalsamy, 2009; Gondil *et al*., 2017).

Table 5. The genetic similarity of isolates K324 and K111 through NCBI database.

No.	Isolate ID	16S rRNA closest relative	Identity (%)
1	K324	*Serratia marcescens* subsp. *sakuensis* strain WTB52	98.96%
2	K111	*Variovorax gossypii* strain HNG12	99.48%

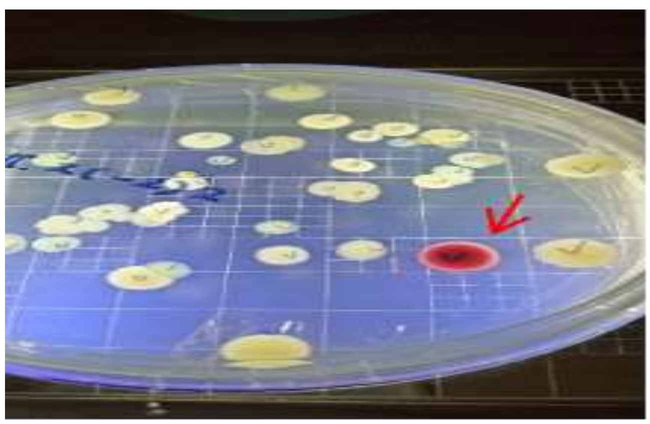

Figure 5. The appearance of isolate K324 colony. The arrow points the red-color colony which may be an indication of *Serratia* species and correspond to the results of similarity analysis.

DISCUSSION

The inoculation of three isolates in the agar media showed significant inhibition. Extreme inhibition occurred in the consortium inoculation of isolates K324 and K111 (Figure 2). Presumably, the inhibition factor on the agar media was due to the red pigment of K324 (Figure 5). A report stated that the red pigment (prodigiosin) of *Serratia* species caused a significant reduction in chlorophyll-a and, consequently, a reduction in the photosystem II electron flow. It means prodigiosin remarkably inhibited the photosynthesis process, with the initial growth of black rice plants subdued similarly (Zhang *et al.,* 2017). Furthermore, the inability of isolate K324 to produce IAA (Table 1) also causes stunted growth, considering IAA's main function is to support plant growth and development (Ngoma *et al.,* 2012).

In addition, the BE2 treatment on black rice plants' growth revealed consortium inoculation of isolates K111 and K324 was somewhat suppressive, especially on root length parameters (Figure 2). Based on the PGP characterization, isolate K111 did not fix N; however, it produced IAA at 72.6 ppm (Table 1). These results indicated that nitrogen fixation is vital during the early stages of plant growth, as stated by Triadiati *et al.* (2012), where plants exhibited active N nutrients absorption during the vegetative stage.

Expectedly, combining the two isolates into a consortium produced PGP character complementation to support the growth of rice plants (Table 1). However, based on the acquired results, the plant's growth appeared hampered on the agar media (Figure 2). The synergism test did not show an inhibitory reaction, yet, the growth with the inoculant K324 was more dominant (Figure 1). Consequently, prodigiosin was in high concentration, and it probably inhibited the photosynthesis process, hindering the black rice's growth (Zhang *et al.,* 2017).

The growth of black rice plants on soil media with BE7/K324 was much superior versus that on water agar (Figure 3), which may refer to prodigiosin produced by isolate K324. The agar media, a sterile medium, can cause a high concentration of prodigiosin, consequently inhibiting black rice growth. On soil media*,* isolate K324 associated with other microbes, subsequently lowering the concentration of prodigiosin and enabling the bacteria to exhibit PGP properties. Prodigiosin production gained impacts from carbon and nitrogen sources, pH, metal ions, temperature, and incubation time (Gondil *et al.*, 2017). This phenomenon is an engaging study on injecting *S. marcescens* in plants grown on sterile and non-sterile media. Based on reference studies, prodigiosin compound exhibits antimicrobial (Lapenda *et al*., 2015; Choi *et al.,* 2021), anticancer **(**Darshan and Manonmani, 2015), and anti-larvae and anti-nematode activities (Lin *et al.,* 2020). A report with similar results showed that *S. marcescens* significantly increased the yield of rice production in the field condition by enhancing zinc content in the rice grain and activating the zinc translocation enzymes (Shakeel *et al*., 2023). However, there are limited reports on the effect of *S. marcescens* inoculation on rice plant growth in sterile and non-sterile media.

The shoot length and number of roots also indicate black rice growth improvement (Figure 3). It relates to the endophytic bacterial colonization initiated at the roots, then spreading to the top of the plants (Kumar *et al.,* 2020). It means the roots respond earlier, compared with the other organs. Therefore, the enhanced root growth reflects shoot growth, indicated by the plant height and fresh weight. It links to the chief function of roots to absorb nutrients from the media as materials for metabolism by plants for growth and development. According to previous studies, the root system is the site of water and nutrient uptake from the soil, a sensor of abiotic and biotic stresses, and a structural anchor to support the shoot development (Hannan *et al.,* 2021)**.** The system communicates with the shoot, and the shoot, in turn, sends signals to the roots (Nibau *et al.*, 2008).

In the generative phase at harvest (140 DAP), the growth parameters showed a significant effect of endophytic bacteria inoculation in almost all the parameters compared with control plants, with the best PGP activities produced by isolate K111, followed by treatment BE3, BE2, BE1, BE5, BE4, BE7, and BE8. Generally, the inoculum treatment in consortium form produced superior results, compared with the single inoculum, except for inoculation using K111 isolate. This inoculum exhibited multi-trait PGP because the treatment produced 72.6 ppm IAA, 41.67% siderophores, and had a PO_4 solubilization index of 2.8 (Table 1). According to previous studies, this isolate served mostly for bioremediation and only a few times as PGP, especially for black rice (Posman *et al.*, 2017; Benedek *et al.,* 2021; Lerner *et al.*, 2021). Further reports on the said genus increased the growth in barley and fenugreek (Toukabri *et al.,* 2021), tomato (Kim *et al*., 2018), and *Pisum sativum* (Jiang *et al.,* 2012). Moreover, based on the yield related traits, the isolate K111 also produced the best results, strengthening the conclusion that inoculant

K111 has the potential for endophytic PGPB in black rice plants.

The treatment BE3, a consortium of K10 and K111 isolates, also exhibited potential as PGP for black rice (Table 3). Based on the PGP characteristics, both strains showed complementary traits (Table 1) and balanced the growth, meaning neither of the isolates was more dominant (Figure 1). Conversely, the consortium with K324 isolate supported the progress and harvest of black rice; however, the grain yield was lower than the treatment without K324 isolate (Tables 3 and 4).

The consortium treatment can increase black rice's growth and grain yield (Figure 4). Although, the single injection using isolate K111 generally showed a dominant influence compared with the control, especially for the traits, i.e., plant height, root length, leaf length, panicle length, root weight, and dry grain weight/clum, with an increase of 13.33%, 6.1%, 21.4%, 6.9%, 49.1%, and 32.1%, respectively (Figure 4). The preparation of the consortium as a bioinoculant considers the complementation of characters as PGP and the inhibitory effect due to the secondary metabolites formed and the density of cells localized in the roots. According to Natsadorj (2019), a strain similar to K111 isolate, *Variovorax* sp. HRRK 170 showed a contradictory effect, with the discovery that the strain required a particular cell density to help the significant growth of Chinese cabbage and green pepper.

The presented results provide critical information that the K324 isolate displayed support for the growth of black rice on the soil media, but not on agar media. Isolate K111 from the "Klutuk" banana plant has the potential to improve black rice growth and yield-related traits and, therefore, is valid and beneficial as a bioinoculant for black rice seeds. Notably, inoculation using single endophytic bacteria showed a significant difference from consortium inoculation. However, the mechanism of this action needs more examination in future research. Also, further research needs implementation to develop a consortium among isolated K111 and other species, considering its potential as PGP.

CONCLUSIONS

Three isolates of endophytic bacteria obtained from "Klutuk" banana showed no antagonistic action through in vitro assay. In addition, single inoculation of isolate K324 significantly increased the shoot length and the number of roots of black rice. The productivity of black rice improved significantly with single inoculation of bacterial isolate K111. Furthermore, consortium injection using K10 and K111 isolates demonstrated promising potential as PGP agents for black rice. The novel results provided authentication of endophytic bacterial isolates obtained from "Klutuk" banana to improve black rice growth and yield-related traits.

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