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Role of rice bacterial endophytes from organic and conventional paddy fields as biofertilizers and bio-resistance inducers against insecticide toxicity

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Abstract: Different management methods of paddy fields affects plant growth and associated microbiota, including endophytes over long periods. This study aimed to isolate and compare the functional capabilities of endophytic bacteria from the roots and leaves of rice plants cultivated in organic and conventional paddy fields. After surface sterilization of the roots and leaves samples the endophytic bacteria were isolated on agar plates using NA, YEMA, and Pikovskaya media. Results showed the higher diversity and population density of endophytic bacteria in rice plants from organic fields compared to conventional fields. A total of 28 distinct bacterial colonies were purified, subcultured, and characterized for multifunctional abilities as biofertilizers and resistance to insecticides (*chlorantraniliprole* 100 g L^{-1} and *thiamethoxam* 200 g L^{-1}). Each of the selected isolates of YEROI-1, PELOI-3, and NEROI-2 that were closely related to *Burkholderia* sp., *Burkholderia seminalis* and *Bacillus paramycoides*, respectively. The isolates and control were applied to rice seedling in agarose medium with and without insecticide (10 x) dose using completely randomized design with three replications. The result showed that isolate NEROI-2 significantly increased rice seedling growth and induced resistance to insecticide toxicity, followed by PELOI-3. In contrast, YEROI-1 inhibited rice seedling growth. The present finding revealed the functional capability of the selected isolates by *in vitro* and *in vivo* (soilless media) assessments. Further studies are needed to confirm the capability of the selected isolates in a greenhouse experiment by using soil media.

Keywords: *in vivo* assessment; multifunctional isolate; resistance test; rice cultivation system; rice seedling.

Abbreviations: DAP_days after planting; NA_nutrient agar; NB_nutrient broth; NFB_nitrogen fixing bacteria; KSI_ potassium solubilization index; PSB_phosphate solubilizing bacteria; PSI_phosphate solubilization index; YEMA_yeast extract mannitol agar.

Introduction

The total area of organic rice land increased sharply from 1,401 ha in 2016 to 53,974 ha in 2018 or around 21% of the 251,631 ha total organic agricultural land in Indonesia (Organic Institute et al., 2019), meaning that it is only around 0.47% of the 11,377,934 ha total area of rice harvested land (BPS-Statistics Indonesia, 2022). This fact reveals that rice cultivation practices in Indonesia are still dominated by conventional farming systems, which rely on manufactured chemical inputs such as various chemical fertilizers and pesticides. The effects of the excessive use of chemical fertilizers are not visible immediately, but the pollution impact emerged over the time, including accumulation of nitrate and phosphate that results in water eutrophication, soil degradation, soil salinity (Chislock et al., 2013), and accumulation toxic heavy metal in agricultural topsoil (Hu et al., 2024). The toxic effects of accumulated of pesticide residues have broad impacts on ecosystems, including soil degradation or decreased soil health (Maggi et al., 2020), adverse effects on the biotic environments of non-target organism (Wandscheer et al., 2017) and decrease in biodiversity (Simarmata et al., 2023). These residues can also cause phytotoxic effect on the cultivated plants (Ajermoun et al., 2022) and impacts non-biotic environments (Abdullah et al., 2017). Consequently, this toxicity effect leads to reduced plant productivity and poses risks to human health (Hernández et al., 2013). Various efforts to overcome the toxic effects of pesticide residues on agricultural lands have been widely reported, either by applying various ameliorant materials such as such as compost (Marín-Benito et al., 2018), biochar (Arridho et al., 2019), plant residue and animal dung (Joshi et al., 2019), using microbiota which act as bioremediation agents or pesticide degraders (Castro-Gutiérrez et al., 2019) or by using a plant-based approach in phytoremediation (Gao et al., 2023). Long term land use with different cultivation systems such as organic and conventional farming systems has been proven to affect the chemical, physical and biological characteristics of the soil, and also to the characteristics of the grown plants and the associated microbiota (Bai et al., 2020). This fact indicates that plants as the productive resources are influenced direct and indirectly by the given inputs to the cultivated land either in the form of healthy organic inputs or toxic chemical inputs, in which consequently affects the abundance, diversity, characteristics and functional abilities of their endophytic bacteria (Shofiyah et al., 2023; Sunaryo et al., 2019).

Bacterial endophytes are a class of endosymbiotic microbiota widespread among plants that live or spend at least parts of their life cycle inside plants or in plant tissues (Hardoim et al., 2015) or colonize intercellular and intracellular spaces of all plant compartments (Miliute et al., 2015). Previous studies reported that endophytic bacteria do not visibly harmful effects on the plants (Hallmann et al., 1997), do not cause plant disease or significant morphological changes (Miliute et al., 2015). However, some other studies reported a range of different lifestyles of endophytic bacteria with their host, the most common endophytes are typed as commensals, with unknown or yet unknown functions in plants, and less common ones are detected to have positive (symbiotic, mutualistic, trophobiotic)

or negative (antagonistic, pathogenic) effects on plants (Brader et al., 2017; Hardoim et al., 2015).

Many studies have successfully isolated rice bacterial endophytes from leaves, roots, stems and seeds of several rice varieties (Kumar et al., 2020; Sun et al., 2008), or from rice plants grown in flooded conditions, dry conditions, phase changes after flooding (Bertani et al., 2016; Ferrando and Fernández Scavino, 2015), and wild rice plants (Koomnok et al., 2007), and organic cultivation (Phetcharat and Duangpaeng, 2012). Furthermore, previous studies reported that the endophytic bacteria of rice plants has the ability to produce IAA and siderophores, dissolve phosphate, fix nitrogen, antagonistic activity against disease and the overall effect of increasing plant growth (Ferrando and Fernández Scavino, 2015; Kumar et al., 2020). Rice endophytes were reported in some studies for their multifunctional abilities as biofertilizers coupled with a function as pesticide degraders, such chlorpyrifos, diazinon, and fungicides of etridiazole, metalaxyl, and tricyclazole (Shen et al., 2019).

There is limited information about the comparison of functional capabilities of rice endophytic bacteria from different cultivation system of organic dan conventional paddy fields. The present study aims to explore, characterize and assess the functional capabilities of endophytic bacteria of rice plants from organic and conventional paddy fields as biofertilizers with high resistance to insecticide toxicity that potentially support plant growth by supplying plant nutrients and inducing plant resistance to insecticide toxicity.

Results and Discussion

Diversity and population density of endophytic bacteria

The diversity and population density of the rice endophytic bacterial colonies on the respective media of YEMA, Pikovskaya, and NA are presented in Table 1. The representative endophytic bacterial colonies grown in each medium are presented in Supplementary Figure 1. As shown on Table 1, the diversity and population density of rice endophytic bacteria isolated from organic paddy field either for general bacteria (grown on NA medium) or specific functional bacteria of PSB (grown on Pikovskaya medium) and NFB (grown on YEMA medium) were higher than those isolated from conventional paddy field. It is important to note that the total PSB presented in Table 1 refers to the total number of bacterial colonies grown on Pikovskaya medium, including both the isolates that were able to form a halo zone of phosphate solubilization and those that were not. The population density of general and specific functional endophytic bacteria isolated from the roots of rice plants was higher than those isolated from the leaves, both on organic and conventional paddy fields. Each morphologically distinct bacterial colony from each medium was chosen and sub-cultured for the further examination. In total we obtained 28 isolates, consisted of 4 isolates from YEMA medium, 13 isolates from NA medium, 11 isolates from Pikovskaya medium. The colony morphology is described in Supplementary Table 1. On YEMA medium, colony morphology of all isolates from rice roots and leaves either from organic or conventional paddy field showing the same type of morphology. On NA medium, there were two isolates from rice roots and leaves on the both paddy field which showed the same colony morphology. No isolate grown on Pikovskaya showed the same colony morphology. Several previous studies have reported the presence and functional capability of endophytic bacteria in rice plant in diverse conditions. Shofiyah et al. (2023) reported that the population density of endophytic bacteria on YEMA and Pikovskaya media isolated from the root of rice plants var. IR 64 grown on Inceptisol of conventional paddy field was higher than those in the leaves. Endophytic diazotrophic bacteria from various tissues (root, stem, and leaf) of six cultivar rice plant showed the highest population density in root tissue at nursery stage (Rangjaroen et al., 2015). Inoculation of endophytic bacteria isolated from the roots and leaves of rice plants on saline land to the rice plant in greenhouse experiment resulted in the higher population density of general endophytic bacteria in the roots compared to those in the shoots (Setiawati et al., 2021). The present study showed the similar results comparing with those three studies that the general and specific nitrogen fixing and phosphate solubilizing endophytic bacteria in the different rice cultivar, soil media, and cultivation system have the higher population density in the root tissues than in leaves. The fact was explained by Chi et al. (2005) that the root is the entrance of endophytic bacteria to enter the shoot plant tissue, so the population of endophytic bacteria in the root is higher than other plant tissues.

As shown in Table 1 Shannon diversity index on all the isolated endophytic bacteria revealed that the diversity index of endophytic bacteria from organic paddy field was higher than that from conventional paddy field. Moreover, the Shannon diversity index of endophytic bacteria isolated from rice roots was higher than those isolated rice leaves in both paddy fields. Similar to the rice endophytes observed in conventional rice fields in this study, other research has also reported that the Shannon diversity index of rice endophytes in the root is the highest when compared to the index in the rice stem and leaf. Shannon diversity index (*alpha*) of endophytic bacteria isolated from *Oryza officinalis* in the flowering phase showed the highest diversity in the roots compared to stems and leaves (Tian et al., 2023). Borah et al. (2018) also reported that Shannon diversity index of endophytic bacteria was higher in cultivated rice compared to wild rice with the highest diversity in roots (H=2,718:H=1,946) compared to stems (H =2,659:H=1,609) and leaves (H=2,393:H=1,386) in both cultivated and wild rice.

The population of endophytic bacteria is influenced by many factors including plant age, species, soil tillage, and environmental conditions (Sulistiyani and Meliah, 2017). Paddy field cultivation with organic systems results in high diversity and colony density. Organic processing systems that use inputs of various types of organic materials provide more diverse nutrients and growth factors that are different from chemical inputs which concentrated for certain nutrients. Microbial composition is supported by the host genotype producing root exudates in the rhizosphere and agricultural practices such as fertilization, tillage and type of cropping system influence this (Sessitsch and Mitter, 2015). Long-term organic cultivation can improve soil structure and functions and increase microbial diversity and population. Sunaryo et al. (2019) reported that addition of liquid fertilizer from goat manure resulted in a high population density of endophytic bacteria in potato and black nightside plants, as well as in those plants that had been treated with cuttings compared to the control treatment. The functional contributions of endophytic colonization depends on many variables, including plant tissue type, plant genotype, plant biotic and abiotic conditions (Hardoim et al., 2015).

Cellular and physiological characteristic

The observation on the cellular and physiological characteristic of 28 isolates was summarized on Table 2. Four bacterial isolates from YEMA medium, 5 out of 13 from NA medium, and 5 out of 11 from Pikovskaya medium were characterized as gram negative bacteria. Kumar et al. (2020) recorded 19 isolates (59.3%) of gram-positive bacteria and 13 isolates (40.6%) gram negative bacteria of endophytic bacteria isolated from roots, stems, leaves and grains of 6 different rice varieties. The endophytic bacteria found in the present study showed a higher proportion of gram-negative (55.1%) bacteria compared to gram-positive bacteria (44.8%) compared to previously reported (Kumar et al., 2020).

All bacterial isolate in three media showed motility activity. The shape of all bacterial isolate from YEMA was coccus, while the shape of bacterial cellular from NA and Pikovskaya media was diverse in coccus, bacillus, and coccobacillus. The diversity of physiological characteristic of endophytic bacterial isolate from YEMA, NA, and Pikovskaya media were as follows: the range of temperature 27-45◦C, the range of pH 5-9, and the range of NaCl tolerance of 2-6%, with the dominant member were found at temperature 27◦C, pH 7, and NaCl tolerance 2%.

Table 1. Population density and diversity index of endophytic bacteria from rice plant on various media.

Sources of isolates	NA			Pikovskaya			YEMA		
	Population density of endophytic bacteria (Log ^{10} CFU g^{-1})	Number of distinct colony morphology	Shannon Diversity Index (H')	Population density of endophytic bacteria (Log ^{10} CFU g^{-1})	Number of distinct colony morphology	Shannon Diversity Index (H')	Population density of endophytic bacteria (Log ^{10} CFU g^{-1})	Number of distinct colony morphology	Shannon Diversity Index (H')
Organic paddy field									
Root	5.86±0.00a	4	1.31±0.16a	4.83±0.05a	4	1.25±0.36a	5.84±0.00a	1	0.00±0.00
Leaf	5.60±0.00b	4	1.16±0.36a	4.12±0.25b	4	1.01±0.45a	5.53±0.00b	1	0.00±0.00
Conventional paddy field									
Root	4.99±0.02c	4	0.87±0.34ab	3.42±0.24c	1	0.00±0.32c	5.40±0.01c	1	0.00±0.00
Leaf	4.95±0.03d	4	0.57±0.47b	3.21±0.32c	2	0.49±0.00b	2.56±0.21d	1	0.00±0.00
ANOVA	0.00**		0.00**	0.00**		0.00**	0.00**		

Remarks: Significant level: ns=no significant, * = p <0.05, ** = p < 0.01. Means followed by same letters are not significantly different at 5% level by DMRT; H'= Shannon-Wienner diversity index, H'<1 = low diversity, 1<H'<3 = medium diversity, H'>1 = high diversity.

Table 2. Cellular and physiological characteristic of endophytic bacteria from rice plant

Isolate code	Cellular Characteristic		Physiological Characteristic				
	Shape	Size	Gram stain	Motility	Optimum temperature	Optimum pH	NaCl resistance
YE_ROI-1	Coccus	1.19 µm	Negative	Motile	27◦C	7	2%
YE_LOI-1	Coccus	1.64 µm	Negative	Motile	45◦C	5	2%
YE_RCI-1	Coccus	2.80 µm	Negative	Motile	45◦C	7	2%
YE_LCI-1	Coccus	1.47 µm	Negative	Motile	27◦C	7	2%
NE_ROI-1	Coccus	1.19 µm	Positive	Motile	27◦C	7	2%
NE_ROI-2	Bacillus	1.67 µm	Positive	Motile	27◦C	7	2%
NE_ROI-3	Bacillus	1.00 µm	Positive	Motile	45◦C	9	2%
NE_ROI-4	Bacillus	1.26 µm	Positive	Motile	27◦C	7	2%
NE_LOI-1	Coccobacillus	1.57 µm	Negative	Motile	27◦C	7	6%
NE_LOI-2	Coccus	0.89 µm	Positive	Motile	27◦C	7	2%
NE_LOI-3	Coccobacillus	1.44 µm	Negative	Motile	45◦C	9	2%
NE_LOI-4	Coccus	1.35 µm	Positive	Motile	27◦C	9	2%
NE_RCI-1	Coccus	1.32 µm	Positive	Motile	27◦C	9	6%
NE_RCI-2	Coccus	0.77 µm	Negative	Motile	27◦C	7	2%
NE_RCI-3	Coccus	1.10 µm	Negative	Motile	45◦C	9	2%
NE_LCI-1	Bacillus	1.26 µm	Positive	Motile	27◦C	9	2%
NE_LCI-2	Coccobacillus	0.76 µm	Negative	Motile	27◦C	9	6%
PE_ROI-1	Coccobacillus	1.44 µm	Positive	Motile	45◦C	5	2%
PE_ROI-2	Coccus	1.13 µm	Positive	Motile	45◦C	7	2%
PE_ROI-3	Coccus	0.91 µm	Positive	Motile	27◦C	7	2%
PE_ROI-4	Bacillus	2.23 µm	Positive	Motile	27◦C	5	2%
PE_LOI-1	Coccus	0.55 µm	Positive	Motile	27◦C	5	2%
PE_LOI-2	Coccus	0.55 µm	Negative	Motile	27◦C	9	2%
PE_LOI-3	Coccus	0.89 µm	Negative	Motile	27◦C	5	2%
PE_LOI-4	Coccus	0.78 µm	Negative	Motile	27◦C	5	2%
PE_RCI-1	Coccus	1.55 µm	Negative	Motile	27◦C	5	2%
PE_LCI-1	Coccus	0.89 µm	Positive	Motile	27◦C	7	2%
PE_LCI-2	Coccus	0.74 µm	Negative	Motile	27◦C	9	2%

Remarks: Y = YEMA medium, N = NA medium, P = Pikovskaya medium, E_R = Endophytic bacteria from root of rice plant, E_L = Endophytic bacteria from leave of rice plant, OI = Organic paddy field, CI = Conventional paddy field.

Multi-functional capability of endophytic bacteria isolates

Of the four isolates obtained from YEMA medium, two demonstrated the ability to solubilize phosphorus (P) and potassium (K) as well as fix nitrogen, one isolate exhibited the ability to solubilize phosphorus and fix nitrogen, while the remaining isolate was capable only of nitrogen fixation. All isolates from NA medium (13 isolates) had functional capability of nitrogen fixation except one isolate of NE_LOI-1, and only two isolates showed the functional capability in the solubilization of P namely isolates NE_ROI-1 and NE_ROI-2. A total of 11 isolates from Pikovskaya medium showed the functional capability for three nutrients, in the solubilization of P and K, and fixation of nitrogen. We suggest that utilization of general agar medium for isolation is not always yield the target specific microbes. The present study revealed that isolation procedure by using specific media directly resulted in the higher isolated target bacteria compared with using general medium. Phosphate solubilization index is assessed based on a scale formulated by Sati and Pant, (2019) values < 2.0 classified as low solver, values 2.0 - 3.0 classified as medium and values > 3.0 classified as high solver. The present study classified 8 isolates as medium-solver and 8 isolates as low-solver based on classification value of PSI (Sati and Pant, 2019). Sudewi et al. (2020) reported that 19 isolates of endophytic bacteria isolated from the roots of rice plants with 6 different locations using NA medium, then sub-cultured on Pikovskaya medium and showed clear zone formation with PSI between 2.00 - 2.53.

Among all the isolates from Pikovskaya medium showed the capability of K solubilization with KSI at the range of 2.3-3.12, while the highest KSI was obtained by isolate PE_LOI-3. Furthermore, two isolates from YEMA showed the value of KSI at 2.21 and 2.83. All isolates capable of K solubilization were also exhibited the P solubilization, whereas the isolates capable of P solubilization were not always indicated the capability in K solubilization. Azizah et al. (2020) reported isolated endophytic bacteria from the roots, stems, and leaves of maize and showed that 4 isolates grown on Pikovskaya medium were also able to form clear zone on Aleksandrov medium as an indication of the

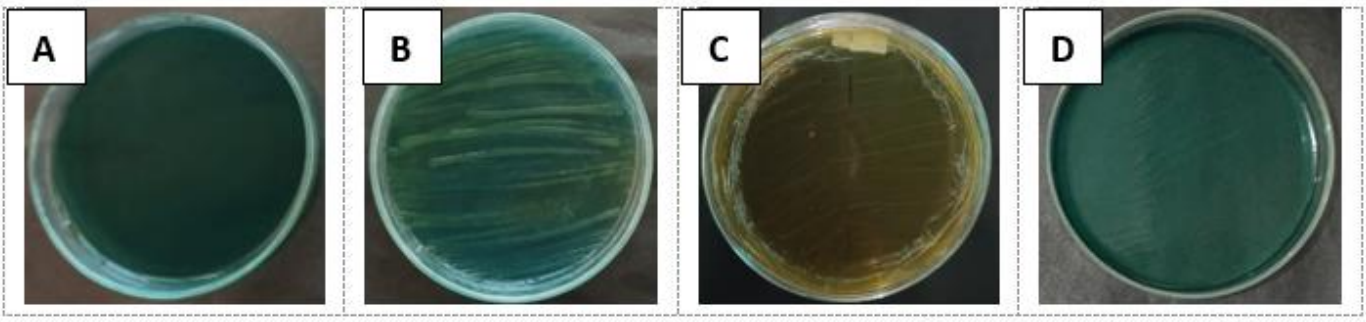

Figure 1. Representation of nitrogen fixation examination with BTB. A = control, B = isolate YEROI-1 (greenish blue to yellowish green and the color of colony became yellowish), C = Isolate PELOI-3 greenish blue to yellow, D = isolate NEROI-2 greenish blue to dark blue.

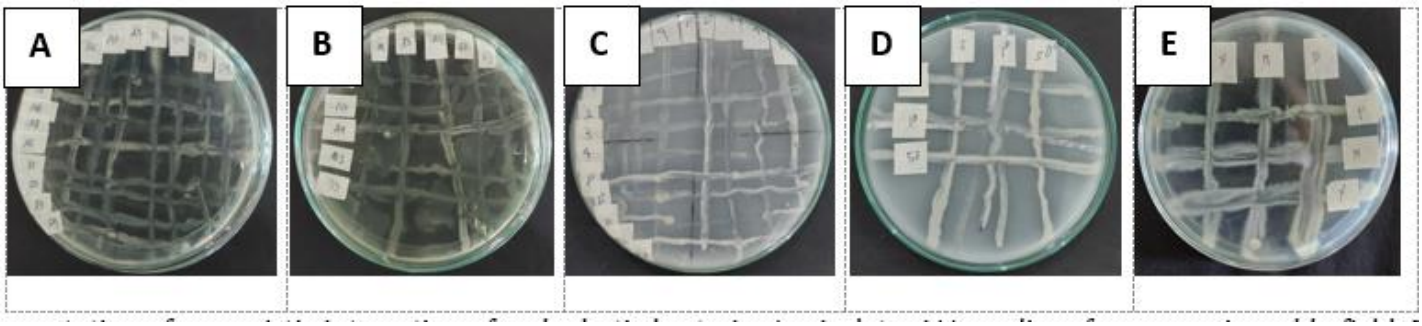

Figure 2. Representation of synergistic interaction of endophytic bacteria. A = isolates NA medium from organic paddy field, B = isolates NA medium from conventional paddy field, C = isolates Pikovskaya medium from organic paddy field, D = isolates Pikovskaya medium from conventional paddy field, E = 3 superior isolates from various media.

solubilization of potassium. The mechanism by which endophytic bacteria solubilize phosphate and potassium involves the secretion of organic acids, such as oxalic acid, tartaric acid, and gluconic acid, which are capable of dissolving insoluble minerals (Eid et al., 2021). To confirm the functional capability in nitrogen fixation for the three selected endophytic bacteria (isolate YEROI-1, PELOI-3, and NEROI-2), the continued examination was conducted by streaking each isolate to YEMA medium with the addition of Bromothymol blue (BTB). As shown in Figure 1, isolate YEROI-1 changed the color of media YEMA+BTB from greenish blue to yellowish green and the color of colony became yellowish. Isolate PELOI-3 affected the color alteration of YEMA+BTB medium and the colony to yellow, whereas the isolate NEROI-2 changed the color of YEMA+BTB medium to dark blue. According to (Howieson and Dilworth, 2016) the three isolates have proved a positive function in nitrogen fixation, by which isolates YEROI-1 and PELOI-3 showed the mechanism of organic acid production and mediated the acidification of the medium. In contrast, the isolate NEROI-2 showed the mechanism ammonium production and mediation of medium (Sulistiyani and Meliah, 2017). This interesting finding showed that endophytic bacteria isolated from the root and leave of rice plant using YEMA medium have functional capability of nitrogen fixation by in-vitro assessment. YEMA medium is designed for cultivation of nitrogen fixing bacteria which are symbiotically associated with legumes. Numerous previous studies have reported the presence of these symbiotic nitrogen-fixing bacteria (NFB) in non-leguminous plants, such as sugarcane (Muangthong et al., 2015), cassava (Zhang et al., 2022), and paddy rice (Alvarez et al., 2020). These bacteria contribute to plant growth by supplying nitrogen through nitrogen fixation in plant tissues (Mahmud et al., 2020) and producing phytohormones like IAA and ethylene (Rangjaroen et al., 2015).

Resistance of endophytic bacteria against insecticide

The result of resistance test of 28 endophytic bacterial against 5x and 10x doses insecticides are presented in Table 4. Among all isolates, isolate NEROI-2 showed the highest resistance capability to insecticide with 5x recommended dose. Isolate NEROI-2 (with insecticide) showed the highest growth with absorbance value of 2.322, thus it was resulted a cell density gap or absorbance different by 0.374. In the second resistance test using 10x dose insecticide, although it did not show the higher absorbance value (the growth of cell density), but isolate NEROI-2 consistently showed the highest absorbance difference or cell density gap (the difference cell density in medium with and without 10x dose insecticide). Based on the high resistance to insecticide performed by isolate NEROI-2, and supported by the multifunctional capabilities in phosphate solubilization and nitrogen fixation (Table 3), the isolate NEROI-2 was selected as one as the superior isolates which then subjected for the further examination. The higher growth of cell density in the treatment with insecticide with high dose (5-10x) clearly revealed the high resistance of the bacterial isolate to insecticide. Moreover, it showed the capability of bacterial isolate to use insecticide in supporting their cellular growth. Some previous reported that some microbia isolate capable use insecticide as the carbon or energy sources. Chia et al. (2024) stated that microbes are capable to break down certain pesticide compounds that serve as carbon and energy sources, thus further paving the way for remediation processes. Growth of *Sphingomonas* sp. strain HJY from the leaves of Chinese chives on MSM medium including 20 mg L^{-1} chlorpyrifos showed an increased cell density on day 3 and then decreased slightly on day 6 to 15 (Feng et al., 2017). These results indicate that strain HJY can use chlorpyrifos as the only carbon source. The decrease of *Sphingomonas* sp. strain HJY after day 3 might be attributed to the fact that endophytic bacteria isolated from plant tissues usually do not experience high levels of stress compared to bacteria from polluted soils or water.

This finding explained that two crucial criteria should be considered to evaluate the resistance capability to insecticide of each bacterial isolate: the first is the growth capacity of each isolate in determined culture medium with insecticide and the second is the comparison of the growth bacteria cell density gap (absorbance different) between the treatment with and without insecticide. In addition, various biotic and abiotic factors affect the resistance capability of bacteria isolate to insecticide, such as the age of isolate, preservation condition of isolate, temperature during incubation, etc.

Table 3. Multi-functional capability of endophytic bacteria from rice plant.

Isolate Code	PSI	KSI	Symbiotic Nitrogen Fixing Bacteria
YE_ROI-1	2.65	2.83	Positive
YE_LOI-1	2.25	nh	Positive
YE_RCI-1	2.75	2.21	Positive
YE_LCI-1	nh	nh	Positive
NE_ROI-1	2.39	nh	Positive
NE_ROI-2	2.24	nh	Positive
NE_ROI-3	nh	nh	Positive
NE_ROI-4	nh	nh	Positive
NE_LOI-1	nh	nh	Negative
NE_LOI-2	nh	nh	Positive
NE_LOI-3	nh	nh	Positive
NE_LOI-4	nh	nh	Positive
NE_RCI-1	nh	nh	Positive
NE_RCI-2	nh	nh	Positive
NE_RCI-3	nh	nh	Positive
NE_LCI-1	nh	nh	Positive
NE_LCI-2	nh	nh	Positive
PE_ROI-1	1.07	2.84	Positive
PE_ROI-2	1.43	2.45	Positive
PE_ROI-3	1.00	3.03	Positive
PE_ROI-4	1.00	3.05	Positive
PE_LOI-1	1.60	3.1	Positive
PE_LOI-2	2.52	2.3	Positive
PE_LOI-3	2.14	3.12	Positive
PE_LOI-4	2.00	3.02	Positive
PE_RCI-1	1.51	3.08	Positive
PE_LCI-1	1.00	2.74	Positive
PE_LCI-2	1.56	3.08	Positive

Remarks: Y = YEMA medium, N = NA medium, P = Pikovskaya medium; E_R = Endophytic bacteria from root of rice plant, E_L = Endophytic bacteria from leave of rice plant; OI = Organic paddy field, CI = Conventional paddy field; nh = no halo zone; PSI values < 2.0 classified as low solver, values 2.0 - 3.0 classified as medium and values > 3.0 classified as high solver.

Synergism test

Before preparing a formula of bacterial culture as consortium consisted of several endophytic bacterial isolate, it is important to evaluate the synergic and antagonistic interaction among all the isolates by synergism test. If there is an inhibition zone that appears in the interaction of among the isolates, it indicates that one or more of individual isolate show antagonistic interaction among others (Ethica et al., 2019). The synergy streak method can evaluate the inhibitory activity of one bacterial isolate to produce antagonistic properties against several other more sensitive bacteria (Dinata et al., 2021).

The present study was arranged to prepare a consortium containing three superior isolates based on the functional capability as biofertilizer and as insecticide bio-resistance inducer. According to the functional capability as biofertilizer, isolate PE_LOI-3 and isolate YE_ROI-1 was selected as the superior of phosphate solubilizing and nitrogen fixing endophytic bacteria, respectively. As insecticide bio-resistance inducer, isolate NE_ROI-2 was selected for its superior endophytic bacteria activity. The three selected isolates were then subjected for the synergism test on NA medium. The results of the synergism test (Figure 2) of the three isolates on NA medium showed synergic interaction among other and there was no antagonistic interaction. The three isolates proved the capability to grow together in synergic interaction with no inhibitory activity among other. Synergism between two or more microbes occurs when they do not compete for the same nutrient source (Setiawati et al., 2021) and there is inhibition or antagonistic activity among the microbes (Ethica et al., 2019). Similarly, microbial consortia allow for synergistic interactions between microbes through physical or biochemical activities, thus increasing viability of each individual microbes (Wang et al., 2019). The ability of bacteria to synergize among each other is affected by several factors including type of bacteria tested, charge distribution, bacterial cell wall content, and diffusion of bacterial molecules into the growth medium (Gurunathan and Dhamotharan, 2014). In addition, component of bacterial consortium was capable to synergize one another due to the collaboration activity to utilize nutrients or to obtain energy from the same media. Many interactions occur between different types of bacteria and result in either growth promotion or resistance in plants (Dinata et al., 2021).

Effect of endophytic bacteria inoculation on rice seedling growth

Table 5 and Figure 3 showed that the interaction between inoculation of endophytic bacteria and insecticide (10x dose) significantly affected the root length and shoot height of the rice seedling (7 DAP). The highest root length was yielded by the I_3P_0 treatment followed by I_3P_1, while the highest shoot height was observed by the I_2P_1 treatment followed by I_2P_0. These finding showed that isolate PE_LOI-3 from Pikovskaya medium has the functional capability to increase root development. The in-vivo assessment on rice seedling using Agarose medium was conducted according the method of Verma et al. (2018). In the present study, the basal nutrition was not added to Agarose medium. Thus, the role of the isolate PE_LOI-3, that is characterized as effective PSB in increasing root length, was not related to phosphate solubilization since no P source exist in the medium. This phenomenal proposed the other mechanism that might played by isolate PE_LOI-3 in increasing root length. Verma et al. (2018) reported significant effects of 23 endophytic bacterial isolates from *Leersia oryzoides* seeds on rice grown on petri dish with 0.7% agarose media. After 5 days of incubation, 10 isolates showed activity to increase root and shoot length in rice seedlings, whereas 13 isolates showed inhibitory activity on rice seed growth. Krishnamoorthy et al. (2020) isolated 9 endophytic bacteria from in vitro grown callus of two aromatic rice cultivars and inoculated rice seeds of IR64. Only 4 isolates among them showed significant increases in root and shoot length compared to the control treatment. Endophytic bacteria can enhance seed germination efficiency by biosynthesizing hormones such as gibberellin that can stimulate the activity of specific enzymes

Table 4. Resistance test of endophytic bacterial isolates against insecticides (5-10x doses)

Isolate code	Resistance to insecticide 5x dose		Resistance to insecticide 10x dose	
	Absorbance value (Cell density in medium with insecticide 5x dose)	Absorbance difference (Cell density gap = the difference cell density in medium with and without insecticide 5x dose)	Absorbance value (Cell density in medium with insecticide 10x dose)	Absorbance difference (Cell density gap = the difference cell density in medium with and without insecticide 10x dose)
Control	0.000±0.00n	0.000±0.00bcde	0.000±0.00l	0.000±0.00fgh
YE$_R$OI-1	1.572±0.02fghij	-0.165±0.07cdefgh	1.492±0.09bc	0.115±0.00bcdef
YE$_L$OI-1	1.621±0.03efgh	-0.243±0.14efgh	1.576±0.09abc	0.289±0.00abcd
YE$_R$CI-1	1.492±0.05ghijk	-0.129±0.15cdefgh	1.612±0.12ab	0.388±0.21a
YE$_L$CI-1	1.580±0.05efghi	-0.910±0.22cdefgh	1.472±0.08bcd	0.404±0.18a
NE$_R$OI-1	1.584±0.10efghi	-0.101±0.17cdefgh	1.018±0.09hi	-0.296±0.01ij
NE$_R$OI-2	2.322±0.32a	0.374±0.27a	1.095±0.11ghi	0.432±0.23a
NE$_R$OI-3	1.344±0.10k	-0.304±0.09gh	0.652±0.11j	0.012±0.01efgh
NE$_R$OI-4	1.669±0.07def	0.029±0.17bcd	1.296±0.11def	-0.002±0.00fgh
NE$_L$OI-1	1.427±0.10ijk	0.039±0.00bcd	1.541±0.11abc	0.253±0.00abcde
NE$_L$OI-2	0.621±0.10m	-1.404±0.19k	1.191±0.10fgh	-0.148±0.00hi
NE$_L$OI-3	1.561±0.10fghij	0.083±0.20bc	0.988±0.11i	0.352±0.00ab
NE$_L$OI-4	1.942±0.09b	-0.019±0.00cde	1.193±0.07fgh	0.098±0.16cdefg
NE$_R$CI-1	1.869±0.12bc	0.349±0.19a	0.733±0.11j	-0.691±0.01k
NE$_R$CI-2	1.918±0.10bc	0.348±0.17a	1.182±0.17fgh	0.405±0.17a
NE$_R$CI-3	1.407±0.09jk	-0.054±0.00cdefg	1.407±0.09cde	0.325±0.10abc
NE$_L$CI-1	1.659±0.10defg	-0.104±0.09cdefgh	1.136±0.09fghi	-0.261±0.00ij
NE$_L$CI-2	1.476±0.10hijk	-0.076±0.00cdefg	1.256±0.13efg	0.320±0.10abc
PE$_R$OI-1	1.436±0.10ijk	0.048±0.00bcd	1.537±0.10abc	0.245±0.00abcde
PE$_R$OI-2	1.344±0.10k	-0.785±0.00i	1.411±0.11cde	0.247±0.20abcde
PE$_R$OI-3	1.506±0.10fghijk	0.323±0.17a	0.971±0.11i	-0.145±0.19ghi
PE$_R$OI-4	1.576±0.10fghij	-0.221±0.09efgh	1.566±0.09abc	0.032±0.19efgh
PE$_L$OI-1	1.366±0.07k	-0.170±0.10defgh	1.701±0.10a	0.278±0.00abcd
PE$_L$OI-2	1.794±0.10bcd	-0.327±0.17h	0.484±0.11k	-0.402±0.01j
PE$_L$OI-3	1.746±0.10cde	-0.052±0.00cdef	1.463±0.07bcd	-0.139±0.03ghi
PE$_L$OI-4	0.768±0.10l	-1.102±0.00j	1.627±0.11ab	0.089±0.17cdefgh
PE$_R$CI-1	1.560±0.10fghij	0.230±0.00ab	1.651±0.09ab	0.050±0.09defgh
PE$_L$CI-1	1.555±0.10fghij	-0.080±0.16cdefgh	1.706±0.01a	0.345±0.08ab
PE$_L$CI-2	1.590±0.10efghi	-0.258±0.00fgh	1.625±0.10ab	0.121±0.19bcdef
ANOVA	0.00**	0.00**	0.00**	0.00**

Remarks: Significant level: ns=no significant, * = p <0.05, ** = p < 0.01. Means followed by same letters are not significantly different at 5% level by DMRT. Y = YEMA medium, N = NA medium, P = Pikovskaya medium, E$_R$ = Endophytic bacteria from root of rice plant, E$_L$ = Endophytic bacteria from leave of rice plant, OI = Organic paddy field, CI = Conventional paddy field.

such as α-amination. In this condition, endophytic bacteria are transmitted vertically through seeds. Endophytic bacteria as PGPB live in the vital organs of plants that are obtained from seeds (vertically from the host plant) or soil (horizontally from the environment) (Frank et al., 2017). Seed endophytic bacteria play important roles in germination such as hormone production, seedling resistance, maintaining plant health, nutrient mobilization, phytopathogen control, and antioxidant activity (Pal et al., 2019).

Molecular identification of endophytic bacteria

The sequences of the 16 rDNA of the three superior isolates of YE$_R$OI-1, PE$_L$OI-3, and NE$_R$OI-2 were submitted to DNA Data Bank of Japan (DDBJ) and recorded with the accession number LC790726, LC790728, and LC790727, respectively. The length of sequence of DNA fragments of the three isolates was 1375 – 1405 bp.

Two isolates were closely related with the genus *Burkholderia* but different species, and one isolate closely related with *Bacillus* (Table 6). Many members of genus *Burkholderia* are detected as beneficial e.g. *Burkholderia cepacia* from root of rice (Singh et al., 2013) and sugarcane (Luvizotto et al., 2010), *Burkholderia vietnamensis* from rice and shoot of rice (Govindarajan et al., 2008). The authors demonstrated their functions in nitrogen fixation, production of phytohormone (IAA production and acetylene reduction), phosphate solubilization, siderophore production. Many members of genus *Bacillus* were also recorded as beneficial bacteria. Shahzad et al. (2016) reported *Bacillus amyloliquefaciens* as an endophyte derived from rice seeds that plays a role in the regulation of endogenous phytohormones (salicylic acid, abscisic acid, and jasmonic acid) and gibberellin production. Thus, based on the characteristic of closest relatives of the three superior isolates in the present study, it is known that the three isolates have potential to be used as beneficial member of *Burkholderia* (for isolate YE$_R$OI-1 and PE$_L$OI-3) and *Bacillus* (for isolate NE$_R$OI-2). Further studies of in-vivo test are needed to confirm the beneficial function of these three isolates.

Materials and Methods

Sampling of rice plant and rhizosphere soils

Healthy rice plant cultivar Inpari 32 were grown at maximum vegetative phase on 45 DAP and their rhizosphere soils were collected from organic paddy field in Kuto Village (7°32.998'S; 111°01.760'E) and from conventional paddy field in Tamansari Village (S7°33'47.8'S; 111°03'07.7'E), Kerjo District, Karanganyar Regency, Central Java, Indonesia in May 2023. The root and leaf of the rice plants were used as the sources of endophytic bacteria. Both of the organic and conventional paddy fields were classified as Alfisols with chemical characteristics as follows: pH H_2O 5.32 and 4.76, organic-C 3.39 % and 1.37 %, total-N 0.60 % and 0.75 %, C/N ratio 5.67 and 1.84, available-P 5.49 ppm and 4.64 ppm, available-K 0.08 cmol(+) kg^{-1} and 0.19 cmol(+) kg^{-1}, respectively.

Isolation, colony observation, and purification

All steps of the laboratory research were conducted in the Laboratory of Biology and Biotechnology, Faculty of Agriculture, Sebelas Maret University, Surakarta, Indonesia from May to August 2023. The samples of leaf and root of rice plants were

Table 5. Effect of inoculation of endophytic bacterial and insecticide to root length and shoot height of rice seedling.

Combination treatment	Root length of rice seedling (cm)	Root hairs (*)	Shoot height of rice seedling (cm)
I0P0	2.96±0.67cd	+++	4.16±0.30bc
I0P1	3.26±0.25bcd	+++	4.43±0.37ab
I1P0	3.29±0.25bc	+	3.29±0.20bcd
I1P1	3.76±0.83abc	+++	3.71±0.62bcd
I2P0	3.66±0.26abc	+++	5.36±0.57a
I2P1	3.21±0.42bcd	+++	5.37±0.54a
I3P0	4.42±0.76a	+++++	4.16±1.01bc
I3P1	4.01±0.32ab	+++++	4.20±0.68bc
I4P0	3.06±0.45bcd	+++	3.24±0.95cd
I4P1	2.27±0.62d	+++	2.98±0.31d
F-Values			
I	0.00**		0.00**
P	0.39ns		0.65ns
I x P	0.00**		0.00**

Remarks: Significant level: ns=no significant, * = p <0.05, ** = p < 0.01. Means followed by same letters are not significantly different at 5% level by DMRT. I_0P_0 = Control, I_0P_1 = Without isolates, add insecticide, I_1P_0 = Isolate YERoI-1, I_1P_1 = Isolate YERoI-1+insecticide, I_2P_0 = Isolate NERoI-2, I_2P_1 = Isolate NERoI-2+insecticide, I_3P_0 = Isolate PELoI-3, I_3P_1 = Isolate PELoI-3+insecticide, I_4P_0 = Combination of three isolates, I_4P_1 = Combination of three isolates + insecticide. (*) + = short root hairs, +++ = moderate root hairs, +++++ = long and abundant root hairs.

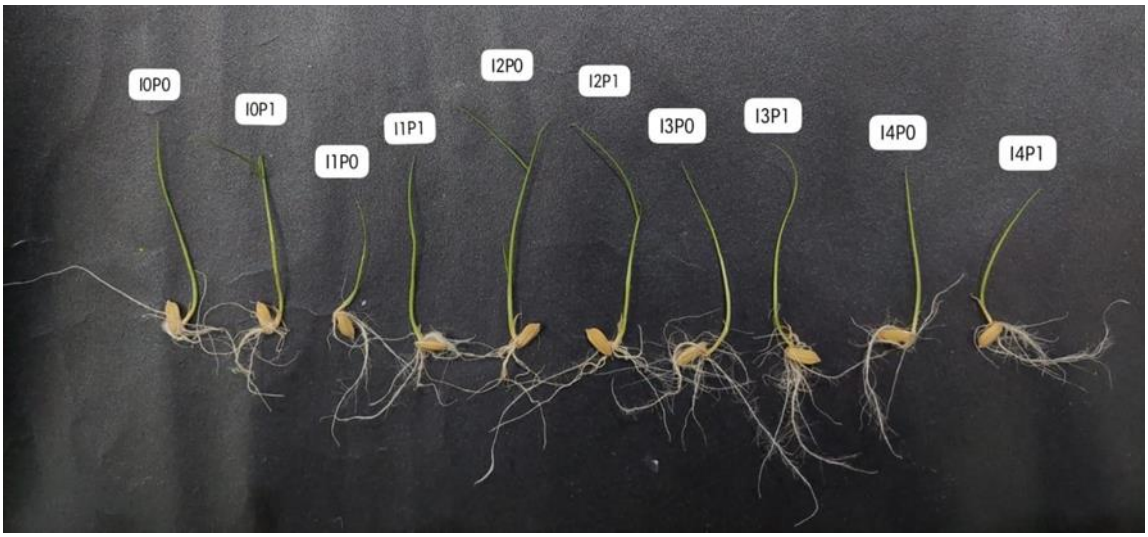

Figure 3. The growth of rice seedling as affected by the combination treatment of endophytic bacteria and insecticide.

subjected for the isolation of bacterial endophytes. At first, the leaves and roots were washed under running tap water, then their surfaces were sterilized by a consecutive soaking in 70% ethanol (1 min), 3.25% NaOCl (6 min), 70% ethanol (1 min), and rinsed with sterile water (1 min). The sterilization procedure was repeated five times (Yan et al., 2018). After drying, the samples were cut and mashed using a sterile mortar and pestle, then weighed and subjected for a serial dilution of spread plate method with six replications (Sanders, 2012).Three types of media were prepared, namely YEMA for symbiotic NFB (Birhan et al., 2018), Pikovskaya for PSB (Naik et al., 2008), and NA for general bacteria (Pathmanathan et al., 2016). After incubation for two days at room temperature, the colony morphology and population density were observed (Sousa et al., 2013). Morphologically distinct bacterial colonies were selected and purified using the same media in the slant agar for the stock culture and in the new petri dish to be used for further examination.

Physiological and cellular characterization of bacterial isolate

Each bacterial isolate was tested for NaCl resistance, temperature, and pH tolerance. NaCl resistance was assessed in nutrient broth (NB) with 2%, 6%, and 10% NaCl (Hena et al., 2022). Temperature tolerance was tested at 10°C, 27°C, and 45°C (Katipoglu-Yazan et al., 2023). pH tolerance was examined in NB media at pH 5, 7, and 9 (Katipoglu-Yazan et al., 2023). All cultures were incubated for 4 days at room temperature, and growth was measured at 590 nm with a spectrophotometer. Cell shape, size, and wall characteristics were observed using gram staining (Kandi, 2015).

Assessment of capability for phosphate and potassium solubilization

All isolates from the three media of YEMA, Pikovskaya, and NA were examined for their multifunctional capability as biofertilizer by cross culturing each isolate to Pikovskaya and Aleksandrov media to evaluate the capability in the solubilization phosphate and potassium. The formation of halo zone on the Pikovskaya and Aleksandrov media was measured on day 5 of the incubation for the PSI and KSI (Bouizgarne et al., 2023).

Resistance test of endophytic bacteria grown on media with and without insecticide addition

Testing of resistance capability of endophytic bacteria to insecticide with active ingredients *chlorantraniliprole* 100 g L^{-1} and *thiamethoxam* 200 g L^{-1} was conducted by growing each isolate on the NB medium with and without insecticide addition. The resistance test was conducted in two steps, the first step used 5x dose and the second step used 10x dose. In each step, the experiment was performed in a completely randomized design with single factor of isolates treatment, consisting of 29 levels including the control (treatment with no isolate) with 3

Table 6. Identity of three superior endophytic bacterial isolates.

The code and molecular identity of the three selected 3 isolates								
Isolate Code	Source of isolation	Sequence bp	Accession Number	Closest Relatives				Similarity
				Microorganism	Phylogenetic affiliations	Accession Number	Source	
YE$_R$OI-1	Rice root tissue on organic paddy field	*1375*	LC790726	*Burkholderia* sp.	Phylum: Pseudomonadota Class: Betaproteobacteria	KY357354.1	heavy metal-contaminated soils	100%
				Burkholderia seminalis	Phylum: Pseudomonadota Class: Betaproteobacteria	CP072521.1	surface-sterilized vetiver roots	100%
PE$_L$OI-3	Rice root tissue on organic paddy field	*1379*	LC790728	*Burkholderia cepacia*	Phylum: Pseudomonadota Class: Betaproteobacteria	OL469786.1	Cd-contaminated soil	100%
				Burkholderia seminalis	Phylum: Pseudomonadota Class: Betaproteobacteria	MH748603.1	the root nodules of the vegetable cowpea	100%
NE$_R$OI-2	Rice leaf tissue on organic paddy field	*1405*	LC790727	*Bacillus paramycoides*	Phylum: Bacillota Class: Bacilli	OQ970651.1	Salt polluted-soil	99.93%

replications. Each isolate was cultured in 5 mL NB medium per tube with 3 replicates. In first step, 0.5 µL of insecticide (concentration 0.1 ppm) was added to each tube and the other tube was without insecticide (as positive control). One loopful of endophytic bacterial colony was inoculated to each tube. All those cultured NB medium were incubated at room temperature and shaken at 150 rpm for 4 days, then the cell density was observed using a spectrophotometer at a wave length of 590 nm (Andriani et al., 2017). The length of incubation (4 days) was determined based on preliminary test by culturing several isolates on NB medium in the tubes, until the cell density of all the isolates reached 10^8 cells m L^{-1} after 4 days.

The second step of the testing resistance capability was conducted after the isolate was sub-cultured three times during three months. This testing is important to evaluate the consistency of the resistance capability of each isolate after six months preservation in the laboratory condition. In second step, insecticide was applied with 10x of recommended usage dose (concentration 0.2 ppm). The design and procedure of the second strep of resistance test was the same as conducted in the first step. The resistance bacterial isolate against insecticide was indicated by the capability to grow in the insecticide treatment as demonstrated by the increase of cell density as determinate by absorbance value. To compare the resistance level among all the isolates, it was needed to measure the cell density gap (the difference cell density in medium with and without insecticide) as detected from the absorbance difference. The obtained data then was analyzed statistically using Statistical Analysis of Variance (ANOVA) and continued with Duncan Multiple Range Test (DMRT) with significance of 5%. Based on the resistance capability, one isolate was selected to be used for the greenhouse experiment.

Synergy test between endophytic bacterial isolates

All of the isolates from NA (13) and from Pikovskaya (11) were examined for the synergy interaction on the respective media. The four isolates from YEMA, which obtained from rice leaves and roots in both organic and conventional paddy fields and exhibited similar colony morphology, were not tested for synergistic interactions. Synergy test was conducted by the cross streak method on the respective media. In first step, each isolate was streaked in straight line at 1 cm of the distance between the line. After incubation, 3x24 hours at room temperature, the second streak from each isolate was made across the first line, and the incubation was continued for 3x24 hours. Then, the presence or the absence of the clear zone among the streaked line was observed. The synergistic interaction among the isolates was analysed by the absence of an inhibition zone (clear zone) around the cross between colony streak lines (Ethica et al., 2019). The presence of clear zones is a sign that some bacteria are antagonistic to each other.

Selection of isolates for in-vivo assessment

Out of the 28 rice endophytic bacterial isolates from organic and conventional paddy fields, obtained from YEMA, Pikovskaya, and NA media, one isolate from each medium was selected based on its superior functional capability. Isolate YE$_R$OI-1 from YEMA, isolate PE$_L$OI-3 from Pikovskaya, and isolate NE$_R$OI-2 from NA were selected based on the multifunctional capability. The three selected isolates were evaluated for the synergistic interaction by streaking on NA medium.

The examination of the capability for the nitrogen fixation

Additional examination was performed to assess capability of nitrogen fixation for the three selected isolates. The examination was conducted by cross culturing to YEMA medium with the addition of Bromothymol Blue (BTB) to evaluate the capability of growth of isolates in the media with the absence of N source (Begom et al., 2021). As explained by Sulistiyani and Meliah, (2017) the color changes of YEMA+BTB medium from greenish blue to dark blue indicated the increase of medium pH (alkaline reaction) due to the ammonium production by the isolates which indicate the activity of nitrogen fixation. On the other hand, the color alteration of media YEMA+BTB from greenish blue to yellow indicate acid production by the fast grown rhizobia (Somasegaran and Hoben, 1994).

In vivo assessment to examine the effect of endophytic bacteria inoculation on rice seedling growth

In vivo assesment was designed using a completely randomized design with two factors: first factor was inoculation of isolates consisting of 5 levels: I_0=no isolate; I_1=Isolate YE$_R$OI-1; I_2=Isolate NE$_R$OI-2; I_3=Isolate PE$_L$OI-3; I_4=mixture of three isolates. The second factor was insecticide application (P) consisting of 2 levels: P_0=no insecticide; P_1= with insecticide 10x dose (active ingredient *chlorantraniliprole* 100 g L^{-1} and *thiamethoxam* 200 g L^{-1}). There were 10 combination treatments with 3 replications.

For in vivo assessment, first rice seeds var. Inpari 32 were sterilised with 4% NaOCl for 5 minutes with constant stirring, 70% ethanol for 3 minutes, then the seeds were washed with sterile water 5 times to remove the remaining NaOCl (Ferreira et al., 2021). Then isolate inoculation was performed by soaking rice seed into the treatment of endophytic bacterial culture (control, selected endophytic bacterial isolates of YE$_R$OI-1, PE$_L$OI-3,

NEROI-2 and a mixture of the three isolates, respectively) with cell density 10^8 cells m L^{-1} and incubated for 24 hours. The agarose medium was prepared with and without insecticide. For the insecticide treatment, the insecticide was added to agarose medium just before plating (the temperature at 35-38◦C) in petri dish. The rice seeds were sown on agarose medium (5 seeds in each petri dish) according to respective treatment. The variables of rice seedling development consisted of root and shoot length that were observed 7 days after sowing (Verma et al., 2018). The obtained data was analyzed for Statistical Analysis of Variance (ANOVA) and continued with Duncan Multiple Range Test (DMRT) with significance of 5%.

Molecular identification of endophytic bacteria

Among 28 endophytic bacterial isolates, 3 isolates which indicated superior functional ability were selected to be identified by 16S rDNA sequencing. Total genomic bacterial DNA was extracted using Quick-DNA Magbead Plus Kit (Zymo Research, D4081) and the 16s rDNA was amplified using the eubacterial set of two primers, 27F primer (5'–AGAGTTTGATCMTGGCTCAG–3') and 1492R primer (5'–TACGGYTACCTTGTTACGACTT–3'). PCR was performed in the GeneAmp@PCR system 9700 (Applied Biosystems, Foster City, CA). The component of PCR master mix (total volume 25 µL) contained of 12.5 µL of My Taq HS Red Mix (2x), 1 µL of 27F Primer (10 µM), 1 µL of 1492R Primer (10 µM), 1 µL DNA template (about 25 ng), and 9.5 µL of dd H2O. The PCR conditions was set up as follows: initial denaturation at 95◦C for 3 min, 35 cycles of denaturation at 95◦C for 15 sec, annealing at 52◦C for 30 sec, extension at 72◦C for 45 sec, and final extension at 72◦C for 2 min. After amplification, the PCR products were assessed by electrophoresis with 1% TBE Agarose. The PCR product was then subjected for Bi-directional Sequencing using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with 27F and 1492R Primers according to the manufacturer's instructions (Cahyani et al., 2009). Sequencing was performed using ABI PRISM® 3100-Avant™ Genetic Analyzer (Applied Biosystems, Foster City, CA). The sequences of the DNA fragments of the selected isolates were compared to NCBI database (www.ncbi.nlm.nih.gov) with the BLAST search to determine closest relatives of the isolates, and then the sequences were submitted to DNA Data Bank of Japan (DDBJ) (https://www.ddbj.nig.ac.jp/submission-navigation-e.html). The 16S rDNA sequences of the selected isolates obtained in this study are available in the DDBJ database with the accession number LC790726-LC790728.

Conclusion

The differences in cultivation system affected the diversity and population density of endophytic bacteria of rice plants. On organic paddy field, the diversity and population density of rice endophytic bacteria was higher than those on conventional paddy field. The general endophytic bacteria in rice leaves showed higher population than those in rice roots. On the contrary, the specific endophytic bacteria of NFB and PSB in rice leaves indicated lower population density compared to those in rice roots. Among a total of 28 isolates from YEMA, Pikovskaya, and NA media, three isolates of YEROI-1, PELOI-3, and NEROI-2 were closely related to *Burkholderia* sp., *Burkholderia seminalis* and *Bacillus paramycoides*, respectively. They were selected based on their superior functional capability as biofertilizer and bio-resistance inducer to insecticide in *in vitro* assessment. Isolate NEROI-2 yielded the highest effect in increasing the growth of rice seedling as indicated the highest shoot height and root length either with and without insecticide treatments, followed by isolate PELOI-3. On the contrary, isolate YEROI-1 showed inhibition effect toward the growth of rice seedling in the treatments with and without insecticide. Further assessments are required to elucidate the comprehensive characteristics of isolate YEROI-1 or any other factors, causing inhibition effect for the plant growth. Evaluation study is needed to confirm the effect of these endophytic bacteria in supporting plant growth, inducing insecticide resistance, and increasing soil fertility and health in a greenhouse experiment.

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