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Rice growth and chlorpyrifos residue on an Alfisol as affected by endophytic and rhizospheric bacteria isolated using Aleksandrov medium

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ABSTRACT

The intensive use of insecticides and chemical fertilizers in rice cultivation is a common practice which leads to environmental and health concerns. This study isolated endophytic and rhizospheric bacteria from organic and conventional paddy fields using Aleksandrov medium, to find bacteria which basically having functional capabilities as potassium solubilizers and screening them for further capability as plant growth promoters and insecticide biodegradation agents for supporting rice growth on an Alfisol treated with multivariant insecticides. Consecutive assessments were conducted after isolation, involved (1) in vitro assessment of the functional capabilities and the resistance to insecticide, (2) in vivo assessment for the effect of the selected to the growth of rice seedling on agarose medium treated with multivariant insecticides (chlorpyrifos, chlorantraniliprole, thiamethoxam, and carbofuran), and (3) greenhouse pot experiment to confirm the effect of selected isolates to rice growth on an Alfisol treated with multivariant insecticides. Nine out of 11 isolates with distinct colony morphotypes, demonstrated the capability as plant growth promoters which also resistant to insecticide at the in vitro assessment. It was found that the three isolates of AElC-1, AElO-1, and ARsC-3 showed the consistent higher performance during consecutive assessments. The highest level of chlorpyrifos residue in soil and in plant tissue was indicated in the control (+) treatment, while in the NPK treatment which showed the highest plant biomass, indicated the lowest chlorpyrifos residue in plant tissue, but the second high level of chlorpyrifos residue in soil after the control (+). The treatment of 3 superior isolates demonstrated the range of chlorpyrifos residue in plant tissue was 0.098–0.101 mg/kg or higher than that in the NPK treatment but lower than that in the control (+), while in soil the residue at the range 0.132–0.135 mg/kg which was lower than that in the NPK treatment and in the control (+). The 3 superior isolates demonstrated superior capabilities in suppressing chlorpyrifos which potential to be used as soil and plant protecting agents especially in agricultural environments. Molecular identification by 16S rDNA sequencing revealed AElO-1 as *Microbacterium* sp., AElC-1 as *Caulobacter* sp., and ARsC-3 as *Sphingomonas trueperi*.

Keywords: biofertilizer, insecticide degradation, multivariant insecticides, potassium solubilizing bacteria, resistance inducer.

INTRODUCTION

The extensive use of pesticides and chemical fertilizers in rice farming has raised serious concerns regarding environmental and health impacts (Charaslertrangsi et al., 2024; Smith et al., 2021). Among various groups of pesticides, such as insecticides, rodenticides, herbicides, bactericides, fungicides, it is well known that insecticide are the most numerous and diverse in types that have been intensively and extensively used to control diverse rice pests in various growth phases. Various insecticide for paddy field such as methomyl, fipronil, buprofezin, chlorantraniliprole, deltamethrin, dimehypo, alpha-cypermethrin, cypermethrin, carbofuran, and chlorpyrifos were commonly applied without controlling the doses and the frequency of application which causing harmful impacts on the environment including to soil fertility and to non-target organisms (Kaur et al., 2017; Tam et al., 2018). Studies show that the continuous and simultaneous use of various insecticides can disrupt soil microbial communities (Yu et al., 2020; Zhang et al., 2021), influence soil pH levels (Wu et al., 2020; Zou et al., 2018), inhibit plant growth (Shahid and Khan, 2022; Wu et al., 2020), and impact to consequences for the non-target organisms (Mamy et al., 2023). Additionally, the accumulation of insecticide residues in both soil and plant tissues presents significant threats to environmental and human health (Rani et al., 2021).

To address the negative impacts of conventional agricultural practices, researchers have explored various strategies, including the use of compost, biochar, and beneficial microbial agents (Ali et al., 2014; Aziz et al., 2021; Ma et al., 2022; Yaseen et al., 2016). Recent studies have highlighted the potential of endophytic bacteria in rice cultivation. *Enterobacter cloacae* ATCC 13047 and *Phytobacter diazotrophicus* LS 8 that isolated from rice have the ability to solubilize potassium as indicated by the ability to form clear zone in the Aleksandrov medium with potassium feldspar (Liu et al., 2022). Jena et al. (2024) reported that endophytic bacteria from rice roots that have been identified as *Priestia megaterium* and *Priestia aryabhattai* have the ability to solubilize phosphate (40.91–83.70 μg/mL) and produce indole acetic acid (28.10–60.18 μg/mL), which can promote plant growth. Regarding insecticide toxicity, *Bacillus cereus* G-H27 has the ability to promote the growth of wheat by colonize root surface, produce biofilms, and reduce chlorpyrifos concentration in wheat roots (Zhang et al., 2024). Comparative studies between organic and conventional rice fields have revealed that the bacterial communities indicated the similar composition which both of organic and conventional paddy soils showed the presence of *Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes,* and *Proteobacteria* as dominant phyla (Jung et al., 2024). Despite these promising findings, our understanding of the capabilities of endophytic and rhizospheric bacteria derived from organic and conventional rice fields remains limited, particularly regarding their function as potassium solubilizers which can enhance plant growth, and at the same time contribute in reducing insecticide toxicity.

The present study aimed to screening endophytic and rhizospheric bacteria isolated from organic and conventional paddy fields using Aleksandrov medium to find the superior isolates with basic functional capability as potassium solubilizers which capable to contribute multifunctions as plant growth promoters, plant resistance inducer to insectide toxicity, and insectide biodegradation agents in soil. On the other words, the present study took efforts to find superior bacterial agents which be able to play roles in protecting soil and plant health in agricultural environments. Three insecticide products which totally contained four active ingredients (chlorpyrifos, carbofuran, chlorantraniliprole, and thiamethoxam) were used for the in vitro, in vivo, and greenhouse assessment, however the present study focused on the assessing chlorpyrifos residues in soil and plant tissues. We postulated that exploration from rice plant tissue and their rhizosphere in organic and conventional paddy fields by isolation using Aleksandrov medium followed a successive screening procedures can be found superior endophytic and rhizospheric bacteria which capable to play multifunctions as biofertilizers, plant growth promoters, plant resistance inducers against insecticida toxicity, and also as insecticide degraders in soils.

Comprehensive screening was performed to evaluate the functional capabilities of the isolated bacteria. In vitro evaluations were conducted to examine the functional capabilities including the functions as plant growth promoters and the resistance capability against the toxicity of monovariant and multivariant insecticides of all isolates, followed by two stages of in vivo evaluations to the selected isolates. The first stage examined the effect of isolate inoculation on the growth of rice seedlings on agarose medium supplemented with multivariant insecticides. Then, the second stage examined the effect of isolate inoculation on the growth of rice plants in a greenhouse using an Alfisol soil media supplemented with multivariant insecticides. A soil type of Alfisol was selected for this study to be used as a medium for plant growth based on its nature, as the mineral composition and high cation exchange capacity of Alfisol usually limit potassium availability, leading to its immobilization (Li et al., 2021).

MATERIALS AND METHODS

Sampling of rice plant and rhizospheric soil

Healthy rice plants (*Oryza sativa* L.) variety Inpari-32 and their rhizospheric soil as the source of endophytic and rhizospheric bacteria were taken from organic rice field in Jungke Village (7°35'59''S 110°55'59'E), Karanganyar District and from conventional rice field in Tamansari Village (7°35'14''S 111°2'8''E), Kerjo District, Karanganyar Regency, Jawa Tengah Province, Indonesia.

Isolation and purification of endophytic and rhizospheric bacteria

Prior to the isolation, rice leaf and roots were subjected for surface sterilization (Muhtari et al., 2024). Each sample of rice leaf and roots were diluted in physiological saline solution (0.9%) until 10^{-3} and inoculated to Aleksandrov medium (Sun et al., 2020).

As for rhizospheric bacteria, at first the rhizospheric soil sample was subjected for preparing soil extract agar, by extracting 500 gr soil with 1 liter water then filtering using filter paper. The filtrate was used as agar medium (15 gr/liter filtrate) (Norman, 1958). The transparent and halozone forming colonies that were grown on the soil extract agar were selected and subculture to Aleksandrov medium. The isolates were selected based on distinct colony morphology and growth criteria, and this procedure was repeated several times until pure colonies were obtained. All colonies of endophytic rice and rhizospheric soil that formed halozone were observed, calculated, and purified to the new Aleksandrov medium.

Assessment for the functions as biofertilizers and plant growth promoters

Potassium and phosphate solubilization

All purified colonies were again subcultured in point streak on new other Aleksandrov medium. The formation of a clear zone or halozone around the bacterial colony demonstrates potassium solubilization activity. This clear zone was measured to determine the potassium solubilization index (KSI) of each isolate (Khanghahi et al., 2018). Similar procedure was conducted to determine the capability in phosphate solubilization, by subculturing in point streak of each isolate from Aleksandrov medium to Pikovskaya medium. The formation of halozone around the colony was measured for phosphate solubilization index (PSI) (Fatimah et al., 2023; Shah et al., 2022).

Nitrogen fixation

The isolates were tested for their nitrogen-fixing ability by culturing the bacteria on Jensen agar medium supplemented with Bromothymol Blue (BTB) as a colour indicator for nitrogen fixation (Jain et al., 2021). The colour changes from dark green to yellow indicated acid production while from dark green to blue or bluish green indicated alkaline production (Sulistiyani et al., 2024; Sulistiyani and Meliah, 2017).

IAA production

A mixture of Nutrient broth and L-tryptophan were used as media to identify the production of Indole-3-Acetic Acid (IAA) by cultures of endophytic and rhizospheric bacterial isolates. The bacterial culture was incubated for 4 × 24 hours until it reached a cell density of 10^8 CFU/mL. Subsequently, each broth culture was centrifuged at 10,000 rpm for 15 minutes. Afterwards, the supernatant was measured colorimetrically at 530 nm using 1 ml of Salkowski reagent. All IAA determinations were performed in triplicate (Chandra et al., 2018).

Assessment of resistance capability against insecticides or potential function as insecticide-biodegradation agent

Insecticide resistance testing was conducted by culturing each endophytic and rhizospheric bacteria to three tubes containing 5 ml of liquid NB medium supplemented with insecticide by applying 10x of recommended dose either for monovariant or multivariant insecticides. The first tube supplemented with an insecticide product (monovariant) containing the active ingredient chlorpyrifos 400 EC (added 9 µl/L NB), the second tube supplemented with multivariant insecticides containing an insecticide product with the active ingredients chlorpyrifos 400 EC (added 9 µl/L NB), a product containing carbofuran 3% (added 0.4 g/L NB), and a product which containing two types active ingredients of chlorantraniliprole 100 g/L with thiamethoxam 200 g/L (added 3 µl/L NB), and the third tube with no addition of insecticides. In addition, the medium without any isolate and without insecticide was used as control to check the possibility of contamination. The cultures were incubated by shaking at 150 rpm for four days at room temperature. Cell density was measured using a spectrophotometer at a wavelength of 600 nm (Shahid et al., 2019a).

In vivo assessment for the selected isolates to the growth of rice seedling on agarose medium treated with multivariant insecticides

At first each selected bacterial isolate was cultured in NB medium until it reached a cell density of 10^8 CFU/mL, then the seeds of rice (*Oryza sativa* L.) variety Inpari 32 were soaked in these cultures and incubated for 24 hours. The seeds were inoculated onto agarose medium supplemented with a combination of multivariant insecticides (chlorpyrifos, carbofuran, and a mixture of chlorantraniliprole with thiamethoxam) at 10x of recommended dose, except for the control (-) treatment (no isolate and no insecticide). Some additional treatments were prepared, namely: Control (+) treatment which no isolate but with insecticide application and consortium isolates treatments which consisted of a group of isolates with high capability as biofertilizer (MABf), a group of isolates with exhibiting a high level of resistance to insecticide (MABr), and a mixture of those two groups (MABfBr). The growth of rice seedling was observed and measured on day 10 for seedling height, fresh weight, root length, and chlorophyll content.

Effect of inoculation of the selected isolates toward rice growth on Alfisol soil treated with multivariant insecticides in pot experiment

The same treatment as conducted for in vivo assessment on agarose medium, was also performed for in vivo assessment in a pot experiment in a greenhouse. The pot experiment was set up in a completely randomized design (CRD) with a single factor consisting of 15 levels of treatment with 4 replications, total there were 60 pots. The Alfisol soil used for the pot experiment was collected from Bakaran Village, Jumantono District, Karanganyar Regency, Jawa Tengah Province, Indonesia (7°38'12"S 110°56'46"E). The soil was characterized by a clay texture (11% sand, 33% silt, and 62% clay), with pH H_2O 5.99, organic C 1.05%, total N 0.14%, C/N ratio 7.87, available P 0.79 mg/kg, available K 0.01 mg/kg, cation exchange capacity 12.91 cmol(+)/kg, and base saturation 66%. The seeds of rice (*Oryza sativa* L.) variety Inpari 32 were soaked in each culture of selected bacterial isolates that had cell density of 10^8 CFU/mL by shaking at 80 rpm in room temperature for 24 hours. Two types of control were prepared, the first control was NB medium with no isolate and no insecticide (control (–)), the second control was NB medium with no isolate but with insecticide (control (+)). As the comparison treatment, the chemical fertilizer (NPK) addition was applied dose urea 350 kg ha^{-1}, SP-36 75 kg ha^{-1}, and KCl 100 kg ha^{-1} according to the recommendation of Declare of Indonesian Ministry of Agriculture No.13 FY 2022. With the exception for control (–), all pots were added with the basal chemical fertilizer (1/3 of recommended dose).

The chemical fertilizers were applied a week before transplanting. The multivariant insecticides (chlorpyrifos 400 EC (0.018 ml/pot), carbofuran 3% (0.75 g/pot), a mixture of chlorantraniliprole 100 g/l and thiamethoxam 200 g/l (0.006 ml/pot)) were first applied at 10× dose on 3 days before transplanting to all pots, except for the control (–) treatment. One rice seedling per pot was transplanted 14 days after sowing. The second application of multivariant insecticide was applied 30 days after transplanting at 5× dose (chlorpyrifos 400 EC (0.009 ml/pot), carbofuran 3% (0.375 g/pot), a mixture of chlorantraniliprole 100 g/l with thiamethoxam 200 g/l (0.003 ml/pot)). The rice growth was observed and measured until maximum vegetative or 95 days after transplanting. Soil chemical characteristics were analyzed : pH (electrophotometri method), organic-C (Walkley and Black method), total-N (Kjeldahl method), available-P (Olsen method), available-K (ammonium acetate extraction method), chlorpyrifos residue (gas chromatography-mass spectrometry (GC–MS) method).

Data analysis

All data including the population and diversity of the isolated bacteria, data from in vitro assessment for functional capabilities, in vivo assessment on rice seedling growth, and data from greenhouse experiment were analysed for ANOVA and continued with Duncan's multiple range test (DMRT) at significance level 5%, and Pearson correlation analysis.

Moleculer identification of the superior isolates

The selected bacterial isolates which showed the consistent highest effect in vitro and in vivo assessment and increasing soil nutrients status (C, N, P, K) and rice growth on Alfisol supplemented with multivariant insecticides were sequenced to determine the species identity. At first the bacterial isolates were subjected for the DNA extraction using Quick-DNA Magbead Plus Kit (ZymoResearch, D4082). Then, bacterial DNA was amplified using MyTaq HS Red Mix, 2x (Bioline, BIO-25048). After confirmation by electrophoresis of the PCR product of 16S rDNA, the two directions of sequencing was conducted by the method of Sanger DNA sequencing by using *Capillary Electrophoresis.* The sequences of bacterial isolates were compared to the bacterial 16s rDNA from a database from https://ncbi.nlm.nih.gov with BLAST search to determine the closest relatives. The bacterial 16s rDNA sequences obtained in the present study were deposited in the DNA Data Bank of Japan (DDBJ) database (http://getentry.ddbj.nig.ac.jp/) with the accession number LC848126, LC848127, and LC848128.

RESULTS AND DISCUSSION

Population density and diversity of rice endophytic and rhizospheric bacteria isolated with Aleksandrov medium

Population density, number of bacterial morphotypes, and the Shannon diversity index of endophytic and rhizospheric bacteria in organic paddy fields were found in the present study to be significantly higher than those in conventional paddy fields. These results are presented in Table 1. Lin et al. (2020) reported similar results that the dynamics of endophytic bacterial community structure in rice roots vary under different field management systems, with organic farming exhibiting a greater diversity of endophytic bacteria compared to conventional practices. Furthermore, it was observed that the population and diversity of rhizospheric bacteria exceeded those of endophytic bacteria in both organic and conventional fields. Putrie et al. (2020) supported that the number of bacterial isolates from the pineapple (*Ananas comosus* L. Merr) rhizosphere was significantly greater than those from endophytic sources. Additionally, the study by Suzuki et al. (2019) indicated that the bacterial community composition in organic fields diverse substantially from that in conventional systems, a difference primarily attributed to the application of organic fertilizers, which enhances microbial diversity through improved nutrient cycling and soil health.

In the present study, from all isolation sources, a total of 11 isolates with distinct colony morphotypes were selected and subcultured onto new media for further assessment.

Functional capability as biofertilizers and plant growth promoters

The 11 selected isolates which originally isolated from Aleksandrov medium with initial screening on their capability as potassium solubilizer, at the further assessments showed multifunctional capabilities in phosphate solubilization, N-fixation, and IAA production. All isolates (Table 2) showed as potential multifunctional biofertilizers and plant growth promoters with different levels of superiority.

As presented in Table 2, isolate AEIC-1 showed the highest potassium solubilization

Table 1. Population density and diversity of rice endophytic and rhizospheric bacteria isolated using Aleksandrov medium

Type of paddy field	Source of Isolation	Number of colony morphotype	Population density (CFU/gr sample)	Shannon diversity index
Organic paddy field	rice leaf	2	7.100±9.56c	0.07±0.00c
	rice root	1	1.000±3.00e	0.00±0.00d
	rhizospheric soil	4	114.400±8.07a	0.46±0.00a
Conventional paddy field	rice leaf	1	6.000±5.32d	0.00±0.00d
	rice root	no growth	no growth	no growth
	rhizospheric soil	3	111.400±9.12b	0.36±0.00b
ANOVA			0.00**	0.00**

Note: significant level: ns – no significant, * – $p < 0.05$, ** – $p < 0.01$. Average values ± standard deviation within a column followed by the same letters are not significantly different at 5% level by DMRT.

Table 2. Functional capability as biofertilizer and plant growth promoter

Isolate*	KSI*	PSI*	N-Fixation*	IAA production
F-Values	0.00**	0.00**	colour changes	0.00**
AErO-1	2.89±0.01c	1.60±0.01d	+ (Blue)	3.88±0.03e
AElO-1	7.38±0.00b	1.01±0.00h	+ (Blue)	3.85±0.05ef
AElO-2	1.17±0.01g	1.44±0.01g	+ (Blue)	6.93±0.08c
ARsO-1	1.26±0.02f	5.31±0.01a	+++ (Yellow)	3.73±0.05h
ARsO-2	1.17±0.00g	5.02±0.00b	+++ (Yellow)	1.00±0.00k
ARsO-3	1.26±0.02f	3.80±0.00c	+++ (Yellow)	4.94±0.06d
ARsO-4	2.55±0.02d	1.00±0.00i	++ (medium Blue, colony Yellow)	3.91±0.01e
AElC-1	9.63±0.02a	1.01±0.00i	+++ (Yellow)	10.42±0.05a
ARsC-1	1.12±0.01h	1.53±0.02f	+ (Blue)	1.37±0.00j
ARsC-2	1.16±0.01g	1.57±0.01e	-	2.88±0.00i
ARsC-3	1.69±0.01e	1.60±0.01d	+++ (Yellow)	8.12±0.00b

Note: the acronym of isolate name (A – Aleksandrov medium; Er – Endophytic bacteria from root, El – Endophytic bacteria from leaf; Rs – rhizosphere soil bacteria; O – organic paddy field; C – conventional paddy field); KSI – potassium solubilization index, PSI – phosphate solubilization index; significant level: ns – no significant, * – $p < 0.05$, ** – $p < 0.01$. Average values ± standard deviation within a column followed by the same letters are not significantly different at 5% level by DMRT.

index (KSI) and IAA production, significantly higher than the other isolates. The highest phosphate solubilization index (PSI) value of 5.31 was indicated by the isolate ARsO-1, followed by isolate ARsO-2 with a PSI value of 5.02. Nitrogen fixation ability was assessed qualitatively based on the change of medium colour. Among 11 isolates, 5 isolates (ARsO-1, ARsO-2, ARsO-3, AElC-1, and ARsC-3) turned the medium from green to yellow completely in petridish. On the other side 4 isolates (AErO-1, AElO-1, AElO-2, and ARsC-1) turned the medium from green to blue, 1 isolate (ARsO-4) showed the turning of medium to blue but the colony in yellow, and 1 isolate (ARsC-2) showed no change of colour.

The findings align with those reported by Yang et al. (2024) who isolated endophytes from rice that were capable of nitrogen fixation, iron carrier production, potassium dissolution, and IAA synthesis. Similarly, Devi et al. (2022) reported the bacteria *Erwinia persicina* EU-A3SK3 (P-solubilizer), *Halomonas aquamarina* B2RNL2 (K-solubilizer), and *Pseudomonas extremorientalis* EU-B1RTR1 (N-fixer) that isolated from the rhizosphere and roots of wheat, demonstrating their capability to enhance nutrient availability and promote chilli (*Capsicum annum* L.) plant growth. A study by Pang et al. (2022) identified several endophytic bacterial isolates from wheat roots, stems, leaves, and seeds, highlighting their potential as biofertilizers through IAA production, nutrient solubilization (potassium and phosphate), and nitrogen fixation. Potassium solubilizing bacteria (KSB) showed diverse solubilization mechanisms and produced plant growth-promoting substances (Saheewala et al., 2023).

Resistance capability against insecticide toxicity

The absorbance difference value of bacterial growth in the two treatments of insecticide (monovariant and multivariant) is shown in Figure 1. The white histogram indicated the absorbance difference between the absorbance value of bacterial culture supplemented with monovariant insecticide with absorbance value of bacterial culture without insecticide. The black histogram indicated the absorbance difference between the absorbance value of bacterial culture supplemented with multivariant insecticide with absorbance value of bacterial culture without insecticide.

The highest value of absorbance difference under monovariant insecticide treatment was obtained by isolate ARsC-3 followed by isolate AElC-1 and AElO-1. On the other hand, the highest value of absorbance difference under multivariant was yielded by isolate AElO-1 followed by isolate AElC-1 and ARsC-3. These findings revealed a variety of bacterial responses to the insecticide treatment, most bacteria showed positive value of absorbance difference which indicated

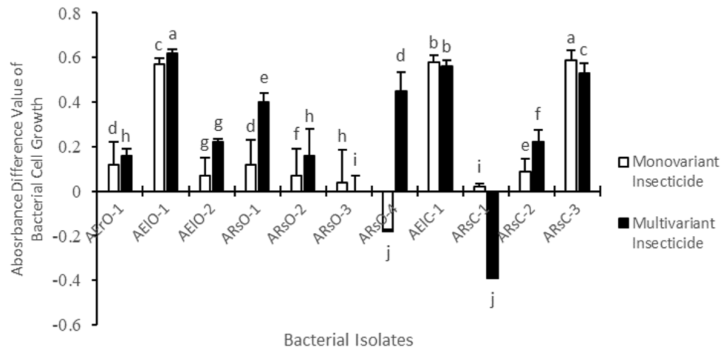

Figure 1. The absorbance difference value indicates the difference in absorbance values of bacterial cell growth in medium supplemented with monovariant or multivariant insecticides compared to growth in medium without insecticides

the growth of bacteria was higher in the culture supplemented with insecticide either monovariant or multivariant insecticide, while there were only two isolates that showed negative values, indicating that most bacteria exhibited resistance ability against insecticides toxicity, or suggesting their capability to utilize insecticides as nutrients or energy sources for growth.

Previous studies have demonstrated the effects of insecticide on bacterial growth. A study by Shahid et al. (2019) highlights the high toxicity of various active ingredients, particularly glyphosate and atrazine, which adversely affect both bacterial growth and plant development by causing structural damage at the cellular level and reducing the production of essential growth regulators such as IAA. Kumar et al. (2022) found that strains *Pseudomonas* degraded chlorpyrifos, achieving 65.77% degradation by the 7th day and complete degradation (100%) by the 30th day of incubation in MS medium. Moreover, Lamilla et al. (2021) explained the mechanisms mediated by *Pseudomonas* strains which capable of producing biosurfactants that enhance the solubility and bioavailability of hydrophobic pesticides like chlorpyrifos, leading to significantly improve degradation efficiency. Conde-Avila et al. (2021) reported that *A. vinelandii* ATCC 12837 was capable of degrading chlorpyrifos into nutrients through enzymatic hydrolysis. Bacterial growth in insecticide-contaminated environments is influenced by factors such as the active ingredients of the insecticide, environmental conditions, nutrient availability, and the inherent resistance of specific bacterial strains (Shahid and Khan, 2022; Kebede et al., 2021). Bacteria can degrade pesticides into less toxic compounds by secreting enzymes that transform these chemicals into carbon and water, which are safer and serve as energy sources (Ahmad et al., 2022).

The effect of selected isolates to the growth of rice seedling on agarose medium treated with multivariant insecticides

Based on the capability as biofertilizers and plant growth promoters, and the resistance against insecticide, 9 out of 11 isolates were selected to assess their effect on the growth of rice seedling on agarose medium until 10 days after showing. Among all observed variables, the isolates AElC-1 (isolate 1 of rice leaf endophytic bacteria from conventional paddy field) and AElO-1(isolate 1 of rice leaf endophytic bacteria from organic paddy field) showed the highest growth of rice seedlings as represented from the plant fresh weight, plant height, and chlorophyll content (Table 3). These two isolates had high capability in potassium solubilization, IAA production (Table 2), and also high resistance to mono-variant and multi-variant insecticides (Figure 1). The capability of both isolates to solubilize potassium and produce IAA contributed to enhanced plant growth as indicated by the increased fresh weight, plant height, and chlorophyll content of rice seedlings. Ahumada et al. (2022) and Edelmann (2022) reported that IAA production is an essential phytohormone that stimulates root elongation and enhances plant growth development. Combining potassium solubilization with IAA production amplifies the benefits for plant growth and can significantly enhances biomass and resilience under stress, including insecticide exposure (Tian et al., 2024; Patani et al., 2023). The results of this study were consistent with the findings of Prodhan et al. (2023), who reported that certain rice endophytic bacteria can degrade chlorpyrifos, an organophosphate insecticide, by breaking down toxic compounds into non-toxic metabolites, while also promoting plant growth by producing growth hormones such as P-solubilization, N-fixation, and IAA production.

Meanwhile isolate ARsO-1, despite showing lower fresh weight (Table 3) and insecticide resistance (Figure 2) than AElC-1 and AElO-1, promoted significantly the highest root length (Table 3) which is related to the higher phosphate-solubilizing ability and capable to produce IAA (Table 2). This result is consistent with Ibrahim and Ikhajiagbe (2021), who reported that seed inoculation with the bacteria capable of solubilizing-phosphate (*Bacillus cereus* GGBSU-1) enhanced root length, chlorophyll content, and biomass significantly. Similarly, Mohamed et al. (2019) found that strains such as *Enterobacter* and *Pantoea,* capable of solubilizing-phosphate, also produce IAA, which supports cell elongation and division, thus stimulating root development. Cheng et al. (2023) confirm that bacteria capable of solubilizing phosphate promote root growth by secreting organic acids, which lower soil pH and increase phosphorus availability.

Potassium-solubilizing bacteria (KSB) play a vital role in improving potassium availability, which is critical for plant growth and stress tolerance (Nawaz et al., 2023). KSB strains play a mechanisme to produce organic acids such as citric acid, that solubilize potassium from insoluble mineral forms, making it more accessible to plants (Olaniyan et al., 2022). This process is crucial for maintaining plant health, especially under stress conditions like insecticide exposure.

The effect of selected isolates toward soil chemical characteristics treated with multivariant insecticides

A comparison of the treatment of control (+) and control (–) indicated that the application of multivariant insecticides resulted in a slight increase in pH and available P, with no significant effect on other soil variables. Among all the treatments, the highest increase of soil nutrient status (C, N, P, K) was demonstrated by the NPK treatment (Table 4). This result indicated that all the isolates inoculation treatments gave lower effect on soil fertility compared to the NPK treatment. The isolate AElC-1 (isolate 1 of rice leaf endophytic bacteria from conventional paddy field) showed the highest effect in increasing soil fertility compared to all other isolate treatments as indicated by the increase of total-N, available-P, and available-K. The treatment of NPK increased total-N, available-P, and available-K by 19.4%, 45.2%, and 60% compared to the control (+), respectively. In the meanwhile, the treatment isolate AElC-1 increased those three soil macro nutrients by 16.6%, 10.7%, and 50% compared to the control (+), respectively (Table 4). The lowest effect of isolate inoculation either in the individual or consortium treatments to the increased soil nutrients was detected on the increase of available-P which at the range 1.9–10.7%, while the

Table 3. The effect of selected isolates to the growth of rice seedling on agarose medium treated with multivariant insecticides

Treatment	Plant height (cm)	Plant fresh weight (gr)	Root length (cm)	Chlorophyll content (mg/kg)
F-values of ANOVA	0.00**	0.00**	0.00**	0.00**
Control (-)	5.58±0.76d	0.031±0.011de	3.30±0.89d	0.41±0.06cd
Control (+)	5.38±1.38d	0.028±0.013e	3.26±0.85d	0.34±0.14d
AErO-1	11.48±0.86bc	0.048±0.007abc	4.40±0.65bc	0.72±0.06ab
AElO-1	12.00±1.43a	0.050±0.006ab	5.30±1.22ab	0.85±0.25a
AElO-2	8.46±2.58abcd	0.034±0.003de	4.20±0.89bcd	0.56±0.02bc
ARsO-1	10.08±3.64abc	0.048±0.009abc	6.04±1.36a	0.58±0.28bc
ARsO-2	6.16±3.64cd	0.035±0.009de	4.32±1.86bcd	0.46±0.12cd
ARsO-3	10.14±4.74abc	0.046±0.003abc	3.48±0.93cd	0.55±0.08bcd
ARsO-4	9.16±1.68abcd	0.041±0.006bcd	4.66±1.00bcd	0.51±0.33bcd
AElC-1	11.84±1.59a	0.053±0.008a	5.04±1.74ab	0.81±0.06a
ARsC-3	10.72±0.56ab	0.046±0.003abc	5.24±0.54ab	0.78±0.01a
MABf	8.6±4.41bcd	0.039±0.007cde	4.12±1.55cd	0.51±0.13cd
MABr	6.88±3.76abcd	0.038±0.008bcde	3.88±1.37cd	0.53±0.06bcd
MABfBr	6.98±4.41bcd	0.038±0.008cde	4.44±1.12bcd	0.57±0.07bc

Note: significant level: ns=no significant, * = $p < 0.05$, ** = $p < 0.01$. Average values ± standard deviation within a column followed by the same letters are not significantly different at 5% level by DMRT.

Figure 2. The growth of rice seedling on agarose medium treated with multivariant insecticides (10 days after sowing). Control (–) – (no isolate and no insecticide), control (+) – (no isolate but with insecticide), and the treatments of individual isolate of AElC-1, AElO-1, and ARsO-1 which exhibited higher growth of rice seedling compared to other isolate treatments

increase of total-N at the range 14.2–16.6% and available-K at the range 20–50% (Tabel 4). This finding revealed that the selected isolates in the present study were superior as biofertilizers especially in K solubilization. The research by Liu et al. (2021) supports the findings of this study, showing that inoculation of endophytes isolated from *Phytolacca acinosa* tissue grown in Cd-contaminated soil increased the availability of soil nutrients (available-N 14.5%, available-P 20.1%, available-K 18.7%, and organic matter content 7.4%) compared to the control on soil that initially was characterised as follows: pH 7.87 (1:2.5 soil-water suspension), available-N 27.02 mg/kg, available-P 60.81 mg/kg, available-K 99.02 mg/kg, organic matter 18.1 g/kg, and Cd concentration 56.72 mg/kg.

The treatment of NPK and the control (+) showed the highest level of chlorpyrifos residue in soil, at the level of 0.174 and 0.166 mg/kg, respectively. The treatments of bacterial isolate inoculation affected to reduce the chlorpyrifos residue in soil to the levels at 0.132–0.147 mg/kg for individual isolate inoculation and at the levels 0.152–0.153 for consortium isolate inoculation. The highest decrease of chlorpyrifos residue in soil was indicated by isolate ARsC-3 treatment, followed by isolate AElC-1 and AElO-1. Several studies have confirmed the ability of endophytic and rhizospheric bacteria to degrade pesticide.

Table 4. The effect of selected isolates toward the characteristics of Alfisol soil treated with multivariant insecticides

Treatment	pH H_2O	Organic-C (%)	Total-N (%)	C/N ratio (%)	Available-P (ppm)	Available-K (cmol(+)/kg)	Chlorpyrifos residue in soil (mg/kg)
F-values of ANOVA	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Control (-)	5.58±0.01f	1.61±0.02g	0.41±0.01e	3.94±0.12c	7.71±0.04cd	0.12±0.14d	0.000±0.00l
Control (+)	5.85±0.05e	1.69±0.00fg	0.42±0.02de	4.02±0.19c	7.69±0.03d	0.10±0.12e	0.154±0.00b
NPK	6.39±0.31a	3.40±0.70a	0.50±0.00a	6.77±1.42a	11.17±0.80a	0.16±0.05a	0.176±0.00a
AErO-1	6.20±0.21bc	2.09±0.01cd	0.48±0.02ab	4.35±0.22c	8.50±0.38b	0.13±0.25b	0.141±0.00i
AElO-1	6.35±0.02ab	2.22±0.00bcd	0.49±0.03ab	4.47±0.31c	8.52±0.07b	0.14±0.25b	0.135±0.00j
AElO-2	6.03±0.03cde	1.74±0.00efg	0.43±0.02cde	3.98±0.26c	8.02±0.05bcd	0.14±0.12b	0.147±0.00f
ARsO-1	6.19±0.18bc	2.08±0.00cd	0.46±0.03bc	4.45±0.30c	8.24±0.01bc	0.13±0.13c	0.142±0.00h
ARsO-2	5.94±0.04de	1.73±0.00efg	0.43±0.02cde	3.99±0.23c	7.84±0.29cd	0.12±0.08d	0.150±0.00e
ARsO-3	6.11±0.05cd	2.15±0.02cd	0.46±0.02bc	4.41±0.20c	8.48±0.25b	0.13±0.08c	0.143±0.00g
ARsO-4	5.93±0.03de	1.76±0.01efg	0.45±0.00bcd	3.90±0.29c	8.01±0.06bcd	0.13±0.11c	0.147±0.00f
AElC-1	6.37±0.03ab	2.56±0.02b	0.49±0.01ab	5.22±0.12b	8.52±0.64b	0.15±0.04a	0.134±0.00h
ARsC-3	6.31±0.07ab	2.18±0.00c	0.48±0.02ab	4.41±0.22c	8.51±0.39b	0.14±0.09b	0.132±0.00k
MABf	5.98±0.02de	1.86±0.00defg	0.45±0.02bcd	4.11±0.19c	8.50±0.12b	0.12±0.09c	0.153±0.00c
MABr	6.04±0.13cd	1.93±0.02cdfg	0.45±0.00bcd	4.20±0.04c	8.04±0.04bcd	0.14±0.02b	0.152±0.00d
MABfBr	6.02±0.03cde	1.87±0.04defg	0.45±0.02bcd	4.10±0.22c	8.50±0.29b	0.13±0.80b	0.153±0.00c

Note: significant level : ns = no significant, *= $p < 0.05$, ** = $p < 0.01$. Average values ± standard deviation within a column followed by the same letters are not significantly different at the 5% level by DMRT.

Research by Nasrollahi et al. (2020) showed that rice endophytic bacteria such as *Bacillus altitudinis* DB26-R and *B. subtilis* B6-L can degrade diazinon in vivo, reducing pesticide residues in rice plants. A study by Farhan et al. (2021) showed that *Bacillus cereus* Ct3 isolated from cotton-growing soil effectively degraded 88% of chlorpyrifos, as revealed by GC–MS analysis that chlorpyrifos is first converted into diethyl thiophosphoric acid and 3,5,6-trichloro-2-pyridinol (TCP), which was then completely mineralized without any toxic byproducts. In the present study the highest degradation of chlorpyrifos (14.2%) contributed by isolate ARsC-3 was lower compared to the capability of *Bacillus cereus* Ct3 reported by Farhan et al. (2021).

The rice growth on the treatment of control (+) was lower compared to control (–) as indicated by all variables (Table 5). This phenomenon demonstrated that the application of multivariant insecticides inhibited rice growth. The treatment of NPK showed the highest growth of rice as represented by all variables, indicating that the treatment of NPK chemical fertilizer at the recommended dose (350 kg/ha, SP-36 75 kg/ha, and KCl 100 kg/ha) gave higher effect in increasing plant growth compared to the inoculation of endophytic or rhizospheric bacteria (Table 5). Among all the isolate treatments, the isolate AElC-1 showed the highest increase of rice growth as indicated by all variables of plant biomass and nutrient uptake (N, P, K), followed by isolate AElO-1 which showed higher effect on plant weight and K-uptake. The increase of plant fresh and dry weights by NPK treatments were 212.2% and 261%, while the increase by isolate AElC-1 were 59.4% and 96.7% compared to the control (+). Thus, the increase of rice plant fresh and dry weight by isolate AElC-1 was 28% and 37% of the increase by NPK treatment.

The previous study has shown that combining endophytic bacteria with reduced levels of chemical fertilizers can lead to substantial increases in rice grain yields. Fatema et al. (2024) reported that using both *Alcaligenes faecalis* (BTCP01) from *Eleusine indica* (Goosegrass) and *Metabacillus indicus* (BTDR03) from *Cynodon dactylon* (Bermuda grass) with 50% of the recommended N, P, and K fertilizer doses resulted in higher yields compared to using 100% of the recommended doses. In this context, endophytic bacteria significantly contribute to supplying plant nutrients, potentially reducing the need for chemical fertilizers. However, this study found that the contribution of endophytic bacteria in substituting chemical fertilizers was lower than in Fatema et al. (2024), with isolate AElC-1 providing only 25.5% of N-uptake, 44% of P-uptake, and 49.2%

Table 5. The effect of selected isolates toward rice growth on Alfisol soil treated with multivariant insecticides

Treatment	Plant height (cm)	Plant fresh weight (g/pot)	Plant dry weight (g/pot)	N-uptake (mg/plant)	P-uptake (mg/plant)	K-uptake (mg/plant)	Chlorophyll content (mg/g)	Chlorpyrifos residue in plant (mg/kg)
F-values of ANOVA	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Control (-)	76.2±2.47cde	39.78±2.79bcd	12.75±3.84cd	0.59±0.08c	2.42±1.13e	0.40±0.12de	0.80±0.09de	0.000±0.00o
Control (+)	73.1±1.37e	32.86±3.06d	9.28±0.38d	0.55±0.03c	2.37±0.23e	0.34±0.11e	0.74±0.14e	0.294±0.00a
NPK	90.8±5.17a	102.43±23.82a	33.34±7.28a	1.91±0.35a	13.14±2.58a	1.00±0.58a	1.48±0.36a	0.074±0.05n
AErO-1	76.7±2.50bcde	47.20±5.52bc	15.15±2.09bc	0.70±0.11c	3.79±1.77cde	0.60±0.12cd	0.97±0.36c	0.195±0.00j
AElO-1	80.2±2.52bcd	50.64±4.24b	17.99±2.57b	0.76±0.09bc	4.46±0.44bc	0.62±0.13bcd	1.20±0.13ab	0.101±0.00l
AElO-2	78.1±2.27bccde	40.41±4.64bcd	12.61±1.79cd	0.68±0.11c	3.10±0.66cde	0.57±0.09cd	0.99±0.32c	0.199±0.00h
ARsO-1	78.2±1.39bcde	42.84±1.36bcd	13.65±1.33c	0.68±0.10c	3.73±0.26cde	0.55±0.13cde	0.96±0.46c	0.196±0.00i
ARsO-2	75.1±2.37de	35.02±4.41cd	11.34±1.28cd	0.61±0.06c	2.42±0.34e	0.42±0.09de	0.87±0.36d	0.207±0.00e
ARsO-3	77.8±3.41bcde	41.36±4.76bcd	14.32±1.72bc	0.61±0.05c	3.54±0.71cde	0.53±0.13de	0.91±0.55cd	0.201±0.00g
ARsO-4	80.5±2.02bc	41.79±2.07bcd	14.71±0.30bc	0.60±0.14c	2.66±0.53de	0.50±0.05de	0.98±0.61c	0.202±0.00f
AElC-1	81.8±0.43a	52.32±6.82b	18.13±1.16b	0.93±0.12b	5.81±1.09b	0.67±0.13b	1.22±0.60ab	0.098±0.00m
ARsC-3	80.3±5.45bcd	44.55±4.57bcd	15.40±1.78bc	0.71±0.07c	4.26±0.57cd	0.64±0.13bc	1.11±0.13b	0.193±0.08k
MABf	76.4±1.76cde	37.45±4.16cd	11.56±0.68cd	0.62±0.02c	2.73±0.45de	0.48±0.04de	0.89±0.65d	0.215±0.00c
MABr	76.6±3.66cde	40.30±4.72bcd	11.60±1.60cd	0.65±0.19c	2.85±0.41cde	0.46±0.07de	0.87±0.24d	0.209±0.00d
MABfBr	77.6±2.40cde	47.31±8.24bc	12.34±1.32cd	0.67±0.08c	3.01±0.84cde	0.44±0.12de	0.82±0.24de	0.217±0.00b

Note: significant level: ns = no significant, *= $p < 0.05$, ** = $p < 0.01$. Average values ± standard deviation within a column followed by the same letters are not significantly different at the 5% level by DMRT.

of K-uptake compared to the full recommended doses of chemical fertilizers.

Previous studies have also reported that endophytic bacteria enhance plant growth. For instance, Liu et al. (2021) showed that all experimental endophytes used in the study could stimulate plant growth and increase Cd accumulation in *P. acinosa* plants in soil compared to the control, endophyte inoculation increased dry weight and Cd concentration by 7.96–25.13% and 3.34–19.54%, respectively. Yang et al. (2024) reported that inoculation of the endophytic actinobacteria strain Ahn65 isolated from rice stem significantly increased rice yield, root length, stem length, and number of tillers elevated by 12.01%, 18.00%, and 16.00%, respectively, after 60 days of culture. Additionally, the dry weights of roots and stems in the treated group were 14.55% and 3.35% higher than those in the control group after 30 days.

The rice growth showed a significantly positive correlation with soil nutrient status, as demonstrated by Pearson correlation analysis. Plant fresh and dry weights were strongly correlated with soil organic-C ($r = 0.833$, $p < 0.01$; $r = 0.875$, $p < 0.01$), with total-N ($r = 0.746$, $p < 0.01$; $r = 0.474$, $p < 0.01$), available-P ($r = 0.902$, $p < 0.01$; $r = 0.852$, $p < 0.01$), and available-K ($r = 0.428$, $p < 0.01$; $r = 0.486$, $p < 0.01$). Thus, increasing soil nutrients affected significantly in supporting plant growth as indicated by the enhancement of plant biomass.

Rice plants treated with *Aquabacter pokkalii* sp. nov. (strain L1I39T), isolated from salt-tolerant pokkali rice in brackish environments, exhibited significant plant growth-promoting properties, including biological nitrogen fixation and ACC deaminase activity, which enhanced root and shoot length, increased fresh and dry weights, and elevated nitrogen content by day 18 compared to untreated control (Sunithakumari et al., 2024).

The highest level of chlorpyrifos residue in rice plants was detected in the treatment of control (+) (with insecticide application but without isolate inoculation) (Table 5). Although the NPK treatment showed the highest chlorpyrifos residue in soil but indicated the lowest chlorpyrifos residue in rice plants. Similar to the NPK treatment, the bacterial isolate inoculation which showed higher chlorpyrifos residue in soil indicated lower chlorpyrifos residue in rice plants. The Pearson correlation analysis revealed significant correlations between chlorpyrifos residues in soil and in plant tissue with plant fresh and dry weights. Chlorpyrifos residues in the soil showed a significant positive correlation with plant fresh weight ($r = 0.240$, $p < 0.01$) and dry weight ($r = 0.213$, $p < 0.01$) of plants. In contrast, chlorpyrifos residues in plant tissue exhibited significant negative correlations with fresh weight ($r = -0.404$, $p < 0.01$) and dry weight ($r = -0.444$, $p < 0.01$).

Feng et al. (2017) isolated various endophytic bacteria from chlorpyrifos-treated rice plants, including *Pseudomonas aeruginosa, Bacillus megaterium, Sphingobacterium siyangensis, Stenotrophomonas pavanii,* and *Curtobacterium plantarum*. Inoculation with these bacteria resulted in a significant reduction of chlorpyrifos residues on rice grains, with up to 80% reduction compared to the control. The isolate AElC-1 in the present study resulted in chlorpyrifos reduction 66.6% in rice tissue (a mixture of rice shoot and root at vegetative phase), which was lower compared to the chlorpyrifos reduction in rice grains reported by Feng et al. (2017).

Molecular identification of the superior isolates

Molecular identification revealed that isolates AElO-1, AElC-1, and ARsC-3 exhibited high sequence similarity with *Microbacterium* sp., *Caulobacter* sp., and *Sphingomonas trueperi*, respectively (Table 6). The detected closest relative reported play a significant functional role in the agricultural environment either as plant growth promoter or as bio-degradation agent of pesticide. *Microbacterium* sp. D-2 isolated from agricultural soil contaminated with the organochlorine insecticide dicofol showed a degradation ability of

Table 6. Identification of the superior isolates by 16s rDNA sequencing

Isolate code	Isolate source	Closest relatives (Accession number)	Similarity	Accession number of isolate
AEIO-1	Rice leaf tissue from organic paddy field	*Microbacterium* sp. (MN410660.1)	99.93%	LC848126
AEIC-1	Rice leaf tissue from conventional paddy field	*Caulobacter* sp. (MH392625.1)	99.26%	LC848127
ARsC-3	Rice rhizospheric soil from conventional paddy field	*Sphingomonas trueperi* (MF686447.1)	100%	LC848128

81.9% in soil (Lu et al., 2019). *Caulobacter* sp. fulfills diverse ecological roles, including symbiotic associations with nitrogen-fixing cyanobacteria and enhancement of plant growth in *Arabidopsis thaliana* (Garcıa et al., 2018; Luo et al., 2019).

In the context of degradation and plant growth promotion, members of the *Sphingomonas* genus have been isolated from various environments, including desert soils, agricultural soil, abandoned lead-zinc mines, and wetland soil (Dong et al., 2022; G. Da Feng et al., 2019; Lee et al., 2024; Siddiqi et al., 2023). For instance, *Sphingomonas trueperi* CW3 has been demonstrated to effectively degrade allethrin, a synthetic pyrethroid insecticide, achieving optimal degradation (93%) under specific conditions (pH 7.0, 30 °C, inocula concentration 100 mg/L), with successful application in soil bioaugmentation (Bhatt et al., 2020). Other species have also effectively degraded the pesticides fipronil and thiobencarb through oxidative and hydrolytic processes (Faridy et al., 2024) and exhibit multiple plant growth-promoting characteristics (Mazoyon et al., 2023). These findings highlight the potential applications of these bacterial genera in bioremediation strategies and agricultural improvement.

CONCLUSIONS

The population density and diversity of endophytic and rhizospheric bacteria isolated from the organic paddy fields utilizing Aleksandrov medium exhibited significantly higher levels compared to those from conventional paddy fields. This finding indicates that organic paddy fields create environments that are more conducive to the proliferation of more diverse associated microbiota. The Aleksandrov medium facilitated the selective proliferation of bacterial isolates with potassium-solubilizing ability. However, this study reveals that most isolates also possess multifunctional potential, functioning as biofertilizers and plant growth promoters. Furthermore, the majority of these isolates exhibited significant resistance to insecticide-induced toxicity. The isolate treatments either as a single or a consortium treatments gave significant effect to induce plant bio-resistance to insecticide toxicity and to contribute in the degradation of chlorpyrifos in soil as indicated by the comparison level of chlorpyrifos residue in plant tissue and in soil as represented by the level of residue in control (+) at 0.294 and 0.154 mg/kg, in the NPK treatment at 0.074 and 0.176 mg/kg, the isolate treatments at the range of 0.098–0.217 and 0.132–0.153 mg/kg, respectively. Although the isolate treatments exhibited a lower impact on plant growth and insecticide resistance compared to NPK chemical fertilizer, they contributed more significantly to the degradation of chlorpyrifos in soil, highlighting their potential as agents for protecting soil and plant health in agricultural environments. This study identifies three isolates with the highest potential among the 11 initially selected strains: two rice leaf endophytic bacteria, AElC-1 and AElO-1, and one rhizosphere bacterium, ARsC-3. In both laboratory and greenhouse pot experiments, the rice leaf endophytic isolates AElC-1 and AElO-1 showed superior efficacy in promoting plant growth compared to the rhizosphere bacterium ARsC-3. However, ARsC-3 outperformed AElC-1 and AElO-1 in enhancing chlorpyrifos degradation in soil. Molecular identification showed 99.26–100% sequence similarity of isolates AElO-1, AElC-1, and ARsC-3 to *Microbacterium* sp., *Caulobacter* sp., and *Sphingomonas trueperi*, respectively.

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