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Screening of indole-3-acetic acid PGPB from three agricultural systems at Nakhon Pathom, Thailand

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Abstract. *Homthong M, Kaewpuk W, Yamsuan S, Thongsima A, Mokkapan K, Pikulthong V. 2022. Screening of indole-3-acetic acid PGPB from three agricultural systems at Nakhon Pathom, Thailand. Biodiversitas 23: 5935-5941.* Plant growth-promoting bacteria (PGPB) are associated with plant roots and enhance growth via a variety of mechanisms, including fixation of atmospheric nitrogen, solubilization of phosphorus, phytohormone synthesis, and plant phenolic compound stimulation. Sixty-five isolated bacteria from the three agricultural systems in Nakhon Pathom were screened for IAA production. The results showed that seven isolates—C01-28, C02-48, C02-51, G01-34, G03-25, G03-38, and O02-46—produced IAA under L-tryptophan as a precursor at values of 9.08 ± 0.51, 68.64 ± 29.07, 40.67 ± 18.04, 10.65 ± 1.07, 63.30 ± 1.15, 37.73 ± 1.80, and 121.28 ± 15.20 mg mL^{-1}, respectively. Capability on plant growth-promoting traits revealed that none of the isolates stimulated ten-morning glory (*Ipomoea aquatica* Forsk.) seedling growth, which was not significantly different from the control. Isolates O02-46 enhanced salicylic acid (SA) in ten-morning glory seedlings the most, showing 7.18 ± 1.78 µg g^{-1} of fresh weight, while isolates G01-34 enhanced phenolic compound (PC) production the most showing 218.18 ± 29.55 mg GAE g^{-1} extract, respectively. The 16S rRNA analysis revealed that isolates O02-46, C02-48, G03-25, and C02-51 were identified as similar to *Paenibacillus alvei* at 99.20% (Accession number AY826588.1), *Bacillus megaterium* at 79.91% (Accession number MH031358.1), *Bacillus* sp. at 98.57% (Accession number JF322976.1), and *Lysinibacillus fusiformis* at 99.61% (Accession number HE610782.1).

Keywords: Agricultural systems, indole-3-acetic acid (IAA), plant growth promoting bacteria

INTRODUCTION

Plant growth promoting bacteria (PGPB) naturally associate with plants and facilitate growth through a variety of mechanisms, including the ability to modulate the concentrations of phytohormones in plants (Beneduzi et al. 2012). Plant growth promoting rhizobacteria (PGPR) are a heterogeneous group of bacteria that promote plant growth by colonizing the root. They improve the extent or quality of the plant via direct or indirect mechanisms (Dewi et al. 2015; Gupta et al. 2015; Santosa et al. 2018). PGPR directly confers various beneficial mechanisms including the production of phytohormones (indole-3-acetic acid or IAA, gibberellic acid, cytokinins, and ethylene), solubilization of phosphate, and, asymbiotic nitrogen fixation. Indirect mechanisms are associated with biological control through the production of antibiotics, lytic enzymes, hydrogen cyanide, catalase, and siderophore. PGPR also enhances plant tolerance to salinity and drought and lessen heavy metal stress (Ahemad and Kibret 2013; Vurukonda et al. 2016; Leontidou et al. 2020). Previous studies reported that *Pseudomonas*, *Azotobacter*, *Klebsiella*, *Enterobacter*, *Alcaligens*, *Arthobacter*, *Burkholderia*, *Bacillus*, *Serratia*, and *Streptomyces* sp. enhanced plant growth, while more than 80% of bacteria isolated from the rhizosphere produced IAA via different biosynthesis pathways (Sasirekha et al. 2012; Duan et al. 2013; Mohite 2013; Majeed et al. 2015; Zheng et al. 2018; Khamna et al. 2010). Nutaratat et al. (2015) found that the yeast *Rhodosporidium* produced IAA.

Indole-3-acetic acid (IAA) is an important secondary metabolite produced by bacteria, fungi, and plants (Fu et al. 2015; Kumla et al. 2014; Kumla et al. 2020). Generally, IAA affects plant cell division, extension, and differentiation by stimulating seed, tuber germination, phototropism, geotropism, and fluorescence. IAA promotes the production of longer roots with increased numbers of root hairs and root laterals that are involved in nutrient uptake (Glick 2012). PGPR also produce IAA using the pathway with and without L-tryptophan as a precursor (Myo et al. 2019). However, more than one pathway is often present in a bacterium depending on the presence of a phytohormone precursor (Patten and Glick 1996). Bharucha (2013) found that *Pseudomonas putida* UB1 produced higher IAA when the L-tryptophan medium for IAA production was supplemented with sucrose 0.5 %, $(NH_4)_2SO_4$ 10 mg mL^{-1} and tryptophan 0.2 mg mL^{-1} at pH 7.5. Similarly, Yasmin et al. (2007) revealed that L-tryptophan did not enhance IAA production in some isolates, while other strains showed a maximum of 9-fold increase in the presence of L-tryptophan. Under natural conditions, the narrow zone of plant roots accumulates a

variety of chemical compounds secreted by plant roots including L-tryptophan which is utilized by PGPR for IAA production (Flores et al. 1999). Sitlaothaworn et al. (2022) found that *Staphylococcus edaphicus* of IAA-producing bacteria exhibited the properties of potential plant growth-promoting. Wongchindakhun et al. (2015) found that *Pseudomonas fluorescens* cell-free filtrate through biochemical changes brings about an accumulation of plant growth regulators that relates to the biosynthesis of IAA. Furthermore, plant hormones have been implicated in plant defense mechanisms that interact in complex networks with other secondary metabolites including salicylic acid (SA) and phenolic compounds (PC) (Ncube et al. 2012; Denance et al. 2013). Salicylic acid is considered to be a plant signal molecule that is involved in biotic and abiotic stress responses by activating many defense compounds including phenolic acid, coumarins, flavonoids, and lignin (Al-Wakeel et al. 2013). Mendoza et al. (2018) revealed that SA and methyl jasmonate (MeJA) increased PC and flavonoid compound production in plant cell suspension cultures. Currently, biological approaches to manage pests, diseases and crop production are gaining interest for ecological sustainability. Many saprophytic bacteria, yeast, filamentous fungi, and PGPR in soils have also been evaluated as naturally occurring biocontrol agents (Pliego et al. 2011; Wang et al. 2021). Beneduzi et al. (2012) revealed that PGPR strains can promote plant growth and biocontrol agents. Several substances produced by antagonistic rhizobacteria have been related to pathogen control and indirect promotion of growth in many plants, such as siderophores and antibiotics. Induced systemic resistance (ISR) in plants resembles pathogen-induced systemic acquired resistance (SAR) under conditions where the inducing bacteria and the challenging pathogen remain spatially separated. The target groups of the research include Organic farmers, in Don Tum District, Kamphaeng Saen District, and Mueang District. The research instruments to survey the topic of research include 1) a questionnaire 2) interviews 3) study visits. Data analysis was used to analyze the topic of research on local needs. Here, IAA-producing bacteria were screened from three agricultural systems 1) organic, 2) good agricultural practices (GAP), and 3) chemical-based in three districts of Nakhon Pathom Province; 1) Don Tum District, 2) Kamphaeng Saen District, and 3) Mueang District. Their potential to enhance salicylic acid and phenolic compound production in plants was investigated to control plant diseases and mitigate future chemical usage.

MATERIALS AND METHODS

Materials

Nutrient agar (NA) (Hi-MEDIA#M001) and Nutrient Broth (NB) medium were purchased from Himedia (India). Van Urk Salkowski reagent, standard IAA, *gallic acid, Folin-Ciocalteu reagent, ferric ammonium sulfate*, and agarose were obtained from Sigma-Aldrich (St. Louis, MO, USA). Reaction agents for DNA extraction and 16S rRNA amplification were purchased from Thermo Fisher (Invitrogen; Sao Paulo, Brazil).

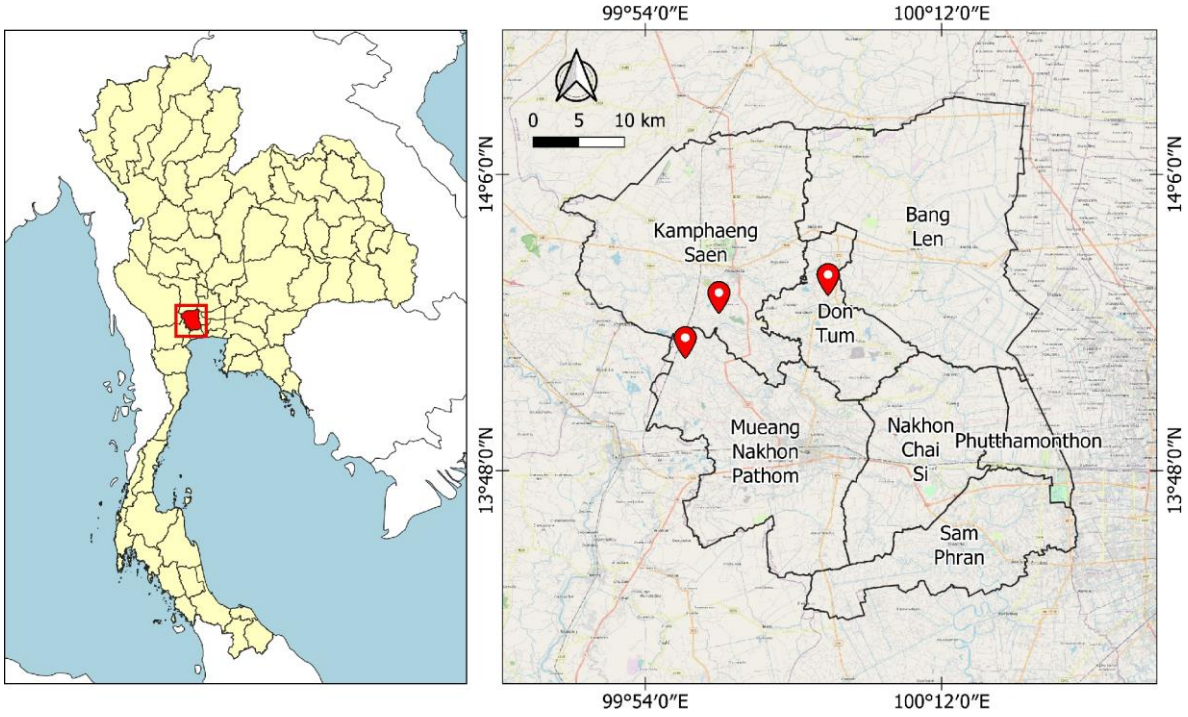

Figure 1. Map showing the location of sample collection sites in Don Tum District, Kamphaeng Saen District, and Mueang District of Nakhon Pathom Province, Thailand

Isolation of IAA-producing bacteria from soil

Nine soil samples of each agriculture system from Don Tum District, Kamphaeng Saen District, and Mueang District of Nakhon Pathom Province, Thailand were collected (Figure 1). Soils are sampled by using a spade, making a V-shaped cut to the desired soil depth of 3-5 cm thick taken from the V-shape hole, usually to 10-15 cm depth. Collect at least 10 to 15 spots/locations from each soil sample and place them in a tray. One gram of soil was mixed in 9 mL of sterile distilled water and incubated in a rotary shaker at 120 rpm for 2 h. One microliter of suspension sample was serially diluted (10^{-2}-10^{-5}) and 1 mL of each diluted sample was then spread on NA and incubated under the aerobic condition at room temperature for 3-7 days. Colonies of different shapes and colours were selected and transferred to NA media.

IAA production

IAA production was determined using the Salkowski method (Gordon and Weber 1950). All isolates were grown in NB containing 50 mg l^{-1} of *L-tryptophan* and incubated at 28°C for 72 h. The culture broth was centrifuged at 8,000 rpm for 20 minutes after incubation. The supernatant was reserved while the cell pellet was discarded. A 2 mL aliquot of supernatant was mixed with 2 mL of Salkowski's reagent and kept in the dark. The reaction was assessed for IAA production after incubation in the dark for 30 and 120 minutes. The amount of IAA was measured by the spectrophotometric method at 535 nm. The concentration of IAA was calculated using the standard curve and measured in the range of 100-900 µg mL^{-1}.

Effect of IAA-producing isolates on plant growth

To study the effect of IAA-producing bacteria on plant growth. The seven IAA-producing isolates were selected for evaluation in the soil pot trial with Ten-morning glory. We assigned all studies to different conditions between seeds incubated with bacterial supernatant of the seven IAA-producing isolate conditions and control conditions, treated with distilled water. Ten-morning glory (*Ipomoea aquatica* Forsk.) seeds were germinated for each treatment in triplicate. Surface sterilized ten-morning glory seeds were incubated with bacterial supernatant of the IAA-producing isolate for 3 h, while the control was treated with distilled water and NB. Seeds were dried and sowed into sterile soil as a carrier. Pots were irrigated with sterile distilled water every day and kept in sunlight. After 8 days, the seedlings were measured for shoot height, root length, and biomass weight.

Content of total salicylic acid compounds

The SA analysis of 8-day-old ten-morning glory seedlings was adapted from the protocol of Raskin et al. (1989). One gram of fresh tissue samples was ground in 2.5 mL of 90% methanol and centrifuged at 12,000 rpm for 15 minutes. Five hundred microliters of supernatant were added to 500 µL of 0.02 M ferric ammonium sulfate. Colorimetric determination was measured by the spectrophotometric method at 535 nm after the reaction was incubated at 30°C for 5 minutes. The amount of SA (µg/g fresh weight) was calculated using the standard curve of SA.

Content of total phenolic compounds

Total PCs from fresh 8-day-old ten-morning glory seedlings were extracted using the Folin-Ciocalteu reagent as described by Onanong et al. (2011). Three grams of fresh tissues were ground in 12 mL of 80% ethanol and centrifuged at 12,000 rpm for 20 minutes. The supernatant (0.5 mL) was removed and mixed with 0.5 mL of 10% Folin-Ciocalteu phenol reagent (1:1) and then added with 1 mL of saturated sodium carbonate solution (7% w v^{-1}). The reaction was kept in the dark for 40 minutes. Absorbances of blue color from different samples were measured at 760 nm. The phenolic content was calculated as gallic acid equivalents GAE g^{-1} of dry plant material based on a standard curve at 2.5-90 µg mL^{-1} of gallic acid.

Identification of bacterial strains

The purified isolates were initially classified based on their molecular characteristics using 16S rRNA analysis, and kept at 4°C and -80°C (in nutrient broth containing 20% glycerol) until required for further study. Genomic DNA was extracted following the adapted method of Wilson (2001). Species were identified by 16S rRNA analysis using universal primers with the following sequences: forward primer 5′-AGAGTTTGATCCTGGCT CAG-3′ (primer fD1); reverse primer 5′-AAGGAGGTGA TCCAGCC-3′ (primer rD1) from Weisburg et al. (1991). These primers are designed to yield nearly full-length 16S rDNA from most bacteria. The PCR product was purified using Favor PrepTM GEL/PCR purification kit (Favorgen, Taiwan) and sent to 1st BASE (Malaysia) for 16S rRNA sequencing. All sequences were compared with the GenBank database using the BLAST program from NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

All experiments were performed in triplicate with values expressed as mean ±SD. Analysis of variance and significance of difference among means were tested by one-way ANOVA using Duncan's Multiple Range Test (DMRT) by using SPSS ver. 17.0 statistical software.

RESULTS AND DISCUSSION

IAA producing bacteria selection

Sixty-five purified isolates were selected from three different agricultural systems (organic, GAP, and chemical-based) in Nakhon Pathom Province using the spread plate method, and tested for quantitative estimation of IAA production in the presence of 50 mg l^{-1} of L-tryptophan. IAA production was checked by forming pink solution samples with Salkowski's reagent. Results indicated that seven isolates as C01-28, C02-48, C02-51, G01-34, G03-25, G03-38, and O02-46 produced IAA showing 9.08 ± 0.51, 68.64 ± 29.07, 40.67 ± 18.04, 10.65 ± 1.07, 63.30 ± 1.15, 37.73 ± 1.80, and 121.28 ± 15.20 µg mL^{-1}, respectively. One (O02-46) isolate, three (G01-34, G03-25,

and G03-38) isolates, and three (C01-28, C02-48, and C02-51) isolates were selected from the soil of organic, GAP, and chemical-based systems, respectively. Isolate O02-46 showed the highest IAA production significantly higher than the others, followed by C02-48, G03-25, C02-51, G03-38, G01-34, and C01-28 (Figure 2).

Plant growth promoting traits

Capabilities for plant growth promoting metabolites of the seven IAA-producing isolates were evaluated by pot assay. Shoot heights, root lengths, and weights of seedlings of each treatment were measured after seed germination for 8 days. Results exhibited that C01-28, G01-34, G03-25, and O02-46 stimulated ten-morning glory seedling growth (shoots and roots) and seedling fresh weights of all treatments but were not significantly different from the control. Our findings results revealed that isolates C02-48, C02-51, and G03-38 stimulated ten-morning glory shoot heights at the highest values of 8.4 ± 1.2, 8.2 ± 0.4, and 8.1 ± 1.7 cm, respectively while the longest ten-morning glory root lengths of 11.9 ± 3.5, 11.5 ± 2.4, and 11.2 ± 1.1 cm were enhanced by isolates G03-38, C02-48, and C02-51, respectively (Figure 3). Seedling fresh weights of 0.45 ± 0.07, 0.43 ± 0.06, and 0.42 ± 0.07 cm were enhanced by isolates G03-38, C02-51, and C02-48, respectively (Table 1).

Salicylic acid stimulation in morning ten-glory seedling

Salicylic acid production of 8-day-old ten-morning glory seedling tissues was extracted with 90% methanol and assayed according to the protocol of Raskin et al. (1989). The SA accumulation in ten-morning glory seedling tissues induced by isolate O02-46 showed the highest value of 7.18 ± 1.78 µg g^{-1} fresh weight and was significantly higher than the others ($p \leq 0.05$) (Table 2). This result suggested that the high concentration of IAA in bacterial supernatant produced by isolate O02-46 was related to SA accumulation in ten-morning glory seedlings. However, the amount of SA in ten-morning glory seedling tissues incubated with G03-25 did not relate to their IAA production, showing as 1.55 ± 0.59 and significantly lower than the others. Production of SA in seedlings inoculated with isolates C01-28, C02-48, C02-51, G01-34, and G03-38 also showed no significant differences from the control.

Phenolic compound contents in ten-morning glory seedlings

Phenolic compound contents of 8-day-old ten-morning glory seedling tissues were 218.18 ± 29.55, 213.97 ± 77.93, 212.11 ± 38.41, 210.01 ± 24.41, 167.24 ± 31.04, and 163.94 ± 18.89 mg GAE g^{-1} extract for seeds incubated with bacterial supernatant of G01-34, G03-38, C02-51, C02-48, O02-46, and G03-25, respectively (Table 2). Isolates G01-34, G03-38, C02-51, C02-48, O02-46, and G03-25 enhanced PC contents above the control but with no significant differences ($p \geq 0.05$). Isolate C01-28 was significantly lower than isolates G01-34, G03-38, C02-51, and C02-48 ($p \leq 0.05$) and gave 122.80 ± 32.07 mg GAE g^{-1} extract. Isolate O02-46 enhanced SA to the maximum value but the trend of PC content of ten-morning glory seedling tissues (incubation with a high concentration of IAA) did not relate to their SA accumulations.

Table 1. Effect of IAA on shoot height, root length, and biomass weight of ten-morning glory seedlings

Isolate	Shoot height (cm)	Root length (cm)	Weight (g)
Control	7.3 ± 0.3^{ab}	8.6 ± 1.8^{ab}	0.30 ± 0.07^{ab}
C01-28	8.0 ± 1.4^{ab}	8.6 ± 1.4^{ab}	0.40 ± 0.04^{ab}
C02-48	8.4 ± 1.2^{b}	11.5 ± 2.4^{b}	0.42 ± 0.07^{b}
C02-51	8.2 ± 0.4^{b}	11.2 ± 1.1^{b}	0.43 ± 0.06^{b}
G01-34	7.7 ± 1.3^{ab}	8.0 ± 2.5^{ab}	0.31 ± 0.03^{ab}
G03-25	7.7 ± 0.2^{a}	7.0 ± 3.0^{a}	0.31 ± 0.05^{a}
G03-38	8.1 ± 1.7^{b}	11.9 ± 3.5^{b}	$0 .45 \pm 0.07^{b}$
O02-46	7.7 ± 0.8^{ab}	9.0 ± 0.2^{ab}	0.39 ± 0.04^{ab}

Table 2. Total salicylic acid compounds and total phenolic compounds

Isolate	Salicylic acid compounds (µg g^{-1} fresh weight)	Phenolic compounds (mg GAE g^{-1} extract)
Control	3.54 ± 1.44^{b}	146.73 ± 43.13^{ab}
C01-28	2.76 ± 0.79^{ab}	122.80 ± 32.07^{a}
C02-48	4.28 ± 1.37^{b}	210.01 ± 24.41^{b}
C02-51	2.68 ± 0.57^{ab}	212.11 ± 38.41^{b}
G01-34	2.49 ± 0.16^{ab}	218.18 ± 29.55^{b}
G03-25	1.55 ± 0.59^{a}	163.94 ± 18.89^{ab}
G03-38	3.02 ± 0.69^{ab}	213.97 ± 77.93^{b}
O02-46	7.18 ± 1.78^{c}	167.24 ± 31.04^{ab}

Note: In each column, superscripts (a, b) represent significant differences ($p \leq 0.05$) using DMRT.

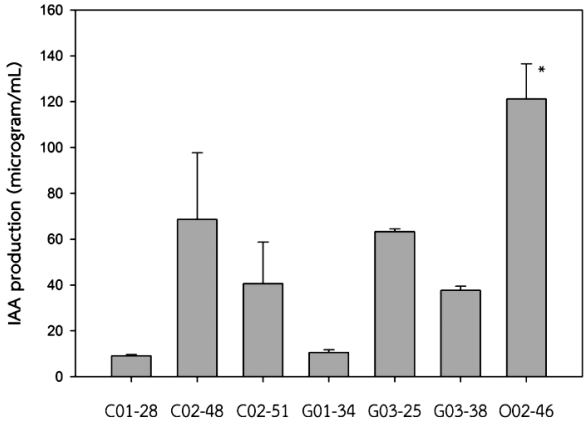

Figure 2. Production of indole-3-acetic acid (IAA) (µg mL^{-1}) of bacterial supernatant after growth in NB containing 50 mg l^{-1} of L-tryptophan for 72 h. Note: In figure represents (*) represent significant differences ($p \leq 0.05$) using DMRT. The first character = agricultural systems (C = chemical-based, G = good agricultural practices (GAP), O = organic) the second character = sample collection sites (01 = Don Tum District, 02 = Kamphaeng Saen District, 03 = Mueang District) the third number = separable order (1 = Isolate No. 1, 2, 3, ... , 65)

Table 3. Sequences producing significant alignments

Sequencing	Scientific Name	E-value	Per. Ident	Accession
O02-46	*Paenibacillus alvei*	0.0	99.20%	AY826588.1
C02-48	*Bacillus megaterium*	1e-103	79.91%	MH031358.1
G03-25	*Bacillus* sp.	0.0	98.57%	JF322976.1
C02-51	*Lysinibacillus fusiformis*	0.0	99.61%	HE610782.1

A **B**

Figure 3. Efficacy of C02-48 on shoot and root lengths (A) after incubation for eight days compared with untreated seeds (B). Note: In each column, superscripts (a, b) represent significant differences ($p \leq 0.05$) using DMRT

16S rRNA identification

Four isolates that showed high IAA production were selected from seven isolates capable of producing IAA. Four isolates were classified based on their molecular characteristics using 16S rRNA analysis. Sequencing displayed that O02-46, C02-48, G03-25, and C02-51 were similar to *Paenibacillus alvei* at 99.20% (Accession number AY826588.1), *Bacillus megaterium* at 79.91% (Accession number MH031358.1), *Bacillus* sp. at 98.57% (Accession number JF322976.1), and *Lysinibacillus fusiformis* at 99.61% (Accession number HE610782.1), respectively (Table 3).

Discussion

The rhizosphere is the narrow zone of plant roots that acts as a microbe storehouse where the biological and chemical features of the soil are influenced by the roots (Kundan et al. 2015). Compounds released by plants play a vital role in large heterogeneous microbial diversity. Agricultural management also influences the biological composition of soil microorganisms and the amount, diversity, and function of microorganisms (Vacheron et al. 2013). Here, soil microorganisms from organic, GAP, and chemical-based agricultural systems in Nakhon Pathom Province were collected and screened for IAA-producing bacteria. Results showed that one strain, three strains, and three strains of IAA-producing isolates were found in the organic, GAP, and chemical-based agricultural systems, respectively. These seven isolates produced IAA ranging from 9.08 ± 0.51 to 121.28 ± 15.2 μg mL^{-1}. Although only one IAA-producing strain (O02-46) was obtained from the organic system, this produced IAA at the highest value of 121.28 ± 15.2 μg mL^{-1} which corresponds to Deejing and Buntor (2018) found that bacteria RSTSA-6 (*Paenibacillus alvei*) could produce indole-3-acetic acid (IAA) 20.55 mg/L. The 16S rRNA analysis revealed that the IAA-produced strains isolated from the soil were *Paenibacillus alvei*, *Bacillus aryabhattai*, and *Bacillus amyloliquefaciens* while Niazi et al. (2014) found that *Bacillus amyloliquefaciens* subsp. *plantarum* strain UCMB5113 can colonize plant roots and stimulate plant growth, and *Lysinibacillus* sp., while Boonnadakul et al. (2019) found that *Pseudomonas nitroreducens* PY7-6 provided the highest amount of IAA.

The efficacy of the seven isolates on plant growth promoting traits revealed that some treatments stimulated ten-morning glory seedling growth that was significantly different from the control. Inconsistent with our results, previous studies found that *Pseudomonas fluorescens* SP007s and *Brevibacillus agri* promoted plant growth by producing and releasing plant hormones (IAA or GA3) (Chuaboon and Athinuwat 2014; Inthasan et al. 2017). Furthermore, results suggested that high IAA production of O02-46 was also related to SA accumulation in ten-morning glory seedlings, showing 7.18 ± 1.78 μg g^{-1} fresh weight. Similarly, Ton et al. (2009) revealed that phytohormones including auxin and abscisic acid (ABA) stimulated SA accumulation in plants and were also involved with plant-pathogen interaction. Salicylic acid has been widely reported as promoting the production of phenolic acids in plants (Serghini et al. 2001; Katoch et al. 2005; Dong et al. 2010). In our study, the trend of PC contents did not relate to SA accumulation in ten-morning glory seedlings. Results exhibited that the PC

content of O02-46 stimulated ten-morning glory seedlings at lower than ten-morning glory seedlings stimulated by G01-34, G03-38, C02-51, and C02-48. However, the role of SA not only involved an increase in PC but also enhanced other defense compounds such as coumarins, flavonoids, and lignin (Al-Wakeel et al. 2013). Pot experiments were also assayed using an aseptic technique. Thus, it was probably reasonable that the trend of SA accumulation did not relate to PCs. To understand these complex relations, secondary metabolite defense compounds such as PCs, coumarins, flavonoids, and lignin should be estimated under biotic or abiotic stress. These seven IAA-producing isolates are also gaining interest regarding other plant growth promoting traits such as properties to produce 1-aminocyclopropane (ACC) deaminase (Penrose and Glick 2003), siderophore (Schwyn and Neiland 1987), solubilization of phosphate (Pikovskaya 1948), and modes of action against plant pathogens for the appliance of these strains into agriculture.

In conclusion, as many as 65 isolates were isolated from 3 agricultural systems, found 7 isolates capable of producing IAA. The efficacy of these seven isolates on plant growth-promoting traits revealed that all treatments were not significantly different from the control. Isolates O02-46 and G01-34 enhanced the highest salicylic acid (SA) and phenolic compound (PC) production in ten-morning glory seedlings, respectively. High IAA production of isolates O02-46 and C02-48 were also related to SA accumulation in ten-morning glory seedlings. However, the trend of PC contents in treated ten-morning glory seedlings did not relate to SA accumulation. These seven IAA-producing isolates play an expedient role by stimulating plant growth and inducing systemic resistance.

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REFERENCES

Ahemad M, Kibret M. 2013. Mechanisms and applications of growth promoting rhizobacteria: Current perspective. J King Saud Univ Sci 26: 1-20. DOI: 10.1016/j.jksus.2013.05.001.

Al-Wakeel SAM, Moubasher H, Gabr MM, Madany MMY. 2013. Induced systemic resistance: an innovative control method to manage branched broomrape (*Orobache ramose* L.) in tomato. IUFS J Biol 72 (1): 9-21.

Beneduzi A, Ambrosini A, Passaglia MPL. 2012. Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. Genet Mol Biol 35: 1044-1051. DOI: 10.1590/S1415-47572012000600020.

Bharucha U, Patel K, Trivedi BU. 2013. Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth-promoting rhizobacteria on mustard (*Brassica nigra*). Agric Res 2 (3): 215-221. DOI 10.1007/s40003-013-0065-7.

Boonnadakul C, Cheunbarn S, Cheunbarn T, Klayraung S, Aumtong S. 2019. Selection of free-living nitrogen fixing bacteria to promote on rice growth. Naresuan Phayao J 12: 32-40.

Chuaboon W, Athinuwat D. 2014. Plant hormone produced from *Pseudomonas fluorescens* SP007s to enhance growth of organic kale. SNRUJST 3 (3): 196-205.

Deejing S, Buntor A. 2018. Screening and identification of endophytic bacteria from organic red jasmine rice soil. JOA 35 (1): 49-60.

Denance N, Sanchez-Vallet A, Goffner D, Molina A. 2013. Disease resistance or growth: the role of plant hormones in balancing immune responses and fitness costs. Front Plant Sci 4 (155): 1-12. DOI: 10.3389/fpls.2013.00155.

Dewi TK, Arum ES, Imamuddin H, Antonius S. 2015. Karakterisasi mikroba perakaran (PGPR) agen penting pendukung pupuk organik hayati. Pros Sem Nas Masy Biodiv Indon 1 (2): 289-295. DOI: 10.13057/psnmbi/ m010220. [Indonesian]

Dong J, Wan G, Liang Z. 2010. Accumulation of salicylic acid-induced phenolic compounds and raised activities of secondary metabolic and antioxidative enzymes in *Salvia miltiorrhiza* cell culture. J Biotechnol 148: 99-104. DOI: 10.1016/j.jbiotec.2010.05.009.

Duan J, Jiang W, Cheng Z, Heikkila JJ, Glick RB. 2013. The complete genome sequence of the plant growth-promoting bacterium *Pseudomonas* sp. uw4. Plos One 8 (3): E58640. DOI: 10.1371/journal.pone.0058640.

Flores HE, Vivanco JM, Loyola-Vargas VM. 1999. "Radicle" biochemistry: the biology of root-specific metabolism. Trends Plant Sci 4: 220-226. DOI: 10.1016/s1360-1385(99)01411-9.

Fu S-F, Wei J-Y, Chen H-W, Liu Y-Y, Lu H-Y, Chou J-Y. 2015. Indole-3-acetic acid: A widespread physiological code in interactions of fungi with other organisms. Plant Signal Behav 10 (8): e1048052. DOI: 10.1080/15592324.2015.1048052.

Glick BR. 2012. Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012: 1-15. DOI: 10.6064/2012/963401.

Gordon AS, Weber RP. 1950. Colorimetric estimation of indole acetic acid plant. Plant Physiol 26 (1): 192-195. DOI: 10.1104/pp.26.1.192.

Gupta G, Parihar SS, Ahirwar KN, Snehi KS, Singh V. 2015. Plant growth promoting rhizobacteria (PGPR): current and future prospects for development of sustainable agriculture. J Microb Biochem Technol 7 (2): 96-102. DOI: 10.4172/1948-5948.1000188.

Inthasan J, Dechjirarattanasiri C, Boonmee P. 2017. Effect of bacteria-producing indole-3-acetic acid (IAA) on growth and nutrient contents of bird chili (*Capsicum annuum* L.). J Agric 33 (3): 333-344.

Katoch R, Mann APS, Sohal BS. 2005. Enhanced enzyme activities and induction of acquired resistance in pea with elicitors. J Veg Sci 11: 67-83. DOI: 10.1300/J484v11n01_07.

Khamna S, Yokota A, Peberdy FJ, Lumyong S. 2010. Indole-3-acetic acid production by *Streptomyces* sp. isolated from some Thai medicinal plant rhizosphere soils. EurAsia J BioSci 4: 23-32. DOI: 10.5053/ejobios.2010.4.0.4.

Kumla J, Suwannarach N, Bussaban B, Matsui K, Lumyong S. 2014. Indole-3-acetic acid production, solubilization of insoluble metal minerals and metal tolerance of some sclerodermatoid fungi collected from northern Thailand. Ann Microbiol 64: 707-720. DOI 10.1007/s13213-013-0706-x.

Kumla J, Suwannarach N, Matsui K, Lumyong S. 2020. Biosynthetic pathway of indole-3-acetic acid in ectomycorrhizal fungi collected from northern Thailand. Plos One 15 (1): e0227478. DOI: 10.1371/journal.pone.0227478.

Kundan R, Pant G, Jado N, Agrawal PK. 2015. Plant growth promoting rhizobacteria: mechanism and current prospective. J Fertil Pestic 6(2): 1-9. DOI: 10.4172/2471-2728.1000155.

Leontidou K, Genitsaris S, Papadopoulou A, Kamou N, Bosmali I, Matsi T, Madesis P, Vokou D, Karamanoli K, Mellidou I. 2020. Plant growth promoting rhizobacteria isolated from halophytes and drought-tolerant plants: genomic characterisation and exploration of phyto-beneficial traits. Sci Rep 10: 14857. DOI: 10.1038/S41598-020-71652-0.

Majeed A, Abbasi MK, Hameed S, Imran A, Rahim N. 2015. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. Front Microbiol 6 (198): 198. DOI: 10.3389/fmicb.2015.00198.

Mendoza D, Cuaspud O, Arias JP, Ruiz O, Arias M. 2018. Effect of salicylic acid and methyl jasmonate in the production of phenolic compounds in plant cell suspension cultures of *Thevetia peruviana*. Biotechnol Rep E00273. DOI: 10.1016/j.btre.2018.e00273.

Mohite B. 2013. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its effect on plant growth. J Soil Sci Plant Nutr 13 (3): 638-649. DOI: 10.4067/S0718-95162013005000051.

Myo ME, Ge B, Ma J, Cui H, Liu B, Shi L, Jiang M, Zhang K. 2019. Indole-3-acetic acid production by *Streptomyces fradiae* NKZ-259 and its formulation to enhance plant growth. BMC Microbiol 19: 1-14. DOI: 10.1186/s12866-019-1528-1.

Ncube B, Finnie JF, Van Staden J. 2012. Quality from the field: the impact of environmental factors as quality determinants in medicinal plants. S Afr J Bot 82: 11-20. DOI: 10.1016/j.sajb.2012.05.009.

Niazi A, Manzoor S, Asari S, Bejai S, Meijer J, Bongcam-rudloff E. 2014. Genome analysis of *Bacillus amyloliquefaciens* subsp. *plantarum* UCMB5113: a rhizobacterium that improves plant growth and stress management. Plos One 9 (8): E104651. DOI: 10.1371/journal.pone.0104651.

Nutaratat P, Amsri W, Srisuk N, Arunrattiyakorn P, Limtong S. 2015. Indole-3-acetic acid production by newly isolated red yeast *Rhodosporidium paludigenum*. J Gen Appl Microbiol 61: 1-9. DOI 10.2323/jgam.61.1.

Onanong K, Sirithon S, Natthida W. 2011. Phenolic compounds and antioxidant activities of edible flowers from Thailand. J Funct Foods 3: 88-99. DOI: 10.1016/j.jff.2011.03.002.

Penrose DM, Glick BR. 2003. Methods for isolating and characterizing ACC deaminase-containing plant growth promoting rhizobacteria. Plant Physiol 118: 10-15. DOI: 10.1034/j.1399-3054.2003.00086.x.

Pikovskaya RI. 1948. Mobilization of phosphorus in soil connection with the vital activity of some microbial species. Microbiology 17: 362-370.

Pliego C, Ramos C, Vicente AD, Cazorla FM. 2011. Screening for candidate bacterial biocontrol agents against soilborne fungal plant pathogens. Plant Soil 340: 505-520. DOI: 10.1007/s11104-010-0615-8.

Raskin I, Turner IM, Melander WR. 1989. Regulation of heat production in the inflorescence of an *Arum lily* by endogenous salicylic acid. Proc Natl Acad Sci USA 86 (7): 2214-2218. DOI: 10.1073/pnas.86.7.2214.

Santosa S, Sutarno, Purwanto E, Suranto, Sajidan. 2018. Molecular characterization of Plant Growth Promoting Rhizobacteria using 16S rRNA sequences in the organic rice field of Sukorejo Village, Central Java, Indonesia. Biodiversitas 19: 2157-2162. DOI: 10.13057/biodiv/d190623.

Sasirekha B, Shivakumar S, Sullia SB. 2012. Statistical optimization for improved indole-3-acetic acid (IAA) production by *Pseudomonas aeruginosa* and demonstration of enhanced plant growth promotion. J Soil Sci Plant Nutr 12 (4): 863-873863. DOI: 10.4067/S0718-95162012005000038.

Schwyn B, Neiland JB. 1987. Universal chemical assays for the detection and determination of siderophores. Anal Biochem 160 (1): 47-56. DOI: 10.1016/0003-2697(87)90612-9.

Serghini KA, Perez-de-Luque A, Castejon-Munoz M, Garcia-Torres L, Jorrin, JV. 2001. Sunflower (*Helianthus annuus* L.) response to broomrape (*Orobanche cernua* Loefl.) parasitism: Induced synthesis and excretion of 7-hydroxylated simple coumarins. J Exp Bot 52: 2227-2234. DOI: 10.1093/jexbot/52.364.2227.

Sitlaothaworn K, Busabun T, Dechkla M, Thaipratum R, Khunphaderm M. 2022. Isolation and optimization of efficient indole acetic acidproducing *Staphylococcus edaphicus* from *Saccharum officinarum* rhizosphere with their influence on multiple plant growth-promoting traits. Intl J Agric Technol 18 (5): 2255-2270.

Ton J, Flors V, Mauch-Mani B. 2009. The multifaceted role of ABA in disease resistance. Trends Plant Sci 14: 310-317. DOI: 10.1016/j.tplants.2009.03.006.

Vacheron J, Desbrosses G, Bouffaud ML, Touraine B, Moënne-Loccoz Y, Muller D, Legendre L, Wisniewski-Dyé F, Combaret CP. 2013. Plant growth promoting rhizobacteria and root system functioning. Front Plant Sci 4 (356): 1-19. DOI: 10.3389/fpls.2013.00356.

Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A. 2016. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiol Res 184: 13-24. DOI: 10.1016/j.micres.2015.12.003.

Wang H, Liu R, You PM, Barbetti JM, Chen Y. 2021. Pathogen biocontrol using plant growth-promoting bacteria (PGPR): role of bacterial diversity. Microorganisms 9 (9): 1-18. DOI: 10.3390/microorganisms9091988.

Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173 (2): 697-703. DOI: 10.1128/jb.173.2.697-703.1991.

Wilson K. 2001. Preparation of genomic DNA from bacteria. Curr Protoc Mol Biol 56 (1): 2.4.1-2.4.5. DOI: 10.1002/0471142727.mb0204s56.

Wongchindakhun A, Chuaboon W, Athinuwat D. 2015. Efficacy of the cell-free filtrate of *Pseudomonas fluorescens* to enhance growth of para-rubber seedling. Thai J Sci Technol 4 (2): 155-164. DOI: 10.14456/tjst.2015.16.

Yasmin F, Othman R, Saad MS, Sijam K. 2007. Screening for beneficial properties of rhizobacteria isolated from sweet potato rhizophere. Biotechnol 6: 49-52. DOI: 10.3923/biotech.2007.49.52.

Zheng W, Zeng S, Bais H, Lamanna MJ, Hussey SD, Jacobson LD, Jin Y. 2018. Plant growth-promoting rhizobacteria (PGPR) reduce evaporation and increase soil water retention. Water Resour Res 54 (5): 3673-3687. DOI: 10.1029/2018WR022656.