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Identification and plant growth-promoting activity of endophytic bacteria from sugarcane roots and genome analysis of *Gluconacetobacter* strain PS25

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ABSTRACT: Fifty endophytic bacteria isolated from sugarcane (*Saccharum officinarum* L.) roots in Thailand were identified and evaluated for plant growth-promoting capability using rice as a plant model. They were identified as *Gluconacetobacter* (37 isolates), *Pantoea* (8 isolates), *Burkholderia* (2 isolates), and each of *Nguyenibacter*, *Pseudomonas*, and *Aureimonas* based on the phenotypic characteristics and 16S rRNA gene analyses. Strain PS25 has genome size of 4.4 Mbp with DNA G+C content of 64.7 mol%. The average nucleotide identity (ANIb and ANIm) values of PS25 and *G. dulcium* LMG1728^T were 93.77% and 98.74%, respectively, and the digital DNA-DNA hybridization (dDDH) value was 88.80%. The PS25 was identified as *G. dulcium*. Most strains were found to fix nitrogen, solubilize P (SI=1.15–4.40) and Zn (SI=1.59–5.60), but only 9 strains could produce indole-3-acetic-acid (67.25–202.25 μg/ml) in the medium with L-tryptophan. Furthermore, *G. liquefaciens* and *G. dulcium* were found as new strains with the ability to fix nitrogen. *A. phyllosphaerae* SK2, *G. dulcium* PS25, and *G. liquefaciens* LSG1 significantly increased root length (3.09–3.49 cm), shoot length (10.14–11.21 cm), number of lateral roots, and biomass of rice seedlings. This work indicates that these endophytic bacteria could be applied to enhance plant growth.

KEYWORDS: acetic acid bacteria, endophytic bacteria, indole-3-acetic acid, nitrogen fixation, phosphate solubilization, zinc solubilization

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is one of agricultural crops that is crucial to economy for producing sugar, biochemicals, and biofuel in more than 120 countries such as India, Brazil, Vietnam, China, Australia, and Thailand [1, 2]. The development for increasing crop yield using potential microorganisms as plant growth promotor, bioinoculant, or biofertilizer to decrease the use of chemical fertilizer is still needed. Endophytic bacteria associated with sugarcane that are colonized in root tissues can enhance plant growth via several mechanisms, for example nitrogen fixation, solubilization of insoluble elements, production of antimicrobial substances and phytohormones. Many endophytic bacteria referred to as plant growth promoting bacteria (PGPB) were isolated from sugarcane such as *Bacillus*, *Pseudomonas*, *Rhizobium*, *Azotobacter*, *Burkholderia*, *Azospirillum*, *Herbaspirillum*, and *Gluconacetobacter* [3].

Some acetic acid bacteria (AAB) have been reported to colonize the epidermis of sugarcane stem and root and could fix nitrogen in the air and convert it to ammonia by nitrogenase enzyme [4]. Besides nitrogen fixation, AAB were also reported to produce indole-3-acetic acid (IAA), solubilize P and Zn such as *Acetobacter diazotrophicus*, *A. nitrogenifigens*, *A. peroxydans*, *Gluconacetobacter azotocaptans*, *G. johannae*, *Asaia bogorensis*, and *A. siamensis* [5–7]; however, there are a few studies on AAB isolated from sugarcane in Thailand. The aim of this study was to isolate and identify endophytic bacteria from sugarcane root and evaluate their IAA production, nitrogen fixation, and P and Zn solubilization. Selected strains have also been tested as a bioinoculant for *in vitro* rice growth enhancement. The diversity of the isolates was analyzed by partial 16S rRNA gene sequence and genome technology.

MATERIALS AND METHODS

Sample collection and bacteria isolation

Seven root samples of sugarcane were collected from 6 Provinces in Thailand (Table 1). Root samples were washed with tap water and surface sterilized by soaking in series of solution as follows: 2% sodium

Table 1 Characteristics of bacterial strains from different collecting locations.

Province	Strain no.	Group	N_2 fixation	Solubilization		Similarity (%)	Accession no.	Nearest type strain
				P	Zn			
Si Sa Ket	SK1	A	+	1.25	2.79	99.93	OM742987	*Pantoea dispersa* LMG 2603^T
Sa Kaeo	KG1	A	++++	3.89	5.60	99.85	OM742989	*Pantoea dispersa* LMG 2603^T
	KG2	A	++	1.38	2.44	99.93	OM742990	*Pantoea dispersa* LMG 2603^T
	KG3	A	++	-	3.29	99.78	OM742991	*Pantoea dispersa* LMG 2603^T
	KG4	A	++	1.97	1.59	100	OM742992	*Pantoea dispersa* LMG 2603^T
	KG5	A	++	-	3.51	100	OM742993	*Pantoea dispersa* LMG 2603^T
	PK1	A	++	-	3.08	99.93	OM742999	*Pantoea dispersa* LMG 2603^T
Chumphon	CH2	A	+	1.83	2.42	100	OM743002	*Pantoea dispersa* LMG 2603^T
Nong Khai	LSG1	B1	+++	2.67	3.50	100	LC618513	*Gluconacetobacter liquefaciens* IFO 12388^T
	LSS3	B1	+++++	1.71	4.54	100	LC618514	*Gluconacetobacter liquefaciens* IFO 12388^T
	LSS4	B1	+	2.28	4.60	100	LC618515	*Gluconacetobacter liquefaciens* IFO 12388^T
Phetchaburi	AM1	B1	++	1.18	3.09	100	OM742994	*Gluconacetobacter liquefaciens* IFO 12388^T
	AM2	B1	++	1.43	3.08	100	OM742995	*Gluconacetobacter liquefaciens* IFO 12388^T
	AM4	B1	+++	3.25	4.10	99.85	OM742997	*Gluconacetobacter liquefaciens* IFO 12388^T
	AM5	B1	++	1.54	3.42	100	OM742998	*Gluconacetobacter liquefaciens* IFO 12388^T
Sa Kaeo	PK2	B1	++	2.31	4.83	100	OM743000	*Gluconacetobacter liquefaciens* IFO 12388^T
Chumphon	CH1	B1	++	2.14	5.09	100	OM743001	*Gluconacetobacter liquefaciens* IFO 12388^T
	CH3	B1	++	2.28	5.40	100	OM743003	*Gluconacetobacter liquefaciens* IFO 12388^T
	CH4	B1	+++	2.13	4.15	100	OM743004	*Gluconacetobacter liquefaciens* IFO 12388^T
Prachuap	PS1	B1	++	1.46	2.67	100	LC618520	*Gluconacetobacter liquefaciens* IFO 12388^T
Khiri Khan	PS2	B1	++	1.57	2.92	100	LC618521	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS3	B1	+	4.00	3.25	99.85	OM742965	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS4	B1	++	3.90	3.40	100	OM742966	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS5	B1	+++	3.73	3.40	100	OM742967	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS6	B1	++	4.40	3.36	100	OM742968	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS7	B1	++	3.64	3.40	100	OM742969	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS8	B1	++	1.73	3.80	100	OM742970	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS9	B1	++	3.57	4.20	100	OM742971	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS10	B1	++	1.55	3.90	100	OM742972	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS11	B1	++	3.23	2.71	100	OM742973	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS12	B1	++	2.69	3.60	100	OM742974	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS13	B1	++	3.54	3.41	100	OM742975	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS14	B1	+++	1.15	3.50	100	OM742976	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS15	B1	+	4.20	3.80	100	OM742977	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS16	B1	++	1.83	3.80	100	OM742978	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS17	B1	++	2.31	3.75	100	OM742979	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS18	B1	++	2.57	3.42	100	OM742980	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS19	B1	++	1.70	3.90	100	OM742981	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS20	B1	++	1.33	3.58	100	OM742982	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS21	B1	++	2.83	3.70	100	OM742983	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS22	B1	++	2.53	3.42	100	OM742984	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS23	B1	+++	2.18	3.45	100	OM742985	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS24	B1	++	3.00	3.75	100	LC618522	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS25	B1	+++	2.64	3.42	99.64	OM742986	*Gluconacetobacter liquefaciens* IFO 12388^T
	PS26	B1	++	2.60	3.42	100	LC618523	*Gluconacetobacter liquefaciens* IFO 12388^T
Nong Khai	LTS2	B2	++	2.62	5.38	100	LC618516	*Nguyenibacter vanlangensis* TN01LGI^T
Nong Khai	LGF5	B3	++	1.67	3.00	100	LC618517	*Burkholderia anthina* R-4183^T
Phetchaburi	AM3	B3	+++	2.14	3.67	99.86	OM742996	*Burkholderia territorii* LMG 28158^T
Nong Khai	LRF6	B4	+++	1.54	2.92	100	LC618518	*Pseudomonas oryzihabitans* NBRC 102199^T
Si Sa Ket	SK2	B5	++++	-	-	97.01	OM742988	*Aureimonas phyllosphaerae* L9-753^T

+ very low, ++ low, +++ moderate, ++++ high, or +++++ very high positive reaction showing ammonia synthesis compared with control (NF broth) measured at 560 nm.

hypochlorite for 3 min, sterile distilled water for 3 min, and 70% ethanol for 1 min, and finally washed 3 times with sterile distilled water. Sterile roots were cut and then ground in a sterilized mortar. One hundred microliters of the dilution was spread on glucose/ethanol/yeast extract (GEY) agar plate [8] containing 0.3% $CaCO_3$ and nitrogen-free LGI medium [9] and incubated at 30 °C for 3–7 days. Bacterial colonies were picked and purified, then the isolates were preserved in 20% (v/v) glycerol by freezing at −20 °C for further study.

Strain identification

Phenotypic characteristics

Morphology and physiological and biochemical characteristics were used for grouping of bacterial strains based on dendrogram illustration, which was constructed by IBM SPSS statistical software (version 22) (Fig. S1). Bacterial strains were tested for Gram stain, catalase and oxidase activities and hydrolysis of aesculin, arginine, casein, gelatin, and starch. Growth at pH 5, 6, 8, and 9 using buffer system in GEY broth and with 1%, 3%, and 5% NaCl (w/v) in GEY broth were examined at 30 °C for 48 h [10], except growth at 40 °C and 45 °C on GEY agar plate. Acid formation from sugars was performed as previously described [11]. The ability to oxidize acetate and lactate was investigated for AAB.

16S rRNA gene sequence analysis

For molecular analysis, the bacterial strains were identified by 16S rRNA gene sequence. The amplification of the 16S rRNA gene was performed with universal primers: 20F (5′-GAGTTTGATCCTGGCTCAG-3′) and 1500R (5′-GTTACCTTGTTACGACTT-3′), and the PCR products [12] were sequenced by Macrogen®Korea with 4 universal primers: 800R, 27F, 518F, and 1492R. All 16S rRNA gene sequences were blasted with the database obtained from NCBI GenBank (24/7/2022). The sequences of reference strains were selected from maximum identity score and further aligned by a multiple alignment software program, ClustalW. A phylogenetic tree was constructed by neighbor-joining method with the program MEGA11. The confidence values of individual branches in the phylogenetic tree were determined using the bootstrap analysis with 1000 replications. The values for sequence similarity among the closest strains were determined using the EzTaxon server (https://www.ezbiocloud.net).

Genome sequence analysis

Genomic DNA of strain PS25 was extracted using the GenepHlow™ Gel/PCR kit (Geneaid Biotech Ltd., Taiwan). The genome sequencing was performed using Illumina MiSeq sequencer (Illumina Inc., USA) at Omics sciences & Bioinformatics Center, Chulalongkorn University. The genome of strain PS25 was annotated and predicted by Prokka version 1.13 [13]. Circular genomic map was constructed by CG view sever [14]. Whole genome sequences of strain PS25 and the closest type strains obtained from GenBank database were estimated for overall genome relatedness indices (OGRIs) including average nucleotide identity (ANI) on Jspecies (http://imedea.uib-csic.es/jspecies) online service and digital DNA-DNA hybridization (dDDH) by using the genome-to-genome distance calculator (GGDC) with the recommended formula.

Potential plant growth-promoting activity

Nitrogen fixation activity and determination of ammonia production

Bacterial strains were preliminary screened for nitrogen fixation using Nessler's reagent assay [15]. Each strain was inoculated (log 8 CFU/ml) in 9 ml of NF broth [16] and incubated at 30 °C for 48 h. The broth culture was centrifuged at 3,000 rpm for 10 min, and 60 µl of Nessler's reagent was added into 3 ml of supernatant [17]. The strain showing yellow-orange color was marked as nitrogen fixer, and its absorbance at 560 nm was used for calculation of ammonia production compared to standard curve of ammonia sulfate (expressed as mmol/l) [18].

Qualitative analysis of phosphate and zinc solubilization

The strains were tested for an ability to solubilize P and Zn on Pikovskaya's medium [19] (containing 0.5% $Ca_3(PO_4)_2$) and mineral salt agar (containing 0.1% ZnO) [20], respectively. The strains were grown on calcium carbonate agar at 30 °C for 24 h, then the culture-grown agar was cut into 6 mm diameter and placed on Pikovskaya's medium and mineral salt agar and incubated at 30 °C for 7 days and 1 day, respectively. A clear zone appeared around colony on agar considered a positive result, and the diameter of the clear zone was measured. Solubilization index (SI) of the strains was determined by clear zone diameter/-colony diameter [21].

Screening of IAA production

All strains were screened for indole-3-acetic-acid (IAA) production using colorimetric assay by culturing each strain (log 8 CFU/ml) in 90 ml of nitrogen free (NF) broth supplemented with 100 mg/l of L-tryptophan and incubated in the dark at 30 °C, 200 rpm for 48 h. The culture was centrifuged at 8,000 rpm for 20 min, and then 70 µl of supernatant and 140 µl of Salkowski's reagent were mixed and incubated in

the dark at room temperature for 20 min [22]. Appearance of pink color confirmed IAA production, and absorbance at 530 nm was compared to standard curve of IAA. Strain SK2 was selected for confirmation of IAA production by reverse phase HPLC using C18 column equipped with diode-array detector at 280 nm. The solvent system was deionized water: methanol (55:45) at a flow rate of 1 ml/min [23].

Rice growth-promoting ability of selected strains

The ability of IAA of strain SK2, LSG1, and PS25 on promoting *in vitro* rice seed germination was determined. Ten milliliters of culture (log 8 CFU/ml) was inoculated into 90 ml of NF broth supplemented with 100 mg/l of L-tryptophan (for IAA-production) and incubated in the dark at 30 °C, 200 rpm for 48 h. Rice seeds (Khao Dawk Mali 105) were surface sterilized by the procedure described in previous report [24] and placed on filter paper soaked with sterilized water in petri dish for germination (24–48 h). The resultant germinated seeds were inoculated with strain SK2, PS25, or LSG1 by dipping into the culture for 3 h [25]. The germinated seed was transferred into a test tube (25×200 mm) containing 35 ml of MS (Murashige and Skoog) solid medium and incubated in the dark at 25 °C. Distilled water and 10 μg/ml of standard IAA solution were used as control and positive control, respectively. Ten replicates were performed for each treatment. Growth parameters of rice seedlings including total length, root length, shoot length, number of lateral roots, root fresh weight, shoot fresh weight, root dry weight, and shoot dry weight were recorded daily for 15 days. The data were analyzed, and the differences between treatments were compared by Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Strain identification

Fifty endophytic bacteria isolated from roots of 7 different sugarcane samples obtained from 6 Provinces in Thailand are shown in Table 1. They were rod-shaped, Gram-negative, oxidase-negative, and catalase-positive; they grew at pH 5 and 6 and on 1% NaCl medium but did not hydrolyze starch and arginine. The characteristics of the strains used in the experiment including sample collecting locations, strain number, plant growth-promoting activity, 16S rRNA gene sequence similarity and differential phenotypic characteristics are shown in Tables 1 and 2.

Group A contained 8 strains of rod-shaped. All strains showed a high degree of 16S rRNA gene sequence similarity of 99.78–100% with *Pantoea dispersa* LMG 2603^T (Table 1); therefore, they were identified as *P. dispersa*.

Group B contained 42 strains of rod-shaped which divided into 5 subgroups including subgroup B1 (37 strains), B3 (2 strains), and 1 strain each for B2, B4, and B5. All strains in subgroup B1 oxidized lactate and acetate. They were closely related to *Gluconacetobacter liquefaciens* IFO 12388^T with 98.94–100% 16S rRNA gene sequence similarity.

Subgroup B2, B4, and B5 included strain LTS2, LRF6, and SK2, respectively. All strains did not hydrolyze casein, gelatin, and aesculin. Strain LRF6 and SK2 grew on 3% and 5% NaCl. Strain LTS2 and LRF6 produced acid from trehalose and L-arabinose but could not produce acid from lactose, raffinose, and D-ribose. Based on 16S rRNA gene sequence, strain LTS2, LRF6, and SK2 were closely related to *Nguyenibacter vanlangensis* TN01LGI^T (100%), *Pseudomonas oryzihabitans* NBRC 102199^T (100%), and *Aureimonas phyllosphaerae* L9-753^T (97.01%), respectively. Therefore, strain LTS2 was identified as *N. vanlangensis*, and LRF6 was identified as *P. oryzihabitans*. The strain SK2 was closely related to *A. phyllosphaerae* (97.01%) which has similarity lower than 98.70%, thus it is possible to be a new species as described by Stackebrand and Ebers [26]. Therefore, the further study is required.

Subgroup B3 included 2 rod-shaped, LGF5 and AM3, respectively. These strains grew at 40 °C, pH 9, and in 3% NaCl. They hydrolyzed aesculin and casein. Strain LGF5 and AM3 showed 100% and 99.86% similarity of 16S rRNA gene sequence with *Burkholderia anthina* R-4183^T and *Burkholderia territorii* LMG 28158^T, respectively. Therefore, they were identified as *B. anthina* and *B. territorii*, respectively. In this study, *Gluconacetobacter* species is dominant in sugarcane roots and in different locations (Fig. S2 and Table 1) except sugarcane from Si Sa Ket Province.

Genome sequence analysis

The assembled genome of strain PS25 was 4.4 Mbp in length with 98 contigs and 64.7 mol% G+C content and has been deposited at GenBank under accession PRJNA808755 (Table S1). Genomic sequences of strain PS25 and related type strains of *Gluconacetobacter* were selected for genome comparison shown as ANI using either BLASTn (ANIb) or MUMMER (ANIm) software and dDDH values. ANIb value between strain PS25 and *G. dulcium* LMG 1728^T was 93.77% whereas ANIm and dDDH values were 98.74% and 88.80%, respectively (Table S2). According to the ANI criteria [27], this indicated that strain PS25 belonged to the same species with *G. dulcium* LMG 1728^T and it was identified as *G. dulcium*. The *G. dulcium* PS25 had potential in fixing nitrogen (Table 1). Moreover, strain PS25 contained *nif* genes related to nitrogen fixation in genome circular map (Fig. S4).

Table 2 Differential phenotypic characteristics of strains.

Characteristic	A (8)	B				
		B1 (37)	B2 (1)	B3 (2)	B4 (1)	B5 (1)
Cell form	Rod	Rod	Rod	Rod	Rod	Rod
Growth in						
3% NaCl	+	+(−7)	−	+	+	+
5% NaCl	+	+(−7)	−	+(−1)	+	+
Growth in						
pH 8	+(−1)	+	+	+	+	+
pH 9	+(−1)	+(−4)	+	+	+	−
Growth at						
40 °C	+	+(−4)	+	+	−	+
45 °C	+	+(−12)	+	+(−1)	−	+
Citrate utilization	+	+(−9)	−	+	+	−
Hydrolysis of						
Aesculin	+	−	−	+	−	−
Casein	−	−	−	+	−	−
Gelatin	−	−	−	+(−1)	−	−
Lipid	+(−2)	−	−	+(−1)	−	+
Acid from						
L-Arabinose	+	−(+7)	+	−	+	−
Lactose	+	−	−	−	−	−
Maltose	+	−	−	−	+	−
Raffinose	+(−6)	−	−	−	−	−
D-Ribose	+	−	−	−	−	−
D-Sorbitol	+(−1)	−	−	+(−1)	+	−
Trehalose	+	+	+	+(−1)	+	−

+, positive reaction; −, negative reaction. Numbers in parentheses indicating the number of strains showing the reaction.

Potential of plant growth-promoting activity

Nitrogen fixation activity and determination of ammonia production

All bacterial strains were positive in fixing nitrogen (Table 1). Twelve strains produced ammonia in a range of 4.41–11.40 mmol/l (Fig. S5). These results indicated that bacterial strains that colonized sugarcane root exhibited majority of nitrogen fixation through nitrogenase activity by converting of N_2 in the air to ammonia, which was supported by *nif* genes found in the genome (Fig. S4). Several researchers suggested that stem, root, leaves, and rhizosphere soil of sugarcane were sources of nitrogen-fixing bacteria, especially *G. diazotrophicus* [28, 29]. Our work revealed that nitrogen-fixing bacteria, *G. liquefaciens* and *G. dulcium*, were other species of *Gluconacetobacter* that were dominantly found in sugarcane roots.

Qualitative analysis of phosphate and zinc solubilization

In this study, 50 strains were examined for P and Zn solubilization ability using solid medium containing 0.5% $Ca_3(PO_4)_2$ and 0.1% ZnO, respectively. As shown in Table 1, all strains were able to solubilize tricalcium phosphate (except strain KG5, SK2, and PK1) and zinc oxide (except strain SK2). Four phosphate-solubilizing bacteria (PSB): PS3, PS6, PS15, and KG1 were classified as high phosphate solubilizer as their SI values were in a range of 3.89–4.40 whereas high zinc solubilization activity was observed in strain LTS2, KG1, and CH1 at SI value of 5.38, 5.60 and 5.09, respectively (Table 1). Strain KG1 had strong solubilization activity for both P and Zn. P and Zn are essential elements for plants as they are required for cell synthesis, cell activity, protein and vitamin productions. In soil, P and Zn form insoluble complex with aluminum and iron minerals, [30, 31] which limits plant availability. The PSB and zinc-solubilizing bacteria (ZSB) had a potential to increase P and Zn availability for plant through various mechanisms such as production of phosphatase enzymes and chelating agents and secretion of organic acids [32]. Organic acids such as oxalic, citric, butyric, malonic, lactic, succinic, malic, gluconic, acetic, and fumaric were reported to chelate cations bound to phosphate or zinc and their hydroxyl and carboxyl groups [33]. These results revealed that bacterial strains produced acids as shown by clear zone around their colonies on agar medium (Fig. S6); similarly, *G. diazotrophicus* has been previously reported to produce acid during plate assay [34].

Screening of IAA production

The 50 bacterial strains were screened for IAA production. It was found that only 9 strains (18%) could produce IAA in NF medium supplemented with L-tryptophan, a main precursor for IAA biosynthesis in Trp-dependent pathway of bacteria. Strain KG5 gave maximum IAA production (202.25 μg/ml) followed by strain KG4 (152.79 μg/ml), SK1 (141.18 μg/ml),

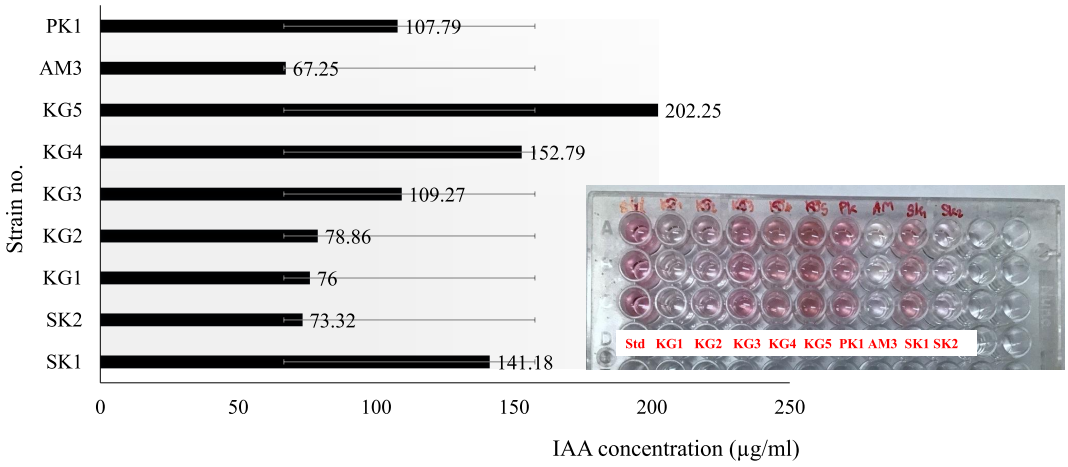
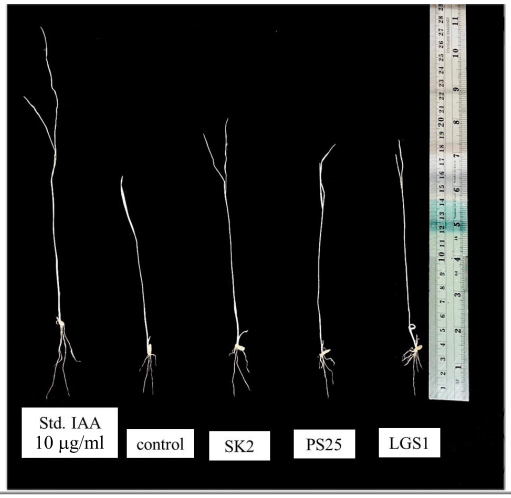


Fig. 1 Qualitative production of IAA of strains cultivated in NF broth containing 100 mg/l of L-tryptophan. The data representing the average of independent experiments ($n = 3$), each with mean ± standard deviation (SD) from 3 measurements.



Treatment	Length (cm)			Number of lateral root	Fresh weight (g)		Dry weight (g)	
	Total	Root	Shoot		Root	Shoot	Root	Shoot
10 µg/ml IAA	29.81^{a}	6.16^{a}	11.96^{a}	9^{a}	0.037^{a}	0.060^{a}	0.0051^{a}	0.0059^{a}
Control	15.51^{b}	3.14^{b}	7.25^{b}	7^{b}	0.011^{b}	0.022^{b}	0.001^{b}	0.0021^{b}
SK2	19.86^{c}	3.49^{c}	11.04^{c}	8^{a}	0.031^{c}	0.043^{c}	0.0028^{c}	0.0038^{c}
PS25	18.22^{d}	3.17^{b}	10.14^{d}	9^{a}	0.033^{c}	0.030^{d}	0.0026^{c}	0.0038^{c}
LSG1	18.05^{d}	3.09^{b}	11.21^{c}	15^{c}	0.036^{a}	0.040^{c}	0.0041^{d}	0.0050^{d}

Fig. 2 Plant growth parameters of Khao Dawk Mali 105 treated with the selected strains measured after 15 days. The same alphabets in each column indicating no significant differences ($p \leqslant 0.05$) according to the Duncan's multiple range test.

KG3 (109.27 µg/ml), and PK1 (107.79 µg/ml), respectively (Fig. 1). Most of the IAA-producing bacteria isolated were in group A which were identified as *P. dispersa* (Table 1). These results coincided with *Pantoea* sp. KRZ5 isolated from rhizosphere of the RB 867515 sugarcane variety which produced IAA at 69.36 µg/ml [35]. Moreover, *Pantoea* has been described as a plant growth promoter. Furthermore, other species including *A. phyllosphaerae* (strain SK2) and *B. territorii* (strain AM3) could produce IAA at 78.86 and 67.25 µg/ml, respectively (Fig. 1). In this experiment, *G. liquefaciens* found as dominant species in different samples of sugarcane could not produce IAA. Several factors such as bacterial species, IAA biosynthetic pathway, precursor concentration, media composition, temperature, and growth stage

affected IAA biosynthesis of bacteria [36]. The strain SK2 (*A. phyllosphaerae*) was selected to confirm for the presence of IAA (73.32 μg/ml) by HPLC analysis. Crude extract of strain SK2 showed peak at 2.74 min retention time while peak of standard IAA was at 2.63 min retention time (Fig. S7). This result confirmed that the strain SK2 produced IAA in the presence of L-tryptophan which agreed well with other previous report [37]. Khakipour et al [38] suggested that HPLC is a more assuring method in recognition and analysis of IAA than the mass spectrophotometry.

Rice growth-promoting ability of selected strains

Three selected strains were examined for an ability to promote rice growth, including *A. phyllosphaerae* SK2, a representing IAA-producing bacterium, and *G. dulcium* PS25 and *G. liquefaciens* LSG1, representing non-IAA producing bacteria. After incubation of treated germinated rice seed for 15 days, all 3 strains significantly increased total length, shoot length, and number of lateral roots of rice seedlings more than control whereas the increase of root length was observed with strain SK2 and PS25. However, the highest total length, root length, and shoot length were observed in rice seedlings treated with IAA (10 μg/ml). Strain SK2, an IAA-producing bacteria, increased total length (19.86 cm), root length (3.49 cm), and fresh shoot weight (0.043 g) of rice seedlings more than strain PS25 and LSG1, which did not produce IAA. No differences of total length and root length of rice seedlings treated with strain PS25 and LSG1 were observed while rice seedlings treated with strain LSG1 had the highest number of lateral roots (Fig. 2). The results indicated that endophytic bacteria, both IAA-producing and non-IAA-producing bacteria, had potential to promote rice growth, and they were beneficial in increasing of crop yield as biofertilizer or bioinoculant. Furthermore, *A. phyllosphaerae* SK2, *G. dulcium* PS25, and *G. liquefaciens* LSG1 were isolated from sugarcane roots but provided positive effect on rice which was similar to previous reports by Deivanai et al [39] and Khan et al [40]. Nevertheless, further studies on mechanisms between endophytic bacteria and plants are required for a better understanding of their relationship.

CONCLUSION

Among 50 endophytic bacteria isolated from sugarcane roots, 94%, 98%, 24%, and 18% were able to solubilize P and Zn, fix nitrogen, and produce IAA, respectively. Based on 16S rRNA gene sequence, they belonged to genera *Gluconacetobacter*, *Pantoea*, *Nguyenibacter*, *Burkholderia*, *Pseudomonas*, and *Aureimonas*. *G. liquefaciens* was dominant species. The genome analysis of the strain PS25 was used to confirm its taxonomic position, and it was clearly identified as *G. dulcium*. This is the first report to isolate *G. liquefaciens* and *G. dulcium* from sugarcane roots and to present their nitrogen fixation capability. Three endophytic bacteria, strain SK2, PS25, and LSG1, exhibited potential rice growth-promoting ability and may play a crucial role in plant growth promoting as a bioinoculant in the future.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2022.145.

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Appendix A. Supplementary data

Table S1 General genomic characteristics of strain PS25 and the related type strains of *Gluconacetobacter* species.

No.	Type strain	Accession number	Size (Mbp)	G+C content (mol%)	Number of contig	Number of gene
1	PS25	PRJNA808755	4.44	64.7	98	4,417
2	*Gluconacetobacter dulcium* LMG 1728^{T}	NZ_JABEQN000000000	4.42	64.7	76	3,993
3	*Gluconacetobacter liquefaciens* NBRC 12388^{T}	NZ_BJMI00000000.1	4.16	64.4	78	3,728
4	*Gluconacetobacter takamatsuzukensis* LMG 27800^{T}	NZ_JABEQK000000000	3.78	67.0	35	3,377
5	*Gluconacetobacter sacchari* LMG 19747^{T}	NZ_JABEQJ000000000	4.83	66.0	114	4,570
6	*Gluconacetobacter tumulicola* LMG 27725^{T}	NZ_JABEQL000000000	4.30	65.1	90	3,875
7	*Gluconacetobacter asukensis* LMG 27724^{T}	NZ_JABEQE000000000	4.39	65.2	64	3,940
8	*Gluconacetobacter aggeris* LMG 27801^{T}	NZ_JABEQD000000000	4.32	65.2	61	3,908

Table S2 ANIb, ANIm, and dDDH values among the draft genomes of strain PS25 and the related type strains of *Gluconacetobacter* species.

No.	Reference genome	Hit strain	ANIb (%)	ANIm (%)	DDH (formula2[a])	Prob. DDH⩾70%
1	*Gluconacetobacter dulcium*	LMG 1728^{T}	93.77	98.74	88.80	95.38
1	*Gluconacetobacter liquefaciens*	NBRC 12388^{T}	89.13	95.31	62.40	59.89
1	*Gluconacetobacter takamatsuzukensis*	LMG 27800^{T}	82.97	88.53	32.90	0.32
1	*Gluconacetobacter sacchari*	LMG 19747^{T}	80.39	86.85	28.90	0.07
1	*Gluconacetobacter tumulicola*	LMG 27725^{T}	82.63	89.23	35.80	0.84
1	*Gluconacetobacter asukensis*	LMG 27724^{T}	84.12	89.61	36.00	0.90
1	*Gluconacetobacter aggeris*	LMG 27801^{T}	84.26	89.52	36.00	0.91

1 = Strain PS25.

[a] Recommended formula (identities/HSP length), which is liberated of genome length and is thus prosperous against the use of incomplete draft genomes.

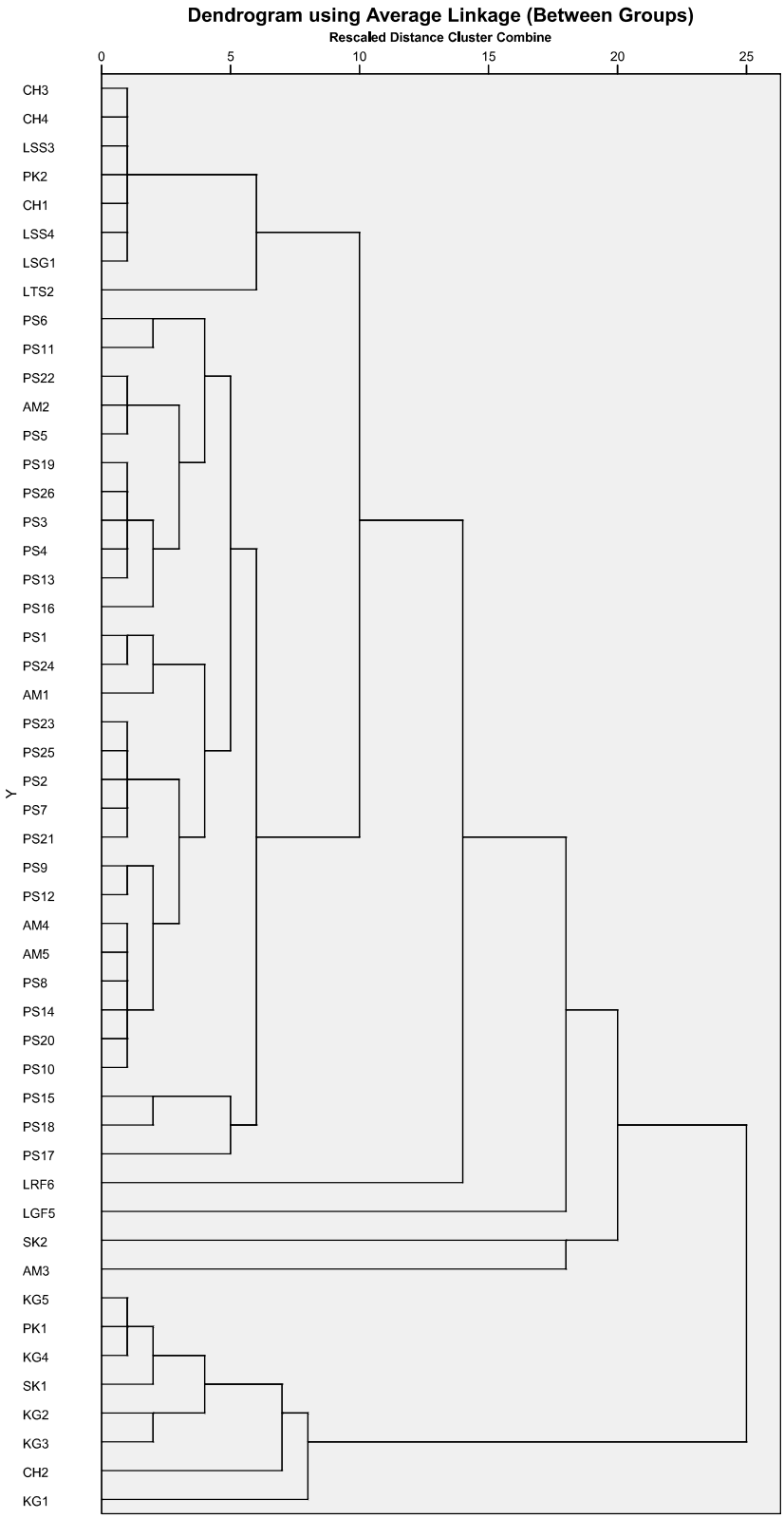

Fig. S1 Dendrogram illustration showing clustering and relationship of bacterial strains. The analysis performed by calculating the Squared Euclidean distance and the associations of strains constructed using the average linkage method (between groups) in the IBM SPSS Statistic version 22.

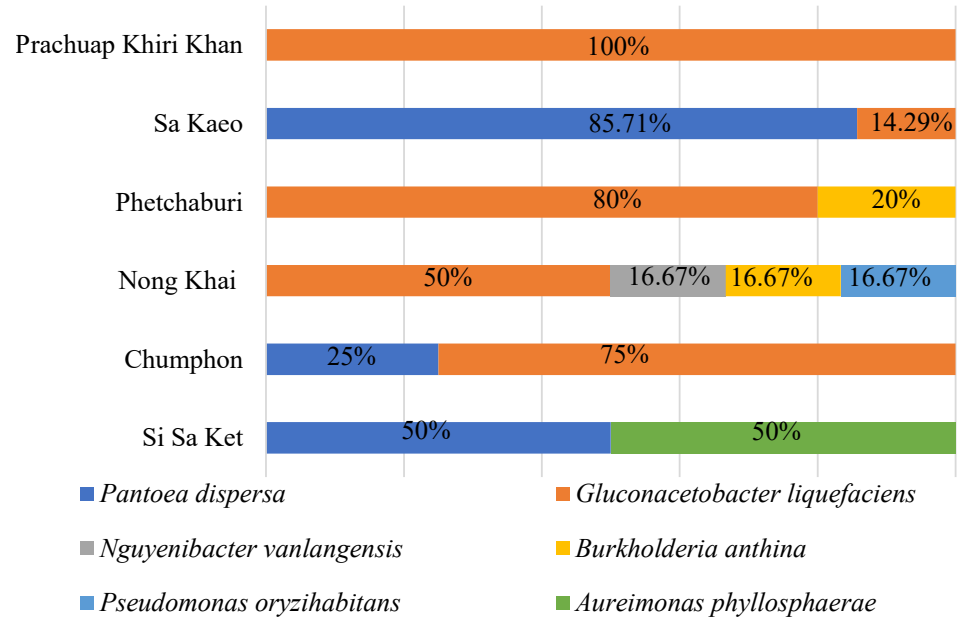

Fig. S2 Dominant species (%) of strains from sugarcane roots collected from different Provinces. The total strains found in each Province: Prachuap Khiri Khan (26 strains), Sa Kaeo (7 strains), Phetchaburi (5 strains), Nong Khai (6 strains), Chumphon (4 strains), and Si Sa Ket (2 strains).

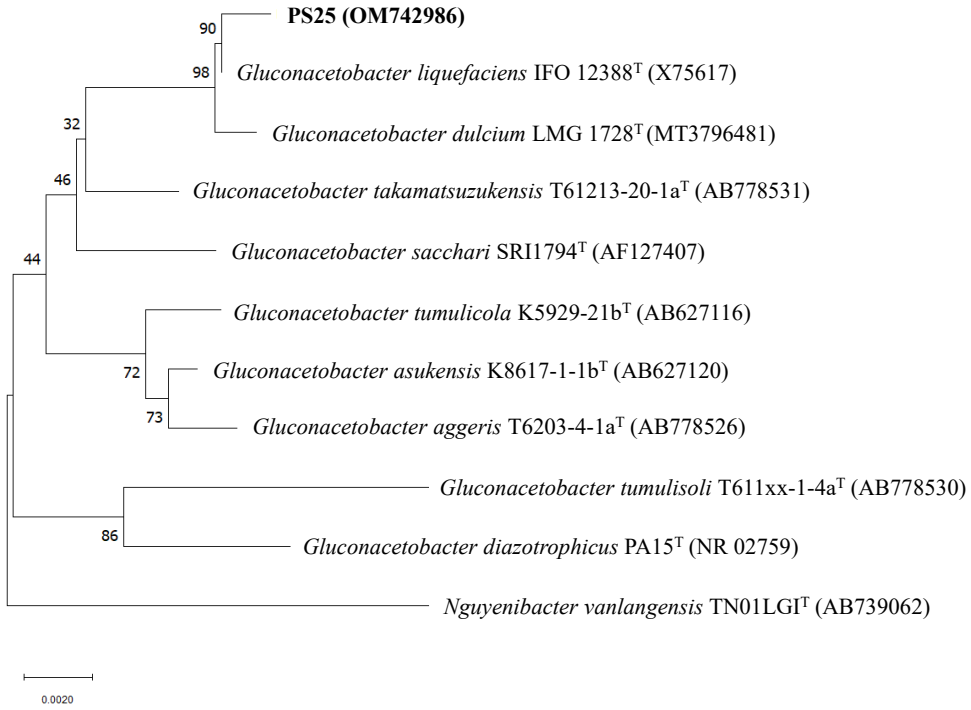

Fig. S3 Phylogenetic tree of strain PS25 and *Gluconacetobacter* species based on the 16S rRNA gene sequences. The numbers on the branches indicating the percentage bootstrap values of 1,000 replicates. Bar, 0.002 substitutions per nucleotide position.

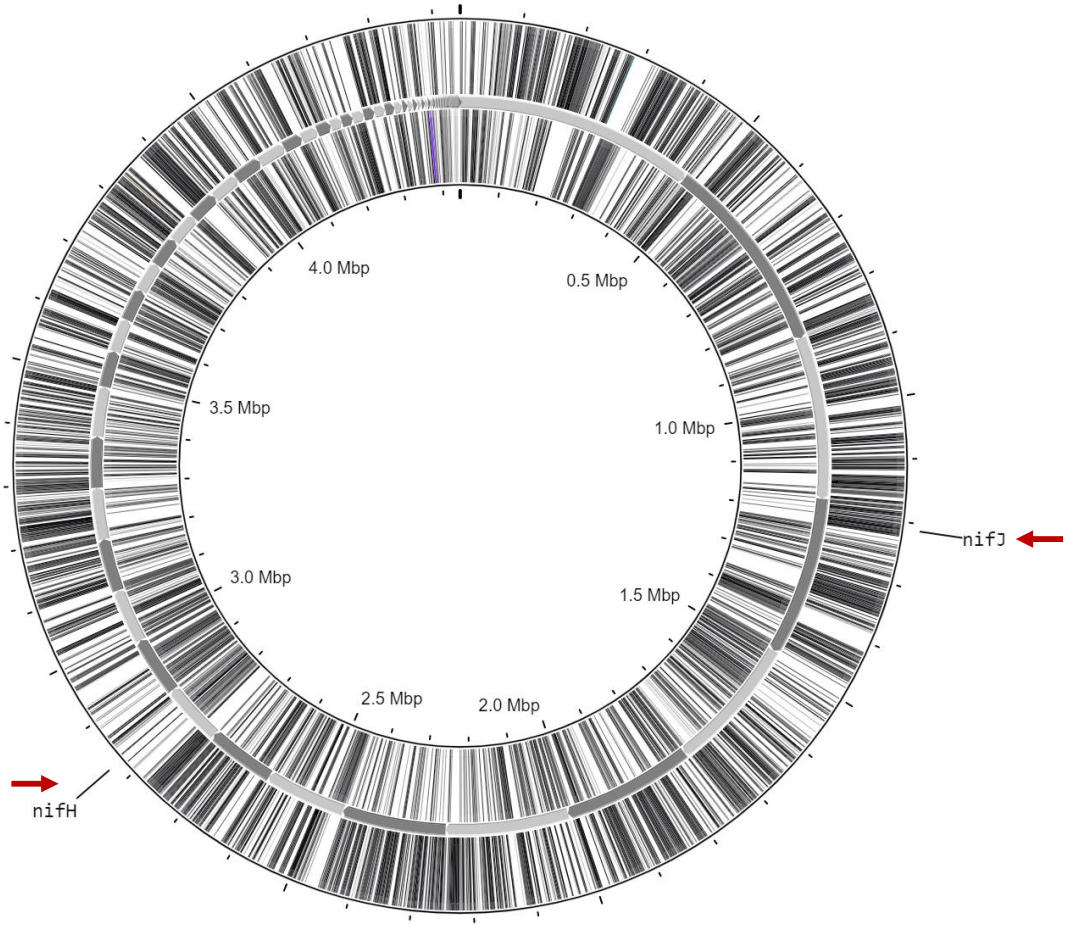

Fig. S4 Circular genomic map of strain PS25 with *nif* genes (red arrow).

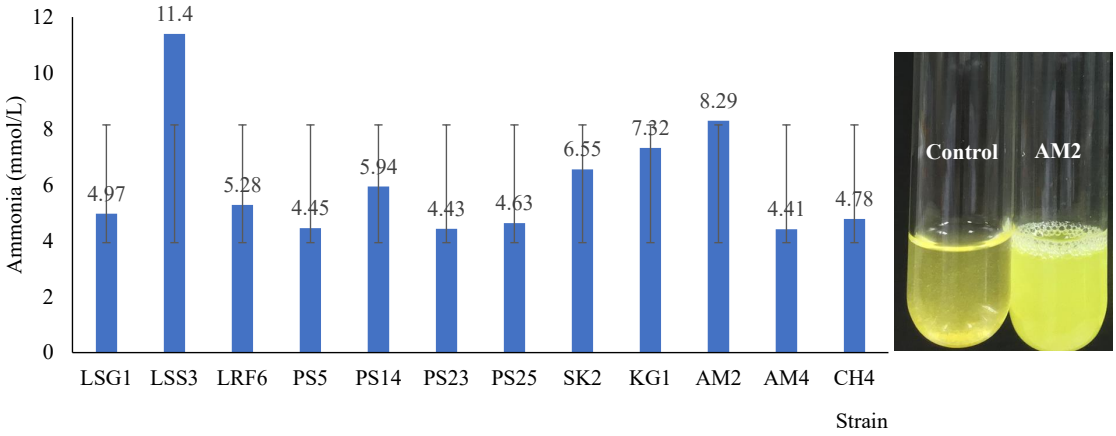

Fig. S5 Ammonium primarily tested using Nessler's reagent and ammonia concentration of strains. The data representing the average of independent experiments ($n = 3$), each with mean ± standard deviation (SD) from 3 measurements.

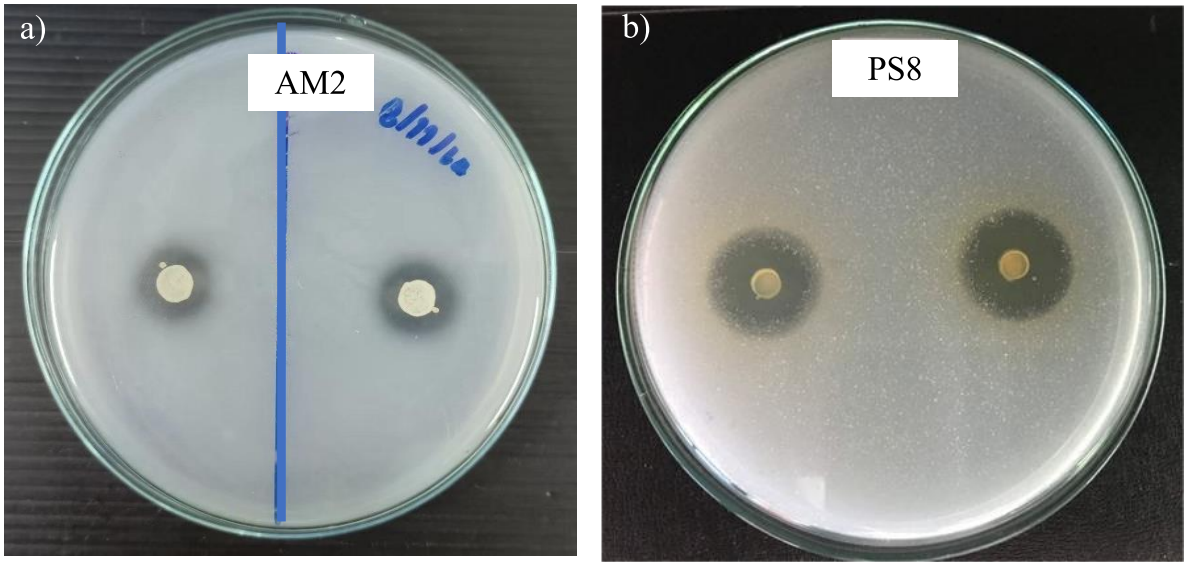

Fig. S6 Analysis of phosphate and zinc solubilization. (a) Solubilization zone of tricalcium phosphate by strain AM2 and (b) solubilization zone of zinc oxide by strain PS8 [21].

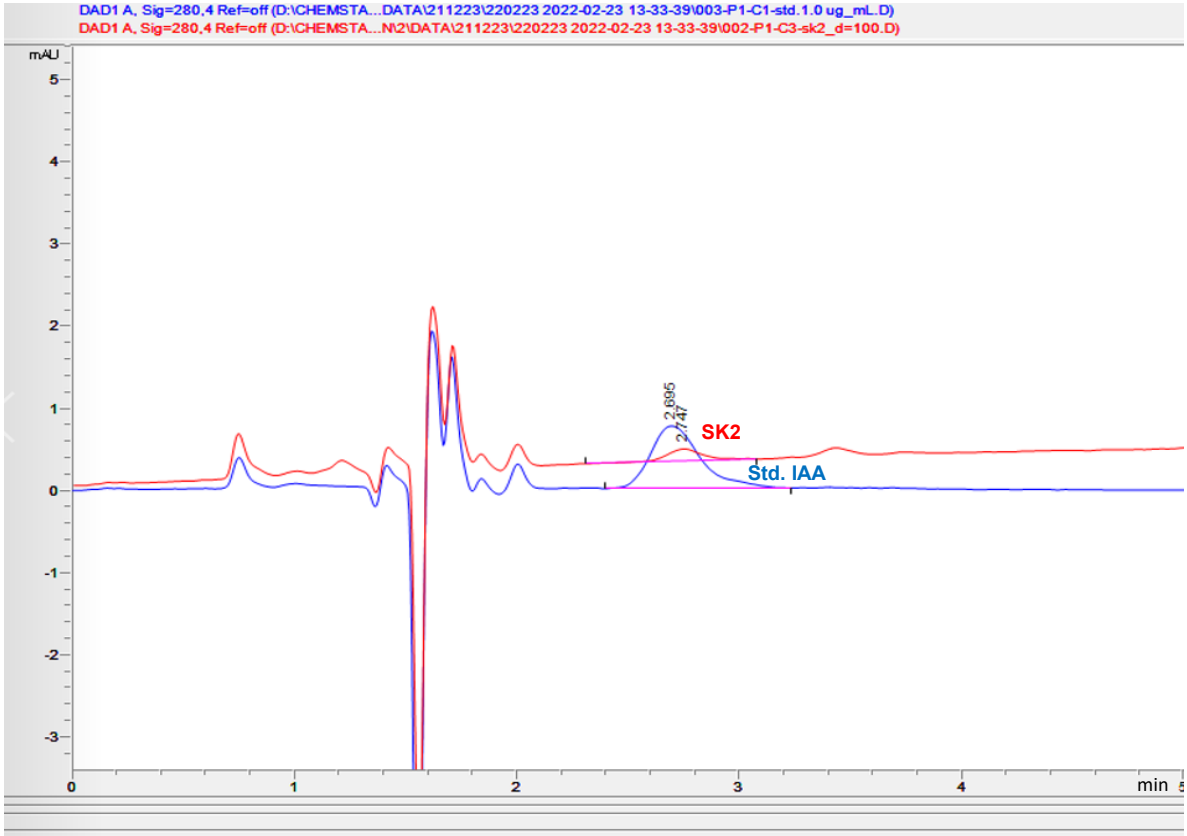

Fig. S7 Overlay IAA production peak of strain SK2 and standard IAA as analyzed by HPLC using water and methanol as mobile phase.