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Isolation and optimization of efficient indole acetic acid-producing *Staphylococcus edaphicus* from *Saccharum officinarum* rhizosphere with their influence on multiple plant growth-promoting traits

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Abstract Bacterial endophytes are microorganisms that promote plant growth and synthesize indole-acetic acid (IAA), modulating nitrogen fixation, producing phytohormones, and solubilizing some soil minerals. The microbes were isolated from the rhizosphere and root plants of *Saccharum officinarum* (sugar cane) and *Citrus aurantifolia* (lime) collected from Phetchaburi province, Thailand. Twenty-one strains of endophytic bacteria were isolated and examined for their potential IAA production. Eight isolated strains were identified as IAA-producing endophytic bacteria in the presence of L-tryptophan precursor. Three isolated strains of SR8, LR18, and LS13 were shown to produce high levels of IAA of around 126.35 µg/mL, 56.62 µg/mL, and 53.49 µg/mL, respectively. The optimized IAA production conditions were shown at 30 ℃, pH 7, 0.5% L-tryptophan concentration, and 48 hours incubation. Furthermore, all three isolated strains of IAA-producing bacteria exhibited the properties of potential plant growth-promoting effects such as phosphate solubilization, zinc solubilization, and ammonium production. Sequencing and evolutionary analysis of the 16S rRNA gene from SR8 isolates showed 99.6% similarity to *Staphylococcus* sp.; these rhizospheric bacteria were identified as *Staphylococcus edaphicus*, a new member of the plant growth-promoting bacteria from sugar cane and lime. Results could be useful for the application of the IAA-producing endophytic bacterium, *S. edaphicus*, isolated from sugar cane rhizosphere, as a biofertilizer for plant growth.

Keywords: Endophytic bacteria, Indole-acetic acid, L-tryptophan concentration, Optimization condition, *Staphylococcus* sp.

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Introduction

Endophytic bacteria live within the inner plant tissues without harming their host plants. Bacterial endophytes can be isolated from surface-sterilized plant organs, such as plant roots and root surfaces (Chanway *et al*., 1998; Ahemad and Kibret**,** 2014). The microbes promote plant growth and development by producing indole-acetic acid (IAA), solubilizing soil minerals (such as phosphorus and iron), fixing nitrogenas well as stimulating shoot and root elongation (Becana and Rodriguez-Barrueco, 1989; Bergersen *et al*., 1991; Marschner, 1995). However, the endophytic bacteria species found in root plants vary among plant species such as wheat, rice, potato, soybean, and corn. The root and soil immediately surrounding root plants are important areas for the synthesis and uptake of metabolic compounds in plant growth and metabolism, due to abundant plant growth-promoting bacteria (PGPB), including IAA-producing microorganisms. IAA is a member of the auxins, an essential plant hormone that stimulates plant growth by cell division, tissue differentiation, cell elongation, lateral root formation, and seedling growth (Kuklinsky-Sobral *et al*., 2004; Herlina *et al*., 2017). IAA production levels vary between different species and strains of endophytic bacteria, depending on the availability of suitable precursors. In the presence of tryptophan, a primary precursor of IAA formation, IAA biosynthesis in the soil surrounding plant roots may be established via a tryptophan-dependent pathway by rhizosphere microbes and bacterial endophytes (Longfei *et al*., 2018). In recent years, endophytic bacteria have been used as biological fertilizers to enhance plant growth, replace chemical fertilizers, and increase economic crop production (Luo *et al*., 2012).

Plant growth-promoting bacteria (PGPB) are known as effective organisms in biological fertilizers for supplying many inorganic nutrients to promote plant growth and optimize crop yields. To date, many genera, including *Azotobacter, Azosprillum, Bacillus, Enterobacteria, Pseudomonas*, *Staphylococcus,* and *Rhizobium* have been isolated from plant roots and root surfaces and identified as PGPB that act as IAA-producing bacteria, phosphate-solubilizing bacteria (PSB), potassium-solubilizing bacteria (KSB), zinc-solubilizing bacteria (ZSB) and nitrogen-fixing bacteria (Han *et al*., 2006; Zul *et al*., 2022b). Inorganic substances, such as phosphorus (P), potassium (K), zinc (Zn), and nitrogen (N), are essential nutrients required as multi-functional components in several biological processes, for example, the synthesis of proteins and sugar, the enzyme cofactor of IAA biosynthesis, and in biomolecules of the photosynthesis process. Numerous soil microorganisms promote plant growth through cell elongation of roots and stems, mineralization

and solubilization of inorganic compounds and nitrogen fixation which have beneficial effects from both economic and environmental perspectives. However, the ability of microbes to solubilize several inorganic elements and generate different organic nutrients is dependent on the plant genotype and bacterial species (Giassi *et al*., 2016; Zhang *et al.*, 2019).

In the present study, endophytic bacteria were isolated from the rhizospheric soils of *Saccharum officinarum* (sugar cane) and *Citrus aurantifolia* (lime), the economically important industrial crops with high nutritional values, from Phetchaburi province in Thailand. All of the rhizobacterial isolates were then identified and characterized for their production of indole acetic acid (IAA). The different growth parameters of IAA microbial biosynthesis were further optimized to achieve the highest IAA production levels from the bacterial isolates. The IAA-producing endophytic bacteria isolated from the rhizosphere of sugar cane and lime, were also examined for their plant growth-promoting (PGP) abilities. Based on the 16S rRNA gene and phylogenetic analysis, this study reports a novel isolate collected from the root plants of *S. officinarum* belonging to *Staphylococcus edaphicus*; which may influence the growth and development of plants and can provide the useful information for improving the growth of other economic crops.

Materials and methods

Sample collection

The roots and rhizosphere soil of sugar cane (*S. officinarum*) and lime (*C. aurantifolia*) were collected from the Pa Daeng sub-district, Kaeng Krachan, Phetchaburi province, Thailand (12.91° N, 99.65° E). The root samples were kept in a polyethylene bag and stored at 4 ℃ for bacterial isolation.

Isolation of endophytic bacteria

The root samples were washed with tap water to remove adhering dust and soil particles. The samples were then surface sterilized to remove most surface microbes by immersion in 70% (v/v) ethanol for 1 min and sodium hypochlorite solution for 15 min. The root samples were then crushed and inoculated in tryptic soy broth at 30 ℃ and 200 rpm for 24–48 hours. The suspension and soil samples were then diluted in 0.85% NaCl, followed by spreading on nutrient agar (NA) medium plates. The plates were incubated at

30 ℃ for three days. The individual bacterial colonies were then selected and purified for further analysis.

Isolation of IAA-producing endophytic bacteria

IAA production was investigated according to previously described methods (Patel and Patel, 2014; Giassi *et al*., 2016). Briefly, the isolated endophytic bacteria were cultured in nitrogen-free (NF) broth containing 0.01% L-tryptophan at 30 ℃ and 150 rpm for 24 hours. The bacterial cells were then removed by centrifugation at 3,000 rpm for 10 min. The supernatant was mixed with Salkowski's reagent, followed by incubation in dark conditions for 20 min. The development of a pink color was considered a positive test for IAA production. The level of IAA production was further quantified by measuring at OD_{530} with a spectrophotometer and analyzing the result with an IAA standard curve.

Optimization of IAA production parameters

Four different parameters involved in IAA production (i.e., temperature, pH, L-tryptophan concentration, and incubation time) were chosen for investigation in the isolated endophytic bacteria (Patel and Patel, 2014; Bhutani *et al*., 2018). The bacterial isolates were grown in NF broth supplemented with different concentrations of L-tryptophan (0.5%, 1%, 1.5%, and 2%), followed by incubation at various temperatures (25–45 ℃), pH values (5–9), and incubation times (24–120 hours). IAA production was quantified using Salkowski's reagent and estimated through a standard plot.

Phosphate and zinc solubilization of IAA-producing bacteria

The determination of PSB and ZSB was performed according to previously described methods (Bhutani *et al*., 2018; Bhatt and Maheshwari, 2019). Pikovskaya's agar, containing 0.5% tricalcium phosphate [$Ca_3(PO_4)_2$], and mineral salts agar, containing 0.1% zinc oxide (ZnO), were used to screen for PSB and ZSB, respectively. The cultured endophytic bacteria were spreaded on NF agar and incubated at 30 ℃ for 24 hours. Pikovskaya's agar and mineral salt agar were then placed on the NF agar plate and further incubated at 30 ℃ for 5–7 days. The halo zone of phosphate and zinc solubilization was quantified from the bacterial colonies that produced clearing zones on an agar plate. The phosphate and zinc-solubilizing activity values were calculated and

reported as a solubilization index (SI), as previously described in Giassi *et al*. (2016).

Ammonia production of IAA-producing bacteria

Ammonia production was analyzed using the previously described qualitative methods (Ahmad *et al*., 2008; Bhutani *et al*., 2018). The isolated endophytic bacteria were inoculated in 5 mL of NF broth and incubated at 30 ℃ for 24 hours, followed by the addition of 0.5 ml of Nessler's reagent in culture supernatant. A positive test for ammonia production was identified by a yellow to brownish-orange color.

Morphological and biochemical characterizations

The morphological and biochemical characteristics of the isolated endophytic bacteria were examined for preliminary identification. The bacterial isolates were grown on an NA medium and incubated for 48 hours at room temperature. The morphology of bacterial colonies was observed to identify the bacterial cell type and form. Biochemical identification was performed using a biochemical tests (Biochemical test kits, HiMedia). The tested isolates were analyzed according to identify various bacterial species. Furthermore, bacterial cultures were plated on NF media containing different carbon sources and further incubated at 30 ℃ for three days. The bacterial isolates were then examined for their ability to grow on different biochemical media.

Genomic DNA extraction and sequence analysis

For molecular analysis, the isolated endophytic bacteria were inoculated and cultured overnight at 30 ℃ with vigorous shaking at 250 rpm. DNA extraction was performed using a bacterial genomic DNA isolation kit (Qiagen, Germany) and used for analysis of the 16S ribosomal RNA (rRNA) gene sequencing. The 16S rRNA gene was amplified by PCR with 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 800R (5′-TACCAGGGTATCTA ATCC-3′) primers. According to the manufacturer's protocol, the amplification was performed in a thermocycler with PCR conditions of 35 cycles at 95 ℃ for 15 sec, 50 ℃ for 30 sec, and 68 ℃ for 1 min, with initial denaturation at 95 ℃ for 2 min and final extension at 68 ℃ for 5 min (KOD DNA polymerase, Toyobo). The PCR product was further purified using a QIAquick purification kit (Qiagen, Germany) and subjected to DNA sequencing analysis (Center of Excellence for Medical Genomics, Thailand). The partial sequence of PCR

products was analyzed through the NCBI database (https://www.ncbi.nlm.nih.gov) for homologous sequences of 16S rRNA genes (BLASTN analysis). The sequence of SR8, LS13, and LR18 strains was deposited into the DDBJ database (https://www.ddbj.nig.ac.jp) under the accession numbers of LC618526, LC618527 and LC618528, respectively. The nucleotide sequences were aligned using ClustalW, and the phylogenetic tree was constructed based on neighbor-joining methods in MEGA7 software with 1,000 bootstrap replicates.

Statistical analysis

The data were reported as mean ± standard deviation for each sample and subjected to analysis of variance (ANOVA). The data analysis was based on three independent replications of the experiments. The differences in parameters were compared using Duncan's Multiple Range Test (DMRT) at the $P \leq 0.05$ significance level using SPSS software package version 16.0.

Results

Isolation of endophytic bacteria from root plants and screening for IAA production

A total of 21 strains of endophytic bacteria were successfully isolated from plant roots and rhizosphere soil from *S. officinarum* and *C. aurantifolia* in Phetchaburi province, Thailand. Among these strains, nine isolates were found in *S. officinarum* and 12 were found in *C. aurantifolia* (Table 1). All 21 isolates were screened for their IAA production ability using Salkowski's reagent supplemented with tryptophan precursor. As a result, eight isolated strains, designated as SS1, SS2, SS3, SR6, and SR8 from sugar cane and LS13, LR18, and LR21 from lime, showed IAA production ability in the range 9.05–126.35 μg/mL (Table 2). In particular, SR8, LR18, and LS13 produced high levels of IAA, with SR8 showing the highest IAA production of 126.35 μg/mL, followed by LR18 (56.62 μg/mL) and LS13 (53.49 μg/mL).

Identification of IAA-producing endophytic bacteria

The 16S rRNA sequences of the eight strains of IAA-producing bacteria were identified by BLASTN analysis through the NCBI database. The results showed that the eight isolates of IAA-producing bacteria from root plants of sugar cane and lime were classified into six groups. In detail, SS1, SS2, SS3, SR6, SR8, LS13, LR18, and LR21 were found to be closely related to *Stenotrophomonas*, *Arthrobacter*, *Staphylococcus*, *Pseudomonas*, *Bacillus*, and

Acinetobacter with 84–100% similarity in their 16S rRNA sequences (Table 2). Three isolates (~2.7%) were found to be closely related to the genus *Arthrobacter*, indicating *Arthrobacter sp.* represents a major group of IAA-producing bacteria from the roots and soil surrounding sugar cane and lime plants.

Table 1. Twenty-one endophytic bacteria isolated from root and soil surrounding root plants of sugar cane and lime

Sample No.	Name of bacterial isolates	Plant species	Source of isolates
1	SS1	*Saccharum officinarum*	Soil surrounding root plant
2	SS2	*Saccharum officinarum*	Soil surrounding root plant
3	SS3	*Saccharum officinarum*	Soil surrounding root plant
4	SS4	*Saccharum officinarum*	Soil surrounding root plant
5	SS5	*Saccharum officinarum*	Soil surrounding root plant
6	SR6	*Saccharum officinarum*	Root plant
7	SR7	*Saccharum officinarum*	Root plant
8	SR8	*Saccharum officinarum*	Root plant
9	SR9	*Saccharum officinarum*	Root plant
10	LS10	*Citrus aurantifolia*	Soil surrounding root plant
11	LS11	*Citrus aurantifolia*	Soil surrounding root plant
12	LS12	*Citrus aurantifolia*	Soil surrounding root plant
13	LS13	*Citrus aurantifolia*	Soil surrounding root plant
14	LS14	*Citrus aurantifolia*	Soil surrounding root plant
15	LR15	*Citrus aurantifolia*	Root plant
16	LR16	*Citrus aurantifolia*	Root plant
17	LR17	*Citrus aurantifolia*	Root plant
18	LR18	*Citrus aurantifolia*	Root plant
19	LR19	*Citrus aurantifolia*	Root plant
20	LR20	*Citrus aurantifolia*	Root plant
21	LR21	*Citrus aurantifolia*	Root plant

Table 2. Molecular characterization of eight isolated strains of IAA-producing endophytic bacteria

Bacterial isolates	IAA production (µg/ml)	16S rRNA sequence length (bp)	Closet relative sequences in NCBI database		Highest identity (%)
			Microorganisms	NCBI accession no.	
SS1	32.3	775	*Stenotrophomonas rhizophila*	KU360268	84.1
SS2	33.9	751	*Arthrobacter* sp.	EU423299	99.2
SS3	16.4	747	*Arthrobacter enclensis*	MK396577	99.8
SR6	9.1	696	*Pseudarthrobacter* sp.	MK089842	99.4
SR8	126.4	707	*Staphylococcus* sp.	MK534012	99.6
LS13	56.6	707	*Pseudomonas* sp.	JN630861	98.9
LR18	53.5	712	*Bacillus altitudinis*	MN907672	100
LR21	43.1	740	*Acinetobacter schindleri*	LC623762	100

Morphological and biochemical characterizations of IAA-producing bacteria

All eight isolated endophytic IAA-producing bacteria were investigated for their cellular characteristics of shape and form, enzymatic activity on different substrates, and hydrolysis activity with various carbon sources (Table 3). The results showed that seven of the isolates were bacilli (rod-shaped bacteria), while only the SS2 isolate was a coccus (spherical-shaped bacteria). All isolates were non-spore-forming bacteria and negative for oxidase activity, except for LS13. In detail, seven isolates were positive for catalase activity, six isolates were negative for citrate utilization, and five isolates were positive for gelatin hydrolysis. Only LS13 was positive for both the oxidase test and lipase hydrolysis. However, the ability to use different carbon sources on the culture medium varied among the bacterial isolates. Among all the strains, only SR8 isolates were able to grow on culture media with the most carbon sources, followed by SS2, three isolates of SS3, SR6, and LS13, and three isolates of SS1, LS18, and LR21, respectively. Among the various carbon sources, raffinose and ribose were found to be the most suitable carbon sources for the growth of all eight strains of IAA-producing bacteria.

Table 3. Morphological and biochemical characteristics of IAA-producing endophytic bacteria

Biochemical test	**Bacterial isolates**							
	SS1	**SS2**	**SS3**	**SR6**	**SR8**	**LS13**	**LR18**	**LR21**
Cell shape	rods	coccus	rods	rods	rods	rods	rods	rods
Endospore formation	-	-	-	-	-	-	-	-
Oxidase activity	-	-	-	-	-	+	-	-
Catalase activity	+	+	+	+	+	-	+	+
Citrate utilization	-	+	-	+	-	-	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-
Casein hydrolysis	-	-	-	-	-	-	+	-
Gelatin hydrolysis	+	-	+	+	+	-	+	-
Lipase hydrolysis	-	-	-	-	-	+	-	+
Aesculin hydrolysis	-	-	-	-	-	-	-	-
Arginine hydrolysis	-	-	-	-	-	-	-	-
Moltose utilization	-	+	-	-	+	-	-	-
Lactose utilization	-	-	-	-	+	-	-	-
Raffinose utilization	+	+	+	+	+	+	+	+
Ribose utilization	+	+	+	+	+	+	+	+
Sorbitol utilization	-	-	-	+	-	-	-	-
Trehalose utilization	-	+	+	+	+	-	-	+
Arabonose utilization	-	-	+	-	-	-	-	-
Fructose utilization	-	+	+	+	-	-	+	+
Galactose utilization	+	+	-	-	+	+	+	-
Cellubiose utilization	-	-	-	-	+	+	-	-
Xylose utilization	-	-	-	-	+	+	-	-
Sucrose utilization	-	-	-	-	+	-	-	-

The results of oxidase test, catalase test, utilization tests and hydrolysis activities were given as; + : Tested Positive, - : Tested Negative.

Optimization of IAA production from bacterial root endophytes

Three isolated strains of IAA-producing bacteria (SR8 from sugar cane and LS13 and LR18 from lime) showed high levels of IAA production and were selected for further analysis. IAA production optimization from culturable root endophytic bacteria was carried out at different temperatures (25, 30, 35, 40, and 45 ℃), pH (5, 6, 7, 8, and 9), L-tryptophan concentrations (0.5%, 1%, 1.5%, and 2%), and incubation times (24, 48, 72, 96, and 120 hours). The results of a qualitative comparison indicated that the optimum conditions for IAA production were 30°C, pH 7, 0.5% L-tryptophan concentration, and 48 hours of incubation time (Figure 1, a-d). The SR8 strain exhibited the highest levels of IAA production at all optimization parameters, followed by LR18 and LS13 strains.

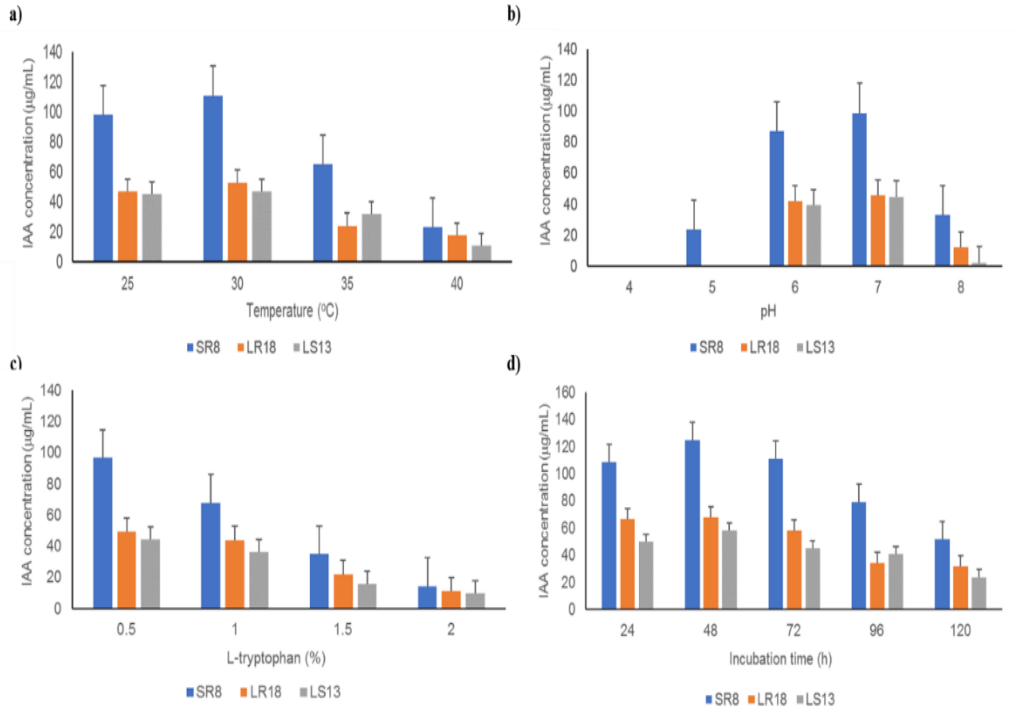

Figure 1. The optimized conditions of (a) temperature, (b) pH, (c) tryptophan concentration, and (d) incubation time for IAA production of three isolated strains (SR8, LR18, and LS13) from plant roots. Each bar represents the mean ± standard deviation of each optimized condition. The error bars represent standard errors from three independent replications

Qualitative estimation of plant growth-promoting traits in IAA-producing bacteria

All three strains of IAA-producing bacteria were examined for their ability to dissolve P and Zn using Pikovskaya's agar plates containing tricalcium phosphate and mineral salt agar with zinc oxide, respectively. The solubility index showed that only SR8 was capable of solubilizing both phosphate and zinc, while LS13 and LR18 were capable of solubilizing either phosphate or zinc (Table 4). As a result, SR8, which showed the highest level of IAA production, could function as both PSB and ZSB. In contrast, LS13 and LR18 could function as either PSB or ZSB. However, LR18 was the only strain to also show ammonia production activity among three strains of IAA-producing bacteria. The results showed that SR8, LS13, and LR18 can promote plant growth and development through their ability to solubilize inorganic compounds and mediate nitrogen fixation.

Table 4. Characteristics of PSB, ZSB, and ammonia production from three isolated strains of IAA-producing bacteria

Bacterial strains	P Solubility Index (SI)	Zn Solubility Index (SI)	Ammonia production
SR8	2.20	2.17	-
LS13	1.53	-	-
LR18	-	2.67	+

Evaluation of the tests: positive (+) shows the presence of activity, while negative (-) shows the absence of activity.

Overall, regarding plant growth-promoting traits, the SR8 strain showed multiple properties of PGPB such as high levels of IAA production and the capability to solubilize P and Zn. Furthermore, comparative analyses of the amplified 16S rRNA sequence fragments revealed that the SR8 isolate was closely related to *Staphylococcus* sp., with 99.6% identity by BLAST identification, and was clustered into *Staphylococcus edaphicus* by phylogenetic tree analysis (Figure 2).

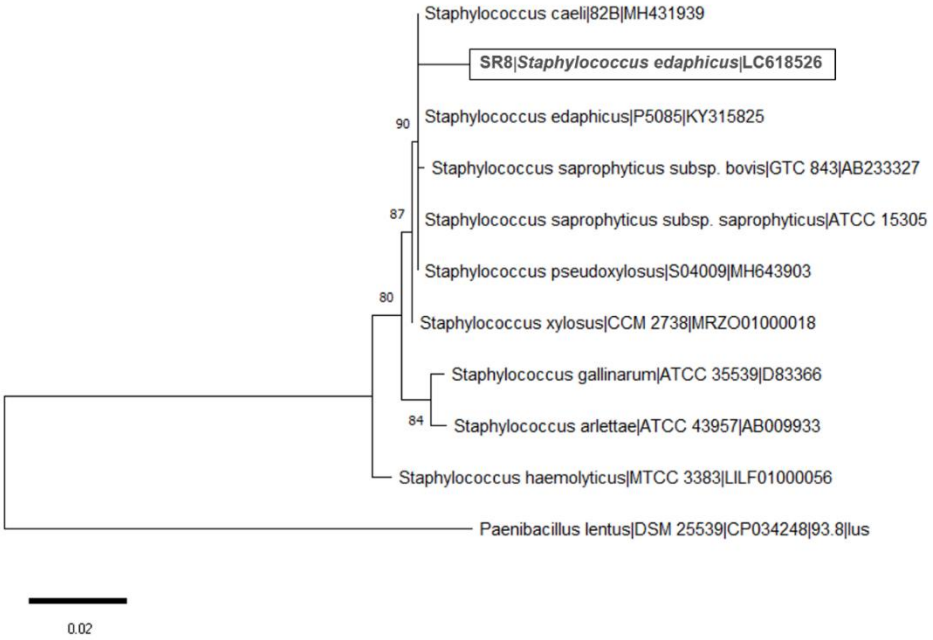

Figure 2. Phylogenetic analysis of SR8 isolate from sugar cane roots based on 16S rRNA sequences with related type strains. The tree was constructed based on the neighbor-joining method in MEGA7 software. The accession numbers of the 16S rRNA sequences from related type strains are indicated. Bootstrap values are shown next to the branches as percentages of 1,000 replicates. The tree is drawn to scale with branch lengths similar to those of the evolutionary distances used to infer the phylogenetic tree

Discussion

A total of 21 bacterial isolates collected from the rhizosphere and roots of *S. officinarum* and *C. aurantifolia* were screened for their potential to produce IAA and examined to determine their optimal IAA production conditions based on a range of different parameters. Among the 21 isolates, eight were identified as IAA-producing endophytic bacteria. Of these, strains SR8, LS13, and LR18 were able to utilize exogenous tryptophan in the culture medium and produce high levels of IAA. The study's results indicate that around 38% of all the bacterial endophytes were able to produce IAA. The optimal IAA production conditions for the three isolated strains were found to be 30 ℃, pH 7, 0.5% L-tryptophan concentration, and 48 hours of incubation time. Strain SR8 showed the highest level of IAA production, followed by

LR18 and LS13, respectively. Molecular characterization of the 16S rRNA sequences revealed that the SR8, LS13, and LR18 strains were clustered in different genera and were identified as *Staphylococcus* sp., *Pseudomonas* sp., and *Bacillus altitudinis*, respectively. Isolates SR8, LS13, and LR18 were capable of producing IAA and solubilizing inorganic compounds, exerting a positive effect on plant growth and development; however, only strain LR18 showed the ability to mediate nitrogen fixation. Consistent with the present study, the rhizobacteria *Pseudomonas putida* and *Staphylococcus* sp. exhibited high levels of IAA production and stimulated growth and development of root plants in both canola seeds and mustard (Patten and Glick, 2002; Bharucha *et al*., 2013). Furthermore, *Pseudomonas* spp. and *Bacillus* spp. exhibited the abilities of phosphate solubilization and nitrogen fixation, were able to promote plant growth by stimulating shoot and root elongation in both maize and citrus rootstock (Viruel *et al*., 2014; Giassi *et al*., 2016). In addition, the salt-tolerant pathogenic bacteria, such as *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus* and *Bacillus cereus*, were found to retain the ability to produce IAA and exhibited positive effects on promoting plant growth (Egamberdieva *et al*., 2008). Taken together, the results from the present study were in good agreement with those from previous research, which have classified *Staphylococcus* sp., *Pseudomonas* sp. and *Bacillus* sp. as the rhizosphere bacteria capable of producing organic compounds and promoting plant growth and development through IAA production. (Mike-Anosike *et al*., 2018; Zul *et al*., 2022a).

Among the three IAA-producing bacterial strains, SR8 showed the highest level of IAA production, in the range of ~100–126.35 µg/mL, with the ability to solubilize inorganic compounds and utilize various carbon sources. For the SR8 isolate, IAA production was found to gradually increase in the range of 25–30 ℃, pH 6–7, 0.5% tryptophan concentration, and 24–48 hours of incubation time. The maximum IAA production level was observed at 30 ℃, pH 7, 0.5% tryptophan concentration, and 48 hours incubation time. These results are consistent with those of previous studies, which revealed the optimum conditions of IAA production observed at: (i) 30 ℃, pH 7.5, 0.2% tryptophan concentration, and 72–96 hours incubation time and (ii) 37 ℃, pH 7, 0.5% tryptophan concentration, and longer incubation periods of up to 5 days (Bharucha *et al*., 2013; Tallapragada *et al*., 2015). Consistent with the findings of previous research, IAA production was observed to decrease in a wide range of conditions at 40–45 ℃, pH 8–9, 1% tryptophan concentration, and longer incubation periods of up to 6 days (Herlina *et al*., 2017; Chandra *et al*., 2018). Molecular analysis of the SR8 strain showed that the results of BLASTN identification were positively correlated with those of phylogenetic analysis,

indicating the SR8 strain as *Staphylococcus edaphicus*. Current findings indicate that *S. edaphicus*, an IAA-producing bacterium in the presence of a tryptophan precursor, is a recently described new member of the PGPB that can regulate shoot and root elongation for the growth and development of maize crops (Prudêncio de Araújo *et al*., 2020). Furthermore, *S. edaphicus* has demonstrated the properties of bacterial resistance and tolerance to extreme environmental stresses, indicating its potential suitability for the application of biofertilizers to improve plant growth, supplement nutrients, and increase crop yields (Pantůček *et al*., 2018).

In summary, the present study was carried out to evaluate the IAA production of bacterial endophytes isolated from sugar cane and lime rhizospheres. Three isolates (SR8, LS13, and LR18) exhibited high levels of IAA production and were further examined in terms of their morphological and biochemical characterization, P and Zn solubilization activity, and ammonia production. All three IAA-producing bacterial isolates exhibited different levels of plant growth-promoting activity. In particular, SR8 strain identified as *S. edaphicus*, showed the highest yield of IAA production based on the optimization of temperature, pH, tryptophan concentration, and incubation period. Furthermore, the SR8 isolate was able to utilize various carbon sources during its growth, which may account for its higher IAA production levels relative to LS13 and LR18 isolates. In conclusion, SR8, LS13, and LR18 isolates could be used as the rhizosperic bacteria to improve plant growth and development in organic agriculture due to their IAA production traits and multiple growth-promoting characteristics. The LS13 and LR18 strains exhibited phosphate solubilization and ammonium production capabilities, may be suitable for improving plant height and weight to increase biological mass and crop yield (Mehrvarz *et al*., 2008), while SR8 strain has the potential to be used for indirectly promoting plant growth, for example, in the development of pathogen-resistant plants under stress condition (Orbovic *et al*., 2008). In future research, further investigation of the newly identified *S. edaphicus* in biological fertilizer mixtures will be carried out to evaluate the influence of rhizobacterial isolates on plant growth, plant productivity, and environmental stress resistance in sugar cane, lime, and other economic crops.

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