Identification and Plant Growth-Promoting Activities of Proteobacteria Isolated from Root Nodules and Rhizospheric Soils

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Abstract

Phosphate solubilization, zinc solubilization, nitrogen fixation and indole-3-acetic acid (IAA) production are plant growth-promoting activities that occur in certain kinds of bacteria. In particular, the rod-shaped proteobacteria isolated from root nodules and rhizospheric soil are considered to be plant growth-promoting. This study aimed to screen and identify plant growth-producing bacteria and included a study of the IAA optimization of the selected isolates. A total of twelve Gram-negative, rod-shaped bacteria were isolated from *Leguminosae* root nodules and *Leguminosae* rhizospheric soils. The samples were collected in Udon Thani, Nong Bua Lumphu and Nakhon Phanom provinces, Thailand. Based on the phenotypic characteristics and 16S rRNA gene sequence similarities (99.2-100% similarity), the isolates were characterized as relatives of *Rhizobium pusense* (2 isolates), *Ochrobactrum oryzae* (1 isolate), *Pseudomonas aeruginosa* (1 isolate), *Acinetobacter pittii* (1 isolate), *Klebsiella pneumoniae* subsp. *rhinoscleromatis* (1 isolate), and *Pseudomonas geniculata* (6 isolates). The isolates were screened for their plant-growth-promoting activities. The results revealed that 8 isolates exhibited phosphate solubilization (10.9 ± 0.00 to 17.0 ± 1.40 Solubilization Index, SI), zinc solubilization (21.5 ± 0.70 to 33.0 ± 1.40 SI) and indole-3-acetic acid production (1.0 ± 0.2 to 113.4±3.5 µg/ml), and 2 isolates showed their nitrogen-fixing activities. The isolates SN1, SN3-3, SN5, PN1-1 and LS1 were selected and optimized for IAA production.

Keywords: proteobacteria, phosphate solubilizing, zinc solubilizing, indole-3-acetic acid
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1. Introduction

Proteobacteria including Gram-negative, rod-shaped bacteria in genera *Acinetobacter*, *Achromobacter*, *Aerobacter*, *Agrobacterium*, *Azospirillum*, *Burkholderia*, *Chryseomonas*, *Curtobacterium*, *Enterobacter*, *Erwinia*, *Flavimonas*, *Ralstonia*, *Pseudomonas*, *Enterobacter*. *Ralstonia*, *Rhizobium*, *Pantoea*, and *Sphingomonas* strains were found in soils, soybean cultivars, legume tissues and root nodules [1, 2]. They are plant growth promoters and can be used in place of chemicals in agriculture, horticulture, silviculture, and environmental clean-up [3]. Solubilized phosphate and zinc sulfate, which can be supplied via fertilization, are essential nutrients for plant growth [4, 5]. Indole-3-acetic acid (IAA) is one of the most physiologically active auxins produced by plant growth-promoting rhizobacteria [6]. *Rhizobium* strains that present within the root nodules are involved in fixing atmospheric nitrogen for the host plant [3]. This research deals with the identification, screening and optimization of plant growth-promoting activities of bacteria from root nodules and rhizospheric soils.

2. Material and Methods

2.1 Sample collection and isolation of isolates

Eight plant root nodules and 2 rhizospheric soils were randomly collected from Udon Thani, Nong Bua Lamphu and Nakhon Phanom provinces in Thailand (Table 1). All samples were separately kept in sterile plastic bags and then preserved at 4°C. The plant root samples were pretreated as described by Riker and Riker [7]. Each rod nodule was squeezed and streaked on yeast extract-mannitol-congo red (YMC) agar plates and incubated at 30°C for 3-5 days. Then, a 10-fold (10^{-3} and 10^{-4}) dilution of each soil sample was prepared, and 0.1 ml of each dilution was spread onto YMC agar plates. After incubation, the colonies were selected and purified on nutrient agar (NA, Difco) plates. The selected isolates were preserved in 20% (v/v) glycerol at -20°C and in 10% (w/v) skim milk as lyophilized ampoules.

2.2 Identification methods

2.2.1 Phenotypic characterization

Cell morphology, colony appearance, oxidase, catalase, hydrolysis of aesculin, arginine, gelatin, casein, starch and Tween 80; indole, Methyl Red-Voges-Proskauer (MR-VP), nitrate reduction, citrate utilization, and acid production, and growth in NaCl (3%, 5%, w/v) at pH (pH 5 and 9) and temperature at 30, 37, 40 and 48ºC, of the isolates were determined as reported by Barrow and Feltham [8] and Tanasupawat *et al*. [9].

2.2.2 Genotypic characterization

The 16S rRNA gene sequences were amplified using primers 27F (5'-AGAGTTTGATCMTGGC TCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3')[10], and their PCR products were analyzed by Macrogen, Korea. The EzBiocloud server [11] was used for the analysis of sequence similarity among the closest strains.

The sequences of isolates were aligned with selected type strain sequences obtained from GenBank. Multiple alignments of the sequences determined were performed using CLUSTAL_X version 1.81. Gaps and ambiguous bases were eliminated prior to construction of a phylogenetic

tree. A phylogenetic tree was reconstructed by the neighbor joining method [12] with the program MEGA 6 [13], and the confidence values of individual branches were also determined using bootstrap analyses based on 1000 replications as described by Felsenstein [14].

2.3 Screening of plant promoting activities

The culture, cultivated in nutrient broth (NB) and incubated at 30ºC for 24 h, was used as an inoculum in this study.

2.3.1 Phosphate solubilizing activity on Pikovskaya's (PVK) agar

The suspension of cultures in NB was swabbed on nitrogen free (NF) agar plates [15] and incubated at 30ºC for 2 days. The culture on the NF agar plates was prepared as inoculum using a cork-borer technique and then inoculated on PVK agar plates [16**]** and incubated at 30ºC for 3-5 days. Phosphate solubilization ability was observed by the formation of a transparent halo around the colonies on the PVK agar plates supplemented with tricalcium phosphate [$Ca_3(PO_4)_2$]. After incubation, a clear halo formed, and the colony diameter and clear zone were measured. The solubilization index was calculated per the following equation [17]:

$$\text{Solubilization index (SI)} = \frac{\text{Colony diameter + clear zone diameter (mm)}}{\text{Colony diameter (mm)}}$$

2.3.2 Zinc solubilizing activity on Mineral salt agar (MSA)

The suspensions of cultures were swabbed on NF agar plates [15] and incubated at 30°C for 2 days. The culture on NF agar was prepared as the inoculum using a cork-borer technique as mentioned above. Then it was inoculated on MSA as described by Saravanan *et al*. [5] and incubated at 30ºC for 3-5 days. The isolates produced transparent halos around the colonies indicating positive zinc solubilization ability. The clear zones formed by the isolates were determined as described by Saravanan *et al*. [5] using the equation mentioned above.

2.3.3 Nitrogen fixing characterization

The nitrogen fixing activity was determined using Nessler's reagent assay [18]. The isolates were cultivated in NF broth and incubated on a shaker (180 rpm) at 30ºC for 48 h. The broth was centrifuged at 3000 rpm for 10 min and the supernatant was tested with Nessler's reagent under dark conditions. It was then subject to colorimetric analysis by microplate reader (CLARIOstar Plus, BMG Labtech) at 560 nm after 20 min incubation.

2.3.4 Indole-3-acetic acid (IAA) production

The isolates were cultivated in NF broth with 1% tryptophan on a shaker (180 rpm) at 30ºC for 48 h. IAA production was evaluated by the Salkowski's method [19] with Salkowski's reagent. The broth was centrifuged at 4000 rpm for 10 min and the supernatant was estimated using colorimetric technique, in the dark. The optical density (OD) of samples was measured at 530 nm after 120 s by microplate reader [20]. The production of the isolate was determined and expressed as µg/ml based on a standard curve of IAA [21].

2.4 Optimization of IAA production

The carbon sources (glucose, mannitol, sucrose, glucose with mannitol and glucose with sucrose), nitrogen sources ($NaNO_3$, KNO_3, peptone, $NaNO_3$ with peptone or KNO_3 with peptone), and tryptophan concentration (0.1%, 0.5%. 1% and 1.5%) were optimized. Effects of pH (5-9) and temperature (30-37°C) on IAA production were also tested. Phosphate buffer was used to maintain the pH value of the medium. Yeast extract-Malt Extract-Dextrose (YMD) broth supplemented with tryptophan was used as basal medium for optimization of IAA production [22].

2.5 Statistical analysis

The data were statistically analyzed by Statistical Package for the Social Sciences (SPSS, Statistics version 24.0.0.0) using one-way analysis of variance (ANOVA) and the grouping was obtained by Duncan's multiple range tests at *p*-value 0.05 [23]. The data were expressed as mean values of triplicates ± standard deviation.

3. Results and Discussion

3.1 Identification of isolates

Twelve Gram-negative, rod-shaped bacteria were isolated from the root nodules of 8 selected plant species and 2 rhizospheric soil samples (Table 1). They were divided into 6 groups based on their phenotypic characteristics (Figure 1). All the isolates grew at pH 5 and 9. They were positive for catalase, nitrate reduction and arginine hydrolysis but were negative for MR-VP. They produced acids from glucose, mannitol and sucrose but did not produce acids from lactose and xylose. The various characteristics are presented in Table 2. The isolates that belonged to genera *Rhizobium* [24], *Ochrobactrum* [25], *Pseudomonas* [26, 27], *Acinetobacter* [28] and *Klebsiella* [29] were designated as Group I to VI (Table 1).

Group I consisted of 2 isolates, LN4-2 and LS1. Colonies were creamy-white, irregular, smooth, flat and opaque on NA agar. All isolates grew in 3% NaCl and at 37°C. They were positive for oxidase, starch, indole, nitrate, and Simmons citrate utilization (Table 2). Based on 16S rRNA gene sequence similarity (100%) and phylogenetic tree analysis (Figure 2), isolate LN4-2 (1299 bp) was identified as *Rhizobium pusense*, while isolate LS1 (1340 bp) was most closely related to *Rhizobium pusense* LMG 25623^T with 99.5% sequence similarity.

Group II consisted of one isolate, which was SN1. The colonies were white, irregular, smooth, flat and opaque on NA agar. The isolate was positive for oxidase. It did not grow in 3 or 5% NaCl and at 40°C. Based on 16S rRNA gene sequence and phylogenetic tree analysis (Figure 2), isolate SN1 (1278 bp) was most closely related to *Ochrobactrum oryzae* MTCC 4195^T with 99.5% sequence similarity.

Group III consisted of one isolate, SN2. The colonies were white, irregular, smooth, flat and opaque on NA agar. All isolates grew in 3 or 5% NaCl and at 40°C. The isolate was positive for oxidase, indole, nitrate reduction, hydrolysis of casein and gelatin, and Simmons citrate utilization. Based on 16S rRNA gene sequence and phylogenetic tree analysis (Figure 2), isolate SN2 (1310 bp) was closely related to *Pseudomonas aeruginosa* JCM 5962^T with 100% sequence similarity. Therefore, it was identified as *Pseudomonas aeruginosa* [26].

Table 1. Samples, location, isolate number, 16S rRNA gene similarity (%) and nearest relatives

Plant/ Soil	Province	Isolate no.	Group	Similarity (%)	Nearest relatives
Indigofera tinctoria	Nong Bua Lamphu	LN4-2	I	100	*Rhizobium pusense*
Macroptilium lathyroides (L.) Urb. Soil	Nong Bua Lamphu	LS1	I	99.5	*Rhizobium pusense*
Sesbania javanica	Udon Thani	SN1	II	99.5	*Ochrobactrum oryzae*
Samanea saman	Udon Thani	SN2	III	100	*Pseudomonas aeruginosa*
Vigna unguiculata sesquipedalis (L.) Verdc	Udon Thani	SN6-2	IV	100	*Acinetobacter pittii*
Tamarindus indica	Udon Thani	SN3-3	VA	99.2	*Pseudomonas geniculata*
Arachis hypogaea	Udon Thani	SN5	VA	100	*Pseudomonas geniculata*
Mimosa pudica	Nong Bua Lamphu	LN1-4	VA	99.9	*Pseudomonas geniculata*
Mimosa pudica	Nong Bua Lamphu	LN1-2	VA	99.4	*Pseudomonas geniculata*
Mimosa pudica	Nong Bua Lamphu	LN1-8	VB	100	*Pseudomonas geniculata*
Mimosa pudica Soil	Nakhon Phanom	PS1-5	VB	99.8	*Pseudomonas geniculata*
Pueraria phaseoloides (Roxb.) Benth	Nakhon Phanom	PN1-1	VI	99.9	*Klebsiella pneumoniae* subsp. *rhinoscleromatis*

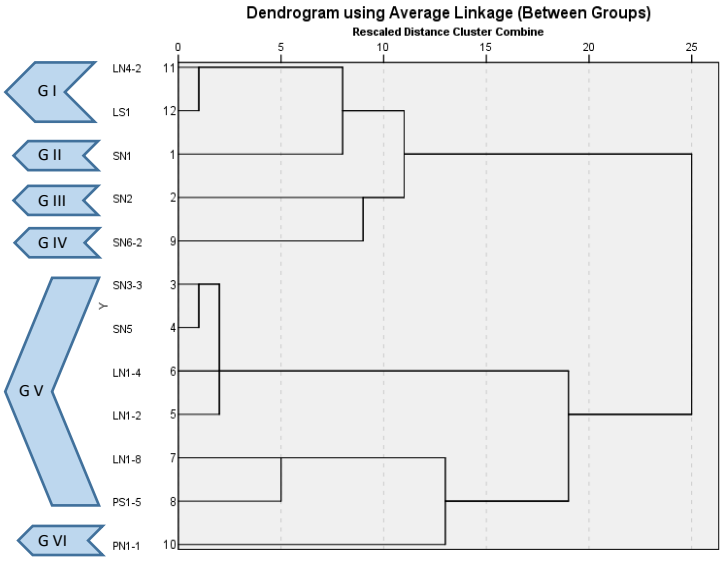

Figure 1. Dendrogram analyzed by SPSS version 24.0.0.0 showing the hierarchical cluster of isolates based on their phenotypic characteristics

Table 2. Phenotypic characteristics of the isolates

Characteristic	I	II	III	IV	VA	VB	VI
	2	1	1	1	4	2	1
Colony color							
Growth in NaCl 3%	+	-	+	+	+	+	+
NaCl 5%	-	-	+	+	+	+	+
Growth at 40°C	-	-	+	+	+	+	+
at 48°C	-	-	-	-	-	-	+
Oxidase	+	+	+	-	-	+	-
Indole	+	-	+	+	+	+	+
Tween 80	-	-	-	-	-	+	-
Citrate utilization	+	-	+	+	+	+	+
Hydrolysis of:							
Aesculin	-	-	-	+	+	+	+
Casein	-	-	+	-	+	+	+
Gelatin	-	-	+	-	+	+	-
Starch	+	-	-	-	-(+2)	+(-1)	+
Acid production from:							
Arabinose	-	-	-	-	+	+(-1)	+
Cellobiose	-	-	-	-	+	+	+
Fructose	-	-	-	-	+	+	+
Galactose	-	-	-	-	+	+	+
Maltose	-	-	-	-	+	+	+
Raffinose	-	-	-	-	-	-	+
Ribose	-	-	-	-	+	-	+
Sorbitol	-	-	-	-	+	+(-1)	+
Trehalose	-	-	-	-	+	-	+

+ positive reaction, - negative reaction

Group IV consisted of one isolate, SN6-2. Its colonies were white, irregular, smooth, flat and opaque on NA agar. It grew in 3 or 5% (w/v) NaCl and at 40ºC. It was positive for indole, nitrate reduction, aesculin hydrolysis and Simmons citrate utilization. Based on 16S rRNA gene sequence and phylogenetic tree analysis (Figure 2), isolate SN6-2 (1383 bp) was closely related to *Acinetobacter pittii* CIP 70.29^T with 100% sequence similarity. Therefore, it was identified as *A. pittii* [28].

Group V consisted of 6 isolates (VA for SN3-3, SN5, LN1-4 and LN1-2; VB for LN1-8 and PS1-5). Colonies were creamy-white, irregular, smooth, flat and opaque on NA agar. All isolates grew in 3 or 5% NaCl and at 40ºC. They were positive for aesculin, casein and gelatin hydrolysis, indole, nitrate reduction and Simmons citrate utilization. Oxidase, starch, and Tween hydrolysis were variable. Four isolates in Groups VA and VB showed differences for oxidase activity, hydrolysis of Tween 80 and acid production from trehalose. On the basis of their 16S rRNA gene sequence and phylogenetic tree analysis (Figure 2), isolates SN3-3 (1207 bp), SN5 (1297 bp), LN1-4 (1249 bp), LN1-2 (1237 bp), LN1-8 (1170 bp) and PS1-5 (1403 bp) were most closely related to *Pseudomonas geniculata* ATCC 19374^T with 99.2, 100, 99.4, 99.3, 100 and 99.8% sequence similarity, respectively [27]. Isolates SN3-3, LN1-2 and LN1-4 exhibited low sequence similarity (99.2%, 99.3% and 99.4%); however, their taxonomic positions were in the same cluster. Further genomic sequence analysis is required for identification.

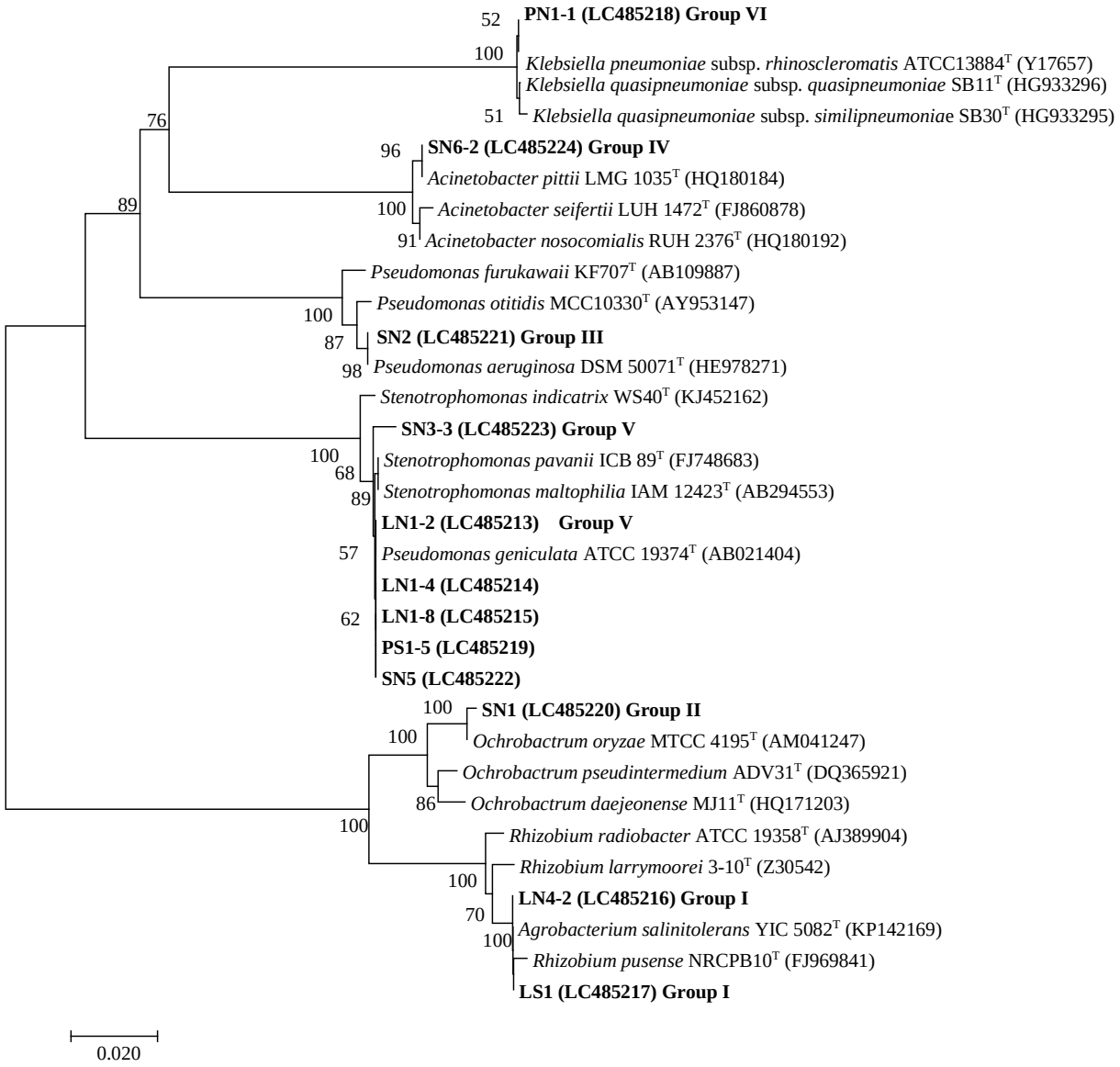

Figure 2. Neighbor-joining tree based on the 16S rRNA gene sequences showing relationships of the isolates. The numbers on the branches indicate the percentage bootstrap values of 1,000 replicates; only values higher than 50% are indicated. Bar, 0.02 substitutions per nucleotide position

Group VI consisted of one isolate, PN1-1. Its colonies were white, irregular, smooth, flat, and opaque on NA agar. The isolate was able to grow in 3 or 5% NaCl and at 40 or 48ºC. It was positive for catalase, indole, nitrate reduction, Simmons citrate utilization, aesculin, casein and starch hydrolysis. On the basis of 16S rRNA gene sequence and phylogenetic tree analysis (Figure 2), isolate PN1-1 (1383 bps) was most closely related to *Klebsiella pneumoniae* subsp. *rhinoscleromatis* 01A030^{T} with 99.9% sequence similarity.

3.2 Screening of plant-growth-promoting activities

The phosphate solubilizing ability in Pikovskaya medium varied from 12.8 ± 7.00 to 17.0 ± 1.40 (Table 3). Isolate LN1-8 exhibited the highest level of solubilized phosphorus (17.0 ± 1.40 mm). Zinc solubilizing ability varied from 21.5 ± 0.70 to 33.0 ± 1.40 mm using ZnO^{3+} as a source of insoluble Zn. Isolate PS1-5 exhibited the highest solubilized zinc (33.0 ± 1.40) (Table 2). The isolates produced IAA (1.0 ± 0.2 to 113.4±3.5 µg/ml) in NF medium with 1% L-tryptophan. Isolates PN1-1 and SN3-3 produced the highest levels of IAA, 113.4±3.5 and 110.8±17.1µg/ml, respectively (Table 2). Among the 12 isolates, only LN1-4 and LN1-8 were able to fix nitrogen (Table 3).

Table 3. Phosphate solubilizing, zinc solubilizing, indole-3-acetic acid (IAA) production and nitrogen fixing activities of the isolates

Isolate no.	Group	Phosphate solubilizing SI*	Zinc solubilizing SI*	IAA (µg/ml)	N_2 fixing (NH_4^+)
LN4-2	I	-	-	27.3±2.3^{b,c}	-
LS1	I	-	-	33.7±8.8^{c}	-
SN1	II	12.8±7.00	-	56.8±2.4^{d}	-
SN2	III	10.9±0.00	21.5±0.70	1.8±0.2^{a}	-
SN6-2	IV	-	21.5±0.70	2.9±0.8^{a}	-
SN3-3	VA	14.6±0.70	29.5±0.70	110.8±17.1^{e}	-
SN5	VA	-	30.5±0.70	12.6±7.6^{a,b}	-
LN1-2	VA	13.4±6.40	21.5±2.10	8.1±0.3^{a}	-
LN1-4	VA	14.6±1.40	-	8.1±0.3^{a}	+
LN1-8	VB	17.0±1.40	-	-^{a}	+
PS1-5	VB	14.9±0.00	33.0±1.40	1.0±0.2^{a}	-
PN1-1	VI	13.0±1.40	30.0±0.00	113.4±3.5^{e}	-

SI*, solubilization index. +, positive reaction; -, negative reaction or no activity.
The clustering result of IAA (µg/ml) from each isolate was a group of a, b, c, d and e, respectively, and was separated by multiple comparisons with the statistical program by Duncan's method at *p*-value 0.05.

3.3 Optimization of IAA production

3.3.1 Effect of different carbon and nitrogen sources on IAA production

The significant differences of IAA production from various carbon sources of each isolate revealed that isolate SN1 produced the maximum IAA (11.8±0.6 µg/ml) when glucose was used, but a low yield of IAA was obtained when sucrose was used (Figure 3). Based on the statistical analysis, glucose was a suitable carbon source for IAA production. However, the amount of IAA produced was not significant when glucose coupled with mannose, and glucose coupled with sucrose were

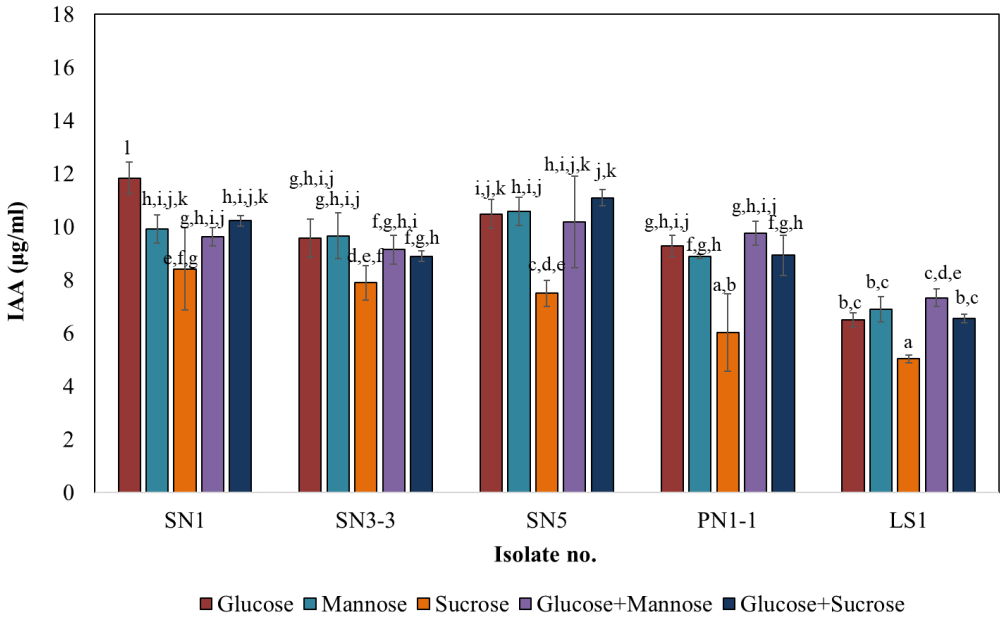

Figure 3. Effect of various carbon sources on IAA production by the selected isolates. The vertical bars represent the standard deviation with triplicates experiment. The clustering result of carbon sources on IAA production defined groups a, b, c, d, e, f, g, h, i, j, k, and l. The values marked by the same letter are not significantly different (*p*-value more than 0.05)

used as carbon sources. In addition, among 5 sources of nitrogen, peptone was clearly the best nitrogen source for IAA production for all isolates. The maximum IAA (13.7±1.6 µg/ml) was obtained from isolate SN5, followed in decreasing order of IAA production by LS1, SN1, SN3-3, and PN1-1 (11.4±0.3, 10.9±0.1, 10.6±0.2, and 10.5±0.1 µg/ml, respectively) (Figure 4). The results from multiple comparisons of different nitrogen sources were divided into 3 groups, and it was confirmed that peptone was the best nitrogen source. The degree of IAA production depending on the isolates, the carbon sources (glucose, mannitol, and sucrose) and the nitrogen sources (peptone, $NaNO_3$ and KN O_3) was also reported by Mohite [22].

3.3.2 Effect of L-tryptophan concentration on IAA production

L-Tryptophan concentrations, 0.1% to 1.5%, affected the production of IAA (Figure 5). Tryptophan was an important variable factor for promoting IAA production of proteobacteria. This result was supported by the research of Costacurta and Vanderleyden [30], who noted that tryptophan was generally considered as an IAA precursor used by bacterial cultures to enhance IAA biosynthesis. However, when the concentration of tryptophan was maintained at 1.0%, isolate PN1-1 was able to produce sufficient IAA with the maximum of 56.7±1.0 µg/ml (Figure 5).

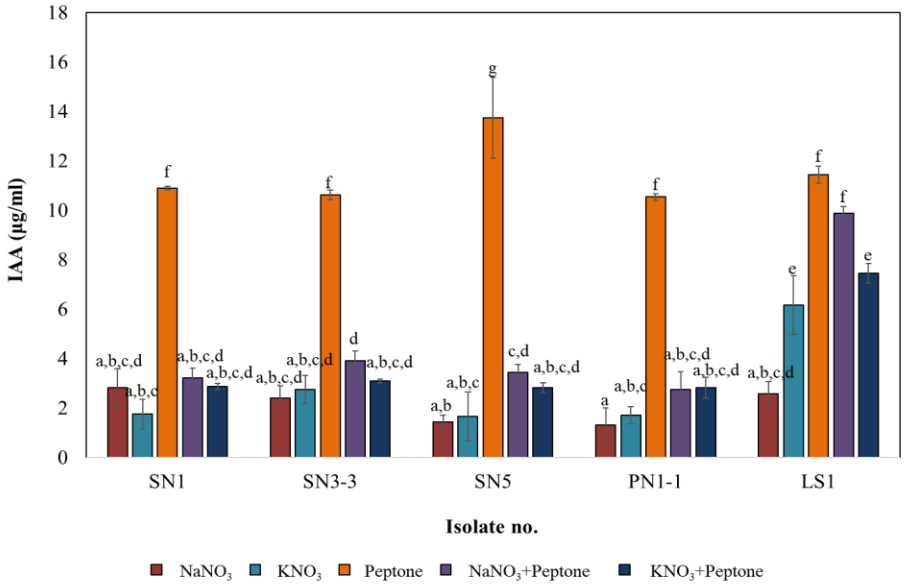

Figure 4. Effect of various nitrogen sources on IAA production by the selected isolates. The vertical bars represent the standard deviation with triplicates experiment. The clustering result of carbon sources on IAA production defined groups a, b, c, d, e, f, and g. The values marked by the same letter are not significantly different (*p*-value more than 0.05).

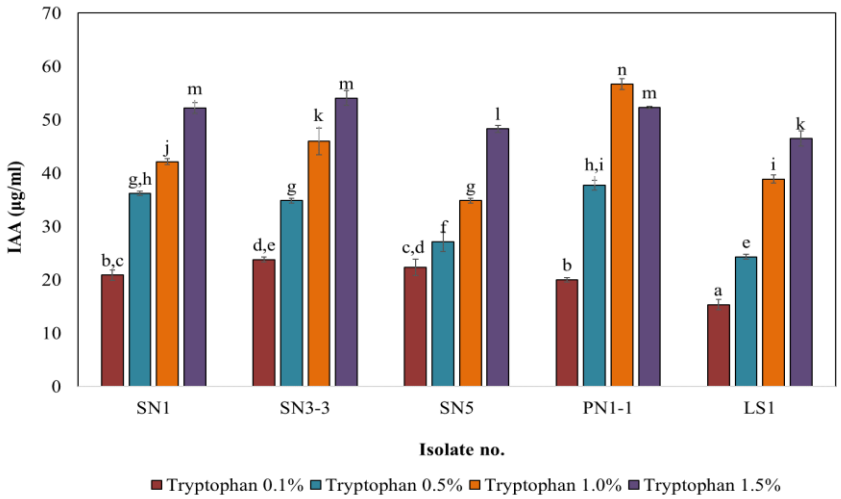

Figure 5. Effect of various tryptophan concentrations on IAA production by the selected isolates. The vertical bars represent the standard deviation with triplicates experiment. The clustering result of carbon sources on IAA production defined groups a, b, c, d, e, f, g, h, i, j, k, l, m, and n. The values marked by the same letter are not significantly different (*p*-value more than 0.05).

3.3.3 Effect of pH and temperature on IAA production

The maximum amount of IAA (70.1±2.2 µg/ml) was produced by isolate SN5 at pH 9. Similarly, IAA was also produced at high concentration at pH 9 by isolates PN1-1, SN1, SN3-3, and LS1 (66.5±2.3, 65.0 ±1.3, 64.6±0.2, and 64.6±0.4 µg/ml, respectively). IAA production was reduced when the pH value was reduced to 8, and further suppressed when the pH value decreased to 5-7. (Figure 6). Therefore, the result was elucidated that pH value is a significant factor for IAA production. The weak alkaline (pH value of 8-9) supported the production of IAA of selected isolates. The result from statistical analysis confirmed that a low concentration IAA was produced at pH value in the range 5-7 and was not significantly different within the group (*p*-value 0.052).

In the comparison of the IAA production at 30 and 37 °C, the results showed that IAA production was favored at 37 °C compared to 30 °C. The isolate SN5 produced the maximum IAA concentration of 8.6±0.9 µg/ml, and it was followed in decreasing order by isolates PN1-1, SN3-3, LS1, and, SN1, which produced 8.4±0.9, 7.1±1.0, 6.5±0.9, and 6.3±1.3 µg/ml of IAA, respectively at 37°C (Figure 7).

According to Sudha *et al*. [31] and Mandal *et al*. [32], *Rhizobium* strain VMA 301 produced high concentrations of IAA in a medium at pH 7.2, while Khamna *et al*. [33] reported that the conditions most suitable for maximum IAA production by *Streptomyces* sp. were at 30°C and pH 7.0. Low pH limits plant growth because the concentrations of metals (Al^{3+} and Mn^{2+}) in the soil solution can reach toxic levels. In addition, *Bacillus* strains produced the maximum amount of IAA when the pH of the medium was at 7, 8 and 9 [22, 34].

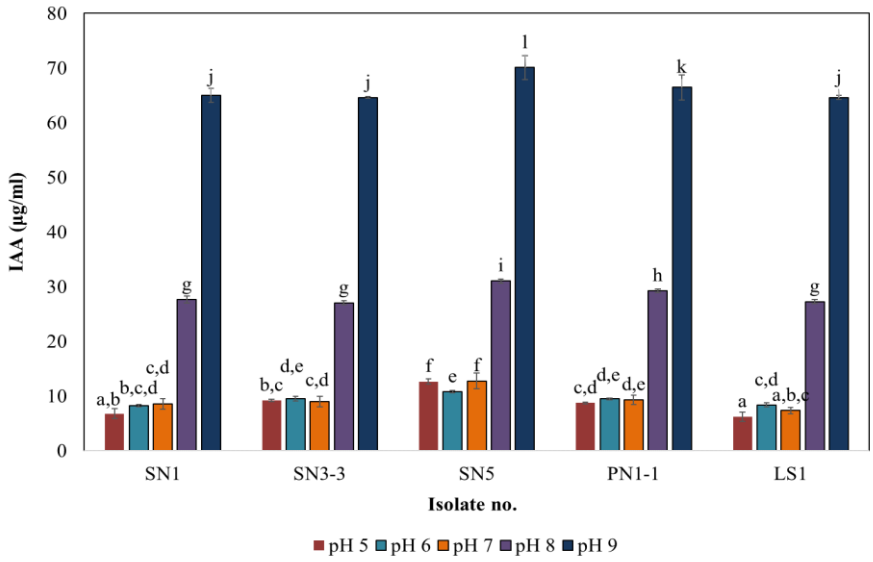

Figure 6. Effect of various pH on IAA production by the selected isolates. The vertical bars represent the standard deviation with triplicates experiment. The clustering result of carbon sources on IAA production defined groups a, b, c, d, e, f, g, h, i, j, k, and l. The values marked by the same letter are not significantly different (*p*-value more than 0.05).

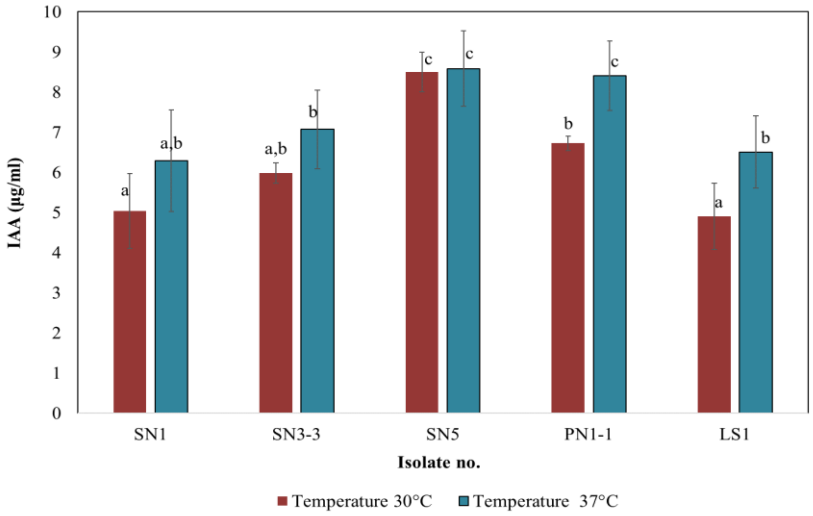

Figure 7. Effect of various temperatures on IAA production by the selected isolates. The vertical bars represent the standard deviation with triplicates experiment. The clustering result of carbon sources on IAA production defined groups a, b, and c. The values marked by the same letter are not significantly different (*p*-value more than 0.05).

Gram-negative bacteria, *Pantoea, Pseudomonas, Rahnella* and *Rhizobium* strains, were reported to produce IAA [35, 36], and they were isolated from rhizospheric soil [22]. Our strains produced different amounts of IAA that was influenced by culture conditions and substrates as reported in *Streptomyces* strains by Matsukawa *et al*. [37]. Moreover, the isolates from the rhizospheric soil were found to be more effective in auxin production, a result which was in agreement with the conclusions of Sarwar and Kremer. [38].

In this study, SN1, SN3-3, SN5, PN1-1, and LS1 proved to be potential isolates for IAA production. The study of the effects of medium components (YMD) on IAA production by selected isolates using statistical analysis indicated that glucose and peptone were the most suitable sources of carbon and nitrogen, respectively. The optimum physical factors at pH 9 and 37ºC were elucidated. In addition, IAA production was further optimized when the culture medium was supplemented with 1.0% L-tryptophan.

4. Conclusions

In this study, isolates LN4-2 and LS1 in Group I, isolate SN1 in Group II, isolate SN2 in Group III, isolate SN6-2 in Group IV, isolates SN3-3, SN5, LN1-4, LN1-2, LN1-8 and isolate PS1-5 in Group V, and isolate PN1-1 in Group VI, were most closely related to *Rhizobium pusense, Ochrobactrum oryzae, Pseudomonas aeruginosa, Pseudomonas geniculata, Acinetobacter pittii* and *Klebsiella pneumoniae* subsp. *rhinoscleromatis*, respectively. They belong to the gamma proteobacteria group. The SN1, SN5 and SN3-3, PN1-1 and LS1 isolates were able to produce IAA effectively when optimized for media components, pH, and temperature.

5. Acknowledgements

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