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Genome analysis and plant growth promoting activity of *Pseudonocardia* strain DR1-2 from the root of *Dendrobium christyanum* Rchb.f.

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ABSTRACT

Aims: The objective of this study was to analyze the genome of endophytic actinomycete associated with orchids and evaluate its plant hormone activities, including phytohormone, siderophore, ammonia production, zinc and phosphate solubilization.
Methodology and results: Strain DR1-2 isolated from the roots of the Thai orchid, *Dendrobium christyanum* Rchb.f., was closely related to *Pseudonocardia alni* DSM 44104^T, *P. antarctica* DSM 44749^T and *P. carboxydivorans* Y8^T (99.93-100% similarity) based 16S rRNA gene sequence. This strain exhibited IAA production (294.10 ± 12.17 µg/mL), phosphate solubilization (2.20 ± 0.08 solubilization Index, SI), positive for siderophore production and ammonia production (36.99 ± 2.24 µg/mL). It showed a maximum IAA of 489.73 ± 8.90 µg/mL, when optimized using 0.5% L-tryptophan, pH 6 and incubated at 30 °C for 7 days. The IAA of strain enhanced the root length, shoot length, number of roots and fresh weight of rice seedlings (*Oryza sativa* L. cv. RD49). The draft genome of strain DR1-2 was 6,077,423 bp in 23 contigs with G+C content of 74.6%. The average nucleotide identity-Blast (ANIb) and average nucleotide identity-MUMmer (ANIm) values of strain DR1-2 and related type strains were 95.81 to 97.25% and the digital DNA-DNA hybridization (dDDH) values were 72.60 to 74.00%, respectively. Genomic analysis of strain DR1-2 revealed that the gene encodes the enzyme involved in the phytohormones biosynthesis and gene clusters involved in the biosynthesis of bioactive metabolites.
Conclusion, significance and impact of study: Endophytic actinomycete, *Pseudonocardia* strain DR1-2 from Thai orchid, *D. christyanum* Rchb.f., exhibited significant IAA production and affected the growth of the plant, which was the potential source of plant hormones for agricultural applications.

Keywords: Endophytic actinomycetes, indole-3-acetic acid, plant growth-promoting activity, *Pseudonocardia*, *Dendrobium christyanum* Rchb.f.

INTRODUCTION

Endophytic bacteria are the biological agent that promotes plant growth. They colonize the root system and create phytohormones to stimulate the development of the plant, including indole-3-acetic acid (IAA), gibberellic acid, cytokinin and ethylene and supply nutrients through siderophores production, nitrogen fixation and phosphate solubilization (Jiang *et al*., 2021). The interaction between plants and bacteria could provide the basis for future crop management by using less fertilizer and pesticides. Recently, actinobacteria are attractive among plant-associated microbes and have become a more common practice in an agricultural system due to their ability to produce a variety of secondary metabolites and their plant growth-promoting potential (Yadav *et al*., 2018).

Endophytic actinomycetes have been proven to promote host plant growth and reduce disease in various environmental conditions by producing multiple secondary metabolites. For example, *Streptomyces* sp. PT2 from *Panicum turgidum* produced a high amount of IAA and increased seed germination and root extension in tomato cv. Marmande (Goudjal *et al*., 2013). In the greenhouse experiment, *Streptomyces* sp. 34 produced phytohormones (IAA), solubilized inorganic phosphate, siderophores and ammonia, leading to increased shoot and root length of chili plant and also protecting the plant from phytopathogens (Passari *et al*., 2015). The inoculation of mung beans (*Vigna radiata*) with *Streptomyces thermocarboxydus* S3 made increasing the

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fresh weight, root length and total length significantly (Lasudee *et al*., 2018). Furthermore, *Streptomyces fradiae* NKZ-259 produced nearly four times of IAA when it was optimized and the root, shoot length, fresh weight and dry weight of tomato seedlings were increased dramatically (Myo *et al*., 2019).

In addition, members of the genus *Pseudonocardia* strains have been reported and recovered from different habitats, such as soil, insect and plant tissue. For example, *P. antimicrobica* YIM 63235 from stems of *Artemisia annua* L. (Zhao *et al.*, 2012), *P. zijingensis* YIM 61043 from root of *Maytenus austroyunnanensis* (Qin *et al*., 2009) and *P. sichuanensis* KLBMP 1115 from root of *Jatropha curcas* L. (Qin *et al.*, 2011). Some *Pseudonocardia* strains have been developed to improve agricultural crop yields. For instance, *P. carboxydivorans* T1LA3, isolated from *Camellia sinensis* L. showed the highest IAA and ammonia production (Borah and Thakur, 2020), *Pseudonocardia* sp. W34 obtained from wheat roots could produce siderophore and IAA (Gangwar *et al*., 2012). However, there have been few studies on the potential of *Pseudonocardia* in agricultural applications and no genome analysis to provide insights into the physiological properties and potential of the strain. The genome sequencing analysis proved helpful for identifying their critical metabolites (Zerikly and Challis, 2009). In this study, *Pseudonocardia* strain from *D. christyanum* Rchb.f. orchid was characterized based on their phenotypic and genotypic characteristics. The plant growth-promoting potential of this strain is evaluated and its genome analysis is discussed.

MATERIALS AND METHODS

Isolation and cultivation

Strain DR1-2 was isolated from the roots of the Thai orchid, *D. christyanum* Rchb.f. collected from Loei Province, Thailand (14°03’20.8’’N 102°17’21.2’’E). The roots of *D. christyanum* Rchb.f. were washed with running tap water and dried at room temperature and prepared according to our previously reported protocols (Tedsree *et al*., 2021). The suspension of the sample was serially diluted ten times. Each diluted suspension was spread on starch casein gellan gum (SCG) (Tedsree *et al*., 2021) supplemented with nalidixic acid (25 µg/mL) and cycloheximide (50 µg/mL). The bacterial colony was selected and purified after 3-4 weeks of incubation at 30 °C. The purified strain was preserved on ISP 2 slants and freeze-dried for long-term storage.

Identification

Phenotypic characterization

The morphological characteristics of strain DR1-2 cultivated on yeast extract-malt extract (ISP 2) agar plates at 30 °C for 14 days were investigated by light microscopy (CX41; Olympus) and scanning electron microscopy (JSM-IT500HR, Jeol). Cultural characteristics were observed on ISP 2 agar, oatmeal agar (ISP 3), inorganic salts-starch agar (ISP 4), glycerol-asparagine agar (ISP 5), peptone-yeast extract iron agar (ISP 6), tyrosine agar (ISP 7) (Shirling and Gottlieb, 1966) and nutrient agar after incubation at 30 °C for 14 days. The colors of aerial mycelia, substrate mycelia and diffusible pigment were determined using the NBS/IBCC color system (Kelly, 1964). Physiological characteristics were evaluated by cultivating on ISP 2 agar at different temperatures (20-45 °C) and NaCl concentrations (0-10%, w/v), while the effect of pH for growth, at a pH range of 4-12 (at intervals of 1 pH unit) was evaluated in ISP 2 broth at 30 °C for 14 days by using the following buffer system: acetate buffer (pH 4-5), phosphate buffer (pH 6-8) and glycine-sodium hydroxide buffer (pH 9-12). Carbon utilization on ISP 9 supplemented with 1% (w/v) carbon source, starch hydrolysis, nitrate reduction, coagulation, peptonization, gelatin liquefaction and H_2S production were examined as previously mentioned by Arai *et al.* (1975). These biochemical characteristics were detected after incubation at 30 °C for 14 days.

Genotypic characterization

Genomic DNA was prepared using the technique earlier described (Kudo *et al*., 1998). The 16S rRNA gene sequence was amplified according to a well-established method (Suriyachadkun *et al*., 2009) and then sequenced on a DNA sequencer (Macrogen) using universal primers, 27F forward (5’-AGAGTTTGATCMTGGCTCAG-3’) and 1492R reverse (5’-TACGGYTACCTTGTTACGACTT-3’). The sequence similarity values between the isolates and their related neighbors were calculated using the EzBiocloud service (Yoon *et al*., 2017). The Kimura-2-parameter (Kimura, 1980) was used to create a phylogenetic distance matrix. A phylogenic tree was constructed through the neighbor-joining (NJ) technique (Saitou and Nei, 1987) using MEGA 7.0 (Kumar *et al*., 2016) based on 1000 replications of bootstrap value.

Determination of plant growth-promoting activities

The strain was prepared by cultivation in ISP 2 broth and incubated at 30 °C for 3 days, and then was subsequently used for plant growth-promoting assays.

Ammonia production

The seed culture of strain DR1-2 was cultured in peptone water and incubated at 30 °C with shaking (180 rpm) for 7 days. The bacterial culture was centrifuged at 4 °C for 15 min. The supernatant was mixed with 0.5 mL of Nessler’s reagent. A positive test for ammonia production was observed by the change of color from pale yellow to dark brown (Cappuccino and Sherman, 1992). A spectrophotometer was used to measure the absorbance at 450 nm and the results were compared to the standard curve of $(NH_4)_2SO_4$ and expressed in µg/mL.

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Phosphate solubilization

Qualitative phosphate solubilization activity of strain DR1-2 was determined using Pikovskaya medium (PVK agar). The 20 µL of seed culture of strain DR1-2 was spotted onto PKV agar and incubated at 30 °C for 7 days. A clear halo zone around a bacterial colony indicated the phosphate solubilization. The solubilization index (SI) was calculated as the ratio of the solubilization zone diameter to the colony diameter (Pande *et al*., 2017).

Solubilization index (SI) = [Colony diameter + Clear zone diameter (mm)]/Colony diameter (mm)

Zinc solubilization

Qualitative zinc solubilization activity of strain DR1-2 was determined using Tris-mineral salts medium with glucose (1% w/v). The medium was separately supplemented with 0.1% insoluble zinc compounds, including zinc sulfate ($ZnSO_4$), zinc chloride ($ZnCl_2$) and zinc oxide (ZnO). Subsequently, twenty µL of seed culture was spotted onto the agar plate and incubated at 30 °C for 7 days. After incubation, the diameter of the halo zone around the colony and bacterial colony was determined, and the values were used to calculate the SI. The clear zone formed by the isolates was determined using the equation above**.**

Siderophore production

Siderophore production was assessed using the universal procedure modified by Schwyn and Neilands (1987). Twenty microliters of seed culture were dropped on Chrome azurol S (CAS) agar plates and incubated for seven days at room temperature. Siderophore production was determined by the formation of a yellow-orange halo zone around the spot colony.

Indole acetic acid production

IAA production was estimated according to Gordon and Weber (1951). The seed culture was transferred to ISP 2 broth (0.2% L-tryptophan, pH 7.0) and incubated at 30 °C with shaking (180 rpm) for 7 days. The culture was centrifuged at 6,500 rpm for 5 min and the supernatant was used to determine the amount of IAA production. One mL of supernatant was added to two mL of Salkowski reagent [0.5 M of $FeCl_3$ in 35% $HClO_4$ in a proportion of 1:50 (v/v)] and kept in the dark for 30 min (Sameera *et al*., 2018). A UV-Vis spectrophotometer was used to detect IAA at an absorbance of 530 nm. The uninoculated medium with reagent was used as a control. The amount of IAA was calculated based on the calibration curve of IAA and expressed in µg/mL.

Optimization of IAA production

IAA production was optimized based on the effects of incubation time, temperature, pH and L-tryptophan level. Strain DR1-2 was grown in a 500 mL flask with 100 mL of ISP 2 medium plus 0.2% L-tryptophan with shaking (180 rpm). Salkowski's method was used to evaluate IAA production. The same cultural conditions were employed as mentioned above. The effect of incubation period on IAA production was investigated at 48 h intervals for 15 days. The concentration of L-tryptophan (0.1, 0.2, 0.3, 0.4, 0.5, 1.0 and 1.5%), pH (4, 5, 6, 7, 8, 9 and 10) and temperature (25, 30, 35, 37 and 40 °C) were determined for 7 days. The one-factor-at-a-time (OFAT) method was used to optimize all experiments (Czitrom, 1999).

Extraction, purification and detection of IAA

The seed culture of 1 mL of strain DR1-2 was used to inoculate 100 mL of ISP-2 broth supplemented with 0.5 µg/mL of L-tryptophan and incubated at 30 °C with 180 rpm shaking for 7 days. After 7 days of incubation, cells were separated from the supernatant by centrifugation at 6,500 rpm for 10 min. 1 N HCl was used to acidify the supernatant to pH 2 before extracting it twice with ethyl acetate. The fraction of ethyl acetate was evaporated at low temperature by a rotary evaporator. The crude extract was dissolved with 1 mL of methanol and stored at 20 °C. The C18 SPE column was used to partially purify IAA from the crude extract by slightly modified from the method of Zhu (2020). The cartridges were activated with 6.0 mL of methanol and equilibrated with 6.0 mL of methanol/water/formic acid (10/95/0.1, v/v/v). The sample was loaded into a cartridge and washed with 3.0 mL of methanol/water/formic acid (10/95/0.1, v/v/v), then the plant hormones were eluted with 3.0 mL of methanol/water/formic acid (80/20/0.1, v/v/v) (Zhu *et al*., 2020). The eluates were dried using rotary evaporators and dissolved with 1 mL methanol. The purified IAA was confirmed by thin-layer chromatography (TLC). The ethyl acetate extract was spotted on a TLC plate and developed using the mobile phase propanol and distilled water in the ratio of 8:2 (v/v) (Sameera *et al*., 2018). After development, the TLC plate was dried and sprayed with Salkowski reagent. The spots with Rf values will be compared with authentic IAA.

Growth-promoting activity of DR1-2 on rice

To investigate the impact of IAA produced by the strain DR1-2 on seed germination and root elongation in rice (*Oryza sativa* L. cv. RD49), surface sterilization of rice seeds was performed by soaking in 10% sodium hypochlorite (NaOCl) for 1 min and 95% ethanol for 3 min, followed by thorough washing in sterile distilled water. The treatment was carried out by soaking rice seeds in a standard IAA and IAA from DR1-2 with a concentration of 50 µg/mL. The control group was soaked in sterile distilled water. Seeds were placed in sterilized Petri dishes coated with two sheets of filter paper and soaked with 10 mL of sterile water (three replicates, ten seeds/plate). All the plates were incubated in a chamber with light at 30 °C for 16 h daily. Seed germination, root length, shoot length, number of roots, fresh weight and

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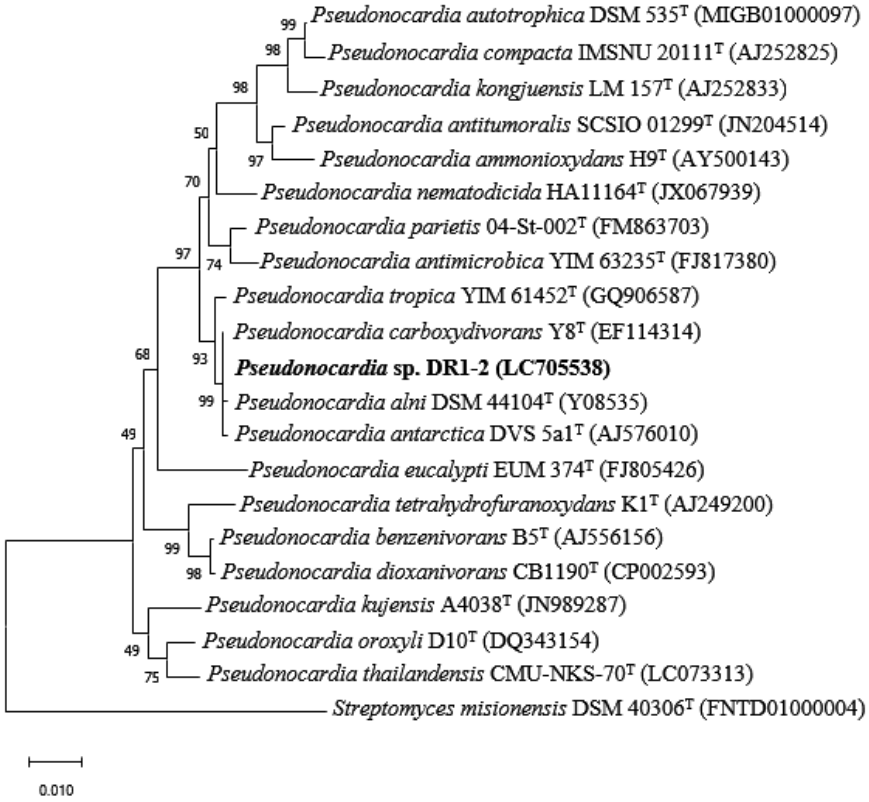

Figure 1: Phylogenetic tree of strain DR1-2 based on 16S rRNA sequences using the neighbor-joining method. Bootstrap percentages at nodes were calculated with 1,000 replicates.

dry weight were measured after 7 days.

Genome sequence analysis

Whole genome sequence analysis of strain was performed with an Illumina Miseq platform (Illumina, Inc., San Diego, US-CA) using 2× 250 bp paired-end reads. The assembling of the reads to contigs was managed using SPAdes 3.12 (Bankevich *et al*., 2012). The draft genome of strain DR1-2 was determined using the antiSMASH server (Blin *et al*., 2019) to detect putative biosynthetic gene clusters (BGCs). Gene prediction was accomplished using the Rapid Annotation using Subsystem Technology SEED viewer (Aziz *et al*., 2008). All genomes were annotated on Prokka software 1.13 (Seemann, 2014) in line with the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). Average nucleotide identity (ANI), ANI-Blast (ANIb) and ANI-MUMmer (ANIm) values between strain DR1-2 and closely related type strains were calculated pairwise using the JSpeciesWS web service (Richter *et al*., 2016). The digital DNA-DNA hybridization (dDDH) was evaluated using the Genome-to-Genome Distance Calculator (GGDC 2.1) with the BLAST+ method (Meier-Kolthoff *et al*., 2013) and the results were dependent on recommended formula 2 (identities/HSP length), which is proposed for use with incomplete whole-genome sequences.

Statistical analysis

Data were statistically analyzed by ANOVA using the SPSS software package (SPSS 28 for Windows). The grouping was performed by Duncan's multiple range tests at $p<0.05$ on each of the significant variables measured. The data were reported as the standard deviation of the mean values of triplicates.

RESULTS AND DISCUSSION

Isolation and identification

Strain DR1-2 was recovered from the roots of Thai orchid *D. christyanum.* The strain was aerobic and stained Gram-positive, with yellowish-white aerial mycelium and deep orange-yellow substrate mycelium on ISP 2 agar after 7 days of incubation. The strain grew very well on all media. Scanning electron micrograph of strain DR1-2 revealed the aerial mycelium fragmented into rod-shaped spores and the spore surface was smooth (Figure 1). The optimum temperature of strain was 30 °C and pH range 5-10. The strain utilized various sugars and nitrogen for growth and grew in 5-10% NaCl concentrations. The cultural, physiological and biochemical characteristics of the strain are shown in Table 1.

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Table 1: Phenotypic characteristics of the strain DR1-2.

Characteristics	Results	Characteristics	Results
Growth	Filamentous aerial	Carbon source utilization:	
Diffusible pigment production	-	Fructose	+
Growth temperature range	25 °C to 37 °C	Glycerol	+
Growth optimal temperature	30 °C	Raffinose	+
Growth pH range	5-10	Mannose	+
Optimal pH for growth	7.0	Mannitol	+
Production of H_2S	-	Xylose	+
NaCl tolerant	2% to 10%	Cellobiose	+
Peptonization	-	Galactose	+
Coagulation	-	Maltose	+
Starch hydrolysis	-	Melezitose	+
Gelatin liquefaction	-	Lactose	+
Nitrate reduction	+	*myo*-inositol	+
Colour of aerial mycelium	Colour of substrate mycelium	Rhamnose	+
Yellowish white on ISP 2	Deep orange yellow on ISP 2	Sucrose	+
Yellowish white on ISP 3	Moderate yellow ISP 3	Melibiose	+
Pale yellow on ISP 4	Light olive brown ISP 4	Nitrogen source utilization:	
Yellowish white on ISP 5	Moderate orange yellow ISP 5	L-Arginine	w
Yellowish white on ISP 6	Strong yellowish brown ISP 6	L-Asparagine	w
Yellowish white on ISP 7	Deep orange yellow ISP 7	L-Proline	+
Yellowish white on NA	Deep orange yellow on NA	L-Valine	w
		L-Cysteine	w
		L-Tyrosine	+
		L-Arabinose	+

All grew very good on all media. +, positive; w, weakly positive; -, negative.

The pairwise alignment of DR1-2 showed relatively high 16S rRNA gene similarity to strains of *P. alni* DSM 44104^T (99.93%), *P. antarctica* DSM 44749^T (99.93%) and *P. carboxydivorans* Y8^T (100%). The phylogenetic tree based on the neighbor-joining algorithm, the strain DR1-2 and the 3 closest relatives were placed in the same position (Figure 2). The 16S rRNA gene sequence of this strain has been deposited in NCBI database, with accession numbers LC705538.

Determination of plant growth-promoting activities

Strain DR1-2 was evaluated for plant growth-promoting activities, including phytohormone, siderophore, ammonia production, zinc and phosphate solubilization. IAA production by DR1-2 was determined on ISP 2 broth 0.2% L-tryptophan, pH 7.0 at 30 °C with 180 rpm shaking for 7 days. IAA production obtained was 294.10 ± 12.17 µg/mL. The strain was able to dissolve phosphate in plates by producing a clear halo around the colony on Pikovskaya medium (2.20 ± 0.08) but lacked zinc solubilizing ability in all of the sources of insoluble zinc. Strain DR1-2 was positive for ammonia production at 36.99 ± 2.24 µg/mL. Siderophore production was detected on CAS agar media by forming a clear orange halo zone around the colonies. Our results concurred with Borah and Thakur (2020), who discovered *P. carboxydivorans* T1LA3, an endophytic actinobacteria isolated from *Camellia sinensis* L. showed IAA, ammonia and siderophore production (Borah and Thakur, 2020).

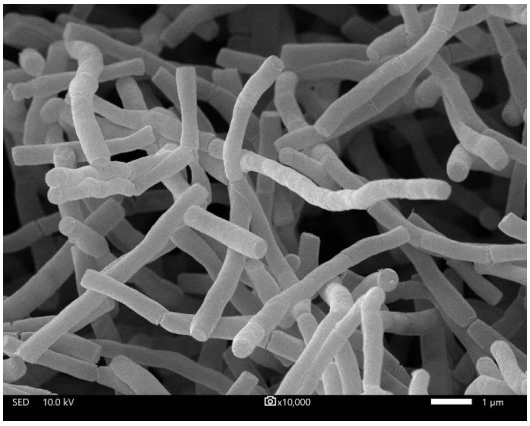

Figure 2: Scanning electron micrograph of strain DR1-2.

Extraction, purification and detection of IAA

The crude extract of IAA of strain DR1-2 was extracted using ethyl acetate and partial purification of IAA by C18 SPE column concentrated and then was detected on the TLC plate. The chromatograms were examined in visible and ultraviolet light (254 nm). Pink-colored spots were observed after spraying with Salkowski's reagent, with an Rf value of 0.82, identical to the standard IAA. The results show that the purified compound was identified as IAA using thin-layer chromatography and compared to the Rf value of the standard IAA.

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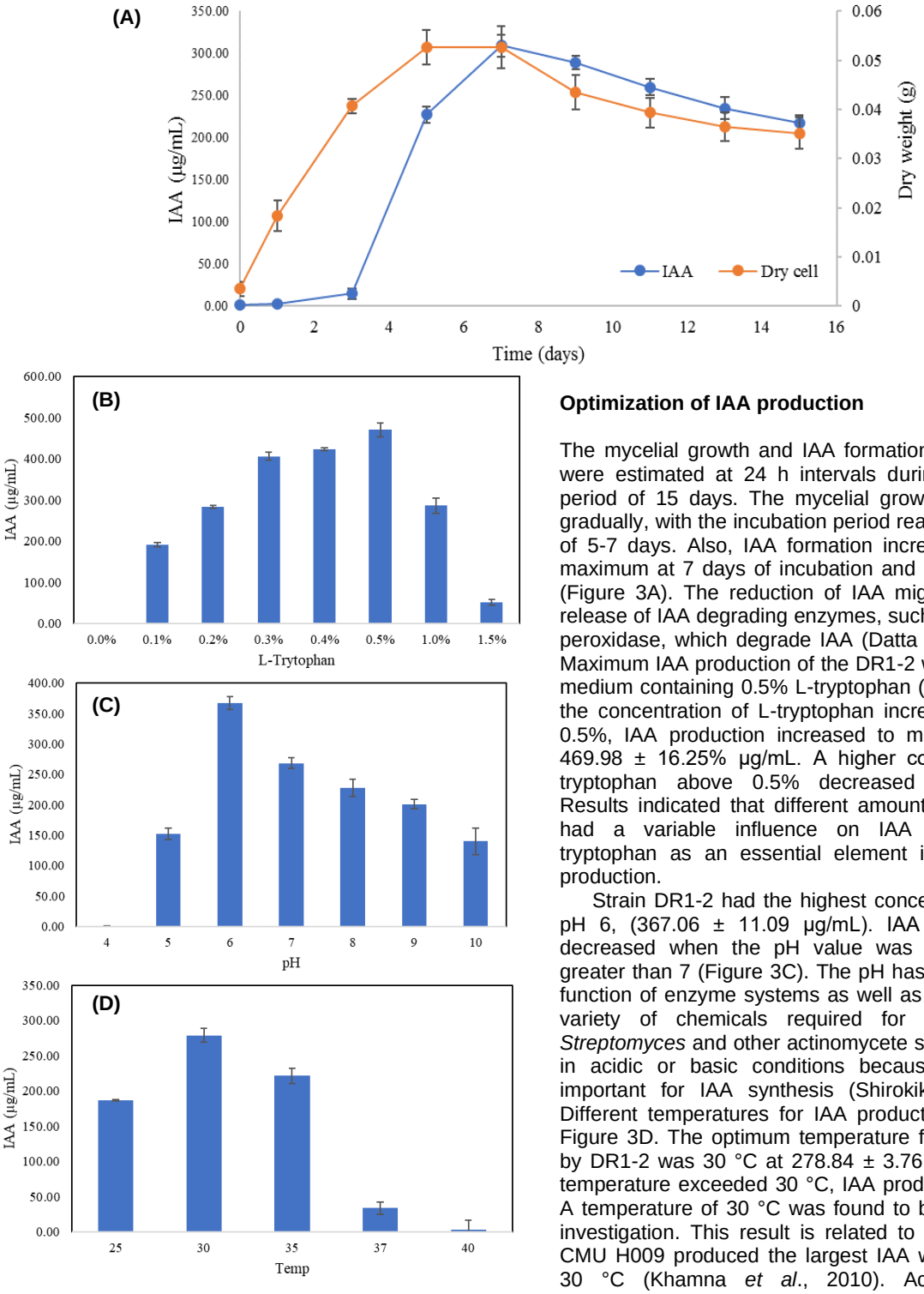

Figure 3: (A) Effect of incubation time (day), (B) Effect of L-tryptophan concentrations, (C) Effect of pH and (D) Effect of temperature on IAA production by the selected isolates. Vertical bars represent standard deviation from triplicate experiments.

Optimization of IAA production

The mycelial growth and IAA formation by strain DR1-2 were estimated at 24 h intervals during the incubation period of 15 days. The mycelial growth was increased gradually, with the incubation period reaching a maximum of 5-7 days. Also, IAA formation increased, reaching a maximum at 7 days of incubation and decreasing slowly (Figure 3A). The reduction of IAA might be due to the release of IAA degrading enzymes, such IAA oxidase and peroxidase, which degrade IAA (Datta and Basu, 2000). Maximum IAA production of the DR1-2 was observed in a medium containing 0.5% L-tryptophan (Figure 3B). When the concentration of L-tryptophan increased from 0.1 to 0.5%, IAA production increased to maximum levels at 469.98 ± 16.25% µg/mL. A higher concentration of L-tryptophan above 0.5% decreased IAA production. Results indicated that different amounts of L-tryptophan had a variable influence on IAA production, with tryptophan as an essential element in increasing IAA production.

Strain DR1-2 had the highest concentration of IAA at pH 6, (367.06 ± 11.09 µg/mL). IAA levels of DR1-2 decreased when the pH value was less than 6 and greater than 7 (Figure 3C). The pH has an impact on the function of enzyme systems as well as the solubility of a variety of chemicals required for bacterial growth. *Streptomyces* and other actinomycete strains grew slowly in acidic or basic conditions because pH levels are important for IAA synthesis (Shirokikh *et al*., 2007). Different temperatures for IAA production are shown in Figure 3D. The optimum temperature for IAA production by DR1-2 was 30 °C at 278.84 ± 3.76 µg/mL; when the temperature exceeded 30 °C, IAA production decreased. A temperature of 30 °C was found to be optimal for this investigation. This result is related to *Streptomyces* sp. CMU H009 produced the largest IAA when cultivated at 30 °C (Khamna *et al*., 2010). Accordingly, OFAT optimization experiments showed that the highest IAA production required cultivation in ISP 2 broth with 0.5% L-tryptophan, pH 6 at 30 °C for 7 days. The maximum IAA value of DR1-2 was 489.73 ± 8.90 µg/mL, which increased almost equal IAA production after optimization.

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Table 2: Effect of strain DR1-2 on the growth of rice (*O. sativa* L. cv. RD49).

Isolate	Growth parameters of actinomycetes treated rice					
	Root length (cm)	Shoot length (cm)	Number of roots	Seedling fresh weight (g)	Seedling dry weight (g)	% Seed germination
DR1-2	5.45 ± 0.64[a]	5.90 ± 0.83[a]	3.30 ± 0.67[a]	0.81 ± 0.07[a]	0.30 ± 0.05[a]	100
IAA	5.10 ± 0.52[a]	5.25 ± 0.68[ab]	3.80 ± 0.79[a]	0.80 ± 0.08[a]	0.30 ± 0.06[a]	100
Control	4.40 ± 0.66[b]	4.90 ± 0.74[b]	2.40 ± 0.52[b]	0.56 ± 0.08[b]	0.25 ± 0.03[b]	100

Different superscripts indicate significantly different (P<0.05) mean ± SD.

Table 3: Genome statistics, ANIb, ANIm and dDDH values among the draft genomes of strain DR1-2 and closely related strains. Strains: 1, DR1-2; 2, *P. alni* DSM 44104^T; 3, *P. antarctica* DSM 44749^T. The genome of type strains was obtained from GenBank.

Genome of	Features				
	Accession no.	Genome size (bp)	G+C content (%)	No. of contigs	Protein coding genes
1	JAMQOF000000000	6,077,423	74.6	23	5834
2	PHUJ01000000	5,994,807	74.2	3	5777
3	JACCCZ000000000	6,242,493	74.1	2	6015
Genome of	Reference genomes	ANIb%	ANIm%	%dDDH (formula2[a])	Prob. DDH>=70%
1	2	95.91	97.12	72.60%	82.51%
1	3	95.81	97.25	74.00%	84.49%

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

According to earlier studies by Myo *et al*. (2019), *S. fradiae* NKZ-259 could be able to produce more IAA after being optimized.

Growth-promoting activity of DR1-2 on rice

The effects of IAA purified from DR1-2 on rice seed germination, root length and shoot length were determined. Rice seeds soaked under various conditions exhibited significant differences in root lengths, shoot lengths and quantity of roots compared to the controls (Table 2). Treatments with IAA from DR1-2 significantly influenced the quality of seedling root, with no significant differences identified between standard IAA. The fresh and dry weights of seedlings after treatment with IAA produced by DR1-2 and standard IAA were significantly different from the control. However, all treatments did not affect seed germination. Endophytic actinomycetes utilize root exudates to generate a variety of plant compounds. IAA from DR1-2 increased shoot length, root length and the number of roots, showing that DR1-2 can produce plant growth regulators such as IAA. Our study results related to screening bacteria for plant growth-promoting agents on rice seedling growth, as reported by Etesami *et al*. (2015) and IAA-producing bacteria isolated from orchid rhizoplanes of *D. moschatum* (Tsavkelova *et al*., 2007) and *Cymbidium eburneum* (Faria *et al*., 2013) also have been reported to improve plant seed germination. Our strain indicated the presence of IAA production as a good option for use as plant growth enhancement in both economic and agricultural systems.

Genome sequence analysis

Genome analysis of strain DR1-2 revealed the size of 6,077,423 bp distributed in 23 contigs with G+C content of 74.6%. The phylogenetic analysis based on whole-genome sequences indicated that strain DR1-2 was phylogenetically closely related to *P. alni* DSM 44104^T and *P. antarctica* DSM 44749^T. The ANIb and ANIm values of the draft genomes between strain DR1-2, *P. alni* DSM 44104^T and *P. antarctica* DSM 44749^T were 95.91 and 97.12%, and 95.81 and 97.25%, respectively (Table 3). The digital DNA-DNA hybridization (dDDH) values between strain DR1-2 and its closest strains, *P. alni* DSM 44104^T and *P. antarctica* DSM 44749^T were 72.60 and 74.00%, respectively (Table 3). For genome comparison, ANI and dDDH values are considered well correlated when the values were ≥95% (ANI) and ≥70% (dDDH), respectively (Fitch, 1971; Seemann, 2014). Since the dDDH and the ANI values between strain DR1-2 and its closest strains were higher than the species cut off, strain DR1-2 should be the same species as *P. alni*, *P. antarctica* and *P. carboxydivorans*. Further taxonomic studies on *P. antarctica* and *P. carboxydivorans* should be described.

Gene function annotation and secondary metabolism gene clusters

The draft genome of DR1-2 has 5,834 protein-coding sequences (CDS), 51 transfer RNA (tRNA) genes and 3 ribosomal RNA (rRNA) genes. The annotation included

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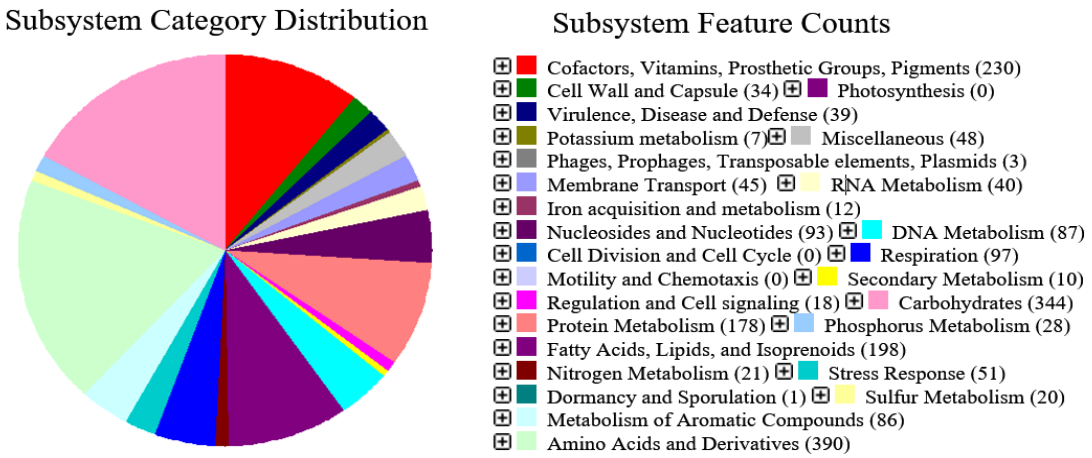

Figure 4: Subsystems distribution statistic of strain DR1-2 based on RAST annotation server.

Table 4: Biosynthetic gene clusters (BGCs) and secondary metabolites of strain DR1-2 based on the analysis of genome mining with AntiSMASH 5.0.

Region	Type	Most similar known cluster
Region 1	NAPAA	Stenothricin
Region 2	Terpene	SF2575
Region 3	NAPAA	Streptobactin
Region 4	Ectoine	Ectoine
Region 5	NAPAA	CC-1065
Region 6	RiPP-like	-
Region 7	NAPAA, T1PKS, NRPS	-
Region 8	Terpene	Isorenieratene
Region 9	Redox-cofactor	Lankacidin C
Region 10	Ranthipeptide	-
Region 11	RiPP-like	-
Region 12	NRPS, T1PKS, NRPS-like	Amychelin

NAPAA-non-alpha poly-amino acids like e-Polylysin; RiPP-like-Other unspecified ribosomally synthesized; NRPS-Nonribosomal peptide synthetases; NRPS-like-NRPS-like fragment; T1PKS-type 1 polyketide synthetases.

2,293 hypothetical proteins and 3,541 proteins with functional assignments. The proteins with functional assignments included 1,246 proteins with Enzyme Commission (EC) numbers (Schomburg *et al*., 2004), 1,091 with Gene Ontology (GO) assignments (Ashburner *et al*., 2000) and 983 proteins that were mapped to KEGG pathways (Kanehisa *et al*., 2016). RAST server annotation of the whole genome describes the subsystem distribution of strain DR1-2. Among the subsystem categories, there were genes for amino acids and derivatives (390 ORFs), carbohydrate metabolism (344 ORFs), cofactors, vitamin, prosthetic groups, pigment (230), fatty acids, lipids and isoprenoids (198 ORFs) and protein metabolism (178 ORFs). Nucleosides and nucleotides (93), respiration (97), DNA metabolism (97) and stress response genes (51) were also found in Figure 4.

For phytohormone IAA production, the gene associated with the tryptophan synthase alpha and beta chain (EC 4.2.1.20), which catalyzes the final stage in tryptophan biosynthesis was discovered. The gene encoding enzymes, aromatic-L-amino-acid decarboxylase (EC 4.1.1.28), anthranilate phosphoribosyl transferase (EC 2.4.2.18), monoamine oxidase (EC 1.4.3.4) and phosphoribosylanthranilate isomerase (EC 5.3.1.24) involved in auxin biosynthetic pathways were found in strain DR1-2. PATRIC annotation revealed *iaaM* gene in genome DR1-2 that encodes the enzyme tryptophan-2-monooxygenase, which converts tryptophan to the indole-3-acetamide (IAM), intermedia in IAM pathway. In bacteria, the IAM pathway is the best-characterized pathway (Sekine *et al*., 1989) and has been suggested to convert exogenous tryptophan to IAA (Perley and Stowe, 1966). Mevalonic acid pathway (MVA) and methylerythritol phosphate pathway (MEP), which produced a class of plant growth regulators, brassinosteroids (BRs) and gibberellins (GAs) were also found, respectively. Results indicated that DR1-2 might be a promising source of plant hormones for agricultural applications.

The draft genome of strain DR1-2 was determined using the antiSMASH server to detect putative biosynthetic gene clusters (BGCs). The 12 gene clusters were observed on DR1-2 genome related to various

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BGCs, mainly non-alpha poly-amino acids like e-Polylysin (NAPPA), terpene and ectoine (Table 4). The secondary metabolite biosynthetic gene clusters (smBGCs) exhibited similar genetic relatedness to the known clusters producing stenothricin (Liu *et al*., 2014), lankacidin C (Ahsan *et al*., 2017) and amychelin (Xie *et al*., 2020) have antimicrobial activity. Strain DR1-2 is predicted to produce anticancer, antitumor and antioxidant agents such as SF2575 (Pickens *et al*., 2009), CC-1-65 (Cacciari *et al*., 2000) and isorenieratene (Chen *et al*. 2019), respectively. In addition, ectoine was predicted in DR1-2, which protects proteins and cellular membranes against damage caused by severe environments like heat, UV light, strong osmolarity or dryness (Richter *et al*., 2019; Bilstein *et al.*, 2021). Thus, strain DR1-2 might be one of the sources of biological compounds used in pharmaceutical applications. Moreover, the predicted secondary metabolites, streptobactin (Matsuo *et al.*, 2011) and amychelin (Seyedsayamdost *et al.*, 2011) can be used as siderophore, compounds that have a high-affinity iron-chelating ability in agricultural farming. In addition, the genome of strain DR1-2 contained four smBGCs that displayed no similarity to any known smBGCs in antiSMASH (Table 4). These results suggested that strain DR1-2 might be a source of novel secondary metabolites.

CONCLUSION

In this study, strain DR1-2 associated with roots of *D. christyanum* orchid was closely related to *P. carboxydivorans*. The strain showed high IAA, ammonia, siderophore production and phosphate solubilizing activity. This strain could promote the number of roots, shoot length, root length and fresh weight of rice seedlings. The draft genome sequence analysis of strain DR1-2 indicated that gene clusters are involved in plant hormone biosynthesis. This strain will be helpful as phytohormone-producing bacteria for seed germination and plant growth improvement.

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