Research article

Genome Insights into the Plant Growth Promoting Features of a Newly Found *Microbispora* sp. SCL1-1

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Abstract

Plant growth-promoting (PGP) actinobacteria can be used to promote plant growth. Their use is a promising strategy that can be employed instead of agricultural chemical fertilizers. An actinobacterium strain, designated SCL1-1, was collected and isolated from a soil sample in a herbal garden at Pathum Thani province, Thailand. Analysis revealed that the SCL1-1 strain was a Gram-positive bacterium that formed longitudinal paired spores that were borne directly on aerial mycelia. It contained *meso*-diaminopimelic acid in its cell wall peptidoglycan. Moreover, madurose, which is a diagnostic sugar, was present in its whole-cell hydrolysates. *16S rRNA* gene analysis revealed that the SCL1-1 strain was a member of the *Microbispora* and showed a close relationship to *Microbispora rosea* ATCC 12950^T (99.6%), followed by *Microbispora hainanensis* DSM 45428^T (99.2%). However, a genome-based polyphasic study revealed that strain SCL1-1 had a low average nucleotide identity (ANI) (<95%), and digital DNA–DNA hybridization (dDDH) value (<70%) with *M. rosea* ATCC 12950^T and *M. hainanensis* DSM 45428^T, indicating that strain SCL1-1 was a different species to its close relatives. Genome mining of strain SCL1-1 showed the presence of genes related to the production of indole-3-acetic acid (IAA), and siderophore, which are agents that promote plant growth. In addition, the genome of strain SCL1-1 was found in several secondary metabolite biosynthetic gene clusters, which were possibly encoded for a broad range of remarkable natural products and antibiotics.

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1. Introduction

Microorganisms are classified as critical natural resources. Microbial secondary metabolites can be used for medical, agricultural, and industrial applications [1, 2]. Some bacteria, especially Actinobacteria, can produce secondary metabolites with various types of chemical structures and biological activities [3]. *Microbispora* spp. are classified as actinobacteria of the family *Streptosporangiaceae* and are considered a rare actinomycete group because the opportunity to isolate *Microbispora* spp. by conventional methods is much lower than that of *Streptomyces* spp. which are the common actinomycetes. The genus *Microbispora* is characterized as a Gram-positive filamentous bacterium that forms branching substrates and aerial mycelia. They always produce longitudinally paired spores on aerial mycelia [4]. All *Microbispora* species contain *meso*-diaminopimelic acid (*meso*-DAP) in their peptidoglycan. Madurose is a diagnostic sugar found in their cell-hydrolysates. Moreover, the previous study has revealed that this genus, *Microbispora*, consists of the series of MK-9 as significant menaquinones and phospholipid pattern type PIV (ninhydrin-positive glycophospholipid) [4]. Several *Microbispora* species such as *Microbispora corallina* [5], *Microbispora hainanensis* [6], *Microbispora rosea* [7], *Microbispora siamensis* [8], *Microbispora sitophila* [9], *Microbispora soli* [10], and *Microbispora cellulosiformans* [11] were isolated from various types of soil samples.

Genus *Microbispora* has been recognized as a promising resource of bioactive compounds or secondary metabolites. For example, a diterpene compound (2-α-hydroxy-8(14), 15-pimaradien-17, 18-dioic acid), bispolides, linfuranone A (a polyketide), and microbiaeratin (an indole alkaloid) are produced by *Microbispora hainanensis* strain CSR-4 [12], *Microbispora* sp. A34030 [13], plant derived *Microbispora* sp. GMKU 363, and *Microbispora aerata* [14], respectively. *Microbispora* spp. play a significant role as the producer of humus, plant nutrients and hormone, and bioactive compounds or secondary metabolites in the soil [15]. Recently, various secondary metabolites with plant growth-promoting activity were reported to be produced by the genus *Microbispora*. For example, indole acetic acid (IAA), a plant growth hormone, was found in the culture broth of *Microbispora hainanensis* CSR-4. Many genes associated with IAA production, such as the genes encoding for indole-3-glycerol phosphate synthase (*trp*C) [16], anthranilate phosphoribosyl transferase (*trp*D), anthranilate synthase (*trp*E), and aminase (*trp* A and B) were detected in the genome of the genus *Microbispora* [17]. Furthermore, the gene involved in the production of anthranilate phosphoribosyl transferase (*trp*F), which is a gene associated with IAA production, was found in many actinomycete genera [18]. In addition, *Microbispora* spp. were revealed to produce siderophores, to solubilize phosphate, to inhibit phytopathogenic fungal growth, and to produce indole-3-acetic acid products [15]. Phosphate solubilization is one mechanism for plant growth promotion. *Microbispora* spp. play a role in making phosphorus available to plants. They contained a gene responsible for the solubilization of inorganic polyphosphate, *ppx-gppA* gene [19]. The *pstS* gene found in the genome of *Microbispora* species was reported as an important gene associated with the production of the phosphate ABC transporter [20]. These preliminary studies indicated that the genus *Microbispora* is a rich-microbial resource for producing plant growth-promoting metabolites. Thus, an attempt to investigate *Microbispora* spp. in nature is still ongoing. In this study, we focused on the search for actinobacterial resources from nature, and a *Microbispora*-like strain designated SCL1-1 was isolated from the soil. We performed a genome-based taxonomic characterization of *Microbispora* strain SCL1-1, investigated its plant growth-promoting properties, and did genome mining of its plant growth-promoting genes.

2. Materials and Methods

2.1 *Microbispora* strain SCL1-1 isolation and preservation

The SCL1-1 strain was isolated from a soil sample that collected from a herbal garden (14° 6' 52.9194" N and 100° 23' 8.1234" E) in Pathum Thani province, Thailand. The soil sample was air-dried at room temperature for three days. To isolate the strain, the isolation method was used as previously described by Kittisrisopit *et al*. [10]. Modified starch-casein nitrate seawater (SCN) agar containing 10 g soluble starch, 1 g sodium caseinate, 0.5 g KH_2PO_4, 0.5 g $MgSO_4$, and 15 g agar (one liter of distilled water was used instead of seawater), supplemented with 50 mg L^{-1} nalidixic acid and 200 mg L^{-1} nystatin, at pH 7.2, was used as the isolation medium [21]. The isolation plate was incubated at 30°C for 21 days. A pinkish-white colony was selected and transferred to yeast extract-malt extract agar (International *Streptomyces* Project, ISP 2 medium) [22]. The colony was purified on ISP 2 agar using the cross-streak technique. To preserve the strain, the pure culture was maintained in glycerol solution (20 %, v/v) at -80°C.

2.2 Phenotypic characterizations

2.2.1 Morphological observation

To prepare the SEM samples, strain SCL1-1 was cultured on the medium as described in previous research [23]. Spore formation was initially observed by light microscopy (ECLIPSE E200; Nikon). A previous described method was used to prepare the SEM samples [24]. Then, the morphology of the spore was determined using a scanning electron microscope (SEM, model JSM-6610 LV; JEOL).

2.2.2 Cultural, physiological, and biochemical characterizations

To determine the cultural characteristics of strain SCL1-1, ISP media (ISP 2-7) was used [22]. The ISCC-NBS color chart was used for assigning the color of aerial and substrate mycelia [25]. Various physiological and biochemical characterizations such as the temperature and pH range for growth (10, 15, 20, 25, 30, 37, 40, 45, 50, and 55°C), pH (4.0-11.0), NaCl tolerance (0-10%, w/v), starch hydrolysis, nitrate reduction, urease production, milk peptonization, gelatin liquefaction, and decomposition of adenine, cellulose, hypoxanthine, tyrosine, and xanthine, were investigated using standard methods of Arai [26], Williams and Cross [27] and Gordon *et al*. [28]. The ability of the strain to utilize carbon was also analyzed as described in previous research [29].

2.3 Chemotaxonomic tests

Biomass for the chemotaxonomic studies was prepared as described by Phongsopitanun *et al*. [30]. Cells were harvested by centrifugation, washed five times in sterile distilled water, and dried using a freeze-dryer. The typical chemotaxonomic properties of the genus *Microbispora* were evaluated in this study. Isomers of diaminopimelic acid (DAP), and whole-cell sugars were done using one dimensional TLC as described by Komagata and Suzuki [31]. The acyl type of muramic acid was performed using the standard protocol [32]. Isoprenoid quinones were carried out using a previously described protocol [33]. Cellular fatty acids were prepared and analyzed using the Sherlock Microbial Identification (MIDI) system and the ACTIN version 6 database [34, 35].

2.4 *16S rRNA*, genome-based taxonomy, and the biosynthetic gene clusters (BGCs) analyses

To prepare the genomic DNA (gDNA) of the SCL1-1 strain for *16S rRNA* gene amplification and whole-genome sequencing, three-day-old cultures grown in ISP 2 broth at 30ºC were used and purified following the GeneJET Genomic DNA purification Kit (Thermo Scientific) protocol. The *16S rRNA* gene amplification and sequencing were done using a method previously described [36]. The nearly complete *16S rRNA* gene sequence of strain SCL1-1 was submitted to the EzBioCloud server [37] for analysis of sequence similarity. The *16S rRNA* gene trees, neighbor-joining (NJ) [38], maximum-likelihood (ML) [39], and maximum-parsimony (MP) [40], were reconstructed using the online server, MEGA version X [41]. For whole-genome sequencing, the genomic DNA sequencing library was prepared using QIAGEN FX kits. Genomic DNA was sequenced using an Illumina Miseq platform (Illumina, Inc., San Diego, US-CA). The assembled genomes were evaluated using SPAdes [42]. The draft genome was annotated by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). The average nucleotide identity (ANI) and average amino acid identity (AAI) values of strain SCL1-1 and its related type strains were analyzed by the JSpecies Web Server [43, 44] and the Kostas Lab AAI calculator [45], respectively. Digital DNA–DNA hybridization (dDDH), and phylogenomic tree were calculated using the genome-to-genome distance calculator (GGDC 2.1; blast+method) [46]. Phylogenomic tree was done using an automated multi-locus species tree (autoMLST) pipeline [47]. *Actinomadura madurae* DSM 43067^T (GCA_900115095) was used as an outgroup. We used gene clusters (smBGCs) in the genome of strain SCL1-1, its closely related type strains, and the anti-SMASH platform to predict secondary metabolite biosynthesis [48]. The genome assembly of strain SCL1-1 was submitted to GenBank as accession number VIRK00000000.

2.5 Genome mining for plant growth-promoting genes of strain SCL1-1

To evaluate the genes relating to enzyme production, and plant growth promotion, the genome of strain SCL1-1 was analyzed using blastp on the Uniprot database with matrix; blosum62 [49].

2.6 Evaluation for plant growth-promoting (PGP) traits of strain SCL1-1

2.6.1 Nitrogen fixation

The nitrogen fixation was analyzed according to the previous protocol [50]. A five days-old SCL1-1 strain was inoculated on NF agar and incubated at 30°C for 14 days. The nitrogen fixation ability was observed from the blue coloration zone around the colony. The diameter of the zone was measured.

2.6.2 The production of siderophore and phosphate solubilization

The production of siderophore and phosphate solubilization was evaluated by culturing the strain on chrome azurol S (CAS) agar [51] and on pikovskaya's agar (HiMedia, Mumbai, India), respectively at 30°C for 14 days. To observe the positive result of the production of siderophore, the yellow halo zone around the colony was measured, while the formation of the halo zone around the colony was recorded for the ability of phosphate solubilization.

3. Results and Discussion

3.1 Phenotypic and chemotaxonomic characterizations of strain SCL1-1

Soil typically contains essential nutrients such as humus and mineral salts that are required for actinobacterial growth, and researchers have found a higher diversity of actinomycetes in soil than in other environments. In this study, an isolated actinomycete from a herbal garden soil was classified and named SCL1-1. The SCL1-1 strain was able to grow well on the ISP 2, ISP 3, and ISP 6 media. The strain formed pinkish brown to reddish-orange substrate mycelia. A pinkish-white aerial spore mass was detected after 21 days of cultivation. Longitudinally paired spores (0.6-1.0 x 0.8-1.1 µm) borne directly on aerial mycelia with a smooth surface were observed (Figure 1). This morphology was commonly found in the genus *Microbispora* [52].

In additional study as described in Table 1, strain SCL1-1 could not grow at the temperature of 45°C but it could grow at NaCl concentrations of up to 3% (w/v). The range of pH that strain SCL1-1 could grow was between pH 5 to 8; however, we found that the optimum pH for SCL1-1 was pH 7 (data not shown). Moreover, we found that strain SCL1-1 was able to decompose cellulose and utilize a range of nitrogenous compounds except for hydroxy proline. However, the strain was unable to reduce nitrate, decompose xanthine, and utilize carbon. Strain SCL1-1 was found to contain *meso*-diaminopimelic acid (*meso*-DAP) in its cell wall peptidoglycan. Glucose, ribose, and madurose were found in the whole-cell hydrolysates. Madurose (3-O-methyl-D-galactose) is a diagnostic sugar detected in most *Microbispora* species [48]. Acetyl, the acyl type of muramic acid, was detected. Strain SCL1-1 contained iso-$C_{16:0}$ (52.7 %) and $C_{16:0}$ (11.9%) as the major fatty acids (>10%) in the cell (Table 2). It was previously observed that iso-$C_{16:0}$ was usually found in most of the members of *Microbispora* spp. [4]. In addition, we found that the characteristic isoprenoid quinones of strain SCL1-1 were MK-9 (H_4) (46.6%), MK-9 (H_2) (22.1%), MK-9 (H_6) (18.2%), and MK-9 (H_0) (9.1%) (Table 1). These chemotaxonomic results showed that strain SCL1-1 was clearly defined as a member of the genus *Microbispora* [52].

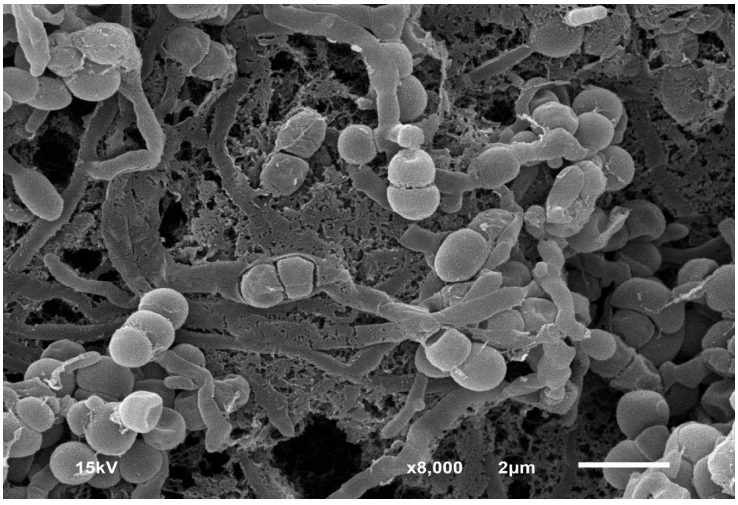

Figure 1. The SCL1-1 strain was grown on ISP 2 media at 30°C for 21 days and photographed by scanning electron microscope (SEM). Bar, 2 µm

Table 1. Differential characteristics of the SCL1-1 strain and closely related type strains. +, Positive; -, Negative; w, Weakly positive

Characteristics	Strain SCL1-1	*M. hainanensis* DSM 45428^T	*M. rosea* ATCC 12950^T
Decomposition of xanthine	w	-	-
Decomposition of CM cellulose	+	-	-
Nitrate reduction	-	+	+
Urease production	+	-	-
Maximum NaCl tolerance (%w/v)	3	1	1
Growth at 45 °C	-	-	+
The pH range for growth	5-8	5-10	5-10
Carbon utilization:			
L-arabinose	-	+	+
D-raffinose	-	+	+
D-mannitol	-	w	+
D-fructose	-	+	w
L-rhamnose	w	+	-
Glycerol	-	w	+
Lactose	-	+	+
Nitrogen utilization:	+		+
DL-2-aminobutyric acid	+	-	-
L-arginine	+	-	-
L-cysteine	+	-	-
Hydroxy proline	-	+	-
L-methionine	+	-	-
L-phenylalanine	+	-	+
Menaquinones	MK-9 (H_4), MK-9 (H_2), MK-9 (H_6), and MK-9 (H_0)	MK-9 (H_4), MK-9 (H_2), and MK-9 (H_0)	MK-9 (H_4), MK-9 (H_2), and MK-9 (H_0)

3.2 Genome features, genome-based taxonomy, and *16S rRNA* gene sequence analysis

To determine the taxonomic position of the SCL1-1 strain, its *16S rRNA* gene sequence was analyzed. The results revealed that the strain belonged to the genus *Microbispora* and showed the highest similarity to *Microbispora rosea* ATCC 12950^T (99.6%), followed by *Microbispora hainanensis* DSM 45428^T (99.2%). In addition, strain SCL1-1 formed a clade with *M. rosea* ATCC 12950^T in all tree-making algorithms, i.e. NJ, MP, and ML trees (Figures 2-4).

In this study, the genome-based taxonomy was used to clarify the taxonomic position at the species level of strain SCL1-1, as shown in Table 3. As described in Table 3, the genome sequence of SCL1-1 was submitted in to the GenBank database under the accession number VIRK00000000. The genome assembly contained 8.81 Mbp that included 272 contigs and N50 of 172 kbp. The sequences showed a genomic G+C content of 71.2 %. In addition, the genome sequences consisted of 8,137 predicted genes, which were possibly included 8,075 protein-coding genes, 56 tRNA genes, 3 rRNA genes (5S, 16S, and 23S). The phylogenomic tree based on multi-locus sequence alignment of 100 conserved single-copy genes indicated that strain SCL1-1 formed a clade together with the type strain of *M. hainanensis* DSM 45428^T with 100% bootstrap values (Figure 5). The average nucleotide

Table 2. Cellular fatty acid composition of strain SCL1-1. Cultures were grown in yeast extract-malt extract broth on a rotary shaker at 30°C for 5 days. *Data were obtained in this study.

Fatty acids*	Strain SCL1-1	*M. hainanensis* DSM 45428^T	*M. rosea* DSM 43839^T
Saturated fatty acids			
$C_{12:0}$	0.2	2.7	0.1
$C_{13:0}$	-	-	0.2
$C_{14:0}$	0.8	1.5	1.1
$C_{15:0}$	-	4.1	2.3
$C_{16:0}$	11.9	21.6	10.1
$C_{16:0}$ 3-OH	0.6	0.9	-
$C_{17:0}$	2.7	6.1	14.6
$C_{17:0}$ 3-OH	0.2		
$C_{18:0}$	4.3	3.2	2.9
Unsaturated fatty acids			
$C_{17:1}$ ω8c	0.5	1.4	-
$C_{16:1}$	-	-	2.5
iso-$C_{16:1}$	0.4	-	-
anteiso-$C_{17:1}$ ω9c	0.1	-	-
$C_{18:1}$ ω9c	0.4	1.7	0.2
Branched fatty acids			
iso-C12:0	0.1	-	-
iso-C13:0 3OH	0.1	-	-
iso-C14:0	0.7	1.1	-
iso-C15:0	3.7	5.3	2.7
anteiso-C15:0	1	2.2	-
iso-C16:0	52.7	19.7	41.9
anteiso-C16:0	0.2	-	-
iso-C17:0	1.9	2.7	1.8
anteiso-C17:0	4.3	3.2	8.6
iso-C17:0 3OH	0.1	-	-
iso-C18:0	2.1	-	9.2
iso-C19:0	-	-	1.7
anteiso-C19:0	0.1	-	-
C17:0 10-methyl	6.6	5.3	0.3
C18:0 10-methyl, TBSA	1.4	2.6	-

Figure 2. Neighbor-joining tree based on *16S rRNA* gene sequencing shows the relationship between strain SCL1-1 and related taxa. The numbers written on the branches indicate the percentage bootstrap values of 1,000 replicates; only values ≥50 % are shown. Bar, 0.05 substitutions per nucleotide position

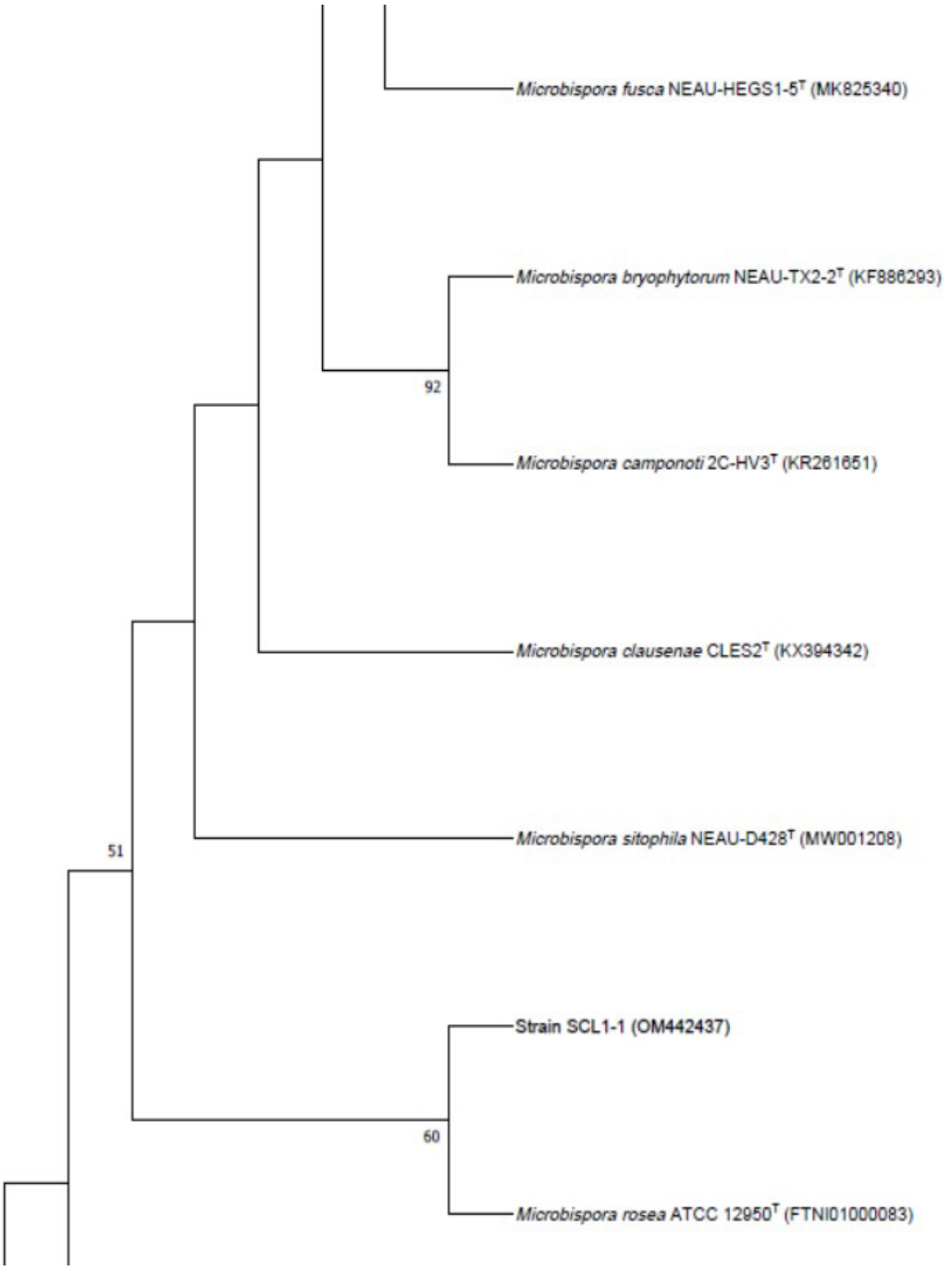

Figure 3. Maximum parsimony tree based on *16S rRNA* gene sequences shows the relationship between strain SCL1-1 and related taxa. The numbers on the branches indicate the percentage bootstrap values of 1,000 replicates; only values ≥50 % are shown.

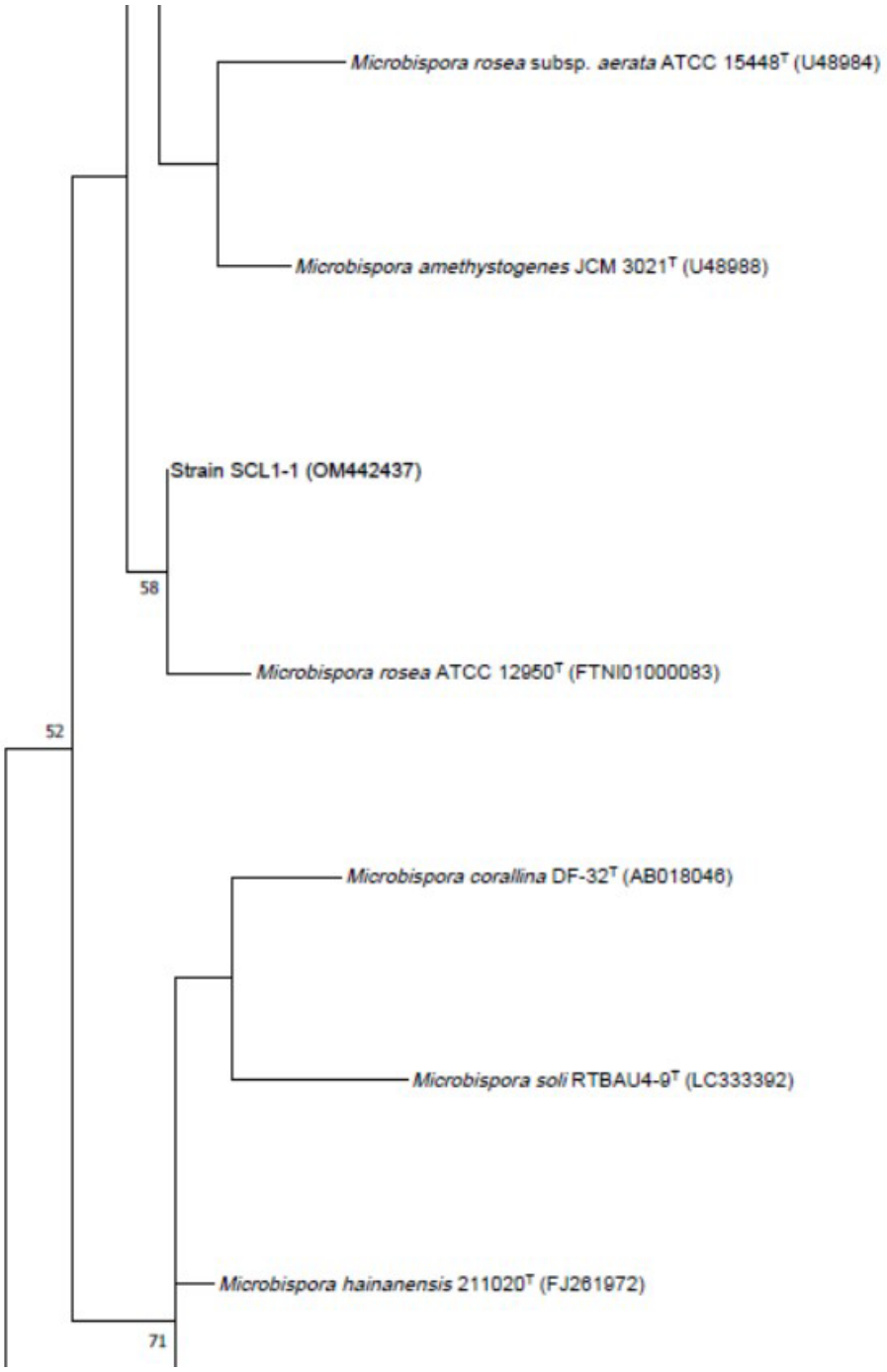

Figure 4. Maximum-Likelihood tree based on 16S rRNA gene sequences shows the relationship between strain SCL1-1 and related taxa. The numbers on the branches indicate the percentage bootstrap values of 1,000 replicates; only values ≥50 % are shown. Bar, 0.02 substitutions per nucleotide position.

identity (ANI), average amino acid identity (AAI), and digital DNA–DNA hybridization (dDDH) values between strain SCL1-1 and its closely related type strains, *M. rosea* ATCC 12950^T and *M. hainanensis* DSM 45428^T were in the ranges of 91.9 to 94.3%, 91.0-94.2%, and 48.2-58.3% for ANI, AAI and dDDH, respectively. The analyzed results for ANI values between strain SCL1-1 and its close phylogenetic neighbors are shown in Table 4. The comparative genome data analysis revealed that strain SCL1-1 was probably a different species to *M. rosea* ATCC 12950^T and *M. hainanensis* DSM 45428^T because the overall genome relevance and the ANI, AAI, and dDDH indices were below the threshold values proposed for delineation of genomic species [43, 53, 54].

Table 3. The 13 features of the genome sequences of strain SCL1-1 and its related type strains

Features	**Strain SCL1-1**	***M. hainanensis* DSM 45428^T**	***Microbispora rosea* ATCC 12950^T**
Bioproject	PRJNA552306	PRJNA552327	PRJEB18889
Accession no.	VIRK000000000	VIRM0000000	FTNI01000000
Genome coverage	98x	95x	164x
N50	172,635	121,286	200,586
Number of Contigs	272	251	95
Genome size (Mbp)	8.81	8.71	8.85
DNA G+C content (%)	71.2	71.3	71.2
Number of genes	8,137	8,061	8,886
Protein coding genes	8,075	7,692	8,620
Number of RNAs	62	62	63
rRNA	3	4	3
tRNA	56	57	57
Other RNA	3	1	3

Table 4. ANIb, AAI, and digital DNA-DNA hybridization relatedness values of strain SCL1-1 and its related type strains

Query genome	**Reference genome**	**ANIb (%)**	**AAI (%)**	**Digital DNA-DNA hybridization relatedness**				**G+C difference**
				Formula 2*				
				% dDDH	**Model C.I. (%)**	**Distance**	**Prob. DDH ≥ 70 (Same species)**	
Strain SCL1-1	*Microbispora rosea* ATCC 12950^T	91.9	91.0	48.2	45.6–50.8	0.0760	14.47	0.1
	Microbispora hainanensis DSM 45428^T	94.3	94.2	58.3	55.5–61.1	0.0545	46.72	0.1

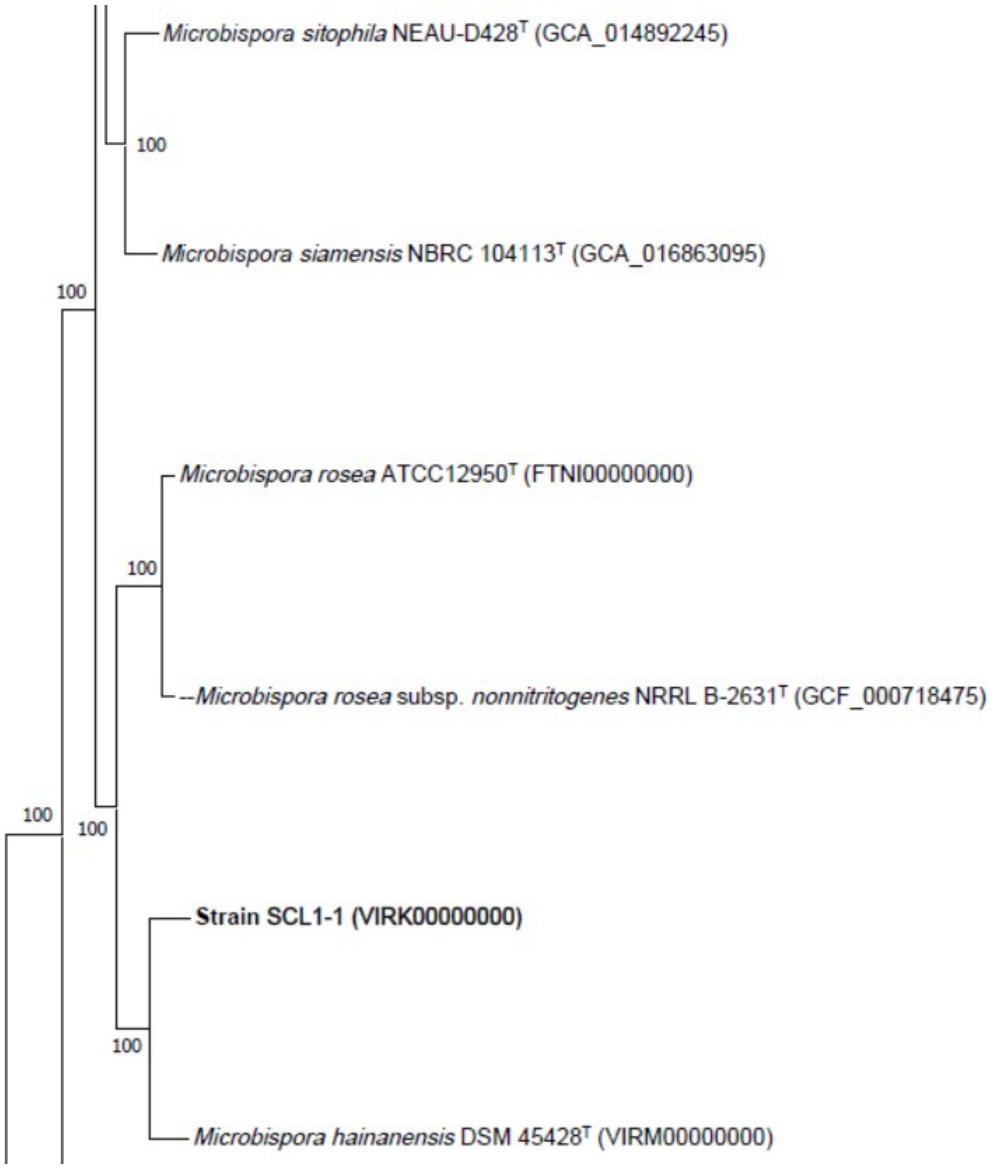

Figure 5. Phylogenomic analysis of strain SCL1-1 and type strains affiliated with the genus *Microbispora,* and its related taxa based on 100 bacterial conserved single copied gene sets of the members. The bootstrap values on the nodes are displayed by >50.

3.3 Differential phenotypic traits between strain SCL1-1 and its closely related type strains

The result of *16S rRNA* gene and genome analyses indicated that the type strains, *M. hainanensis* DSM 45428^T, and *M. rosea* ATCC 12950^T were closely related type strains of strain SCL1-1. Thus, we chose these type strains to perform the comparative phenotypic study because strain SCL1-1 could be distinguished from its closest type strains using various phenotypic traits. From the results, we found that strain SCL1-1 was different from *M. hainanensis* DSM 45428^T, and *M. rosea* ATCC 12950^T in terms of its xanthine and CM cellulose decomposition, nitrate reduction, urease production, highest NaCl concentration, and highest temperature for growth conditions. Interestingly, strain SCL1-1 could not utilize L-arabinose, D-raffinose, D-mannitol, D-fructose, glycerol, and lactose, but both *M. hainanensis* DSM 45428^T, and *M. rosea* ATCC 12950^T could utilize all those compounds. Moreover, strain SCL1-1 could grow in the presence of L-arginine, L-cysteine, and L-methionine, but both *M. hainanensis* DSM 45428^T and *M. rosea* ATCC 12950^T could not grow under those conditions. These significantly different phenotypic characteristics suggested that strain SCL1-1 was a different species to *M. hainanensis* DSM 45428^T and *M. rosea* ATCC 12950^T.

3.4 Genome mining of plant growth-promoting *Microbispora* sp. SCL1-1

Microorganisms, especially actinomycetes, are remarkable in that they produce plant hormones and siderophores and make phosphorus available for plants [55]. In this study, the plant growth-promoting (PGP) abilities of strain SCL1-1 were evaluated. The *in vitro* study of the PGP ability showed that strain SCL1-1 could produce siderophores and indole acetic acid (IAA) products, and solubilize phosphate, but it was unable for nitrogen fixation. As previously reported, phytohormones, especially indole-3-acetic acid (IAA), play a crucial role in plant growth [56]. It is known that IAA and other auxins are generally produced by soil microorganisms [57]. The genome of strain SCL1-1 was found to have genes associated with indole-3-glycerol phosphate synthase. This enzyme is involved in the production of IAA via the tryptophan biosynthetic pathway [58]. In addition, the genes encoding for the synthesis of aminase (*trp*A and *trp*B), anthranilate phosphoribosyl transferase (*trp*D), and anthranilate synthase (*trp*E) were also detected in the genome of strain SCL1-1. These genes are associated with the production of other components of the tryptophan biosynthetic pathway [59] (Table 5).

The rhizosphere actinomycetes are promising microbial resources for converting insoluble organic and inorganic phosphorus sources into soluble forms and producing siderophores for plants [60]. In this study, several genes associated with phosphate regulation and metabolism were detected in the genome of strain SCL1-1. These included genes encoding for the production of alkaline phosphatase, dITP/XTP pyrophosphatase, inorganic pyrophosphatase (*ipp*), polyphosphate kinase (*pp*K), NAD^+ transhydrogenase subunit beta (THb), and phosphate-specific transport system accessory protein (*pho*U). Moreover, genes associated with the production of phosphatase (*ppx*/*gpp*A), and phosphate transporter (*pst*S) were also found in the genome of strain SCL1-1. It is known that *ppx*/*gpp*A and *pst*S genes are responsible for the solubilization of inorganic polyphosphate compounds [61] and the production of the phosphate ABC transporter [62], respectively. In the case of siderophore production, strain SCL1-1 comprised genes that are associated with the synthesis of iron ABC transporter permease, siderophore biosynthesis protein, isochorismatase (*icm*), and isochorismate synthase (*pch*A). Besides, the genome of strain SCL1-1 was found to contain the genes *iuc*A/*iuc*C, which are involved in aerobactin biosynthesis via the NIS pathway [63], as shown in Table 5.

Table 5. Biosynthetic gene clusters (≥50 % similarity with known bioclusters) found in strain SCL1-1^T and closely related type strains

Region	Type	From	To	Most similar known cluster to	MIBiG BGC-ID
***Microbispora* sp. strain SCL1-1**					
Region 46.3	Terpene	142,067	164,259	Geosmin (100% of genes show similarity)	BGC0000661
Region 49.1	TransAT-PKS, NRPS, Terpene	1	104,519	Guangnanmycin (71% of genes show similarity)	BGC0001611
Region 57.1	Phenazine	15,613	36,101	Phenazine SA (56% of genes show similarity)	BGC0002561
Region 59.1	NI-siderophore	23,207	35,042	FW0622 (62% of genes show similarity)	BGC0002690
Region 119.1	T3PKS, Lanthipeptide-class-iii	164,028	205,095	Alkylresorcinol (100% of genes show similarity)	BGC0000282
Region 130.1	T1PKS	40,376	113,760	Abyssomicin M-Z (71% of genes show similarity)	BGC0001492
***Microbispora rosea* ATCC 12950^T**					
Region 33.1	Terpene	149,847	172,141	Geosmin (100% of genes show similarity)	BGC0000661
Region 34.1	Other, Lanthipeptide-class-ii	106,206	146,796	Actinokineosin (63% of genes show similarity)	BGC0001496
Region 41.1	T3PKS, Lanthipeptide-class-iii	162,048	203,115	Alkylresorcinol (100% of genes show similarity)	BGC0000282
Region 77.1	Lanthipeptide-class-ii	50,068	72,971	Duramycin (50% of genes show similarity)	BGC0001579
***Microbispora hainanensis* DSM 45428^T**					
Region 13.1	T3PKS, Lanthipeptide-class-iii	135,165	175,880	Alkylresorcinol (100% of genes show similarity)	BGC0000282
Region 57.1	NI-siderophore	105,865	117,727	FW0622 (62% of genes show similarity)	BGC0002690
Region 95.1	Phenazine	37,345	57,833	Phenazine SA (56% of genes show similarity)	BGC0002561
Region 130.1	Terpene	105,448	127,640	Geosmin (100% of genes show similarity)	BGC0000661

3.5 Specialized metabolite biosynthetic gene clusters

To determine the secondary metabolite biosynthetic gene clusters (smBGCs) in the genomes of strain SCL1-1 and the closely related type strains, *M. hainanensis* DSM 45428^T and *M. rosea* ATCC 12950^T, antiSMASH version 6.0 was used. The genome of strain SCL1-1 was rich in clusters of Type I PKS (Polyketide synthase), and terpenes and non-ribosomal peptide synthases (NRPS) (Figure 6). In comparison to *M. hainanensis* DSM 45428^T and *M. rosea* ATCC 12950^T, strain SCL1-1 was not found to have the clusters of TIIPKS, lanthipeptide-class-ii, and thioamitides (Figure 6). Six smBGCs in the genome of strain SCL1-1 revealed high similarity (>50%) to known compounds and are equipped to synthesize terpene, transAT-PKS, NRPS, phenazine, *iuc*A/*iuc*C-like siderophores (NI-siderophores), Type I PKS (Polyketide synthase), Type III PKS (Polyketide synthase), and class III lanthipeptides-like labyrinthopeptin (lanthipeptide-class-iii). These smBGCs were found to have the genetic ability to produce products related to geosmin (100% gene similarity), a natural bicyclic terpene with an earthy odor produced by soil microorganisms [64], alkylresorcinol (100% gene similarity), which is a lipophilic polyphenol compound synthesized by bacteria that possess various biological activities [65], guangnanmycin (71% gene similarity), a promising anticancer drug that is commonly produced by actinobacteria [66], phenazine SA (56% gene similarity), a nitrogen-containing heterocyclic redox agent with broad-spectrum antibiotic activities [67], and FW0622 compound, a desferrioxamine-like siderophore produced by *Verrucosispora* strain [68]. Strain SCL1-1 also contains bioclusters that are predicted to synthesize abyssomicin M-Z (71% gene similarity), which shows anti-*Bacillus cereus* and anti-*Mycobacterium tuberculosis* activity [69]. Furthermore, the genome of strain SCL1-1 contained many bioclusters that are predicted to encode for a broad range of specialized metabolites albeit with low levels of gene identity (<50%), as shown in Table 6. Thus, the gene clusters that were observed in the SCL1-1 genome indicated that strain SCL1-1 had the genotypical ability to produce several valuable compounds with potential use in agriculture and pharmaceutics.

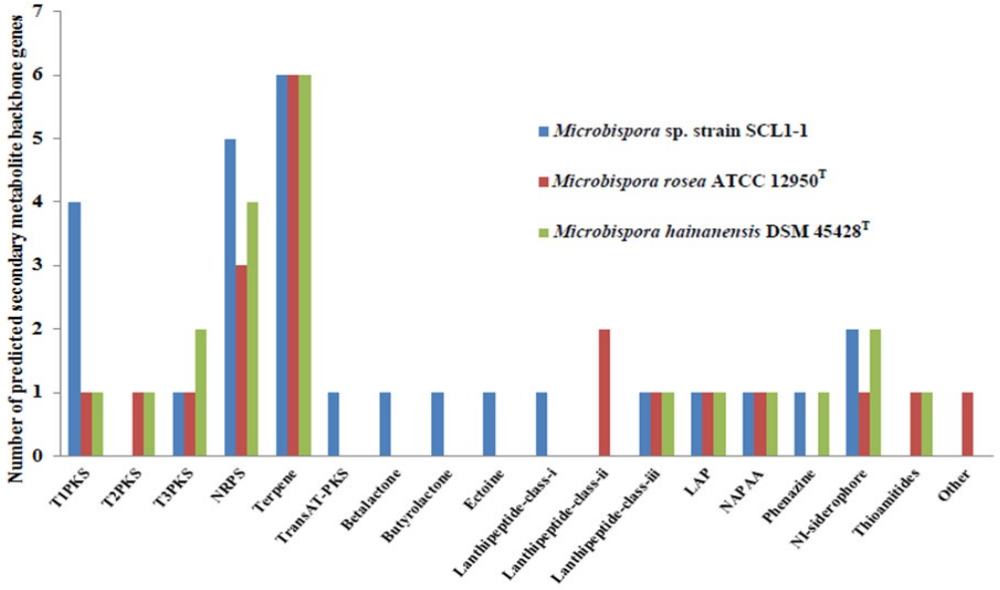

Figure 6. Gene cluster prediction of biosynthetic compounds from strain SCL1-1, *M. hainanensis* DSM 45428^T and *M. rosea* ATCC 12950^T performed by antiSMASH 6.0

Table 6. Biosynthetic gene clusters (≥50 % similarity with known bioclusters) found in strain SCL1-1 and the closely related type strains

Region	Type	From	To	Most Similar Known Cluster To	MIBiG BGC-ID
***Microbispora* sp. strain SCL1-1**					
Region 1.1	Butyrolactone	494,282	505,292	Colabomycin E (4% of genes show similarity)	BGC0000213
Region 7.1	T1PKS	1	3,522	Linfuranone B (40% of genes show similarity)	BGC0001653
Region 24.1	NRPS	60,608	118,461	GP6738 (9% of genes show similarity)	BGC0002702
Region 35.1	Terpene	151,207	176,278	Hopene (38% of genes show similarity)	BGC0000663
Region 46.1	T1PKS	1	38,507	Caniferolide A (4% of genes show similarity)	BGC0001856
Region 46.2	NAPAA	71,423	105,445	Madurastatin A2 (11% of genes show similarity)	BGC0002718
Region 48.1	T1PKS	150,318	174,869	Akaeolide (12% of genes show similarity)	BGC0001199
Region 52.1	NRPS	87,914	133,919	Tetronasin (3% of genes show similarity)	BGC0000163
Region 63.1	NRPS	18,596	63,920	Desertomycin B (3% of genes show similarity)	BGC0002523
Region 64.1	Terpene	41,536	62,573	K-252a (5% of genes show similarity)	BGC0000814
Region 68.1	NI-siderophore	44,636	57,893	Peucechelin (15% of genes show similarity)	BGC0002466
Region 75.2	LAP	346,757	370,594	Hexacosalactone A (4% of genes show similarity)	BGC0002497
Region 82.1	Terpene	34,290	55,183	Frankiamicin (14% of genes show similarity)	BGC0001197
Region 85.2	Ectoine	26,069	36,443	Showdomycin (47% of genes show similarity)	BGC0001778
Region 97.1	Betalactone	241,528	274,367	Frankobactin A1 (12% of genes show similarity)	BGC0002409
***Microbispora rosea* ATCC 12950^T**					
Region 23.1	T1PKS	39,702	87,522	Indigoidine (6% of genes show similarity)	BGC0000727
Region 29.1	NRPS	70,810	128,335	Tetronasin (5% of genes show similarity)	BGC0000163
Region 33.2	NAPAA	216,841	250,893	Madurastatin A2 (11% of genes show similarity)	BGC0002718
Region 39.1	T2PKS	1	56,836	Tetarimycin A (31% of genes show similarity)	BGC0000274
Region 40.1	Lanthipeptide-class-v	41,162	83,224	Pristinin A3 (29% of genes show similarity)	BGC0002303
Region 42.2	NRPS	226,504	271,714	Desertomycin B (3% of genes show similarity)	BGC0002523
Region 48.1	Terpene	178,798	204,004	Hopene (38% of genes show similarity)	BGC0000663
Region 52.1	Terpene	1	15,632	K-252a (5% of genes show similarity)	BGC0000814
Region 60.1	LAP	10,358	34,147	Hexacosalactone A (4% of genes show similarity)	BGC0002497
Region 67.1	Thiopeptide, Thioamitides	48,351	77,537	Clipibicyclene (11% of genes show similarity)	BGC0002697
Region 70.1	NI-siderophore	43,319	56,588	Peucechelin (15% of genes show similarity)	BGC0002466
Region 71.1	Terpene	34,182	55,078	Frankiamicin (14% of genes show similarity)	BGC0001197

Table 6. Biosynthetic gene clusters (≥50 % similarity with known bioclusters) found in strain SCL1-1 and the closely related type strains (continued)

Region	Type	From	To	Most Similar Known Cluster To	MIBiG BGC-ID
***Microbispora hainanensis* DSM 45428^T**					
Region 2.1	T1PKS	1	28,674	Tetronasin (5% of genes show similarity)	BGC0000163
Region 2.2	Terpene	30,719	55,874	Hopene (38% of genes show similarity)	BGC0000663
Region 60.1	Terpene	88,555	109,451	Frankiamicin (14% of genes show similarity)	BGC0001197
Region 61.1	Terpene	72,213	93,184	K-252a (5% of genes show similarity)	BGC0000814
Region 67.1	NI-siderophore	76,493	89,741	Peucechelin (15% of genes show similarity)	BGC0002466
Region 69.1	NRPS	1	39,165	Bosamycin A (33% of genes show similarity)	BGC0002581
Region 84.1	NRPS	20,973	67,023	Tetronasin (3% of genes show similarity)	BGC0000163
Region 86.1	NRPS	144,821	190,175	Desertomycin B (3% of genes show similarity)	BGC0002523
Region 86.2	T2PKS, T3PKS	223,559	279,649	Pradimicin-A (39% of genes show similarity)	BGC0000256
Region 130.2	NAPAA	106,473	188,936	Madurastatin A2 (11% of genes show similarity)	BGC0002718

4. Conclusions

Plant growth-promoting (PGP) actinobacteria have been used as alternatives to agricultural chemical fertilizers. In this study, we isolated an actinobacterium strain called SCL1-1 from a soil sample collected from a herbal garden in Pathum Thani province, Thailand. We did morphological study and *16S rRNA* analysis and found that strain SCL1-1 was a member of *Microbispora* and showed a close relationship with *Microbispora rosea* ATCC 12950^T (99.6%), followed by *Microbispora hainanensis* DSM 45428^T (99.2%). However, genome-based polyphasic study indicated that strain SCL1-1 was different from the type strains, *M. rosea* ATCC 12950^T and *M. hainanensis* DSM 45428^T. The results of genome mining of strain SCL1-1 indicated the presence of genes associated with indole-3-acetic acid (IAA), and siderophore production, both of which are currently used as PGP agents. Thus, this study suggested that the *Microbispora* SCL1-1 strain could be used as a promising resource of PGP compounds. To confirm these findings, the co-cultivation of strain SCL1-1 and plants needs to be investigated.

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