Antonie van Leeuwenhoek (2022) 115:911–920
https://doi.org/10.1007/s10482-022-01744-6

ORIGINAL PAPER

Diversity of the culturable lichen-derived actinobacteria and the taxonomy of *Streptomyces parmotrematis* sp. nov.

Achiraya Somphong · Vasun Poengsungnoen · Kawinnat Buaruang · Chanwit Suriyachadkun · Paranee Sripreechasak · Somboon Tanasupawat · Wongsakorn Phongsopitanun

Received: 10 December 2021 / Accepted: 1 May 2022 / Published online: 30 May 2022

Abstract A total of 37 actinobacteria were isolated from eighteen lichen samples collected in Thailand. Based on the 16S rRNA gene sequences, they were identified into five genera including *Actinoplanes* (1 strain), *Actinomadura* (1 strain), *Pseudosporangium* (1 strain), *Wangella* (1 strain) and *Streptomyces* (33 strains). Among these isolates, strain Ptm05^T, Ptm01 and Ptm12 showed low 16S rRNA gene similarity and was selected for the further taxonomic study using the polyphasic approach. These strains showed the highest 16S rRNA gene sequence similarity with *Streptomyces sparsogenes* ATCC 25498^T (97.44–97.72%). Strain Ptm05^T was selected for the type strain. The chemical cell composition of the strain was similar to the members of *Streptomyces* genus. LL-diaminopimelic acids were detected in the peptidoglycan. Menaquinones were MK-9(H_8) and MK-9(H_6). Phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, one unidentified phospholipid, one unidentified glycolipid and one unidentified lipid were detected as the polar lipids. The predominant cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$ and $C_{16:0}$. The dDNA-DNA hybridization values among strain Ptm05^T and its closely related *Streptomyces* type strains were 17.2–18.0%. In

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10482-022-01744-6.

The DDBJ accession number for the 16S rRNA gene sequence of strain Ptm05^T, Ptm01 and Ptm12 are, MZ922479, OL770019 and OL774519, respectively. The DDBJ/ENA/GenBank accession number for the draft genome and SRA of strain Ptm05^T is JAINVZ000000000.

A. Somphong · S. Tanasupawat · W. Phongsopitanun (✉)
Department of Biochemistry and Microbiology, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok 10330, Thailand
e-mail: Wongsakorn.p@chula.ac.th

V. Poengsungnoen · K. Buaruang
Lichen Research Unit, Department of Biology, Faculty of Science, Ramkhamhaeng University, Bangkok 10240, Thailand

C. Suriyachadkun
National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand Science Park, Pathumthani 12120, Thailand

P. Sripreechasak
Department of Biotechnology, Faculty of Science, Burapha University, Chonburi 20131, Thailand

W. Phongsopitanun
Natural Products and Nanoparticles Research Unit (NP2), Chulalongkorn University, Bangkok 10330, Thailand

addition, the ANIb and ANIm between strain Ptm05^T and related *Streptomyces* type strains were ranged from 75.69 to 76.13% and 85.21 to 85.35%, respectively. Based on phenotypic and genomic evidence, strain Ptm05^T (=TBRC 14546^T=NBRC 115203^T) represents the novel species of the genus *Streptomyces* for which the name *Streptomyces parmotrematis* sp. nov. is proposed. This study showed that the lichens are the promising source of the novel actinobacterial taxa.

Keywords Actinobacteria · Actinomycetes · Lichen · *Parmotrema* · *Streptomyces parmotrematis*

Abbreviations

ANI	Average nucleotide identity
dDDH	Digital DNA-DNA hybridization
GGDC	Genome-to-genome distance calculator

Introduction

Actinobacteria are the phylum of high DNA G+C content Gram-positive bacteria. A well-known genus of actinobacteria is *Streptomyces*, producing important bioactive secondary metabolites, especially antibiotics. Actinobacteria produce not only antibiotics but also antifungal agents, antiviral agents, antiparasitic agents, insecticides, antitumor agents, immunosuppressants, enzyme inhibitors and plant hormones (Bérdy 2005). Many actinobacterial secondary metabolites have been applied to clinical treatment. Examples are actinomycin, ivermectin, platensimycin, streptomycin, vancomycin and tacrolimus. Although actinobacteria have been driving drug discovery for a century, the new lead bioactive compounds isolated from actinobacteria have been decreasing over time. The isolation of the soil actinobacteria leads to the re-isolation of the previously known actinobacteria. To overcome this problem, the new habitats become the alternative source of actinobacterial isolation.

Streptomyces, the largest genus of the actinobacteria, was first described by Waksman and Henrici (1943). The members of genus *Streptomyces* are Gram-stain-positive bacteria that form extensively branched substrate and aerial mycelia. *Streptomyces* generally produce chains of spores on the aerial mycelia. Some strains can produce a wide variety of pigments and antibiotics. Up to date, *Streptomyces* have more than 670 validly published names (Parte et al. 2020). The members of *Streptomyces* widely distribute in various habitats including soil, plant material, marine sediment, sponges, corals, insects, and lichens. Lichen is a symbiosis between mycobiont (fungi) and photobiont (green algae or cyanobacteria) as well as internal bacterial communities that integral to lichen thalli. However, the knowledge about these bacterial communities is still limited (Bates et al. 2011). In the past decade, several new actinobacteria were isolated from the lichens. These include *Actinoplanes lichenis*, *Actinoplanes lichenicola*, *Actinoplanes ovalisporus* and *Streptomyces lichenis* (Phongsopitanun et al. 2016; Saeng-In et al. 2018, 2021). This study aims to investigate the diversity of culturable lichen-associated actinobacteria in Thailand and describe the taxonomic position of the selected novel *Streptomyces* candidate.

Materials and methods

Lichen collection and isolation of actinobacteria

Eighteen lichen samples were collected from four different sampling sites of Thailand, including Doi Suthep-Pui National Park (Chiang Mai), Lumpini park (Bangkok), Ramkhamhaeng (Bangkok), Khao Yai National Park (Nakhon Ratchasima), and Pho Si Somphon temple (Chaiyaphum). The lichen samples were preserved and identified by the lichen culture collection, Ramkhamhaeng University, Thailand (Table 1). For the isolation of actinobacteria, the lichen samples were air-dried for 7 days on the clean bench. Then, the lichen thalli were dissected into the small pieces subsequently by making a tenfold dilution using basic-lauryl sulfate solution, with 0.1 ml of the resultant solution spread on humic acid vitamin agar (Hayakawa & Nonomura 1987) and starch casein nitrate agar (Sripreechasak & Athipornchai 2019) supplemented with cycloheximide (50 mg l^{-1}) and nalidixic acid (25 mg l^{-1}) and incubated at 30 °C for 14 days. The colonies of actinobacteria were selected and transferred to the yeast extract-malt extract agar (ISP 2) (Shirling & Gottlieb 1966), after incubation at 30 ℃ for 14 days. The purified cultures were preserved as working cultures on ISP 2 slants.

Table 1 Location of lichen collection and the total number of the actinobacterial isolates

No	Lichen species	Type of lichen	Location	Number of isolates	Isolate name
1	*Usnea* sp.	Fruticose	Doi Suthep-Pui National Park, Chiangmai	0	–
2	*Usnea* sp.	Fruticose		0	–
3	*Usnea* sp.	Fruticose		0	–
4	*Ramalina* sp.	Fruticose		0	–
5	*Parmotrema tinctorum*	Foliose		4	Ptm01, Ptm05, Ptm07, Ptm08
6	*Parmotrema* sp.	Foliose		1	Ptm12
7	*Pyxine cocoes*	Foliose	Lumpini park, Bangkok	2	Lp01, Lp03
8	*Pyxine cocoes*	Foliose		7	Lp04, Lp05-1, Lp05-2, Lp06, Lp07, Lp08, Lp09
9	*Pyxine cocoes*	Foliose		3	Lp10-3, Lp11, Lp13
10	*Dirinaria picta*	Foliose	Ramkhamhaeng University, Bangkok	1	Ram02
11	*Lecanora helva*	Foliose		1	Ram04-1
12	*Parmotrema praesorediosum*	Foliose	Pho Si Somphon Temple, Chaiyaphum	2	Pm05-1, Pm05-3
13	*Parmotrema* sp.	Foliose		1	Pm01
14	*Parmotrema* sp.	Foliose		9	Pm02-2, Pm02-3, Pm02-4, Pm02-5, Pm02-7, Pm02-8, Pm02-9, Pm02-13, Pm02-14
15	*Parmotrema* sp.	Foliose		1	Pm03-1
16	*Parmotrema* sp.	Foliose		2	Pm04-1–1, Pm04-4
17	*Parmotrema chozoubae*	Foliose	Khao Yai National Park, Nakhon Ratchasima	1	Un01-10
18	*Cardonia fruticulosa*	Fruticose		2	Un02-1–1, Un02-8
		Total		37	

16S rRNA gene analysis and phylogenetic trees

The genomic DNA was extracted using DNA purification kits (Purelink™). The 16S rRNA gene was amplified using the primers 20F (5´-GAGTTTGATCCTGGCTCAG-3´) and 1500R (5´-GTTACCTTGTTACGACTT-3´) (Suriyachadkun et al. 2009). The nucleotide sequence of the PCR product was carried out using the universal primers (Macrogen) (Lane 1991). BLAST analysis was performed using EZBioCloud database (www.ezbiocloud.net/) (Yoon et al. 2017). The phylogenetic trees, neighbour-joining (NJ), maximum-parsimony (MP), maximum-likelihood (ML), were constructed using MEGA X (Kumar et al. 2018). The evolutionary distances for NJ and ML were analysed using Kimura's two-parameter, while the subtree-pruning-regrafting method was used for MP. The confidence of nodes was evaluated using the bootstrap analysis with 1,000 replications (Felsenstein 1985).

Genome and bioinformatics

The genome of the selected strain Ptm05^T was sequenced using the Illumina MiSeq using 2×250 bp paired-end reads. The quality of raw reads was checked using FASTQC (Babraham Bioinformatics). The adaptors and low-quality reads were removed using Trim Galore. The assembled genome was accomplished using Unicycler (Wick et al. 2017). Genome annotation was carried out using PATRIC 3.6.7 (Davis et al. 2020). The whole genome shotgun project was deposited at DDBJ/ENA/GenBank under the accession number JAINVZ000000000. The phylogenetic tree based on whole genome data sequences of strain Ptm05^T was analysed using the TYGS server (http://tygs.dsmz.de) (Meier-Kolthoff & Göker 2019). The G+C content was determined from whole genome sequences. The digital DNA–DNA hybridization (dDDH) analysis was performed by using the Genome-to-Genome Distance Calculator (GGDC)

web service at DSMZ (http://ggdc.dsmz.de). In addition, JSpeciesWS was used to calculate the average nucleotide identity (ANI) (Ritcher et al. 2016). The biosynthetic secondary metabolite gene clusters in the genome of the type strain were determined using antiSMASH version 6.0.1 (Medema et al. 2011).

Phenotypic studies

The morphology of strain Ptm05^T was observed after the strain was cultivated on ISP3 agar at 30 °C for 14 days using a light microscope (Olympus, CH2) and scanning electron microscope (JSM-5600, JEOL). Samples for scanning electron microscopy were prepared as described by Itoh et al. (1989). Cultural characteristics were determined on several International *Streptomyces* Project (ISP) media (Shirling and Gottlieb 1966) and nutrient agar (NA). The colour of aerial mycelia, substrate mycelia and soluble pigment was observed after the strain grown at 30 °C for 14 days using the National Bureau of Standards/Inter-Society Colour Council (NBS/ISCC) colour chart (Kelly 1964).

The growth of different pH (4.0–11.0, at intervals of 1 pH unit) was observed on ISP 2 broth with pH adjusted by using acetate buffer (pH 4.0–5.0), phosphate buffer (pH 6.0–7.0), Tris–HCl (pH 8.0–9.0), and carbonate-bicarbonate buffer (pH 10.0–11.0). The growth temperature (4, 30, 37, and 55 °C) and NaCl tolerance (1.0–9.0% (w/v), at 1.0% intervals) were determined using ISP 2 agar medium. The carbon utilization was determined using basal mineral salt medium (containing 0.0064 g $CuSO_4{\cdot}5H_2O$, 0.0011 g $FeSO_4{\cdot}7H_2O$, 0.0079 g $MnCl_2{\cdot}4H_2O$, 0.0015 g $ZnSO_4{\cdot}7H_2O$, 2.64 g $(NH_4)_2$ SO_4, 2.38 g KH_2PO_4, 5.65 g $K_2HPO_4{\cdot}3H_2O$, 1.00 g $MgSO_4{\cdot}7H_2O$ 1 L of distilled water pH 7.0). Nitrogen sources utilization was determined using basal medium (containing 0.5 g d-glucose, 0.05 g $MgSO_4{\cdot}7H_2O$, 0.05 g NaCl, 0.001 g $FeSO_4{\cdot}7H_2O$, 0.01 g K_2HPO_4 and 1 L of distilled water pH 7.2). Both basal media used for carbon and nitrogen utilization were supplemented with 1% (w/v) each of the sugars and nitrogen sources to be tested, respectively (Shirling & Gottlieb 1966; Arai et al. 1975). Starch hydrolysis, gelatin liquefaction, peptonization of skimmed milk, and nitrate reduction were determined using the standard methods (Williams & Cross 1971; Gordon et al. 1974; Arai et al. 1975). All phenotypic properties were recorded the results after the strains were grown at 30 °C for 14 days. Enzymatic activities were determined using API ZYM strips (bioMérieux) by following the instruction of the manufacturer.

Chemotaxonomy

The freeze-dried cells were used for all chemotaxonomic studies. The isomers of diaminopimelic acid and whole-cell sugars were analyse using the TLC method (Staneck & Roberts 1974). The menaquinones were extracted according to the method of Collins et al. (1997) and analysed using the high performance liquid chromatography (HPLC). Two-dimensional TLC was used to analyse the polar lipids (Minnikin et al. 1984). Cellular fatty acid methyl esters were analysed using MIDI microbial identification system (Sasser 1990).

Results

Diversity of the lichen-derived actinobacteria

A total of 37 actinobacterial isolates were obtained from 18 lichen samples (Table 1). 19 and 18 isolates were obtained from the starch casein nitrate agar and humic acid vitamin agar, respectively (Supplementary Table S1). Most isolates were obtained from foliose lichen, especially *Parmotrema* sp. and *Pyxine cocoes*. No isolates were obtained from *Usnea* lichens. Based on the BLAST result of the 16S rRNA gene sequences phylogenetic tree, these actinobacterial strains were classified into five genera including *Actinoplanes* (1 strain), *Actinomadura* (1 strain), *Pseudosporangium* (1 strain), *Wangella* (1 strain) and *Streptomyces* (33 strains) (Supplementary Fig. S1; Table S1). Among these isolates, strain Ptm05^T, Ptm01 and Ptm12 showed low 16S rRNA gene sequence similarity compared to the valid publish type strains found in the ExBiocloud database. Therefore, these three isolates were selected for further taxonomic study.

Taxonomic studies the new actinobacterial species

Phylogenetic trees and genomic studies

Based on the EzbioCloud database, strain Ptm05^T, Ptm01 and Ptm12 showed the highest 16S rRNA gene sequence similarity values of 99.24–99.7%

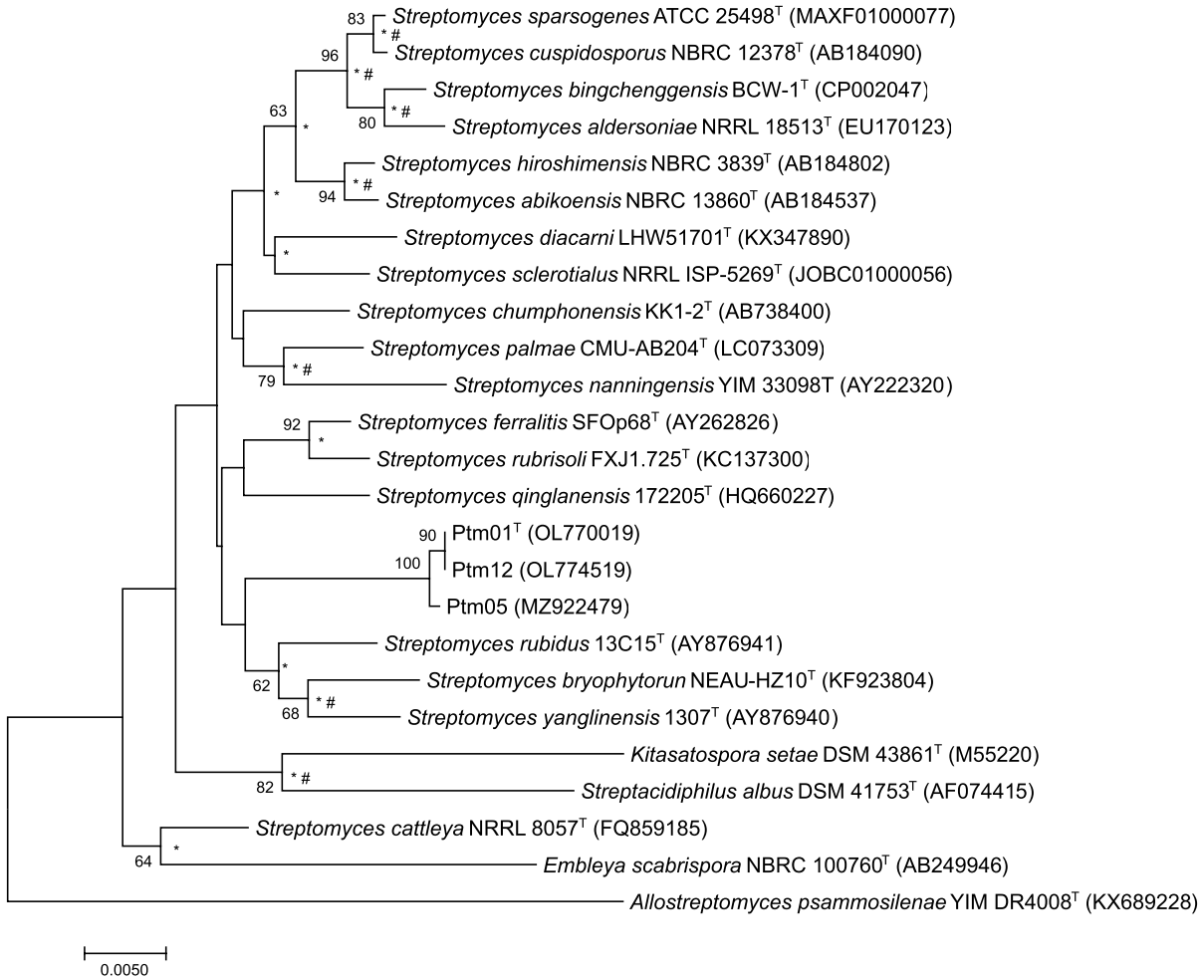

Fig. 1 Neighbour-joining tree based on 16S rRNA gene sequences showing the relationships between strains Ptm05^T, Ptm01, Ptm12 and related type strains of the genus *Streptomyces*. Type species of the family *Streptomycetaceae* were used as the out group. The numbers at branch nodes indicate bootstrap percentages derived from 1000 replications. Asterisk (*) and sharp (#) represented the node that recovered in the ML and MP, respectively. Bar, 0.0050 substitutions per nucleotide position

with the phylotype NSKS_s strain ICBB 8177 (NSKH01000023). When compared with only validly published species, these strains showed the highest 16S rRNA gene sequence similarity with *Streptomyces sparsogenes* ATCC 25498^T with the value of 97.44–97.72%. The phylogenetic based on 16S rRNA gene sequences exhibited that strain Ptm05^T, Ptm01 and Ptm12 shared relative with *Streptomyces rubidus* JCM 13277^T, *Streptomyces bryophytorum* DSM 42138^T, and *Streptomyces yanglinensis* JCM 13275^T (Fig. 1). The strain Ptm05^T was selected for the representative type strain among these three isolates. The assembled genome of strain Ptm05^T had 83 contigs, with of 7,401,816 bp with G + C content of 72.13 mol%. The Ptm05^T genome has 6,801 protein-coding sequences (CDS), 56 transfer RNA genes and 3 ribosomal RNA genes. The phylogenomic tree showed that strain Ptm05^T shared the same node with *S. rubidus* CGMCC 4.2026^T and *S. yanglinensis* CGMCC 4.2023^T (Fig. S2). Based on phylogenetic trees and BLAST results of the 16S rRNA gene, *S. sparsogenes* ATCC 25498^T, *S. rubidus* JCM 13277^T, *S. bryophytorum* DSM 42138^T, and *S. yanglinensis* JCM 13275^T were selected for further taxonomic comparison.

The dDNA-DNA hybridization values among strain Ptm05^T and its closely related *Streptomyces* type strains including, *S. rubidus* CGMCC 4.2026^T, *S. bryophytorum* DSM 42138^T, *S. yanglinensis* CGMCC 4.2023^T, and *S. sparsogenes* DSM 40356^T, were 17.2–18.0%. This value is lower than the 70% cut-off level for assigning strains to the same species (Wayne, 1987). In addition, the ANIb and ANIm between strain Ptm05^T and related *Streptomyces* type strains (*S. rubidus* CGMCC 4.2026 ^{T}, *S. bryophytorum* DSM 42138^T, *S. yanglinensis* CGMCC 4.2023^T, and *S. sparsogenes* DSM 40356^T) were ranged from 75.69 to 76.13% and 85.21 to 85.35%, respectively. These values were lower than the threshold cut-off value 95–96% (Goris et al. 2007) (Supplementary Table S3). Based on both dDDH and ANI, strain Ptm05^T represents the novel species of the genus *Streptomyces* for which the name *Streptomyces parmotrematis* sp. nov. is proposed.

When compared with the phylotype NSKS_s strain ICBB 8177, strain Ptm05^T showed ANIb, ANIm and dDDH values of 92.63, 93.25 and 65.2%, respectively. This confirmed that the genome of strain Ptm05^T different from this phylotype. The genome of strain Ptm05^T contained several secondary metabolites biosynthetic gene clusters (BGC) including terpene, polyketide, lanthipeptide, non-ribosomal peptide (NRP) and non-alpha poly-amino acids like e-Polylysin (NAPAA) (Supplementary Table S4). Among these BGCs, three of them showed 100% similarity to geosmin, ectoine, limazepine and 2-methylisoborneol.

Chemotaxonomy

Strain Ptm05^T showed chemotaxonomic properties similar to the *Streptomyces* genus. It contained LL-diaminopimelic acid in the cell-wall peptidoglycan. The strain had no diagnostic sugars. Only glucose and ribose were detected in the whole-cell hydrolysate. Based on this data, strain Ptm05^T had cell wall type I according to Lechevalier & Lechevalier (1971). The menaquinones of strain Ptm05^T were MK-9(H_8) (82.3%) and MK-9(H_6) (17.7%). The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, one unidentified phospholipid, and one unidentified lipid and unidentified glycolipid (Supplementary Fig. S3). Based on the presence of only one nitrogenous phospholipid, phosphatidylethanolamine, the phospholipid type PII was characterized for the strain (Lechevalier et al. 1977). The predominant cellular fatty acids (> 10%) were anteiso–$C_{15:0}$ (20.0%), iso–$C_{15:0}$ (18.5%), iso–$C_{16:0}$ (16.4%), iso–$C_{17:0}$ (11.4%), $C_{16:0}$ (10.2%). The minor cellular fatty acids were iso–$C_{13:0}$ (0.3%), anteiso–$C_{13:0}$ (0.2%), iso–$C_{14:0}$ (1.6%), $C_{14:0}$ (0.6%), iso G–$C_{13:0}$ (0.1%), $C_{15:1}$ ω8c (0.2%), $C_{15:1}$ ω6c (0.5%), iso H-$C_{16:1}$ (0.3%), anteiso-$C_{17:1}$ ω9c (0.2%), $C_{17:0}$ (0.9%), iso-$C_{18:0}$ (0.4%), $C_{18:1}$ ω9c (0.2%), $C_{18:0}$ (0.3%), 3OH iso-$C_{17:0}$ (0.1%), 3OH-$C_{17:0}$ (0.1%) and 2OH-$C_{18:1}$ (0.3%).

Phenotypic properties

Strain Ptm05^T, Ptm01 and Ptm12 could grow on all media used in this study. The aerial mycelia were observed on ISP 2 and ISP 3 media. The aerial mycelia were white and light greenish yellow. The substrate mycelia were pale greenish yellow. The detail of the cultural characteristics was summarized in Supplementary Table S2. The pale greenish yellow pigment was observed on ISP 2. These strains produced the spiral type of spore chain on the aerial mycelia (Fig. 2). The spores were cylindrical in shape and had smooth surfaces. Strain Ptm05^T, Ptm01 and Ptm12 grew well on the media containing 0–4% NaCl. Poor growth was observed at the presence of 5–7% NaCl. No growth was observed at 8% of NaCl. The pH range for growth was 4.0–9.0 with the optimum growth pH of 6. The optimum growth temperature was 37°C.

Strain Ptm05^T, Ptm01 and Ptm12 showed similar biochemical test and other phenotypes. However, the phenotypic characters could be used for distinguishing these strains from its closely related *Streptomyces* type strains. The strains showed negative result for the starch hydrolysis, but other type strains showed the positive result. All type strains used in this study tolerated the sodium chloride up to 2% (w/v), however strain Ptm05^T, Ptm01 and Ptm12 could grow at 7% of sodium chloride. The liquefy of gelatin and nitrate reduction could be distinguished strains Ptm05^T, Ptm01 and Ptm12 from *S. rubidus* JCM 13277^T and *S. yanglinensis* JCM 13275^T. In addition, the utilization of D-mannitol, creatine, *myo*-inositol, glycerol, D-mannose, D-maltose, D-galactose, L-arabinose, L-valine, L-proline, L-tyrosine, and ammonium sulfate could also be used for distinguished the

strains from their closely related *Streptomyces* species (Table 2).

Fig. 2 Scanning electron micrograph of strain Ptm05^T grew on ISP 3 at 30 °C for 14 days. Bar, 1 µm

Discussion and conclusion

Based on the culture-dependent technique used in this study, the actinobacteria could be isolated from both humic acid vitamin agar and starch casein nitrate agar. The ratio of *Streptomyces* and the rare actinobacteria obtained from these media was not significantly different. It should be noted that no actinobacteria were obtained from all *Usnea* samples. This might be because both isolation media may not be suitable for isolating the actinobacteria associated with *Usnea* lichens. Another reason is the *actinobacteria* may not be associated with this lichen. To answer this point, the bacterial microbiome of the *Usnea* lichen should be determined in future study. The bacterial communities associated with lichen symbiosis have been reported for a decade. However, the understanding of the role of these bacterial associations is still limited. In 2009, Grube et al. studied bacterial communities in three lichen species including *Cladonia arbuscula, Lecanora polytropa* and *Umbilicaria cylindrica* and found that the predominant bacterial group was *Alphaproteobacteria*. Later, Bates et al (2011) reported that each lichen species harbored a distinct bacterial

Table 2 Differential characteristics of strain Ptm05^T and closely related type strains

Characteristics	Strain						
	1	2	3	4	5	6	7*
Strach hydrolysis	−	−	−	+	+	+	w
Maximum NaCl tolerance (%, w/v)	7%	7%	7%	2%	2%	2%	nd
Gelatin liquefaction	−	−	−	+	−	−	w
Nitrate reduction	+	+	+	−	−	+	−
pH ranges	4–9	4–9	4–9	5–10	6–11	5–7	6–8
Carbon source utilization:							
L-Arabinose	−	−	−	+	−	+	+
D-Mannitol	−	−	−	+	−	−	+
Creatine	−	−	−	+	+	+	nd
myo-inositol	w	w	w	−	−	−	−
Glycerol	+	+	+	−	−	−	nd
D-Mannose	+	+	+	+	w	−	−
D-Maltose	w	w	w	w	−	−	nd
D-Galactose	+	+	+	+	−	−	−
Nitrogen source utilization:							
L-Valine	w	w	w	+	+	+	−
L-Proline	−	−	−	+	+	+	+
L-Tyrosine	+	+	+	−	+	w	nd
Ammonium sulfate	+	+	+	w	−	w	nd

Strains 1, Ptm05^T; 2, Ptm01; 3, Ptm12; 4, *S. rubidus* JCM 13277^T; 5, *S. bryophytorum* DSM 42138^T; 6, *S. yanglinensis* JCM 13275^T; 7, *S. sparsogenes* NRRL 2940^T, +, positive; w, weakly positive; −, negative; nd, no data

All data were obtained from this study except * were obtained from Ping et al. (2004) and Goodfellow et al. (2007)

community but dominated by *Alphaproteobacteria* including *Rhizobiales* and nitrogen fixers such as *Azospirillum*, *Brandyrhizobium* and *Frankia*. In 2020, Sierra et al. used the 16S rRNA gene amplicon sequencing to study bacterial communities in seven lichens genera. They found that the lichen microbiome varied in the diversity indexes and number of OTUs. However, the predominant bacterial phyla were *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Proteobacteria*, and *Verrucomicrobia*. Based on the study of Sierra et al. (2021), Actinobacteria is one of the bacterial communities found in the lichens. There are several novel actinobacteria species isolated from lichen samples in the past five years. These included *Actinoplanes lichenis*, *Actinoplanes lichenicola*, *Actinoplanes ovalisporus* and *Streptomyces lichenis* (Phongsopitanun et al. 2016; Saeng-In et al. 2018, 2021). However, the role of actinobacteria associated with the lichen is still unknown and need more investigation. According to Kim et al. (2014), the 98.65% of 16S rRNA gene sequence similarity value can be used as the threshold for differentiating two species. In this study, seven isolates showed 16S rRNA gene similarity lower than 98.65%. Based on this study, lichen samples should be a new promising source of novel actinobacterial taxa. For both phenotypic and genomic evidence, strain Ptm05^T represents a novel species of the genus *Streptomyces*, for which the name *Streptomyces parmotrematis* sp. nov. is proposed.

Description of *Streptomyces parmotrematis* sp. nov.

Streptomyces parmotrematis (par.mo.tre.ma´tis. N.L. gen. n. *parmotrematis,* of *Parmotrema*, referring to the isolation of the type strain from the lichen *Parmotrema* sp.)

An aerobic, Gram positive actinomycetes, produce spiral chains of spores with smooth surfaces. White and gray aerial mycelia are formed on ISP 2 and ISP 3. The aerial mycelia colour was white and light greenish yellow. The colony colour and substrate mycelia is pale greenish yellow. Pale greenish yellow pigment is observed on ISP 2 agar. Grows well on ISP 2 and ISP 3. Moderate growth is observed on ISP 6, ISP 7 and NA and poor growth is observed on ISP 4 and ISP 5. Tolerates up to 7% of sodium chloride (w/v). pH range for growth is 4.0–9.0 with the growth optimum of pH 6. The optimum growth temperature was 37°C . No growth was observed at 45 °C. Nitrate is reduced to nitrite. Starch hydrolysis, gelatin liquefaction, skim milk peptonization, and skim milk coagulation are negative. Utilizes, D-fructose, glycerol, D-xylose, D-mannose, D-galactose, and D-glucose, weakly utilizes on D-maltose and *myo*-inositol but does not utilize creatine, D-mannitol, D-cellobiose, lactose, D-melezitose, L-rhamnose, sucrose, D-raffinose, and D-melibiose. Utilizes L-tyrosine and ammonium sulfate, weakly utilizes L-asparagine, L-cysteine, L-arginine, and L-valine, but does not utilizes L-proline and L-arabinose, as a sole nitrogen source. Positive results of enzymatic activity are observed for leucine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, *N*-acetyl-*β*-*D*-glucosaminidase, alkaline phosphatase, esterase (C4), valine arylamidase, *α*-mannosidase, cystine arylamidase and *α*-chymotrypsin, esterase lipase (C8) and α-glucosidase. Lipase (C14), *α*-galactosidase, *β*-galactosidase, *β*-glucuronidase, *β*-glucosidase, and *α*-fucosidase are negative.

The cell-wall peptidoglycan contains LL-diaminopimelic acid. Sugars in the whole-cell hydrolysates are glucose, and ribose. The polar lipids are phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, one unidentified phospholipid, and one unidentified lipid and one unidentified glycolipid. The menaquinones are MK-9(H_8) and MK-9(H_6). The predominant cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{17:0}$ and $C_{16:0}$.

The type strain, Ptm05^T (=TBRC 14546^T=NBRC 115203^T), was isolated from the lichen sample, *Parmatrema tinctorum* collected from Doi Suthep-Pui National Park, Chiang Mai Province, Thailand. The G+C content of the type strain is 72.13 mol%. The GenBank accession numbers for the 16S rRNA gene sequence and the draft genome sequence of the type strain are MZ922479 and PRJNA758018, respectively.

Acknowledgements We thank the Pharmaceutical Research Instrument Center, Faculty of Pharmaceutical Sciences, Chulalongkorn University, for providing research facilities. We thank Professor Aharon Oren for the etymology of the strain.

Author's contribution AS carried out the experiment including isolation of the actinobacteria, sequencing the 16S rRNA gene sequences and the polyphasic taxonomy of the novel strain. VP and KB collected, preserved and identified the lichen samples. CS and PS analysed the chemotaxonomic study. ST provide the conceptual advice. WP decided the study, proofread the manuscript and provided the conceptual advice.

Funding This project was funded by the National Research Council of Thailand.

Declarations

Conflicts of interest The authors declare that there are no conflicts of interest.

References

Arai T (1975) Culture media for actinomycetes. The Society for Actinomycetes Japan, Tokyo

Bates ST, Cropsey GW, Caporaso JG, Knight R, Fierer N (2011) Bacterial communities associated with the lichen symbiosis. Appl Environ Microbiol 77:1309–1314

Bérdy J (2005) Bioactive microbial metabolites. J Antibiot 58:1–26

Collins M, Pirouz T, Goodfellow M, Minnikin D (1977) Distribution of menaquinones in actinomycetes and corynebacteria. Microbiology 100:221–230

Davis JJ, Wattam AR, Aziz RK, Brettin T, Butler R et al (2020) The PATRIC bioinformatics resource center: expanding data and analysis capabilities. Nucleic Acids Res 48:D606–D612

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Goodfellow M, Kumar Y, Labeda DP, Sembiring L (2007) The *Streptomyces violaceusniger* clade: a home for Streptomycetes with rugose ornamented spores. Antonie Van Leeuwenhoek 92:173–199

Gordon RE, Barnett DA, Handerhan JE, Pang CHN (1974) *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. Int J Syst Bacteriol 24:54–63

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P et al (2007) DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol 57:81–91

Grube M, Cardinale M, de Castro JV, Berg MHG (2009) Species-specific structural and functional diversity of bacterial communities in lichen symbioses. ISME J 3:1105–1115

Hayakawa M, Nonomura H (1987) Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. J Ferment Technol 65:501–509

Itoh T, Kudo T, Parenti F, Seino A (1989) Amended description of the genus *Kineosporia*, based on chemotaxonomic and morphological studies. Int J Syst Bacteriol 39:168–173

Kell K (1964) Color-name charts illustrated with centroid colors. Inter-Society Color Council-National Bureau of Standards, Chicago

Kim M, Oh HS, Park SC, Chun J (2014) Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351

Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 35:1547–1549

Lane D (1991) 16S/23S rRNA sequencing nucleic acid techniques in bacterial systematics. Wiley, London, Chichester, pp 115–175

Lechevalier HA, Lechevalier MP, Gerber NN (1971) Chemical composition as a criterion in the classification of actinomycetes. Adv Appl Microbiol 14:47–72

Lechevalier MP, DeBiévre C, Lechevalier HA (1977) Chemotaxonomy of aerobic actinomycetes: phospholipid composition. Biochem Syst Ecol 5:249–260

Medema MH, Blin K, Cimermancic P, de Jager V, Zakrzewski P, Fischbach MA, Weber T, Takano E, Breitling R (2011) antiSMASH: rapid identification, annotation and analysis of secondary metabolite biosynthesis gene clusters in bacterial and fungal genome sequences. Res Spec Publ 39:W339–W346

Meier-Kolthoff JP, Göker M (2019) TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nat Commun 10:2182

Minnikin D, O'donnell A, Goodfellow M, Alderson G, Athalye M, Schaal A, Parlett J, (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M (2020) List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. Int J Syst Evol Microbiol 70:5607–5612

Phongsopitanun W, Matsumoto A, Inahashi Y, Kudo T, Mori M, Shiomi K, Takahashi Y, Tanasupawat S (2016) *Actinoplanes lichenis* sp. nov., isolated from lichen. Int J Syst Evol Microbiol 66:468–473

Ping X, Takahashi Y, Seino A, Iwai Y, Ōmura S (2004) *Streptomyces scabrisporus* sp. nov. Int J Syst Evol Microbiol 54:577–581

Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J (2016) JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. Bioinformatics 32:929–931

Saeng-In P, Phongsopitanun W, Savarajara A, Tanasupawat S (2018) *Streptomyces lichenis* sp. nov., isolated from lichen. Int J Syst Evol Microbiol 68:3641–3646

Saeng-In P, Kanchanasin P, Yuki M, Kudo T, Ohkuma M, Phongsopitanun W, Tanasupawat S (2021) *Actinoplanes lichenicola* sp. nov. and *Actinoplanes ovalisporus* sp. Nov., isolated from lichen in Thailand. Int J Syst Evol Microbiol. https://doi.org/10.1099/ijsem.0.004921

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids, MIDI technical note 101. Newark, DE: MIDI inc.

Shirling ET, Gottlieb D (1966) Methods for characterization of *Streptomyces* species. Int J Syst Bcteriol 16:313–340

Sierra MA, Danko DC, Sandoval TA, Pishchany G, Moncada B, Kolter R, Mason CE, Zambrano MM (2020) The microbiomes of seven lichen genera reveal host specificity, a reduced core community and potential as source of antimicrobials. Front Microbiol 11:398. https://doi.org/10.1099/ijsem.0.004921

Sripreechasak P, Athipornchai A (2019) Potential antimicrobial properties of *Streptomyces* isolated from Sammuk Mountain soil, Chonburi Province, Thailand. J Adv Pharm Technol 10:195–199

Staneck JL, Roberts GD (1974) Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl Microbiol 28:226–231

Suriyachadkun C, Chunhametha S, Thawai C, Tamura T, Potacharoen W, Kirtikara K, Sanglier JJ (2009) *Planotetraspora thailandica* sp. nov., isolated from soil in Thailand. Int J Syst Evol Microbiol 59:992–997

Waksman SA, Henrici AT (1943) The nomenclature and classification of the actinomycetes. J Bacteriol 46:337–341

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kadler O et al (1987) International committee on systematic bacteriology. report of the *ad hoc* committee on the reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Wick RR, Judd LM, Gorrie CL, Holt KE (2017) Unicycler: Resolving bacterialgenome assemblies from short and long sequencing reads. PLOS Comput Biol 13:e1005595

Williams S, Cross T (1971) Chapter XI actinomycetes. Method Microbiol, Academic Press 4:295–334

Yoon SH, Ha SM, Kwon S, Lim J, Kim Y, Seo H, Chun J (2017) Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int J Syst Evol Microbiol 67:1613–1617