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# *Micromonospora solifontis* sp. nov., an actinobacterium isolated from hot spring soil

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**Abstract**

An actinobacterium strain, PPF5-17$^T$, was isolated from hot spring soil collected from Chiang Rai province, Thailand. The strain exhibited morphological and chemotaxonomic properties similar to those of members of the genus *Micromonospora*. Colonies of PPF5-17$^T$ were strong pinkish red and turned black after sporulation in ISP 2 agar medium. Cells formed single spores directly on the substrate mycelium. Growth was observed from 15 to 45 °C and at pH 5–8. Maximum NaCl concentration for growth was 3% (w/v). PPF5-17$^T$ was found to have *meso*-diaminopimelic acid, xylose, mannose and glucose in the whole-cell hydrolysate. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol and phosphatidylinositolmannosides were observed as the membrane phospholipids. MK-10($H_6$), MK-9($H_6$), MK-10($H_4$) and MK-9($H_4$) were the major menaquinones. The predominant cellular fatty acids were iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. PPF5-17$^T$ shared the highest 16S rRNA gene sequence similarity with *Micromonospora fluminis* LMG 30467$^T$ (99.3%). A genome-based taxonomic study revealed that PPF5-17$^T$ was closely related to *Micromonospora aurantinigra* DSM 44815$^T$ in the phylogenomic tree with an average nucleotide identity by BLAST (ANIb) of 87.7% and a digital DNA–DNA hybridization (dDDH) value of, 36.1% which were below the threshold values for delineation of a novel species. Moreover, PPF5-17$^T$ could be distinguished from its closest neighbours, *M. fluminis* LMG 30467$^T$ and *M. aurantinigra* DSM 44815$^T$, with respect to a broad range of phenotypic properties. Thus, PPF5-17$^T$ represents a novel species, for which the name *Micromonospora solifontis* sp. nov. is proposed. The type strain is PPF5-17$^T$ (= TBRC 8478$^T$ = NBRC 113441$^T$).

The genus *Micromonospora* is one of the most prominent lineages in the family *Micromonosporaceae* [1]. This genus was first described by Ørskov [2] for Gram-positive bacteria that produce single spores borne directly on substrate hyphae. Recently whole-genome sequencing (WGS) technologies have been developed to clarify the taxonomic position of prokaryotes, including actinomycetes. This generates crucial taxonomic information, such as digital DNA–DNA hybridization (dDDH, <70%) [3], average nucleotide identity (ANI, <95–96%) [4–6], average amino acid identity (AAI, <95–96%) [7, 8] and tetranucleotide

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**Keywords:** Actinomycete; hot spring soil; *Micromonospora*; whole-genome sequence analysis; 16S rRNA gene.

**Abbreviations:** AAI, average amino-acid identity; ANIb, average nucleotide identity-BLAST; ANIm, average nucleotide identity-MUMmer algorithm; DAP, diaminopimelic acid; dDDH, digital DNA–DNA hybridization; DPG, diphosphatidylglycerol; DPG, diphosphatidylglycerol; GBDP, genome blast distance phylogeny; GC, gas chromatography; GGDC, Genome to Genome Distance Calculator; GL, glycolic acid; HPLC, high performance liquid chromatography; ISCC-NBS, Inter-Society Color Council-the National Bureau of Standards; L, unidentified lipid; MK, menaquinone; ML, maximum-likelihood; NJ, neighbor-joining; NPL, ninhydrin-positive lipid; NRPS, nonribosomal peptide synthases; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PKS, polyketide synthase; PL, phosphatidylinositol; PLs, unidentified phospholipids; TLC, thin layer chromatography; TYGS, Type Strain Genome Server.

The DDBJ accession number for the 16S rRNA gene sequence of strain PPF5-17$^T$ is LC383890. The draft genome of the strain has been deposited at DDBJ/ENA/GenBank under the accessionJAAHBY000000000.Clean sequence reads have been deposited in the Sequence Read Archive (SRA) database under the accession number SRR22860063.

Five supplementary figures and six supplementary tables are available with the online version of this article.

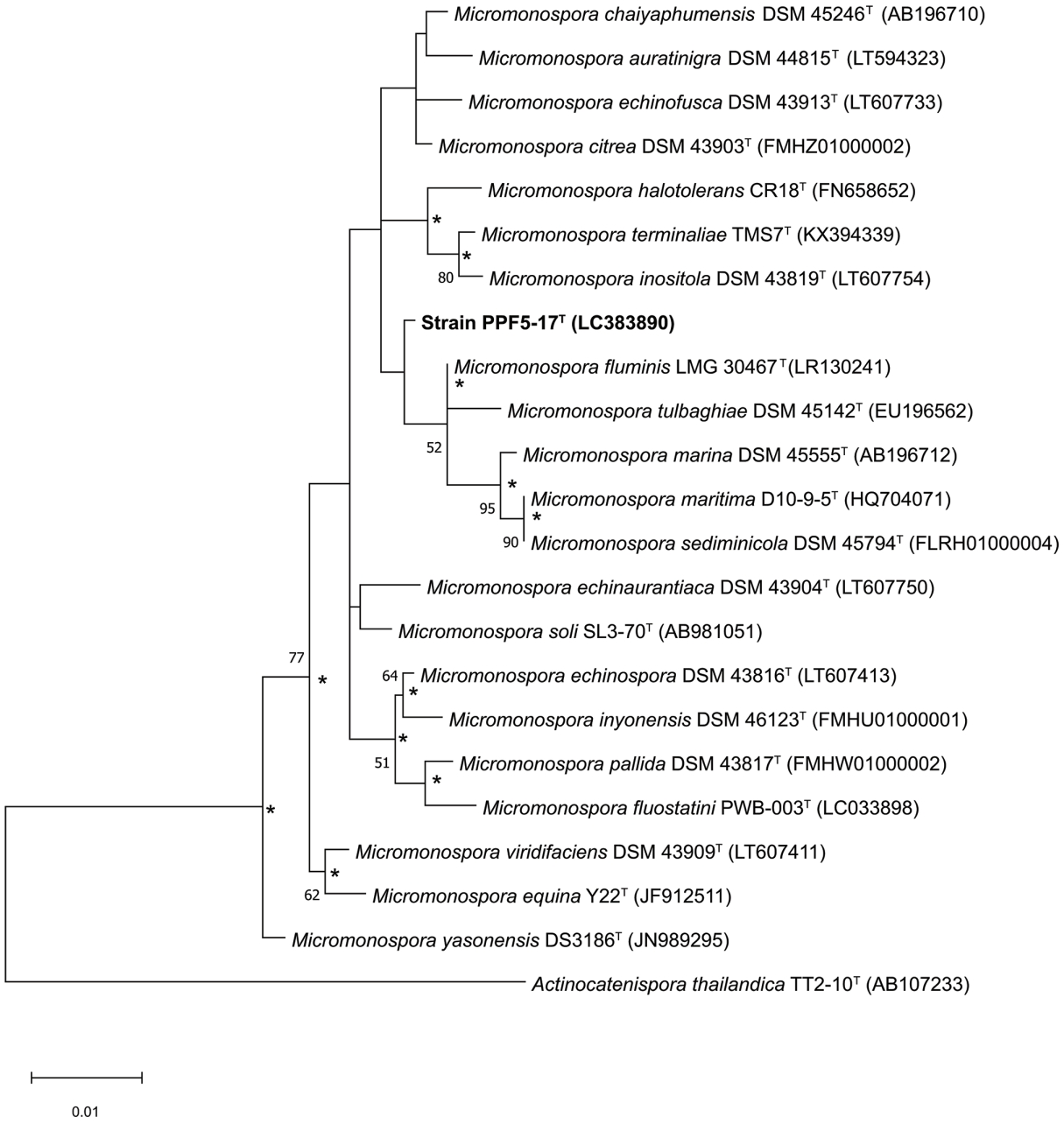


**Fig. 1.** Maximum-likelihood tree based on 16S rRNA gene sequences showing the relationship between PPF5-17$^T$ and related taxa. Asterisks (*) indicates the branches of the tree that were also found using the neighbor-joining and maximum-parsimony methods. The numbers on the branches indicate the percentage bootstrap values of 1000 replicates; only values of 50% or more are shown. Bar, 0.01 substitutions per nucleotide position.

signature correlation index (Tetra, <0.989) [9], that are used as taxonomic criteria for species discrimination. As the result of genome-based taxonomy, the members of the genera *Verrucosispora*, *Xiangella* and *Jishengella* have been transferred to the genus *Micromonospora* [10–12]. At the time of writing, the genus *Micromonospora* contains 113 species with validly published names that have been recorded [13]. Generally, micromonosporae have been isolated from terrestrial soil, water and marine sediment [1]. It is known that extreme environments, such as hot springs, are attractive places for investigating novel species of microorganisms. At present, only *Micromonospora caldifontis* has been reported as a novel species discovered in a hot spring pond [14]. During a continuing programme investigating novel actinomycetes from the extreme environment, a *Micromonospora*-like strain, designated strain PPF5-17, was successfully isolated, and polyphasic taxonomic approaches were used to characterize the taxonomic position of the strain.

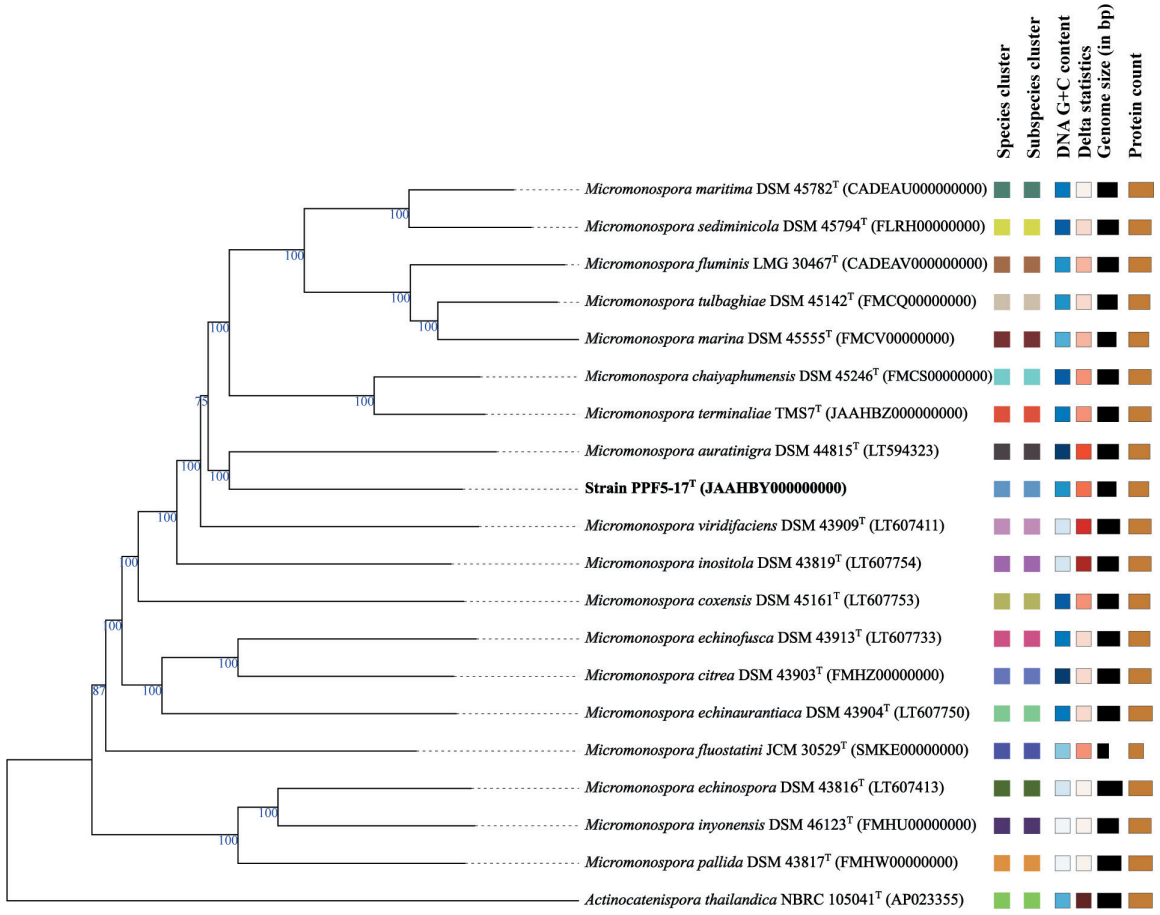


**Fig. 2.** Phylogenomic tree of PPF5-17$^T$ and the related type strains of species of of the genus *Micromonospora* obtained from TYGS. Tree inferred with FastME 2.1.6.1 [53] from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula $d_5$. The numbers above branches are GBDP pseudo-bootstrap support values exceeding 60% from 100 replications, with average branch support of 96.1%. The tree was rooted at the midpoint [54]. Leaf labels are annotated by affiliation to species and subspecies clusters, DNA G+C content, Delta statistic values, overall genome sequence length and the number of proteins [29].

## ISOLATION AND ECOLOGY

PPF5-17$^T$ was isolated from a soil sample collected from a hot spring pond (42.8 °C, pH 6.2, sampling date: 19 November 2017) (GPS location: 19°58′36″ N 99° 50′00″ E), Chiang Rai province, Thailand. The soil sample was air-dried for 7 days. Ten grams of dried soil was added to 0.01% tween 80 solution (90 ml) and was pretreated by heating at 70 °C for 10 min in the water bath. Then the soil solution was serially diluted to $10^{-3}$ with sterile normal saline. Then an aliquot of 0.1 ml of the $10^{-3}$ solution was spread onto modified Zhang's starch soil extract (ZSSE) medium [15] (5 g soluble starch, 1 g $KNO_3$, 15 g agar, 1000 ml soil extract solution, pH 7.1) supplemented with nystatin (100 mg $l^{-1}$) and nalidixic acid (50 mg $l^{-1}$). After incubation at 40 °C for 21 days, a dark red colony of strain PPF5-17$^T$ was picked and immediately purified on yeast extract–malt extract agar (International Streptomyces Project, ISP 2 medium) [16]. The working culture of PPF5-17$^T$ was maintained on an ISP 2 agar slant, and another was kept at 4 °C as a stock culture. For long-term preservation, PPF5-17$^T$ was preserved using the freeze-drying technique in 10% skim milk.

## 16S rRNA GENE PHYLOGENY

Cells grown in ISP 2 broth at 40 °C for 5 days were lysed and the genomic DNA was extracted using the protocol suggested by Tamaoka [17]. The 16S rRNA gene amplification and sequencing were performed as previously described by Nakajima *et al.* [18]. A BLAST search using EzBioCloud server [19] was used for comparing the 16S rRNA gene similarities between the strain and the most closely related type strains. The taxonomic positions in 16S rRNA gene trees of PPF5-17$^T$ and its closely related type strains were preliminarily reconstructed using the standard tree-making algorithms, neighbor-joining (NJ) [20], maximum-parsimony (MP) [21] and maximum-likelihood (ML) [22] in MEGA X [23]. The confidence values of nodes were evaluated using 1000 replications of the bootstrap resampling method [24].

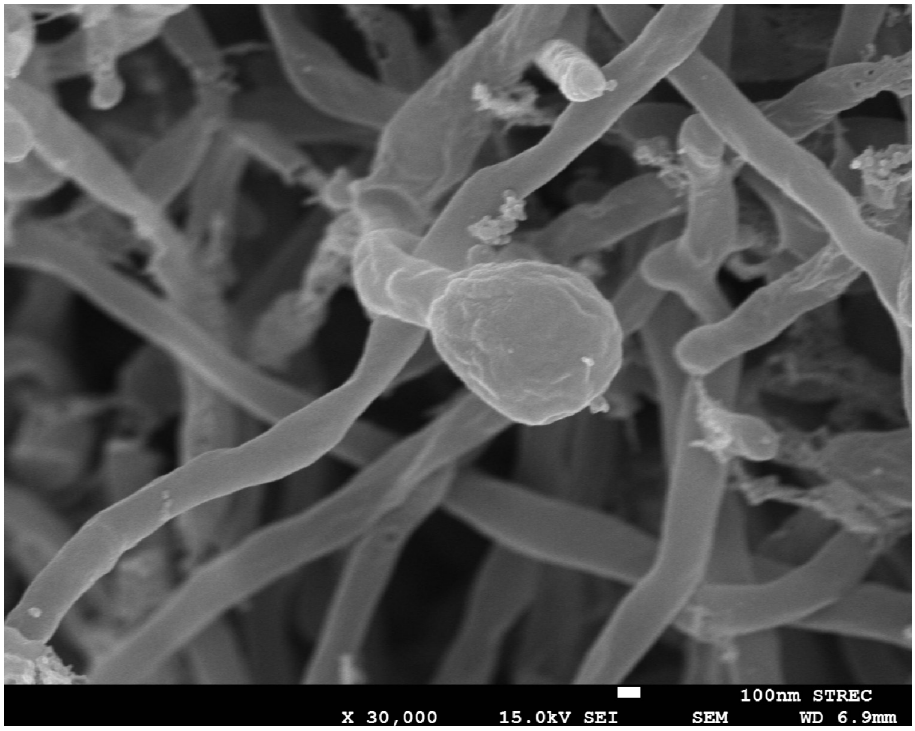


**Fig. 3.** Scanning electron micrograph showing the single spore on a substrate mycelium of strain PPF5-17$^T$ grown on ISP 2 medium at 40 °C for 21 days. Bar, 100 nm.

The results of 16S rRNA gene sequence analysis indicated that *Micromonospora fluminis* LMG 30467$^T$ was the closest relative and shared 99.3% 16S rRNA gene sequence similarity with PPF5-17$^T$, followed by *Micromonospora terminaliae* TMS7$^T$ (99.2%) and *Micromonospora echinaurantiaca* DSM 43904$^T$ (99.1%). A phylogenetic analysis of PPF5-17$^T$ and the closely related species of the genus *Micromonospora* revealed that PPF5-17$^T$ formed a distinct clade separated from *M. fluminis* LMG 30467$^T$ and other members of the genus *Micromonospora*. The position of PPF5-17$^T$ in the ML tree was similar to that in the NJ tree (Figs 1 and S1, available in the online version of this article) while PPF5-17$^T$ formed a distinct branch in another part of the MP tree (Fig. S2).

## GENOME FEATURES

Genomic DNA for genome sequencing of PPF5-17$^T$ was extracted from 3-day-old cultures grown in ISP 2 broth at 40 °C. The GeneJET Genomic DNA purification Kit (Thermo Scientific) was used for purification. The PCR-free library was prepared using the QIAseq FX DNA Library Kit (Qiagen). A Miseq platform (Illumina) with Reagent Kit V3 (600 cycles; 2×250 bp paired-end reads) was applied for sequencing genome at Chulalongkorn University, Thailand. *De novo* assembly of the genome was done using SPAdes version 3.0 [25]. The average nucleotide identity (ANI), ANI-BLAST (ANIb) and ANI-MUMmer (ANIm), values of the genome of PPF5-17$^T$ and its closest relatives were calculated using the JSpeciesWS web service [26]. Digital DNA–DNA hybridization (dDDH) values were obtained with the Genome-to-Genome Distance Calculator version 3.0 (available at https://ggdc.dsmz.de/ggdc.php) [27]. The average amino acid identity (AAI) value was evaluated using the Kostas Lab AAI calculator [28]. To reconstruct the phylogenetic tree based on the genomes of PPF5-17$^T$ and related taxa, the Type (strain) Genome Server (TYGS) available at https://tygs.dsmz.de/ [29] was used. The biosynthetic gene clusters (BGCs) for secondary metabolite synthesis were predicted using Secondary metabolite Analysis Shell (antiSMASH) version 6.0 [30]. To determine the genes relating to enzyme production, the genome of PPF5-17$^T$ was analysed using BLASTp on the Uniprot database with matrix; blosum62 (https://www.uniprot.org/blast) [31].

The draft genome of PPF5-17$^T$ had 227 contigs, with a total length of 5 908 624 bp with an average DNA G+C content of 73.1% (Table S1). The genome of PPF5-17$^T$ contained 5571 protein-coding sequences (CDS), 50 tRNA genes and 3 rRNA genes. The phylogenomic tree inferred with Genome BLAST Distance Phylogeny (GBDP) indicated the taxonomic position of PPF5-17$^T$ among the members of the genus *Micromonospora* and forming a tight clade with *Micromonospora auratinigra* DSM 44815$^T$ (Fig. 2). In addition, PPF5-17$^T$ showed the highest ANIb (87.7%), ANIm (89.8%), AAI (86.3%) and dDDH (36.1%) values with *M. auratinigra* DSM 44815$^T$. All genome-based taxonomic values were clearly lower than the cut-off criteria for species

**Table 1.** Differential characteristics of PPF5-17$^T$ and closely related type strains

Strains: 1, PPF5-17$^T$; 2, *M. auratinigra* DSM 44815$^T$; 3, *M. fluminis* LMG 30467$^T$. All results were determined during this study. +, Positive; –, negative; w, weakly positive.

| Characteristics | 1 | 2 | 3 |
|---|---|---|---|
| Colour of colonies on ISP 2 (7 days) | Strong pinkish red | Vivid orange | Deep orange |
| Maximum NaCl tolerance [% (w/v)] | 3 | 2 | 3 |
| Temperature range for growth (°C) | 15–45 | 15–40 | 20–45 |
| pH range for growth | 5–8 | 5–10 | 6–9 |
| Milk peptonization | + | w | + |
| Nitrate reduction | + | – | + |
| Carbon source utilisation (1.0% w/v): | | | |
| L-Arabinose | – | + | + |
| D-Fructose | – | + | + |
| Glycerol | – | – | + |
| D-Galactose | – | + | + |
| *myo*-Inositol | – | – | + |
| D-Mannitol | + | – | + |
| L-Rhamnose | + | – | + |
| Sucrose | + | – | + |
| D-Xylose | – | + | + |
| Decomposition [1.0% (w/v)] of: | | | |
| Cellulose | – | – | + |
| L-tyrosine | + | – | – |

delineation, 95–96% for ANI [4], 95–96% for AAI [6] and 70% for dDDH [32] (Table S2). Also, *M. fluminis* LMG 30467$^T$, a closely related type strain according to the results of 16S rRNA gene analysis, displayed high ANIb (85.1%), ANIm (87.8%), AAI (83.0%) and dDDH (30.7%) values to PPF5-17$^T$. It can be clearly concluded from genome-based taxonomic criteria that PPF5-17$^T$ may represent a novel member of the genus *Micromonospora*. We used antiSMASH version 6.0 to determine and compare the putative biosynthetic gene clusters in the genome of PPF5-17$^T$ and closely related type strains of species of the genus *Micromonospora*. It was found that the genomes of all species of the genus *Micromonospora* examined in this study were particularly rich in clusters of type I polyketide synthase (PKS), terpene and nonribosomal peptide synthases (NRPS). In contrast, the beta lactone gene cluster was found to be present only in the genome of strain PPF5-17$^T$ (Fig. S3). Additionally, the genome of PPF5-17$^T$ contained biosynthetic gene clusters which exhibited similarities (>50%) against those for the synthesis of (i) alkyl-*O*-dihydrogeranyl-methoxyhydroquinones, a secondary metabolite produced by *Actinoplanes missouriensis* 431 (this gene cluster was also found in the genomes of *M. fluminis* LMG 30467$^T$ and *M. auratinigra* DSM 44815$^T$) [33]; (ii) neocarzilin A produced by *Streptomyces carzinostaticus*, a potent cytotoxic agent against K562 chronic myelogenous leukaemia cells [34]; and (iii) rakicidin A, a macrocyclic depsipeptide produced by *Micromonospora* sp. M42 [35] (Table S3). Furthermore, the genome of PPF5-17$^T$ contained genes associated with heat adaptation and heat response. It was found to have many genes associated with the production of spermidine synthase and agmatinase which play an important role in microbial cell growth; genes associated with the production of translation elongation factor (*Lep*A), heat shock protein (*Grp*E) and chaperone proteins (*Dna*K and *Dna*J), which have important functions in maintaining protein homeostasis under thermal stress conditions. These genes were also found in *M. auratinigra* DSM 44815$^T$ and *Micromonospora* sp. MH33 [36]. In the case of genes involved in extracellular enzyme production, it was found that the PPF5-17$^T$ genome contained genes that code for alpha-1,4-glucan:maltose-1-phosphate maltosyltransferase, alpha-galactosidase, alpha-glucosidase, cellulose 1,4-beta-cellobiosidase, beta-xylanase, alpha-glucuronidase, pectate lyase, pectinesterase, aminopeptidase N and extracellular small neutral protease (Table S4).

## PHYSIOLOGY AND CHEMOTAXONOMY

Morphological characteristics of PPF5-17$^T$ grown on ISP 2 medium at 40 °C for 30 days were examined by scanning electron microscope (model JSM-6610 LV; JEOL). Samples for scanning electron microscopy were prepared by cutting a block from an agar plate and then fixing it in 1% $OsO_4$ in 0.1 M phosphate buffer (pH 7.2) at room temperature for 4 h. The samples were dehydrated in a graded series of ethanol [30, 50, 70 and 95%, followed by 100% ethanol (10 min) three times] and then were dried with a critical point dryer (model EM CPD300; Leica). The samples were placed onto a stub-bearing adhesive and spatter-coated with gold under a vacuum. The cultural characteristics were tested using 14-day cultures grown at 40 °C on various agar media, International Streptomyces Project (ISP) media (ISP 2–7) [16], Czapek's sucrose agar, glucose–asparagine agar (ISP 5 with 1% glucose replaced with glycerol) and nutrient agar (Difco). The Inter-Society Color Council–National Bureau of Standards (ISCC–NBS) colour charts were used for determining colour designations [37]. The effects of temperature (10, 15, 20, 25, 30, 37, 40, 42, 45, 50 and 55 °C) and different concentrations of NaCl [0–10% (w/v) at increments of 1%] on growth were evaluated on ISP 2 agar after incubation for 14 days. The effect of pH on growth (4.0–12.0 at an increment of 0.5 pH units) was tested by cultivation at 40 °C in ISP 2 broth for 14 days. Carbon utilisation was tested using ISP 9 medium supplemented with a final concentration of 1% (w/v) of the tested carbon sources. Nitrogen utilisation (1%, w/v) was examined using the method of Gordon *et al.* [38]. Hydrolysis of starch, peptonization of milk, nitrite production, decomposition of insoluble compounds, adenine, cellulose, hypoxanthine, L-tyrosine and xanthine and gelatin liquefaction were determined by cultivation on various media as described by Arai [39] and Williams and Cross [40]. Melanin and hydrogen sulphide production were tested on tyrosine agar and peptone iron agar supplemented with 0.1% (w/v) yeast extract, respectively. Catalase and oxidase tests were performed using 3% (v/v) hydrogen peroxide and 1% *N, N, N′,N′*-tetramethyl-*p*-phenylenediamine dihydrochloride solutions, respectively. A motility test was carried out by inoculating the spores of PPF5-17$^T$ into ISP 1 broth. This was then incubated at 40 °C for 30 min and observed under 100× magnification through the microscope to check motility.

The freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in ISP 2 broth on a rotary shaker at 40 °C. After cultivation for 5 days, cells were harvested and washed five times with sterilised distilled water by centrifugation (6500 ***g*** at 4 °C). The cells of PPF5-17$^T$ were dried in a vacuum freeze-dryer. The isomers of diaminopimelic acid (DAP) and reducing sugar in whole-cell hydrolysates were determined according to the methods of Hasegawa *et al.* [41] and Komagata and Suzuki [42], respectively. The acyl group of muramic acid in the peptidoglycan was determined using the protocol described by Uchida and Aida [43]. The phospholipids were extracted and analysed according to the method of Minnikin *et al.* [44] and Collins and Jones [45]. Menaquinones were extracted according to the method of Collins *et al.* [46] and analysed with HPLC equipped with a Cosmosil 5 $_{C18}$ column (4.6 by 150 mm; Nacalai Tesque). A mixture of methanol and 2-propanol (2:1, v/v) was used as the elution solvent. Fatty acid methyl ester analysis was performed by GLC according to the instructions of the Microbial Identification System (MIDI) with the ACTIN version 6 database [47, 48]. Mycolic acids were extracted and analysed by TLC method following the procedure of Minnikin *et al.* [49].

The chemotaxonomic characteristics of PPF5-17$^T$ coincided with those of members of the genus *Micromonospora*. The isomer of DAP of PPF5-17$^T$ was *meso*. The strain showed a positive result on the glycolate test, indicating that the strain has *N*-glycolyl muramic acid in the peptidoglycan. The whole cell hydrolysates contained xylose, mannose and glucose. PPF5-17$^T$ was therefore characterised by cell wall type II and whole-cell sugar pattern D of Lechevalier and Lechevalier [50]. The presence of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI) phosphatidylinositol-mannosides (PIMs), unidentified glycolipid (GL), five unidentified phospholipids (PLs) and unidentified ninhydrin-positive lipid (NPL) (Fig. S4) was observed but not phosphatidylcholine, corresponding to phospholipid type II of Lechevalier *et al.* [51]. The major fatty acids (>10%) of PPF5-17$^T$ were iso-$C_{15:0}$ (25.9%), iso-$C_{17:0}$ (16.8%), anteiso-$C_{17:0}$ (12.9%) and iso-$C_{16:0}$ (11.2%) (Table S5). This pattern corresponds to fatty acid type 3b of Kroppenstedt [52]. Mycolic acids were absent. The major menaquinones were MK-10($H_6$) (28.8%), MK-9($H_6$) (27.4%), MK-10($H_4$) (14.9%) and MK-9($H_4$) (13.8%), while minor amounts of MK-10(H8) (8.5%) and MK-9(H8) (6.1%) were also present.

PPF5-17$^T$ showed morphological characteristics typical of members of the genus *Micromonospora*. It produced a strong pinkish-red, well-developed and branched substrate hyphae on ISP 2 but no aerial hyphae (Fig. S5). The colonies turned black after sporulation. Spores were borne singly on substrate hyphae and were oval, rough on the surface (Fig. 3) and non-motile. The strain grew well on ISP 2 and ISP 3, grew moderately on ISP 6 and poorly on ISP 4, ISP 5, ISP 7, Czapek's sucrose and nutrient agar. No growth was observed on glucose–asparagine agar. A pale yellow diffusible pigment was detected on ISP 2 (Table S6). Other phenotypic characteristics are listed in the species description and in Table 1. Compared with *M. fluminis* LMG 30467$^T$ and *M. auratinigra* DSM 44815$^T$, PPF5-17$^T$ exhibited a strong pinkish-red substrate mycelium when it was cultivated on ISP 2 medium for 7 days, while the colour of substrate mycelium of *M. fluminis* LMG 30467$^T$ and *M. auratinigra* DSM 44815$^T$ was orange. Furthermore, PPF5-17$^T$ was not able to use L-arabinose, D-fructose, glycerol, D-galactose, *myo*-inositol and D-xylose as sole carbon sources, while *M. fluminis* LMG 30467$^T$ did. In the case of the decomposition of insoluble compounds, the inability to decompose cellulose and the ability to decompose L-tyrosine and the ability to reduce nitrate effectively discriminate PPF5-17$^T$ from closely related type strains (Table 1). On the basis of these phenotypic and genome-based taxonomic data, it is suggested that strain PPF5-17$^T$ should be recognised as representing a novel species of the genus *Micromonospora* and we propose the name *Micromonospora solifontis* sp. nov. for the strain.

## DESCRIPTION OF *MICROMONOSPORA SOLIFONTIS* SP. NOV.

*Micromonospora solifontis* (so.li.fon'tis. L. neut. n. *solum*, soil; L. masc. n. *fons*, *fontis*, spring; N.L. gen. n. *solifontis* of a spring soil, referring to the soil as the isolation source)

Cells are aerobic, Gram-stain-positive, mesophilic, non-motile actinomycetes that form well-developed and branched substrate hyphae. Colonies are strong pinkish red in ISP 2, turning to black after sporulation. Single spores are formed on the substrate hyphae. Aerial mycelium is absent. The spores are non-motile, and their surfaces appear rough. Growth is observed from 15 to 45 °C, with optimum growth at 40 °C. Maximum NaCl concentration for growth is 3% (w/v). Growth occurs at pH 5–8, with optimum growth at pH 7. Catalase, starch hydrolysis, gelatin liquefaction, decomposition of L-tyrosine, nitrate reduction and peptonization of milk are positive. Urease production, decomposition of hypoxanthine, adenine, cellulose, xanthine and oxidase activity are negative. Utilises adonitol, glucose, trehalose, D-mannitol, D-mannose, raffinose, L-rhamnose, and sucrose as sole carbon sources, but does not utilise L-arabinose, D-fructose, glycerol, D-galactose, *myo*-inositol, D-ribose, salicin, xylitol, and D-xylose. Weakly utilises L-arginine and L-asparagine as sole nitrogen sources but not DL-2-aminobutyric acid, L-cysteine, L-histidine, 4-hydroxyproline, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine and L-valine. Cell wall peptidoglycan contains *meso*-diaminopimelic acid. Xylose, mannose and glucose are detected as whole-cell sugars. The polar lipid profile contains diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylinositol, phosphatidylinositolmannosides, an unidentified glycolipid, five unidentified phospholipids and an unidentified ninhydrin-positive lipid. The main fatty acids (>10%) are iso-$C_{15:0}$, iso-$C_{17:0}$, anteiso-$C_{17:0}$ and iso-$C_{16:0}$. The major menaquinones are MK-10($H_6$), MK-9($H_6$), MK-10($H_4$) and MK-9($H_4$).

The type strain, PPF5-17$^T$ (= TBRC 8478$^T$ = NBRC 113441$^T$), was isolated from a soil sample collected from a hot spring pond in Chiang Rai province, Thailand. The total genome size of the type strain is about 5.90 Mb, with a DNA G+C content of 73.1%. The GenBank/EMBL/ DDBJ accession number for the 16S rRNA gene sequence of strain PPF5-17$^T$ is LC383890. The whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number JAAHBY000000000.

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

References

1. Genilloud O. Genus *Micromonospora*. In: Goodfellow M, Kämpfer P, Busse HJ, Trujillo ME, Suzuki K, et al. (eds). *Bergey's Manual of Systematic Bacteriology* (*The Actinobacteria vol 2*), vol. 5. New York: Springer; 2012. pp. 1039–1057.
2. Ørskov J. Investigations into the Morphology of the Ray Fungi. Copenhagen: Levin and Munksgaard, 1923.
3. Auch AF, von Jan M, Klenk H-P, Göker M. Digital DNA–DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. *Stand Genomic Sci* 2010;2:117–134.
4. Richter M, Rosselló-Móra R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc Natl Acad Sci* 2009;106:19126–19131.
5. Chun J, Rainey FA. Integrating genomics into the taxonomy and systematics of the *Bacteria* and *Archaea*. *Int J Syst Evol Microbiol* 2014;64:316–324.
6. Konstantinidis KT, Rosselló-Móra R, Amann R. Uncultivated microbes in need of their own taxonomy. *ISME J* 2017;11:2399–2406.
7. Thompson CC, Chimetto L, Edwards RA, Swings J, Stackebrandt E, *et al*. Microbial genomic taxonomy. *BMC Genomics* 2013;14:913.
8. Konstantinidis KT, Tiedje JM. Towards a genome-based taxonomy for prokaryotes. *J Bacteriol* 2005;187:6258–6264.
9. Teeling H, Meyerdierks A, Bauer M, Amann R, Glöckner FO. Application of tetranucleotide frequencies for the assignment of genomic fragments. *Environ Microbiol* 2004;6:938–947.
10. Nouioui I, Carro L, García-López M, Meier-Kolthoff JP, Woyke T, *et al*. Genome-based taxonomic classification of the phylum *Actinobacteria*. *Front Microbiol* 2018;9:2007.
11. Li L, Zhu HR, Xu QH, Lin HW, Lu YH. *Micromonospora craniellae* sp. nov., isolated from a marine sponge, and reclassification of *Jishengella endophytica* as *Micromonospora endophytica* comb. nov. *Int J Syst Evol Microbiol* 2019;69:715–720.
12. Kuncharoen N, Kudo T, Yuki M, Okuma M, Pittayakhajonwut P, *et al*. *Micromonospora radicis* sp. nov., isolated from roots of *Azadirachta indica* var. *siamensis* Valenton, and reclassification of *Jishengella zingiberis* as *Micromonospora zingiberis* comb. nov. *Int J Syst Evol Microbiol* 2019;69:2884–2891.
13. Parte AC, Sardà Carbasse J, Meier-Kolthoff JP, Reimer LC, Göker M. List of Prokaryotic names with Standing in Nomenclature (LPSN) moves to the DSMZ. *Int J Syst Evol Microbiol* 2020;70:5607–5612.
14. Thawai C, Tanasupawat S, Kudo T. *Micromonospora caldifontis* sp. nov., isolated from hot spring soil. *Int J Syst Evol Microbiol* 2019;69:1336–1342.
15. Zhang J, Zhang L. Improvement of an isolation medium for *Actinomycetes*. *MAS* 2011;5:124–127.
16. Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 1966;16:313–340.
17. Tamaoka J. Determination of DNA Base Composition. In: Goodfellow M and O'Donnell AG (eds). *Chemical Methods in Prokaryotic Systematics*. Chichester: John Wiley and Sons; 1994. pp. 463–470.
18. Nakajima Y, Kitpreechavanich V, Suzuki K, Kudo T. *Microbispora corallina* sp. nov., a new species of the genus *Microbispora* isolated from Thai soil. *Int J Syst Bacteriol* 1999;49 Pt 4:1761–1767.

19. Yoon S-H, Ha S-M, Kwon S, Lim J, Kim Y, *et al*. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. *Int J Syst Evol Microbiol* 2017;67:1613–1617.
20. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
21. Fitch WM. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* 1971;20:406.
22. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
23. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Mol Biol Evol* 2018;35:1547–1549.
24. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
25. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
26. Richter M, Rosselló-Móra R, Oliver Glöckner F, Peplies J. JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 2016;32:929–931.
27. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Res* 2022;50:D801–D807.
28. Rodriguez-R LM, Konstantinidis KT. Bypassing cultivation to identify bacterial species. *Microbe Magazine* 2014;9:111–118.
29. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. *Nat Commun* 2019;10:2182.
30. Blin K, Shaw S, Kloosterman AM, Charlop-Powers Z, van Wezel GP, *et al*. antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Res* 2021;49:W29–W35.
31. The UniProt Consortium. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res* 2019;47:D506–D515.
32. Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, *et al*. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81–91.
33. Awakawa T, Fujita N, Hayakawa M, Ohnishi Y, Horinouchi S. Characterization of the biosynthesis gene cluster for alkyl-*O*-dihydrogeranylmethoxyhydroquinones in *Actinoplanes missouriensis*. *Chembiochem* 2011;12:439–448.
34. Otsuka M, Ichinose K, Fujii I, Ebizuka Y. Cloning, sequencing, and functional analysis of an iterative type I polyketide synthase gene cluster for biosynthesis of the antitumor chlorinated polyenone neocarzilin in "*Streptomyces carzinostaticus*." *Antimicrob Agents Chemother* 2004;48:3468–3476.
35. Tsakos M, Clement LL, Schaffert ES, Olsen FN, Rupiani S, *et al*. Total synthesis and biological evaluation of Rakicidin A and discovery of a simplified bioactive analogue. *Angew Chem Int Ed Engl* 2016;55:1030–1035.
36. Schoch CL, Ciufo S, Domrachev M, Hotton CL, Kannan S, *et al*. NCBI Taxonomy: a comprehensive update on curation, resources and tools. *Database* 2020;2020:baaa062.
37. Kelly KL. *Inter-Society Color Council-National Bureau of Standard Color Name Charts Illustrated with Centroid Colors*. Washington, DC: US Government Printing Office; 1964.
38. Gordon RE, Barnett DA, Handerhan JE, Pang CHN. *Nocardia coeliaca*, *Nocardia autotrophica*, and the nocardin strain. *Int J Syst Bacteriol* 1974;24:54–63.
39. Arai T. Culture media for actinomycetes. Tokyo: The Society for Actinomycetes Japan, 1975. pp. 1–131.
40. Williams ST, Cross T. *Actinomycetes*. In: Booth C (eds). *Methods in Microbiology*, vol. 4. London: Academic Press;1971; . pp. 295–334.
41. Hasegawa T, Takizawa M, Tanida S. A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* 1983;29:319–322.
42. Komagata K, Suzuki KI. Lipid and cell-wall analysis in bacterial systematics. *Methods Microbiol* 1987;19:161–207.
43. Uchida K, Aida K. An improved method for the glycolate test for simple identification of the acyl type of bacterial cell walls. *J Gen Appl Microbiol* 1984;30:131–134.
44. Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, *et al*. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol Methods* 1984;2:233–241.
45. Collins MD, Jones D. Lipids in the classification and identification of coryneform bacteria containing peptidoglycans based on 2, 4-diaminobutyric acid. *J Appl Bacteriol* 1980;48:459–470.
46. Collins MD, Pirouz T, Goodfellow M, Minnikin DE. Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100:221–230.
47. Sasser M. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. Newark: Microbial ID, Inc; 1990
48. Kämpfer P, Kroppenstedt RM. Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* 1996;42:989–1005.
49. Minnikin DE, Hutchinson IG, Caldicott AB, Goodfellow M. Thin-layer chromatography of methanolysates of mycolic acid-containing bacteria. *J Chromatog A* 1980;188:221–233.
50. Lechevalier MP, Lechevalier HA. Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* 1970;20:435–443.
51. Lechevalier MP, De Bievre C, Lechevalier H. Chemotaxonomy of aerobic actinomycetes: phospholipid composition. *Biochem Syst Ecol* 1977;5:249–260.
52. Kroppenstedt RM. Fatty acid and menaquinone analysis of actinomycetes and related organisms. In: Goodfellow M and Minnikin DE (eds). *Chemical Methods in Bacterial Systematics*. London: Academic Press; 1985. pp. 173–199.
53. Lefort V, Desper R, Gascuel O. FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. *Mol Biol Evol* 2015;32:2798–2800.
54. Farris JS. Estimating phylogenetic trees from distance matrices. *Am Nat* 1972;106:645–668.