

Gandjariella thermophila gen. nov., sp. nov., a new member of the family *Pseudonocardiaceae*, isolated from forest soil in a geothermal area

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Abstract

Six mycelium-forming actinomycete strains were isolated from forest soil near the Cisolak geysers in West Java, Indonesia. The 16S rRNA gene sequences of these strains showed high similarity to members of genera in the family *Pseudonocardiaceae* with values less than 96.0%, and most closely related to the genus *Thermotunica*, *T. guangxiensis* AG2-7^T (94.6–95.2% similarity). The type strain, designated SL3-2-4^T, was aerobic, thermophilic, Gram-stain-positive that formed branched, non-fragmented substrate mycelia and unbranched aerial mycelia with long-chain, oval-shaped spores on International *Streptomyces* Project (ISP) 3 medium. It produced light-orange substrate mycelia and light-orange diffusible pigments on ISP 3 medium with 2% gellan gum, grown at 30–55°C, with optimum growth at 45°C. The pH range for growth was 4.0–8.0, with optimum growth at pH 7.0. Strain SL3-2-4^T was able to hydrolyze casein, esculin, gelatin, guanine, hypoxanthine, starch, L-tyrosine, and xanthine, but not adenine, carboxymethyl-cellulose, cellulose, chitin, Tween 20, or xylan. The major fatty acid was *iso*-C_{16:0}, and the major menaquinone was MK-8 (H₄). The detected polar lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidyl-*N*-methylethanolamine, unidentified aminophospholipids, unidentified glycolipids, and unidentified phospholipids. The cell wall hydrolysate of SL3-2-4^T contained *meso*-2,4-diaminopimelic acid. The whole cell sugars were arabinose and galactose. The DNA G+C content was 71.6 mol%. Phenotypic features and phylogenetic data differentiated SL3-2-4^T from members of the family *Pseudonocardiaceae*. Therefore, the strain SL3-2-4^T is proposed as a representative of a novel species in a novel genus, *Gandjariella thermophila* gen. nov., sp. nov. The type strain is SL3-2-4^T (=UICC B-83^T=NRRL B-67478^T=InaCC A981^T).

Labeda et al. [1] proposed the elimination of the family *Actinosynnemataceae* and emended the description of the family *Pseudonocardiaceae*, based on 16S rRNA gene sequence analysis, to include the genera formerly found in this family. As a result, Labeda and Goodfellow [2] proposed the order *Pseudonocardiales* ord. nov., which contains a single family, and the description and signature nucleotides of the 16S rRNA gene are that of the family *Pseudonocardiaceae*. The current study reports the findings on a new thermophilic actinomycete genus belonging to the family *Pseudonocardiaceae* based on phenotypic data and the

phylogenetic analysis of 16S rRNA gene sequences. Characterisation of the new genus was also performed by comparing it with the phylogenetically closely related genera *Thermotunica* [3], *Goodfellowiella* [4, 5], *Actinoalloteichus* [6], *Streptoalloteichus* [7], *Crossiella* [8], and *Kutzneria* [9].

In the frame work to investigate the thermophilic actinomycete diversity in an Indonesian geothermal area, we collected the soil sample (0 to 10 cm depth) from forest soil (under bamboo plants) near the Cisolak Geysers, a geothermal area in West Java, Indonesia (lat 6° 57' 4.8" S, long 106° 28' 6.6" E), in September 2015. The sample was placed in a

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Keywords: thermophilic actinomycete; forest soil; geysers; polyphasic taxonomy.

Abbreviations: APL, unidentified aminophospholipid; CM-cellulose, carboxymethyl cellulose; GL, unidentified glycolipid; ISP, International *Streptomyces* Project; MCC, microcrystalline cellulose; *meso*-DAP, *meso*-2,4-diaminopimelic acid; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PL, unidentified phospholipid.

The GenBank/EMBL/DDJB accession for the 16S rRNA gene sequence of six strains (SL3-2-4^T, SL3-2-5, SL3-2-6, SL3-2-7, SL3-2-9, SL3-2-10) are LC203478 and LC469351–LC469355).

Three supplementary figures and five supplementary tables are available with the online version of this article.

plastic bag, transported to the laboratory and stored at 4 °C until use.

During the work of isolation, the strain SL3-2-4^T showed unusual colonies that were observed under a digital microscope (KH-8700; Hirox). Then, its morphological, physiological, biochemical, chemotaxonomic characteristics and 16S rRNA gene sequences was examined to determine its taxonomic position. Later, another five strains (designated strains SL3-2-5, SL3-2-6, SL3-2-7, SL3-2-9, and SL3-2-10) were isolated from the same source as SL3-2-4^T. The result of the 16S rRNA gene sequences indicated that all strains should be placed in a novel species of a new genus belonging to the family *Pseudonocardiaceae*. However, the later five strains (SL3-2-5, SL3-2-6, SL3-2-7, SL3-2-9, and SL3-2-10) have not yet been fully characterised taxonomically. Only the observation of morphological appearance on International *Streptomyces* Project (ISP) 3 [10], with 2 % gellan gum instead of agar, medium and phylogenetic analyses based on 16S rRNA gene sequences have been performed for these five strains.

Isolation of strains was performed using 1 % ISP 1 [10] with 2 % gellan gum, and 2 % gellan gum media at 50 °C. Soil samples were air-dried for a few hours, spread on the media, and incubated at 50 °C for 3 to 4 weeks. The unusual colonies, forming white aerial mycelia on 2 % gellan gum, were observed under a digital microscope (KH-8700; Hirox). The single colonies were isolated and purified on 2 % gellan gum medium several times. The pure isolates were maintained on ISP 3 medium [10] with 2 % gellan gum at 4 °C, in 20 % (v/v) glycerol as a mycelial-fragment suspension at –80 °C, and as lyophilised cells for long-term preservation.

The morphology of cells grown at 45 °C for 5 days on ISP 3 agar was observed using a scanning electron microscope (JSM-6700F; JEOL) as described previously [11]. Cultural characteristics were observed on ISP 1, ISP 2, ISP 3, ISP 4, ISP 5, ISP 6, and ISP 7 media [10], as well as Bennett's agar [12], FS1V medium [13], Sauton's agar [14], Czapek solution agar (Difco), tap-water agar [15], and 2 % gellan gum

medium after 14 days (Table S1) at 45 °C. The Gram-reaction was determined as described by Magee *et al.* [16]. Growth temperatures at 4, 16, 25, 28, 30, 37, 40, 45, 50, 55, 60, and 65 °C; tolerance to NaCl, between 0 % and 2 % (with intervals of 0.5 % w/v); and optimum growth between pH 4.0 and 11.0 (adjusted with HCl and NaOH at room temperature) were tested on modified Bennett's agar at 45 °C and observed after 7 and 14 days of incubation. Catalase and oxidase activities, hydrogen sulfide (H₂S) formation, and hydrolysis of adenine, casein, aesculin, gelatin, guanine, hypoxanthine, starch, Tween 20, L-tyrosine, urea, xanthine, and xylan were evaluated as previously described by Williams *et al.* [17]. The hydrolysis of carboxymethylcellulose (CM-cellulose; 0.5 % w/v), chitin (1 % w/v), and microcrystalline cellulose (MCC; 0.5 % w/v) was observed on modified Bennett's agar at 45 °C after 7 and 14 days incubation by the presence of a clear zone around the colony. Acid fastness was examined using the method described by Gordon *et al.* [15]. The ability to utilise various carbon sources was tested as described by Shirling and Gottlieb [10]; however, ISP 4 medium without a carbon source [3] was used because the SL3-2-4^T strain did not grow on ISP 9 medium. Growth under anaerobic conditions on modified Bennett's agar plates was observed using an anaerobic chamber (Mitsubishi Gas Chemical) at 45 °C after 7 and 14 days incubation.

Strain SL3-2-4^T was Gram-reaction positive, aerobic, and showed optimum growth at 45 °C, and pH 7.0, with <0.5 % NaCl in the medium. The strain grew well on ISP 3 agar, ISP 3 with 2 % gellan gum, Bennett's agar, modified Bennett's agar, Bennett's medium with 2 % gellan gum, ISP 5 with 2 % gellan gum, ISP 7 agar, ISP 7 with 2 % gellan gum, 2 % gellan gum medium, and FS1V with 1.5 % gellan gum. It showed moderate growth on ISP 1 agar, ISP 1 with 2 % gellan gum, and FS1V-agar media. It showed poor growth on 2 % tap water agar, ISP 2 agar, ISP 2 with 2 % gellan gum, ISP 5 agar, ISP 6 agar, and ISP 6 with 2 % gellan gum. It did not grow on Czapek solution agar, ISP 4 medium, or Sauton's agar (Table S1, available in the online version of this article). Unbranched aerial mycelia were white with

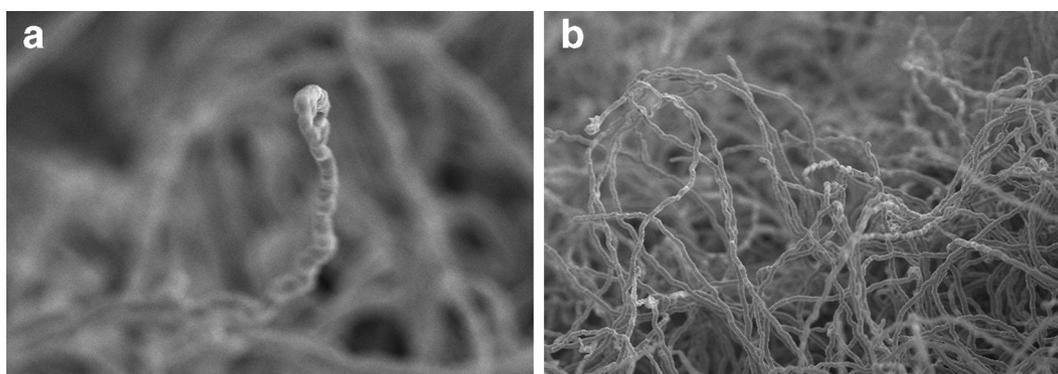


Fig. 1. Scanning electron micrograph of strain SL3-2-4^T showing unbranched aerial mycelia and indicating the formation of long chains of spores after incubation in ISP 3 medium for 5 days at 45 °C. Bars, (a) 5 μm; (b) 10 μm.

indications of long spore chains containing oval-shaped spores, and branched, non-fragmented substrate mycelia were observed on ISP 3 agar (Fig. 1). The strain produced light-orange diffusible pigments on ISP 3 gellan gum medium (ISP 3 medium with 2% gellan gum instead of agar). Detailed physiological and biochemical properties are listed in the species description. A comparison of the phenotypic features of strain SL3-2-4^T with those of closely related genera in the family *Pseudonocardiaceae* is given in Table 1. A comparison of the morphological appearance of the strain SL3-2-4^T and other related strains in ISP 3 gellan gum medium are given in Fig. S1 and Table S2. Unfortunately, one strain, SL3-2-5, could not be recovered for morphological examination. The five strains (SL3-2-4^T, SL3-2-6, SL3-2-7, SL3-2-9, and SL3-2-10) showed different types of colour of substrate mycelia (white, white transparent, venetian red, and grey green) and pigment production (cinnamon, pompeian red, sanguine, or no soluble pigment) after 7 days incubation on ISP 3 gellan gum medium at 45 °C.

Freeze-dried cells were collected for chemotaxonomic analysis from SL3-2-4^T culture grown in baffled flasks of modified Bennett's liquid medium at 45 °C with a shaking frequency of 100 r.p.m. for 5 days. Fatty acid analysis of strain SL3-2-4^T was performed according to the instructions of the Sherlock Microbial Identification System (version 6.0; MIDI, USA) [18] with the TSBA6 database. Cell walls were prepared using a previously described method [19]. Menaquinones were extracted as described by Collins *et al.* [20]; the menaquinone analyses were recorded in positive ion mode on a liquid chromatography-mass spectrometry system (6530 Q-TOF; Agilent) equipped with a 4.6×250 mm packed column (COSMOSIL 5C18-AR-II; Nacalai Tesque, Japan) for atmospheric pressure chemical ionisation. The amino acid composition of complete cell wall hydrolysates was determined by developing preparations on cellulose thin-layer chromatography (TLC) plates (Merck) using two-dimensional descending chromatography [21]. The polar lipids of strain SL3-2-4^T were determined using two-dimensional TLC, as described by Minnikin *et al.* [22, 23]. The DNA G+C content of strain SL3-2-4^T was determined by HPLC according to Tamaoka and Komagata's [24] method. The whole cell sugars were analysed using TLC, as described by Hasegawa *et al.* [25].

The cell wall hydrolysate of strain SL3-2-4^T contained meso-diaminopimelic acid (*meso*-DAP) as the diagnostic diamino acid, and the whole cell sugars were galactose and arabinose as diagnostic sugars. According to Lechevalier and Lechevalier [26], the cell-wall chemotype of this strain is type IV; this chemotype is comparable with those of some genera in the family *Pseudonocardiaceae*, such as *Actinobispora*, *Actinokineospora*, *Actinopolyspora*, *Amylocolatopsis*, *Kibdelosporangium*, *Pseudonocardia*, *Saccharopolyspora*, *Saccharomonospora* [6], and *Thermotunica* [3]. The fatty acid profile of strain SL3-2-4^T (Table S3) clearly distinguishes it from its nearest phylogenetic neighbouring genera; the major fatty acid (>10%) of this strain was *iso*-C_{16:0}

(55.6%). The major menaquinone of strain SL3-2-4^T was MK-8 (H₄), and the minor menaquinones were MK-8 (H₂), MK-8 (H₆), and MK-8 (H₈). The major menaquinones differed from those of this strain's closely related genera, being MK-9 (H₄), MK-9 (H₆), MK-10 (H₄) or MK-10 (H₆) (Table 1). Based on Lechevalier *et al.* [27], the genera *Pseudonocardia* and *Amycolata* are the only members of the family *Pseudonocardiaceae* that contain MK-8 (H₄) as the major menaquinone. However, in 1994, Warwick *et al.* [28] classified the genus *Amycolata* in an emended genus *Pseudonocardia*. Before this study, only the genus *Pseudonocardia* was known to contain MK-8 (H₄) as the major menaquinone. The polar lipids of strain SL3-2-4^T consisted of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), phosphatidyl-*N*-methylethanolamine (PME), unidentified aminophospholipids (APL), unidentified glycolipids (GL), and unidentified phospholipids (PL) (Fig. S2). As shown in Table 1, the DPG, PE, and PME have also been found in other genera of the family *Pseudonocardiaceae*. However, the major polar lipid of strain SL3-2-4^T (phosphatidylethanolamine) differed from that of the other genera of this family, with the exception *Actinoalloteichus* (Table 1).

Genomic DNA for PCR amplification of the 16S rRNA gene was prepared using the method as described by Yabe *et al.* [11]. The 16S rRNA gene of six strains was amplified using PCR with universal primers 9F (5'-GAGTTT-GATCCTGGCTCAG-3') and 1510R (5'-GGCTACCTTGT-TACGA-3'). The PCR conditions involved an initial denaturation at 94 °C for 5 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 72 °C for 2 min. This was followed by a final extension at 72 °C for 3 min. The purified PCR product samples were sequenced using DNA sequencing service of Hokkaido System Science Co., Japan (www.hssnet.co.jp) and the 1st BASE DNA sequencing service (<http://base-asia.com/dna-sequencing-services>). The 16S rRNA gene sequences of six strains were deposited in the GenBank/EMBL/DDBJ under accession numbers LC203478, and LC469351-LC469355. All of the type strain sequences for all of the type species used in the phylogenetic analysis were retrieved from the DDBJ/EMBL/GenBank databases.

A nearly full-length sequence of the 16S rRNA gene sequences of three strains (SL3-2-4^T, 1443 nt; SL3-2-6, 1481 nt; and SL3-2-9, 1480 nt) and partial sequence of three strains (SL3-2-5, 1371 nt; SL3-2-7, 1388 nt; and SL3-2-10, 1293 nt) were used for a sequence-similarity search against all related species in the database through EzTaxon-e (www.ezbiocloud.net/; Kim *et al.* [29]) and the phylogenetic analysis. The sequences of six strains were aligned with the sequences of type strains retrieved from the DDBJ/EMBL/GenBank databases using CLUSTAL X (version 1.83) [30]. The 16S rRNA gene sequences of six strains aligned with the most closely related type strains were analysed using MEGA (Version 7.0.26) [31]. Phylogenetic trees were reconstructed based on nearly full-length 16S rRNA gene sequences of

Table 1. Differential characteristics of strain SL3-2-4^T and related genera of the family *Pseudonocardiaceae*

Taxa: 1, *Gandjariella* gen. nov. (SL3-2-4^T; this study); 2, *Thermotunica* [3]; 3, *Goodfellowiella* [4, 5]; 4, *Actinoalloteichus* [6]; 5, *Streptoalloteichus* [7]; 6, *Crossiella* [8]; 7, *Kutzneria* [9].

Gal, galactose; Rib, ribose; Man, mannose; Glc, glucose; Rha, rhamnose. DPG, diphosphatidylglycerol; OH-PE, phosphatidylethanolamine with hydroxy fatty acids; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIM, phosphatidylinositol mannosides; PME, phosphatidyl methylethanolamine; PL, unidentified phospholipids; APL, unidentified aminophospholipids; GL, unidentified glycolipid; NPG, ninhydrin-positive glyco-phospholipid; GluNu, unidentified glucosamine-containing phospholipid. ai, *anteiso*-branched; i, *iso*-branched. v, variable; +, present; –, absent.

	1	2	3	4	5	6	7
Aerial mycelium	v	v	v	+	+	v	+
Fragmented mycelia	+	–	+	+	–	+	+
Sporangium-like structure	–	–	–	–	+	+	+
Temperature range (°C)	30–55	37–65	15–45	20–37	20–54	10–33	25–37
Whole-cell sugars	Gal, Ara	Gal, Rib	Gal, Rib	Glc, Gal, Man, Rib	Gal, Man	Gal, Man, Rha, Rib	Gal, Rha,
DNA G+C content (mol%)	71.6	63.6	68.2	72–73	71.6	71.4	70.3–70.7
Phospholipids	PE, PME, DPG, PG, PL, GL, APL	DPG, NPG, PE, PME, PI, PIM, PL, PG, GluNu	DPG, PE, OH-PE, PME	PE, PIM, PI, PG, DPG, PME	DPG, PE, PI, PIM, DPG, PME	DPG, PE, PI, PIM, PME	DPG, PE, OH-PE, PI, PG†, PME†
Major menaquinones	MK-8 (H ₄)	MK-9 (H ₄)	MK-9 (H ₄), MK-10 (H ₄)	MK-9 (H ₄)	MK-9 (H ₆), MK-10 (H ₆)	MK-9 (H ₄)	MK-9 (H ₄)
Major fatty acids	i-C _{16:0}	ai-C _{17:0} , i-C _{17:0} , i-C _{16:0}	i-C _{16:0} , ai-C _{17:0}	ai-C _{17:0} , i-C _{16:0} , i-C _{15:0} , ai-C _{15:0}	i-C _{15:0} , ai-C _{17:0}	i-C _{15:0} , 9-methyl-C _{16:0} , i-C _{17:0}	i-C _{16:0} , 10-methyl-C _{16:0} , 2-OH-i-C _{16:0}

type strains of taxa closely related to the six strains using the neighbour-joining [32], minimum-evolution [33], and maximum-likelihood [34] methods of the MEGA software package [31], with bootstrap values based on 1000 replications (NJ, ME, and ML methods) [35]. Evolutionary distances were computed using the Kimura two-parameter method [36].

The 16S rRNA gene sequence of strain SL3-2-4^T (1443 nt) showed the greatest similarity to members of genera in the family *Pseudonocardiaceae* (*Goodfellowiella*, *Streptoalloteichus*, *Thermotunica*, and *Actinoalloteichus*) with similarity values of 96.1%; 95.6–96.2%; 94.9%; and 94.4–94.4%, respectively. The 16S rRNA gene sequence similarity values between six strains and type strains of other members of the family *Pseudonocardiaceae* were less than 96.0%.

A detailed phylogenetic analysis based on 16S rRNA gene sequences (Fig. 2) indicated that six strains are located in the *Pseudonocardiaceae* family clade and most closely related to the genus *Thermotunica*, *T. guangxiensis* AG2-7^T (94.6–95.2% similarity, Table S4), with low bootstrap support. They are separated from other closely related genera, including *Streptoalloteichus* (*S. hindustanus* NBRC 15115^T), *Goodfellowiella* (*G. coeruleoviolacea* NRRL B-24058^T), and *Actinoalloteichus* (*A. cyanogriseus* IFO 14455^T) (Fig. 2). As shown in Fig. 2, the type strain SL3-2-4^T and another five strains seem to be closely related in a monophyletic group

and make a distinct lineage from *Thermotunica guangxiensis*. These six strains are divided into three groups, with very strong bootstrap support (99%). The percentage of similarities among the strains *Gandjariella thermophila* based on NCBI BLAST results was ranging from 99.7–100% (Table S4). The sequence differences among strains only by one or two deletion. Considering the 16S rRNA gene sequence of three strains have not yet fully sequences, the conclusion whether all six strains belong to single species or not could not be justified. The genome sequences of the later five strains (SL3-2-5, SL3-2-6, SL3-2-7, SL3-2-9, and SL3-2-10) should be determined to clarify the taxonomic position of these strains.

Phylogenetic relationships between the type strain SL3-2-4^T and all type species in the family *Pseudonocardiaceae* based on nearly full-length 16S rRNA gene sequences is shown in Fig. S3. The close relationship between strain SL3-2-4^T and *Thermotunica* is also supported by a minimum-evolution algorithm by a low bootstrap value (data not shown). Phylogenetic analysis using the neighbour-joining method with the same set of OTUs with three different outgroups (i.e. *Micrococcus luteus* ATCC 381^T, *Streptomyces ambofaciens* ATCC 23877^T, and *Actinopolyspora halophila* ATCC 27976^T) in the MEGA software package consistently indicated that the closest related genus of strain SL3-2-4^T was *Thermotunica* (*T. guangxiensis* AG2-7^T) (data not shown). The low sequence similarity values between six strains and type

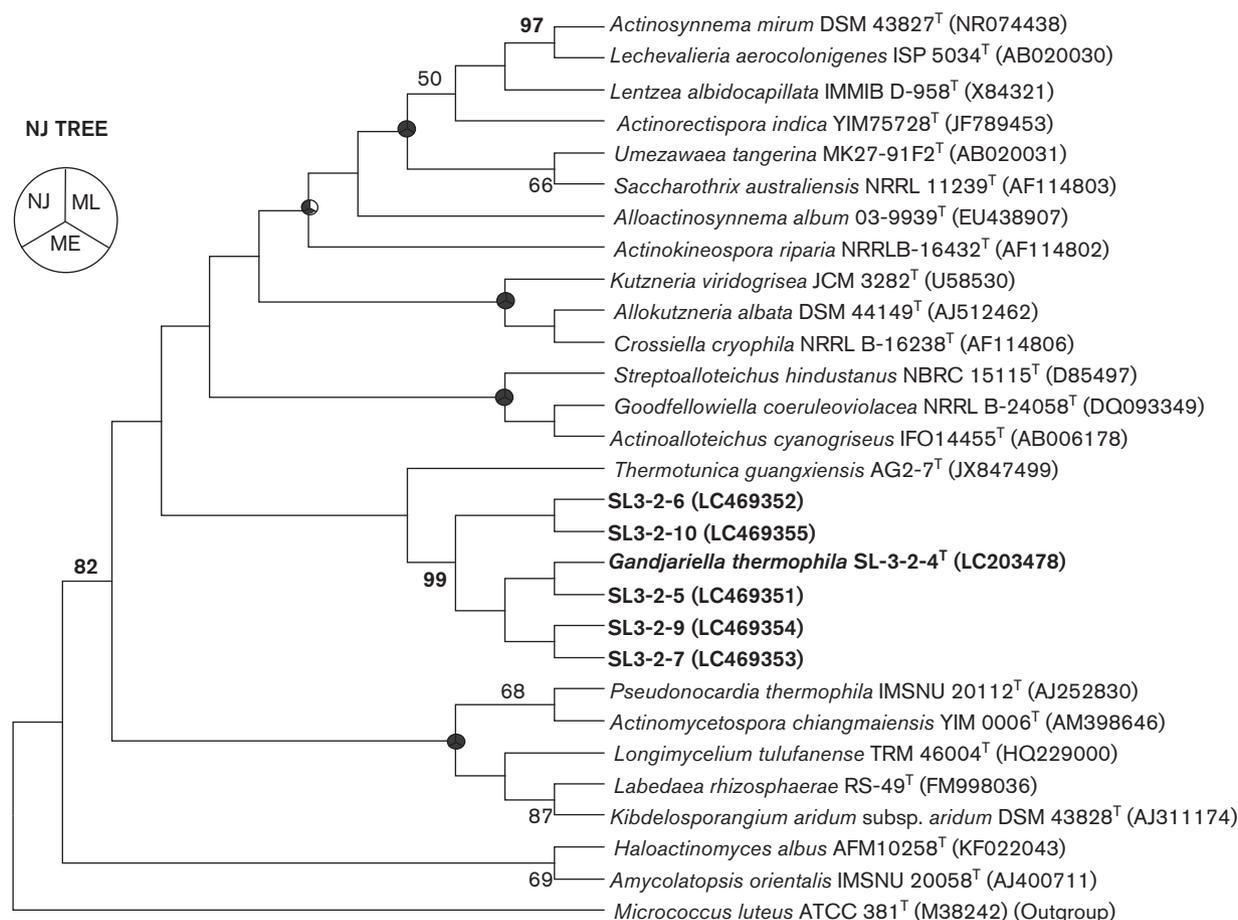


Fig. 2. Phylogenetic position of the type strain *G. thermophila* SL3-2-4^T and other five strains among the known taxa of the family *Pseudonocardiaceae*. The evolutionary history was inferred using the Neighbour-Joining method. The bootstrap consensus tree inferred from 1000 replicates. All positions containing gaps and missing data were eliminated. There were a total of 1244 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

strains of other members of the family *Pseudonocardiaceae*, along with the phylogenetic position of six strains, suggested that these strains represent a new taxon in the family *Pseudonocardiaceae*.

As shown in Figs 2 and S3, the interrelationships among the genera in the family *Pseudonocardiaceae* generally exhibited weak bootstrap support. This result is in good agreement with those of previous analyses [3, 4, 7, 28] that found that the relationship within the family *Pseudonocardiaceae* are unstable, depending on which sequence are analysed. The relationships in this family may receive strong support from the 16S rDNA sequences if additional closely related sequences are included in the analysis.

The genome sequences of SL3-2-4^T was obtained using the Illumina platform (MacRogen, Japan). *De novo* assembly was used to analyse the reads using SPAdes (<http://cab.spbu.ru/software/spades/>). The draft genome sequence was then submitted to the DDBJ Fast Annotation and Submission Tool server (<https://dfast.nig.ac.jp>) for annotation [37].

The antiSMASH (<https://antismash.secondarymetabolites.org>) tool was used to analyse the secondary metabolic gene clusters within the genome [38].

The draft genome features of strain SL3-2-4^T are summarised in Table S5; the genome consists of 6.12 Mb with 5740 protein-coding genes. The DNA G+C content of strain SL3-2-4^T was 71.6 mol% (Table 1). The genome contains 22 biosynthetic gene clusters for polyketide synthase, nonribosomal peptide synthase, and ribosomally synthesized and post-translationally modified peptide family clusters.

The morphological, physiological, and chemotaxonomic properties that distinguish the strain SL3-2-4^T from related genera of the family *Pseudonocardiaceae* are shown in Table 1. Phylogenetically, strain SL3-2-4^T and other five strains appear to be distinct from other genera of the family *Pseudonocardiaceae* within the suborder *Pseudonocardineae* (Fig. 2). The phenotypic data and phylogenetic tree provide evidence to demonstrate that these strains represent a novel genus of the family *Pseudonocardiaceae*, therefore, a new

genus, to be named *Gandjariella* gen. nov., and a novel species, to be named *Gandjariella thermophila* sp. nov. are proposed.

DESCRIPTION OF GANDJARIELLA GEN. NOV.

Gandjariella (Gan.dja.ri.e'l'a. N.L. fem. dim. n. *Gandjariella*, named for Indrawati Gandjar, a microbiologist at Universitas Indonesia, in recognition of her contributions to microbiology in Indonesia).

A Gram-positive, thermophilic, aerobic, non-acid-fast, non-motile actinomycete. It forms a branched, non-fragmented substrate mycelium. Unbranched aerial mycelia are white, with indications of long spore chains containing oval-shaped spores on ISP 3 media, however, sporangium-like structures are not produced. The diagnostic diamino acid in the cell-wall peptidoglycan is *meso*-DAP. The whole cell sugars are arabinose and galactose. The major menaquinone is MK-8 (H₄), and the minor menaquinones are MK-8 (H₂), MK-8 (H₆), and MK-8 (H₈). The major cellular fatty acid is *iso*-C_{16:0}. The polar lipid profile contains diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidyl-*N*-methylethanolamine, unidentified aminophospholipids, unidentified glycolipids, and unidentified phospholipids. Phylogenetically, the genus belongs to the family *Pseudonocardiaceae* (suborder *Pseudonocardineae*). The type species is *Gandjariella thermophila*.

DESCRIPTION OF GANDJARIELLA THERMOPHILA SP. NOV.

Gandjariella thermophila (ther.mo'phi.la. Gr. fem. n. *thermê* heat; N.L. masc. adj. *philus* [from Gr. masc. adj. *philos*] friend, loving; N.L. fem. adj. *thermophila* loving heat, thermophilic).

In addition to the morphological and chemotaxonomic characteristics given in the genus description, the species properties can be described. It grows well on ISP 3 agar, ISP 3 with 2 % gellan gum, ISP 5 with 2 % gellan gum, ISP 7 agar, ISP 7 with 2 % gellan gum, Bennett's agar, Bennett's medium with 2 % gellan gum, modified Bennett's agar, 2 % gellan gum, and FS1V with 1.5 % gellan gum. It grows moderately on ISP 1 and FS1V agar and poorly on ISP 2 agar, ISP 2 with 2 % gellan gum, ISP 5 agar, ISP 6 agar, ISP 6 with 2 % gellan gum, and tap water agar. However, it does not grow on ISP 4 medium, Sauton's agar, or Czapek solution agar. The aerial mycelium is white, unbranched, and abundantly produced on ISP 3 with agar and 2 % gellan gum. Substrate mycelia are branched, non-fragmented, and whitish orange to brown, depending on the medium. Light-orange diffusible pigments are produced on ISP 3 with 2 % gellan gum medium. It grows at 30–55 °C (optimum, 45 °C), at pH 4–8 (optimum, pH 7) with <1 % w/v NaCl (optimum 0 % NaCl). It utilises D-galactose, D-glucose, D-mannitol, D-mannose, *myo*-inositol, D-ribose, and D-xylose as the sole carbon sources, but not L-arabinose, D-fructose, lactose, maltose, raffinose, L-rhamnose, sorbitol, or sucrose. It is

positive for catalase activity but negative for H₂S production and oxidase and urease activities. It hydrolyses casein, aesculin, gelatin, guanine, hypoxanthine, starch, L-tyrosine, and xanthine, but not adenine, carboxymethyl cellulose, cellulose, chitin, Tween 20, or xylan. The G+C content of the genomic DNA was 71.6 mol%.

The type strain is SL3-2-4^T (=UICC B-83^T=NRRL B-67478^T=InaCC A981^T), and other five strains were isolated from the forest soil of a geothermal area in Cisolok, West Java, Indonesia.

The GenBank/EMBL/DDBJ accession for draft whole genome sequence of the type strains SL3-2-4^T are BJFL01000001-BJFL01000131; and for the 16S rRNA gene sequence of the type strain SL3-2-4^T and other five strains are LC203478 and LC469351-LC469355, respectively.

Funding information

This work was funded by Hibah Penelitian Unggulan Perguruan Tinggi (PUPT), Direktorat Jenderal Pendidikan Tinggi (DIKTI) 2017, Kementerian Riset Teknologi dan Pendidikan Tinggi Republik Indonesia Research Grant contract no. 2718/UN2.R3.1/HKP05.00/2017 to W. S., and by the Institute of Fermentation Osaka (IFO) to S. Y. and F. N.

Acknowledgements

We thank Eiji Aoyagi (Institute for Materials Research, Tohoku University) for conducting the scanning electron microscopy.

Conflicts of interest

The authors have no conflict of interest related to the content of this article.

Ethical statement

No experiment was conducted with human or animal subjects.

References

- Labeda DP, Goodfellow M, Chun J, Zhi XY, Li WJ. Reassessment of the systematics of the suborder *Pseudonocardineae*: transfer of the genera within the family *Actinosynnemataceae* Labeda and Kroppenstedt 2000 emend. Zhi et al. 2009 into an emended family *Pseudonocardiaceae* Embley et al. 1989 emend. Zhi et al. 2009. *Int J Syst Evol Microbiol* 2011;61:1259–1264.
- Labeda DP, Goodfellow M. *Pseudonocardiales* ord. nov. *Bergey's Manual of Systematics of Archaea and Bacteria*. Bergey's Manual Trust: John Wiley & Sons, Inc; 2015.
- Wu H, Lian Y, Liu B, Ren Y, Qin P et al. *Thermotunica guangxiensis* gen. nov., sp. nov., isolated from mushroom residue compost. *Int J Syst Evol Microbiol* 2014;64:1593–1599.
- Labeda DP, Kroppenstedt RM. *Goodfellowia* gen. nov., a new genus of the *Pseudonocardineae* related to *Actinoalloteichus*, containing *Goodfellowia coeruleoviolacea* gen. nov., comb. nov. *Int J Syst Evol Microbiol* 2006;56:1203–1207.
- Labeda DP, Kroppenstedt RM, Euzéby JP, Tindall BJ. Proposal of *Goodfellowiella* gen. nov. to replace the illegitimate genus name *Goodfellowia* Labeda and Kroppenstedt 2006. *Int J Syst Evol Microbiol* 2008;58:1047–1048.
- Tamura T, Zhiheng L, Yamei Z, Hatano K. *Actinoalloteichus cyanogriseus* gen. nov., sp. nov. *Int J Syst Evol Microbiol* 2000;50:1435–1440.
- Tamura T, Ishida Y, Otaguro M, Hatano K, Suzuki K. Classification of '*Streptomyces tenebrarius*' Higgins and Kastner as *Streptoalloteichus tenebrarius* nom. rev., comb. nov., and emended description of the genus *Streptoalloteichus*. *Int J Syst Evol Microbiol* 2008;58:688–691.
- Labeda DP. *Crossiella* gen. nov., a new genus related to *Streptoalloteichus*. *Int J Syst Evol Microbiol* 2001;51:1575–1579.

9. Suriyachadkun C, Ngaemthao W, Chunhametha S, Tamura T, Sanglier JJ. *Kutzneria buriramensis* sp. nov., isolated from soil, and emended description of the genus *Kutzneria*. *Int J Syst Evol Microbiol* 2013;63:47–52.
10. Shirling EB, Gottlieb D. Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 1966;16:313–340.
11. Yabe S, Aiba Y, Sakai Y, Hazaka M, Yokota A. *Thermosporothrix hazakensis* gen. nov., sp. nov., isolated from compost and description of *Thermosporothricaceae* fam. nov. within the class *Ktedonobacteria*. *Int J Syst Evol Microbiol* 2010;60:1794–1801.
12. Jones KL. Fresh isolates of actinomycetes in which the presence of sporogenous aerial mycelia is a fluctuating characteristic. *J Bacteriol* 1949;57:141–145.
13. Stott MB, Crowe MA, Mountain BW, Smirnova AV, Hou S et al. Isolation of novel bacteria, including a candidate division, from geothermal soils in New Zealand. *Environ Microbiol* 2008;10:2030–2041.
14. Mordarska H, Mordarski M, Goodfellow M. Chemotaxonomic characters and classification of some nocardioform bacteria. *J Gen Microbiol* 1972;71:77–86.
15. Gordon RE, Barnett DA, Handerman JE, Pang CH-N. *Nocardia coeliaca*, *Nocardia autotrophica*, and the Nocardin Strain. *Int J Syst Bacteriol* 1974;24:54–63.
16. Magee CM, Rodeheaver G, Edgerton MT, Edlich RF. A more reliable gram staining technic for diagnosis of surgical infections. *Am J Surg* 1975;130:341–346.
17. Williams ST, Goodfellow M, Alderson G, Wellington EM, Sneath PH et al. Numerical classification of *Streptomyces* and related genera. *J Gen Microbiol* 1983;129:1743–1813.
18. Sasser M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids*, MIDI Technical Note 101. Newark, DE: MIDI Inc; 1990.
19. Schleifer KH, Kandler O. Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* 1972;36:407–477.
20. Collins MD, Pirouz T, Goodfellow M, Minnikin DE. Distribution of menaquinones in actinomycetes and corynebacteria. *J Gen Microbiol* 1977;100:221–230.
21. Harper JJ, Davis GHG. Two-dimensional thin-layer chromatography for amino acid analysis of bacterial cell walls. *Int J Syst Bacteriol* 1979;29:56–58.
22. Minnikin DE, Collins MD, Goodfellow M. Fatty acid and polar lipid composition in the classification of *cellulomonas*, *oerskovia* and related taxa. *J Appl Bacteriol* 1979;47:87–95.
23. 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.
24. Tamaoka J, Komagata K. Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* 1984;25:125–128.
25. Hasegawa T, Takizawa M, Tanida S. A rapid analysis for chemical grouping of aerobic actinomycetes. *J Gen Appl Microbiol* 1983;29:319–322.
26. Lechevalier MP, Lechevalier H. Chemical composition as a criterion in the classification of aerobic actinomycetes. *Int J Syst Bacteriol* 1970;20:435–443.
27. Lechevalier MP, Prauser H, Labeda DP, Ruan J-S. Two new genera of nocardioform actinomycetes: *Amycolata* gen. nov. and *Amycolatopsis* gen. nov. *Int J Syst Bacteriol* 1986;36:29–37.
28. Warwick S, Bowen T, McVeigh H, Embley TM. A phylogenetic analysis of the family *Pseudonocardiaceae* and the genera *Actinokineospora* and *Saccharothrix* with 16S rRNA sequences and a proposal to combine the genera *Amycolata* and *Pseudonocardia* in an emended genus *Pseudonocardia*. *Int J Syst Bacteriol* 1994;44:293–299.
29. Kim OS, Cho YJ, Lee K, Yoon SH, Kim M et al. Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* 2012;62:716–721.
30. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997;25:4876–4882.
31. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 2016;33:1870–1874.
32. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987;4:406–425.
33. Rzhetsky A, Nei M. A simple method for estimating and testing minimum-evolution trees. *Mol Biol Evol* 1992;9:945–967.
34. Felsenstein J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol Evol* 1981;17:368–376.
35. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 1985;39:783–791.
36. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980;16:111–120.
37. Tanizawa Y, Fujisawa T, Nakamura Y. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. *Bioinformatics* 2018;34:1037–1039.
38. Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ et al. antiSMASH 4.0-improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res* 2017;45:W36–W41.

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