Actinaurispora siamensis gen. nov., sp. nov., a new member of the family *Micromonosporaceae*

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Two actinomycete strains, CM2-8^T and CM2-12, were isolated from temperate peat swamp forest soil in Chiang Mai Province, Thailand. Their taxonomic positions were determined using a polyphasic approach. Chemotaxonomic characteristics of these strains coincided with those of the family *Micromonosporaceae*, i.e. cell wall chemotype II, *N*-glycolyl type of muramic acid, and type II phospholipids. Phylogenetic analysis based on 16S rRNA gene sequence data also indicated that these strains fell within the family *Micromonosporaceae* and formed a distinct taxon in the *Micromonosporaceae* phylogenetic tree. On the basis of phylogenetic analysis, characteristic patterns of 16S rRNA gene signature nucleotides and chemotaxonomic data, it is proposed that the novel isolates belong to a new genus, *Actinaurispora* gen. nov. The type species of the genus is proposed as *Actinaurispora siamensis* sp. nov., with strain CM2-8^T (=JCM 15677^T=BCC 34762^T) as the type strain.

The family *Micromonosporaceae* was first described by Krasil'nikov (1938), and amended by Koch *et al.* (1996), Stackebrandt *et al.* (1997) and Zhi *et al.* (2009), who classified 17 genera [*Actinocatenispora* (Thawai *et al.*, 2006), *Actinoplanes* (Couch, 1950), *Asanoa* (Lee & Hah, 2002), *Catellatospora* (Asano & Kawamoto, 1986), *Catenuloplanes* (Yokota *et al.*, 1993), *Couchioplanes* (Tamura *et al.*, 1994), *Dactylosporangium* (Thiemann *et al.*, 1967), *Longispora* (Matsumoto *et al.*, 2003), *Micromonospora* (Ørskov, 1923),

Pilimelia (Kane, 1966), *Polymorphospora* (Tamura *et al.*, 2006), *Planosporangium* (Wiese *et al.*, 2008), *Pseudosporangium* (Ara *et al.*, 2008a), *Salinispora* (Maldonado *et al.*, 2005), *Spirilliplanes* (Tamura *et al.*, 1997), *Verrucosispora* (Rheims *et al.*, 1998) and *Virgisporangium* (Tamura *et al.*, 2001)] within this family on the basis of 16S rRNA gene sequence analysis. Two actinomycete genera, *Luedemannella* (Ara & Kudo, 2007a) and *Krasilnikovia* (Ara & Kudo, 2007b), have since been described as additional members of the family. Recently, *Catellatospora koreensis* and *Catellatospora tsunoense* were transferred to the genera *Catelliglobospora* (as *Catelliglobospora koreensis*) and *Hamadaea* (as *Hamadaea tsunoensis*), respectively, based on 16S rRNA gene sequence analysis and chemotaxonomic data (Ara *et al.*, 2008b).

During an investigation of the actinomycete diversity of soils in northern Thailand, two strains, CM2-8^T and CM2-12, which showed chemotaxonomic characteristics that were typical of members of the family *Micromonosporaceae*

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CM2-8^T and CM2-12 are AB454379 and AB465341, respectively.

Phylogenetic trees based on almost complete 16S rRNA gene sequences constructed using the neighbour-joining, maximum-parsimony and minimum-evolution methods and tables giving the fatty acid composition and 16S rRNA gene signature nucleotide positions of strains CM2-8^T and CM2-12 and related strains/species are available with the online version of this paper.

but which represented a novel phylogenetic lineage, were isolated. In this report, these strains were characterized using a polyphasic approach and it is proposed that they be classified as representatives of a novel species in a new genus.

Strains CM2-8^T and CM2-12 were isolated from temperate peat swamp forest soil in Chiang Mai Province, Thailand. Samples were taken from the soil surface and kept at 4 °C. The sampling and isolation methods have been described by Thawai *et al.* (2004) and pure cultures were kept at 4–10 °C on yeast extract-malt extract agar (ISP 2 medium) slants.

Strains CM2-8^T and CM2-12 were grown for 14 days at 30 °C on humic acid-vitamin agar and observed by light and scanning electron microscopy (model JSM-5410 LV; JEOL). Samples for scanning electron microscopy were prepared as described previously (Itoh *et al.*, 1989).

Cultural, physiological and biochemical characteristics were examined by using several standard methods; cultural characteristics were tested using 21 day cultures grown at 30 °C on various media. The Jacal Colour Card L2200 (Japan Colour Research Institute) was used for determining colour designations and names. Decomposition of various compounds was examined using the basal medium recommended by Gordon *et al.* (1974). Temperature, NaCl and pH tolerance were determined on ISP 2 medium. Carbon source utilization was tested using ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with a final concentration of 1 % of the tested carbon sources and 0.05 % Casamino acids. Gelatin liquefaction, peptonization of milk, nitrate reduction and starch hydrolysis were determined by cultivation on various media as described by Arai (1975) and Williams & Cross (1971). Melanin and hydrogen sulphide production were examined on tyrosine agar and peptone iron agar slants supplemented with 0.1 % (w/v) yeast extract.

Freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in yeast extract-malt extract broth (ISP 2 broth) on a rotary shaker at 30 °C for 5 days. Cell wall peptidoglycan was prepared and hydrolysed according to Kawamoto *et al.* (1981) and the amino acid composition was analysed with an automatic amino acid analyser. The cell wall diaminopimelic acid (DAP) isomers were determined by the method of Stanek & Roberts (1974). The acyl group of the muramic acid in the peptidoglycan was determined by the method of Uchida & Aida (1984). Reducing sugars from whole-cell hydrolysates were analysed by the HPLC method of Mikami & Ishida (1983). Cell phospholipids were extracted and analysed by the method of Minnikin *et al.* (1984). Fatty acid methyl ester analysis was performed by GLC according to the instructions of the Microbial Identification System (MIDI) (Sasser, 1990; Kämpfer & Kroppenstedt, 1996). The presence of mycolic acids was investigated by using the method of Minnikin *et al.* (1975). Isoprenoid quinones were extracted by the method of Collins *et al.*

(1977) and analysed by HPLC equipped with a Cosmosil 5C₁₈ column (4.6 × 150 mm; Nacalai Tesque). The elution solvent was a mixture of methanol and 2-propanol (2:1, v/v).

Chromosomal DNA was isolated from cells grown in ISP 2 broth according to the method of Tamaoka (1994). The DNA G+C content was determined using the HPLC method of Tamaoka & Komagata (1984). An equimolar mixture of nucleotides for analysis of DNA base composition (Yamasa Shoyu) was digested by bacterial alkaline phosphatase into nucleosides and used as the quantitative standard. DNA–DNA hybridization was conducted in microdilution plates, as reported by Ezaki *et al.* (1989). Hybridization was carried out at 55 °C for 2 h. DNA–DNA relatedness (%) was determined by using the colorimetric method (Verlander, 1992).

Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene and sequencing of PCR products were carried out as described by Nakajima *et al.* (1999). The 16S rRNA gene sequence was multiply aligned with selected sequences obtained from the GenBank/EMBL/DDBJ databases using CLUSTAL W version 1.81 (Thompson *et al.*, 1994). Alignment was verified manually and adjusted prior to the construction of a phylogenetic tree. The phylogenetic tree was constructed by using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Kluge & Farris, 1969) and minimum-evolution (Felsenstein, 1997) methods in the MEGA program version 2.1. Confidence values of branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings. Sequence similarity values between closely related strains were determined using the EzTAXON server (Chun *et al.*, 2007).

Strains CM2-8^T and CM2-12 produced well-developed and branched substrate hyphae on ISP 2 agar, but no aerial hyphae. Spores were borne singly on the substrate hyphae and were 0.4–0.6 mm in diameter (Fig. 1). Spores had a rough surface and were non-motile. Colonies of strains CM2-8^T and CM2-12 were strong reddish orange in colour and did not darken after sporulation; soluble pigments were not produced on most of the media tested, with the exception of a pale-yellow pigment on ISP 2 agar. Physiological and biochemical characteristics of the strains are presented in the species description.

Cell-wall hydrolysates of the two novel isolates contained glutamic acid, glycine, alanine and *meso*-DAP, indicating that these strains have wall chemotype II (described by Lechevalier & Lechevalier, 1970); the peptidoglycan was of type A1γ (described by Schleifer & Kandler, 1972). The acyl moiety of murein contained glycolyl residues. The isolates contained glucose, galactose, xylose and mannose as whole-cell sugars, but not arabinose or ribose. The characteristic phospholipids were diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannosides and phosphatidylethanolamine; phosphatidylcholine was not present.

Fig. 1. Scanning electron micrograph of strain CM2-8^T. Bar, 1 µm.

This pattern corresponds to phospholipid type II of Lechevalier *et al.* (1977). The cellular fatty acid compositions are shown in Supplementary Table S1, available in IJSEM Online. The major fatty acids of CM2-8^T and CM2-12 were iso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0}, anteiso-C_{15:0} and iso-C_{17:0}; 10-methyl-C_{17:0} was also found. This pattern corresponds to fatty acid type 3b described by Kroppenstedt (1985). Mycolic acids were not detected. The predominant menaquinones found in the isolates were MK-9(H₆) (41.7%), MK-10(H₆) (15.9%), MK-9(H₈) (9.4%) and MK-10(H₈) (5.4%); small amounts of MK-9(H₄) (1.3%), MK-10(H₄) (0.7%) and MK-9(H₂) (0.5%) were also present. The DNA G+C contents for strains CM2-8^T and CM2-12 were 72.6 and 72.2 mol%, respectively.

These chemotaxonomic characteristics, in particular the cell-wall type (type II), the muramic acid acyl type (N-glycolyl), the absence of mycolic acids and the phospholipid type (type II), suggest that strains CM2-8^T and CM2-12 are members of the family *Micromonosporaceae*.

Almost complete 16S rRNA gene sequences of isolates CM2-8^T (1446 nt) and CM2-12 (1450 nt) were compared against 16S rRNA gene sequences of all members of the genus *Micromonospora* and all members of the family *Micromonosporaceae*. Phylogenetic analysis based on this large dataset revealed that the novel isolates belonged to a cluster of the family *Micromonosporaceae* (see Supplementary Figs S1, S2 and S3, available in IJSEM Online). When the sequences of CM2-8^T and CM2-12 were compared with selected 16S rRNA gene sequences of all genera in the family *Micromonosporaceae* and the 16S rRNA gene sequence of *Streptomyces ambofaciens* (used as an outgroup), it was observed that they represented a new phyletic line that was different from all described genera in this family (Fig. 2). The highest levels of similarity were with *Micromonospora eburnea* JCM 12345^T (97.9%), *Micromonospora auratinigra* JCM 12357^T (97.5%), *Salinispora arenicola* CNH-643^T (97.4%), *Micromonospora nigra* DSM 43818^T (97.3%) and *Salinispora tropica* CNB-440^T (97.3%). Although the two novel isolates showed highest levels of 16S rRNA gene sequence similarity to members of the genus

Micromonospora, phylogenetic analysis indicated that the isolates did not cluster within the genus *Micromonospora* but were related to members of the genus *Salinispora*. Signature nucleotides of the 16S rRNA gene sequence of the isolates were shared in all but one position (840:846 according to the *Escherichia coli* numbering scheme) with genera of the family *Micromonosporaceae* (Zhi *et al.*, 2009). However, signature nucleotides of the novel isolates differed from those of their closest neighbours, members of the genera *Micromonospora* and *Salinispora*, in several positions (Supplementary Table S2, available in IJSEM Online) and clearly differentiate the novel strains from both of these genera. The gene sequence similarity between isolates CM2-8^T and CM2-12 was 100%. The DNA–DNA relatedness value between the two novel isolates was 88.9±0.8%.

Members of the two genera *Micromonospora* and *Salinispora* seem to be most closely related to isolates CM2-8^T and CM2-12. Members of the genus *Salinispora* require seawater for growth. The initial colour of colonies is orange to deep orange and can become darkened after sporulation. They have arabinose, galactose and xylose as diagnostic sugars in whole-cell hydrolysates, MK-9(H₄) as the major menaquinone component and fatty acid type 3a. Members of the genus *Micromonospora* have arabinose and xylose as the major sugars, and MK-10(H_{4,6}) and MK-9(H_{4,6}) as the predominant menaquinone components. All members of this genus exhibit yellowish white to orange brown substrate mycelia and turn greyish black to black after sporulation. Isolates CM2-8^T and CM2-12 can be clearly distinguished from members of these two genera in terms of colony colour, physiological characteristics (i.e. the requirement of seawater for growth), characteristic whole-cell sugars, predominant menaquinones and fatty acid composition.

These data suggest that the novel isolates do not belong to any known genera of the family *Micromonosporaceae*. Differential characteristics for the isolates and members of related genera in the family *Micromonosporaceae* are shown in Table 1.

On the basis of phenotypic, chemotaxonomic and genotypic data, isolates CM2-8^T and CM2-12 are readily distinguishable from the members of all established genera in the family *Micromonosporaceae*. Therefore, it is proposed that isolates CM2-8^T and CM2-12 should be classified as representatives of a novel species in a new genus, for which the name *Actinaurispora siamensis* gen. nov., sp. nov. is proposed.

Description of *Actinaurispora* gen. nov.

Actinaurispora [Ac.tin.au.ri.spo'ra. Gr. n. *actis* -inos ray; L. n. *aurum* gold, the colour of gold; N.L. fem. n. *spora* (from Gr. n. *spora* seed) a spore; N.L. fem. n. *Actinaurispora* ray (fungus) producing orange spores].

Gram-positive, non-acid-fast and aerobic. Form extensively branched substrate hyphae. Colonies are strong

Fig. 2. Neighbour-joining tree (Saitou & Nei, 1987) based on almost complete 16S rRNA gene sequences showing the relationships between CM2-8^T, CM2-12, and strains of all type species of recognized representative members of the family *Micromonosporaceae*. The 16S rRNA gene sequence of *Streptomyces ambofaciens* ATCC 23877^T was used as an outgroup. Asterisks indicate branches of the tree that were also found using the maximum-parsimony (Kluge & Farris, 1969) and minimum-evolution (Felsenstein, 1997) methods. Bootstrap values (%) of 1000 replicates are indicated at branch-points; only values >50 % are shown. Bar, 0.01 substitutions per nucleotide position.

reddish orange in ISP 2 medium and do not darken after sporulation. Single spores with rough-surfaced spores (0.4–0.6 µm in diameter) are formed on substrate hyphae. Aerial mycelium is absent. Spores are non-motile. A pale-yellow pigment is produced in ISP 2. Cell wall contains glutamic acid, glycine, alanine and *meso*-DAP. The *N*-acyl group of the cell wall muramic acid is glycolyl. Glucose, xylose, mannose and galactose are detected as whole-cell sugars. Contains phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides as cellular phospholipids (phospholipid pattern type II). Predominant menaquinones are MK-9(H₆), MK-10(H₆), MK-9(H₈) and MK-10(H₈); minor components are MK-9(H₄), MK-10(H₄) and MK-9(H₂). The major fatty acids are iso-C_{15:0}, iso-C_{16:0}, anteiso-C_{17:0}, anteiso-C_{15:0} and iso-C_{17:0}; 10-methyl-C_{17:0} is also found. Mycolic acids are not detected. Isolated from soil. The type species is *Actinaurispora siamensis*.

Description of *Actinaurispora siamensis* sp. nov.

Actinaurispora siamensis (si.am.en'sis. N.L. fem. adj. *siamensis* pertaining to Siam, the old name for Thailand, the country of origin of the soil from which the type strain was isolated).

Morphological, chemotaxonomic and general characteristics are as described for the genus. Utilizes D-glucose, L-rhamnose, melibiose, lactose, D-galactose, cellobiose, D-fructose and D-xylose, but not D-mannitol, D-ribose, raffinose, glycerol, salicin or L-arabinose. Weakly positive for peptonization of milk and gelatin liquefaction. Negative for hydrolysis of starch, formation of melanin, H₂S production and reduction of nitrate. The optimal temperature for growth is 25–30 °C. No growth occurs above 40 °C. Minimum pH tolerated is 5. Maximum NaCl concentration for growth is 1.5 %. Does not require seawater for growth.

Table 1. Differential characteristics of the genus *Actinaurispora* and related genera

Taxa: 1, *Actinaurispora* gen. nov.; 2, *Actinocatenispora*; 3, *Actinoplanes*; 4, *Asanoa*; 5, *Catellatospora*; 6, *Catenuloplanes*; 7, *Couchioplanes*; 8, *Dactylosporangium*; 9, *Longispora*; 10, *Luedemannella*; 11, *Micromonospora*; 12, *Pilimelia*; 13, *Polymorphospora*; 14, *Salinispora*; 15, *Spirilliplanes*; 16, *Verrucospora*; 17, *Virgisporangium*; 18, *Planosporangium*; 19, *Pseudosporangium*; 20, *Krasilnikovia*; 21, *Hamadaea*; 22, *Catelliglobospora*. Data are from Thawai *et al.* (2006), Couch (1950), Goodfellow *et al.* (1990), Lee *et al.* (2000), Lee & Hah (2002), Asano & Kawamoto (1986), Ara & Kudo (2006, 2007a, b), Yokota *et al.* (1993), Kudo *et al.* (1999), Tamura *et al.* (1994, 1997, 2001, 2006), Thiemann *et al.* (1967), Matsumoto *et al.* (2003), Ørskov (1923), Kane (1966), Maldonado *et al.* (2005), Rheims *et al.* (1998), Stackebrandt & Kroppenstedt (1987), Vobis (1989), Wiese *et al.* (2008) and Ara *et al.* (2008a, b). +, Present; –, absent; ND, not determined. Ara, Arabinose; Gal, galactose; Rham, rhamnose; Rib, ribose; Man, mannose; Xyl, xylose.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Spore chains	—	+	—	+	+	+	+	—	+	—	—	—	+	—	+	—	—	—	—	ND	+	+
Spore motility	—	—	+	—	—	+	+	+	—	—	—	+	—	—	+	—	+	+ / —	—	—	—	—
Seawater requirement for growth*	—	—	—	—	—	—	—	—	—	ND	—	—	ND	+	—	—	—	ND	ND	ND	ND	ND
Diamino acid	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	L-Lys	L-Lys	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP	<i>m</i> -DAP, 3-OH-DAP	<i>m</i> -DAP	<i>m</i> -DAP, 3-OH-DAP	<i>m</i> -DAP
Major menaquinone(s) (MK-)	9(H ₆), 10(H ₆)	9(H _{4,6})	9(H ₄), 10(H ₄)	10(H _{6,8}), 9(H _{4,6})	10(H _{8,6}), 9(H _{4,6})	9(H ₈), 10(H ₈)	9(H ₄)	9(H _{4,6,8})	10(H _{4,6})	9(H _{4,6})	10(H _{4,6}), 9(H _{2,4})	10(H _{6,4}), 9(H _{6,4})	9(H ₄)	10(H ₄)	9(H ₄)	10(H _{4,6})	9(H ₄), 10(H ₄)	9(H ₆)	9(H _{6,4,8})	9(H ₆)	10(H ₄)	
Phospholipid type†	PII	PII	PII	PII	PII	PIII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII	PII
Fatty acid type‡	3b	3b	2d	2d	3b	2c	2c	3b	2d	2d	3b	2d	2a	3a	2d	2b	2d	3b	2d	2d	3b	3b
Characteristic whole-cell sugars	Xyl, Man, Gal	Ara, Gal, Xyl	Ara, Xyl	Ara, Gal, Xyl	Ara, Gal, Xyl/Xyl only	Xyl	Ara, Xyl, Gal	Ara, Xyl	Ara, Gal, Xyl	Ara, Gal, Man, Rham, Rib, Xyl	Ara, Xyl	Ara, Xyl	Xyl	Ara, Gal, Xyl	Gal, Xyl	Man, Xyl	Ara, Gal, Xyl	Ara, Xyl	Gal, Rib, Man, Xyl, Ara	Gal, Man, Xyl, Ara, Rib	Xyl, Gal, Man, Rib, Ara, Rham	Rham, Man, Xyl, Gal
DNA G+C content (mol%)	72	72	72–73	71–72	71–72	71–73	70–72	72–73	70	71	71–73	ND	71	70–73	69	70	71	71.4	73	71	70	70

*Data from Maldonado *et al.* (2005).

†According to the classification of Lechevalier *et al.* (1977).

‡According to the classification of Kroppenstedt (1985).

The type strain is CM2-8^T (=JCM 15677^T=BCC 34762^T), isolated from temperate peat swamp forest soil in Chiang Mai Province, Thailand. The DNA G+C content of the type strain is 72.6 mol%.

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