**Microbispora siamensis** sp. nov., a thermotolerant actinomycete isolated from soil

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An actinomycete, strain DMKUA 245ᵀ, isolated from soil, was investigated using a polyphasic approach. The isolate formed longitudinally paired spores on the tips of short sporophores that branched alternately from aerial hyphae. The morphological and chemotaxonomic properties clearly demonstrated that the new isolate belonged to the genus *Microbispora*. 16S rRNA gene sequence analysis supported the assignment of the novel strain to the genus *Microbispora*. The gene sequence similarity values between the novel strain and the closely related species *Microbispora corallina*, *Microbispora rosea* subsp. *rosea*, *Microbispora rosea* subsp. *aerata* and *Microbispora amethystogenes* were 98.4%, 97.4%, 97.0% and 96.9%, respectively. The DNA–DNA hybridization values and some physiological and biochemical properties indicated that strain DMKUA 245ᵀ could be distinguished from its phylogenetically closest relatives. Based on these genotypic and phenotypic data, strain DMKUA 245ᵀ represents a novel species in the genus *Microbispora* for which the name *Microbispora siamensis* sp. nov. is proposed. The type strain is strain DMKUA 245ᵀ (≡ BCC 14407ᵀ = NBRC 104113ᵀ). In addition, DNA–DNA relatedness values in reciprocal hybridization experiments showed that *M. amethystogenes* was a separate genomic species from *M. rosea* subsp. *rosea*. A combination of genotypic and phenotypic data supported the classification of *M. amethystogenes* as a separate species.

The genus *Microbispora* was proposed by Nonomura & Ohara (1957) for actinomycetes that form obvious aerial mycelia bearing longitudinal pairs of spores. Ten species were described as members of this genus in the Approved Lists of Bacterial Names (Skerman et al., 1980). Subsequently, *Microbispora viridis* (Miyadoh et al., 1985), *Microbispora indica* and *Microbispora karnatakensis* (Rao et al., 1987) have been proposed, while *Microbispora echinospora* and *Microbispora viridis* have been transferred to the genus *Actinomadura* as *Actinomadura echinospora* comb. nov. (Kroppenstedt et al., 1990) and *Actinomadura rugatobispora* comb. nov., respectively (Miyadoh et al., 1990). On the basis of DNA–DNA hybridization experiments, Miyadoh et al. (1990) suggested that 10 species of the genus *Microbispora* should be combined into the type species *Microbispora rosea* with two subspecies, *M. rosea* subsp. *rosea* and *M. rosea* subsp. *aerata*. Subsequently, Wang et al. (1996) transferred *Microbispora bispora* to the new genus *Thermobispora* as *Thermobispora bispora* comb. nov. based on its 16S rRNA gene sequence. *Thermomonospora mesophila* was reclassified by Zhang et al. (1998) as *Microbispora mesophila* comb. nov. *Microbispora corallina* was proposed as a new member of the genus by Nakajima et al. (1999). During a study of the diversity of actinomycetes from Thai soil, an actinomycete strain, DMKUA 245ᵀ, was isolated that exhibited the typical morphological characteristics of the genus *Microbispora*. Further study of this strain, using a polyphasic taxonomic approach, confirmed its position as a representative of a novel species within the genus *Microbispora*.

Strain DMKUA 245ᵀ was isolated from a soil sample collected in the Sakaerat Biosphere Reserve in Nakhonratchasima, Thailand. This strain was picked from colonies present on humic acid–vitamin agar (Hayakawa & Nonomura, 1987) after incubation at 30 °C for 21 days. The strain was then purified and maintained on yeast extract–malt extract agar (ISP 2 medium). Its cultural characteristics were determined using 14 day cultures grown at 30 °C on various agar media. The utilization of
carbon sources was tested using ISP 9 medium (Shirling & Gottlieb, 1966) supplemented with 1% (w/v) final concentration of the tested carbon sources. Utilization of organic acids was tested according to the method of Gordon et al. (1974). Gelatin liquefaction, skimmed milk peptonization, nitrate reduction and cellulosomal degradation were determined as described by Hamada (2000). Growth temperature and tolerance of NaCl were tested on ISP 2 medium. Melanin pigment formation was examined on tyrosine agar (ISP 7 medium). The presence of isomers of medium. Melanin pigment formation was examined on tyrosine agar (ISP 7 medium). The presence of isomers of

diaminopimelic acid (A2pm) and whole-cell sugars was determined following the method of Lechevalier & Lechevalier (1970). Phospholipids were extracted according to the method of Minnikin et al. (1979) and identified using two dimensional TLC. Cellular fatty acid methyl esters were prepared and analysed according to the protocol of the MIDI Sherlock Microbial Identification System (Sasser, 1990; MIDI, 2002). Menaquinones were extracted by chloroform/methanol (2:1, v/v) (Minnikin et al., 1984) and analysed by LC/MS (QP8000s; Shimadzu LCMS, www.shimadzu.com.br/analitica/applicacoes/cromatografos/lc_ms/lcms010.pdf).

DNA was extracted using a phenol/chloroform solution according to the method of Hopwood et al. (1985) and RNA was removed using the protocol of Saito & Miura (1963). The G+C content of DNA was determined using the HPLC method of Tamaoka & Komagata (1984). DNA–DNA relatedness was measured fluorometrically using the microplate hybridization method devised by Ezaki et al. (1989). The 16S rRNA gene was amplified using the PCR with a Taq DNA polymerase and primers 9F (position 9–27, Escherichia coli numbering, Brosius et al., 1978) and 1541R (1541–1525). Amplification was carried out using a DNA thermal cycler. The amplified 16S rRNA gene fragment was purified and subjected directly to cycle sequencing using a BigDye Terminator v3.1 Cycle sequencing kit on an ABI 3100 automated DNA sequencer (Applied Biosystems). The following primers were used for sequencing: 9F, 9–27; 785F, 785–805; 1541R, 1541–1525; 802R, 802–785.

The sequence was aligned with selected sequences obtained from the GenBank/EMBL/DDBJ database using the CLUSTAL_X v.1.81 program (Thompson et al., 1997). Alignments were manually verified and adjusted prior to the construction of a phylogenetic tree. The phylogenetic tree was constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony methods in the MEGA 3 program (Kumar et al., 2004). Distance matrices for the aligned sequences were calculated by the two-parameter method of Kimura (1980). The confidence values for branches of the phylogenetic tree were determined using bootstrap analyses (Felsenstein, 1985) based on 1000 resamplings.

Strain DMKUA 245T exhibited a range of phenotypic properties typical of members of the genus Microbispora. This strain produced branched and non-fragmented substrate mycelia. Non-motile spores in characteristic longitudinal pairs were borne on short sporophores branching from aerial hyphae. Each spore was oval and its surface was smooth. Neither sporangium-like bodies nor any other special structures were observed. Whole-cell hydrolysates of strain DMKUA 245T contained meso-diaminopimelic acid as the diagnostic diamino acid of the cell-wall peptidoglycan and madurose as the diagnostic sugar. Diphosphatidylglycerol, phosphatidylinositol, phosphatidylycerol, phosphatidylmethylethanolamine, phosphatidylino-sitol mannosides and ninhydrin-positive glycopospholipids were detected as polar lipids. This phospholipids pattern was similar to that of type IV (Lechevalier et al., 1977). The major cellular fatty acids were iso-C16:0, iso-C16:1 and iso-C15:0. The predominant menaquinones were MK-9(H4), MK-9(H2) and MK-9. The G+C content of the DNA of the new isolate was 68 mol%.

The almost complete 16S rRNA gene sequence of strain DMKUA 245T, consisting of 1470 nucleotides, was compared with sequences from members of the genus Microbispora and other strains selected from the family Streptosporangiaceae (Fig. 1). Strain DMKUA 245T and the other members of the genus Microbispora formed a coherent cluster that was supported by high bootstrap values. The 16S rRNA gene similarities between strain DMKUA 245T and the type strains of recognized species of the genus Microbispora ranged from 98.4% (M. corallina) to 96.9% (M. amethystogenes). The results of the physiologicoal and biochemical characteristics of strain DMKUA 245T and the other strains used for comparison are summarized in Table 1. The results clearly demonstrated that strain DMKUA 245T could be differentiated from all other recognized species of the genus Microbispora. Furthermore, DNA–DNA relatedness values determined by reciprocal hybridizations were much lower than 70% between strain DMKUA 245T and the other members of the genus Microbispora (19–46%). Thus, it was concluded that strain DMKUA 245T represents a separate genomic species.

It is evident from the genotypic and phenotypic data that strain DMKUA 245T can be distinguished from previously described species of the genus Microbispora. It is therefore proposed that strain DMKUA 245T represents a novel species of the genus Microbispora, for which the name Microbispora siamensis sp. nov. is proposed.

Miyadoh et al. (1990) proposed that M. amethystogenes is a later heterotypic synonym of M. rosea subsp. rosea. In this study, however, DNA–DNA relatedness values (from reciprocal hybridizations) between M. amethystogenes NBRC 101907T and M. rosea subsp. rosea NBRC 14044T were in the range of 49 to 53%. Since these values are lower than the 70% cut-off value, it is suggested that Microbispora amethystogenes is a separate genomic species from M. rosea subsp. rosea (Fig. 1). In addition, M. amethystogenes NBRC 101907T and M. rosea subsp. rosea NBRC 14044T could be differentiated by characteristics
such as gelatin liquefaction, milk peptonization, and the utilization of maltose, melezitose, D-sorbitol, fumaric acid and L-malic acid (Table 1). *M. amethystogenes* is therefore considered to merit separate species status on the basis of genotypic and phenotypic data (Nakajima *et al.*, 1999).

**Description of Microbispora siamensis sp. nov.**

*Microbispora siamensis* (si.a.men’s.is. N.L. fem. adj. siam-ensis pertaining to Siam, the old name of Thailand, the source of the soil from which the type strain was isolated).

An aerobic, Gram-positive, non-motile and thermotolerant actinomycete that forms non-fragmented branched vegetative hyphae. Substrate mycelia are colourless to yellow. During sporulation, the surface of the colony is pale pink. Yellow and green soluble pigments are produced in ISP 2 and ISP 3 media, respectively. Spores arranged in longitudinal pairs are formed on short sporophores that branch alternately from aerial hyphae. Spores are oval and have smooth surfaces. Substrates utilized as sole carbon sources include: L-arabinose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose and sucrose, but not dulcitol, inositol, maltose, melezitose, raffinose, L-rhamnose or D-sorbitol. Citric acid, L-malic acid and succinic acid are used, but not benzoic acid, fumaric acid or mucic acid. Tests for cellulose degradation, gelatin liquefaction, nitrate reduction and milk peptonization are negative. The temperature range for growth is 25–50 °C, but no growth.

**Table 1.** Physiological and biochemical characteristics of strain DMKUA 245<sup>T</sup> and four other species of the genus Microbispora

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<td>Milk peptonization</td>
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<td>Carbon utilization:</td>
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<td>DNA G+C content (mol%)</td>
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Taxa: 1, DMKUA 245<sup>T</sup>; 2, *M. corallina* NBRC 16418<sup>T</sup>; 3, *M. rosea* subsp. *rosea* NBRC 14044<sup>T</sup>; 4, *M. rosea* subsp. *aerata* NBRC 14624<sup>T</sup>; 5, *M. amethystogenes* NBRC 101907<sup>T</sup>; +, Positive; –, negative; w, weak reaction.
occurs at 55 °C. No growth occurs in the presence of 3% (w/v) NaCl. Strain DMKUA 245\textsuperscript{T} contains meso-diaminopimelic acid as the diagnostic diamino acid. Madurose is detected in whole-cell hydrolysates, indicating a cell wall chemotype IIIB (Lechevalier & Lechevalier, 1970). The phospholipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol mannosides and inositol-glycophospholipids and indicates a phospholipid type IV chemotype. The major cellular fatty acids are iso-C\textsubscript{16:0}, iso-C\textsubscript{16:1}, and iso-C\textsubscript{15:0}. The predominant menaquinones are MK-9(H\textsubscript{4}), MK-9(H\textsubscript{2}) and MK-9.

The type strain, DMKUA 245\textsuperscript{T} (=BCC 14407\textsuperscript{T}=NBRC 104113\textsuperscript{T}), was isolated from soil. The DNA G+C content of the type strain is 68 mol%.

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References


