Herbidospora sakaeratensis sp. nov., isolated from soil, and reclassification of *Streptosporangium claviforme* as a later synonym of *Herbidospora cretacea*

Antika Boondaeng,¹ Chanwit Suriyachadkun,² Yuumi Ishida,³
Tomohiko Tamura,³ Shinji Tokuyama⁴ and Vichien Kitpreechavanich^{1,5}

Correspondence

Vichien Kitpreechavanich
fsciwck@ku.ac.th

¹Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

²BIOTEC Culture Collection National Center for Genetic Engineering and Biotechnology, Pathumthani 12120, Thailand

³NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation, Chiba 292-0818, Japan

⁴Faculty of Agriculture, Shizuoka University, Shizuoka 422-8529, Japan

⁵Center for Advanced Studies in Tropical Natural Resources, KU Institute for Advanced Studies, Kasetsart University, Bangkok 10900, Thailand

An actinomycete strain, DMKUA 205^T, was isolated from a soil sample collected from the Sakaerat Biosphere Reserve in Nakhonratchasima Province, Thailand. The novel strain produced short chains of non-motile spores on the tips of long sporophores branching from the vegetative hyphae. The morphological and chemotaxonomic properties of this new isolate corresponded to those of members of the genus *Herbidospora*. Furthermore, 16S rRNA gene sequence analysis showed that the strain was closely related to members of the genus *Herbidospora*. Phenotypic properties and DNA–DNA relatedness values differentiated the new strain from its closest phylogenetic relatives *Herbidospora yilanensis* 0351M-12^T (35–54 % DNA–DNA relatedness) and *Herbidospora daliensis* 0385M-1^T (58–65 % relatedness). On the basis of phenotypic, genotypic and phylogenetic data, strain DMKUA 205^T could be clearly distinguished from the type strains of *H. yilanensis* and *H. daliensis*. Therefore, strain DMKUA 205^T represents a novel species, for which the name *Herbidospora sakaeratensis* sp. nov. is proposed. The type strain is strain DMKUA 205^T (=BCC 11662^T=NBRC 102641^T). In addition, the DNA–DNA hybridization results from this study revealed that *Streptosporangium claviforme* is a later synonym of *Herbidospora cretacea*.

The genus *Herbidospora* was described by Kudo *et al.* (1993) for actinomycete strains that produce (short) chains of spores (10–30 spores per chain) on the tip of sporophores from vegetative mycelia. This genus belongs to the family *Streptosporangiaceae* and currently encompasses four species with validly published names, *Herbidospora cretacea* and the recently described species *H. yilanensis*, *H. daliensis* and *H. osyris*. In the course of a study on the diversity of actinomycetes in Thailand, we isolated an actinomycete, strain DMKUA 205^T, having a similar morphology to members of the genus *Herbidospora*. In the present study, conducted using a polyphasic approach, strain DMKUA 205^T is proposed as representing

a novel species of the genus *Herbidospora*. In addition, the taxonomic position of *Streptosporangium claviforme* was investigated in this study because of its close phylogenetic relationship with *H. cretacea*.

Strain DMKUA 205^T was isolated from a soil sample collected from the Sakaerat Biosphere Reserve in Nakhonratchasima, Thailand. The strain was isolated using a dilution plate technique on humic acid-vitamin agar (Hayakawa & Nonomura, 1987) after incubation at 30 °C for 21 days. The utilization of carbon sources was tested using International *Streptomyces* Project (ISP) medium 9 (ISP 9; Shirling & Gottlieb, 1966) supplemented with 1 % (w/v) final concentration of the tested carbon sources. Utilization of nitrogen sources was examined on a basal medium containing 10 g glucose, 0.5 g MgSO₄·7H₂O, 0.5 g NaCl, 0.01 g FeSO₄·7H₂O, 1.0 g K₂HPO₄ and 12 g agar in 1 l distilled water (Locci, 1989). Utilization of

Abbreviation: ISP, International *Streptomyces* project.

The GenBank/EBML/DDBJ accession number for the 16S rRNA gene sequence of strain DMKUA 205^T is FJ199995.

organic acids was tested by using the medium and method of Gordon *et al.* (1974). Gelatin liquefaction, skimmed milk peptonization, nitrate reduction and cellulose degradation were determined as described by Miyadoh (2001). Growth temperature and NaCl tolerances were determined on ISP 2. Melanin pigment formation was examined on tyrosine agar (ISP 7). The presence of isomers of diaminopimelic acid (A₂pm) in the cell-wall hydrolysate was detected according to the methods described by Hasegawa *et al.* (1983). Samples for analysis of whole-cell sugars were prepared as described by Lechevalier & Lechevalier (1970) and were analysed by the HPLC technique of Mikami & Ishida (1983). 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 (LCMS QP8000 α ; Shimadzu). The acyl type of the muramic acid was determined by the method of Uchida & Aida (1977). Mycolic acids were analysed by the method of Minnikin *et al.* (1975).

DNA was extracted by using a phenol/chloroform mixture and the method of Hopwood *et al.* (1985). RNA was removed following the method of Saito & Miura (1963). The G+C content of the 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 method with a *Taq* DNA polymerase and primers 9F (position 9–27, *Escherichia coli* numbering, Brosius *et al.*, 1978) and 1541R (1541–1525). Amplification was performed 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 ABI3100 automated DNA sequencer (Applied Biosystems). The following primers were used for sequencing: 9F, 9–27; 785F, 785–805; 1541R, 1541–1525; 802R, 802–785 (Nakagawa *et al.*, 2001).

The sequence was aligned with the selected sequences obtained from the GenBank/EMBL/DDBJ database by using the CLUSTAL_X program v.1.81 (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 method (Saitou & Nei, 1987) in the MEGA3 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 205^T exhibited a range of phenotypic properties typical of members of the genus *Herbidospira*. The novel strain produced well-developed and non-fragmented branched substrate mycelia but true aerial mycelium was not formed. Straight, relatively short chains of non-motile spores were borne on the tips of long sporophores branching from the vegetative hyphae. The strain contained *meso*-diaminopimelic acid as the diagnostic diamino acid in the cell wall. Whole-cell hydrolysates contained glucose, mannose, ribose and madurose (3-O-methyl D-galactose) (cell-wall type IIIB of Lechevalier & Lechevalier, 1970). Phosphatidylethanolamine, phosphatidylmethylethanolamine and glucosamine-containing phospholipids were detected. This result suggested that the phospholipid pattern was type PIV of Lechevalier *et al.* (1977). The fatty acid profiles contained C_{13:0} (5.2%),

Fig. 1. 16S rRNA gene sequence-based neighbour-joining tree showing the relationship between strain DMKUA 205^T, species of the genus *Herbidospira* and representatives of the family *Streptosporangiaceae*. *Marinactinospira thermotolerans* SCSIO 00652^T (GenBank accession no. EU698029) was used as an outgroup. The numbers on the branches indicate the percentage bootstrap values of 1000 replicates (only values >50% are indicated). Bar, 0.01 substitutions per nucleotide position.

Table 1. Differential characteristics of strains DMKUA 205^T, *H. yilanensis* 0351M-12^T and *H. daliensis* 0385M-1^T

Strains: 1, DMKUA 205^T; 2, *H. yilanensis* 0351M-12^T; 3, *H. daliensis* 0385M-1^T. All strains were positive for utilization of D-fructose, D-galactose, D-glucose, D-mannitol and sucrose and for growth at 40 °C. All strains were negative for cellulose degradation and for utilization of benzoic acid, dulcitol, mucic acid, D-ribose and D-sorbitol. +, Positive; –, negative; w, weak reaction.

Characteristic	1	2	3
Carbon utilization			
L-Arabinose	+	–	+
Maltose	–	+	+
Mannose	+	+	–
Melezitose	–	+	+
myo-Inositol	–	+	–
Raffinose	–	+	+
Rhamnose	–	+	+
Xylose	+	–	–
Organic acid utilization			
Fumaric	+	+	–
DL-Lactic	–	+	–
L-Malic	+	–	–
Succinic	+	+	–
NaCl tolerance (% w/v)	1.5	1	5

i-C_{14:0} (3.5 %), C_{15:0} (29.1 %), i-C_{16:0} (17.4 %), 2-OH-C_{15:0} (3.7 %), cis9-C_{17:1} (12.1 %), i-2-OH C_{16:0} (4.3 %), C_{17:0} (8.2 %) and 10-methyl C_{17:0} (16.5 %). Mycolic acids were absent. The major menaquinones were MK-10(H₄) and MK-10(H₆). The acyl type of the cell-wall muramic acid was acetyl. The G+C content of the DNA was 73 mol%.

The phylogenetic tree (Fig. 1) showed that strain DMKUA 205^T was phylogenetically closely related to *H. yilanensis* and *H. daliensis*. The 16S rRNA gene sequence of strain DMKUA 205^T shared 99 % sequence similarity with *H. yilanensis* and *H. daliensis*. The physiological and biochemical characteristics of strain DMKUA 205^T enabled the novel strain to be differentiated from the phylogenetically closest species *H. yilanensis* 0351M-12^T and *H. daliensis*

0358M-1^T (Tseng *et al.*, 2010; Table 1). Furthermore, DNA–DNA relatedness values in reciprocal hybridizations were much lower than 70 % between strain DMKUA 205^T and *H. yilanensis* 0351M-12^T (35–54 %, reciprocal) and *H. daliensis* 0358M-1^T (58–65 %, reciprocal), indicating that strain DMKUA 205^T represents a separate genomic species (Table 2).

It is evident from the genotypic and phenotypic data that strain DMKUA 205^T can be distinguished from previously described species of the genus *Herbidospora*. It is therefore proposed that this strain represents a novel species of the genus *Herbidospora* for which the name *Herbidospora sakaeratensis* sp. nov. is proposed.

DNA–DNA relatedness values in reciprocal hybridizations were >70 % between *Streptosporangium claviforme* and *H. cretacea* JCM 8553^T (82.1 and 89.3 %, reciprocal), indicating that *S. claviforme* NBRC 15623^T was indistinguishable from *H. cretacea* (Table 2). Therefore, the name *S. claviforme* should be treated as a later synonym of *H. cretacea*.

Description of *Herbidospora sakaeratensis* sp. nov.

Herbidospora sakaeratensis (sa.ka.e.ra.ten'sis. N.L. fem. adj. *sakaeratensis* pertaining to Sakaerat Biosphere Reserve, the source of the soil from which the type strain was isolated).

Aerobic, Gram-positive, mesophilic, non-motile actinomycete that forms non-fragmented branched vegetative hyphae, but true aerial mycelium is not formed. Spores are short rods and have smooth surfaces. Temperature range for growth is 20–40 °C. Growth occurs between pH 6.0 and 9.0. The maximum NaCl concentration for growth is 1.5 % (w/v). Utilizes L-arabinose, D-fructose, D-galactose, D-glucose, D-mannitol, D-mannose, starch, sucrose and D-xylose as sole carbon sources, but not dulcitol, inositol, maltose, melezitose, raffinose, L-rhamnose, D-ribose or D-sorbitol. Utilizes fumaric acid, L-malic acid and succinic acid, but not benzoic acid or mucic acid. Tests for cellulose degradation, gelatin liquefaction, nitrate reduction, milk peptonization and melanoid pigment formation are negative. Cell walls contain meso-diaminopimelic acid as the diagnostic diamino

Table 2. Levels of DNA–DNA relatedness among strain DMKUA 205^T and related taxa

Taxa: 1, DMKUA 205^T; 2, *H. yilanensis* 0351M-12^T; 3, *H. daliensis* 0385M-1^T; 4, *Streptosporangium claviforme* NBRC 15623^T; 5, *H. cretacea* JCM 8553^T. The data are mean ± standard deviations from the mean. ND, not determined.

Strain	DNA G+C content (mol%)	% DNA–DNA relatedness with labelled DNA from:				
		1	2	3	4	5
1. DMKUA 205 ^T	73.0	100	35.0 ± 13.4	58.0 ± 4.9	43.7 ± 2.6	46.5 ± 0.7
2. 0351M-12 ^T	70.6	54.0 ± 13.4	100	53.6 ± 0.9	ND	ND
3. 0385M-1 ^T	70.7	65.0 ± 4.9	54.9 ± 0.9	100	ND	ND
4. NBRC 15623 ^T	ND	47.4 ± 2.6	ND	ND	100	82.1 ± 5.0
5. JCM 8553 ^T	70.0	45.5 ± 0.7	ND	ND	89.3 ± 5.0	100

acid and *N*-acetylated muramic acid. Whole-cell hydrolysates contain madurose (chemotype IIIB). The phospholipid profile contains phosphatidylethanolamine, phosphatidyl-methylethanolamine and glucosamine-containing phospholipids (phospholipid type PIV). The major cellular fatty acids are C_{17:1}ω9c, 10-methyl C_{17:0}, iso-C_{16:0} and C_{15:0} (fatty acid type 3c). Mycolic acids are absent. The predominant menaquinones are MK-10(H₄) and MK-10(H₆).

The type strain, DMKUA 205^T (=BCC 11662^T=NBRC 102641^T), was isolated from soil. The DNA G+C content of the type strain is 73 mol%.

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