Herbidospora yilanensis sp. nov. and Herbidospora daliensis sp. nov., from sediment

M. Tseng, S.-F. Yang and G.-F. Yuan

Bio-resource Collection and Research Center, Food Industry Research and Development Institute, HsinChu, 300, Taiwan

Two actinomycete strains, 0351M-12T and 0385M-1T, were isolated from sediment samples collected from Yilan County and Dali City in Taiwan, respectively. The two isolates displayed characteristics which were similar to those of the genus Herbidospora. They both produced branched and unfragmented substrate mycelia, and aerial hyphae were not produced on Bennett’s, glucose-asparagine, Hickey-Tresner, sucrose-nitrate, yeast extract-malt extract, or inorganic salts-starch agars. Short spore-chains were borne on the tips of sporophores arising directly from the agar surface. The isomer of diaminopimelic acid (A2pm) is the meso isomer. The whole-cell sugars are glucose, mannose, ribose and a trace amount of madurose. The acyl type of muramic acid in the cell wall is the acetyl type. The phospholipid pattern is the type PIV pattern of Lechevalier et al. (1977). The major fatty acids are straight, iso-C16 : 0 and 10-methyl C17 : 0. The DNA G + C contents of the two new isolates were 70.6 mol% (0351M-12T) and 70.7 mol% (0385M-1T), respectively. The relatedness between the isolates and Herbidospora cretacea NBRC 15474T was 40.7–48.5%. The value of DNA–DNA hybridization between strain 0351M-12T and strain 0385M-1T was 53.6–54.9%. These two isolates could also be distinguished from each other and from H. cretacea NBRC 15474T by differences in several phenotypic characteristics. We therefore propose the names Herbidospora yilanensis sp. nov. and Herbidospora daliensis sp. nov. for these novel bacteria, with strains 0351M-12T (=FIRDI 003T =BCRC 16875T =LMG 24337T) and 0385M-1T (=FIRDI 004T =BCRC 16876T =LMG 24336T) as the type strains, respectively.

The genus Herbidospora was described by Kudo et al. (1993) and contains only one species, Herbidospora cretacea, at the time of writing. H. cretacea produces branching substrate mycelia, but no distinct aerial hyphae. Short chains of spores (10–30 spores per chain) are borne on the tips of sporophores arising directly from the agar surface. The isomer of diaminopimelic acid (A2pm) is the meso isomer. The whole-cell sugars are glucose, mannose, ribose and a trace amount of madurose. The acyl type of muramic acid in the cell wall is the acetyl type. The phospholipid pattern is the type PIV pattern of Lechevalier et al. (1977). The major fatty acids are straight, iso-C16 : 0 and 10-methyl C17 : 0. The DNA G + C contents of the two new isolates were 70.6 mol% (0351M-12T) and 70.7 mol% (0385M-1T), respectively. The relatedness between the isolates and Herbidospora cretacea NBRC 15474T was 40.7–48.5%. The value of DNA–DNA hybridization between strain 0351M-12T and strain 0385M-1T was 53.6–54.9%. These two isolates could also be distinguished from each other and from H. cretacea NBRC 15474T by differences in several phenotypic characteristics. We therefore propose the names Herbidospora yilanensis sp. nov. and Herbidospora daliensis sp. nov. for these novel bacteria, with strains 0351M-12T (=FIRDI 003T =BCRC 16875T =LMG 24337T) and 0385M-1T (=FIRDI 004T =BCRC 16876T =LMG 24336T) as the type strains, respectively.

During our search for novel actinomycetes, we isolated some novel strains from sediments in Taiwan. In this study, we describe two isolates, 0351M-12T and 0385M-1T, belonging to the genus Herbidospora. On the basis of polyphasic taxonomic characterization that combined phenotypic properties, phylogenetic and genetic data, we propose that the two strains should be classified as novel species of genus Herbidospora as Herbidospora yilanensis sp. nov. and Herbidospora daliensis sp. nov.

Strains 0351M-12T and 0385M-1T were isolated from sediment samples from a dry lake in Yilan county and a river in Dali city in Taiwan, respectively. The wet clay sediment samples were dried at room temperature for 7 days. Approximately 2 g of each sediment sample was suspended in 18 ml of sterile distilled water, subjected to shaking for 1 h and allowed to settle. The suspensions (100 μl) were plated on humic acid-vitamin (HV) agar
Herbidospora yilanensis sp. nov. and H. daliensis sp. nov.

(Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg I\(^{-1}\)) and nalidixic acid (20 mg I\(^{-1}\)) and incubated at 28 °C for 4 weeks. The two strains were purified and maintained on oatmeal agar slants and as suspensions of spores or mycelial fragments in glycerol (20%, v/v) stored at −20 °C.

Morphological characteristics of the two strains were observed by scanning electron microscopy (S-4700, Hitachi) following incubation on oatmeal agar for 14 days at 28 °C and fixation in 4% osmium tetroxide solution. The samples were then dehydrated through a series of ethanol-acetone solutions and critical point dried. Cultural characteristics were tested by using 21-day-old cultures grown at 28 °C on various media (Supplementary Table S1 available in IJSEM Online). The ISCC-NBS Colour-Name Charts (Kelly, 1964) were used for determining colour designations of the substrate mycelium. Media and procedures used for determination of physiological and biochemical features and carbon source utilization were those described by Shirling & Gottlieb (1966), Goodfellow (1971) and Gordon et al. (1974).

The isomer of A\(_2\)pm and the sugars in whole-cell hydrolysates were determined by the methods of Hasegawa et al. (1983). The presence of mycolic acids was examined by TLC following Minnikin et al. (1975), and phospholipids were extracted and identified following the method of Minnikin et al. (1984). Menaquiones were extracted and purified by the method of Collins et al. (1977), then analysed by HPLC (model 600, Waters) using a Nova-Pak C18 column. The cellular fatty acid compositions of strains 0351M-12\(^T\) and 0385M-1\(^T\) were determined by using the Sherlock Microbial Identification System (MIDI). Extracts of the methylated fatty acids were prepared according to the protocol provided by the manufacturer and analysed with a 5890 gas chromatograph equipped with a flame-ionization detector and an automatic injector with a G1512A controller (Hewlett Packard). Identification of the peaks was made by comparing the results with the built-in TSBA 40 database (MIDI).

Total DNA was extracted from 14-day-old cells by using the Qiagen genomic DNA kit. The G+C content of the DNA was determined by the HPLC method of Tamaoka & Komagata (1984). The 16S rRNA gene was PCR-amplified by using the method of Nakajima et al. (1999) and was sequenced directly on an ABI model 3730 automatic DNA sequencer by using a PRISM ready reaction dye primer cycle sequencing kit (Applied Biosystems). Phylogenetic analysis was performed using the software packages PHYLIP (Felsenstein, 1993) and MEGA version 2.1 (Kumar et al., 2001) after multiple alignment of the sequences by CLUSTAL_X (Thompson et al., 1997). Genetic distances were calculated using the Kimura two-parameter model (Kimura, 1980, 1983) and clustering was performed by using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis was used to evaluate the tree topology of the neighbour-joining data by performing 1000 resamplings (Felsenstein, 1985).

Strains 0351M-12\(^T\) and 0385M-1\(^T\) produced branched and unfragmented substrate mycelia; no distinct aerial hyphae were found. Short spore chains were borne on the tips of sporophores arising from the substrate mycelia. The spore chains were composed of 10–20 non-motile, smooth-surfaced, oval spores. Scanning electron micrographs of strains 0351M-12\(^T\) and 0385M-1\(^T\) are shown in Fig. 1 and Fig. 2. The cultural characteristics of strains 0351M-12\(^T\) and 0385M-1\(^T\) are shown in Supplementary Table S1. Growth was good on most of the media tested and the substrate mycelia on most media were yellow. Sporulation occurred on oatmeal-nitrate, yeast-extract-starch (only strain 0351M-12\(^T\)), oatmeal, inorganic salts-starch (only strain 0385M-1\(^T\)) and glycerol-asparagine (only strain 0351M-12\(^T\)) agar. No soluble pigments were produced on any of the media tested. The results of the physiological and biochemical tests are indicated in Table 1 and in the species descriptions.

Strains 0351M-12\(^T\) and 0385M-1\(^T\) contained glucose, galactose, mannose, ribose and a trace amount of madurose in whole-cell hydrolysates, and the cell-wall peptidoglycan contained meso-A\(_2\)pm. The predominant menaquinone was MK-10(H\(_4\)) and minor components were MK-10(H\(_6\)) and MK-9(H\(_4\)). Mycolic acids were not detected. The phospholipids were phosphatidyldiethanolamine (PE), phosphatidy-l-N-monomethylethanolamine (PME) and a phospholipid of unknown structure containing glucosamine (GlcNU) (phospholipid type PIV of Lechevalier et al., 1977). The fatty acid methyl ester profiles of strains 0351M-12\(^T\) and 0385M-1\(^T\) are shown in Supplementary Table S2. The G+C contents of the genomic DNA were 70.6 and 70.7 mol%, respectively. The almost-complete 16S rRNA gene sequences of strains 0351M-12\(^T\) (1517 nt) and 0385M-1\(^T\) (1515 nt) were determined. Preliminary comparison of the sequences against

![Fig. 1. Scanning electron micrograph of strain 0351M-12\(^T\) after growth on oatmeal agar at 28 °C for 14 days. Bar, 10 μm](http://ijs.sgmjournals.org)
the GenBank database indicated that the two isolates belonged to the family Streptosporangiaceae (Fig. 3) and were most closely related to *H. cretacea* NBRC 15474T. The binary similarities of the two isolates to *H. cretacea* were 98 %. The results of DNA–DNA hybridization among isolates 0351M-12T, 0385M-1T and *H. cretacea* NBRC 15474T are shown in Supplementary Table S3. The relatedness between the isolates and *H. cretacea* NBRC 15474T was 40.7–48.5 %. The value of DNA–DNA hybridization between strain 0351M-12T and strain 0385M-1T was 53.6–54.9 %.

The DNA–DNA relatedness values of the isolates to each other and to the type strain of *H. cretacea* were lower than 70 %, which is the recommended threshold value for the delineation of genomic species (Wayne et al., 1987). The physiological characteristics also showed (Table 1) that the isolates were distinguishable from *H. cretacea* NBRC 15474T. Therefore, we propose that the isolates represent novel species of the genus *Herbidospora*, for which we propose the names *Herbidospora yilanensis* sp. nov. and *Herbidospora daliensis* sp. nov.

**Table 1.** Phenotypic properties useful for distinguishing strains 0351M-12T and 0385M-1T from *H. cretacea* NBRC 15474T

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decomposition of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypoxanthine</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Carbon source utilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L-Arabinose</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>myo-Inositol</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Mannose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Salicin</td>
<td>+</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Trehalose</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Requirement for:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Riboflavin</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Thiamine</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Organic acid utilization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumaric acid</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>DL-Lactic acid</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Mucic acid</td>
<td>−</td>
<td>−</td>
<td>+</td>
</tr>
<tr>
<td>Succinic acid</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>NaCl tolerance (%)</td>
<td>1</td>
<td>5</td>
<td>1</td>
</tr>
</tbody>
</table>

Strains: 1, 0351M-12T; 2, 0385M-1T; 3, *H. cretacea* NBRC 15474T. +, Positive; −, negative.

**Description of *Herbidospora yilanensis* sp. nov.**

*Herbidospora yilanensis* (yi.lan.en’sis. N.L. fem. adj. yila-nensis pertaining to Yilan county, Taiwan, where the micro-organism was isolated).

Cells are Gram-positive, aerobic, non-acid-fast and mesophilic. Produces branched and unfragmented substrate mycelia; no aerial hyphae are found. Short spore-chains are borne on the tips of sporophores arising from the substrate mycelium. Spore chains are composed of 10–20 non-motile, smooth-surfaced, oval spores. The substrate mycelium is yellowish white to yellow. No soluble pigments are produced. Good growth occurs on most media, but sporulation only occurs on oatmeal-nitrate, yeast-extract-starch, oatmeal and glyceral-asparagine agars. Growth occurs between 20 and 40 °C; NaCl tolerance is 1 % on nutrient agar. Decomposes starch but not adenine, aesculin, hypoxanthine, tyrosine or xanthine. Fructose, fumaric acid, galactose, glucose, glycerol, DL-lactic acid, myo-inositol, maltose, mannitol, mannose, melibiose, melezitose, raffinose, rhamnose, salicin, sucrose, succinic acid and trehalose are utilized. Adonitol, L-arabinose, benzoic acid, cellobiose, cellulose, dulcitol, erythritol, L-malic acid, mucic acid, propionic acid, D-ribose, D-sorbitol, L-tartaric acid and xylose are not utilized. p-Aminobenzoic acid, myo-inositol and thiamine are required for growth. Biotin, nicotinic acid, pantothentic acid and pyridoxine are not required for growth. Melanin is not produced. Lysozyme sensitive. Glucose, galactose, mannose, ribose and a trace amount of madurose occur in whole-cell hydrolysates. The cell-wall peptidoglycan contains meso-A2pm. The predominant menaquinone is MK-10(H4) and the minor components are MK-10(H2) and MK-9(H2). The phospholipids are PE, PME and GlcNU. The cellular fatty acid profile contains 10-methyl C17:0 (41.94 %), iso-C16:0 (18.44 %), C17:1 8c (6.34 %), C17:0 (4.47 %), C15:0 2-OH (4.39 %), C16:0 (3.88 %) and C17:1 9c (3.83 %). The DNA G+C content of the type strain is 70.6 %.

The type strain is 0351M-12T (=FIRDI 003T =BCRC 16875T =LMG 24337T), which was isolated from sediment from Yilan, a county in the north of Taiwan.
Description of *Herbidospora daliensis* sp. nov.

*Herbidospora daliensis* (da.li.en’sis. N.L. fem. adj. daliensis pertaining to Dali city, Taiwan, where the micro-organism was isolated).

Cells are Gram-positive, aerobic, non-acid-fast and mesophilic. Produced branches and unfragmented substrate mycelia; no aerial hyphae are found. Short spore-chains are borne on the tips of sporophores arising from the substrate mycelium. The spore chains are composed of 10–20 non-motile, smooth-surfaced, oval spores. Growth occurs between 20 and 40°C; NaCl tolerance is 5% on nutrient agar. Decomposes starch but not adenine, hypoxanthine, tyrosine or xanthine. L-Arabinose, fructose, galactose, glucose, maltose, mannitol, melezitose, raffinose, rhamnose and sucrose are utilized. Adonitol, benzoic acid, cellobiose, cellulose, dulcitol, erythritol, fumaric acid, glycerol, *myo*-inositol, DL-lactic acid, L-malic acid, mannose, melibiose, mucic acid, propionic acid, D-ribose, salicin, D-sorbite, succinic acid, L-tartaric acid, trehalose and xylose are not utilized. Requires p-aminobenzoic acid and *myo*-inositol for growth. Biotin, nicotinic acid, pantathenic acid, pyridoxine, riboflavin and thiamine are not required for growth. Melanin is not produced. Lysozyme sensitive. Glucose, galactose, mannose, ribose and a trace amount of madurose occur in whole-cell hydrolysates. The cell-wall peptidoglycan contains *meso*-Apm. The predominant menaquinone is MK-10(H₉) and the minor components are MK-10(H₈) and MK-9(H₉). The phospholipids are PE, PME and GlcNU. The cellular fatty acid profile contains 10-methyl C₁₇ : 0 (36.28%), C₁₇ : 1v8c (16.09%), isoC₁₆ : 0 (10.83%), C₁₇ : 0 (9.21%), C₁₅ : 0 2-OH (6.66%), C₁₇ : 1v6c (3.63%) and C₁₆ : 0 (3.03%). The DNA G + C content of the type strain is 70.7%.

The type strain is 0351M-1² (=FIRDI 004² =BCRC 16876² =LMG 24336²), which was isolated from sediment from central Taiwan.

Acknowledgements

This research was supported partially by the Ministry of Economic Affairs (project no. 93-EC-17-A-17-R7-0525). We thank Mr J. H. Chien and Dr S. Y. Hsieh for assistance with sample collection. We also thank Dr Wen-Jun Li for correcting this manuscript.

References


Felsenstein, J. (1993). *PHYLIP* (phylogeny inference package), version 3.5c. Distributed by the author, Department of Genome Sciences, University of Washington, Seattle, USA.

http://ijs.sgmjournals.org


