Herbidospora gen. nov., a New Genus of the Family *Streptosporangiaceae* Goodfellow et al. 1990

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Eight actinomycete strains originally isolated from soil and plant samples were studied to determine their taxonomic status. All isolates produced branching substrate mycelia, but no distinct aerial hyphae. Relatively short chains of nonmotile spores (10 to 30 spores per chain) were borne on the tips of sporophores arising directly from the agar surface. The chemotaxonomic characteristics of the isolates, with the exception of the menaquinone profile, coincided with those of members of the family *Streptosporangiaceae* Goodfellow, Stanton, Simpson, and Minnikin 1990. Furthermore, the results of a phylogenetic analysis performed with 5S rRNA support the conclusion that the isolates should be classified in this family. The isolates differed from members of the constituent genera of the *Streptosporangiaceae* in morphological characteristics and menaquinone composition. Therefore, we propose a new genus for the strains, *Herbidospora*. The type species and type strain are *Herbidospora cretacea* sp. nov. and strain K-319 (= JCM 8553), respectively.

The tentative name “maduromycete group” was used by Goodfellow and Cross in 1984 (12) for the chemotaxonomically defined actinomycete group which has cell wall type III and whole-cell sugar pattern B of Lechevalier and Lechevalier (28). At that time, this group included the genera *Actinomadura*, *Microbispora*, *Microtetraspora*, *Planobispora*, *Planomonospora*, *Spirillospora*, and *Streptosporangium*. Subsequently, the genera *Actinomadura* and *Spirillospora* were eliminated from this group (11) on the basis of cellular lipid profiles (1, 2, 23, 29) and phylogenetic relationships (9). In 1990, the family *Streptosporangiaceae* was proposed by Goodfellow et al. (13) for the redefined maduromycete group. The taxonomic properties of the genera in this family are similar to each other. These organisms are characterized by the following common features. Substrate and aerial mycelia are well developed and branched. Spore chains or sporangia are formed on the tips of sporophores branching from the aerial hyphae. *meso*-Diaminopimelic acid is present in the cell walls, but glycine is not (wall chemotype III). The *N*-acyl type of muramic acid in the cell walls is an acetyl type (21). The cellular fatty acids consist of straight, iso-branched, and 10-methylated acids (23, 25). The predominant isoprenoid quinones are unsaturated, dihydrogenated, and tetrahydrogenated menaquinones with nine isoprene units [MK-9, MK-9(H₂), and MK-9(H₄), respectively] (1, 2, 23, 25, 26, 32); the tetrahydrogenation occurs at the sites of isoprene units III (the third unit from the 2-methyl-1,4-naphthoquinone moiety) and VIII [MK-9(III,VIII-H₄)] (4, 25). Phosphatidylethanolamine and glucosamine-containing phospholipids are present as diagnostic polar lipids (phospholipid type PIV of Lechevalier et al. [27]). At present, these genera are distinguished by morphological features, including the existence of sporangia and the number of spores per sporangium or chain.

We isolated eight actinomycete strains with unusual morphological features from soil and plant samples in Japan.

Although these isolates differed in morphology from the previously described members of the family *Streptosporangiaceae*, all of their chemotaxonomic characteristics, except their menaquinone profiles, agreed with those of members of this family. Furthermore, the results of a phylogenetic analysis performed with 5S rRNA sequences supported the classification of these organisms in the *Streptosporangiaceae*. In this paper we describe our isolates as members of a new species of a new genus in the family *Streptosporangiaceae* on the basis of morphological, chemical, physiological, and biochemical characteristics, levels of DNA-DNA relatedness, and 5S rRNA sequences. The names *Herbidospora* gen. nov. and *Herbidospora cretacea* sp. nov. are proposed for the new genus and species.

MATERIALS AND METHODS

Bacterial strains. Eight strains isolated from plant and soil samples in Japan (Table 1) were investigated. The plant samples from which strains I-186, I-385, I-404, and I-405 were isolated were desiccated at 28°C for at least 1 week and were ground with a blender after sterile water was added. The suspension was incorporated into yeast extract agar containing 0.2 g of yeast extract (Difco Laboratories, Detroit, Mich.), 50 mg of cycloheximide, 50 mg of nystatin, and 15 g of agar in 1,000 ml of distilled water, and this preparation was poured into a petri dish. After incubation at 28°C for more than 2 weeks, colonies that appeared were picked with a sterile needle under a stereomicroscope. Strains K-83, K-319^T (T = type strain), SF2625, and SF2742 were isolated by the dilution agar plating method by using yeast extract-starch agar (2 g of yeast extract [Difco], 10 g of starch, and 15 g of agar in 1,000 ml of distilled water; pH 7.3) and the humic acid-vitamin agar of Hayakawa and Nonomura (15).

The following type strains were used for the comparative studies: *Actinomadura madurae* JCM 7436, *Microbispora rosea* JCM 3006, *Microtetraspora glauca* JCM 3300, *Nocardopsis dassonvillei* JCM 7437, *Planobispora longispora* JCM 3092, *Planomonospora parantospora* JCM 3093, *Planomonospora venezuelensis* JCM 3167, *Saccharothrix aus-*

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TABLE 1. List of the isolates used in this study and their sources

Strain	Source
I-186 (= JCM 8548) ^a	Decayed leaves collected in Saitama, Japan
I-385 (= JCM 8549)	Bark of Japanese red pine (<i>Pinus densiflora</i>) collected in Saitama, Japan
I-404 (= JCM 8550)	Petal of daisy fleabane (<i>Erigeron annuus</i>) collected in Saitama, Japan
I-405 (= JCM 8551)	Petal of daisy fleabane (<i>Erigeron annuus</i>) collected in Saitama, Japan
K-83 (= JCM 8552)	Soil collected in Saitama, Japan
K-319 ^T (= JCM 8553 ^T)	Soil collected in Saitama, Japan
SF2625 (= JCM 8554)	Soil collected in Tokyo, Japan
SF2742 (= JCM 8555)	Soil collected in Okinawa, Japan

^a JCM, Japan Collection of Microorganisms, RIKEN, Wako, Saitama, Japan.

traliensis JCM 3370, *Streptosporangium roseum* JCM 3005, and *Streptosporangium viridigriseum* JCM 3282.

Morphology. Cultures were grown on oatmeal agar (International *Streptomyces* Project [ISP] medium 3) for 14 days at 28°C and observed with a light microscope and a scanning electron microscope (model JEM 100C-ASID; JEOL, Ltd., Tokyo, Japan). Samples for scanning electron microscopy were prepared by cutting an agar block on which the culture grew, fixing it in osmium tetroxide vapor at room temperature for 2 h, and then freeze-drying it. The specimen was sputter-coated with gold under a vacuum.

Cultural, physiological, and biochemical characteristics. Cultural characteristics were tested by using 21-day cultures grown at 28°C on various agar media (Table 2). The *Color Harmony Manual* (18) was used to determine colony colors. Decomposition of various compounds was examined by using the basal media of Gordon et al. (14) and Goodfellow (10). Utilization of carbohydrates as sole carbon sources was tested by using yeast nitrogen base without amino acids (Difco) as a basal medium and the method described by Stevenson (40). Utilization of organic acids was examined on the defined medium of Gordon et al. (14) supplemented with the vitamin B complex solution used in humic acid-vitamin agar (15). The requirements for vitamins (each at a concentration of 0.5 µg/ml) were examined on a defined medium containing 10 g of glucose, 2 g of vitamin assay Casamino Acids (Difco), 0.5 g of KCl, 0.5 g of MgSO₄ · 7H₂O, 0.3 g of K₂HPO₄, and 15 g of purified agar (Difco) in 1,000 ml of distilled water (pH 7.2). Formation of a melaninlike pigment was examined on peptone-yeast extract-iron agar (ISP medium 6) and tyrosine agar (ISP medium 7). Growth temperature was determined on yeast extract-starch agar by using a

temperature gradient incubator (model TN-3; Advantec Toyo, Tokyo, Japan). Bennett's agar medium was used to determine tolerance to sodium chloride, lysozyme, and antibiotics (Table 3).

Chemotaxonomy. Freeze-dried cells for chemotaxonomic analyses were obtained from cultures grown in yeast extract-glucose broth (containing 10 g of yeast extract and 10 g of D-glucose in 1,000 ml of distilled water; pH 7.2) on a rotary shaker at 30°C. Cell wall peptidoglycan was prepared and hydrolyzed by the methods of Kawamoto et al. (21), and the amino acid composition was analyzed with an automatic amino acid analyzer (model 835; Hitachi Ltd.). The isomers of diaminopimelic acid in cell walls were determined by the method of Stanek and Roberts (39), and whole-cell sugars were detected by the methods of Stanek and Roberts (39) and Meyertons et al. (30). The cell wall acyl type was determined by the method of Uchida and Aida (45). Phospholipids in cells were extracted and identified by the method of Minnikin et al. (31). Methyl esters of cellular fatty acids were prepared by the direct transmethylation method with methanolic hydrochloride (41) and were analyzed by using a gas-liquid chromatograph (model GC-7A; Shimadzu Corp., Kyoto, Japan) equipped with a fused-silica capillary column coated with OV-1 (0.24 mm by 25 m; Shimadzu Corp.). The temperature of the column was programmed to increase from 180 to 220°C at a rate of 1°C/min, and the injector was kept at 250°C. The peaks were identified by comparison with authentic samples and by using a gas-liquid chromatograph-mass spectrometer (model QP-1000; Shimadzu Corp.) equipped with the column described above. The quantitative fatty acid data were numerically analyzed by using the NTSYS-pc program package (Applied Biostatistics

TABLE 2. Cultural characteristics of *H. cretacea*

Agar medium ^a	Strain K-319 ^{Tb}		Strain SF2742	
	Growth (reverse side color) ^c	Sporulation (aerial mass color) ^c	Growth (reverse side color) ^c	Sporulation (aerial mass color) ^c
Bennett	Good (light amber [3ic])	Poor (white)	Good (camel [3ie])	None
Glucose-asparagine	None to poor (colorless)	None	None to poor (colorless)	None
Glycerol-asparagine	Poor (colorless)	None	Poor (colorless)	None
Hickey-Tresner	Good (cinnamon [3le])	Poor (white)	Good (light amber [3ic])	Poor (white)
Inorganic salts-starch	Poor (colorless)	None	Poor (colorless)	None
Oatmeal	Good (bamboo [2gc])	Good (white)	Good (light tan [3gc])	Good (bamboo [2fb])
Oatmeal-nitrate	Moderate (colorless)	Good (white)	Moderate (camel [3ie])	Good (bamboo [2fb])
Sucrose-nitrate	None to poor (colorless)	None	None to poor (colorless)	None
Yeast extract-malt extract	Good (mustard gold [2pe])	Poor (white)	Good (cinnamon [3le])	Poor (white)
Yeast extract-starch	Good (light tan [3gc])	Moderate (white)	Good (camel [3ie])	Good (bamboo [2fb])

^a No diffusible pigments were produced on any of the media.

^b All of the other isolates except strain SF2742 have characteristics similar to those of strain K-319^T.

^c Color names and designations were taken from the *Color Harmony Manual* (18).

TABLE 3. Physiological and biochemical characteristics of *H. cretacea*^a

Characteristic	Reaction ^b
Decomposition of:	
Adenine.....	—
Arbutin.....	—
Casein.....	+
DNA.....	+
Esculin.....	+
Guanine.....	—
Hypoxanthine.....	+
Keratin.....	—
Starch.....	+
Testosterone.....	v
Tyrosine.....	—
Urea.....	v
Xanthine.....	—
Growth on the following compounds as sole carbon sources (1%, wt/vol):	
Adonitol.....	—
L-Arabinose.....	+
Cellobiose.....	+
Cellulose.....	—
Dulcitol.....	—
<i>i</i> -Erythritol.....	—
D-Fructose.....	+
D-Galactose.....	+
D-Glucose.....	+
Glycerol.....	—
<i>myo</i> -Inositol.....	—
Maltose.....	+
D-Mannitol.....	+
D-Mannose.....	+
Melezitose.....	—
Melibiose.....	±
Raffinose.....	—
L-Rhamnose.....	v
D-Ribose.....	+
Salicin.....	v
D-Sorbitol.....	±
Starch.....	+
Sucrose.....	+
Trehalose.....	v
D-Xylose.....	+
Utilization of:	
Benzoic acid.....	—
Citric acid.....	—
Fumaric acid.....	+
DL-Lactic acid.....	+
L-Malic acid.....	+
Mucic acid.....	—
Oxalic acid.....	—
Propionic acid.....	—
Succinic acid.....	+
L-Tartaric acid.....	—
Requirement for:	
<i>p</i> -Aminobenzoic acid.....	—
Biotin.....	—
Inositol.....	—
Nicotinic acid.....	—
Pantothenic acid.....	—
Pyridoxine.....	—
Riboflavin.....	—
Thiamine.....	+
Formation of melaninlike pigment in:	
Peptone-yeast extract-iron agar.....	NG
Tyrosine agar.....	—
Growth in the presence of:	
Lysozyme (0.005%, wt/vol).....	—
Gentamicin (50 µg/ml).....	—
Novobiocin (20 µg/ml).....	—
Rifampin (20 µg/ml).....	—
Streptomycin (50 µg/ml).....	—
Vancomycin (50 µg/ml).....	—

^a The data are based on the reactions of our eight isolates. In addition, *H. cretacea* tolerates NaCl concentrations of <3% and grows at temperatures ranging from 13 to 37°C.

^b +, positive; ±, doubtful; —, negative; v, variable; NG, no growth.

tics, Inc., Setauket, N.Y.), and the results are presented below as a dendrogram drawn by using unweighted pair-group arithmetic average clustering (38). Methyl esters of cellular mycolic acids were prepared and detected as described by Tomiyasu (44). Isoprenoid quinones were extracted by the method of Collins et al. (5) and were analyzed by using a high-performance liquid chromatograph (HPLC) (model LC-6A; Shimadzu Corp.) (42) and a mass spectrometer (model QP-1000). The HPLC was equipped with a Cosmosil 5C₁₈ column (4.6 by 150 mm; Nacalai Tesque, Inc., Kyoto, Japan), and the elution solvent was a mixture of methanol and 2-propanol (2:1, vol/vol). The positions of hydrogenation of MK-10(H₄) obtained from the isolates were determined with a linked scan-fast atom bombardment mass spectrometer (model JMS SX102; JEOL, Ltd.).

Preparation and sequence analysis of 5S rRNAs. 5S rRNAs were prepared as described by Park et al. (33). The nucleotide sequence was determined by both chemical (35) and enzymatic (7) methods. Both terminal bases at the 3' and 5' ends were determined by thin-layer chromatography as described by Dekio et al. (6). The sequence alignment was based on the secondary structures constructed by the method described by Hori and Osawa (16). Evolutionary distances were represented by the *K*_{nuc} value of Kimura (22), and a phylogenetic tree was constructed by the neighbor-joining method of Saitou and Nei (37).

Preparation of DNA, DNA base composition, and DNA-DNA hybridization. DNA was isolated from cells grown in yeast extract-glucose broth by using the method of Saito and Miura (36). The guanine-plus-cytosine (G+C) content of the DNA was determined by the HPLC method (20, 43). An equimolar mixture of nucleotides for analysis of DNA base composition (Yamasa Shoyu Co., Ltd., Choshi, Japan) was used for calculation of G+C content. DNA-DNA relatedness was measured fluorometrically by using the microplate hybridization method described by Ezaki et al. (8).

Nucleotide sequence accession numbers. The new nucleotide sequence data reported in this paper appear in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under the following accession numbers: *Actinomyces madurae* JCM 7436^T, D13615; *Microtetraspora glauca* JCM 3300^T, D13616; *N. dassonvillei* JCM 7437^T, D13617; *Planobispora longispora* JCM 3092^T, D13618; *Saccharothrix australiensis* JCM 3370^T, D13619; *Streptosporangium roseum* JCM 3005^T, D13620; *Streptosporangium viridogriseum* JCM 3282^T, D13621; and *Herbidospira cretacea* K-319^T (= JCM 8553^T), D13622.

RESULTS

Morphology. All of the isolates produced well-developed, branched, non-fragmented substrate mycelia. Macroscopically, they formed apparent aerial masses on some media, but no distinct aerial hyphae were observed with light and scanning electron microscopes (Fig. 1 and 2). Straight, relatively short chains of nonmotile spores (10 to 30 spores per chain) were borne on the tips of sporophores branching from the vegetative hyphae. In an old culture incubated for more than 3 weeks, the tips of the spore chains were often entangled, resulting in globular forms. Each spore was oval, and its surface was smooth. No sporangia were observed.

Cultural, physiological, and biochemical characteristics. Cultural characteristics of our isolates are shown in Table 2.

FIG. 1. Light micrograph of *H. cretacea* SF2625 grown on oatmeal agar (ISP medium 3) for 14 days at 28°C.

All isolates except strain SF2742 had similar characteristics. Generally, the vegetative mycelium was colorless to yellowish brown, and no distinct pigments were produced. When sporulation occurred, the surface of the colony was white.

No diffusible pigments were produced in any agar medium, and growth on defined media did not occur or was very poor. The strain SF2742 colony surface became brownish yellow on some media.

FIG. 2. Scanning electron micrographs of *H. cretacea* SF2625 grown on oatmeal agar (ISP medium 3) for 14 days at 28°C. (B) Bar = 10 µm.

TABLE 4. Cellular fatty acid compositions of *H. cretacea* strains and selected type strains

Strain	Fatty acid composition (%)																					
	Normal acids										Iso acids					10-Methyl acids						
	14:0 ^a	16:0	16:1	2-OH 16:0	18:0	18:1	13:0	15:0	17:0	17:1	2-OH 17:0	19:1	14:0	16:0	2-OH 16:0	18:0	18:1	15:0	17:0	10-Me 16:0	10-Me 17:0	10-Me 18:0
<i>H. cretacea</i> strains																						
I-186	1	6	1	2	4	1	1	5	8	6	2	1	2	15	4	1	3	1	1	1	28	3
I-385	2	5	2	1	4	1	1	9	6	4	tr ^b	tr	tr	21	2	1	3	1	1	3	27	4
I-404	3	13	1	3	5	2	1	5	8	6	1	1	1	7	2	tr	2	2	2	3	24	6
I-405	3	13	1	3	5	2	1	5	8	6	1	1	1	7	2	tr	2	2	2	4	23	6
K-83	3	11	2	3	3	2	1	6	9	8	1	1	1	6	2	tr	1	3	3	3	24	5
K-319 ^c	3	10	1	2	4	3	1	6	7	7	2	2	1	7	2	tr	2	2	2	3	27	6
SF2625	1	9	1	2	3	2	1	7	14	12	2	1	1	8	2	1	1	2	2	1	22	3
SF2742	3	16	3	5	3	2	1	7	5	7	1	tr	tr	7	2	tr	1	1	1	5	22	5
Members of the <i>Streptosporangiaceae</i>																						
<i>Streptosporangium roseum</i> JCM 3005 ^T	tr	5	1	1	1	1	1	7	8	9	1	8	20	1	1	2	tr	tr	1	1	30	3
<i>Microbispora rosea</i> JCM 3006 ^T	tr	4	1	1	1	1	1	7	17	8	tr	tr	29	1	1	tr	tr	3	2	3	17	1
<i>Microbispora glauca</i> JCM 3300 ^T	1	4	2	1	2	2	3	5	5	8	tr	tr	1	34	8	2	3	3	1	2	14	3
<i>Planobispora longispora</i> JCM 3092 ^T	4	14	3	1	2	14	4	7	14	31	tr	tr	1	1	tr	1	tr	tr	tr	3	3	1
<i>Planomonospora parentospora</i> JCM 3093 ^T	tr	8	2	tr	5	7	1	5	32	24	tr	tr	tr	5	tr	1	tr	tr	tr	tr	3	1
<i>Planomonospora venezuelensis</i> JCM 3167 ^T	2	6	1	tr	1	6	5	10	15	28	tr	tr	2	8	tr	2	tr	tr	tr	10	2	2
<i>A. madure</i> JCM 7436 ^T	2	39	3	tr	10	22	1	2	2	1	tr	tr	4	4	1	tr	tr	tr	tr	1	1	14
<i>N. dassonvillei</i> JCM 7437 ^{Tc}	1	1	tr	tr	9	8	1	1	1	1	1	1	1	29	4	6	2	23	11	1	2	17
<i>Saccharothrix australiensis</i> JCM 3370 ^{Td}	1	1	tr	tr	1	1	1	1	1	1	tr	tr	1	12	4	tr	tr	tr	tr	1	1	1

^a Number of carbon atoms:number of double bonds.^b tr, trace (less than 0.5%).^c This strain also contains 21% anteiso-C_{17:0}.^d This strain also contains 15% anteiso-C_{17:0}, 8% iso-C_{17:0} and 4% anteiso-C_{15:0}.

FIG. 3. Dendrogram showing relationships among strains of *H. cretacea* and related organisms based on fatty acid compositions (Table 5). Data were analyzed by using the NTSYS-pc program package, and clustering was performed by using unweighted pair-group arithmetic average analysis.

All of the isolates also resembled each other in physiological and biochemical characteristics (Table 3).

Cell chemistry. The isolates were homogeneous in their chemotaxonomic properties. The molar ratio of glutamic acid to alanine to diaminopimelic acid to glycine in the hydrolysates of the purified cell walls was 1.00:1.46:0.99:0.14 (calculated by defining the amount of glutamic acid as 1.00). The isomer of diaminopimelic acid was the *meso* isomer. As the amount of glycine was not sufficient to determine the cell wall type of Lechevalier and Lechevalier (28), we concluded that the cell wall type was type III. The acyl type of muramic acid in the cell walls was determined to be the acetyl type. Glucose, mannose, ribose, and a trace amount of madurose (3-*O*-methyl-D-galactose) were detected as whole-cell sugars, but galactose, arabinose, xylose,

and rhamnose were absent (whole-cell sugar pattern B of Lechevalier and Lechevalier [28]).

The polar lipid profile was characterized by the presence of phosphatidylethanolamine and its derivatives and ninhydrin-positive glycerophospholipids. This result suggests that the phospholipid pattern of the isolates is the type PIV pattern of Lechevalier et al. (27). The cellular fatty acid compositions of the isolates and of some type strains used for comparison are shown in Table 4. The fatty acids of the isolates consisted of straight, iso-branched, 10-methyl, and 2-hydroxyl acids, and the members of the family *Streptosporangiaceae* tested had a similar pattern (type 3c of Kroppenstedt [23]). The results of a cluster analysis based on fatty acid compositions are shown in Fig. 3. Mycolic acids were not detected in the cells of the isolates. The predominant menaquinone was MK-10(H₄); small amounts of MK-10(H₆), MK-10(H₂), MK-10(H₀), and MK-9(H₄) were also present (Table 5). The retention time of the MK-10(H₄) molecule obtained from the isolates in the HPLC analysis was manifestly different from that of the previously known MK-10(H₄) molecule, and this fact suggested that these two MK-10(H₄) molecules were isomeric. Figure 4 shows the linked scan mass spectrum of MK-10(H₄) prepared from strain K-319^T, and the fragment ion peaks of this spectrum indicate that the molecule is saturated at the sites of units III and IX [MK-10(III,IX-H₄)]. These positions of hydrogenation have not been reported previously. The G+C contents of our isolates were 69 to 71 mol% (Table 6).

5S rRNA sequence. The nucleotide sequences of the 5S rRNAs from one of our isolates (strain K-319^T) and some authentic type strains are shown in Fig. 5. The sequence of *Streptomyces griseus* was taken from the data of Park et al. (34). Figure 6 shows an unrooted phylogenetic tree based on the sequence data, which indicates that the 5S rRNA sequence of strain K-319^T is very similar to the 5S rRNA sequences of members of the family *Streptosporangiaceae*, particularly *Streptosporangium roseum* and *Planobispora longispora*. The phylogenetic tree also indicates that

TABLE 5. Menaquinone compositions of *H. cretacea* strains and selected type strains

Strain	Menaquinone composition (%)										
	MK-9 (H ₀)	MK-9 (H ₂)	MK-9 (II,III-H ₄) ^a	MK-9 (III,VIII-H ₄) ^a	MK-9 (H ₆)	MK-9 (H ₈)	MK-10 (H ₀)	MK-10 (H ₂)	MK-10 (II,III-H ₄) ^a	MK-10 (III,IX-H ₄) ^b	MK-10 (H ₆)
<i>H. cretacea</i> strains											
I-186				5	tr ^c		4	12		72	7
I-385				4	tr		5	11		69	10
I-404				7	1		3	9		67	12
I-405				7	1		3	10		66	11
K-319 ^T				6	1		2	7		67	17
K-83				6	1		4	12		65	12
SF2625				3			2	12		74	8
SF2742				4			6	12		71	6
Members of the <i>Streptosporangiaceae</i>											
<i>Microbispora rosea</i> JCM 3006 ^T	7	44		41	3	1		2			
<i>Microtetraspora glauca</i> JCM 3300 ^T	16	18		57	6	tr					
<i>Planobispora longispora</i> JCM 3092 ^T	5	55		31	4	tr					
<i>Planomonospora parontospora</i> JCM 3093 ^T	10	74		10	tr						
<i>Planomonospora venezuelensis</i> JCM 3167 ^T	9	55		30	2						
<i>Streptosporangium roseum</i> JCM 3005 ^T	2	42		48	7	tr					
<i>A. madurae</i> JCM 7436 ^T			6		73	20					
<i>N. dassonvillei</i> JCM 7437 ^T	3	3					26	30	23		6
<i>Saccharothrix australiensis</i> JCM 3370 ^T	3	3	56					2	28		

^a Positions of hydrogenation were determined by comparing retention times with retention times of previously described structures.

^b Positions of hydrogenation were determined by mass spectrometry by the linked scan method (Fig. 3).

^c tr, trace (less than 1%).

FIG. 4. Linked scan-fast atom bombardment mass spectrum of MK-10(H₄) obtained from *H. cretacea* K-319^T (= JCM 8553^T) and assignment of the fragment peaks.

Streptosporangium viridogriseum is located apart from the other members of the family.

DNA-DNA hybridization. The levels of DNA relatedness are shown in Table 6. There were three homology groups defined at a level of more than 70% among our isolates. The first group contained strains I-186, I-404, I-405, K-83, and K-319^T. The second group consisted of strains I-385 and SF2742, and the third group was a single-member group containing strain SF2625. However, the levels of relatedness among these three groups were relatively high, ranging from 44 to 68%.

DISCUSSION

The family *Streptosporangiaceae* is a taxon mainly defined by chemotaxonomy, and our isolates shared chemotaxonomic characteristics with members of this family in

many ways (Table 7), including a type III cell wall composition, *N*-acetylated muramic acid in the cell walls, and a type PIV phospholipid composition. Fatty acid type 3c, characterized by iso-hexadecanoic, *n*-heptadecenoic, 10-methyl heptadecanoic, and 2-hydroxy acids (24), is also the fatty acid type. The dendrogram based on the quantitative fatty acid analyses (Fig. 3) shows that the isolates and the members of the family are not clearly separated by this cluster analysis, while they are distinguished from other strains having cell wall type III which are not in the family *Streptosporangiaceae* (*A. madurae*, *N. dassonvillei*, and *Saccharothrix australiensis* strains). On the other hand, our isolates have a novel menaquinone, MK-10(III,IX-H₄), which is different from the menaquinone of the previously described members of the family *Streptosporangiaceae*. The previously described members of this family have MK-9(III,VIII-H₄) exclusively as their tetrahydrogenated

TABLE 6. DNA base compositions and levels of DNA relatedness among *H. cretacea* strains

Strain	G+C content (mol%)	% of DNA complementarity with labeled DNA from:								
		K-319 ^T	I-186	I-404	I-405	K-83	I-385	SF2742	SF2625	JCM 3005 ^T
<i>H. cretacea</i> strains										
K-319 ^T	70	100	102	89	80	100	57	69	57	26
I-186	70	84	100	93	88	82	61	65	53	26
I-404	70	82	87	100	96	91	61	62	54	25
I-405	70	77	90	104	100	83	60	64	51	26
K-83	70	88	94	88	87	100	65	68	57	27
I-385	71	54	64	64	68	56	100	81	58	29
SF2742	70	44	62	56	63	45	70	100	44	20
SF2625	69	49	68	68	64	58	57	66	100	26
<i>Streptosporangium roseum</i> JCM 3005 ^T	70	14	22	25	27	15	20	21	18	100

TABLE 7. Comparison of the genus *Herbidospora* with related genera of actinomycetes having type III cell walls

Genus	Morphology			Chemotaxonomy			
	Aerial mycelium	Sporangium	Motile spores	Whole-cell sugar pattern	Phospholipid type	Fatty acid type	Predominant menaquinones
<i>Herbidospora</i>	—	—	—	B	PIV	3c	MK-10(III,IX-H ₄), MK-10(H ₆), MK-10(H ₂)
Members of the <i>Streptosporangiaceae</i>							
<i>Microbispora</i>	+	—	—	B	PIV	3c	MK-9(III,VIII-H ₄), MK-9(H ₂), MK-9(H ₀)
<i>Microtetraspora</i>	+	—	—	B or C	PIV	3c	MK-9(III,VIII-H ₄), MK-9(H ₂), MK-9(H ₀)
<i>Planobispora</i>	+	+	+	B	PIV	3c	MK-9(III,VIII-H ₄), MK-9(H ₂), MK-9(H ₀)
<i>Planomonospora</i>	+	+	+	B	PIV	3c	MK-9(III,VIII-H ₄), MK-9(H ₂), MK-9(H ₀)
<i>Streptosporangium</i>	+	+	—	B	PIV	3c	MK-9(III,VIII-H ₄), MK-9(H ₂), MK-9(H ₀)
<i>Actinomadura</i>	+	—	—	B	PI	3a	MK-9(II,III,VIII-H ₆), MK-9(II,III,VIII,IX-H ₈)
<i>Nocardiopsis</i>	+	—	—	C	PIII	3d	MK-10(II-H ₂), MK-10(II,III-H ₄)
<i>Saccharothrix</i>	+	—	—	C	PII	3f	MK-9(II,III-H ₄), MK-10(II,III-H ₄)
<i>Spirillospora</i>	+	+	+	B	PI or PII	3a	MK-9(H ₄), MK-9(H ₆)

Aerobic, gram-positive, non-acid-fast actinomycetes that form stable substrate mycelia. Aerial mycelia are variable. When aerial hyphae are formed, spores or sporangia are borne on sporophores derived from aerial hyphae. When aerial hyphae are not formed, the sporophores are derived from vegetative mycelia. Spores are nonmotile or motile.

Cell walls contain *meso*-diaminopimelic acid and *N*-acetylated muramic acid, but lack a significant amount of glycine (cell wall type III). The peptidoglycan type is type A1 γ . Madurose in whole cells is variable (whole-cell sugar pattern B or C). Glucosamine-containing phospholipids are present as diagnostic polar lipids (phospholipid type PIV). The cellular fatty acids include straight, iso- and anteiso-branched, and 10-methyl acids. The major proportion of the unsaturated, di- and tetrahydrogenated menaquinones have 9 or 10 isoprene units. Saturation of the tetrahydrogenated menaquinones occurs at the sites of unit III and the second unit from the ω terminus [MK-9(III,VIII-H₄) or MK-10(III,IX-H₄)]. Mycolic acids are absent. The G+C content of the DNA ranges from 66 to 74 mol%.

Description of *Herbidospora* gen. nov. *Herbidospora* (Her. bi.do.spo'ra. L. adj. *herbidus*, grassy; Gr. n. *spora*, a seed; M.L. f. n. *Herbidospora*, organism forming spores like grass). Nonfragmented branched vegetative hyphae are formed, but true aerial hyphae are not formed. Short chains of spores (10 to 30 spores per chain) are borne at the tips of sporophores, which are derived from the vegetative mycelia in clusters. Spores are nonmotile.

Cell walls contain *meso*-diaminopimelic acid and *N*-acetylated muramic acid, but lack a significant amount of glycine (cell wall type III). Whole-cell hydrolysates contain a trace amount of madurose. Contains phosphatidylethanolamine and its derivatives and glucosamine-containing phospholipids (phospholipid type PIV). The cellular fatty acid composition is characterized by iso-hexadecanoic, *n*-hexadecanoic, *n*-heptadecanoic, 10-methyl heptadecanoic, and 2-hydroxy acids (fatty acid type 3c). The predominant isoprenoid quinone is a tetrahydrogenated menaquinone with 10 isoprene units, and the hydrogenation occurs at units III and IX [MK-10(III,IX-H₄)]. MK-10(H₂), MK-10(H₀), MK-10(H₆),

and MK-9(III,VIII-H₄) are also present as minor components. The type species is *H. cretacea*.

Description of *Herbidospora cretacea* sp. nov. *Herbidospora cretacea* (cre.ta'ce.a. L. fem. adj. *cretacea*, chalk white). Vegetative mycelia are yellow to brown on most media. When sporulation occurs, the surface of the colony is white or brownish yellow. A diffusible pigment is not produced. Decomposes casein, DNA, esculin, hypoxanthine, and starch, but not adenine, arbutin, guanine, keratin, tyrosine, and xanthine. Utilizes L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, maltose, D-mannitol, D-mannose, D-ribose, starch, sucrose, and D-xylose as sole carbon sources, but not adonitol, cellulose, dulcitol, *i*-erythritol, glycerol, *myo*-inositol, melezitose, and raffinose. Utilizes fumaric acid, DL-lactic acid, L-malic acid, and succinic acid, but not benzoic acid, citric acid, mucic acid, oxalic acid, propionic acid, and L-tartaric acid. No growth occurs in the presence of 3% NaCl. Susceptible to lysozyme, gentamicin, novobiocin, rifampin, streptomycin, and vancomycin. Requires thiamine for growth. Mesophilic. The G+C content of the DNA is 69 to 71 mol%. The type strain is strain K-319 (= JCM 8553).

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