Sphaerisporangium flaviroseum sp. nov. and *Sphaerisporangium album* sp. nov., isolated from forest soil in China

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Two Gram-positive, aerobic actinomycete strains, designated YIM 48771^T and YIM 48782^T, were isolated from virgin forest soil samples collected in Hunan Province, China. 16S rRNA gene sequence similarities of the two novel isolates ranged from 96.3 to 97.6 % with species of the genus *Sphaerisporangium* with validly published names but, in the tree based on 16S rRNA gene sequences, the isolates formed distinct phyletic lines. The level of 16S rRNA gene sequence similarity between the two novel isolates was 97.1 %. DNA–DNA hybridization of strains YIM 48771^T and YIM 48782^T with recognized species of the genus *Sphaerisporangium* revealed that the level of DNA–DNA relatedness was below 70 %. The DNA G+C contents of strains YIM 48771^T and YIM 48782^T were 67.1 and 71 mol%, respectively. Chemotaxonomic data [major menaquinone, MK-9(H_4); major polar lipids, diphosphatidylglycerol, phosphatidylinositol mannoside, phosphatidylethanolamine and phosphoglycolipids; major fatty acids, iso-$C_{16:0}$ and 10-methyl $C_{17:0}$] supported the affiliation of the two isolates with the genus *Sphaerisporangium*. The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of the two isolates from recognized *Sphaerisporangium* species. Based on morphological, chemotaxonomical and phylogenetic data, strains YIM 48771^T and YIM 48782^T are considered to represent two novel species of the genus *Sphaerisporangium*, for which the names *Sphaerisporangium flaviroseum* sp. nov. (type strain, YIM 48771^T=DSM 45170^T=KCTC 19393^T) and *Sphaerisporangium album* sp. nov. (type strain, YIM 48782^T=DSM 45172^T=CCTCC AA 208026^T) are proposed.

The genus *Sphaerisporangium* was described by Ara & Kudo (2007) and was affiliated with the family *Streptosporangiaceae* (Goodfellow *et al.*, 1990). Currently, the genus comprises four species, *Sphaerisporangium melleum*, *Sphaerisporangium rubeum*, *Sphaerisporangium cinnabarinum* and *Sphaerisporangium viridialbum* (Ara & Kudo, 2007). In the course of an investigation of the actinomycete diversity of Wuling Mountain, China, we isolated two novel strains. Based on the results of the polyphasic taxonomic study, strains YIM 48771^T and YIM 48782^T should be classified as representing two novel species of the genus *Sphaerisporangium*.

Strains YIM 48771^T and YIM 48782^T were isolated, respectively, from soil samples collected from virgin forest at Jinbian Rivulet and Tianzi Mountain, Hunan Province, by using the improved glycerol-asparagine agar [per litre: glycerol, 10 g; asparagine, 1 g; $K_2HPO_4.H_2O$, 1 g; $MgSO_4.7H_2O$, 0.5 g; $CaCO_3$, 0.3 g; vitamin mixture powder, 3.7 mg (Hayakawa & Nonomura, 1987); potassium dichromate, 50 mg; agar, 20 g; pH 7.2]. The morphology of spore vesicles grown for 21 days at 28 °C on ISP 2 medium was observed using light microscopy (BH-2; Olympus) and scanning electron microscopy (Philips XL30; ESEM-TMP). The cultural characteristics of the two strains were determined using ISP 2, ISP 3, ISP 4 and ISP 5 media (Shirling & Gottlieb, 1966) and Czapek's agar (Pridham & Lyons, 1980) at 28 °C. The colony colour was determined by means of the ISCC-NBS colour charts (Kelly, 1964). The physiological and biochemical characteristics were determined after incubation at 28 °C for

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains YIM 48771^T and YIM 48782^T are EU499338 and EU499344, respectively.

An extended phylogenetic tree based on 16S rRNA gene sequences, constructed using NJ and ML, showing the relationships of strains YIM 48771^T and YIM 48782^T and representative species of genera of the family *Streptosporangiaceae* is available as supplementary material with the online version of this paper.

15 days according to Smibert & Krieg (1994). Carbon and nitrogen source utilization was assessed by using the media and methods of Gordon *et al.* (1974). Growth at various pH values was carried out according to Xu *et al.* (2005) and NaCl tolerance was examined after incubation at 28 °C for 7–15 days on ISP 2 medium. Enzyme activities were determined by using API ZYM test kits (bioMérieux). Catalase activity was detected based on bubble formation in 3 % (v/v) H_2O_2 solution. Oxidase activity was determined from the oxidation of 1 % *p*-aminodimethylaniline oxalate.

Cells of strains YIM 48771^T and YIM 48782^T for chemotaxonomic analysis were grown in ISP 2 medium, with shaking, at 28 °C and harvested. Analysis of the cell-wall amino acids and sugars of whole-cell hydrolysates was carried out as described by Staneck & Roberts (1974). Polar lipids were extracted, examined by using two-dimensional TLC and identified using published procedures (Minnikin *et al.*, 1979; Collins & Jones, 1980). Menaquinones were determined using the method of Collins *et al.* (1977) and analysed by HPLC as described by Tamaoka *et al.* (1983). Fatty acid analysis was performed using the standard protocol of the MIDI/Hewlett Packard Microbial Identification system (Sasser, 1990; Kämpfer & Kroppenstedt, 1996) after growth on TSB agar plates [trypticase soy broth (BBL), 3 % (w/v); Bacto agar (Difco), 1.5 % (w/v)] for 7 days at 28 °C. The DNA G+C contents were determined by using HPLC (Mesbah *et al.*, 1989).

The 16S rRNA gene sequences were analysed as described by Li *et al.* (2007). Phylogenetic analysis was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 3.1 (Kumar *et al.*, 2004) after multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997). Distances (using distance options according to the Kimura two-parameter model; Kimura, 1980, 1983) were calculated. The phylogenetic tree was constructed using the neighbour-joining (NJ) (Saitou & Nei, 1987), maximum-parsimony (MP) (Fitch, 1971) and maximum-likelihood (ML) methods by using PHYLIP v3.6 (Felsenstein, 1993). The stability of relationships was assessed by performing bootstrap analyses based on 1000 resamplings (Felsenstein, 1985).

The results of the cultural characterization of strains YIM 48771^T and YIM 48782^T are shown in Table 1. Morphological, physiological and biochemical characterization, utilization of carbon and nitrogen sources, amino acids of the cell wall, sugars of whole-cell hydrolysates, polar lipids, fatty acids, menaquinones and DNA G+C contents are given in Table 2 and the species descriptions.

The phylogenetic tree (Fig. 1) constructed using the three methods (NJ, MP and ML) showed that strains YIM 48771^T and YIM 48782^T belonged to the genus *Sphaerisporangium*. 16S rRNA gene sequence similarity matrix analyses showed that the sequence similarities of strain YIM 48771^T with *S. viridialbum*, *S. cinnabarinum*, *S. melleum*, *S. rubeum* and YIM 48782^T were, respectively, 97.3, 97.4, 97.2, 96.3 and 97.1 %, and those of strain YIM 48782^T with the type strains of the four recognized species were 96.9, 97.6, 96.9 and 97.1 %, respectively.

DNA–DNA hybridization was carried out to determine whether the two strains represent novel species by using the microwell method (Ezaki *et al.*, 1989; He *et al.*, 2005), with the type strains of *S. melleum* (JCM 13064^T), *S. rubeum* (JCM 13067^T), *S. cinnabarinum* (JCM 3291^T) and *S. viridialbum* (JCM 3027^T), which were kindly provided by the Japan Collection of Micro-organisms (JCM; Hirosawa, Japan). DNA–DNA reassociation values between strain YIM 48771^T and *S. viridialbum, S. cinnabarinum, S. melleum, S. rubeum* were 50, 41, 52 and 44 %, respectively, whereas the values for strain YIM 48782^T were 51, 46, 48 and 48 %, respectively. The DNA–DNA relatedness between strains YIM 48771^T and YIM 48782^T was 64 %. These values were lower than the cut-off point recommended for the circumscription of bacterial genomic species (Wayne *et al.*, 1987).

Comparison of strains YIM 48771^T and YIM 48782^T with recognized species of the genus *Sphaerisporangium* (Table 2)

Table 1. Cultural characteristics of strains YIM 48771^T and YIM 48782^T

Diffusible pigments were not produced on any of the media listed. ISP, International *Streptomyces* project. −, No growth; +, growth; ++, good growth.

Medium	YIM 48771^T				YIM 48782^T			
	Aerial mycelium		Substrate mycelium		Aerial mycelium		Substrate mycelium	
	Formation	Colour	Growth	Colour	Formation	Colour	Growth	Colour
Czapek's agar	−	−	+	White	+	White	+	White
Yeast extract-malt extract (ISP 2)	+	White	++	Deep yellow pink	++	White	++	Pale grey
Oatmeal agar (ISP 3)	+	White	++	Soft yellow pink	++	White	++	Yellow white
Inorganic salt-starch agar (ISP 4)	−	−	+	White	+	White	+	White
Glycerol-asparagine (ISP 5)	−	−	+	White	+	White	+	White

Table 2. Differential characteristics between the four recognized species of the genus *Sphaerisporangium* and strains YIM 48771^T and YIM 48782^T

Strains: 1, *S. flaviroseum* sp. nov. YIM 48771^T; 2, *S. album* sp. nov. YIM 48782^T; 3, *S. viridialbum* JCM 3027^T; 4, *S. cinnabarinum* JCM 3291^T; 5, *S. melleum* JCM 13064^T; 6, *S. rubeum* JCM 13067^T. ND, Not determined; +, positive; −, negative; (+), weak growth.

Characteristic	1	2	3	4	5	6
Substrate mycelium colour on agar						
ISP2	Deep yellow pink	Pale grey	Light tan	Bamboo	Honey gold	Coral red
ISP3	Soft yellow pink	Yellow white	Bamboo	Light amber	Mustard gold	Light coral red
Major menaquinones	MK-9(H_4), MK-9(H_2), MK-9	MK-9(H_4), MK-9(H_2), MK-9	MK-9(H_4), MK-9(H_2)	MK-9(H_4), MK-9(H_6)	MK-9(H_4), MK-9(H_6)	MK-9(H_6), MK-9(H_4)
Major fatty acid (%)	$C_{16:0}$ (15.0), iso-$C_{16:0}$ (11.3), 10-methyl $C_{17:0}$ (10.2)	iso-$C_{16:0}$ (56.2), 10-methyl $C_{17:0}$ (15.8)	iso-$C_{15:0}$ (20.2), $C_{17:0}$ (13.1), iso-$C_{16:0}$ (11.5), $C_{15:0}$ (11.2)	iso-$C_{16:0}$ (49.4), 10-methyl $C_{17:0}$ (17.5)	iso-$C_{16:0}$ (47.6), 10-methyl $C_{17:0}$ (15.8)	iso-$C_{16:0}$ (14.6), 10-methyl $C_{17:0}$ (12.9), $C_{15:0}$ (12.3), $C_{17:0}$ (10.8)
DNA G+C content (mol%)	67.1	71	72	70	71	70.4
Nitrate reduction	−	+	−	−	−	ND
Starch hydrolysis	−	+	−	−	−	−
Oxidase activity	−	+	−	−	−	−
Assimilation of:						
(+)-L-Arabinose	+	(+)	+	+	−	−
Cellobiose	−	(+)	+	+	+	−
D-Galactose	+	+	+	+	+	−
Raffinose	(+)	+	−	−	ND	(+)
Maltose	+	+	+	+	+	−
L-Rhamnose	+	+	+	−	+	−
Sucrose	+	(+)	(+)	(+)	ND	+
Fucose	+	+	−	+	+	+
Lactose	(+)	+	+	+	ND	+
D-Fructose	+	(+)	+	+	ND	+
D-Ribose	+	+	+	−	+	(+)
D-Xylose	(+)	(+)	+	+	+	−
Sorbose	+	−	−	+	(+)	−
Inositol	(+)	+	(+)	+	−	(+)
Mannitol	(+)	+	−	−	ND	+
Dextrin	+	+	−	+	ND	−
Urea	+	+	−	−	−	−
L-Histidine	−	+	+	−	−	+
L-Proline	(+)	+	+	+	−	+
L-Serine	(+)	+	(+)	+	−	+
L-Tryptophan	−	−	(+)	(+)	−	−
Xanthine	−	−	(+)	+	−	(+)
L-Arginine	(+)	+	+	+	−	−
L-Lysine	−	+	(+)	+	−	−
DL-Methionine	−	−	+	(+)	(+)	−
L-Valine	+	+	+	+	−	−

showed that the amounts of MK-9 for the two strains were, respectively, 28.1 and 29.0 %, but were present in smaller amounts (<10 %) in the recognized species. The amounts of the fatty acids $C_{15:0}$, iso-$C_{15:0}$ and $C_{17:0}$ of the two strains were less than 10 %, and the fatty acid $C_{17:1}\omega 8c$ was not present, whereas the amounts of these fatty acids for *S. viridialbum* and *S. rubeum* were greater than 10 %. The amount of $C_{16:0}$ for YIM 48771^T was 15.02 %, but those for recognized species of *Sphaerisporangium* and YIM 48782^T were less than 10.3 %. Strains YIM 48771^T and YIM 48782^T contained diphosphatidylglycerol, phosphatidylinositol mannoside, phosphatidylethanolamine and phosphoglycolipids, similar to recognized species of the genus *Sphaerisporangium*. In addition, strain YIM 48771^T contained phosphatidylmethylethanolamine and phosphatidylinositol, and strain YIM 48782^T phosphatidylinositol. The DNA G+C content of YIM 48771^T was 67.1 %, which was less than those of recognized species of *Sphaerisporangium*

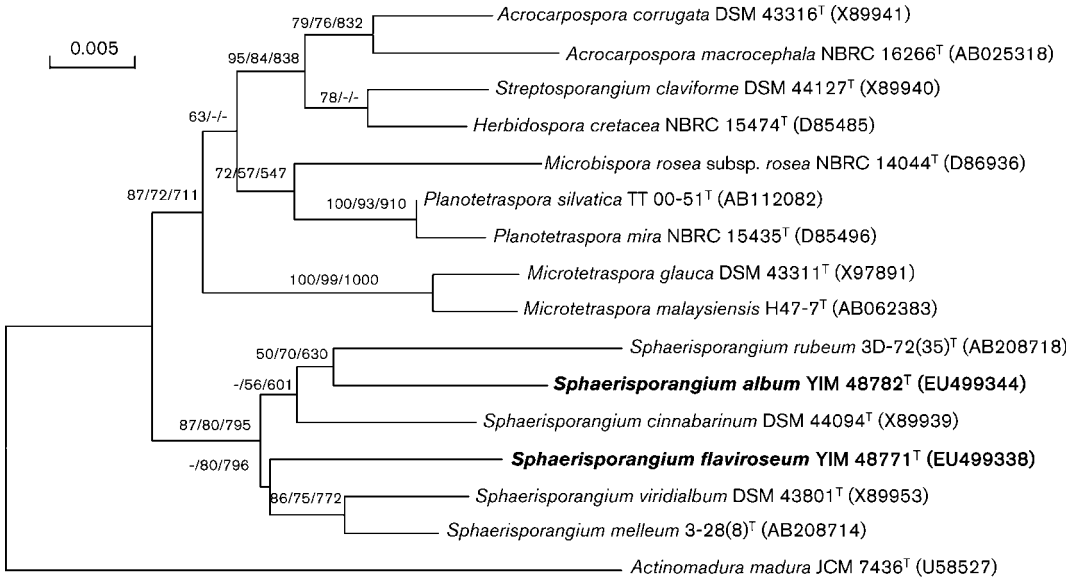

Fig. 1. Phylogenetic dendrogram derived from 16S rRNA gene sequences showing the relationships between strains YIM 48771^T and YIM 48782^T and representative species of genera of the family *Streptosporangiaceae*. The dendrogram was constructed by using the NJ, MP and ML methods. Numbers (NJ/MP/ML) on branch nodes are bootstrap values (based on 1000 resamplings; only values greater than 50 %/500 are given). Bar, 0.5 % sequence divergence. An extended version of this tree is available as Supplementary Fig. S1 (in IJSEM Online).

and strain YIM 48782^T (71 %). Therefore, strains YIM 48771^T and YIM 48782^T should be considered as representing two novel species of the genus *Sphaerisporangium*, for which the names *Sphaerisporangium flaviroseum* sp. nov. and *Sphaerisporangium album* sp. nov. are proposed.

Emended description of the genus *Sphaerisporangium* Ara and Kudo 2007

In addition to the description given by Ara & Kudo (2007), major menaquinones are MK-9(H_4), MK-9(H_6), MK-9(H_2) and MK-9. The DNA G+C contents are 67–72 mol%.

Description of *Sphaerisporangium flaviroseum* sp. nov.

Sphaerisporangium flaviroseum (fla.vi.ro′se.um. L. adj. *flavus* yellow; L. adj. *roseus* rose; N.L. neut. adj. *flaviroseum* yellowish-rose coloured).

Gram-positive. Forms yellow-pink substrate mycelia and white aerial mycelia. No diffusible pigment is produced on any of the media tested. Spherical and pyriform spore vesicles are borne on aerial mycelia. Grows at pH 6–8 and in 1 % NaCl. Catalase- and oxidase-negative. Activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase are positive. Activities of β-glucuronidase, cystine arylamidase, α-galactosidase and lipase (C14) are negative. Gelatin liquification, milk coagulation and peptonization, hydrolysis of starch, nitrate reduction, H_2S production and hydrolysis of cellulose are negative. Glucose, fructose, galactose, mannose, arabinose, xylose, ribose, rhamnose, sucrose, lactose, maltose, melibiose, raffinose, starch, sorbose, dextrin, fucose, inositol, mannitol, aesculin, galactose are utilized as sole carbon sources, but cellobiose, xylitol, erythritol and amygdalin are not. Hydrolyses urea, proline, L-phenylalanine, L-arginine, L-valine, serine and ornithine, but not glycine, L-tryptophan, histidine, methionine, lysine or xanthine. Major menaquinones are MK-9(H_4) (31.9 %), MK-9(H_2) (29.8 %) and MK-9 (28.1 %). Cellular fatty acids are iso-$C_{15:0}$ (6.8 %), $C_{15:0}$ (5.0 %), iso-$C_{16:0}$ (11.3 %), $C_{16:1}$ (5.0 %), $C_{16:0}$ (15.0 %), $C_{17:1}$ (7.6 %), $C_{17:0}$ (9.0 %), 10-methyl $C_{17:0}$ (10.2 %), anteiso-$C_{18:0}$ (5.0 %) and $C_{18:1}$ (6.3 %). The diagnostic amino acid of the peptidoglycan is *meso*-DAP. Whole-cell hydrolysates contain ribose, madurose, galactose, glucose and mannose. Phospholipids consist of diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, phosphatidylethanolamine, phosphatidylmethylethanolamine and phosphoglycolipids. The G+C content of the DNA of the type strain is 67.1 mol%.

The type strain, YIM 48771^T (=DSM 45170^T=KCTC 19393^T), was isolated from soil of Hunan, China.

Description of *Sphaerisporangium album* sp. nov.

Sphaerisporangium album (al′bum. L. neut. adj. *album* white).

Gram-positive. Forms pale-grey substrate mycelia and white aerial mycelia. No diffusible pigment is produced on any of the media tested. Spherical and pyriform spore vesicles are borne on aerial mycelia. Grows in 2 % NaCl. Catalase- and oxidase-positive. Activities of alkaline phosphatase, esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase and α-mannosidase are positive. Cystine arylamidase, α-galactosidase, β-glucuronidase and α-fucosidase are negative. Hydrolysis of starch and nitrate reduction are positive, but gelatin liquification, milk coagulation and peptonization, H_2S production and hydrolysis of cellulose are negative. Glucose, fructose, galactose, mannose, arabinose, xylose, ribose, rhamnose, sucrose, lactose, maltose, melibiose, raffinose, cellobiose, starch, dextrin, fucose, inositol, mannitol, aesculin, galactose are utilized, but sorbin, xylitol, erythritol or amygdalin are not. Hydrolyses urea, proline, serine, ornithine, L-phenylalanine, L-arginine, L-valine, histidine and lysine, but not glycine, L-tryptophan, methionine or xanthine. Major menaquinones are MK-9(H_4) (32.5 %), MK-9(H_2) (31.3 %) and MK-9 (29.0 %). Cellular fatty acids are iso-$C_{15:0}$ (4.8 %), iso-$C_{16:0}$ (56.2 %), and 10-methyl $C_{17:0}$ (15.8 %). Diagnostic amino acid of peptidoglycan is *meso*-DAP. Whole-cell hydrolysates contain ribose, madurose, galactose, glucose and mannose. Phospholipids consist of diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, phosphatidylethanolamine and phosphoglycolipids. The G+C content of the DNA of the type strain is 71 mol%.

The type strain, YIM 48782^T (=DSM 45172^T=CCTCC AA 208026^T), was isolated from soil of Hunan, China.

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