A new genus of the order Actinomycetales, *Cryptosporangium* gen. nov., with descriptions of *Cryptosporangium arvum* sp. nov. and *Cryptosporangium japonicum* sp. nov.

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Four strains that form sporangia with motile sporangiospores and mycelia were isolated from soil samples. Their many sporangia were covered by mycelia. They had glutamic acid, glycine, alanine and meso-diaminopimelic acid as cell wall amino acids (wall chemotype II), acofriose (3-O-methylrhamnose) as a characteristic whole-cell sugar, and menaquinone 9(H₆). The taxonomic characteristics of these strains differ from those of the previously described motile actinomycetes. On the basis of the morphological, physiological, chemotaxonomic and phylogenetic analyses, a new genus is proposed, *Cryptosporangium*, and two new species, *Cryptosporangium arvum* sp. nov. (type strain IFO 15965^T) for strain YU 629-21^T and *Cryptosporangium japonicum* sp. nov. (type strain IFO 15966^T) for strains YU 636-3^T, YU 655-31 and YU 656-31.

Keywords: *Cryptosporangium* gen. nov., *Cryptosporangium arvum* sp. nov., *Cryptosporangium japonicum* sp. nov., suborder Frankineae, 16S rRNA

INTRODUCTION

Phylogenetic studies on the basis of 16S rRNA sequences are useful in the classification of actinomycetes. Recently, Stackebrandt *et al.* (1997) suggested reclassification at the family level of actinomycetes on the basis of the 16S rRNA sequences phylogenetic clustering and the presence of taxon-specific 16S rRNA/DNA signature nucleotides. According to their reclassification (Stackebrandt *et al.*, 1997), actinomycetes with motile sporangiospores belong to the families *Streptosporangiaceae* [*Planomonospora* (Thiemann *et al.*, 1967a), *Planobispora* (Thiemann & Beretta, 1968) and *Planotetraspora* (Runmao *et al.*, 1993)], *Micromonosporaceae* [*Actinoplanes* (Couch, 1950), *Dactylosporangium* (Thiemann *et al.*, 1967b) and *Pilimelia* (Kane, 1966)], *Pseudonocardiaceae* [*Streptoalloteichus* (Tomita *et al.*, 1978)], *Thermomonosporaceae* [*Spirillospora* (Couch, 1963)], *Dermatophilaceae* [*Dermatophilus* (Gordon, 1964)] and *Geodermatophilaceae* [*Geodermatophilus* (Luedemann, 1968)].

During our taxonomic studies of motile actinomycetes isolated from natural sources, we isolated four strains, YU 629-21^T, YU 636-3^T, YU 655-31 and YU 656-31, which produced round and irregularly shaped sporangia and mycelia. In studying the taxonomic position of the four strains, we found that they are motile-sporangiospore-bearing actinomycetes that contain menaquinone 9(H₆) [MK-9(H₆)] and have meso-diaminopimelic acid and glycine in their cell walls (wall chemotype II). The members of the family *Micromonosporaceae*, except for the genera *Couchioplanes* (Tamura *et al.*, 1994) and *Catenuloplanes* (Yokota *et al.*, 1993), and the genus *Glycomyces* (Labeda *et al.*, 1985) are known to have chemotype II. However, the isolates differ from previously described actinomycetes in various characteristics.

In this paper, we describe the characterization and classification of strains YU 629-21^T, YU 636-3^T, YU 655-31 and YU 656-31, and propose that these organisms should be included in a new genus, *Cryptosporangium*, as members of *Cryptosporangium arvum* sp. nov. for YU 629-21^T and *Cryptosporangium japonicum* sp. nov. for YU 636-3^T, YU 655-31 and YU 656-31.

The GenBank/EMBL/DBJ accession numbers for the sequences in this paper are listed in Table 1.

METHODS

Micro-organisms and culture conditions. The isolates are listed in Table 1. Strains YU 629-21^T, YU 636-3^T, YU 655-31 and YU 656-31 were isolated from soil samples collected at Kofu, Yamanashi, and Miyako Island, Okinawa Prefecture, Japan, on humic acid/vitamin (HV) agar (Hayakawa & Nonomura, 1987) using the capillary method (Hayakawa *et al.*, 1992). To obtain freeze-dried mass for chemotaxonomic analyses, the isolates were grown at 28 °C for 5 d in yeast extract/glucose broth (containing 10 g yeast extract and 10 g D-glucose in 1000 ml distilled water, pH 7.0) on a rotary shaker.

Cultural characteristics were recorded after 14 d incubation at 28 °C according to the International *Streptomyces* Project (ISP) method (Shirling & Gottlieb, 1966). Colours are described in common terminology, but exact colours were determined by comparison with colour chips of the Mycological Colour Chart (Rayner, 1970).

Morphological and physiological characteristics. Morphological features were observed on HV agar, and physiological features were observed on media by the methods of Shirling & Gottlieb (1966). Motility was observed with a light microscope using culture grown on HV agar for 7–10 d at 28 °C, then incubated at 28 °C for 1 h in 0.01 M phosphate

Table 1. Strains investigated in this study and accession numbers for 16S rRNA sequences

Strain	Source	Accession no.
YU 629-21 ^T (= IFO 15965 ^T)	Soil from a vegetable field in Yamanashi Prefecture, Japan	D85465
YU 636-3 ^T (= IFO 15966 ^T)	Soil from a sugar cane field in Okinawa Prefecture, Japan	D85466
YU 655-31	Soil from a vegetable field in Yamanashi Prefecture, Japan	AB006168
YU 656-31	Soil from a vegetable field in Yamanashi Prefecture, Japan	AB006166

Fig. 1. Light micrograph (a) and scanning electron micrographs (b–d) of isolate YU 629-21^T grown on HV agar for 14 d at 28 °C. Bars, 50 µm (b), 10 µm (c) and 5 µm (d).

Fig. 2. Light micrograph (a) and scanning electron micrographs (b–d) of isolate YU 636-3^T grown on HV agar for 14 d at 28 °C. Bars, 50 µm (b), 10 µm (c) and 5 µm (d).

buffer (pH 7.0) containing 10% soil extract. Culture grown on HV agar for 14 d at 28 °C was observed with a scanning electron microscope (model JSM-5400; JEOL). Samples for scanning electron microscopy were prepared by cutting a block of agar with growth of the strain, fixing the block in osmium tetroxide vapour at room temperature for 4 h, dehydrating cells through a graded ethanol series followed by critical point drying (model HCP-2; Hitachi Koki), then sputter coating the preparation with palladium under vacuum.

Analyses of chemotaxonomic characteristics. Analyses of whole-cell sugars, polar lipids, cellular fatty acids, mycolic acids, isoprenoid quinones and amino acid composition of the cell walls and the glycolate test were performed as described previously (Tamura *et al.*, 1994).

DNA base composition. DNA was obtained by the method of Saito & Miura (1963). The G+C content of the DNA was determined by the method of Mesbah *et al.* (1989) after treatment with P1 nuclease and alkaline phosphatase and by HPLC, using a model LC-6AD apparatus (Shimadzu) equipped with a Cosmosil 5C₁₈-AR column (4.6 × 150 mm; Nacalai Tesque).

DNA homology experiment. DNA–DNA homology was studied using the photobiotin microplate hybridization method of Ezaki *et al.* (1989).

PCR amplification and sequencing of 16S rDNA. 16S rDNA was amplified by PCR (Saiki *et al.*, 1988) using TaKaRa Taq (Takara Shuzo) and a pair of primers, 9F (5'-GAGTTTG-

ATCCTGGCTCAG) and 1541R (5'-AAGGAGGTGATC-CAACC). The conditions for thermal cycling were denaturation of the target DNA at 96 °C for 2 min followed by 30 cycles of denaturation at 95 °C for 1 min, primer annealing at 55 °C for 1 min and primer extension at 72 °C for 2 min. At the end of cycling, the reaction mixture was held at 72 °C for 5 min, then cooled to 4 °C.

The 1.5 kb amplified 16S rDNA was subjected to cycle sequencing by using a Thermo Sequenase fluorescently labelled primer cycle sequencing kit with 7-deaza dGTP (Amersham) according to the manufacturer's protocol with the following primers: 5'-CTCCTACGGGAGGCAGC-AG-3' (339F), 5'-GTATTACCGCGGCTGCTG-3' (536R), 5'-GCAACGAGCGCAACCC-3' (1099F) and 5'-GCGGT-GTGTACAAGGCC-3' (1382R). The conditions for thermal cycling were denaturation of the target DNA at 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 0.5 min, primer annealing at 55 °C for 0.5 min and primer extension at 72 °C for 1 min. The products were analysed with a Pharmacia ALF DNA Sequencer II according to the manufacturer's protocol.

Phylogenetic analysis. 16S rRNA sequences of the strains examined and reference organisms were aligned against that of *Streptomyces ambifaciens* (Pernodet *et al.*, 1989). The sequences were aligned with published sequences from DDBJ, GenBank and EMBL by the K_{nuc} value of Kimura (1980), and the phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) from K_{nuc} values. The topology of the phylogenetic tree was evaluated

by the bootstrap resampling method of Felsenstein (1985) with 1000 replicates.

RESULTS

Morphological observations

Morphological observation of the 14-d-old cultures of isolates grown on HV agar revealed the presence of round or irregularly shaped sporangia and mycelia. Some sporangia, especially on the central part of the colony, were submerged under thick mycelia (Figs 1b, c and 2b, c), making them difficult to observe by light microscopy (Figs 1a and 2a). In strain YU 629-21^T, many swollen structures resembling globose bodies or immature sporangia were observed on the mycelium (Fig. 1a, d). Sporangia were 3–10 µm in diameter.

After incubation at 28 °C for 1 h in water or 0.01 M phosphate buffer (pH 7.0) containing 10 % soil extract, many spores showed motility.

Growth characteristics

As shown in Table 2, isolates developed yellow to orange colonies on most media tested. Isolates showed good growth on yeast extract/malt extract agar (ISP medium 2), and produced greyish reddish orange soluble pigment on tyrosine agar (ISP medium 7). Isolates developed sporangia on inorganic salts/starch agar (ISP medium 4), glycerol/asparagine agar (ISP medium 5), tyrosine agar (ISP medium 7), nutrient agar and HV agar.

Physiological and biochemical characteristics

The biochemical properties of isolates are shown in Table 3. All isolates utilized D-glucose, inositol, raffinose, saccharose, D-mannitol, L-rhamnose, D-xylose, L-arabinose, lactose, maltose and D-mannose. Strains YU 629-21^T and YU 636-3^T did not utilize D-sorbitol, but strains YU 655-31 and YU 656-31 did utilize it. Only strain YU 629-21^T did not utilize D-fructose. The

Table 2. Cultural characteristics of the strains studied

The colour codes in parentheses correspond to those in the Mycological Colour Chart (Rayner, 1970).

Medium	Description of growth, and colour of colony for strain:			
	YU 629-21 ^T	YU 636-3 ^T	YU 655-31	YU 656-31
ISP medium 2	Good Strong orange (7) to light to moderate orange-yellow (10)	Good Light to moderate orange-yellow (10)	Good Strong orange (7) to light to moderate orange-yellow (10)	Good Strong orange (7) to light to moderate orange-yellow (10)
ISP medium 3	Poor Light to moderate orange-yellow (10)	Poor Moderate yellowish pink (41)	Poor Light to moderate orange-yellow (10)	Poor Light to moderate orange-yellow (10)
ISP medium 4	None Light yellow (11)	Poor Moderate yellowish pink (41)	None Light yellow (11)	None Light yellow (11)
ISP medium 5	Moderate Moderate orange-yellow (44) to light to moderate orange-yellow (10)	Moderate Light to moderate orange-yellow (10) to light yellow (11)	Poor	Poor Light yellow (11)
ISP medium 6	Poor Moderate orange-yellow (44)	Poor Moderate orange-yellow (44)	Poor Moderate orange-yellow (44)	Poor Moderate orange-yellow (44)
ISP medium 7	Poor Dark greenish yellow (40) to strong brown (9)	Poor Strong brown (9) to light greyish brown (106)	Poor Dark greenish yellow (40) to strong brown (9)	Poor Dark greenish yellow (40) to strong brown (9)
Nutrient agar	Poor Pale yellow (45)	Poor Pale yellow (45)	Poor Light yellowish brown (86)	Moderate Moderate orange-yellow (44)
Brain Heart Infusion (Difco)		Good Moderate orange-yellow (44)		

Table 3. Phenotypic characteristics of the strains studied

++ , Good; + , moderate; ± , poor; – , none.

Characteristic	Strain			
	YU 629-21 ^T	YU 636-3 ^T	YU 655-31	YU 656-31
Utilization of:				
Glucose	++	++	++	++
Inositol	++	++	++	++
Raffinose	++	++	++	++
Saccharose	++	++	++	++
D-Fructose	–	++	+	+
D-Mannitol	++	++	++	++
L-Rhamnose	+	+	+	+
D-Xylose	++	++	++	+
L-Arabinose	+	+	+	+
Glycerol	+	±	+	+
Lactose	++	++	++	++
D-Galactose	±	++	+	++
Maltose	++	++	++	++
D-Mannose	++	++	++	++
D-Sorbitol	–	–	++	++
Inulin	–	–	–	–
Growth on NaCl (%):				
0	++	++	++	++
4	–	–	–	+
7	–	–	–	–
Calcium malate hydrolysis	+	+	+	+
Starch hydrolysis	–	–	–	–
Nitrite from nitrate	–	–	–	–
Peptonization of milk	+	+	+	+
Gelatin liquefaction	–	–	–	–
Pigmentation in:				
ISP-1	–	–	–	–
ISP-6	–	–	–	–
ISP-7	+, Greyish reddish orange	+, Greyish reddish orange	+, Greyish reddish orange	+, Greyish reddish orange
Growth at (°C):				
10	–	–	–	–
15	++	+	++	++
20	++	++	++	++
25	++	++	++	++
30	+	+	++	++
37	–	+	–	+
45	–	–	–	–

isolates were positive for peptonization of milk and decomposition of calcium malate, and negative for gelatin liquefaction, hydrolysis of starch and reduction of nitrate. The optimum temperature for growth of all isolates was 20–25 °C.

Chemotaxonomic characteristics

The menaquinone composition, whole-cell sugar pattern, amino acid composition of the cell wall, phospholipid type and G + C content of DNA are shown in

Table 4. The major menaquinone was MK-9(H₆), and small amounts of MK-9(H₄) and MK-9(H₈) were also present. The cell walls contained *meso*-diaminopimelic acid, glycine, glutamic acid and alanine, indicating that the wall chemotype of the isolates is II according to Lechevalier & Lechevalier (1970), and the type of peptidoglycan is suggested to be A1γ according to Schleifer & Kandler (1972). Isolates contained large amounts of acofriose (3-*O*-methylrhamnose) and glucose in the whole cells. As diagnostic phospholipids, phosphatidylethanolamine was detected, but phos-

Table 4. Chemotaxonomic characteristics of the strains studied

Characteristic	Strain			
	YU 629-21 ^T	YU 636-3 ^T	YU 655-31	YU 656-31
Major menaquinone (%)				
MK-9(H ₄)	11	13	8	13
MK-9(H ₆)	54	53	45	56
MK-9(H ₈)	35	34	47	31
Sugar composition of whole cells (%)				
Glucosamine	47	46	41	30
Acofriose	11	14	9	13
Rhamnose		4	1	
Ribose	2			
Mannose	4	4	2	2
Arabinose	5	7	3	4
Galactose	5	7	4	5
Xylose	4	4		
Glucose	22	15	40	45
Amino acid composition of peptidoglycan (molar ratio)*				
Glutamic acid	1.00	1.00	1.00	1.00
Glycine	2.15	2.51	1.85	2.59
Alanine	0.90	0.84	0.81	0.97
meso-Diaminopimelic acid	0.78	0.86	0.43	0.71
Phospholipid composition†				
Phosphatidylglycerol	—	—	—	—
Unidentified phospholipid including glucosamine	—	—	—	—
Phosphatidylcholine	—	—	—	—
Phosphatidylethanolamine	+	+	+	+
Phospholipid type	PII	PII	PII	PII
Mycolic acid†	—	—	—	—
Glycolyl test	Acetyl	Acetyl	Acetyl	Acetyl
DNA G + C content (mol%)	70.1	70.3	70.2	70.4

* Calculated by defining the amount of glutamic acid as 1.00.

† +, Present; —, absent.

Table 5. Percentage cellular fatty acid composition of the strains studied

Strain	Iso-branched		Anteiso-branched	Saturated					Unsaturated			Unidentified
	C _{16:0}	C _{17:0}		C _{15:0}	C _{16:0}	C _{17:0}	C _{18:0}	C _{19:0}	C _{17:1}	C _{18:1}	C _{19:1}	
YU 629-21 ^T	24.0	0.9	4.4	1.8	10.1	13.3	6.0	1.1	14.1	22.3	1.4	0.6
YU 636-3 ^T	50.1	1.6	4.8	1.0	5.5	6.1	1.8		11.1	12.7	1.8	3.5
YU 655-31	41.6	1.0	3.9	1.7	6.7	9.2	2.4	0.5	16.3	13.6	0.8	2.4
YU 656-31	50.7	1.3	3.7	1.2	5.0	6.5	1.6		16.7	11.1	2.3	

phatidylglycerol, phosphatidylcholine and unidentified phospholipid including glucosamine were not detected. This pattern corresponds to phospholipid type II (Lechevalier *et al.*, 1977). The G + C contents of

DNAs were 70 mol %. Mycolic acid was absent. The fatty acids are shown in Table 5. The major cellular fatty acids of strain YU 629-21^T were iso-C_{16:0}, C_{18:1}, C_{17:1}, C_{17:0} and C_{16:0}, and those of strains YU 636-3^T,

Table 6. Levels of DNA relatedness among the strains studied

Strain	Percentage of DNA complementary to labelled DNA from strain:			
	YU 629-21 ^T	YU 636-3 ^T	YU 655-31	YU 656-31
YU 629-21 ^T	100	44	45	42
YU 636-3 ^T	38	100	40	48
YU 655-31	49	47	100	72
YU 656-31	40	57	69	100

YU 655-31 and YU 656-31 were iso- $C_{16:0}$, $C_{18:1}$ and $C_{17:1}$. Both cellular fatty acid patterns correspond to fatty acid type 2a (Kroppenstedt, 1985). The acyl-type of cell wall polysaccharides was acetyl.

DNA-DNA hybridization

The results of DNA-DNA hybridization studies indicated that the four strains were divided into three DNA homologous groups: strain YU 629-21^T, strain YU 636-3^T and strains YU 655-31 and YU 656-31 (Table 6). Strains YU 655-31 and YU 656-31 exhibited species level DNA relatedness (i.e. > 70% relatedness). DNA reassociation between strains YU 629-21^T, YU 636-3^T and YU 655-31 ranged from 38 to 57%.

Phylogenetic position

16S rDNA sequences were determined for YU 629-21^T [positions 28–1524, according to the *Escherichia coli* numbering system of Brosius *et al.* (1978); total 1463 bases], YU 636-3^T (positions 28–1513; total 1459 bases), YU 655-31 (positions 28–1524; total 1462 bases) and YU 656-31 (positions 28–1524; total 1462 bases). The positions at which secondary structures varied between strains and the positions at which sequences were not determined in some reference organisms were excluded from the analysis. For phylogenetic analysis, the total number of nucleotides compared was 1119 after elimination of all sites which were not determined in any sequence. The phylogenetic tree obtained by applying the neighbour-joining method to K_{nuc} values is depicted in Fig. 3. 16S rDNA analysis revealed that the isolates represent a line of descent distinct from previously described actinomycetes (Fig. 3), and similarity values between isolates and other organisms were under 93.8%. The isolates are the closest neighbours with the members of suborder *Frankineae* (Stackebrandt *et al.*, 1997) which consists of the genera *Sporichthya* (Lechevalier *et al.*, 1968; similarity values 92.7–93.8%), *Frankia* (Lechevalier & Lechevalier, 1989; similarity values 92.9–93.7%), *Geodermatophilus* (Luedemann, 1968; similarity values 92.2–93.3%), *Acidothermus* (Mohagheghi *et al.*, 1986; similarity values 91.1–92.0%), *Blastococcus* (Ahrens & Moll, 1970; similarity values 91.0–92.2%) and *Microsphaera* (Yoshimi *et al.*, 1996; similarity values 92.2–93.0%).

DISCUSSION

The strains YU 629-21^T, YU 636-3^T, YU 655-31 and YU 656-31 developed sporangia and mycelia. Characteristics of the isolates and related genera forming sporangia with motile spores are shown in Table 7. The genera *Spirillospora*, *Planobispora*, *Planomonospora* and *Planotetrastora* have *meso*-diaminopimelic acid in the cell wall (cell wall type III) and madurose as a characteristic whole-cell sugar (sugar pattern B). The genus *Streptoalloteichus* has cell wall type III. However, the isolates described here have glycine and *meso*-diaminopimelic acid in the cell wall (cell wall type II) and acofriose (3-*O*-methylrhmannose) as the characteristic whole-cell sugar. The known actinomycetes with cell wall type II are members of the family *Micromonosporaceae* and the genus *Glycomyces*. Among the genera with wall chemotype II, *Actinoplanes*, *Dactylosporangium* and *Pilimelia* have sporangia and motile elements. However, the isolates are distinguished from these genera in their morphological characteristics and in having acofriose as a whole-cell sugar and the acyl-type of peptidoglycan. Isolates can also be distinguished from *Dactylosporangium* and *Pilimelia* by the menaquinone system. Members of the genus *Catellatospora* (Asano & Kawamoto, 1986) are known to have acofriose as the whole-cell sugar. However, this organism does not form a sporangium and motile spores, and has a different phylogenetic position to the isolates. Other actinomycetes with chemotype II, *Spirilliplanes* and *Glycomyces*, are distinguishable from the isolates in morphology, acyl type and menaquinone composition. Thus in their morphological and chemotaxonomic characteristics, the isolates are distinct from the known actinomycetes with chemotype II and other previously described organisms (Table 7).

The phylogenetic tree indicates that isolates are distinct from all actinomycetes whose sequences are available in databases. The isolates are phylogenetically most closely related to members of the genera *Sporichthya* (Lechevalier *et al.*, 1968), *Frankia* (Lechevalier & Lechevalier, 1989), *Geodermatophilus* (Luedemann, 1968), *Acidothermus* (Mohagheghi *et al.*, 1986), *Blastococcus* (Ahrens & Moll, 1970) and *Microsphaera* (Yoshimi *et al.*, 1996). Recently, these organisms have been assigned to the suborder *Frankineae* (Stackebrandt *et al.*, 1997). Members of this suborder have various morphological and chemotaxonomic charac-

Fig. 3. Phylogenetic tree derived from 16S rRNA sequences of actinomycetes. *E. coli* was used as the outgroup. Bar, 0.01 K_{nuc} in nucleotide sequences. The numbers on the branches are confidence limits (expressed as percentages) estimated from a bootstrap analysis with 1000 replicates (only percentages above 50% are indicated).

teristics which are distinct from those of the isolates described here. The genus *Sporichthya* shows motility and has LL-diaminopimelic acid as diamino acid in the cell wall. The genus *Geodermatophilus* develops zoospores and rudimentary mycelia, but does not produce aerial mycelium. The genus *Frankia* does not form aerial mycelium or zoospores.

The results of DNA-DNA hybridization of the four strains revealed that they were genetically divided into three groups. Strain YU 629-21^T was distinguished from other strains by the cellular fatty acid pattern; however, strains YU 636-3^T and YU 655-31 and YU 656-31 did not have any diagnostic phenotypic differences. Phenotypic characteristics may be reflected by

only a few genes. Diagnostic differences between the last three strains may be found by further study.

On the basis of morphological, physiological, chemotaxonomic and phylogenetic criteria, strains YU 629-21^T, YU 636-3^T, YU 655-31 and YU 656-31 are readily distinguishable from the motile actinomycetes mentioned above and warrant the formation of a new taxon. Therefore, we propose that strains YU 629-21^T, YU 636-3^T, YU 655-31 and YU 656-31 be classified in a new genus, *Cryptosporangium* gen. nov., with the type species *Cryptosporangium arvum* sp. nov. (type strain IFO 15965^T).

From the phylogenetic analysis, the genus *Cryptosporangium* can be considered to belong to the sub-

Table 7. Differential characteristics of the genus *Cryptosporangium* and related genera

Genus	DNA G + C content (mol %)	Motility of spore*	Sporangium formation*	Fatty acid type†	Glycolate	Cell wall type‡	Major menaquinones	Phospholipid type§	Characteristic sugar in whole cells¶
<i>Cryptosporangium</i>	70	+	+	2a	Acetyl	II	MK-9(H ₄)	PII	Aco
<i>Actinoplanes</i>	72–73	+	+	2d	Glycolyl	II	MK-9(H ₄), 10(H ₄)	PII	Ara, Xyl
<i>Dactylosporangium</i>	72–73	+	+	3b	Glycolyl	II	MK-9(H ₄ , H ₆ , H ₈)	PII	Ara, Xyl
<i>Pilimelia</i>	ND	+	+	2d	Glycolyl	II	MK-9(H ₄ , H ₆)	PII	Ara, Xyl
<i>Spirillospora</i>	69–71	+	+	3a	ND	III	MK-9(H ₄ , H ₆)	PI	Mad
<i>Streptoalloteichus</i>	ND	+	+	ND	Acetyl	III	MK-9(H ₄), 10(H ₄)	PII	Gal, Man
<i>Planomonospora</i>	72	+	+	3c	Acetyl	III	MK-9(H ₄ , H ₆)	PIV	Mad
<i>Planobispora</i>	70–71	+	+	3c	Acetyl	III	MK-9(H ₄)	PIV	Mad
<i>Planotetraspora</i>	ND	+	+	ND	ND	III	ND	ND	Ara, Xyl
<i>Micromonospora</i>	71–73	–	–	3b	Glycolyl	II	MK-10(H ₄ , H ₆)/9(H ₄ , H ₆)	PII	Ara, Xyl
<i>Catellatospora</i>	71–72	–	–	ND	Glycolyl	II	MK-10(H ₄ , H ₆)/9(H ₄ , H ₆)	PII	Ara, Xyl
<i>Spirilliplanes</i>	69	+	–	2d	Glycolyl	II	MK-10(H ₄)	PII	Xyl, 3-O-M-Man
<i>Catenuloplanes</i>	71–73	+	–	2c	Glycolyl	VI	MK-9(H ₄), 10(H ₄)	PIII	Xyl
<i>Couchioplanes</i>	70–72	+	–	2c	Glycolyl	VI	MK-9(H ₄)	PII	Ara, Xyl
<i>Glycomyces</i>	71–73	–	–	2c	Glycolyl	II	MK-9(H ₄), 10(H ₄ , H ₆)	PI	Ara, Xyl

ND, Not determined.

* +, Present; –, absent.

† According to the classification of Kroppenstedt (1985).

‡ According to the classification of Lechevalier & Lechevalier (1970).

§ According to the classification of Lechevalier *et al.* (1977).

¶ Aco, acofriose; Ara, arabinose; Xyl, xylose; Mad, madurose; Gal, galactose; Man, mannose; 3-O-M-Man, 3-O-methylmannose.

order *Frankineae*. Further study will be needed to clarify the relationship of the genus *Cryptosporangium* with other members of the suborder *Frankineae*.

Description of *Cryptosporangium* gen. nov.

Cryptosporangium [cryp'to.spo.ran'gi.um. Gr. adj. *kryptos* hidden; Gr. n. *spora* a seed, spore; Gr. n. *argeion* vessel; M.L. neut. n. *Cryptosporangium* an organism with spore-containing vessels (sporangia) covered or hidden by mycelium].

Gram-positive, non-acid-fast, aerobic organisms with branching hyphae. Non-fragmentary substrate mycelia are present. The sporangia and aerial mycelia aggregate, and sporangiospores show motility when they are suspended in water. Morphology of a 14-d-old culture grown on inorganic salts/starch agar shows the presence of aerial mycelia. Strictly aerobic. Good growth occurs at 20–25 °C. The organism shows good growth on oatmeal agar, inorganic salts/starch agar and peptone/yeast extract iron agar. In general, the vegetative mycelia are yellow to orange and the aerial mycelia are white. Cell walls contain glutamic acid, glucosamine, glycine, alanine and *meso*-diaminopimelic acid. Wall chemotype is II according to Lechevalier & Lechevalier (1970), and the peptidoglycan type is suggested to be A1γ according to Schleifer & Kandler (1972). Glucose and acofriose are detected as whole-cell sugars. As major cellular fatty acids, C_{17:1}, C_{18:1} and iso-C_{16:0} are present. The G + C content of DNA is 70 mol%. The major menaquinone is MK-9(H₆), and small amounts of MK-9(H₄) and 9(H₆) are also present. Phosphatidylethanolamine and phosphatidylinositol are present as diagnostic phospholipids

(phospholipid pattern type PII). The acyl-type of cell wall polysaccharides is acetyl. Habitat: soil. The type species is *Cryptosporangium arvum*.

Description of *Cryptosporangium arvum* sp. nov.

Cryptosporangium arvum (ar.vum. L. n. *arvum* arable land, pertaining to isolate from arable land).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. Pale reddish-brown soluble pigment is produced on tyrosine agar (ISP medium 7). Gelatin liquefaction is negative. Does not hydrolyse starch. Decomposes calcium malate. Coagulates milk. Xylose, glucose, inositol, raffinose, rhamnose, mannitol, sucrose, arabinose, glycerol, lactose, maltose and mannose are utilized, but sorbitol and inulin are not. No growth at 37 °C. As major cellular fatty acids, iso-C_{16:0}, C_{18:1}, C_{17:1}, C_{17:0} and C_{16:0} are present. The G + C content of DNA is 70 mol%. Habitat: cultivated soil. The type strain is YU 629-21^T (= IFO 15965^T).

Description of *Cryptosporangium japonicum* sp. nov.

Cryptosporangium japonicum (ja.po'ni.cum. M.L. masc. adj. *japonicum* pertaining to Japan where the organisms were isolated).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. Pale reddish brownish soluble pigment is produced on tyrosine agar (ISP medium 7). Gelatin liquefaction is negative. Does not hydrolyse starch. Decomposes calcium malate. Coagulates milk. Xylose, glucose, inositol, raffinose, rhamnose, mannitol, sucrose, fructose, arabinose, lactose, galactose, maltose and mannose are utilized,

but sorbitol and inulin are not. Grows at 37 °C. The G+C content of DNA is 70 mol%. Habitat: cultivated soil. The type strain is YU 636-3^T (= IFO 15966^T).

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