Four New Species of the Genus *Actinokineospora*: *Actinokineospora inagensis* sp. nov., *Actinokineospora globicatena* sp. nov., *Actinokineospora terrae* sp. nov., and *Actinokineospora diospyrosa* sp. nov.

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The taxonomic positions of motile actinomycetes that were isolated from soil and fallen leaves obtained from around a pond and a lake and from fallen leaves of persimmons were studied. The aerial mycelia of all of the isolates exhibited fragmentation during growth, and motile spores arranged in chains were produced within the mycelia. Sporangia were not observed. These isolates contained menaquinone MK-9(H₄), their DNA G+C contents were 69 to 70 mol%, they contained glutamic acid, glycine, alanine, and meso-diaminopimelic acid as cell wall amino acids, and arabinose and galactose were found in their whole-cell hydrolysates. These taxonomic characteristics are the same as those of *Actinokineospora riparia*. On the basis of morphological, physiological, and chemotaxonomic characteristics and DNA-DNA hybridization data, we propose the following four new species of the genus *Actinokineospora* for these strains: *Actinokineospora inagensis* for a single isolate, type strain YU4-1 (= IFO 15663); *Actinokineospora globicatena* for isolates YU5-1, YU6-1, YU6-2, YU7-1, and YU7-2 (type strain, YU6-1 [= IFO 15664]); *Actinokineospora terrae* for a single isolate, type strain YU6-3 (= IFO 15668); and *Actinokineospora diospyrosa* for a single isolate, type strain YU8-1 (= IFO 15665).

Hasegawa (3) described *Actinokineospora riparia* as a motile arthrospore-bearing actinomycete. This organism has meso-diaminopimelic acid as a cell wall diamino acid and arabinose and galactose in its whole-cell hydrolysate, which makes it a chemotype IV organism. However, Takeuchi et al. (14) stated that the cell wall chemotype of *A. riparia* was type III/A, because arabinose was not the major sugar in the cell wall. The following five genera of motile arthrospore-bearing actinomycetes have been described previously: *Sporichthya* (7), *Actinosynnema* (4), *Actinokineospora* (3), *Catenuloplanes* (17), and *Couchioplanes* (15). Additional new species of the genera *Sporichthya*, *Actinokineospora*, *Catenuloplanes*, and *Couchioplanes* have not been described.

During a study of motile actinomycetes, we obtained eight isolates from soil and fallen leaves. All of these isolates formed motile arthrospores, but sporangia were not observed. These isolates contained menaquinone MK-9(H₄), had DNA guanine-plus-cytosine (G+C) contents of 69 to 70 mol%, were phospholipid type PII organisms, had meso-diaminopimelic acid as a cell-wall diamino acid, and contained arabinose and galactose in their whole-cell hydrolysates. These morphological, physiological, and chemotaxonomic characteristics and DNA-DNA hybridization data indicated that these isolates belong to the genus *Actinokineospora* and are taxonomically distinct from *A. riparia*.

In this paper we describe the characterization and classification of the motile actinomycete isolates mentioned above and propose the following four new species of the genus *Actinokineospora* for them: *Actinokineospora inagensis*, *Actinokineospora globicatena*, *Actinokineospora terrae*, and *Actinokineospora diospyrosa*.

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MATERIALS AND METHODS

Microorganisms and culture conditions. The strains which we used are shown in Table 1. Strains YU4-1^T (T = type strain), YU5-1, YU6-1^T, YU6-2, YU6-3^T, YU7-1, YU7-2, and YU8-1^T were isolated from soil and fallen leaves collected beside a pond and a lake and in a persimmon field in Yamanashi Prefecture, Japan, on humic acid-vitamin (HV) agar (5) by using the modified method of Makkar and Cross (9). *A. riparia* IFO 14541^T (Institute for Fermentation, Osaka, Japan) was used as a reference strain.

Cultural observations. Cultural characteristics were recorded after 14 days of incubation at 28°C by the International *Streptomyces* Project (ISP) method (13). Colors are described below by common terms, but exact colors were determined by comparison with color chips in the *Color Harmony Manual* (1).

Morphological and physiological characterization. Morphological features were observed on HV agar, and physiological features were observed on media commonly used for identification of members of the order *Actinomycetales* (13). Motility was observed with a light microscope by using cells grown for 7 to 10

TABLE 1. Bacterial strains studied

Strain	Source and/or other designation(s)	Species
YU4-1 ^T	Fallen leaves on the shore of a lake in Yamanashi Prefecture; IFO 15663 ^T	<i>A. inagensis</i>
YU5-1	Soil on the shore of a lake in Yamanashi Prefecture	<i>A. globicatena</i>
YU6-1 ^T	Soil around a pond in Yamanashi Prefecture; IFO 15664 ^T	<i>A. globicatena</i>
YU6-2	Soil around a pond in Yamanashi Prefecture	<i>A. globicatena</i>
YU6-3 ^T	Soil around a pond in Yamanashi Prefecture; IFO 15668 ^T	<i>A. terrae</i>
YU7-1	Fallen leaves around a pond in Yamanashi Prefecture	<i>A. globicatena</i>
YU7-2	Fallen leaves around a pond in Yamanashi Prefecture	<i>A. globicatena</i>
YU8-1 ^T	Fallen persimmon leaves in Yamanashi Prefecture; IFO 15665 ^T	<i>A. diospyrosa</i>
IFO 14541 ^T	ATCC 49499 ^T , JCM 7471 ^T	<i>A. riparia</i>

FIG. 1. Scanning electron micrographs of isolate YU4-1^T grown on HV agar for 10 days at 28°C.

FIG. 2. Scanning electron micrographs of isolates YU6-1^T (A and B) and YU6-2 (C and D) grown on HV agar for 10 days at 28°C.

FIG. 3. Scanning electron micrographs of isolate YU6-3^T grown on HV agar for 10 days at 28°C.

days at 28°C on HV agar and then incubated at 28°C for 1 h in 0.01 M phosphate buffer (pH 7.0) containing 10% soil extract. Cultures grown on HV agar for 14 days at 28°C were observed with a scanning electron microscope (model JSM-5400; JEOL, Ltd.). Samples for scanning electron microscopy were prepared by cutting a block from the agar on which a culture grew, fixing the block in osmium tetroxide vapor at room temperature for 4 h, dehydrating the cells through a graded ethanol series and then in a Hitachi model HCP-2 critical point drying apparatus, and sputter coating the preparation with palladium under a vacuum.

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 7.0) on a rotary shaker at 28°C.

Analyses of chemotaxonomic characteristics. Cell wall sugar, polar lipid, cellular fatty acid, isoprenoid quinone, and peptidoglycan analyses were performed as described previously (15).

DNA base composition. DNA was obtained by the method of Saito and Miura (11). The G+C content of DNA was determined by the method of Mesbah et al. (10) after treatment with P_i nuclease and alkaline phosphatase, using a model LC-6AD high-performance liquid chromatography apparatus (Shimadzu Co., Ltd., Kyoto, Japan) equipped with a Cosmosil 5C₁₈-AR column (4.6 by 150 mm; Nacalai Tesque, Inc., Kyoto, Japan). The mobile phase was 0.2 M ammonium phosphate-acetonitrile (40:1, vol/vol).

DNA-DNA hybridization. Levels of DNA-DNA relatedness were determined fluorometrically by the method of Ezaki et al. (2), using biotinylated DNA.

RESULTS AND DISCUSSION

Morphological observations. All of the isolates were gram-positive, non-acid-fast, aerobic organisms with branched hyphae and therefore belong to the order *Actinomycetales* Buchanan. Nonfragmenting, pale orange to tan substrate mycelia were formed. Morphological observations of 10-day-old cultures grown on HV agar revealed the presence of aerial mycelia with spore chains. The spores were rod shaped (0.3 to 0.5 by 0.5 to 0.8 µm) and were formed by fragmentation of the hyphae (arthrospores); the spores had smooth surfaces, as revealed by scanning electron microscopy (Fig. 1 through 4). In isolates YU5-1, YU6-1^T, YU6-2, YU7-1, and YU7-2, the spore chains and aerial mycelia often aggregated into clusters resembling sporodochia (Fig. 2).

Cultural characteristics. As shown in Table 2, all of the isolates had nearly the same cultural characteristics. The colonies were yellow to orange. All of the isolates exhibited good growth on all of the media tested except peptone-yeast extract-

FIG. 4. Scanning electron micrographs of isolate YU8-1^T grown on HV agar for 10 days at 28°C.

TABLE 2. Cultural characteristics of *A. ingensis* YU4-1^T, *A. globicatena* YU6-1^T, *A. terrae* YU6-3^T, and *A. diospyrosa* YU8-1^{Ta}

Medium	Characteristic	Strain YU4-1 ^T	Strain YU6-1 ^T	Strain YU6-3 ^T	Strain YU8-1 ^T
Yeast extract-malt extract agar (ISP medium 2)	Growth	Moderate, light melon yellow (3ca) to light amber (3ic)	Moderate, amber (3pe), clove brown (3ni) to light melon yellow (3ca)	Good, bright melon yellow (3ia)	Good, toast tan (4lg) to light rose beige (4cc)
	Aerial mycelium	Absent	Absent	Absent	Absent
	Soluble pigment	Absent or golden brown (3pg)	Golden brown (3pi)	4ne	Dark luggage tan (4pg) or 4pn
	Reverse color	Light tan (3gc) to light amber (3ic)	Light amber (3ic) to golden brown (3pi)	Bright yellow (3pa)	Spice brown (4ni)
Oatmeal agar (ISP medium 3)	Growth	Moderate, eggshell (2ca) to shell (3ca)	Moderate, light wheat (2ea)	Moderate, amber (3pc) to light amber (3ic)	Moderate, light wheat (2ea) to colonial yellow (2ga)
	Aerial mycelium	Absent	Absent	Absent	Absent
	Soluble pigment	Absent	Absent	Absent	Absent or 2ea
	Reverse color	Shell (3ca) to light amber (3ic)	Light wheat (2ea)	Light amber (3ic) to light wheat (2ea)	Colonial yellow (2ga) to light wheat (2ea)
Inorganic salts-starch agar (ISP medium 4)	Growth	Fair, shell (3ca) to light amber (3ic)	Moderate, light wheat (2ea) to colonial yellow (2ga)	Fair, eggshell (2ca)	Moderate, light wheat (2ea) to bamboo (2gc), mustard tan (2lg)
	Aerial mycelium	Absent	White	Absent	Absent
	Soluble pigment	Absent	Absent	Absent	3gc
	Reverse color	Shell (3ca)	Light wheat (2ea)	Eggshell (2ca)	Bamboo (2gc)
Glycerol-asparagine agar (ISP medium 5)	Growth	Moderate, shell (3ca) to melon yellow (3ga)	Good, light wheat (2ea) to colonial yellow (2ga), light gold (2ic)	Moderate, light wheat (2ea)	Moderate, light wheat (2ea) to bamboo (2gc), mustard tan (2lg)
	Aerial mycelium	Absent	Absent	Absent	Absent
	Soluble pigment	Absent	Absent	Absent	Mustard gold (2pg) or 3ic
	Reverse color	Shell (3ca) to melon yellow (3ga)	Light wheat (2ea)	Light wheat (2ea) to eggshell (2ca)	Bamboo (2gc) to light gold (2ic)
Peptone-yeast extract-iron agar (ISP medium 6)	Growth	Fair, shell (3ca)	Moderate light wheat (2ea) to light gold (2ic)	Moderate, light amber (3ic)	Moderate, light wheat (2ea) to colonial yellow (2ga)
	Aerial mycelium	Absent	Absent	Absent	Absent
	Soluble pigment	Absent	Absent	Absent	Absent
	Reverse color	Colorless	Light gold (2ic) to mustard gold (2ne)	Mustard gold (2pe) to bright gold (2nc)	Light wheat (2ea) to colonial yellow (2ga)
Tyrosine agar (ISP medium 7)	Growth	Moderate, light melon yellow (3ca) to light tan (3gc)	Good, light wheat (2ea) to colonial yellow (2ga), bright yellow (3pa)	Moderate, light gold (2ic) to light wheat (2ea)	Moderate, light mustard tan (2ic)
	Aerial mycelium	Absent	Absent	Absent	Absent
	Soluble pigment	Absent	Absent	Absent	Absent
	Reverse color	Light melon yellow (3ca) to light tan (3gc)	Light wheat (2ea) to gold (2ic)	Light wheat (2ea) to light gold (2ic)	Mustard gold (2ne) or 4ng
Bennett's agar	Growth	Good, light amber (3ic)	Moderate, light wheat (2ea) to light gold (2ic)	Good, light amber (3ic) to colonial yellow (2ga)	Good, toast tan (4lg)
	Aerial mycelium	Absent	Absent	Absent	Absent
	Soluble pigment	Golden brown (3pi) or 3pe	Golden brown (3pg)	3pe	Light brown (4ng) or 4pi
	Reverse color	Melon yellow (3ga) to light amber (3ic)	Light gold (2ic)	Amber (3pe)	Spice brown (4ni)
Bennett's agar containing maltose	Growth	Moderate, eggshell (2ca)	Moderate, light amber (3ic)	Good, light wheat (2ea)	Good, light beige (3cc)
	Aerial mycelium	Absent	Absent	Absent	Absent
	Soluble pigment	Absent	Mustard gold (2pe)	Bright gold (2pc) or absent	Yellow maple (3le) or 4pi
	Reverse color	Eggshell (2ca)	Light amber (3ic)	Light wheat (2ea) to bamboo (2gc), light gold (2ic)	Light gold (2ic)
Nutrient agar	Growth	Fair, light wheat (2ea)	Good, light amber (3ic) to light wheat (2ea)	Fair, light melon yellow (3ea)	Fair, light wheat (2ea) to colonial yellow (2ga)
	Aerial mycelium	Absent	Absent	Absent	Absent
	Soluble pigment	Absent	Mustard gold (2ne) to light orange (2ia)	Butter yellow (1 1/2ga)	Light wheat (2ea) to colonial yellow (2ga)
	Reverse color	Light wheat (2ea)	Fair dull white, colorless	Fair, white, colorless	low (3ga)
Water agar	Growth	Fair, dull white, colorless	Fair dull white, colorless	Fair, white, colorless	Fair, dull white, colorless
	Aerial mycelium	Absent	Absent	Absent	Absent
	Soluble pigment	Absent	Absent	Absent	Absent
	Reverse color	Dull white, colorless	Dull white, colorless	Dull white, colorless	Dull white, colorless

^a The color codes correspond to the color codes in the *Color Harmony Manual* (1).

TABLE 3. Phenotypic characteristics of *A. inagensis*, *A. globicatena*, *A. terrae*, *A. diospyrosa*, and *A. riparia* strains

Characteristic	<i>A. inagensis</i> YU4-1 ^T	<i>A. globicatena</i> strains					<i>A. terrae</i> YU6-3 ^T	<i>A. diospyrosa</i> YU8-1 ^T	<i>A. riparia</i> IFO 14541 ^T
		YU5-1	YU6-1 ^T	YU6-2	YU7-1	YU7-2			
Utilization of:									
Inositol	— ^a	—	—	—	—	—	—	—	—
D-Fructose	±	+	++	++	++	+	++	++	±
L-Arabinose	—	±	±	—	+	+	±	+	—
Sucrose	—	+	+	+	++	+	+	+	—
D-Xylose	—	—	—	—	—	—	—	—	—
D-Mannitol	—	—	—	—	—	—	—	—	—
Raffinose	—	—	—	—	—	—	—	—	—
L-Rhamnose	±	±	±	±	±	±	±	±	±
D-Mannose	—	+	+	+	+	+	+	±	±
Maltose	—	±	+	++	+	+	+	+	±
D-Galactose	—	—	—	—	—	—	—	—	—
Lactose	—	—	—	—	—	—	—	—	—
D-Sorbitol	—	—	—	—	—	—	—	—	—
Growth temp range (°C)	14–33	13–36	11–35	12–34	13–34	14–34	12–34	12–32	NT
Optimum temp for growth (°C)	22–27	23–30	25–30	22–29	23–27	22–28	20–26	22–29	NT
Reduction of nitrate	P	P	P	P	P	P	P	P	A
Salt tolerance (%)	<2	<4	<4	<4	<4	<4	<4	<4	<3
Peptonization of milk	A	A	A	A	A	A	P	P	A
Hydrolysis of:									
Starch	A	P	P	P	P	P	P	P	A
Gelatin	A	P	P	P	P	P	P	P	A
Cellulose	A	A	A	A	A	A	A	A	A
Decomposition of calcium malate	A	P	P	P	P	P	P	A	A

^a —, none; ++, good; +, moderate; ±, poor; P, present; A, absent; NT, not tested.

iron agar (ISP medium 6) and water agar. All of the isolates produced a soluble pigment on Bennett's agar and yeast extract-malt extract agar (ISP medium 2). All of the isolates except isolate YU4-1^T produced soluble pigments on Bennett's agar containing maltose. Isolates YU7-1 and YU8-1^T produced a soluble pigment on glycerol-asparagine agar (ISP medium 5) and tyrosine agar (ISP medium 7).

Physiological and biochemical characteristics. Biochemical properties of the isolates are shown in Table 3. All of the new isolates reduced nitrate, but IFO 14541^T did not. All of the strains except YU4-1^T and IFO 14541^T liquefied gelatin. Isolates YU5-1, YU6-1^T, YU6-2, YU6-3^T, YU7-1, and YU7-2 solubilized calcium malate.

Chemotaxonomic characteristics. The whole-cell sugar patterns, cell wall amino acid compositions, phospholipid types, menaquinone compositions, and cellular fatty acid compositions of the isolates are shown in Tables 4 through 6. The cell

walls of the isolates contained *meso*-diaminopimelic acid, glycine, D-glutamic acid, and L-alanine, indicating that the peptidoglycan type of these organisms is type A1γ as described by Schleifer and Kandler (12). All of the isolates contained galactose and glucose as their major whole-cell sugars in addition to small amounts of arabinose, mannose, and rhamnose, indicating that the whole-cell sugar pattern of these isolates is pattern A as described by Lechevalier and Lechevalier (6). All of the isolates contained galactose as the major cell wall sugar, as well as trace to small amounts of arabinose (data not shown). The major menaquinone was MK-9(H₄), and small amounts of MK-7(H₄) and MK-8(H₄) were also present (Table 5). Although Hasegawa (3) identified the major menaquinone of *A. riparia* IFO 14541^T as MK-10(H₀), a gas-liquid chromatography-mass spectrometry study indicated that it was MK-9(H₄) (5a). All of the isolates contained iso-C_{16:0} as the major cellular fatty acid, as well as smaller amounts of iso-C_{15:0}, C_{16:0},

TABLE 4. Cell wall amino acid and whole-cell sugar compositions of strains of five *Actinokineospora* species^a

Strain	Amino acid composition of peptidoglycan (molar ratio) ^b					Sugar composition of whole cells						
	Glu	Mur	Gly	Ala	<i>meso</i> -DAP	GlcN	Rha	Man	Ara	Gal	Xyl	Glc
YU4-1 ^T	1.00	0.43	1.31	2.09	0.72	+ ^c	+	+	+	++	—	+
YU5-1	1.00	0.55	0.87	1.77	0.48	+	+	+	+	++	—	++
YU6-1 ^T	1.00	0.75	1.43	2.34	0.87	+	+	+	+	++	—	++
YU6-2	1.00	0.48	1.33	2.30	0.94	+	+	+	+	++	—	++
YU6-3 ^T	1.00	0.74	1.62	2.17	0.61	+	+	+	+	+	—	+
YU7-1	1.00	0.53	1.56	2.55	1.15	+	+	+	+	++	—	++
YU7-2	1.00	0.54	1.20	2.15	0.51	+	+	+	+	++	—	++
YU8-1 ^T	1.00	0.54	0.70	2.37	0.87	+	+	+	+	++	—	++
IFO 14541 ^T	1.00	0.39	1.51	1.73	1.19	+	+	+	+	++	—	++

^a Abbreviations: Glu, glutamic acid; Mur, muramic acid; Gly, glycine; Ala, alanine; *meso*-DAP, *meso*-diaminopimelic acid; GlcN, glucosamine; Rha, rhamnose; Man, mannose; Ara, arabinose; Gal, galactose; Xyl, xylose; Glc, glucose.

^b Calculated by defining the amount of glutamic acid as 1.0.

^c +, present (minor peak); ++, present (major peak); —, absent.

TABLE 5. Phospholipid types and menaquinone compositions of strains of five *Actinokineospora* species

Strain	Phospholipid types ^a	Menaquinone composition					
		MK-7(H ₄)	MK-8(H ₂)	MK-8(H ₄)	MK-9(H ₀)	MK-9(H ₂)	MK-9(H ₄)
YU4-1 ^T	P II	+ ^b	tr	tr	tr		+++
YU5-1	P II						+++
YU6-1 ^T	P II	tr		tr			+++
YU6-2	P II	+		+			+++
YU6-3 ^T	P II	+		+	tr	tr	+++
YU7-1	P II	tr		tr			+++
YU7-2	P II			+			+++
YU8-1 ^T	P II			+			+++
IFO 14541 ^T	P II	+			tr		+++

^a Phosphatidylethanolamine was detected, but phosphatidylglycerol, an unidentified phospholipid, and phosphatidylcholine were not detected.

^b +++, >50%; ++, >10%; +, ≤10%; tr, trace.

and C_{16:1} (Table 6). Mycolic acids were absent. Phosphatidylethanolamine was detected, but phosphatidylglycerol, an unidentified phospholipid, and phosphatidylcholine were not detected; this pattern corresponds to phospholipid type II of Lechevalier et al. (8). The G+C contents of the DNAs were 68.9 to 70.0 mol% (Table 7).

DNA-DNA hybridization. As shown in Table 7, DNA-DNA hybridization studies indicated that the eight isolates and *A. riparia* IFO 14541^T can be divided into five groups on the basis of DNA relatedness data. The first group has a single member, isolate YU4-1^T; the second group contains five isolates, YU5-1, YU6-1^T, YU6-2, YU7-1, and YU7-2; the third and fourth groups each contain a single isolate (YU6-3^T and YU8-1^T, respectively); and the fifth group is a single-member group containing *A. riparia* IFO 14541^T. The levels of DNA relatedness between isolate YU4-1^T and members of other groups were less than 29%; the levels of DNA relatedness among isolates YU5-1, YU6-1^T, YU6-2, YU7-1, and YU7-2 were more than 72%; the levels of DNA relatedness between isolate YU6-3^T and isolates belonging to the second group ranged from 29 to 57%; the levels of DNA relatedness between isolate YU8-1^T and members of other groups were less than 33%; and the levels of DNA relatedness between IFO 14541^T and all of the other isolates were less than 35%.

Our isolates form aerial mycelia with motile arthrospores, have meso-diaminopimelic acid as a cell wall diamino acid, contain arabinose and galactose in their whole-cell hydrolysates, have MK-9(H₄) as their major menaquinone, and are

phospholipid type II organisms. Therefore, these isolates are considered members of the genus *Actinokineospora*. On the basis of their biochemical characteristics and DNA-DNA similarity values, they can readily be distinguished from *A. riparia* IFO 14541^T.

Therefore, we propose the following classification: isolate YU4-1 (= IFO 15563) is the type strain of *Actinokineospora inagensis* sp. nov.; isolates YU5-1, YU6-1^T (= IFO 15564^T), YU6-2, YU7-1, and YU7-2 are members of *Actinokineospora globicatena* sp. nov.; isolate YU6-3 (= IFO 15668) is the type strain of *Actinokineospora terrae* sp. nov.; and isolate YU8-1 (= IFO 15565) is the type strain of *Actinokineospora diospyrosa* sp. nov. Differential characteristics of these four species and *A. riparia* are summarized in Table 8.

Species of the genera *Sporichthya*, *Actinosynnema*, *Actinokineospora*, *Catenuloplanes*, and *Couchioplanes* have been described previously as actinomycetes that form motile arthrospores. On the basis of the results of a comparative analysis of 16S ribosomal DNA sequences, the genera *Catenuloplanes* and *Couchioplanes* were placed in the family *Micromonosporaceae* (15), and it was found that the genus *Actinokineospora* is closely related to species of the genus *Saccharothrix*, but should not be placed in the family *Pseudonocardaceae* (16). Previous taxonomic studies of the genus *Actinokineospora* have been carried out with a single strain of a single species, *A. riparia*. The new species described below might be of help in more detailed studies on the phylogenetic positions of these organisms. Additional studies, including a comparative analysis of

TABLE 6. Cellular fatty acid compositions of strains of five *Actinokineospora* species

Strain	Fatty acid composition																	
	Nonpolar fatty acids															iso-branched 2-OH fatty acids		
	anteiso-C _{17:0}	iso-branched					Saturated					Unsaturated				10-methyl-C ₁₆	iso-C _{15:0}	iso-C _{16:0}
		iso-C _{14:0}	iso-C _{15:0}	iso-C _{16:0}	iso-C _{17:0}	iso-C _{18:0}	C _{14:0}	C _{15:0}	C _{16:0}	C _{17:0}	C _{18:0}	C _{15:1}	C _{16:1}	C _{17:1}	C _{18:1}			
YU4-1 ^T	2.4 ^a	1.2	9.4	61.3	4.6		0.1	0.5	8.9	0.6		0.4	4.4	0.7	0.3	2.3	± ^b	++
YU5-1	5.7	1.2	14.9	35.8	5.2	0.4	0.4	0.4	17.0	2.2	1.0		10.0	1.2	0.5	3.7	+	++
YU6-1 ^T	3.5	1.1	17.9	38.1	8.1	0.8	0.3	0.4	12.7	3.5			10.8	1.8			+	++
YU6-2	2.4	1.8	19.8	35.0	9.4	0.5	0.3	0.6	15.0	2.8	0.6		10.5				+	++
YU6-3 ^T	5.1	1.6	18.2	38.6	6.3		0.2	0.1	12.5	3.6			4.3	2.2		2.9	+	++
YU7-1	4.1	1.7	18.4	39.2	8.2		0.3	0.5	12.4	3.2			8.3	2.3	0.7		+	++
YU7-2	4.0	1.3	13.8	38.4	4.6	0.2	0.4	0.5	17.5	2.7	0.9		12.5	1.7	0.5		+	++
YU8-1 ^T	4.7	1.6	20.3	36.5	11.1			0.8	12.6	3.6	0.5		1.5	1.6	0.4	3.7	+	++
IFO 14541 ^T	7.3	2.2	16.7	43.1	3.9		0.3	1.1	8.0	2.4	0.3		2.3	1.0		5.2	+	++

^a The values are the percentages of the nonpolar fatty acids.

^b ±, <10%; +, 10 to 30%; ++, >30%.

TABLE 7. G+C contents and levels of DNA relatedness among strains of five *Actinokineospora* species

Strain	G+C content (mol%)	% of DNA complementary to labelled DNA from strain:								
		YU4-1 ^T	YU5-1	YU6-1 ^T	YU6-2	YU6-3 ^T	YU7-1	YU7-2	YU8-1 ^T	IFO 14541 ^T
YU4-1 ^T	69.1	100	10	15	6	14	3	8	9	9
YU5-1	69.8	23	100	108	84	55	97	93	28	12
YU6-1 ^T	69.7	19	100	100	92	52	110	86	28	3
YU6-2	69.8	15	75	100	100	41	87	72	27	7
YU6-3 ^T	70.0	12	41	41	38	100	44	29	33	8
YU7-1	69.5	18	102	121	91	57	100	85	25	35
YU7-2	69.8	25	115	115	95	54	91	100	33	12
YU8-1 ^T	69.3	29	29	39	21	37	17	30	100	27
IFO 14541 ^T	68.9	10	3	5	3	6	2	7	6	100

16S rRNA sequences, will be performed to determine the relationship of the genus *Actinokineospora* to other actinomycetes which have motile arthrospores.

Description of *Actinokineospora inagensis* sp. nov. *Actinokineospora inagensis* (in.ag.en'sis. M. L. adj. *inagensis*, pertaining to Lake Inaga, the lake from which the organism was isolated) is a gram-positive, non-acid-fast, aerobic organism with branching hyphae. Nonfragmenting, yellow to tan vegetative mycelia are present. Spores are formed in chains containing several members and are motile. The spores are oval or short rods (diameter, 1.3 to 2.0 µm) and exhibit motility when they are suspended in sterile distilled water. Strictly aerobic. The temperature range for growth is 14 to 33°C, and good growth occurs at temperatures between 22 and 27°C. The organism grows well on Bennett's agar. In general, the vegetative mycelia are yellow to tan, and the aerial mycelia are white to gray. Hydrogen sulfide is produced. Reduces nitrate to nitrite. Gelatin liquefaction negative. Does not hydrolyze starch. Does not decompose calcium malate. Does not grow in the presence of 2% NaCl. Peptonization of milk is negative. Fructose and rhamnose are slightly utilized as carbon sources, but inositol, arabinose, sucrose, xylose, mannitol, raffinose, mannose, maltose, galactose, lactose, and glucitol are not utilized.

Cell walls contain glutamic acid, glycine, alanine, and meso-diaminopimelic acid (molar ratio, ca. 1:1:2:1). The peptidoglycan type is type A (probably A1γ) according to the classification of Schleifer and Kandler (12). Arabinose and galactose are the characteristic whole-cell sugars. iso-Hexadecanoic acid (iso-C_{16:0}) is the major cellular fatty acid. The G+C content of the DNA of the type strain is 69.1 mol%. The major menaquinone is MK-9(H₄), and a small amount of MK-7(H₄) is also present. Phosphatidylethanolamine is the diagnostic phospho-

lipid, and phosphatidylglycerol, phosphatidylcholine, and an unidentified phospholipid are absent (type PII). Habitat: fallen leaves.

The type strain is IFO 15663 (= YU4-1).

Description of *Actinokineospora globicatena* sp. nov. *Actinokineospora globicatena* (glo.bi.ca.te'na. L. n. *globus*, a ball, sphere; L. n. *catena*, chain; *globicatena*, ball of chains, referring to the mass of spore chains that aggregate into balls). Morphological, physiological, and chemotaxonomic characteristics are the same as those given above for *A. inagensis*. In addition, the spore chains and aerial mycelia often aggregate into clusters. The organism grows well on yeast extract-malt extract agar (ISP medium 2), glycerol-asparagine agar (ISP medium 5), tyrosine agar (ISP medium 7), Bennett's agar, and Bennett's agar containing maltose. The temperature range for growth is 11 to 36°C, and good growth occurs at temperatures between 20 and 30°C. In general, the vegetative mycelia of the strains are yellow to golden brown, and the aerial mycelia are white to gray. Gelatin liquefaction positive. Hydrolyzes starch. Decomposes calcium malate. Does not grow in the presence of 4% NaCl. Peptonization of milk is negative. Fructose, arabinose, sucrose, mannose, maltose, and rhamnose are utilized as carbon sources, but inositol, xylose, mannitol, raffinose, galactose, lactose, and glucitol are not utilized.

The G+C content of the DNA ranges from 69.5 to 69.8 mol%; the G+C content of the DNA of the type strain is 69.7 mol%. The major menaquinone is MK-9(H₄). Habitat: soil and fallen leaves.

The type strain is IFO 15664 (= YU6-1).

Description of *Actinokineospora terrae* sp. nov. *Actinokineospora terrae* (ter'rae. M. L. gen. n. *terrae*, of the earth). Morphological, physiological, and chemotaxonomic characteristics are the same as those given above for *A. inagensis*. In addition, the organism grows well on yeast extract-malt extract agar (ISP medium 2), glycerol-asparagine agar (ISP medium 5), tyrosine agar (ISP medium 7), Bennett's agar, and Bennett's agar containing maltose. The temperature range for growth is 11 to 36°C, and good growth occurs at temperatures between 20 and 30°C. In general, the vegetative mycelia are yellow to golden brown, and the aerial mycelia are white to gray. Gelatin liquefaction positive. Hydrolyzes starch. Decomposes calcium malate. Does not grow in the presence of 4% NaCl. Peptonization of milk is positive. Fructose, arabinose, sucrose, mannose, maltose, and rhamnose are slightly utilized as carbon sources, but inositol, xylose, mannitol, raffinose, galactose, lactose, and glucitol are not utilized.

The G+C content of the DNA is 70.0 mol%. The major menaquinone is MK-9(H₄). Habitat: soil and fallen leaves.

The type strain is IFO 15668 (= YU6-3).

TABLE 8. Differential characteristics of *Actinokineospora* species

Characteristic	<i>A. riparia</i>	<i>A. inagensis</i>	<i>A. globicatena</i>	<i>A. terrae</i>	<i>A. diospyrosa</i>
Utilization of:					
Sucrose	— ^a	—	+	+	+
D-Mannose	±	—	+	+	+
Reduction of nitrate	—	+	+	+	+
Growth in NaCl (%)	<3	<2	<4	<4	<4
Peptonization of milk	—	—	—	+	+
Hydrolysis of:					
Starch	—	—	+	+	+
Gelatin	—	—	+	+	+
Decomposition of calcium malate	—	—	+	+	—
Formation of clusters	—	—	+	—	—

^a —, none; ++, good; +, moderate; ±, poor.

Description of *Actinokineospira diospyrosa* sp. nov. *Actinokineospira diospyrosa* (di.o.spy'ros.a. M. L. adj. *diospyrosa*, pertaining to the fruit tree *Diospyros kaki*). Morphological, physiological, and chemotaxonomic characteristics are the same as those given above for *A. inagensis*. In addition, the organism grows well on yeast extract-malt extract agar (ISP medium 2), Bennett's agar, and Bennett's agar containing maltose. The temperature range for growth is 12 to 32°C, and good growth occurs at temperatures between 22 and 29°C. In general, the vegetative mycelia are tan, and the aerial mycelia are white to gray. Gelatin liquefaction positive. Hydrolyzes starch. Does not decompose calcium malate. Does not grow in the presence of 4% NaCl. Peptonization of milk is positive. Fructose, arabinose, sucrose, mannose, maltose, and rhamnose are slightly utilized as carbon sources, but inositol, xylose, mannitol, raffinose, galactose, lactose, and glucitol are not utilized.

The G+C content of the DNA of the type strain is 69.3 mol%. The major menaquinone is MK-9(H₄), and a small amount of MK-8(H₄) is also present. Habitat: fallen leaves.

The type strain is IFO 15665 (= YU8-1).

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