Acrocarpospora gen. nov., a new genus of the order Actinomycetales

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The taxonomic position of two actinomycete strains isolated from soil was studied. The isolates contained glutamic acid, alanine and meso-diaminopimelic acid as cell-wall amino acids and menaquinone MK-9(H₄) and madurose in the whole-cell hydrolysate. Phylogenetic analysis revealed that the isolates belonged to the family Streptosporangiaceae, but not to any known genus, and formed a monophyletic cluster with Streptosporangium corrugatum. On the basis of morphological characteristics, phylogenetic analysis and DNA–DNA hybridization, the name Acrocarpospora gen. nov. is proposed for a new genus containing the isolates and Streptosporangium corrugatum, and Acrocarpospora pleiomorpha sp. nov. R-31^T (= IFO 16267^T), Acrocarpospora macrocephala sp. nov. R-55^T (= IFO 16266^T) and Acrocarpospora corrugata comb. nov. IFO 13972^T are described.

Keywords: *Acrocarpospora* gen. nov., *Streptosporangium corrugatum*, family Streptosporangiaceae, 16S rDNA

INTRODUCTION

The family Streptosporangiaceae (Stackebrandt *et al.*, 1997) includes the following genera: *Microtetraspora* (Zhang *et al.*, 1998), *Microbispora* (Zhang *et al.*, 1998), *Streptosporangium* (Stackebrandt *et al.*, 1994), *Herbidospira* (Kudo *et al.*, 1993), *Planomonospira* (Thiemann *et al.*, 1967), *Planobispora* (Thiemann & Beretta, 1968), *Planotetraspora* (Runmao *et al.*, 1993) and *Nonomuraea* (Zhang *et al.*, 1998). Most members of the family have cell wall type III, whole-cell sugar pattern B/C, fatty acid pattern 3c, major menaquinone MK-9(H₀, H₂, H₄, H₆) and phospholipid type IV. Each genus has distinguishable morphology with the exception of *Microtetraspora* and *Nonomuraea*.

The genus *Streptosporangium* develops stable, branched mycelium and produces globose sporangia (usually 10 µm in diameter) on aerial mycelium. Sporangiospores are formed by septation of coiled, unbranched hyphae within the sporangium. However, *Streptosporangium viridogriseum* subsp. *viridogriseum* (Okuda *et al.*, 1966), *Streptosporangium viridogriseum* subsp. *kofuense* (Nonomura & Ohara, 1969), *Streptosporangium albidum* (Furumai *et al.*, 1968), *Strepto-*

sporangium viridialbum (Nonomura & Ohara, 1960), *Streptosporangium claviforme* (Petrolini *et al.*, 1992) and *Streptosporangium corrugatum* (Williams & Sharples, 1976) differ from the ordinary members of the genus *Streptosporangium* in the morphological properties of sporangia detected by scanning electron microscopy, electrophoretic mobility of ribosomal protein AT-L30 (Ochi & Miyadoh, 1992), 16S rDNA (Kemmerling *et al.*, 1993; Ward-Rainey *et al.*, 1996) and 5S rRNA (Kudo *et al.*, 1993). Stackebrandt *et al.* (1994) transferred *S. viridogriseum* subsp. *viridogriseum*, *S. viridogriseum* subsp. *kofuense* and *S. albidum* to the new genus *Kutzneria* as *Kutzneria viridogrisea*, *Kutzneria kofuensis* and *Kutzneria albida* on the basis of chemotaxonomic characteristics.

Two actinomycete strains that formed club-shaped or spherical structures at the heads of the aerial mycelia were isolated from soil. They contained menaquinone MK-9(H₄), phospholipid type IV, meso-diaminopimelic acid as a cell-wall diamino acid and madurose in the whole-cell hydrolysate. Phylogenetic analysis based on 16S rDNA sequences indicated that the isolates were distinct from previously described genera and were closely related to *S. corrugatum* (Williams & Sharples, 1976).

In this paper, we describe the characterization and classification of strains R-31^T and R-55^T and *S. corrugatum* and we propose the creation of *Acrocar-*

The GenBank accession numbers for the 16S rRNA gene sequences of *Acrocarpospora pleiomorpha* sp. nov. strain R-31^T and *Acrocarpospora macrocephala* sp. nov. strain R-55^T are AB006174 and AB025318.

Fig. 1. Light micrograph (a) and scanning electron micrographs (b, c and d) of strain R-31^T grown on HV agar for 14 d at 28 °C. Bars, 50 µm (a) and 10 µm (b–d).

pospora gen. nov., with *Acrocarpospora pleiomorpha* sp. nov. for R-31^T (=IFO 16267^T), *Acrocarpospora macrocephala* sp. nov. for R-55^T (=IFO 16266^T) and *Acrocarpospora corrugata* comb. nov. for *S. corrugatum* (type strain IFO 13972^T)

METHODS

Micro-organisms and culture conditions. Strain R-31^T was isolated from soil in Louisiana, USA, and strain R-55^T was isolated from soil in Saitama prefecture, Japan. *S. corrugatum* IFO 13972^T was used.

Morphological and physiological characteristics. Cultures grown on HV agar and yeast extract/starch agar for 3–14 d at 28 °C were observed with a light microscope and a scanning electron microscope (model JSM-5400; JEOL). Samples for scanning electron microscopy were prepared by cutting a block from an agar plate containing the strain, fixing the block in osmium tetroxide vapour at room temperature for 4 h, dehydrating cells through freeze-drying

and sputter-coating the preparation with palladium under vacuum.

Motility was observed with a light microscope using cells grown on HV agar for 7–10 d at 28 °C and then incubated at 28 °C for 30–60 min in sterile water.

Cultural characteristics on the International *Streptomyces* project (ISP) media (Shirling & Gottlieb, 1966) and nutrient agar (Difco Laboratories) were recorded after 14 d incubation at 28 °C. Colours are described in common terminology, but exact colours were determined by comparison with colour chips of a mycological colour chart (Rayner, 1970).

ISP medium 9 was used as the basal medium for utilization of sugars. Inorganic salts/starch agar (ISP medium 4) was used for determined of starch hydrolysis. Growth temperature was determined on yeast extract/starch agar. Calcium malate medium (1% calcium malate, 0.05% NH₄Cl, 0.05% K₂HPO₄, 1.8% agar, pH 7.0) was used for the determination of solubilization of calcium malate. ISP medium 8 was used for the determination of nitrate

Fig. 2. Light micrograph (a) and scanning electron micrographs (b, c and d) of strain R-55^T grown on HV agar for 14 d at 28 °C. Bars, 50 µm (a) and 10 µm (b–d).

reduction. Gelatin medium (20% gelatin, pH 7.0) and glucose/peptone/gelatin medium (2% glucose, 0.5% peptone and 20% gelatin, pH 7.0) were used for the determination of gelatin liquefaction. Skimmed milk (10%; Difco Laboratories) was used for determination of milk peptonization.

Analysis of chemotaxonomic characteristics. The analysis of whole-cell sugar composition, amino acid composition of the cell wall, menaquinone composition, cellular fatty acid composition, isomers of diaminopimelic acid, acyl type of peptidoglycan, mycolic acid and DNA base composition was performed as described previously (Tamura *et al.*, 1994).

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

PCR amplification and sequencing of 16S rDNA. 16S rDNA was amplified by PCR (Saiki *et al.*, 1988) using protocols described previously (Tamura *et al.*, 1998). The PCR products were sequenced directly using protocols described previously (Tamura *et al.*, 1998). The products were analysed with a Pharmacia ALF DNA Sequencer II according to the manufacturer's protocol.

Phylogenetic analysis. 16S rDNA sequences of the strains and reference organisms were aligned against that of *Streptomyces ambofaciens* (Pernodet *et al.*, 1989). The CLUSTAL W software version 1.7 (Thompson *et al.*, 1994) was used to generate evolutionary distances (the K_{nuc} value of Kimura, 1980), similarity values, neighbour-joining analysis (Saitou & Nei, 1987) from K_{nuc} values and bootstrap resampling method of Felsenstein (1985) with 1000 replicates for evaluation of the topology of the phylogenetic tree. NJPLOT (Perrière & Gouy, 1996) was used for plotting the phylogenetic tree.

Measurement of DNA similarity. The microplate-hybridization method developed by Ezaki *et al.* (1988, 1989)

with minor modifications (Tamura *et al.*, 1999) was applied to determine DNA similarity.

RESULTS

Morphological observations

The isolates were Gram-positive, non-acid-fast and aerobic organisms with branched hyphae. A non-fragmenting substrate mycelium was formed. Morphological observations by light microscope of a 14-d-old culture grown on HV agar revealed the presence of a few club-shaped and many spherical structures at the pointed heads of the aerial hyphae (Figs 1a and 2a). Observation by scanning electron microscope indicated that the structures were variable in size: spherical (2.0–5.0 µm) or club-shaped (0.7–1.5 × 3.5–8.0 µm) (Figs 1b–d and 2b–d). Both structures appeared to be covered by a wrinkled envelope and the undulations of the envelope suggested that spores might be present within (Figs 1c and d, 2c and d). The spores were oval

Table 1. Phenotypic characteristics of isolates R-31^T and R-55^T

Characteristics are scored as negative (–), positive (+) or strongly positive (++).

Characteristic	R-31 ^T	R-55 ^T
Utilization of:		
Glucose	++	++
D-Xylose	–	–
Inositol	–	–
Sucrose	–	++
Raffinose	++	+
Rhamnose	+	+
D-Mannitol	++	++
D-Fructose	–	–
L-Arabinose	–	–
Melanin formation on:		
ISP medium 1	–	–
ISP medium 6	–	–
ISP medium 7	–	–
Gelatin liquefaction	–	–
Peptonization of milk	Weak	+
Starch hydrolysis	+	+
Calcium malate solubilization	–	–
Nitrite from nitrate	+	–
Growth at:		
10 °C	–	–
15 °C	–	+
20 °C	++	++
30 °C	++	++
37 °C	–	–
Growth on:		
0 % NaCl	+	+
4 % NaCl	–	–

Table 2. Chemotaxonomic characteristics of isolates R-31^T and R-55^T

Characteristic	R-31 ^T	R-55 ^T
Amino acid composition of peptidoglycan (molar ratio):*		
Glutamic acid	1.00	1.00
Alanine	1.80	2.04
meso-Diaminopimelic acid	1.09	0.69
Sugar composition of whole cells (%):†		
Rhamnose	ND	trace
Madurose	++	++
Galactose	+	+
Xylose	trace	trace
Glucose	++	++
Major menaquinone:‡		
9(H ₂)	+	+
9(H ₄)	++	++
9(H ₆)	+	+
Phospholipid composition:		
Phosphatidylglycerol	–	–
Unidentified phospholipid including glucosamine	+	+
Phosphatidylcholine	–	–
Phosphatidylethanolamine	+	+
Phospholipid type	IV	IV
Mycolic acid	ND	ND
Acyl type of peptidoglycan	Acetyl	Acetyl

* Relative to glutamic acid as 1.0.

† Scored as ++, major amount; +, minor amount; trace, trace amount.

‡ Scored as ++, >20%; +, <20%.

ND, Not detected.

to rod-shaped (0.8–1.0 × 1.0–1.5 µm) and had a smooth surface.

Cultural characteristics

Both strains exhibited good growth on oatmeal agar (ISP medium 3), moderate growth on inorganic salts/starch agar (ISP medium 4) and poor growth on yeast extract/malt extract agar (ISP medium 2), peptone/yeast extract iron agar (ISP medium 6), tyrosine agar (ISP medium 7) and nutrient agar. On glycerol/asparagine agar (ISP medium 5), isolate R-31^T grew poorly and isolate R-55^T grew moderately. Colonies on most media tested were pale yellow. Only isolate R-55^T sporulated on oatmeal agar (ISP medium 3). Neither isolate produced soluble pigment on any medium tested. Both isolates formed aerial hyphae and sporulated on HV agar (data not shown).

Physiological and biochemical characteristics

Biochemical properties of the isolates are shown in Table 1. Both isolates actuated starch hydrolysis but not gelatin liquefaction or calcium malate

Table 3. Cellular fatty acid compositions of strains R-31^T and R-55^T

Abundances of individual fatty acids are shown as percentages. ND, Not detected.

Fatty acid	R-31 ^T	R-55 ^T
Iso-branched fatty acids		
C _{14:0}	12.0	8.4
C _{16:0}	30.4	26.1
Saturated fatty acids		
C _{13:0}	1.1	1.0
C _{14:0}	ND	1.1
C _{15:0}	5.4	5.8
C _{16:0}	3.9	5.4
C _{17:0}	8.4	8.1
C _{18:0}	1.3	1.6
Unsaturated fatty acids		
C _{17:1}	6.5	6.3
C _{18:1}	0.7	0.9
10-Methylated fatty acids		
C _{16:0}	1.2	1.9
C _{17:0}	27.3	27.7
C _{18:0}	2.2	3.2

solubilization. Both utilized glucose, raffinose, rhamnose and D-mannitol, and isolate R-55^T also utilized sucrose. Isolate R-31^T reduced nitrate, but isolate R-55^T did not. Both isolates grew well at 20 and 30 °C, but neither could grow at 10 or 37 °C. Neither isolate could grow on 4% NaCl.

Chemotaxonomic characteristics

The whole-cell sugar pattern, cell-wall amino acid composition, phospholipid type, menaquinone composition and cellular fatty acid composition of the isolates are shown in Tables 2 and 3. Their cell walls contained *meso*-diaminopimelic acid, D-glutamic acid and L-alanine, indicating that the peptidoglycan is type A1_γ, according to Schleifer & Kandler (1972). The isolates contained madurose and glucose as the major whole-cell sugars in addition to small amounts of galactose and xylose, indicating that the whole-cell sugar pattern of these isolates is B, according to Lechevalier & Lechevalier (1970). The major menaquinone was MK-9(H₄), and small amounts of MK-9(H₂) and MK-9(H₆) were present (Table 2). The isolates contained iso-C_{16:0} and 10-methyl C_{17:0} as the major cellular fatty acids, with small amounts of iso-C_{14:0}, C_{17:0}, C_{17:1}, C_{15:0} and C_{16:0} (Table 3). Mycolic acids were absent. Phosphatidylethanolamine and an unidentified phospholipid including glucosamine were detected, but phosphatidylglycerol and phosphatidylcholine were not detected, corresponding to phospholipid type IV of Lechevalier *et al.* (1981). The acyl type of the cell wall polysaccharides was acetyl. The G + C

contents of DNA were 68.6 mol% for isolate R-31^T and 68.1 mol% for isolate R-55^T.

Phylogenetic analysis

16S rDNA sequences were determined for R-31^T (positions 28–1525 of the *Escherichia coli* numbering system of Brosius *et al.*, 1978) and R-55^T (positions 28–1519). Positions at which secondary structures varied between strains and positions at which sequences were not determined in some reference organisms were excluded from the analysis. The phylogenetic tree obtained by applying the neighbour-joining method to *K*_{nuc} values is depicted in Fig. 3. The 16S rDNA analysis revealed that the isolates fell in the cluster of the family *Streptosporangiaceae*. The isolates were closely related to *S. corrugatum* (similarity levels 97.6–98.8%), *Herbidospira cretacea* (97.2–97.3%), *S. claviforme* (96.9–97.5%) and *Planotetraspora mira* (96.9–97.1%). Binary similarity values of the 16S rDNA between this cluster and other members of the family *Streptosporangiaceae* are 90.0–96.9%.

DNA–DNA hybridization

The results of DNA–DNA hybridization among isolates R-31^T and R-55^T, *S. corrugatum* IFO 13972^T, *H. cretacea* IFO 15474^T, *S. claviforme* IFO 15623^T and *P. mira* IFO 15435^T are shown in Table 4. Relatedness between the isolates was 45–49%. The relatedness between the isolates and *S. corrugatum* IFO 13972^T was 12–24%. In contrast, low levels of relatedness (3–9%) were found between these three strains and the other strains belonging to related species used for comparison.

DISCUSSION

Isolates R-31^T and R-55^T form club-shaped or spherical sporangia at the heads of the aerial mycelia and have *meso*-diaminopimelic acid as a cell-wall diamino acid, madurose in the whole-cell hydrolysate, MK-9(H₄) as the major menaquinone and phospholipid type IV. The isolates produce spores by fragmentation of hyphae and the hyphal sheath, which either expands to form the sporangial envelope or remains around the spore chains. Although the morphological characteristics and spore development of isolates were similar in many respects to those of *Streptosporangium* species, there were differences in the irregular shape and variable size of spore vesicles. Under the light microscope, the isolates appeared similar to *Nonomuraea pusilla*, which develops pseudosporangia, rather than to the majority of *Streptosporangium* species. Similar morphological features are also shown by *S. corrugatum*. Williams & Sharples (1976) reported that, as the club-shaped structures matured, partial or complete removal of their outer, wrinkled envelope revealed chains of three to eight spores, which were prominently ridged. Spores were 0.8–1.0 µm in length and 0.6–0.7 µm in breadth. Ridges were mostly longi-

Fig. 3. Phylogenetic tree, based on neighbour-joining (Saitou & Nei, 1987), derived from 16S rRNA sequences for members of the family Streptosporangiaceae. *Streptomyces ambofaciens* ATCC 23877^T (M27245) was used as the root organism. 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).

tudinal, with annular ridges between adjacent spores. These spores, therefore, had the same form as those found inside the spherical bodies. Although the

surfaces of the spherical bodies were smooth at first, they became wrinkled or remained around the spore chains as spores developed.

Table 4. DNA association between isolates R-31^T and R-55^T and related strains

The data are percentage DNA–DNA reassociation values between DNA of the taxa shown.

Taxon	1	2	3	4	5	6
1. Isolate R-31 ^T	100	45±18	12±4	8±1	3±1	6±1
2. Isolate R-55 ^T	49±11	100	17±5	6±1	4±1	7±2
3. <i>Streptosporangium corrugatum</i> IFO 13972 ^T	17±6	24±3	100	6±1	5±2	7±1
4. <i>Planotetraspora mira</i> IFO 15435 ^T	2±1	3±0	3±1	100	3±1	2±0
5. <i>Herbidosporea cretacea</i> IFO 15474 ^T	5±1	6±0	9±1	5±0	100	80±17
6. <i>Streptosporangium claviforme</i> IFO 15623 ^T	6±1	6±1	7±3	4±1	80±6	100

Table 5. Morphological features and chemotaxonomic characteristics of the new taxon (isolates R-31^T and R-55^T and *S. corrugatum*) and the family *Streptosporangiaceae*

Characteristic	New taxon	<i>Herbidosporea</i>	<i>Planotetraspora</i>	<i>Strepto- sporangium</i>	<i>Microtetraspora</i>	<i>Nonomuraea</i>	<i>Microbispora</i>	<i>Planomonospora</i>	<i>Planobispora</i>
Sporangium formation	Club or globose sporangia on aerial mycelium	Spore chains on sporophore	Sporangia, containing quartet of spores	Globose sporangia on aerial mycelium	Spore chains, typically containing four spores on short aerial mycelium	Spore chain or pseudosporangium on aerial mycelium	Spores in characteristic longitudinal pairs on aerial mycelium	Cylindrical to clavate sporangia, containing a single spore	Cylindrical to clavate sporangia, containing longitudinal pair of spores
Cell-wall type	III	III	III	III	III	III	III	III	III
Whole-cell sugar pattern	B, C	B	D, A	B	B, C	B, C	B, C	B	B
Fatty-acid type	3c	3c	NT	3c	3c	3c	3c	3c	3c
Major menaquinone	MK-9	MK-10	NT	MK-9	MK-9	MK-9	MK-9	MK-9	MK-9
	(H ₂ , H ₄ , H ₆)	(H ₄ , H ₆ , H ₂)		(H ₂ , H ₄)	(H ₀ , H ₂ , H ₄)	(H ₀ , H ₂ , H ₄)	(H ₀ , H ₂ , H ₄)	(H ₂)	(H ₂ , H ₄)
Phospholipid type	IV, II	IV	NT	IV	IV	IV	IV	IV	IV

NT, Not tested.

The phylogenetic position of the isolates is within the confines of the family *Streptosporangiaceae*, adjacent to *S. corrugatum*, which occupies a phylogenetic position distinct from other species of *Streptosporangium* (Kemmerling *et al.*, 1993; Ward-Rainey *et al.*, 1996).

S. corrugatum was reported as a species with some unusual morphological features (Williams & Sharples, 1976): it possesses the major menaquinones MK-9(H₂) and MK-9(H₄) (Kudo & Seino, 1987; Collins *et al.*, 1988; Stackebrandt *et al.*, 1994), major fatty acids C_{17:1} and C_{17:0} (Kudo & Seino, 1987; Stackebrandt *et al.*, 1994) and galactose and a trace amount of madurose as whole-cell sugars (Stackebrandt *et al.*, 1994). It contains phosphatidylethanolamine but not ninhydrin-positive or sugar-positive phospholipids (Stackebrandt *et al.*, 1994), indicating that the phospholipid type is II, according to Lechevalier *et al.* (1981). Isolates R-31^T and R-55^T, on the other hand, contained phosphatidylethanolamine and ninhydrin-positive and sugar-positive phospholipids, indicating that the phospholipid type is IV (Lechevalier *et al.*, 1981). Some chemotaxonomic characteristics of the isolates and *S. corrugatum* were therefore different. However, the results of morphological observation and phylogenetic analysis indicate that they belong to the same group.

Isolates R-31^T and R-55^T, *S. corrugatum*, *H. cretacea*, *S. claviforme* and *P. mira* appear to be closely related to each other, as shown by 16S rDNA sequence similarities ranging from 96.9 to 98.8%. However, the isolates are distinguished from the genus *Herbidosporea* in their morphological features and major menaquinone and from the genus *Planotetraspora* in their morphological features and sugar composition (Table 5). Both morphological features and chemotaxonomic characteristics indicate that they are distinct from known actinomycetes belonging to the family *Streptosporangiaceae* (Table 5).

On the basis of their morphological characteristics and our phylogenetic analysis, isolates R-31^T and R-55^T are readily distinguishable from the actinomycetes mentioned above and warrant the creation of a new taxon. Therefore, we propose the classification of these isolates and *S. corrugatum* in the new genus *Acrocarpospora* gen. nov., with *Acrocarpospora pleiomorpha* sp. nov. for isolate R-31^T, *Acrocarpospora macrocephala* sp. nov. for isolate R-55^T and *Acrocarpospora corrugata* comb. nov. for *S. corrugatum*. These strains have been deposited in the Institute for Fermentation, Osaka, as strains IFO 16267^T (*A. pleiomorpha* R-31^T), IFO 16266^T (*A. macrocephala* R-55^T) and IFO 13972^T (*A. corrugata*). Table 5 summarizes the differential characteristics of the new

genus and other members of the family *Streptosporangiaceae*.

The morphological features of *S. claviforme* are similar to those of *H. cretacea*. Furthermore, their high DNA relatedness (80%) indicates that *S. claviforme* is a subjective synonym of *H. cretacea*. The taxonomic position of *S. viridialbum* remains ambiguous.

Description of *Acrocarpospora* gen. nov.

Acrocarpospora (A.cro.car.po.spo'ra. Gr. adj. *Akros* pointed, sharp, highest; Gr. n. *karpos* fruit, harvest; Gr. fem. n. *spora* a seed; M.L. fem. n. *Acrocarpospora* an organism forming spores like fruits on the terminal mycelium).

Cells are Gram-positive and non-acid-fast and organisms form branching hyphae. Non-fragmentary substrate mycelia are present. Spherical and club-shaped structures are borne on the tips of the aerial mycelium. These structures contain coiled spore chains. Spores are oval or short rod-like ($0.6\text{--}0.8 \times 0.7\text{--}1.0\ \mu\text{m}$) with a smooth surface and non-motile.

Strictly aerobic. Good growth occurs between 20 and 30 °C. The organism shows good growth on oatmeal agar. In general, the vegetative mycelia are pale yellow and aerial hyphae are white.

Cell walls contain glutamic acid, alanine and meso-diaminopimelic acid. Wall chemotype is III (Lechevalier & Lechevalier, 1970) and the peptidoglycan type is presumed to be A1 γ (Schleifer & Kandler, 1972). Madurose, glucose and galactose are detected in whole-cell sugars. Major cellular fatty acids are iso-C_{16:0}, 10-methyl C_{17:0}, C_{17:1} and C_{17:0}. The G+C content of DNA is 68–69 mol%. The major menaquinones are MK-9(H₄) and MK-9(H₂) and small amounts of MK-9(H₆) are present. Phosphatidylethanolamine is present as a diagnostic phospholipid. Mycolic acids are absent. The acyl type of cell-wall polysaccharide is acetyl. The type species is *Acrocarpospora pleiomorpha*.

Description of *Acrocarpospora pleiomorpha* sp. nov.

Acrocarpospora pleiomorpha (ple.i.o.mor'pha. Gr. adj. *pleion* more; Gr. n. *morphe* form; M.L. fem. adj. *pleiomorpha* pleiomorphic, in various shapes).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. Gelatin liquefaction is negative. Hydrolyses starch. Does not decompose calcium malate. Coagulation of milk is weak. Reduction of nitrate is positive. Glucose, raffinose, rhamnose and mannitol are utilized, but xylose, arabinose, sucrose, fructose and inositol are not. As diagnostic phospholipids, phosphatidylethanolamine, ninhydrin-positive and sugar-positive phospholipids are present. The G+C content of DNA is 69 mol%. Habitat is soil. The type strain is R-31^T (=IFO 16267^T).

Description of *Acrocarpospora macrocephala* sp. nov.

Acrocarpospora macrocephala (ma.cro.ce'pha.la. Gr. adj. *makros* large; Gr. fem. n. *kephale* head; M.L. fem. nom. n. *macrocephala* large head).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. Gelatin liquefaction is negative. Hydrolyses starch. Does not decompose calcium malate. Coagulates milk. Glucose, raffinose, rhamnose, mannitol and sucrose are utilized, but xylose, arabinose, fructose and inositol are not. As diagnostic phospholipids, phosphatidylethanolamine and ninhydrin-positive and sugar-positive phospholipids are present. The G+C content of DNA is 68 mol%. Habitat is soil. The type strain is R-55^T (=IFO 16266^T).

Description of *Acrocarpospora corrugata* (Williams and Sharples 1976) comb. nov.

Acrocarpospora corrugata (cor.ru.ga'ta. L. fem. adj. *corrugata* wrinkled).

Descriptions of this taxon have been published previously by Williams & Sharples (1976), Stackebrandt *et al.* (1994), Kudo & Seino (1987), Collins *et al.* (1988) and Whitham *et al.* (1993). As diagnostic phospholipids, phosphatidylethanolamine is present, but ninhydrin-positive and sugar-positive phospholipids are absent. The type strain is IFO 13972^T.

ACKNOWLEDGEMENTS

We thank Professor Dr Hans G. Trüper, Institut für Mikrobiologie und Biotechnologie, Rheinische Friedrich-Wilhelms-Universität Bonn, for help with the Latin names.

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