Kibdelosporangium albatum sp. nov., Producer of the Antiviral Antibiotics Cycloviracins

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A new species of the genus *Kibdelosporangium* is described. This soil organism forms long straight spore chains and numerous sporangiumlike structures on the aerial mycelium. The new species has type IV cell walls and pattern A whole-cell sugars (*meso*-diaminopimelic acid, arabinose, and galactose are present), type PII phospholipids, MK-9(H₄) as the major menaquinone, and no mycolic acids. On the basis of morphology and chemotaxonomy, the single isolate is assigned to the genus *Kibdelosporangium*. The isolate differs from two previously described species of the genus in fatty acid composition, the absence of melanin formation, and many physiological and biochemical characteristics and is identified as a new species. Accordingly, the name *Kibdelosporangium albatum* sp. nov. is proposed for this isolate. The type strain is R761-7 (= ATCC 55061).

In the course of isolating uncommon actinomycetes for our antibiotic screening program, strain R761-7^T (T = type strain) was isolated from a tropical soil sample. This strain produces a complex of new acyl saccharide antibiotics, cycloviracins active against herpes simplex virus (16, 17).

The genera of actinomycetes with type IV cell walls are divided into taxa (possibly two families) on the basis of presence or absence of mycolic acids (6). The mycolate-containing taxon is comprised of the genus *Nocardia* and related genera. The mycolateless wall IV actinomycetes are a recently expanded taxon because of the reclassification or the discovery of new genera, including the genera *Actinopolyspora*, *Amycolata*, *Amycolatopsis*, *Faenia*, *Pseudonocardia*, *Saccharomonospora*, and *Saccharopolyspora* (6). The genus *Pseudoamycolata* was recently added to this taxon (1). The eight genera are distinguished from each other on the basis of morphology, chemotaxonomy, and a few physiological characteristics (1, 7), but form a distinct group on the basis of the results of an analysis of 16S rRNA sequences from representative strains (3, 5). For this group, a new family name, *Pseudonocardiaceae*, was proposed (3, 5).

The genus *Kibdelosporangium*, described by Shearer et al. (13), also belongs to the mycolateless wall IV actinomycete group. *Kibdelosporangia* are characterized by the abundant formation of pseudosporangia with enveloping hyphae that contain no spores, together with long chains of aerial spores. In this paper we describe strain R761-7^T and, on the basis of its distinctive characteristics, propose the name *Kibdelosporangium albatum* sp. nov. for this organism.

MATERIALS AND METHODS

Strain R761-7^T was isolated from a soil sample collected on Mindanao Island, Philippines. The isolation procedure used for the strain was as follows. The pulverized dry soil sample was transferred with a nylon sponge to an agar medium, which contained (per 1,000 ml of distilled water) 5.0 g of soluble starch, 5.0 g of glucose, 1.0 of yeast extract, 2.0 g of NZ-case, 1.0 g of CaCO₃, 20 ml of V-8 juice (Campbell Japan Corp.), and 16 g of agar (pH 7.2). The agar medium

was supplemented with 4 µg of ampicillin per ml, 10 µg of nalidixic acid per ml, and 80 µg of nystatin per ml. The agar plate was incubated at 43°C. This strain has been deposited with the American Type Culture Collection, Rockville, Md., under accession number ATCC 55061. *Kibdelosporangium aridum* Shearer, Colman, Ferrin, Nisbet, and Nash strain ATCC 39323^T (13) was used for a comparative study, and data for *Kibdelosporangium philippinense* Mertz and Yao were obtained from the literature (10).

The cultural and physiological characteristics of the strain were examined by the methods of Shirling and Gottlieb (15) and Shearer et al. (13). The diaminopimelic acid isomer and sugar in the whole-cell hydrolysate and the amino acids in purified cell walls were analyzed by the methods of Lechevalier (8) and Becker et al. (2), respectively. The phospholipids were identified by the procedure of Lechevalier et al. (9). The menaquinone samples were prepared by the procedure of Collins et al. (4) and were analyzed by mass spectroscopy (model JMS-AX505H; JEOL Corp., Tokyo, Japan). The detection of mycolates and the glycolate test were carried out by the methods of Minnikin et al. (11) and Uchida and Aida (18), respectively. The composition of methyl-esterified

FIG. 1. Photograph of abundant sporangiumlike bodies beneath the aerial hyphae (culture grown on ISP medium 2 at 28°C for 3 weeks). Bar = 20.0 µm.

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FIG. 2. Scanning electron micrograph of spore chains (culture grown on ISP medium 4 at 28°C for 3 weeks). Bar = 3.0 μm.

FIG. 3. Scanning electron micrograph of a sporangiumlike body beneath the covering of spore chains (culture grown on ISP medium 3 at 28°C for 3 weeks). Bar = 5.0 μm.

cellular fatty acids was analyzed by gas chromatography with an SPB-1 fused-silica capillary column (0.25 mm by 30 m) and by gas chromatography (model 5890; Hewlett-Packard, Pittsburgh, Pa.)-mass spectrometry.

RESULTS

Morphology. Strain R761-7^T is a gram-positive, non-acid-fast organism that forms well-branched substrate and aerial mycelia. The substrate mycelium exhibited various degrees of fragmentation and bore a specialized structure which appeared to be dichotomously branched and contained fused hyphae. The aerial mycelium formed long, straight chains of spores and numerous sporangiumlike globular bodies, 8 to 20 μm in diameter. The spores were cylindrical (0.4 by 0.8 to 2.0 μm) and had smooth surfaces without a distinct sheath (Fig. 1 through 3). Globular bodies were observed initially as a droplet (balloonlike) at the tip of a stalk, which eventually became a sporangiumlike body. The mature sporangiumlike bodies were surrounded by a membranous envelope and contained irregularly curved hyphae but no spores. Cells of all structures were nonmotile.

Cultural characteristics. The degrees of growth and colors of the aerial and substrate mycelia of strain R761-7^T on various media are shown in Table 1. White or yellow aerial mycelia were formed on most agar media. The substrate mycelium was colorless to orange-yellow or yellowish brown. Melanin and other distinct pigments were not produced.

Physiological and biochemical characteristics. Physiological and biochemical characteristics of strain R761-7^T are presented in Table 2. Growth of R761-7^T occurred between 17 and 45°C. Optimal growth was at 37°C. No growth was observed at 14 or 48°C. The strain tolerated up to 4% NaCl.

Chemotaxonomy. Whole-cell hydrolysates contained *meso*-diaminopimelic acid, rhamnose, arabinose, glucose,

and galactose. Madurose was not detected. The purified cell wall contained *meso*-diaminopimelic acid, glutamic acid, and alanine. The phospholipids contained phosphatidylethanolamine, phosphatidylmethylethanolamine, phosphatidylglycerol, and phosphatidylinositol. Therefore, strain R761-7^T had type IV cell walls with sugar pattern A and type PII phospholipids. The major menaquinone was MK-9(H₄). The menaquinone composition of strain R761-7^T was as follows: MK-9(H₀), 6%; MK-9(H₂), 13%; MK-9(H₄), 67%; MK-9(H₆), 10%, and MK-9(H₈) 4%. Mycolates were absent. The glycolate test was negative; hence, the *N*-acyl type of peptidoglycan was acetyl. The major fatty acids were hexadecanoic acid (10%), heptadecanoic acid (13%), 15-methylpentadecanoic acid (38%), and 14-methylhexadecanoic acid (10%), there were also minor amounts of unsaturated fatty acids, but 10-methyl or 2-hydroxy fatty acids were not detected. The fatty acid composition of strain R761-7^T was as follows: straight-chain 15:0 acid (pentadecanoic acid), 5%; straight-chain 16:0 acid, 10%; straight-chain 17:0 acid, 13%; straight-chain 18:0 acid, 4%; branched-chain i-14 acid, 3%; branched-chain i-15 acid, 8%; branched-chain i-16 acid (15-methylpentadecanoic acid), 38%; branched-chain i-17 acid, 3%; branched-chain i-18 acid, 1%; branched-chain a-15 acid, 2%; branched-chain a-17 acid (14-methylhexadecanoic acid), 10%; unsaturated *cis*-9 15:1 acid, 2%; and unsaturated i-16:1, 1%.

DISCUSSION

Identity of genus and species. Strain R761-7^T forms aerial sporangiumlike bodies containing hyphae but not spores, along with long aerial spore chains, and has the following cellular composition: type IV wall, pattern A whole-cell sugars, type PII phospholipids, MK-9(H₄) menaquinone, iso- and anteiso fatty acids as the major fatty acid components, and no mycolates. On the basis of these morphological and chemical characteristics, strain R761-7^T is identified as a member of the genus *Kibdelosporangium*. This genus

TABLE 1. Cultural characteristics of strain R761-7^{Ta}

Medium	Growth	Aerial mycelium	Substrate mycelium	Diffusible pigment
Sucrose-nitrate agar (Czapek-Dox agar)	Good	Pale yellow (89) ^b	Orange-yellow (71)	Light yellowish brown (76)
Tryptone-yeast extract broth (ISP medium 1)	Moderate, pellicle, not turbid	None	Light yellowish brown (76)	None
Yeast extract-malt extract agar (ISP medium 2)	Good	White	Deep yellow (85)	None
Oatmeal agar (ISP medium 3)	Moderate	White to light yellow (86)	Colorless to light brown (57)	None
Inorganic salts-starch agar (ISP medium 4)	Good	Yellowish white (92)	Yellowish brown (77)	None
Glycerol-asparagine agar (ISP medium 5)	Moderate	Yellowish white (92)	Brilliant orange-yellow (67)	None
Peptone-yeast extract-iron agar (ISP medium 6)	Moderate	White	Vivid yellow (82)	Pale yellow (89)
Tyrosine agar (ISP medium 7)	Moderate	White	Brilliant orange-yellow (67)	Light orange-yellow (70)
Glucose-asparagine agar	Poor	White	Pale yellow (89)	None

^a Cultures were observed after incubation at 28°C for 3 weeks.

^b The color names and designations (in parentheses) were determined by comparison with ISCC-National Bureau of Standards (U.S. Department of Commerce, Washington, D.C.) color name charts.

includes two other species, *K. aridum* Shearer, Colman, Ferrin, Nisbet, and Nash (13, 14) and *K. philippinense* Mertz and Yao (10).

As shown in Table 3, strain R761-7^T differs from the previously described *Kibdelosporangium* species. There-

fore, this strain is proposed as a new species, *Kibdelosporangium albatum*.

Description of *Kibdelosporangium albatum* sp. nov. *Kibdelosporangium albatum* (al' ba. tum. L. adj. *albatum*, clothed in white, referring to the color of the aerial mycelium and the

TABLE 2. Physiological and biochemical characteristics of strain R761-7^T

Characteristic	Reaction ^a	Characteristic	Reaction
Acid produced from:			
Adonitol.....	+	Hydrogen sulfide	+
L-Arabinose	+	Melanin	-
Cellobiose	+	Hydrolysis of:	
Dextrin	+	Potato starch	+
Dulcitol.....	-	Gelatin.....	+
i-Erythritol	-	Utilization of:	
D-Fructose	+	Benzoate.....	-
D-Galactose	+	Citrate	-
D-Glucose	+	Mucate	-
Glycerol.....	+	Succinate	+
i-Inositol	+	Tartrate	-
Lactose.....	+(w)	Decomposition of:	
Maltose.....	+	Adenine	-
D-Mannitol	+	Casein	+
D-Mannose	+	L-Tyrosine.....	+
Melezitose	-	Hypoxanthine	-
Melibiose	-	Xanthine	-
α-Methyl-D-glucoside	-	Cellulose	-
Raffinose	-	Urea.....	+
L-Rhamnose.....	+	Esculin	+
D-Ribose	+	Hippurate.....	+
Salicin	+	Temp range for growth (°C).....	17-45
D-Sorbitol.....	-	Growth in the presence of:	
L-Sorbitol.....	-	Lysozyme (0.01%).....	+(w)
Sucrose.....	+	NaCl (4%)	+
Trehalose	+		
D-Xylose	+		
Production of:			
Nitrate reductase	-		
Catalase	+		

^a +, positive; -, negative; +(w), weakly positive.

TABLE 3. Characteristics differentiating the species of the genus *Kibdelosporangium*

Characteristic	R761-7 ^T	<i>K. aridum</i> ATCC 39323 and <i>K. aridum</i> subsp. <i>largum</i> ATCC 39922 ^a	<i>K. philippinense</i> NRRL 18198
Acid produced from:			
Adonitol	+	—	—
L-Arabinose	+	+	—
Dextrin	+	+	—
Melezitose	—	+	+
Melibiose	—	+	+
α-Methyl-D-glucoside	—	+	+
Raffinose	—	+	—
Salicin	+	v	—
Sucrose	+	+	—
Production of:			
Nitrate reductase	—	—	+
Melanin	—	+	+
Hydrolysis of potato starch	+	—	—
Utilization of citrate	—	+	+
Decomposition of hypoxanthine	—	+	+
NaCl tolerance (%)	4	5–7	2
Antibiotic(s) produced	Acyl saccharide (cycloviracin)	Glycopeptides (aridicin and kibdelin) ^b	Glycopeptide (A-80407) ^c
Cell chemistry			
PME ^d	Present	Present	Absent
Saturated straight-chain fatty acids	Major component	Minor component	Minor component

^a See reference 14.^b See references 12 and 14.^c See reference 10.^d PME, phosphatidylmethylethanolamine.

nonchromogenicity of the organism). Aerobic actinomycete with substrate and aerial mycelia. The substrate mycelium exhibits various degrees of fragmentation. The aerial mycelium bears long straight chains of cylindrical spores with smooth surfaces, and sporangiumlike globular bodies 8 to 20 μm in diameter. The globules are surrounded by a membranous envelope and contain irregularly coiled hyphae but no spores. Motile spores are not formed by the strain.

The color of the aerial mycelium is white or yellow. The substrate mycelium penetrates the agar and is yellow, orange-yellow, or light brown depending on the medium. Melanoid pigments are formed. Acid is formed from various carbon compounds (Table 2). Potato starch is hydrolyzed. Grows at temperatures between 17 and 45°C; the optimal temperature is 37°C. Tolerates up to 4% NaCl.

Antagonistic properties: weakly active on *Bacillus subtilis* but inactive on *Micrococcus luteus* and fungal strains. Produces the antiviral agents cycloviracins (16, 17). Habitat: soil.

The type strain is strain R761-7 (= ATCC 55061). Monotypic.

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