Pseudosporangium ferrugineum gen. nov., sp. nov., a new member of the family *Micromonosporaceae*

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An actinomycete strain 3-44-a(19)^T was isolated from sandy soil collected in Bangladesh. The strain formed irregular pseudosporangia directly from aggregated spore chains above the rudimentary aerial mycelium. The pseudosporangia developed singly. Each pseudosporangium contained many small, non-motile, spherical, smooth-surfaced spores in chains. Strain 3-44-a(19)^T contained meso- and 3-hydroxydiaminopimelic acid in the cell wall and MK-9(H₈) as the major menaquinone and arabinose, galactose, glucose, mannose, ribose and xylose were present in the whole-cell hydrolysate. The diagnostic phospholipid was phosphatidylethanolamine and iso-C_{15:0} (24.6%), C_{18:1ω9c} (15.5%), C_{16:0} (10.6%), C_{18:0} (9.4%), iso-C_{16:0} (8.6%) and anteiso-C_{15:0} (6.0%) were detected as the major cellular fatty acids. The acyl type of the peptidoglycan was glycolyl and mycolic acids were not detected. The G + C content of the DNA was 73.6 mol%. The chemotaxonomic data indicated that the strain belonged to the family *Micromonosporaceae*. Phylogenetic analysis based on 16S rRNA gene sequence data also suggested that strain 3-44-a(19)^T fell within the family *Micromonosporaceae*. On the basis of phylogenetic analysis and characteristic patterns of 16S rRNA gene signature nucleotides as well as morphological and chemotaxonomic data, this strain should be classified as a member of a new genus and species, *Pseudosporangium ferrugineum* gen. nov., sp. nov., in the family *Micromonosporaceae*. The type strain of *Pseudosporangium ferrugineum* is 3-44-a(19)^T (=JCM 14710^T =MTCC9007^T).

The family *Micromonosporaceae* was described by Krasil'nikov (1938) on a morphological basis and its description was emended by Goodfellow *et al.* (1990). Koch *et al.* (1996) further emended the description of the family to reflect its phenotypic and chemotaxonomic heterogeneity. Later, Stackebrandt *et al.* (1997) emended the description of the family again on the basis of phylogenetic clustering of 16S rRNA gene sequences and the presence of taxon-specific 16S rRNA gene signature nucleotides. At present, the family *Micromonosporaceae* comprises 17 genera: *Micromonospora* (Ørskov, 1923), *Actinoplanes* (Couch, 1950), *Pilimelia* (Kane, 1966),

Dactylosporangium (Thiemann *et al.*, 1967), *Catellatospora* (Asano & Kawamoto, 1986), *Catenuloplanes* (Yokota *et al.*, 1993), *Couchioplanes* (Tamura *et al.*, 1994), *Spirilliplanes* (Tamura *et al.*, 1997), *Verrucosipora* (Rheims *et al.*, 1998), *Virgisporangium* (Tamura *et al.*, 2001), *Asanoa* (Lee & Hah, 2002), *Longispora* (Matsumoto *et al.*, 2003), *Salinispora* (Maldonado *et al.*, 2005), *Actinocatenispora* (Thawai *et al.*, 2006), *Polymorphospora* (Tamura *et al.*, 2006), *Luedemannella* (Ara & Kudo, 2007a) and *Krasilnikovia* (Ara & Kudo, 2007c). Each genus has distinctive morphological and/or chemotaxonomic features. *Micromonospora*, *Salinispora* and *Verrucosipora* species form single spores on short or long sporophores; *Actinoplanes*, *Dactylosporangium*, *Luedemannella*, *Pilimelia* and *Virgisporangium* species form sporangia; *Actinocatenispora*, *Asanoa*, *Catellatospora*, *Catenuloplanes*, *Longispora*, *Polymorphospora* and *Spirilliplanes* species form spore chains; *Couchioplanes* and *Krasilnikovia* species form spore chains that turn into pseudosporangium-like structures; *Actinoplanes*, *Catenuloplanes*, *Couchioplanes*, *Dactylosporangium*, *Pilimelia* and

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 3-44-a(19)^T is AB302183.

Details of cultural and physiological characteristics and the fatty acid profile of strain 3-44-a(19)^T and a 16S rRNA gene sequence similarity matrix are available as supplementary material with the online version of this paper.

Spirilliplanes spores show motility; and *Catenuloplanes* and *Couchioplanes* species have lysine instead of *meso*-diaminopimelic acid as the diamino acid of the peptidoglycan.

During bioactivity screening studies of rare actinomycetes, strain 3-44-a(19)^T was isolated from sandy soil collected at a forest waterfall in Chokoria, Cox's Bazar, Bangladesh. The strain formed irregular pseudosporangial structures on rudimentary aerial mycelia. On the basis of 16S rRNA gene sequence comparison with other related strains, the isolate falls phylogenetically within the family *Micromonosporaceae*, adjacent to the genera *Couchioplanes* and *Krasilnikovia*. On the basis of the results of a polyphasic approach to bacterial classification, we propose the creation of the genus *Pseudosporangium* gen. nov.

Strain 3-44-a(19)^T was isolated from soil using the standard dilution plate method and grown on humic acid-vitamin (HV) agar (Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg l⁻¹), nystatin (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹) (Ara & Kudo, 2007b, d). After 21 days of aerobic incubation at 28 °C, the strain was transferred and purified on yeast extract-malt extract agar (ISP medium 2) (Shirling & Gottlieb, 1966) and maintained as working cultures on yeast extract-starch agar (JCM medium 61) containing 15.0 g soluble starch, 4.0 g yeast extract, 0.5 g K₂HPO₄, 0.5 g MgSO₄ · 7H₂O and 15.0 g agar in 1 l distilled water (pH 7.2).

Strain 3-44-a(19)^T was grown on tap-water agar, sucrose-nitrate agar (Waksman medium 1), oatmeal agar, Seino agar and HV agar at 28 °C for 21 days and then observed by light and scanning electron microscopy (model S-2400; Hitachi). The sample for scanning electron microscopy was prepared using a freeze-drying technique. Agar blocks were fixed overnight in osmium tetroxide vapour. The prepared specimen was rapidly frozen using liquid N₂ and immediately freeze-dried for 5 h to overnight. The specimen was gold-coated and examined under the microscope. For the spore motility test, the isolate was inoculated on oatmeal agar, tap-water agar, Seino agar and sucrose-nitrate agar at 28 °C for 21 days. Spore suspensions were obtained by gentle scraping of the agar surface bearing pseudosporangia and mixing with 10 ml sterile tap water. The spore suspension was incubated at 28 °C for about 1 h. The suspension was shaken at irregular intervals and allowed to settle down. About 0.1 ml supernatant was taken with a sterile Pasteur pipette, spread onto a clean slide and covered with a coverslip. The motility of spores was observed under a light microscope at ×100 and ×200 magnifications (model ITC-370M; Nikon).

Cultural and physiological properties were examined using several standard methods. For cultural characterization, the isolate was grown for 21 days at 28 °C on various agar media as described by Waksman (1950, 1961), Shirling & Gottlieb (1966) and Asano & Kawamoto (1986). The *Color Harmony Manual* (Jacobson *et al.*, 1958) was used to determine the names and designations of colony colours. The temperature range and NaCl tolerance for growth were

determined on yeast extract-starch agar (JCM medium 61). Utilization of carbohydrates as sole carbon sources was tested using ISP medium 9 as basal medium according to the method of Shirling & Gottlieb (1966). Production of melanoid pigments was examined using tyrosine agar (ISP medium 7).

The freeze-dried cells used for chemotaxonomic analysis were obtained from cultures grown in yeast-glucose broth and trypticase soy broth on a rotary shaker for 21 days at 28 °C. Isomers of diaminopimelic acid (DAP) were determined by TLC as described by Stanek & Roberts (1974). Reducing sugars from whole-cell hydrolysates were analysed by the paper chromatography method of Becker *et al.* (1965). The *N*-acyl group of muramic acid was determined by the method of Uchida & Aida (1984). Phospholipids in cells were extracted and identified by the method of Minnikin *et al.* (1984). Methyl esters of cellular fatty acids were analysed by TechnoSuruga Co., Ltd according to the instructions of the Microbial Identification System (MIDI) using a gas chromatograph (model HP6890; Hewlett Packard) (Sasser, 1990). Isoprenoid quinones were extracted by the method of Collins *et al.* (1977, 1984) and were analysed using an HPLC (model 802-SC; Jasco) and a CAPCELL PAK C18 column (Shiseido) (Tamaoka *et al.*, 1983). Preparation and detection of methyl esters of mycolic acids were performed as described by Tomiyasu (1982).

Genomic DNA was extracted by the method of Ara & Kudo (2006). PCR-mediated amplification of the 16S rRNA gene and sequencing of the PCR products were performed as described by Nakajima *et al.* (1999). The sequences were multiply aligned with selected sequences obtained from the GenBank/EMBL/DDBJ databases using the CLUSTAL_X program (Thompson *et al.*, 1997). The alignment was verified manually and adjusted before construction of a phylogenetic tree. The phylogenetic tree constructed by the neighbour-joining method (Saitou & Nei, 1987) in the CLUSTAL_X program (Thompson *et al.*, 1997) was based on a comparison of 1337 nucleotides present in all strains following the elimination of gaps and ambiguous nucleotides using the Se-Al v2.0a11 alignment editor (A. Rambout, unpublished; available at <http://tree.bio.ed.ac.uk/software/seal/>). *Streptomyces ambofaciens* ATCC 23877^T was used as an outgroup. Confidence values for branches of the phylogenetic tree were determined using bootstrap analyses based on 1000 resamplings (Felsenstein, 1985).

DNA was isolated from biomass by the method of Tamaoka (1994) and Saito & Miura (1963) with minor modifications. Cells that failed to be lysed by the enzymes were freeze-dried and ground mechanically as described by Raeder & Broda (1985). The G + C content of the DNA was determined using the HPLC method of Tamaoka & Komagata (1984). An equimolar mixture of nucleotides for analysis of DNA base composition (Yamasa Shoyu) was digested by bacterial alkaline phosphatase and used as the quantitative standard. Nucleotides and nucleotide pairs in

the 16S rRNA gene of the novel strain and closely related members of the family *Micromonosporaceae* were determined after manual verification of the CLUSTAL_X sequence alignment. Nucleotide positions were numbered according to the corresponding position in the 16S rRNA gene sequence of *Escherichia coli* (Brosius *et al.*, 1978).

Isolate 3-44-a(19)^T comprised Gram-positive, non-acid-fast, aerobic and branched hyphae. A non-fragmenting rusty- to clove brown-coloured substrate mycelium was formed. Strain 3-44-a(19)^T formed irregular pseudosporangia on rudimentary aerial mycelium on yeast-starch agar (Seino agar) (Fig. 1). Chains of long, coiled and aggregated sporogenous hyphae appeared as clumps of spores and sporangium-like structures under the light microscope. Under the scanning electron microscope, however, these structures were found to be highly aggregated spore chains or spores, not true sporangia. Spores in the pseudosporangium were non-motile, small and spherical in shape with a smooth surface. The physiological properties of strain 3-44-a(19)^T can be summarized as follows. The strain exhibited good growth on glucose-asparagine agar, glycerol-asparagine agar (ISP medium 5), inorganic salts-starch agar (ISP medium 4), ISP medium 7, oatmeal-nitrate agar (JCM medium 52), oatmeal agar (ISP medium 3), ISP medium 2, Bennett's agar, Hickey-Tresner agar and glucose-yeast extract agar (see Supplementary Table S1, available in IJSEM Online). The strain utilized L-arabinose, cellobiose, D-glucose, maltose, D-mannose, D-mannitol, raffinose, L-rhamnose, sucrose, trehalose, xylitol and D-xylose (Table 1). It grew well at 20–37 °C and pH 5–9 and could not grow on 3 % NaCl (Supplementary Table S2).

Chemotaxonomic properties have been found to make an important contribution to the polyphasic approach to bacterial systematics, especially in the circumscription of phylogenetically coherent actinomycete taxa (Kroppenstedt & Goodfellow, 1991; Goodfellow, 1991). The family *Micromonosporaceae* is defined mainly by chemotaxonomy and our isolate 3-44-a(19)^T shared chemotaxonomic characteristics with members of the family as follows. The cell walls contained *meso*- and 3-OH-DAP and arabinose, galactose, glucose, mannose, ribose and xylose as the whole-cell sugars, indicating a whole-cell sugar pattern D according to Lechevalier & Lechevalier (1970). The major menaquinone was MK-9(H₆). iso-C_{15:0} (24.6 %), C_{18:1ω9c} (15.5 %), C_{16:0} (10.6 %), C_{18:0} (9.4 %), iso-C_{16:0} (8.6 %) and anteiso-C_{15:0} (6.0 %) were the major cellular fatty acids, with small amounts of iso-C_{17:0} (3.5 %), anteiso-C_{17:0} (3.4 %), C_{17:0} (2.9 %), C_{17:1ω8c} (2.4 %), C_{14:0} (1.9 %), C_{16:1 iso G} (1.7 %) and iso-C_{14:0} (1.4 %), indicating fatty acid type 2d of Kroppenstedt (1985) (Table 1 and Supplementary Table S3). Mycolic acids were absent. Phosphatidylethanolamine was detected, corresponding to phospholipid type PII of Lechevalier *et al.* (1981). The acyl type of the cell-wall polysaccharides was glycolyl. The G+C content of the DNA was 73.6 mol%. The predominant menaquinone

Fig. 1. Scanning electron micrographs of irregular pseudosporangia formed by the aggregation of rudimentary aerial mycelia of strain 3-44-a(19)^T grown on Seino agar medium for 21 days at 28 °C. Bars, 10 µm.

pattern, DAP and fatty acid pattern of our isolate differed from those of the genus *Couchioplanes* of the family *Micromonosporaceae* (Tables 1 and 2).

Table 1. Cultural, physiological and chemotaxonomic characteristics and G+C content of strain 3-44-a(19)^T and members of the phylogenetically closely related genus *Couchioplanes*Abbreviations: ++, good; +, moderate; ±, poor; –, none; m, moderate amount; t, trace. Data for reference strains were taken from Tamura *et al.* (1994).

Characteristic	Strain 3-44-a(19) ^T	<i>C. caeruleus</i> subsp. <i>caeruleus</i> NBRC 13939 ^T	<i>C. caeruleus</i> subsp. <i>azureus</i> NBRC 13993 ^T
Growth on:			
Yeast extract-malt extract agar (ISP 2)	Good, rusty to clove brown (3pl)	Good, yellow (2ga) to deep teal blue (17pe)	Good, yellow (3ga) to dark blue (13pi to 14pi)
Oatmeal agar (ISP 3)	Good, rusty to clove brown (3pl)	Moderate, shell (2ca) to dark grey blue (14li)	Moderate, yellow (3ea) to ivy (24ml)
Inorganic salts-starch agar (ISP 4)	Good, rusty to clove brown (3pl)	Good, shell (2ca) to dark grey blue (14li)	Good, shell (3ca) to dusty jade (21ec)
Glucose-asparagine agar	Good, rusty to clove brown (3pl)	Moderate, wheat (2ea) to dark blue (17pi)	Fair, wheat (3ea) to dark grey blue (16li)
Pigmentation on:			
Yeast extract-malt extract agar (ISP 2)	Present	Absent	Present
Glycerol-asparagine agar (ISP 5)	Present	Present	Absent
Glucose-asparagine agar	Present	Present	Absent
Spore motility	Absent	Present	Present
Growth in 2% NaCl	Present	Absent	Present
Cell-wall diamino acid(s)	<i>meso</i> - and 3-OH-DAP	L-Lys	L-Lys
Polar lipid(s)*	PE	PG, PE	PG, PE
Menaquinone(s)	MK-9(H ₆)	MK-9(H ₄), MK-9(H ₆) (t), MK-9(H ₂) (t)	MK-9(H ₄), MK-9(H ₆) (m), MK-9(H ₈) (t), MK-9(H ₂) (t)
Major fatty acids (%)	iso-C _{15:0} (24.6%), C _{18:1ω9c} (15.5%), C _{16:0} (10.6%), C _{18:0} (9.4%), iso-C _{16:0} (8.6%)	iso-C _{16:0} (47%), anteiso-C _{15:0} (16%), anteiso-C _{17:0} (14%)	iso-C _{16:0} (47%), anteiso-C _{17:0} (20%), anteiso-C _{15:0} (19%), C _{16:1} (14.0%), C _{18:1} (14.0%)
Cell-wall sugars†	Gal, Glc, Rib, Man, Xyl (t), Ara (t)	Xyl, Glc, Man, Ara (t), Gal (t), Rib (t)	Ara, Xyl, Gal, Glc, Man, Rib (t)
DNA G+C content (mol%)	73.6	69.9	72.1
Utilization of:			
L-Arabinose	++	–	–
D-Fructose	+	++	+
<i>myo</i> -Inositol	+	++	++
Mannitol	++	++	±
L-Rhamnose	+	++	–
Raffinose	++	–	–
D-Xylose	++	–	–

*PE, Phosphatidylethanolamine; PG, phosphatidylglycerol.

†Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rib, ribose; Xyl, xylose.

Table 2. Morphological features and chemotaxonomic characteristics of strain 3-44-a(19)^T (*Pseudosporangium* gen. nov.) and genera of the family *Micromonosporaceae*

Data for reference genera were taken from Vobis (1987) and subsequent chapters, Stackebrandt & Kroppenstedt (1987), Horan & Brodsky (1986), Goodfellow *et al.* (1990), Yokota *et al.* (1993), Tamura & Hatano (2001), Tamura *et al.* (1994, 1997, 2001, 2006), Rheims *et al.* (1998), Kudo *et al.* (1999), Lee *et al.* (2000), Lee & Hah (2002), Matsumoto *et al.* (2003), Maldonado *et al.* (2005), Ara & Kudo (2006, 2007a, c) and Thawai *et al.* (2006). +, Present; –, absent; ND, no data available. Rha, Rhamnose; see Table 1 for remaining sugar abbreviations. Fatty acid types are classified according to Kroppenstedt (1985) and phospholipid types according to Lechevalier *et al.* (1981).

Genus	Sporangium	Spore motility	Diamino acid(s)	Diagnostic sugars	Fatty-acid type	Major menaquinone(s)	Phospholipid type	G + C content (mol%)
<i>Pseudosporangium</i> gen. nov.	–	–	meso- and 3-OH-DAP	Gal, Glc, Rib, Man, Xyl, Ara	2d	9(H ₆)	PII	73
<i>Couchioplanes</i>	–	+	L-Lys	Ara, Gal, Xyl	2c	9(H ₄)	PII	70–72
<i>Krasilnikovia</i>	–	–	meso-DAP	Gal, Man, Xyl, Ara, Rib	2d	9(H _{6,4,8})	PII	71
<i>Actinoplanes</i>	+	+	meso-DAP	Ara, Xyl	2d	9(H ₄), 10(H ₄)	PII	72–73
<i>Luedemannella</i>	+	–	meso-DAP	Xyl, Gal, Man, Rha, Rib, Ara	2d	9(H _{6,4,2,8})	PII	71
<i>Polymorphospora</i>	–	–	meso-DAP	Xyl	2a	10(H _{6,4}), 9(H _{6,4})	PII	71
<i>Salinispora</i>	–	–	meso-DAP	Ara, Gal, Xyl	3a	9(H ₄)	PII	70–73
<i>Longispora</i>	–	–	meso-DAP	Ara, Gal, Xyl	2d	10 (H _{4,6})	PII	70
<i>Actinocatenispora</i>	–	–	meso-DAP	Ara, Gal, Xyl	3b	9(H _{4,6})	PII	72
<i>Micromonospora</i>	–	–	meso-DAP	Ara, Xyl	3b	10(H _{4,6}), 9(H _{4,6})	PII	71–72
<i>Asanoa</i>	–	–	meso-DAP	Ara, Gal, Xyl	2d	10 (H _{6,8})	PII	71–72
<i>Catellatospora</i>	–	–	meso-DAP	Xyl, Man, Gal, Ara, Rha, Rib	3b	9(H _{4,6}) or 10(H _{4,6})	PII	70–71
<i>Catenuloplanes</i>	–	+	L-Lys	Xyl	2c	9(H ₈), 10(H ₈)	PIII	71–73
<i>Dactylosporangium</i>	+	+	meso-DAP	Ara, Xyl	3b	9(H _{4,6,8})	PII	71–73
<i>Pilimelia</i>	+	+	meso-DAP	Ara, Xyl	2d	9(H _{4,2})	PII	ND
<i>Spirilliplanes</i>	–	+	meso-DAP	Ara, Xyl	2d	10(H ₄)	PII	69
<i>Verrucospora</i>	–	–	meso-DAP	Man, Xyl	2b	9(H ₄)	PII	70
<i>Virgisporangium</i>	+	–	meso-DAP	Ara, Gal, Xyl	2d	10(H _{4,6,8})	PII	71

The almost-complete 16S rRNA gene sequence (1511 nt) of strain 3-44-a(19)^T was determined in this study and the phylogenetic position of the isolate based on the 16S rRNA gene sequence is within the confines of the family *Micromonosporaceae* (Fig. 2), near to the genera *Couchioplanes* and *Krasilnikovia* and *Actinoplanes globisporus* (Fig. 2). The maximum sequence similarities for the genera *Couchioplanes* and *Krasilnikovia* and *Actinoplanes globisporus* were 98.5, 97.8 and 96.5 %, respectively (Supplementary Table S4). Table 2 summarizes the differential characteristics of the new genus represented by strain 3-44-a(19)^T and other members of the family *Micromonosporaceae*. The pattern of nucleotides and nucleotide pairs in the 16S rRNA gene of the novel strain and the closest genera in the family *Micromonosporaceae* are presented in Table 3. There are several nucleotide-pair differences from members of the family *Micromonosporaceae* and particularly *Couchioplanes*, *Krasilnikovia* and *Actinoplanes globisporus* (Table 3).

Strain 3-44-a(19)^T developed pseudosporangia on rudimentary aerial mycelia on Seino agar. Characteristics of the

isolate, related genera and other genera of the family *Micromonosporaceae* are shown in Table 2. Members of the closest genus, *Couchioplanes*, have L-lysine in the cell wall, MK-9(H₄) as the major menaquinone component and fatty acid type 2c (Tables 1 and 2). Members of the genus *Krasilnikovia* have MK-9(H_{6,4,8}) as menaquinone components. Strain 3-44-a(19)^T is clearly distinguished from the genus *Couchioplanes* in morphological characteristics, cell-wall diamino acid, menaquinone and fatty acid components (Table 1). Strain 3-44-a(19)^T can also be distinguished from the genus *Krasilnikovia* (Ara & Kudo, 2007c) by the menaquinone system. Members of the genus *Krasilnikovia* have pseudosporangium-like structures on substrate mycelium; however, members of the genus *Krasilnikovia* do not form pseudosporangium-like structures by aggregated spore chains on rudimentary aerial mycelium (Fig. 1). Further, members of the genus *Krasilnikovia* have a phylogenetic position distinct from that of strain 3-44-a(19)^T (Fig. 2). Other genera in the family *Micromonosporaceae* are clearly distinguishable from strain 3-44-a(19)^T in morphological, chemotaxonomic and

Fig. 2. Neighbour-joining tree based on nearly-complete 16S rRNA gene sequences showing relationships between strain 3-44-a(19)^T, species of the genus *Couchioplanes* with validly published names and representatives of other genera classified in the family *Micromonosporaceae*. *Streptomyces ambofaciens* ATCC 23877^T was used as an out-group. Numbers at nodes indicate levels of bootstrap support (%) based on a neighbour-joining analysis of 1000 resampled datasets; only values above 20% are shown. Bar, 0.01 substitutions per site.

phylogenetic characteristics (Table 2). Thus, the distinctness of the 16S rRNA gene sequence, phylogenetic position and specific nucleotide patterns of the 16S rRNA gene of strain 3-44-a(19)^T differentiate it from known actinomycetes belonging to the family *Micromonosporaceae* and thus this isolate merits new genus status. Therefore, we propose that strain 3-44-a(19)^T be classified in a new genus, *Pseudosporangium* gen. nov., the type species of which is *Pseudosporangium ferrugineum* sp. nov.

Description of *Pseudosporangium* gen. nov.

Pseudosporangium (Pseu.do.spo.ran'gi.um. Gr. adj. *pseudes* false; Gr. n. *spora* seed; Gr. neut. n. *angeion* a vessel; N.L. neut. n. *Pseudosporangium* false sporangium).

Cells are Gram-positive, non-acid-fast and aerobic with branching hyphae. Non-fragmenting, rusty- to clove brown-coloured substrate mycelium is present. Large and irregularly shaped structures contain aggregations of spore chains on scanty and rudimentary aerial mycelium resembling sporangia, but true sporangia with a sporangial wall are not observed. The cell wall contains *meso*-DAP and has arabinose, galactose, glucose, mannose, ribose and xylose as the whole-cell sugars, indicating whole-cell sugar pattern D. The menaquinone pattern is MK-9(H₆). The major cellular fatty acids are iso-C_{15:0}, C_{18:1}ω9c, C_{16:0}, C_{18:0}, iso-C_{16:0} and anteiso-C_{15:0} (fatty acid type 2d). Mycolic acids are absent. Phosphatidylethanolamine is detected, corresponding to phospholipid type PII. The acyl type of the cell-wall polysaccharide is glycolyl. The G + C content of the DNA of the type strain of the type species is 73.6 mol%. The type species is *Pseudosporangium ferrugineum*.

Description of *Pseudosporangium ferrugineum* sp. nov.

Pseudosporangium ferrugineum (fer.ru.gi'ne.um. L. neut. adj. *ferrugineum* rusty brown, referring to the orange-dark brown- to clove brown-coloured substrate mycelium).

Morphological, chemotaxonomic and general characteristics are as given above for the genus. On oatmeal agar and Seino (yeast-starch) agar, rudimentary aerial mycelium develops with short spore chains that are highly aggregated. The spores are non-motile, small, spherical to oval (~0.3–0.5 μm) with smooth surfaces. Grows well at 20–37 °C and pH 5–9, and can not grow on 3% NaCl. Exhibits good growth on glucose-asparagine agar, ISP media 2, 3, 4, 5 and 7, oatmeal-nitrate agar, Bennett's agar, Hickey-Tresner agar and glucose-yeast extract agar. Shows moderate to poor growth on nutrient agar, sucrose-nitrate agar, Waksman agar and tap-water agar. Melanin pigment is not produced on ISP medium 7, but pale pigmentation occurs on glucose-asparagine agar, ISP media 2, 3, 5 and 7 and oatmeal-nitrate agar. In general, the vegetative mycelium is rusty to clove brown in colour. Utilizes L-arabinose, cellobiose, D-glucose, maltose, D-mannose, D-mannitol, raffinose, L-rhamnose, sucrose, trehalose, xylitol and D-xylose. Adonitol, D-fructose, *myo*-inositol and melibiose are poorly utilized and D-ribose, glycerol and cellulose are not utilized. The major cellular fatty acids are iso-C_{15:0} (24.6%), C_{18:1}ω9c (15.5%), C_{16:0} (10.6%), C_{18:0} (9.4%), iso-C_{16:0} (8.6%) and anteiso-C_{15:0} (6.0%), with small amounts of iso-C_{17:0} (3.5%), anteiso-C_{17:0} (3.4%), C_{17:0} (2.9%), C_{17:1}ω8c (2.4%), C_{14:0} (1.9%), C_{16:1} iso G (1.7%) and iso-C_{14:0} (1.4%).

The type strain, 3-44-a(19)^T (=JCM 14710^T =MTCC9007^T), was isolated from soil.

Table 3. 16S rRNA gene nucleotide positions that differentiate strain 3-44-a(19)^T from closely related genera of the family *Micromonosporaceae*

Nucleotides that clearly differentiate the genera *Couchioplanes* and *Krasilnikovia* and *Actinoplanes globisporus* from strain 3-44-a(19)^T are shown in bold. Nucleotide positions are given according to the *E. coli* numbering (Brosius *et al.*, 1978).

Position(s)	Strain 3-44-a(19) ^T	<i>Couchioplanes</i>	<i>Krasilnikovia</i>	<i>Actinoplanes globisporus</i>
129	C	C	U	U
138:225	U-C	U-C	C-G	U-G
153:168	C-G	G/C-G	C-G	C-G
154:167	C-G	G/C-G	C-G	C-G
144:178	U-A	U-G	U-G	U-G
182	U	C	U	C
183	U	G	G	G
187	A	A/C	U	U
188	C	C/U	U	A
189	A	G	C	C
190	G	C	U	C
186:191	C-U	C-C/G	C-G	C-G
192	C	U/C	G	G
194	C	C	C	U
204	U	C/U	G	G
205	G	G	G	U
206:213	U-A	U/G-A	G-A	G-A
445:489	G-C	G-C	C-G	G-C
446:488	G-C	G-C	C-G	G-C
457	G	A	G	G
478	G	A	G	G
603:635	U-A	U-A	U-A	C-G
611	C	C/U	C	C
614:626	G-C	G-C	G-C	A-U
631	C	U/C	C	U
632	U	G/U	U	U
1011:1018	C-G	C-G	U-A	C-G
1010:1019	G-U	G-C	G-C	G-C
1119:1154	U-A	U-A	U-A	C-G
1121:1152	G-C	G-C	G-C	C-G
1133:1141	G-C	G-C/U	G-C	A-U
1134:1140	C-G	C-G	G-C	A-U
1136	U	U	U	C
1137	A	A	A	G
1253:1284	G-C	G-C	G-C	C-G
1254:1283	G-C	G-C	G-C	C-G
1262	U	U	U	G
1263:1272	G-U	A-U	G-U	C-G
1362	A	U/A	A	A
1363	A	U/A	A	A
1424:1476	C-G	U/C-G	C-G	C-G
1452	U	U	U	G

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