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Two new species of the genus *Micromonospora*: *Micromonospora chokoriensis* sp. nov. and *Micromonospora coxensis* sp. nov., isolated from sandy soil

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Two actinomycete strains, 2-19(6)^T and 2-30-b(28)^T, which produced single, non-motile nodules to warty spore surfaces, were isolated from sandy soil in Chokoria, Cox's Bazar, Bangladesh. A polyphasic study was carried out to establish the taxonomic position of these strains. Morphological and chemotaxonomic characteristics of these strains coincided with those of the genus *Micromonospora*. Phylogenetic analysis using 16S rDNA sequences indicated that these strains should be classified in the genus *Micromonospora*. The 16S rDNA sequence of strain 2-19(6)^T showed closest similarity to the type strains of *M. mirobrigensis* (98.9%) and *M. carbonacea* (98.8%), and the strain 2-30-b(28)^T to the type strains of *M. purpureochromogenes* (99.4%), *M. halophytica* (99.3%) and *M. aurantiaca* (99.2%). Furthermore, a combination of DNA-DNA hybridization results and some differential physiological and biochemical properties indicated that these strains were distinguished from the phylogenetically closest relatives. These strains therefore represent two novel species, for which the name *Micromonospora chokoriensis* sp. nov. and *Micromonospora coxensis* sp. nov. are proposed. The type strains are 2-19(6)^T (=JCM 13247^T=MTCC 8535^T) and 2-30-b(28)^T (=JCM 13248^T=MTCC 8093^T).

Key Words——actinomycetes; *Micromonospora*; *Micromonosporaceae*; polyphasic taxonomy

Introduction

The genus *Micromonospora* Ørskov (1923) family *Micromonosporaceae* are reported to inhabit soil, water, marine environments and sediments (Kawamoto, 1989; Luedemann and Brodsky, 1964) and produce spores singly borne on sporophores branched from substrate hyphae. *Micromonospora endolithica* sp. nov., isolated from an extreme Antarctic sandstone

environment, has been described (Hirsch et al., 2004), showing that this genus is distributed in very different environments. Kasai et al. (2000) redefined the genus to fourteen species based on DNA-DNA relatedness, 16S rRNA gene and *gyrB* gene phylogenetic data. Recently, Kroppenstedt et al. (2005) proposed eight new species and currently 27 validly described species are encompassed under the genus *Micromonospora*.

During investigation of novel actinomycetes from sandy soil near a waterfall in Chokoria, Bangladesh, we isolated two strains, 2-19(6)^T and 2-30-b(28)^T, that showed typical morphological characteristics of the genus *Micromonospora*. Here we report on the taxonomic characterization and classification of these isolates to determine their taxonomic positions and propose two new species, *Micromonospora chokoriensis* sp. nov. and *Micromonospora coxensis* sp. nov., for

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the strains 2-19(6)^T and 2-30-b(28)^T, respectively.

Materials and Methods

Isolation. Strains 2-19(6)^T and 2-30-b(28)^T were isolated using the standard dilution plate method and grown on humic acid-vitamin agar (HV) (Hayakawa and Nonomura, 1987) supplemented with cycloheximide (50 mg L⁻¹), nystatin (50 mg L⁻¹) and nalidixic acid (20 mg L⁻¹) (Ara and Kudo, 2006). After 21 days of aerobic incubation at 30°C, the strains were transferred and purified on yeast extract-malt extract agar [(medium 2 of the International *Streptomyces* Project (ISP medium 2)] and maintained as working cultures on yeast-starch agar containing soluble starch, 15.0 g; yeast extract, 4.0 g; K₂HPO₄, 0.5 g; MgSO₄·7H₂O, 0.5 g; and agar, 15.0 g in 1 L of distilled water (pH 7.2).

Morphology. Strains 2-19(6)^T and 2-30-b(28)^T were grown on sucrose-nitrate agar (Waksman medium 1) and 1/5 yeast-starch agar media at 30°C for 21 days and then observed by light and scanning electron microscope (model S-2400 Hitachi, Tokyo, Japan). The sample for scanning electron microscopy was prepared as described by Ara and Kudo (2006).

Cultural, physiological and biochemical characteristics. The phenotypic properties were examined using several standard methods. For cultural characterization, the isolates were grown for 21 days at 30°C on various agar media as described by Waksman (1950, 1961); Shirling and Gottlieb (1966) and Asano and Kawamoto (1986) (Table 1). The *Color Harmony Manual* (Jacobson et al., 1958) was used to determine the names and designations of colony colors. The temperature range and NaCl tolerance for growth were determined on yeast-starch agar (JCM medium 61). Utilization of carbohydrates as sole carbon sources was tested by using neutralized yeast nitrogen base without amino acids as a basal medium according to the method of Stevenson (1967). Production of melanoid pigments was examined using tyrosine agar slants (ISP medium 7).

Chemotaxonomy. The freeze-dried cells used for chemotaxonomic analyses were obtained from cultures grown in yeast-starch broth (JCM medium no. 61) on a rotary shaker at 30°C. The isomers of diaminopimelic acid (A₂pm) in the cell wall peptidoglycan were determined by using TLC as described by Stanek and Roberts (1974). Reducing sugars from whole-cell hydrolysates were analyzed by the HPLC

method of Mikami and Ishida (1983). The *N*-acyl group of muramic acid in peptidoglycan was determined by the method of Uchida and Aida (1984). Phospholipids in cells were extracted and identified by the method of Minnikin et al. (1984). Methyl esters of cellular fatty acids were prepared and analyzed according to the instructions of the Microbial Identification System (MIDI) (Sherlock Microbial Identification System; MIDI, Hewlett Packard, Palo Alto, CA, USA) (Sasser, 1990). Isoprenoid quinones were extracted by the method of Collins et al. (1977, 1984) and were analyzed by a HPLC equipped with a Cosmosil 5C₁₈ column (4.6 by 150 mm; Nacalai Tesque, Kyoto, Japan). Preparation and detection of methyl esters of mycolic acids were carried out as described by Tomiyasu (1982).

Preparation of DNA, DNA base composition and DNA-DNA hybridization. Genomic DNA extraction, PCR-mediated amplification of the 16S rRNA gene, and sequencing of the PCR products were carried out as described in Ara and Kudo (2006). DNA was isolated from biomasses by the method of Tamaoka (1994) and Saito and Miura (1963) with minor modification as follows: achromopeptidase crude (Wako Pure Chemicals), *N*-acetylmuramidase SG (Seikagaku Kogyo) and lysozyme were used for lysing cells (Kudo et al., 1998). In case that cells failed to be lysed by these enzymes, the cells were freeze-dried and mechanically ground as described by Raeder and Broda (1985). The G+C content of the DNA was determined using the HPLC method of Tamaoka and Komagata (1984). An equimolar mixture of nucleotides for analysis of DNA base composition (Yamasa Shoyu, Choshi, Japan) was digested by bacterial alkaline phosphatase and used as the quantitative standard. DNA-DNA relatedness was measured fluorometrically using the microplate hybridization method devised by Ezaki et al. (1989). Hybridization was carried out at 55°C for 2 h.

16S rDNA sequence analysis. The sequences were multiply aligned with selected sequences (Fig. 1) obtained from the GenBank/EMBL/DDBJ databases by using the CLUSTAL X program package (Thompson et al., 1997). The alignment was manually verified and adjusted prior to construction of a phylogenetic tree. A phylogenetic tree constructed by the neighbor-joining method (Saitou and Nei, 1987) in the PAUP (Phylogenetic Analysis Using Parsimony) program package (version 4.0 b10) (Swofford, 2001) was based on the comparison of 1,344 nucleotides present in all the strains as a result of elimination of gaps and am-

Fig. 1. Neighbor-joining tree (Saitou and Nei, 1987) based on almost-complete 16S rDNA sequence analysis showing relationships among strains 2-19(6)^T and 2-30-b(28)^T, the 27 validly described *Micromonospora* species and representatives of other genera of the family *Micromonosporaceae*.

Numbers at nodes indicate the level (%) of bootstrap support based on neighbor-joining analysis of 1,000 resampled datasets. Only values >45% are shown. Bar, 0.01 nucleotide substitution per 100 nucleotides.

ambiguous nucleotides from the sequences (*Escherichia coli* position number) and *Streptomyces ambofaciens* was used as an outgroup. The confidence values of branches of the phylogenetic tree were determined using bootstrap analyses based on 1,000 resamplings (Felsenstein, 1985). The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession numbers listed in Fig. 1.

Results and Discussion

Strains 2-19(6)^T and 2-30-b(28)^T produced well-developed and branched substrate hyphae on yeast extract-starch agar medium, but no aerial hyphae. Spores were borne singly on the substrate hyphae, and were 0.5 to 0.8 µm diameter. Spores had rough to nodular surfaces and were non-motile. The color of the strains 2-19(6)^T and 2-30-b(28)^T was golden brown to dark brown or cinnamon brown. Table 1 shows cultural characteristics of strains 2-19(6)^T and 2-30-b(28)^T on various agar media. The morphological and chemical

Table 1. Cultural characteristics of isolates 2-19(6)^T and 2-30-b(28)^T.

Agar medium	2-19(6) ^T	2-30-b(28) ^T
	Growth/Reverse color/Sporulation	Growth/Reverse color/Sporulation
Glucose-asparagine	++/Amber(3lc)/+	+ /Oatmeal sand(2ec)/+
Glycerol-asparagine	±/Colorless/++	+ /Covert tan(2ge)/++
Salts-starch	+ /Bamboo(2gc)/++	++ /Melon yellow(3ga)/±
Tyrosine	±/Light ivory(2ca)/++	++ /Covert tan(2ge)/++
Nutrient	±/Melon yellow(3ia)/±	±/Lt. melon yellow(2ea)/±
Yeast-malt extract	++ /Chocolate brown(4pn)/+	++ /Light amber(3ic)/±
Oatmeal	+ /Bamboo(2gc)/+	+ /Light amber(3ic)/±
Bennett	++ /Dark brown(2pn)/+	++ /Golden brown(2pg)/++
Glucose-yeast extract	++ /Golden brown(3pi)/+	++ /Light amber(3ic)/++
Hickey-Tresner	++ /Dark brown(2pn)/+	++ /Cinnamon(3le)/±
Water	±/Colorless/+	±/Colorless/+
Sucrose-nitrate	±/Colorless/+	±/Colorless/++
Yeast-starch	++ /Golden brown(3pg)/+	++ /Cinnamon(3le)/++
Oatmeal-nitrate	+ /Oatmeal sand(2ec)/++	+ /Oatmeal sand(3ec)/+
1/5 yeast-starch	+ /Oatmeal sand(2ec)/++	+ /Light wheat(2ea)/+
Waksman	++ /Golden brown(3pg)/+	++ /Cinnamon(3lc)/+
Seino*	++ /Dark brown(2pn)/++	++ /Cinnamon(3lc)/++

Cultures were incubated at 30°C for 3 weeks. Aerial mycelium and diffusible pigments were not formed on all of the agar media tested. Color designations and codes in parentheses were taken from the *Color Harmony Manual* (Jacobson et al., 1958). Growth and sporulation on aerial mycelium are scored as: (++), good; (+), moderate; (±), weak/poor; –, no growth and no spore formed; (Lt), light.

*Seino agar medium: (N-Z amine, 0.3%; yeast extract, 0.1%; meat extract, 0.1%; CaCO₃, 0.3% and agar, 1.2%).

properties of strains 2-19(6)^T and 2-30-b(28)^T are consistent with their classification in the genus *Micromonospora* (Kawamoto, 1989). The results of physiological and biochemical tests are shown in Table 2.

Strains 2-19(6)^T and 2-30-b(28)^T exhibited chemotaxonomic characteristics typical of members of the genus *Micromonospora*, i.e. the presence of *meso*-A₂pm and *N*-glycolyl muramic acid in the peptidoglycan, whole cell sugar pattern D (Lechevalier and Lechevalier, 1970), phospholipid type II (Lechevalier et al., 1977), and cellular fatty acid type 3b (Kroppenstedt, 1985) (Tables 3 and 4). The major menaquinone of strain 2-19(6)^T is MK-10(H₄), which is identical to *M. mirobrigensis* DSM 44839^T; that of strain 2-30-b(28)^T is MK-10(H₆, H₈) and this menaquinone profile differs from that reported for most of the closely related type strains of the genus *Micromonospora* (Table 5). The menaquinone composition within the genus *Micromonospora* has been reported to be complex and heterogenous in comparison to the menaquinone composition found in other actinomycete genera, which

show similar high levels of intragenetic relatedness (Koch et al., 1996). However, in this study, most of the data were in agreement with the previous data. The G+C composition of strains 2-19(6)^T and 2-30-b(28)^T was 71 and 73 mol% respectively, as shown in Table 3.

The almost complete 16S rDNA sequence of strains 2-19(6)^T and 2-30-b(28)^T consisting of 1,503 and 1,511 nucleotides, respectively, was determined. Phylogenetic analysis based on the 16S rDNA sequences indicated that strain 2-19(6)^T formed a coherent and monophyletic clade with the closest relatives *M. mirobrigensis* (98.9%) and *M. carbonacea* (98.8%) (which was supported by the bootstrap value of 57% (Fig. 1). The strain 2-30-b(28)^T is most closely related to *M. purpureochromogenes* (99.4%), *M. halophytica* (99.3%), *M. aurantiaca* (99.2%) and *M. chalcone* (98.7%) (Table 6). Differential characteristics shown in Table 5 clearly indicate that strains 2-19(6)^T and 2-30-b(28)^T possesses distinct phenotypic and menaquinone profiles that separate these strains from the phylogenetically closest species. In particular the

Table 2. Physiological characteristics of the isolates 2-19(6)^T and 2-30-b(28)^T.

Characteristic	2-19(6) ^T	2-30-b(28) ^T
Utilization of		
D-Glucose	+	+
Glycerol	—	+
Erythritol	—	—
Adonitol	—	w
L-Arabinose	+	+
D-Ribose	w	+
D-Xylose	+	+
D-Galactose	+	+
D-Fructose	+	+
D-Mannose	+	+
L-Rhamnose	—	w
myo-Inositol	—	—
D-Mannitol	—	—
α-Methyl-D-glucoside	—	+
Salicin	+	+
Lactose	+	+
α-D(+)Melibiose	+	+
Sucrose	+	+
D(+)Raffinose	+	+
Maltose	+	+
Trehalose	+	+
Temperature for growth (°C):	20–37	15–37
pH for growth (pH):	5–9	5–9
Growth on 3% NaCl:	+	+
Melanin pigment:	—	—

Characteristics are scored as (+), positive; (w), doubtful/poor; (—), negative.

Table 4. Cellular fatty acid compositions of 2-19(6)^T and 2-30-b(28)^T.

Fatty acid ^a	2-19(6) ^T	2-30-b(28) ^T
Saturated		
C _{15:0} ^b	1.39	
C _{16:0}	2.98	
C _{17:0}	6.61	1.75
C _{18:0}	3.71	3.55
Unsaturated		
2OH-C _{16:1}		4.13
C _{17:1} (ω6c)		1.67
C _{17:1} (ω8c)	2.99	4.02
C _{18:1} (ω9c)	1.83	3.08
Branched		
i-C _{14:0}		1.36
i-C _{15:0}	32.98	12.26
a-C _{15:0}	6.46	1.57
i-C _{16:0}	12.35	
i-C _{16:1}		7.94
i-C _{17:0}	7.63	3.71
a-C _{17:0}	5.80	8.67
i-C _{17:1} (ω9c)	4.55	12.59
a-C _{17:1} (ω9c)		1.83
i-C _{18:0}		2.53
i-C _{18:1}		1.97
10-Methylated		
C _{17:0}	4.36	21.58
C _{18:0}	1.85	2.13

^a Values are percentages of total cellular fatty acids. Trace amounts (less than 1.0%) are not shown.

^b Number of carbon atoms : number of double bonds.

Abbreviations: i, iso; a, anteiso.

Table 3. Chemotaxonomic characteristics of isolates 2-19(6)^T and 2-30-b(28)^T.

Characteristic	2-19(6) ^T	2-30-b(28) ^T
Cell wall diamino acid	meso-DAP	meso-DAP
Characteristic sugar(s)	Ribose, mannose, xylose, galactose, glucose, arabinose	Glucose, rhamnose, xylose, galactose, mannose, ribose, arabinose
Phospholipid(s)	DPG, PE, PG, PI, PIMs	DPG, PE, PG, PI, PIMs
Menaquinone(s)	MK-10(H ₄), MK-10(H ₆), MK-9(H ₄), MK-9(H ₆)	MK-10(H ₆), MK-10(H ₈), MK-10(H ₄), MK-9(H ₆), MK-9(H ₄), MK-9(H ₈)
N-acyl group of muramic acid	Glycolyl	Glycolyl
Mycolic acid	—	—
G+C mol%	71	73

Abbreviations: DAP, diaminopimelic acid; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; NPG, ninhydrin-positive phosphoglycerolipids; PIMs, phosphatidylinositol mannosides; Abbreviations for menaquinones are exemplified by MK-9(H₄): a tetrahydrogenated menaquinone with nine isoprene units; (—), scored as negative.

Table 5. Characteristics that differentiate strains 2-19(6)^T and 2-30-b(28)^T from their closest neighbors.

Characteristic*	1	2	3 ^a	4 ^a	5 ^a	6 ^{b,c}	7 ^{b,c}	8 ^b	9 ^b	10 ^d
Max. NaCl Tolerance (%)	3	3	3	3	3	4	4	5	1.5	5
Growth at: (°C)	37	37	37	40	40	40	40	40	40	40
Major menaquinone	MK-10(H ₄)	MK-10(H ₆), MK-10(H ₈)	MK-10(H ₄)	MK-9(H ₄)	MK-10(H ₆)	MK-9(H ₄)	MK-10(H ₄)	MK-10(H ₄)	MK-10(H ₄)	MK-10(H ₄), MK-10(H ₆)
Utilization** of:										
Glycerol	—	w	ND	+	—	—	—	—	—	—
L-Arabinose	+	+	+	—	+	+	w	w	—	+
D-Ribose	w	+	ND	—	w	—	—	—	—	—
D-Xylose	+	+	ND	+	+	+	+	+	+	+
D-Fructose	+	+	ND	+	+	+	+	w	+	w
L-Rhamnose	—	w	—	—	—	w	—	—	—	+
Salicin	+	+	ND	+	—	+	—	—	—	+
Lactose	+	+	ND	+	+	+	—	+	w	+
α-D(+)Melibiose	+	+	+	—	—	+	+	+	+	+
D(+)Raffinose	+	+	+	+	+	+	—	+	w	+

Strains: 1, *M. chokoriensis* sp. nov. 2-19(6)^T; 2, *M. coxensis* sp. nov. 2-30-b(28)^T; 3, *M. mirobrigensis* WA201^T; 4, *M. carbonacea* DSM 43168^T; 5, *M. matsumotoense* ATCC 49364^T; 6, *M. halophytica* DSM 43171^T; 7, *M. aurantiaca* DSM 43813^T; 8, *M. chalicea* DSM 43026^T; 9, *M. purpureochromogenes* DSM 43821^T; 10, *M. siamensis* JCM 12769^T.

* All strains were positive for utilization of D-glucose, D-xylose, D-galactose and negative for D-mannitol.

**Characteristics are scored as (+), positive; (w), weakly positive/poor; (—), negative; ND, not determined.

^aTrujillo et al., 2005; ^bThawai et al., 2004; ^cThawai et al., 2005a; ^dThawai et al., 2005b.

Table 6. 16S rDNA sequence similarity (%) of the strain 2-19(6)^T and 2-30-b(28)^T and other closely related valid species of the genus *Micromonospora*.

	1	2	3	4	5	6	7	8	9	10
1	100									
2	98.4	100								
3	98.4	98.6	100							
4	97.6	97.6	97.8	100						
5	99.2	98.7	98.6	98.2	100					
6	98.4	98.1	98.8	96.9	98.4	100				
7	99.1	98.6	97.9	97.7	98.5	97.9	100			
8	99.2	98.3	98.6	96.9	99.4	98.1	98.7	100		
9	98.4	98.5	98.4	96.6	98.6	98.9	97.8	98.1	100	
10	99.1	98.5	98.6	97.6	99	98.2	98.7	99.3	98.4	100

Strains: 1, *M. aurantiaca* DSM 43813^T; 2, *M. siamensis* JCM 12769^T; 3, *M. carbonacea* DSM 43168^T; 4, *M. matsumotoense* ATCC 49364^T; 5, *M. purpureochromogenes* DSM 43821^T; 6, *M. chokoriensis* sp. nov. 2-19(6)^T; 7, *M. chalicea* DSM 43026^T; 8, *M. coxensis* sp. nov. 2-30-b(28)^T; 9, *M. mirobrigensis* WA201^T; 10, *M. halophytica* DSM 43171^T.

maximum NaCl concentration for growth, growth temperature, menaquinone pattern and utilization of glycerol, L-arabinose, D-ribose, D-fructose, L-rhamnose, salicin, lactose, α-D-melibiose (Table 5) were effective

for discrimination between strains 2-19(6)^T, 2-30-b(28)^T and closely related species. On the basis of the physiological data as well as the morphological and chemotaxonomic data, it is evident that the two strains

should be classified in the genus *Micromonospora*.

The DNA-DNA hybridization technique is used to resolve the taxonomic relationships at the species level. In the present study, it is shown that the levels of DNA-DNA relatedness among strain 2-19(6)^T and the closely related type strain *M. mirobrigensis* of the genus *Micromonospora*, ranged from 37.0% to 44.1% (Table 7). The DNA-DNA similarity (37.0%) clearly indicated that isolate 2-19(6)^T does not belong to the species *M. mirobrigensis* JCM 13240^T as this value is well below the threshold value of 70% for definition of bacterial species according to Wayne et al. (1987). Furthermore, physiological, chemical, morphological, 16S rDNA similarity (%) and phylogenetic data indicated that strain 2-19(6)^T represents a different species when compared with the phylogenetically neighboring species *M. carbonacea*, *M. matsumotoense* and *M. siamensis* (please see Table 5). On the other hand, the levels of DNA-DNA relatedness among strain 2-30-b(28)^T and the closely related type strains of the genus *Micromonospora*, ranged from 10% to 47% (Table 8). It is evident from the genotypic and phenotypic data that strains 2-19(6)^T and 2-30-b(28)^T are distinguished from one another and previously described species of the genus *Micromonospora*. There-

fore, the name *Micromonospora chokoriensis* sp. nov., and *Micromonospora coxensis* sp. nov., respectively, are proposed for the strains.

Description of *Micromonospora chokoriensis* sp. nov.

Micromonospora chokoriensis (cho.ko.ri.en'sis N.L. fem adj. *chokoriensis* pertaining to Chokoria, Bangladesh, the origin of the soil where the type strain was isolated).

Forms a well-developed, branched substrate mycelium. An aerial mycelium is absent. Sporulations are abundant and spores are borne directly on substrate mycelium. Spores are spherical and non-motile. Spore surface is rough to nodular. The color of substrate mycelium is golden brown to dark brown on yeast extract-starch agar. A soluble pigment is not produced. Aerobic. Gram-positive. Growth at pH 5–9. The temperature range for growth is between 20°C and 37°C. Utilizes D-glucose, L-arabinose, D-xylose, D-galactose, D-fructose, D-mannose, salicin, lactose, α-D(+)-melibiose, sucrose, D(+)-raffinose, maltose and trehalose. Weak utilization of D-ribose and no utilization of glycerol, erythritol, adonitol, D-fructose, L-rhamnose, *myo*-inositol, D-mannitol or α-methyl-D-glucoside. Production of melanoid pigments is negative. Growth on 3% NaCl. The isomer of A₂pm was *meso* and ribose, mannose, xylose, galactose, glucose and arabinose in the whole-cell hydrolysates. The *N*-acyl type of muramic acid is glycolyl. Mycolic acids are absent. Contains phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. Glucosamine containing phospholipid and phosphatidylcholine are absent. The major menaquinone is MK-10(H₄) with minor amounts of MK-10(H₆), MK-9(H₄) and MK-9(H₆). The fatty acid profile is characterized by significant amounts of iso-C_{15:0} (33.0%), iso-C_{16:0} (12.3%), iso-C_{17:0} (7.6%),

Table 7. DNA base composition and DNA-DNA relatedness of the strain 2-19(6)^T and closely related valid species *M. mirobrigensis* of the genus *Micromonospora*.

Strain	G+C content (mol%)	DNA-DNA binding (%) with label from:	
		2-19(6) ^T	<i>M. mirobrigensis</i>
2-19(6) ^T	71.0	100	44.1
<i>M. mirobrigensis</i> JCM 13240 ^T	68.6	37.0	100

Table 8. DNA base composition and DNA-DNA relatedness of the strain 2-30-b(28)^T and other closely related valid species of the genus *Micromonospora*.

Strain	G+C content (mol%)	DNA-DNA binding (%) with label from: 2-30-b(28) ^T
2-30-b(28) ^T	73.0	100
<i>M. chalcone</i> JCM 3031 ^T	72.0	10
<i>M. halophytica</i> JCM 3125 ^T	72.5	47
<i>M. purpureochromogenes</i> JCM 3156 ^T	73.0	10
<i>M. aurantiaca</i> JCM 10878 ^T	72.0	21

C_{17:0} (6.6%) and anteiso-C_{15:0} (6.5%). Small amounts of anteiso-C_{17:0} (5.8%), iso-C_{17:1}(ω 9c) (4.5%), 10-methylated C_{17:0} (4.4%), C_{18:0} (3.7%), C_{17:1} (ω 8c) (3.0%) and C_{16:0} (3.0%) acids are also present. The G+C content of the DNA was 71 mol%. Isolated from sandy soil collected at a forest-side waterfall, Chokoria, Bangladesh. The type strain is 2-19(6)^T (=JCM 13247^T=MTCC XXX^T).

Description of *Micromonospora coxensis* sp. nov.

Micromonospora coxensis (*cox.en'sis* N.L. fem adj. *coxensis* pertaining to Cox's Bazar, Bangladesh, the origin of the soil where the type strain was isolated).

Forms a well-developed, branched substrate mycelium. An aerial mycelium is absent. Sporulations are abundant and spores are borne directly on substrate mycelium. Spores are spherical and non-motile. Spore surface is nodular to warty. The color of substrate mycelium is light brown to cinnamon brown on yeast extract-starch agar. A soluble pigment is not produced. Aerobic. Gram-positive. Grows at pH 5–9. The temperature range for growth is between 15°C and 37°C. Utilizes D-glucose, glycerol, L-arabinose, D-ribose, D-xylose, D-galactose, D-fructose, D-mannose, salicin, lactose, α -D(+)-melibiose, sucrose, D(+)-raffinose, maltose and trehalose. Weak utilization of L-rhamnose and adonitol, and no utilization of erythritol, myo-inositol or D-mannitol. Production of melanoid pigments is negative. Grows on 3% NaCl. The isomer of A₂pm was *meso*, and glucose, rhamnose, xylose, galactose, mannose, ribose and arabinose were detected in the whole-cell hydrolysates. The *N*-acyl type of muramic acid is glycolyl. Mycolic acids are absent. Contains phosphatidylethanolamine, diphosphatidylglycerol, phosphatidylinositol and phosphatidylinositol mannosides. Glucosamine containing phospholipid and phosphatidylcholine are absent. The major menaquinone are MK-10(H₆) and MK-10(H₈) with minor amounts of MK-10(H₄), MK-9(H₆), MK-9(H₄) and MK-9(H₈). The fatty acid profile is characterized by significant amounts of 10-methylated C_{17:0} (21.6%), iso-C_{17:1} (ω 9c) (12.6%), iso-C_{15:0} (12.3%), anteiso-C_{17:0} (8.7%) and iso-C_{16:1} (7.9%). Small amounts of 2OH-C_{16:1} (4.1%), C_{17:1} (ω 8c) (4.0%), iso-C_{17:0} (3.7%) and C_{18:0} (3.5%) acids are present. The G+C content of the DNA was 73 mol%. Isolated from sandy soil collected at a forest-side waterfall, Chokoria, Bangladesh. The type strain is 2-30-b(28)^T (=JCM 13248^T=MTCC 8093^T).

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