

NOTE

Proposal to transfer *Catellatospora ferruginea* and '*Catellatospora ishikariense*' to *Asanoa* gen. nov. as *Asanoa ferruginea* comb. nov. and *Asanoa ishikariensis* sp. nov., with emended description of the genus *Catellatospora*

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The taxonomic position of *Catellatospora ferruginea* and '*Catellatospora ishikariense*' was investigated by phylogenetic, chemotaxonomic and physiological characterization. The 16S rDNA sequences of the organisms were compared with those of members of the genus *Catellatospora* and other genera of the *Micromonosporaceae* and phylogenetic trees were inferred by using distance-matrix and parsimony methods. The organisms formed a distinct cluster within the radiation of this family that was supported by a high bootstrap value, of 100%. The nearest neighbours were members of the genera *Catenuloplanes* and *Verrucosispora*. The organisms were readily differentiated from all of the validly described genera of the family *Micromonosporaceae* by using a battery of chemical and morphological characters, and the name *Asanoa* gen. nov. is proposed. On the basis of phenotypic and DNA–DNA hybridization data, *Asanoa ferruginea* comb. nov. (type strain IMSNU 22009^T = IFO 14496^T = DSM 44099^T) and *Asanoa ishikariensis* sp. nov. (type strain IMSNU 22004^T = IFO 14551^T) are described.

Keywords: *Asanoa ferruginea*, *Asanoa ishikariensis*, actinomycetes, polyphasic taxonomy

The genus *Catellatospora*, which was described by Asano & Kawamoto (1986), originally contained two species, *Catellatospora citrea* and *Catellatospora ferruginea*. Subsequently, *C. citrea* subsp. *methionotrophica* (Asano & Kawamoto, 1988), *Catellatospora matsumotoense* and *Catellatospora tsunoense* (Asano *et al.*, 1989) were added. This genus is chemotaxonomically heterogeneous, especially in menaquinone composition (Asano & Kawamoto, 1986; Asano *et al.*, 1989; Lee *et al.*, 1999). The chemical heterogeneity between members of the genus *Catellatospora* has also been reflected in comparative analysis of 16S rDNA sequences. Phylogenetic analysis indicated that members of the genus *Catellatospora* were true members of the family *Micromonosporaceae* but were phylogenetically heterogeneous (Koch *et al.*, 1996; Lee *et al.*, 1999). *Catellatospora matsumotoense* has been

transferred to the genus *Micromonospora* as *Micromonospora matsumotoense* on the basis of 16S rDNA sequence studies and phenotypic characters (Lee *et al.*, 1999) and the description was also validated (Lee *et al.*, 2000a). On the other hand, the recent description of a novel species, *Catellatospora koreensis* (Lee *et al.*, 2000b), has increased the chemical heterogeneity yet further among members of this genus. A polyphasic approach based on phenotypic and genotypic classification methods needs to be performed for the precise evaluation of taxa. The aim of this study was to evaluate the taxonomic status of *C. ferruginea* and '*Catellatospora ishikariense*' using a polyphasic approach and to emend the description of the genus *Catellatospora*. The test strains were received from the Culture Collection Center of the Institute of Microbiology, Seoul National University (IMSNU, Korea), and maintained on yeast extract/malt extract agar (medium 2 of the International *Streptomyces* Project; ISP medium 2) at 4 °C and as suspensions in 20% (v/v) glycerol at –20 °C. For morphological obser-

The EMBL accession numbers for the 16S rDNA sequences of ten *Actinoplanes* species, strain IMSNU 22004^T and two *Catenuloplanes* species reported in this study are AJ277565–AJ277574 and AJ294715–AJ294717.

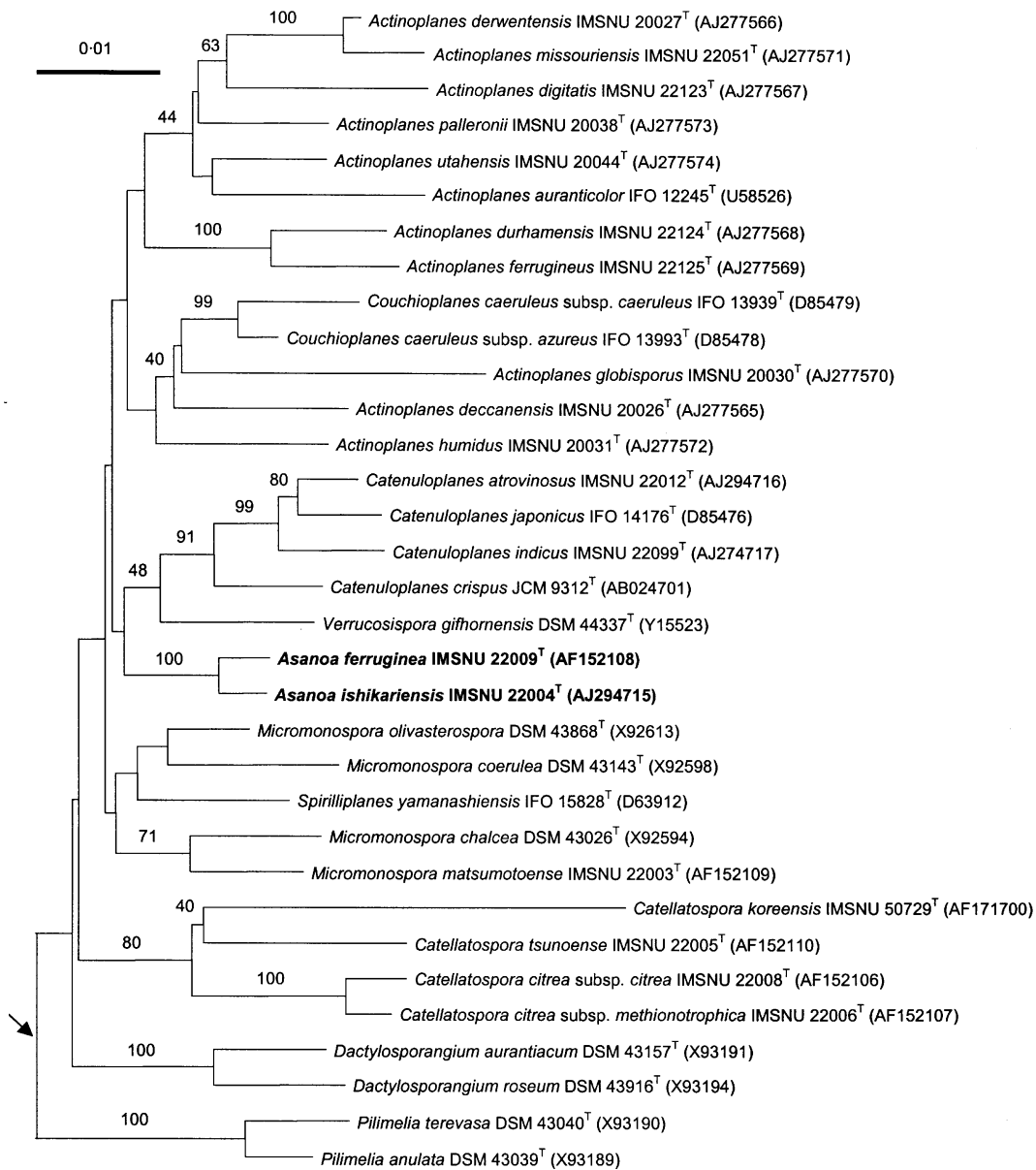


Fig. 1. Phylogenetic dendrogram indicating the placement of the genus *Asanoa* gen. nov. within the radiation of representatives of the family *Micromonosporaceae*. *Nocardia asteroides* was used as the outgroup. The tree was reconstructed by the neighbour-joining method. Bootstrap values indicated at branching points are expressed as percentages of 1000 replications. Bar, 1 nucleotide substitution per 100 nucleotides.

vation, the strains were grown on oatmeal agar (ISP medium 3), inorganic salts/starch agar (ISP medium 4) and tap-water agar at 28 °C for 21 days. Morphology, physiological and chemical characteristics were investigated by methods described previously (Lee *et al.*, 2000b). For DNA isolation, the test strains were cultivated on yeast extract/glucose broth for 3 days at 30 °C, harvested by centrifugation and washed twice with distilled water. Examinations of genotypic properties, such as the determination of G+C content, DNA-DNA hybridization studies and 16S rDNA sequencing, were performed by methods described previously (Lee *et al.*, 2000c).

Almost-complete 16S rDNA sequences of strain IMSNU 22004^T, two strains of *Catenuloplanes* and ten strains of *Actinoplanes* determined in this study were aligned and compared by using the program CLUSTAL X (Thompson *et al.*, 1997) with those of all the type strains of the family *Micromonosporaceae* and related actinomycete taxa. The final phylogenetic tree was represented on the basis of sequences of 33 representatives of the family *Micromonosporaceae* and, as an outgroup member, *Nocardia asteroides* DSM 43757^T (X80606). The programs contained in the PHYLIP package (Felsenstein, 1993) were used to calculate evolutionary distances by the method of

Jukes & Cantor (1969) and a phylogenetic tree was reconstructed by the neighbour-joining method (Saitou & Nei, 1987). The reliability of the resultant tree topology was evaluated by bootstrap analysis of the data with 1000 resamplings. Parsimony analysis was performed with the PAUP program for the Macintosh (Swofford, 1998). Evolutionary trees were displayed and printed using TREEVIEW version 1.5 (Page, 1996).

16S rDNA sequence studies

The almost complete 16S rDNA sequences of 13 test strains determined in this study corresponded to 94.1–97.7% of the *Escherichia coli* sequence (Brosius *et al.*, 1978) and were compared with those of representatives of the family *Micromonosporaceae* (Koch *et al.*, 1996; Tamura *et al.*, 1997; Rheims *et al.*, 1998; Lee *et al.*, 1999, 2000b). The phylogenetic dendrogram based on the neighbour-joining algorithm (Fig. 1) showed that *C. ferruginea* and '*C. ishikariense*' formed a distinct clade within the radiation encompassed by the family *Micromonosporaceae* and thus were well separated from members of the genus *Catellatospora* and the other genera of the *Micromonosporaceae*. This relationship was supported by a bootstrap value of 100%. Parsimony analysis showed similar topologies for a tree obtained with a distance-based algorithm. These organisms were loosely associated with members of the genera *Verrucosipora* and *Catenuloplanes*, to which the levels of sequence similarity were respectively 96.9–97.2% and 97.2–97.4%. On the other hand, these organisms showed low sequence similarity of 93.7–96.1% to other members of the genus *Catellatospora*, 96.9–97.0% to *M. matsumotoense* (formerly *C. matsumotoense*) and 95.4–97.3% to members of the genus *Actinoplanes* investigated in this study.

Chemical characteristics

The chemical properties of *C. ferruginea* IMSNU 22009^T and '*C. ishikariense*' IMSNU 22004 support the conclusion that they have been misclassified as members of the genus *Catellatospora*. Both of the organisms are characterized by the possession of MK-10(H₆, H₈) as major menaquinones (Asano & Kawamoto, 1986; this study). *C. ferruginea* IMSNU 22009^T contained 3-*O*-methylrhannose as a diagnostic component of the cell-wall sugars. This additional cell-wall sugar is also found in the cell wall of *M. matsumotoense* (formerly *C. matsumotoense*) (Asano *et al.*, 1989), but was not detected in the cell wall of '*C. ishikariense*' IMSNU 22004 in our study. On the other hand, other members of the genus *Catellatospora* have the major menaquinones MK-9(H₄, H₆) or MK-10(H₄) and are characterized by the absence of 3-*O*-methylrhannose in the cell wall (Asano & Kawamoto, 1986, 1988; Asano *et al.*, 1989; Lee *et al.*, 2000b). Concerning the fatty acid profiles, *C. ferruginea* and '*C. ishikariense*' are readily differentiated from the other members of the genus *Catellatospora*. The fatty acid profiles of

members of the genus *Catellatospora* (Lee *et al.*, 2000b) showed slight differences from the results of another worker (R. M. Kroppenstedt, unpublished results) in that none of the strains of the genus *Catellatospora* contained anteiso-branched fatty acids with 17 carbon atoms and 10-methyl branched fatty acids were not detected in strains of *C. citrea*. The fatty acid profiles of *C. ferruginea* and '*C. ishikariense*' are characterized by considerable amounts of anteiso- and iso-branched fatty acids. Significant amounts of unsaturated and saturated fatty acids were also present, but 10-methyl-branched acids were not detected. This profile matches the diagnostic fatty acid type 2d (Kroppenstedt, 1985). On the other hand, the other members of the genus *Catellatospora* contained large amounts of iso-C_{15:0} and fatty acids with 17 carbon atoms. In addition, 10-methyl-branched acids were also detected in *C. tsumoense*. This combination of fatty acids corresponds to fatty acid type 3b (Kroppenstedt, 1985).

C. ferruginea IMSNU 22009^T and '*C. ishikariense*' IMSNU 22004 can be distinguished from members of all other genera of the family *Micromonosporaceae* by using a combination of chemical characters (Table 1) and phylogenetic evidence, supporting the conclusion that these organisms merit the recognition of a new genus.

Morphological characteristics

It was reported previously that sporulation of *C. ferruginea* was poor and could be observed only in tap-water agar and glycerol/calcium malate agar media (Asano & Kawamoto, 1986). This morphological characteristic was also confirmed in our study, in that *C. ferruginea* IMSNU 22009^T and '*C. ishikariense*' IMSNU 22004 did not form any spores in inorganic salt/starch agar or oatmeal agar but occasionally produced short chains of spores in tap-water agar. Globose bodies that are found in some species of the genus *Catellatospora* were not observed in these organisms.

Physiological characteristics

C. ferruginea and '*C. ishikariense*', which showed 16S rDNA sequence similarity of 99.2%, can be differentiated physiologically from each other by their utilization of gluconate, salicin and dulcitol, hydrolysis of elastin, resistance against crystal violet and tetracycline and growth at 37 °C. DNA–DNA hybridization indicated that the level of DNA relatedness between the two organisms was 41%. Concerning the fatty acid profiles, these organisms showed significant differences in the amounts of iso-C_{15:0}, iso-C_{16:0}, C_{17:1} and C_{17:0} acids. In addition, *C. ferruginea* contained iso-C_{14:0} acid as a minor component, whereas C_{19:0} acid was detected in the fatty acid profile of '*C. ishikariense*'.

The phylogenetic, chemical and morphological evidence shows that *C. ferruginea* IMSNU 22009^T

Table 1. Diagnostic characteristics that differentiate the genus *Asanoa* from the other genera of the family *Micromonosporaceae*

Data were taken from the following references: Asano & Kawamoto (1986, 1988), Asano *et al.* (1989), Goodfellow (1989), Yokota *et al.* (1993), Tamura *et al.* (1994, 1995, 1997), Rheims *et al.* (1998), Kudo *et al.* (1999) and Lee *et al.* (2000c). +, Present; -, absent; ND, not determined; Xyl, xylose; Ara, arabinose; Gal, galactose; Man, mannose. The abbreviation of menaquinones is illustrated by the following examples: MK-9(H₉), menaquinone with two of nine isoprene units hydrogenated. Spores are present in all genera.

Characteristic	<i>Asanoa</i>	<i>Catellatospora</i>	<i>Catenuloplanes</i>	<i>Actinoplanes</i>	<i>Conchioplanes</i>	<i>Dactylosporangiium</i>	<i>Micromonospora</i>	<i>Pilimelia</i>	<i>Spirilliplanes</i>	<i>Verrucospora</i>
Spore motility	-	-	+	+	+	+	-	+	+	-
Sporangium formation	-	-	-	+	-	+	-	+	-	-
Cell-wall type*	II	II	VI	II	VI	II	II	II	II	II
Characteristic sugar(s) in whole cells	Xyl, Ara, Gal	Xyl, Ara, Gal or Xyl	Xyl	Xyl, Ara	Xyl, Ara, Gal	Xyl, Ara	Xyl, Ara	Xyl, Ara	Xyl, Gal	Xyl, Man
Phospholipid type†	PII	PII	PIII	PII	PII	PII	PII	PII	PII	PII
Major menaquinone(s)	MK-10(H ₆ , H ₈)	MK-10(H ₆) or MK-9(H ₆ , H ₈)	MK-10(H ₆), MK-11(H ₇)	MK-9(H ₆ , H ₈)	MK-9(H ₆)	MK-9(H ₆ , H ₈)	MK-10(H ₆ , H ₈), MK-9(H ₆ , H ₈)	MK-9(H ₆ , H ₈)	MK-10(H ₆)	MK-9(H ₆)
Fatty-acid type‡	2d	3b	2c	2d	2c	3b	3b	2d	2d	2b
G+C content (mol %)	71-72	70-72	70-73	72-73	70-72	72-73	71-73	ND	69	70

* According to the classification of Lechevalier & Lechevalier (1970).

† According to the classification of Lechevalier *et al.* (1977).

‡ According to the classification of Kroppenstedt (1985).

(= IFO 14496^T = DSM 44099^T) and '*C. ishikariense*' IMSNU 22004 (= IFO 14551) can be differentiated from members of the genus *Catellatospora* and the other genera of the family *Micromonosporaceae*. It is therefore proposed that these organisms be classified in the new genus *Asanoa* gen. nov. On the basis of phenotypic and genotypic data, *Asanoa ferruginea* comb. nov. and *Asanoa ishikariensis* sp. nov. are described.

Description of *Asanoa* gen. nov.

Asanoa (As.a.no'a. N.L. fem. n. *Asanoa* of Asano, named after Kozo Asano, the Japanese microbiologist who made the original description of the genus *Catellatospora*).

The description is based on data taken from earlier studies (Asano & Kawamoto, 1986; Lee *et al.*, 2000b) and our own studies. Aerobic. Gram-positive. Catalase-positive. Urease-negative. Nitrate is not reduced to nitrite. H₂S is not produced. Mesophilic. Vegetative hyphae are branched but not fragmented. Orange colony mass. Sporulation is poor and occurs only on tap-water agar and glycerol/calcium malate agar. Other morphological features, such as aerial mycelium and globose bodies, are not observed. Contains meso-diaminopimelic acid and 3-hydroxydiaminopimelic acid as diamino acids, a glycolylated peptidoglycan and whole-cell sugars of arabinose, rhamnose, ribose, xylose, galactose, mannose and glucose. An additional sugar, 3-*O*-methylrhamnose, is variable according to the species. The predominant menaquinones are hexa- and octa-hydrogenated menaquinones with ten isoprene units [MK-10(H₆, H₈)]. The diagnostic phospholipid is phosphatidylethanolamine (a phospholipid type PII pattern). The fatty acid pattern is 2d type. Mycolic acids are not present. The G+C content of the DNA is 71.1-71.5 mol% (HPLC). The genus belongs to the family *Micromonosporaceae*. The type species is *Asanoa ferruginea*.

Description of *Asanoa ferruginea* comb. nov.

Asanoa ferruginea (fer.ru.gin'e.a. L. adj. *ferruginea* rust-coloured).

Basonym: *Catellatospora ferruginea* Asano & Kawamoto 1986.

The description is based on data taken from previous studies (Asano & Kawamoto, 1986; Lee *et al.*, 2000b) and our own studies. Utilizes D-arabinose, L-arabinose, dextran, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannose, melibiose, methyl α -D-glucoside, D-raffinose, L-rhamnose, D-ribose, salicin, starch, sucrose, D-trehalose, D-xylose, adonitol and D-mannitol as carbon sources. No growth on gluconate, inulin, D-melezitose, L-sorbose, dulcitol, butanol, meso-erythritol, ethanol, glycerol, meso-inositol, 2-propanol, 1-propanol, D-sorbitol or D-xylitol. Does not decompose adenine, hippurate,

hypoxanthine, DL-tyrosine or xanthine. Hydrolyses elastin and starch but not casein, DNA or gelatin. Grows on 0.001% brilliant green and 0.0001% crystal violet. No growth on 3% NaCl or 0.01% lysozyme. Susceptible to 50 µg gentamicin ml⁻¹, 5 µg neomycin ml⁻¹ and 100 µg streptomycin ml⁻¹ but resistant to 50 µg novobiocin ml⁻¹, 20 µg vancomycin ml⁻¹ and 10 µg tetracycline ml⁻¹. Grows at 37 °C. The G+C content of the DNA of the type strain is 71.5 mol%. The phospholipid profile comprises diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, phosphatidylethanolamine, phosphatidylglycerol and an unknown phospholipid. The fatty acid profile is characterized by significant amounts of anteiso-C_{15:0} (21.0%), C_{17:0} (20.3%), iso-C_{16:0} (18.3%), C_{17:1} (15.4%) and iso-C_{15:0} (9.5%) acids. Small amounts of C_{15:0} (4.9%), C_{18:0} (4.0%), C_{16:0} (3.1%), iso-C_{14:0} (1.8%) and iso-C_{17:0} (1.1%) acids are also present.

Isolated from soil. The type strain is strain IMSNU 22009^T (= IFO 14496^T = DSM 44099^T).

Description of *Asanoa ishikariensis* sp. nov.

Asanoa ishikariensis (ish.i.k.a.ri.en'sis. N.L. deriv. adj. *ishikariensis* of Ishikari-gun, Hokkaido, Japan, the origin of the soil sample from which the type strain was isolated).

Originally described as '*Catellatospora ishikariense*'. Utilizes D-arabinose, L-arabinose, dextran, D-cellobiose, D-fructose, D-galactose, gluconate, D-glucose, D-lactose, maltose, D-mannose, melibiose, methyl α-D-glucoside, D-raffinose, L-rhamnose, D-ribose, starch, sucrose, D-trehalose, D-xylose, adonitol, dulcitol and D-mannitol as carbon sources. No growth on inulin, D-melezitose, salicin, L-sorbose, butanol, *meso*-erythritol, ethanol, glycerol, *meso*-inositol, 2-propanol, 1-propanol, D-sorbitol or D-xylitol. Does not decompose adenine, hippurate, hypoxanthine, DL-tyrosine or xanthine. Hydrolyses casein, gelatin and starch but not DNA or elastin. Grows on 0.001% brilliant green. No growth on 0.0001% crystal violet, 3% NaCl or 0.01% lysozyme. Susceptible to 50 µg gentamicin ml⁻¹, 5 µg neomycin ml⁻¹, 100 µg streptomycin ml⁻¹ and 10 µg tetracycline ml⁻¹ but resistant to 50 µg novobiocin ml⁻¹ and 20 µg vancomycin ml⁻¹. No growth at 37 °C. The G+C content of the DNA of the type strain is 71.1 mol%. The phospholipid profile consists of diphosphatidylglycerol, phosphatidylinositol, phosphatidylinositol mannoside, phosphatidylethanolamine and an unknown phospholipid. A trace amount of phosphatidylglycerol is also detected. The fatty acid profile is characterized by significant amounts of anteiso-C_{15:0} (24.5%), C_{17:1} (23.8%), iso-C_{15:0} (15.8%) and C_{17:0} (11.8%) acids. Small amounts of iso-C_{17:0} (6.3%), iso-C_{16:0} (4.4%), C_{15:0} (2.8%), C_{18:0} (1.8%), C_{16:0} (1.1%) and C_{19:0} (1.0%) acids are also present.

Isolated from soil. The type strain is strain IMSNU 22004^T (= IFO 14551^T).

Emended description of *Catellatospora Asano & Kawamoto 1986*

Aerobic. Gram-positive. Catalase-positive. Urease-negative. Nitrate is reduced to nitrite. H₂S is not produced. Mesophilic. Vegetative hyphae are branched but not fragmented. Bright yellow or creamy colony mass. Aerial mycelium is not formed. Chains of non-motile spores arise singly or in tufts from vegetative hyphae on the surface of agar media. Globose bodies are produced in some species. Contains *meso*-diaminopimelic acid and 3-hydroxydiaminopimelic acid as diamino acids, a glycolylated peptidoglycan and whole-cell sugars of rhamnose, ribose, xylose, mannose and glucose. Arabinose and galactose are variable according to the species. The predominant menaquinones are tetra- and hexa-hydrogenated menaquinones with nine isoprene units [MK-9(H₄, H₆)] or tetra-hydrogenated menaquinone with ten isoprene units [MK-10(H₄)]. Phosphatidylethanolamine or phosphatidylmethylethanolamine is present as a diagnostic phospholipid. The fatty acid pattern is type 3b. Mycolic acids are absent. The G+C content of the DNA is 70.4–71.5 mol%. Members of this genus form a coherent group within the radiation of family *Micromonosporaceae* on the basis of 16S rDNA sequence data. The genus contains three species, *C. citrea* (with the subspecies *C. citrea* subsp. *citrea* and *C. citrea* subsp. *methionotrophica*), *C. koreensis* and *C. tsunoense*. The type species is *Catellatospora citrea*.

Acknowledgements

This work was supported by the Brain Korea 21 Project for the School of Biological Sciences, Seoul National University. The authors are indebted to Dr Reiner M. Kroppenstedt for the determination of the fatty acid type.

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