Allocatelliglobosispora scoriae gen. nov., sp. nov., isolated from volcanic ash

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A novel actinomycete, designated strain Sco-B14^T, was isolated from volcanic ash collected near Darangshi Oreum (a parasitic or satellite volcano) in Jeju, Republic of Korea. The organism formed well-developed, branched substrate mycelium, on which short chains of non-motile spores were arranged singly or in clusters. Aerial mycelium was not produced. Globose bodies were observed. The reverse colour of colonies was light brown to brown. Diffusible pigments were produced on ISP medium 3 and oatmeal-nitrate agar. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain Sco-B14^T formed a lineage within the family *Micromonosporaceae* and was distinct from established genera. The 16S rRNA gene sequence similarity of strain Sco-B14^T to members of related genera of the family was 95.0–95.7 % to type strains of *Catellatospora* species, 94.7 % to *Hamadaea tsunoensis* IMSNU 22005^T, 94.7 % to *Longispora albida* K97-0003^T and 94.0 % to *Catelliglobosispora koreensis* LM 042^T. 3-Hydroxy-diaminopimelic acid was the diagnostic diamino acid in the cell-wall peptidoglycan. Whole-cell sugars were glucose, rhamnose, ribose, xylose, arabinose, galactose and mannose. The polar lipids included diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. The menaquinone profile contained MK-10(H₄) (49 %), MK-9(H₄) (24 %), MK-10(H₆) (18 %) and MK-9(H₆) (9 %). The predominant fatty acids were iso-C_{15:0} and C_{17:0}. The DNA G + C content was 70.1 mol%. The combination of chemotaxonomic and phylogenetic data clearly separated the isolate from the type strains of all genera in the family *Micromonosporaceae*. On the basis of the phylogenetic and chemotaxonomic data presented in this paper, strain Sco-B14^T is considered to represent a novel species of a new genus in the family *Micromonosporaceae*, for which the name *Allocatelliglobosispora scoriae* gen. nov., sp. nov. is proposed. The type strain of *Allocatelliglobosispora scoriae* is Sco-B14^T (=KCTC 19661^T =DSM 45362^T).

The family *Micromonosporaceae* Krasil'nikov 1938 emend. Koch *et al.* 1996, emend. Stackebrandt *et al.* 1997 is a member of the suborder *Micromonosporineae*, and contained seven genera according to Stackebrandt *et al.* (1997) (*Micromonospora*, *Actinoplanes*, *Dactylosporangium*, *Catellatospora*, *Catenuloplanes*, *Couchioplanes* and *Pilimelia*). Subsequently, the genera *Spirilliplanes*, *Verrucosipora*, *Virgisporangium*, *Asanoa*, *Longispora* and *Salinispora* have been recognized as members of the family *Micromonosporaceae* on the basis of chemotaxonomic characteristics and phylogenetic relationships (Koch *et al.*, 1996; Stackebrandt *et al.*, 1997). Recently, several new genera have been added to the family: *Actinocatenispora* (Thawai *et al.*, 2006), *Polymorphospora* (Tamura *et al.*, 2006), *Luedemannella* (Ara & Kudo, 2007b),

Krasilnikovia (Ara & Kudo, 2007c), *Catelliglobosispora* (Ara *et al.*, 2008a), *Hamadaea* (Ara *et al.*, 2008a), *Pseudosporangium* (Ara *et al.*, 2008b), *Planosporangium* (Wiese *et al.*, 2008), *Rugosimonospora* (Monciardini *et al.*, 2009), *Plantactinospora* (Qin *et al.*, 2009) and *Actinaurispora* (Thawai *et al.*, 2010). *Catelliglobosispora* and *Hamadaea* were erected by Ara *et al.* (2008a) by the reclassification of *Catellatospora koreensis* Lee *et al.* 2000 and *Catellatospora tsunoensis* Asano *et al.* 1989, respectively.

During a study of microbial diversity and bioactive compounds from scoria, a vesicular pyroclastic rock with basaltic composition, strain Sco-B14^T was isolated from volcanic ash collected near Darangshi Oreum (a parasitic volcano or satellite cone) in Jeju, Republic of Korea. A volcanic ash sample (1 g) was ground to a powder with a pestle and suspended in 10 ml distilled water. Aliquots of serial dilutions were spread onto starch-casein agar (1 % soluble starch, 0.03 % casein, 0.2 % KNO₃, 0.2 % NaCl, 0.2 % KH₂PO₄, 0.002 % CaCO₃, 0.005 % MgSO₄·7H₂O, 0.001 % FeSO₄·7H₂O and 1.8 % agar in distilled water,

Abbreviation: DAP, diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain Sco-B14^T is FN386736.

A supplementary table and a supplementary figure are available with the online version of this paper.

pH 7.2) and the plates were incubated at 30 °C for 2 weeks. A single colony was selected and further streaked on ISP medium 2 (Shirling & Gottlieb, 1966). The pure culture was maintained at –20 and –80 °C in 20 % (v/v) glycerol. For cellular fatty acid analyses, *Catelliglobosispora koreensis* LM 042^T and *Hamadaea tsunoensis* IMSNU 22005^T were grown on ISP medium 2 at 30 °C.

Morphological characteristics of strain Sco-B14^T were observed by scanning electron microscopy (model JSM 6700F; JEOL) using a culture grown on ISP medium 3 for 21 days at 30 °C. An agar block with growth was fixed for 1 h with 1 % OsO₄ and dehydrated by using a graded series of ethanol or a mixture of ethanol and isoamyl acetate. After critical-point drying with liquid carbon dioxide, the specimen was coated with platinum before observation. For cultural characterization, strain Sco-B14^T was grown for 21 days at 30 °C on various agar media as described by Nonomura & Ohara (1969), Prauser & Bergholz (1974) and Shirling & Gottlieb (1966).

Strain Sco-B14^T grew well on ISP media 2, 3, 4 and 5 and oatmeal-nitrate agar and showed poor growth on ISP media 6 and 7. The organism formed well-developed, branched substrate mycelium, on which short chains of non-motile spores were arranged singly or in clusters without the formation of aerial mycelium (Fig. 1a). Globose bodies were observed (Fig. 1b). The reverse colour of colonies was light brown to brown. Soluble pigments were brown on ISP medium 3 and oatmeal-nitrate agar.

The ranges of temperature and pH for growth were determined on ISP medium 2 at 4–42 °C and pH 4.1–12.1 (at intervals of 1.0 pH unit). Tolerance of NaCl for growth was tested in the presence of 0–9 % NaCl (at intervals of 1 %). Utilization of carbohydrates as sole carbon sources was tested using ISP medium 9 (Shirling & Gottlieb, 1966) as the basal medium. Carbon sources were filter-sterilized and added to final concentrations of 1 % (for carbohydrates and alcohols) or 0.1 % (for organic acids). Gram staining, oxidase and catalase activities and degradation abilities were determined as described by Lee *et al.* (2008). Results of these physiological tests are given in the species description.

Genomic DNA was extracted and purified as described previously (Hopwood *et al.*, 1985). PCR-mediated amplification and sequencing of the 16S rRNA gene were performed as described by Lee & Lee (2008). The 16S rRNA gene sequence of strain Sco-B14^T was aligned with corresponding sequences obtained from GenBank/EMBL databases using the CLUSTAL_X program (Thompson *et al.*, 1997). Phylogenetic analyses were performed using several programs contained in the PHYLIP suite (Felsenstein, 2008). A phylogenetic tree was constructed by the neighbour-joining method (Saitou & Nei, 1987) from an evolutionary distance matrix calculated with the model of Jukes & Cantor (1969). The tree topology was evaluated by bootstrap analyses based on 1000 resamplings (Felsenstein, 1985).

Fig. 1. Scanning electron micrographs of cells of strain Sco-B14^T grown for 21 days at 30 °C. (a) Spore chains borne singly or in clusters on vegetative mycelium (on ISP medium 3). (b) Globose bodies (on ISP medium 2). Bars, 1 µm.

The partial 16S rRNA gene sequence (1424 nt) of strain Sco-B14^T determined in this study was compared with those of members of the family *Micromonosporaceae*. A phylogenetic tree based on 16S rRNA gene sequences indicated that strain Sco-B14^T belonged to the family *Micromonosporaceae* and formed a distinct phyletic line, remotely related to the genera *Catellatospora*, *Catelliglobosispora* and *Hamadaea* (Fig. 2). This relationship was supported by a bootstrap value of 70 % and was also found in trees obtained by using the maximum-likelihood and maximum-parsimony algorithms. The 16S rRNA gene sequence identity of strain Sco-B14^T to members of related genera of the family *Micromonosporaceae* was 95.0–95.7 % to *Catellatospora*, 94.7 % to *Hamadaea*, 94.7 % to *Longispora*, 94.4 % to *Plantactinospira*, 94.3 % to *Actinaurispora*, 94.2 % to *Polymorphospira*, 94.1–94.2 % to *Rugosimonospora*, 94.0 % to *Catelliglobosispora* and 94.0 % to *Luedemannella*. Levels of 16S rRNA gene sequence similarity between strain Sco-B14^T and members of other genera of the family *Micromonosporaceae* were less than 94.0 %.

Table 1. Characteristics that differentiate strain Sco-B14^T from other genera of the family *Micromonosporaceae*

Data for reference genera were taken from Couch (1950), Thiemann *et al.* (1967), Sharples & Williams (1974), Vobis (1989), Stackebrandt & Kroppenstedt (1987), Horan & Brodsky (1986), Asano & Kawamoto (1986, 1988), Asano *et al.* (1989), Goodfellow *et al.* (1990), Yokota *et al.* (1993), Kane (1966), Tamura & Hatano (2001), Tamura *et al.* (1994, 1997, 2001, 2006), Rheims *et al.* (1998), Kudo *et al.* (1999), Lee *et al.* (1999, 2000), Lee & Hah (2002), Matsumoto *et al.* (2003), Maldonado *et al.* (2005), Ara & Kudo (2006, 2007a, b, c), Thawai *et al.* (2006, 2010), Ara *et al.* (2008a, b), Wiese *et al.* (2008), Monciardini *et al.* (2009) and Qin *et al.* (2009). +, Present; –, absent; ND, not described; *m*-DAP, *meso*-diaminopimelic acid. Fatty acid types are given according to the classification of Kroppenstedt (1985) and phospholipid types according to the classification of Lechevalier *et al.* (1981).

Taxon	Sporangia	Spore motility	Diamino acid(s)	Whole-cell sugar(s)*	Fatty-acid type	Major menaquinone(s)	Phospholipid type	DNA G + C content (mol%)
Sco-B14 ^T	–	–	3-OH DAP	Glc, Rhm, Rib, Xyl, Ara, Gal, Man	3b	10(H ₄), 9(H ₄), 10(H ₆)	PII	70.1
<i>Actinaurispora</i>	–	–	<i>m</i> -DAP	Glc, Xyl, Man, Gal	3b	9(H ₆), 10(H ₆), 9(H ₈), 10(H ₈)	PII	72.6
<i>Actinocatenispora</i>	–	–	<i>m</i> -DAP	Ara, Gal, Xyl	3b	9(H ₄), 9(H ₆)	PII	72
<i>Actinoplanes</i>	+	+	<i>m</i> -DAP	Ara, Xyl	2d	9(H ₄), 10(H ₄)	PII	72–73
<i>Asanoa</i>	–	–	<i>m</i> -DAP	Ara, Gal, Xyl	2d	10(H ₆), 10(H ₈)	PII	71–72
<i>Catellatospora</i>	–	–	<i>m</i> -DAP, 3-OH DAP	Xyl, Man, Gal, Ara, Rhm, Rib, Glc	3b	9(H ₄)	PII	70–71
<i>Catelliglobospora</i>	–	–	<i>m</i> -DAP	Rhm, Man, Xyl, Gal, Glc	3b	10(H ₄)	PII	70.4
<i>Catenuloplanes</i>	–	+	L-Lys	Xyl	2c	9(H ₈), 10(H ₈)	PIII	71–73
<i>Couchioplanes</i>	–	+	L-Lys	Ara, Gal, Xyl	2c	9(H ₄)	PII	70–72
<i>Dactylosporangium</i>	+	+	<i>m</i> -DAP	Ara, Xyl	3b	9(H ₄), 9(H ₆), 9(H ₈)	PII	71–73
<i>Hamadaea</i>	–	–	<i>m</i> -DAP, 3-OH DAP	Xyl, Gal, Man, Rib, Ara, Rhm, Glc	3b	9(H ₆)	PII	70
<i>Krasilnikovia</i>	–	–	<i>m</i> -DAP	Gal, Man, Xyl, Ara, Rib	2d	9(H ₆), 9(H ₄), 9(H ₈)	PII	71
<i>Longispora</i>	–	–	<i>m</i> -DAP	Ara, Gal, Xyl	2d	10(H ₄), 10(H ₆)	PII	70
<i>Luedemannella</i>	+	–	<i>m</i> -DAP	Xyl, Gal, Man, Rhm, Rib, Ara	2d	9(H ₆), 9(H ₄), 9(H ₂), 9(H ₈)	PII	71
<i>Micromonospora</i>	–	–	<i>m</i> -DAP	Ara, Xyl	3b	10(H ₄), 10(H ₆), 9(H ₄), 9(H ₆)	PII	71–72
<i>Pilimelia</i>	+	+	<i>m</i> -DAP	Ara, Xyl	2d	9(H ₄), 9(H ₂)	PII	ND
<i>Planosporangium</i>	+	+ / –	<i>m</i> -DAP	Ara, Xyl	3b	9(H ₄), 10(H ₄)	PII	71.4
<i>Plantactinospora</i>	–	–	<i>m</i> -DAP	Ara, Gal, Xyl	2d	10(H ₆), 10(H ₈), 10(H ₄)	PII	69.7
<i>Polymorphospora</i>	–	–	<i>m</i> -DAP	Xyl	2a	10(H ₆), 10(H ₄), 9(H ₆), 9(H ₄)	PII	71
<i>Pseudosporangium</i>	–	–	<i>m</i> -DAP, 3-OH DAP	Ara, Gal, Glc, Man, Rib, Xyl	2d	9(H ₆)	PII	73.6
<i>Rugosimonospora</i>	+	–	3-OH DAP	Gal, Ara, Xyl	2d	9(H ₈)	PII	72–73
<i>Salinispora</i>	–	–	<i>m</i> -DAP	Ara, Gal, Xyl	3a	9(H ₄)	PII	70–73
<i>Spirilliplanes</i>	–	+	<i>m</i> -DAP	Ara, Xyl	2d	10(H ₄)	PII	69
<i>Verrucosipora</i>	–	–	<i>m</i> -DAP	Man, Xyl	2b	9(H ₄)	PII	70
<i>Virgisporangium</i>	+	–	<i>m</i> -DAP	Ara, Gal, Xyl	2d	10(H ₄), 10(H ₆), 10(H ₈)	PII	71

*Ara, Arabinose; Gal, galactose; Glc, glucose; Man, mannose; Rhm, rhamnose; Rib, ribose; Xyl, xylose.

DAP and 3-OH DAP as the diagnostic diamino acids in the peptidoglycan and *Catelliglobospora koreensis* contains *meso*-DAP, in contrast to strain Sco-B14^T, which contains 3-OH DAP as the only diamino acid. 3-OH DAP is also found in the cell-wall peptidoglycan of members of the genera *Pseudosporangium* and *Rugosimonospora*, but they differ from strain Sco-B14^T in their menaquinone profiles (Table 1). Differential morphological and chemotaxonomic properties between strain Sco-B14^T and genera of the family *Micromonosporaceae* are given in Table 1.

On the basis of the phylogenetic, chemotaxonomic and morphological data presented here, strain Sco-B14^T is considered to represent a novel species of a new genus of the family *Micromonosporaceae*, for which the name *Allocatelliglobospora scoriae* gen. nov., sp. nov. is proposed.

Description of *Allocatelliglobospora* gen. nov.

Allocatelliglobospora (Al.lo.ca.tel'li.glo.bo.si.spo'ra. Gr. adj. *allo* another, the other; N.L. fem. n. *Catelliglobospora* a bacterial generic name; N.L. fem. n. *Allocatelliglobospora* the other *Catelliglobospora*, an organism that is phylogenetically close to *Catelliglobospora* but chemotaxonomically distinct).

Aerobic, oxidase-negative, catalase-positive and Gram-stain-positive. Substrate mycelium is well-developed, branched and light brown to brown in colour. Short chains of non-motile spores are borne singly or in clusters from the vegetative mycelium without the formation of aerial mycelium. Globose bodies are observed. 3-OH DAP is the diagnostic diamino acid in the cell-wall peptidoglycan. Whole-cell sugars are glucose, rhamnose, ribose, xylose, arabinose, galactose and mannose. The polar lipids include diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. The major menaquinone is MK-10(H₄). The predominant cellular fatty acids are iso-C_{15:0} and C_{17:0}. The DNA G + C content of the type strain of the type species is 70.1 mol%. Phylogenetically, the genus belongs to the family *Micromonosporaceae*. The type species is *Allocatelliglobospora scoriae*.

Description of *Allocatelliglobospora scoriae* sp. nov.

Allocatelliglobospora scoriae (sco.ri'ae. N.L. gen. n. *scoriae* of scoria, a type of volcanic ash, referring to the site at which the type strain was isolated).

The morphological and chemotaxonomic characteristics are the same as those given in the genus description. Grows well on ISP media 2, 3, 4 and 5 and oatmeal-nitrate agar; poor growth on ISP media 6 and 7. Soluble pigments are brown and are produced only on ISP medium 3 and oatmeal-nitrate agar. Growth occurs at pH 7.1–8.1 and 20–30 °C. Optimal temperature and pH for growth are 30 °C and pH 7.1. No growth in the presence of 1 % NaCl. Nitrate is not reduced to nitrite. H₂S production is not observed. Aesculin and starch are degraded but CM-

cellulose, casein, DNA, hypoxanthine, tyrosine and xanthine are not. Acetate, L-arabinose, citrate, raffinose, DL-tartrate and D-xylitol are utilized as sole carbon and energy sources. Adonitol, D-arabinose, benzoate, cellobiose, dextran, dulcitol, *meso*-erythritol, formate, D-fructose, D-galactose, D-glucose, glycerol, *myo*-inositol, lactose, malate, maltose, D-mannitol, D-mannose, melezitose, melibiose, methyl α -D-glucoside, methyl α -D-mannoside, L-rhamnose, L-ribose, salicin, D-sorbitol, L-sorbose, succinate, sucrose, trehalose and D-xylose are not utilized.

The type strain, Sco-B14^T (=KCTC 19661^T =DSM 45362^T), was isolated from volcanic ash collected in Jeju, Republic of Korea.

Acknowledgements

This work was supported by the 21C Frontier Microbial Genomics and Application Center Program, Ministry of Science & Technology, Republic of Korea.

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