Actinocorallia cavernae sp. nov., isolated from a natural cave in Jeju, Korea

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A novel actinomycete, strain N3-7^T, was isolated from a natural cave in Jeju, Republic of Korea, using a dilution method and was subjected to characterization using polyphasic taxonomy. A 16S rRNA gene sequence analysis revealed that the organism belonged to the phylogenetic cluster of the genus *Actinocorallia* and was most closely related to *Actinocorallia glomerata* and *Actinocorallia longicatena* (97.6 and 97.5 % similarity, respectively). The main chemotaxonomic properties of strain N3-7^T, such as the principal amino acid of the peptidoglycan, the predominant menaquinone and the polar lipid profile, supported classification in the genus *Actinocorallia*. The organism was readily differentiated from *Actinocorallia* species with validly published names on the basis of a broad range of phenotypic properties. Thus the isolate represents a novel species of the genus *Actinocorallia*, for which the name *Actinocorallia cavernae* sp. nov. is proposed. The type strain is strain N3-7^T (=JCM 13278^T=NRRL B-24429^T).

The genus *Actinocorallia*, which was originally proposed by Inuma *et al.* (1994), was recently emended on the basis of a combination of phylogenetic analyses and major chemotaxonomic properties (Zhang *et al.*, 2001) and currently contains five species with validly published names: *Actinocorallia aurantiaca*, *Actinocorallia glomerata*, *Actinocorallia herbida*, *Actinocorallia libanotica* and *Actinocorallia longicatena*. All of the *Actinocorallia* species with validly published names, with the exception of the type species, *A. herbida*, were transferred from the genus *Actinomadura* on the basis of 16S rRNA gene sequence studies and chemotaxonomic characterization (Kroppenstedt *et al.*, 1990; Itoh *et al.*, 1995; Zhang *et al.*, 1998, 2001).

During investigations of the biodiversity of cave bacteria, a novel actinomycete, strain N3-7^T, was isolated from a soil sample from a natural cave on Jeju Island (Republic of Korea) using starch/casein agar and was subjected to polyphasic characterization to unravel the taxonomic status. A soil sample (1 g) was suspended in 9 ml sterile distilled water and mixed in a tube rotator for 30 min at moderate speed. Aliquots (100 µl) of the serial diluent of the samples were transferred onto the starch/casein agar plates. The isolate was maintained as spores or mycelial fragments in a 20 % (v/v) glycerol suspension at –70 °C.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain N3-7^T is AY966427.

A complete neighbour-joining tree showing the relationships between strain N3-7^T and representatives of the genus *Actinocorallia* is available as a supplementary figure in IJSEM Online.

Cultural and morphological characteristics were observed in the organism grown on ISP 2, ISP 3 and ISP 4 agar media (Shirling & Gottlieb, 1966) for 2–3 weeks at 28 °C. For electron microscopy, the organism was grown on oatmeal agar for 14 days at 28 °C. Agar blocks containing growth were fixed with 1 % osmium tetroxide, dehydrated through a graded series of ethanol and isoamyl acetate and then critical point-dried. The gold-coated specimen was observed using a scanning electron microscope (model JSM 5410LV; JEOL). Strain N3-7^T showed good growth on ISP 2 and ISP 3 media, and moderate growth on ISP 4 medium. The organism was characterized by the formation of smooth spores that were arranged in straight or flexuous chains on aerial mycelium. Colonial pigmentation is reported in the species description and in Table 1. The colour of the substrate mycelium can be used as a cultural marker distinguishing the isolate from other *Actinocorallia* species.

For genotypic characterization, the organism was cultivated on YMG broth (yeast extract, 0.4 %; malt extract, 1 %; glucose, 0.4 %; pH 7.0) for 3 days at 30 °C with shaking. The chromosomal DNA was extracted using a method described previously (Hopwood *et al.*, 1985). The 16S rRNA gene of strain N3-7^T was obtained by PCR amplification as described previously (Lee *et al.*, 2000a) and then purified by using Wizard PCR Preps (DNA Purification System; Promega). It was directly sequenced using an ABI PRISM BigDye Terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 3730xl; Applied Biosystems). The sequence determined in this study was aligned with available reference sequences by using the CLUSTAL X program (Thompson *et al.*, 1997) and was manually optimized by comparison with the secondary

Table 1. Characteristics that differentiate strain N3-7^T from other representatives of the genus *Actinocorallia*

Strains: 1, N3-7^T; 2, *A. aurantiaca* INA 1933^T; 3, *A. glomerata* JCM 9376^T; 4, *A. herbida* IFO 15485^T; 5, *A. libanotica* IMET 9616^T; 6, *A. longicatena* JCM 9377^T. Data for the reference strains are from Iinuma *et al.* (1994), Itoh *et al.* (1995), Lavrova & Preobrazhenskaya (1975), Meyer (1979) and Zhang *et al.* (2001). +, Positive; –, negative; ND, not determined; v, variable.

Characteristic	1	2	3	4	5	6
Morphology						
Spore-chain arrangement	Straight or flexuous	Hooks, spirals	Pseudosporangium	Synnemata	Hooks	Straight or flexuous
Spore-surface ornamentation	Smooth	Warty	Smooth	Smooth	Folded, warty	Smooth
Growth on ISP 2 medium						
Degree of growth	Good	Poor	Good	Good	Good	Good
Aerial mycelium	Sparse	White	White	White to pale yellow	Pink	White
Substrate mycelium	Olive black	Yellow–brown	Dull gold	Ivory to yellow–brown	Yellow–brown	Oak brown
Diffusible pigment	None	None	None	None	None	None
Nitrate reductase	–	+	ND	–	+	ND
Degradation of:						
Aesculin	+	–	ND	ND	+	ND
Casein	+	–	+	–	–	+
DNA	–	v	ND	ND	–	ND
Gelatin	+	+	–	–	–	–
Hypoxanthine	+	–	+	ND	–	+
Starch	+	–	+	+	+	+
Tyrosine	+	–	–	ND	–	–
Xanthine	–	–	–	ND	–	–
Tolerance of 1 % NaCl	–	ND	+	+	ND	+
Madurose in whole-cell sugars	–	+	+	+	+	+
DNA G + C content (mol%)	70.1	ND	71.7	73	66.2	70.9
Source of isolation	Cave	Soil	Decayed leaf	Soil	Soil	Plant root

structure of an *Escherichia coli* sequence (Brosius *et al.*, 1978). Evolutionary distances were computed as previously described (Jukes & Cantor, 1969). A phylogenetic tree was constructed using the neighbour-joining method (Saitou & Nei, 1987). The reliability of the tree topology was evaluated by bootstrap analysis (Felsenstein, 1985). The 16S rRNA gene sequence of strain N3-7^T was a continuous stretch of 1407 nt. A total of 1337 unambiguous aligned positions present in all strains between 57 and 1471 (*E. coli* positions) were used for tree reconstruction. A phylogenetic tree (Fig. 1) based on 16S rRNA gene sequence studies revealed that the organism formed a separate line of descent in the phylogenetic cluster of the genus *Actinocorallia*. Sequence similarity calculations after neighbour-joining analysis showed that the strain was closest to *A. glomerata* and *A. longicatena* (97.6 and 97.5 % sequence similarity, respectively). The 16S rRNA gene sequence similarity values between the isolate and other members of the genus *Actinocorallia* were lower (97.0–97.2 %).

The DNA G + C content, determined by HPLC according to the method of Mesbah *et al.* (1989), was 70.1 mol%.

Analyses of diaminopimelic acid isomers and the acyl type of the muramic acid were carried out using methods described previously (Staneck & Roberts, 1974; Uchida & Aida, 1984).

Fig. 1. Abbreviated neighbour-joining tree based on 16S rRNA gene sequences showing the relationships between strain N3-7^T and representatives of the genus *Actinocorallia*. (The full version of this tree is available as Supplementary Fig. S1 in IJSEM Online.) The tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). The bootstrap values indicated at the branching points are expressed as percentages of 1000 replications (only values greater than 50 % are shown). Bar, 1 substitution per 100 nt.

Whole-cell sugar compositions were analysed by GC, as described previously (Saddler *et al.*, 1991). Phospholipid profiles were investigated using the method of Minnikin *et al.* (1977). Menaquinones were analysed by HPLC as described previously (Kroppenstedt, 1985). Analysis of the mycolic acids was performed using the method of Minnikin *et al.* (1980). Cellular fatty acid compositions were analysed as described previously (Lee *et al.*, 2000b) with an Agilent 6850 gas chromatograph. The organism was cultivated on trypticase soy broth (Difco) for 3 days at 30 °C with shaking. The chemotaxonomic properties determined supported the assignment of strain N3-7^T to the genus *Actinocorallia*. The results of chemotaxonomic analyses are given in the species description. The whole-cell sugar profiles obtained using GC comprised glucose, mannose and a minor amount of galactose (but not xylose, arabinose or madurose) as the characteristic sugars, indicating that the organism has sugar pattern type C (Lechevalier & Lechevalier, 1970). All of the type strains of the five previously recognized *Actinocorallia* species contain madurose as a characteristic sugar (sugar pattern type B) (Zhang *et al.*, 2001), though *A. herbida* was originally described as having no characteristic sugars (Iinuma *et al.*, 1994). Thus the whole-cell sugar composition can be used as a diagnostic characteristic for distinguishing strain N3-7^T from other members of the genus *Actinocorallia*. The fatty acids were a mixture of saturated, unsaturated and branched-chain acids, with a predominance of C_{16:0} (25.9%), C_{18:1} (19.2%), C_{17:0} (11.7%), 10-methyl C_{18:0} (tuberculostearic acid, 9.4%), ai-C_{17:0} (7.4%) and C_{18:0} (6.7%) acids. The other components (occurring in small amounts) were C_{14:0} (1.5%), C_{15:0} (3.7%), i-C_{16:0} (3.0%), C_{16:1} (3.5%), ai-C_{19:0} (1.8%) and 10-methyl C_{17:0} (2.9%) acids.

Carbohydrate utilization was tested using ISP 9 medium (Shirling & Gottlieb, 1966) that included each filter-sterilized compound at a final concentration of 1% (w/v). Urease activity was determined by a colour change in Bacto urea broth (Difco). The production of H₂S was tested on peptone iron agar (Difco). Nitrate reduction, gelatin liquefaction and degradation of elastin and starch were examined by using previously described methods (MacFaddin, 1980). Decomposition of adenine, hypoxanthine, casein, DL-tyrosine and xanthine was examined by using the methods of Gordon *et al.* (1974). Tolerance of NaCl was determined on ISP 2 medium at NaCl concentrations in the range 0–7% (w/v). The temperature range for growth was tested on yeast extract/malt extract agar at 10–45 °C; the pH range for growth was determined on yeast extract/malt extract agar adjusted to pH 4.1–10.1 at intervals of 1.0 pH unit. The results of the physiological characterization are given in the species description and in Table 1.

Strain N3-7^T can be readily distinguished from other *Actinocorallia* species by a range of phenotypic properties (Table 1). It is evident from the phenotypic and genotypic data that strain N3-7^T is a novel member of the genus *Actinocorallia*.

The name *Actinocorallia cavernae* sp. nov. is proposed, with strain N3-7^T as the type strain.

Description of *Actinocorallia cavernae* sp. nov.

Actinocorallia cavernae (ca'ver.nae. L. gen. n. *cavernae*, of a cavern, the site from which the type strain was isolated).

Gram-positive. Forms a well-developed, branched, non-fragmenting vegetative mycelium. Aerial mycelium is sparse on ISP 2 and ISP 4 media but abundant and greyish white in colour on ISP 3 medium. Substrate mycelium is olive black in colour on ISP 2 medium, dark yellowish brown on ISP 3 medium and cream on ISP 4 medium. No diffusible pigments are produced. Smooth spores are formed in the aerial mycelium and form straight to flexuous chains. The optimum temperature range for growth is 25–30 °C. Growth does not occur at 10 or 37 °C. The pH range for growth is 5.1–10.1. Growth does not occur at pH 4.1. Degradation of hypoxanthine and DL-tyrosine is observed but not that of elastin and xanthine. Aesculin, casein, starch and urea are hydrolysed, but DNA is not. Gelatin liquefaction is observed. H₂S is not produced. Nitrate is not reduced to nitrite. Growth occurs in the absence of NaCl but not on 1–7% NaCl. L-Arabinose, D-arabinose, D-cellobiose, D-fructose, D-galactose, D-glucose, D-lactose, maltose, D-mannose, D-melezitose, L-rhamnose, D-ribose, salicin, sucrose, D-trehalose and D-xylose are utilized as sole carbon sources, but inulin, melibiose, methyl D-glucoside, methyl D-mannoside, D-raffinose, L-sorbose, adonitol, 2,3-butanediol, dulcitol, *meso*-erythritol, glycerol, *myo*-inositol, D-mannitol, 1,2-propanediol, D-sorbitol and D-xylitol are not. The cell wall is of type III/C (*meso*-diaminopimelic acid and no diagnostic sugar in the cell wall). The *N*-acyl type of the muramic acid is acetyl. The major menaquinones are MK-9(H₄) and MK-9(H₆). The phospholipid pattern is of type PII (phosphatidylethanolamine, diphosphatidylglycerol and phosphatidylinositol). Mycolic acids are not present. The predominant cellular fatty acids are C_{16:0} (25.9%), C_{18:1} (19.2%), C_{17:0} (11.7%), 10-methyl C_{18:0} (tuberculostearic acid, 9.4%), ai-C_{17:0} (7.4%) and C_{18:0} (6.7%). The DNA G + C content is 70.1 mol%.

The type strain, strain N3-7^T (=JCM 13278^T=NRRL B-24429^T), was isolated from soil inside a natural cave on Jeju Island, Republic of Korea.

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