Classification of '*Sarracenospora aurea*' Furihata *et al.* 1989 as *Actinocorallia aurea* sp. nov.

Tomohiko Tamura, Kazunori Hatano and Ken-ichiro Suzuki

Correspondence

Tomohiko Tamura

tamura-tomohiko@nite.go.jp

NITE Biological Resource Center (NBRC), National Institute of Technology and Evaluation,
2-5-8 Kazusakamatari, Kisarazu, Chiba 292-0818, Japan

Phylogenetic analysis of '*Sarracenospora aurea*' NBRC 14752 and strain NBRC 15120, based on 16S rRNA gene sequences, revealed that these organisms are related to members of the genus *Actinocorallia*. These organisms contained glutamic acid, alanine and meso-diaminopimelic acid as cell-wall amino acids and the menaquinones MK-9(H₄), MK-9(H₆) and MK-9(H₈). The chemotaxonomic characteristics of the strains were consistent with those of the genus *Actinocorallia*. However, DNA–DNA hybridization and phenotypic characteristics revealed that the strains differed from the recognized species of the genus *Actinocorallia*. Therefore, we propose that '*Sarracenospora aurea*' NBRC 14752 and strain NBRC 15120 be reclassified in the genus *Actinocorallia* as a novel species, *Actinocorallia aurea* sp. nov. (type strain NBRC 14752^T=DSM 44434^T).

The genus *Actinocorallia*, with a single species *Actinocorallia herbida*, was proposed by Iinuma *et al.* (1994). Subsequently, Zhang *et al.* (2001) enlarged the monotypic genus by reclassifying four species of the genus *Actinomadura* and emending the genus description. At the time of writing, the genus comprises six species with validly published names, including the recently described species *Actinocorallia cavernae* (Lee, 2006).

During a taxonomic study of strains assigned to genera with non-validly published names, it was revealed that two strains, NBRC 14752 and NBRC 15120, described as '*Sarracenospora aurea*' (Furihata *et al.*, 1989) formed a phylogenetic cluster with the members of the genus *Actinocorallia*. In this paper, we describe the results of polyphasic taxonomic studies of the two strains and propose to assign them to a novel species of the genus *Actinocorallia*.

PCR-amplification and sequencing of the 16S rRNA gene and phylogenetic analysis were performed as described previously (Tamura & Hatano, 2001). Strains NBRC 14752 and NBRC 15120 had identical 16S rRNA gene sequences and formed a monophyletic cluster with members of the genus *Actinocorallia* (Fig. 1). 16S rRNA gene sequence similarity calculations after a neighbour-joining analysis

indicated that the closest relatives of both strains were *Actinocorallia herbida* (98.9% gene sequence similarity), *Actinocorallia aurantiaca* (98.6%), *Actinocorallia glomerata* (98.3%), *Actinocorallia longicatena* (98.1%) and *Actinocorallia libanotica* (98.1%). Lower sequence similarities were found with *Actinocorallia cavernae* (97.3%).

Analyses of whole-cell sugar patterns, cell-wall amino acids, menaquinones, cellular fatty acids, the acyl type of the peptidoglycan, mycolic acids and DNA G + C content were performed as described previously (Tamura *et al.*, 1994). Freeze-dried cells for chemotaxonomic analyses were obtained from a culture grown in yeast extract-glucose broth (1.0% yeast extract; 1.0% D-glucose; pH 7.0) on a rotary shaker at 28 °C. The major menaquinones were MK-9(H₄), MK-9(H₆) and MK-9(H₈) and a small amount of MK-9(H₂) was also present. The organisms have meso-diaminopimelic acid (meso-DAP), glutamic acid and alanine as cell-wall amino acids and glucose, galactose and small amounts of madurose and xylose as the whole-cell sugars (wall chemotype/sugar pattern III/B *sensu* Lechevalier & Lechevalier, 1970). The data differed from those reported by Furihata *et al.* (1989), in which the presence of arabinose and a trace of glycine were revealed and the cell-wall chemotype was designated as II/D. The diagnostic phospholipids phosphatidylethanolamine and phosphatidylglycerol were detected in strains NBRC 14752 and NBRC 15120, but phosphatidylcholine and some other phospholipids, including glucosamine, were not detected (phospholipid type PII *sensu* Lechevalier *et al.*, 1977). The DNA G + C contents of strains NBRC 14752 and NBRC 15120 were 71.6 and 72.2 mol%, respectively. Mycolic acids were not detected. The acyl type of the muramic acid was acetyl. The fatty acid profile of strains NBRC 14752

Abbreviation: ISP, International Streptomyces Project.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences of *Actinocorallia aurea* sp. nov. NBRC 14752^T and strain NBRC 15120 are AB006177 and AB006160, respectively.

A table detailing the cellular fatty acid contents of *Actinocorallia aurea* sp. nov. NBRC 14752^T and selected members of the genus *Actinocorallia*, and a scanning electron micrograph of cells of the strain are available with the online version of this paper.

Fig. 1. Phylogenetic tree derived from 16S rRNA gene sequences of strains NBRC 14752^T and NBRC 15120 and species of the genus *Actinocorallia*. The tree was constructed by using the neighbour-joining method (Saitou & Nei, 1987). The numbers on the branches are confidence limits (expressed as percentages) estimated from a bootstrap analysis with 1000 replicates. Bar, 0.005 K_{nuc} in nucleotide sequences.

and NBRC 15120 (see Supplementary Table S1 in IJSEM Online) contained C_{16:0} (21 % and 22 %, respectively), C_{18:1} (19 % and 18 %), 10-methyl C_{18:0} (10 % and 14 %), C_{17:1} (13 % and 9 %), C_{16:1} (8 % and 12 %), 10-methyl C_{16:0} (4 % and 10 %), 10-methyl C_{17:0} (7 % and 5 %) and iso-C_{16:0} (6 % and 5 %) (fatty acid type 3a *sensu* Kroppenstedt, 1985). No significant differences in the fatty acid profiles were found between strains NBRC 14752 and NBRC 15120 and *A. herbida*.

Morphological characteristics were observed by using scanning electron microscopy as described previously (Tamura *et al.*, 2000). Strains NBRC 14752 and NBRC 15120 formed a straight to flexuous spore chain on the tip of coralloid sporophore as seen in *A. herbida* (Inuma *et al.*, 1994). A structure of the sheath, similar in shape to a North American pitcher plant of the genus *Sarracenia*, was occasionally observed at the tip of the spore chains as previously reported by Furihata *et al.* (1989) (see

Table 1. Differential characteristics of strains NBRC 14752^T, NBRC 15120 and type strains of closely related species of the genus *Actinocorallia*

Strains: 1, *A. aurea* sp. nov. NBRC 14752^T and strain NBRC 15120; 2, *A. herbida* (data from this study); 3, *A. aurantiaca*; 4, *A. libanotica* [data from Goodfellow (1989), Trujillo & Goodfellow (2003) and this study]. +, Positive; –, negative; ND, no data. The colour codes in parentheses correspond to the colour codes in *A Mycological Colour Chart* (Rayner, 1970). All strains utilize D-glucose, L-rhamnose and D-xylose but not D-fructose, myo-inositol, lactose, D-mannitol, glycerol, D-mannose, melibiose, raffinose or D-sorbitol.

Characteristic	1	2	3	4
Culture				
Yeast extract-starch agar:				
Vegetative mycelium	Colourless to light yellow (11)	Moderate to strong yellow (47)	Colourless to light yellow (11)	Pale yellow (45)
Aerial spore mass	Pale greenish yellow (46)	Pale greenish yellow (46)	White	White
Maltose-Bennett's agar:				
Vegetative mycelium	Moderate yellow (64) to pale yellow (45)	Moderate to strong yellow (47)	Colourless to light yellow (11)	Moderate yellow (64)
Aerial spore mass	Pale greenish yellow (46)	None	White	White
1/4 Yeast extract-malt extract agar:				
Vegetative mycelium	Moderate yellow (64) to pale yellow (45)	Moderate to strong yellow (47)	Colourless to light yellow (11)	Moderate yellow (64)
Aerial spore mass	Pale greenish yellow (46)	Pale greenish yellow (46)	White to pinkish white	White
Tyrosine agar (ISP medium 7):				
Diffusible pigment	None	Pale brown	None	None
Spore chain morphology	Straight to flexuous	Straight to flexuous	Hook to spiral of 1–2 turns	Hook or curled
Physiological				
Liquefaction of gelatin	+	–	+	+
Hydrolysis of starch	+	+	–	+
Utilization of:				
L-Arabinose	+	+	–	+
Maltose	+	+	–	+
Sucrose	–	–	–	+
Growth at 37 °C	+	–	–	ND

Supplementary Fig. S1 in IJSEM Online). The tip of the sheath was similar in appearance to the operculum of a pitcher plant and the spore chain resembled the pitcher tube.

Determination of the cultural and physiological characteristics was performed as described previously (Gordon *et al.*, 1974; Seino *et al.*, 1985; Shirling & Gottlieb, 1966; Yokota *et al.*, 1993). Detailed results are provided in the species description and in Table 1. The colour of the aerial spore mass of strains NBRC 14752 and NBRC 15120 was nearly identical to that of *A. herbida*, but the colour of the vegetative mycelium was different. The two strains did not produce a soluble pigment on tyrosine agar (ISP medium 7). Gelatin liquefaction and growth were observed at 37 °C (Table 1).

The microplate-hybridization method developed by Ezaki *et al.* (1988, 1989) was applied to determine DNA–DNA relatedness. The DNA–DNA relatedness of strain NBRC 14752 with strain NBRC 15120 was 60 and 67 % (reciprocal values), 27 % and 34 % with *A. herbida* NBRC 15485^T, and less than 18 % with *A. aurantiaca* NBRC 16147^T, *A. glomerata* NBRC 15960^T and *A. longicatena* NBRC 15961^T. The two strains are distinguishable from the other members of the genus *Actinocorallia* by several phenotypic properties as shown in Table 1. Therefore, on the basis of morphological, physiological and chemotaxonomic data, DNA–DNA hybridization and phylogenetic distinctiveness, '*Sarracenospora aurea*' NBRC 14752 and strain NBRC 15120 should be placed in the genus *Actinocorallia* as members of a novel species, for which the name *Actinocorallia aurea* sp. nov. is proposed.

Description of *Actinocorallia aurea* sp. nov.

Actinocorallia aurea (au're.a. L. fem. adj. *aurea* golden).

The description is based on the data obtained in this study and also reported by Furihata *et al.* (1989). Aerobic, Gram-positive, non-acid-fast actinomycete that produces branching, non-fragmented vegetative hyphae. Straight to flexuous chains of aerial spores are formed on the tip of coralloid sporophores. Spores are short, non-motile rods (0.6–0.9 × 1.0–1.5 µm). Vegetative mycelium is yellow on maltose-Bennett's agar, yeast extract-malt-extract agar (ISP medium 2), inorganic salts-starch agar (ISP medium 4), glycerol-asparagine agar (ISP medium 5) and peptone-yeast extract-iron agar (ISP medium 6) and greyish white on oatmeal (ISP medium 3) and tyrosine agars (ISP medium 7). The aerial spore mass is yellow on yeast extract-starch agar, maltose-Bennett's agar, 1/4 strength ISP medium 2 and ISP medium 3. Tests for starch hydrolysis, nitrate reduction and gelatin liquefaction are positive. Does not produce a soluble pigment on ISP medium 7. Starch is weakly hydrolysed. Calcium malate is not dissolved. Tests for the coagulation and peptonization of milk give negative results. Optimum temperature for growth is 20–30 °C. Grows at 37 °C, but does not grow at 45 °C. Does not grow on 4 % NaCl. D-Glucose, L-rhamnose, D-xylose and maltose are utilized. The cell-wall peptidoglycan contains *meso*-DAP as the diagnostic

diamino acid and the whole-cell sugars contain madurose as the diagnostic sugar, indicating that the cell-wall chemotype is III/B. The major fatty acids are C_{16:0}, C_{18:1}, 10-methyl C_{18:0} and C_{17:1}. The major menaquinones are MK-9(H₄), MK-9(H₆) and MK-9(H₈). The major polar lipids are phosphatidylglycerol and phosphatidylethanolamine. The DNA G+C content is 71–73 mol% (determined by HPLC).

The type strain, NBRC 14752^T (=DSM 44434^T), was isolated from soil.

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