Full Paper

Schumannella luteola* gen. nov., sp. nov., a novel genus of the family *Microbacteriaceae

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An actinobacterial strain KHIA^T, isolated from lichen in Tokyo, was taxonomically characterized using a polyphasic approach. The isolate is a Gram-positive, anaerobic, non-motile and rod-shaped bacterium. Phylogenetic analyses based on 16S rRNA gene sequences revealed that the isolate is represented as an independent lineage distinctive from the *Microbacteriaceae* genera. The G+C content of DNA was 58.7 mol%. The chemotaxonomic characteristics of the isolate are cell wall peptidoglycan type (2,4-diaminobutyric acid), major cellular fatty acids (anteiso-C_{15:0} and iso-C_{16:0}) and quinone type (MK-11 and MK-10). On the basis of the phenotypic and phylogenetic distinctness, it is proposed that strain KHIA^T represents a novel species in a new genus of the family *Microbacteriaceae*, *Schumannella luteola* gen. nov., sp. nov. The type strain is KHIA^T (=JCM 23215^T=TISTR 1824^T).

Key Words—*Schumannella luteola* gen. nov., sp. nov.

Introduction

The family *Microbacteriaceae* currently contains 24 genera, which are characterized by unsaturated major menaquinones (Collins and Jones, 1981) and B-type peptidoglycan (Park et al., 1993; Schleifer and Kandler, 1972; Stackebrandt et al., 1997). The presence of 2,4-diaminobutyric acid in the cell wall (B-type peptidoglycan) is characteristic of species in genera *Agreia* (Groth et al., 1996), *Agrococcus* (Groth et al., 1996), *Clavibacter* (Davis et al., 1984), *Cryobacterium* (Suzuki et al., 1997), *Leifsonia* (Evtushenko et al., 2000), *Leucobacter* (Takeuchi et al., 1996), *Plantibacter* (Behrendt et al., 2002), *Rathayibacter* (Zgurskaya et al., 1993), and *Subtercola* (Männistö et al., 2000) of family *Microbacteriaceae*.

An actinobacterial strain KHIA^T, which was isolated from lichen in Tokyo, was found to have 2,4-diamino-

butyric acid in the cell wall. Analyses of the 16S rRNA gene sequences from strain KHIA^T showed that it was related to genera such as *Leifsonia*, *Rhodoglobus*, and *Salinibacterium* of the family *Microbacteriaceae*.

The aim of the present study is to elucidate the taxonomic position of the isolate, using polyphasic taxonomy (phenotypic characteristics, chemotaxonomic data and phylogenetic analysis of 16S rRNA gene sequence). Based on this substantial data, it is proposed that the isolate represents a novel species within a new genus *Schumannella* as *Schumannella luteola* gen. nov., sp. nov.

Materials and Methods

Bacterial strain. Strain KHIA^T was isolated from lichen in Tokyo (Japan). A lichen sample was washed five times with sterilized water and was crushed in sterilized water. The suspension was diluted serially and spread on modified Detmer medium [1.0 g KNO₃, 0.25 g MgSO₄ · 7H₂O, 0.25 g K₂HPO₄, 0.1 g NaCl, 10 mg CaCl₂ · 2H₂O, 1.0 ml Fe solution (1.0 g FeSO₄ · 7H₂O,

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0.5 L distilled water, 2 drops conc. H_2SO_4), 1.0 ml A5 solution (2.86 g H_3BO_3 , 2.5 g $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.222 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 79.0 mg $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 21.0 mg Na_2MoO_4 , 1 L distilled water), in 1 L distilled water pH 8.0] and incubated at 30°C. The colony was selected and further purified using nutrient agar medium.

Culture media and electron microscopy. Growth tests of the temperature and pH were carried out in nutrient broth. The cell morphology was observed by transmission electron microscopy (JSM-1011 apparatus; JEOL, Tokyo, Japan) after negative staining with uranyl acetate for the cells grown on nutrient agar for 2 days. Motility of the cells was examined by the hanging drop method using phase-contrast microscopy (BX60 microscope; Olympus, Tokyo, Japan). The growth under anaerobic conditions was determined after a week of incubation in an AnaeroPack (Mitsubishi Gas Chemical Co., Inc., Tokyo, Japan).

Biochemical analyses. Catalase activity was tested by adding a drop of 3% H_2O_2 to a single colony and was recorded as positive when development of bubbles was observed. Oxidase activity was determined by cytochrome oxidase paper (Nissui Pharmaceutical Co., Inc., Tokyo, Japan). Growth was measured at various temperatures (5–40°C). pH range for growth was determined in nutrient broth which had been adjusted to various pH values (initial pH 4.5–9.5 at intervals of 0.5 pH units). Prior to sterilization the pH was adjusted to various levels by using HCl and NaOH. Growth at various NaCl concentrations was investigated in nutrient broth lacking NaCl. API 20E and API 50CH microtest galleries (bioMérieux, Marcy-l'Étoile, France) were used to determine the physiological and biochemical characteristics according to the manufacturer's instructions. The API tests were recorded after 2 days of incubation at 30°C.

Analysis of sequence data and construction of a phylogenetic tree. The DNA was prepared according to the method of Marmur (1961). The 16S rRNA gene was amplified and sequenced (Xie and Yokota, 2003). The DNA sequence of the strain KHIA^T was used for a Blast search via NCBI (National Centre for Biotechnology Information). The obtained nucleotide sequences were aligned with the CLUSTAL X software package ver. 1.83 (Thompson et al., 1997), and the evolutionary distances and K_{nuc} value (Kimura, 1980) were generated. Alignment gaps and ambiguous bases were not taken into consideration when the 1,252 bases of the 16S rRNA gene nucleotide were compared. The phy-

logenetic tree was constructed using the neighbor-joining method (Saitou and Nei, 1987). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1,000 replicates. The similarity values were calculated using MEGA3 (Kumar et al., 2004).

Chemotaxonomic investigation. The G+C content of the total DNA was measured by HPLC according to the method described by Mesbah et al. (1989). Respiratory quinone analysis was performed according to the method described by Collins and Jones (1981). The quinone fractions were separated with thin-layer chromatography (TLC) developed with hexane:diethyl ether (85:15, v/v). The quinone spot was detected under UV light, the spot was scraped off, and quinones were extracted with acetone. After concentration, the quinone samples were analyzed with high-performance liquid chromatography (HPLC), model LC-10AD VP (Shimadzu, Kyoto, Japan). The cell wall of the strain KHIA^T was prepared by the method described by Schleifer and Kandler (1972), and the amino acid composition of complete wall hydrolysates was determined by two-dimensional chromatography on cellulose TLC (Harper and Davis, 1979) and by HPLC, as their phenylthiocarbamoyl derivatives (Wako Pure Chemical Ind., Osaka, Japan), with a model LC-10AD VP HPLC apparatus. Cellular fatty acid methyl esters were prepared, separated and identified with the Microbial Identification System (MIDI, Inc., Newark, DE, USA).

Nucleotide sequence accession number. The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequences determined in this study is AB362159 (JCM 23215^T=TISTR 1824^T).

Results

Morphological characteristics

The cells of the strain KHIA^T are short, straight, single rods, approximately 0.5–0.6 μm in diameter and 1.1–1.6 μm in length (Fig. 1). The cells are non-motile. The colonies grown on nutrient agar medium are circular, convex, gummy and yellow.

Phylogenetic analysis

An almost complete 16S rRNA gene sequence of the strain KHIA^T was determined and subjected to comparative analysis. Pairwise analysis revealed that the new isolate exhibited the highest similarity values

Fig. 1. The cells of strain KHIA^T visualized by transmission electron microscope.
Scale bar=1 μ m.

Fig. 2. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequence showing the positions of strain KHIA^T and other related taxa.

The numbers at the nodes indicate the percentages of occurrence in 1,000 bootstrapped trees; only values greater than 50% are shown.

to the genera *Plantibacter* (97.1–97.4% similarity), *Leifsonia* (95.9–97.0%), *Rhodoglobus* (96.4%), *Salinibacterium* (96.3%), and *Okibacterium* (96.2%). The strain KHIA^T showed similarity values lower than 95.2% to the other genera of the family *Microbacteriaceae* such as *Microbacterium*, *Cryobacterium*, *Clavibacter*, and

Agromyces. A phylogenetic tree constructed by using the neighbor-joining method showed that the isolate was closely related to the genera *Plantibacter*, *Okibacterium*, *Rhodoglobus*, *Salinibacterium* and *Leifsonia* (Fig. 2), but formed a distinctive lineage independent from other taxa. The topologies of phylogenetic trees

built using the maximum-likelihood and maximum-parsimony algorithms were similar to those of the tree constructed by neighbor-joining analysis (data not shown).

Physiological and chemotaxonomic characteristics

The DNA G+C content of strain KHIA^T was 58.7 mol%, which is lower than that of the other related taxa. The cellular fatty acid composition of strain KHIA^T was anteiso-C_{15:0} (49.7%), iso-C_{16:0} (34.5%), anteiso-C_{17:0} (6.1%), iso-C_{14:0} (5.7%), iso-C_{15:0} (2.8%) and iso-C_{17:0} (0.5%). Cell wall peptidoglycan of strain KHIA^T contained glutamic acid, glycine, alanine, and 2,4-diaminobutyric acid in a molar ratio of about 1.0 : 1.4 : 2.7 : 0.65. Isoprenoid quinones contained were MK-11 (59%) and MK-10 (28%) and small amounts of MK-9 (13%). The conditions for growth and physiological characteristics of the isolate are given in the genus and the species description.

Discussion

Strain KHIA^T exhibited the closest phylogenetic affiliation to genera such as *Plantibacter*, *Leifsonia*, *Rhodoglobus*, *Salinibacterium*, and *Okibacterium* of the family *Microbacteriaceae*, but it formed a distinctive lineage independent from other genera. Moreover, the isolates can be differentiated from its phylogenetically related neighbors by some phenotypic characteristics (Table 1) such as motility, diamino acid in murein, major quinone and G+C content. DNA G+C content of the isolate was 58.7, which is 9% lower than for the genus *Plantibacter* (68–70 mol%) even though they

share high 16S rRNA gene sequence similarity. Concerning other neighboring genera *Rhodoglobus*, *Salinibacterium*, and *Okibacterium* are differentiated from diamino acid in murein. The isolate has DAB, whereas those genera have Orn or Lys.

In summary, based on the physiological, chemotaxonomic, and phylogenetic characteristics, we conclude that strain KHIA^T belongs to a novel species within a new genus of the family *Microbacteriaceae*, for which the name *Schumannella luteola* gen. nov., sp. nov. is proposed.

Description of *Schumannella* gen. nov.

Schumannella (Schu.man'nel.la. N.L. m. n. *Schumannella* named after P. Schumann, a German microbiologist, who contributed to the taxonomy of actinobacteria)

Cells are non-motile, Gram-positive and aerobic rods. Endospores are not formed. Catalase activity is positive. Oxidase activity is negative. Cell wall peptidoglycan contains glutamic acid, glycine, alanine, and 2,4-diaminobutyric acid. The major fatty acids are anteiso-C_{15:0} and iso-C_{16:0}. Respiratory quinones are MK-11, MK-10. The G+C content of the DNA is 58.7 mol%. The type species of the genus is *Schumannella luteola*.

Description of *Schumannella luteola* sp. nov.

Schumannella luteola (lu.te'o.la. L. adj. *Luteus* yellow; L. dim. adj. *luteola* yellowish, somewhat yellow)

The characteristics are the same as those given in the description of the genus with the following additions. Cells are 0.5–0.6 × 1.1–1.6 μm in size. The colonies grown on NA are circular, convex, gummy and

Table 1. The differential characteristics of *Schumannella* gen. nov. and phylogenetically related genera.

	1	2	3	4	5	6
Motility	–	–	+,–	+	–	–
Growth temperature (°C)	8–35	–2–30	0–42	–2–21	4–37	7–37
Diamino acid in murein ^a	DAB	DAB	DAB	Orn	Lys, Orn	Lys
Major quinone	MK-11, 10	MK-10	MK-10, 11, 12	MK-11, 12	MK-11	MK-10, 11
Major fatty acid	anteiso-15:0 iso-16:0	anteiso-15:0 anteiso-17:0 iso-16:0	anteiso-15:0 iso-16:0 anteiso-17:0	anteiso-15:0 iso-16:0 anteiso-17:0	anteiso-15:0 iso-16:0 iso-14:0	anteiso-15:0 anteiso-17:0
G+C content (mol%)	58.7	68–70	61–71	62	61	67

^aDAB, 2,4-diaminobutyric acid; Lys, lysine; Orn, ornithine.

1, *Schumannella* gen. nov.; 2, *Plantibacter* (data from Behrendt et al., 2002; Lin and Yokota., 2006); 3, *Leifsonia* (Evtushenko et al., 2000; Qiu et al., 2007; Reddy et al., 2003; Suzuki et al., 1999); 4, *Rhodoglobus* (Sheridan et al., 2003); 5, *Salinibacterium* (Han et al., 2003); 6, *Okibacterium* (Evtushenko et al., 2002).

yellow. The conditions for growth are 8–35°C and pH 5.0–9.0. NaCl is not required for growth, but cells are tolerant up to 3% (w/v) NaCl. Catalase and gelatinase activities, and citrate utilization are positive. Acetoin is produced but H₂S and indole are not produced. Oxidase, urease, β -galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and tryptophan deaminase activities are negative. Nitrate is not reduced. Inositol, mannitol, cellobiose, maltose, lactose and sucrose are utilized, and ribose, sorbose, rhamnose, trehalose and D-fucose are not utilized. Acid is produced from glucose and arabinose but not from sucrose. Major menaquinones are MK-11 and MK-10. The cellular fatty acids are composed of iso- and anteiso-branched fatty acids predominated by anteiso-C_{15:0} and iso-C_{16:0}. The diamino acid in cell wall is 2,4-diaminobutyric acid. The G+C content of DNA of the type strain is 58.7%.

Type strain is strain KHIA^T (JCM 23215^T=TISTR 1824^T), which was isolated from a lichen collected at Tokyo, Japan.

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