Plantibacter auratus sp. nov., in the family Microbacteriaceae

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Strain NCIMB 9991^T is a Gram-positive, short rod-shaped, yellow-pigmented bacterium, with a high DNA G + C content, and was originally deposited in 1967 as *Arthrobacter* sp. The bacterium is aerobic, non-motile, catalase-positive and oxidase-negative. Comparative 16S rRNA gene sequencing studies demonstrated that this strain was highly related genealogically to *Plantibacter flavus* DSM 14012^T. Strain IAM 14817^T (= NCIMB 9991^T) has the following characteristics: the predominant menaquinones are MK-9 and MK-10, the DNA G + C content is 68 mol%, the diamino acid in the cell wall is 2,4-L-diaminobutyric acid and the muramic acid in the peptidoglycan is of an acetyl type. The major fatty acid is 12-methyl tetradecanoic acid (anteiso-C_{15:0}), followed by 14-methyl hexadecanoic acid (anteiso-C_{17:0}), 14-methyl pentadecanoic acid (iso-C_{16:0}) and hexadecanoic acid (C_{16:0}). On the basis of morphological, physiological and chemotaxonomic characteristics, together with DNA–DNA hybridization and 16S rRNA gene sequence comparison, strain IAM 14817^T represents a novel species within the genus *Plantibacter*, for which the name *Plantibacter auratus* sp. nov. is proposed, with the type strain IAM 14817^T (= NCIMB 9991^T = NBRC 15702^T).

Members of the genera of the family Microbacteriaceae that contain 2,4-L-diaminobutyric acid (2,4-L-DAB) in their B-type cross-linked peptidoglycan (Schleifer & Kandler, 1972) differ from each other in menaquinone type, cell-wall sugars, G + C content of their DNA and physiological features (Lin *et al.*, 2004). The genus *Plantibacter* was proposed to accommodate a Gram-positive, non-motile rod, with cell-wall peptidoglycan containing 2,4-L-DAB, D-glutamic acid, D-alanine and glycine, with the menaquinones MK-10 and MK-11 and with a G + C content of about 70 mol% (Behrendt *et al.*, 2002). The type species is *Plantibacter flavus*, which was isolated from the phyllosphere of grasses.

Strain IAM 14817 was originally deposited in 1967 by V. I. Kudriavtsev as *Arthrobacter* sp. NCIMB 9991. The strain was grown on peptone/yeast extract agar supplemented with brain heart infusion [PY-BHI, comprising 1 % peptone, 0.2 % yeast extract, 0.2 % NaCl, 0.2 % D-glucose, 0.2 % brain heart infusion (Difco Laboratories), 1.5 % agar (pH 7.0)] and nutrient agar medium [0.5 % peptone, 0.3 % meat extract, 0.3 % NaCl, 1.5 % agar (pH 7.0)]. It was cultured aerobically at 30 °C. *P. flavus* DSM 14012^T was used as the reference strain.

Abbreviation: DAB, diaminobutyric acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of IAM 14817^T is AB177868.

Gram staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was determined using cells grown on PY-BHI agar. Each sample used for scanning electron microscopy (model S-4500; Hitachi) was prepared by fixing cells with 1 % glutaraldehyde, followed by dehydration using a graded acetone series and a Hitachi model HCP-2 critical point drying apparatus. Motility was determined by the hanging drop method. Unless otherwise indicated, all tests were performed at 25 °C. Catalase activity was determined by bubble formation in 3 % H₂O₂ solution. Oxidase activity was determined by the oxidation of 1 % tetramethyl-*p*-phenylenediamine on filter paper. Acid production from carbohydrates, assimilation of carbohydrates, nitrate reduction and hydrolysis of aesculin and gelatin were studied using API 50 CH and API 20NE (bioMérieux).

Cell walls were prepared from ~500 mg (dry weight) bacterial cells as described by Schleifer & Kandler (1972). Amino acids in an acid hydrolysate of the cell walls were identified by two-dimensional ascending chromatography on cellulose TLC plates (Tokyo Kasei Co.) by the method of Harper & Davis (1979) and by HPLC as phenylthiocarbonyl derivatives with a model LC-6AD HPLC apparatus (Shimadzu Co.) equipped with a Wakopak WS-PTC column (Wako Pure Chemical Industries, 1989). Analysis of enantiomeric diamino acid isomers was performed according to Sasaki *et al.* (1998). Cell-wall sugars were analysed as described by Mikami & Ishida (1983). Fatty acids were extracted from dried cells, purified and examined as

Fig. 1. 16S rRNA gene dendrogram obtained by distance matrix (neighbour-joining) analysis showing the position of strain IAM 14817^T. Bar, 0.01 substitutions per position.

described previously (Yokota *et al.*, 1993). The glycolate test was performed by the method of Uchida & Aida (1977).

Isolation and purification of chromosomal DNA and determination of DNA G+C content were performed by the methods of Takagi *et al.* (1993). DNA–DNA relatedness values were determined as described by Ezaki *et al.* (1989). Extraction of genomic DNA, PCR amplification of the 16S rRNA gene and sequencing of the purified PCR product were carried out according to the methods of Lin *et al.* (2004). The 16S rRNA gene was aligned with published sequences retrieved from DDBJ using CLUSTAL W (Thompson *et al.*, 1994) and edited using BIOEDIT (Hall, 1999). The phylogenetic tree was constructed on the basis of the neighbour-joining method (Saitou & Nei, 1987); distances were estimated by the method of Jukes & Cantor (1969) using MEGA version 2.1 (Kumar *et al.*, 2001). The GenBank/EMBL/DDBJ accession numbers used in this analysis are given in Fig. 1. *Arthrobacter citreus* DSM 20133^T was used as an outgroup.

Strain IAM 14817^T formed visible colonies on PY-BHI agar after 48 h at 30 °C. The colonies were yellowish, shiny, convex and round. Cells were Gram-positive, catalase-positive, oxidase-negative, non-motile, non-spore-forming, short rods. Cells were 0.3–0.4 µm wide and 0.5–1.1 µm long. The strain was negative for nitrate reduction and for alkaline phosphatase, but positive for β-galactosidase and α-galactosidase. Hydrolysis of aesculin was positive. Acid was produced from adonitol, rhamnose, inositol, maltose, lactose and melezitose, but not from ribose, L-sorbose, sorbitol, melibiose, inulin or raffinose. The physiological and biochemical characteristics are summarized in Table 1.

The major menaquinones of strain IAM 14817^T were MK-8 (12 % of total quinones), MK-9 (24 %), MK-10 (55 %) and MK-11 (8 %); the major menaquinones of the type species *P. flavus* are MK-10 and MK-11 (Table 2). Cellular fatty acids detected were anteiso-C_{15:0} (57.7 % of total fatty acids), anteiso-C_{17:0} (18.3 %), iso-C_{16:0} (13.4 %), iso-C_{15:0} (4.9 %) and C_{16:0} (3.1 %). The peptidoglycan of strain IAM 14817^T contained D-glutamate, glycine, D-alanine and 2,4-L-DAB in a molar ratio of 1.0:1.0:0.8:1.9. The cell-wall

sugars contained rhamnose, 6-deoxytalose and fucose. The acyl type of the muramic acid in the peptidoglycan was acetyl (Table 2).

Nearly complete 16S rRNA gene nucleotide sequences were determined. The phylogenetic tree constructed using the neighbour-joining method and *K*_{nuc} values clearly showed that strain IAM 14817^T was in the same cluster as *P. flavus* DSM 14012^T (Fig. 1). The level of 16S rRNA gene sequence similarity between strain IAM 14817^T and *P. flavus* DSM 14012^T was 99.7 %. DNA–DNA hybridization levels between strain IAM 14817^T and *P. flavus* DSM 14012^T were 49–55 %. Hence, strain IAM 14817^T can be differentiated phenotypically from *P. flavus* DSM 14012^T (Table 2).

Based on the phenotypic and genotypic data, strain IAM 14817^T is proposed as the type strain of a novel species within the genus *Plantibacter*, for which the name *Plantibacter auratus* sp. nov. is proposed.

Description of *Plantibacter auratus* sp. nov.

Plantibacter auratus (au.ra'tus. L. masc. part. adj. *auratus* gold-coloured, yellow).

Table 1. Differential biochemical characteristics of strain IAM 14817^T and *P. flavus* DSM 14012^T

Both strains were positive for catalase, β-galactosidase, α-galactosidase, hydrolysis of aesculin and acid formation from rhamnose, maltose, lactose and melezitose and negative for oxidase, motility, nitrate reduction, alkaline phosphatase and acid formation from L-sorbose, sorbitol, melibiose and inulin.

Characteristic	<i>P. auratus</i> IAM 14817 ^T	<i>P. flavus</i> DSM 14012 ^T
Acid production from:		
Ribose	–	+
Adonitol	+	–
Raffinose	–	+
Inositol	+	–

Table 2. Chemotaxonomic characteristics of strain IAM 14817^T and *P. flavus* DSM 14012^T

Data for *P. flavus* DSM 14012^T were from Behrendt *et al.* (2002). Both strains have 2,4-L-DAB as the diamino acid in the cell wall and have muramic acids of the acetyl type. —, Not detected.

Characteristic	<i>P. auratus</i> IAM 14817 ^T	<i>P. flavus</i> DSM 14012 ^T
Menaquinone composition (%)		
MK-8	12	—
MK-9	24	9
MK-10	55	60
MK-11	8	16
MK-12	—	2
Fatty acid composition (%)		
C _{16:0}	3.1	2.1
iso-C _{16:0}	13.4	19.1
iso-C _{15:0}	4.9	4.2
anteiso-C _{15:0}	57.7	59.1
anteiso-C _{17:0}	18.3	12.6
Other	2.6	2.9
DNA G+C content (mol%)	68	70
DNA–DNA hybridization (%) with:		
IAM 14817 ^T	100	49
DSM 14012 ^T	55	100

Cells are Gram-positive, aerobic rods, 0.3–0.4 × 0.5–1.1 µm, non-sporulating and non-motile. Colonies are circular, convex, smooth and pale yellow on PY-BHI agar. Optimal temperature for growth is generally 30 °C. Catalase-positive, oxidase-negative. Positive for β-galactosidase, α-galactosidase and hydrolysis of aesculin. Acid is produced from adonitol, rhamnose, inositol, maltose, lactose and melezitose. The following tests are negative: reduction of nitrate or nitrite, nitrate respiration, alkaline phosphatase and production of acid from ribose, L-sorbose, sorbitol, melibiose, inulin and raffinose. The diamino acid in the cell wall is 2,4-L-DAB and the muramic acid in the peptidoglycan is of an acetyl type. The major fatty acid is 12-methyl tetradecanoic acid (anteiso-C_{15:0}), followed by 14-methyl hexadecanoic acid (anteiso-C_{17:0}), 14-methyl pentadecanoic acid (iso-C_{16:0}) and hexadecanoic acid (C_{16:0}). The predominant menaquinones are MK-9 and MK-10. The DNA G+C content is 68 mol%.

The type strain is IAM 14817^T (=NCIMB 9991^T=NBRC 15702^T). The type strain was originally deposited in the NCIMB in 1967 by V. I. Kudriavzev as *Arthrobacter* sp. The source of the strain is unknown.

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