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. Kudriavzev 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.

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 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 phenylthiocarbamoyl 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
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). 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 20133T was used as an outgroup.

Strain IAM 14817T 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 14817T 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-C15:0 (57.7% of total fatty acids), anteiso-C17:0 (18.3%), iso-C16:0 (13.4%), iso-C15:0 (4.9%) and C16:0 (3.1%). The peptidoglycan of strain IAM 14817T contained N-glutamate, glycine, δ-alanine and 2,4-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 14817T was in the same cluster as P. flavus DSM 14012T (Fig. 1). The level of 16S rRNA gene sequence similarity between strain IAM 14817T and P. flavus DSM 14012T was 99.7%. DNA–DNA hybridization levels between strain IAM 14817T and P. flavus DSM 14012T were 49–55%. Hence, strain IAM 14817T can be differentiated phenotypically from P. flavus DSM 14012T (Table 2).

Based on the phenotypic and genotypic data, strain IAM 14817T 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.**


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>P. auratus IAM 14817T</th>
<th>P. flavus DSM 14012T</th>
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<tr>
<td>Acid production from:</td>
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<tr>
<td>Ribose</td>
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<td>Adonitol</td>
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<td>Raffinose</td>
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<tr>
<td>Inositol</td>
<td>+</td>
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Fig. 1. 16S rRNA gene dendrogram obtained by distance matrix (neighbour-joining) analysis showing the position of strain IAM 14817T. Bar, 0-01 substitutions per position.
The type strain is IAM 14817T (=NCIMB 9991T =NBRC 15702T). 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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### References


