Ornithinibacter aureus gen. nov., sp. nov., a novel member of the family Intrasporangiaceae

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A novel strain of the class Actinobacteria was isolated from a seawater sample collected in the South China Sea using modified R2A agar plates. The strain was a Gram-stain-positive, non-motile, non-spore-forming, catalase-positive, irregular rod-shaped bacterium. The strain grew at 4–45 °C and pH 5.0–10.2, and tolerated 5 % (w/v) NaCl. Based on its 16S rRNA gene sequence, the organism was related phylogenetically to members of the genera Fodinibacter (96.7 % similarity), Lapillicoccus (96.5 %), Knoellia (95.0–95.8 %), Oryzihumus (95.6 %) and Humibacillus (95.6 %). The cell-wall contained l-ornithine as the major diagnostic diamino acid in the peptidoglycan. MK-8(H4) was the predominant menaquinone. Major cellular fatty acids were iso-C18:1ω9c, iso-C16:0, iso-C15:0 and C17:0. The G+C content of the DNA was 69.6 mol%.

Phenotypic and phylogenetic data revealed that this strain represents a novel species in a new genus of the family Intrasporangiaceae, for which the name Ornithinibacter aureus gen. nov., sp. nov. is proposed; the type strain of Ornithinibacter aureus is HB09001T (=CGMCC 1.10341T =DSM 23364T).

The description of the family Intrasporangiaceae (Stackebrandt et al., 1997; Stackebrandt & Schumann, 2000) was emended by Zhi et al. (2009) and the family contains a variety of actinomycetes, besides the type genus Intrasporangium (Kalakoutskii et al., 1967), that have L-1-diaminopimelic acid (DAP), meso-DAP or L-ornithine (L-Orn) in the cell-wall peptidoglycan and MK-8(H4) or MK-8 as the major menaquinone (Martin et al., 1997; Maszenan et al., 2000; Groth et al., 2001, 2002; Hanada et al., 2002; Kageyama et al., 2005, 2007, 2008a, b; Jung et al., 2006; Lee & Lee, 2007). At the time of writing, the family contained 18 genera with validly published names, including 16 genera summarized by Zhi et al. (2009) and the recently described genera Marihabitans (Kageyama et al., 2008b) and Fodinibacter (Wang et al., 2009). It is easy to distinguish these genera from each other on the basis of phenotypic and genotypic characteristics. In this paper, the classification of an actinomycete, strain HB09001T, that contained L-ornithine (L-Orn) in the cell-wall peptidoglycan and MK-8(H4) as the predominant respiratory quinone was determined by means of a polyphasic approach. Based on morphological, cultural, physiological and biochemical characteristics of strain HB09001T and 16S rRNA gene sequence analysis, it is proposed that this isolate represents a novel species in a new genus.

Strain HB09001T was isolated from a seawater sample collected in the South China Sea (Hainan, China) by the standard dilution-plating technique on modified R2A agar [0.5 g yeast extract, 0.5 g bacterial peptone, 0.5 g casein acid hydrolysate, 0.5 g glucose, 0.5 g soluble starch, 0.3 g sodium pyruvate, 15 g agar, 750 ml seawater and 250 ml distilled water (pH 7.2)]. Plates were incubated for 30 days at 28 °C. Pure cultures were maintained as 20 % (v/v) glycerol suspensions at −70 °C.

Morphological observation of cultures grown on modified R2A agar at 28 °C for 14 days was performed using a scanning electron microscope (model S-3000N; Hitachi). Motility was tested using cells grown in semi-solid agar medium for 2 weeks at 28 °C (Holt & Krieg, 1994). The Gram reaction was determined by using the Gram stain kit (Beijing Land Bridge) according to the manufacturer’s instructions.

Temperature, pH and NaCl tolerances for growth were determined on modified R2A agar. Catalase and oxidase
activities and degradation of Tweens 20 and 80 were determined according to Cowan & Steel (1965). Milk coagulation and peptonization and starch hydrolysis were determined by cultivation on various media as described by Arai (1975) and Williams & Cross (1971). Nitrate reduction and hydrolysis of gelatin and urea were studied as described by Lányi (1987). H₂S production and cellulose hydrolysis were determined according to Smibert & Krieg (1994). The ability of the strain to grow on a range of sole carbon sources at 1% (w/v) was determined by using carbon utilization medium (Pridham & Gottlieb, 1948). Antibiotic sensitivity was tested by spreading a bacterial suspension on modified R2A agar and applying discs containing the following antibiotics (µg per disc; Hanzhou Microbial Reagent): ampicillin (10), rifamycin (30), gentamicin (10), kanamycin (30), neomycin (30), tetracycline (30), erythromycin (15), nalidixic acid (30), novobiocin (30), rifampicin (5) and chloramphenicol (30).

Strain HB09001ᵀ was cultivated (28°C, 10 days, 200 r.p.m.) in modified YMC medium (4.0 g yeast extract, 10.0 g malt extract, 4.0 g glucose, 0.5 g casein acid hydrolysate, 750 ml seawater and 250 ml distilled water) to obtain the cell mass required for cell-wall and menaquinone analyses. Hydrolysates of whole cells and purified cell walls were analysed for DAP isomers by TLC on microcrystalline cellulose (Wang, 1986). Menaquinones were extracted and purified using the method of Minnikin et al. (1984) and analysed by reversed-phase HPLC (model P680; Dionex) with an ODS-BP C18 column (Collins, 1994). Polar lipids were extracted according to Minnikin et al. (1979) and resolved by two-dimensional TLC on silica gel plates (10 × 10 cm) sprayed with specific reagents (Collins & Jones, 1980). For analysis of cellular fatty acid profiles, biomass of strain HB09001ᵀ was harvested from modified R2A agar plates after cultivation for 10 days at 28°C. Fatty acid methyl esters were extracted and prepared as described previously (Sasser, 1990) and determined according to Oliver & Colwell (1973) by GC (model GC-2010; Shimadzu).

To determine the G+C content, chromosomal DNA was extracted and purified as described by Saito & Miura (1963) and analysed according to Mesbah et al. (1989) with the modification that the DNA was hydrolysed and the resultant nucleotides were analysed using reversed-phase HPLC. Genomic DNA for PCR was extracted as described previously (Pitcher et al., 1989). PCR amplification of the 16S rRNA gene and sequencing of the purified PCR product were performed as described by Rivas et al. (2003). In this study, the 16S rRNA gene sequence (1441 bp) of strain HB09001ᵀ was determined and aligned with representative sequences of members of the family Intrasporangiaceae obtained from GenBank using the program CLUSTAL_X 1.8 (Thompson et al., 1997). Phylogenetic analysis was done with the program MEGA version 2.1 (Kumar et al., 2001). Three tree-making algorithms, the neighbour-joining (Saitou & Nei, 1987), minimum-evolution (Kidd & Sgaramella-Zonta, 1971; Rzhetsky & Nei, 1993) and maximum-parsimony (Fitch, 1971) methods, were used to reconstruct phylogenetic trees. A bootstrap analysis (Felsenstein, 1985) was performed by employing 1000 replicated datasets.

Strain HB09001ᵀ formed Gram-stain-positive, non-motile, non-spore-forming, branching hyphae with a size range of 0.57–0.80 µm × 0.75–1.12 µm (see Supplementary Fig. S1, available in IJSEM Online). Colonies were circular, flat, umbilicate, bright yellow in colour and 0.5–1.5 mm in diameter and had coarse surfaces after 7 days of incubation on modified R2A agar plates at 25°C.

Growth was observed at 4–45°C and pH 5.0–10.2. Optimum growth was observed at 34°C and pH 7.2. NaCl was tolerated up to 5% (w/v), with 1% (w/v) NaCl being optimal. No growth occurred in 6% (w/v) NaCl. Catalase-positive, oxidase-negative and negative for nitrate reduction. L-Rhamnose, L-proline and glycine were assimilated as sole carbon sources. Susceptible to ampicillin and rifamycin. Other physiological properties are given in the species description.

Biochemical characteristics of strain HB09001ᵀ are shown in Table 1. Cells of strain HB09001ᵀ contained L-Orn as the major diagnostic diamino acid in the peptidoglycan and MK-8(H₄) as the predominant menaquinone. The major polar lipids were phosphatidylglyitol, phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and an unknown glycolipid. The cellular fatty acid profile contained iso-C₁₈:₀ 9c (16.27%), iso-C₁₆:₀ (15.47%), iso-C₁₅:₀ (14.26%), C₁₇:₀ (11.82%), C₁₈:₁ (6.53%), C₁₆:₁ 9c (6.37%), C₁₇:₁ (6.07%), iso-C₁₄:₀ (5.73%), iso-C₁₇:₀ (4.84%), C₁₅:₀ (2.74%), C₁₄:₀ (2.03%) and anteiso-C₁₅:₀ (1.79%).

The DNA G+C content of the strain was 69.6 mol%. 16S rRNA gene sequence-based phylogenetic analysis of strain HB09001ᵀ demonstrated a close relationship to members of the family Intrasporangiaceae in the suborder Micrococineae. Highest 16S rRNA gene sequence similarities were found to Fodinibacter luteus DSM 21208ᵀ (96.7%), Lapillicoccus jejuensis DSM 18607ᵀ (96.5%), Knoellia subterranea HK1 0120ᵀ (95.8%), Orzyihumus leptocrescens NBRC 100762ᵀ (95.6%) and Humibacillus xanthopolallidis NBRC 101803ᵀ (95.6%). A neighbour-joining phylogenetic tree (Fig. 1) based on 16S rRNA gene sequences revealed a close phylogenetic relationship between strain HB09001ᵀ and members of genera of the family Intrasporangiaceae, with the isolate grouping on a sub-branch with Fodinibacter luteus DSM 21208ᵀ. However, strain HB09001ᵀ could be distinguished from Fodinibacter luteus DSM 21208ᵀ by the presence of iso-C₁₈:₀ 9c and iso-C₁₅:₀ as the major fatty acids, the presence of an unknown glycolipid and the dissimilar diagnostic diamino acid L-Orn. Of the other phylogenetically close neighbours, members of the genera Lapillicoccus, Humibacillus, Terracoccus, Humihabitans, Intraspargumium, Arsenicoccus and Terrabacter are clearly distinguishable from strain HB09001ᵀ as they contain LL-DAP as the
Table 1. Differential characteristics of strain HB09001\(^\top\) (Ornithinibacter aureus gen. nov., sp. nov.) and genera of the family Intrasporangiaceae

| Taxa: 1, strain HB09001\(^\top\) (data from this study); 2, Ornithinococcus (Groth et al., 1999); 3, Ornithinimicrobium (Groth et al., 2001); 4, Seranicoccus (Yi et al., 2004); 5, Lapillicoccus (Lee & Lee, 2007); 6, Humibacillus (Kageyama et al., 2008a); 7, Terracoccus (Prauser et al., 1997); 8, Humihabitus (Kageyama et al., 2007); 9, Intrasporangium (Schumann et al., 1997); 10, Arsenicoccus (Collins et al., 2004); 11, Terrabacter (Collins et al., 1989, unless otherwise indicated); 12, Fodinibacter (Wang et al., 2009); 13, Knueelia (Groth et al., 2002); 14, Tetrasphaera (Maszenan et al., 2000); 15, Janibacter (Martin et al., 1997, unless otherwise indicated); 16, Phychicoccus (Lee, 2006); 17, Terrabacter (Collins et al., 2008a); 18, Kribbia (Jung et al., 2006); 19, Oryzihumus (Kageyama et al., 2005). ND, No data available.

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<td>Irregular rods and cocci</td>
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<td>Cocci</td>
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<td>L-Orn</td>
<td>L-Orn</td>
<td>L-Orn</td>
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*Major fatty acids are defined as constituting >10% of the total fatty acid content. i, iso; ai, anteiso; Me, methyl.
†Data from Montero-Barrientos et al. (2005).
‡Data from Kämpfer et al. (2006).
§APL, Unknown aminophospholipid; DPG, diphostatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIMs, phosphatidylinositol mannosides; PL, unknown phospholipid; PGL, unknown phosphoglycolipid; GL, unknown glycolipid.
diagnostic diamino acid. Furthermore, there are differences in their polar lipid and fatty acid profiles (Table 1). Strain HB09001T could also be differentiated from members of the genera Knoellia, Oryzihumus, Tetrasphaera, Janibacter, Phycicoccus, Marihabitans and Kribbia by the fact that they contain meso-DAP as the diagnostic diamino acid and exhibit differences in their polar lipid and fatty acid profiles (Table 1). In addition, the genera Ornithinibacter, Ornithinimicrobium and Serinicoccus, which contain L-Orn as the major diagnostic diamino acid in the peptidoglycan, do not contain phosphatidylyethanolamine as a diagnostic phospholipid or iso-C18 : 1ω9c as a major fatty acid. Although there may be differences in the proportions of some fatty acids because of different cultivation conditions and extraction procedures (Table 1), the fatty acid iso-C18 : 1ω9c was the major fatty acid in strain HB09001T. Taken together, the phylogenetic data and differential chemotaxonomic properties strongly suggest that strain HB09001T does not belong to any of the genera with validly published names in the family Intrasporangiaceae.

Based on its distinct phylogenetic position within the family Intrasporangiaceae, as well as its morphological, cultural, physiological and biochemical properties, strain HB09001T represents a novel species in a new genus, for which the name Ornithinibacter aureus sp. nov., gen. nov. is proposed.

**Description of Ornithinibacter aureus sp. nov.**

Ornithinibacter aureus (au.re’us. L. masc. adj. aureus golden, referring to the bright yellow colour of the strain). Cells are 0.57–0.80 by 0.70–1.12 μm. Colonies are bright yellow. Growth occurs at pH 5.0–10.2 (optimum, pH 7.2) and 4–45 °C (optimum, 34 °C) and in the presence of 0–5 % (w/v) NaCl (optimum, 1 %). Urease-negative, catalase-positive and oxidase-negative. Does not reduce nitrate. Able to degrade starch, but not cellulose, gelatin and Tweens 20 and 80. Milk coagulation and peptonization are negative. Indole is produced. H2S is not produced. The methyl red test is negative. Among the carbon sources tested, L-rhamnose, L-proline and glycine are utilized, but D-xylose, lactose, D-galactose, cellobiose, D-fructose, raffinose, sorbitol, inositol, L-cysteine, L-arginine, L-asparagine, sorbic acid and 4-aminobutyric acid are not. Susceptible to ampicillin and rifampicin, but not to gentamicin, kanamycin, neomycin, tetracycline, erythromycin, nalidixic acid, novobiocin, rifampicin and chloramphenicol. The DNA G+C content of the type strain is 69.6 mol%.

The type strain is HB09001T (=CGMCC 1.10341T =DSM 23364T), isolated from a seawater sample collected in the South China Sea.
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References


