Acetobacter oeni sp. nov., isolated from spoiled red wine

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A bacterial strain, designated B13T, was isolated from spoiled red wine from the Dão region, Portugal. The strain was Gram-negative, strictly aerobic, rod-shaped and motile. Phylogenetic analysis on the basis of 16S rRNA gene sequences indicated that B13T belonged to the genus Acetobacter within the Alphaproteobacteria. The closest related species was Acetobacter aceti, with 98.4% 16S rRNA gene sequence similarity. DNA–DNA hybridization showed that B13T constituted a taxon separate from the Acetobacter species with validly published names. The DNA G+C content of B13T was 58.1 mol%. Phenotypic characteristics of B13T allowed its differentiation from the recognized Acetobacter species. B13T produced 5-ketogluconic acid from glucose, but no 2-ketogluconic acid. It produced catalase, but no oxidase. It utilized glycerol, but not maltose, ethanol or methanol as carbon sources. On the basis of the results obtained, B13T represents a novel species for which the name Acetobacter oeni sp. nov. is proposed. The type strain is B13T (=LMG 21952T = CECT 5830T).

The genus Acetobacter currently comprises 23 species with validly published names, nine of which have been subsequently transferred to other genera. The Acetobacter species were delineated mainly on the basis of DNA–DNA relatedness and phylogenetic relationships (Sokollek et al., 1998; Lisdiyanti et al., 2000, 2001; Cleenwerck et al., 2002).

During a study of red wine from the Dão region, Portugal, that had been spoiled due to the presence of volatile phenols produced by Dekkera bruxellensis (Silva et al., 2004), a bacterial strain, B13T, was recovered. The strain was isolated by spreading 1 ml spoiled red wine samples aseptically on YEDC plates (1−1=mol 7 g glucose, 3 g yeast extract, 20 g calcium carbonate and 17 g agar) where it grew well and produced large haloes due to the solubilization of calcium carbonate by the large amounts of acid secreted by the strain. On this agar colonies of the novel isolate were mucoid and opaque cream.

Strain B13T was grown on nutrient agar for 48 h at 22 °C to check for motility by phase-contrast microscopy. Gram staining of cells was carried out according to the classical Gram procedure described by Doetsch (1981). Strain B13T was a Gram-negative, rod-shaped and motile organism with peritrichous flagella (0.8–0.9 × 3.6–5.1 μm).

DNA for 16S rRNA gene sequencing was extracted as described by Rivas et al. (2001). Amplification and sequencing of the nearly complete 16S rRNA gene were performed as described previously (Rivas et al., 2003). The sequence determined was compared with sequences from GenBank using the BLAST program (Altschul et al., 1990). Phylogenetic analysis was performed using the BIONUMERICS 4.0 software package (Applied Maths). The sequence determined was aligned with similar sequences retrieved from the EMBL database. Nucleotide substitution rates were calculated using the two-parameter method of Kimura (1980). The phylogenetic tree was inferred using the neighbour-joining method (Saitou & Nei, 1987). Bootstrap analysis based on 1000 replications was undertaken to test the robustness of the phylogenetic tree (Felsenstein, 1985).

The nearly complete 16S rRNA gene sequence of B13T (1484 nucleotides) was obtained. B13T was located in the Alphaproteobacteria, within the genus Acetobacter. The sequence similarities of B13T to the type strains of Acetobacter aceti, Acetobacter tropicalis, Acetobacter estunensis, Acetobacter cerevisiae, Acetobacter indonesiensis, Acetobacter
malorum, *Acetobacter orleanensis*, *Acetobacter orientalis*, *Acetobacter cibinongensis*, *Acetobacter syzygii*, *Acetobacter lovaniensis*, *Acetobacter pomorum*, *Acetobacter pasteurianus* and *Acetobacter peroxydans* were 98.3, 98.1, 98.0, 97.9, 97.9, 97.8, 97.6, 97.6, 97.5, 97.4, 97.1, 97.0, 96.7 and 96.4% respectively. Fig. 1 shows the phylogenetic position of strain B13<sup>T</sup> within the genus *Acetobacter*.

DNA for DNA–DNA hybridizations and DNA base composition analysis was prepared by the method of Wilson (1987) with minor modifications (Cleenwerck et al., 2002). DNA–DNA hybridizations were performed using a modification of the microplate method described by Ezaki et al. (1989) (Goris et al., 1998; Cleenwerck et al., 2002). The hybridization temperature was 46 °C. Reciprocal reactions (e.g. A × B and B × A) were performed and the variation between them was within the limits of this method (Goris et al., 1998). The DNA–DNA relatedness percentages presented are the means of a minimum of four hybridization experiments, including the reciprocal reactions. Strain B13<sup>T</sup> showed low DNA–DNA relatedness (< 20%) with the type strains of *A. aceti* (13%), *A. tropicalis* (14%), *A. estunensis* (20%), *A. cerevisiae* (13%), *A. indonesiensis* (7%), *A. malorum* (5%), *A. orleanensis* (6%), *A. orientalis* (6%), *A. cibinongensis* (7%), *A. syzygii* (10%), *A. lovaniensis* (15%), *A. pomorum* (12%), *A. pasteurianus* (16%) and *A. peroxydans* (13%). The DNA G+C content was determined by HPLC according to the method of Mesbah et al. (1989). Non-methylated phage lambda DNA (Sigma) was used as the calibration reference. The DNA G+C content of B13<sup>T</sup> was 58.1 mol%.

Phenotypic characteristics were examined as described by Cleenwerck et al. (2002). The phenotypic characteristics of B13<sup>T</sup> are given in the species description. Phenotypic characteristics that differentiate B13<sup>T</sup> from recognized *Acetobacter* species are given in Table 1. Strain B13<sup>T</sup> can be differentiated from the other species of the genus *Acetobacter* on the basis of 2- and/or 5-ketogluconic acid production from D-glucose, which are the main diagnostic characters for this genus. The ability of the strain to grow in the presence of 10% ethanol also distinguishes strain B13<sup>T</sup> from most *Acetobacter* species. Moreover, strain B13<sup>T</sup> differs from the closest phylogenetically related species, *A. aceti* and *A. estunensis*, as it is unable to grow with ammonium as the sole nitrogen source with ethanol as the carbon source.

The results presented above allow the genotypic and phenotypic differentiation of B13<sup>T</sup> from the 14 *Acetobacter* species with validly published names. Strain B13<sup>T</sup> should therefore be classified as representing a novel species for which we propose the name *Acetobacter oeni* sp. nov.

**Description of Acetobacter oeni sp. nov.**


Cells are motile, non-spore-forming rods, 3.6–5.1 μm in length and 0.8–0.9 μm in diameter. Gram-negative, strictly aerobic. The optimal growth temperature on YEDC is 28 °C. Colonies on YEDC are circular, convex, cream, opaque and usually 1–2 mm in diameter within 4 days growth at 28 °C.

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**Fig. 1.** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences of *Acetobacter oeni* B13<sup>T</sup> and other related species of the family *Acetobacteraceae*. The significance of each branch is indicated by a bootstrap value (%) calculated for 1000 subsets. Bar, 1 substitution per 100 nucleotides.
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<th>Characteristic</th>
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<td>5-Keto-D-gluconic acid</td>
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<td>Growth in ammonium with ethanol</td>
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<td>DNA G+C content (mol%)</td>
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<td>57-3.5</td>
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<td>58-9</td>
<td>57-6</td>
<td>59-2</td>
<td>52</td>
<td>56-2</td>
<td>59-2</td>
<td>52-0.3</td>
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Produces 5-keto-D-gluconic acid from D-glucose but no 2-keto-D-gluconic acid. Oxidizes D-glucose in media containing ammonium nitrate as the nitrogen source, but is unable to ferment D-glucose in the same media. Produces catalase, but no oxidase. Unable to grow with ammonium as the nitrogen source with ethanol as carbon source. Growth in presence of 10% ethanol. Utilizes glycerol as a carbon source, but not maltose or methanol. No growth in the presence of 30% D-glucose. DNA G+C content of 58.1 mol%.

The type strain, B13\(^{T}\) (= LMG 21952\(^{T}\) = CECT 5830\(^{T}\)), was isolated from spoiled red wine of the Đào region, Portugal.

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### References


