Enterococcus silesiacus sp. nov. and Enterococcus termitis sp. nov.

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Three enterococci constituted two aberrant branches after numerical analysis of (GTG)₅-PCR fingerprints: analogous patterns were found for two water isolates, strains W213 and W442ᵀ, and a separate position was found for an isolate from the gut of a termite, strain LMG 8895ᵀ. 16S rRNA gene sequence analysis classified all three strains in the Enterococcus faecalis species group. Further sequencing analysis of the housekeeping gene pheS (encoding the phenylalanyl-tRNA synthase α-subunit) and whole-cell-protein analysis confirmed a distinct position for the two water isolates and the termite strain, respectively. DNA–DNA hybridization experiments and distinct phenotypic features between the strains studied and representatives of the E. faecalis species group confirmed novel species status, respectively, for the two water isolates, strains W213 and W442ᵀ, and for strain LMG 8895ᵀ. The names Enterococcus silesiacus sp. nov. and Enterococcus termitis sp. nov. are proposed for the novel taxa, with W442ᵀ (=CCM 7319ᵀ =LMG 23085ᵀ) and LMG 8895ᵀ (=CCM 7300ᵀ) as the respective type strains.

Enterococci generally occur as inhabitants of the human and animal intestinal tract, but they are also common in fermented food and are isolated from the environment (Devriese & Pot, 1995). Although enterococci are considered beneficial and safe members of the population of various fermented products (Giraffa, 2002), they are involved in a variety of human nosocomial infections (Teixeira & Facklam, 2003). The genus is phylogenetically subdivided into a number of species groups. Within these species groups, enterococcal species share certain physiological and phenotypical characteristics that may be useful for their identification (Devriese et al., 1993). Although this identification approach is still valuable for the most common species, for some of the more recently described species a combination of phenotypic and molecular methods is required for reliable identification (Domig et al., 2003; Devriese et al., 2002). In the present paper, we describe two novel enterococcal species by using a polyphasic approach.

Strains W213 (=CCM 7318 =LMG 23084) and W442ᵀ (=CCM 7319ᵀ =LMG 23085ᵀ) were isolated from drinking water in the region of Silesia in the Czech Republic during a routine microbiological water analysis performed by filtration of a 10 ml water sample through Millipore filters (max. pore size 0·45 μm) and cultivation of the filters on Slanetz–Bartley agar plates for 24 h at 37 °C as described by Švec & Sedláček (1999). Strain LMG 8895ᵀ (=CCM 7300ᵀ) was isolated from the gut of a termite and was originally described as Lactococcus lactis subsp. lactis. SDS-PAGE of proteins, however, already revealed (results not shown) that the strain was a member of the enterococci. All other type and reference strains included in this study were obtained from the BCCM/LMG Bacteria Collection (http://www.belspo.be/bccm/).

Genotypic characterization was performed using rep-PCR fingerprinting with the (GTG)₅ primer as described by Švec et al. (2005). (GTG)₅-PCR fingerprints obtained were normalized using BioNumerics (version 4.0) and compared with available profiles in an in-house database (BCCM/LMG Bacteria Collection) covering all described enterococcal species. Strains W213 and W442ᵀ showed analogous patterns, and strain LMG 8895ᵀ occupied a separate branch distinct from all other reference strains (Fig. 1).

Analysis of the complete 16S rRNA gene sequence of strains W213, W442ᵀ and LMG 8895ᵀ was performed as described...
by Vancanneyt et al. (2004). The sequences obtained and reference sequences (downloaded from the GenBank database) were aligned by using the BioEdit software (Hall, 1999). Evolutionary distances were calculated using the Jukes–Cantor evolutionary model (Jukes & Cantor, 1969) and a phylogenetic tree was constructed using the neighbour-joining method with the TREECON software (Van De Peer & De Wachter, 1994). The tree topology obtained with the neighbour-joining method was evaluated and confirmed by the maximum-parsimony analysis using BioNumerics (version 4.0). The phylogenetic analysis placed the three strains in the Enterococcus faecalis species group (Fig. 2), which accommodates E. faecalis, Enterococcus haemoperoxidus and Enterococcus moraviensis (Švec et al., 2001). Strains W213 and W442T showed 99-9 % 16S rRNA gene sequence similarity to each other and showed E. haemoperoxidus and E. moraviensis as their closest phylogenetic relatives with similarities ranging from 99-0 to 99-2 %. Similarly, strain LMG 8895T showed 98-9 % 16S rRNA gene sequence similarity with E. haemoperoxidus and 98-8 % with E. moraviensis species. Sequence similarity between strain LMG 8895T and strains W213 and W442T was 99-3 %.

Amplification and partial sequencing of the pheS gene (encodes a phenylalanyl-tRNA synthase) were performed by using pheS primers: pheS-21-F (5′-CAYCCNGCHCGYGAYATGC-3′), pheS-22-R (5′-CCWARVCCRAARGCAARCC-3′) and pheS-23-R (5′-GGRTGRACCATVCCNGCHCC-3′), which enabled the comparison of a 455 bp gene fragment. The pheS primers were designed based on a selection of the most conservative regions of the pheS gene sequence of representative lactic acid bacteria obtained from publicly available data of whole-genome-sequence projects. Sequencing primer designs, amplification conditions and sequencing parameters were performed as described by Naser et al. (2005). Although the sequences obtained represent only about half of the gene, Naser et al. (2005) demonstrated that this region shows sufficient diversity to distinguish individual species. Different enterococcal species have a maximum of 86 % pheS gene sequence similarity and the intraspecies variation showed a high degree of homogeneity of at least 97 % among strains of the same species. This suggested that pheS is a fast-evolving clock and a valuable tool for identification of enterococci; however, the topology obtained in the pheS dendrogram does not reflect

Fig. 1. (GTG)5-PCR fingerprints obtained from strains W213, W442T and LMG 8895T and from the type strains representing all recognized enterococcal species. The dendrogram was calculated with Pearson’s correlation coefficient using UPGMA clustering method (r, expressed for convenience as percentage similarity values).
Fig. 2. Distance-matrix tree based on 16S RNA gene sequence comparisons showing the phylogenetic relationships of strains W213, W442 and LMG 8895 and selected enterococcal species representing phylogenetic neighbours and intraspecies lineages. The Vagococcus fluvialis (X54258) sequence was used as the outgroup. Bootstrap percentage values (500 tree replications) higher than 50 % are indicated at branch points. GenBank accession numbers are stated in parentheses. Bar, 5 % evolutionary distance.

The DNA base composition was determined for strains W213, W442 and LMG 8895. Isolation of high-molecular-mass DNA from bacterial cells grown in Todd–Hewitt broth (Oxoid), degradation of the DNAs into nucleosides and their separation by HPLC were carried out as described by Vancanneyt et al. (2004). The DNA G+C content of strains W213, W442 and LMG 8895 were 35·6, 36·7 and 37·1 mol%, respectively. These results correspond to the DNA G+C content of the E. faecalis species group that range from 34·3 to 37·7 mol% (Svec et al., 2001).

DNA–DNA hybridization experiments were performed between strains W213, W442 and LMG 8895 and confirms that they represent a single species. DNA–DNA-binding levels between strains W213 and W442 were 12, 26 and 30 %, respectively. Binding levels between strain LMG 8895 and E. faecalis LMG 7937, E. moraviensis LMG 19486 and E. haemoperoxidus LMG 19487 were 12 and 13 %, 41 and 43 % and 48 and 46 %, respectively. These results confirm that the water isolates W213 and W442 and strain LMG 8895 represent two novel enterococcal species.

Growth and biochemical tests were carried out by using API 20 Strep and API 50CH commercial kits (bioMérieux) as well as by conventional tests described by Svec et al. (2001). Results are given in the species descriptions below. The species can be differentiated from their phylogenetically closest known relatives by using the tests listed in Table 1.

All results obtained in this study confirmed the analysed strains as members of two novel enterococcal species, for which the names Enterococcus silesiacus sp. nov. and Enterococcus termitis sp. nov. are proposed.
Table 1. Biochemical tests useful for differentiation of Enterococcus silesiacus sp. nov., E. termitis sp. nov. and their phylogenetic relatives assigned in the E. faecalis species group

Taxa: 1, E. silesiacus sp. nov.; 2, E. termitis sp. nov.; 3, E. faecalis; 4, E. haemoperoxidans; 5, E. moravensis. Characteristics scored as: +, positive; −, negative; d, variable. Data described by Švec et al. (2001), de Vaux et al. (1998), Schleifer & Kilpper-Bälz (1984) and Devriese et al. (1983) or obtained in this study.

<table>
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<th>Characteristic</th>
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Description of Enterococcus silesiacus sp. nov.

Enterococcus silesiacus (síl’e’sí-a´cús, N.L. masc. adj. silesiac pertaining to Silesia, the region in the Czech Republic from which the type strain originates).

Cells are Gram-positive, ovoid cocci, occurring in pairs, short chains or small groups. They elongate in the direction of the chains. Colonies on Columbia agar supplemented with sheep blood are non-pigmented, shiny, circular, smooth with entire margins and about 1 mm in diameter after 24 h of cultivation at 37 °C. The type strain of the species grows well on Todd–Hewitt agar and BHI agar; growth on MRS medium is less abundant. Poor growth on Slanetz–Bartley medium containing 0.04% sodium azide in small dark-red colonies. Positive growth with positive aesculin reaction on kanamycin/aesculin/azide agar and bile/aesculin agar. Non-motile. Growth occurs in BHI broth at 10–45 °C, pH 9–6 and in the presence of 6–5% NaCl. Catalase reaction is negative on blood-containing as well as on blood-free media. Produces leucine aminopeptidase. Does not produce pyrrolidinyl arylamidase, arginine dihydrolase, acetoin (Voges–Proskauer test), β-galactosidase, β-glucuronidase or alkaline phosphatase. Hippurate hydrolysis is negative; aesculin hydrolysis is positive. Acid is produced from glycerol, ribose, D-xylene, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-trehalose and gentiobiose. Acid is not produced from erythritol, D-arabinose, L-arabinose, D-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbitose, L-xylose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, D-melibiose, sucrose, inulin, D-melezitose, D-raffinose, starch, glycerogen, xyitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate or 5-ketogluconate. Acid production from L-arabinose (strain W213 is weakly positive in API 50 CH kit, but negative using API 20 Strep; strain W442T is negative) and gluconate (strain W213 is positive, W442T is negative) is variable. The G+C content of strains W213 and W442T is 35–6 and 36.7 mol%, respectively.

The type strain, W442T (=CCM 7319T = LMG 23085T), and the other strain, W213 (=CCM 7318 = LMG 23084), were isolated from surface waters.

Description of Enterococcus termitis sp. nov.

Enterococcus termitis (ter’mi’tis. L. n. termes -itis a worm that eats wood, a woodworm, and in zoology the name of a scientific genus; L. gen. n. termitis of a termite).

Cells are Gram-positive, ovoid cocci, occurring in pairs, short chains or small groups. They elongate in the direction of the chains. Colonies on Columbia agar supplemented with sheep blood are non-pigmented, shiny, circular, smooth with entire margins and about 1 mm in diameter after 24 h of cultivation at 37 °C. The type strain of the species grows well on Todd–Hewitt agar and BHI agar; growth on MRS medium is less abundant. Poor growth on Slanetz–Bartley medium containing 0.04% sodium azide in small dark-red colonies. Positive growth with positive aesculin reaction on kanamycin/aesculin/azide agar and bile/aesculin agar. Non-motile. Growth occurs in BHI broth at 10–45 °C, pH 9–6 and in the presence of 6–5% NaCl. Catalase reaction is negative on blood-containing as well as on blood-free media. Produces leucine aminopeptidase. Does not produce pyrrolidinyl arylamidase, arginine dihydrolase, acetoin (Voges–Proskauer test), α-galactosidase, β-galactosidase or alkaline phosphatase. Hippurate hydrolysis is negative; aesculin hydrolysis is positive. Acid is produced from glycerol, ribose, D-xylene, D-galactose, D-glucose, D-fructose, D-mannose, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, N-acetylglucosamine, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-trehalose and gentiobiose. Acid is not produced from erythritol, D-arabinose, L-arabinose, L-xylose, D-adonitol, methyl β-D-xylopyranoside, L-sorbitose, L-xylose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl β-D-xylopyranoside, methyl β-D-glucopyranoside, D-melibiose, sucrose, inulin, D-melezitose, D-raffinose, starch, glycerogen, xyitol, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketogluconate or 5-ketogluconate. Acid production from L-arabinose (strain W213 is weakly positive in API 50 CH kit, but negative using API 20 Strep; strain W442T is negative) and gluconate (strain W213 is positive, W442T is negative) is variable. The G+C content of strains W213 and W442T is 35–6 and 36.7 mol%, respectively.

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References


