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 W442\(^T\) (=CCM 7319\(^T\)=LMG 23085\(^T\)) 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 8895\(^T\) (=CCM 7300\(^T\)) 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)\(_{5}\)-PCR 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 W442\(^T\) showed analogous patterns, and strain LMG 8895\(^T\) occupied a separate branch distinct from all other reference strains (Fig. 1).

Analysis of the complete 16S rRNA gene sequence of strains W213, W442\(^T\) and LMG 8895\(^T\) 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 haemoperoxidans and Enterococcus moraviensis (Svec et al., 2001).

Strains W213 and W442T showed 99–99% 16S rRNA gene sequence similarity to each other and showed E. haemoperoxidans 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. haemoperoxidans 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'-CCWARVCCRAARGCAARCCG-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
the phylogenetic relationships revealed by 16S rRNA gene sequencing (Naser et al., 2005). The pheS gene sequence analysis indicated that the two water isolates (W213 and W442T) are members of a single species (99-3 % sequence similarity to each other). Comparison with reference strains revealed the highest sequence similarity of 86-8 % with *E. moraviensis* for both strains. Strain LMG 8895T was differentiated from the above-mentioned isolates (sequence similarity of 84-0 %) and from all enterococcal type strains included in the database and constituted a single separate branch with the highest sequence similarity of 82-6 % with *E. moraviensis* as shown in Supplementary Fig. S1 (available in IJSEM Online). The latter data are an indication that both taxa might represent novel species.

Whole-cell-protein analysis was performed with cells grown on MRS agar (Oxoid) for 24 h at 37 °C. Protein extraction, electrophoresis, SDS-PAGE, densitometric analysis and further analysis of the profiles were performed following the procedure described by Pot et al. (1994). Protein profiles were compared to an in-house database (BCCM/LMG Bacteria Collection) comprising multiple representative strains of all described enterococcal species. The similarity between all pairs of traces was expressed by Pearson’s product–moment correlation coefficient. UPGMA (un-weighted pair group method using arithmetic averages) clustering was used for the construction of the dendrogram. Supplementary Fig. S2 (available in IJSEM Online) shows the whole-cell-protein profiles obtained from investigated strains as well as from their nearest phylogenetic neighbours *E. faecalis*, *E. haemoperoxidus* and *E. moraviensis*. The three new isolates were highly similar to *E. haemoperoxidus* and *E. moraviensis* reference strains. Still, the water isolates (strains W213 and W442T) constituted a single cluster and showed minor differences from strain LMG 8895T, *E. haemoperoxidus* and *E. moraviensis*.

The DNA base composition was determined for strains W213, W442T and LMG 8895T. 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, W442T and LMG 8895T 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, W442T and LMG 8895T and *E. faecalis* LMG 7937T, *E. moraviensis* LMG 19486T and *E. haemoperoxidus* LMG 19487T. High-molecular-mass DNA was isolated as described for determination of the DNA base composition and DNA–DNA hybridization experiments were performed in microdilution wells according to Vancanneyt et al. (2004). The hybridization temperature was 32 °C (calculated as described by Svec et al., 2001). A high DNA-binding value of 93 % was found between strains W213 and W442T and confirms that they represent a single species. DNA–DNA-binding levels between strains W213 and W442T and *E. faecalis* LMG 7937T, *E. moraviensis* LMG 19486T and *E. haemoperoxidus* LMG 19487T were 12 and 13 %, 41 and 43 % and 48 and 46 %, respectively. Binding levels between strain LMG 8895T and *E. faecalis* LMG 7937T, *E. moraviensis* LMG 19486T and *E. haemoperoxidus* LMG 19487T were 12, 26 and 30 %, respectively. The water isolates W213 and W442T and strain LMG 8895T showed binding levels between 25 and 26 %. These data confirm that the water isolates W213 and W442T and strain LMG 8895T 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

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine dihydrolase</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Acetoin (VP test)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
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<tr>
<td>β-Galactosidase</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>d</td>
<td>−</td>
</tr>
<tr>
<td>Hippurate hydrolysis</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>d</td>
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<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>L-Arabinose</td>
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<td>+</td>
<td>d</td>
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<td>−</td>
</tr>
<tr>
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<td>d</td>
<td>+</td>
<td>d</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Methyl α-D-glucopyranoside</td>
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<td>+</td>
<td>+</td>
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<td>+</td>
</tr>
<tr>
<td>Methyl α-D-mannopyranoside</td>
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<tr>
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<td>D-Xylose</td>
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<td>+</td>
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<td>−</td>
</tr>
</tbody>
</table>

Description of *Enterococcus silesiacus* sp. nov.

*Enterococcus silesiacus* (si.le’si.a.cus. N.L. masc. adj. *silesiacus* 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 pyrrolidonyl 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, N-acetylgalosamine, amygdalin, arbutin, salicin, D-cellobiose, D-maltose, D-lactose, D-trehalose and gentiobiose. Acid is not produced from erythritol, D-arabinose, L-xylose, D-adenitol, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, D-melibiose, sucrose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketoglucuronate or 5-ketoglucuronate. Acid production from L-rhamnose (strain W213 is weakly positive in API 50 CH kit, but negative using API 20 Strep; strain W442T is negative) and glucuronate (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 pyrrolidonyl 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-xylose, D-adenitol, methyl β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-mannitol, D-sorbitol, methyl α-D-mannopyranoside, methyl α-D-glucopyranoside, D-melibiose, sucrose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, 2-ketoglucuronate or 5-ketoglucuronate. The G + C content of the type strain is 37.1 mol%.

The type strain, LMG 8895T ( = CCM 7300T), originated from the gut of a termite.
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