Enterococcus caccae sp. nov., isolated from human stools

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The National Antimicrobial Resistance Monitoring System Laboratory at the Centers for Disease Control and Prevention (CDC) isolated two enterococcus-like strains that were referred to the CDC Streptococcus Laboratory for further identification. The isolates were recovered from human stool samples collected on different occasions from the same individual in Portland (OR, USA) in July 2000. Conventional physiological tests distinguished these strains from all known species of enterococci. Analyses of whole-cell-protein electrophoretic profiles showed the same unique profile for the two isolates, being most similar those of Enterococcus moraviensis and Enterococcus haemoperoxidus albeit not close enough to allow conclusive inclusion in any enterococcal species. Both isolates gave positive results in tests using the AccuProbe Enterococcus genetic probe, and Lancefield extracts reacted with CDC group D antiserum. Comparative 16S rRNA gene sequencing studies also revealed that these strains were closely related to the species E. moraviensis (99·6 % identity). The results of DNA–DNA relatedness experiments confirmed that these strains represented a single novel taxon. The highest level of DNA–DNA relatedness found between the novel taxon and any of the currently recognized species of Enterococcus was 32 %, for both E. moraviensis and E. haemoperoxidus. On the basis of this evidence, it is proposed that these stool isolates constitute a novel species, for which the name Enterococcus caccae sp. nov. is proposed. The type strain is 2215-02T (= SS-1777T = ATCC BAA-1240T = CCUG 51564T).

The enterococci have undergone considerable changes in taxonomy in recent years. Since the recognition of Enterococcus as a separate genus (Schleifer & Kilpper-Balz, 1984), several novel species have been described as a result of improvements in the methods used for their identification combined with a growing interest in their role as opportunistic pathogens (Teixeira & Facklam, 2003). The differentiation of some of the species belonging to the genus Enterococcus remains problematic because of the overlap of phenotypic characteristics, and questions might arise as to their precise identification (Devriese et al., 2002; Facklam et al., 2002; Teixeira et al., 1995). In order to accomplish the precise identification of these micro-organisms, we systematically applied analysis of whole-cell-protein profiles and 16S rRNA gene sequencing and DNA–DNA reassociation experiments, in conjunction with conventional physiological tests (Merquior et al., 1994; Teixeira et al., 1995; Carvalho et al., 2004). In the present study, these techniques were used to characterize two enterococcus-like isolates recovered from stool samples obtained from a healthy individual in Portland, OR, USA, on different occasions in July 2000, during routine outpatient surveillance carried out by the National Antimicrobial Resistance Monitoring System Laboratory at the Centers for Disease Control and Prevention (Atlanta, GA, USA), and were referred to the CDC Streptococcus Laboratory for further identification.

The strains were characterized phenotypically using conventional biochemical tests, as previously described (Facklam et al., 2002; Teixeira & Facklam, 2003), and the API Rapid ID 32 Strep system (bioMérieux). The AccuProbe Enterococcus identification test (Gen-Probe) was performed according to the manufacturer’s instructions and was positive for both strains. The phenotypic identification scheme initially used subdivides the enterococcal species into five groups, as...
described previously (Facklam et al., 2002; Teixeira & Facklam, 2003). Using this scheme, both stool strains were placed into group IV, which includes Enterococcus asini, Enterococcus cecorum, Enterococcus phoeniculicola and Enterococcus sulfureus. However, their biochemical profiles did not match those of any enterococcal species with validly published names. The results of physiological tests are presented in the species description below. Table 1 shows the phenotypic tests used to differentiate the stool strains from the phylogenetically closest relatives (Enterococcus moraviensis, Enterococcus haemoperoxidus and Enterococcus faecalis) and the species included in phenotypic group IV.

The API Rapid ID 32 Strep system was used according to the manufacturer’s instructions. The profiles generated by the system’s software identified the stool isolates as Enterococcus durans with 93% confidence.

Extract preparation and whole-cell-protein profile analysis using one-dimensional SDS-PAGE were performed as described previously (Merquior et al., 1994). Dice indices were determined for each isolate by using the Molecular Analyst Fingerprint Plus software package, version 1.6 (Bio-Rad), and a dendrogram was constructed by the unweighted pair-group method with arithmetic averages. Fig. 1 shows the SDS-PAGE profiles of whole-cell-protein extracts of the stool isolates and their separate position relative to the phylogenetically closest relatives, E. moraviensis, E. haemoperoxidus and E. faecalis.

The phylogenetic location of the stool strains was determined by 16S rRNA gene sequencing. Generation and analysis of the sequences were performed according to the procedures described by Shewmaker et al. (2004). The sequences obtained for stool isolates 2215-02T and 826-03

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**Table 1. Tests for differentiating Enterococcus caccae sp. nov. from the phenotypically and phylogenetically most closely related enterococcal species**


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
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</thead>
<tbody>
<tr>
<td>Hydrolysis of arginine</td>
<td>−</td>
<td>−</td>
<td>+ (&gt;4 days)</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Tolerance of tellurite</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Growth at 45 °C</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Voges–Proskauer test</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>Lactose</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>AccuProbe assay result</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>32·5</td>
<td>36</td>
<td>35</td>
<td>35·3</td>
<td>39·4</td>
<td>37</td>
<td>36</td>
<td>38</td>
</tr>
</tbody>
</table>

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**Fig. 1. SDS-PAGE profiles of whole-cell-protein extracts of strains 2215-02T and 826-03 (Enterococcus caccae sp. nov.) and type strains of phylogenetically related species and dendrogram resulting from computer-assisted analysis of the protein profiles.**
and reference sequences obtained from GenBank were aligned with CLUSTAL in the program MEGA, version 3.0, before trimming to a 1409 bp consensus. A neighbour-joining tree was created using the Kimura two-parameter model, with bootstrapping based on 1000 replications (Kumar et al., 2004). The resulting phylogram (Fig. 2) was confirmed with the interior branch test and Tajima’s test (Kumar et al., 2004).

For DNA–DNA hybridization studies, harvesting and lysis of the bacterial cells was performed as described previously (Teixeira et al., 1995). Extraction, purification of the DNA and determination of DNA relatedness by the hydroxyapatite hybridization method were performed as described by Brenner et al. (1982). DNA hybridization experiments were performed at 55°C for optimal DNA reassociation conditions and at 70°C for stringent conditions. DNA of type strain 2215-02^T was hybridized with the phenotypically similar isolate, 826-03, and with the type strains of the most closely related species of Enterococcus (Table 2). The relative binding ratio at the optimal reassociation temperature for the two stool isolates was greater than 70% and the divergence was less than 1%. On the basis of these results, these two strains met the criteria for the definition of species relatedness (Wayne et al., 1987). In addition, when strain 2215-02^T was hybridized against the type strains of the species of Enterococcus most closely related phylogenetically, the highest level of DNA–DNA relatedness found was 32% (for E. moraviensis and E. haemoperoxidus), the value for E. faecalis being 9% (Table 2). These data, in combination with the phenotypic results and the origin of the isolates, indicate that the two isolates could represent one strain that is clearly genetically distinct from the other enterococcal species (Stackebrandt et al., 2002; Wayne et al., 1987), representing a novel taxon, for which the name Enterococcus caccae sp. nov. is proposed.

The DNA G+C content was determined using the optical melting temperature (T_m) and equilibrium buoyant density methods according to the procedures of Mandel et al. (1970). The samples were analysed at least three times; DNA from Escherichia coli K-12 was used as a control. The DNA G+C content of strain 2215-02^T was 32.5 mol%.

**Description of Enterococcus caccae sp. nov.**

Enterococcus caccae (cac’cae, Gr. n. kakke faeces; N.L. gen. n. caccae of faeces).

Cells consist of Gram-positive cocci occurring as short chains, in pairs and as single cells. Non-pigmented, x-haemolytic, small colonies up to 0.5 mm in diameter are formed on sheep-blood agar at 37 °C and are unaffected by the absence or presence of 5% CO₂. Strains are catalase-negative, non-motile and susceptible to vancomycin. Growth occurs at 45 °C and in broth containing 6-5% NaCl. Strains are positive for pyrrolidonyl arylamidase activity, leucine aminopeptidase activity, hydrolysis of aesculin in the presence of bile, hippurate hydrolysis, pyruvate utilization and in the Voges–Proskauer test. Acid is produced from glycerol, maltose, methyl aesculin glucopyranoside, ribose, sucrose and trehalose. Acid is not produced from arabinose, inulin, lactose, mannitol, melibiose, raffinose, sorbitol or sorbose. Reactions are negative for production of gas in MRS broth, hydrolysis of arginine and tolerance of 0-04% tellurite. In the API Rapid ID 32 Strep system, acid is produced from maltose, methyl β-D-glucopyranoside, ribose, sucrose, tagatose and trehalose.

**Table 2. DNA–DNA relatedness between E. caccae strains and related type strains**

Labelled DNA from E. caccae 2215-02^T was used in each case. RBR, Relative binding ratio.

<table>
<thead>
<tr>
<th>Source of unlabelled DNA</th>
<th>RBR at 55 °C</th>
<th>RBR at 70 °C</th>
<th>ΔT_m (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. caccae 826-03</td>
<td>100</td>
<td>100</td>
<td>0·5</td>
</tr>
<tr>
<td>E. moraviensis ATCC BAA-382^T</td>
<td>32</td>
<td>11</td>
<td>7·5</td>
</tr>
<tr>
<td>E. haemoperoxidus ATCC BAA-383^T</td>
<td>32</td>
<td>11</td>
<td>7·5</td>
</tr>
<tr>
<td>E. faecalis ATCC 19433^T</td>
<td>9</td>
<td>1</td>
<td>13·0</td>
</tr>
</tbody>
</table>
The enzymes β-glucosidase, glycol-tryptophan arylamidase, β-mannosidase, pyrogglutamic acid arylamidase and N-acetyl-β-glucosaminidase are produced. Arginine dihydrolase, β-galactosidase (both substrates), β-glucuronidase, α-galactosidase, alkaline phosphatase, alanine-phenylalanine-proline arylamidase and urease are not produced. Acetoin is produced and hippurate is not hydrolysed. Acid is not produced from L-arabinose, D-arabitol, cyclodextrin, glycogen, lactose, mannitol, melezitose, melibiose, pullulan, raffinose or sorbitol. The species is distinguished by whole-cell-protein profiling and 16S rRNA gene sequencing. The AccuProbe Enterococcus genetic probe result is positive and Lancefield extracts react with Centers for Disease Control and Prevention group D antiserum. The DNA G+C content of the type strain is 32-5 mol%.

The type strain, strain 2215-02T (= SS-1777T = ATCC BAA-1240T = CCUG 51564T), was isolated from human stool samples, in Portland, OR, USA.

Acknowledgements

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References


