NOTES

Phenotypic and Phylogenetic Characterization of Some
Globicatella-Like Organisms from Human Sources:
Description of Facklamia hominis gen. nov., sp. nov.

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Six strains of a hitherto undescribed gram-positive, catalase-negative coccus from human sources were characterized by phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing studies demonstrated that the unknown strains were genomically homogeneous and constitute a new line closely related to, but distinct from, the genus Globicatella. The unknown bacterium was readily distinguished from Globicatella sanguis, the type species of the genus Globicatella, by the results of biochemical tests and an electrophoretic analysis of whole-cell proteins. Based on phylogenetic and phenotypic evidence, we propose that the unknown bacterium be classified as Facklamia hominis gen. nov., sp. nov. The type strain of Facklamia hominis is CCUG 36813.

The gram-positive, facultatively anaerobic, catalase-negative cocci form a phenotypically very heterogeneous assortment of organisms. These bacteria invariably possess DNAs with low G+C contents. Many but not all of the gram-positive catalase-negative cocci that cause disease in or associated with humans can be readily assigned to the genus Streptococcus or other well-established taxa (e.g., the genus Enterococcus). The taxonomy of the gram-positive catalase-negative cocci has improved a great deal in recent years because of the use of molecular methods. In particular, 16S rRNA sequence comparisons have proved to be invaluable for clarifying the inter- and intrageneric relationships of these bacteria (4, 6, 14) and have provided an immensely powerful means for recognizing new species and genera of gram-positive cocci (1–3, 5–7). In the present study we characterized six strains of a hitherto unknown gram-positive catalase-negative coccus from human sources by using 16S rRNA gene sequencing. Based on the phylogenetic results and the phenotypic distinctiveness of the unknown bacterium, a new species, Facklamia hominis, is described.

Five human isolates (CCUG 28572, CCUG 28830, CCUG 32738, CCUG 28829, and CCUG 28827) were referred to the Culture Collection of the University of Göteborg in Göteborg, Sweden, for identification. Strain CCUG 28572 originated from urine of a 7-year-old female, strains CCUG 28830, CCUG 28829, and CCUG 28827 originated from vaginas, and strain CCUG 32738 originated from blood. The sixth strain (CCUG 36813) was recovered from an abscess on the buttocks of a patient in Toulouse Hospital in Toulouse, France. All strains were cultured on Columbia agar (Difco, Detroit, Mich.) supplemented with 5% sheep blood at 37°C. The strains were biochemically characterized by using API Rapid ID32 strep and API ZYM systems according to the instructions of the manufacturer (API bioMérieux, Marcy l’Etoile, France).

Polyacrylamide gel electrophoresis of whole-cell proteins was performed as described previously (12). For densitometric analysis, normalization, and interpretation of protein patterns the Gelcompar GCW 3.0 software package (Applied Maths, Kortrijk, Belgium) was used. The cell wall muropeptide and DNA base composition of strain CCUG 36813 were determined by using a Taq DyeDeoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolates were determined by performing a database search with the program FASTA of the Genetics Computer Group package (8). The sequences of the closely related organisms and the sequences of other known related strains were retrieved from the EMBL and Ribosomal Database Project libraries and were aligned with the newly determined sequences by using the program PILEUP (8). The resulting multiple sequence alignment was corrected manually, and approximately 100 bases at the 5’ end of the rRNA were omitted from further analyses because of alignment ambiguities. A continuous stretch of 1,320 bases was used for the distance matrix analysis. A distance matrix was constructed by using the programs PRETTY (8) and DNADIST (using the Kimura-2 correction parameter) (9). A phylogenetic tree was constructed by the neighbor-joining method with the program NEIGHBOR (9). The stability of the groups was estimated by performing a bootstrap analysis (500 replications) with the programs DNABOOT, DNADIST, NEIGHBOR, and CONSENSE (9). In addition, a parsimony analysis (9) was performed with the same data set.

Cells of the six isolates from humans were ovoid and formed pairs or groups. All of the strains were gram-positive, catalase-
FIG. 1. Similarity dendrogram based on whole-cell protein patterns of F. hominis sp. nov. and related species. Levels of correlation are expressed as percentages of similarity.

An examination of the cell wall murein of a representative strain (CCUG 36813T) of the unknown coccus revealed that the unknown coccus resembles members of the genera Abiotrophia, Gemella, and Actinomycetales (Fig. 1).

To establish the phylogenetic position of the unknown coccus from humans, the 16S rRNA genes of the six strains were amplified by PCR and were characterized by performing sequence searches of the EMBL and GenBank libraries (>1,400 nucleotides) of strain CCUG 36813T was determined, and sequence searches of the EMBL and GenBank libraries with the FASTA program revealed that the new organism was phylogenetically most closely associated with the lactococci group of bacteria. The sequences of the nearest relatives of the unknown organism were retrieved from the EMBL and GenBank libraries and subjected to a comparative analysis to determine the phylogenetic position of strain CCUG 36813T. A tree constructed by the neighbor-joining method which indicates the phylogenetic affinity of the unknown coccus within the lactococci group of bacteria is shown in Fig. 2, and the levels of sequence similarity between close relatives are given in Table 1. The unknown coccus formed a distinct line that exhibited a specific phylogenetic association (level of 16S rRNA sequence divergence, approximately 6.3%) with G. sanguis. Bootstrap resampling (bootstrap value, 100%) revealed that this association was statistically highly significant. Abiotrophia deflectiva exhibited a somewhat more distant association (level of 16S rRNA sequence divergence, approximately 8%) with the unknown coccus. Bootstrap resampling showed that the grouping of Abiotrophia deflectiva with the unknown organism and the G. sanguis cluster was robust (bootstrap value, 100%). A parsimony analysis was also performed, and all significant associations were confirmed (data not shown).

If we assume that the unknown coccus shares a common ancestor with G. sanguis or Abiotrophia deflectiva, we can infer that the unknown coccus may have a close evolutionary relationship with these organisms. Further studies are needed to confirm these findings.

TABLE 1. Levels of 16S rRNA similarity between F. hominis sp. nov. and some related lactococci bacteria

<table>
<thead>
<tr>
<th>Species</th>
<th>% Sequence similarity with F. hominis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abiotrophia adiacens (DS0540)</td>
<td>91.9</td>
</tr>
<tr>
<td>Abiotrophia deflectiva (DS0541)</td>
<td>91.9</td>
</tr>
<tr>
<td>Aerococcus urinae (M77819)</td>
<td>90.9</td>
</tr>
<tr>
<td>Alloiooccocus otitides (XS57965)</td>
<td>86.5</td>
</tr>
<tr>
<td>Carnobacterium piscicola (X54268)</td>
<td>90.4</td>
</tr>
<tr>
<td>Dolosigranulum pigrum (X70907)</td>
<td>87.0</td>
</tr>
<tr>
<td>Enterococcus faecalis (L16515)</td>
<td>89.0</td>
</tr>
<tr>
<td>Globicatella sanguis (S50214)</td>
<td>93.9</td>
</tr>
<tr>
<td>Lactobacillus sanfrancisco (X76327)</td>
<td>85.9</td>
</tr>
<tr>
<td>Lactosphaera pasteurii (X87150)</td>
<td>91.3</td>
</tr>
<tr>
<td>Leuconostoc mesenteroides (M23035)</td>
<td>84.8</td>
</tr>
<tr>
<td>Streptococcus iniae (X58316)</td>
<td>86.0</td>
</tr>
<tr>
<td>Vagococcus salmoninarum (X54272)</td>
<td>88.7</td>
</tr>
<tr>
<td>Weissella viridescens (X52508)</td>
<td>87.7</td>
</tr>
</tbody>
</table>

* Based on a comparison of approximately 1,320 bases.

The numbers in parentheses are EMBL and GenBank database accession numbers.

FIG. 2. Unrooted tree showing the phylogenetic relationships of F. hominis sp. nov. and some related lactococci bacteria. The tree was constructed by using the neighbor-joining method and was based on the results of a comparison of approximately 1,320 nucleotides. Bootstrap values, expressed as percentages of 500 replications, are indicated at branch points.
Production of acid from:

- Murein type

production of:

- whereas G.

ship is a relationship between two phylogenetically closely re-

arate generic status of the isolates. Support for the sep-

ative species within the lactic acid group of bacteria. From the

classification in a new genus and new species, for which the

type strain produces pyrrolidonyl aryl-amidase but not urease, glycyrtophan arylamidase, pyrrolidonyl arylamidase, and pyroglutamine arylamidase may or may not be produced. Esulin and gelatin are not hydrolyzed. Hipprurate is hydrolyzed. Vogens-Prokaunder and indole neg-

ative. Nitrate is not reduced. The G+C content of the DNA is 41 mol%. The cell wall murein type is L-LYS-D-ASP (type A4α). The type strain of F. hominis is CCUG 36813T. Strain CCUG 36813T was isolated from an abscess on buttocks and has the characteristics of the species. The type strain produces pyrrolidonyl arylamide but not urease, glycyrtophan arylamidase, or pyro-

glutamine arylamidase.

Nucleotide sequence accession number. The 16S rRNA gene sequence of strain CCUG 36813T has been deposited in the GenBank database under accession no. Y10772.

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