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Facklamia ignava sp. nov., Isolated from Human Clinical Specimens

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Two strains of a hitherto-undescribed gram-positive, catalase-negative coccus isolated from human sources were characterized by phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing studies demonstrated that the unknown strains are genealogically identical and constitute a new line close to, but distinct from, *Facklamia hominis*. The unknown bacterium was readily distinguished from *F. hominis* by biochemical tests and electrophoretic analysis of whole-cell proteins. On the basis of phylogenetic and phenotypic evidence, it is proposed that the unknown bacterium be classified as *Facklamia ignava* sp. nov. The type strain of *Facklamia ignava* is CCUG 37419.

It has only been in recent years that clinical microbiologists have started to fully appreciate the considerable diversity of the gram-positive, facultatively anaerobic, catalase-negative cocci encountered in clinical specimens. In addition to previously unrecognized species within well-established genera (e.g., *Streptococcus*), a plethora of organisms belonging to completely unknown genera, such as *Alloiococcus* (1), *Dolosigranulum* (3), *Facklamia* (5), *Globicatella* (4), and *Helcococcus* (6), have recently been described. Central to the recognition of these newly discovered organisms within the clinical environment has been the application of improved diagnostic tools, in particular the combined use of phenotypic approaches such as miniaturized biochemical testing and protein profiling and molecular-based methodologies such as 16S rRNA gene sequencing. In this article, we report the use of such a polyphasic taxonomic approach for the characterization of two strains of a hitherto-unknown gram-positive, catalase-negative coccus from human sources. On the basis of comparative 16S rRNA gene sequence analysis and the phenotypic distinctiveness of the unknown bacterium, a new species, *Facklamia ignava*, is described.

Two strains (164-97 and 1440-97) from human sources were referred to the Centers for Disease Control (Atlanta, Ga.) for identification. Both strains originated from blood. Strain 164-97 was isolated from an 82-year-old woman who was a resident of a long-term nursing facility in Canada. The culture was received from Margurite Lovgren, Streptococcus Reference Laboratory, Edmonton, Alberta, Canada. The patient was ambulatory and able to care for all her personal needs. She was admitted to the hospital with complaints of nausea and vomiting for the previous 48 h. A diagnosis of pneumonia was determined after the blood cultures were positive for the isolate. The patient was treated with antimicrobial agents and discharged from the hospital after 2 weeks. Strain 1440-97 was isolated from an 86-year-old woman living in North Carolina. The culture was received from Wake Medical Center, Raleigh, N.C. The only other clinical information available is that the patient was septic at the time of culture. The cultures have been deposited in the Culture Collection of the University of Göteborg (CCUG), Göteberg, Sweden, under accession no. CCUG 37419 and CCUG 37659, respectively. The strains were cultured on Columbia agar (Difco, Detroit, Mich.) supplemented with 5% horse blood at 37°C. The strains were biochemically characterized by using the API Rapid ID32 Strep and API ZYM systems according to the manufacturer's instructions (API bioMérieux, Marcy l'Etoile, France). Polyacrylamide gel electrophoresis (PAGE) of whole-cell proteins was performed as described previously (11). 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 murein type of each of the two strains was determined as described by Schleifer and Kandler (12). The DNA base composition of strain 164-97 was determined by thermal denaturation as described by Garvie (9). Phylogenetic studies involved comparative 16S rRNA gene sequence analysis. A large fragment of the 16S rRNA gene (corresponding to positions 30 to 1521 of the *Escherichia coli* 16S rRNA gene) was amplified by PCR using conserved primers close to the 3′ and 5′ ends of the gene. The PCR products were purified by using a Prep-A-Gene kit (Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions and directly sequenced by using a *Taq* Dye-Deoxy 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, using the program FASTA of the Genetics Computer Group package (7). These sequences and those of other, related strains were retrieved from the GenBank or Ribosomal Database Project library and aligned with the newly determined sequences by using the program PILEUP (7). 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 distance matrix analysis. A distance matrix was calculated by using the programs PRETTY (7) and DNADIST (using the Kimura-2 correction parameter) (8). A phylogenetic tree was constructed according to the neighbor-joining method with the program NEIGHBOR (8). The sta-

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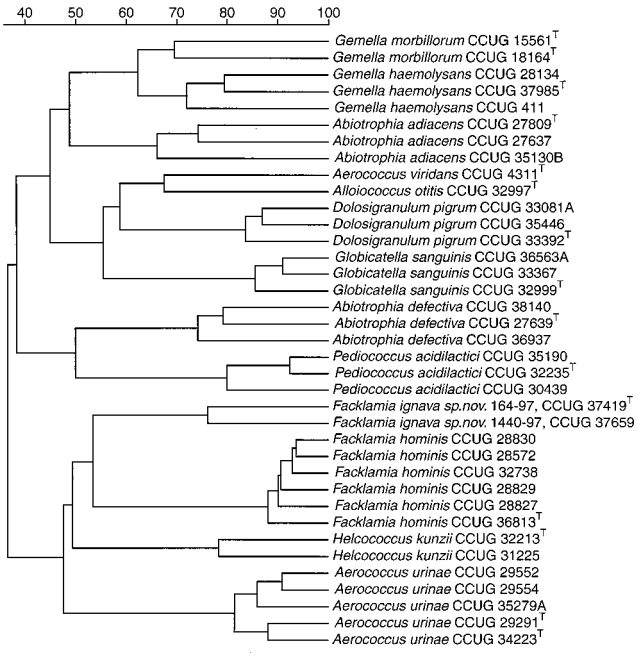

FIG. 1. Similarity dendogram based on whole-cell protein pattern of *F. ignava* sp. nov. and related species. Levels of correlation (top) are expressed as percentages of similarity for convenience.

bility of the groupings was estimated by bootstrap analysis (500 replications), using the programs DNABOOT, DNADIST, NEIGHBOR, and CONSENSE (8).

Cells of the two isolates from humans were ovoid in shape and formed pairs and short chains. Both strains were gram-positive, non-spore-forming, catalase-negative facultative anaerobes which were nonhemolytic. The strains did not grow at 10 or 45°C. They resembled each other in hydrolyzing hippurate and producing alanine-phenylalanine-proline arylamidase and pyroglutamic acid arylamidase. Neither strain produced arginine dihydrolase, alkaline phosphatase, glycyl-tryptophan arylamidase, β-glucuronidase, β-glucosidase, α-galactosidase, β-galactosidase, β-mannosidase, pyrazinamidase, or urease. Both strains weakly fermented glucose but failed to produce acid from amygdalin, L-arabinose, D-arabitol, cellobiose, cyclodextrin, inulin, glycogen, lactose, maltose, D-mannose, melibiose, melezitose, mannitol, lactose, pullulan, D-raffinose, D-ribose, sorbitol, sucrose, D-xylose, L-xylose, D-tagatose, and trehalose. Based on the above characteristics, the unknown isolates appeared to resemble *Facklamia hominis*. However, the unknown bacterium differed significantly from the latter species in not producing arginine dihydrolase, α-galactosidase, β-galactosidase, glycyl-tryptophan arylamidase, and urease. The unidentified bacterium could also be readily distinguished from other catalase-negative cocci (e.g., *Abiotrophia adiacens*, *Abiotrophia defectiva*, and *Globicatella sanguinis*) by its relatively asaccharolytic nature. The close phenotypic affinity between the two clinical isolates was confirmed by PAGE analysis of whole-cell proteins, in which they formed a quite distinct cluster. Consistent with the aforementioned biochemical findings, PAGE analysis showed that the unknown bacterium was phenotypically most similar to, albeit distinct from, *F. hominis*. No close phenotypic resemblance to any other catalase-negative spheroidal organisms studied was shown (Fig. 1). An examination of the cell wall murein compositions of the two strains revealed that lysine was the dibasic amino acid and that the interpeptide bridge was L-Lys–D-Asp (type A4α, according to the nomenclature of Schleifer and Kandler [12]). This murein structure is found in several gram-positive, catalase-negative cocci, including *Dolosigranulum pigrum* (3), *F. hominis* (5), and *Lactosphaera pasteurii* (10), as well as in some enterococci, lactococci, pediococci, and streptococci (12). The cell wall type of the unknown coccus is, however, quite distinct from those of *Alloiococcus otitis* (1), *G. sanguinis* (4), *Abiotrophia defectiva* (5), and aerococci (2), all of which possess murein which is directly cross-linked by lysine (type A1α).

To establish the phylogenetic position of the unknown bacterium isolated from humans, the 16S rRNA genes of both isolates were amplified by PCR and characterized by sequence analysis. The gene sequences of the two strains were determined almost completely (>1,400 nucleotides), and pairwise analysis showed that they were genealogically homogeneous (100% 16S rRNA sequence similarity). Sequence searches of the GenBank and Ribosomal Database Project libraries revealed that the unknown coccus from humans was phylogenetically most closely associated with the lactic acid group

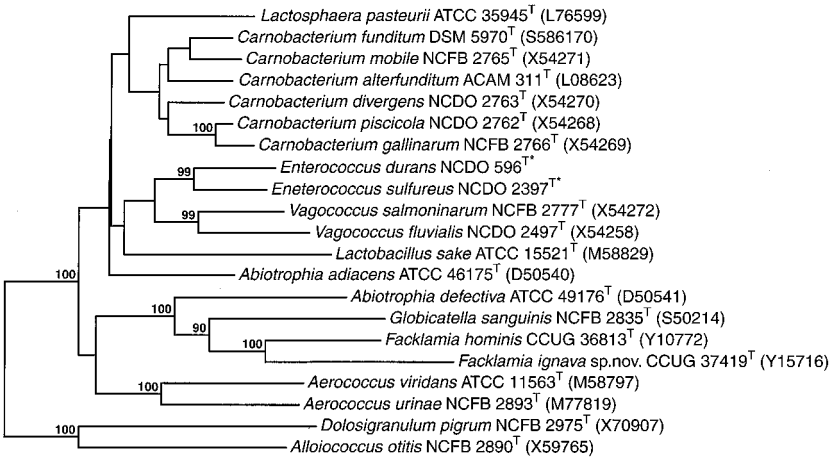

FIG. 2. Unrooted tree showing the phylogenetic relationships of *F. ignava* sp. nov. and some other low-G+C-content gram-positive bacteria. The tree, constructed by the neighbor-joining method, was based on a comparison of approximately 1,320 nucleotides. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points. Asterisks indicate sequences obtained from the Institute of Food Research database.

TABLE 1. Biochemical tests for differentiating *F. ignava* sp. nov. from *F. hominis*

Test[b]	Result for[a]:	
	F. ignava sp. nov.	*F. hominis*
Arginine dihydrolase	−	+
α-Galactosidase	−	+
β-Galactosidase	−	+
Pyroglutamic acid arylamidase	+	V
Glycyl-tryptophan arylamidase	−	V
Urease	−	V

[a] −, negative; +, positive; V, variable result.
[b] Test for the production of the indicated enzyme.

bacteria (data not shown). The sequences of the nearest relatives of the unknown organism were retrieved and subjected to comparative analysis to determine the phylogenetic position of strain 164-97. A tree depicting the phylogenetic affinity of the unknown coccus within the lactic acid bacteria is shown in Fig. 2. The unknown coccus formed a distinct subline exhibiting a specific phylogenetic association (approximately 3% 16S rRNA sequence divergence) with *F. hominis*. Bootstrap resampling (value, 100%) showed this association to be statistically highly significant. The next-nearest relatives of the unknown bacterium were *G. sanguinis* and *Abiotrophia defectiva*, which showed approximately 6 and 8% 16S rRNA sequence divergence, respectively.

From the results of the 16S rRNA gene sequence analysis, it is clear that the unknown coccus from human sources is a member of the recently described genus *Facklamia* (5). Although the phylogenetic association between the unidentified bacterium and *F. hominis* is statistically highly significant, a 16S rRNA sequence divergence of approximately 3% indicates that the two are closely related, but nevertheless quite separate, species. Support for the separateness of the unknown bacterium and *F. hominis* comes from PAGE analysis of whole-cell proteins. In addition, the two taxa can be readily distinguished biochemically (Table 1). Based on both phenotypic and phylogenetic findings, we consider the unknown coccus to merit classification as a new species of the genus *Facklamia*; the name *Facklamia ignava* sp. nov. is proposed.

Description of *Facklamia ignava* sp. nov. *Facklamia ignava* (L. fem. adj. *ignava*, lazy, unreactive) cells are gram positive, ovoid in shape, and occur in pairs or short chains. Cells are nonpigmented, nonhemolytic, and nonmotile. Spores are not produced. Facultatively anaerobic and catalase negative. Grows in 5% NaCl. Does not grow at 10 or 45°C. Weak acid production from glucose. Acid is not produced from amygdalin, L-arabinose, D-arabitol, cellobiose, cyclodextrin, inulin, glycogen, lactose, maltose, D-mannose, melibiose, melezitose, mannitol, lactose, pullulan, D-raffinose, D-ribose, sorbitol, sucrose, D-xylose, L-xylose, D-tagatose, or trehalose. Alanine-phenylalanine-proline arylamidase and pyroglutamic acid arylamidase are produced. Arginine dihydrolase, alkaline phosphatase, glycyl-tryptophan arylamidase, β-glucuronidase, β-glucosidase, α-galactosidase, β-galactosidase, β-mannosidase, pyrazinamidase, and urease are not produced. Esculin and gelatin are not hydrolyzed. Hippurate is hydrolyzed. Voges-Proskauer and indole negative. Nitrate is not reduced. The G+C content of the DNA is 42 mol%. The cell wall murein type is L-Lys–D-Asp (A4α). The type strain of *F. ignava* is CCUG 37419. Isolated from human blood. Habitat unknown.

Nucleotide sequence accession number. The 16S rRNA gene sequence of strain 164-97 (CCUG 37419) has been deposited in GenBank under accession no. Y15716.

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