NOTE***Facklamia sourekii* sp. nov., isolated from human sources**

Matthew D. Collins,¹ Roger A. Hutson,¹ Enevold Falsen²
and Berit Sjöden²

Author for correspondence: Matthew D. Collins. Tel: +44 118 935 7226. Fax: +44 118 935 7222.
e-mail: david.collins@bbsrc.ac.uk

¹ Department of Food
Science and Technology,
University of Reading,
Whiteknights,
Reading RG6 6AP,
UK

² Culture Collection,
Department of Clinical
Bacteriology, University of
Göteborg, Sweden

Two strains of a Gram-positive catalase-negative, facultatively anaerobic coccus originating from human sources were characterized by phenotypic and molecular taxonomic methods. The strains were found to be identical to each other based on 16S rRNA gene sequencing and constitute a new subline within the genus *Facklamia*. The unknown bacterium was readily distinguished from *Facklamis hominis* and *Facklamia ignava* by biochemical tests and electrophoretic analysis of whole-cell proteins. Based on phylogenetic and phenotypic evidence it is proposed that the unknown bacterium be classified as *Facklamia sourekii* sp. nov., the type strain of which is CCUG 28783A^T.

Keywords: *Facklamia sourekii*, taxonomy, phylogeny, 16S rRNA

The Gram-positive catalase-negative cocci constitute a phenotypically heterogeneous group of organisms which belong to the *Clostridium* branch of the Gram-positive bacteria. This broad group of organisms includes many human and animal pathogens (e.g. enterococci, streptococci) and the taxonomy of these organisms has undergone considerable development in recent years. Much of this change has been due to an increased interest in the clinical importance of these organisms in combination with the application of improved molecular identification techniques such as 16S rRNA gene sequencing. The most pronounced development in the taxonomy of the Gram-positive catalase-negative cocci has been the recognition of a plethora of new genera and species, which in the main have been isolated from human sources [e.g. *Abiotrophia elegans* (Roggenkamp *et al.*, 1998), *Alloio-coccus* (Aguirre & Collins, 1992), *Dolosigranulum* (Aguirre *et al.*, 1993) *Gemella bergeriae* (Collins *et al.*, 1998a), *Globicatella* (Collins *et al.*, 1992) and *Helco-coccus* (Collins *et al.*, 1993)]. *Facklamia hominis*, another such example, was proposed to accommodate some unknown catalase-negative cocci from clinical specimens which could not be assigned to any established taxon (Collins *et al.*, 1997). A second species of the genus *Facklamia*, *Facklamia ignava* has recently been characterized for some organisms originating from blood specimens (Collins *et al.*, 1998b). In this article we describe the characteristics of two unknown

Gram-positive coccus-shaped organisms, which constitute a third species of the genus *Facklamia*, *Facklamia sourekii* sp. nov. This report adds to the many new taxa and the diversity of Gram-positive catalase-negative cocci associated with human clinical materials described in the past few years.

Strain CCUG 31976 was received as a presumptive 'α-haemolytic-streptococcus' from the Public Health Laboratory Service in Jönköping (Sweden) in 1993. The strain was isolated from a haemoculture from a 72-year-old man. Initially, the strain could not be identified, but its biochemical profile matched that of a mislabelled strain, CCUG 27618, received from Dr Pavla Kuzemenska in Prague, Czech Republic, as CNCTC Str 2/84 ('SS 1019'). To eliminate any error, the curator of the Czechoslovak National Collection of Type Cultures (CNCTC), Dr Jiri Sourek was asked to deposit Str 2/84 again (= CCUG 28783A^T). Extended phenotyping confirmed that CCUG 31976 and CCUG 28783A^T possessed the same biochemical features. Strain Str 2/84 (CNCTC ← Rotta ← Facklam ← Roguinsky ← de Moore) originated from C. E. de Moore (National Public Health Laboratory, Utrecht, The Netherlands) but no clinical information was available on the organism. The unidentified organisms were cultured on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C, in air plus 5% CO₂. The strains were biochemically characterized by using the API Rapid ID32 Strep and API ZYM systems according to the manufacturer's instructions (API bioMérieux). PAGE analysis of whole-cell proteins was performed as described by Pot *et al.*

The GenBank accession number for the 16S rRNA gene sequence of CCUG 28783^T is Y17312.

Fig. 1. Similarity dendrogram based on whole-cell protein patterns of *F. sourekkii* sp. nov. and related species. Levels of correlation are expressed as percentages of similarity for convenience.

(1994). For densitometric analysis, normalization and interpretation of protein patterns the GelCompar GCW 3.0 software package (Applied Maths) was used. The mol% G+C content of DNA of strain CCUG 28783A^T was determined as described by Garvie (1978). The 16S rRNA genes of the isolates were amplified by PCR and directly sequenced using a *Taq* Dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). The closest known relatives of the new isolates were determined by performing database searches. These sequences and those of other known related strains were retrieved from the GenBank or Ribosomal Database Project (RDP) (Maidak *et al.*, 1997) databases and aligned with the newly determined sequences using the program PILEUP (Devereux *et al.*, 1984). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated using the programs PRETTY and DNADIST (using the Kimura-two correction parameter) (Felsenstein, 1989). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR (Felsenstein, 1989). The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The two isolates were ovoid in shape and formed single cells, pairs or short chains. The strains were Gram-

positive, non-spore-forming, catalase-negative, oxidase-negative facultative anaerobes. Using commercial API systems the isolates produced acid from D-arabitol, maltose, mannitol, sorbitol, sucrose and trehalose. Acid was not produced from L-arabinose, cyclodextrin, glycogen, lactose, melibiose, melezitose, pullulan, raffinose, ribose, tagatose or methyl- β -D-glucopyranoside. Both isolates displayed pyroglutamic acid arylamidase, leucine arylamidase and esterase C-4 (weak reaction) activity. No activity was detected for arginine dihydrolase, alanine-phenylalanine-proline arylamidase, α -galactosidase, β -galactosidase, β -galacturonidase, α -glucosidase, β -glucosidase, β -glucuronidase, lipase C14, α -fucosidase, α -mannosidase, N-acetylglucosaminidase, glycyl-tryptophan arylamidase, chymotrypsin, trypsin, valine arylamidase or urease. They hydrolysed hippurate and were Voges-Proskauer-negative. The close phenotypic affinity between the two unknown isolates was confirmed by PAGE analysis of whole-cell proteins in which the two organisms formed a robust and tight cluster which was quite separate from all other Gram-positive catalase-negative reference organisms examined, including *Abiotrophia* spp., *Aerococcus* spp., *Facklamia* spp. and *Globicatella sanguinis* (Fig. 1). Indeed on the basis of PAGE analysis the unknown isolates clustered, albeit loosely, with *Alloiococcus otitis*. *Aerococcus viridans* and *Pediococcus urinaequei* were the next closest relatives. By contrast, *F. hominis* and *F. ignava* formed distinct clusters which were far removed from the unknown bacterium (Fig. 1).

To assess the phylogenetic affinity between the two isolates and their relationship with other Gram-positive catalase-negative taxa, comparative 16S rRNA gene sequence analyses were performed. Approximately 1400 nucleotides of the 16S rRNA gene of each of the strains was determined and pairwise analysis revealed no base differences (i.e. 100% similarity). Sequence searches of GenBank and RDP databases revealed the unknown bacterium was phylogenetically most closely associated with a group of catalase-negative coccus-forming taxa which included *Abiotrophia*, *Aerococcus*, *Facklamia*, *Ignavigranum* and *Globicatella* (data not shown). A tree constructed by neighbour-joining depicting the phylogenetic affinity of the unknown coccus as exemplified by strain CCUG 28783A^T is shown in Fig. 2. From the branching pattern of the tree the nearest relative of the unknown coccus was *F. ignava*. The clustering together of these organisms occurred in 84% of 500 tree replications. *F. hominis* formed a somewhat deeper subline branching from the unknown coccus/*F. ignava* line (supported by 86% bootstrap value). The next nearest relatives of the aforementioned taxa corresponded to *Ignavigranum ruoffiae* and *Globicatella sanguinis*. Parsimony analysis was also performed and the grouping of the unknown coccus with *F. ignava* and *F. hominis* confirmed.

Biochemical profiling and PAGE analysis of whole-cell proteins demonstrated the two unidentified coccus-

Fig. 2. Unrooted tree showing the phylogenetic relationships of *F. sourekii* sp. nov. and some other low-G+C-content Gram-positive bacteria. The tree, constructed using the neighbour-joining method, was based on a comparison of approx. 1320 nucleotides. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points.

Table 1. Characteristics that differentiate *F. sourekii* sp. nov. from *F. ignava* and *F. hominis*

Character	<i>F. sourekii</i>	<i>F. ignava</i>	<i>F. hominis</i>
Acid from:			
D-Arabitol	+	—	—
Maltose	+	—	—
Mannitol	+	—	—
Sorbitol	+	—	—
Sucrose	+	—	—
Trehalose	+	+	—
Production of:			
Arginine dihydrolase	—	—	+
Alanyl-phenylalanine-proline arylamidase	—	+	+
α -Galactosidase (API Rapid ID32 Strep)	—	—	+
β -Galactosidase (API Rapid ID32 Strep)	—	—	+

shaped organisms were phenotypically similar. This affinity between the isolates was confirmed by comparative 16S rRNA gene sequencing. Phylogenetically the unidentified coccus represents a new subline within the recently proposed genus *Facklamia*. A 16S rRNA gene sequence divergence value of about 7% between the unidentified coccus and *F. ignava* and *F. hominis*, however, unequivocally demonstrated that the bacterium represents a hitherto unknown species. Although the above divergence value indicates the affinity between the unknown coccus and *F. ignava*/*F. hominis* is not particularly close, based on currently described taxa, the genus *Facklamia* is the most appropriate home for this bacterium. It is pertinent to point out that the novel bacterium can be readily distinguished from both *F. ignava* and *F. hominis* by producing acid from D-arabitol, maltose, mannitol, sorbitol and sucrose, and additionally from *F. hominis* in producing acid from trehalose. The unknown coccus

also differs from *F. ignava* and *F. hominis* in being negative for alanyl-phenylalanine-proline arylamidase, and from *F. hominis* in being negative for arginine dihydrolase, α -galactosidase and β -galactosidase. Thus based on the results of 16S rRNA gene sequence and electrophoretic whole-cell protein analyses, and the distinctive biochemical characteristics of the unknown coccus, we consider that this bacterium merits classification as a new species, for which the name *Facklamia sourekii* is proposed. Tests that distinguish *F. sourekii* from *F. ignava* and *F. hominis* are summarized in Table 1.

Description of *Facklamia sourekii* sp. nov.

Facklamia sourekii (Czech pronunciation schou.rek.i.i. *sourekii* named after the late J. Sourek, curator of the CNCTC, Czech Republic).

Cells are Gram-positive, ovoid in shape occurring in

pairs or short-chains. Cells are non-pigmented, α -haemolytic and non-motile. Spores are not produced. Facultatively anaerobic, oxidase-negative and catalase-negative. Grows in 5% NaCl. Acid is produced from D-arabitol, glucose, maltose, mannitol, sorbitol, sucrose and trehalase. Acid is not produced from L-arabinose, cyclodextrin, glycogen, lactose, melibiose, melezitose, pullulan, raffinose, ribose, tagatose or methyl- β -D-glucopyranoside. Pyroglutamic acid arylamidase and leucine arylamidase are produced. Weak activity for esterase C-4 detected. Acid phosphatase, alkaline phosphatase and cystine arylamidase activity may or may not be detected. Arginine dihydrolase, alanine-phenylalanine-proline arylamidase, α -galactosidase, β -galactosidase, β -galacturonidase, α -glucosidase, β -glucosidase, β -glucuronidase, lipase C14, α -fucosidase, α -mannosidase, N-acetylglucosaminidase, glycyl-tryptophan arylamidase, chymotrypsin, trypsin, valine arylamidase and urease are not produced. Hippurate is hydrolysed. Acetoin is not produced. The G+C content of DNA is 41.5 mol%. The type strain of *Facklamia sourekii* is CCUG 28783A^T. The habitat of *Facklamia sourekii* is not known.

Acknowledgements

We thank Dr S. Löfgren for one of the human isolates and Dr R. R. Facklam for providing information on the second strain.

References

- Aguirre, M. & Collins, M. D. (1992). Phylogenetic analysis of an unknown bacterium from human middle ear fluid: description of *Alloiococcus otitis* gen. nov., sp. nov. *Int J Syst Bacteriol* **42**, 79–83.
- Aguirre, M., Morrison, D., Cookson, B. D., Gay, F. W. & Collins, M. D. (1993). Phenotypic and phylogenetic characterization of some *Gemella*-like organisms from human infections: description of *Dolosigranulum pigrum* gen. nov., sp. nov. *J Appl Bacteriol* **75**, 608–612.
- Collins, M. D., Aguirre, M., Facklam, R. R., Shallcross, J. & Williams, A. M. (1992). *Globicatella sanguis* gen. nov., sp. nov., a new Gram-positive catalase-negative bacterium from human sources. *J Appl Bacteriol* **73**, 422–437.
- Collins, M. D., Facklam, R. R., Rodrigues, U. M. & Ruoff, K. L. (1993). Phylogenetic analysis of some *Aerococcus*-like organisms from clinical sources: description of *Helcococcus kunzii* gen. nov., sp. nov. *Int J Syst Bacteriol* **43**, 425–429.
- Collins, M. D., Falsen, E., Lemozy, J., Åkervall, E., Sjöden, B. & Lawson, P. A. (1997). Phenotypic and phylogenetic characterization of some *Globicatella*-like organisms from human sources: description of *Facklamia hominis* gen. nov., sp. nov. *Int J Syst Bacteriol* **47**, 880–882.
- Collins, M. D., Hutson, R. A., Falsen, E., Sjöden, B. & Facklam, R. R. (1998a). *Gemella bergeriae* sp. nov., isolated from human clinical specimens. *J Clin Microbiol* **36**, 1290–1293.
- Collins, M. D., Lawson, P. A., Monasterio, R., Falsen, E., Sjöden, B. & Facklam, R. R. (1998b). *Facklamia ignava* sp. nov., isolated from human clinical specimens. *J Clin Microbiol* **36**, 2146–2148.
- Devereux, J., Haeberli, P. & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12**, 387–395.
- Felsenstein, J. (1989). PHYLIP-phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Garvie, E. I. (1978). *Streptococcus raffinolactis* (Orla-Jensen and Hansen); a group N streptococcus found in raw milk. *Int J Syst Bacteriol* **28**, 190–193.
- Maidak, B. L., Olsen, G. J., Larsen, N., Overbeek, R., McCaughey, M. J. & Woese, C. R. (1997). The RDP (Ribosomal Database Project). *Nucleic Acids Res* **25**, 109–111.
- Pot, B., Vandamme, P. & Kersters, K. (1994). Analysis of electrophoretic whole-organism protein fingerprints. In *Modern Microbial Methods. Chemical Methods in Prokaryotic Systematics*, pp. 493–521. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.
- Roggenkamp, A., Abele-Horne, M., Trebesius, K. H., Tretter, U., Autenreith, I. B. & Belesemann, J. (1998). *Abiotrophia elegans* sp. nov., a possible pathogen in patients with culture-negative endocarditis. *J Clin Microbiol* **36**, 100–104.