NOTE

***Facklamia tabacinasalis* sp. nov., from powdered tobacco**

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An unknown Gram-positive, catalase-negative, facultatively anaerobic, coccus-shaped organism originating as a contaminant of snuff was characterized by phenotypic and molecular taxonomic methods. Comparative 16S rRNA gene sequencing studies demonstrated that the bacterium represents a new subline within the genus *Facklamia*. The unknown bacterium was readily distinguished from *Facklamia hominis* and *Facklamia ignava* 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 tabacinasalis* sp. nov., the type strain of which is CCUG 30090^T.

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

The genus *Facklamia* was proposed by Collins *et al.* (1997) to accommodate a phylogenetically distinct group of Gram-positive, catalase-negative, chain-forming, coccus-shaped organisms encountered in human clinical specimens (including blood, urine, abscesses and vaginal swabs). The genus was originally monospecific, comprising the species *Facklamia hominis*. Two further species, *Facklamia ignava* (Collins *et al.*, 1998) and *Facklamia sourekii* (Collins *et al.*, 1999), also from clinical sources, have recently been assigned to the genus. In this article we report the results of a polyphasic taxonomic study on a biochemically unreactive *Facklamia*-like organism originating as a contaminant of powdered tobacco (snuff). On the basis of the presented findings, we consider that the unknown coccus represents a fourth species of the genus *Facklamia*, for which the name *Facklamia tabacinasalis* is proposed.

Strain CCUG 30090^T originated as an unknown Gram-positive, coccus-forming contaminant of snuff. The unidentified organism was cultured on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C, in air plus 5% CO₂. The strain was biochemically characterized by using the API Rapid ID32S 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.* (1994). For densitometric analysis, normalization and interpretation of protein patterns the GELCOMP GCW version 3.0 software package (Applied Maths) was used. The G + C content of DNA from the isolate was determined by thermal denaturation, as described by Garvie (1978). Phylogenetic analysis was conducted using 16S rRNA gene sequence analysis. A large fragment of the 16S rRNA gene (corresponding to positions 30–1521 of the *Escherichia coli* 16S rRNA gene) was amplified using the PCR with conserved primers close to the 3' and 5' ends of the gene. The PCR products were purified using the Pre-A-Gene kit (Bio-Rad) according to the manufacturer's instructions, and directly sequenced using a Taq Dye-Deoxy terminator cycle sequencing kit (Applied Biosystems) and an automatic DNA sequencer (Applied Biosystems model 373A). The closest known relatives of the new isolate 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) libraries and aligned with the newly determined sequence 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-parameter correction) (Felsenstein, 1989). A phylogenetic tree was constructed according to the neighbour-joining method with the program NEIGHBOR and the stability of the groupings was estimated by bootstrap analysis

The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 30090^T is Y17820.

Table 1. Characteristics that differentiate *F. tabacinasalis* sp. nov. from other *Facklamia* species

Test	<i>F. sourekii</i>	<i>F. ignava</i>	<i>F. hominis</i>	<i>F. tabacinasalis</i>
Acid from:				
D-Arabitol	+	—	—	—
Maltose	+	—	—	—
Mannitol	+	—	—	—
Sorbitol	+	—	—	—
Sucrose	+	—	—	—
Trehalose	+	v	—	—
Production of:				
Arginine dihydrolase	—	—	+	—
Alanine-phenylalanine-proline arylamidase	—	+	+	—
α -Galactosidase	—	—	+	+
β -Galactosidase	—	—	+	—
Hydrolysis of:				
Hippurate	+	+	+	—

v, Variable.

(200 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The unknown bacterium from snuff was ovoid in shape and was mainly formed into pairs; single cells and short chains were also seen. The strain was Gram-positive, non-spore-forming, catalase-negative, oxidase-negative and facultatively anaerobic. Using commercial API systems, the isolate failed to produce acid from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, maltose, mannitol, melibiose, melezitose, pullulan, raffinose, ribose, sorbose, sorbitol, sucrose, tagatose, trehalose and methyl β -D-glucopyranoside. The isolate displayed α -galactosidase and ester lipase C8 (weak reaction) activity. No activity was detected for arginine dihydrolase, acid phosphatase, alkaline phosphatase, alanine-phenylalanine-proline arylamidase, β -galacturonidase, β -galactosidase, β -glucuronidase, α -glucosidase, β -glucosidase, esterase C-4, lipase C14, α -fucosidase, α -mannosidase, β -mannosidase, *N*-acetylglucosaminidase, leucine arylamidase, glycyl-tryptophan arylamidase, cystine arylamidase, chymotrypsin, trypsin, valine arylamidase or urease. The strain did not hydrolyse hippurate and was Voges-Proskauer negative. On the basis of its morphology and general unreactivity in the battery of biochemical tests used, the unknown coccus somewhat resembled *F. hominis* and *F. ignava*. The snuff isolate, however, differed from *F. hominis* by its negative reactions for arginine dihydrolase, alanine-phenylalanine-proline arylamidase and β -galactosidase and in failing to hydrolyse hippurate. Similarly, the unknown bacterium differed from *F. ignava* in producing α -galactosidase, failing to produce alanine-phenylalanine-proline arylamidase and in failing to hydrolyse hippurate (Table 1). PAGE analysis of whole-cell proteins confirmed that the unknown coccus was different from the aforementioned *Facklamia*

spp. and further showed it to be distinct from all other Gram-positive, catalase-negative reference organisms examined, including *Abiotrophia* spp., *Aerococcus* spp., *Gemella* spp., *Alloiococcus otitis*, *Dolosigranulum pigrum* and *Globicatella sanguinis* (Fig. 1). To assess the phylogenetic position of the unknown coccus, its 16S rRNA gene sequence was determined and subjected to comparative analysis. Over 1400 bases were determined, and sequence searches of GenBank and RDP libraries revealed that the unknown bacterium was phylogenetically most closely associated with the lactic acid group of bacteria. A tree constructed by neighbour-joining depicting the phylogenetic affinity of the unknown coccus is shown in Fig. 2. The branching pattern of the tree indicates that the nearest relative of the unknown organism is *F. ignava*. The clustering together of these organisms occurred in 91 % of 200 tree replications. Despite the statistically significant association between the unknown coccus and *F. ignava*, a 16S rRNA sequence divergence value of 6.5 % clearly shows these taxa to be genealogically distinct.

It is evident from comparative 16S rRNA gene sequencing that the unidentified organism from snuff represents a new subline within the recently described genus *Facklamia*. The 16S rRNA sequence divergence values (greater than 6 %) between the unknown coccus and *F. hominis*, *F. ignava* and *F. sourekii* unequivocally demonstrate that the bacterium represents a hitherto unknown species. On the basis of these phylogenetic findings and the distinct electrophoretic whole-cell protein profile and biochemical reactions of the unknown coccus, we consider that this bacterium merits classification as a new species, for which the name *Facklamia tabacinasalis* is proposed. Tests that distinguish *F. tabacinasalis* from other members of the genus *Facklamia* are summarized in Table 1.

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

Fig. 2. Unrooted tree showing the phylogenetic relationships of *F. tabacinasalis* sp. nov. and some other Gram-positive bacteria having a low G+C content. The tree constructed using the neighbour-joining method was based on a comparison of approximately 1320 nucleotides. Bootstrap values, expressed as a percentage of 200 replications, are given at branching points. Bar, 1%.

Description of *Facklamia tabacinasalis* sp. nov.

Facklamia tabacinasalis (ta.ba.ci.na'sa.lis. M.L. n. *tabacum* tobacco; L. adj. *nasalis* pertaining to the nose; M.L. gen. fem. adj. *tabacinasalis* of snuff).

The cells are Gram-positive, non-spore-forming, large

cocci that occur mostly in pairs but also singly or in short-chains. They are α -haemolytic on horse blood. Colonies are of irregular size, reaching a maximum diameter of 0.5 mm after 48 h on blood agar incubated at 30 °C. The colonies are round, smooth and domed (raised) with a sharp border, and are non-pigmented.

The organism is facultatively anaerobic and catalase- and oxidase-negative. Using commercial API systems, acid is not produced from D-arabitol, L-arabinose, cyclodextrin, glycogen, lactose, melibiose, mannitol, maltose, melezitose, methyl β -D-glucopyranoside, pullulan, raffinose, ribose, sorbitol, sucrose, tagatose or trehalose. α -Galactosidase and ester lipase C8 (weak reaction) activity is detected. Activity is not detected for the following: alkaline phosphatase, acid phosphatase, alanine-phenylalanine-proline arylamidase, arginine dihydrolase, *N*-acetyl- β -glucosaminidase, chymotrypsin, trypsin, cystine arylamidase, glycyl-tryptophan arylamidase, α -fucosidase, β -galactosidase, β -galacturonidase, β -glucosidase, β -glucuronidase, esterase C-4, pyroglutamic acid arylamidase, α -mannosidase, β -mannosidase, leucine arylamidase and urease. Acetoin is not produced. Hippurate is not hydrolysed. The G + C content of DNA is 40 mol % (T_m). This organism is isolated from powdered tobacco. The type strain is CCUG 30090^T.

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