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Dolosicoccus paucivorans gen. nov., sp. nov., isolated from human blood

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Phenotypic and phylogenetic studies were performed on a hitherto undescribed Gram-positive, catalase-negative, chain-forming coccus isolated from human blood. Comparative 16S rRNA gene sequencing studies demonstrated that the unknown organism constitutes a new phylogenetic line, close to, but distinct from, *Facklamia* and *Globicatella*. The unknown bacterium was readily distinguished from currently recognized *Facklamia* species and *Globicatella sanguinis* 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 *Dolosicoccus paucivorans* gen. nov., sp. nov. The type strain of *Dolosicoccus paucivorans* is CCUG 39307^T.

Keywords: *Dolosicoccus paucivorans* sp. nov., taxonomy, phylogeny, 16S rRNA

In recent years, the taxonomy of the catalase-negative, Gram-positive cocci has undergone much change and improvement. Central to this development has been the use of phenotypic and molecular genetic methods in concert. In particular, such polyphasic approaches have resulted in much-improved taxonomic information and databases that facilitate not only the identification of established taxa but also the recognition of new diversity. As a result of these developments, combined with a growing interest in the role of the catalase-negative, Gram-positive cocci as opportunistic pathogens, many new genera and species have been described, e.g. *Abiotrophia elegans* (Roggenkamp *et al*., 1998), *Aerococcus urinae* (Aguirre & Collins, 1992a), *Alloiococcus otitis* (Aguirre & Collins, 1992b), *Dolosigranulum pigrum* (Aguirre *et al*., 1993), *Facklamia hominis* (Collins *et al*., 1997), *Facklamia ignava* (Collins *et al*., 1998b), *Globicatella sanguinis* (Collins *et al*., 1992), *Gemella bergeri* (Collins *et al*., 1998a) and *Helcococcus kunzii* (Collins *et al*., 1993). In this paper, we report the characteristics of an unusual biochemically unreactive, Gram-positive, chain-forming coccus from a clinical specimen, for which the name *Dolosicoccus paucivorans* gen. nov., sp. nov. is proposed. This report adds to the plethora of new catalase-negative, Gram-positive cocci described from human clinical sources in the past few years.

The 16S rRNA gene sequence of strain CCUG 39307^T has been deposited in GenBank under accession number AJ012666.

Strain 2992-95^T was sent by the Cleveland Clinic Microbiology Reference Laboratory (Cleveland, OH, USA) to the Centers for Disease Control and Prevention (CDC) (Atlanta, USA) for identification. The organism originated from a 78-year-old male patient who was a resident of a local nursing home. The patient was diagnosed with pneumonia 1 week prior to hospital admission. Physical examination was significant for bilateral exploratory wheeze and bilateral basilar rates. The organism was isolated from one of two sets of blood cultures. The organism was unidentifiable by the Cleveland Clinic Microbiology Laboratory and the Streptococcus Laboratory at the CDC. The patient was treated initially with cefuroxime and later with ceftazidime, and improved as a result until he was discharged in no apparent distress. The strain has been deposited in the Culture Collection of the University of Göteborg under accession number CCUG 39307^T. The unidentified organism was cultured on Columbia agar (Difco) supplemented with 5% horse blood at 37 °C, in air plus 5% CO_2. The strain was characterized biochemically by using the API Rapid ID32 Strep and API ZYM systems according to the manufacturer's instructions (API bioMérieux). The strain was also examined by using conventional biochemical and physiological tests as described by Facklam & Elliott (1995). PAGE analysis of whole-cell proteins was performed as described by Pot *et al*. (1994). The GelCompar GCW 3.0 software package (Applied Maths) was used for densitometric

analysis and normalization and interpretation of protein patterns. The G+C content of DNA of strain 2992-95^T (=CCUG 39307^T) was determined as described by Garvie (1978). The 16S rRNA genes of the isolate were amplified by PCR and sequenced directly 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 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) databases 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 Kimura's two-parameter correction) (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).

Morphologically, the isolate consisted of Gram-positive, ovoid cells that occurred singly, in pairs or in short chains. The organism was α-haemolytic and nonmotile. Using conventional tests employed by CDC, the organism produces pyroglutamic acid arylamidase but not leucine aminopeptidase. Gas production in MRS broth and bile-aesculin reactions were negative. No growth was observed at 10 or 45 °C or in broth containing 6·5% NaCl. No hydrolysis of aesculin, urea, starch or hippurate was observed. Pyruvate was not utilized and 0·04% tellurite was not tolerated. Acid was formed in conventional heart-infusion base medium from raffinose, ribose, sucrose, lactose (weak reaction) and maltose (weak reaction). No acid production was observed with L-arabinose, glycerol, inulin, melibiose, sorbitol, L-sorbose or trehalose. The strain was biochemically somewhat unreactive according to results obtained with the commercial API Rapid ID32 Strep system. Weak acid production was observed from mannitol and lactose but acid was not produced from D-arabitol, L-arabinose, cyclodextrin, glycogen, melibiose, maltose, melezitose, methyl β-D-glucopyranoside, *N*-acetylglucosamine, pullulan, D-raffinose, ribose, sorbitol, sucrose, D-tagatose or trehalose. Hippurate was not hydrolysed and acetoin was not produced. Using API systems, the unknown coccus tested positive for acid phosphatase, pyroglutamic acid arylamidase, esterase C4 (weak reaction) and ester lipase C8 (weak reaction). No activity was observed for alkaline phosphatase, arginine dihydrolase, alanine-phenylalanine-proline arylamidase, chymotrypsin, cystine arylamidase, α-galactosidase, β-galactosidase, β-galacturonidase, α-glucosidase, β-glucosidase, β-glucuronidase, lipase C14, α-fucosidase, α-mannosidase, β-mannosidase, glycyl-tryptophan arylamidase, leucine arylamidase, trypsin, valine arylamidase or urease. On the basis of its general lack of reactivity towards the carbohydrates tested in the API system, the unknown coccus showed some resemblance to *F. ignava* and *F. hominis*. However, the unidentified bacterium differed from *F. ignava* in not hydrolysing hippurate, not producing leucine aminopeptidase and not showing alanine-phenylalanine-proline arylamidase activity. The unknown coccus could also be distinguished from *F. hominis* in the aforementioned tests and by its negative reactions for arginine dihydrolase, α-galactosidase and β-galactosidase. The results of PAGE analysis of whole-cell proteins are shown in Fig. 1. These studies confirmed that the unknown bacterium was quite distinct from *F. ignava* and *F. hominis* and all other reference species of Gram-positive, catalase-negative cocci examined. To establish the phylogenetic position of the unknown coccus, its 16S rRNA genes were amplified by PCR and sequenced. Sequence searches of GenBank and RDP databases confirmed that the unidentified coccus was phylogenetically most-closely associated with the genera *Facklamia* and *Globicatella*. A tree depicting the phylogenetic position of the unknown coccus within the lactic-acid group of bacteria is shown in Fig. 2. The coccus formed a distinct line branching close to, but distinct from, *Facklamia* species and *Globicatella sanguinis*. Sequence divergence values from *F. ignava*, *F. hominis*, *Facklamia sourekii* and *Globicatella sanguinis* were $> 8\%$.

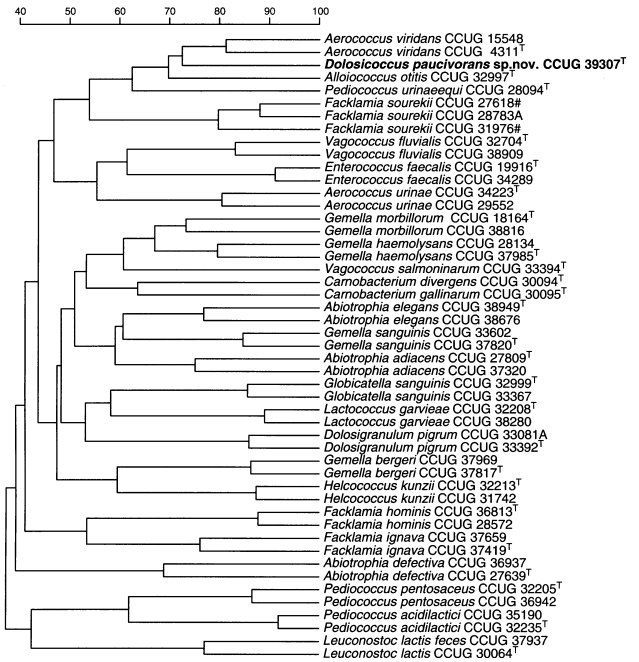

Fig. 1. Similarity dendrogram based on whole-cell protein pattern of *Dolosicoccus paucivorans* sp. nov. and related species. Levels of correlation are expressed as percentages of similarity for convenience.

It is evident from both the phenotypic and phylogenetic investigations that the unidentified coccus

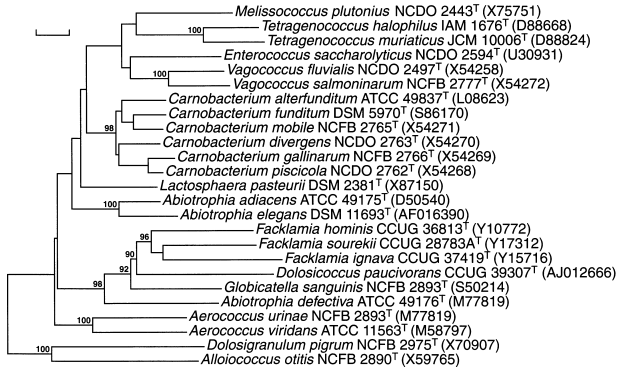

Fig. 2. Unrooted tree showing the phylogenetic relationships of *Dolosicoccus paucivorans* sp. nov. and some other low-G+C-content Gram-positive bacteria. The tree, constructed by the neighbour-joining method, was based on a comparison of approximately 1320 nucleotides. Bootstrap values, expressed as a percentage of 500 replications, are given at branching points. Bar, 1% sequence divergence.

represents yet another unknown bacterium from human clinical specimens. Phylogenetically, the unknown organism exhibits a loose association with *Globicatella sanguinis* and the *Facklamia* clade. However, the unknown coccus forms a deep and distinct subline, branching close to the base of these taxa. Bootstrap resampling showed that this association of the unknown bacterium with *Facklamia* and *Globicatella* is not particularly significant. PAGE analysis of whole-cell proteins showed the unknown coccus to be quite distinct from *Facklamia* species, *Globicatella sanguinis* and other reference Gram-positive cocci. Additionally, the unknown coccus was biochemically readily distinguished from all currently recognized Gram-positive, catalase-negative species. On the basis of both phenotypic and phylogenetic findings, we consider, therefore, that the unknown coccus merits classification in a new genus, for which the name *Dolosicoccus paucivorans* gen. nov., sp. nov. is proposed.

Description of *Dolosicoccus* gen. nov.

Dolosicoccus (Do.lo′si.coc.cus. L. adj. *dolosus* crafty, deceptive; Gr. n. *coccus* a grain; N.L. masc. n. *dolosicoccus* a deceptive coccus).

Cells are Gram-positive, non-spore-forming, non-motile cocci occurring as single cells, in pairs or in short chains. Facultatively anaerobic and catalase-negative. Growth does not occur at 10 or 45 °C or in broth containing 6·5% NaCl. Weak acid production but no gas from glucose. Hippurate, aesculin, gelatin, urea and starch are not hydrolysed. Acid phosphatase and pyroglutamic acid arylamidase are produced as

Table 1. Characteristics that differentiate *Dolosicoccus paucivorans* from *Facklamia* species and *Globicatella sanguinis*

Results were obtained from API Rapid ID32 Strep and API ZYM systems except for leucine aminopeptidase activity and growth in 6·5% NaCl. v, Variable; w, weak.

Characteristic	*D. paucivorans*	*F. ignava*	*F. hominis*	*F. sourekii*	*G. sanguinis*
Production of acid from:					
D-Arabitol	−	−	−	+	−
Glycogen	−	−	−	−	+
Lactose	+ (w)	−	−	−	v
Maltose	−	−	−	+	+
Mannitol	+ (w)	−	−	+	+
Melibiose	−	−	−	−	+
Pullulan	−	−	−	−	v
D-Raffinose	−	−	−	−	+
Ribose	−	−	−	−	+
Sorbitol	−	−	−	+	v
Sucrose	−	−	−	+	+
Trehalose	−	v	−	+	+
Hydrolysis of hippurate	−	+	+	+	+
Production of:					
Alanine-phenylalanine-proline arylamidase	−	+	+	−	+
Arginine dihydrolase	−	−	+	−	−
Leucine aminopeptidase	−	+	+	+	−
α-Galactosidase	−	−	+	−	+
β-Galactosidase	−	−	+	−	+
Growth in 6·5% NaCl	−	+	−	−	+

detected by the API system. Arginine dihydrolase and leucine aminopeptidase are not produced. Voges–Proskauer negative. Nitrate is not reduced. The G+C content of DNA is 40·5 mol% (T_m). The type species of the genus is *Dolosicoccus paucivorans*. As determined by 16S rRNA gene sequence analysis, the genus *Dolosicoccus* belongs to the lactic-acid group of bacteria with low DNA G+C content and is phylogenetically closely related to *Facklamia* species and *Globicatella sanguinis*, but may be distinguished from these taxa by the traits shown in Table 1.

Description of *Dolosicoccus paucivorans* sp. nov.

Dolosicoccus paucivorans (pau.ci′vo.rans. L. adj. *paucus* little; L. v. *vorase* to eat; N.L. adj. *paucivorans* eating little, relating to the observation that the organism utilizes few carbohydrates).

Cells are Gram-positive, ovoid in shape, occurring singly, in pairs or in short chains. Cells are non-pigmented, α-haemolytic and non-motile. Spores are not produced. Facultatively anaerobic, oxidase-negative and catalase-negative. Growth does not occur at 10 or 45 °C or in broth containing 6·5% NaCl. Gas production in MRS broth and bile-aesculin reactions are negative. Pyruvate is not utilized and 0·04% tellurite is not tolerated. No dextrans or levans are formed on 5% sucrose agar or broth. Using conventional heart-infusion base medium, acid is produced from D-raffinose, ribose, sucrose, lactose (weak reaction) and maltose (weak reaction). Acid is not produced from L-arabinose, glycerol, inulin, melibiose, sorbitol, L-sorbose or trehalose. Using the API Rapid ID32 Strep and API ZYM systems, weak acid production is observed from mannitol and lactose. Acid is not produced from L-arabinose, D-arabitol, cyclodextrin, glycogen, melibiose, maltose, melezitose, *N*-acetylglucosamine, pullulan, D-raffinose, ribose, sorbitol, sucrose, trehalose, D-tagatose or methyl β-D-glucopyranoside. Acid phosphatase, pyroglutamic acid arylamidase, esterase C4 (weak reaction) and ester lipase C8 (weak reaction) are produced. Alkaline phosphatase, arginine dihydrolase, alanine-phenylalanine-proline arylamidase, cystine arylamidase, α-galactosidase, β-galactosidase, β-galacturonidase, α-glucosidase, β-glucosidase, β-glucuronidase, leucine aminopeptidase, lipase C14, α-fucosidase, α-mannosidase, β-mannosidase, *N*-acetylglucosaminidase, glycyl-tryptophan arylamidase, chymotrypsin, trypsin, valine arylamidase and urease are not produced. Aesculin, starch and hippurate are not hydrolysed. Acetoin is not produced. No acid or clot is formed in litmus milk. Vancomycin sensitive. The G+C content of DNA is 40·5 mol%. The type and only strain of *Dolosicoccus paucivorans* is 2992-95^T (= CCUG 39307^T). Isolated from a human blood sample. Habitat is unknown.

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