Luteococcus sanguinis sp. nov., isolated from human blood

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An unusual catalase-positive, Gram-positive, coccus-shaped bacterium that originated from a human blood specimen was subjected to a polyphasic taxonomic study. Cell-wall murein and lipid composition analyses indicated that the unknown isolate was a member of the genus *Luteococcus*. The results of comparative 16S rRNA gene sequence analysis were consistent with chemotaxonomic findings and showed that the unidentified bacterium represents a hitherto unknown sublineage within the genus *Luteococcus* that is closely related to, but distinct from, *Luteococcus japonicus*. On the basis of both phenotypic and phylogenetic evidence, it is proposed that the unknown bacterium from human blood should be classified as *Luteococcus sanguinis* sp. nov., with the type strain CCUG 33897^T (=CIP 107216^T).

The genus *Luteococcus* contains two species at the time of writing: *Luteococcus japonicus* (type species of the genus) and *Luteococcus peritonei*. The genus was originally proposed by Tamura *et al.* (1994) to accommodate two Gram-positive, coccus-shaped bacterial strains, originally designated '*Micrococcus aurantiacus*' and *Micrococcus* sp., which were isolated in Japan from soil on Tokara Island and from water used for brewing 'miyamizu', respectively (Oda, 1935). These organisms differed from micrococci in that they contained LL-diaminopimelic acid (LL-DPM) in their walls and were found to represent a distinct lineage within the high-G+C *Actinobacteria*. Recently, a second rod-shaped member of the genus, *L. peritonei*, from a human clinical specimen, was described (Collins *et al.*, 2000). Phylogenetically, *L. japonicus* and *L. peritonei* form a distinct group within the family *Propionibacteriaceae* (Stackebrandt *et al.*, 1997), which embraces the genera *Propionibacterium*, *Propioniferax* and *Microlunatus*. Luteococci resemble most other species of this family in that they possess walls based on LL-DPM, but differ significantly in that they synthesize predominantly mono-unsaturated long-chain cellular fatty acids (Tamura *et al.*, 1994). In contrast, other members of the family *Propionibacteriaceae* produce major amounts of iso- and anteiso-methyl branched cellular fatty acids. In this article, we

report the results of a polyphasic taxonomic study of an unusual coccus-shaped organism isolated from a human blood culture, which resembles luteococci in that it contains exceptionally high levels of monounsaturated long-chain fatty acids. On the basis of biochemical and chemical criteria and the results of 16S rRNA gene sequence analysis, we conclude that the unknown isolate represents a third species of the genus *Luteococcus*, for which the name *Luteococcus sanguinis* sp. nov. is proposed.

Strain CCUG 33897^T was isolated from a blood sample (one out of four bottles) of a 32-year-old man and was submitted to the Culture Collection of the University of Göteborg (CCUG), Sweden, for identification. No other information is available on the source of the isolate. The unidentified bacterium was characterized biochemically by using the API Coryne and API ZYM systems (bioMérieux) according to the manufacturer's instructions. Cells were grown on chocolate horse blood agar (Oxoid) at 37 °C for cellular fatty acid determination and fatty acids were examined by using the MIDI system. Cell-wall murein and lipoquinone compositions of the isolate were determined as described by Schleifer & Kandler (1972) and Collins (1985), respectively. Phylogenetic analysis was performed by comparative 16S rRNA gene sequence analysis. A large fragment of the 16S rRNA gene of the isolate was amplified by PCR, using universal primers pA (5'-AGAGTTTGATCCTGGCTCAG; positions 8–27, *Escherichia coli* numbering) and pH* (5'-AAGGAGGTGATCCAGCCGCA; positions 1541–1522) and sequenced directly by using a *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied

Abbreviations: CCUG, Culture Collection of the University of Göteborg, Sweden; CIP, Collection of Bacterial Strains of the Institut Pasteur, France; LL-DPM, LL-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain CCUG 33897^T is AJ416758.

Biosystems) and an automatic DNA sequencer (model 373A; Applied Biosystems). 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 GenBank and aligned with the newly determined sequence by using the program DNATools (Rasmussen, 1995). The resulting multiple sequence alignment was corrected manually and a distance matrix was calculated with 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 stability of the groupings was estimated by bootstrap analysis (500 replications) by using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989). DNA-DNA reassociation experiments were carried out according to the spectrophotometric method of De Ley *et al.* (1970), using a Gilford System model 2600 spectrophotometer equipped with a Gilford model 2527-R thermoprogammer and plotter.

The unidentified isolate consisted of Gram-positive, non-motile cocci in 'bunches of grapes' formation. The strain was facultatively anaerobic and catalase-positive. When tested by using commercially available API kits, the organism produced acid from glucose, glycogen, mannitol, maltose, lactose and sucrose, but not from ribose or D-xylose. The organism reduced nitrate and hydrolysed aesculin and gelatin. Activities of acid phosphatase, alkaline phosphatase, chymotrypsin, ester lipase C8, cystine arylamidase, α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, leucine arylamidase, phosphoamidase, pyrrolidonyl arylamidase, pyrazinamidase, valine arylamidase (weak reaction) and trypsin were detected. All other enzyme tests gave negative results. Analysis of cell-wall acid hydrolysates of the unknown isolate revealed LL-DPM to be the dibasic amino acid present, whilst tetrahydrogenated menaquinone with nine isoprene units [MK-9(H₄)] was found to be the major respiratory quinone. This combination of chemical features is found in a number of *Actinobacteria*, including members of the family *Propionibacteriaceae*.

The long-chain cellular fatty acid composition of the unknown bacterium was examined and revealed the presence of predominantly monounsaturated fatty acid types (composition: C_{14:0}, 0.8%; C_{15:0}, 5.5%; C_{16:0}, 2.2%; C_{17:0}, 5.2%; C_{14:1}, 2.2%; C_{15:1}, 13.5%; C_{16:1}, 18%; C_{17:1}, 45.1%; C_{18:1}, 7.5%). The presence of such high levels (>80%) of monounsaturated fatty acids in bacteria is very unusual, but has been reported previously in *L. japonicus* (Tamura *et al.*, 1994) and *L. peritonei* (Collins *et al.*, 2000).

To assess the phylogenetic position of the unknown coccus, its partial 16S rRNA gene sequence (1350 bases) was determined and subjected to comparative analysis. Sequence searches of GenBank revealed that the unknown bacterium belonged phylogenetically to the *Actinobacteria* and displayed a specific association with members of the *Propionibacteriaceae*. Highest sequence similarity was shown to *L. japonicus* (96.9%) and *L. peritonei* (94.4%). Significantly lower levels of relatedness were shown to other members of the *Propionibacteriaceae* [*Propioniferax innocua*, *Microlunatus phosphovorus*, *Friedmanniella* spp. and *Propionibacterium* spp. (data not shown)]. A tree constructed by using the neighbour-joining method, depicting phylogenetic relationships of the unidentified isolate, is shown in Fig. 1 and demonstrates that the isolate represents a novel sublineage within the genus *Luteococcus*, with *L. japonicus* as its nearest relative. Branching of strain CCUG 33897^T with *L. japonicus* was found to be statistically significant (100% recovery in bootstrap resampling analysis). Given the close phylogenetic association of the unknown strain and *L. japonicus*, chromosomal DNA-DNA pairing was conducted on these organisms. A reassociation value of 49% was observed between isolate CCUG 33897^T and the type strain of *L. japonicus*, thereby demonstrating that they represent different species.

Polyphasic taxonomic analysis has shown that isolate CCUG 33897^T, which originated from a human blood sample, represents a hitherto unrecognized species within the family *Propionibacteriaceae*. Long-chain fatty acids of the unknown coccus closely resemble those of species of

Fig. 1. Unrooted tree based on 16S rRNA gene sequences, showing the phylogenetic relationships of *Luteococcus sanguinis* sp. nov. Numbers shown at nodes correspond to bootstrap values. Bar, 1% sequence divergence.

the genus *Luteococcus* and serve to distinguish the organism from other members of the family *Propionibacteriaceae* (*Propioniferax*, *Micrococcus*, *Friedmanniella* and *Propionibacterium*), which synthesize major amounts of methyl branched-chain fatty acids (e.g. Pitcher & Collins, 1991; Yokota *et al.*, 1994; Nakamura *et al.*, 1995; Schumann *et al.*, 1997). Treeing analysis of 16S rRNA gene sequence data supports the assignment of the unknown coccus to the genus *Luteococcus*. Both sequence divergence values and branching pattern considerations show that the unidentified organism is related more closely to *L. japonicus* than to *L. peritoneii*. However, 3% sequence difference between the 16S rRNA genes of the clinical isolate and *L. japonicus* demonstrates that these organisms represent separate species, a conclusion that is supported by chromosomal DNA–DNA pairing. Support for the separateness of the blood isolate comes also from its distinct biochemical characteristics. In particular, the production of API Coryne profile 7552177 readily serves to distinguish the blood isolate from *L. japonicus* and *L. peritoneii*, which display profiles 6572375 and 3752377, respectively. Therefore, based on biochemical, molecular chemical and molecular genetic evidence, we propose that isolate CCUG 33897^T from blood should be classified in the genus *Luteococcus* as *Luteococcus sanguinis* sp. nov.

Description of *Luteococcus sanguinis* sp. nov.

Luteococcus sanguinis (san'gui.nis. L. gen. n. *sanguinis* of blood).

Non-motile, Gram-positive cocci. Facultatively anaerobic and catalase-positive. Acid is produced from glucose, glycogen, mannitol, maltose, lactose and sucrose, but not from ribose or D-xylose. Aesculin and gelatin are hydrolysed. When tested by using commercial API Coryne and API ZYM systems, acid phosphatase (weak reaction), alkaline phosphatase, chymotrypsin, ester lipase C8 (weak), cystine arylamidase (weak), α -galactosidase, β -galactosidase, α -glucosidase, β -glucosidase, leucine arylamidase, phosphoamidase (weak), pyrrolidonyl arylamidase, pyrazinamidase, valine arylamidase (weak) and trypsin are detected. Esterase C4, lipase C14, α -fucosidase, β -glucuronidase, N-acetyl- β -glucosaminidase, α -mannosidase, pyroglutamic acid arylamidase and urease are not detected. Voges–Proskauer reaction is negative. Nitrate is reduced to nitrite. Cell wall contains LL-DPM. Long-chain cellular fatty acids are predominantly of the monounsaturated type. MK-9(H₄) is the major menaquinone. DNA G + C content is 64 mol%.

The type strain is CCUG 33897^T (= CIP 107216^T). Habitat is not known. Isolated from human blood. Pathogenic potential is not known.

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