NOTE

***Luteococcus peritonei* sp. nov., isolated from the human peritoneum**Matthew D. Collins,¹ Paul A. Lawson,¹ Natalia Nikolaitchouk^{2,3} and Enevold Falsen²Author for correspondence: Matthew D. Collins. Tel: +44 118 935 7226. Fax: +44 118 926 7917.
e-mail: m.d.collins@reading.ac.uk¹ Department of Food Science of Technology, University of Reading, Reading RG6 6AP, UK² Culture Collection, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden³ Department of Microbiology, Russian People's Friendship University, Moscow, Russia**An unusual catalase-positive pleomorphic Gram-positive rod isolated from a human clinical specimen was subjected to a polyphasic taxonomic analysis. Comparative 16S rRNA gene sequence analysis revealed the unknown bacterium was a member of the high G+C branch of the Gram-positive bacteria (*Actinobacteria*), and was phylogenetically a member of the family *Propionibacteriaceae*, with *Luteococcus japonicus* as its nearest relative. Based on both phenotypic and phylogenetic evidence, it is proposed that the unknown bacterium be classified in the genus *Luteococcus*, as *Luteococcus peritonei* sp. nov. The type strain of *Luteococcus peritonei* is CCUG 38120^T.****Keywords:** *Luteococcus peritonei*, taxonomy, phylogeny, 16S rRNA

The genus *Luteococcus* was proposed by Tamura *et al.* (1994) to accommodate two strains of a Gram-positive coccus-shaped bacterium which were originally isolated in Japan from soil and from water used for brewing 'miyamizu' (Oda, 1935). Currently only a single species, *Luteococcus japonicus*, is recognized. Phylogenetically *Luteococcus japonicus* is a member of the family *Propionibacteriaceae* (Stackebrandt *et al.*, 1997) which includes *Propionibacterium*, *Propioniferax* and *Microlunatus*. *Luteococcus japonicus* resembles most other species of this family in possessing a cell wall murein based on LL-diaminopimelic acid (LL-Dpm) but differs significantly in synthesizing predominantly (approx. 90% of total acids) mono-unsaturated long-chain cellular fatty acids (Tamura *et al.*, 1994). In contrast other members of the *Propionibacteriaceae* produce major amounts of iso- and anteiso- methyl-branched cellular fatty acids. During the course of a study of unusual *Actinobacteria* that cause or are associated with human disease, we have characterized a LL-Dpm-containing rod-shaped bacterium which resembles *Luteococcus japonicus* in possessing very high levels of monounsaturated cellular fatty acids. Based on the results of a polyphasic taxonomic study, we propose the unknown rod-shaped bacterium be classified in the genus *Luteococcus* as *Luteococcus peritonei* sp. nov.

Abbreviation: LL-Dpm, LL-diaminopimelic acid.The GenBank accession number for the 16S rRNA gene sequence of strain CCUG 38120^T is AJ132334.

Strain CCUG 38120^T was isolated from human peritoneum during a foetal autopsy and submitted to the Culture Collection of the University of Göteborg (CCUG) for identification. The unknown strain was biochemically characterized by using the API Rapid ID32 Strep, API CORYNE and API ZYM systems according to the manufacturer's instructions (API bioMérieux). Cells were grown on chocolate agar at 30 °C for cellular fatty acid determination and fatty acids were examined using the MIDI system. The cell wall composition of the isolate was determined as described by Schleifer & Kandler (1972) and respiratory quinone content as described by Collins (1985). Phylogenetic analysis was performed by comparative 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 by PCR using conserved primers close to the 3' and 5' ends of the gene. The PCR products were purified using a Prep-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 (model 373A; Applied Biosystems). The closest known relatives of the new isolate were determined by performing sequence 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

Fig. 1. Unrooted tree based on 16S rRNA showing the phylogenetic relationships of *Luteococcus peritonei* sp. nov. Branch lengths in the tree are additive and the figures shown at the nodes correspond to bootstrap values. Bar, 1% sequence divergence.

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 1320 bases was used for distance matrix analysis. A distance matrix was calculated using the programs PRETTY and DNADIST (using the Kimura 2-correction parameter) of the PHYLIP package (Felsenstein, 1989), and a tree was constructed by the neighbour-joining method with the program NEIGHBOR of the same package. The stability of the groupings was estimated by bootstrap analysis (500 replications) using the programs DNABOOT, DNADIST, NEIGHBOR and CONSENSE (Felsenstein, 1989).

The unknown isolate consisted of Gram-positive pleomorphic rod-shaped cells which were non-motile. The strain was facultatively anaerobic and catalase-positive. Using commercially available API kits the organism produced acid from glucose, lactose, mannitol, methyl β -D-glucopyranoside and sucrose, but not from L-arabinose, D-arabitol, cyclodextrin, glycogen, melibiose, melezitose, pullulan, ribose, raffinose, sorbitol, trehalose, tagatose or D-xylose. The organism hydrolysed aesculin but not gelatin or hippurate. Activities for α -galactosidase, β -galactosidase, β -galacturonidase, α -glucosidase, β -glucosidase, β -glucuronidase, leucine arylamidase and pyrazinamidase were detected but not for arginine dihydrolase, chymotrypsin, α -fucosidase, glycyl-tryptophan arylamidase, *N*-acetylglucosaminidase, lipase C14, α -mannosidase, β -mannosidase, pyroglutamic acid arylamidase, trypsin, valine arylamidase or urease. The organism reduced nitrate to nitrite and was Voges-Proskauer-negative. Analysis of the cell wall hydrolysates of the unknown isolate revealed LL-Dpm as the dibasic amino acid, whilst MK-9(II, III, H₄) was found to constitute the major respiratory menaquinone. This latter combination of chemical features is found in a number of *Actinobacteria* including members of the family *Propionibacteriaceae*. An examination of the long-chain cellular fatty acids of the unknown bacterium was, therefore, performed and revealed predominantly acids of the monounsaturated types (composition: C_{15:0}, 8%; C_{16:0}, 5%; C_{17:0}, 4.2%; C_{15:1},

21.4%; C_{16:1}, 17.5%; C_{17:1}, 37.5%; C_{18:1}, 6.4%). The presence of such high levels (>80%) of mono-unsaturated fatty acids is very unusual but has been previously reported in *Luteococcus japonicus* (Tamura *et al.*, 1994). Determination of the fatty acid composition of *Luteococcus japonicus* CCUG 38731^T, grown under similar conditions as the unknown organism, revealed a remarkably similar profile (composition: C_{14:0}, 0.8%; C_{15:0}, 8.6%; C_{16:0}, 5.4%; C_{17:0}, 3.9%; C_{18:0}, 0.9%; C_{15:1}, 20.2%; C_{16:1}, 14.2%; C_{17:1}, 44%; C_{18:1}, 2%). To investigate the phylogenetic position of the unknown isolate its almost complete 16S rRNA gene sequence was determined and subjected to a comparative analysis. Sequence database searches revealed the unknown bacterium was phylogenetically a member of the *Actinobacteria* and displayed a specific association with members of the *Propionibacteriaceae*. Highest sequence similarity was shown with *Luteococcus japonicus* (94%; 82 mismatches, two unmatched; 1395 bp), with *Propioniferax innocua* (91.5%; 114 mismatches, five unmatched; 1380 bp), *Micrococcus phosphovorius* (91.2%; 123 mismatches, four unmatched; 1397 bp), *Friedmanniella antarctica* (93.4%; 91 mismatches, two unmatched; 1395 bp) and *Propionibacterium* spp. (90–91.5%) also displaying high levels of relatedness. A tree constructed by the neighbour-joining method depicting the phylogenetic relationships of the unknown isolate is shown in Fig. 1 and demonstrates that it represents a new subline, adjacent to *Luteococcus japonicus*, within the *Propionibacteriaceae*. The branching point of the unknown strain CCUG 38120^T and *Luteococcus japonicus* was supported by 93% recovery in the bootstrap analysis.

The polyphasic taxonomic analysis has shown the unknown clinical isolate CCUG 38120^T originating from a foetal autopsy represents a hitherto unrecognized species within the *Propionibacteriaceae*. Phylogenetically the organism forms a distinct subline and has a statistically significant affinity with *Luteococcus japonicus*. This association between the unidentified rod and *Luteococcus japonicus* is strongly

supported by cellular fatty acid data. Both taxa are highly unusual in synthesizing predominantly mono-unsaturated long-chain fatty acids. All other recognized members of the *Propionibacteriaceae* (viz.: *Friedmanniella*, *Microhunatus*, *Propioniferax* and *Propionibacterium*) synthesize major amounts of methyl-branched-chain fatty acids (e.g. Pitcher & Collins, 1991; Yokata *et al.*, 1994; Nakamura *et al.*, 1995; Schumann *et al.*, 1997). Under other circumstances we would consider differences in cellular morphology combined with a 16S rRNA gene sequence divergence of 6% between the unknown rod and *Luteococcus japonicus* to possibly be indicative of different genera. However, the cellular fatty acid composition of *Luteococcus japonicus* is so unusual, that the occurrence of a virtually identical profile within the unknown rod, is in our opinion, sufficiently compelling to outweigh the aforementioned considerations. Therefore, based on molecular genetic and molecular chemical evidence, we propose the unknown isolate CCUG 38120^T be classified in the genus *Luteococcus*, as *Luteococcus peritonei* sp. nov.

Emended description of the genus *Luteococcus* Tamura, Takeuchi and Yokota

The description of the genus *Luteococcus* (Tamura *et al.*, 1994) should be emended to include the following: some cells consist of Gram-positive pleomorphic rods. Nitrate may or may not be reduced to nitrite.

Description of *Luteococcus peritonei* sp. nov.

Luteococcus peritonei (pe.ri.to.ne'i. L. n. *peritoneum* peritoneum; L. gen. neut. n. *peritonei* of the peritoneum).

Cells consist of pleomorphic Gram-positive rods. Cells are non-pigmented. Facultatively anaerobic and catalase-positive. Acid is produced from glucose, lactose, sucrose, mannitol and methyl β -D-glucopyranoside. Acid may or may not be produced from maltose. Acid is not produced from L-arabinose, D-arabitol, cyclodextrin, glycogen, melibiose, melezitose, pullulan, ribose, raffinose, sorbitol, trehalose, tagatose or D-xylose. Aesculin is hydrolysed but not gelatin or hippurate. α -Galactosidase, β -galactosidase, β -galacturonidase, α -glucosidase, β -glucosidase, β -glucuronidase, leucine arylamidase and pyrazinamidase are produced. Arginine dihydrolase, lipase C14, chymotrypsin, α -fucosidase, glycyl-tryptophan arylamidase, N-acetylglucosaminidase, α -mannosidase, β -mannosidase, pyroglutamic acid arylamidase, trypsin, valine arylamidase and urease are not produced. Activity for alkaline phosphatase and acid phosphatase may or may not be detected. Voges-Proskauer-negative. Nitrate is reduced to nitrite. Cell wall contains LL-Dpm. MK-9(H₄) is the predominant menaquinone. Synthesizes predominantly monounsaturated cellular fatty

acids. Habitat is not known. Isolated from human peritoneum. Pathogenic potential is not known. The G+C content of DNA is 65 mol %. The type strain is CCUG 38120^T.

Luteococcus peritonei can be distinguished from *Luteococcus japonicus* by its pleomorphic rod-shaped morphology. Additionally, using API systems and identical test conditions, *Luteococcus peritonei* differs from *Luteococcus japonicus* in producing acid from lactose and methyl β -D-glucopyranoside, producing β -glucuronidase, failing to produce pyrrolidonyl arylamidase, and by reducing nitrate.

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