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***Turicibacter sanguinis* gen. nov., sp. nov., a novel anaerobic, Gram-positive bacterium**

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An unknown, strictly anaerobic, Gram-positive, rod-shaped bacterium (strain MOL361^T) was isolated from a blood culture of a febrile patient with acute appendicitis and characterized using phenotypic and molecular methods. Fatty acid analysis and biochemical examination indicated that the isolate most closely resembles members of the Gram-positive bacteria with low DNA G+C content. 16S rDNA sequencing revealed a relatively high overall similarity (97%) to an uncultured bacterium, but these two strains both exhibit low (<87%) 16S rDNA similarity to other bacteria. Phylogenetic analysis with different treeing methods showed that this strain forms a novel line of descent within the Gram-positive bacteria with low G+C content. Strain MOL361^T is described as the type strain of a novel species within a new genus, *Turicibacter sanguinis* gen. nov., sp. nov.

Keywords: *Turicibacter sanguinis*, 16S rDNA

We report here on a novel bacterial species within a new genus. Strain MOL361^T was originally isolated from a blood culture (BacT/Alert) of a febrile, 35-year-old man with acute appendicitis and sent to our laboratory in an anaerobic liquid medium (thioglycollate) for characterization. Traditional bacteriology failed in identification; therefore, the strain was characterized using routine molecular methods in our lab (Goldenberger *et al.*, 1997). 16S rDNA sequencing is a very powerful tool for the identification of bacteria. Tang *et al.* (1998) showed that 16S rDNA sequencing of 72 unusual aerobic, Gram-negative bacteria was an excellent and rapid method for identification to the genus (97%) and species (89%) levels. Drancourt *et al.* (2000) used 16S rDNA analysis for a large collection of 177 bacterial isolates unidentifiable by phenotypic means. Thus, 90 and 79% were respectively identified to the genus and species level. Sequencing of the 5' end of the 16S rDNA of strain MOL361^T showed very low similarities (< 76% in 492 bp) to known bacteria. We decided to investigate this strain by biochemical and molecular means.

Strain MOL361^T was cultivated on different standard media under anaerobic and aerobic conditions. Presumptive identification was performed according to Engelkirk *et al.* (1992a). A chopped meat carbohydrate broth was inoculated for the detection of fermentation products. Biochemical characteristics were determined by using the RapID Ana II (Remel) and API 50 CHB (bioMérieux) systems according to the manufacturers' instructions. Consumption of additional carbohydrates such as gluconate and glucuronate was tested in a defined minimal broth containing 1% (w/v) of these compounds. The minimal broth contained 2 g K₂HPO₄, 1 g KH₂PO₄, 1 g (NH₄)₂SO₄, 0.5 g MgSO₄ · 7H₂O, 0.5 g NaSO₄ and 0.01 g FeSO₄ l⁻¹ (pH 7.4). The optimum pH for growth was determined with minimal broth containing maltose. Cellular fatty acid compounds were analysed with the MIDI system (Microbial Identification) as described previously (von Graevenitz *et al.*, 1994). Spore formation was tested by stress due to starvation and due to penicillin and with sheep blood and chocolate agar, each containing 0.5 and 10 mg manganese ions l⁻¹. Sporulation was also tested with the heat test and alcohol test as described in Engelkirk *et al.* (1992b). Determination of the G+C content of DNA was performed according to Mesbah & Whitman (1989).

The 16S rRNA gene was amplified using primers 1 and 12 (Table 1) and the amplicon was sequenced bidirectionally with 12 different primers (Table 1) using the

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A colour Gram-stain image of the novel isolate is available as supplementary material in IJSEM Online (<http://ijs.sgmjournals.org/>).

The GenBank accession number for the 16S rDNA sequence of strain MOL361^T is AF349724.

Table 1. Primers for amplification and sequencing of total 16S rDNA

Primers 1 and 12 were used for amplification and primers 1–12 were used for sequencing. Positions are given according to the *E. coli* numbering.

Primer	Sequence (5'→3')	Position
1. Bact-8s-20	AGAGTTTGATCMTGGCTCAG	8–27
2. Bact-339a-17	GCTGCCTCCCGTAGGAG	355–339
3. Bact-341s-17	CCTACGGGAGGCAGCAG	341–357
4. Bact-536a-18	GTATTACCGCGGCTGCTG	536–519
5. Bact-519s-18	CAGCAGCCGCGGTAATAC	519–536
6. Bact-787a-20	GGACTACHAGGGTATCTAAT	806–787
7. Bact-787s-20	ATTAGATACCCTDGTAGTCC	787–806
8. Bact-907s-20	AAACTCAAAGGAATTGACGG	907–926
9. Bact-1098a-17	GGGTTGCGCTCGTTGCG	1114–1098
10. Bact-1098s-17	CGCAACGAGCGCAACCC	1098–1114
11. Bact-1389s-18	CTTGTACACACCGCCCGT	1389–1406
12. Bact-1525a-17	AAGGAGGTGATCCARCC	1541–1525

Fig. 1. Gram stain (a) and transmission electron micrograph (b) of isolate MOL361^T from a 3 day culture on chocolate agar. Bars, 20 (a) and 2 (b) μ m. A colour version of the Gram-stain image is available as supplementary material in IJSEM Online (<http://ijs.sgmjournals.org/>).

BigDye kit and an automatic DNA sequencer (Applied Biosystems; model 310). The primers, except numbers 6 and 7, were derived from Lane (1991). Most of them were modified so that all have approximately the same melting temperature and are specific for the domain *Bacteria*. Primer 6 was described previously (Fischer-Romero *et al.*, 2000) and primer 7 is the reverse-complement of primer 6. The closest relatives of the novel isolate were determined by performing database searches with the FASTA algorithm. In addition, the entire sequence (homologous to *Escherichia coli* positions 8–1542) was fitted into an alignment of about 6500 full or partial primary structures provided with the ARB software package (Strunk *et al.*, 1999). The sequence was initially aligned by using the ARB automatic aligner and then verified and corrected manually. The sequence was then added to the consensus tree

provided in the ARB database by the maximum-parsimony approach. To avoid possible treeing artefacts caused by multiple mutational changes and/or regions that could not be aligned unambiguously, 50% conservation filters for different taxonomic levels were generated by using the appropriate tool of the ARB package. Only those positions that contained identical residues in at least 50% of all sequences of interest were included in the analysis. In addition, phylogenetic analysis was performed using PHYLIP (Felsenstein, 1989). The 16S rDNA sequences of strain MOL361^T and of 15 reference organisms covering most groups of bacteria were aligned with the program PILEUP (Devereux *et al.*, 1984) using *E. coli* as an outgroup. Phylogenetic trees were constructed with the neighbour-joining, unweighted pair group method with averages (UPGMA) and maximum-parsimony

methods using the programs SEQBOOT, DNADIST, NEIGHBOR, DNAPARS and CONSENSE.

Cells of strain MOL361^T were Gram-positive, non-spore-forming, irregularly shaped rods of approximately 0.5–2.0 × 0.7–7.0 µm, which formed chains up to 30 µm long (Fig. 1). They formed non-haemolytic colonies on sheep-blood agar. On solid media, the colonies were greyish white with a convex elevation, but irregular shape with spreading, undulating margins. When cultivated in thioglycollate medium, the strain behaved like a fungus and the mycelial material could not be sedimented by centrifugation. By microscopy, long (up to 30 µm) filaments with thick bulbs were then detected. Growth was observed anaerobically on sheep-blood, chocolate and Brucella agar and in chopped meat carbohydrate and thioglycollate. Growth was found to be optimal at 37 °C, with a range from 25 to 46 °C. In the minimal broth containing maltose, the pH optimum for growth was 7.5; no growth occurred at pH ≤ 6.5 or pH ≥ 8.0. The novel bacterium could not be recultivated when preserved at –70 °C for 1 day in skim milk, Protector tubes (TSC) or glycerol (10, 40 or 70 %, respectively). Only lyophilized cultures could be revived in appropriate medium.

The strain was negative for catalase and oxidase, susceptible to penicillin, vancomycin and kanamycin, but resistant to colistin. Nitrate was not reduced. Lactate and minimal amounts of acetate were the only fermentation products. In the RapID Ana II system, α-glucosidase, α-galactosidase, glycine and serine were positive, whereas all other tests including β-galactosidase and indole remained negative. In contrast, β-galactosidase was positive in the API 20 E system. This result was confirmed with *o*-nitrophenyl β-D-galactopyranoside discs for detecting β-galactosidase (Sanofi Diagnostics Pasteur). The RapID Ana II system identified the isolate as *Propionibacterium propionicus*, but with a low bioscore (1/46 538). In the API 50 CHB system, strain MOL361^T fermented only maltose and (weakly) 5-ketogluconate. All other reactions remained negative. No additional carbohydrates were utilized when tested in minimal broth. In the API system, the strain was identified as *Brevibacillus brevis* (T = 0.80, %id = 96.6). The fatty acid composition showed predominantly C_{16:0} (37%), C_{18:0} (15.5%) and C_{18:1ω9c} (14.5%). The DNA of strain MOL361^T had a G + C content of 36.9 mol %.

In order to investigate the phylogenetic position of the isolate, comparative 16S rRNA gene sequence analysis was performed. Amplification with primers 1 and 12 (*E. coli* positions 8–1541) produced a 1510 bp fragment. Sequencing with the 12 primers proved to be very well adapted for obtaining full 16S rDNA sequences. Database searches revealed a close relationship (97% overall 16S rDNA sequence similarity) to an uncultured bacterium, S24-10 (unpublished accession no. AJ400262; N. Salzman, Y. Paterson, H. J. M. Harmsen and G. W. Welling). With the next most closely related organisms, e.g. members of the

Fig. 2. 16S rRNA-based tree reflecting the phylogenetic position of strain MOL361^T and the uncultured bacterium S24-10 within the Gram-positive bacteria with low DNA G + C content. The tree is based on the results of an optimized maximum-parsimony analysis of a dataset of about 6500 small-subunit rRNA sequences as provided with the ARB software package (Strunk *et al.*, 1999). The sequences were added to this consensus tree by the maximum-parsimony approach. The numbers of sequences that are represented by each group are quoted. Bar, 10% estimated sequence divergence.

Bacillus group, the strain shared less than 88% sequence similarity.

Different treeing methods resulted in different tree topologies. All trees have in common that strain MOL361^T, together with the uncultured bacterium S24-10, branched deeply in the phylogenetic group of Gram-positive bacteria with low DNA G + C content. Fig. 2 shows the tree that was constructed by the maximum-parsimony approach as implemented in the ARB package. The tree illustrates that the isolate is distantly related to other groups of bacteria. This phylogenetic position of strain MOL361^T was supported by most trees generated with the ARB program using different datasets and different filters. However, in a few cases, strain MOL361^T and the uncultured bacterium S24-10 were placed at other positions within the Gram-positive bacteria with low DNA G + C content, e.g. next to the genera *Gemella* or *Paenibacillus*. Phylogenetic analysis with the PHYLIP package placed strain MOL361^T either next to *Paenibacillus macerans* (UPGMA and maximum-parsimony with bootstrap values of 52 and 39 %, respectively) or next to *Gemella haemolysans* (neighbour-joining with a bootstrap value of 53 %).

The establishment of rapid sequence analysis of 16S rRNA has proved to be very helpful in exploring microbial phylogeny, especially when looking at moderately related species (Stackebrandt & Goebel, 1994; Woese, 1987). However, the order of branching among Gram-positive bacteria (*Firmicutes*) on the basis of 16S rRNA sequences has to be interpreted with care. Ahmad *et al.* (1999) have compared three phylogenetic

trees generated from 16S rRNA, DnaK (Hsp70) and DnaJ (Hsp40). They found significant discrepancies in the branching order of the three trees. In the present study, the phylogenetic position of strain MOL361^T and the uncultured bacterium S24-10 could not be determined with certainty. This indicates that these two strains represent a hitherto unrecognized line of descent within the Gram-positive bacteria with low DNA G+C content. They share very low sequence similarities to other groups and therefore can not be easily placed in phylogenetic trees. The variable positioning also points towards the fact that the relatedness among the *Firmicutes* is not fully established at present.

On the basis of all of the data obtained, we conclude that strain MOL361^T merits classification in a new genus, for which the name *Turicibacter sanguinis* gen. nov., sp. nov. is proposed.

During the course of revision of this paper, a bacterium with an identical 16S rRNA sequence was isolated in Gothenburg (Sweden) from a blood culture of a febrile (38.5 °C), 79-year-old woman with a CRP (C-reactive protein) of 120 (C. W. Olsson, personal communication). After treatment with cefuroxim, she soon recovered.

Description of *Turicibacter* gen. nov.

Turicibacter (Tu.ri.ci.bac'ter; L. neut. n. *Turicum* the Latin name of Zürich; N.L. masc. n. *bacter* equivalent of Gr. neut. n. *bakterion* a small rod; N.L. masc. n. *Turicibacter* a rod-shaped organism from Zürich, Switzerland, where the bacterium was first isolated).

Anaerobic, Gram-positive, non-spore-forming bacteria. So far, only one species, the type species *Turicibacter sanguinis*, has been described.

Description of *Turicibacter sanguinis* sp. nov.

Turicibacter sanguinis (san'gui.nis. L. masc. gen. n. *sanguinis* of blood, indicating that the bacterium was isolated from a blood culture).

Gram-positive, long, irregular rod-shaped, chain-forming cells, 0.5–2.0 × 0.7–7.0 µm, non-spore-forming. Colonies are greyish white with a convex elevation, but irregular form with spreading, undulating margins. Chemo-organotrophic, strictly anaerobic, fermentative metabolism. Growth is observed at 25–46 °C, with an optimum at 37 °C. No growth occurs at pH ≤ 6.5 or pH ≥ 8.0; the pH optimum is 7.5. Catalase and oxidase are negative. Maltose and 5-ketogluconate are the only carbohydrates utilized. Activities for α-glucosidase, α- and β-galactosidase are detected. Nitrites are not reduced. Indole is not produced. Lactate is the main fermentation product. The major cellular fatty acids are C_{16:0} (37%), C_{18:0} (15.5%) and C_{18:1ω9c} (14.5%). The DNA has a G+C content of 36.9 mol %. Phylogenetic analysis shows low relatedness to other bacteria (< 88% 16S rDNA similarity) and reveals that this novel species is deeply branching in the tree of

Gram-positive bacteria with low DNA G+C content, i.e. next to the *Mollicutes*.

The type strain is MOL361^T (= DSM 14220^T = NCCB 100008^T).

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References

- Ahmad, S., Selvapandiyan, A. & Bhatnagar, R. K. (1999). A protein-based phylogenetic tree for Gram-positive bacteria derived from *hrcA*, a unique heat-shock regulatory gene. *Int J Syst Bacteriol* **49**, 1387–1394.
- Devereux, J., Haeberli, P. & Smithies, O. (1984). A comprehensive set of sequence analysis programs for the VAX. *Nucleic Acids Res* **12**, 387–395.
- Drancourt, M., Bollet, C., Carliz, A., Martelin, R., Gayral, J. P. & Raoult, D. (2000). 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J Clin Microbiol* **38**, 3623–3630.
- Engelkirk, P. G., Duben-Engelkirk, J. & Dowell, V. R., Jr (1992a). Presumptive identifications. In *Principles and Practice of Clinical Anaerobic Bacteriology*, pp. 147–180. Belmont, CA: Star Publishing.
- Engelkirk, P. G., Duben-Engelkirk, J. & Dowell, V. R., Jr (1992b). Laboratory procedures. In *Principles and Practice of Clinical Anaerobic Bacteriology*, pp. 331–357. Belmont, CA: Star Publishing.
- Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.
- Fischer-Romero, C., Lüthy-Hottenstein, J. & Altwegg, M. (2000). Development and evaluation of a broad-range PCR-ELISA assay with *Borrelia burgdorferi* and *Streptococcus pneumoniae* as model organisms for reactive arthritis and bacterial meningitis. *J Microbiol Methods* **40**, 79–88.
- Goldenberger, D., Künzli, A., Vogt, P., Zbinden, R. & Altwegg, M. (1997). Molecular diagnosis of bacterial endocarditis by broad-range PCR amplification and direct sequencing. *J Clin Microbiol* **35**, 2733–2739.
- Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.
- Mesbah, M. & Whitman, W. B. (1989). Measurement of deoxyguanosine/thymidine ratios in complex mixtures by high-performance liquid chromatography for determination of the mole percentage guanine + cytosine of DNA. *J Chromatogr* **479**, 297–306.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Strunk, O., Gross, O., Reichel, B. & 11 other authors (1999). ARB: a software environment for sequence data. Department of Microbiology, Technische Universität München, Munich, Germany. Online posting date 25 March 1999. <http://www.mikro.biologie.tu-muenchen.de>
- Tang, Y. W., Ellis, N. M., Hopkins, M. K., Smith, D. H., Dodge, D. E. & Persing, D. H. (1998). Comparison of phenotypic and genotypic techniques for identification of unusual aerobic pathogenic gram-negative bacilli. *J Clin Microbiol* **36**, 3674–3679.
- von Graevenitz, A., Pünter, V., Gruner, E., Pfyffer, G. E. & Funke, G. (1994). Identification of coryneform and other gram-positive rods with several methods. *APMIS* **102**, 381–389.
- Woese, C. R. (1987). Bacterial evolution. *Microbiol Rev* **51**, 221–271.