Ornithinibacillus contaminans sp. nov., an endospore-forming species

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A Gram-stain-positive, endospore-forming rod, designated CCUG 53201^T, was isolated from a human blood sample of a 75-year-old woman. 16S rRNA gene sequence-based phylogenetic analysis showed that strain CCUG 53201^T clustered with the type strains of species of the genus *Ornithinibacillus*. Strain CCUG 53201^T was most closely related to *Ornithinibacillus bavariensis* WSBC 24001^T and *Ornithinibacillus californiensis* DSM 16628^T (97.9 and 98.7 % 16S rRNA gene sequence similarity, respectively). Strain CCUG 53201^T contained a peptidoglycan of type A4β L-Orn–D-Asp. The quinone system was composed of the menaquinone MK-7 and small amounts of MK-6. The polar lipid profile of strain CCUG 53201^T consisted of major amounts of diphosphatidylglycerol and an unidentified phospholipid, moderate amounts of phosphatidylglycerol and another two unidentified phospholipids and minor amounts of several other components. The fatty acid profile comprised mainly anteiso- and iso-branched fatty acids and was in accordance with those of members of the genus *Ornithinibacillus*. The polyamine pattern exhibited the major compounds spermidine and spermine. The results of physiological and biochemical tests and DNA–DNA hybridization allowed the phenotypic and genotypic differentiation of strain CCUG 53201^T from its closest phylogenetic neighbours. We propose a novel species with the name *Ornithinibacillus contaminans* sp. nov., with type strain CCUG 53201^T (=DSM 22953^T).

The genus *Ornithinibacillus* was proposed by Mayr *et al.* (2006) and comprises two species, *Ornithinibacillus bavariensis* from pasteurized milk in Bavaria, Germany, and *Ornithinibacillus californiensis* from coastal surface sediments in California, USA. The genus is characterized by having L-ornithine as the diagnostic diamino acid in the peptidoglycan (which is a major differentiating feature from the genus *Oceanobacillus*) and MK-7 as the characteristic menaquinone.

Strain CCUG 53201^T was isolated as a contaminant from a human blood sample of a 75-year-old woman in Sweden. The strain was isolated on blood agar at 37 °C and maintained on tryptone soy agar (TSA; Oxoid) at 30 °C. Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was examined with a Zeiss

light microscope at ×1000 magnification, using cells harvested from TSA after 24 h at 30 °C. Cells of strain CCUG 53201^T were Gram-stain-positive, strictly aerobic, non-motile rods (0.8–1.0 × 2.0–3.0 µm) that formed spherical endospores in a terminal position.

The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). Amplification of the 16S rRNA gene was performed with the primer pair 27F (5′-GAGTTTGAT-CMTGGCTCAG-3′) and MR 1492R (5′-ACGGYTACCT-TGTTACGACTT-3′) (Lane, 1991) and sequencing was performed using standard sequencing primers for the 16S rRNA gene. The 16S rRNA gene sequence of strain CCUG 53201^T was a continuous stretch of 1254 bp. Phylogenetic analysis was performed using ARB software (version December 2007; Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 95 database (version July 2008; Pruesse *et al.*, 2007). Trees were constructed using the maximum-likelihood method with fastDNaml (Olsen *et al.*, 1994) and

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain CCUG 53201^T is FN597064.

topologies were tested without a conservation filter. Distances were calculated using MEGA (version 4.0; Tamura *et al.*, 2007) using the distance options according to the Kimura-2 model. Maximum-likelihood (Fig. 1) and neighbour-joining (not shown) trees showed that strain CCUG 53201^T clustered consistently within the genus *Ornithinibacillus*. The closest relatives of strain CCUG 53201^T were *O. californiensis* MB-9^T (98.7 % 16S rRNA gene sequence similarity) and *O. bavariensis* WSBC 24001^T (97.9 %).

The peptidoglycan of strain CCUG 53201^T was isolated after disintegrating cells with glass beads in a Vibrogen cell mill (D-72408; Johanna Otto, Bodelshausen, Germany) and subsequent trypsin digestion according to the method of Schleifer & Seidl (1985). The peptidoglycan structure was analysed using the methods of Schleifer (1985). The peptidoglycan contained the amino acids ornithine, alanine, glutamic acid and aspartic acid. The peptide L-Orn-D-Asp, which is stable under the conditions for peptidoglycan hydrolysis (4 M HCl, 100 °C, 16 h), was detected. The partial hydrolysate (4 M HCl, 100 °C, 0.75 h) contained the peptides L-Ala-D-Glu and D-Ala-L-Orn-D-Asp. From these results, it was concluded that strain CCUG 53201^T showed the peptidoglycan type A4 β L-Orn-D-Asp (Schleifer & Kandler, 1972), which was in agreement with its affiliation to the genus *Ornithinibacillus* (Mayr *et al.*, 2006). Polyamines, quinones and polar lipids were extracted from cells of strain CCUG 53201^T after

cultivation on PYE medium (0.3 % peptone from casein, 0.3 % yeast extract; pH 7.2) at 28 °C and analysed as reported elsewhere (Altenburger *et al.*, 1996, 1997; Tindall, 1990a, b). HPLC analysis of polyamines and quinones was carried out using the apparatus reported by Stolz *et al.* (2007). The polyamine pattern of strain CCUG 53201^T was composed of the major amines spermidine and spermine [6.5 and 2.3 μ mol (g dry weight)⁻¹, respectively] and small amounts of putrescine and cadaverine [<0.3 μ mol (g dry weight)⁻¹]. This polyamine pattern is in agreement with those of other mesophilic bacilli, although the relative proportion of spermine is usually lower. In contrast, thermophilic bacilli have been shown to exhibit major amounts of spermine and only moderate amounts of spermidine (Hamana *et al.*, 1989, 1993; Meier-Stauffer *et al.*, 1996). The quinone system of strain CCUG 53201^T consisted of menaquinones MK-7 (93 %) and MK-6 (7 %). This trait is shared by the majority of bacilli, including members of the genus *Ornithinibacillus* (Mayr *et al.*, 2006).

For the detection of polar lipids, spray reagents were applied to separate plates. Molybdatophosphoric acid was used to detect total lipids, ninhydrin and molybdenum blue were used in succession, ninhydrin first to detect lipids with an amino group and molybdenum blue second to detect lipids with a phosphate group, and α -naphthol was used to detect lipids with a sugar moiety. In the polar lipid profile (Fig. 2), diphosphatidylglycerol and an unidentified phospholipid (PL1) predominated. Moderate

Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences showing the position of strain CCUG 53201^T among members of the genus *Ornithinibacillus* and related genera. Bar, 0.10 substitutions per nucleotide position.

Fig. 2. Polar lipid profile of strain CCUG 53201^T separated by two-dimensional TLC. The chromatogram is shown with the first dimension horizontally. DPG, Diphosphatidylglycerol; PG, phosphatidylglycerol; AL, unidentified aminolipid; GL, unidentified glycolipid; PL, unidentified phospholipid; L, unidentified polar lipid.

amounts of phosphatidylglycerol and another two unidentified phospholipids (PL2, PL3) were found and minor to trace amounts of three unidentified glycolipids, a fourth phospholipid, an aminolipid and a polar lipid were detected. This polar lipid profile shared several of the major characteristics reported for *Ornithinibacillus* species, including the major compound diphosphatidylglycerol and the presence of phosphatidylglycerol and an unidentified phospholipid (PL3) that exhibited chromatographic behaviour similar to that of PL10 of *O. bavariensis* WSBC 24001^T (Mayr *et al.*, 2006). The presence of PL1 and PL2 has not been shown before for *Ornithinibacillus* species, but it appeared that the diphosphatidylglycerol spot may have previously overlaid the PL1 spot because of poor chromatographic separation. However, the presence of two unidentified phospholipids with chromatographic behaviour similar to that of PL1 and PL2 has been reported for representatives of genera closely related to the genus *Ornithinibacillus*, such as *Virgibacillus* and *Gracilibacillus* (Wainø *et al.*, 1999).

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain CCUG 53201^T contained anteiso- and iso-branched fatty acids and was very similar to those of type strains of species of the genus *Ornithinibacillus* (Table 1).

Results from comparative physiological characterization of strain CCUG 53201^T and the type strains of *O. californiensis* and *O. bavariensis* are given in Table 2. All of the strains were inactive in most biochemical tests and showed only a few positive results after prolonged incubation. DNA–DNA hybridization was performed according to the method of

Table 1. Comparison of the cellular fatty acids of strain CCUG 53201^T with its closest phylogenetic neighbours

Strains: 1, *Ornithinibacillus contaminans* sp. nov. CCUG 53201^T; 2, *O. bavariensis* DSM 15681^T; 3, *O. californiensis* DSM 16628^T. Values are percentages of total fatty acids and were obtained in this study; fatty acids accounting for <0.5% of the total content in all strains are omitted.

Fatty acid	1	2	3
Saturated			
C _{14:0}	—	1.4	0.6
C _{15:0}	1.1	1.2	—
C _{16:0}	2.3	10.9	4.4
C _{18:0}	0.6	—	—
Unsaturated			
C _{16:1} ω7c alcohol	—	2.4	4.4
C _{16:1} ω11c	—	4.0	1.5
iso-C _{17:1} ω10c	—	1.3	—
Branched			
iso-C _{13:0}	—	0.6	—
iso-C _{14:0}	1.3	4.5	10.0
iso-C _{15:0}	24.2	33.3	31.7
anteiso-C _{15:0}	34.5	16.2	21.9
iso-C _{16:0}	4.0	8.9	18.2
iso-C _{17:0}	6.1	7.0	1.9
anteiso-C _{17:0}	27.5	7.4	5.3
Summed feature 4*	—	0.7	—

*Summed features represent two or three fatty acids that cannot be separated by the Microbial Identification System. Summed feature 4 consisted of iso-C_{17:1} I and/or anteiso-C_{17:1} B.

Ziemke *et al.* (1998). DNA–DNA relatedness between strain CCUG 53201^T and *O. bavariensis* DSM 15681^T and *O. californiensis* DSM 16628^T was 40 and 31%, respectively.

The peptidoglycan type, quinone system and fatty acid and polar lipid profiles, as well as 16S rRNA gene sequence analysis, showed that strain CCUG 53201^T is affiliated with the genus *Ornithinibacillus*. On the basis of the observed phenotypic differences, DNA–DNA relatedness and the differences in the 16S rRNA gene sequences, we propose a novel species, *Ornithinibacillus contaminans* sp. nov.

Description of *Ornithinibacillus contaminans* sp. nov.

Ornithinibacillus contaminans (con.ta'mi.nans. L. part. adj. *contaminans* contaminating, polluting, referring to the isolation of the type strain as a contaminant of a human blood sample).

Cells are Gram-stain-positive, strictly aerobic rods (0.8–1.0 × 2.0–3.0 μm) and non-motile. Spherical endospores are formed in a terminal position. Colonies grown on TSA are circular, convex and beige, becoming brownish after prolonged incubation. On TSA, grows at 20–45 °C (optimum 30 °C), but not at 10 or 50 °C, and at

Table 2. Differential phenotypic and physiological characteristics of strain CCUG 53201^T and its closest phylogenetic neighbours

Strains: 1, *O. contaminans* sp. nov. CCUG 53201^T; 2, *O. bavariensis* DSM 15681^T; 3, *O. californiensis* DSM 16628^T. Data for reference strains were taken from Mayr *et al.* (2006) unless indicated. All strains formed rod-shaped cells, were positive for catalase activity and grew at 30 and 37 °C. +, Positive; w, weak; –, negative.

Characteristic	1	2	3
Colony colour*	BE	BO	BO
Spore position†	C	T	T
Motility	–	+	+
Oxidase	+	–‡	+‡
NaCl tolerance (% w/v)	6	10	12
Growth at:			
pH 5.7	–	–	+
5 °C	–	–	–
40 °C	+	+	–
Optimum pH	7–9	7–9	5–9
Optimum temperature (°C)	30	42	30
Acid production from:			
D-Glucose	+	+‡	+‡
D-Mannitol	w	+‡	–‡
D-Xylose	+	–‡	–‡

*BE, Beige; BO, brownish orange.

†C, Central; T, terminal.

‡Data obtained in this study.

pH 6.5–9.5 (optimum pH 7–9). NaCl is not required for growth but up to 6.0 % (w/v) NaCl is tolerated. Catalase and oxidase-positive. Most carbon sources are not utilized. According to the method of Kämpfer *et al.* (1991), some organic acids, such as pyruvate, fumarate and L-malate, are utilized. Other physiological characteristics are indicated in Table 2. The peptidoglycan is of type A4β L-Orn–D-Asp and the major menaquinone is MK-7. The polar lipid profile consists predominantly of diphosphatidylglycerol and an unidentified phospholipid and also includes moderate amounts of phosphatidylglycerol and another two unidentified phospholipids and minor amounts of three glycolipids, a fourth phospholipid, an aminolipid and a polar lipid. The polyamine pattern is composed of the major components spermidine and spermine. The major fatty acids (>10 %) are anteiso-C_{15:0}, iso-C_{15:0} and anteiso-C_{17:0}; fatty acids iso-C_{14:0}, iso-C_{16:0}, iso-C_{17:0}, C_{15:0}, C_{16:0} and C_{18:0} are detected in small amounts.

The type strain, CCUG 53201^T (=DSM 22953^T), was isolated as a contaminant of a human blood sample in Göteborg, Sweden.

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References

- Altenburger, P., Kämpfer, P., Makristathis, A., Lubitz, W. & Busse, H.-J. (1996). Classification of bacteria isolated from a medieval wall painting. *J Biotechnol* **47**, 39–52.
- Altenburger, P., Kämpfer, P., Akimov, V. N., Lubitz, W. & Busse, H.-J. (1997). Polyamine distribution in actinomycetes with group B peptidoglycan and species of the genera *Brevibacterium*, *Corynebacterium* and *Tsukamurella*. *Int J Syst Bacteriol* **47**, 270–277.
- Gerhardt, P., Murray, R. G. E., Wood, W. A. & Krieg, N. R. (editors) (1994). *Methods for General and Molecular Bacteriology*. Washington, DC: American Society for Microbiology.
- Hamana, K., Akiba, T., Uchino, F. & Matsuzaki, S. (1989). Distribution of spermine in bacilli and lactic bacteria. *Can J Microbiol* **35**, 450–455.
- Hamana, K., Hamana, H., Niitsu, M., Samejima, K., Sakane, T. & Yokota, A. (1993). Tertiary and quaternary branched polyamines distributed in thermophilic *Saccharococcus* and *Bacillus*. *Microbios* **75**, 23–32.
- Kämpfer, P. & Kroppenstedt, R. M. (1996). Numerical analysis of fatty acid patterns of coryneform bacteria and related taxa. *Can J Microbiol* **42**, 989–1005.
- Kämpfer, P., Steiof, M. & Dott, W. (1991). Microbiological characterization of a fuel-oil contaminated site including numerical identification of heterotrophic water and soil bacteria. *Microb Ecol* **21**, 227–251.
- Kämpfer, P., Buczolits, S., Albrecht, A., Busse, H.-J. & Stackebrandt, E. (2003). Towards a standardized format for the description of a novel species (of an established genus): *Ochrobactrum gallinifaecis* sp. nov. *Int J Syst Evol Microbiol* **53**, 893–896.
- 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.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar, Buchner, A., Lai, T., Steppi, S. & other authors (2004). ARB: a software environment for sequence data. *Nucleic Acids Res* **32**, 1363–1371.
- Mayr, R., Busse, H.-J., Worliczek, H. L., Ehling-Schulz, M. & Scherer, S. (2006). *Ornithinibacillus* gen. nov., with the species *Ornithinibacillus bavariensis* sp. nov. and *Ornithinibacillus californiensis* sp. nov. *Int J Syst Evol Microbiol* **56**, 1383–1389.
- Meier-Stauffner, K., Busse, H.-J., Rainey, F. A., Burghardt, J., Scheberl, A., Hollaus, F., Kuen, B., Makristathis, A., Sleytr, U. B. & Messner, P. (1996). Description of *Bacillus thermoaerophilus* sp. nov., to include sugar beet isolates and *Bacillus brevis* ATCC 12990. *Int J Syst Bacteriol* **46**, 532–541.
- Olsen, G. J., Matsuda, H., Hagström, R. & Overbeek, R. (1994). fastDNAm: a tool for construction of phylogenetic trees of DNA sequences using maximum likelihood. *Comput Appl Biosci* **10**, 41–48.
- Pruesse, E., Quast, C., Knittel, K., Fuchs, B. M., Ludwig, W., Peplies, J. & Glöckner, F. O. (2007). SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. *Nucleic Acids Res* **35**, 7188–7196.
- Schleifer, K. H. (1985). Analysis of the chemical composition and primary structure of murein. *Methods Microbiol* **18**, 123–156.
- Schleifer, K. H. & Kandler, O. (1972). Peptidoglycan types of bacterial cell walls and their taxonomic implications. *Bacteriol Rev* **36**, 407–477.
- Schleifer, K. H. & Seidl, P. H. (1985). Chemical composition and structure of murein. In *Chemical Methods in Bacterial Systematics*, pp.

201–215. Edited by M. Goodfellow & D. E. Minnikin. London: Academic Press.

Stolz, A., Busse, H.-J. & Kämpfer, P. (2007). *Pseudomonas knackmussii* sp. nov. *Int J Syst Evol Microbiol* **57**, 572–576.

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovorum* from various sources. *Syst Appl Microbiol* **13**, 128–130.

Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Wainø, M., Tindall, B., Schumann, P. & Ingvorsen, K. (1999). *Gracilibacillus* gen. nov., with description of *Gracilibacillus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. *Int J Syst Bacteriol* **49**, 821–831.

Ziemke, F., Höfle, M. G., Lalucat, J. & Rosselló-Mora, R. (1998). Reclassification of *Shewanella putrefaciens* Owen's genomic group II as *Shewanella baltica* sp. nov. *Int J Syst Bacteriol* **48**, 179–186.