Ochrobactrum pituitosum sp. nov., isolated from an industrial environment

Birgit Huber,¹ Holger C. Scholz,² Peter Kämpfer,³ Enevold Falsen,⁴ Stefan Langer¹ and Hans-Jürgen Busse¹

Correspondence

Hans-Jürgen Busse
hans-juergen.busse@
vetmeduni.ac.at

¹Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

²Bundeswehr Institute of Microbiology and Toxicology, D-80937 Munich, Germany

³Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany

⁴CCUG – Culture Collection University of Göteborg, S-413 46 Göteborg, Sweden

Strain CCUG 50899, a Gram-negative, rod-shaped, non-spore-forming, motile bacterium isolated from industrial environment in Sweden and tentatively assigned to the species *Ochrobactrum anthropi*, was studied in order to clarify its taxonomic status. 16S rRNA gene sequence similarities placed the strain in the genus *Ochrobactrum*, sharing highest similarity with the type strains of *Ochrobactrum rhizosphaerae* (99.3%), *Ochrobactrum thiophenivorans* (98.7%), *Ochrobactrum pseudogrignonense* (98.6%) and *Ochrobactrum grignonense* (98.5%). The fatty acid profile of [*O. anthropi*] CCUG 50899 (major fatty acids C_{18:1}ω7c and C_{19:0} cyclo ω8c and presence of C_{18:1} 2-OH), the polar lipid profile (diphosphatidylglycerol, phosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, two unknown aminolipids and an unknown phospholipid), the presence of the quinone system ubiquinone Q-10 and a polyamine pattern with the major compounds putrescine and spermidine and moderate amounts of sym-homospermidine supported its affiliation to the genus *Ochrobactrum*. DNA–DNA reassociation experiments with the type strains of its closest relatives *O. rhizosphaerae*, *O. pseudogrignonense*, *O. thiophenivorans* and *O. grignonense* demonstrated that [*O. anthropi*] CCUG 50899 should be placed in a novel species, which is distinguishable from related species by a set of biochemical traits. Based on these data, reclassification of [*O. anthropi*] CCUG 50899 as the type strain of a novel species appears to be justified. Hence, we describe a novel species to accommodate this strain, for which we propose the name *Ochrobactrum pituitosum* sp. nov. The type strain is CCUG 50899^T (=DSM 22207^T).

The genus *Ochrobactrum* is affiliated with the family *Brucellaceae* (Garrrity *et al.*, 2005). 16S rRNA gene and *recA* sequence-based phylogenies both suggest that the genera *Brucella* and *Ochrobactrum* could be unified in a single genus (Kämpfer *et al.*, 2008; Scholz *et al.*, 2006, 2008). At the time of writing, the genus comprises 13 species: *Ochrobactrum anthropi* (Holmes *et al.*, 1988), *O. intermedium* (Velasco *et al.*, 1998), *O. tritici* and *O. grignonense* (Lebuhn *et al.*, 2000), *O. gallinifacis* (Kämpfer *et al.*, 2003), *O. oryzae* (Tripathi *et al.*, 2006), *O. lupini* (Trujillo *et al.*,

2005), *O. pseudintermedium* (Teyssier *et al.*, 2007), *O. cytisi* (Zurdo-Piñeiro *et al.*, 2007), *O. haematophilum* and *O. pseudogrignonense* (Kämpfer *et al.*, 2007), *O. rhizosphaerae* and *O. thiophenivorans* (Kämpfer *et al.*, 2008). In a comprehensive study, Scholz *et al.* (2008) examined the phylogenetic relationships of numerous *Ochrobactrum* and *Brucella* strains including strain CCUG 50899, reported as a strain of *O. anthropi* according to the CCUG catalogue but which was shown to be phylogenetically distant from the type strain of *O. anthropi* on the basis of both 16S rRNA gene and *recA* phylogeny. In comparison to the majority of *Ochrobactrum* strains, [*O. anthropi*] CCUG 50899 shows an insertion of 47 nt (positions 124–170) in the 16S rRNA gene sequence (GenBank accession no. AM490609) which so far has only been reported for some strains of *O. intermedium* (Teyssier *et al.*, 2003; Scholz *et al.*, 2008). Corrected for the insertion sequence, [*O. anthropi*] CCUG

Abbreviations: pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDBJ accession numbers for the partial *groEL* and *gyrB* sequences of *O. rhizosphaerae* PR17^T, *Ochrobactrum* sp. CCUG 50899, *O. grignonense* OgA9a^T, *O. pseudogrignonense* CCUG 30717^T and *O. thiophenivorans* DSM 7216^T are respectively FM863819–FM863823 (*groEL*) and FM863818 and FM863814–FM863817 (*gyrB*).

50899 shares highest 16S rRNA gene sequence similarity with *O. rhizosphaerae* PR17^T (99.3%), *O. thiophenivorans* DSM 7216^T (98.7%), *O. pseudogrignonense* CCUG 30717^T (98.6%) and *O. grignonense* OgA9a^T (98.5%). Other *Ochrobactrum* species show lower similarity values. Based on partial *recA* sequence similarities, *O. grignonense* OgA9a^T (93.3%) and *O. rhizosphaerae* PR17^T (91.4%) are the closest relatives of [*O. anthropi*] CCUG 50899 (GenBank accession no. AM422871), with other members of *Ochrobactrum* sharing only 83.5–88.3% similarity in the corresponding partial gene sequences. However, these similarity values demonstrate unambiguously the affiliation of [*O. anthropi*] CCUG 50899 to the genus *Ochrobactrum*. Here, we report the classification of [*O. anthropi*] CCUG 50899, applying biochemical, physiological, chemotaxonomic and genetic characterization and comparison to phylogenetic relatives identified recently based on 16S rRNA gene and *recA* phylogenies (Scholz *et al.*, 2006, 2008).

Recalculation of its 16S rRNA gene sequence-based phylogenetic position placed [*O. anthropi*] CCUG 50899 on a separate branch within *Ochrobactrum* consisting of the type strains of *O. grignonense*, *O. pseudogrignonense*, *O. thiophenivorans* and the closest related species *O. rhizosphaerae* with high bootstrap support (Fig. 1), which is in agreement with the relationship indicated by 16S rRNA gene sequence similarities.

Since 16S rRNA gene and *recA* sequence analyses had identified different *Ochrobactrum* species as the closest relatives, partial *gyrB* (1032 nt) and *groEL* (1169 nt) sequences of [*O. anthropi*] CCUG 50899 were analysed and compared to the corresponding sequences of selected reference strains, including *O. anthropi* LMG 3331^T, *O. grignonense* OgA9a^T, *O. rhizosphaerae* PR17^T, *O. thiophenivorans* DSM 7216^T, *O. pseudogrignonense* CCUG 30717^T and *O. intermedium* LMG 3301^T. DNA was released by subjecting a cell suspension in sterile water to three cycles

of freezing in liquid nitrogen and thawing at 60 °C. Released DNA in the supernatant obtained after a short centrifugation was used as the template in PCRs. Partial *gyrB* and *groEL* sequences were amplified with the primer pairs *GyrB_f* [5'-GATGATGATCTTGTG(AG)TA(AGC)-CGCAG-3'] and *GyrB_r* (5'-CGAGGTCATCATGACCCA-GCTTC-3') and *GroEL_f* (5'-CGGCGAAGACCTGCTG-ATC-3') and *GroEL_r* (5'-GCAACGATACCTTCTTCAA-CCG-3'), respectively. The 50 µl assay contained primers (0.5 pmol µl⁻¹), MgCl₂ (3 mM), dNTPs (0.2 mM each), 10 µl 5 × GoTaq Flexi buffer and GoTaq DNA Polymerase enzyme (1.25 U; Promega), 1–2 µl DNA preparation and sterile water. For both primer pairs, PCR amplification was performed using the following conditions: 5 min initial denaturation at 95 °C followed by 35 cycles of denaturation for 1 min at 95 °C, annealing for 1 min at 64 °C and extension for 90 s at 72 °C with a final extension for 5 min at 72 °C. PCR products were excised from a 1.5% agarose gel and then purified with the QIAquick gel extraction kit (Qiagen). Products were sequenced at VBC Genomics (Vienna, Austria). Primers *GyrB_r*, *GyrB_int_544_r* (5'-GACGCTGACCGGCGATGACTG-3'), *GroEL_f* and *GroEL_r* were used as sequencing primers. Multiple sequence alignment was made with the CLUSTAL_X software, version 1.81 (Thompson *et al.*, 1997), and a similarity matrix was calculated with the software BioEdit (Hall, 1999).

For the partial *groEL* gene sequences, similarities between the reference strains were in the range 91.3–96.0% (Table 1). For the partial *gyrB* sequences, similarity values between the reference strains were significantly lower, ranging from 83.9 to 91.5%. In both partial gene sequences, [*O. anthropi*] CCUG 50899 exhibited highest similarity with *O. grignonense* OgA9a^T (97.9% for *groEL*, 95.0% for *gyrB*), suggesting this species as the closest relative and thus confirming the results from *recA* analyses (Scholz *et al.*, 2008). Similarity values with the reference

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences (1355–1394 bp) available from the EMBL database constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the maximum composite likelihood model) were calculated and clustering with the neighbour-joining method was performed using the software package MEGA version 4.0 (Tamura *et al.*, 2007). Bootstrap values based on 2000 replications are listed as percentages at branching points (the bootstrap consensus tree is shown). Bar, 0.01 substitutions per nucleotide position. *O.*, *Ochrobactrum*; *B.*, *Brucella*; *P.*, *Pseudochrobactrum*. The sequence of *Bradyrhizobium japonicum* USDA 6^T was used as an outgroup.

Table 1. Similarities of partial *groEL* and *gyrB* sequences

groEL similarities are given as percentages above the diagonal, with percentage *gyrB* similarities below the diagonal. Sequences were aligned using CLUSTAL_X (Thompson *et al.*, 1997) and sequence similarities were determined using BioEdit (Hall, 1999). Values for [*O. anthropi*] CCUG 50899 are highlighted in bold.

Strain	1	2	3	4	5	6	7
1. <i>O. anthropi</i> LMG 3331 ^T	–	92.8	92.4	92.3	91.4	93.1	96.0
2. <i>O. grignonense</i> OgA9A ^T	85.8	–	97.9	95.2	94.9	92.4	92.8
3. [<i>O. anthropi</i>] CCUG 50899	86.0	95.0	–	95.6	95.5	92.5	92.4
4. <i>O. rhizosphaerae</i> PR17 ^T	83.9	87.1	87.5	–	95.8	92.8	92.1
5. <i>O. thiophenivorans</i> DSM 7216 ^T	84.6	85.3	83.2	85.1	–	92.6	91.3
6. <i>O. pseudogrignonense</i> CCUG 30717 ^T	87.1	88.9	89.1	85.6	85.4	–	92.8
7. <i>O. intermedium</i> LMG 3301 ^T	91.5	87.2	86.8	85.2	84.8	87.7	–

strains were in the range 92.4–95.6 % (*groEL*) and 83.2–89.1 % (*gyrB*), respectively. Similarity of 92.4 % (*groEL*) and 86.0 % (*gyrB*) between [*O. anthropi*] CCUG 50899 and *O. anthropi* LMG 3331^T does not suggest a relationship at the species level.

In order to clarify whether [*O. anthropi*] CCUG 50899 should be considered a member of a separate species, DNA–DNA hybridizations were carried out with DNAs of the nearest relatives *O. grignonense* OgA9A^T, *O. thiophenivorans* DSM 7216^T, *O. pseudogrignonense* CCUG 30717^T and *O. rhizosphaerae* PR17^T. High-molecular-mass DNA was extracted and purified according to Pitcher *et al.* (1989). DNA concentrations were estimated chemically according to Richards (1974). DNA–DNA hybridizations were performed according to Ziemke *et al.* (1998) except that, for nick translation, 2 µg DNA was labelled during a 90 min incubation at 15 °C. In the hybridization experiments, DNA of [*O. anthropi*] CCUG 50899 exhibited 55 % (reciprocal 56 %) reassociation with *O. grignonense* OgA9A^T, 47 % (reciprocal 37 %) reassociation with *O. thiophenivorans* DSM 7216^T, 62 % (reciprocal 35 %) with *O. pseudogrignonense* CCUG 30717^T and 43 % (reciprocal 46 %) reassociation with *O. rhizosphaerae* PR17^T. These data demonstrate that [*O. anthropi*] CCUG 50899 represents a novel species of the genus *Ochrobactrum*.

For phenotypic characterization, [*O. anthropi*] CCUG 50899 was subjected to the set of methods already employed for classification of *O. haematophilum*, *O. pseudogrignonense*, *O. rhizosphaerae* and *O. thiophenivorans* (Kämpfer *et al.*, 2007, 2008).

Polar lipids and quinones were extracted from biomass grown in PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2) and analysed as described previously (Tindall, 1990a, b; Altenburger *et al.*, 1996; Stolz *et al.*, 2007). Polyamines were extracted and analysed as described by Busse & Auling (1988) and Stolz *et al.* (2007). Analysis of quinones indicated that [*O. anthropi*] CCUG 50899 contains a quinone system with ubiquinone Q-10 (99 %) predominating and minor amounts of ubiquinone Q-9 (1 %). The polar lipid profile of [*O. anthropi*] CCUG 50899 exhibited all the major compounds

reported for other *Ochrobactrum* species, including the characteristic aminolipid AL2 (Kämpfer *et al.*, 2003, 2007, 2008). Differences in the presence of minor, quite hydrophilic components were detected (Fig. 2), but we do not want to give too much emphasis here to this observation because, so far, its significance has not been demonstrated by results from other strains of the same species. The polyamine pattern contained the major compounds putrescine and spermidine [34.2 and 27.6 µmol (g dry weight)^{–1}, respectively], moderate amounts of *sym*-homospermidine [4.6 µmol (g dry weight)^{–1}] and small amounts of spermine and 1,3-diaminopropane [0.9 and 0.4 µmol (g dry weight)^{–1}, respectively], which is characteristic of *Ochrobactrum*

Fig. 2. Total polar lipid profile of [*O. anthropi*] CCUG 50899 after two-dimensional TLC and detection with molybdatophosphoric acid. DPG, Diphosphatidylglycerol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PME, phosphatidylmonomethylethanolamine; AL1–4, unknown aminolipids; L2–4, unknown polar lipids; PL1–4, unknown phospholipids.

species (Kämpfer *et al.*, 2007, 2008). Fatty acids were extracted and analysed according to the instructions of the Microbial Identification System (MIDI Inc.) as described before (Kämpfer & Kroppenstedt, 1996). The fatty acid profile of [*O. anthropi*] CCUG 50899 exhibited the characteristics also reported for other species of the genus (Table 2). The fatty acids C_{18:1}ω7*c* and C_{19:0} cyclo ω8*c* were predominant and the hydroxy acid C_{18:1} 2-OH was detected. Examination of the fatty acid profile from cells grown for only 24 h (all other analyses were carried out from cells grown for 48 h) showed a significantly increased amount of C_{18:1}ω7*c* and decreased amount of C_{19:0} cyclo ω8*c*, but the sum of the two amounts was almost the same as in cells grown for 48 h. This observation demonstrates that, for comparison of fatty acid profiles of *Ochrobactrum* strains, only the sum of the amounts of C_{18:1}ω7*c* and C_{19:0}

cyclo ω8*c* should be considered. In contrast to *O. rhizosphaerae* PR17^T and *O. thiophenivorans* DSM 7216^T, small amounts of the hydroxy acid C_{18:1} 3-OH were also detected.

Tolerance towards NaCl was examined on PYE agar supplemented with 1, 2, 3, 4, 5, 6, 7 and 10 % NaCl (w/v) and temperature tolerance was examined in an adjustable incubator. Other phenotypic characteristics were studied using the methods described by Kämpfer *et al.* (1991).

Physiological and biochemical traits are listed in Table 3 and the species description. Biochemical characterization of [*O. anthropi*] CCUG 50899 showed that this strain is distinguishable from the type strains of the closest related species including *O. rhizosphaerae* PR17^T, *O. grignonense* DSM 13338^T, *O. pseudogrignonense* CCUG 30717^T and *O. thiophenivorans* DSM 7216^T as well as from *O. anthropi* CIP 14970^T based on a set of traits. These data support our view that strain CCUG 50899 is affiliated neither with *O. anthropi* nor with any of the other related species.

Phylogenetically, [*O. anthropi*] CCUG 50899 is affiliated unambiguously with the genus *Ochrobactrum*, and its chemotaxonomic traits such as quinone system, polar lipid profile, fatty acids and polyamine pattern are in agreement with this classification. Results from DNA–DNA hybridizations demonstrate that *Ochrobactrum* sp. CCUG 50899 is a representative of a novel species that differs in several phenotypic traits from phylogenetically closely related species. Hence, we here describe a novel species to accommodate strain CCUG 50899, for which we propose the name *Ochrobactrum pituitosum* sp. nov.

Table 2. Fatty acid compositions of [*O. anthropi*] CCUG 50899 and the type strains of closely related species

Strains: 1, [*O. anthropi*] CCUG 50899; 2, *O. rhizosphaerae* PR17^T (data from Kämpfer *et al.*, 2008); 3, *O. grignonense* DSM 13338^T (Kämpfer *et al.*, 2007); 4, *O. pseudogrignonense* CCUG 30717^T (Kämpfer *et al.*, 2007); 5, *O. thiophenivorans* DSM 7216^T (Kämpfer *et al.*, 2008). All strains were grown on TSA at 28 °C for 48 h prior to fatty acid analysis; [*O. anthropi*] CCUG 50899 was also analysed for fatty acids after growth under the same conditions for 24 h (in parentheses). For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain; *cis* isomers are indicated by the suffix *c*.

Fatty acid	1	2	3	4	5
Saturated fatty acids					
C _{16:0}	4.9 (2.7)	8.3	2.9	9.7	7.3
C _{17:0}	2.3 (1.6)	2.8	1.7	1.6	1.4
C _{18:0}	8.6 (7.7)	7.6	7.2	4.9	2.9
Unsaturated fatty acids					
C _{13:1} at 12–13	—	—	0.7	—	—
C _{17:1} ω6 <i>c</i>	—	—	0.5	—	—
C _{18:1} ω7 <i>c</i>	22.5 (61.3)	61.5	31.6	19.2	40.9
11-Methyl C _{18:1} ω7 <i>c</i>	0.9 (—)	—	1.0	1.7	1.5
C _{20:1} ω7 <i>c</i>	0.4 (0.7)	—	0.8	—	—
C _{20:2} ω6,9 <i>c</i>	0.6 (—)	—	0.8	0.9	1.8
Hydroxy fatty acids					
C _{18:1} 2-OH	0.6 (2.0)	4.7	0.5	1.8	1.5
C _{18:1} 3-OH	0.4 (—)	—	0.5	0.5	—
Summed feature 3*	1.2 (1.5)	4.6	1.0	1.4	5.8
Cyclopropane acids					
C _{17:0} cyclo	1.0	—	—	1.0	2.1
C _{19:0} cyclo ω8 <i>c</i>	56.0 (20.8)	11.2	50.2	57.0	34.9
Unknown 13.957†	0.6	—	0.2	0.3	—
Unknown 14.959†	— (0.7)	—	0.7	—	—

*Summed feature 3 contained C_{15:0} iso 2-OH and/or C_{16:1}ω7*c*. Since no other iso-branched fatty acids were detected, it is most likely that summed feature 3 in fact represents only C_{16:1}ω7*c*.

†Unknown fatty acids not identified by the MIDI system. Values are equivalent chain-lengths.

Description of *Ochrobactrum pituitosum* sp. nov.

Ochrobactrum pituitosum (pi.tu.i.to'sum. L. neut. adj. *pituitosum* slimy, referring to the consistency of the colonies after extended incubation).

Cells stain Gram-negative and also show Gram-negative behaviour in the KOH and aminopeptidase tests. Aerobic. Highly motile in the early exponential growth phase but may become less motile or non-motile when approaching the stationary growth phase. Cells are non-spore-forming, pleomorphic rods (approx. 1.5–2.5 µm long). Good growth is observed on Columbia agar supplemented with sheep blood and on nutrient agar, TSA, PYE agar, R2A agar and MacConkey agar. Growth is observed on PYE agar supplemented with 6 % NaCl but not with 7 % NaCl (w/v). Optimal growth occurs at 25–30 °C; weak growth at 20 and 35 °C. No growth occurs at 4 °C or at temperatures above 35 °C on PYE agar. Small, beige, translucent and shiny colonies up to 1 mm in diameter are formed within 24 h on PYE agar. After extended incubation, colonies become slimy. Oxidase- and catalase-positive. The quinone system consists of the predominant ubiquinone Q-10 and minor amounts of Q-9. The polyamine pattern consists of the major compounds spermidine and putrescine, moderate amounts of *sym*-homospermidine and minor amounts of 1,3-diaminopropane and spermine. Predominant polar

Table 3. Physiological characteristics of [*O. anthropi*] CCUG 50899, *O. anthropi* CIP 14970^T and type strains of four closely related *Ochrobactrum* species

Strains: 1, [*O. anthropi*] CCUG 50899; 2, *O. rhizosphaerae* PR17^T; 3, *O. grignonense* DSM 13338^T; 4, *O. pseudogrignonense* CCUG 30717^T; 5, *O. thiophenivorans* DSM 7216^T; 6, *O. anthropi* CIP 14970^T. +, Positive; –, negative; (+), weakly positive. All strains were positive for acid formation from D-glucose (in some cases, a weak positive result was observed). All strains were negative for acid formation from sucrose, D-mannitol, dulcitol, raffinose, maltose, D-arabitol, D-mannose, trehalose and methyl D-glucoside. All strains were positive for hydrolysis of L-alanine *p*-nitroanilide (pNA) and L-proline pNA and negative for hydrolysis of aesculin, *p*-nitrophenyl (pNP) β -D-galactopyranoside, pNP β -D-glucuronide, pNP phosphorylcholine and 2-deoxythymidine-5'-pNP phosphate. All strains were also positive for assimilation of *N*-acetyl-D-galactosamine, L-arabinose, D-fructose, D-galactose, D-glucose, D-mannose, D-ribose, D-xylose, inositol, D-sorbitol, 4-aminobutyrate, fumarate, glutarate, DL-3-hydroxybutyrate, DL-lactate, L-malate, oxoglutarate, L-alanine, β -alanine, L-aspartate, L-ornithine, L-proline and L-serine. All strains were negative for assimilation of *p*-arbutin, melibiose, salicin, putrescine, adipate, azelate, itaconate, mesaconate, suberate, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate.

Test	1	2	3	4	5	6
Acid production from:						
Lactose	–	–	(+)	–	–	–
L-Arabinose	+	+	+	+	(+)	–
Inositol	–	–	–	–	(+)	–
Rhamnose	–	–	(+)	(+)	–	–
D-Xylose	+	–	+	–	–	–
Cellobiose	–	–	+	–	–	–
Adonitol	–	–	–	–	–	(+)
Erythritol	(+)	–	+	(+)	(+)	(+)
Melibiose	–	–	(+)	–	–	–
Hydrolysis of:						
pNP α -D-glucopyranoside, pNP β -D-glucopyranoside	(+)	–	–	–	–	–
pNP phenylphosphonate	+	+	–	(+)	(+)	–
L-Glutamate- γ -3-carboxy pNA	+	–	–	–	–	–
bis-pNP phosphate	–	(+)	(+)	(+)	(+)	(+)
Assimilation of:						
Cellobiose, maltose, maltitol	+	+	–	–	–	+
Sucrose, trehalose, adonitol	+	+	–	+	–	+
Acetate, propionate, pyruvate	–	+	+	+	+	+
L-Rhamnose, <i>cis</i> -aconitate, citrate, L-histidine, L-leucine	+	+	+	+	–	+
<i>trans</i> -Aconitate	+	+	–	+	–	–
<i>N</i> -Acetyl-D-glucosamine	+	(+)	–	–	(+)	(+)
D-Gluconate	–	–	+	+	+	+

lipids are phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, phosphatidylcholine and diphosphatidylglycerol. Moderate amounts of two unidentified aminolipids (AL1, AL2) and a phospholipid (PL1) and traces of several unknown phospholipids, aminolipids and lipids that have not been characterized further are also present. The fatty acid profile contains mainly C_{19:0} cyclo ω 8c and C_{18:1} ω 7c and the hydroxy acids C_{18:1} 2-OH and C_{18:1} 3-OH. Carbon source assimilation and hydrolysis of chromogenic substrates are listed in Table 3.

The type strain CCUG 50899^T (=DSM 22207^T) was isolated from an industrial environment in Sweden.

Acknowledgements

The work was supported by a Contract Research Project for the Bundeswehr Medical Service.

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.
- Busse, H.-J. & Auling, G. (1988). Polyamine pattern as a chemotaxonomic marker within the *Proteobacteria*. *Syst Appl Microbiol* **11**, 1–8.
- Garrity, G. M., Bell, J. A. & Lilburn, T. (2005). Family III. *Brucellaceae* Breed, Murray and Smith 1957, 394^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, p. 370. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Hall, T. A. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser* **41**, 95–98.
- Holmes, B., Popoff, M., Kiredjian, M. & Kersters, K. (1988). *Ochrobactrum anthropi* gen. nov., sp. nov. from human clinical specimens and previously known as group Vd. *Int J Syst Bacteriol* **38**, 406–416.

- 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.
- Kämpfer, P., Scholz, H. C., Huber, B., Falsen, E. & Busse, H.-J. (2007). *Ochrobactrum haematophilum* sp. nov. and *Ochrobactrum pseudogrignonense* sp. nov., isolated from human clinical specimens. *Int J Syst Evol Microbiol* **57**, 2513–2518.
- Kämpfer, P., Sessitsch, A., Schlöter, M., Huber, B., Busse, H.-J. & Scholz, H. C. (2008). *Ochrobactrum rhizosphaerae* sp. nov. and *Ochrobactrum thiophenivorans* sp. nov., isolated from the environment. *Int J Syst Evol Microbiol* **58**, 1426–1431.
- Lebuhn, M., Achouak, W., Schlöter, M., Berge, O., Meier, H., Barakat, M., Hartmann, A. & Heulin, T. (2000). Taxonomic characterization of *Ochrobactrum* sp. isolates from soil samples and wheat roots, and description of *Ochrobactrum tritici* sp. nov. and *Ochrobactrum grignonense* sp. nov. *Int J Syst Evol Microbiol* **50**, 2207–2223.
- Pitcher, D. G., Saunders, N. A. & Owen, R. J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Lett Appl Microbiol* **8**, 151–156.
- Richards, G. M. (1974). Modification of the diphenylamine reaction giving increased sensitivity and simplicity in the estimation of DNA. *Anal Biochem* **57**, 369–376.
- Scholz, H. C., Tomaso, H., Al Dahouk, S., Witte, A., Schlöter, M., Kämpfer, P., Falsen, E. & Neubauer, H. (2006). Genotyping of *Ochrobactrum anthropi* by *recA*-based comparative sequence, PCR-RFLP, and 16S rRNA gene analysis. *FEMS Microbiol Lett* **257**, 7–16.
- Scholz, H. C., Al Dahouk, S., Tomaso, H., Neubauer, H., Witte, A., Schlöter, M., Kämpfer, P., Falsen, E., Pfeffer, M. & Engel, M. (2008). Genetic diversity and phylogenetic relationships of bacteria belonging to the *Ochrobactrum-Brucella* group by *recA* and 16S rRNA gene-based comparative sequence analysis. *Syst Appl Microbiol* **31**, 1–16.
- 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.
- Teyssier, C., Marchandin, H., Siméon De Buochberg, M., Ramuz, M. & Jumas-Bilak, E. (2003). Atypical 16S rRNA gene copies in *Ochrobactrum intermedium* strains reveal a large genomic rearrangement by recombination between *rrn* copies. *J Bacteriol* **185**, 2901–2909.
- Teyssier, C., Marchandin, H., Jean-Pierre, H., Masnou, A., Dusart, G. & Jumas-Bilak, E. (2007). *Ochrobactrum pseudointermedium* sp. nov., a novel member of the family *Brucellaceae*, isolated from human clinical samples. *Int J Syst Evol Microbiol* **57**, 1007–1013.
- Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.
- Tindall, B. J. (1990a). A comparative study of the lipid composition of *Halobacterium saccharovororum* from various sources. *Syst Appl Microbiol* **13**, 128–130.
- Tindall, B. J. (1990b). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.
- Tripathi, A. K., Verma, S. C., Chowdhury, S. P., Lebuhn, M., Gatteringer, A. & Schlöter, M. (2006). *Ochrobactrum oryzae* sp. nov., an endophytic bacterial species isolated from deep-water rice in India. *Int J Syst Evol Microbiol* **56**, 1677–1680.
- Trujillo, M. E., Willems, A., Abril, A., Planchuelo, A. M., Rivas, R., Ludeña, D., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2005). Nodulation of *Lupinus* by strains of the new species *Ochrobactrum lupini* sp. nov. *Appl Environ Microbiol* **71**, 1318–1327.
- Velasco, J., Romero, C., López-Goñi, I., Leiva, J., Díaz, R. & Moriyón, I. (1998). Evaluation of the relatedness of *Brucella* spp. and *Ochrobactrum anthropi* and description of *Ochrobactrum intermedium* sp. nov., a new species with a closer relationship to *Brucella* spp. *Int J Syst Bacteriol* **48**, 759–768.
- 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.
- Zurdo-Piñeiro, J. L., Rivas, R., Trujillo, M. E., Vizcaino, N., Carrasco, J. A., Chamber, M., Palomares, A., Mateos, P. F., Martínez-Molina, E. & Velázquez, E. (2007). *Ochrobactrum cytisi* sp. nov. isolated from nodules of *Cytisus scoparius* in Spain. *Int J Syst Evol Microbiol* **57**, 784–788.