Ochrobactrum haematophilum sp. nov. and *Ochrobactrum pseudogrignonense* sp. nov., isolated from human clinical specimens

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Three Gram-negative, rod-shaped, non-spore-forming bacteria were isolated from clinical specimens between 1992 and 2000. On the basis of 16S rRNA gene sequence similarities, these strains (CCUG 30717^T, CCUG 43892 and CCUG 38531^T) were shown to belong to the *Alphaproteobacteria*, most closely related to *Ochrobactrum grignonense* (99.0 and 98.2% similarity to the type strain). Chemotaxonomic data (major ubiquinone Q-10; major polyamines spermidine, sym-homospermidine and putrescine; major polar lipids phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol and phosphatidylcholine; major fatty acids C_{18:1}ω7c and C_{19:0} cyclo ω8c) supported the affiliation of the isolates to the genus *Ochrobactrum*. The results of DNA–DNA hybridization and physiological and biochemical tests allowed genotypic and phenotypic differentiation of the isolates from described *Ochrobactrum* species. Isolates CCUG 30717^T and CCUG 43892 were closely related on the basis of DNA–DNA reassociation experiments and therefore represent one novel species, for which the name *Ochrobactrum pseudogrignonense* sp. nov. is proposed, with the type strain CCUG 30717^T (=CIP 109451^T). Isolate CCUG 38531^T was different from these strains and also from other *Ochrobactrum* species. For this strain, the name *Ochrobactrum haematophilum* sp. nov. is proposed, with the type strain CCUG 38531^T (=CIP 109452^T).

The genus *Ochrobactrum* was introduced by Holmes *et al.* (1988) and, at the time of writing, the genus comprises the nine species *Ochrobactrum anthropi* (Holmes *et al.*, 1988), *O. intermedium* (Velasco *et al.*, 1998), *O. tritici* and *O. grignonense* (Lebuhn *et al.*, 2000), *O. gallinifaecis* (Kämpfer *et al.*, 2003b), *O. lupini* (Trujillo *et al.*, 2005), *O. oryzae* (Tripathi *et al.*, 2006), *O. pseudintermedium* (Zurdo-Piñeiro *et al.*, 2007) and *O. cytisi* (Teyssier *et al.*, 2007).

Strains CCUG 30717^T and CCUG 38531^T were isolated in Sweden in 1992 and 1997, respectively, from human blood and strain CCUG 43892 was isolated in Norway in 2000 from a human ear and the strains were presumptively identified as ‘*O. anthropi*-like’. All strains showed beige-coloured colonies

on nutrient agar (Oxoid) at 37 °C. Subcultivation was done on tryptone soy agar (TSA) at 28 °C for 48 h.

Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Zeiss light microscope at ×1000, with cells grown for 3 days at 28 °C on TSA. The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003a). Strains CCUG 30717^T and CCUG 43892 showed identical 16S rRNA gene sequences. Phylogenetic analysis was performed using the ARB software package (Ludwig *et al.*, 2004) and also the software package MEGA version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura-2 model) were calculated and clustering with the neighbour-joining method and maximum-parsimony was performed by using bootstrap values based on 1000 replications. The almost-complete 16S rRNA gene sequences of the three strains were compared by sequence

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

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains CCUG 30717^T and CCUG 38531^T are respectively AM422371 and AM422370.

similarity calculations after a neighbour-joining analysis. The results of these calculations indicated that the closest relative of all strains was *O. grignonense* (99.0 and 98.2 % similarity to the type strain). Lower sequence similarities were found with strains of all other species of the genus *Ochrobactrum* with validly published names. A phylogenetic tree is shown in Fig. 1.

In *recA*-based phylogenetic analyses including the type strains of some *Ochrobactrum* and *Brucella* species, strain CCUG 38531^T clustered as a separate lineage most closely related to *Brucella* species (Scholz *et al.*, 2006). *recA* could not be amplified from strains CCUG 30717^T and CCUG 43892, as described previously for the type strain of *O. grignonense* (Scholz *et al.*, 2006).

For quinone and polar lipid analysis, cells were grown on PYE medium (Busse *et al.*, 2005). Detailed results of chemotaxonomic analyses are given in the species description. The following analytical procedures were performed as described: respiratory quinones (Tindall, 1990; Altenburger *et al.*, 1996) but using an HPLC consisting of a JASCO PU 2080 Plus pump and JASCO UV-2075 Plus UV/Vis detector; polyamines (Busse & Auling, 1988; Busse *et al.*, 1997) using a JASCO PU 2080 Plus pump; polar lipids (Ventosa *et al.*, 1993); and fatty acids (Kämpfer & Kroppenstedt, 1996). The quinone system (Q-10) supports affiliation of the three strains to the *Alphaproteobacteria*, where the majority of species (including *Ochrobactrum* species) have Q-10 as the major quinone (Lechner *et al.*, 1995; Yokota *et al.*, 1992). The polyamine pattern, with the predominant compounds putrescine and spermidine, was in agreement with the patterns reported previously for two species, including *O. anthropi* (Lechner *et al.*, 1995; Hamana & Takeuchi, 1998), and is distinct from the polyamine patterns of members of the genera *Rhizobium*, *Mesorhizobium*, *Sinorhizobium*/*Ensifer*, *Aminobacter* and *Phyllobacterium*, which were shown to contain *sym*-homospermidine as the major compound in their polyamine patterns (Busse & Auling, 1988; Auling *et al.*, 1991; Hamana & Takeuchi, 1998), whereas spermidine is only present in minor to moderate amounts. In contrast, other relatives, such as species of *Pseudaminobacter*, exhibit

polyamine patterns with the predominant compounds putrescine, spermidine and *sym*-homospermidine, and *Mycoplana bullata* shows the presence of spermidine as the major compound with moderate amounts of *sym*-homospermidine (Hamana & Takeuchi, 1998; Kämpfer *et al.*, 1999). However, in the three strains, we were able to detect significant amounts of *sym*-homospermidine [9–17 µmol (g dry weight)⁻¹]. Since the *Ochrobactrum* species examined so far have been reported to lack *sym*-homospermidine, we have reanalysed all type strains of *Ochrobactrum* species for their polyamine patterns, and every strain was found to contain at least moderate amounts [>5 µmol (g dry weight)⁻¹] of this polyamine (not shown). These results suggest that, under the standardized conditions for growth of biomass applied here (growth on PYE medium at 28 °C and harvesting the cells at approximately 70 % of the maximum optical density of the culture), *Ochrobactrum* species are characterized by a polyamine pattern containing the major compound spermidine and moderate to major amounts of putrescine and *sym*-homospermidine. Absence of *sym*-homospermidine in representatives of the closely related genus *Pseudochrobactrum* (Kämpfer *et al.*, 2006, 2007; B. Huber and H.-J. Busse, unpublished results) appears to be a distinguishing trait between the two genera. The polar lipid profiles of CCUG 30717^T (Fig. 2), CCUG 43892 and CCUG 38531^T were almost indistinguishable and exhibited a high degree of similarity to the profile of *O. gallinifaecis* (Kämpfer *et al.*, 2003b). Like this species, the strains showed the presence of aminolipid AL2, but its content in the profile of CCUG 38531^T was lower than in the other two strains (not shown). Since AL2 is lacking in extracts of *Pseudochrobactrum* species (Kämpfer *et al.*, 2006, 2007), its presence is apparently another distinguishing trait between *Ochrobactrum* and *Pseudochrobactrum*. In this context, it is worth mentioning here that other *Ochrobactrum* species also contain this characteristic in their polar lipid profiles (B. Huber and H.-J. Busse, unpublished results).

The fatty acid profiles of the three strains are shown in Table 1. No significant differences in fatty acid profiles have been found for other *Ochrobactrum* species; however,

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses) constructed after multiple alignment of data by CLUSTAL_X (Thompson *et al.*, 1997). The tree was constructed as described in the text. Bootstrap values based on 1000 replications are listed as percentages at branching points. Bar, 0.005 substitutions per nucleotide position.

Fig. 2. Two-dimensional TLC of polar lipids of strain CCUG 30717^T. PME, Phosphatidylmonomethylethanolamine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; DPG, diphosphatidylglycerol; PG, phosphatidylglycerol; AL1–3, unknown aminolipids; PL1–6, unknown phospholipids; APL1–3, unknown aminophospholipids; L2–3, unknown polar lipids.

no hydroxylated fatty acids were detected in strain CCUG 38531^T.

Results of the physiological characterization are given in the species description and in Table 2 (differential characters), using previously described methods (Kämpfer *et al.*, 1991). DNA–DNA hybridization experiments were performed with all three strains and the type strains of all *Ochrobactrum* species using the method described by Ziemke *et al.* (1998), except that, for nick translation, 2 µg DNA was labelled during a 3 h incubation at 15 °C. Results for the novel strains and the four most closely related type strains are shown in Table 3.

Phylogenetic examinations based on almost-entire 16S rRNA gene sequences showed the affiliation of the three strains to the genus *Ochrobactrum*. They also shared the distinguishing characteristics of the genus, the presence of unknown aminolipid AL2 and a polyamine pattern containing significant amounts of *sym*-homospermidine in addition to spermidine and putrescine. The relationship of strains CCUG 30717^T and CCUG 43892 at the species level as suggested by their identical 16S rRNA gene sequences was confirmed by the results of DNA–DNA hybridizations. In the phylogenetic tree, they were placed next to *O. grignonense* Oga9a^T, but their status as a distinct species was confirmed by DNA–DNA hybridizations with *O. grignonense* DSM 13338^T; *O. anthropi* CIP 14970^T, *O. tritici* LMG 18957^T and *O. intermedium* LMG 3301^T. The

Table 1. Major fatty acids (%) of the type strains of species of the genus *Ochrobactrum* closely related to strains CCUG 30717^T, CCUG 43892 and CCUG 38531^T

Strains: 1, *O. pseudogrignonense* sp. nov. CCUG 30717^T; 2, *O. pseudogrignonense* sp. nov. CCUG 43892; 3, *O. haematophilum* sp. nov. CCUG 38531^T; 4, *O. grignonense* DSM 13338^T; 5, *O. gallinifaecis* Iso 196^T; 6, *O. intermedium* LMG 3301^T; 7, *O. anthropi* CIP 14970^T; 8, *O. tritici* LMG 18957^T. All strains were grown on trypticase soy broth agar at 28 °C for 48 h prior to fatty acid analysis. For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain. *cis* and *trans* isomers are indicated by the suffixes *c* and *t*, respectively. –, Not detected.

Fatty acid	1	2	3	4	5	6	7	8
Saturated fatty acids								
C _{14:0}	–	–	–	–	0.7	–	–	–
C _{15:0}	–	0.3	–	–	–	–	–	–
C _{16:0}	9.7	9.2	10.8	2.9	8.9	3.7	6.6	3.7
C _{17:0}	1.6	2.1	2.5	1.7	–	3.1	1.4	0.9
C _{18:0}	4.9	5.0	7.7	7.2	3.7	4.1	8.8	9.6
Unsaturated fatty acids								
C _{13:1} at 12–13	–	–	–	0.7	–	–	0.6	–
C _{17:1ω6c}	–	–	–	0.5	–	1.1	–	–
C _{18:1ω7c}	19.2	24.2	32.7	31.6	28.8	25.8	45.6	77.9
11-Methyl C _{18:1ω7t}	1.7	1.3	–	1.0	1.6	1.5	1.0	–
C _{20:1ω7c}	–	0.2	–	0.8	–	–	–	–
C _{20:2ω6,9c}	0.9	0.3	1.2	0.8	1.1	0.9	0.5	–
Hydroxy fatty acids								
C _{18:1} 2-OH	1.8	2.0	–	0.5	1.5	1.8	0.6	1.4
C _{18:0} 3-OH	0.5	0.6	–	0.5	–	–	–	–
Summed feature 3*	1.4	1.7	1.8	1.0	3.7	0.7	1.1	0.7
Cyclopropane acids								
C _{17:0} cyclo	1.0	1.3	–	–	2.9	–	0.8	–
C _{19:0} cyclo ω8c	57.0	50.8	43.2	50.2	47.2	57.4	32.7	5.9
Unknown 13.957†	0.3	0.2	–	0.2	–	–	–	–
Unknown 14.959†	–	0.3	–	0.7	–	–	0.3	–

*Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contained C_{16:1ω7c} and/or iso-C_{15:0} 2-OH.

†Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified. Values are equivalent chain lengths.

results from DNA–DNA hybridizations also demonstrated that CCUG 38531^T is a representative of another species of the genus *Ochrobactrum*. On the basis of unique phenotypic traits and genotypic characterization, we therefore describe two novel *Ochrobactrum* species.

Description of *Ochrobactrum pseudogrignonense* sp. nov.

Ochrobactrum pseudogrignonense [pseu.do.gri.gno.nen'se. Gr. adj. *pseudēs* false; N.L. neut. adj. *grignonense* a bacterial

Table 2. Physiological characteristics of the type strains of *Ochrobactrum* species closely related to strains CCUG 30717^T, CCUG 43892 and CCUG 38531^T

Strains: 1, *O. pseudogrignonense* sp. nov. CCUG 30717^T and CCUG 43892; 2, *O. haematophilum* sp. nov. CCUG 38531^T; 3, *O. grignonense* DSM 13338^T; 4, *O. anthropi* CIP 14970^T; 5, *O. gallinifaecis* Iso 196^T; 6, *O. intermedium* LMG 3301^T; 7, *O. tritici* LMG 18957^T. +, Positive; –, negative; (+), weakly positive. All strains were positive for hydrolysis of L-alanine *p*-nitroanilide (pNA) and weak hydrolysis of bis-*p*-nitrophenyl (pNP) phosphate. All strains were negative for hydrolysis of aesculin^a, pNP β-D-galactopyranoside^a, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP phosphorylcholine and 2-deoxythymidine-5'-pNP phosphate. All strains were also positive for assimilation of L-arabinose^a, D-galactose^a, gluconate, D-glucose, D-mannose, D-ribose^a, D-xylose, acetate, propionate^a, fumarate^a, glutarate, DL-lactate, L-malate^a, oxoglutarate^a, pyruvate^a, L-alanine, L-proline, L-serine^a and ornithine^a. All strains were negative for assimilation of *p*-arbutin, salicin, putrescine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, adipate^b, itaconate, mesaconate, phenylacetate^a, α-D-melibiose^a and azelate.

Test	1	2	3	4	5	6	7
Hydrolysis of:							
pNP phenylphosphonate	(+)	–	–	–	–	–	(+)
L-Glutamate γ-3-carboxy-pNA	–	–	–	(+)	–	+	(+)
L-Proline pNA ^{b*}	+	+	+	+	+	(+)	(+)
Assimilation of:							
D-Fructose, <i>myo</i> -inositol ^a , L-rhamnose ^a , D-sorbitol ^a , <i>cis</i> -aconitate, citrate ^{ab} , DL-3-hydroxybutyrate ^a	+	+	+	+	–	+	+
4-Aminobutyrate, β-alanine ^a	+	+	+	+	(+)	+	+
D-Maltose ^{ab} , N-acetyl-D-glucosamine ^a	–	+	–	+	–	+	+
Adonitol ^{ac}	+	+	–	+	–	+	+
Sucrose ^{ab} , trehalose	+†	+	–	+	–	–	+
Maltitol	–	+	–	+	–	–	–
<i>trans</i> -Aconitate ^a	+	+	–	–	–	(+)	–
D-Cellobiose ^{ac}	–	–	–	+	–	+	–
N-Acetyl-D-galactosamine	+	–	+	(+)	–	+	+
Suberate ^a	–	–	–	–	–	–	+
L-Aspartate ^a	+	+	+	+	+	+	–
4-Hydroxybenzoate	–	+	–	(+)	–	(+)	+
L-Histidine ^a	+	+	+	+	+	+	(+)
L-Leucine ^a	+	+	+	+	–	+	(+)

*Tests (based on different methods) were also performed in the following studies and gave congruent results: *a*, Holmes *et al.* (1988) with *O. anthropi*; *b*, Velasco *et al.* (1998) with *O. intermedium*; *c*, Lebuhn *et al.* (2000) with type strains of all four previously described species.

†Strain CCUG 30717^T showed only weakly positive results.

species epithet; N.L. neut. adj. *pseudogrignonense* a false (*Ochrobactrum*) *grignonense*].

Cells are non-motile, non-spore-forming rods (approx. 2 µm in length). Gram-negative and oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, nutrient agar and MacConkey agar at 25–30 °C. Growth is observed on PYE agar supplemented with 5 % NaCl (w/v) but different results are observed for the two known strains at 7 % NaCl. Growth is observed on PYE agar

at 4 °C but not at 42 °C. Beige, translucent and shiny colonies with entire edges are formed within 24 h, with a diameter of approximately 2 mm. The quinone system consists of Q-10 (96–99 %) and Q-9 (2–4 %). The polyamine pattern [(g dry weight)^{–1}] consists of the major compounds spermidine (23–35 µmol) and putrescine (27–34 µmol), moderate amounts of *sym*-homospermidine (9–10 µmol) and minor amounts of 1,3-diaminopropane (1–2 µmol) and spermine (1 µmol). Predominant polar lipids are phosphatidylethanolamine, phosphatidylmonomethylethanolamine,

Table 3. Percentage DNA–DNA reassociation values between the novel strains and related *Ochrobactrum* type strains

Strain	1	2	3	<i>O. grignonense</i> DSM 13338 ^T	<i>O. anthropi</i> CIP 14970 ^T	<i>O. tritici</i> LMG 18957 ^T	<i>O. intermedium</i> LMG 3301 ^T
1. CCUG 30717 ^T	100	81	21	49	26	32	19
2. CCUG 43892	100	100	22	45	48	25	13
3. CCUG 38531 ^T	38	13	100	40	18	42	47

phosphatidylglycerol and phosphatidylcholine. Moderate amounts of phosphatidylmethylethanolamine, diphosphatidylglycerol and two unidentified phosphorus-free aminolipids (AL1 and AL2) and small amounts of several unknown phospholipids, aminophospholipids and an aminolipid are also present. The fatty acid profile is composed largely of C_{18:1}ω7c (19.2–24.2 %) and C_{19:0} cyclo ω8c (50.8–57.8 %). Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all *Ochrobactrum* species) are indicated in Table 2.

The type strain, CCUG 30717^T (=CIP 109451^T), was isolated in 1992 from blood of a 28-year-old man in Göteborg, Sweden. Strain CCUG 43892 was isolated in 2000 from the ear of a newborn in Tromsø, Norway.

Description of *Ochrobactrum haematophilum* sp. nov.

Ochrobactrum haematophilum (hae.ma.to.phi'lum. Gr. n. *haema* -atis blood; Gr. adj. *philos* loving; N.L. neut. adj. *haematophilum* blood-loving).

Cells are non-motile, non-spore-forming rods (approx. 2 µm in length). Gram-negative and oxidase-positive, showing an oxidative metabolism. Good growth occurs on R2A agar, TSA, PYE agar, nutrient agar and MacConkey agar at 25–30 °C. Growth is observed on PYE agar supplemented with 5 % NaCl (w/v) but not with 7 %. No growth is observed on PYE agar at 4 or 42 °C. Beige, translucent and shiny colonies with entire edges form within 24 h, with a diameter of approximately 2 mm. The quinone system consists of Q-10 (98 %) and Q-9 (2 %). The polyamine pattern [(g dry weight)⁻¹] consists of the major compounds spermidine (49 µmol), putrescine (19 µmol) and *sym*-homospermidine (17 µmol) and minor amounts of 1,3-diaminopropane (3 µmol) and spermine (2 µmol). Predominant polar lipids are phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol and phosphatidylcholine. Moderate amounts of phosphatidylmethylethanolamine, diphosphatidylglycerol and two unidentified phosphorus-free aminolipids (AL1 and AL2) and small amounts of several unknown phospholipids, aminophospholipids and an aminolipid are also present. The fatty acid profile is composed largely of C_{18:1}ω7c (32.7 %) and C_{19:0} cyclo ω8c (43.2 %). Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all *Ochrobactrum* species) are indicated in Table 2.

The type strain, CCUG 38531^T (=CIP 109452^T), was isolated in 1997 from blood of a 79-year-old man in Falun, Sweden.

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