Pseudochrobactrum lubricantis sp. nov., isolated from a metal-working fluid

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A Gram-negative, rod-shaped, oxidase-positive, non-spore-forming, non-motile bacterium (KSS 7.8^T) was isolated from a water-mixed metal-working fluid. On the basis of 16S rRNA gene and *recA* sequence similarities, the isolate was clearly grouped in the genus *Pseudochrobactrum*. This allocation was confirmed by fatty acid data (major fatty acids: C_{18:2}ω7c and C_{19:0} cyclo ω8c), polar lipid profile (major components: phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylcholine, plus moderate amounts of phosphatidyl-monomethylethanolamine and unknown aminolipid AL1), quinone system (ubiquinone Q-10) and polyamine pattern (spermidine and putrescine predominant). DNA–DNA pairing with the most closely related *Pseudochrobactrum* species showed values ranging from 24.2 to 45.7 %, and physiological and biochemical data clearly differentiated this isolate from described *Pseudochrobactrum* species. This organism represents a novel species of the genus *Pseudochrobactrum*, for which the name *Pseudochrobactrum lubricantis* sp. nov. is proposed, with the type strain KSS 7.8^T (=CCUG 56963^T=CCM 7581^T).

The genus *Pseudochrobactrum* was proposed by Kämpfer *et al.* (2006). At present, it comprises *Pseudochrobactrum saccharolyticum*, *Pseudochrobactrum asaccharolyticum*, *Pseudochrobactrum kiredjianiae* (Kämpfer *et al.*, 2006, 2007) and *Pseudochrobactrum glaciei* (Romanenko *et al.*, 2008). Members of the genus can be clearly differentiated from members of the genera *Ochrobactrum* and *Brucella* on the basis of 16S rRNA gene sequence and *recA* sequence data, as well as chemotaxonomic data (Kämpfer *et al.*, 2006, 2007).

Strain KSS 7.8^T was isolated from a metal-working fluid of a metal-processing company in Germany on nutrient agar at 30 °C. Subcultivation was done on tryptone soy agar at 28 °C for 48 h. On this agar, this organism grew also at 15–45 °C, but not at 10 or 50 °C. Growth at 30 °C was also observed on MacConkey agar and R2A agar (all from Oxoid).

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 30 °C on nutrient agar. The 16S rRNA gene was analysed as described previously (Kämpfer *et al.*, 2003). Sequence analysis and similarity calculations were carried out by using the software programs Bionumerics v. 4.0 (Applied Maths BVBA) and MEGA v. 3.1 (Kumar *et al.*, 2004). The sequenced length of the 16S rRNA gene was 1401 bp (GenBank accession no. FM209496) for strain KSS 7.8^T. Sequence similarities to the three most closely related *Pseudochrobactrum* species were 99.8 % to *P. saccharolyticum* CCUG 33852^T, 99.8 % to *P. asaccharolyticum* CCUG 46016^T and 99.3 % to *P. kiredjianiae* CCUG 49584^T. A lower similarity of 96.2 % was found to *P. glaciei* KMM 3858^T. The 16S rRNA gene-based tree shown in Fig. 1 results from a neighbour-joining reconstruction using the Kimura two-parameter correction and 1000 resamplings for bootstrap analysis.

The partial *recA* sequence of strain KSS 7.8^T was analysed as described previously (Scholz *et al.*, 2006). The PCR was performed with primers RecA-PsOchro-f (5'-AAGGCTC-TGGACGCGGCACT-3') and RecA-PsOchro-r (5'-CGC-AAGGTCAGTTC AATCTCAT-3'). *recA* sequence similarity with *P. kiredjianiae* CCUG 49584^T was 89.2 %; it was

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and the *recA* sequence of strain KSS 7.8^T are FM209496 and FM211811, respectively.

Fig. 1. 16S rRNA gene sequence-based phylogenetic neighbour-joining tree reconstruction, showing the affiliation of *Pseudochrobactrum lubricantis* KSS 7.8^T to its closest relatives. The significance of each branch is indicated by a bootstrap value (%) calculated for 1000 subsets. *Bartonella henselae* M40SHD was used as outgroup to construct a pseudo-rooted tree. Bar, 0.005 divergent residues per site.

88.8 % with *P. saccharolyticum* CCUG 33852^T and 91.7 % with *P. asaccharolyticum* CCUG 46016^T within 743 nt. Lower similarity values were observed to *Brucella melitensis* NCTC 10094^T (80.5 %) and *Ochrobactrum anthropi* LMG 3331^T (82.2 %). Strain KSS 7.8^T formed a separate branch in the phylogenetic tree of *recA* sequences (743 nt) (Fig. 2). The tree was constructed by using CLUSTREE neighbour-joining analysis (Higgins *et al.*, 1996). The *recA* gene of *P. glaciei* KMM 3858^T could not be amplified with the primers described above and hence was not included in the analysis.

The results of the fatty acid analysis are shown in Table 1. Fatty acids were analysed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain KSS 7.8^T was mainly composed of C_{19:0} cyclo ω8c (53.9 %), C_{18:1} ω7c (29.1 %), C_{18:0} (7.8 %) and C_{16:0} (4.5 %) and showed a high similarity to the fatty acid profiles of all other *Pseudochrobactrum* species. Quinones and polar lipids were extracted by an integrated protocol as described previously (Tindall, 1990a, b; Altenburger *et al.*, 1996); quinone composition was analysed by HPLC as reported by Stolz *et al.* (2007). Polyamines were extracted and analysed as reported previously (Busse & Auling, 1988; Busse *et al.*, 1997; Stolz *et al.*, 2007). The quinone system of strain KSS 7.8^T consisted of the major compound ubiquinone Q-10 (97 %) and minor amounts of Q-8, Q-9 and Q-11 (0.7–1.9 %). The polar lipid profile (results not shown) consisted of the major compounds phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylgly-

cerol and phosphatidylcholine, moderate amounts of phosphatidylmonomethylethanolamine and unknown aminolipid AL1, and small to trace amounts of three unknown polar lipids (L3, L4 and L5) corresponding to the lipids shown for *P. asaccharolyticum* CCUG 46016^T (Kämpfer *et al.*, 2006). The polyamine pattern was as follows [in μmol (g dry weight)⁻¹]: spermidine, 63.8; putrescine, 43.9; 1,3-diaminopropane, 0.7; spermine, 2.3. Both polar lipid profile and polyamine pattern are quite similar to those reported for other *Pseudochrobactrum* species and are in good agreement with the characteristics of the genus description (Kämpfer *et al.*, 2006, 2007), although the unknown lipids L1 and L2 were not detectable in strain KSS 7.8^T.

Results of the physiological characterization are given in the species description and in Table 2. The methods used have been described previously (Kämpfer *et al.*, 1991). The organism can be clearly differentiated from the other *Pseudochrobactrum* species on the basis of several tests.

DNA–DNA hybridization experiments were performed with strain KSS 7.8^T and type strains of the three most closely related *Pseudochrobactrum* species by 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. Strain KSS 7.8^T showed relatively low DNA–DNA similarity to *P. asaccharolyticum* CCUG 46016^T (24.8 %), *P. saccharolyticum* CCUG 33852 (45.7 %) and *P. kiredjianiae* CCUG 49584^T (40.9 %).

Fig. 2. Phylogenetic tree reconstruction with *recA* sequences (743 nt) using CLUSTREE neighbour-joining analysis (Higgins *et al.*, 1996). The significance of each branch is indicated by a bootstrap value (%) calculated for 1000 subsets. The tree was rooted by using the sequence of *Bartonella henselae* Houston-1^T as an outgroup. Bar, 0.1 divergent residues per site.

Table 1. Major fatty acid compositions (%) of type strains of *Pseudochrobactrum* species

Taxa: 1, strain KSS 7.8^T; 2, *P. kiredjianiae* CCUG 49584^T; 3, *P. asaccharolyticum* CCUG 46016^T; 4, *P. saccharolyticum* CCUG 33852^T; 5, *P. glaciei* KMM 3858^T. All strains were grown on trypticase soy broth agar at 28 °C for 48 h prior to fatty acid analysis. Data were obtained in the present study except where indicated.

Fatty acid	1	2	3	4	5*
Saturated:					
C _{12:0}				0.7	
C _{14:0}					0.6
C _{16:0}	4.5	2.8	5.5	1.9	8.9 (5.4)
C _{18:0}	7.8	7.7	11.0	7.4	5.5 (4.4)
Unsaturated†:					
C _{18:1ω7c}	29.1	33.4	74.9	74.6	31.2 (41.5)
C _{18:3ω6c} (6,9,12)				0.5	
11-methyl-C _{18:1ω7t}	1.8	1.0			1.0
C _{20:1ω7c}		0.8			
C _{20:2ω6,9c}	1.4	0.9			0.9
Hydroxy:					
C _{18:0} 3-OH					0.6
Summed feature 3‡			0.8		1.6
Cyclo propane acids:					
C _{17:0} cyclo		0.5			1.0
C _{19:0} cyclo ω8c	53.9	53.0	7.3	14.3	47.9 (46.2)
Unknown (ECL 14.959)§	1.2		0.6	0.9	0.9

*Data in parentheses are from Romanenko *et al.* (2008).

†The position of the double bond is located by counting from the methyl (ω) end of the carbon chain.

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

§Unknown fatty acids have no name listed in the peak library file of the MIDI system and therefore cannot be identified. ECL, Equivalent chain length.

From the results of 16S rRNA gene and *recA* sequencing and from the physiological characteristics, it is evident that strain KSS 7.8^T is different from previously described species of the genus *Pseudochrobactrum*. For this reason, *Pseudochrobactrum lubricantis* sp. nov. is proposed to accommodate strain KSS 7.8^T.

Description of *Pseudochrobactrum lubricantis* sp. nov.

Pseudochrobactrum lubricantis [lu.bri.can'tis. L. v. *lubricare* to lubricate; N.L. n. *lubricans* -antis (from L. part. adj. *lubricans*) a lubricant; N.L. gen. n. *lubricantis* of/from a (coolant) lubricant].

Shares all characteristics listed in the genus description (Kämpfer *et al.*, 2006). In addition, good growth occurs on R2A agar, tryptone soy agar, nutrient agar and MacConkey agar at 25–30 °C. Beige, translucent and shiny colonies with

Table 2. Physiological characteristics of the type strains of *Pseudochrobactrum* species

Taxa: 1, strain KSS 7.8^T; 2, *P. kiredjianiae* CCUG 49584^T; 3, *P. asaccharolyticum* CCUG 46016^T; 4, *P. saccharolyticum* CCUG 33853^T; 5, *P. glaciei* KMM 3858^T. Data for strains 1 and 5 are from the present study; data for strains 2–4 are from Kämpfer *et al.* (2007), based on the same methods. +, Positive; –, negative; (+), weakly positive. All strains were positive for hydrolysis of L-alanine-*p*-nitroanilide and L-proline-*p*-nitroanilide and weak hydrolysis of bis-*p*-nitrophenylphosphate. All strains were negative for hydrolysis of aesculin, *p*-nitrophenyl-β-D-galactopyranoside, *p*-nitrophenyl-β-D-glucuronide, *p*-nitrophenyl-α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenylphosphorylcholine, *p*-nitrophenyl phenylphosphonate and 2-deoxythymidine-5'-*p*-nitrophenylphosphate. All strains were also positive for assimilation of acetate, oxoglutarate, L-alanine, L-proline and L-serine. All strains (*P. glaciei* KMM 3858^T was not included in the tests) were negative for assimilation of adonitol, *p*-arbutin, cellobiose, D-gluconate, i-inositol, maltose, D-maltitol, D-mannitol, α-D-melibiose, D-sorbitol, salicin, trehalose, putrescine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, *trans*-aconitate, adipate, citrate, itaconate, mesaconate, L-leucine, phenylacetate, suberate, azelate and 4-hydroxybenzoate.

Characteristic	1	2	3	4	5
Assimilation of:					
L-Arabinose	–	–	–	+	–
D-Xylose	–	–	–	+	+
D-Mannose	+	–	–	+	(+)
D-Glucose	+	+	–	+	(+)
D-Fructose	+	–	–	+	+
DL-3-Hydroxybutyrate	–	–	–	+	–
D-Galactose	+	(+)	–	+	+
Pyruvate, ornithine	+	–	–	+	–
D-Ribose, propionate, glutarate	+	+	–	+	–
Fumarate	+	+	–	+	–
DL-Lactate, L-malate	+	+	–	+	–
L-Rhamnose	–	+	–	–	–
4-Aminobutyrate, β-alanine	+	+	–	+	–
cis-Aconitate	(+)	–	+	+	–
N-Acetyl-D-glucosamine	+	(+)	–	+	+
L-Aspartate	+	(+)	–	+	–
L-Histidine	+	–	–	+	+

entire edges form within 24 h, with a diameter of approximately 2 mm. Carbon-source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all *Pseudochrobactrum* species) are indicated in Table 2. The major fatty acids are C_{18:2ω7c} and C_{19:0} cyclo ω8c. The polar lipid profile consists of the major compounds phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylcholine, moderate amounts of phosphatidylmonomethylethanolamine and unknown aminolipid AL1, and small to trace amounts of three unknown polar lipids. Ubiquinone Q-10 is the major quinone. The polyamine pattern consists of the major compounds spermidine and putrescine and minor amounts of 1,3-diaminopropane and spermine.

The type strain is KSS 7.8^T (=CCUG 56963^T=CCM 7581^T), isolated from a water-mixed metal-working fluid.

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