Description of *Pseudochrobactrum kiredjaniae* sp. nov.

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A Gram-negative, rod-shaped, oxidase-positive, non-spore-forming, non-motile bacterium (strain CCUG 49584^T), isolated from a seafood processing plant sample in New Zealand, was subjected to a polyphasic taxonomic study. On the basis of 16S rRNA and *recA* gene sequence similarities, the isolate was allocated to the genus *Pseudochrobactrum*. This was confirmed by fatty acid data (major fatty acids: C_{18:1}ω7c and C_{19:0} cyclo ω8c), a polar lipid profile exhibiting major characteristics of *Pseudochrobactrum* (phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine), quinone system Q-10 and a polyamine pattern with the predominant compounds spermidine and putrescine. DNA–DNA hybridization with the type strains of the two established species of *Pseudochrobactrum* and physiological and biochemical data clearly differentiated the isolate from established *Pseudochrobactrum* species. As a consequence, this organism represents a novel species, for which the name *Pseudochrobactrum kiredjaniae* sp. nov. is proposed, with the type strain CCUG 49584^T (= CIP 109227^T).

The genus *Pseudochrobactrum* was proposed recently by Kämpfer *et al.* (2006), comprising the species *Pseudochrobactrum saccharolyticum* and *Pseudochrobactrum asaccharolyticum*. This genus could be clearly differentiated from *Ochrobactrum* and from *Brucella* species based on its phylogenetic position and the presence of a combination of certain phenotypic traits (Kämpfer *et al.*, 2006).

Strain CCUG 49584^T was isolated in 1995 from stainless-steel vent covers from a seafood processing plant in Nelson, New Zealand, on standard plate count agar (Oxoid) at 30 °C. Subcultivation was done on tryptone soy agar (TSA) at 28 °C for 48 h. On this agar, the organism also grew 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 by Kämpfer *et al.* (2003). Phylogenetic analysis was performed using the software package MEGA (Molecular Evolutionary Genetics Analysis) version 2.1 (Kumar *et al.*, 2001) after multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model on the basis of 1360 bp) and clustering with the neighbour-joining method were performed by using bootstrap values based on 1000 replications (Fig. 1). The sequenced length of the 16S rRNA gene of strain CCUG 49584^T was 1387 bp (GenBank accession no. AM263420). The sequence similarities with the two *Pseudochrobactrum* species were 99.4 % to *P. saccharolyticum* CCUG 33852^T and 99.5 % to *P. asaccharolyticum* CCUG 46016^T. Nucleotide sequence similarities to all established species of the genus *Bartonella* ranged from 94.2 to 94.3 %. Sequence similarities to established species of

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and *recA* gene sequences of strain CCUG 49584^T are AM263420 and AM263419, respectively.

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from GenBank/EMBL/DBJ (accession numbers are given in parentheses) constructed after multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were performed by using MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap percentages based on 1000 replications are given at branch points. Bar, 0.005 substitutions per nucleotide position.

Brucella and *Ochrobactrum* were 94.6–94.8 and 93.8–94.6 %, respectively. The 16S rRNA gene-based phylogenetic tree shown in Fig. 1 resulted from a neighbour-joining reconstruction using the Kimura two-parameter correction and 1000 resamplings for bootstrap analysis.

A partial *recA* sequence of strain CCUG 49584^T was analysed according to Scholz *et al.* (2006). PCR was performed with primers RecA-PsOchro-f (5'-AAGGCTCTGGACGCGGC-ACT-3') and RecA-PsOchro-r (5'-CGCAAGGTCAGTTC-AATCTCAT-3'). The similarities of *recA* between strain CCUG 49584^T and the two other *Pseudochrobactrum* species

were 90.5 % for *P. saccharolyticum* CCUG 33852^T and 91.3 % for *P. asaccharolyticum* CCUG 46016^T within 897 nt. Lower similarity values were observed with *Brucella* species (76.7 %), *Ochrobactrum* species (77.3–81.4 %), *Bartonella* (71.0 %) and *Rickettsia felis* (62.3 %) (Fig. 2). Strain CCUG 49584^T showed several motifs within *recA* that are absent from *Bartonella*, *Brucella* and *Ochrobactrum* species, but are present in *Pseudochrobactrum* species (Kämpfer *et al.*, 2006).

Strain CCUG 49584^T formed a separate branch in the phylogenetic tree of *recA* sequences (897 nt) (Fig. 2). The tree was constructed as described above for the 16S rRNA gene.

Fig. 2. Phylogenetic analysis based on *recA* gene sequences (909 nt) available from GenBank/EMBL/DBJ (accession numbers are given in parentheses) constructed after multiple alignment of data using CLUSTAL_X (Thompson *et al.*, 1997). Distances (distance options according to the Kimura two-parameter model) and clustering with the neighbour-joining method were performed by using MEGA version 2.1 (Kumar *et al.*, 2001). Bootstrap percentages based on 1000 replications are given at branch points. Bar, 0.1 substitutions per nucleotide position.

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 CCUG 49584^T mainly comprised C_{19:0} cyclo ω8c (53.0 %), C_{18:1}ω7c (33.4 %), C_{18:0} (7.7 %) and C_{16:0} (2.8 %). A clear differentiation from the other *Pseudochrobactrum* species was possible based on more than 2-fold greater amounts of C_{18:1}ω7c in the *Pseudochrobactrum* reference strains and more than 3.5-fold greater amounts of C_{19:0} cyclo ω8c in strain CCUG 49584^T. A quinone system with ubiquinone Q-10 predominant, which was detected after extraction and analysis as reported by Tindall (1990) and Altenburger *et al.* (1996), but using an HPLC-apparatus consisting of a JASCO PU 2080 Plus Pump and a JASCO UV-2075 Plus UV/VIS-Detector, was in agreement with the characteristics of the two established *Pseudochrobactrum* species. Polyamines were extracted and analysed by HPLC according to Busse & Auling (1988),

employing JASCO PU 2080 Plus Pump and a JASCO model 821-FP spectrofluorometric detector. Similar to *P. asaccharolyticum* CCUG 46016^T, the polyamine pattern of strain CCUG 49584^T consisted predominantly of spermidine and putrescine. Polar lipids were analysed according to Ventosa *et al.* (1993) from biomass that was grown overnight in PYE medium (0.3 % peptone from casein, 0.3 % yeast extract, pH 7.2). The polar lipid profile of strain CCUG 49584^T shared the major characteristics reported recently for the two *Pseudochrobactrum* species (Kämpfer *et al.*, 2006). It consisted of major components phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, moderate amounts of phosphatidylmonomethyl ethanolamine and an unknown aminolipid AL1, and small to trace amounts of three unknown polar lipids [L3, L4 and L5, which have been reported to be present in minor amounts in the two established *Pseudochrobactrum* species;

Table 1. Major fatty acid compositions (%) of strain CCUG 49584^T and type strains of *Pseudochrobactrum* and *Ochrobactrum* species

Strains: 1, CCUG 49584^T (*Pseudochrobactrum kiredjianiae* sp. nov.); 2, *P. asaccharolyticum* CCUG 46016^T; 3, *P. saccharolyticum* CCUG 33852^T; 4, *O. gallinifaecis* Iso 196^T; 5, *O. intermedium* LMG 3301^T; 6, *O. anthropi* CIP 14970^T; 7, *O. grignonense* DSM 13338^T; 8, *O. lupini* LUP21^T; 9, *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*	9
Saturated acids:									
C _{12:0}	—	—	0.7	—	—	—	—	—	—
C _{14:0}	—	—	—	0.7	—	—	—	—	—
C _{16:0}	2.8	5.5	1.9	8.9	3.7	6.6	2.9	4.2	3.7
C _{17:0}	—	—	—	—	3.1	1.4	1.7	1.6	0.9
C _{18:0}	7.7	11.0	7.4	3.7	4.1	8.8	7.2	3.0	9.6
Unsaturated acids:									
C _{13:1} at 12–13	—	—	—	—	—	0.6	0.7	—	—
C _{17:1} ω6c	—	—	—	—	1.1	—	0.5	1.4	—
C _{18:1} ω7c	33.4	74.9	74.6	28.8	25.8	45.6	31.6	70.8	77.9
C _{18:3} ω6c (6,9,12)	—	—	0.5	—	—	—	—	—	—
11-Methyl-C _{18:1} ω7t	1.0	—	—	1.6	1.5	1.0	1.0	—	—
C _{20:1} ω7c	0.8	—	—	—	—	—	0.8	—	—
C _{20:2} ω6,9c	0.9	—	—	1.1	0.9	0.5	0.8	—	—
Hydroxy acids:									
C _{18:1} 2-OH	—	—	—	1.5	1.8	0.6	0.5	6.2	1.4
C _{18:0} 3-OH	—	—	—	—	—	—	0.5	1.0	—
Summed feature 3†	—	0.8	—	3.7	0.7	1.1	1.0	2.3	0.7
Cyclopropane acids:									
C _{17:0} cyclo	0.5	—	—	2.9	—	0.8	—	—	—
C _{19:0} cyclo ω8c	53.0	7.3	14.3	47.2	57.4	32.7	50.2	4.3	5.9
Unknown 13.957‡	—	—	—	—	—	—	0.2	—	—
Unknown 14.959‡	—	0.6	0.9	—	—	0.3	0.7	—	—

*Data from Trujillo *et al.* (2005).

†Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contains C_{16:1}ω7c 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; they are referred to by their equivalent chain length.

Table 2. Physiological characteristics of strain CCUG 49584^T and type strains of *Pseudochrobactrum* and *Ochrobactrum* species

Strains: 1, CCUG 49584^T (*Pseudochrobactrum kiredjianiae* sp. nov.), 2, *P. asaccharolyticum* CCUG 46016^T; 3, *P. saccharolyticum* CCUG 33852^T; 4, *O. gallinifaecis* Iso 196^T; 5, *O. intermedium* LMG 3301^T; 6, *O. anthropi* CIP 14970^T; 7, *O. grignonense* DSM 13338^T; 8, *O. lupini* LUP21^T; 9, *O. tritici* LMG 18957^T. +, Positive; –, negative; (+), weakly positive; ND, not determined; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl. All strains (except *O. lupini* LUP21^T, for which only the data given in the table are available) are positive for hydrolysis of L-alanine pNA and weakly positive for hydrolysis of bis-pNP phosphate. All strains (except *O. lupini* LUP21^T) are negative for hydrolysis of aesculin, pNP β -D-galactopyranoside, pNP β -D-glucuronide, pNP α -D-glucopyranoside, pNP β -D-glucopyranoside, pNP phosphoryl choline and 2-deoxythymidine-5'-pNP phosphate. All strains (except *O. lupini* LUP21^T) are positive for assimilation of acetate, oxoglutarate, L-alanine, L-proline and L-serine. All strains are negative for assimilation of *p*-arbutin, salicin, putrescine, L-phenylalanine, L-tryptophan, 3-hydroxybenzoate, adipate, itaconate, mesaconate, phenylacetate, α -D-melibiose and azelate. Hydrolysis of aesculin and pNP β -D-galactopyranoside and assimilation of oxoglutarate, L-serine, phenylacetate and α -D-melibiose were also tested using a different method by Holmes *et al.* (1998) with *O. anthropi*; results were in agreement with those of this study. Assimilation of adipate was also tested using a different method by Velasco *et al.* (1998) with *O. intermedium*; results were in agreement with those of this study.

Characteristic*	1	2	3	4	5	6	7	8†	9
Hydrolysis of:									
pNP Phenylphosphonate	–	–	–	–	–	–	–	ND	(+)
L-Glutamate- γ -3-carboxy pNA	–	–	–	–	+	(+)	–	ND	(+)
L-Proline pNA ^a	+	+	+	+	(+)	+	+	ND	(+)
Assimilation of:									
Gluconate	–	–	–	+	+	+	+	+	+
L-Arabinose ^b , D-mannose, D-xylose	–	–	+	+	+	+	+	+	+
D-Glucose	+	–	+	+	+	+	+	+	+
D-Fructose	–	–	+	–	+	+	+	–	+
DL-3-Hydroxybutyrate ^b	–	–	+	–	+	+	+	ND	+
D-Galactose ^b	(+)	–	+	+	+	+	+	–	+
Pyruvate ^b , ornithine ^b	–	–	+	+	+	+	+	ND	+
D-Ribose ^b , propionate ^b , fumarate ^b , glutarate, DL-lactate, L-malate ^b	+	–	+	+	+	+	+	ND	+
i-Inositol ^b , D-sorbitol ^b	–	–	–	–	+	+	+	–	+
L-Rhamnose ^b	+	–	–	–	+	+	+	+	+
Citrate ^{a,b}	–	–	–	–	+	+	+	+	+
4-Aminobutyrate, β -alanine ^b	+	–	+	(+)	+	+	+	ND	+
cis-Aconitate	–	+	+	–	+	+	+	ND	+
D-Maltose ^{a,b} , adonitol ^{b,c}	–	–	–	–	+	+	–	+	+
N-Acetyl-D-glucosamine ^b	(+)	–	+	–	+	+	–	+	+
Sucrose ^{a,b}	–	–	–	–	–	+	–	ND	+
D-Trehalose	–	–	+	–	–	+	–	–	+
Maltitol	–	–	–	–	–	+	–	ND	–
D-Mannitol	–	–	–	–	–	+	+	+	+
trans-Aconitate ^b	–	–	–	–	(+)	–	–	ND	–
D-Cellobiose ^{b,c}	–	–	–	–	+	+	–	–	–
N-Acetyl-D-galactosamine	–	–	–	–	+	(+)	+	ND	+
Suberate ^b	–	–	–	–	–	–	–	ND	+
L-Aspartate ^b	(+)	–	+	+	+	+	+	ND	–
4-Hydroxybenzoate	–	–	–	–	(+)	(+)	–	ND	+
L-Histidine ^b	–	–	+	+	+	+	+	ND	(+)
L-Leucine ^b	–	–	–	–	+	+	+	ND	(+)

*Tested using a different method in another study; results were in agreement with those of this study. *a*, Velasco *et al.* (1998) with *O. intermedium*; *b*, Holmes *et al.* (1988) with *O. anthropi*; *c*, Lebuhr *et al.* (2000) with *O. anthropi*, *O. grignonense*, *O. tritici* and *O. intermedium*.
†Data from Trujillo *et al.* (2005).

see Supplementary Fig. S2 of Kämpfer *et al.* (2006) in IJSEM Online]. In addition, trace amounts of an unknown amino-lipid were detected, which was not reported to be present in the established *Pseudochrobactrum* species (Kämpfer *et al.*, 2006), and phosphatidylmethylethanolamine, an unknown phospholipid and two unknown polar lipids, present in the reference species, were not detected. In contrast to Kämpfer *et al.* (2006), reanalysis of the polar lipid profile of *P. asaccharolyticum* CCUG 46016^T from newly grown biomass, as for strain CCUG 49584^T, also revealed the absence of phosphatidylmethylethanolamine, the unknown phospholipid, the two unknown polar lipids and the presence of trace amounts of the unknown aminolipid. These observations indicate that the presence/absence of phosphatidylmethylethanolamine, the unknown phospholipid, the unknown aminolipid and the two unknown polar lipids may be related to the physiological conditions of the cells when harvested for polar lipid extraction. Based on these results, strain CCUG 49584^T shares the polar lipid characteristics that differentiate *Pseudochrobactrum* species from *Ochrobactrum* and *Brucella* species (Kämpfer *et al.*, 2006).

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

DNA–DNA hybridization experiments were performed with strain CCUG 49584^T and the type strains of the two established *Pseudochrobactrum* 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. Strain CCUG 49584^T showed relatively low DNA–DNA relatedness to *P. asaccharolyticum* CCUG 46016^T (37.8 %) and *P. saccharolyticum* CCUG 33852^T (22.8 %).

From the results of 16S rRNA gene and *recA* sequencing, DNA–DNA hybridization data, fatty acid analyses and from the physiological characteristics it is evident that strain CCUG 49584^T is different from the two established species of *Pseudochrobactrum*. Hence, strain CCUG 49584^T represents a novel species of the genus *Pseudochrobactrum*, for which we propose the name *Pseudochrobactrum kiredjiana*.

Description of *Pseudochrobactrum kiredjiana* sp. nov.

Pseudochrobactrum kiredjiana (ki.red.ji.a'ni.ae. N.L. fem. gen. n. *kiredjiana* of Kiredjian, named after Martine Kiredjian, a contemporary French microbiologist, for her numerous contributions to the taxonomy of *Ochrobactrum* and related organisms).

Shares all characteristics listed in the genus description. Good growth occurs on R2A agar, TSA, nutrient agar and MacConkey agar at 25–30 °C. Beige, translucent and shiny

colonies with entire edges form within 24 h, with a diameter of approximately 2 mm. Quinone system is ubiquinone Q-10 (99 %) and Q-9 (1 %). The polar lipid profile consists of the major components phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol and phosphatidylcholine, moderate amounts of phosphatidylmonomethylethanolamine and unknown aminolipid AL1 and trace amounts of three unknown lipids. Polyamine profile consists of spermidine [60.3 µmol (g dry weight)^{−1}], putrescine [42.7 µmol (g dry weight)^{−1}], 1,3-diaminopropane [2.0 µmol (g dry weight)^{−1}] and spermine [1.5 µmol (g dry weight)^{−1}]. Carbon source utilization and hydrolysis of chromogenic substrates (including characteristics that differentiate *Ochrobactrum* species) are given in Table 2.

The type strain is CCUG 49584^T (=CIP 109227^T), which was isolated in 1995 from a stainless-steel vent cover, from a seafood processing plant in Nelson, New Zealand.

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