Description of *Pseudochrobactrum kiredjianiae* sp. nov.

Peter Kämpfer,1 Holger Scholz,2 Birgit Huber,3 Kathrin Thummes,1 Hans-Jürgen Busse,3 Elizabeth W. Maas4 and Enevold Falsen5

1Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
2Bundeswehr Institute of Microbiology, D-80937 Munich, Germany
3Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria
4National Institute of Water & Atmospheric Research Ltd, Taihoru Nukurangi Greta Point, PO Box 14-901, Kilbirnie, Wellington, New Zealand
5Culture Collection University Göteborg, Department of Clinical Bacteriology, S-41346 Göteborg, Sweden

A Gram-negative, rod-shaped, oxidase-positive, non-spore-forming, non-motile bacterium (strain CCUG 49584T), 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: C18:1ω7c and C19:0 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 kiredjianiae* sp. nov. is proposed, with the type strain CCUG 49584T (=CIP 109227T).

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 49584T 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).

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

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The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA and recA gene sequences of strain CCUG 49584T are AM263420 and AM263419, respectively.
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 49584T was analysed according to Scholz et al. (2006). PCR was performed with primers RecA-PsOchro-f (5'-AAGGCTCTGGACGGCG-ACT-3') and RecA-PsOchro-r (5'-CGCAAGGTAGTTC-AATCTCAT-3'). The similarities of recA between strain CCUG 49584T and the two other Pseudochrobactrum species were 90.5 % for P. saccharolyticum CCUG 33852T and 91.3 % for P. asaccharolyticum CCUG 46016T 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 49584T 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 49584T 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. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from GenBank/EMBL/DDBJ (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.

Fig. 2. Phylogenetic analysis based on recA gene sequences (909 nt) available from GenBank/EMBL/DDBJ (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 Kämper & Kroppenstedt (1996). The fatty acid profile of strain CCUG 49584\textsuperscript{T} mainly comprised C\textsubscript{19:0} cyclo \textomega 8c (53.0 %), C\textsubscript{18:1\textomega 7c} (33.4 %), C\textsubscript{18:0} (7.7 %) and C\textsubscript{16:0} (2.8 %). A clear differentiation from the other \textit{Pseudochrobactrum} species was possible based on more than 2-fold greater amounts of C\textsubscript{18:1\omega 7c} in the \textit{Pseudochrobactrum} reference strains and more than 3.5-fold greater amounts of C\textsubscript{19:0} cyclo \textomega 8c in strain CCUG 49584\textsuperscript{T}.

A quinone system with ubiquinone Q-10 predominant, A quinone system with ubiquinone Q-10 predominant,

Pseudochrobactrum the other

on more than 2-fold greater amounts of C\textsubscript{18:1} (7.7 %) and C\textsubscript{16:0} (2.8 %). A clear differentiation from

unsaturated fatty acids, the position of the double bond is located by counting from the methyl (\textomega) end of the carbon chain; cis and trans isomers are indicated by the suffixes c and t, respectively.

Fatty acid 1 2 3 4 5 6 7 8* 9
Saturated acids:
C\textsubscript{12:0} – – 0.7 – – – – –
C\textsubscript{14:0} – – – 0.7 – – – –
C\textsubscript{16:0} 2.8 5.5 1.9 8.9 3.7 6.6 2.9 4.2 3.7
C\textsubscript{17:0} – – – – 3.1 1.4 1.7 1.6 0.9
C\textsubscript{18:0} 7.7 11.0 7.4 3.7 4.1 8.8 7.2 3.0 9.6
Unsaturated acids:
C\textsubscript{13:1} at 12–13 – – – – 0.6 0.7 – –
C\textsubscript{17:1\textomega 6c} – – – 1.1 – 0.5 1.4 –
C\textsubscript{18:1\omega 7c} 33.4 74.9 74.6 28.8 25.8 45.6 31.6 70.8 77.9
C\textsubscript{18:3\omega 6c} (6,9,12) – – 0.5 – – – – –
11-Methyl-C\textsubscript{18:1\omega 7t} 1.0 – – 1.6 1.5 1.0 1.0 – –
C\textsubscript{20:1\omega 7c} 0.8 – – – – 0.8 – –
C\textsubscript{20:1\omega 6,9c} 0.9 – – 1.1 0.9 0.5 0.8 – –
Hydroxy acids:
C\textsubscript{18:1} 2-OH – – – 1.5 1.8 0.6 0.5 6.2 1.4
C\textsubscript{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\textsubscript{17:0} cyclo 0.5 – – 2.9 – 0.8 – –
C\textsubscript{19:0} cyclo \textomega 8c 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\textsubscript{16:1\omega 7c} and/or C\textsubscript{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 1. Major fatty acid compositions (%) of strain CCUG 49584\textsuperscript{T} and type strains of \textit{Pseudochrobactrum} and \textit{Ochrobactrum} species

Strains: 1, CCUG 49584\textsuperscript{T} (\textit{Pseudochrobactrum kiredjianiae} sp. nov.); 2, \textit{P. asacharolyticum} CCUG 46016\textsuperscript{T}; 3, \textit{P. saccharolyticum} CCUG 33852\textsuperscript{T}; 4, \textit{O. gallinifica} Isol 196\textsuperscript{T}; 5, \textit{O. intermedius} LMG 3301\textsuperscript{T}; 6, \textit{O. anthropi} CIP 14970\textsuperscript{T}; 7, \textit{O. grignonense} DSM 13338\textsuperscript{T}; 8, \textit{O. lupini} LUP21\textsuperscript{T}; 9, \textit{O. tritici} LMG 18957\textsuperscript{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 (\textomega) end of the carbon chain; cis and trans isomers are indicated by the suffixes c and t, respectively. –, Not detected.
Table 2. Physiological characteristics of strain CCUG 49584\textsuperscript{T} and type strains of *Pseudochrobactrum* and *Ochrobactrum* species

<table>
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<tr>
<th>Characteristic*</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8†</th>
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<td>L-Proline pNA\textsuperscript{a}</td>
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<td>+</td>
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<td>L-Arabinose\textsuperscript{b}, D-mannose, D-xylose</td>
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<td>–</td>
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<td>+</td>
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<td>ND</td>
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<td>–</td>
<td>+</td>
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<td>+</td>
<td>ND</td>
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<td>D-Ribose\textsuperscript{b}, propionate\textsuperscript{b}, fumarate\textsuperscript{b}, glutarate, DL-lactate, L-malate\textsuperscript{b}</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
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<td>D-Maltose\textsuperscript{a,c}, adonitol\textsuperscript{b,c}</td>
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<td>N-Acetyl-D-glucosamine\textsuperscript{b}</td>
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</table>

*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, Lebuhn *et al.* (2000) with *O. anthropi*, *O. grignonense*, *O. tritici* and *O. intermedium*. †Data from Trujillo *et al.* (2005).
Pseudochrobactrum kiredjianiae sp. nov.

Description of Pseudochrobactrum kiredjianiae sp. nov.

Pseudochrobactrum kiredjianiae (ki.red.ji.a’ni.ae. N.L. fem. gen. n. kiredjianiae 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, diphasphatidylglycerol 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.

Acknowledgements

We thank E. R. B. Moore for helpful discussions and Gundula Will and Maria Sowinsky for excellent technical assistance. The work of B. H. and H.-J. B. was supported by a Contract-Research-Project for the Bundeswehr Medical Service.

References


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 phosphatidyldimethylethanolamine, 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 phosphatidyldimethylethanolamine, 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 phosphatidyldimethylethanolamine, 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 the 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 kiredjianiae.


