Paenochrobactrum gallinarii gen. nov., sp. nov., isolated from air of a duck barn, and reclassification of *Pseudochrobactrum glaciei* as *Paenochrobactrum glaciei* comb. nov.

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A Gram-negative, rod-shaped, oxidase-positive, non-spore-forming, non-motile bacterium (Sa25^T) was isolated from air of a duck barn. 16S rRNA gene and *recA* sequence analyses clearly placed the isolate in the vicinity of the *Brucella*–*Ochrobactrum*–*Pseudochrobactrum* group, with the closest relative being *Pseudochrobactrum glaciei* KMM 3858^T. This allocation was confirmed by analyses of the quinone system (ubiquinone Q-10), fatty acid data (major fatty acids C_{18:1}ω7c and C_{19:0} cyclo ω8c) and polar lipid profile (major components diposphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine and unknown aminolipid AL1; moderate amounts of three unknown polar lipids, L1–L3, an unknown aminolipid and an unknown aminophospholipid APL2). The polyamine pattern of Sa25^T exhibited the major compound putrescine and moderate amounts of spermidine; a similar polyamine pattern with the major compound putrescine was also detected in *Pseudochrobactrum glaciei* KMM 3858^T. DNA–DNA hybridization of strain Sa25^T with *Pseudochrobactrum glaciei* KMM 3858^T and the type strains of the other *Pseudochrobactrum* species showed values ranging from 50.3 to 24.8%, and physiological and biochemical data clearly differentiated this isolate from the described *Pseudochrobactrum* species. Since Sa25^T and *Pseudochrobactrum glaciei* KMM 3858^T form a distinct lineage in the 16S rRNA gene sequence-based phylogenetic tree, and this separate position is supported by unique characteristics of their polyamine patterns and polar lipid profiles, we propose the novel genus *Paenochrobactrum* gen. nov., with the type species *Paenochrobactrum gallinarii* sp. nov. (type strain Sa25^T = CCUG 57736^T = CCM 7656^T) and the reclassification of *Pseudochrobactrum glaciei* as *Paenochrobactrum glaciei* comb. nov. (type strain Pi26^T = KMM 3858^T = NRIC 0733^T = JCM 15115^T).

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence and partial *recA* sequence of strain Sa25^T are FN391023 and FM995366, respectively.

A *recA* sequence-based neighbour-joining tree, the total lipid profile of strain Sa25^T and a comparison of its fatty acid profile with those of related type strains are available as supplementary material with the online version of this paper.

The genus *Pseudochrobactrum* was proposed by Kämpfer *et al.* (2006). At present, it comprises the five species *Pseudochrobactrum saccharolyticum*, *Pseudochrobactrum asaccharolyticum*, *Pseudochrobactrum kiredjianiae*, *Pseudochrobactrum lubricantis* (Kämpfer *et al.*, 2006, 2007a, 2009) and *Pseudochrobactrum glaciei* (Romanenko *et al.*, 2008). The genus can be differentiated clearly from *Ochrobactrum* and *Brucella* on the basis of 16S rRNA gene and *recA*

sequence data, as well as chemotaxonomic properties (Kämpfer *et al.*, 2006, 2007a).

Strain Sa25^T was isolated from air collected in a duck barn in Germany on tryptone soy agar (TSA) at 25 °C. The investigated barn accommodated 2500 ducks (each 14 months old). Bioaerosol samples were collected on gelatin filters by sampling 500 l air at a rate of 30 l min⁻¹. Subsequently, filters were dissolved and decimally diluted in isotonic NaCl (up to 10⁻⁸). For cultivation, TSA plates were inoculated in triplicate with 0.1 ml of each dilution. Strain Sa25^T was isolated from a plate inoculated with fluid from the first decimal dilution. The concentration of Sa25^T was approximately 2 × 10³ c.f.u. per m³ air.

Subcultivation was done on TSA at 28 °C for 48 h. On this agar, this organism 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. Isolation of DNA for estimation of the G+C content was done according to Cashion *et al.* (1977) and the G+C content of the DNA was estimated according to Mesbah *et al.* (1989), resulting in a value of 49.7 mol%. The 16S rRNA gene was analysed as described by Kämpfer *et al.* (2003). DNA extraction was carried out using the GenElute plant genomic DNA kit (Sigma-Aldrich) according to the manufacturer's instructions. The 16S rRNA gene was amplified by PCR using the primers 27F (5'-GAGTTTGATCMTGGCTCAG) and 1492R (5'-ACGGYTACCTTGTACGACTT) (Lane, 1991) and the following cycling conditions: 95 °C for 3 min, 28 cycles of 94 °C for 1 min, 57.3 °C for 45 s and 72 °C for 2 min and a final elongation step at 72 °C for 15 min. The PCR product was purified with the QIAquick PCR purification kit (Qiagen) according to the manufacturer's instructions and sequenced with standard sequencing primers for the 16S rRNA gene. Phylogenetic analysis was performed using the ARB software package (December 2007 version; Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 95 database (July 2008 version; Pruesse *et al.*, 2007). Trees were reconstructed using the maximum-likelihood method with fastDNaml (Olsen *et al.*, 1994) and a 30% conservation filter (only alignment columns in which the frequency of the most abundant nucleotide is equal to or more than 30% are included in the calculation). Tree topology was further tested with a 50% filter and without filters. No significant differences could be detected between these trees regarding the grouping of strain Sa25^T. Trees reconstructed with the neighbour-joining and maximum-parsimony methods using the software package MEGA version 4.0 (Tamura *et al.*, 2007) showed similar results (not shown). The almost-complete 16S rRNA gene sequence (1345 bp) of strain Sa25^T was compared by distance calculations (distance options according to the Kimura-2 model) using the software

package MEGA version 4.0 (Tamura *et al.*, 2007). A high similarity of 99.5% was found to *Pseudochrobactrum glaciei* KMM 3858^T. The sequence similarity to the type strains of three other *Pseudochrobactrum* species was 96.6% (*Pseudochrobactrum saccharolyticum* CCUG 33852^T), 96.5% (*Pseudochrobactrum asaccharolyticum* CCUG 46016^T) and 96.7% (*Pseudochrobactrum kiredjianiae* CCUG 49584^T). A maximum-likelihood tree based on 16S rRNA gene sequences is shown in Fig. 1.

The partial *recA* sequence of strain Sa25^T was amplified with degenerate primers *recA*-wob-f and *recA*-wob-r as described by Scholz *et al.* (2006), resulting in a 768 bp *recA* fragment that was compared to available *recA* sequences in the EMBL nucleotide database. The highest sequence similarity (92.7%) within 678 bp was found with *Pseudochrobactrum glaciei* KMM 3858^T, supporting the results of the 16S rRNA gene sequence analysis. A neighbour-joining tree based on partial *recA* gene sequences is shown in Supplementary Fig. S1 (available in IJSEM Online). Markedly lower partial *recA* gene sequence similarities were detected in comparison with other *Pseudochrobactrum* species. The similarity in the *recA* sequence (within 678 nt) to *Pseudochrobactrum kiredjianiae* CCUG 49584^T, *Pseudochrobactrum saccharolyticum* CCUG 33852^T and *Pseudochrobactrum asaccharolyticum* CCUG 46016^T was 85.3, 84.2 and 83.9%, respectively.

Together with *Pseudochrobactrum glaciei* KMM 3858^T, strain Sa25^T formed a separate cluster in the phylogenetic tree of *recA* sequences apart from the other recognized *Pseudochrobactrum* species. Hence, both the 16S rRNA and *recA* gene sequence-based phylogenetic reconstructions support the separate position of *Pseudochrobactrum glaciei* KMM 3858^T and strain Sa25^T in comparison to the other recognized *Pseudochrobactrum* species.

For polyamine, polar lipid and quinone analyses, cells were grown on PYE medium (0.3% peptone from casein, 0.3% yeast extract, pH 7.2). 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 from biomass harvested at the late exponential growth phase and analysed as reported previously (Busse & Auling, 1988; Busse *et al.*, 1997; Stolz *et al.*, 2007). The quinone system of strain Sa25^T consisted of the major compound ubiquinone Q-10 (98%) and minor amounts of Q-8, Q-9 and Q-11 (0.2–1.2%). The polar lipid profile of strain Sa25^T consisted of the major compounds phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, phosphatidylmonomethylethanolamine and unknown aminolipid AL1, moderate amounts of three unknown polar lipids, L1, L2 and L3, unknown aminolipid AL2 and an unknown aminophospholipid APL2 (Supplementary Fig. S2). In addition, an aminolipid that stretched over a long range in the first chromatographic dimension ('stretched aminolipid') was detected, which has not been reported to be present in the profile of

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the EMBL database (accession numbers in parentheses). The phylogenetic tree was constructed using the ARB software package (December 2007 version; Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 95 database (July 2008 version; Pruesse *et al.*, 2007). Tree building was performed using the maximum-likelihood method with fastDNAmI (Olsen *et al.*, 1994) and 30 % conservation filter. Bar, 0.05 substitutions per nucleotide position.

any established *Brucella*, *Ochrobacterum* or *Pseudochrobacterum* species. Minor amounts of other lipids were also detected. The polar lipid profile of *Pseudochrobacterum glaciei* KMM 3858^T shared the major characteristics, including the presence of the stretched aminolipid, but the unknown lipids AL2 and APL2 were not detectable. Furthermore, in the polar lipid profile of *Pseudochrobacterum glaciei* KMM 3858^T, another unidentified aminolipid was detected, which exhibited the same R_f value (i.e. the distance travelled by the analyte divided by the distance travelled by the solvent front) in the second dimension as AL1 but showed a slight smaller R_f in the first dimension (not shown). However, these data demonstrate that the polar lipid profile distinguishes Sa25^T and *Pseudochrobacterum glaciei* KMM 3858^T from each other and both strains from all *Brucella*, *Ochrobacterum* and *Pseudochrobacterum* species examined so far. The polyamine pattern of Sa25^T consisted of the major compounds putrescine [99.7 μmol (g dry weight)⁻¹] and spermidine [12.1 μmol (g dry weight)⁻¹] and minor amounts of spermine [1.8 μmol (g dry weight)⁻¹]. *Pseudochrobacterum glaciei* KMM 3858^T exhibited a similar polyamine pattern, with the major compounds putrescine [56.7 μmol (g dry weight)⁻¹] and spermidine [33.9 μmol (g dry weight)⁻¹] and minor amounts of spermine [0.4 μmol (g dry weight)⁻¹]. This polyamine pattern distinguishes the two strains from *Pseudochrobacterum*, *Ochrobacterum* and *Brucella* species, which have been reported to contain high putrescine concentrations, but the major compound is spermidine (*Pseudochrobacterum*) or sym-homospermidine is present (*Ochrobacterum* and *Brucella*) (Kämpfer *et al.*, 2006, 2007a, b, 2008; Scholz *et al.*, 2008). Table 1

summarizes the major chemotaxonomic features in comparison with those of the genera *Pseudochrobacterum*, *Ochrobacterum* and *Brucella*.

The results of fatty acid analysis are shown in Supplementary Table S1. Fatty acids were analysed according to Kämpfer & Kroppenstedt (1996). The fatty acid profile of strain Sa25^T was composed mainly of C_{19:0} cyclo ω 8c (45.2 %), C_{18:1} ω 7c (29.5 %), C_{18:0} (4.0 %) and C_{16:0} (10.6 %) and showed high similarity to the fatty acid profiles of the type strains of *Pseudochrobacterum kiredjianiae* and *Pseudochrobacterum glaciei*. There were no clear similarities to the fatty acid profiles of the type strains of

Table 1. Characteristics that differentiate the genera *Paenochrobacterum* gen. nov., *Ochrobacterum*, *Pseudochrobacterum* and *Brucella*

Genera: 1, *Paenochrobacterum* gen. nov.; 2, *Ochrobacterum* (data from Huber *et al.*, 2010; Kämpfer *et al.*, 2007b, 2008; B. Huber and H.-J. Busse, unpublished results); 3, *Pseudochrobacterum* (Kämpfer *et al.*, 2006, 2007a, 2009); 4, *Brucella* (Scholz *et al.*, 2008, 2010).

Characteristic	1	2	3	4
Polyamine pattern				
sym-Homospermidine present	—	+	—	+
Major compound*	P	S or S and P	S	S
Polar lipid profile				
Stretched aminolipid present	+	—	—	—

*P, Putrescine; S, spermidine.

Pseudochrobactrum asaccharolyticum or *Pseudochrobactrum saccharolyticum*.

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

DNA–DNA hybridization experiments were performed with Sa25^T and *Pseudochrobactrum glaciei* KMM 3858^T and

Table 2. Physiological characteristics of strain Sa25^T, *Paenochrobactrum glaciei* comb. nov. KMM 3858^T and type strains of *Pseudochrobactrum* species

Strains: 1, Sa25^T; 2, *Paenochrobactrum glaciei* comb. nov. KMM 3858^T; 3, *Pseudochrobactrum kiredjianaie* CCUG 49584^T; 4, *Pseudochrobactrum asaccharolyticum* CCUG 46016^T; 5, *Pseudochrobactrum saccharolyticum* CCUG 33853^T. Data were obtained in this study. +, Positive; –, negative; (+), weakly positive. All strains were positive for hydrolysis of L-alanine p-nitroanilide (pNA) and L-proline pNA and weak hydrolysis of bis-p-nitrophenyl (pNP) phosphate. All strains were negative for hydrolysis of aesculin, pNP β-D-galactopyranoside, pNP β-D-glucuronide, pNP α-D-glucopyranoside, pNP β-D-glucopyranoside, pNP phosphorylcholine, pNP phenylphosphonate and 2-deoxythymidine-5'-pNP phosphate. All strains were also positive for assimilation of acetate and L-alanine. All strains were negative for assimilation of adonitol, p-arbutin, cellobiose, D-gluconate, myo-inositol, maltose, maltitol, D-mannitol, α-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.

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

also with the type strains of three other *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 Sa25^T showed moderate DNA–DNA relatedness to *Pseudochrobactrum glaciei* KMM 3858^T (50.3 %) and lower DNA–DNA relatedness to *Pseudochrobactrum asaccharolyticum* CCUG 46016^T (24.8 %), *Pseudochrobactrum saccharolyticum* CCUG 33852^T (45.7 %) and *Pseudochrobactrum kiredjianaie* CCUG 49584^T (40.9 %). Values given are means of two independent analyses.

From the results of 16S rRNA gene and *recA* sequencing and from the physiological characteristics, it is evident that strain Sa25^T is different from *Pseudochrobactrum asaccharolyticum*, *Pseudochrobactrum saccharolyticum* and *Pseudochrobactrum kiredjianaie*. Furthermore, strain Sa25^T shares with *Pseudochrobactrum glaciei* KMM 3858^T a polyamine pattern and a polar lipid profile containing a stretched aminolipid that are unique among the *Brucellaceae*. For this reason, we propose the novel genus *Paenochrobactrum* gen. nov., with the type species *Paenochrobactrum gallinarii* sp. nov., and the reclassification of *Pseudochrobactrum glaciei* as a second species of this genus, *Paenochrobactrum glaciei* comb. nov.

Description of *Paenochrobactrum* gen. nov.

Paenochrobactrum (Pae'no.chro.bac'trum. L. adv. *paene* nearly, almost; N.L. neut. n. *Ochrobactrum* a bacterial genus name; N.L. masc. n. *Paenochrobactrum* almost *Ochrobactrum*).

Cells are non-motile, non-spore-forming rods (approx. 2 µm long). Gram-negative and oxidase-positive, showing an oxidative metabolism. The polyamine pattern exhibits the major compound putrescine and moderate to large amounts of spermidine, while *sym*-homospermidine is absent. The polar lipids consist of the major compounds phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, phosphatidylcholine, phosphatidylmonomethylethanolamine and unknown aminolipid AL1 and moderate or minor amounts of unknown polar lipids L1 and L2. In addition, an aminolipid that stretches over a long range in the first chromatographic dimension (stretched aminolipid) is detectable (Supplementary Fig. S2). The quinone system is ubiquinone Q-10. Major fatty acids are C_{18:1}ω7c and C_{19:0} cyclo ω8c. On the basis of 16S rRNA gene sequence analysis, most closely related to the genera *Pseudochrobactrum*, *Ochrobactrum* and *Brucella*. Member of the family *Brucellaceae*. The type species is *Paenochrobactrum gallinarii*.

Description of *Paenochrobactrum gallinarii* sp. nov.

Paenochrobactrum gallinarii (gal.li.na'ri.i. L. n. *gallinarium* a coop; L. gen. n. *gallinarii* of a coop, referring to the isolation of the type strain from a duck barn).

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. Carbon source utilization and hydrolysis of chromogenic substrates (including differentiating characters for all *Paenochrobactrum* and *Pseudochrobactrum* species) are indicated in Table 2. In addition to the lipids listed in the genus description, moderate amounts of the polar lipid L3, unknown aminolipid AL2 and unknown aminophospholipid APL2 are present, and other lipids are present in minor to trace amounts. Quinone system and polyamine pattern are as listed in the genus description. In addition, the polyamine pattern contains small amounts of spermine. The G+C content of the DNA of the type strain is 49.7 mol%.

The type strain is Sa25^T (=CCUG 57736^T =CCM 7656^T), isolated from a duck barn in Germany.

Description of *Paenochrobactrum glaciei* comb. nov.

Basonym: *Pseudochrobactrum glaciei* Romanenko *et al.* 2008.

The description of the species is that given in the description of *Pseudochrobactrum glaciei* provided by Romanenko *et al.* (2008) with the following additions. The polar lipid profile exhibits the characteristics listed in the genus description. Furthermore, the polar lipid profile contains an unidentified aminolipid, which exhibits the same R_f as AL1 in the second dimension but a slightly lower R_f in the first dimension. The quinone system and polyamine pattern are as listed in the genus description. In addition, the polyamine pattern contains small amounts of 1,3-diaminopropane and spermine.

The type strain is Pi26^T (=KMM 3858^T =NRIC 0733^T =JCM 15115^T).

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