Paenochrobactrum pullorum sp. nov. isolated from a chicken

Peter Kämpfer,1 Marie T. Poppe,2 Gottfried Wilharm,2 Stefanie P. Glaeser1 and Hans-Jürgen Busse3

1Institut für Angewandte Mikrobiologie, Justus-Liebig-Universität Giessen, D-35392 Giessen, Germany
2Robert Koch-Institut, Bereich Wernigerode, D-38855 Wernigerode, Germany
3Institut für Bakteriologie, Mykologie und Hygiene, Veterinärmedizinische Universität, A-1210 Wien, Austria

A Gram-stain-negative, rod-shaped, oxidase-positive, non-spore-forming, non-motile bacterium (strain 280T) isolated from a chicken was studied for its taxonomic allocation. 16S rRNA gene sequence analyses clearly allocated the isolate in the genus Paenochrobactrum group with a 16S rRNA gene sequence similarity of 98.8% to the currently recognized species, Paenochrobactrum gallinarii and Paenochrobactrum glaciei. This allocation was confirmed by the fatty acid data (major fatty acids: C18:1ω7c and C19:0 cyclo ω8c) and a polyamine pattern with the major compound putrescine and relatively high amounts of spermidine. Also, the polar lipid profile with phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidyglycerol, phosphatidylcholine and the genus-specific ‘stretched aminolipid’ was well in line with the description of the genus Paenochrobactrum. The quinone system consisted predominantly of ubiquinone Q-10 with traces of Q-9 and Q-11. DNA–DNA hybridization of strain 280T with Paenochrobactrum gallinarii Sa25T and Paenochrobactrum glaciei KMM 3858T showed relatedness values of 38.8% (reciprocal 20.2%) and 30.2% (reciprocal 29.8%), respectively. These results in combination with differentiating physiological and biochemical data clearly showed that strain 280T merits species status. We propose the name Paenochrobactrum pullorum sp. nov. to accommodate this strain with the type strain 280T (=LMG 28095=CIP 110700T).

The genus Paenochrobactrum was proposed by Kämpfer et al. (2010). At the time of writing it consists of two species, Paenochrobactrum gallinarii and Paenochrobactrum glaciei (Kämpfer et al., 2010). The latter species was originally described as Pseudochrobactrum glaciei (Romanenko et al., 2008). The genus can be clearly differentiated from the genera Ochrobactrum, Pseudochrobactrum and Brucella on the basis of 16S rRNA gene sequence and recA sequence data (phylogenetic analysis), as well as chemotaxonomic data (Kämpfer et al., 2006; 2007; 2010).

Strain 280T was isolated from faeces of a chicken in Germany on CHROMagar Acinetobacter (CHROMagar) after incubation at 42 °C for 24 h. Subcultivation was done on 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 optical microscope at ×1000, with cells grown for 3 days at 30 °C on nutrient agar (Oxoid).

The 16S rRNA gene of strain 280T was PCR-amplified and sequenced by the Sanger dideoxy method with universal primers f31 and r1 (Weisburg et al., 1991). Phylogenetic analyses based on nearly full-length 16S rRNA gene sequences were performed in ARB release 5.2 (Ludwig et al., 2004) in the ‘All-Species Living Tree’ Project (LTP; Yarza et al., 2008) database release LTPs111 (February 2013). All sequences that were not included in the LTP database were aligned with SINA v. 1.2.11 (Pruesse et al., 2012) and subsequently implemented in the LTP database. The final alignment including all sequences used for tree reconstruction was checked manually based on secondary structure information. Pairwise 16S rRNA gene sequence similarities were calculated in ARB using the ARB neighbour-joining tool without an evolutionary substitution model. Phylogenetic trees were reconstructed with the maximum-likelihood method using RAxML v7.04 (Stamatakis, 2006) with GTR-GAMMA and rapid bootstrap analysis, the neighbour-joining method using ARB neighbour-joining with the Jukes–Cantor correction as an evolutionary model.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 280T is KC494696.
(Jukes & Cantor 1969), and the maximum-parsimony method using DNAPARS v. 3.6 (Felsenstein, 2005). All phylogenetic trees were calculated with 100 resamplings (bootstrap analysis; Felsenstein, 1985) and based on 16S rRNA gene sequences between sequence termini 60 and 1458 (according to Escherichia coli numbering; Brosius et al., 1978).

The sequenced 16S rRNA gene fragment of strain 280T represented a continuous stretch of 1389 nt (termini 56 to 1458; E. coli numbering). The alignment in ARB showed that strain 280T contained a 52 nt-long internal sequence stretch between E. coli positions 148 and 196 (5′-CCCCCCUUAA-AAUUUCAAGGAAGUAUAAAGCCCCGCAUUU-AUGGGGGG-3′), which was also present in Ochrobactrum ciceri, Ochrobactrum daejeonense and Ochrobactrum pituitosum but with two nucleotide differences in the sequences. Secondary structure analysis of the 16S rRNA gene showed that this internal sequence fragment represents an additional loop not present in any of the other sequences. Pairwise sequence similarity to the type strains of O. ciceri, O. daejeonense and O. pituitosum were only 94.1 to 94.7% excluding and 94.2 to 94.7% including the internal sequence stretch of 52 nt. Strain 280T shared the highest 16S rRNA gene sequence similarity of 98.8% with the type strains of Paenochrobactrum glaciei and Paenochrobactrum gallinarii. The next most closely related species was Ochrobactrum oryzae with 96.1% sequence similarity. The 16S rRNA gene sequence similarities to all other type strains of the family Brucellaceae were below 95.6%. The maximum-likelihood tree based on the 16S rRNA gene sequences clearly showed that strain 280T formed a distinct monophyletic cluster with Paenochrobactrum glaciei and Paenochrobactrum gallinarii within the family Brucellaceae (Fig. 1). This was confirmed by the neighbour-joining and maximum-parsimony analysis, both showing the same clustering.

Amplification of a partial recA gene sequence of strain 280T was attempted with primer pairs recA-wob-f and recA-wob-r as described by Scholz et al. (2006), recA-BrucOchro-f and recA-BrucOchro-r as described by Scholz et al. (2008) and

![Fig. 1. Maximum-likelihood tree based on nearly full-length 16S rRNA gene sequences showing the phylogenetic position of strain 280T among type strains of the family Brucellaceae. The tree was reconstructed in ARB with RAxML using rapid bootstrap analysis (100 replications). Nucleotide positions between termini 56 and 1458 (E. coli numbering; Brosius et al., 1978) were included in the analysis. Bootstrap values above 70% are shown at branch nodes. Bartonella bacilliformis ATCC 35685T was used as an outgroup. Bar, 0.1 nucleotide substitutions per site.](http://ijs.sgmjournals.org)
recA-Pg-F and recA-Pg-R as described by Romanenko et al. (2008). However, while recA sequences from reference strains could be amplified, a recA sequence of strain 280T could not be amplified.

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). Cells subjected to polyamine extraction (Busse & Auling, 1988) were harvested at the late exponential growth phase whereas biomass for other analyses was harvested at the stationary growth phase. Polyamines were analysed by HPLC using the conditions described by Busse et al. (1997). Quinones and polar lipids were extracted by applying an integrated procedure (Tindall, 1990a, b; Altenburger et al., 1996). HPLC equipment used was described by Stolz et al. (2007). The polyamine pattern of strain 280T was composed of putrescine [44.5 μmol (gram dry weight)]⁻¹, spermidine [17.4 μmol (gram dry weight)]⁻¹, spermine [0.8 μmol (gram dry weight)]⁻¹ and less than 0.2 μmol (g dry weight)⁻¹ cadaverine, 1,3-diaminopropane and sym-homospermidine. The polar lipid profile was composed of the major lipids phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidyglycerol, phosphatidyglycerol, phosphatidylethanolamine, phosphatidylmonomethylethanolamine, peptidic lipid, the genus-specific ‘stretched aminolipid’ lamine, phosphatidylglycerol, phosphatidylcholine, diphosphatidylethanolamine, phosphatidylmonomethylethanolamine; AL1–4, unknown aminolipids; L1–3, 5–7 unknown polar lipids. Unidentified lipids considered to correspond to lipids detected in *Paenochrobactrum gallinarii* were designated with the same label.

**Fig. 2.** Total polar lipid profile of strain 280T after two-dimensional TLC and detection with 5% ethanolic molybdophosphoric acid. PME, phosphatidylmonomethylethanolamine; PE, phosphatidyglycerol; PC, phosphatidylcholine; DPG, diphosphatidylethanolamine; PG, phosphatidylglycerol; AL1–4, unknown aminolipids; L1–3, 5–7 unknown polar lipids. Unidentified lipids considered to correspond to lipids detected in *Paenochrobactrum gallinarii* were designated with the same label.

**Table 1.** Major fatty acids (%) of strain 280T, *Paenochrobactrum gallinarii* Sa25T and *Paenochrobactrum glaciei* KMM 3858T

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saturated fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C12:0</td>
<td>–</td>
<td>0.3</td>
<td>–</td>
</tr>
<tr>
<td>C14:0</td>
<td>–</td>
<td>2.2</td>
<td>0.6</td>
</tr>
<tr>
<td>C16:0</td>
<td>8.5</td>
<td>10.6</td>
<td>8.9</td>
</tr>
<tr>
<td>C18:0</td>
<td>4.9</td>
<td>4.0</td>
<td>5.5</td>
</tr>
<tr>
<td>Unsaturated fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1ω7c</td>
<td>18.5</td>
<td>29.5</td>
<td>31.2</td>
</tr>
<tr>
<td>11-Methyl C18:1ω7c</td>
<td>3.4</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>C20:1ω6c</td>
<td>2.4</td>
<td>0.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Hydroxy fatty acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:0 3-OH</td>
<td>–</td>
<td>–</td>
<td>0.6</td>
</tr>
<tr>
<td>Summed feature 3†</td>
<td>–</td>
<td>–</td>
<td>1.6</td>
</tr>
<tr>
<td>Cyclopropane acids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C17:0 cyclo</td>
<td>–</td>
<td>2.3</td>
<td>1.0</td>
</tr>
<tr>
<td>C19:0 cyclo ω8c</td>
<td>62.5</td>
<td>45.2</td>
<td>47.9</td>
</tr>
</tbody>
</table>

*For unsaturated fatty acids, the position of the double bond is located by counting from the methyl (ω) end of the carbon chain.
†Summed features are groups of two or three fatty acids that cannot be separated by GLC with the MIDI system. Summed feature 3 contained one or more of the fatty acids C16:1ω7c and C15:0 is 2-OH.
Paenochrobactrum pullorum sp. nov.

Paenochrobactrum pullorum (pul. lo’rum. L. gen. pl. n. pullorum of chickens).

Cells are non-motile, non-spore-forming rods (approx. 2 μm in length). Gram-stain-negative and oxidase-positive, showing an oxidative metabolism. Catalase-positive. Good growth occurs on R2A agar, TSA, nutrient agar and MacConkey agar at 20–30 °C. Beige, translucent and shiny colonies with entire edges are formed within 24 h, with a diameter of approximately 2 mm. Grows on TSA at 15–45 °C, but not at 10 or 50 °C. The following carbon sources are utilized: D-glucose, D-fructose, D-ribose, acetate, propionate, lactate, 4-aminobutyrate, and β- and L-alanine. The following carbon sources are not utilized: N-acetyl-D-glucosamine, L-arabinose, adonitol, p-arbutin, cellobiose, D-galactose, D-glucuronide, α-mannooligosides, xylitol, cello-oligosides, and α-glucosidase. The polar lipid profile contains the major compound putrescine and relatively high amounts of spermidine. Other polyamines are present only in minor amounts. The polar lipid profile consists of the major lipids phosphatidylethanolamine, phosphatidylmonomethylethanolamine, phosphatidylglycerol, phosphatidylcholine, diphosphatidylglycerol, the genus-specific 'stretched aminolipid' (Kämpfer et al., 2010), two unidentified aminolipids (AL1, AL4), and a lipid (L6) not stainable with reagents detecting phosphate- or amino- or sugar moieties. Several minor lipids are present as well. The quinone system consists mainly of ubiquinone Q-10 and traces of Q-9 and Q-11. The G+C content of the genomic DNA is 48.5–48.8 mol%.

The type strain is 280T ( =LMG 28095T= CIP 110700T), isolated from faeces of a chicken in Germany.

ACKNOWLEDGEMENTS

We thank Dr I Romanenko for kindly providing the type strain of Pseudochrobactrum gallicet KMM 3858T and Gundula Will, Maria Sowinsky, Katja Grebing and Evelyn Skibe for excellent technical assistance. We would also like to thank Dr Karin Bohland and Dr Christiane Cuny for providing chicken samples.
REFERENCES


