Psychrobacter lutiphocae sp. nov., isolated from the faeces of a seal

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The taxonomic status of a Gram-negative-staining bacterium, isolated from the faeces of a seal, was investigated using a polyphasic approach. Comparative 16S rRNA gene sequence analysis showed that the novel isolate formed a distinct phyletic line within the genus *Psychrobacter*, displaying >3.3% sequence divergence with other known *Psychrobacter* species. The generic assignment was confirmed by chemotaxonomic data, which revealed a fatty acid profile that included straight-chain saturated, unsaturated and 3-hydroxylated fatty acids, with C_{18:1}ω₉c as the major fatty acid. A ubiquinone with eight isoprene units (Q-8) was the predominant respiratory quinone and spermidine was the predominant polyamine. The novel isolate was distinguished from other members of the genus *Psychrobacter* by using a set of phenotypic properties. On the basis of phenotypic and phylogenetic considerations, it is proposed that the new isolate represents a novel species, for which the name *Psychrobacter lutiphocae* sp. nov. is proposed. Strain IMMIB L-1110^T (=DSM 21542^T=CCUG 56590^T) is the type strain.

The genus *Psychrobacter* was first described by Juni & Heym (1986) to comprise psychrophilic and psychrotolerant, halotolerant, aerobic, non-motile, Gram-negative coccobacilli. Members of the genus *Psychrobacter* are capable of growing at temperatures ranging from –10 to 37 °C and they vary from strict (stenothermal) psychrophiles, e.g. *Psychrobacter frigidicola* (0–22 °C), to eurythermal psychrophiles, e.g. *Psychrobacter okhotskensis* (–5–35 °C). At the time of writing, the genus comprised 29 species, which have been isolated from a variety of low-temperature marine environments (Romanenko *et al.*, 2002, 2004), Antarctic sea ice (Bowman *et al.*, 1997; Bozal *et al.*, 2003; Shivaji *et al.*, 2005; Heuchert *et al.*, 2004), ornithogenic soil and sediments (Bowman *et al.*, 1996), the stomach contents of the Antarctic krill, *Euphausia superba* (Denner *et al.*, 2001), seawater (north-western Pacific Ocean, 300 m depth), the deep sea (Maruyama *et al.*, 2000) and the internal tissues of a marine ascidian. Other sources of *Psychrobacter* include pigeon faeces (Kämpfer *et al.*, 2002), fish, poultry, dairy products and fermented seafood (Yoon *et al.*, 2003, 2005b; Jung *et al.*, 2005), clinical sources and an infected lamb (Vela *et al.*, 2003). During the

characterization of bacterial isolates from clinical sources, we encountered a Gram-negative-staining, oxidase-positive, non-motile, coccoid bacterium, which had been isolated from the faeces of a seal. In this paper, we report on the taxonomic characterization of this isolate, designated strain IMMIB L-1110^T, by using a polyphasic approach. Based on the results of this study, we describe a novel species, *Psychrobacter lutiphocae* sp. nov.

Strain IMMIB L-1110^T was isolated from the faeces of a seal. The strain was grown aerobically at 37 °C on Columbia agar (Oxoid) supplemented with 5% sheep blood. It was characterized biochemically using the API 20NE, API Coryne, API 20 Strep and API ZYM systems (bioMérieux), according to the manufacturer's instructions. Hydrolysis of casein, elastin, guanine, xanthine, hypoxanthine, tyrosine and uric acid was investigated by using previously described methods (Gordon, 1966, 1967; Gordon & Mihm, 1957). To determine the chemotaxonomic characteristics of strain IMMIB L-1110^T, the organism was cultivated in shake flasks containing BHI broth for 1 week at 37 °C. After checking for purity at maximum growth, the organism was killed with formaldehyde (1%, v/v), harvested by centrifugation, washed with distilled water and freeze-dried. Fatty acids were analysed as described previously (Yassin *et al.*, 2007). Polar lipids were determined using TLC as described previously (Yassin *et al.*, 1993). Respiratory quinones were extracted and purified according to Collins *et al.* (1977). Mass spectral

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain IMMIB L-1110^T is FM165580.

Supplementary tables of phenotypic characteristics and cellular fatty acid compositions of strain IMMIB L-1110^T and other recognized species of the genus *Psychrobacter* are available with the online version of this paper.

analyses of the quinones were recorded in positive-ion mode on a Q-TOF 2 mass spectrometer (Micromass) equipped with a nanospray source as described by Yassin & Hupfer (2006). Biomass to be subjected to polyamine analysis was grown on BHI broth and harvested at the late-exponential growth phase. Extraction of polyamines was performed as described by Busse & Auling (1988) and analysis was done using the HPLC equipment described by Stolz *et al.* (2007).

For phylogenetic analysis, the 16S rRNA gene sequence of strain IMMIB L-1110^T was amplified by PCR using procedures described by Rainey *et al.* (1996) and directly sequenced using a *Taq* Dye-deoxy Terminator Cycle Sequencing Kit (Applied Biosystems) and an automatic DNA sequencer (model 310; Applied Biosystems). The closest relatives of the isolate were determined by searching the GenBank database. The 16S rRNA gene sequence of isolate IMMIB L-1110^T and the retrieved sequences were added to the ARB database (Ludwig *et al.*, 2004) and aligned using the respective tool of the ARB package. Phylogenetic trees were reconstructed with neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) methods. The stability of the groupings was estimated by bootstrap analysis (Felsenstein, 1985) of the neighbour-joining method based on 1000 replications.

Strain IMMIB L-1110^T was examined for a range of phenotypic characteristics. The isolate formed non-pigmented, circular, smooth, opaque colonies with a diameter of ~2 mm when grown on Columbia blood agar. Cells were non-motile, Gram-negative-staining, diploid coccobacilli. The organism grew aerobically and was catalase- and oxidase-positive. The growth temperature range of the isolate was 10–37 °C. Although NaCl was not required for growth, strain IMMIB L-1110^T grew in the presence of 6 % NaCl. Strain IMMIB L-1110^T was able to assimilate and hydrolyse a set of organic substrates. The detailed phenotypic characteristics of strain IMMIB L-1110^T are given in the species description and Table 1. Supplementary Table S1 (available in IJSEM Online) shows the phenotypic characteristics of strain IMMIB L-1110^T compared with those of most of the recognized species of the genus *Psychrobacter*.

Chemotaxonomically, strain IMMIB L-1110^T possessed chemical markers that supported its assignment to the genus *Psychrobacter*. Cellular fatty acid analysis revealed the presence of straight-chain saturated fatty acids C_{16:0} (1.7 %), C_{17:0} (1.9 %) and C_{18:0} (3.7 %), mono-unsaturated fatty acids C_{16:1ω7c} (8.3 %), C_{17:1ω8c} (2.6 %), C_{18:1ω9c} (69.3 %), C_{18:19t/11c} (4.7 %) and C_{19:1} (0.8 %) and the 3-hydroxylated fatty acid C_{12:0} 3-OH (5.5 %). The detailed fatty acid profile of strain IMMIB L-1110^T and those of most of the recognizes species of the genus *Psychrobacter* are shown in Supplementary Table S2. Strain IMMIB L-1110^T contained diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as the

Table 1. Phenotypic characteristics of strain IMMIB L-1110^T and the phylogenetically most closely related *Psychrobacter* species

Strains: 1, *Psychrobacter lutiphocae* sp. nov. IMMIB L-1110^T (data from this study); 2, *P. celer* KCTC 12313^T (Yoon *et al.*, 2005a); 3, *P. arenosus* KMM 3659^T (Romanenko *et al.*, 2004); 4, *P. pacificensis* IFO 16270^T (Maruyama *et al.*, 2000); 5, *P. marincola* DSM 14160^T (Romanenko *et al.*, 2002); 6, *P. submarinus* DSM 14161^T (Romanenko *et al.*, 2002); 7, *P. faecalis* DSM 14664^T (Kämpfer *et al.*, 2002); 8, *P. pulmonis* S-606^T (Vela *et al.*, 2003). All strains are negative for assimilation of maltose, D-mannose and D-mannitol, for acid production from maltose, D-mannitol, D-sorbitol, sucrose and trehalose and for arginine dihydrolase activity. +, Positive; –, negative; w, weak; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8
Nitrate reduction	+	–	+	–	–	–	+	+
Assimilation of:								
<i>N</i> -Acetylglucosamine	–	ND	–	–	–	–	+	–
Adipic acid	–	ND	ND	–	–	–	–	–
L-Arabinose	–	–	+	–	–	–	w	–
Capric acid	–	ND	–	–	–	–	ND	–
Citrate	–	–	+	–	–	–	+	–
Gluconate	–	ND	–	–	–	–	–	–
D-Glucose	–	–	–	–	–	–	w	–
Malate	+	+	+	+	–	–	+	–
Phenylacetic acid	–	ND	–	–	–	–	–	–
Acid production from:								
L-Arabinose	–	+	+	–*	–	+	–	–
D-Glucose	–	+	+	–*	–	–	–	–
Lactose	–	+	+	–*	–*	–*	–	–
Raffinose	–	–	–	–*	–*	–*	–	ND
D-Xylose	–	+	–	–*	–	–	–	–
Enzyme activities:								
Acid phosphatase	–	–	ND	+	+	–*	ND	–
Alkaline phosphatase	–	+	ND	+	+	+	ND	–
Cysteine arylamidase	–	–	ND	+	+	+	ND	+
Ester lipase (C8)	+	+	ND	+	+	+	ND	+
Esterase (C4)	–	+	ND	+	+	+	ND	+
β-Galactosidase	–	–	ND	–*	–*	–*	ND	–
Leucine arylamidase	+	+	ND	+	+	ND	ND	+
Lipase (C14)	–	–	ND	–*	–*	–*	ND	–
Naphthol-AS-BI-phosphohydrolase	+	–	ND	+	+	ND	ND	–
Urease	+	–	–	+	–	–	–	–
Valine arylamidase	–	–	ND	+	+	+	ND	+

*Data from Bakermans *et al.* (2006).

characteristic polar lipids. Mass spectral analysis of the main isoprenoid component from strain IMMIB L-1110^T showed a strong peak at *m/z* 727.49 that was attributable to [M+H]⁺ in the high mass region. This corresponds to a ubiquinone with eight isoprene units (Q-8). The polyamine pattern of strain IMMIB L-1110^T consisted of (μmol g^{–1} dry weight) spermidine (45.9) and minor-to-trace amounts of putrescine (3.7), spermine (1.3),

cadaverine (1.2), *sym*-homospermidine (0.3) and 1,3-diaminopropane (0.1). The polyamine pattern was in excellent agreement with those of other *Psychrobacter* species, including *Psychrobacter pacificensis* and *Psychrobacter celer* (Hamana *et al.*, 2007).

To establish the phylogenetic position of strain IMMIB L-1110^T, its 16S rRNA gene sequence was determined in this study (1502 bases). Sequence database searches revealed that strain IMMIB L-1110^T was most closely related to species of the genus *Psychrobacter* (data not shown). Phylogenetic analysis confirmed the association of strain IMMIB L-1110^T with the genus *Psychrobacter*. A tree was constructed using the neighbour-joining method to show the phylogenetic position of strain IMMIB L-1110^T (Fig. 1). Strain IMMIB L-1110^T formed a distinct subline within the genus *Psychrobacter*, which was associated with a subcluster containing *P. celer* SW-238^T, *P. arenosus* DSM 15389^T and *P. pacificensis* IFO

16270^T. The association of strain IMMIB L-1110^T with these strains was not statistically significant, as evident from the bootstrap resampling results. Pairwise sequence comparisons to these species revealed similarities of 96.7, 96.3 and 95.5 %, respectively. Other *Psychrobacter* species displayed substantially lower levels of similarity (data not shown). Although, there is no precise correlation between 16S rRNA gene sequence similarity and species delineation, it is generally recognized that divergence values of 3 % and more are significant (Stackebrandt & Goebel, 1994).

It is clear from this polyphasic taxonomic study that strain IMMIB L-1110^T represents a distinct species within the genus *Psychrobacter*. Therefore, based on both phenotypic and molecular genetic characterizations, we consider that strain IMMIB L-1110^T merits classification in a novel species of the genus *Psychrobacter*, for which the name *Psychrobacter lutiphocae* sp. nov. is proposed.

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences and showing the phylogenetic position of strain IMMIB L-1110^T within the radiation of species in the genus *Psychrobacter*. Percentages (>50 %) at nodes are levels of bootstrap support based on 1000 resamplings. The solid circle indicates that the node was also recovered in maximum-likelihood and maximum-parsimony trees. Bar, 5.0 % sequence divergence.

Description of *Psychrobacter lutiphocae* sp. nov.

Psychrobacter lutiphocae (lu.ti.phoc'ae. L. n. *lutum* faeces; L. n. *phoca* a seal; N.L. gen. n. *lutiphocae* of faeces of a seal).

Cells are Gram-negative-staining, non-spore-forming, non-motile, diploid coccobacilli. Aerobic. Oxidase- and catalase-positive. Ubiquinone Q-8 is the major isoprenoid quinone. Spermidine is the predominant polyamine. The polar lipid profile is characterized by the presence of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The fatty acids C_{18:1}ω₉c and C_{16:1}ω₇c are the major non-hydroxylated fatty acids. The major hydroxylated fatty acid is C_{12:0} 3-OH. Growth occurs with 0–6 % (w/v) NaCl. Grows at 10 and 37 °C, but not at 42 °C. Grows on MacConkey agar after 1 week at 37 °C. On Columbia agar, colonies are non-pigmented, circular, smooth and opaque with a diameter of 2–4 mm. Malate is assimilated but adipic acid, L-arabinose, capric acid, trisodium citrate, N-acetylglucosamine, D-glucose, maltose, phenylacetic acid, potassium gluconate, D-mannose and D-mannitol are not assimilated. Acid is not produced from L-arabinose, glycogen, D-glucose, inulin, lactose, maltose, D-mannitol, raffinose, D-ribose, D-sorbitol, starch, sucrose, trehalose or D-xylose. Hippurate is hydrolysed, but aesculin, adenine, casein, elastin, gelatin, guanine, hypoxanthine, testosterone, tyrosine and xanthine are not hydrolysed. Activity is detected for ester lipase (C8), leucine arylamidase, naphthol-AS-BI-phosphohydrolase and urease. No activity is detected for acid phosphatase, alkaline phosphatase, arginine dihydrolase, chymotrypsin, cysteine arylamidase, esterase (C4), α-fucosidase, α- and β-galactosidase, β-glucuronidase, α- and β-glucosidase, N-acetyl-β-glucosaminidase, lipase (C14), α-mannosidase, pyrazinamidase, pyrrolidonyl arylamidase, trypsin or valine arylamidase. Nitrate is reduced. Indole and acetoin production are negative.

The type strain, IMMIB L-1110^T (=DSM 21542^T=CCUG 56590^T), was isolated from the faeces of a seal.

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