Psychrobacter isolates of human origin, other than *Psychrobacter phenylpyruvicus*, are predominantly *Psychrobacter faecalis* and *Psychrobacter pulmonis*, with emended description of *P. faecalis*

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Human *Psychrobacter* isolates, other than *Psychrobacter phenylpyruvicus*, are predominantly designated *Psychrobacter immobilis*. Phenotypic and genotypic testing of *Psychrobacter* isolates that have been deposited in different culture collections as *P. immobilis* indicates that most of these human isolates belong to the species *Psychrobacter faecalis* and *Psychrobacter pulmonis*.

At present, all *Psychrobacter* isolates from human origin, other than *Psychrobacter phenylpyruvicus* (previously *Moraxella phenylpyruvica*), are designated *Psychrobacter immobilis*. Mostly, these are clinically not very relevant, but sometimes they are considered to be opportunistic pathogens (Gini, 1990; Lloyd-Puryear *et al.*, 1991; Lozano *et al.*, 1994). Many other *Psychrobacter* species have been described, mostly from the environment and from poikilothermic animals, but recently two species, *Psychrobacter faecalis* and *Psychrobacter pulmonis*, were isolated from warm-blooded animals, i.e. from pigeon faeces (Kämpfer

et al., 2002) and from the lungs of a lamb (Vela *et al.*, 2003), respectively. The type strain of *P. immobilis*, ATCC 43116^T, was isolated from poultry. Very recently, clinical isolates have been described as members of a novel species, *Psychrobacter sanguinis* (Wirth *et al.*, 2012).

Since 2003, some human isolates have been deposited in the Culture Collection University of Göteborg (CCUG) collection and presumptively identified as *P. faecalis* or *P. faecalis*-like. Because, to our knowledge, cases of human infection with *P. faecalis* have not yet been published and because none of the cases was caused by infection with *P. immobilis*, we compared our clinical isolates with the human isolates deposited in the CCUG and Laboratorium voor Microbiologie, Universiteit Gent (LMG) culture collections as *P. immobilis* and *Psychrobacter* sp. We examined a total of 26 human *Psychrobacter* isolates that did not belong to *P. phenylpyruvicus* present in two culture collections [CCUG ($n=9$), LMG ($n=11$)] and in the collection of the UCL (Georges Wauters, Brussels, Belgium; $n=6$) (Supplementary Table S1, available in IJSEM Online).

Determination of 16S rRNA gene sequences was carried out as described previously (Wauters *et al.*, 2003) and this approach identified the following species: *Psychrobacter cibarius* ($n=1$; LMG 7085), *P. faecalis* ($n=17$), *Psychrobacter nivimaris* ($n=1$; CCUG 39521) and *P. pulmonis* ($n=7$) (Supplementary Table S1, Fig. 1). Cluster analysis of the 16S rRNA gene sequences (length 1456 bp) was performed using the Kodon software (Applied Maths, Sint-Martens-Latem, Belgium) according to the neighbour-joining method based on the multiple aligned distances algorithm (open gap cost, 100%; unit gap cost, 20%). A similar clustering result was

Abbreviations: CCUG, Culture Collection University of Göteborg; LMG, Laboratorium voor Microbiologie, Universiteit Gent; pNA, *p*-nitroanilide; pNP, *p*-nitrophenyl.

The GenBank/EMBL/DDJB accession numbers for the 16S rRNA gene sequences reported in this paper are HQ698589 (*Psychrobacter immobilis* ATCC 43116^T), HQ698586 (*P. immobilis* LMG 7085), HQ698587 (*P. nivimaris* CCUG 39521), HQ698583 (*P. pulmonis* LMG 7092), HQ698585 (*P. pulmonis* UCL-NF 1589), HQ698584 (*P. pulmonis* UCL-NF 1649), HQ698582 (*P. pulmonis* LMG 1012), HQ698580 (*P. pulmonis* CCUG 41791), HQ698581 (*P. pulmonis* CCUG 51808), HQ698590 (*P. pulmonis* UCL-NF 1791), HQ698579 (*P. pulmonis* CCUG 46240^T), HQ698562 (*P. faecalis* DSM 14664^T), HQ698578 (*P. faecalis* UCL-NF 985), HQ698564 (*P. faecalis* LMG 7062), HQ698569 (*P. faecalis* LMG 6782), HQ698588 (*P. faecalis* UCL-NF 1590), HQ698563 (*P. faecalis* LMG 7060), HQ698565 (*P. faecalis* LMG 7063), HQ698566 (*P. faecalis* LMG 7061), HQ698567 (*P. faecalis* LMG 7059), HQ698568 (*P. faecalis* LMG 5125), HQ698570 (*P. faecalis* LMG 7064), HQ698571 (*P. faecalis* CCUG 45950), HQ698573 (*P. faecalis* CCUG 52122), HQ698575 (*P. faecalis* CCUG 55541), HQ698574 (*P. faecalis* CCUG 54876), HQ698572 (*P. faecalis* CCUG 51295), HQ698577 (*P. faecalis* UCL-NF 322) and HQ698576 (*P. faecalis* CCUG 57827).

A supplementary table is available with the online version of this paper.

Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of the *Psychrobacter* species included in this study. Bootstrap values greater than 80% are shown at the branch points. Sequences determined in this study are given in bold. Species names in parentheses are the final designations as determined in this study. *, Sequence is only 1291 bp long, which probably explains its aberrant position.

obtained using the maximum-parsimony algorithm (with mutation cost 100%).

Three sequences have been deposited in GenBank for the type strain ATCC 43116^T of *P. immobilis*, i.e. U39399 (Bowman *et al.*, 1996), AJ309942 (Romanenko *et al.*, 2002) and HQ698589 (this study). It should be noted that the sequence deposited under accession number U39399 differs at 33 positions from the same strain sequenced at our laboratory, whereas there are only six differences between our sequence and that deposited under accession number AJ309942.

Strains were grown on tryptic soy agar (TSA). Growth and biochemical tests were assessed at 30 °C; growth was also determined at 35 °C. Oxidase activity was determined using dimethyl-*p*-phenylenediamine strips (Merck). Acid production from carbohydrates was detected on low-peptone phenol red (LPPR) agar (Wauters *et al.*, 1998) and Hugh and Leifson's oxidation-fermentation medium (Hugh & Leifson, 1953). For urease determination, liquid Christensen medium was used. Nitrate and nitrite reduction was detected using Griess reagent. A streak was inoculated onto TSA supplemented with 1% Tween 80 and 0.01% CaCl₂ for detection of Tween 80 esterase. For phenylalanine

deaminase, phenylalanine agar was used. Paper discs containing 250 µg desferrioxamine were used on Mueller–Hinton agar for susceptibility testing.

Phenotypic characterization of the *Psychrobacter* isolates indicated that all strains were positive for oxidase, catalase and tributyrin esterase, and susceptible to colistin, but negative for alkaline phosphatase, trypsin, pyrrolidonyl aminopeptidase, production of indole, β-galactosidase (ONPG), gelatin, aesculin hydrolase and arginine dihydrolase, and for growth at 42 °C.

P. faecalis and *P. pulmonis*, although genotypically closely related (99 % similarity between both type strains), as determined on the basis of 16S rRNA gene sequences (Fig. 1), differed markedly in terms of their phenotypic characteristics. *P. faecalis* acidified a wide range of the carbohydrates listed in Table 1, and others not listed, i.e. lactose, galactose, melibiose, cellobiose and maltose (delayed), whereas *P. pulmonis* was asaccharolytic, as are all *P. phenylpyruvicus* isolates. Our finding of strong saccharolytic activity of *P. faecalis* on LPPR agar (Wauters *et al.*, 1998) and Hugh and Leifson's oxidation–fermentation medium (Hugh & Leifson, 1953) contradicts the original description of this species (Kämpfer *et al.*, 2002), which states 'no acid production from carbohydrates'.

P. faecalis and *P. pulmonis* were urease-negative and nitrite reductase-positive, which easily differentiates them from

P. phenylpyruvicus and *P. immobilis*. Furthermore, nitrite reductase was only positive in these two species (except for one *P. pulmonis* strain), in contrast to all other strains tested in this study. Almost all *Psychrobacter* species were positive for Tween hydrolysis; it is of interest to note that growth of *P. phenylpyruvicus* is drastically enhanced by addition of 1 % Tween 80 to the medium. Strain UCL-NF 1590, a genuine *P. faecalis* strain according to its 16S rRNA gene sequence, is the only isolate that is unable to hydrolyse Tween 80 or acidify ethylene glycol in this species.

Apart from the saccharolytic properties of *P. faecalis*, we found no discrepancies with the results already described for this species and the other species tested here.

We conclude that most human *Psychrobacter* isolates of European origin, other than *P. phenylpyruvicus*, belong predominantly to the species *P. faecalis* and *P. pulmonis*, and not to *P. immobilis*. We propose that the description of *P. faecalis* is emended to include the strong saccharolytic activity observed in all the *P. faecalis* strains tested in this study.

Emended description of *Psychrobacter faecalis*

Psychrobacter faecalis (fae.ca'lis. L. n. faex faecis dregs; L. masc. suff. -alis suffix denoting pertaining to; N.L. masc. adj. faecalis fecal).

This description is based on that of Kämpfer *et al.* (2002). Unless marked otherwise, most data are from this study. However, chemotaxonomic data and assimilation/utilization test data are from Kämpfer *et al.* (2002) and are based on the type strain only, which was the only strain available at the time of the original description. Cells are straight rods, 0.8–1.2 × 1.0–2.0 µm. Cells occur singly and are non-motile, Gram-negative, oxidase-positive and catalase-positive, with an oxidative, chemoheterotrophic metabolism. Ubiquinone Q-8 is the major quinone type, with low amounts of ubiquinone Q-9 and Q-7; spermidine is the major polyamine. The polar lipid profile is characterized by the presence of phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol. The fatty acids C_{18:1ω9c}, C_{17:1ω8c} and summed feature 3 (C_{16:1ω7c} and/or iso-C_{15:0} 2-OH), in addition to the hydroxylated fatty acid C_{12:0} 3-OH, are produced. On nutrient agar, colonies are circular, opaque, slightly raised and beige with entire margins. The type strain Iso-46^T grows well at 4–36 °C on different complex media, such as R2A agar and nutrient agar. No growth is observed at 45 or 55 °C on nutrient agar. No growth occurs on *Salmonella/Shigella* agar. H₂S is not produced. Negative for indole production, urease, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase and growth on Simmons' citrate. Saccharolytic with acid production from glucose, arabinose and xylose but not from mannitol. Acid is also produced from ethylene glycol. Utilization of *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *L*-arabinose (weak), *D*-glucose (weak), acetate, propionate (weak), *cis*-aconitate, 4-aminobutyrate, citrate, fumarate, glutarate, *DL*-3-hydroxybutyrate, *DL*-lactate, *L*-malate, 2-oxoglutarate, pyruvate, *L*-alanine, *L*-aspartate, *L*-leucine and *L*-ornithine (weak) is

Table 1. Phenotypic characterization of *Psychrobacter* isolates of human origin, including type strains of *P. faecalis*, *P. immobilis*, *P. phenylpyruvicus* and *P. pulmonis*

Taxa: 1, *P. cibarius* LMG 7085; 2, *P. faecalis* (n=18); 3, *P. immobilis* ATCC 43116^T; 4, *P. nivimaris* CCUG 39521; 5, *P. phenylpyruvicus* (n=7); 6, *P. pulmonis* (n=8).

Characteristic	1	2*	3	4	5*	6*
Acid from glucose	+	+	+	+	–	–
Mannitol	–	–	–	–	–	–
Xylose	+	+	+	+	–	–
Arabinose	+	+	+	+	–	–
Ethylene glycol	+	17†	+	+	4	+
Urease	+	–	+	–	+	–
Nitrate reductase	+	+	+	–	2	+
Nitrite reductase	–	+	–	–	–	7
Tween 80 hydrolase	+	17†	+	+	+	+
Phenylalanine deaminase	–	1	–	–	6	–
Desferrioxamine susceptibility	–	1‡	–	–	–	2
Growth at 35 °C	–	+	+	–	+	+

*Where result is not the same for all strains studied, the number of positive strains is indicated.

†Of the 18 strains studied, only UCL-NF 1590 is negative.

‡Type strain is positive.

positive. The following compounds are not utilized as sole carbon sources: L-arbutin, cellobiose, D-galactose, gluconate, maltose, D-mannose, α -melibiose, L-rhamnose, D-ribose, sucrose, salicin, trehalose, D-xylose, adonitol, *i*-inositol, D-sorbitol, glycerol, D-fructose, D-mannitol, maltitol, putrescine, *trans*-aconitate, mesaconate, adipate, azelate, itaconate, suberate, β -alanine, L-phenylalanine, L-serine, L-proline, L-histidine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The chromogenic substrates *p*-nitrophenyl (pNP)- α -D-glucopyranoside, pNP- β -D-galactopyranoside, ONPG, pNP- β -D-glucuronide, pNP- β -D-glucopyranoside, pNP- β -D-xylopyranoside, 2-deoxythymidine-5'-pNP-phosphate, L-glutamate- γ -3-carboxy-*p*-nitroanilide (pNA), L-proline-pNA and pNP-phosphorylcholine are not hydrolysed. Only hydrolysis of L-alanine-pNA and bis-pNP-phosphate is positive.

The type strain is Iso-46^T (=DSM 14664^T=CIP 107288^T), isolated on MacConkey agar from a bioaerosol originating from pigeon faeces.

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