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

Pieter Deschaght,¹ Michèle Janssens,² Mario Vaneechoutte¹ and Georges Wauters²

¹Laboratory Bacteriology Research (LBR), Department Clinical Chemistry, Microbiology, Immunology, University of Ghent, Ghent, Belgium
²Medical Microbiology, University of Louvain, UCL, Brussels, Belgium

Human *Psychrobacter* isolates, other than *Psychrobacter phenylpyruvicus*, are predominantly designated *Psychrobacter immobile*. Phenotypic and genotypic testing of *Psychrobacter* isolates that have been deposited in different culture collections as *P. immobile* 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 immobile*. Mostly, these are clinically not very relevant, but sometimes they are considered to be opportunistic pathogens (Gini, 1990; Lloyd-Puryear, 1991; Lozano et al., 1994). Many other *Psychrobacter* species have been described, mostly from the environment and from poikilo-thermic 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. immobile*, ATCC 43116⁵, 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. immobile*, we compared our clinical isolates with the human isolates deposited in the CCUG and Laboratorium voor Microbiologie, Universiteit Gent (LMG) culture collections as *P. immobile* 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
obtained using the maximum-parsimony algorithm (with mutation cost 100%).

Three sequences have been deposited in GenBank for the type strain ATCC 43116\textsuperscript{T} of \textit{P. immobilis}, i.e. U39399 (Bowman \textit{et al.}, 1996), AJ309942 (Romanenko \textit{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-\textit{p}-phenylenediamine strips (Merck). Acid production from carbohydrates was detected on low-peptone phenol red (LPPR) agar (Wauters \textit{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 % CaCl\textsubscript{2} for detection of Tween 80 esterase. For phenylalanine

![Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences of the \textit{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.](image-url)
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, asucinilhydrolyze and arginine dihydrolyse, 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. immobulis*. 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. immobulis*. 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 dreggs; L. masc. suff. -alis suffix denoting pertaining to; N.L. masc. adj. faecalis faecal).

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 diphostatidylglycerol. The fatty acids C_{18:1}ω9c, C_{17:1}ω8c and summed feature 3 (C_{16:1}ω7c 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\# 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. Sarcarolytic with acid production from glucose, arabino 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. immobulis*, *P. phenylpyruvicus* and *P. pulmonis*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2*</th>
<th>3</th>
<th>4</th>
<th>5*</th>
<th>6*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid from glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Arabinose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ethylene glycol</td>
<td>+</td>
<td>17†</td>
<td>+</td>
<td>+</td>
<td>4</td>
<td>+</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrate reductase</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Nitrite reductase</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tween 80 hydrolyse</td>
<td>+</td>
<td>17†</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenylalanine deaminase</td>
<td>-</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Desferrioxamine</td>
<td>-</td>
<td>1†</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Susceptibility</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth at 35 °C</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*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, 3-melibiose, L-rhamnose, D-ribose, sucrose, salicin, trehalose, D-xylene, adonitol, D-inositol, D-sorbitol, glycerol, D-fructose, D-mannitol, maltitol, putrescine, trans-aconitate, mesaconate, adipate, azelate, itaconate, suberate, beta-alanine, L-phenylalanine, L-serine, L-proline, L-histidine, L-tryptophan, 3-hydroxybenzoate, 4-hydroxybenzoate and phenylacetate. The chromogenic substrates p-nitrophenyl (pNP)-alpha-D-glucopyranoside, pNP-beta-D-galactopyranoside, ONPG, pNP-beta-D-glucuronide, pNP-beta-D-gluopyranoside, pNP-beta-D-xylopyranoside, 2-deoxythymidine-5'-pNP-phosphate, L-glutamate-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-46T (DSM 14664T=CIP 107288T), isolated on MacConkey agar from a bioaerosol originating from pigeon faeces.

References


