Porphyromonas crevioricanis is an earlier heterotypic synonym of Porphyromonas cansulci and has priority

Mitsuo Sakamoto and Moriya Ohkuma

A DNA–DNA hybridization experiment was carried out to clarify the relationship between Porphyromonas crevioricanis and Porphyromonas cansulci. The taxonomic standing of these two species was unclear so far because of the high 16S rRNA gene sequence similarity value (99.9 %). The DNA–DNA relatedness values between P. crevioricanis JCM 15906\textsuperscript{T} and P. cansulci JCM 13913\textsuperscript{T} were above 91 % (91–99 %). In addition, P. crevioricanis JCM 15906\textsuperscript{T} exhibited high hsp60 gene sequence similarity with P. cansulci JCM 13913\textsuperscript{T} (100 %). The hsp60 gene sequence analysis and the DNA–DNA relatedness values demonstrated that P. crevioricanis JCM 15906\textsuperscript{T} and P. cansulci JCM 13913\textsuperscript{T} are a single species. Based on these data, we propose Porphyromonas cansulci as a later heterotypic synonym of Porphyromonas crevioricanis.

It has been reported that Porphyromonas cansulci JCM 13913\textsuperscript{T} shows a very high hsp60 gene sequence similarity value (100 %) to Porphyromonas crevioricanis JCM 15906\textsuperscript{T} as well as a very high 16S rRNA gene sequence similarity value (99.9 %) (Sakamoto & Ohkuma, 2010), P. crevioricanis (Hirasawa & Takada, 1994) and P. cansulci (Collins \textit{et al.}, 1994) appeared in the same issue (vol. 44, no. 4) of International Journal of Systematic Bacteriology. If the level of DNA–DNA hybridization is sufficient to consider \textit{P}. crevioricanis and \textit{P}. cansulci as members of the same species, \textit{P}. cansulci is a later synonym of \textit{P}. crevioricanis because the name \textit{P}. crevioricanis Hirasawa and Takada 1994 has page priority over the name \textit{P}. cansulci Collins \textit{et al.} 1994. To resolve this issue, further studies were performed.

Porphyromonas crevioricanis JCM 15906\textsuperscript{T} and Porphyromonas cansulci JCM 13913\textsuperscript{T} were used in this study. The strains were maintained on Eggerth Gagnon (EG) agar (Merck) supplemented with 5 % (v/v) horse blood for 5–7 days at 37 °C in an atmosphere containing 100 % CO\textsubscript{2}. P. crevioricanis JCM 15906\textsuperscript{T} and P. cansulci JCM 13913\textsuperscript{T} were obligately anaerobic, black-pigmented, asaccharolytic, non-spore-forming, non-motile, Gram-negative short rods or rods. Cells on EG agar were 0.5 μm × 0.8–1.2 μm. Colonies on EG agar plates after 5 days of incubation at 37 °C under anaerobic conditions were 1–2 mm in diameter, circular, entire, dome-shaped, smooth, and brown or black.

Physiological and biochemical reactions were determined in duplicate with the API 20A anaerobe test kit and the Rapid ID 32A anaerobe identification kit, respectively (bioMérieux), according to the manufacturer’s instructions. The phenotypic and biochemical characteristics of 

Porphyromonas crevioricanis JCM 15906\textsuperscript{T} and P. cansulci JCM 13913\textsuperscript{T} were identical. Both strains failed to produce acid from \lbrack L-arabinose, cellobiose, glucose, glycerol, lactose, maltose, D-mannitol, D-mannose, melezitose, raffinose, L-rhamnose, salicin, D-sorbitol, sucrose, trehalose and D-xylose. Both strains were positive for indole production and gelatin digestion. Both strains were negative for aesculin hydrolysis and urease and catalase activities. In Rapid ID 32A tests, both strains were positive for indole production, alkaline phosphatase, alanine arylamidase, glutamyl glutamic acid arylamidase and leucyl glycine arylamidase activities, but negative for nitrate reduction, urease, β-N-acetylglucosaminidase, α-arabinosidase, arginine dihydrolase, α-fucosidase, α-galactosidase, β-galactosidase, β-galactosidase-6-phosphate, α-glucosidase, β-glucosidase, β-glucuronidase, mannose and raffinose fermentation and glutamic acid decarboxylase, arginine arylamidase, glycine arylamidase, histidine arylamidase, leucine arylamidase, phenylalanine arylamidase, proline arylamidase, pyrogallotinic acid arylamidase, serine arylamidase and tyrosine arylamidase activities. All of these comparable data were in agreement with the original data (Collins \textit{et al.}, 1994; Hirasawa & Takada, 1994), with the exception of catalase activity which differed from the original data of \textit{P}. cansulci.

Fatty acid methyl esters (FAMEs) were obtained from about 40 mg wet cells grown on EG agar at 37 °C for...
Table 1. Cellular fatty acid content of P. crevioricanis and P. cansulci

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>1</th>
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<tr>
<td><strong>Saturated straight-chain</strong></td>
<td></td>
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</tr>
<tr>
<td>C14:0</td>
<td>0.6</td>
<td>0.9</td>
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<tr>
<td>C15:0</td>
<td>tr</td>
<td>tr</td>
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<tr>
<td>C16:0</td>
<td>7.7</td>
<td>5.0</td>
</tr>
<tr>
<td>C18:0</td>
<td>2.3</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Unsaturated straight-chain</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C16:1t07c</td>
<td>tr</td>
<td>–</td>
</tr>
<tr>
<td>C18:1t09c</td>
<td>19.1</td>
<td>11.3</td>
</tr>
<tr>
<td>C18:0t06s, 9c</td>
<td>5.4</td>
<td>2.2</td>
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<tr>
<td><strong>Hydroxy</strong></td>
<td></td>
<td></td>
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<tr>
<td>C16:0 3-OH</td>
<td>1.4</td>
<td>1.2</td>
</tr>
<tr>
<td>C17:0 3-OH</td>
<td>–</td>
<td>tr</td>
</tr>
<tr>
<td>iso-C15:0 3-OH</td>
<td>1.9</td>
<td>1.2</td>
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<td>iso-C17:0 3-OH</td>
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<tr>
<td>anteiso-C17:0 3-OH</td>
<td>0.6</td>
<td>0.8</td>
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<tr>
<td><strong>Saturated branched-chain</strong></td>
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<tr>
<td>iso-C13:0</td>
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</tr>
<tr>
<td>iso-C15:0</td>
<td>28.6</td>
<td>39.6</td>
</tr>
<tr>
<td>anteiso-C15:0</td>
<td>1.5</td>
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<tr>
<td>iso-C16:0</td>
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</tr>
<tr>
<td>iso-C17:0</td>
<td>2.7</td>
<td>2.6</td>
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<tr>
<td><strong>Summed feature</strong></td>
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<tr>
<td>10</td>
<td>2.1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Summed features represent groups of two or three fatty acids that could not be separated using the MIDI system. Summed feature 3 contains one or more of an unknown fatty acid of ECL 13.570 and/or iso-C15:0 ALDE. Summed feature 10 contains one or more of an unknown fatty acid of ECL 17.834 and/or C18:1ω11c9t6t fatty acid methyl ester.

Approximately 1500 bases of the 16S rRNA gene sequence have been determined for both strains (Sakamoto & Ohkuma, 2010). For phylogenetic analysis of 16S rRNA gene sequences, 1429 bp sequences of each species were used. 558 bp nucleotide sequences of the hsp60 gene determined by Sakamoto & Ohkuma (2010) were also used. Phylogenetic analysis was performed as described previously (Sakamoto & Ohkuma, 2010). P. crevioricanis JCM 15906T and P. cansulci JCM 13913T formed a single cluster and a distinct line of descent (Fig. 1) as reported previously (Sakamoto & Ohkuma, 2010). Both strains exhibited 99.9% 16S rRNA gene sequence similarity with each other as mentioned above. There were no discrepancies between the 16S rRNA gene sequence of P. crevioricanis JCM 15906T and the original 16S rRNA gene sequence deposited in the public databases. On the other hand, the 16S rRNA gene sequence of P. cansulci JCM 13913T differs in 3 positions (plus 2 unknown bases) from the originally reported sequence (GenBank accession no. X76260). Since the original 16S rRNA gene sequence of P. cansulci has 2 unknown bases, the quality of this original sequence is doubtful. In addition, hsp60 gene sequence analysis suggested that both strains are the same species (Fig. 1). The hsp60 gene has been found to be a useful alternative phylogenetic marker (Sakamoto & Ohkuma, 2010, 2011; Sakamoto et al., 2010). More recently, Sakamoto & Ohkuma (2012) used the hsp60 gene sequence to reclassify Bacteroides chinchillae (Kitahara et al., 2011).

Chromosomal DNA was extracted using a Genomic-tip 100/G kit (Qiagen). The DNA base composition was determined by the HPLC method of Tamaoka & Komagata (1984). The elution solvent was a mixture of 0.02 M NH4H2PO4 and acetonitrile (20:1, v/v). The DNA–DNA hybridization experiment was carried out in microplate wells, as described by Ezaki et al. (1989). Hybridization was performed at 43 °C for 16 h. The DNA G+C contents of P. crevioricanis JCM 15906T and P. cansulci JCM 13913T were 45.9 and 45.6 mol%, respectively. The value for P. crevioricanis JCM 15906T was slightly higher than the original data (44 to 45 mol% by HPLC method) (Hirasawa & Takada, 1994). On the other hand, the value for P. cansulci JCM 13913T was slightly lower than the original data (49 to 51 mol% by the thermal denaturation method) (Collins et al., 1994). The DNA–DNA relatedness values between P. crevioricanis JCM 15906T and P. cansulci JCM...
13913T are shown in Table 2 and demonstrate that these both strains represent a single species. Consequently, P. cansulci Collins et al. 1994 is a later heterotypic synonym of P. crevioricanis Hirasawa and Takada 1994.

Emended description of Porphyromonas crevioricanis Hirasawa & Takada 1994

The description is as given by Hirasawa & Takada (1994) and Collins et al. (1994) with the following modification: Catalase activity is variable. The predominant respiratory quinones are MK-9 (30.7–33.9 %) and MK-10 (49.6–59.6 %); minor amounts of MK-7 (3.0–3.1 %) and MK-8 (6.7–13.4 %) are present. The DNA G+C content is in the range 44–45.9 mol% (by HPLC method).

Acknowledgements

We thank Ms. Natsuko Suzuki and Ms. Misako Matsuda for their technical assistance. This work was supported by a research grant (2009–2011) of IFO (Institute for Fermentation, Osaka, Japan), and also by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (Grant No. 23580126) to M. S.

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