Paenibacillus faecis sp. nov., isolated from human faeces

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A spore-forming, rod-shaped Gram-strain-positive bacterium, strain 656.84T, was isolated from human faeces in 1984. It contained anteiso-C15 : 0 as the major cellular fatty acid, meso-diaminopimelic acid was found in the cell wall peptidoglycan, the polar lipid profile consisted of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and aminophospholipids as the major components, and the predominant menaquinone was MK-7. The DNA G + C content was 52.9 mol%. The results of comparative 16S rRNA gene sequence studies placed strain 656.84T within the genus Paenibacillus. Its closest phylogenetic relatives were Paenibacillus barengoltzii and Paenibacillus timonensis. Levels of DNA–DNA relatedness between strain 656.84T and Paenibacillus timonensis CIP 108005T and Paenibacillus barengoltzii CIP 109354T were 17.3 % and 36.8 %, respectively, indicating that strain 656.84T represents a distinct species. On the basis of phenotypic and genotypic results, strain 656.84T is considered to represent a novel species within the genus Paenibacillus, for which the name Paenibacillus faecis sp. nov. is proposed; the type strain is 656.84T (= DSM 23593T=CIP 101062T).

The Gram-positive, endospore-forming bacteria were first classified in the genus Bacillus, which has been subsequently separated into several distinct genera. The genus Paenibacillus was proposed by Ash et al. (1993) on the basis of 16S rRNA gene analysis of the members of Bacillus group 3, and its description was then amended by Shida et al. (1997). The genus originally consisted of 11 species, but since then, the number of species belonging to the genus has considerably increased. At the time of writing, there is a total of 165 recognized species (LPSN: http://www.bacterio.net/).

The normal habitat of members of the genus Paenibacillus is soil. However, members of the genus have been isolated from various ecosystems such as rhizosphere (Beneduzi et al., 2010; Hong et al., 2009), water (Chou et al., 2007; Saha et al., 2005), cow faeces (Velázquez et al., 2004), blood culture (Roux & Raout, 2004; Ko et al., 2008), milk (Scheldeman et al., 2004), mural paintings (Smerda et al., 2006), spacecraft assembly facilities (Osman et al., 2006) and Antarctic sediments (Montes et al., 2004) with physiologically diverse characteristics.

Members of the genus Paenibacillus are aerobic or facultatively anaerobic organisms that produce endospores and...
have DNA G+C contents ranging from 35 to 59 mol% (Priet, 2009). Anteiso-C15:0 is the major cellular fatty acid in most cases, and MK-7 is the major isoprenoid quinone (Shida et al., 1997). The genus Paenibacillus has been reviewed by Priet (2009).

Here, we describe the phenotypic and genotypic properties of a strain isolated from human faeces, representing a novel species of the genus Paenibacillus.

Strain 656.84T from the Collection de l’Institut Pasteur (CIP) was isolated from human faeces in 1984 and described as a member of the genus Bacillus. It was grown aerobically at 30 °C on Trypto–Casein–Soy Agar (TSA) (Bio-Rad) and has previously been examined according to the recommended minimal standards for describing novel taxa of aerobic, endospore-forming bacteria (Logan et al., 2009). Morphology of the cells was determined by scanning electron microscopy (JSM-6700F field emission; JEOL) with cells cultured for 24 h on TSA at 30 °C. Endospore formation was observed by transmission electron microscopy (JEM-1010; JEOL). For this purpose, the cells were grown on specific spore-forming medium (1T1: 10 g beef extract, 2 g yeast extract, 0.04 g manganese II sulphate monohydrate, 25 g agar, pH 7.2) for two days at 25 °C. Enzymic reactions and acid productions were studied by means of the API 20 NE, API ZYM and the API 50 CH Systems (bioMérieux). These systems were used as recommended by the manufacturer. Vegetative cells were rod-shaped and motile; measuring 0.3–0.5 × 1.5–2.8 μm. Endospores were ellipsoidal and subterminal (Fig. S1 available in the online Supplementary Material). Growth was observed on TSA for 24 h at 20–50 °C with optimal growth between 30–37 °C. Colonies were round, translucent and brilliant with smooth surfaces and entire margins. In TSA, growth occurred at pH 6.0–9.0, with optimal growth at pH 7.0. Strain 656.84T grew in the presence of 2 % NaCl but not in the presence of 4 % NaCl. Differential phenotypic characteristics between strain 656.84T and the type strains of closely related species of the genus Paenibacillus are presented in Table 1. The extracted DNA was used for PCR amplification of the 16S rRNA gene coding for the 16S rRNA. Primers and PCR conditions were the same as those described by Clermont et al. (2009). Each PCR product was purified by filtration on Bio-Gel P100 (Bio-Rad) and then sequenced using a BigDye terminator cycle sequencing kit and ABI PRISM 3700 DNA sequencer, according to the manufacturer’s instructions (Applied Biosystems). The almost-complete sequence of the gene coding for the 16S RNA of 656.84T (1496 nt) was obtained using the script Assembler Tool kit developed by Eric Deveaud and Betina Setterblad, Centre d’Informatique pour la Biologie, Institut Pasteur. A comparison between this sequence and those of representative species belonging to the genus Paenibacillus showed that the novel organism also fell within this genus. Moreover, the highly specific sequence (5’-TCGAT-ACCCCTTGTTGCGGAAGT-3’) considered to be a signature of the genus Paenibacillus, described by Ash et al. (1993) was found in the 16S rRNA gene of 656.84T (position 799–821). 656.84T was most closely related to Paenibacillus timonensis and to Paenibacillus barengoltzii with 97.3 % and 98.0 % 16S rRNA sequence similarity, respectively. Lower levels of similarity were obtained with Paenibacillus konsidensis (96.6 %), Paenibacillus macerans (96.0 %) and Paenibacillus sanguinis (95.5 %), and similarity to Paenibacillus polymyxa, the type species of the genus, was 95.1 %.

Phylogenetic analysis, based on the 16S rRNA gene sequence of strain 656.84T and 16S rRNA sequences of reference type strains of species belonging to the genus Paenibacillus retrieved from GenBank, was performed after elimination of poorly aligned positions using Gblocks (Castresana, 2000). The sequences were aligned using MUSCLE (Edgar, 2004). A distance matrix was constructed using the Jukes–Cantor algorithm with dnadist. A phylogenetic tree was reconstructed

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*Data taken from Traiwan et al. (2011).
using the neighbour-joining method (Saitou & Nei, 1987) of PHYLIP version 3.6 software and viewed using NJPlot (Perrière & Gouy, 1996). The stability of the grouping was estimated by bootstrap analysis (1000 replicates) using the PHYLIP package (Felsenstein, 1989). The results indicated that strain 656.84T is closely related to \( P. \) barengoltzii and \( P. \) timonensis with which it forms a cluster with 80% bootstrap support (Fig. 1). An extended version of the neighbour-joining tree is shown in Fig. S2. The resulting topology was investigated using the maximum-likelihood method; the tree was reconstructed with PhyML (Guindon & Gascuel, 2003) and the nucleotide substitution model F81 (data not shown). The clade composed of strain 656.84\( ^{T} \), \( P. \) barengoltzii SAFN-016\( ^{T} \) (=CIP109354\( ^{T} \)) and \( P. \) timonensis 2301032\( ^{T} \) (=CIP 108005\( ^{T} \)) was supported by the maximum-likelihood method as indicated in Fig. 1 (filled circles).

As the level of 16S rRNA gene sequence similarity between strain 656.84\( ^{T} \) and \( P. \) timonensis and \( P. \) barengoltzii was above the cut-off value (97%) suggested by Stackebrandt & Goebel (1994) for genomic distinction of species, DNA–DNA relatedness experiments were performed by the Identification Service of the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ),

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\begin{align*}
\text{Paenibacillus faecis sp. nov.} \\
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Braunschweig, Germany. Cells were disrupted using a French pressure cell (Thermo Spectronic) and the DNA in the crude lysate was purified by chromatography on hydroxypatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970) including the modifications described by Huss et al. (1983), using a model Cary 100 Bio UV/VIS-spectrophotometer with a Peltier-thermostat-equipped 6 × 6 multichell changer and a temperature controller with in-situ temperature probe (Varian). The experiments were carried out in 2 × SSC/5 % formamide at 65 °C. Levels of DNA–DNA relatedness between strain 656.84T and P. timonensis CIP 108005T and between strain 656.84T and P. barengoltzii CIP 109354T were 17.3 % (11.7 %) and 36.8 % (41.3 %), respectively (values in parentheses are results of measurements in duplicate). When the recommendation of a threshold value of 70 % DNA–DNA similarity for the definition of bacterial species (Wayne et al., 1987) is considered, these results strongly indicate that this strain is representative of a novel, independent species of the genus Paenibacillus.

Gas chromatographic (GC) analysis of cellular fatty acid methyl esters was performed by the Identification Service of the DSMZ, using the Sherlock MIDI system (Sasser, 1990) following the protocol of the Microbial Identification System (MIDI, 1999) with the TSBA6 library (Hewlett Packard). Strains were incubated for 24 h at 28 °C. The fatty acid profile of strain 656.84T revealed that anteiso-C15:0 (39.7 %), C16:0 (15.8 %), anteiso-C17:0 (11.7 %) and iso-C16:0 (13.6 %) were predominant. This cellular fatty acid profile was consistent with that of the genus Paenibacillus (Table S1). Comparison of fatty acid profiles of 656.84T and its nearest phylogenetic neighbours (P. barengoltzii CIP 109354T, P. timonensis CIP 108005T, P. macerans CIP 66.19T and 656.84T), four strains that formed an independent cluster in the 16S rRNA phylogenetic tree (Fig. 1). Only P. woosongensis CIP 110595T and P. fonticola CIP 110593T also possessed GL3. The G+C content of the DNA was determined by the Identification Service of the DSMZ using reverse-phase HPLC of nucleosides as described by Mesbah et al. (1989). The DNA was purified on hydroxypatite according to the protocol of Cashion et al. (1977) and chromatographic conditions were adapted from those described by Tamaoka & Komagata (1984). The DNA G+C content of the isolate 656.84T was 52.9 mol%, corresponding to those of other members of the genus Paenibacillus that have high G+C contents, ranging from 35 to 59 mol% (Priest, 2009).

On the basis of the results of the polyphasic taxonomy described above and the differences observed with its nearest phylogenetic neighbours (Table 1, Tables S1, S2 and S3), it is proposed that strain 656.84T represents a novel species (based on a single isolate, at the time of writing) within the genus Paenibacillus.

**Description of Paenibacillus faecis sp. nov.**

*Paenibacillus faecis* (fae’cis. L. gen. n. faecis, from faeces, as the organism was found in human faeces). Facultatively anaerobic, Gram-positive, motile, short rods, 0.3–0.5 × 1.5–2.8 μm. Ellipsoidal endospores are located in the subterminal position. Colonies grown on TSA are smooth, circular, translucent and bright and approximately 2 mm in diameter after 24 h at 30 °C. Catalase-positive and oxidase-positive. Gelatin is not decomposed. Aesculin is hydrolysed. Indole production is negative. Nitrate is reduced to nitrite. Activities of amylase, α-galactosidase, β-galactosidase and α- and β-glucosidase are positive.
Negative for N-acetyl-β-glucosaminidase, acid phosphatase, alkaline phosphatase, α-fucosidase, α-mannosidase, arginine dihydrolase, β-glucuronidase, esterase, esterase lipase, leucine arylamidase, naphthol-AS-Bl-phosphohydrolase, trypsin, tween esterase and urease. Acid is produced from N-acetylglucosamine, ascorulin, amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, galactose, β-gentiobiose, gluconate, D-glucose, glycogen, inulin, lactose, maltose, mannotol, D-mannose, melibiose, α-methyl-D-gluco side, β-methyl-xylside, raffinose, rhamnose, salicin, L-sorbitose, starch, sucrose, trehalose, turanose and D-xylene. Utilizes N-acetylglucosamine, arabinose, gluconate, glucose, maltose, mannotol and mannose. Predominant fatty acids are anteiso-C15:0, C16:0, iso-C16:0, anteiso-C17:0, iso-C17:0, and iso-C15:0. The major polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and aminophospholipids. The isoprenoid quinone is MK-7. The diamino acid in the cell wall peptidoglycan is meso-diaminopimelic acid.

The type strain is DSM 5393T (=DSM 23593T=CIP 101062T) and was isolated from human faces, in Bordeaux, France in 1984. The DNA G + C content of the type strain is 52.9 mol%.

References


