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 (Beneduzzi 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 & Raoult, 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

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 656.84T is HM212646.

Four supplementary figures and three supplementary tables are available with the online Supplementary Material.
have DNA G+C contents ranging from 35 to 59 mol% (Priest, 2009). Anteiso-C\textsubscript{15}: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 Priest (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 656.84\textsuperscript{T} 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 (1 \textsuperscript{−1}: 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 656.84\textsuperscript{T} grew in the presence of 2 % NaCl but not in the presence of 4 % NaCl. Differential phenotypic characteristics between strain 656.84\textsuperscript{T} 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 \textit{rrs} gene coding for the 16S rRNA of 656.84\textsuperscript{T}. 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 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-ACCCCTTGGTGCCGAAAGT-3’) considered to be a signature of the genus Paenibacillus, described by Ash et al. (1993) was found in the 16S rRNA gene of 656.84\textsuperscript{T} (position 799–821). 656.84\textsuperscript{T} was most closely related to \textit{Paenibacillus timonensis} and to \textit{Paenibacillus barengoltzii} with 97.3 % and 98.0 % 16S rRNA sequence similarity, respectively. Lower levels of similarity were obtained with \textit{Paenibacillus konsidensis} (96.6 %), \textit{Paenibacillus macerans} (96.0 %) and \textit{Paenibacillus sanguinis} (95.5 %), and similarity to \textit{Paenibacillus polymyxa}, the type species of the genus, was 95.1 %.

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

### Table 1. Differential phenotypic characteristics between strain 656.84\textsuperscript{T} and the type strains of closely related species of the genus \textit{Paenibacillus}

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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 656.84\textsuperscript{T} is closely related to \textit{P. barengoltzii} and \textit{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\textsuperscript{T}, \textit{P. barengoltzii} SAFN-016\textsuperscript{T} (=CIP109354\textsuperscript{T}) and \textit{P. timonensis} 2301032\textsuperscript{T} (=CIP 108005\textsuperscript{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\textsuperscript{T} and \textit{P. timonensis} and \textit{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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**Fig. 1.** Phylogenetic tree based on 16S rRNA gene sequences, reconstructed using the neighbour-joining method, showing relationships between the proposed \textit{P. faecis} strain (656.84\textsuperscript{T}) and some related members of the genus \textit{Paenibacillus}. Tree reconstruction was based on a total of 1447 nucleotides. \textit{Bacillus subtilis} subsp. \textit{subtilis} NCDO 1769\textsuperscript{T} was used as outgroup. Numbers represent bootstrap values of 1000 replicates (only values above 50 shown). Nodes retrieved in neighbour-joining and maximum-likelihood methods are indicated by filled circles. Bar, 1 substitution per 100 nucleotides.
Braunschweig, Germany. Cells were disrupted using a French press cell (Thermo Spectronic) and the DNA in the crude lysate was purified by chromatography on hydroxyapatite 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 multiccull 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 (Heewlett 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) revealed variability in the abundance of fatty acids (Table S1).

The structure of the peptidoglycan was analysed from exponentially growing bacteria using a previously described method for Gram-positive bacteria (Wheeler et al., 2014). Peptidoglycan (750 μg) was digested overnight with 10 μg Streptomyces globisporus mutanolysin (Sigma-Aldrich). Following sodium borohydride reduction, soluble muropeptides were analysed by reverse-phase HPLC using a Thermo Scientific Hypersil GOLD aQ C18 column (250 × 4.6 mm, 5 μm) in H2O/0.05 % TFA with a linear gradient of 0–25 % acetonitrile over 135 min. Muropeptide peaks of interest were collected and identified by mass spectrometry as described previously (Irving et al., 2014). The results are summarized in Fig. S3 and Table S2. Cell wall peptidoglycan was of the A1γ type (Schleifer & Kandler, 1972), with direct cross-linking and meso-diaminopimelic acid as the diagnostic diamino acid. The muropeptide profiles of all strains included in the analysis were highly similar, indicating they are all of the A1γ type (Fig. S3a). D-glutamate was the amino acid present at position two of the peptide stem. Modification of the glycan chain by deacetylation of N-acetyl-D-glucosamine was also prevalent.

Analysis of respiratory quinones and polar lipids was carried out by the Identification Service and Dr Brian Tindall, DSMZ. MK-7 was the only menaquinone present in strain 656.84T which was in agreement with the description of the genus Paenibacillus. Total lipid material was detected using molybdatophosphoric acid, and specific functional groups were detected using spray reagents specific for defined functional groups, as described by Tindall et al. (2007). The polar lipids were composed of diphasphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), aminophospholipids (PN1, PN2), phospholipids (PL1), lipids (L1, L2, L3) and glycolipids (GL1, GL2, GL3, GL4) (Fig. S4). Although 656.84T and strain P. polymyxia CIP 66.22T had some polar lipids in common (DPG, PG, PE, GL1, GL2, L1, L2), they were distinguishable from each other on the basis of the presence or absence of other lipids (Table S3). Indeed, 656.84T possessed PN1, PN2, PL1, L3, GL3 and GL4, which were absent in P. polymyxia CIP 66.22T. In contrast, phosphatidylmethylaminoethanolamine (PMME) was detected in P. polymyxia CIP 66.22T and not in 656.84T. We can note that GL3 was found in 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 hydroxyapatite according to the procedure 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, x-fucosidase, x-mannosidase, arginine dihydrolase, β-glucuronidase, esterase, esterase lipase, leucine arylamidase, napthol-AS-BI-phosphohydrolase, trypsin, tween esterase and urease. Acid is produced from N-acetylglucosamine, ascorcin, amygdalin, L-arabinose, arbutin, cellobiose, D-fructose, galactose, β-gentiobiose, glucose, glycogen, inulin, lactose, maltose, mannotol, D-mannose, melibiose, α-methyl-D-glucoside, β-methyl-xyloside, raffinose, rhamnose, salicin, L-sorbitose, starch, sucrose, trehalose, turanose and D-xylose. 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, phosphatidylethanolamine, phosphatidylycerol and aminophospholipids. The isoprenoid quinone is MK-7. The diamino acid in the cell wall peptidoglycan is meso-diaminopimelic acid.

The type strain is 565.84^T (＝DSM 23593^T＝CIP 101062^T) and was isolated from human faeces, in Bordeaux, France in 1984. The DNA G+C content of the type strain is 52.9 mol%.

References


Roux, V. & Raoult, D. (2004). Paenibacillus massiliensis sp. nov., Paenibacillus sanguinis sp. nov. and Paenibacillus barienis sp. nov., isolated from human faeces, in Bordeaux, France in 1984. The DNA G+C content of the type strain is 52.9 mol%.

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