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Fictibacillus enclensis sp. nov., isolated from marine sediment

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Abstract A novel Gram-positive strain, designated NIO-1003^T, was isolated from a marine sediment sample collected from the Chorao Island, Goa Provence, India. Strain NIO-1003^T was found to be strictly aerobic, motile, endospore-forming rods. Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain NIO-1003^T belongs to the genus *Fictibacillus* and to be most closely related to *Fictibacillus rigui* KCTC 13278^T, *Fictibacillus solisalsi* KCTC 13181^T and *Fictibacillus barbaricus* DSM 14730^T with 98.2, 98.0 and 97.2 % similarity and 25, 28, 39 nucleotide differences respectively. Strain NIO-1003^T was characterized by having cell-wall peptidoglycan based on meso-diaminopimelic acid and MK-7 as the predominant menaquinone. The polar lipid profile exhibited the major compounds diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine. In addition, minor amounts of an aminophospholipid were detected. The major fatty acids were identified as ai-$C_{15:0}$, iso-$C_{15:0}$, ai-$C_{17:0}$ and $C_{16:0}$, supporting the grouping of strain NIO-1003^T into the family *Bacillaceae*. The DNA G+C content of strain NIO-1003^T was determined to be 42.6 mol%. On the basis of phenotypic properties, phylogeny and DNA–DNA hybridisation analysis, strain NIO-1003^T is considered to represent a novel species of the genus *Fictibacillus* for which the name *Fictibacillus enclensis* sp. nov. is proposed. The type strain is NIO-1003^T (= NCIM 5458^T = DSM 25142^T).

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Introduction

The genus *Bacillus* comprises more than 222 species with validated names (http://www.bacterio.net). Therefore, classification and identification of Gram-positive, endospore-forming rods should be performed by using a polyphasic taxonomic approach that integrates phylogenetic analysis based on 16S rRNA gene sequences, genomic relatedness and an extensive range of phenotypic characteristics (Yoon et al. 1998; Tcherpakov et al. 1999). For a long time, most aerobic, endospore forming rods were assigned to the genus *Bacillus* (Claus and Berkeley 1986). Subsequently 16S rRNA gene sequence analyses revealed the presence of several phylogenetically distinct lineages within the genus *Bacillus*. Consequently, some phylogenetic groups have been established as new genera such as *Alicyclobacillus* (Wisotzkey et al. 1992), *Paenibacillus* (Ash et al. 1993), *Aneurinibacillus* (Shida et al. 1996), *Brevibacillus* (Shida et al. 1996), *Virgibacillus* (Heyndrickx et al. 1998), *Salibacillus* (Wainø et al. 1999), *Ureibacillus* (Fortina et al. 2001), *Marinibacillus* (Yoon et al. 2001a), *Alkalibacillus* (Jeon et al. 2005), *Pullulanibacillus* (Hatayama et al. 2006), *Sporolactobacillus* (Hatayama et al. 2006) and *Viridibacillus* (Albert et al. 2007). Moreover, several species of the genus *Bacillus* have been reclassified or transferred to the other genera such as *Gracilibacillus* (Wainø et al. 1999), *Sporosarcina* (Yoon et al. 2001b), *Geobacillus* (Nazina et al. 2001), *Salimicrobium* (Yoon et al. 2007), *Lysinibacillus* (Ahmed et al. 2007) and *Rummeliibacillus* (Vaishampayan et al. 2009). In addition to these, recently Glaeser et al. (2013) further proposed the genus *Fictibacillus* and reclassified *Bacillus nanhaiensis*, *B. barbaricus*, *B. arsenicus*, *B. rigui*, *B. macauensis* and *B. gelatini* as *Fictibacillus nanhaiensis*, *Fictibacillus barbaricus*, *Fictibacillus arsenicus*, *Fictibacillus rigui*, *Fictibacillus macauensis* and *Fictibacillus gelatini* comb. nov., respectively. In the course of our study on bacterial diversity, a Gram-stain positive, endospore-forming bacterial strain, designated strain NIO-1003^T, was isolated from a marine sediment sample collected at Chorao Island, Goa, India and was the subject of a taxonomic investigation.

Materials and methods

Bacterial strains

Strain NIO-1003^T, isolated on marine agar (MA) medium after about 2 weeks incubation at 30 °C, originates from a sediment sample collected from an intertidal region of mangroves at Chorao Island in Goa, India (GPS coordinates 15°32′34″N and 73°55′15″E). The strain was maintained on nutrient agar (NA) slants at 4 °C and as glycerol suspensions (20 %, v/v) at −80 °C. Biomass for chemical and molecular-systematic studies was obtained following growth in shake flasks (about 200 rpm) of Tryptic Soy broth, supplemented with the vitamin mixtures of the HV medium (Hayakawa and Nonomura, 1987) at 30 °C for 5 days.

Fictibacillus rigui KCTC 13278^T and *Fictibacillus solisalsi* KCTC 13181^T were obtained from Korean Type Culture Collection, Daejeon, South-Korea and cultured under comparable conditions as reference strains.

Phenotypic tests

Gram staining was carried out by using the standard Gram reaction and cell motility was confirmed by the development of turbidity throughout a tube containing semisolid medium (Leifson, 1960). Morphological features were determined on NA after 48 h at 30 °C. Strain NIO-1003^T was observed with light microscopy (BH2; Olympus). For scanning electron microscopy examination, 1 ml samples were fixed overnight at 4 °C by adding formaldehyde to a final concentration of 7 %. Nine ml PBS (130 mM NaCl, 10 mM sodium phosphate, pH 7 ± 2) was added to the samples, which were then filtered through 0.2 µm Millipore filters and washed with PBS. The filters were then serially dehydrated in 25, 50, 70 and 100 % ethanol solutions (three times for 10 min at each stage), critical-point dried, mounted on scanning electron microscope stubs, sputter-coated with gold and viewed on a FEI Qunta 200 3D dual beam scanning electron microscope. Transmission electron microscopy was used to observe the flagella. Wet mount preparations were used: bacterial suspensions were settled onto specimen grids, stained with 1 % phosphotungstinate and viewed with a transmission electron microscope (JEOL 1200 EX).

For biochemical and physiological properties, cultures were incubated at 30 °C and properties were

recorded for up to 4 days with 24 h intervals. Growth under anaerobic conditions was determined after incubation in an anaerobic chamber with NA that had been prepared anaerobically. Growth at various NaCl concentrations was investigated on NA broth without NaCl and NaCl concentrations (0–15 %) added separately (at intervals of 1 %, w/v). Growth at various temperatures was measured on NA at 4–45 °C, and pH (pH 5.0–12.0) at an intervals of 0.5 pH units using the following buffer systems: pH 4.0–5.0: 0.1 M citric acid/0.1 M sodium citrate; pH 6.0–8.0: 0.1 M KH_2PO_4/0.1 M NaOH; pH 9.0–10.0: 0.1 M $NaHCO_3$/0.1 M Na_2CO_3.

The utilization of sugars and acid production from carbohydrates as carbon sources was determined using API 50CH kits (bioMérieux) according to the manufacturer's instructions, with API 50CHB as inoculation medium. Fingerprints of enzymic activities were obtained using API ZYM test strips (bioMérieux) according to the manufacturer's instructions. Nitrogen assimilation was assessed using NA broth. Catalase activity was determined by production of bubbles after the addition of a drop of 3 % H_2O_2. Oxidase activity was determined using the API oxidase reagent. Hydrolysis of urea was determined on peptone-glucose agar (L^{-1}): peptone 1 g, glucose 1 g, NaCl 5 g, KH_2PO_4 2 g, containing 2 % (w/v) urea and 0.001 % (w/v) phenol red. Hydrolysis of starch was determined on peptone-beef extract agar containing 0.2 % (w/v) soluble starch by flooding of the plates with iodine solution. Hydrolysis of casein was tested on casein agar by observation of clear zones around the colonies. The incubation period for hydrolysis of urea, starch and casein was 24–48 h d at 30 °C. Gelatin hydrolysis was determined by incubation for one week at 30 °C on peptone-gelatin medium (L^{-1}) (peptone 5 g, gelatin 120 g). Milk coagulation and peptonization was determined using 20 % (w/v) skimmed milk as medium incubation for 48–72 h at 30 °C.

Molecular analyses

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene from strain NIO-1003^T was performed as described by Li et al. (2007). The resulting 16S rRNA gene sequence was compared with available 16S rRNA gene sequences from GenBank using the BLAST program to determine the nearest neighbours of the strain NIO-1003^T. Multiple alignments with sequences of the most closely related bacteria and calculations of levels of sequence similarity were carried out using CLUSTAL_X (Thompson et al. 1997). Phylogenetic analyses were performed using four tree-making algorithms: the neighbour-joining (Saitou and Nei, 1987), maximum-likelihood (Felsenstein, 1981) and maximum-parsimony (Fitch, 1971) methods. A phylogenetic tree was constructed using the neighbour-joining method of Saitou and Nei (1987) from K*nuc* values (Kimura, 1980) using MEGA version 5.0 (Tamura et al. 2011). The topology of the phylogenetic tree was evaluated by the bootstrap resampling method of Felsenstein (1985) with 1,000 replicates.

To further study the genetic relatedness of NIO-1003^T with the two closest type strains, *F. rigui* KCTC 113278^T and *F. solisalsi* KCTC 13181^T, the strains were analyzed by AP-PCR. In this method, arbitrarily selected primers are annealed to template DNA under low stringency conditions for the initial cycles of DNA amplifications, which allows interactions between the primers and target DNA in regions containing base mismatches. The AP-PCR fingerprinting were performed by using the M13F primer [(–20): GTAAAACGACGGCCAGT] and the following PCR program: two cycles of 94 °C for 5 min, 40 °C for 5 min, and 72 °C for 5 min; followed by 40 high-stringency cycles of 94 °C for 1 min, 60 °C for 1 min and 72° for 2 min. Amplified DNA product were resolved by electrophoresis n agarose 2 % w/v gels.

Chemotaxonomic analyses

Sugar analysis of the purified cell walls followed procedures described by Staneck and Roberts (1974). Polar lipids were extracted from freeze-dried cells and separated by two dimensional silica gel thin layer chromatography (Minnikin et al. 1984). The first direction was developed in chloroform/methanol/water (65:25:3.8, by vol.) and the second in chloroform/methanol/acetic acid/water (40:7.5:6:1.8, by vol.), first spraying with ninhydrin and developing at 120–160 °C in an incubator. Reaction sites were outlined with a soft pencil before executing a second spraying treatment with the molybdenum-Blue and drying in an incubator at 100 °C. Menaquinones were isolated according to Minnikin et al. (1984) and separated by reversed phase HPLC (Kroppenstedt 1982). For fatty acids analysis, strains NIO-1003^T *F.*

rigui KCTC 113278^T and *F. solisalsi* KCTC 13181^T were cultured on tryptic soy agar (TSA, Difco) at 30 °C for 48 h. Cellular fatty acids analysis was performed as described by Sasser (1990) according to the MIDI protocols by gas chromatography with flame ionization detector (GC-FID) and identified using the Microbial Identification Software (MIDI Sherlock aerobe method and TSBA library version Aerobic Bacteria Library TSBA6/RTSBA6 v6.10; Newark, DE, USA).

For the determination of G+C composition, genomic DNA was prepared according to the method of Marmur and Doty (1962). Genomic DNA was hydrolysed and the resultant nucleotides were analysed by reversed phase HPLC (Tamaoka and Komagata 1984).

DNA–DNA hybridization

For DNA–DNA hybridization experiments, genomic DNA was extracted and purified according to the method of Marmur (1961) with additional RNAse treatment. DNA–DNA hybridization was carried out based on principles and equations described by De Ley et al. (1970) under the consideration of modifications done by Huss et al. (1983) with optimized fluorimetric procedure evaluated by Loveland-Curtze et al. (2011), using a Step One Plus Real-Time PCR system (Applied Biosystems) fitted with 96 well thermal cycling block. DNA suspended in 2X SSC buffer was used for the analysis in triplicate. The reassociation was carried out at optimum renaturation temperature 70 °C (Gillis et al. 1970, Marmur and Doty 1962). The relatedness values are expressed by the means of the three values and the results DNA–DNA hybridizations were taken from the three means of relatedness values. The reassociation of DNA was carried out at optimum renaturation temperature 70 °C [$Tor = 0.51 \times (\%\ G+C) + 47.0$] according to De Ley et al. (1970).

Results and discussion

Colonies of strain NIO-1003^T were observed to be cream coloured on nutrient agar and tryptone soy agar; no aerial mycelia were observed. No diffusible pigment was observed. Strain NIO-1003^T was found to have morphological characteristics typical of the genus *Bacillus*, including endospore formation. Cells were observed to be Gram-positive short rods, 0.45–0.46 × 3.0–3.2 μm, and motile (Online Supplementary Fig. 1). Strain NIO-1003^T was found to grow at 15–42 °C, pH 5.0–12.0 and with 0–12 % (w/v) NaCl, with optimum growth observed at 30 °C, pH 7.0–7.5 and 0–5 % (w/v) NaCl concentration. No growth was observed below 15 °C, pH 5.0 and above 12 % (w/v) NaCl. The detailed physiological and biochemical characteristics of strain NIO-1003^T are given in the species description and Table 1.

Strain NIO-1003^T was found to contain *meso*-diaminopimelic acid as the diagnostic amino-acid in the cell-wall peptidoglycan and ribose, glucose, galactose as cell-wall sugars. The predominant menaquinone was identified as unsaturated menaquinone with seven isoprene units (MK-7). The major polar lipids detected in strain NIO-1003^T were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unidentified amino lipid (Supplementary Fig. 2). The genomic DNA G+C content of strain NIO-1003^T was determined to be 42.6 mol%. The major fatty acids (>5 %) detected in strain NIO-1003^T and the reference strains *F. rigui* KCTC 13278^T and *F. solisalsi* KCTC 13181^T were anteiso-$C_{15:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$ and $C_{16:0}$. The detailed comparative percent profile of fatty acid analysis is given in Table 2.

The almost complete 16S rRNA gene sequence (1,419 bp) of strain NIO-1003^T was obtained (GenBank/EMBL/DDBJ accession number JF893461). Strain NIO-1003^T shares highest sequence similarity with *F. rigui* KCTC 13278^T, *F. solisalsi* KCTC 13181^T and *F. barbaricus* DSM 14730^T with 98.2, 98.0, 97.2 % and 25, 28, 39 nucleotide differences respectively. A phylogenetic tree, based on 16S rRNA gene sequence data from strain NIO-1003^T and corresponding sequences from the type strains of the genus *Fictibacillus* was constructed using the neighbour-joining (Fig. 1) and maximum parsimony (Suppl. Fig. 3) algorithms. The comparative analysis of 16S rRNA gene sequences and phylogenetic relationships showed that strain NIO-1003^T forms a subclade in the tree with *F. rigui* KCTC 13278^T supported by a high bootstrap value (Fig. 1), with which it shares the highest 16S rRNA gene sequence similarity. The affiliation of strain NIO-1003^T and it closest neighbours was also supported by the maximum parsimony and maximum-likelihood algorithms with high bootstrap values. The determined DNA–DNA relatedness

Table 1 Phenotypic characteristics that differentiate strain NIO-1003 from its phylogenetic neighbours in the genus *Fictibacillus*

Characteristics	NIO-1003^T	*F. rigui* KCTC 13278^T	*F.solisalsi* KCTC 13181^T	*F. barbaricus* KACC 12101^T
Motility	+	+	+	−
Anaerobic growth	+	−	+	+
Growth at/in				
15 °C	−	+	+	−
47 °C	−	−	+	−
5 % (w/v) NaCl	+	+	+	−
10 % (w/v) NaCl	−	−	+	−
Aesculin hydrolysis	+	−	+	−
Malonate utilization	+	+	−	+
Utilization of carbon sources				
Adonitol	W	−	−	−
Fructose	+	−	+	−
Galactose	−	W	−	−
Glucose	+	−	−	−
Inositol	+	−	−	−
Lactose	−	W	−	−
D-Mannitol	−	+	+	−
D-Mannose	+	−	−	−
Maltose	W	+	−	+
Melezitose	+	+	−	−
Melibiose	+	+	−	−
Raffinose	+	+	−	−
Sucrose	+	+	−	−
Xylitol	+	+	−	−
D-xylose	−	+	+	−
Major Fatty acids	ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{17:0}$, $C_{16:0}$	ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{17:0}$, $C_{16:0}$	ai-$C_{15:0}$, i-$C_{15:0}$, ai-$C_{17:0}$, $C_{16:0}$	ai-$C_{15:0}$, i-$C_{15:0}$; i-$C_{14:0}$ and i-$C_{16:0}$
DNA G+C content (mol %)	42.6	41.9[a]	41.8[a]	42.0[a]
Isolated from	Sediment	Fresh water	Saline soil	Wall painting

All data were generated from present study except that for *F. barbaricus* KACC 12101^T which was taken from Täubel et al. (2003)

[a] Data collected from Täubel et al. (2003), Liu et al.(2009) and Baik et al. (2010)

value between strain NIO-1003^T and *F. rigui* KCTC 13278^T and between strain NIO-1003^T and *F. solisalsi* KCTC 13181^T were 53.9 ± 1.2 and 54.8 ± 2.1 % respectively, which is well below the 70 % cutoff point accepted for the recognition of genomic species (Wayne et al. 1987). Depending on the investigated taxo-nomic group, the threshold value of DNA–DNA hybridizations is considered necessary has been increased to between 98.2- 99.0 % 16S rRNA sequence similarity (Stackebrandt and Ebers, 2006; Meier-Kolthoff et al. 2013). The 16S rRNA gene sequence similarity and DNA–DNA relatedness data therefore suggest that the strain NIO-1003^T should be considered as a different genomic species of the genus *Fictibacillus*. Furthermore, AP-PCR amplicon fingerprint profiles showed marked differences in the banding patterns between strains NIO-1003^T, *F. rigui* KCTC 13278^T, and *F. solisalsi* KCTC 13181^T (Supplementary Fig. 4), consistent with the assignment of these strains to separate species.

The phenotypic and chemotypic properties of strain NIO-1003^T, and the 16S rRNA gene sequence

Table 2 Cellular fatty acid profiles (%) of strain NIO-1003^T and its phylogenetically related species of the genus *Fictibacillus*

Fatty acids	1	2	3
Straight-chain saturated			
$C_{12:0}$	–	–	0.6
$C_{14:0}$	1.2	1.0	1.3
$C_{16:0}$	5.0	5.2	7.5
$C_{18:0}$	0.6	1.1	2.1
Branched			
iso-$C_{10:0}$	–	0.4	–
iso-$C_{14:0}$	4.1	1.9	2.7
iso-$C_{15:0}$	13.7	18.6	16.2
iso-$C_{15:1}$ ω 5c	–	0.4	–
iso-$C_{16:0}$	3.3	1.7	3.8
iso-$C_{17:0}$	1.1	1.6	2.7
iso-$C_{17:1}$ ω10c	0.1	0.4	–
anteiso-$C_{15:0}$	60.5	52.0	49.0
anteiso-$C_{17:0}$	7.4	6.7	10.6
anteiso-$C_{17:1}$ ω9c	0.1	–	0.1
Monounsaturated			
$C_{16:1}$ ω 7c	–	0.4	0.2
$C_{16:1}$ ω 11c	–	1.0	–
Summed features[a]			
3	0.3	0.4	0.3
4	–	1.1	–
5	0.1	–	–
6	–	0.4	–
8	–	0.2	0.2

Strains: *1* NIO-1003^T, *2 F. rigui* KCTC 13278^T, *3 F. solisalsi* KCTC 13181^T. *All data* were obtained in this study and are representative of triplicate analyses; cells of all strains were harvested after cultivation at 30 °C on TSA medium after 48 h. '–' not detected

[a] *Summed features* represent groups of two or three fatty acids that could not be separated by GLC with the MIDI system. Summed feature 3 contained C $_{16:1}$ ω6c/$C_{16:1}$ ω7c. Summed feature 4 contained iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B. Summed feature 5 contained ai-$C_{18:0}$/$C_{18:2}$ ω6, 9c. Summed feature 6 contained $C_{19:1}$ ω9c/$C_{19:1}$ ω11c. And summed feature 8 contained $C_{18:1}$ ω 6c/$C_{18:1}$ ω7c

comparison and DDH results, supports the proposal to classify the isolate NIO-1003^T as a novel member of the genus *Fictibacillus*. The phenotypic, genotypic and phylogenetic data distinguish strain NIO-1003^T from other validly named members of the genus *Fictibacillus*. Therefore, we propose that isolate NIO-1003^T represents a novel species within the genus, for which the name *Fictibacillus enclensis* sp. nov. is proposed.

Description of Fictibacillus enclensis sp. nov.

Fictibacillus enclensis (en.clen'sis. N.L. masc. adj. *enclensis* arbitrary name formed from NCL, the acronym for the National Chemical Laboratory, India, where taxonomic studies on this species were performed).

Cells are aerobic, Gram stain-positive, motile rods 0.45–0.46 × 3.0–3.2 μm in size, which are endospore-forming and occur singly or in chains. Endospores are ellipsoidal. After 2 days incubation on nutrient agar, colonies are 0.5– 1.0 mm in diameter, cream in colour, opaque, circular, smooth and convex. Grows at 15–42 °C (optimum, 30 °C) and pH 5–12 (optimum, pH 7–7.5). Tolerates up to 12 % NaCl. Growth does not occur under anaerobic conditions. Growth occurs on nutrient agar, but not on Simmons' citrate agar, cetrimide agar or MacConkey agar. Catalase- and oxidase-positive. Phenylalanine deaminase negative. In Hi25 (Hi-media, Mumbai) tests, positive for glucose, fructose, inositol, D-mannose, beta-galactosidase, lysine decarboxylase and Voges–Proskauer reaction. Negative for arginine dihydrolase, arginine decarboxylase, ornithine decarboxylase, indole and H_2S production, urease and nitrate reduction. Starch, DNA, tyrosine, Tween 20, aesculin and casein are hydrolysed, but Tween 80, gelatin and carboxymethyl cellulose are not. Acid is produced from trehalose, salicin and D-fructose. Utilizes D-xylose, D-fructose, D-mannose, trehalose, D-mannitol, L-arabinose and salicin, but not D-lactose, sucrose, D-galactose, D-glucose, maltose, melibiose, turanose, cellobiose, D-ribose, melezitose, raffinose, L-rhamnose, L-sorbose, adonitol, L-arabitol, i-erythritol, xylitol, D-sorbitol, inositol, dextrin, glycerol, acetate, gluconate, inulin, amygdalin, *N*-acetyl-D-glucosamine, pyruvate or methyl alpha-glucoside. The diagnostic diamino acid of the cell-wall peptidoglycan is meso-diaminopimelic acid and MK-7 is the predominant quinone. The cellular fatty acid profile consists of significant amounts of anteiso-$C_{15:0}$, iso-$C_{15:0}$, anteiso-$C_{17:0}$ and $C_{16:0}$. Major polar lipids are diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and an unidentified aminolipid. The DNA G + C content of the type strain is 42.6 mol%.

The type strain, NIO-1003^T (= NCIM 5458^T = DSM 25142^T), was isolated from a sediment sample taken from the Chorao Island in the Goa Province, India. The GenBank/EMBL/DDBJ accession number

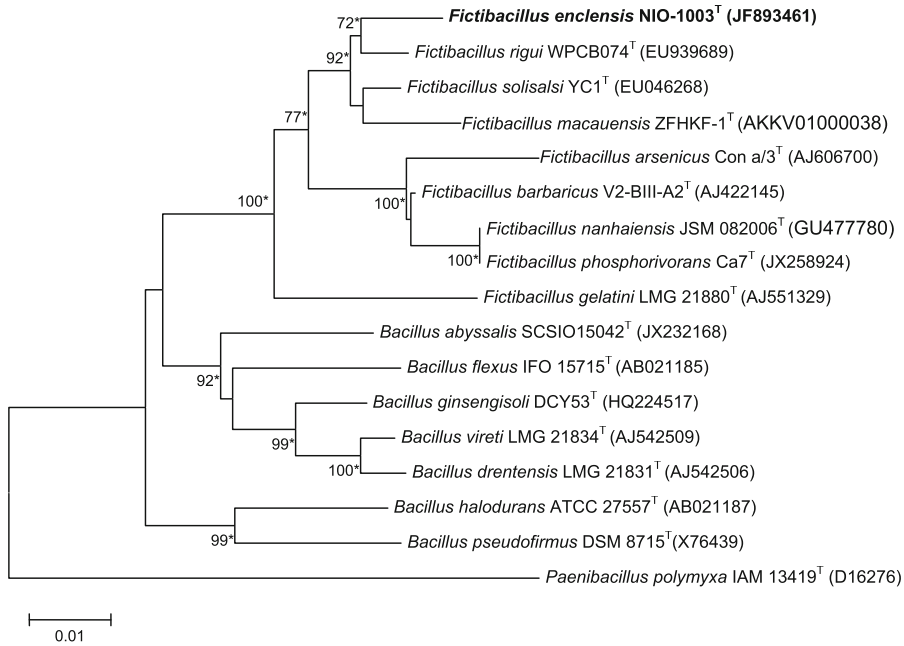

Fig. 1 Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain NIO-1003^T and related members of the genus *Fictibacillus*. *Bootstrap values* (>70 %; 1,000 resamplings) are given at branch points. *Astrikes* indicate that the corresponding nodes (groupings) are also recovered in Fitch–Margoliash, maximum-parsimony and maximum-likelihood trees. *Bar*, 0.01 nucleotide substitutions per position

for the 16S rRNA gene sequence of strain NIO-1003^T is JF893461.

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References

Ahmed I, Yokota A, Yamazoe A, Fujiwara T (2007) Proposal of *Lysinibacillus boronitolerans* gen. nov., sp. nov., and transfer of *Bacillus fusiformis* to *Lysinibacillus fusiformis* comb. nov. and *Bacillus sphaericus* to *Lysinibacillus sphaericus* comb. nov. Int J Syst Evol Microbiol 57:1117–1125

Albert RA, Archambault J, Lempa M, Hurst B, Richardson C, Gruenloh S, Duran M, Worliczek HL, Huber BE et al (2007) Proposal of *Viridibacillus* gen. nov. and reclassification of *Bacillus arvi*, *Bacillus arenosi* and *Bacillus neidei* as *Viridibacillus arvi* gen. nov., comb. nov., *Viridibacillus arenosi* comb. nov. and *Viridibacillus neidei* comb. nov. Int J Syst Evol Microbiol 57:2729–2737

Ash C, Priest FG, Collins MD (1993) Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Proposal for the creation of a new genus *Paenibacillus*. Antonie Van Leeuwenhoek 64:253–260

Baik KS, Lim CH, Park SC, Kim EM, Rhee MS, Seong CN (2010) *Bacillus rigui* sp. nov., isolated from wetland freshwater. Int J Syst Evol Microbiol 60:2204–2209

Claus D, Berkeley RCW (1986). Genus *Bacillus* Cohn 1872. In Bergey's Manual of Systematic Bacteriology, vol. 2, pp. 1105–1140. Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: Williams & Wilkins

Felsenstein J (1981) Evolutionary trees from DNA sequences: a maximum likelihood approach. J Mol Evol 17:368–376

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791

Fitch WM (1971) Toward defining the course of evolution: minimum change for a specified tree topology. Syst Zool 20(4):406–416

Fortina MG, Pukall R, Schumann P, Mora D, Parini C, Manachini PL, Stackebrandt E et al (2001) Ureibacillus gen. nov., a new genus to accommodate *Bacillus thermosphaericus* (Andersson,1995), emendation of *Ureibacillus thermosphaericus* and description of *Ureibacillus terrenus* sp. nov. Int J Syst Evol Microbiol 51:447–455

Gillis M, De Ley J, De Cleene M (1970) The determination of molecular weight of bacterial genome DNA from renaturation rates. Eur J Biochem 12:143–153

Glaeser SP, Wolfgang D, Busse H-J, Kämpfer P (2013) *Fictibacillus phosphorivorans* gen. nov., sp. nov. and proposal to reclassify *Bacillus arsenicus*, *Bacillus barbaricus*, *Bacillus macauensis*, *Bacillus nanhaiensis*, *Bacillus rigui*, *Bacillus solisalsi* and *Bacillus gelatini* in the genus *Fictibacillus*. Int J Syst Evol Microbiol 63:2934–2944

Hatayama K, Shoun H, Ueda Y, Nakamura A (2006) *Tuberibacillus calidus* gen. nov., sp. nov., isolated from a compost pile and reclassification of *Bacillus naganoensis* Tomimura, 1990 as *Pullulanibacillus naganoensis* gen. nov., comb. nov. and *Bacillus laevolacticus* Andersch et al. 1994 as *Sporolactobacillus laevolacticus* comb. nov. Int J Syst Evol Microbiol 56:2545–2551

Hayakawa M, Nonomura H (1987) Humic acid-vitamin agar, a new medium for selective isolation of soil actinomycetes. J Ferment Technol 65:501–509

Heyndrickx M, Lebbe L, Kersters K, De Vos P, Forsyth G, Logan NA (1998) *Virgibacillus*: a new genus to accommodate *Bacillus pantothenticus* (Proom and Knight 1950). Emended description of *Virgibacillus pantothenticus*. Int J Syst Bacteriol 48:99–106

Huss VAR, Festl H, Schleifer KH (1983) Studies on the spectrophotometric determination of DNA hybridization from renaturation rates. Syst Appl Microbiol 4:184–192

Jeon CO, Lim JM, Lee JM, Xu LH, Jiang CL, Kim CJ (2005) Reclassification of *Bacillus haloalkaliphilus* Fritze 1996 as *Alkalibacillus haloalkaliphilus* gen. nov., comb. nov. and the description of *Alkalibacillus salilacus* sp. nov., a novel halophilic bacterium isolated from a salt lake in China. Int J Syst Evol Microbiol 55:1891–1896

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16(2):111–120

Kroppenstedt RM (1982) Separation of bacterial menaquinones by HPLC using reverse phase (RP-18) and a silver-loaded ion exchanger. J Liq Chromatogr 5:2359–2367

Leifson E (1960) Atlas of Bacterial Flagellation. Academic Press, London

Ley De, Cattoir JH, Reynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

Li W-J, Xu P, Schumann P, Zhang Y-Q, Pukall R, Xu L-H, Stackebrandt E, Jiang C-L (2007) *Georgenia ruanii* sp. nov., a novel actinobacterium isolated from forest soil in Yunnan (China), and emended description of the genus *Georgenia*. Int J Syst Evol Microbiol 57:1424–1428

Liu H, Zhou Y, Liu R, Zhang K-Y, Lai R (2009) *Bacillus solisalsi* sp. nov., a halotolerant, halophilic bacterium isolated from soil around a salt lake. Int J Syst Evol Microbiol 59:1460–1464

Loveland-Curtze J, Vanya IM, Jean EB (2011) Evaluation of a new fluorimetric DNA–DNA hybridization method. Can J Microbiol 57:250–255

Marmur J (1961) A procedure for isolation of deoxyribonucleic acid from micro-organisms. J Mol Biol 3:208–218

Marmur J, Doty P (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J Mol Biol 5:109–118

Meier-Kolthoff JP, Göker M, Spröer C, Klenk HP (2013) When should a DDH experiment be mandatory in microbial taxonomy? Arch Microbiol 195:413–418

Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, Schaal K, Parlett JH (1984) An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J Microbiol Methods 2:233–241

Nazina TN, Tourova TP, Poltaraus AB, Novikova EV, Grigoryan AA, Ivanova AE, Lysenko AM, Petrunyaka VV, Osipov GA et al (2001) Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans*, *Bacillus kaustophilus*, *Bacillus thermoglucosidasius* and *Bacillus thermodenitrificans* to *Geobacillus* as the new combinations *G. stearothermophilus*, *G. thermocatenulatus*, *G. thermoleovorans*, *G. kaustophilus*, *G. thermoglucosidasius* and *G. thermodenitrificans*. Int J Syst Evol Microbiol 51:433–446

Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4:406–425

Sasser M (1990) Identification of bacteria by gas chromatography of cellular fatty acids. USFCC Newsl 20:1–6

Shida O, Takagi H, Kadowaki K, Komagata K (1996) Proposal for two new genera, *Brevibacillus* gen. nov. and *Aneurinibacillus* gen. nov. Int J Syst Bacteriol 46:939–946

Stackebrandt E, Ebers J (2006) Taxonomic parameters revisited: tarnished gold standards. Microbiol Today 33:152–155

Staneck JL, Roberts GD (1974) Simplified approach to identification of aerobic actinomycetes by thin-layer chromatography. Appl Microbiol 28:226–231

Tamaoka J, Komagata K (1984) Determination of DNA base composition by reversed-phase high-performance liquid chromatography. FEMS Microbiol Lett 25:125–128

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol Biol Evol 28(10):2731–2739

Täubel M, Kämpfer P, Buczolits S, Lubitz W, Busse H-J (2003) *Bacillus barbaricus* sp. nov., isolated from an experimental wall painting. Int J Syst Evol Microbiol 53:725–730

Tcherpakov M, Ben-Jacob E, Gutnick DL (1999) *Paenibacillus dendritiformis* sp. nov., proposal for a new pattern-forming species and its localization within a phylogenetic cluster. Int J Syst Bacteriol 49:239–246

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The clustal_x windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Vaishampayan P, Miyashita M, Ohnishi A, Satomi M, Rooney A, La Duc MT, Venkateswaran K et al (2009) Description of Rummeliibacillus stabekisii gen. nov., sp. nov. and reclassification of *Bacillus pycnus* Nakamura, 2002 as *Rummeliibacillus pycnus* comb. nov. Int J Syst Evol Microbiol 59:1094–1099

Wainø M, Tindall BJ, Schumann P, Ingvorsen K (1999) *Gracilibacillus* gen. nov., with description of *Gracilibacillus halotolerans* gen. nov., sp. nov.; transfer of *Bacillus dipsosauri* to *Gracilibacillus dipsosauri* comb. nov., and *Bacillus salexigens* to the genus *Salibacillus* gen. nov., as *Salibacillus salexigens* comb. nov. Int J Syst Bacteriol 49:821–831

Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, Krichevsky MI, Moore LH, Moore WEC, Murray RGE, Stackebrandt E, Starr MP, Trüper HG (1987) Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. Int J Syst Bacteriol 37:463–464

Wisotzkey JD, Jurtshuk P Jr, Fox GE, Deinhard G, Poralla K (1992) Comparative sequence analysis on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. Int J Syst Bacteriol 42:263–269

Yoon J-H, Yim DK, Lee J-S, Shin K-S, Sato HH, Lee ST, Park YK, Park Y-H (1998) *Paenibacillus campinasensis* sp. nov., a cyclodextrin-producing bacterium isolated in Brazil. Int J Syst Bacteriol 48:833–837

Yoon J-H, Weiss N, Lee K-C, Lee I-S, Kang KH, Park Y-H (2001a) *Jeotgalibacillus alimentarius* gen. nov., sp. nov., a novel bacterium isolated from jeotgal with L-lysine in the cell wall, and reclassification of *Bacillus marinus* Rüger 1983 as *Marinibacillus marinus* gen. nov., comb. nov. Int J Syst Evol Microbiol 51:2087–2093

Yoon JH, Lee KC, Weiss N, Kho YH, Kang KH, Park YH (2001b) *Sporosarcina aquimarina* sp. nov., a bacterium isolated from seawater in Korea, and transfer of *Bacillus globisporus* (Larkin and Stokes 1967), *Bacillus psychrophilus* (Nakamura 1984) and *Bacillus pasteurii* (Chester 1898) to the genus *Sporosarcina* as *Sporosarcina globispora* comb. nov., *Sporosarcina psychrophila* comb. nov. and *Sporosarcina pasteurii* comb. nov., and emended description of the genus *Sporosarcina*. Int J Syst Evol Microbiol 51:1079–1086

Yoon JH, Kang SJ, Oh TK et al (2007) Reclassification of *Marinococcus albus* Hao, 1985 as *Salimicrobium album* gen. nov., comb. nov. and *Bacillus halophilus* Ventosa et al. 1990 as *Salimicrobium halophilum* comb. nov., and description of *Salimicrobium luteum* sp. nov. Int J Syst Evol Microbiol 57:2406–2411