Paraclostridium benzoelyticum gen. nov., sp. nov., isolated from marine sediment and reclassification of Clostridium bifermentans as Paraclostridium bifermentans comb. nov. Proposal of a new genus Paeniclostridium gen. nov. to accommodate Clostridium sordellii and Clostridium ghonii

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Twenty-three rod-shaped, endospore-forming, Gram-stain-positive, obligately anaerobic bacteria were isolated from different marine sediment samples of Gujarat. All 23 strains shared 16S rRNA gene sequence similarity of ~100%. Strain JC272T was designated the type strain and shared highest sequence similarity with Clostridium bifermentans ATCC 638T (99.8%), Clostridium ghonii JCM 1400T (98.0%), Clostridium sordellii ATCC 9714T (97.9%) and other members of the genus Clostridium (<96.4%). C16 : 0, C18 : 0, C17 : 0, C16 : 1ω9c and iso-C15 : 0 were the major (>5%) fatty acids. Strain JC272T contained diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and two unidentified amino lipids. Genome-based analysis of average nucleotide identity (ANI) and in silico DNA–DNA hybridization (DDH) of strain JC272T with C. bifermentans ATCC 638T yielded values of 94.35 and 58.5 ± 2.8%, respectively. The DNA G+C content of strain JC272T was 28.3 mol%. Strain JC272T together with C. bifermentans were found to fall outside Clostridium rRNA cluster I considered as Clostridium senso stricto. Based on ANI value, in-silico DDH, and distinct morphological and physiological differences from the previously described taxa, we suggest that strain JC272T represents a novel species of a new genus in the family Clostridiaceae, for which the name Paraclostridium benzoelyticum gen. nov., sp. nov. is proposed. The type strain is JC272T (=KCTC 15476T=LMG 28745T). It is also proposed to transfer C. bifermentans to this new genus, as Paraclostridium bifermentans comb. nov. (type strain ATCC 638T=DSM 14991T=JCM 1386T). The genus Paeniclostridium gen. nov. is proposed to accommodate C. sordellii and C. ghonii as Paeniclostridium sordellii comb. nov. (type strain ATCC 9714T=LMG 15708T=JCM 3814T) and Paeniclostridium ghonii comb. nov. (type strain ATCC 25757T=DSM 15049T=JCM 1400T).

In an attempt to discover antibiotics from obligately anaerobic bacteria, we have isolated a large number of these organisms from marine habitats of India which belong to the genus Clostridium. Based on 16S rRNA gene sequence analysis, the majority of these strains shared highest 16S rRNA gene sequence similarity (>99%) with Clostridium bifermentans ATCC 638T. C. bifermentans and Clostridium sordellii are phylogenetically closely related (Nisida et al., 1964; Brooks et al., 1969). Although the type strain C. bifermentans ATCC…

Abbreviations: ANI, average nucleotide identity; DDH, DNA–DNA hybridization; ME, minimum-evolution; ML, maximum-likelihood; NJ, neighbour-joining.

The GenBank/EMBL accession numbers for the 16S rRNA gene sequence and the draft genome sequence of strain JC272T are LN846800 and LBBT00000000 (Version LBBT01000000), respectively.

Two supplementary figures and four supplementary tables are available with the online Supplementary Material.
638T is reported as being non-pathogenic, many still consider some strains of *C. bifermentans* as pathogens (Wong et al., 2014) and most strains of *C. sordellii* are pathogens (Aldape et al., 2006). A re-examination of pathogenic *C. bifermentans* is required to determine the taxonomic affiliation of this species. Furthermore, many strains of *C. bifermentans* have been exploited for the production of industrially important biomolecules (Myszka et al., 2012; Leja et al., 2013) but the production of antimicrobials has not usually been reported. Thus, we have critically looked into the taxonomic status of the new isolates which are capable of producing antimicrobials. The present communication discusses only the taxonomic status of these bacteria and not the nature of the antimicrobial compounds produced by them. Nevertheless, antimicrobials from a few members of the genus *Clostridium* are well documented (Barber et al., 1979; Gebhart et al., 2012). While reclassifying several species of the multiphyletic genus *Clostridium* into five new genera, Gerritsen et al. (2014) also suggested reclassification of the closely related *C. bifermentans, C. ghonii* and *Eubacterium tenue* into a separate novel genus within the family Peptostreptococcaceae. The genus *Clostridium* is thus restricted to the type species *Clostridium butyricum* and clade I species (Lawson & Rainey, 2015). We propose representative strain JC272T as the type strain of a novel species of a new genus in the family Clostidiaceae as strain JC272T together with *C. bifermentans* forms a distinct 16S rRNA gene clade from species of the genus *Clostridium sensu stricto*. We also propose to reclassify *C. sordellii* and *C. ghonii* within a new genus, which has support from protein signatures and genome-based data analysis.

Sediment and soil samples from different marine habitats of Gujarat (Table S1, available in the online Supplementary Material) collected during December 2013 were pasteurized for 1 h at 80 °C and plated on reinforced clostridial medium (HiMedia M443, with 2 % NaCl) agar slants through a crowded plating technique (Waksman, 1945; Myszka et al., 2012; Leja et al., 2013) but the production of antimicrobials has not usually been reported. Thus, we have critically looked into the taxonomic status of the new isolates which are capable of producing antimicrobials. The present communication discusses only the taxonomic status of these bacteria and not the nature of the antimicrobial compounds produced by them. Nevertheless, antimicrobials from a few members of the genus *Clostridium* are well documented (Barber et al., 1979; Gebhart et al., 2012). While reclassifying several species of the multiphyletic genus *Clostridium* into five new genera, Gerritsen et al. (2014) also suggested reclassification of the closely related *C. bifermentans, C. ghonii* and *Eubacterium tenue* into a separate novel genus within the family Peptostreptococcaceae. The genus *Clostridium* is thus restricted to the type species *Clostridium butyricum* and clade I species (Lawson & Rainey, 2015). We propose representative strain JC272T as the type strain of a novel species of a new genus in the family Clostidiaceae as strain JC272T together with *C. bifermentans* forms a distinct 16S rRNA gene clade from species of the genus *Clostridium sensu stricto*. We also propose to reclassify *C. sordellii* and *C. ghonii* within a new genus, which has support from protein signatures and genome-based data analysis.

Cell material for 16S rRNA gene sequencing was taken from 23 colonies grown anaerobically. DNA was extracted and purified by using a Qiagen genomic DNA extraction kit. Recombinant *Taq* polymerase (Genei) was used for PCR, and 16S rRNA gene sequencing was carried out as previously described (Lakshmi et al., 2011). A BLAST search analysis of 16S rRNA gene sequences (1360–1490 bp) was performed using the EzTaxon-e server (Kim et al., 2012). All 23 strains showed highest sequence similarity with *C. bifermentans* ATCC 638T (99.8 %), *C. ghonii* JCM 1400T (98.0 %), *C. sordellii* ATCC 9714T (97.9 %) and other members of the genus *Clostridium* (<96.4 %). 16S rRNA gene sequences of all 23 strains were aligned using the SILVA incremental aligner (SINA; http://www.arb-silva.de), and MEGA 6 (Tamura et al., 2013) software was used for phylogenetic analyses. Distances were calculated by using the Kimura two-parameter model (Kimura, 1980) in a pairwise deletion procedure. Neighbour-joining (NJ), minimum-evolution (ME) and maximum-likelihood (ML) methods in the MEGA 6 software were used to reconstruct phylogenetic trees and a combined phylogenetic tree (NJ, ME, ML) confirmed the clustering of the 23 strains with *C. bifermentans* ATCC 638T (Fig. 1). Among the 23 strains, we designated strain JC272T as a representative for detailed taxonomic characterization and compared it with *C. bifermentans* JCM 1386T (= ATCC 638T) and *C. ghonii* JCM 1400T (= ATCC 25757T) in our laboratory. However, because *C. sordellii* ATCC 9714T (16S rRNA gene sequence similarity >97 %) is pathogenic and subject to restricted culture exchange, data for this strain were taken from the literature (Nisida et al., 1964).

Genomic DNA was extracted and purified according to the method of Marmur (1961) and the G + C content of the DNA was determined for all 23 strains by reversed-phase HPLC (Mesbah et al., 1989). The G + C content of the 23 strains was between 29.8 and 30.5 mol% (strain JC272T 30.2 mol%). The taxonomic relationship between strain JC272T and its most closely related members of the genus *Clostridium* was examined using DNA–DNA hybridization (DDH), which was achieved using a membrane filter technique (Tourova & Antonov, 1988), using a Nick translation kit supplied by BRIT, at the Jonaki Laboratory, CCMB campus, Hyderabad. Hybridization was performed using three replications for each sample (control: reversal of strains was used for binding and labelling). Control in each of the experiments was the self-hybridized DNA. x-P32dCTP was used for labelling the probe. The DNA immobilized on the blots (nylon membranes) was probed with labelled DNA and then exposed to a phosphor imaging screen (Amersham Biosciences). The phosphor imaging screen was scanned and quantified using a TYPHOON (3480) variable mode imager. The per cent hybridization was calculated according to the formula: % hybridization = (counts obtained from heterologous hybridization/counts obtained from homologous hybridization) × 100. When strain JC272T was radioactively labelled, mean levels of DNA–DNA reassociation with *C. bifermentans* JCM 1386T and *C. ghonii* JCM 25757T were 56 ± 1 and 21 ± 2 %, respectively. However, when *C. bifermentans* JCM 1386T and *C. ghonii* JCM 25757T were labelled and used for DDH with JC272T in the reciprocal reaction, the reassociation values were 58 ± 2 and 20 ± 2 %, respectively. Based on the hybridization results, strain JC272T thus represents a novel species according to recommendations for delineating a bacterial species (Wayne et al., 1987; Stackebrandt & Goebel, 1994).

Genome sequence-based comparison was performed to further distinguish strain JC272T from *C. bifermentans*
JCM 1386\textsuperscript{T}. Sequencing of the whole genome of strain JC272\textsuperscript{T} was performed (Tushar \textit{et al.}, 2015) and determination of average nucleotide identity (ANI; Goris \textit{et al.}, 2007) and in-silico DDH (Auch \textit{et al.}, 2010; Meier-Kolthoff \textit{et al.}, 2013) were performed by using the Kostas lab server (http://enve-omics.ce.gatech.edu/ani/) and genome to genome distance calculator (http://ggdc.dsmz.de), respectively, and used for species identification. ANI values between strain JC272\textsuperscript{T} and \textit{C. bifermentans} ATCC 638\textsuperscript{T} and \textit{C. sordellii} ATCC 9714\textsuperscript{T} were 94.03 and 82.77 \%, respectively, which are below the species cut-off (95–96 \%). ANI between \textit{C. bifermentans} ATCC 638\textsuperscript{T} and \textit{C. sordellii} ATCC 9714\textsuperscript{T} was 82.61 \%. Mean genome to genome distance between JC272\textsuperscript{T} and \textit{C. bifermentans} ATCC 638\textsuperscript{T} and \textit{C. sordellii} ATCC 9714\textsuperscript{T} was 58.50 ± 2.8 and 25.1 ± 2.4 \% (in-silico DDH), respectively, which is also lower than the species cut-off (70 \%). The calculated in-silico DDH value (58.50 ± 2.8 \%) between JC272\textsuperscript{T} and \textit{C. bifermentans} ATCC 638\textsuperscript{T} was very close to the experimental value (~57 \%). The genome to genome distance between \textit{C. bifermentans} ATCC 638\textsuperscript{T} and \textit{C. sordellii} ATCC 9714\textsuperscript{T} was 24.4 ± 2.4 \%. Based on ANI and in-silico DDH values, strain JC272\textsuperscript{T}, \textit{C. bifermentans} ATCC 638\textsuperscript{T} and \textit{C. sordellii} ATCC 9714\textsuperscript{T} are distinct from each other at the species level.

For all routine culturing and experiments, unless mentioned otherwise the growth medium as mentioned above.

**Fig. 1.** NJ tree based on 16S rRNA gene sequences showing the phylogenetic relationships between strain JC272\textsuperscript{T} and other closely related members of the genus \textit{Clostridium}. The tree was computed with MEGA6 software (Tamura \textit{et al.}, 2013) and rooted by using \textit{Bacillus subtilis} DSM 10\textsuperscript{T} as the outgroup. Numbers at nodes represent bootstrap values. The GenBank accession numbers for 16S rRNA gene sequences are shown in parentheses. Bar, 0.02 nt substitutions per position. Nodes that were obtained by all treeing methods (NJ, ME and ML) are represented by filled circles and open circles represent nodes that were recovered by the NJ and ME methods. GenBank accession numbers for the 16S rRNA gene sequences of the 22 strains in the clade with strain JC272\textsuperscript{T} are given in Table S1.
was used. Morphological properties such as cell shape, cell size and motility were observed by phase-contrast light microscopy (Leica DM2500). Salinity, pH and temperature ranges for growth were examined in liquid medium. Growth on organic substrates (D-glucose, D-galactose, D-fructose, sucrose, D-ribose, D-rhamnose, lactose, D-mannitol, D-sorbitol, L-arabinose, cellulose, cellobiose, maltose, starch, pyruvate, L-glutamate, crotonate, benzoate, caprate, fumarate, glycerol, malate, glycollate, butyrate and succinate) was tested in a minimal medium described by Subhash et al. (2013). Vitamin requirements were tested by replacing yeast extract with single and also combinations of vitamins as growth factors. Amino acid fermentation was tested in a medium containing (g l\(^{-1}\)) KH\(_2\)PO\(_4\) (4.5), NH\(_4\)Cl (0.5), MgSO\(_4\) · 7H\(_2\)O (0.1), CaCl\(_2\) · 2H\(_2\)O (0.05), ZnSO\(_4\) · 7H\(_2\)O (0.002), FeSO\(_4\) · 7H\(_2\)O (0.009) and vitamin B\(_12\) (100 μg l\(^{-1}\)), and one amino acid each was added at 0.1 % (w/v). Various biochemical tests (starch/gelatin hydrolysis, H\(_2\)S production, nitrate reduction, indole production, citrate utilization, and caseinase, lipase, oxidase and catalase activities) were carried out in the prescribed media to meet the requirements of the standard methods as described previously (Cappuccino & Sherman, 1999; Shivani et al., 2015). Polar lipids and fatty acids were analysed as described previously (Subhash et al., 2013; Shivani et al., 2015).

Cells of all 23 strains were rod-shaped, similar in length and width, and formed pseudo-filaments. Cells of strain JC272\(^T\) were 0.5–0.9 μm in width and 2–4 μm in length while those of C. bifermentans JCM 1386\(^T\) were 0.5 μm in width and 1.5–2 μm in length and did not form pseudo-filaments (Fig. S1). Cells of strain JC272\(^T\) also were distinct from those of C. ghonii JCM 25757\(^T\), which were much longer (0.5–1.5 μm in width and 2–10 μm in length) and did not form pseudo-filaments (Fig. S1). All 23 strains were Gram-stain-positive and produced terminal endospores. Growth of strain JC272\(^T\) occurred at pH 6–8 and with optimum growth at pH 7–8 (Table 1). Strain JC272\(^T\) did not require NaCl for growth, tolerated up to 2 % (w/v) NaCl and grew optimally with 0–1 % NaCl. Optimum growth occurred at 30–35 °C, with a growth range of 25–40 °C. Strain JC272\(^T\) showed negative reactions for catalase, oxidase and indole production and positive reactions for H\(_2\)S production.

### Table 1. Differential characteristics between strain JC272\(^T\) and its closest relatives

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (μm)</td>
<td>0.5–0.9 × 2–4</td>
<td>0.5 × 1.5–2</td>
<td>0.5–1.5 × 2–10</td>
<td>0.5–0.8 × 4.9–20.0</td>
</tr>
<tr>
<td>Optimum (range) pH</td>
<td>7–8 (6–8)</td>
<td>6–7 (6–8.5)</td>
<td>6–7 (6–8.5)</td>
<td>6–7 (6–8.5)</td>
</tr>
<tr>
<td>Optimum NaCl (range) (%)</td>
<td>0–1 (0–2)</td>
<td>0–1 (0–3)</td>
<td>0–1 (0–4)</td>
<td></td>
</tr>
<tr>
<td>H(_2)S production</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Indole production from L-tryptophan</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
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<tr>
<td>NO(_3) reduction</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td></td>
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<tr>
<td>Hydrolysis of:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Starch</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Antimicrobials produced</td>
<td>+</td>
<td>(+)</td>
<td>–</td>
<td>NR</td>
</tr>
<tr>
<td>Organic substrate utilized for growth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Mannitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Starch</td>
<td>+</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Cellobiose</td>
<td>+</td>
<td>–</td>
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</tr>
<tr>
<td>D-Mannose</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Benzoate</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>NR</td>
</tr>
<tr>
<td>Fumarate</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>NR</td>
</tr>
<tr>
<td>DNA G+C content (%)</td>
<td>29.2 (+28.3)</td>
<td>28.5 (+28.4)</td>
<td>30.2</td>
<td>NR</td>
</tr>
</tbody>
</table>

*Calculated DNA G+C content based on draft genome sequence.
gelatin hydrolysis, nitrate reduction and starch hydrolysis. Growth of strain JC272<sup>T</sup> was dependent on yeast extract, while addition of vitamins to the media in place of yeast extract in the medium did not support growth.

Strain JC272<sup>T</sup> was an obligate chemo-organoheterotroph [anaerobically with D-glucose (0.3 %, w/v) as carbon source/electron donor]. Substrates utilized as carbon sources/electron donors under chemo-organoheterotrophic conditions included D-glucose, ascorbate, butyrate, fumarate, starch, benzoate, maltose, cellobiose, D-fructose, bicarbonate, D-sorbitol, D-mannitol, L-arabinose and D-caproate. Those which could not be utilized by strain JC272<sup>T</sup> included sucrose, lactose, lactate, succinate, ethanol and ribose. Benzoate supported growth of all 23 strains but it did not support growth of <i>C. bifermentans</i> JCM 1386<sup>o</sup> or <i>C. ghonii</i> JCM 1400<sup>T</sup>. Benzoyl-CoA reductase (three subunits), required for the degradation of benzoate, was present in the genomes of strain JC272<sup>T</sup> and <i>C. bifermentans</i> JCM 1386<sup>o</sup>. Other genes related to benzoate degradation could not be observed in the draft genomes of either strain. Chemolithoautotrophic growth [anaerobically with HCO<sub>3</sub> as carbon source; Na<sub>2</sub>S . 9H<sub>2</sub>O as electron donor] could not be demonstrated. Ammonium chloride, nitrate, glutamate, glutamate and urea were used as nitrogen sources for growth while nitrite was not utilized. Amino acids arginine, proline, and urea were used as nitrogen sources for growth while glutamine, glutamate, aspartate, and cysteine were not utilized/fermented.

Strain JC272<sup>T</sup> was resistant (μg ml<sup>−1</sup>) to kanamycin (100), fosfomycin (100), rifampicin (100), polymyxin-B (100), nalidixic acid (100), gentamicin (50) and tetracycline (100) but sensitive to ampicillin (100), chloramphenicol (50), penicillin (50), erythromycin (100) and metronidazole (100). C<sub>16 : 0</sub> was the major (31.3 %) fatty acid, with C<sub>18 : 0</sub> C<sub>17 : 0</sub> C<sub>16 : 1</sub> 9c and iso-C<sub>16 : 0</sub> as minor (5–10 %) fatty acids (Table S2). Diphosphatidyglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and two unidentified amino lipids were present in strain JC272<sup>T</sup> (Fig. S2). Haploids were extracted as described by Tushar <i>et al.</i> (2014) and analysed by GC-MS. None of the strains (23 newly isolated strains, <i>C. bifermentans</i> JCM 1386<sup>o</sup> and <i>C. ghonii</i> JCM 1400<sup>T</sup>) showed the presence of detectable haploids. 

<i>C. bifermentans</i> JCM 1386<sup>o</sup>, <i>C. ghonii</i> JCM 1400<sup>T</sup> and the 23 novel strains were harvested in the exponential-phase of growth by centrifugation and the supernatant was evaporated to dryness. The dried supernatant was twice extracted with methanol and used for antimicrobial activity against <i>Bacterium subtilis</i> JC14, <i>Staphylococcus aureus</i> JC114 and <i>Escherichia coli</i> JC 260 according to the protocol of Zhang <i>et al.</i> (2009). Extract from all 23 strains revealed a zone of inhibition against <i>B. subtilis</i> JC14, <i>S. aureus</i> JC114 and <i>E. coli</i> JC260. By contrast, methanolic extract of <i>C. bifermentans</i> JCM 1386<sup>o</sup> showed a poor zone of inhibition and <i>C. ghonii</i> JCM 1400<sup>T</sup> showed no antimicrobial activity. Strain JC272<sup>T</sup> was similar to its nearest phylogenetic neighbours (<i>C. bifermentans</i> JCM 1386<sup>o</sup> and <i>C. ghonii</i> JCM 1400<sup>T</sup>) with respect to the following characteristics: obligate anaerobic growth; motility; methyl red/Voges–Proskauer negative; citrate not utilized; catalase- and oxidase-negative; urease-negative; casein and lipid are hydrolysed; phosphate is not solubilized; organic substrates such as D-glucose, D-fructose, malate, glyceral, butyrate, fumarate, glycolate and ascorbate are utilized; succinate, D-ribose, lactose and sucrose are not utilized; and optimum salinity and pH. However, strain JC272<sup>T</sup> differed from <i>C. bifermentans</i> JCM 1386<sup>o</sup> and <i>C. ghonii</i> JCM 1400<sup>T</sup> with regard to cell morphology (Fig. S1), H<sub>2</sub>S production, indole production, NO<sub>3</sub> reduction, hydrolysis of starch and gelatin, benzoate utilization (Table 1) and polar lipid composition (Fig. S2). The phenotypic differences are supported by genomic differences based on DDH values (experimental and genome-based <i>in-silico</i> analysis) and ANI scores, suggesting that strain JC272<sup>T</sup> represents a novel species. However, phylogenetic analysis (Fig. 1) indicated that the 16S rRNA gene sequence of strain JC272<sup>T</sup> and the type strains of <i>C. bifermentans</i>, <i>C. ghonii</i> and <i>Pae-
phosphatidylethanolamine, phosphatidylcholine and un-
identified amino lipids as the polar lipids. Antimicrobials are produced.

The type species is *Paraclostridium bifermentans.*

**Description of Paraclostridium bifermentans comb. nov.**

**Basonym:** *Clostridium bifermentans* (Weinberg & Séguin, 1918).

The description of *Paraclostridium bifermentans* is identical to that of *Clostridium bifermentans* (Weinberg & Séguin, 1918) except for the following modifications. *C*16 : 0 is the major fatty acid and *C*18 : 1ω7c and *C*18 : 0 are the minor fatty acids. Q-10 is the major quinone. Diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, phosphatidylglycerol, an unidentified aminolipid, an unidentified phospholipid, an unidentified glycolipid and five unidentified lipids are the polar lipids.

**Description of Paraclostridium benzoelyticum sp. nov.**

*Paraclostridium benzoelyticum* [N.L. n. *benzo* (from Arabic *luban dschawi*) benzoic resin; N.L. adj. *lyticum* (from Gr. adj. *lytikos*) dissolving; N.L. neut. adj. *benzoelyticum* degrading benzoate].

Has the following characteristics in addition to those given for the genus. Cells are 0.5–0.9 μm wide and 2–4 μm long, and produce terminal endospores. Obligately anaerobic. Indole is not produced from L-tryptophan. Growth occurs at pH 6.0–8.0 (optimum 7.0–8.0). Tolerates up to 2 % (w/v) NaCl with optimum growth with 0.1 % NaCl. Optimum growth occurs at 30–35 °C (range 25–40 °C). D-Mannitol, cellobiose, cellulose, starch, D-mannose, D-sorbitol, D-mannitol, fumarate and benzoate are utilized for growth. Growth is not possible with sucrose, lactose, lactate, succinate, ethanol or ribose. Traces of yeast extract are required for growth. Added vitamins are not essential for growth. Resistant to kanamycin, fosfomycin, rifampicin, polymyxin-B, nalidixic acid, gentamicin and tetracycline. Sensitive to ampicillin, erythromycin and metronidazole. *C*16 : 0 is the major fatty acid with minor amounts of *C*19 : 0, *C*17 : 0, *C*16 : 1ω9c and *iso*-*C*16 : 0. Diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine and two unidentified amino lipids are the polar lipids. Antimicrobials are produced.

The type strain is JC272T (＝KCTC 15476T=LMG 28745T). The calculated DNA G+C content of the type strain is 28.3 mol% (based on the draft genome sequence). The novel species is based on the analysis of 23 strains.

**Description of Paeniclostridium sordellii comb. nov.**

The properties of *Paeniclostridium sordellii* are as given for *Clostridium sordellii* (Hall & Scott, 1927; Rainey et al., 2009).

**References**


Cells are Gram-stain-positive, rod-shaped, mesophilic and produce endospores. Cells are motile and multiply by binary fission. Catalase- and oxidase-negative. Obligate anaerobes that grow on a number of organic substrates. Growth factors and NaCl are not required for growth. The fatty acid *C*19 : 0 cyclo ω8c is present in minor amounts together with other *C*12 to *C*18 branched and unbranched fatty acids. Five phospholipids (diphosphatidylglycerol, phosphatidylglycerol, phosphotidylcholine, phosphatidylethanolamine and phosphatidylinositol) and two amino lipids (unidentified) are present. Some species are pathogens.


Leja, K., Myszka, K. & Czaczky, K. (2013). The ability of Clostridium bifermentans strains to lactic acid biosynthesis in various environmental conditions. Springerplus 2, 44.


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