**Domibacillus enclensis** sp. nov., isolated from marine sediment, and emended description of the genus *Domibacillus*

Vidya V. Sonalkar,¹ Rahul Mawlankar,¹ Srinivasan Krishnamurthi,² Shan-Kun Tang³ and Syed G. Dastager¹

¹NCIM-Resource Center, CSIR-National Chemical Laboratory, Pune-411008, Maharashtra, India
²Microbial Type Culture Collection and Gene Bank (MTCC), CSIR-Institute of Microbial Technology, Sector-39A, Chandigarh-160036
³Key Laboratory of Microbial Diversity in Southwest China, Ministry of Education and Laboratory for Conservation and Utilization of Bio-resources, Yunnan Institute of Microbiology, Yunnan University, Kunming, Yunnan, 650091, PR China

A novel red-pigmented bacterial strain, designated NIO-1016ᵀ, was isolated from a sediment sample from Chorao Island, India and was investigated by a polyphasic taxonomic approach. The strain was Gram-reaction-positive, strictly aerobic, motile and catalase-positive and produced spherical to slightly ellipsoidal endospores with subterminal position on swollen sporangia. The genomic DNA G+C content was 46.9 mol%. The major fatty acids were anteiso-C₁₅ : 0, anteiso-C₁₇ : 0, iso-C₁₅ : 0 and C₁₆ : 0. The predominant quinones were MK-6 (89.3 %) and MK-7 (8.7 %). The polar lipids consisted of diphosphatidylglycerol, phosphatidylglycerol, and an unidentified phospholipid. *meso*-Diaminopimelic acid (type A₁γ) was present in the cell-wall peptidoglycan and the major whole-cell sugars were glucose and ribose. The closest phylogenetic neighbours were identified as *Domibacillus robiginosus* DSM 25058ᵀ (98.0 % similarity) and *Domibacillus indicus* DSM 28032ᵀ (97.2 % similarity), other species of the genus *Bacillus* displayed below 96 % similarity. Phylogenetic, physiological, biochemical and morphological differences between strain NIO-1016ᵀ and its closest neighbours indicate that this strain represents a novel species in the genus *Domibacillus* in the family *Bacillaceae* for which the name *Domibacillus enclensis* sp. nov. is proposed with the type species NIO-1016ᵀ (≡DSM 25145ᵀ≡NCIM 5462ᵀ≡CCTCC AB 2011121ᵀ).

The genus *Bacillus* comprises a phylogenetically and phenotypically heterogeneous group of species. Recently, the systematics of the bacillus group has been greatly modified. A novel genus of the bacillus family, *Domibacillus*, was described by Seiler et al. (2013) for a bacterium that forms red-pigmented colonies and was isolated from a pharmaceutical clean room in eastern Germany, based on its morphological, chemotaxonomic and phylogenetic differences from closely related members of the genera *Bacillus*, *Jeotgalibacillus* and *Planococcus*. At the time of writing, two species, *Domibacillus robiginosus* (Seiler et al., 2013) and *Domibacillus indicus* (Sharma et al., 2014) with validly published names are included in the genus *Domibacillus*. Members of the genus *Domibacillus* are Gram-stain-positive, spore-forming, oxidative, motile and strictly aerobic rods. The presence of MK-6 as the dominant quinone is one of the characteristic features of the genus. In the current study we propose that strain NIO-1016ᵀ represents a novel species of the genus *Domibacillus*, and have determined its taxonomic position by using a polyphasic approach.

Strain NIO-1016ᵀ was isolated from a marine sediment sample taken from Chorao Island. After primary isolation and purification on marine agar 2216 (Difco) at 28 °C for 2 weeks, the purified strain was subcultured on the same medium and stored as slants at 4 °C and as 20 % (v/v) glycerol suspensions at −70 °C. Biomass for chemical and molecular studies was obtained by cultivation in shake flasks (shaken at about 140 r.p.m.) using trypticase soy broth (TSB, Hi-Media, Mumbai) medium at 28 °C for 1 week. Gram staining was carried out by using the standard Gram reaction; a non-staining method was used to determine the Gram reactions (Buck, 1982) and cell motility was confirmed by the development of turbidity.
throughout a tube containing semisolid medium (Leifson, 1960). Morphological and physiological characterizations were performed. Cells were grown on marine agar (pH 7.0) at 28 °C for 48 h. Cell morphology and surface ornamentation was observed by light microscopy and scanning electron microscopy. Oxidase reagent (bioMérieux) was used for testing oxidase activity and catalase activity was determined by bubble formation in a 3% (v/v) H2O2 solution. Semisolid agar (motility test medium; marine agar) was used to examine motility (Tittsler & Sandholzer, 1936). In order to determine the temperature range for growth, cells were grown in MA broth at 4, 15, 25, 30, 37, 45 and 50 °C for 72 h. Growth at various NaCl concentrations (0, 1, 3, 5, 7, 10, 12 and 15%, w/v) was determined at 28 °C in broth medium that contained all of the constituents of marine agar, except NaCl, supplemented with appropriate concentrations of NaCl. The pH range for growth was determined from pH 4.0 to 12.0 (at intervals of 1.0 pH unit) using the buffer system described by Xu et al. (2005) in marine agar broth. The strain was characterized biochemically using the API CH50 and API ZYM systems (bioMérieux) as recommended by the manufacturer. Nutrient plates were used to examine hydrolysis of starch and Tweens 20, 40, 60 and 80 (final concentration of 1%, v/v).

Extraction of genomic DNA, PCR amplification and sequencing of the 16S rRNA gene from strain NIO-1016T were 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 an approximate phylogenetic affiliation. Multiple alignments with sequences of the most closely related species of the genus bacillus group and calculations of levels of sequence similarity were carried out using CLUSTAL_X (Thompson et al., 1997). Phylogenetic trees were reconstructed using the neighbour-joining (Saitou & Nei, 1987), maximum-parsimony (Fitch, 1971) and maximum-likelihood (Felsenstein, 1981) algorithms with Kimura’s two-parameter calculation model (Kimura, 1980) implemented in the program MEGA version 5.0 (Tamura et al., 2011). The resultant tree topologies were evaluated by bootstrap analysis based on 1000 replicates (Felsenstein, 1985). Results of phylogenetic analysis (Fig. 1) indicated that strain NIO-1016T grouped with members of the genus Domibacillus, i.e. D. robiginosus DSM 25058T (98.0%) and D. indicus DSM 28032T (97.2%). Very similar tree topologies were obtained using the other algorithms.

DNA–DNA hybridizations (DDH) play a key role in microbial species discrimination in cases when 16S rRNA gene sequence is insufficient. Depending on the investigated taxonomic group, the threshold value has been increased to between 98.2 and 99.0% and as recommended by Stackebrandt & Ebers (2006) and Meier-Kolthoff et al. (2013) and this appears reasonable. The nearest neighbours of strain NIO-1016T are noted to share less than ≤98.0% 16S rRNA gene sequence similarity and so DNA–DNA hybridization was not carried out in the present study. For the determination of the DNA G+C content, genomic DNA of strain NIO-1016T was prepared according to the method of Marmur (1961). The G+C content of the DNA was determined by using reversed-phase HPLC (Mesbah et al., 1989).

Whole-cell sugars were analysed according to the procedures developed by Hasegawa et al. (1983). Polar lipids were extracted, examined by using two-dimensional TLC and identified by using standard procedures (Minnikin et al., 1984). Polar lipids were separated by using two-dimensional TLC (silica-gel plate 60; Merck). The first direction was developed in chloroform/methanol/water (65:25:4, by vol.) and the second was developed in chloroform/methanol/acetic acid/water (80:12:15:4, by vol.). Total lipid material and specific functional groups were detected by using Dittmer and Lester reagent (phosphate), ninhydrin (free amino groups), Dragendorff reagent (quaternary nitrogen) and anisaldehyde–sulfuric acid (glycolipids). Menaquiones were isolated according to the protocol of Minnikin et al. (1984) and were separated by using HPLC (Kroppenstedt, 1982). For analysis of fatty acids, strain NIO-1016T was cultured on trypticase soy agar (TSA; Difco) at 28 °C for 72 h. Preparation and analysis of fatty acid methyl esters were performed as described by Sasser (1990) by using the Microbial Identification System (MIDI) and the Microbial Identification software package (Sherlock version 6.1; MIDI database TSBA6).

The colonies of strain NIO-1016T were aerobic, Gram-reaction-positive, motile rods of 0.8–1.15 μm in diameter. Strain NIO-1016T grew well on marine agar media and nutrient agar (NA). Strain NIO-1016T formed opaque, light pink, circular colonies with entire margins after incubation on NA (pH 7.0) at 28 °C for 48 h. Strain NIO-1016T was catalase-positive, but oxidase-negative. It grew at temperatures between 25 and 45 °C (optimum, 28–30 °C), at pH 6.0–12.0 (optimum, pH 7.0–7.5) and in the presence of 0–12% (w/v) NaCl. NaCl was not required for growth. Strain NIO-1016T hydrolysed starch, but not casein, presence of 0–12% (w/v) NaCl. NaCl was not required for growth. Strain NIO-1016T hydrolysed starch, but was negative for oxidase, gelatinase and nitrate reductase.

The nearly complete 16S rRNA gene sequence of strain NIO-1016T (1483 bp) was determined and compared with the corresponding sequences of other bacterial strains in the GenBank database. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain NIO-1016T should be assigned to the genus Domibacillus. The phylogenetic tree, based on 16S rRNA gene sequence data from strain NIO-1016T and corresponding sequences from the type strains of species of the genus Bacillus as well as the genus Domibacillus, was reconstructed using the neighbour-joining algorithm (Fig. 1). The comparative analysis of 16S rRNA gene sequences and phylogenetic relationships

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**Fig. 1.** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences, showing the phylogenetic position of strain NIO-1016ᵀ and strains of related species. The sequence of *Escherichia coli* DSM 30083ᵀ (KF034792) was used as an out-group. Only bootstrap values >50%, expressed as percentages of 1000 replications, are shown at branch points. Bar, 0.02 substitutions per nucleotide position.
Table 1. Characteristics that differentiate strain NIO-1016\textsuperscript{T} and other type strains of species of the genus Domibacillus

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Spore/sporangium shape</td>
<td>S–E, s</td>
<td>S, S–E, s</td>
<td>S, S–E, s</td>
</tr>
<tr>
<td>Spore position</td>
<td>C–S</td>
<td>C, S</td>
<td>T</td>
</tr>
<tr>
<td>Pigmentation (on TSBA)</td>
<td>Red pigmentation (after 72 h)</td>
<td>Reddish</td>
<td>Transparent (reddish after 72 h)</td>
</tr>
<tr>
<td>Temperature range for growth (°C)</td>
<td>25–45</td>
<td>13–45</td>
<td>10–40</td>
</tr>
<tr>
<td>Growth with 12% NaCl</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Hydrolysis of aesculin</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>β-Galactosidase (ONPG)</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Utilization of gluconate as sole carbon source</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Acid production from:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N-Acetyl-β-glucosamine</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>l-Arabinoise</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Glycerol</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Inulin</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>d-Mannitol</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>MK-6, MK-7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major menaquinones</td>
<td>MK-6</td>
<td>MK-6</td>
<td>MK-6</td>
</tr>
<tr>
<td>Major fatty acids (&gt;5%)</td>
<td>ai-C_{15:0}, ai-C_{17:0}, i-C_{15:0}, C_{16:0}, C_{17:0}</td>
<td>ai-C_{15:0}, i-C_{15:0}, i-C_{17:0}</td>
<td>ai-C_{16:0}, i-C_{14:0}, i-C_{16:0}, ai-C_{15:0}, ai-C_{17:0}</td>
</tr>
</tbody>
</table>

Strains: 1, NIO-1016\textsuperscript{T}; 2, \textit{D. robiginosus} DSM 25058\textsuperscript{T} (Seiler et al., 2013); 3, \textit{D. indicus} DSM 28032\textsuperscript{T} (Sharma et al., 2014). +, Positive; –, negative. S, spherical; S–E, spherical to elliptoidal; s, sporangia swollen, C–S, central/subterminal; T, terminal.

showed that strain NIO-1016\textsuperscript{T} lies in a subclade in the tree with \textit{D. robiginosus} DSM 25058\textsuperscript{T} and \textit{D. indicus} DSM 28032\textsuperscript{T} (supported by bootstrap values of 99 and 96 %, respectively, Fig. 1), with which it shares a 16S rRNA gene sequence similarity of 98.0 and 97.2 %, respectively. The affiliation of strain NIO-1016\textsuperscript{T} and its closest neighbour, \textit{D. robiginosus} DSM 25058\textsuperscript{T}, was also supported by the maximum-parsimony and maximum-likelihood algorithms with high bootstrap values (PHYLIP version 3.6), thus indicating that NIO-1016\textsuperscript{T} should be considered as representing a novel member of the recently described genus \textit{Domibacillus}. The genomic DNA G + C content of strain NIO-1016\textsuperscript{T} was 46.9 mol%, which was similar to those of related reference species of the genus \textit{Domibacillus}.

Chemo taxonomically, strain NIO-1016\textsuperscript{T} had \textit{meso}-diaminopimelic acid as the diagnostic diamino acid and the A1\textsubscript{γ} type of peptidoglycan. Ribose and glucose were major cell-wall sugars, while galactose was present in a minor quantity. The polar lipid profile contained diphostatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphoglycolipid (PGL) (Fig S1, available with the online Supplementary Material). MK-6 (89.3 %) and MK-7 (8.7 %) were detected as major and minor menaquinone, respectively. The fatty acids found in strain NIO-1016\textsuperscript{T} were C_{14:0} (1.7 %), iso-C_{15:0} (17.4 %), anteiso-C_{15:0} (20.7 %), C_{16:1}\text{a7(c10)6c} (4.9 %), C_{16:1}\text{a11c} (5.9 %), C_{16:0} (11.2 %), iso-C_{17:0} (2.0 %), anteiso-C_{17:1} (3.7 %), anteiso-C_{17:0} (2.0 %), iso-C_{17:0} (5.9 %), anteiso-C_{17:0} (19.9 %), C_{18:0} (1.7 %) and C_{18:0} (1.0 %). The fatty acid methyl esters profile of strain NIO-1016\textsuperscript{T}, \textit{D. robiginosus} DSM 25058\textsuperscript{T} and \textit{D. indicus} DSM 28032\textsuperscript{T} showed high levels of variability, which indicated that, unlike members of other genera, the fatty acid profile is not a characteristic feature of members of the genus \textit{Domibacillus} and it can vary from species to species. Currently only two species with validly published names are available. The observed chemotaxonomic features support the assignment of strain NIO-1016\textsuperscript{T} to the genus \textit{Domibacillus}.

The phenotypic and chemotaxonomic properties of strain NIO-1016\textsuperscript{T} and the 16S rRNA gene sequence comparison results support a proposal to classify the novel isolate as a member of the genus \textit{Domibacillus}. The phenotypic, genotypic and phylogenetic characteristics distinguish strain NIO-1016\textsuperscript{T} from other members of the genus \textit{Domibacillus} with validly published names. Therefore we propose that this isolate represents a novel species within the genus, for which the name \textit{Domibacillus enclensis} sp. nov., is proposed.

**Emended description of the genus Domibacillus Seiler et al. 2013**

The description is as given previously (Seiler et al., 2013; Sharma et al., 2014), with the addition of the following characteristics from the present study. Cell motility is variable, aerobic. Colonies are red-pigmented. The fatty acids detected include C_{14:0}, iso-C_{15:0}, anteiso-C_{15:0}, C_{16:0}, C_{16:1}\text{a7(c10)6c}, C_{16:1}\text{a11c}, iso-C_{17:0}, anteiso-C_{17:1}, anteiso-C_{17:0}, iso-C_{17:0}, anteiso-C_{17:0}, C_{18:0}, C_{18:0}. The predominant quinones are MK-6 (89.3 %) and MK-7 (8.7 %).

**Description of Domibacillus enclensis sp. nov.**

\textit{Domibacillus enclensis} (en.clen’sis. N.L. masc. adj. \textit{enclensis} arbitrary name formed from NCL, the acronym for the...
National Chemical laboratory, India, where taxonomic studies on this species were performed).

Cells are Gram-reaction-positive, aerobic, motile rods of 0.8–1.15 μm in diameter. Spores are spherical or ellipsoidal arranged at central or subterminal, sporangia was swollen. Grows well on marine agar media and NA. Forms opaque, light pink, circular colonies with entire margins after incubation on NA (pH 7.0) at 28 °C for 48–72 h. Catalase-positive, but oxidase-negative. Grows at temperatures between 25 and 45 °C (optimum 28–30 °C) and in the presence of 0–12% (w/v) NaCl. NaCl is not required for its growth. Hydrolyses starch, but not casein, CM-cellulose, urea or Tweens 20, 40, 60 or 80. Negative for asaccharolytic hydrolysis, gluconate and β-galactosidase. Positive for utilization of lactose, glucose, trehalose, raffinose, sucrose, melibiose, cellobiose, arabinose and xylose, phenyl deamination and methyl red; negative for utilization of rhamnose, malonate and adonitol, indole production, nitrate reduction, urease, ornithine decarboxylase and lysine decarboxylase. Acid is produced from N-acetyl-glucosamine, 1-arabinose, D-galactose, glycerol and mannitol. Acid is not produced from inulin. The fatty acids detected are C14:0, C16:0, C18:1ω7c/ω9c, C16:1ω11c, iso-C15:0, anteiso-C15:0, C16:1ω7c/ω6c, C16:1ω10c, anteiso-C17:1ω10c, anteiso-C17:1ω9c, iso-C17:0, anteiso-C17:0, C18:1ω9c and C18:0. The predominant quinones are MK-6 and MK-7. The polar lipids consist of dihydrophthidglycerol, phosphatidylglycerol and an unidentified phosphoglycolipid. meso-Diaminopimelic acid (type A1γ) is present in the cell-wall peptidoglycan and the major whole-cell sugars are glucose and ribose.

The type strain, NIO-1016T (=DSM 25145T=NCIM 5462T=CCTCC AB 2011121T), was isolated from a marine sediment sample from Chorao Island, Goa Province of India. The DNA G+C content of the type strain is 46.9 mol%.

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References


