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

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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 A1c) 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 pharma-
ceutical clean room in eastern Germany, based on its morphological, chemotaxonomic and phylogenetic differ-
ences 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.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain NIO-1016T is JF893466.
A supplementary figure is available with the online Supplementary Material.
DNA of strain NIO-1016T was prepared according to 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). Menaquinoones 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) 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, CM-cellulose, urea or Tween 20, 40, 60 or 80. A phenotypic comparison of strain NIO-1016T and related species of the genus Domibacillus is presented in Table 1. It is evident from Table 1 that there were phenotypic differences between strain NIO-1016T, D. roignonosus DSM 25058T and D. indicus DSM 28032T. Strain NIO-1016T showed positive reactions for catalase and amylase 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.
The phenotypic and chemotypic 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 Domibacillus. The phenotypic, genotypic and phylogenetic characteristics distinguish strain NIO-1016\textsuperscript{T} from other members of the genus Domibacillus with validly published names. Therefore we propose that this isolate represents a novel species within the genus, for which the name 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, motile. Colonies are red-pigmented. The fatty acids detected include C\textsubscript{14:0} iso, C\textsubscript{15:0}, anteiso-C\textsubscript{15:0}, C\textsubscript{16:0} iso, C\textsubscript{17:0} and anteiso-C\textsubscript{17:0}. The predominant quinones are MK-6 (89.3%) and MK-7 (8.7%).

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

Domibacillus 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 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 Tween 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, citrate utilization, H₂S production, nitrate reduction, urease, ornithine decarboxylase and lysine decarboxylase. Acid is produced from N-acetyl-β-glucosamine, 1-arabinose, D-galactose, glycerol and mannitol. Acid is not produced from inulin. The fatty acids detected are C₁₄ : 0, iso-C₁₅ : 0, anteiso-C₁₅ : 0, C₁₆ : 0, 10MeC, 10MeC, C₁₆ : 1ω9c, iso-C₁₇ : 0, anteiso-C₁₇ : 0, anteiso-C₁₇ : 1ω9c, iso-C₁₇ : 0, anteiso-C₁₇ : 1, C₁₈ : 1ω9c and C₁₈ : 0. The predominant quinones are MK-6 and MK-7. The polar lipids consist of diphosphatidyglycerol, 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


