

Anoxybacillus amylolyticus sp. nov., a thermophilic amylase producing bacterium isolated from Mount Rittmann (Antarctica) [☆]

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Abstract

A new thermophilic spore-forming strain MR3C^T was isolated from geothermal soil located on Mount Rittmann in Antarctica. Strain MR3C^T was Gram-positive, rod-shaped, occurring in pairs or filamentous. Growth was observed between 45 and 65 °C (optimum 61 °C) and at pH 5.0–6.5 (optimum pH 5.6). It was capable of utilizing galactose, trehalose, maltose and sucrose. The microorganism produced an exopolysaccharide and synthesized an extracellular constitutive amylolytic activity. The G + C content of DNA was 43.5 mol%. On the basis of 16S rRNA gene sequence similarity, strain MR3C^T was shown to be related most closely to *Anoxybacillus* species. Chemotaxonomic data (major isoprenoid quinone–menaquinone-7; major fatty acid–*iso*-C15:0 and *iso*-C17:0) supported the affiliation of strain MR3C^T to the genus *Anoxybacillus*. The results of DNA–DNA hybridization, physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain MR3C^T from the validly published *Anoxybacillus* species. MR3C^T therefore represents a new species, for which the name *Anoxybacillus amylolyticus* sp. nov., is proposed, with the type strain MR3C^T (= ATCC BAA-872^T = DSM 15939^T = CIP 108338^T).

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Introduction

The genus *Anoxybacillus* was first described by Pikuta et al. [30]; the genus comprises the seven species *Anoxybacillus pushchinoensis* [30], *Anoxybacillus flavithermus* [30], *Anoxybacillus gonensis* [2], *Anoxybacillus contaminans* [5], *Anoxybacillus voinovskiensis* [38], *Anoxybacillus ayderensis* and *Anoxybacillus kestanbolensis* [8]. The genus *Bacillus* has a long taxonomy history and its

[☆]The EMBL accession number for the 16S rRNA sequence of *Anoxybacillus amylolyticus* strain MR3C^T is AJ618979.

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systematic has undergone major revision by Stackebrandt and Swiderski [35]. Several efforts have been made to identify thermophilic bacteria which produce extracellular amylase, that have been shown to have significant industrial importance [3,19]. Amylolytic enzymes play an important role in the biogeochemical cycle of carbon and they are also among the most important enzymes in current biotechnological food, detergent, and pharmaceutical industries. In the Antarctic continent many extreme environments are present, e.g. geothermal areas, hypersaline, cold and often anaerobic ones [7,12,15,21,26,27].

The prokaryotes of Antarctica are of broad phylogenetic origin with representatives from both *Archaea* and *Bacteria*. Thermophilic bacterial species isolated so far in Antarctica belonged to *Bacillaceae* as *Bacillus* and *Alicyclobacillus* species [7,11,12,15,26,27]. Our studies in the Antarctic continent were devoted to the taxonomical distribution of extremophiles in Antarctica, the classification of new isolates and chemical and biochemical characterization of their molecules [24,28].

In Victoria Land, warm ground is resident on the summit of Mount Rittmann (73°28'S; 165°38'E) and during the Italian Antarctic Expedition in the Austral summer of 2002–2003, samples from the fumaroles heated by geothermal activities were retrieved.

The present paper describes the isolation, morphological, physiological, biochemical profiles and 16S rRNA sequence of a novel amylase producing bacterium belonging to *Anoxybacillus* species. The characteristics of this isolate are compared with its near neighbour and on the basis of DNA–DNA hybridization values, we propose that it represents a novel species of the genus *Anoxybacillus*, *Anoxybacillus amylolyticus* sp. nov.

Materials and methods

Isolation and growth condition

During the austral summer of 2002–2003, samples of geothermal soil were collected aseptically from active fumaroles at an altitude of 2200 m above sea level and at about 400 m from the crater of Mount Rittmann (73°28'S; 165°38'E) in Antarctica. The summer air temperature was –20 °C and the temperature of the ground surface was 70 °C close to the fumaroles vents.

All samples were immediately incubated at the Terra Nova Bay station laboratory in the temperature range 60–75 °C in different enrichment media. They were: (% value are in w/v) medium (A) containing yeast extract Oxoid (varying from 0.3% to 0.6%) and NaCl (0.3–0.6%) at pH 5.6 and at pH 4.0; medium (B) yeast extract Oxoid 0.4%, (NH₄)₂SO₄ 0.2%, KH₂PO₄ 0.3%, 4 ml Solution A and 4 ml Solution B at pH 3.4, 4.0 and

6.0 (Solution A = 125 g/l (NH₄)₂SO₄ plus 50 g/l MgSO₄ · 7 H₂O; Solution B = 62.5 g/l CaCl₂ · 2H₂O). Cultures were purified from the samples grown in the enrichment medium A (yeast extract Oxoid, 0.6%, NaCl 0.6%, pH 5.6 at 61 °C), using the repeated serial dilution technique and repeated at least five times. Several isolates were selected and taxonomic properties of strain MR3C^T will be presented in this note.

Geobacillus toebii DSM 14590^T, *Anoxybacillus contaminans* DSM 15866^T, and *Geobacillus thermoleovorans* DSM 5366^T were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen, Brunswick, Germany (DSMZ), and were grown according to DSMZ catalogue. *Anoxybacillus voinovskiensis* NCIMB 13956^T was obtained from the National Collections of Industrial, Marine and Food Bacteria, Scotland, UK (NCIMB).

Morphological and physiological tests

The temperature range for growth was determined by incubating the isolate from 37 to 75 °C. The pH dependence of growth was tested in the pH range 4.0–7.0. Growth on single carbon source was tested on liquid medium C containing (w/v): yeast extract Oxoid (0.06%) and NaCl (0.6%) in tap water at pH 5.6. The organic compounds (1%, w/v) were: D-glucose, D-lactose, D-maltose, D-fructose, D-arabinose, D-cellobiose, D-galactose, D-mannose, D-ribose, D-trehalose, D-xylose, and L-sorbose, raffinose, glycerol, sucrose, and sodium acetate. All growth tests were done at 61 °C and growth was scored positive if the *A*₅₄₀ was greater than 0.300 after 3 days.

The growth of the microorganism was also tested in the following complex media (w/v): nutrient broth Oxoid 1% and NaCl 0.6% pH 5.6; peptone Oxoid 1% and NaCl 1% pH 5.6.

Cellular morphology was determined by phase-contrast microscopy (Zeiss) and by scanning electron microscopy (SEM) using Philips XL 30 ESEM. For SEM analysis the samples were fixed for 24 h in 2.5% glutaraldehyde. They were subsequently dehydrated in a graded series of ethyl alcohol, critical point dried, gold coated by sputtering.

Colony morphology was determined by Leica M8 stereomicroscope using cultures grown on media A agar (2%) plates for 24 h at 61 °C. Gram staining was performed according to Dussault [9]. KOH test was performed according to Halebian et al. [14].

Unless otherwise stated the strain was characterized using the modified methods of Gordon and Pang [13].

Starch hydrolysis was tested by flooding cultures on solid enrichment medium A containing 0.2% (w/v) starch with Lugol's iodine. For casein hydrolysis a solid medium A plus an equal quantity of skimmed milk was

used. For gelatine hydrolysis and sensibility to lysozyme, enrichment medium A plus 1.0% (w/v) gelatine or 0.001% (w/v) lysozyme was used, respectively. For spore formation test, enrichment medium A plus 0.001% (w/v) $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ was used. Tolerance of NaCl was determined in medium A with varying concentrations of NaCl. Urease activity was determined as described by Lanyi [20].

Aminopeptidase was assayed with Bactident Aminopeptidase Kit of Merck (Germany). Hydrolysis of *N*'-benzoyl-arginine-*p*-nitroanilide (BAPA) stereoisomer was tested according to Oren and Galinski [29]. All tests were carried on cells grown in the enrichment medium A at 61 °C. Anaerobic growth test was performed as described by Schäffer et al. [34].

The EPS production from MR3C^T strain was performed according to Manca et al. [24]. The cells were harvested by cultural broth centrifugation at the stationary grown phase. EPS production on cell-free culture broth was tested by the phenol-sulphuric acid method with glucose as standard [24]. The supernatant was treated with the same volume of cold ethanol added dropwise under stirring. The alcoholic solution was kept at –18 °C overnight and then centrifuged at 15,000*g* for 40 min. The precipitated EPS was dissolved in hot distilled water, dialysed against tap water (72 h) and lyophilized.

Antimicrobial susceptibility testing

Sensitivity of the strain to the antibiotics was tested by using the enrichment-solid medium A and sensi-discs (6 mm, Oxoid). The following antibiotics were used (µg): neomycin (5, 30), erythromycin (30), penicillin G (10 U), chloramphenicol (10, 50), kanamycin (5, 30), tylosin (10, 30), ampicillin (25), gentamicin (10, 30), novobiocin (30), bacitracin (10 U), lincomycin (15), fusidic acid (10), vancomycin (30), streptomycin (25) and tetracycline (30, 50).

Lipid and fatty acid compositions

Lipid and fatty acids analysis were performed as reported by Nicolaus et al. [28]. Polar lipids were achieved from 3.0 g of freeze-dried cells grown in enrichment medium A at 61 °C, by the extraction with 65:25:4 $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$. The lipid extract was analysed by thin layer chromatography (TLC) on silica gel (0.25 mm, F_{254} , Merk) eluted with $\text{CHCl}_3/\text{MeOH}/\text{H}_2\text{O}$ (65:25:4 by vol.). Lipids were detected by spraying the plates with 0.1% $\text{Ce}(\text{SO}_4)_2$ in 2 N H_2SO_4 followed by heating at 100 °C for 5 min. Phospholipids and aminolipids were detected on the plates upon spraying with the Dittmer-Lester and the ninhydrin reagents, respectively, and glycolipids were visualized with α -naphthol [28].

Authentic standards were used for comparing the R_f of substances.

Fatty acid methyl esters (FAMES) were obtained from complex lipids by acid methanolysis [28] and analysed using a Hewlett-Packard 5890A gas chromatograph fitted with a FID detector and equipped with an HP-V column with a flow rate of 45 ml/min using the temperature program of 120 °C (1 min), from 120 to 250 °C at 2 °C/min. Identification of compounds was obtained with standards and by interpretation of mass spectra. Compounds were identified by ¹H-NMR and EI/MS (Electronic Impact) as previously described [28]. NMR spectra, recorded at the NMR Service of Institute of Biomolecular Chemistry of CNR (Pozzuoli, Italy), were acquired on a Bruker DPX-300 operating at 300 MHz, using a dual probe.

Alpha-amylase assay

Alpha-amylase activity was tested according to Lama et al. [19]. MR3C^T strain was grown at 61 °C in the enrichment medium A and the cells were harvested in the stationary phase of the growth by centrifugation at 8000*g* for 20 min. The supernatant (200 ml) was precipitated at 4 °C by ammonium sulphate added to 80% saturation, stirred for 2 h, stored overnight at 4 °C and then centrifuged at 8000*g* for 1 h. The precipitated proteins were dissolved in 2.5 ml of 0.05 M Na-acetate buffer pH 5.6 and dialysed extensively at 4 °C against the same buffer; the dialysed residue was designated Partial Purified Enzyme (PPE, 5.5 ml) and tested for protein content and α -amylase assay. One enzyme unit is defined as the amount of enzyme which causes the loss of 100 optical density units in 30 min, under our experimental conditions.

Phylogenetic analysis

Genomic DNA extraction, PCR mediated amplification of the 16S rDNA and purification of the PCR products were carried out as described previously [31,32]. The primers for 16S rDNA amplification were: 10-30F 5'GAGTTTGATCCTGGCTCAG and 1500R 5'AGAAAGGAGGTGATCCAGCC. The 16S rRNA resulting sequence was manually aligned and compared with representative sequences of organisms belonging to the *Firmicutes* group [23]. The results are presented as a phylogenetic tree. The 16S rRNA gene similarity values were calculated by pairwise comparison of the sequences within the alignment. For construction of the phylogenetic dendrogram operations of the PHYLIP package were used [10]; the pairwise evolutionary distances were computed from percent similarities by the correction of Jukes and Cantor [17] and the phylogenetic tree was constructed by the neighbour-joining method [33]. The

root of the tree was determined by including the 16S rRNA gene sequence of *Brevibacillus centrosporus* into the analysis.

The 16S rRNA gene sequence of MR3C^T strain was submitted to EMBL Nucleotide Sequence Database and has been assigned accession number AJ618979.

G + C content and DNA–DNA hybridization

The % G + C of DNA was determined by the HPLC method [25] and DNA–DNA hybridization was performed according to Sunna et al. [36]. DNA was isolated using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion et al. [4]. DNA–DNA hybridization was carried out as described by De Ley et al. [6] under consideration of the modifications described by Huss et al. [16] using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with in situ temperature probe (Varian).

Results

Morphological and biochemical analysis

Cells of isolate MR3C^T were Gram-positive, motile rods, 2.0–2.5 μm long and 0.5 μm wide (Fig. 1), occurring in pairs or filamentous with terminal, ellipsoidal to cylindrical endospores. Colonies were usually cream, smooth and circular.

MR3C^T strain was facultative anaerobe that grew in enrichment medium A. The temperature growth range was from 45 to 65 °C with an optimum growth at 61 °C. The pH growth range was from 5.0 to 6.5 with an optimum at pH 5.6. The isolate MR3C^T utilized a wide

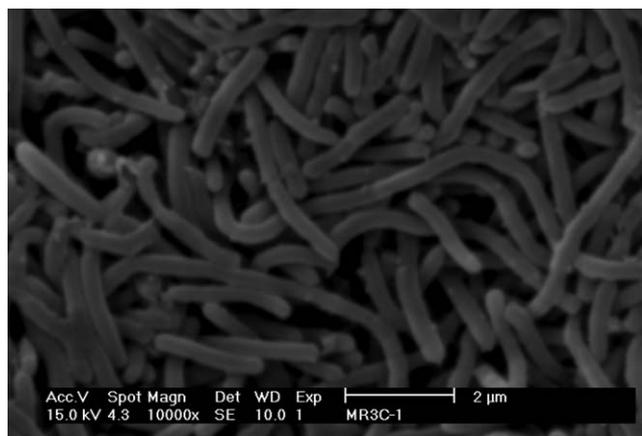


Fig. 1. Scanning electronic micrographs of MR3C^T strain. Bar, 2 μm.

range of carbon sources including galactose, sucrose, trehalose, maltose and raffinose. The isolate was sensitive to penicillin G (10 U), chloramphenicol (10 μg), kanamycin (5 μg), tylosin (10 μg), lincomycin (15 μg), bacitracin (10 U), gentamicin (10 μg), novobiocin (30 μg), fusidic acid (10 μg), streptomycin (25 μg), tetracycline (30 μg) and ampicillin (10 μg). The isolate MR3C^T was catalase and starch hydrolysis positive while it was oxidase, gelatine hydrolysis, casein hydrolysis, indole production and phenylalanine deamination negative. Isolate MR3C^T was able to hydrolyse hippurate, to decompose tyrosine, to reduce nitrate and was sensible to lysozyme (Table 1).

MR3C^T strain, grown on medium C with galactose as sole carbon source, was able to produce EPS reaching in the stationary phase of growth up to 271 mg of EPS/g dry cells.

Lipid and fatty acid composition

The isolate MR3C^T strain possessed complex lipids based on fatty acids. The total lipid content ranged between 8% and 10% of the total dry weight of cells grown at 61 °C in medium A. Under these conditions three major phospholipids, 1,2 diacylglycero-3-phosphorylethanolamine (PEA), 1,2 diacylglycero-3-phosphoryl-glycerol (PG) and cardiolipin (DPG), were identified.

FAMES' composition, determined on cells grown under standard condition, was characterized by the abundance of branched acyl chains. The most abundant was *i*C15:0 (41.2%), other components were *i*C17:0 (31.6%), *ai*C17:0 (8.4%), *i*C16:0 (7.0%) and *ai*C15:0 (2.13%) (Table 2). Chromatographic analysis of quinones revealed the presence of one more abundant UV-absorbing band. ¹H-NMR spectrum showed that the quinone present is of menaquinone (MK) type. The LC/MS analysis gave a molecular peak corresponding to MK7 as major compound (more than 90% of quinones).

Alpha-amylase activity

MR3C^T showed an extracellular constitutive amyolytic activity able to convert starch. Preliminary data on the distribution of this activity in MR3C^T strain indicated that it was present in the culture medium without additional starch during the growth. Maximum activity of the amylase was expressed in the stationary growth phase. PPE (total protein 4.67 mg) of the extracellular fraction presented a specific activity of 170 U/mg of proteins.

Table 1. Comparison of the phenotypic characteristics of *Anoxybacillus amylolyticus* sp. nov. strain MR3C^T and related species

Characteristic	1	2	3	4	5
Cell morphology	Rod	Curved-rod	Rod	Rod	Rod
Gram-stain	+	v	+	+	+
Relation to O ₂	Aerobe/facultative anaerobe	Aerobe/facultative anaerobe	Anaerobe/facultative aerobe	Aerobe/facultative anaerobe	Obligate aerobe
Temperature range (°C)	45–65	50–60	30–64	30–72	39–67
Optimum temperature (°C)	61	50	54	60–65	55
pH range	5.0–6.5	4.5–10.0	7.0–8.0	5.5–9.0	6.0–9.0
Optimum pH	5.6	7.0	7.0–8.0	7.0	7.0
Motility	+	±	–	+	+
NaCl (3%, w/v)	–	+	–	–	–
Nitrate reduction	–	+	+	+	+
Tween 60 hydrolysis	–	N.D.	+	N.D.	w
Gelatin hydrolysis	–	+	–	–	–
Starch hydrolysis	+	+	–	+	w
Oxidase	–	–	+	+	N.D.
Utilization of					
Glucose	+	+	+	–	+
Xylose	–	+	+	–	+
Trehalose	+	+	–	N.D.	+

1, *Anoxybacillus amylolyticus*; 2, *Anoxybacillus contaminans* LGM 21881^T DSM 15866^T [5]; 3, *Anoxybacillus voinovskiensis* TH13^T NCIMB 13956^T [38]; 4, *Anoxybacillus flavithermus* DSM 2641^T [30]; 5, *Geobacillus tepidamans* GS5-97^T DSM 16325^T [34].

+ positive; – negative; w weak response; v variable; N.D. not determined.

The data of *Anoxybacillus contaminans*, *Anoxybacillus voinovskiensis*, *Anoxybacillus flavithermus* and *Geobacillus tepidamans* were obtained from De Clerck et al. [5], Yumoto et al. [38], Pikuta et al. [30] and Schaffer et al. [34], respectively.

Phylogenetic analysis and DNA–DNA hybridizations

The DNA of strain MR3C^T had a G+C content of 43.5 mol%. The total 16S rRNA gene sequence of the strain MR3C^T (EMBL nucleotide sequence accession number is AJ618979) showed high similarity (98.2%) to *A. voinovskiensis* and (98.1%) to *A. contaminans*. The bacterium shared a similarity of 96%, and 94% with *Anoxybacillus ayderensis* and *Anoxybacillus kestanbolensis*, respectively. The other related strain *Geobacillus tepidamans* [34] had a similarity of 97.5%. The results were presented as a phylogenetic dendrogram (Fig. 2). MR3C^T was separate from other species within the *Bacillus* radiation, including the phylogenetically related *Anoxybacillus* species [5].

The DNA–DNA reassociation values found between strain MR3C^T and the close strains *A. voinovskiensis* and *A. contaminans* were 32.8% and 30.7%, respectively. The DNA/DNA relatedness between our isolate and the related species *G. tepidamans* was 30.2%. All these values were lower than the recommended threshold value of 70% which is accepted as the definition of a genospecies [37].

Discussion

Recent taxonomic studies on thermophilic bacilli have shown that this biotechnologically important group of bacteria is still in need of further study to improve its classification and identification [1,18,22].

The organism characterized in this study was isolated in Antarctica from an active fumaroles at an altitude of 2.200 m above sea level (Mount Rittmann).

The strain MR3C^T is a moderate thermophilic Gram-positive rod, facultative anaerobe that shows few phenotypic features in common with the other thermophilic species isolated both from geothermal soil of Mount Rittmann and Mount Melbourne in Antarctica [26,27].

The total 16S rRNA sequence of strain MR3C^T falls within the radiation of the genus *Anoxybacillus*. The genotypic and phenotypic properties of strain MR3C^T differ from those of the known species of this genus. The analysis of the almost complete nucleotide sequence of 16S rRNA of strain MR3C^T allowed the phylogenetic tree to be constructed, with the closest neighbours of related species (Fig. 2). Strain MR3C^T is closely related to the species *A. voinovskiensis*. It has been largely described that if the level of 16S rRNA similarity is

Table 2. Fatty acid methyl ester profiles of *Anoxybacillus amylolyticus* MR3C^T strain and closely related *Anoxybacillus* species and *Geobacillus tepidamans* reference strains^a

Fatty acids	1	2	3	4	5
<i>i</i> C13:0	–	–	–	–	0.4
<i>i</i> C14:0	–	–	1.3	–	0.6
C14:0	–	3.0	1.3	2.0	4.1
C14:0 <i>i</i> 3-OH	–	–	–	54.8	–
<i>i</i> C15:0	41.2	52.0	54.7	4.0	44.3
<i>ai</i> C15:0	2.13	7.0	8.0	1.2	6.6
C15:0	0.1	–	–	2.9	–
<i>i</i> C16:0	7.0	5.0	7.1	11.1	3.2
<i>ai</i> C16:0	0.12	–	–	–	–
C16:0	6.3	11.0	1.9	–	15.1
<i>i</i> C17:1 ω 5c	–	–	–	–	0.8
<i>i</i> C17:0	31.6	12.0	3.9	6.1	15.0
C17:0 anteiso-A	–	–	–	17.7	–
<i>ai</i> C17:0	8.4	7.0	–	–	6.1
C17:0	0.7	–	–	–	–
<i>ai</i> C17:1	–	–	7.1	–	–
C17:1	–	–	2.6	–	–
<i>i</i> C18:0	0.09	–	–	–	–
<i>ai</i> C18:0	0.7	–	–	–	–
C18:0	1.9	–	–	–	0.6

Taxa: 1, *Anoxybacillus amylolyticus* MR3C^T; 2, *Anoxybacillus contaminans* LGM 21881^T DSM 15866^T [5]; 3, *Anoxybacillus voinovskiensis* TH13^T NCIMB 13956^T [38]; 4, *Anoxybacillus flavithermus* DSM 2641^T [30]; 5, *Geobacillus tepidamans* GS5-97^T DSM 16325^T [34].

^aValues are shown as a percentage of the total fatty acid content for each strain.

greater than 97% other additional phenotypic and genotypic characteristics should be used for taxonomical purpose. We have also compared our microorganism with *G. tepidamans*, because this bacterium was phylogenetically similar to the *Anoxybacillus* species but different phenotypically [34].

Table 1 indicates the difference in the phenotypic and biochemical characteristics between strain MR3C^T and its closest relatives. Strain MR3C^T differed from the other relatively closely related species in terms of the following phenotypic characteristics: morphology, optimal *T*°C, pH range for growth, reduction of NO₃⁻ to NO₂⁻, utilization of substrates, starch hydrolysis, relation to O₂ (Table 1). Strain MR3C^T possesses a strong amylolytic constitutive activity not reported in other related microorganisms. Moreover, our microorganism was able to produce an exopolysaccharide growing on galactose medium. A comparison of the polar lipid patterns by one-dimensional TLC (data not reported) revealed high levels of similarity between MR3C^T and *Anoxybacillus* reference strains but they differed in fatty acid composition (Table 2).

The low levels of DNA–DNA hybridization between strain MR3C^T and the representatives of the genus

Anoxybacillus (30–33%) allowed us to differentiate MR3C^T from all the other species of this genus into a separate species [37].

On the basis of 16S rRNA analysis, DNA:DNA reassociation values, fatty acid composition, physiological and biochemical characteristics, we propose that the strain MR3C^T represents a new species of the genus *Anoxybacillus*, for which we propose the name *A. amylolyticus* sp. nov.

Description of *Anoxybacillus amylolyticus* sp. nov.

A. amylolyticus (Gr. n. amulon, starch; connecting vowel –o–; Gr. adj. lutikos: able to loosen, able to dissolve; N.L. masc. adj. amylolyticus, starch dissolving). Cells are Gram-positive, sporulating rods (2.0–2.5 μ m long and 0.5 μ m wide), occurring in pairs or filamentous with terminal, ellipsoidal to cylindrical endospores. They are motile and slightly acidophilic. Colonies on medium A (yeast extract Oxoid, 0.6%, NaCl 0.6%, pH 5.6 at 61 °C) are circular, cream, and smooth. It is thermophilic and facultative anaerobe, exhibiting optimum growth temperature of 61 °C, but is able to grow between 45 and 65 °C and at pH 5.6. It is able to utilize D-galactose, D-trehalose, D-maltose, raffinose, and sucrose when the medium was supplemented with 0.06% of yeast extract. Negative reactions for D-glucose, D-lactose, D-fructose, D-arabinose, D-cellobiose, D-mannose, D-ribose, D-xylose, L-sorbose, glycerol, and sodium acetate as carbon sources were obtained. It is positive for catalase, tyrosine decomposition, hippurate hydrolysis, starch hydrolysis, sensitive to lysozyme, nitrate reduction and hydrolysis of hippurate, and negative with respect to casein hydrolysis, gelatine hydrolysis, oxidase, indole production and phenylalanine deamination. On sugar media it is able to produce exopolysaccharide. MR3C^T possesses a constitutive extracellular amylase activity. MK7 is the most abundant MK and the predominant polar lipids are PEA, PG and DPG. *i*C15:0, *ai*C15:0, *n*C15:0, *i*C16:0, *ai*C16:0, *n*C16:0, *i*C17:0, *ai*C17:0, *n*C17:0, *i*C18:0, *ai*C18:0, *n*C18:0, are the main cellular fatty acids. The following antibiotics inhibited the growth of MR3C^T: penicillin G (10 U), chloramphenicol (10 μ g), kanamycin (5 μ g), tylosin (10 μ g), lincomycin (15 μ g), bacitracin (10 U), gentamicin (10 μ g), novobiocin (30 μ g), fusid acid (10 μ g), streptomycin (25 μ g), tetracycline (30 μ g), ampicillin (10 μ g). The G + C content of DNA is 43.5 mol%. MR3C^T was isolated from geothermal soil on Mount Rittmann on the Antarctica continent. Type strain MR3C^T has been deposited in America Type Culture Collection, Manassas, USA (ATCC BAA-872^T), in Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany (DSM 15939^T), and in Institut Pasteur, Paris, France (CIP 108338^T).

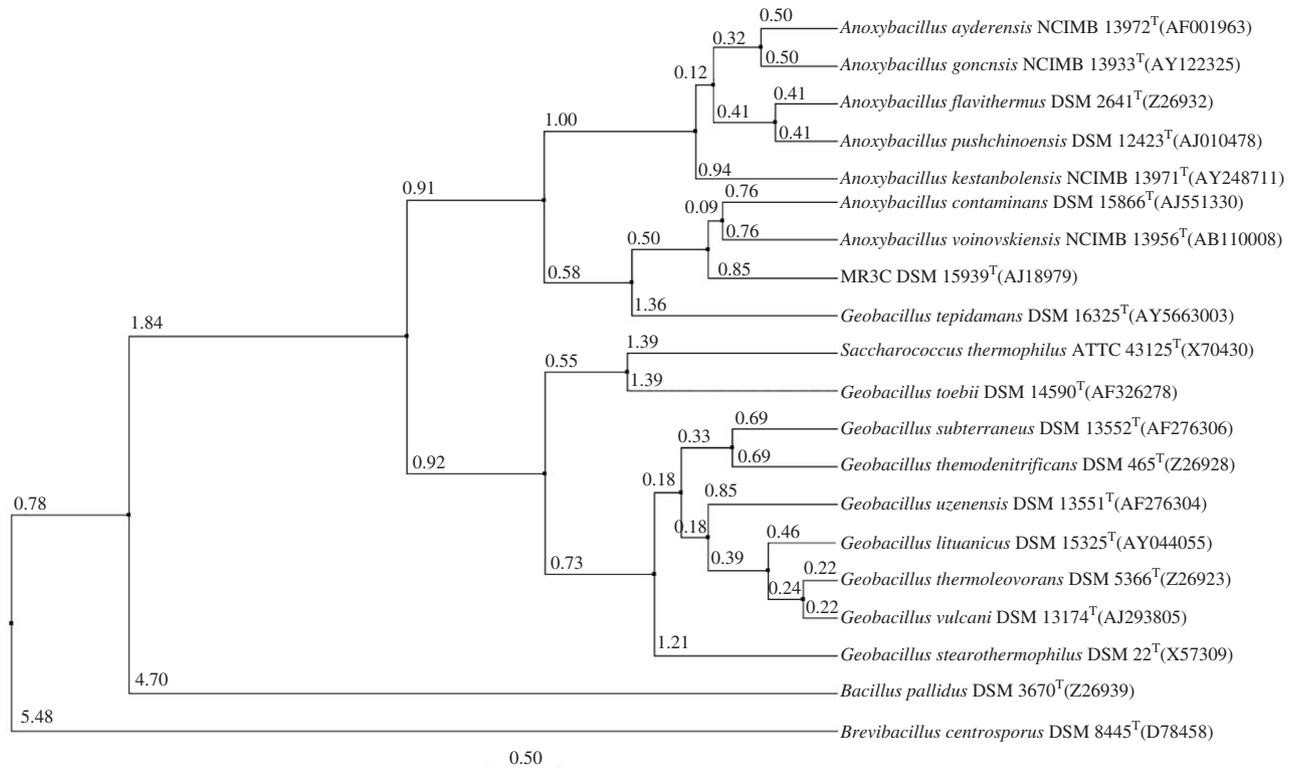


Fig. 2. Phylogenetic dendrogram indicating the position of the strain MR3C^T within the radiation of *Firmicutes*. The root of the tree was determined by including the 16S rRNA gene sequence of *Brevibacillus centrosporus* into the analysis. The branch lengths are given at the nodes. Scale bar represents 0.50 substitutions per nucleotide position.

EMBL Nucleotide Sequence Database accession number [AJ618979](https://www.ebi.ac.uk/EMBL/Sequence/Accession/AJ618979).

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