Anoxybacillus geothermalis sp. nov., a facultatively anaerobic, endospore-forming bacterium isolated from mineral deposits in a geothermal station

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A novel endospore-forming bacterium designated strain GSsed3T was isolated from deposits clogging aboveground filters from the geothermal power platform of Groß Schönebeck in northern Germany. The novel isolate was Gram-staining-positive, facultatively anaerobic, catalase-positive and oxidase-positive. Optimum growth occurred at 60 °C, 0.5 % (w/v) NaCl and pH 7–8. Analysis of the 16S rRNA gene sequence similarity indicated that strain GSsed3T belonged to the genus Anoxybacillus, and showed 99.8 % sequence similarity to Anoxybacillus rupiensis R270T, 98.2 % similarity to Anoxybacillus tepidamans GS5-97T, 97.9 % similarity to Anoxybacillus voinovskiensis TH13T, 97.7 % similarity to Anoxybacillus caldiproteolyticus DSM 15730T and 97.6 % similarity to Anoxybacillus amylolyticus MR3C. DNA–DNA hybridization (DDH) indicated only 16 % relatedness to Anoxybacillus rupiensis DSM 17127T. Furthermore, DDH estimation based on genomes analysis indicated only 19.9 % overall nucleotide similarity to Anoxybacillus amylolyticus DSM 15939T. The major respiratory menaquinone was MK-8. The polar lipid profile consisted of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycerol, one unknown phosphoglycolipid and one unknown phospholipid. The predominant cellular fatty acids were iso-C15:0, iso-C17:0, C16:0, iso-C16:0 and anteiso-C17:0. The peptidoglycan type was A1γ meso-Dpm-direct. The genomic DNA G+C content of the strain was 46.9 mol%. The phenotypic, genotypic and chemotaxonomic characterization indicated that strain GSsed3T differs from related species of the genus. Therefore, strain GSsed3T is considered to be a representative of a novel species of the genus Anoxybacillus, for which the name Anoxybacillus geothermalis sp. nov. is proposed. The type strain of Anoxybacillus geothermalis is GSsed3T (=CCOS808T = ATCC BAA2555T).

The genus Anoxybacillus belongs to the family Bacillaceae and is related to the genus Geobacillus, which explains why, for instance, a former representative of the genus Geobacillus, Geobacillus tepidamans (Schaffer et al., 2004), is now classified as Anoxybacillus tepidamans (Coorevits et al., 2012). The name given to the genus suggests that species assigned to it thrive under anoxic conditions, and, indeed, the first species classified as members of the genus Anoxybacillus were aerotolerant anaerobes (Pikuta et al., 2000). Nevertheless, at the time of writing, from the 22 species of the genus Anoxybacillus, 16 are facultative anaerobes (Atanassova et al., 2011; De Clerck et al., 2011; Cihan et al., 2011, 2014; Dai et al., 2011; De Clerck et al., 2004; Dulger et al., 2004; Kevbrin et al., 2005; Namsaraev

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The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of Anoxybacillus geothermalis GSsed3T is KF722458.
Two supplementary figures and two supplementary tables are available with the online Supplementary Material.
et al., 2010; Poli et al., 2006; Schaffer et al., 2004; Yamoto et al., 2004; Zhang et al., 2011), five are strict aerobes (Coorevits et al., 2012; Derekova et al., 2007; Inan et al., 2013; Poli et al., 2009; Zhang et al., 2013) and one is an aerotolerant anaerobe (Pikuta et al., 2000). Species that belong to the genus *Anoxybacillus* share common characteristics, mainly concerning their major fatty acids (iso-C<sub>17:0</sub> a and iso-C<sub>15:0</sub> 2OH), the rod shape of the vegetative cells and the terminal/subterminal position of their endospores. The DNA G+C content of this genus differs considerably among species, from 37.8 mol % in *Anoxybacillus calidus* (Cihan et al., 2014) up to 57 mol % in *Anoxybacillus gomensis* (Belduz et al., 2003). Besides the phenotypic similarities, species that belong to the genus *Anoxybacillus* share common ecological characteristics, too. All of them are thermophiles or moderate thermophiles, with the majority of the species isolated so far originating from thermal springs (Atanassova et al., 2008; Belduz et al., 2003; Cihan et al., 2011, 2014; Dai et al., 2011; Derekova et al., 2007; Dulger et al., 2004; Inan et al., 2013; Poli et al., 2009; Yamoto et al., 2004; Zhang et al., 2011, 2013). The aim of this study was to describe a novel species of the genus *Anoxybacillus* isolated from a geothermal reservoir.

Samples were taken from deposits at the entrance filters of the geothermal research facility of Groß Schönebeck. The deposits were obtained from coarse filter bags (10–20 μm pore-size) located right after the transport to the surface and degassing of the geothermal fluid. The *in situ* geothermal laboratory of Groß Schönebeck is situated in the North German Basin (52°54′13.15″ N 13°36′5.43″ E). The geothermal fluid is produced from a reservoir (Permian sandstone and volcanic rocks) at about 4200 m depth. At this depth, temperature is around 150 °C. The anoxic hot fluid contains a high salt concentration (total dissolved solids >260 g l<sup>−1</sup>), and the pH measured was close to pH 5.5 at 25 °C. However, under these saline conditions, the measured pH value has to be corrected by about one pH unit, shifting the pH to the neutral range (Feldbusch et al., 2013). The mineral phases identified as precipitates in filter residues were barite (BaSO<sub>4</sub>), laurionite (PbOHC<sub>2</sub>), halite (NaCl), magnetite (Fe<sub>3</sub>O<sub>4</sub>), and occasionally quartz (SiO<sub>2</sub>) and native copper (Regenspur et al., 2013). Isolation was carried out by inoculating 1 g of deposit sample into 5 ml of modified Difco D2216 marine broth. This medium was modified by using 5 g tryptone instead of peptone, omitting the addition of potassium bromide and adjusting the pH to 5.2 with HCl, and it will be referred to as modified D2216 medium. Samples were inoculated at 37 and 60 °C under aerobic conditions. Growth was only observed at 60 °C. After enrichments and several purifications on modified D2216 marine agar with 2 % (w/v) agar instead of 1.5 % (w/v), strain GSsed<sup>3</sup> was isolated. The strain was conserved at −80 °C as a 30 % (w/v) glycerol suspension. In addition, the same procedure of enrichment and isolation was repeated with samples originating from water and a biofilm growing on the pipes of the collection point originating from the geothermal facility of Bruchsal. The municipal Bruchsal geothermal power plant is located in the southern part of Germany, near the German–French border (49°07′31.23″ N 08°34′8.00″ E). Its main purpose is to generate electricity; however, water is also used for heating purposes. The water is pumped from a depth of 2500 m. The pH of the water varies from pH 5.4 to 5.8, and the pressure inside the drilling pipes is 22 bar. Temperature at the deepest drilling point is around 132 °C; however, there is a 10 °C loss in temperature as water reaches the surface. Before sampling, the plant had been circulating for 24 h, pumping 25 l of water per second. Gases are dissolved in the water, with high CO<sub>2</sub> concentration. After enrichment and purification as indicated above, strains B2M1 and B7M1 were also obtained. In this study, a polyphasic taxonomy approach combining genotypic, chemotaxonomic and phenotypic characteristics (Vandamme et al., 1996) was conducted to determine the precise taxonomic position of a novel Gram-staining-positive, endospore-forming bacterium (GSsed<sup>3</sup>) and its related strains (B2M1 and B7M1).

Genomic DNA was extracted using the InnuPrep Bacteria DNA kit (Analytik Jena), according to the manufacturer’s instructions. DNA was quantified fluorometrically with a Qubit dsDNA HS Assay kit and Qubit 2.0 Fluorometer (Invitrogen). To obtain the nearly full-length 16S rRNA gene sequence, PCR amplification was performed using the bacterial universal primer set GM3F (5′-AGA GTT TGA TC (AC) TGG C-3′) and GM4R (5′-TAC CTT AGT ACG ACT T-3′) (Muyzer et al., 1995). The PCR product was purified with a MultiScreen PCRµ96 Filter Plate (Millipore) and sequenced. To obtain a full sequence of the amplicon, PCR products were sequenced in addition with the primers 907r, 926f (Muyzer et al., 1995) and 518r (Ovreis et al., 1997). Sequencing was conducted using the services of Microsynth (Switzerland) and GATC Biotech (Germany). The partial sequences generated were assembled using the online EMBOSS tools revseq and merger, and the consensus sequence was corrected manually for errors. A sequence of 1548 bp for GSsed<sup>3</sup> (B2M1=1247 bp; B7M1=1487 bp) was obtained. Screening of phylogenetic neighbours of strain GSsed<sup>3</sup> was carried out using EzTaxon-e (Kim et al., 2012), taking into account 16S rRNA gene sequences from cultured isolates. Sequences of all species of the genus *Anoxybacillus* along with *Aeribacillus palidus* strain GS3372 (Filippidou et al., 2015), as an outgroup, were obtained and used to build phylogenetic trees using the online tools of the phylogeny.rr website (Dereeper et al., 2008). The tree topology was verified using four independent methods for the reconstruction of phylogenetic trees, neighbour-joining, maximum-likelihood, maximum-parsimony and Bayesian inference (Anisimova & Gascuel, 2006; Castresana, 2000; Dereeper et al., 2008; Elias & Lagergren, 2007; Gascuel, 1997; Goloboff et al., 2000; Guindon et al., 2010; Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Trees were processed (re-rooting, extracting topology and plotting) with the Newick Utilities (Junier & Zdobnov, 2010). The EzTaxon-e
identification of the 16S rRNA gene sequence of strain GSsed3\textsuperscript{T} showed 99.8% similarity to *Anoxybacillus rupiensis* R270\textsuperscript{T}, 98.2% similarity to *Anoxybacillus tepidamans* GS5-97\textsuperscript{T}, 97.9% similarity to *Anoxybacillus voinosvkiensis* TH13\textsuperscript{T}, 97.7% similarity to *Anoxybacillus caldiproteolyticus* DSM 15730\textsuperscript{T} and 97.6% similarity to *Anoxybacillus amylyticus* MR3C\textsuperscript{T}. However, considering that EzTaxon-e uses a Basic Local Alignment Search Tool for identification and that this approach maximizes similarity for partial regions of the alignment, sequence identity for the five strains with similarity values over 97% was verified using the pairwise algorithm Needleman–Wunsch (Needleman & Wunsch, 1970). The results are summarized in Table S1 (available in the online Supplementary Material). Based on pairwise alignments, the 16S rRNA gene of strain GSsed3\textsuperscript{T} has 97.6 and 97.2% identity to *Anoxybacillus rupiensis* R270\textsuperscript{T} and *Anoxybacillus amylyticus* MR3C\textsuperscript{T}, respectively. A high degree of concordance was observed between the clustering within the trees (Fig. 1). The data show that strain GSsed3\textsuperscript{T}, as well as strains B2M1 and B7M1, form a well-supported subline within the genus *Anoxybacillus* that is distinct from all other species within the genus. Furthermore, the branching structure supports the classification of the three isolates as a potentially novel species (Fig. 1).

In order to further support the classification of strain GSsed3\textsuperscript{T} as a representative of a novel species of the genus *Anoxybacillus*, a DNA–DNA hybridization (DDH) test between GSsed3\textsuperscript{T} and *Anoxybacillus rupiensis* DSM 17127\textsuperscript{T} was carried out using the services of DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Germany). Cells were disrupted by using a Constant Systems TS 0.75 KW (IUL Instruments), and the DNA in the crude lysate was purified by chromatography on hydroxyapatite (Cashion et al., 1977). DDH was performed as described by Deley et al. (1970) and Huss et al. (1983) using a model Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multichanger and a temperature controller with in situ temperature probe (Varian). According to the DDH results, strain GSsed3\textsuperscript{T} did not belong to the species *Anoxybacillus rupiensis* (16% DNA–DNA relatedness), when the recommendation of a threshold value of 70% DNA–DNA relatedness is

![Fig. 1. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences. The tree shows the relationship between strains GSsed3\textsuperscript{T}, B2M1 and B7M1 and others members of the genus *Anoxybacillus*. Aeribacillus pallidus* was used as an outgroup. The phylogenetic tree was built online (phylogeny.fr). Multiple alignment was made using MUSCLE, and the phylogenetic tree was reconstructed using BioNJ. Bootstrap values based on 1000 replications were calculated and expressed as percentages. Bar, substitutions per nucleotide position. The NCBI accession number of each sequence is given in parentheses.](image-url)
considered for the definition of bacterial species as suggested by the ad hoc committee (Wayne et al., 1987). Likewise, the comparison of the whole genome sequences of strain GSsed3 with Anoxybacillus amylolyticus DSM 15939T (CP015438-CP015440) using GBDP2_BLASTPLUS (Meier-Kolthoff et al., 2013) indicated a DDH estimate (GLM-based) of 19.9 % (with an error of 2.3 %). These results were below the 70 % (the species DDH cutoff value), which also indicated that strain GSsed3T did not belong to the species Anoxybacillus amylolyticus. Finally, in order to confirm that the related strains (B2M1 and B7M1) belong to Anoxybacillus geothermalis sp. nov., we performed whole genome sequencing using the PacBio technology (CP015435-CP015437), followed by the calculation of average nucleotide identity (ANI) (Rodriguez-R & Konstantinidis, 2014) between strains GSsed3T and B2M1, strains GSsed3 and B7M1, and strains B2M1 and B7M1. The analysis showed an ANI of 99.9 % (SD 0.26 %), 99.9 % (SD 0.27 %) and 100 % (SD 0.04 %), respectively, which is higher than the accepted threshold of 95 % ANI used to define species (Rodriguez-R & Konstantinidis, 2014).

On the basis of the results obtained, GSsed3T is considered to represent a novel species of the genus Anoxybacillus. The novel isolate was characterized by polyphasic taxonomy, and a range of phenotypic and molecular characteristics were determined as recommended by the minimal standards for describing new taxa of aerobic, endospore-forming bacteria (Logan et al., 2009). Moreover, as recommended by the minimal standards, reference strains obtained from the DSMZ, Anoxybacillus caldiproteolyticus DSM 15730T, Anoxybacillus rupiensis DSM 17127T, Anoxybacillus tepidanus DSM 16325T, Anoxybacillus voinovskii DSM 17075T and Anoxybacillus amylolyticus DSM 15939T, were included in this comparative study.

Cell morphology, mean cell size at 24 h in modified D2216 liquid medium, and endospore formation were determined using light and phase-contrast microscopy (Leica DM R; magnification ×1000). Gram staining was performed on an overnight solid culture using the Hucker staining method (Gerhardt, 1994). Mobility was tested according to two different methods (Kohlmeier et al., 2005; Rashid & Kornberg, 2000). The composition of the swimming media were slightly different but both contained 0.3 % agar (w/v). The solid media were dried for 1 h under laminar flow. Colonies from an overnight solid culture were inoculated by thrusting a straight needle into the center of the petri dishes, and incubated for 24 h at 55 °C. Isolate GSsed3T was motile as the colony spread into the medium in comparison with a non-motile strain (Pseudomonas putida ΔfilM). Cells of GSsed3T were rods and Gram-staining-positive. Subterminal to terminal endospores were observed in slightly swollen sporangia (Fig. S1). The strain formed brown, circular colonies, sometimes in spindle form, when grown in modified D2216 medium. They had a diameter of 0.8–1.1 mm and showed spreading after 24 h of growth at 55 °C on modified D2216 solid medium. GSsed3T was motile as shown by the two methods.

Cell growth was monitored at different temperatures (25, 30, 35, 40, 45, 50, 55, 60, 65, 70 and 80 °C), measuring OD600 with a Genesys 10S UV-Vis spectrophotometer (Thermo-scientific). Salt tolerance was tested over 7 days in tryptic soy broth (TSB) medium prepared in-house using 15 g tryptone l−1, 5 g soytone l−1 and 15 g agar l−1, pH 7.0, supplemented with various concentrations of NaCl (0, 0.5, 1, 2, 3, 5, 7, 10 and 20 %, w/v). Cell growth was evaluated at OD600. To determine the pH suitable for growth, cells were inoculated in TSB medium adjusted to pH 3 to 11 (intervals of 0.5 pH unit), using acetate (for pH 3–5.5), phosphate (for pH 6–7.5) or glycine/NaOH (for pH 8.5–11) buffers at concentration 0.05 M, as previously suggested by Derekova et al., 2007. The need for oxygen during growth was verified using the method of thioglycollate medium (Brewer, 1940). According to the thioglycollate test, strains GSsed3T, B2M1 and B7M1 were facultative anaerobes. All growth experiments were performed in triplicate. Regarding the optimal growth conditions, strain GSsed3T, as well as the related strains isolated from another geothermal reservoir (strains B2M1, B7M1), displayed a growth range similar to other species of the genus Anoxybacillus (Table 1). The growth range of the isolates was 40–65 °C, with a temperature optimum at 60 °C. Strain GSsed3T and its related strains could tolerate up to 3 %, w/v, NaCl, with an optimum at 0.5 % NaCl. Growth was detected from pH 5 to 9.5. Optimum growth was observed at pH 7 to 8. Anaerobic growth was observed in contrast to Anoxybacillus rupe
tis DSM 17127T, which is a strict aerobe (Derekova et al., 2007).

All the biochemical tests used for the differentiation of strain GSsed3T were conducted in parallel with closely related species with validly published names, as well as for the other strains isolated from the other geothermal reservoir (Table 1). Nitrate reduction, casein hydrolysis, starch hydrolysis and oxidase activity tests were performed as described by Harrigan & McCance (1987). For the casein hydrolysis assay, the milk agar medium was modified to the following composition: peptone 0.2 % (w/v), glucose 0.2 % (w/v), Na2HPO4·2H2O 0.2 % (w/v), NaCl 1 %, agar 2 % and skimmed milk 10 %. Catalase, citrate utilization, gelatin hydrolysis, urea hydrolysis and aesculin hydrolysis were performed by methods described by Gerhardt (1994). Manitol test and indole production were tested as described by Joffin & Leyral (2001). Bacterial reference strains were used as positive and negative controls for each test. All tests were inoculated with colonies from an overnight solid culture. Incubation was performed at the optimal growth temperature of each individual strain. With the exception of the hydrolysis of gelatin and aesculin and the reduction of nitrate, all the strains from this study (GSsed3T, B2M1, B7M1) were consistent in the results of the tests performed. All the strains were positive for catalase and oxidase, as well as for the hydrolysis of casein and starch. Strain GSsed3T was negative in regards to nitrate reduction, negative for hydrolysis of gelatin and positive for the hydrolysis of aesculin, while strains B2M1 and B7M1 were the opposite for these three tests. The most striking difference between
strains GSsed3$^T$, B2M1, B7M1 and Anoxybacillus rupiensis DSM 17127$^T$ was the negative reaction for oxidase in the latter. Concerning other species of the genus Anoxybacillus, strain GSsed3$^T$ shared the capability to perform the hydrolysis of aesculin with Anoxybacillus caldiproteolyticus DSM 15730$^T$ and Anoxybacillus tepidiprotin DS M 16325$^T$.

Utilization of different carbon sources was assessed with two methods. First, the API 20NE system (bioMérieux) was used according to the manufacturer’s protocol. The API strips were incubated at 55°C in sterile glass Petri dishes containing sterile MilliQ water to prevent evaporation. Complementary analyses for utilization of different compounds as sole carbon and energy source were performed using D-xylan, D-pectin, (−)-D-salvin, dulcitol, (+)-cellibiose, inulin, olive oil, sodium acetate, sodium propionate, (+)-D-mannose, (−)-D-fructose, (−)-D-galactose, D-glucose, (+)-lactose, (+)-melibiose, myo-inositol, ribitol, (+)-raffinose, (−)-D-ribose, (+)-L-rhamnose, (+)-sucrose, D-sorbitol, D-xylene, glycerc, Tween 60, Tween 80, L-arabinose, D-mannitol, maltose, potassium glyconate, adipic acid, malic acid, citrate, N-acetylglucosamine, phenylacetic acid, capric acid, trisodium citrate, L-phenylalanine, L-tyrosine, xanthine and hypoxanthine in modified Adkins basal medium (Adkins

Table 1. Differential characteristics of Anoxybacillus geothermalis sp. nov. strains GSsed3$^T$, B2M1 and B7M1 and related species from the genus Anoxybacillus.

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<tr>
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<td>Sorbitol</td>
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<td>−</td>
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<tr>
<td><em>myo</em>-Inositol</td>
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<td>+</td>
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<tr>
<td>Tween 80</td>
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<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
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<td>Tween 60</td>
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<td>−</td>
<td>+</td>
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<td>−</td>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>46.9</td>
<td>42.5</td>
<td>42.5</td>
<td>41.7$^a$</td>
<td>43.2$^b$</td>
<td>43.5</td>
<td>40.2$^c$</td>
<td>43.9$^d$</td>
</tr>
</tbody>
</table>

*Data from: a, Dereko et al. (2007); b, Coorevits et al. (2012); c, Yumoto et al. (2004).
et al., 1992), containing (per litre of distilled water): 0.8 g of NaCl; 1.0 g NH₄Cl; 0.1 g KCl; 0.1 g K₂HPO₄; 0.2 g MgSO₄.7H₂O; 0.02 g CaCl₂.2H₂O; 0.2 g yeast extract (Merck); 10 g TES; 0.2 g CaCO₃ (precipitated chalk); finally, 5 ml of a trace metal solution and 10 ml of a vitamin solution (Tanner, 1989) were added. The pH of the medium was adjusted to pH 7. Carbohydrates and vitamin solutions were filter-sterilized (0.22 µm pore size). Sugar solutions were added at 5 g l⁻¹ in 10 ml of basal medium (Nazina et al., 2001). Modified D2216 agar culture (100 µl) diluted in physiological water (9 g NaCl l⁻¹) (OD₆₀₀=0.1) was inoculated and incubated under agitation at 55°C. Two successive inoculations were undertaken to confirm assurance (100 µl of culture in 10 ml of medium). Cultures were observed over 7 days. Assimilation was considered positive when turbidity of the culture was different from that of the negative control (Zhao et al., 2009). With the exception of assimilation of (+)-cellobiose, (+)-sucrose and (−)-D-fructose that were positive, and the negative assimilation of D-xylan, L-tyrosine, (−)-melibiose, Tween 80, xanthine and hypoxanthine, the utilization of other carbon sources varied among the three strains studied here (Table 1). Assimilation of (−)-D-fructose and a negative result for the assimilation of xanthine and hypoxanthine were common traits to all the Anoxybacillus strains studied. In contrast, olive oil assimilation by GSSed³ was a unique trait found in the characterization.

DNA G+C content was estimated to be 46.9 mol % based on the draft sequence of the full genome of strain GSSed³. Genome sequence and annotation of this strain has been submitted to Genbank under accession number YCG00000000.1 (Filippidou et al., 2015). The DNA G+C content of GSSed³ was slightly higher than the values reported for the reference strains (Table 1).

Analysis of fatty acid composition, respiratory menaquinones, peptidoglycan structure and polar lipids were performed using the identification services of DSMZ. Fatty acid composition comparison among strain GSSed³, Anoxybacillus rupiensis DSM 17127T, and Anoxybacillus tepidans DSM 16325T is shown in Table S2. The major fatty acids found in GSSed³ corresponded to iso-C₁₅:0 (35.3 %) and iso-C₁₇:0 (26.4 %), confirming the affiliation of the strain to the genus Anoxybacillus. However, in contrast to Anoxybacillus rupiensis DSM 17127T and Anoxybacillus tepidans DSM 16325T, the fatty acids C₁₆:0 and iso-C₁₆:0 represented a significant fraction of the fatty acids of the strain (14.5 and 10.1 %, respectively). In contrast, for Anoxybacillus rupiensis DSM 17127T these fatty acids represented 5.4 and 2.0 %, only. The significant contribution of these two fatty acids (C₁₆:0 and iso-C₁₆:0) is particularly noteworthy, as these fatty acids are rarely found representing 25 % of the total fatty acid content in other species of the genus Anoxybacillus (Cihan et al., 2011; Coorevits et al., 2012; Poli et al., 2009). The respiratory menaquinones found were MK-8 (92 %) and MK-7 (5 %). These appear also to be characteristic of strain GSSed³ compared with other species of the genus Anoxybacillus for which the information is available and that had as major respiratory menaquinone MK-7 (Cihan et al., 2011, 2014; Coorevits et al., 2012; Gul-Guven et al., 2008; Inan et al., 2013). The total hydrolysate (4 M HCl, 16 h at 100°C) of the peptidoglycan contained the amino acids meso-diaminopimelic acid (meso-Dpm), alanine (Ala) and glutamic acid (Glu). The partial hydrolysate (4 M HCl, 0.75 h at 100°C) contained (in addition to the amino acids) the peptides (L-Ala-D-glu and Dpm-D-al). The peptidoglycan type of strain GSSed³ was A1γ meso-Dpm-direct. The amount of peptidoglycan in the cell wall appeared to be rather low because meso-Dpm was also low in the hydrolysate (4 M HCl, 16 h at 100°C) of whole cells. This observation was also reported for Anoxybacillus calidus (Cihan et al., 2014). Analysis of the peptidoglycan structure of other species of the genus Anoxybacillus is still missing and thus defining if a low amount of meso-Dpm is a common feature of the genus Anoxybacillus cannot be assessed at the moment. The analysis of polar lipids, with abundant diphasphatidylglycerol (Fig. S2), showed a polar lipid profile of the strain GSSed³ similar to that of Anoxybacillus caldioxyteuticus, as shown in the supplementary material of Coorevits et al. (2012).

On the basis of the results presented in this study, we consider that strain GSSed³ represents a novel species of the genus Anoxybacillus, for which we propose the name Anoxybacillus geothermalis sp. nov.

**Description of Anoxybacillus geothermalis sp. nov.**

Anoxybacillus geothermalis (ge.o.ther.ma’lis. Gr. n. ge- earth; -thermalis of thermal properties or origin; N.L. masc. adj. geothermalis from hot earth, from a geothermal site).

Gram-stain-positive, motile rod of 0.8–1×2.5–2.7 µm in size. Elliptic terminal or subterminal endospores are observed in slightly swollen sporangia. Colonies on modified D2216 medium are brownish, smooth, opaque and often spreading. The diameter of the colonies is 0.8–1.1 mm after 24 h of growth at 55°C on modified D2216 solid medium. Growth occurs at 40–65°C (optimum 60°C), at pH 5.0–9.5 (optimum pH 7.0–8.0) and with NaCl (0–3 %, w/v; optimum 0.5 %). It is a facultative anaerobe, catalase- and oxidase-positive, hydrolyses casein, starch and aesculin, but not gelatin. Nitrate is not reduced to nitrite. Negative for indole production. It ferments mannitol. Assimilates cellobiose, galactose, glucose, ribose, D-xylene, glycerol, L-arabinose, D-mannitol, maltose, potassium gluconate, adipate, malate, sucrose and pectin. It assimilates fructose and produces gas aerobically, but does not change the pH of the culture medium. It does not assimilate citrate, lactose, myo inositol, rhamnose, sorbitol, Tween 80, N-acetylglucosamide, phenylacetic acid, capric acid, trisdium citrate, salicin, xylan, raffinose, ribitol or inulin. Phenylalanine is not deaminated, tyrosine is not degraded. The major cellular fatty acids are iso-C₁₅:0, iso-C₁₇:0, C₁₆:0 and...
iso-C_{16:0}. The major respiratory menaquinones are MK-8 and MK-7. The peptidoglycan type is A1γ meso-Dpm-direct. The polar lipid profile consists of phosphatidylethanolamine, phosphatidylglycerol, diphosphatidylglycero1, one unknown phosphoglycolipid and one unknown phospholipid.

The type strain, GSsed3^{T} (=CCOS808^{T}=ATCC BAA2555^{T}), was isolated from deposits from a filter in Groß Schönebeck power plant, Germany. The DNA G+C content of the type strain is 46.9 mol%.

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References


