Genome sequence of *Anoxybacillus ayderensis* AB04T isolated from the Ayder hot spring in Turkey

Ali Osman Belduz¹, Sabriye Canakci¹, Kok-Gan Chan², Ummirul Mukminin Kahar³, Chia Sing Chan³, Amira Suriati Yaakop³ and Kian Mau Goh³*²

**Abstract**

Species of *Anoxybacillus* are thermophiles and, therefore, their enzymes are suitable for many biotechnological applications. *Anoxybacillus ayderensis* AB04T (= NCIMB 13972T = NCCB 100050T) was isolated from the Ayder hot spring in Rize, Turkey, and is one of the earliest described *Anoxybacillus* type strains. The present work reports the cellular features of *A. ayderensis* AB04T, together with a high-quality draft genome sequence and its annotation. The genome is 2,832,347 bp long (74 contigs) and contains 2,895 protein-coding sequences and 103 RNA genes including 14 rRNAs, 88 tRNAs, and 1 tmRNA. Based on the genome annotation of strain AB04T, we identified genes encoding various glycoside hydrolases that are important for carbohydrate-related industries, which we compared with those of other, sequenced *Anoxybacillus* spp. Insights into under-explored industrially applicable enzymes and the possible applications of strain AB04T were also described.

**Keywords:** *Anoxybacillus*, *Bacillaceae*, *Bacillus*, *Geobacillus*, Glycoside hydrolase, Thermophile

**Introduction**

The family *Bacillaceae* [1, 2] is one of the largest bacterial families and currently consists of 57 genera [3]. The *Bacillaceae* are either rod-shaped (bacilli) or spherical (cocci) Gram-positive bacteria, the majority of which produce endospores [4]. *Anoxybacillus* [5, 6] is one of the genera within the *Bacillaceae* [1, 2], classified within the phylum *Firmicutes* [7], class *Bacilli* [8, 9], and order *Bacillales* [1, 10].

*Anoxybacillus* spp. are alkalo-thermophiles with optimum growth at temperatures between 50 °C and 65 °C and at pH 5.6–9.7 [4]. Most of the *Anoxybacillus* spp. are found in hot springs [4], but *Anoxybacillus* has also been found in animal manure [5], contaminated diary and meat products [4], animals (i.e., fish gut) [4], insects (i.e., glassy-winged sharpshooter and spiraling whitefly) [11], and plants (i.e., Indian mulberry) [11]. To date, a total of 22 species and two subspecies of *Anoxybacillus* have been described [4, 12, 13].

Almost all members of the *Bacillaceae* are excellent industrial enzyme producers [4, 14, 15]. Members of the genus *Anoxybacillus* exhibit the additional advantage of thermostability compared to the mesophilic *Bacillaceae*. It has been reported that enzymes from *Anoxybacillus* spp. can degrade various substrates such as starches, cellulose, fats, and proteins [4]. Many carbohydrate-encoding genes have been identified in *Anoxybacillus* spp. genomes, and some of the well-studied starch-degrading enzymes are α-amylase [16], pullulanase [17], amylo-pullulanase [18], CDase [19], and xylene-isomerase [20]. In addition, xylanolytic enzymes such as xylanase [21] and α-L-arabinofuranosidase [22] have been characterized from *Anoxybacillus* spp. Apart from their hydrolytic capabilities, *Anoxybacillus* spp. have been proposed as agents for bioremediation of Hg²⁺, Cr²⁺, Al³⁺, As³⁺ ions [4, 23–25], and nitrogen oxide [26], and as possible candidates for biohydrogen production [4].

Among the members of the family *Bacillaceae*, intensive genome sequencing efforts have been undertaken for *Geobacillus* [27] (>80 projects) and *Bacillus* [1, 28] (>1,500 projects), which have been registered in the NCBI BioProject database. In contrast, genomic...
studies on Anoxybacillus are rather limited, with only 16 registered projects. At present, the genome of Anoxybacillus flavithermus WK1 is the only completely sequenced genome (BioProject accession number PRJNA59135) among the Anoxybacillus spp. [5, 29]. Draft genome sequences are available for Anoxybacillus ayderensis AB04T (PRJNA258494; this study) [30], Anoxybacillus sp. BCO1 (PRJNA261743) [31, 32], Anoxybacillus thermarum AF/04T (PRJNA260786) [33–35], Anoxybacillus gongensis G2T (PRJNA264351) [36], Anoxybacillus sp. ATCC BAA-2555 (PRJNA260743), Anoxybacillus sp. KU2-6(11) (PRJNA258246), Anoxybacillus tepidans PS2 (PRJNA214279) [37], A. flavithermus 25 (PRJNA258119) [5, 38], A. flavithermus AK1 (PRJNA190633) [5, 39], Anoxybacillus kamchatken-sis G10 (PRJNA170961) [40–42], A. flavithermus Kn10 (PRJDB1085) [5, 43], A. flavithermus TNO-09.006 (PRJNA169174) [5, 44], Anoxybacillus sp. SK3-4 (PRJNA174378) [45, 46], Anoxybacillus sp. DT-3-1 (PRJNA182115) [45, 46], and A. flavithermus subsp. yunnanensis E13T (PRJNA213809) [35, 47, 48]. Therefore, the genomic study of Anoxybacillus spp. is essential not only to fully understand their biochemical networks, but also to discover their potential applicability in industrial processes.

In the present report, we describe the cellular features of A. ayderensis AB04T and we present a high-quality annotated draft genome of strain AB04T. Additionally, we provide a comparative analysis of the GHs of strain AB04T and other sequenced Anoxybacillus spp. In addition, we discuss the presence of other under-explored industrial enzymes and the potential applications of the bacterium.

Organism information

Classification and features
A. ayderensis AB04T (= NCIMB 13972T = NCCB 100050T) was isolated from mud and water samples from the Ayder hot spring located in the province of Rize in Turkey [30]. Microscopic examination revealed that colonies of strain AB04T were cream-colored, regular in shape with round edges, and 1–2 mm in diameter.

Phenotypic analysis revealed that strain AB04T is a Gram-positive, rod-shaped, motile, and spore-forming bacterium [30]. It is a facultative anaerobe, moderate thermophile that grows well at 30–70 °C (optimum 50 °C) and at pH 6.0–11.0 (optimum pH 7.5–8.5) (Table 1). FESEM showed that cells of the strain AB04T were 0.7–0.8 × 3.5–5.0 μm in size (Fig. 1). The strain gave positive responses for catalase and oxidase activity, and was able to reduce nitrate to nitrite. Strain AB04T was capable of utilizing a wide range of carbon sources including starch, gelatin, D-glucose, D-raffinose, D-sucrose, D-xyllose, D-fructose, L-arabinose, maltose, and D-mannose. The strain grew optimally in the presence of 1.5 % (w/v) NaCl, but it was able to grow in the absence of NaCl. Growth was inhibited in the presence of ampicillin (25 μg/ml), streptomycin sulphate (25 μg/ml), tetracycline (12.5 μg/ml), gentamicin (10 μg/ml), and kanamycin (10 μg/ml). The FAME profile showed that the major fatty acid in AB04T is C15:0 iso (48.17 %), followed by C17:0 iso (20.62 %), C17:0 anteiso (9.22 %), C16:0 (9.10), C16:0 iso (7.47 %), C15:0 anteiso (3.58 %), C14:0 (1.02 %), and C15:0 (0.83 %) [30].

The 16S rRNA-based phylogenetic tree constructed using MEGA6.0 [49] showed that strain AB04T clusters together with Anoxybacillus sp. SK3-4 [45, 46] and A. thermarum AF/04T [33–35] (Fig. 2). Pairwise 16S rRNA sequence similarities among the strains were determined using the EzTaxon server [50], revealing that AB04T shares 99.6 % and 99.2 % similarity with Anoxybacillus sp. SK3-4 [45, 46] and A. thermarum AF/04T [33–35], respectively.

Genome sequencing information

Genome project history
Genomic studies on the genus Anoxybacillus are relatively limited [45]. Hence, the findings of the genomic study on A. ayderensis AB04T presented in this study are important because they contribute to the body knowledge of the Anoxybacillus genomes. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number JXTG00000000. The NCBI BioProject accession number is PRJNA258494. The GOLD Project ID for strain AB04T is Gp0026071. Table 2 presents the project information and its association with MIGS version 2.0 compliance.

Growth conditions and genomic DNA preparation
A. ayderensis AB04T was plated on Nutrient Agar (pH 7.5) and incubated at 50 °C for 18 h. A single colony was transferred into Nutrient Broth (pH 7.5) and incubated at 50 °C with rotary shaking at 200 rpm for 18 h. The cells were harvested by centrifugation at 10,000 × g for 5 min using a Microfuge 16 centrifuge (Beckman Coulter, Brea, CA, USA). Genomic DNA was extracted using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. The purity, quality, and concentration of the genomic DNA were determined using a 6 % (w/v) agarose gel, NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and Qubit 2.0 fluorometer (Invitrogen, Merelbeke, Belgium).

Genome sequencing and assembly
The genome of A. ayderensis AB04T was sequenced using the Illumina MiSeq sequencing platform (Illumina, San Diego, CA, USA) with 300-bp paired-end reads. The adapter sequences were removed and low quality regions and reads were filtered out using Trimmomatic [51] (Phred score = 25 (Q25), sliding window = 4 bp, leading and trailing qualities = 3, and minimum read length = 36 bp),
Scythe (UC Davis Bioinformatics Core, Davis, DA, USA) (prior contamination rate = 0.3, minimum match length argument = 5, and minimum sequence to keep after trimming = 36 bp), and String Graph Assembler (SGA) [52] (k-mer threshold = 3, k-mer rounds = 10, and read error correction = 0.04). Next, the reads were subjected to de novo genome assembly using IDBA-UD 1.0.9 [53] (k_{min} = 3 5 ) .

Genome annotation

Genes, tRNAs and tmRNAs, and rRNAs were predicted with Prodigal [54], ARAGORN [55], and RNAmmer [56], respectively. For functional annotation, the predicted coding sequences were translated and used to search for the closest matches in the NCBI non-redundant database and the UniProt [57], TIGRFAM [58], Pfam [59], CRISPRfinder [60], PRIAM [61], KEGG [62], COG [63], and InterProScan 5 [64] databases. The GHs were identified and verified.
Fig. 2  Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between A. ayderensis AB04\(^\top\) and representative Anoxybacillus spp. The 16S rRNA accession number for each strain is shown in brackets. The 16S rRNA sequences were aligned using ClustalW and the tree was constructed using the ML method with 1000 bootstrap replicates embedded in the MEGA6.0 package [49]. The scale bar represents 0.01 nucleotide substitutions per position. Brevibacillus brevis NCIMB 9372\(^\top\) [77] was used as an out-group. Type strains are indicated with a superscript T. Published genomes are indicated in blue.
using the dbCAN CAZy [65], NCBI BLASTp, and InterProScan 5 [64] databases. Genome comparison was done by the ANI function in the EzTaxon-e database [66].

**Genome properties**

The overall genome coverage was approximately 239-fold. The draft genome was assembled into 74 contigs with a total length of 2,832,347 bp and a G + C content of 41.8% (Fig. 3 and Table 3). The longest and shortest contigs were 448,584 bp and 606 bp, respectively. The mean length of the contigs was 38,275 bp and the N50 contig length was 112,260 bp. We did not detect any additional DNA elements. The genome consisted of 2,998 predicted genes, of which 2,895 were protein-coding sequences and 103 were RNA genes including 14 rRNAs, 88 tRNAs, and 1 tmRNA. A total of 235 (8.1%) genes were assigned a putative function. The remaining annotated genes (1023; 35.3%) were hypothetical proteins. The properties and the statistics of the genome are summarized in Table 3. The distribution of genes into COGs and KEGG functional categories is presented in Table 4 and Fig. 3.

| Table 2 Project information |
|----------------------------|
| MIGS ID | Property | Term |
| MIGS-31  | Finishing quality | High-quality draft |
| MIGS-28  | Libraries used | Illumina Paired-End library |
| MIGS-29  | Sequencing platforms | Illumina MiSeq |
| MIGS-31.2 | Fold coverage | 239 x |
| MIGS-30  | Assemblers | IDBA-UD 1.0.9 |
| MIGS-32  | Gene calling method | Prodigal 2.60 |
| Locus Tag | JV16 |
| Genbank ID | JXTG00000000 |
| Genome Data of Release | February 9, 2015 |
| GOLD ID | Gp0026071 |
| BIOPROJECT | PRJNA258494 |
| MIGS-13  | Source Material Identifier | NCIMB 13972T |
| Project relevance | Biotechnology |

**Fig. 3** A graphical circular map of the *A. ayderensis* AB04T genome. From outside to the center: genes on the forward strand (colored by COG categories), genes on forward strand (red), genes on reverse strand (blue) and genes on the reverse strand (colored by COG categories).
Insights from the genome sequence

Genome features of *A. ayderensis* AB04\(^T\) and other *Anoxybacillus* spp

The genome sizes of the currently sequenced *Anoxybacillus* spp. are shown in Fig. 2. Most of the reported *Anoxybacillus* draft genome sizes are between 2.60 and 2.86 Mb [31, 33, 38–40, 43–45, 47], and the completely sequenced *A. flavithermus* WK1 genome has a size of 2.85 Mb [29]. The incomplete genome sequence of *A. tepidamans* PS2 has a size of 3.36 Mb (Fig. 2), which is the largest *Anoxybacillus* genome sequenced to date [37]. However, cumulative information on the *Anoxybacillus* genomes (Fig. 2) indicates that *Anoxybacillus* has a smaller genome size than the closest genus, *Geobacillus* (~3.50 Mb) [27, 45]. The genomes of other genera within *Bacillaceae* such as *Bacillus* [1, 28] and *Lysinibacillus* [67] are at least 40 % larger than that of *Anoxybacillus* [5, 6, 45]. The average G + C content of the *Geobacillus* spp. genomes (~50.0 %) [27, 45] is slightly higher than that of the *A. ayderensis* [30] genome (Fig. 2), while most *Bacillus* genomes have less than 40 % G + C content [1, 28, 45].

Table 3 summarizes the pairwise ANI values of *Anoxybacillus* spp. [66]. *A. ayderensis* AB04\(^T\) showed the highest ANI of 97.6 % with *Anoxybacillus* sp. SK3-4 [46]. As this ANI value is greater than 95 % [68], *Anoxybacillus* sp. SK3-4 [45, 46] is likely to be a subspecies of *A. ayderensis* [30].

**Analysis of the GHS in *A. ayderensis* AB04\(^T\) and other *Anoxybacillus* genomes**

We detected 14 genes in the AB04\(^T\) genome encoding GH enzymes belonging to GH families 1, 10, 13, 31, 32, 51, 52, and 67 (Table 6). On average, the AB04\(^T\) GHS shared 93.9 % similarity with GHS identified in other *Anoxybacillus* spp. The GHS could be grouped into two types according to their predicted catalytic ability (Table 6). Nine GHS were predicted to be active on α-chain polysaccharides whereas the remaining five GHS were specific for β-linked polysaccharides (i.e., cellulose and xylan).

Interestingly, we found two GH enzymes that were uniquely present in strain AB04\(^T\): endo-1,4-β-xylanase (NCBI locus ID: KIP21668) and α-glucuronidase (KIP 21917) (Table 6). The closest homologs of endo-1,4-β-xylanase and α-glucuronidase were found in *Geobacillus thermoglucosidans* and *Geobacillus stearothermophilus*.

| Attribute                        | Value   | % of Total |
|----------------------------------|---------|------------|
| Genome size (bp)                 | 2,832,347 | 100.00    |
| DNA coding (bp)                  | 2,517,744 | 88.89     |
| DNA G + C (bp)                   |         | 41.83     |
| DNA scaffolds                     | 74      | 100.00    |
| Total genes                      | 2,998   | 100.00    |
| Protein coding genes             | 2,895   | 96.56     |
| RNA genes                        | 103     | 3.44      |
| Pseudo genes                     |         | not determined |
| Genes in internal clusters       |         | not determined |
| Genes with function prediction   | 1,637   | 54.60     |
| Genes assigned to COGs           | 2,349   | 78.35     |
| Genes with Pfam domains          | 2,158   | 71.98     |
| Genes with signal peptides       | 103     | 3.44      |
| Genes with transmembrane helices | 674     | 23.28     |
| Number of CRISPR candidates      | 3       |           |
| Confirmed CRISPR(s)              | 1       |           |
| Unconfirmed CRISPR(s)            | 2       |           |

*The total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.

| Attribute                        | Value   | % of Total |
|----------------------------------|---------|------------|
| Code | Value | % age | Description                                      |
|------|-------|------|-------------------------------------------------|
| J    | 153   | 5.10 | Translation, ribosomal structure and biogenesis |
| A    | 1     | 0.03 | RNA processing and modification                 |
| K    | 169   | 5.64 | Transcription                                    |
| L    | 165   | 5.50 | Replication, recombination and repair            |
| B    | 1     | 0.03 | Chromatin structure and dynamics                 |
| D    | 38    | 1.27 | Cell cycle control, Cell division, chromosome partitioning |
| V    | 27    | 0.90 | Defense mechanisms                               |
| T    | 162   | 5.40 | Signal transduction mechanisms                   |
| M    | 117   | 3.90 | Cell wall/membrane biogenesis                    |
| N    | 80    | 2.67 | Cell motility                                    |
| U    | 53    | 1.77 | Intracellular trafficking and secretion          |
| O    | 99    | 3.30 | Posttranslational modification, protein turnover, chaperones |
| C    | 145   | 4.84 | Energy production and conversion                 |
| G    | 169   | 5.64 | Carbohydrate transport and metabolism            |
| E    | 234   | 7.81 | Amino acid transport and metabolism              |
| F    | 71    | 2.37 | Nucleotide transport and metabolism              |
| H    | 120   | 4.00 | Coenzyme transport and metabolism                |
| I    | 81    | 2.70 | Lipid transport and metabolism                   |
| P    | 140   | 4.67 | Inorganic ion transport and metabolism           |
| Q    | 29    | 0.97 | Secondary metabolites biosynthesis, transport and catabolism |
| R    | 274   | 9.14 | General function prediction only                 |
| S    | 261   | 8.71 | Function unknown                                 |
|     | 409   | 13.64 | Not in COGs                                    |

*The total is based on the total number of protein coding genes in the annotated genome.*
with 81.9 % and 87.1 % sequence similarity, respectively [27].

Genes coding for at least five of the aforementioned GHs including cell-bound α-amylase, pullulanase, CDase, oligo-1,6-glucosidase, and α-glucosidase were consistently found in the genomes of all Anoxybacillus spp. (Table 6). Therefore, these enzymes might play an important role in Anoxybacillus carbohydrate metabolism. A high molecular-mass amylopullulanase (>200 kDa) from Anoxybacillus sp. SK3-4 has been reported previously [18]. We detected this enzyme in other Anoxybacillus spp., for instance A. flavithermus WK1 [5, 29], A. flavithermus subsp. yunnanensis E13 T [35, 47, 48], SK3-4 = Anoxybacillus sp. SK3-4 [45, 46]; DT3-1 = Anoxybacillus sp. DT3-1 [45, 46]; TNO = A. flavithermus TNO-09.006 [5, 44]; G10 = A. kamchatkensis G10 [40–42]; G2 T = A. gonensis G2 T [36]; AT T = A. thermarum AF/04 T [33–35]; AK1 = A. flavithermus AK1 [5, 39]; BCO1 = Anoxybacillus sp. BCO1 [31, 32]; KU2-6 = Anoxybacillus sp. KU2-6(11); Kn10 = A. flavithermus Kn10 [5, 43]; PS2 = A. tepidamans PS2 [37]; 25 = A. flavithermus 25 [5, 38]

### Table 5 Genomic comparison of A. ayderensis AB04 T and 15 other sequenced Anoxybacillus spp. using ANI [66]

|          | AB04 | WK1  | E13  | SK3-4 | DT3-1 | TNO  | G10  | G2 T | AF/04 T | AK1   | BCO1 | KU2-6 | Kn10 | PS2 | 25 |
|----------|------|------|------|-------|-------|------|------|------|--------|-------|------|-------|------|-----|-----|
| AB04     | 100.00 | 87.9 | 87.3 | 97.6  | 94.5  | 87.3 | 94.3 | 94.3 | 94.7   | 85.7  | 97.5 | 89.6  | 88.2 | 72.4 | 97.6 |
| WK1      | 87.9  | 100.00 | 88.4 | 88.0  | 91.8  | 98.2 | 88.2 | 87.8 | 84.8   | 87.7  | 89.8 | 95.0  | 72.5 | 87.5 |
| E13 T    | 87.3  | 88.3  | 100.00 | 87.3 | 87.2  | 88.3 | 86.9 | 87.1 | 86.9   | 85.2  | 87.1 | 89.9  | 89.1 | 72.3 | 87.1 |
| SK3-4    | 97.5  | 88.1  | 87.2  | 100.00 | 94.0  | 87.5 | 93.7 | 93.9 | 94.2   | 85.8  | 96.9 | 89.5  | 88.3 | 72.5 | 96.9 |
| DT3-1    | 94.6  | 88.0  | 87.2  | 94.1  | 100.00 | 87.0 | 98.5 | 98.6 | 94.1   | 85.3  | 94.4 | 89.8  | 88.0 | 72.4 | 94.1 |
| TNO      | 87.5  | 91.8  | 88.4  | 87.7  | 91.0  | 87.1 | 87.0 | 87.3 | 87.5   | 87.4  | 88.6 | 92.5  | 72.5 | 87.3 |
| G10      | 94.3  | 88.2  | 86.8  | 93.8  | 98.5  | 87.0 | 100.00 | 98.8 | 93.7   | 85.3  | 94.3 | 89.7  | 88.2 | 72.6 | 94.0 |
| G2 T     | 94.4  | 88.2  | 87.1  | 94.0  | 98.5  | 87.0 | 98.8 | 100.00 | 93.8  | 85.3  | 94.2 | 89.7  | 88.3 | 72.4 | 93.8 |
| AF/04 T  | 94.8  | 87.9  | 87.0  | 94.2  | 94.1  | 87.2 | 93.8 | 93.8 | 100.00 | 86.1  | 94.1 | 89.1  | 88.1 | 72.7 | 94.0 |
| AK1      | 85.7  | 84.8  | 85.1  | 85.7  | 85.3  | 87.5 | 85.2 | 85.2  | 86.0   | 100.00 | 86.1 | 85.0  | 84.9 | 72.3 | 85.2 |
| BCO1     | 97.6  | 87.6  | 87.1  | 97.0  | 94.4  | 97.2 | 94.3 | 94.1 | 94.2   | 98.6  | 100.00 | 89.4 | 87.9 | 72.4 | 97.1 |
| KU2-6    | 89.5  | 89.8  | 90.0  | 89.5  | 89.7  | 88.6 | 89.5 | 89.6 | 89.0   | 85.0  | 89.4  | 100.00 | 90.8 | 72.5 | 89.3 |
| Kn10     | 88.1  | 94.9  | 89.0  | 88.1  | 88.0  | 92.6 | 88.0 | 88.3 | 87.9   | 84.8  | 87.8 | 90.8  | 100.00 | 72.4 | 87.7 |
| PS2      | 72.4  | 72.4  | 72.2  | 72.4  | 72.6  | 72.5 | 72.4 | 72.6 | 72.3   | 72.5  | 72.3  | 72.5  | 100.00 | 72.5 |
| 25       | 97.6  | 87.5  | 87.1  | 97.0  | 94.0  | 86.9 | 93.9 | 93.8 | 94.0   | 85.2  | 97.0  | 89.3  | 87.8 | 72.7 | 100.00 |

The ANI value (%) shared between genomes (above and below diagonal). AB04 T = A. ayderensis AB04 T [30]; WK1 = A. flavithermus WK1 [5, 29]; E13 T = A. flavithermus subsp. yunnanensis E13 T [35, 47, 48]; SK3-4 = Anoxybacillus sp. SK3-4 [45, 46]; DT3-1 = Anoxybacillus sp. DT3-1 [45, 46]; TNO = A. flavithermus TNO-09.006 [5, 44]; G10 = A. kamchatkensis G10 [40–42]; G2 T = A. gonensis G2 T [36]; AT T = A. thermarum AF/04 T [33–35]; AK1 = A. flavithermus AK1 [5, 39]; BCO1 = Anoxybacillus sp. BCO1 [31, 32]; KU2-6 = Anoxybacillus sp. KU2-6(11); Kn10 = A. flavithermus Kn10 [5, 43]; PS2 = A. tepidamans PS2 [37]; 25 = A. flavithermus 25 [5, 38]

Other A. ayderensis AB04 T enzymes with potential applications

Apart from the GHS, we found that A. ayderensis AB04 T has genes coding for other industrially important enzymes such as xylose isomerase, esterase, and aldolase. Xylose isomerase (EC 5.3.1.5) catalyzes the isomerization of xylose to xylulose and of glucose to fructose, which is important in the industrial production of high-fructose corn syrup [20]. Earlier, a xylose isomerase from A. gonensis G2 T was characterized and the enzyme displays 96.8 % amino acid sequence similarity to the one identified in strain AB04 T (KIP21927) [20].

Previous studies have indicated that A. gonensis G2 T, A. gonensis A4, and Anoxybacillus sp. PDF-1 produce esterase [70–72]. We identified two esterases (KIP19922 and KIP21735) in the genome of strain AB04 T, which shared 96.3 % and 96.0 % amino acid sequence similarity with the esterase from Anoxybacillus sp. PDF-1 [72] and A. gonensis G2 T [70], respectively. In addition, a fructose-1,6-bisphosphate aldolase from A. gonensis G2 T has been described [73]. Strain AB04 T carries two aldolases, KIP21451 and KIP21450, which showed 95.9 % and 99.9 % amino acid similarity to aldolase from A. flavithermus WK1 [5, 29] and A. thermarum AF/04 T [33–35], respectively. We did not biochemically characterize these enzymes from strain AB04 T in the current study.

Thermophilic bacteria are highly sought after for their potential use in bioremediation processes. Several Anoxybacillus spp. efficiently reduce metal ions such as Hg2+. 
### Table 6
List of several glycoside hydrolases (GHs) identified in various *Anoxybacillus* genomes

| GH Enzyme                                      | Similarity within *Anoxybacillus* genome\(^a\) | Number of studied enzyme\(^b\) |
|-----------------------------------------------|-----------------------------------------------|-------------------------------|
|                                               | ABO4  | WK1  | E13  | SK3-4 | DT3-1 | TNO  | G10  | G\(^2\) | AF/04\(^1\) | AK1  | BCO1 | KU2-6 | Kn10 | PS2 |
| 1 β-glucosidase                               | 91.2  | 100  | 90.7 | 39.5  | 92.4  | -    | -    | -       | -    | -    | -    | 91.6 | 92.0 | -    |
| 10 Endo-1,4-β-xylanase                        | 100.00| -    | -    | -    | -    | -    | -    | -       | -    | -    | -    | -    | -    | -    |
| 13 α-amylose (cell-bound)                     | 98.6  | 100.00| 98.4 | 97.4  | 96.2  | 97.2 | 96.2 | 98.2    | 98.6 | 94.5 | 76.6 | 98.8 | 99.8 | 84.3 | 2 [16] |
| 13 α-amylose (extracellular)                  | 77.6  | -    | 100.00| -    | -    | 95.0 | -    | -       | -    | -    | 54.2 | -    | -    | -    | -    |
| 13 Pullulanase                                | 93.4  | 100.00| 93.9 | 90.5  | 89.5  | 95.6 | 89.4 | 91.9    | 93.2 | 88.9 | 59.9 | 94.8 | 98.0 | 67.4 | 1 [17] |
| 13 Amylopullulanase (>200 kDa)                | -     | 100.00| 90.2 | 88.8  | -    | -    | 99.1 | -       | -    | 59.6 | -    | -    | 89.6 | -    | 1 [18] |
| 13 Amylopullulanase (<200 kDa)                | -     | -    | -    | -    | -    | -    | -    | 100     | -    | -    | -    | -    | -    | -    | -    |
| 13 CDase                                      | 95.8  | 100.00| 95.6 | 92.5  | 92.0  | 94.7 | 92.3 | 95.1    | 94.9 | 93.6 | 96.6 | 95.9 | 98.1 | 78.8 | 1 [19] |
| 13 Oligo-1,6-glucosidase                      | 98.2  | 100.00| 61.7 | 96.0  | 96.0  | 98.1 | 96.0 | 53.9    | 97.5 | 97.0 | 53.6 | 96.5 | 98.6 | 90.9 | -    |
| 13 Trehalase-6-phosphate hydrolase            | 95.6  | 100.00| -    | 94.2  | 93.7  | -    | 93.9 | -       | 96.2 | 94.9 | -    | 95.8 | 99.1 | -    | -    |
| 13 1,4-α-glucan branching enzyme              | 93.4  | 100.00| -    | 93.2  | 92.8  | 94.1 | 92.4 | -       | 93.9 | 94.9 | -    | -    | -    | -    | -    |
| 31 α-glucosidase                              | 92.1  | 100.00| 92.9 | 91.0  | 89.2  | 88.1 | 89.2 | 91.5    | 91.7 | 88.5 | 71.1 | 93.4 | 96.9 | 67.4 | -    |
| 32 Sucrase-6-phosphate hydrolase              | 94.7  | 100.00| -    | 91.3  | 91.8  | 91.0 | 91.3 | -       | 93.5 | 92.5 | -    | 93.9 | 93.3 | -    | -    |
| 36 α-galactosidase                            | -     | 91.2  | -    | -    | -    | -    | 79.2 | -       | 100  | 90.5 | 72.3 | 93.7 | -    | 79.5 | -    |
| 51 α-L-arabinofuranosidase                    | 93.6  | 100.00| -    | -    | -    | -    | -    | -       | 99.4 | -    | -    | -    | -    | -    | 1 [22] |
| 52 β-xylanosidase                             | 91.5  | 90.4  | -    | -    | -    | -    | -    | 100     | 99.6 | -    | -    | -    | 89.5 | -    | -    |
| 65 Sugar hydrolase/phosphorylase              | -     | 100.00| -    | 94.9  | 94.1  | -    | 94.0 | -       | 94.1 | -    | -    | 96.6 | -    | -    | -    |
| 67 α-glucuronidase                            | 100.00| -    | -    | -    | -    | -    | -    | -       | -    | -    | -    | -    | -    | -    | -    |

\(^a\)The reference for the protein sequence alignment is denoted as 100 %; \(^b\)The numbers represent the respective cloned, purified, and characterized enzymes from *Anoxybacillus* species. ABO4\(^{1}\) = *A. ayderensis* ABO4\(^{1}\) [30]; WK1 = *A. flavithermus* WK1 [5, 29]; E13\(^{1}\) = *A. flavithermus* subsp. yunnanensis E13\(^{1}\) [35, 47, 48]; SK3-4 = *Anoxybacillus* sp. SK3-4 [45, 46]; DT3-1 = *Anoxybacillus* sp. DT3-1 [45, 46]; TNO = *A. flavithermus* TNO-09.006 [5, 44]; G10 = *A. kamchatkensis* G10 [40–42]; G\(^2\) = *A. gonensis* G\(^2\) [36]; AT\(^{1}\) = *A. thermarum* AF/04\(^{1}\) [33–35]; AK1 = *A. flavithermus* AK1 [5, 39]; BCO1 = *Anoxybacillus* sp. BCO1 [31, 32]; KU2-6 = *Anoxybacillus* sp. KU2-6(11); Kn10 = *A. flavithermus* Kn10 [5, 43]; PS2 = *A. tepidamans* PS2 [37]
Cr\textsuperscript{6+}, Al\textsuperscript{3+}, and As\textsuperscript{3+} [4, 23–25]. The genome of strain AB04\textsuperscript{T} contains at least six heavy metal resistance genes. Four genes are related to mercuric ion reduction; two of these are mercury resistance (mer) operons (KIP20706 and KIP20408) and the other two genes encode mercuric reductases, which catalyze the reduction of Hg\textsuperscript{2+} to Hg\textsuperscript{0} (KIP19952 and KIP20409). In addition, strain AB04\textsuperscript{T} carries genes for an arsenate reductase (KIP20402) and an arsenic efflux pump protein (KIP20401). The function of these genes will be studied in the close future.

Conclusions
Knowledge on the genomics, industrial enzymes, and relevant applications of Anoxybacillus spp. are rather limited compared to that in their closest relatives, Geobacillus and Bacillus. In the present work we presented a whole-genome sequence of \textit{A. ayderensis} AB04\textsuperscript{T} and its annotation. Additionally, we provided insights into several GHS, under-explored enzymes, and putative applications of strain AB04\textsuperscript{T}.

Abbreviations
AN: Average Nucleotide Identity; Cabbage: Cyclomaltodextrinase; FAME: Fatty acid methyl esters; FESFM: Field emission scanning electron microscope; GH: Glycoside hydrolase; ML: Maximum-likelihood.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
AOB, SC, K-GC, UMK, CSC, ASY, KMG carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements
This work was supported by the University of Malaya via High Impact Research Grants (UM/625/1/HIR/MOHE/CHAN/01 [Grant No. A-00000-5001] and UM/625/1/HIR/MOHE/CHAN/14/1 [Grant No. H-50001-A000027]) awarded to K-GC. KMG appreciates the funding from Universiti Teknologi Malaysia (GUP UM.C/625/1/HIR/MOHE/CHAN/14/1 [Grant No. A-00001-50001] and GUP UM.C/625/1/HIR/MOHE/CHAN/01 [Grant No. A-000001-50001]) awarded to UMK. ASY is grateful to UTM Zamalah for providing a graduate scholarship.

Author details
1Faculty of Sciences, Department of Biology, Karadeniz Technical University, 61080 Trabzon, Turkey. 2Division of Genetics and Molecular Biology, Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603 Kuala Lumpur, Malaysia. 3Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia.

Received: 18 March 2015 Accepted: 4 September 2015
Published online: 26 September 2015

References
1. Skerman VBD, McGowan V, Sneath PHA. Approved lists of bacterial names. Int J Syst Bacteriol. 1980;30:225–402.
2. Fischer A. Untersuchungen über bakteriën. Jahrbücher für Wissenschaftliche Botanik. 1895:271–163.
3. Taxon Abstract for the family Bacillaceae. NamesforLife, LLC. Retrieved June 13, 2015. http://dx.doi.org/10.1610/bx.4856.
4. Goh KM, Kahar UM, Chai YY, Chong CS, Chai KP, Ranjani V, et al. Recent discoveries and applications of Anoxybacillus. Appl Microbiol Biotechnol. 2013;97:1475–88.
5. Piikuta E, Lyenka A, Chuliviskayi N, Mendrock U, Hippe H, Suzina N, et al. Anoxybacillus puchinhaensis gen. nov., sp. nov., a novel anaerobic, alkaliphilic, moderately thermophilic bacterium from manure, and description of Anoxybacillus flavithermus comb. nov. Int J Syst Evol Microbiol. 2000;50:2109–17.
6. Piikuta E, Cleland D, Tang J. Aerobic growth of Anoxybacillus puchinhaensis K1\textsuperscript{+}, emended descriptions of \textit{A. puchinhaensis} and the genus Anoxybacillus. Int J Syst Evol Microbiol. 2003;53:1561–2.
7. Gibbons NE, Murray RGE. Proposals concerning the higher taxa of bacteria. Int J Syst Bacteriol. 1978;28:1–6.
8. Ludwig W, Schleifer KH, Whitman WB, et al. Class I Bacilli class nov. In: De Vos P, Garrity G, Jones D, Krieg NR, Ludwig W, Rainey FA, editors. Bergey’s manual of systematic bacteriology, volume 3. 2nd ed. New York: Springer; 2009. p. 19–20.
9. Validation List no. 132. List of new names and new combinations previously effectively, but not validly published. Int J Syst Evol Microbiol. 2013;63:469–72.
10. Prévot AR. In: Hauderoy P, Ehringer G, Guillot G, Magrou J, Prévot AR, Rosset D, et al. Dictionnaire des bacteries pathogènes. 2nd ed. Paris: Masson et Cie; 1953. p. 1–692.
11. Rogers EE, Backus EA. Anteilor forugen microbilia of the glassy-winged sharpshooter explored using deep 16S rRNA gene sequencing from individual insects. PLoS ONE. 2014;9:e106215.
12. Chan AC, Colmus C, Koc M, Ozcak B. Anoxybacillus calidus sp. nov., a thermophilic bacterium isolated from soil near a thermal power plant. Int J Syst Evol Microbiol. 2014;64:211–9.
13. Zhang X-Q, Zhang Z-L, Wu N, Zhu X-F, Wu M. Anoxybacillus vitreomilitaris sp. nov., a strictly aerobic and moderately thermophilic bacterium isolated from a hot spring. Int J Syst Evol Microbiol. 2013;63:460–71.
14. Joshi S, Satyanarayana T. In vitro engineering of microbial enzymes with multifarious applications: prospects and perspectives. Biosens Technol. 2015;17:6273–83.
15. Kanavančůře R, Čtvárlík D. Genetic engineering of Geobacillus spp. J Microbiol Methods. 2015;111:3–9.
16. Chai YY, Rahman RNRA, Illias RM, Goh KM. Cloning and characterization of two novel thermostable and alkalitolerant α-amylases from the Anoxybacillus species that produce high levels of maltose. J Ind Microbiol Biotechnol. 2012;39:731–41.
17. Xu J, Ren F, Huang C-H, Zheng Y, Zhen J, Sun H, et al. Functional and structural studies of pullulanase from Anoxybacillus sp. LM18-11. Proteins: Struct, Funct, Bioinf. 2014;68:1685–93.
18. Kahar UM, Chan K-G, Saleh MM, Hi SM, Goh KM. A high molecular-mass Anoxybacillus sp. SK-34 amylopullulanase: characterization and its relationship in carbohydrate utilization. Int J Mol Sci. 2013;14:11302–18.
19. Turner P, Labes A, Fridjönnson Ó, Hreggvidson GO, Schönheit P, Kristjánsson JK, et al. Two novel cyclodextrin-degrading enzymes isolated from thermophilic bacteria have similar domain structures but differ in oligomeric state and activity profile. J Bacteriol. 2005;187:380–90.
20. Karaghi H, Yannis D, Sal FA, Celik A, Canakci S, Belduz AO. Biochemical characterization of a novel glucose isomerase from Anoxybacillus gossenii G2\textsuperscript{7} that displays a high level of activity and thermal stability. J Mol Catal B. Enzym. 2013;97:215–24.
21. Wang J, Bai Y, Yang P, Shi P, Luo H, Meng K, et al. A new xylanase from thermodualkalinе Anoxybacillus sp. E2 with high activity and stability over a broad pH range. World J Microbiol Biotechnol. 2010;26:917–24.
22. Canakci S, Kacagcan M, Inan K, Belduz AO, Saha BC. Cloning, purification, and characterization of a thermostable o-L-arabinofuranosidase from Anoxybacillus kestanbolensis AC265ari. Appl Microbiol Biotechnol. 2008;81:61–8.
23. Beis FS, De Smet L, Karaghi H, Canakci S, Van Beeumern J, Belduz AO. The ATPas activity of the G2ult gene encoding an aluminum tolerance protein from Anoxybacillus gosseni G2. J Microbiol. 2011;49:46–50.
24. Lim JC, Goh KM, Shamiris MS, Ibrahim Z, Chong CS. Characterization of aluminum resistant Anoxybacillus sp. SK-34 isolated from a hot spring. J Microbiol Basic. 2014;55:514–9.
25. Jiang D, Li P, Jiang Z, Dai X, Zhang R, Wang Y, et al. Chemolithoautotrophic arsenite oxidation by a thermophilic Anoxybacillus flavithermus strain TC7-4 from a hot spring in Tengchong of Yunnan, China. Front Microbiol. 2015;6:360.
26. Chen J, Li Y, Hao H-H, Zheng J, Chen J-M. Fe(II)-EDTA-NO reduction by a newly isolated thermophilic Anoxybacillus sp. HA from a rotating drum biofilter for NOx removal. J Microbiol Methods. 2015;109:129–33.
27. Nazina TN, Tourova TP, Poltarsius AB, Novikova EV, Grigoryan AA, Ivanova AE, et al. Taxonomic study of aerobic thermophilic bacilli: descriptions of
Anoxybacillus ayderensis

T 3.

–

deo novo

– sp. nov. Int J Syst Evol Microbiol. 2008;58:91–16.

Dulger S, Demirbag Z, Belduz AO. Anoxybacillus ayderensis sp. nov. and Anoxybacillus kastrenensis sp. nov. Int J Syst Evol Microbiol. 2004;54:1499–503.

Pilet BD. Draft genome sequence of Anoxybacillus strain BC01, isolated from a thermophilic microbial mat colonizing the outflow of a bore well of the Great Artesian Basin of Australia. Genome Announc. 2015;3:e01457–14.

Ogg CD, Spagnello MD, Pilet BD. Exploring the ecology of the thermophiles from Australia’s Great Artesian Basin during the genomic era. In: Satyanarayana T, Littlechild J, Kawarabayashi Y, editors. Thermophilic microbes in environmental and industrial biotechnology. New York: Springer; 2013. p. 61–97.

Poli A, Nicolaus B, Chan K-G, Kahar UM, Chan CS, Goh KM. Genome sequence of Anoxybacillus thermarius AF/04, isolated from the Euganean Hot Springs in Abano Terme, Italy. Genome Announc. 2015;3:e00490–15.

Poli A, Romano I, Cordella P, Orlando P, Nicolaus B, Berrini CC. Anoxybacillus thermarius sp. nov., a novel thermophilic bacterium isolated from thermal mud in Euganean hot springs, Abano Terme, Italy. Extremophiles. 2009;13:867–74.

Validation List no. 141. List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol. 2011;61:2025–6.

Belduz AO, Dulger S, Demirbag Z. Anoxybacillus gonensis sp. nov., a moderately thermophilic, xylose-utilizing, endospore-forming bacterium. Int J Syst Evol Microbiol. 2003;53:1315–20.

Coorevits A, Dinsdale AE, Halket G, Libeau L, De Vos P, Van Landschoot A, et al. Taxonomic revision of the genus Geobacillus: emendation of Geobacillus, G. steathermophilus, G. jurassicus, G. tobii, G. thermotolerans and G. thermoglucosidasius (nom. corr., formerly ‘thermoglucosidasius’); transfer of Bacillus thermantarcticus to the genus G. thermantarcticus comb. nov.; proposal of Caldibacillus debilis gen. nov., comb. nov.; transfer of G. tepidamans to Anoxybacillus as A. tepidamans comb. nov., and proposal of Anoxybacillus caldiproteolyticus sp. nov. Int J Syst Evol Microbiol. 2011;61:1470–85.

Rozanov AS, Bryanskaya AV, Kotenko AV, Malup TK, Peltek SE. Draft genome sequence of Anoxybacillus flavithermus strain 25, isolated from the Garga hot spring in the Barguzin Valley, Baikal Region, Russian Federation. Genome Announc. 2014;2:e01258–14.

Khalil A, Sivakumar N, Qaraw S. Genome sequence of Anoxybacillus flavithermus strain AK1, a thermophile isolated from a hot spring in Saudi Arabia. Genome Announc. 2013;1:e00644–15.

Lee S-J, Lee Y-J, Ryu N, Park S, Jeong H, Lee SJ, et al. Draft genome sequence of the thermophilic bacterium Anoxybacillus kamchatkensis J. Bacteriol. 2012;194:6684–5.

Kevin W, Zengler K, Lysemko AM, Wieg J. Anoxybacillus kamchatkensis sp. nov., a novel thermophilic facultative aerobic bacterium with a broad pH optimum from the Geyser valley, Kamchatka. Extremophiles. 2005;9:391–8.

Validation List no. 109. List of new names and new combinations previously effectively, but not validly, published. Int J Syst Evol Microbiol. 2006;56:925–7.

Matsutani M, Shirakihara Y, Imada K, Yakushi T, Matsushita K. Draft genome sequence of a thermophilic member of the Bacillaceae family, Bacillus thermodenitrificans. Nucleic Acids Res. 2008;36:W445–W51.

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P. Tiedje JM. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol. 2007;57:819–91.

Ahmed I, Yokota A, Yamazoe A, Fujimura T. Proposal of Lysinibacillus boronitolerans gen. nov. sp. nov., and transfer of Bacillus fusiformis to Lysinibacillus fusiformis comb. nov. and Bacillus spheericus to Lysinibacillus spheericus comb. nov. Int J Syst Evol Microbiol. 2007;57:1117–25.

Richter M, Rossello-Mora R. Shifting the genomic gold standard for the prokaryotic species definition. Proc Natl Acad Sci U S A. 2009;106:19126–31.

Menon V, Rao M. Trends in bioconversion of lignocellulose: biofuels, platform chemicals and biorefinery concept. Prog Energy Combust Sci. 2012;38:522–50.

Sak A, Sişik D, Saglam N, Güner S, Çağan T, Belduz AO. Characterization of a thermookaikophilic esterase from a novel thermophilic bacterium, Anoxybacillus gonensis G2. Bioresour Technol. 2005;96:625–31.

Faiz O, Colak A, Saglam N, Çağan T, Belduz AO. Determination and characterization of thermostable esterolytic activity from a novel thermophilic bacterium Anoxybacillus gonensis A4. J Biochem Mol Biol. 2007;40:588–94.
72. Ay F, Karaoglu H, Inan K, Canakci S, Belduz AO. Cloning, purification and characterization of a thermostable carboxylesterase from Anoxybacillus sp. Protein Expr Purif. 2011;80:74–9.

73. Ertunga NS, Colak A, Belduz AO, Canakci S, Karaoglu H, Sandalli C. Cloning, expression, purification and characterization of fructose-1,6-bisphosphate aldolase from Anoxybacillus gonensis G2. J Biochem. 2007;141:817–25.

74. Field D, Gantty G, Gray T, Morrison N, Selengut J, Sterk P, et al. The minimum information about a genome sequence (MIGS) specification. Nat Biotechnol. 2008;26:541–7.

75. Woese CR, Kandler O, Wheelis ML. Towards a natural system of organisms: proposal for the domains archaea, bacteria, and eucarya. Proc Natl Acad Sci U S A. 1990;87:4576–9.

76. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, et al. Gene ontology: tool for the unification of biology. The gene ontology consortium. Nat Genet. 2000;25:25–9.

77. Shida O, Takagi H, Kadowaki K, Komagata K. Proposal for two new genera, Brevibacillus gen. nov. and Aneurinibacillus gen. nov. Int J Syst Bacteriol. 1996;46:939–46.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit