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EXPERIMENTAL ARTICLES

Anoxybacillus mongoliensis sp. nov., a Novel Thermophilic Proteinase Producing Bacterium Isolated from Alkaline Hot Spring, Central Mongolia[1]

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Abstract—A Gram reaction positive, spore-forming, facultative anaerobic bacterium belonging to the Phylum *Firmicutes,* was isolated from alkaline hot (80°C, pH 9.8) spring Tsenher, Central Mongolia. The cells were rod shaped, feebly motile, peritrichously flagellated. Strain T4^T was moderately thermophilic with optimum growth at 60°C. Maximum temperature for growth was between 70 and 75°C; minimum temperature for growth was between 35 and 30°C. Alkalitolerant, optimum pH for growth was 8.0; minimum pH for growth was between 5.0 and 5.5 and maximum was between 10.5 and 10.8. The growth was observed at NaCl concentrations of 0–5% (w/v) with the optimum at 0.2–0.5%. No growth was observed at 6% NaCl (w/v). Aerobically, the strain utilized proteinaceous substrates, organic acids and a range of carbohydrates including glucose, ribose, sucrose and xylose as well. Anaerobically, only glucose and sucrose were utilized. Strain T4^T produced thermostable alkaline subtilisin-like serine proteinase. The G + C content was 44.2 mol % (td). On the basis of 16S rRNA gene sequence similarity strain T4^T was shown to be closely related to the members of the genus *Anoxybacillus* (family *Bacillaceae,* class "Bacilli"). DNA–DNA hybridization data revealed that strain T4^T had only 38% relatedness to *A. flavithermus* and 28% relatedness to *A. pushchinoensis.* Based on its morphology, physiology, phylogenetic relationship and its low DNA–DNA relatedness values with validly published species of *Anoxybacillus*, it is proposed that strain T4^T represents a novel species *Anoxybacillus mongoliensis* sp. nov., with the type strain T4^T (=DSM 19169 =VKM 2407).

Key words: alkaline hot spring, thermophilic bacterium, *Anoxybacillus*, proteolytic activity.

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The genus *Anoxybacillus* was created by Pikuta et al. [1], and at present it comprises eleven species, *A. pushchinoensis* [1, 2], *A. flavithermus* [1, 3], *A. gonensis* [4], *A. contaminans* [5], *A. ayderensis* and *A. kestanbolensis* [6], *A. voinovskiensis* [7], *A. kamchatkensis* [8], *A. amylolyticus* [9], *A. rupiensis* [10] and *A. bogrovensis* [11]. All species that belong to this genus are thermophilic spore-forming rods capable to utilization of carbohydrates. Although the name of the genus *Anoxybacillus* means "Bacillus living without oxygen", according to the authors [1], most of the species described grow well aerobically and even for some species anaerobic growth was not registered at all [10].

MATERIALS AND METHODS

Isolation and characterization of the strain. Strain T4^T was isolated from combined water–sediment slurry sample taken from alkaline hot spring (80°C, pH 9.8) Tsenher located in Arkhangai aimak, Central Mongolia. Samples were collected in August 2005. Subsample (1 ml) was inoculated into 20 ml of aerobic medium containing (g × l^{-1}): KH_2PO_4, 0.5; NH_4Cl, 0.5; KCl, 0.5; NaCl, 0.5; Na_2SO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; yeast extract, 1; peptone, 1; glucose, 5; trace element solution 1 ml [12]. Enrichment culture of the strain T4^T was incubated at 55°C. The pH 7.5–8.5 was adjusted by adding of 1 M HCl and 1 M NaOH. For strain isolation, samples were transferred onto plates that contained 2% agar. Growth was followed by monitoring the increase in optical density at 550 nm in Hungate tubes (anaerobically) or in plain tubes (aerobically). All runs were done in duplicate. Substrate uti-

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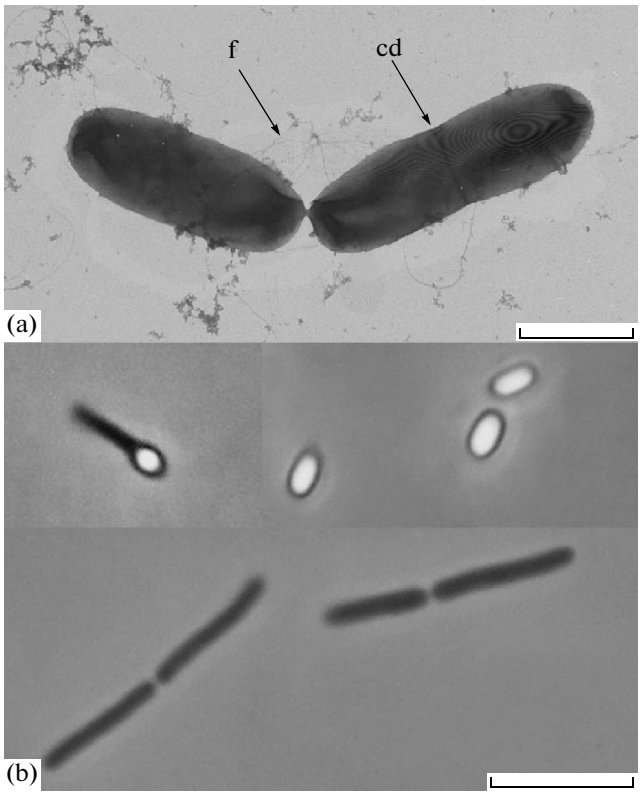

Fig. 1. (a) Scanning electron micrograph of cells of T4^T from the exponential growth phase (f, flagella, cd, cell division). (b) Phase contrast photo of cells of T4^T showing cells from the exponential growth phase without spores, formation of the spore at the end of the cell and free spores. Bars, 1 μm (a) and 5 μm (b).

lization tests were performed in the medium of the following composition (g l^{-1}): yeast extract, 0.05; substrate, 1; mineral stock plus vitamin solution were the same as above. All substrates were prepared as anaerobic stock solutions in distilled water. Catalase and oxidase tests, casein, gelatine, starch hydrolysis, production of urease, H_2S and indole were performed as described by Gerhardt et al. [13].

Proteolytic activity. Proteolytic activity was tested at 410 nm according to Erlanger et al. [14] using synthetic substrates pyroglutamyl-alanyl-alanyl-leucine-*p*-nitroanilide (GlpAAL*p*NA) for chymotrypsin- and subtilisin-like proteinases, N-benzoyl-D,L-arginine-*p*-nitroanilide (BA*p*NA) for trypsin-like proteinases, phenylalanine *p*-nitroanilide (P*p*NA) and L-leucine-*p*-nitroanilide (L*p*NA) for aminopeptidases. The strain was grown at 60°C in the same enrichment medium and the cells were harvested in the stationary phase of the growth by centrifugation at 13000 g for 15 min; the supernatant was used for experiments. Temperature and pH optimum and inhibition studies were performed according to Elpidina et al. [15].

Morphology. Gram-staining was performed as described by Gerhardt et al. [13]. Cell morphology was observed under an Olympus BX-41 phase-contrast microscope at ×1000 with cells grown overnight. Micrographs were taken by C-7070 (Olympus) photo attachment on slides coated by 1% (w/v) ultra pure agar. Negative staining of cells was achieved with 3% uranyl acetate and cells were examined under a transmission electron microscope [16]. A JEM-100CXII electron microscope was used at magnifications of ×10000.

Fatty acid composition. Membrane fatty acids were extracted from the freeze-dried cells with methanol/chloroform and analyzed by GC-MS as described by Pikuta et al. [1]. Bray-Curtis ordination plots were generated by using SBRACO software.

Phylogenetic analysis. DNA was isolated according to Marmur [17]. The G+C content was determined by the thermal denaturation method of Marmur and Doty [18]. DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded as described by De Ley et al. [19]. Genomic DNA extraction and PCR-mediated amplification of the 16S rRNA gene were done as described previously [20]. PCR products were sequenced using the CEQ DCTS dye terminator cycle sequencing kit and run on a CEQ 2000XL DNA sequencing system (Beckman-Coulter). The sequences were aligned using the ClustalX software [21]. An evolutionary-distance matrix was calculated using the Jukes & Cantor algorithm [22]. The phylogenetic tree was constructed using TREECON package [23] by the neighbour-joining algorithm [24]. Bootstrap analyses were based on 500 re-samplings.

RESULTS AND DISCUSSION

General characterization. The cells of strain T4^T were Gram reaction positive rods 2.1–5.5 μm long and 0.4–0.8 μm wide. Endospores were found to be oval (1–1.7 μm long and 0.8–1 μm wide), located terminally and could swell sporangia slightly. Peritrichous flagella were seen on electron micrograph (Fig. 1) but cells were only slightly motile during exponential growth phase.

Maximum temperature for growth was between 70 and 75°C; minimum temperature for growth was between 35 and 30°C; optimum temperature was 60°C. Optimum pH for growth was 8.0; minimum pH for growth was between 5.0 and 5.5 and maximum was between 10.5 and 10.8. The growth was observed at NaCl concentrations of 0–5% (w/v) with the optimum at 0.2–0.5%. No growth was observed at 6% NaCl (w/v) (Fig. 2). The best growth was observed on the media containing peptone (up to 5×10^8 cells ml^{-1}). The doubling times on glucose at optimal growth conditions were 35 min (aerobically) and 42 min (anaerobically).

Physiological and biochemical characteristics of the strain studied are listed in the species description and in Table 1.

Proteolytic activity. Strain T4^T possesses a strong extracellular proteolytic activity not reported in other related microorganisms. The best substrate was GlpAALpNA. Maximum activity of the proteinase was expressed in the stationary growth phase after 24 hours of incubation. The maximum activity of the enzyme with GlpAAL*p*NA was observed at pH values 10.5–10.8. The optimum temperature for the activity of enzyme was 65°C (Fig. 2). The activity was completely inhibited by an inhibitor of serine proteases phenylmethylsulfonyl fluoride (PMSF) and was not inhibited by chymotrypsin specific inhibitor tosyl-L-phenylalanine chloromethyl ketone (TPCK). The results indicate that extracellular enzyme of strain T4^T is a thermostable alkaline subtilisin-like serine proteinase.

Fatty acid composition. The fatty acid profile of strain T4^T was composed of a mixture of straight-chain and branched (*iso* and *anteiso*-) fatty acids found in other species of *Anoxybacillus* (Table 2). The *iso*-$C_{16:0}$ predominates among the fatty acids (41.6%), but *anteiso*-$C_{15:0}$ (9.7%), *anteiso*-$C_{17:0}$ (8.6%), *iso*-$C_{16:0}$ (8.5%), $C_{16:0}$ (7.6%), *iso*-$C_{17:0}$ (7.0%), $C_{14:0}$ (2.7%), $C_{15:0}$ (2.7%), $C_{15:1}$(n-5) (2.4%), $C_{16:1}$(n-7) (2.2%), *iso*-$C_{14:0}$ (1.6%), *anteiso*-$C_{17:1}$ (1.2%) are also present. A Bray-Curtis ordination of the available fatty acids profiles of *Anoxybacillus* species revealed a close association (Euclidean distance of <10.0 U) between strain T4^T and type strains of *A. contaminans* and *A. flavithermus* (Fig. 3). Lower association was observed with the remaining species.

Similarities in phenotypic characteristics support the inclusion of studied strain in the genus *Anoxybacillus*. The major physiological and biochemical properties of the novel isolate differed from those of its closest relatives (*A. pushchinoensis, A. flavithermus* and *A. kestanbolensis*) in its higher salinity range, shape of spore, inability to reduce nitrate, ability to utilize xylose and hydrolyse gelatine, and fatty acids profile (Tables 1, 2).

Genetic characteristics. The G+C content of strain T4^T was 44 mol % (td), in the range of values for recognized *Anoxybacillus* species (Table 1). On the basis of 16S rRNA sequence analysis, the strain T4^T have 99% sequence similarity with *A. pushchinoensis* K1^T and 99.4% similarity with *A. flavithermus* DSM 2641^T (Fig. 4). The levels of sequence similarity with other type strains of *Anoxybacillus* species were: *A. ayderensis* AB04^T—98.6%, *A. kamchatkensis* JW/VK-KG4^1—98.4%, *A. kestanbolensis* K4^T—98.2%, *A. gonensis* G2^T—98.0%, *A. contaminans* DSM 15866^T—97.1%, *A. bogrovensis* BT 13^T—96.6%, *A. amylolyticus* MR3C^T—95.9%, *A. voinovskiensis* TH13^T—95.8%, *A. rupiensis* R270^T—95.7%. The clusters of *Anoxybacillus* species on phylogenetic trees for 16S rRNA sequences obtained by neighbor-joining and maximum-likelihood methods were identical.

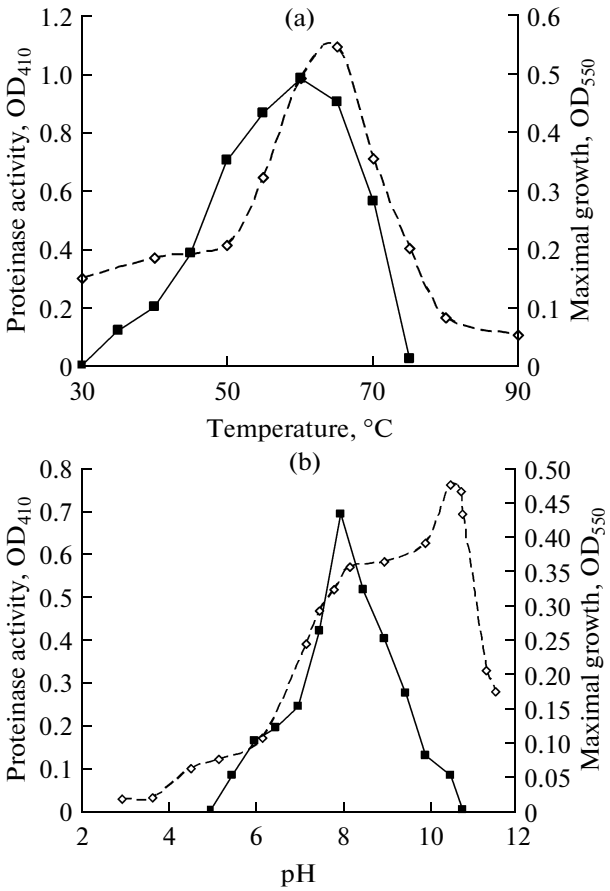

Fig. 2. Effects of temperature (a) and pH (b) on growth of the strain T4^T and proteinase activity. Growth curve of the strain T4^T (solid line). Proteinase activity with 0.5 mM GlpAAL*p*NA (broken line).

DNA–DNA hybridization study with the closest representatives showed that the DNA–DNA relatedness values between strain T4^T and type strains *A. flavithermus* and *A. pushchinoensis* were 38 and 28%, respectively. The DNA–DNA relatedness between type strains of last two species was 59%. None of the pairs yielded the level of DNA–DNA hybridization above 70% [25]. This supports the proposal that strain T4^T belongs to a novel species.

According to the low level of DNA–DNA relatedness between strain T4^T and type strains of the closest *Anoxybacillus* species and on the basis of the above mentioned phenotypic differences, we propose that strain T4^T should be placed in the genus *Anoxybacillus* as the type strain for the novel species, *Anoxybacillus mongoliensis* sp. nov.

Taxonomic Description of *Anoxybacillus mongoliensis* sp. nov.

Anoxybacillus mongoliensis (*mon.go.li.en'sis*. N.L. masc. adj. *mongoliensis*, pertaining to Mongolia, the country of isolation).

Cells are facultatively anaerobic, Gram reaction positive, single or in long chains, peritrichously flagel-

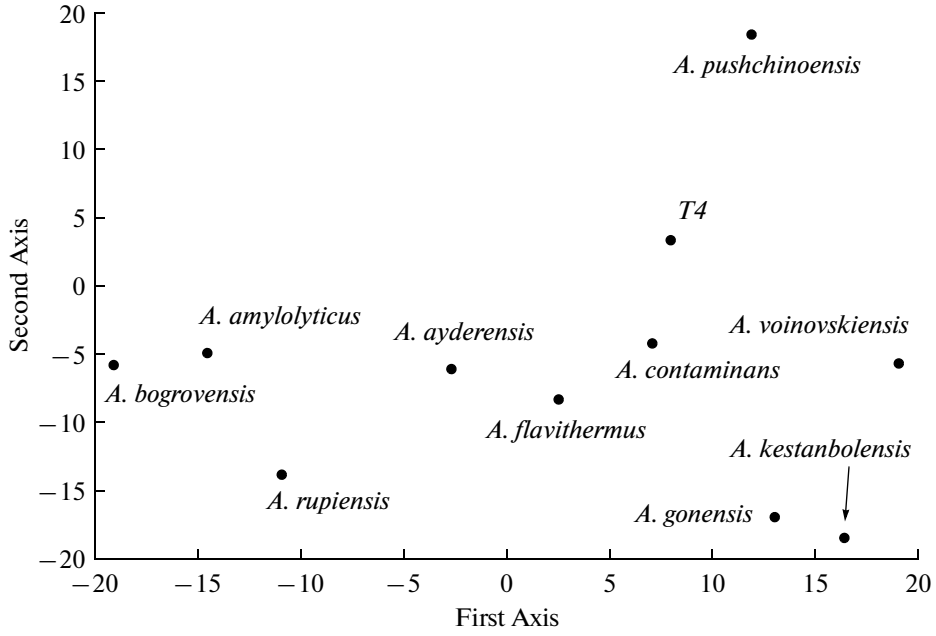

Fig. 3. Bray-Curtis ordination plot of *Anoxybacillus* fatty acids using the Euclidean distance. Note: Before ordination the data on fatty acids composition from Table 2 were normalized, values with amount <1% were eliminated. Data on fatty acid composition of *A. kamchatkensis* are not available.

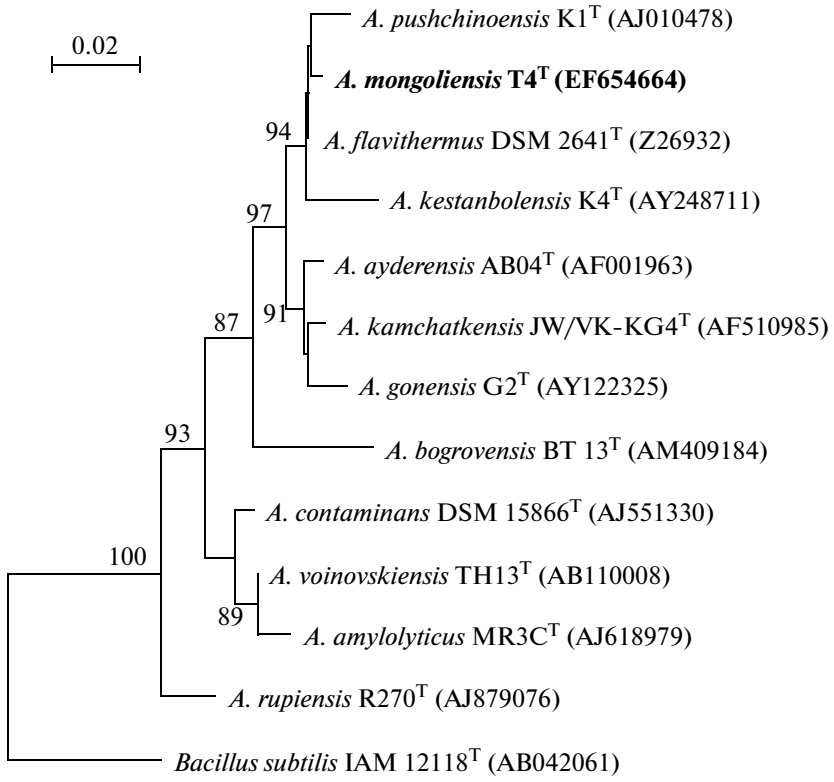

Fig. 4. Phylogenetic relationships of *Anoxybacillus* species, showing the position of the strain T4T. Neighbour-joining tree based on 16S rRNA gene sequences. Bar, 0.02 substitution per nucleotide. Bootstrap value more than 70% is given on appropriate clade. The same clusters were viewed on the maximum likelihood tree constructed in PAUP program version 4.0b.

Table 1. Comparison of strain T4^T with other *Anoxybacillus* species

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
Cell length (μm)	2.1–5.5	2.3–7.1	2.5–3.0	5.0	4–10	4.6	4.75	1.5–5.0	2.5–8.8	2.0–2.5	3.3–7.0	1.4–4.0
Cell diameter (μm)	0.4–0.8	0.85	0.4–0.5	0.75	0.7–1.0	0.55	0.65	0.4–0.6	1.0	0.5	0.7–1.5	0.3–0.6
Spore shape	Oval	Oval	Spherical	Spherical	Oval	Spherical	Spherical	ND	Oval	Oval	Oval	Oval
Motility	+	+	–	+	+	+	+	–	+	+	+	–
Oxygen requirement	FAn	FAn	AAn	FAn	FAn	FAn	FAn	FAn	FAn	FAn	SA	FAn
Temperature (°C)												
Range	35–70	30–72	37–65	40–70	Up to 60	30–70	40–70	30–64	38–67	45–65	35–67	40–69
Optimum	60	60–65	62	55–60	40–50	50	50–55	54	57–62	61	55	65
pH												
Range	5.5–10.5	5.5–9.0	8.0–10.5	6.0–10.0	4.5–9.5	6.0–11.0	6.0–10.5		5.7–9.9	5–6.5	5.5–8.5	6.0–10.0
Optimum	8.0	7.0	9.5–9.7	7.5–8.0	7.0	7.5–8.5	7.5–8.5	7.0–8.0	6.8–8.5	5.6	6.0–6.5	8.0
NaCl (%)												
Range	0–5	Up to 2.5	Up to 3	Up to 4.0	Up to 5	Up to 2.5	0–4	0–3	ND	ND	Up to 1	1.5
Optimum	0.2–0.5		0.5–1			1.5	2.5					0.5
Catalase	+	+	–	w	+	+	+	+	–	+	+	+
Oxidase	+	+	ND	+	–	+	+	+	–	–	ND	ND
Urease	–	–	ND	–	–	–	–	ND	ND	ND	ND	–
Indole production	–	–	ND	–	–	–	–	ND	ND	–	–	–
Nitrate reduction	–	+	+	+	+	–	+	+	ND	+	–	–
Hydrolysis of[1]:												
Starch	+ (–)	+[2]	(+)	+	+	+	+	–	– (–)	+	+	+
Gelatin	+	–	(–)	+	+	+	–	–	– (–)	–	–	+
Casein	+	+	(–)	ND	–	ND	ND	–	–	–	+	–
Pectin	ND	ND	ND	ND	ND	ND	ND	ND	+(–)	ND	–	ND
Substrates used[1]:												
D-glucose	+ (+)	+(+)	+[5](+)	+	+	+	+	+	+ (+)	–	+	+
L-arabinose	+	+	(–)	–	L+, D–	+	–	+	– (–)	–	+	+
D-ribose	+ (–)	ND	(–)	ND	+	ND	ND	ND	w (–)	–	+	–
D-xylose	+	–	(–)	+	D+, L–	+	–	+	– (–)	–	+	–

Table 1. (Contd.)

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12
D-fructose	+	ND	(+)	+	+	+	+	+	+ (+)	–	+	+
D-galactose	–	+[3]	(–)	ND	+	ND	ND	–	+ (–)	+	–	–
D-mannose	–	+	ND	–	+	+	+	+	– (–)	–	+	–
L-rhamnose	+ (–)	+	(–)	–	–	–	–	–	ND	ND	–	+
Mannitol	+	ND	(–)	+	–	+	+	–	+ (+)	ND	+	+
Sorbitol	–	+	(–)	ND	–	ND	ND	+	– (–)	ND	–	–
Sucrose	+ (+)	+	(+)	+	+	+	+	+	+ (+)	+	–	+
Maltose	–	+	ND	ND	+	+	+	+	+ (+)	+	+	+
Lactose	–	ND	(–)	–	–	–	–	–	– (–)	–	–	–
Trehalose	ND	ND	(+)	ND	+	ND	ND	–	+ (+)	+	ND	ND
D-raffinose	+	–	(–)	+	+	+	+	–	– (–)	+	–	–
D-cellobiose	+	ND	ND	ND	–	ND	ND	+	ND	–	ND	+
Yeast extract	+ (–)	ND	+[5](–)	ND	ND	ND	ND	+[6](+[6])	+ (+)	+[7]	+	+[6](+[6])
Peptone	+ (–)	+[4]	(–)	ND	ND	ND	ND	+[6](+[6])	+ (–)	ND	+	+[6](+[6])
Tryptone	+ (–)	ND	ND	ND	ND	ND	ND	ND	+ (–)	ND	ND	ND
Casamino acids	+ (–)	ND	ND	ND	ND	ND	ND	ND	+ (–)	ND	ND	ND
Acetate	+	ND	(–)	ND	ND	ND	ND	ND	ND	–	ND	ND
Lactate	+ (–)	ND	(–)	ND	ND	ND	ND	ND	ND	ND	ND	ND
Pyruvate	+ (–)	ND	(–)	ND	ND	ND	ND	ND	w (–)	ND	ND	ND
Glycerol	–	ND	(–)	ND	+	ND	ND	ND	ND	–	ND	ND
DNA G + C (mol %)	44.2	41.6 (61)[8]	42.2	42.8 (57)[8]	44.4	43.6 (54)[8]	50	43.9	42.3	43.5	41.7	44.1

Strains designations: 1 – T4^T (*A. mongoliensis* sp. nov.), 2 – *A. flavithermus* DSM 2641^T (data from Pikuta *et al.* [1]; Belduz *et al.* [4]), 3 – *A. pushchinoensis* K1^T [1, 2], 4 – *A. gonensis* G2^T [4, 8], 5 – *A. contaminans* LMG 21881^T [5], 6 – *A. ayderensis* AB04^T [6, 8], 7 – *A. kestanbolensis* K4^T [6], 8 – *A. voinovskiensis* TH13^T [7], 9 – *A. kamchatkensis* JW/VK-KG4^T [8], 10 – *A. amylolyticus* MR3C^T [9], 11 – *A. rupiensis* R270^T [10], 12 – *A. bogrovensis* BT 13^T [11].

[1] – aerobic and anaerobic utilization tests are given without or within brackets, respectively.

[2] – assayed as a-amylase activity.

[3] – assayed as b-galactosidase activity.

[4] – tentatively grows because the strain has been maintained on meat bouillon.

[5] – tentatively grows because the strain has been maintained on media with glucose and yeast extract.

[6] – tentatively grows because the strain has been maintained on media with peptone and yeast extract.

[7] – tentatively grows because the strain has been maintained on media with yeast extract.

[8] – data obtained by Pikuta *et al.* [1] and Belduz *et al.* [4], Kevbrin *et al.* [8] and Belduz *et al.* [4], Kevbrin *et al.* [8] and Dulger *et al.* [6] are given without or within brackets, respectively.

+, positive; w, weakly positive; –, negative; ND – not determined. SA – strict aerobe, FAn – facultative anaerobe, AAn – aerotolerant anaerobe.

Table 2. Fatty acid composition of *Anoxybacillus* type strains

Characteristic	1	2	3	4	5	6	7	8	10	11	12
$C_{12:0}$	–	–	6.9	–	–	–	–	–	–	–	–
iso-$C_{13:0}$	0.3	–	–	–	–	–	–	–	–	–	–
$C_{14:0}$	2.7	1.96	7.3	1.18	2.91	1.02	1.29	1.3	–	0.3	0.4
iso-$C_{14:0}$	1.6	–	–	1.25	–	–	0.88	1.3	–	–	0.25
iso-$C_{15:0}$	41.6	54.85	38.7	65.19	51.88	48.17	68.62	54.7	41.2	52.81	40.1
anteiso-$C_{15:0}$	9.7	4.02	2.0	2.64	7.52	3.58	3.56	8.0	2.13	1.64	1.8
$C_{15:0}$	2.7	1.18	0.9	1.12	–	0.83	1.11	–	0.1	0.31	0.4
$C_{15:1}$(n-5)	2.4	–	–	–	–	–	–	–	–	–	–
iso-$C_{16:0}$	8.5	2.97	0.3	5.99	5.07	7.47	6.37	7.1	7.0	2.01	3.9
anteiso-$C_{16:0}$	–	–	–	–	–	–	–	–	0.12	–	–
$C_{16:1}$(n-7)	2.2	–	–	–	–	–	–	–	–	–	–
$C_{16:1}$(n-5)	0.5	–	–	–	–	–	–	–	–	–	–
$C_{16:1}$	–	–	2.6	–	–	–	–	–	–	–	–
$C_{16:0}$	7.6	11.13	14.5	2.38	11.32	9.10	3.47	1.9	6.3	5.44	6.3
10-methyl-$C_{16:0}$	–	–	0.9	–	–	–	–	–	–	–	–
iso-$C_{17:0}$	7.0	17.74	0.8	11.96	11.64	20.62	9.54	3.9	31.6	33.55	36.1
iso-$C_{17:1}$(n-5)	1.0	–	–	–	–	–	–	–	–	–	–
iso-$C_{17:1}$(n-5)c	–	–	–	2.63	–	–	0.59	–	–	–	–
anteiso-$C_{17:1}$	1.2	–	–	–	–	–	–	7.1	–	–	–
anteiso-$C_{17:0}$	8.6	6.15	0.1	3.29	7.02	9.22	3.69	–	8.4	3.94	8.98
anteiso-A-$C_{17:0}$	–	–	–	0.82	–	–	–	–	–	–	–
hydroxy-*iso*-$C_{15:0}$	–	–	0.3	–	–	–	–	–	–	–	–
$C_{17:0}$	0.7	–	0.5	–	–	–	–	–	0.7	–	–
$C_{17:1}$	–	–	–	–	–	–	–	2.6	–	–	–
$C_{18:2}$	–	–	2.2	–	–	–	–	–	–	–	–
$C_{18:1}$(n-7)	0.5	–	–	–	–	–	–	–	–	–	–
$C_{18:1}$(n-9)	–	–	4.3	–	–	–	–	–	–	–	–
$C_{18:1}$(n-11)	–	–	1.0	–	–	–	–	–	–	–	–
iso-$C_{18:0}$	–	–	–	–	–	–	–	–	0.09	–	–
anteiso-$C_{18:0}$	–	–	–	–	–	–	–	–	0.7	–	–
$C_{18:0}$	0.5	–	10.4	–	–	–	–	–	1.9	–	0.5
iso-$C_{19:0}$	–	–	–	–	–	–	–	–	–	–	0.5
$C_{20:0}$	–	–	0.6	–	–	–	–	–	–	–	–

Designations of strains are similar to Table 1. Data are expressed as percentages. For A. kamchatkensis data are not available. For A. contaminans fatty acids with amount <1% are not given. Data from: 1 – this study; 2 – Dulger et al. [6]; 3 – Pikuta et al. [1]; 4 – Belduz et al., [4]; 5 – De Clerck et al. [5]; 6 – Dulger et al. [6]; 7 – Dulger et al. [6]; 8 – Yumoto et al. [7]; 10 – Poli et al. [9]; 11 – Derekova et al. [10]; 12 – Atanassova et al. [11].

lated, feebly motile rods. Cell diameter is 0.4–0.8 μm; cell length is 2.1–5.5 μm Endospores are oval, located terminally and may swell sporangia slightly. Colonies grown on solid medium at 55°C are pale white–yellowish in color with an irregular edge. Colony diameter ranges from 1 to 3 mm. Moderate thermophile with optimum growth at 60°C. Maximum temperature for growth lies between 70 and 75°C; minimum temperature for growth lies between 35 and 30°C. Alkalitolerant, optimum pH for growth is 8.0; minimum pH for growth lies between 5.0 and 5.5 and maximum lies between 10.5 and 10.8. The growth is observed at NaCl concentrations of 0–5% (w/v) with the optimum at 0.2–0.5%; no growth occurs at a salt concentration of 6%. Catalase and oxidase-positive. Chemoorganotrophic. Able to utilize aerobically a wide spectrum of carbon sources: D-glucose, L-arabinose, D-ribose, D-xyloze, D-fructose, L-rhamnose, sucrose, D-raffinose, D-cellobiose, mannitol, inosite, acetate, pyruvate, lactate, glutamate, yeast extract, peptone, tryptone and casamino acids. Able to utilize anaerobically glucose and sucrose. Requires yeast

extract (0.05 g l^{-1}) for growth. Unable to utilize aerobically D-galactose, D-mannose, sorbitol, maltose, lactose, glycerol and glycolate. Unable to utilize anaerobically D-ribose, L-rhamnose, pyruvate, lactate, glutamate, starch, yeast extract, peptone, tryptone and casamino acids. $T4^T$ possesses extracellular proteinase activity. Starch, gelatin and casein are hydrolysed. Indole, H_2S and urease are not produced. Nitrate reduction is negative. The major cellular fatty acid is *iso*-$C_{15:0}$ followed by *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$ and *iso*-$C_{16:0}$. The G+C content of the DNA of the type strain is 44 mol % (td). The type strain, $T4^T$ (=DSM 19169 =VKM 2407) was isolated from alkaline hot spring Tsenher located in Central Mongolia.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain $T4^T$ is EF654664.

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