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Anoxybacillus kamchatkensis sp. nov., a novel thermophilic facultative aerobic bacterium with a broad pH optimum from the Geyser valley, Kamchatka

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Abstract A facultative aerobic, moderately thermophilic, spore forming bacterium, strain JW/VK-KG4 was isolated from an enrichment culture obtained from the Geyser valley, a geothermally heated environment located in the Kamchatka peninsula (Far East region of Russia). The cells were rod shaped, motile, peritrichous flagellated stained Gram positive and had a Gram positive type cell wall. Aerobically, the strain utilized a range of carbohydrates including glucose, fructose, trehalose, proteinuous substrates, and pectin as well. Anaerobically, only carbohydrates are utilized. When growing on carbohydrates, the strain required yeast extract and vitamin B_{12}. Anaerobically, glucose was fermented to lactate as main product and acetate, formate, ethanol as minor products. Aerobically, even in well-aerated cultures (agitated at 500 rpm), glucose oxidation was incomplete and lactate and acetate were found in culture supernatants as by-products. Optimal growth of the isolate was observed at $pH^{25\ C}$ 6.8–8.5 and 60°C. The doubling times on glucose at optimal growth conditions were 34 min (aerobically) and 40 min (anaerobically). The G + C content was 42.3 mol% as determined by T_m assay. Sequence analysis of the 16S rRNA gene indicated an affiliation of strain JW/VK-KG4 with *Anoxybacillus* species. Based on its morphology, physiology, phylogenetic relationship and its low DNA-DNA homology with validly published species of *Anoxybacillus*, it is proposed that strain JW/VK-KG4 represents a new species in the genus *Anoxybacillus* as *A. kamchatkensis* sp. nov. The type strain for the novel species is JW/VK-KG4^T (=DSM 14988, =ATCC BAA-549). The GenBank accession number for the 16S rDNA sequence is AF510985.

Keywords *Anoxybacillus kamchatkensis* · Thermophilic · Facultative aerobic · B_{12}-dependent

Abbreviations TABS: *N*-tris[Hydroxymethyl]methyl-4-aminobutanesulfonic acid · pH** C: The temperature at which the pH was measured and the pH meter calibrated using standards at the indicated temperature in degree C

Introduction

The biologically mediated breakdown of organic substances in anoxic environments is accomplished by the development of a quite complex microbial community involving species of various systematic positions and physiology. This is especially true if the initial substrate is a biopolymer. The first step of decomposition of biopolymers is always performed by the hydrolytic microflora. Some of the occurring dimers, (e.g., cellobiose from cellulose) and monomers usually dissipated into surrounding environment supporting the growth of other microorganisms (dissipotrophs) which do not have the enzymes required for hydrolyzing polymers into oligomers and monomers. This knowledge can be applied for isolation and successive assession of the dissipotrophs' role in particular microbial community.

Here we report on the isolation of a facultative aerobic bacterium which grew on carbohydrates but did not grow on starch which was initially used for obtaining an anaerobic, thermophilic, starch-containing enrichment inoculated with a sample taken from a hot spring in the Geyser Valley (Kamchatka peninsula, Far East region of

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Russia). The phylogenetic 16S rDNA sequence analysis revealed the isolate belongs to the recently established genus *Anoxybacillus* (Pikuta et al. 2000). On the basis of phenotypic features as well as DNA-DNA hybridization studies with the closest known species of *Anoxybacillus*, we propose to classify the new isolate as a new species, *A. kamchatkensis* sp. nov.

Materials and methods

Sample source and enrichment

A combined water — sediment slurry sample was taken from an unnamed hot spring (55°C, $pH^{55\ C}$ 7.0) up the hill at the end of the Geyser valley (Kronotsky National Park, Kamchatka, Far East Russia). Samples were collected in August 2000 and after bringing them to Athens, GA, were kept at +4°C until use in 2001.

Subsamples (2 ml) of the slurry were inoculated into 30 ml of prereduced anaerobic mineral bicarbonate buffered medium containing (in g l^{-1}): KH_2PO_4, 0.3; $(NH_4)_2SO_4$, 0.66; NaCl, 0.29; $MgSO_4{\cdot}7H_2O$, 0.1; $CaCl_2{\cdot}2H_2O$, 0.03; $NaHCO_3$, 8.4; resazurin, 0.001; yeast extract, 0.2; soluble potato starch, 2; $Na_2S{\cdot}9H_2O$, 0.24; cysteine·HCl·H_2O, 0.2. Also, trace element and vitamin solutions, 5 ml/l of each (Freier et al. 1988) were added. The $pH^{25\ C}$ was adjusted to 8.5 by 5 M NaOH before autoclavation.

Isolation and phenotypic characterization

The isolation of pure cultures was carried out strictly anaerobically by serial dilutions in liquid medium of the same composition but with glucose supplement instead of starch. Further, the purity of the obtained isolate was confirmed microscopically and by colonies uniformity formed on solidified medium (plus 2% agar and no reductants) both anaerobically and aerobically.

Growth was followed by monitoring the increase in optical density at 600 nm in Hungate tubes (anaerobically) or in plain tubes (aerobically) using a Spectronic 21 (Bausch & Lomb) spectrophotometer. All runs were done in duplicate. Temperature and pH range determinations were carried out anoxically, i.e., oxygen was removed from the medium by N_2 sparging, however, without the addition of sulfide and cysteine as reducing agents. A shaking temperature gradient incubator (Scientific Industries) was used to determine the temperature range. For the pH range determination, the above medium was used with the following modifications (in g l^{-1}): Na_2CO_3, 1.06; MOPS, 4.18; TABS, 5.14; yeast extract, 0.5; trehalose·$2H_2O$, 2. Trehalose instead of glucose was used due to its higher stability under the autoclavation conditions. The pHs were roughly adjusted at room temperature with 5 M NaOH anaerobically prior to sterilization.. After autoclaving, the pH of each point was measured repeatedly to insure the right pH was plotted. To avoid possible oxygen depletion in standing tubes, the determination of the doubling time and formation of products of incomplete glucose oxidation were carried out in conical flasks closed with cotton plugs and were shaken (500 rpm) in the incubator. Substrate utilization tests were performed at optimal temperature and by using pH-optimal phosphate buffered medium of the following composition (in g l^{-1}): mineral stock plus vitamin solution were the same as above; $Na_2HPO_4{*}7H_2O$, 5.36; yeast extract, 0.5. The $pH^{25\ C}$ after sterilization was 7.4 (anaerobically) and 7.1 (aerobically). All sugars were prepared as anaerobic stock solutions in distilled water: glucose, fructose, galactose, mannose, maltose, cellobiose, inulin, arabinose, ribose, xylose, lactose. Other substrates were autoclaved with the medium. Final concentration for all carbohydrates as well as starch was 2 g l^{-1}, concentration for all proteinous substances was at 5 g l^{-1}.

Cellular characterization

A model Vanox (Olympus) phase-contrast microscope was used for routine examination. Micrographs were taken by PM-10AD photo attachment on slides coated by 2% (w/v) ultra pure agar. Negative staining was carried out as described by Plugge et al. (2000).

Analytical assays

Fermentation products were determined by high-performance liquid chromatography equipped with an Aminex HPX-87H column (Bio-Rad) (Paavilainen and Korpela 1993).

Determination of DNA base ratio and DNA–DNA hybridizations

DNA was isolated according to Marmur (1961). The G+C content was determined by the thermal denaturation method of Marmur and Doty (1962). DNA–DNA hybridization was performed spectrophotometrically and initial renaturation rates were recorded as described by De Ley et al. (1970). The reference microorganisms *Anoxybacillus pushchinensis* $K1^T$ (DSM 12423), *A. flavithermus* DSM 2641^T were obtained from the DSMZ collection, *A. ayderensis* $AB04^T$ — from the NCIMB collection. *A. gonensis* $G2^T$ was a gift from Dr. Ali Belduz (Turkey).

Amplification of the 16S rRNA gene and direct sequencing

Cells were lysed by a proteinase K, lysozyme, and sodium dodecyl sulfate treatment. The extraction of genomic DNA was performed using a standard

chloroform extraction (Murray et al. 1998). In vitro amplification of the 16S rRNA gene sequencing was performed as previously described (Rainey et al. 1996). Sequencing reactions were performed using BigDye Terminator Cycle Sequencing v2.0 Ready Reaction Kits (Applied Biosystems). Sequences were subsequently determined using an ABI 3700 capillary sequencer.

Phylogenetic analysis

The FASTA search engine at the EMBL website was used for finding sequences that were most relevant to strain JW/VK-KG4 (Pearson and Lipman 1988). Retrieved sequences were aligned by ClustalX program (Thompson et al. 1997) and manually edited. An unrooted phylogenetic tree was inferred by means of the TREECON program (Van de Peer and De Wachter 1994). The output set of sequences was bootstrapped and distance matrix was estimated according to Jukes-Cantor formula for each tree. The resulting tree topology was calculated according to the Neighbor-Joining method.

Results and discussion

Enrichment and isolation

Enrichments for thermophilic starch degrading anaerobic bacteria were incubated at 60°C for nearly 2 weeks. After turbidity appeared, subcultures with a variety of mono and disaccharides were inoculated. Tubes with growth were compared by microscopically. Spore forming rods prevailed on glucose- and trehalose-containing media. The glucose grown enrichment was serially diluted in liquid medium with glucose. The culture of the highest dilution exhibiting growth (10^{-9}) was transferred into glucose-containing solidified medium using the agar-shake roll tube technique (Ljungdahl and Wiegel 1986), tubes were subsequently incubated at 60°C. Formation of colonies was observed in 2 days. Anaerobically grown culture formed white, opaque, round colonies with even edge and flat surface. Agar embedded colonies were lens shaped. Later, when growth on air was revealed, colonies on Petri dishes were also obtained. These were pinpoint, yellowish translucent, round with even edge. Being transferred into liquid medium, aerobically or anaerobically grown colonies showed microscopically uniform morphotype. To insure the purity of the culture, we transferred anaerobically grown colonies into aerobic liquid medium. After growth the cells were plated onto solidified aerobic media and aerobically incubated. Subsequently, aerobic and aerobically grown colonies, respectively, were transferred into anaerobic liquid medium with successive plating and isolating colonies several times. In all cases, the appearance of the colonies (aerobically or anaerobically grown) was reproducibly different but the cells were uniform in liquid culture and could be easily transferred from oxic conditions into anoxic and back. No differences in the 16S rRNA gene sequence between the cells from the two treatments were observed. The final isolated strain was termed JW/VK-KG4.

Morphology and cell structure

The cells of the strain JW/VK-KG4 were motile, rod shaped and grew in single rods or in pairs. At early exponential phase, the cells were 1.0 μm in width and 2.5–8.8 μm in length (Fig. 1a). Sometimes, Y-shaped (branch) cells could be seen (Fig. 1b). Earlier, this phenomenon has been observed in other thermophilic bacteria (Engle et al. 1995, 1996; Pikuta et al. 2000) and, possibly, is somehow related to disorders in membrane and/or cell wall syntheses during proliferation at elevated temperatures. The cells stained Gram positive and had Gram positive cell wall structure as revealed by electron microscopy. Peritrichous flagella were seen on electron micrograph (Fig. 2) but cells were only slightly motile during exponential growth phase. At stationary phase, cells formed terminal, oval spores of 0.5 μm in width and 1.5–2.0 μm in length (Fig. 1c). The presence of non mature spores were characteristic in anaerobically grown cultures (Fig. 1d). Mature spores were more abundant in aerobically grown cultures on agar plates or in agitated liquid media. Spores were viable after heat treatment at 100°C for 20 min.

Physiological characteristics

Growth of strain JW/VK-KG4 was observed within a temperature range of 38–67°C, (no growth at 37°C or below and at 68°C or above) with an optimum at 57–62°C. The strain grew within $pH^{25\ C}$ range of 5.7–9.9 (no growth at or below $pH^{25\ C}$ 5.6 and at or above $pH^{25\ C}$ 10) with a broad optimum at $pH^{25\ C}$ 6.8–8.5 (Fig. 3). This classifies the isolate as an alkalitolerant moderate thermophile (Wiegel 1998, Kevbrin et al. 2003; Wiegel and Kevbrin 2004). Although isolated first under strictly anaerobic conditions, strain JW/VK-KG4 could grow well aerobically. Similar to other facultative aerobes, strictly anaerobic conditions (i.e., additions of reductants) are not necessary and the growth rate was even higher when sulfide and cysteine were omitted from the media. Oxidase and catalase tests for aerobically grown colonies were negative. The shortest doubling time on glucose-containing, phosphate buffered medium at 60°C and $pH^{25\ C}$ 7.0 was 34 min (aerobically) and 40 min (anaerobically). Upon transfer into fresh media, a 3 h lag phase was observed.

The substrate spectrum was assayed both, aerobically and anaerobically. The following substrates supported aerobic growth: ribose, glucose, fructose, galactose, mannitol, maltose, trehalose, sucrose, pyruvate, yeast extract, peptone, tryptone, casamino acids, and pectin.

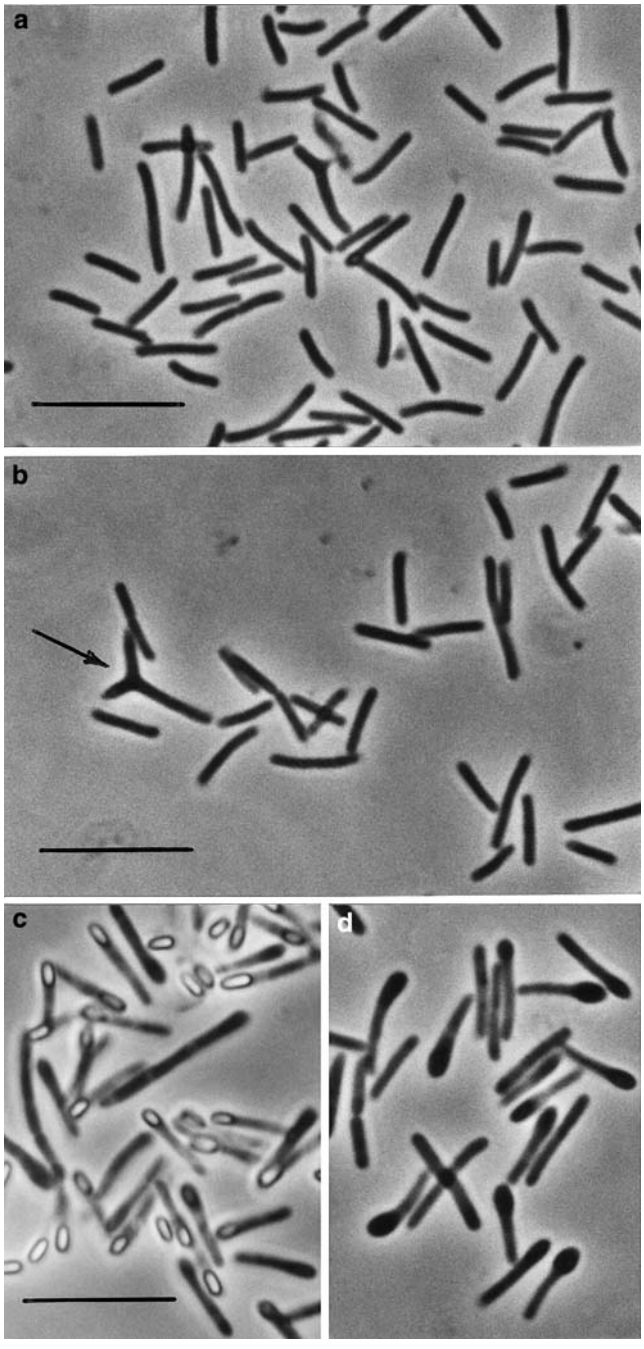

Fig. 1 Phase contrast micrographs of strain JW/VK-KG4 (*bar* 10 μm): **a** Normal view at the exponential growth phase; **b** The same view. Arrow points to Y-shaped cell; **c** Mature spores at the stationary growth phase; **d** View of unmature spores

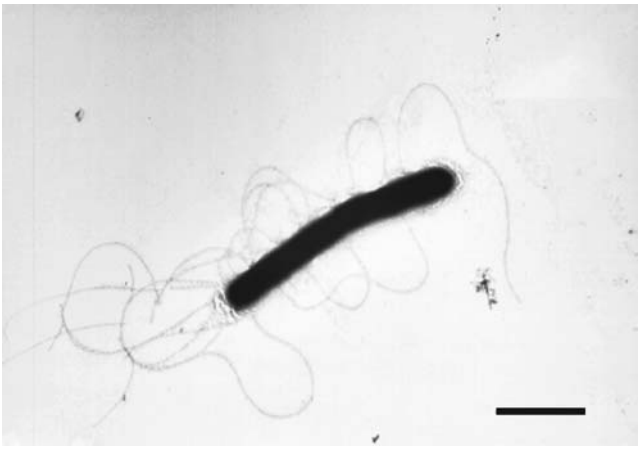

Fig. 2 Negatively stained cell of strain JW/VK-KG4 with peritrichous flagella. *Bar* 2 μm

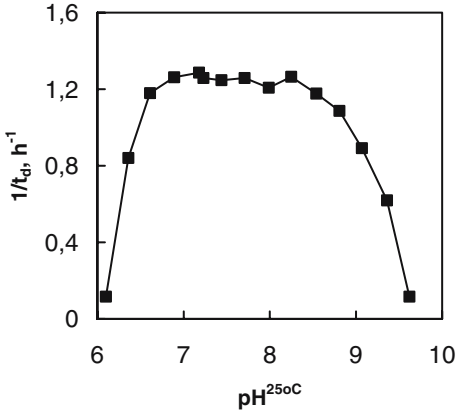

Fig. 3 A pH range of growth for the strain JW/VK-KG4

The substrate range for anaerobically grown cultures was narrower: glucose, fructose, mannitol, maltose, trehalose, sucrose, yeast extract. The following substances did not support growth neither aerobically nor anaerobically after 3 days of incubation: arabinose, xylose, xylitol, mannose, sorbitol, inositol, lactose, raffinose, gluconate, starch, gelatin, and casein. Growth on yeast extract alone can not be regarded as an evidence for proteolytic capabilities as it contains up to 17.5% by weight of carbohydrates (Difco manual 1998). When growing on carbohydrates, the addition of yeast extract was required (0.1 g l^{-1}). Also, addition of vitamin mixture was essential, particularly, vitamin B_{12} (0.02 mg l^{-1}) was required. Growth ceased without extra added B_{12} within 3–4 successive transfers.

Being grown anaerobically, strain JW/VK-KG4 fermented glucose (7.8 mM consumed) into lactate (12.9 mM), formate (2.8 mM), ethanol (2.2 mM), acetate (1.0 mM), and CO_2 (which was not quantified). Incomplete oxidation of glucose occurred when the strain was grown aerobically in shaking flasks. Per 11.6 mM glucose consumed, 6.2 mM of lactate, 7.8 mM of acetate, and traces (~ 0.4 mM) of fumarate, succinate, and ethanol were formed. No formation of formate was observed in this case. For comparison, we also did the same tests for some other *Anoxybacillus* species (Table 1).

Phylogenetic relationship and DNA base composition

The almost complete (1,548 bp) sequence of the 16S rRNA gene of strain JW/VK-KG4 was aligned and

Table 1 Products from glucose for some representatives of *Anoxybacillus* species and the strain JW/VK-KG4

	strain JW/VK-KG4	*A. pushchinoensis* K1^T	*A. flavithermus* DSM 2641^T
Aerobically[a]	Lactate, acetate, traces(< 1 mM) of ethanol, fumarate and succinate	No growth	Acetate only
Anaerobically	Lactate (main), formate, acetate, ethanol	Lactate (main), formate, acetate, ethanol	Lactate (main), formate, acetate, ethanol

[a]Aerobically assayed under cotton plug and at shaker incubator

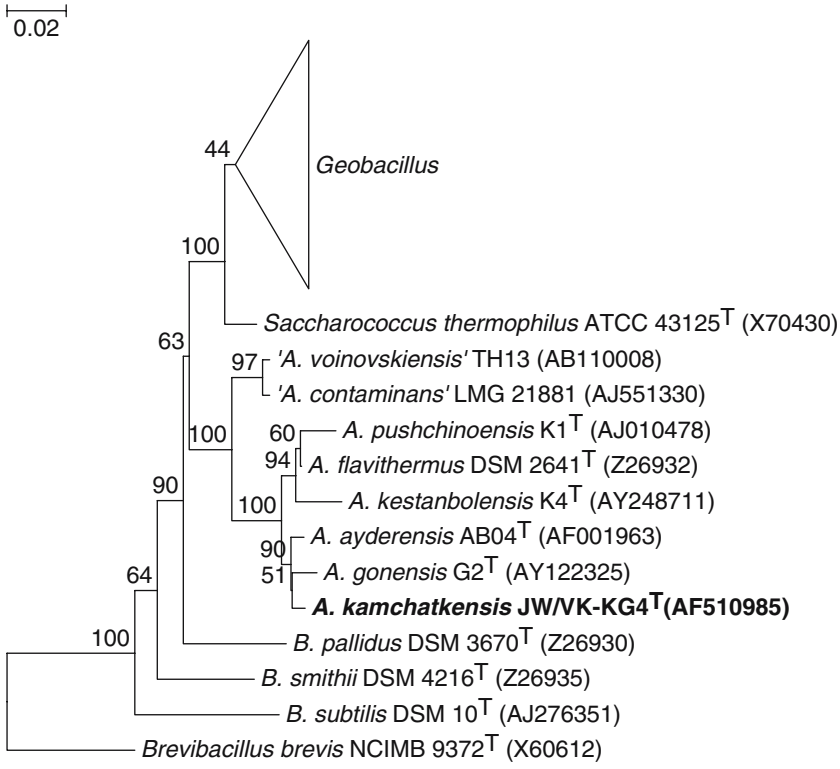

Fig. 4 Phylogenetic dendrogram based on 16S rDNA sequences showing the position of strain JW/VK-KG4^T among *Anoxybacillus* cluster. *A* – *Anoxybacillus*, *B* – *Bacillus*. Bootstrapping was based on 100 resampled datasets. The *scale bar* represents 0.02 nucleotide substitutions per position

compared to sequences of related bacteria. The subsequently constructed phylogenetic tree (Fig. 4) revealed that strain JW/VK-KG4 clusters with representatives of the genus *Anoxybacillus*. To date, this genus has five validly published species, *A. pushchinoensis, A. flavithermus, A. gonensis, A. ayderensis* and *A. kestanbolensis*. All of them are thermophilic, Gram-type positive (Wiegel 1981), spore forming, rod-shaped bacteria with a G + C content of about 42 mol%. The G + C content of strain JW/VK-KG4 was 42.3 mol% (Table 2). We determined the G + C content for all three type strains of *Anoxybacillus*' species at the same run simultaneously and found 42.8 mol% for *A. gonensis* G2^T contrary to the initially published value of 57 mol% (Belduz et al. 2003). Three species isolated in the same laboratory — *A. gonensis, A. ayderensis* and *A. kestanbolensis* — have the G + C content 50–57% (Belduz et al. 2003; Dulger et al. 2004). We belive that our value for *A. gonensis* is more consistent with those of the other members of *Anoxybacillus*, described before *A. gonensis, A. ayderensis* and *A. kestanbolensis* appeared. The levels of 16S rRNA gene sequence similarity between JW/VK-KG4 and the type strains of known *Anoxybacillus* species were: JW/VK-KG4 — *A. pushchinoensis* K1^T 97.7%; JW/VK-KG4 — *A. flavithermus* DSM 2641^T 98.7%, JW/VK-KG4 — *A. gonensis* G2^T 98.9%, JW/VK-KG4 — *A. ayderensis* 99.2%, JW/VK-KG4 — *A. kestanbolensis* 97.6%. Because all of these were higher than 97.5%, we performed DNA-DNA hybridizations studies with some representatives (Table 2). None of the pairs yielded homologies above 70%. According to Stackebrandt and Goebel (1994) this can be regarded as evidence for having a different, novel species.

Comparison to other *Anoxybacillus* species

Strain JW/VK-KG4 differs in some properties to the other *Anoxybacillus* species. Most distinguishable are: oval-shaped spores, lack of catalase and oxidase activity, a significant broader pH optimum for growth, substrates specifities with particularly good growth on pectin and

Table 2 DNA–DNA homology among valid *Anoxybacillus* species and strain JW/VK-KG4

Strain	G + C content (mol %)	DNA–DNA similarity, %			
		K1^T	DSM 2641^T	JW/VK-KG4	G2^T
A. pushchinoensis K1^T	42.2	100			
A. flavithermus DSM 2641^T	41.6	59	100		
JW/VK-KG4	42.3	53	55	100	
A. gonensis G2^T	42.8	54	53	51	100
A. ayderensis AB04^T	43.6	48	52	51	61
E. coli K12 (standard)	51.7				

Table 3 Characteristics of validly published *Anoxybacillus* species and strain JW/VK-KG4

Characteristic	1	2	3	4	5	6
Size (μm)	1.0 × 2.5–8.8	0.55 × 4.6	0.65 × 4.75	0.75 × 5.0	0.4–0.5 × 2.5–3.0	0.85 × 2.3–7.1
Motility	+	+	+	+	–	+
Spore shape	Oval	Spherical	Spherical	Spherical	Spherical	ND
Temperature for growth (°C)						
Range	37–66	30–70	40–70	40–70	37–66	30–72
Optimum	57–62	50	50–55	55–60	62	60
pH for growth						
Range	5.7–9.9	6.0–11.0	6.0–10.5	6.0–10.0	8.0–10.5	5.5–9.0
Optimum	6.8–8.5	7.5–8.5	7.5–8.5	7.5–8.0	9.5–9.7	ND
Catalase	–	+	+	+	–	+
Oxidase	–	+	+	+	ND	+
Substrates used[a]						
Arabinose	– (–)	+	–	–	(–)	+
Ribose	w (–)	ND	ND	ND	(–)	ND
Xylose	– (–)	+	–	+	(–)	ND
Fructose	+ (+)	+	+	+	(+)	ND
Galactose	+ (–)	ND	ND	ND	(–)	+[b]
Mannose	– (–)	+	+	–	ND	+
Rhamnose	ND	–	–	–	(–)	+
Mannitol	+ (+)	+	+	+	(–)	ND
Sorbitol	– (–)	ND	ND	ND	(–)	+
Sucrose	+ (+)	+	+	+	(+)	+
Maltose	+ (+)	+	+	ND	ND	+
Lactose	– (–)	–	–	–	(–)	ND
Trehalose	+ (+)	ND	ND	ND	(+)	ND
Raffinose	– (–)	+	+	+	(–)	ND
Pyruvate	w (–)	ND	ND	ND	(–)	ND
Starch	– (–)	+	+	+	(+)	+[c]
Yeast extract	+ (+)	ND	ND	ND	(–)	ND
Peptone	+ (–)	ND	ND	ND	(–)	+[d]
Tryptone	+ (–)	ND	ND	ND	ND	ND
Casamino acids	+ (–)	ND	ND	ND	ND	ND
Gelatin	– (–)	+	–	+	–	–
Pectin	+ (–)	ND	ND	ND	ND	ND

Strains designations: 1 strain JW/VK-KG4, 2 *A. ayderensis* AB04^T (Dulger et al. 2004), 3 *A. kestanbolensis* K4^T (Dulger et al. 2004), 4 *A. gonensis* G2^T (Belduz et al. 2003), 5 *A. pushchinoensis* K1^T (Pikuta et al. 2000), 6 *A. flavithermus* DSM 2641^T (Heinen et al. 1982)
All strains grow on glucose
+ positive, *w* weakly positive, – negative, *ND* not determined
[a] Aerobic and anaerobic substrate utilization tests are given without or within brackets, respectively
[b] Assayed as β-galactosidase activity
[c] Assayed as α-amylase activity
[d] Tentatively grows because the strain has been maintained on meat bouillon

the lack of growth on starch and raffinose (Table 3). Products of anaerobic fermentation of glucose were quite similar among all strains tested, thus the same metabolism is presumed (Table 1). However, under well-aerated (500 rpm shaking in aerated flasks) conditions, the products of incomplete glucose oxidation were different. *A. flavithermus* DSM 2641^T excreted acetate only while strain JW/VK-KG4 formed a set of products. Interestingly, *A. pushchinoensis* K1^T, the type species of the genus, did not grow at all under these conditions. Growth of *A. pushchinoensis* was only observed under microaerophilic conditions. We observed higher final OD values for strain K1^T at 1–5% of air in nitrogen than under 100% air in non-agitated bottles. This strain was initially described as an obligate anaerobe (Pikuta et al. 2000). Our data showed that *A. pushchinoensis* K1^T is a microaerophile, a fact which was subsequently amended by Pikuta et al. (2003).

Besides common features distinctive from all *Anoxybacillus* species, strain JW/VK-KG4 differs from the nearest species *A. ayderensis* in more higher temperature optimum (10°C difference), 1 pH difference upper limit, differences in capability to grow on arabinose, xylose, mannose, gelatin.

According to the low DNA–DNA homology between strain JW/VK-KG4 and type strains of the validly described species of the *Anoxybacillus* and on the basis of the above mentioned phenotypic differences, we propose that strain JW/VK-KG4 should be placed in the genus *Anoxybacillus* as the type strain for the novel species, *A. kamchatkensis* sp. nov.

Description of *Anoxybacillus kamchatkensis* sp. nov.

Anoxybacillus kamchatkensis (kam.chat.ken'sis L. part. adj. – originating from Kamchatka penninsula, Far East Russia).

Cells are straight rods as single cells or in pairs and 2.5–8.8 μm in length and 1.0 μm in width. Branched cells are infrequently observed. Motile and peritrichously flagellated. Gram staining positive; Gram-type positive cell wall. The strain forms terminal, oval spores in the stationary growth phase. Aerobically grown colonies are pinpoint, yellowish translucent, round with an even edge; anaerobically grown colonies form white, opaque, round colonies with an even edge and flat surface. Catalase and oxidase negative. The strain is an alkalitolerant, moderate thermophile with an optimum growth temperature (Topt) at 60°C, a T_{min} 38 and T_{max} 67°C (no growth at or below 37°C and at or above 68°C). The $pH^{25\ C}$ optimum for growth is broad ($pH^{25\ C}$ 6.8–8.5) with a $pH^{25\ C}{}_{min}$, 5.7 and $pH^{25\ C}{}_{max}$ 9.9 (no growth at or below $pH^{25\ C}$ 5.6 and at or above $pH^{25\ C}$ 10.0). Facultatively aerobic. Aerobic utilization of ribose, glucose, fructose, galactose, mannitol, maltose, trehalose, sucrose, pyruvate, yeast extract, peptone, tryptone, casamino acids, and pectin. Anaerobic utilization of glucose, fructose, mannitol, maltose, trehalose, sucrose, and yeast extract. Starch and gelatin are not hydrolysed. Requires yeast extract (0.1 g l^{-1}) and B_{12} vitamin for growth on carbohydrates. Fermentation products under anaerobic growth conditions from glucose are lactate as main product and acetate, formate, and ethanol as minor products. Products of incomplete glucose oxidation are lactate, acetate, and traces of fumarate, succinate, and ethanol. The G + C content of the DNA is 42–43 mol%, as determined by thermal denaturation. Habitat: volcanically heated thermal fields of the Geyser Valley in Kamchatka, Russia. The type strain is JW/VK-KG4^T (= DSM 14988, = ATCC BAA-549).

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References

Belduz AO, Dulger S, Demirbag Z (2003) *Anoxybacillus gonensis* sp. nov., a moderately thermophilic, xylose-utilizing, endospore-forming bacterium. Int J Syst Evol Microbiol 53:1315–1320

De Ley J, Cattoir H, Raynaerts A (1970) The quantitative measurement of DNA hybridization from renaturation rates. Eur J Biochem 12:133–142

The Difco Manual (1998) 11th edn Sparks, MD: Difco Laboratories, Division of Becton Dickinson and Company

Dulger S, Demirbag Z, Belduz AO (2004) *Anoxybacillus ayderensis* sp. nov. and *Anoxybacillus kestanbolensis* sp. nov. Int J Syst Evol Microbiol 54:1499–1503

Engle M, Li Y, Rainey F, DeBlois S, Mai V, Reichert A, Mayer F, Messner P, Wiegel J (1996) *Thermobrachium celere* gen. nov., sp. nov., a rapidly growing thermophilic, alkalitolerant, and proteolytic obligate anaerobe. Int J Syst Bacteriol 46:1025–1033

Engle M, Li Y, Woese C, Wiegel J (1995) Isolation and characterization of a novel alkalitolerant thermophile, *Anaerobranca horikoshii* gen. nov., sp. nov. Int J Syst Bacteriol 45:454–461

Freier D, Mothershed CP, Wiegel J (1988) Characterization of *Clostridium thermocellum* JW20. Appl Environ Microbiol 54:204–211

Heinen W, Lauwers AM, Mulders JWM (1982) *Bacillus flavothermus*, a newly isolated facultative thermophile. Antonie van Leeuwenhoek J Microbiol Serol 48:265–272

Kevbrin VV, Romanek CS, Wiegel J (2003) Alkalithermophiles: a double challenge from extreme environments. Section VI: Extremophiles and Biodiversity, Vol. 6 "Origins: Genesis, Evolution and the Biodiversity of Life". In: Seckbach J. (ed) Cellular origins, life in extreme habitats and astrobiology (COLE). Kluwer Dordrecht

Ljungdahl LG, Wiegel J (1986) Anaerobic fermentations. Chapter In: Demain AL, Solomon NA (eds) Manual of industrial microbiology and biotechnology. American Society of Microbiology, pp 84–96

Marmur J (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. J Mol Biol 3:208–218

Marmur J, Doty P (1962) Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. J Mol Biol 5:109–118

Murray AE, Preston CM, Taylor LT, Blakis A, Wu K, DeLong EF (1998) Seasonal and spatial variability of bacterial and archaeal assemblages in the costal waters near Anvers Island, Antarctica. Appl Environ Microbiol 64:2585–2595

Paavilainen S, Korpela T (1993) Comparison of high-performance liquid and gas chromatography in the determination of organic acids in culture media of alkaliphilic bacteria. J Chromatogr 634:273–280

Pearson WR, Lipman DJ (1988) Improved tools for biological sequence comparison. Proc Natl Acad Sci USA 85:2444–2448

Pikuta E, Cleland D, Tang J (2003) Aerobic growth of *Anoxybacillus pushchinoensis* K1^T: emended descriptions of *A. pushchinoensis* and the genus *Anoxybacillus*. Int J Syst Evol Microbiol 53:1561–1562

Pikuta E, Lysenko A, Chuvilskaya N, Mendrock U, Hippe H, Suzina N, Nikitin D, Osipov G, Laurinavichius K (2000) *Anoxybacillus pushchinensis* 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 50:2109–2117

Plugge CM, Zoetendal EG, Stams AJM (2000) *Caloramator coolhaasii* sp. nov., a glutamate-degrading, moderately thermophilic anaerobe. Int J Syst Evol Microbiol 50:1155–1162

Rainey FA, Ward-Rainey N, Kroppenstedt RM, Stackebrandt E (1996) The genus *Nocardiopsis* represents a phylogenetically coherent taxon and a distinct actinomycete lineage: proposal of *Nocardiopsaceae* fam. nov. Int J Syst Bacteriol 46:1088–1092

Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA–DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res 25:4876–4882

Van de Peer Y, De Wachter R (1994) TREECON for windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. Comput Applic Biosci 10:569–570

Wiegel J (1981) Distinction between the Gram reaction and the Gram type of bacteria. Int JSyst Bacteriol 31:88

Wiegel J (1998) Anaerobic alkali-thermophiles, a novel group of extremophiles. Extremophiles 2:257–267

Wiegel J, Kevbrin V (2004) Diversity of aerobic and anaerobic alkalithermophiles. Biochem Soc Trans 32:193–198