ISSN 0026-2617, Microbiology, 2009, Vol. 78, No. 1, pp. 102–111. © Pleiades Publishing, Ltd., 2009.
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EXPERIMENTAL ARTICLES

Halomonas chromatireducens sp. nov., a New Denitrifying Facultatively Haloalkaliphilic Bacterium from Solonchak Soil Capable of Aerobic Chromate Reduction

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Received January 15, 2008

Abstract—A heterotrophic bacterial strain AGD 8-3 capable of denitrification under extreme haloalkaline conditions was isolated from soda solonchak soils of the Kulunda steppe (Russia). The strain was classified within the genus *Halomonas*. According to the results of 16S rRNA gene sequencing, *Halomonas axialensis, H. meridiana,* and *H. aquamarina* are most closely related to strain AGD 8-3 (96.6% similarity). Similar to other members of the genus, the strain can grow within a wide range of salinity and pH. The strain was found to be capable of aerobic reduction of chromate and selenite on mineral media at 160 g/l salinity and pH 9.5–10. The relatively low level of phylogenetic similarity and the phenotypic characteristics supported classification of strain AGD 8-3 as a new species *Halomonas chromatireducens.*

Key words: haloalkaliphiles, *Halomonas,* denitrification, chromate reduction, soda marshes.

DOI: 10.1134/S0026261709010135

The soda soils of temperate latitudes are extreme environments with low water activity, high pH, and pronounced fluctuations in temperature and total mineralization. They are specific ecosystems; similar to soda lakes, their functioning depends on the adaptation of their inhabitants to multiple stresses in the environment [1]. Unlike lakes, soils are much more heterogeneous, with the physicochemical characteristics varying due to climatic, geological, and hydrological factors [2]. Halotolerant organisms adapted to the possible drastic desalination, rather than obligate halophiles, are therefore to be expected in saline soils [3]. The members of the genus *Halomonas*, widespread in saline environments, satisfy these requirements; quite a few of them are able to survive salinity fluctuations from 0 to 25% and grow under alkaline conditions [4]. Moreover, Duckworth et al. [5] reported that most of the aerobic organotrophic strains isolated from soda lakes required high pH values for growth; according to the results of phylogenetic analysis, they belonged to the *Halomonadaceae* branch, γ-3 subclass of *Proteobacteria.*

The genus *Halomonas*, type genus of the family *Halomonadaceae*, presently (as of September 21, 2007; Euzeby, J.P., List of Prokaryotic Names with Standing in Nomenclature–Genus *Halomonas*) comprises 45 phenotypically and phylogenetically heterogeneous species [6] and a number of undeclared strains [7]; wide distribution, adaptation to broadly varying values of salinity and pH, and capacity for organotrophic utilization of various organic substrates are their major features. In this genus, only a few neutrophiles are capable of nitrate reduction [7–9]; capacity for anaerobic growth with nitrate, even under conditions of extreme salinity, is much more widespread among its haloalkaliphilic members from soda lakes [9]. A number of strains are highly resistant to such heavy metals as mercury, cadmium, copper, and chromium [2]. The latter feature, as well as aerobic bacterial reduction of chromate under saline alkaline conditions, may be of interest for biological purification of chromate-contaminated industrial wastewaters.

Chromate is presently an important environmental pollutant [10–14]. Under oxidative conditions, it exists as mobile, highly toxic chromate and dichromate oxyanions. The redox reactions transforming these oxyanions into a less toxic trivalent cation occur only at physiological pH values [15]. The known cases of bacterial chromate reduction are also restricted to acidic and near-neutral pH [16]; only recently Cr(VI) reduction under alkaline conditions was reported [17]. Since large amounts of chromium-containing wastes are alkaline solutions with a high mineralization, search for the biological agents reducing chromium in close-to-real conditions is highly important.

The goal of the present work was to isolate and describe the new organism capable of denitrification and aerobic chromate reduction under alkaline conditions and high salinity, in order to achieve detoxifica-

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tion of chromate- and nitrate-containing alkaline solutions.

MATERIALS AND METHODS

Isolation and cultivation of the strain. Strain AGD 8-3 was isolated from anaerobic enrichment under extremely haloalkaline conditions with acetate and nitrate as electron donor and acceptor, respectively; a mixed soil sample of soda solonchak from the Kulunda steppe (Altai krai, Russia) was used as inoculum. Enrichment cultures were obtained in the mineral medium (pH 10) with carbonate buffer (total Na^+ content, 4 M), 20 mM KNO_3, 20 mM CH_3COONa, and 0.1 g/l of yeast extract [9]. The medium was subsequently optimized by substituting 50% of Na^+ with an equimolar amount of NaCl, since nitrite reduction in pure carbonate buffer occurred only after a considerable lag phase. Moreover, solid medium with soda saturation was practically impossible to prepare. The pure culture was isolated on agarized medium with a similar composition; in order to avoid caramelization, the double-strength mineral medium and agarized water (4%) were sterilized separately; after sterilization, the salt solution was then added to the agar (1 : 1) under stirring at about 60°C. The final Na^+ concentration was 2.3 M. For subsequent cultivation the standard medium was used containing the following (g/l): Na_2CO_3, 76; $NaHCO_3$, 12.8; NaCl, 69; K_2HPO_4, 1. After sterilization, the following components were added (g/l): yeast extract, 0.1; KNO_3, 0.5; and sodium acetate, 2.7; as well as 2 ml/l of trace element solution [19] and 1 ml/l of 10% $MgSO_4 \cdot 7H_2O$. The cultures were incubated at 30°C. Significant amounts of nitrite were accumulated at the initial stage of cultivation; nitrate was monitored with the Merckoquant 1.10029 qualitative test. After nitrite consumption, new portions of nitrate were added. Growth stopped after four additions of nitrate (25 mM).

Type strains of *Halomonas campisalis* $4A^T$, *H. desiderata* $FB2^T$, and *H. campaniensis* $5AG^T$, as well as strains *H. campisalis* Z-7398, *'H. kenyensis'* AIR-2, and *'H. natronophila'* Z-7009 were kindly provided by Yu.V. Boltyanskaya.

Morphology and cytology. The size and shape of the cells from the culture grown in the standard liquid medium were determined by light microscopy (Olympus BX 41, Germany, 100×). The intracellular organization and cell wall structure were determined by electron microscopy. For ultrathin sectioning, the culture grown in liquid medium was used. The cells were treated according to Kellenberger, dehydrated, and embedded in Epon. The Reynolds reagent was used for contrasting [20]. Ultrathin sections were examined under a Jeol JEM-100C electron microscope (Japan).

The spectrum of aerobically consumed substrates was determined by cultivating the strain in the standard medium (see above) with 1 g/l of a tested substrates substituting acetate. Growth was monitored as OD_{600}. The result was considered positive when turbidity values were higher than those obtained in the medium containing no substrate except for the yeast extract of the basal medium.

Antibiotic sensitivity. Sensitivity to antibiotics was determined on bacterial lawns on petri dishes. The disks with antibiotics were applied immediately after inoculation. The effect of antibiotics was determined by the width of growth inhibition zones around the disks.

Growth in the range of concentrations of sodium chloride and sulfate was determined under aerobic conditions on the media prepared as follows. Soda solutions were prepared containing 0.1 mol/l of total sodium (pH 10.0); Na_2SO_4 or NaCl were added in order to obtain the required Na^+ molar concentrations; pH was adjusted to 10 with 3N HCl and 2N NaOH. These solutions were then sterilized by filtration and supplemented with other medium components, including acetate as a carbon source. Growth was assessed as OD_{600} after 48 h of incubation.

Growth in a pH range. In order to determine the effect of pH on aerobic growth of the strain, phosphate and carbonate buffer systems were used for pH ranges from 6.0 to 8.0 and from 8.0 to 11.0, respectively. In order to exclude the effect of Cl^- and Na^+ on pH profiles, the concentration of these ions was adjusted to the same level for all pH values. Buffer solutions for the pH 6.0–8.0 range contained 1.18 M NaCl and 0.79 M Na_2SO_4 ($[Na^+]$ = 2.76 M). Required pH values were obtained with 0.2 M solutions of KH_2PO_4/K_2HPO_4. For carbonate buffers, solutions were prepared containing the following: $[Na^+]$, 2.7 M; $[Cl^-]$, 1.2 M; $[HCO_3^-]$ 1 M; $[K_2HPO_4]$ 0.006 M (pH 8.0); and [NaCl], 1.2 M; $[Na_2CO_3]$ 0.75 M (pH 11.0). Solutions with the required pH values were obtained by mixing these two solutions. The media were sterilized by filtration; acetate (1 g/l) was used as an organic substrate. The culture was incubated for 24 h; after this period, growth was assessed and the final pH values were determined.

Reduction of ammonium chromate and sodium selenite by strain AGD 8-3. Growth of strain AGD 8-3 was monitored by the biomass increase and by a decreased chromate concentration in the medium. The initial OD_{600} of the culture at the time of chromate introduction was about 0.030 (approx. 1 μg protein/ml).

To study chromate reduction in suspensions, the culture was grown aerobically on the standard medium with acetate and nitrate. The biomass was separated by centrifugation and resuspended in the standard medium without nutrient components (acetate, nitrate, and yeast extract). The suspension heated for 10 min in a water bath was used for the control. Cell death was confirmed by the absence of growth two days after transfer into fresh medium with all supplements added. Prior to the experiment, suspensions of live and control biomass were stored in a refrigerator (8°C) for 3 days. After this period, chromate was added to the vials.

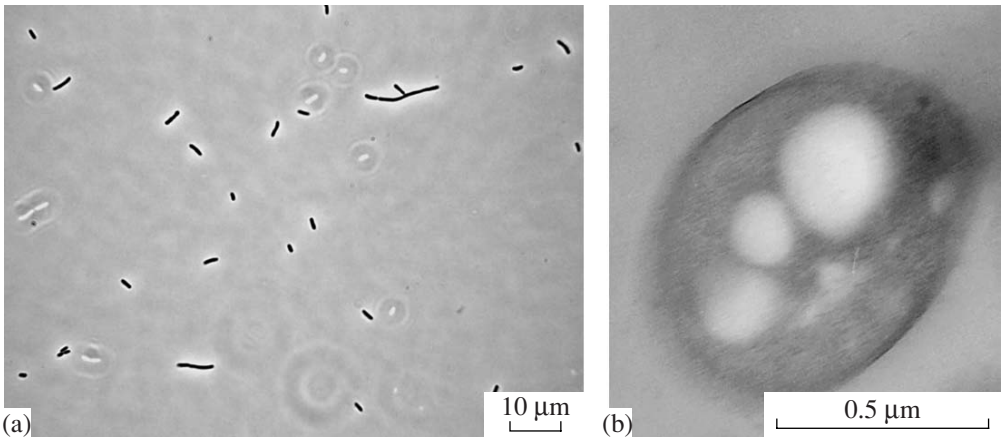

Fig. 1. AGD 8-3 cells under light microscope (a); ultrastructure of AGD 8-3 cells grown aerobically on acetate (b).

Reduction of ammonium chromate by strains *Halomonas campisalis* 4A^T, *H. desiderata* FB2^T, *H. campaniensis* 5AG^T, *H. campisalis* Z-7398, '*H. kenyensis*' AIR-2, and '*H. natronophila*' Z-7009 was determined on the medium of 80 g/l total salinity (pH 9.5) containing the following (g/l): Na_2CO_3, 13; $NaHCO_3$, 4; NaCl, 50; K_2HPO_4, 0.5. After sterilization, the following components were added (g/l): yeast extract, 0.1; NH_4Cl, 0.3; sodium acetate, 2.7; trace elements solution [19], 2 ml/l; $MgSO_4 \cdot 7H_2O$ as 10% solution, 1 ml/l. The initial concentration of ammonium chromate was 5 mg Cr/l.

Selenite reduction was determined in growth experiments in the medium with 5 mM selenite and initial OD of 0.03. Reduction was estimated qualitatively as formation of the red amorphous sediment of elemental selenium.

Analytic techniques. In the experiments on chromate reduction, chromium(VI) was determined with diphenylcarbazide [21]. In order to determine chromate under alkaline conditions, the method was modified as follows: in order to prepare diphenylcarbazide process solution, 4 ml 6N H_2SO_4 was added to 4 ml of the concentrate and water was added to the total volume of 100 ml according to the formulation. In the experiments on chromate reduction, cell protein was determined by the Lowry method with the Folin reagent.

Phylogenetic analysis. DNA isolation, amplification, and sequencing of the 16S rRNA gene were carried out according to the generally accepted procedures; universal bacterial primers were used. The 16S rRNA gene was sequenced in both directions, with forward and reverse universal primers. The CLUSTALX software package was used to align the sequences with the sequences of related bacterial species. Rootless phylogenetic trees were constructed using the TREECON software package. The sequence of strain AGD 8-3 16S rRNA gene was deposited into GenBank (accession no. EU447163).

DNA G+C content. DNA isolation and determination of the G+C base content by thermal denaturation curves (Pye Unicam SP 1800 spectrophotometer) were carried out as described [22, 23].

Fatty acid composition. A sample of dry biomass (5 mg) was treated for 60 min with 0.4 ml of 1N HCl in methanol at 80°C (acid methanolysis). The resulting methyl ethers of fatty acids and dimethyl acetals were extracted with hexane and analyzed on a Sherlock gas chromatograph (Microbial Identification System, MIDI Inc., United States) [24].

RESULTS AND DISCUSSION

General characterization. Strain AGD 8-3 is a facultatively anaerobic heterotroph. It was isolated from enrichments obtained from the mixed soil sample, as an organism capable of denitrification at extreme salinity (4 M Na^+) and pH 10. In the course of isolation, two stages of nitrate reduction were revealed. First, nitrate was almost completely reduced to nitrite; the latter was then reduced to dinitrogen after nitrate consumption. The strain was able to grow, albeit slower, on acetate with nitrite or nitrous oxide as electron acceptors. High carbonate content in the medium decreased the rate of nitrite reduction. This dynamics may result from a high sensitivity of the periplasmic nitrite reductase to extreme concentrations of alkaline salts, unlike the membrane-bound nitrate reductase [9]. Apart from facultatively anaerobic growth on nitrate, strain AGD 8-3 was able to grow aerobically both in liquid and on solid media.

On solid media, small (1–2 mm in diameter) round light beige colonies were formed after 3 days of incubation. In liquid culture, light microscopy revealed motile cells, single or in chains of two to five (Fig. 1a). The cells divided by constriction; spore formation was not detected. Ultrathin sections revealed gram-negative cell wall structures and electron-transparent structures, probably poly-β-hydroxybutyrate (PHB) granules

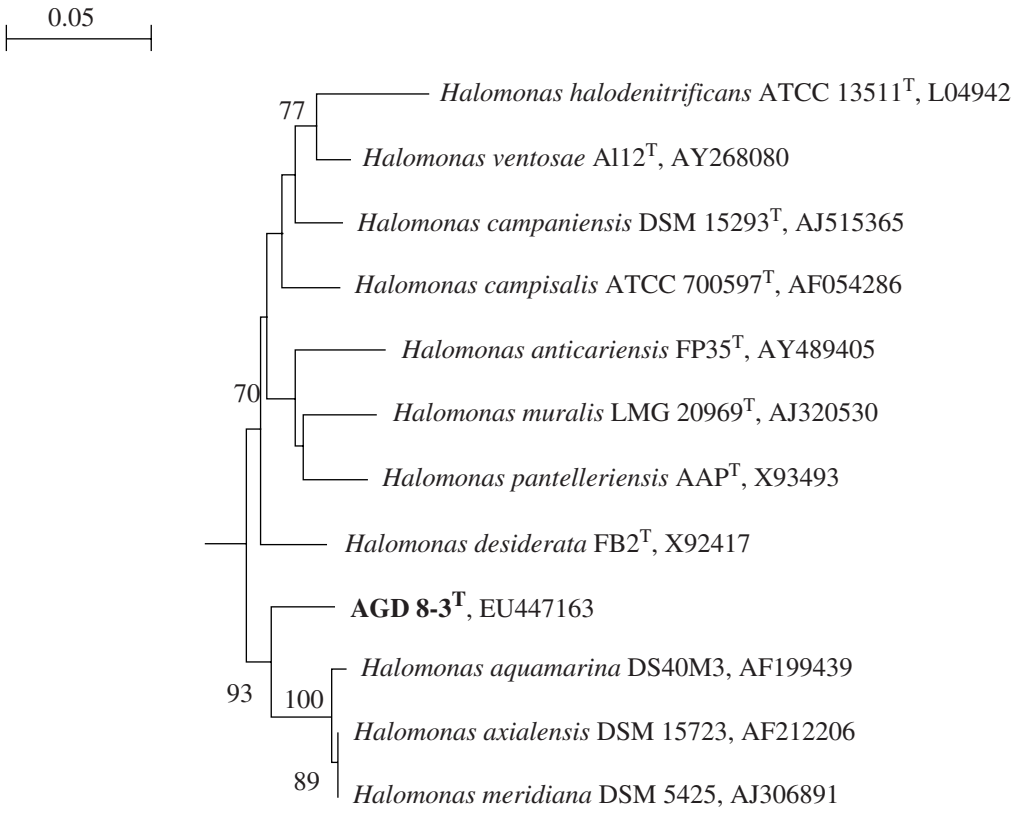

Fig. 2. Position of strain AGD 8-3 on the phylogenetic tree of the genus *Halomonas.*

(Fig. 1b). Up to five peritrichous flagella were revealed in the whole-cell preparations.

Phylogenetic analysis. Phylogenetic analysis supported the classification of strain AGD 8-3 within the genus *Halomonas,* γ-3 subclass of the *Proteobacteria*. The new strain occupies an intermediate position between the cluster comprising *H. meridiana*, *H. axialensis*, and *H. aquamarina* and the species *H. desiderata*, *H. campisalis*, and *H. campaniensis* forming individual branches [6] (Fig. 2). The similarity levels were 96.6% (*H. meridiana, H. axialensis*, and *H. aquamarina*), 95.4% (*H. desiderata*), 95.5% (*H. campisalis*), and *H. campaniensis* (95.8%).

The DNA G+C base content of strain AGD 8-3 was 64.2 ± 0.5 mol %.

Physiological characteristics of strain AGD 8-3 were similar to those of the genus *Halomonas* in general. Growth occurred within a broad range of pH, salinity, and temperature (Fig. 3). The strain grew in the temperature range from 8 to 45°C with the optimum at 35°C; it was therefore a typical mesophile. Stable growth occurred at pH values from 6.7–6.8 to 10.5 (final pH values); the biomass yield was almost constant throughout this interval, with a slight increase at pH 9.0–9.5. Thus, the organism is a facultative alkaliphile.

In respect to salinity, the strain behaved as a facultative halophile. This is demonstrated by its aerobic growth in the range of sodium-determined salinity (as sodium chloride and sulfate). In the case of chloride salinity, active growth occurred within the range of Na^+ concentrations from 0.1 to 3.5 M, with the optimum at 0.1–2.0 M. Salinity resulting from equimolar (by Na^+) concentrations of sodium sulfate presented less problems (Fig. 3). The physicochemical characteristics of sodium sulfate are close to those for sodium carbonate; unlike NaCl, both salts are weak electrolytes. The brines of sodium sulfate and carbonate containing 4 mol/l of total Na^+ are equivalent to 2 M NaCl in conductivity and osmotic pressure. Thus, up to 2 M Na^+, dissolution of sodium sulfate and carbonate results in complete dissociation with the liberation of sodium ions; this is the cause of the first decrease in the growth rate. Unlike NaCl, the subsequent dissolution of sodium sulfate and carbonate occurs via the hydration of whole molecules (unable to dissociate at this ionic strength of the solution); thus, the activity of sodium may actually decrease. Since the load of the energetically expensive osmolyte synthesis does not increase, the culture may grow with the same efficiency; this phenomenon has been described for lithoautotrophic haloalkaliphilic sulfur-oxidizing bacteria [25–27].

Strain AGD 8-3, like other members of this species, is able to utilize a broad range of organic substrates for

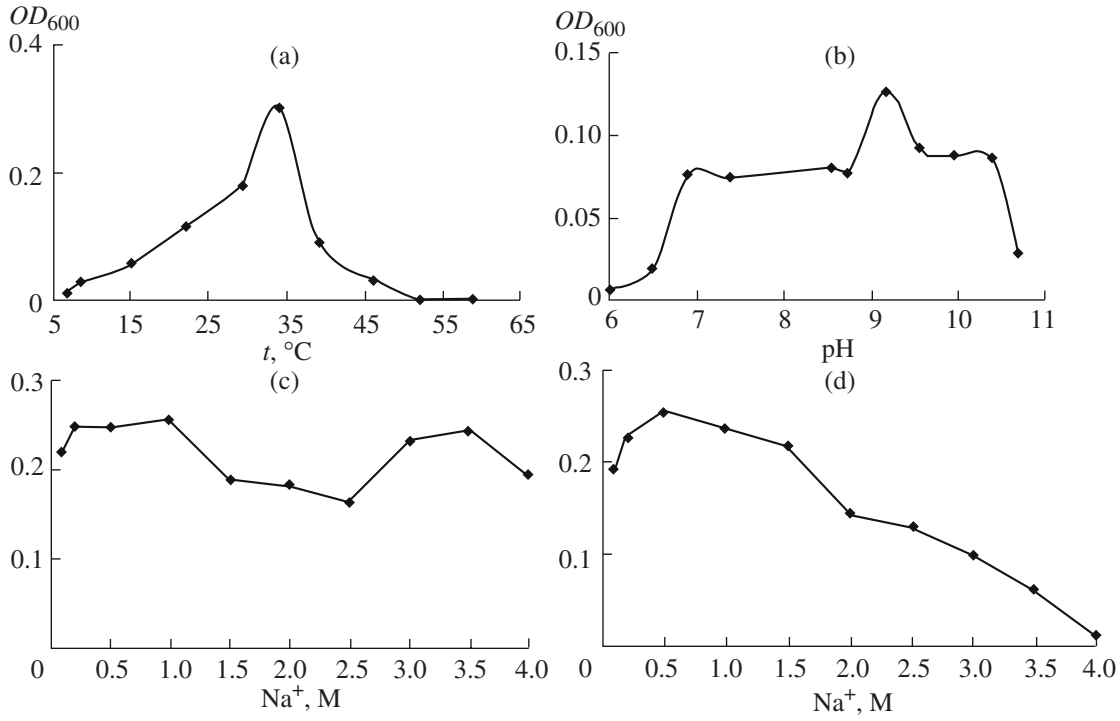

Fig. 3. Growth of strain AGD 8-3 depending of temperature (a), pH (b), Na_2SO_4 salinity (c), and NaCl salinity (d).

growth (table). The highest OD_{600} values were obtained with casamino acids and yeast extract, as well as sucrose, maltose, proline, and trehalose. Acetate, pyruvate, lactate, succinate, fumarate, propionate, butyrate, and citrate were the best growth substrates among organic acids. Sugars were consumed less actively, with maximal growth on disaccharides (maltose, sucrose, and trehalose). Among the alcohols investigated, only ethanol supported active growth; no growth occurred on polyalcohols (glycerol, mannitol, and *meso*-erythritol). Among amino acids, proline, asparagine, lysine, arginine, threonine, and valine were the best substrates among amino acids; peptone yielded comparable growth.

Comparison of strain AGD 8-3 with its closes relatives is presented in the table.

Strain AGD 8-3 was sensitive to amikacin, benzylpenicillin, gentamycin, kanamycin, neomycin, and polymyxin; it was resistant to ampicillin, vancomycin, lincomycin, nalidixic acid, novobiocin, rifampicin, streptomycin, tetracycline, and erythromycin.

The fatty acid composition of the polar lipids of strain AGD 8-3 was generally similar to that of the haloalkaliphilic *H. campisalis* and *H. desiderata* [25]. Among fatty acids, $C_{18:1\omega7}$ (70.69%), $C_{16:0}$ (15.33%), and $C_{16:1\omega7}$ (6.05%) predominated.

Aerobic reduction of selenite and chromate. Strain AGD 8-3 is able to reduce ammonium chromate under aerobic growth conditions in a mineral medium (159 g/l, pH 9.5) with acetate. The maximal amount of chromium reduced under such conditions was 1 mg/(l day). The pattern of chromate reduction was as follows: after inoculation of chromate-containing medium, the lag phase was longer than under standard conditions; then exponential growth commenced. No chromate reduction occurred before an approx. tenfold increase in the biomass; at this stage, active reduction of the oxyanion began. The rate of biomass accumulation decreased; however, after detoxification of over 50% of chromate, the growth rate became comparable to that at the early exponential phase (Fig. 4a). In the presence of an oxidized substrate, chromate reduction could be repeated (Fig. 4b); increasing biomass enabled the reduction of higher concentrations of chromate. This dynamics in the reduction of the toxic oxyanion may be interesting for biotechnological detoxification processes, because the total amount of reduced chromium increases without additional manipulations within the biomass.

When concentrated cell suspensions were used for chromate reduction, initial chromate concentration could be increased to 30 mg/l; only the living active culture was shown to reduce chromate. No chromate reduction occurred in the case of dead cells or living cells not provided with acetate (Fig. 5).

Recent findings revealed that most *Halomonas* species are capable of selenite reduction [4]. It was demonstrated for strain AGD 8-3, which reduced sodium selenite (5 mM) under aerobic conditions in a mineral medium with acetate. Reduction of sodium selenite by strain AGD 8-3 was demonstrated by the qualitative

Comparative characterization of strain AGD 8-3 and related *Halomonas* species

Characteristics	AGD 8-3	*H. axialensis* [28]	*H. meridiana* [29]	*H. aquamarina* [6]	*H. desiderata* [30]	*H. campisalis* [31]	*H. campaniensis* [32]
Source of isolation	Salt marshes, Russia	Hydrothermal spring	Saline lakes, Antarctic	Seawater	Municipal sewage	Salt marshes, United States	Saline basin, Italy
Morphology	Rods	Rods	Rods	Rods	Rods	Rods	Rods
Cell size, μm	0.8–1.0 × 1.6–1.8	1.0 × 2.0–3.0	ND	ND	ND	1.0 × 3.0–5.0	ND
Cell wall	G(–)	G(–)	G(–)	G(–)	G(–)	G(–)	G(–)
Motility	+	+	+	+	+	+	+
Reaction to oxygen	Facultative anaerobe	Facultative anaerobe	Aerobe	Aerobe	Facultative anaerobe	Facultative anaerobe	ND
Cl^- requirement	–	–	ND	ND	–	ND	–
Na^+, M (chloride, int/opt), %	0.1–3.5 / 0.1–2 0.53–20.4 / 3.0	0.5–24 / 4	0–20 / 1–3	0.5–20 / 7.5–10	0–20 / 1–5	0.5–15 / 5	Optimum 10% NaCl, grows without salt
Na^+, M (sulfate, int/opt), %	0.1–4.0 / 0.1–4.0 0.53–28.2						
T, °C (int/opt)	8–47 / 35	–1–35 / 30	–5–45	5–40 / 20–25	10–45	4–50 / 30	10–43 / 37
pH (int/opt)	6.8–10.5 / 9.0–9.5	5–12 / 7–8	5–10	5–10	7–11	8.0–11.0	7–10 / 9
With acetate as electron donor:							
N_2 formation from NO_3^-	+	+	–	–	+	+	ND
NO_2^- formation from NO_3^-	+	+	ND	+	+	+	+
Nitrite reduction	+	– (aerobically)	ND	+	+	+	ND
Substrates utilized for aerobic growth:							
acetate	+	–	+	+	+	+	+
lactate	+	–	ND	+	ND	ND	ND
fumarate	+	+	+/–	+	+	+	ND
pyruvate	+	ND	ND	ND	ND	ND	ND
malate	+/–	+	ND	ND	ND	ND	ND
formate	–	ND	+	–	+	–	ND
succinate	+	–	+	+	+	+	ND
propionate	+	–	+/–	+	–	+	ND
butyrate	+	+	ND	ND	ND	ND	ND
citrate	+	–	–	+	+/–	+	–
L-sorbose	+/–	ND	ND	ND	ND	ND	ND
D-cellobiose	+/–	–	+/–	–	–	+	+
αD-melibiose	+/–	–	ND	+	ND	ND	ND
ribose	+/–	–	+/–	–	+/–	–	ND
D-xylose	+/–	–	+/–	–	+/–	–	–
D-galactose	+/–	–	+	–	+/–	–	–

Table. (Contd.)

Characteristics	AGD 8-3	*H. axialensis* [28]	*H. meridiana* [29]	*H. aquamarina* [6]	*H. desiderata* [30]	*H. campisalis* [31]	*H. campaniensis* [32]
fructose	+/–	+	+/–	–	+	+	+
D-maltose	+	+	+	–	+	+	+
sucrose	+	+	+/–	+	+	+	+
glucose	+/–	+	+/–	+	+/–	+	+
αL-trehalose	+	–	+	+	+	+	ND
D-raffinose	–	–	+/–	–	–	–	ND
L-arabinose	+/–	+	+/–	+	+	–	–
meso-erythritol	–	–	–	–	ND	ND	ND
mannitol	–	ND	+	–	–	–	ND
sorbitol	–	–	+/–	–	–	–	ND
glycerol	+/–	–	+	+	+	+	+
ethanol	+	+	+	+	–	+	ND
DL-alanine	+/–	–	+	+	+/–	+	ND
L-glutamine	+	–	ND	ND	ND	ND	ND
L-asparagine	+	–	+	–	ND	ND	ND
L-lysine	+	–	+	+	–	+	ND
L-histidine	+	–	–	–	–	–	ND
L-cysteine	–	ND	ND	ND	ND	ND	ND
DL-valine	+	–	+/–	+	–	+	ND
L-proline	+	–	+	–	ND	ND	ND
L-arginine	+	–	ND	ND	ND	ND	ND
DL-ornithine	+/–	–	+	+	ND	ND	ND
DL-serine	–	ND	+	+	+	+	ND
DL-threonine	+	–	+	–	ND	ND	ND
DL-tryptophan	–	ND	ND	ND	ND	ND	ND
DL-leucine	–	ND	ND	ND	ND	ND	ND
casamino acids	+	ND	ND	ND	ND	ND	ND
peptone	+	ND	ND	+	ND	ND	ND
yeast extract	+	ND	+	ND	ND	ND	ND
betaine	–	–	ND	ND	ND	ND	ND
G+C, mol %	64.2	57.6	59.0	57.0	66.0	66.0	63.7

Note: "–" indicates absence, "+/–", weak manifestation, and "+", presence of a feature; ND indicates "no data"; "int/opt", interval/optimum.

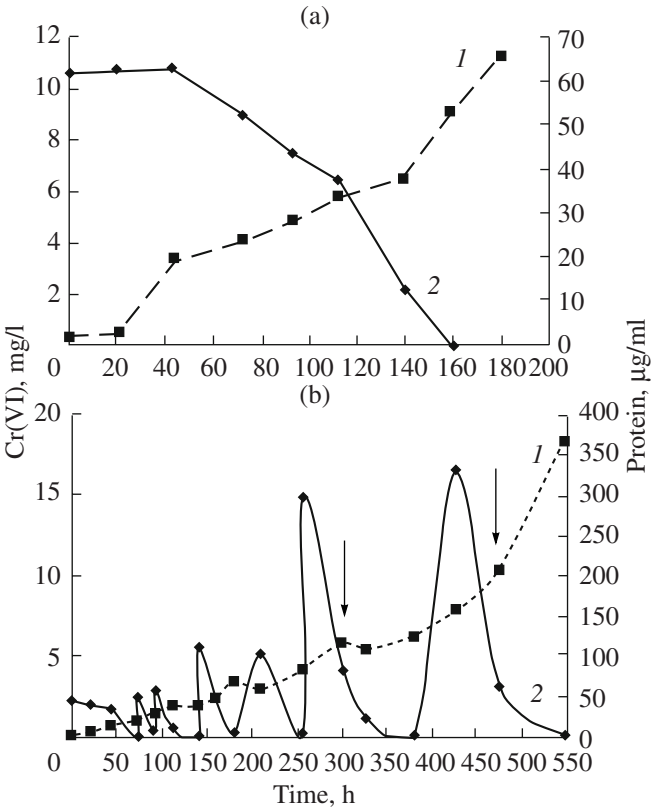

Fig. 4. Kinetics of growth (*1*) and chromate reduction (*2*) by strain AGD 8-3 at single (a) and periodical addition of chromate (b); arrows indicate addition of 20 mM acetate.

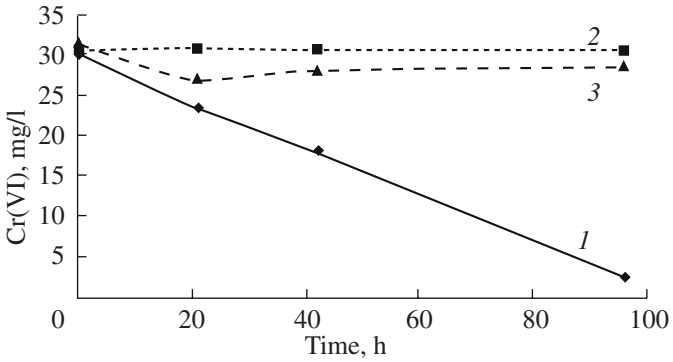

Fig. 5. Aerobic chromate reduction by AGD 8-3 cell suspension: live cells (*1*); dead cells (*2*); live cells without the substrate (*3*).

reaction (formation of red amorphous precipitate of elemental selenium).

Chromate reduction by type strains of *Halomonas* species has not previously been investigated; the capacity for the reduction of this oxyanion by the species most closely related to strain AGD 8-3 (*Halomonas campisalis* 4A^T, *H. desiderata* FB2^T, *H. campaniensis* 5AG^T, *H. campisalis* Z-7398, '*H. kenyensis*' AIR-2, and '*H. natronophila*' Z-7009) was therefore investigated (Fig. 6). Strain AGD 8-3 was the most efficient chromate reducer; *H. campisalis, H. desiderata, 'H. kenyensis'* and *'H. natronophila'* reduced approx. 50, 35, 25, and 25% of chromate, respectively; *H. campaniensis*^{T} reduced the smallest amount of chromate (approx. 5%)

Strain AGD 8-3 isolated from the Kulunda steppe soda solonchak soil is the first representative of *Halomonas* capable of denitrification at extreme salinity. It also has a high potential for reduction of toxic oxyanions. Due to the relatively low level of phylogenetic similarity and phenotypic peculiarities, classification of strain AGD 8-3 as a new species, *Halomonas chromatireducens*, is proposed.

Description of *Halomonas chromatireducens* sp. nov.

(chrom.a.ti.re.du'cens L. n. *chromium*, chrome (element); L. part. adj. *reducens*, converting to a different state; N.L. part. adj. *chromatireducens*, reducing chromate).

Cells are rod-shaped and are variable in size (0.5–0.8 × 2–6 μm); they are single or in chains, motile by means of peritrichous flagella, with a gram-negative cell wall structure. When grown on acetate, PHB-like granules are produced. The organism is an obligate organoheterotroph, it utilizes a broad spectrum of simple organic

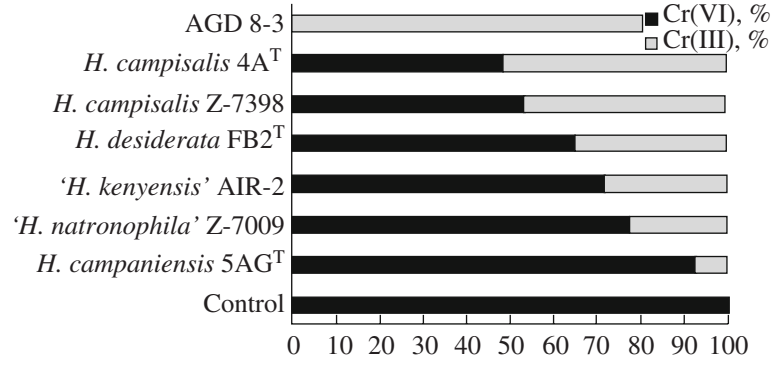

Fig. 6. Chromate reduction by *Halomonas* haloalkaliphilic type strains.

substrates, such as sugars, organic acids, and amino acids as carbon and energy sources. The species is facultatively anaerobic with the capacity for denitrification (with nitrate, nitrite, and N_2O as electron acceptors) at an extremely high pH and salt concentration (pH 10 and 4 M total Na^+). Extremely salt-tolerant (from 0.1 to 4 M total Na^+ with the optimum at 0.5 M) and facultatively alkaliphilic (pH range for growth from 6.8 to 10.5 with the optimum at 9.0–9.5). The organism is capable of chromate reduction to Cr(III) when grown aerobically with acetate. The dominant fatty acids are $C_{18:1\omega7}$, $C_{16:0}$, and $C_{16:1\omega7}$. The DNA G+C content is 64.2 mol % (T_m). The type strain AGD 8-3 (=NCCB100225^T = VKM B-2497^T) was isolated from soda marshes in southwestern Siberia. Accession number of the 16S rRNA gene sequence is EU447163.

ACKNOWLEDGMENTS

The work was supported by the Russian Foundation for Basic Research (projects nos. 07-04-00651, 07-04-00153, and 07-04-10041) and the Molecular and Cell Biology program of the Presidium of the Russian Academy of Sciences.

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