Microbiology, Vol. 69, No. 1, 2000, pp. 75–82. Translated from Mikrobiologiya, Vol. 69, No. 1, 2000, pp. 89–97.

EXPERIMENTAL ARTICLES

Roseinatronobacter thiooxidans gen. nov., sp. nov., a New Alkaliphilic Aerobic Bacteriochlorophyll *a*–containing Bacterium Isolated from a Soda Lake

D. Yu. Sorokin[1], T. P. Tourova, B. B. Kuznetsov, I. A. Bryantseva, and V. M. Gorlenko

Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117811 Russia

Received June 29, 1999

Abstract—Several samples of microbial mat obtained from soda lakes of the Kunkurskaya steppe (Chita region) abundantly populated by purple bacteria were screened for the presence of heterotrophic alkaliphiles capable of oxidizing sulfur compounds to sulfate. This capacity was found in only one pigmented strain, ALG 1, isolated on medium with acetate and thiosulfate at pH 10. The strain was found to be a strictly aerobic and obligately heterotrophic alkaliphile. Growth on medium with acetate was possible within a narrow pH range from 8.5 to 10.4. The strain formed a reddish orange carotenoid and bacteriochlorophyll *a*. Pigments were synthesized only at high concentrations of nitrogen-containing organic compounds (peptone or yeast extract). The production of bacteriochlorophyll *a* was maximal under microaerobic conditions in darkness. Strain ALG 1 could oxidize sulfide, thiosulfate, sulfite, and elemental sulfur to sulfate. In heterotrophically growing culture (pH 10), thiosulfate was not oxidized until the late logarithmic phase. The sulfur-oxidizing activity was maximal at the most alkaline pH values. The notable increase in the efficiency of organic carbon utilization observed in the presence of thiosulfate suggested that the bacterium was a sulfur-oxidizing lithoheterotroph. The phylogenetic analysis of the 16S rRNA gene showed strain ALG 1 to be a member of the α-3 subgroup of Proteobacteria and to constitute a distinct branch located between nonsulfur purple bacteria *Rhodobacter* and *Rhodovulum*. Based on the unique phenotypic properties and the results of phylogenetic analysis, the alkaliphilic isolate ALG 1 was assigned to a new genus and species *Roseinatronobacter thiooxidans* with the type strain DSM-13087.

Key words: alkaliphilic, sulfur-oxidizing, erythrobacteria, bacteriochlorophyll *a*.

INTRODUCTION

Studies of the taxonomic and functional diversity of the microbial community in soda lakes have recently gained new momentum as a result of the increased interest of fundamental and applied microbiology in extremophilic, and in particular alkaliphilic, microorganisms [1–3].

Soda lakes were shown to be populated by microbial communities, represented mainly by specific haloalkaliphilic groups of bacteria that maintain the normal operation of geochemical cycles. The high organic matter production in soda lakes is usually provided by alkaliphilic cyanobacteria and anoxygenic phototrophic bacteria. The latter are dominated by sulfur purple bacteria [4–7]. Alkalitolerant forms of nonsulfur purple bacteria belonging to the genus *Rhodobacter* and including those capable of aerobic heterotrophic growth were found in soda lakes of the Transbaikal region with low mineralization (our unpublished data). However, no obligately aerobic bacteriochlorophyll *a*–containing (erythrobacteria) alkaliphiles have been reported as yet.

The sulfur cycle is one of the most active biogeochemical cycles operating in soda lakes. The reduction of sulfate is carried out by several specific groups of alkaliphiles, mostly at the expense of hydrogen [8]. The light-dependent oxidation of reduced sulfur compounds is performed by anaerobic phototrophs. Chemolithoautotrophic sulfur-oxidizing alkaliphiles are mostly represented by two specific groups of aerobic bacteria belonging to γ-Proteobacteria [9]. One of them is related to the sulfur purple phototrophs of the family *Ectothiorhodospiraceae*, and the other is close to the neutrophilic thitrophic bacteria of the genus *Thiomicrospira*. Among heterotrophic sulfur-oxidizing alkaliphiles, it was mainly the *Halomonas*-like species, oxidizing sulfur compounds to tetrathionate, that were previously cultivated [10]. It was only with a nonsulfur substrate used for enrichment that an alkaliphilic facultatively autotrophic hydrogen bacterium was recently isolated [11], which also appeared to be a sulfur-oxidizing lithoheterotroph.

In this work, we describe the properties of the new alkaliphilic isolate representing, apparently, an intermediate form in evolution from anaerobic nonsulfur purple bacteria (in the sequel, NSPB) to aerobic sulfur-oxidizing lithoheterotrophs. Under certain conditions, this

[1]To whom the correspondence should be addressed (e-mail: soroc@inmi.da.ru).

0026-2617/00/6901-0075$25.00 © 2000 MAIK "Nauka/Interperiodica"

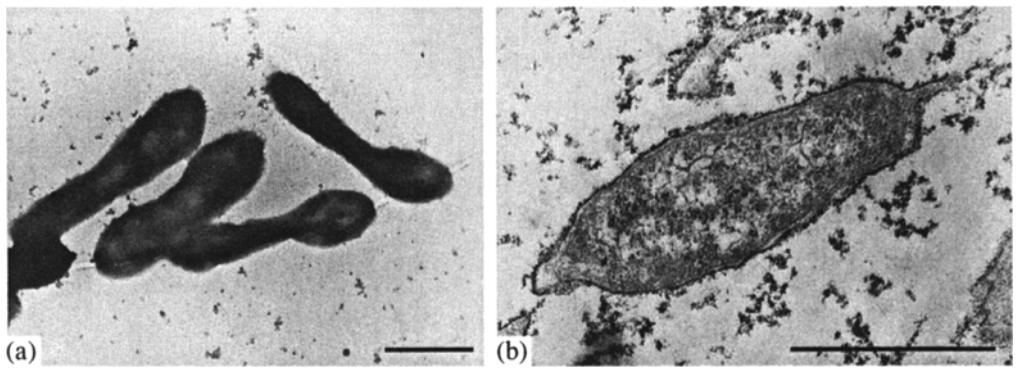

Fig. 1. Morphology of alkaliphilic strain ALG 1: (a) whole cells; (b) ultrathin section of a cell from the stationary phase of a batch culture. Bar, 1 μm.

bacterium can synthesize large quantities of bacteriochlorophyll *a* but is unable to grow photoanaerobically.

MATERIALS AND METHODS

Samples of sediment and microbial mats from the soda lakes of the Kunkurskaya steppe (Chita region) were used to obtain enrichment cultures. The salinity and pH of water in the lakes were 2–40 g/l and 9–10, respectively.

Enrichments of alkaliphilic heterotrophic sulfur-oxidizing bacteria were obtained on medium containing 2 mM acetate and 10 mM thiosulfate at pH 10 and 30°C under constant shaking. The growth of the enrichments was monitored by the increase of turbidity and by thiosulfate utilization. After the complete consumption of thiosulfate, cultures were transferred to a solid medium of the same composition. Following incubation for two weeks, all colored colonies were isolated. The new isolates were tested for their ability to oxidize sulfur compounds and for the presence of bacteriochlorophyll *a*.

In all cases except those explicitly mentioned in the text, the batch cultivation was performed in a standard mineral medium (pH 10.0–10.1) composed of (g/l): Na_2CO_3, 23; $NaHCO_3$, 7; NaCl, 5; K_2HPO_4, 0.5; KNO_3, 0.5; $MgCl_2 \cdot 6H_2O$, 0.1; and 2 ml/l of the trace element solution [12]. During routine cultivation of strain ALG 1, sodium acetate (20 mM), used as a carbon source, thiosulfate (10 mM), yeast extract "Difco" (in the sequel, y.e.), and peptone (1 g/l, each) were added as sterile concentrated solutions. In tests without thiosulfate, $MgSO_4$ was substituted for $MgCl_2 \cdot 6H_2O$. During cultivation, the pH of the medium never dropped by more than 0.2 because of the high capacity of the soda buffer. When culturing at lower pH values, Na_2CO_3 was replaced with 0.1 M HEPES–NaOH (pH 7–8) or $NaHCO_3$–NaOH (pH 8–9) and a constant concentration of Na was maintained by adding NaCl or $NaHCO_3$. Continuous cultivation of strain ALG 1 at pH 10 was performed as previously described [13] by separately supplying the double strength solutions of acidic and alkaline medium constituents.

The respiratory activity of strain ALG 1 cells was measured in a thermostatically controlled cell (vol = 5 ml, 30°C) with a Clark-type oxygen electrode and a polarograph (Yellow Spring Instruments, Ohio, USA). Cells were harvested by centrifugation, washed and suspended in soda buffer (0.6 M of the total Na^+, pH 10) to a final concentration of approximately 10 mg protein/ml. Because, in most cases, the cells showed active endogenous respiration, the cell suspensions prior to measurements were aerated on a shaker for 1–2 h. The final concentrations of substrates added to the cell were (μM): acetate, 500; thiosulfate and sulfide, 100; sulfite, 50; and elemental sulfur, 70. Sulfide and sulfite were added as 0.1 M anaerobic solutions containing 1 mM EDTA. Elemental sulfur was added as a saturated solution in acetone (17 mM). The concentration of oxygen in samples from an ALG 1 batch culture was also determined polarographically.

Thiosulfate at concentrations above 1 mM was determined iodometrically after the neutralization of samples with 50% acetic acid. Lower concentrations of thiosulfate as well as those of tetra- and trithionate were determined by means of cyanolysis [14]. The concentration of sulfate in the medium was measured by turbidimetry after the complete consumption of thiosulfate [15]. The protein content in washed ALG 1 cells was measured by the Lowry method after alkaline hydrolysis. Cell-free extracts were prepared from cells disrupted by sonication. Total pigments were extracted by acetone from the wet cell mass. Bacteriochlorophyll *a* was selectively extracted with methanol. The spectral identification of pigments was performed on a SF-56 spectrophotometer. The content of bacteriochlorophyll *a* was measured spectroscopically in a methanol extract at 770 nm [16].

For electron microscopy of whole cells, samples of liquid culture were fixed with glutaraldehyde and stained with 1% phosphotungstic acid. Material for ultrathin sections was prepared from washed cells suspended in 0.5 M NaCl, pH 9, and fixed with 1% OsO_4

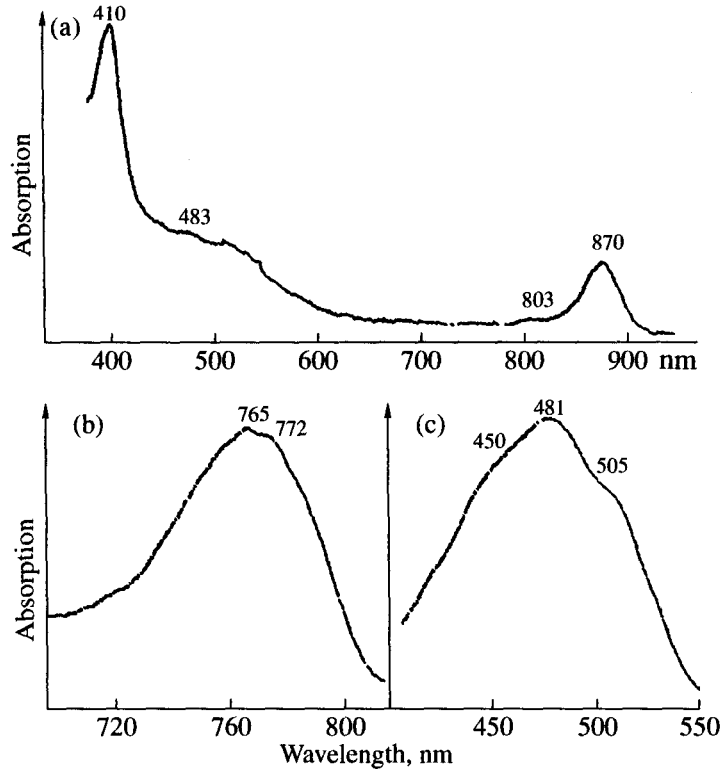

Fig. 2. Absorption spectra of pigments from the alkaliphilic strain ALG 1 grown at pH 10 on medium with acetate (20 mM), y.e. and peptone (1 g/l, each): (a) cell-free extract; (b) bacteriochlorophyll *a* in an acetone extract; (c) the carotenoid fraction in a hexane extract (after acetone).

(w/v) for 8–12 h. Thin sections were stained with lead citrate and uranyl acetate.

Isolation and purification of DNA, determination of the G+C content of DNA, and DNA–DNA hybridization were performed according to Marmur [17] and De Ley [18].

To establish the phylogenetic position of the new isolate, the nucleotide sequence of its 16S rRNA gene was determined. The gene of 16S rRNA was selectively amplified in vitro with universal primers. The buffer used for amplification contained $MgCl_2$, 1.5 mM; KCl, 50 mM; Tris–HCl, 10 mM; and 0.001% gelatin; the pH was 8.3. The reaction mixtures, 100 μl in volume, contained standard concentrations of dNTP and equimolar quantities of the pA and pH primers. Thirty cycles of amplification were performed with the following temperature profile: denaturation of DNA at 94°C for 30 s, annealing of primers at 40°C for 1 min, and elongation at 72°C for 2 min 30 s. After purification accomplished with low melting point agarose and on Promega columns, the 16S rRNA gene was sequenced in both directions with the use of forward and reverse universal primers and Sequenase (Biochemicals, Cleveland, Ohio, USA).

The preliminary analysis of the 16S rRNA gene sequence of the new isolate was done using the data and software of the Ribosomal Database Project. At the next step, the sequence of the 16S rRNA gene of strain ALG 1 was manually aligned with the sequences of the reference representatives of the α-3 subgroup of Proteobacteria using the BIOEDIT sequence editor. Rooted phylogenic trees with *E. coli* as an outgroup were constructed for the bacterium under study by using various algorithms implemented in the TREECON, PHYLIP, and GENEBEE programs.

RESULTS

Isolation and morphology. Out of 48 bacterial strains isolated in pure culture from enrichments on

Table 1. Effect of aeration and light on bacteriochlorophyll synthesis by alkaliphilic strain ALG 1 at pH 10 on medium with acetate (20 mM), y.e., and peptone (1 g/l, each). The microaerobic culture, 200 ml, was grown in a 500-ml flask without mixing; the aerobic culture, 100 ml, in a 300-ml flask on a magnetic shaker with air actively blown through the liquid

Cultivation conditions		Content of bacteriochlorophyll *a*, μg/mg protein
aeration	light	
Microaerobic (static) culture	–	2.72
	+	2.50
Aerobic culture	–	0.45
	+	0.02

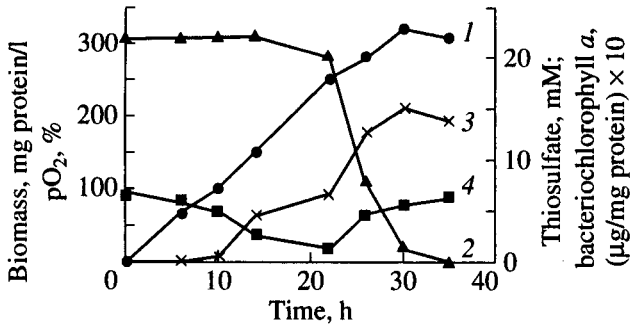

Fig. 3. Dynamics of (*1*) growth, (*2*) thiosulfate oxidation, (*3*) content of bacteriochlorophyll *a*, and (*4*) dissolved O_2 in a batch culture of strain ALG 1 at pH 10. One liter of culture was grown in a 2.5-l conic flask on a shaker at 100 rpm with acetate (20 mM), y.e. and peptone (1 g/l, each), and thiosulfate (22 mM).

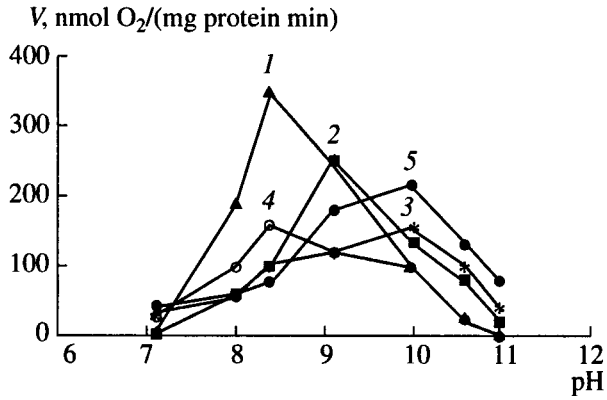

Fig. 4. The effect of pH on oxygen uptake by washed cells of the alkaliphilic strain ALG 1 chemostat-grown on acetate and thiosulfate at pH 10: (*1*) with thiosulfate; (*2*) with sulfide; (*3*) with elemental sulfur; (*4*) with sulfite; (*5*) with acetate. *V* is the rate of oxygen uptake minus endogenous respiration. The buffers used are given in "Materials and Methods."

medium with acetate and thiosulfate at pH 10, only one pink-colored strain ALG 1 from the lake Gorbunka (pH 9.5, mineralization 18 g/l) was able to oxidize thiosulfate to sulfate. Moreover, this bacterium could synthesize bacteriochlorophyll *a* in the dark under aerobic conditions. Exhibiting such a singular combination of properties, strain ALG 1 was selected for further analysis.

Cells of ALG 1 were lemon-shaped, nonmotile, often aggregated in chains, and had beak-like elongations at the ends. The cell wall had a typical gram-negative structure, the cytoplasm was dense, and all cells, including those with the maximum content of bacteriochlorophyll *a*, lacked intracytoplasmic membranes (Fig. 1).

The DNA G+C content in ALG 1 was 61.5 mol%.

Physiology. Strain ALG 1 is an organoheterotroph and obligate aerobe. All attempts to grow the bacterium under autotrophic conditions with thiosulfate and hydrogen or under anaerobic conditions in the light failed. On medium with acetate and y.e., growth was observed only in a narrow interval of alkaline pH from 8.5 to 10.4, with the optimum at 10. On medium with soda buffer at pH 10, growth was observed over the total Na^+ concentration range 0.1–2 M, with optimal growth at 0.4–0.6 M. At pH 10, the following compounds were utilized by the bacterium as a single source of carbon and energy: acetate, pyruvate, malate, glycolate, glyoxylate, lactate, succinate, fumarate, caproate, caprylate, citrate, propionate, valerate, benzoate, sorbitol, mannitol, glycerol, glucose, fructose, glutamate, and aspartate. Among the sources tested, it did not utilize formate, gluconate, butyrate, maleate, malonate, tartrate, methanol, ethanol, propanol, butanol, benzoate, D-ribose, leucine, threonine, or aromatic amino acids. Nitrate, nitrite, ammonium, or amino acids were used as nitrogen sources. Nitrogen-containing organic compounds (peptone, casein hydrolysate, and y.e., in the sequel referred to collectively as N_{org}) stimulated bacterial growth and, especially, pigment synthesis. On a minimal nutritional medium with acetate and 0.05 g/l y.e., no pigmentation at all was noted, whereas at high concentrations of N_{org} (above 0.5 g/l), the biomass of strain ALG 1 had a reddish orange pigmentation. Under microaerobic conditions, the organism reduced nitrate to nitrite.

Pigments. The absorption spectrum of an ALG 1 cell-free extract exhibited two peaks characteristic of bacteriochlorophyll *a*: the minor one at 803–805 nm and the major peak at 870 nm (Fig. 2a). The spectrum of other pigments in vivo was much less pronounced, except for a strong absorption of oxidized cytochrome *c* in the γ-region (410 nm). Bacteriochlorophyll *a*, selectively extracted from wet biomass with methanol, showed its absorption peak at 770 nm. In acetone extract, the absorption peak of chlorophyll was in the 765–772 nm range (Fig. 2b). The largest content of bacteriochlorophyll *a* was determined to be 2.7 μg/mg protein. The reddish orange pigment could be extracted with acetone and showed peaks at 421, 483 (the major one), and 506 nm. When the pigment was transferred from acetone to hexane, the peaks were observed at 450, 481, and 505 nm (Fig. 2c). The specific content of bacteriochlorophyll (and that of carotenoids) during batch cultivation on a shaker at a high background concentration of N_{org} showed a sharp increase at the end of the logarithmic growth phase, which coincided with the local minimum of dissolved oxygen in the culture (Fig. 3). Special experiments undertaken to study the effect of oxygen on bacteriochlorophyll synthesis showed its inhibition by the active aeration of the culture (Table 1). To a lesser degree, the production of pigments was also suppressed by light. Strong aeration combined with illumination resulted in the complete suppression of the synthesis of bacteriochlorophyll. The difference absorption spectrum of the cell-free extract also showed peaks characteristic of the α-region of cytochromes *c* (552 nm) and *b* (558 nm) and cytochrome-oxidase aa_3 (607 nm).

Table 2. Influence of thiosulfate on the growth of the alkaliphilic strain ALG 1 in aerobic batch culture at pH 10. In the variant "Without thiosulfate", a background level of thiosulfate (0.1 mM) was used to remove the effect of assimilation. Y_{thio} is the thiosulfate-dependent biomass yield, mg protein/mmol thiosulfate. The data shown are averages of two tests run in parallel

Substrates	Time, h	Without thiosulfate		With thiosulfate (22 mM)			
		OD_{600}	protein, μg/l	OD_{600}	protein, μg/l	$\Delta S_2O_3^{2-}$, mM	Y_{thio}
Y.e., 1 g/l	20	0.29		0.40		2.5	
	38	0.33	90	0.48	158	19.5	3.5
Peptone, 1 g/l	20	0.15		0.36		1.5	
	38	0.18	64	0.35	110	10.0	4.6
Y.e., 0.5 g/l + peptone, 0.5 g/l	20	0.32		0.50		4.0	
	38	0.38	150	0.65	205	22.0	2.5
Y.e., 0.25 g/l + peptone, 0.25 g/l	16	0.23		0.25		2.0	
	25	0.24		0.30		4.5	
	41	0.24	70	0.36	110	13.0	3.1
The same + acetate, 10 mM	16	0.50		0.65		1.0	
	25	0.80		0.95		6.5	
	41	0.80	208	0.92	236	20.0	1.2
The same +malate, 10 mM	16	0.62		0.60		1.0	
	25	0.65		0.98		2.5	
	41	0.65	124	0.95	200	17.5	4.3
The same + succinate 10, mM	16	0.46		0.48		3.1	
	25	0.58		0.70		5.0	
	41	0.72	200	1.0	200	7.7	2.6
The same + pyruvate, 10 mM	16	0.54		0.54		3.0	
	25	0.56		0.97		8.5	
	41	0.55	190	0.94	220	19.5	1.5
The same + lactate, 10 mM	16	0.52		0.51		2.1	
	25	0.66		0.94		5.0	
	41	0.74	200	1.15	230	10.5	2.9
The same + glucose, 5 mM	16	0.44		0.39		1.5	
	25	0.52		0.59		6.1	
	41	0.70	200	0.90	230	17.9	1.7

Table 3. Influence of thiosulfate on the growth of the alkaliphilic strain ALG 1 in acetate-limited chemostat culture at pH 10 (acetate, 10 mM; y.e., 0.25 g/l; 22°C; D = 0.1 h^{-1}; pO_2 = 95%). The data shown are averages of three "steady-state" measurements

Thiosulfate, mM		Biomass		Y_{thio}	pH
input	output	OD_{600}	protein, g/l		
0.1	0	0.44	132		10.05
2.4	0	0.50	143	4.8	10.0
6.9	0	0.60	162	4.4	9.95
12.5	0	0.75	184	4.2	9.92

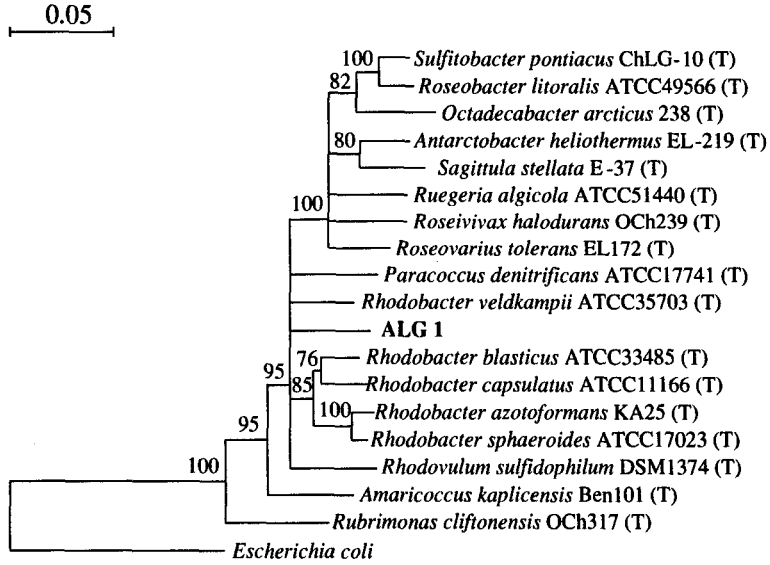

Fig. 5. The phylogenic tree showing the position of alkaliphilic strain ALG 1 in the α-3 subgroup of Proteobacteria.

Growth and oxidation of thiosulfate at pH 10. On medium with acetate and a high concentration of N_{org} at pH 10, the maximum specific growth rate of strain ALG 1 was 0.11 h^{-1}. Thiosulfate oxidation started only at the end of the logarithmic growth phase (Fig. 3). After the complete oxidation of thiosulfate (22 mM), sulfates were detected in the medium at a concentration of 40 mM. Polythionates, sulfite, or elemental sulfur did not accumulate in the growth medium.

Despite the fact that in batch ALG 1 culture, thiosulfate was not oxidized until the final stage of growth, the yields provided by the presence of thiosulfate in media with different organic sources of carbon and energy were positively established (Table 2). The specific thiosulfate-dependent biomass yield increase varied between 1.2 and 4.6 (2.8, on average) mg protein/mmol of oxidized thiosulfate. In a chemostat acetate-limited culture at pH 10, a more stable thiosulfate-dependent increase in the biomass yield of strain ALG 1 was observed, in agreement with the maximum values established for the batch culture (Table 3).

Light had no effect on the growth of a chemostat culture irrespective of the dissolved oxygen concentration and the presence of thiosulfate in the growth medium (data not shown).

The effect of pH on the respiration activity of ALG 1 cells. Washed cells of ALG 1 grown in a chemostat culture at pH 10 with acetate and thiosulfate exhibited an unusually high level of endogenous respiration, apparently, at the expense of stored poly-β-hydroxy butyrate (as suggested by the high refractivity of cells). The specific rate of thiosulfate oxidation was fairly high for a heterotrophic bacterium (Fig. 4). Thiosulfate- and sulfide-dependent oxygen uptake was observed within a narrow pH range (8.5–10), corresponding to the growth interval. At the same time, the pH optimum for oxidation of these compounds, in contrast to that for the oxidation of elemental sulfur and acetate, was somewhat lower than the pH optimum for growth. Also noteworthy was a relatively high level of sulfite-dependent oxygen uptake. Tetrathionate was not oxidized. The stoichiometry of oxygen uptake in the presence of sulfur compounds corresponded to their complete oxidation to sulfate.

Phylogenetic position of strain ALG 1. A total of 1347 nucleotides of the 16S rRNA gene sequence of strain ALG 1 was determined (deposited in the GenBank under the number AF 191225). According to the *E. coli* gene, it corresponded to positions 11 through 1435. As indicated by phylogenetic analysis of the 16S rRNA gene sequence, the alkaliphilic isolate ALG 1 was a member of the α-3 subgroup of Proteobacteria. The sequence similarity with representatives of the α-3 subgroup of Proteobacteria varied between 89.5 and 94.6%. The highest level of sequence similarity (95.4%) was obtained with *Rhodobacter veldkampii*. On the phylogenetic tree constructed by using the neighbor-joining algorithm (Fig. 5) as well as the matrix algorithms and the maximum conservation algorithm (data not shown), the alkaliphilic bacterium under study constituted a distinct branch within the α-3 subgroup of Proteobacteria, closest to the NSPB of the genus *Rhodobacter*.

DISCUSSION

Strain ALG 1 happens to be the first alkaliphilic representative of the aerobic bacteriochlorophyll *a*–containing heterotrophs (erythrobacteria). To date, their neutrophilic and acidophilic representatives were isolated from marine habitats and freshwater sources [19]. Most erythrobacteria species belong to the α-subdivi-

sion of Proteobacteria, including the vast majority of NSPB species. The erythrobacterium described in this work has several unusual properties. The first is its alkaliphily. Another one is the strict dependence of pigment synthesis (including bacteriochlorophyll *a*) on the presence of N_{org} in the medium. It is worth mentioning that most of the other alkaliphilic heterotrophs that we isolated from soda lakes required the presence of N_{org} for their growth. This may have to do with the synthesis of haloprotectants, which requires the participation of amine nitrogen. Strain ALG 1 could grow normally at N_{org} concentrations as low as 0.1 g/l, while the concentrations required to synthesize pigments were higher by at least an order of magnitude. Given that the synthesis of pigments was also stimulated by microaerobic conditions, it can be supposed that the effects of both factors were interrelated: active growth at high concentrations of N_{org} resulted in a rapid consumption of dissolved oxygen, stimulating, therefore, the synthesis of pigments (Fig. 2). The highest observed content of bacteriochlorophyll *a* in the alkaliphilic isolate matched the maximal values reported for neutrophilic erythrobacteria, and was an order of magnitude lower than that in the anaerobic purple bacteria [19]. At the same time, unlike with erythrobacteria [16], light virtually did not inhibit the synthesis of pigments and did not influence the biomass yield of the alkaliphilic strain ALG 1. It was only when illumination was combined with oxygen concentration that the synthesis of pigments was blocked, a feature more typical of anaerobic purple bacteria [19].

Strain ALG 1 is an alkaliphilic heterotrophic bacterium oxidizing sulfur compounds to sulfate. Recently, we isolated an alkaliphilic hydrogen-oxidizing bacterium, strain AHO 1, that could also oxidize sulfur compounds to sulfate during heterotrophic growth and was also phylogenetically close to NSPB in the α-3 subgroup of Proteobacteria [11]. In contrast to ALG 1, however, thiosulfate had virtually no influence on the growth of strain AHO 1. An increased biomass yield was noted only in the acetate limited chemostat culture of the strain AHO 1 in the presence of sulfide. In the case of ALG 1, an undisputable increase in the efficiency of organic carbon source utilization, due to the oxidation of thiosulfate, was recorded both in batch and chemostat cultures. The figures of the thiosulfate-dependent increase in the biomass yield observed in the chemostat culture of strain ALG 1 match the average values obtained for chemostat cultures of neutrophilic lithoheterotrophs from the Black Sea [20]. These data attest to the capacity of alkaliphilic bacterium ALG 1 to grow lithoheterotrophically in the presence of sulfur compounds.

The new alkaliphilic isolate ALG 1, together with the hydrogen-oxidizing alkaliphilic strain AHO 1, are phylogenetically close to the NSPB cluster. This fact is of particular interest for the analysis of the evolution of Proteobacteria after the appearance of oxygen, firstly, because it directly suggests possible scenarios of metabolic adaptation of anaerobic NSPB to aerobic conditions and, secondly, because it elucidates the mechanism of the development of heterotrophic aerobes oxidizing sulfur compounds to sulfate [21]. Specifically, NSPB possess a system of anaerobic photosynthesis and autotrophic carbon dioxide fixation and can utilize hydrogen and sulfur compounds as donors of electrons. Therefore, strains ALG 1 and AHO 1 can be considered as two variants of aerobic specialization of NSPB. AHO 1 represents a hydrogen-oxidizing specialization capable of autotrophic growth and retaining the ability to oxidize sulfur compounds exclusively during heterotrophic growth. Its photosynthesizing apparatus is completely lost. ALG 1 realizes another variant of evolution, retaining many NSPB features but having lost the ability to grow anaerobically. This variant is evidently more specialized to the oxidation of sulfur compounds but is no longer able to grow autotrophically. It should be noted that some species of neutrophilic erythrobacteria are able to oxidize sulfur compounds [22], being, apparently, lithoheterotrophs. This capacity is likely to be rather common among erythrobacteria, but most of them were never tested for its occurrence. In case of the loss of both photopigments and RuBPC, the third variant appears, represented by colorless aerobic heterotrophs oxidizing sulfur compounds to sulfate [21]. The loss of photopigments combined with a retained capacity for facultative autotrophy at the expense of hydrogen or sulfur compound oxidation is the fourth scenario found, for example, with some species of the genus *Paracoccus*. Finally, it should be stressed that the α-3 subgroup of Proteobacteria provides an interesting example of a large-scale physiological evolution [23] which does deserve greater attention on the part of evolutionary biology.

Based on essential phenotypic differences between strain ALG 1 and NSPB and its separate phylogenetic position within the α-3 subgroup, we propose that this alkaliphilic bacterium be assigned to a separate genus and species named *Roseinatronobacter thiooxidans*.

Description of *Roseinatronobacter* gen. nov. *Ro.se.i.nat.ro.no.bac'ter.* M. L. adj. *roseus*, pink; M. L. n. *natron*, soda; M. L. masc. n. *bacter*, rod; M. L. n. *Roseinatronobacter* pink rod from soda lake.

Nonmotile rod with elongated ends. Cells are 0.5–0.8 by 0.8–2.2 μm, occurring singly or in chains. Multiplies by binary fission. Gram-negative, containing no intra cytoplasmic membranes. Synthesizes poly-β-hydroxy butyrate as storage material. In the presence of large amounts of organic nitrogen compounds, synthesizes orange carotenoids and bacteriochlorophyll *a*. Strictly aerobic and obligately heterotrophic. Obligately alkaliphilic. Grows on acetate within the pH range 8.5–10.4 with an optimum close to 10. Mesophilic. Moderately halophilic. At pH 10, grows at concentrations of the total Na^+ as carbonates from 0.1 to 2 M (optimum at 0.4–0.6 M). Utilizes simple organic acids, sugar alcohols, glucose, and fructose as carbon and

energy sources and nitrite, nitrate, ammonia, aspartate, and glutamate as nitrogen sources. Reduces nitrate to nitrite. The growth factor requirements are fully provided for by 0.1 g/l yeast extract. Thiosulfate is oxidized to sulfate during heterotrophic growth. Sulfide, elemental sulfur, and sulfite can be oxidized to sulfate at alkaline pH values with the optimum at pH 9.0–10.0. Able to grow lithoheterotrophically with thiosulfate as additional electron donor. The G+C content of DNA is 61.5% (T_m). Member of the α-3 subgroup of Proteobacteria.

Description of *Roseinatronobacter thiooxidans* sp. nov.'thi.o.ox*i.dans.* Gr. n. *thios*, sulfur; M. L. v. *oxido*, oxidize; M. L. part adj. *thiooxidans*, oxidizing sulfur. The description of the species is the same as that of the genus.

The type strain ALG 1 is deposited with the DSM culture collection under the number DSM 13087. Isolated from a soda lake in southeastern Siberia.

ACKNOWLEDGEMENTS

D.Yu. Sorokin acknowledges the support of the Netherlands Organization for Scientific Research, project no. 047.006.018, and T.P. Tourova and B.B. Kuznetsov acknowledge the support of the Russian Foundation for Basic Research, project no. 99-04-48360.

REFERENCES

1. Horikoshi, K., Alkaliphiles—from an Industrial Point of View, *FEMS Microbiol. Rev.*, 1996, vol. 18, pp. 259–270.
2. Jones, B.E., Grant, W.D., Duckworth, A.W., and Owenson, G.G., Microbial Diversity of Soda Lakes, *Extremophiles*, 1998, vol. 2, pp. 191–200.
3. Zavarzin, G.A., Zhilina, T.N., and Kevbrin, V.V., The Alkaliphilic Microbial Community and Its Functional Diversity, *Mikrobiologiya*, 1999, vol. 68, no. 5.
4. Issatchenko, B.L., Chloride, Sulfate, and Soda Lakes of the Kulundinskaya Steppe and the Biogenic Processes Therein, Moscow: Akad. Nauk SSSR, 1951, pp. 143–162.
5. Imhoff, J.F., Sahl, H.G., Soliman, G.S.H., and Trüper, H.G., The Wadi Natrun: Chemical Composition and Microbial Mass Developments in Alkaline Brines of Eutrophic Desert Lakes, *Geomicrobiol. J.*, 1979, vol. 1, pp. 219–234.
6. Tindall, B.J., Phototrophic Bacteria from Kenyan Soda Lakes, *Ph.D. Thesis*, Leicester Univ., 1980.
7. Bryantseva, I.A., Gorlenko, V.M., Kompantseva, E.I., Imhoff, J.F., Suling, J., and Mityushina, L.L., *Thiorhodospira sibirica* gen. nov., sp. nov., a New Alkaliphilic Purple Sulfur Bacterium from a Siberian Soda Lake, *Int. J. Syst. Bacteriol.*, 1999, vol. 49, pp. 697–703.
8. Zhilina, T.N., Zavarzin, G.A., Rainey, F.A., Pikuta, E.V., Osipov, G.A., and Kostrikina, N.A., *Desulfonatronovibrio hydrogenovorans* gen. nov., sp. nov., an Alkaliphilic Sulfate-reducing Bacterium, *Int. J. Syst. Bacteriol.*, 1997, vol. 47, pp. 144–149.
9. Sorokin, D.Yu., Robertson, L.A., and Kuenen, J.G., Isolation and Characterization of Alkaliphilic Chemolithotrophic, Sulphur-oxidizing Bacteria, *Antonie van Leeuwenhoek*, 1999 (in press).
10. Sorokin, D.Yu., Lysenko, A.M., and Mityushina, L.L., Isolation and Characterization of Alkaliphilic Chemoorganotrophic Bacteria Oxidizing Reduced Inorganic Sulfur Compounds to Tetrathionate, *Mikrobiologiya*, 1996, vol. 65, pp. 370–383.
11. Sorokin, D.Yu. and Kuenen, J.G., A New Facultatively Autotrophic Hydrogen-oxidizing Bacterium from Alkaline Environment, *Extremophiles*, 2000 (in press).
12. Pfennig, N. and Lippert, K.D., Uber das Vitamin B_{12}-Bedürfnis phototropher Schwefel Bacterien, *Arch. Mikrobiol.*, 1966, vol. 55, pp. 245–256.
13. Sorokin, D.Yu., *Sulfitobacter pontiacus* gen. nov., sp.nov.—a New Heterotrophic Bacterium from the Black Sea, Specialized on Sulfite Oxidation, *Mikrobiologiya*, 1995, vol. 64, pp. 354–365.
14. Kelly, D.P., Chambers, L.A., and Trudinger, P.A., Cyanolysis and Spectrophotometric Estimation of Trithionate in Mixture with Thiosulfate and Tetrathionate, *Anal. Chem.*, 1969, vol. 41, pp. 898–901.
15. Cypionka, H. and Pfennig, N., Growth Yield of *Desulfotomaculum orientis* with Hydrogen in Chemostat Culture, *Arch. Microbiol.*, 1986, vol. 143, pp. 396–399.
16. Yurkov, V.V. and van Gemerden, H., Impact of Light/Dark Regime on Growth Rate, Biomass Formation and Bacteriochlorophyll Synthesis in *Erythromicrobium hydrolyticum*, *Arch. Microbiol.*, 1993, vol. 159, pp. 84–89.
17. Marmur, J., A Procedure for the Isolation of Deoxyribonucleic Acid from Microorganisms, *J. Mol. Biol.*, 1961, vol. 3, pp. 208–218
18. De Ley, J., Cattoir, H., and Reynaerts, A., The Quantitative Measurements of DNA Hybridization from Renaturation Rates, *Eur. J. Biochem.*, 1970, vol. 12, pp. 133–140.
19. Yurkov, V.V. and Beaty, T., Aerobic Anoxygenic Phototrophic Bacteria, *Microbiol. Mol. Biol. Rev.*, 1998, vol. 62, pp. 695–724.
20. Sorokin, D.Yu., The Effect of Thiosulfate on the Growth of Continuous Cultures of Black Sea Isolates of Heterotrophic Bacteria Oxidizing Sulfur Compounds to Sulfate, *Mikrobiologiya*, 1994, vol. 63, pp. 255–259.
21. Sorokin, D.Yu. and Lysenko, A.M., Characterization of Black Sea Isolates of Heterotrophic Bacteria Oxidizing Sulfur Compounds to Sulfate, *Mikrobiologiya*, 1993, vol. 62, pp. 1018–1031.
22. Yurkov, V.V., Krasil'nikova, E.N., and Gorlenko, V.M., Metabolism of Thiosulfate in Aerobic Bacteriochlorophyll *a*–containing Bacteria, *Mikrobiologiya*, 1994, vol. 63, pp. 181–188.
23. Woese, C.R., Stackebrandt, E., Weisburg, W.G., Paster, B.J., Madigan, M.T., Fowler, V.J., Hahn, C.M., Blanz, P., Gupta, R., Nelson, K., and Fox, G.E., The Phylogeny of Purple Bacteria: The Alpha Subdivision, *Syst. Appl. Microbiol.*, 1984, vol. 5, pp. 315–326.