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EXPERIMENTAL ARTICLES

Phylogenetic Characterization of the Purple Sulfur Bacterium *Thiocapsa* sp. BBS by Analysis of the 16S rRNA, *cbbL*, and *nifH* Genes and Its Description as *Thiocapsa bogorovii* sp. nov., a New Species

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Abstract—Strain BBS, the purple sulfur bacterium assigned initially to the species *Thiocapsa roseopersicina*, is the best studied representative of this species. However, no molecular phylogenetic analysis has been performed to confirm its systematic position. Based on the results of analysis of the sequences of 16S rRNA, *cbbL*, and *nifH* genes, DNA–DNA hybridization with the *T. roseopersicina* type strain, and comparative analysis of the phenotypic characteristics of various species belonging to the genus *Thiocapsa*, we suggest that strain BBS should be assigned to a new species of the genus *Thiocapsa, Thiocapsa bogorovii* sp. nov.

Key words: Chromatiaceae, Thiocapsa, DNA–DNA hybridization, nucleotide sequences, 16S rRNA, *cbbL, nifH.*

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Bacteria of the genus *Thiocapsa* belong to the family *Chromatiaceae*, which includes purple sulfur bacteria that accumulate intracellular granules of sulfur [1]. This genus was described to accommodate *Chromatiaceae* representatives with nonmotile, spherical cells devoid of gas vesicles. Later, phylogenetic analysis of 16S rRNA gene sequences resulted in major taxonomic changes within the family *Chromatiaceae*; some species were withdrawn from the genus *Thiocapsa*, whereas some representatives of the genus *Amoebobacter*, containing gas vacuoles, were introduced [2, 3]. Strain BBS is among the best studied representatives of *Chromatiaceae*. Investigation of this strain led to the discovery of the capacity of phototrophic bacteria for chemolithoautotrophic growth [4]. The genes and enzymes involved in the hydrogen, sulfur, and carbon metabolism, as well as the biosynthesis of photosynthetic pigments, have also been studied in detail [5–8].

Strain BBS was isolated from estuarial sediment of the White Sea and was initially assigned to *T. roseopersicina*, the type species of the genus *Thiocapsa* [9]. However, this strain differs from the type in some phenotypic characteristics (incapacity for assimilatory sulfate reduction, requirement for vitamin B_{12}, a higher salinity optimum, as well as some differences in the spectra of utilized organic compounds). However, its phylogenetic position has not been studied by molecular biological techniques.

Autotrophic CO_2 assimilation in purple bacteria occurs via the Calvin cycle [10]. Although the presence of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), the key enzyme of this cycle, has been detected in many representatives of the family *Chromatiaceae*, including the type strain of *T. roseopersicina* and strain BBS [7, 11, 12], the RuBisCO gene sequence was determined only for *Allochromatium vinosum* [13].

In the majority of purple bacteria, RuBisCO synthesis is suppressed by oxygen [10]. Thus, their ability to grow under autotrophic conditions in the dark manifests itself only under microaerobic conditions [14–16]. RuBisCO synthesis insensitive to molecular oxygen is a specific feature of strain BBS [12]. This strain can therefore grow under autotrophic conditions both in the light and in the dark even at high oxygen concentrations. The White Sea littoral, from which strain BBS was isolated, is characterized by periodical alternations of phototrophic and chemotrophic conditions, as well as by the presence of sulfide, and this provides a selective advantage for the strain. Thus, its natural environmental conditions are favorable for both photoautotrophic and chemolithoautotrophic growth. In addition to strain BBS, the capacity for chemolithotrophic

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growth was detected in other representatives of *Chromatiaceae* [14–16]. The type strain of *T. roseopersicina* was tested for chemolithoautotrophic growth only under microaerobic conditions [15], and the effect of different oxygen concentrations on its growth and regulation of the RuBisCO synthesis were not investigated. Such data, along with the results of phylogenetic analysis of the 16S rRNA genes and functional genes responsible for specific metabolic features of this microbial group are required to reveal the phylogenetic relations between various representatives of the genus *Thiocapsa*.

The capacity of purple sulfur bacteria for dinitrogen fixation (diazotrophy) is an important feature of their metabolism [17]. The nitrogenase enzyme complex (the key one in this process) consists of two subunits: a FeMo protein encoded by the *nifD* and *nifK* genes, and a Fe protein encoded by the *nifH* gene. Among the representatives of the family *Chromatiaceae*, these genes have been studied only in some strains of the species *Marichromatium purpuratum* [18] and in the type strain of *Thermochromatium tepidum* [19].

The aim of the present work was to determine the taxonomic position of strain BBS. Comparative study of the capacities of the strains BBS and *T. roseopersicina* DSM 217^T for chemolithoautotrophic growth was carried out, and genotypic characterization of strain BBS on the basis of DNA–DNA hybridization and phylogenetic analysis of the 16S rRNA, RuBisCO, and nitrogenase (*nifH*) genes was performed.

MATERIALS AND METHODS

Subjects of investigation were *Thiocapsa* sp. BBS and *Allochromatium minutissimum* MSU^T from the culture collection of the Department of Microbiology, Moscow State University (MGU 317 and 306, respectively), as well as *T. roseopersicina* DSM 217^T. The microorganisms were cultivated at 28°C in a modified Pfennig medium [9] containing $NaHCO_3$ (0.2%), $Na_2S \cdot 9H_2O$ (0.1%), sodium thiosulfate (0.2%), and B_{12} (20 μg/ml). For strain BBS, the medium was supplemented with NaCl (2%). Cultivation under phototrophic conditions was carried out in a luminostat at an illumination intensity of 2000 lx in 50-ml vials or 500-ml flasks with ground glass stoppers. Aerobic cultivation in the dark was carried out on a rotor shaker (140 rpm) in 200-ml flasks filled up to 1/3 and closed with cotton stoppers.

Assimilation of labeled substrates. Biomass from the cells suspensions was harvested by centrifugation, washed with mineral medium, and resuspended to 100–200 μg protein/ml. The experiments aimed at determining $NaH^{14}CO_3$ assimilation by cell suspensions were carried out in 10-ml syringes in the light (2000 lx, 30°C) (phototrophic anaerobic conditions) or in the dark in 20-ml vials containing 1.5 ml of suspension on a shaker (250 rpm, 30°C) (chemolithotrophic aerobic conditions). The reaction was initiated by the addition of $NaH^{14}CO_3$ (5 mM, 4 kBq/ml) and terminated at appropriate time intervals by filtering of the cell suspensions (1 ml) through nitrocellulose filters (0.45 μm). The filters were dried, and radioactivity was measured in a RackBeta 1127 liquid scintillation counter (LKB, Sweden).

Obtaining the cell extracts. Bacterial cells were washed with 50 mM Tris–HCl-buffer (pH 7.8), resuspended in 5–8 ml of the relevant buffer required for determination of the activity of a particular enzyme, and disrupted using an X-press (overpressure of 7000–10000 kg). Intact cells and large cell fragments were precipitated by centrifugation (40000 *g*, 20 min, 4°C); the supernatant was used for enzymatic analyses.

Activity of ribulose-l,5-bisphosphate carboxylase was determined radiochemically from ribulose-1,5-bisphosphate-dependent fixation of CO_2.

Isolation of DNA and amplification and sequencing of the genes under study. DNA extraction and purification, as well as amplification of the 16S rRNA genes with universal primers and amplification of the RuBisCO and *nifH* genes using the earlier designed system of oligonucleotide primers, were carried out as described in [20].

The PCR products obtained by amplification of the *cbbL* genes were cloned in the pGEM-T vector (Promega, United States).

Sequencing of the clones and amplification products was performed by the Sanger method with the use of a Big Dye Terminator v.3.1 kit on an ABI 3730 automatic sequencer (Applied Biosystems, United States) according to the manufacturer's recommendations.

Genotypic studies. DNA from microbial cells was extracted as described in [21]. The content of the G+C base pairs was determined according to the technique described by De Ley et al. [22].

Phylogenetic analysis of the sequences. Editing of sequences was carried out using the BioEdit software package [http://jwbrown.mbio.ncsu.edu/BioEdit/bioediTca.html]. The primary comparison of the de novo determined sequences with the sequences within the GenBank database was carried out using the NCBI BLAST software package [http://www.ncbi.nlm.nih.gov/blast/]. The subsequent comparative analysis was conducted using sequences of the 16S rRNA, *cbbL*, and *nifH* genes available from GenBank. The nucleotide sequences and the deduced amino acid sequences of the studied functional genes were aligned with the appropriate sequences from the closest relatives using the CLUSTALX 2.0 software package [http://bips.u-strasbg.fr/fr/Documentation/ClustalX/]. The phylogenetic trees were constructed using the TREECONW software package [http://bioc-www.uia.ac.be/u/yvdp/treeconw.html]. The significance of the branching order (in %) was determined on the basis of the bootstrap analysis of 1000 alternative trees.

Deposition of the sequences. The 16S rRNA, *cbbL*, and *nifH* gene fragments of strain BBS were deposited in the GenBank database under accession numbers EU622781, EU622784, and EU622783, respectively; the sequences of the *cbbL* and *nifH* genes of strain *T. roseopersicina* DSM 217^T were deposited in the GenBank under accession numbers EU622785 and EU622784, respectively; and the sequences of the *cbbL*1-*cbbL*2, and *nifH* genes of strain *A. minutissimum* MSU^T were deposited in the GenBank under accession numbers EU622786, EU622787, and EU622788.

RESULTS AND DISCUSSION

Morphology of *Thiocapsa* sp. BBS. The cells are spherical, 1.0–1.5 μm in diameter (Fig. 1), gram-negative. Cell reproduction occurs by binary fission. The cells lack flagella, but are capable of random impulsive movements in wet mount preparations.

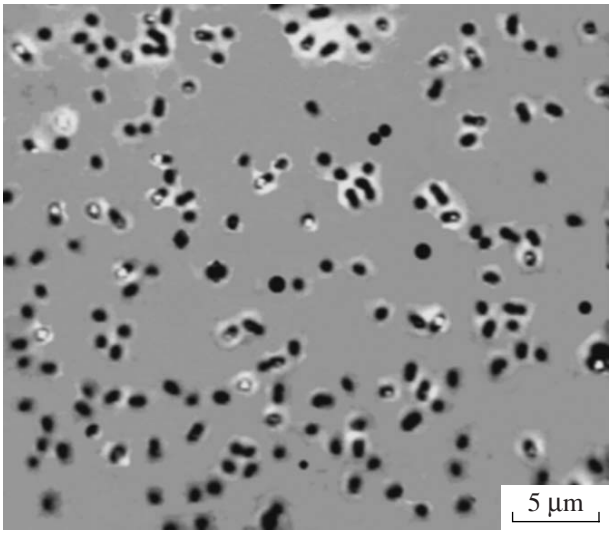


Fig. 1. Micrograph of *Thiocapsa* sp. BBS (light microscopy, phase contrast).

Growth of strain BBS and the type strain of *T. roseopersicina* and CO_2 assimilation under chemolithoautotrophic aerobic conditions. As noted above, the capacity of purple sulfur bacteria for autotrophic growth in the dark under aerobic conditions was first reported for strain BBS. This is a diagnostic characteristics for the representatives of the genus *Thiocapsa*, since not all of them are capable of chemolithotrophic growth. For instance, the inability to grow in the dark is one of the characteristic traits of *T. pendens*. The study of *T. roseopersicina* DSM 217^T showed that both the type strain and strain BBS are able to grow under chemolithoautotrophic aerobic conditions utilizing thiosulfate as an electron donor; the OD_{650} values for the 96-h cultures of these strains grown under chemolithoautotrophic aerobic conditions were 0.27 and 0.17, respectively; the OD_{650} values of the cultures grown under photoautotrophic anaerobic conditions were 1.74 and 0.80, respectively. As in the case of anaerobic growth in the light, the rate of CO_2 assimilation in the cell suspensions depended on the presence of a reducing agent (thiosulfate) in the medium (Table 1). It should be noted that this dependence becomes more pronounced due to the fact that in the dark bacteria utilize thiosulfate both as an electron donor and an energy source. Iodoacetate, an inhibitor of glyceraldehyde phosphate dehydrogenase in the Calvin cycle, inhibits CO_2 assimilation both under anaerobic conditions in the light and under aerobic conditions in the dark (Table 1). In the cell extracts of both strains, the presence of RuBisCO, the key enzyme of the Calvin cycle, was detected (Table 2). The RuBisCO activity was the same in the cells grown under anaerobic conditions in the light and under aerobic conditions in the dark. These data indicate that both strains are able to grow under chemolithoautotrophic aerobic conditions using the Calvin cycle. No differences in this trait were revealed between the two strains. Hence, unlike *Ectothiorhodospira shaposhnikovii*, which is unable to synthesize RuBisCO under aerobic conditions [12], the regulation of RuBisCO synthesis, in both of the considered *Thiocapsa* strains is independent of the presence of oxygen.

Analysis of the 16S rRNA gene sequences and DNA–DNA homology. To establish the phylogenetic position of strain BBS, we determined the nearly complete 16S rRNA gene sequence (1428 nucleotides, corresponding to *E. coli* positions 35–1475). Screening in the GenBank database revealed that this 16S rRNA gene sequence was closest to analogous sequences of representatives of the family *Chromatiaceae* within the *Gammaproteobacteria.* In the constructed phylogenetic

Table 1. Bicarbonate assimilation by the cell suspensions of *Thiocapsa roseopersicina* DSM 217^T and strain BBS grown under autotrophic anaerobic conditions in the light and under chemotrophic aerobic conditions in the dark (nmol min^{-1} (mg protein)$^{-1}$)

The medium contains	*T. roseopersicina* DSM 217^T		*Thiocapsa* sp. BBS	
	Light, anaerobic conditions	Dark, aerobic conditions	Light, anaerobic conditions	Dark, aerobic conditions
CO_2	29.1	1.1	19.2	0.8
$CO_2 + Na_2S_2O_3$	38.4	3.3	35.1	2.6
$CO_2 + Na_2S_2O_3$ + iodoacetate	4.9	0.7	3.5	0.4

Table 2. Activity of ribulose-1,5-bisphosphate carboxylase in the cell extracts of *Thiocapsa roseopersicina* DSM 217^T and strain BBS grown under autotrophic anaerobic conditions in the light and under chemotrophic aerobic conditions in the dark (nmol min^{-1} (mg protein)$^{-1}$)

	T. roseopersicina DSM 217^T		*Thiocapsa* sp. BBS	
	Light, anaerobic conditions	Dark, aerobic conditions	Light, anaerobic conditions	Dark, aerobic conditions
Activity	20.2	19.6	26.6	26.5

Table 3. The level of DNA–DNA hybridization between various strains of the genus *Thiocapsa*

Species and strains	G+C, %	DSM 217^T	OP-3	BM-5^T	BBS
Thiocapsa roseopersicina DSM 217^T	63.6	100			
Thiocapsa roseopersicina OP-3	63.1	100	100		
Thiocapsa litoralis BM-5^T	62.9	22	11	100	
Strain BBS	63.7	34	37	11	100

tree (Fig. 2), strain BBS fell into the monophyletic cluster of *Thiocapsa* species at a maximum possible significance level (a bootstrap value of 100). The similarity level between strain BBS and the representatives of the genus *Thiocapsa* (97.2–99.2% homology) was much higher than between strain BBS and other members of the family *Chromatiaceae* (91.5–96.4%). Strain BBS was found to be most closely related to *Thiocapsa* sp. Mog1 (99.2% homology) isolated from the bacterial community of the relic Lake Mogil'noe [23]. In the phylogenetic tree, these strains formed a separate subcluster within the cluster formed by representatives of the genus *Thiocapsa*. At the same time, the type strain of the species *T. roseopersicina* DSM 217^T fell into another subcluster together with *T. roseopersicina* strains 4210 and 9314. Other species of the genus *Thiocapsa* formed separate subclusters that included some strains identified as members of the species *T. roseopersicina*. The level of 16S rRNA similarity between strain BBS and the type strain of the species *T. roseopersicina* (98.3%) was close to those with *T. marina* (98.8–99.1%), *T. rosea* (98.5%), *T. litoralis* (98.2%), and *T. pendens* (98.3%). Approximately the same level of similarity of 16S rRNA gene sequences (97.7–98.1%) was recorded between the various species of the genus *Thiocapsa* (97.4–98.9%).

Thus, the results of the 16S rRNA gene analysis cast doubts upon the classification of strain BBS as *T. roseopersicina.* According to the recent revision of taxonomic concepts, the quantitative definition of a species in terms of DNA–DNA hybridization level is currently considered to correspond to a new threshold value of similarity between 16S rRNA gene sequences (98.7–99.0%) [24]. Assuming this new criterion, the level of 16S rRNA gene differences between strain BBS and the previously described representatives of the genus *Thiocapsa* (including the type strain *T. roseopersicina*) exceeds the intraspecific one. For more precise determination of the taxonomic status of the strain under study, DNA–DNA hybridization was performed. According to the results obtained (Table 3), the level of DNA hybridization between strain BBS and the strains of the species *T. roseopersicina* and *T. litoralis* (including the type strains) did not exceed 37%; that is, it was much lower than the intraspecific level, which is in accordance with the results of the analysis of the 16S rRNA gene sequences.

Identification and analysis of the genes encoding RuBisCO and nitrogenase. The sequences of the genes encoding RuBisCO and nitrogenase in the majority of *Chromatiaceae* representatives are unknown. Therefore, along with strain BBS and the type strain *T. roseopersicina* DSM 217^T, we included in our study *A. minutissimum* MSU^T as a representative of another genus of the family *Chromatiaceae*. Amplification was performed with the oligonucleotide primers specific to various types and forms of RuBisCO genes [25]. PCR products were obtained only with the primers specific to green-like form I RuBisCO. The use of the primers specific to the genes of red-like form I RuBisCO and form II RuBisCO did not yield specific PCR products, which indicates the absence of the relevant genes in the genomes of the studied bacteria. Sequencing of the cloned PCR products revealed one RuBisCO gene in each of the *Thiocapsa* strains and two RuBisCO genes in *A. minutissimum*, which corresponds to the results previously obtained for *A. vinosum* [13, 26].

Sequencing of the clones obtained yielded DNA fragments about 750 bp long for each of the strains studied. Preliminary screening of the GenBank database for sequences related to the de novo determined ones showed high similarity (at least 79%) of the latter sequences with the *cbbL* gene sequences of other bacteria, thus confirming their affiliation with this gene family.

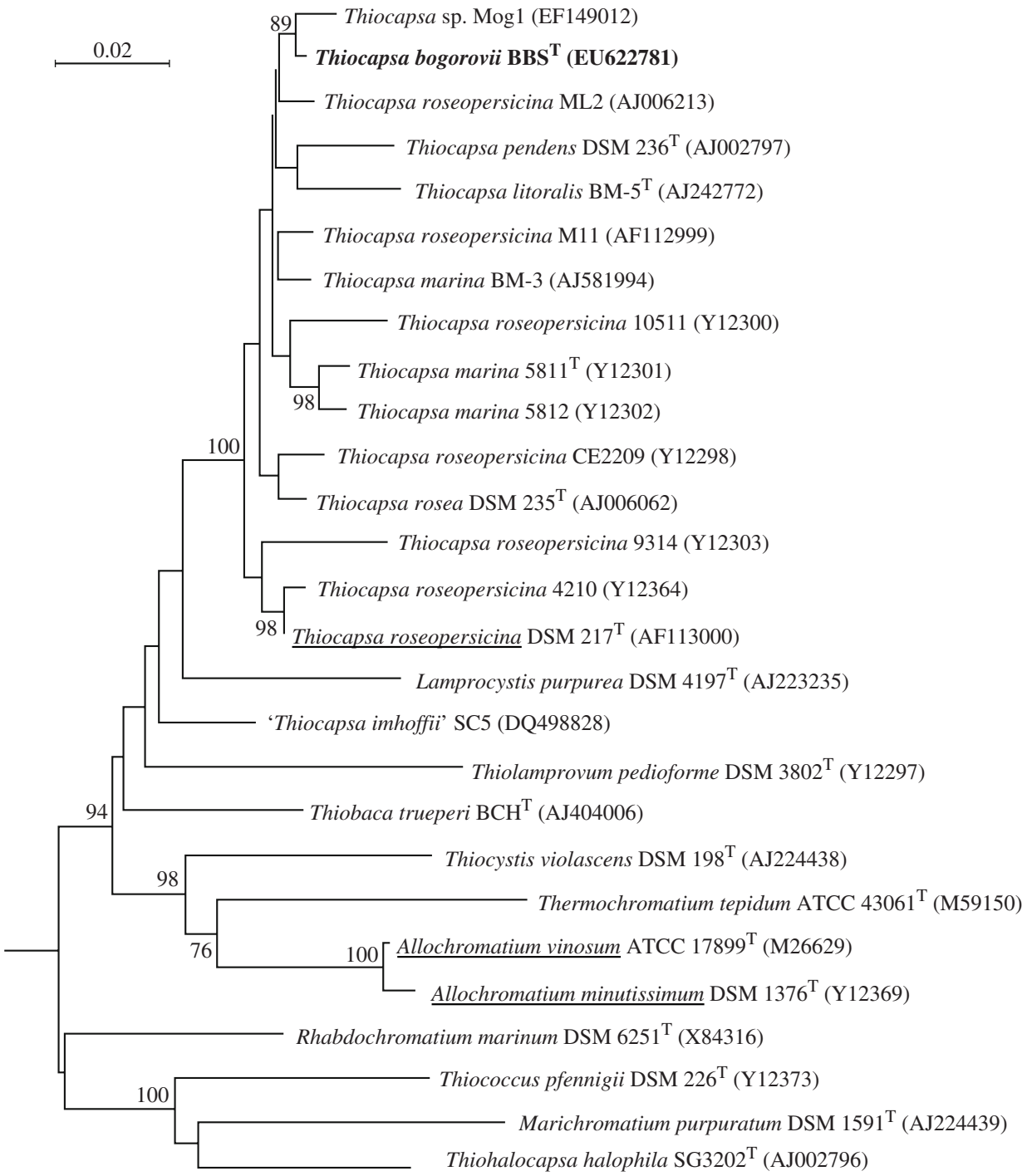


Fig. 2. Phylogenetic position of strain BBS in the phylogenetic tree of purple sulfur bacteria constructed based on the analysis of 16S rRNA gene sequences using the neighbor-joining algorithm with the *E. coli* sequence taken as an outgroup. The sequence determined in this study is bold-typed. The underlined sequences are those of the type strains of the species *T. roseopersicina, A. vinosum*, and *A. minutissimum.* The numerals show the significance of the branching order as determined by bootstrap analysis (values higher than 75 were considered significant). The bar shows the evolutionary distance corresponding to 2 substitutions per 100 nucleotides.

We aligned the de novo determined nucleotide sequences of RuBisCO gene fragments, as well as the deduced amino acid sequences of the corresponding proteins, with analogous sequences of green-like form I RuBisCOs of other bacteria available from the GenBank database and compared 738 positions for nucleotides and 245 positions for amino acid residues. The topologies of the phylogenetic trees constructed based on the analysis of nucleotide (data not presented) and amino acid (Fig. 3) sequences were similar in the region of

interest. In both trees, the strains *T. roseopersicina* DSM 217^T and *Thiocapsa* sp. BBS were relatively close to each other (93.0% and 97.5% identity of nucleotide and amino acid sequences, respectively). It should be noted that the *cbbL*1 and *cbbL*2 genes of *A. minutissimum* were even closer to the *rbcA* [26] and *rbcL* [13] genes of *A. vinosum* (98.4 and 99.3% for nucleotides and 100 and 98.8% for amino acids, respectively). The *cbbL* gene sequences of the studied *Thiocapsa* representatives and the *cbbL*2–*rbcL* gene sequences of the representatives of the genus *Allochromatium* formed a cluster which, as shown earlier [27], was adjoined by two species of alkaliphilic sulfur-oxidizing bacteria, *Thioalkalivibro nitratireducens* and *Tv. paradoxus*, as well as by the nitrifying bacterium *Nitrococcus mobilis*, belonging to another family of purple sulfur bacteria, *Ectothiorhodospiraceae*.

The results that we obtained allow us to suggest a hypothesis concerning the evolution of RuBisCO genes in this group of bacteria. The presence of two copies of the RuBisCO gene (*rbcA* and *rbcL*) in *A. vinosum*, and the fact that these sequences were virtually identical to the analogous sequences of *A. minutissimum* confirm that these organisms are close relatives. Although the homology level of the *rbcA* and *rbcL* nucleotide sequences in *A. vinosum* is only 82%, the codon usage and the content of G+C base pairs are almost identical. Based on the results of this analysis, we suggested that the presence of two copies of the RuBisCO gene in *A. vinosum* is due to gene duplication in the genome of one organism rather than to lateral gene transfer [13, 26]. Later on, a relatively long period of coexistence of these duplicated genes resulted in their functional differentiation: in *A. vinosum,* primarily the RuBisCO encoded by the genes *rbcAB* of the small and large subunits is functional, whereas the level of *rbcLS* gene expression is very low. However, both sets of genes code for enzymes that are potentially functionally active and have different constant values and different affinity to CO_2 [13]. Judging from the high similarity level between the analogous genes of *A. vinosum* and *A. minutissimum*, it may be suggested that the same mechanism is valid for the latter species.

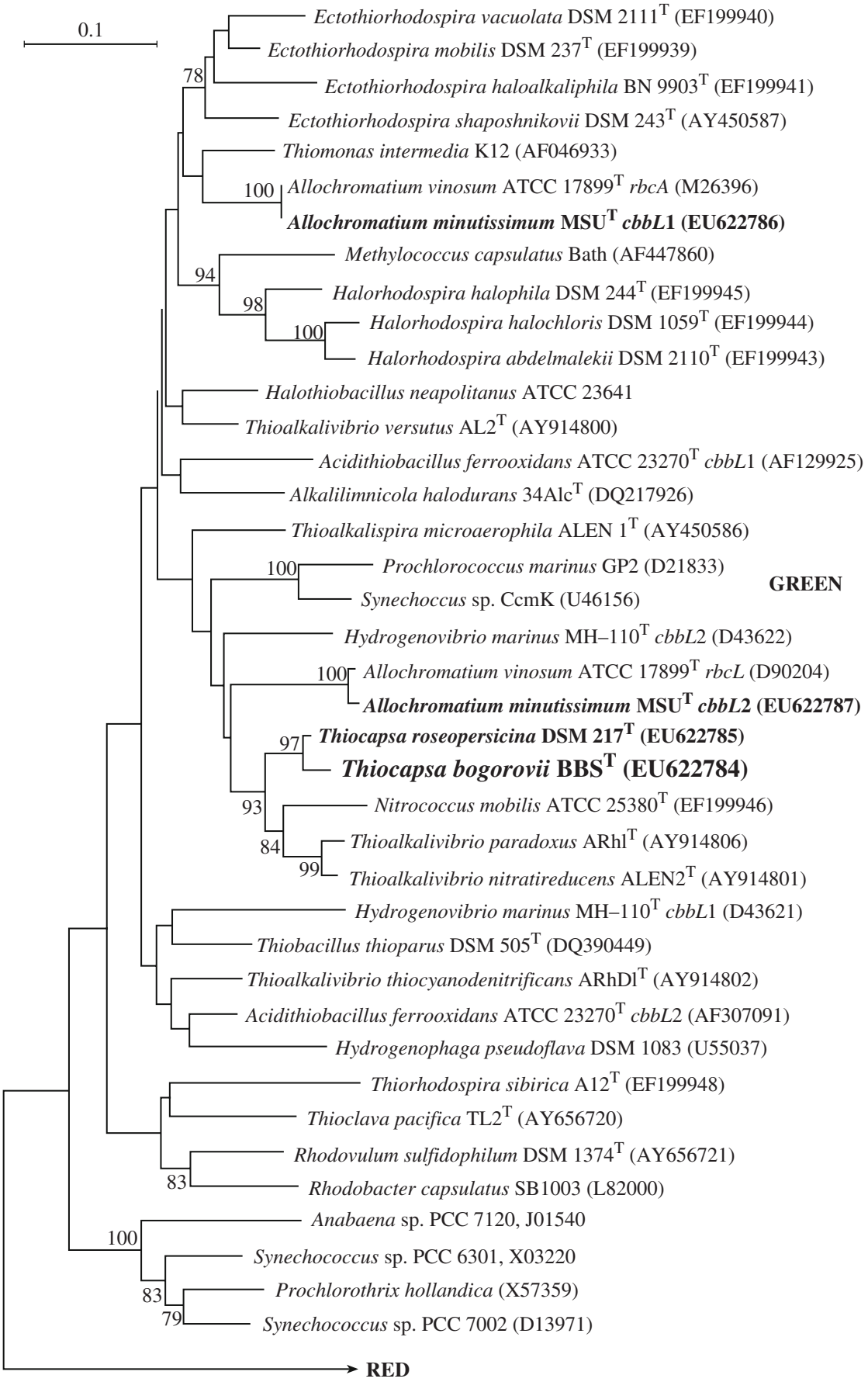
At the same time, the identity level between the *Allochromatium rbcL/cbbL*2 genes and the only (and, therefore, highly physiologically active) *cbbL* gene of *Thiocapsa* allows us to suggest that these genes are derived from the relevant genes of the ancestor of the purple sulfur bacteria of the family *Chromatiaceae*. The presence of their homologs in members of the family *Ectothiorhodospiraceae* can be attributed to horizontal gene transfer, as discussed earlier [27].

The system of specific oligonucleotide primers was then used to detect and sequence the *nifH* gene fragments (about 450 nucleotides) of all the studied strains of purple sulfur bacteria. Preliminary screening using the GenBank database showed the affiliation of the *de novo* determined nucleotide sequences with the *nifH* gene family.

We aligned the obtained nucleotide sequences of the *nifH* gene fragments, as well as the deduced amino acid sequences of the corresponding proteins, with analogous sequences of nitrogen-fixing *Proteobacteria* available from the GenBank database and compared 449 positions for nucleotides and 149 positions for amino acid residues. The topologies of the phylogenetic trees constructed based on the analysis of nucleotide (data not presented) and amino acid (Fig. 4) sequences were similar in the region of interest. The studied representatives of the genus *Thiocapsa*, as well as *A. minutissimum*, formed a separate cluster in these trees. At the same time, the divergence of the nucleotide and amino acid sequences of their *nifH* genes was found to be considerable and virtually the same between the strains BBS and *T. roseopersicina* DSM 217^T (91.5 and 96% identity) and between these strains and *A. minutissimum* (90.2–90.6% and 95.3–96.0% identity). It should be noted that neither representatives of various genera of the family *Chromatiaceae* nor members of the family *Ectothiorhodospiraceae* formed coherent clusters in nitrogenase phylogenetic trees [27].

Although no criteria exist in modern molecular systematics for estimation of the taxonomic significance of the data obtained by functional gene analysis, these data are frequently used as additional criteria in order to establish the level of relatedness. They are especially relevant in cases when the traditional analysis of 16S rRNA genes cannot provide unambiguous results, as it is the case with strain BBS. According to the results of the analysis of the functional *cbbL* and *nifH* genes, strain BBS was indeed found to be most closely related to *T. roseopersicina* DSM 217^T; however, the divergence level between their nucleotide and amino acid sequences was quite significant. This level corresponded to (and, in some cases, exceeded) the level of sequence identity between orthologous genes of the species of the families *Chromatiaceae* and *Ectothiorhodospiraceae.* Hence, the results of comparison of the functional genes do not conflict with the results of 16S rRNA gene comparison and DNA–DNA hybridization, indicating that the strains BBS and *T. roseopersicina* DSM 217^T belong to different species.

Fig 3. Phylogenetic position of strain BBS in the phylogenetic tree of autotrophic bacteria possessing the green-like form I RuBisCO gene. The tree was constructed using the neighbor-joining algorithm with the consensus sequence of red-like form I RuBisCO genes as an outgroup. The sequences determined in this study are bold-typed. The numerals show the significance of the branching order as determined with bootstrap analysis (values higher than 75 were considered significant). The bar shows the evolutionary distance corresponding to 10 substitutions per 100 amino acid residues.


0.1
Ectothiorhodospira vacuolata DSM 2111T (EF199940)
Ectothiorhodospira mobilis DSM 237T (EF199939)
78
Ectothiorhodospira haloalkaliphila BN 9903T (EF199941)
Ectothiorhodospira shaposhnikovii DSM 243T (AY450587)
Thiomonas intermedia K12 (AF046933)
100
Allochromatium vinosum ATCC 17899T rbcA (M26396)
Allochromatium minutissimum MSUT cbbL1 (EU622786)
Methylococcus capsulatus Bath (AF447860)
94
Halorhodospira halophila DSM 244T (EF199945)
98
100
Halorhodospira halochloris DSM 1059T (EF199944)
Halorhodospira abdelmalekii DSM 2110T (EF199943)
Halothiobacillus neapolitanus ATCC 23641
Thioalkalivibrio versutus AL2T (AY914800)
Acidithiobacillus ferrooxidans ATCC 23270T cbbL1 (AF129925)
Alkalilimnicola halodurans 34AlcT (DQ217926)
Thioalkalispira microaerophila ALEN 1T (AY450586)
100
Prochlorococcus marinus GP2 (D21833)
Synechoccus sp. CcmK (U46156)
GREEN
Hydrogenovibrio marinus MH–110T cbbL2 (D43622)
100
Allochromatium vinosum ATCC 17899T rbcL (D90204)
Allochromatium minutissimum MSUT cbbL2 (EU622787)
97
Thiocapsa roseopersicina DSM 217T (EU622785)
Thiocapsa bogorovii BBST (EU622784)
93
Nitrococcus mobilis ATCC 25380T (EF199946)
84
Thioalkalivibrio paradoxus ARhlT (AY914806)
99
Thioalkalivibrio nitratireducens ALEN2T (AY914801)
Hydrogenovibrio marinus MH–110T cbbL1 (D43621)
Thiobacillus thioparus DSM 505T (DQ390449)
Thioalkalivibrio thiocyanodenitrificans ARhDlT (AY914802)
Acidithiobacillus ferrooxidans ATCC 23270T cbbL2 (AF307091)
Hydrogenophaga pseudoflava DSM 1083 (U55037)
Thiorhodospira sibirica A12T (EF199948)
Thioclava pacifica TL2T (AY656720)
Rhodovulum sulfidophilum DSM 1374T (AY656721)
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Anabaena sp. PCC 7120, J01540
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Synechococcus sp. PCC 6301, X03220
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Prochlorothrix hollandica (X57359)
79
Synechococcus sp. PCC 7002 (D13971)
RED

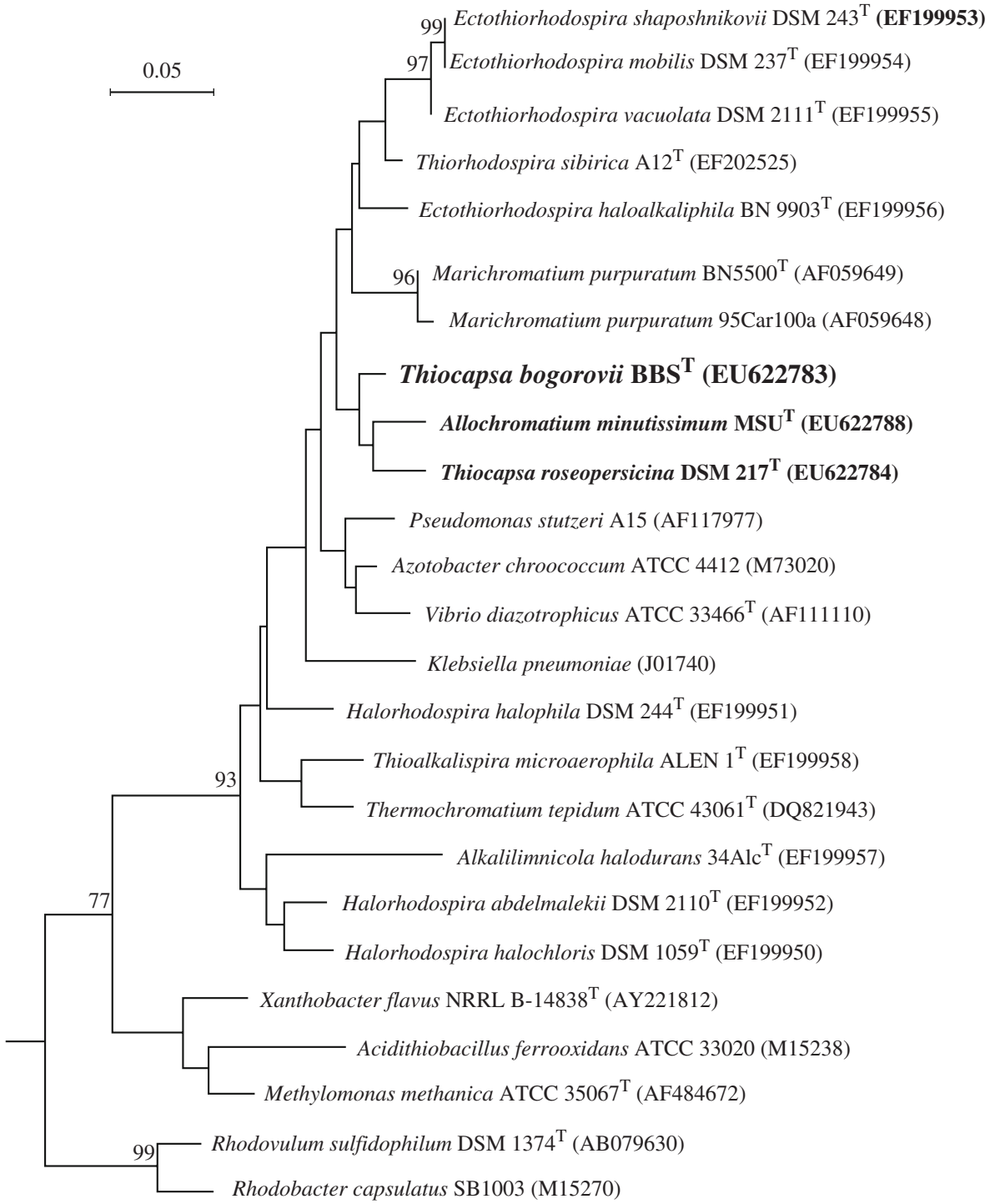

Fig. 4. Phylogenetic position of strain BBS in the phylogenetic tree of diazotrophic proteobacteria constructed using the neighbor-joining algorithm with the *Nostoc* sp. PCC 7120 sequence taken as an outgroup. The sequences determined in this study are bold-typed. The numerals show the significance of the branching order as determined with bootstrap analysis (values higher than 75 were considered significant). The bar shows the evolutionary distance corresponding to 5 substitutions per 100 amino acid residues.

Comparison of the phenotypic characteristics of strain BBS with those of the representatives of the genus *Thiocapsa*. Strain BBS differs from the type strain *T. roseopersicina* DSM 217^T by the incapacity for assimilatory sulfate reduction, high salinity optimum, vitamin B_{12} auxotrophy, as well as by some peculiarities in the spectrum of utilized organic compounds [9] (Table 4).

Table 4. Comparison of the characteristics of strain BBS and the type strains of the species of the genus *Thiocapsa*

Characteristics	Strain BBS	*T. roseopersicina**	*T. litoralis**	*T. pendens**	*T. rosea**	*T. marina**
Cell diameter	1.0–1.5	1.2–3.0	1.5–2.5	1.5–2.0	2.0–3.0	1.5–3.0
Gas vesicles	–	–	–	+	+	–
Color of cell suspension	Pink-red	Pink-red	Pink-red	Pink-red	Pink-red	Purple-red
Major carotenoids	Spirilloxanthin	Spirilloxanthin	Spirilloxanthin	Spirilloxanthin	Spirilloxanthin	Okenone
DNA G+C content (mol %)	63.7	63.3	64.0	65.3	64.3	62.9
Vitamin requirement	B_{12}	–	B_{12}	B_{12}	B_{12}	–
Assimilatory sulfate reduction	–	+	+	–	–	–
NaCl optimum (%)	1–2	0	1	0	0	1–2
pH optimum	7.0–7.5	7.3	6.5	6.7–7.5	6.7–7.5	7.5
Capacity for chemolithoautotrophic growth	+	+	+	–	+	+
Utilized substrates:						
hydrogen	+	+	ND	–	–	+
thiosulfate	+	+	+	+	+	ND
formate	–	–	–	–	ND	ND
acetate	+	+	ND	ND	ND	ND
pyruvate	+	+	ND	ND	ND	ND
propionate	+	+	+	+	+	ND
butyrate	–	–	+	–	–	–
actate	+	+	+	+	+	ND
fumarate	+	+	+	–	–	+
succinate	+	+	+	–	–	+
malate	+	+	+	+	+	ND
fructose	–	+	+	–	+	+
glucose	+	–	+	+	–	–
ethanol	–	–	–	ND	–	ND
propanol	–	–	ND	–	–	ND
glycerol	+	+	–	ND	–	+

Note: “+” in the "Utilized substrates” column means that the substrates stimulated growth in the presence of sulfide and bicarbonate in the medium. In other cases, “+” or “–” mean that the characteristics is positive or negative; ND stands for “no data”; *, data from [28, 29] were used.

Unlike strain BBS, *T. pendens* and *T. rosea* are freshwater organisms; their cells contain gas vacuoles, and they do not grow on H_2. Unlike strain BBS, *T. pendens* and *T. rosea* do not respond to the addition of succinate or fumarate to a sulfide-containing medium by an increase in the biomass yield. In addition, *T. pendens* differs from strain BBS by incapacity for chemolithoautotrophy. The major difference of *T. marina* from strain BBS is the presence of okenone as the main carotenoid. *T. litoralis* differs from strain BBS by the capacity for assimilatory sulfate reduction, as well as by the cell size and spectrum of utilized organic substrates. Table 4 shows the phenotypic differences between strain BBS and the currently recognized species of the genus *Thiocapsa*.

Thus, the comparison of the phenotypic characteristics of strain BBS and those of the other representatives of the genus *Thiocapsa*, together with molecular genetic data, provides sufficient reasons for the revision of the taxonomic status of strain BBS and for its reclassification as an independent species of the genus *Thiocapsa, Thiocapsa bogorovii* sp. nov.

Description of *Thiocapsa bogorovii* sp. nov. (bo.go.ro'vi.i: the last name of the microbiologist who isolated strain BBS).

Cells are spherical, 1.0–1.5 μm in diameter, gram-negative, reproducing by binary fission. The cells lack flagella but are capable of random impulsive movements in wet mount preparations. Storage compounds are represented by polyphosphate granules, poly-β-hydroxybutyric acid, and polysaccharides. When grown in liquid medium, the culture is pink–red or often pale pink due to the accumulation of molecular sulfur, resulting from the oxidation of reduced sulfur compounds. The photosynthetic apparatus consists of vesicular membranes. Bacteriochlorophyll *a* and the carotenoid spirilloxanthin are the major photosynthetic pigments. Catalase and hydrogenase activities are exhibited. Growth occurs in a pH range of 6.0–8.0, with an optimum at pH 7.0–7.5 and at NaCl concentration up to 5%, with an optimum at 1–2%. Photoautotrophic growth occurs in the presence of sulfide, thiosulfate, sulfur, or H_2 as electron donors. Chemolithoautotrophic growth in the dark under aerobic conditions is possible at the expense of thiosulfate used as a sulfur source and as an electron donor and energy source for CO_2 assimilation. Autotrophic CO_2 assimilation occurs via the Calvin cycle; oxygen does not inhibit RuBisCO synthesis. Sulfide and thiosulfate are oxidized to sulfate via the formation of elemental sulfur, which is accumulated within the cells. The cell yield of the cultures grown in the light increases on addition of acetate, pyruvate, lactate, glycerol, or glucose (0.1%) to the mineral medium. Addition of 2-ketoglutarate, propionate, fumarate, succinate, or malate results in an insignificant increase of growth. Other organic compounds, such as formate, butyrate, fructose, galactose, lactose, sorbose, arabinose, rhamnose, xylose, mannose, maltose, sucrose, methanol, ethanol, butanol, propanol, isopropanol, mannitol, sorbitol, and dulcitol, do not affect the biomass yield. Benzoic acid and isobutanol, as well as amyl and isoamyl alcohols, inhibit growth. Capacity for assimilatory sulfate reduction is lacking. Growth occurs only in the presence of S^0, S^{2-}, or $S_2O_3^{2-}$. Cysteine and methionine are not utilized as sulfur sources. Ammonium salts, urea, peptone, casein hydrolysate, and arginine can be utilized as nitrogen sources. No growth occurs with glutamic acid, alanine, hydroxylamine, or KNO_3. Dinitrogen fixation capacity is present. Vitamin B_{12} is required. The content of G+C base pairs in DNA is 66.7 mol %.

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