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EXPERIMENTAL ARTICLES

Fractionation of Stable Carbon Isotopes by Photoautotrophically Growing Anoxygenic Purple and Green Sulfur Bacteria

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Abstract—Fractionation of stable carbon isotopes ^{12}C and ^{13}C by three pure cultures of photoautotrophic purple sulfur bacteria (*Ectothiorhodospira shaposhnikovii, Lamprocystis purpureus*, and *Thiocapsa* sp.) (PSB) and the green sulfur bacterium *Prosthecochloris* sp. (GSB) was investigated in 13–15-day experiments. The cultivation was carried out in a luminostat (2000 lx) on mineral media with 1–1.5 g/l $NaHCO_3$ (inoculum) with the subsequent transfer to the medium with up to 10 g/l $NaHCO_3$. For PSB, the difference in the quantitative characteristics of the isotopic composition of suspended carbon (including bacterial cells) and mineral carbon of the medium ($\Delta^{13}C = \delta^{13}C_{substrate} - \delta^{13}C_{biomass}$) changed from 15.0 to 34.3‰. For GSB, the range of $\Delta^{13}C$ changes was significantly less (18.3–22.7‰). These data suggested the possibility of a pool of soluble mineral carbon in PSB cells. The pool of intracellular mineral carbon was calculated; depending on the PSB species and growth stage, it varied from 0 to 68% of the total cell carbon. The α coefficients reflecting the carbon isotope fractionation by PSB and GBS and calculated from the changes of the bicarbonate carbon isotopic composition in the medium depending on its consumption were 1.029 ± 0.003 and 1.019 ± 0.001, respectively. These α values did not depend on the growth rate. CO_2 fixation on ribulose-bisphosphate was shown to be the major factor determining the carbon isotope fractionation by PSB; at the stage of CO_2 penetration into the cell, fractionation was insignificant. In GSB, fractionation occurred mostly at CO_2 penetration into the cell, while it was insignificant at the stage of carbon dioxide fixation in the reverse TCA cycle. Analysis of the isotopic data of the photosynthesis by PSB and GSB in meromictic lakes also revealed that in PSB-dominated natural communities suspended organic matter was more enriched with light ^{13}C ($\Delta^{13}C$ = 23.4–24.6‰) than in the communities with more active GSB ($\Delta^{13}C$ = 10.2–14.0‰)

Key words: ^{12}C and ^{13}C fractionation, photoautotrophic growth, *Ectothiorhodospira shaposhnikovii, Lamprocystis purpureus, Thiocapsa* sp., *Prosthecochloris* sp., indices of fractionation, $\Delta^{13}C$ (‰) and ε (‰), meromictic water bodies.

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Mass development of anoxygenic phototrophic bacteria is known to occur in many meromictic water bodies [1, 2]. Specific production of organic matter via anoxygenic photosynthesis may sometimes be comparable to or even higher than the values of production by the phytoplankton in the oxic zone [3–5]. Significant differences in the occurrence of ^{12}C and ^{13}C isotopes in the CO_2 utilized and biomass produced (isotope fractionation) was previously demonstrated for anoxygenic phototrophic bacteria [6–9]. Carbon isotope fractionation was found to depend on the biochemical traits of bacteria, including the type of the enzymatic systems responsible for initial CO_2 fixation, as well as on the conditions of photoautotrophic growth (ambient temperature and specific substrate concentration, determined as CO_2 content per cell or per unit biomass) [10].

Purple bacteria are known to utilize the pathway involving initial CO_2 fixation by the interaction of CO_2 with ribulose-bisphosphate (RuBP) due to RuBP carboxylase (RuBisCO) (the Calvin cycle). Green sulfur bacteria fix carbon dioxide as HCO_3^- via the reductive (reverse) TCA (RTCA) cycle involving pyruvate synthase, ketoglutarate synthase, isocitrate dehydrogenase, and phosphoenolpyruvate carboxylase [11–13].

Under the conditions of thermodynamic equilibrium between CO_2 and HCO_3^- in aquatic medium, their carbon isotopic composition is different: depending on temperature, the $\delta^{13}C$ (CO_2) value is 8–10‰ lower than the $\delta^{13}C$ (HCO_3^-) [14]. Moreover, these isotopically different forms of carbon dioxide differ in molecular weight and may exhibit different kinetic isotopic effects when involved in biochemical reactions. Thus,

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purple and green bacteria grown in liquid media utilize isotopically different forms of carbon dioxide; their photosynthetic products should therefore be isotopically different.

It was demonstrated for methanogens [15] and sulfate reducers [16] that fractionation of carbon and sulfur isotopes by the cultures of these microorganisms depended significantly on their metabolic activity. Fractionation of carbon and sulfur isotopes was significantly higher at low specific rates of methane or sulfide formation than at high rates. On the other hand, it was demonstrated that the rates of photoautotrophic growth of the oxygenic algae *Sceletonema costatum* and *Emiliania huxleyi* did not affect the fractionation of carbon isotopes [17].

The mechanisms for concentration of soluble mineral carbon in suspensions containing mineral carbon (CO_2 and HCO_3^-) were previously found in unicellular algae carrying out CO_2 fixation involving RuBisCO [18]. Evidently, accumulation of intracellular mineral carbon may be observed in analyses of the isotopic composition of the biomass carbon, since CO_2 resulting from incineration may originate from at least two sources, viz., cellular organic matter and the intracellular HCO_3^-/CO_2 pool. Existence of a similar mechanism for the concentration of mineral forms of carbon dioxide in the cells of photosynthetic bacteria is presently not established. Although the data on carbon isotope fractionation by anoxygenic phototrophic bacteria exist in the literature [6–10], their physiological and biochemical traits affecting the isotopic differences between organic products of photosynthesis and the major substrate (carbon dioxide) are presently poorly studied.

Due to the important role of photosynthetic sulfur bacteria in the carbon turnover in aquatic ecosystems, investigation of the factors determining the isotopic composition of suspended carbon, including organic matter of the photosynthetic bacteria and the possible intracellular mineral forms of carbon, is of significant interest.

The goal of the present work was to reveal the factors affecting the carbon isotope fractionation by photoautotrophically growing pure cultures of anoxygenic purple and green sulfur bacteria (isolated from meromictic water bodies) under controlled conditions and at different stages of development.

MATERIALS AND METHODS

Microorganisms. The following microbial cultures were used in this work: purple sulfur bacteria (PSB) *Ectothiorhodospira shaposhnikovii* 1C isolated from the silt sediments of a freshwater lake where the content of soluble mineral carbon was determined mainly by the CO_2 concentration in air (the culture was kindly provided by V.M. Gorlenko); *Lamprocystis purpureus* ShAm01 isolated from Lake Shira (concentration of the soluble mineral carbon was 17.8–18.8 mM at 14 g/l salinity in the upper chemocline); *Thiocapsa* sp. Mog 1 isolated from Lake Mogil'noe (concentration of soluble mineral carbon was 2.5–6.3 mM at 28 g/l salinity); and the green sulfur bacterium (GSB) *Prosthecochloris* sp. ShNPe102 isolated from Lake Shunet (concentration of soluble mineral carbon was 19.5–45.0 mM at 45 g/l salinity in the lower chemocline).

The strains of PSB belong to the families *Ectothiorhodaceae* and *Chromatiaceae*; their representatives exhibit high activities of the Calvin cycle enzymes [19, 20]. The GSB strain belongs to the family *Chlorobiaceae*, which fix carbon via the RTCA cycle [8, 21]. The major form of carbon fixed by bacteria via the RTCA cycle is HCO_3^- [13].

Cultivation of phototrophic bacteria. The inoculum was grown in mineral media with 1–1.5 g/l $NaHCO_3$ (13.9–20.8 mM). Carbon fractionation was studied in the same media with the $NaHCO_3$ content increased to 10 g/l (138.9 mM) (Table 1). In the latter case, this $NaHCO_3$ concentration was chosen for two reasons: to reveal the possible bicarbonate concentration inside the cells after transfer from the low-bicarbonate medium to the medium with an elevated $NaHCO_3$ concentration; and to minimize the effect of increased ^{13}C content in the substrate, which occurs at $NaHCO_3$ exhaustion in the course of growth of photoautotrophic bacteria.

Cultivation of phototrophic bacteria was carried out in 30-ml glass vials in two repeats. Homogeneous inoculum (3 ml) was introduced to each vial and fresh medium was added to capacity. For the purple and green sulfur bacteria, pH was 8.3–7.8 and 8.13–7.9, respectively. Bacteria were grown under anaerobic conditions at 20–25°C in a luminostat (2000 lx).

Growth of phototrophic bacteria. Growth kinetics of phototrophic bacteria were determined as an increase of organic carbon in the medium and of the optical density of bacteriochlorophyll in the individual vials depending on exposure time; the carbon introduced with the inoculum did not exceed 10% of the organic matter synthesized by bacteria by the time of their maximal growth. The concentration of bacterial biomass carbon (suspension) was determined from the content of CO_2 after incineration of the material obtained by filtering the suspension through GF/F glass fiber filters.

Apart from determination of suspended carbon, PSB growth was assayed as bacteriochlorophyll *a* (Bchl *a*) content determined as the optical density (OD_{770}) of the acetone–methanol (7 : 2) extract; GSB growth was monitored as bacteriochlorophyll *d* (Bchl *d*) content determined as OD_{654}. Absorption spectra were obtained on a LOMO SF-56 spectrophotometer (Russia).

Table 1. Mineral composition of the media used in the experiments for cultivation of purple and green sulfur bacteria (g/l distilled water)

	Ectothiorodospira shaposhnikovii	*Thiocapsa* sp.	*Lamprocystis purpureus*	*Prosthecochloris* sp.
KH_2PO_4	0.7	0.7	0.7	0.5
NaCl	20	10	20	5.3
$MgSO_4 \cdot 7H_2O$	0.5	0.5	0.5	0.5
NH_4Cl	0.7	0.7	0.7	0.7
KCl	0.33	0.33	0.33	0.33
Na_2SO_4	–	–	–	21
$MgCl_2 \cdot 7H_2O$	–	–	–	4.3
$NaHCO_3$	10	10	10	10
$CaCl_2 \cdot 6H_2O$	0.1	0.1	0.1	0.1
$Na_2S_2O_3 \cdot 5H_2O$	4	1.4	2	–
$Na_2S \cdot 9H_2O$	0.2	0.2	–	–*

* After sterilization and dispensing, 0.1 g Na_2S was added to each vial. All the media were supplemented with vitamin B_{12} (20 μg/l) and trace elements solution [Pfennig, 1965] (1 ml/l).

The rate of increase in Bch *a* and Bchl *d* optical density (relative U day^{-1}) depending on the incubation time (*t*, days) (Fig. 1) were characterized using the two-parameter function for exponential growth: $y_t = b \cdot \exp(c \cdot t)$ where *b* is the aspect ratio and *c* is the constant reflecting the rate of the changes in the bacteriochlorophyll optical density. The values of the parameters *b* and *c* were calculated from the optical density of bacteriochlorophyll at specific (reference) intervals of growth. The calculations were carried out using the SigmaPlot 8 software package.

Quantitative determination of bicarbonate and reduced sulfur compounds. Initial and residual concentrations of reduced sulfur compounds (mg S/l) were determined by direct titration with iodine and back titration with thiosulfate. Residual bicarbonate (mg C/l) was determined by titration with 0.1 N HCl.

Determination of the $\delta^{13}C$ values. Mass spectrometric determination of $\delta^{13}C$ (‰), the parameter characterizing the isotopic composition of mineral carbon in the medium and of organic carbon in bacterial biomass, was carried out with gaseous carbon dioxide (CO_2) as the working gas. All organic products were oxidized to CO_2 by high-temperature incineration (560°C) in the presence of copper oxide as a catalyst. Mineral carbon ($NaHCO_3$) was converted to $BaCO_3$; CO_2 was then obtained by fusion of barium carbonate with tin salts at 560°C. The $\delta^{13}C$ value, which is a relative characteristic of the carbon isotopic composition, was determined on MI-1201B (USSR) and Delta Plus (Thermo Electron Corp., Germany) mass spectrometers and calculated using the accepted mathematical expression:

$$\delta^{13}C = ([^{13}C]/[^{12}C])_{sample}/([^{13}C]/[^{12}C])_{st} - 1) \times 1000‰, \quad (1)$$

where $([^{13}C]/[^{12}C])_{sample}$ and $([^{13}C]/[^{12}C])_{st}$ are the ratios of ^{12}C and ^{13}C content in the sample and in the standard, respectively. The international *PDB* standard with the $[^{13}C]/[^{12}C]$ ratio of 0.001172 was used [22]. The error of $\delta^{13}C$ measurements did not exceed ±0.1‰.

Analysis of the isotope characteristics. The coefficient of carbon isotope fractionation α served as an index of carbon isotope fractionation; it was determined from the changes in the isotopic characteristics of bicarbonate depending on its consumption at a given time *t* ($\delta^{13}C_{(HCO_3)t}$) relative to the initial value ($\delta^{13}C_{(HCO_3)t=0}$). In our model experiments, the α coefficient was determined using the expression (2):

$$\ln((\delta^{13}C_t + 1000)/(\delta^{13}C_0 + 1000)) = (1/\alpha - 1) \cdot \ln(M_t/M_0), \quad (2)$$

where M_0 and M_t are the amount of bicarbonate carbon introduced into the cultivation medium ($t = 0$) and resid-

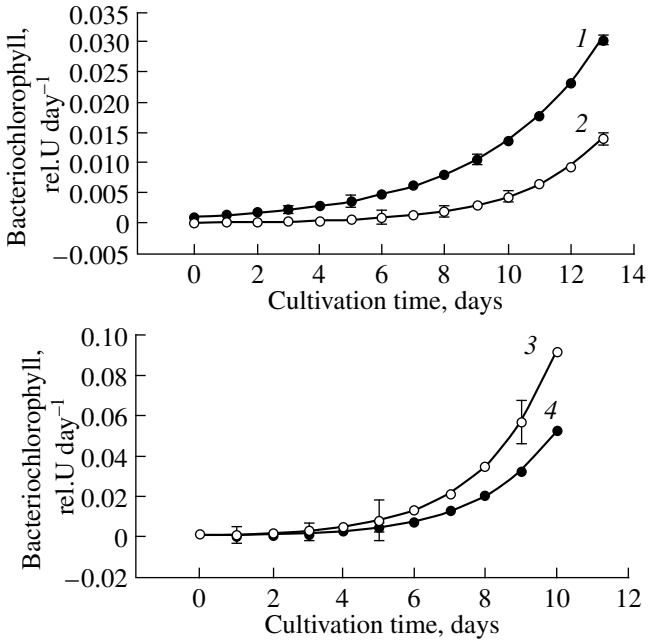


Fig. 1. Rates of increase of Bchl optical density in the cultures of phototrophic bacteria as functions (y_t): *Ectothiorhodospira shaposhnikovii* ($y_t = 10^{-3}\exp(0.2635\,t)$ (*1*); *Thiocapsa* sp. ($y_t = 10^{-4}\exp(0.3813\,t)$ (*2*); *Prosthecochloris* sp. ($y_t = 8 \times 10^{-4}\exp(0.4757\,t)$ (*3*); and *Lamprocystis purpureus* ($y_t = 5 \times 10^{-4}\exp(0.4672\,t)$ (*4*). Vertical lines designate the differences between the experimentally determined rates of increase of Bchl optical density and the calculated values for the reference points.

ual bicarbonate after cessation of bacterial growth at time *t*, respectively.

The value of $\Delta^{13}C = \delta^{13}C_{(\Sigma CO_2)} - \delta^{13}C_{(org)}$, which is the difference between the $\delta^{13}C$ values characterizing the isotopic composition of the total inorganic carbon of the medium (ΣCO_2) and of suspended carbon, including the intracellular carbon dioxide and bacterial organic matter, was also used to estimate the carbon isotope fractionation. Dependence between $\Delta^{13}C$ and the rate of bacterial growth was represented using the three-parameter equation for determination of the maximal $\Delta^{13}C$ values for exponential growth:

$$\Delta^{13}C_v = \Delta^{13}C_0 + a(1 - \exp(-b \cdot v), \quad (3)$$

where v is the rate of increase of the optical density of bacteriochlorophyll. The parameters $\Delta^{13}C_0$, a, and b were determined from the data of $\Delta^{13}C$ measurements for reference v values using the SigmaPlot 8 software package.

Apart from this, the difference in the carbon isotopic composition between the residual substrate (HCO_3^-) and bacterial biomass was calculated using the expression $\varepsilon = ((\alpha - 1)/\alpha) \times 1000‰$. At the values of substrate consumption not exceeding 30%, the value of ε (‰) is qualitatively close to $\Delta^{13}C$ and is used in biogeochemical research on the biological fractionation of carbon isotopes in natural environments [23].

According to (4), the carbon isotopic composition of organic matter of bacterial cells is characterized by the value of $\delta^{13}C_{org}$, which is determined as:

$$\delta^{13}C_{org} = -\varepsilon_{RuBisCo,\,PEP} + \delta^{13}C_{HCO_3(acell)} + \delta^{13}C_{HCO_3(medium)}, \quad (4)$$

where $\delta^{13}C_{HCO_3(medium)}$ and $\delta^{13}C_{HCO_3(cell)}$ characterize the isotopic composition of the carbon dioxide carbon in the mineral medium and inside the cell; $\varepsilon_{carboxylase}$ is fractionation of the carbon isotopes of the substrate (CO_2/HCO_3^-) upon fixation on RuBisCO or in the RTCA cycle. The net carbon isotope fractionation by photoautotrophically growing bacteria relative to bicarbonate of the medium is determined by the value of $\varepsilon = \varepsilon_{carboxylase} + \delta^{13}C_{HCO_3(cell)}$.

In the presence of mechanisms promoting the concentration of soluble mineral carbon in the cell, an intracellular bicarbonate pool may be formed (HCO_3^-/CO_2); its isotopic composition is characterized by the value of $\delta^{13}C_{HCO_3(cell)}$.

Designating the pool of intracellular mineral carbon as x and that of organic cell components as $(1 - x)$ results in the following equation for the material–isotope balance for a suspension containing a mixture of organic carbon ($\delta^{13}C_{(org)}$) and intracellular mineral carbon $\delta^{13}C_{HCO_3(cell)}$:

$$x\,\delta^{13}C_{HCO_3(cell)} + (1-x)\delta^{13}C_{(org)} = \delta^{13}C_{(suspension)}. \quad (5)$$

From Eq. (5), the ratio of carbon (x) representing the relative amount of carbon in the intracellular bicarbonate pool was calculated.

RESULTS AND DISCUSSION

The results of model experiments on carbon isotope fractionation by anoxygenic purple and green sulfur bacteria are presented in Table 2. During the first 4–6 days, relatively low rates of increase in bacteriochlorophyll OD, as well as of consumption of bicarbonate and reduced sulfur compounds, were observed for all investigated cultures of sulfur bacteria. The rate of consumption of sulfur compounds increased on day 6–9, while bacteriochlorophyll OD, on day 5–8. Increased content of suspended carbon correlated with an increase in the optical density of bacteriochlorophyll (Table 2).

As can be seen from Fig. 1, transfer from the low-bicarbonate medium (1–1.5 g/l) to the medium with

Table 2. Growth of anoxygenic phototrophic bacteria, Bchl optical density, content of suspended carbon, reduced sulfur compounds, and mineral carbon, as well as carbon isotopic composition ($\delta^{13}C$, ‰) of the medium (C_{min}) and suspension (C_{org})

Time, days	Optical density, Bchl, relative U	$[HS^-]$, mg S/ml	C_{org}–suspension, mg C/ml	C $[HCO_3^-]$, mg/ml	$\delta^{13}C$, ‰		*$\Delta^{13}C$, ‰
					C_{org}–suspension	C_{min}	
Ectothiorhodospira shaposhnikovii							
0	0	0.289	0.002	1.414		–8.0	
3	0.005	0.286	0.003	1.414	–20.3	–5.3	15.0
5	0.030	0.275	0.052	1.341	–23.6	–2.1	21.5
8	0.056	0.241	0.083	1.131	–25.6	0.5	26.1
9	0.066	0.150	0.154	1.105	–25.7	2.3	28.0
13	0.185	0.041	0.350	1.059	–25.9	6.1	32.0
15	0.168	0.00	–	0.842	–26.0	8.3	34.3
Lamprocystis purpureus, Lake Shira							
0	0	0.116	0.150	1.151		–8.0	
4	0.004	0.095	0.450	1.059	–18.6	–8.2	10.4
6	0.020	0.061	0.430	0.986	–25.0	–4.3	20.7
9	0.120	0.010	0.410	0.914	–30.7	+1.2	31.9
11	0.096	0.003	1.260	0.888	–29.1	+1.5	30.6
13	0.094	0.000	1.490	0.888	–26.7	+1.4	28.1
Thiocapsa sp., Lake Mogiil'noe							
0	0	0.078	0.015	1.44	–	–6.0	
6	0.007	0.065	0.035	1.322	–26.6	–4.7	21.9
8	0.011	0.017	0.062	1.223	–28.2	–4.2	24.0
10	0.026	0.014	0.149	1.223	–30.0	–3.2	26.8
13	0.072	0.010	0.160	1.151	–30.4	–2.5	27.9
16	0.084	0.010	0.141	1.105	–30.3	–1.4	28.9
20	0.09	0.003	0.108	1.078	–30.0	–0.9	29.1
Prosthecochloris sp., Lake Shunet							
0	0	0.197	0.018	1.61		–5.3	
1	0.007	0.136	0.020	1.54	–22.9	–3.9	19.0
3	0.011	0.119	0.080	1.44	–21.3	–2.9	18.4
5	0.026	0.085	0.132	1.30	–19.3	–1.0	18.3
7	0.072	0.075	0.184	1.32	–21.2	–1.6	19.6
9	0.084	0.061	0.148	1.20	–22.3	+0.4	22.7
13	0.09	0.017	0.132	1.32	–21.9	–1.4	20.5

* $\Delta^{13}C = \delta^{13}C_{min} - \delta^{13}C_{org–suspension}$.

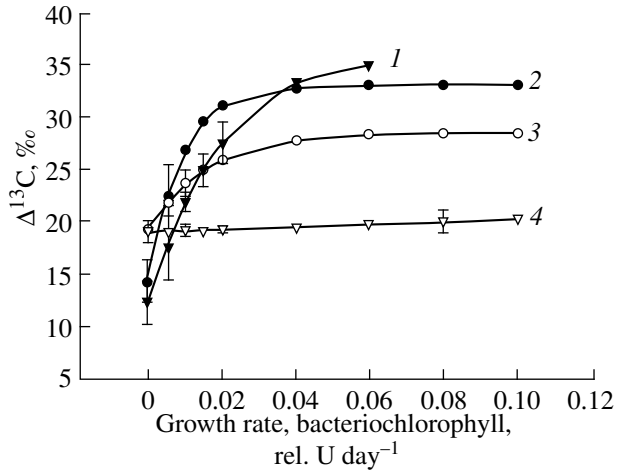


Fig. 2. Carbon isotope fractionation by phototrophic bacteria represented by the function (Δ^{13}C, ‰) depending on the rate of increase of Bchl optical density (v) as a criterion of bacterial growth. *L. purpureus* ($\Delta^{13}C = 12.2438 + 25.003(1 - \exp(-47.1177v))$) (*1*); *E. shaposhnikovii* ($\Delta^{13}C = 14.2878 + 18.8414(1 - \exp(-110.6144v))$ (*2*); *Thiocapsa* sp. ($\Delta^{13}C = 19.2932 + 7.2694(1 - \exp(-61.77v))$ (*3*); and *Prosthecochloris* sp. ($\Delta^{13}C = 18.996 + 12.711v$) (*4*). Vertical lines designate the differences between the experimentally determined Δ^{13}C values and the calculated values for the reference points.

approx. 10 g/l $NaHCO_3$ resulted in a period of adaptation (lag phase). Growth of phototrophic bacteria on media with 10 g/l $NaHCO_3$ was characterized by the *c* values (expressing the rate of bacteriochlorophyll increase) significantly different for different cultures (Fig. 1). For example, the values of the *c* constant were the lowest for *E. shaposhnikovii* isolated from a freshwater basin and *Thiocapsa* sp from Lake Mogil'noe (0.2635 and 0.3818 day^{-1}, respectively), while for the purple bacterium *L. purpureus* and the green bacterium *Prosthecochloris* sp., which were isolated from saline environments, the *c* values were 0.4672 and 0.4756 day^{-1}, respectively. These differences may be due to the physiological and biochemical traits of the phototrophic bacteria depending on bicarbonate concentrations in the sources of their isolation.

The Δ^{13}C values presented in Fig. 2 reflect the differences (‰) between the δ^{13}C of the suspension and of bicarbonate in the medium depending on the growth rate (measured by the OD of bacteriochlorophyll). For PSB, significant changes in the Δ^{13}C values were observed at low rates of Bchl formation during the first 4–6 days after inoculation (lag phase). A slow increase in Bchl OD was then accompanied by increased fractionation of carbon isotopes (Δ^{13}C). During the subsequent period of exponential growth (days 6–13), Δ^{13}C values stabilized at 25–35‰, which is characteristic of PSB fixing carbon dioxide via RuBisCO [6, 9].

Cultivation of the GSB *Prosthecochloris* sp. revealed no significant changes in Δ^{13}C values depending on the growth rate. The Δ^{13}C value was approx. 19‰ and did not change significantly during the 10 days, in spite of a significantly increased growth rate. This Δ^{13}C value is typical of phototrophic bacteria fixing carbon via the RTCA cycle [6, 7].

On Fig. 3, the dependencies demonstrating the changed ratios of ^{12}C and ^{13}C isotopes in residual bicarbonate are presented in the logarithmical coordinates. These linear graphs and expression (2) were used to calculate the coefficients α characterizing carbon isotope fractionation resulting from the different rates of involvement of different forms of mineral carbon in bacterial photosynthesis. The α values were used to determine the ε values (‰) reflecting the difference between the isotopic characteristics of δ^{13}C bicarbonate in the medium and of the organic matter of cell biomass (Table 3).

Growth of all three PSB species was accompanied by fractionation of carbon isotopes of residual bicarbonate in the medium with the coefficients $\alpha = 1.029 \pm 0.003$ and $\varepsilon = 28 \pm 3$‰ (Table 3); these values are typical of purple sulfur bacteria using the Calvin cycle [6, 9]. Fractionation of carbon isotopes by the green sulfur bacterium *Prosthecochloris* sp. was characterized by the coefficients $\alpha = 1.0187$ and $\varepsilon = 18$‰, which are typical of the phototrophs employing RTCA cycle for carbon gas fixation [6, 7]. Characteristically, unlike the Δ^{13}C values, reflecting carbon isotope fractionation, the values α and ε parameters for *E. shaposhnikovii*, *L. purpureus*, and *Thiocapsa* sp. do not depend on bacterial growth rates (Table 3).

Table 2 demonstrates that the $\delta^{13}C_{org}$ values for the suspension used to calculate Δ^{13}C indicate increased ^{13}C content in organic products formed at the initial stages of bacterial growth after transfer from low-$NaHCO_3$ medium to the medium with high bicarbonate content. Further exposure resulted in a gradual decrease of ^{13}C content in the biomass to the values characteristic of bacteria fixing CO_2 via the Calvin cycle. In the case of purple sulfur bacteria, the difference between the isotopic characteristics ε and Δ^{13}C at the initial growth stages suggested that the intracellular bicarbonate pool with the ^{13}C values different from those of carbon of organic products of photosynthesis was responsible for the elevated δ^{13}C content in suspended bacterial cells. The size of the intracellular bicarbonate pool changed depending on the growth rates of bacteria. This hypothesis was supported by (1) the absence of dependence between the ε value and the growth rate of bacteria and (2) the data [24] on the insignificant effect of HCO_3^- concentration on carbon isotope fractionation in the course of CO_2 assimilation via RuBisCO; changing of bicarbonate concentration in the medium from 2.5 to 50 mM resulted in no more than a 3‰ increase in the δ^{13}C value (characterizing the isotopic composition of carbon fixed on RuBisCO [25].

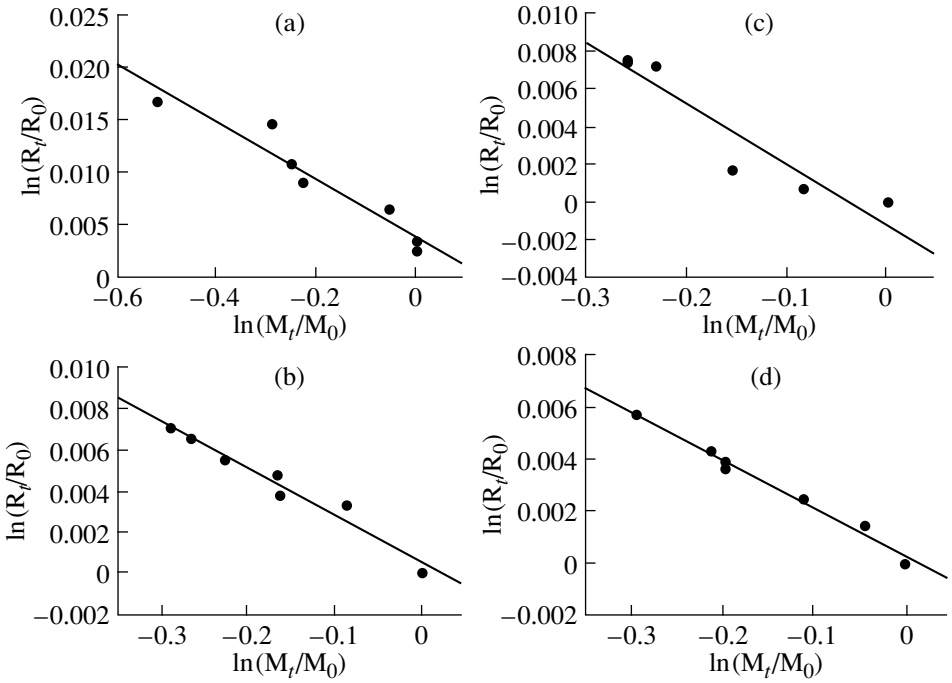


Fig. 3. Changes in the $\ln(R_t/R_0)$ values characterizing the ratio of bicarbonate isotope content relative to the $\ln(M_t/M_0)$ values characterizing bicarbonate decrease in the cultures of purple and green sulfur bacteria as the correlation equations of $\ln(R_t/R_0)$, respectively: *E. shaposhnikovii*, $\log(R_t/R_0) = 3.8 \times 10^{-3}$–0.0275 $\ln(M_t/M_0)$ at R = 0.9598 (a); *L. purpureus*, $\ln(R_t/R_0) = 1.2 \times 10^{-3}$–0.0322 $\ln(M_t/M_0)$ at R = 0.9416 (b); *Thiocapsa* sp., $\ln(R_t/R_0) = 5.0 \times 10^{-4}$–0.023 $\ln(M_t/M_0)$ at R = 0.9789 (c); *Prosthecochloris* sp., $\ln(R_t/R_0) = 3.0 \times 10^{-4}$–0.0185 $\ln(M_t/M_0)$ at R = 0.9946 (d).

It was previously reported [18] that, depending on pH and CO_2 concentration, unicellular phototrophs fixing CO_2 via RuBisCO employ the mechanism for intracellular concentration of mineral forms of carbon (CO_2 and HCO_3^-). This mechanism manifests itself when phototrophic bacteria are transferred from the medium with low bicarbonate content (1–1.5 g/l $NaCO_3$) to the medium with its increased content (10 g/l $NaCO_3$). In the cells of the phototrophic bacteria employing the mechanism for concentrating soluble mineral forms of carbon (HCO_3^-/CO_2), the intracellular bicarbonate pool is evidently formed. Incineration of the suspended bac-

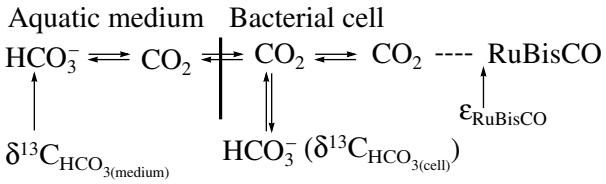
Table 3. Coefficients of carbon isotope fractionation (ε, ‰) calculated from the characteristics of the carbon isotopic composition of residual bicarbonate in the medium ($\delta^{13}C_{min}$, Table 2) depending on its consumption by bacteria ($C[HCO_3^-]$, Table 2)

Bacterial cultures	a*	α	ε, ‰
Ectothiorhodospira shaposhnikovii	–0.0275 (0.0036)	1.028 (0.004)	27 (±2)
Lamprocystis purpureus	–0.0322 (0.0058)	1.033 (0.006)	32 (±3)
Thiocapsa sp.	–0.025 (0.0021)	1.026 (0.0022)	25 (±1)
Prosthecochloris sp.	–0.0184 (0.0009)	1.0187 (0.0009)	18 (±0.5)

In parentheses, standard errors of the calculated values are presented. $\alpha = 1/(a + 1)$, where a* is the parameter determined from the correlation equations according to Fig. 3; $\varepsilon = 1000 \times (\alpha - 1)/\alpha$, ‰.

terial biomass results in the carbon of this bicarbonate being added to the analyzed CO_2. The content of intracellular bicarbonate affects the differences in the isotopic composition between the cellular organic matter and mineral carbon in the growth medium, i.e., on the $\Delta^{13}C = \delta^{13}C_{(\Sigma CO_2 medium)} - \delta^{13}C_{(org)}$ value.

The results of the calculation of the ratio x reflecting the qualitative content of the intracellular bicarbonate pool in the suspension depending on the time of cultivation of phototrophic bacteria are presented in Table 4. In the case of PSB, the ratio of intercellular bicarbonate in the suspension was as high as 68% at the early stages of growth. Further cultivation resulted in an increased ratio of organic carbon in the suspension and a decreased content of carbon of the bicarbonate pool. In the course of bicarbonate consumption from the medium and increasing the ^{13}C content, the isotopic characteristics of organic products ($\delta^{13}C_{(org)}$) also exhibited a relative increase of ^{13}C content in the cell material (Table 4).

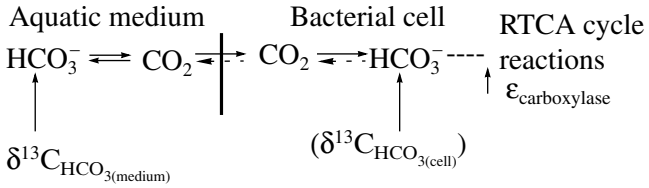


Scheme 1.

In phototrophically growing PSB, carbon isotope fractionation in organic matter relative to bicarbonate of the medium was determined as $\varepsilon = \varepsilon_{RuBisCo} + \delta^{13}C_{HCO_3(cell)}$, where $\varepsilon_{RuBisCo}$ characterizes carbon isotope fractionation in the course of CO_2 fixation via RuBisCO, and $\delta^{13}C_{HCO_3(cell)}$ characterizes carbon isotopic composition of CO_2 arriving into the cell (Scheme 1).

Table 3 demonstrates that the average ε value characterizing the total carbon isotope fractionation by PSB is 28 ± 3‰. Comparison of the ε values obtained in our experiments with native bacterial cells with $\varepsilon_{RuBisCo}$ determined in the reaction of CO_2 fixation on RuBisCO (approx. 27–28‰) [25, 26] revealed an insignificant contribution of $\delta^{13}C_{HCO_3(cell)}$ to the total carbon isotope fractionation by actively growing PSB. This may indicate low bicarbonate content in the intracellular pool of mineral carbon (HCO_3^-/CO_2), although it does not rule out CO_2 concentrations sufficient for the functioning of RuBisCO. Insignificant changes in carbon isotopic composition at the stage of carbon dioxide transport inside the PSB cell evidently result from the steady-state exchange between carbon dioxide in the culture liquid and in the intracellular pool. Carbon isotope fractionation during diffusion of carbon dioxide into the cell is thus compensated by its reverse diffusion from the cell.

In the course of photoautotrophic growth of GSB, carbon is fixed in reactions of the RTCA cycle involving the corresponding carboxylases (Scheme 2). The form of mineral carbon used in these reactions is HCO_3^- [13].



Scheme 2.

Carbon isotope fractionation relative to bicarbonate of the medium is represented as $\varepsilon = \varepsilon_{carboxylase} + \delta^{13}C_{HCO_3(cell)}$, where $\varepsilon_{carboxylase}$ characterizes carbon isotope fractionation at carbon dioxide fixation in the RTCA cycle and $\delta^{13}C_{HCO_3(cell)}$ characterizes fractionation at the stage of its transport into the cell (Scheme 2).

According to [25], fractionation in carboxylation reactions is approx. –2 to –5‰. Considering the overall value of ε = 18‰ determined for photoautotrophic growth of the GSB *Prosthecochloris* sp. (Table 3), fractionation of mineral carbon isotopes at the stage of transportation into the cell is estimated by the value of $\delta^{13}C_{HCO_3(cell)}$, about –16 to –13‰ relative to the bicarbonate carbon in the medium. Thus, carbon transport into the bacterial cell is the major factor determining carbon isotope fractionation by these bacteria.

Table 5 presents the data on $\delta^{13}C$ values for the carbon of bacterial biomass and for the total mineral carbon of lake water from the samples of maximal devel-

Table 4. Isotopic composition of carbon of organic matter ($\delta^{13}C_{org-cell}$, ‰) synthesized by photoautotrophically growing purple and green sulfur bacteria, relative contents of the carbon of the intracellular bicarbonate pool (*x*), and its isotopic characteristics ($\delta^{13}C_{HCO_3(pool)}$, ‰)

Time, days	$\delta^{13}C_{suspension}$, ‰	$\delta^{13}C_{HCO_3-medium}$, ‰	$\delta^{13}C_{HCO_3-pool}$, ‰	$\delta^{13}C_{org-cell}$, ‰	*X*	$\Delta^{13}C$, ‰
Ectothiorhodospira shaposhnikovii						
0.0	–	–8.0	–	–	–	–
3.0	–20.3	–5.3	–5 (±3)	–32.0 (±3)	0.43 (±0.05)	26.7
5.0	–23.6	–2.1	–2 (±3)	–29.0 (±3)	0.18 (±0.06)	26.9
8.0	–25.6	0.5	1 (±3)	–26.0 (±3)	0.00 (±0.10)	26.5
9.0	–25.7	2.3	2 ±3)	–22.0 (±3)	–	22.3
Lamprocistis purpureus						
0.0	–	–8.0	–	–	–	–
4.0	–18.6	–5.2	–10 (±3)	–37 (±3)	0.68 (±0.03)	31.8
6.0	–25.0	–4.3	–9 (±3)	–36 (±3)	0.40 (±0.02)	31.7
9.0	–30.7	1.2	–3 (±3)	–30 (±3)	0.0 (±0.1)	31.2
11.0	–29.1	1.5	–3 (±3)	–30 (±3)	0	31.5
Thiocapsa sp.						
0.0	–	–8.0	–	–	–	–
6.0	–26.6	–4.7	–3 (±1)	–30 (±1)	0.13 (±0.05)	25.3
8.0	–28.2	–4.2	–2 (±1)	–29 (±1)	0.04 (±0.02)	24.3
10.0	–30.0	–3.2	–1 (±1)	–28 (±1)	0.0 (±0.04)	23.8
13.0	–30.4	–2.5	+1 (±1)	–28 (±1)	0	25.5
16.0	–30.3	–1.4	0 (±1)	–27 (±1)	–	25.6
Prosthecochloris sp.						
0.0	–	–8.0		–	–	–
1.0	–22.9	–3.9	–3.9	–21.9 (±1)	0.00 (±0.1)	18.0
3.0	–21.3	–2.9	–2.9	–20.9 (±1)	0.05 (±0.1)	18.0
5.0	–19.3	–1.0	–1.0	–19.0 (±1)	0.06 (±0.08)	18.0
7.0	–21.2	–1.6	–1.6	–19.6 (±1)	–0.05 (±0.2)	18.0
13.0	–21.9	–1.4	–1.4	–19.4 (±1)	–0.14 (±0.2)	18.0

$\delta^{13}C_{org-cell} = -\varepsilon + \delta^{13}C_{HCO_3-medium}$, where the ε value is taken from Table 3.

$\delta^{13}C_{HCO_3-pool} = -\varepsilon_{RuBisCO} + \delta^{13}C_{org}$ (‰), where the $\varepsilon_{RuBisCO} = 27$ (‰) according to [26].

$\Delta^{13}C = \delta^{13}C_{HCO_3-medium} - \delta^{13}C_{org-cell}$.

Table 5. Carbon isotopic composition of suspended organic matter in the water samples collected from the zones of mass development of photoautotrophic bacteria in meromictic lakes Shunet and Mogil'noe

Lake	Lake Shunet							Lake Mogil'noe	
Sample no.	I	II	III	IV	V	VI	VII	VIII	IX
Sampling time	VII.2002	VII.2002	VII.2002	VII.2003	VII.2003	II.2003	II.2003	VI.1999	IX.2001
Sampling depth, m	5.06	5.25	4.50	5.33	5.43	4.75	5.35	9.50	10.25
Light CO_2 fixation rate, μg l^{-1} day^{-1}	271	9	189	1678	14	0	0	814	440
Purple bacteria, cells/ml	4.8×10^5	7.4×5	2×10^5	1.6×10^8	9.5×10^6	6.7×10^3	3.0×10^5	–	–
Green bacteria, cells/ml	9.6×10^6	9.6×10^6	$<10^4$	3.2×10^7	9.7×10^6	2.0×10^6	9.1×10^5	–	–
Bacteriochlorophyll *a*, μg/l	324	–	–	5980	346	0.06	230	Traces	Traces
Bacteriochlorophyll *d* (or *e*), μg/l	1300	–	–	4295	1300	0.36	1500	4600	3200
$\delta^{13}C$ of bacterial biomass, ‰	–25.7	–26.6	–30.8	–27.8	–26.6	–23.1	–23.3	–23.8	–26.2
$\delta^{13}C$ of lake water mineral carbon, ‰	–12.8	–15.8	–6.2	–4.4	–14.3	–10.7	–19.5	–10.8	–12.2
Difference in $\delta^{13}C$ values of biomass and mineral carbon ($\Delta^{13}C$, ‰)	12.9	10.2	24.6	23.4	12.3	12.4	3.8	13.0	14.0
Number of green/number of purple bacteria	2.0	13.0	0.10	0.20	>1	300	3.3	–	–
Bchl *d* (Bchl *e*)/Bchl *a*	4.00	–	–	0.70	3.78	6.0	6.9	>1000	>1000

opment of phototrophic bacteria in the lakes Mogil'noe and Shunet, from which pure cultures of the photoautotrophs used in the present work were obtained. Some parameters of the water samples are also included (rate of photosynthesis, number of purple and green bacteria, and content of various bacteriochlorophylls). The ratio of the content of Bchl *c*, *d*, or Bchl *e* (present in the cells of green bacteria) to the content of Bchl *a* of purple bacteria is the most unbiased criteria characterizing the ratio of green and purple bacteria. In the case of missing bacteriochlorophyll data (samples III and VII), we used the cell ratio of green and purple bacteria.

The biomass of samples IV and III exhibited the lowest $\delta^{13}C$ values of approx. –27.8 and –30.8‰, respectively; in these samples, the number of purple bacteria exceeded the number of green ones (Table 5). In other samples, in which active bacterial photosynthesis was carried out mostly by green photoautotrophic bacteria (I, II, V, VIII, and IX), $\delta^{13}C$ of the biomass varied within the range from –23.8 to –26.6‰. The differences in fractionation in the course of photosynthesis by purple and green sulfur bacteria in their natural habitats becomes more evident when the $\Delta^{13}C$ values are compared. In the water horizons with predominant development of purple sulfur bacteria, $\Delta^{13}C$ of the biomass is 24.6 and 23.4‰ (Table 5, samples III, IV). These values are very close to the theoretical value of carbon isotope fractionation by the organisms employing the Calvin pathway (ε = 27‰) and approach our results obtained on pure PSB cultures (see ε values, Table 3).

In the samples with predomination of GSB, $\Delta^{13}C$ values vary from 10.2 to 14.0 (Table 5, samples I, II, V, VIII, IX). Significant difference of these values from the theoretically calculated for the organisms employing the RTCA cycle and from our experimental data (18‰, see Table 3) is explained by the lighter isotopic composition of mineral carbon (from –10.3 to –15.8‰) resulting from development of the green sulfur bacteria in deeper water layers than the purple sulfur bacteria; in these horizons, mineral carbon is enriched with the light isotope ^{12}C due to anaerobic decomposition of organic matter. The lowest value of $\Delta^{13}C$ (3.8‰) was detected in the February sample from 5.35 m in Lake Shunet (sample VII); in this sample, photosynthesis did not occur and mineral carbon was the most enriched

with ^{12}C (to ~19.5‰) due to anaerobic decomposition of organic matter (Table 5).

The following important conclusions of our research should be named:

1. The degree of fractionation of the carbon isotopes ^{12}C and ^{13}C by photoautotrophically growing cultures of purple sulfur bacteria (*Ectothiorhodospira shaposhnikovii, Lamprocystis purpureus*, and *Thiocapsa* sp.) and the green sulfur bacterium *Prosthecochloris* sp. isolated from meromictic water bodies differed significantly due to involvement of a different enzymatic system of photoassimilation of mineral carbon.

2. The values of the coefficient α characterizing carbon isotope fractionation by phototrophic purple and green sulfur bacteria and calculated from the changes in the isotopic composition of bicarbonate depending on the ratio of its consumption were 1.029 ± 0.003 and 1.019 ± 0.001 and did not depend on the growth rate.

3. For purple sulfur bacteria, the difference between the isotopic characteristics of suspended carbon and of bicarbonate of the medium ($\Delta^{13}C$, ‰) varied depending on the growth rate due to consumption of the intracellular bicarbonate pool. Since photoautotrophic green sulfur bacteria do not have an intracellular bicarbonate pool, this fact precludes the changes in $\Delta^{13}C$ depending on their growth rate.

4. Comparison of the results on carbon isotope fractionation in the environments with mass development of photoautotrophic sulfur bacteria in meromictic lakes and our laboratory data demonstrated that in nature more pronounced carbon isotope fractionation also occurs in the environments with predomination of purple sulfur bacteria; the $\Delta^{13}C$ values vary there from 23.4 to 24.6‰, while in the ecosystems with predominant development of green sulfur bacteria this value is significantly lower, from 10.2 to 14.0‰.

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