Microbiology, Vol. 71, No. 3, 2002, pp. 255–261. Translated from Mikrobiologiya, Vol. 71, No. 3, 2002, pp. 301–307.

EXPERIMENTAL ARTICLES

Carbon and Sulfur Metabolism in Representatives of Two Clusters of Bacteria of the Genus *Leucothrix*: A Comparative Study

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Received August 27, 2001; in final form, October 23, 2001

Abstract—Major pathways of carbon and sulfur metabolisms were studied in representatives of two clusters of bacteria: *Leucothrix thiophila* (cluster I, strains 2WS, 4WS, and 6WS) and *Leucothrix* sp. (cluster II, strains 1WS, 3WS, and 5WS). All strains were capable of chemoorganoheterotrophic growth, as well as of chemolithoheterotrophic growth in the presence of reduced sulfur compounds. The bacteria were found to possess a complete set of the enzymes of the tricarboxylic acid cycle and glyoxylate cycle. The dehydrogenase activity in cells of cluster I strains was an order of magnitude lower than in cluster II strains and in other known heterotrophic bacteria. Cells of bacteria of both clusters exhibited high activity levels of enzymes involved in the energy metabolism of sulfur. The oxidation of sulfur compounds and the operation of the electron-transport chain were shown to be related. Cluster II bacteria more efficiently use organic compounds in their energy metabolism, whereas cluster I bacteria are characterized by more efficient utilization of reduced sulfur compounds. During sulfite oxidation, cluster I bacteria can synthesize ATP both via substrate-level phosphorylation and oxidative phosphorylation, whereas cluster II bacteria synthesize ATP only via the latter process.

Key words: Leucothrix, tricarboxylic acid cycle, glyoxylate cycle, oxidation of thiosulfate and tetrathionate.

The genus *Leucothrix* was until recently represented by a single species, *L. mucor* [1]. In 1996, Dul'tseva *et al.* [2] isolated new strains belonging to the genus *Leucothrix*, and some of them were described as a new species, *L. thiophila*. Phenotypically, these strains are rather similar. The DNA homology of the seven new strains with the type strain *L. mucor* DSM 2157 is only 9–11%. Based on DNA homology, the new strains of *Leucothrix* were divided into two clusters. Cluster I is constituted by strains 2WS, 4WS, 6WS, and 7WS, referred to *L. thiophila*. This cluster is characterized by a fairly close similarity of DNA sequences (89–61%). For the second cluster, represented by strains 1WS, 3WS, and 5WS, this value is almost the same (88–61%). At the same time, the DNA homology between clusters I and II is 42–60%. Given the high phenotypic similarity between the strains of the two groups, the values determined fail to produce a sound basis for referring these clusters to different species. The sequence analysis of 16S rRNA might give a more definite answer as to their taxonomic status.

The studies of the metabolism of the new *Leucothrix* strains and the type strain of *L. mucor* showed that these organisms are able to carry out both chemoorganoheterotrophic and chemolithoheterotrophic metabolisms [3–5]. During chemolithoheterotrophic growth in the presence of sulfur compounds, these bacteria accumulate elemental sulfur in their cells. On these grounds, they can be referred to the group of colorless filamentous sulfur bacteria.

The goal of this work was to study carbon and sulfur metabolism in the new strains belonging to the two clusters of bacteria of the genus *Leucothrix*.

MATERIALS AND METHODS

The subjects of this study were marine filamentous bacteria of the genus *Leucothrix* belonging to two clusters. Cluster I (*L. thiophila*) was represented by strains 2WS, 4WS, and 6WS, and cluster II was represented by strains 1WS, 3WS, and 5WS. All these bacteria are stored in the collection of microorganisms of the Institute of Microbiology, Russian Academy of Sciences, and three strains are also stored in DSMZ: strain 2WS (DSM 13602), strain 4WS (DSM 13603), and strain 5WS (DSM 13604).

Nutrient media. Marine bacteria of the genus *Leucothrix* were cultured on a nutrient medium composed of (g/l) $(NH_4)_2SO_4$, 0.1; $CaCl_2 \cdot 2H_2O$, 0.2; $MgCl_2 \cdot 7H_2O$, 3.0; NaCl, 20.0; $NaHCO_3$, 0.1; and distilled water, 1.0 l. The pH of the medium was 7.2–7.4. Upon

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0026-2617/02/7103-0255$27.00 © 2002 MAIK "Nauka / Interperiodica"

sterilization at 115°C, the medium was supplemented with 10% sterile solutions of a 1 : 4 mixture of KH_2PO_4 and K_2HPO_4, pH 7.2–7.4, (10 ml); $Na_2S_2O_3 \cdot 5H_2O$ (10 ml), and either sodium lactate, 4 mM, or a mixture of sodium lactate and sodium acetate, 4 mM each. *Escherichia coli* was grown on PSS medium [6]. Prior to inoculation, all media were supplemented with Pfennig's solution of vitamins and trace elements [7].

Obtaining cell suspensions and enzyme extracts. Suspensions of bacterial cells were obtained by centrifugation of the culture for 30 min at 6000 *g* at 4°C. The cells were then washed in 0.1 M Tris–HCl buffer (pH 7.5) and precipitated for 20 min by centrifugation under the same conditions. The precipitated cells were resuspended in the same buffer to the required protein concentration. Cell extracts (homogenates) were obtained by disintegrating cells for 2 min in an ice bath in an UZDN-2T ultrasonic apparatus operated at a frequency of 22 kHz (500 W). The supernatant was obtained by centrifugation of cell extracts for 30 min at 9000 *g* at 4°C.

Enzyme activity assays. The activities of enzymes of the tricarboxylic acid (TCA) and glyoxylate cycles were determined on an SF-26 spectrophotometer by conventional methods described elsewhere [8]. The activity of enzymes of sulfur metabolism was determined in supernatant as described in [9, 10]. The activities were expressed in nanomoles of the reaction substrate or product per minute per one milligram of protein.

Determination of the respiration rate. The oxygen uptake of washed cell suspensions was measured at 25°C on a PU-1 polarograph equipped with a 1-ml measuring cell and a standard Clark oxygen electrode.

Chemical analyses. Hydrogen sulfide was determined colorimetrically with the use of dimethyl-*p*-phenylenediamine or by iodometric titration. Thiosulfate was determined by iodometry upon binding sulfite with formaldehyde. For separate determination of thiosulfate and tetrathionate, iodometric titration [11] and the cyanolysis method [12] were used. Elemental sulfur was determined by the method of Morris *et al.* [13]. Sulfates were determined by the chloranilate method [14]. Protein was measured by the method of Lowry *et al.* [15], but prior to that elemental sulfur was extracted from precipitated cells with ethanol for 1 h and the cells were washed in a buffer.

RESULTS

Oxidation of reduced sulfur compounds. The bacteria of the two clusters of the genus *Leucothrix* were able to accumulate elemental sulfur inside their cells in the presence of sulfide, thiosulfate, or tetrathionate. The presence of reduced sulfur compounds in lactate-containing medium was found to increase the cell yield by 20% on average in the case of thiosulfate and by 50–60% in the case of tetrathionate (Fig. 1). The observed dynamics of thiosulfate oxidation and product accumulation by different strains of *Leucothrix* are shown in Fig. 2. With *L. thiophila* 2WS, the concentration of thiosulfate decreased linearly with time for the first three days. In this process, tetrathionate (as the major product) and sulfate accumulated in the medium and elemental sulfur accumulated in cells; the product ratio was 10 : 1.5 : 1. The accumulation of biomass in strains 2WS and 1WS occurred at the same rate; the total protein never exceeded 30–35 mg protein/l. It should be noted that the rate of thiosulfate oxidation by *L. thiophila* 2WS was about two times higher than that shown by cluster II bacteria (strain 1WS). The recorded time courses of the oxidation of tetrathionate and accumulation of its products are shown in Fig. 3. The oxidation of tetrathionate was accompanied by the accumulation of elemental sulfur and sulfates in a 1 : 1 ratio. The accumulation of cell biomass over the test period was similar in the two strains and amounted to 40 mg protein/l on average. The same rates of tetrathionate oxidation were shown by the studied strains of both clusters, equaling 3 mg $S/S_4O_6^{2-}$ per mg protein over the entire experimental period; this value is about half of the oxidation rate of thiosulfate.

Effect of respiration inhibitors. In order to find out the connection between the oxidation processes of sulfur compounds and energy metabolism, we studied how the respiration rates were affected by inhibitors acting at different sites of the electron-transport chain. The polarographically measured respiration rates of cell suspensions in the presence of sulfite are presented in Table 1. The respiration of both groups of bacteria in the presence of sulfite was totally blocked by antimycin A (affecting the cytochrome *b*–cytochrome *c* site) and by HQNO (2-*n*-heptyl-4-hydroxyquinoline-*N*-oxide affecting the flavin–quinone–cytochrome *b* site), inhibited by 87.5% in cluster II and by 74% in cluster I bacteria by myxothiazol (an inhibitor of electron transfer via the cytochrome *b*–cytochrome *c* site), and totally blocked in cluster I bacteria and inhibited by 67% in cluster II bacteria by NEM (*N*-ethylmaleimide). The latter fact suggests the occurrence of sulfhydryl groups in the active center of the corresponding enzyme. The results of this inhibitory analysis indicate that, during sulfite oxidation, electrons enter the transport chain at the flavin–quinone–cytochrome *b* site. The observed connection between the process of sulfite oxidation and the operation of the electron-transport chain in bacteria of both clusters suggests that ATP may be synthesized by means of oxidative phosphorylation.

Activity of enzymes involved in the transformation of sulfur compounds. The results of activity measurements for enzymes participating in the oxidation of inorganic sulfur compounds are given in Table 2. The following enzymes involved in the oxidative metabolism of sulfur compounds were found in the bacteria studied: thiosulfate–ferricyanide oxidoreductase, thiosulfate–cytochrome *c* oxidoreductase, sulfite–ferricya-

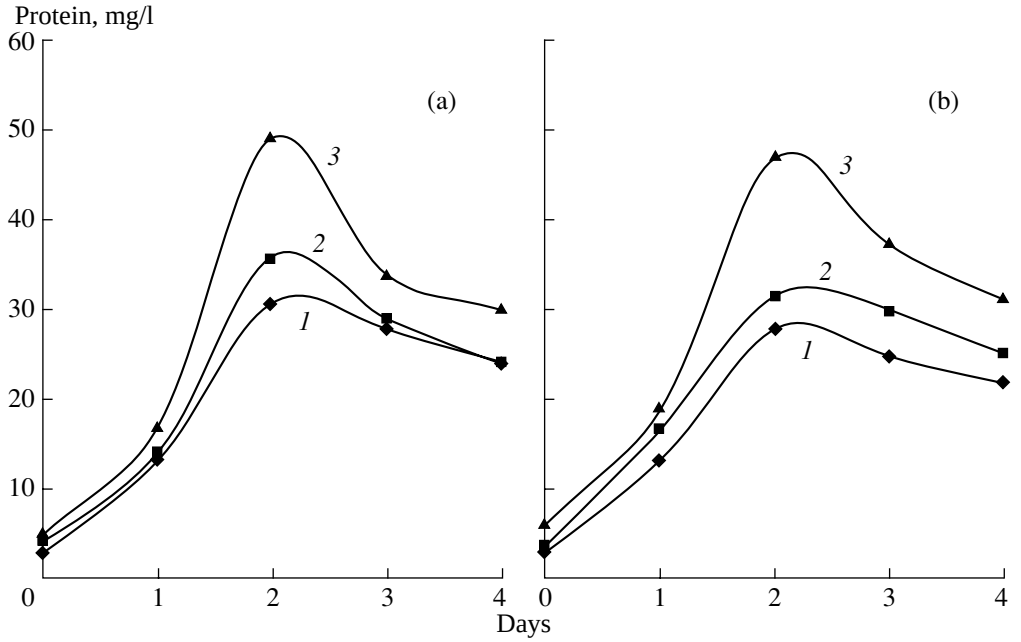

Fig. 1. Effect of thiosulfate on the growth of different strains of the genus *Leucothrix*: (a) *L. thiophila* 2WS and (b) *Leucothrix* sp. 1WS. The lactate concentration in the media was 0.5 g/l. Growth in (*1*) medium without thiosulfate, (*2*) medium with 1 g/l thiosulfate, and (*3*) medium with 1 g/l tetrathionate.

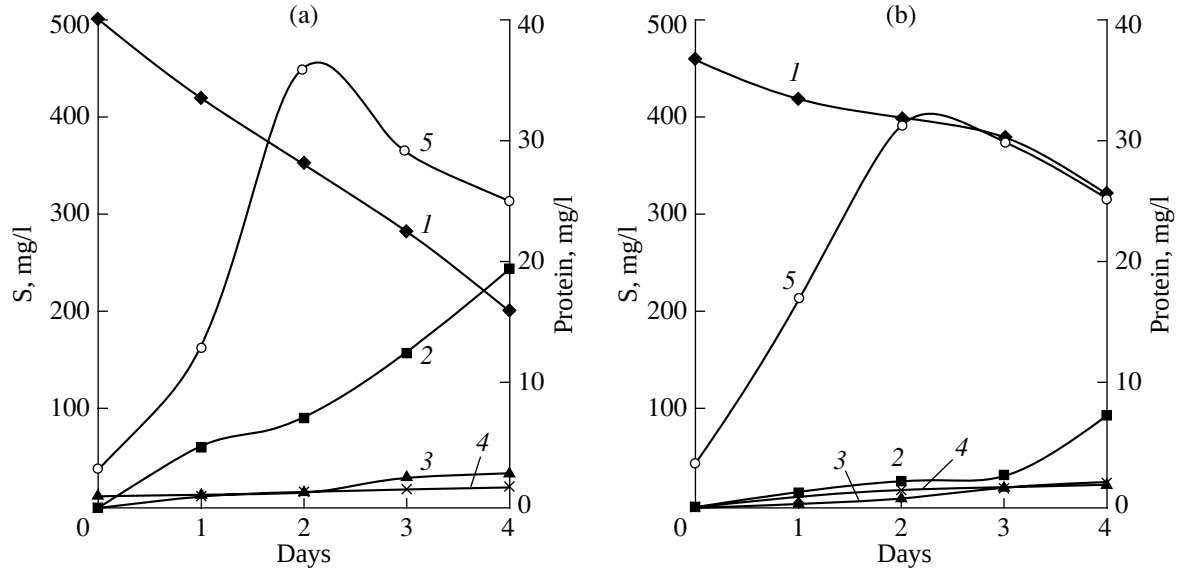

Fig. 2. Oxidation of thiosulfate and accumulation of its oxidation products by strains (a) 2WS and (b) 1WS: (*1*) thiosulfate (S of $S_2O_3^{2-}$); (*2*) tetrathionate (S of $S_4O_6^{2-}$); (*3*) sulfate (S of SO_4^{2-}); (*4*) elemental sulfur (S^0); (*5*) protein.

nide oxidoreductase, sulfite–cytochrome *c*-oxidoreductase, APS reductase, tetrathionate–ferricyanide oxidoreductase, tetrathionate–cytochrome *c* oxidoreductase, sulfur oxygenase, thiosulfate-cleaving complex, and rhodanese (Table 2). The specific activities of these enzymes in the strains studied were comparable to those exhibited by the known sulfur-oxidizing chemolithotrophic bacteria [16]. In cluster I bacteria, the activity of enzymes was higher by a factor of 1.5 to 3 than in cluster II bacteria except for the thiosulfate-cleaving complex, the activity of which in cluster I bacteria was 1.5- to 2-fold lower than in cluster II bacteria. No activity of APS reductase was found in cluster II bacteria, whereas, in cluster I bacteria, the activity of this enzyme was very high. This suggests that the oxidation of sulfite to sulfate in cluster I bacteria may be coupled to substrate-level phosphorylation in addition to oxidative phosphorylation.

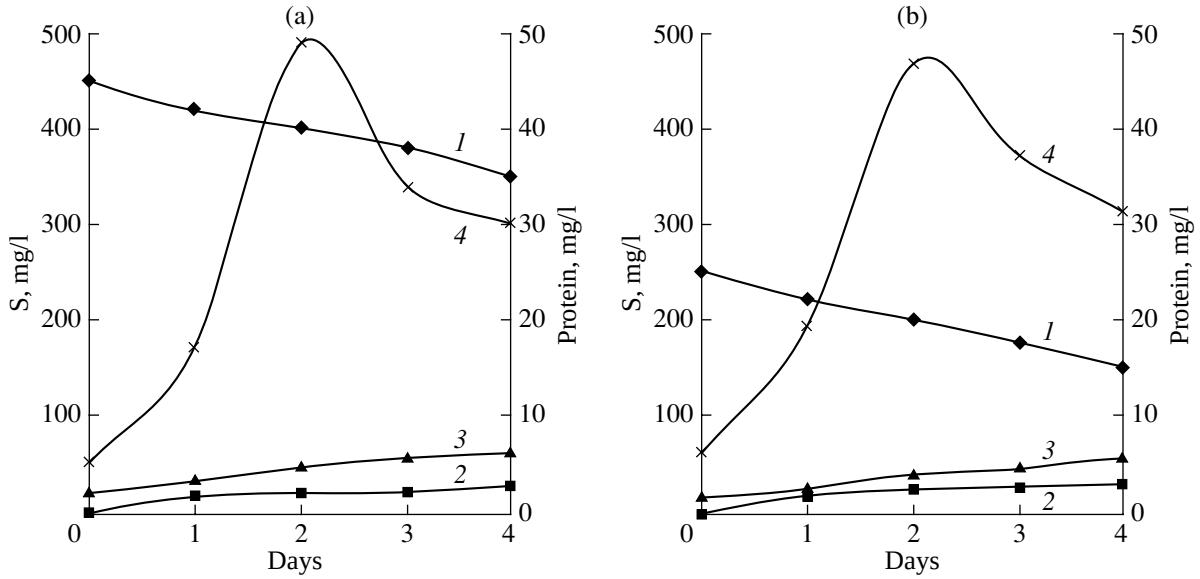

Fig. 3. Oxidation of tetrathionate and accumulation of its oxidation products by strains (a) 2WS and (b) 1WS: (*1*) tetrathionate (S of $S_4O_6^{2-}$); (*2*) elemental sulfur (S^0); (*3*) sulfate (S of SO_4^{2-}); (*4*) protein.

Microscopic examinations of bacteria with intracellular inclusions of elemental sulfur (strains 1WS–6WS) showed that elemental sulfur gradually disappeared from cells when these bacteria were cultured on a medium containing no reduced sulfur compounds. Cells of all strains were shown to contain sulfur oxygenase. At the same time, sulfur reductase was not detected. These finding suggest that elemental sulfur stored in cells is eventually utilized in oxidative metabolism.

Carbon metabolism. All studied strains grown under organotrophic conditions were found to possess all the enzymes of the TCA and glyoxylate cycles (Table 3). The activities of hydratases—aconitate hydratase and fumarate hydratase—were comparable to those in other heterotrophic bacteria. The activities of dehydrogenases—isocitrate dehydrogenase, succinate dehydrogenase, and malate dehydrogenase—in cluster I bacteria were 5- to 10-fold lower than in *E. coli*. In cluster II strains, the activity level of malate dehydrogenase was comparable to that in *E. coli.* The activities of aconitate hydratase, succinate dehydrogenase, and malate dehydrogenase in cluster II bacteria were on average 4 times higher than the enzymatic activities in *L. thiophila.*

The effect of thiosulfate on the activities of enzymes of the TCA and glyoxylate cycles in bacteria of both clusters is illustrated in Table 3. The activities of the

Table 1. Effect of inhibitors on sulfite-dependent respiration in cell suspensions of different strains of the genus *Leucothrix*

Leucothrix strains	Rate of oxygen uptake, nmol/(min mg protein)*	Inhibitor concentration, μM	Rate of oxygen uptake in the presence of inhibitor, nmol/(min mg protein)*	Inhibition, %
L. thiophila 2WS	110.0	Antimycin A (20)	0.0	100
	110.0	Myxothiazol A (25)	28.9	74
	578.0	NEM (50)	0.0	100
	289.0	HQNO (30)	0.0	100
Leucothrix sp. 5WS	731.0	Antimycin A (20)	0.0	100
	731.0	Myxothiazol (25)	81.0	88
	487.0	NEM (50)	163.0	67
	650.0	HQNO (30)	0.0	100

Note: Bacterial biomass for these experiments was grown on medium with thiosulfate. The concentration of sulfite in the measuring cell was 10 μmol/ml.

*The values given are rates of oxygen uptake adjusted for chemical oxidation of SO_3^{2-} (control 1) and endogenous respiration (control 2).

Table 2. Activities of enzymes involved in the conversion of sulfur compounds in the course of bacterial growth on medium with thiosulfate (nmol/(min mg protein))

Enzymes		Cluster I (*L. thiophila* strains)			Cluster II (*Leucothrix* sp. strains)		
		2WS	4WS	6WS	1WS	3WS	5WS
Thiosulfate oxidoreductase	a	65.0	64.0	54.0	40.0	34.0	50.0
	b	15.0	7.0	12.0	5.0	3.0	4.0
Sulfite oxidoreductase	a	0	0	0	9.6	9.7	22
	b	3.0	2.0	6.0	2.0	6.7	3.0
APS reductase		94.0	107.0	100.0	0.0	0.0	0.0
Rhodanese		33.0	30.0	25.0	20.0	21.0	18.0
Sulfur oxygenase		61.0	66.0	66.0	40.0	44.0	38.0
Thiosulfate-cleaving complex		270	260	100	390	540	520
Tetrathionate oxidoreductase*	a	28.0	22.0	29.0	8.0	7.0	13.0
	b	3.0	1.5	4.1	1.2	1.3	1.9

Note: The acceptors in enzymatic reactions were (a) ferricyanide and (b) cytochrome *c*.
* Growth with tetrathionate.

Table 3. Effect of thiosulfate on the activities of enzymes of the TCA and glyoxylate cycles in cells of *L. thiophila* and *Leucothrix* sp. (nmol/(min mg protein)

Enzymes	$-S_2O_3^{2-}$	$+S_2O_3^{2-}$	$-S_2O_3^{2-}$	$+S_2O_3^{2-}$	$-S_2O_3^{2-}$	$+S_2O_3^{2-}$
	L. thiophila 2WS		*Leucothrix* sp. 1WS		*E. coli* VKM B-12	
Aconitate hydratase	37.0	10.0	80.0	55.0	90.0	ND
Fumarate hydratase	104.0	36.0	90.0	92.0	410.0	ND
Isocitrate dehydrogenase (NAD-dependent)	51.0	9.0	14.0	7.0	ND	ND
Isocitrate dehydrogenase (NADP-dependent)	16.0	1.8	10.0	3.0	160.0	145.0
Succinate dehydrogenase	6.4	1.8	30.0	7.4	420.0	400.0
Malate dehydrogenase	51.0	14.0	250.0	160.0	210.0	200.0
2-Oxoglutarate dehydrogenase	ND	ND	130.0	77.0	10.0	ND
Citrate synthase	7.0	5.8	9.7	3.1	ND	ND
Malate synthase	7.0	15.8	6.3	10.8	ND	ND
Isocitrate lyase	20.0	35.0	3.4	8.5	130.0	ND

Note: ND means "not determined." Enzyme activities were determined for all six strains of both clusters but given here are only the data for the two representative strains 1WS and 2WS.

TCA cycle dehydrogenases, such as succinate dehydrogenase, malate dehydrogenase, 2-oxoglutarate dehydrogenase, and isocitrate dehydrogenase, and the activity of citrate synthase in bacteria of both clusters cultured in the presence of thiosulfate decreased 2- to 5-fold and, in some cases, by an order of magnitude as compared to the activities observed after chemoorganoheterotrophic growth. In *E. coli*, the activity of dehydrogenases was not affected by the presence of thiosulfate in the medium. It should be however noted that the activities of aconitate hydratase and fumarate hydratase decreased in the presence of thiosulfate (by a factor of 2 to 4) only in cluster I bacteria, while the activity level of cluster II bacteria remained unchanged. The activities of the key enzymes of the glyoxylate cycle were also affected by the presence of thiosulfate in the medium. Thus, the activities of malate synthase and isocitrate lyase in representatives of both clusters increased twofold in comparison to chemoorganoheterotrophic growth. Our investigation of the effect of thiosulfate on the bacterial metabolism showed that representatives of the genus *Leucothrix* were able to

Table 4. Effect of the growth substrate concentration on activities of the key enzymes of the TCA cycle and sulfur metabolism

Enzymes	Enzyme activity (nmol/(min mg protein))			
	Cluster I (2WS)		Cluster II (1WS)	
	Concentration of organic substrate in the medium			
	a	b	a	b
Aconitate hydratase	25.0	17.0	63.0	57.0
Fumarate hydratase	80.0	57.0	85.0	77.0
Isocitrate dehydrogenase (NAD)	20.0	9.0	11.0	8.0
Isocitrate dehydrogenase (NADP)	10.2	9.8	16.0	12.0
Malate dehydrogenase (NAD)	22.0	7.5	80.0	60.0
Thiosulfate–cytochrome *c* oxidoreductase	12.0	18.0	3.7	17.0
Sulfite–cytochrome *c* oxidoreductase	6.0	15.0	3.0	25.0

Note: The growth substrate was a mixture of acetate and lactate at concentrations of (a) 4 mM each and (b) 0.8 mM each.

change their oxidative metabolism in response to the presence of inorganic electron donors. Specifically, when bacteria were cultured on thiosulfate, the importance of the TCA cycle in their energy metabolism declined and the glyoxylate cycle became effective in providing intermediates for the anabolic reactions.

We also studied the effect of lactate and acetate concentration in the medium on the activities of some enzymes of the TCA cycle and sulfur metabolism. A decrease in the concentration of the organic nutrient in the medium from 4 to 0.8 mM was found to reduce the activities of the TCA cycle enzymes and to concurrently increase the activities of sulfur metabolism enzymes by a factor of 1.5 to 3 in representatives of both clusters (Table 4). The shortage of organic nutrients limits the cell yield and switches on the mechanisms of dissimilatory oxidation of reduced sulfur compounds as an additional energy substrate.

DISCUSSION

Our investigation of the sulfur and carbon metabolism of new strains of *Leucothrix* revealed that, in addition to chemoorganoheterotrophic metabolism, these bacteria are able to carry out chemolithoheterotrophic metabolism in the presence of reduced sulfur compounds. The variation of the enzymatic activities of strains within each cluster was insignificant. Distinctions in the carbon and sulfur metabolism were found between bacteria assigned to two different clusters on genotypic evidence, specifically, on the basis of DNA homology. These distinctions consist in different rates of utilization of sulfur and carbon compounds for energy metabolism and in how the metabolism of sulfur is controlled depending on the presence of an inorganic energy source in the medium and on the concentration of the organic substrate utilized. In cluster II bacteria, the activity of dehydrogenases supplying reducing equivalents from organic compounds to the electron-transport chain (irrespective of the presence of reduced sulfur compounds) is significantly higher than in cluster I bacteria. The latter fact suggests that cluster II bacteria are able to use organic compounds in their energy metabolism more efficiently than cluster I bacteria. At the same time, in the latter bacteria, the activity of dissimilatory enzymes involved in the transformation of sulfur compounds is 1.5 to 3 times higher than in cluster II bacteria. The results of our inhibitory analysis of the electron-transport system and the analysis of the enzyme activities suggest that during sulfite oxidation by cluster I bacteria, ATP can be synthesized both via oxidative and substrate-level phosphorylation. In cluster II bacteria, this occurs only by means of oxidative phosphorylation. It can be argued, therefore, that the ability to utilize reduced sulfur compounds for the energy metabolism is more pronounced in cluster I bacteria than in cluster II bacteria.

A peculiar feature of the carbon and sulfur metabolism in the strains of the two clusters of new bacteria of the genus *Leucothrix*, distinguishing them from the type species of the genus *L. mucor* DSM 2157, is their capacity to utilize reduced sulfur compounds in the energy metabolism irrespective of the concentration of organic compounds in the medium, which affects only the utilization rate of inorganic sulfur compounds. At the same time, *L. mucor* DSM 2157 can utilize sulfur compounds in its energy metabolism only when the concentrations of organic compounds are low [4]. This metabolic feature can be explained by the fact that the activity of the TCA cycle dehydrogenases in strains of both clusters is an order of magnitude lower than in *L. mucor* [17] and in typical heterotrophs, such as *E. coli.* Because of the low activity of dehydrogenases, cells of the bacteria of both clusters may fail to be fully supplied with reducing equivalents and, in addition to organic compounds, have to use inorganic sulfur compounds as donors of electrons for their energy metabolism. In *L. mucor* DSM 2157, the activity of the TCA

cycle enzymes is fairly high and, therefore, sulfur compounds begin to be used in the energy metabolism only when bacterial cells lack a sufficient supply of an organic substrate [4] to sustain their constructive and energy metabolism.

Our comparative study of metabolism of new *Leucothrix* strains and *L. mucor* DSM 2157 shows that physiological and biochemical distinctions between strains of *L. thiophila*, *Leucothrix* sp., and *L. mucor* are in fairly good correlation with the species-level genetic differences between these bacteria.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research (project nos. 99-04-48686 and 99-04-48707) and by the Federal Research and Development Program "Biodiversity 2.1.2."

REFERENCES

1. Brock, T.D., The Genus *Leucothrix*, *The Procaryotes*, 1992, pp. 3247–3255.
2. Dul'tseva, N.M., Dubinina, G.A., and Lysenko, A.N., Isolation of Marine Filamentous Sulfur Bacteria and Description of the New Species *Leucothrix thiophila* sp. nov., *Mikrobiologiya*, 1996, vol. 65, no. 1, pp. 89–98.
3. Dul'tseva, N.M., Taxonomy and Physiology of New Filamentous Sulfur Bacteria, *Cand. Sci. (Biol.) Dissertation*, Moscow, 1996.
4. Grabovich, M.Y., Muntyan, M.S., Lebedeva, V.Y., Ustiyan, V.S., and Dubinina, G.A., Lithoheterotrophic Growth and Electron Transfer Chain Components of the Filamentous Gliding Bacterium *Leucothrix mucor* DSM 2157 during Oxidation of Sulfur Compounds, *FEMS Microbiol. Lett.*, 1999, vol. 178, pp. 155–161.
5. Grabovich, M.Yu., Dubinina, G.A., Dul'tseva, N.M., and Churikova, V.V., Peculiarities of Carbon Metabolism in the Filamentous Sulfur Bacteria *Thiothrix arctophila* and *Leucothrix thiophila* during Chemolitho- and Chemoorganoheterotrophic Growth, *Mikrobiologiya*, 1996, vol. 65, no. 2, pp. 149–153.
6. Hyllemon, P.B., Wells, J.S., Krieg, N.R., and Jannasch, H.W., Genus *Spirillum*: A Taxonomic Study, *Int. J. Syst. Bacteriol.*, 1973, vol. 23, pp. 340–380.
7. Pfennig, N. and Lippert, K.D., Uber das Vitamin B_{12}-Bedurfnis phototrophes Schwefelbakterien, *Arch. Mikrobiol.*, 1966, vol. 55, pp. 245–256.
8. Grabovich, M.Yu., Dubinina, G.A., Churikova, V.V., Glushkov, A.F., and Churikov, S.N., Peculiarities of Carbon Metabolism in the Colorless Sulfur Bacterium *Macromonas bipunctata*, *Mikrobiologiya*, 1993, vol. 62, no. 3, pp. 421–429.
9. Petushkova, Yu.P. and Ivanovskii, R.N., Enzymes Involved in the Metabolism of Thiosulfate in *Thiocapsa roseopersicina* Grown under Various Conditions, *Mikrobiologiya*, 1976, vol. 45, no. 4, pp. 960–965.
10. Grabovich, M.Yu., A Study of the Systematics, Physiology, and Peculiarities of Sulfur Metabolism of Colorless Sulfur Bacteria, *Cand. Sci. (Biol.) Dissertation*, Moscow, 1984.
11. Reznikov, A.A., Mulikovskaya, E.P., and Sokolov, I.Yu., *Metody analiza prirodnykh vod* (Methods for the Analysis of Natural Waters), Moscow: Gosgeoltekhizdat, 1963, pp. 97–103.
12. Kelly, D.P., Chambers, I.A., and Trudinger, P.A., Cyanolysis and Spectrophotometric Estimation of Trithionate in Mixture with Thiosulfate and Tetrathionate, *Anal. Chem.*, 1969, vol. 41, p. 898.
13. Morris, H.E., Lakombe, R.E., and Lane, W.H., Qualitative Determination of Elemental Sulfur in Aromatic Hydrocarbons, *Anal. Chem.*, 1948, vol. 20, pp. 1037–1039.
14. Williams, W.J., *Handbook of Anion Determination*, London: Butteworth, 1979.
15. Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, A.J., Protein Measurement with the Folin Phenol Reagent, *J. Biol. Chem.*, 1951, vol. 193, pp. 265–275.
16. Kelly, D.P., Physiology and Biochemistry of Unicellular Sulfur Bacteria, *Autotrophic Bacteria*, Schlegel, H.G. and Bowien, B., Eds., Madison: Science Tech. Publ., 1989, pp. 193–217.
17. Grabovich, M.Yu. and Mishankova, V.V., Ecologo-Biochemical Peculiarities of the Colorless Filamentous Sulfur Bacterium *Leucothrix mucor, II otkrytaya nauchnaya konferentsiya molodykh uchenykh goroda Pushchino. Tez. dokl.* (II Open Scientific Conference of the Young Researchers of the Town of Pushchino: Abstracts of Papers), 1997, pp. 165–166.