Microbiology, Vol. 74, No. 3, 2005, pp. 258–264. Translated from Mikrobiologiya, Vol. 74, No. 3, 2005, pp. 305–312.

EXPERIMENTAL ARTICLES

Carbon Metabolism of Filamentous Anoxygenic Phototrophic Bacteria of the Family *Oscillochloridaceae*

I. A. Berg[1], O. I. Keppen, E. N. Krasil'nikova, N. V. Ugol'kova, and R. N. Ivanovsky

Microbiology Department, Biological Faculty, Moscow State University, Vorob'evy gory, Moscow, 119899 Russia

Received June 17, 2004

Abstract—The carbon metabolism of representatives of the family *Oscillochloridaceae* (*Oscillochloris trichoides* DG6 and the recent isolates *Oscillochloris* sp. R, KR, and BM) has been studied. Based on data from an inhibitory analysis of autotrophic CO_2 assimilation and measurements of the activities of the enzymes involved in this process, it is concluded that, in all *Oscillochloris* strains, CO_2 fixation occurs via the operation of the Calvin cycle. Phosphoenolpyruvate (PEP), which is formed in this cycle, can be involved in the metabolism via the following reaction sequence: PEP (+CO_2) ⟶ oxalacetate ⟶ malate ⟶ fumarate ⟶ succinate ⟶ succinyl-CoA (+CO_2) ⟶ 2-oxoglutarate. Acetate, utilized as an additional carbon source, can be carboxylated to pyruvate by pyruvate synthase and further involved in the metabolism via the above reaction sequence. Propionyl-CoA synthase and malonyl-CoA reductase, the key enzymes of the 3-hydroxypropionate cycle, have not been detected in *Oscillochloris* representatives.

Key words: phototrophic bacteria, *Chloroflexaceae*, carbon metabolism, ribulose-1,5-bisphosphate carboxylase, carboxylation reactions.

The group of filamentous anoxygenic phototrophic bacteria (FAPB) includes representatives of the genera *Chloroflexus, Oscillochloris, Chloronema, Heliothrix*, and *Roseiflexus* [1, 2]. These microorganisms are multicellular; specifically, they form trichomes. Among FAPB, there are both thermophiles (*Chloroflexus, Heliothrix*, and *Roseiflexus*) and mesophiles (*Oscillochloris* and *Chloronema*). Phylogenetically, this bacterial group is remote from other lineages of phototrophs. According to the latest edition of *Bergey's Manual*, FAPB make up the order *Chloroflexales*, which belongs, together with chemotrophic bacteria of the order *Herpetosiphonales*, to the phylum BVI *Chloroflexi* [2]. Until recently, all FAPB were assigned to the family *Chloroflexaceae.* Currently, based on the results of studies of their phenotypic and genotypic properties, representatives of the genus *Oscillochloris* are assigned to the new family *Oscillochloridaceae* [3]. A phylogenetic study of an enrichment culture of a FAPB identified as "*Chloronema giganteum*" showed that, according to its 16S rRNA sequence, this bacterium falls into a cluster comprising representatives of the family *Oscillochloridaceae* [4]. The recently described "Candidatus *Chlorothrix halophila*," an isolate from a hypersaline microbial mat, belongs, in phylogenetic terms, to the phylogenetic lineage that includes *Chloroflexus* and *Oscillochloris* [5]. *Roseiflexus* is phylogenetically remote from other representatives of the group and can be considered as a separate lineage of FAPB [1].

Among FAPB, only *Chloroflexus, Oscillochloris*, and *Roseiflexus* representatives have been isolated in pure cultures. The capacity for autotrophic growth was shown for *Chloroflexus* and *Oscillochloris*, as well as for "Candidatus *Chlorothrix halophila*." The carbon metabolism was studied only for several strains of *C. aurantiacus* and a single strain of *O. trichoides* (DG6). In *C. aurantiacus*, novel pathways of autotrophic CO_2 fixation were found: the reductive dicarbonic acid cycle [6, 7] and the 3-hydroxypropionate cycle [8]. Despite its autotrophic capacity, *C. aurantiacus* prefers photoheterotrophic conditions; both in the light and in the dark, aerobically or anaerobically, it can utilize a wide range of organic compounds as electron donors and sources of carbon. This diversity of anaerobic metabolism pathways is provided for by the presence of a complete tricarboxylic acid cycle (TCA cycle) and the glyoxylate shunt [9].

Data on the carbon metabolism of "Candidatus *Chlorothrix halophila*" are scarce. Since the activity of propionyl-CoA synthase, the key enzyme of the 3-hydroxypropionate cycle, has not been revealed, it is

[1] Corresponding author; e-mail: ivan_berg@mail.ru

0026-2617/05/7403-0258

thought that this cycle is not involved in autotrophic CO_2 assimilation by this organism [5].

As distinct from *C. aurantiacus*, the autotrophic assimilation of CO_2 in *O. trichoides* DG6 occurs via the Calvin cycle [10]. This is an anaerobic photolithotrophic bacterium that can utilize hydrogen sulfide or molecular hydrogen as electron donors and acetate and pyruvate as additional carbon sources [3]. The TCA cycle is incomplete due to the absence of 2-oxoglutarate dehydrogenase, and, among the glyoxylate shunt enzymes, only malate synthase is synthesized [10]. The carbon metabolism of *O. trichoides* SR1 and *O. chrysea* (cultures now lost) was probably different from that of *O. trichoides* DG6. These bacteria were found to be capable of chemotrophic growth under aerobic and microaerobic conditions [11, 12]; however, their phylogenetic position has not been elucidated.

Thus, although only a few strains of FAPB have been studied, there are grounds to speculate on the high diversity of the carbon metabolism pathways in this group, and further investigation of these pathways in various FAPB representatives is topical area of research. In our laboratory, several new *Oscillochloris* strains were earlier isolated from different habitats, namely, strains R, BM, and KR, which were close to strain DG6 according to the results of phylogenetic analysis of their 16S rRNA genes [3]. The aim of the present work was to study the carbon metabolism of *Oscillochloris* representatives (*O. trichoides* DG6, which is the neotype strain of the species, and the new strains *Oscillochloris* sp. R, KR, and BM) in more detail and to compare it with the carbon metabolism of *C. aurantiacus.*

MATERIALS AND METHODS

Bacteria and cultivation conditions. The subjects under study were four mesophilic FAPB strains of the family *Oscillochloridaceae. O. trichoides* DG6, the neotype strain, was isolated from a hydrogen sulfide spring in the Caucasus; strains *Oscillochloris* sp. R and KR were isolated from water bodies near Moscow, and strain *Oscillochloris* sp. BM was isolated from near-coastal sediments of the White Sea. The thermophilic bacterium *C. aurantiacus* OK-70fl was used for comparative studies. *Oscillochloris* cultures were grown on a modified DGN medium [13] with 0.1% acetate, 0.1% bicarbonate, and 0.07% sulfide. *C. aurantiacus* was grown on the same medium but with molecular hydrogen (autotrophic conditions). Cultivation was carried out anaerobically in light (1000 lx) in magnetically stirred 500-ml flasks with screw caps. The cultivation temperature was 28–30°C for the mesophiles and 55°C for the thermophiles.

Studies of labeled substrate assimilation. Biomass was sedimented by centrifugation, washed with a mineral medium, and resuspended at a density of 100–200 μg of protein/ml. Experiments on the assimilation of $NaH^{14}CO_3$ and [2-^{14}C]acetate by the cell suspensions were carried out in syringes in light (2000 lx). The reaction was initiated by the addition of $NaH^{14}CO_3$ (5 mM, 0.04 MBq) or [2-^{14}C]acetate (10 mM, 0.02 MBq) and terminated by filtering, after certain periods of time, 1 ml of the cell suspension through nitrocellulose filters (0.45 μm). The filters were dried, and their radioactivity was measured in an LKB RacBeta 1127 liquid scintillation counter.

Determination of the rate of $^{14}CO_2$ formation from labeled acetate was performed in flasks with appendages that contained 0.5 ml of an alkaline trapping solution (10% triethanolamine). The experiments were carried out anaerobically in light (2000 lx) as described above. The air in the flasks was replaced with molecular nitrogen. The reaction was terminated after 60 min of incubation by the addition of 0.5 ml of 10% trichloroacetic acid. The radioactivity of the CO_2 absorbed by the trapping solution was measured after 24 h of further incubation at room temperature.

Preparation of cell extracts. The cells were washed with a 50 mM Tris–HCl buffer (pH 7.8), resuspended in 5–8 ml of a buffer appropriate for the determination of a given enzyme, and disrupted either ultrasonically (22 kHz, 3 min, 4°C) or in an X press (excessive pressure of 7000–10000 kg). Undisrupted cells and large cell fragments were sedimented by centrifugation (40000 g, 20 min, 4°C) and discarded, and the supernatant was used for enzymatic studies. The extracts intended for anaerobic determinations of enzyme activities were obtained in an X press sparged with nitrogen. The extracts intended for pyruvate kinase determination were obtained at room temperature.

The determination of enzyme activities was performed at room temperature in the mesophilic *Oscillochloris* strains and at 55°C in *C. aurantiacus.* The reaction mixture contained 0.2–1.5 mg of protein/ml.

Enzymes of the TCA cycle and glyoxylate shunt were assayed spectrophotometrically using standard methods [10].

Malonyl-CoA reductase was determined spectrophotometrically from the malonyl-CoA-dependent oxidation of NADPH [8]. The activity was expressed in nmol of malonyl-CoA reduced per min by 1 mg of protein (the reduction of 1 mole of malonyl-CoA requires 2 moles of NADPH).

Propionyl-CoA synthase was determined spectrophotometrically from the 3-hydroxypropionate-dependent oxidation of NADPH [8, 14].

ATP-citrate lyase was determined spectrophotometrically from NADH oxidation in the presence of malate dehydrogenase in a reaction mixture containing 50 mM Tris–HCl (pH 8.5), 5 mM dithiothreitol, 4 mM $MgCl_2$, 0.2 mM NADH, 0.1 mM CoA, 5 mM citrate, 1 mM ATP, and 0.5 U of malate dehydrogenase.

Pyruvate kinase was determined spectrophotometrically from NADH oxidation in the presence of lactate dehydrogenase in a reaction mixture containing 50 mM

Tris–HCl (pH 7.5), 30 mM $MgSO_4$, 0.25 mM NADH, 5 mM phosphoenolpyruvate (PEP), 10 mM ADP, and 5 U of lactate dehydrogenase.

Pyruvate–phosphate dikinase was determined spectrophotometrically from the oxidation of NADH in the presence of lactate dehydrogenase in a reaction mixture containing 100 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 0.25 mM NADH, 1 mM PEP, 1 mM AMP, 50 mM NH_4Cl, 0.5 mM pyrophosphate, and 5 U of lactate dehydrogenase.

Pyruvate–water dikinase was determined spectrophotometrically from the oxidation of NADH in the presence of lactate dehydrogenase in a reaction mixture containing 100 mM Tris–HCl (pH 7.5), 5 mM $MgCl_2$, 0.25 mM NADH, 1 mM PEP, 1 mM AMP, 2 mM KH_2PO_4, and 5 U of lactate dehydrogenase.

Ribulose-1,5-bisphosphate carboxylase was determined radiochemically from the ribulose-1,5-bisphosphate-dependent fixation of CO_2 [10].

Pyruvate synthase and 2-oxoglutarate synthase were determined spectrophotometrically from the reduction of methyl viologen under strictly anaerobic conditions, as described in [15].

Propionyl-CoA carboxylase was determined radiochemically from the propionyl-CoA-dependent fixation of CO_2 [16].

Pyruvate carboxylase was determined using the same method as propionyl-CoA carboxylase; however, pyruvate (80 mM) was substituted for propionyl-CoA [10].

Phosphoenolpyruvate (PEP) carboxylase, PEP carboxykinase, and PEP carboxytransphosphorylase were determined radiochemically according to the method described in [10]. When GDP-dependent PEP carboxykinase was determined, the reaction mixture contained 5 mM GDP instead of ADP.

Protein determination. Protein was determined by the Lowry method; bovine serum albumin was used as the standard.

RESULTS AND DISCUSSION

Autotrophic fixation of CO_2. All of the *Oscillochloris* strains that we studied are able to grow photoautotrophically. However, as was reported earlier, photoheterotrophic growth on acetate or pyruvate is much better [3]. In *Oscillochloris* sp. R, KR, and BM, as well as in *O. trichoides* DG6, autotrophic fixation of CO_2 was about 80% inhibited by cyanide (Table 1), which is known to inhibit ribulose-1,5-bisphosphate carboxylase [10]. Another Calvin cycle inhibitor, iodoacetate, which acts at the level of glyceraldehyde phosphate dehydrogenase [17], also inhibited CO_2 fixation, although to a lesser extent than cyanide (Table 1). In *O. trichoides* DG6 and *Oscillochloris* sp. R, we demonstrated the presence of ribulose-1,5-bisphosphate carboxylase, the key enzyme of the Calvin cycle (Table 2). The activity of this enzyme was found to be the same under aerobic and anaerobic conditions of determination.

The data obtained indicate that, in *Oscillochloris* sp. R, KR, and BM, the autotrophic fixation of CO_2 occurs via the Calvin cycle; thus, this pathway of CO_2 fixation is universal for all of the *Oscillochloridaceae* representatives studied so far. As was previously assumed [6, 8, 10], in this feature, they differ from *C. aurantiacus* (Tables 1, 2).

The 3-hydroxypropionate cycle is thought to be in operation in the case of *C. aurantiacus* OK-70fl; its key enzymes are malonyl-CoA reductase and propionyl-CoA synthase [8, 14]. We failed to reveal the activity of these enzymes in *O. trichoides* DG6 and *Oscillochloris* sp. R, whereas, in *C. aurantiacus* OK-70fl, it was easily detectable (Table 2).

In the cell extracts of the *Oscillochloris* strains, we found low propionyl-CoA carboxylase activity (Table 2). This enzyme catalyzes one of the carboxylation reactions of the 3-hydroxypropionate cycle. In *Oscillochloris* strains, as in most other organisms, it is apparently involved in fatty acid metabolism rather than in the autotrophic assimilation of CO_2.

No activity of ATP-citrate lyase, which is the key enzyme of the reductive TCA cycle operative in green sulfur bacteria and some chemotrophic bacteria, was detected by us in *O. trichoides* DG6 and *Oscillochloris* sp. R (Table 2).

Metabolism of PEP and pyruvate in bacteria of the genus *Oscillochloris*. In all of the *Oscillochloris* strains studied, several PEP-carboxylating enzymes were found: PEP carboxylase and GDP-dependent PEP carboxykinase, as well as low PEP carboxytransphosphorylase activity (Table 2). In *Oscillochloris* sp. R, pyruvate carboxylase was also found (Table 2). In cells grown in the presence of acetate, the activity of these enzymes was somewhat higher than in cells grown autotrophically. This fact can be explained by the involvement of these enzymes in the assimilation of both PEP formed in the Calvin cycle and pyruvate produced from acetate in the pyruvate synthase reaction (see below). Commonly, PEP carboxylase and PEP carboxytransphosphorylase are thought of as enzymes that carboxylate PEP, whereas their usual in vivo function is oxalacetate decarboxylation [18]. The pyruvate–PEP conversions can be catalyzed by two enzymes found in the cell extracts of *O. trichoides* DG6 and *Oscillochloris* sp. R: pyruvate kinase and pyruvate–phosphate dikinase (Table 2).

The presence of enzymes catalyzing PEP carboxylation explains the pyruvate-induced stimulation of CO_2 fixation that was observed in the presence of the Calvin cycle inhibitors iodoacetate and cyanide (Table 1). Propionate had an analogous effect, which can be explained by the presence of propionyl-CoA carboxylase in the cell extracts of the *Oscillochloris* strains studied (Table 2).

As distinct from the *Oscillochloris* strains, *C. aurantiacus* possesses only PEP carboxylase, which catalyzes one of the carboxylation steps in the reductive dicarbonic acid cycle [7]. The activity of this enzyme

Table 1. The effect of various inhibitors and substrates on the fixation of [^{14}C]bicarbonate by the cells of *Oscillochloris* strains grown under autotrophic conditions

Medium with	Fixation rate, nmol/(min mg protein)			
	O. trichoides DG6	*Oscillochloris* sp. KR	*Oscillochloris* sp. R	*C. aurantiacus*
$*CO_2 + Na_2S$	12.0	14.6	15.3	17.0
$*CO_2 + Na_2S$ + KCN	2.1	3.2	4.7	14.9
$*CO_2 + Na_2S$ + KCN + acetate	2.2	2.4	1.8	ND
$*CO_2 + Na_2S$ + KCN + pyruvate	4.9	4.7	11.7	ND
$*CO_2 + Na_2S$ + KCN + propionate	7.2	5.1	12.3	ND
$*CO_2 + Na_2S$ + iodoacetate	4.5	8.4	6.3	ND
$*CO_2 + Na_2S$ + iodoacetate + acetate	2.3	8.7	7.0	ND
$*CO_2 + Na_2S$ + iodoacetate + pyruvate	8.3	18.4	13.3	ND
$*CO_2 + Na_2S$ + iodoacetate + propionate	9.6	11.1	19.0	ND

Note: The concentrations of acetate, pyruvate, propionate, and bicarbonate were 5 mM; the concentrations of iodoacetate, cyanide, and Na_2S were 10^{-5} M, 1 mM, and 0.05%, respectively. ND stands for "not determined". In the case of *C. aurantiacus*, molecular hydrogen was used as the electron donor instead of sulfide.

Table 2. The activity of enzymes (nmol/(min mg protein)) involved in the assimilation of CO_2 in the *O. trichoides* DG6, *Oscillochloris* sp. R, and *Chloroflexus aurantiacus* OK-70fl cells grown under autotrophic conditions or in an acetate-containing medium

Enzyme	*O. trichoides* DG6		*Oscillochloris* sp. R		*C. aurantiacus* OK-70fl
	$CO_2 + Na_2S$	Acetate + CO_2 + Na_2S	$CO_2 + Na_2S$	Acetate + CO_2 + Na_2S	$CO_2 + H_2$
Malonyl-CoA reductase	0	0	0	ND	30.7
Propionyl-CoA synthase	0	0	0	ND	34.1
Ribulose-1,5-bisphosphate carboxylase	1.9	1.4	3.6	1.2	0
ATP-citrate lyase	<0.5	ND	<0.5	<0.5	<0.5
Pyruvate synthase	95.2	135.1	149.3	365.7	174.9
2-Oxoglutarate synthase	8.5	12.6	3.8	12.0	<1.0
Propionyl-CoA carboxylase	2.0	12.3	2.4	0.5	11.7
Pyruvate carboxylase	<0.5	<0.5	<0.5	3.3	ND
PEP carboxylase	3.6	9.0	0.9	6.6	397.0
PEP carboxykinase (GDP)	5.2	6.6	4.2	15.7	ND
PEP carboxykinase (ADP)	<0.5	<0.5	<0.5	<0.5	0
PEP carboxytransphosphorylase	0.9	1.3	<0.5	0.7	0
Pyruvate kinase	5.1	ND	4.6	3.8	ND
Pyruvate–phosphate dikinase	3.3	ND	1.0	3.2	ND
Pyruvate–water dikinase	<1.0	ND	<1.0	<1.0	ND

Note: ND stands for "not determined".

was almost two orders of magnitude higher in *Chloroflexus* than it was in *Oscillochloris* (Table 2).

Acetate assimilation by bacteria of the genus *Oscillochloris*. As was mentioned above, acetate significantly stimulates the growth of all *Oscillochloris* strains. Earlier, we showed that in *O. trichoides* DG6, the TCA cycle is incomplete due to the absence of 2-oxoglutarate dehydrogenase and that malate synthase is the only synthesized enzyme of the glyoxylate shunt [10]. Analogous data were obtained in the present work in relation to *Oscillochloris* sp. R, KR, and BM (Table 3).

Some bacteria, *Desulfobacter postgatei* in particular, are able to oxidize acetate via a reversed operation of the reductive TCA cycle [19]. The enzymes lacking

Table 3. The activity of enzymes (nmol/(min mg protein)) operating in the tricarboxylic acid cycle and glyoxylate shunt in the cells of *Oscillochloris* strains grown in the presence of acetate

Enzyme	*O. trichoides* DG6	*Oscillochloris* sp. KR	*Oscillochloris* sp. BM	*Oscillochloris* sp. R
Citrate synthase	11.6	26.3	27.6	21.5
Aconitase	63.8	53.0	49.4	81.1
Isocitrate dehydrogenase	34.3	31.8	21.8	43.2
2-Oxoglutarate dehydrogenase	0	0	0	0
Succinate dehydrogenase	11.2	16.8	16.0	8.3
Fumarate hydratase	200.9	73.9	53.4	120.0
Malate dehydrogenase	173.3	200.0	57.3	82.4
Isocitrate lyase	0	0	0	0
Malate synthase	15.2	14.2	28.6	18.6

Table 4. Fixation of [2-^{14}C]acetate by the *O. trichoides* DG6 and *Oscillochloris* sp. R cells grown in the presence of acetate

Medium with	Fixation rate, nmol/(min mg protein)	
	O. trichoides DG6	*Oscillochloris* sp. R
*Acetate	8.5	4.5
*Acetate + CO_2	10.2	6.8
*Acetate + Na_2S	17.7	7.1
*Acetate + Na_2S + CO_2	23.2	7.7
*Acetate + KCN	4.8	3.6
*Acetate + CO_2 + KCN	6.2	2.9
*Acetate + Na_2S + KCN	12.2	4.4
*Acetate + Na_2S + CO_2 + KCN	12.2	5.0
*Acetate + Na_2S + CO_2 + fluoroacetate	25.9	8.8

Note: The concentrations of acetate and bicarbonate were 5 mM, the concentrations of cyanide and fluoroacetate were 1 mM, and the concentration of Na_2S was 0.05%.

Table 5. Fixation of [^{14}C]bicarbonate by the *O. trichoides* DG6 and *Oscillochloris* sp. R cells grown in the presence of acetate

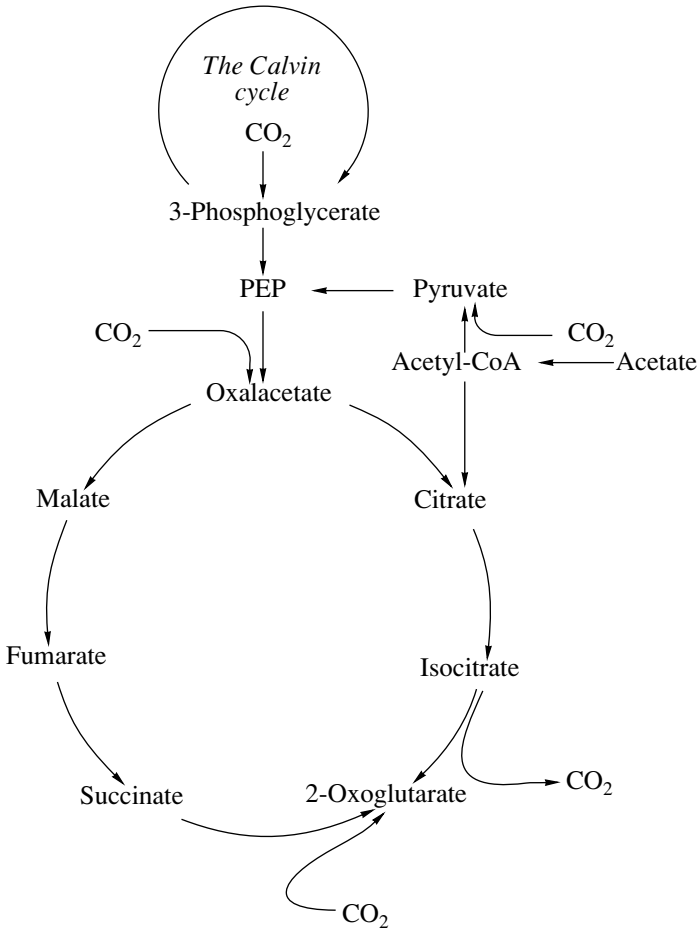
Medium with	Fixation rate, nmol/(min mg protein)	
	O. trichoides DG6	*Oscillochloris* sp. R
*CO_2	0.3	<0.1
*CO_2 + Na_2S	2.5	3.3
*CO_2 + acetate	0.1	0.4
*CO_2 + Na_2S + acetate	2.1	6.1
*CO_2 + Na_2S + iodoacetate	0.2	<0.1
*CO_2 + Na_2S + iodoacetate + acetate	1.9	5.0

Note: The concentrations of acetate and bicarbonate were 5 mM; the concentrations of iodoacetate, cyanide, and Na_2S were 10^{-5} M, 1 mM, and 0.05%, respectively.

in the Krebs cycle are substituted by the enzymes of the reductive TCA cycle that catalyze analogous reactions. Since *Oscillochloris* strains synthesize 2-oxoglutarate synthase (Table 2), which is capable of substituting 2-oxoglutarate dehydrogenase, we checked the ability of the *O. trichoides* DG6 cell suspensions to oxidize acetate. However, it was found that neither the assimilation of [1-^{14}C]acetate nor the assimilation of [2-^{14}C]acetate were accompanied by $^{14}CO_2$ production, irrespective of whether the reaction mixture contained sulfide in addition to acetate and bicarbonate. Fluoroacetate, a TCA cycle inhibitor, did not affect acetate assimilation by the *Oscillochloris* cells (Table 4). The inability of these bacteria to oxidize acetate was indirectly confirmed by the fact that their fixation of acetate was considerably stimulated by sulfide (Table 4), suggesting the necessity of a reductant for acetate involvement in the metabolism.

Acetate assimilation by the *Oscillochloris* cells was stimulated by bicarbonate (Table 4), suggesting the involvement of carboxylation reactions in this process. This may occur via (1) acetate carboxylation that involves pyruvate synthase, PEP carboxylase, and 2-oxoglutarate synthase and proceeds via the following reaction sequence (see the figure): acetyl-CoA (+ CO_2) $\longrightarrow$ pyruvate $\longrightarrow$ PEP (+ CO_2) $\longrightarrow$ oxalacetate $\longrightarrow$ malate $\longrightarrow$ fumarate $\longrightarrow$ succinate $\longrightarrow$ succinyl-CoA (+ CO_2) $\longrightarrow$ 2-oxoglutarate, as occurs in some other phototrophic bacteria, e.g., *Thiocapsa roseopersicina* and *Ectothiorhodospira shaposhnikovii* [17, 20], and via (2) involvement in the acetate assimilation of products of CO_2 fixation in the Calvin cycle. This may occur after the carboxylation of PEP, the main Calvin cycle product, to oxalacetate, whose condensation with acetyl-CoA leads to the formation of citrate, which, via the TCA cycle intermediates aconitate and isocitrate, is then converted into 2-oxoglutarate, the biosynthetic precursor of the glutamate family amino acids (figure).

Most probably, both of the above metabolic variants are realized in *Oscillochloris.* The involvement of pyruvate synthase is indicated by its high activity in the cells grown in the presence of acetate (Table 2); the activity of this enzyme in the autotrophically grown cells is somewhat lower. In addition, in the *Oscillochloris* cells



Carbon metabolism in bacteria of the genus *Oscillochloris.*

grown on an acetate-containing medium, acetate stimulated CO_2 fixation against the background of the inhibition of the Calvin cycle by iodoacetate (Table 5); in contrast, in the autotrophically grown cells, such stimulation was not recorded (Table 1). The involvement of the Calvin cycle in acetate assimilation is confirmed by the suppression of this process by cyanide (Table 4).

During the transition from autotrophic growth conditions to mixotrophic ones, the enzymes involved in acetate assimilation experience a certain regulation, as is confirmed by the fact that the rate of CO_2 fixation by *O. trichoides* DG6 and *Oscillochloris* sp. R is higher in the autotrophically grown cells than in the cells grown in the presence of acetate (Tables 1, 5). The ratio of the rates of assimilation of acetate and bicarbonate in the presence of sulfide is higher in the cells grown in an acetate-containing medium than in the autotrophically grown cells (3.5 and 2.3, respectively, for strain DG6 and 3.2 and 2.0 for strain R).

Acetate assimilation also occurred in the absence of bicarbonate from the medium. This fact can be explained by the incorporation of acetate into the poly-β-hydroxybutyric acid synthesized by *Oscillochloris* [3]. Since the synthesis of this compound is a reductive process, it is stimulated by the addition of sulfide (Table 4).

Thus, all of the *Oscillochloridaceae* representatives studied assimilate CO_2 via the Calvin cycle and use organic compounds only as additional carbon sources, which are necessary due to the incompleteness of the TCA cycle. This property distinguishes *Oscillochloridaceae* representatives from the representatives of *Chloroflexaceae*, which fix CO_2 via a peculiar autotrophic pathway and are able to use a wide range of organic compounds. These profound physiological–biochemical differences support the affiliation of different FAPB to two separate families: *Oscillochloridaceae* and *Chloroflexaceae*.

Interestingly, in their carbon metabolism, *Oscillochloridaceae* representatives are similar to some purple sulfur bacteria, e.g., *Thiocapsa roseopersicina* strain BBS, which can grow only autotrophically and uses only acetate and pyruvate as carbon sources additional to CO_2 [17]. At the same time, the carbon metabolism of *Chloroflexaceae* representatives is similar to that of purple nonsulfur bacteria, which prefer photoorganotrophic growth conditions. A scheme of the carbon

metabolism of *Oscillochloris*, based on the data obtained in this work, is presented in the figure.

ACKNOWLEDGMENT

This work was supported by the Russian Foundation for Basic Research, project nos. 02-04-48974 and 04-04-49377.

REFERENCES

1. Hanada, S., Takaichi, S., Matsuura, K., and Nakamura, K., *Roseiflexus castenholzii* gen. nov., sp. nov., a Thermophilic, Filamentous, Photosynthetic Bacterium That Lacks Chlorosomes, *Int. J. Syst. Evol. Microbiol*, 2002, vol. 52, pp. 187–193.
2. Garrity, G.M and Holt, J.G., Phylum BVI. *Chloroflexi* phy. nov., *Bergey's Manual of Systematic Bacteriology, 2nd ed.*, Boone, D.R. *et al.*, Eds., New York: Springer, 2001, pp. 427–446.
3. Keppen, O.I., Tourova, T.P., Kuznetsov, B.B., Ivanovsky, R.N., and Gorlenko, V.M., Proposal of *Oscillochloridaceae* fam. nov. on the Basis of a Phylogenetic Analysis of the Filamentous Anoxygenic Phototrophic Bacteria and Emended Description of *Oscillochloris* and *Oscillochloris trichoides* in Comparison with Further New Isolates, *Int. J. Syst. Evol. Microbiol*, 2000, vol. 50, pp. 1529–1537.
4. Gich, F., Garcia-Gil, J., and Overmann, J., Previously Unknown and Phylogenetically Diverse Members of the Green Nonsulfur Bacteria Are Indigenous to Freshwater Lakes, *Arch. Microbiol.*, 2001, vol. 177, pp. 1–10.
5. Klappenbach, J.A. and Pierson, B.K., Phylogenetic and Physiological Characterization of a Filamentous Anoxygenic Photoautotrophic Bacterium "Candidatus *Chlorothrix halophila*" gen. nov., sp. nov., Recovered from Hypersaline Microbial Mats, *Arch. Microbiol.,* 2004, vol. 181, pp. 17–25.
6. Ugol'kova, N.V. and Ivanovsky, R.N., On the Mechanism of Autotrophic Fixation of CO_2 by *Chloroflexus aurantiacus, Mikrobiologiya*, 2000, vol. 69, pp. 175–179.
7. Ivanovsky, R.N., Krasilnikova, E.N., and Fal, Y.I., A Pathway of the Autotrophic CO_2 Fixation in *Chloroflexus aurantiacus, Arch. Microbiol.*, 1993, vol. 159, pp. 257–264.
8. Strauss, G. and Fuchs, G., Enzymes of a Novel Autotrophic CO_2 Fixation Pathway in the Phototrophic Bacterium *Chloroflexus aurantiacus*, the 3-Hydroxypropionate Cycle, *Eur. J. Biochem.*, 1993, vol. 215, pp. 633–643.
9. Kondratieva, E.N., Ivanovsky, R.N., and Krasilnikova, E.N., Carbon Metabolism in *Chloroflexus aurantiacus, FEMS Microbiol. Lett.*, 1992, vol. 100, pp. 269–272.
10. Ivanovsky, R.N., Fal, Y.I., Berg, I.A., Ugolkova, N.V., Krasilnikova, E.N., Keppen, O.I., Zakharchuc, L.M., and Zyakun, A.M., Evidence for the Presence of the Reductive Pentose Phosphate Cycle in a Filamentous Anoxygenic Photosynthetic Bacterium, *Oscillochloris trichoides* Strain DG-6, *Microbiology* (UK), 1999, vol. 145, pp. 1743–1748.
11. Gorlenko, V.M. and Korotkov, S.A., Morphological and Physiological Peculiarities of the New Filamentous Gliding Green Bacterium *Oscillochloris trichoides* nov. comb., *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1979, pp. 848–858.
12. Gorlenko, V.M. and Pivovarova, T.A., On the Affiliation of the Blue–Green Alga *Oscillatoria coerulescens* Giclhorn 1921 to a New Genus of Chlorobacteria, *Oscillochloris* nov. gen., *Izv. Akad. Nauk SSSR, Ser. Biol.*, 1977, pp. 396–409.
13. Keppen, O.I., Baulina, O.I., and Kondratieva, E.N., *Oscillochloris trichoides* Neotype Strain DG-6, *Photosynth. Res.*, 1994, vol. 41, pp. 29–33.
14. Alber, B.E. and Fuchs, G., Propionyl-Coenzyme A Synthase from *Chloroflexus aurantiacus*, a Key Enzyme of the 3-Hydroxypropionate Cycle for Autotrophic CO_2 Fixation, *J. Biol. Chem.*, 2002, vol. 277, pp. 12137–12143.
15. Hugler, M., Huber, H., Stetter, K.O., and Fuchs, G., Autotrophic CO_2 Fixation Pathways in Archaea (Crenarchaeota), *Arch. Microbiol.*, 2003, vol. 179, pp. 160–173.
16. Berg, I.A., Filatova, L.V., and Ivanovsky, R.N., Inhibition of Acetate and Propionate Assimilation by Itaconate via Propionyl-CoA Carboxylase in Isocitrate Lyase–Negative Purple Bacterium *Rhodospirillum rubrum, FEMS Microbiol. Lett.*, 2002, vol. 216, pp. 49–54.
17. Kondratieva, E.N., Ivanovsky, R.N., and Krasilnikova, E.N., Light and Dark Metabolism in Purple Sulfur Bacteria, *Soviet Science Review*, Skulachev, V.P., Ed., Guilford: IPC Science and Technology, 1981, pp. 325–364.
18. Lea, P.J., Chen, Z.-H., Leegood, R.C., and Walker, R.P., Does Phosphoenolpyruvate Carboxykinase Have a Role in Both Amino Acid and Carbohydrate Metabolism?, *Amino Acids*, 2001, vol. 20, pp. 225–241.
19. Brandis-Heep, A., Gebhardt, N.A., Thauer, R.K., Widdel, F., and Pfennig, N., Anaerobic Acetate Oxidation to CO_2 by *Desulfobacter postgatei.* 1. Demonstration of All Enzymes Required for the Operation of the Citric Acid Cycle, *Arch. Microbiol.*, 1983, vol. 136, pp. 222–229.
20. Firsov, N.N. and Ivanovsky, R.N., Acetate Photometabolism in *Ectothiorhodospira shaposhnikovii, Mikrobiologiya*, 1975, vol. 44, pp. 197–201.