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Biochemical Properties and Phylogeny of Hydroxypyruvate Reductases from Methanotrophic Bacteria with Different C_1-Assimilation Pathways

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Abstract—In the aerobic methanotrophic bacteria *Methylomicrobium alcaliphilum* 20Z, *Methylococcus capsulatus* Bath, and *Methylosinus trichosporium* OB3b, the biochemical properties of hydroxypyruvate reductase (Hpr), an indicator enzyme of the serine pathway for assimilation of reduced C_1-compounds, were comparatively analyzed. The recombinant Hpr obtained by cloning and heterologous expression of the *hpr* gene in *Escherichia coli* catalyzed NAD(P)H-dependent reduction of hydroxypyruvate or glyoxylate, but did not catalyze the reverse reactions of D-glycerate or glycolate oxidation. The absence of the glycerate dehydrogenase activity in the methanotrophic Hpr confirmed a key role of the enzyme in utilization of C_1-compounds via the serine cycle. The enzyme from *Ms. trichosporium* OB3b realizing the serine cycle as a sole assimilation pathway had much higher special activity and affinity in comparison to Hpr from *Mm. alcaliphilum* 20Z and *Mc. capsulatus* Bath assimilating carbon predominantly via the ribulose monophosphate (RuMP) cycle. The *hpr* gene was found as part of gene clusters coding the serine cycle enzymes in all sequenced methanotrophic genomes except the representatives of the Verrucomicrobia phylum. Phylogenetic analyses revealed two types of Hpr: (i) Hpr of methanotrophs belonging to the Gammaproteobacteria class, which use the serine cycle along with the RuMP cycle, as well as of non-methylotrophic bacteria belonging to the Alphaproteobacteria class; (ii) Hpr of methylotrophs from Alpha- and Betaproteobacteria classes that use only the serine cycle and of non-methylotrophic representatives of Betaproteobacteria. The putative role and origin of hydroxypyruvate reductase in methanotrophs are discussed.

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Keywords: hydroxypyruvate reductase, methanotrophs, serine cycle of carbon assimilation

Aerobic methane utilizing bacteria (methanotrophs) assimilate carbon from methane via three biochemical pathways – the serine pathway and the ribulose monophosphate (RuMP) and the ribulose bisphosphate (RuBP) cycles. The serine pathway is initiated by condensation of formaldehyde (as tetrahydrofolate derivative) and glycine resulting in formation of serine from which hydroxypyruvate is generated after transamination with glyoxylate. The reduction of hydroxypyruvate into glycerate catalyzes hydroxypyruvate reductase (Hpr), considered as an indicator enzyme of the serine pathway. The serine pathway is characteristic for methanotrophs belonging to the Alphaproteobacteria class (type II methanotrophs). In the RuMP pathway, a C–C bond is created by condensation of formaldehyde with ribulose-5-phosphate and forming hexulose-6-phosphate, which is thereafter isomerized into fructose-6-phosphate. Hexosephosphate cleavage leads to synthesis of trioses [1]. The RuMP pathway functions in gammaproteobacterial methanotrophs (type I methanotrophs). In the RuBP pathway realized by methanotrophs of the Verrucomicrobia phylum, CO_2 formed during oxidation of the reduced C_1-substrate is assimilated. After initial deciphering of methylotrophic metabolism half century ago, these pathways were considered as alternatives in each main group of methanotrophic bacteria. Subsequent enzymatic studies provided evidence that in methanotrophs of the *Methylococcus* and *Methylocaldum* genera (type X methanotrophs) there are three simultaneously functioning C_1-assimilation pathways [2-5]. Further-

Abbreviations: Hpr, hydroxypyruvate reductase; RuBP, ribulose bisphosphate; RuMP, ribulose monophosphate.

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more, genes coding for the serine pathway enzymes were revealed in methanotrophs assimilating carbon via the RuMP pathway. However, distribution, functionality, and physiological role of the enzymes in various methanotrophs remain unclear. In this work, we present the results of comparative characterization of Hpr in *Methylomicrobium alcaliphilum* 20Z (representative of type I methanotrophs), *Methylosinus trichosporium* OB3b (type II), and *Methylococcus capsulatus* Bath (type X) and the distribution of *hpr* genes in bacteria.

MATERIALS AND METHODS

Bacteria and growth conditions. *Methylomicrobium alcaliphilum* 20Z (VKM B-2133T = NCIMB 14124T), *Ms. trichosporium* OB3b (VKM B-2117 = NCIMB 11131), and *Mc. capsulatus* Bath (VKM B-2990 = NCIMB 11132) were grown under a methane–air atmosphere (1 : 1) in 2P mineral medium at 30°C (strains 20Z and OB3b) or 37°C (Bath). The medium for strain 20Z was supplemented with 0.1 M $NaHCO_3$ and 0.3 M NaCl [6]. *Escherichia coli* Rosetta (DE3) obtained from Stratagene (USA) was grown in selective LB broth or agar (1.5%; Difco, USA) at 37°C [7]. Kanamycin (100 μg/ml) and chloramphenicol (25 μg/ml) were added if required.

Cloning, expression, and purification of recombinant enzymes. DNA from methanotrophs was prepared by using a ZymoResearch Fungal/Bacterial DNA MiniPrep™ kit (Irvine, USA) according guidelines of the producer. Genes coding for putative Hpr in *Mm. alcaliphilum* 20Z (*hpr20Z*, *MALCv4_3219*), *Ms. trichosporium* OB3b (*hprOB3b*, *ADVE02_v2_12566*), and *Mc. capsulatus* Bath (*hprBath*, *MCA1407*) were amplified from the respective DNA using the primers listed in Table 1. The PCR-products were treated with endonucleases NdeI and HindIII and ligated in expression vector pET30(a)+ (*hpr20Z* and *hprBath*) or pET28b (*hprOB3b*) opened at the respective restriction sites. The resulting plasmids were transferred into *E. coli* Rosetta (DE3), and protein expression was induced by 0.5 mM isopropyl β-D-1-thiogalactopyranoside (Sigma-Aldrich, USA) added in the logarithmic phase of growth (OD_{600} = 0.6-0.8). After 18 h of growth at 18°C, cells were centrifuged (5000*g* for 30 min, 4°C). The cells were disrupted using an ultrasonic disintegrator (Misonix, USA) with 1 min cooling in ice 30 s after each 10-s sonication, then centrifuged for 30 min at 11,000*g* and 4°C. The recombinant proteins were purified on a column with Ni^{2+}-NTA agarose as described earlier [8].

Hpr activity assay. Activity of Hpr was measured by registering velocity of NAD(P)H oxidation at λ = 340 nm using a Shimadzu UV1700 spectrophotometer (Shimadzu, Japan). The assay mixture (1 ml) contained 50 mM Tris-HCl (pH 7.0), 5 mM hydroxypyruvate, and 0.2 mM NADH. Activities of HprOB3b and Hpr20Z were measured at 30°C, and activity of HprBath at 37°C. Values of K_m and V_{max} were determined by measuring the activity with different concentrations of the substrate at saturated concentrations of the other. For calculation, the Enzyme Kinetics Module of SigmaPlot 12 was used, and the data approximated to the Michaelis–Menten equation:

$$A = \frac{A_{max}[S]}{K_m + [S]},$$

or to the equation for substrate inhibition:

$$A = \frac{A_{max}}{1 + \frac{K_m}{[S]} + \frac{[S]}{K_i}}.$$

Influence of pH on Hpr activity was studied using following buffers: MES-KOH (pH 5.5-7.0), Tris-HCl (pH 7.0-9.0), and NaOH-glycine (pH 9.0-10.5).

Quaternary forms of the enzymes were analyzed by non-denaturing gel electrophoresis by using pore-limited gradient polyacrylamide (4-30%) [9]. The reference proteins thyroglobulin (667 kDa), ferritin (440 kDa), amylase (250 kDa), alcohol dehydrogenase (150 kDa), and bovine serum albumin (BSA, 66 kDa) were obtained from Sigma-Aldrich (USA).

RESULTS

Cloning of *hpr* genes and purification of recombinant proteins. In the genomes of *Mm. alcaliphilum* 20Z, *Ms. trichosporium* OB3b, and *Mc. capsulatus* Bath, the open read-

Table 1. Sequences of primers used in this work

Target gene	Primer sequence (5′-3′)	
	forward primer	reverse primer
20Z-hpr	ACATATGAGTAAACCCAAAGTTTTA	GAAGCTTAGCCACTCTGTCTTCAGGTT
OB3b-hpr	TATCATATGTCGCACAAAATCGTCT	GAGAAGCTTCGCGGTCACGAGATTACGCG
Bath-hpr	TATCATATGAGCAAACCCAAAGTACT	TAGAAGCTTGGTCACCTTGTCTCGGGGGGGA

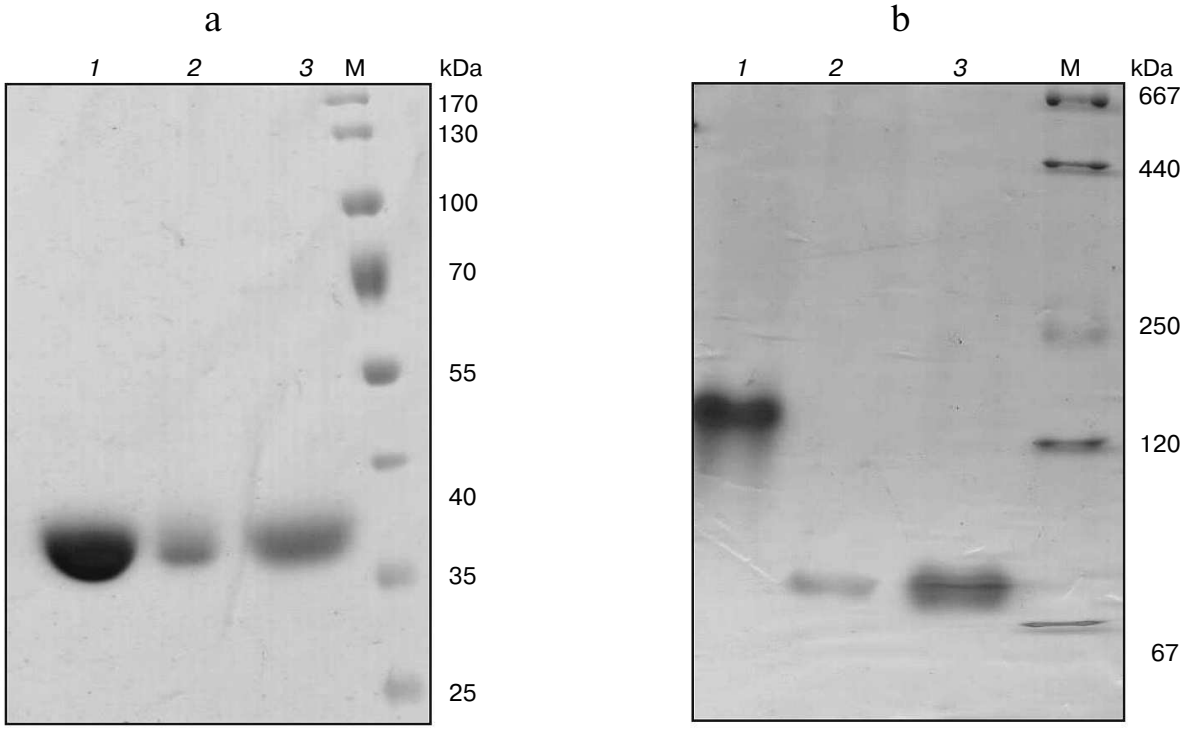

Fig. 1. SDS-PAGE (a) and native electrophoresis in a polyacrylamide gradient (4-30%) (b) of recombinant Hpr from *Ms. trichosporium* OB3b (*1*), *Mm. alcaliphilum* 20Z (*2*), and *Mc. capsulatus* Bath (*3*). *M*, molecular mass marker.

ing frames whose translated amino acid sequences showed homologies with earlier characterized Hpr from *Hyphomicrobium methylovorum* GM2 (37, 36, and 40% identity, respectively) [10] and *Methylobacterium extorquens* AM1 (43, 78, and 46%) [11] were found. These genes were cloned into vectors pET30(a)+ and pET28b appropriated for expression of proteins with six histidines on either N- or C-end under T7 promoter control. The His_6-tagged proteins were purified from extracts of *E. coli* cells by single-step affinity chromatography on Ni^{2+}-NTA agarose. Molecular masses of subunits of Hpr20Z-His_6, HprOB3b-His_6, and HprBath-His_6 estimated by SDS-PAGE were in good agreement with the theoretically calculated subunit sizes (35-37 kDa) (Fig. 1a). Native gradient electrophoresis revealed single bands of 140 kDa for the *M. trichosporium* Hpr, whereas it was 70 kDa for the *Mc. capsulatus* Bath and *Mm. alcaliphilum* 20Z enzymes (Fig. 1b). Hence, the Hpr of strain OB3b exists as a homotetramer, whereas the enzymes from strains Bath and 20Z are homodimers.

Properties of recombinant Hpr. The three recombinant enzymes reduced hydroxypyruvate into glycerate in the presence of either NADH or NADPH, showing maximal activity at pH 7.0 (Fig. 2). Hpr from mesophilic methanotrophs *Mm. alcaliphilum* 20Z and *Ms. trichosporium* OB3b demonstrated unexpectedly high temperature optima, and activity of Hpr from thermotolerant *Mc. capsulatus* Bath increased upon increasing temperature to at least 70°C (Fig. 2).

Besides hydroxypyruvate, all the studied enzymes used glyoxylate as substrate but were not active with pyruvate, lactate, oxaloacetate, and α-ketoglutarate (Table 2). Such substrate specificity has been demonstrated for the Hpr from methylotrophic bacteria *Hyphomicrobium methylovorum* GM2 and *Methylobacterium extorquens* AM1 [10, 11]. However, in contrast to the above-mentioned paralogs, the Hpr of methanotrophs did not catalyze the reverse reaction of glycerate and glycolate oxidation.

The substrate dependence of the activities of the three methanotrophic Hpr on glyoxylate and cofactors obeyed Michaelis–Menten kinetics. However, in the presence of hydroxypyruvate, substrate inhibition of enzyme activity occurred (Table 3). The activity of hydroxypyruvate reduction by the enzyme from *Ms. trichosporium* OB3b was 10-fold higher than those of the Hpr from *Mm. alcaliphilum* 20Z and *Mc. capsulatus* Bath. In the presence of NADH, the catalytic efficiency of HprOB3b with hydroxypyruvate was 7-fold higher than the catalytic efficiency of HprBath and almost 100-fold higher than that of Hpr20Z. However, in the presence of NADPH, the k_{cat}/K_m ratios for the enzymes from *Ms. trichosporium* OB3b and *Mm. alcaliphilum* 20Z were comparable and about 3-fold higher than the catalytic efficiency of HprBath. The efficiency of all three enzymes with glyoxylate as substrate was virtually the same independently of the cofactor used (Table 3). On the other hand, the enzymes had a differ preference

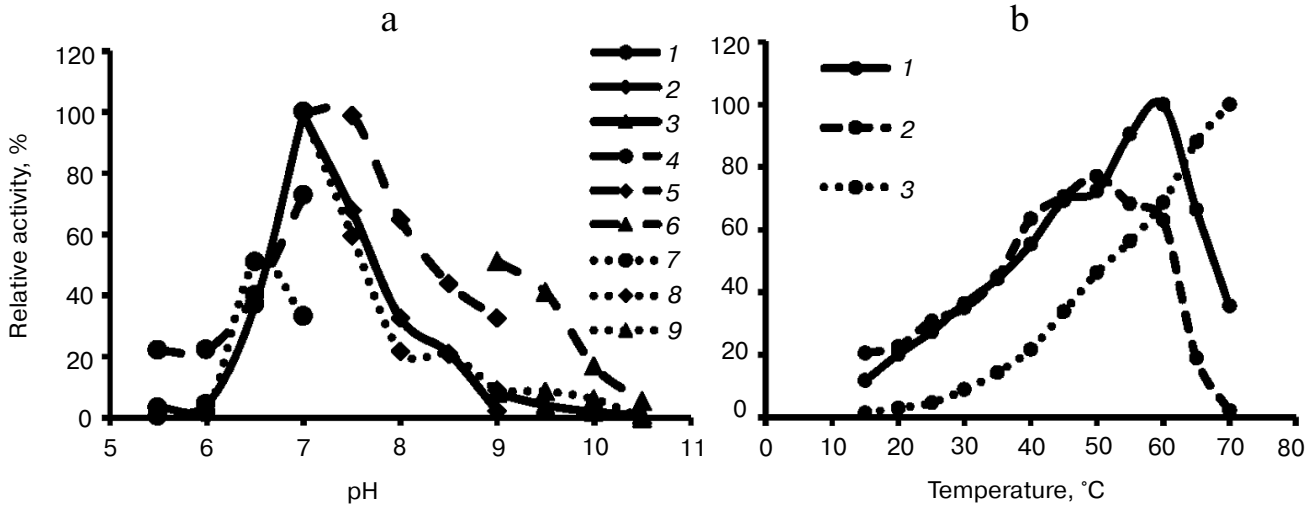

Fig. 2. a) pH dependence of activity of recombinant Hpr from *Mm. alcaliphilum* 20Z (*1-3*), *Ms. trichosporium* OB3b (*4-6*), and *Mc. capsulatus* Bath (*7-9*). Curves: *1*, *4*, *7*) MES-KOH buffer; *2*, *5*, *8*) Tris-HCl buffer; *3*, *6*, *9*) Na-glycine. b) Temperature dependence of activity of recombinant Hpr from *Mm. alcaliphilum* 20Z (*1*), *Ms. trichosporium* OB3b (*2*), and *Mc. capsulatus* Bath (*3*).

depending on the cofactor. Thus, in the presence of NADH, Hpr of *Ms. trichosporium* OB3b used hydroxypyruvate three-orders of magnitude more efficiently than glyoxylate, but in the presence of NADPH only two-orders of magnitude more efficiently. The catalytic efficiency of the enzymes from *Mc. capsulatus* Bath and *Mm. alcaliphilum* 20Z was 10-fold higher with hydroxypyruvate in the presence of NADH, while in the presence of NADPH, HprBath was practically inactive with glyoxylate and Hpr20Z was 35 times more efficient with hydroxypyruvate (Table 3). We propose that the reduction of hydroxypyruvate is the main physiological role of Hpr in type II methanotrophs, while Hpr of type I and type X methanotrophs can also function as glyoxylate reductase. The ratio of these reactions can be regulated by concentrations of NADH and NADPH in cells.

The Hpr from the three methanotrophs differed according to the influence of effectors on their activity. The activity of Hpr20Z was significantly enhanced in the presence of some intermediates of glycolysis and the TCA cycle, as well as serine, ADP, ATP, and inorganic pyrophosphate, but it was inhibited by P_i, pyruvate, acetyl-CoA, phosphoenolpyruvate, and ribulose-5-phosphate. At the same time, only minor stimulatory effect on the activity of Hpr from *Ms. trichosporium* OB3b was revealed in the presence of PP_i, glucose-6-phosphate, ribulose-1,5-bisphosphate, oxaloacetate, and acetyl-CoA, whereas activity of the *Mc. capsulatus* Bath enzyme was dependent on the presence of fructose-1,6-bisphosphate and oxaloacetate only. Thus, HprBath and HprOB3b were inhibited by ADP and ATP (Table 4). The products of the reaction differently affected the activities of the Hpr. All three enzymes were inhibited by glycerate, Hpr20Z to a minor extent. NAD^+ lowered the activity of the enzymes from *Ms. trichosporium* OB3b and *Mc. capsulatus* Bath, but Hpr20Z was inhibited by NAD^+ only in the reaction with NADPH. In contrast, NAD^+ stimulated NADH-dependent activity. $NADP^+$ activated

Table 2. Substrate specificity of Hpr from different methanotrophs

Substrate	Relative activity, %*		
	HprOB3b	HprBath	Hpr20Z
Hydroxypyruvate	100	100	100
Glyoxylate	24	97	62
α-Ketoglutarate	0	0	0
Oxaloacetate	0	0	0
Pyruvate	0	0	0
Lactate	0	0	0

* Activity relatively to activity in the presence of 5 mM hydroxypyruvate and 0.2 mM NADH.

Table 3. Kinetic parameters of Hpr from different methanotrophs

Substrate	A_{max}, U/mg	K_m, mM	k_{cat}, s^{-1}	K_i, mM	k_{cat}/K_m
***Methylomicrobium alcaliphilum* 20Z**					
Hydroxypyruvate					
with NADH	26 ± 7	0.6 ± 0.3	30	9 ± 5	50
with NADPH	41 ± 2	0.17 ± 0.03	48	10 ± 1	282
Glyoxylate					
with NADH	16 ± 1	3 ± 0.4	19		6
with NADPH	75 ± 14	11 ± 4	88		8
NADH					
with hydroxypyruvate	12 ± 1	0.08 ± 0.01	14		175
with glyoxylate	16 ± 1	0.07 ± 0.01	19		271
NADPH					
with hydroxypyruvate	41 ± 2	0.055 ± 0.007	48		873
with glyoxylate	75 ± 14	0.028 ± 0.003	88	–	3142
***Methylococcus capsulatus* Bath**					
Hydroxypyruvate					
with NADH	35 ± 1	0.057 ± 0.006	42	9 ± 1	737
with NADPH	32 ± 8	0.5 ± 0.2	38	1.0 ± 0.4	76
Glyoxylate					
with NADH	34 ± 2	3.9 ± 0.4	41		10.5
with NADPH	trace	–			–
NADH					
with hydroxypyruvate	35 ± 1	0.03 ± 0.01	42		1400
with glyoxylate	34 ± 2	0.015 ± 0.002	41		2733
NADPH					
with hydroxypyruvate	19 ± 2	0.16 ± 0.03	23		143
with glyoxylate	trace	–	–		–
***Methylosinus trichosporium* OB3b**					
Hydroxypyruvate					
with NADH	303 ± 11	0.14 ± 0.01	687	60 ± 20	4907
with NADPH	127 ± 12	1.2 ± 0.2	288	7 ± 1	240
Glyoxylate					
with NADH	73 ± 6	22 ± 4	166		7.5
with NADPH	23 ± 3	18 ± 3	52		2.9
NADH					
with hydroxypyruvate	303 ± 11	0.028 ± 0.004	687		24,535
with glyoxylate	73 ± 6	0.013 ± 0.001	166		12,769
NADPH					
with hydroxypyruvate	125 ± 14	0.11 ± 0.02	284		2582
with glyoxylate	23 ± 3	0.22 ± 0.06	52		236

HprBath and Hpr20Z only in reaction with NADPH (Table 4).

Distribution and phylogenetic analysis. Genes coding for Hpr were found in all available genomes of methanotrophs with except for representatives of the phylum Verrucomicrobia. In *Mm. alcaliphilum* 20Z, the *hpr* gene is located in a single cluster with genes coding for other enzymes of the serine cycle: serine-glyoxylate aminotransferase (*sga*), glycerate-2-kinase (*gck2*), malate thiokinase (*mtkAB*), malyl-CoA lyase (*mcl*), malate dehydrogenase (*mdh*), serine hydroxymethyltransferase (*glyA*), as well as with genes responsible for formaldehyde oxidation: methylene-tetrahydromethanopterin/methylene-tetrahydrofolate dehydrogenase (*mtdA*) and formate-tetrahydrofolate ligase (*ftfL*). An analogous set of genes forming one or two identical gene clusters located in different chromosomal locus occurs in most gammaproteobacterial methanotrophs of genera *Methylomicrobium*, *Methylobacter*, *Methylosarcina*, and *Methylomonas* realizing the RuMP pathway of formaldehyde utilization. The genes of the serine cycle in alphaproteobacterial type II methanotrophs, including *Ms. trichosporium* OB3b, are organized analogously, but they additionally possess genes for phosphoenolpyruvate carboxylase (*ppc*) and methylene tetrahydrofolate cyclohydrolase (*fch*) and have separate location of gene *mdh*. At the same time, in *Mc. capsulatus* Bath the *hpr* gene is co-located with genes of serine-glyoxylate aminotransferase and a putative glycerate-

Table 4. Influence of some metabolites on activity of Hpr from different methanotrophs

Effector	Concentration, mM	Relative activity, %		
		HprOB3b	HprBath	Hpr20Z
ATP	5	57 ± 8	78 ± 8	305 ± 7
ADP	5	78 ± 2	94 ± 6	298 ± 15
Glucose	5	91 ± 8	109 ± 2	112 ± 6
Fructose	5	101 ± 5	107 ± 3	86 ± 9
Glucose-6P	5	134 ± 4	93 ± 6	163 ± 9
Fructose-6P	5	82 ± 2	104 ± 2	99 ± 12
Fructose-1,6P_2	5	95 ± 11	118 ± 4	244 ± 18
Glucose-1P	5	107 ± 1	105 ± 4	82 ± 3
Serine	5	96 ± 2	102 ± 2	180 ± 13
Pyruvate	5	67 ± 3	92 ± 2	78 ± 4
Oxaloacetate	5	119 ± 11	115 ± 3	231 ± 13
α-Ketoglutarate	5	80 ± 5		231 ± 7
Citrate	5	107 ± 1	90 ± 1	399 ± 7
Malate	5	95 ± 10	91 ± 3	125 ± 15
CoA	1	89 ± 7	92 ± 4	92 ± 8
Acetyl-CoA	1	120 ± 1	91 ± 3	58 ± 6
P_i	5	100 ± 2	112 ± 4	67 ± 1
PP_i	1	152 ± 5	100 ± 8	362 ± 8
Phosphoenolpyruvate	5	113 ± 1	107 ± 3	41 ± 9
Ribulose-5-P	1	109 ± 10	96 ± 4	47 ± 8
Ribulose-1,5P_2	1	170 ± 26	92 ± 3	325 ± 25
Glyceraldehyde-3P	5	90 ± 1	107 ± 1	84 ± 3
Glycerate	5	60 ± 3	49 ± 5	84 ± 5
NAD^+ (with NADH)	1	86 ± 5	92 ± 9	157 ± 15
NAD^+ (with NADPH)	1	72 ± 4	54.1 ± 0.5	64 ± 4
$NADP^+$ (with NADH)	1	81 ± 2	104 ± 9	114 ± 9
$NADP^+$ (with NADPH)	1	87 ± 4	200 ± 14	199 ± 10

3-kinase (*gck3*), and the *gck2* gene is absent in the genome. Similar organization of genes for the serine pathway was also revealed in another type X methanotroph, *Methylocaldum szegediense* O12, as well as halophilic type I methanotroph *Methylohalobius crimeensis* 10Ki.

The Hpr from *Mm. alcaliphilum* 20Z and *Mc. capsulatus* Bath shared 58% identity of amino acid sequences, and only 26 and 25.5% identities with the *Ms. trichosporium* OB3b enzyme. Phylogenetic analysis showed the occurrence of two different groups of Hpr presumably of different origin. One group included Hpr of methanotrophs of the Gammaproteobacteria class realizing three pathways of C_1-assimilation (serine, RuMP, and RuBP cycles), as well as non-methylotrophic representatives of the Alphaproteobacteria class. Another phylogenetic group included Hpr of alphaproteobacterial methanotrophs, alphaproteobacterial methylotrophs which unable to grow on methane, as well as methylotrophic and non-methylotrophic representatives of the Betaproteobacteria class (Fig. 3).

DISCUSSION

In this report, we present the comparative characteristics of the Hpr from three methanotrophic bacteria differing in taxonomic position and C_1-assimilation pathways. The recombinant enzymes from *Mm. alcaliphilum* 20Z, *Ms. trichosporium* OB3b, and *Mc. capsulatus* Bath catalyzed NAD(P)H-dependent reduction of hydroxypyruvate and glyoxylate. These enzymes had similar pH optima, while the strains are either neutrophilic (strains OB3b and Bath) or alkalophilic (strain 20Z). Their temperature optima were significantly higher than the optimal growth temperatures for these bacteria. Earlier, unusual high temperature optima were found for malate dehydrogenases from *Mm. alcaliphilum* 20Z and *Ms. trichosporium* OB3b, enzymes which are also involved in the serine cycle [12].

Phylogenetically, Hpr of methanotrophs can be divided on two groups where Hpr of gammaproteobacterial strains 20Z and Bath can be referred to one group, but the enzyme from alphaproteobacterium *Ms. trichosporium*

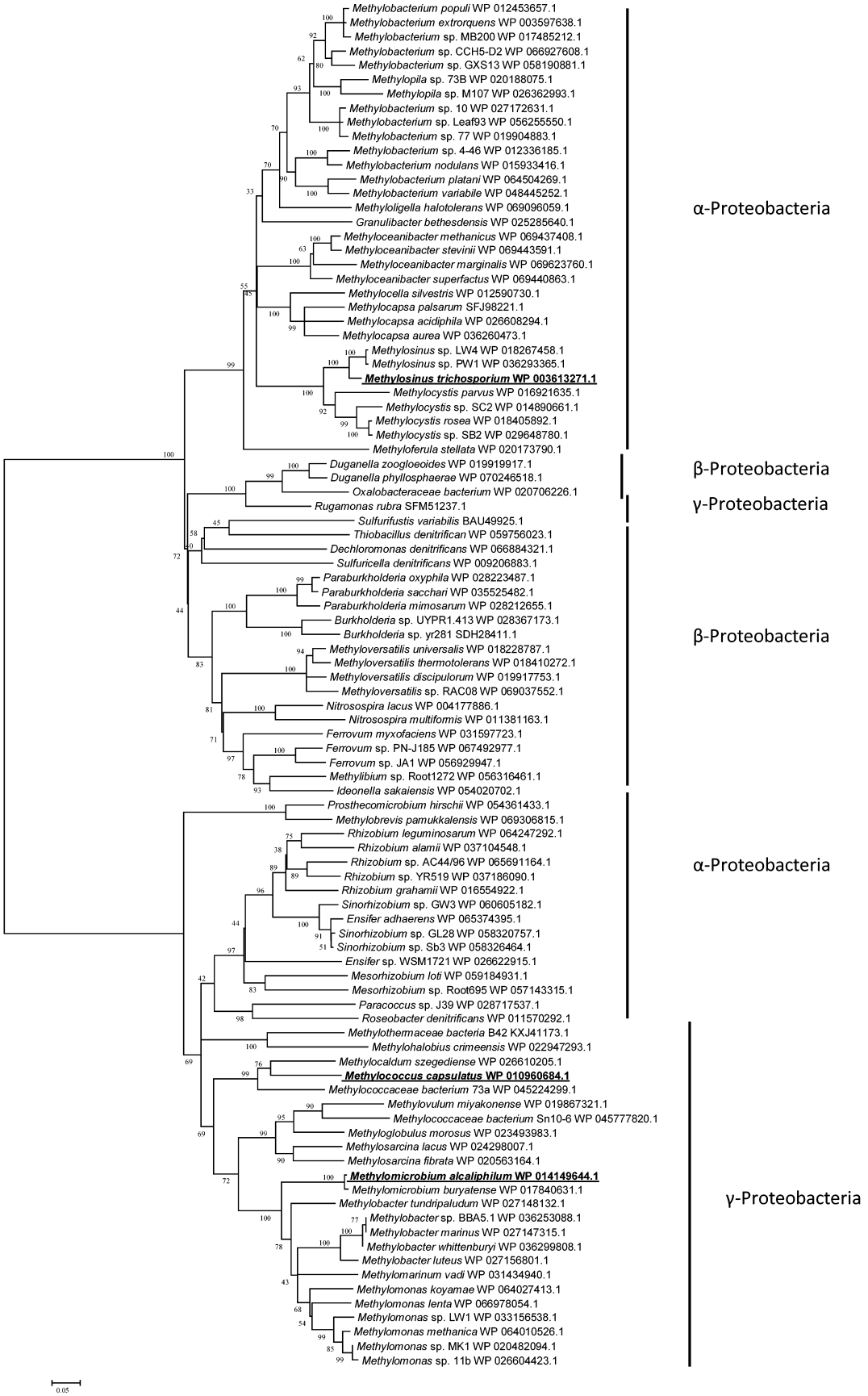

Fig. 3. Phylogenetic tree of translated amino acid sequences of Hpr.

OB3b belongs to another group. In these species, the gene encoding Hpr is a part of a rather conservative gene cluster coding for other serine cycle enzymes, implying "module" organization of the serine pathway in methanotrophs. Overall, topology of the phylogenetic tree corresponds to the taxonomic position of bacterial strains, suggesting an absence or few horizontal gene transfers and vertical evolution of the serine pathway enzymes. On the other hand, a significant divergence of Hpr in different representatives of methanotrophs indicate different functions of the enzyme. Tetrameric subunit structure of HprOB3b was not reported for any described Hpr. In contrast, all known Hprs are dimeric, similar to the enzymes from *Mm. alcaliphilum* 20Z and *Mc. capsulatus* Bath [10, 11, 13].

Activity and affinity to hydroxypyruvate of Hpr from *Ms. trichosporium* OB3b were much higher than of the other two enzymes. Also, its activity underwent less inhibition by hydroxypyruvate. This agrees with the serine cycle in the type II methanotrophs that is the single pathway of methane carbon assimilation. At the same time, the role of the Hpr in methanotrophs of types I and X remains unclear. Earlier, participation of Hpr in the synthesis of serine from phosphoglycerate in *Mc. capsulatus* Bath was proposed [4]. This hypothesis, however, contradicts the irreversibility of the reaction catalyzed by HprBath. Besides methylotrophs, Hpr is found in other bacteria (sometimes the name "glyceraldehyde dehydrogenases" is used). The translated amino acid sequences of methylotrophic enzymes share 37-43% identity with the enzymes from bacteria [14, 15], 32-37% with those from algae [16, 17], 35-65% from plants [18], and 26-46% from animals [13, 19]. It has been proposed that reductase activity of the enzyme is associated with serine degradation while glycerate dehydrogenase activity with biosynthesis of this amino acid [20].

The absence of glycerate dehydrogenase activity in the methanotrophic Hpr confirms involvement of the enzyme in assimilation of C1-substrate via serine and hydroxypyruvate forming in the reactions catalyzed by serine-hydroxymethyltransferase and serine-glyoxylate aminotransferase. In addition, *Mc. capsulatus* Bath has ribulose bisphosphate carboxylase/oxygenase (Rubisco) (MCA2583) that forms phosphoglycolate as a side product, and other enzymes for conversion of phosphoglycolate into glycine (MCA1499-1501) [4]. We suggest that the serine pathway in type X methanotrophs is involved in an analogous process of photorespiration in plants and cyanobacteria and serves for prevention of carbon deprivation as result of the Rubisco oxygenase activity. A stimulation of the activity of Hpr from *Mm. alcaliphilum* 20Z by the central metabolites can imply the mobilization of the serine cycle as an additional assimilation pathway under conditions of carbon source excess.

Activity of glyoxylate reduction was found earlier for most described Hpr, including methylotrophic ones [10, 11, 13, 17, 18]. However, its physiological role is not always clear. It was suggested that the enzyme of *Methylobacterium extorquens* AM1 is needed for growth on either C_1- or C_2-compounds, since the Hpr mutant lost ability to grow on these substrates [11]. Importantly, in the genomes of the three studied methanotrophs there are genes coding for FAD-dependent glycolate oxidase (*ADVE02_v2_14253-14256*; *MCA1499-1501*; *MALCv4_1676-1678*). Perhaps a combination of glyoxylate reducing and glycolate oxidizing activities can form a futile cycle discharging reducing equivalent excess. Further studies using mutagenesis and metabolomic measurements can clarify role of Hpr and the serine pathway in methanotrophs.

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