Antonie van Leeuwenhoek (2019) 112:741–751
https://doi.org/10.1007/s10482-018-1208-4

ORIGINAL PAPER

Serine-glyoxylate aminotranferases from methanotrophs using different C1-assimilation pathways

S. Y. But · S. V. Egorova · V. N. Khmelenina · Y. A. Trotsenko

Received: 7 September 2018 / Accepted: 28 November 2018 / Published online: 3 December 2018
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Abstract The indicator enzyme of the serine pathway of assimilation of reduced C_1 compounds, serine-glyoxylate aminotransferase (Sga), has been purified from three methane-oxidizing bacteria, *Methylomicrobium alcaliphilum* 20Z, *Methylosinus trichosporium* OB3b and *Methylococcus capsulatus* Bath. The native enzymes were shown to be dimeric (80 kDa, strain 20Z), tetrameric (~ 170 kDa, strain OB3b) or trimeric (~ 120 kDa, strain Bath). Sga from the three methanotrophs catalyse the pyridoxal phosphate-dependent transfer of an amino group from serine to glyoxylate and pyruvate; the enzymes from strains 20Z and Bath also transfer an amino group from serine to α-ketoglutarate and from alanine to glyoxylate. No other significant differences between the Sga from the three methanotrophs were found. The three methanotrophic Sga have their highest catalytic efficiencies in the reaction between glyoxylate and serine, which is in agreement with their function to provide circulation of the serine assimilation pathway.The disruption of the *sga* gene in *Mm. alcaliphilum* resulted in retardation of growth rate of the mutant cells and in a prolonged lag-phase after passaging from methane to methanol. In addition, the growth of the mutant strain is accompanied by formaldehyde accumulation in the culture liquid. Hence, Sga is important in the serine cycle of type I methanotrophs and this pathway could be related to the removal of excess formaldehyde and/or energy regulation.

Keywords C1-assimilation · Methanotrophic bacteria · Serine cycle · Serine-glyoxylate aminotransferase

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10482-018-1208-4) contains supplementary material, which is available to authorized users.

S. Y. But (✉) · S. V. Egorova · V. N. Khmelenina · Y. A. Trotsenko
Laboratory of Methylotrophy, Russian Academy of Sciences, Skryabin Institute of Biochemistry and Physiology of Microorganisms, Pushchino, Moscow Region, Russia 142290
e-mail: flash20063@rambler.ru

Y. A. Trotsenko
Pushchino State Institute of Natural Sciences, Pushchino, Moscow Region, Russia 142290
e-mail: trotsenko@ibpm.pushchino.ru

Introduction

Methanotrophs, a specialised group of bacteria utilising methane as a sole source of energy and carbon, play an important role in the environment thus mitigating the hazardous greenhouse effects of CH_4 (Hanson and Hanson 1996). The currently known methanotrophs belong to the phyla *Proteobacteria* and *Verrucomicrobia,* as well as to the obligate anaerobic deep-branching bacterial phylum NC10 represented by *Candidatus* Methylomirabilis oxyfera (Ettwig et al.

2010). Methanotrophs obtain energy for growth mostly by the oxidation of reduced C_1-substrates to CO_2 and assimilate carbon at the level of formaldehyde, formate and/or CO_2 via three biochemical pathways (Trotsenko and Murrell 2008). The methanotrophs belonging to the *Gammaproteobacteria* and classified as type I, use the ribulose monophosphate (RuMP) pathway for carbon assimilation where a C–C bound is formed by condensation of formaldehyde and ribulose-5-phosphate. In contrast, methanotrophic representatives of *Alphaproteobacteria* (type II) assimilate C_1 compounds via the serine pathway where C_3 compounds are the primary products. The methanotrophs of the *Verrucomicrobia* and NC10 clades use methane as a source of energy, oxidising CH_4 to carbon dioxide and then fixing CO_2 via the Calvin-Benson-Bassham (CBB) cycle (Van Teeseling et al. 2014; Khadem et al. 2011; Rasigraf et al. 2014). In some gammaproteobacterial methanotrophs such as *Methylococcus capsulatus* (X type), there are three simultaneously acting pathways where the RuMP cycle is the major carbon assimilation route (Strom et al. 1974; Shishkina et al. 1976; Taylor et al. 1981; Baxter et al. 2002; Eshinimaev et al. 2004). Despite the intensive studies on C_1 metabolism in bacteria, the functionality and role of the serine cycle enzymes in γ-proteobacterial methanotrophs has been poorly studied. Only serine-glyoxylate aminotransferase (Sga), hydroxypyruvate reductase (Hpr) and glycerate kinase (Gck) from the non-methanotrophic methylotroph *Hyphomicrobium methylovorum* GM2 (Izumi et al. 1990a, b; Yoshida et al. 1992) and Hpr from *Methylobacterium extorquence* AM1 have been characterised to date (Chistoserdova and Lidstrom 1991). Very recently, Hpr from the type I, II and X methanotrophs *Methylomicrobium alcaliphilum* 20Z, *Methylosinus trichosporium* OB3b and *Mc. capsulatus* Bath (But et al. 2017) have been obtained as recombinant proteins and characterised. This paper continues the comparative characterisation of the serine cycle enzymes in representatives of type I, type II and type X methanotrophs.

Materials and methods

Bacteria and growth conditions

Mm. 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 methane-air atmosphere in a mineral medium 2P containing (g/L): KNO_3(2), $MgSO_4$ (0.4), $CaCl_2$(0.04), Na_2-EDTA (0.01), $FeSO_4 \times 7H_2O$ (0.004), $ZnSO_4 \times 7H_2O$ (0.0002), $MnCl_2 \times 4H_2O$ (0.00006), $CuCl_2 \times 5H_2O$ (0.008), $CoCl_2 \times 6H_2O$ (0.0004), $NiCl_2 \times 6H_2O$ (0.00004), Na_2MoO_4 (0.00006), H_3BO_3 (0.0006) at 30 °C (strains 20Z and OB3b) or at 37 °C (Bath). The medium for *Mm. alcaliphilum* 20Z was supplemented with 0.1 M $NaHCO_3$ and 0.3 M NaCl (Khmelenina et al. 1999). For growth measurements, *Mm. alcaliphilum* was cultivated in sealed 50-ml vials containing 20 ml of the medium mentioned above. 50 ml of methane was injected into the headspace of the vials or, alternatively, methanol was added up to a concentration of 0.2% (v/v). *Escherichia coli* Rosetta (DE3), obtained from Stratagene (La Jolla, USA), was grown in a selective LB broth or agar (1.5% 'Difco') at 37 °C (Sambrook and Russell 2001). Kanamycin (50 μg/ml) and chloramphenicol (25 μg/ml) were added if required.

Cloning, expression and purification of recombinant enzymes

DNA from the methanotrophs was prepared using a ZymoResearch Fungal/Bacterial DNA MiniPrep™ kit (Irvine, USA) according to the manufacturer's instructions. The genes coding for putative Sga in *Mm. alcaliphilum* 20Z (*sga20Z, MALCv4_3218*), *Ms. trichosporium* OB3b (*sgaOB3b, ADVE02_v2_12567*) and *Mc. capsulatus* Bath (*sgaBath, MCA1406)* were amplified from the respective DNA using the following primers: 20Zsga-F, 20Zsga-R, ob3b-sga-F, ob3b-sga-R, bath-sga-F and bath-sga-R (Table S1). The PCR products were treated with the endonucleases *Nde*I and *HindIII* and ligated in the expression vector pET30(a) + opened at the respective restriction sites. The resulting plasmids were transferred into *E. coli* Rosetta (DE3) and the protein expression was induced by 0.5 mM isopropyl β-D-1-thiogalactopyranoside

(Sigma-Aldrich) added in the exponential growth phase (OD_{600} = 0.6–0.8). After 18-h growth at 18 °C, the cells were centrifuged (5000 g for 30 min, 4 °C). The cells were disrupted during 1 min in an MSE ultrasonic disintegrator (UK) with cooling in ice for 30 s after each 10-s sonication and then centrifuged for 30 min at 11,000 g and 4 °C. The recombinant proteins were purified on a Ni^{2+}-NTA column as described (Reshetnikov et al. 2008).

The purity and subunit molecular masses of enzyme subunits were tested by 12% polyacrylamide gel electrophoresis with sodium dodecyl sulfate (SDS-PAGE) at a field strength of 5 V/cm. Coumassy R-250 staining was used for band visualisation. The quaternary forms of the enzymes were analysed by:

(a) Non-denaturating gel electrophoresis with pore-limited gradient polyacrylamide (4–30%) (Stater 1969) at a field strength of 3 V/cm. The reference proteins thyroglobulin (667 kDa), ferritin (440 kDa), β-amylase (250 kDa), alcohol dehydrogenase (150 kDa) and bovine serum albumin (BSA, 66 kDa) were obtained from Sigma-Aldrich Group.
(b) Gel filtration on a Bio-sil SEC 250 column (300 × 7.8) with 100 mM Tris–HCl buffer (pH 8.0) containing 100 mM NaCl as a mobile phase. The flow rate was 1 ml/min. Ferritin (440 kDa), β-amylase (250 kDa), alcohol dehydrogenase (150 kDa), BSA (66 kDa) obtained from Sigma-Aldrich Group and fructokinase (35 kDa) obtained as a recombinant protein (But et al. 2012) were used as reference proteins.

Aminotransferase activity assays

Serine-glyoxylate aminotransferase (SGAT) activity was measured using hydroxypyruvate reductase as a coupling enzyme. The reaction mixture contained: 50 mM Tris–HCl buffer, pH 7.0 (or pH 7.5 for Sga20Z), 5 mM serine, 5 mM glyoxylate, 25 μM pyridoxal phosphate, 0.25 mM NADPH, and 5 U recombinant hydroxypyruvate reductase from *Mc. capsulatus* (But et al. 2017). The temperature optima were tested by a direct method. The reaction mixture (200 μl) contained: 50 mM Tris–HCl buffer, pH 7.0 (or pH 7.5 for Sga20Z), 5 mM serine, 5 mM glyoxylate, 25 μM pyridoxal phosphate. After 5-min incubation at an appropriate temperature, 20 μl of 100% (m/v) trichloracetic acid was added to terminate the reaction. The formed hydroxypyruvate was measured by HPLC on a Repro-Gel H + column (250x8) with 1 mM H_2SO_4 as a mobile phase.

The serine-α-ketoglutarate aminotransferase (SKAT) and serine-pyruvate aminotransferase (SPAT) activities were measured analogously to the SGAT activity using 5 mM α-ketoglutarate or pyruvate, respectively, instead of glyoxylate.

The alanine-glyoxylate aminotransferase (AGAT) activity was assayed by measuring the rate of pyruvate formation coupled with NADH oxidation by alanine dehydrogenase. The reaction mixture contained 50 mM alanine, 10 mM glyoxylate, 25 μM mM pyridoxal phosphate, 5 mM NH_4Cl, 0.25 mM NADH, and 5 U recombinant alanine dehydrogenase from *Bacillus subtilis* (Ye et al. 2010).

NAD(P)H oxidation rate was monitored at 340 nm using a Shimadzu UV1700 spectrophotometer (Japan). All kinetic parameters were calculated using the Enzyme Kinetics Module for SigmaPlot 12. Apparent K_m and V_{max} values were determined by measuring the activity with different concentrations of one substrate at a saturation concentration of the other (Fig S4). In the case of inhibition by substrate, only non-inhibitory concentrations were used for calculations.

Phylogenetic analysis

The full-length amino acid sequences of Sgas from the protein databases of the National Center for Biotechnology Information (NCBI) were used for phylogenetic analysis. The sequences were aligned using Clustal X software (ver. 1.8) (Thompson et al. 1997) followed by the manual removal of gaps. The phylogenetic tree was generated with the MEGA 5 program using Neighbor-Joining method. The trees generated using Maximum Parsimony and UPGMA methods had similar topologies.

Sga20Z gene disruption

The *sga20Z* gene was knocked out by the insertion of the kanamycin cassette using double homologous recombination as described previously (Mustakhimov et al. 2010). Briefly, the DNA fragments flanking the *sga* gene were amplified by PCR from the genomic DNA using primers sga-up-F and sga-up-R for the upstream region and sga-dw-Fand sga-dw-R for the

downstream region (Table S1). The fragments were cloned into the plasmid pCM184 (Marx and Lidstrom 2002) upstream and downstream of the kanamycin cassette, and the resulting vector was introduced into *Mm. alcaliphilum* 20Z cells by conjugation with *E. coli* strain S17-1 (Simon et al. 1983). Mutant colonies were obtained on selective media with kanamycin and the insertion was additionally confirmed by PCR and sequencing.

Results

Cloning of the *sga* genes and purification of recombinant proteins

Three open reading frames (ORFs) were found by BLAST analysis in the genomes of *Mm. alcaliphilum* 20Z, *Ms. trichosporium* OB3b and *Mc. capsulatus* Bath. Their translated amino acid sequences had an appreciable homology (57-62% of identity) with the previously characterized Sga from *H. methylovorum* GM2 (Izumi et al. 1990a, b, c). The ORFs were expressed in *E. coli* and the appropriate recombinant enzymes were purified. SDS-PAGE revealed a single band of about 43 kDa for each recombinant enzyme, which was in agreement with the theoretically calculated subunit molecular masses of Sga20Z (41.7 kDa), SgaOB3b (43.0 kDa) and SgaBath (43.0 kDa) (Fig S1). The native molecular masses of the enzymes were estimated by non-denaturing pore-limited gradient acrylamide gel electrophoresis and by gel filtration. Sga20Z was found to be a dimer with a molecular mass of about 80-90 kDa, while SgaOB3b displayed a tetrameric structure ($\sim$ 170 kDa). An unexpected molecular mass of about 120 kDa suggested a trimer structure was observed for the *Mc. capsulatus* Sga (Fig S1).

Properties of recombinant serine-glyoxylate aminotransferases

All three enzymes catalysed the pyridoxal phosphate-dependent transamination of serine to hydroxypyruvate in the presence of glycine (SGAT activity), being maximally active at pH 7.0 (SgaBath and SgaOB3b) or 7.5 (Sga20Z). The temperature optima of these enzymes were significantly higher than the optimal growth temperatures of the parental strains (Fig S2). The three enzymes also demonstrated a serine-pyruvate aminotransferase (SPAT) activity; Sga20Z and SgaBath additionally catalysed the transfer of an amino group from serine to α-ketoglutarate (SKAT) (Fig S3). Alanine-glyoxylate aminotransferase (AGAT) activity was also observed for Sga20Z and SgaBath, while SgaOB3b demonstrated only a trace activity with alanine as amino donor (Fig S3). Glyoxylate at a concentration > 2.5 mM inhibited Sga20Z and SgaBath in the AGAT reaction but had no effect on the SGAT reaction. In addition, α-ketoglutarate at a concentration above 12.5 mM inhibited both Sga20Z and SgaBath in the SKAT reaction (Fig. 1).

The kinetic studies showed that the SGAT activity of the enzymes from all three methanotrophs under study were at least 10-fold higher than the AGAT, SKAT or SPAT activities. The enzymes of the three methanotrophs demonstrated rather similar properties during the catalysis of the SGAT reaction. In the SPAT reaction, in contrast, the catalytic efficiency for SgaOB3b was at least fivefold higher than for Sga20Z and SgaBath. At the same time, the parameters of Sga20Z and SgaBath in the AGAT and SKAT reactions were comparable (Table 1, Fig S4). Citrate and inorganic pyrophosphate inhibited the activities of SgaOB3b and SgaBath but had no effect on the Sga20Z activity (Table 2).

The sga20Z mutant characterisation

A *Mm. alcaliphilum* strain with an inactivated *sga* gene was obtained to estimate the significance of the serine cycle in methanotrophs utilising the RuMP pathway. The SGAT activity was not detected in the crude extract of mutant cells but was measured to be 10-20 nmol of hydroxypyruvate formed per minute per mg of protein in the wild type strain. Thus, Sga is the only enzyme providing SGAT activity in *Mm. alcaliphilum* 20Z. The growth rate of the Δsga strain on methane (0.07 ± 0.01 h^{-1}) was significantly lower compared to the wild type strain (0.117 ± 0.006 h^{-1}). During growth on methanol, this effect was even more pronounced: 0.13 ± 0.03 h^{-1} for wild type strain compared to 0.03 ± 0.01 h^{-1} for Δsga (Fig. 2). During growth on methanol, formaldehyde was accumulated up to 2.5–3.5 mM by both strains in the exponential phase. In the stationary phase, the wild type strain completely consumed the produced formaldehyde, while it still could be found in the

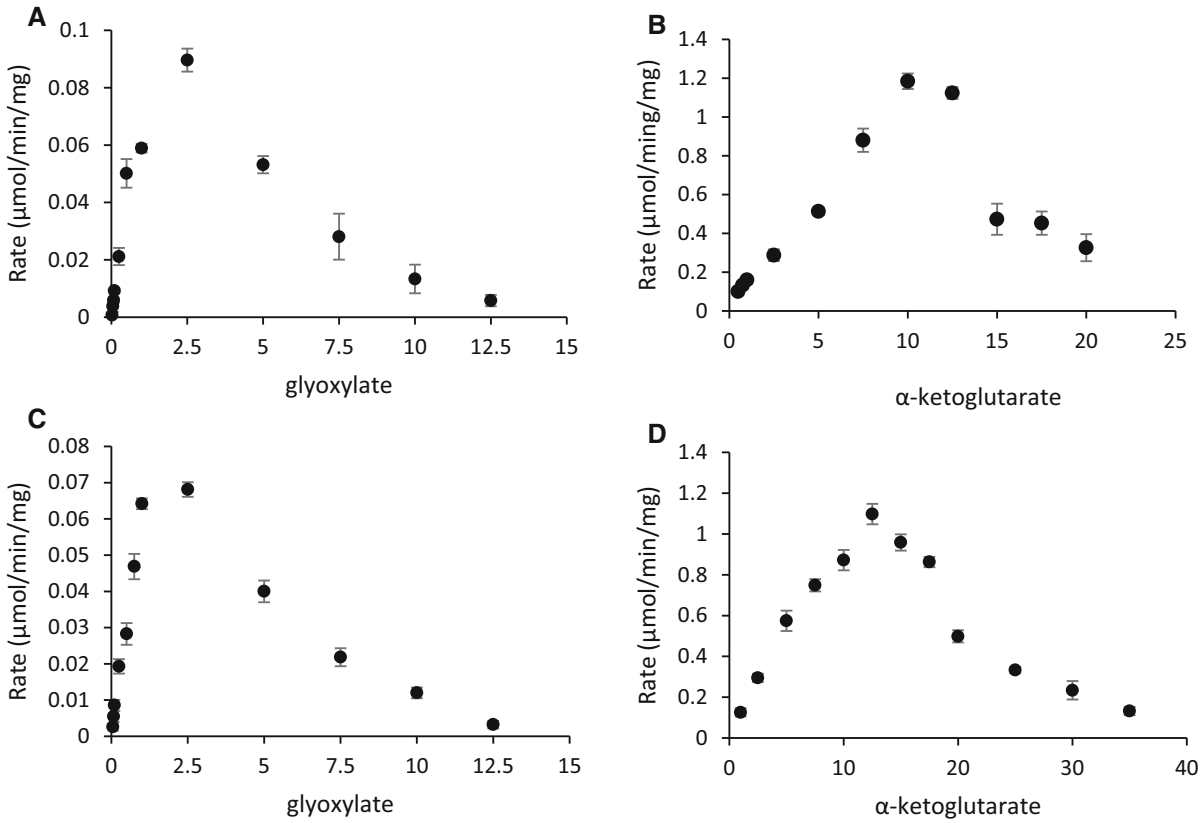

Fig. 1 Dependence of the AGAT and SKAT activities of Sga from *Mm. alcaliphilum* 20Z (**a**, **b**) and *Mc. capsulatus* Bath (**c**, **d**) on glyoxylate and α-ketoglutarate concentrations

growth medium of the mutant (Fig. 2). At the same time, there was neither formaldehyde formation from methanol nor disappearance of added formaldehyde during incubation of the medium without the cells. When transferred from methane to methanol, the mutant cells demonstrated a prolonged lag-phase of about 96 h (data not shown). These data indicate that the serine cycle is apparently involved in the removal of excess formaldehyde formed during growth on methanol.

Phylogeny

The phylogenetic analysis revealed that the Sga of methanotrophs and non-methanotrophic methylotrophs belong to class IV aminotransferases (according to Mechta et al. 1993) along with alanine-glyoxylate aminotransferases (AGT) from plants, animals and bacteria (data not shown). There is 54.4% identity between the amino acid sequences of Sgas from *Mm. alcaliphilum* 20Z, *Ms. trichosporium* OB3b and *Mc. capsulatus* Bath. The AGT from *Arabidopsis thaliana* (Lipman and Olsen 2001) demonstrated a 55–56% amino acid sequence identity with the Sgas from the three methanotrophs under study. The AGT from *Homo sapiens* (Selini et al. 2007) shared a 30–32% identity with these enzymes. In the phylogenetic tree, Sga20Z and SgaBath formed a separate clade together with the enzymes from type I methanotrophs and from the genus *Methylocaldum* (X type) (64–99% identity). The Sgas from *Ms. trichosporium* OB3b and other type II methanotrophs belong to another clade (70-98% identity) clustering together with the enzymes from methylotrophs unable to grow on methane (68-73% identity) and those from representatives of the genus *Paraburkholderia* (69%) (Fig. 3). Interestingly, plant Sga formed a sister clade with the enzymes of type I methanotrophs, while cyanobacterial Sga were an outgroup (Fig. 3).

Table 1 Kinetic properties of the serine-glyoxylate aminotransferases from methanotrophs

Substrate	V_{max}, U/mg	K_m, mM	n[a]	V_{max}/K_m
Methylomicrobium alcaliphulim 20Z				
Serine	13.0 ± 0.3	2.6 ± 0.3		5 ± 0.9
Glyoxylate		0.24 ± 0.01		54.4 ± 0.7
Alanine	0.13 ± 0.01	14 ± 2		0.0088 ± 0.0013
Glyoxylate		1.0 ± 0.2		0.133 ± 0.017
Serine	1.63 ± 0.05	1.17 ± 0.14		1.41 ± 0.13
α-ketoglutarate		5 ± 0.1	1.7	0.326 ± 0.035
Serine	0.226 ± 0.003	0.089 ± 0.008		2.55 ± 0.19
Pyruvate		6 ± 1	0.8	0.039 ± 0.006
Methylosinus trichosporium Ob3b				
Serine	20.7 ± 0.3	2.1 ± 0.1		9.87 ± 0.33
Glyoxylate		0.17 ± 0.01		122 ± 5
Alanine	Trace	–		–
Glyoxylate		–		–
Serine	2.7 ± 0.1	0.18 ± 0.02		15 ± 1
Pyruvate		5.8 ± 0.5	1.4	0.47 ± 0.03
Methylococcus capsulatus Bath				
Serine	20.0 ± 0.2	1.62 ± 0.09		12.3 ± 0.5
Glyoxylate		0.7 ± 0.2		31 ± 9
Alanine	0.10 ± 0.02	23 ± 6		0.0044 ± 0.0003
Glyoxylate		0.59 ± 0.07	1.5	0.165 ± 0.015
Serine	1.16 ± 0.01	1.06 ± 0.05		1.09 ± 0.04
α-ketoglutarate		4.3 ± 0.5	1.8	0.27 ± 0.03
Serine	0.30 ± 0.01	0.089 ± 0.003		3.39 ± 0.02
Pyruvate		4.5 ± 0.3	1.4	0.067 ± 0.002

[a]n—the Hill coefficient. Applied when reaction obeys the Hill kinetic

Discussion

Aerobic methanotrophs assimilate carbon via three pathways: the ribulose monophosphate and ribulose bisphosphate cycles, and the serine pathway. These pathways have been thought to be alternative in methanotrophs belonging to the classes *Alpha-* or *Gammaproteobacteria* or the phylum *Verrucomicrobia*. The exceptions were the so-called type X methanotrophs (*Gammaproteobacteria*), i.e., representatives of the genera *Methylococcus* and *Methylocaldum* simultaneously having three C_1 assimilation pathways (Baxter et al. 2002; Eshinimaev et al. 2004). The BLAST analysis of methanotrophic genomes confirmed the presence of the genes encoding the serine cycle enzymes in all alpha- and gammaproteobacterial methanotrophs, with the single exception of *Methylomagnum ishizawai* possessing genes for the RuMP and CBB cycles but lacking the complete serine cycle genes (Frindte et al. 2017). The methanotrophs of the phylum *Verrucomicrobia* assimilate carbon at the CO_2 level through the CBB cycle and lack the most of the specific serine cycle enzymes such as glycerate kinase, hydroxypyruvate reductase and malyl coenzyme A lyase (Khadem et al. 2012).

This paper is the first report of the functionality and properties of the crucial serine pathway enzyme, serine-glyoxylate aminotransferase, from *Mm. alcaliphilum* 20Z, *Ms. trichosporium* OB3b and *Mc. capsulatus* Bath representing type I, type II and type X methanotrophs, respectively. Sgas from the methanotrophs and non-methanotrophic methylotrophs belong to class IV aminotransferases along with AGAT from plants, animals and bacteria (Mechta et al. 1993). The sequences of the Sgas from strains 20Z, Bath and OB3b have a rather high sequence identity. In the genomes of these strains, the *sga* genes are located in the gene clusters coding for other serine

Table 2 The influence of metabolites on the activities of the recombinant serine-glyoxylate aminotransferases from *Mm. alcaliphilum* 20Z, *Ms. trichosporium* OB3b and *Mc. capsulatus* Bath

	Concentration (mM)	Relative activity (%)[a]		
		Sga20Z	SgaOb3b	SgaBath
ATP	5	15 ± 4	11 ± 2	8 ± 2
ADP	5	17 ± 3	17 ± 3	17 ± 3
Glucose	5	96 ± 5	93 ± 4	102 ± 5
Fructose	5	86 ± 6	93 ± 6	104 ± 4
Glucose-6P	5	73 ± 3	83 ± 1	75 ± 3
Fructose-6P	5	97 ± 3	90 ± 9	87 ± 2
Fructose-1,6P_2	5	100 ± 2	84 ± 7	87 ± 2
Glucose-1P	5	91 ± 2	97 ± 3	91 ± 4
Pyruvate	5	87 ± 4	76 ± 3	76 ± 7
Oxaloacetate	5	81 ± 3	82 ± 1	46 ± 8
α-ketoglutatate	5	96 ± 1	53 ± 4	42 ± 3
Citrate	5	97 ± 4	74 ± 1	62 ± 2
Malate	5	80 ± 3	90 ± 1	92 ± 1
CoA	1	104 ± 6	95 ± 3	100 ± 6
Acetyl-CoA	1	102 ± 6	99 ± 1	103 ± 7
Pi	5	95 ± 3	98 ± 4	104 ± 6
PPi	1	93 ± 7	68 ± 3	60 ± 3
Phosphoenolpyruvate	5	69 ± 12	65 ± 5	23 ± 4
Ribulose-5P	1	98± 3	86 ± 3	90 ± 2
Ribulose-1,5P_2	1	94 ± 2	95 ± 4	95 ± 2
Glycerate	5	76 ± 1	75 ± 2	81 ± 4

[a]Relative activity—percentage of the activity without an effector

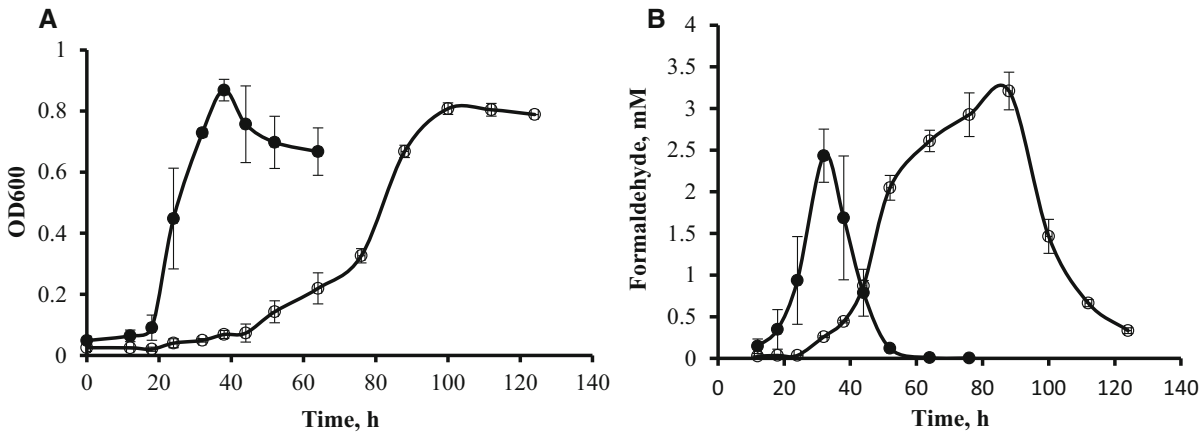

Fig. 2 Growth of the wild type (20Z, solid circles) and mutant (Δsga, empty circles) strains of *Mm. alcaliphilum* on methanol (**a**). Formaldehyde accumulation in the culture media of the strains (**b**)

cycle enzymes (Fig S5). Despite their sequence similarity, SgaOB3b is phylogenetically distant from Sga20Z and SgaBath but close to the enzymes from the methanotrophs and non-methanotrophic methytrophs using solely the serine pathway (Fig. 3). A similar pattern was observed for other enzymes involved in the serine cycle: hydroxypyruvate reductases (But et al. 2017), malate dehydrogenases (Rozova et al. 2015), glycerate kinases, malyl-CoA lyases and malate thiokinases (our unpublished data). We

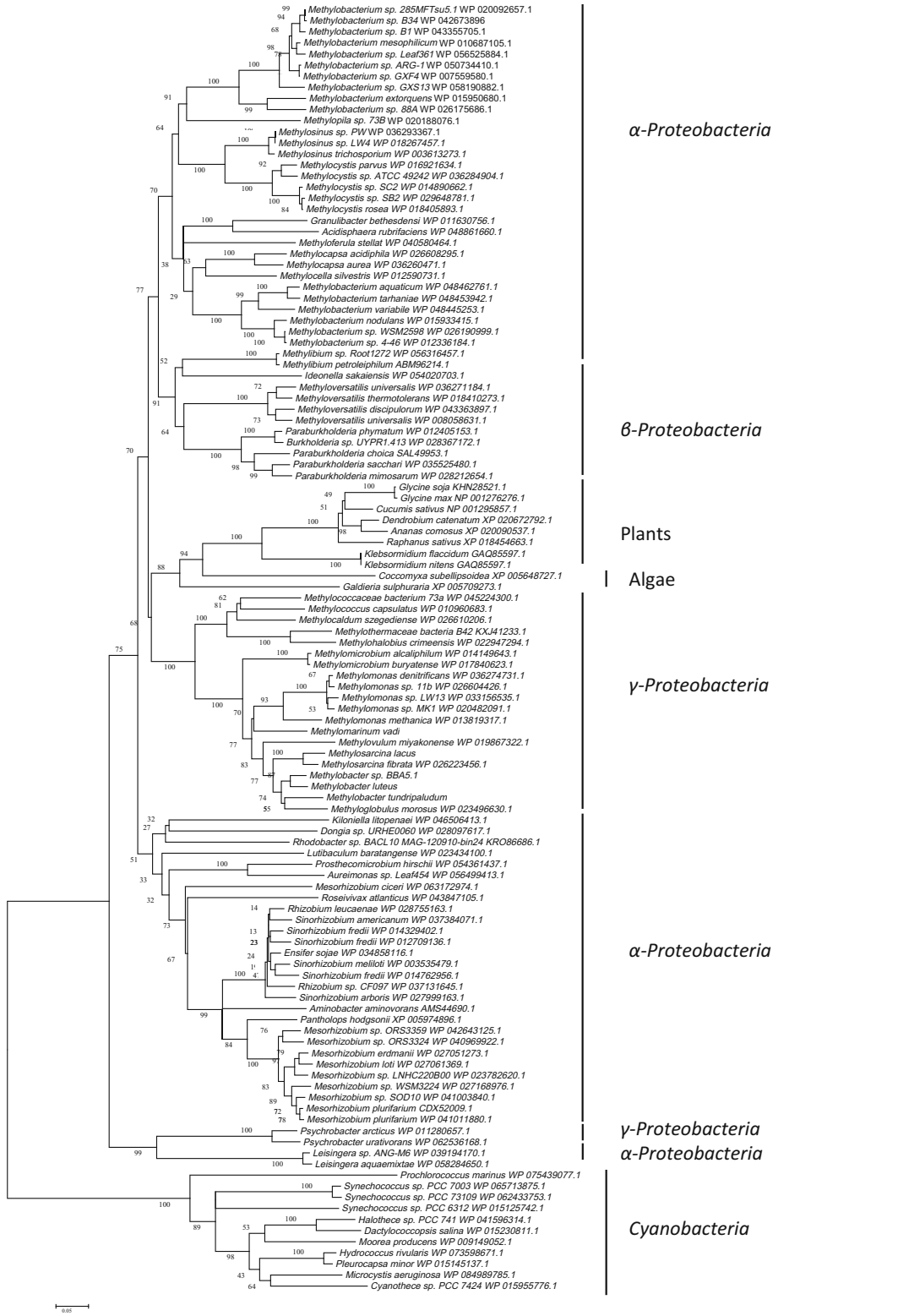

Fig. 3 Phylogenetic tree of serine-glyoxylate aminotransferases

may speculate that the serine cycle genes evolved in different ways in the organisms using the serine cycle as a single assimilation pathway and in those employing the RuMP and/or Calvin cycles. Intriguingly, the plant Sgas are located together with the enzymes from *Gammaproteobacteria* in the phylogenetic tree, thus implying horizontal gene transfer. Probably, plants gained aminotransferases from *Proteobacteria* rather than from *Cyanobacteria*, as was proposed (Kern et al. 2011).

The recombinant Sga obtained from the three methanotrophs demonstrated different subunit structures. Sga20Z was defined as a dimer, while SgaOB3b exhibited a tetramer structure. The molecular mass of SgaBath obtained both by native electrophoresis in pore-limited gels and by gel filtration correlated with the trimer structure. However, the trimer structure has not been found for any Sga characterised to date. At the same time, the Sga from *H. methylovorum* is a tetramer (Izumi et al. 1990a, b, c), while most plant Sgas are dimers (Kendziorek and Paszkowski 2008; Paszkowski and Niedzielska 1990; Truszkiewicz and Paszkowski 2005). Recently it has been shown that Hprs from strains 20Z and Bath, as well as malate dehydrogenase (MaDH) from *Mm. alcaliphilum* 20Z, are dimers, while Hpr and MaDH from *Ms. trichosporium* OB3b are tetramers (But et al. 2017; Rozova et al. 2015). These enzymes are involved in the serine cycle in methanotrophs along with Sga. It is possible that the apparent molecular mass of SgaBath is different from the real one. Crystallographic study may help determine the true enzyme structure, though no crystal structure of Sga has been resolved to date.

In addition to the regeneration of glycine needed for the functional serine cycle, Sga in methanotrophic cells can participate in other reactions, since they display the AGAT, SPAT and SKAT activities. The recombinant enzymes show the highest activity in the reaction between serine and glyoxylate compared to the other activities, which is in agreement with their function to provide circulation of the serine assimilation pathway. It is noteworthy that the kinetic properties of the enzymes in the SGAT reaction were similar. Therefore, we may speculate that Sga is not a point for regulation in the serine cycle in the methanotrophs belonging to different types. On the contrary, earlier we have shown that the properties of hydroxypyruvate reductase, the enzyme catalysing the next step of the serine cycle, significantly vary in the strains 20Z, OB3b and Bath (But et al. 2017).

The disruption of the *sga* gene in *Mm. alcaliphilum* resulted in retardation of the growth rate of mutant cells and in a prolonged lag-phase after passaging from methane to methanol. In addition, the growth of the mutant strain was accompanied by higher formaldehyde accumulation in the culture liquid compared to the wild type (Fig. 2). Hence, the serine cycle in type I methanotrophs could be related to the removal of excess formaldehyde and/or energy regulation. However, the mutant growth retardation might not be a result of the direct toxic effect of formaldehyde. Potentially, the knock-out of the energy-consuming serine cycle causes the excess accumulation of NAD(P)H formed during methanol and formaldehyde oxidation, which in turn causes an imbalance in growth and energy metabolism. Interestingly, the *sga* gene has recently been knocked out in the methanotroph *Methylomicrobium buryatense* 5GB1, which is closely related to *Mm. alcaliphilum* 20Z. The mutant showed no changes in the growth phenotype; however, growth on methanol was not tested (Fu et al. 2017). The role of the serine cycle in metabolism of strains 20Z and 5GB1 does not seem to be drastically different; hence, it might depend on growth conditions including the source of carbon.

It is unclear if the AGAT, SPAT and SKAT activities of the Sga from methantrophs have any physiological effects, because they are too low (at least 10-fold lower than the SGAT activity). In contrast, for the enzyme purified from *A. thaliana* leaves the ratio of AGAT:SGAT activities was 1:1.8 (Kendziorek and Paszkowski 2008). However, this enzymes expressed in *E. coli* demonstrated AGAT and SPAT activity of 14 and 17% of the SGAT activity, respectively (Liepman and Olsen 2001). For human AGT, the SGAT activity was about 30% of its main activity, but affinity to serine was threefold lower than that for alanine (Cellini et al. 2007). In plants, the glycine-producing aminotransferases participate in photorespiration, the process preventing carbon deprivation due to the oxygenase activity of ribulose-bisphosphate carboxylase leading to phosphoglycolate formation. Phosphoglycolate is converted to glyoxylate, which is transformed into glycine by aminotransferase and is involved in further metabolism (Legood et al. 1995; Liepman and Olsen 2001). However, among the methanotrophs tested, only *Mc. capsulatus*

Bath has Rubisco activity. We may speculate that the aminotransferase activities might re-direct the carbon flow to some other reactions. For example, alanine dehydrogenase, which is encoded in the genomes of all the methanotrophs under study, converts pyruvate to alanine. Interestingly, the AGAT activity was observed only for Sga from *Mm. alcaliphilum* 20Z and *Mc. capsulatus* Bath. These methanotrophs implement the RuMP pathway coupled with glycolysis and the Entner–Doudoroff pathway (Kalyuzhnaya et al. 2013; Trotsenko and Murrell 2008), where pyruvate is the central metabolite. Therefore, the AGAT activity could compensate for the excess conversion of pyruvate to alanine. Further comparative studies are in progress to elucidate the exact metabolic roles of the auxiliary serine cycle in different methanotrophs.

Acknowledgements The authors are grateful to all members of the Organization for Methanotroph Genome Analysis for collaboration (OMeGA), the U.S. Department of Energy Joint Genome Institute and Genoscope for the access to methanotrophic genomes for comparative analyses. This work was supported by Russian Foundation for Basic Research# 17-04-01113-a.

Author's contribution SY But designed the experiments, coordinated the study, carried out the mutant generation. SV Egorova carried out purification and characterization of the enzymes, and cultures growth measurements. SY But, VN Khmelenina and YA Trotsenko contributed to data analysis and manuscript preparation. All authors reviewed and approved the final manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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