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Regular paper

Activity and kinetic properties of phosphotransacetylase from intestinal sulfate-reducing bacteria

Ivan V. Kushkevych✉

Laboratory of Molecular Biology and Clinical Biochemistry, Institute of Animal Biology of NAAS of Ukraine, Lviv, Ukraine

Phosphotransacetylase activity and the kinetic properties of the enzyme from intestinal sulfate-reducing bacteria *Desulfovibrio piger* and *Desulfomicrobium* sp. has never been well-characterized and has not been studied yet. In this paper, the specific activity of phosphotransacetylase and the kinetic properties of the enzyme in cell-free extracts of both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 intestinal bacterial strains were presented at the first time. The microbiological, biochemical, biophysical and statistical methods in this work were used. The optimal temperature and pH for enzyme reaction was determined. Analysis of the kinetic properties of the studied enzyme was carried out. Initial (instantaneous) reaction velocity (V_0), maximum amount of the product of reaction (P_{max}), the reaction time (half saturation period, τ) and maximum velocity of the phosphotransacetylase reaction (V_{max}) were defined. Michaelis constants (K_m) of the enzyme reaction (3.36 ± 0.35 mM for *D. piger* Vib-7, 5.97 ± 0.62 mM for *Desulfomicrobium* sp. Rod-9) were calculated. The studies of the phosphotransacetylase in the process of dissimilatory sulfate reduction and kinetic properties of this enzyme in intestinal sulfate-reducing bacteria, their production of acetate in detail can be perspective for clarification of their etiological role in the development of the humans and animals bowel diseases. These studies might help in predicting the development of diseases of the gastrointestinal tract, by providing further details on the etiology of bowel diseases which are very important for the clinical diagnosis of these disease types.

Key words: sulfate-reducing bacteria, phosphotransacetylase, kinetic analysis, inflammatory bowel diseases

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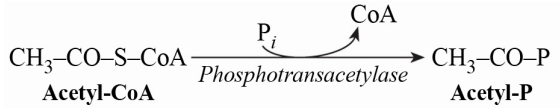
INTRODUCTION

Dissimilatory sulfate-reducing bacteria reduce inorganic sulfate or other oxidized sulfur forms to sulfide (Barton & Hamilton, 2010). This bacteria are heterotrophs and therefore, require an organic carbon source. In the case of *Desulfovibrio* and *Desulfomicrobium* genera, this carbon source can be supplied by simple organic molecules such as lactate, pyruvate, and malate. These are subsequently oxidized to acetate with the concurrent reduction of sulfate to sulfide (Rowan *et al.*, 2009; Kushkevych, 2012a).

The process of organic compounds oxidation is a complex and multistage that provides the bacterial cells with energy (Kushkevych, 2012b). The lactate is the most common substrate used by the species belonging to the sulfate-reducing bacteria (Barton & Hamilton, 2010). This compound is oxidized to acetate *via* pyruvate (Sadana, 1954; Kushkevych, 2012a).

In our previous researches, we have demonstrated that lactate was oxidized incompletely to acetate by the intestinal sulfate-reducing bacteria *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 (Kushkevych, 2013). Lactate oxidation to acetate occurs with the intermediate compounds formation: pyruvate, acetyl-CoA and atsetylfosfat (Kushkevych, 2012a).

One important step in this degradative pathway involves the transfer of an acetyl group from acetyl-S-CoA to orthophosphate to form acetyl-PO_4. The acetyltransferase catalyzing this reaction is phosphotransacetylase (acetyl-S-CoA: orthophosphate acetyltransferase, EC 2.3.18) (Kushkevych, 2012a):



In the presence of sulfate, lactate in human intestine contributes to the intensive bacteria growth and the accumulation of their final metabolism product, hydrogen sulfide, which is toxic, mutagenic and cancerogenic to epithelial intestinal cells (Pitcher & Cummings, 2003; Rowan *et al.*, 2009; Gibson *et al.*, 1991; Kushkevych, 2012a). The increased number of the sulfate-reducing bacteria and intensity of dissimilatory sulfate reduction in the gut can cause inflammatory bowel diseases of humans and animals (Cummings *et al.*, 2003; Gibson *et al.*, 1991; Loubinoux *et al.*, 2000; Kushkevych, 2012b).

As far as it is aware, phosphotransacetylase from intestinal sulfate-reducing bacteria *D. piger* and *Desulfomicrobium* has never been well-characterized. In literature, there are some data on phosphotransacetylase in various organisms as well as in the sulfate-reducing bacteria isolated from environment (Goldman, 1958; Reichenbecher & Schink 1997; Robinson & Sagers, 1972; Sadana, 1954; Shimizu, *et al.*, 1969). However, the data on activity and the kinetic properties of this enzyme from intestinal sulfate-reducing bacteria *Desulfovibrio piger* and *Desulfomicrobium* sp. has not been reported yet.

✉e-mail: ivan.kushkevych@gmail.com
Abbreviations: EDTA, ethylenediaminetetraacetate; SRB, sulfate-reducing bacteria

The aim of this work was to study phosphotransacetylase activity in cell-free extracts of intestinal sulfate-reducing bacteria *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 and to carry out the kinetic analysis of enzymatic reaction.

The aim was accomplished using microbiological, biochemical, biophysical methods, and statistical processing of the results; the obtained data were compared with those from the literature.

MATERIALS AND METHODS

Objects of the study were sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the healthy human large intestine and identified by the sequence analysis of the 16S rRNA gene (Kushkevych, 2013; Kushkevych *et al.*, 2014).

Bacterial growth and cultivation. Bacteria were grown in a nutrition-modified Kravtsov-Sorokin's liquid medium (Kushkevych & Moroz, 2012). Before seeding bacteria in the medium, 0.05 ml/l of sterile solution of $Na_2S{\times}9H_2O$ (1%) was added. A sterile 10N solution of NaOH (0.9 ml/l) in the medium was used to provide the final pH 7.2. The medium was heated in boiling water for 30 min in order to obtain an oxygen-free medium, and then cooled to 30°C. The bacteria were grown for 72 hours at 37°C under anaerobic conditions. The tubes were brim-filled with medium and closed to provide anaerobic conditions.

Obtaining cell-free extracts. Cells were harvested at the beginning of the stationary phase, centrifuged and suspended in 100 ml of 50 mM Tris(hydroxymethyl) aminomethane (Tris)-hydrochloride, pH 7.0 (henceforth referred to as Tris buffer), containing 1 mM ethylenediaminetetraacetate (EDTA). A suspension of cells (150–200 mg/ml) was obtained and homogenized using the ultrasonic disintegrator at 22 kHz for 5 minutes at 0°C to obtain cell-free extracts. The homogenate was centrifuged for 20 min at 16 000 *g* to remove the cell debris. The pellet was then used as the sedimentary fraction, and the supernatant obtained was termed the soluble fraction. The supernatant fluid and a Tris buffer wash of the pellet were subjected to a second centrifugation at 16 000 *g* for 40 min (Robinson & Sagers, 1972). The soluble extract constituted by the supernatant was used as the source of the enzyme. A pure supernatant, containing the soluble fraction, was then used as a cell-free extract. Protein concentration in the cell-free extracts was determined by the Lowry method (Lowry *et al.*, 1951).

Assays for phosphotransacetylase activity. The phosphotransacetylase activity was assayed by measuring acetyl-P arsenolyzed in the presence of CoA as described previously in paper (Shimizu *et al.*, 1969). A reaction mixture, containing 6 μmoles of acetyl-P, 15.8 nmoles of CoA (5 Lipmann units), 5 μmoles of cysteine, 20 μmoles of Tris-HC1 (pH 8.0), 50 μmoles of potassium arsenate (pH 8.0) and the enzyme in a final volume of 1 ml was incubated at 25°C for 12 min. The enzyme was diluted 10–250 times with 50 mM Tris-HC1 (pH 8.0) before incubation, and 50 μl of the solution were added to the mixture. One unit of phosphotransacetylase is defined as the amount of the enzyme which catalyzes the decomposition of μmole of acetyl-P under the specified conditions. Specific enzyme activity was expressed as U × mg^{-1} protein. The specific activity of the studied enzyme in the cell-free extracts of both bacterial strains under the effect of different temperature (20, 25, 30, 35, 40, 45°C) and pH (5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0) in the incubation medium was measured.

Kinetic analysis. Kinetic analysis of the enzyme reaction was performed in a standard incubation medium (as it was described above) with modified physical and chemical characteristics of the respective parameters (the incubation time, substrate concentration, temperature and pH). The kinetic parameters characterizing the phosphotransacetylase reaction are the initial (instantaneous) reaction velocity (V_0), maximum velocity of the reaction (V_{max}), maximum amount of the reaction product (P_{max}) and characteristic reaction time (time half saturation) τ were determined. The amount of the reaction product was calculated stoichiometrically. The kinetic parameters characterizing phosphotransacetylase reactions such as Michaelis constant (K_m) and maximum reaction velocity of substrate decomposition were determined by Lineweaver-Burk plot (Keleti, 1988). For analysis of the substrate kinetic mechanism of phosphotransacetylase, initial velocities were measured under standard assay conditions with different substrate concentrations. The resulting data were also analyzed by global curve fitting in SigmaPlot (Systat Software, Inc.) to model the kinetic data for rapid equilibrium rate equations describing ordered sequential, $V=(V_{max}\ [A]\ [B])/(K_A\ K_B+K_B\ [A]+[A]\ [B])$, and random sequential, $V=(V_{max}\ [A]\ [B])/(a\ K_A\ K_B+K_B\ [A]+K_A\ [B]+[A]\ [B])$, kinetic mechanisms, where V is the initial velocity, V_{max} is the maximum velocity, K_A and K_B are the K_m values for substrates A and B, respectively, and a is the interaction factor if the binding of one substrate changes the dissociation constant for the other (Segal, 1975).

Statistical analysis. Kinetic and statistical calculations of the results were carried out using the software MS Office and Origin computer programs. The research results were treated by the methods of variation statistics using Student's *t*-test. The equation of the straight line that the best approximates the experimental data was calculated by the method of least squares. The absolute value of the correlation coefficient r was from 0.90 to 0.98. The significance of the calculated parameters of line was tested by the Fisher's *F*-test. The accurate approximation was when $P \leq 0.05$ (Bailey, 1995).

RESULTS AND DISCUSSION

Specific activity of phosphotransacetylase, an important enzyme in the process of organic compounds oxidation in sulfate-reducing bacteria, was measured in different fractions obtained from *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 cells (Table 1). Results of our study showed that the highest specific activity of the enzyme was detected in cell-free extracts (1.19 ± 0.122 and 0.37 ± 0.041 U × mg^{-1} protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively). The slightly lower values of activity of phosphotransacetylase were determined in the soluble fraction compared to the cell-free extracts. Its values designated 0.87 ± 0.091 U × mg^{-1} protein for *D. piger* Vib-7 and 0.32 ± 0.036 U × mg^{-1} protein for *Desulfomicrobium* sp. Rod-9. The enzyme activity in sedimentary fraction was not observed.

The effect of temperature and pH of the reaction mixture on phosphotransacetylase activity in the cell-free extracts of the sulfate-reducing bacteria was studied (Fig. 1). The maximum specific activity for both bacterial strains was determined at 30…35°C. The highest enzyme

Table 1. Phosphotransacetylase activity in different fractions obtained from the bacterial cells

Sulfate-reducing bacteria	Specific activity of the enzyme (U × mg^{-1} protein)		
	Cell-free extract	Individual fractions	
		Soluble	Sedimentary
Desulfovibrio piger Vib-7	1.19 ± 0.122	0.87 ± 0.091	0
Desulfomicrobium sp. Rod-9	0.37 ± 0.041***	0.32 ± 0.036**	0

Comment: The assays were carried out at a protein concentration of 41.17 mg/ml (for *D. piger* Vib-7) and 38.12 mg/ml (for *Desulfomicrobium* sp. Rod-9). Enzyme activity was determined after 10 min incubation. Statistical significance of the values M ±m, n = 5; $^{**}P < 0.01$, $^{***}P < 0.001$, compared to *D. piger* Vib-7 strain.

activity of phosphotransacetylase for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 was measured at pH 7.5…8.5. The enzyme activity exhibited typical bell-shaped curves as a function of temperature and pH.

Thus, temperature and pH optimum of this enzyme was 30…35ºC and pH 7.5…8.5, respectively. An increase or decrease in temperature and pH led to a decrease of the activity of studied enzyme in the cell-free bacterial extracts of the sulfate-reducing bacteria.

To study the characteristics and mechanism of phosphotransacetylase reaction, the initial (instantaneous) reaction velocity (V_0), maximum velocity of the reaction (V_{max}), maximum amount of reaction product (P_{max}) and reaction time (τ) were defined. Dynamics of reaction product accumulation was studied for investigation of the kinetic parameters of phosphotransacetylase (Fig. 2).

Experimental data showed that the kinetic curves of phosphotransacetylase activity have tendency to saturation (Fig. 2A). Analysis of the results allows to reach the conclusion that the kinetics of phosphotransacetylase activity in the sulfate-reducing bacteria was consistent to the zero-order reaction in the range of 0–3 min (the graph of the dependence of product formation on the incubation time was almost linear in this interval of time). Therefore, the duration of the incubation of bacterial cells extracts was 5 min in subsequent experiments.

Amount of product of phosphotransacetylase reaction in the *D. piger* Vib-7 was higher (15.43 ± 1.61 μmol × mg^{-1} protein) compared to the *Desulfomicrobium* sp. Rod-9 (4.56 ± 0.47 μmol × mg^{-1} protein) in the entire range of time factor. The basic kinetic properties of the reaction in the sulfate-reducing bacteria were calculated by linearization of the data in the {P/t; P} coordinates (Fig. 2B, Table 2).

The kinetic parameters of phosphotransacetylase from both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were significantly different. Values of initial (instantaneous) reaction velocity (V_0) for the enzyme was calculated by the maximal amount of the product reaction (P_{max}). As shown in Table 2, V_0 for phosphotransacetylase reaction was slightly higher (5.68 ± 0.58 μmol × min^{-1} × mg^{-1} protein) in *D. piger* Vib-7 compared to *Desulfomicrobium* sp. Rod-9 (2.14 ± 0.23 μmol×min^{-1}×mg^{-1} protein). In this case, the values of the reaction time (τ) were more similar for the studied enzyme in both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 strains. Based on these data, there is an assumption that the *D. piger* Vib-7 can consume lactate ion much faster in their cells than a *Desulfomicrobium* sp. Rod-9. Moreover, this hypothetical assumption can be also confirmed by obtained data on maximal velocities of accumulation of the final reaction products, where V_{max} for enzyme reaction in *D. piger* Vib-7 were also more intensively compared to *Desulfomicrobium* sp. Rod-9 (Table 3).

The kinetic analysis of phosphotransacetylase activity dependence on concentration of substrate (acetyl-CoA) was carried out. The increasing of acetyl-CoA concentrations from 0.5 to 5.0 mM caused a monotonic rise of the studied enzyme activity and the activity was maintained on unchanged level (plateau) under substrate concentrations over 3.0 mM. (Fig. 2C). Clearly, the enzyme was saturated with substrate and the higher concentrations (3.0–5.0 mM acetyl-CoA) did not affect its activity, so the activity was maintained on unchanged (plateau) level.

Curves of the dependence {1/V; 1/[S]} were distinguished by the tangent slope and intersect the vertical axis in one point (Fig. 2D). The basic kinetic parameters

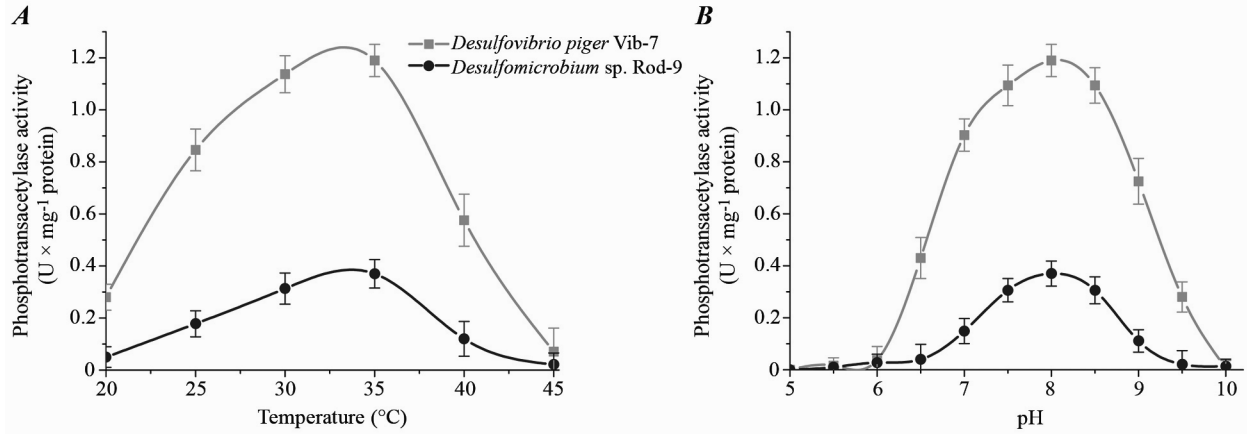


Figure 1. The effect of temperature (A) and pH (B) on the phosphotransacetylase activity in the cell-free extracts of the intestinal sulfate-reducing bacteria

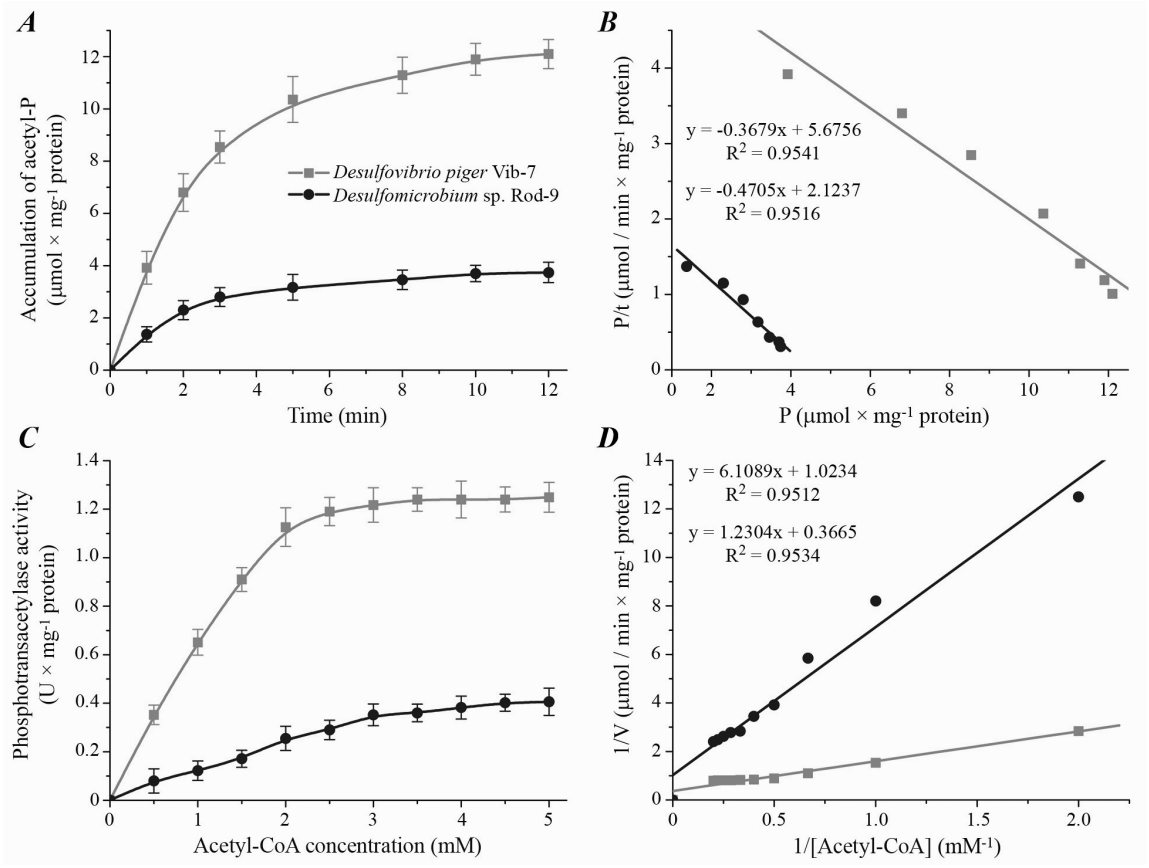


Figure 2. Kinetic parameters of phosphotransacetylase activity in *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9
(**A**) dynamics of product accumulation (M± m, n = 5); (**B**) linearization of curves of product accumulation in {P/t; P} coordinates (n = 5; $R^2 > 0.95$; $F < 0.02$); (**C**) the effect of different concentrations of substrate (Acetyl-coenzyme A) on the enzyme activity (M±m, n = 5); (**D**) linearization of concentration curves, which are shown in Fig. 2C, in the Lineweaver-Burk plot, where V is velocity of the enzyme reaction and [Acetyl-coenzyme A] is substrate concentration (n = 5; $R^2 > 0.95$; $F < 0.005$).

of phosphotransacetylase activity in *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were identified by linearization of the data in the Lineweaver-Burk plot (Table 3).

Calculation of the kinetic parameters of enzyme activity indicates that the maximum velocities (V_{max}) of acetyl-CoA in the *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were significantly different from each other ($P < 0.001$). The values of K_m were also quite different for acetyl-CoA (3.36±0.35, 5.97±0.62 mM) in both *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 strains, respectively.

The described results of the phosphotransacetylase activity and the kinetic properties of the enzyme in cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 strains are new and have never reported in the literature before. These obtained studies were differed significantly from previously described by Sadana (1954). A soluble enzyme system from *Desulfovibrio desufuricans* which catalyses the conversion of two moles of pyruvate to one mole of acetyl phosphate, one mole of ethyl alcohol, and two moles of CO_2 was described. The system required inorganic phosfate for pyruvate dissimilation. Pyrophosphate and arsenate could replace inorganic phosphate. The reaction was most rapid at pH 6.4. The optimum phosphate concentration was 12 M. The requirement of phosphate for the metabolism of pyruvate by the bacterial extract suggests the formation of acetyl coensyme A as an intermediate which is converted to acetyl phosphate and coenzyme A in the presence of inorganic phosphate by transacetylase. The effect is not due to hydrolysis of pyrophosphate to inorganic phosphate since the extracts show no appreciable pyrophosphatase activity (Sadana, 1954).

The phosphotransacetylase activity was also studied in crude extracts of *Escherichia coli* K-12 by Goldman in 1958. A little later, in 1969, Shimizu *et al.* have obtained and purified the phosphotransacetylase from crude extracts of *Escherichia coli* B with the use of ammonium

Table 2. Kinetic parameters of the phosphotransacetylase from intestinal sulfate-reducing bacteria

Kinetic parameters	Sulfate-reducing bacteria	
	Desulfovibrio piger Vib-7	*Desulfomicrobium* sp. Rod-9
V_0 (μmol × min^{-1} × mg^{-1} protein)	5.68±0.58	2.14±0.23**
P_{max} (μmol × mg^{-1} protein)	15.43±1.61	4.56±0.47***
τ (min)	2.72±0.29	2.12±0.22

Comment: V_0 is initial (instantaneous) reaction velocity; P_{max} is maximum amount (plateau) of the product of reaction; τ is the reaction time (half saturation period). Statistical significance of the values M ±m, n = 5; $^{**}P < 0.01$, $^{***}P < 0.001$, compared to the *D. piger* Vib-7 strain.

Table 3. Kinetic parameters of phosphotransacetylase reaction

Kinetic parameters	Sulfate-reducing bacteria	
	Desulfovibrio piger Vib-7	*Desulfomicrobium* sp. Rod-9
$V_{max}^{Acetyl\text{-}CoA}$ (μmol × min^{-1} × mg^{-1} protein)	2.73 ± 0.31	0.98 ± 0.089***
$K_m^{Acetyl\text{-}CoA}$ (mM)	3.36 ± 0.35	5.97 ± 0.62*

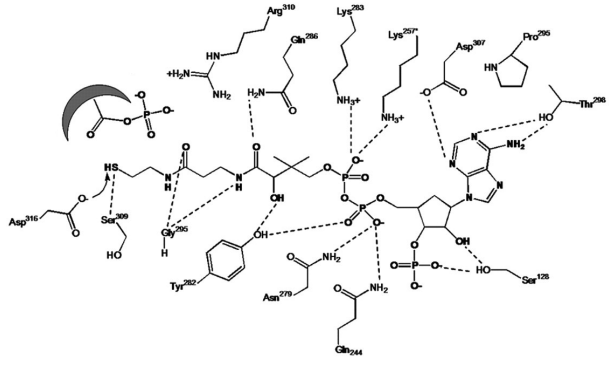
Comment: V_{max} is maximum velocity of the enzyme reaction; K_m is Michaelis constant which was determined by substrate (acetyl-CoA). Statistical significance of the values M ± m, n = 5; *$P < 0.05$, ***$P < 0.001$, compared to the *D. piger* Vib-7 strain.

sulfate as a stabilizer. The purified enzyme was homogeneous in ultracentrifugal analysis and molecular weight was tentatively estimated from the s value. K_m values of this enzyme were 3×10^{-3} and 4×10^{-3} M for acetyl phosphate and 3.2×10^{-4} M for CoA (Shimizu *et al.*, 1969). The values of calculated K_m of acetyl-CoA for phosphotransacetylase reaction in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were slightly different to those previously described by Shimizu *et al.* (1969)

Robinson and Sagers (1972) have measured the activity of phosphotransacetylase from *Clostridium acidiurici*. Authors showed that the phosphotransacetylase has two properties not observed for this enzyme in other bacteria: it required a divalent metal for activity, and it was not subject to uncoupling by arsenate. The enzyme was obtained in highly purified form, with a specific activity 500-fold higher than crude extracts (Robinson & Sagers, 1972).

The described data were also different from results described by Reichenbecher and Schink (1997) for phosphotransacetylase activity from the *Desulfovibrio inopinatus*. Authors demonstrated that the activity was 44 mU (mg protein)$^{-1}$ in extracts of ethanol-grown cells, while it was below 3 mU (mg protein)$^{-1}$ after growth with hydroxyhydroquinone or lactate (Reichenbecher and Schink,1997).

Lawrence and co-authors (2006) have reported structural and functional studies suggesting a catalytic mechanism for the phosphotransacetylase from *Methanosarcina thermophila*. Two crystal structures of phosphotransacetylase from the methanogenic archaeon *M. thermophila* in complex with the substrate CoA revealed one CoA (CoA1) bound in the proposed active site cleft and an additional CoA (CoA2) bound at the periphery of the cleft. Kinetic and calorimetric analyses of site-specific replacement variants indicated that there are catalytic roles for Ser309 and Arg310, which are proximal to the reactive sulfhydryl of CoA1. The reaction is hypothesized to proceed through base-catalyzed abstraction of the thiol proton of CoA by the adjacent and invariant residue Asp316, followed by nucleophilic attack of the thiolate anion of CoA on the carbonyl carbon of acetyl phosphate. Authors have proposed the mechanism of the reaction catalyzed by phosphotransacetylase from *M. thermophila* (Lawrence *et al.*, 2006):



Scheme of the mechanism of the phosphotransacetylase reaction (by Lawrence *et al.*, 2006, modified)

Perhaps, the proposed mechanism for phosphotransacetylase from the methanogenic archaeon *M. thermophila* and the description given by Lawrence and co-authors (2006) may be similar to the studied enzymes from *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9. However, to confirm this hypothetical assumption, the enzyme should be purified from cell-free extracts to study its structure and properties in detail.

CONCLUSIONS

The phosphotransacetylase, an important enzyme in process of dissimilatory sulfate reduction and lactate oxidation in sulfate-reducing bacteria, carries out the central step in oxidative decarboxylation of acetyl-CoA to acetyl-P.

The enzyme activity, V_0 and V_{max} were significantly higher in the *D. piger* Vib-7 cells than *Desulfomicrobium* sp. Rod-9. However, Michaelis constants for acetyl-CoA were quite higher (5.97 ± 0.62 mM) in *Desulfomicrobium* sp. Rod-9 strain compared to *D. piger* Vib-7. The maximum enzyme activity for both strains was determined at +30…35ºC and at pH 7.5…8.5. The kinetic parameters of enzyme reaction are depended on the substrate concentration.

The studies of the phosphotransacetylase in the process of dissimilatory sulfate reduction and kinetic properties of this enzyme in the *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 intestinal strains, their production of acetate and hydrogen sulfide in detail can be perspective for clarification of their etiological role in the development of the humans and animals bowel diseases. Data on the activity and kinetic properties of this enzyme in the strains can be useful to predict the velocity of the accumulation of the final products of metabolism of these bacteria, hydrogen sulfide and acetate, which are formed in the process of dissimilatory sulfate reduction. Assessing rate of formation of these dangerous products in the gut, we are able to predict their toxicity and occurrence of bowel diseases.

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REFERENCES

Bailey NTJ (1995) *Statistical Methods in Biology*. Cambridge: Cambridge University Press, 252 p.

Barton LL, Hamilton WA (2010) *Sulphate-Reducing Bacteria. Environmental and Engineered Systems*. Cambridge: Cambridge University Pres, 553 p.

Cummings JH, Macfarlane GT, Macfarlane S (2003) Intestinal Bacteria and Ulcerative Colitis. *Curr Issues Intest Microbiol* **4**: 9–20.

Gibson GR, Cummings JH, Macfarlane GT (1991) Growth and activities of sulphate-reducing bacteria in gut contents of health sub-

jects and patients with ulcerative colitis. *FEMS Microbiol Ecol* **86**: 103–112.
Goldman DS (1958) Purification of phosphotransacetylase from *Escherichia coli* K-12. *Biochim Biophys Acta* **28**: 436–437.
Keleti T (1988) *Basic Enzyme Kinetics*. Akademiai Kiado, 422 p.
Kushkevych IV (2012a) Sulfate-reducing bacteria of the human intestine. I. Dissimilatory sulfate reduction. *Sci Int J Biol Studies/Studia Biol* **6**: 149–180.
Kushkevych IV (2012b) Sulfate-reducing bacteria of the human intestine. II. The role in the diseases development. *Sci Int J Biol Studies/Studia Biol* **6**: 221–250.
Kushkevych IV, Moroz OM (2012) Growth of various strains of sulfate-reducing bacteria of human large intestine. *Sci Int J Biol Studies/Studia Biol* **6**: 115–124.
Kushkevych IV (2013) Identification of sulfate-reducing bacteria strains of human large intestine. *Sci Int J Biol Studies/Studia Biol* **7**: 115–124.
Kushkevych IV, Bartos M, Bartosova L (2014) Sequence analysis of the 16S rRNA gene of sulfate-reducing bacteria isolated from human intestine. *Int J Curr Microbiol Appl Sci* **3**: 239–248.
Lawrence SH, Luther KB, Schindelin H, Ferry JG (2006) Structural and functional studies suggest a catalytic mechanism for the phosphotransacetylase from *Methanosarcina thermophila*. *J Bacteriol* **188**: 1143–1154.
Loubinoux J, Mory F, Pereira IA, Le Faou AE (2000) Bacteremia caused by a strain of *Desulfovibrio* related to the provisionally named *Desulfovibrio fairfieldensis*. *J Clin Microbiol* **38**: 931–934.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein determination with the Folin phenol reagent. *J Biol Chem* **193**: 265–275.
Pitcher MC, Cummings JH (1996) Hydrogen sulphide: a bacterial toxin in ulcerative colitis? *Gut* **39**: 1–4.
Reichenbecher W, Schink B (1997) *Desulfovibrio inopinatus*, sp. nov., a new sulfate-reducing bakterium that degrades hydroxyhydroquinone (1,2,4-trihydroxybenzene). *Arch Microbiol* **168**: 338–344.
Robinson JR and Sagers RD (1972) Phosphotransacetylase from *Clostridium acidiurici*. *J Bacteriol* **112**: 465–473.
Rowan FE, Docherty NG, Coffey JC, O'Connell PR (2009) Sulphate-reducing bacteria and hydrogen sulphide in the aetiology of ulcerative colitis. *British J Surgery* **96**: 151–158.
Sadana JC (1954) Pyruvate oxidation in *Desulphovibrio desulphuricans*. *J Bacteriol* **67**: 547–553.
Segal IH (1975) *Enzyme kinetics: behavior and analysis of rapid equilibrium and steady-state enzyme systems*. John Wiley & Sons, New York.
Shimizu M, Suzuki T, Kameda K, Abiko Y (1969) Phosphotransacetylase of *Escherichia coli* B, purification and properties. *Biochim Biophys Acta* **191**: 550–558.