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ORIGINAL ARTICLE

ATP sulfurylase activity of sulfate-reducing bacteria from various ecotopes

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Abstract

Sulfate-reducing bacteria (SRB) are widespread in various ecotopes despite their growth and enzymatic features not compared. In this study, the enzymatic parameters of ATP sulfurylase in cell-free extracts of sulfate-reducing bacteria isolated from various ecotopes such as soils, corrosion products and human large intestine were determined. Comparative analysis of both enzyme characteristics and growth parameters were carried out and similar research has not been reported yet. The initial and maximum rates of enzymatic reaction catalyzed by ATP sulfurylase were significantly different ($p < 0.05$) in the bacterial strains isolated from various environmental ecotopes. The specific activity of this enzyme in sulfate-reducing bacteria was determined for corrosive and intestinal strains 0.98–1.56 and 0.98–2.26 $U \times mg^{-1}$ protein, respectively. The Michaelis constants were 1.55–2.29 mM for corrosive and 2.93–3.13 mM for intestinal strains and the affinity range were demonstrated. Based on cluster analysis, the parameters of physiological and biochemical characteristics of sulfate-reducing bacteria from different ecotopes are divided into 3 clusters corresponding to the location of their isolation (soils, heating systems and human intestine). Understanding the enzymatic parameters of the initial stages of sulfate consumption in the process of dissimilatory sulfate reduction will allow the development of effective methods for controlling the production of toxic metabolites, including hydrogen sulfide.

Keywords Sulfate-reducing bacteria · ATP sulfurylase · Cell-free extracts · Ecotopes

Introduction

The sulfate-reducing bacteria (SRB) are widespread in various ecological areas, including marsh, anaerobic depths of reservoirs, sediments (Posgate 1984), as well as intestine of humans and animals (Kushkevych et al. 2019a, Coutinho et al. 2017; Kováč et al. 2018). Sulfate-reduction pathway by SRB is started from the activation of the sulfates that is a nonreactive stable compound and must be activated to participate in subsequent metabolic reactions (Kushkevych 2015b; Kushkevych et al. 2015a, b, 2016). Before sulfate will be reduced, it is transported into bacterial cells through active transport using ATP energy (Barton and Hamilton 2010; Kushkevych et al. 2017a, 2018d). The reaction is catalyzed by the cytoplasmic enzyme ATP sulfurylase (*sat,* sulfate adenylyltransferase, EC 2.7.7.4) which transfers sulfate ions to the adenosine monophosphate moiety of ATP to form adenosine 5′-phosphosulfate (APS) and pyrophosphate (PP_i) (Sperling et al. 1998; Mander et al. 2002; Phartiyal et al. 2006; Kushkevych 2015a, b). The sulfate activation to APS increases the redox potential of the first step in the dissimilatory sulfate reduction from −516 mV to −60 mV (Barton and Hamilton 2010). Among the enzymes of the dissimilatory sulfate reduction pathway, APS reductase (*aps*, EC 1.8.4.9) and dissimilatory sulfite reductase (*dsr,* EC 1.8.99.3) are suitable indicators of this process in the environment. Since the first step is also involved in the assimilatory sulfate reduction, so ATP sulfurylase plays an important role in sulfate

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transport of dissimilatory and assimilatory sulfate reduction (Kushkevych 2015a).

The dissimilatory ATP sulfurylase has been purified and characterized mainly from various SRB such as *Desulfovibrio desulfuricans* ATCC 27,774, *D. gigas* (Gavel et al. 1998), *Desulfotomaculum nigrificans* (Akagi 1981), *D. sulfidodismutans* and *D. desulfuricans* CNS (Kramer and Cypionka 1989). The dissimilatory homo-oligomeric ATP sulfurylase was also described from sulfur-oxidizing purple sulfur bacteria *Allochromatium vinosum* (Parey 2013), from the hyperthermophilic bacteria *Archaeglobus fulgidus* (Sperling et al. 1998) and *Escherichia coli* K-12 (Ming 1997), etc. Structurally, this enzyme appears as a metalloprotein that consists of identical subunits containing cobalt and zinc, they are homotrimers with molecular weights of 147, 141 kDa, respectively. However, this enzyme has different subunits in the bacteria *E. coli* (Barton and Hamilton 2010).

Despite bacterial sulfate dissimilation pathway, ATP sulfurylase is involved in sulfate assimilation. This enzyme has been founded in the cells of many different organisms, such as yeast: *Saccharomyces cerevisiae* and *Komagataella pastoris* (Linder 2017), fungi: *Penicillium duponti* and *Penicillium chrisogenum* (Resonto et al. 1985), plants: cabbage leaves (Osslund et al. 1982), spinach (Resonto et al. 1993), and soybean *Glycine max* (Herrmann 2014). ATP sulfurylase is involved in the transport of sulfate in the cell and in the processes of dissimilation/assimilation of sulfur-containing compounds. Due to large heterogeneity of physiological and adaptation features of SRB (Woordow 1995), the potential ecological function of the SRB in the environments could be differ significantly from SRB isolated from soils and water. They are involved in the global sulfur cycle, especially in the dissimilation processes. In the man-caused systems these bacteria shifted to a corrosive agent and is involved in the microbial corrosion processes (Barton and Hamilton 2010). SRB from the intestine are revealed as competitors of intestinal *Clostridia*, which assimilate sulfate (Coutinho et al. 2017). In view of this fact that the kinetic properties of enzymatic reactions in SRB with different ecological role and isolated from various ecotopes have never been compared and described, therefore this research is relevant. The aim of the research focused on the comparative analysis of enzymatic activity of the ATP sulfurylase from biomass and the growth characteristics of SRB isolated from various ecotopes.

Materials and methods

Bacterial cultures and cultivation

Sulfate-reducing bacteria (SRB) isolated from various ecotopes, including soil, corrosion products, and human feces. Isolation, purification and identification were previously described in the works (Postgate 1966; Asaulenko 2010; Purish 2014). Collection strains *Desulfovibrio desulfuricans* DSM642, *Desulfovibrio vulgaris* DSM644 (GenBank: AF418179.1) were isolated from corrosion products and soil and obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ collection) (Germany) and *Desulfovibrio* sp. 10 (UCM B-11503) (GenBank: KC886400) were isolated from corrosion products of steel construction of DniproHES and obtained from the Ukrainian Collection of Microorganisms at D.K. Zabolotny Institute of Microbiology and Virology of NAS of Ukraine (Kyiv, Ukraine) as well as corrosion strains: *Desulfovibrio* sp. TC2 (UCM B-11504), *Desulfotomaculum* sp. TC3 (UCM B-11505) and *Desulfomicrobium* sp. TC4 (UCM B-11506) were isolated from heat system construction's corrosion products (Kyiv, Ukraine). The intestinal SRB strains, *Desulfovibrio piger* Vib-7 (GenBank: KT881309.1) and *Desulfomicrobium orale* Rod-9 (GenBank: MF939896.1), were isolated from the human large intestine (human feces) and obtained from at the Laboratory of Anaerobic Microorganisms of the Department of Experimental Biology at Masaryk University (Brno, Czech Republic).

Cultivation of SRB was performed in liquid modified Postgate's C medium (Postgate 1984). The highest sulfate concentration from 7.2 to 22.69 mM was used in this modified Postgate's C medium (Kováč and Kushkevych 2017). To adjust the pH (7.2–7.5), sterile 10 N solution of NaOH was used. The redox and anaerobic conditions were controlled by resazurin sodium (Oxoid, BR 0055B). Low redox potential (Eh = −100 to −200 mV) for anaerobic condition have been achieved by the addition of 2% ascorbic acid or 2% solution of sodium sulfide (1 ml per liter of cultivation media). The tubes were filled with medium, which were inoculated with SRB cultures (5% vol/vol.), then closed by rubber plug to provide anaerobic conditions. The corrosive SRB were cultivated at + 28 °C for 7 days, the intestinal bacteria were grown at + 37 °C for 3 days.

Cell-free extracts

Cell-free extracts were prepared from the bacterial cell gained from the exponential phase of growth (1 day for intestinal strains, 7 days for corrosive strains). The bacteria were grown anaerobically in modified Postgate's C liquid medium (Kováč and Kushkevych 2017). The cold extraction buffer (5 M EDTA, 50 mM potassium phosphate buffer, pH 7.5) was added to centrifuged sediment cells to bind and depose heavy metal ions. After this procedure, the suspended bacterial cells containing 0.096–0.927 mg of protein × ml^{-1} were obtained. The cells were homogenized using the ultrasonic homogenizer (Bandelin SONOPULS GM 200, Germany) at 20 kHz for 5 min at 0 °C. The soluble fractions were placed

into centrifugal tubes and cell-free extracts were separated from the cell fragments by centrifugation for 30 min at 14 000 × g and at 4 °C (Hettich EBA 12 Centrifuge, Germany). Supernatant was then used as cell-free extracts. Protein concentration in the cell-free extracts was determined by the Bradford method (1976).

Determination of ATP sulfurylase activity

Enzymatic activity of ATP sulfurylase was assayed by molybdenum-dependent formation of phosphate. The reaction started by adding 5, 10, and 15 mM of MoO_4^{2-} (Na_2MoO_4) to the reaction mixture with 100 µl of cell-free extract. The reaction mixture was consisted of Tris–HCl buffer (for corrosive strains pH 7.5, for intestinal strains pH 8.0), Na_2MoO_4 (5, 10, 15 mM), Na_2ATP (2 mM), $MgCl_2$ (7 mM), and inorganic pyrophosphatase (0.33 U × ml^{-1}) (Sigma, USA). The reaction was stopped after 5, 10, and 15 min of incubation at 27 °C (for intestinal strains 35 °C) at termoblock (Biosan TDB-120, Latvia) by adding 1.0 ml of ice cold 0.5 mM sodium acetate (pH 4.0) and 200 µl of developer solution which consist of L-ascorbic acid (200 mg), Na_2MoO_4 (100 mg) in 10 ml of 0.36 M sulfuric acid. After 10 min it standing the absorbance was read at 660 nm (Spectrosonic Genesis 5) against blank Tris–HCl buffer (Osslund et al. 1982). Temperatures and pH were used accordingly to the properties of cultivation medium and for intestinal samples was described in a paper (Kushkevych 2015a, b; Kushkevych et al. 2015b).

Calculation of growth parameters

The growth dynamics were determined by the protein synthesis in cells and biomass accumulation detection during SRB cultivation. Biomass was determined in the liquid medium (without ferric compounds) by the turbidity of dilute cell suspension using biophotometer (Eppendorf) (λ =340 nm, l = mm) (Kushkevych et al. 2015a). The growth parameters were characterized by the following basic constants (Widdel 2010): generation time (G), relative (specific) rate (µ). Generation time (G) was defined as the time (t) per generation of a number of SRB cells at interval time. The absolute growth rate (or doubling rate) was calculated by the number of divisions (generations) per unit time. The absolute growth rate (R) was defined as the number of cells formed per time. The relative (specific) growth rate (µ) was determined by the absolute growth rate related to the population size (Kushkevych et al 2017a).

Calculation of enzyme kinetic characteristics

The study of kinetic properties of enzymes ATP sulfurylase was performed. All experiments used to study the properties of enzyme were performed using the initial rate V_0 (linear accumulation of product (P) in time). The kinetic indicators that characterize ATP sulfurylase, such as Michaelis constant ($K_m^{sulfate}$) and the maximum reaction rate (V_{max}) were determined by the Lineweaver–Burk plot (Keleti 1988). The obtained concentration dependence of the rate of enzymatic reaction was constructed in the coordinates (1/V on 1/S), where S is the concentration of the reagents, and V is the rate of enzymatic reduction of SO_4^{2-} at a concentration of SO_4^{2-} for ATP sulfurylase.

Statistical analysis

Statistical calculations of the results were carried out using the MS Office (2010), Origin 8.0 (https://www.originlab.com) and Statistica 13 (https://www.statsoft.com) software programs. Cluster analysis was performed by the single linkage method with the calculating of the Euclidean distances. Using the experimental data, the basic statistical parameters (mean: M, standard error: m, M ± m) were calculated. The research results were treated by methods of variation statistics using Student's t-test. The significance of the calculated indicators of the line was tested by the Fisher's F-test. The accurate approximation was when $P \leq 0.05$ (Bailey 1995).

Results

The growth parameters of 8 collections, corrosive-relevant and intestine SRB strains isolated from various ecotopes such as soils, corrosion products, heat city systems and human intestine were studied (Table 1). As shown our research, specific growth rate of SRB strains and division (generation) time depend on ecological properties of environment from where they were isolated. The maximal specific growth rate (µ), which correlated with protein synthesis, was 0.052 and 0.056 h^{-1} for *D. piger* Vib-7 and *D. orale* Rod-9 bacterial strains from human intestine, respectively. The time of generation (1.77–1.93 h) for intestinal strains was significantly lower compared with corrosive-relevant SRB from the environment. These strains were less specific growth rate, which was in the range from 0.016 to 0.022 h^{-1}. Furthermore, the lowest parameters of growth rate (0.0084–0.0098 h^{-1}) were calculated for two strains, *D. vulgaris* DSM644 from the collection and corrosive-relevant *Desulfotomaculum* sp. TC3. These bacterial strains have longest generation time (70.15–82.9 h) among all the studied SRB. The time of generation for corrosive strains were significantly higher from 15 to 49 times than in intestinal strains. SRB isolated from human intestine specific growth rate were highest in 3.25–3.5 times than in *D. desulfuricans* DSM642, but the time of generation for intestine cultures

Table 1 Growth parameters of sulfate-reducing bacteria from various ecotopes

Source of isolation (country of origin)	Bacterial strains	μ (hour^{-1})	T_d (hour)	Protein synthesis (μg/ml)
Corrosion product (DSMZ collection, Germany)	*D. desulfuricans* DSM 642	0.024	28.60	1715
Soil (DSMZ collection, Germany)	*D. vulgaris* DSM 644	0.0084	82.9	1200
Corrosion products of steel construction of DniproHES (Ukraine)	*Desulfovibrio* sp. 10	0.022	33.0	2650
Corrosion products and slime from heat system construction (Kyiv, Ukraine)	*Desulfovibrio* sp. TC2	0.016	41.60	1990
	Desulfotomaculum sp. TC3	0.0098	70.15	1600
	Desulfomicrobium sp. TC4	0.018	38.80	2250
Human feces (Brno, Czech Republic)	*D. piger* Vib-7	0.056	1.77	4493
	D. orale Rod-9	0.052	1.93	3814

μ is specific growth rate, T_d is the division (generation) time

were highest in 15–16 times than in the collection and corrosive SRB strains.

The studied growth parameters above described were performed by kinetic analysis of the ATP sulfurylase (Fig. 1). The enzymatic activity of SRB strains depended on the concentration of sulfate in the medium, it was from 0.5 to 1.5 mM. Duration of sulfate accumulation by the studied enzyme isolated from SRB was 10–15 min. According to the results of our research, the APS accumulation were monotonic reached to 3–7 μmol × mg^{-1} protein (for corrosive strains) and up to 15–40 μmol × mg^{-1} protein (for intestinal strains) (Fig. 1a). The linearization of the effect of different concentrations of sulfate in P/t and P coordinates on ATP sulfurylase activity is presented in Fig. 1b. Obviously, intestinal SRB *D. piger* Vib-7 and *D. orale* Rod-9 are capable to consume of sulfate faster compared with corrosive strains, but according to K_m values, the affinity of the enzyme for a given substrate differs in intestinal and environmental strains. The main kinetic parameters of the studied enzymatic reaction were calculated and presented in Table 2.

The highest specific activity (2.26 ± 0.231 U × mg^{-1} protein) was determined in cell-free extracts of *D. piger* Vib-7. The lowest specific activity 0.98 U × mg^{-1} protein was calculated for both strains *D. orale* Rod-9 and *Desulfovibrio* sp. TC2 which were isolated from various ecotopes. The SRB strains from Germany and Ukrainian collections

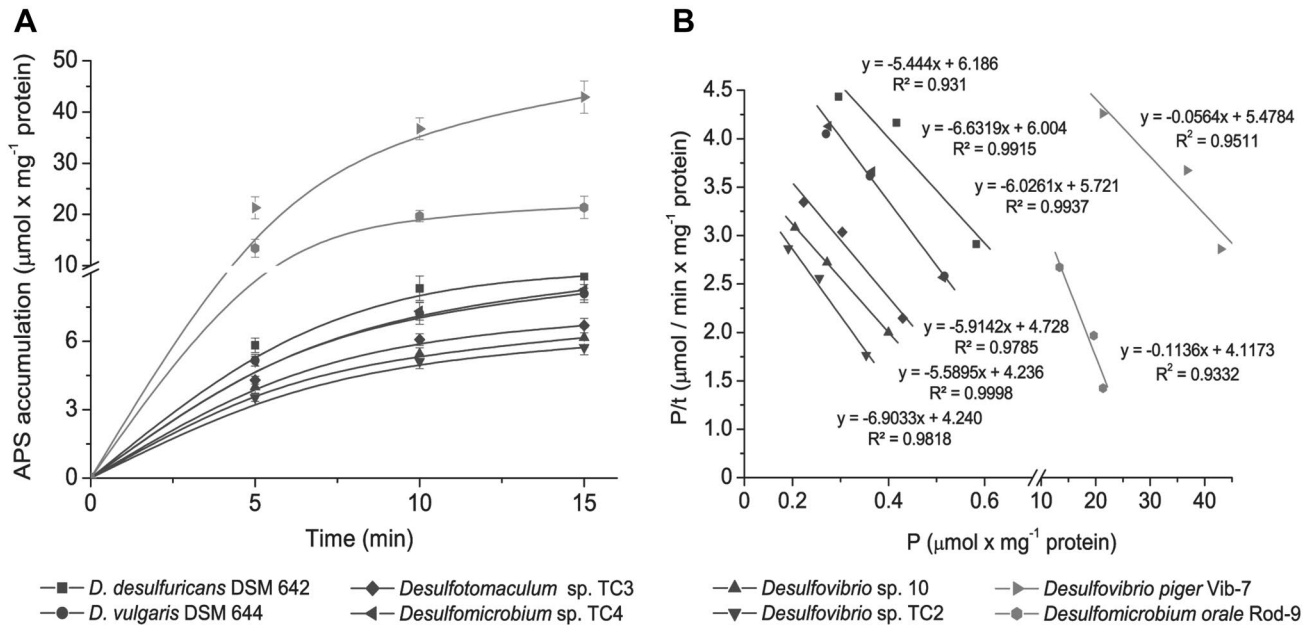

Fig. 1 The dynamics of APS accumulation catalyzed by ATP sulfurylase in cell-free extracts of SRB isolated from different ecotopes (**a**) and the linearization of curves of product accumulation in {P/t; P} coordinates ($n = 3$; $R^2 > 0.93$; $F < 0.02$) (**b**)

Table 2 Kinetic parameters of ATP sulfurylase in cell-free extracts

Bacterial strains	Specific activity (U/mg)	V_0 (umol/min/mg)	V_{max} (umol/min/mg)	K_m (mM)
D. desulfuricans DSM642	1.56 ± 0.019	0.539 ± 0.025	0.78 ± 0.01	2.29 ± 0.24
D. vulgaris DSM644	1.51 ± 0.031	0.54 ± 0.026	0.76 ± 0.015	1.95 ± 0.18
Desulfovibrio sp. 10	1.13 ± 0.0013	0.39 ± 0.001	0.56 ± 0.0067	2.23 ± 0.005
Desulfovibrio sp. TC2	0.98 ± 0.0049	0.37 ± 0.0029	0.49 ± 0.0024	1.60 ± 0.018
Desulfotomaculum sp. TC3	1.27 ± 0.0068	0.48 ± 0.0011	0.64 ± 0.0034	1.55 ± 0.05
Desulfomicrobium sp. TC4	1.36 ± 0.011	0.49 ± 0.0079	0.68 ± 0.0059	1.84 ± 0.048
D. piger Vib-7	2.26 ± 0.231	5.48 ± 0.57	4.73 ± 0.44	2.93 ± 0.26
D. orale Rod-9	0.98 ± 0.0082	4.12 ± 0.38	2.84 ± 0.29	3.13 ± 0.27

V_0 is initial (instantaneous) reaction rate, V_{max} is the maximum rate of the enzyme reaction, K_m is the Michaelis constant which was determined by substrate

of microorganisms had specific enzymatic activity in the range from 1.13 to 0.156 U × mg^{-1} protein; it was less in 30–50% than in *D. piger* Vib-7. Corrosive-relevant SRB isolated from heating system construction had shown average enzyme activity 0.98–1.27 U × mg^{-1} protein among the studied bacteria.

The Michaelis constant (K_m) is the concentration of the substrate at which the reaction rate is equal to half the maximum. This constant is often used to quantify the affinity of the enzyme to the substrate (the smaller the K_m, the greater the affinity) (Sakoda 1976). The data obtained for K_m of ATP sulfurylase reaction were not totally correlated with a specific activity. For example, the highest values of K_m were found in intestinal strains *D. piger* Vib-7 (2.93 ± 0.26 mM) and *D. orale* Rod-9 (3.13 ± 0.27 mM); however strain Rod-9 had lower specific enzyme activity (0.98 U × mg^{-1} protein) and on the other hand Vib-7 had higher specific activity (2.26 U × mg^{-1} protein). Similar data on K_m were obtained in enzymatic reaction of cell-free extract from collection strains of *D. desulfuricans* DSM642, *Desulfovibrio* sp. 10 and *D. vulgaris* DSM644, the values of K_m were 2.29, 2.23 and 1.95 mM, respectively. Thus, the affinity of ATP sulfurylase to sulfate for these bacteria was higher by 27–38% than for intestine strain *D. orale* Rod-9. The high affinity to sulfate had ATP sulfurylase extracted from corrosive strains isolated from heating systems, the K_m values were in a range from 1.55 to 1.84 mM.

The difference between the initial rate (V_0) and the maximum rate (V_{max}) of the enzyme reaction was calculated and shown in the Fig. 2. The initial rate of the catalyzed reaction (V_0)depended on the concentration of the substrate what can be described by the Michaelis–Menten equation (Dowd 1965). The maximum reaction rate (V_{max})observed when the enzyme is completely saturated with the substrate. The difference between maximum and initial enzyme rate were significantly differed for SRB isolated from various environmental biotopes. The enzymatic rate was increased from 0.39–0.54 to 0.56–0.78 umol/min/mg in a reaction catalyzed

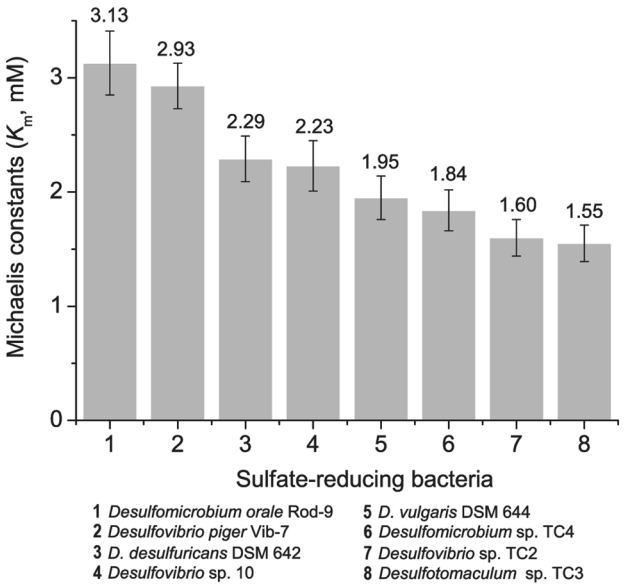

Fig. 2 Affinity range and changes of sulfate accumulation reaction rate for ATP sulfurylase in cell-free extracts from SRB: dV is the difference between the initial rate (V_0) and the maximum rate (V_{max}) of the enzyme reaction; K_m is the Michaelis constant

by ATP sulfurylase for the collection strains of *D. desulfuricans* DSM642, *D. vulgaris* DSM644 and *Desulfovibrio* sp. 10. Similar the trend was observed for corrosive strains of *Desulfovibrio* sp. TC2, *Desulfotomaculum* sp. TC3, *Desulfomicrobium* sp. TC4 isolated from heating systems. In this case, the enzymatic rate was also increased in range from 0.37–0.49 to 0.49–0.68 µmol/min/mg, respectively. However, cardinally other data were obtained from SRB strains of *D. piger* Vib-7 and *D. orale* Rod-9 isolated from human intestine, where enzyme rate decreased from 5.48 to 4.73 umol/min/mg and 4.12 to 2.84 umol/min/mg, respectively. Thus, the maximum rate of the reaction of the collection and corrosive strains was less in 6–8 and 7–10 times, respectively, than in strains isolated from the human intestine. The

initial rate of enzymatic reactions in intestinal SRB was significantly higher ($p < 0.05$) than the maximal rate what may be due to the specificity of this enzyme in intestinal SRB, the rapid consumption of the substrate and, accordingly, saturation of the enzyme by the substrate.

Cluster analysis of all the studied SRB strains was performed using the data gained from a specific growth rate, generation time, protein synthesis, initial and maximum rate and the Michaelis constant. The obtained results showed in the Fig. 3. The studied SRB strains were separated in three clusters: the first one belonged *D. piger* Vib-7 and *D. orale* Rod-9 strains, the second were corrosive strains of *Desulfovibrio* sp. TC2, *Desufomicrobium* sp. TC4 and *Desulfovibrio* sp. 10, and the third cluster joined the collection strains of *D. desulfuricans* DSM642 and *D. vulgaris* DSM644 and corrosion stain of *Desulfotomaculum* sp. TC3.

Thus, a distribution according to the growth and enzymatic characteristics of the strains on the clusters may indicate differences in the functioning and the flow of enzymatic reactions in the bacterial cells. This, in turn, is a consequence of the adaptation of the SRB to the environmental conditions.

Discussion

ATP sulfurylase may function in three different metabolic contexts. In the widely assimilatory sulfate reduction pathway, sulfur is recruited and bounded in amino acids and cofactor biosynthesis. In the dissimilatory sulfate reduction pathways, a few microorganisms consume sulfate in large amounts because this compound can be used as an electron acceptor in energy conversion (Parey 2013; Barton and Hamilton 2010; Kushkevych et al. 2019a). A significant difference in kinetic characteristics of ATP sulfurylase reaction was observed in cell-free extract of SRB studied strains. The kinetic parameters of enzymatic reaction depend on not only

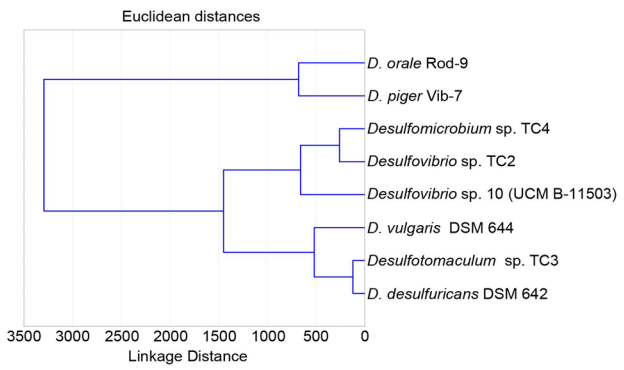

Fig. 3 Cluster analysis between kinetic parameters of ATP sulfurylase reaction and specific growth rate of SRB isolated from different ecotopes

the concentration of substrate, but also the activity of the bacterial strains what could be conditioned by the environment from where bacteria were isolated. Intestinal *D. orale* Rod-9 and corrosive strain of *Desulfovibrio* sp. TC2 had similar activity (0.98 $U \times mg^{-1}$ protein); for other strains, enzyme activity was significantly higher by 13–127% from mentioned above SRB. The enzymatic activities associated with pure ATP sulfurylases have wide range of values from 0.8–1.0 $U \times mg^{-1}$ protein in *D. desulfuricans* and *D. nigrificans* strains (Akagi 1981; Barton and Hamilton 2010) to 1.8–3.8 $U \times mg^{-1}$ protein in soluble fractions from *D. gigas, D. desulfuricans* strains as were described in the paper (Gavel et al. 1998).

According to the Michaelis constant, the high affinity of enzyme to substrate (e.g., sulfate) among SRB studied strains was observed in corrosive-relevant SRB strains *Desulfovibrio* sp. TC2, *Desulfotomaculum* sp. TC3, *Desulfomicrobium* sp. TC4 (K_m was in the range from 1.55 to 1.84 mM). ATP sulfurylase in intestinal SRB strains showed lower affinity to sulfate (K_m was 2.93 and 3.13 mM, see Table 2). Such differences could be explained by the fact that for intestinal SRB strains intend the effect of specific inhibition or unachievable activation site of the enzyme, which characteristics the Michaelis constant. In contrast to corrosive-relevant SRB strains isolated from heating systems for which sulfate was achievable an electron acceptor (Iutynska et al. 2014; Purish et al. 2014) despite intestinal SRB strains more effective used other sulfur-containing compounds (Kushkevych et al. 2017b, 2018a, b, c, d, e, f). It should be noted that for comparing the Michaelis constants (in the range 1.55–3.13 mM) obtained in our research with the literature data, K_m values were 9.5 and 1.03 µM for ATP sulfurylase isolated from *A. vinosum* (Parey et al. 2013) and *Glycine max* (Ravilious et al. 2013). Our data were gained in millimoles and higher in 3 orders compared with the literature results where K_m was mentioned in micromole concentrations. On the other hand, our data on activity of ATP sulfurylases was significantly lower with sulfate-reducing activity in comparison to the activity of plant enzyme isolated from soybeans, K_m values was 14.6 mM (in the reverse reaction of the APS accumulation) (Phartiyal et al. 2006). However, this type of enzyme in the plants works in the opposite direction in the process of the assimilatory sulfate reduction pathway.

A difference between the maximum and initial rate of the enzymatic reaction for SRB strains isolated from different ecotopes was demonstrated. This may be interesting in view of the establishment of mechanisms for the flow of this enzymatic reaction. For strains isolated from human intestine, as it can be seen from our research, the rapid consumption of sulfate and the formation of APS in the initial stages of the process were characteristic. The duration of this process until saturation with the product was determined from 8 to

17 min for intestinal strains. However, the reaction rate for the *D. piger* Vib-7 and *D. orale* Rod-9 strains was only by 69–86% of the initial rate at the end of the enzymatic process. Since this enzyme catalyze both reactions of sulfate activation (dissimilatory/assimilatory sulfate-reduction), it can be assumed that the saturation of direct reaction occurs earlier in intestinal bacteria than in corrosive ones, because the duration process of dissimilatory sulfate reduction was 15–20 min and reverse reaction prevail. This may indicate not only on the rapid consumption of sulfate at the beginning of the process, but also could show to the inhibition of the enzymatic process of sulfate accumulation in the bacterial cells at the end of the reaction. The fact of the specific inhibition, which could be bonded with inactivation of the active site of the enzyme, probably the most raised in intestinal strains and should be also pointed. However, the mechanism of this type of inhibition is still unclear, but the coverage of this issue is relevant.

Also, this may be due to the competition of intestinal SRB with other microorganisms in the intestine as they can compete with methanogens for molecular hydrogen and with other microorganisms for organic compounds which can be for them as energy and a carbon source (Itoh el al. 2002; Kushkevych et al. 2017b, 2018b, 2019b; Černý et al. 2018). Concerning to collection strains of *Desulfovibrio* sp. 10, *D. desulfuricans* DSM642, *D. vulgaris* DSM644 and corrosive-relevant of *Desulfovibrio* sp. TC2, *Desulfotomaculum* sp. TC3, *Desulfomicrobium* sp. TC4, the final enzymatic rate increased by 41–45% and 32–40% compared to initial rate, respectively. Such increasing in the rate of enzymatic reaction in SRB may indicate activation of the process during the reaction. In contrast to intestinal SRB, the strains isolated from soils and heating water systems, undergoing corrosion damage, were dominant in this microbial soil community and may not compete for substrates with methanogens (Iutynska et al. 2014; Itoh et al. 2002). Therefore, it is probably that intestinal SRB strains in the environment where competition with methanogens is possible and also consume sulfate with using ATP sulfurylase have adaptations by increasing the initial rate of sulfate consumption. SRB are biochemically and genetically quite heterogeneous group and they also have a wide range of physiological, adaptive abilities and physiological heterogeneity (Woordow 1995). Thus, the SRB can occupy a lot of habitats with various conditions. Separable classes of ATPase according to their metabolic function are not definable despite the described structural differences and distinguishable kinetic data for the dissimilation and assimilation reaction. Obviously, subtle adjustments in classes of ATP are superimposed by the normal phylogeny of the organisms, including adaptation to the specific environmental conditions (Patron et al. 2008).

However, the question of inhibition of sulfate reduction process by SRB is still relevant. If consider this question in the classical method of sulfate reduction inhibition with the help of nitrites, then it follows that nitrite inhibits the reduction of sulfite to sulfide by *D. vulgaris* in the pathway of enzyme dissimilatory sulfite reductase (encoded by *dsrAB* genes), which slowly reduces nitrite to ammonia, allowing nitrite to serve as a competitive inhibitor. Blockage of *DsrAB* gene may cause accumulation of sulfite in *D. desulfuricans* strain as described by the authors Pires et al. (2003) and Ramos et al. (2012).

Therefore, it is possible that, in addition to competition for substrates with methanogens in the intestinal SRB strains, inhibition of the final stages of sulfate reduction may occur (Kushkevych et al. 2017b, 2018b). In accordance, the accumulation of sulfate activation products in cells and subsequent slow its transformation are carried out. For example, it has been shown that in the intestinal SRB strains, despite the high rate of transport of sulfate through the cell membrane, a low rate of dissimilatory sulfite reductase was observed, compared with the maximum rate of ATP sulfurylase. The elucidation of this issue is currently relevant to further elaborate further the mechanisms and features of the process of sulfate reduction in SRB strains isolated from different ecotops.

Thus, one of the mechanisms of inhibiting the process of dissimilatory sulfate reduction is the non-specific inhibition of the enzymatic reaction of ATP sulfurylase, namely, the slowing or stopping of the transport and the activation of sulfate within the SRB cell, which may result in the switching of SRB from sulfate to nitrate, which excludes the formation of toxic hydrogen sulfide. In addition, for the purpose of sulfate reduction inhibition, there is a promising and perspective to test of activity of newly synthesized compounds against sulfate-reducing bacteria that exhibited high inhibitory effect against SRB (Kushkevych et al. 2015a, 2016, 2018c, e).

Conclusions

The studies of the physiological and biochemical properties of the sulfate-reducing bacteria from different ecotopes, their process of the dissimilatory sulfate reduction, in particular participation of ATP sulfurylase in this process, the activity and kinetic properties of this enzyme as a first step in detail can be a perspective for clarification of the role of these bacteria in the environment in general and their etiological role in human or animal intestine. These studies might help in: I) understanding the mechanisms of the course of enzymatic reactions in the SRB, isolated from different ecotopes, II) predicting of the mechanisms of dissimilatory sulfate reduction, III) the development of methods of control and possible inhibition of the initial stages of sulfate reductase activity, IV) as a consequence

of the reduction or even termination of the production of toxic hydrogen sulfide, which causes undesirable effects.

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Compliance with ethical standards

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

Human and animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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