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DISSIMILATORY SULFITE REDUCTASE IN CELL-FREE EXTRACTS OF INTESTINAL SULFATE-REDUCING BACTERIA

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Dissimilatory sulfite reductase activity in different fractions of the sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from human intestine was studied. Sulfite reductase, an important enzyme in the process of sulfur metabolism in these bacteria, was solubilized from the membrane fraction. The highest activity of the enzyme in the cell-free extract of the bacterial strains was measured (0.032 ± 0.0026 and 0.028 ± 0.0022 U \times mg⁻¹ protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively) compared to other fractions. The optimal temperature (+30...35 °C) and pH 7.0 for sulfite reductase reaction in the extracts of both bacterial strains was determined. The spectral analysis of purified sulfite reductase from cell-free extracts was carried out. The absorption maxima were 284, 391, 412, 583, and 630 nm, as well as 287, 393, 545, and 581 nm for sulfite reductase of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively. Analysis of the kinetic properties of the bacterial sulfite reductase has been carried out. The sulfite reductase activity, initial (instantaneous) reaction rate (V_0) and maximum rate of the sulfite reductase reaction (V_{max}) were higher in the *D. piger* Vib-7 cells than in the *Desulfomicrobium* sp. Rod-9. However, Michaelis constants (K_m) of the enzyme activity were similar for both bacterial strains. The studies of the sulfite reductase activity, the kinetic properties of this enzyme in the intestinal sulfate-reducing bacteria strains, and their production of hydrogen sulfide in detail can be useful for clarification of the etiological role of these bacteria in the development of inflammatory bowel diseases in humans and animals.

Keywords: sulfate-reducing bacteria, sulfite reductase, intestinal microbiocenosis, inflammatory bowel diseases, ulcerative colitis.

INTRODUCTION

Sulfate-reducing bacteria occur in the gut flora of about 50 % of healthy persons where they metabolize hydrogen and low molecular weight organic compounds [4]. High concentration of sulfate in gut creates favorable conditions for the development of the sulfate-reducing bacteria in the human and animal intestine [2, 3]. These conditions are also favorable for process of the dissimilatory sulfate reduction and accumulation of

hydrogen sulfide and acetate which can be cytotoxic to intestinal cells causing various inflammatory bowel diseases. Hydrogen sulfide accumulated in the human intestine is also carcinogenic to its cells and can cause inhibition of cytochrome oxidase, oxidation processes butyrate by colonocytes, and destruction of epithelial cells, develop ulcers, inflammation with subsequent development of colon cancer [2, 10]. The production of highly toxic sulfide from *Desulfovibrio desulfuricans* has been implicated in the onset of a chronic inflammatory large bowel disease, ulcerative colitis, patients with this disease showing elevated levels of sulfide production and a universal carriage rate of the sulfate-reducing bacteria [3, 4].

The process of the dissimilatory sulfate reduction to sulfide occurs due to the formation of many intermediate compounds. One of these intermediates is sulfite, the reduction of which to sulfide is an intermediary step of sulfate reduction in sulfate assimilating organisms [6, 9]. Sulfite reduction is also a terminal step of sulfate reduction and a possible energy-yielding reaction in sulfate-reducing bacteria [7].

Sulfite reductase catalyses the reduction of sulfite to sulfide and forms part of the dissimilatory sulfate reduction pathway [6, 7]. The enzyme from the sulfate-reducing bacteria of the *Desulfovibrio* genus is a hexamer consisting of three different subunits [6, 16, 19, 20]. Contrary to the case of sulfite reduction in sulfate assimilation, Kobayashi et al. have suggested from fractionation experiments that in extracts of *Desulfovibrio vulgaris* sulfite is reduced stepwise to sulfide with intermediary formation of trithionate and thiosulfate [7]. Sulfite reductase, which reacts directly with sulfite in a series of reduction steps from sulfite to sulfide, was purified from *D. vulgaris* and the identity of the enzyme was suggested with a green pigment, desulfovirodin [7, 18]. The demonstration by Lee and Peck (1973) that desulfovirodin is the enzyme responsible for the reduction of sulfite to trithionate raised the problem of the mechanism of sulfite reduction in the Norway strain of *D. desulfuricans* [13, 14]. In this investigation, was reported the purification of a red pigment, which has been provisionally termed desulforubidin, from this anomalous strain of *D. desulfuricans*, and its identification as an enzyme that reduces sulfite mainly to trithionate, analogous to desulfovirodin [6, 7].

As far as it is aware, sulfite reductase from intestinal sulfate-reducing bacteria *D. piger* and *Desulfomicrobium* has never been well-characterized. From literature data, there are a lot of data about sulfite reductase of the sulfate-reducing bacteria isolated from environment [6, 7, 14, 16, 17, 19, 20]. However, the data about activity of this enzyme from intestinal sulfate-reducing bacteria *Desulfovibrio piger* and *Desulfomicrobium* sp. has not been reported yet.

The aim of our work was to study sulfite reductase activity of sulfate-reducing bacteria *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine and to carry out the kinetic analysis of enzymatic reaction.

MATERIALS AND METHODS

Objects of the study were sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine [11] and identified by the sequence analysis of the 16S rRNA gene [12]. The strains are kept in the collection of microorganisms at the Laboratory of Biotechnology, Faculty of Pharmacy, University of Veterinary and Pharmaceutical Sciences Brno (Czech Republic).

Bacterial growth and cultivation. Bacteria were grown in a nutrition-modified Kravtsov-Sorokin's liquid medium with the following composition (g/l): Na₂SO₄ – 0.5; KH₂PO₄ – 0.3; K₂HPO₄ – 0.5; (NH₄)₂SO₄ – 0.2; NH₄Cl – 1.0; CaCl₂×6H₂O – 0.06; MgSO₄×7H₂O – 0.1;

$C_3H_5O_3Na$ – 2.0; yeast extract – 1.0; $FeSO_4 \times 7H_2O$ – 0.004; and sodium citrate $\times 2H_2O$ – 0.3. Before bacteria seeding in the medium, 0.05 ml/l of sterile solution of $Na_2S \times 9H_2O$ (1%) was added. A sterile 10 N 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. Cell-free extracts were prepared from stationary-phase cultures. The cold extraction buffer (50 mM potassium phosphate buffer, pH 7.5, 10^{-5} M EDTA (ethylenediaminetetraacetic acid) was added to centrifuged cells to bind heavy metal ions. A total of 10^{-5} M PMSF (phenylmethylsulfonyl fluoride) for the inhibition of proteases, which is effective at pH above 7.0, was added. After this procedure, a suspension of cells (150–200 mg/ml) was obtained. The cells were homogenized using the ultrasonic disintegrator at 22 kHz for 5 minutes at 0 °C to obtain cell-free extracts. The suspension was displaced into centrifugal tubes and separated from the cells fragments by centrifugation in 30 minutes at 15000 rpm and at +4 °C. The supernatant was used as cell-free extract. Soluble fraction was prepared as described in paper [18]. The spinned cells fragments were used as sedimentary fraction. Protein concentration in the cell-free extracts was determined by the Lowry method [15].

Assays for sulfite reductase activity, desulfovirdin, and desulforubidin. The sulfite reductase activity was assayed manometrically by measuring hydrogen uptake required for sulfite reduction coupled with the hydrogenase methylviologen system. Purification of the enzyme and its spectral analysis were carried out as described in paper [6]. Hydrogen uptake was measured for 5 to 100 min. One unit of enzyme was defined as an amount which consumed 1 μ mole of hydrogen per min in the initial phase of the reaction. Enzyme activity was expressed as $U \times mg^{-1}$ protein. Reaction products (thiosulfate, trithionate, and polythionate containing four or more sulfur atoms) were determined colorimetrically by a modification of the methods described in paper [6, 7]. Desulfovirdin and desulforubidin were measured spectrophotometrically as described in paper [6, 13]. The 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 (4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0) in the medium incubation was measured.

Kinetic analysis. Kinetic analysis of the enzyme reaction was performed in a standard incubation medium [6] with modified physical and chemical characteristics of the respective parameters (the incubation time, substrate concentration, temperature and pH). The kinetic parameters characterizing the sulfite reductase reaction are the initial (instantaneous) reaction rate (V_0), maximum rate of the reaction (V_{max}), maximum amount of the reaction product (P_{max}) and characteristic reaction time (time half saturation) τ were determined [8]. The amount of the reaction product was calculated stoichiometrically. The kinetic parameters characterizing sulfite reductase reactions are Michaelis constant (K_m) and maximum reaction rate of substrate decomposition were determined by Lineweaver-Burk plot [5].

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 calculated by the methods of variation statistics using Student *t*-test. The absolute value of the correlation coefficient *r* was from 0.90 to 0.99. The significance of the calculated parameters of samples was tested by the Fisher's *F*-test. The accurate approximation was when $P \leq 0.05$ [1].

RESULTS AND DISCUSSION

Sulfite reductase, an important enzyme in the process of sulfur metabolism in sulfate-reducing bacteria, was obtained from cells of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9. Activity of sulfite reductase in different fractions including cell-free extract, soluble, and sedimentary was studied (Table 1).

Table 1. Sulfite reductase activity in different fractions obtained from *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 cells

Таблиця 1. Активність сульфїтредуктази у різних фракціях, отриманих з клітин *Desulfovibrio piger* Vib-7 і *Desulfomicrobium* sp. Rod-9

Sulfate-reducing bacteria	Specific activity of sulfite reductase (U×mg ⁻¹ protein)			Desulfovirodin/ *Desulforubidin (Units)
	Cell-free extract	Individual fractions		
		Soluble	Sedimentary	
<i>Desulfovibrio piger</i> Vib-7	0.032±0.0026	0.029±0.0021	0.0012±0.0002	109±9.71
<i>Desulfomicrobium</i> sp. Rod-9	0.028±0.0022	0.024±0.0018	0.0010±0.0001	*102±8.96

Comment: Enzyme activity has been determined after 100 min incubation. Significance of the values M±m, n = 5.

Примітка: Активність ферменту визначена після 100 хв інкубації. Достовірність значень M±m, n = 5.

Results of this study showed that the highest specific activity of the enzyme was measured in cell-free extracts (0.032±0.0026 and 0.028±0.0022 U×mg⁻¹ protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively). Slightly lower activity of sulfite reductase was detected in the soluble fraction compared to cell-free extracts. The lowest enzyme activity was found in sedimentary fraction; its values designated 0.0012±0.0002 and 0.0010±0.0001 U×mg⁻¹ protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively. The level of desulfovirodin and desulforubidin was almost the same in both bacterial strains.

From literature data, it is known that the enzyme activity depends on temperature and pH [2, 7]. The effect of temperature and pH of the incubation medium on the sulfite reductase 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. An increase or decrease in temperature of incubation leads to a decrease of the activity of studied enzyme in the cell-free bacterial extracts. The highest enzyme activity of sulfite reductase was determined in the cell-free extracts of the *D. piger* Vib-7 and the *Desulfomicrobium* sp. Rod-9 at pH 7.0.

Thus, temperature and pH optimum of this enzyme with sulfite as a substrate was +30...35 °C and pH 7.0, respectively. The enzyme activity exhibited typical bell-shaped curves as a function of temperature and pH.

Reaction products (thiosulfate, trithionate, and polythionate containing four sulfur atoms) in the cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were determined. As shown in Table 2, the highest concentration trithionate, 0.54±0.044 and 0.49±0.037 μmoles, was measured in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively.

Accumulation of the final product of the sulfate-reducing bacteria metabolism was 0.47±0.035 μmoles for extracts of *D. piger* Vib-7 and 0.36±0.029 μmoles for *Desulfomic-*

robium sp. Rod-9. The lowest concentration of intermediates was observed for polythionate, its values designated 0.018 ± 0.0014 and 0.012 ± 0.0011 μmoles for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively. Perhaps, trithionate plays an important role in the metabolism of the studied intestinal bacteria among other intermediates. The detection of trithionate and thiosulfate as intermediate compounds during the dissimilatory sulfite reduction process by *Desulfovibrio vulgaris* was first reported by Kobayashi et al. [6]. Subsequently, thiosulfate was shown to accumulate in reaction mixtures containing two fractions isolated from *D. vulgaris* extracts [7].

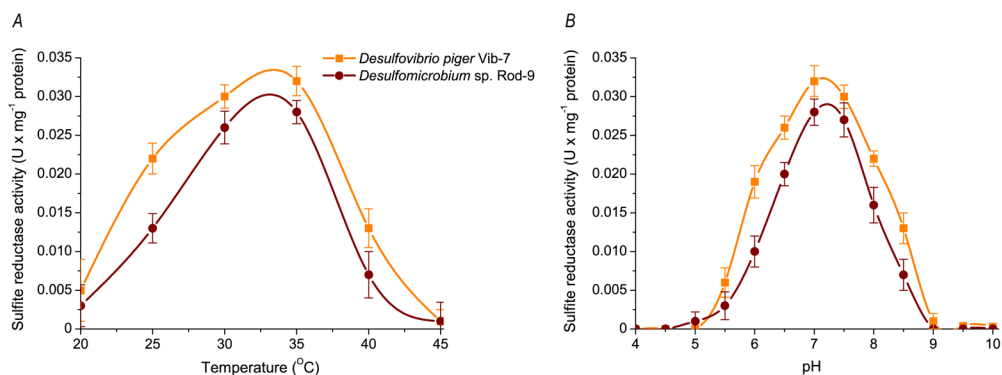


Fig. 1. Effect of temperature (A) and pH (B) on the activity of sulfite reductase in cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9

Рис. 1. Вплив температури (A) і рН (B) на активність сульфїтредуктази у безклїтинних екстрактах *D. piger* Vib-7 і *Desulfomicrobium* sp. Rod-9

Table 2. Sulfite reduction and formation of products reaction in the cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 stains

Таблиця 2. Відновлення сульфїду та утворення продуктів реакції у безклїтинних екстрактах штамів *Desulfovibrio piger* Vib-7 і *Desulfomicrobium* sp. Rod-9

Sulfate-reducing bacteria	Formed compounds (μmoles)				
	$\text{S}_2\text{O}_3^{2-}$	$\text{S}_3\text{O}_6^{2-}$	$\text{S}_4\text{O}_6^{2-}$	H_2S	Total sulfite recovered
<i>Desulfovibrio piger</i> Vib-7	0.21 ± 0.017	0.54 ± 0.044	0.018 ± 0.0014	0.47 ± 0.035	1.24 ± 0.119
<i>Desulfomicrobium</i> sp. Rod-9	0.25 ± 0.019	0.49 ± 0.037	0.012 ± 0.0011	0.36 ± 0.029	1.11 ± 0.107

Comment: 5 μmoles Na_2SO_3 in incubation mixture was added. The formed products were determined at +35 °C and pH 7.0 after 100 min reaction. Significance of the values $M \pm m$, $n = 3$.

Примітка: 5 μmol Na_2SO_3 було внесено до інкубаційної суміші. Утворені продукти визначали за температури +35 °C і рН 7,0 після 100 хв реакції. Достовірність значень $M \pm m$, $n = 3$.

Next task of this study was to carry out a spectral analysis of the purified sulfite reductase from the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9. The absorption maxima were 284, 391, 412, 583, and 630 nm as well as 287, 393, 545, and 581 nm for sulfite reductase of *D. piger* and *Desulfomicrobium* sp. Rod-9, respectively (Fig. 2, A). From literature data, it is known that the visible region of these spectra is quite similar to that of the pigments, desulfovirdin and desulforubidin [6, 13, 18]. Fluorescence spectrum of desulfovirdin in 0.1 N NaOH excited at 365 nm, and absorption

spectrum of desulforubidin (2.47 mg/ml) with 0.2 N NaOH, the absorption maxima at 393 and 547 nm were demonstrated (Fig. 2, B). The ratio of optical density at 284, 412, and 630 as well as 396, 557, and 581 were about 6 : 3 : 1. Addition of alkali or acid caused bleaching of the greenish colour of desulfovirodin and reddish brown color of desulforubidin as well as shifted the absorption peaks toward short wavelengths. The red fluorescence under UV light was quite unstable. The enzyme protein (desulfovirodin) showed a strong red fluorescence with an emission maximum at 600 nm in alkali or 1% SDS solution when excited at 365 nm (Fig. 2).

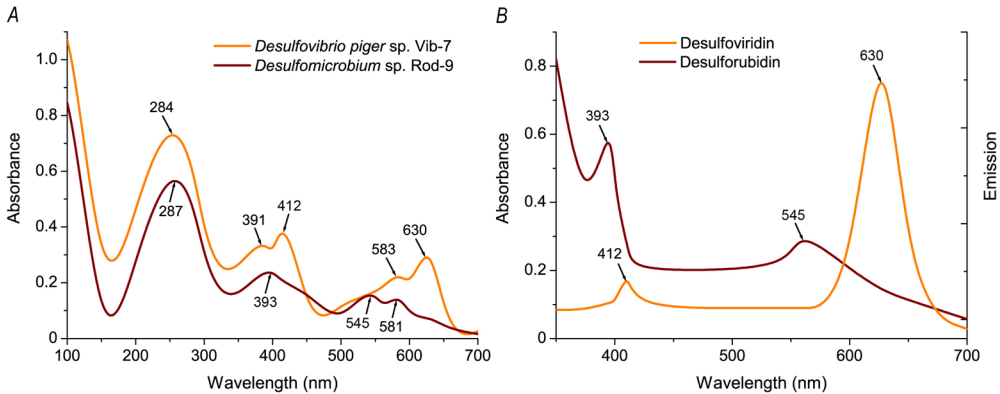


Fig. 2. Absorption spectra of sulfite reductase from *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9. Protein concentration was 3.18 mg (for *D. piger* Vib-7) and 2.84 mg (for *Desulfomicrobium* sp. Rod-9) based on Lowry's method per ml of 0.01 M Tris buffer, pH 7.0 (A). Fluorescence spectrum of desulfovirodin in 0.1 N NaOH excited at 365 nm, and absorption spectrum of desulforubidin (2.47 mg/ml) in 0.2 N NaOH, the absorption maxima at 396 and 557 nm (B)

Рис. 2. Спектри поглинання сульфїтної редуکتаси *D. piger* Vib-7 і *Desulfomicrobium* sp. Rod-9. Концентрація білка 3,18 мг (для *D. piger* Vib-7) і 2,84 мг (для *Desulfomicrobium* sp. Rod-9) визначена методом Лоурі на мл 0,01 М Тріс-буфера, рН 7,0 (А). Спектр флуоресценції десульфівіродину у 0,1 N NaOH при 365 нм та спектр поглинання десульфорубідину (2,47 мг/мл) у 0,2 N NaOH, максимуми поглинання при 396 і 557 нм (В)

Kobayashi et al. (1972) obtained the similar data for *Desulfovibrio vulgaris* sulfite reductase. The absorption spectrum of the purified enzyme preparation had absorption maxima at 630, 585, 410, 390, and 280 nm and a shoulder at 290 nm. The ratio of optical density at 630, 410, and 280 nm was similar to our obtained results, it was about 1 : 3 : 6. The authors have also shown that the enzyme protein had a strong red fluorescence with an emission maximum at 600 nm in alkali solution [6].

A new pigment, purified from extracts of the Norway strain of *Desulfovibrio desulfuricans*, desulforubidin, that has sulfite reducing activity, has been described by Lee et al. The authors registered absorption spectra maxima of desulforubidin at 392, 545, and 580 nm [13].

To study the characteristics and mechanism of sulfite reductase reaction, the initial (instantaneous) reaction rate (V_0), maximum rate of the reaction (V_{max}), maximum amount of reaction product (P_{max}) and reaction time (τ) were defined. Dynamics of hydrogen uptake in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 was studied for investigation of the kinetic parameters of sulfite reductase (Fig. 3).

Experimental data showed that the kinetic curves of sulfite reductase activity have tendency to saturation (Fig. 3, A). Analysis of the results allows to reach the conclusion that the kinetics of sulfite reductase activity in cell-free extracts of the sulfate-reducing bacteria was consistent to the zero-order reaction in the range of 0–10 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 15 min in subsequent experiments.

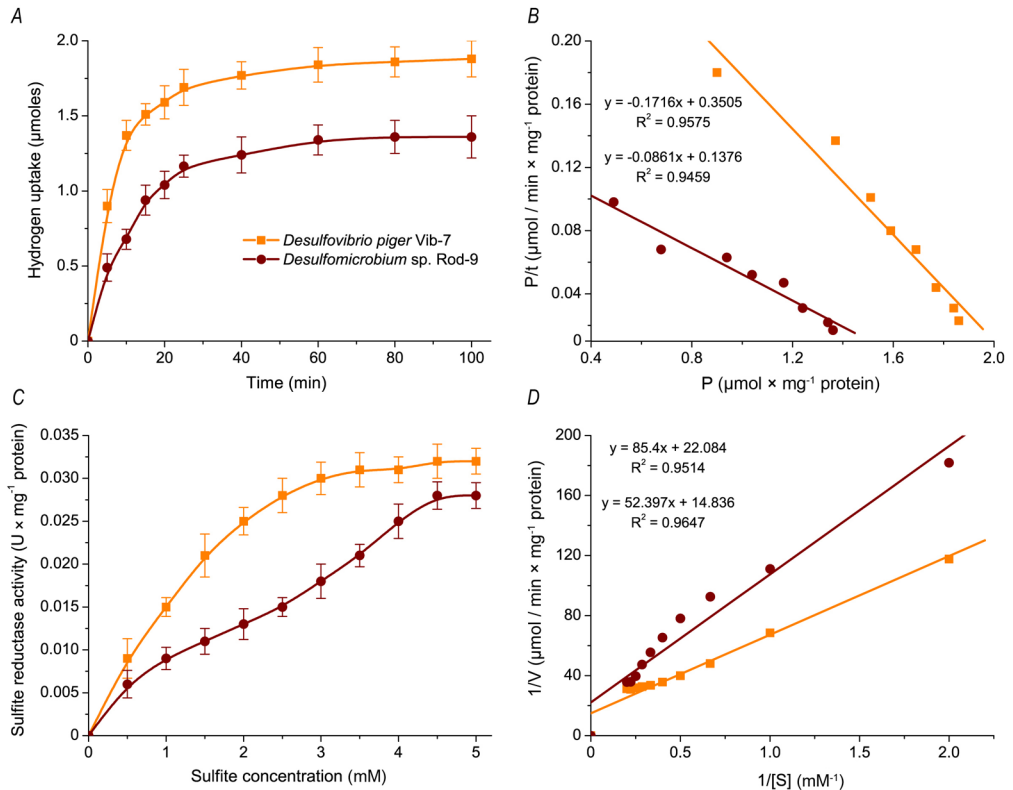


Fig. 3. Kinetic parameters of sulfite reductase activity in cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9: **A** – dynamics of product accumulation ($M \pm m$, $n = 5$); **B** – linearization of curves of product accumulation in $\{P/t; P\}$ coordinates ($n = 5$; $R^2 > 0.95$; $F < 0.02$); **C** – the effect of different sulfite concentrations on sulfite reductase activity ($M \pm m$, $n = 5$); **D** – linearization of concentration curves, which are shown in Fig. 3, C, in the Lineweaver-Burk plot, where V is rate of sulfite reductase reaction and S is substrate concentration ($n = 5$; $R^2 > 0.95$; $F < 0.005$)

Рис. 3. Кінетичні параметри сульфїтредуктази безклїтинних екстрактів *D. piger* Vib-7 і *Desulfomicrobium* sp. Rod-9: **A** – динаміка утворення продукту ($M \pm m$, $n = 5$); **B** – лінеаризація кривих утворення продукту у $\{P/t; P\}$ координатах ($n = 5$; $R^2 > 0.95$; $F < 0.02$); **C** – вплив різної концентрації сульфїту на активність сульфїтредуктази ($M \pm m$, $n = 5$); **D** – лінеаризація концентраційних кривих, які показані на рис. 3, C, у координатах Лайнуївера-Берка, де V – швидкість реакції, каталізованої сульфїтредуктазою, S – концентрація субстрату ($n = 5$; $R^2 > 0.95$; $F < 0.005$)

Amount of product of sulfite reductase reaction in the *D. piger* Vib-7 was higher compared to the *Desulfomicrobium* sp. Rod-9 in the entire range of time factor. The basic kinetic properties of the reaction in the cell-free extracts of the sulfate-reducing bacteria were calculated by linearization of the data in the $\{P/t; P\}$ coordinates (Fig. 3, B, Table 3).

Table 3. Kinetic parameters of hydrogen uptake in cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9

Таблиця 3. Кінетичні параметри поглинання гідрогену безклітинними екстрактами *Desulfovibrio piger* Vib-7 і *Desulfomicrobium* sp. Rod-9

Kinetic parameters	Sulfate-reducing bacteria	
	<i>Desulfovibrio piger</i> Vib-7	<i>Desulfomicrobium</i> sp. Rod-9
V_0 ($\mu\text{mol}/\text{min} \times \text{mg}^{-1}$ protein)	0.351 \pm 0.033	0.138 \pm 0.012***
P_{max} ($\mu\text{mol}/\text{mg}$ protein)	2.042 \pm 0.211	1.598 \pm 0.143**
τ (min)	5.827 \pm 0.562	11.614 \pm 1.036***

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

Примітка: V_0 – початкова (миттєва) швидкість реакції; P_{max} – максимальна кількість (плато) продукту реакції; τ – час реакції (період напівнасичення). Достовірність значень $M \pm m$, $n = 5$; ** $P < 0,01$; *** $P < 0,001$, порівняно до штаму *Desulfovibrio piger* Vib-7.

The kinetic parameters of sulfite reductase in *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 cell-free extracts were significantly different. Values of initial (instantaneous) reaction rate (V_0) for sulfite reductase activity in the cell-free extracts of both bacterial strains was calculated by the maximum amount of the product reaction (P_{max}). As shown in Table 3, V_0 for sulfite reductase reaction was higher in the cell-free extracts of *D. piger* Vib-7 (0.351 \pm 0.033 $\mu\text{mol}/\text{min} \times \text{mg}^{-1}$ protein) compared to *Desulfomicrobium* sp. Rod-9 (0.138 \pm 0.012 $\mu\text{mol}/\text{min} \times \text{mg}^{-1}$ protein). Based on these data, there is an assumption that the *D. piger* Vib-7 can consume sulfite much faster in their cells than a *Desulfomicrobium* sp. Rod-9. Moreover, this hypothetical assumption can be also confirmed by previously obtained data on the accumulation of intermediates and the final products of their metabolism because *D. piger* Vib-7 accumulated trithionate, polythionate and hydrogen sulfide more intensively compared to *Desulfomicrobium* sp. Rod-9 (see Table 2).

The kinetic analysis of sulfite reductase activity dependence on the substrate concentration was carried out. According to the obtained results, increasing of sulfite concentrations from 0.5 to 5.0 mM causes a monotonic rise of the studied enzyme activity and the activity was maintained on unchanged level (plateau) under substrate concentrations over 5.0 mM (Fig. 3, C). Curves of the dependence $\{1/V; 1/[S]\}$ were distinguished by the tangent slope and intersect the vertical axis in one point (Fig. 3, D). The basic kinetic parameters of sulfite reductase activity in *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 cell-free extracts were identified by linearization of the data in the Lineweaver-Burk plot (Table 4).

The K_m values are millimolar concentration ranges which are consistent with similar constants from the literature data [7]. Calculation of the kinetic parameters of sulfite reductase activity indicates that the maximum rate (V_{max}) of hydrogen uptake in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 was similar to each other. Michaelis constants (K_m) of sulfite reductase for both bacterial strains were also approximately similar: 3.53 \pm 0.334 and 3.86 \pm 0.341 mM for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively.

Table 4. Kinetic parameters of sulfite reductase activity dependence on sulfite concentration in the incubation medium

Таблиця 4. Кінетичні параметри сульфїтредуктази залежно від концентрації сульфїту в інкубаційному середовищі

Kinetic parameters	Sulfate-reducing bacteria	
	<i>Desulfovibrio piger</i> Vib-7	<i>Desulfomicrobium</i> sp. Rod-9
V_{\max} ($\mu\text{mol}/\text{min} \times \text{mg}^{-1}$ protein)	0.067 ± 0.0053	0.045 ± 0.0039
K_m (mM)	3.53 ± 0.334	3.86 ± 0.341

Comment: V_{\max} is maximum rate of the enzyme reaction; K_m is Michaelis constant which was determined by sulfite. Significance of the values $M \pm m$, $n = 5$.

Примітка: V_{\max} – максимальна швидкість ензиматичної реакції; K_m – константа Міхаеліса, визначена за сульфїтом. Достовірність значень $M \pm m$, $n = 5$.

The obtained parameters of sulfite reductase reaction in the cell-free extracts of *D. piger* Vib-7 are consistent to Michaelis constant (K_m 3.6×10^{-3} M) defined previously by Kobayashi et al. for sulfite reduction in the extract of *Desulfovibrio vulgaris*. The authors have also investigated the biochemical characteristics of sulfite reductase from sulfate-reducing bacterium, *D. vulgaris*. Trithionate, thiosulfate, and sulfide were detected even in the early phase of sulfite reduction and the amount of each compound did not decrease during the reaction or after hydrogen uptake ceasing. Kobayashi et al. have shown that trithionate and thiosulfate are not reduced by the enzyme, indicating that these three compounds are produced by sulfite reductase. At high concentrations of sulfite and low concentrations of methyl viologen, trithionate was the dominant product. Under the opposite conditions, the accumulation of relatively large amounts of sulfide or thiosulfate was observed. On the basis of these findings, a mechanism of reaction was proposed, taking into consideration labile intermediates, presumably sulfoxylate and elemental sulfur, which accept electrons from reduced methyl viologen to form sulfur and sulfide or react with sulfite to produce trithionate and thiosulfate, respectively [7].

CONCLUSIONS

Based on the obtained studies results and according to the kinetic parameters of sulfite reductase reaction for both bacterial strains, we have concluded that the activity of sulfite reductase, V_0 and V_{\max} were significantly higher in the *D. piger* Vib-7 cells than *Desulfomicrobium* sp. Rod-9. However, Michaelis constants (K_m) of the sulfite reductase were similar and designated 3.53 ± 0.334 and 3.86 ± 0.341 mM for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively. The maximum sulfite reductase activity for both strains has been determined at $+30 \dots 35$ °C and at pH 7.0. The intermediate products of the bacterial metabolism (thiosulfate, trithionate, and polythionate) were determined in the cell-free extracts for both strains. The spectral analysis of the purified sulfite reductase from the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 was carried out. The absorption maxima were 284, 391, 412, 583, and 630 nm as well as 287, 393, 545, and 581 nm for sulfite reductase of *D. piger* and *Desulfomicrobium* sp. Rod-9, respectively. The kinetic parameters of sulfite reductase reaction depended on the substrate concentration. According to the obtained results, increasing of sulfite concentrations from 0.5 to 5.0 mM causes a monotonic rise of studied enzyme activity and the activity was maintained on an unchanged level (plateau) under substrate concentrations over 5.0 mM.

According to these data, *D. piger* Vib-7 strain can be more dangerous and have some pathogenic significance in inflammatory bowel diseases development, consuming sulfite and producing hydrogen sulfide at a higher rate. The studies of the physiological and biochemical properties of the intestinal sulfate-reducing bacteria, the process of the dissimilatory sulfate reduction, in particular participation of sulfite reductase in this process, the activity and kinetic properties of this enzyme in the *D. piger* Vib-7 and *Desulfovibrio* sp. Rod-9 bacterial strains, their production of hydrogen sulfide in detail can be perspective for clarification of the etiological role of these bacteria in the development of various diseases. The data on the concentration of hydrogen sulfide, produced by the isolates is supposed to help in establishing and assessing a toxicity effect of these compounds on the epithelial cells of the human and animal intestine. 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.

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ДИСИМІЛЯЦІЙНА СУЛЬФІТРЕДУКТАЗА БЕЗКЛІТИННИХ ЕКСТРАКТІВ КИШКОВИХ СУЛЬФАТВІДНОВЛЮВАЛЬНИХ БАКТЕРІЙ

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Досліджено активність дисиміляційної сульфїтредуктази у різних фракціях сульфатвідновлювальних бактерій *Desulfovibrio piger* Vib-7 та *Desulfomicrobium* sp. Rod-9, ізольованих із кишечника людини. Сульфїтредуктазу, важливий фермент процесу метаболізму сірки цих бактерій, солюбілізовано з мембранної фракції. Найвища активність ферменту виявлена у безклітинних екстрактах бактеріальних штамів ($0,032 \pm 0,0026$ і $0,028 \pm 0,0022$ $U \times mg^{-1}$ білка для *D. piger* Vib-7 та *Desulfomicrobium* sp. Rod-9, відповідно), порівняно з іншими фракціями. Визначено оптимальну температуру ($+30 \dots 35$ °C) і рН 7,0 для реакції сульфїтредуктази в екстрактах обох бактеріальних штамів. Проведено спектральний аналіз очищеної сульфїтредуктази з безклітинних екстрактів. Максимуми поглинання були 284, 391, 412, 583, 630 нм, а також 287, 393, 545, 581 нм для сульфїтредуктази *D. piger* Vib-7 та *Desulfomicrobium* sp. Rod-9, відповідно. Встановлено, що активність сульфїтредуктази, початкова (миттєва) швидкість (V_0) і максимальна швидкість (V_{max}) реакції сульфїтредуктази вища у клітин *D. piger* Vib-7, ніж у *Desulfomicrobium* sp. Rod-9. Проте константи Міхаеліса (K_m) для активності ферменту були близькими для обох штамів бактерій. Дослідження активності сульфїтредуктази, кінетичні властивості цього ферменту у штамів кишкових сульфатвідновлювальних бактерій, а також детальне вивчення

аккумуляції ними гідроген сульфїду, перспективні для з'ясування етіологічної ролі цих бактерій у розвитку запальних захворювань кишечника людини і тварин.

Ключові слова: сульфатвідновлювальні бактерії, сульфїтредуктаза, мікробіоценоз кишечника, запальні кишкові захворювання, виразкові коліти.

ДИССИМИЛЯЦИОННАЯ СУЛЬФИТРЕДУКТАЗА БЕСКЛЕТОЧНЫХ ЭКСТРАКТОВ КИШЕЧНЫХ СУЛЬФАТВОССТАНАВЛИВАЮЩИХ БАКТЕРИЙ

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Исследована активность диссимиляционной сульфитредуктазы в различных клеточных фракциях сульфатвосстанавливающих бактерий *Desulfovibrio piger* Vib-7 и *Desulfomicrobium* sp. Rod-9, изолированных из кишечника человека. Сульфитредуктаза, важный фермент процесса метаболизма серы сульфатвосстанавливающих бактерий, была солюбилизована из мембранной фракции. Наивысшая активность фермента обнаружена в бесклеточных экстрактах бактериальных штаммов ($0,032 \pm 0,0026$ и $0,028 \pm 0,0022$ U \times mg⁻¹ белка для *D. piger* Vib-7 и *Desulfomicrobium* sp. Rod-9, соответственно) по сравнению с другими фракциями. Определены оптимальные значения температуры (+30...35 °C) и pH 7,0 для реакции сульфитредуктазы в экстрактах обоих бактериальных штаммов. Проведен спектральный анализ очищенной сульфитредуктазы из бесклеточных экстрактов. Максимумы поглощения были 284, 391, 412, 583, 630 нм, а также 287, 393, 545, 581 нм для сульфитредуктазы *D. piger* Vib-7 и *Desulfomicrobium* sp. Rod-9, соответственно. Проведен анализ кинетических свойств этого фермента у исследуемых бактерий. Установлено, что активность сульфитредуктазы, начальная (мгновенная) скорость (V_0) и максимальная скорость (V_{max}) реакции сульфитредуктазы выше у клеток *D. piger* Vib-7, чем в *Desulfomicrobium* sp. Rod-9. Однако константы Михаэлиса (K_m) для активности фермента были близкими для обоих штаммов бактерий. Исследование активности сульфитредуктазы, кинетические свойства этого фермента у штаммов кишечных сульфатвосстанавливающих бактерий, а также изучение аккумуляции ими гидроген сульфида, перспективны для выяснения этиологической роли этих бактерий в развитии воспалительных заболеваний кишечника человека и животных.

Ключевые слова: сульфатвосстанавливающие бактерии, сульфитредуктаза, микробиоценоз кишечника, воспалительные кишечные заболевания, язвенные колиты.

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