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Catalase Activity of Sulfate-Reducing Bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 Isolated from Human Large Intestine

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ABSTRACT

Catalase activity of the sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine was studied. The high activity of the enzyme in cell-free extracts of both bacterial strains was determined (1745.21±154.67 and 873.11±72.23 U×mg^{-1} protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively). The effect of different temperature and pH as well as H_2O_2 concentration and time of incubation on the catalase activity in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were examined. The maximum catalase activity for both bacterial strains at +30ºC temperature was determined. The highest activity of the studied enzyme in the cell-free extracts of *D. piger* Vib-7 at pH 7.5 and *Desulfomicrobium* sp. Rod-9 at pH 7.0 was measured. Based on experimental data, the analysis of the kinetic properties of the catalase by the studied bacteria was carried out. Increasing of hydrogen peroxide 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. The catalase activity, initial (instantaneous) reaction rate (V_0) and maximum rate of the catalase reaction (V_{max}) were significantly higher in *D. piger* Vib-7 than in *Desulfomicrobium* sp. Rod-9 cells. Michaelis constants (K_m) of the catalase reaction were 8.01±0.77 and 10.33±0.98 mM for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively.

Key Words: Sulfate-reducing bacteria, Catalase activity, Toxicity, Intestinal microbiocenosis.

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Introduction

Sulfate-reducing bacteria *Desulfotomaculum*, *Desulfobulbus*, *Desulfomicrobium*, *Desulfomonas*, and *Desulfovibrio* belong to the intestinal microbiota of humans and animals (Barton *et al.*, 2007; Cummings *et al*., 2003). They carry out the dissimilatory sulfate reduction in process of anaerobic respiration (Kushkevych *et al.*, 2012). Besides the sulfates, they can consume different electron acceptors (thiosulfates, sulfites, fumarate, malate, nitrate, nitrite, dimethyl sulfoxide, Mn (IV), Fe (III), Cr (VI) and others (Postgate, 1984). Aerobic conditions inhibit dissimilatory sulfate reduction in the most of the sulfate-reducing bacteria (Barton *et al*., 2007; Postgate, 1984). Precisely because they grow, dissimilating sulfate only in the absence of molecular oxygen in the environment. These bacteria are strictly anaerobic microorganisms and presented in anoxic environments where are high sulfate concentrations (Brenner *et al*., 2005; Postgate, 1984). Such conditions are characteristic for the intestine of humans and animals (Dzierzewicz *et al*., 2003). The high concentration of sulfate creates favorable conditions for the sulfate-reducing bacteria development in the human intestine. These conditions provide the dissimilatory sulfate reduction and accumulation of hydrogen sulfide and acetate which can be cytotoxic for intestinal cells causing various inflammatory bowel diseases (Cummings *et al*., 2003; Dzierzewicz *et al*., 2003). Hydrogen sulfide accumulating in the human intestine is also carcinogenic to its cells and can cause inhibition of cytochrome oxidase, oxidation butyrate in colonocytes, and destruction of epithelial cells, develop ulcers, inflammation with subsequent development of colon cancer (Pitcher *et al*., 1996; Kushkevych, 2012b).

The sulfate-reducing bacteria coexist with many other microorganisms (*Bacteroides*, *Proteus*, *Clostridium*, *Escherichia* etc.) in the human intestine (Gibson *et al.*, 1991; Barton *et al*., 2007). These intestinal microorganisms often compete with sulfate-reducing bacteria for substrate or consume other electron donors. The final products of their fermentation (amino acids, pyruvate, ethanol, valeriat, succinate, hydrogen, butyrate, propionate, lactate, pyruvate), are electron donors for sulfate-reducing bacteria in process of the dissimilatory sulfate reduction (Brenner *et al*., 2007). The sulfate-reducing bacteria in process of the oxidation of organic compounds support required for other microorganisms low partial pressure at which they use hydrogen as electron acceptor. Under these conditions endothermic reactions occur (Kushkevych, 2012a).

Some species of the sulfate-reducing bacteria can widely vary in aerotolerance: from extreme anaerobes to species that retain viability for a long time under aerobic conditions (sulfate reducers of the *Desulfovibrio* genus) (Postgate, 1984). Protection against toxic and mutagenic compounds in aerobes and facultative anaerobes is provided by enzymes of antioxidative defense, the most important of them being superoxide dismutase (SOD) and catalase (Brioukhanov *et al*., 2002; 2004). Catalase has been widely studied in aerobic microorganisms, whereas less is known about monofunctional catalases of anaerobes. In early stages of studies on the enzymes of antioxidative defense, Macleod and Gordon supposed that obligate anaerobes could not grow under aerobic conditions because they lack catalase and their cells accumulate H_2O_2 (McLeod *et al*., 1923). Catalase activity has been found in some *Desulfovibrio* species: *D. desulfuricans* strain Norway 4

(*Desulfomicrobium norvegicum*), *D. vulgaris* (Hatchikian *et al*., 1977), *D. oxyclinae* (Krekeler *et al*., 1998), and *D. gigas* (Dos Santos *et al*., 2000).

Catalase (EC 1.11.1.6) catalyzes disproportioning of H_2O_2 to H_2O and O_2 and thus protects the cells against the oxidative effect of H_2O_2. This enzyme is present in all aerobes and many aerotolerant anaerobes. Two types of phylogenetically remote heme catalases are known: monofunctional catalases and bifunctional catalases–peroxidases, which for the catalase activity use H_2O_2 (K_m ~ 2.5…6.5 mM) as an electron donor and for the peroxidase activity (*K*m ~ 0.2…0.7 mM) use various organic compounds (pyrogallol, diaminobenzidine, dimethoxy-benzidine, dianizidine, NADH, NADPH, etc.) (Brioukhanov *et al*., 2002; 2004). Monofunctional catalases are found in all three empires of living nature, whereas the distribution of bifunctional heme catalases is limited (with rare exceptions) to bacteria and archaea (Loewen, 1997). Fridovich *et al*. also thought that in most cases anaerobes had no catalase activity (Fridovich, 1995; McCord *et al*., 1971). But some obligate anaerobes are known to contain catalase. It is unclear, whether the lack of catalase activity in some microorganisms is associated with the absence of the corresponding gene or with its expression only under certain conditions (Brioukhanov *et al*., 2002; 2004). There are a lot of data about antioxidant systems of the anaerobic bacteria. However, the data about catalase activity of sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine has not been studied yet.

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

Materials and Methods

Objects of the studies were the sulfate-reducing bacteria *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 isolated from the human large intestine (Kushkevych, 2013; Kushkevych *et al*., 2014).

Bacterial growth and cultivation. The bacteria were grown in nutrition modified Kravtsov-Sorokin's liquid medium of following composition (g/l): Na_2SO_4 – 0.5; KH_2PO_4 – 0.3; K_2HPO_4 – 0.5; $(NH_4)_2SO_4$ – 0.2; NH_4Cl – 1.0; $CaCl_2{\times}6H_2O$ – 0.06; $MgSO_4{\times}7H_2O$ – 0.1; $C_3H_5O_3Na$ – 2.0; yeast extract – 1.0; $FeSO_4{\times}7H_2O$ – 0.004; 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. The 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 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, suspended in extracting buffer (50 mM potassium phosphate buffer, pH 7.5, 10^{-5}M EDTA (ethylenediaminetetraacetic acid) was added to centrifuged sedimented 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 0ºC to obtain cell-free extracts. The suspension was displaced into centrifugal tubes and cell-free extract was separated from the cells fragments by centrifugation in 30 minutes at 15000 rpm and at +4ºC.

Protein concentration in the cell-free extracts was determined by the Lowry method (Lowry *et al.*, 1951).

Measuring catalase activity. The catalase activity was determined spectrophotometrically by the concentration of fermented hydrogen peroxide (Luck, 1963; Goldblith and Proctor, 1950). The reaction mixture for the measurement of catalase activity contained: 2.8 ml of 0.05% H_2O_2 (1.47 mM), and 0.1–0.2 ml of the cell-free extracts which was diluted in *n* times. The time of incubation was 7 min. The reaction was stopped by 1.0 ml 6% $(NH_4)_2MoO_4$. The sample which contained H_2O, instead of the cell-free extracts, was used as a control. The enzyme activity was calculated using the formula:

$$A = \frac{\Delta E_{410} \times 4 \times n}{110.6 \times t \times V \times C}$$

where ΔE_{410} is the difference between the E_{410} of the control and E_{410} of the samples; 4 are total volume of the reaction mixture (ml); *n* is dilute of the permeabilized cells (times); 110.6 are coefficient determined by the calibration curve; *t* is time incubation (min); *V* is the volume of cell-free extract which was added into the reaction mixture (ml); *C* is concentration of the protein in the sample (mg/ml).

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… +45°C) and pH (4.0…9.0) in the incubation medium was measured.

Kinetic analysis. The study of the kinetic properties of the enzyme reaction was performed in a standard incubation medium, which was modified by physical and chemical characteristics or the respective components (the incubation time, substrate concentration, temperature and pH). The kinetic parameters characterizing the reaction of O_2 release in process of H_2O_2 decomposition are the initial (instantaneous) reaction rate (V_0), maximum amount of the reaction product (P_{max}) and characteristic reaction time (time half saturation, τ) were determined as described in paper (Valente *et al.*, 2003; Kosterin and Burchynskaya, 1987). The amount of the reaction product was calculated stoichiometrically by reaction of H_2O_2 decomposition. The kinetic parameters characterizing catalase reactions are Michaelis constant (K_m) and maximum reaction rate of H_2O_2 decomposition were determined by Lineweaver-Burk plot (Lineweaver and Burk, 1934; Keleti, 1988). The dependence of the rate of H_2O_2 decomposition on the H_2O_2 concentration was constructed in the coordinates {1/V on 1/S}, where S is the concentration of the substrate (H_2O_2), and V is the rate of enzymatic H_2O_2 decomposition at a H_2O_2 concentration.

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 methods of variation statistics using Student *t*-test. The equation of the straight line that the best approximates the experimental data was calculated by method of least squares. The absolute value of the correlation coefficient *r* was from 0.90 to 0.98. The statistical 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

The activity of catalase in different fractions (cell-free extract, soluble, and sedimentary) was studied (Table 1). Our results showed that the highest enzyme activity in cell-free extracts (1745.21±154.67 and 873.11±72.23 U×mg^{-1} protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively) was measured. Slightly less activity of studied enzyme in the soluble fraction compared to cell-free extracts was detected. The lowest catalase activity was found in sedimentary fraction, it values designated 244.32±28.53 and 102.76±11.28 U×mg^{-1} protein.

Our next task was to determine the activity of the studied enzyme in for 7 min at intervals of one minute time of the measurement. The cell-free extracts of sulfate-reducing bacteria were incubated in the standard incubation medium in different time periods (from 1 to 7 minutes).

Table 1. Catalase activity of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 measured in different fractions

Fractions	Catalase activity (U×mg^{-1} protein)	
	D. piger	*Desulfomicrobium* sp.
Cell-free extract	1745.21±154.67	873.11±72.23**
Individual fractions		
soluble	1169.15±97.21	584.91±68.03**
sedimentary	244.32±28.53	102.76±11.28**

Comment: Enzyme activity was determined at five-minute incubation. Statistical significance of the values M±m, n = 5; **P<0.01, compared to *D. piger* Vib-7 strain.

As the results of our study, catalase activity of the bacteria *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 cell-free extracts depended on time of the enzyme incubation with the substrate. The enzyme activity was increased in proportion to the time of incubation. The highest activity of the studied enzyme was found for 3–7 min of incubation and it was almost unchanged in the time (Fig. 1).

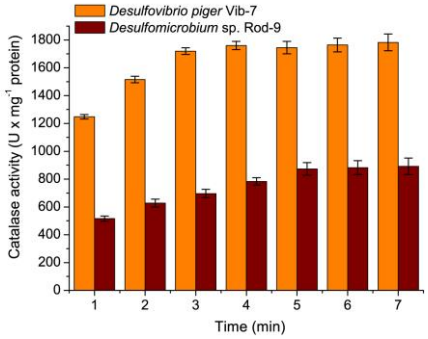

Fig 1. Catalase activity in the cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 (M ± m, n = 5).

From literature data, it is known that the enzymatic activity depends on temperature and pH (Brioukhanov *et al*., 2002; 2004). Therefore the effect of temperature and pH in the incubation medium on the catalase activity in the sulfate-reducing bacteria was studied (Fig. 2, 3).

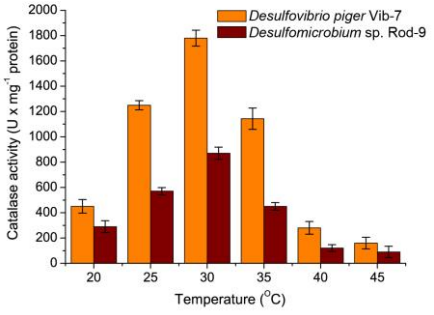

Fig 2. The effect of different temperature on the catalase activity in the cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9.

The maximum catalase activity for both bacterial strains was determined at +30ºC temperature. An increase or decrease in temperature of incubation leads to a decrease of the activity of the studied enzyme in the cell-free bacterial extracts. The highest activity of catalase was determined in the cell-free extracts of the *D. piger* Vib-7 at pH 7.5 and *Desulfomicrobium* sp. Rod-9 at pH 7.0 (Fig. 3).

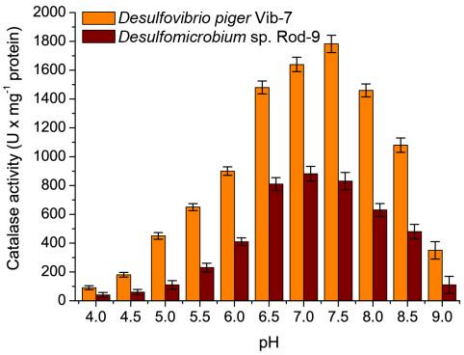

Fig 3. The effect of different pH on the catalase activity in the cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9.

Thus temperature and pH optimum for catalase activity in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 was determined.

To study the characteristics and mechanism of catalase reaction, the initial (instantaneous) reaction rate (V_0), maximum decomposition of hydrogen peroxide (P_{max}) and reaction time (τ) was defined. The dynamics of the hydrogen peroxide decomposition by the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 to establishment the kinetic catalase parameters was studied (Fig. 4).

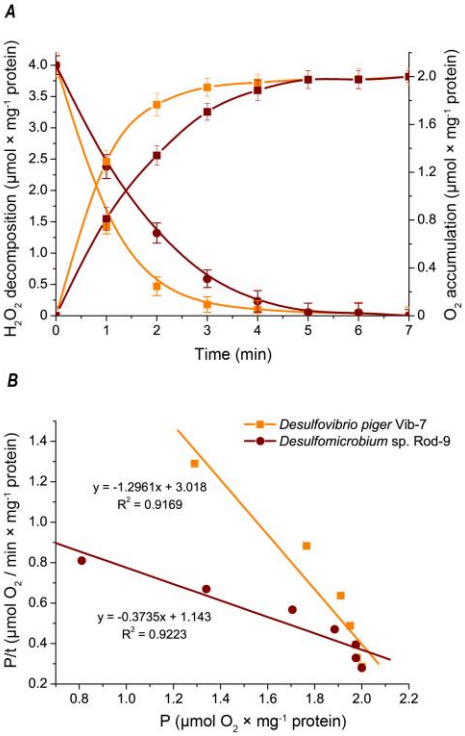

Fig 4. Kinetic parameters of the dynamics of hydrogen peroxide decomposition and product reaction accumulation (***A***) in the cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 (M ± m, n = 5) and linearization of the curves of hydrogen peroxide decomposition (***B***) in {P/t; P} coordinates (n = 5; $R^2 > 0.9$; F <0.02).

Our experimental data showed that the kinetic curves of catalase activity have tendency to saturation (Figure 4*A*). Analysis of the results allowed to reach the conclusion that the kinetics of catalase activity in cell-free extracts of the studied bacteria was consistent to the zero-order reaction in the range of 0–3 min (the graph of the dependence of P on the incubation time was almost linear in this interval of time). Therefore the incubation duration

in the bacterial cells extracts was 3 min in subsequent experiments.

The amount of O_2 released in catalase reaction in cell-free extracts of *D piger* Vib-7 was higher compared to the extracts of *Desulfomicrobium* sp. Rod-9 in the entire range of time factor. The basic kinetic properties of catalase 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. 4*B*, Table 2).

Table 2. Kinetic parameters of hydrogen peroxide decomposition in the cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9

Kinetic parameters	Sulfate-reducing bacteria	
	D. piger	*Desulfomicrobium* sp.
V_0 (μmol O_2/min×mg^{-1} protein)	3.018±0.312	1.144±0.098**
P_{max} (μmol O_2×mg^{-1} protein)	2.329±0.244	3.062±0.327*
τ (min)	0.772±0.081	2.677±0.28***

Comment: V_0 is initial (instantaneous) reaction rate; P_{max} is maximum amount of the reaction product; τ is the reaction time (half saturation period). Statistical significance of the values M±m, n = 5; *P<0.05, **P<0.01, ***P<0.001, compared to *D. piger* Vib-7 strain.

The kinetic parameters of catalase in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were significantly different. Values of the initial (instantaneous) reaction rate (V_0) for catalase reaction in the cell-free extracts of both bacterial strains were calculated by the maximum amount of the product reaction (P_{max}). As shown in Table 2, V_0 for catalase reaction was higher in the *D piger* Vib-7 strain (3.018±0.312 μmol O_2/min×mg^{-1} protein) compared to *Desulfomicrobium* sp. Rod-9 (1.144±0.098 μmol O_2/min×mg^{-1} protein). Based on these data, we suppose that the *D. piger* Vib-7 bacterial cells are more resistant to molecular oxygen effect compared to the cells of *Desulfomicrobium* sp. Rod-9.

The next task of our study was to carry out the kinetic analysis of catalase reaction dependence on the substrate concentration. According to the obtained results, increasing of hydrogen peroxide 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 (Fig. 5*A*). Curves of the dependence [1/V; 1/(H_2O_2)] were different by tangent slope and intersect the vertical axis in one point (Fig. 5*B*). The basic kinetic parameters of the catalase activity in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were identified by linearization of the data in the Lineweaver-Burk plot (Table 3).

Thus based on the obtained studies results and according to the kinetic parameters of catalase activity determined by hydrogen peroxide decomposition for both bacterial strains, we can conclude that the *D. piger* Vib-7 was more resistant to the action of molecular oxygen compared to the *Desulfomicrobium* sp. Rod-9. The activity of catalase, V_0 and V_{max} were significantly higher in *D. piger* Vib- 7 cells than *Desulfomicrobium* sp. Rod-9.

Table 3. Kinetic parameters of hydrogen peroxide decomposition in the cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 dependence on H_2O_2 concentration in incubation medium

Kinetic parameters	Sulfate-reducing bacteria	
	D. piger	*Desulfomicrobium* sp.
V_{max} (μmol/min×mg^{-1} protein)	5000±489	1667±168***
K_m (mM)	8.01±0.77	10.33±0.98

Comment: V_{max} is maximum rate of the catalase reaction; K_m is Michaelis constant determined by H_2O_2 decomposition. Statistical significance of the values M±m, n = 5; ***P<0.001, compared to *D. piger* Vib-7 strain.

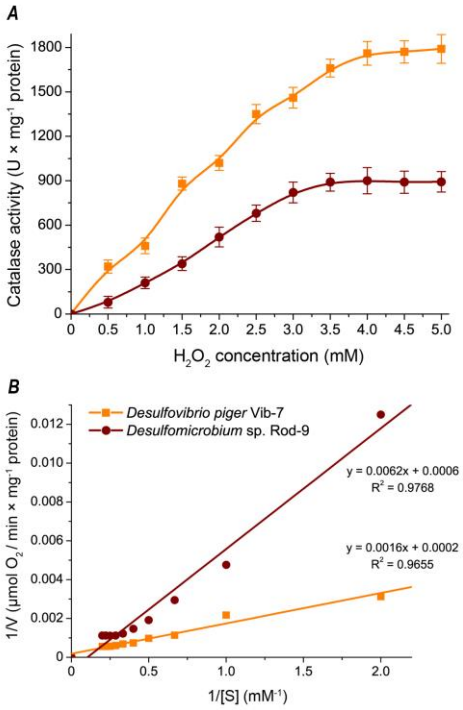

Fig. 5. Kinetic parameters of the effect of different concentration of hydrogen peroxide on the catalase activity (***A***) in the cell-free extracts of *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 (M ± m, n = 5) and linearization of concentration curves, which are shown in fig. 5*A*, in the Lineweaver-Burk plot (***B***), where V is rate of catalase reaction and S is substrate concentration (n = 5; $R^2 > 0.95$; $F < 0.005$).

The K_m values are millimolar concentration range that was consistent with similar constants from the literature data (Gudelj *et al.*, 2001). Calculation of the kinetic parameters of catalase activity indicates that the maximum rate (V_{max}) of hydrogen peroxide decomposition in the cell-free extracts of *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 was different from each other. However, the Michaelis constants (K_m) of catalase reaction in both bacterial strains were almost similar to each other: 8.01±0.77 and 10.33±0.98 mM for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively.

According to these data, *Desulfomicrobium* sp. Rod-9 strain was more sensitive. Probably, this sensitivity makes impossible for survival of studied bacteria in environmental conditions where the effect of molecular oxygen is toxic. Therefore, the bacteria of *Desulfomicrobium* genus was less often isolated from the feces of humans and animals compared to the *Desulfovibrio* bacteria.

It is well known that sulfate-reducing bacteria are strict anaerobes; their growth is suppressed by low concentrations of O_2, which is caused not only by generation of reactive oxygen species but also by its competition with SO_4^{2-} as an acceptor of electrons (Ito *et al.*, 2002). However, many sulfate reducers are aerotolerant (Storz *et al.*, 1990). Moreover, in some *Desulfovibrio* (*D. vulgaris* and *D. desulfuricans*) O_2 is reduced to water during respiration (Sies, 1993; Fridovich, 1995). The *Desulfovibrio* strains generally exhibit O_2 reduction rates comparable to those of aerobes but these rates often decrease by increasing the O_2 concentration and slow down after repeated oxygen additions (Krekeler *et al.*, 1998; Dannenberg *et al.*, 1992). The highest O_2 reduction rate detected so far have been observed in *Desulfovibrio termitidis*, isolated from termite guts, with more than 1570 nmol O_2/min×mg^{-1} protein (Kuhnigk *et al.*, 1996; Brioukhanov *et al*., 2002; 2004).

Procaryotes more often have catalases consisting of two subunits but catalases with four and six subunits are also known. Catalase of *D. gigas* is very sensitive to H_2O_2 and CN^- and less sensitive to S^{2-} (Schramm *et al.*, 1999). Catalase activity, disproportioning of H_2O_2 to O_2 and H_2O, was detected for the first time in *Desulfovibrio* (Hatchikian *et al.*, 1977). The specific activity of the purified catalase of *D. gigas* is unusually low which seems to be associated with a low content of the heme per enzyme molecule (Schramm *et al.*, 1999). Catalases of sulfate-reducing bacteria of the genus *Desulfotomaculum* (*D. nigrificans* subsp. *Salinus* and *D. kuznetsovii*) displayed a high activity not stimulated by addition of hemin to the medium (Johnson *et al.*, 1997). Species of *Desulfotomaculum* are dominating sulfate reducers in soils flooded from time to time and occur in freshwater and marine deposits and in animals' intestine, i.e., in locations with periodic aeration (Brioukhanov *et al.*, 2002, 2004).

The results of our study are consistent to previous kinetic constants that characterize the activity of catalase obtained from various microorganisms (Gudelj *et al.*, 2001; Singh *et al.*, 2008).

Peter Jones and A. Suggett (1968) have shown that the catalase-hydrogen peroxide reaction at high substrate concentrations; reveals a more complex kinetic behaviour than that previously reported. At constant reaction time the catalytic process obeys Michaelis-Menten kinetics but the apparent Michaelis constant is markedly time-dependent, whereas the conventional catalase activity is independent of time. These authors also reported that the kinetics of the "time effect" were analysed and it is suggested that the effect derives from the formation of an inactive species. The process shows Michaelis-Menten kinetics, with a Michaelis constant equal to that for the catalytic reaction in the limit of zero reaction time. It has been confirmed that certain buffer components have marked inhibitory effects on the catalytic reaction and that, in unbuffered systems, catalytic activity is substantially independent of pH (Jones and Suggett, 1968).

Conclusions

Catalase activity of the *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 in cell-free extract of the bacterial strains was detected (1745.21±154.67 and 873.11±72.23 $U \times mg^{-1}$ protein for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively). Optimal temperature for catalase activity was +30ºC in cell-free extract of both bacterial strains. Optimum pH 7.5 and pH 7.0 for the activity was determined for *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9, respectively. The catalase activity, V_0 and V_{max} were significantly higher in *D. piger* Vib- 7 cells than *Desulfomicrobium* sp. Rod-9. However, Michaelis constants (K_m) of the catalase reaction were almost similar with each other (8.01±0.77 mM for *D. piger* Vib-7 and 10.33±0.98 mM for *Desulfomicrobium* sp. Rod-9 strains). Probably, this sensitivity makes impossible survival of studied bacteria in environmental conditions where the action of molecular oxygen is toxic.

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