Biol. Stud. 2016: 10(1); 197–228 • DOI: https://doi.org/10.30970/sbi.1001.560
www.http://publications.lnu.edu.ua/journals/index.php/biology

UDC: 579.846.2:22

DISSIMILATORY SULFATE REDUCTION IN THE INTESTINAL SULFATE-REDUCING BACTERIA

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The study of the intestinal sulfate-reducing bacteria, the process of dissimilatory sulfate reduction and accumulation of hydrogen sulfide, as well as their role in the inflammatory bowel diseases, including ulcerative colitis, in animals and human have increasingly attracted the attention of scientists. New opportunities for studying inflammatory bowel disease and the assessment of the effectiveness of its treatment is an urgent problem of modern biology and medicine. In this review, brief characteristics of these bacteria and their mechanism of dissimilatory sulfate reduction were described based on modern literature data and own research. The characteristics of substrates for intestinal sulfate-reducing bacteria and the thermodynamic properties of their electron donors were also described. Special attention was paid to the mechanism and stages of sulfate dissimilation including role of enzymes involved in this process. Based on our results, general scheme of dissimilatory sulfate reduction showing the activity of each enzyme of the process was demonstrated. The described physiological and biochemical parameters are important for a more detailed understanding of sulfate dissimilation in the human and animal bowel, as well as studying the mechanisms of action of the antimicrobial prophylactics and the therapy against specific components involved in the pathogenesis of the disease. It is also essential for understanding the mechanisms of bowel diseases and for evaluating the effectiveness of its therapy.

Keywords: sulfate reducing bacteria, dissimilatory sulfate reduction, hydrogen sulfide, intestinal microflora.

INTRODUCTION

Sulfate-reducing bacteria (SRB) are common in anaerobic areas of soils, wetlands, fresh and marine waters, and available in the microbiocenosis of large intestine of humans and animals [1, 63]. These microorganisms, dissimilating sulfate to hydrogen sulfide, are involved in the process of biogeochemical sulfur cycle in nature [63]. The sulfate dissimilation process is called the „dissimilatory sulfate reduction" or „sulfate respiration" [54]. Intensive sulfate reduction by SRB, and accordingly the accumulation of toxic hydrogen sulfide in the intestine, is leading to the development of various diseases [12, 16, 46,

49, 55]. SRB and the products of their metabolism are often found during bloody diarrhea and abdominal pain [51, 55]. It is believed that they can cause weight loss, frequent defecation, arthritis, rheumatic diseases, increased intestinal permeability, ulcerative colitis, and malaise, in general [12, 13, 15, 17, 46, 49, 55].

Studying the process of dissimilatory sulfate reduction in the natural SRB strains and those that are isolated from the animal and human intestine during various diseases as well as comparing their biochemical, physiological, genetic and morphological properties is necessary for clarifying of the role of SRB in the development of various human diseases. It is also important to study the thermodynamic properties of electron donors, trophic relations in different species and genera of SRB, and their diversity in natural conditions. The research of SRB from intestines of humans and animals is conducted only in several leading laboratories in the world [12, 13, 48, 49, 50, 55]. The isolation and identification of new strains of intestinal SRB, the study of their physiological and biochemical characteristics, the development of the basic criteria for assessing the aggressiveness of the strains, the toxicity of products of their metabolism for the intestinal mucosa and the clarifying of their role in the disease development are currently most important.

The estimated number of SRB and the level of hydrogen sulfide accumulation in human feces can predict the progress of inflammation in the intestines. Analyzing the sulfate dissimilation process in the intestinal SRB strains allows a better understanding of their temporal dynamics at different stages of its reduction. The measuring of the aggressiveness of SRB and the intestinal mucosal toxicity of the products of their metabolism may be proposed as indicators. Experimental data allow developing basic criteria for the assessment of the course of the inflammatory process and establishing of the level of disease risk in order to prevent it. Such research is also promising for the development of methods of prevention against inflammatory bowel disease. The described physiological and biochemical parameters are important for creating animal models of inflammatory bowel disease involving SRB and using these models to study the mechanisms of the action of antimicrobial prophylactics and the therapy against specific components involved in the pathogenesis of the disease. New opportunities for studying of the inflammatory bowel disease and the assessment of the effectiveness of its treatment are extremely urgent problems in modern biology and medicine.

The aim of this review was to summarize the results of current research and generalize new data on the process of dissimilatory sulfate reduction in the intestinal sulfate-reducing bacteria based on own results and those from recent literature.

1. Intestinal sulfate-reducing bacteria and the bowel diseases. Sulfate-reducing bacteria *Desulfotomaculum*, *Desulfobulbus*, *Desulfomicrobium*, *Desulfomonas,* and *Desulfovibrio* genera belong to the intestinal microbiocenosis in humans and animals [1, 22, 23, 24, 46]. The knowledge on the interaction of SRB with other microorganisms in intestines is not sufficient. Bacteria living on the surface of the colon mucosa are in close relationship with the human body. They interact with the cells of the immune and neuroendocrine system more closely than microorganisms in the intestine lumen [12, 13, 19, 20]. It is believed that the species composition and the number of SRB on the surface of the intestinal mucosa differ from microorganisms in its lumen. The presence of sulfate ions promotes the growth of intestinal SRB which use molecular hydrogen and compete for this substrate with methanogenic bacteria [12, 13].

Among genera of SRB, the species of the *Desulfovibrio* genus in human and animal diseases are the most often isolated (Fig. 1). These bacteria are also isolated in the mono- and polymicrobial infections of the gastrointestinal tract [6, 20, 48, 51, 52, 55, 63].

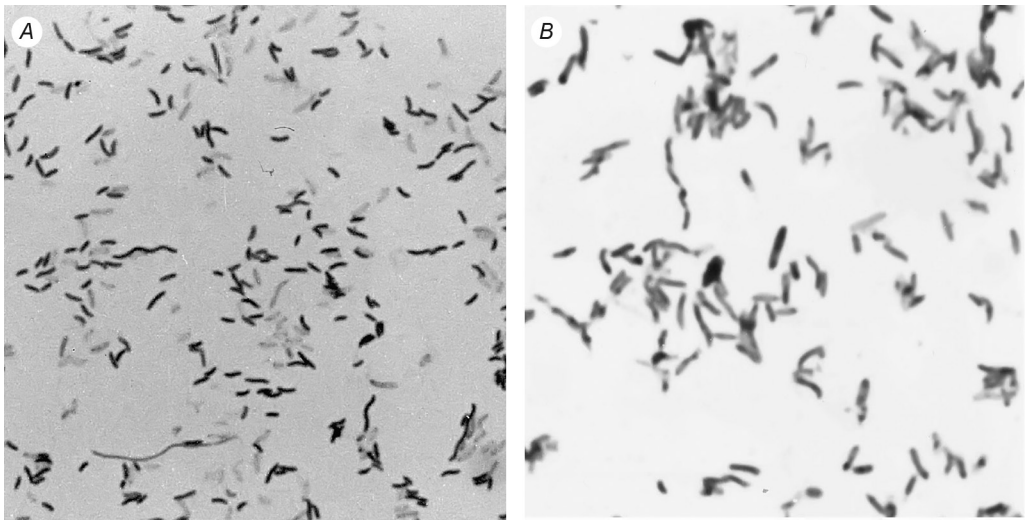

Fig. 1. Bacteria *Desulfovibrio* genus isolated from different objects (light microscopy, ×1,000): *A* – isolate caused a pyogenic liver abscess (photo by Tee et al. 1996) [71]; *B* – isolate caused bacteremia (photo by McDougall et al. 1996) [55]

Рис. 1. Бактерії роду *Desulfovibrio* ізольовані від різних об'єктів (світлова мікроскопія, ×1000): А – ізолят, який спричиняє гнійний абсцес печінки (фото за Tee et al. 1996) [71]; *B* – ізолят, який спричиняє бактеріємію (фото за McDougall et al. 1996) [55]

In 1976, W.E.C. Moore isolated SRB from human feces for the first time and identified the bacteria as *Desulfomonas pigra* which was subsequently reclassified to *Desulfovibrio piger* [1, 4]. Similar research was carried out by J. Loubinoux et al. who isolated bacteria *Desulfomonas* and *Desulfovibrio* genera from the human intestine [48–51].

It is believed that SRB are not pathogenic in humans and animals [1]. However, they can cause various diseases together with other infections [12, 19, 20]. The most often isolated genus among SRB during the disease is the *Desulfovibrio* genus, including *D. fairfieldensis*. These bacteria may be pathogenic more than other species of SRB [51]. Bacteria *D. fairfieldensis* are isolated during mono- and polymicrobial infections of the gastrointestinal tract [51]. Loubinoux J. et al. found that 12 of 100 samples of purulent abdominal and pleural cavities contained human *Desulfovibrio piger*, *D. fairfieldensis* or *D. desulfuricans* [50]. Bacteria *D. desulfuricans* causing bacteremia was isolated from bleeding microvilli of the colon [51]. This research shows that the main way SRB penetrate the blood vessels is through damaged intestinal microvilli and then bacteria cause an infection. SRB is also detected in oral cavity [1, 20]. Similarly to some methanogens, they can cause the development of other diseases, including cholecystitis, abscesses of the brain and abdomen, ulcerative enterocolitis, cancer, etc. [1, 20, 46, 49].

Bacteria of *Desulfotomaculum*, *Desulfobulbus*, *Desulfomicrobium*, *Desulfomonas,* and *Desulfovibrio* genera in the anaerobic respiration, in addition to sulfate, can consume other electron acceptors, including elemental sulfur, fumarate, nitrate, dimethyl sulfoxide, Mn (IV) and Fe (III) [1, 19, 63]. Bacteria *Desulfovibrio gigas* are even capable

of aerobic respiration [1]. However, aerobic conditions inhibit the process of dissimilatory sulfate reduction in most SRB genera [47]. Therefore, SRB grow using sulfate reduction only in the environment with the absence of molecular oxygen [19]. They are strictly (obligate) anaerobic microorganisms present in anoxic environments that are rich in sulfates [1, 19, 63]. Such conditions are characteristic for wetlands, silt ponds and intestines of humans and animals [19, 63]. Thus, the high concentration of sulfate in marine and fresh waters as well as in human and animal intestine is creating favorable conditions for the SRB growth [5]. Under these conditions, sulfide formed in the SRB, is oxidized to sulfate by the chemolithotrophic or photolithotrophic bacteria, which are providing constant level of sulfate in the natural environments [63].

According to the nutritional requirements and the carbon and energy source, SRB may be divided into groups of chemoorganoheterotrophs, chemolithoheterotrophs and chemolithoautotrophs (Fig. 2) [1]. In the chemolithoheterotrophic or chemolithoautotrophic conditions, the bacterial nutrition is provided by the oxidation of mainly hydrogen [90].

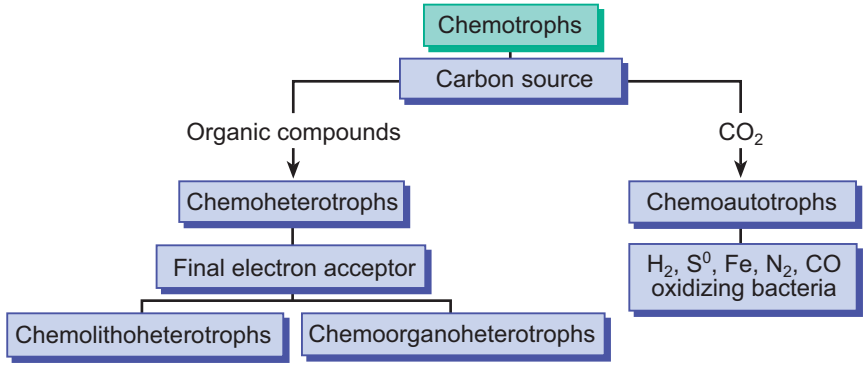

Fig. 2. The SRB groups divided based on carbon and energy source [1]

Рис. 2. Групи СВБ за джерелом карбону та енергії [1]

The chemolithoheterotrophic SRB include some species of the *Desulfovibrio* and *Desulfotomaculum* genera [4]. Bacteria of the *Desulfovibrio* genus grow due to the oxidation of molecular hydrogen using acetate and CO_2 to build carbon containing metabolites [19]. Bacteria *D. vulgaris* use acetate and CO_2 in the interrupted Krebs cycle with formation of acetyl which is transformed to pyruvate. Pyruvate in the presence of CO_2 is then transformed to oxaloacetate [1].

Chemolithoautotrophic type of nutrition is described in some species of the *Desulfotomaculum*, *Desulfobacter*, *Desulfococcus* and *Desulfonema* genera, and *Archaeoglobus* genus [1, 19].

Sulfate-reducing bacteria can use compounds such as lactate, pyruvate, formate, acetate, propionate, butyrate, fatty acids, ethanol, fructose, acetone, dicarboxylic acids and amino acids as a source of carbon and energy [19, 20, 63]. This way of getting nutrition is called chemoorganoheterotrophy. Besides these compounds, SRB can sometimes use carbon (IV) oxide which may be the only source of carbon for autotrophic growth. The dominant among SRB in human feces is the genus *Desulfovibrio* (*D. fairfieldensis*, *D. desulfuricans*) [1, 19]. In some cases and with some frequency, bacteria *Desulfobacter*, *Desulfotomaculum*, and *Desulfobulbus* were also isolated. However,

the species of *Desulfotomaculum* genus were seldom isolated and in small quantities compared to other SRB [19]. Prevalence of SRB varies in different people. These microorganisms were found in the feces of 70% of healthy people in the United Kingdom and only in 15 % of the inhabitants of Africa. SRB number observed in the stool of 143 healthy people ranged from 10^2 to 10^{11} cells/g of feces [1].

Another study with 87 healthy people found that the number of SRB ranged from 10^7 to 10^{11} cells/g of feces, and it differs among residents of different areas [12, 13]. As already mentioned, the species of the *Desulfovibrio* genus is dominant among SRB in the gut. They account for 67–91 % of total SRB number. Significantly fewer bacteria are found from *Desulfobacter* (9–16 %), *Desulfobulbus* (5–8 %) and *Desulfotomaculum* (2 %) genera [19]. SRB producing the largest number of hydrogen sulfide were isolated from feces of human distal colon. It is probably due to the reaction of the environment because the proximal part of the colon is acidic (pH<5.5) and the distal part is neutral [12, 13].

It has been found that SRB are available not only in feces but they also colonize the intestinal wall [1]. As a result, the samples from men and women acquired by the rectal biopsy contain from 10^6 to 10^7 CFU/g of bioptate. In the mucosa of some people, the number of *Desulfovibrio* bacterial genus changed by several orders during the period of 12 months [19]. It probably depended on the nutrition of these individuals. SRB colonize the intestines of humans right from the beginning of their lives [1]. The presence of bacteria of *Desulfovibrio* genus was detected in the feces of infants under the age of six months. The number of *Desulfovibrio* bacteria in these children which had been breastfed or bottle-fed was 3.7×10^3 and 4.5×10^4 cells/g of feces, respectively [12, 13, 19].

Intestinal microflora plays an important role in physiology of humans and animals and their metabolism. Microorganisms are directly involved in the process of food digestion including the metabolization of short chain fatty acids (SCFA). Intestinal bacteria have effect on the human physiological functions and health [1]. For example, the colonization in the gut provides resistance to pathogens and activation or neutralization of mutagenic compounds such as hydrogen sulfide [20].

In spite of the above, the definitive role of SRB in the development of intestinal diseases has not been well characterized and studied yet. That is why it is important to isolate new strains of intestinal SRB, investigate their substrates and the process of sulfate dissimilation in detail, and consequently, the accumulation of hydrogen sulfide as well as the role of these microorganisms in the development of diseases.

2. Substrates of sulfate-reducing bacteria in animals and humans intestine. The cells of the intestinal mucosa, mucin and other secretions are permanently destroyed and can be used by the intestinal bacteria as a source of energy. However, human nutrition has a significant effect on the species composition of these microorganisms and their metabolic activity [1, 20]. The main sources of carbon and energy for bacteria of the intestine are polysaccharides, namely starch and cellulose. They also use a significant amount of oligosaccharides and proteins. The main products of metabolism in the colon are acetate, short chain fatty acids (SCFA), propionate, butyrate, H_2 and CO_2. Among other products of fermentation, lactate, succinate, ethanol, and CH_4 are found in some people. Branched SCFA, amines, phenols, indoles, H_2S and thiols formed during the fermentation are present in the human gut [1]. Most of these products of fermentation are further metabolized by the intestinal microorganisms (*Escherichia*, *Bifidobacterium*, *Lactobacillus*, and *Enterococcus*) [19]. The study of SRB isolated from feces of people

showed that these organisms are able to use a variety of substrates as electron donors with lactate, pyruvate, acetate and ethanol being the most often used [1].

Sulfates are poorly absorbed in the human intestine. In total, 2–9 mmol of sulfate from food reaches the colon daily. Most of them are reduced in the intestine because sulfates are usually detected in fecal secretions in a quantity of less than 0.5 mmol per day [19]. A large number of sulfate may be in the water and vegetables. Moreover, sulfur dioxide, sulfite, bisulfite, metabisulfite and sulfate are used as food additives. In many food products (beer, cheese, wine, bread, canned meat and vegetables, pickled products), sulfur dioxide (SO_2) can be detected where it serves as a conservator, antioxidant or whitening agent. Studies *in vitro* have shown that intestinal bacteria can also get sulfates from depolymerization and desulfurization of glycoproteins which have a high content of sulfates [12, 13].

Another sulfate containing molecule is chondroitin sulfate, an acidic mucopolysaccharide. It is distributed in the tissues of mammals and considered to be an important source of carbon and energy in the colon. This polymer also stimulates the growth of SRB and the accumulation of sulfide in fecal material [12].

Chondroitin sulfate and mucin are not directly absorbed by SRB. This process depends on saccharolytic activity of some intestinal microorganisms, for example *Bifidobacterium*, *Lactobacillus*, and *Enterococcus*. The most of the SRB are in microparticles of intestine containing goblet cells [1].

Lactate is a product of fermentation in the gut by the *Bifidobacterium* and *Lactobacillus* genera. In healthy people, the concentration of this metabolite is not more than a few mmol/kg of feces. The research of digestive mass content which was obtained directly from the intestine during the autopsy showed that lactate is synthesized mainly in the proximal part of intestine [12, 13]. Lactate is well absorbed in the colon and intestinal bacteria can metabolize this compound and keep its concentration low. The formation of lactate in the gut is mainly caused by fermentation of carbohydrates such as starch. A small quantity is formed by etching other polysaccharides. Lactate is an electron donor for SRB in the human intestine. Other microorganisms of the intestine use it much less compared to SRB [1]. A positive correlation between the concentrations of lactate and starch in the human intestine was observed. It is believed that food containing starch can be used by intestinal SRB in the presence of sufficient concentration of sulfates [19].

Hydrogen is one of the products of fermentation in the colon. Intestinal bacteria use protons for splitting sugars, amino acids and carbohydrates [13, 66]. According to theoretical calculations, the daily production of H_2 in human colon is more than 1 liter in the presence of 40–50 grams of carbohydrates. This parameter depends on the food consumed by humans. The total volume of gas in healthy people does not exceed this value. In total, 2.5–14 % of H_2 is formed in the fermentation process. This discrepancy between theoretical and practical H_2 level allocation is the result of activities of many microbial communities using H_2 in the gut [1].

In the United Kingdom, SRB were either not found or their number was very little in about 30 % of people who had a high intensity methanogenesis in the large intestine [13]. Hydrogen is the only electron donor for intestinal methanogenic bacteria *Methanobrevibacter smithii*. Therefore, there is a competition for molecular hydrogen between the SRB and methanogenic organisms. If sulfates are present in sufficient quantities, SRB inhibit the use of hydrogen by the methanogens in the dissimilatory sulfate reduction process [13, 19].

The amount of sulfate in the diet can have an effect on competition for the substrate (molecular hydrogen, lactate) between SRB and methanogenic organisms in the colon. In people with elevated levels of methane, the inclusion of 15 mmol of sulfate per day in the diet causes a reduced intensity of methanogenesis. Under these conditions, the number of methanogenic bacteria decreases by three orders, while the number of SRB in feces increases by three orders of magnitude. In the absence of sulfate in the diet, SRB was not found. Thus, the intensity of methanogenesis can be regulated by the introduction of sulfate, even if SRB are in the low concentration in the intestine [13].

Ability of SRB to use the H_2 as the electron donor can have a significant effect on fermentation in the colon. Sulfate at a concentration of 15 mM stimulates the growth of SRB in the gut. It also stimulates acetate and propionate fermentation, and inhibits butyrate fermentation. Under these conditions, lactate does not accumulate [12].

The dominant species among SRB in the intestine is *Desulfovibrio desulfuricans* which belongs to human colon microbiocenosis [12, 19, 21]. Analysis of biofilm from human bioptate showed that this species was mixed with many types of bacteria. After injecting the *Desulfovibrio* genus in biofilm, the changes of biofilm's metabolism were observed, including the formation of carbon dioxide, a significantly decreased total content of SCFA and acetate accumulation which are typical for the SRB activity. Under these conditions, lactate was not accumulated in the medium because it was used by the *Desulfovibrio* bacteria as an electron donor. In addition to increasing concentrations of acetate, butyrate content was reduced threefold. The syntrophic interactions were observed between *D. desulfuricans* and saccharolytic bacteria (*Lactobacillus*, *Bifidobacterium*, *Enterococcus*) localized in the colon. The reasons for the formation of such biofilms together with SRB are unclear. They form also on digestion remains in the intestinal lumen and mucosal surface [12, 19].

Thus, the most common substrates for SRB in human colon are lactate, pyruvate, acetate and ethanol which can be electron donors in the process of dissimilatory sulfate reduction. The presence of sulfate in the human diet suppresses methanogenesis and, accordingly, the number of methanogenic bacteria, and increases the amount of sulfate-reducing bacteria in the gut. To clarify the role of sulfate-reducing bacteria and their participation in various human and animal diseases, the process of dissimilatory sulfate reduction is necessary to be studied in the natural strains of SRB and the species of SRB isolated from human and animal colon during diseases and from healthy subjects. It is also important to compare their biochemical, physiological, genetic and morphological properties, and to investigate the possibility of using electron donors and their thermodynamic properties in the process of sulfate dissimilation by SRB, in general.

3. Electron donors of intestinal sulfate-reducing bacteria. From a wide range of many electrons donors, which SRB use in the process of dissimilatory sulfate reduction, formate is probably the only also oxidized in periplasm [1, 69]. Biochemical and genetic studies have shown that formate dehydrogenases from bacteria *D. vulgaris* are localized in the periplasm. They use polyheme cytochrome *c* as an electron acceptor. Oxidation of all other electron donors occurs in the cytoplasm or on the inside of the cytoplasmic membrane [19]. In the large intestine, the most common electron donors for SRB are lactate, acetate and propionate. They are formed by fermentation of substrates which humans consume [12]. The oxidation of electron donor can be divided for three process (Fig. 3).

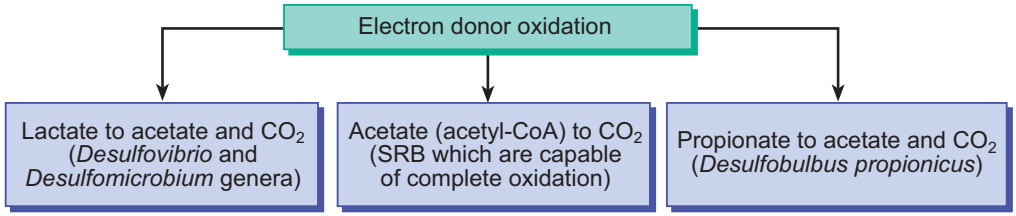

Fig. 3. The oxidation of electron donor in SRB [19]

Рис. 3. Окиснення донора електронів СВБ [19]

Lactate oxidation. Bacteria *D. vulgaris* grow using sulfate and lactate as an energy source. Lactate is not fully oxidized to acetate and the formation of intermediate compounds occurs: pyruvate, acetyl-CoA and acetyl phosphate [1, 40].

$$2\text{Lactate}^- + SO_4^{2-} + H^+ = 2\text{Acetate}^- + CO_2 + HS^- + 2H_2O \quad (1)$$
$$\Delta G^{o\prime} = -196.4 \text{ kJ/mol}$$

This reaction probably consists of the following stages of reduction:

$$\text{Lactate}^- + 2\text{cyt } c_3(\text{ox}) + \Delta mH^+ = \text{Pyruvate}^- + 2\text{cyt } c_3(\text{red}) \quad (2)$$

the catalysis of this reaction is carried out by membrane specific complex of lactate dehydrogenase with active centers localized in the cytoplasm [19];

$$\text{Pyruvate}^- + \text{CoA-SH} + Fd_{ox} = \text{Acetyl-S-CoA} + CO_2 + Fd_{red}^{2-} + 2H^+ \quad (3)$$

the catalysis of pyruvate in the cytoplasm is carried out by pyruvate : ferredoxin oxidoreductase, EC 1.2.7.1 [44];

$$Fd_{red}^{2-} + 2H^+ = Fd_{ox} + H_2 + \Delta mH^+ \quad (4)$$

this reaction is catalyzed by one of the two membrane specific complexes of hydrogenases, EchABCDEF or CooMKLXUHF, which are ferredoxin specific and present in the cytoplasm [1, 56]. Redox potential (E^0 = -500 mV) of the reaction of acetyl-CoA with CO_2/pyruvate is significantly lower than that of H^+/H_2. Hydrogen is formed (reaction 4), diffuses in periplasm and reacts with cytochrome c_3;

$$H_2 + 2\text{cyt } c_3(\text{ox}) = 2\text{cyt } c_3(\text{red})^{-1} + 2H^+ \quad (5)$$

electrons formed by oxidation of lactate (E^0 = -190 mV) are transferred through cytoplasmic membrane with the formation of ΔmH^+ to periplasmic cytochrome c_3 (reaction 5). Reaction 5 is catalyzed by one of four periplasmic cytochrome specific hydrogenases [19]:

$$4\text{cyt } c_3(\text{red})^{-1} + 0.5\ SO_4^{2-} = 4\text{cyt } c_3(\text{ox}) + 0.5\ H_2S \quad (6)$$

Cytochrome c_3 is reduced in cytoplasm, transferred to periplasm via transmembrane electron transfer and then oxidized again (reaction 6).

The transfer of electrons from lactate to cytochrome c_3 causes the formation of ΔmH^+ (reactions 2 and 4). H_2 formation in the cytoplasm and re-oxidation in periplasm are together called the intraspecific transfer of hydrogen or the hydrogen cycle [1, 57].

The sequence of reactions occurs in the presence of specific enzymes and electron carriers. Formed hydrogen is used again for growth of *D. vulgaris* in the medium with lactate and sulfate [64, 69]. In the absence of sulfates, formed H_2 is not used again.

The formation of H_2 from lactate is energy-dependent. The scheme of lactate oxidation to CO_2 is presented in Fig. 4. This process is inhibited by protonophores and arsenates. However, H_2 formation from pyruvate, which is oxidized to acetate and CO_2,

is not inhibited by protonophores and arsenates and it does not require energy [19].

Intraspecific transfer of H_2 is probably also involved in the dissimilatory sulfate reduction in the presence of CO. Cytoplasmic carbon monoxide dehydrogenase which catalyzes ferredoxin reduction in the presence of CO is described in some SRB [63].

In addition to intraspecies hydrogen transfer, formate is also capable of the electron transport from the cytoplasm to periplasm. *D. vulgaris* genome contains genes which are responsible for the synthesis of formate C-acetyltransferase (pyruvate formate-lyase, EC 2.3.1.54) that catalyzes the formation of acetyl-S-CoA and formate from pyruvate and CoA-SH [19].

Pyruvate formate-lyase is a cytoplasmic enzyme. Bacteria *D. vulgaris* have another enzyme, formate dehydrogenase, EC 1.2.2.1, localized in periplasm [64]. Formate formed from pyruvate penetrates through the cytoplasmic membrane due to proton symport. Before that, formate can be used as the electron donor in the dissimilatory sulfate reduction or the reduction of protons to H_2 [66]. This process is called the intraspecies formate transfer [1].

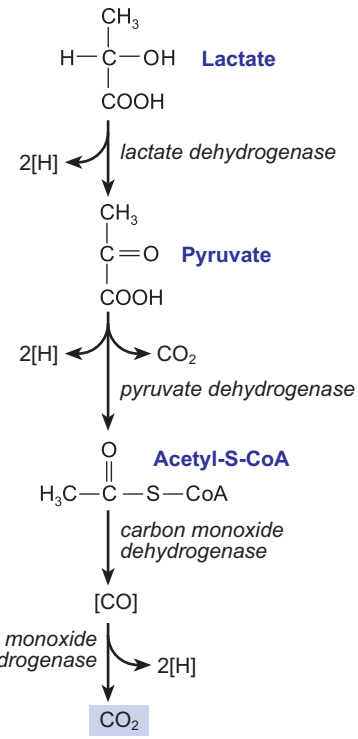

Fig. 4. Pathway of the oxidation of lactate to CO_2 [57]

Рис. 4. Шлях окиснення лактату до CO_2 [57]

Oxidation of acetate (acetyl-CoA) to CO_2. Some thermophilic sulfate-reducing archaea oxidize organic compounds, such as acetate, lactate, fatty acids, alkanes, benzoic acid etc., completely to CO_2 using sulfate as an electron acceptor [19]. Acetyl-S-CoA is an intermediate compound which forms from CO_2. Some SRB, including *Desulfobacter postgatei*, use citric acid cycle (CAC) for oxidation of acetyl-S-CoA to CO_2 [1]. Therefore, they can use intermediates of CAC, namely citrate, aconitate, isocitrate, 2-oxoglutarate, succinyl-CoA, succinate, fumarate, malate and oxaloacetate [63]. However, most SRB, including *Archaeoglobus fulgidus*, use oxidizing acetyl-CoA synthase (decarbonylase)/carbon monoxide dehydrogenase, tetrahydrofolate (H_4F) or tetrahydromethanopterin (H4MPT) as C1-carrier. In this way, acetyl-CoA is oxidized while using carbon (II) oxide via intermediates: methyl-H_4F (methyl-H_4MPT) $\rightarrow$ methylene-H_4F (methylene-H_4MPT) $\rightarrow$ methenyl-H_4F (methenyl-H_4MPT) $\rightarrow$ N^{10}-formyl-H_4F (N^5-formyl-H_4MPT) $\rightarrow$ formate (formyl-methanofuran) (Fig. 5) [57].

Oxidizing acetyl-S-CoA synthase (decarbonylase), EC 2.3.1.169/carbon monoxide dehydrogenase, EC 1.2.99.2 plays an important role in the process of dissimilatory sulfate reduction. Oxidation of organic compounds is accompanied by the redox potential which is more negative than those of adenosine-5′-phosphosulfate (APS)/HCO_3^- (-60 mV) and HCO_3^-/HS^- (-116 mV). Citric acid cycle is only one way of oxidation of succinate to fumarate with the formation of redox potential +33 mV. Oxidation of succinate to fumarate with sulfate as the final electron acceptor needs energy directing reverse electron transport [1, 19].

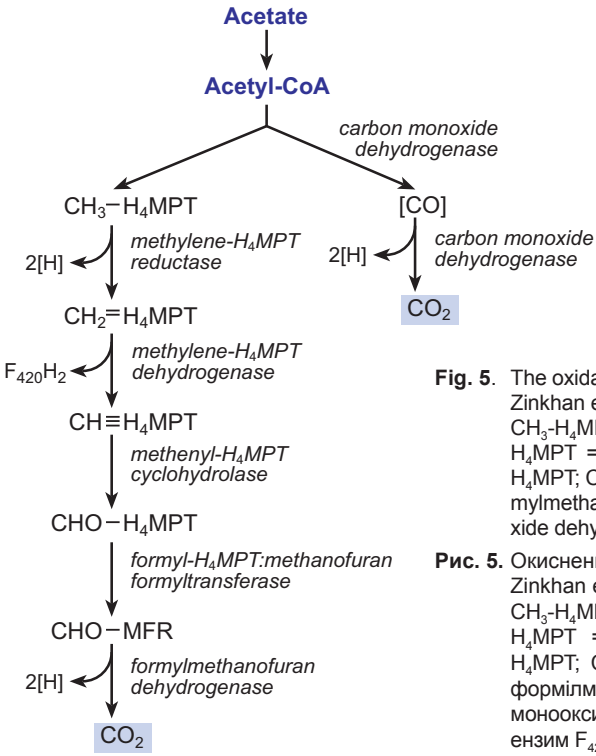

Fig. 5. The oxidation of acetate (acetyl-CoA) to CO_2 (by Möller-Zinkhan et al. 1990) [57]:
CH_3-H_4MPT = methyltetrahydromethano-pterin; CH_2=H_4MPT = methylene-H_4MPT; CH≡H_4MPT = methenyl-H_4MPT; CHO-H_4MPT = formyl-H_4MPT; CHO–MFR = formylmethanofuran; [CO] = CO bound to carbon monoxide dehydrogenase; $F_{420}H_2$ = reduced coenzyme F_{420}

Рис. 5. Окиснення ацетату (ацетил-КоА) до CO_2 (за Möller-Zinkhan et al. 1990) [57]:
CH_3-H_4MPT = метилтетрагідрометано-птерин; CH_2=H_4MPT = метилен-H_4MPT; CH≡H_4MPT = метеніл-H_4MPT; CHO-H_4MPT = форміл-H_4MPT; CHO-MFR = формілметанофуран; [CO] = CO зв'язаний з кабон монооксидом дегідрогенази; $F_{420}H_2$ = відновлений коензим F_{420}

SRB involve phosphotransacetylase and acetate kinase in the oxidation of acetate to CO_2. Phosphotransacetylase, EC 2.3.1.8 catalyzes the phosphorylation of acetyl-CoA after which acetyl phosphate is formed and then changed into acetate by acetate kinase, EC 2.7.2.1 [43, 45].

Oxidation of propionate. Propionyl-S-CoA, which is formed from propionate or during the oxidation of fatty acids, is oxidized through intermediates: methylmalonyl-S-CoA, succinyl-S-CoA, succinate, fumarate, malate, oxaloacetate, pyruvate and acetyl-S-CoA (Fig. 6) [19].

Bacteria *Desulfobulbus propionicus* grow using propionate and sulfur [1]. Obviously, oxidation of methylmalonyl-S-CoA is more energetically favorable than the oxidation of propionyl-S-CoA through acrylic-S-CoA. Since the redox potential of acrylic-S-CoA/propionyl-S-CoA is +69 mV, it is more disadvantageous than that of fumarate/succinate (+33 mV) [19].

Thus, lactate, acetate and propionate are important electron donors for the SRB growth in human and animal intestines. Perhaps, the presence of electron donors and sulfate in the intestine can cause intense development of SRB which in turn will probably heighten the risk of ulcerative colitis due to the formation of hydrogen sulfide. Thermodynamic characteristics of the donor electrons are important for the study of the vital activity of SRB and the process of dissimilatory sulfate reduction.

Thermodynamic properties of electron donors. Reactions occurring with a change in the oxidation state of atoms or reactions between the oxidizing and reducing agent are called redox reactions. The power of oxidant and reductant is determined by the redox potential (E^0). It depends on the changes in the concentrations of ions of H^+ and OH^- in the

medium. This value is measured in millivolts (mV). If the value is more positive, the oxidant is stronger, and conversely, the lesser value means the stronger reducing agent [63].

Sulfate-reducing bacteria which oxidize organic compounds or hydrogen are capable of using various electron acceptors with mainly low redox potential [19]. These acceptors can be sulfate, thiosulfate, sulfite and elemental sulfur [1, 29, 64]. SRB are isolated from anaerobic environments with high redox potential. High concentration of oxidized iron in these conditions causes increasing E^0 and it promotes SRB growth [63].

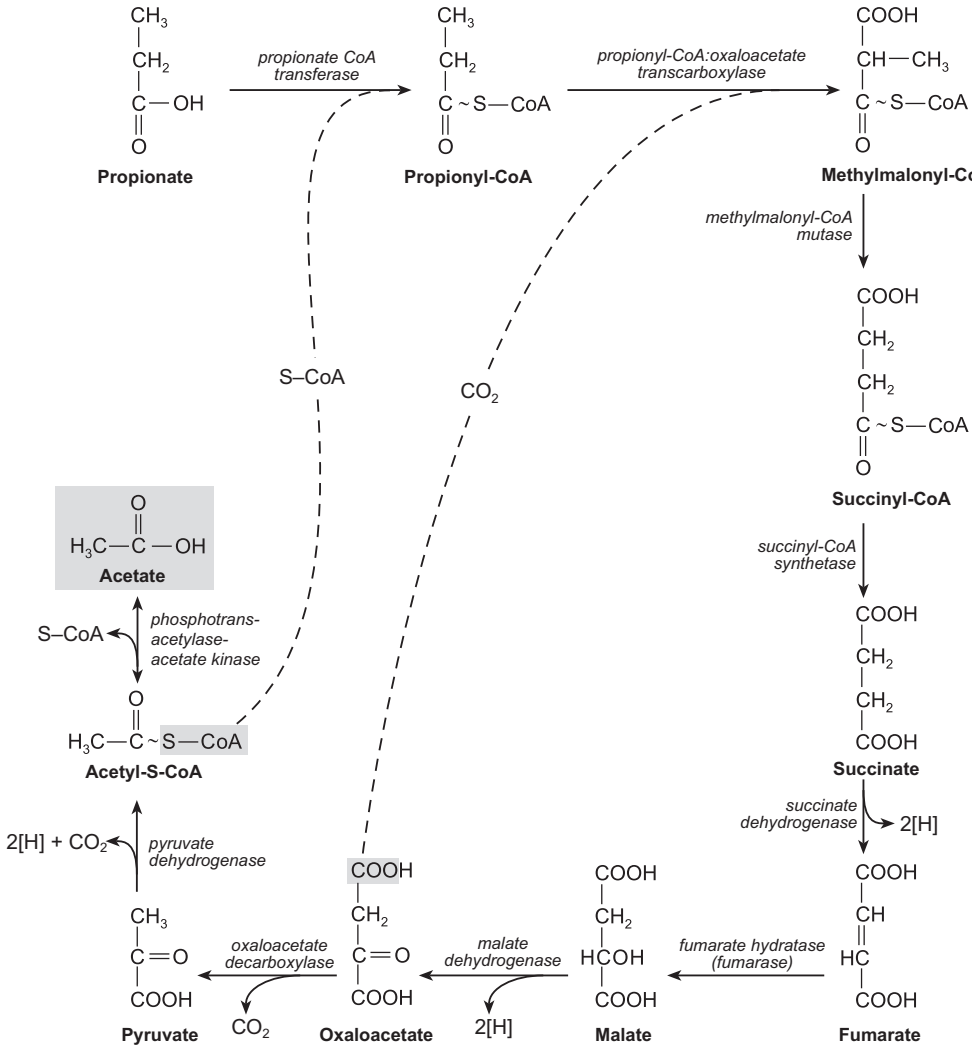

Fig. 6. The scheme of propionate oxidation in *Desulfobulbus propionicus* [19]

Рис. 6. Схема окиснення пропіонату *Desulfobulbus propionicus* [19]

Redox potential of sulfate/HS^- in the presence of 1 M sulfate ions, 1 M HS^-, pH 7.0 and temperature +25 °C is -217 mV. It is slightly larger (-200 mV) for the concentration of SO_4^{2-} < 30 mM and HS^- < 1 mM. Organic compounds of plant and animal origin (carbo-

hydrates, fatty acids, alkanes, aromatic hydrocarbons) can be oxidized completely to CO_2 by some SRB (*Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, *Desulfonema* genera). Other SRB (*Desulfotomaculum* and *Desulfovibrio* genera), which have only some enzymes of the Krebs cycle, oxidize these organic compounds only partially into acetate. Redox potential is changing in the process of oxidation of organic compounds which can be electron donors for SRB. Each of these potential donors of electrons is much more negative than -200 mV of the pair sulfate / HS^- [19].

In the natural environment, E^0 (SO_4^{2-}/HS^-) is greater than $\Delta E'$ of the reducing agent and they are used together. However, there are exceptions, for example H^+/H_2 and S^0/HS^-. Redox potential of H^+/H_2 at pH 7.0 (concentration of H^+ is 10^{-7} M and constant) increases from -414 mV (partial pressure of H_2 is 10^5 Pa) to -270...-300 mV (partial pressure of H_2 from 1 to 10 Pa). Therefore, the oxidation of acetate to CO_2 (E^0 = -290 mV) with H^+ as an electron acceptor (E^0 = -270 mV at H_2 1 Pa) is thermodynamically possible. Microorganisms are able to grow in this range of the redox potential [1].

Redox potential of S^0/HS^- increases from -270 to -120 mV under different conditions. In this case, SRB can be found in sulfur containing environments where they can grow through the S^0 reduction [19, 64].

Growth of SRB via dissimilatory sulfate reduction is accompanied by oxidation of the substrate together with the synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate [36, 39].

In the process of substrate phosphorylation of organic compounds, „energy rich" intermediates are formed [1]. Transport of electrons causes the formation of transmembrane electrochemical proton gradient or gradient of sodium ions. It leads to the phosphorylation of ADP by membrane-bound ATP synthase [19, 36].

For many years, it has been believed that SRB can grow only in the presence of organic substrates which serve as electron donors for the dissimilatory sulfate reduction. However, in 1978 a discovery was made that *Desulfovibrio vulgaris* can grow in the presence of H_2 and sulfate as a single source of energy [2]. It is also believed that energy is produced in these organisms largely as a result of the substrate phosphorylation [1, 63].

Substrate phosphorylation is possible only by oxidation of organic substrates. One exception from this rule is the oxidation of bisulfite to sulfate through energy-rich intermediate products including adenosine-5′-phosphosulfate (APS) [41]. Using this reaction, some SRB grow in the presence of bisulfite and oxidize it to sulfate which is then reduced to hydrogen sulfide [1, 34].

Thus, sulfate-reducing bacteria grow using organic compounds that serve as a source of carbon and energy as well as the electron donors. Sulfate is the primary final electron acceptor. Oxidation of organic compounds causes a change of redox potentials. SRB can grow in the presence of H_2 and sulfate as the sole energy source. Substrate phosphorylation is possible only by oxidation of organic substrates.

4. Sulfate dissimilation and accumulation of hydrogen sulfide. The dissimilatory sulfate reduction is a complex and multistage process providing SRB cells with energy in the form of ATP. As mentioned before, they consume sulfate as a terminal electron acceptor and obtain energy for their growth due to the oxidation of organic compounds and hydrogen [1, 19, 36]. The final product of sulfate reduction is hydrogen sulfide [33].

The reduction of sulfate to hydrogen sulfide occurs through many intermediates and is an eight-electron process [63]. However, these intermediates are not released by SRB into the environment [19].

The enzymes of SRB involved in the process of dissimilatory sulfate reduction are localized in the cytoplasm and periplasm. At the beginning stages of sulfate reduction, absorption of sulfate occurs in bacterial cells [36]. While sulfate can be transported into the cells simultaneously with protons, some halophilic species of SRB can absorb sulfate together with the flow of sodium ions [1].

Dissimilatory sulfate reduction can be divided into six stages (Fig. 7).

Sulfate activation. Before sulfate is reduced, it is transported into bacterial cells and activated by reaction catalyzed by the enzyme ATP sulfurylase, EC 2.7.7.4 which transfers sulfate to the adenine monophosphate moiety of ATP to form adenosine 5′-phosphosulfate (APS) and pyrophosphate (PP_i). The reaction is also reversible, and therefore, ATP can be formed from APS and PP_i [39, 42, 68].

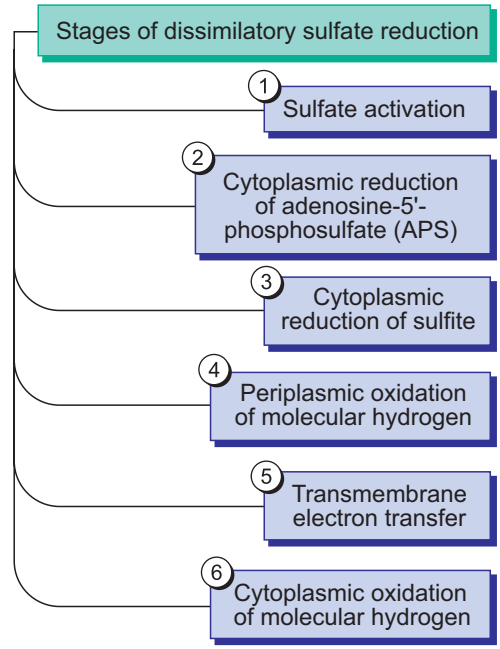

Fig. 7. The stages of dissimilatory sulfate reduction [19]
Рис. 7. Етапи дисиміляційної сульфатредукції [19]

The redox potential of SO_4^{2-}/HSO_3^- is -516 mV and that of APS/HSO_3^- is -60 mV. ATP sulfurylase catalyzes the following reaction [1]:

$$SO_4^{2-} + ATP + 2H^+ = APS + PP_i; \quad \Delta G^o = -46 \text{ kJ/mol} \qquad (7)$$

$$PP_i + H_2O = 2P_i; \quad \Delta G^o = -21.9 \text{ kJ/mol} \qquad (8)$$

ATP sulfurylase can be found in the cells of many different organisms and it differs by its molecular weight and mono-, di-, tetra- or hexameric structure. Most ATP sulfurylases consist of identical subunits containing cobalt and zinc ions (Fig. 8, *A*) [19]. However, in the bacteria *E. coli*, this enzyme has different subunits. ATP sulfurylases of *D. desulfuricans* and *D. gigas* are homotrimers with molecular weights of 141 and 147 kDa, respectively.

Bacteria of the *Desulfovibrio* genus contain cytoplasmic pyrophosphatase, EC 3.6.1.1 which catalyzes the cleavage of pyrophosphate to two phosphate ions (Fig. 8, *B*) [39]. In the process of pyrophosphate hydrolysis, energy is released in the form of a transmembrane proton potential [1].

Cytoplasmic reduction of adenosine-5′-phosphosulfate (APS). Sulfate activation leads to an increase of the redox potential from -516 mV to -60 mV [1]. Increase of E^o provides the reduction of APS which serves as an electron acceptor. Bacteria of the *Desulfovibrio* genus contain cytoplasmic APS reductase (adenylyl-sulfate reductase, EC 1.8.99.2) that promotes the reduction of APS to sulfite or bisulfite and AMP [9, 11, 41]. APS reductase is also present in the cells of some purple and green bacteria and the *Thiobacillus* genus [19].

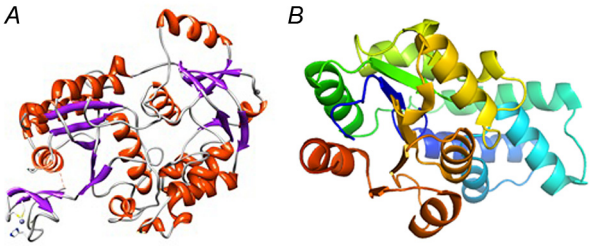

Fig. 8. The structure of Zn-containing ATP sulfurylase of *D. desulfuricans* (*A*) [74] and the structure of pyrophosphatase (*B*) [75]

Рис. 8. Структура Zn-вмісної АТФ сульфурилази *D. desulfuricans* (*A*) [74] і структура пірофосфатази (*B*) [75]

APS reductase is a nonheme iron-sulfur containing flavoprotein with a molecular weight of 95 kDa which consists of α- and β-subunits (Fig. 9). The first (α) subunit contains a molecule of flavin adenine dinucleotide (FAD) and the second (β) contains two [4Fe–4S]-centers [1, 9, 11]. This enzyme reduces APS to sulfite in the position N(5)-FAD. The substantially increased number of polar interactions between the protein matrix and cluster *B* compared to cluster *C* can explain the differences in the redox potential [59].

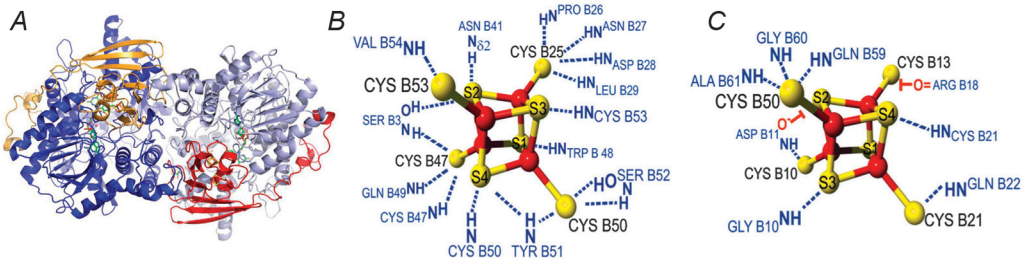

Fig. 9. The structure of APS reductase $\alpha_2\beta_2$ heterotetramer and the [4Fe–4S] electron transfer sites of the enzyme (*A*): clusters (*B*) and (*C*) are covalently linked to the sulfhydryl group of four cysteines (modified by Parey et al. 2013) [59]

Рис. 9. Структура АФС-редуктази $\alpha_2\beta_2$ гетеротетрамера і [4Fe–4S] ділянки ензиму електронного переносу (*A*): кластери (*B*) і (*C*) ковалентно зв'язані з сульфгідрильною групою чотирьох цистеїнів (модифіковано за Parey et al. 2013) [59]

APS reductase can be isolated from the cells of *D. desulfuricans* and *D. vulgaris*, and found in phototrophic and denitrifying bacteria. In the denitrifying bacteria, enzyme converts sulfite and AMP to APS in the process of photosynthesis or denitrification [63]. The activity of this enzyme of bacteria *D. vulgaris* depends on various chemical and physical factors, particularly on adding salts in concentrations of 0.5–1.0 M which leads to its inactivation. Kinetics of direct and reverse reaction also depends on the concentration of the enzyme [41]. The reverse reaction is described by Michaelis-Menten kinetics. Increase of AMP concentration in the environment leads to the inhibition of the reverse reaction and the concentration of 1.8 mM AMP and more causes the reaction to be terminated [1, 19].

The dissimilatory sulfate reduction to H_2S in bacteria *D. vulgaris* occurs through the formation of sulfite as an intermediate product [64].

Cytoplasmic reduction of sulfite. The next important stage in the process of dissimilatory sulfate reduction is sulfite which is the product of the reduction of APS [19]. Sulfite (SO_3^{2-}) is more reactive than sulfate. Reduction of SO_3^{2-} to S^{2-} is carried out by the enzyme called dissimilatory sulfite reductase, EC 1.8.99.1 (Fig. 10) [34, 59].

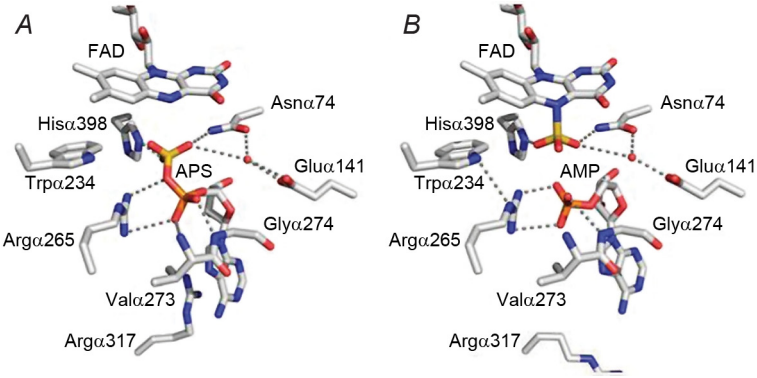

Fig. 10. The structures of APS reductase (*A*) and sulfite reductase (*B*) (modified by Parey et al. 2013) [59]

Рис. 10. Структури АФС-редуктази (*A*) і сульфітредуктази (*B*) (модифіковано за Parey et al. 2013) [59]

This enzyme is usually composed of two α- and β-subunits (α2β2). However, the bacteria *D. vulgaris* and *D. desulfuricans* Essex contain a third subunit (γ). It has been proven that dissimilatory sulfite reductase in these microorganisms is a hexamer (α2β2γ2) [1].

Active centers of sulfite reductases have two metal ion cofactors, siroheme and [FeS]-cluster [7, 19] (Fig. 11). They are involved in the transport of electrons. Six electrons are transported in the process of the reduction of sulfite to sulfide [8, 10, 63].

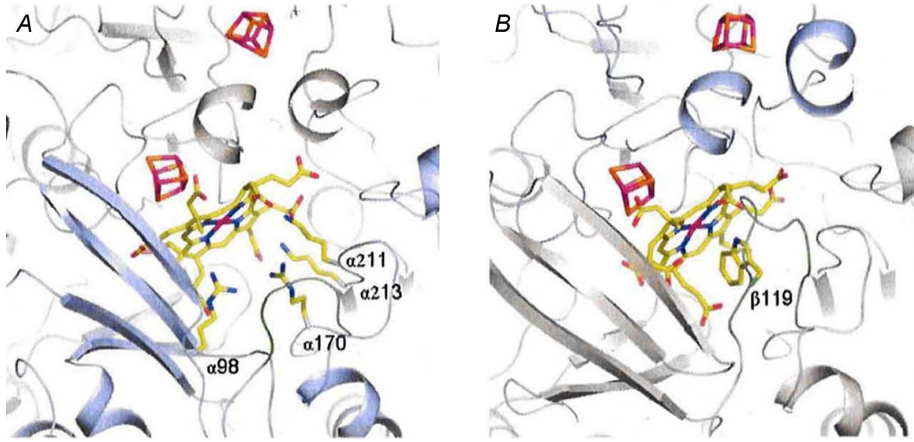

Fig. 11. The structures of the functional (*A*) and the structural centers (*B*) coupled with siroheme-[4Fe-4S] (modified by Parey et al. 2013) [59]

Рис. 11. Структура функціонального (*A*) і структурно поєднаного сірогем-[4Fe-4S] центрів (*B*) (модифіковано за Parey et al. 2013) [59]

SRB have the following main types of dissimilatory sulfite reductases: desulfoviridine, desulforubidine, desulfofuscidine, and protein P_{582} [19].

Bisulfite is one form of sulfite. Some scientists believe that the actual substrate in the process of dissimilatory sulfite reduction to sulfide is bisulfite rather than sulfite [1]. That is why sulfite reductase is often also called bisulfite reductase [63].

For many years, there has been a controversy around the following equation:

$$HCO_3^- + 6\bar{e} + 6H^+ = HS^- + 3H_2O;\ E^0 = -116\ mV \quad (9)$$

because bisulfite reductase also catalyzes reactions 9 and 10 in the high concentration of HCO_3^- [1].

$$3HSO_3^- + 2\bar{e} + 3H^+ = S_3O_6^{2-} + 3H_2O; \quad E^0 = -173\ mV \quad (10)$$

$$S_3O_6^{2-} + 2\bar{e} + H^+ = S_2O_3^{2-} + HCO_3^-; \quad E^0 = +225\ mV \quad (11)$$

According to one of the hypotheses, SRB contain thiosulfite reductase which catalyzes the reaction [19]:

$$S_2O_3^{2-} + 2\bar{e} + H^+ = HS^- + HCO_3^-; \quad E^0 = -402\ mV \quad (12)$$

Two hypotheses regarding sulfite reduction have been suggested [1, 64]:

- Consistent reduction through three two-electron steps with the formation of trithionate and thiosulfate as intermediate compounds;
- Direct six-electron reduction without the formation of trithionate and thiosulfate as intermediates.

$$3SO_3^{2-} \xrightarrow{H_2} S_3O_6^{2-} \xrightarrow[-SO_4^{2-}]{H_2} S_2O_3^{2-} \xrightarrow[-SO_4^{2-}]{H_2} S^{2-}$$

Sulfite reductase plays an important role in the process of assimilation of sulfur. The enzyme promotes the formation of sulfide for synthesis of sulfur-containing amino acids including methionine and cysteine. This enzyme found in the cells of *Desulfovibrio* genus as well as in many other SRB [19, 64].

The mechanism of the six-electron sulfite reduction involves Fe^{2+} which connects sulfur atom with sulfite ion [1]. The two-electron reduction causes the oxygen atom in SO-bond to be protonated and then hydroxyl anion can be eliminated [7, 10]. Sulfide is formed after repeated reduction by two electrons and the subsequent protonation of oxygen atoms which are then gradually removed from the atoms of sulfur (Fig. 12).

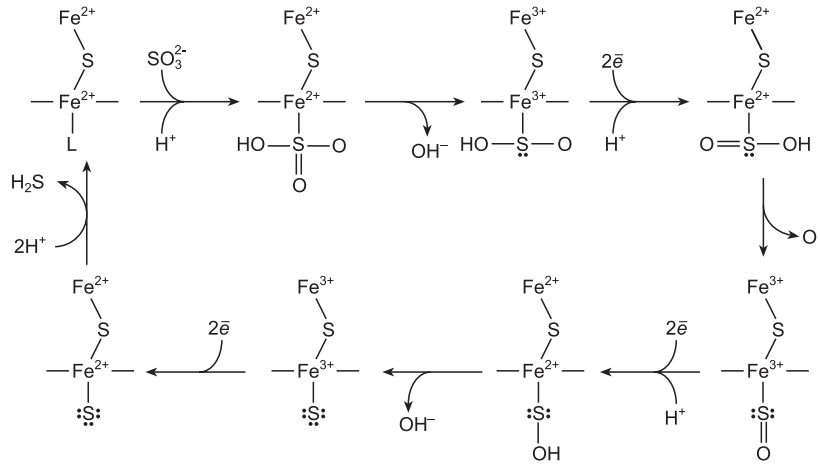

Fig. 12. The mechanism of sulfite reduction through successive two-electron steps: [4Fe–4S]-cluster bound to the iron atom of siroheme via the sulfur atom is represented by only one Fe^{2+} ion; L is a protein ligand that is bound to Fe^{2+} of siroheme via coordinate bond and replaced by sulfite in the catalysis [19]

Рис. 12. Механізм відновлення сульфіту двохелектронними послідовними кроками: [4Fe–4S]-кластер, що зв'язаний з атомом феруму сирогему через сульфур, представлений тільки одним Fe^{2+}. L – білковий ліганд, що зв'язаний з Fe^{2+} сирогему через координацію зв'язків і заміщується сульфітом у процесі каталізу [19]

Bisulfite reduction through three two-electron steps can be faster than only one step using six electrons [1]. If SRB are grown in the presence of bisulfite or thiosulfate, it is possible that they might not use sulfate as the primary electron acceptor [3]. However, it has been shown that when *D. vulgaris* have genetic disorders of the mechanisms of thiosulfate reduction, it does not affect the ability of these bacteria to grow in the medium with sulfate and molecular hydrogen. Under such conditions, bisulfite concentration in the medium is reduced [63].

Even though the main intermediate products in the process of the dissimilatory sulfate reduction are APS and sulfite, there is evidence that other intermediates might be produced, as well, for example three- and tetrathionate [19].

Periplasmic oxidation of molecular hydrogen. Oxidation of molecular hydrogen occurs in the periplasm and involves periplasmic hydrogenases. Hydrogenases are the enzymes that catalyze the reversible redox reaction in the presence of hydrogen. They play an important role in anaerobic respiration [31]. H_2 oxidation is caused by the reduction of the terminal electron acceptor (oxygen, nitrate, sulfate, carbon (IV) oxide, fumarate) [1].

Reduction of H_2 is important for transforming pyruvate. Some molecules and proteins (ferredoxin, cytochrome c_3 and cytochrome c_6) can be physiological donors (D) or acceptors (A) of electrons for hydrogenases [19]:

$$H_2 + A_{ox} \rightarrow 2H^+ + A_{red}$$

$$2H^+ + D_{red} \rightarrow H_2 + D_{ox}$$

Hydrogenases are involved in the absorption and formation of molecular hydrogen. This process occurs through the reaction [63]:

$$H_2 \rightleftharpoons H^+ + H^- \rightleftharpoons 2H^+ + 2\bar{e}.$$

There are four classes of hydrogenases: [NiFe], [FeFe], [NiFeSe] and [Fe]. In all these hydrogenases, metal ions play an important role for the functioning of the active centers (Fig. 13, 14) [19, 73].

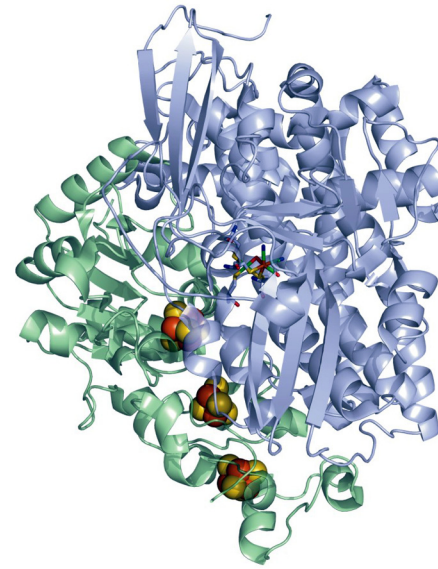

Fig. 13. The structure of [NiFe]-hydrogenase from *D. fructosovorans* [73]

Рис. 13. Структура [NiFe]-гідрогенази *D. fructosovorans* [73]

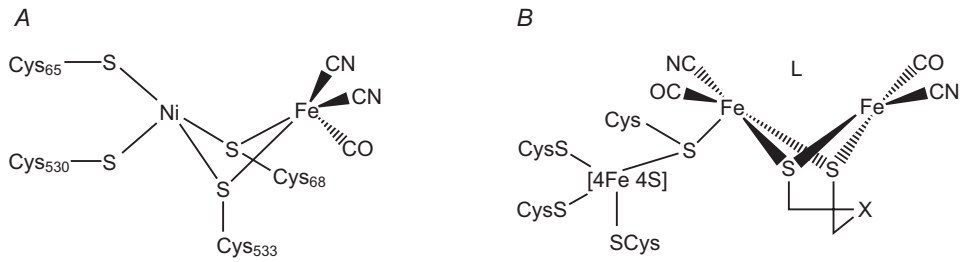

Fig. 14. The structure of [NiFe]- and [FeFe]-active site [73]

Рис. 14. Структура [NiFe]- і [FeFe]-активної ділянки [73]

D. vulgaris bacteria contain four periplasmic hydrogenases, including three [NiFe]-hydrogenases, EC 1.12.99.6 and one [FeFe]-hydrogenase, EC 1.12.7.2 [1, 58]. While the three [NiFe]-hydrogenases are bound with the main periplasmic polyheme cytochrome *c*-type (TpI-c3), one of them, [NiFe]-hydrogenase 2, is probably also bound with the second polyheme cytochrome c (TpII-c3). If bacteria grow in the medium which contains a small amount of nickel and in the presence of hydrogen and sulfate then only [FeFe]-hydrogenase is synthesized. Under these conditions, the level of biomass accumulation is practically unchanged [19]. It has been established that removing of gene of [FeFe]-hydrogenase or one of [NiFe]-hydrogenases also do not have an effect on the growth of bacteria *D. vulgaris* [14, 18]. These data show that the four hydrogenases can completely functionally change each other, especially when growing the bacteria in the medium with high concentrations of H_2 [62].

Transmembrane electron transfer. In the periplasm, protons are transported to the cytochrome c_3 by periplasmic hydrogenases [1]. Subsequently, electrons from cytochrome c_3 are transferred through the cytoplasmic membrane to the recovered APS and HSO_3^- in cytoplasm. Electron transport through membranes involves a protein hmc-complex [19]. This complex is on the one side associated with periplasmic region of polyheme cytochrome *c*, and on the other with the cytoplasmic side containing FeS-protein (Fig. 15). It has a structure similar to heterodisulfite reductase. The hmc-complex is the most studied in bacteria *D. vulgaris* [60]. After removing *hmc* genes, bacteria *D. vulgaris* grew only in the medium with lactate and sulfate, and the growth significantly slowed down in the presence of only H_2 and sulfate. The genome of *D. vulgaris* encodes transmembrane protein complexes (TpII-c3 and Hme) [1, 62].

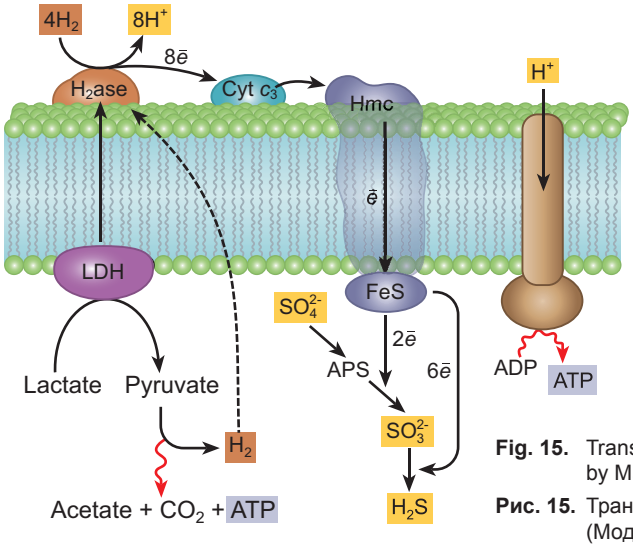

Fig. 15. Transmembrane electron transfer (Modified by Madigan et al. 2006) [53]

Рис. 15. Трансмембранне перенесення електронів (Модифіковано за Madigan et al. 2006) [53]

They are similar to the hmc-complex and located on periplasmic side of cytochrome *c* and cytoplasmic side with FeS-proteins [53]. The sequences of these proteins also resemble those of heterodisulfite reductase [19]. Three transmembrane complexes (Hmc, Hme and TpII-c3) can likely carry out similar functions to four periplasmic hydrogenases [64].

M.S. Sim et al. (2013) have tested mutant strains lacking one or two periplasmic (Hyd, Hyn-1, Hyn-2, and Hys) or cytoplasmic hydrogenases (Ech and CooL), and a mutant strain lacking type I tetraheme cytochrome (TpI-c_3). They have shown that wild-type *D. vulgaris* and its hydrogenase mutants had comparable growth kinetics and produced the same sulfur isotope effects. In continuous culture, wild-type *D. vulgaris* and the CycA mutant produced similar sulfur isotope effects, underscoring the influence of environmental conditions on the relative contribution of hydrogen cycling to the electron transport. The schematic representation of two proposed pathways for electron transport during sulfate reduction in *D. vulgaris* Hildenborough is below presented in Fig. 16 [67].

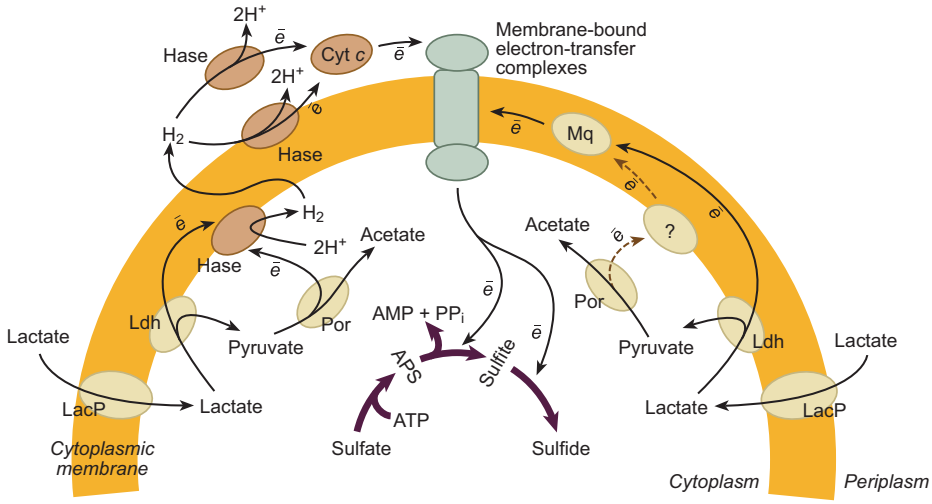

Fig. 16. The schematic representation of two proposed pathways for electron transport during sulfate reduction in *D. vulgaris* Hildenborough: LacP is lactate permease, Ldh is lactate dehydrogenase, Por is pyruvate-ferredoxin oxidoreductase, Hase is hydrogenase, Cyt *c* is cytochrome *c*, Mq is menaquinone pool, APS is adenosine 5′-phosphosulfate. Dashed lines and the question mark indicate currently hypothetical pathways and components (Modified by Sim et al., 2011) [67]

Рис. 16. Схематичне зображення запропонованих двох шляхів транспорту електронів під час сульфатредукції *D. vulgaris* Hildenborough: LacP – лактатпермеаза, Ldh –лактатдегідрогеназа, Por – піруват:ферредоксиноксидоредуктаза, Нase – гідрогеназа, Cyt *c* – цитохром *c*, Mq – менахінон, APS – аденозин 5′-фосфосульфат. Пунктирні лінії та знак питання вказують на гіпотетичні шляхи і компоненти (модифіковано за Sim et al., 2011) [67]

In *D. vulgaris* genome, a cluster of gene encoding transmembrane protein complex (Qmo complex) was found. There are no genes encoding periplasmic cytochrome *c* [1, 19]. The complex Qmo is involved in the reduction of APS (Fig. 17) [65]. Heterodisulfide reductase, EC 1.8.98.1 of methanogens catalyzes the reduction of heterodisulfide of CoM-SS-CoB to coenzyme M (HS-CoM) and coenzyme B (HS-CoB). Both coenzymes are missing in the SRB. Cell extracts of SRB do not catalyze the reaction of CoM-SS-CoB and oxidation of CoM-SH + CoB-SH [60, 61].

Ramos A.R. et al. (2012) have reported the first direct evidence that QmoABC and AprAB interact in *Desulfovibrio* spp., using co-immunoprecipitation, cross-linking Far-Western blot, tag-affinity purification, and surface plasmon resonance studies. They showed that the QmoABC–AprAB complex has a strong steady-state affinity, but has a transient character due to a fast dissociation rate. Far-Western blot identified QmoA as

the Qmo subunit most involved in the interaction. Nevertheless, electron transfer from menaquinol analogs to APS through anaerobically purified QmoABC and AprAB could not be detected. Authors propose that this reaction requires the involvement of a third partner to allow electron flow driven by a reverse electron bifurcation process. This process is deemed essential to allow coupling of APS reduction to chemiosmotic energy conservation [65].

Ramos A.R. et al. (2012) have proposed a schematic representation of the QmoABC–AprAB interaction and the involvement of third partners. In the hypothesis of an electron bifurcation process, the putative electron acceptor of QmoB with a high redox potential is represented by a question mark (Fig. 17*A*). In the hypothesis of an electron confurcating, mechanism several possible co-electron donors for the Qmo complex are considered: ferredoxin (Fd), hydrogenase (Hase), formate dehydrogenase (Fdh) or NADH dehydrogenase (Nox) (Fig. 17*B*). The soluble HdrABC–MvhGAD complex (Fig. 17*C*) and the membrane-bound HdrED (Fig. 17*D*) of methanogens are shown for comparison. The gray dashed arrows represent electron bifurcation in (*A*, *C*), or electron confurcation in (*B*). The gray boxes represent the cytoplasmic membrane with + indicating the periplasm and − the cytoplasm (Fig. 17) [65].

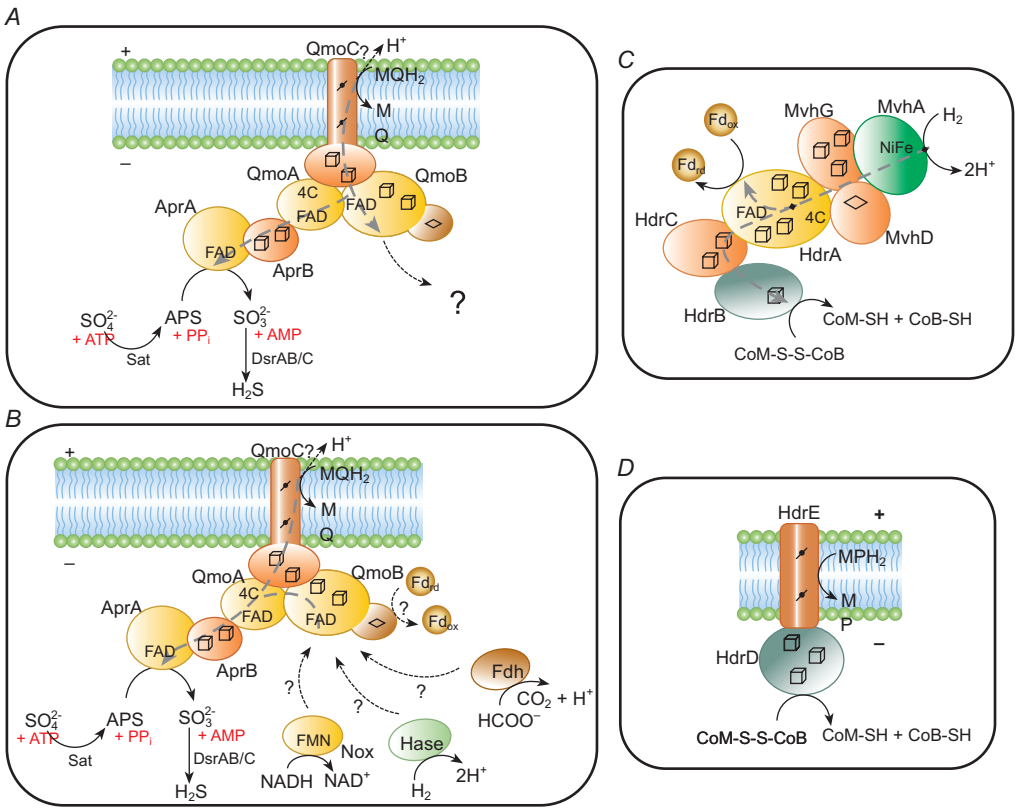

Fig. 17. The schematic representation of the QmoABC–AprAB interaction and the proposed involvement of third partners (modified by Ramos et al., 2012) [65]

Рис. 17. Схематичне представлення взаємодії QmoABC–AprAB і запропонована участь третіх партнерів (модифіковано за Ramos et al., 2012) [65]

FeS proteins are a group of proteins involved in the processes of electron transport (ferredoxins) and some enzymes that catalyze different redox reaction [19]. Depending on the structural features of FeS centers, ferredoxins can carry out simultaneous transfer of one or two electrons [7, 10]. Redox potential of ferredoxins is preferably in the range of -490 to -310 mV. However, there have been described some FeS proteins with positive redox potential of +350 mV [1].

Ferredoxins play an important role in the metabolism of SRB, combining catabolic processes together with biosynthetic reactions. Physiological reactions in SRB cells occur at the negative redox potentials. Under these conditions, FeS proteins are important for the functioning of enzymes, and used as carriers of electrons (Fig. 18) [19].

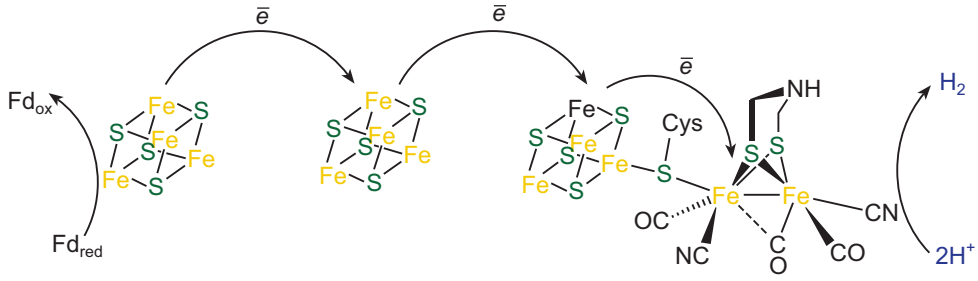

Fig. 18. [Fe_4S_4] cluster-mediated electron transfer pathway in *Desulfovibrio gigas* hydrogenase (shown in the direction of proton reduction): Fd_{red} and Fd_{ox} represent ferredoxin in the reduced and oxidized state, respectively X = CH_2, NH, O [70]

Рис. 18. [Fe_4S_4] кластер опосередкованого переносу електронів гідрогеназ *Desulfovibrio gigas* (показаний в напрямку редукції протонів): Fd_{red} і Fd_{ox} фередоксин у відновленому й окисненому стані, відповідно, X = CH_2, NH, O [70]

FeS proteins in SRB have an amino acid sequence similar to heterodisulfide reductase. Perhaps, they have different substrate specificity and can be involved in other functions. In methanogenic archaea, the reduction of H_2 occurs through oxidation of methyl-coenzyme M. The concentration of H_2 under these conditions decreases and methane is produced. It is believed that disulfide/HS^- couple in SRB can be also involved in the transfer of electrons from hydrogen to sulfite [1].

Cytoplasmic oxidation of molecular hydrogen. The process of molecular hydrogen oxidation occurs by involving cytoplasmic hydrogenase and FeS proteins [19]. Bacteria *D. vulgaris* contains two membrane complexes of hydrogenase, EchABCDEF and CooMKLXUHF, which are interrelated [14, 18, 56]. They catalyze the reduction of ferredoxins in the presence of H_2 or protons to H_2 through ferredoxin reduction. Both of these reductions cause the formation of proton electrochemical potential (ΔmH^+) (energy controlled reverse electron transfer) [58].

Hydrogenase catalyzes the oxidation of H_2 and reduction of ferredoxin [66]. For the studying hydrogenases of SRB, it is necessary to consider the bacterial growth in H_2 and sulfate in the presence of acetate and CO_2 as carbon sources. They are used by the cells forming acetyl-phosphate, acetyl-S-CoA and pyruvate [19]. Acetyl-S-CoA is formed from pyruvate in the reduction reaction of carboxylation involving pyruvate:ferredoxin oxidoreductase [44]. Redox potential (E^0) of acetyl-S-CoA + CO_2/pyruvate is -500 mV and, therefore, considerably more negative than H^+/H_2 pairs (-270 to -300 mV), especially if the partial pressure of H_2 is very low (1 to 10 Pa) [1]. For the synthesis of pyruvate from

acetyl-CoA, CO_2 and H_2 are necessary so that electrons from H_2 may have a more negative potential. This is achieved by a reverse energy transfer of electrons from H_2 to ferredoxin involving hydrogenases [60]. This pattern is characteristic for other reduction reactions such as reductive carboxylation of succinyl-S-CoA to 2 oxoglutarate (-500 mV) or reduction of CO_2 to CO (-520 mV) [1, 19].

Reverse electron transfer is important in anaerobic respiration where it is often a necessity and using reduced equivalents at low redox potential or regulation of the redox reactions. If bacteria *D. vulgaris* metabolize organic substrates such as pyruvate (E^o = -500 mV) or CO (E^o = -520 mV) by oxidation of reduced ferredoxin, then two hydrogenases are involved in the formation of H_2. For example, the reduced cytoplasmic NADP hydrogenase is found in bacteria *D. fructosovorans*. However, it has not been detected in *D. vulgaris* [63].

Bacteria *D. vulgaris* Hildenborough produces a burst of metabolites such as H_2, formate and CO (Fig. 19). This observation led to a proposal of the hydrogen-cycling model which tries to explain the growth of this microorganism despite the energetic constraints that are associated with sulfate reduction [72].

Zhou et al. (2011) proposed a model of hydrogen-cycling. According to this model, hydrogen equivalents that are generated by the oxidation of organic compounds are hypothesized to be cycled to the periplasm via the activities of the cytoplasmic hydrogenases *E. coli* hydrogenase 3 (Ech) [56] and CO-dependent hydrogenase (Coo). In the periplasm, the H_2 is re-oxidized to protons and electrons by the periplasmic hydrogenases, such as the iron-only hydrogenase, and the electrons pass through the cytochrome c_3 network. From here, electrons are proposed to be transferred to the menaquinone-linked quinone reductase complex (Qrc), then to the quinone-interacting membrane-bound oxidoreductase (Qmo) complex and finally to the adenosine phosphosulphate reductase for sulfate reduction. Concurrently, electrons are passed by an unknown mechanism to the dissimilatory sulphite reductase (Dsr) transmembrane complex and then to bisulphite reductase. In this way, sufficient electrons are made available for complete reduction of sulfate to hydrogen sulfide. The process is made energetically favourable by the activity of inorganic pyrophosphatase which removes the pyrophosphate that is generated by sulphate activation. Protons that are generated in the periplasm produce the proton-motive force that is necessary for the generation of additional ATP for growth. CO is metabolized in the cytoplasm by CO dehydrogenase, and formate is cycled to the periplasm, where it is metabolized by formate dehydrogenase, EC 1.2.1.2 (Fdh). Hydrogen cycling is not necessary when H_2 is used as the electron donor, as periplasmic metabolism of H_2 directly establishes the electrochemical gradient that is necessary for ATP synthesis [72] (Fig. 19).

Thus, dissimilatory sulfate reduction is a process consisting of many stages, including transport of sulfate in the SRB cells and its activation, the formation of APS and its reduction to sulfite, periplasmic oxidation of H_2, transmembrane transport of electrons, and cytoplasmic oxidation of H_2.

In previous studies, the intestinal SRB *Desulfovibrio piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were isolated from the healthy human large intestine and identified as described [25, 26]. The strains have since been kept in the collection of microorganisms at the Department of Molecular Biology and Pharmaceutical Biotechnology of Pharmacy Faculty at the University of Veterinary and Pharmaceutical Sciences Brno (Czech Republic) and their physiological and biochemical properties have been studied [21–24, 27–30, 32–35]. The activity and kinetic analysis of main enzymes involved in dissimilatory sulfate

reduction and the enzymes of antioxidant system including catalase [37] and superoxide dismutase [38] in the intestinal sulfate-reducing bacteria *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 were studied in detail. For each of these enzymes were determined: activity (A, U×mg^{-1} protein), initial (instantaneous) reaction rate (V_0, μmol×min^{-1}×mg^{-1} protein), the maximum of enzymatic reaction (V_{max}, μmol×min^{-1}×mg^{-1} protein), Michaelis constant (K_m) determined by the concentration of substrate (Table).

The activity of these enzymes is significantly higher in *D. piger* Vib-7 than in *Desulfomicrobium* sp. Rod-9. The peaks of the enzymatic activity occurred at the temperature of +35ºC. Maximum activity of the enzymes was at pH 8.0 which is consistent with the condition of the human colon. Obviously, such conditions provide their intensive development. The initial and maximum rates of enzymatic reactions and the maximum amount of product were significantly higher in *D. piger* Vib-7 compared to *Desulfomicrobium* sp. Rod-9. Probably, *D. piger* Vib-7 can be more dangerous and have some pathogenic role in the development of inflammatory bowel disease, the dissimilation of sulfate and lactate and, accordingly, accumulating acetate and sulfide with a higher rate.

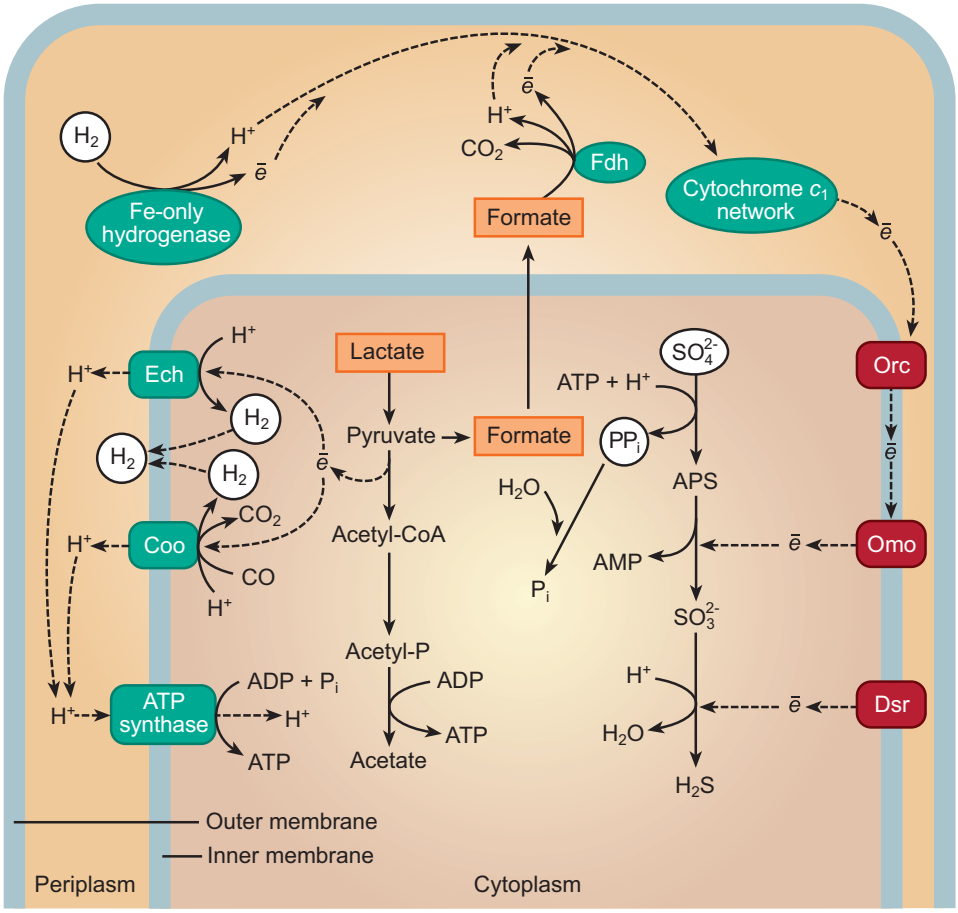

Fig. 19. The hydrogen-cycling model by Zhou et al. (2011) [72]

Рис. 19. Модель циклу гідрогену за Zhou et al. (2011) [72]

Enzymatic characteristics of the intestinal SRB strains
Ензиматичні характеристики кишкових штамів СВБ

Enzymes of dissimilatory sulfate reduction			
Kinetic characteristics [reference]		Strains of intestinal sulfate-reducing bacteria	
		D. piger Vib-7	*Desulfomicrobium* sp. Rod-9
ATPase [36]	A	16.11±1.87	7.31±0.98**
	V_0	15.95±1.58	10.69±0.93***
	V_{max}	36.10±2.87	16.64±1.73***
	K_m	2.24±0.21	2.06±0.18
ATP sulfurylase [42]	A	2.26±0.231	0.98±0.0082**
	V_0	5.48±0.57	4.12±0.38
	V_{max}	4.87±0.55	2.11±0.22**
	K_m	1.98±0.21	1.07±0.12*
APS reductase [41]	A	0.34±0.029	0.11±0.012**
	V_0	0.675±0.062	0.231±0.022***
	V_{max}	0.862±0.084	0.282±0.027***
	K_m	4.33±0.47	3.57±0.32
Sulfite reductase [34]	A	0.032±0.0026	0.028±0.0022
	V_0	0.351±0.033	0.138±0.012***
	V_{max}	0.067±0.0053	0.045±0.0039
	K_m	3.53±0.334	3.86±0.341
Pyrophosphatase [39]	A	24.27±2.47	8.16±0.82***
	V_0	18.24±1.92	5.81±0.52***
	V_{max}	43.86±4.24	13,74±1,32***
	K_m	2.53±0.27	2.60±0.21
Periplasmic hydrogenase [31]	A	1421.4±123.7	568.7±45.6***
	V_0	205.67±18.91	58.16±5.38***
	V_{max}	2500±219	1111±107***
	K_m	864±73	669±62
Lactate dehydrogenase [40]	A	0.472±0.037	0.153±0.014***
	V_0	0.114±0.012	0.026±0.022***
	V_{max}	1.20±0.11	0.65±1.73***
	K_m	0.83±0.07	1.54±0.14***

The end of the Table

Pyruvate:ferredoxin oxidoreductase [44]	A	1.24±0.127	0.48±0.051**
	V_0	4.15±0.43	1.37±0.12***
	V_{max}	2.54±0.261	0.89±0.092***
	K_m	2.72±0.283	2.55±0.245
Phosphotransacetylase [39]	A	1.19±0.122	0.37±0.041***
	V_0	5.68±0.58	2.14±0.23**
	V_{max}	2.73±0.31	0.98±0.089***
	K_m	3.36±0.35	5.97±0.62*
Acetate kinase [45]	A	1.52±0.163	0.46±0.044***
	V_0	6.16±0.63	1.39±0.14***
	V_{max}	3.12±0.32	1.03±0.098***
	K_m	2.54±0.26	2.68±0.25
Main enzymes of antioxidant system			
Catalase [37]	A	1745.21±154.67	873.11±72.23**
	V_0	3.018±0.312	1.144±0.098**
	V_{max}	5000±489	1667±168***
	K_m	8.01±0.77	10.33±0.98
Superoxide dismutase [38]	A	1326.43±142.76	1120.72±88.56
	V_0	0.0086±0.00073	0.0069±0.00055
	V_{max}	1666.67±174.92	833.33±88.54**
	K_m	0.833±0.071	0.750±0.068

Comment: Statistical significance was M±m, n=3–5; *P<0.05; **P<0.01; ***P<0.001 compared to the *D. piger* Vib-7 strain

Примітка: Статистична достовірність M±m, n = 3–5; *P<0,05; **P<0,01; ***P<0,001 порівняно зі штамом *D. piger* Vib-7

Based on the described activities of the enzymes in *D. piger* Vib-7 and *Desulfomicrobium* sp. Rod-9 and the kinetic analysis of enzymatic reactions, the generalized scheme showing the hypothetical model of dissimilatory sulfate reduction in intestinal SRB and the activity of the enzymes in their cells at each stage of this process was demonstrated for the first time. The proposed scheme summarizes already existing data on sulfate reduction and it is especially important for a more detailed understanding of the dissimilation of sulfate in the human and animal intestines (Fig. 20).

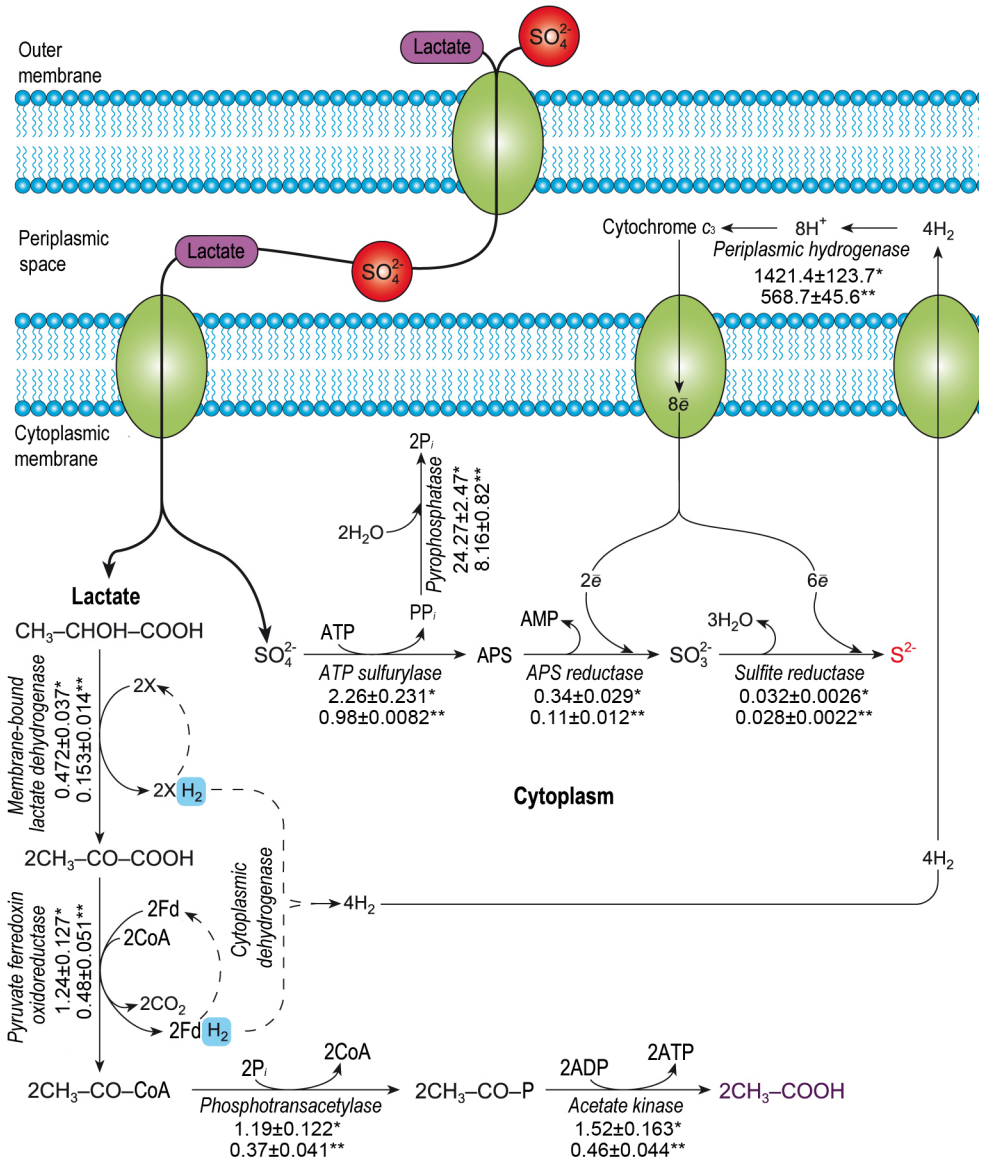

Fig. 20. The proposed metabolic model of the dissimilatory sulfate reduction in intestinal sulfate-reducing bacteria and the activity of the enzyme in *D. piger* Vib-7* and *Desulfomicrobium* sp. Rod-9**: X is unknown hydrogen carrier; Fd is ferredoxin

Рис. 20. Запропонована модель метаболізму дисиміляційного відновлення сульфату кишковими сульфатвідновлювальними бактеріями й ензиматичні активності *D. piger* Vib-7* і *Desulfomicrobium* sp. Rod-9**: X – невідомий переносник гідрогену; Fd – фередоксин

Summarizing the above described studies based on literature data and own research, it can be stated that sulfate-reducing bacteria belong to the human and animal intestinal microflora. The number of these microorganisms in the intestine depends on

the diet. The presence of sulfate induces the increased SRB level which can cause an excessive production of hydrogen sulfide. This compound is the final product of the dissimilatory sulfate reduction and may be mutagenic and toxic. The biochemical and physiological properties of intestinal SRB, their possible role in inflammatory bowel diseases, including ulcerative colitis, are summarized and analyzed. This is important for the understanding of the mechanisms behind these diseases and for the evaluation of the effectiveness of the therapy based on inhibition of SRB growth, accordingly, their production of hydrogen sulfide and acetate in gut.

CONCLUSIONS

Sulfate-reducing bacteria carry out the dissimilatory sulfate reduction. Sulfate is used in this process as a final electron acceptor. Organic compounds which enter the human and animal intestine and are formed in the fermentation process can be electron donors for SRB in the dissimilatory sulfate reduction. This process is complex and multi-staged.

The oxidation of organic substrates leads to a change of redox potentials. It depends on the nature of compounds that are oxidized or reduced, as well as the environmental conditions. SRB can grow in a wide range of oxidation-reduction potentials.

Lactate, pyruvate, acetate and ethanol are the most widespread substrates for SRB in the gut. The presence of sulfate in the food in the increased concentration can stimulate SRB growth and their competition with methanogenic organisms by the substrate in the gut.

ACKNOWLEDGEMENTS

The author expresses his gratitude to Marek Večeřa (Department of Biochemistry at Faculty of Science at Masaryk University, Czech Republic) for his reading, comments and editing of the manuscript. Gratitude is also his expressed to the reviewers for their reading, analysis and comments that have significantly increased the quality of this review.

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ДИСИМІЛЯЦІЙНА СУЛЬФАТРЕДУКЦІЯ КИШКОВИМИ СУЛЬФАТВІДНОВЛЮВАЛЬНИМИ БАКТЕРІЯМИ

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Вивчення кишкових сульфатвідновлювальних бактерій, здійснюваного ними процесу дисиміляційного відновлення сульфату, накопичення гідроген сульфіду, а також їхньої ролі у запальних захворюваннях кишечника, в тому числі виразкових колітах, у тварин і людини дедалі частіше привертає увагу вчених. Нові можливості для вивчення запального захворювання кишечника й оцінка ефективності його лікування є надзвичайно актуальною проблемою сучасної біології та медицини. У цьому огляді на основі даних сучасної літератури та результатів власних досліджень коротко характеризовано ці бактерії й описано їхній механізм дисиміляційної сульфатредукції. Подано характеристики субстратів кишкових сульфатвідновлювальних бактерій і термодинамічних властивостей їхніх донорів електронів. Особливу увагу приділено механізму й етапам дисиміляції сульфату, зокрема ензимів, що залучені у цей процес. На основі власних результатів представлено узагальнену схему дисиміляційного відновлення сульфату, що відображає активність кожного з ензимів у цьому процесі. Описані фізіологічні та біохімічні параметри є важливими для більш детального розуміння процесу дисиміляції сульфату в кишечнику людини і тварин, а також для вивчення механізмів дії антимікробних профілактик та терапії проти конкретних компонентів, залучених у патогенез захворювання. Це також може бути важливим для розуміння механізмів захворювань кишечника і для оцінки ефективності його лікування.

Ключові слова: сульфатвідновлювальні бактерії, дисиміляційне відновлення сульфату, гідроген сульфід, мікрофлора кишечника.

ДИССИМИЛЯЦИОННАЯ СУЛЬФАТРЕДУКЦИЯ КИШЕЧНЫХ СУЛЬФАТВОССТАНАВЛИВАЮЩИХ БАКТЕРИЙ

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Изучение кишечных сульфатвосстанавливающих бактерий, осуществляемого ими процесса диссимиляционого восстановления сульфата, накопления гидроген сульфида, а также их роли в воспалительных заболеваниях кишечника, в том числе язвенных колитах, у животных и человека все чаще привлекает внимание ученых. Новые возможности для изучения воспалительного заболевания кишечника и оценка эффективности его лечения являются чрезвычайно актуальной проблемой современной биологии и медицины. В этом обзоре на основе данных современной литературы и результатов собственных исследований коротко охарактеризованы эти бактерии и описан их механизм диссимиляционой сульфатредукции. Даны характеристики субстратов кишечных сульфатвосстанавливающих бактерий и термодинамических свойств их доноров электронов. Особое внимание было уделено механизму и этапам диссимиляции сульфата, в частности энзимов вовлеченных в этот процесс. На основе собственных результатов представлена обобщающая схема диссимиляционного восстановления сульфата, отражающая активность каждого из энзимов в этом процессе. Описанные физиологические и биохимические параметры важны для более детального понимания процесса диссимиляции сульфата в кишечнике человека и животных, а также для изучения механизмов действия антимикробных профилактик и терапии против конкретных компонентов, вовлеченных в патогенез заболевания. Это также может быть важным для понимания механизмов заболеваний кишечника и для оценки эффективности его лечения.

Ключевые слова: сульфатвосстанавливающие бактерии, диссимиляционное восстановления сульфата, гидроген сульфид, микрофлора кишечника.

Одержано: 24.11.2015