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REVIEW

Catalase and Superoxide Dismutase: Distribution, Properties, and Physiological Role in Cells of Strict Anaerobes

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Abstract—This review considers the distribution of the main enzymes of antioxidative defense, superoxide dismutase (SOD) and catalase, in various groups of strictly anaerobic microorganisms: bacteria of the genus *Clostridium*, *Bacteroides*, sulfate-reducing and acetogenic bacteria, methanogenic archaea, etc. Molecular and biochemical properties of purified Fe-containing SODs, cambialistic SODs, and heme catalases are presented. The physiological role and origin of the enzymes of antioxidative defense in strict anaerobes are discussed. Physiological responses (induction of SOD and catalase) to factors provoking oxidative stress in the cells of strict anaerobes able to maintain viability under aerobic conditions are also considered.

Key words: anaerobes, catalase, superoxide dismutase, oxidative stress

Products of the reduction of oxygen, such as superoxide radical, hydrogen peroxide, and especially hydroxyl radical, are toxic for cells and result in modification of amino acid residues and oxidation of sulfhydryl groups in proteins, breakage of peptide bonds, loss of metals in metalloproteins, depolymerization of nucleic acids, point mutations, and also in oxidation of polysaccharides and polyunsaturated fatty acids [1, 2]. Protection against these 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. Aerobic microorganisms possess an effective cooperatively functioning complex of protective enzymatic and nonenzymatic systems responsible for elimination of reactive oxygen species and, besides, also synthesize enzymes of DNA repair and regulators of antioxidative defense.

For a long time the absence of effective mechanisms of defense against the toxic effect of products of incomplete reduction of O_2 in the cells of anaerobes was thought to explain their sensitivity to oxygen and death in air [1].

The growth and survival of anaerobes, i.e., organisms sensitive to oxygen at partial pressure <0.2 atm and unable to grow in air, are inhibited just by O_2 and not by the high redox potential (E_h). Nevertheless, anaerobes widely vary in aerotolerance: from extreme anaerobes (methanogenic archaea) to species that retain viability for a long time under aerobic conditions (sulfate reducers of the genus *Desulfovibrio*) [3, 4]. Within the last 30 years, numerous data have been obtained about the presence of catalase and SOD in the cells of strict and the strictest anaerobes. Unlike catalase, the wide distribution of SOD in obligate anaerobes posed the question about the physiological role and origin of this enzyme in such organisms. Probably, synthesis of the enzymes of antioxidative defense is induced by O_2 or products of its incomplete reduction. SOD is a significant factor of virulence because it neutralizes $O_2^{\cdot-}$ produced during the cell contact with O_2 and thus promotes the existence of pathogenic anaerobes in living tissues during the initial period of infection, prior to development of the anaerobic conditions necessary for growth of these anaerobes. Little is known about molecular mechanisms of the adaptive response in strict anaerobes that provide for their survival under aerobic conditions. Problems of regulation of the antioxidative defense system in anaerobes and the number of genes involved in the adaptive response are also open. The purpose of the present review was to generalize rather contradictory data on the enzymes of antioxidative defense in strict anaerobes.

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CATALASE OF OBLIGATE ANAEROBIC MICROORGANISMS

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, dimethoxybenzidine, dianizidine, NADH, NADPH, etc.). 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 [5].

Unlike mono- and dimeric bifunctional catalases–peroxidases, monofunctional catalases are mainly tetrameric proteins characterized by higher temperature stability, wide pH optimum (5.5-10.5), and lack of inactivation with ethanol/chloroform. However, 3-amino-1,2,4-triazole is a specific inhibitor of monofunctional catalases [5]. In addition to heme catalases, there are Mn-catalases with a unique primary structure and resistance to N_3^-, which have been found in some facultative anaerobes such as the lactic-acid bacterium *Lactobacillus plantarum* [6, 7] and the hyperthermophilic archaeon *Pyrobaculum calidifontis* [8].

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 [9] supposed that obligate anaerobes could not grow under aerobic conditions because they lack catalase and their cells accumulate H_2O_2 [9]. Fridovich et al. [4] also thought that in most cases anaerobes had no catalase activity. 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.

The majority of known butyric fermentation bacteria of the *Clostridium* genus lack catalase and are peroxide-sensitive; however, some of them contain catalase [10]. In addition to vegetative cells, catalase has been found in spores of *Clostridium butyricum* strain 35/11 [11]. The

Table 1. Specific activity of catalase in cell extracts from anaerobic bacteria

Microorganism	Specific catalase activity, units/mg protein		References
	+ hemin (50 μM)	– hemin	
Clostridium butyricum strain 35/11	n.t.	3.0	[11]
C. acetobutyricum strain 7	112.0	1.0	[12]
C. formicoaceticum	6.0	<0.01	[12]
Bacteroides distasonis strain ATCC 8503	214.0	4.4	[16, 23]
B. fragilis strain 638	n.t.	1.4	[19]
Acetobacterium woodii	5.0	0.8	[12]
A. poludosum strain Z-7390	9.0	0.3	[12]
Desulfovibrio gigas strain ATCC 19364	n.t.	52.6	[24]
Desulfotomaculum nigrificans subsp. *salinus* strain 435	189.0	180.0	[12]
D. kuznetsovii strain 17	39.0	39.0	[12]
Thermohydrogenium kirishiense strain 360	58.0	60.0	[12]
T. lactoethylicum strain 149	8.0	18.0	[12]
Methanosarcina barkeri strain Fusaro	42.0	40.0	[25]
Methanobrevibacter arboriphilus strain DH1	300.0	7.0	[26]

Note: n.t., not tested.

Table 2. Physicochemical properties of purified catalases of anaerobic microorganisms

Microorganism	Molecular weight, kD	Number of subunits	Specific activity, units/mg protein	Heme content calculated per molecule	References
D. vulgaris	232	4	50000	1.8	[27]
D. gigas	186	3	4200	0.9	[24]
B. fragilis	130	2	8000	1.0	[19]
M. barkeri	190	4	61500	0.5	[25]
M. arboriphilus	260	4	120000	1.0	[26]

catalase activity is low in *C. butyricum* strain 21 and *C. acetobutyricum* strain 6, but, on addition of hemin into the nutrient medium, the catalase activity of *C. acetobutyricum* strain 6 increased about 100-fold [12] (Table 1). The catalase apoenzyme is probably synthesized also in cells growing on hemin-free media, and the addition of hemin results in formation of the holoenzyme and induction of synthesis of the catalase apoenzyme. Low catalase activity has been also recorded in the strict anaerobes *Bifidobacterium* [13] and the syphilitic agent (the spirochete *Treponema pallidum*) [14].

Many species of the genus *Bacteroides* are catalase-positive: *B. fragilis*, *B. distasonis*, *B. thetaiotaomicron*, *B. ovatus*, *B. eggerthi* [15-19] (Table 1). Some strains of the gram-negative human opportunistic pathogen *B. fragilis*, which is one of the most aerotolerant strict anaerobes, can withstand the influence of O_2 for 7 days [20]. The low sensitivity of *B. fragilis* to O_2 is an important factor of virulence [21], which protects the cells of the pathogen against toxic oxygen derivatives generated during the host's cell metabolism or produced by phagocytes as protective agents. The catalase-free *katB*-mutant of *B. fragilis* is more sensitive to exogenous H_2O_2 than the parent strain [22].

The heme catalase of *B. distasonis* is a thermolabile enzyme sensitive to CN^- and N_3^- with the molecular weight of 250 kD [16] and properties similar to those of the catalase from bovine erythrocytes. The catalase of *B. fragilis* is a homodimer containing one heme molecule (protoheme IX) per enzyme molecule, and its molecular weight is 130 kD [19] (Table 2). Thus, catalases of *Bacteroides* are different in structural features, and this is also supported by hybridization analysis of the *Sph*I-*Ssp*I fragment (0.79 kb oligonucleotides) of the *B. fragilis katB* and of DNA from various *Bacteroides*; some variations in the structure of the catalase gene existing in the same species [19].

The catalase of *B. fragilis* is highly homologous to the HktE catalase of *Haemophilus influenzae* and to catalases of the majority of gram-positive bacteria and mammals. Amino acid residues of the active site, NADPH-binding region, and heme-binding ligands of the known monofunctional catalases are highly conservative, which has been shown by comparing the enzymes from bovine erythrocytes, *Escherichia coli*, and *B. fragilis* [19]. The catalase of *B. fragilis* also has nonspecific peroxidase activity with pyrogallol (but not with dithionite) as an electron donor, but its primary structure is not similar to that of bacterial catalases–peroxidases [19].

Composition of the nutrient medium influences the synthesis of catalase by the *Bacteroides* cells. Thus, when the cells were grown on yeast extract, synthesis of the enzyme was repressed upon addition of glucose and other fermentable carbohydrates [15]. An addition of 0.5-5.0 μM hemin to the medium (this is significantly higher than the trace concentrations necessary for growth of *Bacteroides*) increased 40-50-fold the synthesis of catalase by the cells of *B. fragilis* and *B. distasonis* [15, 18, 28]. Even high concentrations of $FeSO_4$ and $C_6H_{11}FeNO_7$ did not affect the synthesis of catalase by the *B. distasonis* cells [23]. This was associated with the transport of iron in the tetrapyrrole porphyrin ring into the *Bacteroides* cells with involvement of the heme-binding transport complex of the membrane [17].

The maximum catalase activity of *B. distasonis* was recorded at the late log phase, and the specific activity of catalase in cell extracts (96 and 214 units/mg protein on addition to the medium of 7.7 and 77 μM hemin, respectively) was comparable to that of aerotolerant bacteria [16, 23] (Table 1). Thus, the level of catalase in the *Bacteroides* cells varies over wide limits and depends on the concentration of hemin added to the medium [18]. An increase in the catalase activity in *B. distasonis* cells increases their resistance to H_2O_2 and O_2 [23].

The catalase activity was also found in some *Porphyromonas*: *P. gingivalis*, *P. circumdentaria*, *P. salivosa* [29]. The properties of their enzymes are the same as those of bacterial monofunctional catalases: they retain the activity in the pH range from 5 to 10 and are not inhibited by chloroform/ethanol, but their activity is irreversibly inhibited with 3-amino-1,2,4-triazole and inactivated at temperatures higher than 57°C. The molecular weight of these catalases is 200-216 kD [29].

Acetogenic bacteria studied have low catalase activity, except for *Acetobacterium wieringae*, which possesses the catalase activity of ~9 units/mg protein [12]. Nevertheless, on addition of hemin to the nutrient medium catalase activity was recorded in *C. formicoaceticum*, and in *A. woodii* and *A. poludosum* the catalase activity significantly increased [12] (Table 1).

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 [30]. However, many sulfate reducers are aerotolerant [31]. Moreover, in some *Desulfovibrio* (*D. vulgaris* and *D. desulfuricans*) O_2 is reduced to water during respiration [32, 33].

Catalase activity has been found in some *Desulfovibrio*: *D. desulfuricans* strain Norway 4 (*Desulfomicrobium norvegicum*) [27], *D. vulgaris* [27], *D. oxyclinae* [34], and *D. gigas* [24] (Table 1). The gene of *D. vulgaris* catalase has been expressed in *E. coli* [35]. A monofunctional catalase of *D. gigas* is a constitutively expressed enzyme [24] consisting of three 61-kD subunits [24] (Table 2). 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-} [24]. 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 [24] (Table 2).

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 [12] (Table 1). 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.

Methanogens are strictest anaerobes, unable to grow or produce CH_4 in the presence of O_2. Nevertheless, they can be tolerant to O_2. Methanogens can dwell in places with aerobic microzones or transitory aerobic conditions [36, 37]. Archaea of the *Methanobrevibacter* genus isolated from the intestine of termites harbor the catalase activity of 54 units/mg protein [37]. *Methanobrevibacter arboriphilus* (strains AZ and DH1) has low catalase activity. An addition of hemin to the nutrient medium increased by 30-fold the catalase activity [26] (Table 1), with the maximum in the stationary growth phase [12]. Archaea that are phylogenetically related to *Methanobrevibacter* are unable to synthesize heme and do not contain hemoproteins, thus, the *M. arboriphilus* gene *kat* is thought to have been acquired due to lateral gene transfer. The monofunctional heme catalase of *M. arboriphilus* strain AZ has been purified and characterized [26] (Table 2). The enzyme is a homotetramer, and its activity is repressed with CN^- and N_3^-, the 50% inhibition occurring at the concentrations of 80 and 1 μM, respectively. The corresponding gene *kat* has been cloned and sequenced [26]. The primary structure of the *M. arboriphilus* catalase is highly homologous to the known monofunctional heme catalases [26].

The cytochrome-synthesizing methanogen *Methanosarcina barkeri* has high catalase activity similar to that of other aerotolerant anaerobes [25] (Table 1). The maximum catalase activity is recorded in the stationary growth phase with acetate as a substrate; in the presence of methanol or H_2/CO_2 the enzyme activity is fourfold lower [12]. The monofunctional catalase of *M. barkeri* is also a homotetramer [25] (Table 2). Most of all, the enzyme is homologous to the monofunctional catalase of *Dictyostelium discoideum* and also of *Bos taurus*, *Homo sapiens*, and *Bacillus subtilis* [25]. The *kat* gene is supposed to have repeatedly migrated from eukaryotes to prokaryotes [38]. The *M. barkeri* catalase gene is distinct from other genes of this organism by the ratio of DNA bases and also by codons determining Arg, Asn, His, Phe, and Tyr [25]. The *M. barkeri* catalase is inactivated by H_2O_2 ($[S]_{50}$ = 25 mM) and inhibited by N_3^- ($[I]_{50}$ = 1 μM), CN^- ($[I]_{50}$ = 5 μM), and 3-amino-1,2,4-aminotriazole. The enzyme has wide pH and temperature optimums [25]. The amino acid sequence of the *M. barkeri* catalase is similar to that of the *M. arboriphilus* catalase. It seems that the presence of the heme catalase in archaea is restricted to *Methanosarcina* and *Methanobrevibacter*, and this additionally supports the acquisition of the gene *kat* by these organisms through lateral gene transfer. The genome of the sulfate-reducing archaeon *Archaeoglobus fulgidus* phylogenetically related to *Methanosarcinaceae* contains an open reading frame that is supposed to encode a bifunctional catalase–peroxidase [39].

However, not all methanogens possess a pronounced catalase activity; thus, the catalase activity is low in some strains of *Methanobrevibacter arboriphilus* and *Methanothermobacter marburgensis* [12]. There is no catalase in *Methanopyrus kandleri* and *Methanococcus voltae*. No open reading frames encoding mono- and bifunctional catalases have been found in genomes of the cytochrome-free *Methanococcus jannaschii* [40] and *Methanobacterium thermoautotrophicum* strain ΔH [41].

SUPEROXIDE DISMUTASE OF OBLIGATE ANAEROBIC MICROORGANISMS

Distribution of SOD in obligate anaerobes. SOD (EC 1.15.1.1) catalyzes the dismutation of the superoxide anion-radical ($O_2^{\cdot-}$) (which is generated during the one-electron reduction of oxygen) to O_2 and H_2O_2 [1].

Fridovich et al. [1, 4] found no SOD activity in some anaerobic bacteria and supposed that this enzyme is inherent to aerobic and aerotolerant microorganisms, while anaerobes need no SOD and, therefore, are sensi-

tive to O_2. However, a little later other authors showed that many strict anaerobes possess SOD activity, in particular representatives of *Desulfovibrio* [3, 10, 42], *Clostridium* [3, 10, 42, 43], and *Chromatium* [44].

Because obligate anaerobes can grow only in oxygen-free media, functions of SOD and other enzymes of antioxidative defense are unclear. SOD is likely to be a significant virulence factor that provides persistence of anaerobes in aerobic tissues until the appearance of favorable conditions due to development in the tissues of O_2-absorbing concomitant aerobes, accumulation of gaseous products of tissue decomposition, and decrease in redox potential [42, 45]. Most likely SOD plays the same role in anaerobic and aerobic microorganisms, i.e., it neutralizes $O_2^{\cdot-}$ produced during an accidental transitory exposition of the cells in air as a result of interaction of O_2 with reduced flavins, catecholamines, quinones, and Fe-S-proteins [1, 2, 46]. There are two hypotheses about the origin of SOD in strict anaerobes. According to the first hypothesis, ancestors of obligate anaerobes (urkaryotes) acquired SOD for defense against superoxide generated in ancient oceans during the UV-induced photolysis of water or during the catalytic activity of flavins [47, 48]. According to the second hypothesis, SOD was acquired by strict anaerobes later, and this allowed them to endure transitory contacts with atmospheric oxygen [49, 50]. The occasional distribution of SOD among sulfate reducers [27] and *Clostridium* [3] seems to support the plasmid transfer of the *sod* gene from microaerophilic organisms [49].

Anaerobes that can endure only a short-term contact with O_2 need to have SOD activity in the cells, but, unlike aerobic organisms, their need for catalase is not indispensable, because H_2O_2 generated in the course of dismutation or other reactions is decomposed spontaneously or with involvement of nonenzymatic mechanisms of defense, and its level is insufficient to impair cell structures [1, 3, 45]. Some authors [3, 42, 43, 51-53] even in the 1970s proposed to classify microorganisms as extreme anaerobes, anaerobes, and aerotolerant anaerobes just depending on the SOD level in the cell. Species with a significant SOD activity were thought to have a moderate or high aerotolerance as compared to strictly anaerobic species, which lacked this enzyme or displayed its low activity. Thus, *B. fragilis* strain VPI 2553, containing 4.2 units SOD per mg protein, is significantly more resistant to O_2 than *B. vulgatus* and *B. fragilis* strain VPI 2393, containing 0.5 unit SOD per mg protein [42, 45]. The available information about SOD distribution among various anaerobes (*Bacteroides*, *Clostridium*, sulfate reducers, and methanogens) confirms a close correlation between the cell sensitivity to O_2 and the SOD activity, but there are also some exceptions.

Among obligate anaerobes, high SOD activity was first found in *Chlorobium thiosulfatophilum* [54] (Table 3), *Chromatium vinosum* [44], some *Clostridium* species [3, 42, 55] (Table 3), in particular in *C. perfringens* [3, 42] and *C. butyricum* [11] (Table 3). Spores of *C. butyricum* strain 35/11 have higher SOD activity than the vegetative cells [11]. The cyanide-resistant SOD of *C. perfringens* was partially purified [3], and later the *sod* gene was cloned and sequenced [56].

The amino acid sequence of the SOD from *C. perfringens* [56] is highly homologous to that of the Mn-SOD from *B. subtilis* and *E. coli*. The level of *sod* mRNA in *C. perfringens* increased during the exponential growth phase, surmounted tenfold the maximum on transition to the stationary phase, and then the level of the *sod* transcript decreased fivefold [56].

The SOD activity is low in the cells of some *Bifidobacterium* species, except *B. adolescentis*, which has an extremely high activity of SOD [13]. It is strange that *B. adolescentis* is sensitive to O_2. Authors [13] have supposed that SOD is not involved in detoxification of O_2 and *Bifidobacterium* uses other systems, such as NADH oxidase and NADH peroxidase, to protect against O_2. Later, a nonenzymatic dismutation of $O_2^{\cdot-}$ was shown in seven *Bifidobacterium* strains in the presence of high concentrations of Mn^{2+} and Fe^{2+} [57].

Most *Bacteroides* species have SOD activity [42, 45, 52] (Table 3). Some *P. gingivalis* strains have a very high SOD activity and are the most tolerant to O_2 among *Porphyromonas* and *Bacteroides* studied [58]. The enzymes of antioxidative defense, especially SOD, are important virulence factors in pathogenesis of infectious periodontitis caused by *P. gingivalis* [59, 60]. The SOD activity does not change during the growth cycle of *B. distasonis*, but the composition of the nutrient medium influences its activity [16].

The acetogens studied exhibit pronounced SOD activity [12] (Table 3). Taking into account that all known homoacetogens are strict anaerobes, it is difficult to explain the high SOD activity of *A. woodii* and *A. wieringae*. Some obligate anaerobes living in water under the constant risk of appearance of O_2 have highly active enzymes of antioxidative defense.

Low SOD activities were found in some sulfate reducers, such as *Desulfotomaculum nigrificans* [3, 12] (Table 3), *D. kuznetsovii* strain 17 [12], *Desulfovibrio gigas*, *D. vulgaris*, *D. desulfuricans* [24, 27] (Table 3), and also in other *Desulfovibrio* species [10]. The majority of strains with SOD activity also have catalase activity [27]. The SOD activity of *D. desulfuricans* reaches its maximum in the stationary growth phase and depends on the culture conditions [65]. *D. vulgaris* strains mutant in the *sod* gene were more sensitive to O_2 than the wild type strain [66]. Sulfate reducers use as electron carriers in the respiratory chain cytochromes *c* and *d*, ferredoxin, flavodoxin, desulforedoxin, rubredoxin, and menaquinone, which can generate $O_2^{\cdot-}$ similarly to hydrogenase and adenylyl sulfate reductase containing non-heme iron [50].

Table 3. Specific activity of SOD in cell extracts of some anaerobic bacteria

Microorganism	Specific activity of SOD, units/mg protein	References
Chlorobium thiosulfatophilum strain NCIB 8346	14.0	[3, 54]
Chromatium sp. strain NCIB 8348	0.6	[3]
Clostridium acetobutyricum strain 8	15.0	[12]
Clostridium pasteurianum strain ATCC 6013	0.5	[3]
Clostridium perfringens strain NCIB 11105	15.6	[3]
Clostridium butyricum strain 35/11	9.0	[11]
Clostridium butyricum strain 21	3.0	[12]
Bacteroides distasonis strain ATCC 8503	3.2	[42]
Bacteroides vulgatus strain ATCC 8482	0.5	[42]
Bacteroides fragilis strain VPI 2393	1.5	[45]
Bacteroides melaninogenicus strain 9846	7.5	[53]
Bacteroides thetaiotaomicron	0.9	[61]
Porphyromonas gingivalis strain 381	3.4	[59, 62]
Sporomusa sphaeroides	11.0	[12]
Acetobacterium woodii	26.0	[12]
Acetobacterium wieringae	38.0	[12]
Acetobacterium poludosum strain Z-7390	3.0	[12]
Desulfotomaculum nigrificans strain NCIB 8395	2.6	[3]
Desulfotomaculum nigrificans subsp. *salinus* strain 435	4.0	[12]
Desulfovibrio desulfuricans strain NCIB 8307	0.6	[3]
Desulfovibrio desulfuricans strain Norway 4	3.8	[50]
Desulfovibrio vulgaris	1.0	[27]
Desulfovibrio gigas strain ATCC 19364	3.4	[24]
Thermohydrogenium kirishiense strain 360	5.0	[12]
Thermohydrogenium lactoethylicum strain 149	15.0	[12]
Methanobacterium bryantii	8.5	[63]
Methanobrevibacter arboriphilus strain AZ	22.0	[12]
Methanosarcina barkeri strain Fusaro	11.0	[64]

Some methanogens, in particular *Methanobacterium bryantii* [63, 67] (Table 3), and some *Methanomicrobium* species [67] have low SOD activities. SOD activity was found in *Methanobacterium thermoautotrophicum* [68], *M. barkeri* [64] (Table 3), and *M. arboriphilus* [12] (Table 3). The SOD activity of *M. barkeri* and *M. arboriphilus* is maximal in the stationary growth phase and in *M. barkeri* it depends on the substrate used [12].

For a long time attempts to isolate the corresponding *sod* genes from SOD-positive anaerobes were unsuccessful; however, these studies resulted in detection of the gene of rubredoxin oxidoreductase (*rbo*) in *D. vulgaris* [30] and of the gene of rubrerythrin (*rbr*) in *C. perfringens* [69]. Both proteins have SOD activity, as has been shown on a SOD-deficient *sodA-sodB* mutant of *E. coli*, which lost SOD [56], but the function of these proteins remains

Table 4. Physicochemical features of purified Fe-SODs from *E. coli* and anaerobic microorganisms

Microorganism	Molecular weight, kD*	Specific activity, units/mg protein	Iron content, atom/mol	References
E. coli	38.7 (2 × 17.8)	2470	1.0	[85]
C. thiosulfatophilum	44.0 (2 × 22.0)	4136	1.8	[54]
B. fragilis	42.0 (2 × 21.0)	1200	1.8	[87, 89]
B. thetaiotaomicron	46.0 (2 × 23.0)	1220	1.1	[61]
P. gingivalis	46.0 (2 × 23.0)	1020	1.8	[62, 88]
D. desulfuricans	43.0 (2 × 21.5)	2060	1.6	[50]
D. gigas	43.0 (2 × 22.0)	1900	1.0	[24]
M. bryantii	91.0 (4 × 26.0)	2060	2.7	[63]
M. thermoautotrophicum	105.0 (4 × 24.0)	855	1.7	[68]
M. barkeri	70.0 (3 × 25.0)	1500	1.0	[64]

* The number of subunits and molecular weight of the subunit are given in parentheses.

unclear. The cloning and complete sequencing of *sod* genes of such strict anaerobes as *M. thermoautotrophicum* [48, 70], *P. gingivalis* [71, 72], *B. fragilis* [EMBL M96560], and *C. perfringens* [56] are promising for elucidation of the function of SOD in obligate anaerobes. The amino acid sequence of the protein encoded by the *sod* gene of *M. thermoautotrophicum* strain Marburg is 55.5% homologous to that of the subunit of alkylhydroperoxide reductase (encoded by the *ahpC*-gene) of *Salmonella typhimurium* [70]. Cloning of the *sod* gene of *Desulfoarculus baarsii* in the *sodA-sodB* mutant of *E. coli* revealed a chromosome region similar to the *rbo-rub* operon of *D. vulgaris* encoding rubredoxin oxidoreductase and rubredoxin [30]. To produce the *sod*-phenotype, only *rbo* is required [73].

Types of superoxide dismutases. The active site of SODs contains metal ions. Depending on their type, Cu,Zn-, Fe-, and Mn-SODs are discriminated. The majority of SODs studied include two identical subunits, each containing a metal ion, disulfide bridge, sulfhydryl group, and acetylated terminal amino group [1]. Recently Ni-SOD has been detected in *Streptomyces griseus* and *S. coelicolor* [74].

The molecular weights of Cu,Zn-SODs are ~32 kD, and they are homodimers [1, 51]. Prokaryotes were earlier thought to have Fe- and/or Mn-SODs, which, unlike Cu,Zn-SODs of eukaryotes, are insensitive to CN^- [1]. Later Cu,Zn-SODs were also found in prokaryotes, in particular, in *E. coli* [75], and in the sulfate reducer *D. desulfuricans* strain ATCC 27774 [76], the enzyme of which was inhibited with CN^-. Sulfate-reducing bacteria are known to lack copper-containing proteins, and finding of the Cu,Zn-SOD in *D. desulfuricans* is the first report of such kind. However, the expression of this enzyme is very low [76].

Fe- and Mn-SODs are highly homologous [77-79], have similar three-dimensional structure [78, 79], molecular weights of ~40 kD (2 × 20 kD) [1, 49], and similar metal-binding ligands in the active site of the enzyme [70, 80]. Fe- and Mn-SODs are supposed to have a common origin, different from that of Cu,Zn-SODs of eukaryotes [78, 79]. It seems that Mn-SODs were separated from Fe-SODs of anaerobic archaea after their phylogenetic separation from bacteria, which had a similar but independent differentiation of SODs [68]. However, SODs of these two types are different in sensitivity to azide: 1 mM N_3^- causes the 70-90% inhibition of Fe-SODs, whereas Mn-SODs are 50% inhibited only in the presence of 20 mM N_3^- [1]. H_2O_2 (5 mM) irreversibly inactivates Cu,Zn- and Fe-SODs [1, 8] but not Mn-SODs [1, 45, 81].

Mn- and Fe-SODs may be divided into two subclasses: one of them needs to have either Fe or Mn in the active site [1, 82], while SODs of the other subclass (the so-called cambialistic SODs) are active with both Fe and Mn, which are bound by the same active site [59, 61, 62, 80, 83, 84]. Fe- and Mn-SODs are found in different phylogenetic groups of microorganisms. Moreover, SODs of the different classes can occur in the same organism [84].

Fe-containing SODs (Fe-SODs). Fe-SODs are found only in prokaryotes, and they were first isolated from *E. coli* cells [85]. Fe-SODs are either dimers (40 kD) or tetramers (90 kD) [86]. Nearly all SODs isolated from the cells of strict anaerobes are Fe-SODs or cambialistic SODs [86].

The first anaerobes that were found to have Fe-SOD are the purple sulfur bacterium *Chromatium vinosum* [44] and the green sulfur bacterium *Chlorobium thiosulfatophilum* [54] (Table 4). By absorption spectra, the presence of metal in the active site, and subunit structure (homodimers) both SODs are similar to Fe-SODs of aerobic bacteria.

Fe-SOD was also purified from the cells of obligate anaerobes *Bacteroides* and *Porphyromonas*: *B. fragilis* [87],

P. gingivalis [62, 88], and *B. thetaiotaomicron* [61]. The SOD from *B. fragilis* is a homodimer with noncovalently bound subunits [87] (Table 4), with iron content of 1.8-1.9 g-atom and zinc content of 0.2 g-atom per mol dimer. The Fe-SOD of *B. fragilis* is inhibited by N_3^- (1-2 mM), inactivated by H_2O_2 (5 mM), but is resistant to CN^- (1-5 mM), similarly to other known Fe-SODs [87, 89].

The SOD from the anaerobically grown *B. thetaiotaomicron* is also a dimer consisting of two identical noncovalently bound monomers and has one metal-binding site per subunit [61] (Table 4). The enzyme contains 1.1 g-atom Fe, 0.6 g-atom Zn, and <0.05 g-atom Mn per mol dimer and is 50% inhibited by 0.2 mM NaN_3 [61]. The Fe-SOD of *B. distasonis* is a cyanide-resistant enzyme with molecular weight of 40 kD [16]. The activity of the SOD from *B. distasonis* did not change during the development of the culture but depended on the composition of the medium [16].

The Fe-SOD isolated from anaerobically grown *P. gingivalis* (anaero-SOD) consists of 191 amino acid residues (per subunit); three Fe-isoenzymes have been revealed [59, 62, 88] (Table 4). This enzyme contains 1.79 g-atom Fe and 0.28 g-atom Mn [62]. The SOD from *P. gingivalis* is 80% inhibited with 5 mM N_3^- and 68% inactivated on incubation for 20 min with 1 mM H_2O_2 [62]. The SOD from *E. coli* (the *sodA-sodB* mutant) with the introduced *P. gingivalis sod* gene contains iron and a small amount of manganese. This SOD is cambialistic, and the resulting Fe-enzyme without Mn in the active site is 95% inactivated after incubation for 15 min with 10 mM H_2O_2 [79]. Guanidine (5 M) and 8-hydroxyquinoline (20 mM) reversibly inhibit the Fe-SOD of *Bacteroides*. Activities of the Fe-SOD from *B. fragilis* [83, 89], *P. gingivalis* [59, 62], and *B. thetaiotaomicron* [61] are restored by dialysis of the denatured apoenzyme against buffer containing 1 mM $Fe(NH_4)_2(SO_4)_2$.

Fe-SOD has been isolated from some sulfate-reducing bacteria, in particular *D. desulfuricans* strain Norway 4 [27, 50], *D. vulgaris* [27], and *D. gigas* [24]. The enzyme from *D. desulfuricans* resembles the Fe-SOD of aerobic microorganisms [50] (Table 4) and also the SOD of *C. vinosum* [44] in molecular weight, subunit structure (a homodimer with noncovalently bound subunits), and absorption spectrum. The native enzyme from *D. desulfuricans* contains two high-spin iron atoms, which is specific for Fe-SODs of anaerobic microorganisms [50].

The SOD of *D. vulgaris* (its gene was cloned and expressed in *E. coli*) is a dimer that contains one iron atom per monomer and has specific activity of 850 units/mg protein [35, 66]. The Fe-SOD of *D. gigas* [24] (Table 4) is a constitutive enzyme expressed under anaerobic conditions, and its molecular and biological features are very like those of the SOD from *D. desulfuricans*. Similarly to other Fe-SODs, the enzyme from *D. gigas* is sensitive to H_2O_2 and N_3^- but not to CN^-. The gene encoding the SOD of *D. gigas* is supposed to be a part of an O_2-sensitive operon [24].

The SOD of *Methanobacterium bryantii* ranges to about 0.4% of extractable proteins. The enzyme has been purified and characterized [63] (Table 4). Its amino acid composition, some physicochemical features, EPR-spectra, and sensitivity to H_2O_2 are similar to those of the known Fe-SODs [63]. Nevertheless, the Fe-SOD of *M. bryantii* is more resistant to N_3^- (50% inhibition in the presence of 15 mM N_3^-) than other Fe-SODs, the majority of which have $[I]_{50}$ for N_3^- equal to 4 mM. Moreover, the known Fe-SODs, with rare exceptions, are dimeric and not tetrameric proteins [63]. The SOD from *M. bryantii* contains 2.7 Fe atoms, 1.7 Zn atoms, and less than 0.2 Mn atom per tetramer; the presence of Zn is unusual for Fe-SODs [63].

The *sod* gene of *Methanobacterium thermoautotrophicum* (the first case of sequencing of the *sod* gene of a strict anaerobe) was cloned [48] and expressed in *E. coli* [68]. The protein synthesized was more than 30% of all soluble proteins of *E. coli*. The purified SOD, like the SOD from *M. bryantii*, is a tetrameric protein [68] (Table 4) with low specific activity similar to activities of the known Mn-SODs of anaerobic archaea and with amino acid sequence similar to that of Mn-SODs of both prokaryotes and eukaryotes [48]. Nevertheless, the SOD of *M. thermoautotrophicum* is a Fe-containing enzyme. Features of the *M. thermoautotrophicum* enzyme are unique for Fe-SODs but specific for Mn-SODs: it is not inactivated during incubation for 24 h with 0.5 mM H_2O_2 and is resistant to N_3^- [68]. The only exception from all known Fe-SODs is the enzyme from *M. bryantii* which is also partially inhibited by N_3^- [63]. Probably the insensitivity of the Fe-SOD from *M. thermoautotrophicum* to H_2O_2 is caused by substitution of Trp79 (adjacent to the metal-binding His) which, unlike Trp of Mn-SODs, is conservative for all known Fe-SODs, for Val [68, 78]. It is likely that this SOD can be cambialistic [68]. Having in mind the resemblance of enzymatic features of the SOD from *M. thermoautotrophicum* with those of Mn-SODs, an interesting hypothesis has been proposed: some archaea adapted to aerobiosis due to the presence of the H_2O_2-resistant Mn-SOD and living strictly anaerobically recreated the Fe-SOD from the Mn-SOD [48, 68]. This Fe-SOD with amino acid sequence "intermediate" between those of Fe- and Mn-SODs retained the low sensitivity to H_2O_2. It has been supposed [48, 68] that SODs of archaea existing under conditions of strict anaerobiosis are ancient enzymes and direct "descendants" of SODs of urkaryotes [90]. In the region of DNA between the promotor and initiation codon of the *sod* gene of *M. thermoautotrophicum*, a palindrome sequence GGTGG (CCACC) and a loop of two-three A/T have been found. Sequences of these inverted repeats seem to regulate the *sod* transcription in *M. thermoautotrophicum* [48].

Fe-SODs from *M. barkeri* strain Fusaro [64] (Table 4) and from *M. arboriphilus* strain AZ have been recently isolated and purified. The SOD from *M. barkeri* is similar

to other SODs of methanogens in the molecular weight of subunits, but the structure as a trimer is unusual. The SOD from *M. barkeri* [64] and the SOD from *M. arboriphilus* are inhibited by N_3^- and inactivated by H_2O_2, but significantly less than the Fe-SOD of *E. coli*. As mentioned above, the low sensitivity to N_3^- and H_2O_2 was also shown for Fe-SODs of other archaea: *M. bryantii* [63], *M. thermoautotrophicum* [68], and *Sulfolobus solfataricus* [91]. The primary structure of the SOD from *M. barkeri* determined from the nucleotide sequence had the highest homology with the Fe-SOD of *M. thermoautotrophicum* and other archaea [48, 64, 70]. The homology with SODs from bacterial and eukaryotic cells is significantly lower. This seems to indicate that the *sod* gene could occur in archaea even before the evolutionary division into phylogenetic branches [64, 70].

The amino acid sequence of the SOD from *M. barkeri* has a motif [64], which seems to be conservative for the active site region of all Fe- and Mn-SODs analyzed so far [78]. However, at least five significant differences in the amino acid residues located in the limits of 8 Å from the metal to be bound determine the Fe- or Mn-specificity of SOD [78]; therefore, the SOD from *M. thermoautotrophicum* should be a Mn-SOD because three of five amino acid residues are present in the known Mn-containing bacterial SODs [48]. In the case of SOD of *M. barkeri*, two residues of five are the same as in Mn-SOD and one residue is the same as in Fe-SOD [64]. However, these SODs are Fe-containing enzymes. Obviously, SODs of bacteria are significantly different from SODs of archaea in both the response to inhibitor and the sequence of amino acid residues in the active site. Based on the available information about the N-terminal sequence of amino acids, the gene encoding the SOD from *M. barkeri* was found in the databank, cloned in *E. coli*, and sequenced [64]. The presence of SOD in *M. barkeri* is worth attention because the gene encoding it (*sod*) has not been found in the genome of the sulfate-reducing archaeon *A. fulgidus* [39], which is phylogenetically the closest to *Methanosarcinaceae*.

Mn-containing SODs are cambialistic SODs of anaerobic microorganisms. SOD of this type was first isolated from *E. coli* and was found to be unlike the corresponding enzymes of eukaryotes in all parameters except the catalytic activity [92]. Grown under anaerobic conditions, *E. coli* contains only Fe-SOD, but grown aerobically, *E. coli* contains Fe-SOD, Mn-SOD, and hybrid SOD [1, 93]. The apoenzyme of the Fe-SOD from *E. coli* binds only iron and apoenzyme of the Mn-SOD from *E. coli* binds only manganese [81], but some bacteria (*Propionibacterium shermanii* and *Streptococcus mutans*) have a cambialistic SOD with the apoenzyme producing Fe- or Mn-SOD depending on presence of the corresponding metal in the medium [84].

Mn-SOD (aero-SOD) was also found in strictly anaerobic bacteria, in particular, such an enzyme was isolated from O_2-treated cells of *B. fragilis* [83, 89], *B. thetaiotaomicron* [61], and *P. gingivalis* [59]. The SOD from *B. fragilis* has molecular weight of 43 kD and is a homodimer with noncovalently bound subunits [83]. The enzyme with specific activity of 1760 U/mg contained 1.1 g-atom Mn, 0.3 g-atom Fe, and 0.2 g-atom Zn per mol dimer [83]. The possibility of Fe substitution with Mn in the active site of the SOD from *B. fragilis* and *B. thetaiotaomicron* was studied by dialysis of the apoenzyme treated with guanidine and 8-hydroxyquinoline against Tris buffer supplemented with 1 mM $MnCl_2$. The reconstructed Mn-containing enzyme was 50% inhibited with 20 mM N_3^- in the case of *B. fragilis* [83, 89] and 60% inhibited with 10 mM N_3^- in the case of *B. thetaiotaomicron* [61] but was not inactivated by H_2O_2, which is in common with other known Mn-SODs. Zn^{2+} inhibits the repair of the denatured apoenzyme with both manganese and iron [83, 89]. A possibility of synthesis of the azide-resistant Mn-SOD by *B. fragilis* cells grown on medium supplemented with desferrioxamine and manganese was also shown. The activity of the Mn-SOD in the cell extracts sharply increased with increase in the Mn concentrations added to the medium [94]. The amino acid sequence of the Mn-SOD (aero-SOD) from *B. fragilis* and features of this enzyme are identical to those of the Fe-SOD (anaero-SOD) [83]. The SOD of *P. gingivalis* [59, 62] and of *B. thetaiotaomicron* [61] also can use Fe or Mn as a cofactor.

The enzyme isolated from aerated *B. thetaiotaomicron* cells has molecular weight of 43 kD, consists of two subunits of the same size, and contains 1.0 g-atom Mn, 0.55 g-atom Fe, and 0.3 g-atom Zn per mol dimer [61]. As in the case of the Fe-SOD from the same organism, the activity of the denatured enzyme can be restored by dialysis against buffer containing Fe or Mn [61]. However, the activity of the enzyme from *B. thetaiotaomicron* is restored much worse than the activity of the SOD from *B. fragilis*. The apoenzyme can bind both Fe and Mn; the sensitivity of the holoenzyme to N_3^- is different depending on the metal [61]. Aerated *B. thetaiotaomicron* cells are supposed to contain Fe in the Mn-SOD as it occurs in Fe-Mn-hybrid dimers. Such heterodimeric SODs are indistinguishable in physical properties from both homodimers but have unique characteristics with respect to N_3^-: they are extremely resistant to it [61].

The Mn-SOD from aerated *P. gingivalis* cells (aero-SOD) has the same molecular weight of ~46 kD as the Fe-containing enzyme from the same organism (anaero-SOD), is a homodimer with one metal-binding site per monomer, and consists of 191 amino acid residues per subunit [59, 62]. Both SODs have similar amino acid composition and identical N-terminal amino acid sequences [59, 62]. Data of electrophoresis and isoelectrofocusing and also results of the SOD inactivation during incubation with 1 mM H_2O_2 and of inhibition with N_3^- have shown that aero-SOD from *P. gingivalis* consists of a

single Mn-SOD and a small amount of two Fe-SODs [59]. The SOD from aerated *P. gingivalis* cells contained 1.08 g-atom Mn and 0.36 g-atom Fe; dialysis against buffer with $MnCl_2$ restored the activity of the apoenzyme denatured by treatment with guanidine and 8-hydroxyquinoline [59]. The amino acid sequence of SODs from *P. gingivalis* is similar to that of other bacterial Fe- and Mn-SODs; however, this resemblance is much less than the similarity observed inside the Fe-SOD or Mn-SOD classes [59, 62].

In the region of the supposed second ligand (where the difference between Fe- and Mn-SODs is the most pronounced) the amino acid sequence of the enzyme from *P. gingivalis* is intermediate relative to that in Fe- and Mn-SODs [59, 71]. All three isoenzymes of anaero-SOD and aero-SOD from *P. gingivalis* (similarly to the SOD from *B. fragilis* and *B. thetaiotaomicron*) are suggested to be generated from a single apoenzyme with a single metal-binding site per subunit and ligands able to bind both Fe and Mn, the latter mainly under conditions of aeration [59, 61, 62, 83]. The more sophisticated model suggests the presence of different binding sites specific for each metal [61]. The difference in configuration of glutamine residues in SOD is shown [80] to be partly responsible for the metal-specific activity of the cambialistic Mn,Fe-enzyme from *P. gingivalis*; and, depending on the metal bound, Gln70 located near the metal of the active site in the Fe-SOD from *P. gingivalis* is changed for Gln142 in the Mn-SOD, and amide groups of glutamine residues are located similarly in the spatial structure of the Fe- and Mn-SOD. Moreover, Tyr77 of the Fe-SOD is substituted by phenylalanine in the Mn-SOD [80]. The two enzymes are virtually identical in amino acid sequence and three-dimensional structure and are highly homologous to the corresponding enzymes from *E. coli* [72]. This confirms the hypothesis about the generation of cambialistic SODs in bacterial cells from a single apoenzyme [58].

In *Bacteroides*, the type of cambialistic SOD seems to be regulated on the post-transcriptional level [61]. Thus, a Fe-specific repressor protein, which controls the formation of the Mn-SOD, has been found in *E. coli* [93]. A differentiated transport of metal ions into the cell of *Bacteroides* in the course of aeration can also play a certain role [61].

Actinomyces naeslundii is believed to be the only strict anaerobe that possesses the genuine Mn-SOD [53]. The enzyme is insensitive to 1 mM N_3^-, resistant to H_2O_2 like Mn-SOD from *E. coli*, and its calculated molecular weight is ~130 kD, i.e., significantly higher than that of the SODs of *Bacteroides* and *E. coli* [53].

In the cells of some strict anaerobes proteins have been recently found containing non-heme iron which are different from SOD but having SOD activity: neelaredoxin in the sulfate-reducing bacterium *D. gigas* [95, 96], in the hyperthermophilic archaea *Pyrococcus furiosus* [97-99] and *Archaeoglobus fulgidus* [100]; rubrerythrin in *Clostridium perfringens* [69] and desulfoferredoxin in *Desulfoarculus baarsii* [34, 101], *Desulfovibrio desulfuricans* [76, 98], and *D. vulgaris* [102, 103]. Neelaredoxin of *P. furiosus* is a homotetramer consisting of 14.3-kD subunits, each containing the only one-core site with the non-heme iron (0.5 Fe atom per mol) [97-99]. Neelaredoxins of the hyperthermophilic archaebacteria *P. furiosus* [97-99] and *A. fulgidus* [100] are bifunctional proteins that exhibit both superoxide dismutase and superoxide reductase activities. Desulfoferredoxin (Dfx) of *D. baarsii* and *D. vulgaris* acts as superoxide reductase, i.e., reduces $O_2^{\cdot-}$ to H_2O_2 without generation of O_2, and effectively removes endogenous superoxide radicals [101-103]. Rubredoxin, which is abundant in cells of sulfate reducers, is the predominant electron donor [101]. It is important that superoxide reductases which contain the unique region of Fe[His(4)Cys] [104-106] and are undoubtedly a very ancient and powerful biological mechanism of antioxidative defense can be a preferential mechanism of the fast detoxification of $O_2^{\cdot-}$ in the strictest anaerobes under conditions of short-term strong aeration [101, 105, 107, 108]. Desulfoferredoxins are also advantageous due to the immediate termination of oxidation of electron carriers after the effect of $O_2^{\cdot-}$ has come to end (this prevents the loss of reducing equivalents [101]) and also to the uselessness of sophisticated regulatory systems of response to $O_2^{\cdot-}$ in the presence of Dfx [101]. However, unlike the $O_2^{\cdot-}$ detoxification with SOD, its detoxification with superoxide reductase depends on redox status of the cell [100, 106].

OXIDATIVE STRESS IN STRICT ANAEROBES: INDUCTION OF SOD AND CATALASE

Products of one-, two-, and three-electron reduction of oxygen are toxic for the cell. If the intracellular concentrations of such products surpass the abilities of constitutive protective systems, the cells will be subjected to oxidative stress and respond by derepression of a number of collaboratively regulated genes the proteins of which are responsible for elimination of reactive compounds from the cell or for repair of its damages.

In the cells of microorganisms, the physiological response to oxidative stress factors is complicated and regulated finely. Thus, *E. coli* contains a periplasmic Cu,Zn-SOD, constitutive Fe-SOD (SodB), and Mn-SOD (SodA), and the synthesis of the latter is controlled by at least six regulatory proteins [77]. Catalases of *E. coli* HPI (KatG) and HPII (KatE) are regulated independently: the synthesis of the first is induced by H_2O_2, whereas the synthesis of the other is induced by nutrient limitation at the beginning of stationary growth phase with involvement of the regulatory system RpoS [5, 77]. In *E. coli* ~40 proteins have been found whose syntheses are

induced by reactive oxygen species, and two regulatory systems, SoxRS and OxyRS, which control operons of oxidative stress [77].

For strictly anaerobic microorganisms, there is significantly less information about adaptive molecular mechanisms of oxidative stress (and, in particular, about the regulation of catalase and SOD expression) that ensure their aerotolerance and survival in the presence of O_2 or products of its incomplete reduction than for aerobes. Strict anaerobes have no respiratory (electron transport) chain and, therefore, do not need protection against reactive products of the intracellular reduction of O_2 to H_2O. Nevertheless, they need mechanisms of defense against toxic oxidizing compounds generated in the environment or intracellularly during oxidation of substrates and various xenobiotics and also under the influence of mutagens or UV irradiation. Pathogens of animals can also encounter products of oxygen reduction in macrophages. Oxidative stress in anaerobic microorganisms has to be very fast and fully induced; otherwise, damage to the cell macromolecules by toxic oxygen derivatives will prevent the further expression of the protective mechanisms [109].

Experiments with exponentially growing *B. fragilis* and *B. vulgaris* have revealed a three-fivefold increase in the specific activity of SOD in the cells incubated in an atmosphere of 2% O_2 and 98% N_2 [19, 45, 83, 89]. The increase in the SOD activity accompanied by the enzyme synthesis *de novo* continued for 90 min after the treatment with O_2. The treatment of the cells with O_2 increased their resistance to O_2 as compared to the cells not pre-exposed to oxidative stress [45, 53].

By two-dimensional electrophoresis, *B. fragilis* was shown to have a powerful and complicated regulatory system of defense, which enables the cells to maintain for a long time viability under aerobic conditions [22], and this system has some features in common with the systems of oxidative stress in aerobic and facultative anaerobic bacteria. On treatment with O_2, paraquat, or H_2O_2, *B. fragilis* immediately and in concord synthesizes *de novo* more than 28 proteins involved in detoxification of the reduction products and in defense of the cell macromolecules [22, 110]. These proteins include SOD [83] and catalase [109], as well as thioredoxin peroxidase, thiol peroxidase P20, cytochrome *c* peroxidase, alkylhydroperoxide reductase, O_2-activated ribonucleotide reductase, rubrerythrin, aspartate decarboxylase, ferritin, etc. [22, 110]. The qualitative composition of the proteins synthesized (with molecular weights from 12 to 79 kD, p*I* from 5.1 to 7.2) somewhat varies depending of the *B. fragilis* strain under study [20, 22, 110]. Eleven newly synthesized oxidative stress proteins are similar in properties, with p*I* of 5.1-5.8 and molecular weight of 17-23 kD [22, 110]. Moreover, adaptive responses induced by H_2O_2 or paraquat are similar but not identical to the response induced by O_2 [22, 110]. In particular, three of four proteins of *B. fragilis* induced by H_2O_2 are similar to proteins synthesized under the influence of O_2 [111]. Such a degree of overlapping of adaptive responses to stress is a rare phenomenon. Thus, in *E. coli* only three of fourteen paraquat-induced proteins are found in H_2O_2-treated cells [77]. In the case of *B. fragilis*, there are also unique genes that are expressed only under the influence of a certain factor of oxidative stress. Such adaptive responses with complex regulation suggest the involvement of many regulators, one of which, OxyR, controlling gene expression in response to H_2O_2 or O_2, has been identified [110]. The maximum expression of the OxyR regulon genes was observed in the presence of 50 μM H_2O_2 and 2% O_2, which corresponds to the concentrations of oxidizers in tissues infected with *Bacteroides* [110].

Aeration induces the synthesis of both Mn- and Fe-SOD, increases twofold its specific activity in the O_2-tolerant *P. gingivalis* [59, 112, 113], and increases fourfold the specific activity of the Mn-SOD of *B. thetaiotaomicron* [61]. The amount of SOD isolated from aerated *Bacteroides* cultures in the majority of cases was threefold higher than its amount isolated from anaerobic cultures [59]. Aeration (and also nitrates, decrease in pH, and heat shock) induced the SOD synthesis in *P. gingivalis* [58, 113, 114], and the cells with the high synthesis of SOD (threefold increase in the enzyme activity and the level of the corresponding mRNA) change their virulence and acquire resistance to phagocytic activity of polymorphonuclear leukocytes, which are specified by a high oxidative metabolism [58]. Molecular oxygen induces the major Mn-SOD and two minor Fe-SODs in *P. gingivalis* [59], which may be explained by different regulation of SOD at the post-translational level. As a result of the structural posttranslational change in apo-SOD of *P. gingivalis*, two minor isoenzymes are capable for binding only Fe, and the major isoenzyme binds Fe or Mn [62].

Under anaerobic conditions, catalase activity of *B. fragilis* was found in the stationary growth phase; thus, not only O_2 but also cell starvation resulted in synthesis of the antioxidative defense enzymes [18, 19, 109]. An increase in the expression of catalase of *B. fragilis* in response to aeration and to entrance into the stationary growth phase, but not to H_2O_2, is similar to adaptive response with involvement of hydroperoxidase II (encoded by *katE*) in *E. coli* [5, 77]. The level of monocistronic mRNA of *katB* (the gene encoding catalase) increased more than 15-fold during oxidative stress induced by treatment with O_2, paraquat, or H_2O_2 in the mid-logarithmic anaerobic culture of *B. fragilis*. It seems that the strong induction of *katB* mRNA by oxidative stress is a part of the complicated coordinated response of *B. fragilis* to aerobiosis [109]. Probably the transcription of the genes *katB* and *ahpCF* (alkylhydroperoxide reductase) in *B. fragilis* is controlled by a common regulatory mechanism [115] similar to the regulator of peroxide-induced stress in *E. coli*; at least, an *oxyR* homolog has been found

in *B. fragilis* [110]. Under anaerobic conditions, low levels of *katB* mRNA increased in the course of periodic culture, reaching the maximum expression at the late logarithmic or early stationary growth phase, and decreased during the stationary phase [109]. This indicates an important role of catalase during cell starvation for defense against the effect of O_2 and also H_2O_2 generated in the cells during the stationary growth phase with decrease in metabolic activity. Under anaerobic conditions, the synthesis of catalase in *B. fragilis* was strongly suppressed by glucose (the level of *katB* mRNA was decreased fivefold 30 min after the glucose addition) and other carbohydrates fermented by this microorganism [15, 109] when added to the culture limited by carbon or energy source. Nevertheless, the glucose-induced repression was completely abolished under aerobic conditions or on addition of H_2O_2, and this confirms the dominating role of oxidative stress in the control of catalase synthesis by *B. fragilis* [109]. Phosphate, nitrogen, and hemin limitation, as well as addition of non-fermentable sources of carbon, did not affect the expression of *katB* mRNA. Consequently, the control of *katB* expression in *B. fragilis* was restricted by two independent mechanisms: oxidative stress or carbon and energy source limitation on shifting to the stationary growth phase [109]. In both cases, *katB* uses the same (or overlapped) promotor, but the transcription is initiated from two different nucleotides separated by three or four bases [109]. Such a mechanism of the double regulation of the catalase expression has been earlier shown for the *katA* gene of *Bacillus subtilis* catalase where the transcription is initiated from the same site by two independent mechanisms: the H_2O_2-induced regulation and the genes *spo0A* and *abrB* regulating sporulation [116].

The inverted repeat with the 6-bp sequence in the regulatory region of *B. fragilis katB* is present in the same region of the *sod* gene of *B. fragilis* superoxide dismutase. Probably, this sequence (TGAAATnnnnnATTTGA (the so-called "rot" box, response to oxygen toxicity) is the recognition site for a DNA-binding protein involved in the regulation of oxidative stress genes in this microorganism [19, 109]. Thus, the regulation of catalase in the anaerobe *B. fragilis* phylogenetically remote to other bacteria is complicated, but molecular mechanisms that control the synthesis of catalase and also signals initiating it need to be studied further. It is supposed that in different phylogenetic groups of bacteria the fine regulation of defense mechanisms, in particular catalase, against toxic effect of O_2 evolved independently and strongly varies in various species depending on their needs and environment [5, 19].

Peroxide and oxygen stresses in *B. fragilis* are independent: a mutant with constitutive expression of catalase and alkylhydroperoxide reductase was resistant to H_2O_2 (100 mM, 15 min) and organic hydroperoxides (5 mM, 15 min) but was sensitive to atmospheric oxygen similarly to the parent strain [115]. This can probably be explained by different toxic effects of O_2 and H_2O_2 on *B. fragilis* cells.

In *D. desulfuricans*, O_2 induces SOD expression (tenfold increase in activity) and NADH-oxidase expression that improves the cell viability under oxidizing conditions [117]. Oxygen is suggested to induce the expression of SOD, catalase, or NADH-oxidase also in *D. gigas* [24], at least it has been experimentally shown for catalase [118]. Hydrogen peroxide insignificantly induced catalase in *D. vulgaris* [35, 119]. But the character of protective mechanisms of sulfidogens under the influence of H_2O_2 and O_2^- and the number of genes involved in these responses remain unclear.

Aerobes and facultative anaerobes possess an effective complex of protective enzymatic and nonenzymatic systems for elimination of toxic oxygen derivatives. Anaerobic microorganisms cannot generate reactive oxygen species; nevertheless, they also have the enzymes of antioxidative defense, such as SOD and catalase, but the latter is less distributed among various anaerobes than SOD.

The origin and physiological role of SOD and catalase in anaerobic microorganisms are not elucidated definitely. It is suggested that synthesis of the antioxidative enzymes is induced by O_2 or its derivatives only under unfavorable aerobic conditions. Many anaerobes, including the strictest ones, are aerotolerant and can endure contact with oxygen. Although there are some data on oxidative stress obtained on *Bacteroides*, the problem of regulation of antioxidative defense in strict anaerobes and of the number of genes involved in it remains open. In this connection, the importance of studies on the antioxidative defense enzymes and their regulation in methanogens, which retain features of initial ancestors and exist under conditions of extremely strict anaerobiosis is obvious. There is a hypothesis that Fe-SODs found in methanogens are precursors of the closely related Fe- and Mn-SODs of prokaryotes and direct "descendants" of the initial SODs of urkaryotes. On the other hand, there are hypotheses about parallel pathways of SOD evolution in some methanogens and bacterial Fe-SODs.

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