Antioxidative defense systems of anaerobic sulfate-reducing microorganisms

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Sulfate-reducing bacteria (SRB) are traditionally considered as strict anaerobic microorganisms. But there are a lot of data up to date about survival and metabolic activity of SRB in biotopes periodically exposed to oxygen. The main factor of O_2 toxicity is the action of products of oxygen incomplete reduction, so called the reactive oxygen species (superoxide anion radical, hydrogen peroxide and hydroxyl radical). The main targets of them are cell macromolecules. Also molecular oxygen is able to directly inactivate key enzymes of sulfate reduction metabolism. The SRB have thus developed complicated and tightly regulated systems of physiological and enzymatic mechanisms of oxidative stress response which provide their relative aerotolerance. They can form aggregates in the presence of O_2, establish symbiotic relationships with aerobic microorganisms and possess aerotaxis. In addition to classical enzymes (superoxide dismutase, catalase, peroxidases) which are usual for aerobes, the cells of many SRB possess also unique alternative enzymes with superoxide reductase (desulfoferrodoxin, neelaredoxin) and peroxidase (rubrerythrin, nigerythrin) activities.

Keywords : sulfate-reducing bacteria; oxidative stress; aerotolerance; reactive oxygen species; superoxide dismutase; catalase; desulfoferrodoxin; neelaredoxin; rubrerythrin; nigerythrin.

1. Introduction

Sulfate-reducing bacteria (SRB) are traditionally considered as strict anaerobic microorganisms. But there are a lot of data up to date about survival and metabolic activity of SRB in such zones of biotopes where oxygen exposure temporarily exists – surface sea waters, cyanobacterial mats, activated sludge, water sediments, intestines of termites, etc. SRB represent a group of prokaryotes, morphologically and metabolically highly diverse, which gain energy by coupling the oxidation of great variety of low-molecular mass organic compounds or molecular hydrogen to reduction of sulfate (SO_4^{2-}) in sulfide (H_2S, HS^-) [1]. One of the most abundant biotopes in which sulfate reduction coexists with oxic conditions is present in cyanobacterial mats, where photosynthetic oxygen synthesis, sulfate reduction and microbial sulfide oxidation overlap and create steep opposed gradients of oxygen and sulfide fluctuating in the diurnal cycle [2, 3]. It should be noticed that the sulfate reduction rates in mats under oxic conditions during the daytime often exceed those observed at night under anaerobic conditions [4].

In the case of anaerobic organisms, the toxicity of oxygen is a combination of at least three factors. The main factor is the action of products of oxygen incomplete reduction so called the reactive oxygen species (ROS) – superoxide anion radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^\bullet$). The main targets of ROS are cell macromolecules: nucleic acids, lipids and proteins. Secondly, the high redox potential induced by the presence of O_2 represents an additional reason restricting the availability of anaerobes in oxygenated environments because of displacement of thermodynamic equilibrium and the following failure of metabolic processes with initiation of adverse reactions. Finally molecular O_2 is able to directly inactivate key enzymes of sulfate reduction metabolism.

SRB have developed complicated and tightly regulated systems of behavioral and enzymatic mechanisms of antioxidative defense which are responsible for a relative aerotolerance of SRB and elimination of ROS. Behavioral responses of SRB to oxygen include aggregation, symbiotic relationships with aerobic microorganisms, migration to anoxic zones and aerotaxis [5]. Enzymatic mechanisms involve O_2 reduction that uses cytoplasmic, periplasmic and membrane-bound oxygen reduction chains as well as ROS scavenging reactions. In addition to classical enzymes of antioxidative defense (superoxide dismutase, catalase, peroxidases) which are usual for aerobic microorganisms and eukaryotes, the SRB cells, and specially *Desulfovibrio* species, possess also unique highly effective alternative enzymes with superoxide reductase (desulfoferrodoxin, neelaredoxin) and NADH-dependent peroxidase (rubrerythrin, nigerythrin) activities [6, 7].

2. Ecological aspect of physiological responses of SRB to oxygen exposure

2.1 Effects of oxygen on sulfate-reducing bacteria

Some species of SRB are able to survive after a short-time of exposure to O_2 and they exhibit high oxygen tolerance even when grown in pure cultures [8]. The high number of SRB, found in the oxic environments, indicates that these organisms are able to deal with temporary exposures to elevated O_2 concentrations up to 1.5 mM [9].

Thus, *Desulfovibrio desulfuricans* NCIB 8301 grown on agar media in the presence of oxygen survived well over the first 24 h but the cell viability decreased rapidly for longer exposure times; thereafter, live cells were still detected in colonies for one month [10]. *D. desulfuricans* is capable to grow slowly at low O_2 partial pressures (10-15 mm Hg); the cell yield fell drastically with an increase of the oxygen partial pressure, which was correlated with a decrease of both lactate utilization and hydrogen sulfide production [10].

The growth of *Desulfovibrio gigas* in a lactate/sulfate containing medium is suppressed in the presence of 5 μM O_2; the cell division resumed as soon as anaerobic conditions were restored, even if air exposure lasted for 24 h [11]. Under the aerobiosis, non dividing cells of *D. gigas* were still capable of oxidizing pyruvate to acetate. When grown in a lactate/sulfate medium, *D. gigas* utilizes O_2 and synthesizes NTPs. Nevertheless, the observed increase in NTP levels may indicate that, under the aerobic conditions, O_2 inhibits processes leading to ATP dissipation [11].

Cells of *Desulfovibrio vulgaris* Hildenborough also are able to consume oxygen and they possess aerotaxis in the direction of preferred sub-microaerophilic O_2 concentrations (0.02-0.04%), a level which also supported growth [12]. The air exposure for 2 h has an insignificant effect on survivability of *D. vulgaris* [13] whereas 1 h exposure to 100% O_2 causes five-fold decrease of the cell viability [14].

It has been shown [15] that in homogeneously aerated cultures of several SRB species, the rate of sulfide formation from the reduction of either sulfate, sulfite or thiosulfate decreases as the O_2 concentration increases, and is abolished above 15 μM O_2. Viability, cell motility and ability to cell division decrease with time when cells are exposed to oxygen. In continuous cultures of *Desulfovibrio oxyclinae* sulfate reduction is inhibited in the presence of 1% O_2 [16]. Moreover, oxygen induces morphological changes as it has been observed in the case of *Desulfovibrio* strains, developed atypically elongated cells [17]. This toxicity of O_2 might be related to the sensitivity of several proteins isolated from SRB: hydrogenases (key enzymes of *Desulfovibrio* energetic metabolism), L(+)-lactate dehydrogenase, NAD-dependent alcohol dehydrogenase, aldehyde dehydrogenase [18], some enzymes involved in protein and nucleic acids synthesis, and in cell division [14].

2.2 Sulfate-reducing bacteria in cyanobacterial mats

SRB are often found in the photooxic zone of cyanobacterial mats, where the gas ratio ranges from total O_2 saturation during the daytime to anaerobic conditions and high level of H_2S in the night-time. Cyanobacterial mats provide an interesting stratified system to study the behavioral patterns of SRB in response to oxygen. It have been shown, that during the day the most probable number (mpn) count of SRB in the upper layer of cyanobacterial mats from the Solar Lake (Sinai, Egypt) is 20-times lower than at dark in the absence of O_2 [19].

Within the oxic surface layer of the hypersaline cyanobacterial mat of the Solar Lake and in the mat layers below, diurnally migrating cells of *Desulfonema* are found in variable densities of 10^4 to 10^6 cells ml^{-1}. The number of cultured SRB in the surface aerobic layer and the level of sulfate reduction during the daytime are comparable, and sometimes even higher than identified in the interior of the mat. Facultative aerobic respiration, filamentous morphology, motility, migration to deep anoxic mat layers, and aggregates formation are the main adaptations of SRB from the Solar Lake to the mat matrix and to diurnal oxygen stress [20].

D. oxyclinae D22, isolated from the upper layer of cyanobacterial mats from the Solar Lake during the day, does not show any migration reaction while *D. oxyclinae* strains N24 and N13, dominate in the dark, migrate depending on the O_2 concentration. Strain N13 demonstrates aerotaxis inside cyanobacterial mats, forming bands around the O_2 bubbles on the boundary of aerobic and anaerobic zones. Cells perform circular motion in the bands which is uncharacteristic for anaerobic environment. In a full aeration all isolates are able to respire with oxygen as electron acceptor and hydrogen, lactate or sulfide as electron donors. Ethanol, which can be used for sulfate reduction, is not metabolized with the participation of O_2. Strain N13 supports growth under aerobic conditions up to 2 days, but cell division is inhibited and the length of cells increased to 5 times [19]. Thus, oxygen has the toxic effect on substrate oxidation as well as on anabolic processes.

A chemostat culture of *D. oxyclinae* isolated from the oxic layer of a hypersaline cyanobacterial mat was grown in anaerobic conditions and then subjected to exposure with 1% O_2 [9]. At the onset of aerobic gassing, sulfate reduction decreased by 40%, although viable cell numbers did not decrease. After 42 h, the sulfate reduction rate returned to the level observed in the anaerobic culture. At this stage the growth yield increased by 180% compared to the anaerobic culture. The O_2 consumption rate measured in washed cell suspensions increased by 80%, and the thiosulfate reduction rate of the same samples increased by 29% with lactate as the electron donor. These observations indicated possible oxygen-dependent enhancement of growth. After 140 h of growth under oxygen exposure, the presence of cell aggregates 0.1 to 3 mm in diameter was observed. Formation of SRB cell clusters occurs within a cyanobacterium *Microcoleus chthonoplastes*-dominated layer of a cyanobacterial mat under daily exposure to O_2 concentrations of up to 900 μM [9]. Aggregates can only consist of bacteria, or include particles of inorganic nature, such as FeS, which provide additional mechanical strength and resistance to O_2.

In natural habitats, *D. oxyclinae* exists in close relationship with aerobic microorganisms, consuming O_2, in particular with heterotroph *Marinobacter* sp. Probably, SRB *in vivo* form small aggregates surrounding layers inhabited by symbiotic aerobes. A chemostat coculture of *D. oxyclinae* and *Marinobacter* sp. strain MB, isolated from the oxic zone of a cyanobacterial mat, was grown for 1 week under anaerobic conditions and then exposed to a flux with 5% O_2 [21].

Sulfate reduction persisted under these oxic conditions, though the amount of reduced sulfate decreased by 45%. The coculture consumed oxygen effectively, and no residual O_2 was detected during the growth. The proportion of *D. oxyclinae* cells in the coculture decreased from 86% under anaerobic conditions to 70% in the microaerobic sulfate-reducing mode and 34% in the microaerobic sulfate-depleted mode. The patterns of consumption of electron donors and acceptors suggested that when oxygen was supplied in the absence of either sulfate or thiosulfate, *D. oxyclinae* performed incomplete aerobic oxidation of lactate to acetate. This is the first observation of oxygen-dependent growth of a SRB. Nevertheless, pure cultures of SRB, capable of aerobic growth in the absence of SO_4^{2-}, have not been obtained, so the oxygen respiration is attributed, primarily, the defensive function in microaerophilic conditions [21].

Bacteria belonged to genera D*esulfonema* and *Desulfococcus* are dominant forms of photooxic and chemocline zones of the mats from the Solar Lake [4, 19, 20]. The representatives of *Desulfobacter* are most prevalent at depths corresponding to the chemocline zone during the day and the anaerobic zone at night. Profile of sulfate reduction in the bacterial community is heterogeneous: local anaerobic microniches populated by metabolically active SRB are formed in aerobic zone [4, 19]. Thus, recognized genera of SRB are not necessarily restricted by high O_2 levels in the mat community, and significant sulfur cycling within the chemocline is possible. Glycolate, secreted by cyanobacteria during the photosynthesis, stimulates the respiration of *Desulfonema* in the aerobic surface layer. The distribution of SRB allows associated sulfur-oxidizing chemo-and phototrophic microorganisms to take more sunlit areas with better O_2 access [4, 9, 19]. In the cyanobacterial mat from the saline evaporation pond in Baja California, *Desulfobacter* and *Desulfobacterium* are also restricted to greater depths while the representatives of *Desulfococcus* and *Desulfovibrio* are predominant in the upper part of the well-aerated photosynthetic zone [22].

SRB in the fully oxic photic zone of mats have to compete with numerically dominant and metabolically more diverse aerobic heterotrophic bacteria for organic substrates. Aerobic respiration by SRB contributes to organic carbon mineralization in the oxic zone of microbial mats as daytime porewater concentrations of low-molecular-weight carbon compounds are above typical half-saturation constants. Thus, the strain SRB D2, isolated from the photic zone of a hypersaline microbial mat (Lake Chiprana, Spain), respired pyruvate, alanine, and α-ketoglutarate but not formate, lactate, malate, succinate, and serine at significant rates under fully oxic conditions. Dehydrogenases of only the former substrates are likely oxygen-tolerant enzymes as all substrates supported anaerobic sulfate reduction. Although strain SRB D2 appeared phylogenetically closely related to the oxygen-tolerant *D. oxyclinae*, substrate spectra were essentially different [23].

2.3 Sulfate-reducing bacteria in activated sludge

SRB are wide-spread microorganisms in activated sludge of wastewater treatment facilities, periodically exposed to O_2. The presence of SRB in large bacterial flocs was demonstrated by FISH and PCR-based detection of genes coding for the dissimilatory sulfite reductase. In contrast, sulfate reduction could not be detected in any sludge in either the microsensor or the batch investigation, not even in anaerobic microniches. Probably, electron acceptors like NO_3^- or O_2 are used instead of SO_4^{2-} as more thermodynamically preferable [24].

Washed cell suspensions of *D. desulfuricans* strain DvO1, isolated from the highest sulfate reduction positive mpn dilution of an activated sludge sample, were aerated at 50% atmospheric O_2 saturation in sulfide-free media for 33 h in the presence or the absence of lactate as external electron donor. The cell suspension aerated in the absence of lactate showed insignificant endogenous O_2 reduction rates. In contrast, the cell suspension aerated in the presence of lactate sustained high rates of O_2 reduction during the entire aeration period. Strain DvO1 remained viable throughout the 33-h aeration irrespective of the presence of lactate; however, the O_2 exposure resulted in a dose-dependent reversible metabolic inactivation. Lactate-dependent anaerobic sulfate-reducing activity recovered quickly upon anaerobiosis, and was more aerotolerant than lactate-dependent oxygen-reducing activity [25].

2.4 Sulfate-reducing bacteria in wastewater biofilms

The SRB activity in biofilms is considered as the main reason of corrosion of industrial water pipe-lines and water supply facilities. Taking into consideration the fact that SRB are able to extract smallest Zn particles from water with the formation of ZnS pellet, the water quality control systems can be created on SRB basis [26].

The vertical distribution of SRB in photosynthetic biofilms from the trickling filter of a sewage treatment plant was investigated with 16S rRNA-targeted oligonucleotide probes. A negative correlation between the distribution of SRB and the measured O_2 profiles was found. It was also shown that SRB were located in the biofilms irregularly, being present in all states from single cells to dense clusters of several thousand cells. The distribution differed in light- and dark-incubated samples; in both cases the SRB were largely restricted to anoxic layers [27]. Microsensor measurements for O_2 showed that anoxic zones developed in the aerobic bacterial biofilm within a week and that oxygen level decreased during all stages of biofilm development. Sulfate reduction was first detected after 6 weeks of growth, although favourable conditions for SRB growth were presented from the first week. *In situ* hybridization with a 16S rRNA probe for SRB revealed that they were present in high numbers (~10^8 cells/ml) in all stages of biofilm development, both in the oxic and anoxic zones. Hybridization analysis of the DGGE profiles with taxon-specific oligonucleotide probes showed that *Desulfobulbus*, *Desulfovibrio* and *Desulfomicrobium* were the most numerically

important SRB in all biofilm samples as well as in the bulk activated sludge. It was found that not all SRB detected by molecular methods in the wastewater biofilm were metabolically active [28].

The investigation of successive development of SRB community structure and *in situ* sulfide production activity within a biofilm, growing under microaerophilic conditions, showed that the most active sulfate reduction zone moved upward to the oxic-anoxic interface and intensified with time. This result corresponded with an increase in SRB populations in the surface layer of the biofilm. The number of cells, hybridized with SRB385 and SRB660 probes, significantly increased in the biofilm surface layer during eight-week cultivation, while those populations were relatively unchanged in the deeper part of the biofilm, probably due to substrate transport limitation [29]. The phylogenetic identification and substrate uptake patterns of SRB inhabiting a sewer biofilm with oxygen, nitrate, or sulfate as an electron acceptor was performed with the use of MAR-FISH analysis. It was demonstrated that *Desulfobulbus*, hybridized with SRB660 probe, was a dominant SRB subgroup in the biofilm, accounting for 23% of the total SRB. Approximately 9 and 27% of detected *Desulfobulbus* cells could take up labelled propionate with O_2 and NO_3^-, respectively, as an electron acceptor, which might explain the high abundance of this species in various oxic environments [30].

3. Molecular mechanisms of SRB responses to oxygen

3.1 Toxic effects of molecular oxygen

Products of incomplete reduction of oxygen – superoxide radical ($O_2^{-\bullet}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($HO^\bullet$) – are highly toxic to cells. For instance, these reactive oxygen species (ROS) induce peptide bond breaks and the formation of cross-linked protein aggregates, depolymerization of nucleic acids, impair of sulfhydryl groups and [4Fe–4S] clusters (with concomitant iron release into the cytosol) in enzymes, oxidation of membrane lipids, polysaccharide molecules, and polyunsaturated fatty acids [31-33]. Inhibition of cell growth and DNA synthesis by oxygen was demonstrated in *D. oxyclinae and D. vulgaris* cells [11, 19]. In addition to the ROS effects, oxidative stress is also a consequence of the direct inactivation of key enzymes of sulfate reduction (hydrogenase or lactate dehydrogenase) by O_2 and of the modification of environmental parameters like redox potential and pH.

Hydrogen peroxide is the most stable intermediate in the process of O_2 reduction. H_2O_2 is capable of reacting with ferrous iron and other transition metals, forming more power oxidants, such as hydroxyl radicals (in the course of Fenton reaction: $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^- + HO^\bullet$), as well as alkoxyl radicals and iron-oxygen complexes. In addition to H_2O_2, organic peroxides may be formed in the cells, initiating a chain reaction of lipid oxidation and leading to membrane and DNA molecule damages [34]. Superoxide radical which is the most widespread free radical damages whole cells, in addition to subcellular structures and macromolecules. Moreover, $O_2^{-\bullet}$ is characterized by considerable diffusion capacity and exhibits greater selectivity in its reactions with intracellular proteins than other ROS. In addition, $O_2^{-\bullet}$ and H_2O_2 may be formed in the course of auto-oxidation of reduced cytochromes or flavoproteins, and as a result of UV irradiation [33]. Besides, the auto-oxidation of H_2S, normally produced by SRB, could also result in the formation of reactive species, which therefore increases the cell sensitivity to O_2 [8].

Usually, oxidative stress in microorganisms is induced by $O_2^{-\bullet}$ and H_2O_2 at relatively low intracellular concentrations (nmolar and μmolar, respectively) [35]. Superoxide dismutase (SOD), catalase, peroxidases and oxidases represent the first line of efficient enzymatic antioxidative defense, which prevent the accumulation of ROS and eliminate of those which production cannot be avoided. The expression of certain genes encoding the components of such systems is controlled by general regulatory mechanisms [31].

3.2 Oxidative stress responses of SRB on transcriptional level

Little data has been accumulated on the regulation of oxidative stress mechanisms in strict anaerobes; nevertheless some of them possess enzymes, which are induced under unfavorable oxic conditions [5]. Multiple antioxidative defense pathways were identified in SRB and proposed to be specifically expressed under different concentrations of oxidizing agents, contributing to ability of SRB to survive periodic aerobiosis in their econiches.

The global expression levels of oxidative stress response genes in *D. vulgaris* Hildenborough differ depending on ROS concentration. Concerted up-regulation of the genes belonged to predicted peroxide stress response regulon (PerR) was observed in response to low O_2 levels (0.1%) [36]. In contrast, long air exposure was highly detrimental to the cell viability and caused dramatic changes at the transcriptome level (393 genes were up-regulated and 454 genes were down-regulated) [36]. The expression levels of many genes, encoding enzymes of central metabolic pathways and antioxidative defense systems, decreased significantly during 4-h air exposure, but abundance of proteases and chaperons increased [36]. Transcripts analyses revealed that the PerR regulon of *D. vulgaris* also exhibited opposite regulation in the presence of 0.1 mM and 0.3 mM H_2O_2 and revealed that genes encoding a SOD, a superoxide reductase, a nigerythrin and a thiol peroxidase, in addition to the PerR regulon, belong to the H_2O_2 stimulon [37]. A global transcriptomic analysis pointed out that H_2O_2 as well as redox potential shift increased the expressions of the genes of *D. vulgaris* involved in ROS detoxification, thioredoxin-dependent reduction system, protein and DNA repair,

and decreased those involved in sulfate reduction, lactate oxidation and protein synthesis [38]. In addition, deletion mutants analysis suggested that PerR and Fur (ferric uptake regulator) regulons are functionally overlapped in response to H_2O_2 stresses [38].

A genome-wide transcriptomic analysis of *D. vulgaris* showed that 130 genes were responsive to 1-h air exposure [39]. For instance, amino acid biosynthetic pathways of *D. vulgaris* were induced by oxidative stress. The genes, encoding rubrerythrin (*rbr*) and thioredoxin reductase (*trxB*) were also up-regulated, suggesting an important antioxidative role of these proteins. In contrast, the expression of rubredoxin oxidoreductase (*rbo*), superoxide dismutase (*sodB*) and catalase (*katA*) genes were not changed in response to oxidative stress. Comparison of cellular reactions to oxidative stress and heat shock identified 66 genes that showed a similar drastic response to both of them, implying that these genes might be part of the general stress defensive system in *D. vulgaris* [39].

When *D. vulgaris* cells were exposed to the highest concentration of oxygen (100% O_2, 1 h) that SRB are likely to encounter in natural habitats, 307 genes were responsive, with cellular roles in energy metabolism, protein and nucleic acid synthesis, cell division, transport and regulatory functions, including multiple genes encoding heat shock proteins and peptidases [40]. The oxidative defense response concentrated on damage repair by metal-free enzymes. These data, together with the down-regulation of the Fur operon, which restricts the availability of iron in order to decrease the possibility of ROS generation, and the lack of response of the H_2O_2-sensing regulator operon, suggest that a major effect of high oxygen stress is the inactivation of multiple metalloproteins as a consequence of oxidative damage to their metal clusters [14, 40]. Thus, transcriptional responses of SRB to oxidative stresses are flexible and correlate with cell needs under specific conditions.

3.3 Molecular mechanisms of oxygen reduction by SRB

SRB not only survive oxygen exposure for at least days, but many of them even reduce O_2 to H_2O. It was shown that 14 tested freshwater or marine strains were able to reduce oxygen with at least one substrate as electron donor, including lactate, formate, pyruvate, ethanol, acetate, propionate, butyrate, hydrogen as well as sulfite or thiosulfite. The capability of O_2 reduction may be related to an adaptation to sulfate limitation in corresponding biotopes [41].

In *D. vulgaris* and *D. desulfuricans*, oxygen reduction was coupled to proton translocation and ATP conservation. In these species, the periplasmic fraction, which contains hydrogenases and low redox multiheme *c*-type cytochromes, was found to catalyze reduction of O_2 with high rates [42]. In *D. gigas*, a cytoplasmic rubredoxin oxidase was identified as an oxygen-reducing terminal oxidase (Table 1).

Desulfovibrio strains generally exhibit O_2 reduction rates comparable to those of aerobes, but these rates often decrease by increasing the O_2 concentration and slow down after repeated oxygen additions [19, 41]. The highest O_2 reduction rate detected so far have been observed in *Desulfovibrio termitidis*, isolated from termite guts, with more than 1570 nmol O_2 min^{-1} mg^{-1} [43]. Nevertheless, despite a high respiration rate and energy coupling, aerobic growth of pure cultures is very poor or absent because O_2 blocks sulfate reduction. The oxygen respiratory activity of SRB seems to be a protective mechanism against toxic effects of O_2, also providing considerable ecological advantage over aerobic microorganisms through the recovery of anoxic conditions if the habitat turns transiently oxic.

Table 1 Proteins involved in cytoplasmic and membrane-bound pathways of oxygen reduction in SRB.

Protein	Amount of subunits	Molecular mass of the subunits, kDa	Prosthetic groups	Cell localization	Microorganism
NADH:rubredoxin oxidoreductase	2	26; 32	FMN; FAD	Cytoplasm, soluble	*D. gigas, D. vulgaris, D. desulfuricans*
Rubredoxin	1	6	Fe		
Rubredoxin:oxygen oxidoreductase	2	43; 43	Fe-Fe; FMN		
Cytochrome *bd* oxidoreductase	2	29; 40	Heme; Cu	Membrane-bound	*D. gigas, D. vulgaris, Desulfotalea psychrophila*
Cytochrome *c* oxidase	4	11; 23; 47; 60	Heme; Cu	Membrane-bound	*D. vulgaris* Hildenborough, *D. vulgaris* Miyazaki, *D. desulfuricans* G20

Membranes from *D. gigas* grown in a fumarate/sulfate medium have a fully competent canonical respiratory chain, which allows the reduction of O_2 to H_2O using NADH and succinate [44]. A membrane-bound terminal oxidoreductase of the cytochrome *bd* family possesses a significant quinol oxidase activity. The ETC between the fumarate reductase and the cytochrome *bd* oxidase appears to be able to couple the succinate oxidation with the O_2 reduction to H_2O. It was suspected that, in addition to cytochrome *bd* oxidase, *D. gigas* contained at least another one membrane-bound oxygen

reductase of the heme-copper superfamily [44]. Genomes analysis of *D. vulgaris* Hildenborough, *Desulfotalea psychrophila* and *D. desulfuricans* reveals the existence of a terminal cytochrome *bd* oxidase in all of them suggesting that it could be a common key mechanism to SRB for O_2 reduction [18].

In addition to the oxygen reductase of the *bd*-family, a gene encoding cytochrome *c* oxidase has been found in *D. vulgaris* Miyazaki, downstream the gene encoding cytochrome c_{553} [45]. Genes encoding a homologous enzyme, annotated as a heme-copper cytochrome *c*-type oxidase, have also been detected in *D. vulgaris* Hildenborough [46] and *D. desulfuricans* G20 (genome.jgi-psf.org) genomes. These oxygen reductases would be structurally composed of four subunits. *In silico* sequence analyses suggest that the cytochrome *c* oxidase would be unique in the living world so far described as it would exhibit two additional *c*-type hemes in a subunit II. This proposal has been recently confirmed by Lobo et al. [47] who reported that the cytochrome *c* oxidase from *D. vulgaris* Hildenborough contained two hemes *c* in the subunit II.

Reduction of O_2 by SRB may occur not only with electron acceptors directly utilized from the medium, but also with storage compounds. It has been shown that the endogenous O_2 reduction rate of *Desulfovibrio salexigens* due to a NADH oxidase activity was proportional to the polyglucose content and was highest at air saturation [48]. NADH-linked ETC of *D. gigas* enables the transfer of reducing power, derived from polyglucose, to O_2, and involves three proteins (Fig. 1A): a NADH-rubredoxin oxidoreductase (NRO), a rubredoxin (Rub) and a rubredoxin-oxygen oxidoreductase (ROO) [49]. Rubredoxins are small cytoplasmic proteins that contain a single iron atom coordinated to four cysteinyl sulfur atoms [50]. This protein acts as an electron shuttle between the NRO and the ROO. NRO contains both FMN and FAD, and is able to reduce rubredoxin from NADH [51]. Rub in turn gives electron to its other redox partner, ROO. Each ROO monomer is composed of one lactamase-like domain (with Fe-Fe center, where the reduction of O_2 to H_2O has been proposed to occur via hydroperoxide intermediate) and one flavodoxin-like domain (which contains a FMN cofactor, the intermediate bridge in the electron transfer between Rub and the ROO di-iron center) [49]. In the presence of ROO, electrons are transferred directly from Rub to O_2 to form H_2O and no H_2O_2 is formed [52]. A cluster of genes encoding desulfoferrodoxin, Rub and ROO is present in the *D. vulgaris* Hildenborough [46] and *D. desulfuricans* G20 genomes. Thus, such type of cytoplasmic oxygen reduction chain appears to be a conserved and efficient mechanism of O_2 elimination among *Desulfovibrio* species [18]. It was suggested that O_2 reduction by ROO prevents the formation of ROS under microaerophilic conditions [53]. A role for rubredoxin in diverting electron flow from the electron transport chain of *D. vulgaris* Hildenborough to ROO, rubrerythrin and superoxide reductase under oxidative stress has been proposed [53].

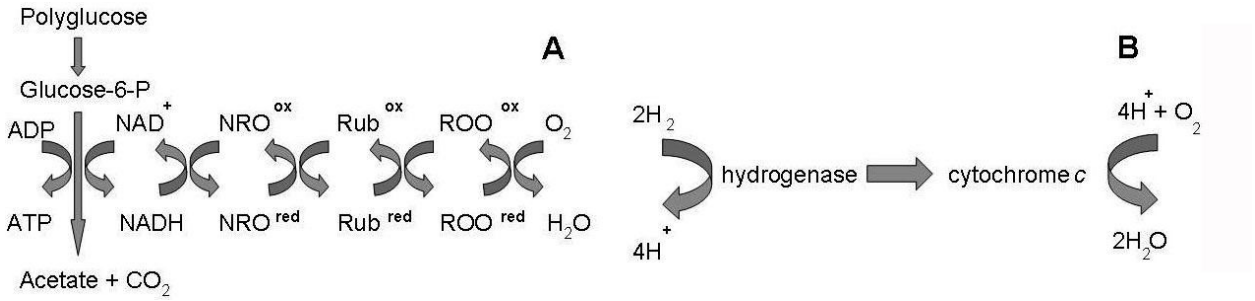

Fig. 1 (A) Cytoplasmic pathway of O_2 reduction of *D. gigas* [49]; (B) periplasmic pathway of O_2 reduction of *D. vulgaris* [54].

Another mechanism of oxygen reduction has been reported for *D. vulgaris* Marburg which reduces O_2 to H_2O with H_2 as electron donor and 90% of this O_2 reduction activity was found in the periplasmic fraction [54]. The maximum hydrogen oxidation rate was 253 nmol O_2 min^{-1} mg^{-1} at low oxygen concentrations (up to 30 µM). Hydrogenases and cytochrome *c* were shown to be involved in this reaction (Fig. 1B). Since this system works with higher rates than sulfate reduction, its antioxidative defense function in *D. vulgaris* and *D. termitidis* could be crucial [54]. An increase in the [Fe] hydrogenase and the cytochrome *c* content in the periplasm of *D. vulgaris* Hildenborough was observed following either O_2 exposure or chromate addition that increased the redox potential of the medium [55]. Viability of the strain lacking the genes, encoding [Fe] hydrogenase (*hydAB*), after 1-h exposure to oxygen was lower than that of the wild-type [55]. A similar behavior has been also described in *D. desulfuricans* NCIB 8301 *;* when the cells were exposed to oxygen, the same increase in cytochrome *c* content was detected [10]. Hydrogenases and cytochromes could be either directly involved in the elimination of O_2 by its reduction to H_2O or involved in a mechanism decreasing the environmental redox potential in order to get the optimal conditions for growth [56].

3.4 Aerotaxis regulation of SRB

In an oxygen gradient, *D. vulgaris* Hildenborough forms a focused band at an estimated O_2 concentration of 0.02–0.04% and *D. desulfuricans* – ring-shaped bands around air bubbles [12]. The fact that bands are formed at some distance from the air bubble shows that the cells are able of negative aerotaxis. Such cell accumulation also suggests the existence of positive aerotaxis [57].

Cells of *D. vulgaris* Hildenborough possess a chemoreceptor (DcrA) that serves as a sensor of the O_2 concentration or redox potential of the environment [58, 59]. A DcrA homolog was also found in the genome of *D. desulfuricans* G20 [18]. DcrA has the structural design of methyl-accepting chemotaxis proteins from enteric bacteria with an N-terminal periplasmic sensing domain which contains a *c*-type heme, and a C-terminal cytoplasmic signaling domain [59, 60]. The level of methylation of the signaling domain is influenced by the redox state of the heme group and decreased upon addition of O_2 [59]. The full chemotactic response of *D. vulgaris* is complicated and controlled by at least 15 genes from the *dcr* family [61]. In response to 1-h exposure to 100% O_2, the positive regulation of three genes that encode chemotaxis proteins was observed, among which one belongs to the *dcr* family and one corresponds to the protein of the basal body of flagellum [40].

Mutants lacking *dcrA* show greater resistance to the toxic effects of O_2 than the wild-type cells. The reason for this unusual phenotype is probably a parallel increase in the transcriptional level of the *rbo-rub* operon that encodes for the superoxide reductase and rubredoxin, located immediately downstream from *dcrA*. Thus, DcrA may act under anaerobic conditions as the repressor of transcription of the *rbo-rub* operon [62].

4. Classical enzymes of ROS detoxification

4.1 Superoxide dismutase (SOD)

Superoxide dismutase eliminates $O_2^{-\bullet}$ by dismutation to H_2O_2 and O_2. The presence of a SOD activity in anaerobic bacteria has been demonstrated for the first time in *D. desulfuricans* and *Desulfotomaculum nigrificans* [63]. At the same time, SOD was isolated from *D. desulfuricans* Norway 4. The enzyme exhibited similar physico-chemical properties as compared to the Fe-SODs found in aerobic microorganisms [64]. More recently, a Fe-SOD has been purified and characterized from *D. gigas.* This SOD, isolated as a homodimer of 22-kDa noncovalently bound subunits (Table 2), has an EPR spectrum characteristic of high-spin ferric iron in a rhombically distorted ligand field. Like other Fe-SODs, the enzyme of *D. gigas* is sensitive to H_2O_2 and azide but not to cyanide [65].

Table 2 Properties of purified Fe-SODs and heme-containing catalases of SRB.

Microorganism	Molecular mass, kDa		Number of subunits		Specific activity, U/mg	
	SOD	catalase	SOD	catalase	SOD	catalase
D. desulfuricans Norway 4	43.0	-	2	-	2060	-
D. desulfuricans B-1388	43.0	-	2	-	1000	-
D. gigas ATCC 19364	43.0	186.0	2	3	1900	4200
D. vulgaris Miyazaki F	43.5	122.0	2	2	850	50000

The genes, encoding SOD, have been cloned from *D. vulgaris* strains Hildenborough [66] and Miyazaki F [67]. Genomes analysis of *Desulfovibrio desulfuricans* and *Desulfotalea psychrophila* reveals the presence of a single *sod* gene per genome [18]. In *Desulfovibrio* species, the N-terminal sequence of the SODs contains a twin-arginine motif typical for signal peptides of periplasmic redox proteins, allowing the SOD holoenzyme to be translocated from the cytoplasm into the periplasm using the TAT system [66, 67].

No significant differences in SOD activity have been observed between cells of *D. gigas* exposed to 120 μM O_2 for different times and anaerobic cultures [11]. Likewise, the amount of SOD does not change when *D. vulgaris* is exposed to air for 1 h [13, 14]. On the other hand, O_2 in low concentration (10 mm Hg) induced SOD activity (10-fold increase) in *D. desulfuricans* that improved cell viability under temporary microaerophilic conditions [10].

The deletion of the *sod* gene in *D. vulgaris* Hildenborough induced an increase in the strain sensitivity to externally produced $O_2^{-\bullet}$ while exposure to air does not demonstrate any difference [68]. The periplasmic space of *Desulfovibrio* species contains a lot of low redox potential enzymes [69] that might generate $O_2^{-\bullet}$ in the presence of O_2. It has been shown that, when cells are exposed to 100% O_2 for a short time, the [Fe] hydrogenase activity is higher in the wild-type strain of *D. vulgaris* than in the Δ*sod* mutant [55]. The periplasmic SOD could be therefore mainly involved in the protection of sensitive Fe-S enzymes against superoxide-induced damages.

4.2 Catalase

Catalase activity, disproportioning of H_2O_2 to O_2 and H_2O, was detected for the first time in *Desulfovibrio* [64]. The enzyme from *D. gigas* is composed of three subunits of 61 kDa (Table 2) and contains only one heme group in an active center per molecule, which may explain for its relatively low specific activity [65]. While *D. vulgaris*, *D. gigas* and *Desulfomicrobium norvegicum* are catalase positive, *D. salexigens* and many strains of *D. desulfuricans* are catalase negative [65]. The activities of three enzymes – catalase, NADPH peroxidase and NADH peroxidase, participating in

H_2O_2 decomposition, were detected in cell-free extracts of *D. desulfuricans* ATCC 27774 [48]. *Desulfotomaculum* species possess high catalase activity comparable to such in aerobic bacteria [70].

The *kat* gene, encoding a cytoplasmic catalase in *D. vulgaris* Miyazaki F, has been cloned and expressed in *E. coli* [71]. Genomes analysis reveals the presence of the *kat* gene in *Desulfotalea psychrophila* and *Archaeglobus fulgidus* [18]. In the case of *D. vulgaris*, it is located on a *nif* gene-containing plasmid that is lost during cell cultivation on a medium with ammonium chloride [68]. In *D. gigas*, catalase activity increased as cells are exposed to increasing oxygen concentration (up to 120 μM) and long exposure times led to higher catalase activity [11].

5. Alternative enzymes of antioxidative defense

5.1 Desulfoferrodoxin and neelaredoxin

In 2000, Lombard et al. reported [72] that desulfoferrodoxin (Dfx) from *Desulfoarculus baarsii*, which had been already shown to functionally complement an *E. coli* mutant deficient in cytoplasmic SODs [73-74], exhibited a superoxide reductase (SOR) activity, catalyzing the reduction of $O_2^{-\bullet}$ to H_2O_2. This protein has been also purified and characterized from *D. desulfuricans* and *D. vulgaris* Hildenborough [75]. Dfx is a monomer of 14-16 kDa that contains two separate mononuclear non-heme iron centers. Center I is a ferric site with distorted tetrahedral sulfur coordination [$Fe(SCys)_4$] and center II is a ferrous site with a unique square pyramidal structure [$Fe(NHis)_4(SCys)$]. Kinetics and spectroscopics experiments identified center II as the active site for the SOR activity [75-79].

Another enzyme with high SOR activity, named neelaredoxin (Nlr), which contains only one active site, identical to center II of Dfx, has been characterized from *D. gigas* [80]. Both two non-heme iron proteins have been also found in a hyperthermophilic sulfate-reducing archaeon *A. fulgidus* [81]. SOR activity of Nlr may allow cells to eliminate $O_2^{-\bullet}$ quickly in a NAD(P)H-dependent pathway. Both enzymes (Nlr, especially) can also act as SOD in conditions of high cellular redox potential in a lack of available electron donor [82, 83].

In the case of Nlr from *D. gigas* [84] and *A. fulgidus* [81, 85], as well as Dfx from *D. vulgaris* [78, 86], superoxide reduction is a fast bimolecular reaction resulting in the formation of a single Fe(III)-hydroperoxide intermediate. In the reaction involving Dfx from *D. baarsii*, a Fe(III)-peroxide species is formed at the first stage, which then undergoes protonation by the solvent and transition into the Fe(III)-hydroperoxide intermediate [87, 88]. During the repeated protonation (by water or a stronger exogenous acid, HX), H_2O_2 is released, and the vacant sixth iron coordination site is occupied by an anion (OH^- or X^-), which may be replaced subsequently by Glu47, a ligand with a higher affinity [86]. While Glu47 might serve to help H_2O_2 release, Lys48 plays an important role during guiding and binding of $O_2^{-\bullet}$ to the iron center II of Dfx (Fig. 2) [77, 79]. Recovery of the SOR leads to a conformation change and breaking the bond with Glu, which promotes more effective interaction with $O_2^{-\bullet}$ [81, 82].

Rubredoxins have been shown to be efficient electron donors for SOR in *D. vulgaris* [89] and *A. fulgidus* [90]. In *D. vulgaris* Hildenborough and *D. desulfuricans* G20, the genes, encoding desulfoferrodoxin (SOR), rubredoxin and rubredoxin-oxygen oxidoreductase, are in the same genes cluster. Such gene organization proves that SOR and ROO may collaborate for the detoxification and reduction of O_2, entering the cytoplasm, through the use of Rub as a common intermediary electron donor [18, 53].

Comparison of the sensitivity of Δ*sor* and Δ*sod* mutants of *D. vulgaris* to various oxidative stresses indicates that under fully aerated conditions, cytoplasmic SOR is the key oxygen defense enzyme. Whereas SOD is involved in the removal of $O_2^{-\bullet}$ in the periplasm under microaerophilic conditions to protect oxygen-sensitive enzymes [66, 68], SOR and SOD are thus complementary components of an efficient superoxide-scavenging cellular system.

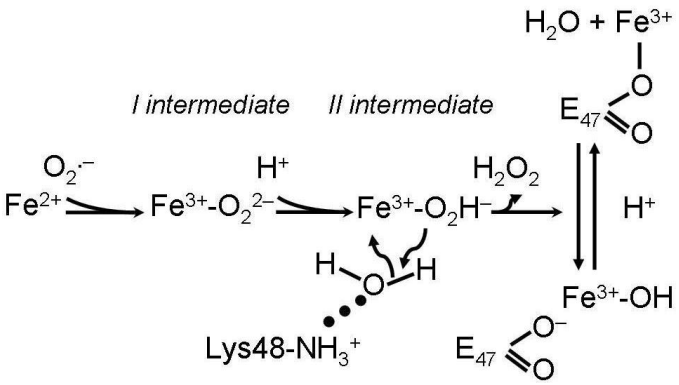

Fig. 2 Superoxide reduction mechanism of desulfoferrodoxin from *Desulfoarculus baarsii* [77, 88].

5.2 Rubrerythrin and nigerythrin

Rubrerythrin and nigerythrin, homodimeric cytoplasmic proteins which exhibit NADH peroxidase activity *in vitro*, have been isolated from *D. vulgaris* Hildenborough [91, 92]. Rubrerythrin (Rbr) contains a non-sulfur, hemerythrin-like carboxylate-bridged diiron site and a rubredoxin-like [Fe(SCys)$_4$] site in each of two 23-kDa subunits [91, 93, 94]. The mechanism of hydrogen peroxide reduction by rubrerythrin is shown on Fig. 3. Nigerythrin (Ngr) is a larger protein (54-kDa) that contains the same types of iron sites as rubrerythrin, but has different domain conformation providing higher peroxidase activity [91, 95]. In *D. vulgaris*, while the *rbr* gene has been shown to be co-transcribed with two other genes (encoding a Fur-like protein and a rubredoxin-like protein), the *ngr* gene is monocistronic [96]. A catalytic electron transfer *in vitro* between rubredoxin and rubrerythrin has been described [89].

The deletion of the *rbr* gene in *D. vulgaris* does not alter the sensitivity of the strain to any oxidative stress [68]. However, this can be explained by the presence of genes encoding a nigerythrin and a second homolog to rubrerythrin that may compensate for the deletion [68]. The expression of *rbr* and, especially, *ngr* from *D. vulgaris* in Δ*katG* or Δ*katE* strains of *E. coli* significantly increased their resistance to H_2O_2 [66]. It should be noticed that under drastic oxidative stress conditions, *D. vulgaris* reduces the amount of SOR and rubrerythrins in order to limit free Fe^{2+}, which is involved in the ROS generation. But two cytoplasmic proteins with a thiol-peroxidase activity are up-regulated in these stress conditions, compensating for this decrease [14, 68].

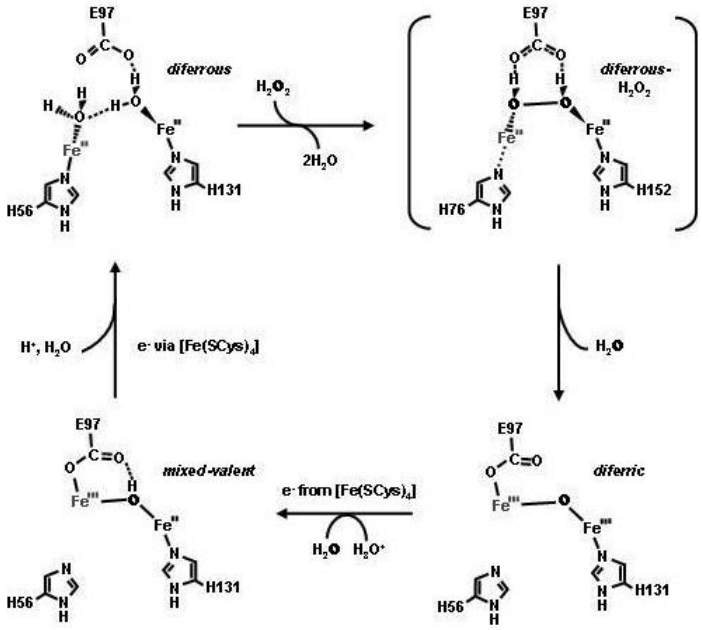

Fig. 3 Supposed highly-specific peroxidase catalytic cycle in a Fe-Fe center of rubrerythrin [97].

6. Conclusion

While cellular mechanisms of defense against toxic ROS in aerobic organisms are well described, relatively few data are available about anaerobes. SRB belong to strict anaerobic bacteria that often encounter oxic conditions in their different natural biotopes, and some SRB possess protective aerobic respiration even. They thereby represent organisms of choice to study the variety of antioxidative defense systems in anaerobes which provide a competitive advantage and survival in changing environments. Ecological, biochemical and genetics studies have brought information on the way by which SRB are able to survive temporary oxygen exposure. Efficient response to the oxidative stresses in the SRB cells includes specific behavior (aerotaxis, aggregation) and complicated molecular mechanisms involving classical as well as unique key antioxidative enzymes like superoxide reductase or rubrerythrin. Some of these highly effective enzymes, characterized for the first time in SRB, are in fact largely distributed in the anaerobic forms of life. The main advantage of these systems is the lack of production of oxygen during the catalytic cycle. The differential proteomics and transcriptomics approaches developed on *D. vulgaris* Hildenborough [14, 36] identified several proteins of unknown functions, differentially expressed under drastic oxidative conditions, that might constitute new systems of ROS detoxification to be further studied.

References

[1]. Sass H, Cypionka H, Babenzien HD. Vertical distribution of sulfate-reducing bacteria at the oxic-anoxic interface in sediments of the oligotrophic Lake Stechlin. *FEMS Microbiol. Ecol.* 1997:22:245-255.
[2]. Canfield DE, Des Marais DJ. Aerobic sulphate reduction in microbial mats. *Science.* 1991:251:1471-1473.
[3]. Visscher PT, Prins RA, van Gemerden H. Rates of sulfate reduction and thiosulfate consumption in a marine microbial mat. *FEMS Microbiol. Ecol.* 1992:86:283-294.
[4]. Minz D, Fishbain S, Green SJ, Muyzer G, Cohen Y, Rittmann BE, Stahl DA. Unexpected population distribution in a microbial mat community: sulfate-reducing bacteria localized to the highly oxic chemocline in contrast to a eukaryotic preference for anoxia. *Appl. Environ. Microbiol*. 1999:65:4659-4665.
[5]. Bryukhanov AL, Netrusov AI. Aerotolerance of strictly anaerobic microorganisms and factors of defense against oxidative stress: a review. *Appl. Biochem. Microbiol*. 2007:43:567-582.
[6]. Brioukhanov AL, Netrusov AI. Catalase and superoxide dismutase: distribution, properties and physiological role in cells of strict anaerobes. *Biochemistry (Moscow)*. 2004:69:949-962.
[7]. Brioukhanov AL. Nonheme iron proteins as an alternative system of antioxidant defense in the cells of strictly anaerobic microorganisms: a review. *Appl. Biochem. Microbiol.* 2008:45:335-348.
[8]. Cypionka H, Widdel F, Pfennig N. Survival of sulfate-reducing bacteria after oxygen stress, and growth in sulfate-free oxygen sulfide gradients. *FEMS Microbiol. Ecol.* 1985:31:39-45.
[9]. Sigalevich P, Meshorer E, Helman Y, Cohen Y. Transition from anaerobic to aerobic growth conditions for the sulfate-reducing bacterium *Desulfovibrio oxyclinae* results in flocculation. *Appl. Environ. Microbiol.* 2000:66:5005-5012.
[10]. Abdollahi H,Wimpenny JWT. Effects of the oxygen on the growth of *Desulfovibrio desulfuricans*. *J. Gen. Microbiol.* 1990:136:1025-1030.
[11]. Fareleira P, Santos BS, Antonio C, Moradas-Ferreira P, LeGall J, Xavier AV, Santos H. Response of a strict anaerobe to oxygen: survival strategies in *Desulfovibrio gigas*. *Microbiology*. 2003:149:1513-1522.
[12]. Johnson MS, Zhulin IB, Gapuzan ME, Taylor BL. Oxygen dependent growth of the obligate anaerobe *Desulfovibrio vulgaris* Hildenborough. *J. Bacteriol.* 1997:179:5598-5601.
[13]. Zhang W, Culley DE, Hogan M, Vitiritti L, Brockman FJ. Oxidative stress and heat-shock responses in *Desulfovibrio vulgaris* by genome-wide transcriptomic analysis. *Ant. van Leeuwen*. 2006:90:41-55.
[14]. Fournier M, Aubert C, Dermoun Z, Durand MC, Moinier D, Dolla A. Response of the anaerobe *Desulfovibrio vulgaris* Hildenborough to oxidative conditions: proteome and transcript analysis. *Biochimie.* 2006:88:85-94.
[15]. Marschall C, Frenzel C, Cypionka H. Influence of oxygen on sulphate reduction and growth of sulphate-reducing bacteria. *Arch. Microbiol.* 1993:159:168-173.
[16]. Krekeler D, Sigalevich P, Teske A, Cypionka H, Cohen Y. Sulfate-reducing bacterium form the oxic layer of a microbial mat from Solar lake (Sinai), *Desulfovibrio* sp. nov. *Arch. Microbiol*. 1997:167:369-375.
[17]. Sass H, Berchtold M, Branke J, Konig H, Cypionka H, Babenzien HD. Psychrotolerant sulfate-reducing bacteria from an oxic freshwater sediment, description of *Desulfovibrio cuneatus* sp. nov. and *Desulfovibrio litoralis* sp. nov. *Syst. Appl. Microbiol.* 1998:21:212-219.
[18]. Dolla A, Fournier M, Dermoun Z. Oxygen defense in sulfate-reducing bacteria. *J. Biotechnol.* 2006:126:87-100.
[19]. Krekeler D, Teske A, Cypionka H. Strategies of sulfate-reducing bacteria to escape oxygen stress in a cyanobacterial mat. *FEMS Microbiol. Ecol.* 1998:25:89-96.
[20]. Teske A, Ramsing NB, Habicht K, Fukui M, Kuver J, Jorgensen BB, Cohen Y. Sulfate-reducing bacteria and their activities in cyanobacterial mats of Solar Lake (Sinai, Egypt). *Appl. Environ. Microbiol.* 1998:64:2943-2951.
[21]. Sigalevich P, Cohen Y. Oxygen-dependent growth of the sulfate-reducing bacterium *Desulfovibrio oxyclinae* in coculture with *Marinobacter sp.* Strain MB in an aerated sulfate-depleted chemostat. *Appl. Environ. Microbiol.* 2000:66:5019-5023.
[22]. Risatti JB, Capman WC, Stahl DA. Community structure of a microbial mat: the phylogenetic dimension. *Proc. Natl. Acad. Sci. USA*. 1994:91:10173–10177.
[23]. Jonkers HM, Koh IO, Behrend P, Muyzer G, de Beer D. Aerobic organic mineralization by sulfate-reducing baceria in the oxygen-saturated photic zone of a hypersaline microbial mat. *Microb. Ecol.* 2005:49:291-300.
[24]. Schramm A, Santegoeds CM, Nielsen HK, Ploug H, Wagner M, Pribyl M, Wanner J, Amann R, de Beer D. On the occurance of anoxic microniches, denitrification, and sulfate reduction in aerated activated sludge. *Appl. Environ. Microbiol.* 1999:65:4189-4196.
[25]. Kjeldsen KU, Joulian C, Ingvorsen K. Effects of oxygen exposure on respiratory activities of *Desulfovibrio desulfuricans* strain DvO1 isolated from activated sludge. *FEMS Microbiol. Ecol.* 2005:53:275-284.
[26]. Vasconcelos C, McKenzie JA. Sulfate reducers - dominant players in a low-oxygen world? *Science.* 2000:290:1711-1712.
[27]. Ramsing NB, Kuhl M, Jorgensen BB. Distribution of sulfate-reducing bacteria, O_2, and H_2S in photosynthetic biofilms determined by olygonucleotide probes and microelectrodes. *Appl. Environ. Microbiol.* 1993:59:3840-3849.
[28]. Santegoeds CM, Ferdelman TG, Muyzer G, de Beer D. Structural and functional dynamics of sulfate-reducing populations in bacterial biofilms. *Appl. Environ. Microbiol.* 1998:64:3731-3739.
[29]. Ito T, Okabe S, Satoh H, Watanabe Y. Successional development of sulfate-reducing bacteria populations and their activities in a wastewater biofilm growing under microaerofilic conditions. *Appl. Environ. Microbiol*. 2002:68:1392-1402.
[30]. Ito T, Nielsen JL, Okabe S, Watanabe Y, Nielsen PH. Phylogenetic identification and substrate uptake patterns of sulfate-reducing bacteria inhabiting an oxic-anoxic sewer biofilm determined by combining microautoradiography and FISH. *Appl. Environ. Microbiol.* 2002:68:356-364.
[31]. Storz G, Tartaglia LA, Farr SB, Ames BN. Bacterial defenses against oxidative stress. *Trends Genet.* 1990:6:363–368.
[32]. Sies H. Strategies of antioxidant defense. Eur. J. Biochem. 1993:215:213–219.
[33]. Fridovich I. Superoxide radical and superoxide dismutases. *Annu. Rev. Biochem.* 1995:64:97–112.

[34]. Imlay JA. How oxygen damages microbes: oxygen tolerance and obligate anaerobiosis. *Adv. Microb. Physiol.* 2002:46:111–153.
[35]. Imlay JA. Pathways of oxidative damage. *Annu. Rev. Microbiol.* 2003:57:395–418.
[36]. Mukhopadhyay A, Redding AM, Joakhimiak MP, Arkin AP, Borglin SE, Dehal PS, Chakraborty R, Geller JT, Hazen TC, He Q, Joyner DC, Martin VJJ, Wall JD, Koo Yang Z, Zhou J, Keasling JD. Cell-wide responses to low-oxygen exposure in *Desulfovibrio vulgaris* Hildenborough. *J. Bacteriol.* 2007:189:5996-6010.
[37]. Brioukhanov AL, Durand MC, Dolla A, Aubert C. Response of *Desulfovibrio vulgaris* Hildenborough to oxidative stress: enzymatic and transcriptional analyses. *FEMS Microbiol. Lett.* 2010:310:175-181.
[38]. Zhou A, He Z, Reddling-Johanson AM, Mukhopadhyay A, Hemme CL, Joachimiak MP, Luo F, Deng Y, Bender KS, He Q, Keasling JD, Stahl DA, Fields MW, Hazen TC, Arkin AP, Wall JD, Zhou J. Hydrogen peroxide-induced oxidative stress responses in *Desulfovibrio vulgaris* Hildenborough. *Environ. Microbiol.* 2010:12:2645-2657.
[39]. Zhang W, Culley DE, Hogan M, Vitiritti L, Brockman FJ. Oxidative stress and heat-shock responses in *Desulfovibrio vulgaris* by genome-wide transcriptomic analysis. *Ant. van Leeuwen.* 2006:90:41-55.
[40]. Pereira PM, He Q, Xavier AV, Zhou J, Pereira IA, Louro RO. Transcriptomical responce of *Desulfovibrio vulgaris* Hildenborough to oxidative stress mimicking environmental conditions. *Arch. Microbiol.* 2008:189:451-461.
[41]. Dannenberg S, Kroder M, Dilling W, Cypionka H. Oxidation of H_2, organic compounds and inorganic sulfur compounds coupled to reduction of O_2 or nitrate by sulfate-reducing bacteria. *Arch. Microbiol*. 1992:158:93–99.
[42]. Cypionka H. Oxygen respiration by *Desulfovibrio* species. *Annu. Rev. Microbiol.* 2000:54:827-848.
[43]. Kuhnigk T, Branke J, Krekeler D, Cypionka H, Koenig H. A feasible role of sulfate-reducing bacteria in the termite gut. *Syst. Appl. Microbiol.* 1996:19:139–149.
[44]. Lemos RS, Gomes CM, Santana M, LeGall J, Xavier AV, Teixeira M. The "strict anaerobe" *Desulfovibrio gigas* contains a membrane-bound oxygen-reducing respiratory chain. *FEBS Lett.* 2001:496:40–43.
[45]. Kitamura M, Mizugai K, Taniguchi M, Akutsu H, Kumagai I, Nakaya T. A gene encoding a cytochrome *c* oxidase-like protein is located closely to the cytochrome c_{553} gene in the anaerobic bacterium, *Desulfovibrio vulgaris* (Miyazaki F). *Microbiol Immunol.* 1995:39:75-80.
[46] Heidelberg J, Seshadri R, Haveman S. *et al.* The genome sequence of the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. *Nat Biotechnol.* 2004:22:554-559.
[47] Lobo S, Almeida C, Carita J, Teixeira M, Saraiva L. The haem-copper oxygen reductase of *Desulfovibrio vulgaris* contains a dihaem cytochrome *c* in subunit II. *Biochim Biophys Acta* 2008:1777:1528-1534.
[48]. van Niel EW, Gottschal JC. Oxygen consumption by *Desulfovibrio* strains with and without polyglucose. *Appl. Environ. Microbiol.* 1998:64:1034–1039.
[49]. Frazao C, Silva G, Gomes CM, Matias P, Coelho R, Sieker L, Macedo S, Liu MY, Oliveira S, Teixeira M, Xavier AV, Rodrigues-Pousada C, Carrondo MA, Le Gall J. Structure of a dioxygen reduction enzyme from *Desulfovibrio gigas*. *Nat. Struct. Biol.* 2000:7:1041–1045.
[50]. Adman ET, Sieker LC, Jensen LH. Structure of rubredoxin from *Desulfovibrio vulgaris* at 1.5 Å resolution. *J. Mol. Biol.* 1991:217:337–352.
[51]. Chen L, Liu MY, LeGall J, Fareleira P, Santos H, Xavier AV. Rubredoxin oxidase, a new flavo-hemo-protein, is the site of oxygen reduction to water by the "strict anaerobe" *Desulfovibrio gigas*. *Biochem. Biophys. Res. Comm.* 1993:193:100–105.
[52]. Gomes CM, Silva G, Oliveira S, LeGall J, Liu MY, Xavier AV, Rodrigues-Pousada C, Teixera M. Studies of the redox centers of the terminal oxidase from *Desulfovibrio gigas* and evidence for its interaction with rubredoxin. *J. Biol. Chem.* 1997:272:22502–22508.
[53]. Wildschut JD, Lang RM, Voordouw JK, Voordouw G. Rubredoxin:oxygen oxidoreductase enhances survival of *Desulfovibrio vulgaris* Hildenborough under microaerofilic conditions. *J. Bacteriol.* 2006:188:6253-6260.
[54] Baumgarten A, Redenius I, Kranczoch J, Cypionka H. Periplasmic reduction by *Desulfovibrio* species. *Arch. Microbiol.* 2001:176:306–309.
[55]. Fournier M, Dermoun Z, Durand MC, Dolla A. A new function of the *Desulfovibrio vulgaris* Hildenborough [Fe] hydrogenase in the protection against oxidative stress. *J. Biol. Chem.* 2004:279:1787–1793.
[56]. Dolla A, Pohorelic BKJ, Voordouw J, Voordouw G. Deletion of the *hmc* operon of *Desulfovibrio vulgaris* Hildenbrough hampers hydrogen metabolism and low-redox-potential niche establishment. *Arch. Microbiol.* 2000:174:143–151.
[57]. Eschemann A, Kühl M, Cypionka H. Aerotaxis in *Desulfovibrio*. *Environ. Microbiol.* 1999:1:489–494.
[58]. Dolla A, Fu R, Brumlik MJ, Voordouw G. Nucleotide sequence of *dcrA*, a *Desulfovibrio vulgaris* Hildenborough chemoreceptor gene and its expression in *Escherichia coli*. *J. Bacteriol.* 1992:174:1726–1733.
[59]. Fu R, Wall JD, Voordouw G. DcrA, a *c*-type hemecontaining methyl accepting protein form *Desulfovibrio vulgaris* Hildenborough, senses the oxygen concentration or the redox potential of the environment. *J. Bacteriol.* 1994:176:344–350.
[60]. Deckers HM, Voordouw G. Membrane topology of the methyl-accepting chemotaxis protein DcrA from *Desulfovibrio vulgaris* Hildenborough. *Ant. van Leeuwen.* 1994:65:7–12.
[61]. Deckers HM, Voordouw G. Identification of a large family of genes for putative chemoreceptor in an ordered library of the *Desulfovibrio vulgaris* Hildenborough genome. *J. Bacteriol.* 1994:176:351–358.
[62]. Fu R, Voordouw G. Targeted gene-replacement mutagenesis of *dcrA,* encoding an oxygen sensor of the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. *Microbiol.* 1997:143:1815-1826.
[63]. Hewitt J, Morris JG. Superoxide dismutase in some obligately anaerobic bacteria. *FEBS Lett.* 1975:50:315-318.
[64]. Hatchikian EC, Henry YA. An iron-containing superoxide dismutase from the strict anaerobe *Desulfovibrio desulfuricans* (Norway 4). *Biochimie.* 1977:59:153–161.
[65]. Dos Santos WG, Pacheco I, Liu MY, Teixeira M, Xavier AV, LeGall J. Purification and characterization of an iron superoxide dismutase and a catalase from the sulfate-reducing bacterium *Desulfovibrio gigas*. *J. Bacteriol.* 2000:182:796–804.
[66]. Lumppio HL, Shenvi NV, Summers AO, Voordouw G, Kurtz DM. Rubrerythrin and rubredoxin oxidoreductase in *Desulfovibrio vulgaris*: a novel oxidative stress protection system. *J. Bacteriol.* 2001:183:101–108.

[67]. Nakanishi T, Inoue H, Kitamura M. Cloning and expression of the superoxide dismutase gene from the obligate anaerobic bacterium *Desulfovibrio vulgaris* (Miyazaki F). *J. Biochem. (Tokyo)* 2003:133:387–393.
[68]. Fournier M, Zhang Y, Wildschut JD, Dolla A, Voordouw JK, Schriemer DC, Voordouw G. Function of oxygen resistance proteins in the anaerobic, sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough. *J. Bacteriol.* 2003:185:71–79.
[69]. ElAntak L, Dolla A, Durand MC, Bianco P, Guerlesquin F. Role of the tetrahemic subunit in *Desulfovibrio vulgaris* Hildenborough formate dehydrogenase. *Biochemistry.* 2005:44:14828–14834.
[70]. Brioukhanov AL, Thauer RK, Netrusov AI. Catalase and superoxide dismutase in the cells of strictly anaerobic microorganisms. *Microbiol.* 2002:71:281-285.
[71]. Kitamura M, Nakanishi T, Kojima S, Kumagai I, Inoue H. Cloning and expression of the catalase gene from the anaerobic bacterium *Desulfovibrio vulgaris* (Miyazaki F). *J. Biochem.* 2001:129:357–364.
[72]. Lombard M, Fontecave M, Touati D, Niviere V. Reaction of the desulfoferrodoxin from *Desulfoarculus barsii* with superoxide anion. *J. Biol. Chem.* 2000:275:115–121.
[73]. Pianzolla M.J, Soubes M, Touati D. Overproduction of the *rbo* gene products form *Desulfovibrio* species suppresses all deleterious effects of lack of superoxide dismutase in *Escherichia coli*. *J. Bacteriol.* 1996:178:6736–6742.
[74]. Liochev SI, Fridovich I. A mechanism for complementation of the *sodA sodB* defect in *Escherichia coli* by overproduction of the *rbo* gene product (desulofoferrodoxin) from *Desulfoarculus baarsii*. *J. Biol. Chem.* 1997:272:25573-22575.
[75]. Tavares P, Ravi N, Moura JJ, LeGall J, Huang YH, Crouse BR, Johnson MK, Huynh BH, Moura I. Spectroscopic properties of desulfoferrodoxin from *Desulfovibrio desulfuricans* (ATCC 27774). *J. Biol. Chem.* 1994:269:10504–10510.
[76]. Coelho AV, Matias P, Fülöp V, Thompson A, Gonzales A, Coronado MA. Desulfoferrodoxin structure determined by MAD phasing and refinement to 1.9 Å reveals a unique combination of a tetrahedral FeS_4 centre with a square pyramidal $FeSN_4$ centre. *J. Biol. Inorg. Chem.* 1997:2:680–689.
[77]. Lombard M, Houee-Levin C, Touati D, Fontecave M, Niviere V. Superoxide reductase from *Desulfoarculus baarsii*: reaction mechanism and role of glutamate 47 and lysine 48 in catalysis. *Biochemistry.* 2001:40:5032–5040.
[78]. Emerson JP, Coulter ED, Cabelli DE, Phillips RS, Kurtz Jr DM. Kinetics and mechanism of superoxide reduction by two-iron superoxide reductase from *Desulfovibrio vulgaris*. *Biochemistry.* 2002:41:4348–4357.
[79]. Mathe C, Mattioli TA, Horner O, Lombard M, Latour JM, Fontecave M, Niviere V. Identification of iron(III) peroxo species in the active site of the superoxide reductase SOR from *Desulfoarculus baarsii*. *J. Am. Chem. Soc.* 2002:124:4966–4967.
[80]. Silva G, Oliveira S, LeGall J, Xavier AV, Teixeira M, Roudigues-Pousada C. Molecular characterization of *Desulfovibrio gigas* neelaredoxin, a protein involved in oxygen detoxification in anaerobes. *J. Bacteriol.* 2001:183:4413–4420.
[81]. Abreu IA, Saraiva LM, Carita J, Huber H, Stetter KO, Cabelli D, Teixeira M. Oxygen detoxification in the strict anaerobic archaeon *Archaeoglobus fulgidus*: superoxide scavenging by neelaredoxin. *Mol. Microbiol.* 2000:38:322–334.
[82]. Abreu IA, Xavier AV, LeGall J, Cabelli DE, Teixeira M. Superoxide scavenging by neelaredoxin: dismutation and reduction activities in anaerobes. *J. Biol. Inorg. Chem.* 2002:7:668-674.
[83]. Romao CV, Liu MY, LeGall J, Gomes CM, Braga V, Pacheco I, Xavier AV, Teixeira M. The superoxide dismutase activity of desulfoferrodoxin from *Desulfovibrio desulfuricans* ATCC 27774. *Eur. J. Biochem.* 1999:261:438-443.
[84]. Emerson JP, Coulter ED, Phillips RS, Kurtz Jr DM. Kinetic of the superoxide reductase catalytic cycle. *J. Biol. Chem.* 2003:278:39662-39668.
[85]. Rodrigues JV, Abreu IA, Cabelli D, Teixeira M. Superoxide reduction mechanism of *Archaeoglobus fulgidus* one-iron superoxide reductase. *Biochemistry.* 2006:45:9266-9278.
[86]. Huang VW, Emerson JP, Kurtz Jr DM. Reaction of *Desulfovibrio vulgaris* two-iron superoxide reductase with superoxide: insights from stopped-flow spectrophotometry. *Biochemistry.* 2007:46:11342-11351.
[87]. Niviere V, Asso M, Weill CO, Lombard M, Guigliarelli B, Favaudon V, Houee-Levin C. Superoxide reductase from *Desulfoarculus baarsii*: identification of protonation steps in the enzymatic mechanism. *Biochemistry.* 2004:43:808-818.
[88]. Mathe C, Niviere V, Mattioli TA. Fe^{3+}-Hydroxide ligation in the superoxide reductase from *Desulfoarculus baarsii* in asociated with pH dependent spectal changes. *J. Am. Chem. Soc.* 2005:127:16436-16441.
[89]. Coulter ED, Kurtz Jr DM. A role for rubredoxin in oxidative stress protection in *Desulfovibrio vulgaris*: catalytic electron transfer to rubrerythrin and two-iron superoxide reductase. *Arch. Biochem. Biophys.* 2001:394:76–86.
[90]. Rodrigues JV, Abreu IA, Saraiva LM, Teixeira M. Rubredoxin acts as an electron donor for neelaredoxin in *Archaeoglobus fulgidus*. *Biochem. Biophys. Res. Commun.* 2005:329:1300–1305.
[91]. Pierik AJ, Wolbert RB, Portier GL, Verhagen MF, Hagen WR. Nigerythrin and rubrerythrin from *Desulfovibrio vulgaris* each contain two mononuclear iron centers and two dinuclear iron clusters. *Eur. J. Biochem.* 1993:212:237–245.
[92]. Coulter ED, Shenvi NV, Kurtz Jr DM. NADH peroxidase activity of rubrerythrin. *Biochem. Biophys. Res. Com.* 1999:255:317–323.
[93]. Gupta N, Bonomi F, Kurtz Jr DM, Ravi N, Wang DL, Huynh BH. Recombinant *Desulfovibrio vulgaris* rubrerythrin. Isolation and characterization of diiron site. *Biochemistry.* 1995:34:3310-3318.
[94]. deMare F, Kurtz Jr DM, Nordlund P. The structure of *Desulfovibrio vulgaris* rubrerythrin reveals a unique combination of rubredoxin-like FeS_4 and ferritin-like diiron domains. *Nat. Struct. Biol.* 1996:3:539–546.
[95]. Iyer RB, Silaghi-Dumitrescu R, Kurtz Jr DM, Lanzilotta WN. High-resolution crystal structures of *Desulfovibrio vulgaris* (Hildenborough) nigerythrin: facile, redox-dependent iron movement, domain interface variability, and peroxidase activity in the rubrerythrins. *J. Biol. Inorg. Chem.* 2005:10:407–416.
[96]. Lumppio HL, Shenvi NV, Garg RP, Summers AO, Kurtz Jr DM. A rubrerythrin operon and nigerythrin gene in *Desulfovibrio vulgaris* Hildenborough. *J. Bacteriol.* 1997:179:4607–4615.
[97]. Kurtz Jr DM. Avoiding high-valent intermediates: superoxide reductase and rubrerythrin. *J. Biol. Inorg. Chem.* 2006:100:670-693.