

## HOW DO ANAEROBIC SULFATE-REDUCING BACTERIA COPE WITH OXIDATIVE STRESSES?

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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 (sea waters, cyanobacterial mats, activated sludge, shallow water sediments, wastewater biofilms, etc.). SRB represent a diverse group of prokaryotes, which gain energy by coupling the oxidation of great variety of low-molecular mass organic compounds or molecular hydrogen to reduction of sulfate ( $\text{SO}_4^{2-}$ ) to sulfide.

In the case of anaerobic microorganisms, 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 ( $\text{O}_2^{\cdot-}$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and hydroxyl radical ( $\text{HO}^{\cdot}$ ). The main targets of ROS are nucleic acids, lipids and proteins. Secondly, the high redox potential induced by the presence of  $\text{O}_2$  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 oxygen is able to directly inactivate key enzymes of sulfate reduction metabolism, for instance, hydrogenases and lactate dehydrogenase.

SRB have thus developed complicated, highly effective and tightly regulated systems of behavioral and enzymatic mechanisms of antioxidative defense which are responsible for a relative aerotolerance of the majority of SRB. Behavioral responses to oxygen include cell aggregation, symbiotic relationships with aerobic microorganisms, migration to anoxic zones and aerotaxis. Enzymatic mechanisms often involve  $\text{O}_2$  elimination that uses cytoplasmic, periplasmic and membrane-bound oxygen reduction chains containing rubredoxin : oxygen oxidoreductase, cytochrome *c* oxidase and quinol *bd* oxidase. So many SRB not only survive oxygen exposure for at least several days, but some of them even reduce  $\text{O}_2$  to  $\text{H}_2\text{O}$ . In addition to classical enzymes of ROS scavenging (superoxide dismutase, heme monofunctional catalase, peroxidases) which are usual for aerobic microorganisms and eukaryotes, SRB, and specially *Desulfovibrio* species, possess also unique alternative nonheme iron proteins with superoxide reductase (desulfoferrodoxin,

neelaredoxin) and NADH-dependent peroxidase (rubrerythrin, nigerythrin) enzymatic activities. The main advantage of these alternative systems is the lack of production of oxygen during the catalytic cycles.

Comparison of the sensitivity of  $\Delta sor$  and  $\Delta sod$  mutants of *Desulfovibrio vulgaris* Hildenborough to various oxidative stresses indicates that under fully aerated conditions, cytoplasmic superoxide reductase (SOR) is the key oxygen defense enzyme. Superoxide dismutase (SOD) is involved in the removal of  $O_2^{\cdot -}$  in the periplasm under microaerophilic conditions to protect oxygen-sensitive enzymes. SOR and SOD are thus complementary components of an efficient superoxide-scavenging cellular system.

Little data has been accumulated on the regulation of oxidative stress mechanisms in strict anaerobic microorganisms; nevertheless some of them possess enzymes, which are induced under unfavorable oxic conditions. The global expression levels of oxidative stress response genes in *D. vulgaris* Hildenborough differ depending on ROS nature, concentration and exposure time. Coordinated up-regulation of the genes belonged to predicted peroxide stress response regulon (PerR) was observed in response to low  $H_2O_2$  levels (0.1 mM). In contrast, stronger  $H_2O_2$  stress (0.3 mM) was highly detrimental to the cell viability and caused dramatic changes at the transcriptome level. Transcripts analyses revealed that key genes of antioxidative defense encoding a superoxide dismutase (*sodB*), a superoxide reductase (*dfx*), two rubrerythrins (*rbr1* & *rbr2*), a nigerythrin (*ngr*), a thiol peroxidase (*tpx*) and an alkyl hydroperoxide reductase (*ahpC*), in addition to the PerR regulon, belong to the  $H_2O_2$  stimulon. A global transcriptomic analysis pointed out that  $H_2O_2$  as well as redox potential shift increased the expression of the genes of *D. vulgaris* involved in ROS detoxification, thioredoxin-dependent reduction system and DNA repair, and decreased those involved in sulfate reduction, lactate oxidation and protein synthesis.

#### References:

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