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OXIDATIVE STRESS AND PROTECTION AGAINST IT IN BACTERIA

Olha Maslovska, Solomiia Komplikevych, Svitlana Hnatush
Ivan Franko National University of Lviv, 4 Hrushevsky St., Lviv 79005, Ukraine

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Microorganisms are exposed to reactive oxygen species (ROS) that are formed in various ways, in particular, as a result of respiration or other intracellular processes, during metal-catalyzed Fenton reactions, as a result of the action of UV- and X-radiation, under the influence of some antimicrobial drugs, or during the host immune oxidative-burst response against infection agents. In this review, we take a look at the mechanisms of microbial cell damage, including damage of lipids and proteins. Lipid peroxidation (LPO) is one of the main molecular mechanisms involved in oxidative damage to cellular structures. A variety of products are formed during LPO reactions: alkoxyl radicals, peroxyl radicals, hydroperoxides, diene conjugates, carbonyl compounds, aldehyde adducts with biopolymers, alcohols, esters, etc. These products include cytotoxic and highly reactive compounds. Free radical reactions of protein damage occur via hydrogen atom abstraction from α-carbon or SH-, NH_2-groups of aminoacids and electron abstraction from nucleophile centers of proteins resulting in the fragmentation of proteins, their denaturation and the formation of amino acid radicals. Bacteria show a significant adaptive potential to the influence of stress agents, including ROS. We summarized the data on bacterial antioxidant protection, ROS redox sensors, and regulators of bacterial cell response to ROS exposure, focusing on the features of anaerobic microorganisms, as their responses to the oxidative damage are the least studied, and many problems remain unsolved. This review contains information about changes in fatty acid composition of lipids of the plasma membrane to maintain the necessary fluidity, and, thus, counteract the effects of various stressing agents, including ROS. The main modifications of the fatty acid composition of lipids important for the regulation of membrane fluidity are described, in particular, via changes in the degree of lipid saturation, *cis*/*trans* isomerization, and synthesis of cyclopropane fatty acids.

Keywords: bacteria, oxidative stress, reactive oxygen species, antioxidant protection, fatty acid composition of lipids, lipid damage, protein damage

INTRODUCTION

In order to survive, bacteria effectively resist a variety of stressing agents, and oxidative stress is one of those that is widespread and difficult to adapt to (Imlay, 2015). The reactive oxygen species (ROS), in particular, superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($HO^{\bullet}$), demonstrated different reactivity and under certain conditions cause oxidative stress (Seixas *et al.*, 2022). Aerobic bacteria use O_2 as the final electron acceptor for aerobic respiration, during which ROS are formed endogenously, as a result of functioning of respiratory flavoproteins of the respiratory chain, or due to other processes in which non-respiratory flavoproteins are involved (Imlay, 2019, Fasnacht & Polacek, 2021). One of the reasons for ROS formation is a long-term action of transition metals, in particular, iron, copper, chromium, cobalt, and others, which even in small amounts catalyze Fenton reactions, which leads to the formation of $OH^{\cdot}$ (Repetto *et al.*, 2012). In addition to ROS, bacterial cells are affected by a wide range of reactive chlorine and nitrogen species, which act as powerful oxidants and are formed during a host's immune response against pathogenic microorganisms (Imlay, 2015, Ezraty, 2017). Microorganisms are also a subject of oxidative damage in the process of wastewater treatment (Li *et al.*, 2022) because they contain many compounds that are precursors or catalysts for the reactions taking place at the formation of free radicals. Biotechnologies of wastewater treatment based on anaerobic microorganisms have certain advantages compared to traditional aerobic processes of wastewater treatment (Hnatush *et al.*, 2020; Malovanyy *et al.*, 2022; Li *et al.*, 2022; Malovanyy *et al.*, 2023). However, the molecular mechanisms of influence of free radical compounds and the physiological consequences of this action on the cells of anaerobic microorganisms are not fully clarified. On the contrary, the bactericidal effects of some drugs, in particular some antibiotics, is based on the destruction of microbial cells by ROS. Thus, managing antimicrobial resistance is one of the most important medical challenges of our time (Hong *et al.*, 2019). The number of publications on studies of oxidative stress in bacteria has increased since 2000. In 2000, there were 150 publications on oxidative stress in bacteria in the Scopus database, whereas in 2022, the number of such publications reached 1874.

ROS possess the ability to attack organic molecules. Lipids undergo peroxidation (LPO) to form a range of cytotoxic compounds, protein molecules – oxidative modifications, which cause changes in their structure, aggregation, and fragmentation, nucleic acids (DNA and all types of RNA) – oxidation of nitrogen bases, ribose, or deoxyribose, leading to mutations, chain breaks and others. As a result of oxidation, the structure and functions of these macromolecules change, and potentially toxic compounds are formed, which can eventually lead to the death of microorganisms as well as cells in mammalian tissues and organs of multicellular organisms (Bhattacharjee, 2014; Fasnacht & Polacek, 2021).

Microorganisms have developed two strategies to protect themselves from oxidants: the first is to eliminate free radical compounds which is realized by ROS scavenging enzymes, such as superoxide dismutases (SODs) and catalases/peroxidases and non-enzymatic antioxidants (Imlay, 2019). The second one ensures the repair of damaged macromolecules after oxidation (Aussel & Ezraty, 2021). The activity of these enzymes and their regulation has been well-studied in *Escherichia coli*, *Salmonella typhimurium*, and *Bacillus subtilis* (Imlay, 2019). For a long time, it was believed that anaerobic metabolism does not produce ROS. However, many obligate anaerobes, such as *Clostridium acetobutylicum*, *Clostridium perfringens*, and *Chlorobaculum tepidum*, contain enzymes that scavenge ROS and oxidation damage repair systems (Sheng *et al.*, 2014; Fu *et al.*, 2015; Imlay, 2019).

A common mechanism in microorganisms to counteract the influence of various stressors is to change the fatty acids (FAs) composition in cellular lipids in order to maintain an appropriate level of fluidity of plasma membrane (Chwastek *et al.*, 2020). In response to stress agents, the structure of lipids in bacterial cells is modified via changes in the content of unsaturated FAs (UFAs), *cis*/*trans* isomerization of double bonds, the length of FAs chain, synthesis of branched/unbranched FA and cyclopropane FA (CFA). It was suggested that oxidative damage of cellular macromolecules also results in modifications of the fatty acid composition of cellular lipids.

In this review, we describe the damage that occurs in bacterial cells exposed to ROS or compounds that cause ROS formation, and the main mechanisms of protection against these radicals. We focused at the antioxidant protection of facultative anaerobic bacteria and modifications of FA composition of lipids as one of the mechanisms to counteract the influence of ROS.

DAMAGES THAT OCCUR IN BACTERIAL CELLS UNDER THE ACTION OF REACTIVE OXYGEN SPECIES

Lipid peroxidation as a result of the effect of free radicals. LPO is a process that includes the formation and propagation of lipid radicals, oxygen absorption, and rearrangement of double bonds in UFA, which causes a destruction of membrane lipids and formation of various degradation products (Repetto *et al.*, 2012). LPO occurs during enzymatic oxidation, photooxidation, autooxidation, or free radical oxidation. Lipoxygenases that catalyze the removal of hydrogen and the addition of molecular oxygen to double bonds in molecules of polyunsaturated fatty acids (PUFAs), formation of hydroperoxides, which are quickly reduced to alcohols or other lipid intermediates, are involved in the enzymatic LPO (Chrisnasari *et al.*, 2022). Lipoxygenases were detected in *Pseudomonas aeruginosa*, *Shewanella woodyi*, *Streptomyces massasporeus,* and bacteria of *Nitrosomonas* and *Nitrospira* genera (Hansen *et al.*, 2013). Photooxidation of PUFA occurs under the influence of UV radiation or visible light on bacterial cells. Photooxidation of lipids is the basis of photodynamic inactivation of a wide range of pathogenic microorganisms, including *Staphylococcus aureus*, *Streptococcus pyogenes*, *P. aeruginosa*, and *Acinetobacter baumannii* (Rontani, 2012).

In the process of non-enzymatical free radical-induced LPO, a significant number of primary, secondary, and advanced products are generated (**Fig. 1**). PUFAs are more susceptible to oxidation (Repetto *et al.*, 2012), and the transition metals are involved in the propagation of the chain reaction (Repetto *et al.*, 2012; Lahir, 2015). The intensity of LPO processes in purple photosynthetic bacteria *Rhodopseudomonas yavorovii* cells under the influence of ferric citrate and cobalt (II) chloride (Hnatush *et al.*, 2022a), in green photosynthetic bacteria *Chlorobium limicola* under the influence of copper (II) sulfate (Sehin *et al.*, 2020), in sulfur-reducing bacteria *Desulfuromonas acetoxidans* under the influence of ferric citrate (Hnatush *et al.*, 2022b) increase significantly. Fe^{2+} and Cu^{2+} stimulate LPO by a single-electron reduction of lipid LOOH to the corresponding alkoxyl ($LO^{\bullet}$) and peroxyl ($LOO^{\bullet}$) radicals that initiate new radical reactions (Repetto *et al.*, 2012; Lahir, 2015). $LO^{\bullet}$ can directly remove hydrogen from other lipids and initiate new chains of LPO, undergo β-cleavage with the formation of aldehydes and alkyl radicals, attach oxygen with the formation of epoxyallyl peroxyl radicals ($OLOO^{\bullet}$), which initiate new chain reactions of lipids more efficiently than the $LO^{\bullet}$ (Guéraud *et al.*, 2010).

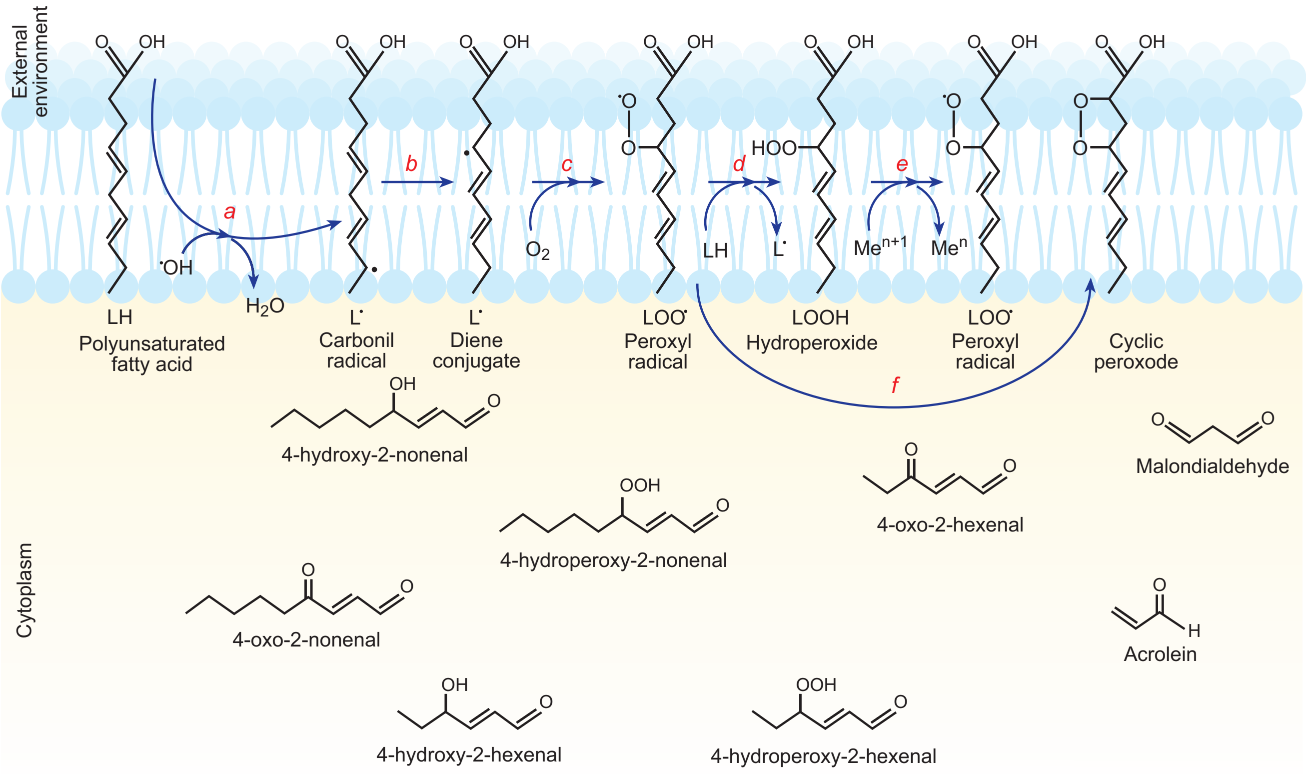

Fig. 1. Oxidation of polyunsaturated fatty acids. Oxidation of PUFA occurs by abstraction of hydrogen by OH$^{\cdot}$, FA radical, or another free radical froms a methylene group and a carbon-centered radical (L$^{\cdot}$) is formed (reaction *a*). L$^{\cdot}$ is located inside the FA molecule and stabilized due to the rearrangement of double bonds and the formation of diene conjugates (reactions *b*). The formed L$^{\cdot}$ reacts with O_2, turning into a peroxyl radical (LOO$^{\cdot}$) (reaction *c*). The LOO$^{\cdot}$ is reduced to lipid hydroperoxide (LOOH) as a result of abstraction of hydrogen from another FA molecule (reaction *d*). In the last reaction, a new radical L$^{\cdot}$ is formed which enters the next reaction and causes a chain reaction that can continue even without initiating substances. Also, LOO$^{\cdot}$ can be oxidized with the formation of strongly oxidized products – dicyclic endoperoxides, monocyclic peroxides, and other cyclic peroxides are formed (reaction *e, f*). Most of these products are unstable and quickly turn into LPO end products. Free radical chain reaction ends when the formed radicals L$^{\cdot}$ are conjugated with other FA radicals, transition metals, or antioxidant molecules (α-tocopherol, reduced glutathione, etc.) (modified from Semchyshyn & Lushchak, 2012)

As a result of breaking of the C–C bond in the RO$^{\bullet}$ molecule, secondary LPO products are formed in cells – short-chain compounds (from C_2 to C_{12} with a wide range of functionality) and lipids with a modified carbon chain. Among the decomposition products of LOOH or LO$^{\bullet}$, various classes of compounds were identified: aldehydes, alkenes, ketones, alkadienals, alcohols, and furans. Many of these compounds do not show a strong toxic effect on the cell. Among them aldehydes are cytotoxic and highly reactive, and, unlike free radicals, are stable compounds capable of diffusing over long distances and covalently modifying proteins and nucleic acids (Gueraud *et al.*, 2010). Among the numerous aldehydes that are formed in the LPO process, the best studied are malondialdehyde and 4-hydroxyalkenals, in particular, 4-hydroxy-2-nonenal (Ayala *et al.*, 2014).

The physiological level of the intensity of OMP and LPO in cells is controlled by the enzymes of the antioxidant defense system. With a significant increase in the intensity of LPO, the degree of oxidative damage to macromolecules exceeds the cell's ability to repair action, which leads to its death (Ayala *et al.*, 2014).

Oxidative modification of proteins. Free amino acids or amino acid residues (ARs) in proteins are very sensitive to oxidation by ROS (**Fig. 2**).

The formed alkyl, peroxyl, and alkoxy radical products can react with other ARs of the same or other protein molecules, which leads to the formation of new carbonyl radicals (Stadtman & Levine, 2003). Under anaerobic conditions, the alkyl peroxide radical is not formed, but the interaction of two carbonyl radicals occurs with the formation of covalent bonds, which causes the accumulation of dimers, trimers, tetramers, and oligomers of polypeptides with a high molecular weight. Carbonyl radicals are capable of cleaving hydrogen from other molecules. This reaction occurs at a low O_2 concentration or when steric factors prevent the dimerization of carbonyl radicals (Stadtman & Levine, 2003, Hawkins & Davies, 2019).

During ROS-mediated oxidation of proteins, side groups of ARs are damaged. During free radical oxidation, methionine residues are transformed into methionine sulfoxide (Ezraty *et al.*, 2017), which is reduced by methionine sulfoxide reductases (Msr) MsrA and MsrB, located intracellulary in most bacteria. Extracytoplasmic MsrAB were found in some bacteria of *Neisseria* and *Streptococcus* genera. Repair of methionine sulfoxide in the periplasmic space of Gram-negative bacteria is provided by the MsrPQ system: the periplasmic MsrP functions in tandem with MsrQ – cytochrome *b* that acts as both a membrane anchor and a redox partner for MsrP (Ezraty *et al.*, 2017; Aussel & Ezraty, 2021; Loiseau *et al.*, 2022). Bacteria *E. coli*, *Xanthomonas campestris*, *P. aeruginosa*, *Streptococcus pneumoniae* (Singh *et al.*, 2018), and *Corynebacterium glutamicum* (Si *et al.*, 2015) with inactivated Msr genes are more sensitive to the influence of oxidative stress inducers than the wild type of these bacteria.

The sulfhydryl group of cysteine is one of the targets for ROS and active electrophilic metabolites (quinones, aldehydes, epoxides, diamides, and α,β-unsaturated dicarbonyl compounds that contain an electron-deficient center) attacks and a substrate for reversible and irreversible post-translational modifications. Under the influence of ROS, the thiol group can be reversibly oxidized and form disulfide bonds with another oxidized thiol group, be reversibly oxidized to sulfinic and sulfonic acids, or undergo S-alkylation under the influence of active electrophilic metabolites (Hillion & Antelmann, 2015; Ezraty *et al.*, 2017; Garrido Ruiz *et al.*, 2022). The conversion of sulfhydryl groups

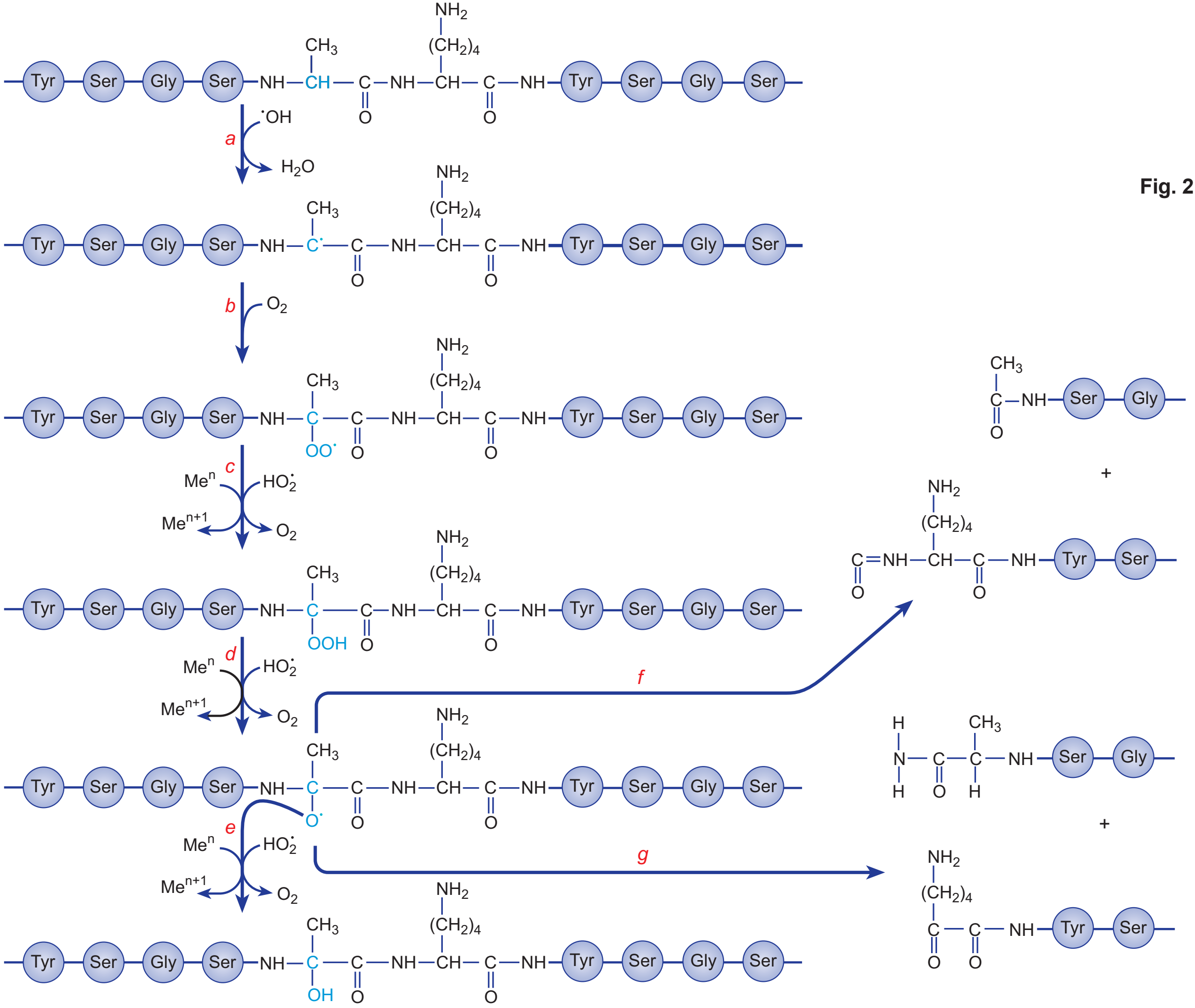

Fig. 2. Oxidative modification of proteins. Oxidation of the polypeptide skeleton of a protein molecule begins with the $OH^{\bullet}$-dependent abstraction of hydrogen from the α-carbon of amino acids or the protein polypeptide backbone (reaction *a*), which leads to the formation of a carbon-centered radical. In the presence of oxygen (reaction *b*), this radical quickly turns into a peroxyl radical that interacts with the protonated form of $O_2^{\bullet-}$ – hydroperoxyl radical ($HO_2^{\bullet}$) or splits off hydrogen from another molecule, as a result of which it turns into a protein hydroperoxide (reaction *c*). Subsequent reactions of the protein hydroperoxide with $HO_2^{\bullet}$ lead to the formation of an alkoxyl radical, which turns into a hydroxyl-derived compound (reactions *d, e*). The formation of an alkoxyl radical can cause a polypeptide chain to break in diamide (reactions *f*) or α-amide pathways (reactions *g*) (modified from Stadtman & Levine, 2003)

into oxidized or alkylated forms occurs post-translationally and plays a key role in functioning of enzymes and redox sensors of bacteria. A well-studied representative of thiol-mediated redox sensors in bacterial cells is OxyR protein which is conserved in bacteria and has been studied in detail in *Salmonella typhimurium* and *E. coli* (Chiang & Schellhorn, 2012; Hillion & Antelmann, 2015). Bacterial thiol-mediated redox sensors also include PerR protein (peroxide sensor in *B. subtilis*), NemR (redox sensor for active electrophilic metabolites, in particular, quinones and aldehydes, and HOCl), SpxA (an unusual transcription factor without typical DNA binding domains that respond to various stress conditions), and others (Hillion & Antelmann, 2015). Various oxidoreductases function in bacterial cells to repair oxidized forms of cysteine, in particular thioredoxins and glutaredoxins (Ezraty *et al.*, 2017).

Aromatic ARs are targets for oxidation by ROS. Residues of tryptophan are oxidized to formylkynurenine and kynurenic acid and various hydroxyl derivatives. Oxidation of phenylalanine and tyrosine residues leads to the formation of hydroxylated derivatives of these ARs, the tyrosyl radical, and the formation of dityrosine linkages. As a result of interaction of histidine with ROS, for example, with $HO^{\bullet}$, 2-oxohistidine, asparagine, and aspartic acid are formed (Hawkins & Davies, 2019).

Oxidation of side groups of asparagine, glutamine, and proline by ROS can cause breaking of peptide bonds in a protein molecule. $OH^{\bullet}$-mediated hydrogen splitting from the γ-carbon atom of glutamine and asparagine residues leads to the breaking of the peptide bond. Under the influence of ROS, proline residues are oxidized to 2-pyrrolidone, which turns into 4-aminobutyl acid, which causes fragmentation of a protein molecule (Stadtman & Levine, 2003, Hawkins & Davies, 2019).

Protein carbonylation is considered as the intramolecular addition of a carbonyl group to side chains of amino acids, mainly to arginine, threonine, proline, and lysine. However, the formation of an additional carbonyl group can also occur during some oxidative reactions, in particular, in the process of metal-catalyzed oxidation of proteins (Matallana-Surget *et al.*, 2013). Carbonyl groups (CG) in proteins are formed during direct interaction with ROS, oxidative cleavage of proteins in the α-amide pathway, or oxidation of side groups, which causes the formation of a peptide in which N-terminal amino acid contains an α-ketoacyl group (Dalle-Donne *et al.*, 2003).

Another way of formation of CG in proteins is the interaction of nucleophilic side groups of cysteine, histidine, and lysine with aldehydes, which are formed in the process of LPO or interaction with reactive carbonyl compounds (methylglyoxal, glucosone, deoxyglucosone), which are formed during the reduction or oxidation of carbohydrates (glycation and glycooxidation processes) (Semchyshyn & Lushchak, 2012). It is also known that the formation of CG in proteins is a result of direct metal-catalyzed oxidation of proline, arginine, lysine, and less often threonine (Borysiuk *et al.*, 2022). The formation of CG in proteins of *R. yavorovii* under the influence of ferric citrate and cobalt (II) chloride and *D. acetoxidans* under the influence of ferric citrate was revealed (Hnatush *et al.*, 2022a; Hnatush *et al.*, 2022b). In the presence of additional CG, the hydrophobicity and surface charge of the protein molecule change, which affects its structure. Such oxidized ARs are chemically stable, therefore CGs are often used as indicators of the oxidative stress in bacterial cells. To investigate the origin of CG in proteins, additional methods of analysis have been developed, for example, the adducts of LPO products and amino acids can be identified using specific antibodies (Dalle-Donne *et al.*, 2003).

CGs are formed in the molecules of alcohol dehydrogenase, elongation factor G, heat shock proteins, enolase, outer membrane protein Omp A, β-subunit of F_0F_1-ATPase, glutamine synthetase, glutamate synthase, aconitase, malate dehydrogenase and pyruvate kinase of *E. coli* (Nystrom, 2005). Under the influence of UV radiation, CGs were formed in the enzymes of glycolysis and the citric acid cycle of *Photobacterium angustum*. These enzymes are sensitive to oxidation, as they are localized close to the sites of ROS formation. The formation of CGs in *P. angustum* was shown in molecules of DNA gyrase, DNA polymerase, chaperones GroEL, DnaK, and proteins involved in transcription and translation. However, they were not detected in molecules of the enzymes involved in DNA repair, in particular, RecA. It has been suggested that the carbonylation of key enzymes functions as a trigger that induces protein turnover, since the CG is one of the tags for the degradation of proteins that are no longer needed for the functioning of a cell or are damaged, and the amino acids are released after cleavage are involved in protein synthesis *de novo* (Nystrom, 2005; Matallana-Surget *et al.*, 2013). Probably, protein carbonylation in bacterial cells performs the role of eukaryotic ubiquitination for further degradation in proteasomes. It was established that carbonylated proteins from *E. coli* cells are recognized by eukaryotic proteasomes *in vitro* (Shringarpure *et al.*, 2003). So, carbonylation of proteins is not only a consequence of oxidative stress but also functions as a signal to activate protein metabolism to maintain the required level of functional proteins (Matallana-Surget *et al.*, 2013).

The heat shock regulon plays an important role in protecting the cell from the accumulation of significant amounts of carbonylated proteins. In *E. coli*, the homolog of the eukaryotic proteasome 20S – HslV (heat shock locus *V*) and the chaperone system DnaK/DnaJ and GroEL/GroES are involved in the neutralization of oxidatively modified proteins (OMP) (Fredriksson *et al.*, 2005; Kim *et al.*, 2021).

ANTIOXIDANT PROTECTION AND CHANGES IN THE FATTY ACID COMPOSITION OF PHOSPHOLIPIDS AS AN ADAPTATION OF FACULTATIVE ANAEROBIC BACTERIA TO STRESS

Antioxidant protection. To reduce the influense of ROS, the genes whose products ensure the prevention of generation or interruption of free radical chain reactions, repair, and degradation of damaged macromolecules are expressed in the bacterial cells (Fu *et al.*, 2015). Protection of bacteria from the effects of ROS is provided by low-molecular antioxidants and antioxidant defense enzymes (SODs, catalases, glutathione peroxidases, alkyl hydroperoxidases, cytochrome *c* peroxidase, etc.) (Lemire *et al.*, 2017). Maintaining of intracellular redox status in the cell and neutralizing ROS under the oxidative stress is carried out by the glutathione redox system, which includes glutathione (GSH), glutaredoxin, and glutathione reductase (Ulrich & Jakob, 2019). At physiological pH GSH (L-γ-glutamyl-L-cysteinyl glycine) has two negatively charged carboxyl groups and a positively charged amino group. The presence of a γ-glutamyl bond protects GSH from degradation by intracellular peptidases, and sulfhydryl groups of cysteine give GSH the properties of a reducing agent and the ability to remove free radicals (Masip *et al.*, 2006). GSH is an important antioxidant that directly interacts with ROS and active nitrogen metabolites, resulting in the formation of a thiyl radical ($GS^{\cdot}$), which dimerizes with another $GS^{\cdot}$ radical to form the glutathione disulfide (GSSG).

GSH is also involved in the detoxification of free radical lipid products (malondialdehyde and 4-hydroxy-2-nonenal) and transition metal ions (Hg^{2+}, Ag^{+}, Cd^{2+}, Cu^{2+}). In *C. limicola* cells under the influence of Cu^{2+}, the total glutathione content increased with increasing Cu^{2+} content, however, the ratio between GSH and GSSG sharply decreased under these conditions. We suggested that *C. limicola* synthesize GSH to maintain the appropriate redox potential of the cytoplasm and neutralize ROS, formed as a result of an exposure to Cu^{2+} (Sehin *et al.*, 2020). However, not all microorganisms synthesize GSH to maintain the redox homeostasis of the cell: bacteria of the genera *Mycobacteria*, *Corynebacteria*, and *Streptomyces* synthesize mycothiol, Gram-positive bacteria *Bacillus* spp., *Staphylococcus* spp., and *Streptococcus agalactiae* – bacillithiol (Ulrich & Jakob, 2019).

Glutaredoxins catalyze the restoration of disulfide bonds in proteins using GSH and are involved in the transduction of intracellular regulatory signals and redox regulation of transcription factors, such as OxyR. A significant increase in the expression of the gene encoding glutaredoxin was found in *Geobacter sulfurreducens* and *Geobacter uraniireducens* under the influence of U(VI). It was assumed that the reduction of U(VI) in cells is mediated by GSH with the formation of GSSG and H_2O_2 as byproducts (Orellana *et al.*, 2014). A reduction of GSSG is carried out by glutathione reductase which catalyzes the transfer of reducing equivalents from the NADPH to GSSG (Yan *et al.*, 2013) and, thus, provides the only known mechanism of GSH restoration from its oxidized form that significantly reduces the need in GSH synthesis (Masip *et al.*, 2006).

GSH-dependent reduction of peroxides is provided by the GSH peroxidase and GSH transferase. GSH peroxidase is involved in the detoxification of H_2O_2 and LOOH and, thus, terminates the LPO chain reaction and protects cell membranes. The affinity of GSH peroxidase for H_2O_2 is higher than that of catalase, so, the former functions more effectively at low substrate concentrations, while catalase plays a key role in protecting cells from the oxidative stress caused by high concentrations of H_2O_2 (Fu *et al.*, 2007). GSH transferase catalyzes the conjugation of GSH with the electrophilic groups of xenobiotics and LPO products. GSH transferase restores hydrophobic hydroperoxides with a relatively large molecular mass: PUFA and phospholipid hydroperoxides, mononucleotide and DNA hydroperoxides (Allocati *et al.*, 2009).

SOD and catalase are the main enzymatic components of bacterial cell protection during oxidative defence. SOD is a metal-containing enzyme catalyzing the disproportionation reaction of $O_2^{\bullet-}$ with the formation of O_2 and H_2O_2 (Miller, 2012; Mishra *et al.*, 2020). Fe-SODs genes have been identified in the genomes of many facultative anaerobic bacteria, in particular, *Desulfovibrio*, *Geobacter*, *Desulfuromonas*, etc. (Holmes *et al.*, 2009). In *E. coli,* Mn-SODs and Fe-SODs are cytosolic enzymes, Cu,Zn-SODs are periplasmatic enzymes (Broxton & Culotta, 2016).

The decomposition of H_2O_2, formed as a result of the dismutation of $O_2^{\bullet-}$, into water and oxygen is catalyzed by heme-containing enzyme catalase (Yuan *et al.*, 2021). Catalase of *Desulfovibrio gigas* contains three subunits and has no peroxidase activity. This low-specificity enzyme is very sensitive to H_2O_2 and cyanide and contains one heme in the active center. Catalase with peroxidase activity was isolated from *G. sulfurreducens* grown with ferric citrate, while no activity was detected during the growth with fumarate, which highlights the important role of the enzyme in the protection of bacteria (Dos Santos *et al.*, 2000).

Non-heme proteins, which are components of protection against the toxic influence of O_2 and ROS, function in the cells of obligate anaerobes. Molecular mechanisms of neutralizing these oxidants differ from those involving SODs and catalases. An alternative way of neutralizing ROS in the cells of anaerobic bacteria is the superoxide reductase (SOR) pathway (Mishra *et al.*, 2020). In the process of detoxification of $O_2^{\bullet-}$ ($e^- + O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2$) with the participation of SOR, unlike SOD, oxygen is not formed, which gives a significant advantage to SOR in the cells of strict anaerobes.

Desulfoferredoxin is a non-heme iron-containing protein that catalyzes single-electron reduction of superoxide anion to H_2O_2. An overexpression of the desulforedoxin gene of *D. gigas* in *E. coli* mutants with a deletion of *sod*A and *sod*B genes completely compensated the negative effect of *sod* mutation and restored the wild-type phenotype (Liu *et al.*, 2014). A reduction of the oxidized form of SORs is catalyzed by a reduced rubredoxin, a protein containing one $[Fe(SCys)_4]$ center (Sheng *et al.*, 2014). Probably, during the oxidative stress, rubredoxin diverts electrons from the electron transport chain of bacteria to rubrerythrin and SORs, thereby protecting redox enzymes that are autooxidizing and causing a decrease in the intracellular content of H_2O_2 and $O_2^{\bullet-}$ (Coulter & Kurtz Jr, 2001).

H_2O_2 is neutralized by peroxiredoxin and NADH-dependent peroxidase or the unique non-heme protein of anaerobes rubrerythrin which functions as a terminal component of NADH-dependent peroxidase and catalyzes a reduction of H_2O_2 to H_2O. Enzymes providing detoxification of $O_2^{\bullet-}$ are important for the protection of facultative anaerobic bacteria under the influence of transition metals. An increase in the activity of SOD or SOR enzymes and enzymes with catalase activity was detected in the cells of *C. limicola* under the influence of copper ions (Sehin *et al.*, 2020), *R. yavorovii* under the influence of Fe^{3+} and Co^{2+} (Hnatush *et al.*, 2022a).

According to GenBank data, the genomes of green photosynthetic bacteria do not contain genes encoding catalase, however, they contain genes encoding proteins and enzymes of the thioredoxin system. In *C. tepidum*, thioredoxin is a donor of reducing equivalents for enzymes of reducing citric acid cycle, enzymes involved in sulfur metabolism, glutamate synthase, etc. In addition to redox regulation of metabolism, thioredoxin is involved in the antioxidant protection of anaerobic bacteria (Hosoya-Matsuda *et al.*, 2009). Taking into account the physiological importance of the thioredoxin system in cells of green photosynthetic bacteria, it is possible that in *C. limicola*, the reduction of peroxides formed due to an exposure to Cu^{2+} is provided by thioredoxin.

The response to oxidative stress includes the activation of specialized redox-sensitive regulators, up-regulation of the expression of genes encoding enzymes and non-enzymatic defence factors, and repair systems that are specific to certain ROS species and provide a precise regulation and specificity of protection (Fu *et al.*, 2015).

The regulation of metabolism with the participation of sigma factors was studied in sulfur-reducing bacteria of the *Geobacter* genus (Aklujkar *et al.*, 2010). The sigma factor RpoS is a regulator of the general response of a bacterial cell to various stresses (osmotic, thermal, oxidative stress, and starvation), which are often observed under natural conditions (Aklujkar *et al.*, 2010; Battesti *et al.*, 2015). In addition to RpoS (σ^{38}), the sigma factors RpoD (σ^{70}), RpoH (σ^{32}), RpoF (σ^{28}), and RpoN (σ^{54}), which are involved in the transcription of “housekeeping” genes, flagellum protein biosynthesis, heat shock proteins, others were found in *G. sulfurreducens* (Aklujkar *et al.*, 2010; Battesti *et al.*, 2015). Also, in *G. sulfurreducens,* the sigma factor RpoE (σ^{24}) was detected, which is involved in the cell’s response to oxidative stress. The RpoS regulon in *G. sulfurreducens* cells is a global regulator of gene expression (DiDonato *et al.*, 2006). In *G. sulfurreducens* under the oxidative stress, RpoS functions as a positive transcriptional regulator of genes encoding for ferredoxin, rubredoxin, desulfuredoxin, SOD, and putative rubredoxin-oxygen-oxidoreductase (DiDonato *et al.*, 2006, Battesti *et al.*, 2015).

Some facultative anaerobic bacteria do not contain homologous regulators OxyR and SoxR/SoxS of *E. coli* which are involved in the response of cells to oxidative stress caused by H_2O_2 or $O_2^{\bullet-}$. In *D. vulgaris*, *Desulfovibrio desulfuricans*, *G. metallireducens*, and *Desulfuromonas* sp., homologs of the peroxide sensor, namely, PerR of *B. subtilis* were identified (Holmes et al., 2009). The H_2O_2 sensor PerR protein belongs to Fur superfamily of transcriptional regulators (Dubbs & Mongkolsuk, 2012). The PerR of *D. vulgaris* regulate an operon with genes *rbr1* and *rdl*, which encode the rubreritin 1 and rubredoxin-like protein, as well as genes *ahpC* (encodes alkyl hydroperoxidase), *rbr2* (encodes rubreritin 2) (Antelmann & Helmann, 2011).

Changes in the fatty acid composition of phospholipids for maintaining an appropriate level of membrane fluidity. According to modern fluid-mosaic model of Singer and Nicolson, biological membranes are built of a lipid bilayer in which protein molecules are immersed (Nicolson, 2014). Fluidity is a property of the membrane that involves a combination of different types of mobility of membrane components and is determined by the ratio between PUFA and saturated fatty acid (SFA) in the composition of membrane lipids and the constant mobility of FA tails. Fluidity of the cytoplasmic membrane (CPM) is the most important parameter that determines cell survival under stressful conditions (Murinova & Dercová, 2014).

Protective procecces that occur in membrane lipids and FAs are called the „homeoviscous adaptation". Cells control membrane fluidity via changing the FA composition of lipids to compensate for the environmental factors' influences, for example, changes in temperature or exposure to toxic, membrane-active compounds. However, in most cases, microorganisms are unable to fully compensate for the changes in membrane fluidity caused by external factors (Eberlein *et al.*, 2018; Chwastek *et al.*, 2020).

In plasma membranes, lipids are located at physically separated microdomains possessing different biochemical properties. Therefore, it was proposed to expand the term "homeoviscous adaptation" (membrane fluidity maintenance) and use the term "homeophasic adaptation" (membrane fluidity regulation), which reflects the maintenance of the lamellar phase.

It was established that the main role in the homeophasic adaptation of bacteria belongs to the FA part of lipids, changes in the structure of which are influenced by the phase state of lipids. Adequate membrane fluidity of bacterial membranes is maintained by a change in the degree of lipid unsaturation, shortening or lengthening of the FA chain, changes in the content of branched or CFAs, and *cis/trans* isomerization of the double bond in FA molecule (Duldhardt *et al.*, 2010; Murinova & Dercová, 2014; Eberlein *et sl.*, 2018; Chwastek *et al.*, 2020).

Changes in the degree of lipid saturation under the influence of toxic compounds refer to the main mechanisms of adaptation of bacteria to stress. An increase in the saturation of membrane phospholipids and, accordingly, an increase in the rigidity of the membrane in cells of sulfur-reducing bacteria *G. sulfurreducens* was shown under the influence of toxic organic compounds (2,4-dichlorophenol, benzene, toluene, xylene), and in anaerobic nitrate-reducing bacteria *Thauera aromatica* under the influence of ethylbenzene (Duldhardt *et al.*, 2010).

Aromatic organic solvents can accumulate in the membrane bilayer between FAs chains, which causes an excessive increase in membrane fluidity. To counteract this effect of organic solvents, the acyl chains of FAs in phospholipids must be packed more tightly to increase the rigidity of the membrane, as a result of which the permeability of aromatic compounds decreases (Duldhardt *et al.*, 2010; Murinova & Dercová, 2014). The reason for the dense packing of SFAs is their high melting temperature (T_M) and the spherical configuration of phospholipids that contain SFAs, in contrast to the conical configuration of phospholipids with UFAs. T_M for long-chain SFAs is very high (for example, for palmitic acid, T_M is 63 °C). This means that palmitic acid is in an ordered state at temperatures below 63 °C. Therefore, with an increase in the content of long-chain SFAs, the level of the orderliness of phospholipids and their denser packing in the membrane increases, which leads to a decrease in CPM fluidity and prevents the penetration of toxic substances into the cell (Murinova & Dercová, 2014; Eberlein *et al.*, 2018).

An increase in the degree of lipid saturation in bacterial CPM occurs under the influence of transition metals. The elevation of this degree was demonstrated in *Klebsiella pneumoniae* and *Enterobacter intermedius* under the influence of cadmium, nickel, zinc, and copper ions. Probably, an increase in the content of SFAs with a decrease in the content of UFA protects lipids from damage at the site of the double bond (Markowicz *et al.*, 2010).

Changes in the degree of lipid saturation play an important role in maintaining the adequate fluidity of CPM under the influence of high or low temperatures. With a sharp rise in temperature of the environment, the fluidity of the CPM increases and it becomes "hyper fluid". Low temperatures cause a decrease in the fluidity of the membrane which leads to a loss of functionality of the membrane. Under the influence of high temperatures, the content of SFA in lipids increases in bacterial cells. An increase in the degree of lipid saturation in the *Desulfovibrio indonesiensis* cells under the influence of high temperatures was reported (Fichtel *et al.*, 2015). Maintenance of CPM fluidity under the influence of low temperatures occurs via increasing in the content of UFA or FAs with similar properties in the lipids. An increase in the content of *cis*-11-octadecenoic acid and a decrease in the content of palmitic acid were shown in *E. coli* due to a decrease in the ambient temperature (Mansilla *et al.*, 2004).

The isomerization of *cis*-isomers into *trans*-isomers of UFA is an alternative mechanism of the membrane fluidity regulation under the influence of toxic compounds (organic solvents, osmotic stress, heavy metal ions, heat shock, and membrane targeting antibiotics). The double bond in the *cis*-configuration forms a chain band with an angle of 30°, which prevents tight packing of phospholipids in the CPM and its low T_M. CPMs that contain *cis*-isomers of UFAs are characterized by relatively high fluidity. In contrast, FAs in the *trans* configuration lose bend in the chain and become embedded into the membrane, just like SFAs. The conversion of *cis*-isomers of UFA into *trans*-isomers reduces the fluidity of CPM. The *cis*/*trans* isomerase (Cti) catalyzes the isomerization of UFA in the absence of *de novo* lipid synthesis and does not require hydrolysis of ATP, $NADPH_2$, or GSH (Eberlein *et al.*, 2018).

The reactions of CFA synthesis are important for stabilizing the bacterial membrane under stressful environmental conditions (Poger & Mark, 2015). These acids are synthesized by transferring a methyl group from S-adenosyl-L-methionine to a double bond in the UFA chain. This reaction is catalyzed by Cfa synthase, which has been identified in the cells of many bacteria (Poger & Mark, 2015). To convert one molecule of UFA into CFA, it is necessary to use the energy of three molecules of ATP. Such a transformation plays an important role in the adaptation of bacterial cells to sudden changes in environmental conditions. For *E. coli*, a correlation between the content of CFAs in the membranes and resistance to lethal pH values was established. The conversion of oleic acid to an acid with a cyclopropane ring has been described in *Lactobacillus helveticus* during cold and acid stress (Montanari *et al.*, 2010). It was assumed that an increase in the rigidity of membranes in the presence of CFAs is due to a higher T_M of these acids compared to UFAs. CFAs are more resistant to ozone and singlet oxygen. It is considered that the primary function of CFAs in bacterial cells is to change chemical properties of membranes without significant changes in their physical properties (Chen & Gänzle, 2016, Poger & Mark, 2015; Karlinsey *et al.*, 2022). However, the role of CFAs in the stabilization of CPM fluidity is ambiguous and requires more detailed study.

Mechanisms of regulation of CPM fluidity by *cis/trans* isomerization or conversion of UFA into CFAs lead to a decrease in its fluidity. Lipids of the anaerobic bacteria contain a significant amount of *iso*- and *anteiso*-branched FAs. A high content of these FAs was found in sulfate-reducing bacteria of the *Desulfovibrio* genus: the content of branched FAs in lipids of *D. africanus* is 30%, in *D. gigas* – 57%, in *D. desulfuricans* – 61% (Murinova & Dercová, 2014).

The *iso*-FAs differ from *anteiso*-FAs in physicochemical properties, molecular structure, and T_M (Kaneda, 1991). This difference leads to significant changes in the CPM fluidity due to an increase in the content of one of the types of branched FAs. A change of *antheiso/iso* degree was shown in *Arthrobacter chlorophenolicus* under the influence of high temperatures and different doses of phenol (Murinova & Dercová, 2014). A response of *D. indonesiensis* under the influence of high temperatures, besides an increase in the degree of lipid saturation, also includes a replacement of the branched FAs by straight carbon chain FAs (Fichtel *et al.*, 2015). At a temperature of 20 °C, in the cells of the mesophilic bacteria *Bacillus megaterium*, the content of *iso*-12-methyl-tetradecenoic acid was 25%, while the content of *antheiso*-13-methyl-tetradecenoic acid was 50%. When the temperature rose to 60 °C, the content of *iso*-12-methyl-tetradecenoic acid increased to 35% and the content of antheiso-13-methyl-tetradecenoic acid decreased to 15% (Koga, 2012).

An increase in the content of long-chain SFA in *C. limicola* cells under the influence of Cu^{2+} was revealed. It was assumed that one of the first reactions of adaptation of *C. limicola* cells under the influence of Cu^{2+} is the *cis/trans* isomerization of monounsaturated FA and the synthesis of CFA. Maintenance of the required level of CPM fluidity under these conditions is provided by the branched FA (Segin *et al.*, 2018). In *D. acetoxidans* IMV B-7384 under the influence of ferric citrate, the level of saturation of lipids is regulated first by converting UFAs to CFAs. However, this modification of the FA composition of phospholipids leads to a decrease in the level of fluidity of the CPM.

Therefore, the branched FA and *cis*-UFA are synthesized in the cells, which increases the fluidity of the CPM. Probably, the regulation of lipid saturation in *D. acetoxidans* IMV B-7384 cells under the influence of ferric ions occurs both at the biosynthetic level (through the synthesis of FA with a branched chain) and the post-synthetic level (through the formation of CFAs).

CONCLUSIONS

Oxidative damage affects the structure of protein polypeptide backbone and amino acid side groups, resulting in the formation of radical and non-radical amino acid derivatives. The toxic effect of protein radicals involves the protein-mediated initiation of LPO and radical centres from one amino acid residue to another. During LPO in cells, cytotoxic compounds are formed, which can covalently modify cellular proteins, lipids and, thus, affect the activity of cellular enzymes and CPM fluidity. The cell's response to toxic products is realized during oxidative modification of proterins and LPO depends on the metabolic conditions and reparative potential, determining the cell's survival or death. Cell membranes are one of the main targets for stress agents, so, the adaptation mechanisms to the effects of these compounds maintain an appropriate state of membrane fluidity.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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AUTHOR CONTRIBUTIONS

Conceptualization, [M.O.; K.S.; H.S.]; methodology, [-]; validation, [-]; formal analysis, [-]; investigation, [-]; resources, [M.O.; K.S.; H.S.]; data curation, [-]; writing – original draft preparation, [M.O.; K.S.; H.S.]; writing – review and editing, [M.O.; K.S.]; supervision, [M.O.; K.S.; H.S.]; project administration, [H.S.]; funding acquisition, [+].

All authors have read and agreed to the published version of the manuscript.

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ОКСИДАТИВНИЙ СТРЕС І ЗАХИСТ ВІД НЬОГО У БАКТЕРІЙ

Ольга Масловська, Соломія Комплікевич, Світлана Гнатуш
Львівський національний університет імені Івана Франка
вул. Грушевського, 4, Львів 79005, Україна

Мікроорганізми зазнають впливу активних форм кисню (АФК), які утворюються в різний спосіб, зокрема, у результаті дихання чи інших внутрішньоклітинних процесів, під час металокаталізованих реакцій Фентона, внаслідок дії ультрафіолетового чи рентгенівського випромінювань, впливу деяких протимікробних препаратів, чи внаслідок "кисневого вибуху" під час імунної відповіді хазяїна на інфекційні чинники. У цьому огляді ми розглядаємо механізми пошкоджень клітин мікроорганізмів, зокрема, пошкодження їхніх ліпідів і білків. Перекисне окиснення ліпідів (ПОЛ) є одним із основних молекулярних механізмів, залучених в окисне пошкодження клітинних структур. У ході реакцій ПОЛ утворюється широкий спектр продуктів: алкоксильні, пероксильні радикали, гідропероксиди, дієнові кон'югати, карбонільні сполуки, адукти альдегідів з біополімерами, спирти, прості ефіри тощо. Серед цих продуктів є цитотоксичні та високореактивні сполуки. Вільнорадикальні реакції пошкодження білків відбуваються внаслідок відщеплення гідрогену від α-карбону, SH-, NH_2-груп амінокислот і відщеплення електронів від нуклеофільних центрів білків, унаслідок чого спостерігають фрагментацію білків, їхню денатурацію й утворення амінокислотних радикалів. Бактерії виявляють значний адаптивний потенціал до впливу стресових чинників, зокрема, АФК. Ми підсумовуємо дані щодо антиоксидантного захисту бактерій, редокс-сенсорів АФК і регуляторів відповіді бактерійних клітин за впливу АФК, акцентуючи увагу на особливостях анаеробних мікроорганізмів, оскільки їхня реакція на окисне пошкодження є найменш вивченою і залишається чимало нез'ясованих питань. Цей огляд містить інформацію про зміну жирнокислотного складу ліпідів для підтримання необхідної плинності плазматичної мембрани, а відтак і протидії впливу різних стресорних чинників, зокрема, АФК. Схарактеризовано основні модифікації жирнокислотного складу ліпідів для регуляції плинності мембрани, зокрема, зміни ступеня насиченості ліпідів, їхню *цис*/*транс* ізомеризацію, синтез жирних кислот із циклопропановим кільцем.

Ключові слова: бактерії, оксидативний стрес, активні форми кисню, антиоксидантний захист, жирнокислотний склад ліпідів, пошкодження ліпідів, пошкодження білків

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