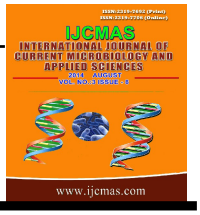
*Int.J.Curr.Microbiol.App.Sci* (2014) 3(9) 34-40

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706*** **Volume 3 Number 9 (2014) pp. 34-40**
**http://www.ijcmas.com**




**Review Article**

# Free radicals and oxidative stress in bacteria

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**Keywords**

Oxidative stress, Reactive oxygen species,

**A B S T R A C T**

Aerobic bacteria use molecular oxygen ($O_2$) for respiration or oxidation of nutrients to obtain energy. Reactive oxygen species (ROS) are necessary for various physiological functions but an imbalance in favor of reactive oxygen species results in oxidative stress (OS). As in humans, the exposure of bacteria to ROS causes damage to a variety of macromolecules, resulting in mutations and often in cell death. However, ROS may also be considered to be beneficial compounds, as they function as signaling molecules that lead to a coordinated response of bacteria under redox-stress conditions. This paper reviews about free radical, generation of reactive oxygen species in bacteria and defense mechanisms responses to oxidative stress.

## Introduction

### 1. Free radicals

Free radicals are a group of short-lived reactive chemical intermediates, which contain one or more electrons with unpaired spin and can oxidatively modify biomolecules that they encounter. This causes them to react almost instantly with any substance in their vicinity (Warren *et al.*, 1987). Generally, free radicals attack the nearest stable molecule, "stealing" its electron (Fig.1). When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade and ultimately lead to the disrupting of living cells. They are highly reactive and oxidize lipids, amino acids and carbohydrates as well as causing DNA mutations.

### 2. Types of free radicals

Reactive oxygen species (ROS) represent the collection of number of molecules and free radicals derived from molecular oxygen in addition, there is another class of free radicals that are nitrogen derived called reactive nitrogen species (RNS) (Sikka, 2001). These reactive species are readily converted into reactive non-radical species by enzymatic or non-enzymatic chemical reactions that in turn can give rise to new radicals (Table 1 and Fig. 2).

### 3. Cellular Source of ROS's

In a living system there are various sources of ROS such as byproduct of cellular respiration (presence of redox cycling compounds), synthesized by enzyme systems – phagocytic cells, neutrophils and

macrophage (NADPH oxidase, myeloperoxidases), exposure to ionizing radiation, production: a) Chain reaction, a free radical steals an electron from a nearby compound forming a new free radical. Free radicals may steal electrons from cellular structures or molecules, b) by normal cellular respiration – electron transport system – often oxygen is the terminal electron acceptor in the cell mitochondria to ROS. Also smoking, herbicides, pesticides, fried foods as well as industrial contaminants that are widespread in soils and on the surfaces of plants are sources of ROS. Recent work has demonstrated that ROS have a role in cell signaling, including; apoptosis; gene expression; and the activation of cell signaling cascades. It should be noted that ROS can serve as both intra- and intercellular messengers (Hancock *et al.,* 2001).

## 4. Oxidative stress

Oxidative stress (OS) is the term applied when oxidants outnumber antioxidants. Interference in the balance between the environmental production of reactive oxygen species (ROS), including hydroxyl radicals (•OH) and hydrogen peroxide ($H_2O_2$), and the ability of biological systems to readily detect and detoxify them, or repair the resulting damage are oxidative stress (de Oru´e Lucana *et al.*, 2012).

It is a common condition caused by biological systems in aerobic conditions such that antioxidants cannot scavenge the free radicals. This causes an excessive generation of ROS, which damages cells, tissues and organs. Highly reactive radicals cause the oxidative damage of different macromolecules—proteins, DNA, and lipids— leading to loss of function, an increased rate of mutagenesis, and ultimately cell death.

## 5. Physiological role of ROS

Aerobic bacteria use molecular oxygen ($O_2$) for respiration or oxidation of nutrients to obtain energy. Bacteria are in continuous contact with ROS over the course of their life cycle generated both endogenously, as a product of aerobic metabolism, or exogenously during ionizing ($\gamma$) and nonionizing (UV) irradiation leading to the production of a number of radical and peroxide species through the ionization of intracellular water (Cabiscol *et al.,* 2000 and de Oru´e Lucana *et al.,* 2012). Reactive by-products of oxygen, such as superoxide anion radical ($O_2^-$), hydrogen peroxide ($H_2O_2$), and the highly reactive hydroxyl radicals (·OH), are generated continuously in cells grown aerobically (Cabiscol *et al.,* 2000). These species cause damage to proteins, lipids, and nucleotides, negatively impacting the organism (de Oru´e Lucana *et al.,* 2012).

## 6. Cellular defense or antioxidant defense mechanisms against ROS

Living organisms have to build up mechanisms to protect themselves against oxidative stress, with enzymes such as catalase and superoxide dismutase, small proteins like thioredoxin and glutaredoxin, and molecules such as glutathione. Bacterial genetic responses to oxidative stress are controlled by two major transcriptional regulators (OxyR and SoxRS). ROS damage a variety of cellular macromolecules and thus elicit adaptive oxidative stress responses in bacteria intended to permit survival in the presence of this stressor (Storz and Imlay, 1999; James and Imlay, 2008). Expression of a number of multidrug efflux systems is positively impacted by agents of oxidative stress, these efflux systems possibly playing a role in ameliorating the effects of this stress.

Similarly, antioxidant mechanisms are recruited in response to antimicrobial exposure, antimicrobials being known to generate ROSs that are key to the often lethal effects of these agents (Dwyer *et al.,* 2009; Kolodkin-Gal *et al.,* 2008). As such oxidative stress responses have the potential to contribute to antimicrobial resistance in a variety of ways.

Cells have a variety of defense mechanisms to ameliorate the harmful effects of ROS. Superoxide dismutase catalyzes the conversion of two superoxide anions into a molecule of hydrogen peroxide ($H_2O_2$) and oxygen ($O_2$) (McCord and Fridovich, 1968). As studies the biological targets for these highly reactive oxygen species are DNA, RNA, proteins and lipids. Much of the damage is caused by hydroxyl radicals generated from $H_2O_2$ via the Fenton reaction, which requires iron (or another divalent metal ion, such as copper) and a source of reducing equivalents (possibly NADH) to regenerate the metal. Lipids are major targets during oxidative stress. Free radicals can attack directly polyunsaturated fatty acids in membranes and initiate lipid peroxidation.

A primary effect of lipid peroxidation is a decrease in membrane fluidity, which alters membrane properties and can disrupt membrane-bound proteins significantly. This effect acts as an amplifier, more radicals are formed, and polyunsaturated fatty acids are degraded to a variety of products. Some of them, such as aldehydes, are very reactive and can damage molecules such as proteins (Humpries and Sweda, 1998). Unlike reactive free radicals, aldehydes are rather long lived and can therefore diffuse from the site of their origin and reach and attack targets which are distant from the initial free-radical event, acting as "second toxic messengers" of the complex chain reactions initiated. DNA is a main target; active species attack both the base and the sugar moieties producing single- and double-strand breaks in the backbone, adducts of base and sugar groups, and cross-links to other molecules, lesions that block replication (Sies and Menck, 1992, Sies, 1993).

Presence of oxygen in the atmosphere led to the development of defense mechanisms that either kept the concentration of the $O_2$-derived radicals at acceptable levels or repaired oxidative damages. Iron plays a significant role in biology (transport, storage and activation of molecular oxygen, reduction of ribonucleotides, activation and decomposition of peroxides, and electron transport) and $Fe^{2+}$ is required for the growth of almost all living cells. Due to its potential damaging effects, in bacteria, iron solubilization and metabolism is strictly regulated at two levels:

(i) the entrance to the cell by specific membrane-bound receptors, and

(ii) inside the cell, by two proteins, bacterioferritin and ferritin, very similar to the eukaryotic ferritin, but presenting ferroxidase activity. Some molecules are constitutively present and help to maintain an intracellular reducing environment or to scavenge chemically reactive oxygen. Among these molecules are nonenzymatic antioxidants such as NADPH and NADH pools, β-carotene, ascorbic acid, α-tocopherol, and glutathione (GSH). GSH, present at high concentrations, maintain a strong reducing environment in the cell, and its reduced form is maintained by glutathione reductase using NADPH as a source of reducing power. In addition, specific enzymes decrease the steady-state levels of reactive oxygen (Cabiscol *et al.,* 2000).

**Fig.1** Formation of a free radical (Agarwal *et al.*, 2008)

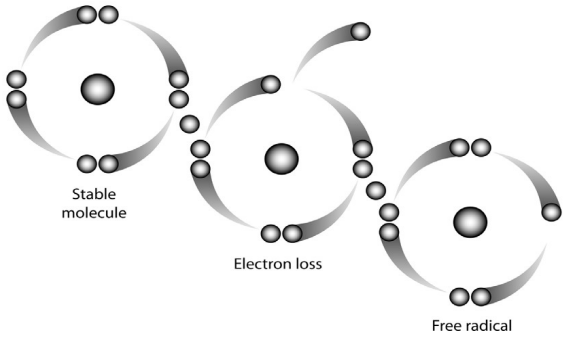



**Fig.2** Free radicals

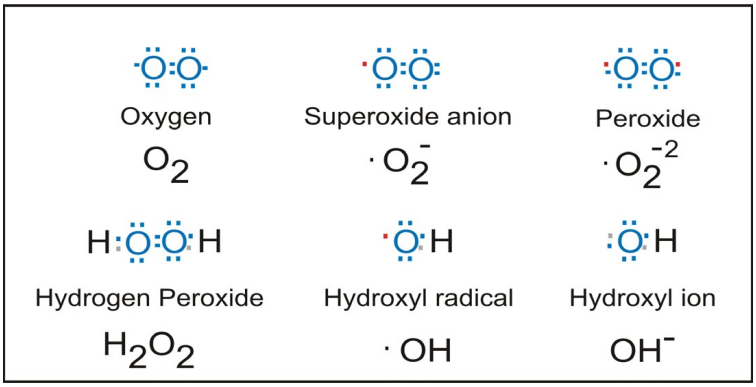
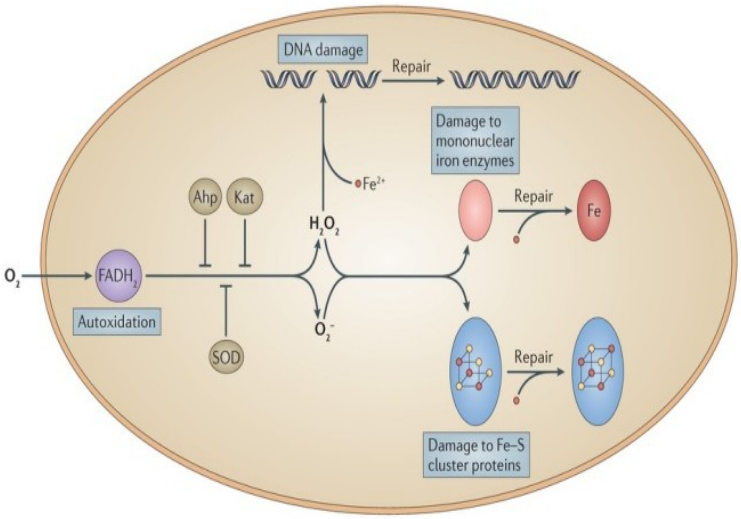



**Table.1** Examples of ROS and RNS

| **Reactive oxygen species** | **Reactive nitrogen species** |
|---|---|
| Superoxide anion ($O_2$• - ) | Nitric oxide (NO•) |
| Hydrogen peroxide ($H_2O_2$) | Nitric dioxide ($NO_2$• ) |
| Hydroxyl radical (OH •) | Peroxynitrite (ONOO-) |

**Fig.3** Overview of damage caused by reactive oxygen species in *Escherichia coli*




Two superoxide dismutases (SOD), which convert $O_2$ – to $H_2O_2$ and $O_2$, have been described in *Escherichia coli* (fig.3) an iron-containing enzyme, whose expression is modulated by intracellular iron levels (Niederhoffer *et al.*, 1990), and a manganese containing SOD, the predominant enzyme during aerobic growth, whose expression is transcriptionally regulated by at least six control systems (Compan and Touati, 1993). A third SOD activity with properties like eukaryotic CuZn-SOD has been found in the *E. coli* periplasmic space (Benov and Fridovich, 1994). In *E. coli*, $H_2O_2$ is removed by two catalases (yielding $H_2O$ and $O_2$): hydroperoxidase I (HPI), which is present during aerobic growth and transcriptionally controlled at different levels (Finn and Condon, 1975), and hydroperoxidase II (HPII), which is induced during stationary phase (von Ossowski *et al.*, 1991). Glutathione peroxidase and DT-diaphorase are also scavenging enzymes.

Secondary defenses include DNA-repair systems and proteolytic and lipolytic enzymes. DNA repair enzymes (Demple and Harrison, 1994) include endonuclease IV, which is induced by oxidative stress, and exonuclease III, which is induced in the stationary phase and in starving cells. Both enzymes act on duplex DNA cleaning up DNA 3' termini. Prokaryotic cells contain catalysts able to repair directly some covalent modifications to the primary structure of proteins. One of the most frequent modifications is the reduction of oxidized disulfide bonds: (i) thioredoxin reductase transfers electrons from NADPH to thioredoxin via a flavin carrier, (ii) glutaredoxin is also able to reduce disulfide bonds, but using GSH as an electron donor and, (iii) protein disulfide isomerase facilitates disulfide exchange reactions with large inactive protein substrates, besides having chaperone activity. Oxidation of methionine to methionine sulfoxide can be repaired by methionine sulfoxide reductase. Recent experimental data described that surface exposed methionine residues surrounding the entrance to the active site are preferentially oxidized without loss of catalytic activity, and

suggested that methionine residues could function as a "last-chance" antioxidant defense system for proteins (Levine *et al.*, 1996).

Reactive oxygen species (ROS) damage DNA, RNA, proteins and lipids, resulting in cell death when the level of ROS exceeds an organism's detoxification and repair capabilities. Despite this danger, bacteria growing aerobically generate ROS as a metabolic by-product, a risk balanced by an increased efficiency and yield of energy from growth substrates. Two possible mechanisms can be used to manipulate bacterial ROS metabolism and increase sensitivity of bacteria to oxidative attack i) amplification of endogenous ROS production and ii) impairment of detoxification and repair systems. Whereas removal of their detoxification and repair system has been shown to make bacteria more susceptible to oxidants (Carlioz and Touati, 1986; Loewen, 1984), antibiotics (Dwyer *et al.*, 2007) and immune attack (Hebrard *et al.*, 2009 and Liu, 2008), manipulation of endogenous bacterial ROS production remains largely unexplored.

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