Universidade de São Paulo
Instituto de Biociências

Unraveling the biological role of LsfA, a 1-Cys Prx involved in the *P. aeruginosa* virulence

“Desvendando o papel biológico de LsfA, uma 1-Cys Prx envolvida na virulência de *Pseudomonas aeruginosa”*

Supervisor: Dr. Luis Eduardo Soares Netto

Rogério Luis Aleixo Silva

São Paulo

2023

Unraveling the biological role of LsfA, a 1-Cys Prx involved in the *P. aeruginosa* virulence

“Desvendando o papel biológico de LsfA, uma 1-Cys Prx envolvida na virulência de *Pseudomonas aeruginosa”*

Thesis submitted to the Institute of Biosciences of the University of São Paulo, as a partial requirement to obtain the doctoral degree in Biological Sciences.

Supervisor: Dr. Luis Eduardo Soares Netto

Rogério Luis Aleixo Silva

São Paulo

2023

Cataloging Data

Aleixo-Silva, Rogério Luis / Aleixo Silva Rogerio Luis; orientador Netto Luis Eduardo Soares -- São Paulo

Unraveling the biological role of LsfA, a 1-Cys Prx involved in the P. aeruginosa virulence

153 pag.

Tese (Doutorado) -- Instituto de Biociências da Universidade de São Paulo. Programa de Pós-Graduação em Ciências Biológicas (Biologia Genética).

1. Peroxiredoxin 2. Bacterial virulence 3. Inflammatory response.

Thesis committee:

X

Prof. Dr.

X

Prof. Dr.

X

Prof. Dr.

X

Prof. Dr.

X

Prof. Dr. Luis E. S. Netto
Supervisor

“Empty your mind. Be formless. Shapeless. Like water. You put water into a cup, it becomes the cup. You put water into a bottle, it becomes the bottle. You put it in a teapot, it becomes the teapot. Water can flow, or it can crash. Be water, my friend.”

Bruce Lee

“Do or do not. There is no try.”

Master Yoda

Acknowledgements

After so many years of work, I have a lot of people to thank.

First of all, I need to thank my family, also built during this PhD, my wife and kids. Always giving me strength and motivation to go further.

I also would like to thank my mom, who gave me full support during my whole life to reach this point.

A special thanks for Professor Luis Netto, who accepted me in his lab back in 2016. Proving as a great mentor scientifically and personally, whom I also thanks for the different experiences that I had, which I would never imagine.

Thanks for the whole team of the LPBR during these years, mainly the ones whom I cohabit during my whole period here as Simone, Fernando, and Thiago. The last ones who became especially helpful during the end of my period in the lab. Thanks for that!

I thank to Professor Jesmond Dalli, who received me in his lab, even after a fast and no previous contact. Which became an incredible experience for my personal life and generated amazing results. I also would give a special thanks for his team, being absolutely supportive and making me feel part of it really fast. Thanks for Esteban, Ana, Maria, Robi, Duco, Soumik, Viny, Rosangela and Cristian.

Would like to thank all of the collaborators whom made this thesis and the future scientific publications possible.

Finally, I would like to thank CNPq and FAPESP by funding my PhD (Process 157752/2018-6 and 2020/00845-4), and FAPESP by the BEPE program in which I spent four months at Professor Dalli`s lab (Process 2021/11077-0).

Index

1. General Introduction

1.1. *Pseudomonas aeruginosa*

1.1.1. General Aspects

Pseudomonas aeruginosa is a gamma-proteobacteria, that acts as an opportunistic pathogen in immunocompromised patients. The origin of the word *Pseudomonas* is from the Greek word Pseudo = false, and monas = only unit; whilst *aeruginosa* is from the Latin word aerūgō = rusty copper. *P. aeruginosa* is a bacterium similar to a rod, with 1-5 µm length and 0,5-1 µm height, that presents a green-blue color. It grows as a facultative aerobic bacterium and is able to use more than a hundred different molecules as carbon sources to obtain energy. The ideal temperature of growing is around 37°C, but it can survive between 4-42 °C; in the soil, consuming aromatic hydrocarbons or in water reservoir, as in sewer. The versatility of *P. aeruginosa* is remarkable as this bacterium can occupy several niches, colonizing several different organisms as: plants, ameba, nematodes, and vertebrates (DIGGLE; WHITELEY, 2020; LYCZAK; CANNON; PIER, 2000; WILLIAMS; DEHNBOSTEL; BLACKWELL, 2010).

This bacterium can cause a wide range of pathological processes, being involved in wounds, pulmonary and eye infections; strongly associated with cystic fibrosis patients, which develop pulmonary infection. *P. aeruginosa* infections are often related to the decline of pulmonary functions and patients' mortality. Biofilm formation and drug-resistant strains represent serious threats that make the corresponding infections almost impossible to eradicate (CIRZ *et al.*, 2006; COURTNEY *et al.*, 2007; WINNIE; COWAN, 1991).

P. aeruginosa is among the main causes of nosocomial infections in Brazil and in the world (ANVISA, 2016; GALES *et al.*, 2001; SADER *et al.*, 2015). The beginning of the infection occurs by the colonization of the impaired respiratory epithelium; as the ones suffering with cystic fibrosis or immunocompromised (STREETER; KATOULI, 2016). The contamination occurs mainly due to contaminated hospital material, where this bacterium is capable of adhering, mainly because of its ability to form biofilm (DONLAN, 2001).

Between 2012 and 2013, *P. aeruginosa* was the most abundant gram-negative bacteria found in patients hospitalized in intensive care units in USA (SADER *et al.*, 2015); with 32600 estimated cases in hospitalized patients, causing 2700 deaths in 2017, according to the Center for disease control and prevention (https://www.cdc.gov/drugresistance/biggest-threats.html#pse). In Latin America, this bacterium was the most prevalent among bacterial pathogens on pneumonia patients, the fifth on cases of blood infections and the third most abundant in wound infections, accordingly to SENTRY (Resistance vigilance program) (GALES *et al.*, 2012). In Brazil, *P. aeruginosa* was the fifth etiologic agent found in blood related with central venous catheter at adult, pediatric and new-born intensive care units between 2012-2016; additionally, up to 43% of the isolates presented carbapenem resistance (ANVISA, 2016). *P. aeruginosa* isolates which present antibiotic resistance are another aggravating in infections by this pathogen (MAGIORAKOS *et al.*, 2012). An international observation showed that, in intensive-care units, *P. aeruginosa* represents 16.2% of patient infections and was the cause of 23% of all ICU-acquired infections, mainly related to respiratory infections (VINCENT *et al.*, 2020). Taken all these facts together, the World Health Organization (WHO) classified *P. aeruginosa* in the list of critical priorities for research and Discovery of new drugs (HARBARTH *et al.*, 2017).

1.1.2. Oxidative Stress and Bacterial Antioxidant systems

The initial oxidative stress concept was formulated in 1985 as a disbalance favoring the prooxidant in contraposition to the antioxidant side of a balance, which can provoke cell damage. More recently, this concept evolved in two different situations. Eustress is the oxidative stress generated under normal physiological conditions, while distress is the supraphysiological challenge imposed by high levels of oxidants, generating a pathological condition (reviewed by SIES, 2018).

Different oxidants can be generated during both eu/distress, the most famous ones are the reactive oxygen species (ROS), which includes hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and superoxide anion radical ($O_2^{-\bullet}$). Despite their capacity to damage biological molecules, such as lipids, proteins, and DNA (CROSS *et al.*, 1987), their involvement with the transduction of signals in the cells has been revealed as another relevant role played by these molecules. Indeed, dysregulation of ROS formation and/or decomposition may impair redox signaling, generating a disease (FINKEL, 2011).

ROS molecules can be generated by different sources. In a eukaryotic cell under physiological conditions, one of the main sources is mitochondria. Initially, superoxide is formed by monoelectronic reduction of molecular oxygen, mainly at complex I and III, but also in complex II, which can then be converted to H_2O_2 by mitochondrial SOD (NAPOLITANO; FASCIOLO; VENDITTI, 2021).

Other important sources of oxidants are the NADPH-dependent oxidases that take part of seven-members family (NOX 1-5 and Duox 1-2), which are involved in the oxidative burst in phagocytic cells and also in other physiological processes (DI MEO *et al.*, 2016; FINKEL, 2011). Superoxide formed by the action of NOX cannot penetrate the bacterial membranes under physiological pH, due to its negative charge. However, superoxide can be dismutated to H_2O_2, which can cross membranes, as this peroxide displays a permeability coefficient similar to water. If the exogenous levels of H_2O_2 exceed 0.2 μM, the influx ratio of H_2O_2 becomes higher than the endogenous amount formed; which happens inside the phagosome during infections, where the levels of H_2O_2 can be elevated by one order of magnitude (HOPKINS, 2017; MISHRA; IMLAY, 2012; SEAVER; IMLAY, 2001).

Nitric oxide ($NO^•$), a reactive nitrogen species (RNS), can be generated by the activity of nitric oxide synthase (NOS), from the metabolism of the amino acid L-arginine. Three NOS isoforms were identified in mammals: the neuronal (nNOS), the endothelial (eNOS) and the inducible (iNOS), the last one is present in phagocytes (ALDERTON; COOPER; KNOWLES, 2001). While nNOS and eNOS are calcium-dependent, iNOS is not (NATHAN; XIE, 1994; XUE *et al.*, 2018). $NO^•$ produced by nNOS is important for cellular communication between neurons, while $NO^•$ produced by eNOS is involved in the relaxation of endothelial cells. In these cases, $NO^•$ is generated at low levels. In contrast, $NO^•$ is generated in high amounts by iNOS in macrophages, which is involved in killing pathogens. Therefore, depending on its levels, $NO^•$ presents distinct properties (NATHAN; XIE, 1994).

Reaction between $NO^•$ and superoxide anion can form peroxynitrite, another powerful oxidant capable to potentialize the killing capacities of phagocytes (RADI, 2018). Peroxynitrite has a short half-life in physiological pH, but it is membrane permeable, making this molecule capable of interfering in the surrounding cells. Noteworthy, peroxynitrite can oxidize/nitrate many biomolecules, as proteins, low molecular weight thiols, DNA, unsaturated fatty acids and enzymatic cofactors (CALCERRADA; PELUFFO; RADI, 2011). This molecule is also capable to interfere in many inflammatory, cardiovascular and neurodegenerative conditions (RADI, 2018). It is important to note that different peroxiredoxins are able to reduce peroxynitrite very efficiently (BRYK; GRIFFIN; NATHAN, 2000; MANTA *et al.*, 2009; SANDER *et al.*, 2002; TRUJILLO; FERRER-SUETA; RADI, 2008).

Pathogenic microorganisms evolved strategies to overcome the oxidative challenge imposed by these molecules. Indeed, bacterial defenses were recently analyzed, based on the KEGG database, (https://www.genome.jp/kegg/), comprising 26 different types of proteins, across ~24k bacterial genomes (JOHNSON; HUG, 2019).

Thioredoxin system: Thioredoxin is one of the most prevalent thiol disulfide oxidoreductases (JOHNSON; HUG, 2019), displaying essential cellular functions such as the reduction of ribonucleotides into desoxyribonucleotides and modulation of transcription factors' activity (HIROTA *et al.*, 1999; LUTHMAN; HOLMGREN, 1982; MOORE; REICHARD; THELANDER, 1964). Upon reduction of target disulfides, thioredoxin is oxidized, being re-reduced by thioredoxin reductase, a flavoenzyme that uses electrons from NADPH. Thioredoxin reductases are divided as high molecular weight enzymes and are present in mammals; while low molecular weight thioredoxin reductases are present in organisms such as prokaryotes, fungi and plants (DE OLIVEIRA *et al.*, 2021; WILLIAMS *et al.*, 2000).

Glutaredoxins: Other oxidoreductases capable of catalyzing thiol-disulfide exchange reactions, being also involved with the formation of deoxyribonucleotides for DNA synthesis, maintaining the levels of reduced sulfur, signal transduction and oxidative stress defense (reviewed by FERNANDES; HOLMGREN, 2004). In contrast to thioredoxins, glutaredoxins display high affinity for glutathione and their activities are also coupled with glutathione reductase and NADPH. Glutaredoxins are also widespread, enzymes of glutaredoxin 2 subgroup are present only in three bacterial phyla, predicted only in 3.7% of bacterial genomes. The other three glutaredoxins groups described in KEGG are glutaredoxin 1, 3 and the monothiolic ones; all of them more prevalent than Glutaredoxin 2 group (JOHNSON; HUG, 2019). These enzymes can display both a monothiolic or a dithiolic mechanism, which are distinct by the differences in deglutathionylation steps (MASHAMAITE; ROHWER; PILLAY, 2015).

Superoxide scavenging enzymes: Superoxide dismutase (SOD) are enzymes that catalyze superoxide dismutation, leading to H_2O_2 production. These enzymes can be classified according to the nature of its metal cofactor, SOD 1 (Cu/Zn), SOD 2 (Fe/Mn) and SOD N (Ni). Among all bacterial genomes, SOD 2 is the most abundant one (72.9%), while SOD 1 and SOD N were only present in 29.2% and 7.9% of genomes respectively (JOHNSON; HUG, 2019). Additionally, several SODs were already related with both antioxidant defense and virulence in different bacteria (IIYAMA *et al.*, 2007; KANAFANI; MARTIN, 1985; KANG; KIM; LEE, 2007; PIDDINGTON *et al.*, 2001; SEYLER; OLSON; MAIER, 2001).

Hydroperoxide scavenging enzymes: Catalase are enzymes capable of reducing H_2O_2 to water and oxygen; and peroxynitrite to nitrite (GEBICKA; DIDIK, 2009). The KEGG divide catalases in three groups: catalase, Mn catalase and catalase-peroxidase, whose prevalence are 41.4%, 13.1% and 33.4% among all bacterial genomes, respectively (JOHNSON; HUG, 2019; NICHOLLS; FITA; LOEWEN, 2000). Noteworthy, some catalases are related with virulence in bacteria, and display compensatory effects with other peroxidases (COSGROVE *et al.*, 2007; LEE *et al.*, 2005; MANDELL, 1975; WOOD *et al.*, 2003; XU; PAN, 2000).

Glutathione peroxidases are seleno-Cys or sulfur-Cys based proteins that reduce peroxides in a glutathione (GSH), thioredoxin or even PDI (Protein disulfide isomerase) dependent manner (CONRAD; FRIEDMANN ANGELI, 2018; TRUJILLO *et al.*, 2022). In mammals, these proteins are mainly seleno-Cys peroxides, reacting very efficiently towards several oxidants, such as H_2O_2, peroxynitrite, cholesterol and phosphatidyl choline hydroperoxides (reviewed by TRUJILLO *et al.*, 2022). In bacteria, these proteins are sulfur-Cys based, and their genes were found in 54.5% of the genomes analyzed (JOHNSON; HUG, 2019).

Ohrs (organic hydroperoxide resistance proteins) are enzymes that reduce organic hydroperoxides highly efficiently in comparison with H_2O_2. Ohrs are also highly efficient in reducing peroxynitrite and their catalytic power is also based on a reactive cysteine residue (so called peroxidatic Cys or C_P), which takes part of a catalytic triad together with fully conserved arginine and glutamate residues (ALEGRIA *et al.*, 2017; DOMINGOS *et* *al.*, 2020). Another fully conserved residue among Ohrs is a cysteine residue (resolving Cys or C_R) involved in the condensation reaction with a sulfenic acid (Cys-SOH) in C_P, resulting in an intra-molecular disulfide bond formation. Afterwards, Ohr is reduced by a lipoylated protein (CUSSIOL *et al.*, 2010). The expression of Ohr genes is regulated in several bacteria by OhrR, a redox, Cys based transcriptional repressor (reviewed by MEIRELES *et al.*, 2022).

Peroxiredoxins (Prx) are widespread, very abundant and highly efficient enzymes, therefore, representing a major group of antioxidant proteins responsible to scavenge different hydroperoxides. These enzymes react very efficiently with a wide range of peroxides with extremely high rate constants (10^6-10^8 $M^{-1}.s^{-1}$), being present in all the three domains of life (RHEE, 2016). These enzymes are also Cys based peroxidases, and the reactive cysteine (also named C_P) takes part of a conserved PxxxT/SxxC motif. Another conserved feature among all Prxs is the presence of a catalytic triad composed of C_P, a Thr/Ser and an Arg (HALL *et al.*, 2011). For some Prxs, a second cysteine residue (so-called resolving cysteine or C_R) forms a disulfide bond with C_P. Additionally, the reduction step varies among Prxs and can involve glutathione (GSH), thioredoxin or ascorbate (FISHER *et al.*, 1999; MONTEIRO *et al.*, 2007; PEDRAJAS *et al.*, 2010, 2016a).

Prxs display different functions in different organisms, such as: defending pathogens from the oxidative burst and its involvement with microorganism virulence (DE OLIVEIRA *et al.*, 2021); sensing and transducing signals (RHEE; WOO; KANG, 2018) and even as PAMPS (pathogen-associated molecular pattern) and DAMPS (host-derived damage- associated molecular patterns) (RHEE, 2016). One sub-family of peroxiredoxins (AhpC/Prx1) is found in 67.2% of all bacterial phyla, but almost exclusively in aerobic bacteria (JOHNSON; HUG, 2019).

1.2. Peroxiredoxins

1.2.1. Overview and the classification

Prxs is part of a large and widespread family of Cys-based peroxidases, involved in functions like regulation of cell proliferation, differentiation, and apoptosis. Prxs can achieve high concentrations in mammalian cells, up to 1% of all soluble protein content; while in *Escherichia coli,* these peroxidases are among the top ten most abundant proteins (HANSCHMANN *et al.*, 2013).

Beyond Prxs abundancy and reactivity (NETTO; ANTUNES, 2016), mammalian Prxs can reduce different types of hydroperoxides, including the fatty acids products derived from lipoxygenases and cyclooxygenases activities (CORDRAY *et al.*, 2007); which are involved in multiple inflammatory pathways (reviewed by KNOOPS *et al.*, 2016). Notably, some cytosolic Prxs are released from necrotic brain cells to the extracellular space, inducing the expression of inflammatory cytokines in macrophages, thereby promoting neural cell death. In contrast, intracellular Prxs are neuroprotective (SHICHITA *et al.*, 2012). Therefore, the extracellular pool of Prxs represents danger signals in the ischemic brain activating Toll-like receptors (SHICHITA *et al.*, 2012).

In general, the catalytic cycle of Prxs consists in the two-electron oxidation of C_P, which is stabilized as a thiolate (C_P-S^-); resulting in sulfenic acid formation and the reduction of the hydroperoxide substrate and releasing a water molecule, nitrite, or the corresponding alcohol, depending on the peroxide used as substrate. For their turnover, thioredoxins (Trxs) (RHEE; CHAE; KIM, 2005) glutathione (GSH) (FISHER, 2011; PEDRAJAS *et al.*, 2016b) or ascorbate (MONTEIRO *et al.*, 2007) among other species can be utilized as reducing agents. Alternatively, C_P-SOH can react with a second peroxide molecule, being hyperoxidized to sulfinic (C_P-SO_2H) or sulfonic (C_P-SO_3H) acid. These hyperoxidized species can only be reduced in 2-Cys Prxs (AhpC/Prx1 subfamily) in an ATP dependent manner (HYUN *et al.*, 2005). The high reactivity of the Prxs is related with the fact that these enzymes stabilize the transition state of the nucleophilic substitution reaction between C_P and the peroxide (HALL *et al.*, 2010).

Peroxiredoxins can be classified based on their catalytic mechanism as 1-Cys Prx and 2-Cys Prxs. The 1-Cys Prxs presents only one Cys residue (C_P) throughout the catalytic cycle. Thereby, after the oxidation, C_P in the sulfenic acid form is directly reduced by its reductant (MONTEIRO *et al.*, 2007; PEDRAJAS *et al.*, 2016b) (Fig. 1A). In the other hand, the 2-Cys Prxs presents a second Cys residue (C_R) participating in catalysis (PERKINS *et al.*, 2015). After the oxidation, the C_P-SOH condenses with C_R, generating a disulfide bond that can be intra-subunit (for the so-called atypical 2-Cys Prxs) or inter-subunit (for the so-called typical 2-Cys Prxs) (PERKINS *et al.*, 2015) (Fig. 1B).

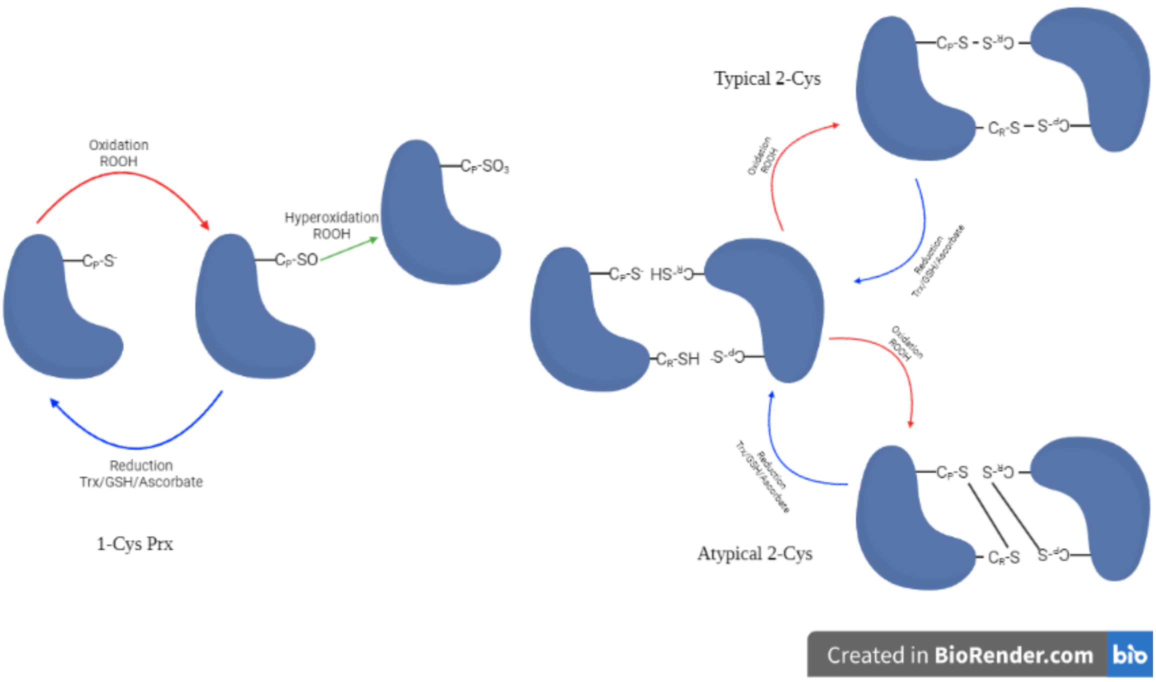

Fig. 1: Peroxiredoxins catalytic mechanism, A) 1-Cys Prxs. B) 2-Cys Prxs, divided into typical and atypical. Highlighting the sulfur atom (S) from the Cys residues part of the reaction (C_P and C_R).

In the reduced state of 2-Cys Prxs, the C_P and C_R are more than 10Å apart. Therefore, a structural rearrangement is required for disulfide formation. The last turn of the α helix containing C_P, unfolds, generating the so-called locally unfolded (LU) state. In contrast, the conformation of 2-Cys Prxs in the reduced and highly reactive state is called fully folded (FF). This FF-LU structural switch is proposed to occur in all Prxs, however is largely studied only in 2-Cys Prxs (KARPLUS, 2015).

Beyond this division based on their mechanisms, Prxs are frequently classified according to their primary sequences and structures (POOLE; NELSON, 2016). In this way, six subfamilies are considered: Prx1/AhpC (or only Prx1), Prx5, Prx6, Tpx, BCP/PrxQ (or only PrxQ) e AhpE (POOLE; NELSON, 2016). Here, we describe studies with the PaLsfA protein, which belongs to the Prx6 subfamily, and presents the 1-Cys Prx mechanism.

1.2.2. Prx6 subfamily

Enzymes from the Prx6 subfamily present mostly the 1-Cys Prx mechanism (POOLE; NELSON, 2016), and are one of the less studied Prx groups. This fact is reflected by the low number (around ten) of structures available on the *Protein Data Bank* (PDB: https://www.rcsb.org/) (HALL *et al.*, 2011). Moreover, the identities of Prx6 biological reductants are mostly unknown. Our research group showed that 1-Cys Prxs can be reduced by ascorbate, representing a breakthrough in the thiol specific activity paradigm of these peroxidases (MONTEIRO *et al.*, 2007). However, the relevance of this reaction in biological systems is still to be demonstrated.

The best characterized Prx6 enzyme is the mammalian isoform, which the reductive pathway is mostly attributed to glutathione (GSH) and requires a heterodimerization with π-glutathione transferase (ZHOU *et al.*, 2016). Although, it is possible that in some tissues where Prx6, but not π-glutathione transferase, is present, ascorbate might be a relevant reductant.

In the case of *Saccharomyces cerevisiae* 1-Cys Prx, which is named ScPrx1, several reductants were proposed: (i) Thioredoxin (Trx) or GSH (PEDRAJAS *et al.*, 2016b), (ii) glutaredoxin (Grx) with GSH (PEDRAJAS *et al.*, 2010) and ascorbate (MONTEIRO *et al.*, 2007). Noteworthy, ScPrx1 is located both into the matrix and the intermembrane space of yeast mitochondria (GOMES *et al.*, 2017), where distinct reductants might act.

Regarding the oxidative pathway, the human Prx6 (or Prdx6) is well characterized, being capable to reduce very efficiently (~10^7 $M^{-1}.s^{-1}$), not only H_2O_2, but also low molecular weight hydroperoxides, such as tert-butyl hydroperoxide (t-BOOH) and cumene hydroperoxide and peroxynitrite. The Prdx6 has a unique capacity among the Prxs to reduce phospholipids hydroperoxides, making the Prdx6 one of the main enzymes responsible for the reduction of oxidized phospholipids in lungs and other organs (reviewed by FISHER, 2017). Additionally, the reduction of some lipid hydroperoxides by Prdx6 were already described, such as: 9-HpODE ((±)9-hydroperoxy-10E,12Z-octadecadienoic acid), 13-HpODE ((9Z,11E)-13-hydroperoxyoctadeca-9,11-dienoic acid) and 15-HpETE ((5Z,8Z,11Z,13E)-15-hydroperoxyicosa-5,8,11,13-tetraenoic acid). In addition, there is a correlation between the peroxidatic activity of Prdx6 and the FAHFAs (fatty acid ester of hydroxy fatty acid) levels in adipose tissue (PALUCHOVA *et al.*, 2022).

A characteristic that makes Prdx6 unique among Prxs is its capacity to hydrolyze glycerophospholipids at the sn-2 position, exhibiting a phospholipase A_2 (PLA_2) activity. This activity is calcium independent, with a clear preference for phosphatidylcholine, which is more active under acidic pH and regulated by phosphorylation. It is dependent on the S^{32}-D^{140}-H^{26} triad, which is conserved in several members of Prx6 subgroup, but not in 2-Cys Prxs. Noteworthy, both peroxidatic and PLA_2 active sites are distinct (FISHER, 2017; WU *et al.*, 2009). Additionally, another exclusive activity of mammalian Prdx6 is the capacity to acylate lysophosphatidylcholine with a free fatty acid, also called lysoPCacyl CoA transferase activity (LPCAT). This activity is governed by the ^{26}HxxxxD^{31} motif that presents a preference for choline. It is important to note that LPCAT activity acts continuously with the PLA$_2$ activity, without the release of the intermediate substrate (FISHER, 2017).

All of these three activities acting together are responsible for preventing: (i) oxidative damage by repairing peroxidized lipids (MANEVICH *et al.*, 2002) and (ii) changes in lipid metabolism in the lungs and, consequently, affecting its surfactant composition. Prdx6 capacity to scavenge PLOOH in mouse lungs is crucial for protection against oxidative stress (LIU *et al.*, 2010). Besides the direct reduction of the damaged phospholipid, the PLA$_2$ together with the LPCAT activity could hydrolyze the phospholipid and reacylate using a non-oxidized fatty acid. Null mutants' mice for Prdx6 or chemically treated with the PLA$_2$ activity inhibitor (MJ33), exhibited a diminished turnover of the lung surfactant phospholipids, whilst the opposite was observed when overexpressing Prdx6 (FISHER *et al.*, 2005, 2006). Prdx6 can regulate NOX2, which is closely dependent on the PLA$_2$ activity and Prdx6 phosphorylation (CHATTERJEE *et al.*, 2011). Together, those activities can completely regenerate the oxidized phospholipids into the membrane (Fig. 2).

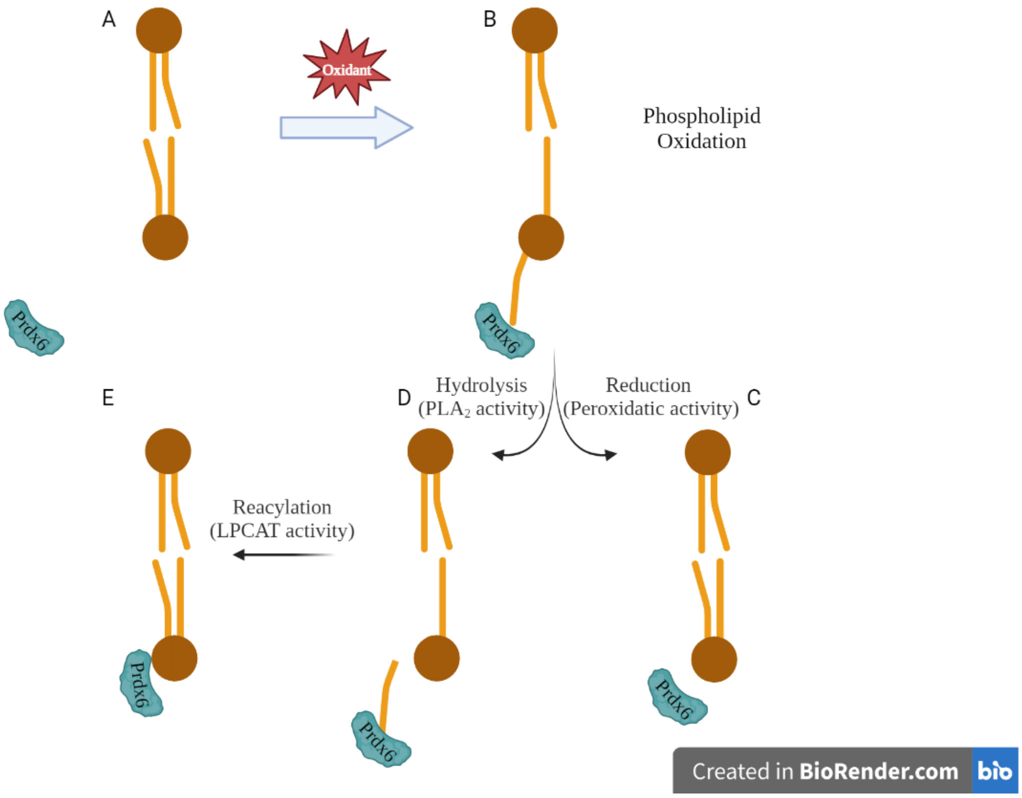

Fig. 2: Scheme of the HsPrdx6 activity in the membrane repair. A) represents a phospholipid membrane in resting state. B) Oxidation of one of the lipidic chain, enabling the action of HsPrx6, which can C) Directly reduce the phospholipid hydroperoxide by the peroxidatic activity, or D) remove the oxidized chain, by the PLA2 activity and then reinsert the reduced lipidic chain by the LPCAT activity, going back to the original state of the phospholipid membrane (Adapted from FISHER, 2017).

1.2.3. The Prx6 from *Pseudomonas aeruginosa*

Pseudomonas aeruginosa possess several antioxidant proteins: SOD, heme-peroxidases, and thiol peroxidases, including several Prxs. PaLsfA is the only enzyme that belongs to the Prx6 subgroup, displaying a molecular mass of 24 kDa and only one cysteine residue, C_{45}, which is the C_P. Besides, PaLsfA also possesses the motif, which in mammalian Prx6 enzymes is involved with their phospholipase activity independent of calcium (aiPLA$_2$) and their LPCAT activity (KAIHAMI *et al.*, 2014; KIM; LEE; KIM, 2016).

The expression of PaLsfA was analyzed in genome wide studies, being induced by sulfate (TRALAU *et al.*, 2007) and iron starvation (HEIM *et al.*, 2003); sodium hypochlorite (SMALL *et al.*, 2007) or paraquat challenge (HARE *et al.*, 2011a). In the other hand, PaLsfA expression was diminished when exposed to tributyltin, an immune system inhibitor and endocrine disruptor in humans (DUBEY; TOKASHIKI; SUZUKI, 2006). PaLsfA was also found during plancktonic growth of this bacterium (PARK *et al.*, 2014). Additionally, a ChIP-chip analysis identified the promoter region of *lsfA* interacting with the H_2O_2-responsive transactivator, OxyR; which is capable to promote the expression of several antioxidant proteins, such as: katA, katB, ahpB and ahpCF (WEI *et al.*, 2012).

The only study specifically focused in PaLsfA revealed an important role of this protein in *P. aeruginosa* virulence, protecting the bacteria against the oxidative burst generated by macrophages (KAIHAMI *et al.*, 2014). The phagocytosis of bacteria lacking PaLsfA (Δ*lsfA*) by J774 macrophages (representing the M1 subtype that is more pro-inflammatory) was similar than the wild-type bacterial strain. However, Δ*lsfA* strain had their survival impaired within macrophage, which was related to the protection afforded by PaLsfA against the oxidative burst imposed by NOX2. In the wild-type strain, TNF-α and IFN-γ production were inhibited by PaLsfA, via the MAPK and NF-kB pathways. In addition, the absence of PaLsfA also affected the recruitment of macrophages and neutrophils, and neutrophils activation in mice's lungs. Finally, the survival rate of the mice infected by the mutant bacteria to PaLsfA were higher when compared with the mice infected by the wild-type strain. All these data strongly related the peroxidatic activity of PaLsfA with the virulence of *P. aeruginosa* (KAIHAMI *et al.*, 2014). Although it was clearly shown that PaLsfA interferes with the regulation of inflammatory pathways, the molecules and mechanisms underlying this process remain to be elucidated.

1.3. Inflammatory response to an invading pathogen

1.3.1. Inflammatory response

Inflammatory process represents a protective response by the host immune system that generates some cardinal signs, such as: rubor, calor, tumor and dolor (SERHAN, 2017). Inflammation has been known since the ancient civilizations and occurs in response to a harmful stimulus, including pathogen invasion or damaged cells (CHEN *et al.*, 2018). It is important to note that the inflammatory response involves two distinct phases: acute inflammation and resolution. A natural course of this process is composed by a first, acute inflammation followed by a resolutive step that will lead the organism back to the basal homeostasis, mediated by a concerted temporal production of lipid mediators (SERHAN *et al.*, 2015), among other molecules (Fig. 3). The common inflammatory response involves inflammatory inducers, which will start the cascade. Then, sensor cells detect these inducers and produces the inflammatory mediators, which will affect the target tissues. For bacterial pathogens, after their detection by the host, through Toll-like receptors (TLRs), macrophages produce inflammatory cytokines and chemokines (e.g., TNFα, IL-1, IL-6, CCL2 and CXCL8) and other mediators, such as the prostaglandins (MEDZHITOV, 2010).

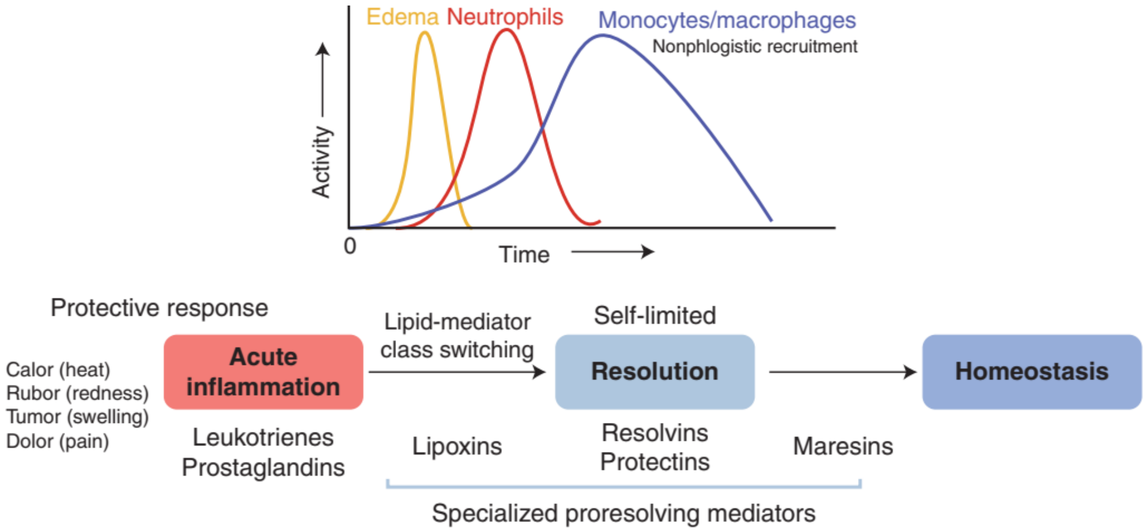

Fig. 3: Time course of the inflammatory process, highliting the different stages of the inflammation and the lipid mediators presence in each stage (SERHAN *et al.*, 2015).

The observed immune response can vary according to the bacterial strain (clinical or lab isolates), mouse model, dose of the inoculum, and the time post-infection that is analyzed. However, a lethal challenge to mice occurs in conditions where bacteria generate a hyperinflammatory response, generating a septic shock (LIN; KAZMIERCZAK, 2017). To invade host lungs, pathogens need to bypass the airway mucus, which possesses a myriad of strategies to combat bacterial invasion, such as: antimicrobial peptides, opsonization, antimicrobial proteins and the alveolar macrophages (LIN; KAZMIERCZAK, 2017). Macrophages represent the first line of the immune response. These phagocytic cells respond to several signals and kill pathogens by the release of several oxidants and by signaling to the recruitment of other phagocytes, such as neutrophils (GWINN; VALLYATHAN, 2006).

Besides its microbicidal activities, oxidants (especially H_2O_2) can also act as second messengers in the NF-κβ and MAPK dependent pathways, triggering a pro-inflammatory response (GWINN; VALLYATHAN, 2006; MORGAN; LIU, 2011; SON *et al.*, 2011). In this way, multiple redox processes are involved in the regulation of NF-κβ (Fig. 4). For instance, NF-κβ regulates the expression of genes encoding antioxidant enzymes (SODs and thioredoxins); and, NADPH Oxidase (NOX2), Cyclooxygenase-2 (COX-2), 12-lipoxygenase (LOX-12) and LOX-5 (MORGAN; LIU, 2011).

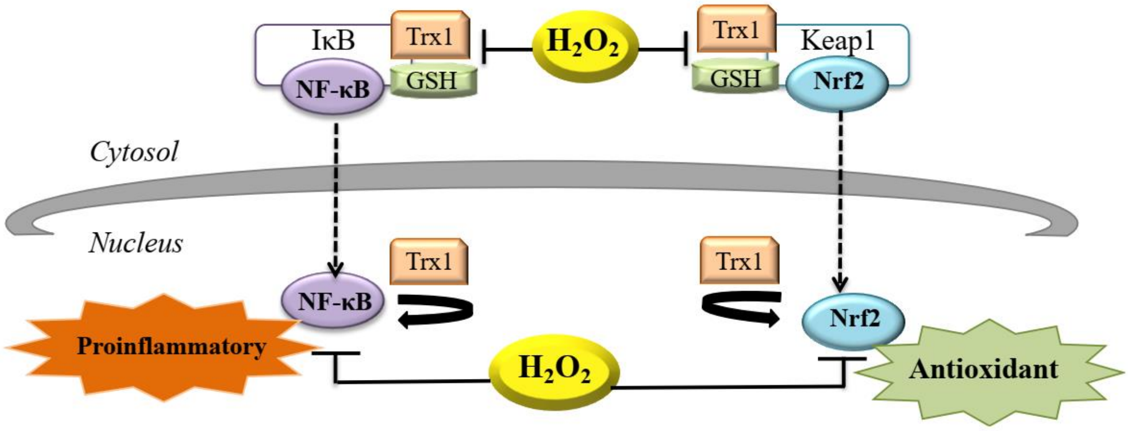

Fig. 4: Proinflammatory and antioxidant signaling relating H_2O_2 and NF-κβ (DI MARZO; CHISCI; GIOVANNONI, 2018).

In addition, lipid molecules promote signaling in both acute inflammation and resolution phases. For instance, prostaglandins and leukotrienes, which are derived from arachidonic acid oxidation through the activities of cyclooxygenase (COX) and 5-lipoxygenase (5-LOX), promote the initiation of the inflammatory process. In contrast, specialized pro-resolving mediators (SPM) stop acute inflammation and thereby promote the resolution step (SERHAN, 2017).

Furthermore, Prxs can interfere in signaling pathways related to both inflammatory and resolution processes. For instance, lipopolysaccharide (LPS) increases the expression of human Prx1 that contributes to alter the levels of pro-inflammatory mediators, resulting in NF-κβ activation (LIU *et al.*, 2014). Meanwhile, the gene expression of Prx6 (human orthologue of PaLsfA) is regulated by cyclooxygenases and prostaglandin E2 in primary macrophages (BAST *et al.*, 2010). Curiously, Prdx6 is capable of activating NOX2 and detoxifying lipid hydroperoxides, by its phospholipase and peroxidase activities (reviewed by (AREVALO; VÁZQUEZ-MEDINA, 2018; ELKO *et al.*, 2019).

Prxs are also present in pathogenic bacteria, but less is known about their involvement in acute inflammation and resolution. Previously, the involvement of PaLsfA was described in *P. aeruginosa* virulence (KAIHAMI *et al.*, 2014). Because of its peroxidase activity, PaLsfA decreases the macrophage oxidative state and protects *P. aeruginosa* from NAPDH oxidase-generated oxidants (KAIHAMI *et al.*, 2014). Additionally, PaLsfA inhibits TNF-α production, influences the recruitment of macrophages and neutrophils and their activation; thus, playing an important role in virulence in acute pneumonia model (KAIHAMI *et al.*, 2014).

1.3.2. Resolutive response

As mentioned above, the inflammatory process is composed of two phases: acute inflammation and resolution (Fig. 3). Problems related to the occurrence of the resolutive phase generate a state of chronic inflammation, often associated with diseases such asthma, cardiovascular diseases, diabetes, and rheumatoid arthritis (LEVY; SERHAN, 2014). Lipid mediators (LM) are involved in regulation of the resolutive phase, such as those derived from arachidonic acid, Eicosapentaenoic acid (EPA), Docosahexaenoic acid (DHA) and n-3 docosapentaenoic acid (n-3 DPA). Their synthesis is precisely and temporally regulated, starting with the increased levels of leukotrienes and prostaglandins during the acute inflammation, followed by a switch to the production of specialized pro-resolving mediators (SPMs), including lipoxins, resolvins and protectins, and maresins, in a spatio-temporal sequence during the resolutive phase (SERHAN *et al.*, 2015). Specialized pro-resolving mediators are a class, derived from ω-6 and ω-3 essential polyunsaturated fatty acids (PUFA) as arachidonic acid (AA), docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), and eicosapentaenoic acid (EPA) capable to sophisticatedly regulate the resolution of inflammation by the formation of four main classes of lipid mediators: lipoxins, resolvins, maresins, and protectins (LEUTI; MACCARRONE; CHIURCHIÙ, 2019).

Lipoxins, generated from arachidonic acid, have both anti-inflammatory and pro-resolving properties, downregulating neutrophil transmigration and increasing the uptake and removal of apoptotic neutrophils by macrophages (LEVY; SERHAN, 2014). Resolvins, derived from EPA (E-series) and DHA (D-Series) with resolutive properties such the capacity to reduce the polymorphonuclear leukocyte (PMN) infiltration and pro-inflammatory cytokine/chemokine production (ARITA *et al.*, 2006), and are capable to enhances bacterial killing/clearance (CODAGNONE *et al.*, 2018; SPITE *et al.*, 2009). Protectins are derived from DHA and are capable of decreasing leukocyte infiltration in murine peritonitis and reduce neutrophil trans endothelial migration, and enhance human macrophage efferocytosis (LEVY; SERHAN, 2014). Finally, maresins are formed from DHA, capable to restore tissue homeostasis after inflammation, counter regulating the proinflammatory cytokines such as IL-1β, IL-6, and TNF-α limiting the recruitment of PMNs and neutrophils, and stimulating phagocytosis and efferocytosis (TANG *et al.*, 2018).

All those SPMs have initial steps in which a hydroperoxide is formed into the PUFA by lipoxygenases (15-LOX, 12-LOX and 5-LOX) or cyclooxygenases (COX-2), as exemplified for the DHA pathway (Fig. 5). In the case of the cysteinyl-SPMs, the conjugation with glutathione is required, which is catalyzed by glutathione transferase or leukotriene C4 synthase, with the generations of: resolvin-CTR (RCTRs), maresin conjugates in tissue regeneration (MCTRs) and protectin-CTR (PCTRs). All these SPMs at pico/nanomolar concentration are capable to protect organs by the stimulation of tissue regeneration (JORDAN; WERZ, 2022; SERHAN; CHIANG, 2023; SERHAN; CHIANG; DALLI, 2018).

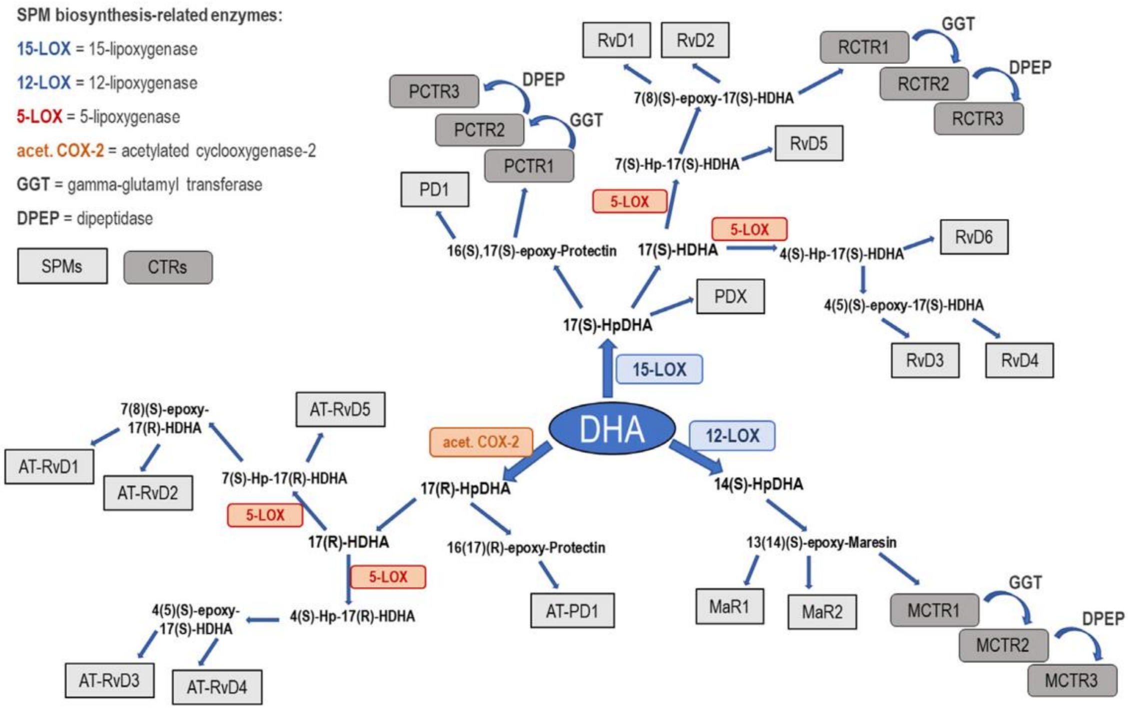

Fig. 5: Scheme of the formation of SPMs derived from DHA, which involves a formation of a hydroperoxide in the first step by 15-LOX, 12-LOX or COX-2 (JORDAN; WERZ, 2022).

Noteworthy, these SPMs are capable of influencing host protection during bacterial infection, decreasing the requirements for antibiotics (reviewed by SERHAN; LEVY, 2018). Of note, lipoxin A_4 can reduce the virulence of *P. aeruginosa* through the inhibition of quorum sensing and increase the phagocytic ability of the neutrophils (WU *et al.*, 2016). Also, lipoxin A_4 and resolvin (Rv)D2 reduced biofilm formation and virulence gene expression (THORNTON *et al.*, 2021).

Therefore, we hypothesize that PaLsfA can regulate both NF-κβ and MAPK pathways, probably by interfering with both the H_2O_2 and fatty acid hydroperoxides levels. Thus, as PaLsfA can control NF-κβ (KAIHAMI *et al.*, 2014), which in turn regulates the expression of LOX-5 and COX2 genes, possibly the levels of these oxygenases might be affected by PaLsfA, and consequently their products.

In this way, we aimed to deeply characterize PaLsfA on its biological influence in *P. aeruginosa*. First, we investigate its relationship with the only reductant agent described so far (by us), ascorbate. Then characterize the roles of PaLsfA in the oxidative defense of *P. aeruginosa* using different techniques. Finally, using macrophages, we intend to verify how the bacterial protein – PaLsfA – could interfere with the inflammatory/resolutive pathways, looking at the lipid mediators.

2. General Methodology

2.1. Table of Primers

Name	Sequence (5’-3’)
PaLsfA^{His37Lys} Foward	Gtgaagtcggccggcttggagaacagcacgc
PaLsfA^{His37Lys} Reverse	Gcgtgctgttctccaagccggccgacttcac
PaLsfA^{His37Phe} Forward	Tgaagtcggccgggaaggagaacagcacgc
PaLsfA^{His37Phe} Reverse	gcgtgctgttctccttcccggccgacttca
PaLsfA^{Thr120Ser} Foward	Cgccaacgacacgctgagcgtgcgttcgctgttc
PaLsfA^{Thr120Ser} Reverse	gaacagcgaacgcacgctcagcgtgtcgttggcg

2.2. PaLsfA mutants’ generation and production

Using the pET15b-PaLsfA^{WT} plasmid as template, the following mutants were generated: PaLsfA^{His37Lys}, PaLsfA^{His37Phe}, PaLsfA^{Thr120Ser}. Primers were constructed using the Agilent web tool (https://www.agilent.com/store/primerDesignProgram.jsp). All the reactions were carried using the QuikChange II Site-Directed Mutagenesis Kit (Agilent) following the manufacturer instructions. The procedure used for expression and purification of PaLsfA mutants was the same as for the PaLsfA^{WT}, previously established by me during my masters (ALEIXO-SILVA, 2018).

2.3. Competition assay between DCPIP and PaLsfA for ascorbate.

Recombinant PaLsfA was first reduced overnight using an 20x excess of DTT (1,4-Dithiothreitol), that was after removed using two HiTrap™ Desalting coupled and the reduced protein was collected following the UV spectra in the FPLC. To obtain the oxidized form (sulfenic acid), PaLsfA was treated using a ratio of 1:1 of H_2O_2. Oxidation state of PaLsfA was confirmed using DTDPy (aldrithiol, Cat. 143057 - Sigma-Aldrich). Both DCPIP (ε_{600nm} = 20500 M^{-1} cm^{-1}) and ascorbate (ε_{265nm} = 14500 M^{-1} cm^{-1}) solutions were prepared fresh and quantified spectrophotometrically in the assay buffer (50 mM potassium phosphate pH 7.2, 50 mM NaCl). Reaction mixtures contained 4 µM ascorbate (injected to start the kinetics), DCPIP (45 µM) and variable protein concentrations (nine replicates per concentration) in the same potassium phosphate buffer described above. In some points of the dataset, we attempted to use the highest possible protein concentration, so the difference between the initial and final absorbance would be higher. Reactions were monitored at 600 nm (oxidized DCPIP) in a Synergy H1 (BioTek® Instruments, Inc.) plate reader with automated injection for 2 minutes at 25 ºC.

2.4. Disk diffusion assay.

This assay was followed according a pre-established protocol (KAIHAMI *et al.*, 2014). An overnight culture of PA14 WT or Δ*lsfA* (both harboring the empty PJN105) were diluted to O.D.$_{600nm}$=0.1 and grown until O.D.$_{600nm}$=1. In a petri dish containing solid LB, 3 mL of 0.7% soft LB agar + 200 µL bacterial culture was added, in order to form a thin layer of cells. After solidification, sterile paper disks (~6mm diameter), three per plate, were saturated with 2.5% H_2O_2 or 1% t-BOOH and placed on the plate. Plates were incubated for 16 hours/37ºC. and photographed. Inhibition halos were measured using imageJ software, in cm. Experiments performed in triplicates and the results were expressed as a percentage of the WT halo.

2.5. CFU (colony-forming unit) assay.

This experiment was performed as described before by our group (ALEGRIA *et al.*, 2017). Bacterial strains -WT or Δ*lsfA* (both harboring the empty PJN105) - were grown in LB broth + 50µg/mL gentamycin 37ºC to O.D.$_{600nm}$=1, washed twice with PBS and diluted to O.D.$_{600nm}$=0.1. 200 µL aliquots of cell suspensions were treated with 5mM ATZ for 10 minutes at room temperature, to inhibit catalase. Then, 3 mM SIN-1 or 2.5 mM paraquat were added and incubated for 30 min at 37ºC. Treated cells were serial diluted in 10 mM MgSO4 and plated on solid LB broth (without antibiotic). Colonies-forming units (CFU) were counted after 16 hours/37ºC. The experiments were expressed as a percentage in relation to the untreated cells. Experiments performed at least in triplicates.

2.6. Ascorbate/Dehydroascorbate *in vivo* quantification.

PA14 WT was grown overnight in LB media, washed and diluted to O.D. = 1 in 50 mL of PBS containing 10 mM of ascorbate and incubate at 37 C for seven hours, which represented time zero. After 1, 3 or 7 hours, cells were collected by centrifugation and ascorbate/dehydroascorbate content was analysed in the supernatant. In order to quantify the intracellular content, the pellet was washed twice with PBS and resuspended into 2mL of PBS. Cells were disrupted by 1-minute cycles of sonication (15s ON/45s OFF) in ice. Then, the methanol method to quantify both dehydroascorbate and ascorbate were employed as described (BADRAKHAN *et al.*, 2004).

2.7. Hyper7 in *P. aeruginosa.*

Freshly transformed cells of PA14 WT and ΔlsfA strains, containing both plasmid pUCP18-Hyper7 and pUCP18-Empty, were grown overnight in LB media containing 300 ug/mL of carbenicillin. In the morning, cells were pelleted and diluted to $O.D._{600nm}$=7 in PBS. Using a Synergy H1(BioTek® Instruments, Inc.) plate reader with automated injection, 180 µL of the cells were added in duplicates into a 96 wells black plate, with transparent bottom. A 2-minute baseline was measured before the addition of H_2O_2, which was automatically added in different concentrations. After addition, the fluorescence was measured for about 20 minutes using excitation at 408 nm and 488 nm and emission at 520 nm (KRITSILIGKOU; SHEN; DICK, 2021), reading the plate from the bottom. Results were expressed as the 488 nm/408 nm ratio of the fluorescence excitations. For the measures of ascorbate reduction, after the pulse of 4mM of H_2O_2 for 1-minute, different concentrations of ascorbate was added.

2.8. Phosphorylation Treatments of Non-Mammalian Prdx6.

Using the human kinase Erk2 (GI: 119554) to phosphorylate the recombinant Prdx6 proteins (150 ng/µL), we performed the assay in a 50 mM Tris-Cl pH 7.5, 20 µM EGTA buffer, containing 10 mM MgCl2 and 2 mM ATP in the presence or absence, of active Erk2 kinase (10 ng/µL). Reactions were carried out for 90 minutes at 30 ºC with shaking. For Erk2 dilution, we followed the manufacturer instruction, using the buffer 50 mM Tris-

Cl pH 7.5, 0.1 mM EGTA, with or without 0.1 mM Na_3VO_4, 0.1% 2-mercaptoethanol and 1 mg/mL bovine serum albumin (BSA).

2.9. Reduction, Alkylation and Tryptic Digestion for MS Samples.

Phosphorylated proteins were mixed with 100 mM ammonium bicarbonate buffer pH 8 plus 8 M urea (1:1) to denaturate them, reduced with 10 μL of 10 mM dithiothreitol (DTT) for 1 h at 30º C. Afterwards, samples were alkylated in an amber tube using the Eppendorf ThermoMixer (Eppendorf, Hamburg, Germany) by adding 10 μL of 500 mM iodoacetamide for 30 min at 25º C (final volume 140 μL). Samples were then diluted with 540 μL of 100 mM ammonium bicarbonate buffer pH 8 and digested by adding 3 μL of Trypsin Gold (Promega, Madison, USA) 40 ng/μL (protein/enzyme ratio of 50:1) for 16 h at 37º C. Resulting peptides were neutralized by adding 0.1% trifluoroacetic acid and completely dried in speed vac. Finally, samples were concentrated using ZipTip resin (Merck, Darmstadt, Germany) according to the manufacturer's protocol, dried again and stored at -20º C until use.

2.10. Evaluation of Thr Phosphorylation by MS

To check the Phosphorylation of Prdx6 proteins, we used liquid chromatography-tandem mass spectrometry (LC-MS/MS) using a nanoACQUITY UPLC system (Waters Corporation, Milford, USA) coupled to a TripleTOF 6600 mass spectrometer (AB SCIEX, Framingham, USA). Analysis was conducted under trap and eluted mode using a nanoACQUITY UPLC-Symmetry (Waters Corporation, Milford, USA) containing a C18 trap column (20mm × 180μm; 5μm) and a separation column (75 μm × 150mm; 3.5 μm). Trapping was done at 10μL/min with 2% of solvent B. Peptides were separated with mobile phase A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) at a flow rate of 0.4 μL/min using the following gradient: 2–35% B from 0 to 60 min; 35–85% B from 60 to 61 min; isocratic elution with 85% B from 61 to 65 min; 85–2% B from 65 to 66 min; isocratic elution with 2% B from 66 to 85 min. Nano-electrospray ion source was operated at 2.2 kV (ion spray voltage floating, ISVF), curtain gas 20, interface heater (IHT) 120, ion source gas 1 (GS1) 3, ion source gas 2 (GS2) zero, declustering potential (DP) 80V. Time-of-flight mass spectrometry (TOF-MS) and mass spectrometry analysis (MS/MS) data were acquired using information-dependent acquisition (IDA) mode. For IDA parameters, a 250ms survey scan in the m/z (mass-to-charge ratio) range of 300–2000 was followed by 25 MS/MS ions in the m/z range of 100–2000 acquired with an accumulation time of 100ms (total cycle time 2.8 s). Switch criteria included intensity greater than 150 counts and charge state 2–5. Former target ions were excluded for 4 s. Software used for acquisition and data processing were Analyst®TF 1.7 (AB SCIEX, Framingham, USA) and PeakView®2.2 (AB SCIEX, Framingham, USA), respectively. For the analysis of protein modification, MASCOT 2.4 software (Matrix Science Ltd., London, United Kingdom, Redoxoma-FAPESP user license 10.10.1.46/Mascot) was used with mass tolerance of 10ppm for MS experiments and 0.05 Da for MS/MS experiments.

2.11. Hydroperoxides Biosynthesis and Isolation.

Comercial 15-LOX (Soybean, type V, sigma (L6632)) was used to produce hydroperoxides derived from fatty acids as described before (DALLI; COLAS; SERHAN, 2013). Briefly, 30 U of 15-LOX was incubated with 0.5 ug/µl of arachidonic acid (AA)/ 100 ng of n-3 docosapentaenoic acid (n-3 DPA) / 75 ng of docosahexaenoic acid (DHA)/ 75 ng of eicosapentaenoic acid (EPA) and submitted to sonication/vortexing cycles (30 seconds each) every four minutes. The reaction was carried for 20 min/ice in a 5 mL of borate buffer, to produce: 15-HpETE, 17-HpDPA, 17-HpDHA, and 15- HpEPE respectively. Then, reaction was stopped by adding one volume of methanol. Samples were submitted to a liquid-liquid extraction by adding 2 volumes of diethyl ether, transferring the top layer, and fully evaporating; at the end, the samples were resuspended in 50 uL of methanol. Finally, the samples were purified using a C18 column in an HPCL system and quantified spectrophotometrically at 234 nm. After the biosynthesis, all the hydroperoxides were confirmed by LC-MS-MS analysis, using NaBH4 to reduce the hydroperoxide and generate the corresponding reduced product, that is more stable to be identified.

2.12. Enzymatic Assays.

To obtain the reduced protein, LsfA was first incubated with 20x excess of DTT (dithiothreitol) overnight in the fridge and then the DTT was removed. Incubation of reduced LsfA (0.2 µM) and different concentrations (indicated in the text) of the hydroperoxides was performed for 1 minute at room temperature, in the protein buffer (Hepes 20 mM, NaCl 150 mM). The reaction was stopped by adding two volumes of methanol+internal standard (d85HETE). The reduced product for each hydroperoxide was measured by mass spectrometry, comparing samples in presence of LsfA or without this enzyme. The obtained pg/mL concentration was converted to velocity (Mol/second) to obtain the individual k for each hydroperoxide (Hp) concentration, using the following equation:

$$k = \frac{v\, M/s}{[LsfA]M * [Hp]M} \qquad k = \frac{M/s}{M^2} \quad k = \frac{s * M/s}{s * M^2} \qquad k = \frac{M}{s * M^2} \qquad k = \frac{}{s * M} \qquad k = M^{-1} * s^{-1}$$

Finally, to determine the k of LsfA with each hydroperoxide, the individual k were plotted against the hydroperoxide concentration, the slope of the resulting equation is the final k.

2.13. Macrophages culture.

Differentiated macrophages were obtained from peripheral blood mononuclear cells (PBMCs) as described (DALLI; SERHAN, 2012). Briefly, isolated human monocytes were used to obtain M1 macrophages by incubating with GM-CSF (20 ng/ ml) for 6 days in RMPI 1640 (supplemented with 10% fetal bovine serum, 2 mmol/l L-glutamine, and penicillin/streptomycin), followed by LPS (100 ng/ml) plus INF-γ (20 ng/ml) treatment for 48 h. To M2 20 ng/ml M-CSF in RPMI 1640 is used for 6 days followed by polarization with 20 ng/ml IL-4 for 48 h.

2.14. Co-incubation of macrophages with *P. aeruginosa.*

PA14 WT and Δ*lsfA* strains were grown overnight in LB media containing gentamicin. In the morning, these cultures were harvested by centrifugation and resuspended into PBS and the optical density was measured. One million differentiated macrophages M1 or M2 were added to the wells of an 12/6 well plate in the RPMI 1640 media without serum. For all the experiments we used a multiplicity of infection (MOI) of 1:50 cells:bacteria. The co-incubation was carried out for 45 minutes or 24 hours and stopped by the addition of methanol or perchloric acid, depending on the subsequent experiment.

2.15. Lipid mediator profiling.

At the end of each co-incubation, was added two volumes of methanol + deuterium-labelled internal standards (IS), and the macrophages were scraped from the wells and stored at -80°C until analysis. Extraction procedure and LC–MS–MS were performed as previously described (COLAS; GOMEZ; DALLI, 2020). Resulting peaks were identified and integrated using the SCIEX OS software; Partial Least-Squares Discriminant Analysis (PLS-DA) was performed in the MetaboAnalist web server (https://www.metaboanalyst.ca/), whilst the pathway analysis figure was generated using the Cytoscape software.

7. General Discussion and Conclusion

Until now, the only description about PaLsfA was describing its involvement with the virulence of *P. aeruginosa* (KAIHAMI *et al.*, 2014) and in proteomic/transcriptomic studies revealing the changes in its levels in response to sulfate or some oxidants (HARE *et al.*, 2011b; QUADRONI *et al.*, 1999; TRALAU *et al.*, 2007). In this way, the work presented in chapter 3 strongly contributed to the understanding of how this antioxidant protein works in *P. aeruginosa*. The kinetic aspect of PaLsfA was the initial step to understand the capacity of this protein in the antioxidant defense. It is well known that the reactivity of the Prxs with different oxidants is in the range $10^6 – 10^8$ $M^{-1}.s^{-1}$ (RHEE, 2016), so despite the very efficient reaction between PaLsfA and H_2O_2, t-BOOH, peroxynitrite (chapter 3), 15 HpETE and 17 HpDPA (chapter 6) this results was not a surprise. Several Prxs were related with the antioxidant defense of microorganisms and with its virulence (DE OLIVEIRA *et al.*, 2021). As an example of its versatility, *P. aeruginosa* possess an outstanding number of antioxidant proteins and systems to support its activity. To note, this bacterium has two superoxide dismutase (SodM and SodB); five heme-peroxidases (chloroperoxidase, cytochrome c peroxidase and the catalases, KatA, KatB and KatE); eleven thiol peroxidases (thiol peroxidase - Tpx, cytoplasmic glutathione peroxidase - GPx, periplasmic GPx2 and GPx3, osmotically inducible protein C – OsmC, organic hydroperoxide resistance protein – Ohr, bacterioferritin comigratory protein – Bcp, 1-Cys-peroxiredoxin PaLsfA and the 2-Cys-peroxiredoxins alkyl hydroperoxide reductases AhpA, AhpB and AhpC) (https://www.pseudomonas.com). Among them, some have been already characterized and related with its virulence, as the KatA in acute infection in Drosophila (APIDIANAKIS; RAHME, 2009) and peritonitis in mice (LEE *et al.*, 2005). Beyond PaLsfA, another Prx was already related with the virulence of *P. aeruginosa* - AhpC1 – capable to protect the bacterium against the inflammatory oxidative burst caused by urate hydroperoxide (ROCHA *et al.*, 2021). Noteworthy, PaLsfA also contributed to the resistance of *P. aeruginosa* against HOCl (ROCHA *et al.*, 2021).

Members of the Prx6 subgroup typically possess a dimeric or decamer/dodecamer organization, which is shared with the Prx1 subgroup (POOLE; NELSON, 2016). In general, archaeal Prxs tends to present a higher oligomerization state, while the others tend to form only dimers, at least for the Prx6 subgroup. As shown in chapter 3, PaLsfA is a Prx6 member that forms only dimer in all the oxidation states analyzed.

As our group first described the reaction of ascorbate with 1-Cys Prxs (MONTEIRO *et al.*, 2007), the next step was to kinetically characterize this reaction. Using different 1-Cys Prxs, and a 1-Cys like (a Tsa2, from *S. cerevisiae,* with a mutation in the resolution cysteine), we showed a very consistent second order rate constant of 10^3 $M^{-1}.s^{-1}$ for all of them, as described in chapter 4. We further characterize this reaction with PaLsfA using the solved crystallographic structures and a molecular docking approach. This strategy revealed some amino acids which frequently interact with the ascorbate molecule. The same approach used in chapter 4 was used to characterize this reaction with the mutants for these amino acids. Surprisingly, the resulting second order constant rate was higher in those mutants of the His37 and Thr120. Revealing ascorbate as the only reductant of PaLsfA until now, however the biological implications of this molecule remained unclear.

Another putative activity of several Prx6 is the phospholipase A_2 calcium independent activity, which is well described for the mammalian homologue (CHEN *et al.*, 2000), but never described for any other member of this subgroup before. Here, we characterized, in chapter 5, the phospholipase activity of PaLsfA 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), the main component of the pulmonary surfactant (VELDHUIZEN *et al.*, 1998), as 3.38 ± 0.1 at acidic pH and 0.04 ± 0.002 at neutral pH; additionally to others non-mammalian Prdx 6. Despite the phosphorylation of the *Triticum aestivum*, *Arabidopsis thaliana* and *Aspergillus fumigatus* Prdx6, we could not identify PaLsfA phosphorylation by the human Erk2 kinase, even with the high conservation of the Thr residue in the primary and tertiary structure of these proteins. It is reasonable to propose that this Thr in PaLsfA might be phosphorylated by some endogenous kinase from *P. aeruginosa*, but not the human Erk2 kinase. For instance, the ppkA protein from *P. aeruginosa* – a Ser/Thr protein kinase – was already related to environmental adaptation and virulence, variations of biofilm formation, pyocyanin production, tolerance to stress, cell invasion and plant virulence; curiously the response to oxidative stress is also affected by the lack of this protein (PAN *et al.*, 2017). This protein shares ~30% identity with human Erk2.

Biological implications of the phospholipase activity from PaLsfA remains unclear in *P. aeruginosa*; however, under the tested conditions, it is not related with the role of PaLsfA in the virulence of *P. aeruginosa* (KAIHAMI *et al.*, 2014). *P. aeruginosa*, as an extremely versatile pathogen, has several phospholipases, among them is ExoU, which is associated with the type III secretion system. Upon its release, ExoU is capable to disrupt the integrity of eukaryotic cell membranes and lead to their rapid lysis; and consequently being related with bacterial virulence (VASIL, 2006). Curiously, the cytosolic phospholipase A_2 from epithelial cells is activated during the *P. aeruginosa* infection, resulting in a release of arachidonic acid (KIRSCHNEK; GULBINS, 2006).

In addition to the capacity of protection of *P. aeruginosa* against different stresses, this bacterium is algo capable to sabotage the host signaling pathways to disrupt and interfere into the inflammatory response, specially within the lipid mediators. An epoxide hydrolase, cystic fibrosis transmembrane conductance regulator inhibitory factor (Cif), is capable to disrupt 15-epi LXA_4 transcellular biosynthesis and function by the hydrolyzation of 14,15-EET, suppressing the transepithelial neutrophil migration (FLITTER *et al.*, 2017). *P. aeruginosa* can also produce LoxA – a 15-LOX -, which can catalyze the peroxidation of several free fatty acids (including arachidonic acid) with positional specificity. This enzyme cannot react with 5- or 15-HETEs, and is required biofilm growth in association with the host airway epithelium (DESCHAMPS *et al.*, 2016; VANCE *et al.*, 2004). Further, the activity of LoxA decrease the recruitment of immune cells in the airspace, modulate the cell response of macrophages and neutrophils, and can ultimately induce the generation of LXA4 by neutrophils (MORELLO *et al.*, 2019). ExoU, a bacterial secreted phospholipase, is capable to promote a release of arachidonic acid and the production of prostaglandins PGE_2 and PGI_2, in addition to the increase in the mono/polymorphonuclear cells recruitment (SALIBA *et al.*, 2005).

Despite the clear capacity of *P. aeruginosa* interfering into the host inflammatory response, it is unknown to us the involvement of a bacterial antioxidant protein in this process. Here, in chapter 6, we first described the involvement of a peroxiredoxin in the production/signalization of lipid mediators related to the inflammatory and resolutive response. The strong influence of PaLsfA can be noted in the increased release of free PUFAs in the initial steps of co-incubation, for both macrophage subtypes and the lower levels of hydroxides in Δ*lsfA* strain, revealing the antioxidant capacity of PaLsfA interfering into the signalization of the macrophages. Results from the disbalance caused by PaLsfA are noted after these cells were subjected to a lipid mediator profiling, clearly showing differences in several analytes present in all the pathways analysed. Curiously, maresin 1 – MaR1 – in all cases, was downregulated in the Δ*lsfA* co-incubation. MaR1 can limit polymorphonuclear neutrophil (PMN) infiltration and enhance human macrophage uptake of apoptotic PMNs, as stimulate efferocytosis; acting as a potent anti-inflammatory and pro-resolving molecule (SERHAN *et al.*, 2012). It is known that *P. aeruginosa* can survive in a hyperinflammatory state, as the one faced during cystic fibrosis infections. (COHEN; PRINCE, 2012).

Another possible step affected by PaLsfA is through its antioxidant activity and the consequent reduction in macrophages oxidative state (KAIHAMI *et al.*, 2014). Our data revealed a strong impact into the mediators which requires a glutathione transferase activity to be formed, including cysteinyl leukotrienes, PCTRs and MCTRs. The biosynthesis of these compound could be impaired by the alterations in the redox state of the macrophages and consequently in the glutathione pool. Finally, the overall changes in the pathways analysed could suggest a broad interference of PaLsfA by the change in the spatio-temporal production of the initial signalization molecules, disturbing the whole inflammatory/resolutive process.

In conclusion, our findings revealed PaLsfA as a very efficient antioxidant protein, capable to react with w wide range of hydroperoxides, and protect *P. aeruginosa* from superoxide and H_2O_2. We newly described two crystallographic structures of this protein, which enabled us to deeper characterize the reduction of PaLsfA by ascorbate, its only reductant until now. The description of a PLA_2 activity for PaLsfA includes this protein in a group of peroxiredoxins with this activity which included only the mammal isoforms before. Finally, the influence of PaLsfA in the inflammatory lipid mediator biosynthesis opens a new perspective about host-pathogen interaction affected by bacterial antioxidant proteins.

8. References

ALDERTON, W. K.; COOPER, C. E.; KNOWLES, R. G. Nitric oxide synthases: Structure, function and inhibition. **Biochemical Journal**, v. 357, n. 3, p. 593–615, 2001.

ALEGRIA, T. G. P.; MEIRELES, D. A.; CUSSIOL, J. R. R.; HUGO, M.; TRUJILLO, M.; DE OLIVEIRA, M. A.; MIYAMOTO, S.; QUEIROZ, R. F.; VALADARES, N. F.; GARRATT, R. C.; RADI, R.; DI MASCIO, P.; AUGUSTO, O.; NETTO, L. E. S. Ohr plays a central role in bacterial responses against fatty acid hydroperoxides and peroxynitrite. **Proceedings of the National Academy of Sciences**, v. 114, n. 2, p. E132–E141, 10 jan. 2017. Disponível em: <http://www.pnas.org/lookup/doi/10.1073/pnas.1619659114>.

ALEIXO-SILVA, R. L. **Caracterização estrutural e bioquí-mica de LsfA, uma 1-Cys Prx envolvida na virulência de Pseudomonas aeruginosa**. 2018. Universidade de São Paulo, São Paulo, 2018. Disponível em: <http://www.teses.usp.br/teses/disponiveis/41/41131/tde-20082018-130732/>.

ANVISA. Boletim de Segurança do Paciente e Qualidade em Serviços de Saúde nº 14: Avaliação dos indicadores nacionais das Infecções Relacionadas à Assistência à Saúde (IRAS) e Resistência microbiana do ano de 2015. **Segurança do Paciente e Qualidade em Serviços de Saúde**, v. 16, p. 82, 2016. Disponível em: <http://portal.anvisa.gov.br/documents/33852/3074203/Boletim+de+Segurança+do+Paciente+e+Qualidade+em+Serviços+de+Saúde+nº+14+Avaliação+dos+indicadores+nacionais+das+Infecções+Relacionadas+à+Assistência+à+Saúde+%28IRAS%29+e+Resistência+microbiana/dbd57c96-9>.

APIDIANAKIS, Y.; RAHME, L. G. Drosophila melanogaster as a model host for studying Pseudomonas aeruginosa infection. **Nature Protocols**, v. 4, n. 9, p. 1285–1294, 2009.

AREVALO, J. A.; VÁZQUEZ-MEDINA, J. P. The role of peroxiredoxin 6 in cell signaling. **Antioxidants**, v. 7, n. 12, 2018.

ARITA, M.; OH, S. F.; CHONAN, T.; HONG, S.; ELANGOVAN, S.; SUN, Y. P.; UDDIN, J.; PETASIS, N. A.; SERHAN, C. N. Metabolic inactivation of resolvin E1 and stabilization of its anti-inflammatory actions. **Journal of Biological Chemistry**, v. 281, n. 32, p. 22847–22854, 2006. Disponível em: <http://dx.doi.org/10.1074/jbc.M603766200>.

BADRAKHAN, C. D.; PETRAT, F.; HOLZHAUSER, M.; FUCHS, A.; LOMONOSOVA, E. E.; DE GROOT, H.; KIRSCH, M. The methanol method for the quantification of ascorbic acid and dehydroascorbic acid in biological samples. **Journal of Biochemical and Biophysical Methods**, v. 58, n. 3, p. 207–218, 2004.

BAST, A.; ERTTMANN, S. F.; WALTHER, R.; STEINMETZ, I. Influence of iNOS and COX on peroxiredoxin gene expression in primary macrophages. **Free Radical Biology and Medicine**, v. 49, n. 12, p. 1881–1891, 2010. Disponível em: <http://dx.doi.org/10.1016/j.freeradbiomed.2010.09.015>.

BRYK, R.; GRIFFIN, P.; NATHAN, C. Peroxynitrite reductase activity of bacterial peroxiredoxins. **Nature**, v. 407, n. 6801, p. 211–215, 2000.

CALCERRADA, P.; PELUFFO, G.; RADI, R. Nitric oxide-derived oxidants with a focus on peroxynitrite molecular targets, cellular responses and therapeutic implications. **Current Pharmaceutical Design**, v. 17, p. 3905–3932, 2011.

CHATTERJEE, S.; FEINSTEIN, S. I.; DODIA, C.; SOROKINA, E.; LIEN, Y.-C.; NGUYEN, S.; DEBOLT, K.; SPEICHER, D.; FISHER, A. B. Peroxiredoxin 6 Phosphorylation and Subsequent Phospholipase A 2 Activity Are Required for Agonist-mediated Activation of NADPH Oxidase in Mouse Pulmonary Microvascular Endothelium and Alveolar Macrophages. **Journal of Biological Chemistry**, v. 286, n. 13, p. 11696–11706, 1 abr. 2011. Disponível em: <http://www.jbc.org/lookup/doi/10.1074/jbc.M110.206623>.

CHEN, J. W.; DODIA, C.; FEINSTEIN, S. I.; JAIN, M. K.; FISHER, A. B. 1-Cys peroxiredoxin, a bifunctional enzyme with glutathione peroxidase and phospholipase A2 activities. **Journal of Biological Chemistry**, v. 275, n. 37, p. 28421–28427, 2000.

CHEN, L.; DENG, H.; CUI, H.; FANG, J.; ZUO, Z.; DENG, J.; LI, Y.; WANG, X.; ZHAO, L. Inflammatory responses and inflammation-associated diseases in organs. **Oncotarget**, v. 9, n. 6, p. 7204–7218, 23 jan. 2018. Disponível em: <www.impactjournals.com/oncotarget/>.

CIRZ, R. T.; O’NEILL, B. M.; HAMMOND, J. A.; HEAD, S. R.; ROMESBERG, F. E. Defining the Pseudomonas aeruginosa SOS response and its role in the global response to the antibiotic ciprofloxacin. **Journal of Bacteriology**, v. 188, n. 20, p. 7101–7110, 2006.

CODAGNONE, M.; CIANCI, E.; LAMOLINARA, A.; MARI, V. C.; NESPOLI, A.; ISOPI, E.; MATTOSCIO, D.; ARITA, M.; BRAGONZI, A.; IEZZI, M.; ROMANO, M.; RECCHIUTI, A. Resolvin D1 enhances the resolution of lung inflammation caused by long-term Pseudomonas aeruginosa infection. **Mucosal Immunology**, v. 11, n. 1, p. 35–49, 2018. Disponível em: <http://dx.doi.org/10.1038/mi.2017.36>.

COHEN, T. S.; PRINCE, A. Cystic fibrosis: A mucosal immunodeficiency syndrome. **Nature Medicine**, v. 18, n. 4, p. 509–519, 2012.

COLAS, R. A.; GOMEZ, E. A.; DALLI, J. Methodologies and Procedures Employed in the Identification and Quantitation of Lipid Mediators via LC-MS/MS. **Research Square**, p. 1–16, 2020.

CONRAD, M.; FRIEDMANN ANGELI, J. P. **Glutathione Peroxidases**. [s.l.] Elsevier, 2018. v. 10–15260–276 p.

CORDRAY, P.; DOYLE, K.; EDES, K.; MOOS, P. J.; FITZPATRICK, F. A. Oxidation of 2-cys-peroxiredoxins by arachidonic acid peroxide metabolites of lipoxygenases and cyclooxygenase-2. **Journal of Biological Chemistry**, v. 282, n. 45, p. 32623–32629, 2007. Disponível em: <http://dx.doi.org/10.1074/jbc.M704369200>.

COSGROVE, K.; COUTTS, G.; JONSSON, I. M.; TARKOWSKI, A.; KOKAI-KUN, J. F.; MOND, J. J.; FOSTER, S. J. Catalase (KatA) and alkyl hydroperoxide reductase (AhpC) have compensatory roles in peroxide stress resistance and are required for survival, persistence, and nasal colonization in Staphylococcus aureus. **Journal of Bacteriology**, v. 189, n. 3, p. 1025–1035, 2007.

COURTNEY, J. M.; BRADLEY, J.; MCCAUGHAN, J.; O’CONNOR, T. M.; SHORTT, C.; BREDIN, C. P.; BRADBURY, I.; ELBORN, J. S. Predictors of mortality in adults with cystic fibrosis. **Pediatric Pulmonology**, v. 42, n. 6, p. 525–532, jun. 2007. Disponível em: <http://doi.wiley.com/10.1002/ppul.20619>.

CROSS, C. E.; HALLIWELL, B.; BORISH, E. T.; PRYOR, W. A.; AMES, B. N.; SAUL, R. L.; MCCORD, J. M.; HARMAN, D. Oxygen radicals and human disease. Davis conference. **Annals of Internal Medicine**, v. 107, n. 4, p. 526–545, 1987.

CUSSIOL, J. R. R.; ALEGRIA, T. G. P.; SZWEDA, L. I.; NETTO, L. E. S. Ohr (organic hydroperoxide resistance protein) possesses a previously undescribed activity, lipoyl-dependent peroxidase. **Journal of Biological Chemistry**, v. 285, n. 29, p. 21943–21950, 2010.

DALLI, J.; COLAS, R. A.; SERHAN, C. N. Novel n-3 Immunoresolvents: Structures and Actions. 2013.

DALLI, J.; SERHAN, C. N. Specific lipid mediator signatures of human phagocytes: Microparticles stimulate macrophage efferocytosis and pro-resolving mediators. **Blood**, v. 120, n. 15, p. 60–72, 2012.

DE OLIVEIRA, M. A.; TAIRUM, C. A.; NETTO, L. E. S.; DE OLIVEIRA, A. L. P.; ALEIXO-SILVA, R. L.; CABRERA, V. I. M.; BREYER, C. A.; DOS SANTOS, M. C. Relevance of peroxiredoxins in pathogenic microorganisms. **Applied Microbiology and Biotechnology**, v. 105, n. 14–15, p. 5701–5717, 2021.

DESCHAMPS, J. D.; OGUNSOLA, A. F.; JAMESON, J. B.; YASGAR, A.; FLITTER, B. A.; FREEDMAN, C. J.; MELVIN, J. A.; NGUYEN, J. V. M. H.; MALONEY, D. J.; JADHAV, A.; SIMEONOV, A.; BOMBERGER, J. M.; HOLMAN, T. R. Biochemical and Cellular Characterization and Inhibitor Discovery of Pseudomonas aeruginosa 15-Lipoxygenase. **Biochemistry**, v. 55, n. 23, p. 3329–3340, 2016.

DI MARZO, N.; CHISCI, E.; GIOVANNONI, R. The role of hydrogen peroxide in redox-dependent signaling: Homeostatic and pathological responses in mammalian cells. **Cells**, v. 7, n. 10, 2018.

DI MEO, S.; REED, T. T.; VENDITTI, P.; VICTOR, V. M. **Role of ROS and RNS Sources in Physiological and Pathological ConditionsOxidative Medicine and Cellular Longevity**2016.

DIGGLE, S. P.; WHITELEY, M. Microbe Profile: Pseudomonas aeruginosa: opportunistic pathogen and lab rat. **Microbiology**, v. 166, n. 1, p. 30–33, 1 jan. 2020. Disponível em: <https://www.microbiologyresearch.org/content/journal/micro/10.1099/mic.0.000860>.

DOMINGOS, R. M.; TEIXEIRA, R. D.; ZEIDA, A.; AGUDELO, W. A.; ALEGRIA, T. G. P.; DA SILVA NETO, J. F.; VIEIRA, P. S.; MURAKAMI, M. T.; FARAH, C. S.; ESTRIN, D. A.; NETTO, L. E. S. Substrate and product-assisted catalysis: Molecular aspects behind structural switches along organic hydroperoxide resistance protein catalytic cycle. **ACS Catalysis**, v. 10, n. 12, p. 6587–6602, 2020.

DONLAN, R. M. Biofilms and device-associated infections. **Emerging Infectious Diseases**, v. 7, n. 2, p. 277–281, 2001.

DUBEY, S. K.; TOKASHIKI, T.; SUZUKI, S. Microarray-mediated transcriptome analysis of the tributyltin (TBT)-resistant bacterium Pseudomonas aeruginosa 25W in the presence of TBT. **Journal of microbiology (Seoul, Korea)**, v. 44, n. 2, p. 200–5, 2006. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/16728957>.

ELKO, E. A.; CUNNIFF, B.; SEWARD, D. J.; CHIA, S. B.; ABOUSHOUSHA, R.; VAN DE WETERING, C.; VAN DER VELDEN, J.; MANUEL, A.; SHUKLA, A.; HEINTZ, N. H.; ANATHY, V.; VAN DER VLIET, A.; JANSSEN-HEININGER, Y. M. W. Peroxiredoxins and Beyond; Redox Systems Regulating Lung Physiology and Disease. **Antioxidants & Redox Signaling**, p. 1–62, 2019.

FERNANDES, A. P.; HOLMGREN, A. Glutaredoxins: Glutathione-Dependent Redox Enzymes with Functions Far Beyond a Simple Thioredoxin Backup System. **Antioxidants & Redox Signaling**, v. 6, n. 1, p. 63–74, fev. 2004. Disponível em: <http://www.liebertpub.com/doi/10.1089/ars.2007.1903>.

FINKEL, T. Signal transduction by reactive oxygen species. **Journal of Cell Biology**, v. 194, n. 1, p. 7–15, 2011.

FISHER, A. B. Peroxiredoxin 6: A Bifunctional Enzyme with Glutathione Peroxidase and Phospholipase A $_2$ Activities. **Antioxidants & Redox Signaling**, v. 15, n. 3, p. 831–844, 2011. Disponível em: <http://www.liebertonline.com/doi/abs/10.1089/ars.2010.3412>.

FISHER, A. B. Peroxiredoxin 6 in the repair of peroxidized cell membranes and cell signaling. **Archives of Biochemistry and Biophysics**, v. 617, p. 68–83, mar. 2017. Disponível em: <http://dx.doi.org/10.1016/j.abb.2016.12.003>.

FISHER, A. B.; DODIA, C.; FEINSTEIN, S. I.; HO, Y. S. Altered lung phospholipid metabolism in mice with targeted deletion of lysosomal-type phospholipase A2. **Journal of Lipid Research**, v. 46, n. 6, p. 1248–1256, 2005. Disponível em: <http://dx.doi.org/10.1194/jlr.M400499-JLR200>.

FISHER, A. B.; DODIA, C.; YU, K.; MANEVICH, Y.; FEINSTEIN, S. I. Lung phospholipid metabolism in transgenic mice overexpressing peroxiredoxin 6. **Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids**, v. 1761, n. 7, p. 785–792, 2006.

FISHER, A.; DODIA, C.; MANEVICH, Y.; CHEN, J.; FEINSTEIN, S. Phospholipid hydroperoxidase are substrates for non-selenium glutathione peroxidase. v. 274, n. 30, p. 21326–21334, 1999. Disponível em: <http://apps.webofknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=43&SID=4BcFMIHo7agCd8icjdg&page=1&doc=1>.

FLITTER, B. A.; HVORECNY, K. L.; ONO, E.; EDDENS, T.; YANG, J.; KWAK, D. H.; BAHL, C. D.; HAMPTON, T. H.; MORISSEAU, C.; HAMMOCK, B. D.; LIU, X.; LEE, J. S.; KOLLS, J. K.; LEVY, B. D.; MADDEN, D. R.; BOMBERGER, J. M. Pseudomonas aeruginosa sabotages the generation of host proresolving lipid mediators. **Proceedings of the National Academy of Sciences of the United States of America**, v. 114, n. 1, p. 136–141, 2017.

GALES, a C.; JONES, R. N.; TURNIDGE, J.; RENNIE, R.; RAMPHAL, R. Characterization of Pseudomonas aeruginosa isolates: occurrence rates, antimicrobial susceptibility patterns, and molecular typing in the global SENTRY Antimicrobial Surveillance Program, 1997-1999. **Clin.Infect.Dis.**, v. 32 Suppl 2, n. 1058–4838, p. S146–S155, 2001.

GALES, A. C.; CASTANHEIRA, M.; JONES, R. N.; SADER, H. S. Antimicrobial resistance among Gram-negative bacilli isolated from Latin America: results from SENTRY Antimicrobial Surveillance Program (Latin America, 2008–2010). **Diagnostic Microbiology and Infectious Disease**, v. 73, n. 4, p. 354–360, ago. 2012. Disponível em: <http://dx.doi.org/10.1016/j.diagmicrobio.2012.04.007>.

GEBICKA, L.; DIDIK, J. Catalytic scavenging of peroxynitrite by catalase. **Journal of Inorganic Biochemistry**, v. 103, n. 10, p. 1375–1379, 2009. Disponível em: <http://dx.doi.org/10.1016/j.jinorgbio.2009.07.011>.

GOMES, F.; PALMA, F. R.; BARROS, M. H.; TSUCHIDA, E. T.; TURANO, H. G.; ALEGRIA, T. G. P.; DEMASI, M.; NETTO, L. E. S. Proteolytic cleavage by the inner membrane peptidase (IMP) complex or Oct1 peptidase controls the localization of the yeast peroxiredoxin Prx1 to distinct mitochondrial compartments. **Journal of Biological Chemistry**, v. 292, n. 41, p. 17011–17024, 2017.

GWINN, M. R.; VALLYATHAN, V. Respiratory burst: Role in signal transduction in alveolar macrophages. **Journal of Toxicology and Environmental Health - Part B: Critical Reviews**, v. 9, n. 1, p. 27–39, 2006.

HALL, A.; NELSON, K.; POOLE, L. B.; KARPLUS, P. A. Structure-based Insights into the Catalytic Power and Conformational Dexterity of Peroxiredoxins. **Antioxidants & Redox Signaling**, v. 15, n. 3, p. 795–815, ago. 2011. Disponível em:

<http://www.liebertonline.com/doi/abs/10.1089/ars.2010.3624>.

HALL, A.; PARSONAGE, D.; POOLE, L. B.; KARPLUS, P. A. Structural Evidence that Peroxiredoxin Catalytic Power is Based on Transition-State Stabilization. **Journal of Molecular Biology**, v. 402, n. 1, p. 194–209, 2010.

HANSCHMANN, E.-M.; GODOY, J. R.; BERNDT, C.; HUDEMANN, C.; LILLIG, C. H. Thioredoxins, glutaredoxins, and peroxiredoxins--molecular mechanisms and health significance: from cofactors to antioxidants to redox signaling. **Antioxidants & redox signaling**, v. 19, n. 13, p. 1539–605, 2013. Disponível em: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3797455&tool=pmcentrez&rendertype=abstract>.

HARBARTH, S.; KAHLMETER, G.; KLUYTMANS, J.; MENDELSON, M.; HOSPITAL, G. S.; TOWN, C.; AFRICA, S.; PULCINI, C.; SINGH, N.; THEURETZBACHER, U.; FOOD, C. U. S.; SPRING, S.; GRAYSON, L.; HOUCHENS, C.; MONNET, D. L.; OUELLETTE, M.; PATEL, J. B.; ZEALAND, N.; CARRARA, E.; SAVOLDI, A.; KATTULA, D.; BURKERT, F. **Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics**. Disponível em: <http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1>.

HARE, N. J.; SCOTT, N. E.; SHIN, E. H. H.; CONNOLLY, A. M.; LARSEN, M. R.; PALMISANO, G.; CORDWELL, S. J. Proteomics of the oxidative stress response induced by hydrogen peroxide and paraquat reveals a novel AhpC-like protein in Pseudomonas aeruginosa. **Proteomics**, v. 11, n. 15, p. 3056–3069, 2011a.

HARE, N. J.; SCOTT, N. E.; SHIN, E. H. H.; CONNOLLY, A. M.; LARSEN, M. R.; PALMISANO, G.; CORDWELL, S. J. Proteomics of the oxidative stress response induced by hydrogen peroxide and paraquat reveals a novel AhpC-like protein in Pseudomonas aeruginosa. **PROTEOMICS**, v. 11, n. 15, p. 3056–3069, ago. 2011b. Disponível em: <http://doi.wiley.com/10.1002/pmic.201000807>.

HEIM, S.; FERRER, M.; HEUER, H.; REGENHARDT, D.; NIMTZ, M.; TIMMIS, K. N. Proteome reference map of Pseudomonas putida strain KT2440 for genome expression profiling: Distinct responses of KT2440 and Pseudomonas aeruginosa strain PAO1 to iron deprivation and a new form of superoxide dismutase. **Environmental Microbiology**, v. 5, n. 12, p. 1257–1269, 2003.

HIROTA, K.; MURATA, M.; SACHI, Y.; NAKAMURA, H.; TAKEUCHI, J.; MORI, K.; YODOI, J. Distinct roles of thioredoxin in the cytoplasm and in the nucleus. A two-step mechanism of redox regulation of transcription factor NF-κB. **Journal of Biological Chemistry**, v. 274, n. 39, p. 27891–27897, 1999. Disponível em: <http://dx.doi.org/10.1074/jbc.274.39.27891>.

HOPKINS, R. Z. Hydrogen Peroxide in Biology and Medicine: An Overview. **Reactive Oxygen Species Cell Med Press**, v. 26, n. 37, p. 1–12, 2017. Disponível em: <http://www.aimsci.com/ros/index.php/ros/article/view/72>.

HYUN, A. W.; JEONG, W.; CHANG, T. S.; KWANG, J. P.; SUNG, J. P.; JEONG, S. Y.; SUE, G. R. Reduction of cysteine sulfinic acid by sulfiredoxin is specific to 2-Cys peroxiredoxins. **Journal of Biological Chemistry**, v. 280, n. 5, p. 3125–3128, 2005.

IIYAMA, K.; CHIEDA, Y.; LEE, J. M.; KUSAKABE, T.; YASUNAGA-AOKI, C.; SHIMIZU, S. Effect of superoxide dismutase gene inactivation on virulence of Pseudomonas aeruginosa PAO1 toward the silkworm, Bombyx mori. **Applied and Environmental Microbiology**, v. 73, n. 5, p. 1569–1575, 2007.

JOHNSON, L. A.; HUG, L. A. Distribution of reactive oxygen species defense mechanisms across domain bacteria. **Free Radical Biology and Medicine**, n. October 2018, p. 1–10, 2019. Disponível em: <https://doi.org/10.1016/j.freeradbiomed.2019.03.032>.

JORDAN, P. M.; WERZ, O. Specialized pro-resolving mediators: biosynthesis and biological role in bacterial infections. **FEBS Journal**, v. 289, n. 14, p. 4212–4227, 2022.

KAIHAMI, G. H.; DE ALMEIDA, J. R. F.; DOS SANTOS, S. S.; NETTO, L. E. S.; DE ALMEIDA, S. R.; BALDINI, R. L. Involvement of a 1-Cys Peroxiredoxin in Bacterial Virulence. **PLoS Pathogens**, v. 10, n. 10, 2014.

KANAFANI, H.; MARTIN, S. E. Catalase and superoxide dismutase activities in virulent and nonvirulent Staphyloccus aureus isolates. **Journal of Clinical Microbiology**, v. 21, n. 4, p. 607–610, 1985.

KANG, I. H.; KIM, J. S.; LEE, J. K. **The virulence of Vibrio vulnificus is affected by the cellular level of superoxide dismutase activityJournal of Microbiology and Biotechnology**2007.

KARPLUS, P. A. A primer on peroxiredoxin biochemistry. **Free Radical Biology and Medicine**, v. 80, p. 183–190, 2015. Disponível em: <http://dx.doi.org/10.1016/j.freeradbiomed.2014.10.009>.

KIM, K. H.; LEE, W.; KIM, E. E. K. Crystal structures of human peroxiredoxin 6 in different oxidation states. **Biochemical and Biophysical Research Communications**, v. 477, n. 4, p. 717–722, 2016. Disponível em: <http://dx.doi.org/10.1016/j.bbrc.2016.06.125>.

KIRSCHNEK, S.; GULBINS, E. Phospholipase A 2 Functions in Pseudomonas aeruginosa - Induced Apoptosis. **Infection and Immunity**, v. 74, n. 2, p. 850–860, fev. 2006. Disponível em: <https://journals.asm.org/doi/10.1128/IAI.74.2.850-860.2006>.

KNOOPS, B.; ARGYROPOULOU, V.; BECKER, S.; FERTÉ, L.; KUZNETSOVA, O. Multiple roles of peroxiredoxins in inflammation. **Molecules and Cells**, v. 39, n. 1, p. 60–64, 2016.

KRITSILIGKOU, P.; SHEN, T. K.; DICK, T. P. A comparison of Prx- And OxyR-based H2O2 probes expressed in S. cerevisiae. **Journal of Biological Chemistry**, v. 297, n. 1, p. 100866, 2021. Disponível em: <https://doi.org/10.1016/j.jbc.2021.100866>.

LEE, J. S.; HEO, Y. J.; LEE, J. K.; CHO, Y. H. KatA, the major catalase, is critical for osmoprotection and virulence in Pseudomonas aeruginosa PA14. **Infection and Immunity**, v. 73, n. 7, p. 4399–4403, 2005.

LEUTI, A.; MACCARRONE, M.; CHIURCHIÙ, V. Proresolving lipid mediators: Endogenous modulators of oxidative stress. **Oxidative Medicine and Cellular Longevity**, v. 2019, 2019.

LEVY, B. D.; SERHAN, C. N. **Resolution and Regulation of Inflammation**. [s.l.] Elsevier Inc., 2014. 332–348 p.

LIN, C. K.; KAZMIERCZAK, B. I. Inflammation: A Double-Edged Sword in the Response to Pseudomonas aeruginosa Infection. **Journal of Innate Immunity**, v. 9, n. 3, p. 250–261, 2017.

LIU, D.; MAO, P.; HUANG, Y.; LIU, Y.; LIU, X.; PANG, X.; LI, Y. Proteomic analysis of lung tissue in a rat acute lung injury model: Identification of PRDX1 as a promoter of inflammation. **Mediators of Inflammation**, v. 2014, 2014.

LIU, G.; FEINSTEIN, S. I.; WANG, Y.; DODIA, C.; FISHER, D.; YU, K.; HO, Y. S.; FISHER, A. B. Comparison of glutathione peroxidase 1 and peroxiredoxin 6 in protection against oxidative stress in the mouse lung. **Free Radical Biology and Medicine**, v. 49, n. 7, p. 1172–1181, 2010.

LUTHMAN, M.; HOLMGREN, A. Rat Liver Thioredoxin and Thioredoxin Reductase: Purification and Characterization. **Biochemistry**, v. 21, n. 26, p. 6628–6633, 1982.

LYCZAK, J. B.; CANNON, C. L.; PIER, G. B. Establishment of Pseudomonas aeruginosa infection: Lessons from a versatile opportunist. **Microbes and Infection**, v. 2, n. 9, p. 1051–1060, 2000.

MAGIORAKOS, A. P.; SRINIVASAN, A.; CAREY, R. B.; CARMELI, Y.; FALAGAS, M. E.; GISKE, C. G.; HARBARTH, S.; HINDLER, J. F.; KAHLMETER, G.; OLSSON-LILJEQUIST, B.; PATERSON, D. L.; RICE, L. B.; STELLING, J.; STRUELENS, M. J.; VATOPOULOS, A.; WEBER, J. T.; MONNET, D. L. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. **Clinical Microbiology and Infection**, v. 18, n. 3, p. 268–281, 2012. Disponível em: <http://dx.doi.org/10.1111/j.1469-0691.2011.03570.x>.

MANDELL, G. L. Catalase, superoxide dismutase, and virulence of Staphylococcus aureus. In vitro and in vivo studies with emphasis on staphylococcal leukocyte interaction. **Journal of Clinical Investigation**, v. 55, n. 3, p. 561–566, 1975.

MANEVICH, Y.; SWEITZER, T.; PAK, J. H.; FEINSTEIN, S. I.; MUZYKANTOV, V.; FISHER, A. B. 1-Cys peroxiredoxin overexpression protects cells against phospholipid peroxidation-mediated membrane damage. **Proceedings of the National Academy of Sciences**, v. 99, n. 18, p. 11599–11604, 2002. Disponível em: <http://www.pnas.org/content/99/18/11599%5Cnhttp://d360prx.biomed.cas.cz:2191/content/99/18/11599%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/12193653%5Cnhttp://www.pnas.org/content/99/18/11599.full.pdf>.

MANTA, B.; HUGO, M.; ORTIZ, C.; FERRER-SUETA, G.; TRUJILLO, M.; DENICOLA, A. The peroxidase and peroxynitrite reductase activity of human erythrocyte peroxiredoxin 2. **Archives of Biochemistry and Biophysics**, v. 484, n. 2, p. 146–154, 2009. Disponível em: <http://dx.doi.org/10.1016/j.abb.2008.11.017>.

MASHAMAITE, L. N.; ROHWER, J. M.; PILLAY, C. S. The glutaredoxin mono- and di-thiol mechanisms for deglutathionylation are functionally equivalent: implications for redox systems biology. **Bioscience Reports**, v. 35, n. 1, p. 1–10, 2015. Disponível em: <http://bioscirep.org/cgi/doi/10.1042/BSR20140157>.

MEDZHITOV, R. Inflammation 2010: New Adventures of an Old Flame. **Cell**, v. 140, n. 6, p. 771–776, 2010.

MEIRELES, D. A.; DA SILVA NETO, J. F.; DOMINGOS, R. M.; ALEGRIA, T. G. P.; SANTOS, L. C. M.; NETTO, L. E. S. Ohr – OhrR, a neglected and highly efficient antioxidant system: Structure, catalysis, phylogeny, regulation, and physiological roles. **Free Radical Biology and Medicine**, v. 185, n. March, p. 6–24, 2022.

MISHRA, S.; IMLAY, J. Why do bacteria use so many enzymes to scavenge hydrogen peroxide? **Archives of Biochemistry and Biophysics**, v. 525, n. 2, p. 145–160, 2012. Disponível em: <http://dx.doi.org/10.1016/j.abb.2012.04.014>.

MONTEIRO, G.; HORTA, B. B.; PIMENTA, D. C.; AUGUSTO, O.; NETTO, L. E. S. Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C. **Proceedings of the National Academy of Sciences of the United States of America**, v. 104, n. 12, p. 4886–91, 2007. Disponível em: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1829234&tool=pmcentrez&rendertype=abstract>.

MOORE, E. C.; REICHARD, P.; THELANDER, L. ENZYMATIC SYNTHESIS OF

DEOXYRIBONUCLEOTIDES.V. PURIFICATION AND PROPERTIES OF THIOREDOXIN REDUCTASE FROM ESCHERICHIA COLI B. **The Journal of biological chemistry**, v. 239, n. 10, p. 3445–52, out. 1964. Disponível em: <http://dx.doi.org/10.1016/S0021-9258(18)97743-4>.

MORELLO, E.; PÉREZ-BEREZO, T.; BOISSEAU, C.; BARANEK, T.; GUILLON, A.; BRÉA, D.; LANOTTE, P.; CARPENA, X.; PIETRANCOSTA, N.; HERVÉ, V.; RAMPHAL, R.; CENAC, N.; SI-TAHAR, M. Pseudomonas aeruginosa Lipoxygenase LoxA Contributes to Lung Infection by Altering the Host Immune Lipid Signaling. **Frontiers in Microbiology**, v. 10, n. August, p. 1–16, 2019.

MORGAN, M. J.; LIU, Z. G. **Crosstalk of reactive oxygen species and NF-κB signalingCell Research**2011.

NAPOLITANO, G.; FASCIOLO, G.; VENDITTI, P. Mitochondrial management of reactive oxygen species. **Antioxidants**, v. 10, n. 11, p. 1–29, 2021.

NATHAN, C.; XIE, Q. wen. Nitric oxide synthases: Roles, tolls, and controls. **Cell**, v. 78, n. 6, p. 915–918, 1994.

NETTO, L. E. S.; ANTUNES, F. The Roles of Peroxiredoxin and Thioredoxin in Hydrogen Peroxide Sensing and in Signal Transduction. **Molecules and cells**, v. 39, n. 1, p. 65–71, 2016. Disponível em: <http://www.molcells.org/journal/view.html?doi=10.14348/molcells.2016.2349>.

NICHOLLS, P.; FITA, I.; LOEWEN, P. C. Enzymology and structure of catalases. *In*: [s.l: s.n.]p. 51–106.

PALUCHOVA, V.; CAJKA, T.; DURAND, T.; VIGOR, C.; DODIA, C.; CHATTERJEE, S.; FISHER, A. B.; KUDA, O. The role of peroxiredoxin 6 in biosynthesis of FAHFAs. **Free Radical Biology and Medicine**, v. 193, n. P2, p. 787–794, 2022. Disponível em: <https://doi.org/10.1016/j.freeradbiomed.2022.11.015>.

PAN, J.; ZHA, Z.; ZHANG, P.; CHEN, R.; YE, C.; YE, T. Serine/threonine protein kinase PpkA contributes to the adaptation and virulence in Pseudomonas aeruginosa. **Microbial Pathogenesis**, v. 113, n. October, p. 5–10, 2017. Disponível em: <http://dx.doi.org/10.1016/j.micpath.2017.10.017>.

PARK, A. J.; MURPHY, K.; KRIEGER, J. R.; BREWER, D.; TAYLOR, P.; HABASH, M.; KHURSIGARA, C. M. A Temporal Examination of the Planktonic and Biofilm Proteome of Whole Cell *Pseudomonas aeruginosa* PAO1 Using Quantitative Mass Spectrometry. **Molecular & Cellular Proteomics**, v. 13, n. 4, p. 1095–1105, 2014. Disponível em: <http://www.mcponline.org/lookup/doi/10.1074/mcp.M113.033985>.

PEDRAJAS, J. R.; MCDONAGH, B.; HERNANDEZ-TORRES, F.; MIRANDA-VIZUETE, A.; GONZALEZ-OJEDA, R.; MARTINEZ-GALISTEO, E.; PADILLA, C. A.; BARCENA, J. A. **Glutathione Is the Resolving Thiol for Thioredoxin Peroxidase Activity of 1-Cys Peroxiredoxin Without Being Consumed during the Catalytic CycleAntioxidants and Redox Signaling**2016a.

PEDRAJAS, J. R.; MCDONAGH, B.; HERNÁNDEZ-TORRES, F.; MIRANDA-VIZUETE, A.; GONZÁLEZ-OJEDA, R.; MARTÍNEZ-GALISTEO, E.; PADILLA, C. A.; BÁRCENA, J. A. Glutathione Is the Resolving Thiol for Thioredoxin Peroxidase Activity of 1-Cys Peroxiredoxin Without Being Consumed During the Catalytic Cycle. **Antioxidants & Redox Signaling**, v. 24, n. 3, p. 115–128, 2016b. Disponível em: <http://online.liebertpub.com/doi/10.1089/ars.2015.6366>.

PEDRAJAS, J. R.; PADILLA, C. A.; MCDONAGH, B.; BÁRCENA, J. A. Glutaredoxin participates in the reduction of peroxides by the mitochondrial 1-CYS peroxiredoxin in

Saccharomyces cerevisiae. **Antioxidants & redox signaling**, v. 13, n. 3, p. 249–258, 2010.

PERKINS, A.; NELSON, K. J.; PARSONAGE, D.; POOLE, L. B.; KARPLUS, P. A. Peroxiredoxins: guardians against oxidative stress and modulators of peroxide signaling. **Trends in biochemical sciences**, v. 40, n. 8, p. 435–45, 2015. Disponível em: <http://www.sciencedirect.com/science/article/pii/S0968000415000961>.

PIDDINGTON, D. L.; FANG, F. C.; LAESSIG, T.; COOPER, A. M.; ORME, I. M.; BUCHMEIER, N. A. Cu,Zn superoxide dismutase of Mycobacterium tuberculosis contributes to survival in activated macrophages that are generating an oxidative burst. **Infection and Immunity**, v. 69, n. 8, p. 4980–4987, 2001.

POOLE, L. B.; NELSON, K. J. Distribution and Features of the Six Classes of Peroxiredoxins. **Molecules and cells**, v. 38, n. 9, p. 53–59, 2016.

QUADRONI, M.; JAMES, P.; DAINESE-HATT, P.; KERTESZ, M. A. Proteome mapping, mass spectrometric sequencing and reverse transcription-PCR for characterization of the sulfate starvation-induced response in Pseudomonas aeruginosa PAO1. **European journal of biochemistry**, v. 266, n. 3, p. 986–96, 15 dez. 1999. Disponível em: <http://doi.wiley.com/10.1046/j.1432-1327.1999.00941.x>.

RADI, R. Oxygen radicals, nitric oxide, and peroxynitrite: Redox pathways in molecular medicine. **Proceedings of the National Academy of Sciences of the United States of America**, v. 115, n. 23, 2018.

RHEE, S. G. Overview on Peroxiredoxin. **Molecules and cells**, v. 39, n. 1, p. 1–5, 2016. Disponível em: <http://www.molcells.org/journal/view.html?doi=10.14348/molcells.2016.2368>.

RHEE, S. G.; CHAE, H. Z.; KIM, K. Peroxiredoxins: A historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. **Free Radical Biology and Medicine**, v. 38, n. 12, p. 1543–1552, jun. 2005. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0891584905000985>.

RHEE, S. G.; WOO, H. A.; KANG, D. The Role of Peroxiredoxins in the Transduction of H 2 O 2 Signals. **Antioxidants and Redox Signaling**, v. 28, n. 7, p. 537–557, 2018.

ROCHA, L. S.; SILVA, B. P. da; CORREIA, T. M. L.; SILVA, R. P. da; MEIRELES, D. de A.; PEREIRA, R.; NETTO, L. E. S.; MEOTTI, F. C.; QUEIROZ, R. F. Peroxiredoxin AhpC1 protects Pseudomonas aeruginosa against the inflammatory oxidative burst and confers virulence. **Redox Biology**, v. 46, p. 102075, 2021. Disponível em: <https://doi.org/10.1016/j.redox.2021.102075>.

SADER, H. S.; CASTANHEIRA, M.; FLAMM, R. K.; MENDES, R. E.; FARRELL, D. J.; JONES, R. N. Ceftazidime/avibactam tested against Gram-negative bacteria from intensive care unit (ICU) and non-ICU patients, including those with ventilator-associated pneumonia. **International Journal of Antimicrobial Agents**, v. 46, n. 1, p. 53–59, 2015. Disponível em: <http://dx.doi.org/10.1016/j.ijantimicag.2015.02.022>.

SALIBA, A. M.; NASCIMENTO, D. O.; SILVA, M. C. A.; ASSIS, M. C.; GAYER, C. R. M.; RAYMOND, B.; COELHO, M. G. P.; MARQUES, E. A.; TOUQUI, L.; ALBANO, R. M.; LOPES, U. G.; PAIVA, D. D.; BOZZA, P. T.; PLOTKOWSKI, M. C. Eicosanoid-mediated proinflammatory activity of pseudomonas aeruginosa ExoU. **Cellular Microbiology**, v. 7, n. 12, p. 1811–1822, 2005.

SANDER, P.; SPRINGER, B.; DERETIC, V.; BOETTGER, E. C.; MASTER, S. S.; TIMMINS, G. S. Oxidative stress response genes in Mycobacterium tuberculosis: role of ahpC in resistance to peroxynitrite and stage-specific survival in macrophages. **Microbiology**, v. 148, n. 10, p.

3139–3144, 1 out. 2002. Disponível em: <http://mic.microbiologyresearch.org/content/journal/micro/10.1099/00221287-148-10-3139>.

SEAVER, L. C.; IMLAY, J. A. Hydrogen peroxide fluxes and compartmentalization inside growing Escherichia coli. **Journal of Bacteriology**, v. 183, n. 24, p. 7182–7189, 2001.

SERHAN, C. N. Treating inflammation and infection in the 21st century: New hints from decoding resolution mediators and mechanisms. **FASEB Journal**, v. 31, n. 4, p. 1273–1288, 2017.

SERHAN, C. N.; CHIANG, N. Resolvins and cysteinyl-containing pro-resolving mediators activate resolution of infectious inflammation and tissue regeneration. **Prostaglandins and Other Lipid Mediators**, v. 166, n. February, p. 106718, 2023. Disponível em: <https://doi.org/10.1016/j.prostaglandins.2023.106718>.

SERHAN, C. N.; CHIANG, N.; DALLI, J. New pro-resolving n-3 mediators bridge resolution of infectious inflammation to tissue regeneration. **Molecular Aspects of Medicine**, v. 64, p. 1–17, dez. 2018. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0098299717300912>.

SERHAN, C. N.; CHIANG, N.; DALLI, J.; LEVY, B. D. Lipid mediators in the resolution of inflammation. **Cold Spring Harbor Perspectives in Biology**, v. 7, n. 2, 2015.

SERHAN, C. N.; DALLI, J.; KARAMNOV, S.; CHOI, A.; PARK, C.; XU, Z.; JI, R.; ZHU, M.; PETASIS, N. A. Macrophage proresolving mediator maresin 1 stimulates tissue regeneration and controls pain. **The FASEB Journal**, v. 26, n. 4, p. 1755–1765, 2012.

SERHAN, C. N.; LEVY, B. D. Resolvins in inflammation: Emergence of the pro-resolving superfamily of mediators. **Journal of Clinical Investigation**, v. 128, n. 7, p. 2657–2669, 2018.

SEYLER, J.; OLSON, J. W.; MAIER, R. J. Superoxide dismutase-deficient mutants of Helicobacter pylori are hypersensitive to oxidative stress and defective in host colonization. **Infection and Immunity**, v. 69, n. 6, p. 4034–4040, 2001.

SHICHITA, T.; HASEGAWA, E.; KIMURA, A.; MORITA, R.; SAKAGUCHI, R.; TAKADA, I.; SEKIYA, T.; OOBOSHI, H.; KITAZONO, T.; YANAGAWA, T.; ISHII, T.; TAKAHASHI, H.; MORI, S.; NISHIBORI, M.; KURODA, K.; AKIRA, S.; MIYAKE, K.; YOSHIMURA, A. Peroxiredoxin family proteins are key initiators of post-ischemic inflammation in the brain. **Nature Medicine**, v. 18, n. 6, p. 911–917, 2012. Disponível em: <http://dx.doi.org/10.1038/nm.2749>.

SIES, H. On the history of oxidative stress: Concept and some aspects of current development. **Current Opinion in Toxicology**, v. 7, p. 122–126, 2018. Disponível em: <https://doi.org/10.1016/j.cotox.2018.01.002>.

SMALL, D. A.; CHANG, W.; TOGHROL, F.; BENTLEY, W. E. Comparative global transcription analysis of sodium hypochlorite, peracetic acid, and hydrogen peroxide on Pseudomonas aeruginosa. **Applied Microbiology and Biotechnology**, v. 76, n. 5, p. 1093–1105, 14 set. 2007. Disponível em: <http://link.springer.com/10.1007/s00253-007-1072-z>.

SON, Y.; CHEONG, Y.-K.; KIM, N.-H.; CHUNG, H.-T.; KANG, D. G.; PAE, H.-O. Mitogen-Activated Protein Kinases and Reactive Oxygen Species: How Can ROS Activate MAPK Pathways? **Journal of Signal Transduction**, v. 2011, p. 1–6, 2011.

SPITE, M.; NORLING, L. V.; SUMMERS, L.; YANG, R.; COOPER, D.; PETASIS, N. A.; FLOWER, R. J.; PERRETTI, M.; SERHAN, C. N. Resolvin D2 is a potent regulator of leukocytes and controls microbial sepsis. **Nature**, v. 461, n. 7268, p. 1287–1291, out. 2009. Disponível em: <https://www.nature.com/articles/nature08541>.

STREETER, K.; KATOULI, M. Pseudomonas aeruginosa : A review of their Pathogenesis and Prevalence in Clinical Settings and the Environment. v. 2, n. 1, p. 25–32, 2016.

TANG, S.; WAN, M.; HUANG, W.; STANTON, R. C.; XU, Y. Maresins: Specialized proresolving lipid mediators and their potential role in inflammatory-related diseases. **Mediators of Inflammation**, v. 2018, 2018.

THORNTON, J. M.; WALKER, J. M.; SUNDARASIVARAO, P. Y. K.; SPUR, B. W.; RODRIGUEZ, A.; YIN, K. Lipoxin A4 promotes reduction and antibiotic efficacy against Pseudomonas aeruginosa biofilm. **Prostaglandins and Other Lipid Mediators**, v. 152, n. September 2020, p. 106505, 2021. Disponível em: <https://doi.org/10.1016/j.prostaglandins.2020.106505>.

TRALAU, T.; VUILLEUMIER, S.; THIBAULT, C.; CAMPBELL, B. J.; HART, C. A.; KERTESZ, M. A. Transcriptomic analysis of the sulfate starvation response of Pseudomonas aeruginosa. **Journal of Bacteriology**, v. 189, n. 19, p. 6743–6750, 2007.

TRUJILLO, M.; FERRER-SUETA, G.; RADI, R. Kinetic Studies on Peroxynitrite Reduction by Peroxiredoxins. *In*: **Methods in Enzymology**. [s.l: s.n.]p. 173–196.

TRUJILLO, M.; TAIRUM, C. A.; DE OLIVEIRA, M. A.; NETTO, L. E. S. **Thiol- and selenol-based peroxidases: Structure and catalytic properties**. [s.l.] Elsevier Inc., 2022. 277–305 p.

VANCE, R. E.; HONG, S.; GRONERT, K.; SERHAN, C. N.; MEKALANOS, J. J. The opportunistic pathogen Pseudomonas aeruginosa carries a secretable arachidonate 15-lipoxygenase. **Proceedings of the National Academy of Sciences of the United States of America**, v. 101, n. 7, p. 2135–2139, 2004.

VASIL, M. L. Pseudomonas aeruginosa Phospholipases and Phospholipids. *In*: **Pseudomonas**. Boston, MA: Springer US, 2006. p. 69–97.

VELDHUIZEN, R.; NAG, K.; ORGEIG, S.; POSSMAYER, F. The role of lipids in pulmonary surfactant. **Biochimica et Biophysica Acta - Molecular Basis of Disease**, v. 1408, n. 2–3, p. 90–108, 1998.

VINCENT, J. L.; SAKR, Y.; SINGER, M.; MARTIN-LOECHES, I.; MACHADO, F. R.; MARSHALL, J. C.; FINFER, S.; PELOSI, P.; BRAZZI, L.; ADITIANINGSIH, D.; TIMSIT, J. F.; DU, B.; WITTEBOLE, X.; MÁCA, J.; KANNAN, S.; GORORDO-DELSOL, L. A.; DE WAELE, J. J.; MEHTA, Y.; BONTEN, M. J. M.; KHANNA, A. K.; KOLLEF, M.; HUMAN, M.; ANGUS, D. C. Prevalence and Outcomes of Infection among Patients in Intensive Care Units in 2017. **JAMA - Journal of the American Medical Association**, v. 323, n. 15, p. 1478–1487, 2020.

WEI, Q.; LE MINH, P. N.; DÖTSCH, A.; HILDEBRAND, F.; PANMANEE, W.; ELFARASH, A.; SCHULZ, S.; PLAISANCE, S.; CHARLIER, D.; HASSETT, D.; HÄUSSLER, S.; CORNELIS, P. Global regulation of gene expression by OxyR in an important human opportunistic pathogen. **Nucleic Acids Research**, v. 40, n. 10, p. 4320–4333, 2012.

WILLIAMS, B. J.; DEHNBOSTEL, J.; BLACKWELL, T. S. Pseudomonas aeruginosa: Host defence in lung diseases. **Respirology**, v. 15, n. 7, p. 1037–1056, 2010.

WILLIAMS, C. H.; DAVID ARSCOTT, L.; MÜLLER, S.; LENNON, B. W.; LUDWIG, M. L.; WANG, P. F.; VEINE, D. M.; BECKER, K.; HEINER SCHIRMER, R. Thioredoxin reductase: Two modes of catalysis have evolved. **European Journal of Biochemistry**, v. 267, n. 20, p. 6110–6117, 2000.

WINNIE, G. B.; COWAN, R. G. Respiratory tract colonization withPseudomonas aeruginosa in cystic fibrosis: Correlations between anti-Pseudomonas aeruginosa antibody levels and pulmonary function. **Pediatric Pulmonology**, v. 10, n. 2, p. 92–100, 1991. Disponível em: <http://doi.wiley.com/10.1002/ppul.1950100210>.

WOOD, Z. A.; SCHRODER, E.; HARRIS, J. R.; POOLE, L. B.; SCHRÖDER, E.; ROBIN HARRIS, J.; POOLE, L. B. Structure, mechanism and regulation of peroxiredoxins. **Trends in biochemical sciences**, v. 28, n. 1, p. 32–40, 2003.

WU, B.; CAPILATO, J.; PHAM, M. P.; WALKER, J.; SPUR, B.; RODRIGUEZ, A.; PEREZ, L. J.; YIN, K. Lipoxin A4 augments host defense in sepsis and reduces Pseudomonas aeruginosa virulence through quorum sensing inhibition. **FASEB Journal**, v. 30, n. 6, p. 2400–2410, 2016.

WU, Y.; FEINSTEIN, S. I.; MANEVICH, Y.; CHOWDHURY, I.; PAK, J. H.; KAZI, A.; DODIA, C.; SPEICHER, D. W.; FISHER, A. B. Mitogen-activated protein kinase-mediated phosphorylation of peroxiredoxin 6 regulates its phospholipase A 2 activity. **Biochemical Journal**, v. 419, n. 3, p. 669–679, 1 maio 2009. Disponível em: <http://biochemj.org/lookup/doi/10.1042/BJ20082061>.

XU, X. Q.; PAN, S. Q. An Agrobacterium catalase is a virulence factor involved in tumorigenesis. **Molecular Microbiology**, v. 35, n. 2, p. 407–414, 2000.

XUE, Q.; YAN, Y.; ZHANG, R.; XIONG, H. Regulation of iNOS on immune cells and its role in diseases. **International Journal of Molecular Sciences**, v. 19, n. 12, 2018.

ZHOU, S.; SOROKINA, E. M.; HARPER, S.; LI, H.; RALAT, L.; DODIA, C.; SPEICHER, D. W.; FEINSTEIN, S. I.; FISHER, A. B. Peroxiredoxin 6 homodimerization and heterodimerization with glutathione S-transferase pi are required for its peroxidase but not phospholipase A2 activity. **Free Radical Biology and Medicine**, v. 94, p. 145–156, maio 2016. Disponível em: <http://dx.doi.org/10.1016/j.freeradbiomed.2016.02.012>.

9. Abstract

9.1. Abstract

Pseudomonas aeruginosa is a ubiquitous, gamma-proteobacteria, which is the main cause of nosocomial infection among all pathogens related to pneumonia in the Intensive Care Unit. We are interested in the redox aspects involved in host-pathogen interactions. PaLsfA belongs to the peroxiredoxin (Prx) family and to the subgroup that contains only one catalytic cysteine (so-called 1-Cys Prx). Prxs are enzymes capable of removing peroxides (including peroxynitrite) at very high rates. As PaLsfA is related to the *P. aeruginosa* virulence we intended in the present thesis, further advance the characterization of this protein, regarding its biological roles in *P. aeruginosa.* We first evaluated the rate constant between ascorbate and PaLsfA, the only reductant of this protein until now. Also, the importance of some amino acids related to the reaction between them, which, surprisingly, for some mutants, increased the reactivity around ten times. By different microbiological approaches, we revealed the importance of PaLsfA for the bacterial defense against H_2O_2 and paraquat (a superoxide generator), but not against SIN-1 (peroxynitrite generator). Using the genetically encoded probe – Hyper7 – we revealed the capacity of PaLsfA protecting *P. aeruginosa* against H_2O_2 and the capacity of ascorbate to act as an intracellular reductant of PaLsfA in this bacterium. Additionally, we first described a phospholipase activity for a bacterial Prx, however the biological implication of this activity remains unclear. Finally, the capacity of PaLsfA influencing the inflammatory process in macrophages is a first description of a bacterial Prx influencing the host inflammatory response, correlating to its involvement with *P. aeruginosa* virulence. In this way, our findings can enlighten the understanding about how this protein affects the virulence of *P. aeruginosa* and make possible future insights into inhibitory mechanisms.

9.2. Resumo

Pseudomonas aeruginosa é uma gamma-proteobactéria ubiqua, principal causa de infecção nosocomial entre todos os patógenos relacionados à pneumonia na Unidade de Terapia Intensiva (UTI). Estamos interessados nos aspectos redox envolvidos nas interações hospedeiro-patógeno. A PaLsfA pertence à família da peroxirredoxinas (Prx) e ao subgrupo que contém apenas um cisteína catalítica (chamada 1-Cys Prx). As Prxs são enzimas capazes de remover peróxidos (incluindo peroxinitrito) em altas velocidades. Como a PaLsfA está relacionada à virulência de *P. aeruginosa*, pretendemos na presente tese avançar ainda mais na caracterização dessa proteína, abordando seus papéis biológicos em *P. aeruginosa*. Primeiro, avaliamos a constante de velocidade entre a ascorbato e PaLsfA, o único redutor desta proteína descrito até o momento. Além da importância de alguns aminoácidos relacionados à reação entre eles, que, surpreendentemente, para alguns mutantes, aumentou a reatividade em torno de dez vezes. Por diferentes abordagens microbiológicas, revelamos a importância de PaLsfA para a defesa bacteriana contra H_2O_2 e paraquat (um gerador de superóxido), mas não contra SIN-1 (gerador de peroxinitrito). Usando a sonda geneticamente codificada - Hyper7 - revelamos a capacidade de PaLsfA de proteger *P. aeruginosa* contra o H_2O_2 e a capacidade da ascorbato de atuar como um redutor intracelular de PaLsfA nesta bactéria. Além disso, descrevemos pela primeira vez uma atividade fosfolipase para uma Prx bacteriana, no entanto a implicação biológica desta atividade ainda necessita ser investigada. Finalmente, a capacidade de PaLsfA em influenciar o processo inflamatório em macrófagos é uma primeira descrição de uma Prx bacteriana influenciando a resposta inflamatória do hospedeiro, correlacionando-se com sua participação na virulência de *P. aeruginosa*. Dessa forma, nossos achados podem esclarecer a compreensão de como essa proteína afeta a virulência da *P. aeruginosa* e possibilitar futuro desenvolvimento sobre mecanismos inibitórios.