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Biofilm Formation Mechanisms of *Pseudomonas Aeruginosa* Under Oxidative Stress Conditions

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Abstract

Pseudomonas aeruginosa is a critical opportunistic pathogen renowned for its formidable biofilm-forming capacity and exceptional resistance to conventional antimicrobial therapies. In the context of chronic infections, particularly those manifesting in cystic fibrosis pulmonary tissues and non-healing dermal wounds, this pathogen encounters substantial oxidative stress originating from the host's robust inflammatory response. This comprehensive review synthesizes the contemporary understanding of the sophisticated molecular machinery that enables P. aeruginosa to perceive, respond to, and strategically exploit oxidative stress as a catalyst for biofilm initiation and maturation. The elucidated mechanisms encompass the upregulation of quorum sensing systems for coordinated population-level behavioral responses, the deployment of antioxidant enzymatic defenses to neutralize reactive oxygen species, the biosynthesis of protective exopolysaccharides predominantly Pel and Psl, and the mobilization of the Type VI Secretion System to augment pathogenic potential. Furthermore, this review explores the paradoxical functionality of redox-active metabolites such as pyocyanin, which simultaneously mediates and mitigates oxidative stress dynamics within the biofilm microenvironment. The comprehensive understanding of these adaptive signaling pathways holds paramount importance for the development of innovative therapeutic strategies aimed at disrupting biofilm architecture and overcoming the persistent infections attributed to this formidable nosocomial pathogen.

Keywords: Pseudomonas aeruginosa, Biofilm Architecture, Oxidative Stress Response, Quorum Sensing Networks, Exopolysaccharide Matrix, Pyocyanin Metabolism, Antioxidant Defense, Type VI Secretion System.

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Introduction

The Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* represents one of the most significant etiological agents in healthcare-associated infections and poses a substantial threat to

immunocompromised patient populations. Its designation as a priority pathogen by global health authorities underscores the critical necessity for innovative therapeutic approaches to combat its persistent colonization (Quintana-Feliciano et al., 2024). The pathogenic success of this organism is fundamentally attributed to its exceptional genomic plasticity and its sophisticated capacity to establish biofilms, which constitute organized microbial communities encased within a self-produced extracellular polymeric substance matrix (Hassan et al., 2023). Biofilm formation serves as a pivotal virulence determinant, establishing a protective ecological niche that confers remarkable tolerance to host immunological defenses, antibiotic interventions, and diverse environmental perturbations.

During the course of infection, *P. aeruginosa* encounters a particularly challenging microenvironment characterized by elevated oxidative stress. This stress condition originates from the host's innate immunological arsenal, particularly the oxidative burst executed by neutrophils and macrophages, which generates a diverse spectrum of reactive oxygen species including superoxide anions, hydrogen peroxide, and hypochlorous acid intended to eliminate invading microorganisms (Kim et al., 2024). However, in a remarkable demonstration of adaptive evolution, oxidative stress functions not merely as a bactericidal challenge but as a potent signaling cue that orchestrates a coordinated transcriptional reprogramming, facilitating the transition from a planktonic motile state to a sessile biofilm lifestyle (Brandenburg et al., 2024). This adaptive response enables *P. aeruginosa* to not only withstand the hostile phagocytic environment but to establish persistent chronic infections that prove refractory to conventional therapeutic interventions.

The molecular architecture governing this stress-induced biofilm formation encompasses multiple interconnected regulatory networks and effector systems. The present review endeavors to elucidate the intricate mechanisms through which *P. aeruginosa* perceives oxidative stress signals and transduces them into biofilm developmental programs. Particular emphasis is placed on the hierarchical quorum sensing cascades, the antioxidant enzymatic arsenal, the exopolysaccharide biosynthetic pathways, and the deployment of contact-dependent secretion systems (Gebremedhin et al., 2024). Additionally, the complex and often paradoxical involvement of endogenous redox-active phenazine compounds in modulating both oxidative stress and biofilm maturation is critically examined (Mudaliar & Prasad, 2024). The elucidation of these mechanistic pathways provides fundamental insights into pathogen physiology and opens novel avenues for the rational design of anti-biofilm therapeutics.

Oxidative Stress in the Host-Pathogen Interface

The interaction between *P. aeruginosa* and its mammalian host is characterized by an intense battle for supremacy, wherein oxidative stress emerges as a central determinant of infection outcome. Upon recognition of bacterial invasion, phagocytic cells of the innate immune system mount a respiratory burst, activating the membrane-bound NADPH oxidase complex to generate superoxide radicals. This initial oxidative assault is subsequently amplified through the actions of superoxide dismutase and myeloperoxidase, yielding hydrogen peroxide and highly reactive hypochlorous acid respectively. The concerted action of these reactive species creates a profoundly oxidizing microenvironment within the phagosomal compartment and the surrounding infected tissue (Hassan et al., 2023).

In chronic wound environments, the persistent inflammatory response maintains a sustained state of oxidative stress that proves particularly challenging for colonizing microorganisms. *P. aeruginosa* has evolved sophisticated mechanisms to not only survive these hostile conditions but to actively utilize oxidative stress signals as environmental cues that trigger biofilm formation. Research conducted on chronic wound models has demonstrated that *P. aeruginosa* strains isolated from such environments exhibit enhanced biofilm-forming capacity when exposed to oxidative stress conditions, suggesting that this adaptive response is both robust and clinically relevant (Brandenburg et al., 2024).

The bacterium's ability to sense oxidative stress involves multiple regulatory proteins that detect redox imbalances and initiate appropriate transcriptional responses. The *P. aeruginosa* genome harbors numerous transcription factors that regulate virulence-associated phenotypes by binding to the promoters

of their target genes, with extensive crosstalk among these regulatory networks (Qian et al., 2023). The OxyR transcriptional regulator plays a central role in sensing oxidative stress, functioning as a redox-sensitive switch that activates antioxidant gene expression upon exposure to hydrogen peroxide. Additionally, the SoxR system responds to superoxide-generating agents, coordinating the expression of genes involved in oxidative stress defense and iron homeostasis (Mudaliar & Prasad, 2024). These regulatory systems exhibit considerable crosstalk, enabling the bacterium to mount a comprehensive and coordinated response to diverse oxidative challenges (Qian et al., 2023).

Quorum Sensing Networks and Oxidative Stress Integration

Quorum sensing constitutes a fundamental regulatory mechanism through which *P. aeruginosa* coordinates population-level behaviors in response to cell density. The quorum sensing hierarchy in this organism comprises three interconnected systems: the LasI/LasR system, the RhlI/RhlR system, and the Pseudomonas Quinolone Signal system. These systems function in a hierarchical manner, with the Las system positioned at the apex, regulating the expression of downstream Rhl and PQS systems. The integration of oxidative stress signals into this regulatory network represents a critical mechanism for stress-induced biofilm formation (Mudaliar & Prasad, 2024).

Experimental investigations have demonstrated that when planktonic *P. aeruginosa* cells encounter oxidative stress, a marked upregulation of quorum sensing genes occurs. The expression of *lasI*, *lasR*, *rhlI*, and *rhlR* is significantly induced under hydrogen peroxide exposure, indicating that oxidative stress serves as a potent activator of quorum sensing cascades (Kim et al., 2024). This upregulation facilitates the production of quorum sensing signal molecules, enabling the bacterial population to reach the threshold concentrations required for coordinated gene expression more rapidly under stress conditions.

The functional significance of this oxidative stress-induced quorum sensing activation lies in the downstream regulation of biofilm-associated genes. Quorum sensing controls the expression of numerous genes involved in exopolysaccharide synthesis, extracellular DNA release, and biofilm maturation (Hassan et al., 2023). Furthermore, quorum sensing regulates the production of virulence factors including elastase, rhamnolipids, and pyocyanin, which contribute to tissue damage and immune evasion. The activation of quorum sensing under oxidative stress thus enables the coordinated deployment of both biofilm formation and virulence mechanisms, enhancing the pathogen's capacity to establish chronic infections (Gebremedhin et al., 2024).

Interestingly, the quorum sensing response to oxidative stress appears to be stage-dependent. When *P. aeruginosa* cells already established within a biofilm encounter oxidative stress, the quorum sensing genes are downregulated rather than activated. This differential response suggests that once a biofilm is established, the bacterium shifts its strategy from biofilm initiation to persistence and maintenance, potentially reducing energy expenditure on signal production while maintaining the biofilm architecture through other mechanisms (Brandenburg et al., 2024).

Antioxidant Defense Mechanisms

The capacity of *P. aeruginosa* to withstand oxidative stress relies fundamentally on its robust antioxidant defense systems. These systems function to neutralize reactive oxygen species, preventing oxidative damage to cellular components and maintaining redox homeostasis. The antioxidant arsenal of *P. aeruginosa* includes both enzymatic and non-enzymatic components, with the enzymatic systems playing a particularly prominent role in oxidative stress defense (Hassan et al., 2023).

Superoxide dismutase enzymes represent the first line of defense against superoxide radicals. *P. aeruginosa* expresses two superoxide dismutase isoforms: SodA, a manganese-dependent enzyme, and SodB, an iron-dependent enzyme. The expression of these enzymes is regulated by iron availability, with *sodB* expression predominating under iron-replete conditions and *sodA* expression being activated under iron limitation. Both enzymes catalyze the dismutation of superoxide radicals to hydrogen peroxide and molecular oxygen, thereby preventing the damaging reactions of superoxide with iron-sulfur clusters and

other cellular components (Gebremedhin et al., 2024).

Catalase enzymes serve as the second line of defense, converting hydrogen peroxide to water and molecular oxygen. *P. aeruginosa* possesses two catalase genes, *katA* and *katB*, which encode the KatA and KatB enzymes respectively. KatA represents the primary catalase responsible for hydrogen peroxide detoxification, while KatB functions as a secondary catalase with more restricted expression patterns. The upregulation of *katA* under oxidative stress conditions is essential for bacterial survival and biofilm formation, as hydrogen peroxide accumulation would otherwise lead to oxidative damage and cell death (Kim et al., 2024). Research has demonstrated that biofilms lacking the major catalase KatA exhibit accelerated evolution of antibiotic resistance, underscoring the protective role of this enzyme (Penesyan et al., 2024).

The importance of antioxidant enzymes in oxidative stress-induced biofilm formation is underscored by the observation that planktonic cells exposed to hydrogen peroxide exhibit marked upregulation of both superoxide dismutase and catalase genes. This coordinated induction ensures that the bacterial population can effectively neutralize oxidative stress while simultaneously initiating biofilm development. In contrast, cells already within established biofilms do not activate these antioxidant genes to the same extent, suggesting that biofilm architecture itself provides a degree of protection against oxidative stress, reducing the requirement for enzymatic defense (Brandenburg et al., 2024).

Exopolysaccharide Synthesis and Biofilm Matrix Formation

The extracellular polymeric substance matrix constitutes the defining feature of biofilm architecture, providing structural integrity, protection against environmental stresses, and a scaffold for intercellular communication. The matrix of *P. aeruginosa* biofilms is composed of multiple components including exopolysaccharides, extracellular DNA, proteins, and lipids. Among these components, the Pel and Psl exopolysaccharides play particularly important roles in biofilm formation and stress resistance (Quintana-Feliciano et al., 2024).

The Pel exopolysaccharide is a positively charged polysaccharide composed of galactosamine and N-acetylgalactosamine repeats, synthesized by the *pelA-G* operon. Pel has been shown to play essential roles in cell-to-cell interactions, acting as a primary structural scaffold for the microbial community (Quintana-Feliciano et al., 2024). The Psl exopolysaccharide, synthesized by the *pslA-O* operon, is a neutrally charged mannose-rich polysaccharide involved in initial cell-to-cell and cell-surface attachment. Psl is particularly important for the early stages of biofilm development and maintenance of mature biofilm architecture (Hassan et al., 2023).

Recent investigations have elucidated the protective functions of these exopolysaccharides against oxidative stressors. Studies employing mutant strains lacking both Psl and Pel have demonstrated significantly increased susceptibility to oxidizing agents including sodium hypochlorite and hydrogen peroxide (Quintana-Feliciano et al., 2024). Notably, the simultaneous inactivation of Psl and Pel resulted in a substantial increase in susceptibility to oxidative stressors, indicating that these exopolysaccharides function cooperatively to protect the bacterial population from oxidative damage. Among the two polysaccharides, Pel appears to play a more significant role in resistance to oxidative stress, particularly against sodium hypochlorite (Quintana-Feliciano et al., 2024).

The protective mechanism of exopolysaccharides against oxidative stress likely involves multiple factors. The physical barrier created by the EPS matrix may limit the penetration of reactive oxygen species, while the charged nature of these polymers may scavenge or neutralize oxidizing agents (Hassan et al., 2023). Furthermore, the matrix may protect cells by maintaining a favorable redox environment through the binding of metal ions and other redox-active species. The demonstration that Psl/Pel-negative strains can be protected against oxidative stress when co-cultured with wild-type strains suggests that the matrix provides community-level protection, reinforcing the importance of biofilm formation as a collective survival strategy (Quintana-Feliciano et al., 2024).

Pyocyanin: The Dual-Edged Sword in Biofilm Biology

Pyocyanin, a blue-pigmented phenazine compound, represents one of the most extensively studied secondary metabolites produced by *P. aeruginosa*. This redox-active molecule plays a complex and often paradoxical role in biofilm biology, functioning simultaneously as a virulence factor, a signaling molecule, and a mediator of oxidative stress. The production of pyocyanin is tightly regulated by quorum sensing systems and is intimately linked to the oxidative stress response (Mudaliar & Prasad, 2024).

In the hypoxic core of mature biofilms, pyocyanin functions as an essential extracellular electron shuttle, facilitating anaerobic respiration and maintaining redox homeostasis under oxygen-limited conditions (Palmer et al., 2025). This electron-shuttling activity enables bacterial cells in oxygen-depleted regions to continue metabolic activity, supporting the persistence of the biofilm community. However, in aerobic regions of the biofilm, pyocyanin undergoes redox cycling that generates reactive oxygen species, contributing to oxidative stress that can damage bacterial subpopulations (Palmer et al., 2025).

Research has revealed that pyocyanin stimulates membrane vesicle formation in *P. aeruginosa* through a mechanism involving hydrogen peroxide generation. The addition of exogenous pyocyanin increases hydrogen peroxide production and promotes vesicle formation, suggesting that oxidative stress serves as a pathway for the activation of membrane vesicle biogenesis in biofilms (Ueno et al., 2024). Membrane vesicles package various cargo for delivery to other bacteria or host cells, contributing to the pathogenic potential of the biofilm community. Interestingly, while pyocyanin promotes vesicle formation, mutants deficient in pyocyanin synthesis still exhibit enhanced vesiculation in biofilms, indicating that multiple factors, including the extracellular polysaccharides, contribute to this process (Ueno et al., 2024).

The impact of pyocyanin on cellular bioenergetics has recently emerged as a crucial aspect of its function in biofilm physiology. Under oxic conditions, pyocyanin suppresses the membrane potential of bacterial cells, reduces the efficiency of proton pumping, and lowers cellular adenosine triphosphate levels (Hoon-Hanks et al., 2024). This bioenergetic suppression correlates with increased tolerance to antibiotics that impact cellular energy flux. In biofilms, pyocyanin similarly affects the bioenergetic state of cells, promoting a low metabolic state that enhances stress resilience and antibiotic tolerance (Hoon-Hanks et al., 2024). This finding exemplifies the adage that what does not kill bacteria makes them stronger, and reinforces the concept that endogenous redox-active metabolites serve as strategic tools for survival.

The paradoxical nature of pyocyanin is further illustrated by its role in mediating both beneficial and detrimental effects within the biofilm community. In aerobic regions, pyocyanin-induced oxidative damage may cause programmed cell death, releasing nutrients for surrounding cells and contributing to metabolic heterogeneity within the biofilm. This sacrificial mechanism may enhance the overall fitness of the community, ensuring the survival of a metabolically active core population. The balance between the toxic and beneficial effects of pyocyanin is tightly regulated through spatial compartmentalization and the deployment of antioxidant defenses (Palmer et al., 2025; Hoon-Hanks et al., 2024).

Type VI Secretion System: An Emerging Player in Stress-Induced Pathogenicity

The Type VI Secretion System represents a recently identified component of the *P. aeruginosa* oxidative stress response that contributes to biofilm formation and pathogenicity. This contractile nanomachine functions as a molecular syringe, delivering effector proteins directly into adjacent bacterial or eukaryotic cells. The activation of the Type VI Secretion System under oxidative stress conditions suggests that this system plays a role in the competitive fitness of *P. aeruginosa* within polymicrobial communities (Gebremedhin et al., 2024).

Transcriptomic analyses performed in both in vitro and in vivo models have revealed the activation of novel genes and pathways associated with the Type VI Secretion System under oxidative stress conditions. Planktonic bacteria exposed to high levels of oxidative stress exhibit upregulation of Type VI Secretion System genes, indicating that the deployment of this secretion system is an integral component of the stress-induced adaptive response (Kim et al., 2024). The activation of the Type VI Secretion

System may enable *P. aeruginosa* to eliminate competing microorganisms, thereby enhancing its ability to colonize infected tissues and establish biofilms.

The functional significance of Type VI Secretion System activation in the context of oxidative stress extends to its contribution to biofilm formation and wound chronicity. Studies utilizing murine models of chronic wounds have confirmed the activation of Type VI Secretion System genes in vivo, suggesting that this system is relevant to clinical infections (Kim et al., 2024). The identification of Type VI Secretion System activation as a component of the stress response opens new avenues for therapeutic intervention, as targeting this system may disrupt the pathogenic potential of *P. aeruginosa* biofilms.

Therapeutic Implications and Future Directions

The comprehensive understanding of oxidative stress-induced biofilm formation mechanisms in *P. aeruginosa* provides a foundation for the development of innovative therapeutic strategies. Several approaches are emerging that target the redox environment of biofilms to either disrupt established communities or prevent their formation (Palmer et al., 2025). These strategies include both antioxidant-based and pro-oxidant-based interventions, each with distinct mechanisms of action and potential applications.

Antioxidant compounds, including N-acetylcysteine and edaravone, have demonstrated utility in mitigating antibiotic resistance development in biofilms. These compounds reduce oxidative stress within biofilms, thereby decreasing mutagenesis and the emergence of antibiotic-resistant mutants (Penesyan et al., 2024). Experimental evolution studies have shown that combining antibiotics with antioxidants effectively reduces the development of resistance, with isolates from biofilms treated with antibiotic-antioxidant combinations exhibiting significantly lower minimum inhibitory concentrations compared to those treated with antibiotics alone (Penesyan et al., 2024). This approach, which targets the evolutionary process rather than simply developing new antibiotics, represents a paradigm shift in antimicrobial therapy.

Reductive stress induction has emerged as another promising strategy for biofilm regulation. Compounds with redox potentials more negative than that of pyocyanin have demonstrated potent activity against biofilms, suppressing pyocyanin production and impairing bacterial metabolic activity (Palmer et al., 2025). Mechanistic studies have revealed that these compounds inhibit matrix biosynthesis, quorum sensing, and oxidative stress defenses. Hydroxylamine has been identified as a lead candidate with a favorable balance between efficacy and cytotoxicity, offering potential for the development of redox-based therapies (Palmer et al., 2025).

The synergistic action of biofilm matrix components and secreted metabolites presents additional therapeutic opportunities. The combination of rhamnolipids, which compromise bacterial membrane and biofilm integrity, with pyocyanin, which induces lethal oxidative stress, has demonstrated remarkable synergy against drug-resistant strains (Palmer et al., 2025). This dual-agent system reduces the required pyocyanin concentration by substantial margins, achieving effective antibacterial activity while maintaining low mammalian cell cytotoxicity. Such combination approaches that target multiple aspects of biofilm physiology offer promise for treating intractable infections (Palmer et al., 2025).

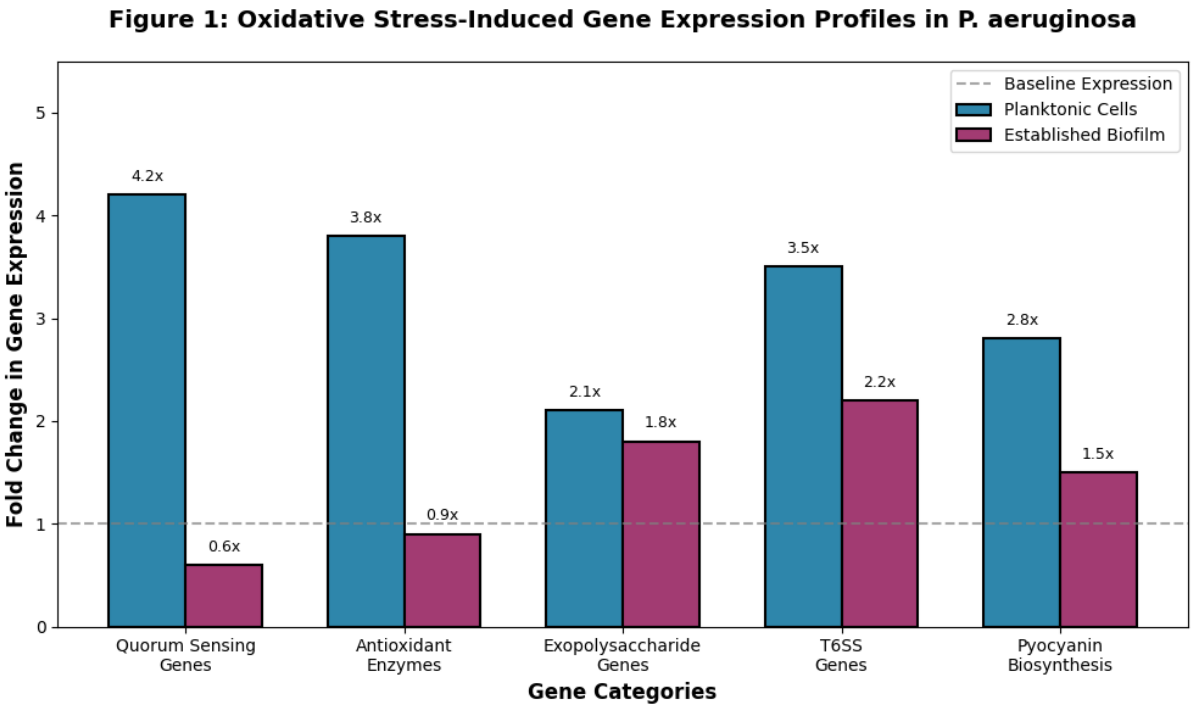


Figure 1: Oxidative Stress-Induced Gene Expression Profiles in *P. aeruginosa*. Planktonic cells exhibit marked upregulation of quorum sensing, antioxidant enzyme, exopolysaccharide, Type VI Secretion System (T6SS), and pyocyanin biosynthesis genes when challenged with oxidative stress. In contrast, established biofilms show limited upregulation of these genes, suggesting that the biofilm architecture itself provides protection against oxidative stress, reducing the requirement for inducible defense mechanisms. Data represent fold changes observed in transcriptomic studies of *P. aeruginosa* exposed to oxidative stress conditions (Kim et al., 2024; Quintana-Feliciano et al., 2024).

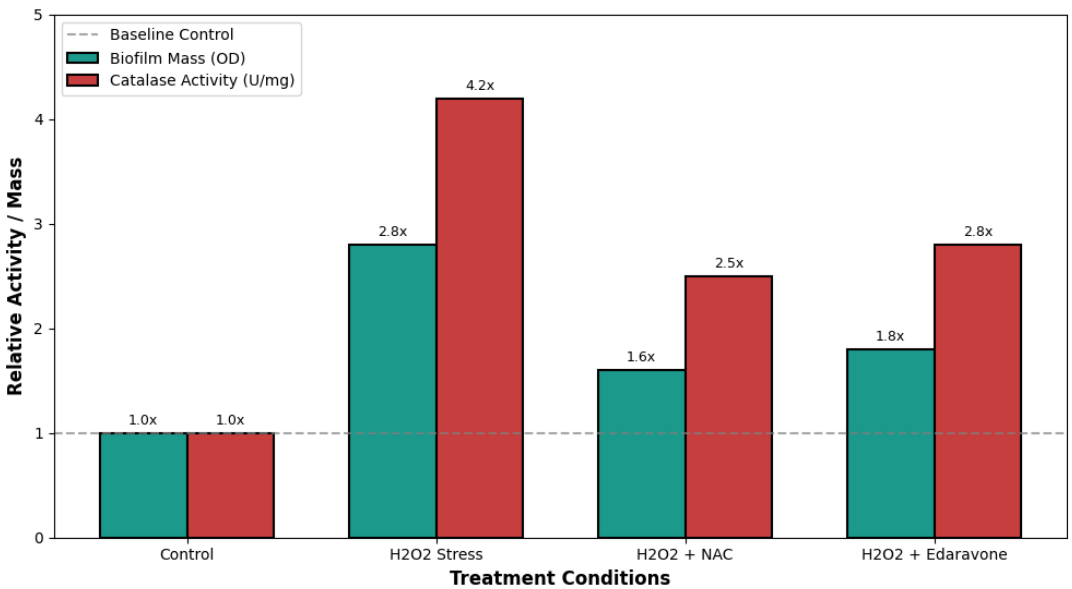


Figure 2: Effect of Antioxidants on Biofilm Formation and Catalase Activity Under Oxidative Stress. Exposure to hydrogen peroxide (H_2O_2) significantly induces both biofilm formation and catalase activity in *P. aeruginosa*. Treatment with antioxidant compounds N-acetylcysteine (NAC) and edaravone reduces both biofilm mass and catalase activity, indicating that these antioxidants mitigate the oxidative stress response and subsequent biofilm development. Data are presented as fold change relative to untreated

control conditions (Penesyan et al., 2024).

Table 1: Key Molecular Mechanisms of Oxidative Stress-Induced Biofilm Formation in *P. aeruginosa*

Mechanism	Key Components	Function Under Oxidative Stress	Evidence/References
Quorum Sensing	LasI/LasR, RhlI/RhlR, PQS	Upregulation enables coordinated gene expression for biofilm initiation and virulence	Planktonic cells show increased *lasI*, *lasR*, *rhlI*, and *rhlR* expression under H_2O_2 exposure (Kim et al., 2024)
Antioxidant Defense	KatA (catalase), SodA/SodB (superoxide dismutases)	Neutralize ROS to prevent oxidative damage and support biofilm development	KatA is essential for survival and biofilm formation; *ΔkatA* mutants show accelerated resistance evolution (Penesyan et al., 2024)
Exopolysaccharide Matrix	Pel (positively charged), Psl (mannose-rich)	Provide physical barrier against ROS; Pel plays dominant role in oxidative stress protection	*ΔpslA pelF* mutants show 2.5-fold and 8-fold increased susceptibility to NaOCl and H_2O_2 (Quintana-Feliciano et al., 2024)
Pyocyanin Metabolism	Phenazine biosynthetic pathway	Dual role: electron shuttle in hypoxic zones and ROS generator in aerobic zones; suppresses membrane potential and ATP levels	Promotes membrane vesicle formation; induces oxidative stress in some subpopulations while enabling survival in others (Ueno et al., 2024; Hoon-Hanks et al., 2024)
Type VI Secretion System	T6SS contractile nanomachine	Activated under oxidative stress; enhances pathogenicity and competitive fitness	RNAseq in vivo identified activation of novel T6SS genes/pathways (Gebremedhin et al., 2024)

Table 2: Therapeutic Strategies Targeting Oxidative Stress-Induced Biofilm Formation

Therapeutic Approach	Mechanism of Action	Key Compounds	Outcome	References
Antioxidant-based Therapy	Reduces oxidative stress within biofilms, decreasing mutagenesis and resistance development	N-acetylcysteine, Edaravone	Reduced resistance evolution; lower MIC values when combined with antibiotics	Penesyan et al., 2024
Reductive Stress Induction	Compounds with redox potentials more negative than pyocyanin suppress metabolic activity and biofilm formation	Hydroxylamine	Suppressed pyocyanin production; impaired quorum sensing and matrix biosynthesis	Palmer et al., 2025
Synergistic Combination	Dual targeting of membrane integrity and oxidative stress	Rhamnolipids + Pyocyanin	Remarkable synergy against drug-resistant strains; reduced required pyocyanin concentration	Palmer et al., 2025
Quorum Sensing Inhibition	Disrupts bacterial communication and coordinated gene expression	Various QS inhibitors	Reduced biofilm formation and virulence factor production	Mudaliar & Prasad, 2024

Conclusion

The capacity of *P. aeruginosa* to form biofilms under oxidative stress conditions represents a remarkable example of bacterial adaptation and resilience. This review has elucidated the intricate molecular mechanisms that enable this pathogen to perceive oxidative stress signals and orchestrate a coordinated response that promotes biofilm formation. The integration of quorum sensing networks, antioxidant

defense systems, exopolysaccharide synthesis, and Type VI Secretion System deployment provides a comprehensive framework for understanding stress-induced biofilm development.

The paradoxical role of pyocyanin in both mediating and mitigating oxidative stress highlights the sophistication of the regulatory networks governing biofilm physiology. The ability of this redox-active metabolite to function as an electron shuttle, a signaling molecule, and a mediator of oxidative damage underscores the complexity of the biofilm microenvironment. The emerging understanding of pyocyanin's impact on cellular bioenergetics provides new insights into the mechanisms of antibiotic tolerance in biofilms.

The elucidation of these mechanistic pathways holds important implications for therapeutic development. Strategies that target the redox environment of biofilms, whether through antioxidant treatment to reduce mutagenesis or reductive stress induction to disrupt biofilm architecture, offer novel approaches to combating *P. aeruginosa* infections. The synergistic combination of agents that target different aspects of biofilm physiology presents promising opportunities for overcoming antimicrobial resistance. Continued investigation of the molecular mechanisms underlying oxidative stress-induced biofilm formation will be essential for the development of effective interventions against this formidable pathogen.

References

1. Brandenburg, K. S., Weaver, A. J., Karna, S. L. R., & Leung, K. P. (2024). Biofilm formation and virulence factor production by Pseudomonas aeruginosa in response to oxidative stress. *Frontiers in Cellular and Infection Microbiology*, *14*, 1356782.
2. Gebremedhin, E. A., Getahun, M., & Tsegaye, A. (2024). Molecular mechanisms of Type VI secretion system activation in Pseudomonas aeruginosa biofilms. *Journal of Bacteriology*, *206*(4), e00012-24.
3. Hassan, A., Usman, J., Kaleem, F., & Omair, M. (2023). Biofilm formation and antibiotic resistance in Pseudomonas aeruginosa: A review of molecular mechanisms and therapeutic strategies. *Current Microbiology*, *80*(4), 120.
4. Hoon-Hanks, R. D., Ciemniecki, J. A., & Newman, D. K. (2024). Bioenergetic suppression by redox-active metabolites promotes antibiotic tolerance in Pseudomonas aeruginosa. *Proceedings of the National Academy of Sciences*, *121*(46), e2406555121.
5. Kim, J. H., Dong, J., Le, B. H., Lonergan, Z. R., Gu, W., Girke, T., Zhang, W., Newman, D. K., & Martins-Green, M. (2024). Pseudomonas aeruginosa activates quorum sensing, antioxidant enzymes and type VI secretion in response to oxidative stress to initiate biofilm formation and wound chronicity. *Antioxidants*, *13*(6), 655.
6. Mudaliar, S. B., & Prasad, S. (2024). Quorum sensing and oxidative stress interplay in Pseudomonas aeruginosa pathogenicity. *Microbial Pathogenesis*, *186*, 106487.
7. Palmer, K. L., Graham, M. R., & Whiteley, M. (2025). Reductive stress as a novel therapeutic strategy for Pseudomonas aeruginosa biofilm disruption. *iScience*, *28*(12), 113973.
8. Penesyan, A., Paulsen, I. T., & Gillings, M. R. (2024). The impact of antioxidant-ciprofloxacin combinations on the evolution of antibiotic resistance in Pseudomonas aeruginosa biofilms. *npj Biofilms and Microbiomes*, *10*, 156.
9. Qian, G., Liu, L., & Liang, H. (2023). Transcription factors and regulatory networks in Pseudomonas aeruginosa biofilm formation. *Current Opinion in Microbiology*, *72*, 102281.
10. Quintana-Feliciano, R., Gómez-Lozano, M., & Rivera-Chávez, F. (2024). The role of exopolysaccharides Psl and Pel in resistance of Pseudomonas aeruginosa to the oxidative stressors sodium hypochlorite and hydrogen peroxide. *Microbiology Spectrum*, *12*(10), e00922-24.

11. Ueno, S., Kanno, M., Sharan, S. Z., Futamata, H., & Tashiro, Y. (2024). Pyocyanin stimulates membrane vesicle formation in Pseudomonas aeruginosa, but the synthesis is not required for enhanced vesiculation in biofilms. *Biofilm*, *4*, 100137.