Regulation Mechanism of Bacterial Quorum Sensing System in Biofilm Formation and Development of Inhibitors

Lin Wu, Sulan Yin, Zixuan Wang, Hailong Yu, Qin Wang*

Hainan Vocational University of Science and Technology, Haikou 571137, Hainan, China

Abstract: The quorum sensing (QS) system is a crucial signaling mechanism in bacteria, consisting of multiple domains. It regulates gene expression within bacterial cells through complex signaling pathways, thereby influencing the growth, metabolism, and physiological processes of bacteria. Research has shown that the QS system can regulate the expression of various signaling molecules, which in turn affects the formation of bacterial biofilms. This article reviews the regulatory mechanisms and research progress of the QS system in biofilm formation, and provides a summary of the small molecule inhibitors reported to date for inhibiting the QS system, focusing on inhibitor screening and development. It aims to provide new ideas and methods for the development of QS system inhibitors. The article will provide a detailed introduction to the QS system and explore potential directions for inhibitor development.

***Keywords*:** bacterial population; induction system; biofilm formation; regulatory mechanism; inhibitor

1. Introduction

The misuse of antibiotics has led to an increasing number of drug-resistant bacterial strains, posing a significant threat to human health. Therefore, there is an urgent need to develop new antibiotics to combat these emerging resistant strains. Bacterial biofilms are complex structures composed of multiple layers, including bacteria, extracellular matrices (such as polysaccharides), and intracellular metabolic products (such as lipopolysaccharides). These biofilms exhibit strong water retention and adhesion properties, enabling them to evade the lethal effects of antibiotics. Research has shown that the bacterial quorum sensing system plays a crucial role in the formation of biofilms and can inhibit this process through various mechanisms. This article reviews the bacterial quorum sensing system and explores the impact of inhibitors on the formation of bacterial biofilms, as well as the development directions for inhibitors, aiming to provide new ideas and methods for the future development of inhibitors.

2. Introduction to bacterial quorum sensing system

The quorum sensing (QS) system in bacteria is a crucial signaling mechanism, consisting of signal molecules and regulatory factors. Signal molecules can be one or more types, which bind to receptor proteins on the bacterial membrane, inducing the synthesis of specific substances like indole, tyrosine, and histidine within the bacterial cells. Regulatory factors control the expression of a series of genes, thereby regulating bacterial growth, metabolism, and physiological processes. The QS system influences these processes by regulating the expression of multiple genes. It can regulate the growth rate of bacteria, the rate of intracellular respiration, the formation of intracellular biofilms, and changes in cell wall components, thus affecting the formation of bacterial biofilms and the development of multi-layer biofilms within them.

3. Regulation mechanism of biofilm formation

3.1 The process of biofilm formation

A biofilm is a complex structure formed by bacteria on various surfaces. It consists of bacteria attached to specific surfaces and exhibits biological activity. In this biofilm, bacteria can move freely on the surface without interference from external factors, and they can also appear as an independent structure on the surface. The biofilm can adapt to changes in its environment, including time, temperature, and pressure. During the formation of a biofilm, the bacterial quorum sensing system plays a crucial role. This system identifies and responds to signaling molecules both within and outside the bacterial population, influencing the formation of the biofilm by altering the structure, chemical composition, and physiological state of the bacterial cell membrane.

3.2 Key factors regulating biofilm formation

The formation of bacterial biofilms requires the coordinated action of two or more factors. First, bacteria must produce

specific biofilm-related substances to sustain their survival, such as reactive oxygen species (ROS), lipopolysaccharides (LPS), and biofilm proteins. Second[1] , bacteria need to grow in a specific environment to form a stable biofilm structure. Thus, the various factors involved in the formation of bacterial biofilms interact in a complex manner, with the most critical factors being the bacteria themselves, the external environment, and environmental factors. The bacteria's characteristics include their size, shape, and density; the external environment includes temperature, pH, and ion concentration; and environmental factors include light intensity, chemical composition, and physical conditions.

3.3 The regulatory role of bacterial quorum sensing system in biofilm formation

The quorum sensing system in bacteria is a signaling mechanism that converts environmental signals into a series of molecules essential for bacterial growth and survival, thereby regulating their growth, metabolism, and pathogenic capabilities. This system plays a crucial role in the formation of bacterial biofilms. The quorum sensing system includes genes involved in energy metabolism, such as β-galactosidase, β-lactamase, and N-methyl-D-aspartate (NDMA) receptor. These genes can enhance bacterial survival in the external environment by interacting with proteins in the quorum sensing system, thus influencing biofilm formation. Research indicates that during biofilm formation, the quorum sensing system can regulate cell growth and biofilm formation by activating specific signaling molecules.

4. Research progress on the regulation mechanism of bacterial quorum sensing system

4.1 Research methods of bacterial quorum sensing system regulation mechanism

The quorum sensing system plays a crucial role in the life activities of bacteria, and its research has long been a focal point and challenge in biology. Currently, two primary methods are used to study the regulatory mechanisms of the quorum sensing system using genetic manipulation techniques: one involves gene knockout and the expression of heterologous genes to investigate the regulatory mechanisms of the quorum sensing system, while the other focuses on studying the interactions between regulatory factors and target proteins to understand their regulatory mechanisms[2] . Both methods enable the knockout or expression of key functional genes in the quorum sensing system, allowing for the theoretical prediction and understanding of the functions and mechanisms of key proteins in this system.

4.2 Related research results and findings

Recently, a research team in our country has made significant progress in studying the quorum sensing system of a specific bacterium. This bacterium can detect chemical signals in its environment and use signaling molecules to regulate its quorum sensing system, thereby adapting to its surroundings. The bacterium induces quorum sensing by producing a signaling molecule called 4-hydroxy-2-butanone, which activates the quorum sensing system. This signaling molecule not only regulates the activation of the quorum sensing system but also enhances a series of metabolic activities within the cells, influencing the formation of bacterial biofilms. Analysis of the signaling molecule in this bacterium reveals that 4-hydroxy-2-butanone can activate multiple signaling pathways, including redox, energy metabolism, DNA replication, and RNA polymerase activity.

4.3 Future research direction of induction system regulation mechanism

(1) Research on bacterial quorum sensing regulation mechanisms has made some progress, but further in-depth studies are needed on the overall regulatory network of quorum sensing systems, the structure of these systems, and their regulatory mechanisms; (2) During the formation of biofilms, how does the quorum sensing system regulate the expression of quorum sensing proteins and downstream genes, and how does it inhibit their expression to reduce biofilm formation? Current research primarily focuses on key regulatory factors related to biofilm formation within the quorum sensing system; (3) In the study of bacterial quorum sensing regulation mechanisms, most research has focused on individual genes or single genes, and the network regulation mechanisms of quorum sensing systems, as well as the relationships between genes within the network, are not sufficiently explored[3].

5. Effects of inhibitors on bacterial quorum sensing system

5.1 Mechanism of action of inhibitors

LQs are crucial signaling molecules within bacterial cells, directly regulating bacterial growth and biofilm formation, and playing a significant role in bacterial pathogenicity and drug resistance. Research indicates that LQs significantly impact the virulence, biofilm formation, antibacterial activity, and drug resistance of Gram-positive bacteria. Given the critical role of quorum sensing systems in the interaction between bacteria and their hosts, the development of inhibitors is crucial for

treating Gram-positive bacteria. Several types of quorum sensing inhibitors have been reported, which alter the signaling pathways of LQs, thereby affecting the regulation of biofilm formation by the quorum sensing system.

5.2 Effects of known inhibitors on the induction system

Since the quorum sensing system plays a crucial role in the formation of bacterial biofilms, many inhibitors have been identified that can interfere with the function of this system. These inhibitors can interfere with the quorum sensing system in two ways: (1) by inhibiting the synthesis of signaling molecules, such as through antibiotics, thereby reducing the quorum sensing system in bacteria. (2) by directly inhibiting signaling molecules or suppressing their synthesis, thus reducing the accumulation of signaling molecules within bacterial cells. For example, NarR is a typical inhibitor of the quorum sensing system, but it does not affect the synthesis of signaling molecules[4]. Therefore, although NarR may be an effective inhibitor of the quorum sensing system, its potential toxicity and the risk of resistance make it an unsuitable inhibitor.

5.3 Application prospect of inhibitors in inhibiting biofilm formation

Through the study of quorum sensing systems, we can see that these systems play a crucial role in the formation of bacterial biofilms. Therefore, inhibiting quorum sensing can help prevent biofilm formation. However, due to the diversity of bacteria and the complexity of the environment, using a single inhibitor or target to inhibit biofilm formation presents several challenges. Thus, developing multi-target inhibitors by leveraging the interactions between quorum sensing systems is essential for effectively inhibiting biofilm formation. Currently, research has identified a method that involves using different sub-module inhibitors from quorum sensing systems in combination to inhibit biofilm formation.

6. Development and prospect of inhibitors

6.1 Screening methods of inhibitors

Through the study of bacterial quorum sensing systems, many natural products and small molecules have been found to exhibit quorum sensing system inhibitory activity. However, due to the complexity of these systems and the varying sensitivities of cells to inhibitors, a single screening method cannot effectively identify compounds with quorum sensing system inhibitory activity. Therefore, a multi-faceted, multi-level combined screening approach is currently used for large-scale, high-throughput drug screening. For example, combining small molecule inhibitors targeting quorum sensing system-related proteins with signaling molecules based on these proteins can help identify compounds with quorum sensing system inhibitory activity. Additionally, using microbial metabolomics technology for screening can also yield compounds with quorum sensing system inhibitory activity.

6.2 Analysis of advantages and disadvantages of existing inhibitors

Currently, some quorum sensing inhibitors, such as ciprofloxacin and daptomycin, inhibit bacterial biofilm formation by disrupting the signaling pathways within bacterial cells. However, this approach can lead to the development of bacterial resistance. Moreover, these inhibitors are ineffective in preventing biofilm formation both in vitro and in vivo, thus failing to effectively control biofilms. Additionally, while these inhibitors are safe for humans, they are toxic to animals. Therefore, researchers are exploring new quorum sensing inhibitors that can inhibit bacteria but do not effectively control biofilm formation[5].

6.3 Future development direction of inhibitor development

In recent years, the bacterial quorum sensing system, as a novel mechanism for regulating biofilms, has become a research hotspot with broad application prospects. However, due to its complex role in biofilm formation and the lack of effective inhibitors, further research is still needed. Developing inhibitors targeting the bacterial quorum sensing system remains a key direction for future development. This includes using gene knockout technology to reduce the activity of the quorum sensing system and further investigate its relationship with biofilm formation; and employing bioinformatics and molecular biology techniques for large-scale screening to identify potential new inhibitors.

Acknowledgments

This paper was supported by the University-Industry Collaborative Education Program (No. 250401414035833).

References

[1] Zhang Jinhua, Ge Shujing, Ren Jingyi. Research Progress on Quorum Sensing Systems and Bacterial Biofilm Forma-

tion [J]. Chinese Journal of Hospital Infection Control, 2025,35(10):1589-1594.
[2] Zhong Huachen and He Yinfeng. Research Progress on the Regulation of Bacterial Biofilm by the Population Induction System [J]. Animal Husbandry and Feed Science, 2020,41(05):7-12.
[3] Zhang Tianzhen, Liu Lingpu, Li Wenchao, et al. Research Progress on the Formation of Bacterial Biofilms Mediated by Quorum Sensing Systems [J]. Bioprocess Technology, 2020,18(02):177-183.
[4] Wang Dexu, Luo Xiaojie, Chen Xiaotao. Research Progress on the Impact of Quorum Sensing Systems on Periodontal Pathogenic Bacteria [J]. Oral Medicine, 2023,43(03):267-272.
[5] Xia Lexin, Zhou Hui, Xu Feng. The Mechanism of Quorum Sensing Regulating Bacterial Drug Resistance [J]. Chinese Journal of Tuberculosis and Respiratory Diseases, 2022,45(12):1260-1266.