Quorum sensing and its inhibition mechanisms

Evgeniya Prazdnova[1,2], *Anzhelica* Bren[1,2], *Lilia* Golovko[1*], *Alexander* Teperin[2], *Delin* Xu[3], *Xinqing* Zhao[4], *Michael* Chikindas[1,5,6], and *Dmitry* Rudoy[1]

[1] Don State Technical University, Gagarin sq. 1, Rostov-on-Don, 344003, Russia
[2] Academy of Biology and Biotechnology, Southern Federal University, 344090 Rostov-on-Don, Russia
[3] Department of Ecology, Institute of Hydrobiology, School of Life Science and Technology, Key Laboratory of Eutrophication and Red Tide Prevention of Guangdong Higher Education Institutes, Engineering Research Center of Tropical and Subtropical Aquatic Ecological Engineering, Ministry of Education, Jinan University, Guangzhou, 510632, PR China
[4] State Key Laboratory of Microbial Metabolism and School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai, 200240, PR China
[5] Health Promoting Naturals Laboratory, School of Environmental and Biological Sciences, Rutgers State University, New Brunswick, NJ 08901, USA
[6] Department of General Hygiene, I.M. Sechenov First Moscow State Medical University, 119435 Moscow, Russia

Abstract. The article is a brief literature review. This article provides an overview of the Quorum Sensing system in bacterial communities, highlighting the peculiarities of the system for gram-positive and gram-negative microorganisms. Basic information about the three existing Quorum Sensing systems is presented. Information is also given about different types of autoinducers, which are signaling molecules that trigger a cascade of behavioral reactions. The importance of the Quorum Sensing system as one of the fundamental mechanisms in the formation and regulation of bacterial biofilms is described, emphasizing the significance of biofilm microorganisms for modern clinical medicine and their impact on aggravating the issue of antibiotic resistance. The main mechanisms of inhibiting bacterial quorum, including by other microorganisms, are presented. The work discusses enzymatic and non-enzymatic methods of inhibiting the Quorum Sensing system, points of application and mechanisms of action. Some microorganisms with confirmed enzymatic activity by Quorum Quenching are indicated. Also presented are registered cases of suppression of other bacteria by microorganisms through the Quorum Sensing inhibitors system.

1 Introduction

Currently, various bacterial processes such as biofilm formation, secretion of virulence factors, bioluminescence, and production of antibiotic substances are extensively studied in

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* Corresponding author: liliya_s_golovko@mail.ru

the scientific community [1, 2, 3]. These processes enhance the survival of microorganisms and their resistance to various inhibitors, including antibacterial agents [4]. The efficient functioning of microbial communities largely depends on interbacterial communication, with one of the key mechanisms being Quorum Sensing (QS) [5, 6, 7, 8]. QS enables microorganisms within biofilms to chemically interact and respond to environmental changes by coordinating their activities, akin to multicellular organisms [9]. Bacteria, in turn, synthesize and export signaling molecules known as autoinducers (AIs) [10]. When extracellular AI molecules reach a certain concentration, biofilm bacteria perceive the presence of these signaling compounds, leading to gene expression changes and behavioral modifications within biofilms. The cellular cascade is activated simultaneously in multiple bacteria, benefiting the bacterial population as a whole by promoting the mass expression of various virulence and pathogenicity factors [11]. The property of altering biofilm behavior due to changes in gene expression resulting from AI accumulation is known as the QS system. It is worth noting that gram-negative and gram-positive bacteria possess different QS systems [8, 12]. There are three main QS systems in bacteria. At the intraspecific level, gram-negative bacteria communicate via AI molecules called acyl-homoserine lactones, whereas in gram-positive microorganisms, AIs are peptides that mediate intercellular signal transduction. The third system of interspecies signal transduction, common to both gram-positive and gram-negative organisms, is based on a derivative of 4,5-dihydroxy-2,3-pentanedione (DPD) known as autoinducer-2 (AI-2) [13].

2 Quorum Sensing System in Gram-negative bacteria

The Gram-negative system is based on the Lux regulon. The term "lux" refers to the luminescence genes. The first group of AI includes N-acyl-homoserine lactones (AHL, or AI-1), consisting of a lactone ring and an aliphatic acyl chain, varying in length and modifications. The receptor proteins interacting with AHL and AHL synthases are homologous to LuxR and LuxI proteins of Vibrio fischeri and belong to LuxR and LuxI-like protein families[7]. LuxI-LuxR type QS systems have been described in many Gram-negative bacteria[8]. LuxR is a protein regulator, while LuxI is an enzyme necessary for AHL biosynthesis. AHL in complex with LuxR acts as a transcription factor, binding to the lux regulon in a series of nucleotides known as the lux box[7]. This binding initiates the expression of LuxI, LuxR, and proteins involved in bioluminescence in certain bacteria, as well as bacterial virulence in specific pathogens[14].

3 Quorum Sensing System in Gram-positive bacteria

For most Gram-positive bacteria, the autoinducers are precursor autoinducing peptides (AIP) (oligopeptides), a transmembrane protein channel for exporting AIP into the extracellular environment, and a sensor histidine kinase that initiates signal transduction by causing adenosine triphosphate (ATP)-mediated phosphorylation of the response regulator protein, which then acts as a transcription factor. Signal transduction occurs through a cascade of phosphorylation mechanisms [1, 7, 8].

4 Autoinducers of Gram-positive and Gram-negative bacteria

AI-2 molecules are heterocyclic compounds - signaling furanones. Two related compounds are included in this category - 2,2,6,6a-tetrahydroxy-3a-furanone and its boronated derivative - furanosyl borate diester [8]. The synthesis of AI-2 depends on the enzyme LuxS, which catalyzes the conversion of S-ribosylhomocysteine to DPD and homocysteine

during the methylation stage of proteins and nucleic acids of bacterial cells. AI-2 is a molecule that binds ribose-like structures. It has been found that AI-2 in Vibrio harveyi is a bi-cyclic borate derivative of DPD, a molecule capable of assuming different cyclic configurations depending on its environment. In other bacteria, for example, in Salmonella typhimurium, AI-2 is a monocylic form of DPD, not containing boron [7]. These autoinducers, used for interspecies communication, are found in both Gram-negative and Gram-positive bacteria [8].

Detailed study by the scientific community of the mechanisms of QS system operation is driven by the fact that it is one of the fundamental factors regulating the growth, development, maturation, and spread of biofilms [7, 15, 16]. Bacterial biofilms are a serious problem in the world today [17]. Bacterial microorganisms that form biofilms secrete extracellular polymeric substance (EPS) at the microcolony development stage [15]. Subsequently, biofilm bacteria are encased in their own self-produced EPS matrix, causing a significantly increased resistance to both antibacterial drugs and the immune system of the host organism compared to planktonic bacteria [18]. The higher resistance of microorganisms within biofilms, coupled with the irrational and excessive use of antimicrobial drugs, leads to a marked rise in antibiotic resistance, which is a significant issue for modern healthcare worldwide [19, 20]. Considering the trend towards increasing multidrug-resistant strains, the long road to finding, synthesizing, and bringing new antibacterial drugs to market [7, 21], the focus of scientific research is shifting towards the study of QS system inhibitors, which could potentially become an alternative strategy for treating infectious processes [22].

5 Quorum-sensing inhibition (QSI) or Quorum-quenching (QQ)

Studying the ways of inhibiting the Quorum-sensing system (Quorum-sensing inhibition (QSI)) or quorum quenching (Quorum-quenching (QQ)) is of great importance in the modern world. Identifying such molecules is promising for both preventing the development of bacterial diseases among plants, animals, or humans and for the therapy of established infectious processes [23]. The development and application of QSI in the clinical field can help address several issues: overcoming antibiotic resistance, more selectively influencing bacterial survival, reducing the dosages of antimicrobial agents, thereby reducing the likelihood of adverse drug reactions [12, 22, 23]. Among the numerous molecules that suppress the QS system, QSI (non-enzymatic methods) and QQ enzymes (enzymatic methods) are distinguished [23]. QSI typically involve compounds that can inactivate AI synthases or receptors by competitive binding/structural modification, while QQ enzymes disrupt signal transmission by degrading the signal [23, 24]. The first major disruption strategy of QS studied is the interference with AI detection, and the second is inactivation/degradation of signaling molecules [24]. Halogenated furanones represent the first group of encountered QSI and were isolated from the red marine algae Delisea pulchra. These algae are one of the organisms well studied for the production of QSI [25]. QSI can be of natural or synthetic origin. In nature, they can be found in terrestrial, marine, or freshwater ecosystems. Synthetic compounds can be derived from existing chemical libraries or designed based on drug development principles (mostly signal mimics and furanone analogs). In nature, QSI are generated by a wide range of living organisms, such as plants, animals, fungi, or bacteria. Most known QSI are predominantly identified in plants and bacteria. This may be due to the fact that both plant extracts and bacteria have undergone more thorough screening for anti-quorum activity [23, 25].

Production of QQ enzymes, which degrade QS signals, has been found in both eukaryotic and prokaryotic organisms. Eukaryotic-derived QQ enzymes have been identified in mammals such as humans and pigs, as well as in other vertebrate and

invertebrate organisms. The ability to suppress QS signals with enzymes is widespread among bacteria. Expression of QQ enzymes has been described in α-proteobacteria, β-proteobacteria, and γ-proteobacteria, as well as in some gram-positive species. Bacterial species with confirmed QQ enzymatic activity include Bacillus sp., Bacillus thuringiensis, Bacillus cereus, Bacillus mycoides, Bacillus anthracis, Bacillus licheniformis, Bacillus amyloliquefaciens, Bacillus megaterium, Agrobacterium tumefaciens, Arthrobacter sp., Klebsiella pneumoniae, P. aeruginosa, Pseudomonas syringae, Rastonia sp., Acinetobacter baumannii, Variovorax paradoxus, Rhodococcus erythropolis, Mycobacterium tuberculosis, Muricauda olearia, and others. The majority of QQ enzymes are involved in AHL degradation, which can be divided into three types based on catalytic mechanisms: lactonase/paraoxonase AHL (lactone hydrolysis), acylase AHL (amidohydrolysis), and oxidase/reductase AHL (oxidoreductase). Most described strategies for inhibiting the QS system primarily target AI-1, followed by AI-2. The first is aimed at combating infections caused by specific individual species, while the second allows for simultaneous inhibition and modulation of QS pathways in many microbial species.

As competing bacteria, representatives of the Enterobacteriaceae family, such as E. coli O157, commensal E. coli K12, Salmonella typhimurium, and Salmonella meliloti, can isolate and disrupt the cell-to-cell communication of other bacterial microorganisms [30]. The presence of these pathogens prevents other participants from using AI-2 signals to regulate their behavior, as it depletes AI-2 from the environment. Studies have shown that when E. coli is cultivated with V. harveyi, bioluminescence mediated by QS signals decreases by 18%. Conversely, the use of a mutant strain of E. coli containing constitutively inhibitable LsrK reduces bioluminescence by 90%, as this mutant E. coli strain disrupts the QS system of V. harveyi [30].

Lactobacillus reuterii, a member of the normal vaginal microbiota, produces cyclic dipeptides that inhibit the Agr-dependent system of staphylococci, thereby suppressing the expression of the toxic shock syndrome toxin (TSST). Currently, about 1000 peptides with similar targeted activities have been discovered through various mechanisms, exhibiting activity against forming and mature (to a lesser extent) biofilms of gram-positive microorganisms [31].

Staphylococcus hominis, often isolated from human skin, is believed to protect the skin barrier from opportunistic pathogens. S. hominis produces six unique autoinducing peptides (AIPs) that inhibit the QS system of Staphylococcus aureus, the regulator of the key virulence factor (agr) [32].

6 Conclusion

The conduct of prospective, both preclinical and subsequently clinical studies on effective molecules that affect the inhibition of the QS system, leading to the development of pharmaceuticals, is an acute niche for scientific exploration. Data on various types of bacterial quorum sensing inhibitors attest to the importance of further research, including efforts aimed at addressing the issue of antibiotic resistance.

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