〔MINI REVIEW〕

The Beginning of Application of Quorum Quenching for Replacing Traditional Antibiotics

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Abstract

Communication among bacteria is achieved via the production, detection and response to chemical signaling molecules, such as N-acyl homoserine lactones (AHLs) known as autoinducers (AI). Detected AIs lead to quorum sensing (QS) gene regulation, when a threshold concentration of them is reached. It is known that the QS with AIs regulates bacterial virulence factors, antibiotic production and biofilm formation. Recently, the AHL-degradation enzymes and QS-inhibitors have been identified in a range of living bacteria and plants. Interfering with the QS system, quorum quenching (QQ), has been suggested as a potential strategy for control of infectious disease. In this review, we discuss the function of QQ enzymes and QS-inhibitors for host diseases and their application in resistance against microbial diseases.

Quorum Sensing and Autoinducer

It has been cleared that bacterial cell can communicate with each other and respond to a changing environment. The cell-cell communication system known as quorum sensing (QS) plays essential roles in regulating gene expression and functional adjustment among bacterial communities as shown in Figure 1. The QS bacteria release, detect and respond to accumulation of small signal molecules in a cell density-dependent manner for regulating the expression of target genes. Several signals for bacterial cell-cell communication have been identified previously, such as acyl homoserine lactone (AHL), cyclic thiolactone, hydroxylpalmitic acid methyl ester, furanosylborate and methy dodecenoic acid.

Among these signals, AHLs called as autoinducer-1 (AI-1) are the best-characterized cell-cell communication signals. And more than a dozen AHL derivatives have been identified in range of Gram-negative bacterial species, which vary in length or substitution at the acyl side chain. And, it has been reported that these AHL signals are involved in the regulation of important biological functions, antibiotic production, motility, production of virulence factors and biofilm formation. Still there are many bacterial species known to produce AHL signals, but the corresponding biological functions remain to be un-cleared.

Also, autoinducer-2 (AI-2) has been determined as member of extracellular signaling molecules used in QS. AI-2 is a furanosyl borate diester derived from the recycling of S-adenosyl-homocystein. AI-2, originally discovered in the QS

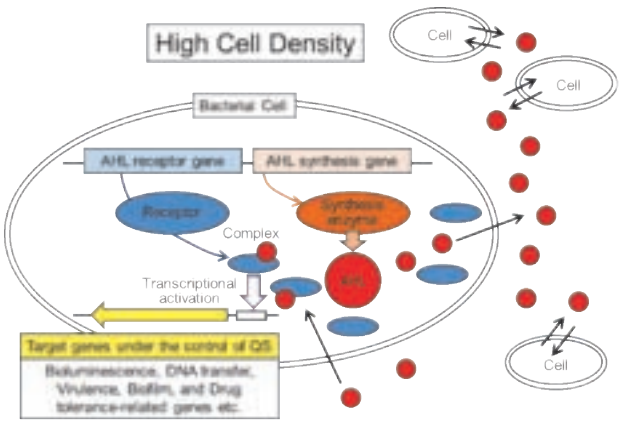

Figure 1：Quorum sensing system of AHL type.

bacterium *Vibrio harveyi*, is made by many species of Gram -negative and Gram-positive bacteria. In every case, production of AI-2 is dependent on the LuxS autoinducer synthase. Also, AI-2 is a species-nonspecific signal used by both Gram-negative and Gram-positive bacteria. And, it is reported that AI-2 has been found to influence biofilm formation in a mixed-species biofilm between *Streptococcus gordonii* and *Porphyromonas gingivalis*.

Dental plaque has been known to be three-dimensional oral biofilm. And it is reported that oral *Veillonella* species as the early colonizers play important roles at the early stage of oral biofilm formation with *Streptococcus* species as the initial colonizers. Recently, it is confirmed that *Veillonella tobetsuensis*, established in our laboratory in 2013, produce AI-1 and AI-2 in the culture supernatant, and these AIs may regulate biofilm formation with *Streptococcus gordonii*.

Quorum Quenching Enzyme

Over the past decade, a range of quorum quenching (QQ) enzymes and inhibitors have been identified from different sources, including prokaryotic and eukaryotic organisms as shown in Figure 2.

It is known partially that these QQ enzymes attenuate the effectiveness of the QS signal molecule, render the QS molecule incapable of binding to target regulator, affect the specificity and recognition of the AHL signal, or disturb the activation of the QS-mediated genes regulated by AHLs.

These enzymes and inhibitors are the key molecules for establishing the concept of QQ, anti-pathogenic and signal interference. But, a limited number of QQ enzymes that interfere with bacterial QS molecules are known, although the mechanisms of the QS system are well understood.

The QQ enzyme for AHLs may be grouped into three types based on degradation pattern of AHLs. (1) Lactonase cleaves the molecule AHLs to open the homoserine lactone ring as shown in Figure 3(1). These lactonases, such as AiiA (Riaz et al., 2008) and AttM (Uroz et al., 2008), are Zn^{2+}-dependent lactonases that produced in the genera *Bacillus* and *Agrobacterium*. (2) Acylase hydrolyzes the amide linkage between homoserine moiety and acyl chain, and releases homoserine lactone and fatty acid as shown in Figure 3(2). Previously, various acylases have been reported including AiiD in *Ralstonia*, AhlM in *Streptomyces* and AiiC in *Anabaena* (Dong et al., 2001). (3) Oxidoreductase catalyzes modification of the chemical structure of the acyl side chain but not degradation as shown in Figure 3(3). It is reported that *Bacillus* (Chowdhary et al., 2007), *Pseudomonas* (Bijtenhoorn et al., 2011) and *Rhodococcus* (Uroz et al., 2005) produce these kind of oxidoreductases.

Recently, the interfering with microbial QS system by QQ has been suggested as a novel strategy for control of infectious disease because QQ aims to shut down the virulence expression in pathogenic bacteria rather than restrict cell growth. Although the roles of QQ enzymes in native environments are not always certain, their ability and availability in potential therapeutic applications are prospective, considerably.

Quorum Sensing Inhibitor from Natural Plants

Although there are some chemically synthesized QS inhibitors, many inhibitors have been discovered in natural plants extracts. Because plants are constantly exposed to bacterial infections like humans and animals, it makes sense to expect that plants have developed good chemical and/or physiological mechanisms to combat pathogenic bacteria. Furthermore, since the plants, such as vegetables, can be consumed by humans, the compounds having QS inhibitory activity from the natural plant products may be deemed as

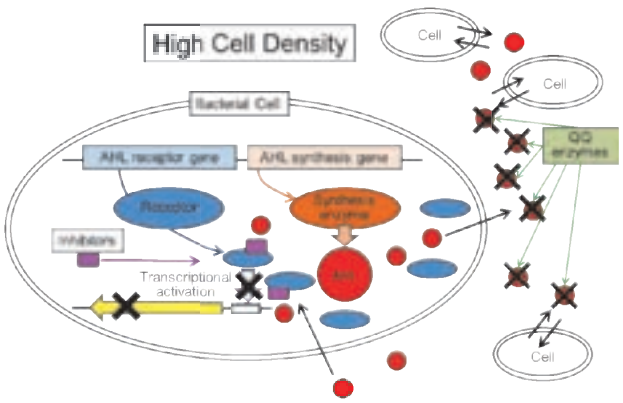

Figure 2 : The effects of Quorum quenching (QQ) enzymes and inhibitors.

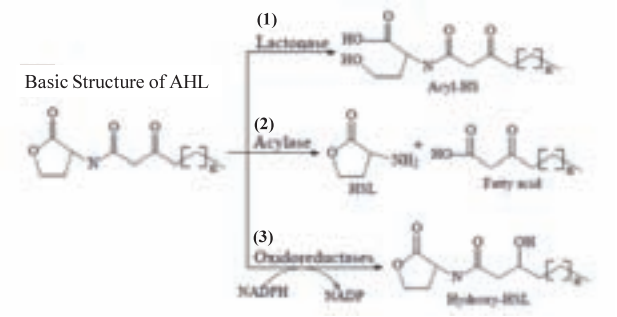

Figure 3 : Basic structure of AHL and patterns of degradation by QQ enzyme

safe may not cause toxicity against human cells. Of course toxicity studies on the components are indispensable before use.

One of the most extensively studied natural products is Australian seaweed as known *Delisea pulchra* (Manefield et al., 1999). This seaweed appears to be able to inhibit bacterial colonization by interfering with the AHL system. Halogenated furanones from *D. pulchra* inhibit the QS regulated reactions by competitively bind to the LuxRs that are receptor proteins for AHLs. It is also demonstrated that the furanones are strong inhibitors for both AI-1 and AI-2-mediated QS systems and the inhibition is partially relieved according to increase of AHL concentration (Manefield et al., 2002).

Recently, it is reported that a non-competitive compound, malabaricone C, which is extracted from nutmeg (*Myristica cinnamomea*) and is not similar to AHL in structure, inhibit the QS system in *Pseudomonas aeruginosa* (Ganin et al., 2013). Also, vegetables including carrot, chamomile, water lily and pepper have been proven to have anti-QS activity.

Application of Quorum Quenching for Bacterial Infection

Due to the extensive emergence of antibiotic-resistant strains of bacteria, the exploitable drugs to treat bacterial infections have become circumscribed considerably. Therefore, the search for novel antibacterial therapy is important and has received greater attention.

Under this severe condition, QQ may be used to control infectious disease in a QS system by triggering the pathogenic pattern. Since the QQ strategy does not intend to kill the pathogen or reduce bacterial cell, but to shut down the expression of pathogenic genes, the intercept with the QS system by QQ enzyme or QS inhibitor is a potential strategy instead of conventional antibiotics. That is to say, the subsistent of QQ enzyme or QS inhibitor in QS system can turn down their QS, leading to blocking disadvantageous gene expression.

Up to now, two strategies have been reported as novel therapeutic tools for controlling infectious disease. (1) In a co-culture of QS-dependent pathogens, the QQ enzymes produced by QQ microbes and/or QS inhibitors extracted from plants could limit the accumulation of QS signal molecules, resulting in a significant reduction in QS-mediated infection. (2) When QQ enzymes and/or QS inhibitors were challenged with QS-mediated pathogen, the enzymes and/or inhibitors showed an elevated tolerance to the pathogen.

Dental plaque is a typical natural biofilm causing oral infectious disease, such as dental caries and periodontal disease. Biofilm infection is difficult to exterminate because of its protection against antibiotics and macrophages compared with planktonic cells. The QS signal molecule has been shown to mediate biofilm formation to protect against antibiotics.

Since it was also shown previously that acylase I, one of QQ enzyme, reduced significantly the biofilm formation by environmental strains of bacteria, it may be possible to control oral biofilm formation at an early stage using QQ enzymes or QQ inhibitors, leading to establish a novel preventative method for dental caries and periodontal diseases. Actually, grapefruit extract contains bioactive compounds such as furocoumarins, limonoids and coumarin that have antibacterial activity. Also, the furocoumarins were shown to have strong inhibition against AI-1 and AI-2 activities, as well as hinder the formation of biofilm. In addition to that, obacunone, contained in many plants as one of limonoids, has been proven to have strong activity as QS inhibitor against both AHL and AI-2 systems and biofilm formation.

In the future, the external addition of the QQ enzymes or QS inhibitors may represent a novel general antibacterial therapy, especially against antibiotic-resistant strains of bacteria, and highlights the potential value of QQ enzymes and QS inhibitors to protect against bacterial infection.

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