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Biotechnological applications of quorum quenching enzymes

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ABSTRACT

Numerous bacteria use quorum sensing (QS) to synchronize their behavior and monitor their population density. They use signaling molecules known as autoinducers (AI's) that are synthesized and secreted into their local environment to regulate QS-dependent gene expression. Among QS-regulated pathways, biofilm formation and virulence factor secretion are particularly problematic as they are involved in surface-attachment, antimicrobial agent resistance, toxicity, and pathogenicity. Targeting QS represents a promising strategy to inhibit undesirable bacterial traits. This strategy, referred to as quorum quenching (QQ), includes QS-inhibitors and QQ enzymes. These approaches are appealing because they do not directly challenge bacterial survival, and consequently selection pressure may be low, yielding a lower occurrence of resistance. QQ enzymes are particularly promising because they act extracellularly to degrade AI's and can be used in catalytic quantities. This review draws an overview of QQ enzyme related applications, covering several economically important fields such as agriculture, aquaculture, biofouling and health issues. Finally, the possibility of resistance mechanism occurrence to QQ strategies is discussed.

1. Introduction

Bacterial communication, referred to as quorum sensing (QS), is the molecular mechanism by which bacteria sense their overall population density, allowing them to synchronize their behavior [1]. Bacteria produce small molecules known as autoinducers (AI's) which are secreted in the environment and can be perceived by specific receptors within neighboring cells. This mechanism regulates gene expression patterns [2]. The response of microorganisms to QS is organism-dependent, but some traits are commonly regulated through QS, such as: production of antibiotics, exopolysaccharides, or exoenzymes, expression of secretion systems, swarming motility, and biofilm formation.

This review first summarizes the main aspects of bacterial QS and its implications in virulence and biofilm formation. Consequently, disrupting QS is particularly promising to modify bacterial behavior and moderate their undesirable traits. Different strategies have been considered for this purpose, including the use of QS inhibitors (QSI's) or quorum quenching (QQ) enzymes. Special attention is then dedicated to applications involving QQ enzymes in various fields such as agriculture, animal and human health, and antifouling. The phosphotriesterase-like lactonase (PLL) family is then discussed as many of these enzymes have been found in extreme environments conferring attractive biotechnological capabilities. In addition, the possibility of resistance mechanisms to QQ strategies is discussed. The strengths and the weaknesses of this approach are emphasized in light of recently published research.

2. Quorum sensing

Several autoinducers have been identified as QS molecules. Gram-positive bacteria mainly use autoinducing peptides (AIP's), also referred to as peptide-pheromones, which are specific to species and strains. Gram-negative bacteria use different types of QS systems: (i) acyl homoserine lactones (AHL's), also known as autoinducer-1 (AI-1), are mostly produced by Gram-negative bacteria: it is a molecule composed of a lactone ring and an aliphatic chain whose length and nature may vary (e.g. *Pseudomonas* spp., *Acinetobacter* spp., *Burkholderia* spp.), (ii) autoinducer-2 (AI-2), a

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furanosyl-borate diester which is found in a wide range of both Gram-negative and Gram-positive bacteria (e.g. *Vibrio* spp., *Pectobacterium* spp.), (iii) AI-3 (epinephrine and norepinephrine) are commonly found in human opportunistic pathogens (e.g. *Enterobacter* spp., *Escherichia* spp., *Klebsiella* spp., *Salmonella* spp.). Other molecules such as (iv) fatty acids (*Xanthomonas* spp.), (v) esters (*Ralstonia* spp.), (vi) α-hydroxy-ketones (*Legionella* spp., *Vibrio* spp.) or (vii) quinolones (*Pseudomonas* spp.) have also been reported [3–9]. Numerous Gram-negative bacteria utilize more than one QS system and may combine these systems either in additive models [10,11], or in hierarchical models when one system induces a second one [12], or with distinct or partially overlapping systems [13]. Considering the variety of signals and complexity of signaling networks, QS is a sophisticated communication system used by bacteria to sense their population density and their surrounding environment [14].

Bacterial pathogens represent increasing concern to human health due to the rapid dissemination of antibiotic-resistant strains. Infections with these pathogens result in increased lethality risks and greater costs for health care systems. In several bacterial pathogens, QS is involved in the switch between commensal, or saprophytic lifestyles, to pathogenic cycles. This is the case for *Pseudomonas aeruginosa* which is naturally present in water and humid environments. Moreover, *P. aeruginosa* is an opportunistic human pathogen that can proliferate in wounds; in such confined environments, QS signals accumulate and QS is triggered leading to the expression of virulence factors and the development of disease [15].

Bacterial pathogens also represent great financial burdens in industries other than health care. For example, bacterial infections of plants result in significant economic losses in agriculture [16]. The most widespread plant bacterial pathogens were recently listed according to their economic or scientific impact (e.g. *Ralstonia solanacearum*, *Xanthomonas* spp., *Pseudomonas syringae* pv., *Erwinia amylovora*) [17,18]. All of the selected bacteria use complex regulation networks where QS plays a central role to induce virulence. Additionally, fish or crustacean bacterial pathogens (e.g. *Vibrio* spp. [19,20]) have economic impacts in aquaculture, causing losses in livestock and contaminations that may be spread to humans.

QS also regulates the formation of biofilms. Biofilms are a specific mode of life where bacteria adhere to a surface and stick together. They build communities embedded in extracellular polymeric substances mainly made of DNA, proteins and polysaccharides that confer protection to environmental stresses (UV, desiccation, antimicrobial compounds). Biofilms are particularly challenging as they can be formed on a wide range of surfaces, biotic or abiotic, and they contribute to the virulence and resistance of bacteria affecting numerous industries, spanning health care (contamination of medical devices), fisheries, and the oil industry [21–25].

Interfering with QS is an attractive strategy to inhibit biofilm formation and limit the pathogenicity of bacteria. This strategy was first described in 2000 through the identification of an enzyme that degrades AHL QS signal molecules [26]. Two QQ strategies can be distinguished: (i) to prevent bacteria from producing or perceiving QS signals and (ii) to degrade QS signals. The first strategy is mainly based on the identification of molecules QSI's by screening natural compounds that will inhibit QS by different means. Halogenated furanones are one of the most common families of QSI's and they were first isolated from a red macroalga, *Delisea pulchra* [27]. Further studies showed that they both target AHL's or AI-2 mediated QS with distinct modes of action: they reduce the stability or binding affinity of the LuxR regulator and they inhibit the synthase, LuxS, by covalent interaction to prevent AI-2 synthesis [28–30]. Many screens have already been performed to identify such molecules. Most results were obtained in laboratory conditions but few direct applications using QQ compounds have been described. Following the example of QSI's, QQ enzymes have also been investigated for their ability to disrupt QS without the need to enter the bacterial cell. Among these, AHL-lactonases, acylases, or oxidoreductases have proved to display QQ activities. The next section is focused on the description of QQ biotechnological applications.

3. Applications

3.1. Plant pathogens

Bacterial plant pathogens rely on sophisticated regulation networks to synchronize the infection process and induce specific virulence factors when in contact with the host plant. Besides the perception of plant signals or nutrient availability, QS plays an essential role in the establishment of the pathogenic cycle. Therefore, QQ strategies are now considered as possible alternatives or complementary strategies to the use of pesticides [17]. Depending on the bacterial pathogens, different QS signaling molecules are produced: AHL's for *Agrobacterium tumefaciens*, *Dickeya* spp., *Erwinia* spp., *Pantoea* spp., *Pectobacterium* spp. and *P. syringae*; AI-2 for *Erwinia* spp., *Pantoea* spp., *Pectobacterium* spp., 3-hydroxypalmitate methyl ester (3-OH-PAME) for *R. solanacearum*, and diffusible signal factors (DSF family) for *Xanthomonas* spp. and *Xylella fastidiosa* [31]. Most of these signals can be degraded by QQ enzymes: an esterase produced by the soil bacterium *Ideonella* sp. 0-0013 degrades 3-OH-PAME from *R. solanacearum*, the enzyme CarAB (a carbamoyl phosphate synthetase) produced by several *Pseudomonas* spp. degrades DSF signals. Lactonases or acylases are produced by many organisms to degrade AHL signals [32,33].

Some soil bacteria such as *A. tumefaciens* or *Bacillus* sp. naturally produce lactonases to degrade AHL's [26,34,35]. For example *Bacillus thuringiensis* was shown to produce a lactonase, called AiiA, which degrades the AHL's produced by *Pectobacterium carotovorum*, thereby reducing its pathogenicity on potato slices [36]. In order to improve the efficiency of the *B. thuringiensis* lactonase AiiA, a fusion with a secretive protein was generated to enhance the dispersion of the lactonase in the environment, resulting in an increased tolerance to *P. carotovorum* on potato [37]. Since the 1960s, *B. thuringiensis* is commonly used as a biological pesticide against insects due to its natural ability to produce endotoxins lethal to moths, butterflies or mosquitoes [38]. Currently, its use against bacterial pathogens in fields has, to our knowledge, not been reported.

Another QQ strategy was also tested against bacterial plant pathogens: some plants were genetically modified using bacterial genes from *Bacillus* spp. or *A. tumefaciens* to produce lactonases. The first transgenic lines were reported in 2001, transforming tobacco and potato lines with the *aiiA* gene from *Bacillus*. The resulting transgenic lines showed an increased tolerance to *P. carotovorum* with symptoms only appearing after inoculation with very high bacterial concentrations [39].

These results showed that QQ has been used as a successful approach to protect plants from bacterial pathogens in laboratory conditions. Nevertheless, this demonstration was only achieved using plant GMO producing lactonases. QQ enzymes that may be used to treat and protect plants from bacterial infections is an attractive alternative to genetically modified plants but is however impaired by the poor stability of enzymes. To circumvent this issue, the development of environmentally stable and chemical-resistant enzymes is crucial.

Another possible drawback in the use of QQ strategies for pest control could be the impact on beneficial or symbiotic bacteria that are naturally found in the environment. The ecological impact of