

Quorum Quenching Enzymes and Biofouling Control

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Bacterial cell to cell communication strategies called quorum sensing (QS) using small diffusible signaling molecules (auto-inducers) govern the expression of various genes dependent on their population density manner. As a consequence of synthesis and response to the signaling molecules, individual planktonic cells synchronized group behaviors to control a diverse array of phenotypes such as maturation of biofilm, production of extra-polymeric substances (EPS), virulence, bioluminescence and antibiotic production. Many studies indicated that biofilm formations are associated with QS signaling molecules such as acyl-homoserine lactones (AHLs) mainly used by several Gram negative bacteria. The biofilm maturation causes undesirable biomass accumulation in various surface environments anywhere water is present called biofouling, which results in serious eco-technological problems. Numerous molecules that interfere the bacterial QS called quorum quenching (QQ), have been discovered from various microorganisms, and their functions and mechanisms associated with QS have also been elucidated. To resolve biofouling problems related to various industries, the novel approach based on QS interference has been emerged attenuating multi-drug resisting bacteria appearance and environmental toxicities, which may provide potential advantages over the conventional anti-biofouling approaches. Therefore this paper presents recent information related to bacterial quorum sensing system, quorum quenching enzymes that can control the QS signaling, and lastly discuss the anti-biofouling approaches using the quorum quenching.

Key words : Acyl-homoserine lactone, anti-biofouling, biofouling, quorum quenching enzymes, quorum sensing (QS)

Introduction

Biofouling is a phenomenon caused by undesirable accumulation of biomass composed of microorganisms, plants, algae, or invertebrate animals in a surface contacting with water. This can occur almost anywhere water is present such as aquatic transportation vessels, aquaculture, petroleum industries, medical devices, bioreactors or water distribution network and wastewater plant see recent reviews [24, 28, 83]. Bacteria are the most common biofouling agents and the feature of biofouling is related to the bacterial community composed of one or more species able to generate biofilms. The major cost of biofouling is associated with the increased of operating cost or fuel consumption [75]. The traditional anti-biofouling strategies rely on physical clean-

ing, or the use of antibacterial compounds (cooper salts), detergent and oxidizing agents such as hydrogen peroxide, chlorinated compounds [10]. The traditional antifouling approaches to the confined environments have resulted in serious health and environmental problems due to their eco-toxicities to many aquatic organisms [10].

Many bacterial species are commonly known to control their expression of gene circuits in a population dependent manner through the release of extracellular signaling molecules to monitor their population density in various environments called quorum sensing. The small diffusible molecules called autoinducers (AIs), also known as a quorum sensing molecules, reaches the threshold level in proportion to the cell density, and then the signal molecules bind to a specific receptor protein and activates the expression of specific genes. Synthesizing, secreting, and responding to these small diffusible molecules as a part of regulating the synchronizing behaviors trigger a diverse array of phenotypes such as bioluminescence [21], antibiotic production [66], virulence [1], sporulation [30], and biofilm formation [14]. There are many types of QS molecules have been discovered such as acylhomoserine lactones (AHLs), auto-inducer peptides (AIPs), hydroxyl-palmitic acid methyl ester

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(PAME), fumocylborate (AI-2) and ethyl dodecenoic acid (DSF) [27, 67, 80, 93, 94]. Among them, Gram positive bacteria mainly use the peptide-based signaling, whereas Gram negative bacteria use the AHLs based signaling.

The process interfering QS communication systems has been generally referred as quorum quenching (QQ) [19]. QQ mechanisms so far described in nature use two strategies. One is the enzymes that inactivate QS signals are called QQ enzymes and the other is the chemicals disturbing the pathway of QS system called QS inhibitors (QSI). The production of QQ enzyme and QSI have been reported from various organisms including algae and invertebrates, terrestrial plants, bacteria, and mammalian cells [27, 73]

This review focuses on the novel strategies to overcome the conventional anti-fouling approaches relying on AHLs mediated QS sensing interference of QQ enzymes so far discovered and the application for the quorum quenching enzymes in anti-fouling strategy.

AHLs molecules mediated quorum sensing

Among the QS molecules, AHLs are the most well characterized, and share common chemical structure features with an acyl chain and homoserine lactone ring. Gram negative bacteria have a conserved QS system, with two central components, where a LuxR-type (R) regulator and LuxI-type (I) protein serve as the signal receptor and an AHL synthase, respectively (Fig. 1). These signal molecules are synthesized by LuxI synthase from S-adenosyl-methionine and an acyl chain carried by an acyl carrier protein. Once the AHL molecules recognized by Lux R-type regulators, and then the regulators control the gene expression associated with various phenotypes. Structural variants of the basic AHL molecules have been discovered and they vary in length and degree of saturation of the acyl side chain as well as in the functional group located at C₃ [87]. These QS signals have been identified and characterized in over 100 species of Gram-negative bacteria belonging to Proteobacteria, other bacteria and archaea such as the haloalkaliphilic archaeon *Natronococcus occultus* [61] and the acidophilic gamma-proteobacterium *Acidithiobacillus ferrooxidans* [70], the cyanobacterium *Gloeotheca* [78] and different marine *Bacteroidetes* [71]. In particular, the bacteria that use AHL mediated QS for biofilm formations and other phenotypes are summarized in Table 1.

Quorum quenching enzymes

Since the quorum quenching strategy prevents the ex-

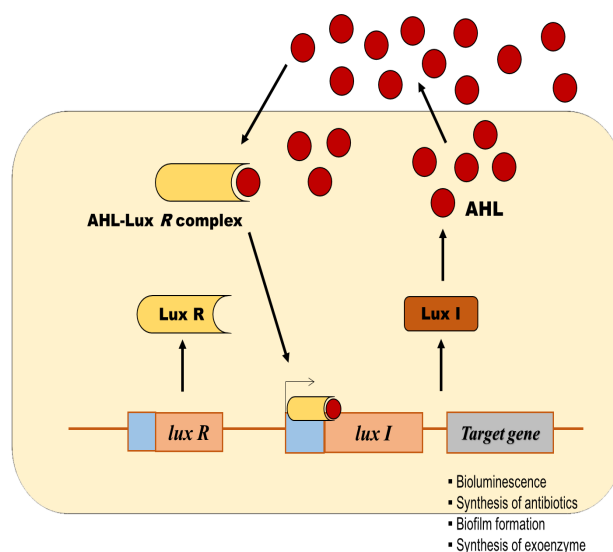


Fig. 1. Schematic diagram of the AHL/LuxR/LuxI type quorum sensing system in Gram-negative bacteria. The *luxR* is a gene encoding LuxR type transcriptional factors and *luxI* is a gene coding for LuxI type AHL synthases. The expression of target genes for such as bioluminescence and biofilm formation are transcriptionally regulated by LuxR homologue proteins when the high concentration AHL depending on a threshold level of bacterial cell density is present

pression of the biofilm formation related genes have been discovered, various biological origins of quorum quenching enzymes or QS inhibitors have been identified from quorum sensing and non-quorum sensing microbes [27].

Based on the chemical structure of AHLs, three different ways of degradation have been discovered as shown in Fig. 2; the lactonases that open the homoserine lactone ring, the acylases (also referred as deaminase) that cleave AHLs at the amide bond and release fatty acid and homoserine lactone. In addition, signal confusing QQ enzymes: the reductases that convert 3-oxo-substituted AHL to their 3-hydroxyl-substituted AHL, and cytochrome oxidase that catalyze oxidation at the acyl chain have also discovered [20].

AHL lactonase

Lactonase activities against AHL signal have been discovered from diverse microorganisms as summarized in Table 2. Based on amino acid sequence and structure of AHL lactonases, the QQ enzymes belong to amidohydrolase superfamily and further subclassified into four different lactonase families such as metallo-lactamase-like lactonase (MBL), phosphotriesterases-like lactonase (PLL), paraoxonase, and α/β -hydrolase fold lactonase (HFL) [49, 53].

Table 1. Summary of various Gram negative bacteria using AHLs based QS and their molecules that mediate biofilm formation

Synonyms	Producing organisms	Phenotypes controlled	Reference
C ₄ -HSL	<i>Aeromonas hydrophila</i> , <i>Aeromonas salmonicida</i> , <i>Pseudomonas aeruginosa</i> , <i>Serratia liquefaciens</i> MG1	Biofilm formation, proteases, virulence factor, and swimming motility	[26, 40, 84]
C ₆ -HSL	<i>Chromobacterium violaceum</i> , <i>Edwardsiella tarda</i> , <i>Burkholderia cepacia</i> , <i>Serratia marcescens</i> SS-1, <i>S. liquefaciens</i> MG1, and <i>Pseudomonas chlororaphis</i>	Biofilm formation, exoenzyme, pigment, virulence factor, pigment prodigiosin, swimming motility, and antibiotic synthesis	[40, 45, 52, 57, 66, 69]
C ₈ -HSL	<i>Burkholderia cepacia</i> , <i>Rhodospirillum rubrum</i>	Biofilm formation, siderophore production, virulence factor, photosynthetic membrane production	[4, 45, 69]
3-oxo-C ₁₀ -HSL	<i>Pseudomonas aeruginosa</i> , <i>Pseudomonas putida</i>	biofilm formation, and virulence factor,	[26, 82]
3-oxo-C ₁₂ -HSL	<i>Pseudomonas aeruginosa</i> , <i>P. putida</i> , and <i>Rhodospirillum rubrum</i>	Biofilm formation, virulence factor, and photosynthetic membrane production	[4, 35, 82]

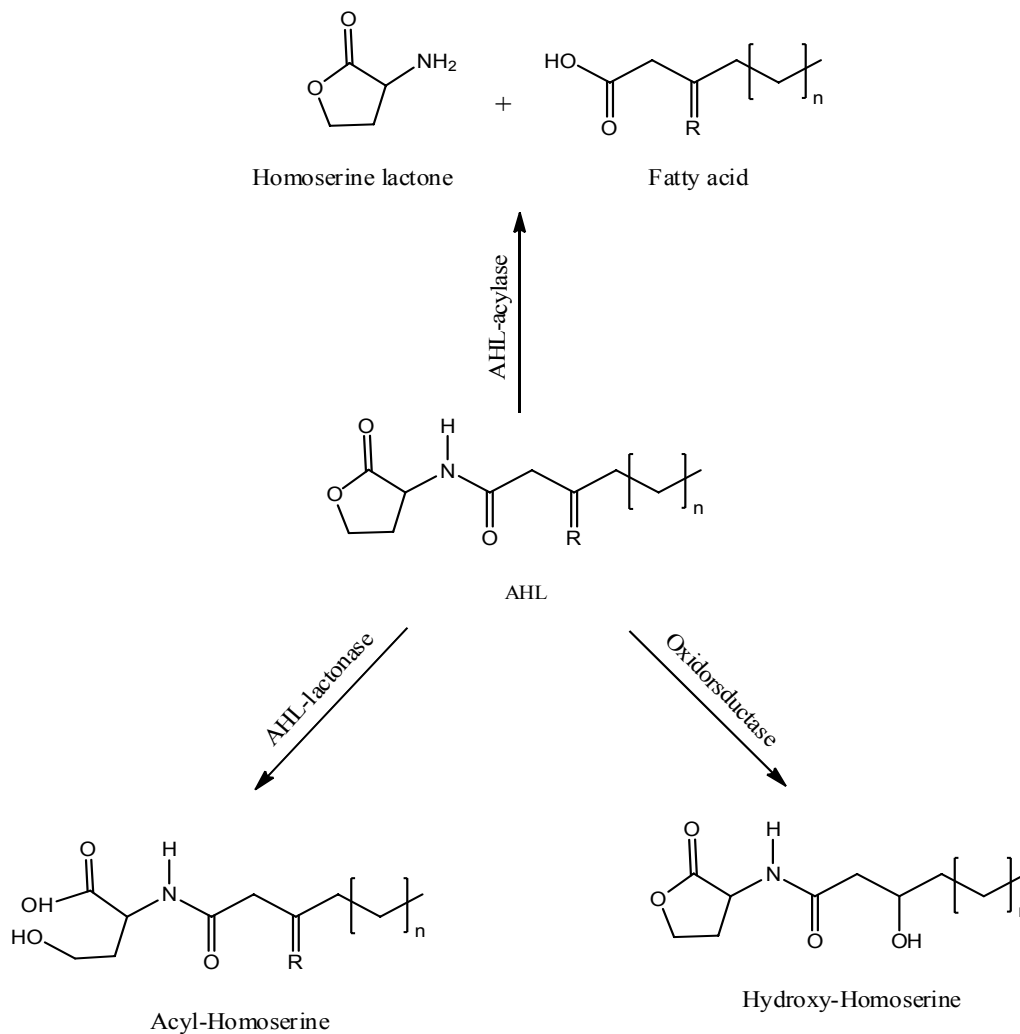


Fig. 2. AHL-degradation or modification mechanism of quorum quenching enzymes.

Table 2. Summary of AHL lactonase enzymes produced by various organisms

Species/Source	QQ enzyme	Protein family	Substrates	Metal Ion	Reference
<i>Bacillus thuringiensis</i> subsp. <i>kurstaki</i>	AiiA	MBL	3-oxo-C ₆ -HSL	Zn ²⁺	[18]
<i>Geobacillus kaustophilus</i> HTA426	GkaP	PLL	C ₄ -, C ₆ -, C ₈ -, C ₁₀ -HSL; 3-oxo-C ₆ -, 3-oxo-C ₈ -, 3-oxo-C ₁₂ -HSL	Zn ²⁺	[95]
<i>Agrobacterium tumefaciens</i> c58	AttM	MBL	3-oxo-C ₈ -HSL	Zn ²⁺	[5]
<i>Agrobacterium tumefaciens</i> c58	AiiB	MBL	C ₆ -, C ₇ -, C ₈ -HS; 3-oxo-C ₆ -, 3-oxo-C ₈ -HSL	Zn ²⁺	[49]
<i>Arthrobacter</i> sp. IBN110	AhID	MBL	C ₄ -, C ₆ -, C ₈ -, C ₁₀ -HSL; 3-oxo-C ₆ -, 3-oxo-C ₁₀ -HSL	Zn ²⁺	[63]
<i>Rhodococcus erythropolis</i> W2	QsdA	PLL	C ₆ -, C ₈ -, C ₁₀ -, C ₁₂ -, C ₁₄ -HSL; 3-oxo-C ₆ -, 3-oxo-C ₈ -, 3-oxo-C ₁₀ -, 3-oxo-C ₁₂ -, 3-oxo-C ₁₄ -HSL	Zn ²⁺	[86]
<i>Flaviramulus ichthyenteri</i> Th78T	FiaL	MBL	C ₆ -HSL; 3-oxo-C ₆ -, 3-oxo-C ₁₀ -HSL	Zn ²⁺	[96]
<i>Sulfolobus islandicus</i>	SisLac	PLL	C ₄ -, C ₈ -, C ₁₂ -HSL; 3-oxo-C ₈ -, 3-oxo-C ₁₀ -, 3-oxo-C ₁₂	Co ²⁺ , Fe ³⁺	[22, 29]
<i>Sulfolobus solfataricus</i> P2	SsoPox	PLL	C ₈ -HSL; 3-oxo-C ₈ -, 3-oxo-C ₁₀ -, 3-oxo-C ₁₂ -HSL	Co ²⁺ , Fe ³⁺	[15]
Soil metagenomics clone	QlcA	MBL	C ₆ -, C ₇ -, C ₈ -HSL; 3-hydroxy-C ₆ -, 3-hydroxy-C ₈ -HSL; 3-oxo-C ₈ -HSL	Zn ²⁺	[68]
<i>Ochrobactrum</i> sp. T63	AidH	HFL	C ₄ -, C ₆ -, C ₁₀ -HSL; 3-oxo-C ₆ -, 3-oxo-C ₈ -HSL	Mn ²⁺	[53]
Human	PONs	Paraoxonase	C ₆ -HSL; 3-oxo-C ₁₂ HSL	Ca ²⁺	[9]

The QQ enzymes, AiiA isolated from *Bacillus thuringiensis* BTK [37] and AiiB from *Agrobacterium tumefaciens* [49] belong to the metallolactamase-like lactonase family with the characteristics requiring the Zn²⁺ binding conserve motif "HXHXDH-H" at the active center of the enzyme which is involved in the cleavage of the ester bond on the lactone ring and the proper folding of the AHL lactonase [37]. Several other lactonase not relying on zinc or other ions for activity have also been reported such as AttM from *Agrobacterium tumefaciens* [5], AhID from *Arthrobacter* [63] and QlcA from metagenomes [68], although their motifs are similar to the Zn²⁺ binding motif (HXHXDH) of several metallohydrolases [19].

Phosphotriesterase-like lactonase (PLL) containing a triosephosphate isomerase (TIM) like fold consisting of eight α -helices and eight parallel β -strands have been discovered from many eubacteria and several archaea [48, 95]. The lactonase belonging to this family, in particular, appeared to have important biotechnology aspects [3, 58]. Several enzymes from this family have interestingly shown thermostable lactonase activities such as GkaP from *Geobacillus kaustophilus* HTA426 [95], SisLac from *Sulfolobus islandicus* [29] and SsoPox from *S. solfataricus* [15, 58]. Although quorum quenching lactonases have great potential to prevent the

pathogenicity from various pathogenic bacteria, broad AHLs substrate ranges and thermostabilities of the enzyme are important characteristics in anti-fouling section. Chow et al. [7] reported directed evolution strategy to isolate thermostable quorum quenching lactonase from *Geobacillus kaustophilus* HTA426. In addition Seo et al. [77] reported a thermostable quorum quenching lactonase from *Geobacillus caldoxylosilyticus* YS-8 with broad substrates specificity.

Despite different amino acid sequence and architecture, the lactonases are all belonging to amidohydrolase super-families with a similar catalytic mechanism using metal ions and key active site [23]. Besides the zinc binding motifs, possible other catalytic motifs for metal binding to lactonase have been elucidated indicating that the diversity and polymorphism of lactonase are exist [56]. Their characteristics associated with substrate usage and metal ion requirements are summarized in Table 2.

Acylase

AHL acylases degrade the amide bond in AHL molecules yielding homoserine lactones and their corresponding acyl chain of fatty acids which severe as the carbon energy and nitrogen source respectively for bacterial growth as shown in Fig. 2. Since the first AHL acylase AiiD has been reported

from *Variovorax paradoxus* VAI-C [43], various bacteria are reported to produce this enzyme family. Currently the porcine kidney and several bacterial enzymes such as AiiD, PvdQ, ahlM, AiiC and QuiP belong to this family known N-terminal nucleophile (Ntn) hydrolase family. Table 3 summarizes the QQ acylase enzyme characterized so far with their substrate preference. In contrast to the AHL lactonase which belongs to various protein families, the majority of the characterized AHL acylase belongs to the Ntn hydrolase family. Most of AHL acylases in general exhibit a preference for long-chain AHLs (with or without a substituent at C-3 of the acyl chain). In particular, AiiC from *Anabaena* sp. PCC7120 [72] showed a broad range of AHLs hydrolyses, which has high potential for anti-biofouling control agents.

Compared to AHL lactonases, AHL acylases are considered as advantageous for anti-biofouling applications, because in contrast to the lactonase product N-acyl homoserine, which can re-circularize to the AHL at acidic pH, the acylase reaction products cannot spontaneously regenerate a functional QS signal. Also several AHL acylase from various bacteria have been reported to reduce biofilm formation which is summarized in Table 4.

AHL oxidoreductase

AHL reductase is considered as QS confusing enzymes which modify the signal molecules but not destroy as shown in Fig. 2. It has been reported that an AHL reductase produced from *Rhodococcus erythropolis* W2 converts 3-oxo-sub

Table 3. Summary of AHL acylase enzymes produced by various organisms

Species/Source	QQ enzyme	Protein family	Substrates	Reference
<i>Variovorax paradoxus</i> VAI-C	ND	ND	C ₄ -, C ₆ -, C ₈ -, C ₁₀ -, C ₁₂ -, C ₁₄ -HSL; 3-oxo-C ₆ -HSL	[43]
<i>Ralstonia</i> sp. XJ12B	AiiD	Ntn-hydrolase	3-oxo-C ₆ -, 3-oxo- C ₈ , 3-oxo-C ₁₂ -HSL	[47]
<i>Pseudomonas aeruginosa</i> PAO1	PvdQ	Ntn-hydrolase	C ₇ -, C ₈ -, C ₁₀ -, C ₁₁ -, C ₁₂ -, C ₁₄ -HSL; 3-oxo-C ₁₀ -, 3-oxo-C ₁₂ HSL	[81]
<i>Pseudomonas aeruginosa</i> PAO1	QuiP	Ntn-hydrolase	C ₆ -HSL, C ₈ -HSL, C ₁₀ -HSL, and C ₁₂ -HSL	[32]
<i>Streptomyces</i> sp. M664	AhlM	Ntn-hydrolase	C ₈ -, C ₁₀ -HSL; 3-oxo-C ₁₂ HSL	[65]
<i>Anabaena</i> sp. PCC7120	AiiC	Ntn-hydrolase	C ₄ -, C ₆ -, C ₈ -, C ₁₀ -, C ₁₂ -, C ₁₄ -HSL; 3-oxo-C ₄ , 3-oxo-C ₆ -, 3-oxo-C ₈ -, 3-oxo-C ₁₀ -, 3-oxo-C ₁₂ -, 3-oxo- C ₁₄ -HSL; 3-hydroxo-C ₄ -, 3-hydroxo-C ₆ -, 3-hydroxo-C ₈ -, 3-hydroxo-C ₁₀ -, 3-hydroxo-C ₁₂ -, 3-hydroxo-C ₁₄ -HSL	[72]
<i>Rhodococcus</i> sp. BH4	ND	ND	C ₆ -, C ₈ -, C ₁₀ -, C ₁₂ -HSL; 3-oxo-C ₆ -, 3-oxo- C ₈ , 3-oxo- C ₁₀ -, 3-oxo-C ₁₂ -HSL	[59]
<i>Ochrobactrum</i> sp.A44	AiiO	α/β-Hydrolase	C ₁₀ -, C ₁₂ -HSL; 3-oxo- C ₁₀ -, 3-oxo-C ₁₂ -, 3-oxo-C ₁₄ -HSL; 3-hydroxo-C ₁₂ -HSL	[13]
<i>Rattus norvegicus</i>	AcyI	ND	C ₄ -, C ₈ -HSL	[89]

Table 4. Summary of AHL acylase enzymes produced from various bacteria that have been used in the inhibition of biofilm formation

Quenching bacteria	Protein	AHLs degraded	Phenotypes regulated
<i>Bacillus pumilus</i> S8-07	ND	3-Oxo-C ₁₂ -HSL	Inhibit biofilm formation in <i>P. aeruginosa</i> PAO1
<i>Pseudomonas aeruginosa</i>	QuiP	C ₆ -, C ₈ -, C ₁₀ -, C ₁₂ -HSL	Inhibits biofilm formation in <i>Aeromonas</i> sp.
<i>Pseudomonas syringae</i> B728a	HacA	C ₈ -, C ₁₀ -, C ₁₂ -HSL	Influence biofilm formation
<i>P. syringae</i> B728a	HacB	C ₆ -, C ₈ -, C ₁₀ -, C ₁₂ -HSL; 3-oxo-C ₆ -, 3-oxo-C ₁₂ -HSL	Influence biofilm formation
<i>Pseudomonas</i> sp. 1A1	Unknown	C ₆ -, C ₈ -, C ₁₀ -, C ₁₂ -HSL; 3-oxo-C ₈ -, 3-oxo-C ₁₀ -, 3-oxo-C ₁₂ -HSL	Inhibit biofilm formation in membrane bioreactor (MBR)
<i>Rhodococcus</i> sp. BH4	ND	C ₆ -, C ₈ -, C ₁₀ -, C ₁₂ -HSL; 3-oxo-C ₆ -, 3-oxo- C ₈ , 3-oxo- C ₁₀ -, 3-oxo-C ₁₂ -HSL	Inhibit biofilm formation in MBR
<i>Shewanella</i> sp. MIB015	Aac	C ₈ -, C ₁₀ -, C ₁₂ -HSL	Reduces biofilm formation in <i>Vibrio anguillarum</i>

stituted AHLs to 3-hydroxy derivatives with a high substrate preference against long-chain 3-oxo-AHLs (> Carbon number 8) [64]. This enzyme showed a broad stereospecificity capable of reduction on both D- and L- isomers of 3-oxo AHL substrates as well as on other AHL analogs such as an aromatic acyl chain substituent, N-(3-oxo-6-phenylhexanol) and 3-oxododecanamine which lacks the homoserin lactone ring. Recently another QQ reductase BpiB09 from a metagenomic library has been reported to attenuate *Pseudomonas aeruginosa* biofilm formation [2].

The cytochrome P450 oxidase CYP102A1 from *Bacillus megaterium* catalyzing the oxidation of acyl homoserine and fatty acids at the ϵ -1, ϵ -2, ϵ -3 positions [8, 62]. The AHL oxidation products still act as QS auto-inducers, but are significantly less active than the parent compounds [8]

Application of QQ as anti-biofouling strategy

Marine biofouling

Marine biofouling is the undesirable biomass accumulation of microorganisms, plants, algae and invertebrate animals on any structures in contact with seawater, such as boats, fish nets, or pipelines. This is causative for friction on boats resulting in excessive fuel consumption, increased maintenance costs, and generates considerable economic losses annually [75, 76]. Since the use of toxic organotin tributyltin based paint products has been banned due to its toxicities caused to marine organisms, various novel approaches searching for non-toxic alternatives have been attempted for either preventing biofouling formation or destroying biofilms using plant extracts [11, 17], bacteriophage [54], macroalga extract [25, 50] and new surfaces materials using new coating and paint and silver nanoparticles [6, 34, 46, 51, 55]. A detail summary of the known novel anti-biofouling strategies used is provided by other review [74]. Despite of relatively abundant data, anti-QS strategies have not been widely investigated in particular in the marine industry.

QS disrupting strategies using QQ enzymes would be of promising strategy as QQ enzymes could reduce QS-regulated biofilm formation involved in microfouling, but may also prevent the attraction and fixation of macrobiofouling species, which have demonstrated the influence of bacterial biofilm on the settlement of spores from algae or others [16, 85]. Several reviews discuss the potential of quorum quenching enzymes such as AHL-acylase, AHL-lactonase, and oxidoreductases to control biofilm formation as an anti-biofoul-

ing agents or their incorporation into paints or coatings [12, 39, 60]. Even though QQ enzymes have been considered as efficient anti-fouling agents, no comparable work has been reported for marine biofouling. The main limitation would be the stability of QQ enzymes within paints as well as possible limited activities in seawater. QQ enzymes from extremophile organisms or halophile organisms would be of promising source as they usually are highly robust and may be active in marine environments.

Membrane biofouling

Membrane filtration has been widely used in food industry, fresh water or wastewater treatment industries as an efficient solid/liquid separation technology. However biofilm formation on membrane altering their functions is one of inevitable problems. Quorum quenching (QQ) enzyme as an effective antifouling strategy for membrane biofouling has recently been adapted in this field in membrane bioreactor (MBR) in advance wastewater treatment due to many advantages over conventional activated sludge treatments [31, 42, 44, 79, 88, 91]. Simpler steps, higher volumetric loading, lower sludge production and better effluent quality provide substantial benefits from the MBR base process over conventional waste water processes [92]. However, the performance of MBR filtration inevitably decreases with operation time due to the formation of biofilm onto the membrane. This phenomena cause by bacterial community developed through the quorum sensing. Therefore, this remains one of the most challenging issues for further MBR development process. In addition, studies to attenuate membrane biofouling have suggested that biofilm formation is mostly associated with Gram-negative bacteria and their secreted metabolites [14, 41]

To resolve this technical issue, several studies addressed quorum quenching enzyme as an anti-biofouling strategies with immobilized QQ enzymes or immobilized QQ bacteria. Yeon et al. [90] applied a QQ technique for MBRs. When the QQ enzyme (porcine kidney acylase I) was added to an MBR, a great reduction in membrane fouling was observed. Synergistic or combined effects of physical cleaning modes with QQ were also been investigated in MBRs. Further improvement in this innovative approach was achieved by immobilizing QQ enzymes on various matrixes such as magnetic particles [91] or sodium alginate capsules [33], nanofiltration membrane [36]. These techniques found to prevent membrane biofouling caused by QS signal molecules

due to a reduced secretion of extracellular polymeric substances (EPS). A significant improvement in membrane permeability was observed without any negative impact on effluent quality. However, the enzyme production cost and enzyme stabilities for long-term use constrain this technology in this MBR based wastewater plants.

In this respect, Oh et al. [59] proposed an alternative method, where QQ bacteria *Rhodococcus sp.* BH4 were used instead of QQ enzymes. When a microbial vessel containing QQ bacteria was placed in an MBR, QS was inhibited while mitigating the formation of a biofilm on the membrane surface. Further improvement of this approach was made by Kim et al. [38] who fabricated free-moving beads for entrapping QQ bacteria, and they found that biofouling was controlled by both physical and biological actions. The EPS production was decreased due to QQ and the bombardment of beads onto the membrane surface enabled the biofilm to slough off from the membrane surface more easily. However, above mentioned studies were investigated under synthetic wastewater at lab scales. In this respect, recently Lee et al. [44] have reported a similar strategy of entrapped QQ bacteria of *Rhodococcus sp.* BH4 in beads under pilot-scale MBRs at a pilot scale wastewater treatment plant with feeding real municipal wastewater. They reported effectiveness of this bacterium for membrane fouling control and the amount of energy savings due to reduction in the rate of transmembrane pressure built-up in QQ-MBR without compromising the effluent water quality compared to that in a conventional-MBR. Furthermore, the QQ activity and mechanical stability of QQ-beads were well maintained indicating QQ-MBR has good potential for practical applications [44].

Conclusions

Large amount of literature indicates that QQ enzymes interfering AHLs based QS have been identified not only limited to Gram negative and positive bacteria, but also found in several archaea and eukaryotic animals. This indicates their various roles in nature. One of important biological functions of QQ enzymes from nature can possibly applied to anti-biofouling strategies where biofilm formation mediated by AHL based quorum sensing causes serious economic and pathological problems. In particular, to prevent biofilm formation observed in various industries, alternative novel strategies have been search for. However, still conventional

anti-biofouling techniques such as copper based agents incorporated to paints are still in use. Although several QSI from various organisms showed great potential alternatives, the application of QQ enzymes in this field are still limited due to their compatibility issues with paints or coatings and the activities in sea water. In this respect, the QQ enzymes from thermophilic or halophilic archaea would be important sources in this field as future studies. Another important applicable area for QQ enzymes is to prevent biofilm formations cooperated to the membrane as anti-fouling strategies. In this respect the immobilization techniques using QQ enzymes or QQ bacteria can be practically applied in the advanced wastewater treatment using MBR systems even in large scale wastewater treatment plants. Obviously, the important applicability of QQ enzyme or bacteria in the advanced wastewater treatment using MBR systems needs to be further confirmed by life cycle assessment. Despite of many types of QQ enzymes existed, only AHL acylases mainly used this field which indicates the importance of biotechnological sources. Moreover, QQ enzymes are highly attractive as these molecules are usually not toxic and may be integrated into various matrices without being released. The proofs of concept have been widely described and further investigations would obviously permit to develop concrete applications in order to address the issues of bacterial virulence and biofouling.

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초록 : 정족수 제어효소와 biofouling 제어

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정족수 인식 체계라 불리는 세균들의 세포간 의사교환 전략은 다양한 유전자의 발현조절을 통해, 생물막 성숙, 세포 외 고분자물질의 생산, 병원성 발현 및 항생제 생산 등과 같은 다양한 표현형을 조절하는 세균의 다세포성 행동 양식을 제어한다. 다수의 연구에 의하면 많은 종류의 그람 (Gram) 음성 세균들이 정족수 인식체계에 필요한 신호전달 물질로 acyl-homoserine lactones (AHLs)를 사용하고 있으며, 이들은 생물막 형성에 중요한 인자로 작용함을 시사하였다. 이러한 정족수 인식체계에 의한 생물막의 형성은 물이 존재하는 모든 표면환경에서 불필요한 바이오매스 축적이라는 심각한 기술적, 경제적 문제를 초래하고 있다. 최근 정족수 인체 체계를 교란하는 다수의 물질들이 다양한 미생물로부터 발견되어, 그들의 정족수 인식 체계와 관련된 주요 기능과 기작들이 밝혀지고 있다. 이러한 정족수 제어 물질들은 최근 다양한 산업에서 발생하는 생물 부착현상들을 제어할 수 있는, 환경 친화적이며 세균의 항생제 다제 내성을 완화시킬 수 있는 새로운 방법으로 대두되고 있다. 따라서 본 논문은 세균의 정족수 인식 체계와 관련된 최근 정보, 정족수 인식 신호를 제어할 수 있는 정족수 제어 효소와 이러한 기술을 이용한 생물 부착 저해 방법 등을 논의하고자 한다.