QUORUM QUENCHING BACTERIA ISOLATED FROM WASTEWATER TREATMENT PLANT, AND GENES FOR *N*-ACYL-HOMOSERINE LACTONE DEGRADING ENZYMES FROM THE ISOLATES

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Introduction. Quorum sensing (QS) is a regulatory mechanism employed by bacteria to coordinate the behavior of their community in response to population density. Bacteria recognize the changes in population density by sensing the concentration of signal molecules, *N*-acyl-homoserine lactones (AHLs) [1]. As AHL-mediated QS plays a key role in biofilm formation [2], the interference of QS, referred as quorum quenching (QQ) has received great deal of attention [3]. The concept of QQ can be applied to membrane bioreactors (MBR) for advanced wastewater treatment as a new strategy to control biofouling [4]. The close correlation between QS and biofilm formation on membranes has been studied in detail. In addition, the effective reduction of biofouling by quorum quenching enzymes that inactivate the AHLs has been reported recently [5].
The purpose of our study is to isolate quorum quenching bacteria, which could inhibit biofilm formation, and eventually could reduce biofouling in MBR system of waste water treatment plant.

Methods. To isolate AHL-degrading bacteria efficiently, enrichment culture method was used. The screening medium is a minimal medium containing AHL as the sole carbon source. The sludge samples from MBR plant were cultivated in the AHL-minimal medium. After three rounds of enrichment culture, the cell suspension was spread on LB agar plate. The isolated strains were identified by 16s rDNA sequence analysis. For bioassay of the AHL-degrading activity, samples were mixed with synthetic AHL and after reaction, based on the color zone size of reporter strain (*Chromobacterium violaceum* CV026) overlaid agar plates, the residual AHL contents in reaction mixture were determined. To investigate whether isolated QQ strains could inhibit biofilm formation, biofilm-forming strains and QQ strains were co-cultured for 16 hrs on LB broth in microtitre plates. In addition, inhibition of biofilm formation on slide glass was tested using biofilm forming strains and BH4 cell extract. The adherent biofilm on microtitre plates and slide glasses were stained with crystal violet.

Results. Diverse AHL-degrading strains including *Rhodococcus* sp., *Pseudomonas* sp., *Paenibacillus* sp., *Geobacillus* sp., *Enterobacteria* sp., *Micrococcus*, sp., etc. were isolated from waste water treatment plant by enrichment culture. These quorum quenching isolates were characterized, and it was found that they could effectively degrade various AHLs, although the activity and substrate specificities were quite different. Among isolates, *Rhodococcus* sp. BH4 and *Pseudomonas* sp. 1A1 exhibited the most rapid growth rate and consumption of *N*-hexanoyl-L-homoserine lactones (HHL) from the HHL-minimal medium. Strain BH4 produced the intracellular QQ enzyme, while strain 1A1 produced the extracellular QQ enzyme. Biofilm formation by AHL-producing bacteria was reduced by isolated quorum quenching bacteria, when both the strains were co-cultured. AHL-lactonase gene from *Rhodococcus* sp. BH4 was cloned and expressed in *E. coli*. The cloned gene from strain BH4 shared 90% DNA sequence identity with AHL-lactonase gene (*qsdA*) of *Rhodococcus erythropolis* W2. Three genes encoding putative AHL-acylases were identified in *Pseudomonas* sp. 1A1. One of three genes encoding AHL-acylase from strain 1A1 was cloned and expressed heterologously in *Bacillus thurigiensis,* and the recombinant AHL-acylase was produced extracellularly in *B. thuringiensis.* It shared 52% amino acids sequence identity with AHL-acylase, PvdQ from *P. aeruginosa* PAO1. And it belongs to Ntn hydrolase superfamily, including a signal peptide followed by α-subunit, spacer sequence and β-subunit.

Conclusions. We isolated diverse AHL-degrading bacteria from MBR plant using the enrichment cultures. *Rhodococcus* sp. BH4 and *Pseudomonas* sp. 1A1 were shown to have high potential to inhibit biofilm formation. We have cloned an AHL-lactonase gene form *Rhodococcus* sp. BH4 in *E. coli. Pseudomonas* sp. 1A1 produced extracellular QQ enzyme, and three putative AHL-acylase encoding genes were identified. The gene encoding AHL-acylase (PvdQ homologue) from 1A1 was expressed in *B. thuringiensis,* and the recombinant AHL-acylase was produced extracellularly.

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