변형 Cross-feeding assay를 이용한 혼합 배양 시스템에서의
$N$-acylHSL 정족수 감지 미생물 종 분리

Isolation of $N$-acyhomoserine lactone quorum sensing bacteria from mixed culture using modified cross-feeding assay method

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1. Introduction

Many physiological properties of microorganism depends on the quorum sensing mechanism which is gene regulating system in level of bacteria’s signalling molecules (autoinducer). However, all quorum sensing researches have used single model microorganism or mutant bacteria whose target genes were deleted artificially. Therefore, it is difficult to apply these experimental techniques directly to the study of biological wastewater treatment system consisting of various microbial communities. To overcome this problem, we tried to develop the experimental protocol which can isolate the quorum sensing bacteria from mixed culture system by modifying the cross-feeding assay. We studied reliability of the new method with the defined mixed culture of *Pseudomonas auriginosa*, *Bacillus subtilis* and then finally applied this techniques to the lab-scale membrane bioreactor for wastewater treatment and reuse.

2. Result and Discussion

*Agrobacterium tumefaciens* A136(Ti)(pCF218)(pCF372)(Fuqua et. al., 1996) was used as an indicator strain for exogenous AHL autoinducers. This *A. tumefaciens* biosensor is highly sensitive to a variety of AHLs with chains ranging from C6 to C14(Zhu et al., 1998). *Pseudomonas auriginosa*, *Bacillus subtilis* was selected as a model microorganism of gram negative and gram positive bacteria respectively. Activated sludge was obtained from domestic wastewater treatment plant.

For long term storage, all cultures were suspended in a mixture of LB Broth and glycerol and frozen at $-80^\circ$C using glycerol as a cryoprotectant. Prior to use, frozen cultures were removed.
from storage, and incubated on LB medium or LB medium supplemented with spectinomycin and tetracycline (Strain A136(Ti)(pCF218)(pCF372). All cultures were incubated at 30°C.

Isolation protocol was made based on the soft agar method proposed by the McLean et al. (2005). Briefly, *A tumefaciens* A 136 biosensor was grown overnight in LB broth and was placed on the LB agar plate. After solidification, 16㎕ X-gal (50 mg/mL stock solution in dimethyl formamide) was added to the *A tumefaciens* A 136 assay plates.

Then, microorganism sample, defined culture of mixed environmental culture was streaked on the assay plates and incubated for 2 days. If microorganism which can produce acyl-homoserine lactone molecules was exist at the sample, the colony of this microorganism will shows blue coloration due to lacZ expression and X-gal hydrolysis in the *A tumefaciens* biosensor. As a result of this, we can isolate AHL quorum sensing single microorganism. Following selection blue color colony, this single microorganism was cultured in LB medium overnight then finally its quorum sensing activity was confirmed again using conventional cross feeding assay method described by McLean et.al. (1997) and Sticker et al.(1998)

At first, we check the reliability of newly developed method at the defined culture which consists of *Pseudomonas auriginosa*, *Bacillus subtilis*. Gram negative and positive bacteria use different signalling molecules, that is peptide and acyl-homoserina lacton compounds respectively, at the quorum sensing (Miller et al., 2001). After isolation of each strains, we checked the results by both gram staining and cross-feeding assay.

Next, we applied this protocol to the biological wastewater treatment system. Among many biological treatment system, we selected membrane bioreactor. We operated the lab-scale membrane bioreactor with synthetic wastewater and monitored the transmembrane pressure during the operation. The reactor volume was 1 L, the total COD was 2,000 ppm with glucose as a main carbon source. Zeeweed membrane with the pore size of 0.004 micrometer and membrane area of 0.004 m² was operated at the flux of 25 L/min-m². When the transmembrane pressure was reached at the certain level, which indicates the increased biofouling, biofilm formed on the membrane surface was detached by vortexing and quorum sensing active bacteria species was separated using by the same procedure described above.

Finally, we analysed the total microbial community of membrane–biofilm and compared this with isolated quorum sensing active bacteria using PCR–DGGE (Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis) and investigated the quorum sensing activity in membrane bioreactor system.

**References**

Fuqua C. and Winans, S.C., Conerved cis–acting promoter elements are required for