Development of *in vitro* Screening Method of AHL Antagonist by Immobilized TraR and LasR

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Abstract

Acylhomoserine lactones (AHL) are known to be the triggering molecules in the quorum sensing mechanism of many gram-negative bacteria. In order to detect AHL inhibitors that are potential biofilm inhibitors, a sensitive *in vitro* bioassay was developed based on two transcriptional activator proteins, TraR from *Agrobactrium tumefaciens* and LasR from *Pseudomonas aerugenosa*. Both proteins were produced in a His_6 tagged form in recombinant *E. coli* strains harboring the corresponding genes and purified using a Ni^{2+} affinity column. When tested against several AHLs and fimbrolide, a competitive inhibitor of AHL, both proteins exhibited a strong binding affinity with a high specificity. The AHLs and fimbrolide captured by the transcriptional activator proteins could be recovered by denaturing the proteins in urea and analyzed by high performance liquid chromatograph.

References

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