Abstract No: 161 ***Life Sciences***

SOLUBILITY ENHANCEMENT OF THE AHL LACTONASE (jsvC) IDENTIFIED FROM *DERMACCOCUS NISHINOMIYAESNSIS*

G.R.L.R. Goonawardana*, C.Y. Kim, V. Mikolaityte and J.K. Lee

Department of Biotechnology, Pai Chai University, Daejeon, Republic of Korea.
**ruuth.1991@gmail.com*

Quorum sensing and quorum quenching are two key phenomena in bacterial cell signalling mechanisms. In many bacterial populations, gene expression is regulated by the quorum sensing mechanism using N-acylhomoserine lactone (AHL) as the signalling molecule. Quorum quenching refers to all processes involved in the disturbance of quorum sensing. AHL lactonase breaks down the AHL and leads to quorum quenching. The gene coding for AHL-lactonase (jsvC), which belongs to the phosphotriesterase family, was identified in *Dermacoccus nishinomiyaensis* isolated from the faeces of Carp fish. For the overexpression of AHL-lactonase (jsvC), the gene was cloned into the pET-28a vector and protein was expressed in *Escherichia coli* BL21 (DE3). However, the expressed protein was obtained as an inclusion body. Considering the codon bias and high GC content (> 65%) of the coding sequence, the expression host was changed to *E. coli* Rosetta™ and *E. coli* BL21-PGro7, which has additional chaperon vector assisting protein folding. Protein expressions were conducted under different conditions by varying temperatures and IPTG concentrations. Irrespective of these optimization attempts, proteins were obtained as inclusion bodies. A newly modified pET-28a/MBP vector with an in-frame maltose binding protein (MBP) coding sequence was used to overcome the solubility issue. The resulting protein contains an MBP fusion tag at the C terminal of the AHL-lactonase (jsvC) protein. The recombinant MBP-jsvC protein was obtained in soluble form in *E. coli* BL21 (DE3) and purified using the amylose resin. To determine the activity of AHL-lactonase (jsvC), N-hexanoyl-HSL (HHL) was used as the substrate and a soft agar overlay assay was performed with the reporter strain *Chromobacterium violaceum* CV026. HHL degradation by AHL-lactonase (jsvC) was confirmed by the colour chromaticity of violacein production.

Keywords: AHL-degradation, AHL lactonase, *Dermacoccus nishinomiyaensis,* Inclusion bodies**,** Quorum quenching