Enhanced expression of L1 lipase from *Bacillus stearothermophilus* L1 in *Escherichia coli* BL21(DE3) by inserting three peptide (threonine-serine-serine) into its N-terminus

안정오[1], 장형욱[2], 이홍원[4], 최의성[3], 함승주[1], 정준기[4]
연세대학교 화학공학과[1], 에이스 바이오텍[2], 한국생명공학원 미생물유전체연구실[3],
한국생명공학원 산업화지원실[4]
전화(042)860-4522, FAX (042)860-4516

Cellulose binding domain (CBD) and linker sequences of endoglucanase II from *Trichoderma harzianum* were isolated by polymerization chain reaction (PCR), and they were fused to N-terminal of *Bacillus stearothermophilus* L1 lipase for effective immobilization to cellulose matrix and easy purification of it. L1 lipase and fused to CBDLinker(CBDLinker-lipase) were expressed under T7*lac* promoter in *Escherichia coli* BL21(DE3) respectively. A growth inhibition effect was observed in case of the mature BSL expression, not in case of CBDLinker-lipase expression. As a result, the expression of CBDLinker-lipase was dramatically increased about 4 times than it of the mature L1 lipase.

To investigate this improved expression and growth. three kinds of expression vectors were constructed; CBD fused to N-terminal of lipase (CBD-lipase), C-terminal of lipase (lipase-CBD) without linker, and linker fused to N-terminal of lipase (Linker-lipase) without CBD. The enhanced expression of lipase was observed in all expression vectors but the expression characteristics were different. When CBD was fused to N- or C-terminal of lipase, a large portion was observed in the insoluble fraction without loss of its activity. However, when only linker was fused to lipase, most of fusion protein was observed as an active soluble form. Linker sequence consists of 32 amino acids rich in serine, proline and threonine. To investigate the function of amino acids among linker sequences, the subdivided linkers were fused to N-terminal of lipase and were expressed respectively. All subdivided linkers containing theronine-serine-serine amino acids enhanced expression of L1 lipase as same as it of linker (full length)-lipase. Furthermore, we are investigating whether the three amino acids affect the folding or the structure or the enzyme characteristics of lipase.

References

1. Hans-Dietmar Beeer, Gerd Wohlfahrt, Rolf d. Schmid and John E.G. MccarthyKerri, "The folding and activity of the extracellular lipase of *Rhizopus oryzae* are modulated by a prosequence" (1996), Biochemical journal, 319, 351-359
2. Patrick argos, " an investigation of oligopeptides linking domains in protein tertiary structures and possible candidates for general gene fusion" (1990), Molecular biology, 211, 943-958