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Optimization of the Culture Condition for the Production of Recombinant Lipocortin-1-2 in *Escherichia coli* HB101.

이경일*, 이계준
서울대학교 자연과학대학 미생물학과

Lipocortin-1-2 (LC-1-2) was derived by deleting the C-terminal two domains of Lipocortin-1 (LC-1) which is a phospholipase A2 inhibitor originated from human placenta, whose gene was cloned into expression vector pHT2. pHT2 was found to be so unstable that the plasmid was cured thoroughly in a few generations without selection pressure. Ampicillin 100 μg/ml, glucose 1%, KH_2PO_4 1.13 g/l and $MgSO_4 \cdot 7H_2O$ 0.232 g/l were added to Luria broth. Addition of several other minerals did not increase the biomass more. The optimum temperature was 28℃ for seed culture and 40℃ for main culture. The productivity of LC-1-2 was the highest at the optimum temperature although the biomass is less than that at 37℃. Plasmid amplification occurred at the optimum temperature. Main culture was performed at pH 6, 7, 8 and without pH-adjustment. Biomass production at pH 6 was slightly higher than that at pH 7, but the productivity of LC-1-2 at pH 7 was much higher than that at any other pH-operation. Large fraction of glucose in the media was turned into acetate which is toxic to cell and decreases $Y_{x/s}$ and $Y_{p/s}$. Fermentation of acetate-free mutant increased $Y_{x/s}$ and produced lactate instead of acetate.

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Genetic Organization of the Genes Encoding 2,3-DHBP Dioxygenase and HOPDA Hydrolase from 4-Chlorobiphenyl-Degrading *Pseudomonas* sp. DJ-12.

김은희*, 채종찬, 서동인, 김치경
충북대학교 자연과학대학 미생물학과

The *pcbABCD* genes of *Pseudomonas* sp. DJ-12 are responsible for biodegradation of biphenyl/4-chlorobiphenyl. The *pcbC* and *pcbD* genes code for 2,3-dihydroxybiphenyl (2,3-DHBP)/2,3-dihydroxy-4'-chlorobiphenyl dioxygenase and 2-hydroxy-6-oxo-phenyl-hexa-2,4-dienoic acid (HOPDA) hydrolase, respectively. In this study, we determined the nucleotide sequence of the *pcbCD* genes and compared them with those from other related species. The *pcbC* gene composed of 960 bps encodes 2,3-DHBP dioxygenase of 320 amino acids with a calculated molecular weight of 35,000 and *pcbD* gene encodes HOPDA hydrolase of 283 amino acids with a molecular weight of 31,000. The two genes were arranged in the order of *pcbD-pcbC* and the ribosome binding sites were shown to precede the start codon of each gene. A promoter-like sequence was not present upstream of *pcbC* gene and the clone carrying only *pcbC* did not have 2,3-DHBP dioxygenase activity without induction of the *lac* promoter. Therefore, the *pcbD* and *pcbC* genes were thought to be transcribed by using a common promoter located upstream of the *pcbD* gene. Comparison of the deduced amino acid sequences of the PcbC and PcbD with those from other strains indicated that PcbC has 30 to 40% and PcbD has 50 to 60% sequence homology.