Cloning of β-glucosidase gene from *Cellulomonas* sp. into *E.coli*

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To clone β-glucosidase gene from Cellulomonas sp. a gene library was constructed using E. coli JM83 pUC9. Among 2,500 pseudotransformants obtained, 20 clones developed yellow color on the p-nitrophenyl- -D-glucopyranoside filter paper. These 20 clones were classified into three groups based on the results of activity staining using nondenaturating polyacrylamide gel electrophoresis and restriction enzyme digestions. Among the three groups, only one group containing pCEl plasmid has specificity for cellobiose.

Purification and reaction pattern of cephalexin synthesizing enzyme from *Acetobacter turbidans*

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Cephalexin synthesizing enzyme (α amino acid ester hydrolase) was partially purified from the culture broth of *Acetobacter turbidans* ATCC9325 through ammonium sulfate fractionation, DEAE, CM, and Sephacryl S-200 gel filtration. The enzyme has optimum pH 6.0 and temperature, 40°C respectively. From the analysis of reaction mixtures by thin layer chromatographic and high performance liquid chromatographic techniques, it was confirmed this enzyme catalyzed simultaneously the following reactions:

1) Synthesis of cephalexin from D- α -phenylglycine methylester (PGM) and 7-amino 3-deacetoxycetoxycephalosporanic acid (7-ADCA)
2) Hydrolysis of cephalexin to form 7-ADCA and phenylglycine (PG)
3) Hydrolysis of PGM to form PG and methanol.

Base on the above experimental observations, the reaction model of this enzyme was identical with that of the enzyme from *Xanthomonas citri.*

Fermentation of carboxymethylcellulase using recombinant DNA-Bacillus megaterium

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For the analysis of fermentation characteristics and productivity of plasmid coded product, carboxymethylcellulase in a recombinant DNA cell fermentation system, batch and continuous fermentations were carried out using a *Bacillus* megaterium ATCC 14945 transformed with a plasmid, pCK 108 haboring carboxymethyl cellulase gene. The effects of carbon and nitrogen sources and of temperature and pH on cell growth, product yield, plasmid stability, specific plasmid contents of cell,

and gene expression efficiency were carefully studied. These experimental results will be discussed in some details.

*Preteus mirabilis*가 생산하는 cysteinylglycine 분해효소의 정제에 관한 연구

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세균내 glutathione의 동태를 연구하기 위한 일환으로 *P. mirabilis*로부터 cysteinylglycine 분해효소를 정제 검토하였다. 본 균이 생산하는 cysteinylglycine 분해효소의 정제는 무세포추출액에 비해 비활성이 10배 증가하였고 0.68%의 낮은 수율을 나타내었다.

본 효소는 $(NH_4)_2SO_4$ 침전과정에서 활성을 크게 손실하는등 정제과정에서 불안정하였으며 투석중에 형성하는 불용성 침전물은 4% Trition X-100 처리에 의해 효과적으로 용해되었다.

*Proteus mirabilis*가 생산하는 cysteinylglycine 분해효소의 성질 및 세포내 분포에 관한 연구

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*P. mirabilis*로부터 정제한 cysteinylglycine 분해효소의 성질 및 세포내 국재성을 검토하였다. 본 효소의 일반적 성질은 pH가 7.3 온도 37℃에서 최대 활성을 나타내었으며 pH 8.0에서 안정하였고, 열안정성은 50℃, 30분처리에 30%의 활성 손실을 보였다.

또한 Mn^{+2} 이온과 Mg^{+2} 이온에 의해 활성이 촉진되었으며 본 효소를 반응전 30분 preincubation 하므로서 최대 활성을 보였다.

본 효소는 glutathione 일단계 분해효소인 γ-glutamyltranspeptidase와 마찬가지로 세포내의 periplasmic space에 존재하였다.