

Lac 변이주를 MRS-lactose 배지의 plate에 도말하여 배양하면 4일 이후 부터는 revertant가 나타났으나 Lac Gal Cell에서는 인정되지 않았다. 얻어진 Lac 변이주의 plasmid를 분리하여 parent와 비교한 결과 plasmid DNA가 소실되었음을 확인하였다.

***Flavobacterium multivorum* dextranase gene cloning에 관한 연구**

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토양으로부터 dextranase를 분비하는 균을 분리하였으며, 그 균이 *Flavobacterium multivorum*으로 동정되었다. 이 *F. multivorum*의 배치조건에 따른 dextran 이용과 dextranase 분비능을 조사하였고 아울러 성장조건도 알아보았다. 한편 위의 균은 plasmid를 갖고 있지 않았으며, 그에따라 chromosomal DNA를 추출하여 Sau3AI 절단한 후

Factors affecting the final antibiotic titer of sisomicin fermentation

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Since sisomicin which is produced by *Micromonospora inyoensis* is an intracellular antibiotic, the final antibiotic titer to be attained depends significantly on the cell mass in fermentation broth. Cobalt ion in medium was indispensable for getting a high antibiotic titer. However, in the presence of cobalt ion in medium, the antibiotic production proceeded up to about 4 days and thereafter stopped. From the experiments on the addition of cobalt ion to culture medium, it was shown that the antibiotic production stopped due to the other physiological properties of cells rather than the accumulation of antibiotic in cells.

On-Line Induction of Fermentation with recombinant cells: Optimization and Data Acquisition

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λ PL promoter와 Influenza virus의 NS1 Structural gene이 있는 pAS1 EH801 plasmid를 *E. coli* host NS⁺과 AR120에 각각 transformation하여 온도와 nalidixic acid로 각각 induction하여 보았다.

N5151의 경우, O.D. 600 1.2에서 42°C로 induction 하였을때 maximum productivity를 보였으며 AR120의 경우는 O.D. 600 1.2, 37°C, 40 µg nalidixic acid/ml induction 하였을때 maximum yield를 보여주었다. 이때 pH, DO, temperature, O₂%, CO₂%를 A/D converter 통해 computer에 연결시켜 data acquisition을 한 결과, 접종후 ON-line induction이 가능함을 알 수 있었다.

Studies on Thermostable Tryptophanase from a Symbiotic Thermophile

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Thermostable tryptophanase was extracted from a thermophilic bacterium, strain T which was absolutely symbiotic with strain S. The enzyme was purified 14.7 fold with 5.8% yield by chromatographies using ion exchange, gel filtration, and hydrophobic interaction columns, followed by high performance liquid chromatography on hydroxyapatite column. The purified enzyme has a molecular weight of approximately 210,000 estimated by gel filtration column chromatography, and the molecular weight of subunit was determined by SDS polyacrylamide gel electrophoresis to be 46,000, which indicates that the native enzyme is made of four homologous subunits. The tryptophanase was stable at 65°C and the optimum temperature for the enzyme activity for 20 min reaction was 70°C. The purified enzyme activity for 20 min reaction was 70°C. The purified enzyme catalyzed the degradation of L-tryptophan into indole, pyruvate and ammonia in the presence of pyridoxal phosphate. 5-Hydroxy-Ltryptophan, 5-methyl-DL-tryptophan, L-cysteine, S-methyl-L-cysteine, 5-methyl-DL-tryptophan, L-cysteine, S-methyl-Lcysteine, and L-serine were also used as substrates to form pyruvate. The amino acid composition of the tryptophanase was determined, and found to contain a high percentage of hydrophobic amino acids, especially in the proline content, which was much higher than that of *Escherichia coli* tryptophanase. In addition, the 35N-terminal amino acid sequence of the tryptophanase was completely different from that of *E. coli* tryptophanase.

Cloning and Expression of *B. Aphaericus* insecticidal toxin gene in *E. coli*

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B. sphaericus 1593 K-5 synthesize a potent entomocidal toxin against mosquito larvae. *B. sphaericus* EcoRI DNA fragment carrying the biocidal activity was cloned and expression in *E. coli* JM83. For the construction of a recombinant plasmid bearing the toxin activity the DNA of *B. sphaericus* was partially digested by the EcoRI. The EcoRI DNA fragments were ligated to plasmid pUC 8-EcoRI site. The transformants were selected on LB plates containing X-gal and ampicilline. The transformants were bioassayed against mosquito larvae of which two clones showed biocidal activity. The two clones were redigested with Eco R1 and analyzed by 0.7% agarose gel electrophoresis.