N5151의 경우, O.D.600 1.2에서 42℃로 induction하였을때 maximum productivity를 보였으며 AR120의 경우는 O.D.600 1.2, 37℃, 40 μg nalidixic acid/ml induction 하였을때 maximum yield를 보여주었다. 이때 pH, DO, temperature, O_2%, CO_2%를 A/D converter 통해 computer에 연결시켜 data acquision을 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 entomicidal toxin against mosquito larvae. B. sphaericus EcoRl DNA fragment carrying the biocidal activity was clonied 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 EcoRl. The EcoR1 DNA fragments were ligated to plasmid pUC 8-EcoR1 site. The transformants were selected on LB plates containing X-gall 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 electrophorsis.