The apparent Km values of the purified xylanase I and II were 7.0 and 2.5 ㎎/㎖ respectively against oat spelt xylan. The purified enzyme is surpposed to endo-type xylanase by the of TLC analysis of reaction products.

H308

Development of Method of Multiplex PCR for Specific Detection of *L.ivanovii* in Food

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Study of development of multiplex PCR method was conducted to develop a rapid and precise detection method in foods using specific primer of *iap*(invasion-associated protein) gene encoding p60 protein that commonly exist in all of *Listeria spp.* We ensured that *L. ivanovii* and other *Listeria* species could be detected with the multiplex PCR method. We should demonstrate that this method is superior to biochemical detection by reinspecting biochemically classified species. This multiplex PCR is simple, precise and economic. Also, this detection method have various application potential.

H309

Purification of the *E.coli* Expressed p60 Protein from *Listeria welshimeri*II by Amylose Resin Based Affinity Chromatograpy

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The *Listeria welshimeri*II is an animal and human pathogen and its p60 protein is a major extracellular protein, which is encoded in *iap*(invasion associated protein) gene. These proteins are believed to be involved in the invasion of these bacteria into their host cells. To produce p60 in *E.coli*, the *iap* gene was recombinantly cloned and overexpressed. A purification protocol was developed for MBP(maltose binding protein)-p60 fusion protein by amylose-resin based affinity chromatograpy. The purified MBP-p60 was detected either as denaturated or neutralized form using a specific p60 monoclonal antibody. The method might be an easy alternative to common purification protocols of p60 from *Listeria spp.* for antibody production.

H310

Enhanced Production of Avermectin B1a with *Streptomyces avermitilis* by Medium Optimization and Glucose Feeding

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Avermectin is a group of potent anthelmintic and insecticidal antibiotics produced by *Streptomyces avermitilis.* Productivity of avermectin B1a was enhanced by medium optimization and intermittent glucose feeding. Response

surface methodology was used to optimize the concentration of nitrogen sources. A strong stimulation on avermectin B1a production in *S. avermitilis* was observed by 3% glucose feeding at the time of residual glucose being 1%. The avermectin B1a productivity could be further improved by another glucose feeding at 206 hour of cultivation. Avermectin B1a titer was increased by 86.3% and the proportion of avermectin B1a in the total avermectins was increased from 38% to 45% through medium optimization and glucose feeding process. These results would be very useful for enhancing productivity of avermectin B1a in up-scaled processes.

H311

Development of a Simplified Purification Process of Avermectin B1a from Fermentation Broth of *Streptomyces avermitils* YA99-40

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Avermectin B1a is the most potent anthelmintic and insecticidal antibiotic among eight avermectin derivatives produced by *Streptomyces avermitilis*. To circumvent difficulty in the purification of avermectin B1a from the other avermectins, especially avermectin B1b, a new *S. avermitilis* mutant YA99-40 was developed by mutagenesis through ultraviolet light irradiation and protoplast fusion. Avermectins were extrated with 5:1 mixture of ethylacetate and acetone, then applied to a column packed with XAD-2000 resin. The avermectins were eluted with 95% methanol. The pooled avermectins B1a was concentrated and further purified by crystallization. Analysis result of the purified avermectin B1a showed that the proportion of avermectin B1 was more than 95%. This process was simple and applicable to up-scaled purification processes

H312

Synthesis of 7a-hydroxycephalosporin C by Immobilized Enzyme

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In the chemical synthesis of 7a-methoxylated cephalosporins from cephalosporins substrate, difficulties have been experienced such as low conversion yield and generation of toxic wastes. Enzymatic 7a-methoxylation has been studied to alleviate these problems. The methoxylation of cephalosporin C by enzymes was reported to proceed by two step reactions which involves the formation of 7a-hydroxy cephalosporin C with subsequent methylation to yield 7a-methoxycephalosporin C. We have attempted to synthesize 7a-hydroxycephalosporin C from cephalosporin C as the first step with immobilized cephalosporin 7a-hydroxylase. First of all, the conversion of cephalosporin C to 7a-hydroxycephalosporin C was examined with the cell-free extract of several cephamycin producing strains for the selection of converting strain. *Streptomyces clavuligerus* ATCC 27064 was the most potent strain for the activity of cephalosporin 7a-hydroxylase. The cephalosporin 7a-hydroxylase was purified to near homogeneity through DEAE-sephacel chromatography, ammonium sulfate