Intergeneric protoplast fusion between *Bacillus pumilus* and *Cellulomonas fimi*

D. M. Kim*, K.H. Lee
Dept. Food Sci. & Technol., College of Agri.,
Seoul National Univ.

Cellulose utilising hybrids between *Cellulomonas fimi* and *Bacillus pumilus* were isolated after PEG mediated protoplast fusion. 33% (w/v) PEG #6,000 and 50mM Ca++ were optimum concentration. The intergeneric fusion frequency was 3.2 x 10^{-7}. Extracellular CMCase and β-glucosidase activities were detected from one hydrid unlike only CMCase was detected from *Cellulomonas fimi*.

*Penicillium verruculosum*의 세포융합에 관한 연구

정기철 · 박창렬 ○ · 배 석 · 전순배 · 김기청
전남대학교 생물공학연구소

섬유소 분해효소를 생산하는 *P. verruculosum*으로부터 유도된 영양요구성 돌연변이주간의 원형질체 융합을 위한 조건을 검토하였다. 18-20시간 배양한 각 영양요구성 돌연변이주 균사체에 Novozym 234를 처리하여 원형질체를 추출할 수 있었으며, 원형질체 생성량은 각 영양요구성 돌연변이주의 균사체 40mg(dry weight)당 $2.4-3.0 \times 10^7$수준이었고, 원형질체 재생 완전 배지상에서의 환원율은 26.6-42.4% 수준이었다. 원형질체 융합을 위한 Polyethylene glycol (PEG) 6000의 최적농도는 20%였으며, PEG 최적 처리시간은 10분, $CaCl_2$의 최적 첨가농도는 10mM, 최적 pH는 5.5였고, 원형질체 융합 최적조건하에서의 융합율은 $1.8 \times 10^{-3}-3.5 \times 10^{-3}$ 수준으로 나타났다.

모균주와 융합체로부터 측정된 DNA 양의 차이로 보아 융합체의 염색체는 aneuploid 상태임을 알 수 있었으며, filter paper를 기질로 한 Cellulase 활성측정에서 융합체가 야생형 균주보다 2배이상, 모균주 보다는 1-3배 이상 향상되었다.

A spore-forming bacterium as a typical contaminant in aminogly-coside antibiotic fermentation process

이찬용* · 이재홍 · 조영제 · 길광훈 · 유무영
제일제당(주)종합연구소

항생물질 발효중 자주 오염을 일으키는 원인균을 분리하여 그 특성과 오염방지를 위한 연구를 수행하였다. 오염원인균을 분리한 결과, 열저항성 포자를 형성하며, Gram 양성, Catalase 양성, 간균인 *Bacillus* sp. 이었다. 이 오염균은 여러종류의 항생물질에 대하여

내성을 가지고 있었으며, R-Plasmid는 갖고 있지 않았다. 항생물질 발효시 매 24시간마다 인위적으로 오염을 시켜본 결과 초기 2일내에 오염이 되었을 경우에는 항생물질 생산이 거의 이루어지지 않았으나, 발효 3일 이후 즉 항생물질 생성시기 (idiophase)에는 오염이 되었다 하더라도 항생물질 생성에 크게 영향을 못 미쳤다. 또한 초기 오염억제의 방법으로 낮은 농도의 젠타마이신을 발효 초기에 첨가한 결과 항생물질 발효에는 영향을 주지 않고서도 오염을 억제할 수 있었다.

Molecular Cloning of a CMCase Gene from Alkalophilic sp. and Its Expression in *Escherichia coli*

Ju-Hyun Yu, In-Soo Kong,
Jin-Man Kim, Yoon-Suk Park
Yonsei Univ., Dept. of Food Eng.

For isolation of the CMCase gene of the alkalophilic *Bacillus sp.* strain N-4 to analyze their genetic information for the multicomponents of the cellulase, *Bscherichia coli* K12 and plasmid DNA pBR322 was used as host-vector system.

After the digestion of purified chromosomal DNA and plasmid DNA pBR322 with HindIII, these were ligated. The ligated DND were transformed into *Escherichia coli,* and recombinant plasmid 107 carried the gene coding for CMCase was constructed. The CMCase produced by *Escherichia coli* cells containing plasmid DNA pYBC107 was found in the cells as intracellular enzyme and nearly 60% of the total CMCase activity was localized in cellular fraction. Also, the optimum pH for the reaction of CMCase produced by *Escherichia coli* was appeared at pH 8.0 and the enzyme was stable between pH 7.0 and pH 8.0.

Expression of Tunicamycin Resistance in *Bacillus subtilsls* by Several Transfroming Plasmids

In-Soo Kong* and Makari Yamasaki
*Department of Food Engineering, Yonsei University
Department of Agricultural Chemistry, The University of Tokyo

pSp-Si (1.6 kbp) was originally found in pediococcus halophilus to be a cryptic multicopy-plasmid. Hoping that the plasmid can also replicate in Bacillus subtilis, protoplast transformation of strain 207-25 (recE) was performed using pSP-Sl onto which was added the marker of tmrB8 (on 4.9 kbp EcoRI fragment) or tmrB+ (on 0.9 kbp xbaI fragment) gene. Though the tmrB8 gene can expres tunicamycin-resistance at the single copy state, and the tmrB+ gene exerts the resistance only at the multicopy state, we could not confirm the replication of pSP-Sl (tmrB8) or pSP-Sl (tmrB+) in B. subtilis. During the experiment, however, we unexpectedly found that the circularized 0.9 kbp xgaI fragment (tmrB+) itself, which had no replication origin, could transform strain 207-25 to tunicamycin-resistant by protoplast transformation. Southern hybridization analyses with tmrB+ and other probes revealed the integration of the fragment at a single copy state into a position other than the homologous tmrB gene. This recE independent integration of another tmrB+ gene into the chromosome may contribute to the tunicamycinresistance in the transformants.