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

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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 $3.2 \times 10^{-7}$. Extracellular CMCase and $\beta$-glucosidase activities were detected from one hybrid unlike only CMCase was detected from *Cellulomonas fimi*.

*Penicillium verruculosum*의 세포융합에 관한 연구

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섬유소 분해효소를 생산하는 *P. verruculosum*으로부터 유도된 영양요구성 돌연변이주간의 원형질체 융합을 위한 조건을 검토하였다. 18-20 시간 배양한 각 영양요구성 돌연변이주 균주사체에 Novozym 234를 처리하여 원형질체를 추출할 수 있었으며, 원형질체 생성량은 각 영양요구성 돌연변이주의 균주사체 40mg (dry weight) 당 2.4-3.0 $\times 10^{7}$ 수준이었고, 원형질체 재생 완전 배지상에서의 활성율은 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 최적조건 하에서의 융합율은 $1.8 \times 10^{-3}$-3.5 $\times 10^{-3}$ 수준으로 나타났다.

모균주와 융합체로부터 추정된 DNA 양의 차이로 보아 융합체의 염색체는 aneuploid 상태임을 알 수 있었으며, filter paper를 기질로 한 Cellulase 활성측정에서 융합체가 약생형 군주보다 2배이상, 모균주보다는 1-3배 이상 향상되었다.

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

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항생물질 발효중 자주 오염을 일으키는 원인균을 분리하여 그 특성과 오염 방지를 위한 연구를 수행하였다. 오염원균을 분리한 결과, 염증항성 포자를 형성하며, Gram 양성, Catalase 양성, 간균인 *Bacillus* sp. 이었다. 이 오염균은 여러종류의 항생물질에 대하여
Molecular Cloning of a CMCase Gene from Alkalophilic sp. and Its Expression in *Escherichia coli*

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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, *Escherichia 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 subtilis* by Several Transfoming Plasmids

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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-SI 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-SI (tmrB8) or pSP-SI (tmrB+) in *B. subtilis*. During the experiment, however, we unexpectedly found that the circularized 0.9 kbp xbaI 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 tunicamycin-resistance in the transformants.