High Production of Thermostable Beta-galactosidase of *Bacillus stearothemophilus* in mesophiles

Hirosuke Okada, Haruhisa Hirata and Seiji Negoro
Department of Fermentation Technology, Osaka University, Yamada-oka, Suita-shi, Osaka 565, Japan.

Recent advances in recombinant DNA techniques have provided a tool for breeding of microorganisms of hyper production. Enzyme production by cloned microorganism has some advantages. They are i) Enzymes can be produced by a microorganism easily cultured ii) Hyper production. iii) In some cases, such as thermophilic enzyme gene is cloned in a mesophilic bacteria, the enzyme purification procedure can be simplified. One examaple, production of thermophilic β-galactosidase in *B. subtilis* will be presented.

Bacillus stearothermophilus IAM11001 produced three β-galactosidases, β-galactosidase I, II and III (β-gal-I, II and III). By connecting restriction fragments of the chromosomal DNA to plasmid vector, followed by transformation of *Escherichia coli,* two β-galactosidase genes (*bgaA and bgaB)* located close to each other on the chromosome were cloned.

By recloning the gene of β-gal-I into *B. subtilis* using pUB110, the enzyme production was enhanced 40 times. *B. subtilis* (pHG5) produced γ-gal-I about 6% of the total parotein in the cell extract while *B. stearothermophilus* produced the enzyme at 0.15% of total protein or 10% of th total -galactosidase activity. As most of proteins of *B. subtilis* can be denatured at 70°C this enzyme could be purified to 80% homogeniety by incubating the cell extract of *B. subtilis* at 70°C for 15 min followed by centrifugation off the denatured proteins. The DNA sequence of *bgaB* gene and its franking reagions were determined. From the results, it has a typical promoter useful for *x55* of RNA-polymerase of *B. subtilis,* TATAAT for -10 region and TTGACA for -35 region in addition to the strong S.D. sequence. From the advantages of the enzyme characteristics, heat stability, low Km for lactose, relatively high Ki value for galactose, high production and easy purfication, this enzyme is suitable for industrial use.

Factos affecting the production of butanol and acetone by *Clostridium acetobutylicum*

G. Gottschalk, Institut fur Mikrobiologie, Universitat Gottingen, Federal Republic of Germany

Owing to the growing interest in the production of fuels and chemicals from biomass, the well-know butanol-acetone fermentation as carried out by *Clostridium acetobutylicum* has been intensely studied again in recent years. Several solvent-yielding fermentation processes were established wnich are operated by using batch cultures or continuous cultures. I could be shown that under conditions of phosphate limitation an asporogenous mutant of *C. acetobutylicum* establishes itself in a chemostat which produces the solvents continuously. Attempts have been made to change the butanol/acetone ratio in favor of butanol production.

A corresponding shift of the product spectrum can be achieved by carbon monoxide addition to the head space of the fermentation (B.H. Kim et al., App. Envioron. Microbiol. 48, 764-770, 1984) or by iron limitation.

Progress has been made in understanding the mechanism underlying the shift from acid to solvent prodcction. Experimental results are in agreement with the view that intracellular accumulation of acetic and butyric acid results in a shortage of phosphate and coenzyme A. This shortage may serve then as signal for the synthesis of the enzymes involved in the formation of acetone and butanol.