STABILITY AND STORAGE OF ESCHERICHIA COLI MUTANTS HYPERPRODUCING β-GALACTOSIDASE

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SUMMARY

Enzyme activity of the hyperproducing mutants isolated from a chemostat decreases by passaging under nonselective conditions to about one half of the original value, and then remains stable. High activity can be quickly restored by transfer to chemostat selective conditions. The elaborated storage method prevents the decrease of enzyme activity after 2-3 years.

INTRODUCTION

An important problem in microbiology is the stability and storage of microbial strains especially when they overproduce a particular compound. These strains are often functionally crippled and suffer from one or more genetic disorders or, in case of an extra inserted gene or plasmid, they suffer from the extra genetic burden, which they must carry. Such strains may survive only in a growth environment which does not select against them; in other words the improved property of the strain must offer a growth advantage for the overproducer (Neijssel and Tempest, 1979). The selection of enzyme hyperproducing mutants in chemostat under specific nutrient limitation is based on this principle (Horiuchi et al., 1962). In this paper we present a study of the stability of two hyperproducing mutants for β-galactosidase and their storage.

MATERIALS AND METHODS

Bacterial strains: Escherichia coli B-28 and K-12, both inducible for β-galactosidase, were used as parental strains for selection of constitutive hyperproducing mutants.

Medium: Mineral medium M 56 (Monod et al., 1951) with appropriate carbon source (for selection lactose 1.5 mM, for batch cultivation and single colony isolation glycerol 20 mM).
Chemostat selection procedure (Horiuchi et al., 1962) was used for selection of hyperproducing mutants for β-galactosidase. Assay of β-galactosidase was performed according to Lederberg (1950) and the specific enzyme activity was expressed as nkat per mg dry cell mass.

RESULTS AND DISCUSSION

Mutants with elevated synthesis of β-galactosidase selected in chemostat synthesize the enzyme constitutively with about 7-fold increased specific activity (285 nkat) in comparison with the fully induced parental strain (40 nkat). The increased specific enzyme activity of *E. coli* B-28 mutant occured only in the first passage in a batch culture grown on glycerol. During further ten passages under these conditions the enzyme level decreased to less than one half of the original value i. e. to about 120 nkat and remained stable and constitutive (Fig. 1). Similar results were achieved with *E. coli* K-12.

![Figure 1. Decrease of β-galactosidase level during passaging of hyperproducing population in a batch culture.](image)

The genetic analysis of β-galactosidase hyperproduction showed that the increased enzyme synthesis resulted from the amplification of lac genes. The extra copies of lac genes are tightly associated with the lac region on the bacterial chromosome (Horiuchi et al., 1963). No lac genes are present in extra-chromosomal vectors.