CATABOLITE REPRESSION IN MICROBIAL TRANSFORMATION OF DRUG MOLECULES

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The use of microbial transformation to prepare intermediates in drug synthesis has been shown to provide an alternative to chemical methods that is potentially safer and more efficient. Preliminary screening experiments with Streptomyces and Cunninghamella species have indicated that transformation may be dependent on the primary carbon source in the growth medium. Catabolite repression, particularly by glucose, has been demonstrated in β-galactosidase enzyme systems in E.coli (Paigen and Williams 1979), and has been shown to prevent utilisation and transformation of simple amines by methylotrophic Pseudomonads, (Sewell et al 1979). We report experiments carried out to determine whether N-dealkylation by a Cunninghamella species is subject to catabolite repression or is simply a function of the growth rate of the organism.

The carbon sources examined were glucose, maltose, sucrose, lactose and succinate. The test organism was a codeine transforming Cunninghamella isolate (GJS14). This was grown in a semi defined liquid medium containing 1% of the test carbon source. Codeine phosphate was added to a secondary culture after 24 hours incubation at 27^{o}, to give a concentration of 10^{-3} Mol.L^{-1}, and incubation was continued for 6 days. The liquor was basified, extracted with 1,2,dichloroethane, evaporated to dryness, and the residue dissolved in tetrahydrofuran (1.0ml), from which 100µl aliquots were taken for analysis. Phenazocine 1mg/ml in tetrahydrofuran (100µl) was added as internal standard to each aliquot and derivatisation was carried out with acetic anhydride in pyridine. After drying under nitrogen the residue was redissolved in tetrahydrofuran (100µl) and analysed by GLC using a 2m glass column packed with 3% SE 30 Ultraphase on Chromosorb W HP 100/120 mesh. A measure of cell growth was obtained by determining the dry cell weight of the inoculum and of the final cell mass of the secondary culture prior to extraction. The data obtained are summarised in Table 1.

Table 1. Cell growth and transformation yields for a Cunninghamella isolate grown with different carbon sources.

Carbon source	increase in dry cell weight (g)	% codeine transformed
Glucose	0.40	1.42
Sucrose	0.52	3.19
Maltose	0.62	3.90
Lactose	0.13	4.37
Succinate	0.15	11.27
None	0.25	8.95

Limited growth of the test organism was observed in the absence of a primary carbon source, presumably because it was able to utilise as a carbon source the amino acids in the growth medium. A high yield of nor-codeine was obtained under these conditions. The presence of glucose, sucrose, or maltose resulted in enhanced cell growth but lower yields of transformed codeine. Reduced cell growth with a significant increase in the yield of nor-codeine was observed with succinate as the primary carbon source whereas the presence of lactose inhibited cell growth and also reduced transformation yield. The data indicate that the yield of transformed drug is not a direct function of cell growth. Care must be exercised in the selection of the primary carbon source, particularly in screening procedures where important transformations may not be detected because of repression by specific sugars.

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