OBTENTION AND CHARACTERIZATION OF A S. clavuligerus gut 1 MUTANT ABLE TO UTILIZE GLUCOSE FOR GROWTH AND CEPHAMYCIN AND CLAVULANE ACID PRODUCTION

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We are studying the molecular mechanism of carbon catabolite regulation exerted by glucose on cephamycin C biosynthesis in Streptomyces. S. clavuligerus is able to use glycerol but does not grow on glucose, due to lack of glucose kinase activity. Four clones able to utilize glucose were obtained by mutagenesis with NTG and plating of 1.5x10^6 spores of S. clavuligerus in minimal media with glucose as only carbon source. One of these mutants, S. clavuligerus gut 1, was characterized biochemically.

Glycerol, maltose and starch were used efficiently as carbon source by the wild type and the gut 1 mutant. In addition, the mutant was able to grow on glucose and galactose both in solid and liquid medium. 2-deoxyglucose (2-DOG) and 6-deoxyglucose inhibited the growth of both strains on maltose and that of the mutant on glucose.

The gut 1 mutant showed a linear rate of glucose uptake and incorporation for at least 30 minutes. The wild type showed only a 3% of uptake in relation to the gut 1 mutant. The uptake of glucose by the mutant was inhibited by azide (2mM) (65%), 2-4 dinitrophenol (2mM) (98%) and oligomycin (0.125mM) (93%). Glucose competed with the uptake of 2-DOG and galactose in the mutant, but no with the uptake of glycerol.

ATP-dependent glucose kinase activity was not detected in the wild type but it was present in the gut 1 mutant to the same level as in Streptomyces coelicolor (41 mUnits/mg of protein).