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INHIBITION OF CI857-CONTROLLED RECOMBINANT GENE EXPRESSION IN *Escherichia coli* AT VERY LOW CONCENTRATIONS OF GLUCOSE.

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Summary. The effects of glucose on biomass and temperature-mediated recombinant gene expression has been studied in *Escherichia coli* batch cultures. Glucose, above 2 g/L, has inhibitory effects on aerobic cell growth and specially on gene expression. Although lower concentrations have no influences on biomass, a tenuous, but still remarkable concentration-dependent reduction of gene expression levels was observed even at about 0.1 g/L.

INTRODUCTION

Composition of culture media is of essential importance regarding productivity of recombinant proteins in bacteria. Different formulations have been proposed, improved and adapted to specific products and to particular fermentation strategies. Glucose is the most universal sugar used as carbon source in recombinant bacterial cultures. However it has been shown that some of its by-products released during aerobic growth of *E. coli*, mainly acetate (Reiling et al., 1985). have inhibitory effects on cell metabolism (Andersen and von Meyenburg, 1980) and recombinant protein production (Rinas et al., 1989). Therefore, researchers using this supplement in fermentation experiments try to keep its concentration under the inhibitory levels, considered to be about 1 g/L (Cutayar and Poillon, 1989; Chen et al, 1992).

In this work, we have studied bacterial growth and thermal induction of recombinant gene expression in *E. coli* batch cultures supplemented with different glucose concentrations (from 0.1 to 40 g/L). In any case, glucose addition improves protein and biomass production, and the inhibitory effects on gene expression evidenced at high concentration are still detectable at 0.1 g/L.

MATERIALS AND METHODS

The strain used in all the experiments was MC1061, *E. coli* K12 *hsdR, mcrB, araD139, (araABC-leu)7679, LacX74, galU, galK, rpsL, thi*, harboring the plasmid pJCO46. This construct derives from pJLA602 (Schauder et al., 1987), and expresses the *E. coli lacZ* gene under the control of $p_L p_R$ promoters and the temperature-sensitive CI857 repressor. Details of this plasmid will be given elsewhere (Vila et al., manuscript in preparation). The culture media was always Luria-Bertani (LB) broth (Sambrook et al., 1989) with 100 μg/ml ampicillin. After an 1:50 inoculum from overnight cultures, cells were grown in 100 ml shaker flasks at 250 rpm and at 28°C. Thermal induction was done by shifting to 42°C when the OD_{550} reached 0.4. Glucose, when present, was added to the medium just before the inoculum, from a stock of 400 g/L in LB medium. ß-Galactosidase was assayed as described (Miller, 1972) after cell lysis mediated by chloroform and 0.1 % SDS.

All the experiments were performed at least by duplicate and the results were very consistent. Representative ones are depicted in Figure 1.

RESULTS AND DISCUSSION

Biomass evolution and recombinant gene expression after thermal induction was studied in cells growing in LB medium supplemented with different glucose concentrations. The main objectives were to determinate the minimal doses in which the "Crabtree effect" was detectable in our system, and to evaluate the putative stimulating effects of lower glucose concentrations on gene expression, in order to incorporate this supplement in batch production experiments. At more than 50 g/L, glucose is considered to be inhibitory of *E. coli* cell growth (Riesenberg, 1991). However, only moderate effects on optical density were detected at 40 g/L, that became no significant at 20 g/L or less (Figure 1A). Moreover, cell viability was not essentially affected at these glucose concentrations and plasmid segregation was not detected. However, *lacZ* gene expression was dramatically diminished (Figure 1B). To determine if at lower doses, glucose could improve cell growth or production of the recombinant enzyme, concentrations were progressively reduced until 0.1 g/L. Surprisingly, in any of the performed experiments we were able to find the expected stimulatory effects. On the contrary, glucose, even at the lowest doses we tested, had some light but consistent inhibitory, dose-dependent influences (Figure 1D, F), although below 0.5 g/L, the pH of the culture was not distinguishable from that of the non supplemented cultures after 5 hours of induction (not shown).

These results indicated that the undesirable "Crabtree effect" is not restricted to high concentrations of glucose, and that in batch cultures, glucose addition to rich media is not advantageous regarding productivity of thermally induced recombinant proteins.

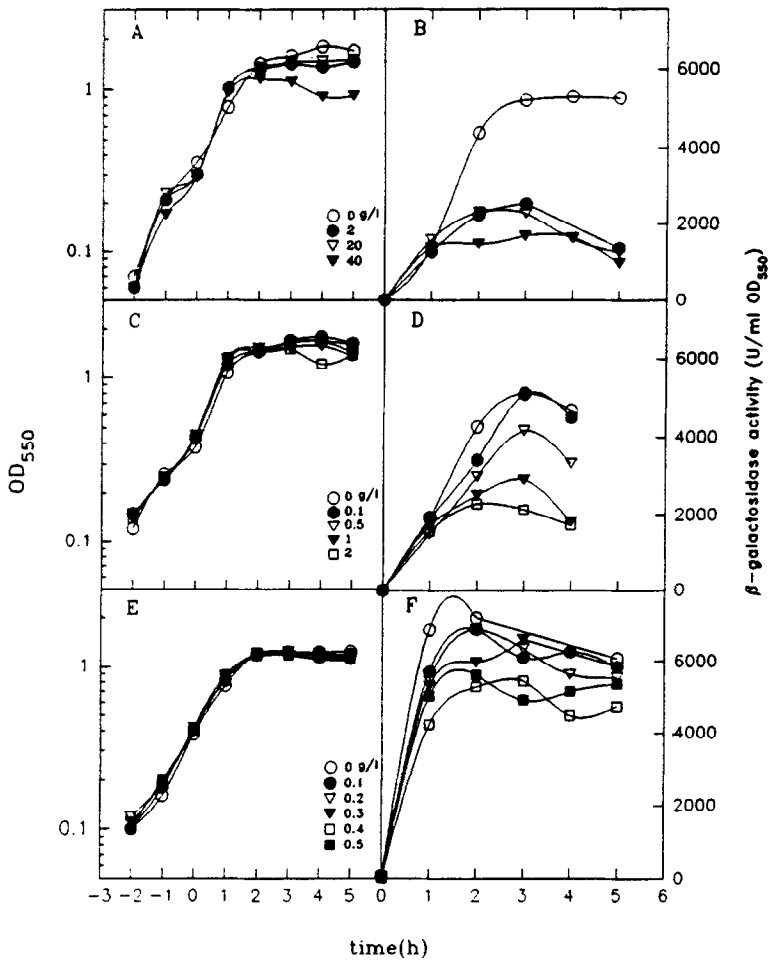

Figure 1. Biomass (left panels) and recombinant gene expression (right panels) in induced cultures growing in presence of different glucose concentrations (2 to 40 g/L in A, B; 0.5 to 2 g/L in C, D; 0.1 to 0.5 g/L in E, F). Time 0 indicates the temperature shift.

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