CHLORAMPHENICOL PRODUCTION IN CARBON-LIMITED MEDIA: EFFECT OF METHYL α-GLUCOSIDE

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Streptomyces venezuelae grows rapidly with glucose as a source of carbon and energy but fails to grow when glucose is replaced with methyl α-glucoside[1]. The methylated sugar induces an α-glucosidase for which it has been shown to serve as a substrate. Failure to metabolize the glycoside may be due either to inability of the mycelium to transport methyl α-glucoside at a rate sufficient to support growth, or to a modification of the glycoside during or immediately after uptake, which renders it inaccessible to the enzyme. The latter situation occurs in *Escherichia coli* where methyl α-glucoside is phosphorylated during transport by the phosphoenol-pyruvate: sugar phosphotransferase system[2]. Experiments in which mixtures of glucose and methyl α-glucoside were used as the carbon source in cultures of *S. venezuelae* indicate that the methylated sugar interferes with glucose utilization, creating carbon-limited growth conditions. These caused a decrease in the excretion of acidic metabolites and an increase in the specific rate of chloramphenicol synthesis.

Cultures grown in shaken 500-ml Erlenmeyer flasks containing 50 ml of a medium with 2% glucose, 30 mM potassium nitrate and the salts composition of medium B[3] excreted large amounts of acid. By 48 hours the pH of the cultures fell below 5.5, preventing further growth. Addition of increasing amounts of methyl α-glucoside to the medium resulted in a progressive decrease in the rate of glucose consumption (Fig. 1). At 1% glycoside concentration, acidification of the cultures was less rapid but the pH still reached values that terminated growth prematurely and slowed utilization of the carbon and nitrogen sources. At 2% and above, no decrease in pH was observed and growth yields became nitrogen-limited. Cell mass doubling times were

Fig. 1. Effect of methyl α-glucoside on the time required to reach maximum biomass (□), exhaust the nitrogen source (△) and consume 30% of the carbon source (○) in cultures of *Streptomyces venezuelae* grown in glucose - nitrate medium.

The final pH of cultures in media with less than 2% methyl α-glucoside was 4.4~4.6.

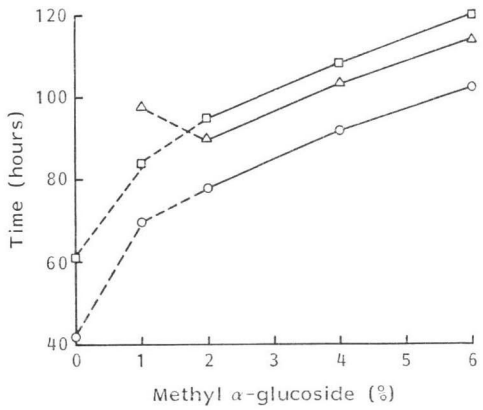

Fig. 2. Biomass accumulation (○), nitrate utilization (△), glucose utilization (□) and chloramphenicol synthesis (●) in cultures of *Streptomyces venezuelae* grown in a buffered glucose - nitrate medium alone (——), and supplemented with 6% methyl α-glucoside (------).

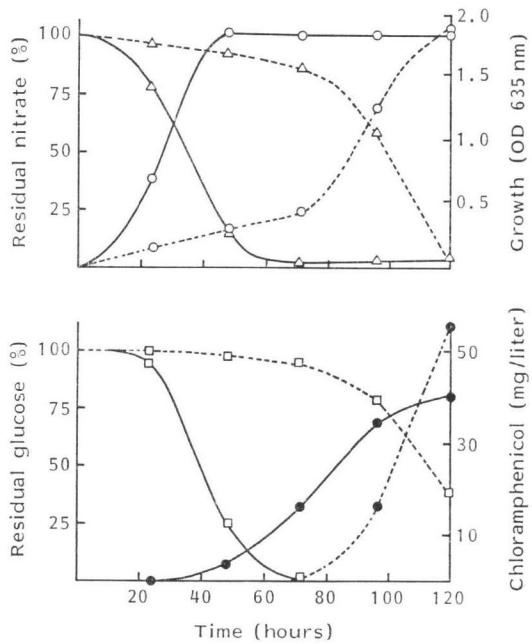

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Table 1. Specific rate of chloramphenicol synthesis (μg/ml/hour/OD_{640nm}) in media containing methyl α-glucoside.*

Methyl α-glucoside (%)	Age of culture			
	48 hours	72 hours	96 hours	120 hours
Nil	0.21	0.33	0.29	0.13
1	0.25	0.42	0.50	0.27
6	0	0.75	1.02	0.98

* The basal medium contained (per liter): glucose 20 g, KNO_3 3.03 g, KH_2PO_4 4.5 g, K_2HPO_4 10.5 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, NaCl 90 mg, $CaCl_2$ 90 mg, $FeSO_4 \cdot 7H_2O$ 9 mg, $ZnSO_4 \cdot 7H_2O$ 3.96 mg, $CuSO_4 \cdot 5H_2O$ 180 μg, $MnSO_4 \cdot H_2O$ 27 μg, H_3BO_3 26 μg and $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 17 μg.

restricted by the rate of glucose utilization, which decreased with increasing methyl α-glucoside concentration. Measurements of the concentration of methyl α-glucoside in the medium during growth of the cultures, obtained by difference from assays of total carbohydrate[4] and reducing sugar[5], confirmed that methyl α-glucoside was not metabolized by *S. venezuelae*.

The rapid fall in pH resulting from acid production in weakly buffered medium could be prevented by increasing the potassium phosphate concentration to 107 mM. The effect of a 6% methyl α-glucoside supplement on biomass accumulation and substrate utilization patterns for cultures grown under these conditions with 2% glucose as the carbon source is shown in Fig. 2. In the absence of methyl α-glucoside, chloramphenicol was produced at the end of exponential growth; in the presence of the glucoside, antibiotic biosynthesis became more closely associated with growth. In addition, the specific rate of synthesis increased (Table 1).

In cultures where potassium nitrate was replaced with proline, a more slowly assimilated source of nitrogen[3], addition of methyl α-glucoside at concentrations up to 8% had little effect on growth or on the rate at which glucose or proline was metabolized. These cultures did not produce appreciable quantities of acid and, even in a weakly buffered medium with 7 mM phosphate, 2% glucose and 30 mM proline, the pH remained close to 7.0. Other results (Z. U. AHMED and S. SHAPIRO, unpublished) indicate that copious acid excretion occurs during nitrogen-limited growth of *S. venezuelae* in glucose-containing media. Failure to produce acid and the insensitivity to methyl α-glucoside in glucose-proline medium suggest that carbon compounds generated endogenously during metabolism of proline may restrict the assimilation of glucose in a manner mimicking the action of methyl α-glucoside.

In the glucose-proline medium chloramphenicol was produced during late exponential growth and the rate of synthesis decreased sharply after proline was depleted. Addition of methyl α-glucoside to the medium gave a concentration-dependent increase in antibiotic production only at supplement concentrations of 4% and above. Overall, the results of these experiments support the previous conclusion[6] that chloramphenicol production is negatively correlated with growth rate. They supplement observations of increased synthesis during nitrogen-limited growth[7] with evidence that carbon limitation yields a similar pattern in which synthesis is enhanced and occurs in close association with biomass formation. Thus restricted growth *per se* rather than growth limitation through depletion of a specific nutrient appears to regulate the rate of chloramphenicol synthesis. Whether control is exerted at the level of gene expression or whether the availability of precursors is the chief determinant of chloramphenicol output is now under investigation.

Acknowledgment

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