MUTANTS OF ESCHERICHIA COLI AFFECTED IN "INDUCER EXCLUSION"

Eugene B. Ackerman

INTRODUCTION

The uptake of carbohydrates by Escherichia coli is affected by two processes: active transport, in which the substrate appears inside the cell chemically unchanged; and by the PEP dependent phosphotransferase system, in which the carbohydrate is phosphophorylated as it enters the cell. Although the mechanisms of active transport are entirely different from those of the PT-system, it is known that mutants in the PT-system may show reduced rates of growth on sugars taken up by active transport. Furthermore, it is known that glucose and non-catabolizable analogs of glucose may inhibit the induction of sugars which are taken into the cell by active transport; this phenomenon is known as "inducer exclusion".

The present work attempts to analyze inducer exclusion on a genetic level by directly generating mutants in inducer exclusion and then locating the locus of the mutation on the chromosome by phage transduction.

METHODS

E. coli K12 strain HK743 was incubated overnight at 41°C on the following medium:

0.2 ml 10 mM glucose and 10 ml Basal Medium containing 50 mM Tris, pH 7.4, 190 mM NH_4 Cl, 0.33 mM $K_2H\ PO_4 \cdot 3H_2O$, 0.1 mM $FeSO_4 \cdot 7H_2O$, half strength ASW, (0.4 M $MgSO_4 \cdot 7H_2O$, 0.02 M KCl, and 0.02 M $CaCl_2 \cdot 2H_2O$) and 0.1 ml tHALT. This medium permitted a high OD_{680} for plating the next day on a 5 mM lactose tHalt plate. The bacteria were spread on the plate then a well of (0.1 M) 3-deoxy-3-fluoroglucose was made in the center. The next day a zone of inhibition occurred nd the following day mutants arose within the zone of inhibition. These mutants were screened for their continued ability to grow on glucose and to take up [^{14}C] glucose and methyl-α-D-[^{14}C] glucoside. The picked mutants were grown overnight on the glucose medium previously described. The next day 2.0 ml of cells were spun down for 5 min at 10,000 rpm then were resuspended in a solution containing 10 ml basal medium, 0.15 ml tHALT, 0.1 ml 0.5 M glucose, 0.1 ml 0.25 M lactose, and 50 µl [^{14}C]-lactose (6 µci/ml). Growth curves and [^{14}C]-lactose uptake was noted for both the parent and the mutant by shaking the cell suspension at 30° then filtering 20 µl of cell suspension for radioactive assay. This uptake study was repeated using 0.1 ml of 0.5 ml N-acetyl glucosamine.

Examination of the lac operon was conducted when glucose grown cells were further grown in media containing 2.5 mM lactose and either 5 mM glucose of 5 mM N-acetyl glucosamine.

Growth of phage:

1) 3.5 ml of soft (.75%) nutrient agar at 42°C. Add 0.125 ml 50 mM $CaCl_2$. Add 0.1 ml of mutant culture. Pore over nutrient agar plate and set.

2) Put 0.1 ml of phage plus 0.3 ml nutrient broth on plate. Spread over all but control area.

3) Incubate 4-6 hrs at 37°C.

1) Scrape soft agar off into sterile tube and wash with 2.5 ml 0.9% NACl and add to soft agar then centrifuge.

5) Decant supernatant into sterile tube, add 2 drops of chloroform, leave at room temperature for 15 min.

Transduction:

1) Subculture H1C761 (1:10) for 60-75 min in nutrient.

2) Incubate 0.1 ml phage nd 0.1 ml 50 mM $CaCl_2$ at 37°C for 30 min to remove chloroform.

3) Add HK761 (≈1 ml).

4) Incubate for 20 min at 37°C.

5) Plate 0.05, 0.2, and 0.2 ml onto appropriate plates. Control is 0.2 ml of HK761 and phage on separate plates.

RESULTS AND DISCUSSION

The first objective was to directly generate mutants in inducer exclusion. By using a ptsM⁻ strain as the parent and screening out ptsG mutants which could no longer take up glucose we obtained mutants in inducer exclusion or some other regulatory property. Figures 1 and 2 indicates that it is inducer exclusion that is affected, for the parents functioned while the mutant did not function in inducer exclusion.

Our mutants readily induced the lactose operon while the parent strain failed to do so. Figure 3 shows that the mutant also lost "catabolite inhibition" since neither glucose nor N-acetyl glucosamine was used in preference to other sugars taken up via the PEP-dependent phosphotransferase system such as fructose.

CONCLUSIONS

These results show that we have a novel mutation which affects the regulatory role of the PT-system in controling the uptake and utilization of sugars, whether taken up by the PT-system or not.

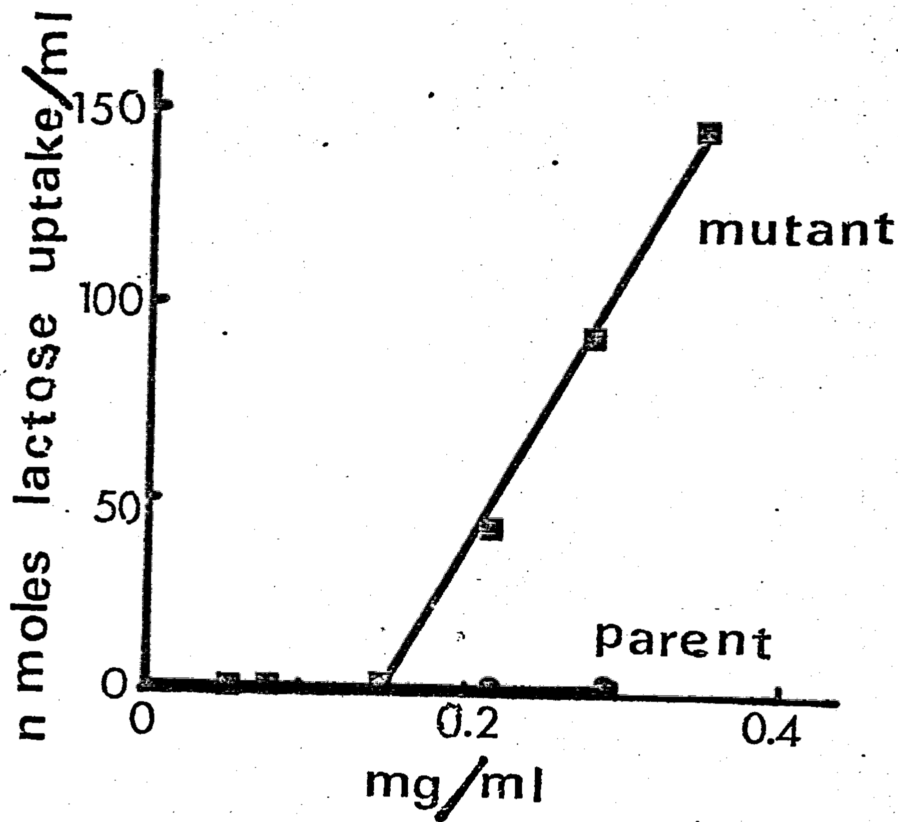

Figure 1. Mutant and parent (HK 743) grown on glucose overnight, then suspended in 5 mM glucose

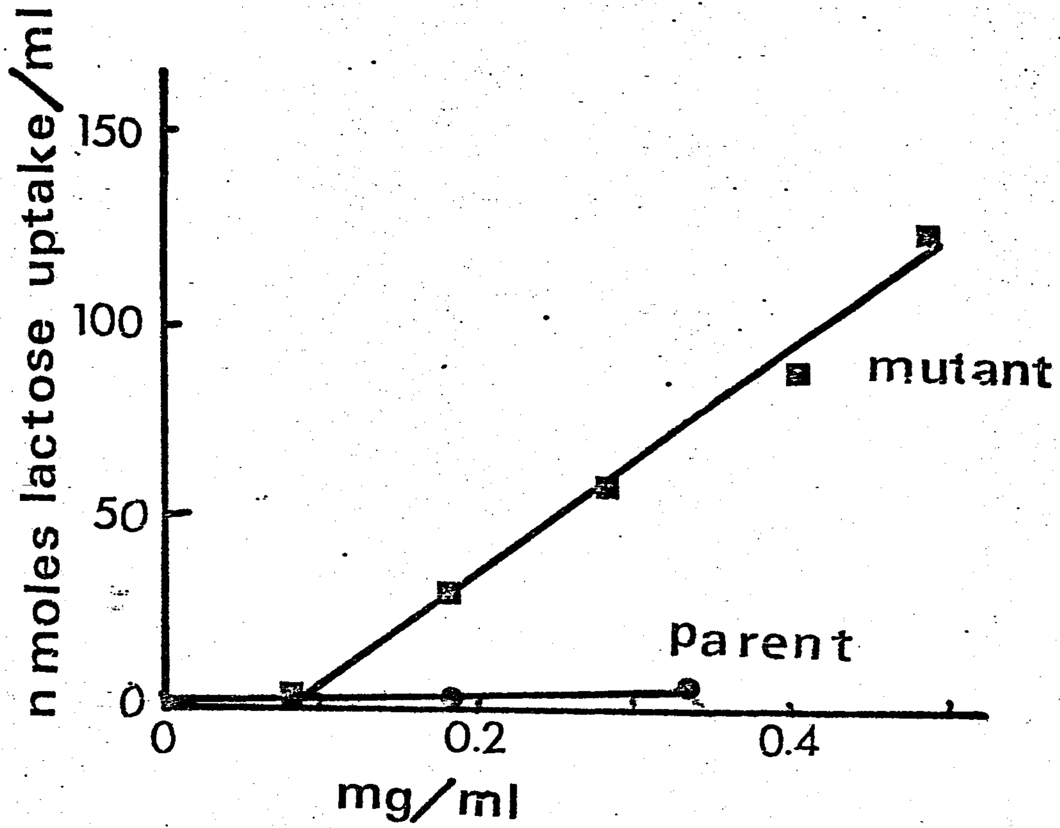

Figure 2. Mutant and parent (HK 743) grown on glucose overnight then suspended in 5 mM N-acetyl glucosamine

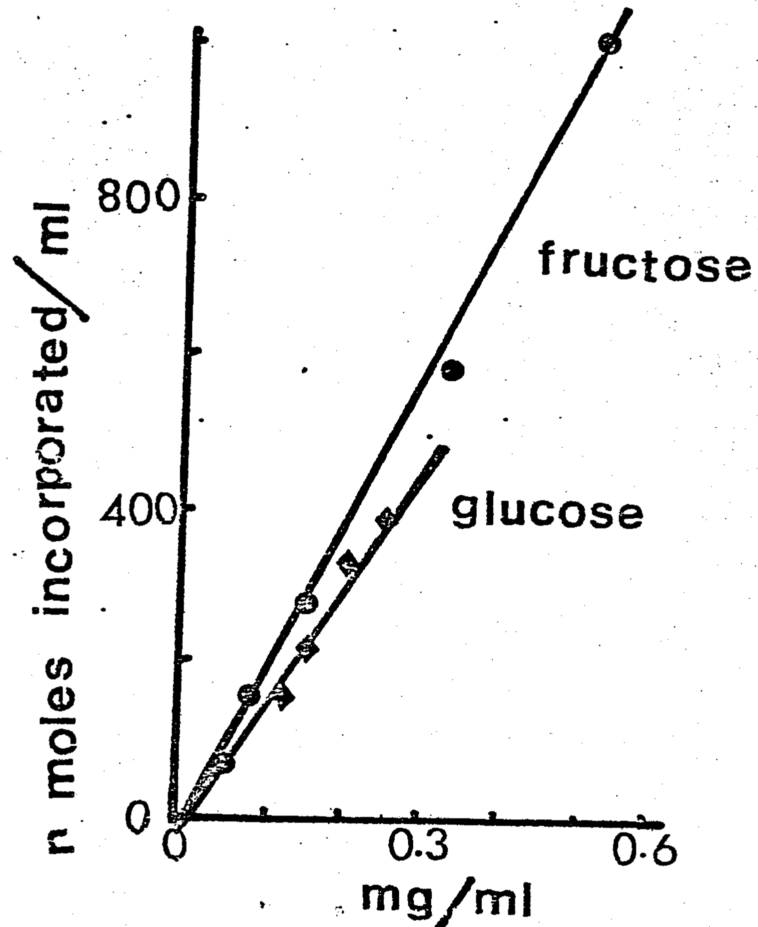

Figure 3. Mutant of HK 743 shows "catabolite inhibition" for glucose does not supress the uptake of other PT-sugars