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Mutations Affecting the Dissimilation of Mannitol by *Escherichia coli* K-12[1]

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Mutants of *Escherichia coli* K-12 defective in the mannitol-specific enzyme II complex of the phosphoenolpyruvate phosphotransferase system (PTS) or lacking mannitol-1-phosphate dehydrogenase have been isolated. These mutants fail only to grow on mannitol. Growth of the dehydrogenase-negative mutant on casein hydrolysate can be abruptly inhibited by exposure to mannitol. A mutant with constitutive expression of both of these enzymes has also been isolated. All three mutations are clustered in a region represented at min 71 of the Taylor map. In a mutant with less than 5% of the activity of enzyme I of the PTS, both the enzyme II complex and the dehydrogenase remain inducible by mannitol. In the mutant defective in the enzyme II complex, mannitol is able to induce the dehydrogenase. Thus, mannitol, rather than its phosphorylated product, seems to be the inducer.

The process of bringing mannitol from the external medium into the cytoplasm of *Escherichia coli* K-12 involves phosphorylation of the substrate by the phosphoenolpyruvate phosphotransferase system as represented by reactions 1 and 2.

$$\text{HPr} + \text{phosphoenolpyruvate} \xrightleftharpoons[Mg^{2+}]{\text{enzyme I}} \text{P-HPr} + \text{pyruvate} \quad (1)$$

$$\text{P-HPr} + \text{mannitol} \xrightarrow{\text{enzyme II complex}_{mtl}} \text{mannitol-1-phosphate} + \text{HPr} \quad (2)$$

Where HPr represents a low-molecular weight, histidine-containing protein; P-HPr is its high-energy phosphate derivative; and enzyme II complex_{mtl} is the membrane-associated phosphorylation system for mannitol.

Mannitol-1-phosphate (mannitol-1-P) delivered inside the cells is then converted to fructose-6-phosphate (fructose-6-P) by a pyridine nucleotide-linked dehydrogenase.

$$\text{mannitol-1-P} + \text{NAD} \xrightleftharpoons{\text{mannitol-1-P dehydrogenase}} \text{fructose-6-P} + \text{NADH} \quad (3)$$

Where NAD represents nicotinamide adenine dinucleotide and where NADH is the reduced form of NAD.

The phosphotransferase system probably has a broad role yet to be fully described, but it is clear that the generation of P-HPr, as shown in reaction 1, is necessary for the uptake and dissimilation of a number of sugars which serve the cell as carbon and energy source (15, 22).

The levels of enzyme I and HPr are increased by growth on a number of carbohydrates (23, 26). In contrast, enzyme II complex_{mtl} is known to be inducible only by mannitol and is specific for the phosphorylation of this polyhydric alcohol (4, 26). Mutations affecting enzyme I and HPr had previously been analyzed in *E. coli* and found to lie at about 45 min on the chromosome (5, 7, 29). This paper describes the isolation and mapping of mutations affecting enzyme II complex_{mtl}, mannitol-1-P dehydrogenase, and a constitutive mutant which produces both of these enzymes in the absence of inducer. The three loci are designated as *mtlA*, *mtlD*, and *mtlC*, respectively.

MATERIALS AND METHODS

Bacteria. The origins and genotypes of the *mtl* mutants isolated in this study are summarized in Table 1. Strain 11 was isolated from *E. coli* K-12, strain AB313, as previously described (4). Strain X7082-M (F^-, *his*, *malA*, *xyl*) used in mapping enzyme II complex_{mtl} was constructed by mating strain X7082 (F^-, *his*, *malA*, *xyl*, *mtl*) with strain 11 (Hfr, mtl^+) and selecting mannitol-positive recombinants.

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TABLE 1. *Origin and genotype of mtl mutants*

Strain[a]	Parent	Source	*mtl* Markers					
			mtlA	*mtlD*	*mtlC*	*thr*	*leu*	*thi*
AB313			+	+	+	−	−	−
11	AB313	Spontaneous	+	+	+	+	−	−
236	11	EMS[b]	−	+	+	+	−	−
237	236	Spontaneous	+	+	+	+	−	−
238	11	EMS	+	+	c[c]	+	−	−
239	238	EMS	+	−	c	+	−	−
250	239	Spontaneous	+	+	c	+	−	−

[a] All strains are Hfr.
[b] Ethyl methanesulfonate mutagenesis.
[c] Mannitol constitutivity.

Strain 204 (Hfr, *xyl*, *lct*, *mtl*$^+$) used in phage P1 transductions was constructed from strain 560 (F$^-$, *xyl*, *lct*, *mtl*), provided by J. Puig (21), by transduction because a spontaneous mannitol-positive revertant of 560, strain 158, did not have normal levels of enzyme II complex$_{mtl}$. Therefore, the *xyl*, *lct*, *mtlC*$^+$ and *mtlD*$^+$ markers from strain 158 were transduced into strain 239 (*xyl*$^+$, *lct*$^+$, *mltC*^{c}, *mtlA*$^+$, *mtlD*), and *mtlD*$^+$ transductants were selected. One of these was strain 204 (Hfr, *xyl*, *lct*, *mtlC*$^+$, *mtlA*$^+$, *mtlD*$^+$).

The following strains, isolated in other laboratories, were used for examining the *mtl* locus (28): PA201 (*thr*, *leu*, *lac*, *gal*, *his*, *strA*, *mal*, *xyl*, *mtl*, *arg*, *thi*); AB1320 (*pro*, *lac*, *gal*, *his*, *aro*, *str*$^+$, *xyl*, *mtl*, *thi*), and X7082. PA201 is a strain from the collection at the Pasteur Institute in Paris. Strain AB1320 is from the collection of E. A. Adelberg. Strain X7082 was described above.

Culture media and growth conditions. The basal mineral medium used for growth of cells has been previously described (26). Casein acid hydrolysate (CAA; Nutritional Biochemicals; salt-free, vitamin-free) was added to mineral medium to give a final concentration of 1%. Unless otherwise indicated, carbon sources were added to give a final concentration of 0.2%. When required, supplements were added to give the following concentrations: leucine, 50 μg/ml; thiamine, 20 μg/ml; and histidine, 50 μg/ml. Solid medium was prepared by the addition of 15 g of agar (Difco) per liter of mineral medium. Growth was monitored turbidimetrically with a Klett colorimeter (no. 42 filter). One Klett unit equals 4×10^6 cells of an exponentially growing culture per ml.

Enzyme assays. The preparation of cell extracts for enzyme assays has been previously described (27). These extracts are stable to freezing and thawing for both enzyme II complex$_{mtl}$ and mannitol-1-P dehydrogenase activity. Assay of enzyme II complex$_{mtl}$ activity was performed by measuring the rate of formation of ^{14}C-mannitol-1-P from D-[*1*-^{14}*C*]mannitol (The Radiochemical Centre, Amersham, England) in the presence of excess enzyme I and HPr (4). Enzyme I and HPr were prepared from strain 236 which lacks enzyme II complex$_{mtl}$.

Mannitol-1-P dehydrogenase activity was assayed by following the rate of reduction of NAD at 25 C at 340 nm (30). The assay mixture contained 0.2 ml of 0.01 M mannitol-1-P (Sigma), 0.1 ml of 0.02 M NAD, 0.1 ml of 1 M sodium carbonated buffer (*p*H 9.5), crude extract, and water to a final volume of 1.0 ml. Substrate was omitted from the blank. Alternatively, the dehydrogenation was monitored at *p*H 7.5 with phenazine methosulfate and [3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl tetrazolium bromide] as the electron acceptors (26).

Protein concentrations were measured with the biuret reagent (9).

Uptake assay. Washed cells were suspended in mineral medium to a density of 100 Klett units. Uptake of labeled substrate was measured at 25 C by mixing 0.4 ml of the suspended cells with 1 ml of mineral medium containing 5×10^{-6} M ^{14}C-substrate and 40 μg of chloramphenicol per ml. Incubation was generally continued for 1 min, during which time the reaction remained linear. The reaction was terminated by delivering 1 ml of the incubation mixture onto a membrane filter (Millipore Corp.; 0.45-μm pore size) which had been previously wet with mineral medium. The filter was then washed with 10 ml of mineral medium and dried, and the radioactivity was determined by scintillation counting.

Genetics. The mating procedure was essentially that of Adelberg and Burns (1). The transduction procedure was modified from that of Luria, Adams, and Ting (17) and Arber (2).

RESULTS

Induction of the mtl system. Enzyme II complex$_{mtl}$ and mannitol-1-P dehydrogenase were both induced in cells of the wild-type strain grown on mannitol either aerobically or anaerobically (Table 2). When tested aerobically with sorbitol, glucose, and fructose, all of which serve as sole source of carbon and energy, no significant induction of either enzyme was observed. (The slight induction observed with sorbitol could be attributed to contamination of the preparation by mannitol.)

Since mannitol-1-P apparently is unable to enter the cell, there was no direct way of testing its ability to induce these enzymes. However, mannitol-1-P dehydrogenase cata-

TABLE 2. *Induction of mtl enzymes in wild-type strain 11*

Growth condition	Enzyme II complex$_{mtl}$	Mannitol-1-P dehydrogenase
Aerobic growth		
CAA[a]	0.19[b]	0.055[c]
CAA + mannitol	5.00	3.1
+ sorbitol	0.85	0.404
+ glucose	0.16	0.12
+ fructose	0.23	0.11
+ fructose-6-P	0.15	0.12
Anaerobic growth		
Mannitol	16.7	2.6
Glucose	0.16	0.046

[a] Casein hydrolysate.
[b] Nanomoles per minute per milligram.
[c] Micromoles per minute per milligram, *p*H 7.5 assay.

lyzes the reduction of fructose-6-P to mannitol-1-P, as well as the reverse reaction. It is therefore possible that when a cell is growing in the presence of fructose-6-P a certain amount of mannitol-1-P may be generated inside the cell. If this quantity of mannitol-1-P were sufficient and this compound were the inducer, perhaps growth in the presence of fructose-6-P would result in raised levels of the *mtl* enzymes. As can be seen from Table 2, however, this did not occur. Therefore, either the level of mannitol-1-P was not sufficiently high or the phosphorylated substrate is not the inducer.

Helle and Klungsøyr (11) have shown that, when *E. coli* is grown anaerobically on glucose minimal medium, mannitol-1-P is excreted. Their explanation for this phenomenon is that the reduction of fructose-6-P to mannitol-1-P serves as a means of regenerating NAD from NADH. Cells of our wild-type strain were therefore grown anaerobically on glucose to see whether enzymes of the mannitol pathway could be induced. The result (Table 2) shows unambiguously that, whereas minimal mannitol medium gave excellent induction under anaerobic conditions, minimal glucose medium gave none. Again, perhaps either mannitol-1-P did not accumulate (because of strain difference), or it is not the inducer. It might be argued that glucose prevented the appearance of the *mtl* enzyme activities through catabolite repression. This possibility is excluded, however, by the direct observation that glucose did not interfere significantly with the induction of the *mtl* system when mannitol was provided.

To determine whether mannitol must necessarily be converted to mannitol-1-P to be effective as an inducer, mutants that are defective in the phosphorylation process were used. As is shown in Table 3, both enzyme II complex$_{mtl}$ and mannitol-1-P dehydrogenase remained inducible by mannitol in a mutant, strain 223, defective in enzyme I. Moreover, in a mutant which is defective in enzyme II complex$_{mtl}$, the dehydrogenase was still inducible, although to a level which is lower than expected. The failure of mannitol-1-P dehydrogenase to reach the level expected in wild-type cells could be due either to a polarity effect of the particular mutation (in case both genes belong to the same operon and the gene for the phosphorylating enzyme is transcribed prior to the gene coding for the dehydrogenase) or to the poor permeability of the cell to mannitol in the absence of the enzyme II complex$_{mtl}$.

Enzyme II complex$_{mtl}$-negative mutant. A mutant unable to utilize mannitol as a carbon source was isolated by mutagenesis of wild-type strain 11 with ethyl methanesulfonate (16), followed by two cycles of penicillin selection in mannitol minimal medium (8). To eliminate mutants with lesions affecting the other nonspecific components of the phosphotransferase system, namely enzyme I and HPr, the cells were cycled twice in glucose minimal medium. Mannitol-negative mutants were selected as the white colonies on MacConkey indicator plates in which mannitol was substituted for lactose. Strain 236, one of these colonies, was further tested for growth on glucose, fructose, and glycerol and was found to grow normally on these compounds. A spontaneous mannitol-positive revertant of strain 236,

TABLE 7. *Mapping of mtlD by transduction*

Strain	Mutation	Enzyme II complex$_{mtl}$		Mannitol-1-P dehydrogenase	
		CAA	CAA + mannitol	CAA	CAA + mannitol
11	*mtl*$^+$	0.28[b]	6.3[b]	0.058[c]	2.7[c]
223	*ptsI*	0.34	10.5	0.02	1.26
236	*mtlA*	<0.1	<0.1	0.10	0.96
237	*mtl*$^+$	0.11	8.0	0.086	3.7
238	*mtlC*[e]	15	ND[d]	2.9	ND
239	*mtlD*	6.6	ND	<0.01	ND
250	*mtl*$^+$	15	14	0.36	0.55

[a] Cells were grown exponentially either on casein hydrolysate (CAA) or on this carbon source in the presence of mannitol (CAA + mannitol).
[b] Nanomoles per minute per milligram.
[c] Micromoles per minute per milligram.
[d] Not determined.

strain 237, was selected on minimal mannitol medium. As shown in Table 3, strain 236 is missing enzyme II complex$_{mtl}$ activity, and strain 237 has regained this activity.

Mannitol-constitutive mutant. A method devised earlier for the isolation of mutants constitutive for specific catabolic pathways (16) was not found useful for the detection of colonies constitutive for mannitol metabolism, probably because contaminating mannitol could not be eliminated from the agar preparations employed. Another method based upon the assumption that, if a mixed culture of wild-type and constitutive cells was transferred from a glucose minimal medium to a minimal medium with mannitol as a sole source of carbon and energy at a concentration near or below the threshold of induction of the necessary enzymes, the constitutive cells would have an advantage, at least during the adaptive phase, over the wild-type cells (12). Tentatively, 10^{-6} M mannitol was assumed to be near the desired concentration and yet high enough to give a 1,000-fold growth from an adequate inoculum (200 cells/ml). Leucine and thiamine were added at nonlimiting concentrations. Cells of wild-type strain 11 were mutagenized with ethyl methanesulfonate and grown for several cycles in glucose minimal medium, after which 2×10^5 cells were suspended in 1 liter of 10^{-6} M mannitol medium. After 1 day of incubation at 37 C, full growth occurred. The culture was diluted into fresh 10^{-6} M mannitol medium. Similar cycling was continued for 7 days, with decreasing numbers of cells being transferred on each successive day. At the end of this time, several colonies were tested for constitutive uptake of ^{14}C-mannitol. Strain 238 was thus identified. As can be seen in Table 4, this constitutive uptake is specific for the mannitol system and does not involve general constitutive uptake of phosphotransferase system substrates such as fructose and sorbitol. Table 3 shows that the uninduced enzyme levels of the *mtl* system in this strain are comparable to induced levels in strain 11.

Mannitol-1-P dehydrogenase-negative mutant. The basis for the selection of a mannitol-1-P dehydrogenase-negative mutant was the well known observation that the accumulation of certain sugar-phosphates, such as galactose-1-phosphate (14, 20, 25, 32) or L-α-glycerophosphate (6) in mutant strains of *E. coli* that are blocked in the further metabolism of these compounds, leads to growth stasis. It was assumed that the loss of mannitol-1-P dehydrogenase in cells would render them susceptible to growth inhibition by mannitol as a result of the accumulation of mannitol-1-P. The constitutive strain 238 was therefore mutagenized with ethyl methanesulfonate and cycled twice in glucose minimal medium, as in the previous selections. The desired mutants, those colonies which were white (mannitol-nonfermentive) and also very small (presumably owing to their mannitol sensitivity), were selected from MacConkey mannitol plates. Strain 239 was one of these colonies that upon further testing was found to grow normally on CAA or fructose, but not on mannitol.

TABLE 4. *^{14}C-Substrate uptake by strains 11 and 238*

Strain	Growth medium	^{14}C-Mannitol (10^3 counts/min)	^{14}C-Fructose (10^3 counts/min)	^{14}C-Sorbitol (10^3 counts/min)
11	CAA[a]	1.1	0.13	0.23
11	CAA + inducer[b]	5.7	1.6	0.56
238	CAA	4.1	0.23	0.21

[a] Casein hydrolysate.

[b] Inducer is mannitol, fructose, or sorbitol for measurement of uptake of ^{14}C-labeled mannitol, fructose, or sorbitol, respectively.

Figure 1 demonstrates the effect of the addition of mannitol to a culture of strain 239 which was growing on CAA. Growth was severely inhibited. Four and one-half hours after the addition of mannitol, the viability of the inhibited culture was tested. It was found that growth stasis, rather than killing, had occurred. As seen in Table 3, this strain lacks mannitol-1-P dehydrogenase activity but still produces enzyme II complex$_{mtl}$. During the course of this work, a mannitol-1-phosphate dehydrogenase mutant of *Salmonella typhimurium* was also isolated on the basis of mannitol sensitivity (3).

General location of mtlA by conjugation. The approximate position of the mutation affecting enzyme II complex$_{mtl}$ was determined by conjugation of strain 236 (Hfr, *mtlA*, *xyl*$^+$, *malA*$^+$, *his*$^+$) with a multiply marked female strain X7082-M (F$^-$, *mtlA*$^+$, *xyl*, *malA*, *his*) for 1 hr. Recombinants were selected for the single markers *xyl*$^+$, *malA*$^+$ and *his*$^+$ (Fig. 2). These recombinants were then scored for inheritance of the unselected *mtlA* marker. Table 5 shows the results of this cross. The linkage is clearly greatest with *xyl* and rapidly drops off with *malA* and *his*. Hence, *mtlA* should be between the origin of strain Hfr AB313 (74 min) and *malA* (64 min), and is nearer to *xyl* than *malA*.

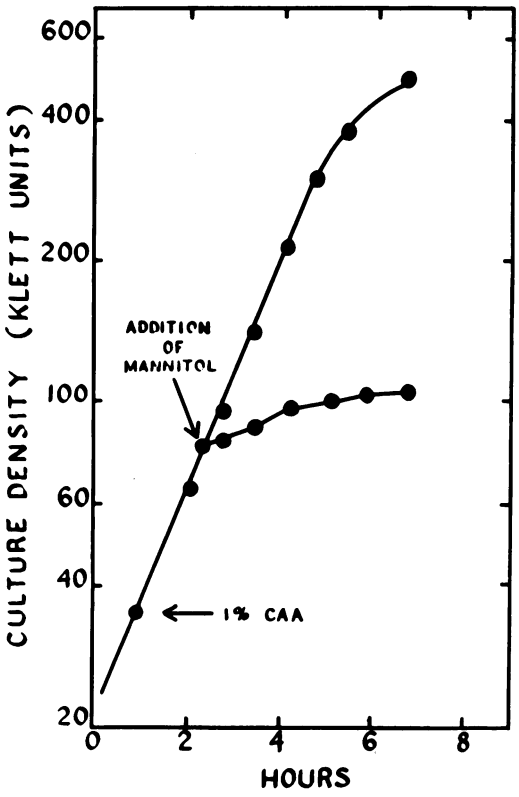


FIG. 1. *Inhibition of growth of strain 239 by mannitol. An exponentially growing culture of strain 239 was divided in two, and 0.2% mannitol was added to one flask. Growth stasis is seen in this culture.*

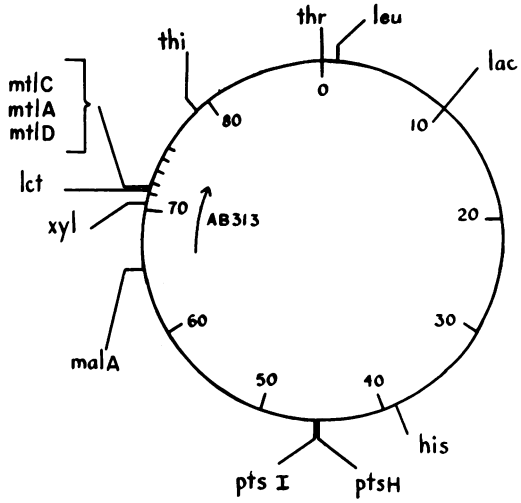


FIG. 2. *Pertinent markers on the genetic map of E. coli, after Taylor (28). The map is graduated in 10-min intervals. The polarity and origin of Hfr AB313 is denoted by the arrow. The following abbreviations for genetic markers are used: his, dependence on histidine; lac, utilization of lactose; lct, L-lactate dehydrogenase; leu, dependence on leucine; malA, utilization of maltose; ptsI, enzyme I of the phosphotransferase system; ptsH, HPr of the phosphotransferase system; thr, dependence on threonine; and thi, dependence on thiamine. The abbreviations for the mtl system are: mtlA, enzyme II_{mtl}; mtlD, mannitol-1-phosphate dehydrogenase; and mtlC, mtl control gene.*

TABLE 5. *General location of mtlA*

Selected marker	No. of mannitol recombinants		Linkage (%)
	+	−	
xyl$^+$	28	72	72
malA$^+$	59	41	41
his$^+$	97	3	3

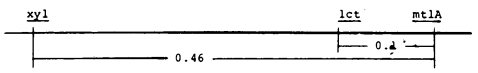
Location of mtlA by transduction. A more precise location for *mtlA* was obtained by determining the cotransduction frequencies of this marker with *xyl* and *lct* (21) in a three-factor cross. Phages grown on strain 204 (*xyl, lct, mtlA*$^+$) were used to infect strain 236 (*xyl*$^+$, *lct*$^+$, *mtlA*) and transductants selected on minimal mannitol plates. Six hundred of these mannitol-positive transductants were then scored on xylose and lactate. The results are shown in Table 6.

First, the frequency of cotransduction of *mtlA* with *lct* (i.e., total *mtlA*$^+$, *lct* transductants) is 72%, whereas the frequency with *mtlA* and *xyl* (i.e., total *mtlA*$^+$, *xyl* transductants) is only 15%. The marker *mtlA* thus is considerably closer to *lct* than to *xyl*. The distances between these markers as seen on the top of Table 6 are expressed in fractions of the length of the transducing particle after an equation of Wu (31).

Second, the finding of the least frequent class of transductants, *xyl, lct*$^+$, *mtlA*$^+$, is consistent with the order *xyl-lct-mtlA*. Formation of this class requires four crossover events. Were the order *xyl-mtlA-lct*, none of the *mtlA* II$^+$ classes of transductants would require four recombinational events, and a low-frequency class would not have been found.

Location of mtlD and mtlC by transduction. The positions of the mannitol-1-P dehydrogenase mutation (*mtlD*) and the control locus mutation (*mtlC*) in strain 239 were determined by a four-factor cross. Phages grown on strain 204 (*xyl, lct, mtlC*$^+$, *mtlD*$^+$) were used to infect strain 239 (*xyl*$^+$, *lct*$^+$, *mtlC*^{c}, *mtlD*), and mannitol-positive (i.e., *mtlD*$^+$) transductants were selected on minimal man-

TABLE 6. *Mapping of mtlA by transduction*



Recombinant Genotype*	Number Found	% Distribution
xyl⁺ lct mtlA⁺	355	59
xyl⁺ lct⁺ mtlA⁺	155	26
xyl lct mtlA⁺	78	13
xyl lct⁺ mtlA⁺	12	2

* The donor is represented by the upper bar; the recipient is represented by the lower. The crossovers between the donor and the recipient required by the order *xyl*, *lct*, *mtlA* are indicated by the dotted lines.

nitol plates. Three hundred of these recombinants were scored on lactate and xylose. The results are given in Tables 7 and 8.

As with the *mtlA* marker, the total number of *mtlD*⁺, *lct* transductants (57.4%) is greater than the total number of *mtlD*⁺, *xyl* transductants (18.4%), so that the dehydrogenase gene is closer to *lct* than *xyl*. Also, the least frequent class of transductants, *xyl*, *lct*⁺, *mtlD*⁺, is consistent with the order *xyl-lct-mtlD*.

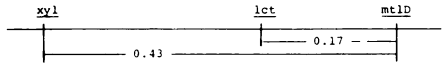
Of these 300 transductants, 100 were tested for inheritance of the unselected *mtlC* marker. This was done by growing the transductants overnight in 1% CAA liquid medium and testing ^{14}C-mannitol uptake by the cells directly without harvesting. Strains 239 and 204 were used as controls for constitutive and noninduced uptake, respectively. The results are shown in Table 8. Of 100 colonies tested, 94 gave the *mtlC*⁺ phenotype, indicating 94% cotransduction of the dehydrogenase gene and *mtlC*.

Inheritance of *mtlC*⁺ along with *mtlD*⁺ does not significantly change the probability of acquiring *lct*, i.e., cotransduction between *mtlD* and *lct* is still about 55%. However, inheritance of *mtlC*^{c} with *mtlD*⁺ lowers the inheritance of *lct*, so that the cotransduction between *mtlD* and *lct* becomes 1%. This is consistent with the order *lct-mtlC-mtlD*. Thus, all these mutations affecting mannitol utilization are closely clustered at min 71 on the chromosome as represented by the Taylor map.

mtl Mutations reported in previous studies. Previous to our study, a number of *mtl* markers have been mapped in F⁻ strains of *E. coli*. These mutations, too, were found to be located at min 71 on the chromosome (28). However, none of the mutants has been characterized enzymologically, and strains bearing a genetic lesion at this locus are classified simply as "unable to utilize mannitol." An examination of cell extracts of some of these mutants in the present study revealed that they do not all have the same defect; instead, they fall into several categories regarding expression of the *mtl* genes. An example of one strain in each of these categories is given in Table 9. Surprisingly, they are not simply missing either enzyme II complex$_{mtl}$ or mannitol-1-P dehydrogenase.

Strain PA201 produces neither of these enzyme activities. Strain AB1320 has no enzyme II complex$_{mtl}$ activity, but does have dehydrogenase activity. The levels of the dehydrogenase, however, are rather unusual. The basal level is quite high, yet this level cannot be raised to a normal induced level by growth in the presence of mannitol. Hence, the dehydro-

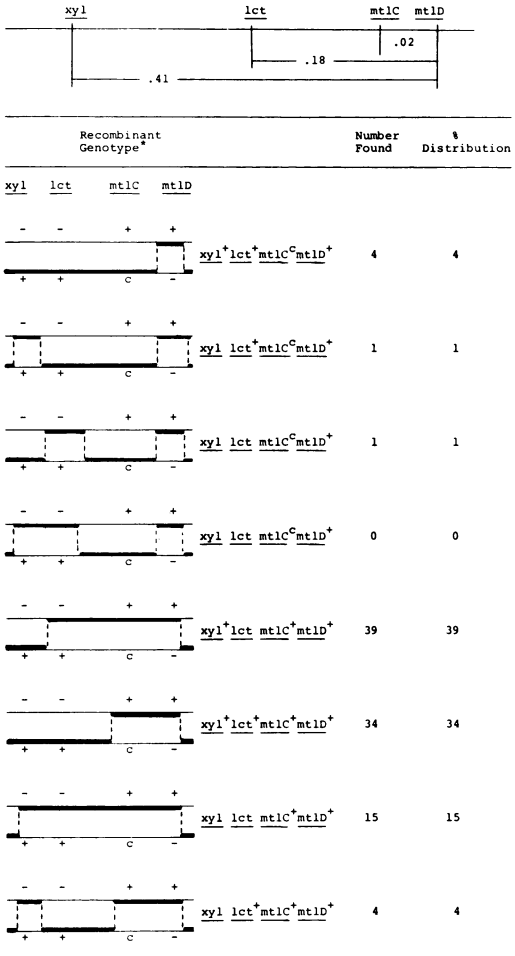
TABLE 7. *Mapping of ntlD by transduction*



Recombinant Genotype*	Number Found	% Distribution
xyl⁺ lct mtlD⁺	128	42.7
xyl⁺ lct⁺ mtlD⁺	117	39
xyl lct mtlD⁺	44	14.7
xyl lct⁺ mtlD⁺	11	3.7

* The donor is represented by the upper bar, the recipient by the lower. The crossovers between the donor and the recipient required by the order *xyl*, *lct*, *mtlD* are indicated by the dotted lines.

TABLE 8. *Mapping of mtlD and mtlC by transduction*



* The donor is represented by the upper bar, the recipient by the lower. The crossovers between the donor and the recipient required by the order *xyl*, *lct*, *mtlC*, *mtlD* are indicated by the dotted lines. A total of 100 transductants were analyzed.

genase activity appears to be expressed constitutively, but at a low level. The third mutant, X7082, does not contain either enzyme II complex$_{mtl}$ or dehydrogenase activity and does not revert on minimal mannitol medium. The exact nature of all of these mutants is yet to be established.

DISCUSSION

Although direct evidence that the site of mutation in strain 236 is in the structural genes for enzyme II complex$_{mtl}$ is not yet available, it is not likely that the mutation is of the regulatory kind. First, strain 236 retains an inducible mannitol-1-P dehydrogenase activity. Second, its mannitol revertant, strain 237, produces both the enzyme II complex$_{mtl}$ activity and mannitol-1-P dehydrogenase inducibly, and not constitutively. If the mutation in strain 236 were in a repressor gene resulting in failure of the protein to interact properly with the inducer, the most frequent class of "revertant" would be expected to be constitutive as a consequence of random inactivation of the repressor protein for operator binding activity. It should be noted, however, that the dehydrogenase level in strain 236 is not induced to the wild type level by mannitol (Table 2). This may be an indication of a nonsense mutation in enzyme II complex$_{mtl}$, which has a polar effect on the dehydrogenase level. An additional complication arises in the interpretation of the lesion of strain 236 in that enzyme II complex$_{mtl}$ is probably a multicomponent enzyme with at least two protein subunits as well as a lipid portion (10, 13, 24). Inactivity of this enzyme could therefore result from mutations in several possible structural genes. More exact specification would require the resolution of this membrane-associated enzyme complex, a task not yet achieved.

Strain 239, which lacks dehydrogenase activity, retains the constitutive production of enzyme II complex$_{mtl}$. Hence, in this case too a mutation in the structural gene (or in the operon in which the dehydrogenase is coded) is the most likely defect.

Strain 238 produces both enzyme II$_{mtl}$ and the dehydrogenase constitutively. In view of the close proximity of this mutation site to

TABLE 9. *Characteristics of some mtl mutants*

Strain	Reverts on mannitol[a]	Enzyme II complex$_{mtl}$		Mannitol-1-P dehydrogenase	
		CAA[b]	CAA[b] mannitol	CAA	CAA + mannitol
11		0.28[c]	6.3[c]	0.058[d]	2.7[d]
PA201	Yes		<0.1		0.031
AB1320	Yes	<0.1	<0.1	1.2	1.2
X7082[e]	No		<0.1		0.030

[a] The possibility of extra-cistronic suppression has not been explored.

[b] Cells were grown exponentially either on casein hydrolysate (CAA) or on this carbon source in the presence of mannitol (CAA + mannitol).

[c] Nanomoles per minute per milligram.

[d] Micromoles per minute per milligram.

[e] 260 R 9 notation of W. Maas.

those affecting the individual activities of enzyme II_{mtl} and mannitol-1-P dehydrogenase, it is not possible to decide with the available data whether *mtlC* (C for "control") represents the operator region controlling the expression of both structural genes or a locus specifying a regulator protein. The inducer of the mannitol system (subject to the reservation that the mutants affected in enzyme I or the enzyme II $_{mtl}$ complex are not too leaky) appears to be mannitol rather than mannitol-1-phosphate. This inducer specificity is in contrast to the control of the glycerol kinase gene and the other elements of the *glp* regulon for which the phosphorylated product, L-α-glycerophosphate, rather than glycerol, acts as the inducer.

Results of genetic crosses indicate that the *mtl* genes are on the far side of *lct* from *xyl*. This is in contradiction to the order, *lct-mtl-xyl*, found by Pascal *et al.* (21). The *mtl* locus that was mapped by these workers, however, was not defined biochemically. Moreover, it is possible that they have mapped a mutation that is different from those in strains 236 and 239. Secondly, their transduction data are based on the examination of 100 recombinant colonies, whereas this study used 600 in one case and 300 in another. The order they obtained was determined by the number of recombinants in two classes, one of which contained two colonies and the other, zero. These numbers may be too small to be reliable, especially since the *lct* marker is leaky and rather difficult to score.

Not surprisingly, the *mtl* genes which have a specific function for mannitol utilization are not closely linked to the genes for enzyme I and HPr which play much broader metabolic roles. The genes controlling the specific phosphorylation of mannitol and the dehydrogenation of mannitol-1-phosphate are also found to be linked in *Salmonella typhimurium* (3) and in the gram-positive organism *Staphylococcus aureus* (18, 19). The loci controlling a similar pathway for sorbitol in *E. coli* also appear to be clustered (J. Lengeler, *personal communication*). It would be interesting to look at some of the other carbohydrate pathways of the phosphotransferase system in this regard to see whether the genes show similar clustering.

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