LOSS OF "GALACTOSE PERMEASE" AND GALACTOSE SENSITIVITY OF E. COLI ASSOCIATED WITH MUTATION IN LAC OPERON

David Rosenberg* and Albert S. Keston,

The Institute for Medical Research and Studies, New York, N.Y. & Graduate School of Biological Sciences, Mt. Sinai School of Medicine, C.U.N.Y.

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SUMMARY

E.coli W3092C which possesses a constitutive "galactose permease" and is galactose sensitive loses its galactose sensitivity concurrently with a mutation in the lac operon. ML32400 which likewise possesses a constitutive "galactose permease" and is galactose sensitive loses both "permease" and sensitivity concurrently with a mutation in the lac operon.

The experimental results presented here clearly show that there is a direct relationship between galactose resistance, "galactose permease," and the lac operon. E.coli ML32400 and W3092C may have genetically different "galactose permeases".

We reported previously that galactose inhibited the growth of galactokinase negative strains of E. coli (1) although these organisms cannot make Gal-1-P.

Galactose sensitive E. coli strains, ML32400 (gal K^-T^-) and W3092C (gal K^-T^+) possess a constitutive "galactose permease," which is demonstrated by accumulation of large amounts of galactose against a very high concentration gradient (2)(3). Mutant strains, whose growth was not markedly inhibited

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*Present Address: Department of Biological Sciences, Douglass College, Rutgers University, the State University of New Jersey

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by galactose, have been isolated from the original strains. We found that the galactose resistance in most of these mutants was associated with loss of function of genes in the lac operon. Mutation in the lac operon was found to be associated with the loss of the "galactose permease" in ML32400. However, mutation in the lac operon of W3092C did not result in loss of the "galactose permease."

Galactose sensitive revertants where the lac operon was restored were also studied. Such revertants derived from the MLR strains did not exhibit reappearance of the "galactose permease" activity. These data are difficult to reconcile with previously known properties of the lac operon and indeed with the general operon theory. The finding that loss of at least an important component of the "galactose permease" system is associated with the mutation in lac operon is surprising. The finding that the two strains of E. coli show differences in this regard is also unexpected. This raises the possibility that the "galactose permease" may not be a single entity or system.

Materials and Methods

Bacterial Strains: ML32400 was provided by B. L. Horecker and W3092C by H. M. Kalckar. These strains were labelled by reisolation resistant to streptomycin and to various coliphages.

Media: BTB-agar (4) and M63S (5), a mineral salts medium with 0.4% succinate were as described previously (1). Growth on various sugars was tested with minimal A agar (6) supplemented with 1% sugar, or tetrazolium lactose agar (7)

Isolation of Galactose Resistant Bacteria: Single colonies of ML32400 and W3092C on BTB-gal agar were incubated 2-3 days at 37°C. Galactose resistant papillae, growing out from the sensitive colonies, were picked and cloned several times on BTB-gal agar.

Assays:

Galactose Permease: Washed, logarithmically growing cells were suspended in M63S with chloromycetin (50 μg/ml) at optical density of 1.0 at 600 mμ and

incubated with 3.5 x 10^{-5}M galactose-C^{14} or H^3 (15,000 cpm/ml) for 15 minutes at 37°C. Samples were filtered (0.45 micon diameter, Millipore) and washed with iced medium. Galactose retained by the cells was determined by scintillation counting of the radioactivity on the dried filters.

TMG Permease:

a) Minimal A agar plates containing 0.2% melibiose were streaked with the W3092C and WCR strains and incubated at 43°C. This assay could not be used for ML32400 since it does not metabolize melibiose (8).

b) Induced and non-induced logarithmic cultures grown, washed and resuspended in M63S with chloromycetin as described above were incubated with 1 x 10^{-4}M TMG-C^{14} (1 x 10^5 cpm total) at 15°C. for 15 minutes. The entire incubation mixture was filtered and washed with 1 ml cold medium. Standard scintillation counting of the dried filters was added to determine the uptake of the TMG.

Enzyme Assays: Induction was done with 5 x 10^{-3}M IPTG for 2-3 hours. Dr. P. Lengyel (personal communication) kindly provided the details of his modification of the assay of thiogalactoside transacetylase devised by Alpers, 1965 (9). The O-nitrophenyl galactoside hydrolysis method of Paigen, 1963 (10) was used with toluene treated cells. The growth inhibition of sensitive strains on BTB-Gal agar plates is unambiguous when compared to resistant strains after 1-2 days incubation at 37°C.

Results and Discussion

Galactose Resistance: The primary result of this investigation was the discovery that most of the galactose resistant bacteria were mutated in the lac operon (Tables 1 and 2). MLR-1 and WCR-5 are completely negative for β-galactosidase, galactoside permease, and thiogalactoside transacetylase. In contrast, MLR-2 can produce normal amounts of β-galactosidase, but has lost the galactoside permease and transacetylase (Tables 1, 2). The $z^-y^-a^-$, $z^+y^-a^-$ resistant strains conform to the previously described cases of polar mutations in the lac operon (11). The majority (20 of 24) of the

MLR strains could not ferment lactose, but 17 of the 20 lac$^-$ could produce β-galactosidase. These 17 MLR strains appear to be mutants in the lac y gene (cryptic mutants). The remaining two lac$^-$ MLR strains are similar to MLR-1 since they are missing β-galactosidase. All the lac$^-$ MLR

TABLE 1

LAC OPERON FUNCTIONS OF GALACTOSE RESISTANT BACTERIA

PARENT STRAIN	# ISOLATED	# LAC$^+$	#LAC$^-$	#z$^+$y$^-$	#z$^-$y$^-$
ML32400	24	4	20	18	2
W3092C	20	0	20	18	2

TABLE 2

PROPERTIES OF TYPICAL GALACTOSE RESISTANT MUTANTS

	GALACTOSE		LACTOSE		
STRAIN	GROWTH INHIBITION	PERMEASE	z	y	a
ML32400	S	+	+	+	+
MLR-1	R	-	-	-	-
MLR-2	R	-	+	-	-
MLR-1R	S	-	+	+	+
MLR-2R-A	S	-	+	+	+
F13/MLR-1	S	-	+	+	nt
W3092C	S	+	+	+	+
WCR-5	R	+	-	-	-

+=normal; -=deficient; S=sensitive; R=resistant; nt=not tested.

TABLE 3

GALACTOSE PERMEASE OF RESISTANT STRAINS

STRAIN	COUNTS PER MINUTE ON FILTER
Galactose-C^{14} added	15,000/ml
ML32400	7,060
MLR-1	264
MLR-1R	228
MLR-2	281
MLR-2R	260
W3092C	6,125
WCR-5	6,679

8 ml total volume of cell suspension
1 ml filtered thru Millipore

strains grow normally on glucose minimal medium but not at all or very slowly on lactose minimal agar.

Although 4 MLR strains could grow on lactose minimal agar, none were lac^+ among more than 50 galactose resistant strains isolated from W3092C.

There is little doubt that some element of the lac operon is responsible for the galactose sensitivity of galactokinase-less strains. Galactose resistance results from mutation in the lac operon, while sensitivity invariably follows restoration of the lac operon lesion, either by spontaneous reversion or by introducing a functional lac operon by F-duction (see F13/MLR-1 in Table 2). Several other WCR strains were lac^- and could not grow on melibiose, but showed normal levels of β-galactosidase and thiogalactoside transacetylase after induction. Therefore, these strains would seem to be mutated in only the lac y gene. The data are suggestive that the galactose sensitivity of galactokinase mutants in the W strains is primarily due to the action of the lac y gene product, the TMG permease, and that resistance to galactose probably is associated with the absence of TMG permease. We are still investigating in order to establish this point more firmly and also whether this conclusion may also apply to the MLR strains.

A striking difference between the parental strains is that the galactose resistant mutants of W3092C and 4 lac^+ MLR strains retained galactose permease activity (Table 3), while all of the lac^- MLR's lost the galactose permease.

D-fucose, a gratuitous inducer of the galactose operon, did not stimulate galactose uptake in MLR-1.

There are several puzzling aspects to these findings:

1. The gene for "galactose permease" appears to be within the lac operon of ML32400 since polar mutations, such as MLR-1 and MLR-2, lose this permease as well as the lac functions.

2. The lac operon in ML32400 is inducible while the galactose permease is constitutive. Furthermore, induction of the lac operon did not stimulate

galactose uptake.

3. Galactose sensitive lac$^+$ revertants of both MLR-1 and MLR-2 failed to regain the galactose permease (Table 3).

4. Polar mutations in the lac operon of W3092C do not result in loss of galactose permease.

Thus, these strains of E. coli have genetically different "galactose permeases."

Abbreviations:
K, galactokinase; T, galactose-1-phosphate transferase; Lac z, y, a, lactose operon genes for β-galactosidase, TMG permease and thiogalactoside transacetylase; MLR, galactose resistant bacteria from ML32400; WCR galactose resistant bacteria from W3092C; MLR-R and WCR-R, lactose fermenting revertants of corresponding lac$^-$, galactose resistant strains; BTB, brom thymol blue: TMG, methyl β thiogalactoside; IPTG, isopropyl β thiogalactoside.

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