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Escherichia coli K-12 *tolZ* Mutants Tolerant to Colicins E2, E3, D, Ia, and Ib: Defect in Generation of the Electrochemical Proton Gradient

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Spontaneous *Escherichia coli* K-12 mutants tolerant to colicin E3 were isolated, and on the basis of their tolerance patterns to 19 kinds of colicins, a new phenotypic class of *tolZ* mutants was found. The *tolZ* gene was located between min 77 and 78 on the *E. coli* K-12 genetic map. The *tolZ* mutants were tolerant to colicins E2, E3, D, Ia, and Ib, and showed an increased sensitivity to ampicillin, neomycin, and EDTA, but not to deoxycholate; they were able to grow on glucose minimal medium, but not on nonfermentable carbon sources (succinate, acetate, pyruvate, lactate, malate, etc.). The pleiotropic phenotype of the *tolZ* mutant was due to a single mutation. Both respiration and membrane ATPase activity of the *tolZ* mutant were normal. The *tolZ* mutant had a defect in the uptake of proline, glutamine, thiomethyl-β-D-galactoside, and triphenylmethylphosphonium ion; these uptake systems are driven by an electrochemical proton gradient ($\Delta\bar{\mu}_H+$) or a membrane potential ($\Delta\psi$). In contrast, the uptake of methionine and α-methyl-D-glucoside, which is not dependent on $\Delta\bar{\mu}_H+$ and $\Delta\psi$, was normal in the *tolZ* mutant. Glucose 6-phosphate uptake at pH 5.5, which is driven by a transmembrane pH gradient, in the *tolZ* mutant was similar to the parent level. These results indicate that the *tolZ* mutant has a defect in the generation of $\Delta\bar{\mu}_H+$ and $\Delta\psi$.

The initial interaction of colicins with a sensitive cell is by attachment to a specific receptor on the cell surface. Mutation to colicin resistance alters this receptor and prevents adsorption of colicin. A colicin-tolerant mutant has an apparently unchanged receptor and adsorbs colicin as well as its parent does. However, the mutant is not killed by the colicin. Many kinds of *Escherichia coli* mutants have been reported which have defects in cell membrane and are tolerant to some colicins (21).

Colicin E2 kills *E. coli* by catalyzing DNA degradation (32, 33), whereas colicin E3 degrades rRNA (5, 6, 36). To exert the killing effect, each colicin, or a part of it, has to enter the cell to act on its target. Since both colicins are macromolecular proteins, it is reasonable to suppose that colicins E2 and E3 would utilize some bacterial membrane function(s) that is essential to cell growth. It has been reported that colicins E2, E3, and K require an energized state of cytoplasmic membrane for the initiation of colicin action (18), which suggests the possibility of isolating *E. coli* mutants that might be blocked in an energy-dependent step in the process of translocation of colicins E2 and E3 across the cytoplasmic membrane. Such mutants would be useful in the characterization of membrane functions required for their translocation.

Davies and Reeves (10) reported that colicins are divided into two groups, group A and group B, based on cross-sensitivity of colicin-insensitive mutants among 19 kinds of colicins. Group A consists of colicins E1, E2, E3, K, A, L, N, S4, and X, and group B consists of colicins B, D, G, H, Ia, Ib, M, Q, S1, and V. Davies and Reeves (9, 10) could not find any mutants that showed cross-tolerance between colicins of the two groups, although there are many phenotypic classes showing cross-tolerance within group A (10) or group B (9).

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In this study we have isolated colicin E3-tolerant mutants of *E. coli*, and on the basis of their sensitivity to colicins of group A and group B we found a new class of mutant, *tolZ*. The *tolZ* mutants showed cross-tolerance between group A colicins E2 and E3 and group B colicins D, Ia, and Ib. Growth properties and transport activities of the *tolZ* mutant are described in this paper.

MATERIALS AND METHODS

Bacterial strains. The non-colicinogenic *E. coli* K-12 strains used are shown in Table 1. The standard indicator strain for the colicin assay was strain W2252, and all colicin-resistant and -tolerant mutants were derived from this strain. The colicinogenic strains used are shown in Table 2.

Media and growth of cells. Bacteria were usually cultured at 37°C with shaking in modified L broth supplemented with 20 mg of thymine per liter (ML broth) (25). K10 minimal medium (46 mM Na_2HPO_4, 23 mM NaH_2PO_4, 8 mM $(NH_4)_2SO_4$, 6 μM $FeCl_3$, 0.4 mM $MgSO_4$, 10 mM KCl; pH 7.1) (25) or Davis medium (25), to which 0.5% glucose or 30 mM nonfermentable carbon source was added, was used for growth and genetic experiments. EMB medium (25) and EM medium (25) were used for determination and selection, respectively, of sugar-fermentable strains. The amino acids and nucleotides required were added at final concentrations of 50 μg/ml, thiamine was added at 1 μg/ml, and biotin was added at 0.1 μg/ml. Media were solidified with 1.5% agar.

For experiments on growth yields, cells were grown at 37°C in K10 minimal medium containing limiting concentrations of glucose. For aerobic growth, cultures were aerated by shaking. For anaerobic growth conditions, 10 ml of medium in a test tube was gassed with nitrogen. Growth was considered complete when two successive readings of optical density at 660 nm, taken at 1-h intervals, were similar.

Preparation and assay of colicins. Colicin E1 was prepared from *Salmonella typhimurium* OS19 (ColE1-K30) as previously described (35), except that the culture induced with

TABLE 1. Strains of *E. coli* K-12 used

Strain	Sex or plasmid[a]	Chromosomal markers[b]	Source
W2252	HfrC	*metB1 relA1 spoT1*	Y. Sugino
UM21	HfrC	Like W2252, but *tolZ21*	This paper
UM79	HfrC	Like W2252, but *btuB79*	This paper
KM101	HfrC	Like UM21, but *rpsL101*	This paper
JC1553(MAF1)	F140	*leuB6 hisG1 recA1 argG6 metB1 lacY1 gal-6 xyl-7 mtl-2 malA1 rpsL104 tonA2 tsx-1 supE44* λ^r	K. B. Low via CGSC[c]
KL708	F141	Like JC1553(MAF1)	K. B. Low via CGSC
KL14	Hfr	*thi-1 relA1 spoT1*	K. B. Low via CGSC
PK3	Hfr	*thr-1 leuB6 thi-1 lacY1 azi-15 tonA21 supE44*	P. Kahn via CGSC
KL228	Hfr	*thi-1 leuB6 gal-6 lacY1* or *lacZ4 supE44*	K. B. Low via CGSC
X7014a	F^-	*pyrC46 purB51 thi-1 malA1 mtl-2 xyl-7 lacZ43* or *lacZ13 rpsL125*	J. Beckwith via CGSC
Hfr3000 U482	HfrH	*asd-1 thi-1 relA1 spoT1*	F. Jacob via CGSC
K3141	Hfr	*kdgK2 metB1 relA1 spoT1*	Pouysségur and Stoeber via CGSC

[a] The points of origin and directions of F-prime factors and Hfr strains used as donors are shown in Fig. 4 and in reference 2.
[b] The genetic symbols used are those described in reference 1. For a definition of *tolZ*, see the text.
[c] From B. J. Bachmann, Coli Genetic Stock Center (CGSC), Yale University School of Medicine, New Haven, Conn.

mitomycin C (0.5 μg/ml) was incubated for an additional 3 h. A crude sample obtained from cell extracts by ammonium sulfate fractionation (40 to 60% saturation) was used. Colicins E2 and E3 were purified from *E. coli* W3110 *rpsL*(ColE2-P9) and *E. coli* W3110 *rpsL*(ColE3-CA38), respectively, by guanidine-hydrochloride extraction, ammonium sulfate precipitation, and ion-exchange chromatography as described previously (15, 27). Colicin K was prepared from *E. coli* K235(ColK-K235), which was induced with mitomycin C (1 μg/per ml) at the midlogarithmic phase of growth and was incubated for an additional 4 h. Crude colicin K was prepared from the culture supernatant by ammonium sulfate fractionation (0 to 60% saturation).

Colicin activity was determined by serially diluting the colicin sample with a solution of 0.1 mg of bovine serum albumin per ml and spotting 10 μl of each dilution on ML agar freshly seeded with 10^8 cells of the indicator strain W2252 in 3 ml of ML soft agar (0.7%). The plate was incubated overnight at 37°C. The number of colicin units per milliliter was defined as the highest dilution giving a clear zone of growth inhibition. The colicin activity (units per milliliter) of the prepared samples was as follows: colicin E1, 1×10^4; colicin E2, 1×10^6; colicin E3, 1×10^6; and colicin K, 8×10^3.

Isolation of colicin E3-tolerant mutants. Colicin E3-tolerant mutants were isolated spontaneously from *E. coli* K-12 strain W2252. An overnight culture (ca. 10^8 cells per plate) was spread on ML agar on which colicin E3 (ca. 10^4 units per plate) was previously spread. The plates were incubated at 37°C overnight, and colicin E3-insensitive colonies, including both resistant and tolerant mutants, were obtained. Colicin E3-insensitive colonies thus obtained were scored on ML agar on which BF23 phage lysates (ca. 10^{10} phage per plate) were previously spread, and the plates were incubated at 37°C overnight. Since phage BF23 shares the same receptor with group E colicins (13), phage BF23-sensitive strains were regarded as colicin E3-tolerant mutants with normal receptors. After purification of the mutants, they were used for the following experiments.

Sensitivity to colicins. Sensitivity of isolated colicin E3-tolerant mutants to colicin E1, E2, E3, or K was examined by a cross-streak method. One loop of colicin solutions was streaked across ML agar, and then one loop of overnight culture of the strain to be tested was streaked across the agar at a right angle to the colicin streak. The plate was incubated at 37°C overnight, and tolerance to colicin E1, E2, or E3 was determined by whether the strain was able to grow in the cross part with the colicin. Tolerance to colicin K of colicin K-insensitive strains was determined by a triple-layer agar method described previously (10). The colicin activity (units per milliliter) of the colicin solutions was as follows: colicin E1, 1×10^4; colicin E2, 1×10^5; colicin E3, 1×10^5; and

TABLE 2. Colicins and colicinogenic strains used

Colicins produced	Strain	Original source of plasmid	Source
E1	*Salmonella typhimurium* OS19	K30	H. Ozeki
E1	*E. coli* Y20	K30	S. E. Luria
E2	*E. coli* W3110 *rpsL*	P9	M. Nomura
E3	*E. coli* W3110 *rpsL*	CA38	D. R. Helinski
K, X[a]	*E. coli* K235	K235	M. Nomura
K	*E. coli* K216	K216	P. Reeves
A	*Citrobacter freundii* CA31	CA31	P. Reeves
A[b]	*E. coli* 23	23	P. Reeves
L	*Serratia marcescens* JF246	JF246	J. Foulds
N, E3	*E. coli* 284	284	P. Reeves
N, E3	*E. coli* 285	285	P. Reeves
S4	*Shigella dispar* P15	P15	P. Reeves
X	*E. coli* K-12 185II NxII S7a	K235	P. Reeves
B	*E. coli* T20	K260	P. Reeves via PRC[c]
B	*E. coli* BZB2102	K260	PRC
D	*E. coli* W3110 *rpsL*	CA23	P. Reeves
G	*E. coli* CA46	CA46	P. Reeves via PRC
H	*E. coli* CA58	CA58	P. Reeves via PRC
Ia	*E. coli* W3110 *rpsL*	CA53	J. Konisky
Ib	*E. coli* W3110 *rpsL*	P9	J. Konisky
M	*E. coli* CL142 *met rpsL*	K260	PRC
Q, E1, D, I[d]	*E. coli* II	II	PRC
S1	*Shigella boydii* P1	P1	P. Reeves via PRC
V	*E. coli* CL138	K94	PRC

[a] Produces little detectable colicin X (10).
[b] Some evidence of a second, undefined colicin (10).
[c] From E. M. Lederberg, Plasmid Reference Center (PRC), Department of Medical Microbiology, Stanford University, Stanford, Calif.
[d] Four colicins are produced, but colicin Q shows the largest zone (E. M. Lederberg, personal communication).

colicin K, 8×10^3. The sensitivity of colicin E2- and E3-tolerant mutants to other colicins was examined by the triple-layer agar method.

Sensitivity to drugs. One loop of overnight culture of the strain to be tested was streaked on ML agar containing different concentrations of drug. The plates were incubated at 37°C overnight, and the growth of each strain was recorded.

Oxygen consumption. Rates of oxygen consumption by cells were measured at 37°C with a Clark electrode in conjunction with a Yellow Springs Instruments model 53 oxygen monitor as described previously (22).

ATPase assay. Inverted membrane vesicles were prepared as described previously (43). ATPase activity was measured at 37°C by the spectrophotometric method of Ebel and Lardy (11), with slight modifications. The assay mixture contained the following in a final volume of 0.5 ml: 50 mM Tris-hydrochloride (pH 7.9), 5 mM ATP, 2.5 mM $MgCl_2$, 2.5 mM potassium phosphate, 0.4 mM NADH, 0.6 mM phosphoenolpyruvate, 5 mM KCN, 1 unit of pyruvate kinase, and 1 unit of lactate dehydrogenase. The reaction was initiated by the addition of ATP and was followed by monitoring the decrease of absorption at 340 nm.

Uptake of amino acids, sugars, and triphenylmethylphosphonium ion. The procedure of uptake experiments in whole cells was essentially based on that of Lieberman et al. (22). Cultures grown at 30°C in K10 medium containing 0.5% glucose were harvested at mid-logarithmic phase of growth. The cells were washed twice with K10 medium minus carbon source and Fe^{3+} ($K10C^-Fe^-$), and the washed cells were suspended in $K10C^-Fe^-$ containing chloramphenicol (100 μg/ml) at 1×10^9 to 2×10^9 cells per ml. For triphenylmethylphosphonium ion uptake, Tris-EDTA-treated cells were prepared as described previously (22), and the cells were suspended as described above. For thiomethyl-β-D-galactoside uptake, cells were induced for the *lac* operon by growth in K10 medium containing 0.5% glycerol and 0.5 mM isopropyl-β-D-thiogalactoside.

To 0.3 ml of cell suspension, 0.1 ml glucose or D-lactate was added at a final concentration of 20 mM, and then the mixture was preincubated at 37°C for 10 min with shaking. The uptake reaction was initiated by the addition of radioactive substrate (0.1 ml). At intervals, 0.1-ml samples were withdrawn, filtered on 25-mm membrane filters, and washed with 5 ml of $K10C^-Fe^-$. Nitrocellulose filters (0.45-μm pore size; Sartorius) were used for amino acid or sugar uptake, and cellulose acetate filters (0.5-μm pore size; Millipore Corp.) were used for triphenylmethylphosphonium ion uptake. The filters were dried and counted as described previously (3). Nonspecific binding of radioactive substrate to the cells and filters was measured, and these blank values were subtracted from the uptake data obtained.

TABLE 3. Sensitivity of *tolZ* mutants to colicins[a]

Strain	Genotype	Sensitivity to colicin:					
		E2	E3	D	Ia	Ib	S1
W2252	Wild type	S	S	S	S	S	S
UM21	*tolZ21*	T	T	T	T	T	pT
UM31	*tolZ31*	T	T	T	T	T	pT
UM34	*tolZ34*	T	T	T	T	T	pT
UM37	*tolZ37*	T	T	T	T	T	pT

[a] Colicin sensitivity was examined by a cross-streak method and a triple-layer agar method as described in the text. Abbreviations: S, sensitive; T, tolerant; and pT, partially tolerant. All strains were sensitive to colicins E1, K, A, L, N, S4, X, B, G, H, M, Q, and V.

TABLE 4. Quantitation of colicin tolerance

Strain	Genotype	Endpoint dilution of colicin prepn[a]			
		E1	E2	E3	D
W2252	Wild type	3	7	12	6
UM79	*btuB79*	0	0	0	NT
UM21	*tolZ21*	3	0	0	0

[a] The numbers given in the table are n, where $1/3^n$ is the last dilution of the colicin preparation to give a complete inhibition zone for growth on a lawn of each strain. Where $n = 0$, no clearing of the bacterial growth could be seen with an undiluted colicin preparation. NT, Not tested.

The experiment of D-glucose 6-phosphate (Glc-6-P) uptake was carried out by the method of Tokuda and Konisky (41). Cells were grown in K10 medium containing 10 mM Glc-6-P as the sole carbon source, and washed with $K10C^-Fe^-$ minus Mg^{2+}. The washed cells were suspended in 50 mM potassium phosphate buffer (pH 5.5, 6.5, or 7.6) and washed once with and suspended in the same buffer. After a 10-min preincubation at 37°C, potassium ascorbate, phenazine methosulfate, and, immediately thereafter, [^{14}C]Glc-6-P were added (final concentrations: ascorbate, 20 mM; phenazine methosulfate, 0.1 mM; and Glc-6-P, 0.4 mM). The buffer used for filtration was 50 mM potassium phosphate (pH 5.5, 6.5, or 7.6). Other experimental procedures were similar to those described above.

The specific activities and final concentrations of the radioactive substrates used were as follows: L-[*U*-^{14}C]proline (19.6 Ci/mol), 10 μM; L-[*U*-^{14}C]glutamine (33.7 Ci/mol), 9.2 μM; L-[*methyl*-^{14}C]methionine (20 Ci/mol), 10 μM; [*methyl*-^{14}C]thiomethyl-β-D-galactoside (19.6 Ci/mol), 49.4 μM; [D-glucose-*U*-^{14}C]α-methyl-D-glucoside (20.2 Ci/mol), 50 μM; D-[*U*-^{14}C]glucose 6-phosphate (17.9 Ci/mol), 0.4 mM; and [*methyl*-^{3}H]triphenylmethylphosphonium bromide (17.9 Ci/mol), 0.4 mM.

Protein determination. Protein was determined by the method of Lowry et al. (23) with bovine serum albumin as a standard.

Procedures for mating and transduction. The procedures used for mating and transduction were essentially those described previously (25), except that a recipient of mating, an HfrC-derivative *tolZ* strain, was cultured overnight in ML broth (ca. 2×10^9 cells per ml) and used for the mating experiment after shaking vigorously with a mixer for 3 min.

Reagents. D-[*U*-^{14}C]glucose 6-phosphate was a product of the Radiochemical Centre, Amersham, England, and the other radioactive compounds were obtained from New England Nuclear Corp., Boston, Mass. Pyruvate kinase and lactate dehydrogenase was obtained from Boehringer Mannheim Biochemicals, Indianapolis, Ind. All other chemicals used were reagent grade.

RESULTS

Isolation of a new phenotypic class mutant *tolZ*. Fifteen spontaneous mutants tolerant to colicin E3 were isolated independently. Four mutants were tolerant to colicins E2 and E3 and were sensitive to colicins E1 and K (Table 3). The other 11 were 2 *tolA*-type mutants (4) tolerant to colicins E2, E3, and K, and 9 *tolB*-type mutants (4) tolerant to colicins E1, E2, E3, and K.

For the four mutants tolerant to colicins E2 and E3, sensitivity to the other group A colicins (colicins A, L, N, S4, and X) and group B colicins (colicins B, D, G, H, Ia, Ib, M, Q, S1, and V) was examined. The mutants were tolerant to colicins D, Ia, and Ib, and were partially tolerant to colicin

S1 (Table 3). The tolerance shown by a triple-layer agar method (10) was confirmed by a cross-streak method with crude samples of colicins D (39), Ia, and Ib (20) prepared as described previously. The colicin Q producer *E. coli* II also produces colicins E1, D, and I in small amounts. Therefore, sensitivity to colicin Q of these mutants may reflect the sensitivity to colicin E1 being present together. We propose to designate the gene concerned with the tolerance to colicins E2, E3, D, Ia, and Ib tentatively as *tolZ*; the gene locus was determined to be located between min 77 and 78 on the *E. coli* K-12 genetic map as described below.

Quantitation of colicin tolerance (Table 4) showed that the *tolZ* type strain UM21 was fully tolerant to colicins E2, E3, and D. The extent of tolerance to colicins E2 and E3 was the same as that of resistance of *btuB* strain UM79.

Sensitivity to drugs and bacteriophages. Table 5 shows the sensitivity of the *tolZ* mutants to ampicillin, neomycin, and EDTA; the mutants were more sensitive to these agents than was the parental strain, W2252. The *tolD* (7) and *tolE* (12) mutations are known to cause resistance to ampicillin as well as tolerance to colicins E2 and E3. Thus, the increased sensitivity of the *tolZ* mutants to ampicillin presents a remarkable contrast. The *tolZ* mutants were able to grow as well as the parent up to 0.5% concentration of sodium deoxycholate.

The *tolZ* mutants were sensitive to phages T5, TuIa, TuIb, TuII*, P1, and φ80. The plaque formation of λ on strain UM21 was about 2×10^{-4} as much as that on the parent.

Growth properties. When cells were cultured in ML broth at 37°C, the doubling times of the *tolZ* strain UM21 and the parental strain W2252 were 28 and 22 min, respectively. When cultured in K10 minimal medium at 37°C, the *tolZ* mutants were able to grow with glucose as a carbon source, and the doubling time of strain UM21 was 70 min, longer than that of the parent, 55 min. However, unlike the parent, the *tolZ* mutants were all unable to grow with succinate, acetate, pyruvate, D,L-lactate, malate, isocitrate, aspartate, or alanine as the sole source of carbon. The *tolZ* strain UM21 showed temperature-sensitive growth. After the culture grown in glucose minimal medium at 30°C was shifted to 42°C, the growth of strain UM21 stopped soon, and cell viability decreased exponentially (data not shown).

Aerobic and anaerobic growth yields were examined at 37°C in minimal medium containing limiting concentrations of glucose. The aerobic and anaerobic growth yields of strain UM21 were 45 and 60%, respectively, of those of the parent (Fig. 1). This indicates that the lesion caused by the *tolZ* mutation affects both aerobic and anaerobic growth of the mutant.

TABLE 5. Sensitivity of *tolZ* mutants to drugs

Drug	Concn	Growth[a]	
		W2252 (wild type)	UM21 (*tolZ21*)
Ampicillin (μg/ml)	1	++	++
	3	++	−
	6	−	−
Neomycin (μM)	5.5	++	−
	11	+	−
	28	−	−
EDTA (mM)	1	++	++
	5	++	++
	10	++	−

[a] Additional *tolZ* strains UM34 and UM37 showed identical growth patterns to strain UM21. ++, Normal growth; +, poor growth; −, no growth.

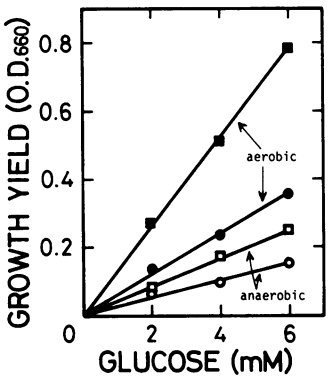

FIG. 1. Growth yields for strains W2252 and UM21 grown on limiting concentrations of glucose. Symbols: ■, strain W2252, aerobic; □, strain W2252, anaerobic; ●, strain UM21, aerobic; ○, strain UM21, anaerobic.

Revertants of *tolZ*. The pleiotropic phenotype of the *tolZ* mutants was due to a single mutation. Revertants of the *tolZ* strains, which were selected on succinate minimal plates, simultaneously regained all tested parental properties. Succinate-utilizing revertants of strain UM21 were obtained at a frequency of about 10^{-8} per cell. The revertants exhibited parent-like sensitivity to colicins E2, E3, D, Ia, and Ib, were able to grow on D,L-lactate and malate, and were no longer temperature sensitive.

Respiration and ATPase activity. Since the *tolZ* mutants were unable to grow on nonfermentable substrates, it may be possible that the lesion resides in the component of the electron transport chain or in the H^+-ATPase complex. When oxygen consumption dependent on glucose, succinate, or D,L-lactate was measured at 37°C, the respiratory rates in whole cells were similar in the parent and the four *tolZ* mutants (data not shown). Moreover, membranes prepared from the parent and strain UM21 also exhibited similar levels of ATPase activity at 37°C, 1.4 and 1.8 U/mg of protein, respectively. Thus, respiration and ATPase activity of the *tolZ* mutant seem to be normal.

Uptake of amino acids and sugars. The inability of cells to grow on nonfermentable substrates could result from the defect in the generation of electrochemical gradient of protons ($\Delta\bar{\mu}_H+$) (26). $\Delta\bar{\mu}_H+$ is composed of a membrane potential ($\Delta\psi$) and a transmembrane pH gradient (ΔpH) (26). Whether the *tolZ* mutant is able to generate $\Delta\bar{\mu}_H+$, $\Delta\psi$, and ΔpH was examined by measuring the uptake of amino acids and sugars. Uptake of proline (3, 31) and thiomethyl-β-D-galactoside (37) is dependent on $\Delta\bar{\mu}_H+$, and both $\Delta\psi$ and ATP (or acetyl phosphate [17]) are essential to glutamine uptake in *E. coli* cells (29). Uptake of proline (Fig. 2A), glutamine (Fig. 2B), and thiomethyl-β-D-galactoside (Fig. 2D) was much lower in the *tolZ* strain UM21 than in the parent. On the other hand, uptake of α-methyl-D-glucoside (Fig. 2E) in strain UM21 cells was normal; this is driven by phosphoenolpyruvate:hexose phosphotransferase system (14). Methionine uptake (Fig. 2C), which is driven by ATP (19) (or acetyl phosphate [17]), in strain UM21 cells seems to be normal, although this uptake was a little lower in strain UM21 than in the parent. These results suggest that the *tolZ* mutant is unable to generate sufficient $\Delta\bar{\mu}_H+$ and $\Delta\psi$.

Generation of Δψ and ΔpH. The accumulation of triphenylmethylphosphonium ion in cells is dependent on Δψ (34), and the transport system for Glc-6-P is driven by ΔpH at pH 5.5 in *E. coli* cells (31, 41). Therefore, the generation of Δψ and ΔpH can be detected by measuring the uptake of these compounds. Figure 2F shows that the accumulation of triphenylmethylphosphonium ion in strain UM21 cells was about 25% of that in the parental cells. In contrast, strain UM21 was able to transport Glc-6-P at pH 5.5 nearly as well as the parent (Fig. 3A). Figure 3 also shows that Glc-6-P uptake by strain UM21 decreased progressively as the pH was raised from 5.5 to 7.6; at pH 7.6 the uptake by strain UM21 was less than a half of that by the parent. At pH 7.6 the uptake of Glc-6-P is driven by Δψ (31), and thus these results suggest that the cytoplasmic membrane of the *tolZ* strain UM21 is permeable to some ion(s) other than protons.

Mapping the *tolZ* locus. To map the *tolZ* locus, at first we used the Coli Genetic Stock Center F′ kit consisting of 18 strains carrying F′ factors that span the *E. coli* genetic map (2). Although *tolZ* strain UM21 is a derivative of HfrC and thus should be a poor recipient in F conjugation, this did not interfere with the experiment. By selection of recombinants on succinate minimal agar, only two strains, F140-carrying strain JC1553(MAF1) and F141-carrying strain KL708, gave strong succinate-positive transfer. Succinate-positive recombinants appeared at a frequency of about 4×10^{-5} per recipient cell in conjugation with either F′ factor. In crosses with Hfr strain KL14, PK3 or KL228, succinate-positive recombinants of *tolZ rpsL* strain KM101, were also obtained at frequencies of more than 10^{-5} per recipient cell. These results suggest that there should be an overlapping region between the terminal end of F141 and the starting end of PK3, where the *tolZ* gene seems to be located (Fig. 4).

To test for cotransduction of *tolZ* with the markers *malA*, *asd*, *kdgK*, *xyl*, and *mtl* (Fig. 4), P1 phage lysates were prepared on *malA xyl mtl* strain X7014a, *asd* strain Hfr3000 U482, or *kdgK* strain K3141 and were used to transduce $tolZ^+$ into strain UM21 by selection on succinate minimal agar. No donor markers were found in any $succinate^+$ transductants tested for *malA*, *xyl*, and *mtl* (each 0 out of 416 transductants) and *asd* (0 out of 520 transductants). However, *kdgK* was cotransducible with *tolZ* at a frequency of 10% (10 $kdgK^-$ of 100 $tolZ^+$ transductants). Considering that the *pit* gene (77.0 min) (Fig. 4) is cotransducible with *asd* at a frequency of 2%, but not with *xyl* (38), and that the *kdgK* gene (78.3 min) is cotransducible with *xyl* at a frequency of 1.5 to 6.5% (30), the results described above strongly suggest that the *tolZ* gene is located between min 77 and 78 on the *E. coli* K-12 genetic map (Fig. 4).

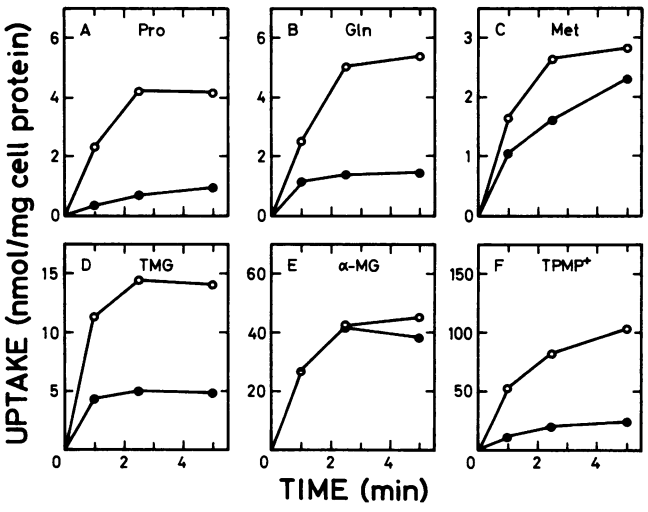

FIG. 2. Uptake of amino acids, sugars, and triphenylmethylphosphonium ion by cells of strains W2252 and UM21. For α-methyl-D-glucoside uptake, D-lactate was used as the sole carbon source; for the others glucose was added as the sole carbon source. Symbols: ○, strain W2252; ●, strain UM21. Panels: A, proline; B, glutamine; C, methionine; D, thiomethyl-β-D-galactoside; E, α-methyl-D-glucoside; F, triphenylmethylphosphonium ion.

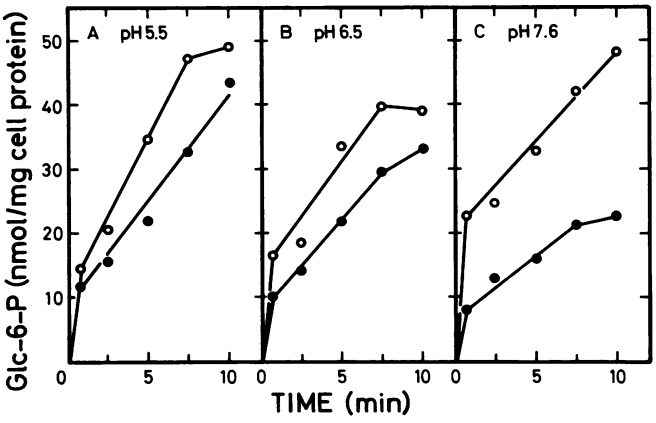

FIG. 3. Glc-6-P uptake by cells of strains W2252 and UM21. Incubation was carried out at pH 5.5 (A), 6.5 (B), or 7.6 (C). Symbols: ○, strain W2252; ●, strain UM21.

DISCUSSION

Colicins fall into two well-defined groups, A and B (10), and no colicin-tolerant mutants have been found which show cross-tolerance between colicins of the two groups (9, 10). One of the major findings in this study is that the *tolZ* mutants showed cross-tolerance between group A colicins E2 and E3 and group B colicins D, Ia, and Ib (Table 3). The targets of colicins E2 (32, 33) and E3 (5, 6, 36) are chromosomal DNA and rRNA, respectively, and colicin D specifically causes an inhibition of protein synthesis in sensitive cells (40). Since every target of these colicins exists inside the cells, the colicins, or parts of them, have to enter the cells to exhibit their killing effects. On the other hand, colicin Ia is known to make cytoplasmic membrane permeable to ion(s) other than protons and induce membrane depolarization, which is brought about by direct interaction of the colicin molecules with bacterial cytoplasmic membranes (41, 42).

The *tolZ* mutants were unable to grow on nonfermentable carbon sources, whereas both respiration and membrane ATPase activity of the *tolZ* type strain UM21 were normal. Analysis of the generation of $\Delta\bar{\mu}_H+$, Δψ, and ΔpH by measuring the uptake of amino acids, sugars, and triphenylmethylphosphonium ion indicates that the $\Delta\bar{\mu}_H+$ and Δψ in the *tolZ* mutant are not sufficient to carry out coupling of energy to active transport, and suggests that the cytoplasmic membrane of the *tolZ* mutant is permeable to some ion(s) other than protons.

Several colicin-tolerant mutants of *E. coli*, which have a defect in growing on nonfermentable carbon sources, were reported, and their mutation loci were determined as follows: *tolI* (1, 8), tolerant to colicins Ia and Ib, 0 min; and *efcA* (1, 16) and *ecfB* (1, 28) tolerant to colicin K, 65 and 88

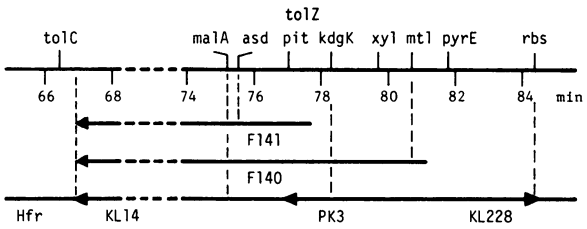

FIG. 4. Genetic map and the origins and directions of F′ factor transfer for F140 and F141 and of chromosome transfer for Hfr strains KL14, PK3, and KL228. The positions of the genes listed and the data for F′ and Hfr are derived from references 1 and 2, respectively. The position of the terminal end of F141 is obscure. The presumed site of the *tolZ* gene is shown.

min, respectively. The *ecfA* (16) and *ecfB* (28) mutants are unable to transport proline and thiomethyl-β-D-galactoside, like the *tolZ* mutant. All of these mutants except *tolZ* are sensitive to colicins E2 and E3. Therefore, inability to generate $\Delta\tilde{\mu}_H+$ does not necessarily cause tolerance to colicins E2 and E3. It is possible that the *tolZ* protein is a component of some ion-transport system with which colicins E2, E3, and D interact to translocate to the interior of the cells. The ion transport system may be the interaction site of colicin Ia and Ib molecules on the cytoplasmic membrane.

We have already cloned the *tolZ* gene into plasmid RP4, and the plasmid was able to complement the *tolZ* mutation (Y. Koyama, H. Matsuzawa, and T. Ohta; unpublished data). This may suggest that the *tolZ* mutation is recessive.

ADDENDUM

Some colicin K used previously (16, 18, 28) may be colicin A, judging from reference 24.

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