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New Class of Conditional Colicin-tolerant Mutants

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A new class of temperature-dependent *tol* mutants of *Escherichia coli* expresses tolerance to colicins only after growth at higher temperatures. Colicin sensitivity or tolerance, acquired by growth at 30 or 41 C, respectively, is not lost upon incubation at the other temperature in the presence of chloramphenicol. The kinetics of conversion from sensitivity to tolerance and vice versa have been analyzed. In one instance, the *tol VIII* temperature-dependent phenotype was due to the modifying role of a *str-r* mutation on the suppression of a *tol VIII* amber mutant by the suppressor mutation *su*II. The bearing of the present findings on the role of the *tol* genes is discussed.

Several types of colicin-tolerant mutants (*tol*), which adsorb colicin but are not killed by it, have been isolated and characterized (2, 6, 10, 11, 14). Thus, the *tol II* mutants, insensitive to colicins K, A, and those of group E, have fragile cells, are slow growers, and are sensitive to deoxycholate; the *tol VIII* mutants are tolerant to colicin E1 and hypersensitive to deoxycholate and to several dyes. These characteristics of the *tol II* and *tol VIII* mutations have been interpreted as indicative of changes in structural components of the surface layers, presumably in the cytoplasmic membrane (10).

In this paper, we report the isolation and study of mutants of the *tol II* and *tol VIII* types that express their tolerant phenotype at high temperatures only. These temperature-dependent mutants have been analyzed genetically, and their behavior after shifts in temperature has been followed to gain some knowledge of the properties of the affected cellular constituents.

MATERIALS AND METHODS

Bacteria and bacteriophages. The bacterial strains used are listed in Table 1. Phages T4, T4 amber mutants, BF23, and P1 were from our laboratory collection. Defective phage hybrid 80-λ carrying the *su*III gene was obtained from E. Signer's collection.

Media. The composition of the media employed has been given previously (10). DOC agar contained LB broth with 2% Difco agar and 0.5% sodium deoxycholate (DOC).

Methods. Most of the methods employed (procedures for matings, P1 transductions, preparation of colicins, tests of response to colicins) have been described (10).

Mutagenesis. A log culture of strain LA261 grown in LB broth was treated with nitrosoguanidine by the procedure of Adelberg et al. (1); the treated cells were permitted to grow at 30 C to allow phenotypic expression. The culture was then equilibrated at 42 C and left at this temperature for 30 min; colicins E1 and K were added at high multiplicity (about 50 killing units per bacterium) and, after 15 minutes, trypsin was added at a final concentration of 250 μg/ml. After 30 min, the culture was diluted into fresh LB broth and allowed to grow at 30 C. The entire procedure of colicin and trypsin treatment at high temperature was repeated once more. Then, after a second cycle of growth at low temperature, the cells were plated on LB agar and incubated at 30 C; colonies were picked and checked for growth on DOC plates at 30 and 42 C. Those colonies that were resistant to DOC (DOC-r) at low temperature and sensitive (DOC-s) at high temperature were purified and tested by spot test for response to colicins of groups E, K, and A at 30 and 42 C.

RESULTS

Properties of mutant strain LA639. One strain, designated LA639, was isolated from strain LA 261 after mutagenesis. It turned out to be DOC-s at 41 C and DOC-r at 30 C in tests performed on plates or in liquid media. Typically, LA639 grows in broth with 1% DOC at 30 C but does not grow with 0.1% DOC at 41 C.

Spot tests indicated that strain LA639 was sensitive to colicins A, E1, E2, E3, and K at 30 C and resistant or almost resistant to these colicins at 41 C. Detailed measurements of colicin resistance will be presented in a later section. Appropriate tests showed that mutant LA639 grown at 41 C does adsorb colicin E1; hence, its resistance is not due to lack of adsorption.

The growth of the mutant and parental strains was followed at 30 and 41 C (Fig. 1). Strain LA 639 grows at the same rate as the parental strain at the lower temperature but more slowly at 41 C.

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TABLE 1. *Bacterial strains*

Strain	Genotype[a]	Source or reference
La261	F⁻ *pro⁻ arg⁻ his⁻ lac⁻ gal⁻ str-r*	*str-r* mutant from Adelberg's AB1122
W602	F⁻ *leu⁻ thi⁻ bio⁻ gal⁻ str-r*	E. L. Wollman
W3101	*str-s* (F′ *suc⁺ gal⁺ bio⁺*)	E. L. Wollman
LA610	*thr⁻ leu⁻ thi⁻ tol II* (F′ *suc⁺ gal⁺ bio⁺ tol II*)	(10)
W4032	Hfr(Cavalli) *pro⁻ met⁻ str-s T6-r*	J. Lederberg
Hfr C × 1	Hfr(Hayes) *try⁻ ade⁻ ilv⁻ lac⁻ gal⁻ str-r suII⁺*	E. R. Signer
WD7002	F⁻ lac^{-}_{am} *glt⁻ str-r su⁻*	J. Beckwith

[a] Symbols: *ade*, adenine; *am*, amber; *arg*, arginine; *bio*, biotin; *gal*, galactose; *glt*, glutamate; *his*, histidine; *ilv*, isoleucine and valine; *lac*, lactose; *leu*, leucine; *met*, methionine; *pro*, proline; *str*, streptomycin; *suc*, succinate; *thi*, thiamine; *thr*, threonine; *tol*, tolerant; *try*, tryptophan.

Genetic characterization of strain LA639. The pattern of resistance to colicins, the sensitivity to DOC, and the slow growth rate of LA639, all properties manifested at 41 C, as well as its capacity to adsorb colicins, suggested that this strain carries a temperature-dependent mutation of the *tol II* type. The three experiments described below were performed to characterize it genetically.

Transduction. A *gal⁺* revertant from LA639 was isolated, and a P1 lysate was prepared on it and used to transduce *gal⁺* to strain W602. Ten *gal⁺* transductants were isolated, purified, and tested for response to DOC and to colicin E1. Three of them were sensitive to colicin E1 and resistant to DOC, like the recipient strain; the other seven showed at 41 C the characteristics of DOC sensitivity and colicin resistance of the donor. Thus, the mutation present in strain LA 639, like *tol II*, is cotransduced at high frequency with galactose (10).

Dominance test. An F-*gal* factor was transferred from strain W3101 to LA639 by mixed cultivation followed by plating on eosin methylene blue (EMB)-galactose-streptomycin-agar. Several *gal⁺* str-r colonies were tested, and all had acquired at 41 C the DOC resistance and colicin sensitivity characteristic of the donor strain. This indicates that the F-*gal* factor carries the dominant wild-type allele of the LA639 mutation. These are the same relations that exist between this F-*gal* factor and the *tol II* mutation (10).

Complementation. Strain LA639 was used as recipient for the F-*gal* factor from the homogenote strain *tol II* (F-*gal⁺ tol II*). *Gal⁺* colonies were selected on EMB-galactose-agar with streptomycin and were found to be sensitive to the male phage MS2 and sensitive to colicins at 30 C; at 41 C, these partial diploids showed the colicin resistance and the DOC sensitivity characteristic of the endogenote. This indicates that the *tol II* mutation carried by the F-*gal* factor and the mutation of LA639 do not complement; it also shows that the temperature-sensitive mutation is dominant over the *tol II* mutation. As expected, during growth at 30 C the partial diploids segregated many colicin-resistant recombinants. Thus, all the results of these genetic tests lead to the inclusion of the mutation of strain LA639 in the *tol II* gene. This mutation will be designated as *tol II td* (= temperature-dependent).

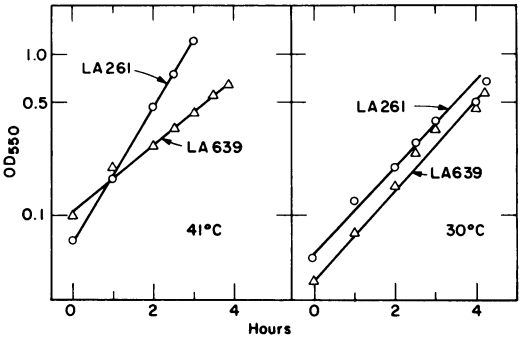

FIG. 1. *Growth of mutant LA639 and of its parent strain LA261 at 30 and 41 C. The bacteria were grown in LB broth, and optical density was read at intervals in a Zeiss spectrophotometer.*

Temperature dependence of the tol II td phenotype. The response of strains LA261 and LA639 to colicin E1 was determined quantitatively at 30 and 41 C. As shown in Fig. 2, strain LA261 was about equally sensitive to E1 at both temperatures; the mutant strain LA639, on the other hand, was as sensitive as LA261 at 30 C, but almost completely resistant at 41 C.

The effect of the temperature at which bacteria are plated is also illustrated in Fig. 2. The parental strain LA261 showed no difference in sensitivity to colicin E1 at different plating temperatures. Unexpectedly, when cells of strain LA639 grown at 41 C and treated with colicin E1 at 41 C were

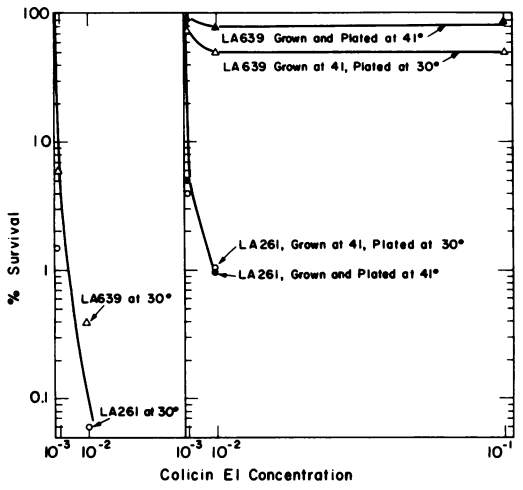

FIG. 2. *Sensitivity of strains LA261 and LA639 to colicin E1 at 30 and 41 C. Cells of strains LA261 and LA639 grown in LB broth at 30 or 41 C to an optical density at 550 nm of 0.6 were treated with E1 for 10 min, diluted, and plated at the same temperature as that of previous growth. The cells grown and treated at 41 C were also plated on plates incubated at 30 C.*

then plated at either 30 or 41 C, they behaved as tolerant irrespective of the plating temperature. There was some difference in survival at the two temperatures (for example, 50% versus 80% in the experiment of Fig. 2), but these differences were slight compared with the sensitivity of cells grown at 30 C.

The significant fact is that cells of the *tol II td* mutant grown and treated at 41 C remained viable when transferred to 30 C. This indicates that most of the colicin molecules attached to receptors synthesized at 41 C are unable to exert their killing action even when the bacterium-colicin complexes are transferred to low temperatures after adsorption. It appears that those portions of the cell envelope that have been synthesized at the high temperature and have thereby acquired the tolerant property do not lose tolerance after being shifted to 30 C. Temperature-shift experiments performed in liquid media, following the changes in optical density of broth cultures after colicin treatment, confirmed the results of platings; again, cells grown and treated at 41 C survived and grew after transfer to 30 C.

The next set of experiments was performed to determine the effect of temperature shifts made before colicin treatment.

If the shift from 30 to 41 C or vice versa was made in the presence of chloramphenicol to prevent protein synthesis, little change in the response of LA639 to colicin E1 occurred (see Table 2): cells grown at 30 C remained sensitive; cells grown at 41 C remained tolerant (although a slight decrease in resistance was always present). It appears that the changes in colicin sensitivity following temperature shifts do not result from activation or inactivation of preexisting structures.

The findings are very different when temperature shifts are done under growth conditions and then the cells are challenged with colicin. Typical results are shown in Table 2. In the shift down from 41 to 30 C, the cells of strain LA639 became progressively more sensitive to colicin E1. On bacteria that had undergone one doubling in cell mass, a given amount of the colicin acted as if it had about one-half as much killing activity as it had on sensitive cells grown at 30 C; after two doublings, the activity was about three-fourths

TABLE 2. *Effects of temperature shifts on the response of LA639 to colicin E1*[a]

Sample	Treatment	"Killing multiplicity" of E1
I	30 C control	10
II	30 → 41 C with chloramphenicol	10
III	30 → 41 C	
	After one doubling	1.1
	After two doublings	0.3
I	41 C control	<0.5
II	41 → 30 C with chloramphenicol	<0.5
III	41 → 30 C	
	After one doubling	4.9
	After two doublings	8.0

[a] Two cultures of strain LA639 were grown in LB broth at 30 and at 41 C to an optical density at 550 nm of 0.6 to 0.7. From each culture, sample I (= control) was treated for 10 min with different concentrations of colicin E1, diluted, and plated, all operations being performed at the original growth temperature. A second sample (II) was shifted from 30 to 41 C or vice versa in the presence of chloramphenicol (100 μg/ml), kept at the new temperature for 1 hr, treated for 10 min with colicin E1, diluted, and plated. A third sample (III) was diluted in fresh broth, shifted to the new temperature, and allowed to grow; samples were removed at an optical density (at 550 nm) corresponding to one and two doublings, treated with colicin E1, diluted, and plated. The "killing multiplicity" m was calculated from the survival in those mixtures of bacteria and colicin that gave between 10 and 50% survivors, from the first term of the Poisson distribution: $N = N_0 e^{-m}$, where N_0 and N are the initial and final number of colony formers. The values of m for a given sample were normalized to colicin concentration and averaged.

that on cells grown at 30 C. These results suggest that during growth after the temperature shift-down there is a simple dilution of colicin-tolerant sites on the cell surface. The simplest interpretation is that the only receptors from which a colicin molecule can cause a lethal event are those located in those portions of the cell envelope that were synthesized after the shift to 30 C.

A different situation was observed, however, in the appearance of E1 tolerance after shifting from 30 to 41 C under growth conditions. Tolerance appeared much faster than one would expect from the calculated amount of cell mass synthesized at the high temperature. This suggests that a certain proportion of the sensitive sites synthesized at 30 C become unresponsive during growth following the shift to the higher temperature.

Experiments on the effects of temperature shifts on the sensitivity of strain LA639 to other colicins were performed in a similar way. The results with colicin K, whose mode of action resembles that of colicin E1, were quite similar to those with E1. With colicins E2 and E3, the picture was somewhat different. Again, there was no loss of sensitivity or tolerance after temperature shifts in the presence of chloramphenicol. With these colicins, however, the kinetics of appearance of sensitivity or tolerance upon growth after temperature shifts followed more closely what was expected if sites made at either temperature, 30 or 41 C, retained their sensitivity or tolerance and were simply diluted by growth after the temperature shifts.

Because a certain range of variation is observed from experiment to experiment in this kind of test, it is difficult to assess in a precise quantitative way the early kinetics of appearance of sensitivity or tolerance after shifts in temperature. An additional source of uncertainty derives from the fact that, after temperature shift-ups, viable counts, total cell counts, and optical density measurements do not always keep constant ratios with each other. Deviations as high as a factor of 2 are observed, suggesting that cell division may lag behind cell growth.

Despite these limitations, it appears that with all the colicins investigated the acquisition of sensitivity by strain LA639 after a temperature shift-down follows the pattern expected from a simple addition of newly formed colicin-sensitive surface components. For colicins E1 and K, but not for E2 and E3, after a temperature shift-up the colicin-sensitive sites are lost more rapidly than by simple dilution.

Mutant strain LA641. After nitrosoguanidine mutagenesis of strain LA261, another mutant strain was isolated which proved to be a mutant of the *tol VIII* type, partially sensitive to colicin E1 at low temperature and tolerant at 41 C. Genetic analysis of this mutant, called LA641, showed that in this case the temperature dependence was not due to a mutation in the *tol VIII* gene, but to an effect of temperature on suppression on a *tol VIII* amber mutation by a suppressor gene.

Properties of strain LA641. This mutant forms colonies on DOC agar plates at 30 and 37 C but not at 42 C. In LB broth, growth occurs with concentrations of DOC up to 1% at 30, 37, and 41 C, but definite inhibition by DOC is present at 43 to 44 C. On EMB plates, LA641 grows at 30 and 37 C but not at 42 C. As with the *tol VIII* mutants (10), the inhibition of LA641 on EMB plates is due to the methylene blue (MB) as shown by tests in liquid media at 41 C with different concentrations of MB. Additional tests confirmed that sensitivity to MB is manifested at lower temperatures than that to DOC.

The sensitivity of mutant LA641 to colicin E1 was determined at both 30 and 41 C and was compared with that of the parental strain (Fig. 3). The parental strain was sensitive to colicin E1 at both temperatures, whereas mutant LA641 was somewhat sensitive at 30 C and almost completely tolerant at 41 C. Strain LA641 did not show any indication of tolerance to colicins K, E2, or E3 at any of the temperatures tested. The adosrption of colicin E1 by cells of LA641 grown at 41 C was comparable to that by parent cells.

All the characteristics of LA641—tolerance to colicin E1 only, sensitivity to DOC, and sensitivity to MB—suggested that it was a temperature-dependent mutant of the *tol VIII* type.

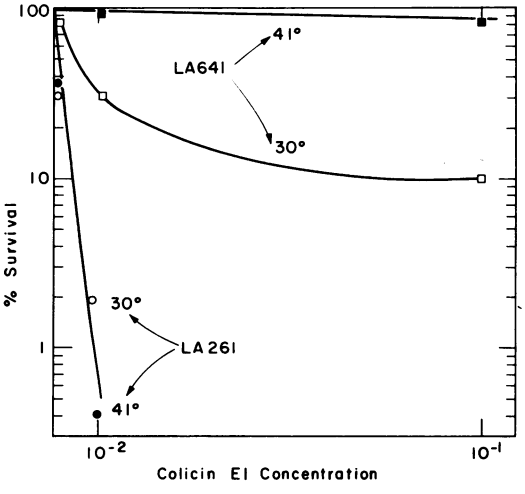

FIG. 3. *Sensitivity of strains LA261 and LA641 to colicin E1 at 30 and 41 C. Experimental conditions were similar to those in Fig. 2.*

Genetic characterization of strain LA641. The first indication that the temperature-dependent phenotype of strain LA641 was due, not to a mutation in the *tol VIII* gene, but to temperature-sensitive suppression of a *tol VIII* mutation, came from a mating experiment between LA641 and the Hfr strain W4032. As shown in Table 3, the analysis of *his*$^+$ *met*$^+$ recombinants from this cross revealed the appearance of a new phenotype identical to *tol VIII* (tolerant to colicin E1 and sensitive to MB and DOC at both 30 and 41 C) different from the phenotypes of the F$^-$ and the Hfr parents. All of the *his*$^+$ *met*$^+$ recombinants with the *tol VIII* temperature-dependent phenotype were str-r (Table 3).

The hypothesis that a suppressor was involved was tested by plating several T4 amber mutants on strain LA261 and on its mutant LA641. Both these strains proved to be permissive (*su*$^+$), whereas the Hfr strain W4032 was *su*$^-$. The suppressor activity of several *his*$^+$ *met*$^+$ recombinants from the mating W4032 × LA641 was also tested. The *tol*$^+$ *str-s* recombinants were either *su*$^+$ or *su*$^-$, but all the *tol VIII* recombinants tested (14 of 14) were *su*$^-$, irrespective of whether they carried the *str-r* or *str-s* genes.

These results indicated that the *tol VIII* temperature-dependent phenotype of strain LA641, which is *su*$^+$, might be due to suppression of a *tol VIII am* mutation, and that the *tol VIII* phenotype was expressed irrespective of temperature only in those recombinants that combined the *su*$^-$ allele from the Hfr parent and the *tol VIII* mutation from LA641.

The suppressor gene present in strain LA261 was identified as *su*II by the pattern of suppression of different T4 amber mutants (12) and by the fact that a P1 lysate prepared on strain LA261 can cotransduce, with a frequency of about 13%, the *glt*$^+$ character and the suppressor activity to strain WD7002, *glt*$^-$ *lac*$^+$ amber. The cotransduction of *glt* and *su*II has been observed by B. M. Ohlsson (Ph.D. Thesis, Harvard Univ., Cambridge, Mass., 1968).

TABLE 3. *Analysis of his*$^+$ *met*$^+$ *recombinants from a mating between W4032 and LA641*[a]

Class	Total	*strs-s*
tol VIII	45	8
tol VIII td	224	0
tol VIII$^+$	435	134
All classes	704	142

[a] The *his*$^+$ *met*$^+$ recombinant colonies were selected on minimal plates containing arginine, proline, and thiamine, transferred to master plates of the same selective medium, and replica-plated both to LB agar plates with streptomycin and to duplicate DOC plates. Sets of DOC plates were incubated at 30 C or at 41 C. The following classes are recognized on the DOC plates: *tol VIII*$^+$ is DOC-resistant at 30 and 41 C; *tol VIII* is DOC-sensitive at 30 and 41 C; *tol VIII td* grows on DOC agar at 30 C but not at 41 C.

As shown in Table 3, the *tol VIII td* phenotype did not appear in any of 142 *str-s his*$^+$ *met*$^+$ recombinants from the cross between Hfr W4032 and F$^-$ LA641. There was a correlation, therefore, between the expression of this phenotype and the presence of the *str-r* mutation. To analyze this situation, two F$^-$ *tol VIII su*$^-$ strains, one *str-s* and the other *str-r*, both recovered as recombinants from the cross between Hfr W4032 and LA641, were used as recipients for the *su*II$^+$ gene. This gene was transferred from strain HfrCxl in a mating experiment interrupted at 40 min in order to prevent entry of the *str-s* gene of the donor. Thus, two new strains were derived, one *tol VIII su*II$^+$ *str-s* and the other *tol VIII su*II$^+$ *str-r*. As shown in Fig. 4, the *tol VIII* phenotype in the *su*II$^+$ *str-r* strain was temperature-dependent, whereas the *tol VIII su*II$^+$ *str-s* strain was sensitive to E1 at both low and high temperatures. This result clearly indicates that the suppression of the *tol VIII* phenotype by suppressor *su*II in a *str-s* strain is complete or almost complete, so that the strain behaves as *tol*$^+$, whereas suppression by *su*II in a *str-r* strain is only partial and the *tol VIII* phenotype is manifested in a "leaky," temperature-dependent way.

Interactions between suppression by the *su*II suppressor and certain *str-r* mutations have been reported in other systems (3, 4, 5, 8, 9, 13). The restriction in the efficiency of suppression of the *tol VIII* amber mutation in a *str-r* strain is specific for the *su*II suppressor. The *su*III gene, introduced by lysogenization with a λ-80 hybrid phage that carries the *su*III$^+$ gene, restored the *tol*$^+$ phenotype in both the *str-r* and the *str-s* strains.

Behavior of strain LA641 in temperature shifts. As with mutant LA639, no large changes in colicin sensitivity were observed when cells treated with colicin E1 were plated at either low or high temperature (Table 4). Cells grown and treated at 30 C were sensitive; cells grown and treated at 41 C were tolerant whether they were plated at 30 or 41 C. Experiments of temperature shift after colicin treatment were also done in broth. They essentially confirmed the observations made in the plating tests: cells grown and treated at the high temperature are not killed when shifted to low temperature.

Again, as with mutant LA639, little change from sensitivity to tolerance or vice versa occurred after a shift in temperature of LA641 cells in the

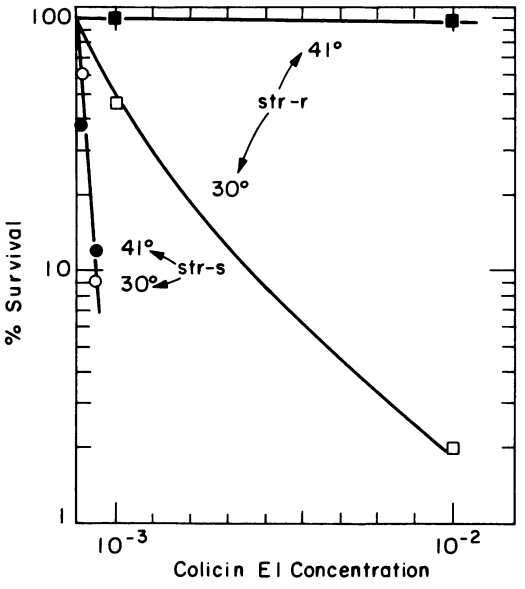

FIG. 4. *Effect of colicin E1 on cells of tol VIII am strains, either str-s or str-r, grown, tested, and plated at 30 or 41 C.*

TABLE 4. *Effects of plating temperature and temperature shifts on the response of strain LA641 to colicin E1*[a]

Sample	Treatment	Relative colicin concn	Per cent survivors	
			Plated at 30 C	Plated at 41 C
I	30 C control	1×	20	23
		10×	7.5	6.5
II	30 → 41 C, 1 hr in chloramphenicol	1×	13	12
		10×	2.8	5.0
III	30 → 41 C, 1 hr of growth	1×	72	70
		10×	52	59
I	41 C control	10×	100	100
II	41 → 30 C, 1 hr in chloramphenicol	1×	89	100
		10×	78	72
III	41 → 30 C, 1 hr of growth	1×	50	50
		10×	26	32

[a] The colicin concentration given as 1× corresponded to a killing multiplicity of about 8 measured on strain LA261. The experimental conditions were like those given in Table 2.

presence of chloramphenicol. Acquisition of sensitivity at 30 C and of tolerance at 41 C took place with growth at the new temperature after a shift.

DISCUSSION

Two types of temperature-dependent tolerant mutants have been analyzed. In one strain, LA 639, the temperature dependence was due to a mutation in the *tol II* gene. In the other strain, LA641, the temperature-dependent phenotype resulted from incomplete suppression of a *tol VIII* amber mutation by the *su*II suppressor in the presence of an *str-r* mutation.

The process of conversion of these mutants from the tolerant to the sensitive condition and vice versa after temperature shifts differs from that observed with other temperature-dependent tolerant mutants previously investigated (7, 11).

With the *tol IV td* mutant, which is tolerant to E2 and E3 at 40 C (11), conversion from the sensitive to the tolerant state took place during the shift from low to high temperature in the absence of protein synthesis; tolerance did not disappear when cells grown at 40 C were incubated at 30 C in the presence of chloramphenicol. This indicates that colicin action requires the integrity of a component, probably a protein, which in the td mutant is inactivated irreversibly at 40 C.

Another type of td mutant studied (7, 11) is tolerant to colicin E2 at low temperature and partly sensitive at 40 C. This mutant too showed a rapid change in response to colicin after temperature shifts, even in the absence of protein synthesis.

With the *tol II td* and *tol VIII td* mutants described in the present paper, the phenotype depends only on the temperature at which the bacteria have grown; no changes in response to colicin occur after temperature shifts in the absence of protein synthesis. This suggests that in these mutants a component necessary for colicin action either is not made or is made in inactive form at the higher temperature. Once made, this component is not activated or inactivated by shifts in temperature alone.

That the primary products of the *tol II* and *tol VIII* genes are protein is made evident by the existence both of temperature-dependent mutants and of amber mutants. Terek Schwarz (*unpublished observations*) has isolated many amber mutants of the *tol II*, *tol III*, and *tol VIII* types. The normal products of these genes may be enzymes needed to make or modify some components of the cell envelope essential for colicin molecules adsorbed to cell receptors to act on their targets, or they may be structural proteins of the cell envelope. If they were structural proteins, the observed temperature dependence could be explained by assuming that in the mutants studied here the synthesis of these proteins is temperature-sensitive, but the proteins, once made at the lower temperature, are heat-stable. Alternatively, these proteins, once made at low temperature, might

determine specific irreversible configurations of the cell surface, leading to colicin sensitivity, even if the proteins themselves were damaged by heating.

The changes in sensitivity following temperature shifts under growth conditions indicate that colicin adsorbed to "tolerant" receptors synthesized at the higher temperature never becomes functional, even after the cells have grown at the lower temperature. The kinetics of appearance of sensitivity after a shift from 41 to 30 C suggest that colicin can act only if adsorbed on receptors located on portions of the cell surface synthesized after the shift. This would occur if the receptors and some critical components controlled by the *tol* genes segregated together during cell growth. Synthesis or positioning of these components on the cell envelope—wall or membrane—might occur in a mosaic pattern, so that receptors made on a "tolerant" portion of the surface would never get connected to normal components and would remain nonfunctional. The rather fast disappearance of sensitivity to colicins E1 and K during growth after a shift to higher temperature suggests that some functional receptors synthesized at low temperature are made inactive or "disconnected" when normal components are not synthesized.

The isolation of different types of tolerant mutants with different patterns of response to colicins is revealing the existence of several intermediate steps between adsorption of colicin to receptors and its action on the target. Whatever the chemical nature of the components involved might be, we can conclude, at least for the *tol II* mutants and probably also for *tol VIII*, that the affected components are constituents of the cell envelope, probably located close to the colicin receptor sites and affecting some early post-adsorption steps in colicin action.

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