Genetics and Physiology of a $tolE$ Mutant of
Escherichia coli K-12 and Phenotypic
Suppression of Its Phenotype by Galactose

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The $tolE$ mutation causes tolerance to colicins E2 and E3 as well as other
effects on the phenotype of Escherichia coli K-12. The lipopolysaccharide of
the mutant shows a reduction in the content of galactose, glucose, and rhamnose.
The phenotype of the mutant, including the composition of the lipopolysaccha-
ride, is suppressed by galactose. The map position is shown by the gene order
$trp$-$purB$-$tolE$-$tolD$-$galKETO.

In a study of ampicillin-resistant mutants of
Escherichia coli K-12, we found a class of
mutants that were changed in the properties of
the outer envelope. These mutants show a
pleiotropic phenotype, and they can be divided
into many subclasses (5). Two of these contain
mutants ($tolD$ and $tolE$) that are tolerant to
colicins E2 and E3. The $tolD$ type has been
described before (3). In this paper we describe
the $tolE$ mutant.

The strains used in this study are listed in
Table 1. The $tolE$ mutant G11e6 differs in many
respects from its parent strain G11al, but the
main features are tolerance to colicins E2 and
E3, sensitivity to phage C21 (but resistance in
the presence of galactose), and that the mutant
is galactose negative when tested on purple base
agar after pregrowth in the presence of glucose
(5) (see Table 4). The $tolE$ mutant was found to
produce uridine 5'-diphosphate galactose-4-
epimerase activity (tested as described in ref. 7).

The $tolE$ mutation is transferred as a late
marker in conjugations with HfrC. Since the
mutant phenotype cannot be directly selected for,
all genetic data are based on linkage to
other genes. Strain G11e6 ($tolE$) was conjugated
with strain MS31 ($galE$). Out of 92 $Trp^+$ recom-
binsants, 6 had obtained the $tolE$ gene, whereas
only one had lost the $galE$ gene. Hence, the gene
$tolE$ cannot be closely linked to $galE$. The gene
is transferred between $trp$ and $galE$. Strain
G11e6 ($tolE$) was conjugated with strain X195.
The gene order was found to be $pyrF$-$trp$-$purB$
$tolE$ (Table 2).

To map the $tolE$ mutation in relation to $tolD$,
the conjugation reported in Table 3 was per-
formed. $Trp^+$ recombinants were selected, and
four different phenotypes were obtained. Two of
these were identical to the parental phenotypes
and one was wild type with respect to $tolD$ and
$tolE$. This suggests that the fourth phenotype
has the genotype $tolD$, $tolE$. Since wild-type
recombinants were obtained, $tolE$ cannot be
allelic to $tolD$. Wild-type recombinants were
much less frequent than the double mutants,
which locates $tolE$ between $trp$ and $tolD$. Thus
the gene order is $trp$-$purB$-$tolE$-$galKETO$.

The locus $tolE$, as well as $tolD$, is genetically

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**Table 1. E. coli K-12 strains used and their relevant
categories**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Parent strain</th>
<th>Origin (reference no.)</th>
<th>Sex</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>G11al</td>
<td>G11</td>
<td>4</td>
<td>HfrC</td>
<td>$ilo$, $metB$, $ampA1$</td>
</tr>
<tr>
<td>G11el</td>
<td>G11al</td>
<td>4</td>
<td>HfrC</td>
<td>$ilo$, $metB$, $ampA1$, $tolD$</td>
</tr>
<tr>
<td>G11e6</td>
<td>G11al</td>
<td>5</td>
<td>HfrC</td>
<td>$ilo$, $metB$, $ampA1$, $tolE$</td>
</tr>
<tr>
<td>MS31</td>
<td>MS3</td>
<td>3</td>
<td>F-</td>
<td>$pyrD$, $trp$, $galE$, $strA$</td>
</tr>
<tr>
<td>RE103</td>
<td>RC711</td>
<td>12</td>
<td>F-</td>
<td>$proA$, $trp$, $his$, $cmlA$, $strA$</td>
</tr>
<tr>
<td>AS2</td>
<td></td>
<td>12</td>
<td>F-</td>
<td>$proA$, $trp$, $tolD$, $strA$</td>
</tr>
<tr>
<td>X195</td>
<td>?</td>
<td>11</td>
<td>F-</td>
<td>$his$, $met$, $pro$, $purB$, $pyrF$, $trp$, $tyr$, $strA$</td>
</tr>
</tbody>
</table>

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* All strains carry prophage $\lambda$.

* Abbreviations: $amp$, ampicillin; $cml$, chloramphenicol; $gal$, galactose; $his$, histidine; $ilo$, isoleucine-valine; $met$, methionine; $pro$, proline; $pur$, purine; $pyr$, pyrimidine; $str$, streptomycin; $tol$, tolerance to colicins E2 and E3; $trp$, tryptophan; $tyr$, tyrosine. The capital letters after some of the symbols refer to the genetic map of Taylor and Trotter (14).

* A His$^+$/Str$^+$-recombinant from a cross between G11e6 ($tolD$) and RE103 (cmlA). The strain AS2 is Cml$^+$ and contains the gene $tolD$. 

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NOTES

**Table 2. Genotypes of recombinants from a cross between G11e6 and X195**

<table>
<thead>
<tr>
<th>Donor gene selected</th>
<th>Genotype of recombinant</th>
<th>% of recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pyrF</td>
<td>trp</td>
</tr>
<tr>
<td>pyrF+</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>trp+</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>purB+</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>1</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Conjugations were performed as described before (5). In each case, 115 recombinants were tested. Streptomycin was used as counter selection. The genotypes of the parents are as follows:

In the body of the table, the donor allele is always labelled "1" and the recipient "0."

**Table 3. Results of conjugations between the donor strain G11e6 (tolE) and the recipient strain AS2 (tolD)**

<table>
<thead>
<tr>
<th>Recombinant class</th>
<th>Phenotype</th>
<th>% of Trp+ recombinants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistance to:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholate (mg/ml)</td>
<td>Chlor-amphenicol (μg/ml)</td>
<td>Growth on galactose</td>
</tr>
<tr>
<td>Recipient type</td>
<td>&lt;10</td>
<td>5</td>
</tr>
<tr>
<td>Donor type</td>
<td>&lt;10</td>
<td>2</td>
</tr>
<tr>
<td>Wild type</td>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>Double recombinant</td>
<td>50</td>
<td>5</td>
</tr>
</tbody>
</table>

*Conjugations were performed as described before (5). Trp+-recombinants (175) were tested. Streptomycin was used as counter selection.

*Tested on purple base agar containing galactose.

distinct from other loci, giving tolerance to colicins of the E group (3).

Sensitivity to phage C21 is due to a reduction of the galactose content of the lipopolysaccharide (13). The lipopolysaccharide of strain G11e6 (tolE) contained a reduced amount of galactose, glucose, and rhamnose (Table 4). Sensitivity to phage C21 was lost when the cells were pregrown in the presence of galactose. This loss was accompanied by a restoration of the carbohydrate composition of the lipopolysaccharide. Furthermore, all other phenotypic properties were suppressed by galactose. This sugar had no effect on the tolD mutant G11e1.

Mutations in the galU, lpsA, or lpsB genes result in a reduction of the glucose, galactose, and rhamnose content of the lipopolysaccharide (5). However, none of these three mutations affects the response to colicins of the E group. To our knowledge, it has never been reported that colicin tolerance can be due to changes in the outer membrane. This strengthens the view that colicin tolerance can be a nonspecific consequence of changes in the cell envelope and due to impaired diffusion of colicin molecules through the cell wall into their targets (3). This is even more likely after the demonstration that colicin E3 acts as an enzyme on the ribosomes.
**Table 4. Effect of galactose on the carbohydrate composition of the lipopolysaccharide (LPS) and on some phenotypic properties of tolE and tolD mutants**

<table>
<thead>
<tr>
<th>Strain and carbon source added to LB medium</th>
<th>Lipopolysaccharide (µg/mg of LPS)*</th>
<th>Survival in presence of colicin E2a</th>
<th>Uptake of gentian violet (%)b</th>
<th>Resistance to sodium cholate (µg/ml)c</th>
<th>Response to phage C21d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rhamnose</td>
<td>Galactose</td>
<td>Glucose</td>
<td>Heptose</td>
<td>Hexosamine</td>
</tr>
<tr>
<td>G11a1 (wild type)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>12</td>
<td>32</td>
<td>52</td>
<td>108</td>
<td>59</td>
</tr>
<tr>
<td>Galactose</td>
<td>12</td>
<td>30</td>
<td>53</td>
<td>97</td>
<td>65</td>
</tr>
<tr>
<td>G11e6 (tolE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>6</td>
<td>16</td>
<td>28</td>
<td>76</td>
<td>70</td>
</tr>
<tr>
<td>Galactose</td>
<td>11</td>
<td>32</td>
<td>69</td>
<td>105</td>
<td>59</td>
</tr>
<tr>
<td>G11e1 (tolD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>19</td>
<td>27</td>
<td>62</td>
<td>95</td>
<td>56</td>
</tr>
<tr>
<td>Galactose</td>
<td>8</td>
<td>24</td>
<td>58</td>
<td>85</td>
<td>58</td>
</tr>
</tbody>
</table>

* Lipopolysaccharide was determined as described before (5). Hexosamine was determined as described by Ghuysen et al. (6).

* The bacteria were grown in LB medium (1) or in LB medium in which glucose was replaced by galactose. In both cases the media were supplemented with medium E (15) and glucose, 0.2% (wt/vol). At a cell density of 4 \times 10^8 cells per ml, colicin E2 was added at a multiplicity of 8 killing units per bacterium. After 10 min at 37 C, samples were taken, diluted 100 times in ice-cold LB, and viable count was determined. Survival was defined as the ratio between viable count of the sample and that of a parallel sample to which no colicin was added. Colicins were prepared by the method of Nagel de Zwaag and Luria (8).

* The bacteria were grown as in footnote b. At a cell density of 2 \times 10^8 cells per ml, they were chilled on ice, centrifuged, and resuspended in glucose minimal medium (15) containing 0.2% glucose and 10 µg of gentian violet. After 10 min on a rotary shaker at 37 C, the bacteria were removed by centrifugation, and the remaining gentian violet was determined at 590 nm (10).

* Overnight cultures in minimal medium E (15) with either glucose or galactose as carbon sources were incubated in fresh media, and optical density was followed until 100 Klett units. The cells were diluted and 100 to 200 cells were spread on plates containing different concentrations (every 2.5 mg/ml from 0–55 mg/ml) of sodium cholate (single cell test) and the same medium as in the pregrowth medium. Resistance is given as the highest concentration at which the plates plated gave rise to the same size of colonies as on the control plates without cholate (9).

Furthermore, survival of the tolE strain in presence of colicin E2 was far from complete (Table 4).

The gene product of the tolE gene is not known. However, one tempting possibility is that tolE is involved in the regulation of the galU and pgm genes. These genes are involved with the biosynthesis of galactose uridine 5'-diphosphate and uridine 5'-diphosphate galactose, which are substrates for the biosynthesis of lipopolysaccharide. It is suggested that galactose acts by derepressing the galU and pgm genes to normal activity.

In conclusion, we have discovered a previously undescribed gene, tolE, which genetically and phenotypically is distinct from other genes causing tolerance to colicins of the E group (4). The effect of the mutation is phenotypically suppressed by galactose, which indicates that the tolE mutant is defective in the regulation of some reaction(s) that is (are) involved both in galactose catabolism and in the biosynthesis of lipopolysaccharide.

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