Mutation Affecting Plasmolysis in
Escherichia coli

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A temperature-sensitive mutant of Escherichia coli is described that at the restrictive
temperature has lost the ability to plasmolyze. The mutation is located near pyrF.

By using N-methyl-N'-nitro-N-nitrosoguanidine, 51 heat-sensitive mutants of Escherichia
coli K-12 were isolated that stopped growth when brought to 42 C, but grew normally at 28 C
(A. Rösch, unpublished data). They were registered as TKG; to the thermostensitive
mutation present the provisional symbol gts (for growth temperature-sensitive) was applied.
Because some of the TKG strains might be potential membrane mutants, their ability to
plasmolyze in 20% sucrose was investigated, at both 28 and at 42 C. In TKG 49 (Fig. 1A) plas-
molysis appeared to be absent after 3 hr of incubation at 42 C (Fig. 1B). When plated at
28 C all cells were still viable. Cells of TKG 49 grown at 28 C show normal plasmolysis.
Because the spontaneous heat-tolerant revertant TKG 49 gts+ (Fig. 1A) simultaneously re-
covered the ability to plasmolyze at 42 C (Fig. 1B), it is concluded that the gts-49 mutation is
responsible for both the effect on growth and the effect on plasmolysis.

Plasmolysis is related to the semipermeability of the cytoplasmic membrane. If the gts
mutation only affected permeability to sucrose, it should not cause bacteriostasis in sucrose-
free medium at 42 C. Accordingly, the possibility was considered that the mutation changed a
general structure in the membrane. Some membrane mutants have been reported to
differ from the wild type in their growth response to the presence in the medium of
several dyes, ethylenediaminetetraacetate (EDTA), or sodium deoxycholate (1-4, 6). In
TKG 49 such effects could not be demonstrated for methylene blue, eosin, acridine
orange, and EDTA in broth-agar plates. Deoxycholic acid at concentrations of 0.1 to 0.4%
prevented the growth of TKG 49 at 28 C, although only in the presence of at least 1% NaCl.
However, deoxycholate sensitivity must be the result of a second mutation, independently
induced together with gts-49, because it is also found in the spontaneous gts+ revertant.

In some heat-sensitive membrane mutants, 1 to 2% NaCl can restore growth at the restric-
tive temperature (5). TKG 49 was tested and compared with its wild type and to the sponta-
neous gts+ revertant. It was found, indeed, that concentrations of NaCl as low as 0.5%
prevented the lethal effect of 42 C.

The plasmolysis mutation can be mapped by virtue of the inability to form colonies at 42 C
it confers upon the cell. The frequency of recombinants for gts-49 in conjugation with HfrH
was about the same as for pyrF. Therefore, cotransduction with pyrF was expected and
found, as well as with trp (Table 1). This cotransduction was demonstrated for gts+ as well
as for gts-, indicating that the locus found does not involve a suppressor gene. From the
cotransduction frequencies a gene order trp-pyrF-gts is deduced. As a further test, a three-
point transductional cross was made (Table 1). Of the recombinant types possible, the class
trp+ pyrF- gts- was the rarest; in fact they were not found at all among trp+ transduc-
tants. With the interpretation that for formation of this type of recombinant at least four
crossing-over events are required, only the gene order trp-pyrF-gts is compatible.

Membrane genes in this region have not yet been published.

I thank A. Rösch for providing the temperature-sensitive strains, Conrad Woldringh for suggesting plasmolysis as a
screening tool and for many a discussion, and Christien Vos
for expert technical assistance.

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Table 1. Cotransduction of gts with pyrF and trp

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<th>Donor</th>
<th>Recipient</th>
<th>Selected marker</th>
<th>No. of transductants</th>
<th>Unselected markers</th>
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<td>TKG 49 pyrF⁻ gts⁻</td>
<td>gts⁺</td>
<td>30</td>
<td>pyrF⁺ 26 (87%)</td>
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<tr>
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<td></td>
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<td>trp⁺</td>
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<td>gts⁻ 10 (10%)</td>
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<td>GIA 55 trp⁻ pyrF⁻ gts⁺</td>
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<td>gts⁻</td>
<td>10</td>
<td>pyrF⁻ gts⁻ 0</td>
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</tbody>
</table>

*Heat-tolerant (gts⁺) transductants were selected on minimal medium at 42 C after 4 hr at 28 C to allow for recombinant expression. For transduction ϕ 363 was used. Full genotypes of the bacterial strains are: KMBL 171 HfrH met; TKG 49 F⁻ thr leu his ilvA arg thi pyrF thyA lac tonA tax gts-49; GIA 55 trp pyrF.

Abbreviation: gts = growth temperature-sensitive.

LITERATURE CITED


Fig. 1. (A) Growth of TKG 49 (●) and TKG 49 gts⁺ (○) in aerated broth after a shift to 42 C. (B) Percentage of cells plasmolyzing in 20% sucrose in TKG 49 (●) and TKG 49 gts⁺ (○) following various periods of growth at 42 C. At the indicated time 5-ml samples were centrifuged, concentrated in 0.5 ml of 40% sucrose, and examined by phase-contrast microscopy.