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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*Escherichia coli* K-12 were isolated that stopped growth when brought to 42 C, but grew normally at 28 C (A. Rörsch, *unpublished data*). They were registered as TKG; to the thermosensitive 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) plasmolysis 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 recovered 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 restrictive temperature (5). TKG 49 was tested and compared with its wild type and to the spontaneous *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*$^+$ transductants. 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.

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TABLE 1. *Cotransduction of gts with pyrF and trp*[a]

Donor	Recipient	Selected marker	No. of transductants tested	Unselected marker(s)	
				Gene	No.
KMBL 171 *pyr*$^+$ *gts*$^+$	TKG 49 *pyrF*$^-$ *gts*$^-$	*gts*$^+$	30	*pyrF*$^+$	26 (87%)
		pyrF$^+$	66	*gts*$^+$	48 (73%)
TKG 49 *trp*$^+$ *pyr*$^+$ *gts*$^-$	GIA 55 *trp*$^-$ *pyrF*$^-$ *gts*$^+$	*trp*$^+$	100	*pyrF*$^+$	32 (32%)
				gts$^-$	10 (10%)
			100	*pyrF*$^-$ *gts*$^+$	68
				pyrF$^+$ *gts*$^+$	22
				pyrF$^+$ *gts*$^-$	10
				pyrF$^-$ *gts*$^-$	0

[a] 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 tsx gts-49;* GIA 55 *trp pyrF.* Abbreviation: *gts* = growth temperature-sensitive.

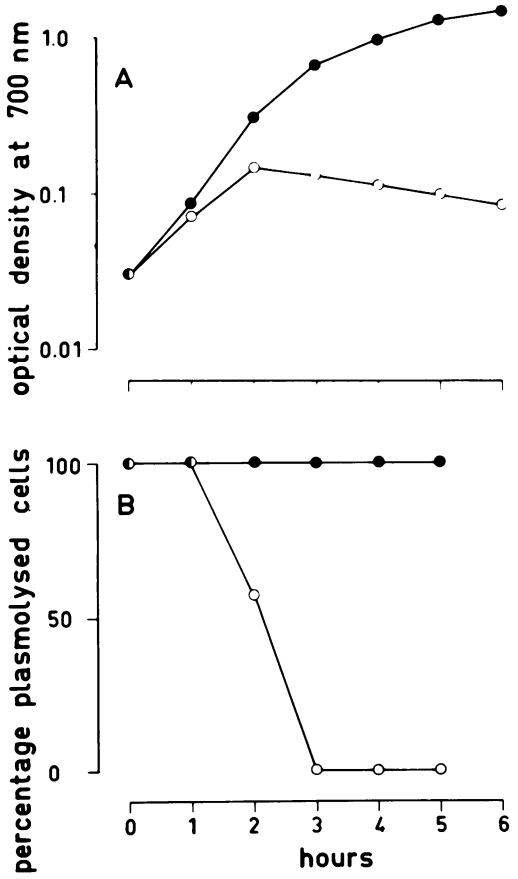

FIG. 1. *(A) Growth of TKG 49 (○) and TKG 49 gts*$^+$ *(●) in aerated broth after a shift to 42 C. (B) Percentage of cells plasmolyzing in 20% sucrose in TKG 49 (○) and TKG 49 gts*$^+$ *(●) following various periods of growth at 42 C. At the indicated time 5-ml samples were centrifuged, concentrated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

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