

Transfectability of Rough Strains of *Salmonella typhimurium*

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Cells of rough (but not smooth) strains of *Salmonella typhimurium* become competent for transfection by phage P22 deoxyribonucleic acid after treatment with 0.1 M CaCl₂. The yield of infectious centers is about 10⁻⁸ per genome equivalent of deoxyribonucleic acid. However, different sorts of rough strains vary in their ability to become competent in a fashion that can be correlated with the level of the genetic block in cell wall lipopolysaccharide synthesis. The most amenable strains are blocked by defects in the addition of galactose units I and II of the lipopolysaccharide by the inability to synthesize uridine 5'-diphosphate-galactose (*galE* point mutants and *gal* deletion mutants). Strains blocked only in the addition of galactose I, glucose I, or heptose II have low levels of transfectability, whereas strains with either more complete or more deficient lipopolysaccharide core are not competent for transfection. When normal lipopolysaccharide synthesis is restored either genetically or by furnishing exogenous galactose (*galE* point mutants that can still use it), the cells are no longer competent for transfection.

Mandel and Higa (10), Cohen et al. (3), and Oishi and Cosloy (11) have described the transfectability of *Escherichia coli* cells treated with CaCl₂. We report here the induction of competence to transfection by phage P22 deoxyribonucleic acid (DNA) as a result of exposure of *Salmonella typhimurium* cells to CaCl₂. Operationally, two classes of *S. typhimurium* strains can be defined (15): smooth strains able to adsorb P22 phage and rough strains unable to adsorb P22 phage. Wilkinson et al. (14) described various classes of rough mutants of *S. typhimurium*, all lacking the O antigenic specificity of their smooth parent as a consequence of alterations in the polysaccharide of the somatic lipopolysaccharide (LPS).

After a treatment with 0.1 M CaCl₂, certain rough strains of *S. typhimurium*, but not smooth ones, can be transfected by the DNA extracted from the smooth-specific bacteriophage P22, a temperature-sensitive C2 mutant (13). An overnight inoculum is diluted 200-fold into L (7) or Penassay (Difco antibiotic medium 3) broth and grown at 37 C with aeration to an optical density at 600 nm of 0.6 per centimeter (ca. 2 × 10⁸ cells/ml). The cells are chilled, recovered by centrifugation at 12,000 × *g* at 4 C, and suspended to original volume in 0.1 M

MgCl₂. The suspension is spun-down and the cells are resuspended in 0.5 of the original volume of 0.1 M CaCl₂ and kept at 0 C for 20 min. Finally, the cells are pelleted and resuspended in 1/10 of the original volume of 0.1 M CaCl₂ at 0 C ("treated" cells). DNA was prepared by phenol extraction of CsCl-purified phage particles (13). No obvious heterogeneity was noted by electron microscopy; less than one break per five molecules was detected by sedimentation in alkaline sucrose gradients. The transfection was performed by mixing native P22 DNA, diluted in 0.1 ml of SSC (0.15 M NaCl plus 0.015 M sodium citrate), with 0.2 to 0.4 ml of "treated" cells. A preincubation of 5 to 60 min at 0 C followed by a thermal treatment, 2 min at 42 C or 5 min at 37 C (0.3 to 0.5 ml in a 10 by 100 nm tube was transferred to 42 or 37 C water bath and left there for 2 or 5 min, respectively), was required to bring about optimal induction of competence for transfection. It was found that no significant difference was observed by varying the length of the incubation period at 0 C or that of the thermal treatment.

Because these rough mutants do not adsorb P22 phage, the appearance of plaques requires the presence of smooth cells as indicators. At the end of the thermal treatment, 2 × 10⁸ to 5 × 10⁸ stationary phase smooth indicator cells (*proC90* [5] or SL1027 [12]) were added to the treatment mixture, 2.5 ml of soft agar was

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added, and the suspension was plated on tryptone agar. The plaques were counted after overnight incubation at 37 C.

Omission of the thermal treatment caused a 30-fold decrease in the number of plaques. If the thermal treatment was at 22 C, the plaques produced were less than half of those obtained at 42 C.

The DNA concentration dependence of the transfection process is reported in Fig. 1. Plaques are evidently increasing at DNA concentrations higher than 0.1 $\mu\text{g}/\text{plate}$. A plateau appears to be reached at a concentration of approximately 1 $\mu\text{g}/\text{plate}$, but its level varied from one batch of cells to another. If the CaCl_2 treatment was omitted, no plaques were detected. In the DNA range 0.1 to 1.0 $\mu\text{g}/\text{plate}$, the efficiency of transfection equals 5×10^{-8} plaques per genome equivalent. Recently, a high efficiency of transfection of spheroplasts

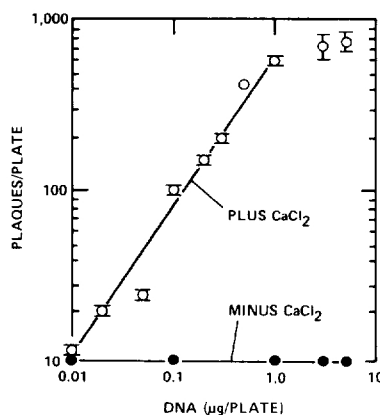


FIG. 1. Effect of DNA concentration on CaCl_2 -dependent transfection. Cells of *S. typhimurium* TA1659 were grown, harvested, and treated as reported in the text. In the control, the CaCl_2 solution was replaced by *L* broth.

TABLE 1. Comparison of the transfectability of CaCl_2 -“treated” cells

Strain no.	Description	Inferred LPS character ^a	No. of plaques per 10^8 cells at 1 μg of DNA
1. TA1659 ^b	LT2 <i>chl-1013</i> (Δgal , <i>bio</i> , <i>uvrB</i>)	Rc	700-3,000
2. SL1694	TA1659 carrying F'8- <i>gal</i> ⁺	+	<1
3. SL1694 Gal ⁻	Gal ⁻ segregant of SL1694	Rc	300-2,000
4. SL1657 ^c	LT2 <i>galE496</i>	Rc	100-400
5. TA1701	LT2 <i>hisC3076</i> , <i>chl-1038</i> (Δgal , <i>bio</i> , <i>uvrB</i> , <i>aroG</i> , <i>nic</i>)	Rc	100-200
6. TA 1701-H ⁺	His ⁺ spontaneous revertant of TA1701	Rc	100-200
7. TA1657	LT2 <i>chl-1011</i> (Δgal , <i>bio</i> , <i>uvrB</i>)	Rc	100-400
8. TA1662	LT2 <i>chl-1016</i> (Δgal , <i>bio</i> , <i>uvrB</i>)	Rc	90-200
9. SL1181 ^c	<i>rfaF511</i>	Rd ₁	10-100
10. EL199	His mutant of SL4213 (2) <i>galE496</i>	Rc	50-300
11. <i>his-519</i>	LT2 deletion of <i>his</i> and <i>rfb</i> clusters	Ra	5
12. <i>his-520</i>	LT2 deletion of <i>his</i> and <i>rfb</i> clusters	Ra	5
13. TA1674	LT2 <i>chl-1028</i> (Δgal , <i>bio</i> , <i>uvrB</i> , <i>nic</i> , <i>dhb</i> , <i>aroG</i>)	Rc	200
14. SL1747	LT2 <i>metE47</i> , <i>galE161</i>	Rc	40
15. SL1752	SL1747 carrying F'8- <i>gal</i> ⁺	+	<1
16. <i>galE503</i>	LT2-M1 <i>galE503</i>	Rc	150-300
17. SL1717	LT2 <i>galE503</i> carrying F'8- <i>gal</i> ⁺	+	<1
18. SL1670	Q1 <i>ndx-101</i>	+	<1
19. SL3613	LT7 <i>proAB47</i> <i>purE66</i>	+	<1
20. SL3759	LT2/7 <i>argE116</i>	+	<1
21. SL1746	LT2 <i>metE47</i> <i>galE160</i>	Rc	590
22. SL1751	SL1746 carrying F'8- <i>gal</i> ⁺	+	<1
23. TA1656	LT2 <i>chl-1010</i> (Δgal , <i>bio</i> , <i>uvrB</i> , <i>dhb</i> , <i>aroG</i>)	Rc	250
24. SL1102 ^c	<i>rfaE543</i>	Re	<1
25. SL4507 ^c	<i>galU455</i>	Rd ₂	<1
26. SL1032 ^c	<i>rfaG471</i>	Rd ₂	30-100
27. SL1060 ^c	<i>rfaH487</i>		160

^a For definitions of LPS chemotypes, see reference 9 and Table 2.

^b TA strains were obtained from Bruce Ames through B.A.D. Stocker.

^c SL strains were obtained from the collection of B.A.D. Stocker, and EL199 was obtained from Esther Lederberg. For other genetic markers of SL1181 and SL1102, see reference 14; for SL1657, SL4507, SL1032, and SL1060, see reference 12; for EL199, see reference 4.

has been reported for recombination deficient mutants (2).

The above procedure has little or no effect on the viability of the cells, but it is apparently able to induce competence only in a limited fraction of the "treated" cells, not higher than 2×10^{-6} . Table 1 compares the transfectability of CaCl_2 -"treated" cells from a number of strains that carry different mutations affecting their LPS character. Thus, strains 1, 3, 4, 5, 6, 7, 8, 10, 13, 14, 16, 21, and 23 (all Gal^- and thought to be deficient of uridine 5'-diphosphate-galactose epimerase) gave a positive response to the CaCl_2 treatment. Strains 11 and 12, phenotypi-

cally rough, i.e., lacking the repeating polysaccharide unit attached to the LPS core, gave few plaques. Most of the other tested strains, all Gal^+ , gave no positive response, except strains 9, 26, and 27. Compare strains no. 1 (TA1659), containing a deletion through the galactose operon, which yielded the highest transfectability, and no. 2 (SL1694), derived from the former by introduction of an F'8 gal^+ episome. Strain SL1694 is smooth and is no longer transfectable in contrast to its Gal^- segregants, which are phenotypically rough and transfectable (strain no. 3).

Rough mutants EL199 and *galE503*, both

TABLE 2. Structure of somatic lipopolysaccharide and its relation to transfectability

LPS ^a component present	LPS ^a character	Enzyme defect	Can ^b synthesize galactose	Can ^b assimilate exogenous galactose	No. of plaques per 10^8 cells at $1 \mu\text{g}$ of DNA	Strain no.
Complete core and O chains	-	None	+	+	<1	SL1694
						SL1752
						SL1670
						SL3613
						SL3759
						SL1751
KDO ^c and lipid only	Re	<i>rfaE</i> : defect in heptose addition	+	+	<1	SL1102
						SL1181
KDO, lipid, and heptose I	Rd ₁	<i>rfaF</i> : defect in addition of heptose II	+	+	10-100	SL1181
KDO, lipid, and heptose I and II	Rd ₂	<i>galU</i> : UDP-glucose pyrophosphorylase deficient	-	-	<1	SL4507
KDO, lipid, and heptose I and II	Rd ₂	<i>rfaG</i> : LPS glucosyl transferase deficient	+	+	30-100	SL1032
KDO, lipid, heptose, glucose I, and galactose II	Rd ₂	<i>rfaH</i> : LPS galactosyl transferase deficient	+	+	160	SL1060
KDO, lipid, heptose, and glucose I	Rc	<i>galE</i> : lack UDP-galactose epimerase, <i>gal</i> point mutation	-	+	100-400	SL1657
					50-300	EL199
					150-300	<i>galE503</i>
					40	SL1747
					600	SL1746
KDO, lipid, heptose, and glucose I	Rc	<i>galΔ</i> : lack UDP-galactose epimerase, galactose kinase, and galactose phosphate uridyl transferase, by a deletion covering the entire <i>gal</i> operon	-	-	700-3,000	TA1659
					300-2,000	SL1694 gal^-
					100-200	TA1701
					90-200	TA1662
					200	TA1674
					250	TA1656
Complete core	Ra	<i>rfb</i> : <i>his</i> ⁻ <i>rfb</i> deletion	+	-	5	his-519
					5	his-520

^a See Fig. 2.

^b - + category is rough, is deficient only in the synthesis of UDP-galactose from UDP-glucose, and becomes smooth when grown with galactose; - - category is unable to make UDP-galactose from exogenous galactose, is rough, and does not become smooth when grown with galactose.

^c 2-keto-3-deoxy-octonate.

carrying different *galE* point mutations, gave 160 and 300 plaques, respectively, in the standard assay. When they were grown on media supplemented with 0.5% galactose, they were no longer competent for transfection. This accords with other observations that these mutants become smooth when grown in the presence of galactose (12, 14). Galactose exerted this effect during growth before CaCl₂ treatment; exposure of the cells to galactose in the agar did not affect the results.

TA1659, a *gal* deletion mutant lacking UDP-galactose epimerase, galactose kinase, and galactose-phosphate uridyl transferase, was still transfectable after growth in the presence of galactose to about half the control experiment. These findings are consistent with those reported by Wilkinson et al. (14), who showed that other mutants deficient in the same enzymes remained phenotypically rough when grown in the presence of galactose. Table 2 summarizes the structure of the LPS (outlined in Fig. 2) and the specific galactose gene involved and its relationship to transfectability.

Figure 2, adapted from Ornellas and Stocker (12) and Lindberg and Svensson (8), shows the order of addition of subunits in the biosynthesis of the LPS and the genes mediating each step. We have shown that the highest level of transfectability corresponds to the LPS core of type Rc, lacking both galactose units I and II produced by *galE* mutants and unable to synthesize UDP galactose and by *galΔ* mutants (in

Fig. 2 included in the *galE* box). There is a lower level of transfectability when synthesis of the LPS core is blocked at the level of addition of galactose I only (class *rfaH*), glucose I (*rfaG*), or heptose II (*rfaF*), and there is no transfectability either with a more deficient or with a more complete core.

Strain SL4507, *galU*, deficient in UDP-glucose pyrophosphorylase, was not competent for transfection, even though its LPS is expected to be similar to that of SL1032, *rfaG*, which is deficient in transferase for addition of glucose I unit (Fig. 2). The enzyme defect of SL4507, when tested in vitro, also appears incomplete.

In conclusion, our data show that some rough strains of *S. typhimurium* may be transfectable by P22 DNA, but that smooth strains (either genetically smooth or made phenotypically smooth by growth with galactose) are not competent. Defects in synthesis of the LPS core of *E. coli* or *S. typhimurium* cause increased sensitivity to various antibiotics, mutagenic chemicals, and bile salts by causing increased permeability of the outer membrane (1; R. J. Roantree, T. Kuo, D. G. MacPhee, and B. A. D. Stocker, *Bacteriol. Proc.*, p. 79, 1969). The deepest defect, failure to add heptose units, caused the greatest change. By contrast, maximum transfectability was found in mutants making LPS cores with an intermediate degree of incompleteness, i.e., failure to add the galactose units of the core. The *Salmonella* classes

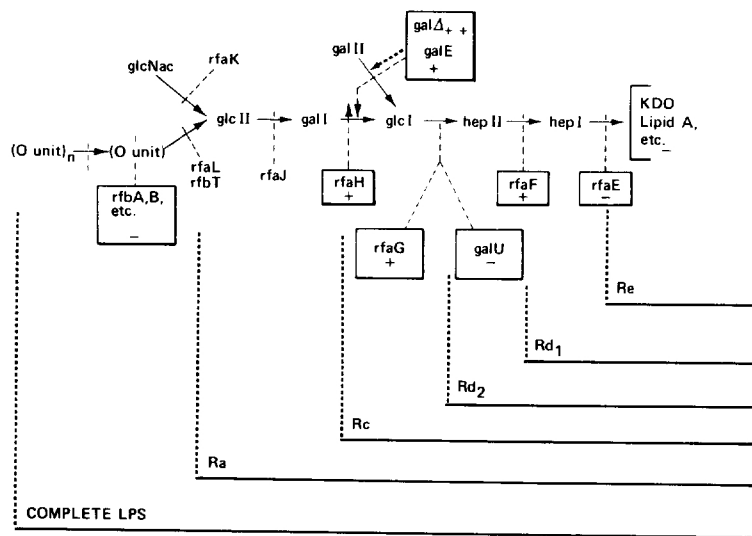


FIG. 2. Level of transfectability of different rough mutants of *S. typhimurium*. Symbols: -, +, and ++, no, poor, and higher competence for transfection, respectively.

competent for transfection correspond almost exactly to those able to adsorb phages C21 and P1 (12).

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