

Characterization of Plasmids in a Sucrose-Fermenting Strain of *Escherichia coli*

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A multiply drug-resistant strain of *Escherichia coli* isolated from a patient in Bangladesh was shown to carry four types of plasmids based on size differences. One type carries a gene or genes for sucrose fermentation.

Genetic determinants for sucrose fermentation in several strains of *Salmonella* have been shown to be located on plasmids (3, 7). We examined a sucrose-fermenting (Scr⁺) strain of *Escherichia coli* for a possible plasmid location of the *scr*⁺ gene(s). The strain we used, *E. coli* 15833-1, was isolated from a patient with diarrhea in Dacca, Bangladesh, and was characterized by Evans and Evans as Scr⁺ (2). It does not produce enterotoxin (Ent⁻). These authors found that most of their Ent⁺ isolates were Scr⁻ and most of the Scr⁺ isolates were Ent⁻. Since the genes for enterotoxin production are always located on a plasmid, a possible explanation for their findings is that the gene(s) for sucrose utilization is also located on a plasmid and that there is mutual exclusion between Ent plasmids and the postulated Scr plasmid.

The media, strains (except strain 15833-1), growth conditions, mating procedures, curing by acridine orange, and tests for sensitivity to male- and female-specific phages and to antibiotics have been described previously (5, 6). Presence or absence of the Scr⁺ character was determined on MacConkey Base agar plates with 1% sucrose or on minimal medium A agar plates with 2% sucrose as the sole carbon source. Transformation experiments were carried out by the method of Cohen and Chang (1), by using as recipient a derivative of strain 15833-1 (see below) that had been cured of all but one cryptic plasmid. The methods for isolation of plasmid deoxyribonucleic acid (DNA) and characterization by electron microscopy have also been described (4).

From a 500-ml culture of strain 15833-1 in exponential phase, DNA was extracted by our usual procedure (4) and centrifuged to equilibrium in a cesium chloride-ethidium bromide density gradient. A "satellite" component rep-

resenting about 5% of the total DNA was seen at a position of higher density than the bulk (chromosomal and open circular) DNA. Such a band is characteristic of double-stranded, covalently closed circular DNA. The DNA of this band was examined with an electron microscope after nicking with X rays. Four sizes of open circular molecules were distinguished with contour lengths of 25.4, 16.5, 2.2, and 1.4 μ m (Table 1).

To determine which, if any, of the four plasmids is responsible for sucrose utilization, strain 15833-1, which besides being Scr⁺ is also resistant to ampicillin, tetracycline, chloramphenicol, streptomycin, and sulfonamides, was grown in the presence of 50 μ g of acridine orange per ml and tested for sucrose fermentation. Five Scr⁻ colonies were isolated, three of which had also become sensitive to all the drugs (R⁻), whereas the other two retained complete resistance (R⁺). The types of plasmids found in the partially cured (Scr⁻ R⁺) and completely cured (Scr⁻ R⁻) strains are shown in Table 1. The results indicate that the 25.4- μ m plasmid is associated with the Scr⁺ phenotype and that the two small plasmids (2.2 and 1.4 μ m) are associated with drug resistance. The 16.5- μ m plasmid, which was not removed after

TABLE 1. Plasmids present in strain 15833-1 and its derivatives

Source of plasmid DNA	Size of plasmid molecules (μ m)			
	I	II	III	IV
<i>E. coli</i> 15833-1 (Scr ⁺ R ⁺)	25.4 (16) ^a	16.5 (27)	2.2 (42)	1.4 (42)
<i>E. coli</i> 15833-1 (Scr ⁻ R ⁺)	Absent	17.0 (13)	2.3 (19)	1.4 (18)
<i>E. coli</i> 15833-1 (Scr ⁻ R ⁻)	Absent	17.0 (35)	Absent	Absent
<i>E. coli</i> 15833-1 (Scr ⁺ trans-formant, R ⁻)	25.6 (50)	17.1 (50)	Absent	Absent

^a The numbers in parentheses indicate the number of molecules measured.

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growth in the presence of acridine orange, appears to be a cryptic plasmid. To confirm the association of the Scr^+ phenotype with the 25.4 μm plasmid, transformation with plasmid DNA of strain 15833-1 was carried out by using a cured $Scr^- R^-$ derivative of strain 15833-1 as recipient. With selection for Scr^+ the frequency of transformants was 2.4×10^{-6} per recipient cell. The two types of plasmids present in the transformants (25.6 and 17.1 μm) were as expected (Table 1).

Some tests were carried out with a cured $Scr^- R^-$ strain and a $Scr^+ R^-$ transformant for genetic traits known to be plasmid controlled. No conjugal transfer of the Scr^+ character was found. However, transfer inhibition was observed when a *finO traO⁺* derepressed R factor, R1*drd-19*, was introduced into either the $Scr^+ R^-$ or the $Scr^- R^-$ strain. No such inhibition was observed with another derepressed R factor, R386, which is *finO⁺ traO^c*. The conclusion drawn from these findings is that the cryptic 16.5- μm plasmid present in both recipient strains produces a repressor of the *tra* operon of these R factors. No instability among the plasmid combinations was observed, as tested by biological or physical methods. It can therefore be concluded that the two plasmids in the $Scr^+ R^-$ strain do not belong to incompatibility group FII (R1*drd-19*) or FI (R386). Strain 15833-1 and all its derivatives studied here are resistant to male-specific phage R17 and sensitive to female-specific phage ϕ II.

Our studies thus show that the genes for sucrose fermentation of *E. coli* strain 15833-1 are located on a plasmid of size 25.4 μm . A Scr^+ plasmid of similar size was described in *Salmonella* (7), but in contrast to our plasmid, the *Salmonella* plasmid was transmissible by conjugation, and its presence promoted the growth of phage R17. Although we did not find conjugal transfer, it is possible that our Scr^+ plasmid is a conjugative one and that our failure to demonstrate conjugal transfer may have been due to transfer inhibition by the 16.5- μm cryptic plas-

mid. We attempted to transfer the Scr^+ plasmid by transformation to other K-12 strains that do not carry the 16.4- μm plasmid or any other recognizable plasmid, but we were unable to do so. Thus far we cannot say whether or not the Scr^+ plasmid is self-transmissible by conjugation. Furthermore, we have not been able to transfer an Ent plasmid into a strain carrying the Scr^+ plasmid or determine a possible mutual exclusion between the Scr^+ plasmid and Ent plasmids.

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LITERATURE CITED

1. Cohen, S., and A. Chang. 1973. Recircularization and autonomous replication of a sheared R-factor DNA segment in *E. coli* transformants. Proc. Natl. Acad. Sci. U.S.A. 70:1293-1297.
2. Evans, D. J., and D. G. Evans. 1973. Three characteristics associated with enterotoxigenic *Escherichia coli* isolated from man. Infect. Immun. 8:322-328.
3. Minor, L. L., C. Coynault, R. Rohde, B. Rowe, and S. Aleksic. 1973. Localisation plasmidique du determinant genetique du caractere atypique "saccharose +" des salmonella. Ann. Microbiol. (Inst. Pasteur) 124B:295-306.
4. Palchaudhuri, S., E. Bell, and M. R. J. Salton. 1975. Electron microscopy of plasmid deoxyribonucleic acid from *Neisseria gonorrhoeae*. Infect. Immun. 11:1141-1146.
5. Palchaudhuri, S., W. K. Maas, and E. Ohtsubo. 1976. Fusion of two F-prime factors in *E. coli* studied by electron microscope heteroduplex analysis. Mol. Gen. Genet. 146:215-231.
6. Santos, D., S. Palchaudhuri, and W. K. Maas. 1975. Genetic and physical characteristics of an enterotoxin plasmid. J. Bacteriol. 124:1240-1247.
7. Wohlhieter, J. A., J. Lazere, N. J. Snellings, E. M. Johnson, R. M. Synenki, and L. S. Baron. 1975. Characterization of transmissible genetic elements from sucrose-fermenting *Salmonella* strains. J. Bacteriol. 122:401-406.