

Transformation of *Salmonella typhimurium* by Plasmid Deoxyribonucleic Acid

ESTHER M. LEDERBERG AND STANLEY N. COHEN

Departments of Medical Microbiology and Medicine, Stanford University School of Medicine,
Stanford, California 94305

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A modified transformation procedure that is effective for the introduction of plasmid deoxyribonucleic acid at high frequency into *Salmonella typhimurium*, as well as into *Escherichia coli*, is described. Transformed bacteria acquire a circular deoxyribonucleic acid species having the genetic and molecular characteristics of the transforming plasmid.

In 1970, Mandel and Higa reported that calcium chloride-treated *Escherichia coli* can take up phage λ deoxyribonucleic acid (DNA), resulting in the production of viable phage particles (11). Subsequently, it was shown that calcium chloride treatment of *E. coli* strain K-12 renders this host susceptible to genetic transformation by purified bacterial plasmid (4) or chromosomal (6, 17) DNA, and that bacteria that have been transformed with plasmid DNA contain replicons having the genetic and physical properties of the parent plasmid molecules. Transformation has since proved to be useful for investigating the characteristics of discrete molecular species of plasmid DNA that are separable in vitro by various fractionation procedures, and for introducing nonconjugative plasmids into specific *E. coli* recipient strains in the absence of transducing phage or conjugative transfer plasmids (7, 13, 16). In addition, certain fragments of larger plasmids generated by shearing (2) or by treatment with restriction endonuclease (3, 9) can be introduced by transformation into *E. coli*, where they can become functional replicons (3). Finally, transformation has enabled the cloning of plasmid chimeras constructed in vitro from genes originating on different *E. coli* plasmids (3) or in diverse bacterial species such as *E. coli* and *Staphylococcus aureus* (1), and has enabled the introduction of eukaryotic DNA sequences into a (prokaryotic) *E. coli* host (12).

During investigations of the molecular structure of R plasmids in different *Enterobacteriaceae*, it became apparent that a procedure for introduction of discrete plasmid DNA species into *Salmonella* would also be useful. The transformation procedure described earlier (4) yields a frequency of approximately 10^{-3} trans-

formants per viable cell per microgram of DNA in *E. coli*, using the small tetracycline resistance plasmid pSC101 as donor DNA, but the frequency of transformation observed in *Salmonella typhimurium* LT2 is less than 10^{-7} , which is the lower limit of detection. This communication reports a modified and somewhat simplified transformation procedure which is effective for the introduction of plasmid DNA species into *S. typhimurium* at a frequency comparable to that observed previously for *E. coli*, and which is also satisfactory for the latter host.

The bacterial strains and plasmids used in these experiments are shown in Table 1. EL226 is a mutant of *S. typhimurium* LT2 which is defective in its lipopolysaccharide coat as the result of a deletion covering the *gal* operon. This mutant was shown to be competent in transfection by *Salmonella* phage P22 (H. Burszstyn, unpublished data), and for this reason was used initially in these experiments. However, we subsequently observed that a *gal*⁺ "smooth" (LPS⁺) strain could be transformed by plasmid DNA at a similar frequency. The procedures used for isolation and sucrose gradient centrifugation of plasmid DNA have been described previously (4, 5). Bacteria were made competent for transformation as follows: a 50-ml culture inoculated with 0.5 ml of an overnight L broth (10) culture was grown in L broth to an optical density at 600 nm = 0.6. The cells were chilled, sedimented, and resuspended in 50 ml of cold 0.1 M MgCl₂. After a second centrifugation at 4 C, the cell pellet was resuspended in 25 ml of cold 0.1 M CaCl₂ and chilled in an ice bath for 20 min. A third centrifugation in the cold was carried out, and the cell pellet was resuspended in 2.5 ml of cold 0.1 M CaCl₂. For transformation, 0.1-ml volumes of chilled DNA

TABLE 1. *Bacterial strains and plasmids*

Bacterial strains	Relevant properties	Source or reference
<i>S. typhimurium</i> LT2 (EL226)	Nalidixic acid-resistant mutant of TA 1659; <i>chl-1013</i> ($\Delta gal, bio, uvrB$)	B. Ames and M. Alper (TA 1659)
<i>S. typhimurium</i> LT2 (EL291)	Tetracycline-resistant transformant of EL226 carrying pSC101	This laboratory
<i>E. coli</i> K-12 (C600)	<i>E. coli</i> K-12	A. D. Kaiser
<i>E. coli</i> K-12 (SC227)	Tetracycline-resistant transformant of C600 carrying pSC101	2,3
Plasmid (pSC101)	Nonconjugative plasmid derived from R6-5 and carrying tetracycline resistance	2,3

samples in TEN buffer [0.02 M tris(hydroxymethyl)aminomethane (pH 8.0)–1 mM ethylenediaminetetraacetic acid (pH 8.0)–0.02 M NaCl] were added to a 0.2-ml volume of competent cells in an ice bath, and the mixture was incubated for 30 min at 0 C (2, 4). As described previously (4), competent cells were then subjected to a heat pulse at 42 C for 2 min to enable DNA uptake and then chilled, diluted 10-fold into prewarmed L broth, and incubated with shaking at 37 C for 90 to 120 min to allow full expression of antibiotic resistance before plating on selective media. Drug resistance was assayed on nutrient agar plates containing tetracycline (20 μ g/ml) (2). The substitution of a magnesium chloride wash and the use of higher concentrations of calcium chloride to achieve competence in this bacterial species were suggested by the results of experiments carried out by H. Bursztyn (personal communication) and by Sgaramella et al. (*Genetics* 74:s249, 1973) involving transfection of *Salmonella* by phage P22 DNA.

The transformation frequency observed in a representative experiment with the procedure described here using the nonconjugative tetracycline resistance plasmid pSC101 isolated from *E. coli* (5) or from *S. typhimurium* is shown in Table 2. Transformation was accomplished in both bacterial species, and in each instance was 10-fold higher when the transforming plasmid DNA was isolated from the species being transformed (Table 2). This finding is consistent with earlier observations (18) indicating that conjugal transfer of antibiotic resistance plasmids between different species of *Enterobacteriaceae* occurs at a reduced frequency, as compared with transfer between bacteria of the same species.

DNA was isolated from a *S. typhimurium* LT2 parent strain and from a transformed tetracycline-resistant clone of the strain by the procedure indicated earlier for *E. coli* (4, 5).

TABLE 2. *Frequency of transformation of S. typhimurium or E. coli with pSC101^a*

Recipient cells	Source of plasmid DNA	
	<i>E. coli</i> (pSC101)	<i>S. typhimurium</i> (pSC101)
<i>E. coli</i> C600	1.5×10^{-3}	3×10^{-3}
<i>S. typhimurium</i> EL226	1.5×10^{-4}	3×10^{-2}

^a Isolation of plasmid DNA from *S. typhimurium* or *E. coli* was done as described previously (5) and transformation of both bacterial species was carried out as indicated in the text. Transformation frequency is expressed as the average number of transformants per viable cell per microgram of pSC101 plasmid DNA. No colonies were observed when between 10^7 and 10^9 viable bacteria were assayed on media containing tetracycline in the absence of DNA.

Covalently closed circular DNA obtained from both parent and transformant clones by cesium chloride-ethidium bromide gradient centrifugation (14) was analyzed in 5 to 20% neutral sucrose gradients in the presence of 34S bacteriophage λ DNA marker (5) (Fig. 1). Both the parent strain and the transformant clone contain DNA species having sedimentation properties consistent with those reported for the covalently closed and open circular DNA forms of the previously described 60×10^6 dalton cryptic plasmid carried by *S. typhimurium* LT2 (Fig. 1) (15). In addition, the tetracycline resistance transformant contains an additional circular DNA species having sedimentation properties (27S) characteristic of the pSC101 plasmid (2, 3). This DNA was itself able to transform both *E. coli* and *Salmonella* for tetracycline resistance as indicated in Table 1. Moreover, the conjugative transfer plasmid SPI (8) was able to mobilize pSC101 from the *Salmonella* transformant to a recipient strain, as reported earlier for *E. coli* (2).

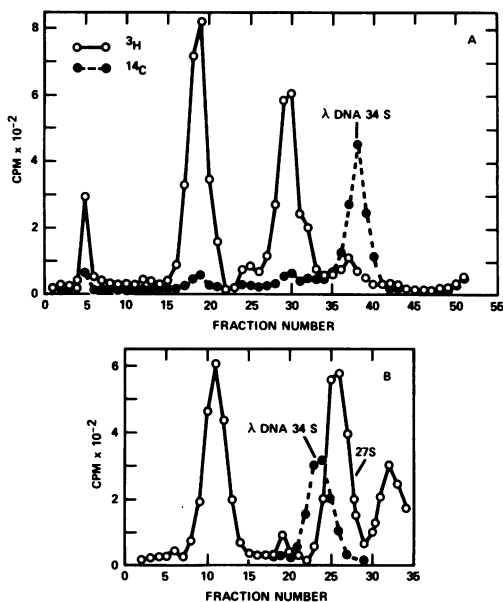


FIG. 1. Sucrose gradient centrifugation of covalently closed circular (CCC) DNA species isolated from a tetracycline-resistant *Salmonella* transformant and from the parent strain. Conditions for isolation of plasmid DNA by cesium chloride-ethidium bromide centrifugation have been described previously. DNA species were analyzed by centrifugation in 5 to 20% linear sucrose gradients for 75 min at 39,000 rpm in an SW50.1 rotor. (A) CCC DNA peak isolated from *S. typhimurium* LT2 parent strain. (B) CCC DNA peak isolated from tetracycline-resistant transformant of above.

These experiments report a procedure that is potentially useful for the introduction of various natural bacterial plasmids and constructed plasmid chimeras into both *Salmonella* and *E. coli*.

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