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High efficiency transformation of *Salmonella typhimurium* and *Salmonella typhi* by electroporation

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Summary. *Salmonella typhimurium* and *S. typhi* were transformd with high efficiency by electroporation. Transformation efficiencies of up to 10^{10} transformants per μg of pBR322 were obtained. In contrast to chemical transformation methods, neither the smooth lipopolysaccharide of *S. typhimurium* nor the Vi capsular polysaccharide of *S. typhi* greatly affected transformation efficiency. The introduction of a *galE* mutation slightly improved transformation efficiency in *S. typhimurium* (<tenfold) while the Vi antigen of *S. typhi* had no detectable effect. The transformation efficiency of *S. typhimurium* with DNA derived from *Escherichia coli* was increased greatly by the removal of the *hsd* restriction system (100-fold). Under these conditions electroporation can be used for the routine and direct transformation of *Salmonella* strains with partially purified (alkaline lysis) plasmid DNA from *E. coli*.

Key words: Electroporation – *Salmonella typhimurium* – *Salmonella typhit*

Introduction

Transformation of bacteria with DNA is a key method in genetics. Enteric bacteria, such as *Escherichia coli* and *Salmonella*, are not naturally competent for transformation. However, methods involving chemical treatment of the cells (Hanahan 1985) can induce competence. Transformation has been studied mostly in *E. coli* and optimal methods can yield over 10^8 transformants per μg plasmid DNA. Similar methods have been applied to *Salmonella* but with lower yields (Sanderson and Stocker 1987 and references therein).

Recently, it has become possible to transform bacteria by electroporation i.e. exposure to a brief, intense electrical field which permits direct entry of DNA into the cell. Electroporation has been used to transform a wide variety of gram-positive and gram-negative bacteria, including several previously thought untransformable (Wirth et al. 1989; Chassy et al. 1988). A recent report has shown that *E. coli* strains can be transformed with efficiencies of up to 10^{10} transformants per μg plasmid DNA (Dower et al. 1988). *S. typhimurium*, however, has been reported to be untransformable by electroporation or transformable only at comparatively low levels (10^2 or 10^5 transformants per μg plasmid; (Wirth et al. 1989; Taketo 1988). In this paper we show that using a procedure developed for *E. coli*, *S. typhimurium* can be transformed with frequencies as high as those reported for *E. coli*. We also report, for the first time, high frequency transformation of *S. typhi*.

Materials and methods

Bacterial strains. S. typhimurium strains used are listed in Table 1. They include LT2 and three derivatives with mutations in either all three host restriction systems (*hsd SA*, *hsd LT*, *hsd SB*) or/and *galE*. *S. typhimurium* HWSH is a virulent strain in mice and HWSH*aroA* is an attenuated derivative (O'Callaghan et al. 1988). *S. typhi*

Table 1. Effect of restriction and *galE* mutations on transformation efficiency of *Salmonella typhimurium* strains by electroporation with pBR322 derived from *Escherichia coli*

Strain	Restriction phenotype	*galE*	Ampicillin resistance transformation efficiency[a]
LT2[b]	+	+	$2.55 \times 10^7 \pm 22\%$
SL1306[c]	+	–	$4.2 \times 10^7 \pm 75\%$
LB 5000[b]	–	+	$1.9 \times 10^9 \pm 56\%$
LB 5010[b]	–	–	$8.3 \times 10^9 \pm 32\%$

[a] Results are mean of three experiments ± SD. Selection for tetracycline resistance yielded 2-5 fold fewer transformants
[b] Sanderson and Stocker (1987)
[c] From B.A.D. Stocker

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541Ty and 543Ty (Edwards and Stocker 1988) were provided by B.A.D. Stocker. *S. typhi* 541Ty is an *aroA*, *purA*, *his*, Vi^+ derivative of a wild-type *S. typhi* CDC 10–80, and 543Ty is a spontaneous Vi^- mutant of 541Ty (Edwards and Stocker 1988).

Media. L agar and L broth were as described (Miller 1972). SOC medium was as described by Hanahan (1985). When appropriate, media were supplemented with ampicillin (100 μg/ml) or tetracycline (12.5 μg/ml).

Plasmids. pBR322 was purchased from Pharmacia and diluted to 10 ng/μl in distilled water.

Electroporation. Electroporation was performed using a Bio-Rad Gene Pulser™ as described by Dower et al. (1988). Briefly, bacteria were grown in 100 ml L broth to A_{600} of 0.6, chilled on ice, and harvested by centrifugation (15 min, $1000 \times g$ at 4° C). The pellet was washed twice with 100 ml ice-cold distilled water, and once with 20 ml ice-cold 10% glycerol (BRL, Ultra Pure) in distilled water. The bacterial pellet was suspended in a final volume of 200 μl in 10% glycerol. Aliquots (40 μl) were mixed with 1–2 μl DNA in a chilled microcentrifuge tube and transferred to chilled cuvettes (0.2 cm electrode gap). A single pulse of 12.5 kV/cm (2.5 kV, 200 Ω, 25 μF) was applied and 1 ml of pre-warmed SOC was immediately added. The bacteria were transferred to 17×100 mm polypropylene tubes and shaken for 1 h at 37° C before plating onto selective media. Percentage survival was assessed by plating a suitable dilution of bacteria on L agar before and after electroporation. Dower et al. (1988) found that optimal transformation of *E. coli* required a post-pulse survival rate of approximately 30% and a pulse time constant of 5 ms. We found that a 12.5 kV/cm pulse gave survival rates of between 20%–50% for both *E. coli* and *Salmonella* with a pulse time constant of 4 ms.

Agglutination. Transformants were tested for their lipopolysaccharide (LPS) and Vi polysaccharide by slide agglutination using Salmonella typing sera kindly provided by M. Popoff (Institut Pasteur, Paris). Vi^+ strains were boiled for 1 h before testing for O-antigen.

Results and discussion

Electroporation of S. typhimurium *and* S. typhi *strains*

The low electroporation efficiencies previously reported for *S. typhimurium* (Wirth et al. 1989; Taketo 1988) used conditions which were unlikely to be optimal i.e. large sample volume, low cell density, long incubations post-pulse. We therefore decided to examine the efficiency of electroporation in various mutants of *S. typhimurium* LT2 using a protocol optimised for *E. coli* (Dower et al. 1988).

Bacteria were transformed with 10 ng (1 μl) pBR322 prepared from *E. coli* K12. Mean transformation efficiencies of up to 8.3×10^9 transformants per μg were obtained when selecting for ampicillin resistance (Table 1, line 4). In individual experiments, efficiencies of up to 1.2×10^{10} transformants per μg were obtained with LB5010. These efficencies are 10^3–10^4 times higher than with chemical methods, and similar to those with *E. coli*. Alkali lysis mini-preparation DNA also proved to be sufficiently pure for successful electroporation. The efficiency was somewhat lower than that observed with highly pure DNA, but gave more than enough transformants for routine use with wild-type strains such as HWSH and HWSH*aroA*.

These high levels were obtained with r^- *Salmonella* strains mutated in the *hsd* restriction system (LB5000, LB5010; Table 1). To assess the effects of *hsd* restriction we also electroporated r^+ *Salmonella* strains. Both strains with r^+ phenotypes transformed with efficiencies about two orders of magnitude lower (LT2, SL1306; Table 1). However, this is still over 10^7 transformants/μg: an improvement of up to one millionfold on the chemical transformation method.

The role of restriction was further evident when an attenuated vaccine strain of *S. typhimurium* (HWSH *aroA*) was transformed. HWSH*aroA* transformed at lower efficiency with pBR322 derived from *E. coli*, yielding only 3.3×10^4 transformants/μg plasmid. To confirm that this low efficiency was due to restriction systems, possibly highly active in HWSH (a recent field isolate), pBR322 which had been modified in *Salmonella* was purified from LB5010 and used to retransform LB5010 and HWSH *aroA*. Transformation frequencies were equally high in both strains (data not shown) showing that the low efficiency in HWSH*aroA* was indeed due to restriction.

The smooth LRS of *S. typhimurium* is known to inhibit transformation by chemical techniques (Sanderson and Stocker 1987). When chemical methods are used, smooth strains transform poorly, with less than 10^3 transformants per μg plasmid. Rough strains with Rc (*galE*) chemotypes have been shown to give up to 10^6 transformants/μg plasmid (Tsai et al. 1989). Chemical transformation of smooth strains may lead to selection of rough variants. We found the *galE* mutation had little or no effect on the electroporation efficiency (Table 1). All HWSH*aroA* transformants retained their smooth phenotype as judged by agglutination and P22 sensitivity.

The two *S. typhi* strains were transformed with pBR322 derived from *E. coli* at efficiencies similar to that of LT2, with 541Ty and 543Ty giving 9×10^6 and 1.17×10^7 transformants/μg, respectively. This suggests that the Vi antigen (present on 541Ty but not 543Ty) does not greatly affect the entry of DNA during electroporation. Twenty transformants of each strain were tested for Vi and O-9 by agglutination. All possessed their parental phenotype.

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