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Novel Selection for Tetracycline- or Chloramphenicol-Sensitive *Escherichia coli*

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A method for selecting tetracycline- or chloramphenicol-sensitive *Escherichia coli* cells from a population of predominantly resistant cells is described. This method depends on the inability of drug-sensitive cells to induce λ receptors in the presence of chloramphenicol or tetracycline, protein synthesis-inhibiting drugs. The addition of bacteriophage λ *vir* to a mixture of drug-sensitive and drug-resistant cells, induced for λ receptors in the presence of tetracycline or chloramphenicol, preferentially kills the drug-resistant cells (which are capable of inducing λ receptors). The result is a culture enriched for the sensitive cells. Several common strains used for transformation were compared for their ability to be selected. *E. coli* 294 was found to be superior.

Drug-resistant transposons have proved to be very useful tools for genetic manipulations. These transposons can serve as selective markers for the presence of plasmids, allowing the easy recovery of plasmid-containing bacteria after transformation (4). Plasmid vectors have been constructed which contain more than one type of drug resistance (2). With these vectors, a segment of DNA can be cloned by insertion into the structural gene of the transposon to inactivate it, while the other transposon, encoding a different drug resistance, remains intact. By screening for the loss of the first drug resistance, there is a high probability that the plasmid will be a hybrid molecule. It would be convenient, however, to be able to select for the loss of drug resistance and consequently greatly reduce the work of screening.

A method for selecting for tetracycline-sensitive cells has been described by Bochner et al. (1). In this method, fusaric acid is used to kill the resistant bacteria. Maloy and Nunn (5) have described a modification of the fusaric acid technique by which the background of tetracycline-resistant bacteria is reduced. However, this procedure may not be suitable for all of the *Escherichia coli* strains commonly used for transformation. In addition, this method is specific for tetracycline. The selection scheme described here provides an alternative method which can be used to select for tetracycline- or chloramphenicol-sensitive cells from a population of predominantly resistant bacteria.

The *E. coli* strains used in this study were 294 (*endA hsdR thi pro hsdR hsdM hsm*), HB101 (*endA hsdR pro leu lacY ara-14 galK2 xyl-5 thi mtl-1 rpsL20 recA supE44*), C600 (*hsdR hsdM*$^+$ *thr leu thi lac tonA*), GM31 (*dcm thr leu lac gal ara xyl thi tonA tsx str*), and GM48 (*dam-3 dcm-6 thr leu thi lacY galK2 galT22 ara-14 tonA31 tsx78 supE44*). The strains were obtained from H. Boyer. The plasmids used were pBR322 (Amp^r Tet^r), pCB1 (pBR322 with a segment of DNA inserted in the ampicillin gene), kindly provided by C. Benicourt, pSF2124 (Amp^r), and pBR325 (Amp^r Tet^r Cm^r). The bacteriophage λ *vir* was a gift from J. Hedgepeth. All cells were grown at 37°C in M9 minimal medium (6) with glucose as a carbon source, supplemented with Casamino Acids and thiamine. Cells were plated on Luria agar plates (6). The plates contained tetracycline (10 μg/ml), ampicillin (20 μg/ml), or chloramphenicol (150 μg/ml).

Cells were transformed by the procedure of Cohen and Chang (3), except that the cells were grown in Luria broth (6) rather than H1 medium (3). The cells were transformed with 0.2 μg of pBR322 DNA and scored for tetracycline resistance.

The selection for drug-sensitive cells is based on the preferential killing of resistant bacteria with bacteriophage λ *vir*. This is accomplished by inducing the synthesis of λ receptors with the addition of cAMP and maltose in the presence of chloramphenicol or tetracycline, protein synthesis-inhibiting drugs. The resistant cells will synthesize large numbers of receptors, whereas the sensitive cells will be unable to respond to induction because protein synthesis is blocked. After induction, the phage λ *vir* is added to the culture and kills the resistant cells preferentially.

The preferential killing of the drug-resistant

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cells can be demonstrated by determining the survival of a mixture of *E. coli* 294 cells carrying either the plasmid pSF2124 (Amp^r) or pCB1 (Tet^r). These cells can be differentiated by plating on selective medium. A culture of cells in which 1% of the cells harbored pSF2124 (Amp^r) and the remainder harbored pCB1 (Tet^r) was incubated with tetracycline for 10 min; maltose (0.01 M) and cAMP (4 mM) were added. The λ receptors were induced by growth in this medium for 1 h at 37°C, and the cells were plated on selective medium.

The survival curves (Fig. 1) clearly demonstrate that the drug-resistant cells were much more sensitive to killing by λ *vir*. The survival of the cells can be expressed by the equation $N = N_o e^{-K\phi}$, where ϕ is the average multiplicity of infection (MOI), N is the number of surviving bacteria at the particular MOI, N_o is the original number of bacteria, and K is a constant characteristic of the state of the cell that reflects the number of λ receptors on the cell surface. To obtain a good selection of drug-sensitive cells, the ratio of the K value for the sensitive cells to that of the resistant cells (K_s/K_r) should be less than 0.2. This condition is met for these cells; the ratio of K_s/K_r is 0.05 (Fig. 1). It should be noted that at high MOIs (i.e., 8 to 9) the resistant cells exhibit a subpopulation of cells which are less sensitive to λ *vir*. This reduces the effectiveness of selection at high multiplicity.

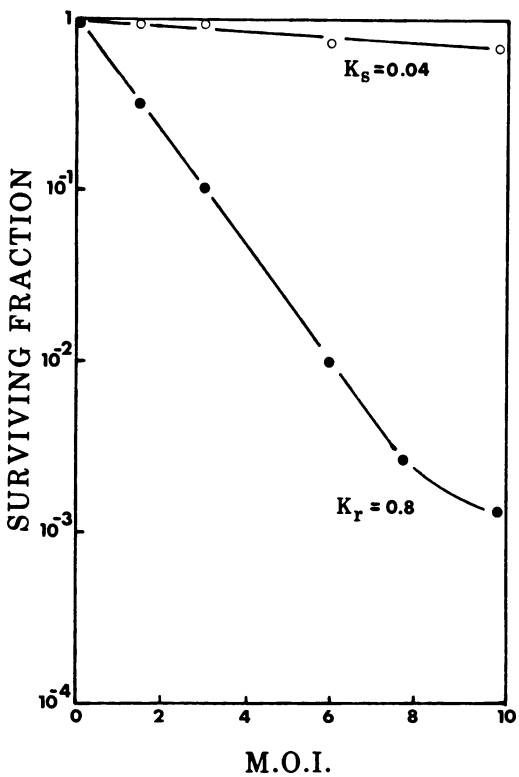

FIG. 1. Survival curves of *E. coli* 294 induced for λ receptors in the presence of tetracycline. A population of 294 in which 1% of the cells harbored pSF2124 (Amp^r Tet^s) and the remainder harbored pCB1 (Amp^s Tet^r) at an absorbance at 460 nm of 0.5 was treated with tetracycline for 10 min. The culture was then induced for λ receptors by the addition of maltose (0.01 M) and cAMP (4 mM) and incubation at 37°C for 1 h. λ *vir*, at the indicated MOI, was adsorbed for 7 min at 37°C; the cells were then diluted and plated on selective medium to determine the survival of the cells that harbored pSF2124 (○) or pCB1 (●). The K values determined from the linear portion of the plots are indicated.

The ability to select for drug-sensitive cells was tested with mixed cultures of *E. coli*. *E. coli* 294 cultures in which 1 or 15% of the cells harbored pSF2124 (Amp^r Tet^s) and the remainder harbored pBR322 (Amp^r Tet^r) were induced for λ receptors and treated with increasing phage concentrations as described in the legend to Fig. 1. These cells were plated on nonselective medium. The resultant colonies were then replica plated on tetracycline-containing plates to determine the ratio of drug-sensitive cells to drug-resistant cells (N_s/N_r). As expected, the ratio increased rapidly (note that N_s/N_r is a log scale) in response to the phage challenge (Fig. 2). The value of N_s/N_r at an MOI of 0 was below the initial value of 0.01; during the drug treatment the resistant cells continued to grow, whereas the sensitive cells did not. At the highest MOI used (about 30), the fraction of tetracycline-sensitive cells increased from 1 to 69% and from 15 to 96%. This means that after phage selection very few colonies must be screened to get a large number of tetracycline-sensitive cells.

A similar experiment was performed with a culture of *E. coli* 294 in which 1% of the cells harbored pBR322 (Amp^r Tet^r Cm^s) and the remainder harbored pBR325 (Cm^r Amp^r Tet^r). The cells were treated with chloramphenicol in a manner identical to the tetracycline treatment. The value of N_s/N_r increased in a fashion essentially identical to that observed with the tetracycline-sensitive cells (Fig. 3). This would be expected, since the ratio is a reflection of the host cell and not the plasmids involved. These results show that preferential phage killing of at least two types of drug-resistant cells can lead to an effective selection for the drug-sensitive phenotype.

The success of this method depends on the ability to induce the λ receptors in the host cell. Therefore, the K values for some strains commonly used for transformation were determined. The K values were determined for cells which had been induced for λ receptors (K_I) or not induced (K_{NI}). The K_{NI}/K_I (corresponding to K_s/K_r) ratios for 294 and GM48 were low and

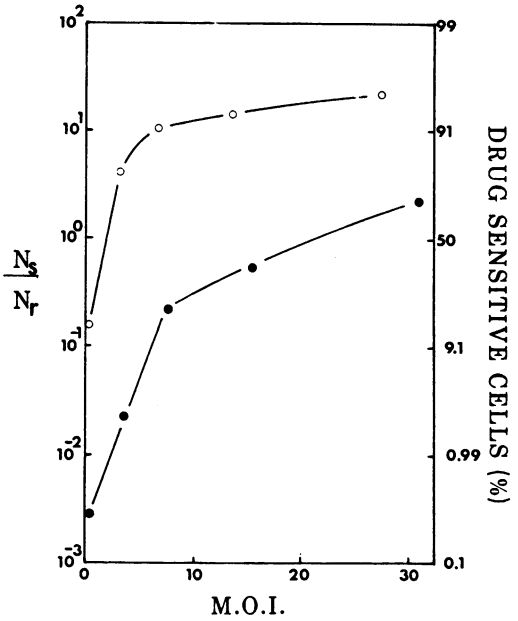

FIG. 2. Enrichment for tetracycline-sensitive cells. Cultures of *E. coli* 294 in which 1% (●) or 15% (○) of cells harbored pSF2124 (Amp^r Tet^s) and the remainder harbored pBR322 (Amp^r Tet^r) were treated with tetracycline for 10 min and then induced for λ receptors, as described in the legend to Fig. 1. λ *vir* was adsorbed for 7 min at the indicated MOI. The cells were pelleted, suspended in fresh medium, and plated on Luria broth plates. Single colonies were then replica plated onto plates with either no tetracycline or 10 μg of tetracycline per ml to allow for the calculation of N_s/N_r.

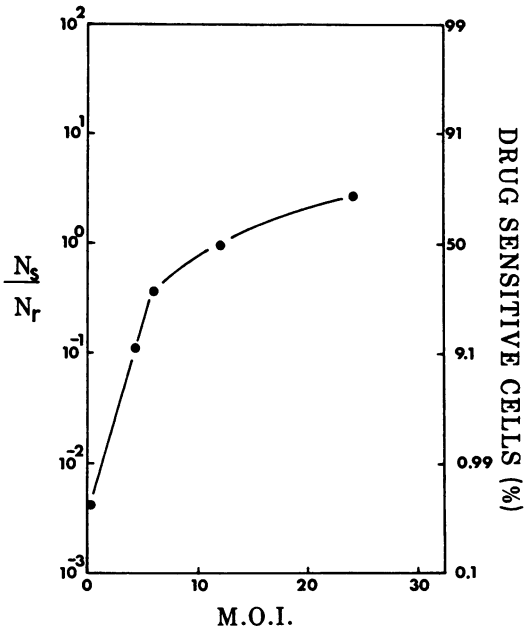

FIG. 3. Enrichment for chloramphenicol-sensitive cells. Cultures of *E. coli* 294 in which 1% of cells harbored pBR322(Cm^s Amp^r Tet^r) and the remainder harbored pBR325(Cm^r Amp^r Tet^r) were treated with chloramphenicol for 10 min and then induced for λ receptors, as described in the legend to Fig. 1. λ *vir* was adsorbed for 7 min at the indicated MOI. The cells were pelleted, suspended in fresh medium, and plated on Luria broth plates. Single colonies were then replica plated onto plates with either no chloramphenicol or 150 μg of chloramphenicol per ml to allow for the calculation of N_s/N_r.

should allow for excellent selection for drug-sensitive cells (Table 1). Strain HB101, which carries the *mtl-1* mutation, fails to induce the λ receptors effectively and is not suitable for this selection procedure. Strain GM31 does not induce the receptors and is also unsuitable. Strain C600 does not appear to induce the λ receptors as effectively as does 294 or GM48 under these conditions. However, the K_{NI}/K_I ratio of 0.12 should allow for some enrichment of drug-sensitive cells.

It has been demonstrated that *E. coli* cells which are sensitive to tetracycline or chloramphenicol can be selected from a population of predominantly resistant cells. The selection is accomplished by inducing λ receptors in the presence of the drug and killing the drug-resistant cells preferentially with the bacteriophage λ *vir*. This is possible since *E. coli* grown on medium with glucose as a carbon source has very few λ receptors per cell (7). Induction with maltose and cAMP leads to about a 1,000-fold increase in the number of λ receptors after 1 h of induction (7).

The expected exponential increases in the ratio of N_s to N_r with increasing MOI are observed only up to an MOI of about 8 or 9. At these MOIs, a subpopulation of the drug-resistant cells which has a smaller K_r becomes apparent. These cells, therefore, reduce the effectiveness of selection at the higher MOI. However, a high MOI (i.e., about 30) can be used without significant losses of drug-sensitive cells. To reduce the number of phage present on the agar

TABLE 1. K_I and K_{NI} values and transformation efficiencies for some *E. coli* host strains[a]

Strain	K_I	K_{NI}	K_{NI}/K_I	Transformants (per 0.2 μg of DNA)	Relative transformation frequency
HB101	0.05	0.05	1.0	4.8×10^5	1
294	0.8	0.04	0.05	4.8×10^6	10
C600	0.33	0.04	0.12	2.8×10^6	5.8
GM31	0.05	0.05	1.0	5.6×10^5	1.2
GM48	0.9	0.05	0.06	1.0×10^5	0.2

[a] K values were determined for the strains as described the legend to Fig. 1.

plate, it is desirable to centrifuge the cells before dilution and plating. This simplifies obtaining phage-free isolates of the resultant colonies. The dilution of cells for plating is sufficient to reverse the lethal effects of the drugs; therefore, no special efforts are needed to eliminate the drugs.

This method is suitable for use in cloning by insertional inactivation of the tetracycline gene or chloramphenicol gene. For example, by plating a phage-treated population of pBR322-transformed 294 (after induction for λ receptors in the presence of tetracycline) on ampicillin-containing plates, it is possible to select plasmid-containing cells with an insertion in the tetracycline gene in one step. Similarly, it would be possible to select for insertion in the chloramphenicol gene.

Another possible use of the method described here is the selection for loss of chloramphenicol "cassette" expression (R. L. Rodriguez, personal communication). This would allow for the selection of mutations which inactivate the promoter function of DNA segments placed next to the cassettes. In addition, this method could be used to select for bacterial mutants which lose tetracycline or chloramphenicol resistance.

The data presented in Table 1 clearly show that the preferred bacterial host strain for this selection procedure is 294. The ratio of K_{NI} to K_I is very low, allowing for excellent selection, and 294 also exhibits the highest transformation frequency of the strains tested. Strain C600 has a high transformation frequency, but the K_{NI} to K_I ratio is not as favorable for selection. Strain GM48 does not transform at a high frequency but does have a very low K_{NI}/K_I ratio, which would allow for good selection. This strain could be used effectively when it is desirable to have plasmid DNA which is not methylated. Strain HB101 is not suitable for this procedure.

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LITERATURE CITED

1. **Bochner, B. R., H.-C. Huang, G. L. Schieven, and B. N. Ames.** 1980. Positive selection for loss of tetracycline resistance. J. Bacteriol. **143**:926–933.
2. **Bolivar, F., R. L. Rodriguez, P. J. Greene, M. B. Betlach, H. L. Heyneker, H. W. Boyer, J. H. Crosa, and S. Falkow.** 1977. Construction and characterization of new cloning vehicles. II. A multipurpose cloning system. Gene (Amst.) **2**:95–113.
3. **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.
4. **Cohen, S. N., A. C. Y. Chang, and L. Hsu.** 1972. Nonchromosomal antibiotic resistance in bacteria: transformation of *Escherichia coli* by R-factor DNA. Proc. Natl. Acad. Sci. U.S.A. **69**:2110–2114.
5. **Maloy, S. R., and W. D. Nunn.** 1981. Selection for loss of tetracycline resistance by *Escherichia coli*. J. Bacteriol. **145**:1110–1112.
6. **Miller, J. H.** 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Ryter, A., H. Shuman, and M. Schwartz.** 1975. Integration of the receptor for bacteriophage lambda in the outer membrane of *Escherichia coli*: coupling with cell division. J. Bacteriol. **122**:265–301.