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Efficient curing of an *Escherichia coli* F-prime plasmid by phenothiazines

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SUMMARY

Chlorpromazine and several other phenothiazines at sub-bacteriocidal concentrations were found to cure an *Escherichia coli* F′ *lac*$^+$ strain of its plasmid efficiently. Curing was most efficient at high pH and in complex medium when 70 % or more of the bacteria were plasmid-free after 24 h growth of cultures in the presence of the drug.

1. INTRODUCTION

Phenothiazines are widely used in medicine as sedatives and in particular in the treatment of schizophrenia. Although their precise mode of action is unknown, many studies (Clement-Courmier *et al.* 1974; Miller, Horn & Iverson, 1974) indicate that their relative potencies as drugs closely parallels their ability to inhibit dopamine-sensitive adenylate cyclase in the brain.

Recently Feinberg & Snyder (1975) have proposed that chlorpromazine, acting as a structural analogue of dopamine, prevents the binding of this molecule to specific surface receptors in synaptic areas. In contrast, phenothiazines have also been reported to have antibacterial properties (for example, Bourdon, 1961; De Court, Gastal & Grenet, 1953) and in our previous study, chlorpromazine (CP), levomepromazine (LE), promethazine (PR) and parkazine (PA) were all shown to be bacteriocidal for both Gram-positive and Gram-negative bacteria (Molnár, Király & Mándi, 1975). In this previous study it was also observed that chlorpromazine caused curing of an R-factor from a strain of *E. coli*. In order to optimize conditions for curing and to test the effect of phenothiazines on another plasmid we have now extended these studies to an F′ *lac*-bearing strain of *E. coli* K 12.

2. MATERIALS AND METHODS

Bacterial strain *E. coli* K12, LE 140 (*lac*Δ, *mal*, *str*^{R}, λ^R) carrying F′ *lac*$^+$ was kindly provided by Mr P. A. Meacock. Bacterial cultures were grown in either minimal M 9. glucose medium (Clowes & Hayes, 1968) or in MTY medium containing M 9 supplemented with tryptone and yeast extract as described by Alföldi, Rasko & Kerekes (1968). To distinguish between *lac*$^+$ colonies from colonies formed by *lac*$^-$, plasmidless bacteria, cultures were plated on EMB-lactose agar (see Clowes & Hayes, 1968). The phenothiazines, chlorpromazine (CP), levomepromazine (LE), promethazine (PR) and parkazine (PA) were obtained from EGYT, Budapest, Hungary. For the curing of plasmid-bearing bacteria an overnight culture of *E. coli* LE 140 was diluted to 10^3 bacteria/ml in either M 9 or MTY medium, chlorpromazine or other phenothiazines was added to a

final concentration of 10–80 μg/ml and incubation continued with gentle shaking for 24 h at 37 °C. Cultures were then diluted and plated to obtain individual colonies on EMB-lactose agar. The plates were scored after 24–48 h incubation at 37 °C.

3. RESULTS AND DISCUSSION

Cultures of *E. coli* K 12 LE 140 were grown in the presence of various concentrations of chlorpromazine in M 9-minimal glucose medium and plated out for survivors after 24 h. Table 1 shows that although the plasmid is stably inherited in the absence of the

Table 1. *Elimination of F′* lac *plasmid by chlorpromazine*

	pH 7·2		pH 7·6	
Chlorpromazine (μg/ml)	Lac$^-$ colonies (%)	Viable cells after 24 h	Lac$^-$ colonies (%)	Viable cells after 24 h
0	0	$2{\cdot}0 \times 10^9$	0	$1{\cdot}0 \times 10^9$
10	2	$2{\cdot}0 \times 10^9$	5	$5{\cdot}0 \times 10^8$
20	5	$1{\cdot}5 \times 10^9$	13	$1{\cdot}0 \times 10^8$
40	8	$5{\cdot}0 \times 10^8$	20	$6{\cdot}0 \times 10^7$
60	15	$2{\cdot}0 \times 10^8$	0	$< 10^5$

E. coli K 12 LE 140 carrying an F′ *lac* factor and deleted for the *lac* region of the chromosome was grown in M 9-minimal media, pH 7·2 or 7·6, for 24 h at 37 °C in the presence of different concentrations of chlorpromazine. Cultures were then plated on EMB-lactose agar and the viable count and proportion of *lac*$^-$ colonies determined. Between 200 and 2000 colonies were screened in each case.

Table 2. *Curing action of chlorpromazine in complete medium, MTY*

Chlorpromazine (μg/ml)	No. of colonies tested	Lac$^-$ colonies	Viable cells after 24 h
0	620	0	$1{\cdot}0 \times 10^9$
10	800	3	$2{\cdot}0 \times 10^8$
20	600	10	$1{\cdot}5 \times 10^8$
40	800	30	$4{\cdot}0 \times 10^7$
60	1200	70	$3{\cdot}0 \times 10^7$

The F′ *lac* strain was grown in M 9 medium supplemented with tryptone and yeast extract at 37 °C for 24 h in the presence of CP. Cultures were finally plated on EMB-lactose agar.

drug, in the presence of 60 μg/ml CP, 15 % of bacteria lose the plasmid. Identical results were obtained with the phenothiazines, LE, PR and PA. As in the case of curing with acridine dyes (Hirota, 1960), elimination of the F′ *lac* factor by CP was more effective at pH 7·6 in M 9 medium (Table 1). Under these conditions, however, high concentrations (> 40 μg/ml) of the drug were extremely bacteriocidal and few bacteria survived. The curative action of CP was also tested with cultures grown in MTY-complete medium at pH 7·2. As shown in Table 2, curing was now extremely effective, with at least 70 % of bacteria showing loss of the plasmid after growth in the presence of 60 μg/ml CP. Virtually identical results were obtained with the other phenothiazines under these conditions. However, in some experiments with promethazine at 60 μg/ml, up to 90 % of the bacteria were found to be *lac*$^-$ after treatment.

The mechanism of plasmid curing by chlorpromazine and other phenothiazines is unknown although at least two possible alternatives may be envisaged. Thus initial binding of the drug to specific surface receptors may occur which then either leads to

differential inhibition of plasmid DNA replication, or death if plasmid bearing bacteria are simply more susceptible than normal bacteria to some lethal effect of the bound drug. Alternatively, since as shown in Fig. 1 acridines, which are known to intercalate into DNA molecules (Schreiber & Danne, 1974), and phenothiazines share a similar chemical structure, direct interaction of the drug with plasmid DNA is a possibility. In this case too a contribution to its own elimination by the plasmid may be envisaged, since recent evidence indicates that plasmids promote extensive changes in the host cell surface (Levy, McMurray & Palmer, 1974; Iyer & Holland, 1975), which could lead to increased permeability to the drug.

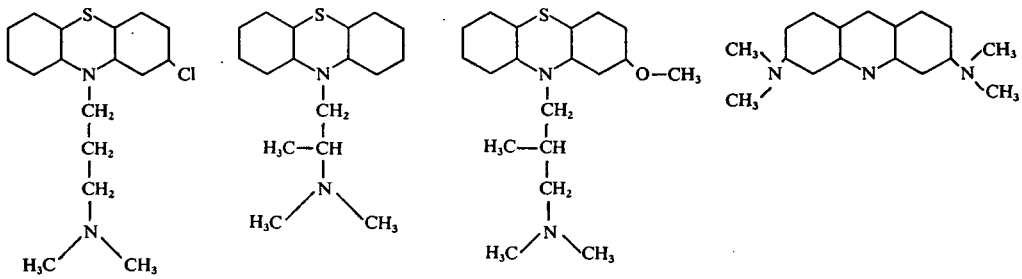

Fig. 1. Structural formulae of acridine orange and some phenothiazines.

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