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Preferential Inhibitory Action of Sodium Cholate on an *Escherichia coli* Strain Carrying a Plasmid in an Integrated State

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Sodium cholate was shown to be preferentially more active on *Escherichia coli* strains carrying an integrated plasmid, i.e., on Hfr strains, than on their parental strains with or without a plasmid in an autonomous state.

In an Hfr strain, a specific cointegrate composed of the host chromosome and a conjugative plasmid, the replication system is substituted by the integrated plasmid when the chromosomal replication is not normally functioning (3). In our previous studies (4, 6), an Hfr strain was constructed by the use of integrative suppression with a plasmid whose replication was affected differentially from that of the host chromosome by some inhibitory condition, such as the addition of chloramphenicol (1) or the elevation of growth temperature. These studies suggested that chromosome replication is governed by the replication system of the chromosome itself unless it is some way inhibited. The integration of a plasmid into the host chromosome seems to have a profound biochemical effect, so that some chemical agents may become extremely active when the plasmid is integrated as opposed to being autonomous. If this speculation is correct, then the use of this type of chemical may be a convenient method of clarifying the regulatory interaction between the two component replicons forming the cointegrate.

Twelve nonionic and four anionic detergents were tested. The R plasmids used were R386 (Tc), N3 (Sa Sm Tc), and R6K (Ap Sm) (Tc, Sa, Sm, and Ap indicate resistance to tetracycline, sulfonamide, streptomycin, and ampicillin, respectively). These plasmids belong to incompatibility groups FI, N, and X, respectively (2). Strain YC294 (6), made by transduction of *ilv dnaA*(Ts) strain CRT46 to *ilv*$^+$ *dnaA*$^+$, was used as a plasmid-free strain and as the host carrying a plasmid autonomously. The Hfr strains were isolated as integratively suppressed, temperature-resistant revertants of strain CRT46 carrying one of the plasmids autonomously (Table 1). When selected for the chromosomal marker, all of the Hfr strains showed a recombination frequency higher than that for selection with the plasmid drug resistance marker. Conversely, their respective isogenic partners carrying the same plasmid autonomously showed a plasmid transfer frequency far higher than that of the chromosomal marker. An Hfr strain made by a similar method with R6K has already been described (4).

Detergents were screened for inhibitory action on plasmid-free strain YC294. Of the 16 nonionic and anionic detergents tested, Emulgen p120T (minimal inhibitory concentration [MIC], 10%), Brij 35 (MIC, 2.5%), sodium cholate (MIC, 5%), sodium dodecyl sulfate (MIC, 0.05%), and sodium lauryl benzene sulfonate (MIC, 0.05%) effectively inhibited growth of strain YC294. These five detergents were tested for their

TABLE 1. *Frequency of transconjugant formation*[a]

Donor		Selection		Frequency of transconjugant formation
Strain	Property	Donor	Recipient	
YC606	Hfr(R386)	*arg*$^+$	*thr*$^+$ *leu*$^+$	9.5×10^{-5}
	dnaA(Ts)	*tet*^{r}	*thr*$^+$ *leu*$^+$	4.5×10^{-7}
YC607[b]	Hfr(R386)	*arg*$^+$	*thr*$^+$ *leu*$^+$	9.6×10^{-5}
	dnaA$^+$	*tet*^{r}	*thr*$^+$ *leu*$^+$	$<10^{-7}$
YC603	CRT46	*arg*$^+$	*thr*$^+$ *leu*$^+$	$<10^{-7}$
	(R386)	*tet*^{r}	*thr*$^+$ *leu*$^+$	6.1×10^{-2}
YC608	Hfr(N3)	*arg*$^+$	*thr*$^+$ *leu*$^+$	6.6×10^{-5}
	dnaA(Ts)	*tet*^{r}	*thr*$^+$ *leu*$^+$	7.5×10^{-6}
YC604	CRT46(N3)	*arg*$^+$	*thr*$^+$ *leu*$^+$	$<10^{-7}$
		tet^r	*thr*$^+$ *leu*$^+$	1.3×10^{-3}

[a] AB1450 was used as the recipient. Mating was interrupted at 90 min.

[b] YC294 and Hfr strains were phenotypically isogenic with respect to the chromosomal genetic constitution, but the Hfr strains genotypically were not isogenic with respect to *dnaA*. For the Hfr strain made with R386, the *dnaA*(Ts) genotype was converted to *dnaA*$^+$ by transducing *ilv*$^+$ *dnaA*$^+$ from W3104.

growth inhibitory concentration against three sets of isogenic strains: YC294, YC294 (R^+), and Hfr (R^+). Only sodium cholate was shown to be preferentially more active on the Hfr strain than on the strains not containing the plasmid or containing it autonomously. No detectable difference in susceptibility was observed with four other detergents used. This lack of preferential susceptibility of the Hfr strain to the latter four agents suggests that sodium cholate is specifically active against strains carrying the integrated plasmid. Figure 1 shows growth kinetics (monitored turbidimetrically with an automatic biophotometer [Sakagami Co., Chiba Prefecture, Japan]) of these strains in the presence and absence of sodium cholate. In all of the Hfr strains tested, irrespective of their *dnaA* genotype, the beginning of a detectable turbidity increase was delayed markedly. Also, the final attainable growth turbidity of the strains bearing the plasmid autonomously was slightly, but reproducibly, inhibited compared with that of the plasmid-free strain. This slight difference was more marked for another host, W677, and its plasmid-bearing strains (Fig. 2). Thus, the strain carrying the plasmid autonomously showed either a delayed onset of detectable growth increase or a reduced growth rate. These effects were not observed for some of the strains made by curing the plasmid from strain W677 carrying R386 autonomously (Fig. 2C). When this latter strain was grown in the presence of sodium cholate, curing was stimulated appreciably.

The supposition that the activity of this type of detergent is related to the mechanism by

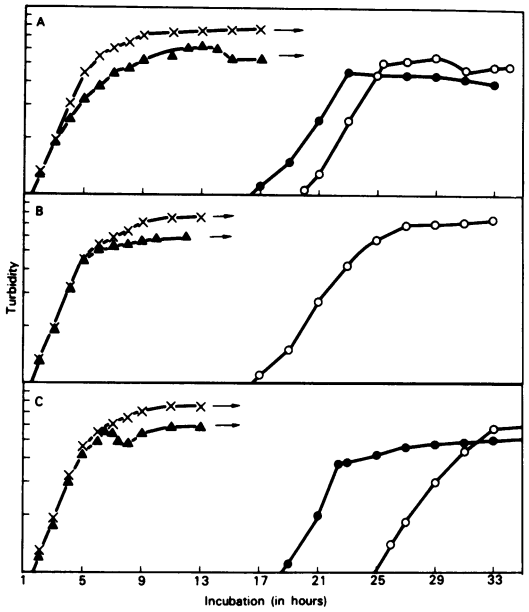

FIG. 1. *Effect of sodium cholate on growth of isogenic strains with or without a plasmid in the autonomous and integrated state. Cultures at the late logarithmic phase were diluted 10^{-2} with fresh Penassay broth (Difco), containing 10 μg of thymine per ml and 2.5% sodium cholate (pH 7.0), and incubated at 30°C with shaking. The optical density was recorded with an automatic photometer. (A) ×, YC294 (plasmid-free); ▲, YC600, a YC294 strain carrying R386 autonomously; ○, YC606, an Hfr strain made with R386 [dnaA(Ts)]; ●, YC607, same as YC606 but dnaA⁺. (B) ×, YC294 (plasmid-free); ▲, YC601, a YC294 strain carrying N3 autonomously; ○, YC608, an Hfr strain made with N3 [dnaA(Ts)]. (C) ×, YC294 (plasmid-free); ▲, YC602, a YC294 strain carrying R6K autonomously; ○, YC609, an Hfr strain made with R6K [dnaA(Ts)]; ●, YC610, same as YC609 but dnaA⁺.*

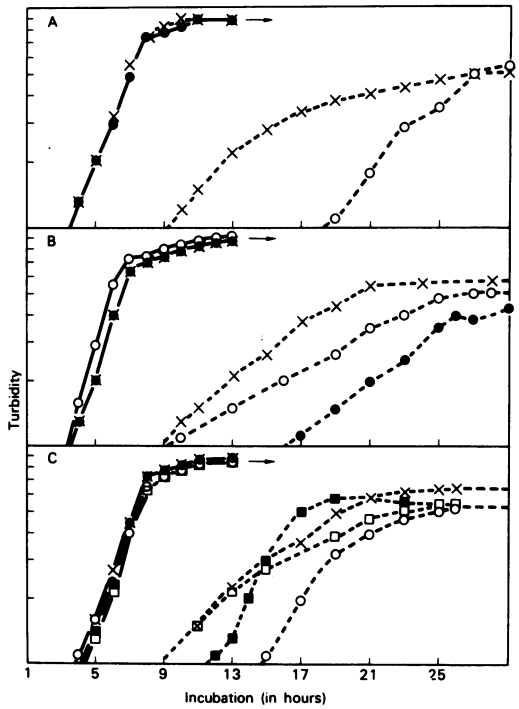

FIG. 2. *Effect of 5.0% sodium cholate on growth of W677 carrying a plasmid autonomously. Cultures at the late logarithmic phase were diluted 10^{-4} with fresh Penassay broth with or without 5.0% sodium cholate and incubated at 30°C with shaking. The optical density was recorded with an automatic photometer. (——) Without sodium cholate; (---) with 5.0% sodium cholate. (A) ×, W677; ○, W677 carrying N3 autonomously. (B) ×, W677; ○ and ●, W677 carrying R6K autonomously. (C) ×, W677; ○, W677 carrying R386 autonomously; □ and ■, two representative derivatives made by curing R386 from strain W677 carrying the plasmid autonomously.*

which the integrated plasmid function is suppressed seems to have been verified by our recent isolation of a sodium cholate-resistant mutant from an Hfr strain made with Rts1. This mutant was shown to carry Rts1 in an integrated state but to be thermosensitive as if the plasmid existed autonomously (5).

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