ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1977, p. 423-426

Vol. 12, No. 3
Printed in U.S.A.

NOTES

Elimination of Plasmids from Several Bacterial Species by Novobiocin

GAIL L. McHUGH AND MORTON N. SWARTZ*
Infectious Disease Unit, Massachusetts General Hospital, Boston, Massachusetts 02114

Received for publication 5 April 1977

Certain plasmids can be eliminated by exposure to growth-inhibiting concentrations of novobiocin. Novobiocin cured 8 of 14 plasmids (13 R-plasmids and an F' *lac*) among one or another of four different bacterial hosts.

A variety of compounds (acridine orange, ethidium bromide, acriflavine, sodium dodecyl sulfate, etc.) have been reported as curing agents for plasmids in various bacterial species (19, 3, 13, 20). Many of these are intercalating compounds thought to act by inhibition of plasmid replication through selective binding to plasmid deoxyribonucleic acid (DNA) (11). The recent discovery of an enzyme, DNA gyrase, which introduces negative superhelical turns into double-stranded relaxed closed circular DNA (colicin E1, simian virus 40, phage λ), suggested the possibility of a plasmid-specific site for the action of curing agents (7). Two antibiotics, coumermycin and novobiocin, have been shown to inhibit in vitro the activity of DNA gyrase purified from *Escherichia coli* (8). For this reason, the possible activity of novobiocin as a curing agent has been investigated by using a variety of plasmids in four different bacterial species.

Five of nine plasmids tested in *E. coli* J53 (4) were cured at maximal frequencies of 15 to 37% after 18 h of incubation with novobiocin (Table 1). Concentrations of novobiocin (50 to 175 μg/ml) sufficient to inhibit multiplication or to produce minimal killing appeared necessary to effect plasmid elimination. Curing was defined as the loss on subculture in the absence of novobiocin of all the phenotypic characteristics associated with the plasmid. Susceptibility to curing by novobiocin was shown to individual plasmids belonging to several incompatibility groups (H, FI, FII). Of two H-group plasmids examined, one (R726) could be cured and another (R27) could not. A resistance plasmid (pMG101) originally found in *Salmonella typhimurium* and bearing resistance to $AgNO_3$ and to multiple antibiotics was highly resistant in *E. coli* J53 to curing by novobiocin, whereas a derivative plasmid (pMG102) was quite susceptible in the same *E. coli* host (17).

Transferable resistance plasmids have been found in *Streptococcus faecalis* (14). One such plasmid (pJH1) is extremely stable, showing only 0.04 to 0.20% loss of its antibiotic resistance determinants either spontaneously or on treatment with acriflavine, acridine orange, or ultraviolet irradiation (14). Treatment with novobiocin (8 to 10 μg/ml) eliminated this plasmid at a frequency of 10 to 34% (Table 2). Another transferable resistance plasmid (D. J. Krogstad and R. C. Moellering, personal communication) in a clinical isolate (*Enterococcus* EBC22) could be eliminated by similar treatment with novobiocin. A nontransferable plasmid in a clinical isolate of *Enterobacter cloacae* bearing a Lac marker as well as determinants for resistance to $AgNO_3$ and $HgCl_2$ could be eliminated by concentrations of novobiocin about 30-fold higher than were effective in enterococcal strains (Table 2). This enterobacter plasmid appears to be similar if not identical to that described by Annear et al. (1). Attempts to cure two plasmids (FP2, pMG1) in *Pseudomonas aeruginosa* (Table 2) were unsuccessful, as were attempts to eliminate from *E. coli* a widely transmissible plasmid (RP1) originally found in pseudomonas (Table 1). Lowering of pH levels below 7, previously demonstrated to increase the antibacterial effect of novobiocin (6), did not augment curing activity.

The concentrations of novobiocin that eliminated plasmids in the foregoing experiment were comparable to those inhibiting growth of the host bacterial species. In *E. coli* J53, plasmids were cured by novobiocin concentrations ranging from 50 to 175 μg/ml; the minimal inhibitory concentration of novobiocin for clinical isolates of *E. coli* is about 400 μg/ml (range

TABLE 1. *Effect of novobiocin on plasmids in E. coli*

Strain	Plasmid reference	Plasmid characteristics[a]	Incompatability group	Novobiocin (μg/ml)	Viable colonies/ml[b]	Colonies cured/ colonies examined	% Cured
J53 (R726)	5	Cm Tc	H	0	1.6×10^9	0/200	0
				100	1.2×10^8	1/193	0.5
				125	1.2×10^7	1/20	5.0
				150	2.1×10^6	11/100	11.0
				175	5.9×10^4	15/93	16.1
J53 (R386)	12	Tc	FI	0	1.4×10^9	0/170	0
				150	3.3×10^8	0/50	0
				200	3.0×10^5	24/160	15.0
J53 (R1-16)	12	Cb Cm Sm	FII	0	1.4×10^9	0/100	0
				175	5.0×10^6	34/100	34.0
J53 (pMG102)	—[c]	Sm Tc Ag Te		0	1.5×10^9	12/500	2.4
				20		28/230	12.2
				50	2.0×10^6	60/296	20.3
				75	2.7×10^5	5/143	3.5
				100	4.0×10^4	57/387	14.7
				150	2.9×10^4	11/150	7.3
ECO (Fts-114 *lac*$^+$)	2	F′*lac*		0	8.0×10^8	0/208	0.0
				50	6.0×10^7	1/72	1.4
				150	9.5×10^6	15/104	15.0
				175	2.0×10^6	65/228	29.0
				200	3.0×10^6	101/276	36.6
J53 (R27)	5	Tc	H	100–200	1.5×10^9–4.1×10^4	0/72	0
J53 (R124)	12	Tc	FIV	100–200	1.4×10^9–6.5×10^4	0/100	0
J53 (pMG101)	17	Ap Cm Sm Su Tc Ag Hg Te		50–225	1.3×10^9–1.6×10^5	0/300	0
J53 (RP1)	9	Cb Km Tc	P	100–200	1.3×10^9–3.0×10^4	0/64	0
K12 (RP1)	9	Cb Km Tc	P	50–150	1.0×10^9–6.5×10^5	0/210	0

[a] Antibiotic and metal ion resistance markers: Ap, ampicillin; Cb, carbenicillin; Cm, chloramphenicol; Em, erythromycin; Gm, gentamicin; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline; Ag, silver nitrate; Hg, mercuric chloride; Te, potassium tellurite.

[b] Inocula were overnight cultures (Penassay broth[Difco]) diluted to 10^5 cells/ml. Standing cultures were then incubated for 18 h with varying concentrations of novobiocin, at 37°C, except ECO (Fts-114 *lac*$^+$), which was incubated at 30°C. After appropriate dilutions, cultures were plated on TYE plates and tested for the loss of plasmid markers (21).

[c] G. L. McHugh and M. N. Swartz, unpublished observations on derivative plasmid from pMG101 (17).

TABLE 2. *Effect of novobiocin on plasmids in genera other than E. coli*

Strain	Plasmid reference	Plasmid characteristics[a]	Incompatability group	Novobiocin (μg/ml)	Viable colonies/ml	Colonies cured/ colonies examined	% Cured
Enterobacter sp. L-1 (pMG150)	This paper	Ag Hg Lac		0	2.0×10^9	17/242	7.0
				225	1.7×10^8	21/40	52.5
				250	1.0×10^7	46/149	30.9
				275	1.7×10^7	44/189	23.3
Streptococcus faecalis JH1-18 (pJH1)	14	Em Km Sm		0	6.6×10^8	0/100	0
				6	1.6×10^7	0/50	0
				8	6.0×10^4	17/50	34.0
				10	6.0×10^3	6/58	10.3
Enterococcus EBC22 (pDR1)	—[b]	Km Sm		0	9.0×10^8	0/100	0
				3	6.0×10^5	1/50	2.0
				6	3.0×10^3	6/27	22.2
				10	7.0×10^3	28/100	28.0
				25	3.0×10^3	14/50	28.0
P. aeruginosa PU21 (FP2)	16	Hg	P-8	100–500	3.0×10^9–2.0×10^3	0/150	0
P. aeruginosa PU21 (pMG1)	15	Gm Sm Su Hg	P-2	100–500	1.2×10^9–2.2×10^3	0/150	0

[a] For antibiotic and metal iron resistance markers, see footnote *a*, Table 1.

[b] D. J. Krogstad and R. C. Moellering, unpublished observations.

of 1 to >400) (6). In enterococcal strains JH1-18 and EBC 22 the curing concentrations of novobiocin ranged from 6 to 25 μg/ml; the minimal inhibitory concentration of novobiocin for clinical isolates of enterococci is about 25 μg/ml (range 1 to 50) (6).

To establish that the elimination of resistance determinants represented eradication of

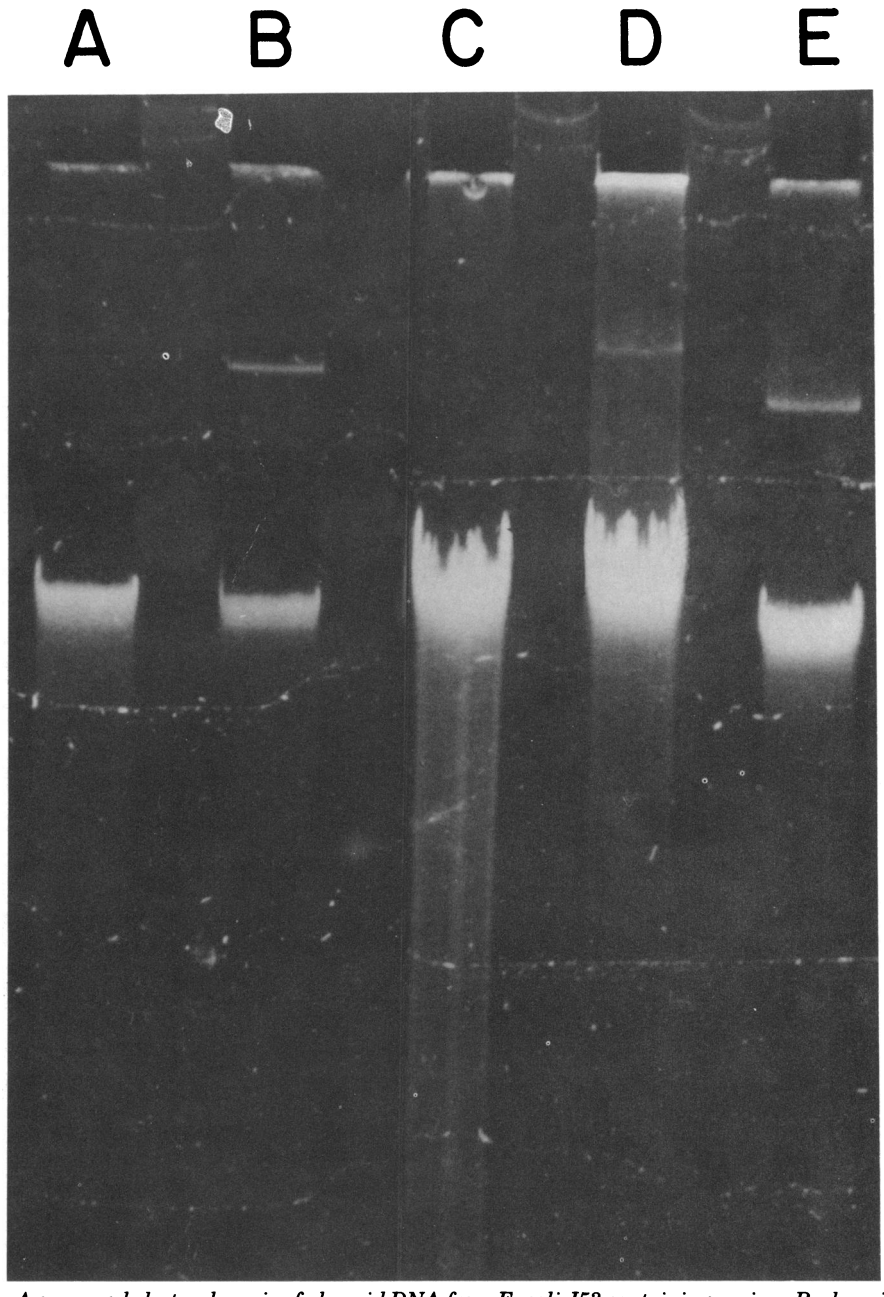

FIG. 1. *Agarose gel electrophoresis of plasmid DNA from E. coli J53 containing various R-plasmids. Forty microliters of ethanol-precipitated DNA from cleared lysates was subjected to electrophoresis (70 to 100 mA, 70 V, for 7 h) in 1.0% agarose using a vertical slab gel (17.5 by 13.5 cm) apparatus. The gel was stained in a solution of ethidium bromide (0.5 μg/ml). (A) Cured E. coli J53 (R1-16); only band consists of chromosomal fragments from E. coli. (B) E. coli J53 (R1-16); upper band, R1-16. (C) Cured E. coli J53 (R386); only band consists of chromosomal fragments from E. coli. (D) E. coli J53 (R386); upper band, R386. (E) E. coli J53 (R1033); upper band, R1033 (molecular weight, 45 × 10^6); second band, chromosomal fragments from E. coli J53.*

the plasmid in its entirety rather than of only specific antibiotic resistance markers, physical evidence for curing was sought. Crude lysates were made from both plasmid-containing and cured *E. coli* J53 (R1-16) and J53 (R386) by a modification of the method of Guerry et al. (10), and were then prepared and subjected to agarose gel electrophoresis by a modification of the

method of Meyers et al. (18). In J53 (R386) a discrete plasmid band is observed, whereas such a band is absent from the same *E. coli* strain cured of its plasmid by treatment with novobiocin (Fig. 1). The plasmid band in J53 (R1-16) has been eliminated in the same *E. coli* strain similarly cured by novobiocin.

Although it is tempting to relate the curing effect of novobiocin to its capacity to inhibit the DNA gyrase, it should be emphasized that there is, as yet, no direct evidence to relate the two activities in vivo. Whether a plasmid-mediated DNA gyrase separate from a host gyrase exists to account for the resistance of certain plasmids to the curing action of novobiocin is unknown and must await purification of DNA gyrase from *E. coli* containing such plasmids.

This work was aided by Public Health Service training grant 5 T01 AI00215-15 from the National Institute of Allergy and Infectious Diseases.

We thank G. A. Jacoby, A. O. Summers, T. R. Korfhagen, and D. J. Krogstad for valuable discussions and suggestions.

LITERATURE CITED

1. **Annear, D. I., B. J. Mee, and M. Bailey.** 1976. Instability and linkage of silver resistance, lactose fermentation and colony structure in *Enterobacter cloacae* from burn wounds. J. Clin. Pathol. 29:441–443.
2. **Beckwith, J. R., and E. R. Signer.** 1966. Transposition of the *Lac* region of *Escherichia coli*. I. Inversion of the *Lac* operon and transduction of *Lac* by ϕ 80. J. Mol. Biol. 19:254–265.
3. **Bouanchaud, D. H., M. R. Scavizzi, and Y. A. Chabbert.** 1969. Elimination of ethidium bromide of antibiotic resistance in enterobacteria and staphylococci. J. Gen. Microbiol. 54:417–425.
4. **Clowes, R. C., and D. Rowley.** 1954. Some observations on linkage effects in genetic recombination in *Escherichia coli* K-12. J. Gen. Microbiol. 11:250–260.
5. **Datta, N., and J. Olarte.** 1974. R-factors in strains of *Salmonella typhi* and *Shigella dysenteriae* I isolated during epidemics in Mexico: classification by compatibility. Antimicrob. Agents Chemother. 5:310–317.
6. **Finland, M., and R. L. Nichols.** 1957. Novobiocin. Antibiotica et Chemotherapia. S. Karger Publisher, Basel, Switzerland.
7. **Gellert, M., K. Mizuuchi, M. H. O'Dea, and H. A. Nash.** 1976. DNA gyrase: an enzyme that introduces superhelical turns into DNA. Proc. Natl. Acad. Sci. U.S.A. 73:3872–3876.
8. **Gellert, M., M. H. O'Dea, T. Itoh, and J. Tomizawa.** 1976. Novobiocin and coumermycin inhibit DNA supercoiling catalyzed by DNA gyrase. Proc. Natl. Acad. Sci. U.S.A. 73:4474–4478.
9. **Grinsted, J., J. R. Sanders, L. C. Ingram, R. B. Sykes, and M. H. Richmond.** 1972. Properties of an R-factor which originated in *Pseudomonas aeruginosa* 1822. J. Bacteriol. 110:529–537.
10. **Guerry, P., D. J. LeBlanc, and S. Falkow.** 1973. General method for the isolation of plasmid deoxyribonucleic acid. J. Bacteriol. 116:1064–1066.
11. **Hahn, F. E., and J. Ciak.** 1976. Elimination of resistance determinants from R-factor R1 by intercalative compounds. Antimicrob. Agents Chemother. 9:77–80.
12. **Hedges, R. W., and N. Datta.** 1972. R 124, an Fi^+ R-factor of a new compatibility class. J. Gen. Microbiol. 71:403–405.
13. **Hirota, Y.** 1960. The effect of acridine dyes on mating type factors in *Escherichia coli*. Proc. Natl. Acad. Sci. U.S.A. 46:57–64.
14. **Jacob, A. H., and S. J. Hobbs.** 1974. Conjugal transfer of plasmid-borne multiple antibiotic resistance in *Streptococcus faecalis* var. *zymogenes*. J. Bacteriol. 117:360–372.
15. **Jacoby, G. A.** 1974. Properties of R-plasmids determining gentamicin resistance by acetylation in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 6:239–252.
16. **Jacoby, G. A., and J. A. Shapiro.** 1977. Plasmids studied in *Pseudomonas aeruginosa* and other pseudomonads. *In* A. T. Bukhari, J. A. Shapiro, and S. Adhya (ed.), DNA insertion elements, plasmids, and episomes. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
17. **McHugh, G. L., R. C. Moellering, C. C. Hopkins, and M. N. Swartz.** 1975. *Salmonella typhimurium* resistant to silver nitrate, chloramphenicol, and ampicillin. Lancet i:235–239.
18. **Meyers, J. A., D. Sanchez, L. P. Elwell, and S. Falkow.** 1976. Simple agarose gel electrophoretic method for the identification and characterization of plasmid deoxyribonucleic acid. J. Bacteriol. 127:1529–1537.
19. **Riva, S., A. Fietta, M. Berti, L. G. Silvestri, and E. Romero.** 1973. Relationships between curing of the F episome by rifampin and by acridine orange in *Escherichia coli*. Antimicrob. Agents. Chemother. 3:456–462.
20. **Tomoeda, M., M. Inuzuka, N. Kubo, and S. Nakamura.** 1968. Effective elimination of drug resistance and sex factors in *Escherichia coli* by sodium dodecyl sulfate. J. Bacteriol. 95:1078–1089.
21. **Watanabe, T., and T. Fukasawa.** 1961. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. II. Elimination of resistance factors with acridine dyes. J. Bacteriol. 81:679–683.