PREFERENTIAL EFFECTS OF ANTIBIOTICS ON *E. COLI* STRAINS CARRYING PLASMIDS

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R Factors, capable of conferring multiple drug resistance on their host bacteria, are distributed among many species of bacteria isolated from human beings, livestock[1,2] and cultured fish[3,4], creating problems in practical medicine and hygiene. It is therefore an urgent problem to find agents that can eliminate R factors from their host bacteria or that can preferentially kill bacteria possessing R factor.

We have reported previously that the antibiotic macarbomycin perferentially kills *Escherichia coli* strains carrying an R factor, resulting in the elimination of resistant bacteria from mixed cultures consisting of both drug-resistant and sensitive cells[5,6]. This result raised the possibility that bacterial strains carrying R factors may be removed as a result of their susceptibility to certain chemotherapeutic agents.

We have examined various antibiotics for their ability to inhibit the growth of bacteria carrying R factors. The method was similar to that previously described[5]; *Escherichia coli* K12 strain, W2241 (*lac*$^-$, lactose non-fermentable) and W2241 carrying R-factor R100 (or F-*lac.tet* factor) were used. R-factor R100 confers resistance to tetracycline (TC), chloramphenicol (CM), streptomycin (SM) and sulfanilamide (SA) on its host. F-*lac.tet* is a recombinant between an F-*lac* episome and the TC-resistance determinant (*tet*) of R_{10}(TC. CM. SM. SA)[7]. Antibiotics to be

Table 1. Effect of antibiotics on the growth of *E. coli* carrying plasmids

Antibiotics	Mixed culture					
	W2241/F-*lac. tet*$^+$			W2241/R100$^+$		
	Added (μg/ml)	Growth	Percent of sensitive cells in population*	Added (μg/ml)	Growth	Percent of sensitive cells in population*
Althiomycin	1	##	48	0.25	##	40
Azomycin	20	#	65	20	#	—
Daunomycin	0.5	#	88	0.5	#	—
Diumycin	100	#	84	100	#	—
Griseolutein A	1	##	30	3	##	90
Iyomycin	5	##	64	5	##	16
Kasugamycin	5	#	68	10	#	—
Mitomycin C	0.1	##	34	0.2	##	80
Neopluramycin	5	##	68	5	##	28
Netropsin	2	##	41	10	#	—
Panosialin	200	##	22	400	#	—
Phleomycin	0.5	#	—	0.2	##	22
Streptothricin A-249	10	#	98	10	#	—
Xanthomycin	5	##	44	10	#	—

One ml of mixed culture in BHI broth was prepared by inoculating a mixture of *E. coli* W2241 carrying a plasmid (10^8 cells) and W2241 without plasmid (10^5 cells) and by incubating with or without antibiotic at 37°C. After overnight cultivation, the growth level of the culture was noted, and the number of W2241 cells without plasmid counted as drug-sensitive cells by the replica plating method[8].

Growth; ##, $10^9 \sim 10^9$; #, $10^7 \sim 10^9$; +, $10^8 \sim 10^7$ cells per ml.

* The ratio in a control culture without antibiotic was less than 10%. —, less than 10%.

examined were added to a mixed culture of W2241 and W2241 carrying R100 (or F-*lac. tet*), and their effect was examined by comparing the number of drug-sensitive survivors with drug-resistant ones. The results are shown in the Table 1. Among 14 antibiotics, 5 were found to have an inhibitory effect on both W2241 R100$^+$ and W2241 F-*lac.tet*$^+$, 8 were only effective against W2241 F-*lac. tet*$^+$ and the remaining one was only effective against W2241 R100$^+$. The strain carrying F-*lac.tet* was more susceptible to antibiotics than that carrying R100. Three antibiotics, streptothricin, daunomycin and diumycin efficiently inhibited the growth of W2241 F-*lac. tet*$^+$ but not W2241 R100$^+$. On the other hand, mitomycin C and griseolutein were especially effective against W2241 R100$^+$, and also inhibited the growth of W2241 F-*lac.tet*$^+$ to some extent. The difference in susceptibility to antibiotics may be due to changes in structure of bacterial cells subsequent to infection with either an R or F factor.

Next, we examined the plasmid-curing effect of these antibiotics on *E. coli* W2241 carrying a plasmid. W2241 R100$^+$ or W2241 F-*lac. tet*$^+$ was cultured at 37°C in BHI (Difco) broth supplemented with various concentrations of each antibiotic. After 18-hour incubation, the culture which contained the highest concentration of drug showing bacterial growth, was diluted and spread on EMB-lactose agar[8]; colonies were replica-plated[9] onto antibiotic plates and scored for resistance characters. It was found that about 1% of W2241 F-*lac.tet*$^+$ cells lost the plasmid after incubation with mitomycin C, the other antibiotics failed to eliminate F-*lac.tet* or R100 factor from their hosts. This indicates that the preferential drug-inhibition of W2241 carrying a plasmid in the mixed culture can be explained by a preferential killing or static effect on bacteria carrying a plasmid.

There are two modes by which a plasmid can be eliminated from a bacterial population; one is the elimination of plasmid from its host by inhibition of plasmid DNA replication[10,11,12], and the other is preferential inhibition of the growth of bacterial cells carrying a plasmid, resulting in selection for cells which had spontaneously lost the plasmid[13,14]. Using the mixed-culture method as described above, it is possible to test drugs for their ability to eliminate bacterial cells carrying an R factor from a bacterial population.

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