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ELIMINATION OF R PLASMIDS PRESENT IN POULTRY BACTERIA BY ACRIDINE ORANGE

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ABSTRACT

R-plasmids of Gram negative bacteria isolated from poultry in Karachi were studied for their curing by acridine orange in *E. coli* AB712. Of 16 R-plasmids studied, 4 were lost by treatment with acridine orange.

INTRODUCTION

Some bacteria possess extrachromosomal genetic determinants known as R plasmids that confer resistance to one or several antimicrobial drugs (Khatoon & Mohammad, 1986). Plasmid genes for antimicrobial resistance often control the formation of enzymes capable of destroying the antimicrobial drugs.

R plasmids may be transferrable or non transferrable. Transferrable R plasmids are detected by conjugation where as the existence of non transferrable R plasmids is determined by their loss from host cell spontaneously due to some errors in replication or segregation (Derylo & Lorkiewicz, 1970; Novick, 1969). Such losses are increased when the cells are treated with certain chemical agents such as acridine orange or ethidium bromide (Iyer & Iyer, 1969; Mitsuhashi et al., 1961; Watanabe & Fukasawa, 1961). This phenomenon is referred to as curing and has been used to ascertain the plasmid associated nature of genes (Bouanchand et al., 1969; Hirota, 1960; Michel-Briand & Laporte, 1985; Ott et al., 1971; Salisbury et al., 1972; Watanabe & Lyang, 1962). However, the extent of spontaneous loss or segregation is a property of a particular plasmid. The losses are also more frequent when Shigella or Salmonella host strains are used (Mitsuhashi et al., 1961; Watanabe & Fukasawa, 1961). Susceptibility to curing agents also varies among plasmids. Some plasmids are not curable by ethidium bromide or acridine orange (Amir & Khatoon, 1976; Khatoon, 1987).

Sixteen R plasmids of Gram negative bacteria isolated from poultry in Karachi

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(Ansari & Khatoon, 1994) were studied for their curing by acridine orange. Previously, we found these R plasmids to be incurable by spontaneous segregation (Ansari & Khatoon, unreported data). Four of these R plasmids were cured by treatment with acridine orange (Table 1).

Some of the R plasmids incurable by treatment with acridine orange were cured by treatment with ethidium bromide and will be reported elsewhere.

MATERIALS AND METHODS

Bacterial Strain

All the R plasmids were studied for their curing by acridine orange in *E. coli* AB712 provided by E. Adelberg of U.S.A.

Media

MacConkey's agar (M.A.) was from E. Merck, Germany. Nutrient agar (N.A.) had the following composition: Beef extract 3 gms, peptone 10 gms, NaCl 5 gms, agar agar 20 gms, distilled water 100ml, pH 7.Nutrient broth (N.B.). It had the composition similar to N.A. except that agar agar was not added.

Antibiotics and other chemicals

The antibiotics used were: ampicillin trihydrate, chloramphenicol levo (Opal Laboratories Ltd., Karachi), furazolidone (Risma Laboratories, Karachi), gentamycin sulphate (Aspro Nicholas Ltd., Karachi), kanamycin sulphate (Continental Pharma, Belgium), neomycin sulphate (Glaxo Laboratories, Karachi), polymyxin B, streptomycin sulphate (Pfizer Laboratories, Karachi) and tetracycline HCl (Lederle Laboratories, Karachi).

All the antibiotics except chloramphenicol and furazolidone were dissolved in distilled water to give a concentration of 10 mg/ml. Chloramphenicol and furazolidone were dissolved in ethyl alcohol and dimethyl formamide respectively.

Ethyl alcohol was from E. Merck, Germany and dimethyl formamide came as a gift from Prof. Salim-uz-Zaman Siddiqui FRS of H.E.J. Research Institute of Chemistry, University of Karachi.

Antibiotics were sterilized by millipore filtration and kept frozen when not in use. The concentration of working solution of antibiotics was 50 μg/ml.

Curing of R-plasmids by acridine orange

Techniques were similar as described by Khatoon (1971). The R plasmids were studied for curability in *E. coli* AB 712. L.B. Broth (5 ml) tubes containing 60 μg/ml of acridine orange were inoculated with log phase cultures of *E. coli* host bearing R plasmid to give a 20 fold dilution. A control tube lacking acridine orange was also included. All the tubes were incubated overnight at 37°C. The contents of the tube with acridine orange were plated on M.A. to obtain isolated colonies. Some 100 colonies from each plating were grided onto M.A. plate. After overnight incubation at 37°C, these were replicated on antibiotic containing plates alongwith the control to check for the loss (or it's absence) of antibiotic resistance determinants.

RESULTS AND DISCUSSION

Table-1 lists the R plasmids of Gram negative bacteria isolated from poultry in Karachi which were cured by treatment with acridine orange. Of the 16 R plasmids studied, 4 were lost by treatment with acridine orange. All of these R plasmids were earlier found to be stable during spontaneous segregation (Ansari & Khatoon, unreported data). Some of the R-plasmids incurable by treatment with acridine orange were found to be stable even after treatment with ethidium bromide. Results of this findings will be reported elsewhere.

The stability of R plasmids after treatment with chemical agents reflects that these are very dangerous from epidemiologic point of view as the diseases caused by organisms bearing these R plasmids (by conjugation) will be difficult to control.

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Table 1
Curing of R plasmids in *Escherichia coli* AB 712 with acridine orange

R plasmids	*Antibiotic Resistance Determinants	Conc. of Acridine Orange	No. of Colonies Tested	Number of Colonies with Lost Marker(s)	*Markers Lost	% Loss
pFK-1	KNP	60 μg/ml	100	87	KNP	87.0
pFK-2	KN	60 μg/ml	100	-	-	0.0
pFK-4	KN	60 μg/ml	100	1	KN	1.0
pFK-5a	A	60 μg/ml	100	-	-	0.0
pFK-5b	KN	60 μg/ml	100	-	-	0.0
pFK-6	KT	60 μg/ml	100	1	KT	1.0
pFK-7	KNT	60 μg/ml	100	-	-	0.0
pFK-8a	AT	60 μg/ml	100	-	-	0.0
pFK-10a	KN	60 μg/ml	100	1	KN	1.0
pFK-10b	T	60 μg/ml	100	-	-	0.0
pFK-13	T	60 μg/ml	100	-	-	0.0
pFK-17	T	60 μg/ml	100	-	-	0.0
pFK-18	KNT	60 μg/ml	100	-	-	0.0
pFK-24	T	60 μg/ml	100	-	-	0.0
pFK-29	T	60 μg/ml	100	-	-	0.0
pFK-30	FKN	60 μg/ml	100	-	-	0.0

*A = ampicillin
F = furazolidone
K = kanamycin
N = neomycin
P = polymyxin B
T = tetracycline

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