

CURING OF R PLASMIDS PRESENT IN GRAM NEGATIVE BACTERIA ISOLATED FROM POULTRY IN KARACHI BY ETHIDIUM BROMIDE

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ABSTRACT

R plasmids of Gram negative bacteria isolated from poultry in Karachi were studied for their curing by ethidium bromide (EBr) in *E. coli* AB 712. Of 12 R plasmids studied. 3 were lost by treatment with ethidium bromide.

INTRODUCTION

Bacteria often contain in addition to a large, circular chromosome, various small, autonomous replicating cyclic DNA molecules, called plasmids. Among various plasmids present in bacteria R plasmids are very significant as they confer resistance to one or several antibiotics and thus pose a threat to chemotherapy (Khatoon & Mohammed. 1986).

R plasmids may be classified as transferrable or non transferable. Transferable R plasmid are detected by conjugation where as non transferrable R plasmids are detected by their loss from host cell spontaneously due to some errors in replication or segregation (Derylo & Lorkiewiez, 1970; Novick, 1969). Treatment of cells with certain chemical agents such as acridine orange or ethidium bromide enhances the elimination of the plasmid from host cell (Iyer & Iver, 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-Brand & 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 treatment with acridine orange or ethidium bromide (Amir & Khatoon, 1976; Khatoon, 1987).

Twelve R plasmids of Gram negative bacteria isolated from poultry in Karachi (Ansari & Khatoon, 1994) were studied for their curing by ethidium bromide (EBr). Previously we found these R plasmids to be incurable by acridine orange (Ansari & Khatoon, 1996). Three of these R plasmids were cured by treatment with ethidium bromide (Table 1).

All these R plasmids were also incurable by spontaneous segregation and will be reported elsewhere.

MATERIALS AND METHODS

Bacterial strain:

All the R plasmids were studied for their curing by ethidium bromide 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 compositions Beef extract 3 gms. peptone 10 gms. NaCl 5 gms. agar agar 20 gms, distilled water 1000 ml. pH 7. Nutrient broth (N.B.) had the same composition as N.A. except that agar agar was not added.

Antibiotics and other chemicals:

The antibiotics used were: ampicillin trihydrate (Opal Laboratories Ltd., Karachi), kanamycin sulphate, neomycin sulphate (Glaxo Laboratories, Karachi), polymyxin B (Pfizer Laboratories, Karachi) and tetracycline HCl (Lederle Laboratories, Karachi).

All the antibiotics were dissolved in distilled water to give a concentration of 10 mg/ml and 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 ethidium bromide:

Techniques were similar as described by Khatoon (1971). The R plasmids were studied for curability in *E. coli* AB712. N.B. broth (5 ml) tubes containing graded concentration of ethidium bromide (EBr) were incubated with log phase cultures of *E. coli* host bearing R plasmids to give a 20 fold dilution. A control tube lacking EBr was always included. All the tubes were incubated overnight at 37°C. The contents of the control tube and of the EBr-containing tubes were plated on M.A., to obtain isolated colonies. Some 100 colonies from each plating were streaked onto M.A. plates. After overnight incubation at 37°C, these were replicated on antibiotics containing plates to check for the loss (or its 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 ethidium bromide (EBr). Of the 12 R plasmids studied, 3 were lost by treatment with EBr. All of these R plasmids were earlier found to

be stable during spontaneous segregation (Ansari & Khatoon, unreported data) and treatment with acridine orange (Ansari & Khatoon, 1996).

The stability of R plasmids after treatment with chemical agents is alarming from view point of chemotherapy as diseases caused by organisms bearing these R plasmids will be difficult to control.

Table I
Curing of R plasmids in *E. coli* AB712 with Ethidium bromide¹

R-plasmid	Antibiotic* Resistance Determinants	% of colonies with lost marker(s) ²	Markers lost
pFK-2	KN	0.0	-
pFK-5a	A	0.0	-
pFK-5b	KN	0.0	-
pFK-7	KNT	0.0	-
pFK-8a	AT	0.0	-
pFK-10b	T	5.0	T
pFK-13	T	0.0	-
pFK-17	KNT	9.0	KNT
pFK-18	KNT	0.0	-
pFK-24	T	2.0	T
pFK-29	T	0.0	-
pFK-30	PKT	0.0	-

*A=ampicillin, K=kanamycin, N=neomycin, P=polymyxin B and T=tetracycline.

¹EBr was used in concentrations of 30, 50, 100, 200 & 300 µg/ml.

²100 colonies were tested.

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REFERENCES

- Amir A.S. and Khatoon H. (1976). *J. Sc. Kar. Univ.* **3**: 84.
- Ansari F.A. and Khatoon H. (1994). *Indian J. Exp. Biol.* **32**: 211.
- Ansari F.A. and Khatoon H. (1996). *Pakistan Journal of Pharmacology.* **13**(1): 13.
- Bouanehand D.H., Scavizzi M.R. and Chabbert Y.A. (1969). *J. Gen. Microbiol.* **54**: 417.
- Dorylo M. and Lorkiewiez Z. (1970). *Acta Microbiologica Polonica Ser.* **19**: 165.
- Hirota Y. (1960). *Proc. Natl. Acad. Sc. U.S.A.* **46**: 57.
- Iyer R.V. and Iyer V.N. (1969). *J. Bacteriol.* **100**: 605.
- Khatoon H. (1971). Ph.D. thesis, Department of Microbiology & Immunology, University of Ottawa, Canada.
- Khatoon H. and Mohammad S.B.A. (1986). *Indian J. Exp. Biol.* **24**: 1.
- Khatoon H. (1987). *Indian J. Exp. Biol.* **25**: 710.
- Michel-Briand Y. and Laporte J.M. (1985). *Microbiol. Hung.* **32**: 87.
- Mitsuhashi S., Harada K. and Kameda M. (1961). *Japan J. Exp. Med.* **31**: 119.
- Novick R.P. (1969). *Bacteriol. Rev.* **33**: 210.
- Ott J.L., Short L.J. and Holmes D.H. (1971). *Annals N.Y. Academy of Sciences.* **182**: 312.
- Salisbury V., Hedges R.W. and Datta N. (1972). *J. Gen. Microbiol.* **70**: 443.
- Watanabe T. and Fukasawa T. (1961). *J. Bacteriol.* **81**: 679.
- Watanabe T. and Lyang K.W. (1962). *J. Bacteriol.* **84**: 422.