ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1976, p. 762-764

Vol. 10, No. 4
Printed in U.S.A.

NOTES

Inhibition of Bacteriophage Lambda, T1, and T7 Development by R Plasmids of the H Incompatibility Group

DIANE E. TAYLOR* AND ROBERT B. GRANT

Research Institute and Department of Bacteriology, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8

Received for publication 7 June 1976

R plasmids from chloramphenicol-resistant salmonella from Ontario are shown to belong to the H_2 incompatibility subgroup and to mediate a broad-spectrum, phage inhibition function.

In the Mexican typhoid epidemic of 1972 to 1973 and in typhoid outbreaks in India and Vietnam, many isolates of the causative organism were found to be resistant to chloramphenicol (3, 7, 8). This fact has obvious medical significance in view of the previous, long-standing position of chloramphenicol as the drug of choice for the treatment of typhoid fever. The resistance was mediated by transmissible drug resistance factors, which were found to belong to a new incompatibility group, designated "H" (6). Subsequently, two subgroups of H plasmids were identified: H_1 plasmids, which are incompatible with F factors in the autonomous state, and H_2 plasmids, which are compatible with F (10). Both H_1 and H_2 plasmids are fi^- and exhibit thermosensitive transfer (10, 11). Both types have been found to have a wide distribution, occurring in various salmonella isolates throughout the world (1).

Recent studies in our laboratory of antibiotic resistance in nontyphoid salmonella from the province of Ontario in Canada have revealed a high incidence of drug resistance plasmids. In Ontario during the summer 1974, 12% of 589 human salmonella and 37% of 204 animal salmonella isolates were resistant to chloramphenicol; the majority of the resistance was transferable to *Escherichia coli* and mediated by a plasmid that also coded for resistance to kanamycin, streptomycin, and tetracycline (Tc), although a few plasmids had determinants for chloramphenicol and Tc only (Grant and Dimambro, unpublished data). In this communication, we demonstrate that these plasmids from Ontario salmonella belong to the H_2 subgroup and that they mediate an unusual phenotype of phage inhibition; they inhibit the growth of a number of bacteriophages in a manner phenotypically similar to the way plasmid F inhibits phage T7.

The plasmids employed in this study are listed in Table 1. The thermosensitive transfer phenotype of each plasmid was demonstrated by simultaneous overnight matings at 26 and 37°C with an *E. coli* donor and an *E. coli* recipient strain by a method described previously (4). In each case the frequency of recombinants expressed as a fraction of total recipient cells was 500- to 50,000-fold greater at 26°C (10^{-1} to 10^{-4}) than at 37°C (10^{-5} to 10^{-8}). This kind of thermosensitive transfer is characteristic of H plasmids (6).

Since the chloramphenicol resistance plasmids from Ontario all exhibit a similar thermosensitivity of transfer, incompatibility experiments were performed between a single member of the Ontario sample, a Tc-sensitive segregant of pAS251-2, pAS251-2Tc$^-$, obtained by mutagenesis with ethyl methane sulfonate, and a standard H plasmid, R27 (Tc$^+$) (5). Transfers were performed in both directions, and the segregation of plasmids from doubly R$^+$ clones was monitored during the growth of the recombinants in nonselective medium. The H_1 standard plasmid and pAS251-2Tc$^-$ were found to be incompatible. The absence of incompatibility with plasmid F places the Ontario H plasmid in the H_2 subgroup.

We have screened for the phage inhibition phenotype with nine different bacteriophages (Table 2). Titrations were performed concurrently with R$^+$ and R$^-$ cells, using three different hosts, namely, two *E. coli* K-12 derivatives and one *E. coli* C strain. All of the H_2 but none of the H_1 subgroup plasmids express the "phage reduction" phenotype. The lambdoid phages ϕ80 and ϕ21 show the greatest reduction in the

TABLE 1. *H plasmids employed in this study*

Plasmid designation	Resistance pattern[a]	Subgroup	Original host	Place and data of origin	Source[b]
pSD12	Cm Tc	H_2	*Salmonella typhimurium*	Canada, 1974	OPHL
pSD50	Cm Tc	H_2	*S. typhimurium*	Canada, 1974	OPHL
pSD32	Cm Ka Sm Tc	H_2	*S. typhimurium*	Canada, 1974	OPHL
pSD62	Cm Ka Sm Tc	H_2	*S. typhimurium*	Canada, 1974	OPHL
pSD88	Cm Ka Sm Tc	H_2	*S. typhimurium*	Canada, 1974	OPHL
pSD114	Cm Ka Sm Tc	H_2	*S. anatum*	Canada, 1974	OPHL
pAS251-2	Cm Ka Sm Tc	H_2	*S. typhimurium*	Canada, 1974	HSC
pRG1241	Cm Sm Sp Su Tc	H_1	*S. typhi*	Mexico, 1972	H.W.S.
pRG1252	Cm Sm Sp Su Tc	H_1	*S. typhi*	Vietnam, 1972	H.W.S.
R27	Tc	H_1	*S. typhimurium*	England, 1961	N.D.

[a] Cm, Chloramphenicol; Ka, kanamycin; Sm, streptomycin; Sp, spectinomycin; Su, sulfonamide; Tc, tetracycline.

[b] OPHL, Ontario Public Health Laboratory; HSC, The Hospital for Sick Children, Toronto; H.W.S., H. Williams Smith; N.D., N. Datta.

TABLE 2. *Effect of H_2 plasmids on bacteriophage infection of host*

Phage	Relative EOP (R^+/R^-)	Plaque morphology
λ[a]	10^{-4}	Pinpoint
φ80	10^{-5}	Pinpoint
21	10^{-5}	Pinpoint
P2	10^{-1}-10^{-4}[b]	Pinpoint
T1	10^{-1}	Small center, small halo
T7	10^{-1}	Small, no halo
T4	1	Normal
P1	1	Normal

[a] λ*vir*; λ*cI*.

[b] Varies with host strain.

efficiency of plating (EOP), whereas phages T4 and P1 are unaffected. The phage reduction effect, however, is not the usual restriction and modification phenomenon encountered in plasmids of both fi^+ and fi^- specificities (9), since lysates of the inhibited phages prepared from confluent lysis plates of R^+ cells do not overcome the restriction. The phages do not become modified or adapted to the R^+ host.

When λ infection in R^+ and R^- hosts was compared, adsorption was normal in both hosts, 65 to 80% after 5 min starting with 10^6 phage and 5×10^7 cells/ml, the unadsorbed particles being measured after centrifugation of the infected cells. One-step growth experiments with anti-λ serum showed that abortive infection occurred in 90 to 95% of phage-infected R^+ cells, whereas 5 to 10% had a normal latent period (45 min) and burst size (about 100 plaque-forming units). To investigate the possibility that the cells that had a normal burst of phage had lost their plasmid before being infected, MacConkey plates were inoculated with R^+ cells immediately prior to phage infection. The colonies were subsequently replica plated to a medium that contained 16 μg of chloramphenicol per ml, and it was determined that none of the 1,000 colonies tested had lost the plasmid.

The H_2 plasmids reduce the EOP of T7 by 10-fold and change the plaque size, producing an effect resembling that of a particular mutant of the F factor (2). In addition, unlike the F factor, they markedly reduce the EOP of lambdoid phages. The defect is not in adsorption, but in some other stage of infection. One fi^- R factor has been described that inhibits the growth of T1 and λ by a mechanism thought to be restriction without modification (12, 13). Further studies are required to determine whether the H_2 phage reduction phenomenon depends on the restriction of phage deoxyribonucleic acid without modification, permeability effects, or some other unrelated mechanism. Whatever the mechanism, the phage reduction function provides a valuable genetic marker for H_2 plasmids. Together with thermosensitivity of transfer it serves to identify preliminarily this important group of resistance plasmids found in salmonella.

We thank Aina Shapley for technical assistance, S. Toma and H. Lior of the Ontario Public Health Laboratory for salmonella strains, C. Fuerst for anti-λ serum and phage 21, and H. Williams Smith and N. Datta for plasmids.

Diane E. Taylor is a recipient of a postdoctoral fellowship from the Medical Research Council of Canada.

LITERATURE CITED

1. **Anderson, E. S.** 1975. The problem and implications of chloramphenicol resistance in the typhoid bacillus. J. Hyg. 74:289-299.
2. **Blumberg, D. D., C. T. Mabie, and M. H. Malamy.** 1976. T7 protein synthesis in F-factor-containing

cells: evidence for an episomally induced impairment of translation and its relation to an alteration in membrane permeability. J. Virol. 17:94–105.
3. **Butler, T., N. N. Linh, K. Arnold, and M. Pollack.** 1973. Chloramphenicol-resistant typhoid fever in Vietnam associated with R factor. Lancet ii:983–985.
4. **Coetzee, J. N., N. Datta, and R. W. Hedges.** 1972. R factors from *Proteus rettgeri*. J. Gen. Microbiol. 72:543–552.
5. **Datta, N.** 1975. Epidemiology and classification of plasmids, p. 9–15. *In* D. Schlessinger (ed.), Microbiology–1974. American Society for Microbiology, Washington, D.C.
6. **Grindley, N. D. F., J. N. Grindley, and E. S. Anderson.** 1972. R factor compatibility groups. Mol. Gen. Genet. 119:287–297.
7. **Olarte, J., and E. Galindo.** 1973. *Salmonella typhi* resistant to chloramphenicol, ampicillin, and other antimicrobial agents: strains isolated during an extensive typhoid fever epidemic in Mexico. Antimicrob. Agents Chemother. 4:597–601.
8. **Paniker, C. K. J., and K. N. Vimala.** 1972. Transferable chloramphenicol resistance in *Salmonella typhi*. Nature (London) 239:109–110.
9. **Roulland-Dussois, D., R. Yoshimori, P. Greene, and M. Betlach, H. M. Goodman, and H. W. Boyer.** 1975. F factor-controlled restriction and modification of deoxyribonucleic acid, p. 187–198. *In* D. Schlessinger (ed.), Microbiology–1974. American Society for Microbiology, Washington, D.C.
10. **Smith, H. R., N. D. F. Grindley, G. W. Humphreys, and E. S. Anderson.** 1973. Interaction of group H resistance factors with the F factor. J. Bacteriol. 115:623–628.
11. **Smith, H. W.** 1974. Thermosensitive transfer factors in chloramphenicol-resistant strains of *Salmonella typhi*. Lancet ii:281–282.
12. **Watanabe, T., H. Nishida, C. Ogata, T. Arai, and S. Sato.** 1964. Episome-mediated transfer of drug resistance in Enterobacteriaceae. VII. Two types of naturally occurring R factors. J. Bacteriol. 88:716–726.
13. **Watanabe, T., T. Takano, T. Arai, H. Nishida, and S. Sato.** 1966. Episome-mediated transfer of drug resistance in *Enterobacteriaceae*. X. Restriction and modification of phages by fi^- R factors. J. Bacteriol. 92:477–486.