Loss of Colicinogeny in Escherichia coli Strains
Infected by Certain Resistance Factors

V. KRČMÉRY, C. HURWITZ, AND P. FREDERICQ

Research Institute of Hygiene, Bratislava, Czechoslovakia, Research Service, Veterans Administration Hospital, Albany, New York, and University of Liège, Belgium

Received for publication 2 March 1970

The original observation that in wild-type colicinogenic Escherichia coli strains the introduction of some R factors abolish their colicin production was studied in certain col" strains bearing well-defined col factors. Two resistance (R) factors were used and introduced by conjugation in these strains, namely the 222 factor of Watanabe and a Salmonella typhimurium ST factor (coding for resistance to streptomycin and tetracycline only). The introduction of above mentioned R factors abolished the colicin production of col" strains most probably by elimination of col factors. All col factors, however, were not equally susceptible to elimination by the R factors tested, since colicin production in ML strains was abolished by infection by the 222 factor but not by the R factor of S. typhimurium ST, which is able to eliminate other col factors.

During an investigation of the incidence of tetracycline resistance caused by resistance-transfer factors in enterobacteria isolated from swine feces (7), a loss of colicinogeny was observed in a number of Escherichia coli strains after infection with resistance (R) factors carrying resistance to streptomycin (S) and tetracycline (T). We have extended these findings by studying the effect of two different R factors on colicinogeny of other col" strains.

MATERIALS AND METHODS

Strains. Colicinogenic strains were as follows. (i) E. coli 2H 1327, a wild-type strain isolated from swine feces, was used. This strain was found to produce colicin B. As recipient strain for transfer of R factors, we used a nalidixic acid-resistant mutant isolated from this strain. (ii) E. coli Y 20 (Col E 1), a lac- strain which produces colicin E 1, was obtained from S. E. Luria. (iii) The following variants of E. coli ML 35 were tested, which were all found to produce colicin E 1: E. coli ML 35 Ns, a nalidixic acid-resistant mutant isolated from lac- sensitive strain, obtained from H. Rosenkranz; E. coli ML 35 Cn, a S-resistant mutant isolated from a S-dependent strain obtained from H. Rosenkranz, resistant to 1,000 µg of S per ml; E. coli ML 35 Cc, ile- tyr+, a deficient mutant isolated from E. coli ML 35 Cc.

R-factor-bearing donor strains were as follows. (i) E. coli CHS-2 (222), resistant to S, T, chloramphenicol (C), and the sulfonamides (Su) was used. This is T. Watanabe's donor strain which usually transfers these resistances as a unit (9). It is sensitive to phages T1, T5, and BF3, which can be used for its identification. The resistance levels of this strain on MacConkey agar are 50 µg of T per ml, 15 µg of S per ml, and 100 µg of chloramphenicol per ml. Sulfinamide resistance was not estimated nor checked after transfer. (ii) Salmonella typhimurium 5445, isolated from pig feces (8), transfers an R-factor ST at a frequency of about 10^-4 to E. coli 2H 1327 and Y 20. (iii) E. coli K-12 185 ST, which received its R factor from 5445, was used to transfer this ST-determinant to E. coli Y 20 and to E. coli ML strains which are lac-. It is sensitive to phages T1, T5, and BF3, whereas none of the col" strains used in this study is sensitive to these phages.

Transfer of R factors to col" strains. Transfer of R factors to col" strains was performed essentially by the method of Anderson and Lewis (2) in the following way. The strains tested were inoculated from stock cultures on Dorset Egg medium into Nutrient Broth (Difco) and shaken for 6 hr. Two milliliters of donor and two milliliters of recipient strain cultures were then mixed and incubated overnight at 37°C. Afterwards, the mixture was diluted 10^5-fold and one standard loop (0.01 ml) of each dilution was plated on MacConkey agar (Difco) containing: (i) in the cross 5445 ST × 1327 Ns col+ or 222 SsuCT × 1327 Ns col+, 20 µg of S, 10 µg of T, and 20 µg of nalidixic acid per ml; and (ii) in the crosses 185 ST × Y 20 or 222 SsuCT × Y 20, the same amounts of S and T only. Lac+ colonies on ST plates were scored and tested for insensitivity to phages T1, T5, and BF3. (iii) In the crosses 185 ST × ML 35 Ns or 222 SsuCT × ML 35 Ns the plates were the same as in (i). (iv) In the crosses 185 ST × ML 35 Cn strains or 222 SsuCT × ML 35 Cc, the MacConkey Agar plates contained 1,000 µg of S per ml. The lac- character of the recipient colonies and their insensitivity to the above mentioned phages were checked.

Colicin production. Colicin production by colonies of the col" strains was checked before and after the
receipt of ST-factors in a following way (4): 10 colonies of each recipient strain were transferred to a segment of a Nutrient Agar plate (Difco) and incubated 48 hr to allow colicin production and diffusion. The macrocolonies on Nutrient Agar were then killed by chloroform vapors, and the plates were overlaid with about 10° cells of indicator strain in 0.6% agar. The presence of inhibitory zones around the macrocolonies of the producer strains was taken as proof of colicin production. As indicator strains, E. coli ROW or E. coli K-12 185 (the latter obtained from J. Smarda, Brno), or both strains, were used.

RESULTS

Table 1 summarizes our experiments with the colicin production of the above mentioned col+ strains before and after introduction of two types of R factors. It is evident from the results that, whereas the R factor from 5445 and 222 blocked the expression of col factor in 2H 1327 and Y 20 strains, only R factor from 222 strain had this effect in the ML strains.

To see whether the absence of colicin production caused by introduction of R factor into previously col+ cells is due to elimination of col factors by superinfection or merely to the failure of the col factor to function normally while remaining present in the cell, we tested the cross-immunity of R+ colonies of previously col+ colonies to colicins produced by original col+ clones. The R+col− colonies were tested as indicators against original col+ clones. In the instances tested, i.e., in 2H 1327 ST 5445 or 222 against 2H 1327, and in ML 222 against ML, we observed the cross-immunity of R+col− clones was lost. This indicates that the corresponding col factors are eliminated from the cells.

We also derived R− variants of R+col− clones of previously col+ strains by acriflavine treatment performed exactly as described by Kato et al.

(6). The col factors of the original col+ strains were not eliminated by this procedure (6). There was no resumption of colicin production after elimination of R factors, as tested in two R− colonies obtained after acriflavine treatment of R+col− clones of each previously col+ strain.

DISCUSSION

Kato et al. (6) described the elimination of col factors X and K from strain K 235 by various R-factors. Their experiments indicated that the eliminative factor was in each instance the transfer factor (2) or resistance-transfer factor (9) part of R-factor molecules rather than various R determinants, although the eliminating R factors they used contained T-determinant in each instance. Our results show the elimination of another kind of col factor by two groups of R factors, although one of our Col E1 factors, in the ML strain, was eliminated by only one R factor, i.e., by that of 222.

It is known that more than one extrachromosomal genetic element can exist in a single host cell (5), but the presence of more than one such element in the same host frequently creates an unstable situation resulting in repression or loss of some factors (3). Introduction of certain R factors represses the F function (9). Anderson et al. (1) have proposed a mechanism whereby extrachromosomal elements may compete for a single site on the transfer factor. It has also been shown that the introduction of certain colicinogenic factors into a cell can mobilize for transfer otherwise latent extrachromosomally located R-determinants (2).

In this report, we have shown that introduction from two different donor cells of an R-factor into a number of colicinogenic strains results in inhibition of production of colicins most probably by the elimination of col factors from the R+ cells by the R factors. Kato et al. (6) indicate that R factors interfere with the replication of col factors in such cells, which leads to the elimination of the latter from the cells.

All col factors, however, are not equally susceptible to elimination by R factors, since colicin production in ML strains was abolished by infection with the R-factor of E. coli CSH-2 222 but not with the R factor of S. typhimurium 5445, which is able to eliminate other col factors.

ACKNOWLEDGMENT

V. Křemý was supported by an E.M.B.O. Brussels grant in P. Fredericq's laboratory during the final stages of this study.

LITERATURE CITED

1. Anderson, E. S., M. V. Kelemen, C. M. Jones, and J. S. Pitton, 1968. Study of the association of resistance to two drugs in a


