MOBILIZATION OF NONTRANSMISSIVE EPISOMES

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A technique of mobilizing nontransmissive col-factors was developed experimentally using the principle of formation of a system of high-frequency colicinogeny transmission (HFCT). Mobilization of nontransmissive colicinogeny factors (colD and $colI_a$) incorporated into the structure of various transmissive episomal elements [$colI_b$, Hly(β), R_{222}, R_{64}, F, and col(V+E_1)+F_V] was demonstrated.

Under natural conditions colicinogenic bacteria are widely distributed, but most of the inherited factors controlling ability to synthesize colicins, especially about 20 colicinogenic factors (col-factors), are nontransmissive. For genetic research it is essential to be able to utilize nontransmissive col-factors in genetically marked strains and also in combination with various episomes.

In the present investigation the possibility of mobilizing nontransmissive col-factors for transmission with the aid of transmissive episomes was studied by the authors' method.

EXPERIMENTAL METHOD

In conjugation experiments with Escherichia coli the following strains were used as donors of transmissive episomes: AB-247 ($colI_b$+), inducing colicin I_b (from the museum of the Laboratory of Episomes); CSH-2 (R_{222}+), resistant to the antibiotics tetracycline, streptomycin, and chloramphenicol and to sulfonamides (obtained from Professor Watanabe, Japan), J-5-3 (R_{64}+), resistant to tetracycline and streptomycin (obtained from Dr. Claus, U.S.A.). The resistance of both strains was not higher than to 100 μg/ml of the antibiotic. Other strains of E. coli were obtained from the museum of the Laboratory of Episomes: no. 195 [Hly(β)], producing hemolysin β, and AB-247(F^+), carrying the sex factor, and also strain K-30 [col(V+E_1)+F_V^+] (obtained from Professor Frédéricq, Belgium).

Colicinogenic strains with nontransmissive col-factors were used as intermediate recipients: Ca-23 ($colD_1^+$) (obtained from Professor Frédéricq, Belgium), and 026/14 ($colD_2$+) (from the museum of the authors' laboratory), producing colicins D_1 and D_2 respectively.

The final recipients were strains E. coli, P-678, J-62, and C-600, not carrying the above-mentioned episomes, lac^-, and resistant to high concentrations of streptomycin (1000 units/ml).

Laboratory indicator strains were used to detect and titrate colicinogeny in the resulting recombinants.

The media were prepared with Hottinger's nutrient broth, pH 7.2; His' assortment with carbohydrates (lactose, glucose, mannitol, maltose, sucrose), Endo's medium, and 1.5% agar with 5% rabbit erythrocytes also were used. Antibiotics of Soviet manufacture were added to selective media: streptomycin (calcium chloride complex), chloramphenicol, tetracycline hydrochloride, and naladixic acid in concentrations corresponding to the purpose of the investigation.

Colicinogeny of the bacteria was determined by Frédéricq's method [2]. Conjugation experiments using low (LFT) and high (HFT) frequencies of transmission of episomes were carried out as described by Stocker et al. [5] and Watanabe et al. [6].

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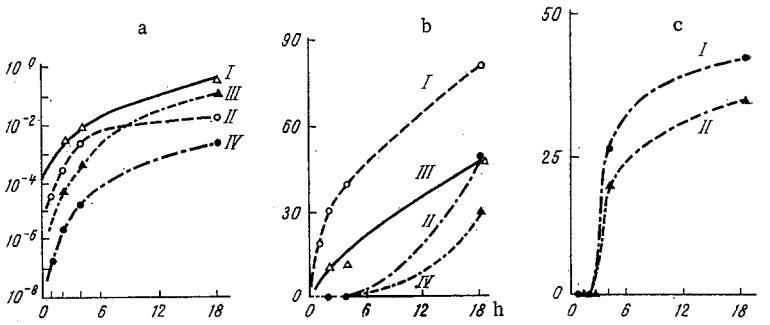

Fig. 1. Kinetics of transmission of transmissive episomes: a) crossing of strains CSH-2 (R_{222}^+) Ca-23 (col D, ×J-62: I) transmission of R_{222}^+-factor, II) transmission of (R_{222}^+, colD_1^+) complex and crossing CSH-2 (R_{222}^+) × 026/14 (colD_2^+) × J-62, III) transmission of R_{222}^+-factor, IV) transmission of (R_{222}, colD_2^+) complex; b) crossing of strains no. 195 (Hly^+) × Ca-23 (colD_1^+) × J-62: I) transmission of Hly^+-factor, II) transmission of Hly^+, colD_1^+) complex and crossing of no. 195 (Hly^+) × 026/14 (colD_2^+) × J-62; III) transmission of Hly^+-factor, IV) transmission of (Hly^+, colD_2+) complex; c) crossing of strains AB-247 (F+) × Ca-23 (colD_1^+) × J-62: I) transmission of (F+, colD_1^+) complex and crossing of AN-247 (F+) × J-62, II) transmission of (F+, colD_2^+) complex. Abscissa, time of incubation with final recipient (in h); ordinate, percentage of transmission.

Development of the mobilization technique. The principle of creation of the HFT system was used as the basis for development of the mobilization technique. Cultures of the donor (with the transmissive episome) and intermediate recipient (with the nontransmissive colD-factor) were incubated together (in the ratio of 1:20). At the beginning of "epidemic" spread of the transmissive episome among the recipient's cells (by 18 h) the mixture of cultures was diluted 1:10 and incubated until the logarithmic phase of growth, after which a culture of the final recipient, to which transmissive episomes were transmitted with high frequency, was added.

To prevent death of the partner cells during conjugation the colicin secreted into the medium was inactivated with trypsin (200 μg/ml). To increase the yield of col^+-recombinants an additional selective agent (homologous coligin) was added before seeding to the conjugation mixture in the ratio of 1:1 and left for 30 min at 37°C, after which the colicin was removed by centrifugation and the mixture was seeded on selective media.

EXPERIMENTAL RESULTS

The experiments showed that if the intermediate recipient contains a nontransmissive episome, this episome "attracts" the transmissive or is mobilized for transmission.

Results for the kinetics of transmission of the transmissive episomes and mobilization of the colD-factors are shown in Fig. 1.

Transmissive episomes $colI^+$, $Hly(\beta)+$, R_{222}^+, R_{64}^+, F^+, and $col(V+E_1)^+ + F_V^+$ possessed mobilizing activity and formed complexes with nontransmissive colD_1^+, colD_2^+, and $colI_\alpha^+$-factors.

The efficiency of transmission of R_{222}^+ (Fig. 1a, I, II), Hly^+ (Fig. 1b, I, III), and F^+ (Fig. 1c, I) was always higher than that of their combined transmission with the colD-factors. It should be noted that the higher the recipient activity of the intermediate recipient (Ca-23 or 026/14) the higher also were the mobilization indices of the corresponding colD-factor.

The resulting recombinants E. coli P-678 ($colI_R$, colD_1); ($colI_R$, colD_2), ($Hly(\beta)$, colD_1), R_{64}, colD_2), ($Hly(\beta)$, colD_1), R_{64}, colD_2), [$col(V+E_1)^+F_V$, $colI_a$]; and E. coli J-62 (F, colD_1), F, colD_2), R_{222}, colD_1), R_{222}, colD_2) were checked for their selective markers and tested for further transmissive activity. The results showed that they all were capable of subsequently transmitting the episomal complex in a specially chosen system (HFT) during conjugation (final recipient E. coli C-600 Nx^R, resistant to naladixic acid, 100 μg/ml).

Anderson [1] obtained a transmissive complex consisting of the transmissivity factor (△) and a nontransmissive marker of resistance to streptomycin, which was capable of further transmission. More recently Frédéricq et al. [3, 4] have shown that nontransmissive extrachromosomal determinants of resistance to streptomycin can be transmitted by transmissive col-factors.

It can be concluded on the basis of this evidence from the literature and the results of the present investigations that mobilization is due to transmission factors incorporated into the structure of the transmissive episomes. This provides an opportunity for studying the mobilization of various nontransmissive episomes and plasmids, especially if their connection with the chromosomal apparatus of the bacteria has not been established.

These facts concerning the mobilization of nontransmissive episomes can help toward the understanding of the mechanisms of distribution of these episomal elements in natural associations of the microbial flora of man and animals.

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