MOLECULAR CLONING OF THE GENETIC REGION DETERMINING THE SURFACE EXCLUSION SYSTEM OF F-LIKE PLASMID pAP42

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The efficacy of spread of plasmids in cells of natural bacterial populations largely depends on the character of functioning of specific surface exclusion (Sfx) systems, present in their genomes [2, 4, 6].

Since the derepressed F-like plasmid (genetic transfer factor) pAP42, which we identified, has been found to be a representative of a new surface exclusion group SfxV [2], the aim of the present investigation was to determine the possible location of the genetic region determining the SfxV system in the genome of this plasmid.

EXPERIMENTAL METHOD

Cells of *E. coli* K-12, strains AP132, C600, and HB101, carrying the test plasmid pAP42 and its transposon-containing derivatives (pAP42::Tn5, pAP42::Tn9) were used.

Surface exclusion was studied in standard conjugative crosses of bacteria containing or not containing the test plasmids. The surface exclusion index (SEI) was determined as the ratio of the number of plasmid transconjugants found by the use of a plasmid-free recipient strain, to their number obtained for an isogenic strain containing the plasmid. Plasmid DNA was isolated by standard methods [3, 5]. For molecular cloning purposes, restriction of DNA of the test plasmids by different endonucleases was carried out, followed by ligation of the isolated DNA fragments with the vector plasmid pBR325 and transformation of the bacterial cells by the usual method [5].

EXPERIMENTAL RESULTS

The work began with the obtaining of recombinant plasmids on the basis of vector plasmid pBR325 (ApCmTc) and of fragments of the DNA of plasmid pAP42, obtained with the aid of restriction endonucleases EcoRI, HindIII, and SalI.

Cloning the SalI-fragment f5 of plasmid pAP42 led to the obtaining of recombinant plasmids, among which two, designated pAP110 and pAP111, were selected for further research.

Experiments to determine the ability of plasmids pAP110 and pAP111 to carry out surface exclusion of plasmid pAP42::Tn5 showed that SEI for plasmid pAP110 was 80, whereas for plasmid pAP111 it was 85 (in similar control experiments with vector plasmid pBR325 SEI was 1.2-1.3).

When these results were analyzed it might be supposed that the genetic region responsible for the SfxV system of plasmid pAP42 is contained in its SalI-fragment f5.

To confirm this hypothesis DNA from recombinant plasmids pAP110 and pAP111 was isolated and subjected to restriction analysis, with the aid of restriction endonuclease SalI.

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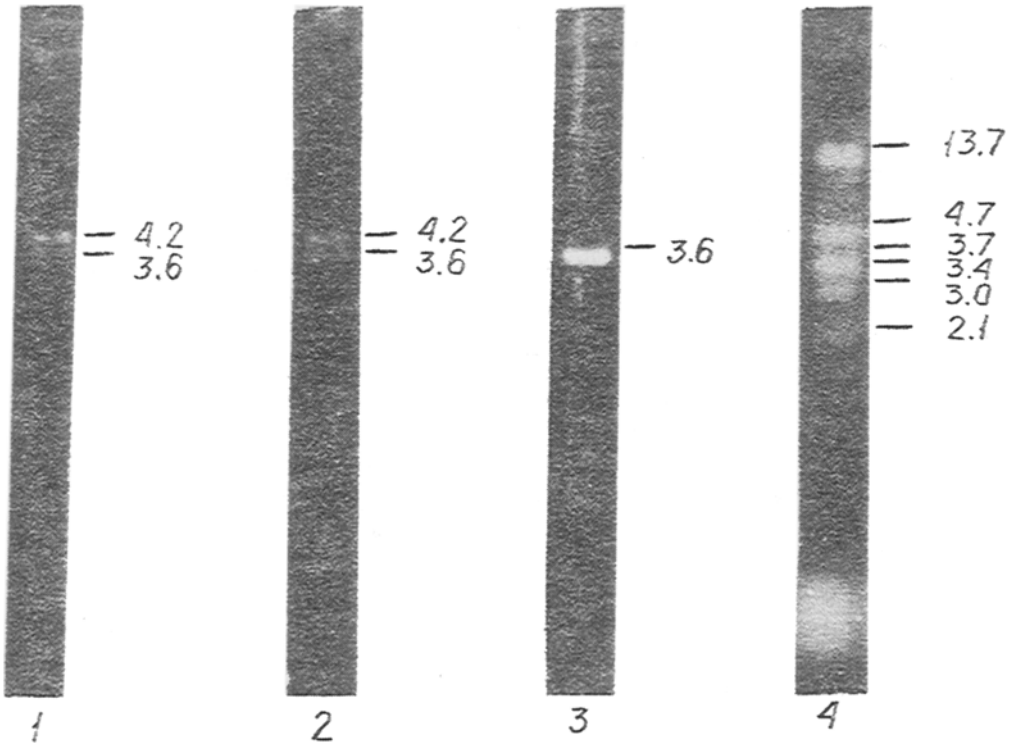

Fig. 1. Electrophoretic fractionation of restriction products of DNA of plasmids pAP110, pAP111, and pBR325 by the enzyme SalI. 1) pAP110, 2) pAP111, 3) pBR325, 4) λ, EcoRI.

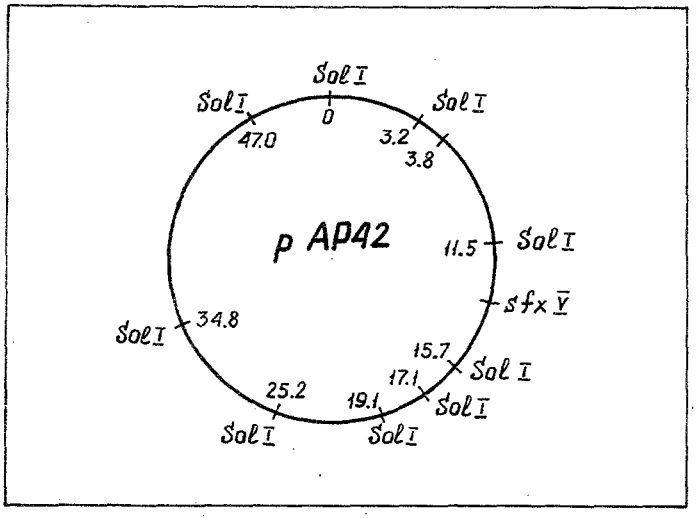

Fig. 2. Location of genetic region SfxV on restriction map of plasmid pAP42.

The results (Fig. 1) showed that besides vector DNA, the recombinant plasmids also contained in their structure the SalI-fragment f5 (4.2 megadaltons) of plasmid pAP42. Consequently, the genetic region determining the surface exclusion system of plasmid pAP42 (the SfxV locus) is in fact located in the SalI fragment f5 of this plasmid. This fragment has coordinates of 11.5-15.7 megadaltons on the restriction map of plasmid pAP42 [1].

REFERENCES

1. R. Pereira, E. V. Shcherbakova, and A. P. Pekhov, Byull. Éksp. Biol. Med., No. 7, 61 (1985).
2. V. P. Shchipkov, S. L. Sokolova, O. B. Gigani, K. S. Krivskaya, et al., Byull. Éksp. Biol. Med., No. 9, 303 (1990).
3. H. C. Birnboim and J. Doly, Nucleic Acids Res., **7**, 1513 (1979).
4. B. S. Hansen, P. A. Manning, and M. Achtman, J. Bacteriol., **150**, 89 (1982).
5. T. Maniatis, E. F. Fritsch, and J. Sambrook, Molecular Cloning: a Laboratory Manual, Cold Spring Harbor, New York (1982).
6. N. Willetts and G. Maule, Genet. Res., **47**, 1 (1985).