# GENETICS

# Molecular Cloning of Determinants of Surface Exclusion of F-Like Plasmid pAP22-2

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Molecular cloning of the SalI-fragment of plasmid pAP22-2 DNA was carried out in order to detect the possible location of surface exclusion genes of this plasmid (Sfx VII system). The results of studying recombinant plasmids indicate that Sfx domain determinants are located in two different fragments of plasmid pAP22-2.

**Key Words:** *plasmid; surface exclusion system; molecular cloning*

The presence of genes controlling surface exclusion (Sfx system genes) in plasmid genomes is an important factor precluding the conjugation transfer of plasmids between cells of natural populations of bacteria [3,6,8].

Derepressed F-like plasmid (genetic transfer factor) pAP22-2, which we identified previously, proved to be a representative of a new group of surface exclusion Sfx VII [4]. Therefore, our task in this study was to elucidate the possible location of genetic determinants of surface exclusion in the genome of this plasmid.

## MATERIALS AND METHODS

*Escherichia coli* K-12 strains AP132, C600, and HB101 containing plasmid pAP22-2 or its derivative variant pAP22::Tn5 were used in the study.

Surface exclusion was determined by performing standard conjugation crossing of bacteria containing or not containing the respective plasmid. The surface exclusion index (SEI) was estimated for each experiment. This index represents the ratio of the number of plasmid transconjugates detected using a recipient of a plasmid-free bacterial strain to the number of transconjugates obtained for the same recipient strain containing the plasmid.

In experiments on molecular cloning, the isolation of plasmid DNA and its restriction with endonuclease SalI followed by ligation of the isolated fragments with the restricted DNA of vector plasmid pBR325, as well as transformation of bacterial cells were carried out as described previously [7]. Restriction fragments of plasmid DNA were separated using horizontal slab-electrophoresis in 0.65% agarose gel (Serva). The sizes of restriction DNA fragments were assessed by comparing their mobility in agarose gel with the mobility of lambda-phage DNA fragments [5].

## RESULTS

In order to detect the possible location of surface exclusion determinants of pAP22-2 plasmid (to identify the genetic locus responsible for the Sfx VII system), we performed molecular cloning of DNA fragments of this plasmid which were obtained after plasmid treatment with SalI restriction endonuclease (Fig. 1). For this purpose, recombinant plasmids containing individual SalI fragments of the tested pAP22-2 plasmid DNA were constructed on the basis of vector plasmid pBR325 (ApCmTc). Since

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the vector plasmid pBR325 possesses the only site of "recognition" by SalI in the locus of the tetracycline resistance (Tc) gene, the insertion of a cloned DNA fragment (if it takes place) may be hypothesized to inactivate this gene, causing transformants to lose tetracycline resistance, that is, leading to the appearance of transformants with the phenotype $Tc^s$ in the appropriate nutrient medium. Hence, transformants with the $Ap^rCm^rTc^s$ phenotype were selected for further studies. Ten to twenty transformants with the above phenotype were obtained for each of the 10 cloned SalI fragments of pAP22-2 plasmid DNA.

The ability or inability of the constructed recombinant plasmids to provide the surface exclusion of pAP22-2::Tn5 plasmid of the Sfx VII group was assessed in further experiments. The results of conjugation experiments helped single out two recombinant plasmids denoted as pAP114 and pAP115, which were characterized by such a capacity (SEI 23-28 and 29-31, respectively). In control experiments with the recipient *E. coli* strain HB101 containing vector plasmid pBR325 the SEI of pAP22-2::Tn5 plasmid varied from 0.9 to 1.2. Since the recombinant plasmids pAP114 and pAP115 were selected as a result of cloning pAP22-2 plasmid SalI fragments f3 and f9, respectively, it may be assumed that the genetic domain of this plasmid responsible for surface exclusion (Sfx VII system) is located in these two fragments.

To verify this hypothesis, we isolated DNA of recombinant plasmids pAP114 and pAP115 and carried out restriction analysis using endonucleases SalI and EcoRI.

The results showed the presence of insertion SalI fragments with molecular weights of 2.7 MD in plasmid pAP114 (Fig. 1, *5*) and 3.3 MD in plasmid pAP115 (Fig. 1, *3*) among the products of SalI hydrolysis of the examined plasmids, besides a fragment coinciding with the linear form of vector pBR325 DNA (3.7 MD). EcoRI restriction of plasmid pAP114 led to the formation of two fragments with molecular weights of 4.5 and 1.9 MD (Fig. 1, *2*). The results confirm the recombinant nature of plasmids pAP114 and pAP115.

Overall, the results of our studies suggest that Sfx VII domain determinants are located in various SalI fragments of plasmid pAP22-2. This allows us to speculate on certain features of the genomic arrangement of plasmid pAP22-2 in comparison with other F-like plasmids we studied [1,2]. Further studies to determine the mutual disposition of the cloned fragments and their possible genetic organization will involve obtaining mutants for the Sfx domain genes and physical mapping of the pAP22-2 plasmid.

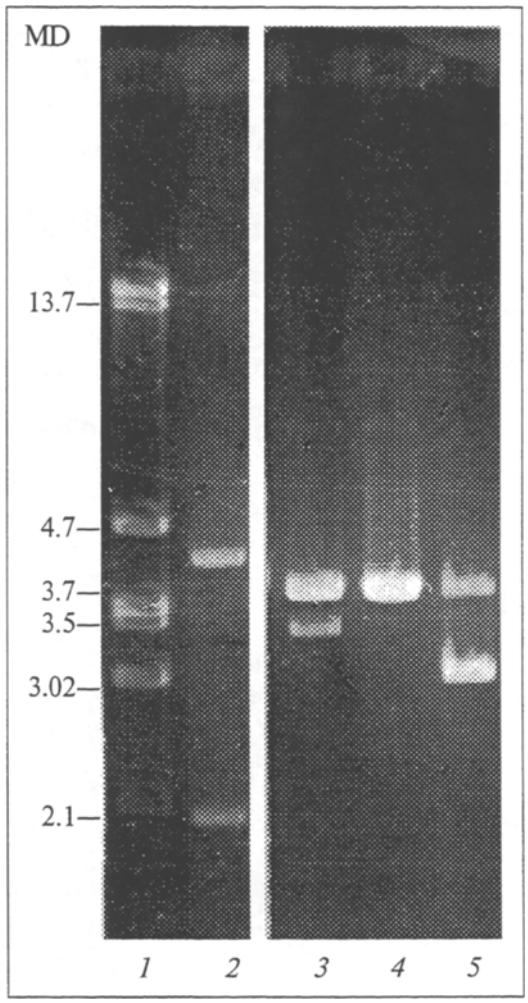


**Fig. 1.** Electrophoregram of DNA restriction products of plasmid pAP22−2. *1*) lambda phage DNA, EcoRI; *2*) pAP114, EcoRI; *3*) pAP115, SalI; *4*) pBR325, SalI; *5*) pAP114, SalI.

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