GENETIC INCOMPATIBILITY REGION OF F-LIKE PLASMID pAP18-1

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The phenomenon of compatibility (incompatibility) of plasmids is widely used for classifying these genetic structures in order to organize the study of their distribution in natural communities of different bacteria [1, 2, 6, 7, 10]. However, the mechanism of genetic control of the incompatibility groups to which individual plasmids belong (Inc-groups) and also the localization of determinants of particular plasmids, controlling group incompatibility, in the genome remain unexplained.

The aim of this investigation was to establish the location of the genetic incompatibility region (the region of Inc-genes) of F-like plasmid pAP18-1 (Tc, ColV), belonging to incompatibility group Inc FXI [4], and its derepressed mutant pAP18-1drd (Inc FVII) [3]. To solve this problem we undertook molecular cloning of DNA fragments of these plasmids. The results are described below.

EXPERIMENTAL METHOD

Standard recipient strains of *E. coli* K-12 with chromosomal markers of resistance to antibiotics (AP115 Na, HB101 Str, J83 Str), containing or not containing standard and test plasmids, were used. Compatibility (incompatibility) of the plasmids was determined by the standard method [6]. Plasmid DNA was isolated by the method of Meagher and co-workers [9] with minor modifications, the purified cell lysates being centrifuged in a CsCl density gradient. Restriction of plasmid DNAs by specific endonucleases EcoRI and SalG1, elution of the restriction fragments from the gel, and subsequent molecular cloning of the isolated DNA fragments in the composition of the vector plasmids pUC19 and pBR325 followed by transformation of *E. coli* J83 and *E. coli* HB101 cells were carried out by the usual methods [8]. Restriction fragments were fractionated in 0.65% agarose gel by horizontal slab electrophoresis. The dimensions of the restriction fragments of DNA were determined by comparing their mobility in agarose gel with the mobility of fragments of DNA from phage λ [5].

EXPERIMENTAL RESULTS

Experiments to study the possible localization of the genes responsible for plasmids pAP18-1 and pAP18-1drd belonging to incompatibility groups Inc FXI and Inc FVII respectively, by molecular cloning, were begun with isolation of the DNA of these plasmids, followed by fractionation of the EcoRI fragments. As the genetic vector for fragments of plasmid pAP18-1 we used plasmid pUC19 (Ap, Lac), and for plasmid pAP18-1drd we used plasmid pBR325 (Ap, Cm, Tc). In the first case, transformants which had lost their ability to ferment lactose, and in the second case, transformants which had lost their resistance to chloramphenicol were selected. Considering that the "cleavage" sites of DNA of the vector plasmids by restriction endonuclease EcoRI used are located in loci of the genes controlling the above-mentioned features, these transformants were regarded as the most probable carriers of recombinant plasmids, i.e., plasmids consisting of the vector and cloned fragment of the test plasmid.

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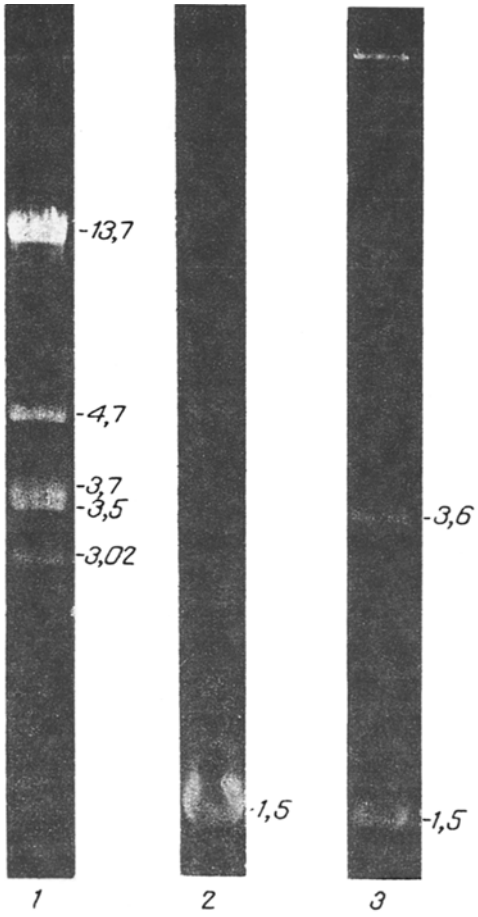

Fig. 1. Electrophoretic fractionation of restriction products of DNA of recombinant plasmid pAP108 by restriction endonuclease EcoRI. 1) DNA of phage λ, EcoRI; 2) pUC19, EcoRI; 3) pAP108, EcoRI.

For the subsequent work two recombinant plasmids, designated pAP108 and pAP109 were selected. Plasmid pAP108 was obtained by cloning EcoRI-fragment f5 of plasmid pAP18-1 into the vector plasmid pUC19, whereas plasmid pAP109 was obtained by cloning the EcoRI-fragment f2 of plasmid pAP18-1drd into vector plasmid pBR325. To determine the compatibility (incompatibility) of the constructed hybrid plasmids, double plasmid transconjugants containing one such plasmid and a reference plasmid belonging to one of the incompatibility groups of F-like plasmids were obtained [2, 4, 7]. As the experiments showed, plasmid pAP108 was found to be completely incompatible with the original plasmid pAP18-1, which is a representative of the Inc FXI group. So far as plasmid pAP109 is concerned, it is incompatible with plasmid pAP38::Tn9, a reference plasmid of the Inc FVII group. Consequently, it can be concluded from these data that plasmid pAP108 contains the genetic region of plasmid pAP18-1, responsible for its belonging to the Inc FXI group, whereas plasmid pAP109 contains the genetic region of plasmid pAP18-1drd, responsible for its belonging to the Inc FVII group.

To study the genomic organization of plasmids pAP108 and pAP109, restriction analysis of these plasmids by endonucleases EcoRI and SalG1 was next carried out. The results of EcoRI restriction showed that plasmid pAP108 in fact consists of the vector plasmid pUC19 (1.5 megadaltons – MD) and EcoRI-fragment f5 (3.6 MD) of plasmid pAP18-1 (Fig. 1), whereas plasmid pAP109 consists of vector plasmid pBR325 (3.6 MD) and EcoRI-fragment f2 (7.2 MD) of plasmid pAP18-1drd (Fig. 2). Conversely, analysis of restriction products of plasmid pAP109, obtained by restriction endonuclease SalG1 revealed three fragments corresponding in mobility to molecular weights of 5.1, 3.6, and 2.1 MD (Fig. 3b). It follows from these data that the SalG1-fragment with molecular weight of 3.6 MD is "hidden" by the SalG1 site of plasmid vector pBR325, and in the EcoRI-fragment f2 there is a "recognition" site for restriction endonuclease SalG1. This led to the formation of two SalG1-fragments with molecular weights of 5.1 and 2.1 MD, equivalent in electrophoretic mobility to SalG1-fragment f4 and SalG1-fragment f5.

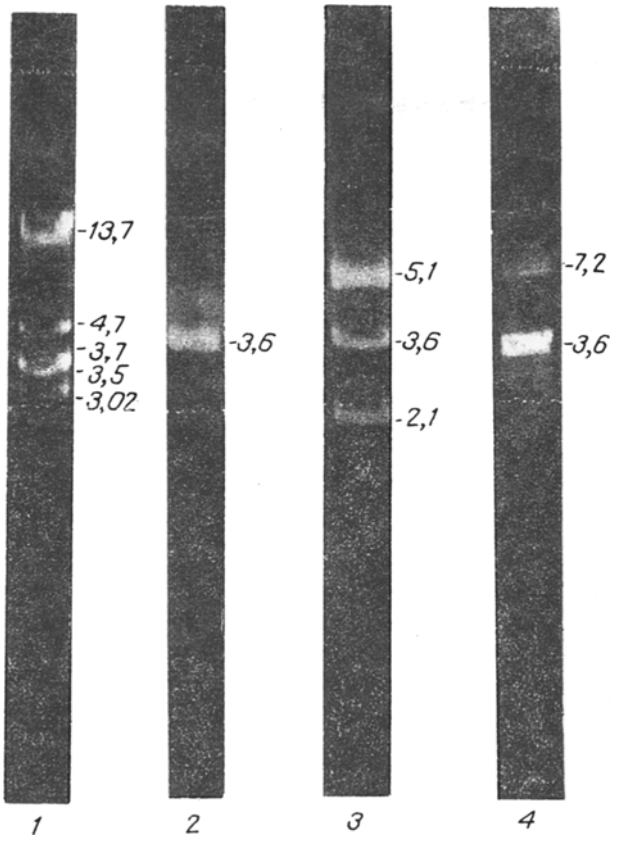

Fig. 2. Electrophoretic fractionation of restriction products of DNA of recombinant plasmid pAP109 by restriction endonucleases EcoRI and SalG1. 1) DNA of phage λ, EcoRI; 2) pBR325, EcoRI; 3) pAP109, SalG1, 4) pAP109, EcoRI.

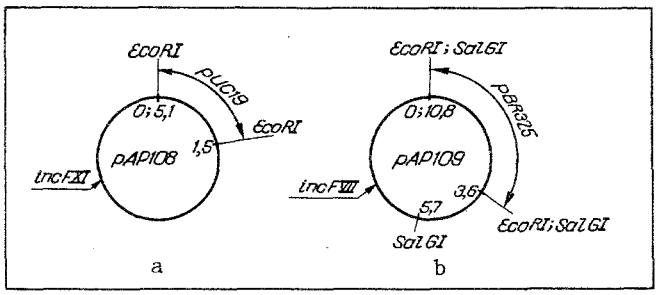

Fig. 3. Restriction maps of recombinant plasmids. a) pAP108. Sites oriented relative to EcoRI site (0), distances from which are given in MD; b) pAP109. Sites oriented relative to EcoRI and SalG1 sites (0), distances from which are given in MD.

The results of restriction of recombinant plasmid pAP108 by restriction endonuclease EcoRI and of plasmid pAP109 by restriction endonucleases EcoRI and SalG1, combined with previous data [4], led to the construction of restriction maps of these plasmids (Fig. 3a, b).

It can be concluded from the data as a whole that the genetic region of plasmid pAP18-1, responsible for its belonging to incompatibility group FXI is located in EcoRI-fragment f5 (3.6 MD), whereas the genetic regions of plasmid pAP18-ldrd, responsible for its belonging to incompatibility group FVII, is located in the EcoRI-fragment f2 (7.2 MD). If

these results are compared with data reflected in the maps of plasmids pAP18-1 and pAP18-ldrd [4] it can be postulated that a change in the incompatibility group Inc FXI of plasmid pAP18-1 to the Inc FVII group in its derepressed mutant is linked with the functioning of different inc genes, located in different regions of the genomes of these plasmids.

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DIAGNOSIS OF ANEUPLOIDY BY IN SITU HYBRIDIZATION: ANALYSIS OF INTERPHASE NUCLEI

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Recent advances in genetics have enabled a fundamentally new approach to be made to the diagnosis of hereditary pathology and, in particular, of chromosomal anomalies. This has become possible through improvements in the technology of obtaining recombinant DNA molecules and using them as DNA-probes for the diagnosis of inherited defects. The discovery of chromosomal pathology is based on the use of cloned fragments of the human genome of a special type, namely chromosome-specific DNA probes. With the aid of these probes several distinguishing features of the chromosomal set can be discovered without direct cytogenetic analysis: by DNA hybridization in situ or blot-hybridization [1, 5].

The method of in situ hybridization for detection of chromosomal pathology has an important advantage over other methods of molecular research, in that it does not require a large quantity of biopsy material, isolation of DNA from the patient's cells, the use of the unstable and biologically dangerous isotope of radioactive phosphorus, and the use of the laborious process of blot hybridization. By the use of this method it is possible also to detect and analyze individual chromosomes at virtually every stage of the cell cycle, including in interphase [2, 6], which is particularly important for prenatal diagnosis, when, on the basis of a small volume of material (in native preparations of chorionic cells), it is possible to determine quickly and accurately the sex of the fetus or to discover aneuoploidy.

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