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VETERINARY SCIENCE

Genetic Polymorphism of DNA of *Fusobacterium necrophorum* Cultures According to RAPD Analysis Data

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Abstract—Dendrograms constructed from the RAPD analysis data are consistent with the results of biological investigations and reflect more completely the degree of similarity of cultures from different territories. Investigations of genomic polymorphism of pathogens of bacterial infections can be used for determining regularities of the occurrence and circulation of epizootic strains in herds as well as for revealing variability of microorganisms under the effect of various factors.

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It is known that RAPD makers are used for genetic mapping and analyzing processes of hybridization and introgression in populations, species identification, and establishing phylogenetic ties and taxonomic relations, and are also used in searching for markers closely linked with morphological and economically valuable genes of crop plants. RAPD markers have Mendelian inheritance, as a rule, of the dominant type [1]. The method of RAPD markers makes it possible to obtain a large number of markers scattered throughout the entire genome; therefore, such markers are convenient for constructing genetic maps [1, 2] or linkage maps with quantitative trait loci, i.e., loci of economically valuable properties. The results of using RAPD-PCR for genetic typing of *Fusobacterium necrophorum* cultures are given in the present work.

METHODS

In the work we used *Fusobacterium necrophorum* cultures nos. 8, 2, 12, 16, 19, 20, 22, 34, 37, and 41. Genomic DNA of the bacteria was obtained by means of proteolytic enzymes, subsequent extraction with a phenol–chloroform mixture, and precipitation with ethanol [3].

Amplification of DNA was carried out in a 25-μl reaction mixture. In the work we used 10-bp "arbitrary" primers constructed after analyzing the nucleotide sequences of leukotoxin and proteins of *F. necrophorum* membrane published in the NCIBI/GenBank. Chemical synthesis of primers nos. 38/1, 60, and 61 for *Fusobacterium* was accomplished in the Natural Compounds Chemistry Department of the Vektor State Virology and Biotechnology Scientific Center. During conduction of RAPD-PCR, the value of Tm was changed depending on the energy of the primer. The reaction products were visualized by electrophoresis in 1.5% agarose gel with ethidium bromide. Documentation was done on Mikrat-300 photographic film.

For statistical analysis, binary matrices were compiled for each of the primers and used for constructing dendrograms by the cluster analysis method (STATISTICA 6). As a result a dendrogram of genetic distances was obtained.

RESULTS AND DISCUSSION

Fragments characteristic for the majority of the investigated isolates were revealed as a result of analyzing the RAPD spectra obtained by means of primer

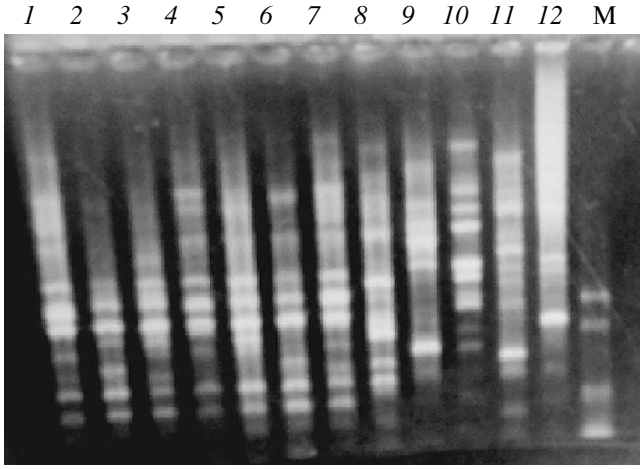

Fig. 1. RAPD spectra of amplified DNA of *F. necrophorum* isolates obtained by means of primer no. 38/1: *1*—no. 2; *2*—no. 8TS; *3*—no. 9; *4*—no. 12; *5*—no. 16; *6*—no. 19; *7*—no. 20; *8*—no. 22; *9*—no. 34; *10*—no. 40; *11*—no. 41; *12*—no. 2B; (M) pUC18/AluI.

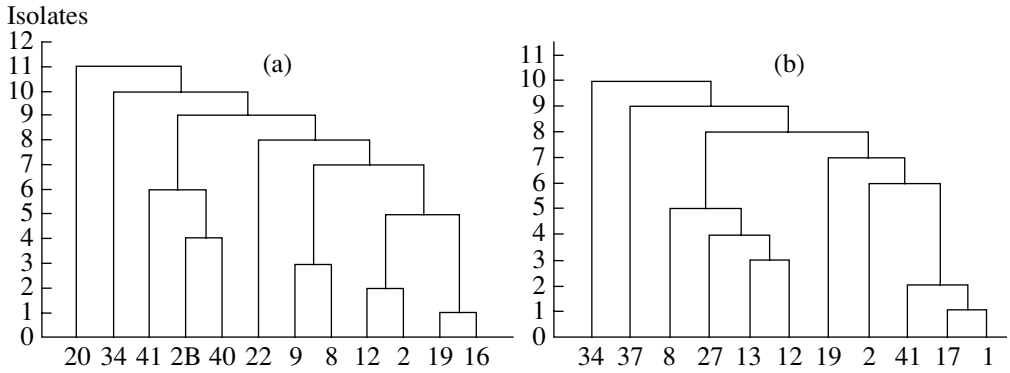

Fig. 2. Dendrograms of *F. necrophorum* isolates constructed from the results of RAPD analysis with primers no. 38/1 (a) and nos. 60 and 61 (b).

no. 38/1 synthesized on the basis of the nucleotide sequences of *F. necrophorum* leukotoxin (Fig. 1). The genetic distances between isolates were determined by the cluster analysis method. Two main clusters are distinguished on the dendrogram (Fig. 2a): the first includes isolates nos. 9, 8, 12, 2, 19, and 16; the second, nos. 41, 2B, and 40. Separate clusters contain nos. 20, 22, and 34. Consequently, the investigated group of isolates of the given species of microorganisms was heterogeneous with respect to the investigated leukotoxin in spite of morphological similarity. Experiments conducted on white mice also revealed a different effect on the animals: dry necrosis of subcutaneous tissues or paralysis of limbs, or emaciation and paralysis of limbs.

On conducting RAPD analysis simultaneously with primers no. 60 and no. 61 selected on the basis of the nucleotide sequences of membrane protein, the following genetics distances were determined among the investigated isolates (Fig. 2b). Two main clusters were distinguished on the dendrogram: the first contained isolates nos. 19, 2, 41, 17, and 1 and the second, nos. 8, 27, 12, and 13. Individual clusters contained nos. 34 and 37.

Each of the main clusters (Fig. 2a, 2b) represents a mixed group. Thus, the degree of similarity (Fig. 2a, primer no. 38/1) between the main clusters is at the 42–54% level, which indicates a considerable polymorphism between isolates of the given sample with respect to pathogenicity. The level of similarity of the two main clusters (Fig. 2b) obtained by means of primers nos. 60 and 61 is 45–63%. Since these primers were selected on the basis of nucleotide sequences encoding membrane proteins and they mainly perform the same functions in anaerobes of different species, the similarity between isolates of the main clusters is higher.

Consequently, the dendrograms constructed from the results of the RAPD analysis are consistent with the results of biological investigations and, in our opinion, reflect more completely the degree of similarity of cultures from different territories. Investigations of genomic polymorphism of pathogens of bacterial infections can help in determining regularities of the occurrence and circulation of epizootic strains in herds and can also reveal variability of microorganisms under the effect of various factors.

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