ON ANTIGENIC PROPERTIES OF GENETIC RECOMBINANTS OF Escherichia coli SEROTYPES

A. P. Pekhov and L. G. Stolyarova

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A study of conjugation between typed strains of Escherichia coli belonging to different O serogroups and of conjugation between typed and untyped strains showed that the genetic determinant controlling synthesis of the O100 antigen is closely linked with the histidine locus. Among recombinants isolated from crosses between typed E. coli cells some were found to have a different serotype from that of the donor and recipient cells.

KEY WORDS: donor; recipient; recombinant; conjugation.

There is evidence in the literature that as a result of conjugation between donor cells of typed strains of Escherichia coli genetic determinants controlling antigenic properties can be transmitted to recipient cells of untyped strains of E. coli [5]. Recombinants formed in these crosses possess antigenic properties characteristic of the donor cells.

To determine the character of the antigenic properties of recombinants formed in crosses in which the donors and recipients are typed strains of E. coli belonging to different serological groups, the antigenic properties of hybrids obtained by conjugating E. coli O100 donor cells with E. coli O86 recipient cells were studied.

EXPERIMENTAL METHOD

As the donor strain in the conjugation experiments the typed strain E. coli AP17 Hfr O100 Str^S, constructed by the writers previously by introducing the F plasmid from E. coli P4x into E. coli F^-O100 trp^- lac^- [2], was used. Cells of this strain transfer genes in the O-pro-thr-leu-arg-his-trp-lac direction. The recipient strains were the typed strain E. coli AP19 F^- O86:K62/L/:H_2 met^- trp^- arg^- Str^r, isolated in the writers' laboratory by L. G. Stolyarova after treatment of cultures of strain E. coli F 1961 O86:K63/L/:H_2, obtained from the All-Union Escherichia Center, with N-methyl-N′-nitro-N-nitrosoguanidine, and the untyped strain E. coli PA 373 F^- met A^- arg F^- thr^- leu^- lac^- nal^r.

The cultures were crossed by the standard conjugation method [1]. The conjugation mixtures were kept for 2 h at 37°C and then seeded on selective media. The sensitivity of the recombinants to specific "male" phage f_2 was determined by the agar layer method. The serological properties of the recombinants were studied in the agglutination test with specific serum on slides and in the expanded reaction in tubes.

EXPERIMENTAL RESULTS

The experiments began with crosses aimed at identifying the location of the genetic determinant controlling synthesis of the antigen by virtue of which the donor bacteria belonged to the serogroup O100. For this purpose, E. coli AP17 Hfr O100 × E. coli PA 373 F^- crosses were carried out. From these crosses 100 each of met^+, arg^+, and thr^+ leu^+ his^+ recombinants were selected and these were then investigated in the agglutination test with specific O100 serum. As these experiments showed, only his^- Str^r recombinants gave positive

Department of Biology and General Genetics, Patrice Lumumba Peoples' Friendship University. Scientific-Research Laboratory of Experimental Immunology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.)
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TABLE 1. Serological Properties of Recombinants Obtained by Crossing E. coli AP17 Hfr O100 × E. coli AP19 F⁻ O86

Class of recombinants	Number of recombinants tested	Number of recombinants agglutinated by O86 serum	Number of recombinants agglutinated by O86 serum	Number of recombinants not agglutinated by O100 and and 086 sera
arg⁺ trp⁺	15	5	10	0
arg⁺	85	45	30	10
met⁺	60	40	15	5
met⁺ trp⁺	40	35	2	3

results in the agglutination test. Consequently, the determinant (determinants) controlling synthesis of the O100 antigens is closely linked with the histidine locus.

On the basis of these results and also of those obtained by other workers [3, 7], showing that antigens O25, O26, O55, O86, and O6 are closely linked with the his genetic locus, it can be postulated that the determinants controlling synthesis of the various antigens of the O serogroup have a complex structure, probably in the nature of a complex locus in which different alleles determine different antigens.

Investigation of the genetic determinant of the O100 serogroup by cells possessing the genetic determinant controlling their membership of the O86 serogroup was carried out in crosses of E. coli AP17 Hfr O100 × E. coli AP19 F⁻ O86. The met⁺ Str^r, arg⁺ Str^r, and trp⁺ Str^r recombinants were selected from these crosses, in which they were formed in frequencies of 2.8×10^{-4}, 2.7×10^{-4}, and 5×10^{-6}, respectively. After purification on similar media, these recombinants were studied in the agglutination test with specific O100 and O86 sera (Table 1).

As Table 1 shows, most of the recombinants were agglutinated by specific O86 serum. Those recombinants which were not agglutinated by this serum were tested in the agglutination test with O100 serum. Some of these recombinants were found not to give a positive agglutination reaction with either serum.

When discussing the nature of the genetic recombinants not agglutinated by these sera one possible explanation is that in this case some other serotype was formed. It can also be postulated that either recombination of alleles of antigens took place in this case, accompanied by the formation of hybrid antigens, or mutation occurred in the rfb region, determining the biosynthesis of the lipopolysaccharide chain, leading to a disturbance of synthesis of the O-specific chains and to modification of the O serogroup [4, 6].

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