# STUDY OF THE GENETIC STRUCTURE OF RECOMBINANTS BRED BY CROSSING PATHOGENIC SEROTYPES 026 : B6 AND 020 : B145 OF Escherichia coli

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When Escherichia coli cells belonging to one serotype are crossed, the recombinants show considerable changes in sensitivity of T-phages and, in particular, in antigenic structure. Besides exhibiting polyagglutinability, the recombinants contain antigens of different serotypes, giving a positive agglutination reaction in high titers.

Sometimes recombinants isolated after crossing typed and untyped strains of Escherichia coli exhibit the formation of new antigens [1-3, 7].

The investigation described below was undertaken to study unselective characters, notably antigenic properties, of recombinants obtained by crossing E. coli cells belonging to serotypes O26 : B6 and O20 : B145.

## EXPERIMENTAL METHOD

Fifteen strains of E. coli belonging to serotypes O26 : B6 and O20 : B145 isolated from sick children were used in the experiments. All strains were prototrophs and were agglutinated by homologous serum in high titers. Strain No. 69 was lysed by phages T2, T4, T5, and T6 and strain No. 334 by phage T2, while the rest were resistant to all T-phages. Ten strains did not ferment sucrose, 4 did not ferment raffinose, and the remainder fermented carbohydrates to acid and gas.

Auxotrophic mutants were isolated by the penicillin method [4] by means of ultraviolet rays from a type BUV-15 lamp.

The bacteria for investigation were crossed by the SRP method [5, 6]. The selective medium used was a minimal agar medium with the addition of corresponding amino acids, 1% glucose, and 300 μg/ml streptomycin.

The antigenic structure of the recombinants was studied in slide agglutination tests with monovalent O20, O26, O55, O111, and 408 sera. Fermentation activity was determined by seeding in Hiss's medium, and motility in 0.75% meat-peptone agar.

## EXPERIMENTAL RESULTS

Of the 15 strains studied, 7 were successfully crossed with standard E. coli K-12, recipients, acting as donor strains. Auxotrophic mutants were isolated from the remaining strains, dependent on one of the following amino acids: threonine, leucine, histidine, proline, and tryptophan. As a result of their crossing with standard donor E. coli Hfr H, two recipient strains were selected.

Subsequently crosses were obtained from strains belonging to the same serotype. Altogether 168 recombinants were obtained, and their antigenic properties, motility, ability to ferment carbohydrates, and behavior toward phages of the T group were investigated.

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TABLE 1. Serologic Properties of Recombinants Bred in Crosses from Serotypes O26 : B6 × O26 : B6 and O20 : B145 × O20 : B145

| Cross | Recombinants | Agglutination titers | | | | | | | | | | Control sera | Strain control |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | living culture | | | | | heated culture | | | | | | |
| | | O111 | O26 | O55 | O20 | 408 | O111 | O26 | O55 | O20 | 408 | | |
| 416 (O20:B145)F+ × 334 (O20:B145) F- | 17 | — | — | — | — | — | 1:800 | 1:25 | 1:25 | — | — | — | — |
| | 82 | — | — | — | — | — | 1:400 | 1:400 | — | — | — | — | — |
| | 83 | — | — | — | — | — | 1:800 | 1:25 | — | — | — | — | — |
| | 96 | — | — | — | — | — | 1:1600 | — | — | 1:800 | — | — | — |
| | 104 | 1:25 | — | 1:25 | 1:25 | — | 1:400 | — | — | — | — | — | — |
| | 105 | — | — | — | 1:200 | 1:25 | — | — | — | 1:800 | — | — | — |
| | 110 | 1:25 | — | — | — | — | — | 1:50 | 1:25 | 1:3 200 | — | — | — |
| | 111 | 1:25 | — | 1:25 | — | — | 1:50 | — | — | 1:1 600 | — | — | — |
| | 113 | — | — | — | 1:50 | — | 1:50 | — | 1:25 | 1:50 | — | — | — |
| 103 (O26:B6) F+ × 69 (O26:B6) F- | 20 | — | 1:25 | 1:25 | 1:200 | — | — | 1:25 | 1:25 | 1:3 200 | — | — | — |
| | 84 | — | — | — | 1:400 | — | — | — | 1:25 | 1:3 200 | — | — | — |
| | 86 | — | — | — | 1:400 | — | — | — | 1:200 | 1:400 | — | — | — |
| | 87 | — | — | 1:25 | 1:25 | — | — | — | 1:400 | 1:3 200 | — | — | — |
| | 100 | 1:25 | 1:50 | 1:25 | 1:25 | — | — | — | — | — | — | — | — |
| | 101 | — | — | — | 1:400 | 1:25 | — | 1:25 | 1:400 | 1:3 200 | — | — | — |
| 211 (O26:B6) F+ × 69 (O26:B6) F- | 62 | 1:100 | 1:200 | 1:50 | 1:400 | — | — | 1:200 | 1:100 | 1:3 200 | — | — | — |
| | 63 | 1:50 | — | — | 1:400 | — | — | 1:200 | 1:100 | 1:3 200 | — | — | — |
| | 65 | — | 1:200 | — | 1:200 | — | — | — | — | 1:3 200 | — | — | — |
| | 67 | — | — | — | 1:400 | — | — | 1:25 | 1:50 | 1:3 200 | — | — | — |
| | 68 | — | 1:200 | — | 1:400 | — | — | 1:100 | — | 1:1 600 | — | — | — |

Legend: Underlined numbers indicate strongly positive agglutination reaction (+++, ++++); numbers not underlined denote positive agglutination reaction (++).

The antigenic properties were studied in 30 recombinant strains obtained by crossing bacteria of the same serotype (Table 1).

Recombinants from the crossing 416(O20 : B145)$F^+$× 334(O20 : B145)$F^-$ were divided into three groups: 1) those which had lost the properties of the original strains and which possessed new antigens; 2) those possessing new antigens and retaining one of the antigens of the original strains; 3) those possessing both parent antigens and characters of a new serotype.

The recombinants of group 1, which had lost the antigens of the parent strains, were agglutinated in the heated state by O111 serum in fairly high titers, and some of them by O26 and O55 sera. Besides the parent antigens, most of the group 2 recombinants possessed somatic antigen of serotype O111 : B4. Five strains were found to have one of the antigens of serotypes O26 : B6 and O55 : B5. The 3rd group contained only two recombinants (105 and 113). Recombinant 105 possessed the surface antigen of serotype 408 and strain 113 the somatic antigens of serotypes O55 : B5 and O111 : B4.

Recombinants from crossing 103(O26 : B6)$F^+$× 69(O26 : B6)$F^-$ and 211(O26 : B6)$F^+$×69(O26 : B6)$F^-$ could be divided into the same three groups, although the detected antigens belonged to another serologic type. Antigens of serotypes O55 : B5 and O111 : B4 were found in the strains obtained from these crosses. However, antigens of serologic type O20 : B145 were most prominent, often giving a positive agglutination reaction in a titer equal to that of the serum.

Tests of motility of the recombinants showed that all 168 strains obtained were motile.

Since the original strains possessed equal fermenting power, the recombinants were studied in order to detect new properties, i.e., loss of the ability to ferment particular carbohydrates. All the studied recombinants were indistinguishable in fermenting power from the original strains.

The behavior of 80 recombinants toward phages of the T group was studied. Resistance only to some of the phages producing lysis of the recipient strain was transmitted to a proportion of the strains. Some strains which retained the sensitivity of the recipient strain were lysed by phages to which the recipient was resistant. Strains were found which were resistant to phages producing lysis of the recipient strain and sensitive to phages to which the recipient was resistant.

The study of the serologic properties of the recombinants thus demonstrated the formation of new antigens in them, of a type which depended on the bacteria crossed. If they were bacteria of serotype O20 : B145, then antigens of serotype O111 : B4 were formed. Crossing of bacteria of serotype O26 : B6 led to the appearance of characters of serotype O20 : B145. Besides the polyagglutinability which was characteristic of the majority of strains, antigens of one of the serotypes were predominant.

## LITERATURE CITED


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