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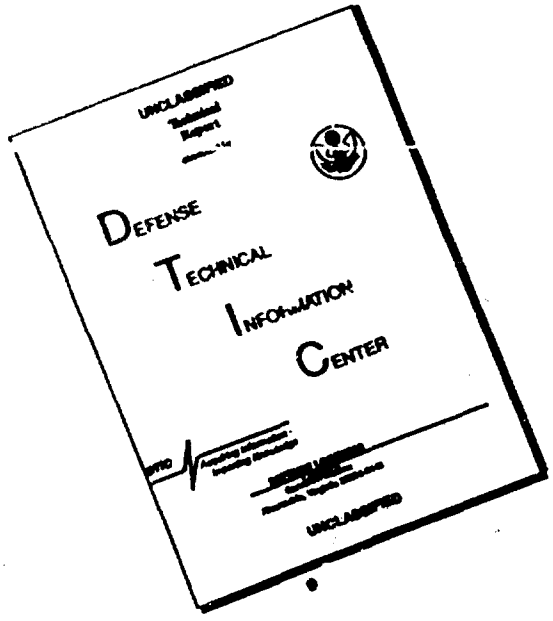

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PROPERTIES OF GENETIC RECOMBINANTS (E. COLI WITH SHIGELLA FLEXNERI) IN THE BODIES OF LABORATORY ANIMALS

[Following is the translation of an article by A. A. Abidov, Doctor of Medical Sciences, and N. A. Zakirov, Ye. F. Kalinina, and M. A. Abdusamatov, Candidates of Medical Sciences, from the Laboratory of Vaccine Strain Genetics of the Tashkent Scientific-Research Institute of Vaccines and Sera, published in the Russian-language periodical Med. Zhur. Uzbek. (Medical Journal of Uzbekistan) 11:41--44, 1967.]

At the present time it is feasible under laboratory conditions to cross coliform bacteria with dysenteric bacilli. Here a number of features of coliform bacteria may be conveyed to dysenteric bacilli. However, this problem has been studied little (Stenzel). Its solution has significance for clearing up the mechanism of formation of atypical cultures.

The purpose of the present work is the isolation of genetic recombinants from the urinary bladder of guinea pigs and the intestines of white mice by means of crossing coliform Hfr strains with Shigella flexneri and studying their biochemical, serological, and pathogenic properties, and also type of nourishment and streptomycin resistance.

As donors we used strains Hfr H ($S+B-T_6^S T_1^S Lac+ Gal+$), Hfr H ($\lambda+B-$), HfrC ($\lambda+me-Lac+Gal$), derived from E. coli K-12. They are all sensitive to streptomycin, lactose positive, auxotrophic mutants.

λ^+ - presence of prophage; B_1 - incapable of syntnesizing thiamine; $T_1^S T_6^S$ - sensitivity to phages T_1 and T_6; Gal+ - fermentation of galactose; λ^- - absence of prophage; mi - incapable of synthesizing methionine; S^r - resistant to streptomycin; Lac+ - fermentation of lactose.

As the receptors we used 5 strains of Shigella flexneri which had been stored under laboratory conditions for 3 years and 14 strains (560, 567, 569, 740, 836, 840, 841, 851, 871, 875, 885, 893, 2750) which were freshly isolated from patients with dysentery. These strains were prototrophic, streptomycin resistant, and did not ferment lactose.

Crossing of coliform bacteria with Shigella flexneri under artificial conditions was done by the method of Lederberg, which was described in detail in the work by A. P. Pekhov and A. A. Abidova.

As the selective medium we used the minimal medium which is used in work with auxotrophic mutants (Lederberg). It includes the following components: K_2HPO_4 - 7 g, KH_2PO_4 - 2 g, Na_3 citrate · $5H_2O$ - 0.5 g, $MgSO_4 \cdot 7H_2O$ - 0.1 g, $(NH_4)_2SO_4$ - 1 g, 1% lactose, eosin - 0.4 g/l, methylene blue - 0.065 g, agar Difco - 20 g, distilled water - 1,000 ml, streptomycin - 100 AU/ml. This medium made it possible to select recombinants Lac+ Sr, i.e., fermenting lactose and resistant to streptomycin.

In addition to this, as a control we used the Ploskirev and Endo medium.

For crossbreeding we used the method of Shteptsel. In an evacuated urinary bladder we introduced 1 ml of a 16-hour broth culture of Shigella flexneri containing $2x10^{10}$ bacteria. In 12 hours, using the same method, we introduced $2x10^{10}$ of shigella bacteria, a washing of a 16-hour culture, and coliform bacteria HfrC, found in 1 ml of meat-peptone broth. In 6 hours the first samples of urine were taken with a sterile catheter and a drop was placed on the surface of the Ploskirev-Endo selective medium and ground with a spatula. Then again $2x10^{10}$ microbial bodies of coliform bacilli HfrC were introduced. In 24 hours after the second superinfection urine was taken again.

The experiments were repeated once in 3 days. After 3 days the animals were sacrificed by using chloroform vapors, the abdominal cavity was autopsied, and seedings made directly from the urinary bladder. After autopsy of the urinary bladder biopsy specimens were taken from the damaged wall.

In carrying out the tests we used the method of Shneyder, Formol, and Baron (1961).

The test mice, weighing 18--20 g, were not fed or given anything to drink for 24 hours. Then, using a needle with metallic olive /?/ on the end we introduced 0.5 ml of distilled water containing 100 AU of streptomycin, 10 AU of erythromycin, and 500 AU of mycostatin.

After 24 hours the mice were divided into groups. The mice of the first group were given an 0.5 ml suspension of Shigella flexneri containing $1x10^{10}$ microbial cells, and the mice of the second group - 0.5 ml of an 18-hour broth culture of E. coli HfrC containing $2x10^{9}$ viable bacterial cells. After 24 hours excrement was seeded on the Ploskirev and Endo media. Then all the mice were nourished with suspensions of the mutual parent culture.

Samples were taken after every 4 hours for 24 hours. After this feces were seeded for 6 days once every 24 hours. Material taken from the intestines was emulsified in physiological solution and 0.1 ml

was seeded on selective medium and incubated for 6 days at 37°. Recombinants appeared on the selective medium on the fourth day.

Preliminary crossing of coliform bacilli was conducted under artificial conditions. For the tests we used 5 strains of coliform bacteria and 5 of Shigella flexneri which had been stored for a long time under laboratory conditions.

Data of the tests, which were repeated 8 times, are presented in the Table.

Table

Data of fertile combinations of strains of dysenteric bacteria with various Hfr strains

№ штамма дизентерийных бактерий	Фертильность комбинаций доноров и реципиентов		
	Штамм кишечной палочки		
	Hfr HSλ+	Hfr HSλ -	Hfr C
2044	1	2	3
2047	13	5	8
2050	—	—	5
2055	—	—	2
3584	—	—	—

Key: (a) No. of strain of dysenteric bacteria; (b) Fertility of combinations of donors and receptors; (c) Strain of coliform bacilli.

As can be seen from the table, all the strains of coliform bacteria HfrC, Hfr H λ^-, and Hfr λ^+ crossed with Shigella flexneri No 2044 and 2047. The HfrC strain of coliform bacteria when crossed with Shigella flexneri No 2050 produced a greater number of recombinants than with strain No 2055. Shigella flexneri strain No 3584 did not cross with one of the donors.

The resulting showed that strains of Shigella flexneri which had been stored under laboratory conditions for more than 3 years possessed the capacity to cross with coliform bacteria. Out of 5 dysenteric strains only one did not display this ability.

Of the various crosses 39 genetic recombinants were obtained. All the resulting recombinants were prototrophic and streptomycin resistant.

An investigation of biochemical properties showed that the recombinants fermented lactose, i.e., inherited this property from the donors.

Investigation of the serological properties of the recombinants showed that they are agglutinated by adsorbed flexneri serum.

On guinea pigs we checked the pathogenic properties of the initial cultures of Shigella flexneri and genetic recombinants with the help of a kerato-conjunctival test. It was established that none of them possessed pathogenic properties. This may be explained by the prolonged storage of the strains under laboratory conditions. The lowering of pathogenic properties in streptomycin resistant dysenteric cultures was pointed out by Stenzel in his work, and he proposed its use for the crossing of the more pathogenic strains of Shigella flexneri.

For this purpose we obtained 32 freshly isolated strains from dysentery patients from the bacteriological laboratory of the Kuybyshev Region of the city of Tashkent and from the sanitary-epidemiological station of the Central Asian Railroad. Out of the 32 cultures we selected 14 lactose negative and streptomycin resistant. By means of crossing them under artificial conditions with the 14 freshly isolated strains of Shigella flexneri we obtained 14 recombinants: from the cross Hfr CX2730 - 12 and from the cross Hfr CX569 - 2. All of these were prototrophic, lactose positive, and streptomycin resistant.

An investigation of the pathogenic properties of initial strains of Shigella flexneri No 2730 and 569, isolated from recombinants No 1, 2, and 3 from cross Hfr CX2780, and recombinant No 4 from the cross Hfr CX569, showed that they possess pathogenic properties.

Thus the resulting data testify that out of the 14 freshly isolated Shigella flexneri only 2 strains in crosses with coliform bacteria Hfr produced the growth of genetic recombinants.

Subsequently, using these strains, we set up tests on the crossing of coliform bacteria with dysenteric in the urinary bladder of guinea pigs.

In guinea pigs, infected with freshly isolated strains of Shigella flexneri No 2730 and 569, we observed a lowering of weight by two times, a lowering of appetite, and transient non-retention of urine.

Six hours after the administration of coliform bacteria Hfr C in the urinary bladder of a guinea pig urine was taken from it with a sterile catheter.

Then a drop of urine was placed on the surface of selective medium, and on the fourth day of incubation at a temperature of 37° two recombinants were isolated from a cross of Hfr CX2730. Both recombinants fermented lactose with the formation of acid without gas and were prototrophic and streptomycin resistant.

Investigation of the serological properties of the recombinants and the initial cultures showed that the recombinants were agglutinated by adsorbed flexneri serum, but in comparison with the initial culture of Shigella flexneri lost the type and sub-type antigens. At the same time group antigens were replaced with 7.8 for 3.4.

A study of the relationship of recombinants of Shigella flexneri to phages λ, T_2, and T_6 showed that the recombinants inherited a sensitivity to these phages from the coliform bacteria.

Subsequently we crossed the coliform bacteria with Shigella flexneri in the intestines of white mice, as a result of which three recombinants were obtained - two from the cross Hfr CX2730 and one from the cross Hfr CX2050. The isolated recombinants were related to the prototrophic type of nourishment and were lactose positive and streptomycin resistant. It is necessary to note that 2 recombinants out of 3 were isolated from a cross, where as the recipient freshly isolated dysenteric bacteria were used.

Thus, under laboratory conditions it is possible to cross coliform bacteria with Shigella flexneri in the body of animals.

For obtaining recombinants under natural conditions it is necessary that the initial cultures possess a high degree of viability. Also necessary are 16-18 hour growth, a lesser number of donors in comparison with recipients, and no less than 4 hours during which the mixed culture is preserved in the body of animals.

Out of the 5 resulting genetic recombinants 4 were isolated from crosses, where as the recipient we used freshly isolated (from patients) strains of Shigella flexneri.

The isolation of a small number of recombinants during the crossing of coliform bacteria with dysenteric from guinea pigs and white mice is explained by the death of the recombinants under the influence of protective forces of the organism and the absence of a full-value laboratory animal, in the intestines of which the coliform bacteria Hfr and Shigella could take root to an equal measure.

Thus, the results of our investigations testify to the feasibility of interspecies hybridization of bacteria in the living organism. They also make it possible to draw the conclusion that the given mechanism partly explains the formation of pathogenic atypical dysenteric cultures.