METHOD FOR PRODUCING GENETIC RECOMBINANTS OF Escherichia coli AND Shigella flexneri

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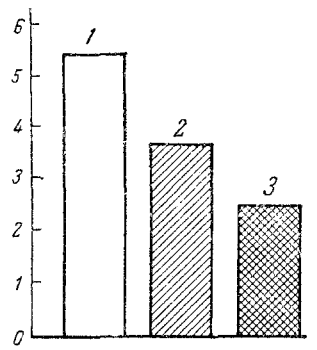
Usually a 0.85% physiological salt solution is used as a medium for conjugation of bacteria when setting up experiments on genetic recombination. Recombinants are elicited on a selective minimal EMG-lactose-streptomycin medium, the recipe of which was worked out as long ago as in 1950 by Laderberg [3]. With all the advantages of this medium, recombinants can be demonstrated on it only if they have required lactose-positivity and streptomycin-resistivity. To elicit other recombinants a careful selection of new selective test substances is required, and this brings up considerable difficulties. Therefore, many recombinant varieties are inaccessible to the researcher.

We attempted to change the method of producing recombinants when crossing Shigella and E. coli on the basis of two factors. First, the best contact between bacteria can occur in a favorable medium with a rigorously determined isoelectric point and electrical conductivity of the latter. On the basis of this we replaced the 0.85% solution of NaCl, which is hypertonic for E. coli and used as a medium for conjugation, by a 0.5% solution of NaCl which is isotonic for the intestinal group of microorganisms. This salt solution promoted the preservation of the optimal turgor of the microbial cells and excluded plasmolytic processes which are possible in a hypertonic medium. Second, it seemed to us that recombinants could be elicited more successfully if we were to use a medium richer in nutrients than the minimal. The ability to ferment lactose which is transferred to the recombinants should be demonstrated on any medium containing lactose. For this purpose we carried out a parallel experiment with a minimal medium, a modified Endo medium, and Hiss' solid carbohydrate medium.



Dependence of the number of recombinants obtained on the NaCl concetration. On the y-axis is the average number of colonies of genetic recombinants on 1 plate with selective medium (the index was derived on the basis of 7 series of experiments); 1) 0.5% solution of NaCl; 2) 0.2% solution of NaCl; 3) 0.85% solution of NaCl.

The Endo medium was prepared in the usual manner, cooled to 45-50°, and supplemented with a solution of streptomycin in an isotonic 0.5% solution of NaCl. It was experimentally established that the best results are obtained when streptomycin is added in a dose of 155-165 units per 1 ml of medium. This was the first variant of the Endo medium. The second variant of the medium had the same composition, but we added a solution of methylene blue to it in a quantity which corresponded to its content in the minimal medium. As the third medium we used Hiss' dry preparation with lactose and indicator VR, from which we prepared a solid medium by mixing 5 g of the preparation with 100 ml of distilled and boiling water. To the medium, cooled to 50°, we added streptomycin,and for contrast with the VR indicator, a solution of eosin. Here, the concentration of eosin was equal to the content of the stain in the minimal medium.

The experiment of recombination was set up simultaneously on several media by the method used at the Laboratory of Genetics at the Institute of Experimental Biology, USSR Academy of Medical Sciences (with the exception of the concentration of salt solution and the ratio of

Effectiveness of Using Various Media to Demonstrate Recombinants

Nutrient medium	M (aver. frequency of eliciting recombinants on 1 plate)	Statistical data			
		σ	m	t	p
Minimal medium with lactose, streptomycin, eosin, and methylene blue	0.43	±0.36	±0.08	5.4	<0.05
Endo medium with streptomycin	5.12	±4.01	±0.89	5.7	<0.05
Endo medium with streptomycin and methylene blue	5.34	±3.87	±0.86	6.2	<0.05
Hiss' medium (solid) with lactose, VR indicator, eosin, and streptomycin	3.21	±2.97	±0.66	4.8	<0.05

the number of donors and recipients). As a donor we used a culture of E. coli, strain HfrH, obtained from the Pasteur Institute (Paris) and kindly forwarded to us for the experiments by Doctor of Medical Sciences A. P. Pekhov. The recipient strains of Shigella flexneri were obtained in part from the laboratories in Moscow (5 strains) and from the municipal sanitation and epidemilogical stations in Vladivostok (10 strains). The main selective indices of the donor culture of E. coli were Lac^+ and S^S and of the recipient cultures, Lac^- and S^r. The ratios (F^+ and F^-) were worked out for the given media experimentally and can be expressed quantitatively as 1:4, i.e., the ratio was explicit,but there was a somewhat smaller predominance of female individuals than that recommended [2].

After 60 min holding in the thermostat and completion of the conjugation process, the mixtures of donors and recipients were cultured on the media: on the minimal medium and on the aforementioned variants of the Endo and Hiss' media. Here, inoculation was done in a volume of 0.1 ml of whole suspension and suspensions diluted by a factor of 10, 100, and 1000. At the same time we set up a control for the survival rate of the donor and recipient cultures. All inoculations were carried out in 4-5 samples for convenience of subsequent statistical analysis of the material and for increasing the possibility of detecting recombinants. There was a five-fold replication of each culture. In all, 75 series of experiments were set up.

The following was observed on the minimal medium with streptomycin, lactose, and the methylene-eosin indicators. The control cultures of E. coli (strain HfrH) and of Shigella flexneri (F^-) did not grow; the streptomycin interfered with the first, and the second could not find nutrients. The recombinants, having acquired from the parents streptomycin-resistance (S^r) and the ability to split lactose (Lac^-), began to utilize this carbohydrate, multiplied, and were elicited as a pink-red colony. Unfortunately, positive finds in the form of single colonies could be obtained only after a five- or six-fold repetition of the experiments and then not in each series of the experiments.

The picture was somewhat different on the variants of the lactose-containing Endo medium which were used parallel with the minimal medium. E. coli (strain HfrH) as usual did not grow owing to the streptomycin-sensitivity (S^S) inherent to this strain; Shigella flexneri grew as a delicate colorless film or uniformly distributed isolated colonies. After 48 h single, but very distinct bright red colonies of recombinants were elicited against this background. We did not observe a later appearance of such colonies nor an earlier growth.

Especially demonstrative was the appearance of recombinants on the Endo medium supplemented with methylene blue: raspberry red colonies appeared against a grayish-blue background of the medium. Furthermore, the methylene blue disturbed the pinking of the medium and for a long time, 6-7 days, remained unchanged.

The use of Hiss' dry preparation with lactose, VR indicator, eosin, and streptomycin as the base for the nutrient medium was ineffective: we observed a rather luxurious growth of the Shigella cultures with many color hues. Among them it was rather difficult to elicit recombinants which produced slightly orange colonies against a brownish-red background of the dysentery bacteria.

An assured harvest of recombinants in each series of experiments and the complete absence of so-called sterile series were common for all the used variants of the media. A second important advantage is the lesser dependence of the results on the quality of the medium itself and the greater standardization of the experimental conditions.

The results of using each medium separately were analyzed by the variance method and are shown in the table.

As is apparent from the data in the table, the best indices are obtained when using the Endo medium supplemented with methylene blue. Hiss' medium proved to be the most unsuccessful: the Shigella recipient culture hampered the growth of the recombinants and their detection. On this medium we obtained satisfactory results when we inoculated mixtures of $F^+ + F^-$ only at dilutions of 100 and 1000.

To check the effect of the 0.5% isotonic solution of NaCl in comparison with the physiological (and hypertonic for the E. coli group) 0.85% solution of NaCl and the 0.2% (hypotonic) solution, experiments were specially set up with the use of just one medium—the Endo medium with streptomycin and methylene blue. These data are shown in the figure.

The data in the figure indicate a small difference in the "yield capacity" of the recombinants when using various conditions of bacterial conjugation. However, we see an explicit tendency toward an increase in the number of recombinants when using an isotonic 0.5% solution. We cannot disregard this if we take into account the difficulty in eliciting recombinants on a starvation minimal medium.

Thus, on the basis of these investigations we can conclude that the use of the Endo medium in the modifications named here considerably facilitates eliciting the recombinants formed in each series of experiments. The best modification of the Endo medium is the second variant—the Endo medium with streptomycin and methylene blue. Distinct colonies of recombinants are seen on this medium and its color remains unchanged. Hiss' medium with lactose, VR indicator, streptomycin, and eosin is unsuitable for recombination experiments.

The replacement of the 0.85% solution of NaCl by a 0.5% isotonic solution of NaCl for E. coli creates optimal conditions for conjugation of the bacteria.

LITERATURE CITED

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