THE PRODUCTION OF FILTERABLE FORMS OF Escherichia coli IN FAVORABLE AND UNFAVORABLE CONDITIONS OF CULTIVATION

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Hitherto, the role of filterable forms of microorganisms in the development of the bacterial cell and in pathological processes has been inadequately studied. The absence of any generally accepted view of the structure and development of microorganisms and of the role of filterable forms in the ontogenesis of microorganisms is due to the considerable variety of methods of production of filterable forms. The most widely held opinion at the present time of the process of formation of filterable forms, as particles without cellular structure and arising as the result of disintegration of cells under the influence of unfavorable factors [1,6,7,8,9,10] has led to the creation of corresponding methods of influencing the bacterial cell: aging, grinding, shaking, repeated freezing and thawing, ultrasound, the action of phage and of antibiotics. Certain authors [2,3,5] regard filterable forms as a stage of the "ontogenetic development of the cell, probably reflecting the phylogenetic history of the particular species" (G.L. Kalina), and claim that filterable forms may be obtained much more frequently in young cultures, growing in favorable conditions.

In the present research, an attempt was made to study comparatively the detection of filterable forms and the subsequent regeneration from these forms of secondary strains, in both young (4-6-hour) broth cultures, grown in conditions favorable for growth, and in old (1-2-month) cultures with signs of autolysis of the bacterial cells, subjected to repeated freezing, thawing and agitation in a shaker.

METHOD

The material used for the investigation consisted of Escherichia coli 844 which, after 3-4 subcultures in an egg medium (2 yolks + 5ml physiological saline), was seeded in a dose of 0.1-1.0 ml in 200 ml of nutrient medium: meat-peptone broth, meat-peptone broth with the addition of 10% of egg medium, or casein broth with 10% of egg medium. Some of the inocula were aerated for 4-6 hours by the passage of sterile air into the medium; others were kept in the refrigerator at a temperature of 4° for 18-20 hours, and all were centrifuged for 10-15 minutes at 3000-4000 rpm before filtration, in order to remove excess bacterial cells, especially those growing on egg media with aeration. After growing for 4-6 hours at a temperature of 37°, all the inocula were filtered through 30-mm Seitz filters with S. F. asbestos pads at negative pressure(−100 mm). At this pressure the original bacterial cells did not pass into the filtrate, as was from time to time observed at a negative pressure greater than −100 mm.

From the young cultures 197 filtrates were obtained.

During the parallel investigation of the old (1-2-month) broth cultures, of which 92 were subjected to agitation for 6 hours in a shaker, and 88 (suspensions of $20 \cdot 10^9$ bacterial cells in distilled water) were twice frozen (18 hours) and thawed, 180 filtrates were obtained.

Thus, altogether, 377 filtrates were obtained. In order to detect filterable forms, 25-30 ml of each filtrate was seeded into equal volumes of nutrient media: meat-peptone broth, 10-20% serum broth, broth containing 1% glucose, broth containing 3% and 5% of a filtered lyzate of Sarcina, obtained by the method of dissolving washings of a suspension of $10 \cdot 10^9$ cells in 0.5% physiological saline with lysozyme [4]. The filtrates were kept at a temperature of 37° throughout the experiment. After different intervals of time (7,14,21 days and over) subcultures were made on Petri dishes containing a feebly alkaline (pH = 7.6) meat-peptone agar, 10% serum agar and agar containing 3-5% filtered lyzate of a Sarcina culture. The dishes with the subcultures of the filtrates were kept at room temperature for 6-7 days or longer, for the filterable forms grew very slowly.

RESULTS

The experimental results given in the table show that the formation of filterable forms in both the young

Results of Production of Filterable Forms from Different Objects

Objects from which filtrates were obtained	Aeration	Number of filtrates investigated	Number of regenerated filterable forms
Young cultures			
4- hour culture in meat-peptone broth	+	31	2
	—	31	3
4- hour culture in meat-peptone broth with addition of egg medium (10%)	+	34	3
	—	27	2
6- hour culture in casein broth with addition of egg medium (10%)	+	34	3
	—	40	4
Total		197	17
Old cultures			
30-60-day broth cultures treated in the shaker	—	92	9
Suspensions of 20 × 10^9 microoganisms in distilled water, frozen and thawed twice	—	88	7
Total		180	16

cultures, grown in conditions favorable to growth (nutrient medium, aeration), and in the old cultures, subjected to the destructive treatment, took place equally rarely (33 of 377). The number of regenerated, secondary strains from filtrates of the young cultures (17 of 197) and from old cultures exposed to cell-destructive action (16 of 180) was roughly equal.

It may also be seen from the table that neither aeration nor cooling had any appreciable effect on the formation of secondary strains. Secondary strains were grown most often (19 of 33) on meat-peptone agar with 3% of filtered lyzate of Sarcina followed by subsequent subculture in 10% serum broth.

The growth of the secondary cultures on agar was hardly visible to the naked eye; they appeared as bead-like, slowly growing, greyish-blue microcolonies, which on subculture in broth gave submerged growth in the form of a precipitate, causing slight clouding of the broth.

Microscopic examination showed that the secondary strains consisted of Gram-negative bacilli with terminal swellings, or of short Gram-negative rods. Biochemically, all the strains isolated were inert and did not decompose sugars (when tested on a small number of various sugars).

The experimental results demonstrate that the frequency of positive subculture of secondary strains from filtrates was insignificant, and was dependent neither on the age of the original culture nor on the conditions of its growth. Filterable forms were obtained in approximately the same number of cases from young cultures grown in favorable conditions and from cultures treated with cell-destructive agents, after repeated subculture on various media.

Of the media which we used, that containing lyzates of Sarcina merits attention and further study.

SUMMARY

Three hundred seventy-seven Escherichia coli filtrates were investigated for the presence of filterable forms. Seventeen secondary cultures were recovered from a total of 197 filtrates obtained from young 4-6 hour cultures grown on egg and egg-casein broth with aeration, i. e., in conditions favorable for growth. Sixteen filterable forms were isolated from the remaining 180 filtrates of old (30-60 day) cultures of the same Escherichia coli strain, subjected to shaking, or double freezing and thawing, i. e., to effects destroying the bacterial cell. Thus, the supposition of some scientists, that the number of isolated secondary cultures depends on favorable growth conditions of the initial culture, was not confirmed by our investigations.

LITERATURE CITED

1. N. N. Zhukov-Verezhnikov and A. P. Pekhov, Zhur. Mikrobiol., Épidemiol. i Immunobiol. 5, 14 (1957).
2. G. P. Kalina, in: Inheritance and Variation in Plants, Animals and Microorganisms[In Russian], (Moscow, 1939) Vol. 1, p. 285.

3. G. P. Kalina, Abstracts of Proceedings of a Conference on "Variation of Microorganisms and Bacteriophage" [In Russian] (Moscow, 1958)p. 35.
4. K. S. Karpuzidi and M. S. Drozhevkina, Zhur. Mikrobiol., Épidemiol. i Immunobiol. 6, 92 (1957).
5. V. A. Krestovnikova, Abstracts of Proceedings of a Conference on "Variation of Microorganisms and Bacteriophage" [In Russian] (Moscow, 1958) p. 37.
6. S. N. Muromtsev, Zhur. Obshchei Biol. 12, 3, 161 (1951).
7. V. D. Timakov, R. S. Mikhel'son, L. S. Kolyaditskaya et al., Zhur. Mikrobiol., Épidemiol. i Immunobiol. 10, 36-40 (1951).
8. V. D. Timakov, R. S. Mikhel'son, L. S. Kolyaditskaya et al., Zhur Mikrobiol., Épidemiol. i Immunobiol. 2, 25 (1952).
9. V. D. Timakov, G. Ya. Kagan, E. I. Kopteleva et al., Transactions of the Institute of Microbiology of the AN SSSR [In Russian], p. 25.
10. V. D. Timakov, G. Ya. Kagan, and E. I. Kopteleva, Transactions of the Institute of Microbiology of the AN SSSR[In Russian], p. 225.