

# MICROBIOLOGY AND IMMUNITY

## GROWTH AND DEVELOPMENT OF BACTERIA IN CONNECTION WITH THE PHENOMENON OF BACTERIOPHAGIA

### COMMUNICATION 3. MICROBIAL SYMBIOSIS AS A FACTOR INDUCING PHAGE-PRODUCTION IN BACTERIA

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The phenomenon of so-called spontaneous bacteriophagia is of great importance for the solution of a number of questions of bacteriophage theory [1].

In 1950 A. Lwoff, L. Siminovitch and N. Kjeldgaard [6] showed that the action of small doses of ultraviolet radiation on a microbial population could induce the production of free phage particles in cultures of certain lyso-genic strains. This effect, called induction, has been intensively studied in recent years by many authors. So far it has been shown that various physical and chemical agents besides ultraviolet rays possess inducing properties; these include x- and  $\gamma$ -rays, thiomalic and ascorbic acids, tertio-butyl peroxide, ethylenimine, nitrogen mustards, sulfathiazole [4, 5, 7].

As we know, these agents are mutagenic or carcinogenic factors, usually absent in natural conditions of life of the microorganisms. Inducing factors of a biological nature have not been described in the literature and the establishment of such factors is of undoubted interest at present.

While carrying out a life study of isolated cells of sarcina and the colon bacillus (the symbiotic relations of which have been revealed by Zeitraffer filming [2]), growing in a joint culture on solid nutrient media, we found that in several cases the colon bacillus cells were lysed in the presence of sarcina packets. The nature of the lysis in this case did not differ from a bacteriophage lysis of these bacteria, studied at the same time.

On the basis of this observation we set up experiments for isolating phage from laboratory cultures of colon bacilli, by cultivating them jointly with the selected sarcina-symbiont to induce phage-production.

#### EXPERIMENTAL METHODS

Flasks containing 25 ml of meat-peptone bouillon were inoculated with 1 ml of a two-billionth suspension of day-old agar culture of *Bact. coli aerogenes* 1321 and with the same quantity of an agar culture of a strain of yellow sarcina 30. After several hours cultivation at 36° the contents of the flasks were centrifuged at 2500-3000 rpm for 45-50 minutes. Free phage particles in the supernate were demonstrated by inoculating it on a dish with indicator strains *Bact. coli aerogenes* 1321 and 1322 by the agar layer method [3]. As a control we used the supernatant liquid from cultures of colon bacilli without sarcina.

#### EXPERIMENTAL RESULTS

In the majority of our experiments we obtained positive results. Usually the plates showed a small number of negative colonies, appearing as small turbid spots of 1 mm diameter. The typical distribution of the spots on

the agar surface was either random, or had the form shown in Fig. 1. The control experiments gave negative results.

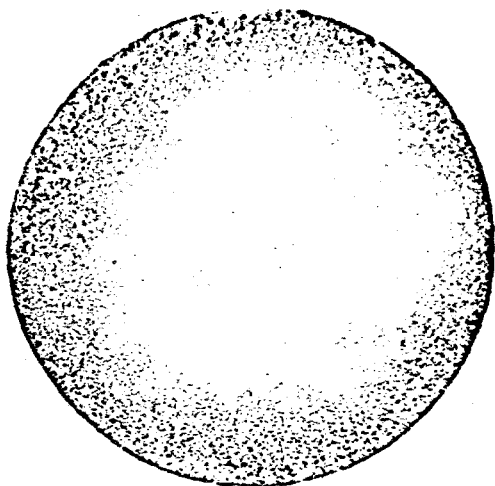


Fig. 1. Sterile spots on indicator plate after adding centrifugate of mixed culture of colon bacilli and sarcina.

After 1-2 passages on the plates the phage particles were separated from the bacteria by filtration of the phage-lysates through asbestos plates SF or membrane filters No.2. The five phage strains isolated in this way were arbitrarily designated S 1/1321, S 1/1322, S 2/1321, S 2/1322 and S 3/1321. In comparison with a reference phage strain 1321 lysing *Bact. coli aerogenes* 1321, the following properties were studied in these 5 phages: 1) titer on indicator strain, 2) morphology of spots (colonies) on agar, 3) serological affinity and 4) range of action.

While the titer of phage 1321 was  $10^{-8}$ , for S 1/1322 it was  $10^{-7}$ , for S 2/1322 and S 3/1321 —  $10^{-9}$  and for S 1/1321 and S 2/1321 —  $10^{-10}$ . All the phages on the agar with the indicator strain gave round clear spots of diameter 1.5-2.5 mm with a turbid zone 0.5-1.5 mm. Photographs of colonies of some of them are shown on Fig. 1, 2 and 3. For revealing the serological affinity of the phages we used antiserum obtained by immunizing rabbits with the reference phage 1321.

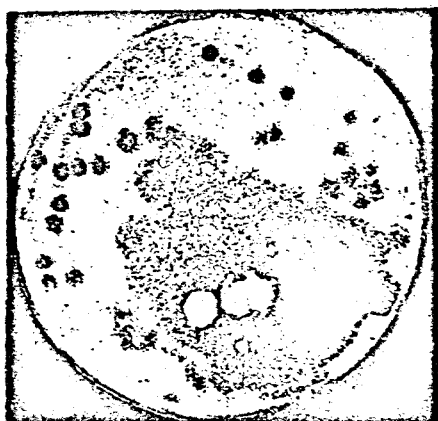


Fig. 2. Sterile spots on indicator plate after adding phage-filtrate S 2/1322.



Fig. 3. Sterile spots on indicator plate after adding phage-filtrate S 3/1321.

We found the value K for the antiserum (i.e. the rate constant of the reaction between phage and antibody, which depends on phage concentration and its activity); K of antiserum in testing with different phages is considered as an index of their degree of affinity [3].

For serum anti-1321 with respect to phage 1321 the constant was equal to 10, with respect to S 1/1321 and S 1/1322 — 15, to S 2/1321 — 20, to S 2/1322 — 7, and to S 3/1321 — 14.5. The absence of difference in the values of K shows that all 6 phages possess serological similarity.

In experiments to reveal the range of action of the phages we used strains of cultures of *Bact. coli aerogenes* 1321 and 1322, *Bact. coli* 675, 1094, 844 and K = 12. It was found that both the reference and isolated strains of phage lysed only strains 1321 and 1322.

Thus, by using the phenomenon of microbial symbiosis, we were able to induce phage-production in the colon bacillus 1321 by means of sarcina. The phage strains isolated by this method were indistinguishable from one another in several properties, and from the reference phage strains which lyse this species of bacteria.

The results of these experiments open up new prospects for further research on the origin of phages. The re-

sults obtained may be considered as part of the evidence for the hypothesis we have put forward, that one of the factors causing bacteria to produce phage in nature is the symbiosis of microorganisms.

#### SUMMARY

A method was developed of inducing phage-production of Bact. coli aerogenes 1321 with the aid of sarcina symbionts. The phages which were isolated have certain properties in common.

#### LITERATURE CITED

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