

3248

Action of the Bacteriophage on a Thermophilic Bacillus.

STEWART A. KOSER.

From the Hygienic Laboratory, University of Michigan, Ann Arbor, and the Department of Bacteriology, University of Illinois, Urbana.

Of the various species of bacteria which have been found susceptible to the action of the lytic principle or bacteriophage,¹ it is noteworthy that all are types which have their optimum temperature for growth at 37° C. or lower. It seemed of interest, therefore, to determine whether organisms capable of growth at high temperature—the thermophiles—would also be susceptible to the action of the lytic principle. Efforts to obtain a “thermophilic bacteriophage” have been successful and a preliminary report will be given here.

The organism employed in these experiments was one of the stock collection of thermophiles maintained in this laboratory. It was isolated originally by Tanner and Harding² from milk, and has been carried in stock under the designation T60. It is a medium-length, slender, Gram-positive rod, forms spores, and stains readily by the ordinary dyes. The culture is capable of growth at all temperatures from 20° to 60° C., the optimum appearing to be from about 45° to 52° C., at which point growth is very rapid and more luxuriant than at 37°. At room temperature a moderate growth develops after 2 to 3 days.

From sewage polluted river water an active lytic principle was obtained against this organism by employing the usual method of alternate feeding and filtration. The incubation temperature during this process was always 37° C. After developing a lytic filtrate at this temperature it was found to be very active, without any gradual adaptation to a higher temperature, when brought in contact with a homologous culture growing at 52° C. The lytic principle was transmissible through series of broth cultures, one series was held at 37°, the other at 52° C. At the higher temperature lysis occurred more quickly and complete clearing was frequently observed in a few hours.

Lytic areas were produced when appropriate dilutions of the active filtrate were spread over agar slants previously inoculated with the T60 culture. Incubation at 55° C. for 16 to 18 hours

resulted in distinct areas 2 to 3 mm. in diameter. The edge of these areas was somewhat irregular, in contrast to the even, round appearance usually shown in the colon-typhoid-dysentery group. Also, the areas showed a central, clear zone of lysis surrounded by a distinct grayish zone of incomplete lysis. Larger areas, 5 or 6 mm. in diameter, were produced by longer incubation. At 37° lytic areas were somewhat smaller than at 55° C. The irregular outline was evident, but the zone of incomplete lysis was absent, or at least so narrow as to escape notice. At room temperature the culture develops slowly and distinct areas are apparent only after 3 or 4 days. Their appearance at this time is quite similar to that shown after overnight incubation at 37° C.

Cross tests in which the T60 lytic principle was allowed to act against three other species of thermophilic bacilli at 52° all proved to be negative. Likewise, similar tests with a Shiga dysentery culture at 37° were negative.

The maximum temperature at which the thermophilic bacteriophage exhibited activity coincided very closely with the limits of growth of the culture. From 45° to 56° C. the culture developed readily, and lysis resulted upon application of a loop of the lytic filtrate. At 58°, although growth was retarded, lysis was evident in those tubes receiving the filtrate. At 60° growth was so scanty as to leave the result of the tests in doubt, while at 62° they were clearly negative.

Tests of inactivation by heat showed that the lytic filtrate resisted temperatures up to and including 70° C., but was destroyed at 75° C. The period of exposure was 30 minutes in each case. It should be noted that in this respect its resistance to heat corresponds very closely to that of the ordinary coli, typhoid, or dysentery bacteriophage. It is an interesting point that the thermophilic lytic principle, which is very active at temperatures fully 20 degrees above the optimum for ordinary bacteria, does not require a correspondingly higher temperature for its destruction.

The present observations may also prove of interest in connection with the discussion of the nature of the bacteriophage. d'Herelle¹ believes it to be a distinct living entity, ultramicroscopic and filterable, and he designates it by a specific name. If the present work is to be reconciled to this viewpoint, we must postulate the existence of thermophilic filterable viruses. Opposed

to d'Herelle's concept we have the theories of an autolytic enzyme or some other product of the cell itself. Since the deportment of the T60 lytic principle in regard to temperature follows closely that of the homologous culture, it would seem to lend more support to the latter opinion.

¹ d'Herelle, F., *The Bacteriophage and its Behavior*. English translation by G. H. Smith, Williams and Wilkins, Baltimore, 1926.

² Tanner, F. W., and Harding, H. G., *Centr. Bakt.*, Abt. II, 1926, lxxvii, 330.

ERRATUM.

Volume XXIII, No. 8, "A method for preserving and counterstaining vitally-stained cells," pp. 624 and 625, should read 1.1 (or 1.2) cc. of formalin, instead of 1.8 cc.