Bacteriophagy of *Streptomyces griseus* as Revealed by Phase Microscope.* (21093)

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Hofer(1) reported observation, but not photography of the lytic action of a rhizobium phage, as seen through the phase microscope. Since certain differences are apparent between his observations and our own with a different phage: host system, this note is offered. The host used is *Streptomyces griseus*, strain 4, and the phage is W2a isolated from soil here.

Methods. A suspension of spores was prepared by pipetting 3-4 ml of liquid medium (3 g yeast extract, 2.5 g glucose, and one g peptone per liter of distilled water) onto a 4-day slant culture on agar medium of similar composition. Approximately one ml of the suspension was removed to a small test tube and held 3 hours to allow incipient germination. A loopful of the germinating spore suspension was then added to about 0.2 ml of phage filtrate of approximately 5 x 10^7 particles per ml. When lysis was anticipated, wet mount preparations under No. 0 coverslips were set up under bright phase-contrast for observation over the critical period: the latent period with this phage : host system is about 1.5 hours. An American Optical (Spencer) phase microscope with turret condenser and Periplan 12X ocular was used. Light was provided by a Bausch and Lomb lamp with 6-volt ribbon filament regulated by a transformer. For the photomicrographs, a green (B) filter and Plus-X film were used. The camera was a Leica III-C with a Micro Ibso attachment.

Results. The general appearance of the cells at the stage used may be seen from the figures, showing the spore outlines from which protrude one or more short tubules or young hyphae. The beginning of lysis is indicated by less opacity and distinctness of outline, particularly at the tip and point of attachment of the hypha to the old spore body. It is to be noted too that the cells in the course of lysis do not appear larger in size than normal cells; they become merely hazy, granular and finally fade from sight. Following lysis of the young hyphal cell the old spore body persists for a time. Fig. 1, location *a*, shows a cell in early stage of lysis and just above it, at location *b*, one in late stage of lysis with the spore body still rather distinct. In succeeding Fig. 2-6 the cell at *a* is seen to be undergoing rapid lysis, since the time lapse between pictures is 5 minutes. Thus the time for disappearance of cell *a* is about 15 minutes from the normal appearance in Fig. 1 to complete lysis before Fig. 4. Traces of the spore body persist, however, nearly twice as long; Fig. 6 was taken 25 minutes after Fig. 1. Meanwhile another cell at *c* is undergoing lysis in Fig. 3-6.

The above phenomena of lysis are remarkable in their rapid sequence, realized in the study of living preparations. Contrary to the report of Hofer, we were unable to see phage particles attacking the cell surface with the optical system here used. Further there was no evidence of either swelling or bursting of the cell during phage multiplication. Although there is a granular stage of the cell (note Fig. 3, *a*), there is no residue suggestive of phage particles when lysis is complete.

The slow final lysis of the spore body suggests that it is of different composition from the young hyphal cell; perhaps it is a spore membrane or wall, slowly autolyzed rather than lysed by phage.

Summary. Lysis by phage of germinating spores of *Streptomyces griseus* has been observed and photographed under the phase microscope, bright phase-contrast. The time

* Published with approval of Director of the Wisconsin Agric. Exper. Station. This work supported in part by grant from Commercial Solvents Corp, Terre Haute, Ind.

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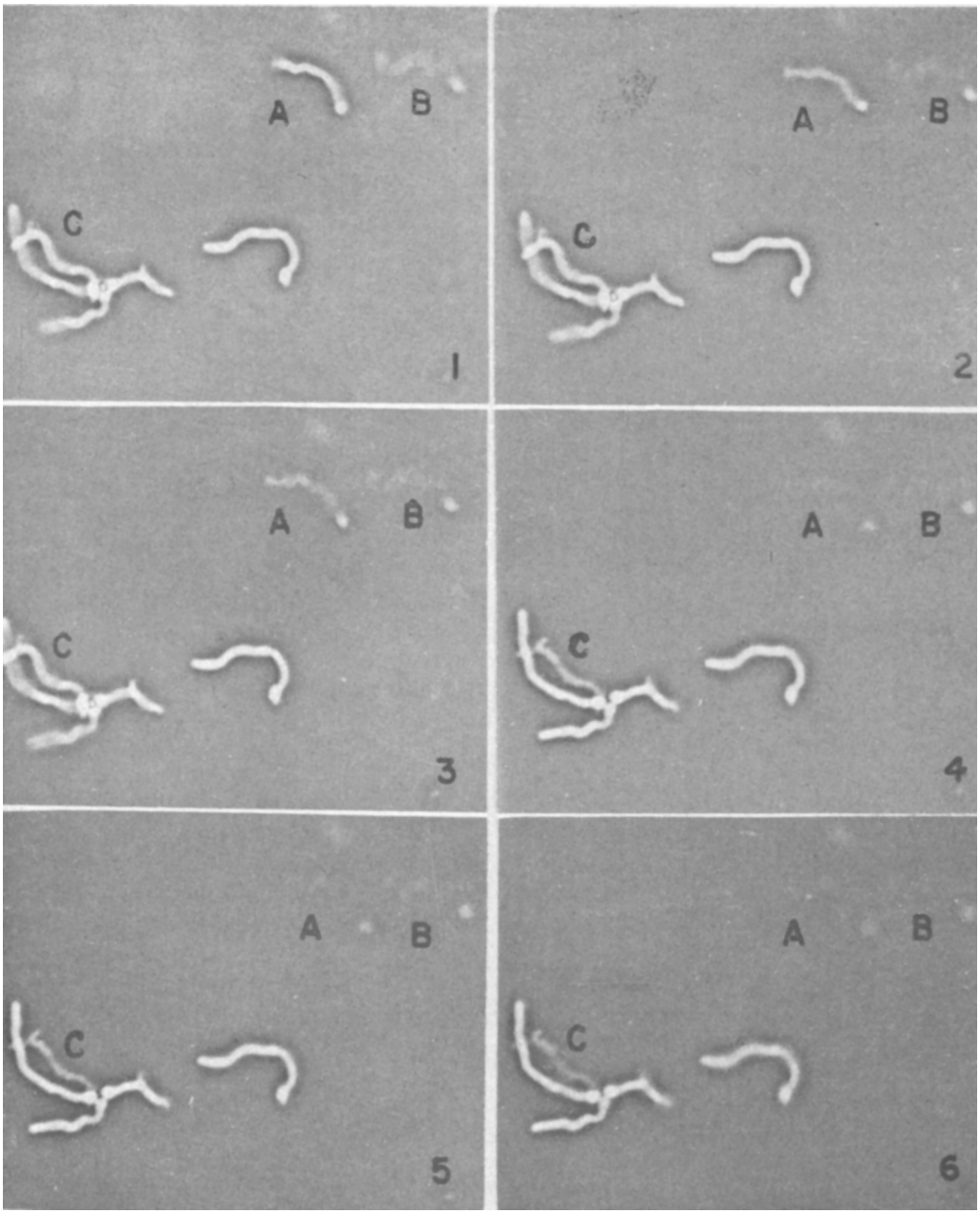

FIG. 1-6. Lysis of *S. griseus* by phage W2a. Photographs under bright phase-contrast, taken at 5 min. intervals. ×862.

required for complete lysis of a young hyphal cell is about 15 minutes.

1. Hofer, A. W., *J. Bact.*, 1947, v53, 781.

Received May 19, 1954. P.S.E.B.M., 1954, v86.