Use of Trypsin to Increase Bacteriophage Yield and to Facilitate the Isolation of Bacteriophage-resistant Mutants

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Lysis of bacteriophage-infected cells is often accompanied by the release of excess phage-induced lytic enzyme into the medium. Presumably, this enzyme facilitates lysis from within at the termination of the latent period, although details of the mechanism are lacking. The release of this enzyme by the first cells to lyse can cause premature lysis of the remaining cells, thus resulting in a reduced phage yield. Moreover, the isolation of phage-resistant, lytic enzyme-sensitive mutants is rendered difficult if not impossible. To overcome these problems, the enzyme must be destroyed or inactivated without affecting the infected or resistant cells.

Micrococcus lysodeikticus strain 1 was grown in 25 ml of 1.5% peptone, 0.7% yeast extract, 0.2% glucose, and 0.2% NaCl (pH 7.5) in 125-ml Erlenmeyer flasks. Incubation was at 35°C on a reciprocal shaker. The N1 bacteriophage used throughout the study was described previously (2) and was quantitated by the overlay method of Adams (1). Lysates were prepared by infecting a 20% inoculum of a 24-hr M. lysodeikticus strain 1 culture with the desired level of N1 phage and by incubating with shaking at 35°C until clearing was complete (usually 3 to 4 hr). It was previously determined that the N1-induced lytic enzyme which appears in the lysate is sensitive to several proteolytic enzymes (2). Since the conditions existing in the growth medium closely approximated ideal conditions for trypsin activity (i.e., pH 7.5), this enzyme was chosen for further study. Addition of filter-sterilized trypsin (1:250 trypsin, Difco) to the 0.04% level in the growth medium had no detectable effect on either the growth of M. lysodeikticus strain 1 or on the adsorption of N1 phage to the cells. However, the presence of trypsin in the growth medium did result in increased phage yields at all phage to cell ratios. The magnitude of the increase varied from 3 to 1,300% in various experiments, but the flask containing trypsin always yielded the largest number of phage particles. It was also noted that the yield of phage particles was seemingly independent of the multiplicity of infection in contrast to the situation when trypsin was omitted from the growth flask.

The titer of lysates prepared in the absence of trypsin could not be increased by adding trypsin after lysis was complete. Sodium citrate (0.05 m) could be substituted for trypsin in the growth vessel insofar as increasing phage yields was concerned. To further substantiate that destruction of the phage-induced lysozyme rather than a phage inhibitor was responsible for increased phage yields, the following experiment was performed. A 20% inoculum was infected with N1 phage and shaken at 35°C. At 30, 60, and 90 min (in a 100-min latent period) after infection, samples were withdrawn and the cells were lysed by adding egg white lysozyme. Each sample was divided in half, and one-half was treated with trypsin. An equal number of N1 phage particles was added to each portion. After 15 min of incubation, the phage content of each sample was determined by the overlay technique. There was no difference in titer between the trypsinized and untreated portions of the lysate. These data indicate that is is unlikely that any phage inhibitor is formed during the latent period; phage inhibitor, if present, would reduce the phage yield in lysates prepared without trypsin.

Continued incubation of the clear lysates containing trypsin often resulted in the growth medium becoming fully turbid within 24 to 48 hr. Flasks without trypsin never became turbid. Streaking a loopful of the turbid culture on plates having the same composition as the broth but including 1.5% agar resulted in the isolation of numerous colonies. Repeated restreaking of these colonies yielded pure cultures free from N1 phage. These isolates were then tested for their ability to adsorb and propagate a set of six different phages to which the parent strain was sensitive. The isolates were unable to adsorb any of the different phages; under the same conditions,

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M. lysodeikticus strain 1 adsorbed at least 90%. Moreover, the addition of $10^6$ phage particles to overlay plates containing the resistant isolates never yielded a single plaque. The phage-resistant isolates were tested for sensitivity to lysozyme and the N1-induced lytic enzyme. The results showed that each isolate was sensitive to both enzymes.

Similar success in increasing phage yields was achieved with two other phages of the N series; i.e., N2 and N6. The benefits derived from the incorporation of trypsin and possibly other proteolytic enzymes in the phage propagation medium are twofold. First, greater phage yields are consistently obtained. Second, phage-resistant, lytic enzyme-sensitive mutants can be isolated for study of the structure of the cell wall or phage receptor sites. It is assumed that if this technique can be applied in other phage-host systems, gram-positive organisms, with their comparatively simple cell walls and relative sensitivity to lysis by phage-induced lysins, would offer the best chance for success.

Presumably, the trypsin destroys the phage-induced lytic enzyme before enzymatic lysis from without occurs, thus allowing (i) phage-infected cells to continue producing phage particles until the normal termination of the latent period, and (ii) the survival and growth of enzyme-sensitive, phage-resistant cells which would otherwise be lysed.

Literature Cited