NOTES

In Situ Lysis of $\phi$29- and SPO1-Infected Bacillus subtilis

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An improved method of in situ lysis of bacteriophage-infected Bacillus subtilis was developed and used to study $\phi$29 and SPO1 phage structures produced by individual cells.

The in situ lysis technique for electron microscopy of bacteriophages was introduced by Kellenberger et al. to preserve and resolve phage T4 structures produced in lysis-inhibited Escherichia coli (6). T4-infected cells were partially lysed with osmium tetroxide, fixed with formaldehyde, and adsorbed onto specimen films where they gently lysed when negatively stained with phosphotungstic acid. Other investigators have also used in situ lysis to study phage (primarily T4)-infected E. coli (1, 3, 8, 9, 10, 11).

Farrell adapted the technique for use with spontaneously lysing SPO2-infected Bacillus subtilis (2), but we were unsuccessful in applying his method to phage $\phi$29-infected cells. The improved method of in situ lysis of $\phi$29- and SPO1-infected B. subtilis reported here is a rapid and simple means of observing viral progeny of individual cells and has proven useful for preserving fragile $\phi$29 particles produced in restrictive infections with conditional lethal mutants (unpublished data). The technique often provides more information than conventional thin section analysis of phage-infected bacteria, because more structural detail can be observed when particles are released from the tightly packed bacterial cytoplasm.

B. subtilis SPOA12 (5) was grown in dialyzed Difco antibiotic medium no. 3 (PB) at 37 C to $2 \times 10^8$ cells, collected by centrifugation, and resuspended to $2 \times 10^8$ cells per ml in adsorption medium (0.15 M NaCl; 0.01 M MgCl$_2$; 6H$_2$O; 0.05 M Tris-hydrochloride, pH 7.4). The concentrated cells were infected with phage at a multiplicity of 20 to 40. A second identical infection was made 5 min later. After 10 min of adsorption at 37 C, the infected cells were diluted 10-fold into prewarmed PB and incubated at 37 C until lysis of the first culture. The second infected culture was then immediately placed on ice and held until the time normal lysis would have occurred. All subsequent operations were performed with this culture. Kellenberger fixative (7) was added to give a final osmium concentration of 0.01%, and the suspension of partially lysed cells was then divided into 0.5-ml portions. At successive 2-min intervals for the next 16 min, formaldehyde (37%, Mallinckrodt) was added to the individual portions to give a final concentration of 2%. Immediately after the formaldehyde addition, grids with carbon-coated Formvar films were brought into contact with drops of the suspension of partially lysed cells. After drawing off the excess liquid with filter paper, the grids were stained by applying a drop of 1.5% neutral phosphotungstic acid. Grids were scanned and micrographs taken with a Philips EM301 electron microscope at 80 kV.

The 5-min interval between identical infections was essential for monitoring the time of spontaneous lysis, which varied slightly under seemingly identical conditions. Moreover, the time of lysis in $\phi$29 mutant infections varied substantially (unpublished data). The time gradient of osmium treatment prior to formaldehyde addition was also critical. Phage-infected B. subtilis are apparently most susceptible to osmium-induced partial lysis during a brief interval just prior to spontaneous lysis.

Figure 1 illustrates a $\phi$29-infected B. subtilis cell lysed in situ. Large numbers of phage heads and complete virus particles are observed. Most preparations contain a wide variety of cells in different stages of lysis, ranging from intact cells packed with phage structures to lysed cells which have released their phage contents.

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Figure 2 demonstrates an in situ lysed SPO1-infected cell. A large number of phage structures are observed; as in the φ29 preparation (Fig. 1), many of the heads appear to contain DNA but lack tails. Several unattached tails are present, and a few tail cores can be seen attached to heads.

Many full heads without tails are observed in
both the φ29 and SPO1 preparations. Unattached, assembled φ29 tails have never been observed with in situ preparations. This observation, in conjunction with other evidence obtained in our laboratory (E. W. Hagen and M. Tosi, unpublished data), indicates that the φ29 tail is sequentially assembled on mature heads. Phage φ29 tail assembly is therefore a very late
process, and some intermediates may be fragile. In contrast, the large number of free SPO1 heads and tails suggests independent assembly pathways for these substructures. These results demonstrate that the in situ lysis technique has been successfully adapted for φ29- and SPO1-infected B. subtilis. Electron microscope observation of phage structures is facilitated by the presence of large numbers of progeny in and around the lysing cells. The presence of a high proportion of DNA-filled heads indicates that preservation is good. Tail structure can be analyzed when the phage particle or tail is free of concentrated cytoplasmic material. The technique has also been used successfully with φ29 infections in minimal medium (4) and with φ29-infected Bacillus amyloliquefaciens (unpublished observations).

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LITERATURE CITED


