Differential Phage Sensitivity of Cell Types in Caulobacter

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The genus Caulobacter has been characterized in detail by Poindexter (1964). Because of the simple yet distinct morphological and physiological changes which occur during the life-cycle of this stalked pseudomonad, it may provide a bacterial example for the study of cellular differentiation among lower life forms. The stalked, non-motile Caulobacter cell gives rise to two different cell types upon division, a motile swarmer cell with flagellum and a cell with the original stalk. The stalked cell divides again following the same pattern. However, the motile cell must lose motility and develop a stalk before cell division. It then gives rise to a motile, non-stalked cell and a stalked cell as did its parent. A key to the study of such a system with two distinct growth stages is the effective separation of the two cell types or the elimination of one or the other so that each may be studied separately. Schmidt (1966) has been able to separate some strains by differential centrifugation. She has also shown that in certain piliated strains apparently only swarmers have pili and may be infected with RNA bacteriophage. The latter observation suggests that perhaps the RNA bacteriophage might be useful in eliminating the swarmer stage from the population. However, we have been unable to separate many of our strains by the centrifugation technique, and the RNA phages isolated to date have a very limited host range. This note reports the characterization of a DNA bacteriophage with a wide host range which adsorbs to and kills only the motile swarmer cells in Caulobacter cultures.

Phage φ6, so designated because it was first isolated on Caulobacter vibrioides cv-6, was shown to be a DNA phage by the acridine orange technique of Bradley (1966). Nineteen of 23 strains tested were sensitive to phage φ6. The burst time on cv-6 was 160 min. and the average burst size, determined by single burst experiments, was 43 phage/infected cell.

Two sensitive strains, cv-6 and cv-113, were selected for further studies. Adsorption rates of phage φ6 to cv-6 and cv-113 are presented in Fig. 1. Multiplicity of infection (m.o.i.) was 0.1. Adsorption was not as efficient as is seen in other phage-host systems. The addition of Mg²⁺ and/or Ca²⁺ (0.01, 0.001, or 0.0001 M) did not enhance adsorption, nor did adsorption improve when different adsorption media were used.

Phase-contrast microscopy of mid-log phase broth cultures of cv-6 and cv-113 revealed that approximately 60% of the cells of a cv-6 culture were of the motile non-stalked type while only 30% of a cv-113 culture were of that type. Direct microscopic examination of such cultures after the addition of phage φ6 showed the complete cessation of motility by 90 min.

Viable counts of cultures of cv-6 and cv-113 after infection with phage φ6 are presented in Fig. 2. Phage, added at an m.o.i. of 1 × 10² when the cultures were in log phase, caused a 50% reduction in viable count within 1 hr in cultures of cv-6, the strain with 60% swarmers, and a reduction of 28% in cultures of cv-113, the strain with 30% swarmers.

Examination of negatively stained cell-phage mixtures by electron microscopy indicated that only non-stalked cells adsorbed the phage.

These observations suggest that phage φ6 selectively infects only the motile, non-stalked stage of Caulobacter and that the remaining viable cells are of the non-motile stalked type.

Arguments in favour of this suggestion may be summarized as follows: (1) the strain with
Fig. 1. Adsorption of phage $\phi 6$ to the several strains of *Caulobacter*. The adsorption of phage $\phi 6$ to the sensitive strains cv-6 and cv-113 was determined by the assay of unadsorbed or free phage at times up to 140 min. cv-5, a non-sensitive *Caulobacter* strain was included as a control. ○-○, cv-6; ●-●, cv-5; △-△, cv-113.

Fig. 2. The effect of phage $\phi 6$ infection of sensitive strains of *Caulobacter*. Broth cultures of cv-6 and cv-113 were incubated for 7 hr and viable counts determined. At 7 hr phage $\phi 6$ was added to the cultures in excess to give an m.o.i. of $1 \times 10^5$. The cultures to which phage was added at 7 hr are indicated by the solid symbols. Parallel uninfected controls are indicated by the open symbols. Viable counts were determined for up to 30 hr; however, only 10 hr are indicated here. The initial drop in viable count after addition of phage reflects the loss of viability of the portion of the population infected. After the initial drop, the viable count remained constant for up to 30 hr. This constant level of viable cells represents the portion of the population that was already in the stalked or phage resistant stage at the time of addition of phage. ○-○, cv-6; ●-● cv-6 + $\phi 6$; △-△, cv-113; △-■, cv-113 + $\phi 6$.

the greater percentage of motile cells adsorbed the greater number of phage; (2) the strain with the greater percentage of motile cells showed the greater decrease in viable count due to phage infection; (3) motility was lost after the addition of phage; (4) viable cell counts after phage infection (apparently phage-insensitive cells) closely approximated the level of non-motile cells in the populations at the time of addition of phage; (5) electron microscopy of cell-phage mixtures showed phage adsorbed only to non-stalked cells.
As described earlier, the stalked cell divides giving rise to a stalked cell and a swarmer. When phage are added, the swarmers become infected and are no longer detectable in the viable counts, which would account for the drop in viable count seen in Fig. 2. The continued constant level of viable cells through 30 hr in the presence of excess phage would represent the original stalked cells present at the time of addition of phage. Any new swarmers produced as a result of cell division would become infected and not develop to the stalked or phage-resistant stage; hence, after the initial decrease, the viable count would remain constant.

The adsorption of phage φ6 only to the swarmer cells might indicate a difference in the cell wall of the two types. However, we have observed phage attachment to the flagellum of the swarmer cells and observe attachment of the phage to the cell wall only in the area immediately adjacent to the flagellum. We suggest that attachment of the phage to the cell wall is preceded by attachment to the flagellum. If such is the case, stalked cells, which have no flagella, cannot be infected by the phage.

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REFERENCES


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