

Identity of the Protoplast Infecting and Cell Infecting Agent Derived from T₂ Bacteriophage¹

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INTRODUCTION

Urea treatment of bacteriophage T₂ yields a new agent (pi) capable of infecting protoplasts of *E. coli*. Recently it was found that the pi also showed a small "background" count when plated with normal cells. Three possibilities were recognized as the possible source. First, some phage had been left intact by the urea treatment. Second, an unrecognized minority type of pi had been left able to attach to cell receptor sites. Third, a bacterial cell existed with some sort of defect in the cell wall and hence susceptible to pi. A combination of these three seemed also possible. If the source of background turned out to be a cell infectable by both phage and pi, it would be very valuable for genetic recombination between the two. This investigation was undertaken in an attempt to find the nature of the infective agent involved in this background count.

Phage and pi have very marked differences of inactivation rates by heat and proteolytic enzymes. Assays of inactivated pi on protoplasts and background cells should show different inactivation rates if the two are different agents.

METHODS AND MATERIALS

In all experiments, the host cell was *E. coli*, strain B, and the bacteriophage was T₂. Cells were grown in 3XD medium (1) with aeration at 37°C. The protoplasting procedure was as described previously (2). All pi (3) dilutions were made in D medium (2), and all protoplast dilutions in broth—bovine serum albumin (2). All heat inactivations were done in a thermostatically controlled, stirred water bath by procedures previously described (4). The proteolytic enzyme was the crystalline product from Worthington Laboratories and the procedure the same used previously (4).

Preparation of pi

T₂ was mixed 1/20 with 8M. urea (pH8.3-8.5). The resulting mixture was placed in a water bath at 37° for one hour. The pi was then dialysed overnight at 3°C against 0.15M saline to remove the urea and was stored under refrigeration.

Plating

In working with background, it is most important to use cultures of *E. coli* at a constant "age" because the background register otherwise varies markedly (5). For plating it is necessary to grow cells from a low inoculum to 5 x 10⁸ cells/ml. (determined by Petroff-Hauser Counter). These cells yield a negligible background.

To obtain maximum background with pi, cells grown to 5 x 10⁸/ml. are

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placed under refrigeration for at least two weeks. This brings the background titer to around 10^3 counts/ml. In this investigation, cells which had been refrigerated for three weeks were used.

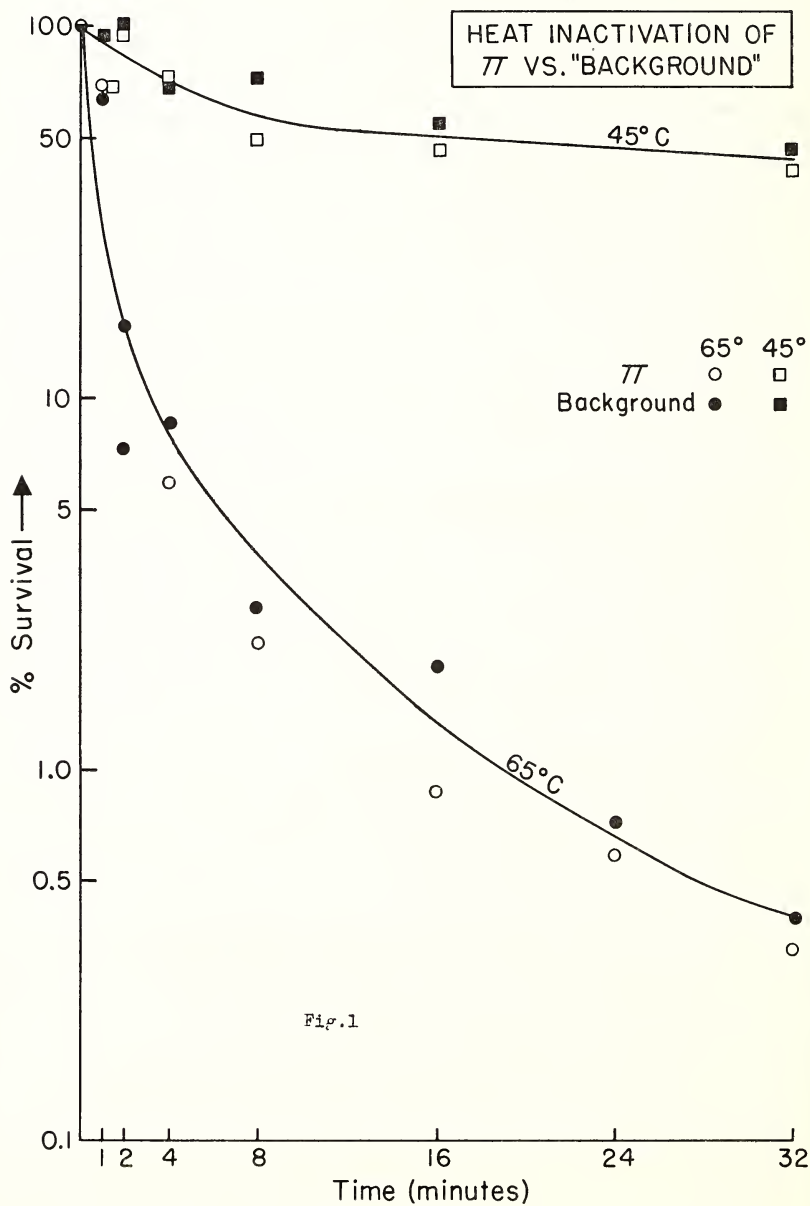


Figure 1

RESULTS

Heat inactivation of pi

Both cell and protoplast assays of pi inactivated at 65°C are shown in Fig. 1. As may be seen, the assays conform reasonably. In order to eliminate freak correspondence of inactivation rates at 65°C, the pi was inactivated at 45°C. Again, the experiment (Fig. 1) showed no difference in inactivation rates.

Trypsin inactivation of pi

Pi and phage also exhibit different inactivation rates when treated by the proteolytic enzyme trypsin (phage being completely stable to the enzyme). The trypsin-inactivated pi was assayed with cells and protoplasts. Again the rates of decline of activity against protoplasts and cells were parallel.

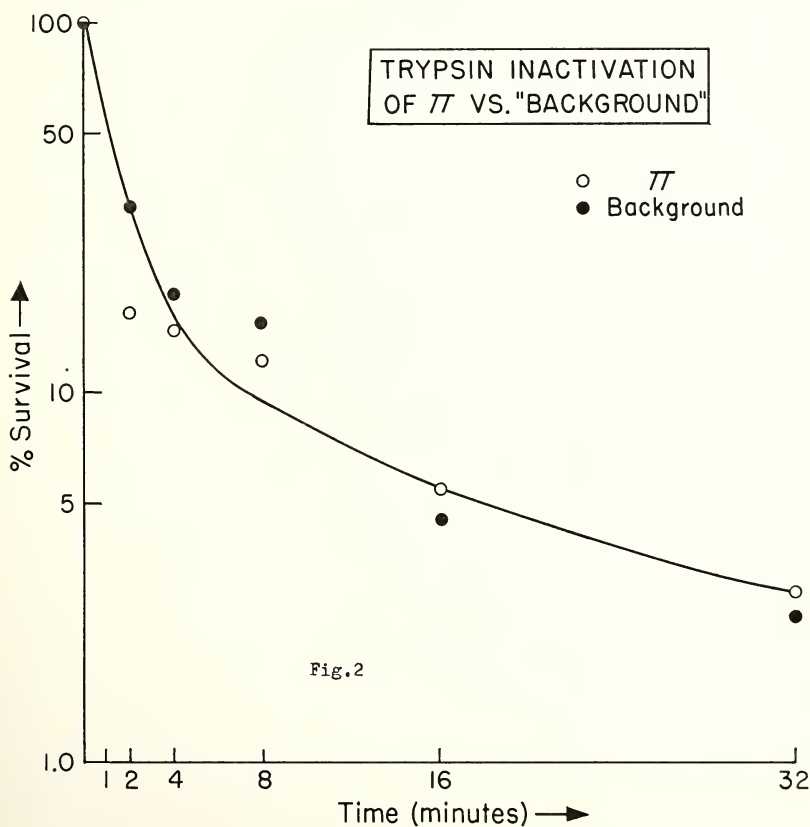


Fig. 2

Figure 2

DISCUSSION AND SUMMARY

The above evidence tends to indicate that pi itself is the infective agent causing background count. The possibility of existence of a bac-

terium with some sort of incomplete cell wall is under investigation. Preliminary evidence points to an "unusual" cell as responsible for backgrounds (5).

Literature Cited

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