Bacteriophage Growth on Stationary Phase Achromobacter cells

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SUMMARY

A new phage-host system is described in which phage $\alpha$ 3a grows on stationary phase Achromobacter mutant strains. Characteristic clear plaques are formed, at an e.o.p. $10^{-1}$ to $10^{-2}$, on already confluent bacterial lawns of the mutant strains. Phage growth is sensitive to aeration and growth only occurs under microaerophilic conditions. Lysates prepared on the mutant strains cannot transduce in contrast to transducing lysates prepared from wild type Achromobacter strains.

INTRODUCTION

Phage growth is dependent on the physiological state of the bacterial host and is inhibited by a decline in the bacterial metabolism (Adams, 1959; Hayes, 1968). Furthermore, plaque development is normally not initiated in confluent, stationary phase bacterial populations (lawns). A new phage-host system involving phage growth on stationary phase Achromobacter cells is described.

Woods & Thomson (1975) reported a transduction system for a collagenolytic Achromobacter strain by four closely related $\alpha$ phages. Some of the transductant colonies (strains 14 and 15) which were originally resistant to phage $\alpha$ 3a, became semi-sensitive (e.o.p. $10^{-1}$ to $10^{-2}$) after 4 cycles of cloning on minimal agar. These strains were investigated further as plaques were only observed after 48 h incubation as compared with overnight incubation for plaque formation using the wild type (w.t.) Achromobacter strain.

METHODS

Media. Nutrient broth (Difco) supplemented with 0.4 M-NaCl was used. For phage growth in liquid media and for double-agar-layer phage assays, the tryptone media of Thomson & Woods (1973) were used. Incubation was at 30 °C.

Bacteria and bacteriophages. Wild-type Achromobacter sp. 2 (Thomson, Woods & Welton, 1972) and two Achromobacter trp+ transductants, strains 14 and 15 (Woods & Thomson, 1975), which were semi-sensitive (e.o.p. $10^{-1}$ to $10^{-2}$) to phage $\alpha$ 3a (Thomson & Woods, 1974) were used.

Phage and transduction techniques. The phage and transduction techniques have been described previously (Thomson & Woods, 1973; Thomson & Woods, 1974; Woods & Thomson, 1975). For phage growth under anaerobic conditions the double-agar-layer plates were incubated under N₂ gas in anaerobic jars and liquid medium was perfused with N₂ gas in Hungate anaerobic tubes (Bellco Glass, Inc.). Samples were added to or withdrawn from the anaerobic tubes by injecting through the rubber seals. Phage adsorption was investigated by the method of Adams (1959). For phage growth on stationary phase cells in liquid medium, Achromobacter w.t. and mutant strains were grown in tryptone broth with aeration by shaking for 1.5 to 4 days prior to the addition of $\alpha$ 3a phage. The cultures were
re-incubated under aerobic or anaerobic conditions, with or without shaking, and were sampled at different time intervals, centrifuged and the supernatant fluids assayed for p.f.u. on the w.t. strain.

**Nucleic acid synthesis.** RNA synthesis was determined by incorporation of $^3$H-uracil ($2 \mu$g/ml, 0.4 $\mu$Ci/ml) into trichloroacetic acid (TCA)-precipitable material by the method of Eichenlaub & Winkler (1974). As the *Achromobacter* strains did not incorporate $^3$H-thymidine, DNA synthesis was determined by incorporation of $^3$H-adenine ($2 \mu$g/ml, 0.4 $\mu$Ci/ml) into NaOH hydrolysed TCA-precipitable material (Friesen, 1968). Exponential and stationary phase (3-day-old) *Achromobacter* strain 15 cultures were added to used (3-day-old) cell-free media containing the appropriate label. Samples were withdrawn after 0, 15, 30, 45 and 60 min and the incorporation of label into RNA and DNA estimated.

**RESULTS**

*Aerobic plaque formation and transduction*

The circular and turbid plaques (Thomson & Woods, 1974) formed by $\alpha 3a$ on w.t. *Achromobacter* sp. 2 were visible after overnight (15 to 18 h) incubation and appeared with the development of the background lawn. The phage $\alpha 3a$ plated with an e.o.p. $10^{-2}$ to $10^{-8}$ on *Achromobacter* strains 14 and 15, and plaques only developed in already confluent lawns after 36 to 48 h incubation. The plaques were clear with an irregular outline. These plaques were not due to the spontaneous release or induction of phage, as control backgrounds of strains 14 and 15 without added $\alpha 3a$ did not give rise to plaques. Furthermore, strains 14 and 15 were not slow-growing mutants as the growth rates of the w.t. and mutant strains were similar. The bacteria reached stationary growth phase after 7 h growth in tryptone medium.

A clear plaque on a mutant background was stabbed, diluted and membrane filtered before plating on the w.t. and mutant strains. Circular turbid plaques were obtained after overnight incubation with the w.t. strain and irregular clear plaques (e.o.p. $10^{-1}$ to $10^{-2}$) after 36 h incubation with the mutant strains. Phage $\alpha 3a$ was therefore not modified by growth on the mutant strains.

High titre lysates prepared from $\alpha 3a$ on w.t. backgrounds transduced an *Achromobacter* trp- mutant to prototrophy at a frequency of $1 \times 10^{-6}$/p.f.u. adsorbed. Identical tests for transduction were negative when $\alpha 3a$ high titre lysates, prepared on *Achromobacter* strain 15, were used.

**Anaerobic plaque formation**

Under anaerobic conditions $\alpha 3a$ plated with an e.o.p. of 0.5 on the w.t. *Achromobacter* strain. The growth rate of the bacterium was slower under anaerobic conditions compared with aerobic conditions, and confluent background lawns were only visible after two days' incubation. Plaques appeared with the development of the w.t. background but were clear with irregular outlines in contrast to the aerobic, circular and turbid plaques.

When $\alpha 3a$ was plated on strains 14 and 15, no plaques were observed even after 34 days' incubation although confluent background lawns were visible after 2 days. After 34 days these plates were removed from the anaerobic jars and incubated aerobically. Clear plaques with irregular outlines developed after a further 2 days' incubation. The e.o.p. was $10^{-1}$ to $10^{-2}$. 
Phage growth on stationary phase cells

Phage growth on stationary phase cells in liquid medium

Stationary phase w.t. and mutant (15) cultures were inoculated with \( \alpha 3a \) and re-incubated aerobically without shaking. The results (Fig. 1a) indicated that the cells were in stationary phase prior to the addition of phage. Viable count determinations paralleled the optical density measurements. Addition of \( \alpha 3a \) to the w.t. strain, after 2 days’ incubation, resulted in a fourfold increase in phage (Fig. 1b). A tenfold decrease in p.f.u. was observed when \( \alpha 3a \) was added to the w.t. strain after 3 days’ incubation. Addition of \( \alpha 3a \) to strain 15 after 2 or 4 days’ incubation resulted in a 10^4-fold increase in phage.

The effect of aeration by shaking on \( \alpha 3a \) growth on stationary phase cultures of Achromobacter strain 15 is shown in Fig. 2. Shaking the cultures inhibited the growth of \( \alpha 3a \) but on standing the phage titres increased between 10^2- to 10^4-fold.

Growth of \( \alpha 3a \) on stationary phase Achromobacter strain 15 was inhibited by stringent
Fig. 2. Effect of aeration, by shaking on x 3a growth on stationary phase cultures of *Achromobacter* 15. Cultures were shaken for 3 (○—○) and 6 (■—■) days before standing at 30 °C. x 3a was added to both cultures after 1.5 days' incubation (↑). ↑, time at which cultures were removed from shaker.

Fig. 3. Growth of phage x 3a on *Achromobacter* 15 under anaerobic conditions. Stationary phase (2-day-old) shaking cultures were perfused with nitrogen, infected with x 3a and then sealed and re-incubated anaerobically with (○—○) and without (△—△) shaking; the cultures were opened, aerated and re-incubated aerobically without shaking after 8 (↑) and 4 (↓) days respectively. ■—■, control culture which was incubated aerobically without shaking after the addition of phage.

anaerobic conditions, with or without shaking (Fig. 3). When the seals were opened and the cultures aerated before being re-incubated aerobically without shaking, the phage titres increased.
Fig. 4. RNA and DNA synthesis by log and stationary phase *Achromobacter* 15 cells in used media. 
(a) Incorporation of $^3$H-uracil into TCA-precipitable material. Log phase cells (○—○), stationary phase shaken cells (□—□) and stationary phase shaken cells which have then stood for 24 h ($\triangleleft\triangleleft\triangleleft$) were added to used cell-free media containing 2 μg/ml, 0.4 μCi/ml $^3$H-uracil. (b) Incorporation of $^3$H-adenine into NaOH hydrolysed TCA-precipitable material. Log phase cells (●—●), stationary phase shaken cells (■—■) and stationary phase shaken cells which have then stood for 24 h ($\triangleleft\triangleleft\triangleleft$) were added to used cell-free media containing 2 μg/ml, 0.4 μCi/ml $^3$H-adenine.

**Phage adsorption**

The adsorption of $\alpha$3a to the w.t. *Achromobacter* strain was rapid and with log and stationary phase cells 99% of the phages were adsorbed after 10 min. With shaking and standing stationary phase mutant cultures, 58% and 53% of the phages respectively were adsorbed after 10 min. With log phase mutant cultures 60% phage adsorption was obtained after 10 min.

**Nucleic acid synthesis**

Studies on nucleic acid synthesis in *Achromobacter* strain 15 indicated that shaken log phase cells on transfer to old, cell-free, labelled media incorporated $^3$H-uracil into RNA and $^3$H-adenine into DNA (Fig. 4). No RNA or DNA synthesis was detected in shaken stationary phase (3-day-old) *Achromobacter* strain 15 cells. However, RNA synthesis was detected when 3-day-old shaken cultures of strain 15 were allowed to stand, at 30 °C, for 24 h before estimating nucleic acid synthesis (Fig. 4). DNA synthesis was not observed in standing resting phase cultures.
DISCUSSION

In contrast to previously described phage systems, phage α 3a is able to grow and form plaques on stationary phase *Achromobacter* strain 15 cultures. This phenomenon is not due to induction of a prophage or contamination by *Bdellovibrio bacteriovorus*. Phage growth on *Achromobacter* strain 15 is very sensitive to aeration and phages grow only under micro-aerophilic conditions. The requirement for a micro-aerophilic environment indicates why plaques do not appear with the development of the background bacterial lawn but appear in already confluent lawns. Growth of the bacteria must first take place to produce the micro-aerophilic environment required for phage growth. The detection of RNA synthesis in micro-aerophilic stationary phase cultures but not in aerated cultures suggests that under micro-aerophilic conditions the bacteria, although in stationary phase and in old medium, are able to re-initiate RNA synthesis.

Adsorption of α 3a to the mutant cells was not affected by shaking but was markedly lower than α 3a adsorption to w.t. cells. This could account for the reduced e.o.p. of α 3a on the mutant strains.

An interesting observation was that high titre lysates from turbid plaques could transduce whereas lysates from clear plaques were non-transducing. Woods & Thomson (1975) described an unstable generalized transducing system in *Achromobacter* and proposed that the generalized transducing particles contained both phage and bacterial DNA as a result of gene pick-up being due to a recombination process rather than phenotypic mixing. The present result supports the hypothesis as transducing particles are only formed under conditions when recombination between the host and phage are favourable. Anaerobic or micro-aerophilic conditions inhibit turbid plaque development and result in the formation of clear plaques with irregular outlines.

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REFERENCES


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