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BACTERIOPHAGE P22 DEVELOPMENT IS TEMPERATURE SENSITIVE IN THIOLUTIN RESISTANT MUTANTS OF Salmonella typhimurium

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SUMMARY

Thiolutin resistant mutants of Salmonella typhimurium can not support the development of phage P22 at high temperature (40°C). Although normal amounts of phage DNA and lysozyme are synthesised, very few infectious particles are formed at higher temperature. The results indicate the involvement of host function in phage development.

INTRODUCTION

In recent years a number of mutants of Escherichia coli that are defective in the development of some RNA as well as DNA coliphages have been reported (1-5). No such mutant is known for S.typhimurium. Mutants of S.typhimurium resistant to the antibiotic thiolutin have been isolated in our laboratory. These are resistant to thiolutin in enriched media but sensitive to the same in minimal media. It will be shown in the present report that these thiolutin resistant mutants are temperature sensitive for the production of infectious phage P22 particles, although the host itself is not temperature sensitive. This effect is independent of the growth medium.

MATERIALS AND METHODS

^{3}H thymidine (65 mci/mMole) was obtained from the Bhabha Atomic Research Centre, Trombay, India. Thiolutin was a gift from D. N.Belchar, Pfizer Central Research, U.S.A. Nitrocellulose filters (0.45 u) were obtained from Schleicher and Schull Co.

The bacterial strains 18/MC2 ..... 18/MC9 are thiolutin resistant mutants isolated from the strain No.18 (wild type) while the strains 153/MC2...153/MC5 are the thiolutin resistant mutants isolated from the strain 153 (histidine requiring). The mutants are spontaneous mutants and have been isolated by

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Table 1

Burst-size of P22 in different thiolutin resistant mutants growing in M9CAA at 30°C and 40°C

Strains	Burst sizes at 30°C	40°C	Strains	Burst sizes at 30°C	40°C
18	300	330	153	340	360
18/MC2	315	36	153/MC2	250	24
18/MC3	360	12	153/MC3	320	20
18/MC4	46	10	153/MC4	52	1
18/MC5	214	14	153/MC5	300	20
18/MC6	243	36			
18/MC7	235	20			
18/MC8	237	25			
18/MC9	250	10			

Cells (2.6×10^8/ml) growing exponentially in M9CAA were infected at 30°C or 40°C with a clear plaque forming mutant (C_1) of phage P22 at an m.o.i. of 10. The burst size was calculated on the basis of the number of infective centres which agreed well with the number of cells. Plating was done on permissive host.

plating about 10^9 cells on tryptone agar plates containing 10-20/ug of thiolutin. The resistant colonies were purified by repeated streaking on thiolutin containing plates. The strain 18 and its derivatives were grown in minimal media (6) whereas strain 153 and its derivatives were grown in Luria broth (7) or minimal media supplemented with 20 ugm/ml of histidine.

The rate of DNA synthesis was measured as described by Smith and Levine (8).

The induction of lysozyme was followed according to the method of Rao and Burma (9).

RESULTS AND DISCUSSION

Burst size of P22 in thiolutin resistant mutants:

The burst-size of P22 in different thiolutin resistant mutants of S.typhimurium growing in the enriched media, M99CAA (10) at two different temperatures i.e., 30°C and 40°C, was determined. The phage has very low burst-size in the thiolutin resistant mutants only at higher temperature (Table 1). The effect is observed in either synthetic or enriched media and

in presence or absence of thiolutin. The two strains 18/MC4 and 153/MC4 have comparatively low burst-size even at 30°C. These two strains may be more defective so far as the host function involved in the production of infective particles is concerned. Hence further studies were mostly carried out with these two strains only.

The kinetics of phage adsorption:

The kinetics of phage adsorption (results not presented) was found to be normal at nonpermissive-temperature (40°C). So the reduction in the burst-size is definitely not due to the inefficient adsorption of phage to the host. This is further supported by the observation that the number of infective centres at 40°C is normal.

Phage DNA synthesis:

The maximum rate of phage DNA-snythesis in the strain 153/MC4 and 40°C (nonpermissive-temperature) is comparable with that in the permissive host (Fig.1). The difference in the kinetics of DNA synthesis in the initial stages may be due to the difference in the growth rate of the hosts. The generation time of 153/MC4 is twice as long as that of the parent strain 153. The rate of phage DNA synthesis in other strains is also comparable to the rate in its parent strain 18 (results not presented).

Kinetics of lysozyme induction:

The amount of phage-induced lysozyme synthesized in 18/MC4 and 153/MC4 following infection at 40°C was found to be equal to that in the control cells (Fig.2). The delay in the lysozyme synthesis in 153/MC4 may be due to longer generation time as discussed before.

Temperature shift experiment:

In order to determine whether any host function is necessary for early or late phage function, the temperature shift experiment was performed with 18/MC2 as host, as this strain produces normal burst of phage at 30°C. As

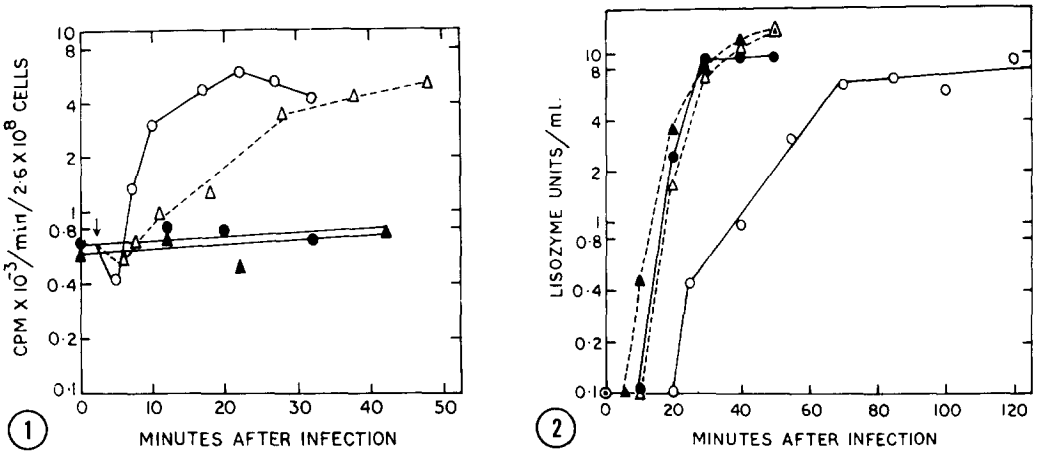

Fig. 1. Rate of DNA synthesis in 153/MC4 following infection at non-permissive temperature (40°C). The cells were grown in M9CAA. Exponentially growing cells (2.6×10^8/ml) were used for the experiment. The rate of incorporation of ^{3}H-thymidine into trichloroacetic acid insoluble fraction was determined as described by Smith and Levine (8). The arrow indicates the time of addition of the phage. (-●-), uninfected 153; (-▲-), uninfected 153/MC4; (-O-), infected 153; (-Δ-), infected 153/MC4.

Fig. 2. Kinetics of lysozyme induction in 18/MC4 and 153/MC4 at nonpermissive temperature (40°C). (-▲-), 18; (-Δ-), 18/MC4; (-●-), 153; (-O-), 153/MC4. Infection was carried out at an m.o.i. of 10.

shown in Fig. 3 infective phages were not formed when the temperature was raised from 30°C to 40°C during the first 25 min post infection. Conversely, the burst-size of the phage was not much reduced when cells grown and infected at 40°C, were shifted down to 30°C during the first 25 min post infection. Similar temperature shift has no such effect on the development of P22 in the wild strain (LT2) of S.typhimurium. The results indicate that at higher temperature phage development in these mutants is not blocked in a very early step. Moreover, the host function is required continuously throughout the late phase of its development.

Noninfectious particles are formed at higher temperature:

To test whether noninfectious P22 like particles are formed in these mutants at nonpermissive temperature the particles were purified from the lysates obtained by infecting 18/MC4 and 153/MC4 at 30°C as well as 40°C

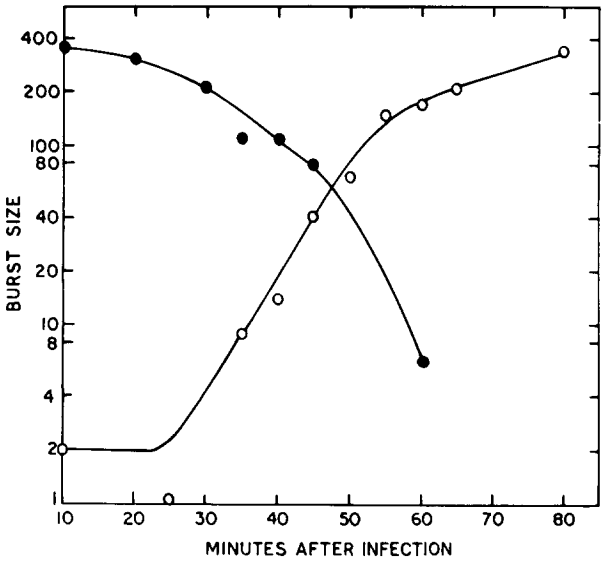

Fig. 3. Effect of the time of temperature shift on the growth of P22 in a mutant cell (strain 18/MC2). Cells (2.6×10^8/ml) growing exponentially in Luria Broth was infected with $P22C_1$ at an m.o.i. of 10 either at 30°C or at 40°C. At different times after infection, the cells infected at 30°C were shifted at 40°C whereas the cells infected at 40°C were shifted down to 30°C. The burst size in each sample was determined at the end of 90 min incubation. (-O-), shifted from 30°C to 40°C; (-●-), shifted from 40°C to 30°C.

Table 2

Amount of defective phage particles produced in thiolutin resistant mutants following infection at nonpermissive temperature

Host	Plaque forming units/ml	Total no. of particles/ml	Ratio of the particles and plaque forming units
18	3.3×10^{12}	6.0×10^{12}	1.8
18/MC4	2.8×10^{11}	1.4×10^{13}	50
153/MC4	1.0×10^{10}	3.5×10^{12}	350

Cells (2.6×10^8/ml) growing exponentially in LB at 40°C were infected with $P22C_1$ at m.o.i. of 10. The phage particles obtained after lysis were purified by differential centrifugation and CsCl gradient centrifugation. The number of phaque forming units was determined by plating on strain 18 (permissive host). The total number of particles was calculated from the absorbancy of the sample at 260 nm (11).

and the number of infective particles (by plating) and total particles (by absorbancy measurement of purified phage preparations) were compared. The results (Table 2) clearly indicate that at nonpermissive-temperature these

mutants produce noninfectious particles. Electron micorgraphs (not presented) of the lysate obtained from 18/MC4 strain revealed that these lysates prepared under nonpermissive condition contain complete particles (normal looking).

The observed effect is definitely due to the change to thiolutin resistance as all the thiolutin resistant mutants isolated at random from different sets of experiments exhibit such property though to a variable extent. The results definitely indicate the involvement of host function in the production of infectious virus particles. Experiments are in progress to elucidate the defect in such particles and the nature of host function for the production of infective particles.

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