CHARACTERIZATION OF TS-MUTANTS OF CYANOPHAGE N-1 BY THEIR INACTIVATION BY PHYSICAL AND CHEMICAL AGENTS

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Summary. – The effect of temperature, ultraviolet (UV) light and ethylenediaminetetraacetic acid (EDTA) on the stability of cyanophage N-1, infecting the cyanobacterium *Nostoc muscorum* was studied. Complete inactivation of the phage occurred at 60 °C in 6 mins. All the temperature-sensitive (ts) mutants exhibited faster inactivation at 50 °C than the wild type. UV light readily inactivated the particles of the wild type giving a survival of 3.44% at a dose of 60 secs. All the ts-mutants were found to be more sensitive to UV light than the wild type. 10^{-4} mol/l EDTA inactivated 40% of the wild type in 60 mins. 5 x 10^{-4} mol/l EDTA inactivated the wild type nearly completely within 2 mins, while a similar inactivation of ts-mutants required only 90 secs.

Key words: cyanophage N-1; ts-mutants; inactivation pattern

Introduction

Cyanophage N-1 which infects the nitrogen fixing cyanobacterium *Nostoc muscorum* resembles bacterial viruses of T-even class with regular polyhedral head, contractile sheathed tails and double-stranded DNA (Adolph and Haselkorn, 1971). The morphology, physical properties and relation between photosynthesis and virus development have been investigated (Adolph and Haselkorn, 1973).

Adams (1959) studied the stability of bacteriophages in the presence or absence of electrolytes and chelating agents at various pH and many environmental stresses. Studies involving cyanophage N-1 mainly pertain to the effects of temperature, chelating agent shock (CAS) and pH on the stability of the phage (Padhy and Singh, 1977a, 1978a), the effect of temperature on adsorption and one-step growth of the phage (Padhy and Singh, 1977b), and the effects of host aging, ions and pH on the adsorption of the phage (Padhy and Singh, 1978b). Sarma and Kaur (1993) reported the isolation of spontaneous and induced host-range mutants of cyanophage N-1. We have earlier reported the isolation of ts-mutants of cyanophage N-1 that differed in their adsorption rates and burst sizes at the permissive and restrictive temperatures (Sarma and Singh, 1994). In order to employ these ts-mutants in genetic complementation studies, their further characterization is needed to establish their genetic distinctiveness. We report here the characterization of ts-mutants of cyanophage N-1 by their inactivation by temperature, UV light and EDTA.

Abbreviations: CAS = chelating agent shock; EDTA = ethylenediaminetetraacetic acid; p.i. = post infection; ts = temperature-sensitive; UV = ultraviolet

Materials and Methods

Bacterium and phages. The nitrogen-fixing cyanobacterium *Nostoc muscorum* ISU (ATCC 27893) and cyanophage N-1 have been used in the present study (Sarma and Kaur, 1993). Axenic cultures of the host were propagated in modified Chu-10 medium (Safferman and Morris, 1964) with A-6 as trace elements (Allen and Arnon, 1955), where calcium nitrate (0.232 g/l) was replaced by the same molar concentration of calcium chloride. Cultures were maintained at 28 ± 1 °C and illuminated with day-light fluorescent tubes (approximate light intensity 2000 lux) for 14 hrs per day. The cultures were transferred after every 12 days into fresh medium and log phase 6 day-cultures were concentrated by centrifugation for phage plating.

Lysates of N-1 phage and its ts-mutants were prepared by infecting concentrated log phase cultures (approximately 10^9 cells/ml) of the host with stock phage lysate (10^3 PFU/ml). After complete lysis which generally occurred after 72 hrs p.i., the lysates were centrifuged at 5000 x g for 10 mins at 4 °C to remove cell debris.

The lysates were further purified by filtering through Milipore membrane filters (porosity 0.45 μm) and stored at 4 °C. The titer of N-1 phage and its ts-mutants was estimated by couting the number of plaques after plating appropriately diluted lysates (Adams, 1959).

Temperature sensitivity. 10 ml portions of N-1 phage lysates of known titer were incubated at 50, 55 and 60 °C in a water-bath for desired time intervals. One ml aliquots were withdrawn at regular intervals and diluted 10 times in fresh medium. For plaque assay, 0.1 ml portions of samples diluted to 10^{-6} were mixed with the host and plated on triplicate plates. The sensitivity of ts-mutants to heat was determined at 50 °C. From the survival curves of N-1 phage and its ts-mutants the LD_{37} values (dose corresponding to 63% survival) were calculated.

UV light. Germicidal lamp with emission peak at 253.7 nm served as the source of UV radiation. Ten ml portions of N-1 phage and its ts-mutants (titer 10^8 PFU/ml) magnetically stirred in 10 cm Petri dishes were irradiated from a distance of 22.5 cm at the intensity of 85 ergs/mm²/sec. At various time intervals, 0.1 ml aliquots were taken and diluted up to 10^{-6}, and 0.2 ml aliquots of each dilution were plated on agar plates for plaque assay. The plates were covered with a black cloth for 24 hrs to avoid photoreactivation and subsequently exposed to light in the culture room. Then plaques were counted and the percentage of survival and LD_{37} values were calculated.

CAS. Lysates of N-1 phage and its ts-mutants of known titer (approximately 10^8 PFU/ml) were diluted 10-fold with 1 x 10^{-4} and 5 x 10^{-4} mol/l EDTA, respectively. Samples (0.1 ml) were taken at various intervals, diluted with fresh medium up to 10^{-5} and plated (0.2 ml) on agar plates for plaque assay. A similar experiment without EDTA served as control. The sensitivity of ts-mutants to CAS was determined with 5 x 10^{-4} EDTA. The percentage of survival and LD_{37} values were calculated.

Results

N-1 phage was found to be very sensitive to temperature and was rapidly inactivated with rise of temperature. At 50 °C, a survival of 62.09% was observed after 60 mins. At 55 °C, a survival of 12.67% was noticed after 30 mins. While at 60 °C, 0.625% of phage particles survived after 3 mins. Complete inactivation of the phage occurred at a dose of 6 mins at 60 °C (Fig. 1).

Generally, all the ts-mutants exhibited faster inactivation than the parental phage at 50 °C. Mutants ts-4, ts-5, ts-19, ts-21, ts-24, ts-40 and ts-143 proved to be highly sensitive to heat, since at 15 mins, a survival of less than 1% could be scored out. It was further observed that ts-15 was highly sensitive to heat and was inactivated completely at 50 °C in 9 mins. Mutants ts-147 and ts-151 showed a survival of 25 and 12.50%, respectively, after 45 mins at 50 °C. Mutants ts-147 and ts-151 exhibited increased tolerance to heat as compared to other ts-mutants.

The LD_{37} values for inactivation by heat revealed that most of the ts-mutants except ts-147 and ts-151 required a markedly shorter time for inactivation by 37% in comparison to the parental phage. LD_{37} value of the latter was 57 mins while it varied between 0.50 and 9 mins for different ts-mutants. Mutants ts-147 and ts-151 showed 21.50 and 17.50 mins LD_{37} values (Table 1). So ts-mutants were found to be more sensitive to heat as compared to the parental phage.

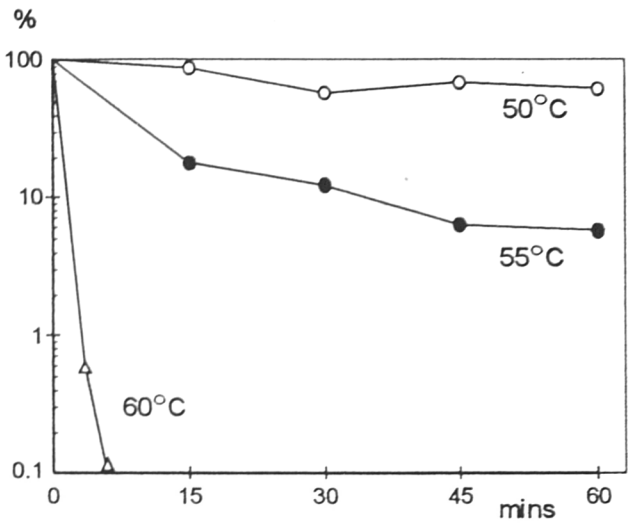

Fig. 1
Inactivation of N-1 phage at 50, 55 and 60 °C
Ordinate: survival (%).

N-1 phage exhibited a survival of 3.44% at 60 secs dose of UV light (Fig. 2). All the ts-mutants were found to be more sensitive to UV light than the wild type. Moreover, mutant ts-72 was found to be more sensitive to UV light as compared to other ts-mutants since it exhibited 1.20% survival at a dose of 40 secs. All the ts-mutants showed a similar pattern of sensitivity to UV light and exhibited approximately same survival values.

The LD_{37} value for N-1 phage was 12 secs and for its ts-mutants it varied from 5 to 7 secs (Table 2). We conclude

Table 1. Inactivation of N-1 phage and its ts-mutants at 50 °C

Phage strain	LD_{37} value (mins)
ts^+ (wild strain)	57.00
ts-4	0.50
ts-5	0.50
ts-10	3.00
ts-19	2.00
ts-21	1.00
ts-24	1.00
ts-40	1.00
ts-41	9.00
ts-72	1.00
ts-125	1.50
ts-143	0.50
ts-147	21.50
ts-151	17.50

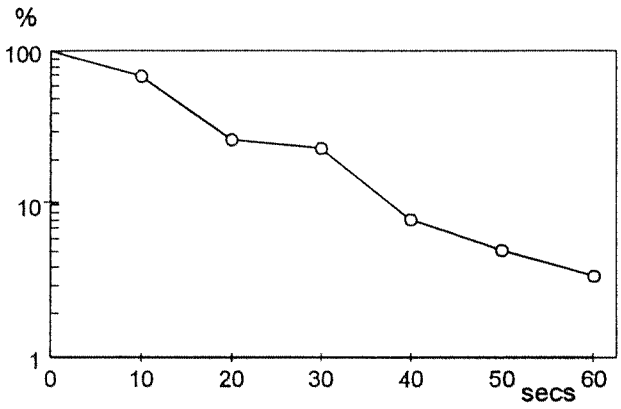

Fig. 2
Inactivation of N-1 phage by UV light
Ordinate: survival (%).

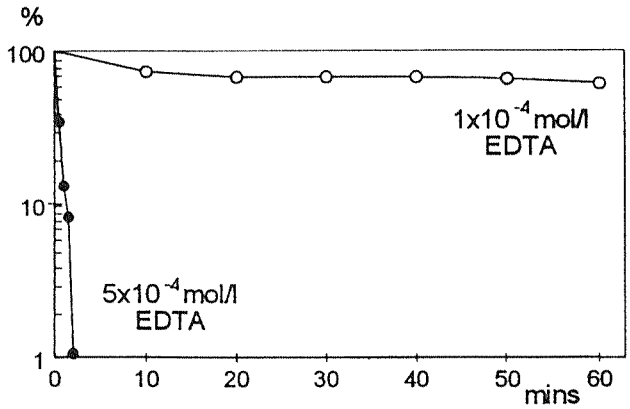

Fig. 3
Inactivation of N-1 phage by EDTA
Ordinate: survival (%).

from these results that ts-mutants are more sensitive to UV light than the wild type.

N-1 was inactivated by EDTA to nearly 40% after 1 hr at a concentration of 10^{-4} mol/l, and when the concentration of EDTA was increased to 5 x 10^{-4} mol/l, almost complete inactivation of the phage occured within 2 mins (Fig 3). All the ts-mutants were inactivated within 90 secs at a concentration of 5 x 10^{-4} mol/l EDTA except ts-125 and ts-151 which showed a survival of 22% and 1.39% at a dose of 1.5 and 4 mins, respectively. So except these two mutants the rest of them was found to be more sensitive to CAS as compared to the wild type.

The LD_{37} values of different ts-mutants for CAS are presented in Table 3. N-1 phage showed LD_{37} of 8 secs while nine ts-mutants exhibited nearly same value, i.e. 4 – 5 secs. Minimum of 3 secs was noted in ts-19 while maximum of 6 secs was noted in ts-143. Two ts-mutants, ts-125 and ts-151 required longer time to become inactivated by 37%, i.e. 38 and 60 secs, respectively. It is clear from these results that except two all the ts-mutants are more sensitive to CAS than the wild type.

Table 2. Inactivation of N-1 phage and its ts-mutants by UV light

Phage strain	LD_{37} value (secs)
ts^+ (wild type)	12.00
ts-4	5.00
ts-5	5.00
ts-10	5.00
ts-19	6.00
ts-21	6.00
ts-24	7.00
ts-40	5.00
ts-41	6.00
ts-72	5.50
ts-125	6.50
ts-143	5.50
ts-147	6.00
ts-151	5.00

Table 3. Inactivation of N-1 phage and its ts-mutants by CAS (5 x 10^{-4} mol/l EDTA)

Phage strain	LD_{37} value (secs)
ts^+ (wild type)	8.00
ts-4	4.00
ts-5	4.50
ts-10	4.00
ts-19	3.00
ts-21	4.00
ts-24	4.00
ts-40	5.00
ts-41	5.00
ts-72	4.50
ts-125	38.00
ts-143	6.00
ts-147	4.00
ts-151	60.00

Discussion

N-1 phage was rapidly inactivated with rise of temperature from 50 to 60 °C. Padhy and Singh (1977a) reported thermal inactivation of N-1 phage at 60 °C, and that of phages LPP-1, SM-1 and AS-1 was tested at 55 and 60 °C by various investigators (Safferman and Morris, 1964; Safferman *et al.*, 1969, 1972). At 55 °C, 90% of N-1 phage particles was inactivated in 20 mins (Padhy and Singh, 1977a). Singh *et al.* (1972) reported that LPP-1, P-5 and P-6 cyanophages were inactivated at 55 °C in 3.5, 19 and 2.5

mins, respectively. After incubation at 40 °C for 60 mins a marked decrease in titer of LPP-1 phage was reported (Safferman and Morris, 1964). Thus N-1 phage was comparatively more temperature-resistant than LPP phages. Singh *et al.* (1972) reported similar observations in phages infecting *P. boryanum*. The temperate phage infecting the bacterium *Eriwinia herbicola* was also heat-resistant (Harrison and Gibbins, 1975).

All the ts-mutants were found to be highly sensitive to heat; they exhibited rapid inactivation at 50 °C in 15 mins as compared to the wild type. Mutants ts-147 and ts-151 exhibited slightly increased tolerance to heat as compared to other ts-mutants, but in comparison to the wild type, they were less heat-resistant.

N-1 phage exhibited a 3.44% survival at 60 secs-dose of UV light. All the ts-mutants were found to be more sensitive to UV light than the wild type.

A concentration of 10^{-4} mol/l of EDTA inactivated 40% of N-1 phage in 60 mins, and when the concentration of EDTA was increased to 5 x 10^{-4} mol/l, nearly complete inactivation of phage particles occurred within 2 mins. Lark and Adams (1953) reported that EDTA inactivated viruses by forming a complex with cations bound to virus particles. EDTA has been reported to inactivate phages infecting *Plectonema boryanum* (Padan *et al.*, 1972; Singh, 1974, 1975).

All the ts-mutants were rapidly inactivated within 90 secs at a concentration of 5 x 10^{-4} mol/l except ts-125 and ts-151 which had increased tolerance to EDTA as compared to the wild type. The majority of ts-mutants differed from the wild type in being more sensitive to CAS. However, ts-125 and ts-151 were found to be less sensitive to EDTA as compared to the wild type. The ts-mutants of LPP-1 have been reported to be quite sensitive to EDTA and photodynamic effect of acriflavine (Singh and Kashyap, 1977). Minute plaque forming mutant of cyanophage AS-1 has been found to be more sensitive to heat (where 40% of particles have been found inactivated in 60 mins), CAS, acriflavine and UV light than its wild type (Amla, 1981). It is interesting that all ts-mutants exhibited differences with respect to LD_{37} values of the three inactivating agents and each of the ts-mutants was distinct in its properties.

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