EFFECT OF REARING AT NON-PERMISSIVE TEMPERATURE ON SILKWORM LARVAE INFECTED WITH TEMPERATURE-SENSITIVE *LEF-8* MUTANT OF BOMBYX MORI NUCLEOPOLYHEDROVIRUS

M. SHIKATA, M. NAKAGAWA, Y. SANO, T. MATSUMOTO, Y. HASHIMOTO*

Department of Applied Biology, Faculty of Textile Science, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan

Received August 3, 1998; accepted August 20, 1998

Summary. – A temperature-sensitive (*ts*) mutant of bombyx mori nucleopolyhedrovirus (BmNPV), ts-S1, contains a mutation in a putative RNA polymerase gene, which is involved in late viral gene expression. When 4th-instar silkworm larvae were infected with 1.0 x 10^5 $TCID_{50}$ of ts-S1 per larva and reared at 33°C, the titre of budded virus (BV) and number of occlusion bodies (OBs) in the haemolymph of the infected larvae were very low in the early stage but markedly increased in the late stage of infection. In contrast, a rapid increase in both BV titre and OB number was detected in the haemolymph of infected larvae reared at 25°C. LD_{50} values of ts-S1 and wild type BmNPV (wtBmNPV) for 4th-instar larvae were 2.41 and 0.96 $TCID_{50}$ per larva at 25°C, and >1.0 x 10^6 and 1.70 $TCID_{50}$ per larva at 33°C, respectively. These results indicate that the virulence of ts-S1 for the larvae reared at 33°C was markedly reduced. To examine further the reduction of ts-S1 virulence at the non-permissive temperature of 33°C, silkworm larvae were infected with ts-S1 at the multiplicity of 1.0 x 10^2 – 1.0 x 10^6 $TCID_{50}$ per larva, reared for various time at 33°C and then shifted to 25°C. Longer rearing periods at 33°C resulted in better survival rates indicating that the reduction of virulence of ts-S1 was proportional to cumulative rearing time at 33°C. When large virus inocula were used, a growth alteration of larvae was preferentially induced. However, when small virus inocula were used, the appearance of abortive infection due to the non-permissive temperature became more evident.

Key words: bombyx mori nucleopolyhedrovirus; silkworm larvae; temperature-sensitive mutant; virulence; abortive infection

Introduction

BmNPV, which belongs to the family *Baculoviridae*, has a closed circular double-stranded DNA genome. The entire nucleotide sequence of BmNPV DNA has been determined (Maeda, 1993), and several genes homologous to those of autographa californica nucleopolyhedrovirus (AcMNPV) genes encoding DNA binding protein, p35, p10, cystein protease, p39, CG30, late expression factors (*lefs*), DNA helicase and p95 have been investigated in terms of expression and function (Maeda *et al.*, 1991; Kamita *et al.*, 1993; Hu *et al*, 1994; Okawa *et al.*, 1994; Chaeychomsri *et al.*, 1995; Lu and Iatrou, 1996; Gomi *et al.*, 1997; Kamita and Maeda, 1997; Lu *et al.*, 1998; Mikhailov *et al.*, 1998). Characterisation of BmNPV genes using virus *ts* mutants has not been carried out extensively in comparison to AcMNPV. Recently, we isolated several *ts* mutants of BmNPV by BrdUrd mutagenesis (Shikata *et al.*, 1998b). When the silkworm cell line BmN4 was infected with the well characterised *ts* mutant ts-S1, normal viral DNA synthesis but defective BV production and polyhedrin

*Corresponding author.

Abbreviations: AcMNPV = autographa californica nucleopolyhedroviurs; BmNPV = bombyx mori nucleopolyhedrovirus; BV = budded virus; EGT = ecdysteroid UDP-glucosyltransferase; lef = late expression factor; LT_{50} = median lethal time; OB(s) = occlusion body(ies); p.i. = post infection; ts = temperature-sensitive; wt = wild type; wtBmNPV = wild type BmNPV

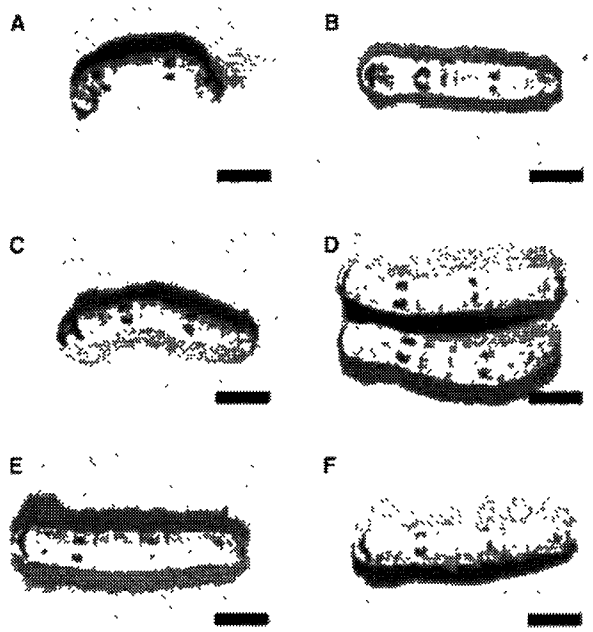

Fig. 1
Symptoms in ts-S1-infected larvae reared at 25°C and 33°C
Fourth-instar silkworm larvae were infected with 1.0 x 10^5 $TCID_{50}$ of ts-S1 or wtBmNPV per larva. (A) wtBmNPV-infected larva reared at 25°C at day 5 p.i. (B) wtBmNPV-infected larva reared at 33°C at day 4 p.i. (C) ts-S1-infected larva reared at 25°C at day 5 p.i. (D) ts-S1-infected larvae reared at 33°C at day 5 p.i. (E) Mock-infected larva reared at 25°C at day 5 p.i. (F) Mock-infected larva reared at 33°C at day 5 p.i Bars represent 10 mm.

synthesis were observed. ts-S1 carries a single nucleotide mutation of a putative RNA polymerase gene, *lef-8* (Passarelli *et al.*, 1994; Todd *et al.*, 1995). When silkworm larvae were infected with ts-S1 at the multiplicity 1.0 x 10^2 $TCID_{50}$ per larva and reared first at a non-permissive temperature for 7 days and then at a permissive temperature, most of them aborted the virus infection. This result showed that the non-permissive temperature defined not only the ts phenotype of ts-S1 in infection of cultured cells but also its virulence for the silkworm. In this study, we examined changes in BV titre and OB number in the haemolymph of ts-S1 or wtBmNPV-infected larvae reared at the permissive temperature of 25°C or non-permissive temperature of 33°C. We also examined the effects of various virus doses injected into silkworm larvae and various times of rearing at 33°C on the virulence of ts-S1 by determining LD_{50} and LT_{50} values, symptoms appearing in the infected larvae, and alterations in their growth patterns.

Our results indicate that the virus causes larval death and/or molting alterations in dependence on dose, and that the rearing temperature of 33°C for infected larvae is involved in the abortion of ts-S1 infection in a time-dependent manner. Composite but separately recognised physiological and pathological responses of the silkworm larvae caused by infection with ts-S1 are discussed in relation to a variety of experimental parameters used in bioassays.

Materials and Methods

Cells, viruses and silkworm. The established silkworm cell line BmN4 (Maeda, 1989) was maintained at 27°C in Grace's Insect Culture Medium (Gibco-BRL) supplemented with 10% heat-inactivated foetal bovine serum, 0.26% tryptose and 0.035% sodium bicarbonate. BmNPV strain D1 (wtBmNPV) (Hashimoto *et al.*, 1994) and *ts* mutant, ts-S1, which was derived from wtBmNPV by BrdUrd-mediated mutagenesis (Shikata *et al.*, 1998b), were propagated in BmN4 cells. BV titre of a culture medium was expressed in $TCID_{50}$. Silkworm eggs of a commercial strain (Kinshu × Showa or Shunrei × Shogetsu) were purchased from Kanebo and maintained at 25°C for several days at high humidity. After hatching, the silkworm larvae were reared on an artificial diet (Silkmate 2S, Nihon-Nosan-Kogyo) at 25°C. Newly ecdysed 4th-instar larvae were used in bioassays.

Determination of BV titre and OB number in larvae. Fourth-instar silkworm larvae were injected with 1.0 x 10^5 $TCID_{50}$ wtBmNPV or ts-S1 per larva and reared at 25°C or 33°C. In time course experiments, the time point 0 hr was defined as the 1st hr post infection (p.i.). The haemolymph was collected from three larvae every 24 hrs until pupation, pooled and put into a pre-chilled microtube containing 10 mmol/l cystein and 10 mg/ml gentamycin sulfate. Haemolymph samples were stored at -80°C until determination of BV titre and OB number. For determination of BV titre, a sample was centrifuged at 3,000 x g for 5 mins to precipitate blood cells and OBs. The supernatant was diluted tenfold and used for titration of BV. To count OBs, the precipitate was resuspended in distilled water and sonicated until OBs were free from cell debris. OBs were counted in a Thoma haemocytometer.

Bioassay. Tenfold dilutions of ts-S1 and wtBmNPV (1.0 x 10^8 – 1.0 x 10^2 $TCID_{50}$/per ml) were used as inocula. Aliquots (10 µl) of each dilution were injected into 20 newly ecdysed 4th-instar larvae. The larvae were individually reared in plastic dishes (Falcon 1058) at 25°C and 33°C. Larvae which died due to nucleopolyhedrosis were counted every 12 hrs and assayed for the presence of OBs in the silk gland. LD_{50} values of virus materials for 4th-instar larvae and LT_{50} values of the infected larvae were determined by probit analysis (Finney, 1978). Mock-infected larvae were reared at 33°C without physiological damage for at least 8 days.

Rearing temperature shift bioassay Aliquots (10 µl) of tenfold dilutions of inocula of ts-S1 or wtBmNPV (1.0 x 10^8 – 1.0 x 10^4 $TCID_{50}$/ml), which induced death in 100% of larvae at 25°C, were injected into newly ecdysed 4th-instar larvae. The latter were reared at 33°C for 1, 3, 5 or 7 days and then shifted to 25°C. For each shift, 20 larvae were used. Each larva was individually reared until death or emergence. Dead larvae were counted every 12 hrs.

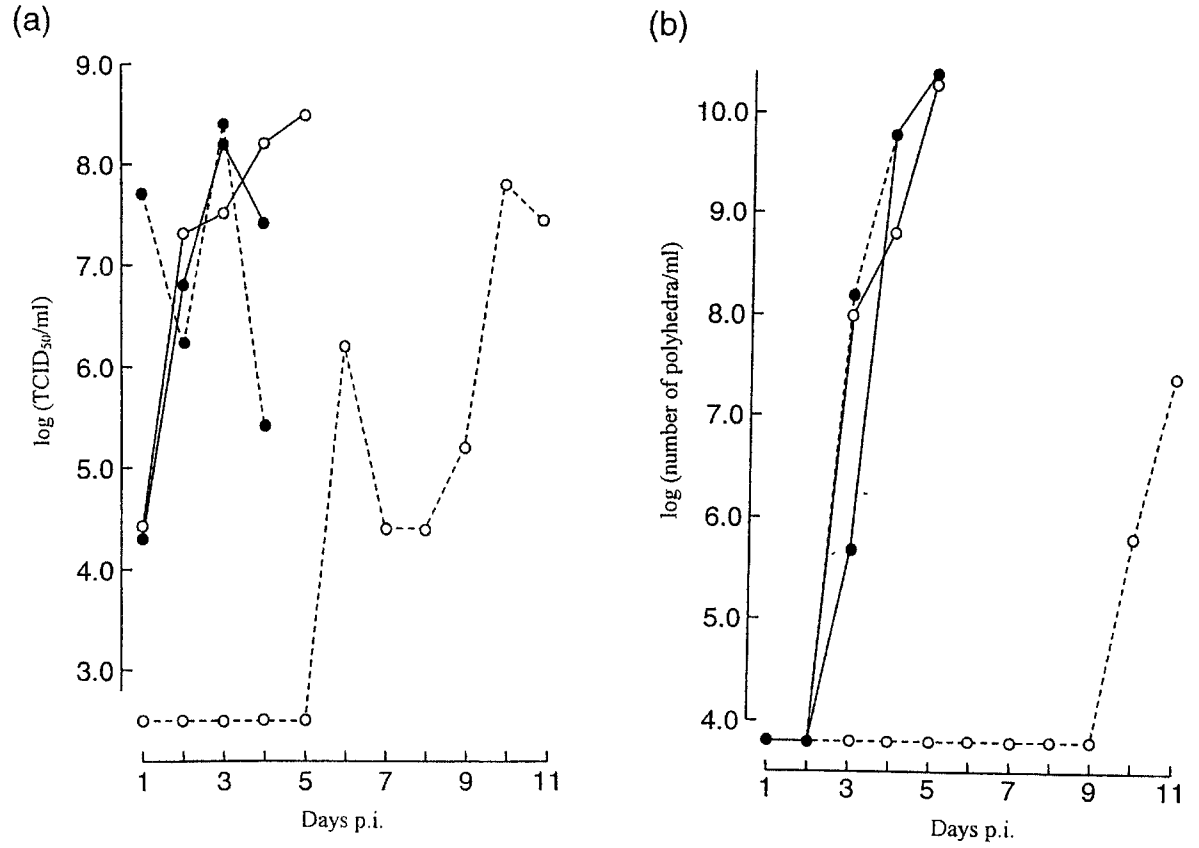

Fig. 2
Time courses of BV and OB production by ts-S1- and wtBmNPV-infected larvae reared at 25°C and 33°C
Fourth-instar silkworm larvae were infected with 1.0 x 10^5 $TCID_{50}$ of ts-S1 or wtBmNPV. (a) BV production by wtBmNPV- and ts-S1-infected larvae reared at 25°C and 33°C. (b) OB production by wtBmNPV- and ts-S1-infected larvae reared at 25°C and 33°C. 25°C (solid line), 33°C (dotted line), wtBmNPV (o) and ts-S1 (•). The circles out of the vertical scale indicate that BV titres or OB numbers could not be determined by the methods used.

Results

Nucleopolyhedrosis symptoms in ts-S1-infected larvae

Fourth-instar silkworm larvae were infected with 1.0 x 10^5 $TCID_{50}$ of ts-S1 per larva and reared at 25°C and 33°C. At 25°C, the larvae infected with either virus indicated symptoms typical of nucleopolyhedrosis and died by day 5 p.i. (Fig. 1a and c). At 33°C, wtBmNPV-infected larvae reared at 33°C died due to nucleopolyhedrosis by day 4 p.i. (Fig. 1b). However, ts-S1-infected larvae reared at 33°C showed no symptoms of nucleopolyhedrosis until day 5 p.i. Half of these larvae molted to the 5th-instar, and the rest remained at the 4th instar with glossy skin (Fig. 1d). Some of the non-molting 4th-instar larvae began spinning, but most of them died before reaching the prepupal stage. Larvae infected with 1.0 x 10^6 $TCID_{50}$ of ts-S1 per larva and reared at 33°C did not molt to the 5th-instar.

Replication of ts-S1 in larvae

ts-S1 is completely defective in BV and OB production at 33°C in BmN4 cells (Shikata *et al.*, 1998b). To determine replication properties of ts-S1 in silkworm larvae at 33°C, newly ecdysed 4th-instar larvae were infected with 1.0 x 10^5 $TCID_{50}$ of ts-S1 per larva and reared at 25°C and 33°C. The haemolymph was collected from surviving larvae every day and assayed for BV titre and OB number. Fig. 2a shows the kinetics of BV production in ts-S1- and wtBmNPV-infected larvae reared at 25°C and 33°C. In wtBmNPV-infected larvae reared at 33°C, the BV titre increased up to 10^8 $TCID_{50}$/ml at day 3 p.i. and the larvae died at day 4 p.i. In ts-S1- and wtBmNPV-infected larvae reared at 25°C, the BV titre rapidly increased from day 1 p.i. and reached 10^8 $TCID_{50}$/ml at day 3 p.i. ts-S1-infected larvae began to die at day 5 p.i. and wtBmNPV-infected larvae at day 4 p.i. In ts-S1-infected larvae reared at 33°C,

Table 1. Bioassay of 4th-instar silkworm larvae infected with wtBmNPV at 25°C and 33°C

Rearing temp.	Virus dose ($TCID_{50}$/larva)	wtBmNPV Number of dead larvae (days p.i.) 3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	Mortality (%)	LT_{50} (days)
	1.0×10^6		1	19						100	4.2
			1	18	1					100	4.3
	1.0×10^5		2	18						100	4.2
				19	1					100	4.4
	1.0×10^4			5	13	1	1			100	4.7
				3	15	2				100	4.7
	1.0×10^3			1	16	3				100	4.8
					15	4	1			100	5.0
25°C	1.0×10^2				8	12				100	5.0
					7	5	8			100	5.3
	1.0 x 10					13	5			90	5.3
					3	5	11			100	5.4
	1.0					7	1			35	–
						3	1			20	–
	1.0×10^{-1}						1			5	–
						1				5	–
	1.0×10^{-2}									0	–
										0	–
	LD_{50} for wtBmNPV at 25°C: 0.96 ± 0.26 $TCID_{50}$/larva										
	1.0×10^6	18	2							100	3.4
		17	1	2						100	3.4
	1.0×10^5	12	6	2						100	3.5
		16	4							100	3.4
	1.0×10^4	4	14	2						100	3.7
		1	12	7						100	3.8
	1.0×10^3	3	5	12						100	3.8
			14	6						100	3.9
33°C	1.0×10^2	5	6	3	6					100	4.0
			13	3	4					100	4.1
	1.0 x 10		2	8	8	1				95	4.6
				13	5	2				100	4.6
	1.0			1	5					30	–
				3						15	–
	1.0×10^{-1}									0	–
										0	–
	1.0×10^{-2}									0	–
										0	–
	LD_{50} for wtBmNPV at 33°C: 1.70 ± 0.04 $TCID_{50}$/larva										

LD_{50} values represent averages from duplicate bioassays.

the BV titre was very low until day 4 p.i. Thereafter, the larvae molted to the 5th-instar and the BV titre gradually increased up to 10^8 $TCID_{50}$/ml at day 10 p.i., but no larvae showed symptoms typical of nucleopolyhedrosis within 11 days p.i. Fig. 2b shows the kinetics in OB production in ts-S1- or wtBmNPV-infected larvae at 25°C and 33°C. In ts-S1-infected larvae reared at 25°C and wtBmNPV-infected larvae reared at 25°C or 33°C, the OB number in haemolymph increased linearly up to 10^{10}/ml until they died due to nucleopolyhedrosis. In ts-S1-infected larvae reared at 33°C, the OB number was very low until day 9 p.i., then it increased to 10^8/ml.

Virulence of ts-S1 for silkworm larvae

To examine the virulence of ts-S1 for the larvae, LD_{50} values of ts-S1 and wtBmNPV for 4th-instar larvae reared at 25°C or 33°C and LT_{50} values of these infected larvae were determined. LD_{50} values of wtBmNPV for larvae reared at 25°C and 33°C and of ts-S1 for larvae reared at 25°C were 0.96 ± 0.26,

Table 2. Bioassay of 4th-instar silkworm larvae infected with ts-S1 at 25°C and 33°C

Rearing temp.	Virus dose ($TCID_{50}$/larva)	ts-S1 Number of dead larvae 3.5	4.0	4.5	5.0 (days p.i.)	5.5	6.0	6.5	7.0	Mortality (%)	LT_{50} (days)
	1.0×10^{6}			16	4					100	4.4
			1	14	4	1				100	4.4
	1.0×10^{5}			9	11					100	4.5
				7	11	2				100	4.7
	1.0×10^{4}				19	1				100	4.9
				4	14	2				100	4.7
	1.0×10^{3}				18	2				100	4.9
				2	12	6				100	4.8
25°C	1.0×10^{2}				6	13	1			100	5.2
					2	13	5			100	5.3
	1.0×10				1	17				90	5.3
					1	9	9	1		100	5.5
	1.0						5	2		35	–
								3		15	–
	1.0×10^{-1}							1		5	–
										0	–
	1.0×10^{-2}									0	–
										0	–
	LD_{50} for ts-S1 at 25°C: 2.41 ± 0.39 $TCID_{50}$/larva										
	1.0×10^{6}			1	3	2	1	1		40	–
					5	2	2			45	–
	1.0×10^{5}					1	1		3	25	–
					1	1	3			25	–
	1.0×10^{4}						1			5	–
					1				2	15	–
	1.0×10^{3}									0	–
										0	–
33°C	1.0×10^{2}									0	–
										0	–
	1.0×10									0	–
										0	–
	1.0									0	–
										0	–
	1.0×10^{-1}									0	–
										0	–
	1.0×10^{-2}									0	–
										0	–
	LD_{50} for ts-S1 at 33°C: > 1.0×10^{6} $TCID_{50}$/larva										

LD_{50} values represent averages from duplicate bioassays.

1.70 ± 0.04 and 2.41 ± 0.39 $TCID_{50}$ per larva, respectively (Tables 1 and 2). The LD_{50} value of ts-S1 for larvae reared at 33°C could not be determined, since the larval mortalities obtained by injection of the largest virus dose (1.0×10^{6} $TCID_{50}$/larva) did not exceed 50%. The highest mortality rate in ts-S1-infected larvae reared at 33°C was 45% for the dose of 1.0×10^{6} $TCID_{50}$ per larva. Therefore, the LD_{50} value of ts-S1 for the larvae reared at 33°C was higher at least by 5 orders than those for the larvae reared at 25°C and wtBmNPV-infected larvae reared at 25°C or 33°C. This result indicated that the virulence of ts-S1 for larvae reared at 33°C was markedly reduced. LT_{50} values increased slightly when less virus was injected into larvae. This tendency was observed for ts-S1-infected larvae reared at 25°C or 33°C. The LT_{50} value for ts-S1-infected larvae reared at 33°C could not be determined in the same reason described for calculation of LD_{50} value for ts-S1-infected larvae reared at 33°C. However, the speed at which ts-S1 killed larvae reared at 33°C was similar to that observed at 25°C. This was in contrast to the case of wtBmNPV which killed larvae faster at 33°C in comparison to 25°C.

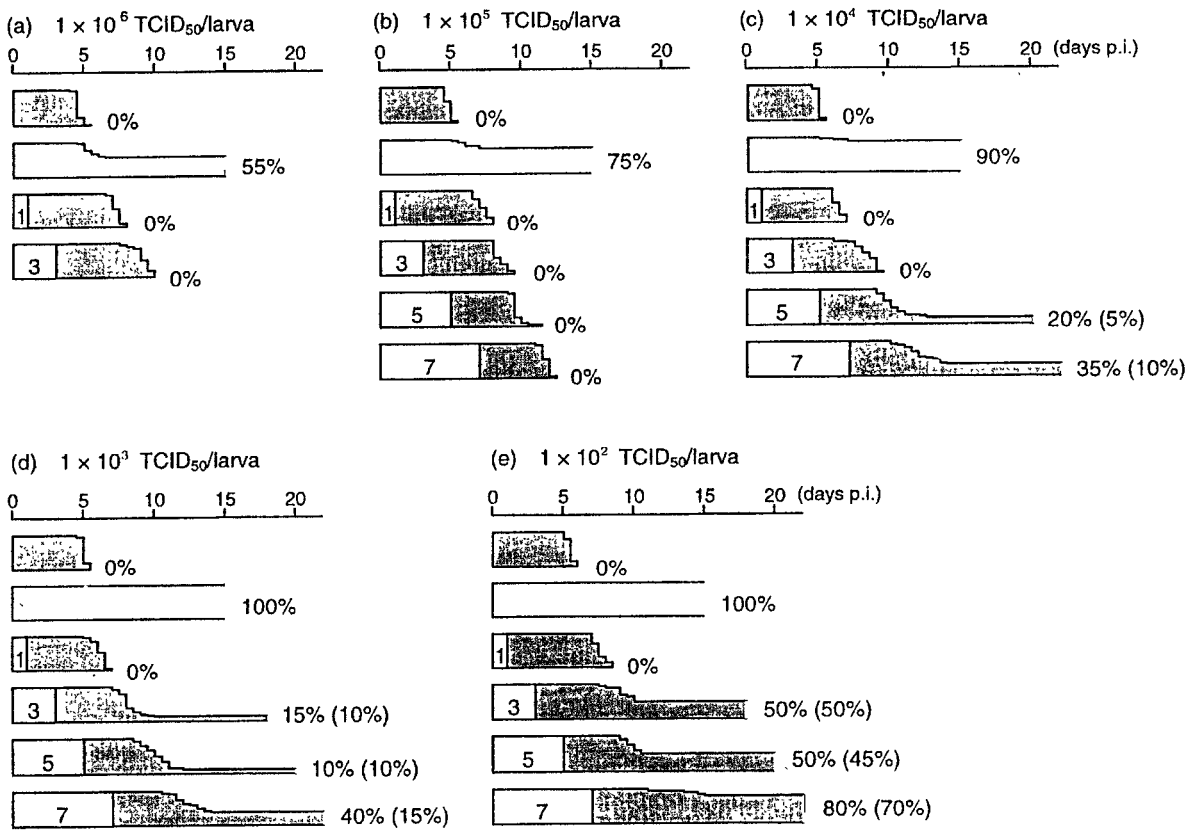

Fig. 3

Effect of rearing at 33°C on 4th-instar silkworm larvae infected with ts-S1 at different multiplicity

Open and shaded areas in the bars represent rearing at 33°C and 25°C, respectively. Numbers in the open bars represent rearing term in days. The height of each box represents number of living larvae, the height at day 0 p.i. indicates 20 of larvae. Horizontal direction from left to right represents cumulative days p.i. Numbers with and without parentheses on the right of the bars represent percentages of living pupae in which virus infection was aborted and percentages of emerged larvae, respectively. In the control experiment, mock-infected larvae reared at 25°C molted to the 5th-instar at day 5 p.i. and pupated at day 16 p.i.

Effect of rearing temperature shift

Long-term rearing at 33°C generally causes physiological dysfunction in silkworm larvae such as incomplete molting, spinning or other homeostasis problems; this prevents clear examination of virulence of *ts* mutants for larvae. To determine the effect of rearing at 33°C, larvae were reared for various time at 33°C and then shifted to 25°C. Larvae infected with 1.0 x 10^6 $TCID_{50}$ of ts-S1 per larva died after rearing at 33°C within 3 days. They did not molt to 5th instar stage and had glossy skin. Larvae infected with 1.0 x 10^5 $TCID_{50}$ of ts-S1 per larva and reared at 33°C for 1.0 – 7 days died after shift to 25°C. Larvae infected with 1.0 x 10^4 $TCID_{50}$ of ts-S1 per larva and reared at 33°C for 1 – 3 days died after shift to 25°C. However, 20 – 35% of the larvae reared at 33°C for 5 – 7 days survived, and 5 – 10 % emerged, respectively. Larvae infected with 1.0 x 10^3 $TCID_{50}$ of ts-S1 per larva and reared at 33°C for 1 day died after shift to 25°C, but 10 – 40% of the larvae reared at 33°C for 3 – 7 days survived and 10 – 15% emerged, respectively. Larvae infected with 1.0 x 10^2 $TCID_{50}$ of ts-S1 per larva and reared at 33°C for 1 day died after shift to 25°C, but 50 – 80% of the larvae reared at 33°C for 3 – 7 days survived and 45 – 70% emerged, respectively. These results showed that in silkworm larvae, a temperature shift from 33°C to 25°C during rearing after infection with various doses of ts-S1, which would cause 100% infection of the larvae at 25°C, a longer period of rearing at 33°C resulted in better survival. This tendency was enhanced in proportion to decreasing virus dose injected.

Discussion

Half of the surviving larvae infected with 1.0 x 10^5 $TCID_{50}$ per larva showed growth alterations at the rearing temperature of 33°C, such as prolongation of 4th instar and glossy skin. These features of ts-S1-infected larvae are very similar to the phenotype of the non-molting glossy (*nm-g*) mutant of the silkworm, which shows abnormal secretion of the molting hormones, ecdysteroids (Tanaka, 1998). Baculoviruses have an early gene encoding the ecdysteroid UDP-glucosyltranferase (EGT), which inactivates ecdysteroids in haemolymph by EGT-mediated sugar conjugation reaction to extend larval instar stages (O'Reilly and Miller, 1989). EGT is necessary as a growth regulator in the infection of the target insects. When reared at 33°C, ts-S1-infected larvae did not show symptoms typical for nucleopolyhedrosis, but showed growth alterations similar to those caused by *egt* expression associated with baculovirus infection (O'Reilly and Miller, 1989; O'Reilly and Miller, 1991; Shikata *et al.*, 1998a). As ts-S1 has a mutation of the *lef-8* gene (Shikata *et al.*, 1998b) which regulates the late gene expression, it is possible that early genes of ts-S1 including the *egt* gene were expressed and that the EGT induced growth alterations in some larvae at 33°C. Since ts-S1 is a non-BV-producing mutant, secondary and further infection cycles should not occur in infected larvae (Shikata *et al.*, 1998b). If the infection dose of ts-S1 is closely related to the amount of EGT secreted from the primarily infected cells into haemolymph, EGT, even in small amounts, may have very powerful effects on the growth of silkworm larvae.

Following the ts-S1 injection into silkworm larvae at 33°C, physiological damage caused by high rearing temperature may partially obscure the appearance of growth alterations. When silkworm larvae were infected with 1.0 x 10^5 $TCID_{50}$ of virus per larva and reared at 33°C, the production of BV and OBs was markedly delayed and reduced in haemolymph. Eventually, the production of BV and OBs in haemolymph of ts-S1-infected larvae reared at 33°C occurred only in those larvae which had molted to 5th instar. This suggested that the injected ts-S1 could be retained in larvae during larval-larval molting and became virulent in 5th-instar larvae and/or that physiological changes occurring in larvae during molting also facilitated the virus propagation. The delayed ts-S1 replication may be a leaky property at the organism level at non-permissive temperature.

LT_{50} values for wtBmNPV- and ts-S1-infected larvae reared at 25°C for different virus doses were similar to each other. The LT_{50} value for ts-S1-infected larvae reared at 33°C could not be determined as the highest mortality rate observed was less than 50%. The distribution peak of dead ts-S1-infected larvae reared at 33°C did not coincide with the LT_{50} value of wtBmNPV-infected larvae reared at 33°C and occurred slightly later than that of ts-S1-infected larvae reared at 25°C. The killing speed of ts-S1 for the larvae at 33°C coincided with the slow accumulation of BV and OBs in haemolymph of ts-S1-infected larvae reared at 33°C. These results indicate that the virulence of ts-S1 was markedly reduced in infected larvae reared at 33°C.

In ts-S1-infected larvae, longer rearing times at 33°C increased the survival time after the temperature shift to 25°C. When the infected larvae were reared continuously at 33°C, higher virus doses caused their lower viability. The highest virus dose (1.0 x 10^6 $TCID_{50}$/larva) failed to decrease the viability below 50%. In contrast, no larvae survived the rearing at 25°C regardless of the virus dose used. Longer rearing at 33°C seemed to shorten the survival time at 25°C. This suggests that the non-permissive temperature of 33°C causes not only a restriction of virus replication but also physiological stress leading to a reduction of the larval lifespan after the shift to 25°C. These phenomena were clearly observed in the case of large virus doses, e.g. 1.0 x 10^5 – 1.0 x 10^6 $TCID_{50}$/larva. The incidence of survivors which aborted the virus infection became marked when lower virus doses were used. The dates of death of larvae that died soon after the shift to 25°C tended to be scattered in proportion to the duration of rearing at 33°C; this was clearly observed in bioassays with virus doses of 1.0 x 10^4 – 1.0 x 10^6 $TCID_{50}$/larva. The scattering of dates of death may have been due to the variation in physiological conditions of individual larvae in response to the rearing temperature of 33°C and the virus dose used.

We have demonstrated here that the virulence of ts-S1 for silkworm larvae was markedly reduced by rearing them at 33°C in proportion to the length of time at this temperature, although the rearing at 33°C disturbed their normal growth. In the case of large doses of ts-S1 we assume that single infection of systemic cells by the injected virus and resulting expression of the *egt* gene were responsible for the death and growth alterations.

Acknowledgments. This study has been supported in part by grants from The Integrated Research Programme for the Development of Insect Technology and The Ministry of Agriculture, Forestries and Fisheries of Japan, and by grant No. (A)(2)10306006 from the Ministry of Education, Science, Sports and Culture of Japan. M.S. has been supported by a research fellowship from the Japan Society for the Promotion of Science for Young Scientists.

References

Chaeychomsri S, Ikeda M, Kobayashi M (1995): Nucleotide sequence and transcriptional analysis of the DNA

polymerase gene of *Bombyx mori* nuclear polyhedrosis virus. *Virology* **206**, 435–447.

Finney DJ (1978): *Statistical Method in Biological Assay.* 3rd ed., Charles Griffin and Co., London, pp. 508.

Gomi S, Zhou CE, Yih W, Majima K, Maeda S (1997): Deletion analysis of four of eighteen late gene expression factor gene homologues of the baculovirus, BmNPV. *Virology* **230**, 35–47.

Hashimoto Y, Kanamori Y, Hayakawa T, Katayama Y, Kamita SG, Maeda S, Matsumoto T (1994): Physical mapping of *Bombyx mori* nuclear polyhedrosis virus strain D1: Identification of novel interspersed homologous regions. *Appl. Entomol. Zool.* **29**, 442–448.

Hu NT, Lu YF, Hashimoto Y, Maeda S, Hou RF (1994): The p10 gene of natural isolates of *Bombyx mori* nuclear polyhedrosis virus encodes a truncated protein with an M(r) of 7700. *J. Gen. Virol.* **75**, 2085–2088.

Kamita SG, Majima K, Maeda S (1993): Identification and characterization of the p35 gene of *Bombyx mori* nuclear polyhedrosis virus that prevents virus-induced apoptosis. *J. Virol.* **67**, 455–463.

Kamita SG, Maeda S (1997): Sequencing of the putative DNA helicase-encoding gene of the *Bombyx mori* nuclear polyhedrosis virus and fine-mapping of a region involved in host range expansion. *Gene* **190**, 173–179.

Lu M, Iatrou K (1996): The genes encoding the P39 and CG30 proteins of *Bombyx mori* nuclear polyhedrosis virus. *J. Gen. Virol.* **77**, 3135–3143.

Lu M, Swevers L, Iatrou K (1998): The p95 gene of *Bombyx mori* nuclear polyhedrosis virus: temporal expression and functional properties. *J. Virol.* **72**, 4789–4797.

Maeda S (1989): Gene transfer vectors of a baculovirus, *Bombyx mori* nuclear polyhedrosis virus, and their use for expression of foreign genes. In Mitsuhashi J (Ed.): *Invertebrate Cell Systems and Applications.* Vol. 1, CRC press, Boca Raton, Fla, pp. 167–181.

Maeda S, Kamita SG, Kataoka H (1991): The basic DNA-binding protein of *Bombyx mori* nuclear polyhedrosis virus: the existence of an additional arginine repeat. *Virology* **180**, 807–810.

Maeda S (1994): Complete DNA sequence of *Bombyx mori* nuclear polyhedrosis virus. Genbank accession No. L33130.

Mikhailov VS, Mikhailova AL, Iwanaga M, Gomi S, Maeda S (1998): *Bombyx mori* nucleopolyhedrovirus encodes a DNA-binding protein capable of destabilizing duplex DNA. *J Virol.* **72**, 3107–3116.

Okawa T, Majima K, Maeda S (1994): A cysteine protease encoded by the baculovirus *Bombyx mori* nuclear polyhedrosis virus. *J. Virol.* **68**, 6619–6625.

O'Reilly DR, Miller LK (1989): A baculovirus blocks insect molting by producing ecdysteroid UDP-glucosyl transferase. *Science* **245**, 1110–1112.

O'Reilly DR, Miller LK (1991): Improvement of a baculovirus pesticide by deletion of the *egt* gene. *Bio-Technology* **9**, 1086–1089.

Passarelli AL, Todd JW, Miller LK (1994): A baculovirus gene involved in late gene expression predicts a large polypeptide with a conserved motif of RNA polymerases. *J Virol.* **68**, 4673–4678.

Shikata M, Shibata H, Sakurai M, Sano Y, Hashimoto Y, Matsumoto T (1998a): The ecdysteroid UDP-glucosyltransferase gene of *Autographa californica* nucleopolyhedrovirus alters the molting and metamorphosis of a non-target insect, the silkworm, *Bombyx mori* (*Lepidoptera, Bombycidae*). *J. Gen. Virol.* **79**, 1547–1551.

Shikata M, Sano Y, Hashimoto Y, Matsumoto T (1998b): Isolation and characterization of temperature-sensitive mutant of *Bombyx mori* nucleopolyhedrosis virus for putative RNA polymerase gene. *J. Gen. Virol.* **79**, 2071–2078.

Tanaka Y (1998): Induction of larval ecdysis by ecdysone in the *non-molting glossy (nm-g)* mutant larvae of *Bombyx mori*. *J. Serie. Sci. Jpn.* **67**, 109–115.

Todd JW, Passarelli AL, Miller LK (1995): Eighteen baculovirus genes, including *lef-11, p35, 39K,* and *p47*, support late gene expression. *J. Virol.* **69**, 968–974.