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EFFECT OF HIGH-TEMPERATURE TREATMENT ON THE BROWN PLANTHOPPER, Nilaparvata lugens, WITH REFERENCE TO PHYSIOLOGICAL ROLES OF ITS YEAST-LIKE SYMBIOTE【Research report】

高溫處理對褐飛蝨酵母狀共生菌之影響【研究報告】

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Abstract

Treatment of the lst-instar nymphs of the brown planthopper, Nilaparvata lugens, at 32℃ or 35℃ for 3-5 days might reduce population of its intracellular yeast-like symbiote (YLS). However, the YLS could regain their population after consecutively treated at 32℃ through 3 generations, suggesting that the symbiote can adapt to the heat treatment. The 3-day-old female adults containing fully developed ovaries were treated with 32℃ for 3 days, resulting in low fecundity and great reduction in the number of YLS in these females, but the number of YLS in eggs remained almost unchanged compared with the normal insects. It seems that the transovarian transmission of the YLS is not inhibited by this heat treatment. The number of YLS in eggs was drastically reduced when treated with 32℃ for 2 days on day 1, 4 or 7 of the egg age, but only those eggs treated on day 7 lowered their hatchability, speculating that YLS is more important to the late embryonic development than the early stages. Although the YLS has been reported to supply its host with sterols, addition of 2.5mg/ml cholesterol to the sterol-free holidic diet did not enhance percentage of adult emergence of N. lugens compared with those insects on the sterol-free diet; therefore, the function of the YLS in the planthopper remains to be studied.

摘要

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Effect of High-Temperature Treatment on the Brown Planthopper, *Nilaparvata lugens*, with Reference to Physiological Roles of Its Yeast-Like Symbiote

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ABSTRACT

Treatment of the 1st-instar nymphs of the brown planthopper, *Nilaparvata lugens*, at 32° or 35°C for 3-5 days might reduce population of its intracellular yeast-like symbiote (YLS). However, the YLS could regain their population after consecutively treated at 32°C through 3 generations, suggesting that the symbiote can adapt to the heat treatment.

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Although the YLS has been reported to supply its host with sterols, addition of 2.5 mg/ml cholesterol to the sterol-free holidic diet did not enhance percentage of adult emergence of *N. lugens* compared with those insects on the sterol-free diet; therefore, the function of the YLS in the planthopper remains to be studied.

Introduction

The brown planthopper, *Nilaparvata lugens*, harbors a great number of yeast-like symbiotes (YLS) in its fat-body cells of abdomen, and transmits the symbiotes to its progeny through ovaries (Chen *et al.*,1981a, b; Nasu *et al.*,1981). The significance of symbiotes in insects may be regarded as supplying their host with certain nutrients in immature stages and essential for reproduction in imago (Richards and Brooks 1958). To study the physiological functions of intracellular symbiotes in insects, one of the feasible methods is to eliminate the symbiotes from their host so that the aposymbiotic insects are produced, and their functional abnormalities in these individuals are indicative of the contributions of symbiotes to their host.

Treatment with elevated temperatures has been reported to be effective in destroying intracellular symbiotes of homopterans (Noda and Saito,1979b; Chen *et al.*,1981a). Using this treatment, Noda *et al.* (1979) proposed the sterol source supplied by symbiotes in the smaller brown planthopper, *Laodelphax striatellus*, and Chang (1974) reported decrease in fecundity when the bed bug, *Cimex lectularius*, was treated at 36°C for 2 weeks. Essentiality of symbiotes for development of embryo abdomen was reported in the leafhopper, *Euscelis plejebus* (Schwemmler, 1974). However, studies on physiological roles of YLS in *N. lugens* are as yet scanty. Although treatment with high temperature causes decrease in YLS number in *N. lugens* (Chen *et al.*,1981a), the question arises as to whether the YLS is adaptable to high temperature if treated for consecutive generations. This paper presents some interactions between the brown planthopper and its YLS under high-temperature treatment.

Materials and Methods

Insects

The stock colonies of the brown planthopper, *N. lugens*, were collected from paddy fields of the vicinity of Taichung city, and were reared for several generations on rice seedlings in the laboratory. Most insects are macropterous type.

Calculation of YLS population

Ten to twenty insects were homogenated in 0.85% NaCl solution using a glass homogenizer (Pyrex No. 7727). A drop of this solution was added onto a Thoma haemacytometer and its YLS number was calculated under a Nikon Biophot microscope. Each calculation was done in 6 replicates, and the number of YLS in each insects was calculated following the formula described by Chen *et al.* (1981b).

The heat treatment of the brown planthopper through generations

The ovipositing females were placed on rice seedlings for one day and then were removed. The newly-hatched nymphs were collected daily from the rice seedlings after incubated for 9 days, and were reared at 32°C for 3-5 days before shifting back to 26°C. This is referred to as "heat treatment". The number of YLS in these treated nymphs was calculated every two days. These insects were regarded as the parental generation, their F_1 and F_2 nymphs were also subjected to the heat treatment as mentioned above. The number of YLS in these insects was calculated as usual.

Artificial rearing of the brown planthopper on holidic diets

The newly-hatched nymphs were fed on a holidic diet formulated by Koyama *et al.* (1981) (Table 1). The feeding device was modified from Hou and Lin (1979). Thirty to forty nymphs were reared in a feeding chamber, the diet sachet was changed daily.

Histological sectioning

The specimens were fixed for 3 hr using the Carnoy's fluid and then were dehydrated thrice with 2, 2-dimethoxypropane. The sections cut in 7μ were stained with Delafield's hematoxylin-Eosin Y system.

Table 1. Composition of MED-4 Diet for *N. lugens* (Modified from Koyama, 1981) (mg/100ml)

I. *Amino acids*		III. Others	
L-alanine	100	Sucrose	5,000
L-asparagine	450	$MgCl_2 \cdot 6H_2O$	200
L-aspartic acid	150	KH_2PO_4	500
L-cysteine	80	$FeCl_3 \cdot 6H_2O$	2.0
L-glutamic acid	300	$CuCl_2 \cdot 2H_2O$	0.3
L-glutamine	900	$MnCl_2 \cdot 4H_2O$	0.8
L-histidine	300	$ZnCl_2$	0.4
L-isoleucine	300	$CaCl_2 \cdot 2H_2O$	3.0
L-leucine	300	pH	6.5
L-lysine hydrochloride	300		
L-methionine	150		
serine	150		
L-threonine	300		
II. Vitamins			
Thiamine hydrochloride	2.5		
Riboflavin	5.0		
Nicotinic acid	10.0		
Pyridoxine hydrochloride	2.5		
Folic acid	1.0		
Calcium pantothenate	5.0		
Inositol	50.0		
Choline chloride	50.0		
Biotin	0.1		
L-ascorbic acid	100.0		

Results

Effect of heat treatment on YLS population through generations

Treatment of the 1 st instar nymphs with 32°C for 5 days through generations was carried out in a plant growth chamber. Table 2 shows that treating temperature at 32°C did not prolong adult emergence compared with those insects reared at 25°C, but adult emergence resulted from treating at 35°C delayed for 3 days, and persisted for 4 days more. It is obvious that growth of the planthopper is not affected by treating at 32°C. The symbiote population of the heat-treated parental generation was reduced to ca. 22% of that of normal insects in the beginning, and down to 18% by 14 days after treatment; while the population in both F_1 and F_2 nymphs which were subjected to the same treatment as the parental generation was increased distinctly (Fig. 1). The YLS population of F_1 insects was found to be ca. 1.5 fold of the parental generation at day 6 after treatment, and ca. 4.4 fold by 14 days. Figure 5 shows that the YLS population of the F_1 progeny from the heat-treated parents restored to as many as the normal insects. A similar result was also obtained from the F_2 progeny reared at room temperature whose parental insects had been subjected to heat treatment. The restoration of the symbiote population can be seen in fat-body cells by histological sectioning (Figs. 2 A-D). The heat-treated parental insects harbored only a few YLS in the sections of abdomens, while their F_1 nymphs that were also subjected to heat treatment had more symbiotes.

Effect of YLS population on reproduction of the planthopper

The number of eggs laid by each female and the YLS number in females and eggs were counted for 6 days on 3-day-old female adults which were subjected to heat treatment at 32°C for 3 days. These females were found to have fully developed ovaries. Figure 3 shows that the YLS number in these females decreased to ca. 27% of that of the normal insects, remaining at a low population during 6-day period without regaining the YLS number. Similarly, the number of YLS in eggs laid by these females was not varied distinctly (Table 3). However, Table 4 shows a relatively lower number of eggs laid by the heat-treated females compared with the normal insects. These results indicated that the low symbiote number resulted from heat treatment did not inhibit transovarian transmission of the YLS but could decrease fecundity of these females.

Table 2. Adult Emergence of *N. Lugens* after Treatment with Various Temperatures

Temperature (°C)	Adult emergence (Days)														
	12	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*	*21*	*22*	*23*	*24*	*25*	*26*
25	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-
17	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-
32	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-
35	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-

Effect of YLS population on embryonic development of the planthopper

The eggs were subjected to heat treatment at 32°C for 2 days by 3 embryonic stages, i.e. egg age: day 1-3, 4-6, 7-9, respectively. Then, the YLS number was counted by sampling every 2 days and their hatchability was recorded. As shown in Figure 4, the YLS number was decreased to below 50% as treated in 1st and 2nd embryonic stage but the YLS could then multiply rapidly. When the late stage was treated, the YLS number decreased drastically to only ca. 22% of that of the normal population. In addition, Table 5 shows that hatchability was lowered when the late embryonic stage was subjected to the heat treatment. It is then visualized that the embryonic development is deterred if subjected to heat treatment at late stage.

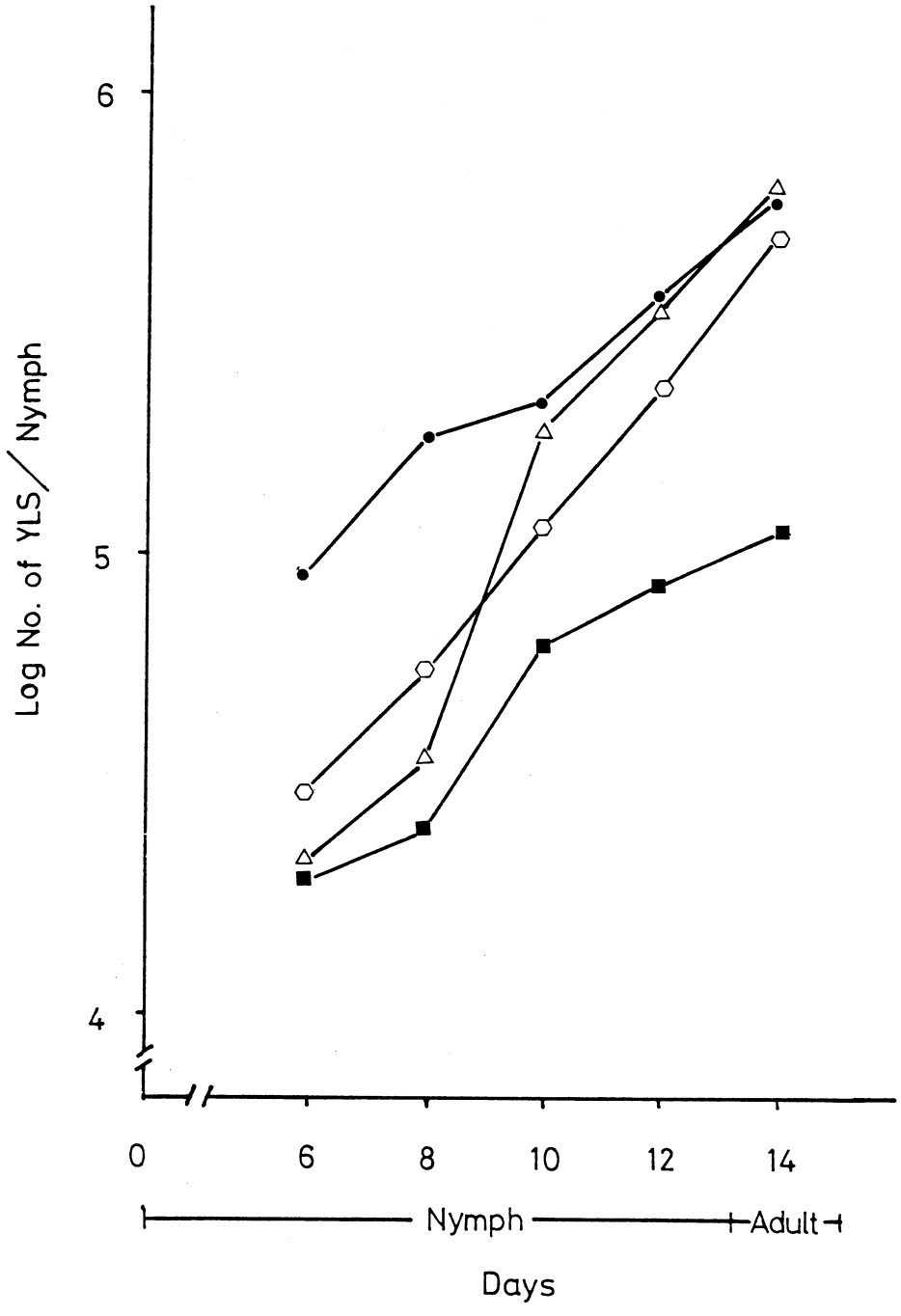

Fig. 1. Population dynamics of the brown planthopper symbiote after heat treatment through 3 generations.

●——● : Normal planthoppers

○——○ : lst-instar nymphs at 32°C for 5 days through 2 generations

△——△ : 1st-instar nymphs at 32°C for 5 days through 3 generations

■——■ : 1st-instar nymphs of parental generation at 32°C for 5 days

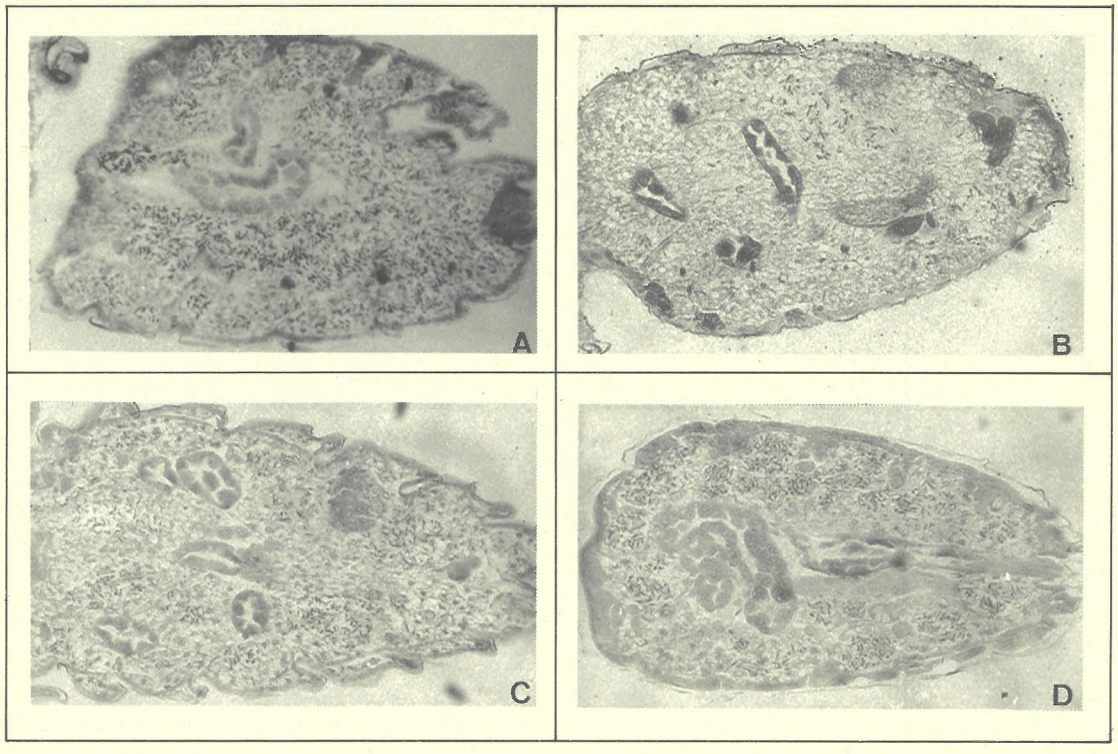

Fig. 2. Sagittal sections through 8-day-old nymphs of the brown planthopper after various temperature treatments at first instar.
A. Normal insect at 26°C
B. Parental generation at 32°C for 3 days
C. Both parental and F_1 generation at 32°C for 3 days
D. Parental generation at 32°C, 3 days; F_1 at 26°C

Table 3. The Number of Yeast-Like Symbiotes in the Egg of *N. lugens* Laid by the Normal and the Heat-treated Female Adults

Insect group	Log No. of YLS/egg post-treatment					
	1st	2nd	3rd	4th	5th	6th day
Normal	3.24	3.21	3.03	2.88	3.15	3.20
Heat-treated	2.69	2.82	3.05	3.24	3.20	3.07

Table 4. The Number of Eggs Laid by the Normal and the Heat-treated Female Adults of *N. lugens*

Insect group	No. of egg/female/day post-treatment					
	1st	2nd	3rd	4th	5th	6th day
Normal	9.78	12.22	13.44	13.00	17.26	14.73
Heat-treated	8.03	10.00	12.87	10.68	5.32	11.53

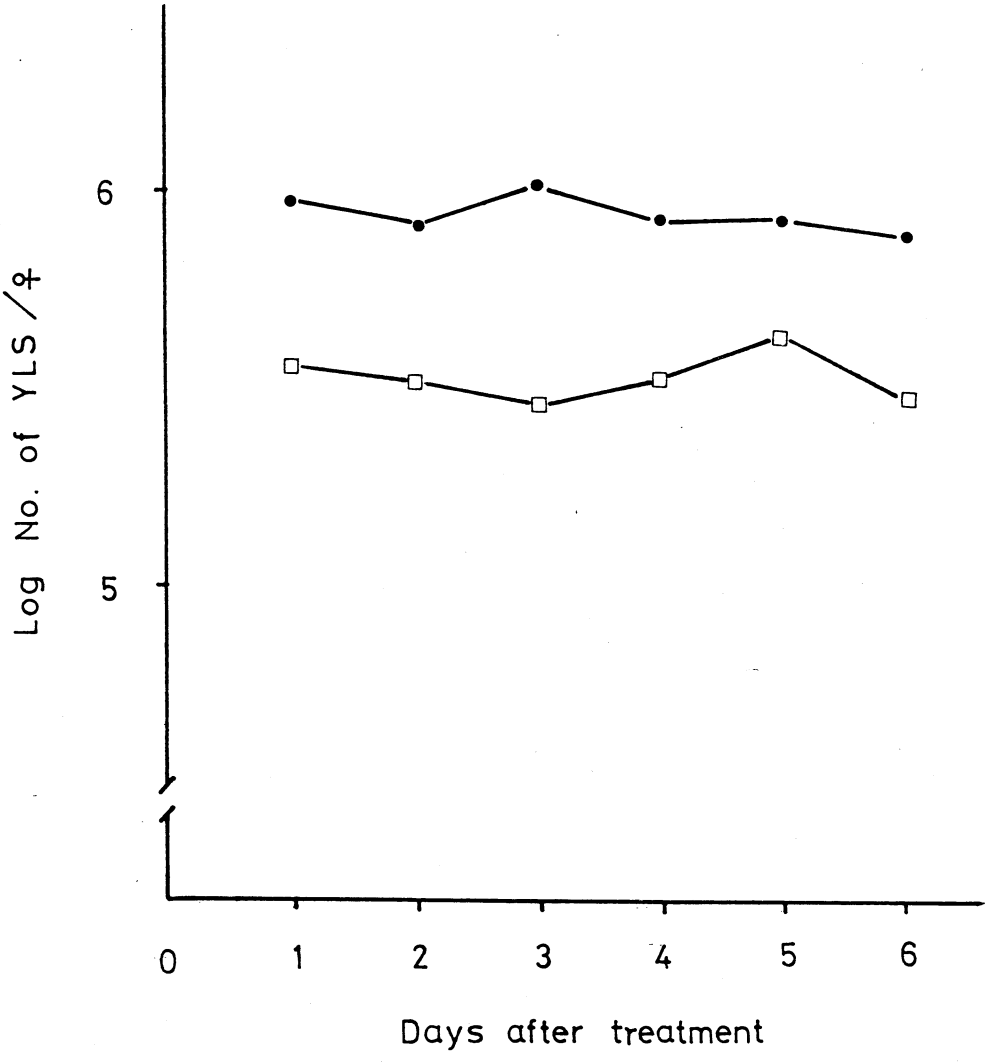

Fig. 3. Population dynamics of the brown planthopper symbiote in the heat-treated females.

●—● : 3-day-old females at 26°C for 3 days

□—□ : 3-day-old females at 32°C for 3 days

Table 5. Heatchability of *N. lugens* after Heat Treatment at Different Larval Ages

Treatment 32°C, 48hr	Hatchablilty (%)			Total hatchability (%)
	1st	2nd	3rd day	
Control	50.00	96.40	96.40	97.20
1st-3rd day	80.00	88.90	94.35	94.80
4th-6th day	76.36	90.08	93.72	94.01
7th-9th day	14.29	39.29	51.79	56.71

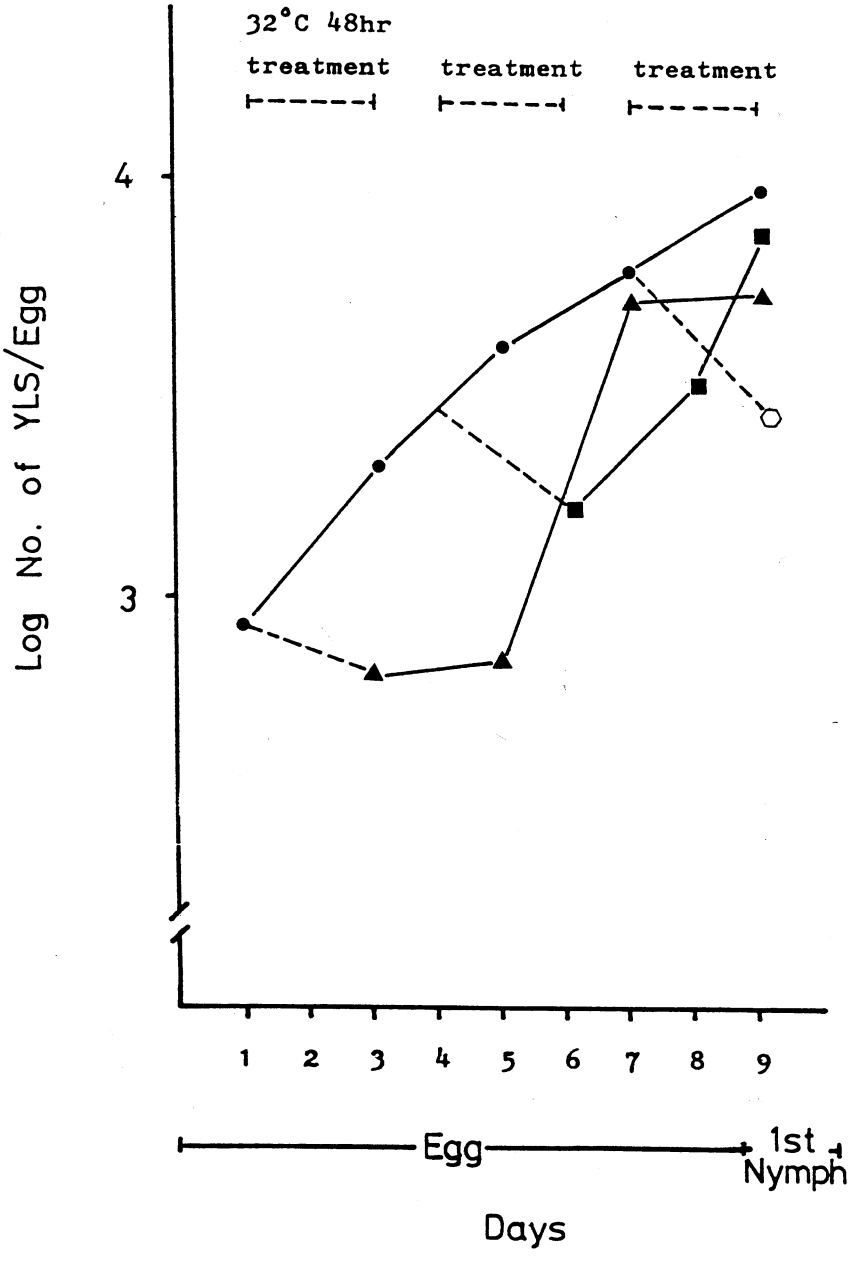

Fig. 4. Population dynamics of the brown planthopper symbiote in the heat-treated eggs.

●—● : All the egg stage at 26°C

▲—▲ : Heat treatment at one-day-old egg

■—■ :Heat treatment at 4-day-old egg

----○ : Heat treatment at 7-day-old egg

Table 6. Cumulative Mortality of *N. lugens* in Each Instar Reared on Different Diets

Diet	Cumulative mortality (%)					Adult emergence (%)
	Instar					
	1st	2nd	3rd	4th	5th	
MED-4	18.85	26.93	31.21	36.71	52.83	42.33
Rice seedling	3.32	6.54	9.76	15.20	18.46	80.65

Relationship between YLS and sterol requirements of the planthopper

The planthoppers were reared on the MED-4 diet formulated by Koyama *et al.* (1981). Emergence of the insects fed on the sterol-deficient diet was ca. 42%, their body size being similar to those insects on rice plant, and the females could lay fertile eggs. The heat-treated planthoppers gained a similar adult emergence on MED-4 diet; however, addition of 2.5 mg/ml cholesterol in this diet did not enhance adult emergence of the heat-treated insects (Table 7). It is therefore doubtful that the intracellular symbiotes may provide their host with sterols as revealed in this feeding test with the heat-treated aposymbiotic insects.

Discussion

The high temperature treatment is effective in destroying intracellular symbiotes of *L. striatellus* (Noda and Saito, 1979a). Chen *et al.* (1981a) also found that the symbiote population in *N. lugens* was greatly reduced when the newly-hatched nymphs were treated at 35°C for 3 days. However, the present study showed that the YLS tended to regain their population after being subjected to the heat treatment for consecutive generations. There are then two possibilities for explaining this restoration of symbiote population: One, some of the YLS are able to adapt to the temperature above 32°C after being pressed for generations; the other, there could be two symbiote groups in this intracellular microflora consisting of the high temperature-sensitive and resistant strain. Thus, only the temperature-resistant strain remains viable after selecting for three generations in this study. The latter hypothesis is supported by our results in which the symbiote number in the eggs laid by the heat-treated females that harbor a low number of YLS was not reduced markedly;

Table 7. Cumulative Mortality of *N. lugens* Treated with Different Temperatures and Diets

Treatment	Cumulative mortality (%)			Adult emergence (%)
	Instar			
	3rd	4th	5th	
26°C, on MED-4	31.21	36.71	52.83	42.33
32°C, 3 days, then 26°C, on MED-4	11.15	20.41	49.12	49.25
32°C, 3 days, then 26°C, on MED-4 + Cholesterol	29.64	41.87	72.33	26.31
26°C, on Rice seedling	0.00	3.30	19.97	80.00

therefore, the number of YLS transmitted through ovaries to the next generation was not reduced either. After the heat selection, the temperature-resistant strain increased from generation to generation, resulting in restoration of the YLS population. It is thus predictable that the heat treatment could lose its ability to suppress YLS population after continuous treatment for many generations.

Our results clearly showed that both YLS population and hatchability of the eggs subjected to heat treatment were much lower than the normal. It was observed that abdominal segmentation of the brown planthopper embryo is completed at day 6 of egg age; meanwhile, its symbiote ball is enveloped into the posterior end of abdomen. Although heat treatment prior to day 6 might decrease YLS number in eggs, the nymphs hatched from these eggs remain normal. Schwemmler (1974) treated newly laid eggs of the leafhopper, *E. plejebus*, with X-ray to kill symbiotes in posterior end of the egg, resulting in abdomenless embryos. He considered the requirement of symbiotes for normal abdominal development in this leafhopper. Our results also showed a low hatchability in *N. lugens* after being subjected to heat treatment at the late embryonic stage, revealing a special significance of symbiote during this period of embryonic development. Noda *et al.* (1979) analysed cholesterol and 24-methylene cholesterol in *L. striatellus* and found much lower contents in the heat-treated planthopper. Since the heat treatment can destroy symbiotes of this planthopper (Noda and Saito,1979a), Noda *et al.* (1979) proposed supply of sterols by symbiotes to their host. The present study showed failure in promoting adult emergence of the heat-treated nymphs when adding cholesterol to feeding diets. Although the diet might not be optimal for the planthopper growth and development, it is still possible that the symbiotes are unable to supply their host with sterols. Hou and Brooks (1977) indicated essentiality of sterols for normal development of the aster leafhopper. *Macrosteles fascifrons*, which also harbors intracellular symbiotes. Campbell and Nes (1983) studied in detail the synthetic pathway of sterols in the aphid, *Schizaphis graminum*, using radioisotope labelling and various chromatographic analyses. They could not detect radioactively labelled desmethyl sterols and other metabolic intermediates when aphids were reared on artificial diets containing (2-C^{14}) -mevalonic acid, concluding that *S. graminum* and/or its symbiotes are unable to synthesize cholesterol. Therefore, there is discrepancy regarding sterol source of insects supplied by symbiotes. We cannot at present draw any definite conclusion as to whether YLS could supply *N. lugens* with sterols. The physiological roles of symbiotes in this planthopper need further investigations, and analyses of cholesterol contents in the heat-treated insects are under way in this laboratory.

Acknowledgements

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