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The Physical Methods for Induction of Anti-Bacterial Substances in the Silkworm Larva, *Bombyx mori*

Zhongzheng Gui*, Jianyi Dai [1] and Dahuan Zhuang

Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212018, Jiangsu, China.
[1]Sericulture Company of Tongxiang City, Tongxiang-314500, Zhejiang, China.

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To understand the physical method for induction of anti-bacterial substances from the silkworm larvae, *Bombyx mori*, three physical methods, *i.e.*, infrared radiation, ultraviolet radiation and ultrasonic wave, have been used in this study. The results have shown that ultrasonic wave can induce anti-bacterial substances effectively than radiations in the *B. mori* larva. The induction of anti-bacterial substances was different from silkworm race to race. Summer-autumn silkworm race (Qiufeng × Baiyu) was easy to induce anti-bacterial substances. It is suggested that the ultrasonic wave is a simple and easy method for induction.

Key words: Anti-bacterial substances, *Bombyx mori* L., Induction, Physical methods

Introduction

Insects have highly effective cell-free immune systems that are induced by either infection of some pathogenic substances or stimulation of non-pathogenic physical or chemical factors like ultrasonic wave, rays, physiological saline etc. As a response, insects produce a series of anti-bacterial substances that can be isolated from haemolymph of the insects (Hoffmann *et al.*, 1981).

Silkworms like other insects have efficient self-defence mechanisms, such as phagocytosis, encapsulation and humoral responses against microbial infection. The humoral response mainly involves production of a variety of anti-bacterial substances such as cecropins, attacins, defensins etc. Several anti-bacterial substances such as anti-bacterial protein, anti-bacterial peptide, lectin, lysozyme etc. have been isolated and purified from a diverse insect (Dai and Zhang, 1999).

It was reported that several methods can induce anti-bacterial substances from silkworms, *i.e.*, *Escherichia coli*, insecticide and γ-radiation for *Philosamia cynthia ricini* (Qu, 1984; Dai *et al.*, 1989, 1991), *E. coli* for the pupa of *Bombyx mori* (Dai *et al.*, 1988), *Hyalophora cecropia* (Hultmark *et al.*, 1982) and *Antheraea pernyi* (Qu, 1982), ultrasonic wave (Qi *et al.*, 1983) and physiological saline (Zhang *et al.*, 1985) for *A. pernyi* etc. In this paper, we studied some physical methods to induce the anti-bacterial substances from the *B. mori* larva.

Materials and Methods

Silkworm

Newly molted larvae of the 5th instar, *Bombyx mori*, were used in this experiment. Spring race (Suzhen × Chunguang), spring-autumn race (871 × 872) and summer-autumn race (Qiufeng × Baiyu) were selected and tested for this study. Twenty larvae (10 females and 10 males) were treated for each treatment. Seventy-two hrs after treatment, the haemolymph was collected from silkworm larvae to find out the induction of anti-bacterial substances.

Physical methods for induction of antibacterial substances

In this experiment, three physical methods were used, *i.e.*, infrared radiation (50°C for 10 min), ultraviolet radiation (30 w for 10 min, 15 min and 20 min) and ultrasonic wave (250 w and 150 w for 5 min and 10 min, respectively). Meanwhile, one batch of normal larvae was used as control.

*To whom correspondence should be addressed.
Sericultural Research Institute, Chinese Academy of Agricultural Sciences, Zhenjiang 212-018, Jiangsu, P. R. China. Tel: +86-511-5616716; Fax: +86-511-5622507; E-mail: srizzgui@hotmail.com
Present address: College of Natural Resources and Life Science, Dong-A University, Pusan 604-714, Korea. Tel: +82-51-200-5672; Fax: +82-51-200-7594; E-mail: srizzgui@hotmail.com

Treatment of the immunized haemolymph

The immunized haemolymph was heated in the boiling water for 30 min and then centrifuged at 10,000 rpm/min for 30 min. The supernatant was stored at –20°C for further use.

Anti-bacterial substances assay

The anti-bacterial substances were assayed according to the method of Hultmark *et al.* (1982). Ten microliter of *E. coli* ($K_{12}D_{31}$, $10^7/mm^3$) was added to 8 ml of LB (Luria-Bertani) culture medium (about 50°C) and plated. After the medium containing *E. coli* cooled down to the room temperature, several holes (d = 3 mm) were made by the disinfected steelless hole-maker. Fivel of treated haemolymph sample were added to each hole and cultured at 37°C for 6 – 8 hrs.

Results and Discussion

Comparison of the induction effect by different physical methods

Three physical methods, infrared radiation, ultraviolet radiation and ultrasonic wave, were used to induce the anti-bacterial substances from the 5th instar larva *B. mori*. The result (Table 1) showed that infrared radiation and ultraviolet radiation did not induce anti-bacterial substances from the larva. On the other hand, ultrasonic wave effectively induced anti-bacterial substances in all treatments from the larvae. It indicates that the larvae were more sensitive to ultrasonic wave than radiations. Qu *et al.* (1982) demonstrated that there is much difference on the anti-bacterial substances in response to different inducers within same insect. Thus, our finding is valuable in that one more immune inducer can be added to the present insect immune inducers.

Induction of anti-bacterial substances in different silkworm races

Fig. 1. Inhibition zone assay. (A) Inhibition zone formed by the haemolymph collected after ultrasonic wave. (B) No inhibition zone formed by the hemolymph collected for control.

To understand the induction of anti-bacterial substances in different silkworm races, we studied the induction effect by ultrasonic wave in spring silkworm race (Suzhen × Chunguang), spring-autumn race (871 × 872) and summer-autumn race (Qiufeng × Baiyu). It was shown that the summer-autumn race was easy to induce anti-bacterial substances than spring race, and spring-autumn race was unvaluable induce (Table 2). This result hints that the induction of anti-bacterial substances is different from silkworm races. Figure 1 shows the inhibition zone formed by the hemolymph collected from the ultrasonic wave treated silkworm (Fig. 1A). The result indicated the silkworm treated with ultrasonic wave truly produce anti-bacterial substances, and resultantly inhibit the growth of *E. coli*. Thus, further research is expected to find out characteristics of the substances induced.

In conclusion, ultrasonic wave can induce anti-bacterial substances effectively from the *B. mori* larva, and the activity was different from the race to race. It is suggested that ultrasonic wave is a simple and easy method for induction. It believed that a novel anti-bacterial substance is a potential substance to develop a kind of new medicine with high effective. Thus, we expect further study can identify the exact molecules induced by ultrasonic wave.

Table 1. Effect of different physical methods on the induction of anti-bacterial substances from the *B. mori* larva

Physical methods	control	infrared radiation	ultraviolet radiation	ultrasonic wave
Anti-bacterial substances	N	N	N	E

*N: none effective, E: effective.

Table 2. Induction effect in different silkworm races by ultrasonic wave

Silkworm race	Suzhen × Chunguang		871 × 872		Qiufeng × Baiyu	
Stimulative power	250 w	150 w	250 w	150 w	250 w	150 w
Anti-bacterial substances	N	E	N	N	E	E

*N: none effective, E: effective.

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