Effect of chitosan solution on the bacterial septicemia disease of *Bombyx mori* (Lepidoptera: Bombycidae) caused by *Serratia marcescens*

Bin LI,* Ting SU, Xiaoling CHEN, Baoping LIU, Bo ZHU, Yuan FANG, Wen QIU and Guanlin XIE*

State Key Laboratory of Rice Biology, Key Laboratory of Molecular Biology of Crop Pathogens and Insects, Ministry of Agriculture, Institute of Biotechnology, Zhejiang University; Hangzhou, 310029, China

(Received 26 May 2009; Accepted 11 September 2009)

Abstract

The *in vitro* antibacterial properties of chitosan solution and its effect in protecting silkworms from bacterial septicemia disease were evaluated. The results showed that chitosan solution at concentrations of 0.01, 0.05 and 0.10 mg/ml exhibited strong antibacterial activity against two strains of *Serratia marcescens*. The antibacterial activity of chitosan solution against strain ZJS0801 of *S. marcescens* increased with the increase of chitosan concentration, while the antibacterial activity against strain ZJS0802 of *S. marcescens* was unaffected by chitosan concentration. The antibacterial activity of 0.10 mg/ml chitosan solution against *S. marcescens* increased with the increase of incubation time regardless of the tested strains. The mortality of larvae inoculated with the mixture of *S. marcescens* ZJS0801 and chitosan solution was significantly reduced compared to bacteria alone. In addition, the larvae mortality of silkworms inoculated with *S. marcescens* ZJS0801 was significantly reduced when silkworm larvae were fed on mulberry leaves treated with chitosan solution. Overall, the results indicated that chitosan solution had the potential for control of bacterial septicemia disease of silkworms.

Key words: Antibacterial activity; bacterial septicemia disease; chitosan; *Serratia marcescens*; silkworm

INTRODUCTION

The domesticated silkworm, *Bombyx mori*, is an economically important insect which has been used for silk production for more than 5,000 years (Goldsmith et al., 2005). Although artificial fibers have replaced the use of silk in much of the textile industry, silk has maintained its appeal and value. It has many unique physical advantages and the luster and drape of silk fabrics make silk the most attractive and highest priced natural fiber. Today, China produces about 75% of the world's raw silk, which is valued at \$1.6 billion. India produces about 15% and Korea and Turkmenistan produce just under 5% each. About 30 other countries produce commercial silk. The worldwide demand for silk is increasing and the silk industry has a commercial value of \$200–\$500 million annually (Dingle et al., 2005).

Silkworm crop loss is mainly attributed to the

occurrence of diseases, rather than to unfavorable weather conditions that lead to poor harvests of mulberry leaves (Watanabe et al., 1988); however, it is difficult to exclude pathogens from the silkworm-rearing environment (Nataraju et al., 1999). Indeed, silkworms are highly susceptible to viral, bacterial and fungal diseases (Choudhury et al., 2002, 2004; Kaito et al., 2002; Kunimi, 2007). The etiology of viral and fungal diseases has been investigated thoroughly whereas the etiology of bacterial diseases is not fully understood because of the multiplicity of factors involved in bacterial infection. Bacteria are ubiquitous in nature and a variety is constantly present on mulberry leaves, rearing tools, dust in the rearing room and on the hands of laborers who handle silkworms (Watanabe et al., 1998).

Several types of bacterial disease are found among silkworms (Weiser and Lysenko, 1956; Choudhury et al., 2004), among which bacterial

* To whom correspondence should be addressed at: E-mail: libin0571@zju.edu.cn; glxie@zju.edu.cn
DOI: 10.1303/aez.2010.145

septicemia is one of the most severe diseases of the silkworm, which is caused by *Serratia marcescens*. It is now well established that this organism is widely distributed in sericulture environments and causes severe damage to the silkworm industry (Krishnaswami et al., 1973). Indeed, *S. marcescens* is a Gram-negative bacterium that causes disease in plants and in a wide range of both invertebrate and vertebrate hosts (Grimont and Grimont, 1978; Fletcher, 2007). When the integument is weak in the molt, this bacterium can invade from the metamere to the fluid, and proliferate rapidly. In less than 48 h, the silkworm dies as a result of septicemia. In addition, coating the parasitoid ovipositor with *S. marcescens* has been suggested as a suitable technique to indicate parasitoid oviposition in host range studies (McNeill, 2000; McNeill et al., 2000; Jackson et al., 2004).

The occurrence of disease might vary due to weather conditions, rearing season, silkworm races, feeding and rearing techniques, and geographical areas; therefore, good hygiene and disinfection can keep growing areas clean but do not prevent the entry of infectious agents. Breeding for resistance is one way of decreasing the effects of infection. An additional hazard for any insect culture is the widespread use of a biological agent, the bacterium *Bacillus thuringiensis*, to control plant pests. This agent will also kill susceptible *B. mori* (Dingle et al., 2005). Prevention of silkworm diseases is one of the most important aspects in the success of commercial sericulture; however, no remedial measures have so far been developed to check the infection and further spread of bacterial septicemia disease in silkworms.

Chitosan is a natural nontoxic biopolymer derived by deacetylation of chitin, a major component of the shells of crustacea, such as crab, shrimp, and crawfish. In recent years, applications of chitosan in the fields of medicine, food, chemical engineering, pharmaceuticals, nutrition, environmental protection and agriculture have received considerable attention (Chung and Chen, 2008; Li et al., 2008b). In addition, chitosan has several advantages over other types of bactericide because it possesses higher antibacterial activity, a broader spectrum of activity, a higher killing rate, and lower toxicity toward mammalian cells (Hu et al., 2007). Interestingly, the growth-promoting effect has recently been observed in silkworms raised

with chitosan feed rather than additive free (Hashida et al., 2006). In addition, the bactericidal activity of chitosan against human colonic bacteria has been reported (Simunek et al., 2006); however, few investigations have been performed on chitosan activity for bacterial diseases in invertebrate animals. In the present study, the possible application of chitosan solution for the control of bacterial septicemia in silkworms was investigated.

MATERIALS AND METHODS

Preparation of chitosan stock. Chitosan (degree of *N*-deacetylation no less than 85%, practical grade, from crab shells) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Stock solution of chitosan (5 mg/ml) was prepared in 1% acetic acid with pH adjusted to 6.0 with NaOH (Liu et al., 2006). After stirring (160 rpm) for 24 h at room temperature, the stock solution was autoclaved at 121°C for 20 min. Sterile deionized water of pH 6.0 was used as a control.

Rearing of silkworms. Hybrid strain larvae of silkworm (commercial name: Qingsong Haoyue) were reared at 25°C in this study. Fresh mulberry leaves (average size: 10 cm×20 cm) were obtained from a local mulberry farm (Hangzhou, China). Silkworm larvae were fed sufficient fresh mulberry leaves until the fifth instar and then used for *in vivo* experiments.

Cultivation of bacteria. Two virulent strains of *S. marcescens* (ZJS0801 and ZJS0802) were kindly provided by the College of Animal Science, Zhejiang University, and were isolated from diseased silkworms in Zhejiang Province and Jiangsu Province, respectively. All bacterial strains involved in this study were deposited in the culture collection of the Institute of Biotechnology, Zhejiang University, China. The bacterial strains were cultured for 48 h on nutrient agar medium (Li et al., 2008a) at 28°C. After incubation, each bacterial suspension was prepared in sterilized water, and the initial concentration of bacteria was adjusted to approximately 10⁸ colony-forming units (CFU)/ml.

Counting surviving cells. Bacterial suspensions were ten-fold serially diluted and 10 µl samples were inoculated on nutrient agar medium in hexaplicate for each dilution and were incubated for 48 h at 28°C. After incubation, surviving cells on the agar were counted based on the colony-forming

units and then the mean value of cells at the lowest dilution was calculated. Each experiment was carried out in duplicate and replicated twice.

In vitro* antibacterial activity of chitosan against *S. marcescens

Effect of chitosan concentration. Chitosan solutions of 5 ml in volume were prepared by adding chitosan stock to sterile deionized water to give final chitosan concentrations of 0.01, 0.05 and 0.10 mg/ml. Bacterial solution was added to 5 ml chitosan solution to give a final bacterial concentration of 10^7 CFU/ml and then the mixture was incubated at 28°C on a rotary shaker (Hualida Company, Taicang, China) at 160 rpm. In the control treatment, chitosan stock was replaced with sterile deionized water of pH 6.0 in order to obtain the same pH. Two hours later, samples were collected from each cell suspension and bacteria were counted as indicated above.

Effect of incubation time. Chitosan solutions of 5 ml in volume were prepared by adding 100 µl chitosan stock to 4.90 ml sterile deionized water to give a final chitosan concentration of 0.10 mg/ml. Bacterial strains were selected and inoculated into chitosan solution as indicated above. In the control treatment, chitosan stock was replaced with sterile deionized water of pH 6.0 in order to obtain the same pH. Antibacterial activity of chitosan solution on the growth of *S. marcescens* was determined after 0.5, 1.0, 2.0 and 4.0 h of incubation, respectively.

Control of bacterial septicemia disease of silkworm by chitosan solution. In order to investigate the potential of chitosan solution to control bacterial septicemia disease of silkworms, two experiments were conducted by inoculating healthy fifth instar larvae with strain ZJS0801 of *S. marcescens*.

Effect of mulberry leaves treated with chitosan solution. In the first experiment, healthy larvae of uniform size and age were randomly selected from the stock colony and placed in trays with mulberry leaves pretreated with chitosan solution or with sterile deionized water as a control treatment. Before bacterial inoculation, silkworm larvae were fed continuously on mulberry leaves immersed in chitosan solution of 0.10 mg/ml for 30 s. The experiment was carried out in a randomized block design with three replicates of each treatment, each of twenty larvae. After application of chitosan, healthy larvae were inoculated by pricking them in

the third abdominal segment with sterile needles that had been dipped into 1.0 ml bacterial suspension (10^7 CFU/ml). Control larvae were inoculated with sterile water. Untreated healthy larvae were used as additional controls. After bacterial inoculation, larvae were examined daily for symptoms of disease or death. In addition, mortality was recorded after 24, 48, and 72 h of rearing, respectively.

Effect of the mixture of *S. marcescens* and chitosan solution. To determine the effect of chitosan solution on bacterial virulence, a second experiment was carried out where bacteria were exposed to chitosan solution. Bacterial inoculum was prepared by adding *S. marcescens* ZJS0801 to 5 ml chitosan solution at 0.10 mg/ml to give a final bacterial concentration of 10^7 CFU/ml, and then the mixture was incubated at 28°C on a rotary shaker at 160 rpm. In the control treatment, chitosan solution was replaced with sterile deionized water of pH 6.0 in order to obtain the same pH. After 0, 1.5, 2.0 and 4.0 h of co-culture, healthy larvae were inoculating by pricking them with sterile needles that had been dipped into 1.0 ml bacterial inoculum. The experiment was carried out in a randomized block design with three replicates of each treatment, each of twenty larvae. Control larvae were inoculated with sterile water. Untreated healthy larvae were used as additional controls. After bacterial inoculation, larvae were examined and mortality was recorded as indicated above.

Statistical analysis. STATGRAPHICS Plus, version 4.0 (Copyright: Manugistics Inc., Rockville, Md., USA) was used to perform statistical analysis. The levels of significance ($p < 0.05$) of the main treatments and their interactions were calculated by analysis of variance after testing for normality and variance homogeneity.

RESULTS

Effect of chitosan concentration on antibacterial activity

The effect of concentration on the antibacterial activity of chitosan solution against two strains of *S. marcescens* is shown in Fig. 1. Chitosan solutions up to 0.10 mg/ml showed stronger antibacterial activity against strain ZJS0801 of *S. marcescens* compared with other treatments. The survival rate of strain ZJS0801 in chitosan solution

Fig. 1. Effect of chitosan concentration on the antibacterial activity of strain ZJS0801 and ZJS0802 of *S. marcescens*. Initial concentration of bacteria was approximately 10^7 CFU/ml. Error bars represent the standard error of the mean ($n=6$). Data are from a representative experiment repeated twice with similar results.

of 0.01 mg/ml was 4.6%, while the survival rate in chitosan solution of 0.10 mg/ml decreased to 0.8% compared to the starting value (Fig. 1). However, chitosan solution at 0.01 mg/ml showed effective antibacterial activity against strain ZJS0802 of *S. marcescens* compared to the starting value after 2.0 h incubation. The survival rate of strain ZJS0802 in chitosan solution of 0.01 mg/ml was 1.1% compared to the starting value. Concentrations higher than 0.01 mg/ml were not significantly different (Fig. 1).

Effect of incubation time on antibacterial activity

The effect of the incubation time on the antibacterial activity of chitosan against strain ZJS0801 of *S. marcescens* is shown in Fig. 2a. In the absence of chitosan, the survival rate of bacteria in sterile deionized water was 34.8% after 0.5 h of incubation compared to the starting value. With the increase in incubation time, the survival rate of bacteria remained stable. In the presence of chitosan, the surviving cell numbers were significantly decreased compared to the starting value. In addition, the antibacterial activity of 0.10 mg/ml chitosan solution increased with the incubation time within 4.0 h. After 0.5 h incubation, the survival rate of bacteria in chitosan solution was 1.9% compared to the starting value; however, the survival rate of bacteria in chitosan solution decreased to 0.2%

Fig. 2. Effect of incubation time on the antibacterial activity of chitosan solution at 0.10 mg/ml against two strains of *S. marcescens*. (a) ZJS0801, (b) ZJS0802. Initial concentration of bacteria is approximately 10^7 CFU/ml. Error bars represent the standard error of the mean ($n=6$). Data are from a representative experiment repeated twice with similar results.

compared to the starting value after 4.0 h incubation.

The effect of the incubation time on the antibacterial activity of chitosan against strain ZJS0802 of *S. marcescens* is shown in Fig. 2b. In the absence of chitosan, the survival rate of bacteria in sterile deionized water was 30.1% after 0.5 h incubation compared to the starting value. With the increase in incubation time, the survival rate of bacteria remained stable. In the presence of chitosan, the surviving cell numbers were significantly decreased compared to the starting value. In addition, the antibacterial activity of 0.10 mg/ml chitosan solution against strain ZJS0802 increased with the incubation time within 4.0 h, which is consistent with the antibacterial effect of chitosan solution against strain ZJS0801. The survival rate of bacteria in chitosan solution was 2.8% after 0.5 h incubation, which decreased to 0.2% after 4.0 h incubation

compared to the starting value.

Control of bacterial septicemia disease of silkworm by chitosan solution

The effect of 0.10 mg/ml chitosan solution on the prevention of bacterial septicemia disease is shown in Table 1. In the absence of chitosan, silkworm larvae inoculated with strain ZJS0801 showed 83.3, 91.7 and 95.0% mortality after 24, 48 and 72 h of rearing, respectively. However, the mortality of larvae treated with sterile water and the uninoculated control was 0% regardless of the time of rearing (Table 1). When silkworm larvae were fed on mulberry leaves treated with chitosan solution, the mortality of larvae inoculated with strain ZJS0801 was significantly reduced by 24.0,

25.5 and 28.1% after 24, 48 and 72 h of rearing, respectively. In addition, application of chitosan solution did not result in a significant change in the mortality of larvae treated with sterile water and the uninoculated control.

The effect of co-culture time on the inhibition of bacterial virulence by 0.10 mg/ml chitosan solution is shown in Table 2. In the absence of chitosan, silkworm larvae inoculated with strain ZJS0801 showed 93.3% mortality after 72 h rearing, while the mortality of larvae treated with sterile water and the uninoculated control was 0%. The mixture of bacteria and chitosan solution resulted in a significant decrease of bacterial virulence compared to bacteria alone. When bacteria were co-cultured with chitosan solution for 0.5 h, the mortality of

Table 1. Effect of mulberry leaves treated with chitosan solution on larvae mortality of silkworms inoculated with *S. marcescens* ZJS0801

Treatment	Mortality (%) ^a		
	24 h	48 h	72 h
Inoculated with ZJS0801 ^b	83.3±4.4 c	91.7±1.7 c	95.0±2.9 c
Inoculated with ZJS0801+Leaves with chitosan ^c	63.3±3.3 b	68.3±4.4 b	68.3±4.4 b
Inoculated with sterile water	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Inoculated with sterile water+Leaves with chitosan	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Uninoculated	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Uninoculated+Leaves with chitosan	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a

^aData are shown as the means±standard error from a representative experiment repeated twice with similar results. All means within a column followed by the same letter are not significantly different ($p<0.05$, Fisher's LSD test). Each value represents the average of three replicates.

^bThe inoculum concentration of bacteria was 10^7 CFU/ml.

^cConcentration of chitosan solution was 0.10 mg/ml.

Table 2. Effect of co-culture time on the virulence of *S. marcescens* ZJS0801 when co-cultured bacteria with chitosan solution

Treatment	Mortality (%) ^a		
	24 h	48 h	72 h
Inoculated with ZJS0801 ^b	73.3±4.4 c	86.7±3.3 c	93.3±1.7 d
Inoculated with ZJS0801 and chitosan ^c (0.5 h co-culture)	6.7±1.7 b	16.7±3.3 b	26.7±3.3 c
Inoculated with ZJS0801 and chitosan (1.0 h co-culture)	0.0±0.0 a	10.0±2.9 b	13.3±3.3 b
Inoculated with ZJS0801 and chitosan (2.0 h co-culture)	0.0±0.0 a	16.7±3.3 b	20.0±2.9 bc
Inoculated with ZJS0801 and chitosan (4.0 h co-culture)	0.0±0.0 a	10.0±5.8 b	13.3±3.3 b
Inoculated with sterile water	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a
Uninoculated	0.0±0.0 a	0.0±0.0 a	0.0±0.0 a

^aData are shown as the means±standard error from a representative experiment repeated twice with similar results. All means within a column followed by the same letter are not significantly different ($p<0.05$, Fisher's LSD test). Each value represents the average of three replicates.

^bThe inoculum concentration of bacteria was 10^7 CFU/ml.

^cConcentration of chitosan solution was 0.10 mg/ml.

larvae inoculated with the mixture was significantly reduced by 90.9, 80.7 and 71.4% compared to bacteria alone after 24, 48 and 72 h of rearing, respectively. When bacteria were co-cultured with chitosan solution for 1.0, 2.0 or 4.0 h, the mortality of larvae inoculated with the mixture was 0% after 24 h of rearing. There was no significant difference in the mortality of larvae between bacteria co-cultured with chitosan solution for 0.5 h and 1.0, 2.0 or 4.0 h after 48 h of rearing. In addition, the mortality of larvae was significantly reduced by 85.7% when bacteria were co-cultured with chitosan solution for 1 or 4 h after 72 h of rearing (Table 2).

DISCUSSION

In this study, chitosan solution at different concentrations showed effective antibacterial activity against two strains of *S. marcescens* from silkworms compared to the control. To the best of our knowledge, this is the first report on the antibacterial effects of chitosan on *S. marcescens*. In addition, the mortality of larvae inoculated with *S. marcescens* was significantly reduced when silkworm larvae were fed on mulberry leaves treated with chitosan solution. Overall, the results indicated that chitosan solution had the potential to protect silkworm larvae from bacterial septicemia disease.

The results of this study indicated that the sensitivity of strain ZJS0801 to chitosan was dose dependent, which is consistent with the result of Liu et al. (2006), who found that the antibacterial activity of chitosan was influenced by its concentration in the solution. However, the lowest concentration of chitosan (0.01 mg/ml) was enough to affect the survival of strain ZJS0802, indicating that strain ZJS0802 was more sensitive to chitosan than strain ZJS0801. The observed difference in chitosan sensitivity for bacteria may be attributed to the physiological state of the micro-organisms and strain diversity. In addition, this result showed that the antibacterial activity of chitosan solution increased with the increase of incubation time regardless of the bacterial strain, showing that a certain incubation time is required for the antibacterial activity of chitosan solution.

Outbreaks of bacterial septicemia disease caused by *S. marcescens* are common in insectary-reared silkworms, which can be attributed to the contami-

nation of mulberry leaves and rearing appliances used for silkworm rearing, improper disposal of bed refuse, inadequate disinfection, continuous rearing of silkworms, simultaneous rearing of different stages, overcrowding, unhygienic rearing conditions, etc. (Bulla Jr et al., 1975). In addition, Sikorowski and Lawrence (1998) demonstrated that healthy moths of *Heliothis virescens* may become infected from *S. marcescens* surface-contaminated pupae; infected moths may contaminate eggs externally or infect them internally.

From this study, it is evident that chitosan solution has strong antibacterial activity against *S. marcescens*, which is also an opportunistic human pathogen, and in the last three decades there has been a steady increase in nosocomial *S. marcescens* infections that can be life-threatening (Hejazi and Falkiner, 1997). As many *S. marcescens* strains are also resistant to multiple antibiotics (Hejazi and Falkiner, 1997), it represents a growing problem for public health. Chloroform extract of *Ferula persica* var. *persica* roots was found to inhibit the red pigment production of *S. marcescens*. However, neither the chloroform extract nor the isolated umbelliprenin fraction showed any antibacterial activity against the test strain at a certain concentration (Iranshahi et al., 2004). Considering the absence of any sort of remedial measures for *S. marcescens* infections, the present investigation may prove helpful in this field.

In our pre-study experiments, which were carried out to select a suitable method for silkworm inoculation, we also found that *S. marcescens* is highly pathogenic when inoculated into the hemocoel of silkworm larvae, while it is only mildly pathogenic when ingested (data not shown). This is consistent with the result of Bucher (1959), who found that the mean lethal dose of *S. marcescens* inoculated into the hemocoel of grasshoppers ranged from 10 to 50 bacterial cells, and the LD₅₀ of *S. marcescens* ingested by grasshoppers was found to be about 3×10^5 – 5×10^5 bacterial cells. Recently, the silkworm genome project was completed by Japanese and Chinese groups, which will facilitate the study of host factors involved in infectious processes (Mita et al., 2004; Xia et al., 2004; Kaito and Sekimizu, 2007).

The results of this study indicated that silkworm larvae fed mulberry leaves treated with chitosan

solution showed significantly reduced mortality when inoculated with strain ZJS0801 of *S. marcescens*. In addition, silkworm larvae inoculated with a mixture of *S. marcescens* and chitosan solution resulted in a significant decrease in mortality compared to bacteria alone. In agreement with our *in vivo* experiment results, we also found that the surviving cell numbers in chitosan solution were decreased significantly after different incubation times compared to the control. Therefore, decreased mortality can be attributed, at least in part, to the antibacterial activity of chitosan solution.

Most studies on the mode of action of chitosan have been conducted with fungal pathogens, and little is known about its action on bacteria (Li et al., 2008b). Several studies have indicated that the interactions between positively charged chitosan molecules and negatively charged residues on the bacterial cell surface play an important role in the inhibitory effect of chitosan on Gram-negative bacteria (Helander et al., 2001). In addition, it has been suggested that the reduction of cell numbers is caused by cell-surface alterations and loss of barrier functions (Helander et al., 2001). Chitosan with a positive charge easily reacts with negatively charged bacteria and further inhibits bacterial growth. Surface interference is a possible mechanism of the bactericidal properties (Helander et al., 2001). Further, Chung and Chen (2008) found that the inactivation of *Escherichia coli* by chitosan occurs via a two-step sequential mechanism: initial separation of the cell wall from its cell membrane, followed by destruction of the cell membrane.

Hejazi and Falkiner (1997) demonstrated that *S. marcescens* mannose-dependent fimbriae can adhere to biological surfaces and form biofilms, which are aggregations of bacteria and extracellular secretions (Queck et al., 2006). Fimbriae are among the virulent cell-surface structures of *S. marcescens* which contain the adhesins mediating adherence to eukaryotic cell surfaces, via recognition of specific carbohydrate moieties on the host cell surface glycoconjugates. Recently, Castro et al. (2007) demonstrated that D-mannose protects *Trypanosoma cruzi* and *Trypanosoma rangeli* from lysis caused by *S. marcescens* variants SM365 and RPH; however, this carbohydrate was unable to interfere with the hemolysis induced by SM365 and RPH variants. Thus, D-mannose bacterial fimbriae are relevant to *S. marcescens* in the lysis of para-

sites. Further studies are required to evaluate the combined effects of chitosan and D-mannose on bacterial septicemia disease of silkworms.

In conclusion, our results clearly demonstrate that chitosan solution has strong antibacterial activity against two strains of *S. marcescens* under various environmental conditions. In addition, it is evident that chitosan solution has potential in the prevention and control of silkworm bacterial disease. Considering the absence of any remedial measures for bacterial septicemia disease of silkworms, the present investigation may prove helpful in a field which is largely dominated by economically restricted silkworm growers.

ACKNOWLEDGMENTS

This project was supported by the Science Foundation of Chinese University (KYJD09022) and Zhejiang Provincial Natural Science Foundation of China (Y3090150).

REFERENCES

- Bucher, G. E. (1959) Bacteria of grasshoppers of western Canada. III. Frequency of occurrence, pathogenicity. *J. Insect Pathol.* 1: 391–405.
- Bulla Jr, L. A., R. A. Rhodes and G. S. Julian (1975) Bacteria as insect pathogens. *Annu. Rev. Microbiol.* 29: 163–190.
- Castro, D. P., C. S. Moraes, E. S. Garcia and P. Azambuja (2007) Inhibitory effects of d-mannose on trypanosomatid lysis induced by *Serratia marcescens*. *Exp. Parasitol.* 115: 200–204.
- Choudhury, A., A. Guha, A. Yadav, B. G. Unni and M. K. Roy (2002) Causal organism of flacherie in the silkworm *Antheraea assama* Ww: isolation, characterization and its inhibition by garlic extract. *Phytother. Res.* 16: 89–90.
- Choudhury, A., A. Guha, A. Yadav, J. Kumari, B. G. Unni and M. K. Roy (2004) Induced immunity in *Antheraea assama* Ww larvae against flacherie causing *Pseudomonas aeruginosa* AC-3. *Exp. Parasitol.* 106: 75–84.
- Chung, Y. C. and C. Y. Chen (2008) Antibacterial characteristics and activity of acid-soluble chitosan. *Bioresource Technol.* 99: 2806–2814.
- Dingle, J. G., E. Hassan, M. Gupta, D. George, L. Anota and H. Begum (2005) *Silk Production in Australia*. Rural Industries Research and Development Corporation, Kingston. 105 pp.
- Fletcher, J. (2007) Niche adaptation by *Serratia marcescens*: A versatile enterobacterial pathogen of many hosts. *Phytopathology* 97: 139.
- Goldsmith, M. R., T. Shimada and H. Abe (2005) The genetics and genomics of the silkworm, *Bombyx mori*. *Annu. Rev. Entomol.* 50: 71–100.
- Grimont, P. A. D. and F. Grimont (1978) Genus *Serratia*. *Annu. Rev. Microbiol.* 32: 221–248.
- Hashida, S., S. Kikukawa, Y. Yuyama, K. Yoshida, H. Yoshimine, H. Shirato and K. Hiasa (2006) Method of

- raising insects until it cocoonizes from hatching with feed for silkworms comprising various concentration of chitosan. Patent number JP2006271243-A.
- Hejazi, A. and F. R. Falkner (1997) *Serratia marcescens*. *J. Med. Microbiol.* 46: 903–912.
- Helander, I. M., E. L. Nurmiäho-Lassila, R. Ahvenainen, J. Rhoades and S. Roller (2001) Chitosan disrupts the barrier properties of the outer membrane of gram-negative bacteria. *Int. J. Food Microbiol.* 71: 235–244.
- Hu, Y., Y. M. Du, J. H. Yang, Y. F. Tang, J. Li and X. Y. Wang (2007) Self-aggregation and antibacterial activity of *N*-acylated chitosan. *Polymer* 48: 3098–3106.
- Iranshahi, M., A. R. Shahverdi, R. Mirjani, G. Amin and A. Shafiee (2004) Umbelliprenin from *Ferula persica* roots inhibits the red pigment production in *Serratia marcescens*. *Z. Naturforsch. C* 59: 506–508.
- Jackson, T. A., M. R. McNeill and D. Madurappulige (2004) Use of *Serratia* spp. bacteria for monitoring behaviour of parasitoids. *Int. J. Pest Manage.* 50: 173–176.
- Kaito, C. and K. Sekimizu (2007) A silkworm model of pathogenic bacterial infection. *Drug Discov. Ther.* 1: 89–93.
- Kaito, C., N. Akimitsu, H. Watanabe and K. Sekimizu (2002) Silkworm larvae as an animal model of bacterial infection pathogenic to humans. *Microb. Pathog.* 32: 183–190.
- Krishnaswami, S., M. N. Narsimhanna, S. K. Suryanarayana and S. Kumarraj (1973) *Sericulture Manual 2—Silkworm Rearing*. Food and Agriculture Organisation of United Nations, Rome. 131 pp.
- Kunimi, Y. (2007) Current status and prospects on microbial control in Japan. *J. Invertebr. Pathol.* 95: 181–186.
- Li, B., L. H. Xu, M. M. Lou, F. Li, Y. D. Zhang and G. L. Xie (2008a) Isolation and characterization of antagonistic bacteria against bacterial leaf spot of *Euphorbia pulcherrima*. *Lett. Appl. Microbiol.* 46: 450–455.
- Li, B., X. Wang, R. X. Chen, W. G. Huangfu and G. L. Xie (2008b) Antibacterial activity of chitosan solution against *Xanthomonas* pathogenic bacteria isolated from *Euphorbia pulcherrima*. *Carbohydr. Polym.* 72: 287–292.
- Liu, N., X. G. Chen, H. J. Park, C. G. Liu, C. S. Liu, X. H. Meng and L. J. Yu (2006) Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli*. *Carbohydr. Polym.* 64: 60–65.
- McNeill, M. R. (2000) Effect of container type on suitability of the pathogen *Serratia marcescens*—*Microctonus hyperodae* (Hym., Braconidae) association to indicate parasitoid oviposition attempts. *J. Appl. Entomol.* 124: 93–98.
- McNeill, M. R., B. I. P. Barratt and A. A. Evans (2000) Behavioural acceptability of *Sitona lepidus* (Coleoptera: Curculionidae) to the parasitoid *Microctonus aethiopoides* (Hymenoptera: Braconidae) using the pathogenic bacterium *Serratia marcescens* Bizio. *Biocontrol Sci. Techn.* 10: 205–213.
- Mita, K. et al. (2004) The genome sequence of silkworm, *Bombyx mori*. *DNA Res.* 11: 27–35.
- Nataraju, B., V. Sivaprasad and R. K. Datta (1999) Studies on the cause of ‘thatteroga’ in silkworm, *Bombyx mori* L. *Indian J. Seric.* 38: 149–151.
- Queck, S. Y., M. Weitere, A. M. Moreno, S. A. Rice and S. Kjelleberg (2006) The role of quorum sensing mediated developmental traits in the resistance of *Serratia marcescens* biofilms against protozoan grazing. *Environ. Microbiol.* 8: 1017–1025.
- Sikorowski, P. P. and A. M. Lawrence (1998) Transmission of *Serratia marcescens* (Enterobacteriaceae) in adult *Heliothis virescens* (Lepidoptera: Noctuidae) laboratory colonies. *Biol. Control* 12: 50–55.
- Simunek, J., G. Tishchenko, B. Hodrova and H. Baptonova (2006) Effect of chitosan on the growth of human colonic bacteria. *Folia Microbiol.* 51: 306–308.
- Watanabe, H., Y. Kurihara, Y. X. Wang and T. Shimizu (1988) Mulberry pyralid, *Glyphodes pyloalis*: Habitual host of nonoccluded viruses pathogenic to the silkworm, *Bombyx mori*. *J. Invertebr. Pathol.* 52: 401–408.
- Watanabe, K., W. Hara and M. Sato (1998) Evidence for growth of strains of the plant epiphytic bacterium *Erwinia herbicola* and transconjugation among the bacterial strains in guts of the silkworm *Bombyx mori*. *J. Invertebr. Pathol.* 72: 104–111.
- Weiser, J. and O. Lysenko (1956) Silk-worm septicaemia. *Folia Microbiol.* 1: 216–222.
- Xia, Q. Y. et al. (2004) A draft sequence for the genome of the domesticated silkworm (*Bombyx mori*). *Science* 306: 1937–1940.