Effects of Probiotic Bacteria on the Growth Parameters of the Thai Silkworm, *Bombyxmori*

S. Suraporn[1,*], W. Sangsuk[1], P. Chanhan[2] and S. Promma[2]

[1]*Department of Biology, Faculty of Science, Mahasarakham University 44150, Thailand*
[2]*Silk Innovation Center, Mahasarakham University 44150, Thailand*

**Corresponding author, Email: siripuk_s@yahoo.com*

Abstract

The effects of *Lactobacillus acidophilus*, on the growth of two strains of Thai silkworm, *Bombyxmori*, Nang Lai and Nang Lai X 108 were studied. A suspension of *L. acidophilus* of 10^8 cells mL^{-1} was topically applied to mulberry leaves and fed during the 2nd, 3rd, 4th and 5th instars. On the other hand, control groups were fed mulberry leaves dipped in water. The effects of *L. acidophilus* treatment were investigated on quality parameters, such as survival ratio, mature larval weight, pupation ratio, cocoon weight and cocoon shell ratio. Silkworms treated with *L. acidophilus* showed positive results compared to the control group. Improvement of growth parameters was recorded in Nang Lai vs control, with a survival ratio of 92.66±1.52% vs 84±1.00, a larval weight of 1.26±0.05 g in the 5th instar (5th day) vs 1.18 ± 0.05, pupation ratio of 91±1.00% vs 82.33±1.52, cocooning ratio of 91.33±1.52% vs 85±1.00, cocoon weight of 1.08±0.09g vs 0.94±0.07, and cocoon shell ratio of 14.95±0.06% vs 12.78±0.15. Likewise, Nang Lai X 108 vs control showed satisfactory quality parameters with a survival ratio of 91±1.73% vs 80.33±0.58, a weight of 1.70±0.09 g in the 5th instar (5th day) vs 1.53±0.05, pupation ratio of 90.33±1.52% vs 83±1.00, cocooning ratio of 90.33±0.57% vs 81±1.00, cocoon weight of 1.18±0.01 g vs 1.15±0.00, and cocoon shell ratio of 16.67±0.51% vs 16.01±0.62. This study indicated that *L. acidophilus* stimulated growth factors leading to an increase in the silk yield and to an improvement of the silk harvest. The study also indicates that mulberry leaves could be used as a nutrient for *L. acidophilus*.

Keywords: Thai silkworm strains, *Bombyxmori*, probiotic, *Lactobacillus acidophilus*

Introduction

Thailand has been known for its traditional sericulture practices for centuries. The production of famous Thai silk has earned the country a considerable amount of foreign exchange (Chewonarin, 1991). In the past, sericulture was practiced only at family level, as a part-time activity, but now it has become a national industry. It is an important alternative, or full-time income generating activity, a chance for farmers in Thailand. The cocoons of these Thai silkworm strains are yellow and tiny but fluffy and rich in sericin. Now-a-days, not only the silk does provides the basic material for making of silk cloth, but also for use in other proposes. For instance, *Cordyceps* sp. which grows on silkworm pupa can protect from HIV and cancer (Suraporn and Siriwattanametanon, 2009). The silkworms of Nang Lai strain reared in Thailand are polyvoltine and are of Thai origin. Nang Lai produces smaller yellow cocoons, while the hybrid strain Nang Lai x108 produces larger cocoons.

Bacteria like, *Lactobacillus* sp and *Bifidobacterium* sp. have been found to be beneficial as probiotics. Probiotic bacteria, or lactic acid bacteria are basically anaerobic and facultative in nature. They live in microbial yoghurt and

dietary supplements benefiting the host by improving the microbial balance in gut (Austin et al., 1995). The *Lactobacillus acidophilus* is the most commonly used probiotic bacterium. It is gram-positive, rod like prokaryotic bacterium inhabiting human and animal gastrointestinal tract. The species ferments lactose only, producing lactic acid, as a final product of fermentation at a temperature of 30°C.It is one of several species of bacteria populating or thriving in the human gastrointestinal tract. The bacterium protects against an intrusion and proliferation of undesired organisms causing the diseases. The beneficial role played by activities of probiotics has been reported by Chan et al. (1985) in human beings, Douillet and Longdon (1994) in aquaculture and by Dillon and Dillon (2004) in insects. The products containing probiotic bacteria are widely explored, thereby increasing the importance of their accurate specializations (Yeung et al., 2002) and the beneficial effects by the activity of gut micro-flora and its influence on mucosal immunity through altering the enzymatic activities, has been extensively studied in human, animals, and many insects (Yeung et al., 2002). Effect of probiotic and neutraceutical agent on growth, development and commercial characteristics of *B. mori,* has recently been reported (Bai and Bai, 2012). Hence, the research has been undertaken to study effects of probiotic bacteria *L. acidophilus* on Thai silkworm strains of *Bombyxmori* that may lead to enhance the growth of silkworm larvae and in turn to increase silk yields.

Materials and Methods

Insects

Eggs of pure polyvoltine Thai silkworm strain Nang Lai and Thai hybrid silkworm strain Nang Lai x108 were obtained from the Sericulture Research Center and Sericulture Experiment Stations respectively located in Khon Kaen Province of Thailand. The eggs in the form of egg sheets were incubated at 25 ˚C and their disinfection was accomplished by dipping them in 3% formalin for 10 min. Then, they were placed into 75% alcohol for 1 min, and finally into 95% for 1 min, respectively. The eggs were then placed into incubator and incubated at 25˚C until hatching. Newly hatched larvae of Nang Lai, and Nang Lai x 108 silkworm strains were then reared by feeding on mulberry leaves in separate groups under the standard rearing conditions at a temperature of 25±3 ˚C with 75 ± 5 % humidity. Young silkworm larvae of both strains fed on mulberry leaves until they molted to the second instar.

Probiotic Bacteria

Theprobiotic bacterium *L. acidophilus* was cultured in Luria Bertani (LB) agar (2% NaCl) and incubated at room temperature overnight. Total of seven colonies were observed. A single colony was picked up and transferred into 10 mLof Luria Bertani (LB) broth, supplemented with 2% NaCl (w/v). The culture was then incubated at 28˚C overnight with agitation. The *L. acidophilus* culture was transferred again into 50 mL of medium DE Man, Rogosa and Sharpe (MRS) broth and incubated under the same conditions overnight. The culture broth was then centrifuged at 5,000 rpm at 4°C for 15 min. The pellet of *L. acidophilus* was collected and washed with distilled water. A suspension of the culture was used to feed the silkworms of 10^8 cells mL^{-1}. The number of cells was measured at an absorbance of 660 nm by comparing to McFarland's standard No.5. (Marques et al., 2006)

Feeding Schedule Bacteria and Assessment of Quality Parameters

After both Nang Lai and Nang Lai x 108 molted to their 2nd instars, 100 larvae were selected each for three replications for a one time feeding of mulberry leaves dipped in *L. acidophilus* suspension (10^8 cells mL^{-1}). This feeding was repeated prior to the 3rd, 4th, and 5thinstars of the larval cycle respectively. The remaining 100 silkworm larvae in each of the three control groups were fed with natural mulberry leaves treated by dipping them in distilled water. The treated groups as well as the control groups consisting of 100 larvae each were subjected for estimation of quality parameters like survival ratio, determined at day 3 pupa, mature larval weight (g) determined at day 6, fifth instar larva, pupation (%) determined at day 3 pupa, cocooning ratio (%) determined at day 3 pupa, cocoon weight (pupa+cocoon shell) determined at day 3 pupa and cocoon shell ratio (%) calculated at day 3 pupa. The data were analyzed by ANOVA

Mulberry Leaves as a Nutrient for *L. acidophilus in vitro*

Probiotic bacteria need access to their food source, a prebiotic or nutrient, to survive in the midgut of the silkworm. To confirm that mulberry leaves were usable as a food source for *L. acidophilus*, the mulberry leaves of 100 g were first ground in 100 mL of distilled water. The mixture was then sterilized by using an autoclave at 121 ˚C, for 20 minutes under 15 pounds-force per square inch (psi). The single colony of *L. acidophilus* was picked up, and suspended in the mulberry leaf juice. The 100 mL of suspension of mulberry juice, containing the bacterium *L. acidophilus,* was incubated at room temperature (37 ˚C) for 48 hours with gentle shaking. The growth of probiotic bacteria, *L. acidophilus* in incubate indicated that mulberry leaves can be used as a prebiotic or a nutrient source for the survival of *L. acidophilus* bacteria.

Results

Characteristics of Thai Silkworm, Nang Lai and the Hybrid Strain Nang Lai x 108

Nang Lai was the original polyvoltine Thai silkworm strain. It had been the traditional strain for sericultural practice in Thailand for a long time. The visible characteristics of the Nang Lai silkworm were a black and white stripe pattern, while Nang Lai x 108, a Thai hybrid strain resulting from a cross between Nang Lai and hybrid strain 108. It had also similar characteristics as Nang Lai. The cocoons and larvae of Nang Lai x 108 hybrid strain were larger in size than Nang Lai. The life cycle of both the strains was approximately 45±5 days. It depended on the temperature, humidity and food. In general, Nang Lai, the original Thai silkworm showed a higher resistance to pathogens than that of the hybrid Nang Lai x 108 (unpublished data). Accordingly, the percentage of survival was found to be higher in Nang Lai than Nang Lai x 108.

Effects of *Lactobacillus acidophilus* as a Supplementary Bacteria for Thai Silkworm

L. acidophilus was used as a supplementary bacteria. It was purified and propagated in a laboratory. The effects of *L. acidophilus* at 10^8 cells mL^{-1} on the quality parameters of Thai silkworm strains, both the original Nang Lai and the hybrid strain, Nang Lai x108, were shown in Table 1. The results revealed that the larvae of the treated groups had a higher larval weight, pupation ratio, cocooning ratio, cocoon shell weight and cocoon shell ratio, respectively, when compared to the control group fed on mulberry leaves alone.

Mulberry leaves treated with *L. acidophilus* were fed to the larvae of Nang Lai and Nang Lai x 108 during 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instars. The results showed that larvae of both silkworm strains fed with *L. acidophilus* treated mulberry leaves showed improved quality parameters under the experimental conditions in comparison to naturally fed control. Nang Lai showed a survival ratio of 92.66±1.52%, a larval weight of 1.26±0.05 g in the 5^{th} instar (5^{th} day), pupation of 91±1.00%, cocooning ratio of 91.33±1.52%, fresh cocoon weight of 1.08±0.09g, and cocoon shell ratio of 14.95±0.06%. Likewise, Nang Lai X 108 showed good quality parameters, when compared to the control group. The results showed a survival ratio of 91±1.73%, a weight of 1.70±0.09 g in the 5^{th} instar (5^{th} day), pupation of 14.95±0.06%, cocoon ratio of 90.33±0.57%, fresh cocoon weight of 1.18±0.01 g, and cocoon shell ratio of 16.67±0.51%, respectively.

Discussion

Improved parameters for survival ratio, larval weight, cocooning ratio, pupation ratio, shell weight and cocoon shell ratio indicated the suitability of the probiotic bacterium, *L. acidophilus* for the silkworm larvae when ingested with mulberry leaves. The amount of food consumed by a larva influences its growth rate, development, final body weight and probably the survival too (Slansky and Scriber, 1985; Singh et al. 2005). The present results showed that growth of *L. acidophilus* in silkworm midgut is beneficial to the host contributing in turn to an incremental rate in quality parameters that increases silk production. Food intake is also regulated by the physical nature of food and the presence of phagostimulants in the food (Dadd, 1970). *B. mori* reared on mulberry leaves supplemented with minerals, proteins, cereal flours, medicinal extracts and plant growth

Table 1 Effect of Lactobacillus acidophilus on the growth parameters of Thai silkworms, Nang Lai and the Thai hybrid, Nang Lai X 108.

Silkworm strains	Treatment	Survival ratio (%)	5th instar larval wt. (g)	Pupation ratio (%)
Nang Lai	Control (mulberry leaves)	84±1.00[b]	1.18±0.05[cd]	82.33±1.52[bc]
	Mulberry leaves+*L. acidophilus* (10^7 cells mL^{-1})	92.66±1.52[a]	1.26±0.05[cd]	91±1.00[a]
Nang Lai x 108	Control (mulberry leaves)	80.33±0.577[c]	1.53±0.05[b]	83±1.00[bc]
	Mulberry leaves+*L. acidophilus* (10^7 cells mL^{-1})	91±1.73[a]	1.70±0.09[a]	90.33±1.52[a]
		Cocoon ratio (%)	Fresh cocoon wt. (g)	Shell ratio (%)
Nang Lai	Control (mulberry leaves)	85±1.00[b]	0.940±0.07[c]	12.78±0.15[d]
	Mulberry leaves+*L. acidophilus* (10^7 cells mL^{-1})	91.33±1.52[a]	1.08±0.09[bd]	14.95±0.06[c]
Nang Lai x 108	Control (mulberry leaves)	81±1.00[c]	1.15±0.00[ad]	16.01±0.62[b]
	Mulberry leaves+*L. acidophilus* (10^7 cells mL^{-1})	90.33±0.57[a]	1.18±0.01[a]	16.67±0.51[a]

hormones (Sunder et al., 2000) were reported to have beneficial effects on quality parameters. Singh et al. (2005) has reported enhanced effects on silk yield and economic parameters using mulberry leaves and the probiotic bacteria *L. plantarum*. In the present study, similar observations may be attributed to an increased efficiency in digestion and assimilation of food materials leading to an increase in protein synthesis. Observations may also be attributed to an accumulation of stored proteins in the body, owing to the activity of probiotic microbial flora in the midgut of silkworm *B. mori* as shown in the earlier report (Bai and Bai, 2012). Our study showed that mulberry leaves are a food source, supplementary for the probiotic *L. acidophilus* bacteria *in vitro*. How probiotic bacteria ingested into the midgut with mulberry leaves affect the silkworm larva and how long the probiotic bacteria live in the midgut are the questions to be studied by further studies. Because the probiotic bacteria are anaerobic in nature, it is likely to live longer in the midgut of the silkworm than expected and make interaction with microflora in the midgut.

Present study indicates beneficial effects of probiotic *L. acidophilus* on quality parameters, and economic traits of polyvoltine Thai silkworm strains. The mechanism of interaction of probiotic bacteria with the host native micro-biota to affect on food assimilation, physiology and innate immunity of the silkworm is the subject of further investigation.

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