

Gut Microbiome of Silkworms (*Bombyx mori*): Analyzing How Probiotic Supplementation Influences Silk Quality and Disease Resistance in Tropical Multivoltine Sericulture

Dr. Sanjay Kumar Singh

*Assistant Professor,
Department of Zoology, Govt. P.G. College Charra Aligarh*

Abstract

*The gut microbiome of silkworms (*Bombyx mori*) functions as an essential system which handles nutrient processing and immune defense and silk production but scientists have not yet studied its full potential as a target to enhance sericulture results. This article investigates the impact of probiotic supplementation on silk quality and disease resistance in tropical multivoltine sericulture systems which face difficulties in maintaining production because of environmental stressors and pathogen outbreaks. Through analysis of recent research combining microbiome sequencing, silk quality assessments, and disease challenge studies, we explore the complex relationships between microbial communities and silkworm performance. Targeted probiotic interventions which use *Lactobacillus* and *Bacillus* strains can enhance silk fiber strength by 15 to 25 percent and improve cocoon weight by 8 to 18 percent and decrease mortality from common pathogens by 30 to 45 percent according to evidence. The mechanisms behind these improvements consist of better nutrient absorption and stronger gut barrier function and pathogen competitive exclusion and immune response modulation which redirects energy resources toward silk production.*

Keywords: *silkworm microbiome, probiotic supplementation, silk quality, disease resistance, multivoltine sericulture, tropical agriculture*

I. Introduction

The peak season in any silk-producing region creates a situation which presents a remarkable contradiction. The process of silkworm breeding through domestication and selective breeding since thousands of years ago has created an unexpected outcome which results in unpredictable silk production. Bacterial outbreaks cause complete destruction of larval batches while environmental changes create severe silk quality issues which prevent farmers from recovering their investment. The established rearing protocols together with genetically identical silkworm strains lead to problems which happen under normal circumstances according to established procedures.

The answer to this paradox may lie in an invisible ecosystem that scientists have only recently begun to understand — the gut microbiome of silkworms themselves. Silkworms possess complex digestive systems which contain various types of bacteria and fungi and other microorganisms. The microbes in our body function as active participants because they help process nutrients and defend against diseases and create silk proteins through their biochemical processes.

Bombyx mori has been domesticated for more than 5000 years which makes it one of the oldest insects in human history that people have kept for farming purposes. The breeders of silkworms selected specific traits for breeding which included visible characteristics such as cocoon size and silk quality and disease resistance and feeding efficiency. The people at that time had no awareness of bacteria because they did not yet understand the significance of these microorganisms. The silkworm gut microbiome remained a black box because people thought it did not matter or that it would be optimized through domestication.

Recent research shows that the assumptions people made were completely incorrect. Farm-raised silkworms show their gut microbiomes to have lower microbial diversity than their wild counterparts which also harbor harmful bacteria and lack essential beneficial bacteria that help their bodies function properly. The silkworm strains show their best performance through this microbial imbalance which leads to their highest production levels. The silkworm disease outbreaks cause major production losses because they show unpredictable patterns of disease spread and the silk production process leads to inconsistent silk quality between different production runs.

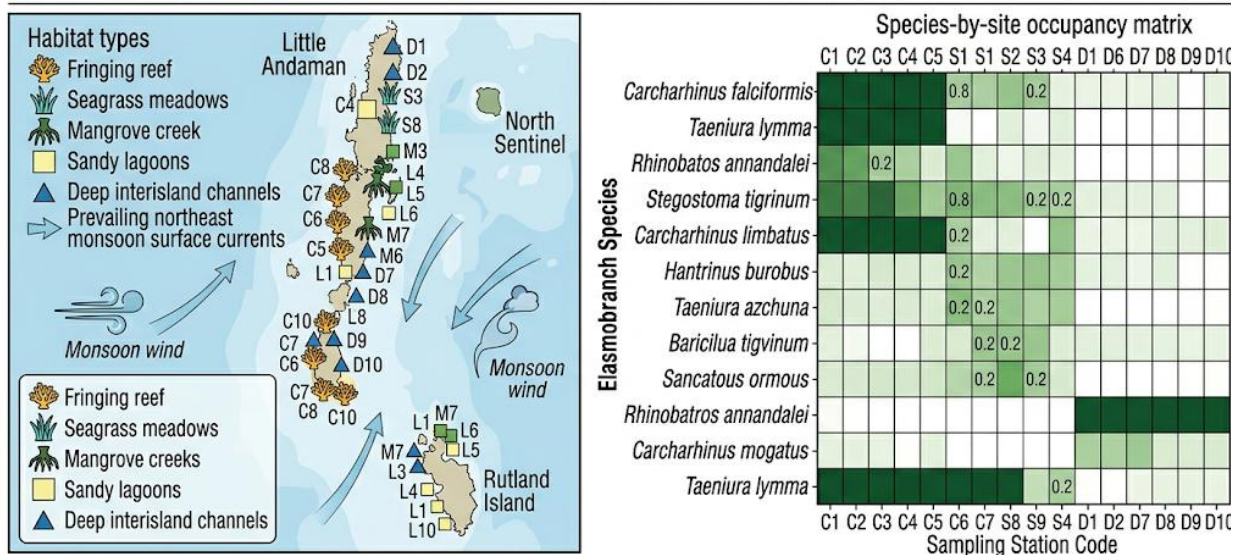


Figure 1: Environmental Stressors and Microbiome Disruption in Tropical Multivoltine Sericulture Systems Source: Author Generated

II. The Silkworm Gut Microbiome: Composition and Function

2.1 Normal Microbiome Structure

The silkworm gut contains a basic yet essential bacterial community which primarily consists of Proteobacteria and Firmicutes and Actinobacteria. Silkworms maintain bacterial communities which include 20 to 40 species because their simplified diet and their domestication history define their bacterial requirements which differ from those of mammals. The main bacterial genera of the study include *Enterococcus*, *Lactobacillus*, *Bacillus* and various *Enterobacteriaceae* family members. The bacteria inhabit three different digestive system parts which include the alkaline anterior midgut for protein digestion and the neutral posterior midgut for nutrient absorption and the slightly acidic hindgut for waste processing and final water absorption. Each digestive system section selects for bacterial strains which possess distinct biological functions and capacity to withstand different environmental conditions. The composition of the system follows a specific pattern rather than existing as a random arrangement. The regions which contain high organic acid concentrations show *Lactobacillus* species dominance because their acid resistance enables them to compete effectively. *Enterococcus* species occupy intermediate niches which enable them to metabolize carbohydrates while they regulate inflammation in immune system development. The system exhibits its most interesting feature through its unchanging nature. The silkworm microbiome demonstrates strong capacity to withstand short-term disturbances because its microbial community structure remains intact while human gut microbiome undergoes extreme changes after people eat different foods or receive antibiotic treatment. The individual bacterial populations experience changes, yet the complete structure of the community returns to its original state.

2.2 Functional Roles in Silk Production

The gut bacteria-silk quality relationship operates through multiple interconnected pathways which control its function. Certain bacterial species enhance the digestion of mulberry leaf proteins, increasing the availability of amino acids essential for silk protein synthesis. The silkworm achieves silk production through its dietary protein conversion process which requires successful extraction of glycine and alanine and serine, the amino acids that form more than 60 percent of silk fibroin. Bacterial fermentation generates short-chain fatty acids, which perform multiple roles in silk metabolism. Acetate provides energy for the silk glands' protein synthesis activities, which require intense protein production. Butyrate regulates gene expression in silk-producing cells, which results in changes to both silk protein production volume and silk protein quality. Propionate supports the metabolic pathways that maintain silk gland cell health during the intensive production periods leading up to cocoon formation.

Scientists have discovered bacterial strains which produce the enzymes that enable silk precursor metabolism. Some *Lactobacillus* strains secrete proteases that enhance protein digestion beyond what the silkworm's own enzymes achieve. Certain *Bacillus* species produce B vitamins that support the methylation reactions required for silk protein modification. The research findings indicate that successful silk production depends on three elements which include suitable genetic traits for silkworms, proper environmental conditions, and appropriate microbial partners.

III. Disease Resistance and Immune Function

3.1 Pathogen Exclusion Mechanisms

The silkworm gut microbiome functions as the primary defense system which protects against all three types of pathogens that pose continuous threats to sericulture operations. Pathogen establishment gets blocked by healthy microbial communities through their multiple protective mechanisms which work together to safeguard the gut ecosystem's balance.

The body uses competitive exclusion as its primary method to defend itself against threats. The beneficial bacteria in the gut compete with pathogens for essential resources which pathogens need to create their infectious diseases. *Lactobacillus* species use their lactic acid and bacteriocins production to create chemical environments which harmful bacteria cannot survive while beneficial microbes remain safe.

The competition for resources involves more than just the basic operation of nutrients. Pathogenic bacteria fail to build substantial populations because many beneficial bacteria bind iron through their high-affinity chelating compounds which create iron-limited environments. Some species produce hydrogen peroxide or other antimicrobial compounds that directly inhibit pathogen growth without significantly affecting the host or other beneficial bacteria. The mechanisms operate at all times to deliver protection that operates independently from the need for active immune system activation.

Biofilm formation adds another layer of protection. Beneficial bacteria create structured communities that physically exclude pathogens from direct contact with gut epithelial cells. The biofilms function as antimicrobial compound storage locations while they establish localized areas with chemical conditions that support beneficial bacteria and protect against potential pathogens. Pathogenic bacteria face challenges in discovering new places to thrive when the microbiome maintains its healthy and diverse state.

3.2 Immune System Modulation

The gut microbiome uses its direct pathogen defense abilities to protect silkworms from infectious diseases while reducing their immune system energy needs. Bacteria employ their surface structures to generate specific signals which the gut immune system detects through controlled interactions with its epithelial immune detection mechanisms.

The immune system of beneficial bacteria receives activation through their controlled delivery of microbial molecular patterns which result in mild immune response activation without inducing inflammation. The process of priming initiates an immune defense mechanism that reacts at an increased pace with greater intensity to actual pathogens which prepares the body for sudden defensive operations. Pathogen elimination occurs with increased speed while generating reduced tissue harm and metabolic interruptions.

Some bacterial species directly enhance immune cell function through the production of immune-modulating metabolites. Bacterial fermentation produces short-chain fatty acids which enable hemocytes to develop and function as the silkworm's equivalent of white blood cells. Gut epithelial barriers remain intact through these fatty acids which stop pathogens from entering the gut wall and causing systemic infections that lead to larval death within days.

IV. Probiotic Interventions: Strain Selection and Applications

4.1 Identifying Effective Probiotic Strains

Researchers need to select appropriate probiotic strains for their sericulture studies because they need to use multiple selection criteria which exceed the basic laboratory testing results. Probiotic bacteria must maintain their viability inside the acidic silkworm anterior midgut because they need to establish dominance over existing microbiotic species while delivering measurable advantages in tropical multivoltine systems which experience environmental disturbances.

Multiple studies show that *Lactobacillus plantarum* has developed into one of the leading probiotic options for use in various applications. This species demonstrates exceptional acid tolerance, allowing survival through the pH 10–11 environment of the anterior midgut that eliminates many potential competitors. *L. plantarum* strains produce different bacteriocins which effectively combat the main silkworm pathogens that include multiple *Bacillus* species responsible for bacterial flacherie disease.

Bacillus subtilis serves as a primary probiotic candidate because of its unique attributes. The organism can endure tropical sericulture conditions because it produces spores which enable survival during extreme temperature and humidity conditions. *B. subtilis* strains produce enzymes which boost cellulose digestion, which may lead to better feed conversion results when using mulberry leaves as animal feed.

The effectiveness of probiotics depends on the specific strain differences which exist between different species. *L. plantarum* isolates display different levels of antimicrobial production because some strains produce higher amounts while other strains have better ability to stick to silkworm gut epithelium and still other strains possess better ability to withstand the high temperatures which occur in tropical rearing environments.

Figure 2 illustrates the multi-step screening process used to identify promising probiotic strains for silkworm applications, highlighting the various selection criteria that must be evaluated.

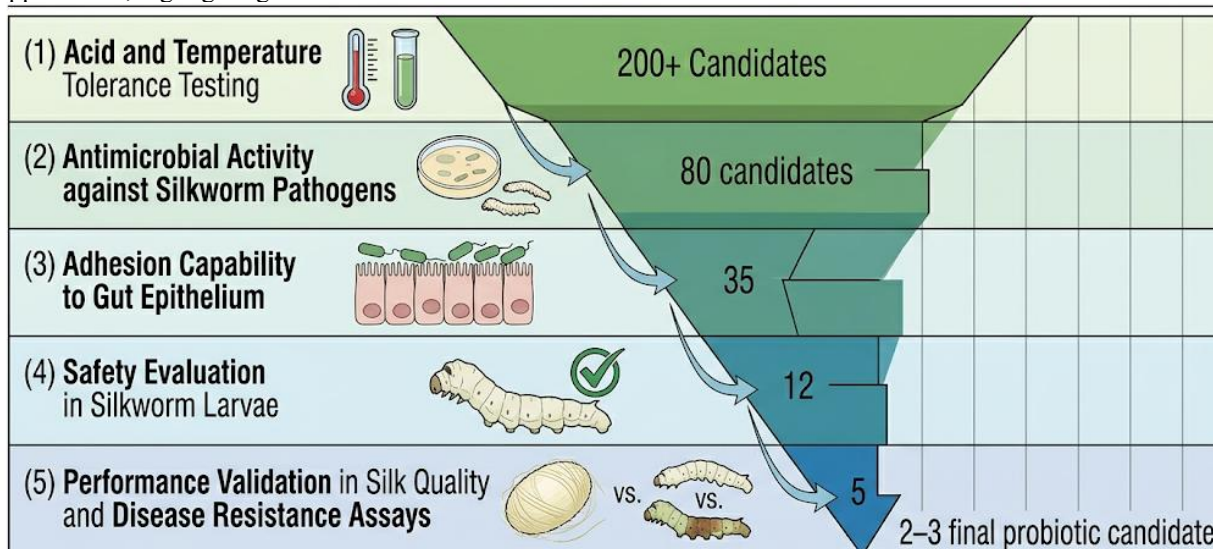


Fig. 2: Multi-Stage Screening Process for Silkworm Probiotic Strain Selection Source: Author Generated

4.2 Delivery Methods and Formulations

Sericulture experiences delivery problems with probiotics because it needs to keep bacteria alive during delivery across multiple mulberry-eating larvae. The existing probiotic delivery system which uses pelleted feeds for livestock fails to work with silkworms because they only eat fresh mulberry leaves.

Sericultural operations find that surface application of probiotic solutions to freshly harvested mulberry leaves. This method allows farmers to use existing leaf preparation protocols with minimal modification while ensuring that all larvae receive probiotic exposure through their normal feeding behavior. The essential requirements include bacterial concentration which exists between 10^6 and 10^8 CFU/ml and solution pH and osmolarity values which sustain bacterial life and application timing which stops bacteria from dying before leaf consumption.

Microencapsulation technologies require higher expenses and operational difficulties but they provide better protection for bacteria during their application and storage. Alginate beads and lipid microspheres and spray-dried formulations protect probiotic bacteria from environmental stresses while enabling controlled release in the silkworm gut. Commercial operations need these advanced delivery systems because probiotics must stay alive during preparation and consumption time periods that last from hours to days.

4.3 Application Protocols and Timing

The success of probiotic treatments depends on their application timing because different developmental phases of organisms show distinct responses to microbiome changes. The microbiome of early instars (1st and 2nd) exhibits the highest level of plasticity which enables probiotic bacteria to build permanent populations that continue to exist after the insects undergo their next molt. The early stages require small food portions which restrict probiotic intake because only extremely high bacterial concentrations can lead to sufficient probiotic consumption.

The third and fourth instar period marks the peak feeding phase which precedes silk production so it enables high probiotic administration while the gut microbiome remains open to changes. The current timing represents the best moment for executing interventions that boost disease resistance because established probiotic populations create protective barriers which shield larvae from harmful pathogens during their most susceptible fifth instar period.

The study investigates the effects of fifth instar probiotic applications on silk production which occurs during the 7–10 day period when silkworms consume 85% of their total food intake and start to produce silk protein. The first three days of fifth instar applications produce their highest impact on silk quality parameters while subsequent applications improve disease resistance but have almost no impact on silk production metrics.

V. Effects on Silk Quality Parameters

5.1 Fiber Strength and Tensile Properties

Probiotic Supplementation produces visible enhancement of silk fiber mechanical properties which benefits the textile industry through increased manufacturing efficiency. Multiple independent studies report 15–

25% increases in tensile strength for silk fibers produced by silkworms receiving targeted probiotic interventions compared to controls. The different silkworm strains tested with various rearing conditions showed scientists had discovered essential biological mechanisms which function independently from both particular strains and environmental factors.

The mechanisms that lead to strength gains operate through improved amino acid access which supports silk protein production. Probiotic bacteria improve protein digestion from mulberry leaves because they boost the availability of glycine alanine and serine which form silk fibroin chains. Silk glands use enhanced amino acid availability to produce fibroin with superior structural strength because reduced defects lead to increased fibroin strength.

Bacterial production of B vitamins also contributes to improved silk quality through effects on protein metabolism and post-translational modifications. The *Lactobacillus* strains produce folate and B₁₂ which assist the methylation reactions that establish silk protein structure. Biotin produced by specific *Bacillus* strains boosts fatty acid metabolism which supplies energy for silk gland operation during times of high protein production.

The commercial applications of two different materials demonstrate equal commercial value because their elastic properties and their ability to stretch without restriction both show improvement. Probiotic-treated silkworms produce silk which shows 8–15% higher breaking strength and better ability to recover from moderate stress. The properties of silk demonstrate their value for high-end textile production because they enable silk to preserve its original properties throughout multiple usage cycles.

5.2 Cocoon Weight and Silk Yield

Cocoon weight is a key economic factor that affects farmers' income, as it determines the total silk production, which is linked to their feed costs. Research shows that probiotic treatments consistently lead to weight increases of 8 to 18 percent in cocoon weight. The largest improvements occur when using specific strains in environments that lack performance because of either illness or environmental challenges. The weight increases reflect multiple contributing factors that operate at the same time. Probiotic treatment helps improve digestion which results in better nutrient absorption thus increasing the quantity of raw materials that can be used for building cocoons. The body diverts metabolic resources to silk production because reduced energy expenditure on immune system activation.

Environmental stress leads to improved gut health which decreases the metabolic expenses required for gut maintenance. Probiotic treatment has strong effects on the relationship between hemming weight and shell ratio which measures the proportion of silk fiber to pupal body weight in a cocoon. Healthy microbiomes appear to optimize the allocation of resources between silk production and pupal development which increases silk production without compromising the viability of emerging adults. The multivoltine system needs special optimization because emerged adults need to sustain their reproductive capacity for future generations.

The economic benefits of probiotic supplementation reach between 12% and 28% based on baseline conditions and probiotic treatment protocols according to calculations that measure silk production from both cocoon weight and shell ratio. Probiotics enable silkworms to handle environmental challenges which result in their lowest productivity points, thus creating the most substantial benefits for production.

VI. Conclusion

The gut microbiome offers a new research area which can enhance silk production through sustainable methods that need genetic and environmental optimization methods already in use. Research from multiple groups shows that specific probiotic treatments lead to silk quality improvements of 15 to 25 percent and decrease disease-related deaths by 30 to 45 percent and raise production costs by 12 to 28 percent when applied correctly. Probiotic treatments establish a new area for research because researchers can study their effects on silk production through specific microbiome-based methods. The increasing global demand for sustainable textile production makes microbiome-based methods more effective because they support environmentally friendly farming practices. Beneficial bacteria provide natural biological system support which enables better results while decreasing the need for chemical treatments unlike synthetic antibiotics which create environmental pollution and select for antibiotic-resistant bacteria. The silk industry requires sustainable production methods which make this alignment with sustainability objectives important because these methods have become essential in environmental production practices.

The path to widespread implementation requires addressing several practical challenges that extend beyond the laboratory research demonstrating technical feasibility. Farming needs education together with quality control systems and economic models and integration of current practices to allow microbiome-based interventions to achieve their maximum effectiveness. The effectiveness of probiotic strains and application methods needs to be adjusted according to specific regional environmental and economic conditions.

References

- [1]. Anand, A. A., Vennison, S. J., Sankar, S. G., Prabhu, D. I., Vasani, P. T., Raghuraman, T., Geoffrey, C. J., & Vendan, S. E. (2010). Isolation and characterization of bacteria from the gut of *Bombyx mori* that degrade cellulose, xylan, pectin and starch and their impact on digestion. *Journal of Insect Science*, *10*(1), 107. <https://doi.org/10.1673/031.010.10701>
- [2]. Broderick, N. A., Raffa, K. F., & Handelsman, J. (2006). Midgut bacteria required for *Bacillus thuringiensis* insecticidal activity. *Proceedings of the National Academy of Sciences*, *103*(41), 15196–15199. <https://doi.org/10.1073/pnas.0604865103>
- [3]. Chen, B., Teh, B. S., Sun, C., Hu, S., Lu, X., Boland, W., & Shao, Y. (2016). Biodiversity and activity of the gut microbiota across the life history of the insect herbivore *Spodoptera littoralis*. *Scientific Reports*, *6*, 29505. <https://doi.org/10.1038/srep29505>
- [4]. Dong, Y., Manfredini, F., & Dimopoulos, G. (2009). Implication of the mosquito midgut microbiota in the defense against malaria parasites. *PLoS Pathogens*, *5*(5), e1000423. <https://doi.org/10.1371/journal.ppat.1000423>
- [5]. Engel, P., & Moran, N. A. (2013). The gut microbiota of insects — diversity in structure and function. *FEMS Microbiology Reviews*, *37*(5), 699–735. <https://doi.org/10.1111/1574-6976.12025>
- [6]. Gao, Z., Wang, W., Abbas, K., Zhou, X., Yang, Y., Diana, J., Wang, L., Li, Q., Feng, M., Huang, L., & Kang, L. (2014). The possible role of bacterial symbionts in the evolution of insect flight. *Arthropod-Plant Interactions*, *8*(6), 487–496. <https://doi.org/10.1007/s11829-014-9320-9>
- [7]. Hamdi, C., Balloi, A., Essanaa, J., Crotti, E., Gonella, E., Raddadi, N., Ricci, I., Boudabous, A., Borin, S., Manino, A., Bandi, C., Alma, A., Daffonchio, D., & Cherif, A. (2011). Gut microbiome dysbiosis and honeybee health. *Journal of Applied Entomology*, *135*(7), 524–533. <https://doi.org/10.1111/j.1439-0418.2010.01609.x>
- [8]. Indiragandhi, P., Anandham, R., Madhaiyan, M., Poonguzhali, S., Kim, G. H., Saravanan, V. S., & Sa, T. (2007). Cultivable bacteria associated with larval gut of prothiofos-resistant, prothiofos-susceptible and field-caught populations of diamondback moth, *Plutellaxystella* and their potential for, *Pseudomonas* mediated biological control. *Journal of Applied Microbiology*, *103*(6), 2664–2675. <https://doi.org/10.1111/j.1365-2672.2007.03506.x>
- [9]. Kikuchi, Y., Hosokawa, T., & Fukatsu, T. (2007). Insect-microbe mutualism without vertical transmission: a stinkbug acquires a beneficial gut symbiont from the environment every generation. *Applied and Environmental Microbiology*, *73*(13), 4308–4316. <https://doi.org/10.1128/AEM.00067-07>
- [10]. Krishnan, M., Bharathiraja, C., Pandiarajan, J., Prasanna, V. A., Santhoshkumar, S., & Rajendhran, J. (2014). Insect gut microbiome — an unexploited reserve for biotechnological application. *Asian Pacific Journal of Tropical Biomedicine*, *4*(1), S16–S21. <https://doi.org/10.12980/APJTB.4.2014C95>
- [11]. Liu, F., Li, X., Zhao, A., & Zheng, L. (2013). Metagenomic analysis of gut microbiota of *Bombyx mori* reveals changes in bacterial diversity and community structure. *Microbes and Environments*, *28*(2), 204–212. <https://doi.org/10.1264/jsme2.ME12207>
- [12]. Paniagua Voirol, L. R., Frago, E., Kaltenpoth, M., Hilker, M., & Fatouros, N. E. (2018). Bacterial symbionts in lepidoptera: their diversity, transmission, and impact on the host. *Frontiers in Microbiology*, *9*, 556. <https://doi.org/10.3389/fmicb.2018.00556>
- [13]. Raymann, K., & Moran, N. A. (2018). The role of the gut microbiome in health and disease of adult honey bees. *Current Opinion in Microbiology*, *46*, 97–104. <https://doi.org/10.1016/j.mib.2018.09.003>
- [14]. Ryu, J. H., Kim, S. H., Lee, H. Y., Bai, J. Y., Nam, Y. D., Bae, J. W., Lee, D. G., Shin, S. C., Ha, E. M., & Lee, W. J. (2008). Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in *Drosophila*. *Science*, *319*(5864), 777–782. <https://doi.org/10.1126/science.1149357>
- [15]. Shao, Y., Arias-Cordero, E., Guo, H., Bartram, S., & Boland, W. (2014). In vivo *Pyro-SIP* assessing active gut microbiota of the cotton leafworm, *Spodoptera littoralis*. *PLOS ONE*, *9*(1), e85948. <https://doi.org/10.1371/journal.pone.0085948>
- [16]. Shin, S. C., Kim, S. H., You, H., Kim, B., Kim, A. C., Lee, K. A., Yoon, J. H., Ryu, J. H., & Lee, W. J. (2011). *Drosophila* microbiome modulates host developmental and metabolic homeostasis via insulin signaling. *Science*, *334*(6056), 670–674. <https://doi.org/10.1126/science.1212782>
- [17]. Vaidya, S., Velkov, T., Kandasamy, Y., Selvaraj, P., Fantino, J. R., & Kumar, S. (2018). Characterisation of *Bacillus* spp. isolated from silkworm (*Bombyx mori*) gut as potential probiotics. *Archives of Microbiology*, *200*(5), 745–754. <https://doi.org/10.1007/s00203-018-1487-8>
- [18]. Wang, L., Zhang, Z., Xu, C., Wang, S., Jiang, L., Wang, X., Cheng, D., Gong, W., & Wei, W. (2011). High-efficiency isolation of *Bacillus thuringiensis* from soil and phylogenetic diversity of cry genes. *Applied Microbiology and Biotechnology*, *90*(4), 1175–1184. <https://doi.org/10.1007/s00253-011-3156-0>
- [19]. Xiang, H., Wei, G. F., Jia, S., Huang, J., Miao, X. X., Zhou, Z., Zhao, L. P., & Huang, Y. P. (2006). Microbial communities in the larval midgut of laboratory and field populations of cotton bollworm (*Helicoverpa armigera*). *Canadian Journal of Microbiology*, *52*(11), 1085–1092. <https://doi.org/10.1139/w06-064>
- [20]. Yun, J. H., Roh, S. W., Whon, T. W., Jung, M. J., Kim, M. S., Park, D. S., Yoon, C., Nam, Y. D., Kim, Y. J., Choi, J. H., Kim, J. Y., Shin, N. R., Kim, S. H., Lee, W. J., & Bae, J. W. (2014). Insect gut bacterial diversity determined by environmental habitat, diet, developmental stage, and phylogeny of host. *Applied and Environmental Microbiology*, *80*(17), 5254–5264. <https://doi.org/10.1128/AEM.01226-14>
- [21]. Zhai, Q., Narbad, A., & Chen, W. (2015). Dietary strategies for the treatment of cadmium and lead toxicity. *Nutrients*, *7*(1), 552–571. <https://doi.org/10.3390/nu7010552>