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PROBIOTICS SUPPLEMENTATION TO MULBERRY SILKWORM *B. MORI*

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INTRODUCTION

- The *Bombyx mori* L. silkworm is an economically important lepidopteran insect for silk production due to its ability to convert protein from mulberry leaves into silk (Yeruva et al. 2020; Hassan 2020). It is known that silkworms, as a monophagous insects, require certain nutrients (proteins, amino acids, essential sugars, fatty acids, vitamins and micronutrients) for their growth and production that are provided by the fresh mulberry leaves (*Morus* spp.) as unique natural source of feed (Saviane et al. 2014; Zhang et al. 2019; Ayandokun and Alamu, 2020). The quality of mulberry leaves depends on environmental conditions and affects the cocoons production and silk quality. Silkworm larvae's nutrition and health status are the main factors influencing silk production.

OBJECTIVE

- This review highlighted the trends in silkworm nutrition by using microbial species as natural ways to supplement the mulberry leaves and improve silkworms' larval and cocoon traits. The definition, characteristics and modes of action of probiotics, the intestinal microbiota, and the functional role of gut bacteria in silkworms are also presented in this review. The studies that used probiotic supplementation of mulberry leaves in silkworms, with special emphases on *Lactobacillus* and *Bacillus* spp., and the main results on productivity and health status were summarised.

RESULTS AND DISCUSSIONS

- Food and Agriculture Organization of the United Nations and World Health Organization (FAO 2002), defined probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host". The selection of microorganisms as probiotics requires specific properties or characteristics related to their safety, performance, and technological aspects (Priyadharshini et al. 2021). The major mechanisms of action of probiotics: i). epithelial barrier improvement, ii). increased adhesion to the intestinal mucosa, iii). inhibition of pathogen adhesion, iv). competitive exclusion of pathogenic microorganisms, v). production of anti-microorganism substances, and vi). immune system modulation (Bermudez-Brito et al. 2012).
- Probiotic microbial flora activity in the host's gut may improve feed absorption and digestion efficiency, boosting the host's production and assimilation of proteins (Rowland et al. 2018). Additionally, probiotics break down easily absorbed substances and can even generate specific vitamins, which may aid in nutritional absorption. According to the theory that beneficial microbial ecology is crucial for eukaryotic metabolism, insects require a variety of enzymes from their gut microbial flora to aid in the digestion of feed components. These enzymes can then help release other molecules, such as fermentable sugars and amino acids, that benefit the insect's growth (Liang et al. 2015).
- The microbial species *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Streptococcus*, and *Saccharomyces* are the most often evaluated probiotics used for insects (Guarner et al 2017).

Role of gut bacteria species in *B. mori* silkworm host

Species	Functions	Reference
Lactobacillus	✓ Stimulated growth factors and improved economic characteristics.	Yeruva et al., 2020; Sing et al., 2005; Suraporn et al., 2015; Suraporn and Terenius, 2021.
Bifidobacterium	✓ Immunomodulatory effect by increasing the activity of protease, amylase and invertase; improve production of raw silk.	Taha et al., 2017
Bacillus subtilis	✓ Release secondary metabolites, vitamin B synthesis, produce antimicrobial peptides (AMP), and increase resistance.	Li et al., 2022
Bacillus aryabhattai and *Bacillus* sp. *Bacillus megaterium* *Bacillus pumilus*	✓ Cellulolytic activity. ✓ Glycolysis of starch. ✓ Digestive system and protection against antiviral agents nuclear polyhedrosis virus (BmNPV).	Pandiarajan and Revathy, 2020 Prasanna et al., 2014 Liu et al., 2018
Bacillus licheniformis	✓ Production of extracellular enzymes.	Mala and Vijila 2018
Bacillus sp., Brevibacterium sp., Corynebacterium sp., Staphylococcus sp., Klebsiella sp., and *Stenotrophomonas sp.*	✓ Digestion and synthesis of lipase enzyme.	Feng et al., 2011
Enterococcus sp. and *Staphylococcus sp.*	✓ Mechanism of defence against infection.	Sun et al., 2016
Enterococcus mundtii	✓ Host metabolism (production of metabolites and lactic acid).	Liang et al., 2018
Enterococcus casseliflavus	✓ Growth and development through the synthesis of L-tryptophan.	Liang et al., 2022
Enterococcus faecalis	✓ Immunity against infection.	Zhang et al., 2010
Staphylococcus gallinarum	✓ Defence mechanism.	Gibson et al., 2019
Staphylococcus gallinarum SWGB 7 and *Staphylococcus arlettae SWGB 16*	✓ Stimulated growth, improved economic traits and immune system.	Saranya et al., 2019
Streptomyces noursei	✓ Antimicrobial activity in relation to disease control.	Subramanian, 2010; Mohanraj et al., 2014
Enterobacter aerogenes, pneumoniae sp. Pneumonia, Yersinia enterocolitica	✓ Growth and development, host resistance, chemical pesticide degradation, and entomopathogen host antagonism interactions.	Ramesh et al., 2009

Effects of probiotics supplementation of mulberry leaves in silkworm

Probiotic	Dose/ Concentration	Instar	Main results	Reference
L. plantarum	ns	ns	↑ larval body weight, cocoon, shell weight and pupation rate.	Sing et al., 2005
L. acidophilus	10^8 cfu/ml	3 to 5^{th} larval instars	↑ survival ratio, mature larval weight, pupation ratio, cocoon weight and cocoon-shell ratio. ↑ silk yield and silk harvest.	Suraporn et al., 2015
L. casei	10^8 cfu/ml	5^{th} instar	↑ larval weight, cocooning and pupation ratio, and economic characters (cocoon weight and size) of larvae infected with microsporidium *Nosema bombycis*. ↑ feed digestion and nutrient absorption.	Suraporn et al., 2021
L. rhamnosus *B. bifidum* and their mixture	3 and 6%	4 to 5^{th} larval instars	*L. rhamnosus + B. bifidum* ↑ larval performance and tissue growth, ↑ feed intake and assimilation, followed by *L. rhamnosus* → *Bifidum.* Both concentrations ↑ economic and silk (length, weight and size) parameters with no significant differences between them. Lower concentrations (3%) are recommended.	Moustafa and Soliman, 2019
Spirulina, *S. cerevisiae,* *L. acidophilus* and *L. sporogens*	300 ppm of each	ns	Spirulina and yeast ↑ cocoon characteristics, fibroin content and silk quality, followed by *L. acidophilus* and *L. sporogens*.	Masthan et al., 2017
B. cereus, *B. subtilis,* *B. amyloliquefaciens, L. casei,* *L. plantarum* and their mixture	ns	3 to 5^{th} larval instars	*B. amyloliquefaciens* ↑ silk gland proteins followed by combined bacterial diet → *L. casei* → *B. cereus* → *L. plantarum* → *B. subtilis.* *B. amyloliquefaciens* ↑ silk gland carbohydrates followed by *B. cereus* → combined bacterial diet → *B. subtilis* → *L. plantarum.* *B. amyloliquefaciens* ↑ silk gland lipids followed by *B. cereus* → combined bacterial diet → *L. casei* → *L. plantarum* → *B. subtilis.* *B. amyloliquefaciens* ↑ fibroin content followed by *B. cereus* = combined bacterial diet → *L. plantarum* → *L. casei* → *B. subtilis.* *B. amyloliquefaciens* ↑ digestion and nutrient absorption, ↑ protein synthesis and deposition in the body, ↑ silk gland and cocoons. *L. casei* ↑ length and weight of larval body and ↑ cocoon weight. *L. casei* ↑ protects against harmful bacteria and promotes growth rather than immune response. This study demonstrates the possible probiotic-boosting effect of *B. amyloliquefaciens, L. casei, B. cereus,* and a microbial community on silkworm physiology, metamorphosis, and silk production.	Sekar et al., 2016
B. licheniformis BMGB42 *B. niabensis BMGB17* and their mixture	10^4 cfu/ml 10^6 cfu/ml 10^8 cfu/ml	5^{th} instar	*B. licheniformis* followed by *B. licheniformis + B. niabensis* (10^6 cfu/ml) ↑ larval weight, effective rate rearing, cocoon weight, shell weight, pupal weight, shell ratio, silk productivity and filament length; ↓ finer denier; ↓ larval mortality due to disease incidence.	Mala and Vijila, 2018
Lact-Act (commercial probiotic based on *L. sporogens, B. thuringiensis*, yeast hydrolysate, a-amylase, vitamin-mineral mix)			↑ larval survival when exposed to bacterial pathogens (*B. thuringiensis* var. sotto. and *S. aureus*).	Rajakumari et al., 2007

CONCLUSIONS

- Enhancing the quality of mulberry leaves is crucial for improving the appetite and/or feed quality, as it is one of the primary factors influencing the silkworms development in larval instars.
- Findings from the literature indicate that probiotics supplementation to mulberry leaves is a sustainable way to boost silkworm health, productivity, and quality of silk.

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