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Probiotics and its role in silkworm growth and development

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Probiotics

Probiotics are the preparation of or a product containing viable, defined microorganisms in sufficient numbers, which alter the microflora by implantation or colonization, in a compartment of the host and by that, exert beneficial effects on host health. Food and Agriculture Organization of the United Nations-World Health Organization (FAO-WHO) officially defined probiotics as “live microorganisms that when administered in adequate amounts confer a significant health benefit on the host”

Probiotics based food products have become now popular due to appreciation of their contribution to good health. These beneficial microorganisms are ingested and thereby introduced into the intestinal microflora intentionally and resulted in high numbers of beneficial bacteria to participate in the competition for nutrients with and starving off harmful bacteria. Probiotics take part in many positive health-promoting activities in human physiology. The beneficial effects of the ingested probiotic bacteria are provided by those organisms that adhere to the intestinal epithelium. The presence and adherence of probiotics to the mucous membrane of the intestines build up a strong natural biological barrier for many pathogenic bacteria.

Characteristics of probiotics

A microbial strain has to fulfil several specific properties to be regarded as a probiotic. These criteria are classified into safety, performance and technological aspects. They should possess GRAS (generally regarded as safe) status, be non-pathogenic, non-toxic, non-allergic, non-mutagenic. It should be able to adhere to and colonise the epithelial cell lining to establish itself in the colon. The ability to adhere to the epithelium secures the strain from being easily flushed out by peristaltic movements. With respect to their performance, potential probiotic strains should be able to survive in sufficient numbers and adhere to the intestinal mucosal surface Probiotic strains should also stimulate an immune response, thereby positively influencing the host. The probiotic should survive the environmental conditions in their target site of action and proliferate in this location. Technologically, a good probiotic strain should be easily, inexpensively reproducible. It must be able to withstand stress during processing and storage, with the process and product application robustness. The organism should be able to survive, in particular, the harsh environmental conditions of the small intestine (e.g. gastric and bile acids, digestive enzymes). Strains should be capable of being prepared on a large scale and should be able to multiply rapidly, with good viability and stability in the product during storage. The strains must not produce off flavours or textures once incorporated into foods. Probiotic strains must be resistant to phages and have good sensory properties.

Mode of action of probiotics:

Enhancement of the epithelial barrier

The intestinal barrier is a major defence mechanism used to maintain epithelial integrity and to protect the organism from the environment. Defences of the intestinal barrier consist of the mucous layer, antimicrobial peptides, secretory IgA and the epithelial junction adhesion complex.

Increased adhesion to intestinal mucosa and inhibition of pathogen adhesion

Adhesion of Probiotic bacteria to the intestinal mucosa is important for its colonization. Lactic acid bacteria (LABs) display surface adhesions that are involved in their interaction with intestinal epithelial cells (IECs) and mucus. Mucin produced by the host intestinal epithelial cells is a complex mixture of glycoprotein that is the principal component of mucous, thereby preventing the adhesion of pathogenic bacteria.

Competitive exclusion of pathogenic microorganisms

The efficacy of a Probiotic can be determined by its ability of binding to receptor sites present in the intestinal tract. The binding of probiotic bacteria inhibit the adhesion of pathogenic bacteria and prevent their colonization. Probiotic bacteria reduce the growth or inhibit the colonization of pathogen using the following mechanism: creation of a hostile microecology, elimination of available bacterial receptor sites, production and secretion of antimicrobial substances and selective metabolites, and competitive depletion of essential nutrients. Competitive exclusion of pathogenic organisms is based on a bacterium-to-bacterium interaction in which bacteria compete for available nutrients and mucosal adhesion sites. Bacteria can modify their environment to make it less suitable for their competitors for example; bacteria produce antimicrobial substances, such as lactic and acetic acid.

Production of antimicrobial substances

Probiotics produce short-chain fatty acids, which lower the intracellular pH, inhibiting the growth of a pathogenic organism. Many Probiotic organisms secrete antimicrobial peptides including bacteriocins and AMPs. Bacteriocin such as Nisin, Plantaricin and lactacin show a narrow activity spectrum against some pathogens.

Modulation of the immune system

Probiotic bacteria can exert an immunomodulatory effect on the host by interacting with epithelial and dendritic cells (DCs) and with monocytes/macrophages and lymphocytes.

Immune effects of probiotics

- Probiotics deliver anti-inflammatory molecules to the intestine
- Reduce inflammatory response by increasing signalling in host cells.
- Switch in the immune response to reduce allergy
- Induce antibody response to reduce infection.
- Decrease the production of inflammatory substances

Common species of probiotics

The most commonly consumed probiotics are strains of two main species, *Bifidobacteria sp. Lactobacillus sp.* and yeast**.** This species of bacteria produce lactase, the enzyme that breaks down lactose, or milk sugar. These bacteria also produce lactic acid. Lactic acid helps control the population of bad bacteria. It also serves as muscle fuel and increases the body's absorption of minerals.

Role of probiotics in silkworm

Silkworm, *B. mori* is a monophagous and host plant-specific insect that feeds solely on mulberry leaves (*Morus alba*, Family: Moracea) produces silk. The growth and development of larvae and subsequent cocoon production are greatly influenced by the nutritional quality of mulberry leaves. In addition, nutritional supplements include vitamins, amino acids, protiens and probiotics when added to larval feed tend to increase nutritional efficiency and economic traits of the silkworm. The products containing probiotic bacteria are widely explored, thereby increasing the importance of their accurate specializations 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 humans, animals, and many insects. No commercial probiotics formulations are specifically designed for sericulture though they are available for human, aquaculture and veterinary medicinal use. Few limited reports on the use of probiotics for silkworm growth and development were reported.

Esaivani *et al.* (2014) attempted to study the impact of mulberry leaf fortification with probiotic microorganism *Saccharomyces cerevisiae* on the enzymatic profile and the quantitative economic parameters of a silkworm, *Bombyx mori*. The results indicate that there was a profound increase in amylase and invertase activity in the digestive juice of the probiotic treated worms than the control with enhanced immunity and quality silk production.

The silkworm larvae were fed on *ricinus communis* leaves treated with 0.5%, 1%, 1.5%, 2%, 2.5%, 3% concentrations of probiotic Darolac from 3rd instar onwards and its effect on food consumption,utilization and economic traits was studied to understand the efficiency of conversions and its potentials to increase the silk yield in eri silkworm. *Ricinus communis* leaves treated with probiotics recorded maximum food assimilation, assimilation rate food conversion rate at 2% concentration while various economic parameters like weight (7.96gms/10 worms) pupal weight (4.06 gms), cocoon weight (4.61) and shell weight (0.57) silk ratio % (12.17) and ERR% (95) also showed an increase when compared with control-treated batch.The probiotic(darolac) treatment showed an increase in nutritional parameters as well as commercial qualities of the cocoon at 2% concentration (Anitha *et al.*, 2015).

The effect of *Lactobacillus acidophilus,* on the growth of two strains of Thai silkworm, *Bombyx mori,* Nang Lai and Nang Lai X 108 were studied by Surapornet *et al.* (2015). *L. acidophilus* @ 10^8 cells mL-1 was topically applied to mulberry leaves and fed to II,III, IV and V instars and observed 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, the 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, the 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.

Mulberry leaves supplemented with commercial probiotics Flora-SB at the concentration of 1%, 3% and 5% were administered, starting with the first day, first feed of each instar. Economical parameters *viz.,* daily measurement of dry matter ingested, faeces produced, biomass gained by the larvae, quantitative and qualitative characters of the cocoon were recorded. Probiotics with a concentration of 3% was very effective and recorded maximum food consumption (1.836 ± 0.07g), assimilation (1.544 ± 0.05g), tissue growth (0.118 ± 0.002g), RGR (54.06 ± 1.84 per cent), cocoon weight (1.98 ± 0.09g), shell weight (0.37 ± 0.01g) and filament length (804.26 ± 23.12m)

An experiment to determine the feed efficiency of silkworm fed mulberry leaves with four types of probiotics, Blue-green algae, spirulina, Yeast, *Lactobacillus acidophilus*, *L. supergenes*. The results suggested that Blue-green algae, Spirulina and yeast had better efficiency of food conversion when compared to other probiotics.

The silkworm larvae were fed with four different kinds of probiotics feed supplements *viz., spirulina*, Azolla, yeast and soy milk at five different concentrations (1, 2, 3, 4 and 5%) each was given to silkworm hybrid, PM × CSR-2 from fourth instar onwards through mulberry leaves every day once in the morning till the spinning stage. Among the probiotics tested Azolla was found to be superior for an effective rate of rearing. cocoon weight on the day of 50 per cent spinning followed by soy milk and yeast in comparison with control (un supplementation) in both the rearings (Shruti *et al.*, 2019).

Saranya *et al.* (2019) investigated economic parameters of silkworm bivoltine double hybrid (CSR6 x CSR26) x (CSR2 x CSR27) fed on mulberry leaves fortified with

*Staphylococcus gallinarum*strain SWGB 7 and *Staphylococcus arlettae*strain SWGB 16. *Staphylococcus gallinarum*Strain SWGB 7 (108 cfu/ml) recorded maximum larval weight (4.12 g), effective rate rearing (96.36 %), cocoon weight (1.97 g), shell weight (0.37 g), pupal weight (1.60 g), shell ratio (18.78 %), silk productivity (4.81 g), filament length (1170.84 m), filament weight (0.31 g) and finer denier (2.38) besides reduced larval mortality (3.64 %) due to disease incidence compared to control. The results indicated that there is a profound increase due to probiotic treatment in larval growth and cocoon characters than the control with enhanced immunity and quality silk production.

Conclusion

Probiotics live microbial feed supplement, beneficially affects the host by improving its microbial balance and enhances body immune system, disease resistance, production of vitamins and increase in enzymatic activity. Administration of probiotics to silkworm through mulberry leaves increase nutritional and economic parameters of the silkworm.

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