CHAPTER-1

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Insects represent a highly significant group of invertebrates within our biosphere (Chapman, 2006), renewed for their intricate influence on human civilization, both positively and negatively. Among these, silkworms, belonging to the silk producing category, hold particular prominence in the silk industry. They are pivotal contributors, furnishing silk fibers integral to the manufacturing of textiles, medical equipment, and various other materials. Taxonomically they fall under order Lepidoptera, alongside other moths and butterflies. Silkworms distinguish themselves through their remarkable life cycle characterized by metamorphosis from larval stage, also known as caterpillar to an adult stage characterized by vibrant pigmentation and advent of wings.

Lepidopteran insects play pivotal role in various ecosystems, serving as pollinator, food source for other organisms, and even as indicator of environment health. Silkworms are primarily used for silk production. Nevertheless, silkworms predominantly occupy a pivotal role in the silk industries, their silk being prized for its exceptional strength and opulent sheen for millennia. Beyond their industrial significance, silkworms have also gained prominence as indispensable subjects in scientific research. They serve as exemplary model organisms for the study of developmental processes, genetics, and diseases due to their amenability to controlled laboratory rearing and their abbreviated life cycle, making them useful tools in understanding intricate biological mechanisms (Xia and Yang, 2004; Xia *et al*., 2009; Nwibo *et al*., 2015; Meng *et al*., 2017).

There are five major types of commercial silk producing silkworms, namely- Mulberry silkworm (*Bombyx mori)*, Tasar silkworm (*Antheraea mylitta*), Oak Tasar silkworm (*Antheraea proylei*), Eri silkworm (*Samia ricini*) and Muga silkworm (*Antheraea assamensis*) which are found globally. India is the home for the production of all the five varieties of silk secreting silkworms (Singh *et al.,* 2017), became world's second-largest producer of silk (Indian Silk, 2022). North-eastern region of India particularly Assam state is also blessed with natural hub of silk producing insects. Among the different commercially exploited silkworm species of India, rearing of adaptable nature and also availability of the food plants, which makes it ideal for rearing (Ahmed and Rajan, 2011; Saikia, 2011).

Ericulture is the practice of cultivation of food plants and rearing of Eri silkworm (*Samia ricini*) for the production of commercial silk and their by-products. Eri silkworm (*S. ricini*), a member of the family Saturniidae, is the most exploited, domesticated and commercialized non mulberry silkworms (Tungjitwitayakul and Tatun, 2017).

Taxonomical classification of Eri silkworm

Phylum - Animalia
Class - Arthropoda
Order - Lepidoptera
Family - Saturniidae
Genus - *Samia*
Species – *S. ricini*

Figure 1.1 Eri silk moth (*Samia ricini*)

In India, Eri silk industry is mainly contributed by North-East India, accounting about 90% of the total Eri silk production in India. Among the various north-eastern states, Assam stands first as the prime producing state. In recent years, Eri culture has also been practiced by the farmers of several other states *viz.* Andhra Pradesh, Madhya Pradesh, Tamil Nadu, Karnataka, Maharashtra, Uttaranchal, Uttar Pradesh, Jharkhand, Bihar, West Bengal, Orissa and Sikkim (Sahu *et al*., 2006). The Eri silkworm (*S. ricini*) is commercially exploited for its proteinaceous pre-pupa/pupa. It is a source of Eri silk, also known as ‘peace silk’ or ‘nonviolent silk’ as the moths are not destroyed in the cocoon but are allowed to emerge and the pierced cocoons are spun rather than reeled to produce the Eri silk yarn (Satarupa, 2016). Eri silk fibre is fine, white wool or cotton like soft in nature and has the thermal properties, thus being used commonly in the production of carpets, blankets, denims, shawls, jackets, and other garments. Eri silk has a great economic influence on the sericulture industry. It has also gain traditional uses in many North-eastern states and is called as “Poor Men’s Silk” (Patil and Savanurmath, 1989; Sarmah, 2011) because a large number of tribal people are engaged in Eri industry. *S. ricini* is multivoltine and polyphagous in nature feeding on different food plants. Eri silkworm primarily feeds on castor (*Ricinus communis*), and Kesseru (*Heteropanax fragrans*), however, Eri silkworm being polyphagous feeds on several other alternative food plants viz. Borkesseru (*Ailanthus excelsa*), Barpat (*Ailanthus grandis*), Tapioca (*Manihot esculenta*), Gulancha (*Plumeria acutifolia*), Gamari (*Gmelina arborea*), Payam (*Evodia flaxinifolia*), Papaya (*Carica papaya*), Jatropha (*Jatropha curcas)*, etc. (Naika *et al.,* 2003; Sannappa *et al.,* 2004; Kumar and Elangovan, 2010).

The nutritional quality and availability of food plants has a great influence on the growth, development and economic parameters of silkworm (Singh and Das, 2006). Eri silkworm being herbivorous, is primarily dependent on the nutrient composition of the food leaves. Different food plants shows variation in the composition and level of nutritional and other bioactive molecules which influences the physiology of organism. This subsequently affects the survival, rate of food intake, digestion and assimilation which directly influences the proper growth and development of silkworms (Babic *et al*. 2008; Sarate *et al*., 2012).

Therefore, different food plants are categorized into primary, secondary and tertiary depending on the preferences of the larvae and their economic parameter. Some food plants are considered as alternative food plant which can be used during the time of scarcity of primary and secondary food plants but not the entire life cycle. Ultimately, the amount and quality of food intake of larvae influences the different parameters like growth rate, larval duration, survival rate, and reproductive potential (Das and Das, 2003).

The gut, as a bodily system, represents one of the primary place to extract and assimilate nutrients from the consumed food. Presently, the significance of the gut is being increasingly recognized, and its immense complexity is only just beginning to be unveiled (Sonnenburg *et al*., 2004). It is estimated that the gastrointestinal tract harbors ten times more microorganisms than total cells and possesses a hundred times more microbial genes than animal genes. Such investigations and figures prompt inquiries into whether animals can be considered 'super-organisms' (Lederberg, 2000), housing both animal and microbial genomes, or if animals are simply successful microbial collectives. The gut of insects serves as an ecologically rich habitat abundant in nutrients, fostering into whether animals can be considered 'super-organisms' (Lederberg, 2000), housing both animal and microbial genomes, or if animals are simply successful microbial collectives. The gut of insects serves as an ecologically rich habitat abundant in nutrients, fostering the proliferation of various microbial groups including viruses, bacteria, archaea, as well as microscopic eukaryotes such as protozoans, fungi, and nematodes. While the diversity and role of bacterial communities within the gut have garnered some attention, research on other microbial groups remains sparse and infrequent.The gut of insect's is inhabited by a wide diversity of microorganisms as a result of its constituting intestinal microbial ecosystem. Studies have revealed that the gut microbiota is able to resist invasion of pathogenic microorganisms and to maintain normal ecological balance. Earlier reports indicate that the intestinal microbiota in insects are involved in the digestion process of insects, and may also assist in insect immunity by forming a persistent infection within their hosts (McKillip *et al.,* 1997; Dillon *et al.,* 2005).

Most insects have a variety of gut bacteria, from simple to composite, that serve vital roles (Lilburn *et al*., 2001). This symbiotic relationship between insects and microbes support physiological functions like photosynthesis, nitrogen fixation, cellulose degradation, hydrogen consumption, and the synthesis of critical nutrients. Symbiotic relationships with intestinal bacteria appear to offer significant advantages for certain insect species. The communities that make up these symbiotic microbes may have distinct effects on host fitness than a single species would. The complex and diverse population of microbes that live in the digestive system of insects is known as the gut flora or gut microbiota; some of them are helpful to the insects, leading to symbiotic relationships. Growth and development of insects are associated with their reside beneficial gut microorganisms that are helpful in aiding the digestion of refractory dietary components, upgrade diets deficient in nutrients, support intra- and interspecies communication, and safeguard against parasites, predators, and diseases (Sun *et al*., 2016; Arora and Douglas, 2017; Gandotra *et al.,* 2018a). The main metabolic sources for insects are their gut bacteria, which increase their capacity for endurance through effective nutrient utilisation and the degradation of complex primary and secondary metabolites of plant substrate into simpler forms to facilitate better digestion (Khyade and Marathe, 2012).

Lepidopteran gut microbiota has garnered comparatively less focus compared to other insect families. Studying the Lepidoptera's gut bacteria and the possible roles they play in larval physiology may lead to new targets for environmentally friendly pest control. The exploration of beneficial gut bacteria may open the way for the development of probiotics to boost the feeding and productivity of beneficial insects (Thangamalar *et al.*, 2009). The gut microbial populations of insects differ depending on the food they consume. Dietary specialists can be inhabited by a specific gut microbial community and also maintain them through their whole life by constantly reintroducing particular microbes, promoting specific metabolism, and maintaining a consistent gut environment (De Filippo *et al.,* 2010; Nicholson *et al.,* 2012). In contrast, a polyphagous insect is inclined to harbour a wide array of microorganisms associated with its varied diet (Jones *et al.,* 2019).

The composition and abundance of gut bacteria in silkworms can vary significantly depending on several factors, such as the host genotype, diet, age, and environment. The genotype and phylogeny of the host insect prove to have a substantial impact on shaping the gut microbiota of silkworms. Studies have found that different individual of silkworm from same species have distinct gut bacterial profile, which may influence their adaptability and overall health (Gandotra *et al*., 2018b). Similarly, diet has also been shown to affect gut diversity of bacteria in silkworms. Feeding on certain artificial and antibiotics treated diet different from their natural diet can alter the gut composition and function, leading to changes in the host’s physiology and silk production (Liang *et al.,* 2014; Dong *et al.,* 2018; Li *et al*., 2022a). Age is another important factor that can affect the diversity and evenness of bacteria in the gut of silkworms. Findings suggest that gut bacterial communities of silkworms undergo significant changes during their growth and development stages. For instances, the gut bacterial diversity tends to increases as larvae attend maturity (Chen *et al*., 2018; Tan *et al.,* 2022). Gender of host silkworms also plays a role in shaping the gut microbiota of silkworms. Research indicates that genders, namely male and female, can have different gut microbiota compositions (Sun *et al.*, 2016). Changes in the composition of the bacterial diversity of gut were also caused by use of certain insecticides and disinfectant that certainly influencing host's physiology (Chen *et al.*, 2020; Lin *et al.*, 2020; Li *et al.,* 2020). The use of insect growth regulator can also be found to alter the diversity of intestinal bacteria (Lu *et al.*, 2022).

Finally the seasonal factor and environmental factors, such as temperature, humidity and infection, can also influence the assortment of bacteria found in the gut of silkworms (Haloi *et al.*, 2016; Sun *et al.*, 2017; Kumar *et al.*, 2019; Sun *et al.*, 2022; Gogoi *et al.*, 2023a). Poor environmental conditions can lead to the proliferation of harmful bacteria and the suppression of beneficial ones, resulting in infection or disease that in turn affect the overall health and reduced silk production (Chen *et al.*, 2018). Overall, understanding the factors that affect the gut bacterial diversity in silkworms is crucial for optimizing their growth, health and silk production, and for developing sustainable Sericulture practices.

The main focus in the aspect of bacterial communities residing in insect gut was on endosymbionts, which inhabit unique cells derived from their hosts. Most of bacterias cannot be cultivated on the microbiological general purpose growth medium. In order to analyse the intestinal bacteria of insects, both culture-independent (Chandler *et al.*, 2011) and culture-dependent (Arias-Cordero *et al.*, 2012) approaches were applied. Culture-dependent approach involves isolating bacteria from the gut of silkworms on culture media and then identifying them based on their morphology, growth characteristics, and biochemical properties. Culture-dependent gut bacteria provide a fresh approach to studying the symbiotic relationship that exists within the insect gut, as well as characterise the functionality of gut bacteria, which allows for their use and genetic modification. Although culture-dependent methods are easy to perform and can provide information on the viability and culturability of the bacteria, they are biased towards the detection of fast-growing and culturable bacteria, and may not accurately reflect the variety within the gut microbiota. Culture-independent techniques involved analysing DNA or RNA extracted directly from the gut of silkworms to identify and quantify the bacterial taxa present. These methods include polymerase chain reaction (PCR) amplification of 16S rRNA genes, metagenomic sequencing, and metatranscriptomic sequencing, allowing more comprehensive analysis of the gut microbiota, including the detection of non-culturable and low-abundance bacteria. This resulted in a better, more inclusive representation of bacterial communities and a significant improvement in our understanding of insect bacterial symbionts (Yun *et al*., 2014).

Gut bacteria exhibit a contribution to the nutrition of the host insect. The interlaced alliance of bacteria and insects is owing to the vast biological modifications of the digestive tract that support diverse microbiota in many orders of insects (Engel and Moran, 2013). Members of the class Insects lack a well-developed enzyme system, thus they rely on gut microbiota to support several metabolic activities in host insects (Liang *et al*., 2018). The gut microbiota extends the lives of insects by improving digestion and nutrition by supplying digestive enzymes, amino acids, and vitamins (Liang *et al.,* 2022; Li *et al*., 2022b). Many gut bacterial have been reported to have significance important in cellulase, lipase, amylase, gelatinase, xylanase, hydrolases in many silkworm species (Anand *et al*., 2010; Feng *et al*., 2011; Gandotra *et al*., 2018a).

The multivorous/ broad feeder nature of insect enable the Eri silkworm to feed on more than one food plants. Eri silkworms also member of herbivore insects depended on the nutrient components of food plants leaves. The cell walls of plants leaves are composed of tough structural components such as of cellulose, xylan and pectin a complex carbohydrates that provides structural support, mechanical strength and chemical stability to the plants cell wall (Brown, 2004). To degrade these complex carbohydrates components of cell wall in order to obtained plant nutrients requires a group of enzymes that work together to degrade them well and are known to be efficiently produced by the gut microbe that hydrolyse, cellulose, xylan and pectin components (Beguin and Aubert, 1994).

The nutrition of the silkworms is the most fundamental and important challenge in the sericulture and the ability of silkworm to secrete digestive enzymes is largely influenced by the nutritional composition of the food plants (Manjula *et al*., 2010).

Many recent studies on gut bacteria of silkworm has provided the evidence on association of gut bacterial species in the complementing host digestive system by producing key digestive enzymes (Anand *et al*., 2010)

Cellulase enzyme is a group of enzymes that catalyse the breakdown of cellulose, a complex polysaccharide that is a major component of plant cell walls including the leaves. These enzymes are produced by variety of organisms including bacteria, fungi, and some animals. Cellulase enzyme work by breaking the 1, 4-glycosidic bonds that link cellulose molecules together, which requires a high degree of substrate specificity and a complex catalytic mechanism. Cellulase is a multienzyme system made up of enzymes such as endoglucanases, exoglucanases and cellobiohydralases, with a variety of isozymes, each of which has a unique role in breaking down cellulose into simpler sugars that can be used for energy or other cellular processes. Cellulase enzymes play important roles in a variety of biological processes, including the digestion of plant materials in the environment and the production of biofuel from the plant biomass. Microbes such as actinomycetes, bacteria, and fungi are among the microbes that are known to produce endo-1, 4-D-glucanase, glucosidase, and exo-1, 4-D-glucanase, three different forms of cellulose components. Microbes play significant roles in cellulose degradation and also facilitate some insects to make use of cellulose-rich substrates that might otherwise be incompatible because of low levels of nitrogen and other important nutrients (Genta *et al*., 2003).

The α-amylase is an enzyme involved in the breakdown of complex carbohydrates such as starch, into simple sugars. It is produced by various organisms including humans, animals, plants and microorganisms. The enzyme plays a crucial role in the breakdown of starch into glucose and maltose by breaking alpha 1-4 glycosidic bond present between glucose units in polysaccharides. The production of α-amylase by the bacteria present in the gut of silkworms is also documented (Anand *et al*., 2010). Studies have revealed that certain gut bacteria have the capability to synthesize α-amylase, which can contribute to the breakdown of complex carbohydrates within the gut from dietary intake. The digestion of complex carbohydrates in the gut, from the dietary intake, diet of host that has direct impact on nutrient digestion process of the host animal. The α-amylase activity is important for proper nutrient acquisition and growth of insect (Mendiola-Olaya *et al*., 2000; Ahmadi *et al*., 2012).

Proteinase is group of enzymes that are required for the digestion of protein molecules into simpler readily absorbable form of peptides and amino acids that can be absorbed by the body for energy and nutrition. Proteases are also produced by various organisms including the animals, plants and microorganism. In insects' proteases plays a crucial role in the breakdown of dietary protein in the gut. In addition gut microbes particularly bacteria can also produce proteases, which can aid in the breakdown of dietary proteins in the gut of host insect. Earlier study have shown that the composition of gut microbes and their protease production can affect the digestion of dietary proteins and the absorption of amino acids, which can impact various aspect of health including immune function, metabolism and disease risk (Shinde *et al*., 2012).

Lipase (EC 3.1.1.3) is an enzyme that can catalyse the hydrolysis of triacylglycerols (TAG), which results in the production of fatty acids. Lipases are the enzymes which play a key role in lipid acquisition, storage, and mobilization (Santana *et al*., 2017). Lipase enzyme is found in many animals, plants, bacteria, and fungus and can be used in a variety of applications including food, detergents, pharmaceuticals, and cosmetics. In lepidopteran insects' unsaturated fatty acids and linolenic acids plays a crucial role in their survivality (Dadd, 1985). These fatty acids are essential components of their diet and are needed to support various biological functions necessary for their survival and lack of which lepidopteran insects may experience nutritional deficiencies that can impact their growth, development and overall health. In silkworms, these enzymes play an important role in the digestion and effective transformation of organic food molecules of the leaves into simpler assimilable nutrients ultimately determining the overall growth and development of the silkworm larvae (Mala and Vijila, 2017). Several researchers such as Anand *et al*. (2010); Feng *et al*. (2011); Prasanna *et al*. (2014); Gandotra *et al*. (2018a); MsangoSoko *et al*.(2021); MsangoSoko *et al*. (2022) have provided evidence on the ability of intestinal bacteria to produce digestive enzymes like amylase, cellulase, proteinase, gelatinase, and lipase in silkworms.

Therefore, in the present study of the gut bacterial community structure based on the food plants and their intricate relationship with the host organism will be a very promising aspect of host micro-ecology. Furthermore, the identification and characterization of important key digestive enzymes producing bacteria may be utilized as a source of probiotics to improve the gut health and overall quality development of Eri silkworms as well for other biotechnical applications.