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Bacterial Pathogens of Silkworm: Impacts and Management Strategies

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ABSTRACT

Bacterial diseases affecting silkworms are collectively known as flacherie. It is one of the infectious diseases reported to cause huge crop loss upto tune of 27–35% with significant cocoon yield reduction of 11 to 15 kg 100 dfls^{-1} annually. It is caused by a wide variety of bacteria belonging to different genera viz. ,*Aeromona, Bacillus, Enterobacter, Pseudomonas, Serratia, Streptococcus, and Staphylococcus.* The poor quality mulberry leaf with low protein, sucrose or high cellulose makes silkworms comparatively more susceptible to infection by pathogens. Many factors *viz.,* improper rearing house, faulty rearing practises, improper disinfection and hygiene, poor quality of nutrition, host susceptibility, host population density, synergistic association of pathogens and host population density lead to bacterial disease outbreak in the silkworm population. The silkworms appear physiologically weak and flacherie outbreak elicits a heavy toll on sericulture every year. The growth of affected silkworm larvae becomes stunted, flaccid and larvae develop different colours, depending on the species of bacteria involved in causing the infection, become rotten and foul smelling. Microscopic tools help in detection of silkworm pathogens and play a significant role in the management aspects. Many prophylactic measures are adopted to reduce the disease outbreak in silkworms. Antibiotics such as erythromycin, kanamycin, streptomycin, chloramphenicol, Aureomycin, neomycin and tetracycline have been reported to be suppressive against bacterial disease of digestive system. The plant extracts bearing anti-microbial activity can be used to control bacterial diseases in silkworm, *B. mori.*

KEYWORDS: Antibiotics, *Bacillus thuringiensis*, bacterial, disease, flacherie, septicemia toxicosis

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1. INTRODUCTION

Bacterial diseases which affect silkworms are collectively known as flacherie which is a syndrome identified by flaccidity, loose elasticity, softening and rotting of larval body (Karthikairaj et al., 2014; Park et al., 2024). Flacherie is considered as most dreadful disease in India identified often causes dysentery in silkworm (Saad et al., 2019; Satish et al., 2024). It may be caused by synergistic effect of different bacteria with viruses such as nuclear polyhedrosis virus (NPV), cytoplasmic polyhedrosis virus (CPV), infectious flacherie virus (IFV), densonucleosis viruses (DNV) (Aruga, 1971; Chitra et al., 1975). Many causal agents may be responsible for mixed infection to cause flacherie (Haloi et al., 2016; Tayal and Chauhan, 2017). During primary infection, the physiological state of the host is altered which often originates secondary infection in the host organism (Suraporn et al., 2025). The primary infection is mostly caused by virus whereas bacteria cause the secondary infection. Flacherie is an infectious disease reported to cause huge crop loss upto tune of 27-35 % with significant cocoon yield reduction of 11 to 15 kg 100 $dfls^{-1}$ annually (Chakravorty and Pandey, 2005; Selvakumar and Savithri, 2012). Savanurmath et al. (1992) reported the flacherie as the most pre-dominant (47.9%) among the diseases of silkworms of *B. mori*. Flacherie is prevalent to the extent of 22.33% to 43.05% throughout the year (Sivaprasad et al., 1995). Samson et al. (1990) reported bacterial flacherie to be highest (57.2%) followed by cytoplasmic polyhedrosis virus (27.88%), nuclear polyhedrosis virus (5.00%), pebrine (2.32%) and muscardine (0.48%) in a three year detailed survey on incidence of silkworm diseases in sericulture. In a commercial rearing, bacterial flacherie incurred loss of 48.9% (Shashidhar et al., 2018). Under different physiological stress conditions, the bacterial propagation and multiplication encourages the flacherie disease development (Mohanta et al., 2015). However, some adverse environmental conditions viz., high temperature, high humidity, polluted air and starvation (Samson et al., 1981) are the predisposing factors for flacherie disease development and cocoon croploss (Hossain et al., 2017). Kumari et al. (2001) reported that with the increase in temperature the metabolic activities of 4th and 5th instar silkworms get accelerated while as the dip in optimal temperature slacken the growth of 4th and 5th instar silkworms which proves the sensitivity of silkworms to temperature fluctuations (Chopade and Raghavendra, 2021). Kumari et al. (2001) reported that exposure of high temperature to 5th instar silkworms reduces their survivability. Flacherie incidence in Jammu and Kashmir was studied by chisti et al. (1991) and the result of the study shown that the intensity of infection was 3–8% in worms reared scientifically and 12–35% in worms reared under unhygienic conditions. The disease incidence was mostly reported in 4th and 5th instars (Manimegalai, 2010). Bhat et al. (2016) reported that disease incidence % was maximum in 5th instar**.** *Bacillus thuringiensis* is a detrimental bacterium which is considered to be causal organism of flacherie. An extreme level of risk to sericulture is enforced when flacherie is caused by B. thuringiensis (Smith, 1999). Even low concentration (0.01%) of bacterial infection in silkworm larvae leads to convulsions and causes more than 50% mortality during 4th instar (Aruga, 1994; Attia et al., 2025). Hence flacherie disease pose a serious challenge to sericulture and it is little bit difficult to manage the disease effectively and eliminate the pathogen load from rearing environment (Park et al., 2024).

2. CAUSAL ORGANISM

A wide range of bacteria belonging to different genera viz., *Aeromona, Bacillus, Enterobacter, Pseudomonas, Serratia, Streptococcus* and *Staphylococcus* are reported to be the causal organisms of flacherie disease (Parbin et al., 2024). The other causes of flacherie such *Proteus vulgaris, Proteus inconstana, Proteus morganii, Aerobacter aerogenes, Micrococcus flavus and micrococcus* species were reported by, many authors from time to time. A bacterial species, coccus and spore forming bacterium *Bacillus bombycis* in addition to a number of pathogenic bacteria including *Bacillus cubonianus* were reported to cause flacherie disease in silkworm (Santha et al., 2007). The flacherie is the most vulnerable disease when it is caused by bacterium, *B. thuringiensis. B. thuringiensis is a* spore forming gram-positive and soil inhabiting bacterium (Nazar et al., 2020) can be found on surface of leaves, midgut of lepidopteran caterpillars and in aquatic environment (Dashora et al., 2017). Many bacteria viz., *Bacillus* spp., *Bacillus thuringiensis, Micrococcus* spp., *Pseudomonas* spp., *Streptococcus bombycis* and *Serratia marcescens* are reported to be associated with flacherie as well which weaken silkworm immunity (Table 1). Therefore, causes significant cocoon crop losses in sericulture (Javaid et al., 2021). *Bacillus megaterium* and *Bacillus ellenbachi* are also reported to be the causal organism of flacherie disease in silkworm (Santha et al., 2007). A chronic type of flacherie known as 'gattine' in French and 'macilenze' in the Italian is caused by *Streptococcus bombysis* which is isolated from the

Table 1: Different types of flacherie and their causal agents

Sl. No.	Flacherie disease	Causal agent	Pathogen shape	reference
1.	Bacteremia	*Streptococcus faecium*	Spherical	Park et al., 2024
2.	Septicaemia	*Serratia marscens*	Rod-shaped	Hanh et al., 2024
3.	Toxicosis	*Bacillus-thuringiensis*	Rod-shaped	Kumar et al., 2016

intestine (Das, 2008; Santha et al., 2008). 'Khuto' disease (multi-bacterial dysentery) was caused by feeding silkworm with leaves grown in shade and deficient in chlorophyll (Das, 2008). In southern china, the spore forming bacterium was isolated from pupae of silkworm (Liang et al., 2015). *Bacillus mycoides* infected into the body and Bacillus *laterosporus* administered were found pathogenic to silkworm (Nesa et al., 2020).Nakasuji and Kodama (1969) found *Aerobacter cloacae* and *Achoromobacter superficialis* to be pathogenic to silkworm. *Streptococcus faecalis, Streptococcus faecicum, Serratia piscatorum, Serratia marcescens, Proteus vulgaris, Proteus inconstana, Proteus morganii, Aerobacter aerogenes, Micrococcus flavus and micrococcus* species were some of the other pathogenic bacteria species (Santha et al., 2007). Chitra et al. (1974) isolated seven bacteria species from flacherie infected worms and distinguished bacterial disease broadly into two classes such as those that are restricted to the intestine (*Achromobacter cloacae, Archromobacter superficial, Archromobacter delmarvae, Pseudomonas boreopolis, Pseudomonas ovalis, Escherichia freundii, and Staphylococus albus*) and those that enter into thehaemolymph (*Aerobacter cloacae, Archromobacter delmarvae, Achromobacter seperficialis*) and to both (septicaemia). The flacherie (sappe) disease might be a syndrome where multiplicity of pathogens is involved (Mohanta et al., 2013). Investigations conducted by Vasantharajan and Munirathnamma (1978) indicated the presence of *Serratia marcescens* in Kenchu disease of silkworm could cause a lethal infection when associated with *Staphylcoccus* species. It is reported several septic bacteria causing septicaemia in silkworm from cocoons and out of the septic bacteria isolated, Serratia was most abundant (Park et al., 2024). Bacteria belonging to different genera were isolated *viz.*, *Bacillus, Staphylococcus and serratia* from disease silkworm (Priyadarshini et al., 2008). The first time *Streptococcus faecalis* was reported from diseased silkworm in India (Pryadarshiniet al., 2008).

3. PATHOGENICITY OF BACTERIA TO SILKWORM

The pathogenicity of bacteria depends on the mode of infection and the type of bacteria involved in causation of disease. Lysenko (1958) found that different strains of Streptococcus faecalis were more pathogenic than *Streptococcus faecium*. The isolates of bacteria such as *Aerobacter cloacae*, *Archromobacter superficialis, Achromobacter delmarvae, Staphylococcus albus, Escherichia freundii, Pseudomonas ovalis* and *Pseudomonas boreopolis* when fed to the silkworm caused flacherie with different rates of mortality (Chitra et al., 1973). Chitra et al. (1974) reported that severe bacterial flacherie was observed during moulting periods and when silkworms were ready for spinning which often leads to severe mortality.

Anitha et al. (1994) found that Serratia species is less toxic than Bacillus and staphylococcus and the pathogenicity of Staphylococcus comparatively higher than both, bacillus and serratia species. The septic bacteria including *Pseudomonas* sp., *Serratia marcescens, Proteus* sp., *Streptococcus faecalis, Aeromonas* sp., and *Bacillus* sp were found highly pathogenic to silkworm and caused Septicaemia in silkworm. The pathogenicity of Serratia showed a high mortality at high humidity and causes death within 18 hours of post infection(Santha et al., 2007). The human pathogenic bacteria were studied with silkworm infection models (Matsumoto and Sekimizu, 2019) viz *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Kaito et al., 2002), *Streptococcus pyogenes* (Kaito et al., 2005), pathogenic *Escherichia coli* (Miyashita et al., 2012), *Listeria monocytogenes* (Castillo et al., 2016), *Serratia marcescens* (Ishii et al., 2012), and *Vibrio cholerae* (Kaito et al., 2002). The injection of extracellular toxins, α-toxin and β-toxin of different *B. thuringiensis*, exotoxin A (*S. aureus*), diphtheria toxin (*P. aeruginosa*) or hemolysin (*B. cereus*) were reported to kill silkworms (Hossain et al., 2006; Usui et al., 2009). The toxins of *Bacillus thuringiensis* acts by producing a protein that blocks the digestive system of larvae and induce vacoulation of midgut cells, swelling and vacuolation in mitochondrial cells and tissues of malphigian tubules in *Bombyx mori* (Zou et al., 2024). The vacuolation and nuclear condensation in other insects due to B. thuringiensis was also reported (Pandey et al., 2009; Badr and Darwish, 2024).

4. DIFFERENT GENERA OF BACTERIA AFFECTING SILKWORM

The enotomopathogenic bacteria quickly infect and easily multiply in silkworm body and causes reduction in silk production. The various genera of bacteria have been found to infect silkworms include *Bacillaceae, Pseudomonadaceae, Enterobacteriaceae, Streptococcaceae, Micrococcaceae, Paenibacillaceae*, *S. marcescens* and *S. entomophila*. Some of the bacteria such as *S. marcescens* and *S. entomophila* impair the feeding behavior and growth of lepidopterna insects. They proliferate in gut of insects and secrete large amounts of proteolytic enzymes such as β-hemolysins, elastases, and chitinases. The bacterial diversity of silkworm gut was proved by the presence of *Serratia marcescens, Pseudomonas aeruginosa,* and *Bacillus halotolerans* isolated from the diseased larvae (Javaid et al., 2021).

The microbiota in the silkworm gut was investigated by dilution culture method of intestinal juice at different developmental stages and 253 strains of bacteria belonging to 16 genera were isolated (Sun et al., 1996). Lu et al. (1999) studied the intestinal content of the healthy silkworm larvae and adult moths and reported 89 Enterococcus species isolates by utilizing numerical taxonomy of bacteria.

Different bacterial populations belonging to different genera viz., Arthrobacter, Lactobacillus, Pseudomonas, Escherichia, Micrococcus, Bacillus and Staphylococcus were identified (Yuan et al., 2006). Xiang et al. (2010) reported the presence of many predominant genera *viz.,* Brevundimonas, Stenotrophomonas, Enterobacter, Aeromonas, Brevibacterium, Citrobacter, Escherichia, Klebsiella,Pseudomonas, Agrobacterium and Staphylococcus in the silkworm gut.

Lu et al. (2003) reported that the distribution of intestinal bacteria changes with respect to the state of health of the silkworm and it was reported that the number of bacterial species of the genus *Enterococci* increases in silkworms which are already infected with Nosema bombycis and causes the epidemic disease pebrine in silkworms. The members of the many bacteria such *Proteobacteria* and *Firmicutes* phyla are present in many lepidopteran insects viz., armyworms, mosquitoes and silkworms. The intestinal bacterial microbiota of insects share similar characteristics and there are differences in the composition and diversity of bacterial microbiota in different insects, stated that the diversity of gut bacteria in insects can be affected by environment, habitat, diet and developmental stage. Tian et al., 2007 reported the predominant genera in the bacterial microbiota of silkworm strain (Dongting×Bibo) were Brevundimonas, Stenotrophomonas, Enterobacter and Staphylococcus. Xiang et al. (2007) reported Enterococcus and Thermus as the predominant genera in the C108 and SCN2 strains of silkworm. Sun et al. (2016) reported the presence of predominant genera *viz.,*Enterococcus, Delftia, Ralstonia, Pelomonas, Tepidimonas,Aurantimonas, Pseudomonas, Aspromonas and Staphylococcus in strain Daizo.

5. ISOLATION AND PURIFICATION OF BACTERIA

Pathogenic bacteria causing flacherie in silkworms are isolated from diseased larvae (Selvakumar, 2013). The diseased larvae are disinfected in 2% sodium hypochlorite for 3 to 5 minutes and the bacterial strains are collected from the gut. The gut material is suspended in sterile saline water and spread on agar medium incubating at 37 degree Celsius overnight for bacterial culture (Li et al., 2022). Streak plate method is used to purify the bacterial culture (Liang et al., 2015). The bacterial strains once isolated can be permanently stored in 15% glycerol for future (Javaid et al., 2021). The cadavers showing bacterial infections are surface sterilized with mercuric chloride (0.1%) and washed three times in distilled water. The larvae are titrated in sterile water blanks with glass rod. Using the inoculation needle, a loopful of the suspension is taken and streaked on Nutrient Agar medium and incubated at room temperature (Govinda et al.,1998). After growth of bacterial pathogens in nutrient agar medium, the isolated pathogens are sub-cultured using streak plate method. The bacterial colonies are purified by streaking on the plate. The isolated single colonies of bacteria are separated by picking and streaked on nutrient agar slants. Once the bacteria attain good growth, the slants are stored in refrigerator at 4°C for further studies (Robert et al., 2002). Anitha et al. (1994) collected infected silkworm of various larval development stages and studied the morphology of bacteria. They reported about 28% of bacteria showed a similar morphology consisting of big gram positive rods with endospores while as 28% consisted of gram-positive cocci morphology, which were seen in clusters while about 34% of cases showed gram-negative tiny rods (Table 2). The biochemical activity and characteristic morphological study identified different bacteria viz.,Bacillus sp., Staphylococcus sp and Serratia sp (Table 3 and 4) (Lakshmi and Jamil, 2002). Anitha et al. (1994) isolated pathogenic bacterium from 125 samples of faecal materials and dusts have been characterized as *Streptococcus faecalis.* Several strains of *Streptococcus faecalis* and *Streptococcus faecium* based on physiological characters were isolated (Santha et al., 2007). Javaid et al. (2021) isolated some bacterial strains showing close relevance with *Serratia* species, *Bacillus* species and *Pseudomonas* spp. Approximately 83.3% of strains were found resistant to Penicillin, Tetracycline, Amoxicillin, Ampicillin, and Erythromycin. Aneja (1996) identified predominent colonies from four samples by various tests which includes biochemical tests, colony morphology, motility, sugar fermentation and staining properties.

Table 2: Identification of bacteria in diseased silkworms (Aneja, 1996)

Bacteria	Gram's staining	Shape	Motility	spore
Bacillus subtilis	+	rods	+	+
Streptococcus pnumoniae	+	cocci	_	_
Staphylococcus aureus	+	cocci	_	_
Escherichia Coli	_	rods	+	_
Pseudomonas fluorescence	_	rods	+	_
Bacillus cerus	+	rods	+	_
Klbsiella cloacae	_	rods	_	_

'+' indicates ' positive and '_' indicates 'negative'

6. MODE OF ACTION

The proliferation of *S. aureus* indicated that the killing effect of bacteria requires bacterial growth in silkworm. The killing effect of *P. aeruginosa* to C. elegans has two

Table 3: Biochemical analysis for identification of bacteria (Aneja, 1996)

Bacteria	Catalase	Coagulase	Starch hydrolysis	Lipid hydrolysis	Gelatin hydrolysis
Bacillus subtilis	–	–	–	–	–
Streptococcus pnumoniae	–	–	–	–	–
Staphylococcus aureus	+	+			
Escherichia.coli	–	–	–	–	–
Pseudomonas fluorescence	–	–	–	–	+
Bacillus cerus	–	–	–	–	–
Klbsiella cloacae	–	–	–	–	–

'+' indicates 'positive and '_' indicates 'negative'

Table 4: Characterization of bacteria for carbohydrate metabolism (Aneja, 1996)

Bacteria	Glucose	Lactose	Sucrose	Mannitol
Bacillus subtilis	+	–	+	–
Streptococcus pnumoniae	–	+	–	–
Staphylococcus aureus	+	+	–	+
Escherichia coli	+	+	+	+
Pseudomonas fluorescence	–	–	–	+
Bacillus cerus	+	–	+	+
Klbsiella cloacae	+	+	+	–

'+' indicates 'positive and '_' indicates 'negative'

different modes of action. One is 'fast killing' which is caused by exotoxins secreted into the culture medium and another one ' slow killing' which occurs after proliferation of bacteria in the insect bodies (Kaito et al., 2002). The bacterial toxins are stimulated to active form by silkworm gut enzymes. The activated toxin binds to the receptor and subsequently gets inserted into the membrane and causes leakage of ions and small molecules (Chen et al., 2020). The silkworms become weak, the metabolism become inactive which causes imbalance in body functions. As the digestive fluid of silkworm gut weakens, the bacteria devoured together with mulberry leaf multiplies in the digestive tract and takes nutrition from the body of the silkworm which destroys the membranous tissue of the intestine (Yuan et al., 2023). The mortality in the larval stages of *B. mori* is attributable to eight genotypes of *Bacillus thuringiensis* within 3 hours of post inoculation when *B. thuringiensis* endotoxin cause damage to gut lining cause gut paralysis and the larval death in silkworm occurs due to starvation (Bhowmik, 2014). The beta endotoxin of *Staphylococcus aureus*, *Bacillus cereus* and *Pseudomonas aeruginosa* causes toxidermia and septicemia disease and eventually death of the silkworm larvae.

The bacteria multiply in silkworm while undergoing different developmental stages inside the body. A typical growth curve shows different phases of growth which includes lag phase, exponential phase and stationary phase.

6.1. Lag phase

It is the latent period or equilibrium phase before the bacterial cell division. It is most poorly understood growth phase and controlled by unknown regulatory mechanism. The lag phase has been assumed to allow the adaptations for exploitation of new environmental conditions and the bacterial cells begin accordingly. This phase is not characterized by physiological or biochemical criteria but it is supposed to be the active phase of bacteria (Madigan et al., 2000).

6.2. Exponential phase

This phase represents the processes of cell divisions and the cessation of divisions respectively. Many factors influence the exponential phase which includes nutrient availability, presence or absence of inhibitory factors and environmental conditions (Navarro-Llorens et al., 2010). The exponential bacterial growth physiology and replication involves DNA synthesis, transcription and translation process for synthesis of DNA-RNA-Protein and some necessary biomolecules (Rolfe et al., 2012).

6.3. Stationary phase

At this stage growth cessation occurs but cells remain metabolically active. This phase comes after exponential growth and conditions become unfavorable for growth of bacteria and they stop replication. This phase leads to many physical and molecular changes. The physical changes includes changes in cell structure, they become spherical in shape and smaller in size surrounded with a rigid cell envelope, the cell wall becomes highly cross-linked,

membrane fluidity gets reduced and cells are activated to the stringent response mechanism to survive (Jaishankar and Srivastava, 2017). During this division, the rate of cell division is directly proportional to the rate of cell death. After this comes the death phase of bacteria and cells lose viability.

The contaminated mulberry leaves with bacteria, exuviae of bacterial infected silkworm, excreta of infected silkworms, pest infested mulberry leaf contains bacterial pathogens and get introduced into a healthy population (Mondal et al., 2025). The unhygienic leaves and wet leaves may consist of bacterial pathogens which get inoculated into silkworm. The physiological starvation is caused when silkworms are fed with unhygienic and poor nutritive mulberry leaves due to which silkworms does not produce anti-bacterial factor (Mir et al., 2018; Dai et al., 2025). The bacteria propagating on the unhygienic rearing environment gets ingested with leaf into the silkworm body and causes flacherie outbreak (Dai et al., 2025). The toxins produced by microorganisms viz., *Staphylococcus aureus* and *Pseudomonas aeruginosa*, provide a selective growth advantage to bacteria in silkworm rearing environments. The toxins produced by bacteria aid in combating the immune system of host silkworm and securing nutrients for better growth. *Staphylococoocus aureus* produces different types of components which enhance the virulence of bacteria by contributing in different mechanism viz., surface associated adhesions, exoenzymes, capsular polysaccharides and exotoxins (Cheung et al., 2021).

Bacteria infect silkworms through two different ways viz., ingestion or wounding. Ingestion refers to entry of bacteria into the midgut of silkworm through mulberry leaves which are contaminated by it (Miyashita et al., 2015). Infection through wounds is caused when bacteria directly invades the haemocoel through wounds on body surface (Wang et al., 2022). The bacterial species causing disease in silkworm mainly belongs to genus Bacillus. Some of the bacillus species causing disease in silkworm are *Bacillus cuboniaus*, *Bacillus bombysepticus*, *Bacillus mycoides*, and *Bacillus leterosporus*. There is one another possible mode of transmission of bacterial disease in silkworm which refers to transovarial transmission and persistence of latent infection in the silkworm eggs. However, no such mode of bacterial infection transmission has been reported earlier in silkworm. Rai et al. (2013) found a bacterium *B. subtilis* which was isolated from the eggs of silkworms and was found prevalent in progeny eggs laid by infected females. It is probably the first report transovarial transmission of Bacillus species in insects which confirms the bacteria inoculated to parents are transmitted to progeny eggs. It indicates the transovarial transmission of bacteria is observed in silkworm, if it survives till the adult stage.

The bacteria invade the silkworm body through mouth or integument and proliferate in the digestive organs of silkworm. Though different pathogens multiply in different parts of the body and the host succumbs to infection inshorter period (Deb et al., 2021). In case of septicaemia, the bacteria penetrate the haemolymph through membranous cells of the alimentary canal of silkworm and propagate into large numbers causing septicaemia disease (Yup-Lian, 1991). Due to damage of midgut membrane, the silkworm starves which makes it wander aimlessly and causes death (Trager, 2012). The cephalothoracic region becomes translucent and in the advanced stage even the posterior region becomes translucent and the larvae show symptoms of diarrhoea and develop foul smell (Mallikarjuna and Balasaraswathi, 2023).

The infection of bacteria lowers the gut pH and provides congenial conditions for growth of other pathogens. Due to faster multiplication of bacteria in gut, the peritrophic membrane degenerates and blocks the absorption of nutrients which causes stoppage of feeding and sluggishness in silkworms (Rajakumari et al., 2007). A change in blood pH also leads to general paralysis in silkworms and leads to leakage of gut contents into the haemocoel. In 1970, the production of 'late moulter' in silkworm rearing was found to be associated with bacterial septicaemia. The changes in protein and fat content in haemolymph and intestinal part of the 5th instar larvae was reported when infected with *Serratia marcescens* (Guo-Ping and Xi-Jie, 2011). The carbohydrate content was found to be lower in intestines and higher in haemolymph (Sam et al., 1994). The bacteria disintegrate fat body, trachea and other tissues (FuKuda and Lwashita, 1988). The bacteria multiplies in attached specific tissue surfaces and releases different components such as immunosuppressive factors, special metabolites and toxin proteins which results in the rapid death of host silkworm (Zhang et al., 2023). The silkworm cadavers infected with bacteria shows slow growth, sluggishness, body shrinkage, oral and anal discharge, swelling of thorax, rupturing of skin, loses appetite and elasticity and oozes a brown fluid with foul smell (Gudimalla et al., 2020). *B. thuringiensis* produces crystal protein or Cry protein that gets attached to epithelial layer of midgut and rupture it (Mullins, 1985; Nazar et al., 2019). The ruptured midgut and larval body liquefies. The haemocyte number significantly decreases and signs of rupture integument increase (Broderick et al., 2010).The bacteria, *Serratia marcescens*, were isolated from exuvium of the cocoons the same bacteria was smeared on body surface of 5th instar larvae before cocooning. It confirms the fact that bacteria adheres to the body surface of silkworms and gets introduced into cocoons when infected in 5th instar. It causes infection, death, and putrefaction in cocoons within an extremely short time (Enomoto et al., 1987).

7. HOST RANGE

Microbes have symbiotic association with various insect hosts and their interactions lead complementary to high pathogenic responses. Bacillus thuringiensis is a soil dwelling micro-organism found to infect a range of lepidopteran insects including *Spodoptera littoralis, Helicoverpa armigera, Spilarctia obliqua* and *Bombyx mori* as well. The vigorous feeding on infected leaves ingests B.thuringiensis toxins which influences the larval stage of susceptible worms (Thakur et al., 2015). Other than bacillus species, there are are many bacteria which infect a wide range of lepidopteran insects. Serratia marcescens isolated from the gut of *Rhynchites bacchus* were found to cause mortality in the same host and silkworm *B. mori* as well. The species of genus Pseudomonas, Bacillus, Staphylococcus, Enterobacter and Enterococcus are found to be most widespread and infect a range of insect hosts. In lepidopteran insects, the gut bacterial families belong to proteobacteria phylum. Around 60% of the lepidopteran species were screened for the presence of bacteria belonging to enterobacteriaceae, bacillaceae, pseudomonacea, staphylococaceae and enterococaccaceae families (Voirol et al., 2018). For instance, the pathogenicity is caused by Enterococcus bacteria present in the midgut of *Manduca sexta* Linnaeus (Mason et al., 2011). Also, Enterobacter cloacae which is commonly found in the intestines of insects when administered orally to Spodoptera litura causes alterations in the intestinal microbial community and leads to pathogenicity (Thakur et al., 2015). The oral administration of Flavobacterium species, Klebsiella species and Serratia marcescens has been confirmed to be pathogenic to insects (Zhang et al., 2020).

8. SYMPTOMS OF BACTERIAL FLACHERIE

The symptoms caused by different bacteria are a typical and are of general type. The growth becomes stunted and the larvae become flaccid (Plate 1). The dead larvae develop different colours, depending on the species of bacteria involved in causing the infection, become rotten and foul smelling (Yup-Lian, 1991; Zhang et al., 2020). The silkworms appear physiologically weak and flacherie outbreak elicits a heavy toll on sericulture every year (Aruga, 1994). The silkworms affected with flacherie lose appetite and become sluggish. At the beginning, no colour change occurs but infected silkworms turn flaccid later with vibrant discolouration and larvae turn dark brown. Larvae wriggle in pain due to rapid pulsation of dorsal vessel. Vomiting brown fluid is also a symptom, excreta becomes soft and sticks to the rearing bed. During moulting, skin is not shed properly. A change in physiological state, alternation in biochemical constituents and haemolymph composition is reported in flacherie infected larvae which is often observed with stoppage of feeding by infected larvae (Pawar and Ramakrishnan, 1977; Begum et al., 2004).In case of septicemic flacherie, larvae turn red or brown in colour depending upon the pathogen infected to silkworm and causes diarrhoea in severe infections (Santha et al., 2007). B. thuringiensis causes shrinking and softening of larval body. Hence, it is also known as as shrinking disease, softening disease or faecal disease. *Bacillus thuringiensis* in silkworm cause abrupt halting of feeding, head movements, spasms, tremors, paralysis, distress, sudden collapse of body and death in case of acute bacterial toxicosis. Death may occur within ten minutes to a few hours (Yup-Lian, 1991). Shortly after death, the silkworm is extended and appears hard for touch with the head retracted so as to assume a hook-shaped appearance. Gradually, the dead silkworm becomes black and rotten, exuding a foul smelling dark brownish fluid (Santha et al., 2007). Chronic bacterial toxicosis is caused by ingestion of small quantity of *Bacillus thuringiensis* crystal toxin (Mitsuhashi and Miyamoto, 2020). The thorax and abdominal tip become transparent and the silkworms are often motionless among the litter. After 10 to 12 hours of infection, the larvae become sluggish, lose clasping power, body turn flaccid and eventual death of larvae (Anitha et al., 1994; Mallikarjuna and Balasaraswathi, 2023). Silkworm infected with bacteria cocci exhibits body shrinkage, skin softness, stunted growth and turn light brown in color (Santha et al., 2007).

Plate 1: Flacherie infected silkworms

9. SUSCEPTIBILITY OF SILKWORMS TO BACTERIAL INFECTION

Silkworms are susceptible to bacterial infections and degree of susceptibility may vary with race and larval instar (Anusha and Bhaskar, 2018). Infection with some bacterial pathogens causes high mortality and maximum deaths occur during moulting and just before spinning. Also irrespective of the age of infection, mortality is maximum in the final instar. The susceptibility of fifth instar larvae to infection is more or less in the same (Tayal and Chauhan, 2017). The younger larvae show varying degree of susceptibility (Rahmathulla, 2012; Tayal and Chauhan, 2017). Kumari et al. (2001) investigated sensitivity of 4th and 5th instar silkworms towards temperature fluctuations. With the increase in temperature, the metabolic functions also increase while as with the decrease in temperature, the

metabolism of silkworms is totally shaken. A stress factor is generated in silkworms when temperature goes higher or lowers than 25°C and it enhances the susceptibility of silkworm to viral infections (Steinhaus, 1958). Kato (1989) investigated that high temperature exposure to 5th instar larvae declines the survival rate of silkworms. The most susceptible instar is third instar as revealed by maximum mortality due to infection at this instar (Santha et al., 2007). The susceptibility of silkworm to bacterial infection is governed by the nutritive factors (Zha et al., 2021). The poor quality mulberry with low protein, sucrose or high cellulose makes silkworms comparatively more susceptible to infection by pathogens (Urbanek et al., 2022). The rise in silkworm rearing temperature accelerates the development of bacterial disease (Huang et al., 2009). *Aerobacter cloacae* and *Staphylococcus albus* are observed to be more lethal to late age larvae than to early instar larvae and the reverse is true with *Escherichia freundii*. A silkworm exposed to small doses of *B. thuringiensis, Var alesti* leads to chronic infection, but on exposure to higher temperature of around 30°C rapidly develops into fatal bacteriosis (Paudel et al., 2021; Sun et al., 2022). High temperature and humidityleads to dysfunction of alimentary canal, which in turn leads to *Enterococcus* multiplication (Santha et al., 2007).

10. DETECTION OF BACTERIAL DISEASE IN SILKWORM

There is no such process in detection of this disease, it is detected by observing the symptoms of disease (Chopade et al., 2021). Morphologically the disease can be identified by presence of soft and lethargic larvae which rapidly stop feeding and rot (Yadav et al., 2016). The larvae with retarded growth vomit gut juices excrete semi-solid faeces and the inner content of gut turn blackish color which emit foul smell (Sakthivel et al., 2012). However for the early detection of disease the microscopic study is important. Direct microscopical and bacterioscopic examinations are carried out to exclude the risk of the presence of pathogens. The developmental stages of silkworm, egg and larval stage are subjected to microscopy and examinations in sericulture. The molecular tools play a profound role in detection of silkworm pathogens in sericulture. These tools have revolutionized the detection of microbes due to its special attributes viz.,early and timely detection, high accuracy and sensitivity, superior specificity and rapid interpretation and analysis. The primary step to detect pathogens relies on the identification of pathogen to control infectious diseases in sericulture. The preparatory steps such as genomic Deoxyribo nucleic acid (DNA) and Ribonucleic acid (RNA) extraction by lyses of tissue sample are required to detect the pathogen. The traditional protocols or commercially available kits are used to achieve the preparatory process. After preparatory the quantification of extracted genomic DNA or RNA is required (Deepika et al., 2024). The 16S rRNA gene is common to all prokaryotes. It is often used as a marker for identifying bacterial species (Schmidt et al., 1994). Hence it is a powerful tool to detect and identify a particular bacteria infecting silkworm. The restriction pattern of 16S rRNA gene amplification of bacterial gut metagenomic DNA of silkworm was used to detect the bacterial population infecting silkworm.Some advanced techniques viz., scanning electron microscopy, florescent microscopy, and phase contrast microscopy and transmission electron microscopy are also used for diagnosis of silkworm diseases (Rabha et al., 2024). However these techniques are sophisticated, expensive and requires technically sound personnel for the maintenance of high costs equipments. These factors limit the use advanced microscopic techniques for diagnosis of silkworm pathogens. The immunological techniques viz., Enzyme linked immunoassay (ELISA), immuno-diffusion assay, immunofluorence assay are widely used for detection of *Nosema* (pebrine) and infectious flacherie virus in sericulture. However no work has been done with detection of bacterial pathogens using immunological diagnostics in sericulture. However, more advanced molecular techniques possess remarkable attributes to be utilized for detection of pathogens. Polymerase chain reaction (PCR) is an efficient detection technique for initial diagnosis of disease. It has been widely utilized for detection of different pathogens including bacteria as well. *Bacillus* species and *Pseudomonas* species are detected through PCR (Subrahmanyam et al., 2023). Unlike PCR, other molecular techniques like restriction fragment length polymorphism (RFLP), Enzyme linked immunosorbent assay (ELISA), western blotting, lateral flow assay (LFA)are also used to detect bacterial pathogens affecting silkworm (Deepika et al., 2024). Restriction fragment length polymorphism (RFLP) analysis and nucleic acid fingerprinting can help to detect differences in the genomes of closely related microbial species. Additionally, it can detect the involvement of a particular pathogen in disease outbreak.

11. MANAGEMENT OF BACTERIAL DISEASE

Improper disinfection of rearing house and environment causes the build-up of bacterial pathogens in the rearing environment and this can be destabilizing factor (Trivedyet al., 2011). Thorough disinfection of rearing room and rearing equipments with recommended general disinfectant is essential to ensure elimination of pathogens from the rearing environment before initiation of rearing (Davies and Wales, 2019). Different bed disinfectant are used in silkworm rearing to curb the bacterial diseases which includes vijetha, hydrated lime, active lime, captan,

Resham Keed Oushad (RKO), Ankush, Jyothi, Labexetc (Shashidhar et al., 2018). The application of chemical based and season specific bed disinfectant ' Sanjeevni' for control of bacterial flacherie is recommended in sericulture.Use surface disinfected quality eggs and rear the early instar of silkworms under optimum conditions. In order to prevent the outbreak of bacterial flacherie, the silkworm should be reared on nutritive mulberry leaves under hygienic and congenial rearing conditions (Gupta and Dubey, 2021). Application of silkworm body and rearing bed disinfectants as per recommended schedule and quantity prevent the spread of bacterial diseases (Habeanu et al., 2024; Chauhan and Tayal, 2017). It is essential to provide required bed space as per the instar in addition to ventilation. In order to prevent flacherie and thatteroga in silkworm rearing, the rearing trays and equipments or tools used for silkworm rearing are to be dipped in an effective disinfectant solution for 10 minutes (Dingle et al., 2005; Tayal and Chauhan, 2017). Ensuring good cross ventilation helps in reducing humidity between the rearing beds. Dusting of slaked lime before resumption of silkworms is helpful in keeping the humidity low especially during moulting reduces the pathogen build-up in the rearing environment (Singh, 2010). Use of antibiotics to suppress bacterial flacherie, especially bacterial disease of digestive organ is found to be effective (Darvekar et al., 2024). Antibiotics such as erythromycin, kanamycin, streptomycin, chloramphenicol, Aureomycin, neomycin and tetracycline have been reported to be suppressive against bacterial disease of digestive system (Kahn, 2016; Montali et al., 2020). Enriching the mulberry leaves with 0.1% gentamycin was found to reduce flacherie (Nanda et al., 2024). As a prophylactic measure, amplicillin at the rate of 500 ppm can be used to treat mulberry leaves and fed to silkworms during the first feeding of 3rd and 5th instars to manage bacterial flacherie in order to harvest better cocoon crops (Manimegalai and Chandramohan, 2008). The plant extracts bearing anti-microbial activity can be used to control bacterial diseases in silkworm, B. mori. The botanicals are used to control the bacterial infections in silkworm. Many plants viz., aloevera, neem, viscum album, were found posses anti-microbial activity (Yusuf et al., 2013; Ujjwala et al., 2006).

12. CONCLUSION

Bacterial flacherie is most outrageous disease causing significant economic losses in sericulture. Many bacterial species are causal organisms. Different diagnosis strategies have been developed, among which the artificial tools using sensors are most reliable to detect the pathogen presence. The implementation of precautions and prevention strategies can be adopted for healthy cocoon crop production. The modern techniques like feeding of antibiotic fortified leaves and application of botanical bed disinfectants may help to sustain healthy cocoon production and promote smart sericulture.

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