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RESEARCH ARTICLE

ISOLATION & IDENTIFICATION OF BACTERIA RESPONSIBLE FOR BACTERIAL FLACHERIE IN SILKWORM (*BOMBYX MORI* L.) & ITS MANAGEMENT BY ANTIBIOTICS

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ABSTRACT

The commercial production of silk is called as "Sericulture" which is a labour intensive, agro-based cottage industry, ideally suited not only to India but to all developing countries of tropical belt. Mulberry silkworm (*Bombyx mori* L.) is a monophagous & multivoltine insect since it is a producer of silk. There are many factors that influence the success of silk production. Bacterial flacherie considered the most important factor. Flacherie is a Syndrome associated with bacterial disease. In the present study, diseased silkworms were collected & the pathogens were isolated and identified based on its morphological, physiological & biochemical features. A gram negative, rod-shaped bacterium was isolated from diseased silkworm. The colony was round, slightly convex, smooth, moist; translucent regular-edged and slightly pinkish on MacConkey Agar confirming the presence of Proteus strain family Enterobacteriaceae. Antibiotics showed promising results for controlling of silkworm diseases & to improve the production of silk & harvesting superior crop. Oral supplementation of antibiotics through mulberry leaves significantly reduced the incidence of Flacherie disease. The antibiotics play a significant role in conferring immunity to the silkworms thereby increasing the effective rate of rearing. The main objectives of this study to identify the pathogen which causes flacherie disease in silkworm (*Bombyx mori* L.) and its management by application of antibiotics.

INTRODUCTION

The art of silk production is called sericulture that comprises cultivation of mulberry silkworm rearing and post cocoon activities leading to production of silk yarn. *Bombyx mori* L. has an economic value because of the commercial value of its silk. Therefore, several trials for developing the biological processes and improving the quantitative and qualitative characters of silk yield took place (Taha, 2002). In China, sericulture is an important part of agriculture. However, bacterial diseases often cause severe economical loss in sericulture. Silkworms are affected by a number of diseases due to various biological, chemical, physical, nutritional and environmental causes. Being poikilotherms silkworms respond very quickly to environmental changes, particularly to temperature and relative humidity (Zhang *et al*., 2013). Bacterial flacherie is a common disease of mulberry silkworm. The aetiology of bacterial diseases is not fully understood because of multiplicity of bacterial types involved in bacterial infections (Choudhary *et al*., 2002).

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Generalized symptoms of insects associated with bacterial infections are loss of appetite, diarrhea (discharge of watery feces) and vomiting then the larvae softening and die emitting a foul odour (Tanada & Kaya, 1993). In sericulture the productivity and quality largely depends on the healthiness, growth of silkworm larvae and the suitable environmental conditions. In this study, a previously unknown pathogenic bacterial strain isolated from a diseased silkworm, *Bombyx mori* L. The larvae of diseased silkworm were collected separately in distilled water and homogenized then, isolation of bacteria was done using agar plating method until the single pure colonies were obtained. The cultured colony was translucent and slightly pinkish in color when grown in MacConkey Agar medium plate. The isolates were identified as Proteus bacterium the family Enterobacteriaceae according to its morphological, physiological & biochemical features. Antibiotics showed promising results for controlling of silkworm diseases & to improve the production of silk & harvesting superior crop. Oral supplementation of antibiotics through mulberry leaves significantly reduced the incidence of Flacherie disease. The antibiotics play a significant role in conferring immunity to the silkworms thereby increasing the effective rate of rearing. The antibiotics such as Gentamycin,

Ampicillin and Tetracycline were found to be effective in reducing the mortality of silkworms by 23-25% without affecting the cocoon parameters. The antibiotics such as Gentamycin, Tetracycline, Ticarcillin and Ampicillin supplemented through mulberry leaves resulted in significant reduction in the occurrence of both grasserie and flacherie diseases (Santha *et al*., 2007). Broad spectrum antibiotics viz., Ampicillin, Terramycin, tetracycline and Chloramphenicol were already tried on silkworm and found successful (Venkatesh and Srivastava, 2010). The beneficial action of the antibiotics has been attributed to the oral feeding of them along with mulberry leaves, which reduced significantly the incidence of flacherie (Radha *et al*., 1980). Ananda Kumar *et al*, (2012) found that the prevention or management of disease is a vital component for successful rearing for higher yield and quality cocoons. Thus, application of antibiotics through leaf freshness technology may through ray of hope in controlling diseases in silkworm indoor cultivation for bumper harvest and quality cocoons.

MATERIAL AND METHODS

Silkworm eggs were obtained from the Silk Seed Board Center, Dehradun (UK).

Collection of samples

Silkworm *Bombyx mori* L. larvae were reared on fresh mulberry leaves under ambient conditions as per the standard rearing method (Krishnaswamy, 1978). While larvae reaching the 4th instar stage worms were infected, sample were collected.

Isolation of Bacteria

Bacterial pathogens were collected from diseased flacherie infected larvae, the diseased larvae were crushed by using mortar and pestle and the solution was filtered. The filtrate was centrifuged at 4000-5000 rpm for 10 minutes. The supernatant was discarded and the pellet was used for bacterial culture after re-suspending in distilled water (Aneja, 2003).

Preparation of MacConkey Agar medium and bacterial culture

After culture preparation, the bacterial sample was streaked in MacConkey Agar under aseptic conditions in a laminar air flow chamber with the help of streaking loop then incubated at 37^{o}C overnight. After 24 hr the bacterial growth was noticed, and further it was sub cultured. A sample of bacteria was taken with the help of a loop and centrifuged for 15 minutes at 4000 rpm. By discarding the supernatant, pellet sedimented at the bottom of the tube was dissolved in distilled water. The presence of bacteria was confirmed by staining with basic dyes like crystal violet and methylene blue (Suparna *et al*., 2011).

Identification of bacterial isolate

The strain was purified and incubated on a MacConkey agar plate at 30^{o}C for 24 hrs. During this period, the colony characteristics including size, color, transparency, humidity, shape, edge were observed the bacterial colony that had been cultured for 24 hrs was subjected to Gram's staining and observed under a microscope (Zhao *et al*., 2000). Samples were subjected for identification by biochemical tests (Aneja K R, 1996).

Antibiotic sensitivity test

Antibiotic test of total 5 samples of silkworm larvae were performed by disc diffusing techniques (Baur *et al*., 1966). Antimicrobial disc used were of Ampicillin (A), Ticarcillin (Ti), Gentamycin (G), Trimethoprim (Tr), Sulphame thoxazole (Sx), Tetracycline (T), Colistin methane sulphonate (Cl). Antimicrobial disc were placed on inoculated plates of isolates then, incubated over night at 37^{o}C. Sensitivity or resistivity of bacterial isolates to antibiotics was determined by observing the absence or presence of growth around the discs.

RESULTS

6 samples of silkworm were used for isolation of Proteus bacterium. Out of 6 samples lactose fermenting smooth pink colonies were produced only in 5 samples confirming the presence of Proteus bacterium on the basis of shape, colony of morphology appeared in agar plates and sugar fermentation and staining properties. Lactose fermenting Pink colored smooth and round colonies was produced on MacConkey agar medium when enriched sample was streaked on medium and incubated at 37^{o} C for 24 hrs and Gram staining of isolates results Proteus bacteria is a gram negative bacterium.

Table 1. Pure colonies of bacteria on MacConkey agar

Sample No.	Incubation period	Pink color colonies	Non-Pink color colonies
worm 1	24 hr	+	-
worm 2	24 hr	+	-
worm 3	24 hr	+	-
worm 4	24 hr	+	-
worm 5	24 hr	+	-

Table 2. Cultural characteristics

Isolates	Colony morphology	habitat
Worm 1	Rounded pink	Anaerobic
Worm 2	Rounded pink	Anaerobic
Worm 3	Rounded pink	Anaerobic
Worm 4	Rounded pink	Anaerobic
Worm 5	Rounded pink	Anaerobic

Biochemical characterization using TSI slants

Blackening of the butt due to H_2S production may mask the acid reaction (yellow) in the butt. Production of hydrogen sulfide gas is indicated either by cracks or bubbles in the media or the media being pushed away from the bottom of the tube confirms the presence of Proteus strain.

Antibiotic test

The sensitivity (S) or the resistivity (R) of Proteus isolates to antibiotics was determined by observing the absence or presence of growth around the discs. Those isolate which show growth around particular antibiotics are resistant to the corresponding antibiotics, whereas the isolates whose growth is inhibited by a particular antibiotic seem to be sensitive to that

particular antibiotic (Baur *et al*., 1966). Ampicillin, Gentamycin, Tetracycline and Ticarcillin shows effective results on the growth of Proteus isolates.

Sample/isolates	Antibiotics reaction						
	G	Tr	Sx	T	Cl	A	Ti
Worm 1	S	R	R	S	R	S	S
Worm 2	S	R	R	S	R	S	S
Worm 3	S	R	R	S	R	S	S
Worm 4	S	R	R	S	R	S	S
Worm 5	S	R	R	S	R	S	S

Conclusion

Anitha *et al*, (1994) has reported that the bacteria as the etiological agent of flacherie in silkworms as early as 1870. In the present study, the organisms identified were strain of Proteus bacterium this result was supported by (Kodama and Nakasuji, 1969). The major fact responsible for bacterial flacherie was the rearing conditions. The rise in temperature and humidity in rearing place leads to dysfunction of alimentary canal which encourages flacherie (Nataraju *et al*., 2005). Rearing condition is followed by mulberry leaves of poor quality is also responsible for bacterial flacherie (Manimegalai and Chandramohan, 2005). The leaves of poor nutritive value will not be able to provide sufficient quality of essential requirement to the larva to produce antibacterial factor, which results in high rate of multiplication of infectious bacteria and development of bacterial flacherie (Nataraju *et al*., 2005). Many authors reported that antibiotics reduce the growth of isolated bacterium in vitro and in vivo in silkworms (Kodama *et al*., 1970; Baig *et al*., 1990). The effective antibiotics for bacteria such as Proteus species are Ampicillin, Tetracycline, and Gentamycin. Oral supplementation of these antibiotics through mulberry leaves resulted in lowering the occurrence of flacherie disease. Radha *et al* (1981) studied the efficacy of antibiotics such as Tetracycline on the bacterial flacherie. Kaito *et al* (2002) found that when Ampicillin was injected in the silkworm larvae after injection of bacterium at least 90% of larvae survived. Broad spectrum of antibiotics viz. Ampicillin, streptomycin, tetracycline and Chloramphenicol were already tried on silkworm and found successful (Venkatesh and Srivastava, 2010). Mahmud *et al*, (2012) found that Gentamycin significantly increase the effective rate of rearing and larval weight (97.33% & 2.63 gm) respectively or found that the effect of antibiotic (Gentamycin) upon the infection with bacterial flacherie.

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