Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

Advances in Applied Science Research, 2012, 3 (6):4066-4068

ISSN: 0976-8610
CODEN (USA): AASRFC

Isolation and identification of bacteria responsible for flacherie in silkworms

S. Sakthivel , C. Angaleswari , P. U. Mahalingam

Department of Biology, Gandhigram Rural Institute- Deemed University, Gandhigram, Dindigul

---

ABSTRACT

The silkworm is the larva or caterpillar of the domesticated silk moth, Bombyx mori. It is an important economic insect since it is a producer of silk. Flacherie is a syndrome associated with bacterial diseases. In the present study diseased silkworms were collected, dissected to separate the contents of foregut, midgut, and hindgut. The samples were serially diluted and from which 10^{-5}, 10^{-6} dilutions were plated onto nutrient agar plates. The predominant colonies were selected and identification procedures such as colony morphology, staining property and biochemical tests. As a result the organisms identified were Bacillus subtilis, Streptococcus pneumoniae, Staphylococcus aureus, E.coli, Pseudomonas fluorescence, Bacillus cereus and Klebsiella cloacae. Hence the study concludes with this bacterial identification which is responsible for bacterial flacherie in silkworms. So if a measure is taken to control these bacterial species the disease incidence in silkworms can be reduced which in turn increase the economics.

Key words: silk moth, *Bombyx mori.* Flacherie

INTRODUCTION

Sericulture means cultivation of silkworms which finally produces SILK The word silk sounds luxury and class. Till today, no other fabric can match it in luster and elegance. As long as human desire for silk garments continues, the demand for sericulture activity remains. Silk is the queen of textile and the naturally produced animal fibre.(Shelagh,2004; Welford, 1969)

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 the environmental changes, particularly to temperature and relative humidity.

One of the major constraints in silk production is the diseases in silkworm rearing Silkworm *Bombyx mori* is domesticated for silk production and are reared in colonial forms. A code of conduct for rearing silkworm is practiced to ensure survival of silkworm and cocooning.

All the major pathogenic microbes cause disease in silkworm and the most common among them are nuclear polyhedrosis, bacterial and viral flacherie, muscardine and pebrine.. Bacterial flacherie are caused primarily by *serretia marcesens. Streptococcus sp, and Staphylococcus sp* of bacteria. Muscardine is caused normally by *Beauveria bassiana and Spicaria prassina*. *Pebrine* a dreaded uncommon disease is caused by Nosema bombycis, and several other microporidians Variomorpha, Pleistophora, Thelophania, etc. Flacherie is a syndrome associated with bacterial diseases. Diseased/dead silkworms, their faecal matter, contaminated mulberry leaves and rearing appliances act as sources of infection. Wide fluctuation in temperature and humidity with poor quality mulberry leaves are the major predisposing factors for flacherie. Disease symptoms. During the intial stages of infection the larva becomes lethargic and stops eating. At an advanced stage infection the larva exhibits retarded growth, vomits gut juices and excretes semi soild faeces. The larva becomes soft and translucent. Finally the larvae ferments and the inner content turns into a black coloured liquid, which emits foul odour. The other symptoms includes flacheria (bacterial) lose appetite, sluggishness of worms with slow growth, shrinkage, swelling of thorax, appearance of

brown speeks on skin, straightened appearance of body, oral and anal discharge, liquefaction of inner organs, rupturing of skin and oozing out of oul smelling brown liquid (Samson, 1995)

MATERIALS AND METHODS

Collection of sample and Isolation of bacteria:
Healthy and diseased silkworms were collected from Nilakottai, Dindigul, Tamil Nadu, India.

The samples were surface sterilized with 0.1%mercuric chloride solution and washed thrice with distilled water. After sterilization the silkworms were dissected to collect the contents from surface area (before sterilization), foregut, midgut, and hindgut. Serial dilution (David A) was carried out from which 10^{-5}, 10^{-6} dilutions were plated on nutrient agar plates in which healthy silkworm sample served as the control and the plates were incubated for 24 hrs at 37° C. (Govindhan *et al*., 1998)

Identification of bacterial isolate:
Six predominant colonies from four samples were selected and subjected for identification by various biochemical tests, motility, colony morphology, and staining properties. (Aneja K.R. 1996)

RESULTS

Six predominant colonies isolated and identified using biochemical tests, motility, colony morphology, and staining properties and sugar fermentation were tabulated in tables 1, 2, and 3.

Table1: Identification of bacteria in diseased silkworms

S.No	Bacteria	Gram's staining	Motility	spore
1	*Bacillus subtilis*	+, rods	+	+
2	*Streptococcus pnumoniae*	+, cocci	−	−
3	*Staphylococcus aureus*	+, cocci	−	−
4	*Escherichia .coli*	−, rod	+	−
5	*Pseudomonas fluorescence*	−, rod	+	−
6	*Bacillus cereus*	+, rod	+	−
7	*Klbsiella cloacae*	−, rod	−	−

Table2: Biochemical tests for identification of bacteria

S.No	Bacteria	Catalase	Coagulase	Starch hydrolysis	Lipid hydrolysis	Gelatin hydrolysis
1	*Bacillus subtilis*	-	-	-	-	-
2	*Streptococcus pnumoniae*	-	-	-	-	-
3	*Staphylococcus aureus*	+	+			
4	*Escherichia .coli*	-	-	-	-	-
5	*Pseudomonas fluorescence*	-	-	-	-	+
6	*Bacillus cereus*	-	-	-	-	-
7	*Klbsiella cloacae*	-	-	-	-	-

Table3: Carbohydrate metabolism

S.No	Bacteria	Sugar fermentation			
		Glucose	Lactose	Sucrose	Mannitol
1	*Bacillus subtilis*	+	-	+	-
2	*Streptococcus pnumoniae*	-	+	-	-
3	*Staphylococcus aureus*	+	+	-	+
4	*Escherichia .coli*	+	+	+	+
5	*Pseudomonas fluorescence*	-	-	-	+
6	*Bacillus cereus*	+	-	+	+
7	*Klbsiella cloacae*	+	+	+	-

DISCUSSION

Anitha *et al* 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 *Bacillus subtilis, Streptococcus pneumoniae, Staphylococcus aureus, E.coli, Pseudomonas fluorescence, Bacillus cereus* and *Klebsiella cloacae* this results were supported by Taumanoff &Vago, 1951, Chitra *et al*., 1973, Anitha *et al*., 1994, Bucher, 1963. 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 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.

REFERENCES

[1]. Aneja K.R. **1996**. Methods of obtaining pure culture of microorganisms. In: Experiments in microbiology, plant pathology tissue culture and mushroom cultivation, 2nd ed, New Age International (P) Ltd., New Delhi. PP. 139-153
[2]. Anitha T., Sironmani P., Meena P. and Vanitha R. **1994**. *Sericologia*, 34 (1): 97 – 102.
[3]. Bucher G.E. **1963**. Non sporulating bacterial pathogens. In insect pathology: An advanced Treatise. (E.A. Steinhaus, ed). Vol.2 PP117-147. Academic press New York
[4]. Chitra C. Bhandarkar A. Karanth N.G.K. Vasantharajan V.N. **1973**. *Current science*, 42:373-376.
[5]. David A. Mickels and Greg A. Freyar. PP 244-245. Cold spring harbor.
[6]. Govindhan R. Narayanaswamy T.K. Devaiah M.C. **1998**. Principles of silkworm pathology. Seri.Scientific publishers, Bangalore. PP.420
[7]. Manimegalai S. and Chandramohan N. **2005**,. *Sericologia* 45 (1): 51-58
[8]. Nataraju B. Sathyaprasad K. Manjunath D. Aswani Kumar C. **2005**. Silkworm crop protection. Central silk board, PP 61-85.
[9]. Toumanoff C. Vago C. 1951Vago, **1951**. L agent pathogen de la flacherie desvers a soie endemique dans la region descevennes. Bacillus ceris var. alest. Var. Nov. compt. *Rent acad. Sci*. 233:1504-1506.
[10]. Shelagh, V. **2004**, Chinese Silk: A Cultural history, London, British Museum Press. pp 6 – 17.
[11]. Samson M.V. **1995**. Flacherie in Bombyx mori L. Indian Silk. 33(11): 31-32.
[12]. Welford, T. (**1969**). The textile student manual. 6th Edition Isaac Pitman and Sons Ltd. Great Britain