Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 6 (12): 891-897, 2011
ISSN 1816-4935 / DOI: 10.3923/jm.2011.891.897

Characterization and Identification of Bacteria Infecting Indian Tropical Tasar Silkworm, *Antheraea mylitta* D.

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ABSTRACT

Tropical tasar silkworm, *Antheraea mylitta* D., an economically important insect is affected by bacteriosis caused by bacteria, which accounts considerable loss of 10-15% to silk cocoon production. The aim of the present investigation was to isolation, characterization and identification of bacteria causing diseases in Indian tropical tasar silkworm, *Antheraea mylitta* D. Total 15 isolates of bacteria in two groups (8 from anal lip sealing diseased silkworms and 7 from rectal protrusion diseased worms) were isolated. The shape, size and colour of bacterial colony were recorded. The gram reaction of vegetative cells, its shape, size and pattern of reaction with different enzymes were observed for characterization of different bacterial isolates. Pathogenecity of these bacteria have shown that only two bacterial isolates coded SA3 and RP2 were responsible for anal lip sealing and rectal protrusion diseases, respectively in tasar silkworm. The bacterial isolates coded SA3 and RP2 on the basis of cultural, morphological and biochemical characters tentatively identified as *Serratia* sp. which were, close to *Serratia nematodiphila* and *Serratia marcescens* sub sp., respectively. The infection of anal lip sealing and rectal protrusion diseases in Indian tropical tasar silkworm caused by *Serratia nematodiphila* and *Serratia marcescens* was reported first time.

Key words: Tasar silkworm disease, anal lip sealing, rectal protrusion, bacteria identification, morphological characters, biochemical characters

INTRODUCTION

The silkworm *Antheraea mylitta* D. produces natural silk. Industrial and commercial use of silk depicts its economic importance and its application all over the world has contributed to the silkworm promotion as a powerful laboratory model for the basic research in biology (Singh *et al*., 2011).

Tropical tasar silk is produced by the larvae of *Antheraea mylitta* Drury. Being wild in nature and reared outdoor on tall host plants of *Terminalia tomentosa*, *T. arjuna* and *Shorea robusta*, these silkworms suffer from various diseases. In India, the extant of cocoon crop loss due to the silkworm diseases is nearly 40% (Sahay *et al*., 2000). The bacteriosis caused by different types of bacteria is common and prevails all through the year in tasar culture regions. The impact is more pronounced in larval stage which affects cocoon productivity and quality along with 10-15 % crop loss (Sahay *et al*., 2000). Three types of distinct features developed in tasar silkworm larva suffered with the infection of bacteria:

- Sealing of anal lips: A soil coloured sticky semisolid fluid seals the anal lips. The larva shrinks lengthwise
- Chain type excreta: The faecal beads are excreted out of with a jelly like substances in the form of chain and hangs
- Rectal protrusion: The rectum protrudes out of the anal opening in the forms of transparent bag filled with haemolymph (Sahay *et al.*, 2000). The anal lips dilate and the body contracts lengthwise

In all the cases the body color becomes darker, larva stops feeding, does not response to external stimuli, fall and die (Singh *et al.*, 2011). The investigations on the various types of bacteria in general and bacteria infecting other sericigenous insects were carried out to develop suitable control measures (Baig *et al.*, 1990; Patil, 1990; Anitha *et al.*, 1994; Kalpana *et al.*, 1994; Nahar, 1995; Matsumoto *et al.*, 1985; Fedhila *et al.*, 2006; Ramesh *et al.*, 2009; Amini *et al.*, 2011; Hamid *et al.*, 2010; Jing *et al.*, 2008). In tropical tasar silkworm, *Antheraea mylitta* some work has been done on breeding aspects (Reddy *et al.*, 2010a, b). Studies on biochemical changes in tasar silkworm related to stress have been carried out by (Pandey *et al.*, 2010; Kumar *et al.*, 2011). Singh *et al.* (2011a, b) have studied on cellular and biochemical changes in virus infected tasar silkworm and oral vaccine against virus infection. Madhusudhan *et al.* (2011) have observed impact of *Nosema mylitta* on larval growth, protein concentration and haemocyte count in tasar silkworm. Reports on the identification and characterization of bacteria causing bacteriosis in tropical tasar silkworm, *A. mylitta* are scanty. Hence, in the present investigation an attempt was made to characterize and identify the bacteria infecting Indian tropical tasar silkworm, *A. mylitta*.

MATERIALS AND METHODS

Collection of samples: The experiment was conducted in 2010 at Central Tasar Research and Training Institute, Ranchi, India. The tasar silkworm of 3rd instar showing the symptoms of bacterial anal lip sealing and rectal protrusion were collected from the silkworm rearing of 2nd crop (September-October 2010).

Isolation of micro-organism from tasar silkworm using serial dilution agar plating method: The hind gut of silkworm infected with anal lip sealing and rectal protrusion bacterial diseases were collected separately in distilled water and homogenized. Then the isolation of the bacteria was done using serial dilution agar plating method (Nataraju *et al.*, 2005).

Cultivation of bacteria: Bacteria were cultured in a liquid broth (beef extract and peptone).

Tests for bacteria identification: The isolated bacteria were identified on the basis of Cultural, Morphological and Biochimical characters. The shape, size and colour of bacterial colony were observed. Morphologically shape, size and gram reaction of vegetative cells of isolated bacteria were considered. Biochemical characters included the pattern of reaction with different enzymes (Conn *et al.*, 1957).

Pathogenesis of bacteria to tasar silkworm: The 2nd instar tasar silkworms were orally inoculated with bacterial suspension (1×10^5 bacteria mL) to test the pathogenesis of the respective bacteria. The disease symptoms and mortality in tasar silkworm was observed.

RESULTS AND DISCUSSION

Isolation of bacteria from diseased silkworm: Total eight different bacteria were isolated from silkworm suffered with anal lip sealing diseased and seven bacteria from silkworm suffered with rectal protrusion on the basis of shape and color of colony appeared in agar plates. The bacteria isolated were coded as shown in Table 1.

Identification of bacterial isolates: The cultural characters of the bacterial isolates are presented in Table 2. In cultural method of identification the bacterial isolates were characterized by the colour (white or yellow), shape (circular, irregular, filamentous and spindle), of growth in slant (beaded, filliform, echinulate and effuse) and habitat (aerobic, anaerobic and facultative).

Table 1: Code of bacterial isolates of SA7 was recorded

Disease symptoms	Sample No.	Code of isolates
Sealing of anal lip (group A)	1	SA1
	2	SA2
	3	SA3
	4	SA4
	5	SA5
	6	SA6
	6	SA7
	8	SA8
Rectal protrusion (group B)	1	RP1
	2	RP2
	3	RP3
	4	RP4
	5	RP5
	6	RP6
	7	RP7

Table 2: Cultural characters of the bacterial isolates

	Cultural characters		
Isolates	Colony morphology	Form of growth in slant	Habitat
SA1	White circular	Beaded	Aerobic
SA2	White filamentous	Echinulate	Anaerobic
SA3	Yellow circular	Beaded	Aerobic
SA4	Yellow circular	Effuse	Facultative aerobic
SA5	White circular	Filliform	Aerobic
SA6	White irregular	Echinulate	Aerobic
SA7	White filamentous	Echinulate	Aerobic
SA8	Yellow circular	Filliform	Facultative anaerobic
RP1	White circular	Filliform	Facultative anaerobic
RP2	White sticky	Filliform	Facultative anaerobic
RP3	Yellow irregular	Filliform	Facultative anaerobic
RP4	White spindle	Echinulate	Aerobic
RP5	White irregular	Echinulate	Aerobic
RP6	White filamentous	Echinulate	Aerobic
RP7	Yellow circular	Effuse	Facultative anaerobic

Fig. 1: Bacteria: small single rods

The vegetative cells of bacterial isolates were rods or coccus shape in single, pair or groups. The smallest cell size 1.15±0.34×0.5±0.22 μm of SA1 and largest 5.28±0.26×1.5±0.25 μm. Gram stain was positive or negative for different bacterial isolates. The lowest temperature range 7-30°C was observed for SA1, SA6, RP2 and RP7 whereas, the highest range 15-45°C was for SA2 and RP6. The pH range of RP1 and RP7 was 5-7 whereas it was 7-8 for SA3 and SA7 Table 3. The different bacterial isolates were subjected for biochemical tests like pattern of reaction with different enzymes and results are presented in Table 4.

Second instar silkworms were orally inoculated with the bacterial suspension of 1×10^5 bacteria mL of different bacterial isolates. The silkworm reared on leaves of *Terminalia tomentosa* up to the spinning of cocoons. Bacterial isolate of code number SA3 developed the symptoms of anal lip sealing disease and RP2 the symptoms of rectal protrusion disease and silkworm died in 5-6 days of post inoculation, while silkworm orally inoculated with the bacterial isolates of other code numbers did not show the symptoms of bacteria disease till the spinning of the cocoons.

On the basis of above, cultural, morphological and biochemical characters bacterial isolates of code number SA3 was tentatively identified *Serratia* sp. which is closely related to *Serratia nematodiphila* and RP2 as *Serratia* sp. which is closely related to *Serratia marcescens* sub sp. (Table 2-4).

SA3 (*Serratia* sp.): Produce white irregular colony, aerobic in habitat, gram +ve, spore was not found, rod in shape (Fig 1), temperature range 15 to 30°C, pH range 7-8, catalyse positive, amylase negative, protolytic positive, citrate negative, MR reduction positive VP reaction positive, Indole production negative, Nitrate production negative, celloulose production negative and H_2S production negative (Table 2-4).

RP2 (*Serratia* sp.): Produce white sticky colony, facultative anaerobic in habitat, gram -ve, spore was not found, rod in shape, temp range 7 to 30°C, pH range 6-8, catalase positive, amylase negative, protolytic positive, citrate positive, MR reduction negative, VP reaction positive, Indole production negative, Nitrate production positive, celloulose production positive and H_2S production negative (Table 2-4).

Bourtzis and Miller (2003) have isolated several species of gut bacteria including *E. aerogens, Y. enterocolitica* in insects and studied their functional role. Visotto *et al.* (2009) have studied the role of gut bacterium in the digestion of the velvet bean caterpillar, *Anticarsia gemmatalis*. Indiragandhi *et al.* (2008) isolated and characterized Serratia marcescens (a gut bacterium) of *P. xylostella* which enhanced the larval growth and development.

Table 3: Morphological characters of bacterial isolates

Isolate code	Vegetative cell structure	Size of cell (μm)	Gram stain	Sporestain	Range of temperature (°C)	Range of pH
SA1	Rod, pair	1.15 ±0.34×0.5 ±0.22	-	-	7-30	6-7
SA2	Long rod, chain	5.12±0.4×0.86±0.22	+	+	15-45	6-8
SA3	Tetroid	1.36±0.32×1.2±0.26	-	-	15-30	7-8
SA4	Small rod, pair	1.75±0.12×1.1±0.19	-	-	15-30	6-7
SA5	Small rod, pair	2.32±0.36×0.64±0.55	-	+	7-45	6-7
SA6	Small rod, single	1.45±0.35×0.68±0.25	+	-	7-30	6-8
SA7	Long rod, chain	5.28±0.26×1.5±0.25	+	-	15-30	7-8
SA8	Coccus, pair	1.23±0.37	-	-	15-30	6-7
RP1	Small rod, chain	2.17±0.3×1.02±0.25	-	-	15-30	5-7
RP2	Moderate rod, single	3.52±0.38×1.43±0.25	-	-	7-30	6-8
RP3	Small rod, pair	1.53±0.25×0.68±0.25	-	-	15-30	6-7
RP4	Long rod, chain	5.17±0.52×1.76±0.25	+	+	15-30	6-8
RP5	Moderate rod, chain	4.14±0.62×1.15±0.22	+	+	15-45	6-8
RP6	Moderate rod, chain	4.51±0.52×1.15±0.22	+	+	15-45	6-8
RP7	Small rod, single	1.57±0.33×0.77±0.35	-	-	7-30	5-7

Table 4: Biochemical characters of bacterial isolates

Isolate code	Catalase Production	Amylase activity	Protolytic activity	Citrate utilization	MR reduction	VP reaction	Indole production	Nitrate production	Cellulose decomposition	H_2S production
SA1	+	-	-	-	+	-	-	+	-	-
SA2	+	-	+	+	-	-	-	+	+	-
SA3	+	-	+	-	+	-	-	-	-	-
SA4	+	-	+	+	-	+	-	+	+	+
SA5	+	+	+	+	-	+	-	+	-	+
SA6	+	-	+	+	-	+	-	-	-	+
SA7	+	-	+	-	-	+	-	+	-	+
SA8	+	-	+	+	-	+	-	+	+	-
RP1	+	-	+	+	-	+	-	+	-	+
RP2	+	-	+	+	-	+	-	+	+	-
RP3	+	-	+	+	-	+	-	+	-	+
RP4	+	+	+	+	+	+	-	-	+	-
RP5	+	+	+	+	+	+	-	+	+	-
RP6	+	+	+	+	+	+	-	+	+	-
RP7	+	+	-	+	-	+	-	+	-	+

+: Positive, -: Negative

Selvakumar *et al*., 1998, 1999; Patil, 1990) reported a new record of a pathogenic bacteria *Streptococcus faecalis* on mulberry silkworm, *Bombyx mori* from India. However, the bacteria infecting Indian tropical tasar silkworm, *Antheraea mylitta* and causing anal lip sealing and rectal protrusion bacterial diseases have not been identified and characterized earlier.

CONCLUSION

Indian tropical tasar silkworm suffers with bacterial diseases which accounts 10-15% loss of cocoon crop. The specific bacteria causing different bacterial diseases have not been identified earlier. In the present investigation two species of *Serratia* responsible for anal lip sealing and rectal protrusion bacterial diseases have been identified and characterized first time.

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