anthesis (9.30–11.30 h) in particular, become restricting factors for the availability of pollinators. This is in all probability, accounts for such an adaptation, whereby the stigma has resorted to a fascinating mechanism to capture self-pollen and assure seed-set.

Thus *T. purpurea* becomes another addition to the species exhibiting movements of stigma and style. Previously some species of *Alpinia*, *Valeriana. Grewia* and *Ajuga* have been reported to exhibit this phenomenon. Flexistyly has been demonstrated in two species of *Alpinia – A. blepharocalyx* and *A. kwangsiensis* (family Zingiberaceae). The former displays stylar movements to facilitate pollen transfer between floral morphs, whereas the latter exhibits the same as a regular feature[17,18]. *Valeriana wallichii* (family Valerianaceae) has also been reported to exhibit such mechanism. This plant species is normally an out-breeder, but when cross-pollen is not available, it resorts to autogamy which is facilitated by curvature movements displayed by the style[19]. Wani *et al.*[20] have reported stigmatic flexing in *Grewia asiatica* (family Malvaceae), which deploys this mechanism for delayed autogamy when cross-pollen is not available. Likewise, Ganie *et al.*[21] have reported this mechanism in *Ajuga bracteosa* (family Lamiaceae), which practices autogamy favoured by stigmatic flexing.

The mechanism of flexistyly in *T. purpurea* is slightly different from the species mentioned above. It displays stigmatic as well as stylar curvature, thereby exhibiting both flexi-stigma and flexistyly. Also, the column of sticky pollen mass entrapped in the stigmatic exudates and especially the long, extending stigmatic hairs capturing self-pollen with great efficiency, make it a unique case.

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Aeromonas caviae CSB04, a causal organism of bacterial flacherie in muga silkworm (*Antheraea assamensis* Helfer)

Muga silkworm, *Antheraea assamensis* Helfer is an indigenous golden silk producing lepidopteran insect endemic to Assam and adjoining north eastern states of India[1]. The silkworm is generally reared outdoor and is thus prone to various infections caused by microorganisms[2]. Bacterial flacherie is one of those diseases rapidly inhibiting the growth of the industry leading to an indiscriminate loss to native farmers of the region. Although many reports are available on the causal organisms of this particular disease, pathogens of human origin may also prove severe in this regard. An attempt has been made to detect the presence of Aeromonads in the gut of flacherie-infected muga silkworm.

Aeromonads are a group of gamma proteo-bacteria belonging to the family Aeromonadaceae. They are mostly rod-shaped, Gram-negative facultative anaerobe and are ubiquitous in all microbial biosphere[3] which includes aquatic habitats, mammals, human, fish, foods, domesticated pets, invertebrate species, birds, ticks, insects, natural soils, etc. According to Janda and Abbott[4], almost 85% of this genus is prone to a majority of human gastrointestinal infections. Although studies have been made worldwide, the factors responsible for its virulence are unknown. However, Sahu *et al.*[5] suggested the lethal effect of homeolytic and proteolytic toxins secreted by the bacteria. Hence, the presence of these bacteria in some economically important insects may also be a major threat to biodiversity.

Diseased cadavers of *A. assamensis* were collected from five localities of upper Assam and tissue homogenate was prepared according to standard methodology[6]. Subsequently, the prevalent bacteria were isolated by culture-dependant technique using nutrient agar medium. Total viable colony count was made according to the standard methodology[7] and the single colonies were isolated after 24–36 h of incubation. The morphological and biochemical characters of the pure isolates were studied according to *Bergey's Manual of Determinative Bacteriology*.

In vivo pathogenicity test was conducted on healthy silkworms (90 in number) in three replications at the institute's farm. The isolates were cultured in nutrient broth medium for 24 h and cells were harvested by centrifugation and resuspended in phosphate buffer solution (PBS). Serial dilution was made in PBS and bacterial cell enumeration was determined as total viable colony count. Fresh bacterial cell suspension at 10^8 cfu/ml was sprayed on *Persea bombycina* leaves to feed the third instar *A. assamensis* larvae. Inoculation was done by spraying the suspension on leaf surface twice daily for three consecutive days from the second day onwards of third instar rearing[8]. Disease symptoms were observed and the mortality rate was recorded each day. Severe infection and mortality were noticed from 10^4 dilution onward (Figure 1), whereas disease symptoms or mortality was not witnessed in control.

The pathogenic bacteria isolate CSB04 is a Gram-negative bacillus with 0.3–0.5 × 1.50–1.80 µm size having smooth, circular and convex colonies during phenotypic characterization. The bacterial cells were collected by centrifugation of culture broth and fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate, 0.05 M NaCl, 0.35 M saccharose for 2 h at pH 7.2. Thereafter, the cells were washed twice in sodium cacodylate buffer and 0.3 M NaCl having pH 7.2 for 1 h and dehydrated in ethanol and transferred to 100% acetone. Later, the samples were dried with liquid carbon dioxide at its critical point and immersed in tetra methyl saline for 5–10 min at 4°C and brought to room temperature for drying. The cells were then mounted on brass stubs and coated with gold of 35 nm thickness. The preparation was then observed in a scanning electron microscope (Carl Zeiss-Sigma) and photographed (Figure 2). The isolate CSB04 was positive for production of hydrogen sulfide, urea, indole, oxidase, catalase, citrate utilization, gelatin liquefaction, nitrate and Voges-Proskaeur. It produces acid from glucose, galactose, maltose, sucrose, mannitol, rhamnose, sorbitol and raffinose. However, it could not utilize glycerol, lactose and mannose (Table 1).

Molecular identification of the isolate CSB04 was carried out by 16S rDNA sequencing and homology study. The genomic DNA was isolated according to standard methodology[9] and purity and quantity of the DNA were confirmed by spectrophotometer at 260 and 280 nm. The partial sequencing of the 16S rDNA gene was carried out through the courtesy of DNA sequencing service, Bioserve, Hyderabad, India. NCBI nucleotide blast result showed that, isolate CSB04 has 99% sequence similarity with *Aeromonas caviae* strain 75a and Neighbour-joining[10] phylogenetic tree after multiple alignments using CLUSTALW was constructed with the closest homologous group of bacteria obtained from NCBI blast (Figure 3). The identified gene sequence was submitted to NCBI GenBank and an accession number (KT982275) was obtained for

Figure 1. Flacherie-infected larvae of *Antheraea assamensis* caused by *Aeromonas caviae* CSB04.

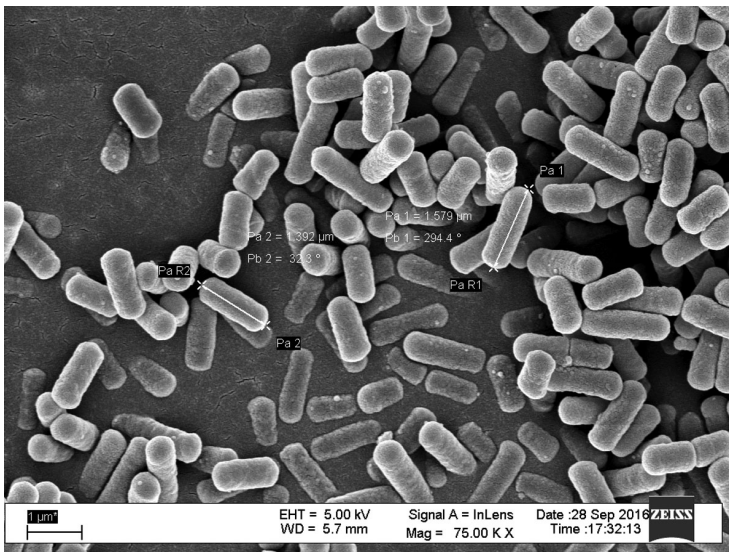

Figure 2. Scanning electron microscopy photograph of *Aeromonas caviae* CSB04.

Table 1. Morphological and biochemical properties of isolate CSB04

Characteristic features	Result	Characteristic features	Result
Gram reaction	Negative	Sugar utilization test	
Morphology	Rod	Glucose	+
H_2S production	+	Galactose	+
Urea	+	Maltose	+
Citrate utilization	+	Sucrose	+
Indole production	+	Mannitol	+
Oxidase	+	Rhamnose	+
Catalase	+	Sorbitol	+
Gelatin liquefaction	+	Raffinose	+
Nitrate	+	Glycerol	–
Voges-Proskaeur	+	Lactose	–
Methyl red	–	Mannose	–

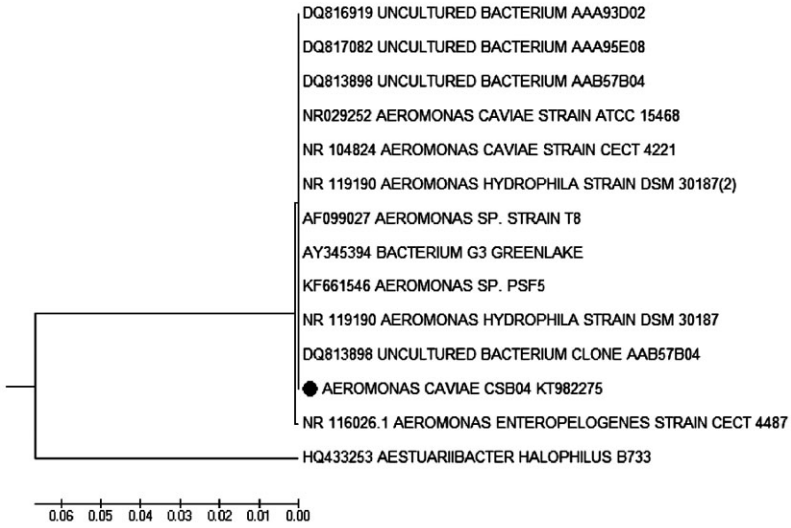

Figure 3. Phylogenetic tree-based on the 16S rDNA sequence indicating the position of strain CSB04 (using Neighbour-joining method).

further reference. The DNA G + C content of the strain was determined as 55.44 mol%.

Being poikilothermic in nature, the muga silkworm is prone to diseases such as flacherie[11], microsporidiosis and occasionally virosis[12]. Virulent strains of *Pseudomonas aeruginosa* causing flacherie in muga silkworms (*A. assamensis*) were resistant to different antibiotics and plant extracts[13]. The major pathogenic microbes that affect the muga silkworm productivity are *P. aeruginosa* strain AC3 (ref. 13), *Bacillus thuringensis*[14], *Fusarium moniliforme*, *Beauveria basiana*, *Penicillium digitatum* and *Aspergillus* sp.[15]. Under tropical climate, out of every 5–6 crops per annum, sericulturists experience 2–3 crop failure due to disease outbreak[16]. Bacteria flacherie is the most threatening disease of muga silkworm with percentage disease prevalence (PDP) up to 70, followed by grasserie caused by viral pathogen (2–47%) and muscardine (10–47%) caused by fungal pathogen[13]. Antibiotic resistance of bacteria associated with flacherrie has been reported earlier[17,18].

Thousands of families in northeast India earn their livelihood by rearing muga silkworm. The production of golden silk is in great demand in the textile industry and is thus needed to be conserved for long. Conversely, the muga silk productivity rate is decreasing due to the microbial diseases[19]. Therefore, studies in this sector are important to save this economically important species from extinction.

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