Biocontrol potential of a newly isolated bacterial agent against *Arctornis submarginata* (Walker) (Lepidoptera: Lymantriidae) occurring in Darjeeling Terai region.

Sangita Khewa (Subba) and Ananda Mukhopadhyay

ABSTRACT

A strain of *Bacillus* causing disease in caterpillars of *Arctornis submarginata*, a defoliator of tea crop, was isolated from Darjeeling terai region. The strain showed positive reaction in lysine decarboxylase, ornithin decarboxylase, Voges-Proskaur, citrate utilization, nitrate reduction and in utilization of trehalose and glucose; difference with *Btk* was observed in ONPG test, and in utilization of citrate, arabinose, xylose, cellobios, melibiose and saccharose. The doubling time was 84 min, which is exactly the double of that of *Btk*. Difference was not evident in protein profile of the strain with that of *Btk*. The LC_{50} value was found to be 398.1 µg/ml with fiducial lower limit 353.06µg/ml and UL 443.14 µg/ml. The LC_{50} value of the new strain was lower than that of *Btk*, which was found to be [537.0 µg/ml; LL 483.63µg/ml and UL, 590.37µg/ml. The LT_{50} values of the new strain were also lower than that of *Btk*. These values were, 7.28 days for 1000 µg/ml and 8.88 days for 750 µg/ml as compared to the LT_{50} values 7.57 days for 1000 µg/ml and 9.5 days for 750 µg/ml of *Btk*. This findings opened up the possibility of developing new strain as microbial pesticide after standardizing its formulations and determining its safety aspects.

Key words: *Arctornis submarginata*, *Camellia sinensis*, biopesticide, crop pest

INTRODUCTION

Arctornis submaginata, commonly called as hairy caterpillar, is emerging as a potential pest of tea (Mukho padhyay and Roy, 2009). Swarms of caterpillars defoliate the mature and maintenance leaves adversely affecting the tea yield at the Darjeeling foothill region (Terai) (Mukhopadhyay *et al.*, 2007). *A. submarginata* has been found in North East Himalaya, Borneo and Sumatra on bamboo and other hosts (Schintlmeister, 1994). To combat the pest problem of tea Chemical pesticides are mainly used, with some backlashes such as, environmental pollution, human health hazard, resistance and resurgence in pests (Sarker and Mukhopadhyay, 2006). Hence, efforts are being made to evolve alternative strategies to manage this pests. One such approach is development and application of microbial bioagents. Commercial formulations of *Bacillus thuringiensis kurstaki* applied for management of this pest could not produce desirable results against *A. submarginata* swarms.

The objective of the present study was aimed to isolate and characterize a naturally occurring bacterial pathogen of *A.submarginata* and to know its potential as biopes ticide through determination of its median lethal concentration and time.

Bacillus thuringiensis kurstaki was used as a reference for comparison. Pest management using microbial pesticides would greatly help in production of export quality tea, free of pesticide residues.

MATERIALS AND METHODS

A bacterial strain was isolated from dead or moribund larvae of *A. submarginata*. For isolation of bacteria, the larvae dying of disease was taken for surface sterilization with 70% alcohol and then washed thrice with double - distilled water. These were then stored in double - distilled water within sterilized eppendorf which was wrapped with parafilm and stored at -20°C (Lacey and Brooks, 1997). The stored dead larvae were taken and thoroughly macerated by glass homogenizer. Crude homogenate was sieved through fine sterilized cotton cloth. The filtrate was centrifuged at 3000 rpm for 30 min. The supernatant was discarded and the precipitate containing bacterial suspension was taken for pure culture isolation by 'dilution streak method' in nutrient agar medium. The infectivity of the pure cultured bacterium was determined following Koch's postulate by infecting healthy second instar larvae. After proving the Koch's postulate the viability of the bacterium was checked by inoculating newly prepared agar medium at weekly intervals.

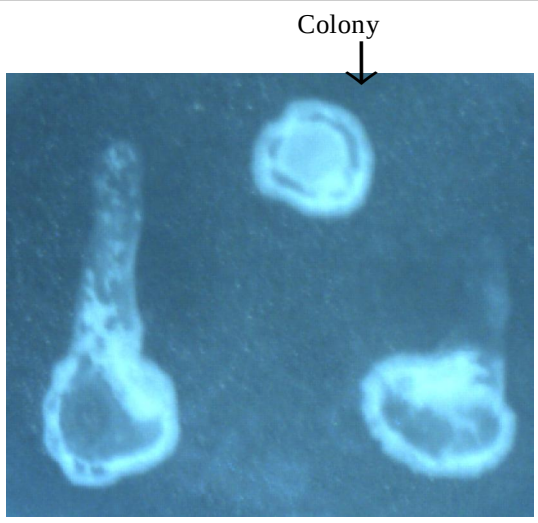

Figure 1a. Colony of the new Strain of *Bacillus*

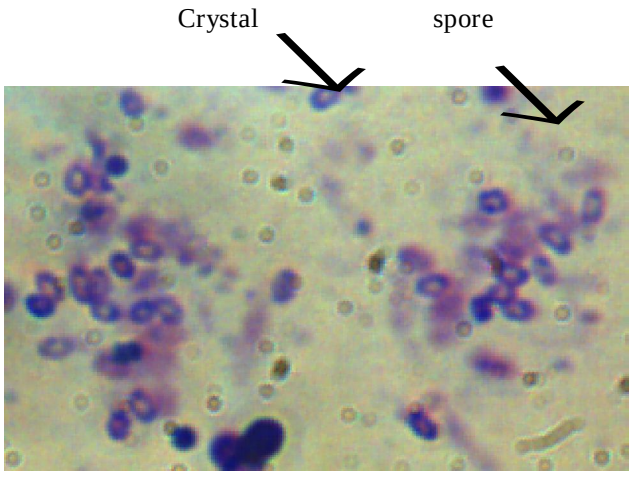

Figure 1b. Spore with crystal of the new strain of *Bacillus*

Cell shape (vegetative body), colony texture and motility were recorded and compared with that of *Bacillus thuringiensis kurstaki* (*Btk*). Biochemical experiments were done with the help of Rapid Biochemical testing kit (Himedia co.). Results were recorded and compared with that of *Btk*.

Doubling time or generation time of the bacterial strain was determined by turbidimetric method developed by Cappuccino and Sherman (1996). The isolated bacterial strain was cultured on Luria- Bertani (LB) agar for 24 h at 37ÚC and the proteins were extracted using 1% lysozyme solution. The lysis buffer contained 4% SDS, 20% 2-marcaptoethanol, 70% Tris-HCL, pH 6.8, and 4% deionized water. SDS-PAGE analysis was done according to the method described by Costas (1992). Gel was fixed in trichloroacetic acid and stained with the Brilliant blue G - 250.

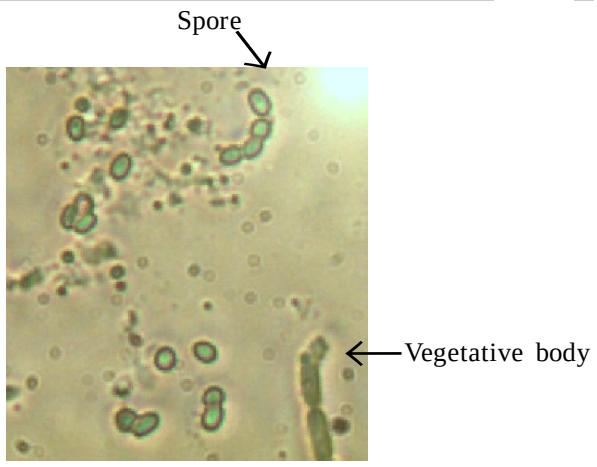

Figure 1c. Phase contrast photograph of spore and vegetative body of the new strain of *Bacillus*

Different concentrations of crude samples containing spores at a concentration of 100, 300, 500, 750, 1000 µg/ml were used in a LC_{50} (median lethal concentration) and LT_{50} (lethal median time) bioassay, by spreading these uniformly on tea leaves and offering as food as per the procedure of Unnamalai and Sekar (1995). In each replicate one hundred second instar caterpillars were used for the experiment and the mortality was recorded at an interval of 24 hrs from the day of inoculation (bioassay testing). The corrected mortality was calculated using Abbott's formula. Data were subjected to probit analysis (Finney, 1971) and median lethal concentration (LC_{50}) value was calculated. Median lethal time (LT_{50}) was also calculated simultaneously following Biever and Hostetter (1971).

RESULTS AND DISCUSSION

All the morphological characteristics of the isolated bacterial strain such as, vegetative body structure (chain like), spore-shape (oval), presence of crystal structure, motility (high), colony texture (smooth), were found to be similar to that of *Bacillus thuringiensis kurstski* (*Btk*) (Fig 1 a,b,c). Its SDS-PAGE of whole body protein profile also exhibited similarity in their banding pattern with the *Btk* (Fig 2). However, in case of biochemical characteristics, it showed some difference with that of *Btk* in respect of ONPG test, and in utilization of citrate, arabinose, xylose, cellobios, melibiose and saccharose. Moreover, its generation or doubling time was exact double (84 min) to that of *Btk* (42 min).

Dose dependent mortality was observed both in Btk. (17,19, 48, 60 and 76%) and the new strain (23, 33, 56, 67 and 78%) for 100, 300, 500, 750 and 1000 µg/ml respectively). The LC_{50} value 398.1 µg/ml for the isolated bacterium was found to be lower than *Btk*, i.e. 537.0 µg/ml with fiducial lower

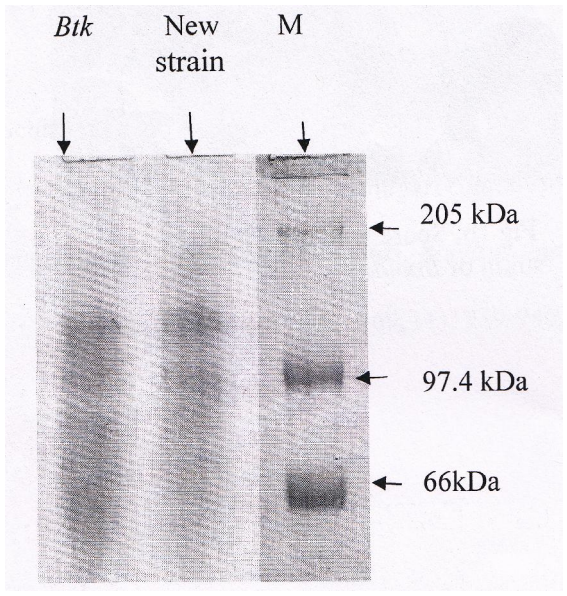

Figure 2. SDS - PAGE of vegatative protein of Btk and the new strain of *Bacillus*

limit 483.63µg/ml and upper limit 590.37µg/ml. Its LT_{50} values 7.28 days for 1000 µg/ml and 8.88 days for 750 µg/ml and 9.45 days for 500 µg/ml were lower than that of *Btk* which were 7.57 days for 1000 µg/ml and 9.5 days for 750 µg/ml of *Btk* .

The low LC_{50} and LT_{50} values of the new strain of *Bacillus* makes it a promising potential entomopathogenic bacterium that can be further developed in to biopesticide with proper formulation and field testing. Sampurna Sattar *et al.* (2008) and Netravathi *et al.* (2009) explained that the variations in efficacy against different pests may be due to varying number of cry genes and the absence of specific binding sites.

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Sangita Khewa (Subba) and Ananda Mukhopadhyay[1]

Entomology Research Unit, Department of Zoology, University of North Bengal, Darjeeling-734013, West Bengal, India

[1]Entomology Research Unit, Department of Zoology, University of North Bengal, Darjeeling-734013, West Bengal, India, Phone : 91-0353-2776353 (O), 91-0353-2581830 (R), Fax: 0353-2699001, E-mail: am_nbu@yahoo.co.in