hydrate requirements for methane production. According to the IPCC (1992), the annual average production of methane from rice paddies is 110 Tg. If the efficiency, as experimentally observed, is taken into consideration, it would require 1100 million tonnes (10% efficiency) or 1000 million tonnes (11% efficiency) of glucose or glucose equivalent for producing 110 million tonnes of methane. Assuming the harvest index of 40%, with an annual rice paddy production of 527 million tonnes, the maximum biomass would be 1300 million tonnes. All estimates made so far suggest that plants can release organic substance up to 10% of their weight through roots[5]. Thus, a maximum amount of 130 million tonnes may be available as substrate for methane production. From this amout only 11 to 13 million tonnes of methane can be produced. Thus an upper limit of 13 Tg methane production from rice paddies can be expected. Any assessment ignoring biological aspects of the mechanism of methane production is an artifact of methodology.

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Bacillus thuringiensis, a biocontrol agent for major tea pests

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A preliminary screening was carried out to determine the efficacy of crystal proteins of nine strains of *Bacillus thuringiensis* to control two lepidopteran pests of tea plants. Among these strains *B. thuringiensis* var. *kurstaki* HD1 and HD73 were highly toxic, *B. thuringiensis* var. *thuringiensis* HD2 was moderately toxic, *B. thuringiensis* var. *aizawai* HD133 showed low toxicity, while the other strains showed no toxicity towards these pests. The LC_{50} values of the three toxic strains and that of the purified crystal protein of the highly toxic strain HD1 were determined by laboratory bioassays.

TEA is an important plantation crop of India and is affected by many pests. The two most economically important lepidopteran insect pests of tea are the leaf-feeding caterpillars, *Caloptilia theivora* (leaf roller) and *Cydia leucostoma* (flushworm), belonging to the family Gracillariidae and Olethreutidae respectively. They cause considerable damage to young leaves of the tea plant by destroying the apical bud and tender leaves, thereby arresting the shoot growth.

The choice of conventional insecticides to be used on this crop is limited because of concerns related to consumer protection and environmental safety. Hence the search for effective biopesticides for the management of tea pests becomes appropriate. We undertook a study on the feasibility of the use of *Bacillus thuringiensis*, a well-known entomopathogen, as a biopesticide in tea plantations.

We carried out a preliminary screening for the efficacy of the crystal proteins of various *B. thuringiensis* strains belonging to class I which are known to be toxic to lepidopterous insects (Table 1). These strains were cultured on nutrient agar plates (peptone, 5 g; NaCl, 5 g; yeast extract, 1.5 g; beef extract, 1.5 g; distilled water, 1000 ml; pH 7.4) for four days at 30°C. One plate was used for each strain. The resultant spore–crystal mixtures were scraped off the plates, washed once in 0.5 M NaCl, followed by three washings in sterile water and resuspended in 1 ml of sterile water containing a protease inhibitor phenylmethylsulphonyl fluoride, at a concentration of 1 mM. These crude preparations were stored at –20°C until further use.

The pure crystal protein preparation was made from the spore–crystal mixture of HD1 (ref. 1). The purity of the crystals was 95% as determined by light microscopy. The level of purity of crystals was also confirmed by SDS–PAGE analysis[2]. Protein concentration of the spore–crystal mixtures of all nine strains and the purified crystal fraction of the strain HD1 were estimated by the dye-binding method[3] after solubilizing the crystal inclusions at 37°C in 10 mM NaOH (pH 10.5) containing 25 mM dithiothretol.

Prior to setting up bioassays, the surface area of the leaves was measured. Preliminary bioassays were conducted using crude spore–crystal samples obtained from the nine strains of *B. thuringiensis* towards larvae of flushworm and leaf rollers. Assays were done at 28°C

Table 1. Various strains of *B. thuringiensis* used in the study

Strain	Serotype
B thuringiensis var *kurstaki* strain HD1	3a 3b
B thuringiensis var. *kurstaki* strain HD73	3a 3b
B. thuringiensis var *thuringiensis* strain HD2	1
B thuringiensis var. *aizawai* strain HD133	7
B. thuringiensis var *indiana* strain HD521	15
B. thuringiensis var *dakota* strain HD551	16
B thuringiensis var *pakistani* strain HD395	13
B thuringiensis var *morrisoni* strain HD12	8a 8b
B thuringiensis var. *galleriae* strain HD207	13

Table 2. Bioassays using the spore–crystal mixtures of various *B. thuringiensis* strains[a]

Strain	Percentage mortality after 72 h[b]	
	Flushworm	Leafroller
HD1	70	60
HD73	60	65
HD2	45	45
HD133	30	25
HD521	0	0
HD511	0	0
HD207	0	0
HD12	0	0
HD395	0	0
Control*	0	0

*Water-coated leaves were used as control.
[a]Leaves coated with 200 ng cm^{-2} of crude crystal protein were used.
[b]Average of three replicates

Table 3. Toxicity of selected crude/purified crystal proteins against flushworm and leafroller

Strain	Flushworm*		Leafroller*	
	LC_{50}** (ng cm^{-2})	95% fiducial limits	LC_{50}** (ng cm^{-2})	95% fiducial limits
HD1	185.36	151.39–226 95	174 97	143 84–212 82
HD73	208 95	171.70–254 28	184 05	150 31–225.35
HD2	221.79	184.39–266 78	296 67	178 60–492 80
Purified HD1	40 97	37.02–45 35	33.93	30.07–38.28

*Larval mortalities scored after 72 h.
**LC_{50} values were calculated from three independent bioassay tests.

by coating fresh individual tea leaves with 200 ng cm^{-2} of protein from crude spore–crystal preparation and placing the 2nd instar larvae over it. In all bioassays, a total of 20 larvae/treatment (5 larvae/leaf) were used. The percentage mortality was scored after 72 h. A control was maintained by coating sterile distilled water on the leaves. All bioassays were repeated three times.

LC_{50} values of crude spore–crystal samples of the three toxic strains were estimated by conducting larval bioassays. Different concentrations of crude samples (100, 150, 200, 250, 300 ng cm^{-2}) and the pure crystal protein of the most toxic strain (20, 30, 50 and 75 ng cm^{-2}) were used in the bioassays. The data were subjected to probit analysis[4].

The results of preliminary bioassays showed that *B. t.* var. *kurstaki* HD1, HD73 and *B. t.* var. *thuringiensis* HD2 were toxic to the pests. *B. t.* var. *aizawai* showed some toxicity, while the other strains showed no toxicity towards these pests (Table 2).

Among these three strains, HD1 and HD73 were most toxic to both flushworms and leafrollers. The LC_{50} value of HD1 towards flushworms was 185.36 ng cm^{-2} with a 95% fiducial limit (FL) of 151.39–226.95 and that of HD73 was 208.95 ng cm^{-2} (FL = 171.70–254.28). The LC_{50} value of HD1 towards the leaf rollers was 174.97 ng cm^{-2} (FL = 143.84–212.82) and that of HD73 was 184.05 ng cm^{-2} (FL = 150.31–225.35) (Table 3). The LC_{50} value of the purified crystal protein of HD1 was 40.97 ng cm^{-2} and 33.93 ng cm^{-2} respectively for flushworms and leafrollers (Table 3). The low LC_{50} values observed for the purified crystal proteins when compared to the crude preparations may be due to the presence of significant amounts of the spore proteins in the crude samples.

Attempts to control pests of tea plant using *B. thuringiensis* were made by Kariya[5] in 1977. Recently, CryIA(a) protein of Fu-2-7 strain was reported to be about half as toxic against smaller tea tortrix as the strain HD1. It is known that *B. thuringiensis* var. *kurstaki* strain HD1 produces several lepidopteran toxic proteins such as CryIA(a), CryIA(b), CryIA(c), CryIIA and CryIIB while, HD73 produces only CryIA(c) protein[7]. Since the LC_{50} values for the strain HD73 (containing CryIA(c) protein only) and HD1 (containing several Cry proteins) were quite similar, it is likely that CryIA(c) may be more toxic. Other CryI proteins such as CryIA(a), CryIA(b) and CryIB produced by the strains HD1 and HD2 may also exhibit some toxicity. Studies to establish the relative efficacy of individual Cry proteins to control leafrollers and flushworms are underway.

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