African Journal of Biotechnology Vol. 9 (4), pp. 496-501, 25 January, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2010 Academic Journals

Full Length Research Paper

Population dynamics of soil microbes and diversity of *Bacillus thuringiensis* in agricultural and botanic garden soils of India

Tushar Kanti Dangar, Y. Kishore Babu and Jyotirmayee Das

Microbiology Laboratory, Division of Crop Production, Central Rice Research Institute, Cuttack 753006, Orissa, India.

Accepted 18 August, 2008

Different microbial guilds of three rice fields (aerobic (dry or wet) laterite and red sandy, and anaerobic (flooded), clay-loam), a botanical garden (wet, aerobic, humus) and a sorghum (dry, aerobic, sandy) soil were investigated. Population ($x10^6$ cfu/g dr. soil) of the aerobic (3.8 - 26.2) and anaerobic (0.91 - 13.6) heterotrophic, aerobic (1.1 - 3.7) and anaerobic (0.8 - 1.3) spore forming, Gram (-)ve (0.3 - 0.7), phosphate solubilizing (0.01 - 0.06), asymbiotic N_2-fixing (0.26 - 0.88), sulfur oxidizing (0.13 - 0.75), sulfate reducing (0.01 - 0.1), nitrifying (0.1 - 0.23) and denitrifying (0.04 - 0.25) bacteria; actinomycetes ($0.01x10^4$ cfu/g dr. soil), fungi ($0.01 - 0.05x10^5$ cfu/g dr. soils) and *Bacillus thuringiensis* (Bt) ($0.13-0.25x10^6$ cfu/g dr. soil) population were variable in different soils. Nevertheless, proportions of Bt in relation to spore forming bacteria were comparable (index (5-9) $x10^{-4}$) and they produced either monotypic (bipyramidal or spherical) or heterotypic (polymorphic-bipyramidal or bipyramidal-rhomboidal) crystals although the bipyramidal crystal producing Bt were predominant in the soils. The bipyramidal crystal producing Bt were diverse in different ecologies, resistant to penicillin group of antibiotics and tolerated 5 - 6% NaCl. Phenotypic characters allowed to group the Bt isolates of botanic garden as *B. thuringiensis* subsp. *coreanensis* and as *B. thuringiensis* subsp. *thompsoni/coreanensis*, sorghum fields as *B. thuringiensis* subsp. *finitimus* and but those of rice fields as *B. thuringiensis* subsp. *thuringiensis/ shandongiensis*, f*initimus* and *thompsoni/coreanensis.* The isolates produced 25.78, 25.78, 86.26, 24.73, 68.0, 26.8 and 26.8 kDa proteins and equivalent to Cyt, Cry5 and Cry2 toxins effective against the insects of Diptera and Lepidoptera/Coleoptera.

Key words: Microbial diversity, stress tolerance, *Bacillus thuringiensis*, rice, soil.

INTRODUCTION

Qualitative and quantitative microbial activities are the key factors for productivity and sustainability of soils health for maintenance of crop production (Pankhurst et al., 1996; Nannipieri et al., 2003; Tilak et al., 2005). Rice is the staple food of about 85% Asian and 40% world population and population growth demands more food. Whereas, the wild ecologies like forests, national parks, botanical gardens etc. play the pivotal role for maintenance of global environment. Analysis of structural and functional microbial diversity would, therefore, reveal their sustainability of both cultivated and wild ecosystems. Although microbial diversity of rice soils has been nominally investigated (Das and Dangar, 2007a,b), Indian wild habitats like forests, botanical gardens etc. remained unattained to date. Insect pests and pathogens are serious threat for agriculture and forestry, which are conventionally controlled by pesticides resulting in severe health hazards and environmental imbalance and warrant augmentation of biopesticides application. Several soil microbes are potent entomopathogens, which naturally suppress the insect pests and disease vectors (Pankhurst et al., 1996). Evidently, Theunis et al. (1998) recorded infection of more than 50% different arthropods of rice ecologies by *Bacillus thuringiensis* (Bt). Therefore, knowledge of microbial diversity and functionality of the

*Corresponding author. E-mail: dangartk@rediffmail.com. Tel: 91-0671-2367768-783 Ext. 207. Fax: 91-0671-2367663.

soils, and entomopathogens are essential to understand soil health conditions and select potent microbes or pathogens to augment soil nutrition status or develop broad-spectrum biocontrol agents.

As every ecosystem has well adapted microbial diversity, exploitation of the native microbiota would be the best method for sustainable development. Bt is the most potent and versatile pathogen capable to infect wide range of organisms viz. protozoa, nematodes, flatworms, mites and insects, which are either pests, pathogens or vectors botanical, animal or human concern (Feitelson, 1993; Joung and Cote, 2000) which resulted in comercialization of about 100 commercial Bt formulations (Flexner and Belnavis, 2000). Nevertheless, selection and exploitation of potent and indigenous Bt would be most desirable to preserve the native ecosystem. In India, diversity, distribution, potency etc. of the microbes of biogeochemical cycles of flooded and irrigated rice fields have been studied significantly but Bt has not received sufficient impetus (Kaur and Singh, 2000; Das and Dangar, 2007a, b). Furthermore, microbiology of sandy desert soils and botanical gardens has been ignored. Limited information on the microbial functionalities of laterite and sandy agricultural soils, wild ecologies (botanical parks) and biopesticides resulted in this investigation. Microbial dynamics of different biogeochemical cycles and diversity of *B. thuringiensis* (Bt) in the wet and humus Botanical Garden soil (Sims, Koonoor, Tamil Nadu), and different rice ecologies viz. dry and red laterite soil (CRRI research station, Hazaribagh, Jharkhand), red and sandy soil (Pratapbagan, West Bengal), flooded clay-loam soil (CRRI research station, Gerua, Assam) and sandy desert sorgham soil (Jodhpur, Rajastan) were assessed to understand their microbial functionalities.

MATERIALS AND METHODS

The soil samples were collected from dry, sandy and aerobic sorghum (*Sorghum vulgare* Pers.) fields of desert ecology at Jodhpur, Rajastan; aerobic and wet humus soil of Sims Botanical Garden of Koonoor, Tamilnadu; dry and aerobic rice fields of laterite soil of CRRI research station, Hazaribagh, Jharkhand; rainfed, wet and aerobic red sandy soil of rice field of Pratapbagan, West Bengal and rainfed, anaerobic and flooded clay-loam soil of low land rice fields of CRRI research station, Gerua, Assam. Top soil (1 cm) was scrapped off and about 100 g soil samples were collected from five locations of each field, mixed thoroughly put in sterile polythene bags, sealed with rubber bands and the samples were analyzed in the laboratory.

Appropriate sterile materials were used and sterile conditions were maintained during the study. The microbial communities were enumerated under a digital colony counter as colony forming units (cfu)/g dr. soil. Viable cells of the aerobic microbiota were assessed from the plates incubated at 30±0.1°C in a BOD incubator. The anaerobic microbes were grown in a BOD contained in an anaerobic jar with anaerogas pack (Himedia, India). To count viable cfu of different bacteria, 10 µl soil suspension (10^{-3}) was mixed with 100 ml of different medium and plated but heated (60 ± 0.1°C, 30 min) soil suspensions were used for spore formers. The fungi, actinomycetes and sulfur oxidizing bacteria were estimated after 7 d, the nitrifying bacteria were recorded from 5-30 d (5 d intervals) but all other organisms were counted after 3 d incubation. The aerobic heterotrophic, Gram (-)ve and spore forming bacteria were enumerated using nutrient agar (NA) medium but 0.01 g/l crystal violet was added to the medium before plating to visualize the Gram (-)ve bacteria which formed violet colonies Pelczar et al. (1957). The nitrifying and denitrifying bacteria were enumerated on Winogradsky's medium containing either $(NH_4)_2SO_4$ (1.0 g/l) or KNO_3 (1.0 g/l), respectively, and the colonies were visualized (pink colour) by flooding the plates with sulphanillic acid reagent Pelczar et al. (1957). The inorganic phosphate solubilizing bacteria were assessed from the halo zone formation around the colonies on the insoluble phosphate ($Ca_3(PO_4)_2$) containing medium Pelczar et al. (1957). The asymbiotic nitrogen fixing bacteria, sulphur-oxidizing (formed brown colonies) bacteria, soil fungi and actinomycetes were counted on the nitrogen free medium Pelczar et al. (1957), *Thiobacillus* medium, mycological broth agar medium and Krainsky's medium Pelczar et al. (1957), respectively. The sulfate reducing bacteria were enumerated (MPN) in 10 ml thioglycolate medium contained in the tubes capped with serum septum, layered with 1 cm liquid paraffin (sterilized) and contained in the anaerobic jars following MPN method Reichardt et al. (2001). *B. thuringiensis* (Bt) was isolated from the heat treated soil suspensions (Das and Dangar, 2007b). An aliquot (100 µl, 10^{-2} dilution) suspension was mixed with 100 ml NA, plated in 5 plates and incubated in a BOD incubator for 3 d. All colonies were checked under a phasecontrast microscope (x 100) and those (107 colonies) produced crystals were isolated, purified and maintained on NA slants at 4 ± 0.1°C. The isolates were identified phenotypically (Sneath, 1986; de Barjac and Frachon, 1990; Smibert and Krieg, 1995). Response of the Bt isolates to oxygen was also checked in thioglycolate medium (without paraffin overlay) contained in capped tubes (20 x 2 cm) inoculated with a loop full of bacteria at the bottom, one set incubated in a BOD incubator and another set within the anaerobic jar.

Crystal protein composition of the *B. thuringiensis* isolates were profiled SDS-PAGE (Attathom et al., 1995). The bacteria were grown for 7 d in 50 ml nutrient broth on a shaker at 100 rpm at 30 ± 0.1°C. The cells were harvested by centrifugation at 10000 g for 10 min at 4 ± 0.1°C. The pellet was washed 3 times in 50 ml of crystal wash (1M NaCl containing 0.1% SDS) solution and finally once with sterile distilled water. The pellet was re-suspended in 3 ml sterile distilled water, mixed with equal volume of alkaline solubilization buffer (50 mM anhydrous Na_2CO_3 containing 10 mM dithiothreitol (DTT), pH 10) and incubated at 37 ± 0.1°C for 12 h on a shaker at 25 rpm. The pH of the solubilized crystal was neutralized with 0.5 M HCl, treated with 1/10 volume aqueous trypsin (200 U/gm potency) solution (1 mg/ml) and incubated for 3 – 4 h at 37 ± 0.1°C. An equal amount of trypsin was added again and incubated for 12 h at 37 ± 0.1°C, centrifuged at 10000 g for 15 min at 4±0.1°C and the supernatant was taken. Protein concentration was determined at 280 nm as bovine serum albumin equivalent. Protein profile of trypsinized crystals was analyzed by SDS–PAGE resolved at 5 V/cm up to end of stacking gel followed by 15 V/cm for the resolving the gel (Janssen, 1994). The protein fragments were developed with coomassie brilliant blue R250 stain (Janssen, 1994) and their MW (kDa) was determined using Photocapt software.

RESULTS AND DISCUSSION

Viable count (cfu) method of microbes reveals only 1-

Table 1. Population dynamics of different microbes in the soil.

Type of organism	Microbial population (x10^6 cfu/g soil)				
	Soil no. 36 (Hazribagh)	**Soil no. 48 (Koonoor)**	**Soil no. 57(Bankura)**	**Soil no. 61(Jodhpur)**	**Soil no. 70 (Gerua)**
Aerobic heterotrophic bacteria	10.06 ± 0.96	20.26 ± 0.97	8.71 ± 1.04	3.84 ± 0.01	26.21 ± 9.06
Anaerobic heterotrophic bacteria	1.41 ± 0.01	3.64 ± 0.05	1.72 ± 0.01	0.91 ± 0.04	13.60 ± 0.01
Aerobic spore forming bacteria	1.72 ± 0.02	3.31 ± 0.01	2.53 ± 0.05	3.70 ± 0.01	1.10 ± 0.02
Anaerobic spore forming bacteria	0.92 ± 0.02	1.12 ± 0.01	0.81 ± 0.04	0.31 ± 0.02	1.31 ± 0.02
Gram (–)ve bacteria	0.30 ± 0.01	0.60 ± 0.04	0.31 ± 0.05	0.36 ± 0.04	0.70 ± 0.07
Nitrifying bacteria	0.23 ± 0.01	0.12 ± 0.01	0.10 ± 0.01	0.14 ± 0.001	0.14 ± 0.01
Denitrifying bacteria	0.05 ± 0.01	0.15 ± 0.01	0.04 ± 0.004	0.06 ± 0.006	0.27 ± 0.02
Phosphate solubilizing bacteria	0.06 ± 0.001	0.04 ± 0.002	0.01 ± 0.001	0.04 ± 0.002	0.06 ± 0.003
Asymbiotic N_2 fixing bacteria	0.26 ± 0.01	0.30 ± 0.005	0.70 ± 0.06	0.84 ± 0.07	0.88 ± 0.08
Sulfur oxidizing bacteria	0.13 ± 0.001	0.16 ± 0.002	0.22 ± 0.004	0.16 ± 0.005	0.75 ± 0.006
Sulfate reducing bacteria	0.01 ± 0.001	0.09 ± 0.002	0.02 ± 0.001	0.01 ± 0.001	0.10 ± 0.004
Actinomycetes	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.001	0.01 ± 0.002	0.01± 0.001
Fungi	0.01 ± 0.001	0.01± 0.001	0.05 ± 0.004	0.01 ± 0.002	0.02 ± 0.003
Bacillus thuringiensis (Bt)	0.13 ± 0.001	0.14 ± 0.002	0.22 ± 0.003	0.15 ± 0.002	0.25 ± 0.002
Bt index* (x 10^{-3})	0.6	0.8	0.9	0.6	0.5

Results are mean of 3 replications ± SE.
cfu = Colony forming units.
*Bt index = Number of Bt cfu/number of spore forming cfu.

10% bacteria but depict the structural and functional composition, ecological role and phenotypic specificity of most active microbial communities unlike the direct microscopic, MPN (ineffective for low population) and genetic analysis techniques which not only fail to reflect microbial functionality but inconsistent also (Liesack et al., 2000; Tabachioni et al., 2000; Reichardt et al., 2001). Therefore, viable count (for aerobic) and MPN (for anaerobic) methods were adopted to assess the active microbial guilds in the soils and the result are interpreted accepting this bias.

In the Gerua (soil no. 70) and Koonoor (soil no. 48) soil population (x10^6cfu/g soil) of aerobic heterotrophs (26.2 and 20.3 and anaerobic (13.6), aerobic (1.1 and 3.3), anaerobic (1.3 and 1.1) spore forming (1.1), Gram (-)ve (0.7) were more than the other soils (Table 1). The nitrifying bacteria and denitrifying bacteria were more (0.23 - 0.25 x10^6 cfu/g soil) at Hazaribagh (soil no. 36) than other soils (Table 1). The fungi and actinomycetes were negligible (0 - 0.05 x 10^6 cfu/g soil) in the soils (Table 1). Population of different microbial guilds in the soils did not follow any common trend (Table 1). The results favoured that microbiology of the soils was very complex and therefore, no correlation between population dynamics and habitat could be obtained. however, pool sizes of the microbes of this study were at least 10 fold lower than (0.01 - 154.11x10^7 cfu/g dr. soil) those of partially aerobic rice fields of brackish water, Himalayan, island and mesophilic habitats in India (Das and Dangar, 2007b). But they were comparable to those of irrigated, fertilized, anoxic rice fields (0.7 x 10^5 cfu/g soil) other than heterotrophs (2.8 x 10^8 cfu/g. dr. soil) recorded by different authors (Chin et al., 1999; Leisack et al. 2001). Heterotrophs would be favoured in Koonoor and Gerua (Table 1) as organic content would be more in humus and clay-loam soils (Lee and Pankhurst, 1992; Das and Dangar, 2007b). On the other hand, anaerobic microbes viz. denitrifying and sulfate reducing bacteria would be more in flooded or water saturated soil of Gerua and Koonoor (Table 1) as the processes are affected in aerobic soils. Probably, higher moisture content of Gerua and Koonoor soils, and lower organic content of sandy soils of Pratapbagan, Hazaribagh and Jodhpur reduced the fungi and actinomycetes (10^4 - 10^5 and 10^3 - 10^4 cfu/g dr. soil, respectively) (Table 1) than those (about 10^8 - 10^6 cfu/gm soil) of the aerobic and non-sandy soils because moisture content has negative and organic content has positive relation with those organisms (Pankhurst et al., 1996; Reichardt et al., 2001).

B. thuringiensis (Bt) was obtained from all soils (Table 1) which supported its ubiquitous distribution i.e. in 50 - 70% soils of 30 different countries, 93% Asian and 87% agricultural soils (Theunis et al., 1998; Kaur and Singh, 2000; Das and Dangar, 2007a, b). Population of Bt ranged from 0.13 - 0.25 x 10^6 cfu/g soil and its population index (ratio) against total spore formers was (0.5-0.9) x 10^{-3} (Table 1) which was relatively lower than previous results (Das and Dangar, 2007a, b).They produced diffe-

Table 2. Phenotypic characters of the bacteria isolated from different soils.

Soil no. (Location)	Total isolate	Strain no. TB	Colony character	Bacterium[1] l x w (µm)	Spore[2] l x w (µm)	Crystal		Antibiotic	
						Morphotype	l x w (µm)	Sensitive	Resistant
Soil 48 (Konoor)	11	175-1	I,CW,M,R,U	2.7x1.1	1.9 x 1.05	Bipyramidal	2.56 x 1.11	b,c,e,g	a,d,f
	8	175-2	I,CW,M,R,L	2.8x1.15	2.09 x 1.07	Bipyramidal	2.22 x 1.12	b,c,d,e,g	a,f
	4	122	I,CW,M,R,L	2.5x1.11	1.8x1.1	Spherical	1.75	b,c,d,e	a,g,f
Soil 57 (Prarapgar)	5	176	I,CW,M,R,U	4.1x1.1	1.70 x 0.92	Bipyramidal	2.31 x 0.94	c,d,e,g	a,b,f,
	2	113-1	I,CW,M,R,U	3.4x1.12	1.65x 0.98	Elliptical	2.22x 0.88	b,c,d,e,g	a,f
	1	114-1	I,CW,M,R,L	3.3x1.22	1.98x 0.91	Spherical	2.68	c,d,e,g	a,b,f,
Soil 61 (Jodhpur)	6	177	I,CW,M,R,L	2.3x1.4	1.7 x 0.92	Bipyramidal	2.32 x 1.01	c,d,e,g	a,b,f,
Soil no.70 (Gerua)	7	178-1	I,CW,M,R,L	3.4x1.6	1.73 x 1.05	Bipyramidal	2.40 x 1.05	b,c,d,e,g	a,f
	5	178-2	I,CW,M,R,U	3.2x1.12	2.06 x 1.02	Bipyramidal	2.69 x 1.13	b,c,d,e,g	a,f
	2	178-3	I,CW,M,R,U	2.1x0.98	1.88x 1.01	Spherical	1.97	b,c,d,e,g	a,f

The isolates were obtained from 100 µl Pasteurized soil suspension of 10^{-3} dilutions.
[1]Rod shaped; and [2]eliptical.
I = Irregular; M = Metallic; CW = Creamish white; R = Raised; U = Undulate; L = Lobate.
a = Penicillin (10 U/disc); b = Bacitracin (10 U/disc); c = Polymyxin B (300 U/disc); d = Triple sulphus (300 µg/disc), e = Streptomycin (10 µg/disc); f = Sulbactum (10 µg/disc); g = Erythromycin (15 µg/disc).

rent (bipyramidal, spherical and rhomboidal) crystal morphotypes which had no relation with habitat and bipyramidal crystals were predominant (Table 2). Bt being a saprophytic and aerobic soil bacteria (Joung and Cote, 2000), its population would be lower in the nutrition poor sandy and anaerobic flooded soils of our study (Lee and Pankhurst, 1992; Kaur and Singh, 2000). Nevertheless, the observations revealed and supported that Bt population in the native and diverse soil habitats were diverse (Theunis et al., 1998; Kaur and Singh, 2000; Das and Dangar, 2007a, b). Physiological and toxin protein diversity of the bipyramidal crystal producers were further assessed to reveal difference/similarities of that predominant group in different habitat. The bipyramidal crystal producing Bt produced different types of colonies, the bacteria were Gram (+)ve motile rods (2.3-4.1x1.1-1.6 µm) with elliptical spores (1.7-2.09 x 0.92-1.07 µm) (Tables 2, 3) and bipyramidal crystals (2.22-2.69 x 0.94-1.13 µm) (Table 2). They were sensitive to streptomycin, polymyxin B and erythromycin but resistant to sulbactum, penicillin and nystatin (Table 2). Based on phenotypic characters of the isolates were identified as genus *Bacillus* (Group 1) and species *thuringiensis* (Table 3) (Sneath, 1986; Smibert and Krieg, 1995). Pending serology, they were tentatively grouped phenol-typically as Bt subspecies (serovar. or variety) *coreanensis* (TB 175-1), *thompsoni*/*coreanensis* (TB 175-2 and TB 178-1), *thuringiensis*/*shandongiensis* (TB 176), TB 177 and *finitimus* (TB 178-2) (Table 4). The results confirmed that Bt population had no relation with the soil ecology and crystal morphotypes has no relation with Bt subsp (Joung and Cote, 2000; Das and Dangar, 2007a, b). The isolates were sensitive to streptomycin (10 µg/ disc), polymyxin B (300 U/disc) and erythromycin (100 U/disc) but resistant to penicillin G (10 U/disc) (Table 2). The results confirm that Bt is generally resistant to the penicillin group of antibiotics (Kaur and Singh, 2000; Das and Dangar, 2007a, b). Tolerance of about 4 - 6% (w/v) NaCl of the Bt isolates (Table 3) depicted that they would be potent for development of salt-tolerant crops or microbes for pest control and nutrition management in saline rice fields (Zahran, 1997; Nanniperi et al., 2003).

SDS-PAGE proteogram of bipyramidal crystal proteins of the Bt isolates revealed 1-5 protein components of 6.5 to 198.03 KDa sizes viz. 175-1 and 175-2 had one each (25.78 kDa), 176 had five (86.27, 48.91, 13.18, 9.58 and 6.5 kDa), 177 had one (24.74), 178-1 had five (198.03, 68, 44.69, 26.84 and 6.84 kDa) and 178-2 had two (217 and 26.84 kDa) proteins components (Table

Table 3. Phenotypic identification of the bipyramidal crystal producing bacteria.

Character	Bacteria (BT)	Observation
Matte colony, rod shape, endospore, motile, Gram stain, crystal, facultative anaerobe/ microaerobic, catalase, oxidase, acidity from glucose	175-1, 175-2, 176, 177, 178-1, 178-2	+
Length (µm)	Do	
Diameter >2.5 µm, cell/filament curved, cocci	Do	-
Filament	Do	-
Sporangium	Do	Not swollen
Nitrate reduction	176, 177, 178-2	+
	175-1, 175-2, 178-1	-
Genus	175-1, 175-2, 176, 177, 178-1, 178-2	*Bacillus*
Species	Do	*thuringiensis*

Table 4. Phenotypic grouping of the bacteria into subspecies.

Characters	Bacteria (BT)			
	175-1	**175-2, 178-1**	**176**	**177, 178-2**
Acetylmethyl carbinol	+	+	+	+
Urease	+	+	-	±
Arginine dihydrolase	-	-	+	-
Pellicle	-	+	-	-
Cellobiose utilization	+	+	+	+
Mannose utilization	+	+	+	+
Starch hydrolysis	+	+	+	+
Sucrose fermentation	+	+	+	+
Tween esterase	+	+	+	+
Citrate utilization	+	+	+	+
Salicin fermentation	-	-	-	-
Lecithinase	+	+	+	+
Gelatinase	+	+	+	+
Casein	-	-	+	+
Esculin fermentation	+	-	-	+
Chitin hydrolysis	-	-	-	-
Bt subspecies group	*coreanensis*	*thompsoni/coreanensis*	*thuringiensis/shandongiensis*	*finitimus*

+ = Positive result. - = Negative result.

Table 5. Composition of proteins of the bipyramidal crystals of the Bt isolates.

Protein no.	Molecular weight (kDa) of protein fractions of the BT isolates					
	175-1	**175-2**	**176**	**177**	**178-1**	**178-2**
1	25.780	25.780	86.268	24.735	198.026	217.000
2			48.913		68.000	26.843
3			13.157		44.689	
4			9.582		26.843	
5			6.500		6.842	

5, Figure 1). The 27 kDa (approx.) proteins of 175-1, 175-2, 177 and 178-2 was identical to cytolysin (27 kDa) toxic to dipteran (mosquito), the 86.27 kDa fraction of 176 was comparable to Cry V toxic to lepidopteran/coleopteran,

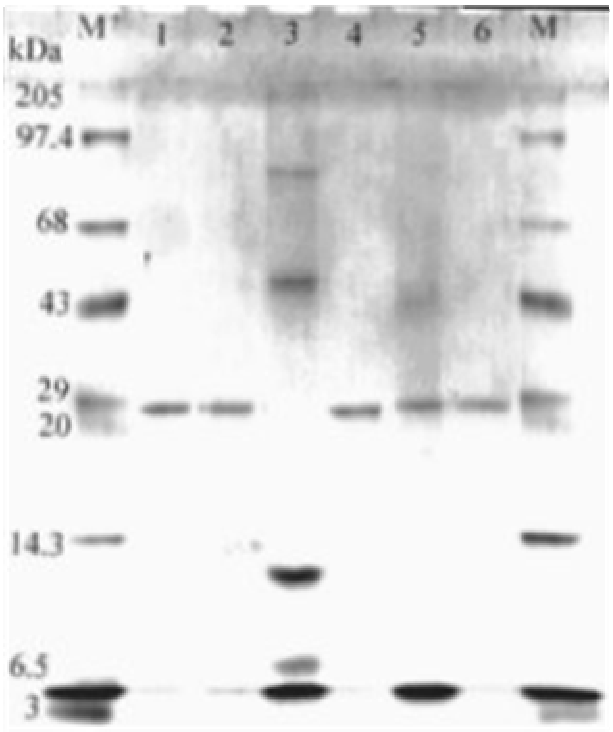

Figure 1. SDS-PAGE of crystal proteins of *Bacillus thuringiensis* isolates. Lane 1 = BT175-1, lane 2 = BT175-2, lane 3 = BT176, lane 4 = BT177, lane 5 = BT178-1, lane 6 = BT178-2, and M = Molecular weight markers.

and the 68 and 26.84 kDa proteins of 178-1 was similar to Cry III toxic to lepidopteran/dipteran insects (Table 5, Figure 1). The observations proved that besides being bipy-ramidal crystals, their composition was different and comparable to known toxins of different insect groups i.e. the native Bt population were not physiologically, functionally and toxigenically correlated (Whitely and Schnef, 1986). Population and crystal protein diversity supported that they can control a wide range of pests and supported Theunis et al. (1998) who observed infection of more than 50% insects in rice environment. The unknown protein fractions of the crystals of Bt 176, 178-1 and 178-2 would, however, be required for crystal structure formation Rang (1997).

REFERENCES

Attathom T, Chongrattanameteekul W, Chanpaisang J, Siriyan R (1995). Morphological diversity and toxicity of delta-endotoxin produced by various strains of *Bacillus thuringiensis*. Bull. Entomol, Res. 85: 67-173.

Das J, Dangar TK (2007a). Diversity of *Bacillus thuringiensis* in the rice field soils of different ecologies in India. Ind. J. Microbiol. 47: 364-368.

Das J, Dangar TK (2007b). Microbial population dynamics, especially stress tolerant Bacillus thuringiensis, in partially anaerobic rice field soils during post-harvest period of the Himalayan, island, brackish water and coastal habitats of India. World J. Microbiol. Biotechnol. DOI 10.1007/s11274-007-9620-3.

Flexner JL, Belnavis DL (2000). Biological and biotechnological control of insect pests. In: Microbial Insecticides Rechicigl JE, Rechicigl NA (eds). Lewis Publishers, NY, USA, pp. 35-60.

Janssen K (1994). Current protocols in molecular biology. Greene Publ. Assoc. Inc. and John Wiley Sons Inc., NY, USA.

Joung KB, Cote JC (2000). A review of the environmental impacts of the microbial insecticide *Bacillus thuringiensis*, Tech Bull no. 29. Hort. Res. Dev. Cent, Canada, p.16.

Kaur S, Singh A (2000). Distribution of *Bacillus thuringiensis* strains in different soil types from North India. Ind. J. Ecol. 27: 52-60.

de Barjac H, Frachon E (1990). Classification of *Bacillus thuringiensis* strains. Entomophaga, 35: 233-240.

Liesack W, Schnell S, Resbech NP (2000). Microbiology of flooded rice paddies. FEMS Microbiol. Rev. 24: 625-645.

Pankhurst CE, Opjel-keller K, Doube BM, Gupta VVSR (1996). Biodiversity of soil microbial communities in agricultural systems. Biodiver. Conserv. 5: 202-209.

Pelczar Jr. MJ, Bard RC, Burnett GW, Conn HJ, Demoss RD, Euans EE, Weiss FA, Jennison MW, Meckee AP, Riker AJ, Warren J, Weeks OB (1957). Manual of Microbiological Methods. McGraw Hill Book Company, Inc., New York. Soc. Am. Bacteriol.

Rang C (1997). Simulataneous production of the 34-kDa and 40-kDa proteins from *Bacilus thuringiensis* subsp. thompsoni is required for the formation of inclusion bodies. FEBS Letters, 412(3): 587-591.

Reichardt W, Briones A, de Jesus R, Padre B (2001). Microbial population shifts in experimental rice systems. Appl. Soil Ecol. 17: 151-163.

Smibert RM, Krieg NR (1995). Phenotypic characterization. In: Gerhardt P, Murray RGE, Wood W, Krieg E (eds). Methods for general and molecular bacteriology. Am. Soc. Microbiol. Washington.

Sneath PHA (1986). Endospore-forming gram-positive rods and cocci. In: Sneath PHA, Main NS, Sharp ME, Holt JG (eds). Bergey's manual of systematic bacteriology. Williams and Wilkins, Baltimore.

Tabachioni S, Bevivino A, Cantale C, Dalmastri C (2000). Bias caused by using different isolation media for assessing the genetic diversity of a natural microbial population. Microbial. Ecol. 40: 169-176.

Theunis W, Aguda RM, Cruz WT, Decock C, Peferoen M, Lambert B, Bottrell, DG, Gould FL, Litsinger JA, Cohen MB (1998). *Bacillus thuringiensis* isolates from the Philippines: habitat distribution, δ-endotoxin diversity and toxicity to tem borers (Lepidoptera: Pyralidae). Bul Entomo Res. 88: 335-342.

Nannipieri P, Ascher J, Ceccherini MT, Landi L, Pietramellara G, Renella G (2003). Microbial diversity and soil functions. Eur. J. Soil Sci. 54: 655-670.

Tilak KVBR, Ranganayaki N, Pal KK, De R, Saxena AK, Nautiyal CS, Mittal S, Tripathi AK, Johri BN (2005). Diversity of plant growth and soil health supporting bacteria. Curr. Sci. 89: 136-150

Feitelson JS (1993). The *Bacillus thuringiensis* family tree. In: ed. Kim L, Marcel Dekker, Inc., NY, Adv. Eng. pesticides, USA, pp. 63-71.

Whitely HR, Schepf HE (1986). The molecular biology of Parasporal crystal body formation in *Bacillus thuringiensis* Ann. Rev. Microbiol. 40: 549-576.

Zahran HH (1997). Diversity adaptation and activity of the bacterial flora in saline environments. Biol. Fert. Soil. 25: 211-223.