Advanced Journal of Microbiology Research Volume 2013
Available online at http://advancedresearchjournals.org/AJMR

Full Length Research Paper

Microbial populations and *Bacillus thuringiensis* diversity in saline rice field soils of coastal Orissa, India

Jyotirmayee Das, Bandana Das and Tushar Kanti Dangar

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

Accepted 11 November, 2012

Different microbial populations and *Bacillus thuringiensis* (Bt) diversity in saline soils of three rice fields and two fallow lands of coastal Orissa, India, were assessed. Populations (x10^5 colony forming units (cfu) g $^{-1}$ dr. soil) of aerobic heterotrophic (4.5 - 47.6), spore-forming (1.00 - 21.2) and Gram-negative (0.8 - 27.8) bacteria were relatively more abundant in the soils than those of nitrifying (0.10 - 1.02), denitrifying (0.15 - 1.31), phosphate-solubilizing (1.4 - 8), sulfur-oxidizing (3.2 – 8.0) and asymbiotic nitrogen-fixing (2.6 – 4.0) bacteria. Populations of fungi (0 - 2) and actinomycetes (0 - 0.02) were low. Bt populations varied between 0.5 - 6.4 x 10^5 cfu g^{-1} dr. soil in the different fields. The Bt isolates (*n* = 406) could be divided into 9 groups (TB 155-161, 163, 164), based on their cultural, morphological and crystal (viz. bipyramidal, spherical and pleomorphic) characteristics. Phenotypically the isolates were similar to *B. thuringiensis* subsp. *galleriae* (TB155), *kurstaki/kenyae/aizawai* (TB156, 159, 164), *darmstadiensis* (TB158, 163), *thompsoni/coreanensis* (TB157) and *entomocidus* (TB161). However, one isolate (TB160) did not match with any subspecies.

Key words: Microbial populations, *Bacillus thuringiensis*, saline soil, rice.

INTRODUCTION

Microbes live in diverse habitats, including soil, where their functions may include maintaining the soil structure and nutritional status, degradation of pollutants and the control of several pests and diseases. These functions, however, are negatively correlated with salinity (Lee and Pankhurst, 1992; Zahran, 1997; Nannipieri et al., 2003; Tilak et al*.,* 2005; Zhau et al*.* 2008). In India, the rice growing area comprises 42.4 mha of which about 53% is irrigated, while 25% has developed salinity and thereby resulted in a decline in microbial activities, soil nutrition status and productivity (Bandopadhyay and Bandopadhyay, 1983; Zaharan, 1997; Tilak et al*.,* 2005). The diversity of microbial populations in rice ecosystems have mainly been studied in net houses (Leisack et al*.,* 2000; Reichardt et al*.,* 2001), but studies regarding mesophilic or saline rice soils, especially along the coastal regions of India, have been neglected (Bandopadhyay and Bandopadhyay, 1983; Tilak et al*.,* 2005; Das and Dangar, 2007; 2008).

Several soil microbes, especially *Bacillus thuringiensis* (Bt), has been reported to control several agricultural pests and diseases (Lee and Pankhurst, 1992; Zhou et al*.* 2008). Indeed, in one study it has been reported that more than 50% of pests in rice fields were infected by this bacterium (Theunis et al*.,* 1998). Their ubiquitous distribution and high numbers (0.5 - 50% of the spore formers) in soils indicate that Bt could play a role in the nutrition cycle (Jong and Cote, 2000; Kaur and Singh, 2000; Das and Dangar, 2007; 2008). In India, research on Bt is mainly centered around transgenic crop production. Bt diversity in Indian rice fields, especially in saline soils, and the ability of Bt to control rice pests have not been investigated in any great detail (Kaur and Singh, 2000; Das and Dangar, 2007; 2008). To date, only one Bt isolate has been reported from a saline mangrove forest (Maeda et al*.,* 2001). In this study, different microbial populations in the soil from saline rice fields of Gadakujang and Bhuiyapada in the Jagatsinghpur district, Orissa, India, were enumerated. The Bt diversity were also investigated to assess their use in pest and disease control, as well as to assess their potential in nutrition management of mesophilic and saline soils.

MATERIALS AND METHODS

Location and period of study

The study was undertaken in two saline areas on the east coast of

*Corresponding author: E-mail: dangartk@rediffmail.com.

India, that is, along the Bay of Bengal. The locations were Gadakujang (20º0'27"N and 85º59'46"E) and Bhuiyapada (20º0'29"N and 85º59'48"E) in the Jagatsinghpur district, Orissa, India. The study was undertaken during the post-harvest period, that is, November 2003 - 2005. These areas have an average rainfall of 1.1 - 7.2 mm.

Determination of soil characteristics

The soil samples were collected (Black, 1965) after harvest from the water-saturated rice fields of Gadakunjang (Paddy field no. 1 and 2) and Bhuiyapada (Paddy field no. 3), and two adjacent fallow lands at Gadakunjang (Fellow land no. 1 and 2). Approximately 100 g of soil was collected from five arbitrarily selected spots in each field by removing 1 cm of the top soil. The samples were mixed, placed in polythene bags and sealed (Black, 1965). To determine pH, 50 g of soil was suspended in 100 ml of distilled and deionized water, stirred for 1 h at 100 rpm on a rotary shaker and then centrifuged at 10 000 x *g* for 5 min. The pH and Eh ($mScm^{-1}$) of the supernatants were recorded with a pH and a conductivity meter, respectively (Black, 1965). For determination of the water content, 1 g of fresh soil was blotted to optimum dryness, air- dried, powdered and then heated at 65°C up to a constant weight. Soil types were determined according to Black (1965).

Determination of organic carbon

One gram air -dried soil (sieved through a 0.25 mm mesh) was suspended in 10 ml of 1 N potassium dichromate in a 500 ml conical flask and 20 ml of concentrated H_2SO_4 was added slowly to digest the soil. The mixture was then cooled to room temperature (25ºC). Within 30 min, 200 ml of distilled water, 10 ml of orthophosphoric acid (88% [w/v]), NaF and 1 ml of diphenylamine (0.5 g of dye dissolved in 20 ml of water and diluted to 100 ml with concentrated H_2SO_4), were added. The solution was titrated with 0.5 N ferrous ammonium sulfate until the colour changed from dark blue to dark green. Two control sets were also included (Black, 1965). The organic carbon content (%) of the soil was calculated as: 3(B-T)/BW, where W = gram of soil, B = volume of ferrous ammonium sulfate required for the control and T = volume of ferrous ammonium sulfate required for titration of the digested soil.

Determination of total nitrogen

The soil (0.5 g) sample was digested in a 30 ml Kjeldahl digestion flask with 1 ml of concentrated H_2SO_4 and a catalyst mixture ($CuSO_4.5H_2O$ 1 g, selenium 1 g, K_2SO_4 20 g) until it became colourless. The mixture was cooled to room temperature (25^oC) and the volume was made up to 10 ml with distilled water. To a 1-ml sample, 1 ml of a silicate mixture (equal volume of 10% [w/v] Na_2SiO_3 and 10% [w/v] NaOH) and 5 ml of Nessler's reagent were added. The absorbance was read at 540 nm and the total nitrogen was estimated as $(NH_4)SO_4$ equivalents (Snell and Snell, 1949).

Viable counts of the different microorganisms

One gram of soil (blotted dry) was mixed with 9 ml of sterilized (121^oC, 15 min) distilled water and diluted serially up to 10^{-3}. The media and ingredients were autoclaved (110 kPa, 121^oC, 15 min) or passed through a membrane filter (0.20 µm) for sterilization. The soil suspensions (100 µl of 10^{-3} dilution) were mixed separately with 100 ml of different media, indicated below (Pelczar et al., 1957), and plated in five plates. Heat-treated (60^o C, 30 min) soil suspensions were included in the experiments to enumerate the spore-forming bacteria. The cultures were incubated at 30^oC in a BOD incubator. Incubation time was 7 days for fungi, actinomycetes and sulfur-oxidizing bacteria, 25 - 30 days for nitrifying bacteria and 3 days for other microbes. The colony forming units (cfu) of the microbes were counted under a colony counter. The heterotrophic, Gram-negative and spore-forming bacteria were enumerated on nutrient agar (NA) (g l^{-1}: peptone 5, beef extract 3, NaCl 3, pH 7.0, agar 20). For Gram-negative bacteria, sterilized crystal violet (0.01 g l^{-1} , aqueous solution) was added with nutrient agar before plating and the violet colonies were counted. The nitrifying and denitrifying bacteria were enumerated on Winogradsky's medium (g l^{-1}: K_2HPO_4 1, NaCl 2, $MgSO_4.7H_2O$ 0.5, $FeSO_4.7H_2O$ trace, $CaCl_2.2H_2O$ 0.02, pH 8.5), containing 1 g l^{-1} $(NH_4)_2SO_4$ or KNO_3, respectively. The colonies were visualized (pink colour) by flooding the plates with sulphanillic acid reagent (equal volume mixture of sulphanillic acid [8 g l^{-1} in 5 M acetic acid] and -naphthyl amine [5 g l^{-1} in 5 M acetic acid]). Colonies that formed haloes on insoluble phosphate-con-taining medium (g l^{-1}: glucose 10, $Ca_3(PO_4)_2$ 5, $MgSO_4.7H_2O$ 0.25, $MgCl_2$ 5, KCl 0.2, $(NH_4)_2SO_4$ 0.1, agar 18) were counted for the phosphate-solubilizing microbes (Nautiyal, 1999). The asymbiotic nitrogen-fixing bacteria were counted on nitrogen-free medium (g l^{-1}: mannitol 10, K_2HPO_4 0.5, $MgSO_4.7H_2O$ 0.2, NaCl 0.2, $MnSO_4.4H_2O$ trace, $FeCl_3$ trace, agar 18, pH 7.2). The sulfur-oxidizing bacteria (brown colonies), soil fungi and actinomycetes were counted on *Thiobacillus* medium (g l^{-1}: $Na_2S_2O_3$ 0.5, $(NH_4)_2SO_4$ 0.4, KH_2PO_4 4, $CaCl_2$ 0.25, $MgSO_4.7H_2O$ 0.5, $FeSO_4$ 0.01, agar 18), mycological agar medium (g l^{-1}: papaic digest of soyabean meal 10, dextrose 40, agar 18, pH 7) and Krainsky's medium (g l^{-1}: glucose 10, asparagine 0.5, K_2HPO_4 0.5, agar 15, pH 7), respectively (Pelczar et al., 1957). All of the experiments were repeated three times.

Isolation and characterization of *Bacillus thuringiensis*

A heat-treated soil suspension (100 l of a 10^{-3} dilution) was mixed with 100 ml of nutrient agar and plated in five plates to enumerate and isolate *Bacillus thuringiensis* (Bt). The plates were incubated for 72 h at 30^o C. All the bacterial colonies were checked under a phase-contrast microscope (x100). Those producing crystal were recorded, isolated, purified and maintained on nutrient agar slants at 4^oC. The phenotypic characteristics of the organisms were studied by making use of standard methods (Sneath, 1984). Response of the Bt isolates to oxygen was checked in thioglycolate medium (g l^{-1}: peptone as pancreatic digest of casein 15, glucose 5, yeast extract 5, L-cysteine 0.75, NaCl 2.5, agar 0.75, sodium thioglycolate 0.1 - 0.5, pH 7.2) by inoculating a loopful of bacteria at the bottom of the tubes (20 cm x 20 mm diameter). The tubes were loosely fitted with a screw cap without a paraffin seal. The isolates were compared with the phenotypic characteristics of Bt subspecies and grouped accordingly (Sneath, 1984; de Barjac and Frachon, 1990).

RESULTS AND DISCUSSION

The soils of Gadakunjang and Bhuiyapada were sandy loam and sandy, respectively. The soils were saline (3.7 - 8.6 mS cm^{-1}), acidic (pH 3.6 - 6.6), possessed 0.6 - 1.1% (w/v) organic carbon and 0.06 - 0.1% (w/v) organic nitrogen (Table 1). The properties of these soils were comparable to those of the saline rice fields of the coastal Bay of Bengal in West Bengal (Bandopadhyay and Bandopadhyay, 1983). However, the acidity and salinity levels were more than the pH (6.08-6.39) and Eh (3.58 - 4.18 $mScm^{-1}$) levels of the Himalayan (Srinagar) island (Port Blair), brackish water (Mahe) and coastal mesophi-

Table 1. Characteristics of the saline soils of Gadakujang and Bhuiyapada.

Soil no.	Location	Site	Soil type	pH	Eh (mS cm^{-1})	Organic C (%)	Organic N (%)
98	Gadakujang	Paddy field no.1	Sandy loam	5.0±0.1	4.6±0.1	1.0± 0.04	0.09 ± 0.02
99	Gadakujang	Fallow land no. 1	Do	3.6±0.2	5.1±0.2	0.8± 0.03	0.09 ± 0.01
100	Gadakujang	Fallow land no. 2	Do	4.9±0.1	6.8±0.2	0.8± 0.06	0.08 ± 0.02
101	Bhuiyapada*	Paddy field no. 3	Sandy	6.6±0.2	3.7±0.3	0.6± 0.02	0.06 ± 0.01
102	Gadakujang	Paddy field no. 2	Sandy loam	5.2±0.2	8.6±0.2	1.1± 0.08	0.1± 0.02

* No fellow land was available around the field. Backward flow of seawater inundates the fields at Gadakujang. Paddy field no. 2 and fellow land no. 2 remain submerged from July to November, whereas Paddy field no. 1 and fellow land no. 1 remain submerged from July to September. The field of Bhuiyapada is periodically submerged by tidal water. Fellow land 1 is located above the Paddy field, away from the flow of seawater and upland. Fellow land 2 lies below Paddy field 2, towards the flow of seawater and lowland.

Table 2. Populations of different microbes in the soil.

Type of organism	Microbial population (cfu x 10^5 g^{-1} dr. soil) in soil no.				
	98	99	100	101	102
Heterotrophic bacteria	27.30 ± 8.02	27.90 ± 8.03	8.10 ± 1.04	4.50 ± 0.01	47.60 ± 15.06
Spore-forming bacteria	11.00 ± 1.15	11.80 ± 1.21	1.70 ± 0.03	1.00 ± 0.01	21.20 ± 7.04
Gram-negative bacteria	26.70 ± 9.06	27.80 ± 8.03	0.80 ± 0.07	2.20 ± 0.04	27.80 ± 8.02
Nitrifying bacteria	0.06 ± 0.005	0.03 ± 0.004	0.02 ± 0.002	0.01 ± 0.001	0.10 ± 0.001
Denitrifying bacteria	11.20 ± 1.21	13.10 ± 1.01	2.50 ± 0.01	1.50 ± 0.02	1.80 ± 0.03
Phosphate-solubilizing bacteria	1.69 ± 0.02	7.30 ± 1.01	8.00 ± 1.04	1.40 ± 0.03	6.60 ± 1.02
Asymbiotic nitrogen-fixing bacteria	40.00 ± 16.5	21.40 ± 2.03	7.40 ± 1.03	2.60 ± 0.04	15.20 ± 1.03
Sulfur-oxidizing bacteria	7.01 ± 1.02	7.41 ± 0.03	4.26 ± 0.01	3.22 ± 0.01	8.01± 0.04
Actinomycetes	0.01 ± 0.001	0.02 ± 0.002	0	0	0.01 ± 0.001
Fungi	0.00 ± 0.001	0.01± 0.001	0.01 ± 0.002	0.01 ± 0.001	0.02 ± 0.002
Bacillus thuringiensis (Bt)	0.70 ± 0.06	0.50 ± 0.05	0	0.70 ± 0.04	6.40 ± 0.1
Bt index*	0.06 ± 0.005	0.04 ± 0.05	0	0.70 ± 0.05	0.30 ± 0.04

cfu = colony forming units. * Bt index = number of Bt cfu/number of spore forming cfu.

lic (Mangalore) rice fields of the coastal Arabian Sea of India (Das and Dangar, 2008).

Viable counts, despite its inherent limitations, were used in our study as it reflects abundance and functional dominance of various microbial communities. In contrast, molecular techniques do not reveal the ecological role of phenotypes (Liesack et al., 2000; Reichardt et al., 2001; Nannipieri et al., 2003). In different soils, the heterotrophic, spore-forming, Gram-negative, denitrifying, phosphate-solubilizing, asymbiotic nitrogen-fixing and sulfur-oxidizing bacterial populations were 4.50 - 47.6, 1.00 - 21.2, 0.80 - 27.8, 1.50 - 13.1, 1.40 - 8, 2.6 - 40 and 3.2 – 8.0 cfu x10^5 g^{-1} dr. soil, respectively (Table 2). Populations (cfu x 10^5 g^{-1} dr. soil) of nitrifying bacteria (0.01 - 1.00), actinomycetes (0.00 - 0.02) and fungi were very low (0.00 - 0.02) (Table 2). Despite the higher salinity, microbial populations of denitrifying and asymbiotic nitrogen-fixing bacteria occurred in higher numbers in Paddy field no. 2 of Gadakujang compared to other soils (Table 2) . This is in contrast to the view that salinity and microbes have a negative correlation (Zahran, 1997; Nannipieri et al., 2003; Tilak et al., 2005). Comparable population size (2, 2 - 6) x 10^6 cfu g^{-1} of non-saline soil (Liesack et al., 2000; Reichardt et al., 2001) and our results of saline soils also disagree to the proposition. Nevertheless, more microbial density (10^6 - 10^9 cfu/g) of relatively lesser saline soils of the Himalayan (Srinagar), island (Port Blair), brackish water (Mahe) and coastal (Mangalore) rice fields at post-harvest period than the present study support that salinity has negative effect on soil microbes (Das and Dangar, 2008). Sulphate may act as an electron acceptor during mineralization in saline soils (Zahran, 1997). Therefore, more sulfur -oxidizing bacteria (Table 2) may have a positive effect on the other soil microbes in saline soils. The relatively higher organic carbon and nitrogen content in the soil of field No. 2 of Gadakujang (Table 1) might have supported more micro-bial growth, thus favouring more microbial populations in the clay-loam soil of Srinagar compared to the sandy loam soils of the rice fields of different parts of India (Das and Dangar, 2008). However, universal correlation of microbial populations with soil salinity, redox potential, nitrogen and carbon content has also not been observed in previous studies (Zahran, 1997; Nannipieri et al ., 2003; Tilak et al., 2005, Das and Dangar 2008). The results (Table 2) implied that microbial functionalities of coastal

Table 3. Phenotypic characteristics of the bacteria isolated from different soils.

Soil no.	Total isolate * (Number)	Isolate no. (TB)	Colony character[#]	Bacterium[§] (l x w, µm)	Spore[‡] (l x w, µm)	Crystal		Tolerance		Antibiotic	
						Morphotype	l x w, m [†]	NaCl(%)	Na-acetate (M)	Sensitive	Resistant
98	16	158	I, GW, M, R, E, G	3±0.54 x1.1± 0.54	1.7±0.44 x 1.1±0.22	Bipyramidal	1.4±0.54 x 0.9±0.29	7	0.4	a, c, e	b, d
	21	159	P, GW, M, F, E, G	2.4±1.48 x 1.2±0.44	1.8±0.44 x 0.92±0.29	Bipyramidal	1.3±0.44 x 0.9±0.29	9	0.1	a, c, d, e	b
99	11	155	F, CW, M, F, U, G	2.6±0.89 x 1.2±0.44	1.92±0.2 x 1.6±0.54	Bipyramidal	1.3±0.27 x0.92±0.29	9	0.25	a, e	b, c, d
	9	156	I, CW, M, R, L, G	3.5±0.5 x 1.2±0.44	1.6±0.54 x 0.92±0.29	Bipyramidal	1.2±0.44 x 0.92	9	0.1	a, c, d, e	b
100	0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
101	28	157	F, CW, M, F, E, G	2.9±0.74 x 1.4±0.54	1.8±0.44 x 1.3±0.44	Bipyramidal	2.1±0.22 x 0.92±0.29	9	0.3	a, c, d, e	b
	18	160	F, CW, M, R, E, G	2.6±0.89 x 0.9±0.29	1.5±0.5 x 1.1±0.22	Spherical	1.4±0.5	18	0.25	a, d, e	b, c
	18	161	F, GW, M, F, E, G	2.6±0.89 x 1.2±0.44	2.1±0.89 x 0.92±0.29	Small spherical	1.1±0.22	18	0.25	a, c, d, e	b
102	46	163	F, GW, M, F, E, G	2.8±0.83 x 1.2±0.44	0.92±0.29 x 1.1±0.22	Bipyramidal	1.1±0.22 x 0.8±0.28	4	0.25	a, c,	b, d, e
	84	164	R, GW, M, R, L, G	3.0±0.7 x 1.1±0.22	1.6±0.54 x 1.1±0.22	Bipyramidal	1.2±0.44 x 0.9±0.29	3	0.1	a, d, e	b, c

*The isolates were obtained from a 100- l pasteurized soil suspension of 10^{-3} dilutions. [#]Colony size in mm. [§]Rod shaped. [‡]Eliptical. [†]For spherical crystals diameter is given in the Table. I = irregular. C = circular. W = white. M = metallic. GW= Gummy white. CW = Creamish white. BW = Brownish white. R = raised. E = Entire. U = Undulate. L = Lobate. G = Gummy. a = Streptomycin, norfloxacin (10 µg/disc), vancomycin (30 g/disc), polymixin (300 U/disc); b = Penicillin G (10 U/disc), ampicillin/sublactum (10/10 g/disc), trimethoprim (30 µg/disc); c = bacitracin (10 U/disc); d = Triple sulph (300 g/disc); e = erythromycin (15 µg/disc). NA = Not applicable..

coastal saline paddy fields were diverse and complex, and that the nutrition status of the soil has a bearing on the populations in these soils (Zahran, 1997; Tilak et al*.,* 2005).

Predominance of the asymbiotic nitrogen-fixing bacteria over other microbial populations and the presence of low numbers of nitrifying bacteria in the soils (Table 2) is in agreement with results of non-saline rice fields (Tilak et al*.,* 2005; Reichardt et al*.,* 2001; Das and Dangar, 2008). This implied that salinity has a negative impact on the nitrification process. Similar to our results (Table 2), more denitrifying than the nitrifying bacteria were also recorded in saline soils in other studies (Zahran, 1997; Das and Dangar, 2008) . The negative impact of salinity on nitrification but its positive effects on denitrification suggests that salinity might cause more nitrogen loss, resulting in a decline of production. However, Bandopadhyay and Bandopadhyay (1983) recorded inhibition of both nitrification and denitrifcation by salinity. Nominal fungal and actinomycetes populations in the soils (Table 2) favoured its negative relationship with water content and salinity (Zahran, 1997; Reichardt et al., 2001; Tilak et al., 2005), which would be an important cause of low nutrition, especially carbon levels, in the saline rice fields.

No *B. thuringiensis* (Bt) isolates could be isolated from soil no. 100 (Table 2). However, the Bt population was highest in soil no. 102, which had high salinity. Despite soil no. 101 being a less saline habitat, its Bt isolates tolerated a higher concentration of salt (9 - 18% [w/v]) compared to that of soil no. 102 (4 - 8% [w/v]), which had a higher salinity (Eh 8.6 ms cm^{-1}) and a smaller Bt population (Tables 1 - 3). This implied that osmotic stress tolerance and population size may not be directly correlated with salinity. The Bt population size (index 0.04 - 0.70) and number of Bt-positive saline soils (80%) (Table 2) were comparable to those of phyllospheric and non- saline soil habitats (Bt index of 0.05 - 0.8, and 70 - 78% Bt-positive soils) (Kaur and Singh, 2000; Das and Dangar, 2007, 2008). However, the Bt population in this study was about 10-times higher than in the relatively lower saline rice fields (Das and Dangar, 2008). This implied that more Bt could have adapted to the osmotic conditions. Phenotypic and crystal morphotypes of the Bt were different within and among the soils. Bt containing bipyramidal crystals were predominant (Table 3), which supported the observation that bipyramidal crystal-producing Bt were more diverse in the rice fields (Attathom et al., 1995; Kaur and Singh, 2000; Das and Dangar, 2007; 2008).

Increased NaCl tolerance (4 - 18% [w/v]) of the Bt (Table 3) compared to that of other rice fields (Das and Dangar 2007; 2008) suggested that these Bt isolates would be better suited to the control of pests and to manage nutrition in saline

Table 4. Identification of the bacteria based on the phenotypic characteristics

Character	Bacteria (TB)	Observation
Matty colony, rod shape, endospore, motile, Gram stain, crystal, facultative anaerobe/ microaerobic, catalase, oxidase	155-161, 163, 164	+
Length (m)	155-161, 163, 164	2.4-3.5
Diameter >2.5 m	155-161, 163, 164	0.9-1.4
Rod/filament curved, cocci in tetrads/packets	155-161, 163, 164	-
Filament	155, 156, 158-161, 163, 164	-
	157	+
Sporangium	155-161, 164	Not swollen
	163	Swollen
Acidity from glucose	155-158, 160, 161, 163, 164	+
	159	-
Nitrate reduction	155, 156, 158, 159, 161, 164	+
	157, 160, 163	-
Genus	155-161, 163, 164	*Bacillus*
Species	155-161, 163, 164	*thuringiensis*

NS= non-swollen, S= Swollen. + = Positive, - = Negative.

Table 5. Phenotypic grouping of the bacteria into different subspecies

Characters	Bacteria (TB)					
	155	**156, 159, 164**	**157**	**158, 163**	**160**	**161**
Acetyl methyl carbinol	+	+	+	+	-	-
Urease	+	+	+	±	-	-
Arginine dihydrolase	+	+	+	+	-	-
Pellicle	-	±	+	-	+	-
Cellobiose utilization	+	+	-	±	+	+
Mannose utilization	-	-	+	+	-	-
Starch hydrolysis	+	+	+	+	+	±
Sucrose fermentation	-	-	-	±	+	-
Tween esterase	+	±	+	+	+	+
Citrate utilization	-	-	-	-	-	-
Salicin fermentation	-	-	-	-	+	+
Lecithinase	-	+	+	-	+	-
Gelatinase	+	+	+	+	+	+
Esculin fermentation	+	+	+	±	+	+
Chitin hydrolysis	+	+	+	±	-	-
Bt subspecies	*galleriae*	*kurstaki/kenyae/aizawai*	*thompsoni/ coreanensis*	*darmstadiensis*	Unidentified	*entomocidus*

+ = Positive result, - = Negative result.

rice fields (Zahran, 1997; Nanniperi et al., 2003).

Altogether 406 Bt colonies were isolated from the soils of Gadakujang and Bhuiyapada, and phenotypically characterized up to subspecies level (Sneath, 1984; de Barjac and Frachon, 1990). Based on the phenotypic characters, the isolates were divided into 9 groups, designated as TB 156-161, 163 and 164 (Table 3). The organisms formed matty colonies; they were Gram-positive motile rods, catalase positive, had oval spores and formed crystals with a non- swollen sporangium (except for the TB 163) (Table 4). The phenotypic characters identified the isolates into Group I of the genus *Bacillus* and species *thuringiensis*, but TB 163 differed due to a swollen sporangium (Sneath, 1984; de Barjac and Frachon, 1990). Bt is typically classified by serotyping (flagellar antigenicity) beyond species level, but some subspecies can be tentatively assigned based on phenotypic characters (de Barjac and Frachon, 1990). Pend-

ing serotyping, the isolates were phenotypically grouped as subspecies *darmstadiensis* (TB 158 and 163), *kurstaki/kenyae/aizawai* (TB 156, 159, 164), *thompsoni/coreanensis* (TB 157), *galleriae* (TB 155) and *entomocidus* (TB 161). However, TB 160 could not be assigned to any subspecies (Table 5). The swollen sporrangium of TB 163 (Table 4) and, based on a report noting occasional swelling of the sporangium of a Bt isolate (Attathom et al*.*, 1995), warrants reconsideration regarding the grouping of *Bacillus* spp. into Group I, II and III based on sporangium character (Sneath, 1984). All Bt isolates were resistant to penicillin, ampicillin and the antifungal agent nystatin (Table 3) . This is in agreement with results reported previously (Kaur and Singh, 2000; Das and Dangar, 2007; 2008). The crystal morphotypes were different in some of the Bt subspecies isolated from the saline soil (Table 3), which implied that the crystal morphotype has no relation with serotype (Attathom et al*.,* 1995; Kaur and Singh, 2000; Das and Dangar, 2007; 2008). The diversity and salt tolerance of the Bt isolates suggests that they may be able to control different arthropod pests in saline rice fields.

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.

Bandopadhyay BK, Bandopadhyay AK (1983). Effect of salinity on mineralization and immobilization of nitrogen in a coastal saline soil of West Bengal. Indian Agric. 27:41-50.

Black CA (1965). Methods of Soil Analysis, Part 2, American Soc. Agron., Madison, Wisconsin.

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

Das J, Dangar TK (2008). 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. Microbial. Bioterchnol. 24: 1403-1410.

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

Jong KB, Cote JC (2000). A review of the environmental impacts of the microbial insecticide Bacillus thuringiensis: Technol. Bull no. 29. Hort. Res. Develop. Centre., Montreal.

Kaur S, Singh A (2000). Natural occurrence of *Bacillus thuringiensis* in leguminous phylloplanes in New Delhi region of India. World J. Microbiol. Biotechnol. 16: 679-682.

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

Lee KE, Pankhurst CE (1992). Soil organisms and sustainable productivity. Australian J. Soil Res. 30**:**855-92.

Maeda M, Mizuki E, Hara M, Tanaka R, Akao T, Yamashita S, Ohba M (2001). Isolation of *Bacillus thuringiensis* from intertidal brackish sediments in mangroves. Microbiol. Res. 156:195-198.

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

Nautiyal CS (1999). An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. FEMS Microbial. Lett. 70: 265-270.

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

Sneath PHA (1986). Endospore-forming Gram Positive Rods and Cocci. In Sneath et al. (eds) Bergey's Manual of Systematic Bacteriology, Williams and Wilkins: Baltimore, pp 1104-1139.

Snell FD, Snell CT (1949). Colorimetric Method of Analysis, Third edn., Vol. 2. Von Nostrand Co., Inc.: Princeton, New Jersey.

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.

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). Bull. Entomol. Res. 88**:**335-342.

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

Zhou Y, Choi YL, Sun M, Yu Z (2008). Novel roles of *Bacillus thuringiensis* to control plant diseases. Appl. Microbiol. Biotechnol. 80: 563-572.