Hindawi Publishing Corporation
International Journal of Ecology
Volume 2016, Article ID 7924258, 10 pages
http://dx.doi.org/10.1155/2016/7924258

Research Article

Seasonal Fluctuation of the Population and Characterization of *Bacillus* spp. Isolated from the Coastal Soils of Digha, West Bengal, India

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Received 19 October 2015; Accepted 7 March 2016

Academic Editor: Panos V. Petrakis

Seasonal fluctuation of the population of *Bacillus* spp. in the coastal soils of Digha, West Bengal, India, was determined and it has been found that, during summer, monsoon, and winter season, the *Bacillus* population density varied in the range of 0.01–0.236 × 10^6, 0.11–0.202 × 10^6, and 0.098–0.155 × 10^6, respectively. Two-way ANOVA, agglomerative hierarchial cluster (AHC) analysis, and principal component analysis (PCA) were performed to determine the diversity of *Bacillus* spp. in both spatial and temporal aspects. During summer season, the population of *Bacillus* spp. reached a comparatively higher density than monsoon or winter. Spatial variation was also exhibited among the *Bacillus* spp. in different coastal villages. A total of 25 strains of *Bacillus* spp. (DSB1–DSB25) were isolated from the coastal soils of different village areas of Digha, during the study period. The isolates were characterized morphologically, physiologically, and biochemically. Colony morphology of each of the isolates was thoroughly studied. Biochemical tests along with fermentation tests, NaCl, pH, and temperature tolerance tests were done. The antibiotic sensitivity of the isolated *Bacillus* spp. against different standard antibiotics was also assessed. The study revealed that the coastal soils of Digha area were rich in different strains of *Bacillus* spp. showing significant differences in the morphophysiological and biochemical properties.

1. Introduction

Diversity is composed of richness and evenness and it occurs in nature across all forms of life at global, regional, and local scale. Microorganisms represent the richest repertoire of molecular and chemical diversity in nature, as they comprise the most diverse form of life and also they are extraordinary reservoir of life in the biosphere that we have to explore and understand [1]. Among the extremophilic microorganisms, bacteria are the most dominant group involved in the biogeochemical cycling mainly mediated by their enzymatic reactions. Nowadays, for sustaining a relatively pollution-free environment, a significant proportion of the bacterial flora which is not harmful to human being is exploited worldwide as biocontrol agents against different hazardous pests and vectors that were previously controlled by chemical pesticides [2]. Among the bacterial groups, *Bacillus* spp. are commercially formulated for biological control of plant pathogens as well as of vectors of several pathogenic agents of deadly diseases [3].

The bacteria of the genus *Bacillus* are generally defined as Gram-positive, aerobic or facultative anaerobic, motile, endospore-forming rod shaped microorganisms [4]. They are found in diverse environments such as soil and clays, rocks, dust, aquatic environments, vegetation, food, and the gastrointestinal tracts of various insects and animals [5]. The genus *Bacillus* is phenotypically and genotypically heterogeneous with its members exhibiting an extremely wide range of nutritional requirements, growth conditions, metabolic diversity, and DNA base composition [6]. Diversity of different strains of *Bacillus* is also exhibited in biochemical properties such as the ability to degrade many different substrates including cellulose, starch, proteins, agar, hydrocarbons, and also biofuels that are derived from plant and animal

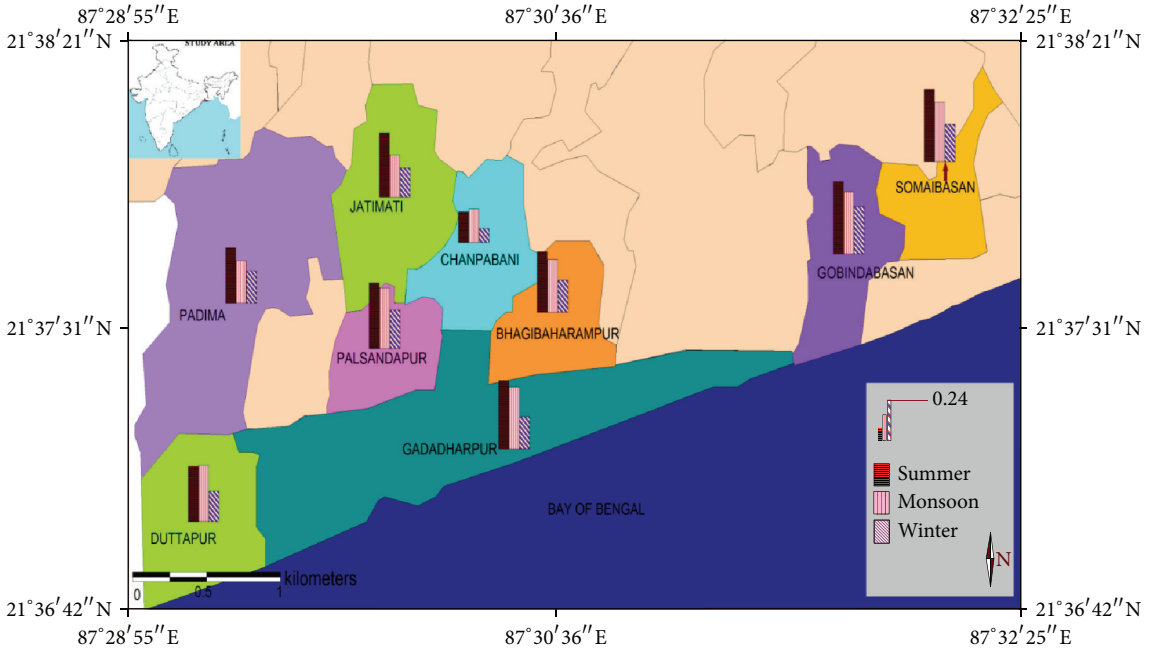

Figure 1: GIS image of the study site showing seasonal diversity of *Bacillus* spp. in bar diagrams.

sources [7]. Furthermore, some *Bacillus* species are heterotrophic nitrifiers, denitrifiers, nitrogen fixers, iron precipitators, selenium oxidizers, oxidizers and reducers of manganese, facultative chemolithotrophs, acidophiles, alkaliphiles, psychrophiles, thermophiles, and halophiles [6, 8]. This diversity in biophysiological characteristics thus allowed the *Bacillus* species to occupy and colonize a wide variety of ecological niches. This ability of wide ecological diversification of *Bacillus* strains is potentiated by the production of spores, which are characterized by their magnificent ability of resistance against environmental stresses and dormancy. Several studies have been done on the *Bacillus* diversity all over the world, but in this context the coastal soils of Digha area remained unexplored till date. The coastal areas of Digha having special environmental condition, such as high humidity, high salinity, and low nutrient availability in soil, may contain bioresource microbes with special adaptable characteristics that would help them to be sustained in adverse environmental condition for a long time. The present piece of work was aimed at studying the seasonal population diversity of *Bacillus* spp. along with their characterization on morphological, biochemical, and physiological aspects in the coastal areas of Digha, West Bengal, India.

2. Materials and Methods

2.1. Soil Collection. The soil samples were collected from nine village areas of Midnapore coastal belt, West Bengal, India (Padima (21°37′39″N, 87°29′28″E), Jatimati (21°37′40″N, 87°29′46″E), Chanpabani (21°37′39″N, 87°30′22″E), Palsandapur (21°37′30″N, 87°30′04″E), Bhagibaharampur (21°37′36″N, 87°30′44″E), Duttapur (21°36′57″N, 87°29′29″E), Gadadharpur (21°37′05″N, 87°30′04″E), Gobindabasan (21°37′36″N, 87°31′18″E), and Somaibasan (21°37′50″N, 87°32′11″E)) during the time period from March 2014 to February 2015 (Figure 1). The topmost soil (1 cm) was scrapped off and then about 100 g of soil from each area (9 samples/area) was collected in sterile polythene bags sealed with rubber bands and taken to the Parasitology and Microbiology Research Laboratory, The University of Burdwan.

2.2. Culture and Enumeration of the Bacillus Colonies from Soil. To study the seasonal diversity of *Bacillus* spp. in coastal areas of Digha, the soil suspensions were made (up to 10^{-5} dilution) and were pasteurized at 60°C for 30 minutes. A 100 μL portion of the pasteurized soil suspensions was mixed with 100 mL of Nutrient Agar (peptone 5 g/L, beef extract 3 g/L, NaCl 5 g/L, and agar 15 g/L, pH 7.4 ± 0.2), KG agar (peptic digest of animal tissue 1 g/L, yeast extract 0.5 g/L, phenol red 0.025 g/L, and agar 18 g/L, final pH 6.8 ± 0.2, after autoclaving, sterile egg yolk emulsion 100 mL/L and polymyxin B), and Hicrome Bacillus agar (peptic digest of animal tissue 10 g/L, meat extract 1 g/L, d-mannitol 10 g/L, sodium chloride 10 g/L, chromogenic mixture 3.2 g/L, phenol red 0.025 g/L, and agar 15 g/L, final pH 7.1 ± 0.2) media separately and plated on Petri dishes and then incubated in a Biological Oxygen Demand (BOD) incubator at 30 ± 0.1°C for 48 hours. After incubation, the colonies grown in those media were observed thoroughly for screening of the *Bacillus* colonies and a portion of each colony was observed under microscope to observe the rod shaped vegetative cells. The *Bacillus* colonies were then enumerated and total colony forming unit (cfu)/g of dry soil was calculated [9, 10].

2.3. Isolation and Characterization of the Bacillus spp. The isolated *Bacillus* colonies were streaked on Nutrient Agar

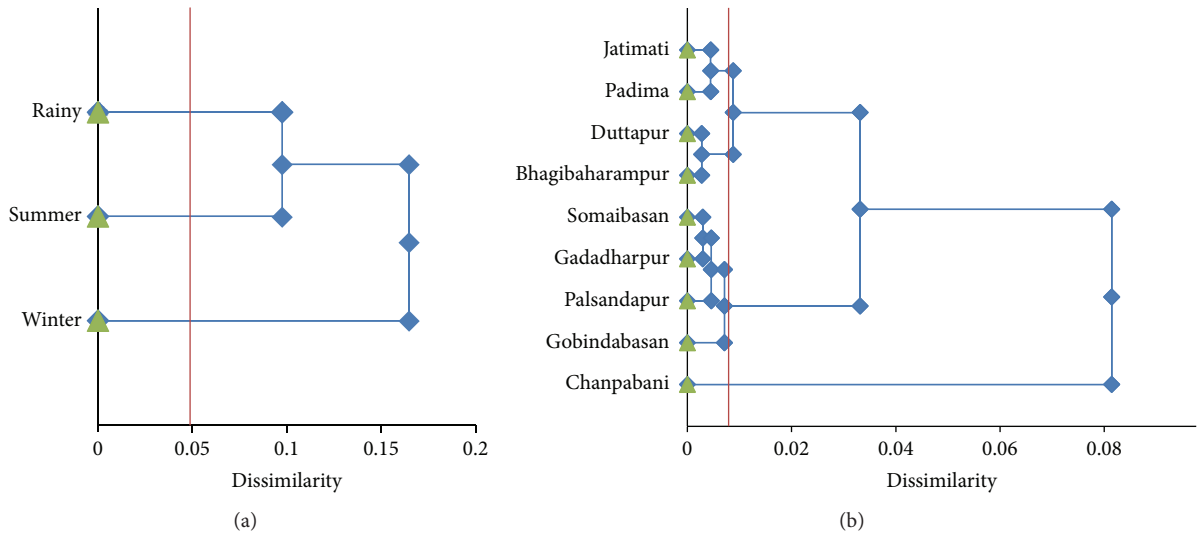

Figure 2: AHC analysis showing distribution pattern of *Bacillus* spp. (a) in different seasons and (b) in different places.

slants and stored at 4°C for further characterization. Morphological characters of the colonies and physiological and biochemical characters of the bacterial isolates were studied following the standard microbiological methods [11–13]. To study the biochemical properties, catalase, citrate utilization, nitrate reduction, indole production, methyl-red, Voges-Proskauer, urease, oxidase, temperature tolerance, pH tolerance, NaCl tolerance, and carbohydrate metabolism (acid gas production) tests were done. Total carbohydrate, protein, and DNA content of the bacterial isolates were estimated to assess the cellular constituents of the isolates [14–16]. For qualitative characterization of enzymes produced, starch hydrolysis, lipase, protein hydrolysis, gelatin hydrolysis, and casein hydrolysis tests were done.

2.4. Antibiotic Sensitivity Test. Antibiotic sensitivity tests of the bacterial isolates against different standard antibiotic discs (Amoxicillin (10 μg), Chloramphenicol (30 μg), Ciprofloxacin (30 μg), Kanamycin (30 μg), Streptomycin (10 μg), Tetracycline (30 μg), Vancomycin (30 μg), polymyxin B (300 units), Bacitracin (10 units), Ampicillin (10 μg), Nystatin (100 U), Erythromycin (15 μg), Penicillin G (10 units), and Rifampicin (5 μg)) were performed following Brown [17].

2.5. Statistical Analysis. Two-way ANOVA, agglomerative hierarchial cluster analysis, principal component analysis, and other statistical analyses of the observed experimental data were performed using Microsoft Excel 2007 and SPSS statistical 17.0 software [18].

3. Result

In the coastal areas of Digha, during summer, monsoon, and winter season, the *Bacillus* population density varied in the ranges of 0.01–0.236 $\times$ 10^6, 0.11–0.202 $\times$ 10^6, and 0.098–0.155 $\times$ 10^6, respectively (Table 1). In soil samples of Padima, Jatimati, Palsandapur, Bhagibaharampur, Gadadharpur, Gobindabasan, and Somaibasan village areas, the *Bacillus* population showed higher density in summer season whereas, in Chanpabani and Duttapur area, the density of *Bacillus* in soil was highest during monsoon. The estimated marginal mean analysis showed that in summer the *Bacillus* population was comparatively higher than other seasons of the year. The winter showed relatively lower population density of *Bacillus* spp. in the villages of coastal areas of Digha, West Bengal, India (Table 1). The two-way ANOVA was carried out to determine the variation of *Bacillus* spp. according to different places and different seasons. It was found that the population of the *Bacillus* spp. significantly varied according to different habitat and as well as different seasons ($p < 0.05$). Agglomerative hierarchial cluster analysis showed that the density pattern of *Bacillus* spp. in summer and rainy season was significantly different from winter season (Figure 2(a)). In terms of density pattern in different places, Jatimati and Padima; Duttapur and Bhagibaharampur; Somaibasan, Gadadharpur, Palsandapur, and Gobindabasan; Chanpabani formed four different clusters (Figure 2(b)). Principal component analysis (PCA) exhibited

Table 1: Seasonal diversity ($\times 10^6 \pm$ S.E cfu/g dry soil) of *Bacillus* spp. in some villages of coastal areas of Digha, West Bengal, India.

Place	Summer	Monsoon	Winter
Padima	0.183 ± 0.010	0.139 ± 0.005	0.104 ± 0.013
Jatimati	0.211 ± 0.025	0.138 ± 0.012	0.098 ± 0.006
Chanpabani	0.010 ± 0.011	0.110 ± 0.006	0.047 ± 0.012
Palsandapur	0.216 ± 0.028	0.197 ± 0.012	0.126 ± 0.011
Bhagibaharampur	0.198 ± 0.020	0.175 ± 0.012	0.107 ± 0.011
Duttapur	0.179 ± 0.032	0.187 ± 0.009	0.099 ± 0.006
Gadadharpur	0.223 ± 0.022	0.201 ± 0.022	0.106 ± 0.013
Gobindabasan	0.234 ± 0.025	0.202 ± 0.022	0.155 ± 0.025
Somaibasan	0.236 ± 0.027	0.194 ± 0.026	0.124 ± 0.023

Results are mean of nine replications. SE: standard error.

Table 2: Colony characteristics* of the *Bacillus* spp. isolated from the coastal areas of Digha, West Bengal, India.

Isolate number	Form	Colour	Elevation	Margin	Size (mm)	Consistency
DSB1	Circular	Off-white	Convex	Undulate	2.0 × 2.5	Gummy
DSB2	Irregular	Off-white	Convex	Entire	1.5 × 2.5	Gummy
DSB3	Circular	Pale yellow	Convex	Entire	1.5 × 2.5	Gummy
DSB4	Circular	Off-white	Convex	Entire	3.5 × 3.5	Gummy
DSB5	Circular	Pale yellow	Convex	Entire	1.0 × 2.0	Gummy
DSB6	Circular	Light yellow	Convex	Entire	1.0 × 2.0	Gummy
DSB7	circular	Off-white	Flat	Undulate	2.0 × 2.5	Gummy
DSB8	Circular	Off-white	Convex	Entire	2.0 × 3.0	Gummy
DSB9	Irregular	Off-white	Convex	Undulate	1.5 × 2.0	Gummy
DSB10	Irregular	Off-white	Flat	Undulate	2.5 × 3.5	Gummy
DSB11	Circular	Off-white	Convex	Entire	4.0 × 4.0	Gummy
DSB12	Spindle	Brownish	Convex	Entire	3.0 × 3.5	Gummy
DSB13	Irregular	Off-white	Convex	Undulate	2.0 × 2.5	Gummy
DSB14	Circular	Off-white	Convex	Undulate	1.5 × 2.5	Gummy
DSB15	Circular	Off-white	Convex	Entire	3.0 × 3.0	Gummy
DSB16	Circular	Pale yellow	Convex	Entire	1.5 × 2.0	Gummy
DSB17	Circular	Off-white	Convex	Slightly serrated	1.0 × 2.0	Gummy
DSB18	Circular	Pale yellow	Convex	Entire	2.5 × 3.5	Gummy
DSB19	Circular	Light yellow	Convex	Entire	2.0 × 2.5	Gummy
DSB20	Spindle	Brownish	Convex	Undulate	3.0 × 3.5	Gummy
DSB21	Irregular	Off-white	Convex	Entire	3.0 × 3.5	Gummy
DSB22	Circular	Pale yellow	Convex	Entire	2.0 × 2.5	Gummy
DSB23	Circular	Off-white	Convex	Slightly serrated	1.5 × 2.5	Gummy
DSB24	Circular	Pale brown	Convex	Entire	1.5 × 2.5	Gummy
DSB25	Circular	Off-white	Convex	Entire	1.0–2.0	Gummy

*Colony characteristics were observed from the colonies grown in Nutrient Agar medium.

two factors having the Eigenvalues of 1.727 and 0.970 that explained more than 89% (component 1, 57.56%, and component 2, 32.32%) of the variance on the microbial abundance in different seasons (Figure 3).

25 strains of *Bacillus* spp. (DSB1–DSB25) were isolated from the soil samples collected from the study area throughout the year. Colony characteristics of the isolated *Bacillus* spp. are shown in Table 2. The scanning electron micrographs of the isolates showed the morphology of the vegetative body, spores, and crystals (if present) (Figure 4). NaCl tolerance, pH tolerance, and temperature tolerance level of the isolates are shown in Figure 5.

Results obtained from the biochemical tests of these 25 isolates were shown in Table 3. All the isolates were positive for catalase test but negative for citrate, indole, chitinase, and lecithinase tests. Fermentation ability of the isolates to different carbohydrate sources was depicted in Table 4. All of the isolated *Bacillus* spp. could ferment glucose, sucrose, mannose, and arabinose, but none of them could ferment xylose and esculin present in the nutrient broth medium. Total carbohydrate, protein, and DNA content of the isolated *Bacillus* spp. were shown in Figure 6. Table 5 represents the antibiotic sensitivity of the isolated *Bacillus* spp. against different standard antibiotics. From the scanning electron images and biochemical characteristics, the isolates were identified as *Bacillus* spp. and among them, the crystal producing DSB1, DSB2, DSB4, DSB6, DSB7, DSB11, DSB12, DSB13, DSB15, DSB17, DSB18, DSB19, DSB20, DSB22, DSB23, and DSB24 was identified as *Bacillus thuringiensis*.

4. Discussion

Bacillus spp. almost inhabit all ecological niche. Numerous species of *Bacillus* inhabit coastal and marine environments [19–21]. The present investigation showed that the coastal soil of Digha comprised different *Bacillus* spp. which was found to be similar with the study of Chatterjee et al. [22] where they assessed the *Bacillus* population and characterized different isolates of *Bacillus thuringiensis* with respect to their morphological, biochemical, and crystal producing aspects. Das et al. [23] studied on different microbial population and assessed *Bacillus thuringiensis* diversity in coastal saline soils of Orissa. Their study revealed that the spore forming bacteria were ubiquitously distributed having diverse crystal morphotypes, salt tolerance, and nitrate reducing capability. Parvathi et al. [24] studied the *Bacillus* diversity and isolated several *Bacillus* spp. via biochemical and molecular characterization in the coastal environment of Cochin. Kumar et al. [25] characterized 12 efficient phosphate producing *Bacillus* strains on the basis of their morphological and biochemical

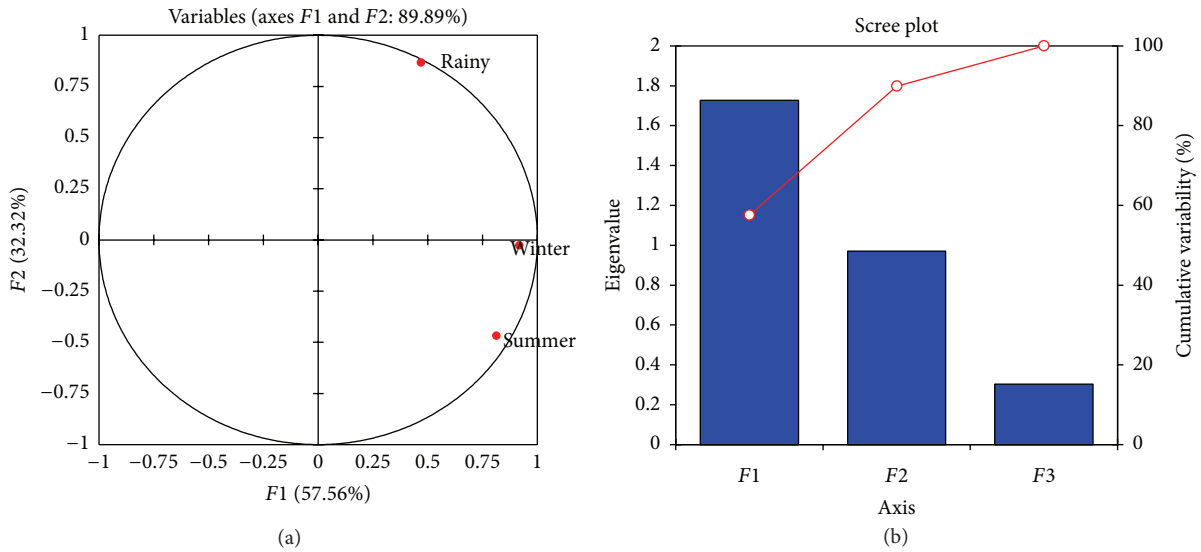

Figure 3: PCA showing (a) the relationship in the distribution pattern of *Bacillus* spp. and (b) Scree plot of the factors extracted.

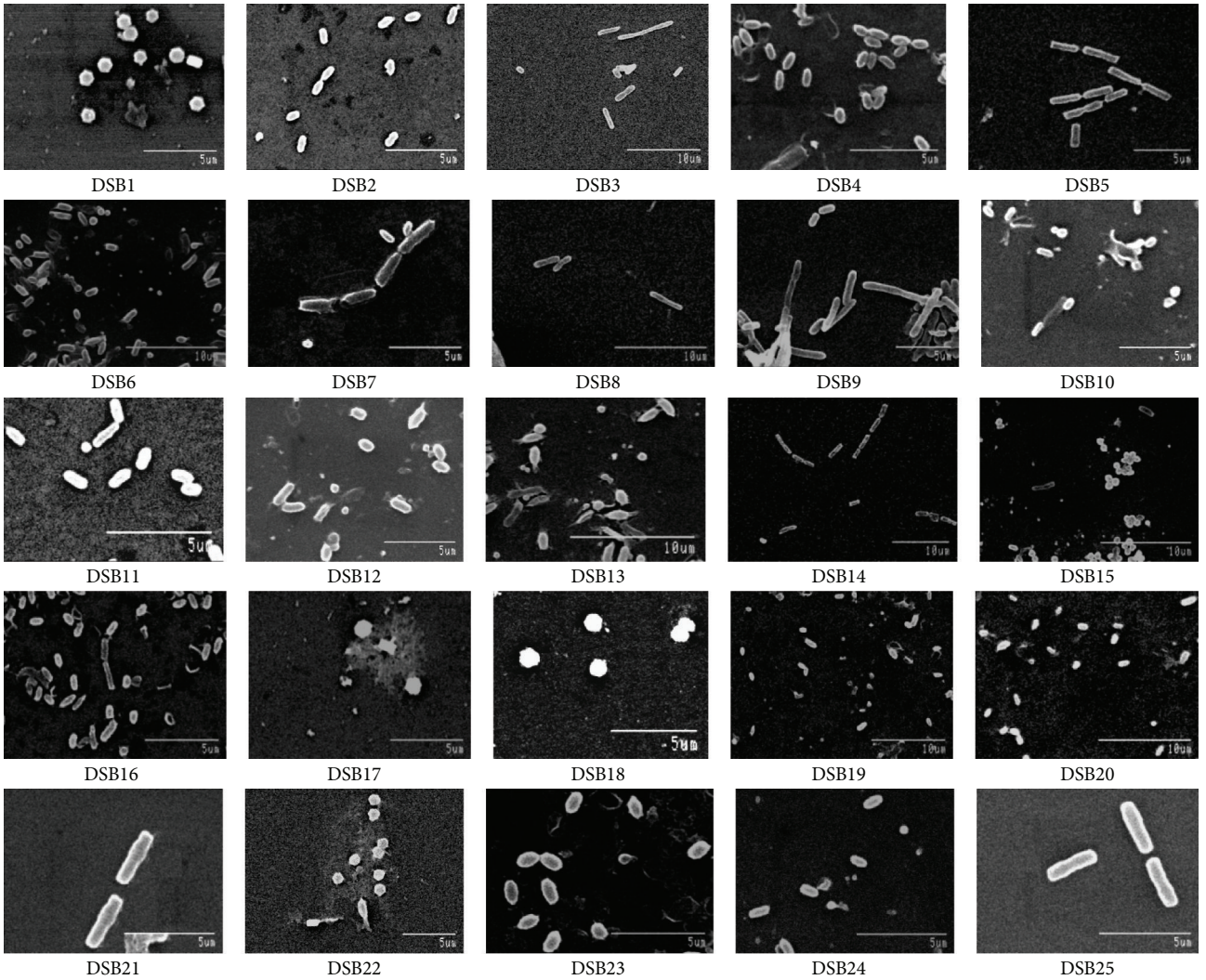

Figure 4: Scanning electron micrographs of the *Bacillus* spp. showing vegetative body, spores, and crystals (if present).

Table 3: Biochemical characterization of the *Bacillus* spp. isolated from the coastal areas of Digha, West Bengal, India.

Isolate number	Catalase	MR	VP	Nitrate	Citrate	Indole	Oxidase	Urease	Starch	Casein	Gelatin	Chitinase	Lecithinase	Lipase	Tween 20	Tween 80
DSB1	+	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−
DSB2	+	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−
DSB3	+	+	−	+	−	−	−	+	−	−	−	−	−	−	−	−
DSB4	+	+	−	−	−	−	−	+	−	−	−	−	−	−	−	−
DSB5	+	+	−	+	−	−	+	+	−	−	−	−	−	−	−	−
DSB6	+	+	−	+	−	−	+	−	+	+	−	−	−	+	−	+
DSB7	+	+	−	+	−	−	+	−	−	−	+w	−	−	−	−	−
DSB8	+	+	−	+	−	−	+	+	−	−	−	−	−	−	−	−
DSB9	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−
DSB10	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
DSB11	+	+	−	+	−	−	−	−	−	−	−	−	−	−	−	−
DSB12	+	+	−	+w	−	−	+	+	−	−	−	−	−	−	−	−
DSB13	+	+	−	−	−	−	+	+	−	−	−	−	−	−	−	−
DSB14	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−
DSB15	+	+	−	+	−	−	+	+	−	−	−	−	−	−	−	−
DSB16	+	+	+	−	−	−	−	−	+	−	+	−	−	+	−	−
DSB17	+	−	−	+	−	−	+	−	+	+	+	−	−	+	+	−
DSB18	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−
DSB19	+	+	+	−	−	−	−	−	−	−	−	−	−	+	−	+
DSB20	+	+	+	+	−	−	−	−	+	−	+	−	−	+	−	−
DSB21	+	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−
DSB22	+	+	+	+	−	−	+	−	−	−	+	−	−	−	−	−
DSB23	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	−
DSB24	+	+	+	+w	−	−	−	−	−	−	+	−	−	+	−	−
DSB25	+	−	−	+w	−	−	+	−	+	−	−	−	−	−	−	−

MR: methyl red, VP: Voges-Proskauer, "+": positive, "−": negative, and "+w": weakly positive.

Table 4: Fermentation tests of the *Bacillus* spp. isolated from the coastal areas of Digha, West Bengal, India.

Carbohydrate source	Positive	Negative
Glucose, sucrose, mannose, and arabinose	DSB1, DSB2, DSB3, DSB4, DSB5, DSB6, DSB7, DSB8, DSB9, DSB10, DSB11, DSB12, DSB13, DSB14, DSB15, DSB16, DSB17, DSB18, DSB19, DSB20, DSB21, DSB22, DSB23, DSB24, and DSB25	
Xylose and esculin		DSB1, DSB2, DSB3, DSB4, DSB5, DSB6, DSB7, DSB8, DSB9, DSB10, DSB11, DSB12, DSB13, DSB14, DSB15, DSB16, DSB17, DSB18, DSB19, DSB20, DSB21, DSB22, DSB23, DSB24, and DSB25
Fructose	DSB2, DSB3, DSB5, DSB6, DSB7, DSB9, DSB10, DSB11, DSB12, DSB13, DSB15, DSB16, DSB17, DSB19, DSB20, DSB21, DSB22, DSB23, and DSB25	DSB1, DSB4, DSB8, DSB14, DSB18, and DSB24
Lactose	DSB12, DSB13, DSB14, DSB15, DSB20, DSB21, DSB22, DSB23, DSB24, and DSB25	DSB1, DSB2, DSB3, DSB4, DSB5, DSB6, DSB7, DSB8, DSB9, DSB10, DSB11, DSB16, DSB17, DSB18, and DSB19
Salicin	DSB2, DSB18, and DSB23	DSB1, DSB3, DSB4, DSB5, DSB6, DSB7, DSB8, DSB9, DSB10, DSB11, DSB12, DSB13, DSB14, DSB15, DSB16, DSB17, DSB19, DSB20, DSB21, DSB22, DSB24, and DSB25
Mannitol	DSB3, DSB11, and DSB18	DSB1, DSB2, DSB4, DSB5, DSB6, DSB7, DSB8, DSB9, DSB10, DSB12, DSB13, DSB14, DSB15, DSB16, DSB17, DSB19, DSB20, DSB21, DSB22, DSB23, DSB24, and DSB25

Table 5: Antibiotic sensitivity tests of the isolated *Bacillus* spp. against some standard antibiotics.

Antibiotic	Sensitive	Resistant
Amoxicillin (10 μg), Chloramphenicol (30 μg), Ciprofloxacin (30 μg), Kanamycin (30 μg), Streptomycin (10 μg), Tetracycline (30 μg), Vancomycin (30 g), polymyxin B (300 units), and Bacitracin (10 units)	DSB1, DSB2, DSB3, DSB4, DSB5, DSB6, DSB7, DSB8, DSB9, DSB10, DSB11, DSB12, DSB13, DSB14, DSB15, DSB16, DSB17, DSB18, DSB19, DSB20, DSB21, DSB22, DSB23, DSB24, and DSB25	—
Ampicillin (10 μg) and Nystatin (100 U)	—	DSB1, DSB2, DSB3, DSB4, DSB5, DSB6, DSB7, DSB8, DSB9, DSB10, DSB11, DSB12, DSB13, DSB14, DSB15, DSB16, DSB17, DSB18, DSB19, DSB20, DSB21, DSB22, DSB23, DSB24, and DSB25
Erythromycin (15 μg)	DSB1, DSB2, DSB6, DSB7, DSB8, DSB9, DSB10, DSB12, DSB13, DSB14, DSB15, DSB16, DSB17, DSB18, DSB19, DSB21, DSB22, DSB23, DSB24, and DSB25	DSB3, DSB4, DSB5, DSB11, and DSB20
Penicillin G (10 units)	DSB2, DSB10, DSB11, DSB12, DSB13, DSB14, DSB15, DSB21, DSB22, and DSB25	DSB1, DSB3, DSB4, DSB5, DSB6, DSB7, DSB8, DSB9, DSB16, DSB17, DSB18, DSB19, DSB20, DSB23, and DSB24
Rifampicin (5 μg)	DSB3, DSB4, DSB5, DSB6, DSB7, DSB8, DSB9, DSB10, DSB12, DSB13, DSB14, DSB15, DSB16, DSB17, DSB20, DSB21, DSB22, DSB23, DSB24, and DSB25	DSB1, DSB2, DSB11, DSB18, and DSB19

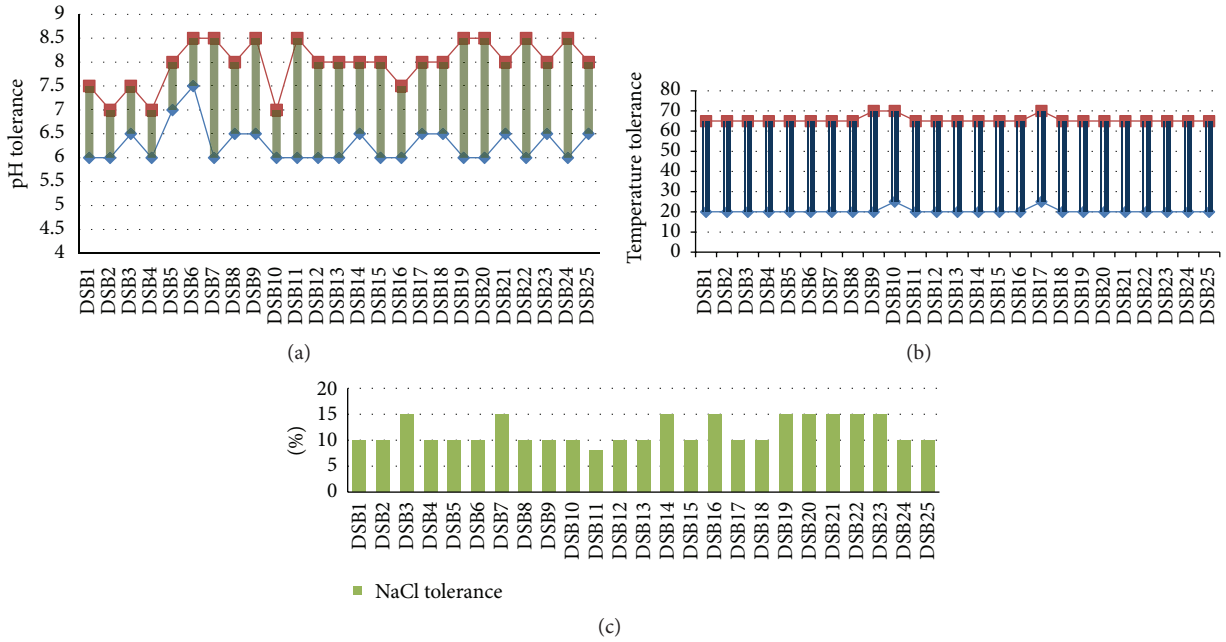

FIGURE 5: Line and bar diagrams showing (a) pH tolerance, (b) temperature tolerance, and (c) NaCl tolerance level of the isolated *Bacillus* spp.

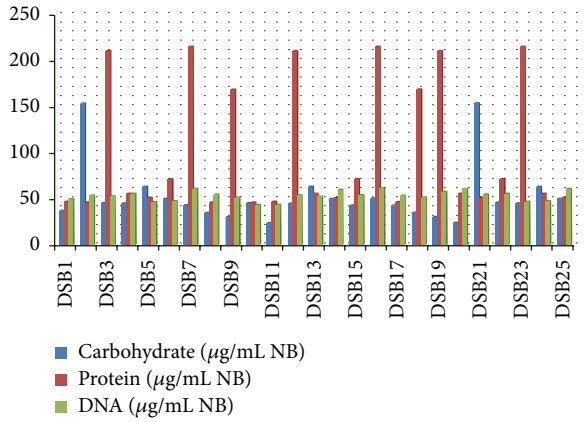

FIGURE 6: Total carbohydrate, protein, and DNA content of the *Bacillus* spp. isolated from the coastal areas of Digha, West Bengal, India.

characteristics. Several colonies of *B. cereus/B. thuringiensis* group were obtained from mangrove sediments and 1.7% of them were allocated to *B. thuringiensis* [26]. Apart from the coastal soils, Das and Dangar [27] have studied the bacterial diversity along with stress tolerant *Bacillus thuringiensis* in the Himalayan region. The present study characterized the *Bacillus* spp. on the basis of their biochemical and physiological properties taking account of the previous works of Park et al. [28], Joshi et al. [29], Al-Allaf [30], Azmi et al. [31], and Mondal et al. [32, 33], who also isolated and characterized different *Bacillus* spp. from different sources by biochemical and physiological analysis in different parts of the world. These *Bacillus* spp. isolated from different sources survive the harsh conditions by producing enzymes which are newly described, stable, and industrially important [34]. Gömöryová et al. [35] studied the distribution of functional groups of microbes via BIOLOG analysis and found organic carbon as an important functional group influencing microbial diversity in soil. Physicochemical properties of soils also influenced the soil microbial diversity pattern [9, 10]. The two-way ANOVA study revealed that in Digha coastal belt the *Bacillus* diversity varied significantly both in spatial and in temporal aspects which may be due to the variation in the microhabitat present in these areas in different seasons. The agglomerative hierarchial cluster (AHC) analysis established the correlation of the diversity of *Bacillus* spp. along with different places as well as different seasons in the study area. PCA analysis revealed that the *Bacillus* population showed variable density in different seasons which supported the observation from AHC analysis.

5. Conclusion

In the present study it was found that the coastal soils of Digha harboured different isolates of *Bacillus* spp., mainly *Bacillus thuringiensis* providing them an appropriate milieu for their living and propagation. The population density of *Bacillus* spp. in the coastal soils of Digha also greatly varied spatiotemporally. *Bacillus* spp. constituted one of the major components of coastal bacterial communities owing to their diverse and flexible physiological and biochemical attributes. Isolation and characterization of *Bacillus* spp. from these soils may help in identifying previously unknown mechanisms such as diverse metabolic activities and production of different enzymes and antimicrobial substances which would be biotechnologically valuable.

Competing Interests

The authors declare that they have no competing interests.

Acknowledgments

The authors are grateful to UGC and DST-FIST for providing financial assistance. The authors acknowledge The University of Burdwan for providing the laboratory facility required for the present study.

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