**International Journal of Experimental Research and Review (IJERR)**

**ISSN: 2455-4855 (Online)** ***Original Article***
**Received:** 10th May, 2020; **Accepted:** 21st June, 2020; **Published:** 30th August, 2020

DOI: https://doi.org/10.52756/ijerr.2020.v22.002

# Spatial variation of valuable bacterial enzymes in soil: A case study from different agro ecological zones of West Bengal, India

**Subarna Bhattacharyya[1*], Jayita Chopra[1], Rashmi Khushboo Minz[1], Mousumi Chakraborty[1], Srimanti Dutta Gupta[1], Malancha Roy[1], Soumi Sarkar[1], Punarbasu Choudhuri[2], Joydeep Mukherjee[1]**

[1]School of Envirnmental Studies, Jadavpur University, 188, Raja S. C. Mallick Road, Kolkata, W.B., India; [2]Department of Environmental Science, University of Calcutta, W.B., India

***Corresponding Author:** barna_kol@yahoo.com

**Abstract**

The spatial variability of cellulase, amylase, protease and pectinase activities were evaluated from four zones of West Bengal, India. The enzyme production data was plotted on the map of the study areas and spatial variability of cellulase, amylase, protease and pectinase activity was obtained. Available nitrogen of the soil was the most variable parameter with changing enzyme activity. It also varied with the available phosphorus but the variation was least with organic carbon content of the soil. Amylase was correlated with pectinase, available nitrogen and phosphorus. Cellulase was correlated with only available nitrogen; protease was correlated with pectinase and Pectinase was correlated with available nitrogen of the soil of the four sampling zone. Except protease activity, other enzymes were significantly correlated with bacterial density of the soil. These findings ultimately develop relationship among soil major nutrients and the map can be used for future enzyme bioprospecting in West Bengal, India.

**Keywords:** Amylase, protease, soil nutrient, bioprospecting, sustainable development.

## Introduction

Soil is a natural resource playing key roles in organic matter decomposition, nutrient cycling, and water retention and release. Soils are subject to natural or environmental degradation, often accompanied by erosion and leaching. The soil quality depends on the environmental stress, availability of soil flora and microorganisms. Soil enzyme activity is a potential indicator of soil quality. The sources of soil enzymes include living and dead microbes, plant roots and residues, and soil animals. Enzymes stabilized in the soil matrix accumulate or form complexes with organic matter (humus), clay, and humus-clay complexes. The enzyme activity is the cumulative effect of long term microbial activity and activity of the viable population at sampling (Tan et al., 2014). Enzyme activity

generally increases with the rise of soil organic matter content. Higher enzyme activity indicates larger microbial communities and greater stability of enzymes adsorbed on humic materials (Marinari and Antisari, 2010). The activities of extracellular enzymes in soil vary significantly with geographical locations (Paz-Ferreiro et al., 2010), as well as soil depth (Wittmann et al., 2004, Alarcón-Gutiérrez et al., 2009). Together these findings indicate that soil enzyme activities have broad-scale spatial variability depending on the environmental conditions of soil. The bacterial extracellular enzymes diversity in the soil may have a pivotal role in nutrient cycling and he decomposition of organic matter (Zimmerman et al., 2013). Cellulase, amylase, protease and pectinase are considered as industrially significant enzymes using at paper and pulp bleaching, food processing unit, textile like industries. Different bacterial enzymatic studies have been carried out in West Bengal, India collecting soil sample from East Calcutta wetlands, Sundarban Biosphere Reserve and Western dry soil (Ghosh et al., 2007; Biswas and Paul, 2013; Thatoi et al., 2013; Mandal, 2015; Basak et al., 2015). However, no correlative study between enzyme activity and soil Physicochemical parameters were undertaken.

Bioprospecting is defined as the exploration of biodiversity for commercially valuable biochemical and genetic resources for achieving economic and conservation goals (Firn, 2003). This holds substantial promise for the development of novel compounds for food production and processes, consumer goods, public health, and environmental and energy uses. To serve these purposes, existing diversity of microorganisms can act as a resource reservoir from which individual species with special traits can be exploited (Bull et al., 2000; Egorova and Antranikian, 2005). Due to the environmental related issues and advances in biotechnology, many chemical processes in different industries such as textile, leather, pulp and paper, fruits and vegetables processing and animal feed are being replaced with biocatalysts i.e., enzymes. Microbial derived enzymes are highly valued in many industrial applications. Enzymes are usually required in small amounts and can be easily obtained by manipulation of the microbes. Enzymes are often used in the processing of food and beverage, paper and pulp, textile, animal feed, detergent, cosmetic and chemical synthesis processes (Headon and Walsh, 1994).

We hypothesized that the soil of different agro-climatic part of West Bengal might be a good source of potential bacteria producing industrially significant enzyme for future application. Therefore, this study was designed to characterize the soil nutrients and quantification of bacterial enzyme isolated from soil. Here we aimed to develop relationship among enzymes with bacterial load and major soil nutrient and also to develop a spatial enzyme map of West Bengal for future use.

**Materials and Methods**

The State of West Bengal is situated in the eastern part of India between $21^{o}20'$ and $27^{o}32'$ latitude and $85^{o}50'$ and $89^{o}52'$ E longitude. The total area of the state is 88,752 sq km which is 2.7% of the total area in the country. The state has two distinct natural divisions the Northern Himalayan region and the Southern Alluvial plains. Three main rivers, namely, Teesta, Torsa and Jaldhaka and other two important rivers Ganga and Hooghly are passing through the state. The state has many shallow marshy depressions

which are the relatively unfilled parts of ancient topographic formations. These are subjected to annual inundation during the monsoon months, having in many instances permanent wetlands in their shallowest parts. In addition to all these, there are many types of landforms flanking the northern Himalayan Mountain as also the western Deccan plateau within West Bengal which had developed originally by the sediments brought by rivers in the ancient geological periods. On a physiographic basis the state can be divided into four physiographic divisions, namely, the Himalayan Region, Eastern fringe of Chotanagpur Plateau, the Deltaic Zone and the Alluvial Plains Remaining areas of the State. The state has international borders with Bangladesh, Nepal and Bhutan while it shares national state boundaries with Sikkim, Assam, Bihar, Jharkhand and Orissa. In its south lies the Bay of Bengal (Bhattacharya and Banerjee, 1979).

West Bengal has 23 districts. On the basis of distribution of climate and soil, the agricultural feasibility of the state is harnessed from 5 distinct agro-climatic zones, and they are the (a) Hill region: covering the districts of Darjeeling district , Coochbehar and Jalpaiguri (b) Old Alluvial Zone : comprising of North Dinajpore, South Dinajpore and Malda (c) Alluvial Zone : covering Murshidabad, Nadia, 24 North Parganas, Hooghly and Burdawan (d) Red and Laterite zone: covering the districts of Birbhum, Bankura, Purulia, West Medinipur (e) Saline Coastal region: covering East Mediinipur, Hooghly, 24 South Parganas, Kolkata (West Bengal Action Plan on Climate Change, 2010).

**Collection of soil sample and isolation of bacteria**

Representative soil sample has been collected from five different agro climatic zones of West Bengal such as, Hill region of Darjeeling, Dooars region/Himalayan foot hills, Arid region, Gangetic plain and Coastal region West Bengal, India.

All the soil samples were collected aseptically using sterile polythene bags and transported to the laboratory. About one gram of soil sample was diluted in 100 ml of sterile distilled water blank and further serially diluted $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$ dilution using 9 ml sterile distilled water. Nutrient agar was used for isolation of bacteria. After incubation, morphologically different colonies were selected for further studies.

**Analysis of physical and chemical parameters of collected soil**

pH, Moisture content and electrical conductivity were measured following standard procedures. Organic carbon content (Walkely and Black, 1934), Available Nitrogen (Subbiah and Asija 1956), and Total Phosphorus (Bray and Kurtz, 1945) were measured in the laboratory after drying the sample. All experiments were performed thrice in duplicate sets.

**Screening of microorganisms for enzyme activity and enzyme assay**

Isolated colonies from five areas were again tested for enzymatic study. The cellulase, amylase, protease and pectinase producing bacterial strain were isolated using carboxy methyl cellulose agar, starch agar, Gelatin-Agar and pectin agar medium respectively (Mondol et al., 2010). Observation was taken after 48 hours incubation and colonies were distinguished and selected based on their morphology. 3,5-dinitrosalicylic acid (DNS) was used to measure the cellulase and amylase enzyme activity of the all isolated

strains (Bailey, 1988). Caesin was used as the substrate for the protease activity according to Rajamani and Hilda (1986) and Pectinase was determined by modified Sigma-Aldrich standard protocol (Khairnar et al., 2009).The activity was calculated in IU/ml.

**Statistical analysis and application of GIS4**

Soil quality data, density of soil bacteria and enzyme activity of bacteria were statistically analyzed using SPSS software (version 16.0). Spearman's Correlations test was performed for correlation studies. "ArcGIS (version 10, ESRI) was applied for determine the spatial distribution of the bacterial enzymes among the agro-ecological zone of the study area (Hossain and Bhuiyan, 2016).

**Results**

**Physical and chemical parameters of collected soil**

Soil moisture content of the Sukna was highest followed by soil of Panchla (Howrah), Darjeeling Sonada, Darjeeling Mahakal hill, Sonamukhi (Bankura), Digha (Medinipur East) and Gosaba (24 Parganas South) whereas the electrical conductivity data shows highest values at Gosaba (24 Parganas South) and lowest at Mahakal Hill (Derjeeling District). Regarding the organic carbon content, Sukna forest shows 7.13% followed by Sonada forest (Darjeeling District), Mahakal Hill (Derjeeling District), Panchla (Howrah), Gosaba (24 Parganas South), Digha (Medinipur East) and Sonamukhi (Bankura).Available Nitrogen content of the soil collected from Panchla (Howrah) and Digha (Medinipur East) are above 100 mg/kg whereas available phosphorus 19 content of all soil varies from 27.35 to 53.09 mg/kg (Table1).

**Bacterial density**

The highest number of cellulase producing bacteria was found at Digha followed by Darjeeling (Sonada), Darjeeling (Mahakal), Sukna (Dooar), Sonamukhi (Bankura) and Panchla (Howrah) of the West Bengal. Load of amylase producing bacteria were highest at Darjeeling (Mahakal) and lowest were Sukna (Dooar) areas. In Sukna (Dooar) the protease producing bacterial load was least but the saline zone showed highest concentration of protease followed by Panchla(Howrah), Sonamukhi (Bankura) and Digha (Medinipur East) whereas pectinase producing bacterial load was highest at Sukna forest area of West Bengal (Table 2).

**Bacterial enzyme activity**

The cellulase levels were highest at Digha followed by Darjeeling Sonada Forest, Gosaba, Darjeeling Mahakal, Sonamukhi, Panchla and Sukna, whereas pectinase level were highest at Sukna and lowest were found at soil of Digha. Amylase level was almost same at Panchla and Darjeeling Mahakal hill. It ranged from 1.413 ± 0.5 to 14.76 ± 3.1 IU/ml. The protease level was varied from 24.85 ± 5.2 to 31.75 ± 7.5 IU/ml (Table 2).

**Development of enzyme map of study area**

The mean concentration value of the bacterial enzyme was interpolated using GIS and visualized on the map of the West Bengal, India (Fig. 1A-D). Fig 1A and 1B showed the reverse spatial distribution of amylase and cellulase of the study area whereas Fig. 1C and D showed almost similar spatial distribution of pectinase and protease production. The northern part of the study area showed higher organic carbon content of the soil (ranging from 2.65 to 7.13 %) than the southern part (ranging from 0.8 to 2.23%) and middle part (ranging from 0.54 to 2.08 %) of the West Bengal (Table 1).

**Table 1. Characterization of Soil chemical parameters.**

| Soil parameter | Moisture content (%) | Electrical conductivity (µS/cm) | Organic carbon content (%) | Available Nitrogen content (mg/kg) | Available Phosphorus content (mg/kg) |
|---|---|---|---|---|---|
| Derjeeling Mahakal | 25±2.5 | 0.13±0.2 | 2.65±0.36 | 49.1±4.6 | 47.35±23.23 |
| Derjeeling Sonada | 28±3.5 | 0.15±0.2 | 3.05±0.47 | 47.1±8 | 47.35±22 |
| Sukna (Dooar) | 253.9±22.5 | 3.539±1.8 | 7.137±2.15 | 81.2±10.45 | 37.35±18.52 |
| Panchla (Howrah) | 67.65±12.5 | 0.26±0.05 | 2.08±0.25 | 117±11.3 | 44.2±16.9 |
| Sonamukhi (Bankura) | 8.43±1.5 | 98.5±5.8 | 0.54±0.12 | 84±12.64 | 53.09±18.25 |
| Digha (Medinipur East) | 18.32±3.5 | 240±24.5 | 0.8±0.14 | 112±12.25 | 56.32±10.25 |
| Gosaba (24 Parganas South) | 4.69±1.2 | 600±30.5 | 2.2375±1 | 75±10.25 | 27.35±8.48 |

**Table 2. Enzyme activity (IU/ml) and bacterial density (CFU/gm soil) of collected soil.**

| Sampling site | Cellulase | Density of Cellulase producing bacteria | Amylase | Density of Amylase producing bacteria | Protease | Density of Protease producing bacteria | Pectinase | Density of Pectinase producing bacteria |
|---|---|---|---|---|---|---|---|---|
| Derjeeling (Mahakal) | 1.09±0.36 | 66 | 14.27±4.14 | 54 | nd | nd | 8.91±2.4 | 10 |
| Derjeeling (Sonada) | 1.86±0.29 | 96 | 3.47±2.4 | 26 | nd | nd | 9.8±5.4 | 25 |
| Sukna (Dooar) | 0.24±0.08 | 39 | 1.413±0.5 | 23 | 28.54±3.1 | 4 | 200±19.5 | 142 |
| Panchla (Howrah) | 0.402±0.2 | 34 | 14.76±3.1 | 35 | 31.75±7.5 | 24 | 155±33.5 | 87 |
| Sonamukhi (Bankura) | 0.404±0.27 | 36 | 6.36±1.9 | 24 | 28.54±7.9 | 56 | 130±31.84 | 29 |
| Digha (Medinipur East) | 3.43±1.1 | 98 | 9.78±3.0 | 26 | 31.54±9.1 | 58 | 1.462±0.5 | 16 |
| Gosaba (24 Parganas South) | 1.22±0.8 | 75 | 6.32±2.8 | 25 | 24.85±5.2 | 42 | 62.89±20.5 | 71 |

Nd: not detected, DCB: Density of Cellulase producing bacteria, DAB: Density of Amylase producing bacteria, DPB: Density of Protease producing bacteria and DPcB: Density of Pectinase producing bacteria

**Table 3. Spearman's rank correlation matrix**

| | Amylase | Cellulase | Protease | Pectinase | Moisture | Organic Carbon | Available Nitrogen | Available Phosphorus | Density of bacteria |
|---|---|---|---|---|---|---|---|---|---|
| Amylase | | | | | | | | | |
| Cellulase | 0.019 | | | | | | | | |
| Protease | 0.090 | 0.045 | | | | | | | |
| Pectinase | -0.290** | -0.134 | 0.241* | | | | | | |
| Moisture | 0.115 | 0.073 | -0.402 | 0.093 | | | | | |
| Organic Carbon | -0.048 | -0.016 | -0.013 | 0.087 | 0.521 | | | | |
| Available Nitrogen | -0.710** | -0.642** | -0.025 | 0.426** | 0.270 | 0.115 | | | |
| Available Phosphorus | -0.212* | 0.102 | -0.216 | 0.132 | 0.213 | -0.041 | 0.033 | | |
| Density of bacteria | 0.893* | 0.758* | 0.012 | 0.841* | 0.484* | 0.451* | 0.583* | 0.653* | |

**Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).

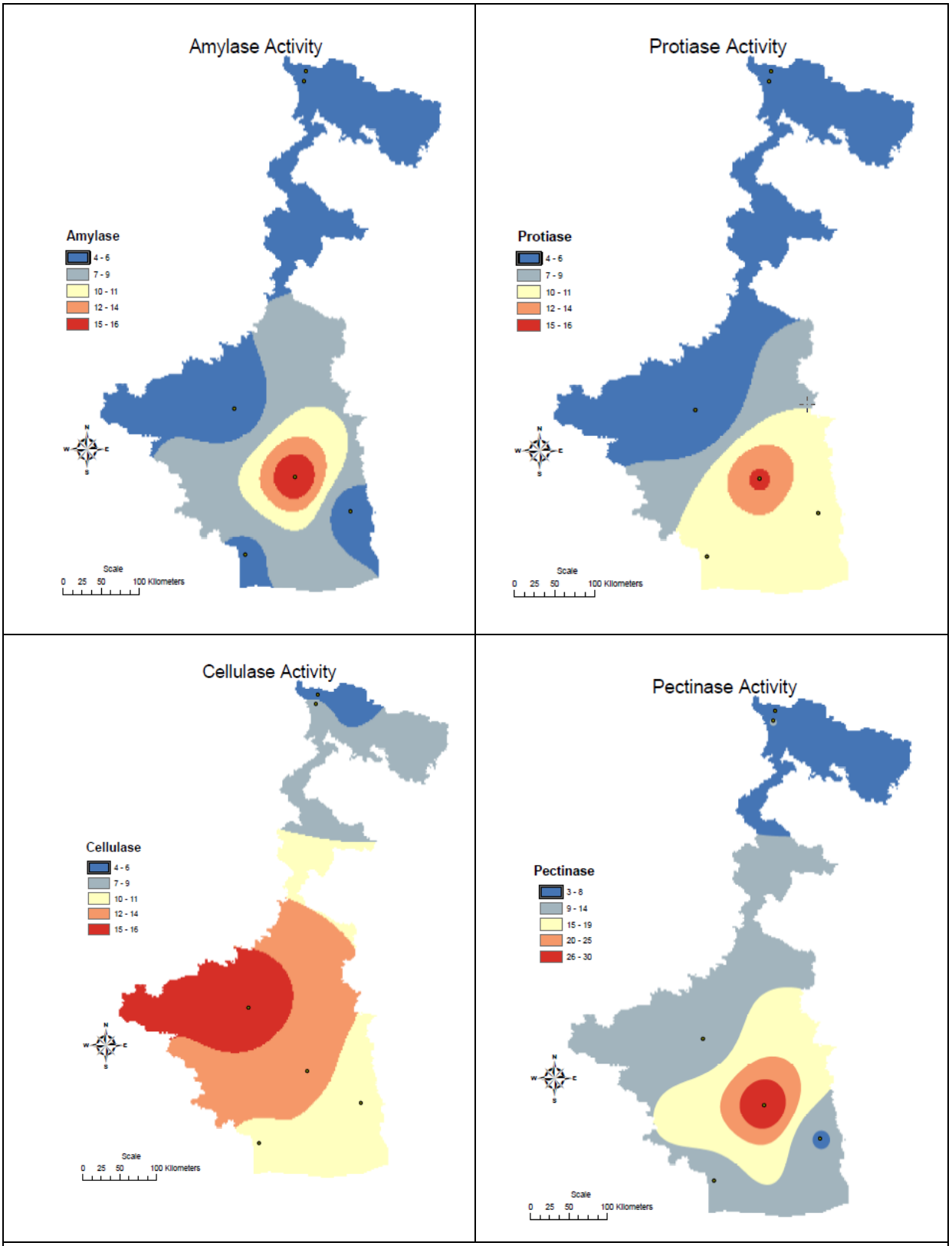


**Fig. 1 (A-D). Spatial Distribution of Mean bacterial enzyme production of soil of West Bengal, India. (A) Amylase Activity (B) protease Activity (C) Cellulase Activity (D) Pectinase Activity.**

The organic carbon did not show significant correlation with the four enzymes (Table 2) whereas Spearman's rank correlation matrix showed significant correlations ($P< 0.01$) between the available nitrogen with amylase ($R^2$=-0.310) and cellulase (-0.384), where increasing available nitrogen load of the soil decreased the concentration of both two bacterial enzyme production.

On the contrary the other bacterial enzyme pectinase ($R^2$ = 0.426) was positively correlated with the available nitrogen of the soil. Amylase production of bacteria also showed negative correlation ($R^2$ = -0.212 at $P<0.05$) with the soil available phosphorus (Table 3). The density of isolated bacteria showed positive correlation with all enzyme production and other abiotic factors (Table 3).

**Discussion**

In this paper the study area was classified into four agro-climatic zones and our aim was to quantify four industrially significant bacterial enzymes isolated from the soil. Usually West Bengal has been classified into five distinct agro ecological zones (West Bengal Action Plan on Climate Change, 2010.). Here we took soil samples from the four zones and overlapped old alluvial zone i.e., Dinajpur and Coochbehar with the Terai zone of Northern part of West Bengal. The distribution of soil bacterial enzymes (Fig. 1A-D) was correlated with some soil available nutrient (Table 3). It has been established that soil nutrient level is depend on the parent rock and drifted materials from where soil has been formed. In the current study, as we collected soil at the depth of 0 to 20 cm, the physicochemical characteristics of that soil mostly controlled by the local factors like decomposition of plants biomass, dead bodies and excreta of animals etc (Berg, 2000). In this study we had determined the four enzymes namely cellulase, amylase, protease and pectinase of the soil. We observed highest cellulase level at the coastal area (Fig 1A) Digha and lowest at Sukhna (Fig. 1A). The correlation data (Table 3) revealed that cellulase activity was inversely varied with the soil nitrogen. Some previous study reported that availability of the nitrogen in the soil affected on the carbon mineralization and enzyme activities during decomposition of the substrate like wheat straw and dry leaves (Dick, 1992; Henriksen and Breland, 1999). Moreover soil organic carbon may considerably influence the activities of soil enzymes (Gianfreda and Bollag, 1996). Iyyemperumal and Shi (2008) reported that the activities of soil enzymes involved in the cycling of a given nutrient are often negatively associated with the availability of that nutrient in the soil. Some another hypothesis suggested that the cellulase enzyme production is depend on the soil temperature and pH; as the temperature increases the activity would also enhance (Nicolardot et al., 1994; Kautz et al., 2004). The average temperature of south Bengal is more than the northern part of the West Bengal (West Bengal Action Plan on Climate Change, 2010) and previous works suggested that the maximum cellulase production was observed at 50$^{o}$C or above in laboratory condition (Gomes et al., 1993; 18 Deswal et al., 2011; Budihal et al., 2016). Some more previous study also reported that cellulase activity of *Clostridium thermocellum* was displayed at 70°C (Johnson et al., 1982). The other factors like organic carbon were least at Digha and highest was Sukna area of northern part of study site (Fig 1). Here we could not find any relationship among organic carbon with cellulase and amylase (Table 3). Both these enzymes produced glucose from cellulose and carbohydrate compound respectively. It has

been reported that polysaccharide biodegradation is not only depend on the cellulase or amylase, lots of other enzymes and structural limitations of substrate influence the complete hydrolysis of lignocellulosic polysaccharides (Mansfield et al., 1999).

The available nitrogen of the soil was higher in both alluvial plain and coastal area of the West Bengal. This data could have been correlated with the both cellulase ($R^2$= -0.64, P<0.01) and amylase ($R^2$= -0.71, P<0.01) production. Similarly the available phosphorus also plays a significant role for both cellulase ($R^2$= 0.41, P<0.05) and amylase ($R^2$= -0.31, P<0.05). Literature reported that soil cellulase activities in deciduous forest depend on the nitrogen available in that ecosystem (Carreiro et al., 2000). Elevated nitrogen level resulted in changes in soil carbon and resulted from the divergent regulatory control of microbial extracellular enzymes by soil nitrogen availability (Waldrop et al., 2004).The phosphorus availability of the soil also depends on the enzyme activities and decomposition rates of organic biomass (Sinsabaugh and Moorhead, 1994), here cellulase activity increased with the presence of available phosphorus, whereas amylase activity decreased with available phosphorus. Weintraub et al. (2013) evaluated six hydrolytic soil enzymes responsible for liberating carbon, nitrogen and phosphorus. Here also phosphorus was negatively correlated with the organic carbon utilization. The protease level was varied from 24.85 to 31.75 I.U. Here we also found higher protease level at central and Southern coastal West Bengal (Fig 1D). The protease level were directly correlated with available nitrogen (R2= -0.134, P<0.01) and phosphorus level ($R^2$= -0.216, P<0.01). The organic carbon was inversely correlated with protease production of the soil. A previous study revealed that out of twenty four soil samples of superficial layer, proteolytic enzyme varied very less with the soil organic carbon (von Steiger et al 1996). The other study also suggested that organic matter degrading oxidative enzymes increase mobilization of soil available nitrogen and dynamics of rhizosphere (Kieloaho et al., 2016).

The pectinase activity level varied widely within the study area. The pectinase level was higher at Sukna forest and lowest at Digha coastal area (Table 3) of West Bengal. Pectinase is 18 only directly correlated with organic carbon ($R^2$= - 0.187, P<0.01) and available nitrogen ($R^2$=0.426 P<0.01) of the soil. The phosphorus level had very small influence on pectinase production. Pectinase is the heterogeneous enzyme that acts on pectin substances. It is present in higher plants & microorganisms. The pectinolytic enzymes have great industrial importance and are required for food processing industries, especially for extraction and clarification of fruit juices, extraction of oils etc. Soil pectinase had role in leaf litter degradation and production of this extracellular enzyme is influenced by various factors including nitrogen limitation, elevated temperature and microbial interactions (Schneider et al., 2010).

The density of cellulase producing bacteria were highest at Dighaand we also found highest cellulase activity at the same place ($R^2$= 0.758, P<0.05). Snajdr et al., (2008) reported that extracellular enzymes of *Quercuspetraea* forest soil and microbial biomass content were correlated. Our work is consistent with the report indicating that other enzymes with the 30 exception of protease, amylase and pectinase was positively correlated with the bacterial density of soil (Table 3). It is likely that enzyme activities fluctuated along with the

microorganism population in the rhizospheric soil of maize plants at different plant growth stages (Li et al., 2002; Pathan et al., 2015). Microbial loads and soil enzymes profiles were also significant reduction in protease activity also altered with the proteolytic bacterial load of their study area. The present study also showed significant positive correlation between bacterial population load and amylase, cellulase and pectinase activity of the soil (Table 3).

**Conclusion**

From the current research study a relationship between four targeted enzymes and (cellulase, amylase, protease and pectinase) and soil nutrients (carbon, nitrogen and phosphorus) and bacterial density could be established. The results of the present research provide important data on the spatial distribution of bacterial enzyme and it is expected to offer expanded capabilities for basic and applied scientific research. It is also indicative of the potential for further bioprospecting. The bacterial enzymes maps can be of great advantage for more extensive exploration in the soil of different agro-climatic landscape in the State of West Bengal, India.

**References**

Alarcón-Gutiérrez, E., Floch, C., Augur, C., Le Petit, J., Ziarelli, F. and Criquet, S. (2009). Spatial variations of chemical composition, microbial functional diversity, and enzyme activities in a Mediterranean litter (Quercus ilex L.) profile. *Pedobiologia*. 52(6): 387-399.

Bailey, M. J. (1988). A note on the use of dinitrosalicylic acid for determining the products of enzymatic reactions. *Applied Microbiology and Biotechnology.* 29(5): 494-496.

Basak, P., Pramanik, A., Roy, R., Chattopadhyay, D. and Bhattacharyya, M. (2015). Cataloguing the bacterial diversity of the Sundarbans mangrove, India in the light of metagenomics. *Genomics Data*. 4: 90-92.

Berg, B. (2000). Litter decomposition and organic matter turnover in northern forest soils. *Forest ecology and Management.* 133(1): 13-22.

Bhattacharya, A. and Banerjee, S. N. (1979). Quaternary geology and geomorphology of the Ajay–Bhagirathi valley, Birbhum and Murshidabad districts, West Bengal. *Indian Journal of Earth Sciences*. 6(1): 91-102.

Biswas, J. and Paul, A. K. (2013). Production of extracellular enzymes by halophilic bacteria isolated from solar salterns. *Pharmaceutical Technology* 4(4):30-36

Budihal, S. R., Agsar, D., & Patil, S. R. (2016). Enhanced production and application of acidothermophilic Streptomyces cellulase. *Bioresource Technology.* 200: 706-712.

Bull, A.T., Ward, A.C. and Goodfellow, M. (2000). Search and Discovery Strategies for Biotechnology: the Paradigm Shift. Microbiol. *Mol. Biol. Rev*. 46(3): 573-606.

Carreiro, M. M., Sinsabaugh, R. L., Repert, D. A. and Parkhurst, D. F. (2000). Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. *Ecology*. 81(9): 2359-2365.

Crecchio, C., Curci, M., Pizzigallo, M. D., Ricciuti, P. and Ruggiero, P. (2004). Effects of municipal solid waste compost amendments on soil enzyme

activities and bacterial genetic diversity. *Soil Biology and Biochemistry*. *36*(10): 1595-1605.

Deswal, D., Khasa, Y. P. and Kuhad, R. C. (2011). Optimization of cellulase production by a brown rot fungus Fomitopsis sp. RCK2010 under solid state fermentation. *Bioresource Technology.* 102(10): 6065-6072.

Dick, R. P. (1992). A review: long-term effects of agricultural systems on soil biochemical and microbial parameters. *Agro Ecosystem. Environ*. 40: 25–36.

Egorova, K. and Antranikian, G. (2005). Industrial relevance of Thermophilic Archaea. *Curr. Opin. Microbiol*. 8: 649-655.

Firn, R. D. (2003). Bioprospecting-Why is it so unrewarding? *Biodiversity and Conservation*. 12: 207-216.

Ghosh, A., Maity, B., Chakrabarti, K. and Chattopadhyay, D. (2007). Bacterial diversity of east Calcutta wet land area: possible identification of potential bacterial population for different biotechnological uses. *Microbial ecology.* 54(3): 452-459.

Gomes, J., Purkarthofer, H., Hayn, M., Kapplmüller, J., Sinner, M. and Steiner, W. (1993). Production of a high level of cellulase-free xylanase by the thermophilic fungus *Thermomyces lanuginosus* in laboratory and pilot scales using lignocellulosic materials. *Applied Microbiology and Biotechnology*. 39(6): 700-707.

Headon, D. R. and Walsh, G. (1994). The industrial production of enzymes. *Biotechnol. Adv*. 12(4): 635-646.

Henriksen, T. M. and Breland, T. A. (1999). Nitrogen availability effects on carbon mineralization, fungal and bacterial growth, and enzyme activities during decomposition of wheat straw in soil. *Soil Biology and Biochemistry*. (8): 1121-1134.

Hossain, G. M. and Bhuiyan, M. A. H. (2016). Spatial and temporal variations of organic matter contents and potential sediment nutrient index in the Sundarbans mangrove forest, Bangladesh. *KSCE Journal of Civil Engineering* 20(1): 163-174.

Iyyemperumal, K. and Shi, W. (2008). Soil enzyme activities in two forage systems following application of different rates of swine lagoon effluent or ammonium nitrate. *Applied Soil Ecology*. 38(2): 128-136.

Johnson, E. A., Sakajoh, M., Halliwell, G., Madia, A. and Demain, A. L. (1982). Saccharification of complex cellulosic substrates by the cellulase system from Clostridium thermocellum. *Applied and Environmental Microbiology*. *43*(5): 1125-1132.

Kautz, T., Wirth, S. and Ellmer, F. (2004). Microbial activity in a sandy arable soil is governed by the fertilization regime. *European Journal of Soil Biology*. 40(2): 87-94.

Kieloaho, A. J., Pihlatie, M., Carrasco, M. D., Kanerva, S., Parshintsev, J., Riekkola, M. L. and Heinonsalo, J. (2016). Stimulation of soil organic nitrogen pool: The effect of plant and soil organic matter degrading enzymes. *Soil Biology and Biochemistry.* 96: 97-106.

Li, C. H., Ma, B. L. and Zhang, T. Q. (2002). Soil bulk density effects on soil microbial populations and enzyme activities during the growth of maize (*Zea mays* L.) planted in large pots under field

exposure. *Canadian Journal of Soil Science*. *82*(2): 147-154.

Mandal, A. (2015). Study of distribution of xylanase producing microbes in soil: a case study in Midnapore town, Paschim Medinipur, West Bengal, India. *Asian Journal of Science and Technology*. *6*(08): 1712-1718.

Mansfield, S. D., Mooney, C. and Saddler, J. N. (1999). Substrate and enzyme characteristics that limit cellulose hydrolysis. *Biotechnology progress*. 15(5): 804-816.

Marinari, S. and Antisari, L. V. (2010). Effect of lithological substrate on microbial biomass and enzyme activity in brown soil profiles in the northern Apennines (Italy). *Pedobiologia*. 53(5): 313-320.

Nicolardot, B., Fauvet, G. and Cheneby, D. (1994). Carbon and nitrogen cycling through soil microbial biomass at various temperatures. *Soil Biology and Biochemistry*. 26(2): 253-261.

Pathan, S. I., Ceccherini, M. T., Pietramellara, G., Puschenreiter, M., Giagnoni, L., Arenella, M. and Renella, G. (2015). Enzyme activity and microbial community structure in the rhizosphere of two maize lines differing in N use efficiency. *Plant and Soil*. 387(1-2): 413-424.

Paz-Ferreiro, J., Trasar-Cepeda, C., Leirós, M. C., Seoane, S. and Gil-Sotres, F. (2010). Effect of management and climate on biochemical properties of grassland soils from Galicia (NW Spain). *European Journal of Soil Biology*. 46(2): 136-143.

Sarkar, S. (2014). Bioprospecting of Bacterial Enzymes Derived from the Soil of the Coastal Region of West Bengal (Doctoral dissertation, Jadavpur University). Pp. 45 - 49.

Schneider, T., Gerrits, B., Gassmann, R., Schmid, E., Gessner, M. O., Richter, A., Eberl, L. and Riedel, K. (2010). Proteome analysis of fungal and bacterial involvement in leaf litter decomposition. *Proteomics*. 10(9): 1819-1830.

Sinsabaugh, R. L. and Moorhead, D. L. (1994). Resource allocation to extracellular enzyme production: a model for nitrogen and phosphorus control of litter decomposition. *Soil Biology and Biochemistry*. *26*(10): 1305-1311.

Šnajdr, J., Valášková, V., Merhautová, V., Herinková, J., Cajthaml, T. and Baldrian, P. (2008). Spatial variability of enzyme activities and microbial biomass in the upper layers of *Quercus petraea* forest soil. *Soil Biology and Biochemistry*. *40*(9): 2068-2075.

Subbiah, B.V. and Asija, C. L. (1956). A rapid method for the estimation of available nitrogen in soil. *Current Science.* 25: 259-260.

Tan, X., Xie, B., Wang, J., He, W., Wang, X. and Wei, G. (2014). County-scale spatial distribution of soil enzyme activities and enzyme activity indices in agricultural land: Implications for soil quality assessment. *The Scientific World Journal.* Volume 2014, Article ID 535768, 11 pages.

Thatoi, H., Behera, B. C., Mishra, R. R. and Dutta, S. K. (2013). Biodiversity and biotechnological potential of microorganisms from mangrove ecosystems: a review. *Annals of Microbiology*. 63(1): 1-19.

von Steiger, B., Nowack, K. and Schulin, R. (1996). Spatial variation of urease activity measured in soil monitoring. *Journal of environmental quality.*

25(6): 1285-1290.

Waldrop, M. P., Zak, D. R., Sinsabaugh, R. L., Gallo, M. and Lauber, C. (2004). Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. *Ecological Applications*. 14(4): 1172-1177.

Weintraub, S. R., Wieder, W. R., Cleveland, C. C. and Townsend, A. R. (2013). Organic matter inputs shift soil enzyme activity and allocation patterns in a wet tropical forest. *Biogeochemistry*, 114(1-3): 313-326.

Wittmann, C., Kähkönen, M. A., Ilvesniemi, H., Kurola, J. and Salkinoja-Salonen, M. S. (2004). Areal activities and stratification of hydrolytic enzymes involved in the biochemical cycles of carbon, nitrogen, sulphur and phosphorus in podsolized boreal forest soils. *Soil Biology and Biochemistry*. 36(3): 425-433.

Zimmerman, A. E., Martiny, A. C. and Allison, S. D. (2013). Microdiversity of extracellular enzyme genes among sequenced prokaryotic genomes. *The ISME journal. 7*(6): 1187-1199.