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Monitoring lipase and protease producing microbes along the sugarcane growing alluvial soil tracts of West Bengal, India : an ecological investigation

SUNANDA CHANDA, KAKALI GHOSH, SUTRIPTA SARKAR, PRIYANKA MAJUMDAR AND SAMARENDRA BARIK

Agricultural and Ecological Research Unit, Indian Statistical Institute, 203 B. T. Road, Kolkata 700108

In view of the significant contribution of the lipolytic and proteolytic microbes in the areas of soil ecology and industrial microbiology, the present investigation on the occurrence of the two groups of microflora along the sugercane growing areas of alluvial soil tracts of West Bengal in relation to the physico-chemical status of the soil was investigated as soil microflora have been known to be greatly influenced by soil type and is physico-chemical properties. Potential lipase producing microorganisms were trapped in specialized media having lipid sources such as Tween 80, Tributyrin and various oils whereas protease producing microbes were screened on the basis of casein digestion and gelatin liquefaction. For identification of the microbes morphological, physiological, biochemical characteristics were taken into consideration. Prominant lipid degrading microbes were identified as *Bacillus licheniformis, B. polymyxa, B. coagulans, Pseudomonas fluorescens, P. fragi, Micrococcus luteus, Staphylococcus aureus, Actinomyces* spp., *Streptomyces* spp., Mucor *hiemalis, Mucor mucedo, Rhizopus arrhizus, Penicillium purpurogenum, Cladosporium* and *Trichosporon* sp. Dominant protein hydrolyzing organisms were identified as *Bacillus licheniformis, B. coagulans, B. megaterium, C. herbarum, Alternaria alternata, Fusarium pallidoroseum, Aureobasidium pullulans Pseudomonas fluorescens, P. aureofaciens, Serratia indica, Streptomyces* spp., *Aspergillus niger, A. nidulans, A. aculeatus, Penicillium frequentans, P. chrysogenum, P. citrinum, Myrothecium rorium, Paecilomyces* sp. and *Fusarium pallidoroseum.*

Frequency (%) and Relative abundance (%) on the occurrence of each microbe were calculated to establish their relative prominence.

Fermentation trials were rendered varying nutritional sources to asses biosynthesis of extra-cellular production of both the enzymes. A good number of microbes produced appreciable amount of lipase (12-25 units/ml) and protease (5-8 units/ml) in culture broth indicating their feasibility in commercial exploitation. Both lipases and proteases of microbial origin are extensively used in medicines as digestive aid, laundry detergents, tannery and dairy industries and too in flavour promotions, waste treatments etc.

Key words : Sugarcane fields, microbes, protease, lipase, ecology, industry

INTRODUCTION

The soil environment contains a vast population of bacteria, actinomycetes, fungi, algae and protozoa. It is the most dynamic site of microbial interaction in nature and it is the region in which occur many of the biochemical reactions concerned in transfer of organic matter into easily available nutrients for the plant life. The soil is composed of five major components—air, water, organic matter, minerals and a living population, the quantity of these constituents is not the same in all soils but varies with the locality. The physical and chemical composition of the soil as well as the environmental condition determine the relative abundance of the microbes at a given time in the soil. In the soil environment biochemical transformations are being carried out through hydrolysis, oxidation, reduction

etc. The reactions are catalysed by a number of enzymes identified as urease, phosphatase, cellulase, amylase, protease, lipase. The nature of enzyme is quite variable depending upon the type of organism and growth medium. The enzymes are either extracellular which are released by the organisms outside the cells or intracellular.

Because of their activities in both aqueous and non-aqueous solvent system (Zaks and Klibanov,1985), it has become evident that lipase have considerable applications in industry and medicine (Macrae and Hammond, 1985; Bjokling, 1991). Recently, attention has been focused on the application of alkaline lipase in household detergents components (Andree *et al.*, 1980; Jaeger *et al.*,1994). Now-a-days proteolytic enzymes have found their applications in food technology, leather technology and medicine (Aunstrup 1980; Sidhu *et al.*, 1998). Proteases specially alkaline proteases from microorganism have a range of biotechnological applications in detergents, leather tanning and food industries (Sizer, 1976; Kelly and Fogarty, 1976; Cowen, 1985; Juhasz and Karka, 1990). In view of the significant contribution of proteolytic and lipolytic microorganisms in the areas of soil ecology and industrial fermentation the present investigation has been undertaken.

MATERIALS AND METHODS

To study the physico-chemical characteristics and microbiological profile of sugarcane fields, soil samples were collected randomly from different locations along various sugarcane growing fields of Malda and Murshidabad district of West Bengal. Soil samples were dried in air and analysed for their physico-chemical properties like organic carbon, nitrogen, pH, CEC and minerals (Black, 1965). For microbiological studies soil suspensions were diluted serially and incorporated in various media having casein and gelatin (for proteolytic organisms) and Tween 80 and Tributyrin (for lipolytic organism). After desired incubation organisms were picked up and subcultured for identification. Identification of fungal isolates was based on microscopical studies following Gilman (1975). Identification of bacteria and actinomycetes was done by (i) micromorphology (ii) cultural, biochemical and physiological characters following Bergey's Manual of Determinative Bacteriology (Hensyl, 1994) and transmission electron microscopical studies. Statistical analysis was done following the methods of Snedecor and Cochran (1967).

On the basis of appearance of individual colony, total colonies, total sites, samples, frequency (%) and relative density were calculated following the method of Clark and Christensen (1981). Microbiological profile studies were conducted counting the total number of bacterial, actinomycetal and fungal colonies per gram of soil by dilution plate method (Warcup 1950). To screen proteolytic microbes, isolates were inoculated to Gelatin Agar medium in Petri dishes. After incubation and development of colonies the plates were flooded with 20% solution of sulphosalicylic acid. A clear transparent zone was found surrounding the colonies with proteolytic activities (Chanda *et al.*, 2003). To detect lipolytic colonies the isolates were inoculated to agar medium containing 4.7 ml Tween 80/L with usual nutrients. The appearance of cloudy zones around the colonies in plates indicated precipitation of Ca-salts of free fatty acids (Chakrabarti *et al.*, 1979).

Soil isolates with promising proteolytic and lipolytic activities were grown in liquid culture media. Fermentation for fungi and actinomycetes were carried on at 30°C for 5-8 days while bacteria were incubated at 37°C for 24 to 72 h. Bacterial cells were removed by centrifugation (12,000 rpm for 20 min at 4°C). The fungal and actinomycetous mycelial mat was removed by filtration. The cell free supernatant was tested for proteolytic and lipolytic activities.

Population dynamics of both proteolytic and lipolytic microflora was recorded by determining the per cent proteolytic and lipolytic bacteria with respect to the total microbes observed. Distribution of total microbe was calculated by frequency (%) and relative abundance (%) of respective microbes in different fields. On the basis of appearance of individual colony with respect to the total colonies and total site samples, frequency (%) and relative abundance (%) were calculated using the formula (Clarke and Christensen, 1981);

$$\text{Frequency (\%)} = \frac{\text{Sites of occurrence of bacteria / fungi / actinomycetes}}{\text{Total site samples}} \times 100$$

$$\text{Relative abundance (\%)} = \frac{\text{Isolates of the given bacteria / fungi / actinomycetes}}{\text{Total isolates (bacteria / fungi / actinomycetes)}} \times 100$$

RESULTS AND DISCUSSION

Soil harbours a dynamic population of microorganisms. This abundance in nature gives an indication of possible rate in decomposition of organic matter, phosphate solubilization, transformation of nitrogen in nature, humification of organic residue etc. Soil microflora have been known to be greatly influenced by soil type and physico-chemical properties of soil (Walksmann, 1952). The data in Tables 1 and 2 showed the physico-chemical properties of soil of sugarcane field, while the data in Tables 3 and 4 exhibited the microbiological profiles of respective fields.

Table 1 : Physico-chemical properties of Alluvial Soil of West Bengal

Sugarcane fields

Location	pH	Electrical Conductivity (dsm^{-1})	Total Organic Carbon (%)	Total Nitrogen (%)	C/N Ratio
Murshidabad	8.25	0.32	0.726	0.069	10.40
Malda	8.10	0.42	0.558	0.055	10.27

Table 2 : Physico chemical properties of Alluvial Soil of West Bengal

Sugarcane fields

Location	Phosphorus	Mechanical Analysis (%)			Cation Exchange	Minerals (me/100g)			
		Sand	Silt	Clay	Capacity [cmol (p+)] kg^{-1}	Na+	K+	Ca++	Mg++
Murshidabad	5.44	50.0	20.0	30.0	41.37	4.03	43.0	7.78	9.77
Malda	5.76	60.0	22.5	17.5	23.57	4.03	25.0	9.30	11.32

Table 3 : Population Dynamics of Proteolytic and Lipolytic Microflora (g^{-1} dry soil)

Sugarcane fields

Location	Bacteria ($x10^6$)			Actinomycetes($x10^5$)			Fungi ($x10^3$)		
	T*	Pr**	L***	T*	Pr**	L***	T*	Pr**	L***
Murshidabad	66.00	8.50	9.25	25.75	3.95	4.60	18.25	1.75	1.22
Malda	49.75	4.45	4.00	21.75	2.47	2.87	16.25	1.39	0.96

The data in Tables 1 and 2 exhibited the physico-chemical status of sugarcane fields of West Bengal where pH was moderately alkaline (8.1-8.25), organic carbon content was higher in Murshidabad (0.72%) than that of Malda (0.55%) whereas phosphorus content was more or less same (5.44-5.76). Cation exchange capacity was higher (41.37) than that of Malda. Both the soils was sandy while clay percentage is higher (30%) in Murshidabad soil.

Among the exchangeable cations Na^+ content was same (4.03) whereas K content was higher in Murshidabad (43.0). Ca^{++} and Mg^{++} value was quite different from each other ranging (7.78-9.30) and (9.77-11.32) me/100 g respectively. Bisht (1986) worked on the nutritional status of soil in grassland of the subtropics. He recorded the different composition of soil and pH, these are organic carbon (0.88-1.52%), total nitrogen (0.11-0.12%), available phosphorus (4-9 ppm), exchangeable potash (55-105 ppm) and pH 5.7-6.7. These results are more or less in accordance with our findings. Maji *et al.*, (1998) worked on morphological and chemical characterization of soils of Sagar island of Sunderban, W.B. Their reported data on various physico-chemical factors are as follows, pH (6.1-8.0), organic carbon (0.23-0.69%), CEC [(18.5-21.6) Cmol(p^+) Kg^{-1}]. This results harmonise our present study. Kimura *et al.*, (1979) studied extensively the rhizosphere of paddy soil in Japan with stress on the effect of anaerobiosis on microbes. Data showed that the total carbon (2.07-2.21%) and nitrogen (0.18-0.19%) contents of

Table 4 : Percentage Composition of Total population

Sugarcane fields

Location	Bacteria (%)		Actinomycetes (%)		Fungi (%)	
	Pr **	L***	Pr **	L***	Pr**	L***
Murshidabad	12.87	14.00	15.33	17.86	9.58	6.68
Malda	8.94	8.04	11.37	13.21	8.53	5.92

T* — Total Microflora, **Pr**** — Proteolytic Microflora
L*** — Lipolytic Microflora

paddy soil are quite higher in respect of our finding. In sugarcane field PR^+ and L^+ bacteria existed in the range of (8.94 -12.87%) and (8.04-14.0%), PR^+ and L^+ actinomycetes as (11.37-15.33%) and (13.2-17.86%), PR+ and L+ fungi as (8.53-9.58%) and (5.92-6.68%) in respect of total respective population.

The soil samples were primarily collected from rhizospheric regions in search of metabolically active microbes The rhizosphere region is important as more than a third of the photosynthate reaching in plant roots is lost in the soil as sloughed off root cap cells, mucilage, soluble exudates, lysates, decaying root hairs and cortical cells (Curl and Truelove,1986). The breakdown of these plant components by extra cellular enzymes such as cellulases, lipases, proteases results in the low molecular weight compounds which serve as nutrient source for indigenous micro flora (Curl and Truelove, 1986).

Mondal and Santra (1994) while working on fertility status and microbiological profile of paddy field soil reported that organic carbon and nitrogen percentage range of 0.5-0.61 and 0.80-1.52 respectively. The results indicated that nitrogen content and microbial count for bacteria (50×10^6 – 45×10^8) and fungi (42×10^3–90×10^7) were higher than that of our findings.

Takeuchi and Hayano (1994) characterized a paddy soil of Japan under monoculture of rice. According to them study of soil pH, organic carbon, nitrogen, clay content, colony forming unit of bacteria and fungi were as 6.6, 2.4%, 0.26%, 19%, 2.5×10^8, and 2.7×10^5.The values are better in comparison to our findings. (Tables 1, 2, 3, and 4).

To summerise the results of findings on microbial community structure (Table 5) exhibited the distribution of lipolytic microflora in different sugarcane growing field. For each microbe, frequency (%) and relative abundance (%) were indicated in the bracket to establish their relative prominence. Among different bacterial population representative of *Bacillus* and *Pseudomonas* dominated. *Bacillus licheniformis* (R. Abundance 15.66), *B. polymyxa* (R.A. 9.27), *B. coagulans* (R.A. 10.05), *P. fluoroscens* (R.A.16.88), *P. fragi* (R.A. 12.18) were important. Other bacterial representatives were *Micococcus luteus* and *Staphylococcus aureus.*

Table 5 : Distribution of Lipolytic microflora along the sugarcane fields of West Bengal, India

Name of organism	Frequency (%)	Relative abundance (%)
A. Bacteria		
Bacillus licheniformis	**40**	15.66
B. coagulans	30	10.05
B. polymyxa	30	9.27
Pseudomonas fluorescens	**40**	**16.88**
P. fragi	30	12.18
Micrococcus luteus	20	10.55
Staphylococcus aureus	20	9.75
B. Actinomycetes		
Actinomycetes sp. *A1*	30	15
Actinomycetes sp. *A5*	30	15
Streptomyces sp. *S2*	**50**	**60**
C. Fungi		
Mucor hiemalis	**40**	15.55
M.mucedo	**40**	16.55
Rhizopus arrhzius	**40**	**18.25**
Penicillium purpurogenum	30	10.26
Cladosporium herbarum	**40**	15.44
Alternaria alternata	30	7.85
Fusarium pallidoroseum	20	8.88
Aureobasidium pullulans	20	4.25
Trichosporon sp.	20	3.00

Among Actinomycetes group two Actinomyces species designated as *Actinomyces* sp. A_1 (R. abundance, 15) and *Actinomyces* sp. A_5 (R. abundance, 15) and *Streptomyces* sp. S_2 (R.A. 60).

A number of lipolytic fungi were screened and identified as *Mucor hiemalis* (R.A. 15.55), *M. mucedo* (R.A. 16.55), *Rhizopus arrhizus* (R.A. 18.25), *Penicillium purpurogenum* (R.A. 10.26), *Cladosporium herbarum* (R.A. 15.44), *Alternaria alternata* (R.A. 7.85), *Fusarium pallidoroseum* (R.A. 8.88), *Aureobasidium pullulans* (R.A. 4.25), *Trichosporon* sp (R.A. 3) were important.

Among the proteolytic microflora a good number of bacteria, Actinomyces and fungi were screened along the sugarcane growing areas of West Bengal (Table 6). Among bacterial representative *Bacillus* and *Pseudomonas* group dominated. Relative abundance (%) was indicated in the bracket. *Bacillus licheniformis* (19.75), *B. coagulans* (14.88), *Pseudomonas fluoroscens* (20.88), *Serratia indica* (8.77), *Micrococcus luteus* (9.54) were important.

Table 6 : Distribution of Proteolytic microflora along the sugarcane field of West Bengal, India

Name of organism	Frequency (%)	Relative abundance (%)
A. Bacteria		
Bacillus licheniformis	**70**	19.75
B. coagulans	50	14.88
B. megaterium	60	14.7
Pseudomonas fluorescens	50	**20.88**
P. aureofaciens	20	5.95
Serratia indica	30	8.77
Micrococcus luteus	30	9.54
B. Actinomycetes		
Streptomyces sp. S1	30	26.49
Streptomyces sp. S13	**70**	**50.55**
Streptomyces sp. S8	30	10.77
C. Fungi and Yeast		
Aspergillus niger	60	12.33
A. nidulans	50	9.49
A. aculeatus	40	7.78
Penicillium frequentans	60	12.99
P. chrysogenmum	30	6.75
P. citrinum	40	7.25
Myrothecium rorium	30	8.25
Paecilomyces sp.	40	7.99
Fusarium pallidoroseum	50	**15.88**

Among actinomycetes the *Streptomyces* species appeared very frequently taxonomically designated as *Streptomyces* sp. S_1 (26.49), *Streptomyces* sp. S_{13} (50.55), and *Streptomyces* sp. S_8 (10.77).

Among protease producing fungi *Aspergillus niger* (12.33), *A. nidulans* (9.49), *A. aculeatus* (7.78), *Penicillium frequentans* (12.99), *Myrothecium rorium* (8.25), *Paecilomyces* sp. (7.99) and *Fusarium pallidoroseum* (15.88) were important.

Ruiling and Wen-Ying (1981) worked extensively on ecological distribution of bacteria in paddy soil of China. They indicated the presence of *Bacillus*, *Arthrobacter*, *Pseudomonas* and *Micrococcus*. Among them *Bacillus*, the prominent genus contains such species like *Bacillus firmus*, *B. megaterium*, *B. pumilus*, *B. circulans*. Klubek *et al.*, (1992) characterized the microbial abundance and activity from three coal ash basins highlighted on the prominence of lipolytic as well as proteolytic microbes along with the cellulolytic, hemicellulolytic and amylolytic microbes.

To sum up, strains of *Bacillus* and *Pseudomonas* (bacteria), *Streptomyces* (actinomycetes) and *Penicillium* and *Aspergillus* (fungi) had the highest prominence when both lipolytic and proteolytic microflora were taken into consideration. Sugarcane fields of West Bengal was also studded with a number of yeasts. Lipolytic yeasts were identified as *Trichosporon* sp., *Candida* sp. and *Torula* sp. For proteolytic yeasts, in addition to these strains, *Chrysosporium* sp. was also identified.

Our results were compared with those of several investigators who worked in the similar field (Lawrence *et al.*, 1967). Chen *et al.*, (1992), through their sustained work on soil screening programme isolated 245 strains of organisms by enrichment culture method using olive oil as carbon source. Out of these organisms a deuteromycetous yeast *Trichosporon fermentans* WU-C12 was the best producer of extracellular lipase. Sangiliyandi and Gunasekharan (1996) isolated and characterized a lipase producing *Bacillus licheniformis* from an oil mill refinery effluent.

Chanda *et al.* (2002) worked on the population dynamics of proteolytic bacteria of rice field of West Bengal and reported the presence of *Bacillus licheniformis*, *B. cereus*, *B. mycoides*, *B. subtilis*, *Pseudomonas aeruginosa*, *Micrococcus roseus* and *M. luteus*.

Sztajer *et al.*, (1988) gave an elaborated account on the production of exogenous lipase production by fungi, actinomycetes and bacteria and they identified the different lipolytic microbes as *Rhizopus* sp., *Pseudomonas fluorescens*, *Penicillium* sp., *B. licheniformis*, *Lipomyces starkeyi*, *Streptomyces fradiae*, *Streptomyces* spp.

Several researchers carried out detailed investiga-

tion on population dynamics of proteolytic micro-organisms. *Bacillus* spp. and Pseudomonads are efficient producer of protease showed by various workers (Cowen *et al.*, 1985; Fogarty and Griffin, 1973; Takami *et al.*, 1989; Thompson *et al.*, 1985; Coolbear, 1991). *Thermoactinomyces* and *Streptomyces* are the two important extracellular protease producer as highlighted by Tsuchiya *et al.* (1991), Kang *et al.*, (1995) and Chanda *et al.*, (2003). Thus the data compared were quite similar to those of ours.

Statistical interpretation on the abundance of total, proteolytic and lipolytic microflora in relation to various physico-chemical parameters of soil taken from different soil tract of sugarcane field.

Soil Characters

Analysis of the variance of the data collected on each of the soil characters-pH, Carbon, Phosphorous, Cation exchange capacity, Potassium, Calcium and Magnesium from two localities in sugarcane growing soil tracts is presented in Table 7. Analysis is not possible for other characters due to the non-existing variance within the localities. As there are two localities, F-test is sufficient to test the significance of the difference between the

Table 7 : Mean values of each soil character measured from two localities in the Sugarcane growing soil tract

Soil character		Location		Mean	Critical difference at Probability level	
Name	Unit	Murshidabad	Malda		5%	1%
pH		8.24	8.10	8.17	0.10	—
Electrical Conductivity	dsm^{-1}	0.32	0.42	0.38	0.02	0.03
Total Organic Carbon	Per cent	0.73	0.57	0.65	0.01	0.04
Nitrogen	Per cent	0.07	0.06	0.06	—	—
Phosphorus	m.e./100g	5.44	5.77	5.60	—	—
Sand	Per cent	50.00	60.00	55.00	—	—
Silt	Per cent	20.00	22.50	21.25	—	—
Clay	Per cent	30.00	17.50	23.78	—	—
Cation exchange capacity	C mol $(p^+)Kg^{-1}$	41.38	27.58	34.47	2.24	3.40
Sodium	m.e/100g	4.04	4.04	4.04	—	—
Potassium	m.e/100g	43.00	25.00	34.00	3.31	5.02
Calcium	m.e/100g	7.78	9.30	8.54	0.83	1.26
Magnesium	m.e/100g	9.77	11.34	10.56	1.47	—

Table 8 : Inter correlation Coefficients matrix for the thirteen soil characters measured from the soil samples of two localities in Sugarcane growing soil tract.

Soil Character	pH	Electrical Conductivity	Total Organic Carbon	Nitrogen	Phosphorus	Sand
pH	1.000					
Electrical Conductivity	–0.764	1.000				
Total Organic Carbon	0.787	–0.968**	1.000			
Nitrogen	0.800	–0.982**	0.990**	1.000		
Phosphorus	–0.367	0.418	–0.375	–0.482	1.000	
Sand	–0.796	0.982**	–0.987**	–0.996**	0.373	1.000
Silt	–0.796	0.982**	–0.987**	–0.996**	0.373	1.000**
Clay	0.796	–0.982**	0.987**	0.996**	–0.373	–1.000**
Cation Exchange Capacity	0.805	–0.979**	0.974**	0.994**	–0.511	–0.987**
Sodium	0.246	0.029	0.065	0.075	–0.333	0.000
Potassium	0.767	0.976**	0.987**	0.989**	–0.348	–0.983**
Calcium	–0.825*	0.820*	–0.795	–0.848*	0.281	0.868*
Magnesium	–0.783	0.779	–0.632	–0.718	0.288	0.732

* — Significant at 5% Probability level. ** — Significant at both 5% and 1% Probability level.

Table 9 : Mean values of abundances of each type of Soil microflora against each locality in the Sugarcane growing Soil tract.

Soil microflora		Localities		Mean	Critical difference at level of significance	
Name	Type	Murshidabad	Malda		5%	1%
Bacteria´(10^6)	Total	66.00	49.75	57.85	5.03	7.62
	Proteolytic	8.50	4.45	6.48	0.87	1.32
	Lipolytic	9.25	4.00	6.62	1.27	1.93
Actinomycetes´(10^5)	Total	25.75	21.75	23.75	3.70	5.61
	Proteolytic	3.95	2.48	3.22	0.76	1.15
	Lipolytic	4.60	2.88	3.74	0.90	1.36
Fungi´(10^3)	Total	18.25	16.25	17.25	–	–
	Proteolytic	1.75	1.39	1.57	0.23	0.34
	Lipolytic	1.22	0.96	1.09	0.06	0.09

Table 10 : Analysis of variance of the data on each of ten Soil character measured from Soils of two localities in the Sugarcane growing Soil tract.

Soil character		Source of variation	Degrees of freedom	Mean Squares	Calculated Variance ratio(F)
Name	Unit				
PH		Locality	1	0.03781	10.37*
		Error	6	0.00365	
Electrical conductivity	dsm^{-1}	Locality	1	0.02101	162.88**
		Error	6	0.00013	
Total Organic Carbon	Per cent	Locality	1	0.04662	233.60**
		Error	6	0.00020	
Phosphorus	m.e/100g	Locality	1	0.21451	
		Error	6	0.22065	
Cation exchange Capacity	Cmol $(p^+)Kg^{-1}$	Locality	1	380.88001	228.83**
		Error	6	1.67917	
Potassium	m.e/100g	Locality	1	648.00000	176.73**
		Error	6	3.66667	
Calcium	m.e/100g	Locality	1	4.62080	18.39**
		Error	6	0.23130	
Magnesium	m.e/100g	Locality	1	5.01178	6.94*
		Error	6	0.72263	

*-Significant at 5%Probability level. **-Significant at 5% and 1% Probability level.

Table 11 : Physiochemical profile of Sugarcane growing Alluvial Soil tract at a glance.

Soil character		Mean	Standard Error	Coefficients of Variation (%)	Minimum	Maximum
Name	Unit					
PH		8.17	0.0327	1.13	8.00	8.25
Electrical Conductivity	dsm^{-1}	0.37	0.0197	14.93	0.31	0.44
Total Organic Carbon	Per cent	0.65	0.0293	12.75	0.56	0.73
Total Nitrogen	Per cent	0.06	0.0028	12.45	0.06	0.07
Phosphorus	m.e/100g	5.60	0.1657	8.37	4.76	6.14
Sand	Per cent	55.00	1.8900	9.72	50.00	60.00
Silt	Per cent	21.25	0.4725	6.27	20.00	22.50
Clay	Per cent	23.75	2.3620	2.81	17.50	30.00
Cation exchange Capacity	$Cmol(p^+)Kg^{-1}$	34.48	2.6420	2.17	27.30	43.20
Sodium	m.e/100g	4.04	0.0430	3.02	3.85	4.25
Potassium	m.e/100g	34.00	3.4590	2.88	23.00	45.00
Calcium	m.e/100g	8.54	0.3308	1.10	7.50	10.20
Magnesium	m.e/100g	10.56	0.4086	1.09	9.52	12.36

Table 12 : Microbiological studies of Sugarcane growing Alluvial Soil tract.

Soil Microflora		Mean	Coefficients of Variation (%)	Minimum	Maximum
Name	Type				
Bacteria ´ (10^6)	**Total**	57.88	15.71	45.00	68.00
	Proteolytic	6.48	34.20	4.00	9.00
	Lipolytic	6.62	43.59	3.50	10.00
Actinomyces ´ (10^5)	**Total**	23.75	12.28	20.00	28.00
	Proteolytic	3.21	27.63	1.75	4.20
	Lipolytic	3.74	27.80	2.00	5.20
Fungi ´ (10^3)	**Total**	17.25	10.16	14.00	20.00
	Proteolytic	1.57	14.55	1.35	1.90
	Lipolytic	1.09	12.97	0.90	1.24

localities. However, the mean value of each character with critical difference are also presented in the Tables 7, 8, 9, 10, 11 and 12.

The date in Table 7 exhibited that Murshidabad soil is richer than Malda soil in respect of pH, Carbon, Cation exchange capacity, Potassium. On the basis of the data on eight localities in respect of each character, pair wise relationship among the character are presented in the table below.

The date in Table 8 revealed that electrical conductivity had no relationship with pH, Phosphorous, Sodium and Magnesium. This character bore +ve relationship with sand, silt and Calcium and –ve relationship with Carbon, Nitrogen, Clay, Cation exchange capacity and Potassium. Carbon had strong +ve relationship with Nitrogen, Clay, Cation exchange capacity and Potassium and strong –ve relationship with electrical conductivity, sand and silt only. Magnesium had +ve relationship with Calcium only. Calcium had strong +ve relationship with sand and silt and -ve relationship with pH, Nitrogen, Clay and Cation exchange capacity. Sodium had no relationship with other character. The total sample size is too small to reflect the true relationship existing among the soil character under study.

The date in Table 9 showed that more colonies of each type of soil microflora was observed from Murshidabad soil.

As the number of sample observations is less than the total number of soil characters, it is not possible to determine the prediction model for each type of microflora with requisite precision for the sugarcane soil-growing tract.

Microflora

Analysis of the variance of the data on abundance of each of nine types of soil microflora is presented in the Table 11.

From the table, it has been observed that, all the type of microflora under study with the exception of fungi-total type, varied between two localities. The mean abundance of each type is furnished against each locality in the Table 9.

Through the sustained study on the distribution pattern of different groups of microflora along the sugarcane fields of West Bengal, we can formulate an opinion that these microbes definitely have a role in a number of biochemical reactions, which ultimately govern the nutrient balance and the physicochemical status of the soil. Moreover the microbial lipase and proteases derived from various selected potential microbes obtained from soil tracts of sugarcane field may be exploited commercially in various industrial fermentations by adopting suitable fermentation technology for production of different value added products.

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