RESEARCH ARTICLE

STUDY OF DISTRIBUTION OF XYLANASE PRODUCING MICROBES IN SOIL: A CASE STUDY IN MIDNAPORE TOWN, PASCHIM MEDINIPUR, WEST BENGAL, INDIA

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ARTICLE INFO	ABSTRACT
	Xylan, one of the most abundant products accumulated in soil is degraded by Endo-1, 4-β-xylanase (Endo-β-1, 4-xylan, xylanohydrolase; EC. 3.2.1.8, commonly called xylanase). The enzymes are produced mainly by soil microbes. Xylanases from fungal and bacterial sources have been extensively studied. The characters of soil and the distribution of such potent xylanase-producing bacteria from the adjoining areas of Midnapore town have been focused. Xylanase producing microbes were isolated on the basis of producing transparent zone around each colony on xylan agar plate and xylan hydrolyzing capacity. Highest number of bacteria (107×10^4 c.f.u) and fungi (4×10^4 c.f.u.) were found in forest soil and the lowest numbers are found from garbage dump soil (45×10^4 c.f.u bacteria and 2×10^4 c.f.u fungi). The highest number of xylanase produces was found in forest soil (14×10^4 c.f.u.). Percentage of xylanase producers among the fungi are is (about 26%) than bacteria (about 12%)

INTRODUCTION

Existence of microbes is well documented from soil, water and atmosphere. They are carried from the earth surface to upper atmosphere by air currents and into lakes and other large water bodies by streams and rivers. Even they are found in the bottom of the ocean at its greatest depth. Soil is the rich source of microorganisms where the interaction between biology and geology is optimum. Microorganisms are indispensable components of soil ecosystem. The balance of organic and inorganic matters in soil eco-systems are maintained by microbes (Paul and Clark, 1989). The prime sources of organic substances in the soil are plant materials. The organic constituents of plant materials deposited in the soil are commonly divided into six broad categories: (i) cellulose, the most abundant chemical constituent, varying in quantity from 15% to 60% of the dry weight, (ii) hemicellulose, commonly comprising 10% to 30% of the dry weight (Subramanium and Prema, 2002), (iii) lignin, usually making up 5% to 15% of the plant dry weight, (iv) water soluble fraction including the simple sugars, amino acids etc., contributing 5 to 20% of the tissue weight, (v) a fraction containing fats, oils, waxes, resins and a number of pigments, (vi) proteins, which have in their structure much of the plants nitrogen and sulfur. These organic substances are the sources of energy necessary for growth and development of the vast quantities of micro-flora.

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Presence of innumerable microbes and their diversified types in the soil constitute a picture like Lilliputian zoo. Generally microbial cells are impermeable to complex molecules and therefore, these molecules are at first simplified by the extra-cellular enzymes of microbes prior to their uptake. Abundance of a particular group of microflora in the soil depends upon the availability of particular substrate in their habitat. Actually these substrates induce microbes to release enzymes for breaking down of the substrates as source of their energy. Thus soil micro flora is used as a source of specific enzymes. Soil microbes are generally non pathogenic. Now-a-days they are widely used in food, chemical and pharmaceutical industries and have gained paramount importance for the production of bread, cheese, beer, antibiotics, vaccines, vitamins, enzymes and other important products.

Enzymes are the catalytic cornerstones of metabolism, and in the applied point of view it is preferred not only by biologists but also by process designers, chemical engineers, and researchers working in other scientific fields. Modern research areas have shown interest in the production of potential enzymes from the microbial sources. Enzymes produced by microbes can easily be extracted because most of these enzymes are extra-cellular. The production and extraction of the enzyme from microbial systems are cost effective as well as time saving (Subramaniyan and Prema, 2002). Scientists are searching for the suitable microorganisms and the fact is that the microbial world is giving a promising number of potential enzymes to make better industries, environment and economy.

In this context bacteria have great importance as they can tolerate more adverse conditions and the metabolic products like enzymes have also more tolerance range. So search for new potent bacteria is generally practiced for obtaining better enzyme producers. Microbial amylase, protease, tannase, lipase, cellulase, xylanase etc. are the examples of the most popular industrially utilized enzymes. Among them xylanase have got paramount importance in last four decades. Xylanases are most extensively used in paper industries for pulping and prebleaching processes (Viikari *et al*., 1986; Bajpai, 1999). Other potential applications are bioconversion of lignocellulose materials to fermentative products (e.g. ethanol), clarification of vegetable and fruit juices, improvement of consistency of beer (Royer and Nakas, 1989) and digestibility of animal feed stock (Khasin *et al*., 1993). Xylanases are reported to be produced mainly by bacteria (Gilbert and Hazlewood, 1993; Sunna et al., 1997), fungi (Beg *et al*., 2000; Kar *et al*., 2006), and actinomycetes (Ball and McCarthy, 1989; Beg *et al*., 2000). The present study deals with the search for xylanase-producing bacteria and fungi from soil of the adjoining areas of Midnapore town. The climate of the selected area is tropical and the land surface is characterized by rocky uplands, lateritic soil. The average annual rainfall of this area is approximately 1656 mm. The soils of the surrounding village areas are fairly fertile. Distribution of xylanase producing bacteria and fungi in various collected soils has been and fungi.

MATERIALS AND METHODS

Collection of soil samples

Soil samples were collected from different adjoining places of Midnapore town, District Paschim Medinipur, West Bengal, India. Soil samples were collected in small plastic packets carefully to avoid any possibility of mixing. Enumeration of xylanase producers was made by direct plate count method on selective media.

Medium preparation

The composition of isolating medium (IM) was (g/l): $(NH_4)_2SO_4$ 1.0; $MgSO_4$ 0.2; K_2HPO_4 0.2; $CaCl_2$ 0.2; $MnCl_2$ 0.02; yeast extract 0.1, xylan 10.0 and agar 20.0. Before sterilization of the media, xylan was completely dissolved in water by sonication (7 hz, 2 min). The pH was adjusted to 7.0 before sterilization. The medium was sterilized for 15 minutes at 121ºC.

Dilution plating

Each soil sample of 10 g was suspended in a 500 ml conical flask containing 100 ml of sterile water. The flask was shaken thoroughly by mechanical shaker for 5 minutes. In each case the suspension was allowed to stand for 15 minutes to settle down heavy particles and stock solution was prepared. Sample from each stock solution was then serially diluted. During dilution 1.0 ml of suspension was taken and added to 9.0 ml of sterilized distilled water in test tube and thus 10 times diluted soil sample was made. Each soil sample was diluted up to 10^{10}. Then 0.1 ml of each suspension was pipetted out and poured aseptically in the respectively labeled sterilized Petri plate

(xylan containing isolating medium) separately and spreaded. For each dilution of every sample three replicas were made.

Study of the soil characters

Water holding capacity

Percent of water holding capacity of the soil samples were determined with the help of Keen-Raczkowski box (Piper, 1967). A soil sample was allowed to soak water for 24h. The saturated soil sample was kept in previously weighed Keen box and weighed. Then it was allowed to dry for 24h in an oven at 105ºC. The weight of the over dried soil samples with the box was taken. The water holding capacity of soil samples were calculated as follows:

$$\text{Water holding capacity of the soil} = \frac{b - c}{c - a} \times 100$$

Where,

a = weight of the Keen box
b = weight of the Keen box (a) + saturated soil
c = weight of the Keen box (a) + dried soil

Soil pH

pH of each soil sample was determined with the help of a glass electrode pH meter (Systronic model 331). Soil sample was mixed with distilled water (1:5) and shaked for half an hour by mechanical shaker. Then the solution was filtered and pH of the filtrate solution was measured directly by the instrument.

Organic carbon

The organic carbon (OC) content of the soil sample was determined following Rapid Titration Method of Walkley and Black (1934). This method has an advantage that it excludes less active elementary carbon of soil and includes only that part of OC, which is involved in maintenance of soil productivity. In this method the soil was digested with 1 N potassium dichromate ($K_2Cr_2O_7$) and sulphuric acid (98% v/v). Two volume of sulphuric acid (H_2SO_4) was mixed with one volume of potassium dichromate. The excess of $K_2Cr_2O_7$, not reduced by soil organic matter, is then determined by titration with 0.4 N ferrous sulphate ($FeSO_4$) solutions. Organic carbon content was calculated as follows:

1 ml of one normal $K_2Cr_2O_7$ = 0.003 gm of carbon

$$\text{Percentage of organic carbon} = \frac{(V_1 - V_2) \times 0.003 \times 100}{W}$$

Where,

V_1 = volume of standard $K_2Cr_2O_7$
V_2 = volume of standard $FeSO_4$ used in titration
W = weight of the soil

Total nitrogen

Total nitrogen content of the soil was done by Macro-Kjeldahl process following Bear (1964). Each soil sample was

subjected to high temperature digestion at 330ºC–350ºC with concentrated H_2SO_4 to convert organic and inorganic forms of nitrogen to $(NH_4)_2SO_4$ (ammonium sulphate), which was distilled with 1N NaOH. The ammonia liberated by this distillation process was absorbed in known amount of standardized H_2SO_4 solution in a receiving flask. By back titration with 1N NaOH solution, the amount of ammonia liberated was calculated. From this the total nitrogen content of the soil was calculated as follows:

$$\text{Percentage of total nitrogen} = \frac{(V_1 - V_2) \times N \times 0.014}{W_1} \times 100$$

Where,

V_1 = volume of alkali (NaOH) consumed to titrate the known amount of standardized H_2SO_4 solution.
V_2 = volume of alkali (NaOH) consumed for titrating excess of H_2SO_4 in the determination.
N = the normality of the standard acid
0.014 = meq. wt. of nitrogen
W_1 = weight of the sample (g)

Available phosphate

Available phosphate content of the soil was extracted following the guideline of Bartlett, (1994). The soil (500 mg) was treated with calcium lactate (0.02 M) dissolved in 0.01 M HCl (25 ml) and shaked for 90 minutes. The extract was filtered through Whatman No 42 filter paper. 1 ml of clear extract plus 3 ml of mixed colouring reagent* were then dispensed into 10 ml colorimetric tubes to make total volume of 5 ml. Concurrently 2, 4, 6, 8, 10 and 12 ppm aliquots of KH_2PO_4 solution (2% w/v), were dispensed into 10 ml colourimeter tubes and mixed colour reagent (for working standards) was added to each tube to make a total volume of 5 ml. The phosphate content of the filtrate was measured using a standard curve prepared by standard KH_2PO_4 solutions of different concentrations.

*[*Mixed Colour Reagent (Samples)*

0.143 g ascorbic acid (C6H8O6) was dissolved in 100 ml water, it was transferred to 250 ml volumetric flask and19 ml reagent A was added.

Reagent A (Ammonium Molybdate-Sulphuric Acid-Sb Solution)

17.14 g ammonium molybdate [(NH₄)₆Mo₇O₂₄.4H₂O] was dissolve in 250 ml distilled water. Separately 0.392 g potassium antimonyl tartrate (KSbO.C₄H₄O₆) was also dissolve in 1 ml distilled water; both solutions were added to 1 l of cool 3.6 M H₂SO₄, and was diluted to 2 l with distilled water and then were mixed thoroughly. The solution was then stored in a dark, cool place in borosilicate glass.]

Soil potassium

Potassium content of the soil was measured using a Digital Flame Photometer (Toshniwal Type PL 01.02). Soil sample was treated with ammonium acetate (CH_3COONH_4) solution,

shaked for 30 minutes and then filtered. Potassium content of the filtrate was measured using a standard curve prepared by standard KCl solutions of different concentrations.

Selection of xylanase producer

Xylan agar plates generally look whitish and opaque in appearance. Xylanase producing microorganisms were isolated on the basis of clear and transparent zone around the colony in xylan agar plate. The area of hollow zones produced surrounding each colony was measured and this was the basis of primary selection. The primarily selected isolates were then transferred to 25 ml xylan containing broth (pH 7.0) in 100 ml of conical flasks and allowed to grow in room temperature for 48h in shaker. Each culture fluid was then centrifuged separately and used for assaying xylanase activity. The effective isolates were further selected on the basis of their enzyme production capability in the submerged condition. The final selection of the isolates was made on the basis of their growth behaviour in wide range of pH as well as enzyme production.

Assay of xylanase

Xylanase activity was assayed by measuring released reducing sugar from birch wood xylan (Fluka) with 3, 5- dinitrosalicylic acid (Miller, 1959). The reaction mixture containing 0.4ml phosphate buffer (0.2M, pH 7.0), 0.3ml of 5% (w/v) xylan and 0.3ml enzyme solution. The enzymatic reaction was carried out at 50ºC and after 30 min 1ml of DNS (3%w/v) was added to stop the reaction. The solution was incubated in a boiling water bath for 15 min for colour development and the absorbency was measured at 540nm (Systronic spectrophotometer 105) against the enzyme blank. The xylanase activity was determined by using a standard calibration curve of D-xylose (Sigma). One unit of xylanase activity (μ/ml) was defined as the amount of enzyme required to produce 1μmol of reducing sugars as xylose by hydrolyzing xylan per minute under the above assay condition.

RESULTS

Distribution of xylanase producing microbes in collected samples

Xylanase producing bacteria was primarily isolated from different soils like garden, forest, saw mill and garbage. All the sites for soil collection were confined within and adjoining areas of the Midnapore town, Paschim Medinipur, West Bengal, India. Distribution of the microbes in the tested soil samples was presented in Table 1. Among them maximum bacteria (107×10^4/g) and fungi (04×10^4/g) were recorded in the forest soil. Almost similar result was observed in garden soil. But the saw mill soil and garbage dump soil had less number of microbial counts. In respect to total microbes the saw mill soil contained higher percent of xylanase producers (18.18% of total microbes). In these soils about 26% fungi and 12% bacteria were xylanase producers (Table 1).

Study of soil samples

The physicochemical properties of soil like pH, organic carbon (OC), nitrogen (N), phosphate (PO_4), potassium (K) content,

Table 1. Distribution of xylanase producing microorganisms from different adjoining sites of Midnapore town

Source	Number of microbes (c.f.u.×10⁴/g)			Number of xylanase producers (c.f.u.×10⁴/g)			% of xylanase producing microbes		
	Fungi	Bacteria	Total	Fungi	Bacteria	Total	% of xylanase producers from total microbes	% of xylanase producing fungi among the total fungi	% of xylanase producing bacteria among the total bacteria
Forest soil	4.00 ± 0.07	107 ± 2.30	111	1.30 ± 0.37	12.00 ± 1.7	14	12.35	33.33	11.56
Garden soil	2.30 ± 0.13	99 ± 3.50	101	0.33 ± 0.43	11.00 ± 2.10	11	10.86	14.29	10.77
Soil from saw mill	3.30 ± 0.13	52 ± 5.00	55	1.00 ± 0.00	9.00 ± 2.00	10	18.18	30	17.42
Garbage dump soil	2.00 ± 0.70	45 ± 5.80	47	0.33 ± 0.43	4.00 ± 2.00	04	09.15	16.67	08.82
Total (approx)	12	303	314	03	36	39	12.41	25.71	11.89

Table 2. Some physicochemical characteristics of soil samples collected from different adjoining sites of Midnapore town

Sources	Soil pH	Organic carbon (OC) (%)	Nitrogen (N) (%)	Phosphate (PO_4) (ppm)	Potassium (K) (ppm)	Water holding capacity (WHC) (%)
Forest soil	6.27	0.40	0.06	5.73	7.02	38.36
Garden soil	5.99	0.39	0.04	7.90	10.60	37.37
Soil from saw mill	6.63	0.28	0.06	5.97	6.08	33.71
Garbage dump soil	5.77	0.32	0.02	8.17	12.42	30.38

water-holding capacity (WHC) were evaluated (Table 2). It was found that all of the soils were slightly acidic; contained organic carbon in the range of 0.28 – 0.40 g%. The range of nitrogen, phosphate and potassium contents and water holding capacity were from 0.02 – 0.06 g%, 5.73 – 8.17 ppm, 6.08 – 12.42 ppm and 30.38 – 38.36 % respectively. Distribution pattern of xylanase producing fungi and bacteria in relation to various edaphic factors was evaluated statistically by non-parametric tests (Wilcoxon Singed Rank Test, Table 3). The relationship between the xylanase producing organisms (fungi and bacteria) and the different edaphic factors is presented by the multiple scatter diagram (Figure 1). The Pearson Correlation Matrix (Table 4) shows that the xylanase producing bacterial population has positive correlation with water holding capacity, soil pH and nitrogen content of the soil. Other parameters were least significant to xylanase producers. Multiple regressions between the xylanase-producing bacteria with different edaphic factors of soil have been done by using same statistical package. Multiple regression equation of xylanase producing bacteria on different soil parameters is as follows:

Xylanasw producers (Xp) = – 37.669+5.190 pH – 33.546 OC – 25.309 N + 1.065 PO_4 – 0.834 K + 0.129 WHC.

With R^2 = 0.850, which shows satisfactory fitting.

Isolation of xylanase producing bacteria

Six bacterial isolates were primarily considered according to larger transparent zone around each colony on xylan agar plate and xylan hydrolyzing capacity. The strains were designated as BSA1 (from garbage dump soil), BSA2 (from forest soil), BSA9, BSA22 and BSA33 (from saw mill soil), BSA23 (from garden soil). Among the isolates BSA1 produced maximum xylanase in liquid cultures (3.23 U/ml). The capability of xylanase production of BSA22 and BSA23 was very close to BSA1 (Table 5). The tolerance of pH, growth kinetics and xylanase production capabilities of these three isolates (BSA1, BSA22, BSA23) were again compared (Table 6). It was found that BSA1 was able to grow in a wide range of pH (3-10). Enzyme production in wide pH range (3-10) was also noticed by BSA1, whereas BSA22 and BSA23 showed xylanase production in comparatively narrow pH range of 6.0-9.0 and 7.0-10.0 respectively.

DISCUSSION

Microbial xylanase have tremendous importance in different industries. Therefore, search for a new type of xylanase producer is an important aspect. This study showed that there were sufficient numbers of micro organisms (fungi: 04×10^4/g and bacteria: 107×10^4/g) present in the soil and among them about12% was xylanase producer.

Table 3. Wilcoxon Signed Rank test result

Variable	Estimated mean	Confidence Interval
pH	6.138	(5.925, 6.400)
OC	0.3475	(0.310, 0.400)
N	0.0475	(0.035, 0.060)
PO$_4$	6.98	(5.96, 7.98)
WHC	34.67	(31.09, 37.91)
K	8.72	(6.59, 10.57)
Fungi (total)	3.00	(2.00, 3.50)
Bacteria (total)	76.0	(52.50, 101.00)
Fungi (Xp)	1.000	(0.50, 1.00)
Bacteria (Xp)	9.50	(6.50, 11.50)

WHC = Water Hilding Capacity, Xp = Xylanase producer

Table 4. Pearson Correlation matrix of different variables of the soil

	pH	OC	N	PO$_4$	K	WHC	Fungi (total)	Bacteria (total)	Fungi (Xp)	Bacteria Xp
pH	1.000	-.384	0.853	-.775	-.865	0.433	0.617	0.061	0.522	0.555
OC	-.384	1.000	-.056	0.061	0.249	0.449	0.165	0.613	0.254	0.331
N	0.853	-.056	1.000	-.877	-.833	0.551	0.631	0.365	0.466	0.617
PO$_4$	-.775	0.061	-.877	1.000	0.898	-.495	-.720	-.237	-.689	-.580
K	-.865	0.249	-.833	0.898	1.000	-.434	-.690	-.225	-.553	-.612
WHC	0.433	0.449	0.551	-.495	-.434	1.000	0.626	0.659	0.627	0.703
Fungi (total)	0.617	0.165	0.631	-.720	-.690	0.626	1.000	0.279	0.776	0.500
Bacteria (total)	0.061	0.613	0.365	-.237	-.225	0.659	0.279	1.000	0.155	0.752
Fungi (Xp)	0.522	0.254	0.466	-.689	-.553	0.627	0.776	0.155	1.000	0.523
Bacteria (Xp)	0.555	0.331	0.617	-.580	-.612	0.703	0.500	0.752	0.523	1.000

Fungi Xp = Xylanase producing fungi, Bacteria Xp = Xylanase producing bacteria, OC = Organic carbon, WHC = Water Holding Capacity, PO$_4$ = Phosphate, N = Nitrogen, K = Potassium

Table 5. Measurement of diameter (mm) of the transparent zone around the bacterial colonies on xylan agar plate and production of xylanase through submerged fermentation after 48h at 37°C

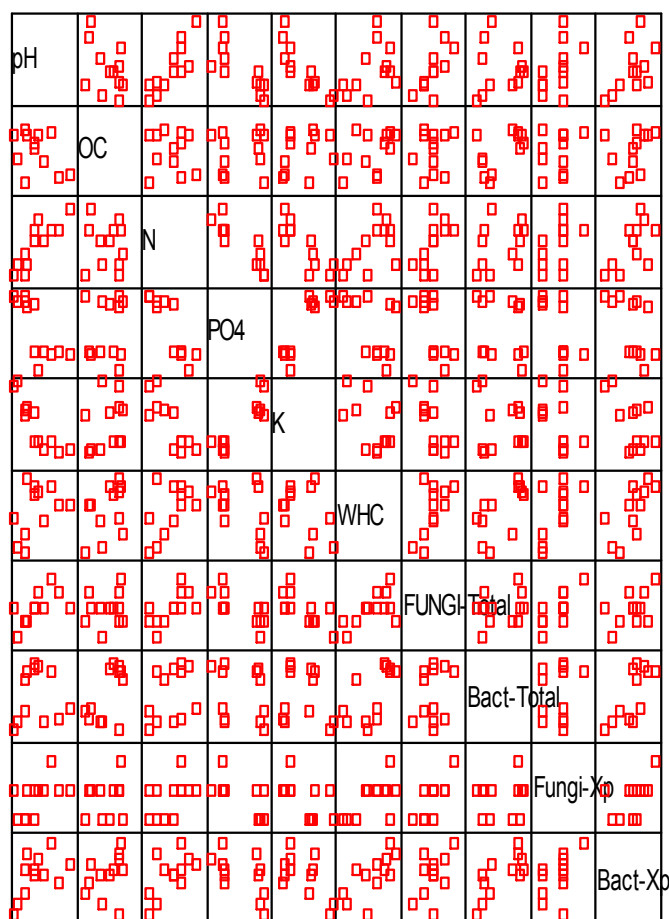| Strain No	Diameter of clear zone (mm)	Xylanase (U/ml)
BSA1	11	3.23
BSA 2	05	1.65
BSA9	07	2.32
BSA22	10	2.97
BSA23	09	2.84
BSA33	06	1.73

Figure 1. Multiple scatter diagrams of the different variables of soil

Occurrence of xylanase in soil microbes plays an active role in the decomposition and recycling of plant materials containing hemicellulose (Pelczar *et al.*, 1993a). There is no such type of report regarding the distribution of xylan degrading bacteria in soil. The pectin degrading bacteria was reported to be about 19% of soil bacterium (Pelczar *et al.*, 1993b). The quantity of xylan degrading microbes was found higher (18.18%) in saw mill soil. In this soil saw dust is always mixed and it is a perpetuated source of wood hemicelluloses. Xylan (one of the major hemicelluloses) is the main component of such woody tissues (Timell, 1967). The abundance of xylan in soil may be the prime cause of increased percentage of xylanase producing microbes in such soil samples (Bernier *et al.*, 1983). Quantity of xylanase producing bacteria was significantly correlated with edaphic factors like water holding capacity, pH and nitrogen content, but not so strongly with organic carbon, phosphate and potassium content of the soil. Soil is a complex mixture of different organic and inorganic matters and microbes and the microbial activity in the soil is responsible

Table 6. Growth and enzyme production by the primarily isolated strains in different pH

Strain No	pH of the media															
	3		4		5		6		7		8		9		10	
	Gr	En	Gr	En	Gr	En	Gr	En	Gr	En	Gr	En	Gr	En	Gr	En
BSA1	1.7	1.4	3.2	2.0	5.7	3.6	5.0	3.8	4.8	3.5	3.2	3.1	1.8	1.1	0.7	0.9
BSA22	ND	ND	ND	ND	ND	ND	0.6	1.4	0.3	3.1	4.1	2.3	3.2	1.8	ND	ND
BSA23	ND	ND	ND	ND	ND	ND	ND	ND	1.1	1.6	3.0	2.9	2.0	1.0	0.5	0.5

Gr = Growth (c.f.u./ml×10^8), En = Enzyme production (U/ml), ND = Not detectable

for establishment of soil pH and *vice versa*. Organic matters after decomposition produce both, organic and inorganic acids. The simplest and perhaps the most widely found acid is carbonic acid. The slow but persistent solvent action of carbonic acid on the mineral constituents of the soil is responsible for the removal of base forming cations and if these are leached or carried by rain water, the soil pH become acidic (Brady, 2000a). The saw mill and garbage dump soil had the pH of 6.63 and 5.77and these contained 18.18% and 9.15% xylanase producers respectively. Thus soil pH is one of the most modulating factors for the association of xylanase producer in the soil. Water is very much essential for every living system. Midnapore is situated in the arid region where the water in soil plays a critical role for soil flora and fauna. Singh and Kashyap (2007) reported that the presence of adequate soil moisture enhanced microbial activity. Water between the soil particles helps the microbes to grow and the microbial network causes accumulation of nutrients produced through decomposition in soil. In the present study, a positive correlation was found between water holding capacity of the soil and xylanase-producing bacteria (r=0.703, Table 4).

Though the proper explanation behind this relationship is not available, it seems that water molecule enhances the biochemical reactions between hemicellulosic organic matters of soil with the microbial cell, thereby the population of xylanase producing bacteria increases. The average value of nitrogen content of surface mineral soil is 0.15% (Brady *et al.*, 2000b) and the examined value was found poor (0.045%). Large amount of soil nitrogen is withdrawn by the plants and it is also lost through rain water which quickly runs away due to the landscape of the locality and it may be a probable cause of low nitrogen content. Nitrogen is assimilated into the cell as amino acids and proteins, and other compounds are utilized in different physiological action. Therefore nitrogen content in a sample primarily promotes microbial growth as well as decomposition (Alexopoulos *et al.*, 1996). This is the probable cause of positive correlation between nitrogen content and soil microbes. It was found that phosphate and potassium content was higher in garbage dump soil in comparison to other samples but the numbers of soil bacteria as well as xylanase producers were less here.

This indicated that phosphate and potassium in municipal garbage remained mostly in inorganic forms and not easily available to the soil microbes. Another plausible cause of lower number of microbes here was that the garbage dump soil is more polluted as it causes accumulation of more harsh chemicals. There are reports that municipal sludges contain organic and inorganic toxic chemicals that can have harmful effects (Furr *et al.*, 1976). Deposition of plant litters here are less and this is the prime cause of containing lower percentage of xylanase producers. In this study the finally selected bacteria *Bacillus cereus* BSA1 was isolated from garbage dump soil and it should be a pollutant tolerant one. In the present study six bacterial strains (BSA1, BSA2, BSA9, BSA22, BSA23 and BSA33) were primarily isolated on the basis of clear zones around the colonies on xylan agar plates. The formation of transparent zones was due to degradation of xylan by the xylanase producers. Roy and Abedin (2002) isolated xylanase-producing bacteria on the basis of clear hollow zones on xylan agar plate. Among the six primary isolates BSA1, BSA22 and BSA23 were again selected on the

basis of the production of higher amount of enzyme. BSA1 was finally selected because it can grow and produce higher amount xylanase in wide range of pH (pH 3.0 - 10.0). During prebleaching process of pulp, chlorinated chemicals are used extensively and the pH of the effluent remains alkaline. If xylanase producing microbes are used as substitute of the chemical prebleaching agent (Sindhu *et al.* 2006), the organism should have an essential criterion of tolerating higher pH. As the newly isolated organism tolerate a wide range of pH and can produce enzyme even in pH 10.0 (Table 6) it can be qualified for use in biobleaching process (Choudhury *et al.* 2006).

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