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In search of xylanase from caves: characterization of xylanase producing bacteria from Meghalaya, India

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Abstract

Microscopic life residing the extreme environments of the caves are increasingly drawing interest of the microbiologists worldwide. Certain bacterial inhabitants of cave are found to produce various extracellular enzymes such as cellulase, mannose, xylanase that can be used to consume lignocellulosic biomass. The xylanase activity is carried on different pretreated lignocellulosic biomass containing high xylan content such as sugarcane baggase. Media optimizations for high xylanase production are performed on LB, AIM, TB followed by extraction and purification of the enzyme. The enzyme xylanase are useful as feed supplements, food improvers, bleaching, lignocellulosic waste degradation leading to the production of bioethanol and several other industrial applications. Highest production of xylanase from Bacillus sp. isolated from the caves was achieved in the TB medium extracellular protein concentration containing xylanase 14U. Partially purified extracellular xylanase displayed optimum pH 6.5 and temperature 50ºC. Thermostability of the xylanase at the elevated temperature showed stability between 80-90ºC retaining its 99% activity. Time dependent enzymatic hydrolysis of beechwood xylan and preprocessed agro waste sugarcane bagasse exhibited the release of xylotetrose, xylotriose and xylobiose oligosaccharide (XOS) significantly high as compared to the other oligosaccharides.

Keywords: Cave; Lignocellulosic Biomass; Sugarcane Baggase; Pretreated; Bioethanol.

Introduction

Cave microbiology deals with the study of microscopic life that resides in caves. Caves are the extreme environments for life. In the interior of caves, the light source is absent; hence the phototrophic microorganisms are unable to synthesize organic matter. The primary productivity of caves relies upon the chemolithoautotrophic microorganisms [1]. The majority of the cave dwelling microorganisms are oligotrophic [2]. The cave microflora is investigated for the production of xylanase in this study.

Microbial xylanases is preferred over plant and animal sources, because of the simple availability and short generation time, structural stability and easy genetic manipulation. The microbes from the pristine environments and the enzymes produced by them can be of superior industrial values due to their adaptations to low nutrient content and other factors so as to cope up with the conditions available for them in the extremities like caves. The use of microbial enzymes for the hydrolysis of polysaccharides in lignocellulosic materials is widely researched because of the importance of hydrolysis products (soluble sugars) in fermentation processes for the production of fuel, chemicals, food and feed. The economics of enzymatic conversion of lignocellulosic materials will be greatly improved if both the cellulose and hemicellulose contents can be utilized[3].

Xylan is the most abundant non-cellulosic polysaccharide, found to be of approximately 20–35%, thus as a whole comprises approximately one third of all renewable organic carbon sources on earth. In annual plants and hardwoods, xylan is the most abundant non-cellulosic polysaccharide which accounts for 20-35% of the total dry weight in biomass[4,5].Depending upon the plant origin, the xylan backbone was found to be covalently bound to varying degree of acetic acid, arabinose, D-glucuronic acid, 4-O-methyl-D-glucuronic acid, ferulic acid and p-cumaric acid[6]. Apart from the three basic chemical compounds (cellulose, hemicellulose, and lignin), lignocellulosic biomass contains water, proteins, minerals and other compounds. The organic component of biomass plays a major role in processing and producing biofuel [7].

Hydrolysis of xylan is an important step towards proper utilization of lignocellulosic material in nature, which otherwise can be a source of pollution when left idle. Chemical hydrolysis of lignocelluloses results in hazardous byproducts, forcing the use of microbial enzymes, which are species in action for xylan hy-

drolysis and is an environment friendly option [3]. Thus microbial xylanase are an important source to minimize environment pollution both by the lignocellulosic biomass and the chemicals that are used for the hydrolysis of this biomass.

Xylanase (endo-1, 4-β-xylanase) and β-xylosidase (β-D-xyloside xylohydrolase) are the major constituents of well known microbial xylanolytic enzyme systems. Cellulases and xylanases have an assortment of industrial applications including pulp & paper, laundry, food, animal feed, brewery and wine, textile, bioenergy industry etc. Xylanases have also gained increasing attention because of their applications in pre-bleaching of Kraft pulps, in paper industries for pulp treatment (improving the effectiveness of conventional bleaching chemicals), fibre modification, the extraction of coffee, plant oils and starch, and the improvement of the nutritional properties of agricultural silage and grain feed, fruit juice and wine clarification, in saccharification of agricultural, industrial, household and municipal wastes[3,8,9].

In order to make the enzyme applications more cost effective at industrial level, its production using low cost substrates such as agro-wastes, food and industrial waste, fruit waste, vegetable waste and weed plants as a low-cost solid substrate for the production of cellulases and xylanases have potential to overcome the problem of high cost enzyme production has been recommended by many workers[10-12].The costs of enzyme production can be reduced by optimizing the fermentation process such as by optimizing medium, temperature, pH is the goal of basic research for industrial applications[13].

Xylanases have been produced either in solid state cultivation/ fermentation (SSC/SSF) or submerged cultivations or fermentation (SmC/SmF). However, SSC has gained renewed interest in recent years and has often been employed for the production of many metabolites due to a number of economical and engineering advantages. The alternative for this is use of solid-state fermentation, which is closer to natural system and has proved to be more efficient in producing certain enzymes and metabolites[14]. Submerged fermentation process is mostly preferable because of more nutrients availability, sufficient oxygen supply and less time required for the fermentation than other fermentation techniques[15].

XOS act as the prebiotic, which can be used as ingredients of functional food, cosmetics, pharmaceuticals or agricultural products and as a plant growth regulator. In addition to the health effects, XOS present interesting physico-chemical properties, they are moderately sweet, and stable over a wide range of pH and temperatures and have organoleptic characteristics suitable for incorporation into foods [16-18]. XOS have importance in decreasing the blood lipids, protecting liver functions, decreasing blood pressure, anti-cancer and regulating blood sugar. Therefore, XOS-containing diets are considered to be beneficial in improving gastrointestinal health.

Thermostable enzymes have an obvious advantage as catalysts in the lignocellulose conversion processes due to better enzyme accessibility and cell-wall disorganization achieved at high-temperature reaction conditions [19]. Longer energetic life under high temperature conditions would make these enzymes approving for improved and competent biomass conversion [20]. Therefore, for an enzyme to be effective, thermostability be the most important aspect for the enzyme utilized under severe bioprocessing conditions [20]. The thermostability of the enzyme was established by testing the enzyme activity of the enzyme after incubation at a range of temperature from 20 ℃ to 100 ℃.

Materials and Methods

Chemical reagents

Nutrient Agar, Nutrient Broth, Luria Bertani broth, Muller Hinton agar, Glucose, Dextrose, Sucrose, Lactose, Mannitol, Phenol red, Starch agar, Gram's Iodine, Yeast extract, Gelatin, Skim milk powder, Agar, Peptone, Tryptone, Kovac's reagent, MR-VP media, Simmons Citrate Agar, Nutrient agar, Nutrient broth, Safranin, Crystal violet, VP reagent I, VP reagent II, Oxidase disc, Ferrous ammonium sulphate, Sodium thiosulphate, Potassium dihydrogen phosphate, Agarose gel, TAE buffer, Bradford's reagent, DNS reagent, Hydrogen peroxide, Petroleum ether, Glacial acetic acid, n-Butanol.

Sample Collection

Soil and Water sample from Cave: Sampling date: 01-Feb-2019, Cave name: Krem Phyllut; Location: Sohra (Cherrapunjee), East Khasi Hills, Meghalaya; GPS parameters (latitude & longitude): N 26°09.202' & E° 91°39.633' Elevation: 1169 metres, Ambient temperature inside cave: 18°C, Relative Humidity (RH) inside cave: 93%.

Codes assigned for the samples collected: PL1: Downstream water sample (temp.: 18.5°C; pH: 7.0), PL7: Upstream floor sediment, PL8: Upstream water sample (temp.: 18.5°C; pH: 7.0), PL10: Downstream floor sediment.

The cave investigated in the present study, Krem Phyllut (in Khasi, cave=krem), is located in Sohra (Cherrapunji), East Khasi Hills, Meghalaya. Water samples (PL1 & PL8) and floor sediments (PL7 & PL10) of a running stream inside the cave were collected aseptically in sterile containers, immediately brought to the laboratory and kept at 4◦C. Routine microbiological work was carried out within 24-48 hours of sample collection.

Lignocellulosic Biomass: The agricultural biomass used in this study is Sugarcane bagasse, which is the left over waste of sugarcane after the removal of the juice. The biomass was collected from the juice sellers of the University campus.

Isolation of Bacterial cultures: The 1 gm of soil sediment and 1ml of the water samples were diluted into 90 ml distilled water and dilutions ($10^{-}3$, $10^{-}4$, $10^{-}5$, 10-6, 10-7), were prepared separately. Then 0.1 ml of suspension from each dilution was transferred into nutrient agar and R2A agar plates by using spread plate or pour plate technique and incubated at 37˚C for 24 hours.

Colony forming Units (CFU) were calculated by counting the total number of colonies that appeared in the plates using the formula:

CFU = (Number of colonies appeared)/(volume of inoculum plated) × Dilution Factor

Morphological, cultural and biochemical tests: The bacterial isolates were then characterized morphologically, physiologically and biochemically following standard protocol as described by Cappuchino and Sherman (1996) [20]. The bacterial isolates were subjected to gram staining n to analyze morphologically and physiologically. Biochemical tests viz. i) Fermentation of sugars: glucose, mannitol, lactose and sucrose;, ii) hydrogen sulphide production test, iii) citrate utilization test, iv) Sugar utilization test was performed with HiCarbohydrate Kit, v) catalase and vi) oxidase tests were performed to detect the preliminary characteristics of the isolated bacterial colonies.

Antibiotic Susceptibility Test: This test was performed with Antimicrobial Susceptibility Test Discs (HiMedia) to select the most appropriate antimicrobial agents for treatment against the infectious organisms. Antibiotic discs were placed on the surface of the agar plates inoculated with test organism. The absence of growth of the organism around the antibiotic disc indicates that the respected organism was susceptible to that antibiotic and the presence of growth around the antibiotic disc indicates the organism was resistant to that particular antibiotic. Muller Hinton Agar plates were prepared. The isolates were inoculated on the agar plates and spreaded uniformly. Disc were placed on the agar plates and incubated at 37 ℃ for 48 hours. The zone of inhibition was measured with a Vernier calliper.

The commercially available antibiotic discs (HiMedia Laboratories Pvt. Ltd.) used was:

Amoxicillin (10 mcg) – AMX; Ampicillin (10 mcg) – AX; Cefalexin/cephalexin (30 mcg) – CN; Cephalothin (30 mcg) – CEP; Chloramphenicol (30 mcg) – C; Clindamycin (2 mcg) – CD; Cloxacillin (5 mcg) – COX; Co-trimoxazole (25 mcg) – COT; Erythromycin (15 mcg) – E; Gentamicin (10 mcg) – GEN; Oxacillin (1 mcg) – OX; Penicillin-G (2 units) – P; Tetracycline (10 mcg) – TE; Vancomycin (30 mcg) – VA.

Antimicrobial activity against enteric pathogens: This antimicrobial activity was performed against 8 enteric pathogens from Microbial Type Culture Collection (MTCC), IMTECH, Chandigarh. The enteric pathogens were in freeze dried form in sealed ampoule. These were inoculated in Lactose Broth and incubated. The eight enteric pathogens were-

1. Proteus vulgaris MTCC 426
2. Salmonella enterica ser. Typhi MTCC 733
3. Salmonella enterica ser. Paratyphi MTCC 735
4. Salmonella enterica typhimurium MTCC 98
5. Enterobacter aerogenes MTCC 8559
6. Escherichia coli MTCC 739
7. Klebsiella pneumoniae subsp. Pneumoniae MTCC 109
8. Staphylococcus epidermidis MTCC 435

9 test isolates were cultured in Lactose Broth. 2ml of it was taken in sterilized Eppendorf tubes and centrifuged at 1000rpm for 15mins. The supernatants were transferred in sterile culture vials. Muller Hinton Agar plates are prepared and 200µl each enteric bacterium was inoculated and spreaded uniformly. The agar was punctured with sterile well borer to form wells. 100µl of each 9 supernatants was inoculated in the wells. The plates were incubated in 37℃ for 48hours.

FESEM analysis of the bacterial isolates: 1.5ml of broth culture was transferred into eppendorf and centrifuged at 13,000 rpm for 10-12 minutes. The supernatant was discarded and the pellet was resuspended in grades of alcohol for dehydration (10%, 30%, 50%, 70%, 90%, 100%). The pellet was then picked and a smear was prepared in a coverslip and was allowed to dry for 2-3 hours.

Screening for xylanase activity of bacterial isolates- Plate assay: In-vitro xylanase activity screening was performed using congo red plate assay method as described elsewhere [21]. The bacterial isolates were cultured overnight in 1% (w/v) birchwood xylan containing LB agar plates at 37 °C. After the bacterial enumeration into unilayer, the culture plates were flooded with 0.1% (w/v) congo red solution and left for 15 min with intermittent shaking. Following the congo red staining, plates were washed with distilled water to remove any additional unbound stain and finally washed with 1M NaCl solution to distain the plates.

Preparation and Pretreatment of Lignocellulosic Biomass: The sugarcane bagasse collected was dried at 60℃ for 24hrs. The fully dried biomass is then grinded and a dry powdery substrate is obtained. The substrate in then pretreated by Alkaline Peroxide (H_2O_2) Pretreatment method. It is an effective method for pretreatment of LCB. The powdered Lignocellulosic biomass (LCB) is substrate was soaked with H_2O_2 at room temperature, pH of 11-12 using NaOH, for a period of 6-24hrs. In this method, a significant removal of lignin (about 70-90%) was reported. The delignified biomass is then filtered and allowed to dry for 24hrs. The powder thus obtained was then used as substrate for further analysis[7].

Production and purification of xylanase: Selected colonies showing exo-xylanase activity in plate assay were cultured in different media containing 1% (w/v) birchwood xylan in order to obtain higher production of xylanase. In order to find the best media for higher production of bacterial cell mass and extracellular xylanase four different sets were prepared viz. Luria Bertani (LB), 5xLB, Auto inducing Media (AIM) and Terrific Broth (TB medium) were prepared as described by Tripathi et al., 2009. Each 100 ml culture broth was inoculated with the bacterial colony showing exo-xylanse activity and incubated at 37 ºC, 200 rpm overnight. The cells were harvested and separated from the culture broth. The 100 ml culture broth was centrifuged at 13,000 rpm for 10 min and the supernatant was separated. Cell free supernatant containing proteins was subjected to 80% (w/v) ammonium sulfate precipitation till the saturation achieved at 4 ºC, 18 h. The partially purified protein was dialyzed against 50 mM sodium phosphate buffer at 4 ºC overnight.

Protein profiling of the enzyme using SDS-PAGE: Denaturing SDS-PAGE was performed according to BioRad specifications. 12% resolving gel was made by mixing 3.4 ml of double distilled water, 4 ml of 30% acrylamide (29.2 g acrylamide and 0.8 g bis-methylene acrylamide in 100 ml), 2.5 ml of 1.5 M Tris buffer pH 8.8 (27.23 g Tris was mixed with 120 ml double distilled water and was adjusted pH to 8.8 using 6N HCl. The total volume was made up to 150 ml using double distilled water), 0.1 ml of 10% SDS (Sodium dodecyl sulphate), 50 µl

freshly prepared 10% ammonium persulfate (APS) and 10 µl of tetramethylethylenediamine (TEMED). Resolving gel was made by mixing all the above chemicals and casted into the glass plate immediately after preparation and was allowed 40 minutes of standing to polymerize. Stacking gel was casted on top of the resolving gel and comb was immediately placed on the top of the glass plate. The stacking gel was prepared by mixing 6.1 ml of double distilled water, 1.3 ml of 30% acrylamide (29.2 g acrylamide and 0.8 g bis-methylene acrylamide in 100 ml), 2.5 ml of 0.5 M Tris buffer pH 6.8 (6 g Tris was mixed with 80 ml double distilled water and adjust pH to 6.8 using 6 N HCl and bring the total volume to 100 ml), 0.1 ml of 10% SDS, 50 µl of 10% APS (freshly prepared) and 10 µl TEMED. The stacking gel was also allowed 40 min to polymerize. Protein was extracted in extraction buffer (50 mM Tris-HCl; pH 7.8, 0.3 mM MgSO4 and 0.1 mM EDTA) and mixed with sample buffer in the ratio of 1:1. The sample buffer contains of 3.55 ml double distilled water, 1.25 ml 0.5 M Tris (pH 6.8), 2.5 ml glycerol, 2 ml 10% SDS, 0.2 ml of 0.5% bromophenol blue. 50 µl of β-mercaptoethanol (βME) was mixed with 950 µl of sample buffer. Then the final sample buffer was mixed with protein sample in the ratio of 1:1. The samples were boiled for 3 min and loaded into the respective wells of the gel. The comb was removed from the gel prior to sample loading. Electrophoresis was performed at room temperature for 1 h at a constant voltage of 150 V in 1X running buffer. The running buffer was prepared by mixing 25 mM Tris, 192 mM glycine and 0.1% SDS (3.03 g Tris, 14.4 g glycine and 1 g SDS were dissolved in double distilled water and brought the final volume of 1000 ml) in double distilled water. 10X running buffer was prepared and stored at 4 °C for later use.

Staining and de-staining

After electrophoresis, the gel was stained with 0.1% Coomassie Brilliant Blue (CBB) 250 dye in 50% methanol, 10% glacial acetic acid (dye was dissolved first in methanol) and incubated overnight. The gel was destained at least three times with double distilled water, methanol and glacial acetic acid in the ratio of 5:3:1. Gel was then viewed under EZ Gel Doc image analyzer.

Enzyme activity assay: Initially, the enzyme assays of partially purified xylanse were carried out in 100 µl of a reaction mixture in 50 mM sodium phosphate buffer pH 7.0 containing 1% (w/v) birchwood xylan using 10 µl of enzyme and incubating at 50 °C for 15 min.. Temperature optimization assays were carried out between 40 °C and 100 °C, respectively, for 15 min. The enzyme activity was measured by estimating the liberated reducing sugar by dinitrosalicylic acid (DNS) method as described elsewhere[22]. The mixture was diluted by adding water to make up the volume to 1 ml and the absorbance at 540 nm (A540) was measured by a UV-Visible. D-Xylose in the range of 10-500 µg/ml was used for generating the standard plot. One unit (U) of enzyme activity is defined as the amount of enzyme that liberates 1 µmole of reducing sugar per min.

Substrate specificity of xylanase against xylose polysaccharides: The enzyme activity of xylanase was determined by using 50 mM sodium phosphate buffer at optimum pH and temperature incubated for 15 min. The 100 µl reaction mixture contained 1.0% (w/v) substrate, 50 µl of partially purified xylanase. The assays were performed in triplicate. The concentration of reducing sugar was estimated using a standard curve of D-Xylose as partially purified protein has xylanase activity. 100 µl of reaction mixture was taken for estimation of enzyme activity which was calculated against birchwood xylan. After analysis of substrate specificity the enzyme assays, kinetic parameters viz. Km, Vmax and Kcat were determined.

Temperature optimization of enzyme activity: Thermal stability is an important parameter for industrially important enzymes for their utilization at elevated temperatures. In this study the temperature optimization of the partially purified xylanase was assessed at varying temperatures from 50°C to 100°C. The partially purified protein (50mM sodium phosphate buffer, pH 7.0) was incubated individually prior to addition of substrate at 50°C, 60°C, 70°C, 80°C, 90°C, and 100°C, for 30 min in a water bath. The best activity of the enzyme was tested by taking the OD of the samples at 540nm.

Thin layer Chromatography (TLC): The qualitative analysis of hydrolyzed products was performed by thin-layer chromatography (TLC) on silica gel-coated aluminium foil (TLC Silica gel 60 F254 20×20 cm, Merck) for detecting sugars. The enzyme with 1% (w/v) in birchwood xylan and the lignocellulosic substrate prepared, 100 µl reaction mixtures were incubated at optimized temperature 60°C and optimized pH 6.9, for 24 hours. The reaction products were then dissolved in alcohol and separated from the solid substrate mixture. Then the samples were dried at 60 ℃ and 0.2 µl of samples were loaded on the TLC plate and kept in the developing chamber saturated with the developing solution (mobile phase) which consisted of acetic acid-n-propanol-water-acetonitrile (4:10:11:14). At the end of the run, migrated sugars were visualized by immersing the TLC plate in a visualizing solution (sulphuric acid/methanol 5:95, v v-1; α-napthol 5.0 %, w/v). The TLC plates were then dried at 80°C for 20 min. The migrated reaction products (sugars) appeared as clear spots on the TLC plate.

Results and Discussion

The resultant bacterial load of the water sample collected from the Krem Phyllut Cave of Meghalaya was observed to be 1.8×10-7CFU/ml. A total of nine bacterial colonies were selected based on their distinct plate morphological characters and are then isolated in pure form and were designated as PL1-3, PL7-2, PL7-3 PL8-2, PL8-3, PL10-1, PL10-5, PL10-6, PL10-7, where PL1 is for spring water upstream, PL8 is for spring water downstream, PL7 is for upstream cave sediment and PL10 is for downstream cave sediment. The biochemical analyses were performed for the preliminary identification of the isolates. All these enumerated bacterial colonies in the plate assay with 1% (w/v) birchwood xylan, displayed positive xylanolytic activity by producing a white halo around the colonies when stained with Congo red. This result suggested that the cultures have the capacity to produce extracellular xylanase. The sugarcane Flasks containing 1% of the substrate prepared and the culture showing high enzyme activity in plate assay was incubated in LB medium at 37 °C and 180 rpm for overnight. The enzyme production was estimated and further media optimization was done with Terrific Broth (TB), Autoinduction Media (AIM), and the protein concentration was measured using Bradford's reagent. The protein concentrations in the other related media were measured as the following order TB>AIM>LB. Therefore TB medium was considered for further enzyme production experiments.

Protein profiling of the enzyme – SDS-PAGE

The SDS-PAGE was performed for the enzymes extracted from the culture PL8-3, PL10-5, PL10-7 and the protein profiling was observed in the gel docing system. The bands were observed in the lanes shown (Figure below).

Temperature optimization of the enzyme activity

The partially purified protein (50mM sodium phosphate buffer, pH 7.0) was incubated individually prior to addition of substrate at 50°C, 60°C, 70°C, 80°C, and 90°C, for 30 min in a water bath. The best activity of the enzyme was tested by taking the OD of the samples at 540nm. The highest activity of the enzyme from PL10-7 was at 50 ℃ with activity of ~1.4U/ml followed by PL8-3 which was at 60°C of ~1.25U/ml and then PL10-5 at 50 ℃ of ~1.0U/ml (in 1% substrate concentration).

Enzyme Kinetics

The determination of enzyme kinetic parameters such as Km and Vmax were obtained from MM plot and Line Weaver Burk Plot, respectively, as displayed in Table 8, Figure 14 - 15. In MM plot the enzyme PL8-3 showed highest enzyme activity at 1.75% (w/v) preprocessed sugarcane bagasse concentration with Vmax = 2U/ml and Km = 3.33mg/ml (Table 8, Figure 14 - 15). Whereas, PL10-5 displayed lower activity at 1.5% (w/v) preprocessed sugarcane bagasse concentration as compared to PL8-3 with Vmax = 1.8U/ml and Km = 3.5mg/ml. In contrast, PL10-7 displayed much lesser activity at 1.5% (w/v) preprocessed sugarcane bagasse concentration Vmax = 1.4U/ml and Km = 4mg/ml.

Figure 1. 12% SDS-PAGE analysis of partially purified proteins from the isolated bacterial species.

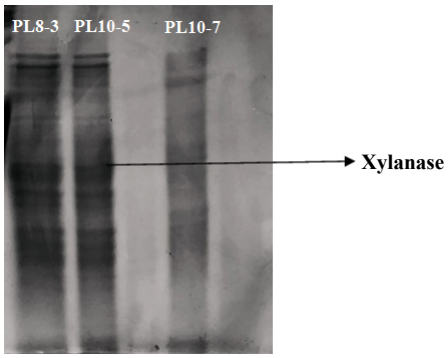


Figure 2. Plot showing enzyme activity at different temperature.

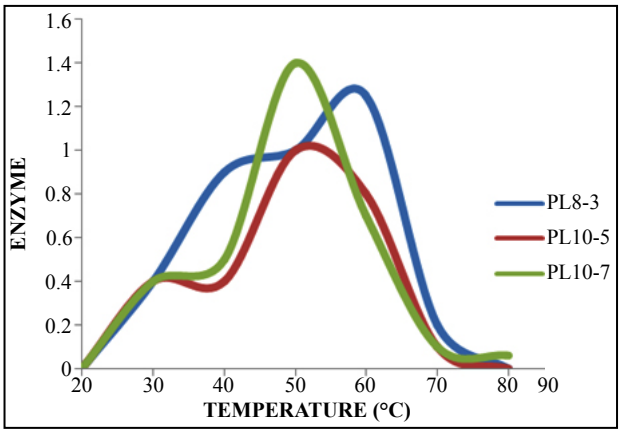


Figure 3. Michaelis Menten Plot for enzyme activity.

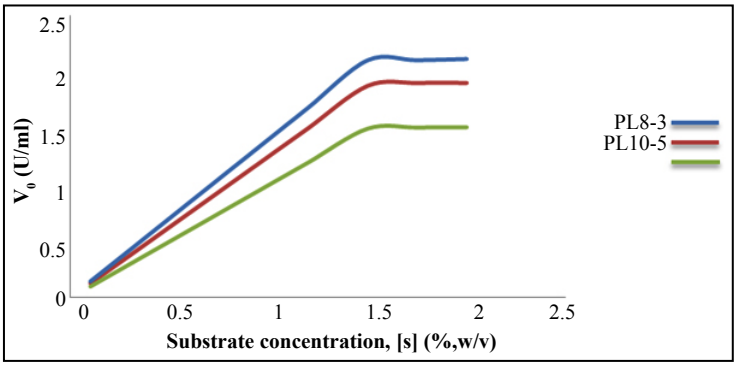


Figure 4. Line Weaver Burk Plot (Vmax) for enzyme activity.

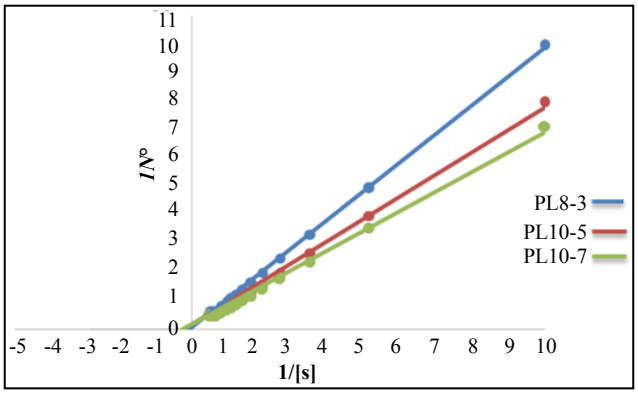
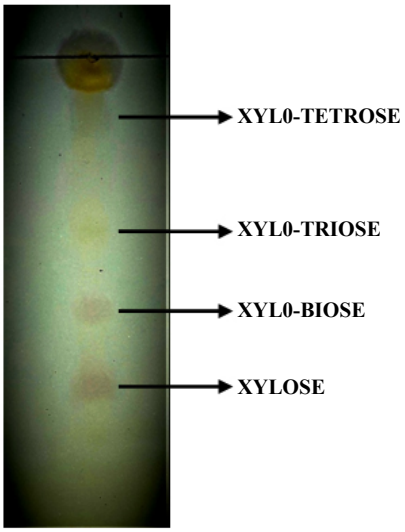


Table 1. Km and Vmax of the enzymes using sugarcane bagasse as substrate.

Substrate – Sugarcane Bagasse		
Culture	**Km(mg/ml)**	**Vmax (U/ml)**
PL8-3	3.33	2
PL10-5	3.5	1.8
PL10-7	4	1.4

Figure 5. Production of xylo-oligosaccharide from sugarcane bagasse by using PL8-3.



Production of xylo-oligosaccharide using TLC

Thin layer chromatography is used to detect the production of the xylo-oligosaccharide by the enzymes of PL8-3, PL10-5, and PL10-7. After 24 hours of incubation with the substrate from sugarcane bagasse and the partially purified enzyme displayed the different degrees of polymerization of substrate after hydrolysis which was appeared in the form of spots on the TLC plates. Predominantly xylose was found as maximum followed by xylo-biose, xylo-triose, and xylo-tetrose.

Conclusion

Cultural characters help in the identification as well as classification of bacteria in different taxonomic groups (Harley, 2008). Most of the colonies studied in the present work were found to be of rod -shaped, Gram positive and endospore forming. The biochemical analysis indicates the colonies were identified as Bacillus sp. Thermophilic xylanases have much industrial significance. The extracellular xylanase from Bacillus sp. exhibited highest enzymatic activity at 50-60°C which is corroborating the nature of thermophilic xylanase active between 40°C-60 °C as per the report of Walia et al., 2014 [23]. Media composition played a significant role in production of extracellular xylanase form Bacillus sp. where the composition of extracellular xylanase production was found maximum in TB medium. The higher cell densities and high protein content in TB medium as compared to TB, LB and 5xLB might be due to the rich source of tryptone, yeast extract and phosphate salts corroborated the finding as described elsewhere[24,25]. Partially purified xylanase from the bacterial isolate exhibited endo-xylanase activity as it replaces maximum amount of reducing sugar xylose as birchwood and beechwood xylan contain 82-85% xylose containing β-(1→4)–glycosidic linkages between the monomers as per the reported of the safety manual of Sigma LLC, CO, USA, where the enzyme cleaves randomly between the linear chains of xylose backbone containing β-(1→4)–glycosidic linkages. Moreover, the pattern of hydrolysis of commercial and agro

waste sugarcane bagasse in TLC showed the production of apparently showing spots of xylopentose, xylotetrose, xylotriose and xylobiose exhibiting endoxylanase activity. The production of pretreated XOS from the hydrolyzed agrowaste sugarcane bagasse was quite significant by extracellular endo-xylanase from the isolated Bacillus sp., which is comparable to the production of XOS by the action of fungal and bacterial xylanase similiarly as reported elsewhere[26,27]. Since enzyme was highly active against the agrowaste biomass – sugarcane bagasse which produced significant degree of polymerization i.e., different degrees of oligosaccharides similarly which has reported by Ghosh et al., 2019[28]. Therefore, the endoxylanase has produced by the Bacillus sp. in this present study may pave many industrial applications and the XOS as functional food in future nutraceutical industries.

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