International Journal of Engineering, Science and Mathematics
Vol. 9 Issue 08, August 2020,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijmra.us, Email: editorijmie@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

A STUDY ON PRODUCTION, PURIFICATION AND INDUSTRIAL APPLICATION OF MICROBIAL XYLANASE

Kottala Ravindar, Research Scholar, Dept. of Boi-Technology, Sikkim Professional University
Dr Aakansha Goswami, Professor, Dept. of Boi-Technology, Sikkim Professional University

ABASTRACT

The most prevalent noncellulosic polysaccharide, xylan makes up 20–35% of the dry weight of the biomass of tropical plants and is found in both hardwoods and annual plants. Xylans are less prevalent in temperate softwoods and can make up as much as 8% of the total dry weight. In the secondary cell wall, where it is most abundant, xylan is thought to be creating an interphase with lignin and other polysaccharides. It is believed that xylan molecules interact with polysaccharides like pectin and glucan as well as the phenolic residues in lignin. Xylans are linear homopolymers that include D-xylose monomers and are connected by -1, 4-glycosyl linkages in their most basic forms. By randomly cleaving -1, 4 glycosidic bonds, xylanase (EC 3.2.1.8) degrades -1, 4 xylan, resulting in xylose and xylo-oligosaccharides like xylobiose. Xylanases play a significant role in industry since they can be used to clarify fruit juices, brighten pulp for animal feed, and bleach paper pulp in the paper making process. Applications for xylanase eliminate the use of pricy, environmentally harmful chemicals. Xylanases are abundant in microorganisms and are produced by numerous bacterial taxa, actinomycetes, and fungal species. High levels of extracellular xylanases are secreted by a number of Bacillus species and filamentous fungi. Xylanase secretion frequently correlates with either a low or high cellulase concentration. It is recommended to employ cellulose-free xylanases when treating pulp because cellulase may negatively impact the quality of the paper pulp. Finding naturally occurring microbial strains that can secrete cellulose-free xylanases under ideal fermentation conditions is the most realistic strategy. Xylanase needs to be stable at high temperatures and an alkaline pH in order to be used prominently in the bleaching process.

KEY WORDS: Noncellulosic Polysaccharide, Xylan, Noncellulosic, Polysaccharide, Xylan.

INTRODUCTION

After cellulose, xylan is the second most bountiful biopolymer, and it is a sustainable non-cellulosic polysaccharide found generally in the optional cell walls of both hardwood and yearly plants. It represents 33% of our planet's natural carbon source (Kamble and Jadhav, 2012). The construction of xylan is very confounded and fluctuates enormously between plant species. D-xylose combined with a - 1,4 linkage (xylopyranose) is the principal part of xylan, with a level

of polymerization going from 150 to 200 different sugars and natural mixtures, for example, arabinose, ferulic corrosive, glucuronic corrosive, etc (Terrasan et. al.,2013; Chakdar et. al., 2016). The promptly open lignocellulosic substance in nature, xylan, which represents 20-40% of all out plant biomass, can be best taken advantage of after hydrolysis (Ninawe et. al., 2007; Kumar et. al., 2015; Walia et. al., 2017). The actual adaptation, dissolvability, and reactivity of the xylan particle with other hemicellulosic parts still up in the air by the side chains. Substance handling, which is broadly utilized in industry, is a work of art and speedier technique for separating these xylan particles. This cycle consumes an enormous number of synthetic compounds, which brings about the union of unsafe parts/items in huge amounts, making it risky to the climate (Beg et. al., 2001). Enzymatic hydrolysis or cleavage of such an intricate construction is confounded and requires synergistic activity by a gathering of compounds, for example, xylanase, - d-xylosidases, - L-arabinofuranosidases, acetyl xylanesterases, and - d-glucuronidases (Terrasan et. al., 2013; Kumar et. al., 2018).

The catalyst xylanase, which deals with various substrates and lignocellulosic parts and has industry-accommodating elements, is the best option in contrast to conventional compound methods. Xylanase is generally utilized in different businesses, including food and feed, materials, pastry shop, paper and mash, etc. Xylanase (cellulose free) has been displayed to work on the presentation of standard dying synthetics in eliminating lignin from hardwood and softwood kraft pulps in the paper and mash industry (Chakdar et. al., 2016; Walia et. al., 2017). Besides, the catalyst xylanase's sturdiness at antacid pH and high temperatures makes it ideal for modern use. Utilizations of microbial xylanases over compound medicines have been utilized in modern enormous scope processes for quite a while and are critical. Because of the growing interest and exploration, novel microorganisms from which xylanase can be disconnected are being accounted for consistently.

To utilize xylanase on a modern scale, we should zero in on the compound's quality and monetary achievability to meet every one of the modern norms while keeping away from the use of customary substance processes. Since the presence of cellulase debases the nature of the completed item by obliterating/harming valuable cellulose (Sindhu et al., 2006), and the greater part of the methods utilized in the business need high temperatures at a basic pH, the presence of cellulase is bothersome. To accomplish these rules, the xylanase should be without cellulase, thermostable, and dynamic at both impartial and soluble pH levels. The worthiness of contagious xylanase is restricted because of the presence of cellulase, low ideal pH, and more unfortunate security, yet bacterial xylanase is by and large acknowledged because of its absence of cellulose, high ideal pH, and more prominent steadiness. The expense of compound blend likewise affects its business use. The expense of compound blend in enterprises differs in view of the expense of development substrate. Since xylanase can be effortlessly fabricated from bounteously accessible agrarian and modern waste, it is more affordable than xylanase produced using monetarily accessible xylan, which is restrictively costly, thus fits modern prerequisites. In the business, enormous scope xylanase assembling can be achieved by maturing the most affordable horticultural squanders. On account of the nearness of the aging framework to the microorganisms' regular territory, strong state maturation enjoys an upper hand over lowered aging with regards to chemical creation productivity (Babu and Satyanarayana, 1995; Jecu, 2000). Different benefits of strong state maturation over lowered maturation incorporate more straightforward item recuperation, lower fluid volume necessities, lower pollution risk, higher item (protein) yield, lower capital venture, and a less complex scale-up process.

In the paper and mash industry, xylanase assumes a significant part in the expulsion of lignin from kraft mash during the pulping and fading processes (Eriksson, 1990a; Beg et. al., 2001; Walia et. al., 2017). The 'Kraft' and 'Sulfate' medicines are two normal synthetic medicines for pulping and blanching. These substance medicines transmit a lot of

perilous synthetics, including unsafe natural mixtures that are cancer-causing and mutagenic (Garg et. al., 1998; Beg et. al., 2000b). Minuscule woody chips are broiled at high temperatures and soluble pH in enormous tanks to eliminate lignin, with most of the lignin dissolving in the pulping fluid however a modest quantity staying to cause cooking of the paper. Compound treatment with chlorine (Cl2), chlorine dioxide (ClO2), and other natural synthetic substances is utilized to totally eliminate the excess lignin content (Damiano et. al., 2003). The activity includes an extremely hazardous outflow that represents a damage to the climate. To forestall wellbeing and ecological dangers, as well as to conform to government administers, the business is progressively changing old treatment processes by carrying out enzymatic natural medicines, which are more secure, more savvy, and harmless to the ecosystem. Natural methodologies including enzymatic treatment with xylanase and different chemicals can specifically dispose of the lignin sugars buildings (LCC) that create during the kraft cycle. Enzymatic treatment in the paper business includes pulping and beating (Poorna and Prema, 2007). Pulping includes drenching and enzymatic hatching to eliminate the filaments from the paper; then again, beating includes first crushing and afterward warming the mash to eliminate the strands and deactivate the protein (Klungness and Ahmed, 2000). Bio blanching is the best non-chlorine fading process since it is more productive and savvy (Koponen, 1991; Ahlawat et. al., 2007).

RESEARCH METHODOLOGY

Xylanase activity estimation

The release of reducing sugars during the enzyme substrate reaction was used to measure xylanase activity. Miller's approach was used to calculate the release of reducing sugars (Miller, 1959).

By adding 1.5 mL dinitrosalicylic acid (DNSA) reagent and boiling the reaction mixture for 15 minutes in a boiling water bath, the reduced sugars released could be determined and calculated. After cooling to room temperature, the color of the resulting combination was measured at 540 nm in comparison to enzyme and substrate controls. Under normal assay conditions, one unit (U) of enzyme activity is defined as the amount of enzyme that catalyzes the release of one gram of reducing sugars from the substrate in one minute. The activity of xylanase was measured in units per milliliter (U/ml). The amount of reducing sugar xylose was calculated using a standard curve plotted with xylose concentrations ranging from 50 to 500 g/ml. All of the experiments were done in triplicate and the findings reported are the average of the three results.

DNSA reagent composition

Components	Quantity/500 ml
Sodium hydroxide	5g
Phenol	1 ml

Sodium potassium tartarate	100 g
Sodium bisulphate	250 g
DNSA	5 g

Final volume of 500 ml was made up with distilled water

Estimation of cellulase activity

The crude extract's cellulase activity was determined using the same procedure that was used to determine xylanase activity. The carboxymethyl cellulose activity was measured at several pH levels ranging from 6 to 12 for the cellulase assay.

The amount of enzyme necessary to release 1 g of reducing sugar (as glucose)/ml/min under standard assay conditions was defined as one unit of cellulase activity, which was expressed as U/ml.

Enzyme assay (qualitative)

The congo red method was used to investigate the microorganisms' ability to produce xylanase (Carder, 1986). The plates were treated with 1% oat spelt xylan and sterilized at 121 oC for 15 minutes. Pure culture inoculum was spotted on these plates, which were then incubated at 37 oC for 24 hours. The zone of hydrolysis was seen by staining with 0.1 percent congo red for 30 minutes and then destining with 1 M sodium chloride solution.

Quantitative enzyme assay

Extracellular xylanase production was tested in modified Horikoshi medium on isolates that tested positive in the plate assay. 2 percent (OD-0.5) of the overnight developed inoculum was inoculated into 250 ml Erlenmeyer flasks containing 50 ml medium and incubated at 37 oC under shaking conditions (120 rpm). The culture broths were centrifuged for 15 minutes at 10,000 g to extract the supernatant, which was then tested for xylanase activity. At alkaline pH and high temperature, the isolate with the highest xylanase activity and no cellulase activity was chosen for future research. Bacillus cereus was identified as the chosen isolate.

RESULTS AND DISCUSSION

Soil samples were gathered from a variety of locations, including Ballarpur Paper Industries Pvt. Ltd. in Yamunanagar, Haryana; National Forest Kalesar forestland; and agricultural soil in several

adjacent regions. A total of 40 samples were gathered from various sources, including 8 samples from soil near the paper mill, 16 samples from forestland, and 16 samples from agricultural area. Collected samples were inoculated for bacterial culture separation, and a total of 68 bacterial cultures were isolated (showed a considerable increase in turbidity, indicating bacterial development). Increased turbidity was followed by a corresponding drop in the medium's true hue.) To obtain pure isolated colonies, bacteria were cultivated in medium containing xylan nutrition broth of pH 8.0 with 0.25 percent oat-spelt xylan and serial dilution procedure.

During initial screening, Congo red staining was used to test xylanolytic activity, which is an important screening approach. Teather and Wood created this approach in 1982, and it is based on the creation of a complex between the dye congo red and the polysaccharide (xylan). After adding dye and destaining with NaCl on plates, a clear halo zone forms due to the loss of polysaccharide around the expanding colony due to microorganisms' use of polysaccharide with xylanase production. The clear zone after de-staining was found within a range of 6-23mm after screening bacterial cultures for xylanolytic activity on a plate technique. Only 18 isolates were found to be appropriate after destaining, with a clear and transparent zone of hydrolsis during qualitative plate assays using congo-red staining (Table-1).

These isolates were then screened for quantitative xylanase production using the DNSA (3,5-Dinitrosalicylic acid) method, with only 8 isolates being found to be positive for xylanase production after quantitative screening (Table -2). XPB 3 and XPB 9 were the isolates that produced the most xylanase. The isolate XPB 9 produced the most xylanase and did not demonstrate any cellulase activity with CM cellulose, hence it was chosen for further investigation. The biochemical characterisation of isolate XPB 9 is shown in Table-3. The findings demonstrated that strains reacted positively to nitrate reduction by converting nitrate to nitrite. When treated with H2O2, the catalase test resulted in the production of oxygen. Citrate consumption as a carbon source was also found to be beneficial in the strain. Voges-proskauers, motility, heamolysis, and KCN were all positive in the isolate. However, gelatin hydrolysis, indole test, methyl red test, oxidase, and pigment all showed a negative result. The isolate tested positive for fructose, glucose, glycerol, glycogen, maltose, ribose, starch, and trehalose in the sugar fermentation test. Bacillus cereus was discovered as the preferred bacterium, which was a gram-positive, moderate thermophile with optimum, minimum, and maximum growth temperatures of 40, 30 and 60 degrees Celsius, respectively, under SmF. (Table 4.3). The strain was an alkalophile that grew best at pH 7.5. The culture was kept on nutrient agar medium slants at 4°C and as a glycerated frozen culture

at -20°C (gl-1 peptone, 10.0; yeast extract, 3.0; sodium chloride, 5.0; agar, 2 percent). Only two potent bacterial strains, Bacillus sp. MCC2728 and Bacillus sp. MCC2727, were found among 24 screened strains from 177 bacterial isolates from soil samples from thick plantation regions and forest land, according to Shanthi (2018). Xylanase-producing Bacillus sp. was discovered from 12 strains taken from soil near the Rajsahi University campus, according to Roy and Rowshanul (2009). Bacillus cereus was isolated from coalmine soil by Dhanjal and Cameotra (2010) for aerobic biogenesis of selenium nanospheres.

TABLE.1: QUALITATIVE PLATE ASSAY OF THE ISOLATES

Sr. No.	Sample	Collection Place	Result
1	XPB 1	Paper Ind.	6mm
2	XPB 2	Paper Ind.	13mm
3	XPB 3	Paper Ind.	22mm
4	XPB 4	Forest Soil	10mm
5	XPB 5	Forest Soil	11mm
6	XPB 6	Forest Soil	12mm
7	XPB 7	Forest Soil	11mm
8	XPB 8	Forest Soil	17mm
9	XPB 9	Forest Soil	23mm
10	XPB 10	Forest Soil	10mm
11	XPB 11	Agriculture Soil	11mm
12	XPB 12	Agriculture Soil	15mm
13	XPB 13	Agriculture Soil	12mm
14	XPB 14	Agriculture Soil	5mm
15	XPB 15	Agriculture Soil	16mm
16	XPB 16	Agriculture Soil	12mm
17	XPB 17	Agriculture Soil	16mm

18	XPB 18	Agriculture Soil	13mm

TABLE 2: QUANTITATIVE ASSAY OF THE ISOLATES

Sr. No.	Sample	Collection Place	Amount (U/ml.)
1	XPB 2	Paper Ind.	202
2	XPB 3	Paper Ind.	310
3	XPB 5	Forest Soil	211
4	XPB 8	Forest Soil	250
5	XPB 9	Forest Soil	354
6	XPB 12	Agriculture Soil	252
7	XPB 15	Agriculture Soil	273
8	XPB 17	Agriculture Soil	268

TABLE 3: BIOCHEMICAL CHARACTERIZATION OF THE *BACILLUS CEREUS*

Basic Characteristics	**Properties**
Catalase	+ve
Citrate	+ve
Gelatin Hydrolysis	-ve
Gram Staining	+ve
Growth in KCN	+ve
Hemolysis	+ve
Indole	-ve
Motility	+ve

MR (Methyl Red)	-ve
Nitrate Reduction	Variable
Oxidase	-ve
Pigment	-ve
Shape Rods Spore	+ve
VP (Voges Proskauer)	+ve
Sugar Fermentation	
Adonitol	-ve
Arabinose	-ve
Arabitol	-ve
Cellobiose	Variable
Fructose	+ve
Galactose	-ve
Glucose	+ve
Glycerol	+ve
Glycogen	+ve
Inositol	-ve
Inulin	-ve
Lactose	-ve
Maltose	+ve
Mannitol	-ve
Mannose	-ve
Melibiose	-ve
Raffinose	-ve

Rhamnose	-ve
Ribose	+ve
Salicin	Variable
Sorbitol	-ve
Starch	+ve
Sucrose	Variable
Trehalose	+ve
Xylose	-ve
Enzymatic Reactions	
Acetate Utilization	Variable
Arginine Dehydrolase	Variable
Casein Hydrolysis	+ve
Esculin Hydrolysis	+ve
Lecithinase	+ve
Lysine	-ve
Ornithine Decarboxylase	-ve
Phenylalanine Deaminase	-ve
Tyrosine Hydrolysis	+ve

Microbial growth cycle

The optical density of the inoculated nutrient broth at 660 nm was measured at regular intervals to determine bacterial growth (2 to 30 h). After a one-hour lag in growth, the bacterium started the exponential phase of growth. The optical density of the broth grew exponentially during the exponential phase (1-18 h). Following this, the bacteria entered a stagnant growth phase in which the optical density remained constant (Fig 1)

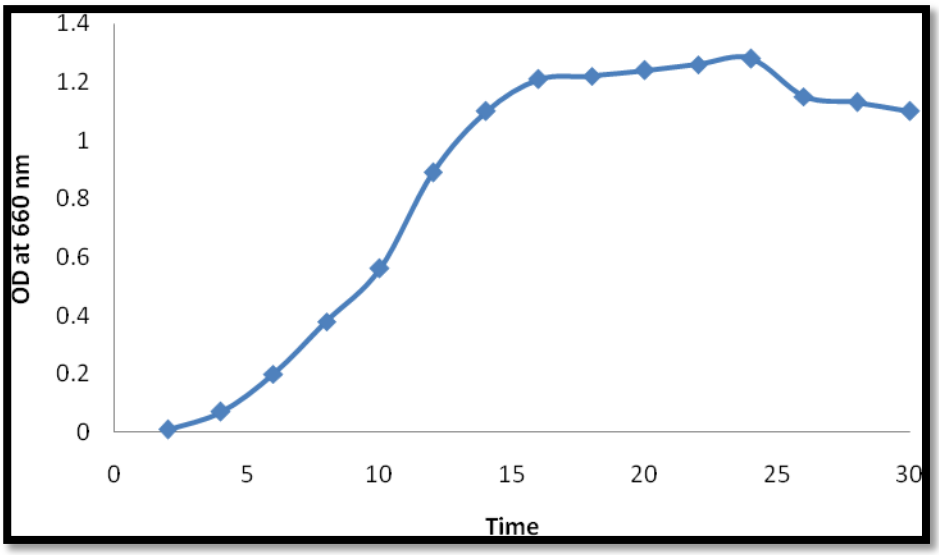

FIG. 1: GROWTH CURVE OF *BACILLUS CEREUS*

CONCLUSION

The investigation's major goal was to isolate a bacterial strain from soil and produce large levels of xylanase that would meet industry-friendly settings such as a broad operational pH and temperature range. The employment of enzyme in diverse industrial strategies to overcome the economic and environmental disadvantages of traditional approaches necessitated the consideration of industry-specific variables.

The investigation's findings and results are summarized below:

Soil samples were taken from various locations for the isolation of xylanase-producing bacteria, including the region of the Yamunanagar paper and pulp plant, the National Forest of Kalesar, and agricultural land in the surrounding area. When cultivated in growth media, the isolated strain produced high levels of xylanase free of cellulosic material. The bacterium was identified as Bacillus cereus during the research using several physiological and biochemical tests. The culture was gram-positive and thermophile, with growth temperatures of 37, 45, and 65 degrees Celsius, respectively. The strain could also grow up to pH11.0, and isolated xylanase was found to be active even at pH10.

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