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OPTIMIZATION STUDIES ON THE PRODUCTION AND ACTIVITY OF XYLANASE OBTAINED FROM PAENIBACILLUS LAUTUS ISOLATED FROM MANGROVE SOIL

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ABSTRACT

Xylanases (endo-1,4-xylanase, EC 3.2.1.8) are hydrolytic enzymes which cleaves the β-1,4- backbone of the complex plant cell wall polysaccharide, xylan which in turn releases xylooligosaccharides and xylose. The screening for xylanase producing bacteria from mangrove soil was carried out on M-9 medium containing 0.5% xylan followed by congo red test using the same medium. The xylanase production was detected by formation of zone of hydrolysis by the xylanase producing organism. Three xylanase producing isolates were obtained. The maximum xylanase producing bacterium was identified as *Paenibacillus lautus* on the basis of morphological, cultural, biochemical and 16S rRNA sequence analysis. Maximum production of xylanase was obtained by the isolate in a medium inoculated with 5% (v/v) of inoculum adjusted to 0.20 O.D. at 540nm containing peptone (10g/L), meat extract (3g/L), NaCl (5g/L) and beechwood xylan (5g/L), pH 7 incubated on a shaker (120 rpm) at 40°C for 48hrs. Maximum xylanase activity was observed at 50°C and pH 7. Metal ions like Ca^{+2}, Mg^{+2}, Mn^{+2}, Cu^{+2}, Fe^{+2} and Zn^{+2} were observed to inhibit enzyme activity. Inhibitors such as SDS, β- mercaptoethanol, Tween 80 and EDTA were seen to inhibit the enzyme activity. The potential action of xylanase as detergent additive was also studied.

KEYWORDS: Xylanase; *Paenibacillus lautus;* mangrove soil; optimization.

INTRODUCTION

Three major components of wood are cellulose (35-50%), hemicellulose (20-30%) and lignin (20-30%). Hemicellulose consists of a group of carbohydrates in which xylan is the second most abundant polysaccharide in nature after cellulose,[1] forms the major class. Xylan is a heteropolysaccharide with a homopolymeric backbone containing β-1,4- linked xylopyranose units. Depending on the source, the xylan backbone may be substituted to varying degrees with glucuronosyl, 4-O-methyl-D glucuronopyranosyl, α-1-arabinofuranosyl, or acetyl residues. However, unsubstituted linear xylans have been isolated from guar seed husk, esparto grass and tobacco stalks.[2] Wood xylan exsists as O-acetyl-4-O-methylglucuronoxylans in hardwoods or as arabino-4-O-methylglucuronoxylans in softwoods. The degree of polymerization of hardwoods is higher than that of softwoods.[3] The cereal xylans are made up of D-glucuronic acid and its 4-O-methyl ether and arabinose.[4]

The complete enzymatic hydrolysis of xylan into its constituent monosaccharides requires synergistic action of a group of xylanolytic enzymes. This is due to the fact that xylans from different sources exhibit a significant variation in composition and structure. The most important enzyme is endo-1, 4-xylanase (EC 3.2.1.8) that initiates the conversion of xylan into xylooligosaccharides. Xylosidase, debranching enzymes (L-arabinoforanosidase and glucuronidase) and esterases (acetyl xylan esterase, feruloyl esterase) allow the complete degradation of the xylooligosaccharides to their monomeric constituents.[5] Microbial xylanases are the preferred catalysts for xylan hydrolysis due to their high specificity, mild reaction conditions, negligible substrate loss and side product generation.[6]

Xylanases have been isolated from a number of micro-organisms such as bacteria, fungi, actinomycetes, yeast, as well as plants, animals etc. However, microorganisms are the major source of such enzymes. Some important microorganisms producing xylanase enzyme are *Paenibacillus curdlanolyticus* B6,[7] *Paenibacillus macquariensis,*[8] *Paenibacillus sp.* XJ18,[9] *Paenibacillus sp.* ASCD2,[10] *Paenibacillus campinasensis* BL 11,[11] *Bacillus circulans* D1,[12] *Bacillus* amyloliquefaciens,[13] *Thermomyces lanuginosus.*[14] *Streptomyces violaceoruber, Aspergillus niger.*[15] *Penicillium* oxalicum.[16]

Xylanase is an industrially important enzyme responsible for the breakdown of xylan (a major component of plant hemicelluloses) has gained importance in different areas such as paper and pulp industry, food and feed industry, chemical industry, waste treatment and production of fermentable substrate, textile industry and detergent industry. Currently, xylanases and the microorganisms that produce them are currently used in the management of waste, to degrade xylan to renewable fuels and chemicals, in addition to their use in food, agro-fiber, and the paper and pulp industries, where the enzymes help to reduce their environmental impact.[17] Oligosaccharides produced by the action of xylanases are further used as functional food additives or alternative sweeteners with beneficial properties.[18]

The current study focuses on isolation and identification of xylanase producing isolate obtained from mangrove soil. It further discusses the optimization of xylanase production. It also reports partial purification, characterization and application as detergent additive of xylanase enzyme.

MATERIALS AND METHODS

Enrichment, isolation and screening and identification

Soil samples obtained from mangrove region were used for the study. The soil samples were collected in sterile flasks. 1 gram of soil sample was enriched in 100 ml liquid M-9 medium with 0.5% xylan for isolating the xylanase producing microorganisms present in them.[19] The flasks were incubated at 30°C under shaker conditions at 120 rpm for 48hrs.

After enrichment in M-9 medium with 0.5% xylan, the microorganisms in broth were isolated on sterile M-9 agar plates with 0.5% xylan and the plates were incubated at 30°C for 48 hrs. Colonies that appeared on M-9 medium agar plates with 0.5% xylan were spot inoculated on M-9 medium agar plates with 0.5% xylan.[19] The plates were incubated at 30°C for 48 hours. Xylanase activity was detected using congo red method.[20] 0.1 % Congo red solution was added for 5mins to the agarified medium after incubation, followed by washing the plates with 1M NaCl solution and plates were incubated for 15mins. Cultures showing yellow colour zone around the colonies indicated hydrolysis of xylan and were selected as xylanase producers.

The promising isolate was identified using morphological, cultural, biochemical and 16S rRNA sequence analysis (Yaazh Xenomics, Mumbai).

Enzyme assay to detect xylanase activity

Xylanase activity was assayed by DNSA method.[21] The culture grown in M-9 medium with 0.5% xylan broth was centrifuged at 10,000 rpm at 4ºC for 10 mins. The clear supernatant was used as crude enzyme for enzyme assay. 1.8 ml of beechwood xylan dissolved in 50mM sodium phosphate buffer (pH 7) was mixed with 0.2 ml of crude xylanase enzyme incubated at 50ºC for 5 mins. After incubation 3 ml of DNSA was added, mixed and kept on a boiling water bath for 5 mins. The tubes were cooled in cold water and the absorbance was recorded at 540 nm.[21,22] Reaction mixture containing heat-inactivated enzyme was used as a Blank. The absorbance of the test supernatant against the blank was obtained and plotted on the standard graph of Xylose (200 - 1000 mcg/ml) to obtain the amount of product formed.

One unit (IU) of xylanase activity is defined as the amount of enzyme that catalyzes the release of 1µmol of reducing sugar as xylose equivalent per minute under the specified assay conditions.

Enzyme assay to detect cellulase activity

Cellulase activity was assayed by DNSA method.[21] The fermented broth was centrifuged at 10,000 rpm at 4ºC for 10 mins. The clear supernatant was used as crude enzyme for enzyme assay. 1.0 ml of CMC dissolved in 50mM sodium phosphate buffer (pH 7) was mixed with 0.5 ml of crude enzyme incubated at 50ºC for 15 mins. After incubation 3 ml of DNSA was added, mixed and kept on a boiling water bath for 5 mins. The tubes were cooled in cold water and the absorbance was recorded at 540 nm.[21,23] Reaction mixture containing heat-inactivated enzyme was used as a Blank. The absorbance of the test supernatant against the blank was obtained and plotted on the standard graph of Glucose (200 - 1000 mcg/ml) to obtain the amount of product formed.

Optimization of culture conditions for maximum xylanase production

For enhancing the xylanase yield, the enzyme production was optimized with respect to various parameters using one variable at-a-time approach. In each case, enzyme was produced at different values of the respective parameters followed by extraction of xylanase from the culture filtrate by centrifugation at 10,000 rpm for 10 minutes at 4°C.[24] The enzyme assay was carried out using DNSA method.[21,22] The experiments were carried out in triplicates The range of different parameters used for their optimization were.

Media screening: The yield of xylanase was studied using four different media. The media used were: nutrient broth + xylan containing 10g/L peptone, 3g/L meat extract, 5g/L NaCl and 5g/L beechwood xylan,[24] Horikoshi basal medium containing 5g/L beechwood xylan, 5g/L peptone, 5g/L yeast extract, 1g/L K_2HPO_4 and 0.2g/L $MgSO_4.7H_2O$.[25] M-9 medium + xylan containing 6g/L Na_2HPO_4, 3g/L KH_2PO_4, 5g/L NaCl, 1g/L NH_4Cl and 5g/L beechwood xylan [19]; Xylan broth containing 5g/L peptone, 2g/L yeast extract, 0.5g/L $MgSO_4.7H_2O$, 0.5g/L NaCl, 0.1g/L $CaCl_2.2H_2O$ and 5g/L beechwood xylan.[26]

Determination of incubation period for maximum xylanase production: The time required for xylanase production was studied over 24, 48, 72, 96, 120, 144 and

168hours. The incubation period giving maximum xylanase yield was selected for further studies.[27]

Effect of aeration on xylanase production: The effect of aeration on xylanase production was studied by incubating inoculated flasks, one under static condition and the other flask under shaker conditions at 30°C for 48hrs respectively.[28]

Effect of variable temperature on xylanase production: Optimization of temperature of incubation for xylanase production was carried out using different temperatures. The temperatures used for the study were 30°C, 35°C, 40°C, 45°C and 50°C.[29]

Effect of variable pH on xylanase production: Optimization of pH of the production medium for xylanase production was performed. The range of pH used for the study was 3-11. The pH of the production medium was adjusted using 1N HCl (3-7) and 1N NaOH (9-11).[29]

Effect of inoculum size on xylanase production: The effect of inoculum size on the production of xylanase was studied by using different concentrations such as 5%, 10%, 15%, 20% and 25% (v/v) of culture at O.D. 0.20 at 540nm.[28]

Effect of different carbon sources (carbohydrates or sugars) on xylanase production: The effect of different carbon sources on xylanase production was studied by replacing 0.5% xylan in the production medium with 0.5% of different sugars. The flasks were incubated at 40°C for 48 hours under shaker conditions.[29] 0.5% each of xylan, xylose, dextrose, maltose, sucrose, lactose and CMC were used as carbon sources for the xylanase production.

Effect of different nitrogen sources on xylanase production: The effect of nitrogen source on xylanase production was studied by replacing 1% peptone in the production medium with 1% of different organic and inorganic nitrogen sources. Similarly, 0.3% of meat extract in the medium was replaced by 0.3% of various nitrogen sources and its effect was checked.[29] The different nitrogen sources used were peptone, meat extract, soyameal, yeast extract, urea, $NaNO_3$ and $(NH_4)_2SO_4$.

Optimization of xylanase activity.

The characterization of xylanase activity was carried out based on stepwise modification of the parameters. For each experiment of optimization, the crude enzyme extract was incubated with pure xylan, after adjusting the parameters. The enzyme activity was detected by DNSA method.[21,22] The experiments were carried out in triplicates. The following parameter was studied for obtaining maximum activity of the enzyme.

Optimization of temperature for xylanase activity: Optimization of temperature for xylanase activity was done for different temperatures. The different temperatures used were 30°C, 40°C, 50°C, 60°C, 70°C, 80°C and 90°C. The assay was carried out by incubating enzyme-substrate mixtures at different temperatures. The activity was then determined.[30]

Optimization of pH for xylanase activity: The effect of pH for xylanase activity was determined by incubating crude enzyme and buffers, adjusted to pH 4.0-11.0, with 1% xylan at optimum temperature. The activity was then determined. The following buffer systems was used for obtaining a range of pH: sodium-citrate buffer (pH 4.0-6.0), sodium-phosphate buffer (pH 7.0-8.0) and glycine-NaOH buffer (pH 9.0-11.0).[30]

Effect of metal ion concentration on xylanase activity: The effect of various metal ions such as Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} on xylanase activity was investigated. The metal ions used were in the form of $CaCl_2$, $MgSO_4$, $FeSO_4$, $CuSO_4$, $MnSO_4$ and $ZnCl_2$.[30] The crude enzyme was pre-incubated with 1mM solutions of the above mentioned metal ions for 1 hour at 30°C. The residual activity (%) was measured by standard xylanase assay.

Effect of inhibitors and activators other than metal ions: The effect of inhibitors and activators other than metal ions such as β-meraptoethanol, EDTA and Tween 80 on xylanase activity was investigated.[31]

The crude enzyme was pre-incubated with 1% solutions of the above mentioned compounds for 1 hour at 30°C. The residual activity (%) was measured by standard xylanase assay.

Potential application of crude xylanase enzyme as a detergent additive

The application of xylanase as a detergent was evaluated on pieces of white cloth (8cm × 8cm) stained with orange juice. The stained cloth pieces were dried overnight. The stained cloth was treated with 50 ml xylanase enzyme in a flask. Simultaneously, a second control flask was maintained in which 50 ml distilled water was added and treated identically. A third flask containing the 50 ml xylanase enzyme along with components of detergent (sodium silicate salt-5.7%, alkyl benzene sulphonate-14.5%, sodium carbonate-12.4%, sodium sulphate-26.8%) except enzymes was also maintained and treated. The flasks were swirled gently to stimulate the agitating washing motion at 120rpm for 30 minutes to 1 hour. The treated and untreated samples were compared visually, to evaluate the efficiency of the enzyme treatments as detergent additive.[32]

RESULT AND DISCUSSION

Sample collection, enrichment, isolation and identification

In the present study, soil samples obtained from mangroves were analyzed for xylanolytic organisms. The environmental samples were tested because organisms are present in these samples, which in order to survive and compete with other organisms in nature produce enzymes like xylanases. Xylanolytic organisms have also been isolated from decaying wood materials, sea sands, stagnant water, field soil, garden soil, soil near hot springs, compost, agricultural wastes and sea water.[33,34,35,36,26] Mohammed (2013) isolated *Tricoderma sp*. and *Aspergillus sp.* from saw dust, soil and wood bark.[37]

The samples were enriched in M-9 medium with 0.5% xylan. The growth obtained in the enriched broth were isolated on M-9 agar plates with 0.5% xylan. Six organisms were isolated from the enriched media. The isolates obtained were maintained on nutrient agar slants. The six cultures obtained from broth were spot inoculated on M-9 medium agar plates with 0.5% xylan and incubated at 30°C for 48 hours.[19] The isolates were subjected to congo red test.[20] Basal medium like M-9 medim with 0.5% xylan have been routinely used to screen for xylanase producers.[36,38,19,39] Other medium like nutrient agar with xylan,[24,26] wheat bran agar[26] and xylan agar[26] are also used for screening of xylanase producing microorganisms using congo red test method. Out of six isolates, three cultures showed xylanase production on M-9 agar plates with xylan, characterized by a zone of hydrolysis around the spot inoculated culture. The xylanase activity of the three isolates obtained was measured quantitatively by DNSA method. The isolate giving maximum xylanase activity was used for further optimization study.

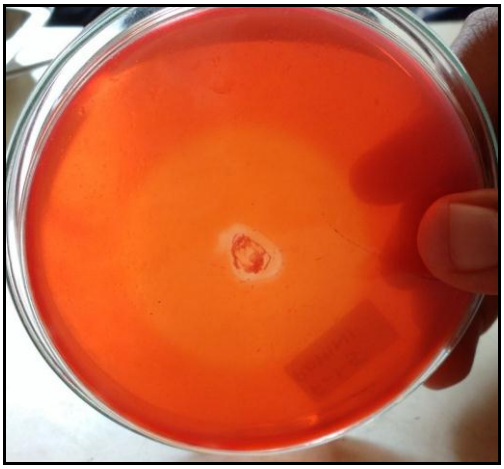

Figure 1: Zone of hydrolysis on M-9 medium with 0.5% xylan.

Xylanase production by each isolate was tested colorimetrically, using beechwood xylan as the substrate.[21,22] The maximum xylanase production was shown by the isolate F-1-5 as shown in Figure 2.

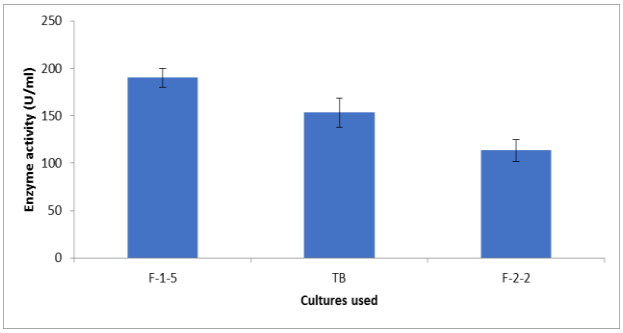

Figure 2: Xylanase activity of different isolates.

Isolates showing minimal cellulase activity or no cellulase activity should be selected for further study. In the present study, the isolate F-1-5 gave minimum cellulase activity as shown in Figure 3. Haltrich *et al.* (1996) reported that xylanases production most of the time was associated with the production of enzyme cellulose.[40] Many industries like paper and pulp industry require cellulase free xylanase enzyme. Cellulase free xylanase will produce superior quality of pulp to be used in paper and pulp industries with minimum damage to the cellulosic components of the pulp.[41] Thus, it has been stated by many authors that cellulase free xylanase producers should be used in these industries.

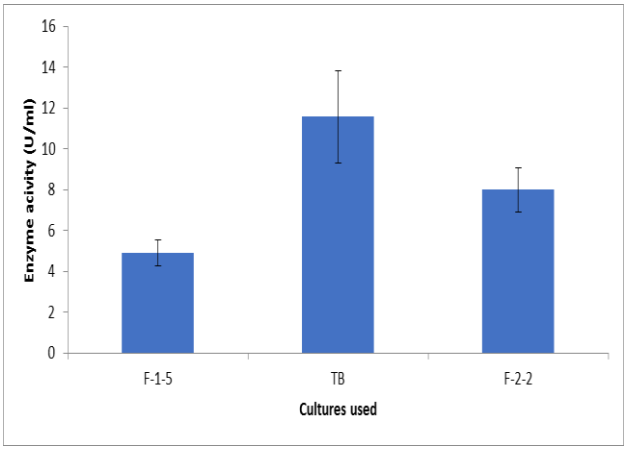

Figure 3: Cellulase activity of different isolates.

The isolate F-1-5 from mangrove soil sample showed maximum xylanase production. Therefore, F-1-5 was used for further studies. The isolate F-1-5 was identified as Paenibacillus lautus by 16S rRNA gene sequencing analysis. Many strains of Paenibacillus have been previously reported to produce extracellular xylanases; for example: Paenibacillus macquariensis,[8] Paenibacillus sp. XJ18,[9] Paenibacillus sp. ASCD2,[10] Paenibacillus campinasensis BL11,[11] Paenibacillus polymyxa ZJ-9,[42] Paenibacillus curdlanolyticus B6,[7] Paenibacillus sp. NF1.[43] Other xylanase producing organisms are Bacillus circulans D1 [12], Bacillus amyloliquefaciens,[13] Thermomyces lanuginosus,[14] Streptomyces violaceoruber.[44] Aspergillus niger,[15] Penicillium oxalicum.[16]

Effect of different media on xylanase production by *Paenibacillus lautus*

The production of xylananse was analysed in different media. The media used were nutrient broth with xylan, M-9 liquid media with xylan, Horikoshi basal medium (HBM) and xylan broth (XB). Maximum xylanase production was found to occur in nutrient broth with xylan as shown in Figure 4. Therefore, nutrient broth with xylan was used for further optimization studies. Many authors have reported the use of minimal salt medium containing xylan for the production of xylanase enzyme.[45] Nagar *et* al., (2012) used nutrient broth, wheat bran broth and xylan broth for production of xylanase enzyme.[26] The wheat bran broth gave the highest production of the enzyme as compared to the other two media used. Thus, Nagar *et* al. (2012) used wheat bran broth for further production of xylanase enzyme.[26] Ghoshal *et al*. (2014) used minimal salt medium for Laboratory scale production of xylanase from *Penicillium citrinum.*[46] Rawashdeh *et al*. (2005) also used a minimal salt medium for the production of xylanase enzyme from *Streptomyces sp.* (Strain lb 24D).[47] Rathee *et al*. (2014) obtained xylanase enzyme from *Bacillus tequilensis* using Horikoshi Basal medium.[25]

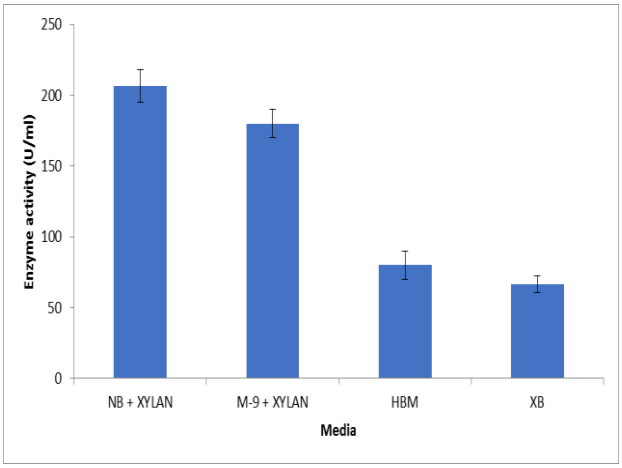

Figure 4: Effect of different media on xylanase production by *Paenibacillus lautus.*

Effect of incubation period on xylanase production by *Paenibacillus lautus*

The production of xylanase was analyzed at different time intervals for 7 days at an interval of 24 hours. Paenibacillus lautus showed xylanase activity starting from 24hrs of growth and reached maximum in 48 hrs and then it declined with further increase in duration of incubation as shown in Figure 5. Paenibacillus lautus produced xylanase in late log phase. Xylanase are released throughout bacterial growth, while peaking by the late log phase.[8] Similar, results were found in the case of Paenibacillus macquariensis, Paenibacillus sp. XJ18 and Bacillus subtilis.[8,9,48] Gaur et al. (2015) also found similar results for Bacillus vallismortis RSPP-15.[49] However, Paenibacillus sp. ASCD2 and Bacillus megaterium showed maximum xylanase activity after an incubation period of 72 hours after which the activity of xylanase enzyme decreased.[10,48] Nagar et al. (2010) and Kamble and Jadhav (2012) reported that the highest enzyme titer from another Bacillus spp. was recorded at 48 hrs and 72 hrs respectively.[50,51] In contrast to these results, Kumar et al. (2013) reported that xylanase production by Bacillus pumilus VLK-1 was maximum in 96 hrs, after which a gradual decrease was observed.[52] It may be due to denaturation or decomposition of xylanase owing to interaction with other components in the medium, as it is reported elsewhere.[51] Incubation time depends on the characteristics of the culture, growth rate, and enzyme production.[51]

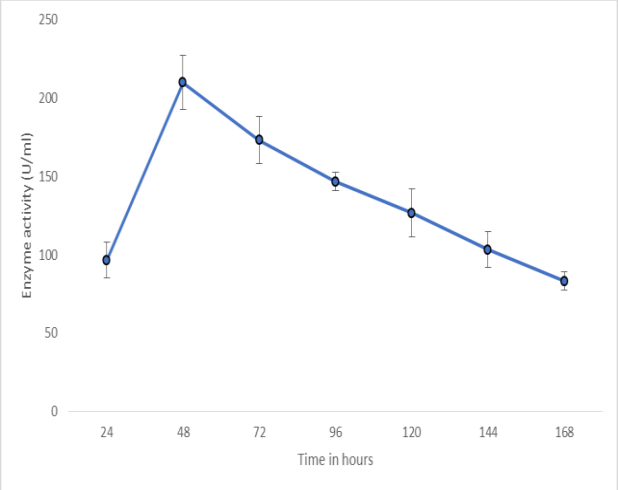

Figure 5: Effect of incubation period on xylanase production by *Paenibacillus lautus*.

Effect of aeration on xylanase production by *Paenibacillus lautus*

Different types of physicochemical conditions could affect the production of xylanase enzyme. Shaker conditions are usually used for the production of extracellular enzymes by aerobic organisms, as it enhances the aeration rate. In our study, aeration has been found essential for xylanase production as shown in Figure 6. Agitation at 120 rpm was carried out to provide aeration. Aeration in culture medium could lead to sufficient supply of dissolved oxygen in the production medium. Xylanase production from *Paenibacillus macquariensis* and *Paenibacillus campinasensis* BL11 was carried out under shaker condition at 120 rpm.[8,11] Similar results were found by Kuhad *et al*. (2009) for *Bacillus subtilis* in which xylanase production was higher under shaking as compared to stationary conditions and increased with agitation rate, reaching a maximum at 200 rpm.[53] The lower enzyme level under low agitation conditions may be attributed to the dissolved oxygen limitation for cell growth, improper mixing of media components and cell clumping. In contrast, xylanase produced by *Aureobasidium pullulans* showed that the optimal agitation rate for the extracellular xylanase activity was 90 rpm rather than 180 rpm, and the amount of extracellular protein apparently increased at this agitation speed.[54] This was

probably because of the deleterious effect of high shear stress on the organism that was due to the higher sheering force, which caused cell lysis and release of proteases that can inhibit xylanase production.[55]

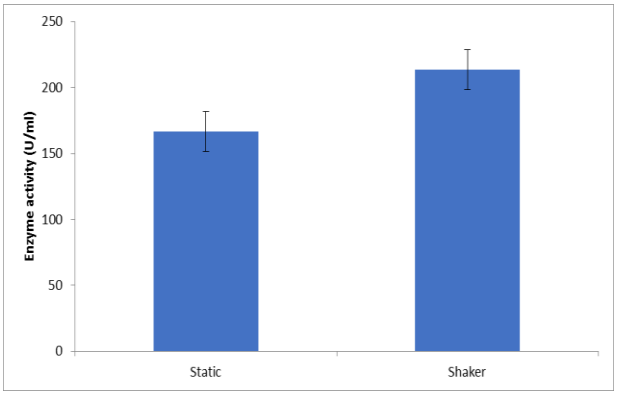

Figure 6: Effect of aeration on xylanase production by *Paenibacillus lautus*.

Effect of variable temperature on xylanase production by *Paenibacillus lautus*

The xylanase production was analyzed at different temperatures ranging from 30-50°C. The xylanase production by *Paenibacillus lautus* was optimum at 40°C and decreased with higher temperature due to reduced growth rate or lower survival as shown in Figure 7. The optimum growth temperature for *Paenibacillus lautus* is 28-30°C, the maximum temperature is 45-50°C and the minimum temperature is 5-10°C.[56] Similar results were obtained for *Bacillus megaterium.*[48] However, in case of *Paenibacillus sp. ASCD2*, *Paenibacillus macquariensis*, *Bacillus halodurans* PPKS-2 and *Thermomyces lanuginosus* maximum xylanase production was observed at 50°C.[8,10,29,57] Irfan *et al*. (2016) obtained a temperature of 35°C for xylanase production in case of *Bacillus subtilis.*[48] The optimum temperature for xylanase production corresponds with the growth temperature of the respective microorganisms. It has been observed that, in general, xylanases are produced in the temperature range of 30°C-75°C.[34]

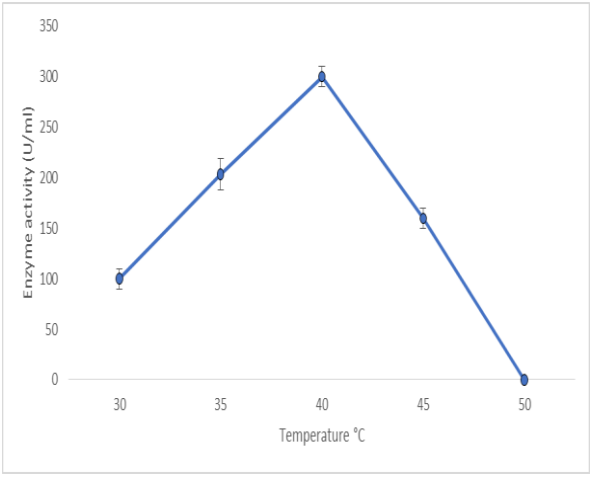

Figure 7: Effect of variable temperature on xylanase production by *Paenibacillus lautus*.

Effect of variable pH on xylanase production by *Paenibacillus lautus*

Xylanase production was tested when the organism was cultured at different pH ranging from 3-11. Paenibacillus lautus grown at pH 7 resulted in maximum xylanase production (Figure 8). Largely, microorganisms prefer neutral pH for best growth and xylanase production,[31] such as Bacillus sp., Penicillium canescens, Streptomyces sp. and Paecilomyces themophila J18.[58] Xylanase production was maximum at pH 7 by Paenibacillus sp. ASCD2.[10] and Paenibacillus campinasensis BL11.[11] These observations were probably seen due to the effect of pH on growth of organism. Irfan et al. (2016) found that the optimum pH for xylanase production is 8.0 for Bacillus subtilis and Bacillus megaterium.[48] The initial pH of the medium may influence many enzymatic systems and the transport of enzymes across the cell membrane. Maximum xylanase activity at higher pH (more than 7.0) has been observed in many cases such as Bacillus sp., Geobacillus sp.[59,31,12]

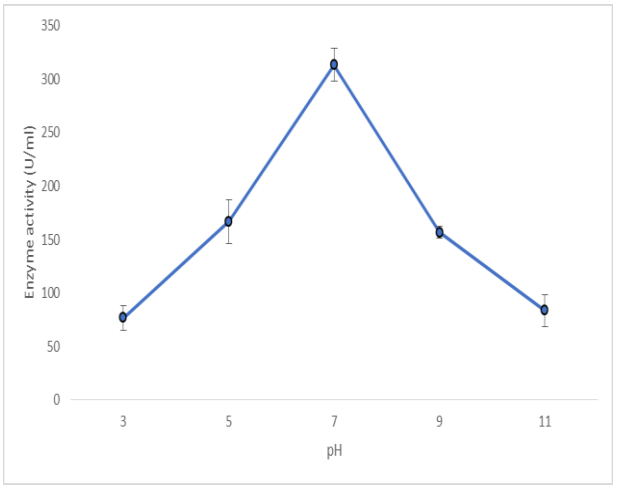

Figure 8: Effect of variable pH on xylanase production by *Paenibacillus lautus*.

Effect of inoculum size on xylanase production by *Paenibacillus lautus*

The xylanase production by *Paenibacillus lautus* was maximum with an inoculum size of 5% at O.D. 0.20 at 540nm (v/v) as shown in Figure 9. The xylanase production decreased with further increase in inoculum size, which caused a decline in the enzyme activity. An optimal inoculum level is necessary for maintaining the balance between proliferating biomass and available nutrients to obtain maximum enzyme yield. A lower enzyme yield at higher inoculum level could result from faster consumption of nutrients. Further, a large inoculum size could lead to formation of thick suspension and hence improper mixing of substrates.[60] It has been suggested that a high level of inoculum is not favourable for its application in industry.[57] Most researchers have reported the use of 1.0-2.0% (v/v) inoculum for hyper production of xylanase in SmF by many organisms like *Bacillus sp., Geobacillus sp., Aspergillus sp.*[26,50,61,62] Irfan *et al*. (2016) found that the

xylanase production was maximum with an inoculum size of 2% and 1.5% for *Bacillus subtilis* and *Bacillus megaterium* respectively.[48]

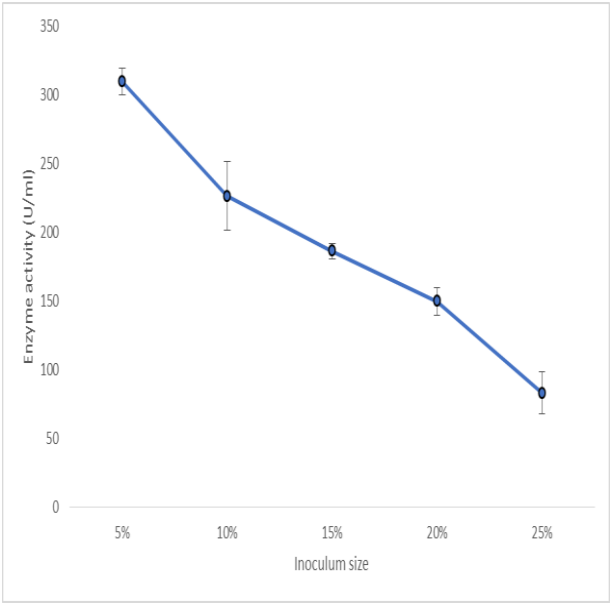

Figure 9: Effect of inoculum size on xylanase production by *Paenibacillus lautus*.

Effect of different carbon sources on xylanase production by *Paenibacillus lautus*

The present study was aimed at optimization of media components which have been predicted to play a significant role in enhancing xylanase production. Different carbon sources have different impacts on xylanse production. In our studies with *Paenibacillus lautus*, highest xylanase activity was observed when 0.5% beechwood xylan was used as a carbon source followed by dextrose, xylose, lactose and maltose whereas sucrose and CMC inhibited xylanase production as shown in Figure 10. On the contrary, sucrose and CMC did not inhibit xylanase production in *Bacillus subtilis* and *Bacillus megaterium*.[48] The xylanase production was maximum when sucrose was used as a carbon source for *Bacillus subtilis* and xylose gave the maximum xylanase production in case of *Bacillus megaterium*.[48] However, maximum xylanase production was observed in case of *Paenibacillus macquariensis* when 2% beechwood xylan was used.[8] Xylanases are also produced using agro residues. *Paenibacillus sp. ASCD2*, *Paenibacillus campinasensis BL11* and *Paenibacillus sp.* XJ 18 produced maximum xylanase using corncobs and rice husk respectively.[9,10,11] Xylan has been reported to have similar effects on the production of xylanase by *Bacillus halodurans* PPKS-2,[29] and *Aspergillus niger*.[15] Xylanase production is significantly influenced by other carbon sources, such as sugars, sugar alcohols, polysaccharides and other complex substances.[47,12,19,63] In contrast to these results, Seyis and Aksoz found that maximum xylanase was produced when sucrose was used as carbon source for *Trichoderma harzianum* 1073 D3.[64]

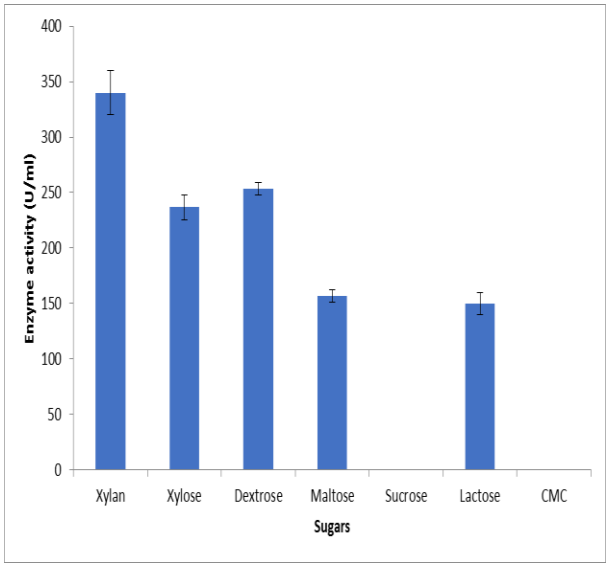

Figure 10: Effect of different carbon sources on xylanase production by *Paenibacillus lautus*.

Effect of different nitrogen sources on xylanase production by *Paenibacillus lautus*

Besides the carbon source, the type of nitrogen source in the medium also influences the xylanase yield in the production broth. The present finding indicated that the supply of organic nitrogen sources like peptone and meat extract resulted in high xylanase production (Figure 11). Similar results were obtained for *Bacillus subtilis* and *Bacillus megaterium*.[48] Generally, microorganisms provide high yield of xylanase when organic nitrogen sources are used, such as yeast extract, peptone or tryptone as in the cases of *Paenibacillus macquariensis*, *Paenibacillus* sp. XJ18**,** *Paenibacillus sp. ASCD2*, *Paenibacillus campinasensis BL11*, *Aspergillus sp.*, *Streptomyces sp.*, *Bacillus sp.*[29,8,9,11,62,64,65,66] However, Ghasemi *et al.* (2014) obtained maximum xylanase activity by *Sphingobacterium sp.* SaH-05 when ammonium sulfate was used as nitrogen source[67]. However, inorganic nitrogen showed stimulatory effect for xylanase production by *Aspergillus niger* and *Trichoderma harzianum* 1073 D3.[68,64] There were other reports that feather hydrolysate supported maximum xylanase production for *Bacillus halodurans* PPKS-2 followed by peptone, casein, beef extract and gelatin.[29]

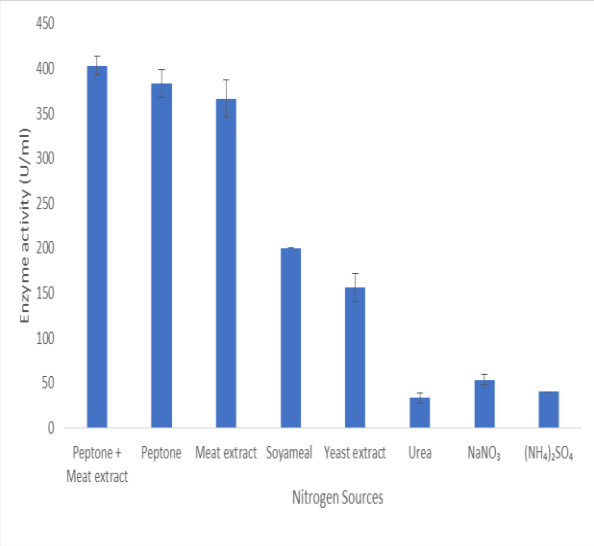

Figure 11: Effect of different nitrogen sources on xylanase production by *Paenibacillus lautus*.

Thus, *Paenibacillus lautus* isolated from mangrove soil sample was shown to be a good xylanase producer. It was grown in nutrient broth with 0.5 % xylan medium containing peptone (1%), meat extract (0.3%), sodium chloride (0.5%) and xylan (0.5%), pH 7 incubated on a shaker (120 rpm) at 40°C for 48h to obtain maximum xylanase yield. The isolate *Paenibacillus lautus* was grown under optimal condition as described above. The extracellular xylanase was then standardized for maximum activity.

Effect of temperature on enzyme activity

In the present study, the optimum temperature for enzyme activity was determined and the enzyme showed maximum activity at 50°C (Figure 12). Similar, results were obtained for *Paenibacillus macquariensi, Bacillus sp.* KS09, *Bacillus subtilis, Bacillus megaterium* and *Furasium heterosporum*.[24,69,48,70] However, *Paenibacillus sp.* ASCD2 showed maximum enzyme activity at 60°C and *Paenibacillus* sp. XJ18 showed maximum enzyme activity at 90°C.[9,10] Xylanase produced by *Bacillus sp.* NG-27 showed highest activity at 90°C. Bacterial xylanase has temperature optima in the range of 20-90°C.[71,38,29,15]

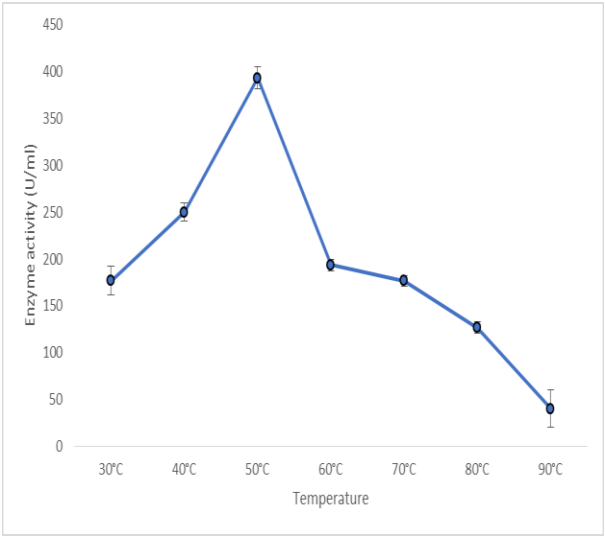

Figure 12: Effect of temperature on enzyme activity.

Effect of pH on enzyme activity

The optimum pH for the enzyme activity was evaluated and it was found that the enzyme showed maximum xylanase activity at pH 7 (Figure 13). However, the enzyme was active at a pH range of 4 – 11. The pH optimum of xylanase purified from different bacterial strains has been reported to vary. The optimum pH observed in this study was similar with the results obtained for *Paenibaillus sp.* ASCD2, *Bacillus* sp. KS09, *Cellulosimicrobium* sp. MTCC 10645 and *Bacillus vallismortis* RSPP-15.[24,10,49,51] In a study carried out for determining the optimum pH for xylanase activity for *Paenibacillus macquariensis* it was found to be 8.6 [8]. The optimum pH of the purified xylanase from *Paenibacillus sp.* XJ18 and *Fusarium heterosporum* was 5.0, *Bacillus subtilis* was found to be 4.5 and *Bacillus megaterium* was 5.5. The enzymes were active within the acidic pH range of 4.5 to 5.5.[9,66,48] Some xylanases exhibited optimum pH in the alkaline range (pH 8-10) in case of *Bacillus sp., Geobacillus sp., Penicillium oxalicum*.[31,35,16]

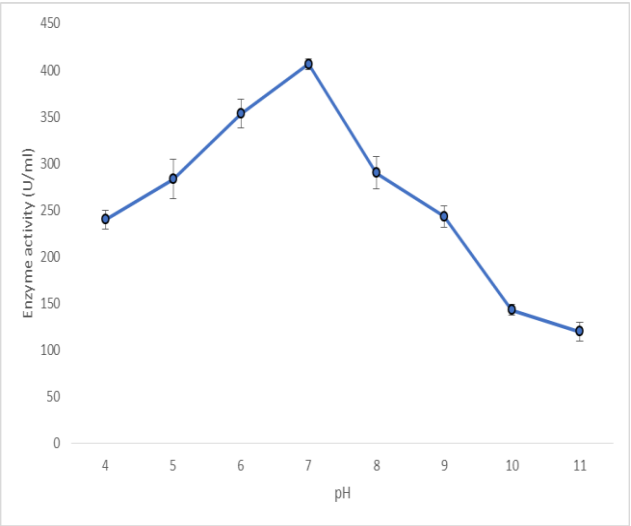

Figure 13: Effect of pH on enzyme activity.

Effect of metal ions on enzyme activity

The effect of some metal ions on enzyme activity were assessed by using various metal ions in1mM concentration. It was seen that metal ions decreased the activity of xylanase as shown in Figure 14. In the presence of Fe^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, Ca^{2+} and Mg^{2+}, 73.77%, 45.08%, 54.10%, 73.77%, 72.95% and 59.01% residual activity was detected respectively. Similar, results were obtained by for *Bacillus sp.* KS09 and *Bacillus halodurans* PPKS-2.[24,29] In contrast to these results, xylanase obtained from *Paenibacillus sp.* ASCD2 showed stimulatory effect to Fe^{2+} and Mg^{2+}.[10] The inhibition by metal ions may occur due to complex formation with reactive groups of the enzyme, for example, metals of group IIb exhibit high affinity for SH, $CONH_2$, NH_2, COOH, PO_4, and this effect is similar to that of mercaptides.[72] In contrast to these results, Heinen *et al.* (2014) reported that metallic ions influenced the xylanase activity of *Fusarium heterosporum*. Ca^{2+}, Ba^{2+} and Mg^{2+} (1 mM) increased the enzyme activity by 20, 28 and 38%, respectively.[70] However, Zn^{2+}, Pb^{2+} and Hg^{2+} were the most effective inhibitors.[70] Irfan and Syed (2012) found that xylanases obtained from *Trichoderme viride* showed stimulatory effect with metal ions.[73]

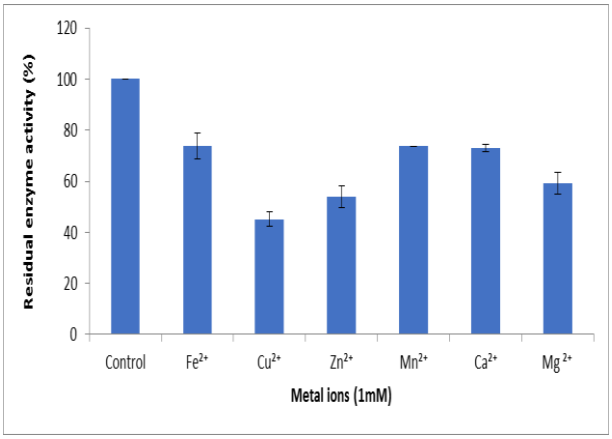

Figure 14: Effect of metal ions on enzyme activity.

Effect of activators and inhibitors on enzyme activity

The effect of activators and inhibitors other than metal ions on enzyme activity were assessed by using various compounds like Tween-80, β-mercaptoethanol, SDS and EDTA in 1% concentration. It was seen that the activity of xylanases got inhibited by these compounds (Figure 15). However, only a slight inhibition was observed in the case of β-mercaptoethanol. It was reported that EDTA inhibited xylanase activity in case of xylanase obtained from *Bacillus sp.* KS09, *Bacillus halodurans* PPKS-2, *Fusarium heterosporum* and *Trichoderma viride*.[24,29,70,73] However, it was found that generally β-mercaptoethanol has a stimulatory effect on xylanase activity obtained from *Fusarium heterosporum*.[70]

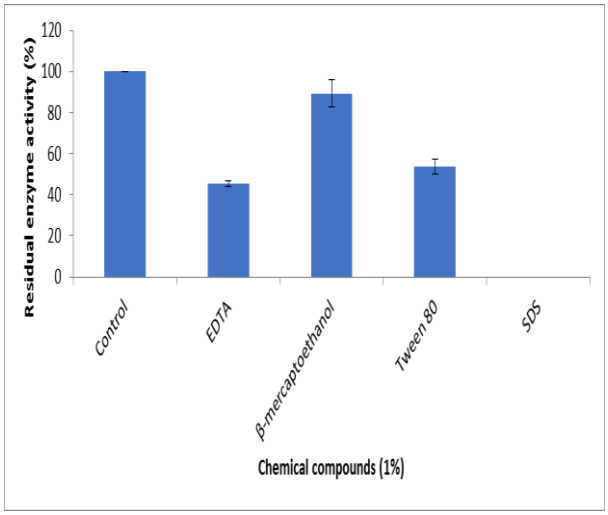

Figure 15: Effect of activators and inhibitors on enzyme activity.

Effect of xylanase enzyme as detergent additive

The application of crude xylanase enzyme as a detergent additive was evaluated in our present study. The results showed that the enzyme xylanase have cleansing action when the crude enzyme treated cloth was compared with the stained cloth and the control cloth treated with distilled water (Figure 16a, 16b and 16c). The cleansing potential of the enzyme was further enhanced when the crude enzyme solution was supplemented with other commonly used detergent ingredients (Figure 16d). Thus, it can be used as a potential additive in detergent industries. Similar, results were obtained by Kumar *et al.* (2004) for alkaline xylanases obtained from an alkaliphilic *Bacillus* NCL (87-6-10).[32]

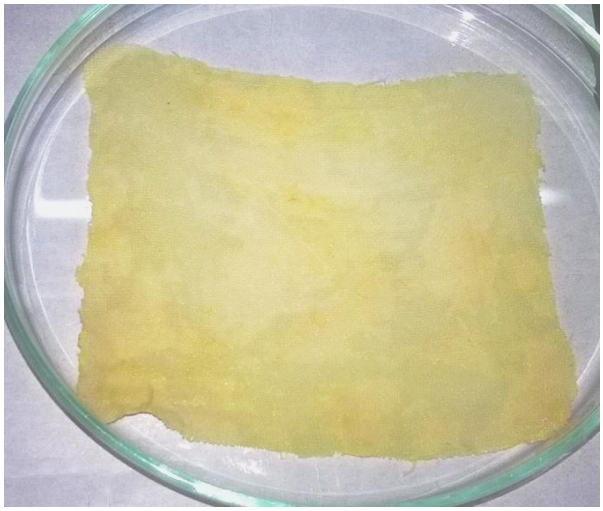

Figure 16a: Cloth stained with juice.

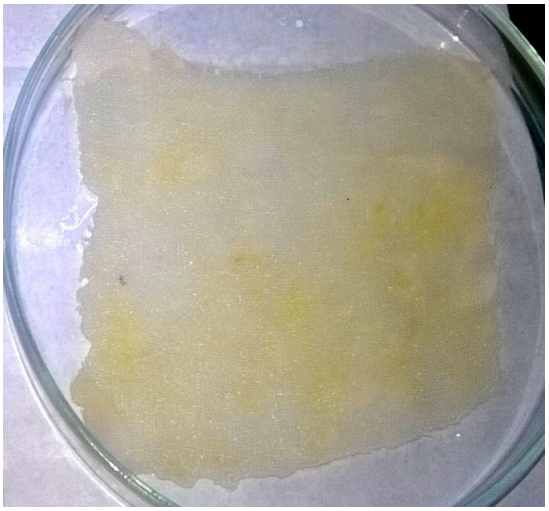

Figure 16b: Control- cloth treated with water.

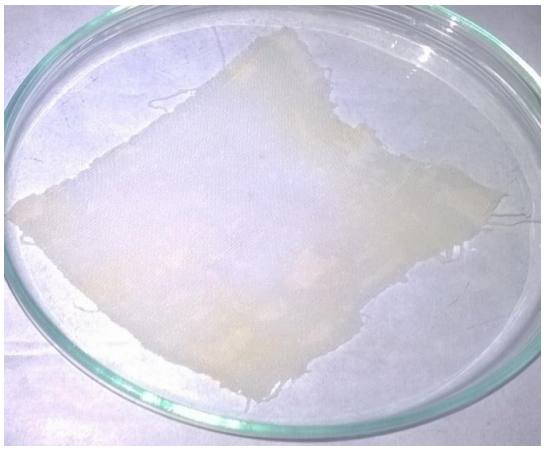

Figure 16c: Cloth treated with crude xylanase enzyme.

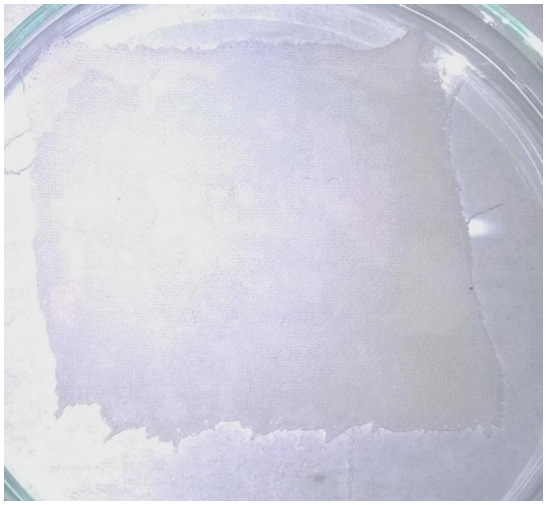

Figure 16d: Cloth treated with crude xylanase enzyme mixed with detergent components other than enzymes.

The importance of xylanases results from their significance in breakdown of hemicelluloses, xylan into xylose and xylooligosaccharides. In the present investigation; optimization of various parameters for maximum production of xylanase by *Paenibacillus lautus* from mangrove soil sample was carried out. The organism showed minimum cellulase activity thus making it suitable for various industrial applications. The enzyme was active at a wide range of temperature (30-90°C) and pH (4-11) giving optimum enzyme activity at 50°Cat pH 7. In conclusion, it can be said that *Paenibacillus lautus* isolate is a good source of xylanase. Thus, can be used for commercial applications in industries such as detergent additive.

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