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Advances in Bioresearch

Production, Optimization, and Purification of Xylanase from agricultural waste by indigenously isolated *Bacillus paralicheniformis* (KJB3)

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ABSTRACT
Xylanase is an industrially important enzyme having several applications for the hydrolysis of xylan – major hemicellulose in most plant species. The main objective of this research was to study submerged fermentation of cost-effective substrate derived from agricultural waste for production of xylanase from bacteria. Production and optimization of xylanase with respect to process parameters such as substrate, temperature, pH, nitrogen source, and metal ion effect have been undertaken here using one factor at a time approach using indigenously isolated Bacillus paralicheniformis (KJB3) identified using 16S rRNA sequencing. Optimized parameters for substrate, temperature, pH, substrate concentration, and nitrogen source were corn cob, 40 ^{0}C, pH 6, 2 g, and peptone, respectively. Additionally, study of metal ion revealed Ca^{2+}, Cu^{2+}, and Fe^{2+} enhanced activity of xylanase, whereas Co^{2+}, and Mn^{2+} had an inhibitory effect and Zn^{2+} had no effect on xylanase activity. Further, purification and characterization of xylanase have been carried out using column and gel electrophoresis (SDS-PAGE), and the molecular weight of xylanase was determined to be 30 KDa.
Keywords: *16SrRNA, metal ions, nitrogen source, temperature, SDS – PAGE*

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INTRODUCTION

Xylanase (E.C 3.2.1.8) one of the non-starch polysaccharides (NSP) degrading enzyme, converts the backbone of the xylan polymeric chain, which is made up of xylose subunits, into xylo-oligosaccharides by hydrolyzing the β-1,4 glycosidic bond [1][2]. Industrial application of xylanases majorly includes those in the production of animal feed, food, and ethanol, pulp, textile, and paper industries, and waste treatment [3]. Owing to its wide applicability, xylanase, and studies pertaining to its efficient production, optimization, and characterization are attracting the interests of researchers [4]. Many microorganisms such as bacteria [5][6], actinomycetes [7][8], fungi [9][10], yeasts [11],[12] and algae [13][14] produce xylanases [15]. Amongst microbial xylanases, bacterial xylanases are majorly focused owing to rapid growth rate, relatively easy handling, and genetic engineering for enhanced production of xylanases [5]. Additionally, bacterial xylanases of high stability and effectiveness under varying extreme environmental conditions are reported [16], [17]. For instance, thermotolerant xylanases active at 60 -70 ^{0}C temperature range have been reported from *Bacillus* spp. [18], [19] *Clostridium* sp. [20], *Stenotrophomonas maltophila* [21] *Thermotoga thermarum* [22], and *Rhodothermus marinus* [23] whereas psychrophilic xylanases, although less reported, have been isolated from *Clostridium* sp. PXLY$_1$ [24], *Flavobacterium sp.* MSY-2 and *Psuedoalteromonas haloplanktis* TAH$_3$A [25]. Likewise, alkali stable xylanases have been produced by *Bacillus* spp. [20], and *Streptomyces althioticus* LMZM [26]. The production of xylanase from microorganisms is affected by choice of substrate, fermentation process, and media components used [27]. These parameters need to be regulated for enhanced production of xylanase and for its application at large scale [28]. With respect to the cost of xylanase production, the substrate used for fermentation accounts for 30 to 40 % of the

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manufacturing cost. For this reason, it is critical to discover low-cost alternatives to the currently used substrates for xylanase production and optimize the process for achieving enhanced production of xylanase [29]. Agricultural wastes are an attractive alternative to conventionally used substrates for xylanase production. In addition to being cost-effective, using agricultural waste offers other advantages, such as being environmentally friendly and easily available [30], [31]. The use of sugarcane bagasse [31], [32], corn cob [31], wheat bran, and wheat husk [1], [31], [33] rice husk [34], rice bran [31], oat bran [34], coconut coir [35], wood pulp [35], [36], fruit and vegetable waste [37], and wood pulp [36], [37].The xylanase production with respect to the fermentation process has been majorly carried out under submerged fermentation (SmF) and solid state fermentation (SSF) [38]. The growth of bacteria requires a large amount of water, therefore, SmF is preferred [39]. Globally about 90% of total xylanase production is carried out using SmF. Advantages of SmF include homogeneous conditions in media, better biomass utilization, and easy scale-up [40]. It has been reported that SmF enables better biomass utilization and therefore results in higher xylanase production [28]. Therefore, considering the need to use SmF for production of xylanase, an attempt has been made to use agricultural waste as substrate using indigenously isolated *B. paralicheniformis* (KJB3) that has been shown to be efficient for xylanase production (data not presented here). The *B. paralicheniformis* was identified using 16S rRNA sequencing [41]. Further optimization to enhance the production of xylanase with respect to process parameters such as substrate, temperature, pH, nitrogen source, metal ion effect has been undertaken here using a one factor at a time approach [41]. Furthermore, purification and characterization of xylanase has been carried out using spin columns and gel electrophoresis [42] (Graphical abstract).

MATERIAL AND METHODS

Procurement of substrates

Several agricultural wastes such as carrot peels, coconut husk, corn cob, rice husk, sugarcane bagasse, and tea waste were bought from Ahmedabad's local market Gujarat, India. Agricultural wastes were soaked in water, sundried for 24 hours, and crushed using a domestic kitchen mixer.

Media preparation

The nutrients employed for isolation were yeast extract, K_2HPO_4, $MgSO_4.7H_2O$, bacto agar and agricultural waste (2 %). Media were prepared and autoclaved as per standard protocols [43].

Bacterial strain

In our previous work, twenty-five isolates were obtained from different soil samples collected from various regions in and around Kalol. KJB3, isolated from the soil of gaushala, Kalol, was found to be an efficient producer of xylanase and was identified as *B. paralicheniformis* by 16S rRNA analysis. The strain was maintained on nutrient agar slant and stored at 4 ^{0}C. Gram's staining and capsule staining of *B. paralicheniformis* carried out for studying cultural and morphological characteristics are presented here. Nutrient agar plates were used for the study of colony characteristics.

Optimization of xylanase enzyme production

Various parameters like pH (2–8), incubation temperature (20 to 50 ^{0}C), substrate concentration (0.5–2.5%), and additional nitrogen supplementation (1%) (ammonium sulfate, peptone, yeast extract, urea, gelatin & glycine) and metal ions (500µg/ml) ($ZnSO_4$, $CaCl_2$, $MnSO_4$, $FeSO_4$, $CoCl_3$, $CuSO_4$) were optimized for enhanced production of xylanase.

Purification and characterization of xylanase

- **SLS One Stop Protein Purification miniprep protocol:**
- The 10 ml supernatant after xylanase production was incubated at 37°C for 30 min and mixed with equal volume of precipitation and binding buffer. The system was incubated on ice for 2-5 min and then centrifuged for 10, 000 RPM for 5 min.
- The supernatant was removed and the pellet was resuspended in a 500µl re suspension buffer. The solution was transferred on SLS One-step protein Spin Column and centrifuge at 10,000 RPM for 1 min.
- The spin column was placed into a brand-new collecting tube and 100µl of elution buffer was added to it. The tube was incubated at room temperature for 1 minute. and then centrifuge at 10,000 RPM for 1 min. The eluted protein (1 ml) was collected for further analysis.

Molecular weight determination

- Molecular weight of xylanase was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using a 3 mm gel slab. Coomassie brilliant blue G-250 was used as a stain. Broad range molecular weight marker was used for molecular weight determination.

RESULTS AND DISCUSSION

Cultural and morphological characters of KJB3

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Table 1. Colony characters of KJB3

Shape	Size	Colony appearance	Margin	Elevation	Texture	Gram's reaction	Pigment
Round	Medium	Smooth	Entire	Raised	Moist	Gram Positive	Nil

Figure: - 1. Colony on the nutrient agar plate

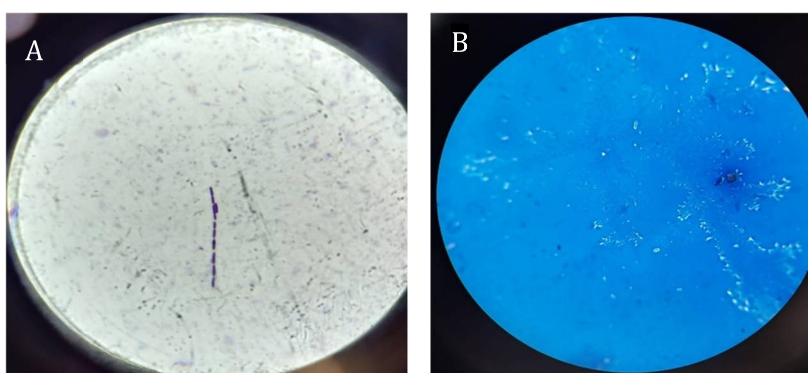

Figure: - 2.Morphological characters of *B. paralicheniformis.* A. Gram staining, B. Capsule staining

The KJB3 colony was smooth, elevated, round, and gram-positive, Table 1 and figure 21. It was butyrous and milky white. The edge had a wavy shape. Based on Bergey's Manual of Systematic Bacteriology [44], this suggested that the isolated KJB3 belonged to a category of rod, spore-forming aerobic/ facultative bacteria, figure 2.

Molecular identification by 16s rRNA gene technology

ABI 3730xl Genetic Analyzer was used to carry out the DNA sequencing reaction of the PCR amplicon with prime r27F using BDT v3.1 Cycle sequencing kit for molecular identification. The BLAST search of 16S rRNA gene sequence against sequences in the nucleotide database showed 100% homology with *B. paralicheniformis* strain KJ-16. Thus, the new indigenously isolated strain was identified as *B. paralicheniformis,* figure 3.

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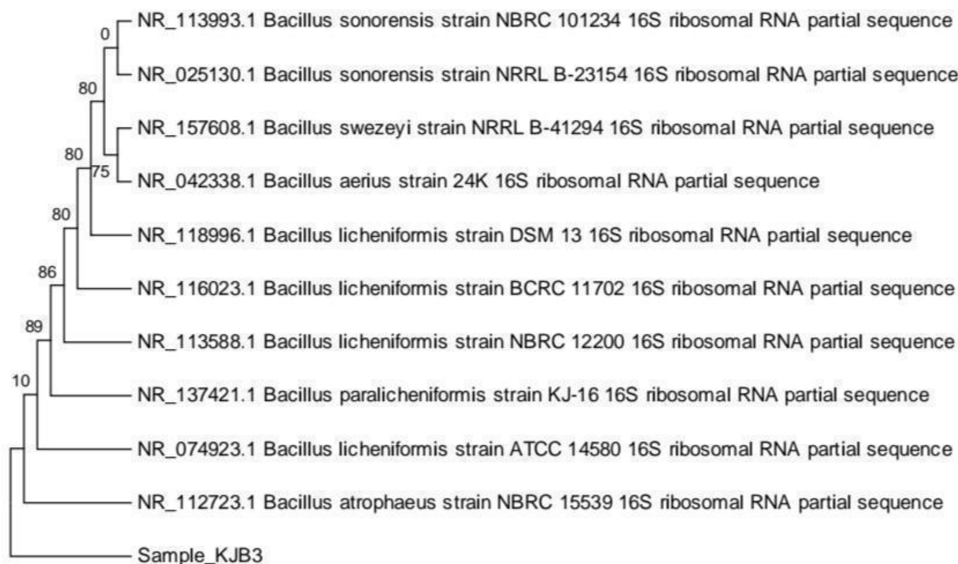

Figure: -3 Phylogenetic tree of *Bacillus paralicheniformis*

Screening of substrate

The ability of *B. paralicheniformis* to produce xylanase from various agricultural wastes, such as rice husk, carrot peel, coconut husk, tea waste, sugarcane bagasse, and corn cobs, was examined during SmF. Findings (figure 4) showed that the maximum sugar concentration was obtained by corn cob (450 µg/ml), followed by coconut husk (350 µg/ml), carrot peel (320 µg/ml), and sugarcane bagasse (300 µg/ml), in that order. Tea waste (250 µg/ml) and rice bran (210 µg/ml) produced the least amount of sugar. Owing to high cost of commercially available xylan substrate several researchers have focused on low-cost agricultural waste and other domestic and industrial organic waste to find alternatives to xylan substrate. Commonly used agricultural waste for xylanase production from *Bacillus* sp. include sugarcane bagasse [31], [32], [34], corn cob [31], wheat bran [1], [31], [33], wheat husk [31], [33], rice husk [34], rice bran [31], oat bran [34], coconut coir [35], wood pulp [35], [36], fruit and vegetable waste [37], wood pulp [36], [37] fruit and vegetable waste. Agro-waste typically consists of dry matter, crude protein, crude fiber and lignocellulosic content [45]. Production of xylanase varies with substrate. Therefore, it is essential to optimize the substrate for a given bacterial species.

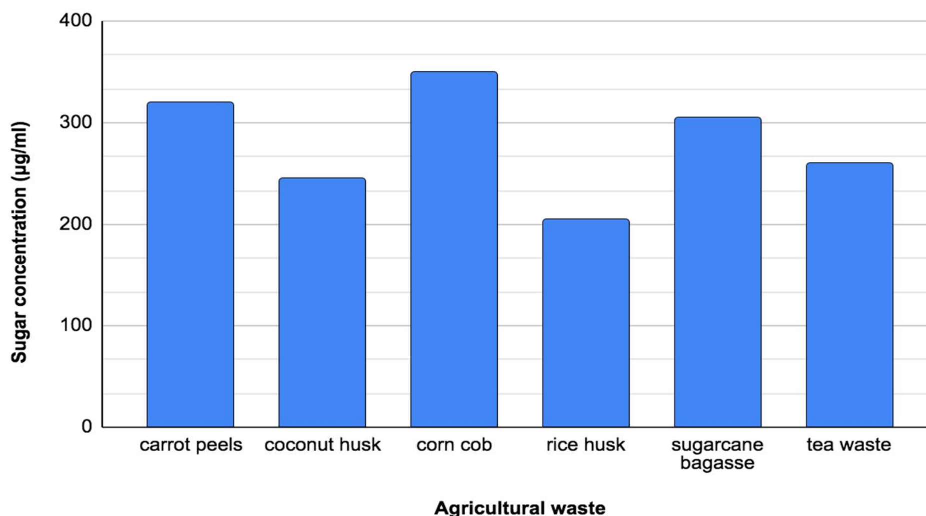

Figure: - 4. Screening of agricultural waste as substrate

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Effect of temperature

The effect of temperature in the range of 20 to 50 ^{0}C; studied for production of xylanase with *Bacillus paralicheniformis* using corn cob as a substrate is depicted in figure 5. In this investigation, the highest sugar concentration (368 µg/ml) was recorded at 40 ^{0}C whereas the lowest sugar concentration (150 µg/ml) was recorded at 50 ^{0}C (figure 5). Similar to our results, Kamble and Jadhav et al., 2012 report xylanase produced from *B. arseniciselenatis* DSM 15340 showed maximum activity at temperatures 30 ^{0}C and 40 ^{0}C [1]. Likewise, xylanase produced from *B. subtilis* BS04 and *B. megaterium* BM07 showed maximum activity at temperature of 35 ^{0}C and 40 ^{0}C, respectively [32]. On the other hand, xylanases produced from *B. halodurans* TSEV1 have reported maximum activity at higher temperature of 70 ^{0}C. Thermal stability of xylanase is due to intrinsic structural properties. Presence of salt bridge, amino acid, and divalent metal ions have been reported to influence thermal stability of xylanase enzyme [28].

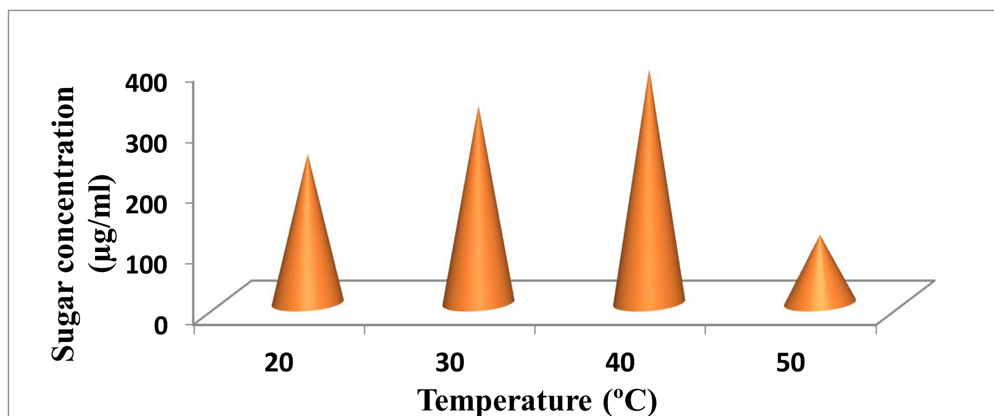

Figure: - 5. Effect of temperature on xylanase production

Effect of pH

Experiments with initial pH levels ranging from 2 to 8 were conducted to determine the ideal initial pH for the synthesis of xylanase using corn cob as substrate at an optimum temperature of 40 ^{0}C. Before sterilization, the pH of the medium was regulated with 0.1 N HCl/NaOH. From the experiments, it was found that the sugar concentration was higher at pH 6 (450 µg/ml), followed by pH 8 (400 µg/ml). The lowest sugar content was found at a pH value of 2 (80 µg/ml) (figure 6). Similar results have been reported by Roy and Habib, 2009, for xylanase produced from *B. cereus*. Likewise, Irfan et al., reported optimum pH of 8.0 for *B. subtilis* BS04 and *B. megaterium* BM07 [32]. Kumar et al., 2014 reported optimum pH of 9.0 for xylanase production from *B. pumilus* VLK-1 [33]. Clearly, optimum initial pH varies from one bacterial strain to another and has an influence on growth and metabolism. pH stability of xylanase has been reported to be influenced by amino acid present near the catalytic surface [28].

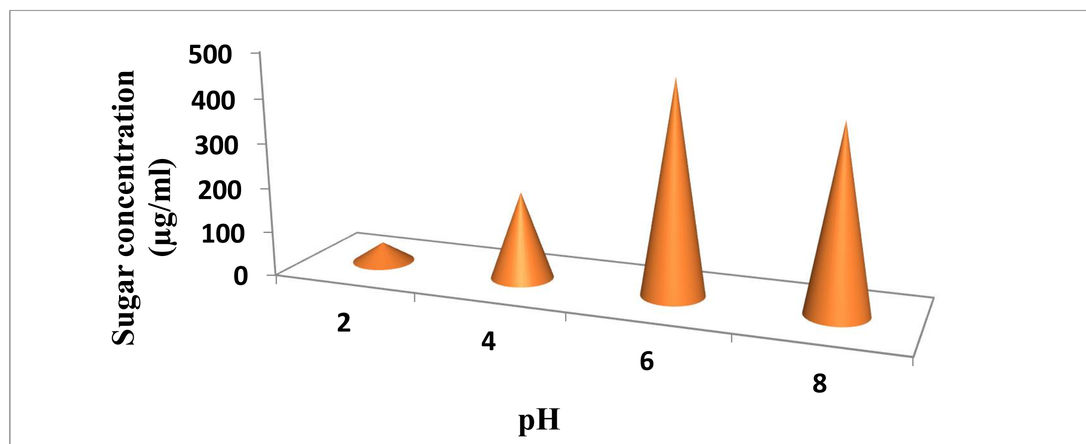

Figure- 6. Effect of pH on xylanase production

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Effect of substrate concentration

By varying the quantity of the chosen substrate (corn cob) in a 500 ml flask (Erlyenmer) from 0.5 to 2.5 g at 40 ^{0}C and pH 6, the optimal substrate concentration for xylanase synthesis was also investigated. Out of all these examined substrate concentrations, 2 g of corn cob in a 500 ml flask demonstrated the highest sugar concentration for the best enzyme production (415 µg/ml), Table 2. Quantity of the substrate has been known to influence xylanase production. As can be seen, with increase in substrate concentration to 2 g, xylanase activity is enhanced. However, further increase of substrate concentration led to decrease in activity of xylanase which may be due to end product inhibition [28]

Table 2: Effect of substrate concentration on xylanase production

Corn cob (g)	Sugar concentration (µg/ml)
0.5	160
1.0	280
1.5	335
2.0	415
2.5	400

Effect of various nitrogen source

Influence of different organic and inorganic nitrogen sources at a concentration of 0.5% was investigated individually for optimum production of xylanase with 2 g corn cob as substrate at 40 ˚C and pH 6 (figure 7). As can be seen, peptone (615µg/ml) and ammonium sulfate (575 µg/ml) enhanced the xylanase activity whereas gelatin (230 µg/ml) and glycine (235 µg/ml) inhibited the activity of xylanase and urea (425 µg/ml) and yeast extract ((430 µg/ml) has no significant effect in comparison to control, figure 8. Prakash et al., reported peptone for significant promotion of xylanase activity of *B. halodurans* PPKS-2 [46]. Likewise, Irfan et al., 2016 reported ammonium sulfate and potassium nitrate as inorganic nitrogen sources and tryptone and malt extract as organic nitrogen sources that positively influenced the xylanase activity of *B. subtilis* and *B. megaterium* respectively [32]. Nitrogen is an essential element required for optimum growth and several metabolic activities including enzyme production in *Bacillus* sp. The optimum nitrogen source varies as per individual species and strain of *Bacillus* sp. [28].

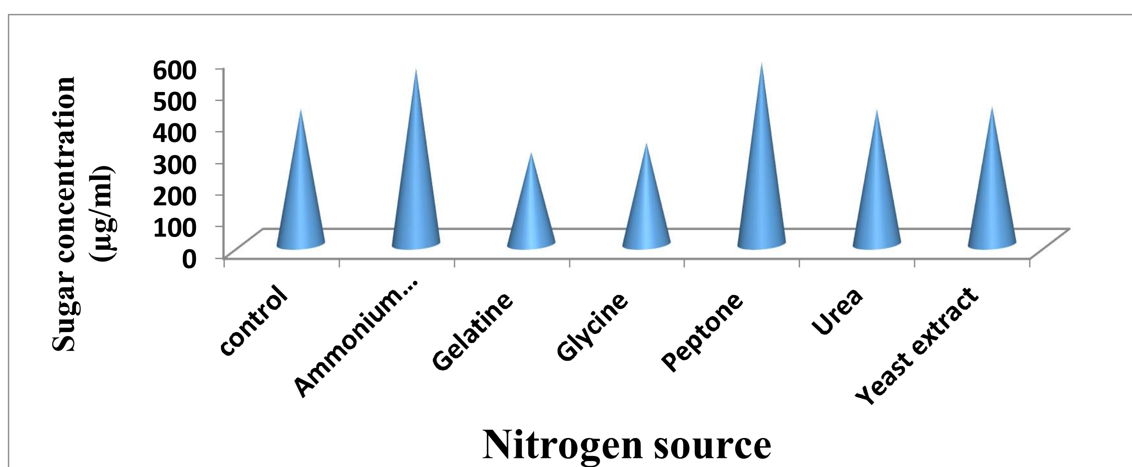

Figure: 7. Effect of various nitrogen sources on xylanase production

Effect of metal ions

Effect of several metal ions on enzyme function was evaluated at a concentration of 0.5 µg/ml for each metal ion individually (figure 8). The experiment was carried out with optimized parameters for each component as discussed above. As can be seen, Ca^{2+}, Cu^{2+} and Fe^{2+} enhanced the activity of xylanase, whereas Co^{2+}, and Mn^{2+} had an inhibitory effect and Zn^{2+} had no effect on xylanase activity. In the literature, positive effects of Ca^{2+} [47], Cu^{2+}, and Fe^{2+} [48] have been reported on xylanase activity by *Bacillus* sp.

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Likewise, Prakash et al., 2011 reported strong inhibition by Mn^{2+} ion on xylanase production by *B. halodurans* PPKS-2 and attributed the negative effect on interaction of Mn^{2+} with cysteine [46].

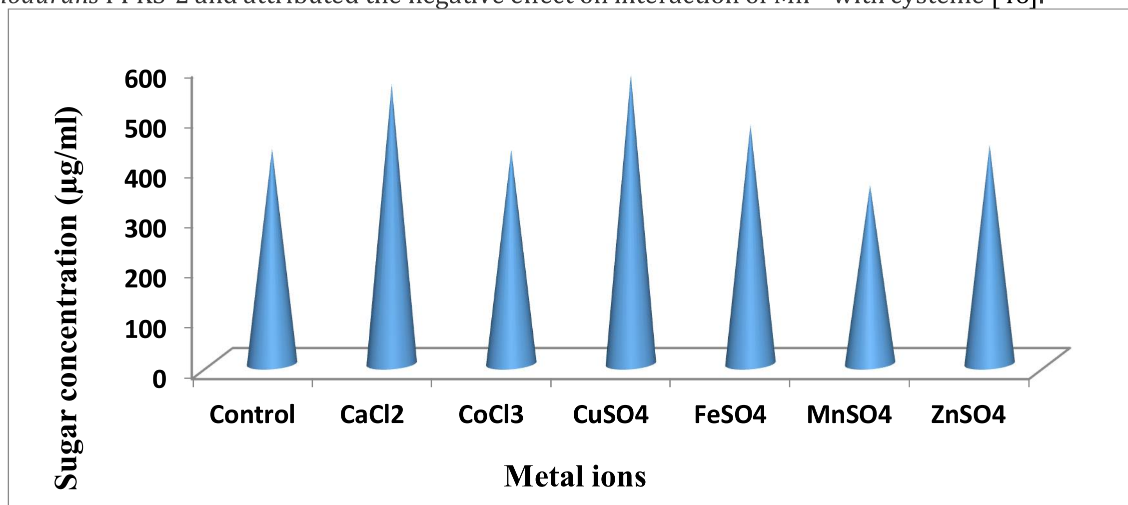

Figure: 8. Effect of various metal ions on xylanase production

Purification and Characterization of Xylanase.

Xylanase was purified using the SLS one-stop nontagged protein purification column per standard protocol. The column is specifically designed with a combination of immobilized metal affinity chromatography and boronic acid based resins for efficient protein purification.

Molecular weight of xylanase enzyme was determined using SDS-PAGE. Xylanase displayed a single protein band. Figure 9 shows that the molecular mass of denatured xylanase was 30 kDa as measured by the comparative movement of proteins on SDS-PAGE. Mittal et al., 2013 reported 27 kDa xylanase from *Bacillus* sp. SV-34S [47]. Luo et al., 2015 reported 31.75 kDa xylanase from *Streptomyces althioticus* LMZM [26].

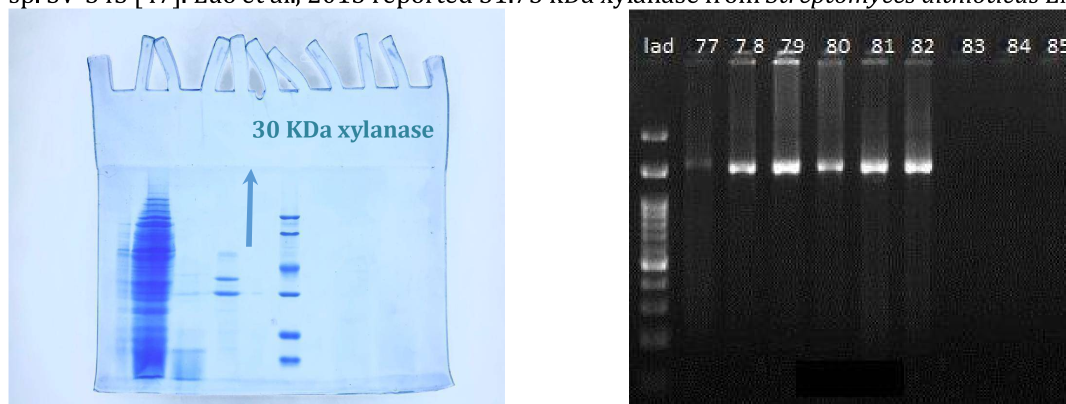

Figure: 9. SDS PAGE for Xylanase.

CONCLUSION

KJB3 is a Gram-positive rod with capsule. KJB3 isolate was identified as *B. paralicheniformis* based on 16S rRNA gene sequencing data. This research demonstrated that *B. paralicheniformis* can synthesize xylanase utilizing corn cob as a substrate. For KJB3, the ideal pH, temperature, and substrate concentration were 6 (470µg/ml), 40 ^{0}C (385µg/ml), and 2 g (415 µg/ml), respectively. In our study, nitrogen sources like peptone, and ammonium sulfate at a concentration of 0.5% enhanced xylanase activity by more than 30%. Likewise, trace amounts (0.5 µg/ml) of metal ions like calcium and copper had a positive effect on xylanase activity. Under optimum conditions (as per our study) total sugar production increased by 49.3% (618 µg/ml) after 48 hours. Molecular weight of xylanase was determined to be 30 KDa using SDS PAGE. Our study investigated production, optimization, and purification of valuable enzyme xylanase from indigenously isolated *B. paralicheniformis* that can be further upscaled and explored for application.

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REFERENCES

1. R. D. Kamble and A. R. Jadhav. (2012). Isolation, Purification, and Characterization of Xylanase Produced by a New Species of *Bacillus* in Solid State Fermentation, Int J Microbiol, 1–8.
2. U. Gupta and R. Kar. (2009). Xylanase production by a thermo-tolerant *Bacillus species* under solid-state and submerged fermentation, Brazilian Archives of Biology and technology, 1363–1371.\
3. Basit, W. Jiang, and K. Rahim. (2020). Xylanase and its industrial applications, in: Biotechnological Applications of Biomass, IntechOpen.
4. G. Guo et al. (2012). Purification and characterization of a xylanase from *Bacillus subtilis* isolated from the degumming line, J Basic Microbiol, 419–428.
5. H. Chakdar et al. (2016). Bacterial xylanases: biology to biotechnology, 1–15.
6. K. S. Sasmitaloka, A. B. Arif, C. Winarti, M. Hayuningtyas, and N. Richana. (2019). Xylan production from corn cobs for isolation of xylanase-producing bacteria, In: IOP Conference Series: Earth and Environmental Science, 012066.
7. Saini, N. K. Aggarwal, A. Sharma, and A. Yadav. (2015). *Actinomycetes*: A Source of Lignocellulolytic Enzymes, Enzyme Res, 1–15.
8. L. Thomas, A. Joseph, M. Arumugam, and A. Pandey. (2013). Production, purification, characterization and over-expression of xylanases from *actinomycetes*.
9. M. de L. T. de M. Polizeli, A. C. S. Rizzatti, R. Monti, H. F. Terenzi, J. A. Jorge, and D. de S. Amorim. (2005). Xylanases from fungi: properties and industrial applications, Appl Microbiol Biotechnol, 577–59
10. Knob, D. Fortkamp, T. Prolo, S. C. Izidoro, and J. M. Almeida. (2014). Agro-residues as alternative for xylanase production by filamentous fungi, Bioresources.
11. K. Šuchová, C. Fehér, J. L. Ravn, S. Bedő, P. Biely, and C. Geijer. (2022). Cellulose-and xylan-degrading yeasts: Enzymes, applications and biotechnological potential, Biotechnol Adv, 107981.
12. C. G. Morais, R. M. Cadete, A. P. T. Uetanabaro, L. H. Rosa, M.-A. Lachance, and C. A. Rosa. (2013). D-xylose-fermenting and xylanase-producing yeast species from rotting wood of two Atlantic Rainforest habitats in Brazil, Fungal Genetics and Biology, 19–28.
13. Y. Hsieh and P. Harris. (2019). Xylans of Red and Green Algae: What Is Known about Their Structures and How They Are Synthesised?, Polymers (Basel), 354.
14. Pasalari and A. Homaei. (2022). Isolation and Molecular Identification of Xylanase-Producing Bacteria from Ulva flexuosa of the Persian Gulf, Processes, 1834.
15. Burlacu, C. P. Cornea, and F. Israel-Roming. (2016). Microbial xylanase: a review, Scientific Bulletin. Series F. Biotechnologies, 335–342.
16. D. Talens-Perales, E. Jiménez-Ortega, P. Sánchez-Torres, J. Sanz-Aparicio, and J. Polaina. (2021). Phylogenetic, functional and structural characterization of a GH10 xylanase active at extreme conditions of temperature and alkalinity, Comput Struct Biotechnol J, 2676–2686.
17. T. Collins, C. Gerday, and G. Feller. (2005). Xylanases, xylanase families and extremophilic xylanases, FEMS Microbiol Rev, 3–23.
18. L. Thomas, M. V. Ushasree, and A. Pandey. (2014). An alkali-thermostable xylanase from *Bacillus pumilus* functionally expressed in *Kluyveromyces lactis* and evaluation of its deinking efficiency, Bioresour Technol, 309–313.
19. V. Kumar and T. Satyanarayana. (2014). Production of endoxylanase with enhanced thermostability by a novel polyextremophilic *Bacillus halodurans* TSEV1 and its applicability in waste paper deinking, Process Biochemistry, 386–394.
20. L. Thomas, A. Joseph, and L. D. Gottumukkala. (2013). Xylanase and cellulase systems of *Clostridium sp.*: an insight on molecular approaches for strain improvement, Bioresour Technol,343–350.
21. Raj, S. Kumar, and S. K. Singh. (2013). A Highly Thermostable Xylanase from *Stenotrophomonas maltophilia*: Purification and Partial Characterization, Enzyme Res, 1–8.
22. H. Shi et al. (2013). A novel highly thermostable xylanase stimulated by Ca2+ from *Thermotoga thermarum*: cloning, expression and characterization, Biotechnol Biofuels, 26.
23. E. N. Karlsson et al. (2004). The modular xylanase Xyn10A from *Rhodothermus marinus* is cell-attached, and its C-terminal domain has several putative homologous among cell-attached proteins within the phylum Bacteroidetes, FEMS Microbiol Lett, 233–242.
24. G. Akila and T. S. Chandra. (2003). A novel cold-tolerant *Clostridium strain PXYL1* isolated from a psychrophilic cattle manure digester that secretes thermolabile xylanase and cellulase, FEMS Microbiol Lett, 63–67.
25. E. Dornez, P. Verjans, F. Arnaut, J. A. Delcour, and C. M. Courtin. (2011). Use of Psychrophilic Xylanases Provides Insight into the Xylanase Functionality in Bread Making, J Agric Food Chem, 9553–9562.
26. L. Luo et al. (2016). Purification and characterization of an alkaliphilic endo-xylanase from *Streptomyces althioticus LMZM* and utilization in the pulp paper industry, Journal of Chemical Technology & Biotechnology, 1093–1098.
27. Pandey, C. R. Soccol, P. Nigam, and V. T. Soccol. (2000). Biotechnological potential of agro-industrial residues. I: sugarcane bagasse, Bioresour Technol, 69–80.

 © 2023 Author

28. N. Bhardwaj, B. Kumar, and P. Verma. (2019). A detailed overview of xylanases: an emerging biomolecule for current and future prospective, Bioresour Bioprocess, 40.
29. Walia, S. Guleria, P. Mehta, A. Chauhan, and J. Parkash. (2017). Microbial xylanases and their industrial application in pulp and paper biobleaching: a review, 3 Biotech, 1–12.
30. D. S. Kocabaş, S. Güder, and N. Özben. (2015). Purification strategies and properties of a low-molecular weight xylanase and its application in agricultural waste biomass hydrolysis, J Mol Catal B Enzym, 66–75.
31. S. Devi, D. Dwivedi, and A. K. Bhatt. (2022). Utilization of Agro-residues for the Production of Xylanase by *Bacillus safensis XPS7* and Optimization of Production Parameters, Fermentation, 221.
32. M. Irfan, U. Asghar, M. Nadeem, R. Nelofer, and Q. Syed. (2015). Optimization of process parameters for xylanase production by *Bacillus sp.* in submerged fermentation, J Radiat Res Appl Sci, 139–147.
33. L. Kumar et al. (2014). Modulation of xylanase production from alkaliphilic *Bacillus pumilus* VLK-1 through process optimization and temperature shift operation, 3 Biotech,345–356.
34. Akhavan Sepahy, S. Ghazi, and M. Akhavan Sepahy. (2011). Cost-Effective Production and Optimization of Alkaline Xylanase by Indigenous *Bacillus mojavensis AG137* Fermented on Agricultural Waste, Enzyme Res, 1–9.
35. da S. V. Rodrigues et al. (2020). Production of xylanases by *Bacillus sp . TC-DT13* in solid state fermentation using bran wheat, Prep Biochem Biotechnol, 91–97.
36. Sindhu, S. Chhibber, N. Capalash, and P. Sharma. (2006). Production of Cellulase-Free Xylanase from *Bacillus megaterium* by Solid State Fermentation for Biobleaching of Pulp, Curr Microbiol, 167–172.
37. Walia, P. Mehta, A. Chauhan, and C. K. Shirkot. (2013). Optimization of cellulase-free xylanase production by alkalophilic *Cellulosimicrobium sp. CKMX1* in solid-state fermentation of apple pomace using central composite design and response surface methodology, Ann Microbiol, 187–198.
38. U. Gupta and R. Kar. (2009). Xylanase production by a thermo-tolerant *Bacillus species* under solid-state and submerged fermentation, Brazilian Archives of Biology and technology, 1363–1371.
39. Z. Mmango-Kaseke, K. Okaiyeto, U. U. Nwodo, L. V Mabinya, and A. I. Okoh. (2016). Optimization of cellulase and xylanase production by *Micrococcus species* under submerged fermentation, Sustainability, 1168, 2016.
40. S. Naz, M. Irfan, and M. U. Farooq. (2017). Xylanase production from *Bacillus subtilis* in submerged fermentation using box-behnken design, Pakistan Journal of Biotechnology, 151–156.
41. Raj, S. Kumar, S. K. Singh, and J. Prakash. (2018). Production and purification of xylanase from alkaliphilic *Bacillus licheniformis* and its pretreatment of eucalyptus kraft pulp, Biocatal AgricBiotechnol, 199–209.
42. N. Boucherba, M. Gagaoua, E. Copinet, A. Bettache, F. Duchiron, and S. Benallaoua. (2014). Purification and characterization of the xylanase produced by Jonesia denitrificans BN-13, Appl Biochem Biotechnol, 2694–2705.
43. Trivedi M.J, Dave S.R. (2012). Microbial Utilization of Cellulosic Material for Organic Acid Production, School of Sciences, 159.
44. Q. Beg, M. Kapoor, L. Mahajan, and G. S. Hoondal. (2001). Microbial xylanases and their industrial applications: a review, Appl Microbiol Biotechnol, 326–338.
45. M. Khanahmadi, I. Arezi, M. Amiri, and M. Miranzadeh. (2018). Bioprocessing of agro-industrial residues for optimization of xylanase production by solid-state fermentation in flask and tray bioreactor, Biocatal Agric Biotechnol, 272–282.
46. P. Prakash, S. K. Jayalakshmi, B. Prakash, M. Rubul, and K. Sreeramulu. (2012). Production of alkaliphilic, halotolerent, thermostable cellulase free xylanase by *Bacillus halodurans PPKS-2* using agro waste: single step purification and characterization, World J Microbiol Biotechnol, 183–192.
47. Mittal, S. Nagar, and V. K. Gupta. (2013). Production and purification of high levels of cellulase-free bacterial xylanase by *Bacillus sp. SV-34S* using agro-residue, Ann Microbiol, 1157–1167.
48. Patel and P. Dudhagara. (2020). Optimization of xylanase production by *Bacillus tequilensis* strain UD-3 using economical agricultural substrate and its application in rice straw pulp bleaching, Biocatal Agric Biotechnol, 101846.

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