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Research Article

PRODUCTION & OPTIMIZATION OF REACTION CONDITIONS FOR XYLANASE FROM *PSEUDOMONAS* SP.XPB-16

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ABSTRACT

The present research investigation reports an attempt to optimize the reaction conditions for maximum activity of crude xylanase from *Pseudomonas* sp. XPB-16. Buffer system, pH, buffer molarity, reaction temperature, incubation time & substrate concentration were the main parameters that were optimized. Furthermore the influence of various metal ions and inhibitors on activity of enzyme was also assessed. Potassium phosphate buffer (50mM), pH 7.0 was reported as the most appropriate for maximal activity of xylanase amongst various buffers tested. The optimum temperature, incubation time, substrate concentration recorded for maximum xylanase activity were 37°C, 25 minutes, 0.6% (w/v) respectively. Sodium azide was reported as the most potent inhibitor of xylanase activity amongst various metal ions and inhibitors. The perspectives of xylan hydrolysis by xylanase from *Pseudomonas* sp. XPB-16 look quite promising. Thus future studies to enhance the xylan hydrolysis rate as well as to assure enhanced process control for increased activity of xylanase would be envisaged.

INTRODUCTION

Xylanase being a hydrolytic enzyme efficiently catalyzes the depolymerisation of xylans, which are single chain glycoproteins having molecular weights ranging from 6–80 kDa. Xylan is a major hemicellulose composed of xylose units interlinked through β -1, 4-glycosidic linkage. Xylan exists as the second most abundant polysaccharide of plant origin behind cellulose (Collins *et al*., 2005). Being a complex molecule with heterogenic nature the thorough enzymatic disintegration of xylan into its individual units necessitates a synergistic action of different xylanolytic enzymes (Subramaniyan & Prema, 2002). Xylanase plays a major role amongst a mixture of enzymes working synergistically for thorough depolymerisation of xylan (Takahashi *et al*., 2013). A broad range of organisms including bacteria, fungi, marine algae, actinomycetes, protozoans, snails, insects, arthropods, gastropods, crustaceans as well as several seeds and plants have been documented as efficient xylanase producers (Motta *et al*., 2013). Xylanases from different sources vary in their requirements for temperature, pH and other important process parameters for optimum activity. Xylan depolymerizing enzymes have wide industrial applications either on their own or in conjunction with other enzymes in various industrial processes. Over the years xylanases have witnessed a significant rise in their utilization in commercial sector. Owing to the surplus availability of hemicellulosic biomass particularly xylan and broad range of applications of xylanases in commercial sector, it was considered meaningful to produce xylanase from *Pseudomonas* sp. XPB-16 under optimized culture conditions and to optimize the reaction conditions for maximum activity of the enzyme.

MATERIAL AND METHODS

Inoculum preparation & Enzyme production

Inoculation of loopful of bacterial isolate *Pseudomonas* sp. XPB-16 isolated from soil sample of Gharuan, Punjab was done in 50 mL seed medium (pH 7.0) containing (% w/v): yeast extract- 0.2 g, dextrose- 0.25 g, peptone- 0.5 g, beef extract- 0.2 g and incubation was done at 37 ˚C for 24 h at 160 rpm. The inoculum(10% w/v) from the seed medium was added to 100 ml of production medium (pH 7.5) containing fructose- 0.75g, ammonium sulphate- 1.0g, yeast extract- 5.0g, KH_2PO_4- 0.15g, $MgSO_4$- 0.015g, xylan- 0.4g (%,w/v) in 250ml Erlenmeyer flask for maximum xylanase production followed by incubation at 37 ˚C for 36h at 160 rpm. Supernatant collected upon centrifugation at 10,000 rpm was further assayed for xylanase activity using the assay method as described by Miller (1959).

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Optimization of Reaction Conditions

Optimization of the best reaction conditions for maximal xylanase activity was executed by stepwise optimizations of various parameters.

Buffer system and pH optimization

Most suitable buffer system & optimum pH for maximum xylanase activity were optimized by using different buffers viz. potassium phosphate buffer (pH 6.0-8.5), sodium phosphate buffer (pH 7.0-8.5), citrate buffer (pH 3.0-5.5) and glycine-NaOH (pH 8.5-10.5) at 50mM concentration in the reaction mixture. Xylanase activity was assayed using the standard assay method as described by Miller (1959) and optimized buffer system& pH were used for further studies.

Optimization of buffer molarity

Optimization of the buffer molarity for maximum xylanase activity was accomplished by using different molar concentrations (25mM to 125mM) of potassium phosphate buffer (pH 7.0) in the reaction mixture. Xylanase activity was assayed using the standard assay method as described by Miller (1959) and optimized buffer molarity was used for further studies.

Optimization of reaction temperature

Optimization of the most appropriate reaction temperature for maximum xylanase activity was accomplished by incubating the reaction mixture in the temperature range 25 °C to 50 °C. Xylanase activity was assayed using the standard assay method as described by Miller (1959) and optimized reaction temperature was used for further studies.

Optimization of incubation time

Optimization of the most appropriate incubation time for maximum xylanase activity was accomplished by incubating the reaction mixture at 37 °C for different time intervals ranging from 5 min to 30 min. Xylanase activity was assayed using the assay method as described by Miller (1959) and the optimized incubation time was used for further studies.

Optimization of substrate concentration

Optimization of the most suitable substrate concentration for maximum xylanase activity was accomplished by using xylan in the range 0.1% to 0.8% (w/v). Xylanase activity was assayed using the assay method as described by Miller (1959) and the optimized substrate concentration was used for further studies.

Effect of metal ions & inhibitors

The effect of metal ions on xylanase activity was investigated by using different metal ions at 1mM concentration in the production medium individually. The enzyme activity was assayed using supernatant harvested after 36 h.

RESULTS AND DISCUSSION

Enzyme production

Xylanase production from *Pseudomonas*sp. XPB-16 was carried out in optimized culture conditions in production medium (pH 7.5) containing fructose- 0.75g, ammonium sulphate- 1.0g, yeast extract- 5.0g, KH_2PO_4- 0.15g, $MgSO_4$- 0.015g, xylan- 0.4g (%,w/v) at 37 °C for 36 h at 160 rpm. Briefly when *Pseudomonas* sp. XPB-16 was cultivated in optimized culture conditions; a 5.586 fold increase in xylanase production was recorded in comparison to xylanase production under unoptimized conditions.

Buffer system and pH optimization

Potassium phosphate buffer (pH-7.0) (Fig 1) proved to be the most suitable for maximum xylanase activity (1.595µmol/min/mL) among various buffers tested. Comparable enzyme activity was also observed in Sodium phosphate buffer. Least xylanase activity was observed in glycine-NaOH buffer. (Sharma & Chand, 2012) recorded maximum enzyme activity for xylanase from *Pseudomonas* sp. XPB-6 in sodium phosphate buffer at pH -7.0.Sharma *et al.*, 2013 reported maximum activity for xylanase from *Paenibacillus macquariensis* at pH- 8.6. (Chang *et al.*, 2017) reported maximum activity for xylanase from Bacillus subtilis Lucky9 in sodium phosphate buffer at pH- 6.5.

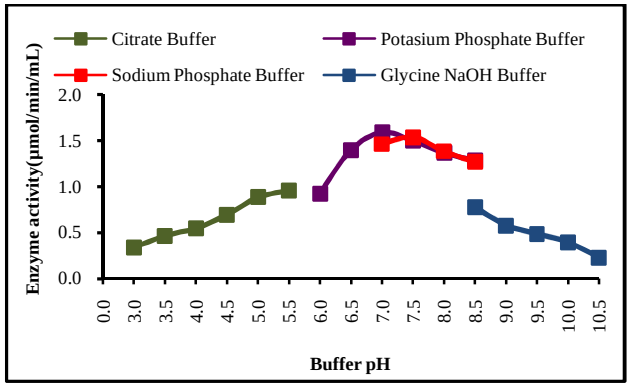

Fig 1 Optimization of buffer system & pH

Optimization of buffer molarity

Maximum xylanase activity (1.641µmol/min/mL) was observed in 50mM potassium phosphate buffer at pH 7.0 (Fig 2) while lowest activity (1.403µmol/min/mL) was observed in 125mM potassium phosphate buffer.(Sharma & Chand, 2012) reported maximum enzyme activity at 100mM Sodium phosphate buffer in *Pseudomonas* sp. (Chang *et al.*, 2017) reported maximum activity for xylanase from *Bacillus subtilis* Lucky9 in 20mM sodium phosphate buffer.

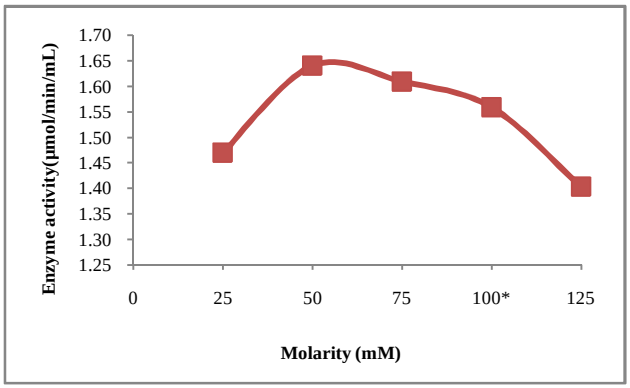

Fig 2 Optimization of buffer molarity

Optimization of reaction temperature

Maximum xylanase activity (1.749µmol/min/mL) was recorded at 37 °C and least activity (0.779µmol/min/mL) was recorded at 50 °C (Fig. 3). Sharma *et al.*, 2013 reported maximum activity for xylanase from *Paenibacillus macquariensis* at 50

°C. Kumar *et al*., (2014) reported maximum xylanase activity at 35 °C for xylanase from *Bacillus atrophaeus* E8. (Chang *et al*., 2017) reported maximum activity at 60 °C for xylanase from *Bacillus subtilis* Lucky9.

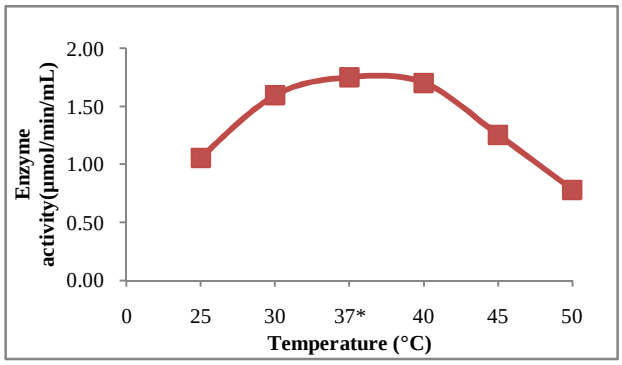

Fig 3 Optimization of reaction temperature

Optimization of incubation time

Maximum enzyme activity (1.788µmol/min/mL) was recorded at 20 min of incubation and least activity was recorded at 5 min of incubation (Fig. 4). Yin *et al*., (2010) reported 30 minutes reaction time optimum for xylanase from *Bacillus* sp. YJ6. Gupta *et al*.,(2012.) reported same incubation time (20min) for xylanase from *Bacillus* sp. KS09.

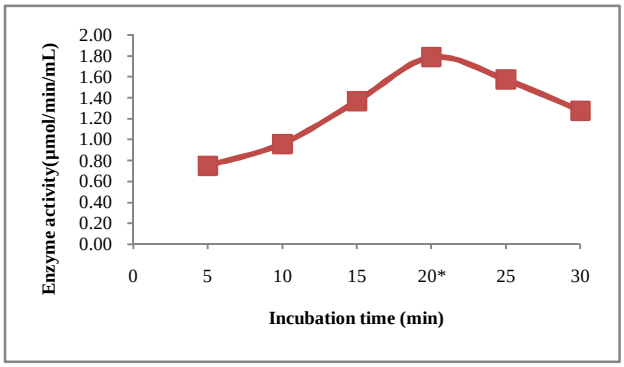

Fig 4 Optimization of incubation time

Optimization of substrate concentration

Maximum xylanase activity (1.877µmol/min/mL) was recorded with 0.6% (w/v) xylan concentration in the reaction mixture while least activity (0.549µmol/min/mL) was recorded with 0.1% (w/v) xylan concentration (Fig. 5).

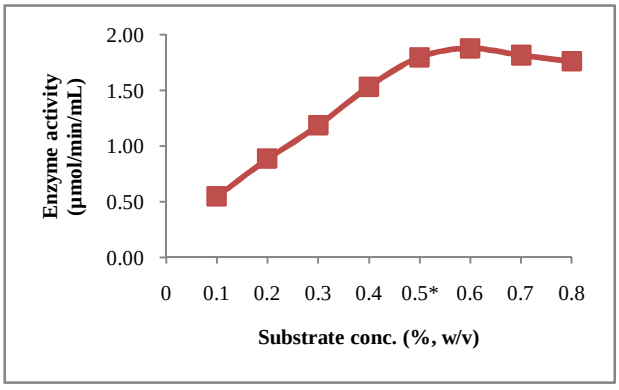

Fig 5 Optimization of substrate concentration

Kowsalya (2011) recorded maximum activity with 2.5% (w/v) xylan concentration for xylanase from *Bacillus cereus.*

Effect of metal ions & inhibitors

The effect of metal ions on xylanase activity was investigated by using different metal ions at 1mM concentration. Addition of Mg^{2+} and Fe^{3+} showed slight decrease in residual activity (87% and 79 % residual activity), Cu^{2+} showed 55 % residual activity whereas sodium azide and mercuric chloride showed considerable inhibition with 12% and 25 % residual activity. None of the metal ions tested showed increase in enzyme activity and maximum activity (1.863µmol/min/mL) was observed in reaction mixture without any metal (Fig. 6). (Kumar *et al*., 2013) reported that metal ions such as Co^{+2} and Mn^{+2} stimulated xylanase enzyme activity whereas Hg^{+2} inhibited the enzyme activity. (Dholpuria *et al*., 2014) reported that metal ions such as $Ca^{2+}$and Zn^{2+} enhanced the enzyme activity whereas Pb^{2+}, $Hg^{2+}$and Mn^{2+} strongly inhibited the enzyme activity.

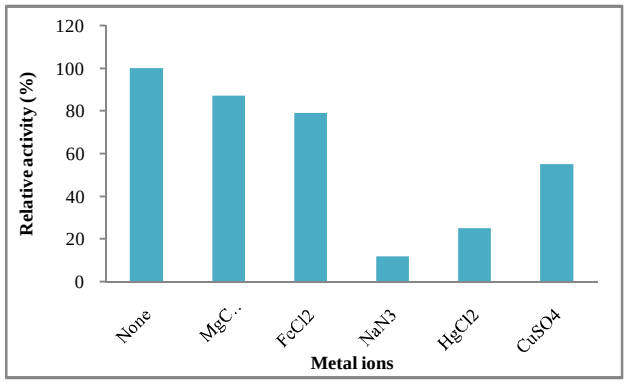

Fig 6 Effect of metal ions and inhibitors

CONCLUSION

The present work entitled "Production & Optimization of reaction conditions for xylanase from *Pseudomonas* sp.XPB-16" was taken up with a view of producing the xylanase enzyme from bacterial isolate *Pseudomonas* sp. XPB-16 under optimized culture conditions. Furthermore reaction conditions were optimized for maximum activity of xylanase. From our study we concluded that the enzyme showed increased activity under optimized reaction conditions. Since the enzyme xylanase has a broad range of applications; thus there is always an opportunity for a xylanase with better and improved characteristics which may find its utility in various applications in the commercial sector.

Conflicts of Interest

The authors declare that there are no conflicts of interest in relation to this research article.

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