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Biological Science

XYLANASE PRODUCTION BY *XYLARIA PRIMORSKENSIS* IN STATIC AND SUBMERGED FERMENTATION CONDITIONS

KEY WORDS: Hydrolytic enzymes; Xylanase; submerged; static; *Xylaria primorskensis*

Mohd Adnan*

Department of Biology, College of Science, University of Hail, Hail, P O Box 2440, Saudi Arabia. *Corresponding Author

ABSTRACT

Fungi are well known and most promising microbes for their ability to produce extracellular hydrolytic enzymes. Xylanases are the group of enzymes that are involved in hydrolysis of xylans, a major constituent of hemicelluloses and have various biotechnological applications. In the present study, *Xylaria primorskensis (X. primorskensis)* screened for xylanase producing ability. It was showing highest xylanase activity (0.398 U/ml) in first week of submerged condition. Comparative study showed that submerged condition was more suitable than static fermentation condition. Based on the results of culture conditions and xylanase activity, *X. primorskensis* was considered as a potential candidate for large scale xylanase production and its use in biotechnological processes.

INTRODUCTION

Xylans are the second most abundant natural polysaccharides, after celluloses in nature. It is the major components of plant cell wall (Collins *et al*., 2005). In dry weight of the woods, almost 20-35% compost with xylan constitutes (Goswami and Pathak 2013). Xylanase [E.C.3.2.1.8] are a class of hydrolytic enzymes which can attacks on β-1, 4 xylan and hydrolyze β-1, 4 glycosidic linkages randomly, which result realize of xylose residue (Tachaapaikoon et al. 2006; Chidi et al. 2008).

Microorganisms such as diverse genera and species of fungi, bacteria and actinomycetes are the potent sources of xylanase enzymes. Out of them, fungal species was known for secretion of large amount of xylanase. Several genuses such as *Aspergillus* and *Trichoderma* which produce high amount of xylanases are also used in industrial scale (Lu et al., 2008; Ramanjaneyulu et al., 2015).

Several other fungal genera including, *Chaeotomium thermophile, Humicola insolens, Thermosmyces lanuginosus* and *Thermoascus aurantiacus* (Ghatora et al., 2006), *Aspergillus Fumigatus* AR1 (Antony et al., 2003), *Penicillium citrinum* (Dutta et al. 2007), *Fusarium* sp. (Jorge et al., 2005; Gupta et al., 2009; Arabi et al., 2011; Sharma et al., 2017) and *Xylaria regalis* (Chang et al., 2005) have been reported for the xylanases production.

Xylanase plays a role in the bioconversion of plant biomass into the production of ethanol via delignification process (Olson and Hahn 1996) and also saccharification of waste material from industries and agricultural operations (Sá-Pereira et al. 2002). Production of enzymes in a large quantity is more important in any industries. In view of industrial and biotechnological applications, we have aimed our focus on screening of fungi for xylanase enzymes and comparisons of static and submerged fermentation for the xylanases production.

MATERIALS AND METHODS

Fungal strains

X. primorskensis used in this study is isolated from natural habitat and, previously identified by Adnan et al., (2018) using 16S rDNA with accession number Mg012860.

Purification of *Xylaria primorskensis*

The mycelia of *X. primorskensis* were cut into small pieces and placed on potato dextrose agar medium supplemented with chloramphenicol (100 mg/l) and incubated at 28°C for 4-5 days. After the growth of fungi, checked for purification, and stored at 4°C for further study.

Screening of *xylanase production*

Purified *X. primorskensis* fungal strains were screened for xylanase activity on potato dextrose agar medium containing 0.5% (w/v) birch wood xylan. Plates were incubated at 28°C for 4-5 days. After incubation, plates were stained with 1% congo red dye and destained with 1 M NaCl. Presence of yellow halo zone against red background indicates positive xylanase activity.

Submerged and solid state fermentation

The xylanase enzyme production was carried out by solid state and submerged fermentation. For the xylanase production, 5% active culture inoculated in 250 ml Erlenmeyer flask containing, 100 ml of MSM medium with 1% of birch wood xylan as carbon source. Incubate one flask on a rotary shaker at 150 rpm and second flask on incubator in static condition at 25°C. Measurement of enzyme activity was assayed at 1st week and 3rd week. 2 ml of the fermented broth was centrifuged at 10,000 rpm for 5 min at 4°C and obtained supernatant was used for enzymatic assay and protein estimation.

Estimation of xylanase enzymes

The clear supernatant (crude extract) was used for enzyme assay. The xylanase activity was assayed according to Bailey et al., (1992) by measuring the amount of reducing sugars (xylose equivalent) liberated from 1 mg/ml birchwood xylan in 0.05M Tris HCL buffer pH 8.5 at 50°C using 3, 5- dinitrosalicylic acid (Miller, 1959). The absorbance of the resulting colour was measured at 540 nm. One unit of enzyme activity was defined as the amount of enzyme liberating 1 µmol of reducing sugars equivalent to xylose per minute under the standard conditions.

Protein estimation

The protein content was estimated according to Lowry et al. (1951). Bovine serum albumin was used as a standard.

RESULTS AND DISCUSSIONS

In nature, degradation and recycling of lignocellulose material by various biotic and abiotic factors. Microorganisms such as bacteria, fungi, actinomycetes etc which produce and secrete necessary extracellular enzymes which help to depolymerization of lignocellulose material. In the present study, results showed that, *X. primorskensis* had xylanolytic activities which was confirmed by qualitative assay and comparative study for quantitative assay in solid state and submerged fermentation conditions.

X. primorskensis was previously reported for xylaranic acid (Adnan et al., 2018). In this study, purification of *X. primorskensis* strain was used for screening of xylanase activity (Figure 1A). In primary screening, clear hydrolyzing halo around the *X. primorskensis* (± mm) colony, (Figure 1B) was seen with further use for fermentation study.

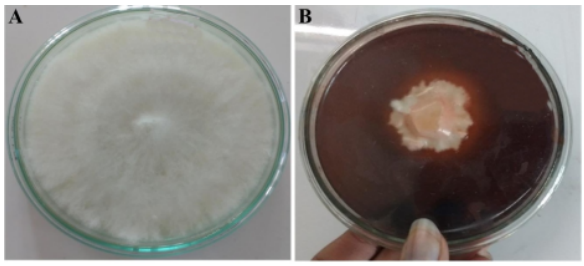

Figure 1: (A) *Xylaria primorskensis* purification on PDA plate and (B) xylanase activity on plate assay.

Two different experimental conditions were applied for xylanase production (Figure 2). After one week fermentation, the xylanase enzyme activity was 0.638 IU/ml and 0.812 IU/ml in static and submerged condition. Whereas, xylanase activity after three weeks 0.482 IU/ml and 0.105 IU/ml in static and submerged condition. Comparative study shown, 24% and 87% of xylanase activity was decreasing in 3rd week of fermentation in static and submerged condition, respectively. Simultaneously, protein estimation was also carried out and data shown the decreasing amount of protein with time period increases (Figure 3).

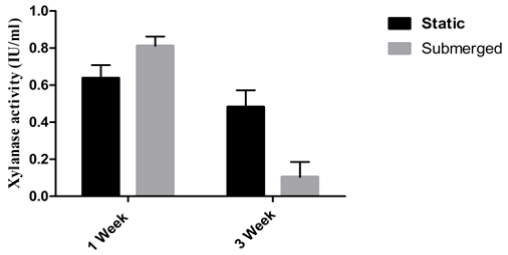

Figure 2: Xylanase activity on static fermentation and submerged fermentation conditions.

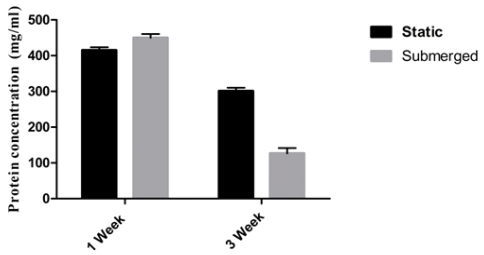

Figure 3: Protein concentrations on static fermentation and submerged fermentation conditions

The xylanase production is depend upon the various parameters such as fungi culture age, nutrients condition in fermentation medium, etc. according to Oyedeji et al., (2018), over incubation period led to decreasing levels of enzyme production, because of increased toxic metabolites and declining essential nutrients levels in the fermentation medium, resultant decreased growth and enzyme production. This type of similar result was reported by strains of *T. viride* (Simoes *et al*., 2009), *A. Niger* (Tallapragada and Venkatesh, 2011) and *T. viride* (Oyedeji *et al*., 2018), for xylanase production. Based on these experimental results, submerged condition is more favourable for short time high xylanases production, and static condition is good too for maintaining xylanases activity for longer period of time.

CONCLUSION

A result of this study has revealed that *X. primorskensis* exhibited extracellular xylanase production. In this comparative study, xylanase production increases during 1st week and decreases at 3rd week of fermentation. But in static condition, production of xylanase was more in the submerged fermentation during 3rd week. As a result of this, relatively high xylanase production, in static condition, *X. primorskensis* acts as a potential sources of xylanases for industrial and biotechnological applications.

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