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ENHANCING EFFECT OF AMINO ACIDS AND VITAMINS ON XYLANASE PRODUCTION BY *PLEUROTUS ERYNGII* THROUGH SUBMERGED FERMENTATION

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ABSTRACT

The purpose of this study was to investigate the effect of vitamins and amino acids on the enhancement of xylanase production synthesized by *Pleurotus eryngii.* Xylanase activity was checked by using oat spelt xylan as a substrate and the reducing group was detected through dinitrosalicylic assay method. The study reveals that vitamins are not good inducers in all cases and reduced the xylanase activity up to 22% when vitamin B_6 was supplemented with 0.6% starch and 1.0% corn steep liquor while amino acid valine enhanced 4% xylanase production.

INTRODUCTION

Xylan is the principal component of hemicelluloses from annual plants and hard woods. It consists of a linear chain of xylose residues joined by β-1-4- glycol-sidic linkages with various types and number of substitutions depending on its origin. Known substituents are O-acetyl groups, L-arabinose, D-O- methyl glucu-ronic acid and phenolic compounds such as coumaric and ferulic acids, xylanases have been produced by many fungi and bacteria and reports clearly indicates that xylanase produced from fungi usually has higher activity (Subramaniyan, *et al.,* 2002, Sa-Pereira, *et al.*, 2003, Collins, *et al.,* 2005) and Filaments fungi are more attractive xylanase producers than bacteria or yeast; because they excrete high level of enzyme in to the medium (Kulkarni, *et al.,* 1999) vitamins and amino acids have been known for their stimulatory effect on the production of number of enzymes such as α-amylase and xylanase (Ikura and Horikoshi 1987, balakrishnan *et al.,* 1997).

The aim of present work was to investigate the effect of various vitamins and amino acids on the growth and production of xylanase by *Pleurotus eryngii*

Material and Methods

Microorganism and culture conditions: In this study, mushroom strain *Pleurotus eryngii* was purchased from Edible fungi Institute, Shanghai Academy of Agri-cultural Sciences Shanghai, China. Stock culture of this fungus was maintained on Potato dextrose agar slants at 4°C. The medium used for xylanase production was composed of (g/l) 6.0g starch, 0.2g yeast extract, 0.5g peptone, 1.0g KH_2PO_4 and 0.5g $MgSO_4 \cdot 7H2O$. The medium was adjusted to pH 8.5 with 2M NaOH. The stock solutions amino acids and vitamins were filter sterilized and added to the sterilized medium at variable concen-trations of 0.05 and o.1% (w/v). The micro-organism was cultured in 50ml of medium in 250ml Erlenmeyer flasks and incubated at 30±2°C on a rotary shaker

(120 rpm). After 96 hours of mushroom cultivation, the fermented broth was separated by filtration through filter paper whatman No.1 and followed by centrifugation (5000*g*, 15min) at 4°C. Therefore, extracellular enzymes were extracted in order to measure enzyme activity.

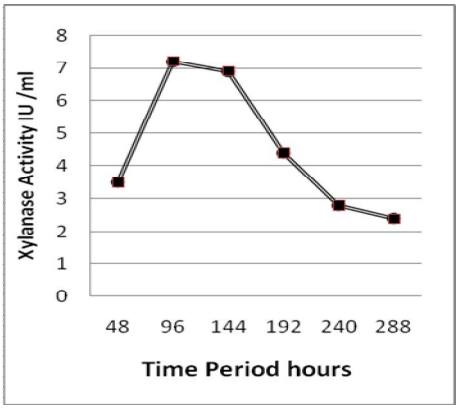
Xylanase assay: Xylanase activity was determined by mixing 0.5ml sample with 0.5ml of oat to xylan (Fluka, Germany) (1% w/v) in 50mM citrate buffer (pH 5.3) at 60°C for 15min (Bailey *et al.,* 1992). Xylose standard curve was used to calculate the xylanase activity. In assay the release of reducing sugars were measured using the dinitrosalicylic acid reagent method (Miller, 1959).

One International unit of enzyme activity was defined as the amount of enzyme, releasing 1µmol of reducing sugars per minute ml^{-1}.

RESULT AND DISCUSSION

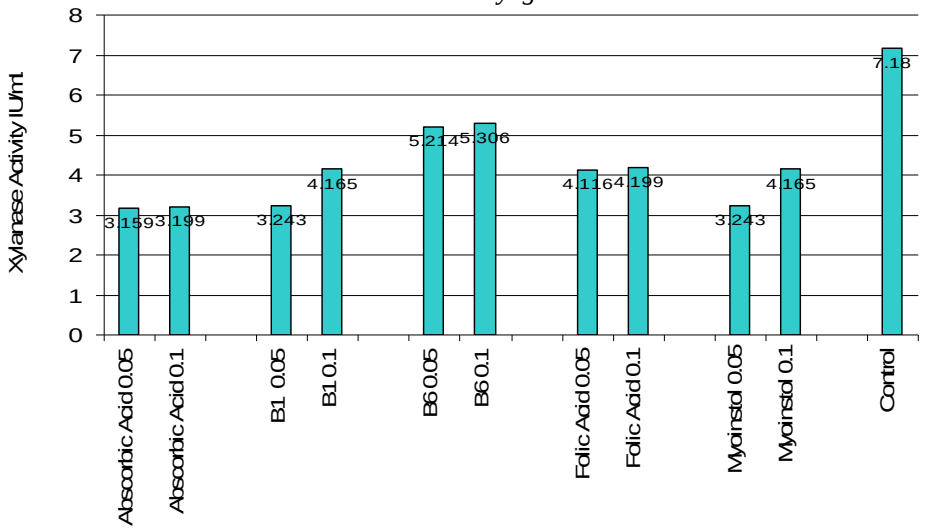
The effect of time period on the production of xylanase by *Pleurotus eryngii* is shown in Figure- 1. It is clear that optimum xylanase production achieved after 96 hours.

Figure-1: Effect of Time Period on the Xylanase production by *Pleurotus eryngii*



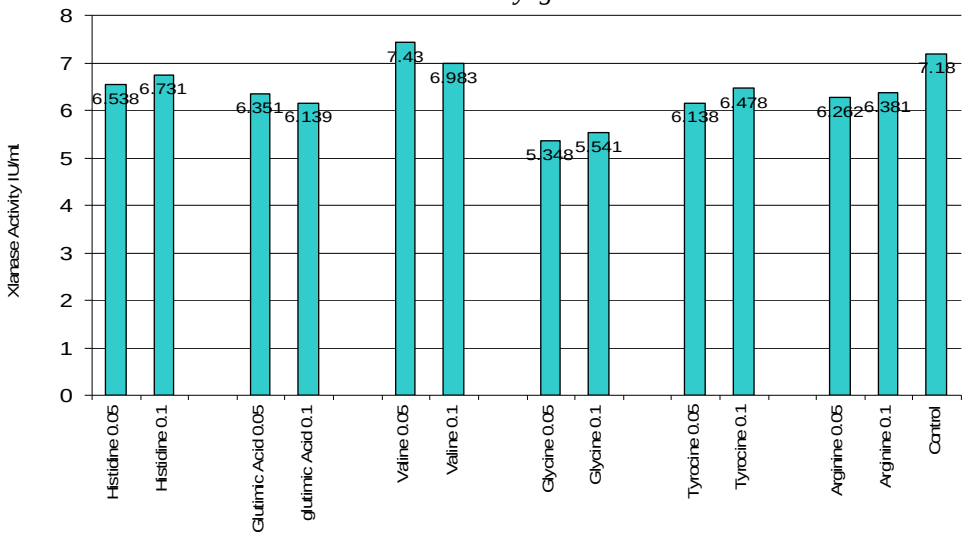
The effect of various vitamins such as ascorbic acid, B_1, B_6, folic acid and myoinstol (0.05 and 0.01% concentrations) in combination with 0.6% starch and 1.0% corn steep liquor was studied. The addition of vitamins in culture media had not proved as good inducer in all cases but vitamin B_6 reduces the xylanase activity up to 27% while other vitamins produced less than 40-50% xylanase activity as sown in Figure -2.

Figure-2: Effect of 0.05 and 0.01% Vitamins on growth and xylanase production by *Pleurotus eryngii*



The effect of amino acids like histidine, glutamic acid, valine, glycine, tyrosine and arganine in 0.05 and 0.01% concentration with 0.6% starch and 1.0% corn steep liquor combination was studied. The addition of amino acids in the culture media proved good for growth and xylanase production. Figure-3 shows that optimum xylanase production achieved when 0.05% valine was added in culture medium but above this concentration, xylanase activity was reduced. It is evident from the results that valine 0.05% posses ability to enhance the xylanase production up to 4%. Ikura and Horkoshi, (1987) have reported that 0.5% (w/v) glycine enhanced the xylanase production by 1.8-fold with Bacillus No. C-125. Balakrishnan *et al.,* (1997) have reported 2 to 5 fold enhancements in xylanase production by *Bacillus sp.* (NCL 87-6-10) with norvaline, glycine and casamino acids. It is reported that increase in linear chain of 2 to 4 carbon atoms stimulated the xylanase production by 5.5 fold but the mechanism behind the stimulation of xylanase production is not clear. The length of carbon chain and the position of $–CH_3$ and $–NH_2$ groups have a significant role in the stimulation of xylanase production (Ikura and Horkoshi, 1987, Balakrishnan *et al.,* 1997).

Figure-3: Effect of 0.05 and 0.01% amino acids on growth and xylanase production by *Pleurotus eryngii*



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