PROCESS STRATEGIES FOR ENHANCED PRODUCTION OF XYLANASE IN SUBMERGED CULTURE BY *Melanocarpus albomyces* IITD3A

by

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...Dedicated to those named and unnamed people
who have inspired me by their undaunted
spirit and zest for life...

CERTIFICATE

This is certify that the thesis entitled **"PROCESS STRATEGIES FOR ENHANCED PRODUCTION OF XYLANASE IN SUBMERGED CULTURE BY *Melanocarpus albomyces* IITD3A"** being submitted by **Ms Ranjita Biswas** to the Indian Institute of Technology, Delhi for the award of the degree of 'DOCTOR OF PHILOSOPHY', is a record of the bonafide research work carried out by her, which has been prepared under our supervision in conformity with the rules and regulations of the "Indian Institute of Technology, Delhi". The research reports and the results presented in this thesis have not been submitted for any degree or diploma in any other University or Institute.

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PROCESS STRATEGIES FOR ENHANCED PRODUCTION OF XYLANASE IN SUBMERGED CULTURE BY *Melanocarpus albomyces* IITD3A

Abstract:

The wild type filamentous fungus, *Melanocarpus albomyces* IIS 68, produces many commercially valuable enzymes like xylanases (approx. 160 $IU.ml^{-1}$) along with xylan-debranching enzymes (Saraswat and Bisaria, 1997). For isolating high xylanase producing mutants, sporulation of the fungus was induced on potato carrot medium. The number of spores obtained was 1.8×10^7 per ml and the size of spores varied from 26 to 40 µm and their shape was mostly oval. The spores were subjected to chemical mutation. The mutant obtained after EMS treatment, *M. albomyces* IITD3A, was stable and produced high xylanase activity (310 $IU.ml^{-1}$) after 72 h at pH 6.0 and 70°C on wheat straw.

The spore count of 3.5×10^5 ml^{-1} was found to be optimum for development of seed culture containing mycelial pellets of 1-2 mm size. The seed culture at 5% v/v was used to inoculate the production medium containing soluble alkaline lignocellulosic extract (SALE) of wheat straw as the sole carbon source (Sahai et al., 2005). The amount of xylan present in the SALE was determined by the amount of reducing sugars released and it was found to be ~10 $g.L^{-1}$. The maximum xylanase activity produced by the mutant, *M. albomyces* IITD3A, was 415 $IU.ml^{-1}$ after 24 h. Urea was the best nitrogen source and C/N ratio of 10:1 was optimum for the production of

xylanase. A Box-Behnken design was applied to analyze the interactive effect of medium components like urea concentration, pH and inoculum size and to arrive at optimum values. A response (xylanase activity) surface plot was generated which showed sensitivity to pH of the production medium. Although the value predicted by the model for optimum production of xylanase was 407 $IU.ml^{-1}$ which matched with the experimental value of 400 $IU.ml^{-1}$, further improvement in activity was realized under controlled pH as predicted by the model.

When the pH of the production medium in 14 L bioreactor was controlled on-line at pH 7.8, the maximum xylanase activity obtained was 415 $IU.ml^{-1}$ after 36 h of fermentation. The maximum biomass yield was 3.2 $g.L^{-1}$ after 36 h and the total protein produced was 9.2 $g.L^{-1}$. Whereas on cycling the pH between 7.8 and 8.2, the maximum xylanase activity obtained was 415 $IU.ml^{-1}$ after 24 h. Hence, the productivity increased from 11,528 $IU.L^{-1}h^{-1}$ to 16,670 $IU.L^{-1}h^{-1}$. The fungal morphology changed from dispersed filaments at 400 rpm to small pellets of size 1-2 mm at 600 rpm. At higher agitation speed (600 rpm), the maximum xylanase activity obtained was 480 $IU.ml^{-1}$ at 24 h compared to 415 $IU.ml^{-1}$ obtained at agitation speed of 400 rpm. Hence, the change in morphology of the fungal biomass seems to have contributed to the improved activity at 600 rpm. Further, at optimum of aeration rate of 0.25 vvm, the maximum xylanase activity of 541 $IU.ml^{-1}$ was attained after 25 h. The overall volumetric productivity of xylanase was thus enhanced to 21,640 $IU.L^{-1}h^{-1}$. There was, thus, 6-fold enhancement in overall volumetric productivity of xylanase produced by the mutant in 14 L bioreactor compared to wild type. The translation of the results to 150 L bioreactor resulted in 450 IU.ml^{-1} activity in 24 h.

The storage stability of an enzyme is intended to enhance their shelf-life before being used in a process. The culture filtrate of xylanase could be stored with only 13% loss in its activity for more than 50 days at 37°C by addition of small amount of bactericide, thiomersal. Also the xylanase could be lyophilized to powder form for long term storage as it retained 100% activity when resuspended in phosphate buffer. The mutant was found to produce much lower protease activity (0.05 IU.ml^{-1}) compared to that produced by the parent (38 IU.ml^{-1}). The presence of low protease activity is also expected to result in longer shelf-life of the xylanase protein.

The xylanase obtained from *M. albomyces* IITD3A was tested on wood Kraft pulp for its pre-bleaching action. Along with improvement in the brightness of the pulp by 3.5 percentage points ISO, there was 15% reduction in consumption of chlorine during bleaching process. There was a little increase in strength properties of the paper also in terms of burst index to 4.10±0.17 kPa.m^2.g^{-1} (over 3.70±0.13 for untreated pulp), tensile index to 64.0±2.80 N.m.g^{-1} (over 59.0±2.70 for untreated pulp) and tear index to 3.60±0.22 mN.m^2.g^{-1} (over 3.20±0.18 for untreated pulp). Characterization of the bleach effluents after enzyme treatment showed a decrease in toxicity level as AOX level was reduced from from 2.12 kg.t^{-1} to 1.7 kg.t^{-1}. Also, the activity of cellulase produced by the mutant was negligible which supports its potential application in pulp and paper industry. The cDNA library of *M. albomyces* IITD3A was constructed in λZAP expression vector in *E. coli*

XL1-Blue MRF' host to fish out the xylanase gene and further overexpress in a suitable host.

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