Original Research Article

Factors Affecting Cellulase Production by *Curvularia pallescens* isolated from textile effluent

Abstract:

Effects of pH, temperature, incubation time, source of carbon and nitrogen were tested in submerged fermentation process in production of cellulose by *Curvularia pallescens* isolated from textile effluent. The production medium was prepared in distilled water, supplemented with 4.5% wheat bran, 0.05% KCl, 0.2% KH_2PO_4, (carbon source), yeast extract (nitrogen source), maintained with pH of 5.5 and incubated at 28^0C for 120h was found optimal for production of cellulose.

Keywords: Cellulase, *Curvularia pallescens*, textile effluent, submerged fermentation, wheat bran

INTRODUCTION

Cellulases are important industrial enzymes and find application in several industrial processes (Kang *et. al.*, 2004). Currently the most important application is the bio-bleaching of pulp, the production of dissolving pulp and, the treatment of wastewater. The cost of production and low yields of these enzymes are the major problems for industrial application. Therefore, investigations on the ability of the cellulose hydrolyzing microbial strains to utilize inexpensive substrate have been done (Kang *et. al.*, 2004; Haltrich *et. al.*, 1996). The enzyme is commercially used after extracting from many microorganisms especially fungal sources (Hanif *et. al.*, 2004; Kang *et. al*., 2004) of mostly terrestrial origin but less from marine sources.

Therefore, in the present study, the enzyme production was attempted in a fungus, *Curvularia pallescens* isolated from textile effluent for maximizing its production under optimal conditions in submerged fermentation by using inexpensive substrate wheat bran.

Comment [U1]: What about peptone, malt and yeast described in results?

MATERIALS AND METHODS

Organism and culture condition

Curvularia pallescens was isolated from textile effluent using serial dilution and spread plate method (Graca *et. al.*, 1997).

All the enzyme production studies were carried out under submerged conditions in the media contain~~ed~~ing ~~W~~wheat bran 4.5%, yeast extract 1.5%, glucose 1%, NH_4Cl 0.25%, Thiamine dichloride 0.05%, KH_2PO_4 0.2%, $MgSO_4.7H_20$ 0.05%, $CaCl_2$ 0.01%, KCl 0.05%.. 10 agar plugs of 8mm diameter of the fungus grown for 7 days on PDA culture plates were inoculated in

100ml of the medium. The flasks were incubated at 28^{0}C under shaker conditions at 120 rpm. Cultures were harvested on 5th day and assayed for cellulase activity.

Optimization of the medium

Standardization of the optimum condition for the growth of the isolated organism as well as for cellulase production was determined by varying temperature and pH of the specially designed media, carbon and nitrogen sources, inoculum size, incubation period, mechanical shaking with different speed during incubation.

Cellulase assay

The test fungus was assayed for total cellulolytic activity by filter paper assay (FPA) (Mandel *et al.*, 1976); endoglucanase (Cx) activity by carboxymethyl assay (CMC), cellobiohydrolase (C1) activity by cotton assay and β-glucosidase activity by using p-nitrophenyl-β-D-pyranoglucosidase (PNPG) method (Rosenberg *et al.*, 1975).

1 unit of FPA , CMCase and cotton activity was defined as the amount of enzyme that releases 1 micromole of glucose from the substrate per minute and 1 unit of β-glucosidase was defined as the amount of enzyme required to liberate 1 micromole of 4-nitrophenol per minute.

RESULTS

Glucose favoured cellobiohydrolase and endoglucanase activity in *C. pallescens* (30.35 and 21.24U/ml respectively) where as sucrose and fructose proved to be best for FPA (61.35 U/ml) activity and β-glucosidase (6.97 U/ml) activity respectively (figure-1).

Organic nitrogen sources used for optimization were peptone, malt and yeast. *C. pallescens* showed maximum FPA activity 38.59 U/ml, cellobiohydrolase activity 30.35 U/ml, β-glucosidase activity 3.08 U/ml in the presence of yeast where as endoglucanase activity 33.71 U/ml reported higher with malt extract (figure-2).

Comment [U2]: What about wheat bran indicated above?

$(NH_4)_2SO_4$ was reported as best inorganic nitrogen source for cellobiohydrolase, endoglucanase and β-glucosidase activities at 57.18, 56.82 and 6.77 U/ml repectively. FPA activity was shown highest at 87.59 U/ml with $NaNO_3$ (figure-3).

The test fungus achieved maximum FPA activity followed by cellobiohydrolase, endoglucanase and β-glucosidase activity at 46.76, 42.06, 26.94 and 3.56 U/ml respectively at pH 5.5 (figure-4). Temperature of 28^0C produced maximum cellulase activity. Highest activity recorded was of FPA (38.94 U/ml), followed by cellobiohydrolase (30.29 U/ml), endoglucanase (22.41 U/ml), and β-glucosidase (3.98 U/ml) (figure-5). FPA activity 38.65 respectively was obtained maximum for *C. pallescens* after 168 hrs whereas cellobiohydrolase, endoglucanase and β-glucosidase activities 40.29, 57.41 and 2.98 U/ml respectively were recorded highest at 120 hrs of incubation (figure-6).

Media containing various amounts of inoculi were used for studying the effect of inoculum size on lignocellulolytic activity. Results are shown in figure-7. Reported maximum FPA, Cellobiohydrolase, endoglucanase and β-glucosidase activities 37.94, 30.01, 22.24 and 3.98 U/ml by inoculation 10 disc of 8mm size in the production medium. *C. pallescens* also gave maximum cellulase production at 120rpm. Endoglucanase activity was observed highest followed by FPA, cellobiohydrolase and β-glucosidase activities as 38.59, 30.35, 27.41 and 1.91 U/ml respectively (figure-8).

DISCUSSION

The media optimization is an important aspect to be considered in the development of fermentation technology. The production of primary metabolites by microorganisms is highly influenced by their growth, which is determined by the availability of the nutrients in the substrates. Garcia *et al*., (2002) reported that submerged fermentation for aerobic microorganisms is well known and widely used method for the production of cellulase and xylanase. Chellapandi and Jani (2009) reported enhanced endoglucanase production by soil isolates of *Fusarium* sp. and *Aspergillus* sp. through submerged fermentation process. Papinutti and Lechner (2008) studied influence of the carbon source on the growth and lignocellulolytic enzyme production by *Morchella esculenta.* Arora and Sehgal (2010) reported production of cellulase and xylanase by *Scopulariopsis acremonium* through submerged fermentation using shake flask cultivation media. The effect of process parameters such as effect of temperature, pH and inoculum size was investigated. Maximum cellulase and xylanase having an enzyme activity of 694.45 and 931.25 IU, respectively, were produced at 30°C incubation temperature. The pH optimum to achieve these enzyme activities was 5.5 with an inoculums size of 1 x 10^5 spores ml^{-1} of tween – 80.

Gupta *et. al*., (1990) studied microbial proteins and cellulase production from cellulosic materials by *Coprinus attramentarius and* reported the optimum pH for protein production and extracellular enzymes (cellulase and xylanase) by *C. attramantarius*, utilizing cellulose to be 6 and optimum temperature 30^{o}C. The resulting enzyme activities were endoxylanase as 7.2 IU ml^{-1}, exoglucanase as 1.0 IU ml^{-1} and xylanase as 5 IU ml^{-1}. Li *et al.*, (2006) reported pH of 4.14 was reported to be optimum for the production of endoxylanase production by *Aspergillus awamori* under submerged fermentation which gave an enzyme activity of 28.25 U ml^{-1}.

Shear stress within the medium, which is directly related to the stirrer speed, has also been shown to have a marked influence on enzyme production by *Thermomyces lanuginosus* SSBP (Reddy *et. al.*, 2002; Singh *et. al.*, 2000). Acharya *et. al.*, (2008) reported maximum cellulase production by *Aspergillus niger* in submerged fermentation at 120 rpm. However Ojumu *et. al.*, (2003) observed maximum cellulase production by *Aspergillus flavus* Linn isolate NSPR 101 at agitation of rate 200 rpm.

Figure-1: Optimization of carbon source for lignocellulases production by *Curvularia pallescens*

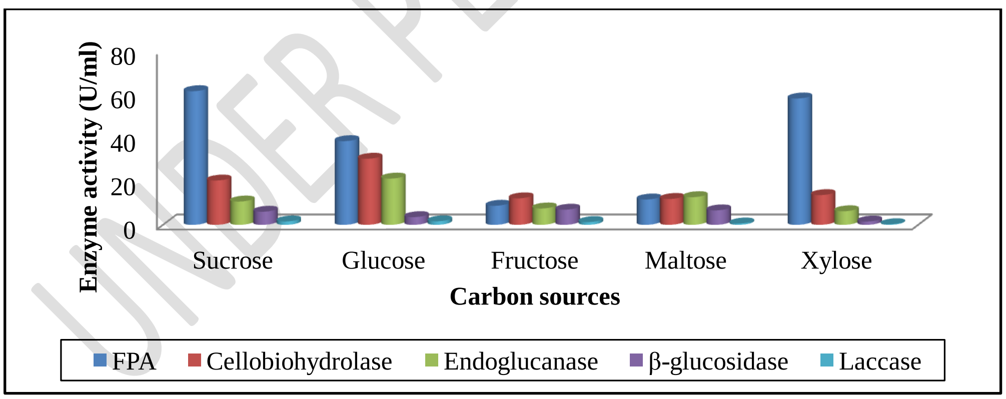


Figure-2: Optimization of nitrogen source (organic) for lignocellulases production by *Curvularia pallescens*

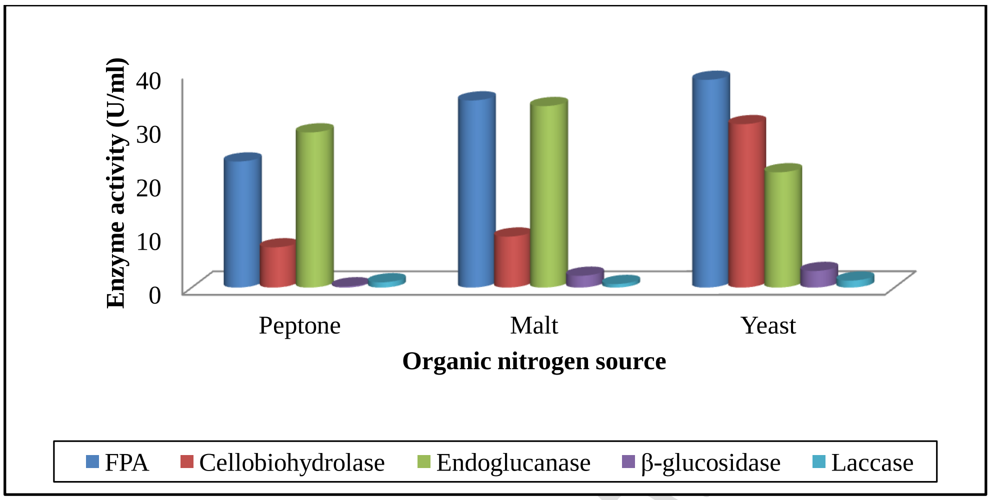


Figure-3: Optimization of nitrogen source (inorganic) for lignocellulases production by *Curvularia pallescens*

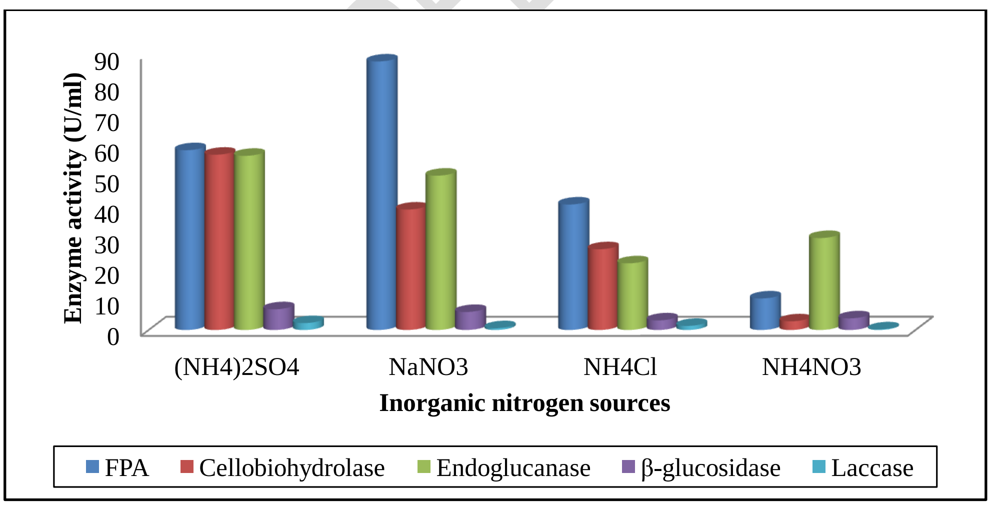


Figure-4: Optimization of pH for lignocellulases production by *Curvularia pallescens*

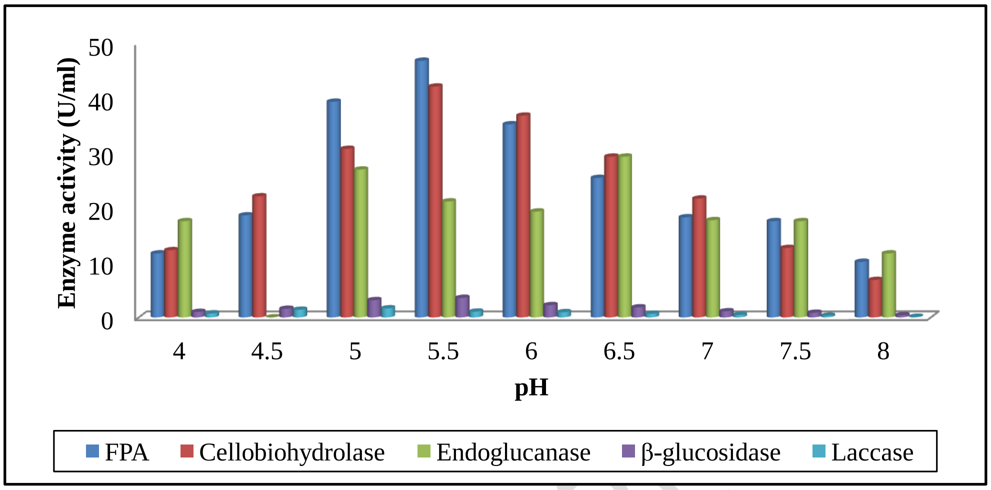


Figure-5: Optimization of temperature for lignocellulases production by *Curvularia pallescens*

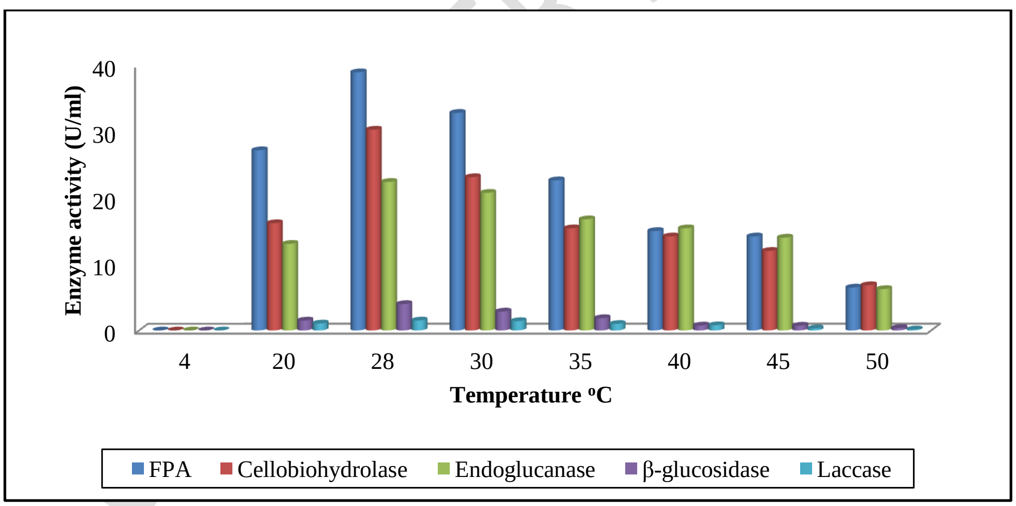


Figure-6: Optimization of incubation time for lignocellulases production by *Curvularia pallescens*

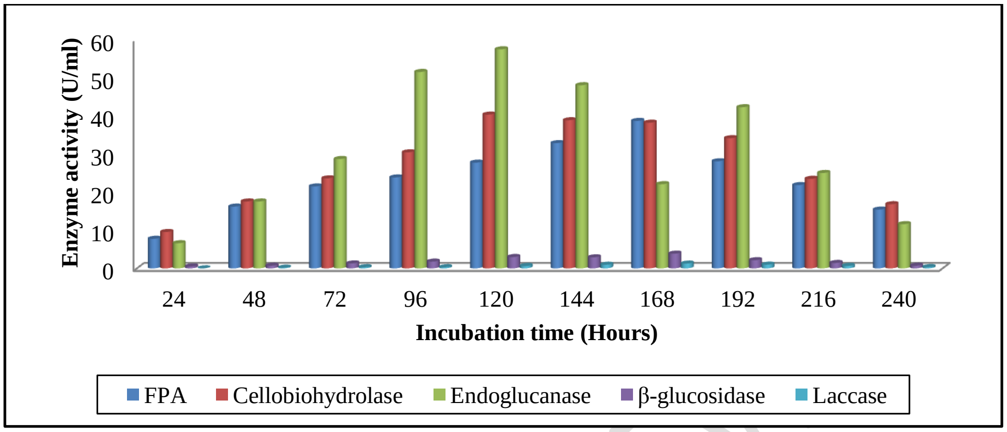


Figure-7: Optimization of inoculum size for lignocellulases production by *Curvularia pallescens*

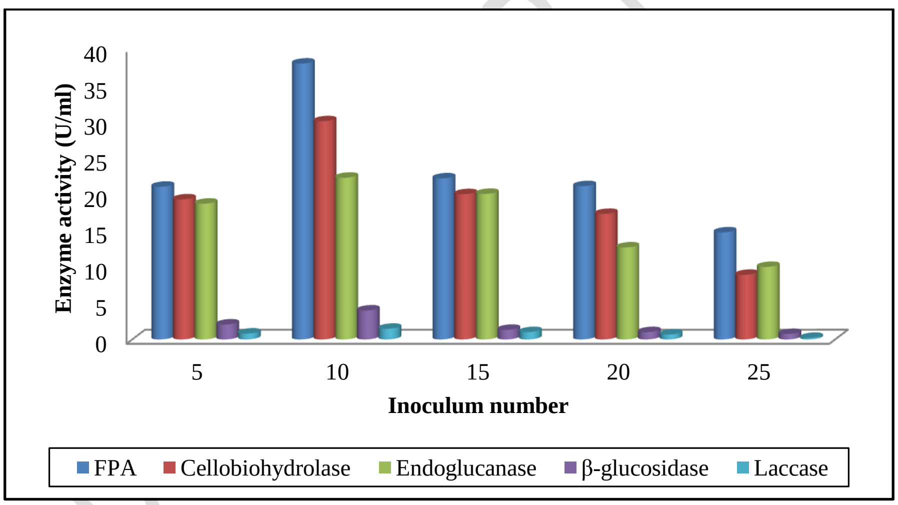
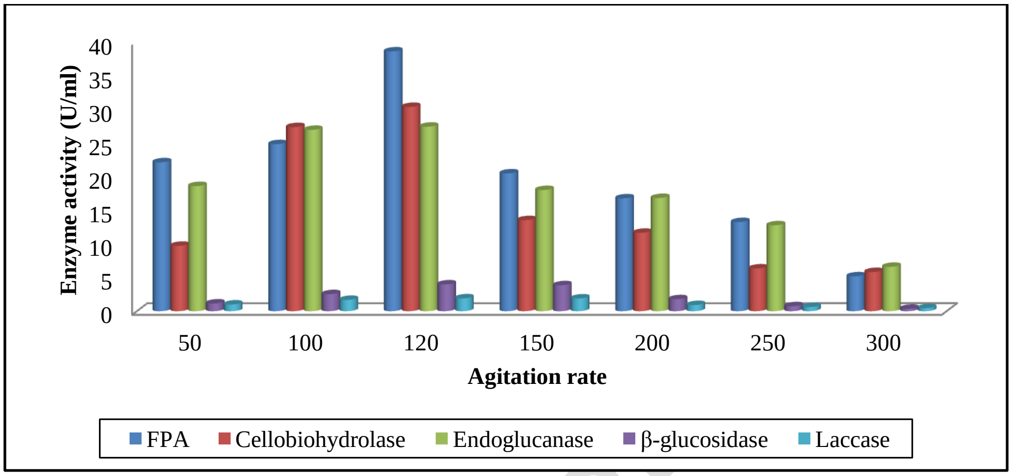


Figure-8: Optimization of agitation rate for lignocellulases production by *Curvularia pallescens*



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