Cotton Saccharifying Activity of Cellulases Produced by Co-culture of
Aspergillus niger and Trichoderma viride

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Abstract: Five different co-culture combinations (1:1 ratio, 2.75×10^7 conidia) of Aspergillus niger MSK-7 (A) and Trichoderma viride MSK-10 (T) i.e., mixing of A and T, mixing of T in 24 and 48 h old monocultures of A, similar mixing of A in 24 and 48 h old monocultures of T and the monocultures of both were evaluated for their potential performance of cellulases production in terms of cotton saccharifying activity. The results of present study showed that the cellulases obtained from compatible mixed cultures i.e., simultaneous mixing of both fungi have 59-66% more cotton saccharifying activity as compared to their pure cultures and other combinations. The fermentation experiments were performed in shake flasks using wheat bran as substrate with E & P as basal nutrition medium. Incubation time (24-168 h), carbon sources with different concentrations and initial pH of fermentation medium was optimized with simultaneous mixed cultures. It was revealed that the cellulose powder (1.0%) at pH 5.5 was the best source of carbon for the enhanced production of cellulases in the compatible mixed culture experiments after 72 h of incubation with 1451 U/h/L.

Key words: Aspergillus niger, Trichoderma viride, cellulases, cotton saccharifying activity, antagonistic effect, inducible enzymes, repressible enzymes

INTRODUCTION

Aspergillus and Trichoderma are the more potent producers of cellulase\textsuperscript{1-4}. The enzymatic degradation of waste cellulose by fungal cellulases has been suggested as a feasible alternate for the conversion of lignocellulosics into fermentable sugars and fuel ethanol\textsuperscript{5,6,10,19}. The cellulase system in fungi is considered to comprise of three hydrolytic enzymes: endo-1, 4-$\beta$-D-glucanase [carboxymethyl cellulase (EC.3.2.1.4)], which cleaves $\beta$-linkage randomly in the amorphous parts of cellulose; exo-1, 4-$\beta$-D-glucanase [celllobiohydrolase (EC.3.2.1.91)], which hydrolyzes celllobiose from either the non reducing or the reducing end, generally from the crystalline parts of cellulose and $\beta$-glucosidase [celllobiase (EC.3.2.1.21)], which releases glucose from celllobiose and short chain cellobioigosaccharides\textsuperscript{6,11}. Although $\beta$-glucosidase has no direct action on cellulose but it is regarded as a component of cellulase system because it completes cellulose hydrolysis\textsuperscript{11}.

The yield and regular supply of desired enzyme can be increased by establishing the methods of fungal cultivation and optimizing the fermentation conditions\textsuperscript{19}. The optimization for the rate of fermentation, carbon source as substrate and pH of the basal fermentation medium play fundamental role in the production of cellulases by Aspergillus niger and Trichoderma viride\textsuperscript{6,7}.

The present study describes the cotton saccharifying activity of cellulases obtained from mono and co-culture fermentation of Aspergillus niger and Trichoderma viride and evaluation of a basal fermentation medium for enhanced production.

MATERIALS AND METHODS

Organisms and culture maintenance: Aspergillus niger MSK-7 and Trichoderma viride MSK-10 strains were obtained from the stock culture of Institute of Industrial Biotechnology, Govt. College University, Lahore and were maintained on 4.0% potato dextrose agar medium. The cultures were maintained by regular transfer onto new slants and were stored at 4°C.

Inoculum preparation: The cultures were grown on PDA slants for 5 days, which were wetted by adding 10 ml of 0.005% sterilized Monoxal O.T (Di-octyl ester of sodium sulpho succinic acid). With the help of inoculating needle,
conidia were scratched and the tubes were shaken vigorously to break the clumps of conidia and to obtain homogeneous suspension. Conidial suspension was used as an inoculum.

**Conidial count:** The conidial count was made on a Haemacytometer slide bridge (Neubauer improved, Precicder HBG. Germany), following the method of Sharma.[13]

**Fermentation procedure:** A total volume of 1.0 ml of conidial suspension containing 2.75×10⁷ conidia/ml (*Aspergillus niger* and *Trichoderma viride*) was used to initiate growth in 250 ml Erlenmeyer conical flask containing 25 ml of E & P medium supplemented with (g/L): KH₂PO₄ (1.0), KCl (0.5), (NH₄)₂SO₄ (0.5), MgSO₄·7H₂O (0.2), L-asparagine (0.5), CaCl₂ (0.1) and yeast extract (0.5) with 1.0% wheat bran and 5.0 pH. After the inoculation, the flasks were incubated for 0-168 hours at 200 rpm in shaking incubator (SANYO, Gallenkamp, PLC, UK) at 30°C. The samples were withdrawn after every 24 h, centrifuged at 10,000×g for 10 minutes and the supernatant was estimated for cotton saccharifying activity of cellulases.

**Optimization of fermentation media:** A total of four different carbon sources e.g., carboxymethyl cellulose (CMC), cellulose powder, rice bran and wheat bran separately (1.0%) were chosen as test substrates on the basis of literature data and availability. All sources were tested in triplicates with 25 ml aliquot of E & P media.

**Saccharogenic activity:** The method described by Takao et al.[16] was used for the determination of cotton saccharifying activity. Sugar was estimated after Miller[40] by spectrophotometer (CECIL CE-7200 Series, UK), using a beam of 550 nm. One unit of cotton saccharifying activity was defined as, “the amount of enzyme required to liberate one micromole of reducing sugar from one gram of cotton in one hour.”

**Statistical analysis:** Treatment effects were compared after Snedecor and Cochrane [15] using computer software Costat, cs6204W.exe. Significance difference among replicates has been presented as Duncan’s multiple range tests in the form of probability (p) values.

**RESULTS AND DISCUSSIONS**

Rate of cellulases production (24-168 h) for CSA was carried out in shake flask fermentation by *Aspergillus niger* and *Trichoderma viride* using wheat bran as substrate with E & P medium (Fig. 1, 2 and 3). The results obtained from monocultures of *Aspergillus niger* and *Trichoderma viride* for CSA were compared with those from co-cultures to evaluate the ability of cotton saccharifying activity of cellulases produced. The maximum activity was found in the simultaneous co-culture of *Aspergillus niger* & *Trichoderma viride* i.e., 657 U/h/L after 72 h of incubation, which was about 59-66% more as compared with their monocultures and other co-cultures. Among the five combinations of two cultures, observations indicated that *Aspergillus niger* and *Trichoderma viride* (simultaneous co-culture) was found to be compatible and of the other four (24A+T, 48A+T and 48T+A) were incompatible due to their antagonistic effect.

It was also found that in all the three parameters tested, further increase in the incubation time decreased the activity of cellulases to degrade cotton. It might be due to the depletion of micro and macronutrients in the fermentation medium, which effected directly on the pH value of the medium and resulted in the inactivation of enzyme synthesis machinery with the passage of time.[15] In addition, the medium components were initially more susceptible to fungal digestion and made a rapid rise in enzymes biosynthesis. But with the prolongation of cultural time, the susceptible portions were completely hydrolyzed by microorganisms, which inhibited the enzyme secretion pathways.[12]

Cellulases are the inducible enzymes so cellulolytic cultures have been investigated with the use of avicel and other crystalline celluloses as carbon sources to induce cellulases production.[9] The nature of carbon sources also plays a vital role in the cotton degrading ability of cellulases. In the present study, a total of four carbon sources i.e., carboxymethyl cellulose (CMC), cellulose powder, wheat bran and rice bran were tested (Fig. 4). Among them, cellulose powder was proved to be the best, which gave 963 U/h/L (68%) cotton degrading ability after 72 h as compared with wheat bran i.e., 657 U/h/L. Cellulases production is directly proportional to the crystallinity of biomass from which it is produced i.e., higher the crystallinility, better will be the yield of cellulases.[18]

Effect of different pH of fermentation medium (ranging from 3.5 to 8.5) on the CSA was also optimized (Fig. 5) with simultaneous co-culturing of *Aspergillus niger* and *Trichoderma viride*. High acidic and high basic pH showed negative effects on CSA as acidic pH precipitate out all the soluble ions in the medium and highly basic pH cause lysis of the substrates like wise detergents, however a medium with slightly acidic pH i.e., 5.5 was ideal for CSA of cellulases produced by
Fig. 1: Rate of CSA of cellulases produced by mono-cultures of A. niger and T. viride and their simultaneous mixed-cultures.

Fig. 2: Rate of CSA of cellulases produced 24A+T and 24T+A mixed-culture combinations.

simultaneous co-culturing of A. niger and T. viride.

Conclusion

Strain compatibility is the determining factor for successful mixed culture fermentation. In the present study, observations revealed that simultaneous mixing of two cultures established compatibility but other four kinds of mix cultures were found to be incompatible due to their antagonistic effect. Therefore, the optimization of
Fig. 3: Rate of CSA of cellulases produced by 48A+T and 48T+A mixed-culture combinations.

Fig. 4: Effect of different carbon sources on the CSA of cellulases in compatible mixed-cultures.

compatible mix culturing of organisms having non-antagonistic behavior can be exploited for further research to establish consolidated bioprocessing with distinctive strengths and challenges, which exhibit the cost effective benefits.

ACKNOWLEDGEMENTS

This work was financially supported by Higher Education Commission of Pakistan.

REFERENCES


