RESEARCH ARTICLE

Analysis of cellulase systems from some fungi

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ABSTRACT

Although a variety of fungi are capable of growth on cellulosic substrates, only a dedicated handful of them can effectively hydrolyse native cellulose through production of cellulases. In the present study, the cellulolytic capabilities of seven cellulolytic fungal organisms viz. *Aspergillus niger* van Tieghem, *Chaetomium crispatum* Fuckel, *C. globosum* Kunze (2 isolates), *C. olivaceum* Cooke and Ellis (2 isolates) and *C. mollicellum* Ames were determined in terms of activities of enzymes, Endo- and Exo-1, 4 β glucanase. *Chaetomium olivaceum* demonstrated maximum activity of Endo- as well as Exo-1,4 β glucanase, followed by *C. crispatum*, *C. globosum* (isolate # g1), *C. olivaceum* (isolate # o2), *C. mollicellum*, *C. globosum* (isolate # g2) and *Aspergillus niger* for Endo-1,4 β glucanase; and *C. globosum* (isolate # g1), *C. crispatum*, *C. olivaceum* (isolate # o2), *C. mollicellum*, *C. globosum* (isolate # g2) and *Aspergillus niger* for Exo-1,4 β glucanase respectively. Enzyme activities were compared with those demonstra- ted by *Aspergillus niger*, a commercially exploited and well known cellulolytic biodeteriogen.

Keywords: *Chaetomium*, Exo-1,4 β glucanase, Endo-1,4 β glucanase, celluloses

INTRODUCTION

Cellulose, the most abundant natural organic compound on earth, makes up an integral part of the plant cell wall, and thus, a major constituent of plant matter. It is a well acknowledged fact that several microorganisms can grow on cellulosic material, however, only a few of these constitute an elite group that can extensively hydrolyse native cellulose by production of extra-cellular enzymes viz. cellulases. Plenty of research attention has been focused on increasing our understanding on the exact mechanisms by which fungi affect cellulose; several concepts and views having been put forward to explain the mechanism from time to time (Streamer *et al*., 1975; Chen, 2014); the phenomenon also having attracted some excellent overviews and reviews (Wood and Garcia-Campyo, 1994: Leschine, 1995).

The enzymatic hydrolysis of cellulose is known to involve synergistic action of three different groups of enzymes viz., endoglucanases (endo 1,4-β-glucanases), exoglucanases (exo 1,4-β-glucanases) and β- glycosidases. Endoglucanase is acknowledged to first act randomly on amorphous cellulose, causing reduction in degree of polymerization, forming cellobiose and glucose; exoglucanase is known to hydrolyse crystalline cellulose, starting from the ends of the chains, whereas cellobiase finally acts by separating the β- 1,4 glycosidic bond of cellobiose and small oligosaccharide molecules accompanied by formation of monomeric sugers (Bhat, 2000).

Recent research has been devoted to understanding the role of these enzymes in saccharification, identification and addition of new, superior fungal organisms in the restricted group of cellulolytic organisms (Jung *et al*., 2015), finding alternative and innovative techniques for higher yields of cellulases (da Silva *et al*., 2014; Hansen *et al*., 2015), experimenting with novel substrates (Singh, *et al*., 2015); and the innovative trend continues. Cellulases have diversified applications and many environment-friendly uses. Recent years have witnessed steps towards enhancement of their efficiency and cost efficacy (Dubey *et al*., 2014). A survey of literature on the subject revealed extensive but scattered and piecemeal data. In the present investigation, cellulose degrading abilities of 7 cellulolytic test organisms were determined in terms of activities of enzymes, Exo 1,4-β-glucanase (C_1 cellulase) and Endo 1,4-β-glucanase (Cx cellulase).

MATERIALS AND METHODS

Seven fungal isolates viz. *Chaetomium crispatum* Fuckel, *C. globosum* Kunze (2 isolates), *C. olivaceum* Cooke and Ellis (2 isolates), *C. mollicellum* Ames and *Aspergillus niger* van Tieghem, obtained from various cellulosic sources (Kolet, 2009; 2011) and characterized using standard literature (Gilman, 1967; Arx *et al*., 1986; Tzean *et al*., 1990) were used for the current study. Cellulase enzyme was obtained from the isolated fungi by shake flask fermentation. Reese liquid medium (Mandels and Weber, 1969) was utilized to determine the amount of production of cellulases by the fungal isolates and methodology as suggested by Bagool (1982) was adopted for the enzyme assay. Soluble proteins were determined as described by Lowry *et al*. (1951). The enzyme activities were determined by estimating reducing sugars, using DNSA reagent (Mandels *et al*.,1976). Activity of Endo-1,4 β glucanase was monitored as expressed in terms of reducing sugars released/mg protein/30 minutes; while that of exo-1,4 β glucanase was expressed as reducing sugars released /mg protein/24 hours. Enzyme activities were compared with that of *Aspergillus niger*, a commercially exploited and well known cellulolytic biodeteriogen.

RESULTS AND DISCUSSION

Analysis of enzyme activities revealed that *Chaetomium olivaceum* (isolate #o1) showed maximum activity of Endo-1,4 β glucanase, articulated in terms of reducing sugars released /mg protein/30 minutes, followed by *C. crispatum*, *C. globosum* (isolate #g1), *C. olivaceum* (isolate #o2), *C. mollicellum*, *C. globosum* (isolate #g2) and *Aspergillus niger*. The maximum activity with respect to Exo-1,4 β glucanase, expressed in terms of reducing sugars released /mg protein/24 hours, was demonstrated by *Chaetomium olivaceum* (isolate #o1), followed by *C. globosum* (isolate #g1), *C. crispatum*, *C. olivaceum* (isolate #o2), *C. mollicellum*, *C. globosum* (isolate #g2) and *Aspergillus niger*. The results, depicted in Fig. 1, are in agreement with those of El-Said *et al*. (2014). Lee (2015) hinted at C_1 cellulase as the entity determining the ultimate

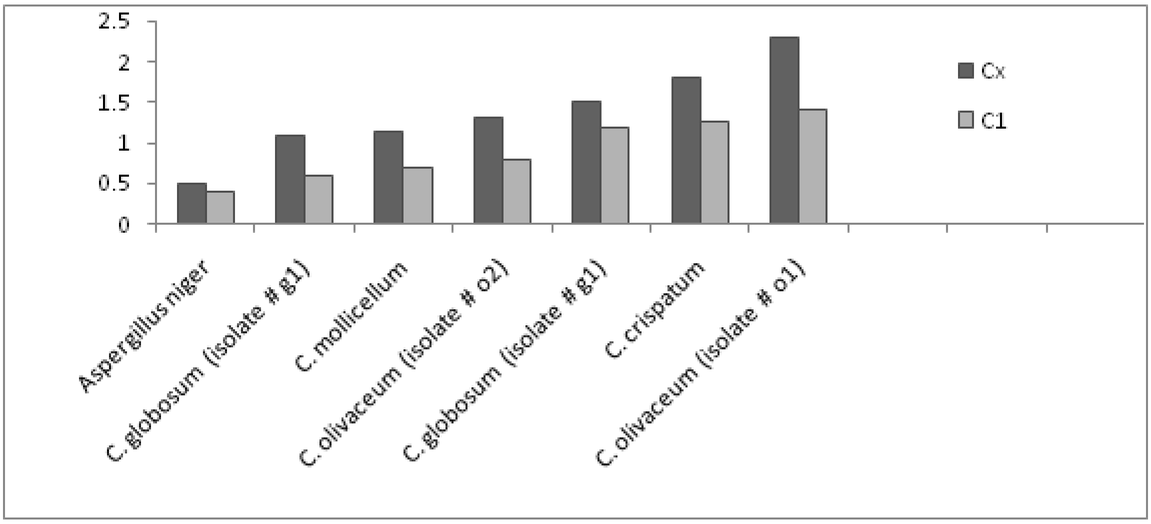

Fig. 1. C_1 and C_x enzyme activities (mg reducing sugars released /mg protein/ 24 hrs and mg reducing sugars released /mg protein/ 30 min) of *Aspergillus niger* and *Chaetomium* sps.

rate of hydrolysis. Earlier, the ratio of activities of enzymes C_1 and Cx cellulases was shown to indicate stability of the enzyme systems in respective organisms (Mandels and Weber, 1969), a key characteristic for their commercial exploitation. In accordance with this criterion, the maximum stability of cellulase complex, in the current investigation, was observed in *Aspergillus niger*. The ratios of activities of the enzymes from isolates studied in the current investigation are indicative of stable enzyme systems which could also be commercially utilized.

CONCLUSION

Seven cellulolytic fungal isolates were utilized for determining activity of cellulases. *Chaetomium olivaceum* (isolate # o1) demonstrated maximum activity of Endo-1,4 β glucanase as well as Exo-1,4 β glucanase, followed by *C. crispatum*, *C. globosum* (isolate # g1), *C. olivaceum* (isolate # o2), *C. mollicellum*, *C. globosum* (isolate # g2) and *Aspergillus niger* for Endo-1,4 β glucanase; and *C. globosum* (isolate # g1), *C. crispatum*, *C. olivaceum* (isolate # o2), *C. mollicellum*, *C. globosum* (isolate # g2) and *Aspergillus niger* for Exo-1,4 β glucanase respectively. The cellulase enzyme complex of *Aspergillus niger* was observed to demonstrate maximum stability.

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