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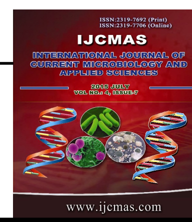

Original Research Article

Isolation and Screening of Cellulolytic *Chaetomium* sp. from Deteriorated Paper Samples

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A B S T R A C T

Useful books and important documents in storage having moldy appearance were analyzed for isolation of cellulolytic fungi. Several fungi were isolated in pure form and identified. The most dominated genus *Chaetomium* with 13 different species was undertaken for screening of their cellulase producing capability by the filter paper degradation ability. Eight *Chaetomium* isolates were chosen for exoglucanase and endoglucanase enzyme activity assay on the basis of percentage loss of filter paper. Five *Chaetomium* species were selected as potentially able to secret high exoglucanase and endoglucanase cellulases. *Chaetomium dolichotrichum*, *C. funiculosum*, *C. globosum*, *C. anguistispirale* and *Chaetomium* sp. were found very good producer of total cellulase and endogluccanase during study.

Keywords

Cellulose degradation, *Chaetomium*, Enzymes

Introduction

The biodeterioration of material is one of the major problems of civilization. From human prospective, biodeterioration is the breakdown of economically important substances mainly due to activities of microorganisms. They cause damage to wood product, pulp and wide range of cellulosic products including various households, books and papers. Paper left on moist ground or other damp condition for a period of time become badly damaged mainly by fungal growth due to their cellulolytic nature, since paper is composed of cellulose fibers of plant origin. In the other aspect these cellulolytic organisms have importance in natural environmental balance and in various industrial applications.

Now a days, cellulases gained significant commercial importance due to their potential applications in food processing, animal feed, detergents, paper and pulp, and textile industries (Penttila *et al.,* 2004; Urlaub, 2002; Oksanan *et al.,* 2000; Ishikuro, 1993; Cavaco-Paulo and Gubitz, 2003).

The demand for more thermostable, highly active and specific cellulases is on increase. Microorganisms, those are capable of producing cellulases have been isolated

(Beguim and Aubert, 1993). Attempts are going on to increase cellulase yields by mutation, protoplast fusion, and genetic engineering techniques (Lachke *et al.,* 1986). Most of these studies have been driven by potential industrial applications of celluloses. Fungal cellulases are inducible enzymes and have extra-cellular activity. The nature of the cellulase complex depends on the nature of cellulose (amorphous or crystalline). The amount of cellulose production varies with the cellulase producing organisms. This present communication deals with cellulase enzymes produced by different species of *Chaetomium* isolated from deteriorated paper collected from different location around Mumbai and screening of hyper-cellulase producer species and strains which can be used for biodegradation of municipal wastes.

Materials and Methods

Samples of deteriorated papers (book, Notebook, Newspaper, paper documents and waste paper) were collected from libraries, Store rooms and paper mills around Bombay. Small pieces of deteriorated portion of samples were cut (1g) and serial dilution method was used for isolation of cellulolytic fungi on selective media Czepek Dox Agar (CZA) ($NaNO_3$ - 3g, K_2HPO_4 - 1g, $MgSO_4.7H_2O$ - 0.5g, KCl - 0.5g, $FeSO_4$ - 0.01g, cellolose - 1%, agar - 15g, Distilled water - 1000 ml. pH 5.5 before autoclaving). A conical flask (150ml) with 50 ml CZA medium (with cellulose powder as the sole carbon source) was also used in addition to plates for isolation of slow growing cellulolytic fungi. After incubation at room temperature the visible fungal colonies were transferred on fresh PDA and CZA plates and purify the isolates using single spore culture. A trace quantity of streptopenicillin was added to suppress bacterial contamination at all stages during isolation and purification of the fungal isolates. Fungi isolated and purified by mono-cultures were identified up to the species level using standard literature and monographs (Pitt, 1979; Ellis, 1971; Samson *et al.,* 2000; Ames, 1969.).The identity of isolates was authentified by Fungus Identification Service MACS, Pune and cultures were deposited in National Facility for Culture Collection of India (NFCCI) at Agharkar Research Institute Pune, India.

Loss in weight of filter paper

The experiment was based on the method described by Fergus (1969). Filter paper disc (Whatman No.1) oven dried and weighed. The experiment was conducted in 90mm Petri plate. One filter paper disc was placed in each of the number of petry plates containing thin surgical cotton mat on the bottom. 10 ml of medium ($NaNO_3$ - 3g, K_2HPO_4 - 1g, $MgSO_4$.7H2O - 0.5g, KCl - 0.5g, $FeSO_4$ - 0.01g, Distilled water - 1000ml, pH - 5.5 before autoclaving). After being autoclave for 20 min. at 121 lb. plates were inoculated with spore suspension 0.5ml ($10^{-6/ml}$) of isolates grown for 10 days on CZA medium. The experiment was conducted in twelve plates for each isolate i.e. triplicates were maintained for monitoring activity at seven days interval up to 28 days (7, 14, 21 and 28 Days). A similar set was maintained in identical conditions as control and an equal amount of D/W was added as inoculums. The plates were incubating at room temperature for 28 days. At the end of respective incubation periods the filter paper discs were removed from the plates and dried in an oven at 80°C for 2 hrs and were allowed to cool to ambient temperature in a desiccator and then weighed. The difference in the weights of each filter paper disc was calculated by comparing it with the original dry weight and also considering the mean difference in weight shown by the control set. The

percentage loss in weight caused by each isolates was calculated by using the formula (Ghewande, 1977).

$$\% \text{ loss in weight} = \frac{\text{Difference in weight}}{\text{Initial weight}} X100$$

Production of extra-cellular enzyme in shake flask

Isolates for the production of extra-cellular enzymes were chosen on the basis of their filter paper degradation ability. The productions of extra cellular enzymes were carried out by shake culture in conical flask. 100ml of Reese liquid medium (KH_2PO_4 - 2.0g, KCl - 0.3g, Urea - 0.3g, NH_4 $(SO)_4$ - 1.4g, $MgSO_4$.7H2O - 0.3g, Peptone - 0.05g, Yeast extract - 0.10g, and traces of $FeSo_4$, $MnSO_4$, $ZnSO_4$, D/W - 1000ml (pH adjusted to 5.5 before autoclaving) was dispensed in 250ml conical flask. 0.5g cellulose powder and 0.1ml Tween 80 were subjected to add separately flasks. The flasks were autoclaved at 15lbs for 20 minutes. Ten days old cultures grown at room temperature on CZA slants were used as source of inoculums. 1 ml spore suspension (10^{-6}) inoculated into conical each flasks. The flasks were incubated at room temperature for seven days on a rotary shaker at 180rpm. After incubation the broth was filtered through glass wool and centrifuged at 6000 rpm for 15 minutes. The supernatants were used as crude enzyme for assayed their enzymatic activity.

Enzyme assay

The cellulolytic activity of the filtrate was determined by using the method described by Ghose (1987). Total cellulose activity was analyzed by measuring the amount of reducing sugar formed from cotton (substrate). Endoglucanase activity was assayed by measuring the amount of reducing sugar from corboxymethyl cellulose (CM-cellulose). The endoglucanase activity was determine by incubating 0.5ml of crude with 0.5ml of 2% CMC gum (Low viscosity CMC, SIGMA) in 0.05M sodium citrate buffer (pH 4.8) at 50^oC for 30 min. Specific cellulose activity was determine by incubating 1.0ml of crude with 50mg of cotton and 1.0ml of 0.05M sodium citrate buffer (pH 4.8) at 50^oC for 24hrs. 1.0ml of uninoculated medium incubated at similar manner serve as control. After incubation the reaction was terminated by adding 3 ml of 3,5-dinitrocalicylic acid (DNSA) reagent. A similar amount of crude was added in control set. Boiling the mixture for 5 min. and determined the absorbance at 550nm. In these test reducing sugars were estimated colorimetrically with DNSA. The enzyme activity of total cellulose and endoglucanase (CMCase) was defined in the International units (IU). One units of enzymatic activity is defined as the amount of enzyme that release 1µmol reducing sugar (measured as glucose) per ml per min. by using glucose as standard.

Results and Discussion

In most investigations, the species of fungal genus *Trichoderma* Pers. have been studied due to their ability to secrete enzyme cellulases. The cellulases secreted by *Trichoderma* have received widespread industrial interest leading to commercial applications (Oksanna *et al.,* 2002; Penttila *et al.,* 2004). The demand for more thermo stable, highly active and specific cellulases is on the increase, therefore cellulase systems of the other fungi also have to be investigated (Hanif *et al.,* 2004; Kamal and Mathur, 2005; Gopinath *et al.,* 2005; Ikram-ul-haq and Khan, 2006). *Chaetomium* is a very strong cellulose decomposer of ubiquitous occurrence. It is more thermo-tolerant than *Trichoderma* spp and other

commonly occurring cellulase degrading fungi.

A total of 48 isolates were obtained from the ten samples collected from store room, Libraries and paper mills. Twelve different genera of fungi were encountered to association with cellulosic paper materials along with three non sporulating (sterile mycelia) forms. Among the identified species 13 belonged to *Chaetomium* Kunze, 10 to *Aspergillus* Link, 6 to *Penicillium* Link, 3 to *Fusarium,* 2 *Syncephelestrum,* 2 *Rhizopus* and 3 *Cladosporium* etc.

The genus *Chaetomium* was found to be associated with all specimens. All the isolates of *Chaetomium* obtained were subjected to the experiment to determining their cellulose degrading ability in terms of loss in weight of filter paper. The species with the greatest cellulolytic activity were (BY-20) *Chaetomium dolichotrichum* 39.87%, (BY 28) *C. globosum* 36.66%, (BY24) *C. funiculosum* 36.6%, (BY17) *C. angustispirale* 35.01%, (BY26) *C. uniporum* 34.66%, (BY-4) *C. seminudum* 34.25% and (BY16) *Chaetomium* sp 33.33% in 28 days. The minimum activity was observed in isolate no BY-26b (18.75%) (Table 1).

Table 1 revealed that progressive decline in percentage of loss in weight in the subsequent weeks was probably related to a simultaneous decline in the available moisture content in the plates. On the basis of weight loss of filter paper 8 superior cellulolytic isolates were selected for comparative study of Total cellulase and CMCase enzyme activities.

Table.1 Screening of isolates for their cellulolytic activity in term of loss in weight of filter paper

Isolate No.	Organisms	Period of Incubation			
		7-Days % loss in Wt.	**14-Days** % loss in Wt.	**21-Days** % loss in Wt.	**28-Days** % loss in Wt.
BY-2	*C. cymbiforme*	10.55%	17.76%	25%	29.25%
BY-3	*C. incomptum*	8.18%	13.8%	17.7%	20.75%
BY-4	*C. seminudum*	11.75%	18.73%	28.03%	34.25%
BY-7	*C. subspirale*	12.6%	18.86%	25.87%	32.58%
BY-8	*C. cochliodes*	11.36%	17.58%	20.55%	26.36%
BY-11	*C. trilaterale*	7.68%	13.03%	18.66%	21.05%
BY-16	*Chaetomium sp*	13.68%	24.25%	29.71%	33.33%
BY-17	*C. angustispirale*	12.55%	25.0%	30.0%	35.01%
BY-20	*C. dolichotrichum*	12.54%	22.5%	32.05%	39.87%
BY-24	*C. funicolum*	11.53%	15.83%	30.0%	36.6%
BY-26	*C. uniporum*	14.53%	25.91%	27.5%	34.66%
BY-26b	*C. bostrychodes*	7.86%	10.91%	13.9%	18.75%
BY-28	*C. globosum*	15.0%	25.36%	30.0%	36.66%
Control	Control set	1.66% (+)	1.66% (+)	1.66% (+)	1.66% (+)

Mean initial weight of filter paper 600mg +gain in weight 10 mg (1.66%) in control set.

The enzyme activities were measured in terms of the production of reducing sugar end groups, which is taken to be indication of cleaves of cellulose molecules. Two standard substrates were used for the determination of cellulase activity in terms of total cellulase i.e. Exoglucanase (C_1) and endoglucanase CMCase (Cx) contents (Ghose, 1987). Pure cotton was used as the substrate to measure the total cellulase activity and reactive form of cellulose (Carboxymethyl cellulose, CMC) was used as a substrate for determining endoglucanase activity. Significant differences in activity were detected for two enzymes (Fig.1).

The C_1 cellulase activity levels were lower than the CMCase (Cx) for all isolates. The observation record revealed that the isolate BY-20 *Chaetomium dolichotrichum* Ames possessed maximum Cx and C1 enzyme activity (7.5 and 2.6 $U.ml^{-1}.ml^{-1}$ glucose). Isolate BY24 *Chaetomium funiculum* Cooke and isolate BY28 *Chaetomium globosum* Kunze exhibited almost similar Cx and C1 enzymes during study. The isolate BY 17 *Chaetomium angustispirale* Sergeeva produced significantly high amount of exoglucanase enzyme (5.1 $U.ml^{-1}.ml^{-1}$) the endoglucanse activity of the isolate less recorded (1.0 $U.ml^{-1}.ml^{-1}$). Isolate BY 16 *Chaetomium* sp. showed good activity both Cx and C1 enzyme (4.2 & 1.8 $U.ml^{-1}.ml^{-1}$). *Chaetomium uniporum* Aue & Muller isolate BY 26 exhibited minimum endoglucanase activity (0.35 $U.ml^{-1}.ml^{-1}$). *Chaetomium semidudum* Ames BY 4 and *Chaetomium subspirale* Chevers BY 7 exhibited lower Cx & C1 enzyme activities. Among the selected isolates the Isolates no. BY-20, BY-24, BY-28, BY-16and BY-17 showed a good filter paper degradation and enzyme Cx, C1 activities within 7 days, not all of them can be recommended as potential hyper cellulose producer.

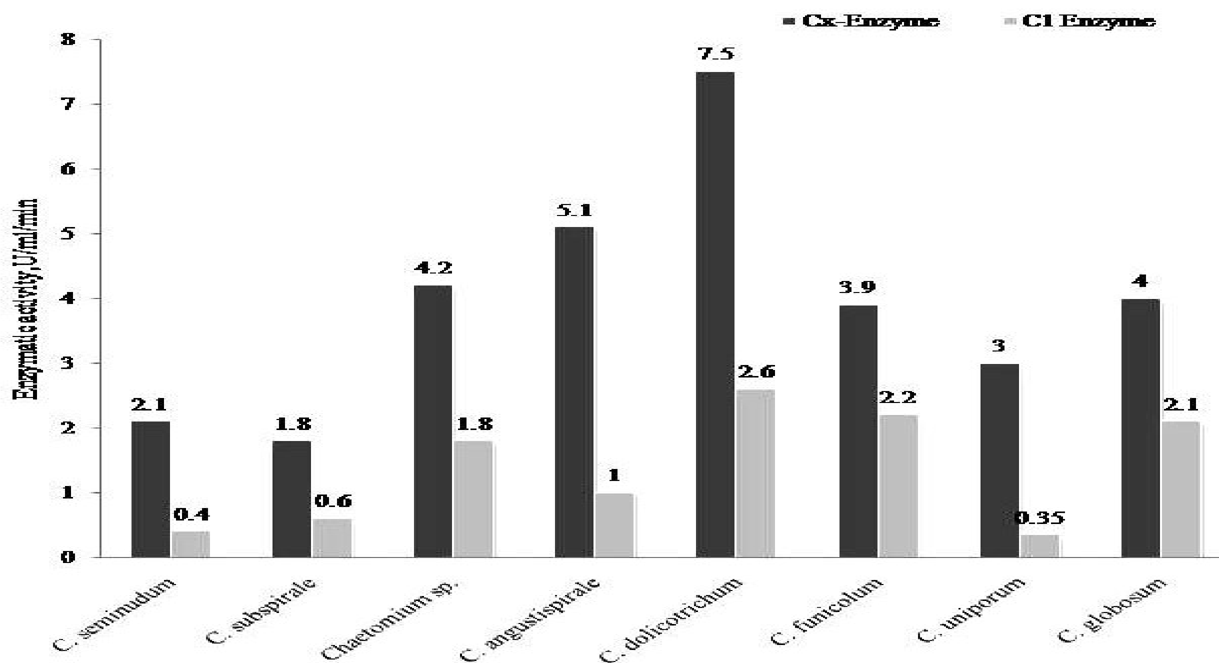

Fig.1 Exocellular endoglucanase (Cx) and total cellulose (C1) activity of *Chaetomium* isolates after 7 days. Each bar represents the mean of three replicates of enzyme activity ($U.ml^{-1}.min^{-1}$)

The isolates which exhibited high percentage of degradation of filter paper during screening of hyper cellulolytic isolates showed maximum activity during final selection on the basis of enzymes activities.

Cellulose degradation is one of the essential and indispensable biochemical cycles occurring in nature. Huge amount of garbage gets deposited on the ground which contains high amount of cellulose. The soil micro-organisms especially the fungi are performing the function of cellulose degradation. However, in the modern and more civilized society the amount of garbage deposited has increased enormously for which the natural process of decomposition is not sufficient. The garbage disposal has reached alarming situation in semi-urban and urban areas. The involvement of high cellulose producers can help in solving the problem to some extent. A formulation of hyper cellulases producer fungi can be used for conversion of garbage into compost, which will benefit the organic farming and restoration of soil fertility.

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References

Ames, L.M. 1969. A monograph of the *Chaetomiaceae*. Pp. 1–30.

Beguim, P., Aubert, J.P. 1993. The biological degradation of cellulose. *EFMS Microbial. Rev.,* 13: 25–58.

Cavaco-Paulo, A.,Gubitz, G. 2003. Catalyst and processing. In: Cavaco- Paul A., Gubitz, G. (Eds), Textile processing with enzymes. Woodhead Publishing Ltd., England. Pp. 86, 119.

Ellis, M.B. 1971. Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, England. Pp. 608.

Fergus, C.L. 1969. The cellulolytic activity of thermophilic fungi and Actinomycetes. *Mycologia,* 61(1): 120–129.

Ghewande, M.P. 1977. Decomposition of cellulose and production of cellulolytic enzymes by pathogenic fungi. *J. Biol. Sci.,* 20(2): 69–73.

Ghose, T.K. 1987. Measurment of cellulose activities. *Pure & Apll. Chem.,* 59(2): 257–268.

Gopinath, S.C., Anbu, P., Hilda, A. 2005. Extra cellular enzymatic activity profiles in fungi isolated from oil-rich environments. *Mycoscience,* 46: 119–126.

Hanif, A., Yasmeen, A., Rajoka, M.I. 2004. Induction, production, respression, and de-respession of exoglucanase synthesis in Aspergillus niger. *Bioresource Technol.,* 94(3): 311–319.

Ikram-ul-Haq, M.M.J., Khan, T.S. 2006. An innovative approch for hyper production of cellulolytic and hemicellulolytic enzymes by consortium of *Aspergillus niger* MSK-7 and *Trichoderma viride* MSK-10. *Afr. J. Biotechnol.,* 5(8): 609–614.

Ishikuro, E. 1993. Feed additives. In: Modern media, Vol. 46, Pp. 289–296.

Kamal, L., Mathur, S.N. 2005. Cellulolytic activities of *Chaetomium globosum* on different cellulosic substrates. *World J. Microbiol. Biotechnol.,* 5(1): 23–26.

Lachke, A.H., Bastawade, K.B., Powar, V.K., Srinivasan, M.C. 1986. Isolation of hyper cellulolytic mutant (Cu-1) *Penicillium funiculosum. Enzyme Microb. Technol.,* 8: 105–109.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J. 1951. Proetin measurement with folin phenol reagent. *J. Biol. Chem.,* 193: 265–275.

Lynd, L.R., Weimer, P.J., Van Zyl, W.H., Pretorius, I.S. 2002. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol. Mol. Biol. Rev.,* 66(3): 506–577.

Oksanan, T., Paavilainen, I., Buchert, J., Viikari, L. 2000. Treatment of recycling craft pulps with *Trichoderma resei* hemicellulases and cellulases. *J. Biotechnol.,* 78: 3–-48.

Penttila, M., Limon, C., Nevalainen, H. 2004. Molecula biology of *Trichoderma* and biotechnological applications. In: Arora, D. (Ed.). Handbook of fungal biotechnology. Marcel Dekker, Inc. Pp. 413–427.

Pitt, J.I. 1979. The genus *Penicillium* and its teleomorphic states *Eupenicillium* and Talaromyces. Academic Press, London. 634 Pp.

Samson, R.A., Hoekstra, E.S., Ftrisvald, O. 2000. Introduction to food and air born fungi. Centraalbureau Voor Schimmelculture, Ultrecht. 383 Pp.

Urlaub, R. 2002. Enzymes in fruits and vegetable juice extaction. In: Whitehurst, R., Law, B. (Eds.) Enzymes in food technology. Sheffied, Academic Press, CRC Press. Pp. 145–183.