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Enhanced Endoglucanase Production by Soil Isolates of *Fusarium* sp. and *Aspergillus* sp. through Submerged Fermentation Process

[Topraktan Batık Kültür Fermentasyonu ile İzole Edilen *Fusarium* sp. ve *Aspergillus* sp'de Artmış Endoglukanaz Üretimi]

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ABSTRACT

Objective: The objective was to optimize the fermentation media components and conditions to improve the production yield of endoglucanase by filamentous fungi isolated from garden soil.

Methods: Cellulolytic fungi were screened from garden soil and identified as *Fusarium sp.* and *Aspergillus sp.* by using conidial morphology. The influences of various culture conditions including incubation time, temperature, pH, carbon, nitrogen, surfactants and fermentation media on endoglucanase production by the isolates were investigated by submerged fermentation process.

Results: The optimum incubation time for maximum enzyme production was recorded between 3-48 hrs. The optimum temperature and pH were 38 ^{o}C and 5.0-5.5, respectively. Besides cellulose, lactose and cellobiose stimulated the enzyme production from both strains. Peptone and yeast extract were found as the best organic nitrogen sources for *Fusarium sp.* and *Aspergillus sp.* respectively. Both isolates yielded maximum endoglucanase (40-43 U/ml), when the growth medium supplemented with 0.06 % (w/v) KNO_3. The surfactants tried had a positive effect on endoglucanase secretion by both strains. Vogel's medium supported the growth of *Aspergillus sp.* and Aspergillus minimal medium also favored *Fusarium sp.* growth.

Conclusion: Optimization of culture conditions are necessary for improved production of endoglucanase by submerged fermentation process. Both isolates appears to be promising for the pilot-scale reactors with the use of our results. Moreover, as *Aspergillus sp.* and *Fusarium sp.* isolates are being rapid growing fungi and synthesizing endoglucanase alone apart from other cellulase components in liquid broth, it will be useful for cellulose bioconversion processes in food and alcohol industries.

Key Words: *Fusarium sp., Aspergillus sp.,* endoglucanase, cellulose, submerged fermentation

ÖZET

Amaç: Çalışmada bahçe toprağından izole edilen filaman mantarların ürettiği endonükleazın miktarını arttırmak amacıyla uygun fermentasyon ortamı bileşenlerinin ve koşullarının araştırılması hedeflenmiştir.

Yöntemler: Bahçe toprağında bulunan ve selülozu hidroliz edebilen mantarlar taranmış ve konidiyum morfolojilerine göre *Fusarium sp.* ve *Aspergillus sp.* tanımlanmıştır. İnkübasyon süresi, sıcaklık, pH, karbon, azot, yüzey aktif madde ve fermentasyon ortamı gibi kültür koşullarının endoglukanaz üretimine etkisi batık kültür fermentasyonu işlemi ile araştırılmıştır.

Bulgular: Batık kültür fermentasyonunda maksimum enzim üretimi için uygun inkübasyon süresi 3-48. saatler arasında; en uygun sıcaklık ve pH ise sırasıyla 38 ^{o}C ve 5.0-5.5 olarak saptanmıştır. Her iki suşta da selüloz, laktoz ve sellobiyoz, enzim üretimini arttırmaktadır. Pepton ve maya özütünün sırasıyla *Fusarium sp.* ve *Aspergillus sp.* için en iyi organik azot kaynağı olduğu bulunmuştur. Besi ortamına % 0.06 (w/v) KNO_3 eklendiğinde en yüksek endoglukanaz aktivitesi (40-43 U/ml) gözlenmektedir. Denenen yüzey aktif maddelerin, her iki suş tarafında üretilen endoglukanaz üzerine olumlu etkisi bulunmaktadır. Vogel's besi ortamı *Aspergillus sp.*'nin büyümesini desteklerken; Aspergillus minimal besi yeri *Fusarium sp.* tarafından tercih edilmektedir.

Sonuç: Batık kültür fermentasyonu ile elde edilen endoglukanazın üretiminin arttırılması için koşulların optimizasyonu gereklidir. Bizim bulgularımızın kullanımı ile her iki örnek de pilot-ölçekli reaktörler için umut verici görünmektedir. Ayrıca, *Aspergillus sp.* ve *Fusarium sp.* hızlı büyüyen mantar türleri olduklarından ve endoglukanaz sentezi sıvı besi ortamında diğer selülaz bileşenlerinden farklı olduğundan, gıda ve alkol endüstrisinde selüloz biyodönüşümü için faydalı olacaktır.

Anahtar Kelimeler: *Fusarium sp., Aspergillus sp.,* endoglukanaz, selülaz, batık kültür fermentasyonu

Introduction

Cellulases are commercially important for saccharification process in textile and alcohol industry. Furthermore, pure cellulase components are used for protoplast fusion and other biochemical techniques (1-2). Enzymatic hydrolysis of cellulose in industrial scales is hindered by the high cost of the enzyme and the low rates of conversion. Cellulase is a synergistic enzyme requiring participants of all components viz., endo 1, 4-β-glucanase (EC.3.2.1.4), exo 1, 4-β-glucanase (EC.3.2.1.91) and β-glucosidase (EC.3.2.1.21) for complete enzymatic hydrolysis of microcrystalline cellulose (3-5). Among these components, endoglucanase in cleaving cellulose is well studied (3-4, 6-7). Therefore, there has been many research directed towards finding new microbial sources with efficient endoglucanase synthesis, particularly amongst fungi (8). The species of *Trichoderma* (5, 9-10), *Penicillium* (11-12), *Aspergillus* (3, 13), *Fusarium* (14), *Curvularia* (6), *Pleurotus* (15), *Lentinula edodes* (Berg.) Pegl. strain CC17 (4), and *Streptomyces* (16) have already been identified as good cellulase producers with considerable commercial interests. *Aspergillus sp.* (13) and *Penicillium sp.* (13, 17) are generally used for the production of β-glucosidase, an enzyme necessary for the conversion of cellobiose to glucose because these fungi produced only low amounts of endoglucanase. Thus, the co-culture of *A.niger* and *Trichoderma viride* was grown by Gupta and Madamwar (18) to overcome the problem. Cellulases production by *P.brasilianum* and *T.reesei* was also comparatively studied to monitor the better performance under submerged fermentation (SMF) (7). However, neither *Aspergillus sp.* nor *Fusarium sp.* have been investigated individually for endoglucanase production capacities in industrial scale yet.

Thus, the present work mainly focused onto optimize different cultural conditions needed for rapid growing fungi *Aspergillus sp.* and *Fusarium sp.* isolated from garden soil in order to enhance the extracellular endoglucanase synthesis under submerged fermentation. Perhaps, this optimized fermentation conditions will be helpful while utilizing these organisms in large scale bioprocess systems.

Materials and Methods

Fungal strains and growth

The fungal strains were isolated from garden soil by using appropriate microbiological techniques. The fungal spores were taken from potato dextrose agar plate with an inoculation loop and then stained with lactophenol-cotton blue staining. Conidial morphology of the fungi was observed on a microscopic slide under a low-power (10x) light microscope. Accordingly, the strains were identified as *Fusarium sp.* and *Aspergillus sp.* as described in standard fungal taxonomy references (20). Among 16 fungal strains isolated from this study, two of the potential strains were selected according to considering maximum cellulolytic activity on Mandels broth (19) supplemented with 1 % (w/v) cellulose.

Inoculum preparation

In a 250 ml flask, one ml conidial suspension (3 x 10^6 spores/ml) from 7 days old potato/dextrose agar slant was transferred to 50 ml Czapek dox liquid medium (21) incorporated with 85 % (v/v) O-phosphoric acid swollen cellulose (Walsath cellulose) and then incubated in an orbital shaker (130 rev/min) at 28 °C. After 4 days incubation, the whole mycelia content was homogenized and then used as inoculum to initiate the fermentation processes in the study.

Fermentation techniques

For cellulase production, both strains were grown in 250 ml Erlenmeyer flasks containing 50 ml of Czapek dox medium (21) and 1 % (v/v) Walsath cellulose. Czapek dox medium (g/L) composed of 10 g sucrose, 10 g yeast extract, 1.0 g K_2HPO_4 and 10 ml Czapek dox salt solution. Czapek dox salt solution (g/L) has 30 g $NaNO_2$, 5.0g KCl and 5.0 g $MgSO_4$. The pH of the medium was initially adjusted to 5.6 with 0.1 M NaOH. One ml (v/v) of the above inoculum was used to initiate fermentation. The inoculated flask was incubated at 30 °C in an orbital shaker (130 rpm) and every 12 hrs aliquots were taken aseptically and then filtered through Whatman no. 1 filter paper. This filtrate was again centrifuged at 6000 rpm for 10 min and preserved at -20 °C until the next use. A study on the influence of different nutrients includes carbon, nitrogen, and detergents on endoglucanase synthesis were examined by replacing appropriate sources in Czapek dox liquid medium supplemented with 1 % (v/v) Walsath cellulose. The effect of pH on endoglucanase production was studied by incubating culture in production medium at different initial pH (4.0-8.5) under shaking condition at 28 °C. The culture broth was also subjected to incubate at different temperature (20, 25, 30 and 37 °C) to study on the effect of incubation temperature for endoglucanase synthesis. The cell-fee culture supernatants were used to estimate enzyme activity.

Endoglucanase assay

Endoglucanase activity was measured by using a reactive mixture containing 0.5 ml of 1 % (w/v) carboxymethylcellulose (CMC) in 0.1 M citrate buffer (pH 4.8) and 0.5 ml culture supernatant (22). The liberated reducing sugars were estimated with 3,5 dinitrosalicyclic acid (DNSA) reagent (23) after incubating the reactive mixture for 30 min at 40 °C. One unit of enzyme activity expressed as the amount of enzyme required to release one μmol reducing sugar/ml under the standard assay condition.

Results and Discussion

Fungal strains were primarily screened from garden soil on the basis of potential growth of mycelia on Mandel's

agar medium supplemented with cellulose as a sole carbon source. Fast growing filamentous fungi that grew on agar plate were selected for secondary screening. Efficient cellulolytic activities in liquid medium as well as rapid growing capabilities were used as criteria to select the isolates for this study. On PDA plates, isolate 1 developed well a flourished aerial growth and black conidiospore while isolate 2 gave a luxuries growth, pale pink, cottony aerial mycelia. According to conidial morphology of these filamentous fungi, we identified isolate 1 as *Aspergillus sp.* and isolate 2 as *Fusarium sp.*

Figure 1 shows the effect of incubation time on endoglucanase production by *Fusarium sp.* and *Aspergillus sp.* by using pre-grown mycelia as inoculants in SMF. Measurements showed that a maximum enzyme activity of 30 U/ml was obtained at 48 hrs growth of both isolates and afterward activity gradually declined. Steiner et al. (17) found the similar result for *P.purpurogen* when pre-grown mycelia used as inocula in SMF. Pre-culture grown (4 days) in Mandel's medium containing mixture of 0.5 % cellulose plus 0.5 % glucose gave dense *T.reesei* growth and more endoglucanase activity (5.5 U/ml) after 12 days when transferred to production medium (10). A rapid cellulase production was obtained in a shortened lag period by using a mycelial inoculum of *T.reesei* (19), and also *Aspergillus* and *Fusarium* genera are generally fast growing fungi in heterotrophic environment. Thus, such pre-grown mycelia of the fungal isolates were supported to synthesis extracellular endoglucanase rapidly at initial growth phase (48 hrs) in this study.

The pH effect on endoglucanase production by both strains was compared. At initial pH of 5.0-5.5 *Aspergillus sp.* had more endoglucanase activity (31.4 U/ml) than *Fusarium sp.* (20 U/ml). In addition, when the effects of different temperatures were checked, there was no major difference found on enzyme yield by *Fusarium sp.* until incubation temperature ranged from 30 to 40 □C. However, raising temperature above 30 □C unfavorably affected the *Aspergillus sp.* growth as well as endoglucanase synthesis. Generally, the optimum temperature and pH of these strains are 28-30 °C and 4.6-5.2, which have been reported as similar to ours in many previous reports (3, 13, 17).

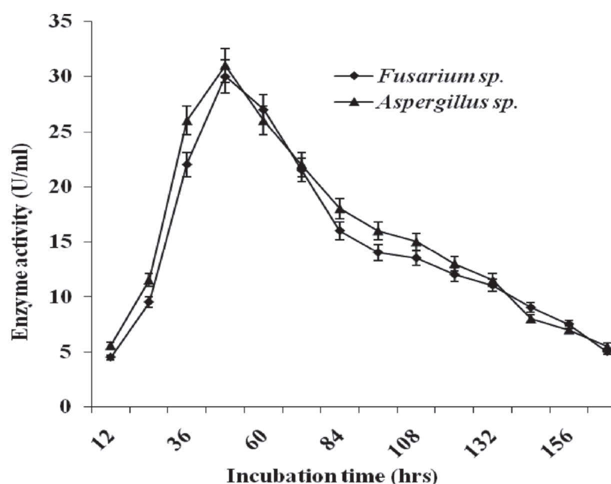

Figure 1. Effect of incubation time on the production of endoglucanase in SMF.

As shown in Table 1, the maximum enzyme yield in the production medium obtained by *Aspergillus sp.* in the presence of 0.5 % cellobiose, which was somewhat comparable with endoglucanase of *Fusarium sp.* Similar results have been obtained in *Aspergillus sp.* for cellulase production under SMF (13, 24-25). Unlike cellobiose, microcrystalline cellulose did not induce the endoglucanase yield. Exceptional stimulation for endoglucanase excretion by *Fusarium sp.* was recorded when production medium supplemented with lactose. The yield was greater than of by *Aspergillus sp.* The effect of different lactose concentrations on endoglucanase production studied further and the results were shown in Table 2. It was found that the optimum concentration of lactose was 0.5 % in fermentation medium for both organisms. In addition, any further increase in concentration of Walsath cellulose above the range of 1.5 to 2.0 % has no measurable effect on cellulase induction suggesting the presence of substrate saturation limit for both organisms. At this concentration, *Fusarium sp.* and *Aspergillus sp.* gave endoglucanase activities of 47.4 U/ml and 26 U/ml respectively. This may be resulted from a desirable saturation of carbon sources and an efficient uptake mechanism of these fungi (5, 26).

Organic nitrogen sources (0.5 %) did favor the dense growth of the organisms and also supported the synthesis of cellulase (Table 3). The concentrations ranged from 0.5 to 1.0 % did not cause any considerable differences in the production yield. Apparently, peptone was a good organic nitrogen source for *Fusarium sp.* (37.7 U/ml) while yeast extract was for *Aspergillus sp.* (37.7 U/ml). In this study the optimum concentration of peptone was found to be 0.25%. Similarly, Gayal and Khandeparkar (11) reported that filamentous fungus *P.funiculosum* produced the highest endoglucanase production (26 U/ml) at this concentration. On comparing both organisms, the endoglucanase yield obtained by *Fusarium sp.* (44.4 U/ml) was similar to that by *Aspergillus sp.* (43 U/ml). Thus, this suggested that organic nitrogen sources are useful of improving production of endoglucanase apart from other nutrients sources examined. KNO_3 was also reported by Hamilton and Was as stimulatory on cellulase activity of *A.terreus* and *A. fumigatus* (13). Likewise, KNO_3 was identified as the best inorganic nitrogen source for enhanced endoglucanase by both organisms in this work. The optimum concentration of KNO_3 was 0.06 % (Table 4). A relative high yield was also observed when $(NH_2)_4H_2SO_4$ added to fermentation medium.

Several investigations have been reported that extracellular cellulase production increased with addition of surfactants in the production medium due to the increasing permeability of cell membranes (27-29). Nevertheless, high surfactants concentration could inhibit the spore

Table 1. Influence of supplementing carbon source (1%, w/v) in the fermentation media for endoglucanase production in SMF.

Carbon source	Enzyme activity (U/ml)	
	Fusarium sp.	***Aspergillus sp.***
Arabinose	26 ± 1.1	21 ± 0.9
Fructose	17 ± 0.4	16 ± 0.6
Cellulose	04 ± 0.2	06 ± 0.3
Sucrose	16 ± 0.6	14 ± 0.4
Glucose	10 ± 0.3	16 ± 0.9
Xylose	26 ± 1.3	16 ± 0.8
Cellobiose	13 ± 0.3	17 ± 0.4
Inulin	11 ± 0.8	11 ± 0.8
CMC	10 ± 0.7	09 ± 0.6
Lactose	30 ± 1.3	27 ± 0.2
Maltose	20 ± 0.8	14 ± 0.4
Starch	15 ± 0.5	16 ± 0.8

Table 2. Effect of concentration of lactose and cellulose in the fermentation media for endoglucanase production in SMF.

Lactose (%)	**Enzyme activity (U/ml)**	
	Fusarium sp.	***Aspergillus sp.***
0.1	01 ± 0.1	07 ± 0.3
0.3	02 ± 0.1	09 ± 0.8
0.5	18 ± 0.5	21 ± 1.0
0.7	12 ± 0.5	12 ± 0.8
0.9	10 ± 0.4	12 ± 0.6
1.1	09 ± 0.8	10 ± 0.8
Cellulose (%)		
0.5	20 ± 0.8	16 ± 0.9
1.0	21 ± 1.1	21 ± 0.9
1.5	27 ± 1.3	22 ± 0.8
2.0	37 ± 1.5	26 ± 1.1
2.5	33 ± 1.1	13 ± 0.7
3.0	33 ± 1.2	14 ± 0.8

Table 3. Influence of supplementing nitrogen source (0.5 %, w/v) in the fermentation media for endoglucanase production in SMF.

Organic nitrogen	**Enzyme Activity (U/ml)**	
	Fusarium sp.	***Aspergillus sp.***
Casein	27 ± 1.4	23 ± 1.7
Yeast extract	31 ± 1.4	33 ± 1.5
Peptone	37 ± 1.7	26 ± 0.9
Meat extract	32 ± 1.2	16 ± 0.5
Inorganic nitrogen		
$(NH_2)_4Cl$	38 ± 1.5	31 ± 1.5
$(NH_2)_4H_2SO_4$	41 ± 1.7	38 ± 1.6
$(NH_2)_4SO_4$	35 ± 1.3	24 ± 1.4
KNO_3	44 ± 1.4	43 ± 1.2

germination so that a fermentation medium with a low concentration of surfactants usually brings about good yield (16, 27-28). A significant endoglucanase yield was obtained by *Fusarium sp.* and *Aspergillus sp.* when surfactant (0.01 %) supplemented in fermentation media in this study (Table 5). A combination of sodium deoxycholate and organic nitrogen source (peptone for *Fusarium sp.* and yeast extract for *Aspergillus sp.*) in fermentation medium supported for a good amount of ezyme production by *Fusarium sp.* and the activity was 34 U/ml. It was comparatively better than that produced by *Aspergillus sp.* However, KNO_3 alone with surfactants did not support cellulase induction by both strains. The results suggest that organic nitrogen sources are essential supplements to endoglucanase production even through surfactants are preferred in shake flask culture.

The results shown in Table 6, we observed that *Fusarium sp.* and *Aspergillus sp.* were capable of producing endoglucanase on lignocellulosic wastes. *Aspergillus sp.* gave 41.8 U/ml cellulase activity as maximum in fermentation medium incorporated with 1 % (w/v) groundnut shell which was much greater than that achieved by *Fusarium sp.* Mineral salts, corn syrup liquor and sugarcane bagasse pith were also tried as the sole carbon sources in a simple medium with moderate shaking at 29 ^{o}C for endoglucanase production (3). Similar results have also been observed for cellulase production by *P.citrinum* on lignocellulosic wastes (30). This suggests that these organisms may be able to excrete cellulase, in the presence of lignocellulosic materials in the fermentation media. Aspergillus minimal medium supported for maximum production of endoglucanase by *Fusarium sp.*; in contrast Vogel's medium was more efficient for the growth as well as cellulase production by *Aspergillus sp.* (Table 7). *Fusarium sp.* grew better in defined fermentation media compared to *Aspergillus sp.*

To produce and increase endoglucanase yield under SMF, our isolates are comparatively better than other fungi such as *Curvularia lunata* (6.7 U/ml) (6), *A.niger* (3.72 U/ml) (31), *P.citrinum* (37 U/ml) (29), *P.purpurogenim* (43 U/ml) (17), *P.funiculos* (27 U/ml) (30) and *Pleurotus sajor-caju* (28 U/ml) (15) reported in earlier studies. Though *Aspergillus sp.* and *Fusarium sp.* are already reported as the potential organisms for □-glucosidase, they have not been reported to favor the endoglucanase production yet (14). The species of *Trichoderma* and *Volvariella* are capable of producing a considerable endo and exoglucanase. However, as these are slow growing fungi, a prolonged fermentation time is needed to make use of such organisms in industrial scale. The isolates investigated in this study are fast growing in optimized fermentation media as well as they have shorter fermentation period with enhanced endoglucanase yield. Thus, we suggest these isolates as potential microbial sources for producing endoglucanase under submerged culture conditions. Both isolates could be more appropriate and reliable to the pilot-scale reactors if such defined

Table 4. Effect of concentration of KNO_3 in the fermentation media for endoglucanase production in SMF.

KNO_3 (%)	Enzyme activity (U/ml)	
	Fusarium sp.	*Aspergillus sp.*
0.01	16 ± 0.3	29 ± 0.8
0.03	18 ± 0.5	33 ± 1.2
0.06	27 ± 0.9	31 ± 1.7
0.09	16 ± 0.8	30 ± 1.5
0.12	18 ± 0.7	29 ± 1.3
0.15	13 ± 0.9	26 ± 0.9

Table 5. Influence of supplementing surfactants (0.01 %, w/v) in the fermentation media for endoglucanase production in SMF.

Surfactant	Enzyme Activity (U/ml)	
With organic nitrogen*	*Fusarium sp.*	*Aspergillus sp.*
SDS	30 ± 0.8	15 ± 0.2
Tween-80	22 ± 0.9	18 ± 0.9
CTAB	31 ± 0.8	14 ± 0.6
Sodium deoxycholate	34 ± 1.3	21 ± 0.9
With KNO_3		
SDS	09 ± 0.2	10 ± 0.3
Tween-80	18 ± 0.4	11 ± 0.6
CTAB	19 ± 0.4	12 ± 0.9
Sodium deoxycholate	13 ± 0.3	09 ± 0.2

*Peptone and yeast extract were used for *Fusarium sp.* and *Aspergillus sp.* respectively.

Table 6. Influence of supplementing different lignocellulosic material (1 %, w/v) in the fermentation media for endoglucanase production in SMF.

Lignocellulosics	Enzyme Activity (U/ml)	
	Fusarium sp.	*Aspergillus sp.*
Groundnut shell	13 ± 0.7	41 ± 0.8
Wheat straw	12 ± 0.9	22 ± 1.2
Newspaper	12 ± 0.6	22 ± 0.6
Filter paper	23 ± 0.7	30 ± 1.3
Cotton waste	12 ± 0.6	26 ± 1.6

Table 7. Influence of supplementing different fermentation media for endoglucanase production in SMF.

Fermentation media	Enzyme Activity (U/ml)	
	Fusarium sp.	*Aspergillus sp.*
Rich medium	17 ± 0.8	16 ± 0.5
Vogel's medium	09 ± 0.6	21 ± 0.8
Aspergillus medium	40 ± 1.7	18 ± 0.6
Mandel's medium	16 ± 0.6	15 ± 0.5
Berg's medium	15 ± 0.5	13 ± 0.6

and optimized media are chosen. Moreover, *Fusarium sp.* and *Aspergillus sp.* isolates will perhaps be useful in cellulose bioconversion processes in food and alcohol industries since they synthesize endoglucanase alone apart from other cellulase components in liquid broth (5-7, 16). Thus, this present work would provide an insight on using the fungal isolates and this optimized fermentation conditions for a large-scale fermentation process.

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References

[1] Lakshmikant. (1990) Cellulose degradation and cellulase activity of five cellulolytic fungi. World J Microbiol Biotechnol. 6: 64-66.

[2] Park EY, Anh PN, Okuda N. (2004) Bioconversion of waste office paper to L (+)-Lactic acid by the filamentous fungus *Rhizopus oryzae*. Bioresource Technol. 93 (1): 77-83.

[3] Garcia-Kirchner O, Munoz-Aguilar M, Perez-Villalya R, Huitron-Vargas C. (2002) Mixed submerged fermentation with two filamentous fungi for cellulolytic and xylanolytic enzyme production. Appl Biochem Biotechnol. 98-100: 1105-1114.

[4] Periera Jr. JAS, Corriea MJ, Oliveira NT. (2003) Cellulase activity of a *Lentinula edades* (Berg.) Pegl. strain grown in media containing carboxyl methylcellulose or microcrystalline cellulose. Brazilian Arch Biol Technol. 46 (3): 333-337.

[5] Das M, Banerjee R, Bal S. (2008) Multivariable parameter optimization for the endoglucanase production by *Trichoderma reesei* Rut C30 from *Ocimum gratissimum* seed. Brazilian Arch Biol Technol. 51(1): 35-41.

[6] Banerjee UC, Chakrabarti S. (1992) Production and properties of carboxymethyl cellulase from *Curvularia lunata*. World J Microbiol Biotechnol. 8: 423-424.

[7] Das MK, Prasad JS, Ahmad SK. (1997) Endoglucanase production by paper-degrading mycoflora. Lett Appl Microbiol. 25 (5): 313-315.

[8] Rosgaard L, Pedersen S, Cherry JR, Harris P, Meyer AS. (2006) Efficiency of new fungal cellulase systems in boosting enzymatic degradation of barley straw lignocelluloses. Biotechnol Prog. 22 (2): 493-498.

[9] Sternberg D. (1976) Production of cellulase by *Trichoderma sp.* Biotechnol.Bioeng.Symp. 6: 35.

[10] Maheswari DK, Jahan J, Paul A, Varma A. (1993) Wheat straw, a potential substrate for cellulase production using *Trichoderma reesei*. World J Microbiol Biotechnol. 9: 120-121.

[11] Gayal SG, Khandeparkar. (1998) Production of cellulase by *Penicillium funiculosum*. Indian J Microbiol. 38: 167-168.

[12] Krogh KB, Morkeberg A, Jorgensen H, Frisyad JC, Olsson L. (2004) Screening genus *Penicillium* for producers of cellulolytic and xylanolytic enzymes. Appl Biochem Biotechnol. 113-116: 389-401.

[13] Hamilton JA, Was D. (1991) Some components of cellulases from two different strains of *Aspergillus fumigates*. Process Biochem. 26: 287-290.

[14] Sulzenbacher G, Schulein M, Davis GJ. (1997) Structure of the endoglucanase I from *Fusarium oxysporum:* native, cellobiose,

and 3, 4-epoxybutyl beta-D-cellobioside-inhibited forms, at 2.3 A resolution. Biochem. 36 (19): 5902-5911.

[15] Kumaran S, Sastry CA,Vikneshwari S. (1997) Laccase, cellulase, and xylanase activities during growth of *Pleurotus sajar-caju* on sago hampas. World J Microbiol Biotechnol. 13: 43-49.

[16] Chellapandi P, Himanshu MJ. (2008) Production of endoglucanase by the native strains of *Streptomyces* isolates in submerged fermentation. Brazilian J Microbiol. 39: 122-127.

[17] Steiner J, Socha C, Eyzaguirre J. (1994) Culture conditions for enhanced cellulase production by a native strain of *Penicillium purpurogen*. World J Microbiol Biotechnol. 10: 280-284.

[18] Gupta A, Madamwar D. (1994) Solid-state fermentation (Editor: Pandey A), s. 130-134, New Age publisher, New Delhi.

[19] Mandels M, Weber J. (1969) Production of cellulases. Adv. Chemical Ser. 95: 391-403.

[20] Dugan FM. (2006) Identification of fungi: An illustrated introduction with keys, glossary, and guide to literature, APS Press, USA.

[21] Atlas RM. (2004) Handbook of Microbiological Media. CRC Press, London.

[22] Mandels M, Andreotti R, Roche C. (1976) Measurement of saccharifing cellulase. Biotechnol Bioeng Symp. 6: 21-31.

[23] Miller GL. (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analyt Chem. 32: 426-428.

[24] Reese ET, Maguire A. (1960) Induction of cellulase in fungi by cellobiose. J Bacteriol. 79 (6): 816-826.

[25] Pakula TM, Slomen K, Uusitala J, Penttila M. (2005) The effect of specific growth rate on protein synthesis and secretion in the filamentous fungus *Trichoderma reesei*. Microbiol. 151: 135-143.

[26] Reese ET, Maguire A. (1957) Induction of cellulase in *Trichoderma viride* as influenced by carbon sources and metals. J Bacteriol. 73 (2): 269-278.

[27] Reese ET, Maguire A. (1967) Increase in cellulase yield by addition of surfactants to cellobiose culture of *Trichoderma viridea*. Develop Indust Microbiol. 12: 221-224.

[28] Reese ET, Maguire A. (1969) Surfactants as stimulants of enzyme production. Appl Microbiol. 17 (2): 242-244.

[29] Okeke BC, Obi SKC. (1993) The production of cellulolytic and xylanolytic enzymes by an *Anthrographiae sp.* World J Microbiol Biotechnol. 9: 345-349.

[30] Kuhad RC, Singh A. (1993) Enhanced production of cellulase by *Penicillium citrinum* in solid-state fermentation of cellulosic residue. World J Microbiol Biotechnol. 9: 100-111.

[31] Prasertsan P, Oi S. (1992) Production of cellulolytic enzymes from fungi and use in the saccharification of palm cake and palm fiber. World J Microbiol Biotechnol. 8: 536-538.