Mycologia Iranica 7(2): 187 – 194, 2020 Original Article DOI: 10.22043/mi.2021.124281

Cellulolytic fungi from degraded woods of Arasbaran forest, Iran

M. Ebadi ✉
S. Yeganeh-Ahmadi
M. Pazhang
F. Mahmoudi Kurdi
Department of Biology, Faculty of Science, Azarbaijan Shahid Madani University, Tabriz, Iran

S. Mollaei
Department of Chemistry, Faculty of Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran

Abstract: Cellulose is the most abundant component of lignocellulose with a vast range of applications in various fields such as nanotechnology, pharmacy, food industry. The cellulase enzyme complex consists of three major enzymes, including endoglucanases, exoglucanases, and beta-glucosidases, which are capable of decomposing cellulose. In this study, various fungal species were isolated from the degraded woods of Arasbaran forests. The activity zone technique was used to screen cellulolytic fungi. Based on the results, three fungal isolates had the highest cellulolytic activity. Phylogenetic analysis was performed based on the Internal Transcribed Spacer (ITS) region and the isolates were classified into two genera as follows: *Trichoderma* and *Aspergillus*. The cellulolytic activity of three fungal isolates was measured by the DNS method and indicated that *T. harzianum* has the highest activity (22.3 U/mg) compared with other isolates. Afterward, the growth condition of *T. harzianum* was optimized by Response Surface Methodology (RSM) to obtain a high amount of cellulase activity. The results indicated that the maximum amount of cellulase production (38.3 U/mg) was observed at 1.5% CMC, 0.51% peptone, pH 5, temperature 26.4°C, and incubation for about 3 days. Therefore, *T. harzianum* could be considered as a potential candidate for genetic improvement and enzyme production.

Keywords: *Aspergillus*, cellulase, optimization, phylogeny, *Trichoderma*

INTRODUCTION

Cellulose is a homopolysaccharide composed of linear chains of *β*-1,4 linked D-glucose subunits. It is an important structural compound of the plant cell wall and is the most abundant organic polymer on the earth (Seddiqi et al. 2021). Cellulose has tremendous economic importance all around the world because of its breakdown into simple sugars, which cause a huge change in the food and natural fuel industries (Chakraborty & Gaikwad 2012; Wüstenberg 2014)

The cellulose polymer could be broken through acid and enzymatic hydrolysis. Enzymatic hydrolysis consumes less energy and is more environmentally friendly than acid hydrolysis (Bhat 2000). The enzymatic hydrolysis of cellulose is carried out by a group of enzymes known as cellulases. Cellulases play mainly in the hydrolysis of cellulose to smaller sugar components that can later be converted to numerous industrial products (Kuhad et al. 2011). Moreover, it has a main role in natural biodegradation processes in which plant lignocellulosic biomasses are efficiently degraded (Peciulyte 2007). Cellulases are a complex group of enzymes composed of endo-1,4-β-D-glucanase, exo-1,4-β-D-glucanase, and β-glucosidase that the synergic acts of these enzymes are necessary for complete hydrolysis of cellulose (Nishida et al. 2007, Dashtban et al. 2009, Sari et al. 2016).

Cellulases are the most important commercial enzymes that are widely used in different industries, including textile, food, paper, and pulp industries, pharmaceuticals, and alcoholic fermentation of cereals (Kumar et al. 2019). Cellulases are produced by different pro- and eukaryotes microorganisms, including both bacteria and fungi (Lederberg 1992). The microorganisms that can produce all three types of cellulases enzymes, and hydrolyze cellulose to glucose, are very important (Béguin & Aubert 2009). Many studies have been carried out aimed at obtaining new micro-organisms producing cellulase enzymes with higher specific activities and greater efficiency (Rathnan et al. 2012).

Fungi are one of the major contributors to the breakdown of organic matters in nature (Lynd et al. 2002). Lignocellulosic biomasses, such as decayed woods, are suitable substrates for cellulolytic fungi and

Submitted 26 Aug. 2020, accepted for publication 9 Dec. 2020
✉ Corresponding Author E-mail: ebadi2023@gmail.com

http://mij.areeo.ac.ir

could be a potential source for the discovery of cellulase-producing fungi.

Structurally fungal cellulase systems are less complex as compared to bacterial cellulase systems and can be more rapidly produced (Maki et al. 2009). Therefore, there is an interesting challenge to obtain cellulase-producing fungi with higher activity (Rathnan et al. 2012). Although different species of fungi can hydrolysis cellulose, only a small number of them produce significant amounts of cellulases capable of complete hydrolysis of cellulose polymers (Rathnan et al. 2012). Many filamentous fungi such as *Aspergillus*, *Humicola*, *Penicillium*, *Neurospora*, and *Trichoderma* are the most studied cellulase producer (Atanasova et al. 2010; Herculano et al. 2011; Hildebrand et al. 2015).

In view of the significance and application of the fungal cellulases, this study aims to isolate and identify cellulose-degrading fungi from decayed wood and optimize the growth factors to improve efficient cellulase production.

MATERIALS AND METHODS

Fungal strains

Fungi were isolated from degraded wood samples which were collected from Arasbaran forests (38°50′N, 46°59′E) in the north-west of Iran in October 2018. The degraded wood samples were segmented into 1 cm size and surface sterilized was done according to the Burgdorf et al. (2014) method. Samples were dipped in 70% ethanol for 1 min, soaked in 0.5% sodium hypochlorite for 1 min, and washed three times in sterile distilled water. The sterilized samples were placed on potato dextrose agar media (PDA, Merck) and incubated at 25°C for 2 weeks. During this time, the growing tips of fungal mycelia were transferred onto a new plate of PDA for obtaining pure culture. The pure culture was conserved on the PDA slant agar at 4°C.

Screening of cellulolytic fungi

The cellulolytic activity of fungal isolates was determined based on the decolourization area on Carboxy Methyl Cellulose (CMC) selective media (Saroj et al. 2018). Selective media contained the following: $NaNo_3$ 2 g, $FeSO_4$ 0.01 g, K_2HPO_4 0.005 g, $CaCl_2$ 0.02 g, $MgSO_4$ 0.5 g, CMC 5 g, and agar 15 g in a liter of distilled water. Single spore isolates were grown in selective media and incubated at 25°C for 5 days. To visualize the decolourization area, the plates were flooded with an aqueous solution of Congo red 1% at room temperature for 15 min on a rotary shaker at 120 rpm and thoroughly washed with 1 M NaCl (Chetna et al. 2015). Fungal isolates which showed the highest cellulolytic activities were used for further research. The formation of a clearing zone around the colonies confirms the secretion of cellulase.

DNA extraction, PCR amplification, and sequencing

To identify the fungal isolates, two disks (0.6 cm diameter) from cellulose-degrading isolates were inoculated into 100 ml of potato dextrose broth (PDB, Difco) media and incubated at 25 °C for 5 days on a rotary shaker at 120 rpm. The mycelia were harvested by filtration, dried for 48 h at 50 °C, and used for DNA extraction using the method described by Zhu et al. (1993).

DNA amplification of ITS-nrDNA region was performed using oligonucleotide primers ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TC CTCCGCTTATTGATATGC-3') (Toju et al. 2012). PCR reaction was conducted in 25 µL of volume containing 10 ng template DNA, 2.5 µL reaction buffer (10X), 0.7 µL $MgCl_2$, 0.5 µL dNTPs mix (10mM), 0.5 U *Taq* DNA polymerase, 0.4 µL each primer (20 pmol of each primer), and 18 µl deionized water. Thermal cycling was performed in a thermocycler (SensoQuest) using the following settings; initial 94 ºC for 5 min, 30 cycles at 94 ºC for 1 min, 55 ºC for 1 min and 72 °C for 1 min, followed by a final extension step of 10 min at 72 °C. The PCR products were confirmed via 1% agarose gel electrophoresis and visualized by a UV transilluminator.

The PCR products were sequenced using the respective primers. The sequences were deposited in the NCBI Gen Bank and a similarity search was done by NCBI-BLAST analysis. Phylogenetic analyses were assessed using MUSCLE and MEGA software ver. 6.0 (Tamura et al., 2013) combined with bootstrap analyses from 1000 replicates.

Cellulase assay

Cellulase production assay was carried out in centrifuge tubes (50 ml) which contained CMC and peptone (Islam & Roy 2018). The media also contained minerals such as KH_2PO_4 (1 g/L), $MgSO_4$ (0.5 g/L), and $CaCl_2$ (0.001 g/L), which were inoculated with one plug (diameter 0.6 cm) from the margin of actively growing isolates on PDA. The growth media was incubated at 30°C for 3 days. After incubation, the media was centrifuged at 10,000×g for 20 min and then the supernatant was collected to study cellulolytic activity using the method described by Miller (1959). In this method, the concentrations of reducing sugars (products of enzyme activity) were measured using the DNS reagent test. The reaction mixture was prepared by adding 0.1 mL of culture supernatant to 0.25 mL of CMC (1%) substrate and incubated at 25°C for 60 min for an enzymatic reaction. Then, 0.35 ml of DNS (3,5-Dinitro salicylic acid) reagent was added and the tubes were boiled in a water bath for 5 min. The absorbance of the reaction mixture was measured at 540 nm by UV spectroscopy. Bradford protein assay method was used to measure the total protein concentration in the culture media (Bradford 1976).

Optimization of enzyme activity process by RSM

In this study, the role of the pH of culture medium, temperature and time of incubation, the concentrations of C and N sources were investigated to determine the optimal conditions for cellulase production.

Box-Behnken Design (BBD), one of RSM designs, with a three-level factorial design, was utilized as the experimental design model for optimization of the influential parameters to obtain the highest enzyme activity (Table 1). Five independent variables were CMC concentration (X_1= 0.5-1.5%), peptone concentration (X_2=0.5-1.5 %), pH (X_3=5-7), temperature (X_4=25-35 ºC), and Time (X_5= 3-7 days), and the enzyme activity was chosen as responses. According to BBD, forty-six randomized experiments, including six replicates at the center points, were conducted.

Table 1. Experimental levels of independent variables.

Variables	Symbol	Variable levels		
		-1	0	+1
CMC concentration (%)	X_1	0.5	1.0	1.5
Pepton concentration (%)	X_2	0.5	1.0	1.5
pH	X_3	5	6	7
Temperature (ºC)	X_4	25	30	35
Time (day)	X_5	3	5	7

RESULTS

In this study, the degraded wood samples were found to be occupied with different fungal isolates. A total of 63 degraded wood segments were processed, and 30 fungal isolates were recovered. The isolates had different cultural characteristics such as colony appearance, mycelia texture, and pigmentation.

The CMC medium was used to screen the potential cellulolytic fungi based on the clear zone formed around their colonies. The presence of clear zones around the colony after the addition of Congo red solution was strong evidence for cellulase secretion (Fig. 1). Out of 30 fungal isolates, three isolates could produce cellulase enzymes and developed a clear zone on the CMC medium. The isolates that hydrolyzed the CMC very efficiently (Td1, T3 and T5c) were selected for molecular identification and cellulolytic activity assay.

The phylogenetic tree was created using the neighbor-joining (NJ) algorithm with a p-distance substitution model and bootstrapping of 1000 replications (Fig. 2). It revealed that the lineages of the isolates could be clustered into three clades, representing different as follows: *Trichoderma* (T5c and T3) and *Aspergillus* (Td1) (Fig. 2). The first clade showed 100% bootstrapping to the *T. harzianum* sequences. The second clade comprised the isolate grouped with 100% bootstrapping to *T. viride* sequences obtained from NCBI. The third clade contained the isolate grouped to *A. flavus* sequences obtained from NCBI.

Fig. 1. Isolate T5c (*Trichoderma harzianum*) on CMC medium after coloring with 1% Congo red. Clear zone indicate cellulolytic activity.

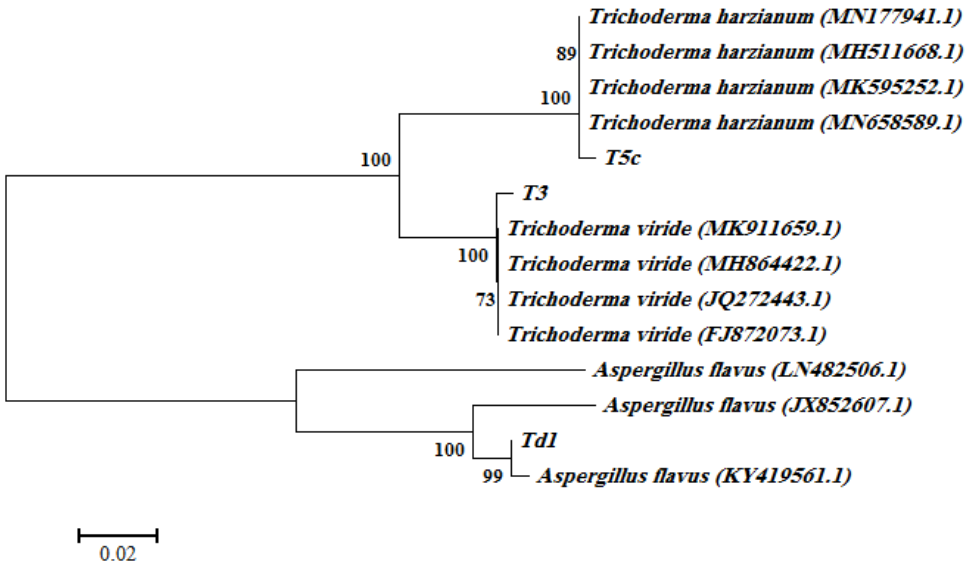

Fig. 2. Phylogenetic analysis of cellulolytic fungi isolated from degraded woods based on ITS-nrDNA region sequences. The phylogenetic tree was constructed using the neighbor-joining method (1000 bootstrap replications).

The cellulolytic activity assay of three selected isolates showed that isolate T5c has the highest activity (22.3 U/mg) compared to other isolates. Hence, T5c was selected to achieve the optimal condition for the highest enzymatic activity.

According to ANOVA, the significance of the Quadratic Model was confirmed by the higher amount of *F* (2.37) and a smaller amount of *p* 0.04). Moreover, the validity of the Quadratic model was confirmed by the lack of fit value (*P*–value of 0.082), which showed that it was non-significant. Furthermore, a good model fit was obtained for the enzyme activity, with "R-Squared" of 0.9840, and "Adj R-Squared" of 0.9635, which show a good demonstration of the variability of the parameters by the models. Additionally, the "Pred R-Squared" of 0.7938 was in good agreement with the "Adj R-Squared". Also, the significance of the model terms was estimated by *p*-value. According to the ANOVA results, X_1X_2, X_1X_3, and X_{12} have significant effects on enzyme activity. So, after eliminating the non-significant factors, a final equation was according to equation 1.

$$Y = 0.1 - 9\ X_1X_2 - 11.77\ X_1X_3 + 7.23\ X_1^2 \text{ (Equation 1)}$$

Equation 1 shows that the interaction between peptone and CMC concentration and also the interaction between pH and CMC concentration have negative effects on the enzyme activity, while the square of CMC concentration has a positive effect on the activity.

To imagine the influence of the independent variables and their interactions on the enzyme activity, 3D response surface graphs were plotted (Fig. 3a, b). Fig. 3a indicated the response surface plot between peptone and CMC concentration on the enzyme activity. According to Fig. 3a, at low CMC concentration (0.5%), the enzyme activity increased significantly by increasing peptone concentration, but at high CMC concentration (1.5%), the enzyme activity decreased significantly by increasing peptone concentration. Therefore, the highest enzyme activity was obtained at low CMC concentration and high peptone concentration, and or inverse.

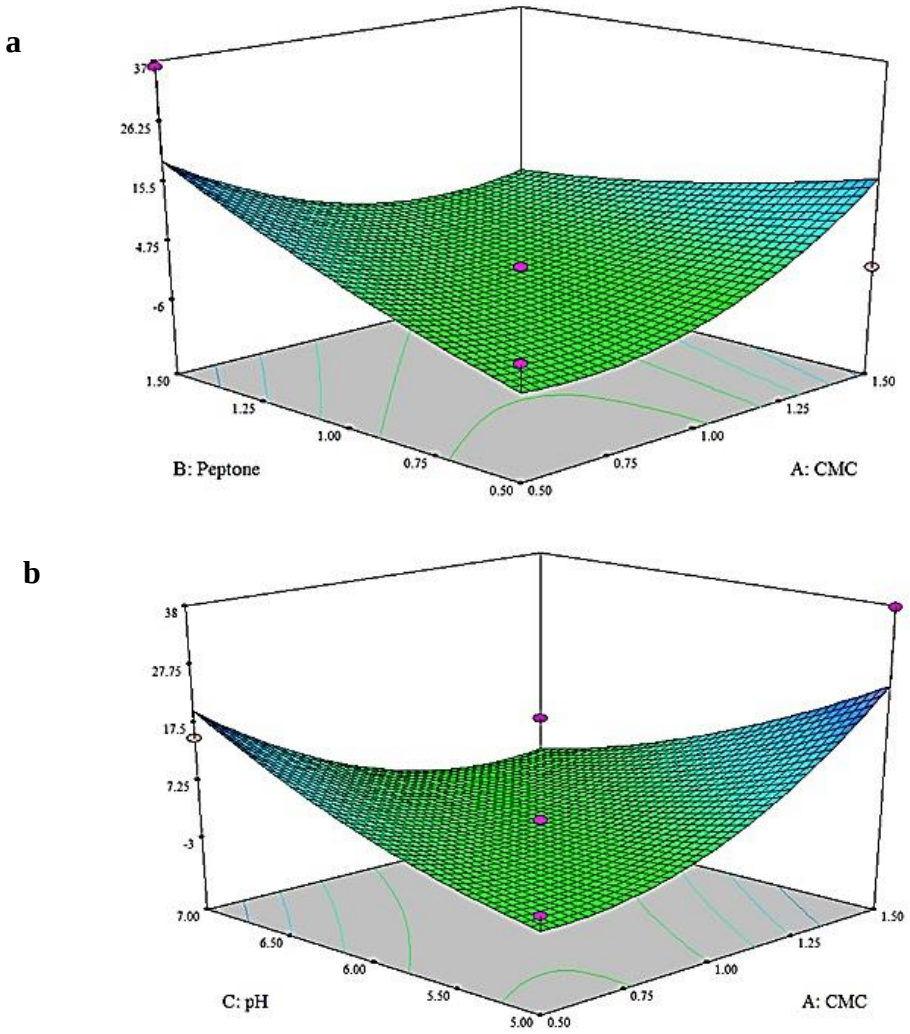

Fig. 3. Response surface plot showing the (a) 3D effect of peptone and CMC concentration; (b) 3D effect of pH and CMC concentration

DISCUSSION

Microorganisms play major roles in the natural biodegradation of organic matters (Shahriarinour et al. 2011). They are usually preferred as efficient sources for enzyme production due to their advantages, such as fast growth, easy handling, and genetic tuning for obtaining the desired products (Nduka 2007). Filamentous fungi have the capability to produce higher quantities of cellulases as compared to other organisms (Imran et al. 2016, Amouri & Gargouri 2006, Gaur & Tiwari 2015). They are used in many industrial processes for the production of enzymes and metabolites. Some of the advantages of employing fungi for enzyme production are low-cost materials with high productivity, faster production, and amenable modified enzymes. Also, fungal cellulases are less complex than that of bacterial origin and they can be more rapidly cloned and produced (Maki et al. 2009). Furthermore, the extracellular enzymes which are normally secreted outside cells can be easily recoverable from the culture media (Vishwanatha et al. 2010, de Souza et al. 2015). Cellulose hydrolyzing enzymes include β-1,4-endoglucanases (carboxymethyl cellulases), *β*-1,4-exoglucanases (cellobiohydrolases), and *β*-1,4-glucosidases. Among them, *β*-1,4-endoglucanases are primary enzymes in cellulose deconstruction and produce substrates for *β* -1,4-exoglucanases, and *β*-1,4-glucosidases (Singh & Khajuria 2018.). Then, *β*-1,4-endoglucanases have more importance rather than others (*β*-1,4-exoglucanases and *β*-1,4-glucosidases) in cellulose degradation (Singh & Khajuria 2018). So, in this work, the carboxymethyl cellulase activity of the fungal isolates were studied using CMC as a substrate.

The result of cellulolytic activity screening based on the clear zone dimension on the CMC media showed that isolates Td1, T3 and T5c had the highest cellulolytic activities. The molecular characterization using the sequence of 18s rDNA showed that each of them was closely related to *Aspergillus* sp. and *Trichoderma* sp. Several studies reported that these fungi can potentially be used as extracellular cellulase producers in the fermentation industry process. Sivakumaran et al. (2014) have successfully recovered 21 isolates of cellulolytic fungi from rice straw and showed that *Aspergillus* and *Trichoderma* are potential isolates in the aspect of cellulase production. In the same studies, *A. flavus* and *T. harzianum* as cellulolytic fungi were isolated from rice straw and degraded wood (Lee et al. 2011; Elbakary & Ageez 2018). However, the cellulolytic activity of studied isolates was varied from previous studies. The study of Pedersen et al. (2009) showed that there was variation between isolates of the same species. Mishra et al. (2002) indicated that the high level of intraspecific variation could be due to point mutations.

In our study, all the studied isolates were recovered from decayed woody samples of oak and maple trees. This might be due to the fact that decayed wood is the best habitat for the growth of such a microbial system. Similar to our study, Bekele et al. (2015) identified potential cellulolytic fungal isolates from degraded wood.

Different parameters such as temperature, pH, time of incubation, and media components such as carbon and nitrogen sources can affect cellulase production by fungal isolates (Sethi & Gupta 2014). Interactions of these components are critical; hence optimization is an essential process in the production and commercial application of such enzymes (Niyonzima 2020).

Among the environmental parameters, the pH of the culture medium has a crucial effect on enzymatic activity. Each enzyme has its own optimum range for pH, where it is most active and the result is determined by the effect of pH on a combination of factors such as binding of the enzyme to substrate, catalytic activity of the enzyme, ionization of the substrate and the variation of protein structure (Robinson 2015). The optimum pH value differs between enzymes of various fungi. In this study, the optimum pH for *T. harzianum* was closer to neutral that showed the preference of *T. harzianum* towards acidic to a neutral environment.

Maximal production of cellulase was observed in cultures of isolate Tc5 on day 3, 1.5% CMC, 0.5% Peptone, pH 5 at 26 °C. In a study of cellulase production by *T. reesei* Rut-C30, maximum production of cellulase occurred after 3 days of incubation (Zhang et al. 2012). Yadav et al. (2016) and El-hadi et al. (2014) showed that among different sources of nitrogen such as yeast, meat extract, urea, peptone, ammonium chloride and ammonium sulfate, the effect of peptone on cellulase production was higher than other nitrogen sources.

CONCLUSION

In this study, 17 fungal isolates were grown on CMC selective media that out of them only three isolates, namely: *A. flavus*, *T. harzianum*, and *T. viride* were efficiently hydrolyzed the CMC. *T. harzianum* (isolate T5c) showed maximum zone of hydrolysis followed by *A. flavus* and *T. viride*. The results of optimization of the culture condition showed that maximal cellulase production of *T. harziaum* was obtained in cultures containing 1.5% CMC, 0.51% Peptone, pH 5 at 26 °C for 3 days. Thus, this isolate should be considered as a potential candidate for genetic improvement and enzyme production.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from Azarbaijan Shahid Madani University.

REFERENCES

Amouri B, Gargouri A. 2006. Characterization of a novel β-Glucosidase from a Stachybotrys Strain. Biochemical Engineering Journal 32: 191-197.

Atanasova L, Jaklitsch WM, Komoń-Zelazowska M, Kubicek CP, Druzhinina IS. 2010. Clonal species Trichodermaparareeseisp. nov. likely resembles the ancestor of the cellulase producer Hypocrea jecorina/T. reesei. Applied and Environmental Microbiology 76: 7259-7267.

Béguin P, Aubert J. 1992 Cellulases. In: Encyclopedia of Microbiology, ed. Lederberg J. 1: 467–477. San Diego: Academic Press, USA.

Bekele A, Abena T, Habteyohannes A, Nugissie A, Gudeta F, Getie T, Berhanu A. 2015. Isolation and characterization of efficient cellulolytic fungi from degraded wood and industrial samples. African Journal of Biotechnology 14: 3228-3234.

Bhat MK. 2000. Cellulase and related enzyme in biotechnology. Biotechnology advances 18: 355-383.

Bradford MM. 1976. A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72: 248-254.

Burgdorf RJ, Laing MD, Morris CD, Jamal-Ally SF. 2014. A procedure to evaluate the efficiency of surface sterilization methods in culture-independent fungal endophyte studies. Brazilian Journal of Microbiology 45: 977-983.

Chakraborty S, Gaikwad A. 2012. Production of cellulosic fuels. Proceedings of the National Academy of Sciences, India Section A: Physical Sciences 82: 59-69.

Chetna G, Priyanka J, Dhermander K, Dixit AK, and Jain RK. 2015. Production of cellulase enzyme from isolated fungus and its application as efficient refining aid for production of security paper. International Journal of Applied Microbiology and Biotechnology Research 3: 11-19.

Dashtban M, Schraft H, Qin W. 2009. Fungal Bioconversion of Lignocellulosic Residues: Opportunities & Perspectives. International Journal of Biological Sciences 3: 378-395.

de Souza PM, de Assis Bittencourt ML, Caprara CC, de Freitas M, de Almeida RPC et al. 2015. A biotechnology perspective of fungal proteases. Brazilian Journal of Microbiology 46: 337-346.

Elbakary M, Ageez A .2018. Identification of endoglucanase gene responsible for cellulose degradation using Aspergillus flavus, Egypt. Egyptian Academic Journal of Biological Sciences 10: 47-57

El-Hadi A, El-Nour S, Hammad A, Kamel Z, Anwar M. 2014**.** Optimization of cultural and nutritional conditions for carboxymethylcellulase production by Aspergillus hortai. Journal of Radiation Research and Applied Sciences 7: 23-28.

Gaur R, Tiwari S .2015. Isolation, production, purification and characterization of an organic solvent thermostable alkalophilic cellulase from Bacillus vallismortis RG-07. BMC Biotechnology 15:19.

Herculano PN, Lima DMM, Fernandes MJS, Neves RP, Souza-Motta CM et al. 2011. Isolation of cellulolytic fungi from waste of castor (Ricinus communis L.). Current Microbiology 62: 1416-1422.

Hildebrand A, Szewczyk E, Lin H, Kasuga T, Fan Z. 2015. Engineering Neurospora crassafor Improved Cellobiose and Cellobionate Production. Applied and Environmental Microbiology 81: 597-603.

Imran M, Anwar Z, Irshad M, Asad MJ, Ashfaq H. 2016. Cellulase Production from Species of Fungi and Bacteria from Agricultural Wastes and Its Utilization in Industry: A Review. Advances in Enzyme Research 4: 44-55.

Islam F, Roy N. 2018. Screening, purification and characterization of cellulase from cellulase producing bacteria in molasses. BMC research notes 11: 1-6.

Kuhad RC, Gupta R, Singh A. 2011. Microbial cellulases and their industrial applications. Enzyme research 2011: 1-10.

Kumar VA, Kurup RSC, Snishamol C, Prabhu GN. 2019. Role of cellulases in food, feed, and beverage industries. Green Bio-processes 323-343.

Lee S, Jang Y, Lee YM, Lee J, Lee H et al. 2011. Rice straw-decomposing fungi and their cellulolytic and xylanolytic enzymes. Journal of Microbiology and Biotechnology 21: 1322-1329.

Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. 2002. Microbial cellulose utilization: fundamentals and biotechnology. Microbiology and Molecular Biology Reviews 66: 506-577.

Maki M, Leung KT, Qin W. 2009. The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. International Journal of Biological Sciences 5: 500-516.

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry 31: 426-428.

Mishra PK, Fox RTV, Culham A. 2002. Restriction analysis of PCR amplified nr DNA regions revealed intraspecific variation within populations of Fusarium culmorum. FEMS Microbiology Letters 215: 291-296.

Nduka O. 2007. Modern Industrial Microbiology and Biotechnology, Science Publishers, Enfield, NH, USA.

Nishida Y, Suzuki KI, Kumagai Y, Tanaka H, Inoue A et al. 2007. Isolation and primary structure of a cellulase from the Japanese sea urchin Strongylocentrotus nudus. Biochimie 89: 1002-11.

Niyonzima FN, Veena SM, More SS. 2020. Industrial Production and Optimization of Microbial Enzymes. Microbial Enzymes: Roles and Applications in Industries 115-35.

Peciulyte D. 2007. Isolation of cellulolytic fungi from waste paper gradual recycling materials. Ekologija 53: 11-18.

Pedersen M, Hollensted M, Lange L, Andersen B. 2009. Screening for cellulose and hemicellulose degrading enzymes from the fungal genus Ulocladium. International Biodeterioration & Biodegradation 63: 484-489.

Rathnan RK, Nair P, Balasaravanan T. 2012. Isolation, identification and characterization of efficient cellulolytic fungi from natural resources. International Journal of Microbial Resource Technology 1: 379-387.

Robinson PK. 2015. Enzymes: principles and biotechnological applications. Essays in biochemistry 59: 1-14.

Sari SLA, Pangstuti A, Susilowati A, PurwokoTj, Mahajoeno E et al. 2016. Cellulolytic and hemicellulolytic bacteria from the gut of Oryctesrhinoceros larvae. Biodiversitas 17: 70-75.

Shahriarinour M, Noor M, Wahab A, Ariff A, Mohamad R. 2011. Screening, Isolation and Selection of cellulolytic fungi from oil palm empty fruit bunch fiber. Biotechnology 10: 108-13.

Saroj P, Manasa P, Narasimhulu K. 2018. Characterization of thermophilic fungi producing extracellular lignocellulolytic enzymes for lignocellulosic hydrolysis under solid-state fermentation. Bioresources and Bioprocessing 5: 1-14.

Seddiqi H, Oliaei E, Honarkar H, Jin J, Geonzon LC, Bacabac RG, Klein-Nulend J. 2021. Cellulose and its derivatives: Towards biomedical applications. Cellulose 28: 1893-1931.

Sethi S, Gupta S. 2014. Optimization of cultural parameters for cellulase enzyme production from fungi. BioLife 2: 989-996.

Singh S, Khajuria R. 2018. Penicillium enzymes for the textile industry. In: New and future developments in microbial biotechnology and bioengineering. 201-215. Elsevier, Netherlands.

Sivakumaran S. 2014. Isolation of cellulolytic fungi and their degradation on cellulosic agricultural wastes. Journal of Academia and Industrial Research 2: 458-463.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 12: 2725-2729.

Toju H, Tanabe AS, Yamamoto S, Sato H. 2012. High-coverage ITS primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. PloS one 7: e40863.

Vishwanatha KS, Rao AG, Singh SA. 2010. Acid protease production by solid-state fermentation using Aspergillus oryzae MTCC 5341: optimization of process parameters. Industrial Microbiology and Biotechnology 37: 129-138.

Wüstenberg T. 2014. Cellulose and cellulose derivatives in the food industry: fundamentals and applications. John Wiley & Sons, USA.

Yadav PR, Chauhan PB, Gahlout M, Prajapati H. 2016. Isolation. Screening and Optimization of process parameters for enhanced production of cellulase by solid state fermentation, International Journal of Advanced Research in Biological Sciences 3: 21-27.

Zhang L, Liu Y, Niu X, Liu Y, Liao W. 2012. Effects of acid and alkali treated lignocellulosicmaterials on cellulase/xylanase production by Trichoderma reesei Rut C-30 andcorresponding enzymatic hydrolysis. Biomass & Bioenergy 37: 16-24.

Zhu H, Qu F, Zhu LH. 1993. Isolation of genomic DNAs from plants, fungi and bacteria using benzyl chloride. Nucleic Acid Research 2: 5279-5280.

قارچ‌های سلولولایتیکی چوب‌های پوسیده جنگل ارسباران، ایران

مصطفی عبادی[1]✉، شهرزاد یگانه احمدی[1]، محمد پاژنگ[1]، فاطمه محمودی کردی[1]، سعید ملائی[2]

۱- گروه زیست شناسی، دانشکده علوم پایه، دانشگاه شهید مدنی آذربایجان، تبریز، ایران

۲- گروه شیمی، دانشکده علوم پایه، دانشگاه شهید مدنی آذربایجان، تبریز، ایران

چکیده: سلولز بیشترین ترکیب تشکیل‌دهنده لیگنوسلولز است با کاربرد فراوان در زمینه‌های مختلفی از جمله نانوتکنولوژی، داروسازی و صنایع غذایی می‌باشد. مجموعه آنزیمی سلولاز از سه آنزیم اصلی اندوگلوکانازها، اگزوگلوکانازها و بتاگلوکزیدازها تشکیل می‌شود که توانایی تجزیه سلولز را دارد. در این پژوهش، گونه‌های مختلف قارچ از چوب‌های پوسیده جداسازی گردید. جهت غربالگری جدایه‌های تولید کننده آنزیم سلولاز از روش آزمون منطقه فعالیت استفاده شد. با توجه به نتایج، سه جدایه قارچ دارای بیشترین فعالیت سلولازی بودند. نتایج آنالیز فیلوژنتیکی بر اساس ناحیه ITS-nrDNA نشان داد که جدایه‌ها به دو جنس *Trichoderma* و *Aspergillus* تعلق دارند. فعالیت سلولازی سه جدایه قارچ به روش DNS سنجیده شد و مشخص شد *T. harzianum* دارای بیشترین فعالیت (۲۲/۳ U/mg) در مقایسه با سایر جدایه‌ها می‌باشد. سپس، برای بدست آوردن مقادیر بالای آنزیم سلولاز، شرایط کشت *T. harzianum* با استفاده از روش سطح پاسخ (RSM) بهینه سازی شد. نتایج حاصل از روش رویه پاسخ نشان داد، حداکثر میزان تولید آنزیم (۳۸/۳ U/mg) در غلظت CMC ۱/۵ درصد، غلظت پپتون ۰/۵۱ درصد، pH برابر ۵، دمای ۲۶/۴ درجه سلسیوس و زمان انکوباسیون حدود ۳ روز مشاهده شد. بنابراین، *T. harzianum* می‌تواند به عنوان یک جدایه بالقوه برای بهبود ژنتیکی و تولید آنزیم مطرح گردد.

کلمات کلیدی: آسپرژیلوس، سلولاز، بهینه سازی، فیلوژنی، تریکودرما

مکاتبه کننده: مصطفی عبادی Email: ebadi2023@gmail.com

تاریخ دریافت:۱۳۹۹/۰۶/۰۵ تاریخ پذیرش: ۱۳۹۹/۰۹/۱۹