http://dx.doi.org/10.22037/afb.v5i1.18651

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2018, 5 (1):11-18
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

Improvement of Cellulase Production and its Characteristics by Inducing Mutation on *Trichoderma reesei* 2414 under Solid State Fermentation on Rice By-products

Nazanin Darabzadeh[1], Zohreh Hamidi-Esfahani[1]*, Parisa Hejazi[2]

1- Department of Food Science and Industry, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.

2- Department of Chemical Engineering, School of Chemical Engineering, Iran University of Science and Technology, Tehran, Iran.

Abstract

Background and objective: Solid State Fermentation is an economic technology to produce value-added products. Also, the use of agricultural by-products, as a waste management strategy, has recently been considered. On the other hand, the new mutants are interesting for the production of enzymes. The aim of this study was to investigate the effect of mutation on the improvement of cellulase quality. Therefore, rice by-products were used under solid state fermentation for production of cellulase. Moreover, the characteristics of the new cellulase produced from the new mutated strain was studied.

Material and methods: Cellulase was produced under solid state fermentation process. Spore suspensions of *Trichoderma reesei* were subjected to Co^{60} γ irradiation and mutated. The activities of cellulases (from parent and mutants) were compared. The effects of temperature and pH on cellulase activity and the stability of cellulase in optimum condition were investigated.

Results and conclusion: Cellulase was successfully produced under solid state fermentation on the mixture of rice by-products as substrate. The results showed that mutation had a significant effect on cellulase activity and Characteristics. *Trichoderma reesei* B (a mutated strain) had about 30% filter Paperase and 23% Carboxymethyl Cellulase higher than its parent. Cellulase activity of *Trichoderma reesei* B was 47% higher than its parent at the optimum temperature (50°C). In other temperatures, the activity of cellulase extracted from *Trichoderma reesei* B was significantly higher than that of the others; for example, at 60°C, the enzyme activity was 120% higher than its parent. It is notable that an 84% increase in the enzyme activity was observed at the optimum pH (4.5) after mutation and cellulase activity increased from 0.72 U g^{-1} dry solid to 1.31 U g^{-1} dry solid.

Conflict of interest: The authors declare no conflict of interest.

Article Information

Article history:

Received 28 Sep 2017
Revised 23 Oct 2017
Accepted 12 Nov 2017

Keywords:

- Cellulase
- Solid State Fermentation
- Mutation
- *Trichoderma reesei*
- Rice by-products

***Corresponding author:**

Zohreh Hamidi-Esfahani,

Department of Food Science and Industry, Faculty of Agriculture, Tarbiat Modares University, P.O. Box: 14115-336, Tehran, Iran.

Tel: +98-21-48292474
Fax: +98-21-48292200
E-mail:
hamidy_z@modares.ac.ir

1. Introduction

As an industrially important enzyme, cellulases play a key role in hydrolyzing the linkage in cellulose. Cellulase is the third industrial enzyme by dollar volume in the world. This enzyme accounts for about 20% of the total enzyme market in the world [1,2]. It has applications in the industry for production of medicines, perfumes, resins and starch. It is further used in baking, malting, brewing, production of bioethanol, pulp and paper, food and beverages, as well as in the textile, animal feed industries and waste treatment [1,3-5]. Cellulase is produced by various bacteria and fungi; but among them, the genus *Trichoderma* is well known for the production of cellulase [6,7]. Also, *Trichoderma* (*T.*) *reesei* is one of the best microorganisms for cellulase production [8]. Although commercial cellulases are produced by *Aspregillus niger* and *T. reesei* under submerged fermentation [1], the high cost of production and low yield of the enzymes are the major problems for their industrial production [8-11]. The high cost of the substrates is one of the most important issues in cellulase production, so finding the cheapest substrates can help reduce the costs. Also the use of other technologies can be helpful in gaining a cost-effective product [12]. Solid state fermentation (SSF) is an alternative process for industrial enzyme production that leads to reduction of costs [13]. SSF is defined as the fermentation consisting of the growth of microorganisms on the surface of wet solid substrate in the absence of free water [14]. This system is expanding with increasing

attention to the importance of production of high value-added products from agricultural by-products [15]. Lack of free water in SSF and less energy consumption causes the decrease of cellulase price to 10-fold compared to submerged fermentation [16,17]. In the previous publications, abundant agricultural residues such as corn stover, wheat straw, rice straw, rice bran, wheat bran, bagasse and soybean hull were used in cellulase production [17-25]. Therefore, SSF is promising due to its numerous advantages [26].

Another approach to increase the cellulase production by fungal SSF is mutation of fungal strains. The purpose of this mutation is to gain higher cellulase production with higher quality [27]. Prior studies have reported improved strains by chemical mutations. These mutated strains can increase the production of higher quality cellulase. For example, the use of UV and microwave radiation can be mentioned followed by ethyl methane sulfonate [27], microwave and UV [28], Co^{60} γ-rays, ultraviolet irradiation and N-methyl-N'-nitro-N-nitrosoguanidine [29].

To the best of our knowledge, the use of combination of rice by-products (rice straw, rice bran and rice husk) as a cheap and suitable substrate in SSF has not been reported. Mutation of *T. reesei* by Co^{60} γ-rays is promising to gain new improved strains. The new cellulase produced from this new mutated strain potentially has higher quality. Which leads to its wide application in different situations in the industries.

So, the aim of this study was to investigate towards the improvement of cellulase activity and its characteristics by mutation induction. Also, application of cheap agricultural by-products to produce value-added products is a strategy of waste management. Our purpose was bio-conversion of rice by-products to high quality cellulase.

2. Materials and methods

2.1 Microorganism

T. reesei CECT 2414 was obtained from Collection Espanola de Cultivos Tipo (CECT, Spain). Then it was inoculated on potato dextrose agar (PDA) slants, and incubated at 25°C for 8 days. A spore suspension was prepared by addition of a sterile saline solution (NaCl 0.9%) to PDA plates and scrapped well. After that, the suspension was shaken on tube shaker to achieve homogeneity. The final concentration was adjusted on 2.5×10^7 spores ml^{-1} [22,27].

2.2 Substrate and Solid State Fermentation

The by-products of rice including rice bran, rice husk and rice straw were obtained from north of Iran. After that, the substrates were milled (Moulinex la picadpra, 700 W, Indonesia) and screened by sieving (mesh size: 14-18). The mixture of these three substrates (at the same ratio) was utilized as substrate for SSF. For cellulase production in each treatment, about 5 g of the substrate was used with the adjusted moisture content at 55% ww^{-1}. Cultivation of *T. reesei* CCT 2414 was carried out in 250 ml flasks. Then 5 g of the solid substrate, after sterilization at 121°C for 20 min was inoculated with 1 ml of spore suspension (2.5×10^7 spores ml^{-1}). Then it was incubated for 6 days at 25°C [19,23,30].

2.3 Cellulase extraction

The weight of the fermented solid substrate was determined then distilled water was added into the flasks, approximately 5 times its solid weight. The obtained mixture was stirred with magnet stirrer for 30 min at 700 rpm (MR 3001 k Heidolph Germany). Then this mixture was centrifuged at 10000 ×g for 15 min (3-30K sigma Germany), and the supernatant was separated as extracted enzyme [23].

2.4 Fungal strain improvement by γ irradiation mutagenesis

First, *T. reesei* CECT 2414 was cultivated on the rice by-products (rice straw, rice bran and rice husk at the same ratio) as fermentation medium. Next, PDA medium was prepared, and the adapted spores were moved from the solid substrate to the PDA, and incubation was done at 25°C for 8 days. A spore suspension (2.5×10^7 spores ml^{-1}) was prepared, and 5 mL of it was poured into sterile screw cap test tubes (12 tubes). Each tube was exposed to different doses (200, 400, 600, and 800 Gy) of Co^{60} γ-rays [29,31,32].

2.5 Percentage of survival

A serial of dilutions were made from each of the treated spore suspensions. Then 0.1 ml of each dilution was pipetted onto the PDA. After 3 days incubation at 25°C, calculation of survival percentage by plate count method was done [31].

2.6 Screening of putative mutants by appearance

After the incubation period (8 days at 25°C), 48 PDA plates of mutated colonies were investigated. Premier colonies were selected. The base of selection was faster growing and therefore having a larger colony diameter or different colony appearance. The selected mutants were cultivated on PDA separately. In this stage, 50 colonies were isolated and incubated at 25°C for 6 days [31].

2.7 Screening of putative mutants by Congo red test

For 50 putative colonies from the prior stage, the Congo red test was done. First, the discs (with the same diameter ~0.5 cm) were removed by a sterile hollow glass rod from the PDA plates and placed on the center of the plates containing the Congo red test medium. This medium contained (g l^{-1}): $NaNO_3$ 3, K_2HPO_4 1, $MgSO_4$ 0.5, KCl 0.5, agar 20, CMC 5 and $FeSO_4$ $7H_2O$ (0.01 mg l^{-1}). Then they were incubated at 25°C for 7 days. 10 ml of the

Congo red dye (2.5 g l^{-1}) was added to each plates. After an overnight, the solution was discarded, and the cultures were washed with 10 ml of 1 mol l^{-1} NaCl. By measuring the diameter of colony and diameter of halo, the ratio was calculated. Enzymatic index (EI) is the ratio of diameter of hydrolysis zone to the diameter of colony. Based on the results of this test, 9 colonies with the most ratio were selected [33,34].

2.8 Screening by measuring filter paper activity (FPase) and carboxymethyl cellulase (CMCase)

In order to choose the colonies with the most cellulase activity, FPase (as the activity of total cellulase) and CMCase were determined according to the procedures presented by Ghose (1987). After 60 min incubation (for FPase) and 30 min incubation (for CMCase) at 50°C, the liberated reducing sugars in the reaction mixture were determined by dinitrosalicylic acid method presented by Miller [35].

For this purpose, 9 colonies selected from the prior stage were cultivated onto the solid substrate, separately. After the incubation period (at 25°C for 6 days), the produced cellulase was extracted and its enzyme activity was measured [35,36].

For FPase measurement, 0.5 ml of the extracted enzyme was added to the filter paper (about 50 mg) in a test tube containing 1 ml of citrate buffer (50 mmol l^{-1}, pH 4.8). The control of enzyme including 0.5 ml enzyme and 1 mL citrate buffer without filter paper and the control of substrate including 1.5 ml citrate buffer and filter paper without enzyme were prepared. Blank samples included 1.5 ml citrate buffer. All tubes were incubated at 50°C for 60 min. Then 3 ml of dinitrosalicylic acid reagent was added to each tube and heated for 5 min in a boiling water bath. Then it was cooled down in the ice bath. Absorbance was read by spectrophotometer at 540 nm (Algilent Technologies Cary 60 UV-Vis). For CMCase measurement, 2% carboxy-methyl cellulose in 50 mmol l^{-1} citrate buffer was used instead of filter paper as substrate and incubation time was 30 min at 50°C. Other parameters were the same as the FPase test.

The enzyme activity was calculated by using the following equation:

$$FPase = A\times (a + b)/0.5 \times C \times t \times 0.18$$

A= glucose concentration (mg / 0.5 ml)
a= water content of the substrate
b= water added during the extraction
C= weight of dry substrate
t= 60 min for FPase (30 min for CMCase)

Enzyme activity was expressed as U g^{-1} dry substrate. One unit (U) of enzyme activity is defined as the amount of enzyme required to liberate 1 µ mol of product per minute at 50°C.

2.9 Confirming of mutated strain enzyme stability

To ensure the stability of produced enzyme from the mutated strains, enzyme activities during 9 generations were investigated. The mutated strains were cultivated on the rice by-products, substrate and incubated at 25°C for 6 days. After that, in a new flask containing the rice by-products substrate, sub-culturing was carried out. Then the produced enzyme of first generation was extracted; this was continued for 9 consecutive generations [27,31,37].

2.10 Optimum temperature for cellulase activity

To determine the optimum temperature for the cellulase activity produced from the mutant strain, measurements were done at 40, 50, 60 and 70°C. For this purpose, FPase was measured according to what was mentioned in the previous sections; however, in this method, incubation temperature (instead of 50°C) was adjusted at 40, 50, 60 and 70°C for each test [31,38].

2.11 Optimum pH for cellulase activity

In terms of pH optimization, a range of pH values from 2.5 to 7.5 were investigated. For this purpose, different pH buffers were prepared using citrate and Na_2HPO_4. Enzyme activities in different pH values were determined by the Ghose method; however, in each test, buffer with special pH was used instead of citrate buffer (pH=4.8). Other conditions were according to the Ghose method [15,31,36, 38].

2.12 Enzyme stability at optimum conditions

After determination of the optimum temperature for the activity of cellulase, stability at this point was investigated. To achieve this goal, the extracted cellulase was kept at optimum temperature for 1, 2, 3, 4, and 5 h. Then FPase was determined for these samples. For pH stability, 1 ml of the extracted enzyme was added to 1 ml of citrate buffer (with optimum pH value). This sample was kept for 1, 2, 3, 4 and 5 h at 4°C. Then the enzyme activities of the samples by Ghose method were evaluated and the result were compared [15,31,38,39].

3. Results and discussion

In this study, a combination of rice by-products was used as substrate for cellulase production. Since rice is the second group of cereals, and the staple food for half of the world population, and on the other hand, word rice production is rising recently as in 2014, the area under rice cultivation was 162,716,862 hectare with production of about 741,477,711 tons [12,40], considering that the most important by-products of rice cultivation and production are bran, straw and husk, the possibility of using these cheap by-products, is economic and attractive.

3.1 Percentage of survival

Mutagenic agents can cause strain improvement, for example gamma radiation induces degradation of cellular components. Its effects depend on the type and dose of energy. The interaction between water and rays generates free radicals that leads to additional effects on DNA [41]. In the present study, mutation by Co^{60} γ irradiation was done on the spore suspension of *T. reesei* 2414.

After gamma irradiation of the spore suspensions of *T. reesei* CCT 2414, the survival percentage was calculated (Figure. 1). The survival percent before gamma irradiation was 100, and after exposure to gamma radiation, it was decreased proportional to the dose of gamma radiation. It means about half of spores of *T. reesei* died under the influence of gamma rays. These results were confirmed by other researches [31].

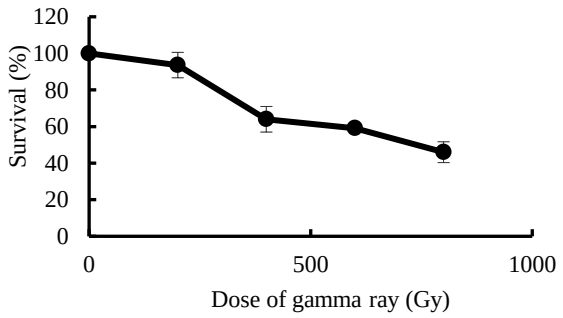

Figure 1. Survival of *T. reesei* CCT 2414 after gamma irradiation of the spore suspension

3.2 Screening of putative mutants

After gamma irradiation, PDA plates containing colonies of mutated spores were investigated. Some of the colonies were different in shape and color or had faster growth; so they had larger colonies. Therefore, 50 colonies were selected and cultivated on PDA medium, separately. After incubation at 25°C for 6 days, the Congo red test was done, the obtained results were calculated. The data for the top 10 colonies among the 50 colonies are shown in Table 1 [34].

Nine colonies with larger ratio than 1.36 (*T. reesei* 2414 before mutation) were selected (Table 1), and their enzyme activities (FPase and CMCase) were measured (Table 2). In case of FPase, the results showed that one of the mutated strains (*T. reesei* B) had significantly more enzymatic activity than its parent. In the following, CMCase values for 9 of the best mutated strains were obtained. CMCase of *T. reesei* before irradiation was 0.553 U g^{-1} dry solid weight; this value for A and B strains was 0.666 and 0.680 U g^{-1} dry solid, respectively. So, the results showed *T. reesei* B about 30 % FPase and 23 % CMCase activity higher than its parent.

Table 1. Congo red test results for the best colonies after irradiation

Name of mutated strains	Diameter of hydrolysis zone/ diameter of colony
T. reesei before mutation	1.36±0.03
A	2.00±0.14
B	1.70± 0.07
C	1.76±0.23
D	2.05±0.04
E	1.79±0.10
F	2.31±0.03
G	2.00±0.28
H	1.89±0.06
I	2.00±0.15

Letters are used just to identify different colonies.

Table 2. FPase and CMCase for the best mutated strains

CMCase (U g^{-1}. dry solid)	FPase (U g^{-1}. dry solid)	Mutated strains
$0.553±0.08^{bc}$	$0.759±0.1^{bc}$	*T. reesei* before mutation
$0.661±0.03^{ab}$	$0.792±0.08^{b}$	*T. reesei* A
$0.680±0.04^{a}$	$0.980±0.02^{a}$	*T. reesei* B
$0.535±0.01^{c}$	$0.777±0.02^{bc}$	*T. reesei* C
$0.503±0.03^{c}$	$0.535±0.02^{e}$	*T. reesei* D
$0.505±0.08^{c}$	$0.659±0.03^{cd}$	*T. reesei* E
$0.310±0.1^{e}$	$0.208±0.05^{f}$	*T. reesei* F
$0.444±0.01^{cd}$	$0.603±0.02^{de}$	*T. reesei* G
$0.359±0.08^{de}$	$0.508±0.16^{e}$	*T. reesei* H
$0.519±0.07^{c}$	$0.733±0.03^{bc}$	*T. reesei* I

Statistically significant at 95% of confidence level.
Different letters indicate significant differences between the means within column.

3.3 Effect of various conditions on the mutant cellulase activity

After extraction of cellulase, the effect of conditions (temperature and pH) on the cellulase activity was investigated. The temperature range varied from 40 to 70°C (Figure 2). The optimum temperature was in the range of 40-50°C. In other words, the extracted cellulases had the highest activity at 40-50°C; for example, the cellulase activity of *T. reesei* 2414 before mutation was in the range of 0.2-0.71 U g^{-1} dry substrate; the lowest activity was at 70°C, and the highest (optimum) was at 50°C. *T. reesei* A was the same as its parent in this regard. The activity of cellulase obtained from *T. reesei* B was in the range of 0.5-1.04 U g^{-1} dry substrate. The activity of *T. reesei* B was 47% higher than that of its parent (at 50°C). As shown in Figure 2, in other temperatures, the activity of cellulase extracted from *T. reesei* B was significantly higher than others; for example, at 60°C, the enzyme activity was 120% higher than its parent.

Khoshnevisan et al. reported that the enzyme activity at 50°C and 60°C was increased. At lower and higher temperatures, the activity was lower [18]. Nataraja et al. observed that the optimum temperature for maximum cellulase activity of Fusarium and Penicillium was 40°C whereas, it was 60°C for Aspergillus [24].

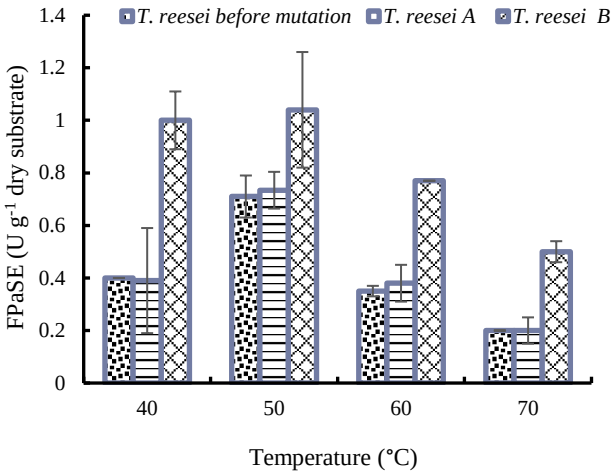

Figure 2. Effect of temperature on the mutants' cellulase activity

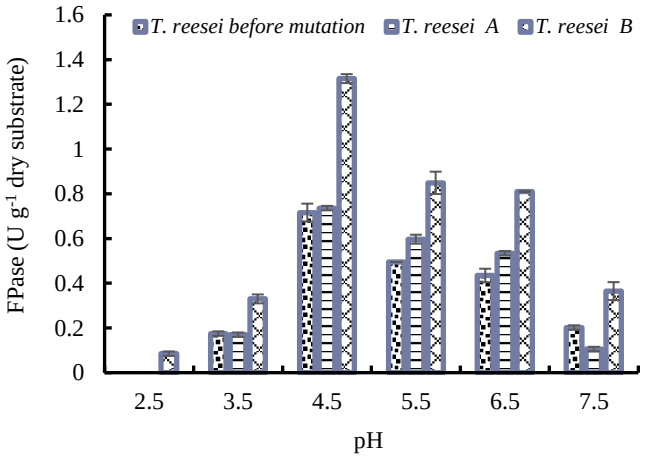

Figure 3. Effect of pH on the mutants' cellulase activity

Also determination of the pH effect on cellulase activity was done the pH range of 2.5-7.5. For this purpose, by using citrate and Na_2HPO_4, different pH values were prepared (Figure 3). The results showed that the optimum pH for all strains was 4.5. The highest activity of cellulase from *T. reesei* B was obtained at pH 4.5 (1.315) that was significantly higher than that of other pH values. It is notable that an 84% increase in cellulase activity (*T. reesei* B) was observed at optimum pH after mutation. In other pH values, the activity of cellulase from *T. reesei* B was significantly higher than that of others. For example, at pH 5.5, about 72% and at pH 7.5, about 81% increase in cellulase activity was obtained post mutation. These results are in agreement with the findings of Khoshnevisan et al [18]. Petchluan et al. reported a downward trend of cellulase activity from pH 5 to 9 [28].

3.4 Enzyme stability at optimum conditions

Due to the importance of enzyme stability, in the present study, the stability of cellulase was investigated at the optimum temperature (50°C). The extracted cellulase were allowed to remain at 50°C for 1 to 5 h. Then their activities were measured. The cellulase from *T. reesei* before mutation, after 1 h remaining at 50°C, retained about 56% of its activity; this amount was 57% after mutation (Table 3). The activity of both of them was gradually reduced and reached zero after 5 h of remaining at 50°C. Khoshnevisan et al. reported the decrease of enzyme activity over 5 h [18].

The stability of cellulase at optimum pH (4.5) was studied. For this purpose the extracted cellulase remained at buffer with pH 4.5 for 1 to 5 h. Then the activity of enzyme was calculated. The results showed that the cellulase obtained from *T. reesei* before and after mutation was totally stable during 5 h remaining at pH 4.5. In other words, they maintained 100% of their activity over 5 h.

Table 3. Enzyme stability in optimum temperature (50°C)

	FPase (U g^{-1} dry substrate)	
Time (h)/ strain	*T. reesei* 2414	*T. reesei* B
0	0.75 ± 0^{a}	0.96 ± 0.02^{a}
1	0.42 ± 0.07^{b}	0.54 ± 0.02^{b}
2	0.26 ± 0.04^{c}	0.27 ± 0.05^{c}
3	0.21 ± 0.03^{c}	0.24 ± 0.04^{c}
4	0.20 ± 0.03^{c}	0.10 ± 0.01^{d}
5	0.00 ± 0.00^{d}	0.00 ± 0.00^{e}

Statistically significant at 95% of confidence level.
Different letters indicate significant differences between the means within column.

Petchluan et al. observed that the cellulase was stable at pH 4-5 with 94% remaining activity [29]. But in the present study, at pH 4.5, cellulase was stable without loss of enzyme activity.

Finally, it is important to note that *T. reesei* B had cellulase activity in a wider range of temperature (40 to 60°C) and pH (4.5 to 6.5) in comparison with its parent; this leads to its wide application in various industries.

4. Conclusion

In this study, for the first time the mixture of rice by-products were used under SSF for cellulase production by the new mutant of *T. reesei*. This process in addition to reducing cellulase price, is also a trick to agricultural waste management. Mutation of *T. reesei* 2414 had a significant effect on the cellulase activity and its features. For example, the optimum temperature and pH were respectively in the range of 40-50°C and 4.5. Also, cellulase produced from the new mutant was more stable (in a wider range of temperature and pH) that leads to its industrial advantages.

5. Acknowledgements

The authors are grateful to Tarbiat Modares University Research Council for the financial support.

6. Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Brijwani Kh, Singh OH, Vadlani PV. Production of a cellulolytic enzyme system in mixed-culture solid state fermentation of soybean hulls supplemented with wheat bran. Process Biochem. 2010; 45: 120-128. doi: 10.1016-j.procbio.-2009.08.015.
2. Behera S, Ray RC. Solid state fermentation for production of microbial cellulases: Recent advances and improvement strategies. Int J Biol Macro Molec. 2016; 86:656-669. doi: 10.1016/j.ijbiomac.2015.10.090.
3. Chahal DS. Production of *Trichoderma reesei* cellulase system with high hydrolytic potential by solid-state fermentation. Enzymes in biomass conversion. 1991; 460: 111-122. ACS Symposium Series. doi: 10.1021/bk-1991-0460.ch009
4. Chandel AK, Chandrasekhar G, Silva MB, da Silva SS. The realm of cellulases in biorefinery development. Crit Rev Biotechnol. 2012; 32: 187-202. doi: 10.3109/07388551.2011-595385.
5. Buck A, Casciatori FP, Thomeo JC, Tsotsas E. Model-based control of enzyme yield in solid-state fermentation. Procedia Eng. 2015; 102:362-371. doi: 10.1016/j.proeng.2015.01.163.
6. Couto SR, Sanroman MA. Application of solid-state fermentation to food industry: A review. J Food Eng. 2006; 76: 291-302. doi: 10.1016/j.jfoodeng.2005.05.022.
7. Shathele MS. Effects of gamma irradiation on fungal growth and associated pathogens. Res J Environ Toxicol. 2009; 3: 94-100. doi: 10.3923/rjet.2009.94.100
8. Deschamps F, Giuliano C, Asther M, Huet MC, Roussos S. Cellulase production by *Trichoderma harzianum* in static and mixed solid-state fermentation reactors under nonaseptic conditions. Biotechnol Bioeng. 1985; 27: 1385-1388. doi: 10.1002/bit.260270917.
9. Elakkiya P, Muralikrishnan V. Cellulase production and purification of mutant strain *Trichoderma viride*. Int J Curr Microbiol App Sci. 2014; 3: 720-727.
10. FAO: http://www.fao.org/faostat/en/#data/QC [Accessed 14 Jun 2017].
11. El-Bakry M, Abraham J, Cerda A, Barrena R, Ponsa S, Gea T, Sanchez A. From wastes to high value added products: Novel aspects of SSF in the production of enzymes. Crit Rev Environ Sci Technol. 2015; 45: 1999-2042. doi: 10.1080/-10643389.2015.1010423.
12. Florencio C, Couri S, Farinas CS. correlation between agar plate screening and solid-state fermentation for the prediction of cellulase production by Trichoderma Strains. Enzyme Res. 2012; 2012:1-7. doi: 10.1155/2012/793708.
13. Ghose TK. Measurements of cellulase activities. Pure Appl Chem. 1987; 59: 257-268. doi: 10.1351/pac198759020257.
14. Holker U, Hofer M, Lenz J. Biotechnological advantages of laboratory-scale solid-state fermentation with fungi. Appl Microbiol Biotechnol. 2004; 64: 175-186.
15. Kang SW, Park YS, Lee JS, Hong SI, Kim SW. Production of cellulases and hemicellulases by *Aspergillus niger* KK2 from lignocellulosic biomass. Bioresour Technol. 2004; 91: 153-156. doi: 10.1016/S0960-8524(03)00172-X
16. Banerjee S, Ghosh U. Production and characterization of glucoamylase by *Aspergillus niger*. Appl Food Biotechnol. 2017; 4(1): 19-26. doi: 10.22037/afb.v4i1.13261.
17. Panesar PS, Kaur R, Singla G, Sangwan RS. Bio-processing of agro-industrial wastes for production of food-grade enzymes: Progress and prospects. Appl Food Biotechnol. 2016; 3(4): 208-227. doi: 10.22037/afb.v3i4.13458.
18. Khoshnevisan K, Bordbar A-Kh, Zare D, Davoodi D, Noruzi M, Barkhi M, Tabatabaei M. Immobilization of cellulase enzyme on superparamagnetic nanoparticles and determination of its activity and stability. Chem Eng J. 2011; 171: 669-673. doi: 10.1016/j.cej.2011.04.039.
19. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959; 31: 426-428. doi: 10.1021/ac60147a030.
20. Kuar PP, Arneja JS, Singh J. Enzymic hydrolysis of rice straw by crude cellulase from *Trichoderma reesei*. Bioresour Technol. 1998; 66:267-269. doi: 10.1016/S09608524(97)-00138-7.
21. Latifian M, Hamidi-Esfahani Z, Barzegar M. Evaluation of culture conditions for cellulase production by two *Trichoderma reesei* mutants under solid-state fermentation conditions. Bioresour Technol. 2007; 98:3634-3637. doi: 10.1016/j.biortech.2006.11.019.
22. Ogel ZB, Yarangumeli K, Dundar H, Ifrij I. Submerged cultivation of *Scytalidium thermophilum* on complex lingocellulosic biomass for endoglucanase production. Enzyme Microb Technol. 2001; 28:689-695. doi: 10.1016-S0141022-9(01)00315-5.
23. Li Xh, Yang Hj, Roy B, Parkb EY, Jianga Lj, Wanga D, Miao Yg. Enhanced cellulase production of the *Trichoderma viride* mutated by microwave and ultraviolet. Microbiol Res. 2010; 165: 190-198. doi: 10.1016/j.micres.2009.04.001.
24. Nataraja S, Chetan DM, Krishnappa M. Effect of temperature on cellulose enzyme activity in crude extracts isolated from solid wastes microbes. Int J Microbiol Res. 2010; 2: 44-47.
25. Liu YT, Lou ZY, Long Ch-N, Wang HD, Long MN, Hu Zh. Cellulase production in a new mutant strain of *Penicillium decumbens* ML-017 by solid state fermentation with rice bran. N Biotechnol. 2011; 28: 733-737. doi: 10.1016/j.nbt.-2010.12.003.
26. Pandey A, Soccol CR, Mitchell D. New developments in solid state fermentation: I-bioprocesses and products. Process Biochem. 2000; 35: 1153-1169. doi: 10.1016/S00329592-(00)00152-7.
27. Pandey A, Selvakumar P, Soccol CR, Nigam P. Solid state fermentation for the production of industrial enzymes. Curr Sci. 1999; 77:149-162.
28. Petchluan P, Pukahuta Ch, Chaikong N. Characterization of xylanase and cellulase from *Lentinus polychrous* Lev. LPPT1. Chiang Mai J Sci. 2014; 41: 1007-1019.
29. Zakipour-Molkabadi E, Hamidi-Esfahani Z, Sahari MA, Azizi MH. Improvement of strain *Penicillium* sp. EZ-ZH190

for tannase production by induced mutation. Appl Biochem Biotechnol. 2013; 6: 1376-1389. doi: 10.1007/s12010-01304-36-3.

30. Rocky-Salimi K, Hamidi-Esfahani Z. Evaluation of the effect of particle size, aeration rate and harvest time on the production of cellulase by *Trichoderma reesei* QM9414 using response surface methodology. Food Bioprod Process. 2010; 88: 61-66. doi: 10.1016/j.fbp.2009.06.006.

31. Rodríguez Couto S, Sanroman M. Application of solid-state fermentation to ligninolytic enzyme production. Biochem Eng J. 2005; 22: 211-219. doi: 10.1016/j.bej.2004.09.013.

32. Shahbazi S, Ispareh Kh, Karimi M, Askari H, Ebrahimi MA. Gamma and UV radiation induced mutagenesis in *Trichoderma reesei* to enhance cellulases enzyme activity. Int J Farm Alli Sci. 2014; 3: 543-554.

33. Shahid M, Anuradha S, Mukesh S, Smita R, Neelam P. Induction of xylanase from *Trichoderma viride* by using different carbon sources. Indian J Agric Biochem. 2012; 25: 163-166.

34. Shahid M, Mukesh S, Neelam P, Smita R, Srivastava AK. Evaluation of antagonastic activity and shelf life study of *Trichoderma viride* (01PP-8315/11). Adv Life Sci. 2012; 1: 138-140.

35. Sharma S, Agarwal L, Saxena RK. Purification, Immobilization and Characterization of Tannase from *Penicillium* variable. Bioresour Technol. 2008; 99: 2544-2551. doi: 10-1016/j.biortech.2007.04.035.

36. Singhania RR, Patel AK, Soccol CR, Pandey A. Recent advances in solid-state fermentation. Biochem Eng J. 2009; 44: 13-18. doi: 10.1016/j.bej.2008.10.019.

37. Singhania RR, Sukumaran RK, Patel AK, Larroche C, Pandey A. Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. Enzyme Microb Technol. 2010; 46: 541-549. doi: 10.1016/j.enzmictec.2010.-03.010.

38. Vu VH, Pham TA, Kim K. Improvement of fungal cellulase production by mutation and optimization of solid state fermentation. Mycobiology. 2011; 39: 20-25. doi: 10.4489-MYCO.2011.39.1.020.

39. Wen Z, Liao W, Chen S. Production of cellulase/b-glucosidase by the mixed fungi culture *Trichoderma reesei* and *Aspergillus phoenicis* on dairy manure. Process Biochem. 2005; 40: 3087-3094. doi: 10.1016/j.procbio.2005.03.044.

40. Xia L, Cen P. Cellulase production by solid state fermentation on lignocellulosic waste from the xylose industry. Process Biochem. 1999; 34: 909-912. doi: 10.1016-/S0032-9592(99)00015-1.

41. Yoon LW, Ang TN, Ngoh GCh, Chua AS. Fungal solid state fermentation and various methods of enhancement in cellulase production. Biomass Bioenergy. 2014; 67: 319-338. doi: 10.1016/j.biombioe.2014.05.013.

http://dx.doi.org/10.22037/afb.v5i1.18651

Research Article

APPLIED FOOD BIOTECHNOLOGY, 2018, 5 (1): 11-18
Journal homepage: www.journals.sbmu.ac.ir/afb

pISSN: 2345-5357
eISSN: 2423-4214

بهبود تولید سلولاز و ویژگی‌های آن با جهش‌زایی *تریکودرما ریزئی* ۲۴۱۴ در سامانه تخمیر جامد با استفاده از فراورده‌های جانبی برنج

نازنین داراب زاده[۱]، زهره حمیدی اصفهانی*[۱]، پریسا حجازی[۲]

۱- گروه علوم و مهندسی صنایع غذایی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران.
۲- دانشکده مهندسی شیمی، نفت و گاز، دانشگاه علم و صنعت ایران، تهران.

چکیده

سابقه و هدف: تخمیر جامد نوعی فناوری اقتصادی برای تولید فراورده‌های با ارزش افزوده می‌باشد. همچنین، استفاده از فراورده‌های جانبی کشاورزی، به عنوان راهبرد مدیریت ضایعات، اخیرا مورد توجه قرار گرفته است. از سوی دیگر، برای تولید آنزیم، جهش‌های جدید جالب توجه می‌باشند. هدف این مطالعه، بررسی اثر جهش‌زایی بر بهبود کیفیت سلولاز بود. بنابراین، فراورده‌های جانبی برنج برای تولید سلولاز با تخمیر جامد مورد استفاده قرارگرفت. به علاوه، ویژگی‌های سلولاز جدید حاصل از گونه‌های جهش یافته بررسی شد.

مواد و روش‌ها: سلولاز با فرایند تخمیر جامد تولید شد. اسپور تعلیقی *تریکودرما ریزئی* تحت پرتوی گامای کبالت۶۰ قرار گرفت و جهش داده شد. فعالیت سلولازها (از والد و جهش یافته) با هم مقایسه شد. اثرات درجه حرارت و pH بر فعالیت سلولاز و نیز پایداری آن در شرایط بهینه مورد بررسی قرار گرفت.

یافته‌ها و نتیجه‌گیری: سلولاز به طور موفقیت‌آمیزی در تخمیر جامد بر روی مخلوطی از فراورده‌های جانبی برنج، به عنوان رشد مایه (Substrate) تولید شد. نتایج نشان داد جهش‌زایی اثر معنی‌داری بر ویژگی‌ها و فعالیت سلولاز دارد. *تریکودرما ریزئی* B (گونه جهش یافته) حدود ۳۰٪ فعالیت فیلتر پی پرآز (filter Paperase) و ۲۳٪ کربوکسی متیل سلولوز بیشتر از نسل والد داشت. فعالیت سلولاز *تریکودرما ریزئی* B در دمای بهینه ۵۰°C تا ۴۷٪ بیشتر از والد بود. در سایر دماها فعالیت سلولاز استخراجی از *تریکودرما ریزئی* B به طور معنی‌دار بیشتر از سایرین بود. به طور مثال، در دمای ۶۰°C فعالیت آنزیمی ۱۲۰٪ بیشتر از والد بود. قابل توجه است که پس از جهش زایی، افزایش ۸۴ درصدی فعالیت آنزیمی در pH=۴/۵ مشاهده شد و فعالیت سلولاز از ۰/۷۲ به ۱/۳۱ واحد در گرم ماده خشک افزایش یافت.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ تعارض منافعی وجود ندارد.

تاریخچه مقاله

دریافت ۲۸ سپتامبر ۲۰۱۷
داوری ۲۳ اکتبر ۲۰۱۷
پذیرش ۱۲ نوامبر۲۰۱۷

واژگان کلیدی

- سلولاز
- تخمیر حالت جامد
- جهش
- *تریکودرما ریزئی*
- فرآورده های جانبی برنج

***نویسنده مسئول**

زهره حمیدی-اصفهانی،
گروه علوم و مهندسی صنایع غذایی، دانشکده کشاورزی، دانشگاه تربیت مدرس، تهران، ایران.

تلفن: ۴۸۲۹۲۴۷۴ ۹۸۲۱+
دورنگار: ۴۸۲۹۲۲۰۰ ۹۸۲۱+

پست الکترونیک:
hamidy_z@modares.ac.ir