Algerian Journal of Chemical Engineering
EISSN: 2773-3068 (Online)
DOI: *BEN HMAD I et al / vol. 01, n°1 - 2024*

Original Article Open Access

Alkalophilic cellulases production from *Stachybotrys microspora* and its potential application in denim biostoning

Ines Ben Hmad[a*], Hafedh Belghith[a], Ali Gargouri[a]

[a]*Laboratory of Molecular Biotechnology of Eukaryotes, Center of Biotechnology of Sfax (CBS)/ University of Sfax, PO Box "1177" 3018, Sfax –Tunisia; inesbenhmad@gmail.com; faouzi.gargouri@cbs.rnrt.tn; hafeth.belghith@cbs.rnrt.tn*

Article history: Received 11/02/2024, Revised 28/03/2024, Accepted 04/05/ 2024

ABSTRACT

The aged look of denim can be the result of the trap of non-homogenous indigo-dyed cellulose microfibers by the mechanical and enzyme actions. However, the major problem is the re-deposition of eliminated indigo dye on the denim fabrics during bio-stoning with acid endoglucanases. This work aims to study the production profile of endoglucanases by Stachybotrys microspora in the presence of lignocellulosic biomass wastes (sugarcane bagasse and macro-algae) at medium initial pH7. The produced endoglucanases by the Stachybotrys strain were monitored by enzymatic assay and zymogram analysis. The best carbon source is sugarcane bagasse, with an optimum production at day 7. More interestingly, the zymogram analysis confirmed that a conditional expression of an alkaline cellulasewas displayed on the sugarcane bagasse based medium and revealed that sugarcane bagasse and macro-algae of the culture medium directed a differential induction of alkalophilic and acidic endoglucanases. Comparing the denim bio-stoning, with our crude enzyme to those with commercial ones showed significantly better results.

This research shows that alkalophilic endoglucanases from Stachybotrys microspora can be considered an efficient additive for denim bio-stoning applications.

Keywords: Alkalophilic endoglucanses; Lignocellulosic waste; *Stachybotrys microspora*; denim biostoning

Recommended Citation
BEN HMAD I, BELGHITH H, GARGOURI A. (2024). Alkalophilic cellulases production from *Stachybotrys microspora* and its potential application in denim biostoning. *Algerian Journal of Chemical Engineering*, 01, 08-17. http://dx.doi.org/10.5281/zenodo.11487313

1. Introduction

During the last decades, the growth of enzymatic technology concerns several sectors: food, pharmaceutical, and industrial (textiles, detergents, paper,...) [1]. Enzymes used in these applications constitute a biological alternative to chemical agents. Indeed, enzymes are eco-friendly and extremely efficient at catalyzing reactions with high substrate specificities under mild reaction conditions with environmental requirements [2]. Cellulose, the most abundant biopolymer of plants, is a macromolecule consisting of D-glucose monomers linked by β-(1,4)-glycosidic bonds [3]. It can be valorized as a valuable substrate for microbial fermentation to produce value-added bio-products. Several microorganisms (fungi and bacteria) synthesize cellulolytic enzymes (endoglucanases, cellobiohydrolases and β-glucosidases) having a synergistic action to complete the hydrolysis of cellulose [4]. Cellulaseswere used to convert cellulosic wastes to glucose and have been implicated in several applications as bioremediation processes, paper and pulp industry, ethanol production, and in brewing industry [5]. Most cellulases are often acidophilic enzymes except a few bacterial and more rarely fungal cellulases [6]. Endoglucanases from *Trichoderma reesei* are the most applied in the textile industry [7]. However, neutral and alkalophilic endoglucanases are biocatalysts with increasing demand in industrial applications such as in the textile industry. Indeed, endoglucanases acting at neutral pH overcome the major problem of indigo back-staining in the application of denim washing (re-deposition of indigo on the tissue at acidic pH due to the use of acidophilic cellulases) [8-9]. For example, neutral endoglucanases from *Humicola insolens* produce less dye re-deposition than cellulases from *Trichoderma reesei* which can be explained by the major factor of back staining control enzyme pH. [10, 11]. Indeed, acidophilic endoglucanases can be greatly adsorbed on cotton tissue, and it seems to be the main cause of the re-deposition of indigo [11]. Furthermore, the experience of alkalophilic endoglucanases from *Chrysosporium lucknowense* strain in denim jeans bio-stoning showed color reduction performance, very low levels of back-staining, and good scarping [12]. Additionally, neutral and alkaline cellulases increase the brightness of the treated denim garment compared with acid cellulases [13].

Stachybotrys microspora is a cellulolytic fungal strain that coproduces acid and alkaline cellulases and can grow on a wide pH range [14], whereas most fungi have an acidic pH within 4–6 [15]. Most interestingly, this strain produces two neutral endoglucanases with the optimum activity being reached at pH=7 [16-17].

The present work aims to study the production of alkalophilic endoglucanases by *Stachybotrys microspora* in the presence of lignocellulosic biomass wastes and to describe the effectiveness of this cellulase for the stoning of denim garments.

2. Materials and Methods

2.1. Materials

This experiment was carried out on the mutant A19 from the *Stachybotrys microspora* (N1) strain isolated by our laboratory group [18]. A19 is slightly improved in the production of cellulases[18]. Carboxymethyl-cellulose (CMC) was purchased from Sigma–Aldrich (St-Louis, USA). Sugarcane bagasse, agroindustrial by-products, was kindly provided by a

local food processing industry (Sfax, Tunisia) and marine macro-algae from the Tunisian coast. All lignocellulosic biomass is conserved at 4°C until use.

2.2. Methods

2.2.1 Growth medium conditions

The growth medium for endoglucanases production from *Stachybotrys microspora* was composed of a modified Mandels medium (g/L) as reported previously by the authors [19, 14], supplemented with 10 g/L lignocellulosic biomass (sugarcane bagasse and macro-algae). Each of the cheap substrates was used at 10 g/L, to study the effects of sugarcane bagasse and macro-algae on the production of endoglucanases. This fungus was incubated at pH 7, 30°C, and with shaking at 150 rpm.

Crude enzyme was obtained by centrifuging at (4500 rpm, 15 min) to remove mycelium. The supernatant was studied for endoglucanase activity and by zymogram. Each culture was carried out in triplicate.

2.2.2 Endoglucanase activity assay

Endoglucanase activity was tested using carboxymethyl-cellulose (CMC) as a substrate. The reaction solution composed of 0.5 ml of crude enzyme diluted with 50 mM citrate buffer at pH 5.0 and 0.5 mL of 1% CMC was incubated at 50°C for 30 min. The amount of reducing sugars released was measured using the 3,5-dinitrosalicylic acid (DNS) way [20]. The reaction solution was heated for 10 min, after adding 3 mL of DNS reagent. After that, 20 mL of distilled water was added to the solution and the absorbance was determined at 550 nm. The blank was set using a denatured enzymatic solution and substrate (1% CMC) by heat.

The unit of endoglucanase activity was determined as the quantity of enzyme demanded to release 1 µmole of reducing sugar per minute under test conditions [20].

2.2.3 Protein quantification assay

The protein concentration was evaluated using the Bradford assay. Bovine serum albumin (BSA) was used as a protein standard for the Bradford assay [21].

2.2.4 Zymogram analysis

Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) [22] was performed on 10 % separating gel and 5 % stacking gel. Protein samples were mixed with a loading dye (1/5 volume). After separation, the gel was incubated in a Tris–HCl buffer, pH 8, 20 mM, for 120 min to eliminate Sodium Dodecyl Sulphate (SDS) allowing protein renaturation. After equilibration for 15 min in either sodium acetate buffer (pH 5; 0.1 M) or in phosphate buffer (pH 8; 0.1 M), the gel was superposed against a gel overlay containing 1 % agar, 1 % CMC (citrate buffer pH 5, 50 mM; phosphate buffer pH 8, 50 mM) and 20 mL citrate buffer (pH 5, 50 mM; phosphate buffer pH 8, 50 mM). After incubation at 50 °C, congo red at 0.1 % was used to stain the overlay for 1 h. After that, it was distained in NaCl solution at 1 M. On the red background, clear bands showed the breakdown of the endoglucanase substrate [14].

2.2.5 Biostoning of denim fabric by endoglucanase

The bio-stoning capacity of the endoglucanase was assessed according to the manufacturer's process. The washing protocol is initiated with the denim garments de-sizing by their treatment in hot water at 100°C for 30 min. After that, the denim garment was treated with the crude endoglucanases (10U) in a 500 mL Erlenmeyer flask: a 6×8-cm denim swatches was submerged into 50 ml of 50 mM sodium phosphate buffer (pH 7.0) rotating at 200 rpm at 50°C for 1 hour. The bio-stoning process involves a detergent wash to eliminate enzymes and prevent any additional loss of fiber strength, followed by rinsing with tap water. Denim watches treated without enzymes are used as a control sample. Among the crude enzymes from *Stachybotrysmicrospora*, we have used two commercial endoglucanases Beizym (commercial enzyme including endocellulolytic enzymes from Swiss manufactory) and EnzySM (commercial enzyme including endocellulolytic enzymes from UAI manufactory).

3. Results and Discussion

3.1. Endoglucanase production

To produce microbial biomolecules via an efficient bioprocess, two types of dried lignocellulosic biomass wastes were used (sugarcane bagasse, and macro-algae) as feedstock. Lignocellulosic biomass, abundant and renewable, derives from plants and is primarily composed of three elements: cellulose, hemicellulose, and lignin [23]. The monitoring of endoglucanase production has been performed in the initial growth medium at pH7 containing sugarcane bagasse, and macro-algae as a carbon source at 10 g/L. As shown in Figure 1, the highest level of production of endoglucanases (0.19 U/mL, and 0.1 U/mL, respectively) by *Stachybotrys microspora* was after 168h of cultivation, after which enzyme activity decreased with time. (Fig.1). Indeed, in fungi, the enzyme expression is affected by factors related to the hierarchy of substrate utilization by the fungal strain, the catabolite regulation of the expression of corresponding proteins [24]. Carbon catabolite repression inhibits the use of less preferred carbon sources by repressing the expression of enzymes [25]. Many researchers who showed that the highest production of endoglucanases was observed after 168h of incubation [26-28] found similar conditions. The best substrate for the production of endoglucanases was sugarcane bagasse. Indeed, sugarcane bagasse is known as one of the highest cellulose-containing lignocellulosic biomass and is utilized for improved cellulase production [29].

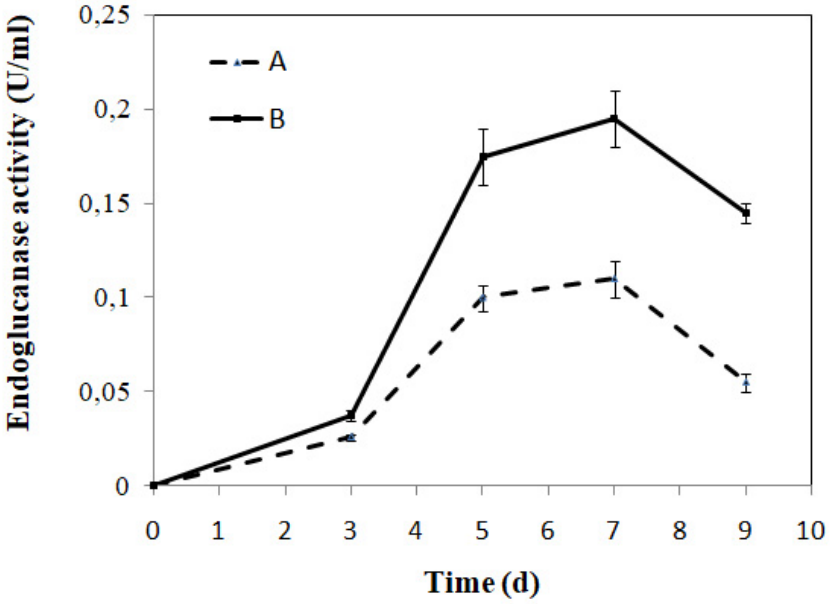

Fig.1. The time course production of endoglucanases by *Stachybotrys microspora* using sugarcane bagasse and macro-algae at 1 %.

To better characterize the alkalophilic endoglucanase activity produced by the *Stachybotrys microspora* strain, we have performed a zymogram analysis of the crude cellulase extracts. A total quantity of proteins of 40 µg, not heated, was deposited on an SDS-PAGE gel. The gel containing the unheated proteins allows the enzymatic activity to be revealed by zymogram at pH 5 and 8. Figure 2 shows that sugarcane bagasse induces more endoglucanase activity than the dried macro-algae. This substrate strongly induces the production of at least two endoglucanases: the first is found in the "staking-gel", which is more active at alkaline pH (Fig 2b) while the second is acidic (Fig.2a). Endoglucanase activity expression relies not only on the substrate utilized in the culture but also on the pH of the zymogram. This behaviour is almost identical to that found on wheat bran produced by the same strain [14]. Figure 2 shows that sugarcane bagasse induces the highest levels of endoglucanase activities but with two different profiles of enzymes. Dried macroalgae induces deficient levels of endoglucanase activity (Fig 2). Furthermore, when comparing zymograms conducted at both pH 5 and 8, distinct disparities in quantitative and qualitative data emerge. Specifically, smaller-sized endoglucanases are evident when overlay is performed at pH 5 using sugarcane bagasse as a culture substrate. These results clearly demonstrate the diversity of our fungal strain in endoglucanases, with some acting at alkaliphilic pH and others acting at acidic pH. It is interesting to note that *Stachybotrys microspora* produces alkalophilic cellulases, while the majority of the fungal endoglucanases are active at acidic pH. For example, the crude endoglucanase solution obtained from *Trichoderma atroviride* 676 and *Penicillium* sp. CR-316 showed an optimum at pH 4, while others from *Myceliophthora thermophila* M.7.7 exhibited an optimum pH of 5.0 [30]. The endoglucanases produced by *Aspergillus fumigatus* revealed maximum activity at pH 5.0 [31].

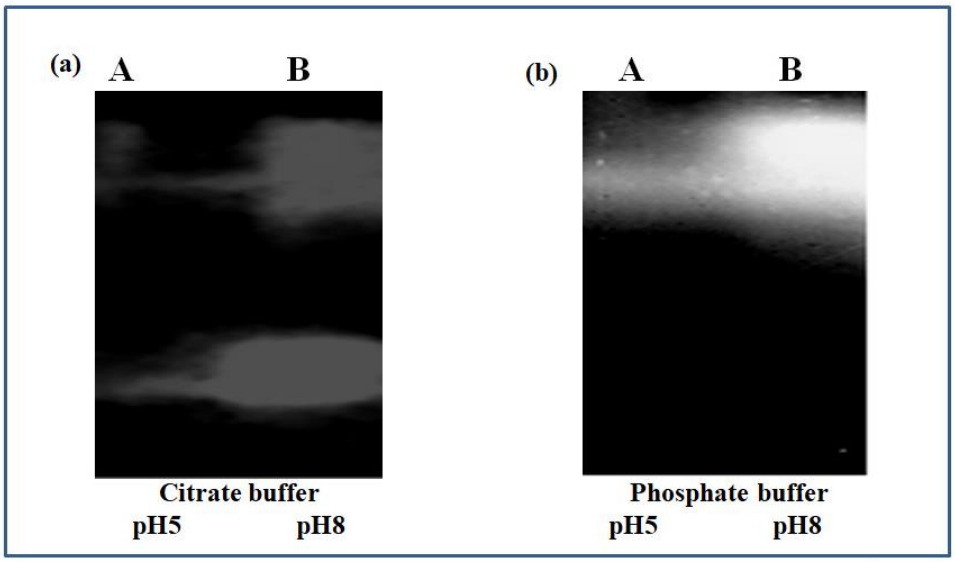

Fig.2. Comparison of zymograms of endoglucanase activities of crops with sugarcane bagasse and macro-algae after 1 h of incubation at 50 °C, at pH 5(a) and pH 8 (b).

3.2. Denim Bio-stoning

The bio-stoning process is conducted using an environmentally friendly process. Compared with only buffer treatment (without enzymes), endoglucanases from *Stachybotrys microspora* increased denim weight and indigo dye removal Fig.3a. Denim garments treated with crude endoglucanase exhibit qualitatively comparable weight loss and indigo dye removal to those treated with commercial neutral cellulase EnzySM (Fig.3b) indicating that it is an excellent potential enzyme for denim bio-stoning application. Similar results were found with cellulases from *Aspergillus* sp. and from Pol6 mutant of *Penicillium occitanis* [32, 33]. The bio-stoning process consists of giving denim a more uniformly aged appearance (Fig.3). These results align well with the fact that endoglucanases play an important role in removing the indigo dye from the fabric surface [33]. Indeed, cellulases act on denim garments and break off the small fibre ends on the tissue, allowing the dye to release after washing [34]. Moreover, the advantage of using cellulases in bio-stoning denim is the absence of streaks, which was very often observed by traditional jeans manufacturers, less damage of fibers, work-effortless, increased productivity of the machines and environmentally benign and homogeneous removal of the indigo [35]. Indeed, the use of endoglucanase for finishing results in back-staining due to the affinity of indigo dye towards the cellulose fiber-bound enzyme. Alkaline condition is preferable for the enzymatic processing of denim since lower back-staining takes place under this condition. Andreaus et al., (2005) suggested that the tissue fabric surface could be highly negatively charged, allowing the dispersion of negatively charged indigo particles by repulsing, whereas, under acid pH, the fabric surface would be less charged [36]. Furthermore, endoglucanases are applied to increase the appearance of cellulosic fabrics by eliminating pills and fuzz fiber from the surface, delivering softening benefits, or reducing pilling propensity [37].

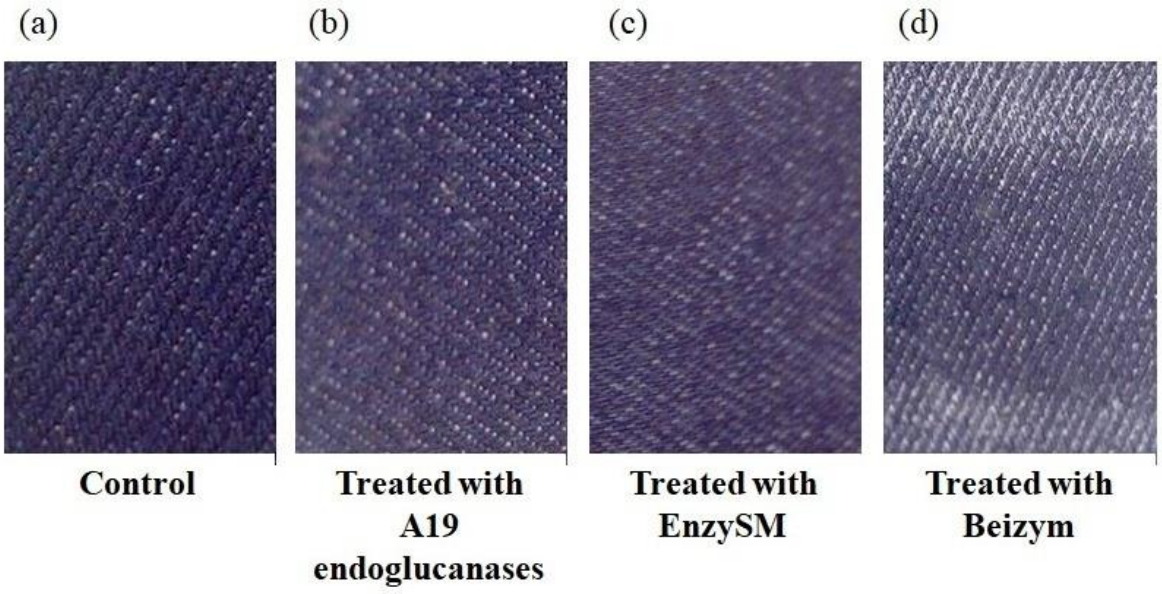

Fig.3. Comparison of biostoning denim by commercial or *Stachybotrys microspora* endoglucanases .(a): control; (b) treated with A19 endoglucanases; (c) treated with EnzySM; (d) treated with Beizym.

4. Conclusion

We shall recall that the mutant A19 from *Stachybotrys microspora* can produce alkalophilic endoglucanases. This a peculiarity of this fungus that it is very required in industrial applications. The sugarcane bagasse is the best lignocellulosic biomass to produce these enzymes. Endoglucanases are highly favored for various industrial applications, including textile, printing and dyeing, pulp and paper, as well as laundry industries. Compared with other commercial endoglucanases, *Stachybotrys microspora* endoglucanases offer an excellent enzyme cocktail for the bio-stoning of blue denim garments and compare very well with commercial endoglucanases. Thus, the *Stachybotrys microspora* strain can produce alkalophilic endoglucanases, which are highly required in bio-stoning in the textile industry.

Acknowledgements

This study was conducted thanks to grants supported by the Ministry of Higher Education and Scientific Research, Tunisia.

Funding Support

The Ministry of Higher Education and Scientific Research, Tunisia sponsor this work.

Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

No data was used for the research described in the article.

References

1. Maghraby, Y. R., El-Shabasy, R. M., Ibrahim, A. H., Azzazy, H. M. E. S. (2023) Enzyme Immobilization Technologies and Industrial Applications. ACS Omega, 8, 5184−96. https://doi.org/10.1021/acsomega.
2. Ostovan, A., Arabi, M., Wang, Y., Li, J., Li, B., Wang, X. Chen L. (2022) Greenificated Molecularly Imprinted Materials for Advanced Applications. Advanced Materials, 34, 1-32. DOI: 10.1002/adma.202203154.
3. Lehrhofer, A. F., Goto, T., Kawada, T., Rosenau, T., Hettegger, H. (2022) The in vitro synthesis of cellulose A mini-review. Carbohydrate Polymers, 285, 119222.https://doi.org/10.1016/j.carbpol.2022.119222.
4. Thapa, S., Mishra, J., Arora, N., Mishra, P., Li, H., O' Hair, J., Bhatti, S., Zhou, S. (2020) Microbial cellulolytic enzymes: diversity and biotechnology with reference to lignocellulosic biomass degradation. Reviews in Environmental Sciences and Biotechnology, 19, 621-648. https://doi.org/10.1007/s11157-020-09536-y.
5. Gupta, A., Gupta, R., Singh, R. L. Microbes and Environment Principles and Applications of Environmental Biotechnology for a Sustainable Future. In Singh, R. L. (ed.). Applied Environmental Science and Engineering for a Sustainable Future. Springer Science+Business Media Singapore, (2017), 43-84. DOI 10.1007/978-981-10-1866-4_3.
6. Balla, A., Silini, A., Cherif-Silini, H, Bouket, A. C., Boudechicha, A., Luptakova, L., Alenezi, F. N., Belbahri, L., (2022) Screening of Cellulolytic Bacteria from Various Ecosystems and Their Cellulases Production under Multi-Stress Conditions. Catalysts, 769, 1-29.https://doi.org/10.3390/catal12070769.
7. Jayasekara, S., Ratnayake, R. Microbial Cellulases: An Overview and Applications. In: Pascual, A. R., Martín, M. E. E. (Eds.). Cellulose. (2019). DOI: 10.5772/intechopen.84531.
8. Ben Hmad, I., Gargouri, A., (2017) Neutral and alkaline cellulases: Production, engineering, and Applications. Journal of Basic Microbiology, 9999, 1-6. DOI: 10.1002/jobm.201700111.
9. Khan, M. K. R., Jintun, S. (2021) Sustainability Issues of Various Denim Washing Methods. Textile and Leather Review, 4, 96-110. https://doi.org/10.31881/TLR.2021.01.
10. Gusakov, A.V., Sinitsyn, A. P., Markov, A.V., Sinitsyna, O.A., Ankudimova, N.V., Berlin, A. G., (2000a) Study of protein adsorption on indigo particles confirms the existence of enzyme–indigo interaction sites in cellulase molecules. Journal of Biotechnology, 87, 83–90.
11. Anish, R., Rahman, M S, Rao M., (2007) Application of CellulasesFrom an *AlkalothermophilicThermomonospora* sp. in Biopolishing of Denims. Biotechnology and Bioengineering, 96, 48-56.
12. Juturu,V., Wu, J. C. (2014)Microbial cellulases: Engineering, production and applications. Renewable and Sustainable Energy Reviews 33, 188-203.https://doi.org/10.1016/j.rser.2014.01.077.
13. Hoque, M. T., Mazumder, N. S., Islam, M. T. Enzymatic Wet Processing. In: Rather, L. J., Shabbir, M., Haji, A. (eds.). Sustainable Practices in the Textile Industry. Scrivener Publishing LLC, (2021), pp. 87–110.
14. BenHmad, I., Abdeljalil, S., Saibi, W., Amouri, B., Gargouri, A., (2014) Medium initial pH and carbon source stimulate differential alkaline cellulase time course production in *Stachybotrysmicrospora*. Applied and Biochemical Biotechnology, 172, 2640–2649.
15. Rousk, J., Brookes, P. C., Bååth, E., (2009) Contrasting Soil pH Effects on Fungal and Bacterial Growth Suggest Functional Redundancy in Carbon Mineralization. Applied and Environmental Microbiology,75, 1589–1596.

16. BenHmad, I., Boudabbous, M., Belghith, H., Gargouri, A., (2017) A novel ionic liquid-stable halophilicendoglucanase from *Stachybotrysmicrospora*. Process and Biochemistry, 54, 59–66.
17. Benhmad, I., Boudabbous, M., Yaîch, A., Rebai, M., Gargouri, A., (2016) A novel, neutral, halophile *Stachybotrysmicrospora*-based endoglucanase active impact on β-glucan. Bioprocess. Biosyst. Eng., 39, 685–693.
18. Amouri, B., Gargouri, A., (2006) Characterization of a novel β-glucosidase from a *Stachybotrys*strain. Biochemical Engineering Journal, 32, 191–97.
19. Mandels, M., Weber, J., (1969) The production of cellulases.Advances in Chemistry,95, 391.
20. Miller, G. L., (1959) Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31, 426–428.
21. Bradford, M. M., (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry,72, 248–254.
22. Laemmli, U. K., Favre, M., (1973) Maturation of the head of bacteriophage T4: I. DNA packaging events. Journal of Molecular Biology, 80, 575–592.
23. Ayadi, I., Belghith, H., Gargouri, A., Guerfali, M., (2019) Utilization of Wheat Bran Acid Hydrolysate by *Rhodotorulamucilaginosa*Y-MG1 for Microbial Lipid Production as Feedstock for Biodiesel Synthesis. BioMed Research International, 2019, 1-11. https://doi.org/10.1155/2019/3213521.
24. Zhang, T., Liu, H., Lv, B., Li, C., (2020) Regulating Strategies for Producing Carbohydrate Active Enzymes by Filamentous Fungal Cell Factories. Frontiers in Bioengineering and Biotechnology, 8, 1-15. doi: 10.3389/fbioe.2020.00691.
25. Kiesenhofer, D., Mach-Aigner, A., Mach, R., Understanding the mechanism of carbon catabolite repression to increase protein production in filamentous fungi. In: Schmoll, M., Dattenböck, C. (Eds). Gene Expression Systems in Fungi: Advancements and Applications. Fungal Biology. (Cham: Springer), (2016), pp 275–288. Doi: 10.1007/978-3-319-27951-0_12.
26. Ladeira, S. A., Cruz, E., Delatorre, A. B., Barbosa, J. B., Martins, M. L. L., (2015) Cellulase production by thermophilic *Bacillus* sp. SMIA-2 and its detergent compatibility. Electron. Journal of Biotechnology 18, 110-115. http://dx.doi.org/10.1016/j.ejbt.2014.12.008.
27. Ellilä, S., Fonseca, L., Uchima, C., Cota, J., Goldman, G. H., Saloheimo, M., Sacon, V., Siika-aho, M., (2017) Development of a low-cost cellulase production process using *Trichoderma reesei* for Brazilian biorefineries. Biotechnology and Biofuels, 30, 1-17. DOI 10.1186/s13068-017-0717-0.
28. Ferreira, F. L., Dall'Antonia, C. B., Shiga, E. A., Alvim, L. J., Pessoni, R. A. B., (2018) Sugarcane bagasse as a source of carbon for enzyme production by filamentous fungi. Hoehnea. 45, 134-142. http://dx.doi.org/10.1590/2236-8906-44/2017.
29. Srivastava, N., Mohammad, A., Singh, R., Srivastava, M., Syed, A., Pal, D. B., Elgorban, A. M., Mishra, P. K., Gupta, V. K., (2021). Evaluation of enhanced production of cellulose deconstructing enzyme using natural and alkali pretreated sugar cane bagasse under the influence of graphene oxide. Bioresour. Technol., 342, 126015. https://doi.org/10.1016/j.biortech.2021.126015.
30. Moretti, M. M. S., Bocchini-Martins, D. A., Da Silva, R., Rodrigues, A., Sette, L. D., Gomes, E., (2012) Selection of thermophilic and thermotolerant fungi for the production of cellulases and xylanases under solid-state fermentation. Brazilian Journal of Microbiology 43, 1062–1071.

31. Luo, Z., Fan, Y., Li, Q., Han, B., Liu, Y., Li, S., Qiu, H., Pang, Z., (2017) Isolation, purification and characterization of 5′-phosphodiesterase from *Aspergillus fumigatus*. PLoS One, 12, 1-13.
32. Ahmed, J.,Taslim, A. U., Raihan, T., Sohag, M. M. H.,Suhani, M. H. S., Qadri, F., Azad, A. K., (2023) Characterization of an endo-beta-1,4glucanase gene from paper-degrading and denim bio-stoning cellulase producing *Aspergillus* isolates. Biotechnology and Applied Biochemistry, 70, 1057–1071. DOI: 10.1002/bab.2420.
33. Belghith, H., Ellouz-Chaabouni, S., Gargouri, A., (2001) Biostoning of denims by *Penicilliumoccitanis*(Pol6) cellulases. Journal of Biotechnology, 89, 257–262.
34. Behera, B.C., Sethi, B.K., Mishra, R.R., Dutta, S.K., Thatoi, H.N., (2017) Microbial cellulases – Diversity & biotechnology with reference to mangrove environment: A review. Journal of Genetic Engineering and Biotechnology, 15, 197-210.
35. Sukumaran, R. K., Singhania, R. R., Pandey, A., (2005) Microbial_cellulases Production, applications and challenges. Journal of Science and Industrial Research, 64, 832-844.
36. Andreaus, J., Campos, R., Gubitz, G. Cavaco-Paulo, A., (2000) Influence of cellulases on indigo backstaining. Textile Research Journal, 70, 628- 632.
37. Anish, R., Rahman, M. S., Rao, M., (2006) Application of CellulasesFrom an Alkalothermophilic*Thermomonospora*sp. in Biopolishing of Denims. Biotechnology and Bioengineering, 96, 48-56.DOI 10.1002/bit.