STUDY ON BIOPRODUCTION ENHANCEMENT OF CELLULASE FROM *Bacillus* sp. D11

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Abstract. Cellulase, an enzyme that hydrolyzes the glycosidic bonds in cellulose to form simple sugar molecules, is widely used in various industrial processes. The finding of new microbial strains producing extracellular cellulase as well as their cellulase biosynthesis conditions has been continuingly reported up to date. In this study, the influence of several factors such as cellulose substrate source, nitrogen source, initial pH, temperature, incubation time for cellulase biosynthesis of *Bacillus* sp. D11 has been identified. *Bacillus* sp. D11 showed strong cellulase biosynthesis with 450.49±12.03 UI/mL in the medium containing 2.0% rice straw (w/v), 1.0% earthworm powder (w/v), 1.0% NaCl (w/v), 0.5% yeast extract (w/v), initial pH 6.0 at 37°C, 150 rpm after 96 hours of incubation. By 16S-rRNA sequencing method, *Bacillus* sp. D11 was identified to be closely related to *Bacillus amyloliquefaciens* MT013383. Overall, the obtained results will contribute to the development of cellulase production processes and applications in different fields.

Key words. *Bacillus*, cellulase, extracellular enzyme, culture condition.

1. INTRODUCTION

Cellulase is the third largest group of enzymes widely used in industry. With the ability to cleave β-1,4 glycosidic bonds in cellulose to form "single sugar" monosaccharides such as β-glucose or into shorter oligosaccharides without the production of toxic by-products [1]. Cellulase is applied in many different fields such as fabric softening in textile technology, production of detergents, environmental treatment, food technology, removing ink in papermaking technology, herbal technology, … [2-5]. In addition, many studies have also shown the potential application of cellulase in the production of biofuel and bio-alcohol from agricultural waste [6-8]. The popular use of cellulase shows its efficacy and sustainable value in the degradation of cellulose, however, the main disadvantage in commercial use of cellulase is its high cost.

Cellulase is a complex enzyme family that is often obtained from diverse groups of microorganisms. Bacteria are one of the most popular production sources due to their ability to proliferate quickly and adapt well to environmental conditions. The search for new sources of microorganisms for cellulase production is still ongoing [8, 9]. Studies on suitable conditions to improve cellulase biosynthesis and reduce production costs are also being conducted around the world [6, 10, 11]. The production of cellulases using *Bacillus* has also been extensively studied due to the outstanding properties of this genus, including robust growth, low nutrient requirements, high extracellular enzyme production, and heat tolerance. These properties make *Bacillus* strains suitable for application in various industries [9, 12-14]. Therefore, in this study, the *Bacillus* sp. D11 was evaluated for its ability to biosynthesize cellulase and suitable culture conditions for enzyme biosynthesis were also investigated.

2. MATERIALS AND METHODS

2.1 Bacterial strain

Bacillus sp. D11 strain was previously isolated from soil in Ho Chi Minh city using dilution-spread plate method on Luria-Bertani agar (10.0 g/L tryptone, 5.0 g/L yeast extract, 10.0 g/L NaCl, 15.0 g/L agar, pH 7.0) at 37°C and the strain was subsequently stored at -70°C in the collection of the Microbiotechnology laboratory, Institute of Food and Biotechnology, Industrial University of Ho Chi Minh city. The strain was reactivated overnight in Luria-Bertani broth with shaking at 150 rpm at 37°C before being used for further studies.

2.2 Examination of cellulase production

Cellulase production of *Bacillus* sp. D11 was examined by spot inoculation on LB agar supplemented with 0.5% CMC at 37℃ for 72 hours. After 72 hours, 2.0 mL of 3.0% Lugol solution was spread evenly over the surface of the plate and the halo zone was recorded. The larger diameter of the halo zone indicated the stronger enzyme activity [15].

2.3 Identification of *Bacillus* sp. D11

The strain *Bacillus* sp. D11 were identified at the molecular level by sequencing the gene fragment encoding 16S-rRNA using the primer pairs 27F 5'-AGAGTTTGATCMTGGCTCAG-3' and 1540R 5'-AAGGAGGTGATCCAACCGCA-3'. The sequence was then used to build a phylogenetic tree based on Neighbor-Joining algorithm with a bootstrap of 1000X using Mega 5.0 software.

2.4 Determination of bacterial growth curve

Overnight culture of *Bacillus* sp. D11 was inoculated in 50 mL of LB broth supplemented with 0.5% CMC (w/v) with shaking at 150 rpm for 16 hours at 37℃. Subsequently, the culture was diluted (1.0% v/v) to obtain 100 ml culture and the bacterial growth was monitored by mesuaring OD_{600nm} every 2 hours for 24 hours. The growth curve was analyzed and plotted by GraphPad Prism 8 (GraphPad Software, USA) [16].

2.5 Cellulase activity assay

Cellulase activity was determined according to the Bernfeld method [17]. The bacterial culture was firstly centrifuged at 13.000 rpm, 4℃ to remove the cells. Obtained supernatant was then used to mix with 1.0% CMC solution and the reaction was set at 50℃ for 30 minutes. DNS reagent was prepared by dissolving 5.0 g of dinitrosalicylic acid in 250 mL of distilled water at 80℃. As this solution cooled down to room temperature, 100 mL of NaOH, 2 N and 150 g of potassium sodium tartarate-4-hydrate were added, and the volume was brought up water to 500 mL with distilled. DNS reagent was then added to stop the reaction and determine the released amount of reduced sugar. The enzyme unit (UI) of cellulase was defined as the amount of enzyme required to catalyze the degradation of cellulose to yield one micromole of reducing sugars in one minute at 50℃.

2.6 Investigation of *Bacillus* sp. D11 cellulase biosynthesis conditions

Effect of supplementation of different cellulose substrates including ground rice straw, ground rice husk, ground sawdust, ground coir, CMC (carboxyl methylcellulose) on cellulase biosynthesis of *Bacillus* sp. D11 was investigated using the basic LB broth at 1.0% (w/v) concentration of dry weight, 1.0% starter culture, pH 7.0, shacking 150 rpm at 37℃. After every 24 hours, the cell-free crude enzyme was collected and used for cellulase activity determination. The type of substrate which yields the highest enzyme activity was selected for further tests. The effect of substrate concentration with various concentrations of 0%, 0.5%, 1.0%, 2.0%, 3.0% (w/v) under the same culture conditions. The substrate concentration which yields highest cellulase activity was selected for further tests.

The effect of different inorganic and organic nitrogen sources such as NH_4NO_3, $(NH_4)_2SO_4$, NH_4Cl, $(NH_2)_2CO$ (urea), peptone, earthworm powder (EP) on cellulase production was investigated at 1.0% (w/v) concentration under the same culture conditions. The substrate concentration which yields highest cellulase activity was selected for further tests.

Effect of initial pH and temperature on cellulase biosynthesis was also studied in culture with suitable cellulose substrate and nitrogen sources adjusted for various initial pH values of 3.0-9.0, and at different temperature of 28℃, 33℃, 37℃, 40℃, 45℃. The culturing process and cellulase activity determination was performed as in previous experiments.

2.7 Data analysis

The results of the experiments are an average of 3 replicates. Raw data were calculated, and graphs were drawn using Microsoft Excel 2010. The results were statistically analyzed by Statgraphics XVI software using ANOVA test with $\alpha = 0.05$

3. RESULTS AND DISCUSSION

3.1 Macroscopic, microscopic characteristics and cellulase production of *Bacillus* sp. D11

Macroscopic characteristics of the *Bacillus* sp. D11 on LB medium was observed after 48 hours of incubation at 37°C with 0.3-0.5 cm round, light yellow dry colonies, wrinkled and convex surface, irregular thick margins, and forming round holes in the center (**Fig**. 1A-a). Microscopic characteristics were observed under optical microscope at 1000X after Gram staining and spore staining. The Gram staining result showed that the strain is Gram-positive due to its color retention with crystal violet and exists as single cells or in pairs (**Fig**. 1A-b). The strain was capable of forming endospores with pink vegetative cells and green spores after staining (**Fig**. 1A-c).

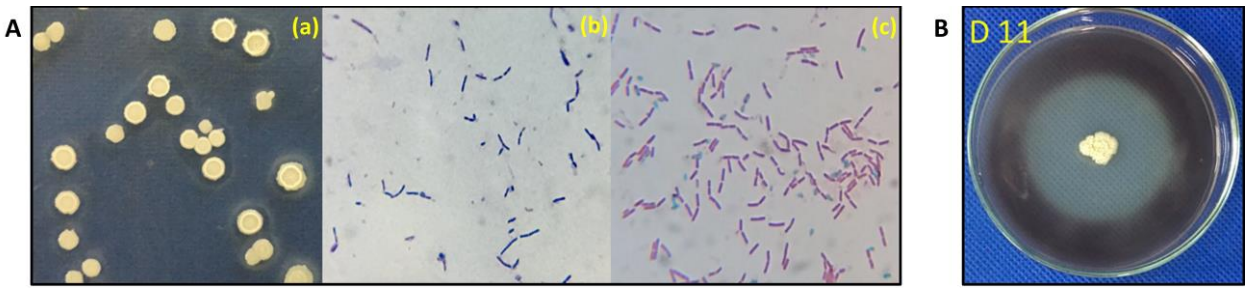

Figure 1. Morphology of *Bacillus* sp. D11. (A), (a) colony morphology (b) Gram staining (c) spore staining. (B) cellulase biosynthesis on LB-CMC.

In addition, decomposition of CMC on agar plate by *Bacillus* sp. D11 was also observed with a 3.2±0.02 cm halo zone (**Fig**. 1B), which was similar to that of *Bacillus* spp. in the study of Huynh Thi Cam Tien and Ho Viet The (2019) with halo zone diameter of 1.2-3.2 cm [18] and *Bacillus* sp. in the study by Mai Thi et al. in 2017 (2.38 cm) [19]. This shows that *Bacillus* sp. D11 has high potential for the production of extracellular cellulase.

3.2 Identification of *Bacillus* sp. D11 and dermination of the growth curve

A part of the gene encoding for 16S-rRNA of *Bacillus* sp. D11 with a length of 1314 nucleotides was sequenced and compared with the data bank on NCBI using the BLASTn tool. The results showed that this strain has the highest similarity (90%) with *Bacillus amyloliquefaciens* species. Additionally, the genetic relationship of *Bacillus* sp. D11, *Bacillus amyloliquefaciens* and other *Bacillus* species were also represented by phylogenetic tree in which *Bacillus* sp. D11 was closely related to *Bacillus amyloliquefaciens* MT013383 (**Fig**. 2).

The growth curve of *Bacillus* sp. D11 was also successfully constructed with an R^2 value of 0.9895. In LB medium, the strain was in the lag phase during the first 2 hours with OD_{600nm} equal to 0.172 and eventually entered the log phase with OD_{600nm} reached 1.068 after 8 hours of incubation. From 12 hours to 24 hours of incubation the bacteria remained in the stationary phase. Therefore, the culture in 12 hours with the most viable and healthy population was chosen as the seed inoculum for subsequent experiments (**Fig**. 3).

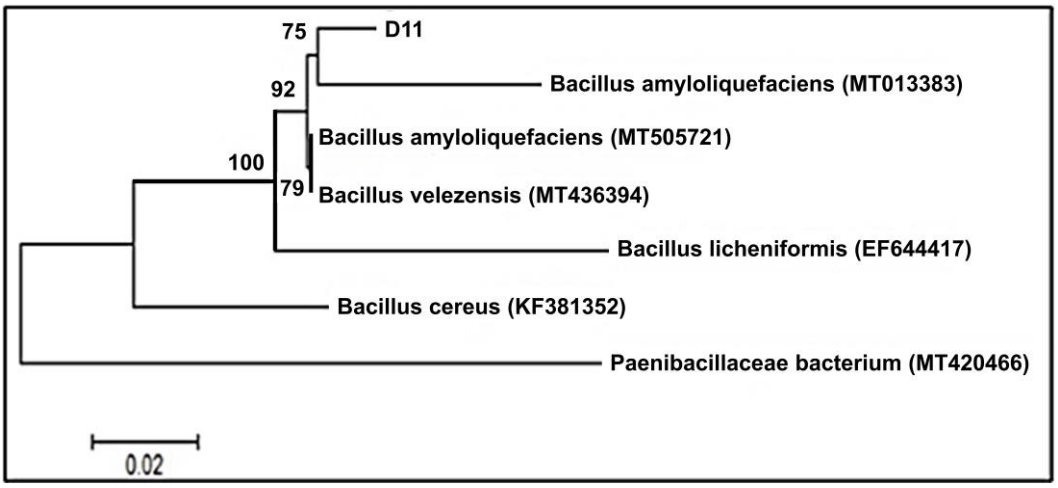

Figure 2: Phylogenetic tree of *Bacillus* sp. D11

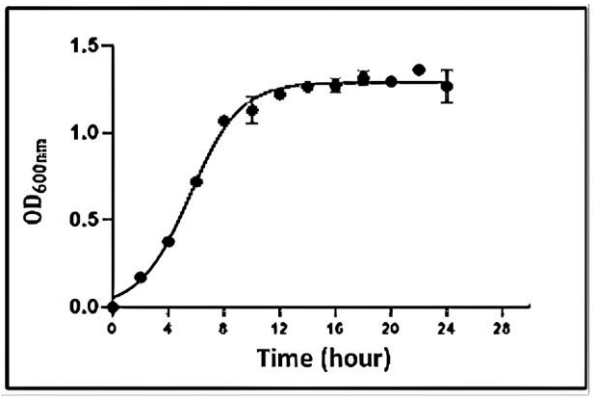

Figure 3: Growth curve of *Bacillus* sp. D11 in LB medium

3.4 Effect of cellulose substrates on cellulase biosynthesis of *Bacillus* sp. D11

The results showed that *Bacillus* sp. D11 was able to produce cellulase in the presence of all 5 types of cellulose substrates, and the enzyme activity gradually increased during 96 hours of incubation. However, when increasing the incubation time to 120 hours, cellulase activity in all experiments was sharply reduced (**Fig**. 4). The cause of enzyme activity decrease could be that the bacteria have entered the final stage of the stationary phase as well as the substrates in the medium were depleted at this point. Comparing among the different substrates, cellulase activity obtained from the medium containing rice straw was highest at all time points (**Fig**. 4A). At 96 hours of incubation, enzyme activity was highest in all experiments with 183.89±0.92 UI/mL, 163.05±15.68UI/mL, 159.09±8.14 UI/mL, 157.64±2.34 UI/mL, and 138.71±9.8 UI/mL respectively with the substrates being rice straw, rice husk, CMC, coir, and sawdust (**Fig**. 4B).
Statistical analysis showed a significant difference in cellulase activity in the case of rice straw compared with other substrate ($p<0.05$). Rice straw was also confirmed to be suitable substrate for several other *Bacillus* strains such as *B. licheniformis* MVS1 and *Bacillus* sp. MVS3 [20], *B. subtilis* [13], *B. circulans* AB [21], and *Bacillus* sp. 313SI [22]. Rice straw was also used as the main substrate for cellulase production by *Bacillus subtilis* subsp. *subtilis* JJBS300 [23]. In addition, rice husk is a suitable substrate after rice straw for cellulase production of *Bacillus* sp. D11 and *Bacillus amyoliquefaciens* DL-3 [24]. Rice straw and rice husk are agricultural by-products, therefore their use in enzyme production would be an economy and eco-friendly approach.

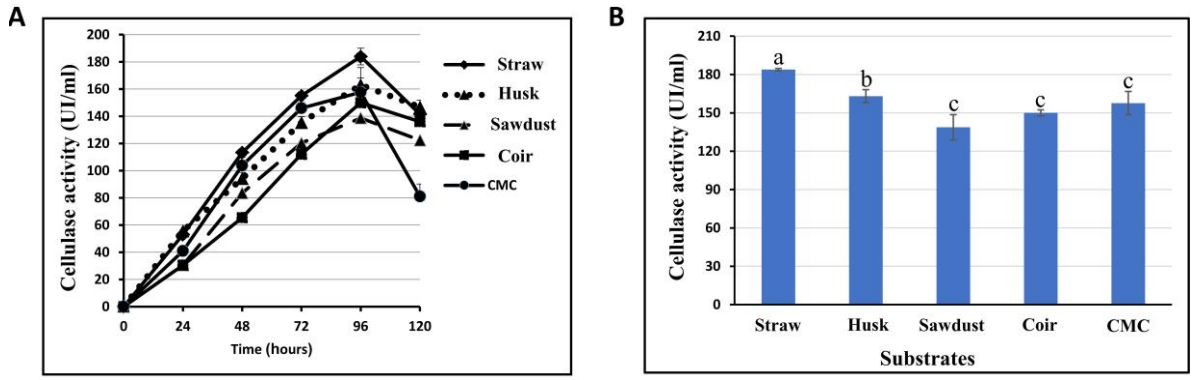

Figure 4. Effect of cellulose substrates on cellulase production of *Bacillus* sp. D11. Time-course of cellulase activity from *Bacillus* sp. D11 in the medium containing various subtrates (A) and enzyme activity at 96 hours of culture (B). Different lower-case letters denote significant difference ($p<0.05$).

Figure 5 showed that the concentration of rice straw also strongly affected the cellulase biosynthesis of *Bacillus* sp. D11. Cellulase activity of all cultures containing different amounts of rice straw strongly increased at 48 hours and reached the highest level at 96 hours of incubation with 2.0% (262.56±10.76 UI/mL) and 3.0% (250.19±14.97 UI/mL) of rice straw concentrations (**Fig**. 5A). These activities were increased by about 1.4-1.5 folds compared to the two concentrations of 1.0% (185.87±2.32 UI/mL) and 0.5% (173.9±1.74 UI/mL) of rice straw, and by about 3.6 folds compared to the control group with no rice straw (72.93±8.02 UI/mL) (**Fig**. 5B).

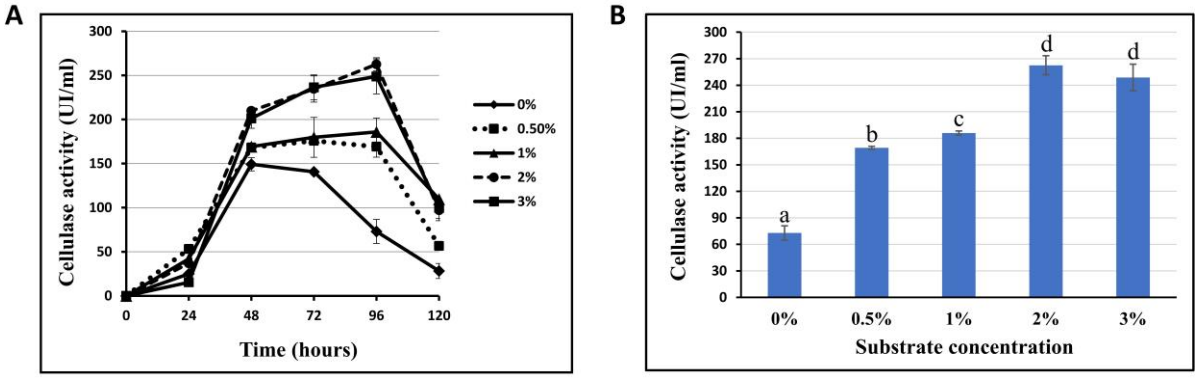

Figure 5. Effect of rice straw concentrations on cellulase production of *Bacillus* sp. D11 (A) and enzyme activity at 96 hours of culture (B). Different lower-case letters denote significant difference ($p<0.05$).

When the concentration of rice straw was increased to 3.0%, the cellulase activity did not increased, possibly because the higher concentration of rice straw would affect the aeration of the culture by shaking, thus affecting the bacterial growth and cellulase biosynthesis. Rice straw was also used as a suitable substrate source for cellulase production of the two strains of *B. licheniformis* MVS1 and *Bacillus* sp. MVS3 isolated from hot springs according to the study of Somen Acharya et al. (2012) [20]. Nevertheless, the the strain *Bacillus* sp. Y3 required 2.0% CMC as the suitable substrate for cellulase production in the study of Lugani et al. in 2015 [25]. Statistical analysis showed that cellulase activity in medium supplemented with 2.0% of rice straw was the highest and therefore, was selected to be used in the fermentation medium for further investigations.

3.6 Effect of nitrogen sources on cellulase biosynthesis of *Bacillus* sp. D11

From the results presented in **Fig**. 6, it could be deduced that different nitrogen sources had different effects on cellulase production of *Bacillus* sp. D11. Cellulase production was significantly higher in the medium supplemented with organic nitrogen sources with a drastic increase at 48 hours of incubation than in the medium supplemented with inorganic nitrogen sources such as NH_4NO_3, $(NH_4)_2SO_4$, and NH_4Cl. In addition, almost no cellulase production was observed in medium supplemented with $(NH_4)_2SO_4$ or NH_4Cl

at any time points, suggesting that the inorganic nitrogen sources were not suitable for cellulase biosynthesis of *Bacillus* sp. D11 (**Fig**. 6A). In the medium supplemented with organic nitrogen sources, cellulase production increased as incubation time increased. At 96 hours of incubation, especially, the medium containing earthworm powder displayed the highest activity of 286.13±6.46 UI/mL, significantly higher than in the case of peptone (233.10±5.59 UI/mL) and is more than 2-folds in comparison with urea (134.74±2.05UI/mL) (**Fig**. 6B).

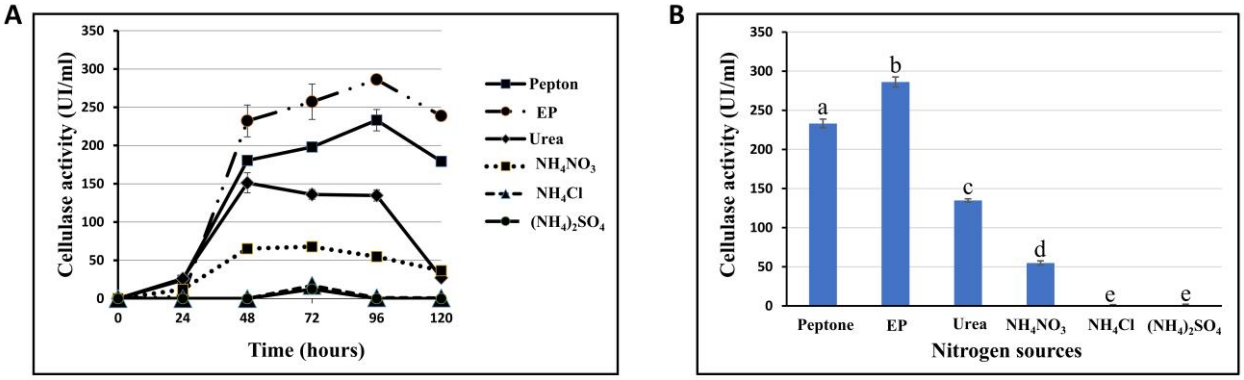

Figure 6: Effect of nitrogen sources on cellulase production of *Bacillus* sp. D11. Time-course of cellulase activity of *Bacillus* sp. D11 in medium supplemented with various nitrogen sources (A) and enzyme activity at 96 hours of culture (B). EP: earthworm powder. Different lower-case letters denote significant difference ($p<0.05$).

Several studies have shown different suitable nitrogen source requirements for cellulase production by vary *Bacillus* strains such as peptone for *Bacillus* sp. Y3 and *B. pseudomycoides* [25, 26], soybean powder for *B. amyloliquefaciens* S1 [27], meat extract for *B. licheniformis* MVS1 and $(NH_4)_2SO_4$ for *Bacillus* sp. MVS3 [20]. In this study, earthworm powder was a suitable nitrogen source for *Bacillus* sp. D11 to synthesize cellulase, possibly because earthworm powder, which is a hydrolyzed earthworm product, contain a variety of oligopeptides, amino acids, and a small amount of minerals which could promote the bacterial growth and cellulase production [28].

3.8 Effect of initial pH, temperature, and incubation time on cellulase biosynthesis of *Bacillus* sp. D11

Bacillus sp. D11 showed different cellulase production capacities under different initial pH values, which is shown in **Fig**. 7. In media with initial pH values of 3.0 to 4.0, very little cellulase activity was detected (0.96 to 31.70 UI/mL). However, cellulase activity increased with increasing pH, reaching a maximum after 96 hours of culture, followed by a strong decrease after 120 hours of culture. The initial pH of 6.0 was the most suitable for cellulase production by *Bacillus* sp. D11, with the highest activity observed at all time points. The cellulase activity at pH 6.0 after 96 hours of culture was 450.49±12.03 UI/mL, which was 1.7-folds higher than the activity at neutral pH (7.0, 266.28±15.59 UI/mL). At pH 5.0, the cellulase activity after 96 hours of culture was 383.73±18.31 UI/mL, which was also 1.44-fold higher than the activity at neutral pH. In alkaline media with pH values of 8.0 to 9.0, the cellulase activity was only about one-half of the maximum, 153.84 to 174.16 UI/mL (**Fig**. 7A). These results indicate that the optimal initial pH for strain D11 to synthesize cellulase was acidic pH (6.0). In other studies, the optimal pH threshold was usually weak acidic or neutral pH (6.5-7.0) for cellulase biosynthetic *Bacillus* strains such as *B. alcalophilus* S39 and *B. amyloliquefaciens* C2 [29], *B. pseudomycoides* [26], *B. licheniformis* BCLLNF-01 [30], *B. subtilis* KO [31]. Some *Bacillus* strains were reported to have the best cellulase biosynthesis ability in the highly acidic initial pH, such as pH 3.5 for *Bacillus* sp. [32], pH 4.0 for *B. subtilis* subsp. *subtilis* JJBS300 [23], pH 5.0 for *B. amyloliquefaciens* D19 [33].

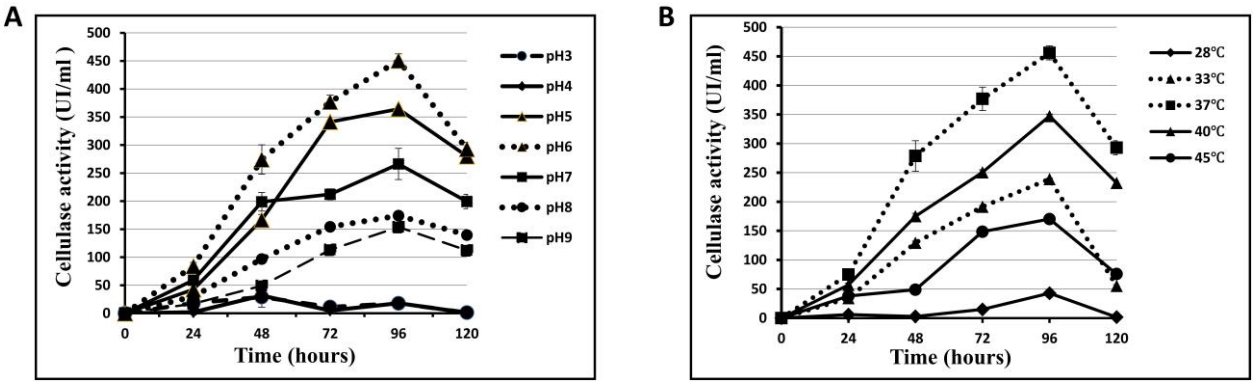

Figure 7. Cellulase activity of crude enzyme from media with different initial pH values (A) and at different growth temperature (B).

The cellulase production of *Bacillus* sp. D11 was also investigated under different temperature conditions (28-45℃). Cellulase activity was significantly affected by temperature conditions after 48-120 hours of culture. A difference of 3-4℃ resulted in a significant decrease in cellulase activity. The highest cellulase activity was observed after 96 hours of incubation at all temperature conditions, followed by a considerable drop after 120 hours of culture which is consistent with the cellulase biosynthesis of *B. licheniformis* BCLLNF-01 [30]. The most suitable temperature for cellulase production by strain D11 was 37℃, with an activity of 450.49±12.03 UI/mL. The activity obtained from culture at 40°C was 347.32±1.41 UI/mL, followed by 33℃ (238.94 UI/mL), 45°C (153.84 UI/mL), and 28°C (62.98±9.83 UI/mL). These results indicate that the optimal temperature and time for strain D11 to synthesize cellulase was 37℃ after 96 hours of incubation (**Fig**. 7B). Likewise, this growth temperature was also proved to be suitable for *Bacillus* sp. Y3 [25], *B. amyloliquefaciens* D19 [33], and *B. brevis* VS-1 [34], to produce extracellular cellulase.

4. CONCLUSION

In this study, macro and micro morphological properties of *Bacillus* sp. D11 were recorded, and the strain was found to be closely related to *B. amyloliquefaciens* MT013383. The suitable culture conditions for cellulase biosynthesis of *Bacillus* sp. D11 has been identified with medium containing 2.0% rice straw, 1.0% EP, 0.5% yeast extract, 1.0% NaCl (w/v), initial pH 6.0, 150 rpm shaking at 37℃ for 96 hours, thereby resulted in a 2.5-fold increase in cellulase activity (450.49±12.03 UI/mL). Variantion of cellulase activity of the culture from tested conditions also suggested that this enzyme may favour weak acidophilic and mesophilic conditions and rice straw as cellulose substrate. Further charaterization of the purified enzyme is in progress and the obtained results provide valuable insights into the development of extracellular cellulase production from the *Bacillus* genus and its applications in related fields.

REFERENCES

[1] S.F. Sulzenbacher G, Morosoli R, Dupont C, Davies GJ, The *Streptomyces lividans* family 12 endoglucanase: construction of the catalytic core, expression, and X-ray structure at 1.75 Å resolution. *Biochemistry*. 36(51): p. 16032-16039. 1997.

[2] V.G. Krishnaswamy, et al., Cellulase enzyme catalyst producing bacterial strains from vermicompost and its application in low-density polyethylene degradation. *Chemosphere*. 288: p. 132552. 2022.

[3] H. Azzaz, et al., Research Article Cellulase Production by *Fusarium graminearumand* its Application in Ruminant's Diets Degradation. 23(1): p. 27-34. 2020.

[4] G. Chen, et al., Application of cellulase treatment in ionic liquid based enzyme-assisted extraction in combine with in-situ hydrolysis process for obtaining genipin from *Eucommia ulmoides* Olive barks. *Journal of Chromatography A*. 1569: p. 26-35. 2018.

[5] C.Y. Hui, K.C. Lee, and Y.P. Chang, Cellulase-Xylanase-Treated Guava Purée by-Products as Prebiotics Ingredients in Yogurt. *Plant Foods for Human Nutrition*. 77(2): p. 299-306. 2022.
[6] M.Y. Areeshi, Microbial cellulase production using fruit wastes and its applications in biofuels production. *International Journal of Food Microbiology*: p. 109814. 2022.
[7] K.N. Rajnish, et al., Immobilization of cellulase enzymes on nano and micro-materials for breakdown of cellulose for biofuel production-a narrative review. *International journal of biological macromolecules*. 182: p. 1793-1802. 2021.
[8] M.S. Samuel, M. Govarthan, and E. Selvarajan, A comprehensive review on strategic study of cellulase producing marine actinobacteria for biofuel applications. *Environmental Research*: p. 114018. 2022.
[9] R. Rajesh and S.N. Gummadi, α-Amylase and cellulase production by novel halotolerant *Bacillus* sp. PM06 isolated from sugarcane pressmud. *Biotechnology and Applied Biochemistry*. 69(1): p. 149-159. 2022.
[10] S. Yan, et al., Alleviating vacuolar transport improves cellulase production in *Trichoderma reesei*. *Applied Microbiology and Biotechnology*. 107(7-8): p. 2483-2499. 2023.
[11] G.B. Santos, et al., Cellulase production by *Aspergillus niger* using urban lignocellulosic waste as substrate: Evaluation of different cultivation strategies. *Journal of Environmental Management*. 305: p. 114431. 2022.
[12] X.S. Shigan Yan, Wencheng Zhang, Liping Zhu, Isolation identification and cellulase-producing condition optimization of *Bacillus subtilis* Q3. *AIP Conference Proceedings* 2110(020004). 2019.
[13] A.A. Mangalanayaki R., Production and optimization of cellulase by *Bacillus subtilis* using coir waste and sawdust under solid state fermentation. *World Journal of Pharmaceutical and medical research*. 4: p. 102-108. 2018.
[14] M. Irfan, et al., Production and Characterization of Organic Solvent-Tolerant Cellulase from *Bacillus amyloliquefaciens* AK9 Isolated from Hot Spring. *Appl Biochem Biotechnol*. 182(4): p. 1390-1402. 2017.
[15] M.J. Leboffe, Microbiology Laboratory Theory & Application 2010: the United States of America. 2010.
[16] R.G. R. C. Kuhad, Y. P. Khasa, Bioethanol production from lignocellulosic biomass: an overview. *Wealth from Waste, B. Lal, Ed., Teri Press*. 2010.
[17] A.P. Bernfeld, Methods in Enzymology. *Methods in Enzymology*. 1: p. 149-158. 1995.
[18] H.T.C. Tiên and H.V. Thế., Phân lập và định danh một số chủng *Bacillus* spp. có hoạt tính cao trong tầng đất mặt được thu thập từ tỉnh Bình Thuận. *Tạp chí Khoa học Công nghệ và Thực phẩm*. 18(2): p. 48-62. 2019.
[19] M. Thi, N.H. Hiệp, and D.N. Thúy, Phân lập, nhận diện vi khuẩn phân hủy cellulose từ sùng (*Holotrichia parallela*) và trùn đất (*Lubricus terrestris*). *Tạp chí Khoa học Trường Đại học Cần Thơ*. 50: p. 81-90. 2017.
[20] S. Acharya and A. Chaudhary, Optimization of Fermentation Conditions for Cellulases Production by *Bacillus licheniformis* MVS1 and *Bacillus* sp. MVS3 Isolated from Indian Hot Spring. *brazilian archives of biology and technology*. 55: p. p. 497-503. 2012.
[21] J.K.G. Ashita Dhillon, B.M. Jauhari, S. Khanna, A cellulase-poor, thermostable, alkalitolerant xylanase produced by *Bacillus circulans* AB 16 grown on rice straw and its application in biobleaching of eucalyptus pulp *Bioresource Technology* 73: p. 273-277. 2000.
[22] A.M. Varsha Goyal, A.K. Bhuwal, G. Singh, A. Yadav and N. Kumar. Aggarwal Parametric Optimization of Cultural Conditions for Carboxymethyl Cellulase Production Using Pretreated Rice

Straw by *Bacillus* sp. 313SI under Stationary and Shaking Conditions. *Biotechnology Research International* 2014. 2013.

[23] Anu, et al., Optimization of cellulase production by *Bacillus subtilis* subsp. *subtilis* JJBS300 and biocatalytic potential in saccharification of alkaline-pretreated rice straw. *Preparative Biochemistry & Biotechnology*. 51(7): p. 697-704. 2021.

[24] Y.J. Lee, et al., Purification and characterization of cellulase produced by *Bacillus amyoliquefaciens* DL-3 utilizing rice hull. *Bioresource technology*. 99(2): p. 378-386. 2008.

[25] Y. Lugani, R. Singla, and B. Singh Sooch, Optimization of Cellulase Production from Newly Isolated *Bacillus* sp. Y3. *Journal of Bioprocessing & Biotechniques*. 5(11). 2015.

[26] S.K. Pramanik, et al., Fermentation optimization of cellulase production from sugarcane bagasse by *Bacillus pseudomycoides* and molecular modeling study of cellulase. *Current Research in Microbial Sciences*. 2: p. 100013. 2021.

[27] M. Ye, et al., The optimization of fermentation conditions for producing cellulase of *Bacillus amyloliquefaciens* and its application to goose feed. *R Soc Open Sci*. 4(10): p. 171012. 2017.

[28] P.T. Việt, et al., Thử nghiệm sản xuất và bước đầu ứng dụng bột đạm từ trùn quế (*Perionyx excavatus*) lên đàn heo con lai (Yourshire và landrace) sau cai sữa. *Journal of Science and Technology-IUH*. 44(02): p. 167-175. 2020.

[29] K.A.A. Abou-Taleb, Mashhoor, W.A., Nasr, Sohair A., Sharaf, M.S. and and H.H.M. Abdel-Azeem, Nutritional and Environmental Factors Affecting Cellulase Production by Two Strains of Cellulolytic Bacilli. *Australian Journal of Basic and Applied Sciences*. 3: p. 2429-2436. 2009.

[30] R.N. da Silva, L.F. de Andrade Melo, and C.L.L. Finkler, Optimization of the cultivation conditions of *Bacillus licheniformis* BCLLNF-01 for cellulase production. *Biotechnology Reports*. 29: p. e00599. 2021.

[31] M. Mandels and E.T. Reese, Induction of cellulase in fungi by cellobiose. *J Bacteriol*. 79: p. 816-26. 1960.

[32] P.K.S. Maidul Islam, Dr. A.K.M. Mohiuddin, Md. Suzauddula, Optimization of fermentation condition for cellulase enzyme production from *Bacillus* sp. *Malaysian Journal of Halal Research Journal (MJHR)*. 2(2). 2019.

[33] N.N. Ẩn, et al., Khảo sát các điều kiện sinh tổng hợp cellulase từ *Bacillus amyloliquefaciens* D19. *Journal of Science and Technology-IUH*. 49(01): p. 67-76. 2021.

[34] V.K. Singh and A. Kumar, Production and purification of an extracellular cellulase from *Bacillus brevis* vs-1. *IUBMB Life*. 45(3): p. 443-452. 1998.

NGHIÊN CỨU GIA TĂNG SINH TỔNG HỢP CELLULASE TỪ *Bacillus* sp. D11

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Tóm tắt. Cellulase là enzyme thủy phân các liên kết glycosyl trong cellulose để tạo thành các phân tử đường đơn và được ứng dụng rộng rãi trong các quy trình công nghiệp khác nhau. Việc tìm ra các chủng vi sinh khả năng sinh tổng hợp cellulase ngoại bào và điều kiện thích hợp để sinh tổng hợp enzyme này vẫn luôn được tiến hành cho đến nay. Trong nghiên cứu này, ảnh hưởng của các yếu tố như cơ chất cảm ứng, nguồn nitrogen, pH ban đầu, nhiệt độ, thời gian nuôi ủ cho quá trình sinh tổng hợp cellulase của *Bacillus* sp. D11 đã được xác định. Chủng vi khuẩn *Bacillus* sp. D11 đã thể hiện khả năng sinh tổng hợp cellulase mạnh với hoạt tính 450,49±12,03 UI/mL trong môi trường có chứa 2,0% rơm (w/v), 1,0% bột trùn (w/v), 1,0% NaCl (w/v), 0,5% cao nấm men (w/v), pH 6,0 ở điều kiện 37°C, lắc 150 vòng/phút sau 96 giờ nuôi ủ. Bằng phương pháp giải trình tự 16S-rRNA, chủng vi khuẩn *Bacillus* sp. D11 được xác định có quan hệ gần với loài *Bacillus amyloliquefaciens* MT013383. Các kết quả đạt được góp phần cho việc phát triển nguồn giống sản xuất cellulase và là cơ sở để nghiên cứu ứng dụng cellulase trong các lĩnh vực khác nhau.

Từ khóa. *Bacillus*, cellulase, enzyme ngoại bào, điều kiện nuôi cấy.

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