Open Access Library Journal
2021, Volume 8, e7291
ISSN Online: 2333-9721
ISSN Print: 2333-9705

Production of α-Amylase by *Bacillus subtilis* QM3 and its Enzymatic Properties

Qingping Hu*, Jingjing Liu

School of Life Science, Shanxi Normal University, Linfen, China
Email: *hqp72@163.com

How to cite this paper: Hu, Q.P. and Liu, J.J. (2021) Production of α-Amylase by *Bacillus subtilis* QM3 and its Enzymatic Properties. *Open Access Library Journal*, **8**: e7291.
https://doi.org/10.4236/oalib.1107291

Received: March 7, 2021
Accepted: March 26, 2021
Published: March 29, 2021

Abstract

Alpha-amylase is a starch hydrolyzing enzyme which has many industrial applications. In this study, *Bacillus subtilis* QM3 has ability to produce α-amylase. Identification was done by iodine test, based on the clear zone around the sample in starch agar plates. Alpha-amylase activity was measured using 3,5-dinitrosalicylic acid (DNS). The result showed *B. subtilis* QM3 can produce α-amylase and activity of α-amylase was 50.58 IU/mL. The study on enzymatic properties was found: the optimum temperature is 70˚C, and its stability is high at 30˚C - 70˚C. The optimum pH is 6.0, and the stability is high under the condition of pH was 6.0 - 7.0. Ca^{2+}, Na^{+}, K^{+} and Mg^{2+} promoted the activity of alpha-amylase, and their relative enzyme activities were 130.1%, 118.1%, 115.9% and 110.4% respectively. Fe^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+} and EDTA inhibited the activity of alpha-amylase. The relative enzyme activities were 86.1% 77.8%, 67.4%, 64.2% and 48.4% respectively.

Subject Areas

Bioengineering, Microbiology

Keywords

Bacillus subtilis QM3, Alpha-Amylase, Enzymological Properties

1. Introduction

Enzymes are macromolecular biocatalysts that initiate and increase the rate of thousands biochemical and metabolic reactions in living cells [1]. Amylase is a general term for enzymes that hydrolyze starch and glycogen. There are many kinds of amylases, and amylases can be divided into α-amylase and β-amylase according to the isomeric types of hydrolysates. Alpha-amylase (1,4-a-D- glucanohydrolase; EC 3.2.1.1) is one of the enzymes that have the ability to hydrolyze

and cleave the α-1,4-glucosidic linkage and catalyze the breakdown of starch and starch derivatives; amylopectin and amylose; oligosaccharides and dextrin, liberating reducing groups [2] [3] [4]. Alpha-amylase represents about 30% of the whole enzyme market in the world. Proteases are the first [5] [6].

Alpha-amylase is widely used in industrial production [7] [8] [9]. α-amylase is used in starch processing industry [10], such as the manufacture of glucose, maltose, various kinds of dextrin, etc. α-amylase is used in food industry, such as bread baking, monosodium glutamate, spices, fruit juice, etc. α-amylase is used in brewing and fermentation industries, such as beer, liquor, soy sauce, vinegar, etc. In textile industry, α-amylase is used in chemical fiber, high-quality silk and man-made cotton desizing process. In pharmaceutical, medical, α-amylase can be made into different kinds of industrial enzymes, medical enzymes, diagnostic enzymes, digestive drugs, etc. In the detergent industry [12], α-amylase acts synergistically with alkaline protease, lipase, etc, to give it a better wash. It is also widely used in paper industry and feed industry.

Plants, animals and microorganisms have the ability to produce amylases [13] [14]. Amylases derived from microbial sources are preferred because of its plasticity, vast availability and they have a great potential in fulfilling industrial demands. *Bacillus* species is considered the best producers of α-amylase that play essential roles in starch processing industry [15]. Amylases isolated from different *Bacillus* species have significantly different properties, such as the optimum pH and temperature as well as metal ion requirements [15].

In this study, taking *Bacillus subtilis* QM3 as the research object, production of α-amylase by *B. subtilis* QM3 was identification by iodine test. The amylase activity of *B. subtilis* QM3 was determined by 3,5-dinitrosalicylic acid (DNS), and the enzymatic properties were studied. It provides a theoretical basis for the application of *B. subtilis* QM3.

2. Materials and Methods

2.1. Materials

2.1.1. Bacteria Materials

Bacillus subtilis QM3 used in the present study come from the microbiological lab, College of Life Science, Shanxi Normal University, China [16].

2.1.2. Media

Basal culture medium (g/100mL): peptone 1.0, beef extract 0.3, sodium chloride 0.5, agar 2.0, pH 7.4-7.6, 121˚C for 20 min.

Screening medium (g/100mL): peptone 0.5, NaCl 0.3, soluble starch 0.2, agar 2.0, pH 7, 121˚C for 20 min.

Seed medium (g/100mL): yeast extract 0.5, peptone 1.0, NaCl 0.5, soluble starch 0.2, monopotassium phosphate 0.1, water, pH 7.0, 121˚C for 20 min.

Fermentation medium (g/100mL): bran 3.0, dipotassium phosphate 0.1, sodium nitrate 0.1, water, pH 7.0, 121˚C for 20 min.

2.2. Methods

2.2.1. Identification of Alpha-Amylase Produced by *B. subtilis* QM3

B. Subtilis QM3 strain was activated and inoculated into culture dish of screening medium and cultured for 48 h. After growing colony, 0.04 g iodine was lightly sprinkled on the cover of the culture dish, Inverted Petri dish, and the iodine was fumigated for 5 minutes, and the appearance of transparent ring was observed [14].

2.2.2. Determination of *α*-Amylase Activity

Preparation of crude enzyme solution: The *B. Subtilis* QM3 were inoculated into seed medium and cultured at 37˚C for 24 h. The fermentation medium was inoculated with 5% inoculum and incubated at 150 r/min and 37˚C for 48 h. The bacterial liquid was centrifuged at 5000 r/min for 10 min. The supernatant is the crude enzyme fluid to be measured.

Alpha-amylase activity was measured using 3,5-dinitrosalicylic acid (DNS) [1]. The unit of enzyme activity was defined as follows: at 70˚C, pH = 6, amylase catalyzed starch hydrolysis for 5 min to produce maltose μmol number, representing one unit of enzyme activity.

2.2.3. Effect of pH on the Enzyme Activity and Stability

The activity of *α*-amylase was measured at pH 3, 4, 5, 6, 7, 8, 9 and 10, respectively. The enzyme solution was placed at pH 3, 4, 5, 6, 7, 8, 9 and 10 for 60 min, and then the activity of *α*-amylase was measured at the corresponding pH. The relative enzyme activity was calculated by comparing the enzyme activity with that of untreated enzyme solution.

2.2.4. Effect of Temperature on the Enzyme Activity and Stability

To investigate the effect of temperature on the alpha-amylase activity, the enzyme assay was carried out over the range of 30˚C - 90˚C. The optimum temperature for the alpha-amylase stability was also considered by incubating the enzyme at the respective temperatures for 1 h. The relative enzyme activity was calculated by comparing the enzyme activity with that of untreated enzyme solution.

2.2.5. Effect of Metal Ions and EDTA on the Enzyme Activity

Various metal ions (Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}) and EDTA were added into the enzyme solution to be tested, respectively, and treated for 60 min. The enzyme activity was measured under the action of different metal ions and EDTA. Enzyme activity in the absence of the chemical agents or the metal ions was considered as 100%.

2.3. Statistics Analysis

All data are the average of three repetitions. The obtained results were analyzed statistically by SPSS (Statistical Product and Service Solutions) statistical software. Data were analyzed by the analysis of variance and treatment mean com-

parison by using least significance difference (LSD; $p < 0.05$).

3. Results and Discussion

3.1. *Bacillus subtilis* QM3 Produces *α*-Amylase

B. Subtilis QM3 grew on the flat plate of amylase medium, and transparent ring appeared on the flat plate by iodine fumigation (Figure 1). The results indicated that *B. subtilis* QM3 could produce *α*-amylase.

3.2. Effect of Temperature on Activity of *α*-Amylase

The influence of temperatures on the activity of *α*-amylase produced by *B. subtilis* QM3 is shown in Figure 2. It can be seen that low temperature can inhibit the activity of the enzyme. At 30˚C - 70˚C, with the increase of temperature, the activity of the enzyme also continues to increase, and the activity of the enzyme reaches the highest at 70˚C. After 70˚C, the enzyme activity decreased continuously, and when 90˚C, the enzyme activity was very low. The results showed that the optimum reaction temperature of amylase was 70˚C.

Figure 1. The starch hydrolysate circle produced by *B. subtilis* QM3.

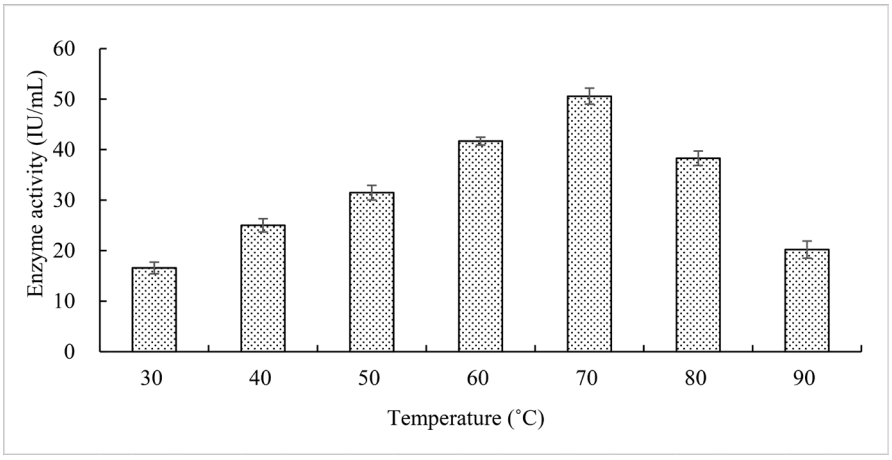

Figure 2. Effect of temperature on *α*-amylase activity.

3.3. Temperature Stability of α-Amylase

As can be seen from Figure 3, when the enzyme solution was left at 30˚C - 90˚C for 60 minutes, the activity of alpha-amylase decreased. The enzyme activity was above 60% at 30˚C - 70˚C. When the temperature exceeded 70˚C, the enzyme activity decreased greatly, and the enzyme activity basically lost at 90˚C. It shows that the highest temperature of amylase tolerance is 70˚C, and then the amylase is inactivated because of high temperature.

3.4. Effect of pH on Activity of α-Amylase

The influence of different pH on the activity of α-amylase produced by *B. subtilis* QM3 is shown in Figure 4. The α-amylase activity increased first and then decreased with the increase of pH value. When PH was 6, the activity of alpha-amylase was the highest, which indicated that the optimum pH of the enzyme was 6.0. Enzymatic reactions have an optimal pH, and the enzyme activity decreases when the pH is above or below the optimal pH. Because the pH of the solution affects the binding ability of the active center of the enzyme and the substrate, and the binding ability and activity is the highest at the optimal pH [2].

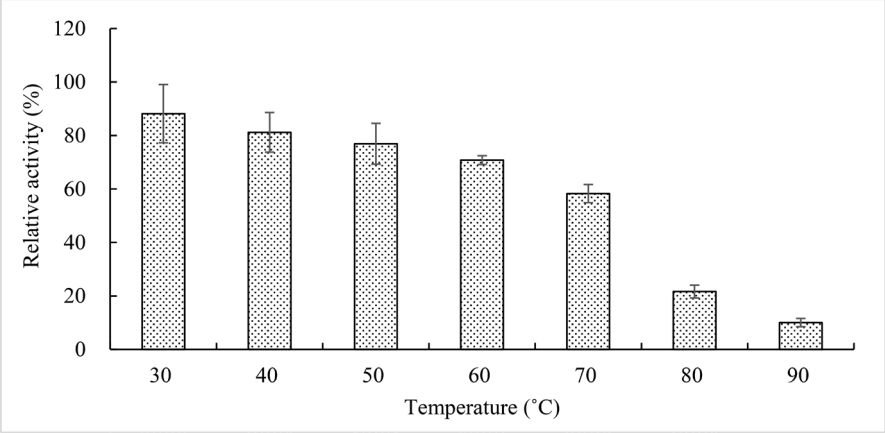

Figure 3. Stability of α-amylase to temperature.

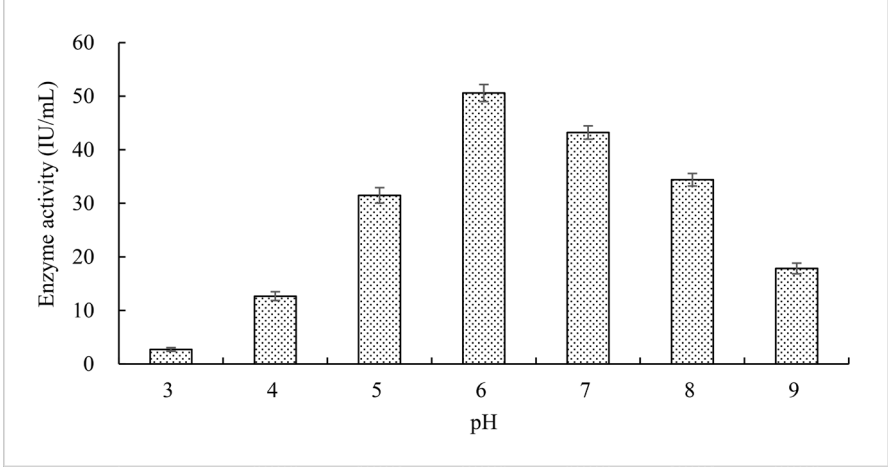

Figure 4. Effect of pH on α-amylase activity.

3.5. pH Stability of *α*-Amylase

As can be seen from Figure 5, when the enzyme solution was placed at a pH of 3 - 9 for 60 min, the activity of *α*-amylase decreased. The activity of *α*-amylase was high at pH 6 - 7, but was low or even partially inactivated when pH was below 5 or above 8.

3.6. Effect of EDTA and Metal ions on Activity of *α*-Amylase

The activity of the *α*-amylase was determined under the action of different metal ions and EDTA as shown in Figure 6. Ca^{2+} could promote the activity of *α*-amylase significantly, the relative enzyme activity was 130.1%. Na^{+}, K^{+} and Mg^{2+} also promoted the activity of alpha-amylase, and relative enzyme activities were 118.1%, 115.9% and 110.4% respectively. Fe^{2+}, Zn^{2+}, Cu^{2+} and Mn^{2+} inhibited the activity of *α*-amylase, the relative enzyme activities were 86.1%, 77.8%, 67.4%, and 64.2% respectively. EDTA inhibited the activity of *α*-amylase significantly, and its relative enzyme activity was less than 50%.

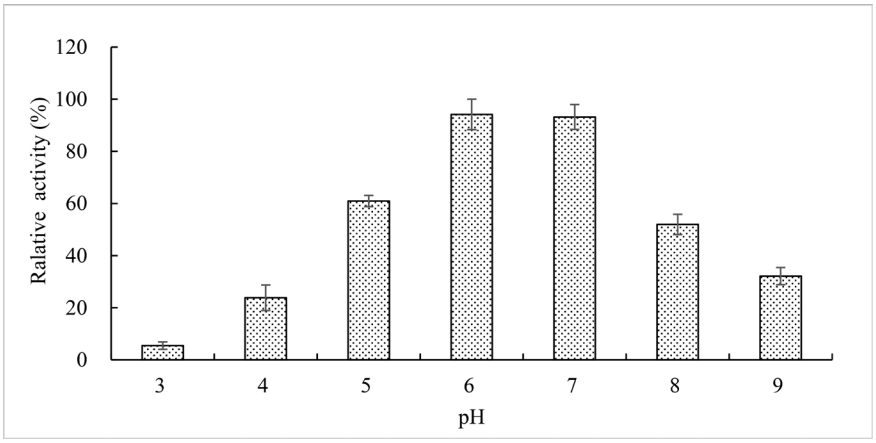

Figure 5. Stability of *α*-amylase to pH.

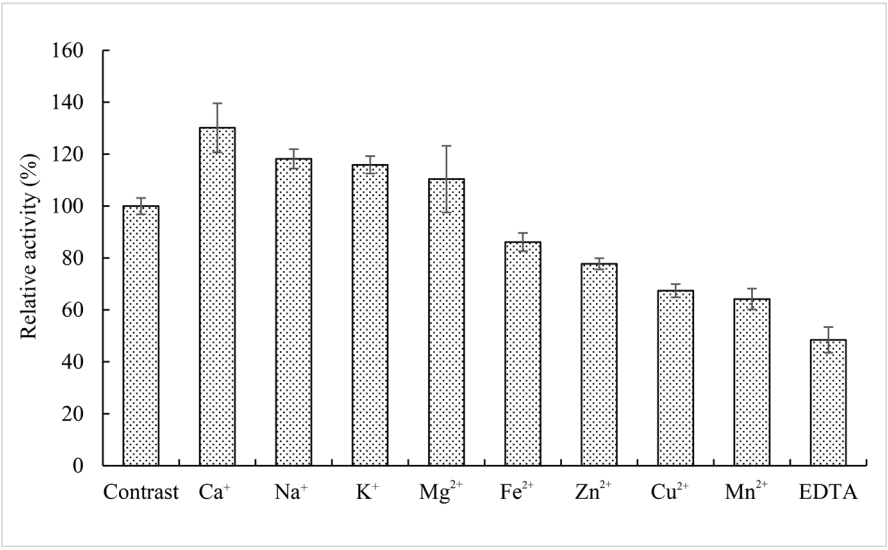

Figure 6. Effect of EDTA and metal ions on activity of *α*-amylase.

Most α-amylase is metalloenzyme. The influence of metal ions on the activity of amylase is mainly due to the metal ions can combine with the active center of α-amylase, thus affect the combination of α-amylase and substrate or the concept of α-amylase, thus affect the activity of α-amylase [2].

4. Conclusion

The results of our study revealed that *B. subtilis* QM3 has ability to produce α-amylase. Activity of α-amylase was 50.58 IU/mL. The optimum temperature is 70˚C, and its stability is high at 30˚C - 70˚C. The optimum pH is 6.0, and its stability is high at pH = 6.0 - 7.0. Ca^{2+}, Na^{+}, K^{+} and Mg^{2+} promoted the activity of alpha-amylase, Fe^{2+}, Zn^{2+}, Cu^{2+}, Mn^{2+} and EDTA inhibited the activity of α-amylase.

Acknowledgements

This work was financially supported by the Natural Science Foundation Project of Shanxi Province (201801D121206) and the Natural Science Foundation of Modern College of Humanities and Sciences of Shanxi Normal University (2018JCYJC01).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Ahmed, M.A., Eman, A.K., Manal, M.G., Maysa, E.M. and Amany, L.K. (2020) Production of a Novel α-Amylase by *Bacillus* Atrophaeus NRC1 Isolated from Honey: Purification and Characterization. *International Journal of Biological Macromolecules*, **148**, 292-301. https://doi.org/10.1016/j.ijbiomac.2020.01.120

[2] David, S., Femi, B., Gbenga, A. and Adeleke, B.S. (2017) Purification and Characterization of α-Amylase from *Bacillus subtilis* Isolated from Cassava Processing Sites. *Journal of Bioremediation & Biodegradation*, **8**, 2-7. https://doi.org/10.4172/2155-6199.1000417

[3] Wu, X.R., Wang, Y.X., Tong, B.D., Chen, X.H. and Jian, H. (2018) Purification and Biochemical Characterization of a Thermostable and Acid-Stable Alpha-Amylase from *Bacillus licheniformis* B4-423. *International Journal of Biological Macromolecules*: *Structure*, *Function and Interactions*, **109**, 329-337. https://doi.org/10.1016/j.ijbiomac.2017.12.004

[4] Aehle, W. (2007) Enzymes in Industry: Production and Applications. Wiley-VCH/Verlag GmbH and Co., Weinheim. https://doi.org/10.1002/9783527617098

[5] Arikan, B. (2008) Highly Thermostable, Thermophilic, Alkaline, SDS and Chelator Resistant Amylase from a Thermophilic *Bacillus* sp. Isolate A3-15. *Bioresource Technology*, **99**, 3071-3076. https://doi.org/10.1016/j.biortech.2007.06.019

[6] Rajagopalan, G. and Krishnan, C. (2008) Alpha-Amylase Production from Catabolite Derepressed *Bacillus subtilis* KCC103 Utilizing Sugarcane Bagasse Hydrolysate. *Bioresource Technology*, **99**, 3044-3050.

https://doi.org/10.1016/j.biortech.2007.06.001

[7] Padma, S. and Pallavi, K. (2016) Isolation and Characterization of Amylase Producing *Bacillus* spp. from Selected Soil Sample. *International Journal of Research in BioSciences*, **5**, 24-29.

[8] Kaur, P.S., Kaur, S., Kaur, H. and Sondhi, S. (2019) Purification and Characterization of a Amylase from *Bacillus licheniformis* MTCC 1483 Having Applications in Textile Industry. *IJEAM*, **54**, 363-368.

[9] Souza, P.M.D. and Pérola, O.M. (2010) Application of Microbial *α*-Amylase in Industry—A Review. *Brazilian Journal of Microbiology*, **41**, 850-861. https://doi.org/10.1590/S1517-83822010000400004

[10] Nielsen, J.E. and Borchert, T.V. (2000) Protein Engineering of Bacterial Alpha-Amylases. *Biochimica Et Biophysica Acta*, **1543**, 253-274. https://doi.org/10.1016/S0167-4838(00)00240-5

[11] Couto, S.R. and Sanroman, M.A. (2006) Application of Solid-State Fermentation to Food Industry—A Review. *Journal of Food Engineering*, **76**, 291-302. https://doi.org/10.1016/j.jfoodeng.2005.05.022

[12] Jaiswal, N. and Prakash, O. (1998) Immobilization of Soybean a-Amylase on Gelatin and its Application as a Detergent Additive. *Asian Journal of Biochemistry*, **6**, 337-346. https://doi.org/10.3923/ajb.2011.337.346

[13] Saideh, A.A., Abdolhamid, B.D. and Namaki, S. (2016) Characterization of a Thermostable, $CaCl_2$-Activated and Raw-Starch Hydrolyzing Alpha-Amylase from *Bacillus licheniformis* AT70: Production under Solid State Fermentation by Utilizing Agricultural Wastes. *Journal of Molecular Catalysis B*: *Enzymatic*, **132**, 98-106. https://doi.org/10.1016/j.molcatb.2016.07.002

[14] Ahmed, S.A., Abdella, M.A., El-Sherbiny, G.M., Ibrahim, A.M. and Atalla, S.M. (2019) Application of One-Factor-at-a-Time and Statistical Designs to Enhance *α*-Amylase Production by a Newly Isolate *Bacillus subtilis* Strain-MK1. *Biocatalysis and Agricultural Biotechnology*, **22**, Article ID: 101397. https://doi.org/10.1016/j.bcab.2019.101397

[15] Raul, D., Biswas, T., Mukhopadhyay, S., Das, S.K. and Gupta, S. (2014) Production and Partial Purification of Alpha Amylase from *Bacillus subtilis* (MTCC 121) Using Solid State Fermentation. *Biochemistry Research International*, **2014**, Article ID: 586141. https://doi.org/10.1155/2014/568141

[16] Li, Y.J. and Hu, Q.P. (2020) Effect of *Bacillus subtilis* QM3 on *β*-Amylase Isoenzyme in Early Germination of Wheat Seed. *South Asian Journal of Research in Microbiology*, **6**, 24-32. https://doi.org/10.9734/sajrm/2020/v6i230146