Purification and Characterization of Cyclodextrin Glucotransferase product from a local isolate of *Mesobacillus jeotgali* HA

Huda Ali Abd Alhussein*, Alaa Jabbar Abd Al-Manhel
Department of Food Science, College of Agriculture, University of Basrah, Basrah Iraq.
*Corresponding author's email: pgs.huda.ali@uobasrah.edu.iq

Email addresses of coauthors: alaa.abd@uobasrah.edu.iq

Abstract

This study aimed to purify and characterize the cyclodextrin glucotransferase (CGTase), produced from a local isolate of *Mesobacillus jeotgali* HA. The enzyme was purified using ammonium sulfate precipitation at 70-80% saturation followed by dialysis. The enzyme yield was 55.90% with a specific activity of 41.71 U/mg protein. Subsequently, gel filtration was performed using an ÄKTA Pure 25 system. This resulted in a specific activity of 68.73 U/mg protein and an enzyme yield of 10.50%. Some important properties of the purified enzyme were investigated. The molecular weight of the enzyme was found to be 66 KDa. The optimum pH for enzyme activity was 7, while the enzyme's stability was 6-8, its optimum temperature was 60°C. The enzyme exhibited thermal stability at temperatures between 30-60°C. The Lineweaver-Burk method was used to determine the values of Km and Vmax. These values were recorded as 2.325 mg/mL and 3.100 µmol/min, respectively.

Keywords: Cyclodextrin glucanotransferase, *Mesobacillus*, Purification, Optimal conditions.

Introduction

Cyclodextrin glucanotransferase is a microbial enzyme, it belongs to the glycoside hydrolase family (GH), or the α-amylase family, with EC number 2.4.1.19 [18]. It catalyzes various types of enzymatic reactions, through which starch and other glucoses linked by α-1,4 bonds are converted into non-reducing maltooligosaccharides, called cyclic dextrins (CDs) [25,1].

The primary source of CGTase enzyme production is microorganisms, these are typically isolated from soil, plants. Bacteria represent the most common microorganisms producing this enzyme, with *Bacillus* leading the way, they constitute approximately 90% of the known CGTase-producing bacteria [25].

The most common types of cyclodextrins produced by the enzyme are α, β, and γ, which are classified based on the number of glucose units they contain, ranging from 6 to 8 glucose units [24]. Of the three types, β-CD is usually the most concentrated in the fermentation medium, constituting approximately 80% of the mixture [25].

The European Food Safety Authority (EFSA) has deemed CGTase enzymes safe for food applications [8]. Its products, α, γ, and β cyclodextrins, have been identified as GRAS (Generally Recognized As Safe) when acting on starch by the US Food and Drug Administration (FDA) [6].

The CGTase enzyme has diverse applications and functions, but its most important function is its ability to produce cyclodextrins (CDs) and various transglycosylation products, which have

ISSN 2072-3857

wide-ranging applications in various industrial sectors, such as food, pharmaceuticals, textiles, cosmetics, and agricultural products [30].

Various methods were used to purify CGTase enzyme produced by bacteria. [29] succeeded in purifying beta-cyclodextrin from *Bacillus flexus* SV 1, using two consecutive steps involving starch adsorption, then, purification was performed using the DEAE-Sepharose ion exchanger, the result was 6.1 purification cycles with an enzyme yield of 44.07%.

Studies have highlighted the importance of characterizing the CGTase enzyme, because it plays a crucial role in determining the optimal conditions for its function and maintaining its activity. Consequently, this positively impacts its potential use in industrial, food, and pharmaceutical applications, [23] reported that the molecular weight of CGTase from *Bacillus licheniformis* was 84.6 kDa, [28] also showed that the optimal pH for CGTase produced from *Microbacterium terrae* KNR 9 was 6.0, [33] also found that the optimal temperature for enzyme activity from *Bacillus sp*.ND1 was 50 °C, with stability ranging between 40–60 °C, [19] indicated that the kinetic constants of CGTase (Vmax) and (Km) using different substrate concentrations were 2500.00 U/mg and 2.75 mg/ml, respectively.

The current study aimed to purify and characterize the CGTase enzyme, produced from a local isolate of *Mesobacillus jeotgali* HA, by determining its physicochemical and kinetic properties, and investigating the optimal conditions for its activity and stability, as well as evaluating its suitability for various industrial applications, particularly in the production of biologically and industrially important cyclodextrins.

Materials and Methods:

Enzyme Production:

Cyclodextrin glucotransferase (CGTase) enzyme was used, produced from the local bacterial isolate (*Mesobacillus jeotgali* HA), isolated from soil samples and identified according to the diagnostic keys mentioned in [11] and [20]. Also identified genetically by PCR, the enzyme suspension was prepared using 250 mL volumetric flasks, containing 50 mL of liquid selective filtration medium (Horikoshi Agar) free of dyes, inoculated with 1% of the activated bacterial isolate for 18 hours, incubated in a shaker incubator at 37°C for 24 hours at 200 rpm, after fermentation, the enzyme was extracted using a cryo-cooled centrifuge at 5000 rpm, one cycle/minute for 5 minutes, the filtrate was collected and used to estimate enzyme activity and protein concentration [10].

Enzyme Assay:

The activity of cyclodextrin glucotransferase was determined using the phenolphthalein method described in [14], 0.1 mL of the crude enzyme extract was transferred to test tubes containing 1 mL of soluble starch solution (40 mg of soluble starch in 1.0 ml sodium phosphate buffer pH 6.0), the tubes were incubated in a water bath at 60°C for 10 minutes. The reaction was stopped by adding 3.5 mL of 30 mM NaOH solution, this was followed by the addition of 0.5 mL of 0.02% (w/v) phenolphthalein solution, the tubes were incubated at room temperature for 15 minutes, the absorbance was measured using a spectrophotometer at 550 nm. A blank sample was prepared using the same method, except for the addition of the enzyme to the solution, and was used as a blank to calibrate the instrument. Enzyme activity was defined as the amount of enzyme that constitutes 1 Micromoles of beta-cyclodextrin per minute under experimental conditions.

Protein determination:

ISSN 2072-3857

The protein concentration was estimated using bovine serum albumin (BSA) as a standard protein according to the method described by [21].

Enzyme Purification:

Ammonium sulfate was added to the crude enzyme extract, gradually at varying saturation levels ranging from 20% to 80%, with continuous stirring at 4°C, using a magnetic mixer, the mixture was then centrifuged at 5000 rpm for 5 minutes, the precipitate was separated, and the enzyme activity and protein concentration of both the filtrate and precipitate were determined for all samples, the filtrate was then discarded, and the precipitate was dissolved in a 0.1 M phosphate buffer (pH 6). The enzyme activity and protein concentration of the precipitate were then determined.

Ammonium sulfate salts were removed from the enzyme by dialysis, Dialysis bags with molecular weights ranging from 12,000 to 14,000 Da were used, these bags were activated at a temperature of 4°C. The solution was replaced every 6 hours, and then the enzyme activity and protein concentration were determined.

The enzyme was purified by gel filtration using an ÄKTA Pure 25 apparatus, A Superdex 200 10/300 GL column with a 10 mm diameter and a volume of 23.562 mL was used, operating at a pressure of 1.5 m Pa, the column was filled with agarose and dextran gel with dimensions of (10 x 300) mm, the column was washed with 5 L of 0.1 M phosphate buffer at pH 6, at a flow rate of 0.5 mL/min, the dialysis sample (after precipitation with ammonium sulfate) was filtered, through a 0.45 µm diameter millipore filter, to remove impurities, then injected into the column at a volume of 0.5 mL, the isolated peaks were monitored at 280 nm on a computer screen, the enzyme activity and protein concentration of the isolated peaks were then measured [3].

Enzyme Purity Test:

[15] method previously used, and described by [10], was employed to determine enzyme purity, by electrophoresis, using polyacrylamide gel, without sodium dodecyl sulfate (SDS-PAGE).

Enzyme Characterization:

Molecular Weight Estimation: The electrophoresis method was used in polyacrylamide gel, with the presence of a denaturant (SDS-PAGE) to estimate the molecular weight of the enzyme according to the method described in [15], which was previously described in [10], based on the relative mobility (Rm) of the protein bundles after staining, and plotting the relationship between the logarithm of the molecular weights of standard proteins and the relative mobility of the gel.

Effect of pH on Enzyme Activity and Stability: The optimum pH for CGTase was determined, by using buffer solutions of varying pH values at a concentration of 0.1 M, the buffer solutions included sodium acetate (pH 4-5), potassium phosphate (pH 6-8), and glycine-sodium hydroxide (pH 9-10). The enzyme activity was then estimated, and the relationship between enzyme activity and pH values was plotted, to determine the optimum pH for enzyme activity, the stability of the enzyme was also studied. Equal volumes of the enzyme were mixed at a 1:1 with buffer solutions of varying pH values and incubated at 60°C for 30 minutes, the solution was then cooled in an ice bath, and the residual enzyme activity (%) was measured, its relationship to pH values was also plotted.

Effect of Temperature on Enzyme Activity and Stability: The effect of temperature on the activity of the CGTase was studied. This

ISSN 2072-3857

was done by incubating the reaction medium at a temperature range of 30-80°C. The relationship between different temperature values and enzyme activity was then plotted, this was done to determine the optimum temperature for the enzyme. the stability of the enzyme was also studied, where a specific volume of the enzyme was incubated in test tubes at different temperatures ranging from (30-80 °C) in a water bath for 30 minutes, then cooled in an ice bath. The relationship between residual enzyme activity (%) and temperature was also plotted, this was done to determine the optimum temperature for enzyme stability.

Enzyme Kinetic Constants: The effect of the substrate on enzyme activity was studied, using different concentrations of the substrate (starch) ranging from 1% to 8% in a phosphate buffer solution, the values of the Michaelis-Menten (Km) and the maximum velocity (Vmax) were estimated, by plotting the relationship between the reciprocal of the initial velocity (V/1) and the reciprocal of the substrate concentration (S/1), according to the method described by [31].

Results and Discussion:

Cyclodextrin glucotransferase (CGTase) was purified in two stages: Ammonium sulfate precipitation followed by gel filtration, this was performed using an ÄKTA Pure 25 apparatus. Various ammonium sulfate saturation levels were used to obtain a greater quantity of CGTase. The results showed that enzyme production was highest at 70-80% saturation, with a specific activity of 41.71 U/mg, the purification yield was 55.90%, with a purification time of 4.40 times, the use of ammonium sulfate is necessary for partial enzyme purification, this is to remove some proteins present in the crude extract. Ammonium sulfate salts are also characterized by their high efficiency and low cost [4].

These results are consistent with several studies, they indicate the importance of using ammonium sulfate in concentrating and purifying the enzyme. [2] used ammonium sulfate to concentrate CGTase from *Thermoactinomyces vulgaris* TA1, achieving 80% saturation and a specific activity of 16.22 U/mg, [26] proved in his study on purifying CGTase enzyme from *Paenibacillus daejeonensis* P-83, demonstrated that the optimal precipitation rate reached 80%, the specific activity reached 4.18 U/mg, and the enzyme yield reached 60%, the number of purification cycles reached 2.00.

The results shown in Figure (1) illustrate the gel filtration step of the CGTase enzyme, using an ÄKTA Pure 25 apparatus, after the dialysis process, two peaks were observed, and the specific activity of each peak was measured, the first peak recorded the highest specific activity at 68.73 U/mg and a filtration rate of 7.26 times, while the yield was 10.50%, as shown in the purification table (Table 1).

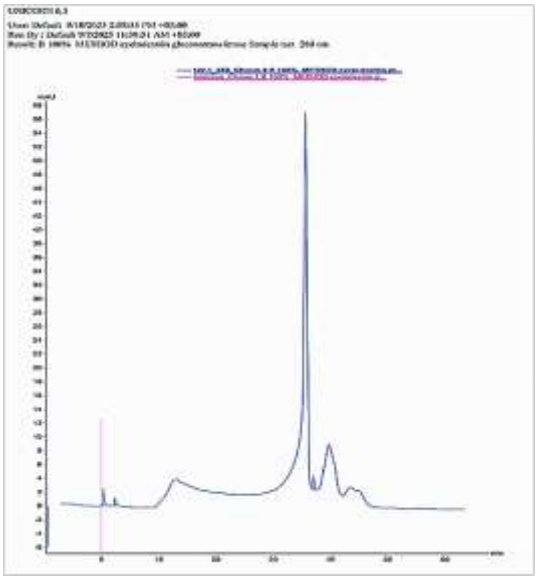


Figure (1) Gel filtration of CGTase enzyme using an ÄKTA Pure 25 apparatus with a Superdex 200 10/300 GL column.

ISSN 2072-3857

The purification steps for CGTase enzyme from microorganisms have varied in numerous studies, among these is the gel filtration technique, in his study, [9] used three consecutive steps to purify the enzyme from *Bacillus lehensis* MLB2, these steps included acetone precipitation and gel filtration using a Sephadex G-100 column. In addition, ion-exchange chromatography was performed on a DEAE-cellulose column, obtained a specific efficiency of 8 U/mg for the gel filtration step, the number of purification cycles reached 38, with a yield of 24%, [13] also purified CGTase produced from *Geobacillus thermoglucosidans* CHB1, this was done by precipitating it with ammonium sulfate, followed by ion-exchange chromatography on a DEAE-Sepharose column, the number of purification cycles was 350.50 times with an enzyme yield of 9.33%. Meanwhile, [26] stated that gel filtration using a Sephadex G-100 column, was one of the steps for enzyme purification from *P. daejeonensis* P-83, the specific activity was recorded at 73.15 U/mg with an enzyme yield of 12%.

Table (1) Steps for purifying the CGTase enzyme produced from the local bacterial isolate *Mesobacillus jeotgali* HA.

Yield (%)	purification	Total activity (U)	Specific activity (U/mg)	Protein (mg/ ml)	Enzyme activity (U/ml)	Volume (ml)	Purification step
100	1	604.4	9.464	0.155	1.467	412	**Crude extract**
55.90	4.40	337.92	41.71	0.135	5.632	60	**Precipitation of $(NH_4)_2SO_4$ (70-80) %, and membrane osmosis**
10.50	7.26	63.514	68.73	0.042	2.887	22	**Gel filtration using ÄKTA Pure 25**

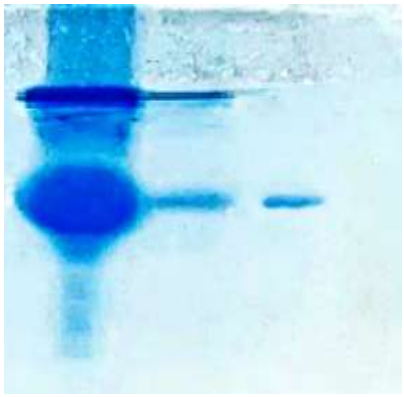


Figure (3) Electrophoresis of the CGTase enzyme produced from the local isolate of *Mesobacillus*

A B C

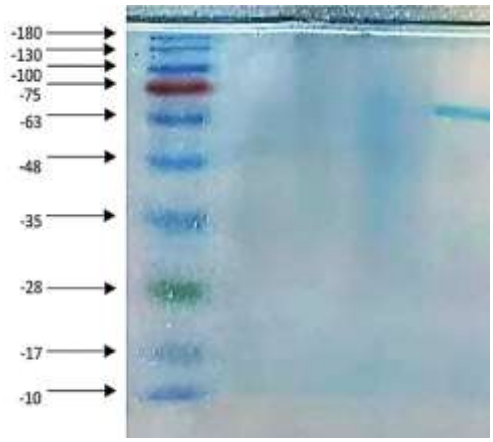
ISSN 2072-3857

jeotgali HA **Electrophoresis:**

The electrophoresis method was used in polyacrylamide gel, to confirm the purity of the enzyme before characterization. Figure (2) shows the protein bands of the CGTase enzyme, produced from the local isolate of *Mesobacillus jeotgali* HA. As shown in the figure, a single protein band of the purified enzyme was observed after the gel filtration step, this indicates the efficiency of the purification processes, which removed all proteins associated with the enzyme, thus achieving high enzyme purity [31]. The researcher [26] used the electrophoresis method in polyacrylamide gel, a single enzyme band was observed after the ammonium sulfate precipitation and gel filtration steps.

Figure (2) Electrophoresis of the enzyme produced from the local isolate of *Mesobacillus jeotgali* HA. (A) Crude enzyme extract. (B) Enzyme after precipitation and dialysis. (C) Purified enzyme after gel filtration using an ÄKTA Pure 25 system.

Figure (3) shows the results of electrophoresis, using polyacrylamide gel with SDS-PAGE denaturant. A marker with standard proteins of varying molecular weights was used, the molecular weight was then estimated based on the relationship between the logarithm of the molecular weight and the relative mobility of the proteins, the results indicated the appearance of a single protein bundle of the CGTase enzyme, produced from the local isolate of *Mesobacillus jeotgali* HA, its molecular weight was approximately 66 KDa, as shown in Figure (4).



ISSN 2072-3857

.

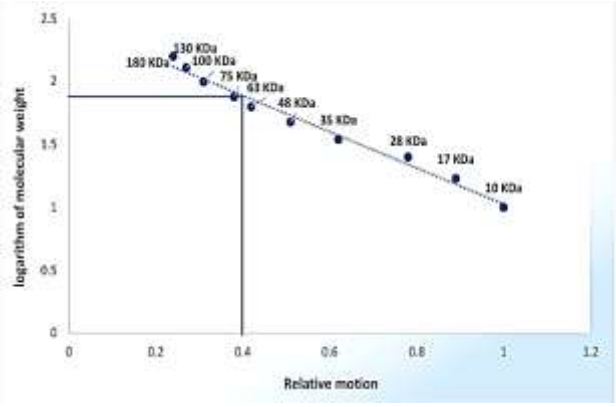


Figure (4) Standard curve for estimating the molecular weight of an enzyme.

[2] ndicated that when estimating the molecular weight of the enzyme, produced by *Thermoactinomyces vulgaris* TA1, using SDS-PAGE, it was 66 KDa, [32] reported that the molecular weight of the enzyme purified from *Bacillus stearothermophilus* NO2 was 66 KDa, Furthermore, [22] and [26] used electrophoresis, to estimate the molecular weight of CGTase, produced by *Caldalkalibacillus mannanilyticus* IB-OR17-B1 and *P. daejeonensis* P-83, respectively, which was approximately 70 KDa.

Optimal pH for Enzyme Activity and Stability:

The results showed that the optimal pH for the purified enzyme was 7, with an enzyme activity of approximately 3.227 U/ml, the values decreased at pH values below 6 and above 9, as shown in Figure 5. The decrease in CGTase activity in the acidic and higher alkaline ranges is attributed to the effect of pH in the reaction medium, as well as the possibility of a change in the ionic state of the reactants [34].

The optimal pH for CGTase enzyme stability was also determined, and the results are shown in Figure 6, Enzyme stability was evident at pH values ranging from 6 to 8, the enzyme retained its full activity within this range. However, a decrease in enzyme activity was observed at acidic pH values. Approximately 55% and 36% of its activity were lost at pH values of 4 and 5, respectively, the decrease in stability at acidic or basic pH values is attributed to a change in the secondary and tertiary structure of the enzyme, as well as its role in altering the ionic state of the enzyme's active site [16].

ISSN 2072-3857

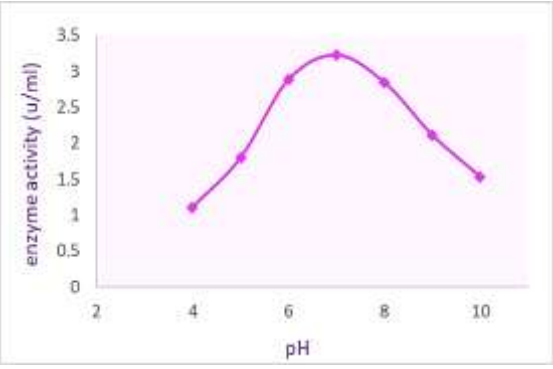


Figure (5) shows the optimal pH curve for the activity of the purified CGTase enzyme produced from the local isolate of *Mesobacillus jeotgali* HA.

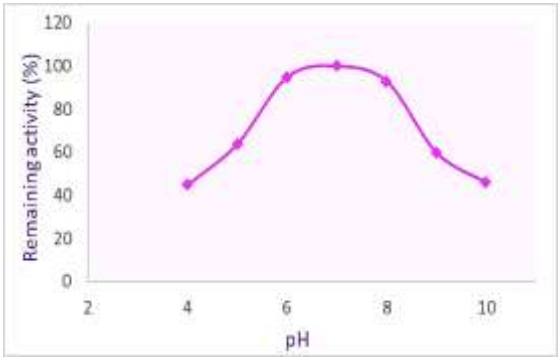


Figure (6) Optimal pH for the stability of CGTase enzyme activity purified from the local isolate of *Mesobacillus jeotgali* HA bacteria.

[9] found that the optimal pH for CGTase enzyme purified from *Bacillus lehensis* MLB2 was 7, [17] studied characterizing CGTase produced from *Paenibacillus sp*, during his study, he observed that the enzyme exhibited high activity at pH 7, the enzyme also showed stability over a pH range of 6-8. Furthermore, the study conducted by [12] on purifying the enzyme from *Bacillus licheniformis*, they indicated that the enzyme's optimal activity was stable at pH 6-8. However, it lost approximately 20% of its activity at pH 9, the enzyme retained only 40% of its activity at pH 10. [26] purified and characterized the enzyme, from *P. daejeonensis* P-83, the results showed that the enzyme's maximum activity was at a pH of 7± 0.2.

Optimal Temperature for Enzyme Activity and Stability:

Figure (7) illustrates the effect of temperature on the activity of the purified enzyme. An increase in enzyme activity was observed with increasing reaction temperature, reaching a maximum at 60 °C with an enzyme activity of 3.187 U/ml. Afterward, the activity gradually decreased with increasing temperature, this may be attributed to an increase in the rate of

ISSN 2072-3857

enzyme reactions, resulting from the higher temperature, this leads to an increase in the kinetic energy of the reactant molecules, which increases the likelihood of collisions between the enzyme and the substrate, the absorption of high energy by the molecules causes a change in the enzyme's tertiary structure (denaturation). Consequently, enzyme activity decreases significantly at higher temperatures [31].

The effect of temperature on the thermal stability of cyclodextrin glucotransferase enzyme, purified from a local isolate of *Mesobacillus jeotgali* HA, was studied, various temperatures ranging from 30 to 80°C were used. The results, shown in Figure 8, indicate that the enzyme retained its full activity within a temperature range of 30 to 60°C. after this, the temperature gradually decreased, and the enzyme lost 20% of its activity at 70°C, it also lost 83% of its activity at 80°C, the reason for the effect of high temperatures on the decrease in enzyme activity is attributed to the occurrence of enzyme denaturation, this rapid change in the enzyme's morphology leads to the breaking of weak hydrogen bonds, this, in turn, causes a complete loss of enzyme activity [7].

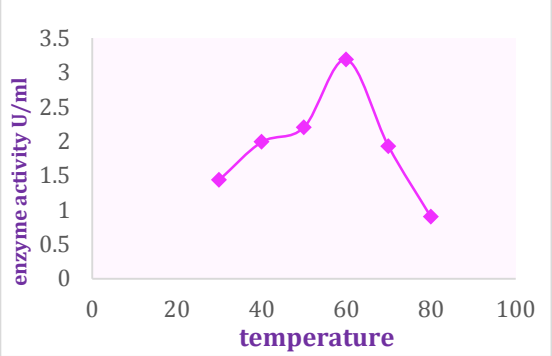
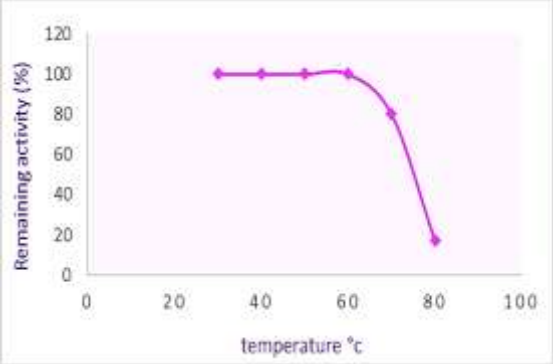
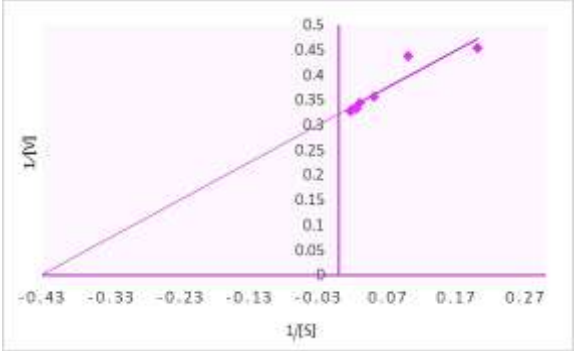


Figure (7) Effect of temperature on the activity of the purified CGTase enzyme produced from the local isolate of *Mesobacillus jeotgali* HA.





F Figure (9) Estimation of the kinetic constants of the CGTase enzyme purified from the local isolate of *Mesobacillus jeotgali* HA bacteria using the Lineweaver-Burk curve.

igure (8) Thermal stability of CGTase enzyme purified from the local isolate of *Mesobacillus jeotgali* HA.

ISSN 2072-3857

A study conducted by [29] showed that CGTase produced from *B. flexus* SV 1 reached its peak activity at 60°C, [12] confirmed that CGTase purified from *B. licheniformis*, retained its activity across a temperature range of 30-60°C, a 42% decrease in enzyme activity was observed at 70°C, while only 30% of its activity was retained at 80°C, [22] indicated that the optimal activity of CGTase, purified from *C. mannanilyticus* IB-OR17-B1, was at 60°C, [5] in characterizing the enzyme from *Alkalihalobacillus lehensis* M136, showed that the enzyme, exhibited thermal stability over a temperature range of 30-80 °C.

Determination of Kinetic Constants:

Various concentrations of soluble starch were used as a substrate to study the kinetic constants of cyclodextrin glucotransferase, the maximum velocity (Vmax) and Michaelis-Menten (Km) were then determined using the Lineweaver-Burk method, the relationship between the maximum velocity and substrate concentrations was plotted, as shown in Figure 9. The results showed that the Km value was 2.325 mg/ml and the Vmax value was 3.100 µmol/min, the variation in the kinetic constant values may be attributed to differences in the operating conditions, such as temperature and pH, as well as differences between enzyme strains and the type of buffer used [35].

The kinetic constants of CGTase have been estimated by several researchers. [33] reported that the Km and Vmax values for CGTase produced from *Bacillus* sp.ND1 were 2.613 ± 0.5 mg/ml and 0.309 ± 0.05 µg/min, respectively, [26] indicated in his study that the Lineweaver-Burk method was used to estimate the kinetic constants of CGTase purified from *P. daejeonensis* P-83, found that the Km value was 126.15 µg and the Vmax value was 526.31 µg/ml.min.

References

1. **Allode, T. D. M., Vodounon, A. C., Dossou, A. G., Honzounnon, N. C., Migan, A. D. F. M., & houenoukpo Hlouedje, W. (2024).** Applications of Cyclodextrin-gluconotransferase in the Biosynthesis of Cyclodextrins: Characteristics, Sources and Production. *American Journal of Microbiological Research*, 12(3), 63-78.
2. **Alsharawi, S. Z., El-Shatoury, E. H., Mohamed, S. T., & Abdalla, N. A. (2018).** Optimization and characterization of cyclodextrin glycosyltransferase produced by *Thermoactinomyces vulgaris* TA1. *Egypt J Exp Biol* (bot), 14(1), 143-152.
3. **Al-Taai, I. H., Al-Fekaiki, D. F., & Jamail, R. M. (2019).** Purification and characterization of alliinase from Iraqi garlic (*Allium sativum*). *Journal of Global Pharma Technology*, 11(5, Suppl.), 437–444.
4. **Beynon, R. &Judith, S. B. (2001).** Proteolytic enzymes. 2nd Edition. Oxford University Press.
5. **Bosito, E. F. M., Montecillo, A. D., Franco, R. A. G., Sabino, N. G., Bertuso, A. L. G., Mendoza, B. C., & Lantican, N. B. (2023).** *Alkalihalobacillus lehensis* M136, a Novel Alkaliphilic, Cyclodextrin Glucanotransferase (CGTase)-producing Isolate from Manleluag Hyperalkaline Spring in Pangasinan, Philippines. *Philippine Journal of Science*, 152(1).
6. **Cid-Samamed A, Rakmai J, Mejuto JC, Simal-Gandara J, Astray G (2022).** Cyclodextrins inclusion complex: Preparation methods, analytical techniques and food industry applications. *Food Chem* 384: 132467.
7. **Dalali, B.K. (1986).** Fundamentals of Biochemistry. University of Mosul/Ministry of Higher Education and Scientific Research (Author).

ISSN 2072-3857

8. **EFSA (2023).** Safety evaluation of the food enzyme cyclomaltodextrin glucanotransferase from the non-genetically modified *Anoxybacillus caldiproteolyticus* strain TCM3- 539. *EFSA Journal*,21(2),e07842.
9. **Elbaz AF, Sobhi A, ElMekawy M (2015).** Purification and characterization of cyclodextrin b-glucanotransferase from novel alkalophilic bacilli. *Bioprocess Biosystems Engineering*,*38*(4), 767–776.
10. **Garfin, D. E. (1990).** One-dimensional gel electrophoresis. In Methods in enzymology (Vol. 182, pp. 425-441). Academic Press.
11. **Harley, J. P., & Prescott, L. M. (2002).** Laboratory exercises in microbiology, 5th ed. The McGraw – Hill companies, U. S. A. 449
12. **Jamil, N., Man, R. C., Shaarani, S. M., Sulaiman, S. Z., Mudalip, S. A., & Arshad, Z. M. (2017).** Characterization of α-Cyclodextrin Glucanotransferase from *Bacillus licheniformis*. Indian J. Sci. *Technol*, 10(10.17485).
13. **Jia, X., Ye, X., Chen, J., Lin, X., Vasseur, L., & You, M. (2018).** Purification and biochemical characterization of a cyclodextrin glycosyltransferase from *Geobacillus thermoglucosidans* CHB1. Starch- Stärke, 70(1-2), 1700016.
14. **Kaneko T, Kato T, Nakamura N, Horikoshi K (1987).** Spectrophotometric determination of cyclization activity of β-cyclodextrin-forming cyclomaltodextrin glucanotransferase. *Journal of the Japanese Society of Starch Science*, 34(1), 45-48.
15. **Laemmli, U. K. (1970).** Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 227, 680 - 285.
16. **Lehmacher,A. & Bisswanger, H. (1990).**Isolation and characterization of an extremely thermostable D- glucose / xylose isomerase from *Thermus aquaticus* HB8. *Journal of General Microbiology* ,136:679-686.
17. **Li, Y., Wei, L., Zhu, Z., Li, S., Wang, J. W., Xin, Q., ... & Qin, H. M. (2017).** Rational design to change product specificities and thermostability of cyclodextrin glycosyltransferase from *Paenibacillus* sp. *RSC Advances*, 7(23), 13726-13732.
18. **Lim, C. H., Rasti, B., Sulistyo, J., & Hamid, M. A. (2021).** Comprehensive study on transglycosylation of CGTase from various sources. *Heliyon*, 7(2), e06305.
19. **Liu Z, Wu G, Wu H (2022).** Molecular cloning, and optimized production and characterization of recombinant cyclodextrin glucanotransferase from *Bacillus* sp. T1. 3 *Biotech* 12(3),58.
20. **Logan, N. A., & Vos, P. D. (2015).** *Bacillus***.** *Bergey's manual of systematics of archaea and bacteria*, 1-163.
21. **Lowry, O., Rosebrough, N., Farr, A. L., & Randall, R. (1951).** Protein measurement with the Folin phenol reagent. *Journal of biological chemistry*, 193(1), 265-275.
22. **Milman, P. Y., Gilvanova, E. A., & Aktuganov, G. E. (2023).** Cyclodextrin glucanotransferase of the alkalophilic strain *Caldalkalibacillus mannanilyticus* IB-OR17-B1. *Applied Biochemistry and Microbiology*, 59(5), 570-579.
23. **Mostafa, Y. S., Alamri, S. A., Alrumman, S. A., Taha, T. H., Hashem, M., Moustafa, M., & Fahmy, L. I. (2021).** Biosynthesis of raw starch degrading β-cyclodextrin glycosyltransferase by immobilized cells of *Bacillus licheniformis* using potato wastewater. *Biocell*, 45(6), 1661-1672.
24. **Muñoz-Shugulí C, Vidal CP, Cantero-López P, Lopez-Polo J (2021).** Encapsulation of plant extract compounds using cyclodextrin inclusion complexes, liposomes, electrospinning and their combinations for food purposes. *Trends Food Sci Technol*, 108, 177-186.

ISSN 2072-3857

25. **Pardhi, D. S., Rabadiya, K. J., Panchal, R. R., Raval, V. H., Joshi, R. G., & Rajput, K. N. (2023).** Cyclodextrin glucanotransferase: fundamentals and biotechnological implications. *Applied Microbiology and Biotechnology*, 107(19), 5899-5907.
26. **Pardhi, D. S., Rabadiya, K., Joshi, R. G., Panchal, R. R., Raval, V. H., & Rajput, K. N. (2025).** Purification and characterization of cyclodextrin glucanotransferase by *Paenibacillus daejeonensis* P-83. *Next Bioengineering*, 1, 100002.
27. **Rabadiya, K., Pardhi, D., Thaker, K., Patoliya, J., Rajput, K., & Joshi, R. (2024).** A review on recent upgradation and strategies to enhance cyclodextrin glucanotransferase properties for its applications. *International Journal of Biological Macromolecules*,259, 129315.
28. **Rajput, K. N., Patel, K. C., & Trivedi, U. B. (2016).** A novel cyclodextrin glucanotransferase from an alkaliphile *Microbacterium terrae* KNR 9: purification and properties. 3 *Biotech*, 6(2), 168.
29. **Reddy, S. V., More, S. S., & Annappa, G. S. (2017).** Purification and properties of beta- cyclomaltodextrin glucanotransferase from *Bacillus flexus* SV 1. *Journal of Basic Microbiology*, 57(11), 974-981.
30. **Saini, K., Pathak, V. M., Tyagi, A., & Gupta, R. (2022).** Microbial cyclodextrin glycosyltransferases: sources, production, and application in cyclodextrin synthesis. *Catalysis Research*, 2(3), 1-69.
31. **Segel, I.H. (1976).** Biochemical Calculations. 2nd. Edition. John Wiley&Sons. Inc. New York.
32. **Tao, X., Su, L., Wang, L., Chen, X., & Wu, J. (2020).** Improved production of cyclodextrin glycosyltransferase from *Bacillus stearothermophilus* NO2 in *Escherichia coli* via directed evolution. *Applied microbiology and biotechnology*, 104(1), 173-185.
33. **Upadhyay, D., Sharma, S., Shrivastava, D., & Kulshreshtha, N. M. (2019).** Production and characterization of β-cyclodextrin glucanotransferase from *Bacillus* sp. ND1. *Journal of basic microbiology*, 59(2), 192-205.
34. **Whitaker, J.R. (1972).** Principles of enzymology for the food science. Mercel Dekker. Inc. New York, USA.
35. **Zhang, L., Yi, H., Du, M., Ma, C., Han, X., Feng, Z., ... & Zhang, Y. (2012).** Enzymatic characterization of transglutaminase from *Streptomyces mobaraensis* DSM 40587 in high salt and effect of enzymatic cross-linking of yak milk proteins on functional properties of stirred yogurt. *Journal of dairy science*, 95(7), 3559-3568.

ISSN 2072-3857