ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Biochemical characteristics of *β*-glucanase from *Penicillium chrysogenum.*

Hamed M. El-Shora[1]*, Metwally A. Metwally[2], and Mohamed A. M. Abd El-Rheem[2].

[1]Department of Botany, Faculty of Science, Mansoura University, Egypt.
[2]Department of Botany, Faculty of Science, Tanta University, Egypt.

ABSTRACT

The optimal incubation time was 40 min. the K_m and V_{max} were 1.08 $mgml^{-1}$ and 32.05 Umg^{-1}protein. The enzyme activity was enhanced by adenosine compounds indicating that *β*-glucanase reaction is endothermic. H_2O_2, EDTA, α-α-dipyridyl, *o*-phenanthroline, succinic anhydride, maleic anhydride, acetic anhydride and citraconic anhydride inhibited *β*-glucanase activity. However, kinetin, gibberellic acid and jasmonic acid were activators. Dithiothreitol (DDT) and thioglycolate as thiol compounds also enhanced the activity at 300 and 100 µmol, respectively.

Keywords: *β*-glucanase, *Penicillium chrysogenum*, Adenosine compounds, Modification, Phytohormones.

https://doi.org/10.33887/rjpbcs/2019.10.3.37

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Enzymes are known as biocatalysts and they have several applications in industrial chemistry [1]. Enzyme levels can be increased by environmental and genetic manipulations [2].

There are many factors affecting the application of biocatalysts including robust computational methods and enzyme promiscuity [3; 4].

β-glucanase enzymes are classified according to the type of *β*-glucosidic linkage they cleave and the mechanism of substrate attack [5].

Commercial *β*-glucanases are produced from *Aspergillus niger* and *Penicillium* species [6]. *P. chrysogenum* was known as *β*-glucanase producer [7; 8].

The stability of enzymes in soluble form is an important to achieve the purification processes as well the storage of purified enzymes [9].

Various methods have been adopted to enhance enzyme stability such as using additives and chemical modifications [10; 11].

The chemical modification of enzymes was used to identify specific residues at the active site involved in substrate binding or chemical catalysis. However, it has been used for tailoring the specificities of enzymes [12; 13; 14; 15].

The aim of the present work was to investigate the influence of H_2O_2, adenosine compounds, chelating agents, thiol compounds, anhydrides and phytohormones on purified *β*-glucanase from *P. chrysogenum*

MATERIAL AND METHODS

Experimental organism

Penicillium chrysogenum was provided by Professor Salwa Abd El-Magid Khalaf, Professor of Microbiology, Botany Department, Faculty of Science, Zagazig University.

Production of β-glucanase

The liquid medium used contained (g/L): 20.0 carboxy methyl cellulose (CMC), 5.0 Yeast extract, 1.0 KCl, 1.0 NH_4NO_3, 1.0 K_2HPO_4, 0.5 $MgSO_4.7H_2O$ and 0.01 $FeSO_4.7H_2O$. The pH of the medium was adjusted to 5.5 with 0.1N HCl before sterilizing in an autoclave for 15 min at 121^oC.

Two ml of the fresh spore suspension (2.0 X 10^6 spores ml^{-1}) from *P. chrysogenum* was inoculated into 100 ml of the respective sterile liquid media in 250 ml Erlenmeyer flask. The culture was incubated at 30^oC for 5 days in dark conditions with continuous agitation at 150 rpm.

Preparation of enzyme extract

The fungal culture was filtered on Whatman no. 1 filter paper and the supernatant was centrifuged to remove cell debris at 10,000 rpm for 10 min at 4^oC. The clear supernatant was used as crude *β*-glucanase source and used to determine its activity [16].

Assay of β-glucanase

β-glucanase activity was assayed by the method of [17] using 3,5-dinitrosalicylic acid. The reaction mixture composed of 0.5 ml of crude enzyme, 0.5 ml CMC (0.5%) and 2 ml citrate buffer (50 mM). The mixture was incubated in water bath at 40˚C for 20 min. The reaction was stopped by adding 3 ml 3,5-dinitrosalicylic

acid and measuring the absorbance spectrophotometrically at 540 nm. One unit (U) of *β*-glucanase activity is the amount of the enzyme that liberates 1µmol glucose per min under the assay condition [18].

Purification of β-glucanase

β-glucanase from *P. chrysogenum* was purified using 85% $(NH_4)_2SO_4$ precipitation, DEAE-cellulose and Sephadex G_{200} with final specific activity of 210 Umg^{-1} protein and 15-fold.

Effect of incubation time on β-glucanase activity

To study the effect of incubation time on *β*-glucanase activity, the activity was measured after 10, 20, 30, 40, 50 and 60 min.

Effect of substrate concentration on β-glucanase activity

The Michaelis constant (K_m) and the maximum velocity (V_{max}) of the *β*-glucanase was evaluated by using various substrate concentrations (2, 4, 6, 8 and 10 % w/v). The reaction mixture was incubated at 45°C for 1 h. Using Lineweaver-Burk plot K_m and V_{max} were determined.

Effect of H_2O_2 on β-glucanase activity

The effect of H_2O_2 on *β*-glucanase activity was tested at different concentrations (5, 10, 15, 20 and 25 % v/v) in the reaction mixture followed by measuring the enzyme activity.

Effect of adenosine compounds on β-glucanase

The effect of ATP, ADP and AMP on *β*-glucanase activity was investigated at 0.4, 0.8, 1.2, 1.6 and 2.0 mM in the reaction mixture followed by determination of *β*-glucanase activity.

Effect of chelating agents on β-glucanase activity

The chelating agents were tested in the reaction medium. EDTA was tested at 1, 2, 3, 4 and 5mM. *α,α*-dipyridyl was used at 5, 10, 15, 20 and 25mM. *O-phenanthroline* was tested at 2, 4, 6, 8 and 10 mM. After incubation for 40 min the enzyme activity was measured.

Effect of thiol compounds on β-glucanase activity

Dithiothreitol (DTT) and thioglycolate were investigated individually at 100, 200, 300, 400 and 500 µmol in the reaction mixture, followed by enzyme assay as mentioned before.

Effect of anhydrides on β-glucanase activity

Anhydrides such as succinic, maleic, acetic and citraconic were dissolved in DMSO at the highest possible concentration. The protein was used at 4 mg/ml concentration in 2 ml of 100 mM borate buffer (pH 8.0). The modification process was followed at room temperature using different concentrations (2, 4, 6, 8 and 10 mM) of each modifier while maintaining the pH of the stirred solution at 8.0 by the addition of 2 M NaOH. The reaction mixtures were kept for another 1 h at 25 °C and then dialyzed extensively against 20 mM phosphate buffer pH (7.0). In control sample the protein had not been modified [19].

Effect of phytohormones on β-glucanase activity

The phytohormones were tested in the growth medium. Kinetin was tested at 30, 60, 90, 120 and 150 µmol. Gibberellic acid was tested at 20, 40, 60, 80 and 100 µmol. Jasmonic acid was examined at 100, 200, 300, 400 and 500 µmol. For 5 days followed by determination of the enzyme activity.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Effect of incubation time on β-glucanase activity from P. chrysogenum

The relation between the enzyme activity and the incubation time resulted in appearance of a graph (Fig. 1) with increasing incubation time up to 40 min which has two distinct regions. Firstly, in initial period of time, the amount of substrate transformed, seems to be directly proportional with the length of time of the reaction. After the initial period, the rate of reaction decreases and shows that the substrate is present in excess [20].

Effect of substrate concentration on β-glucanase activity from P. chrysogenum

The results in Fig. 2a show that there was continuous increase in enzyme activity with increasing the substrate concentration.

A plotting 1/S against 1/V (Fig. 2b) straight line was obtained with K_m of 1.08 %v/v and V_{max} of 32.05 Umg^{-1} protein. V_{max} and K_m values usually are more or less constants independent of the amount of the enzyme present, at least within reasonable limits. It has been reported that K_m value was 4.8 for *Penicillium pinophilum* [21].

Effect of H_2O_2 on β-glucanase activity from P. chrysogenum

The results indicate an increase in the inhibition percentage of the enzyme by H_2O_2 (Fig. 3). This may be due to oxidation of the enzyme protein.

Effect of adenosine compounds on β-glucanase activity

The results in Fig. 4 indicate that there was continuous increase in the enzyme activity with increasing the concentration of ATP, ADP and AMP. ATP was the best followed by AMP and ADP. The increased activity by these compounds indicates that *β*-glucanase reaction is endothermic reaction. Adenosine compounds activated other enzymes such as protease and pullulanase [22; 23].

Effect of chelating agents on β-glucanase activity

Three chelating agents were tested regarding their effect on enzyme activity. They were EDTA, α-α-dipyridyl and *o*-phenanthroline.

The relative activity (Fig. 5a) was reduced under treatment of *β*-glucanase with EDTA and the reduction was concentration dependent. IC_{50} value was 5.1 mM. Other microbial enzymes have been reported to be inhibited by EDTA such as *β*-lactamase [24] and protease [25] (El-Shora and Metwally, 2009).

The results in Fig. 5b show the inhibition of *β*-glucanase by α-α-dipyridyl and IC_{50} was 22.1 mM. *β*-glucanase was inhibited by *o*-phenanthroline (Fig. 5c) and IC_{50} of *o*-phenanthroline was 5.7 mM. The inhibition of *β*-glucanase by the three chelating compounds indicates that *β*-glucanase is metallo-enzyme.

Effect of thiol compounds on β-glucanase activity

There was a continuous increase in the enzyme activity with increasing DTT concentration up to 300 µmol (Fig. 6a) after which there was continuous decline in the enzyme activity. DTT was reported to protect the loss of enzyme activity which occurs by the oxidation of sulfhydryl groups [25; 26; 27].

Also, there was continuous increase in enzyme activity with increasing of thioglycolate up to 100 µmol then the enzyme activity was reduced at the elevated concentrations (Fig. 6b). Other enzymes such protease [28] were enhanced by thioglycolate. It is possible that beside protection of –SH group the thiol compounds may lower the affinity of the enzyme to its substrate and thereby activating the enzyme.

ISSN: 0975-8585

Effect of anhydrides on β-glucanase activity

The four tested anhydrides reduced the enzyme activity in a concentrations-dependent manner. IC_{50} were 8.6, 7.6, 7.4 and 7.7 mM for succinic anhydride (Fig. 7a), maleic anhydride (Fig. 7b), acetic anhydride (Fig. 7c) and citraconic anhydride (Fig. 7d), respectively. It has been reported that the anhydrides can inactivate the enzyme by blocking amino groups in proteins [29].

Effect of phytohormones on β-glucanase activity

Kinetin, gibberellic acid and jasmonic acid induced *β*-glucanase activities from *P. chrysogenum* at the lower concentrations, but the higher concentrations expressed an inhibitory effect (Figs 8a, 8b and 8c).

Kinetin treatment increased the activity of other plant enzymes such as carbonic anhydrase [30]. Applied GA_3 stimulated the development of other enzymes [31] and doubled endo-peptidase activity level [32]. GA_3 induced other enzymes such as phytase [33], phosphoenolpyruvate carboxylase [34], NADH-glutamate synthase [35; 36]. In addition, GA_3 increased the activities of sucrose synthase and sucrose phosphate synthase [37]. It is possible that these compounds induced the enzyme activity through acting either at the level of transcription and/or the stabilization of the transcripts.

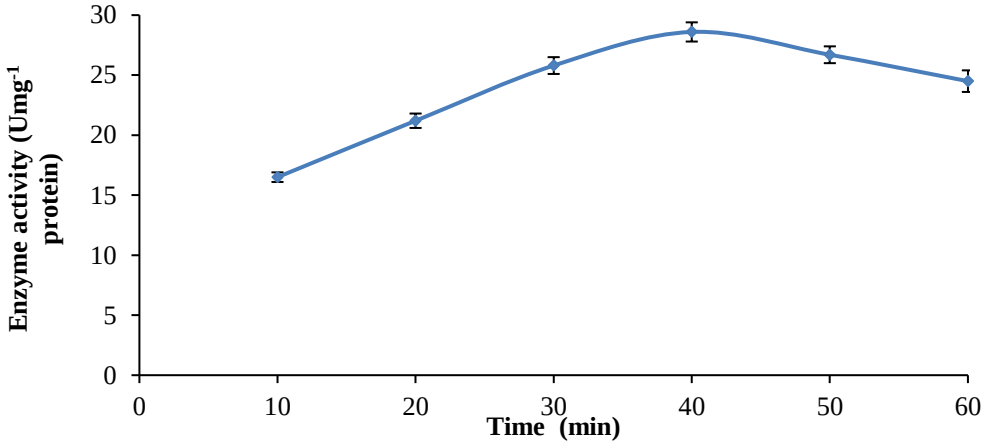


Fig. 1: Effect of incubation time on *β*-glucanase activity from *P. chrysogenum*.

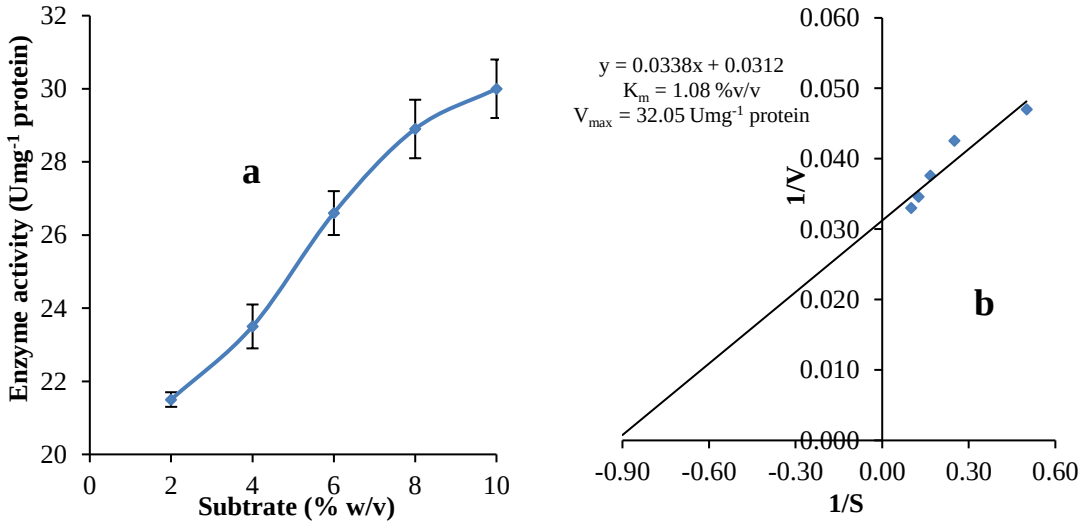


Fig. 2: Effect of substrate concentration (a) and double reciprocal plot of *β*-glucanase from *P. chrysogenum*.

ISSN: 0975-8585

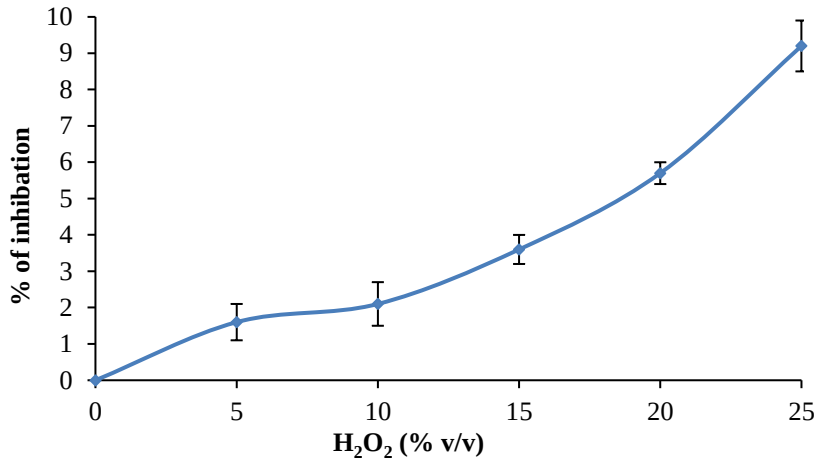


Fig. 3: Effect of H_2O_2 on *β*-glucanase activity from *P. chrysogenum*.

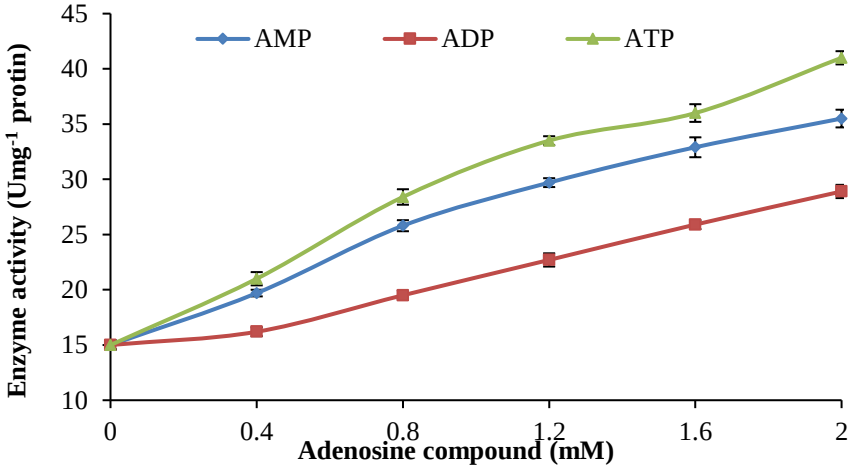
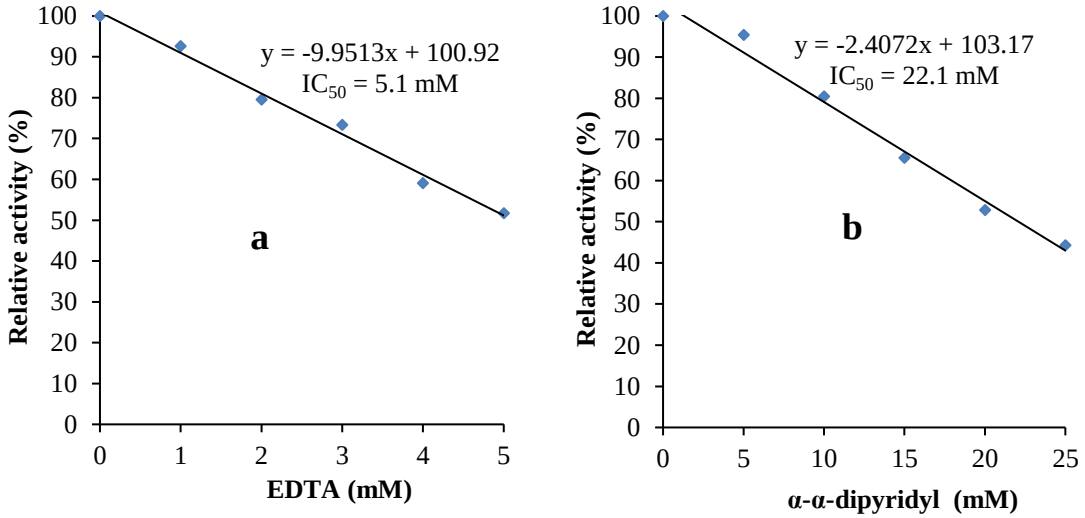


Fig. 4: Effect of adenosine compounds on *β*-glucanase activity from *P. chrysogenum*.



ISSN: 0975-8585

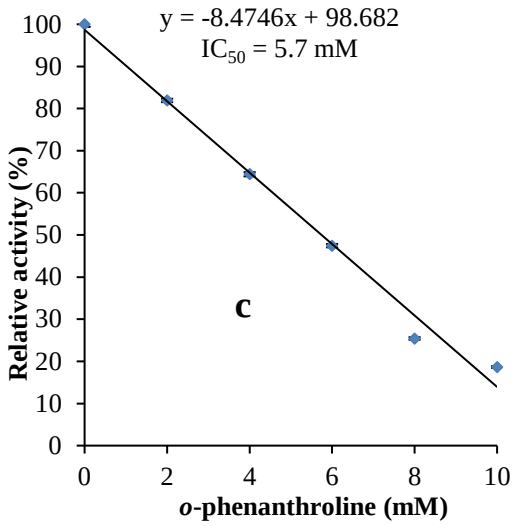


Fig. 5: Relationship between various chelating agents and the relative activity of *β*-glucanase.

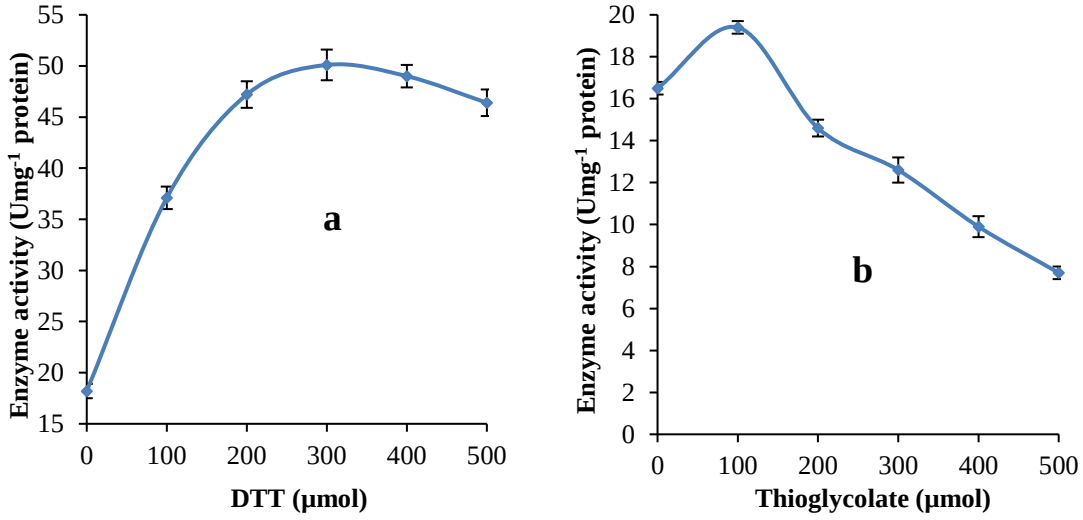
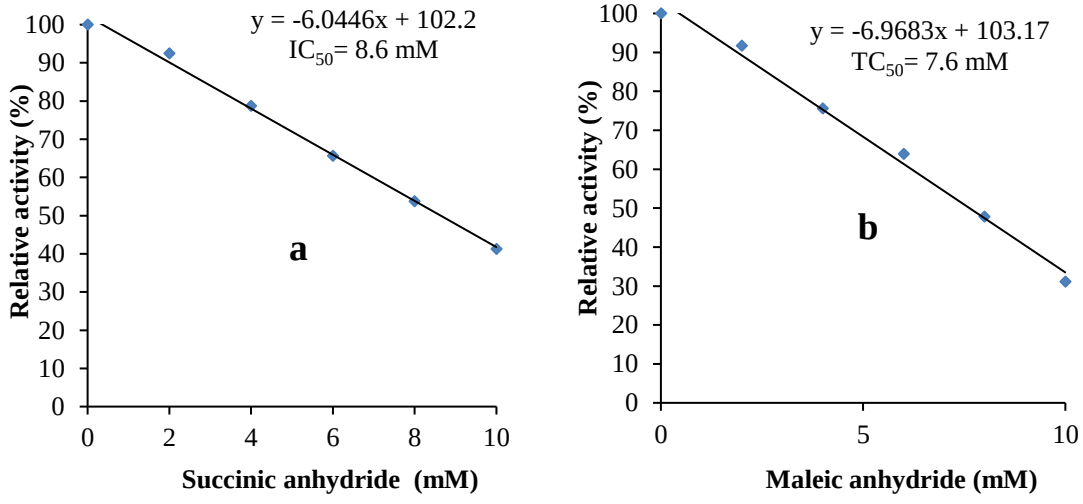


Fig. 6: Effect of thiol compounds on *β*-glucanase activity from *P. chrysogenum*.



ISSN: 0975-8585

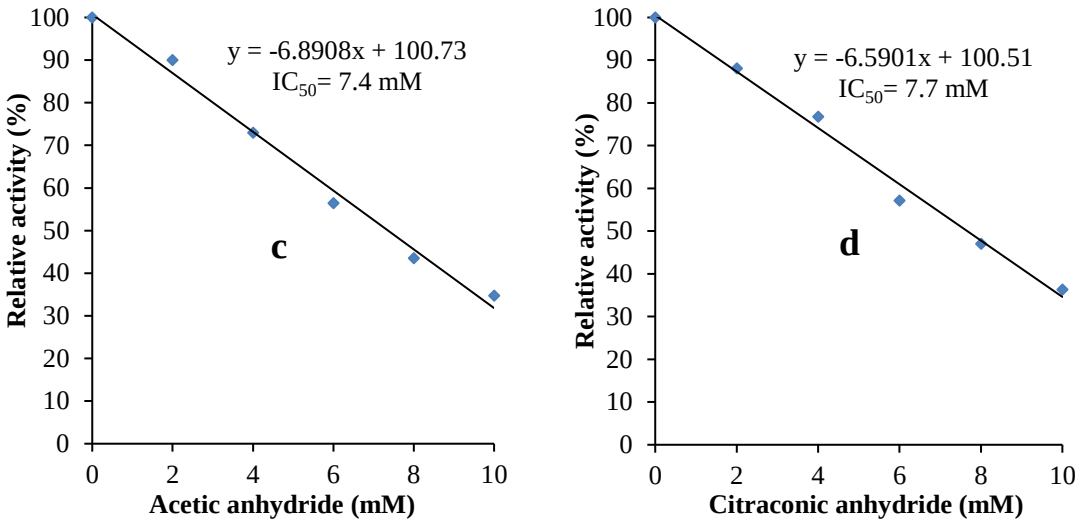


Fig. 7: Relationship between four anhydrides concentration and the relative activity of *β*-glucanase.

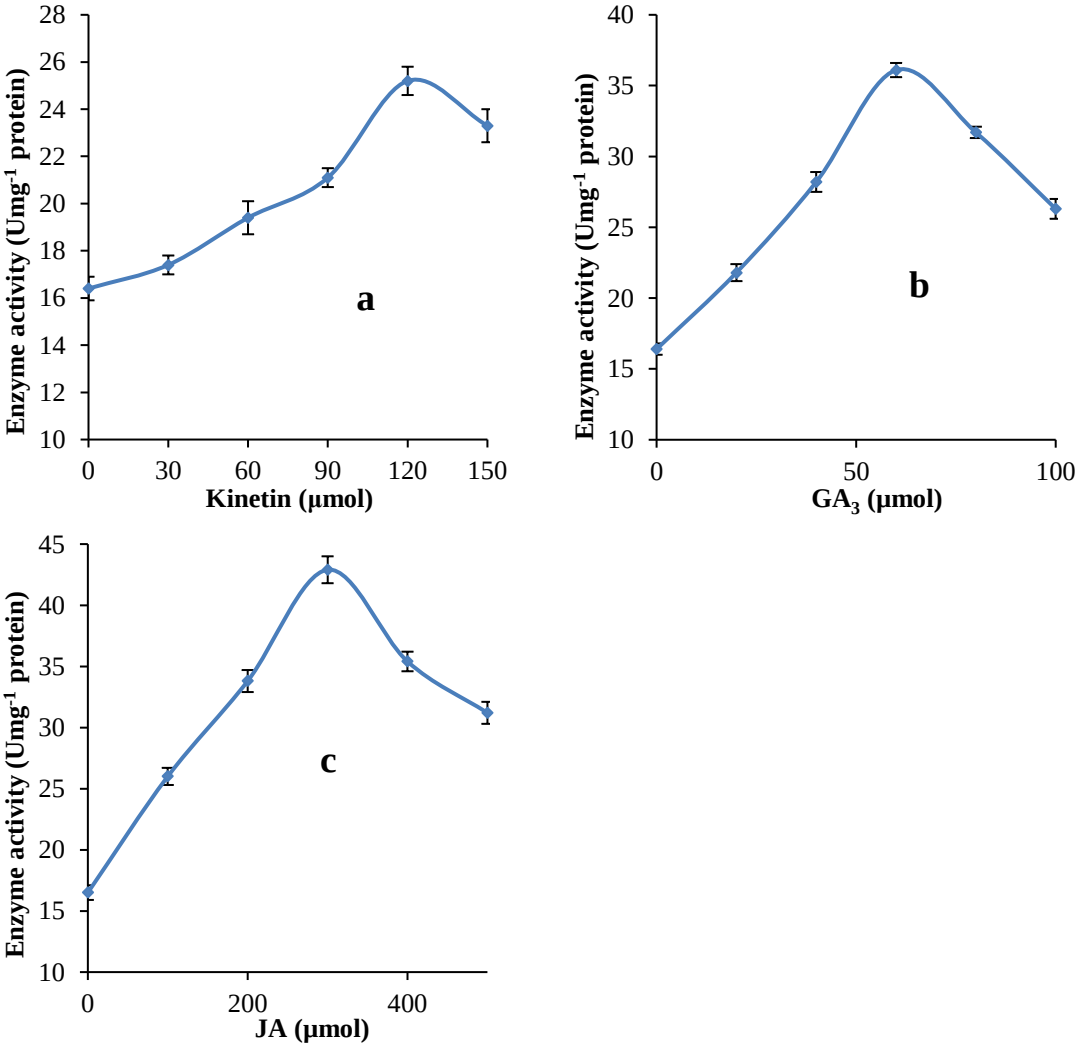


Fig. 8: Effect of phytohormones on *β*-glucanase activity from *P. chrysogenum*.

ACKNOWLEDGMENT

The authors acknowledge the support from Faculty of Science, Tanta University, Egypt.

REFERENCES

[1] Wohlgemuth R. Biocatalysis key to sustainable industrial chemistry. Curr Opin Biotechnol 2010; 21(6): 713-24.
[2] Burns RG, Dick RP. Enzymes in the Environment: Activity, Ecology, and Applications. CRC Press; 2002
[3] Adrio JL, Demain AL. Microbial enzymes: tools for biotechnological processes. Biomolecules 2014; 4(1): 117-39.
[4] Chapman J, Ismail AE, Dinu CZ. Industrial applications of enzymes: recent advances, techniques, and outlooks. Catalysts 2018; 8 (238): 1-26.
[5] Pitson SM, Seviour RJ, Mcdougall BM. Non-cellulolytic fungal *β*-glucanases: their physiology and regulation. Enzyme Microb Technol 1993; 15: 178-92.
[6] Doughari JH. Production of *β*-glucanase enzyme from *Penicillium oxalicum* and *Penicillium citrinum*. Afr J Biotechnol 2011; 10(47): 9657-60.
[7] Karpe AV, Beale DJ, Harding IH, Palomboet EA. Optimization of degradation of winery-derived biomass waste by Ascomycetes. J Chem Technol Biotechnol 2014; 90(10):1793–801.
[8] Karpe AV, Beale DJ, Godhani NB, Morrison PD. Untargeted metabolic profiling of winery-derived biomass waste degradation by Penicillium chrysogenum. J Agric Food Chem 2015; 63(49):10696–704.
[9] Aehle WC. Enzymes in industry: production and applications. John Wiley & Sons; 2007.
[10] Taravati M, Shokrzadeh M, Ebadi AG, Valipour P, Hassan AT, Farrokki F. Various effects of sugar and polyols on the protein structure and function: Role as osmolytes on protein stability. World Appl Sci J 2007; 2: 353-62.
[11] Shelley DM. Enzyme Stabilization and Immobilization: Methods and Protocols, Methods in Molecular Biology. *Springer Science Business Media*; 2011.
[12] Yamashita H, Nakatani H, Tonomura B. Change of substrate specificity by chemical modification of lysine residues of porcine pancreatic alpha-amylase. Biochim Biophys Acta 1993; 1202(1): 129-34.
[13] Qi D, Tann CM, Haring D, Distefano MD. Generation of new enzymes via covalent modification of existing proteins. *Chem Rev* 2001; 101(10): 3081-112.
[14] Davis BG. Chemical modification of biocatalysts. Curr Opin Biotechnol 2003; 14(4): 379-86.
[15] Svendsen A. Understanding Enzymes: Function, Design, Engineering, and Analysis. Pan Stanford; 2016.
[16] Bhatti HN, Saleem N. Characterization of glucose oxidase from *Penicillium notatum.* Food Technol Biotechnol 2009; 47(3): 331-5.
[17] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem 1959; 31(3): 426-8.
[18] Patel AK, Singhania RR, Pandey A. Production, purification, and application of microbial enzymes. Biotechnol Microb Enzymes 2017; 13- 41.
[19] Hassani L. Chemical modification of horseradish peroxidase with carboxylic anhydrides: Effect of negative charge and hydrophilicity of the modifiers on thermal stability. J Mol Catal B: Enzym 2012; 80: 15-19.
[20] Lizotte PA, Henson CA, Duke SH. Purification and characterization of pea epicotyl *β*-amylase. Plant Physiol 1990; 92(3): 615-21.
[21] Pol D, Laxman RS, Rao M. Purification and biochemical characterization of endoglucanase from *Penicillium pinophilum* MS 20. Indian J Biochem Biophys 2012; 49(3): 189-94.
[22] El-Shora HM, Ghada SE, Amany HS. Purification, thermostability and chemical modification of pullulanase from sunflower. Int J Curr Microbiol Appl Sci 2015; 4(12): 284-96.
[23] El-Shora HM, Abou-El-Wafa GS, Kadhim AI. Purification and biochemical characteristic of protease from the red seaweed *Petrocladia capillacea.* Int J Curr Microbiol Appl Sci 2016; 5(2):297-308.
[24] Marchiaro P, Tomatis PE, Mussi MA, Pasteran F, Viale AM, Limansky AS, Vila AJ. Biochemical characterization of metallo-lactamase vim-11 from a *Pseudomonas aeruginosa* clinical strain. Antimicrob Agents Chemother 2008; 52(6): 2250-2.
[25] El-Shora HM, Metwally M. Effect of phytohormones and group selective reagents on acid phosphatase from *Cladosporium cladosporioides*. Asian J Biotechnol 2009; 1(1): 1-11.

[26] Alliegro MC. Effects of dithiothreitol on protein activity unrelated to thiol–disulfide exchange: for consideration in the analysis of protein function with Cleland's reagent. Anal Biochem 2000; 282: 102–6.
[27] El-Shora HM, Abo-Kassem EM. Regulation and characterization of phytase activity in *Latuca sativa cotyledons*. *Bull. Faculty of Science Assiut University*. 2000; 29:389-403.
[28] El-Shora HM, Ibrahim ME, Elmekabaty MR. Immobilization and thermostability of manganese peroxidase from *Trichoderma harzianum*. Microbiol Res J Int 2017; 21(2): 1-11.
[29] Takahashi K. The structure and function of ribonuclease T1. XXIII. Inactivation of ribonuclease T1 by reversible blocking of amino groups with cis-aconitic anhydride and related dicarboxylic acid anhydrides. The J Biochem 1977; 81(3): 641-6.
[30] Hayat S, Ahmad A, Mobin M, Fariduddin Q, Azam ZM. Carbonic anhydrase, photosynthesis, and seed yield in mustard plants treated with phytohormones. Photosynthetica 2001; 39(1): 111-4.
[31] Ramakrishna V, Rao PR. Purification of acidic protease from the cotyledons of germinating Indian bean (*Dolichos lablab* L. var lignosus) seeds. Afr J Biotechnol 2005; 4(7): 703-7.
[32] Taniyama M, Kitamura K, Ban Y, Sugita E, Ito K, Katagiri T. Elevation of circulating proadrenomedullin N, terminal 20-peptide in thyrotoxicosis. Clin Endocrinol 1997; 46: 271-4.
[33] Gabard KA, Jones RL. Localization of phytase and acid phosphatase isoenzymes in aleurone layers of barley. Physiol Plant 1986; 67(2): 182-92.
[34] Bihzad MA, El-Shora HM. Phosphoenolpyruvate carboxylase from *Rumex dentatus*, a C3-plant. J Plant Physiol 1996; 149(6): 669-76.
[35] El-Shora HM. Effect of growth regulators and group-modifiers on NADH-glutamate synthase of marrow cotyledons. J Biol Sci 2001; 7: 597-602.
[36] El-Shora HM. Properties of phenylalanine ammonia lyase from marrow cotyledons. Plant Sci 2002; 162(1): 1-7.
[37] Kaur S, Gupta AK, Kaur N. Effect of GA_3, kinetin and indole-acetic acid on carbohydrate metabolism in chickpea seedlings germinating under water stress. Plant Growth Regul 2000; 30: 61-70.