Ain Shams University
Faculty of Education
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PRODUCTION OF β – GALACTOSIDASE FROM THERMOPHILOUS FUNGI ISOLATED FROM EGYPTIAN SOIL

THESIS
SUBMITTED FOR THE MASTER DEGREE
IN SCIENCE TEACHER'S PREPARATION
(Botany)

BY
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General Diploma For Teacher's Preparation In Science (Botany)-2006
Special Diploma For Teacher's Preparation In Science (Botany)-2007

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إنتاج إنزيم بيتا جالاكتوزيديز من فطريات محبة للحرارة معزولة من التربة المصرية

رسالة مقدمة
للحصول على درجة ماچستير إعداد المعلم في العلوم
(تخصص علم النبات)

من الطالبة
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ABSTRACT

PRODUCTION OF β – GALACTOSIDASE FROM THERMOPHILOUS FUNGI ISOLATED FROM EGYPTIAN SOIL

Two local isolates of thermophilic fungi; *Thermomyces lanuginosus* and *Chaetomium thermophile* were grown on liquid culture containing various carbon sources in addition to lactose.

Maximum extracellular β-galactosidase (3.94 and 2.86 U mg^{-1}, respect ively) was obtained after 7 d at 45°C and initial pH 5.5. Inoculum of 200 CFU was used. The optimal basal solution (OBS) contained (gL-'): 15 polygalacturonic acid; 5.2, casein; 1, K_2HPO4; 1, MgSO4 and 0.01 FeSO4. Under solid substrate cultivation (SSC) on wheat bran and lupine - seed powder (LSP) the yield of the enzyme was highly increased. Each Erlenmeyer flask (250 ml) was charged with 10 g LSP thourghly mixed with 10 ml of OBS (50 % initial moisture content), inoculated with 200 CFU μL^{-1} and incubated at 45 °C .for 7d. SSC increased the enzyme activity about 10 folds. The hyperproduction of the enzyme by the two local isolates is promising in commercial production of β-galactosidase.

Culture filtrates (crude enzyme, CE) for both *Thermomyces lanuginosus* and *Chaetomium thermophile* was dialysed against 50 mM acetate buffer (pH 5.0). The enzyme was partially purified to 4.08 and 109.07 purification fold with 45.16 and 40.86 yield for both experimental fungi respectively , by adsorption on 2.5 % bentonite and eluted with 0.1 M NaCl.

The partially purified enzyme from *Thermomyces lanuginosus* and *Chaetomium thermophile* was optimally active at pH 5.0 and temperature 50 °C and stable between pH 4.5 - 6.5 and temperature 45 - 60 °C.

Alkaline metal ions Na^+, and K^+ in addition to divalent metal ions Ca^{2+} Co^{2+}, Mg^{2+} Mn^{2+}; (5 mM); have no effect on the enzyme activity while Hg^{2+} and Zn^{2+} completely inhibited the enzyme activity. EDTA, PMSF and ME (5 mM) have, also, no effect on the enzyme activity. The enzyme has no metal dependence and is not metalloprotein. The enzyme is suitable for application in lactose hydrolysis in both whey and milk.

Key words:

Thermophilic fungi -*C.themophile-T.lanuginosus*- β- galactosidase Production-partial purification-properties.

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ACKNOLEDGMENTS

Praise and gratitude be to ALLAH ,the GOD of all creatures for directing me the right way in my work.

I am grateful to my advisor, professor *Dr. Ahmed Awad El-Gindy*, for guidance, patience and generosity that he has provided me. This dissertation would not be possible without his support and dedication. I have gained a lot from his insights and I have greatly enjoyed my staying here as his student.

I would like to acknowledge with sincere gratitude to *Dr. Zeinab Mohamed Ibrahim*, for here help and advices on a variety of topics.

I would like also to express my gratitude to Prof. Dr. Mohamed Abdel-Aal, Vice-Dean for higher studies, Faculty of Education, Ain Shams University for his material and moral support during the course of this work.

A great appreciation is given to *prof. Dr. Lila Zaki* Ex-chairman of Biological and Geological Sciences Dept., Fac. of education, Ain shams University; for providing facilities during theoretical studies and practical work.

A great appreciation is also given to *prof. Dr. Nagla Zaki El-Alfy* Chairman of Biological and Geological Sciences Dept., Fac. of education, Ain shams University; for providing facilities during finishing this work.

Special thanks go to my mother for here love and support throughout my entire life. Without her love, I would not be able to deal with ups and downs during my study.

I would also like to thank my father, brothers, sisters, my husband and my sweaty child Taleen for their help, a warm support and always being by my side during the past years.

CHAPTER 1
INTRODUCTION

A- HISTORICAL REVIEW

β-Gal (EC 3.2.1.2.3, β-Gal), commonly known as lactases, catalyse the hydrolysis of lactose to glucose and galactose. In addition, they were shown to catalyse transgalactosylation reactions of various β-D-galacto-pyranosides including lactose as well. Both hydrolase and transferase activity of β-Gals can be useful for different applications **(Domingues, 2005).**

Treatment of milk and other dairy products with β-Gal reduces the content of lactose **(Rao *et al.*, 1988).** Thus, products free of lactose or with low lactose content can be used without problems by people suffering lactose intolerance or galactosemia **(Gekas and Lopez-Leiva, 1985; Novelli and Reichardt, 2000 and Campell *et al.*, 2005).**

Enzymic lactose hydrolysis in whey produces glucose - galactose syrup as a sugar substitute **(Budriene *et al.*, 2005).** Addition of β-Gal during manufacture of certain dairy products, e.g. yoghurt, fresh cheese and ice cream, gives sweeter products and prevents undesired lactose crystallization **(Domingues *et al.*, 2005).**

Galactooligosaccharides (GOS) are commercially produced from lactose by transgalactosylation reaction of β-Gal. GOS have many human health benefits by stimulating the growth and/or activity of bifidobacteria in the colon **(Boon *et al.*, 2000; Hung & Lee, 2002 and Pruksasri, 2007),** and are useful as additives in milk for infants **(Shin and Yang, 1994; Lopez-levia and Guzman, 1995).**

Immobilization of β-Gal will reduce the cost of production of food products and allow for reusage of the enzyme. Novel galactooligosaccharides production by β-Gal will have the way for development of prebiotics that can be used as food supplement. Whey utilization by beta-galactosidase will help to reduce the water pollution caused by lack of downstream processing and lead to production of products like bioethanol and lactose hydrolyzed milk. β-Gal capability has been realized by the pleothera of products like biosensors, digestive supplements. Individual molecule study of β-Gal has shown the various unknown kinetic properties of β-Gal . Thus, research and development of β-Gal finds application in several industries **(Sheik Asraf and Gunasekaran**, **2010)**

Different galactosylation reaction have been reported; production of alkyl galactosides which are suitable substrates for the lipase catalyzed synthesis of surfactants and emulsifiers **(Stevenson *et al.*,1983),** galactosylation of drugs like ergot alkaloids **(Křen *et al.*,1992),** genins **(Ooi *et al.*1985)*,*** and antibiotics **(Scheckermann *et al.*,1997),** beside galactosylation of the thio and hydroxyl groups to produce thio and *o*-galactosides, respectively **(Nakano *et al.*, 2000).**

The occurrence of this enzyme in nature is diverse i.e. in plants, animals, and microorganisms **(Gekas and Lopez-Levia, 1985)**

β-Gals are classified into two families, family 2 (GH2) and 35 (GH35). The ones from GH2 are predominantly found in microorganisms whereas approximately 70% of the ones from GH35 are found in plants **(Alcântara *et al.*, 2006).**

Common sources of exogenous β-Gal are fungal in origin and are used either to reduce the lactose levels in food directly or in vivo by the addition of tablets containing β-Gal **(Kosikowski and Wierzbicki, 1973; Fernando *et al.*,1988).**

Due to the biotechnological utility of β-Gal. there has been considerable investigation on their production from filamentous fungi **(Fantes and Roberts,1973; Akasaki *et al.*,1976; Roa and Dutta,1978; Park *et al.*,1979; Marcis,1981; Castillo *et al.*,1984; Chung *et al.*,1985; Brown *et al.*, 1987; Montero *et al.*, 1989; Gonzalez and Monsan, 1991; Reczey *et al.*, 1992; Rasouli and Kulkarni 1994; Fischer *et al.*,1995; Shaikh *et al.*,1997).** β-Gal from filamentous fungi are generally extracellular having broad stability profiles.

Only few reports exist on β-Gal from thermophilic fungi **(Chung *et al.*, 1985)**, *Thermomyces lanuginosus* **(Fischer *et al.*,1995***), Rhizomucor sp.* **(Shaikh *et al.*,1997)**, *Talaromyces thermophilus* **(Nakkharat and Haltrich, 2006).**

Some reports reported the production of the enzyme from other Fungi e.g. *Aspergillus nidulans* **(Fants and Roberts,1973)**; *Alternaria tenuis* **(Zagustina *et al.*, 1975);**

Aspergillus niger **(Widmer and Leuba, 1979; Greenberg and Mahoney, 1981; Rasouli and Kulkerni,1994**); *Fusarium moniliforme* **(Basil , 1981)** filamentous fungi **(Mckay,1991)**; *Beauveria bassiana* **(Macpherson and Khachatourians, 1991);** *Penicillium chrysogenum* **(Nagy *et al.*, 2001);** *Aspergillus carbonarius* **(El-Gindy, 2003)**, *Aspergillus japonicus* **(Saad,2004);** *Penicillium canescens* **(Budriene *et al.*, 2005)**; *Penicillium notatum* **(Fiedurek *et al.*, 1995).** Yeasts e.g. *Kluyveromyces lactis* **(Dickson, and Markin, 1980,)**; *Kluyveromyces fragilis* **(Sonawat *et al.*,1981)***; Kluyveromyces marxianus* **(Furlan *et al.*, 2000 and Rodriguez *et al.*, 2007).**

β-Gal from bacteria also reported e.g. *Escherichia coli Streptococcus thermphilus* **(Roa and Dutta, 1977);** *Lactobacillus delbrueckii ssp. Bulgaricus* **(Jokar and Karbassi, 2009)** and *Bacillus circulans* **(Imanaka *et al.,* 2011).**

β-Gal from plants e.g. *Tropaeolum majus* L**. (Edwards *et al.*,1988);** *Cicer areietinum* (**Dopico *et al.*,1990);** *Lupinus angustifolius* L (**Buckeridge and Reid, 1994**); ripening mango fruit (**Ali *et al.*,1995);** *Copaifera langsdorffil* (**Alcântara *et al.,*1999**). *Hymenaea courbaril* (**Alcântara *et al.*, 2006).**

Culture Conditions Affecting β-Gal Production by Fungi

Culture and nutrition conditions play an important role in the regulation of β-Gal production by fungi.

1) pH of culture medium:

Microbes grow at a wide pH range. Most fungi prefer slightly acid surrounding, about pH 4.0 - 6.0 and they show relatively broad pH optima 5.0-7.0. A change of pH can affect the net charge of membrane proteins with potential consequences for nutrient uptake. It also affects the degree of dissociation of mineral salts and the balance between dissolved carbon dioxide and bicarbonate ions. Drastic variations in pH can harm microorganism by disrupting the plasma membrane or inhibiting the activity of enzymes and membrane transport proteins, also changes in the external pH might alter the ionization of nutrient molecules and thus reduce their availability to the organism **(Prescott *el al.*, 1999 and Deacon, 2006).**

Miwa *et al.*,1980: reported that *Penicillium frequentans* Westling (identified as FERM-P 3085) produces a β-Gal which is stable at a pH value of 3 to 5 at 4° C., the optimum pH being 3.5 to 4.5; *Penicillium luteum* Sopp (identified as FERM-P 3091) produces a β-Gal which is stable at a pH value of 3.5 to 8 at 4° C., the optimum pH being 4.0 to 5.0; *Penicillium citrinum* ATCC 9849 (identified as FERM-P 3086) produces a β-Gal which is stable at a pH value of 3.5 to 8 at 40° C., the optimum pH being 4.0 to 5.0; *Penicillium glaucum* Link (identified as FERM-P 3090) produces a β-Gal which is stable at a pH value of 3.5 to 8 at 40° C., the optimum pH being 4.0 to 5.0; *Penicillium chrysogenum* Thom IFD 4626 (identified as FERM-P 3088) produces a β-Gal which is stable at a pH value of 3 to 8 at 40° C., the optimum pH being 4.0 to 5.0; and *Penicillium notatum.* Westling (identified as FERM-P 3087) produces a β-Gal which is stable at a pH value of 3.5 to 8 at 40° C., the optimum pH being 4.0 to 5.0.

Mckay (1991) reported that production of β-Gal from *Aspergillus oryzae* and *Scopulariopsis sp*. become optimum at pH 5.0.

Fiedurek *et al.*, (1995) found that an initial pH 5.2 was optimum for β-Gal production by *Penicillium notatum*.

Fischer *et al.*, (1995) found the pH 6.7-7.2 was the optimum for the production of β-Gal from *Thermomyces lanuginosus*. While the enzyme pH stability had a broad range of pH 6-9.

Shaikh *et al.*, (1999) reported that the optimum pH for the enzyme activity from *Rhizomucor* sp. was 4.5.

El-Gindy, (**2003**) found that β-Gal production from *Aspergillus carbonarius* gave optimum values at initial pH 4.5.

Saad, (2004) found that the optimal activity of β-Gal of *Aspergillus japonicu*s occurred at pH 5.2.

Burdiene *et al*.,(2005) reported that the yeast enzymes from *Kluyveromyces* or *Saccharomyces sp*. have neutral pH optima (6-7) making them suitable for the hydrolysis of lactose in milk .and bacterial β-Gal obtained from *E. coli* with pH optima (6.5-7.5) also found that the highest activity of native β-Gal is obtained at about pH 4.5.

Nakkharat and Haltrich (2006) detected that the optimum pH values for β-Gal production from *Talaromyces thermophile* for both oNP Gal and lactose as substrates were 6.0-6.5 and 5.5-6.0

Alcântara *et al*., (2006) found that the optimum pH for β-Gal production from Hymenaea courbaril L was 3.0 while purified enzyme was 4.0.

Aberomand (2009) found that Optimum pH of β-Gal enzyme showed maximum activity for orange (*Citrus sinensis*), onion (*Allium cepa*), garlic (*Allium sativum*) and sweet lemon (*Citrus limon*) at pH 3.6, 4.4, 6.0 and 4.4 were for the selected fruits, respectively. As a conclusion, in order to use fruit as a source of β-Gal activity, the orange with optimum pH 3.6 is a good choice for purification.

2) Temperature of incubation:

Temperature is one of the cardinal factors affecting the growth rate and activity of microbes. There is a minimum temperature, below which growth does not occur. Above the minimum, rate of growth increases due to the increasing in the velocity of any enzyme catalyzed reaction, like that of any chemical reaction. The optimum temperature reached when