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PRODUCTION AND OPTIMIZATION OF GALACTANASE FROM SOME LOCAL ISOLATED FUNGI

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ABSTRACT

Ten fungal strains producing galactanase (E.C 3.2.1.89) were isolated from some local soil samples. Different isolates were tested for galactanase production using submerged culture technique under rotating conditions. Aspergillus fumigatus var. ellipticus and Aspergillus aculeatus were found as the most potent galactanase producers. Maximum galactanase productivity was recorded at pH 5.5& 30^{o} C with arabic gum as a natural carbon source and amixture of yeast extract, $NaNO_3$ as nitrogen source .It was possible to increase the productivity of galactanase after optimization and give high activity at initial6.5 pH , 30^{o} C and showed the maximal galactanase activity after 2 days incubation under shaking conditions.

Key words: Galactanase, Galactan degradation, *Aspergillus fumigatus var*. ellipticus, Aspergillus aculeatus

INTRODUCTION

Plant cell wall polysaccharides are the most abundant organic compounds found in nature. They make up 90% of the plant cell wall and can be divided into three groups: cellulose, hemicellulose and pectin **(McNeil, et al., 1984).** Galactan are mainly carbohydrate chains branched on rhamnogalacturonan belonging to the matrix of pectin **(McCann & Robertes, 1991 and Carpita & Gibeaut, 1993)**. Galactans have an important function in the primary cell wall, where they interact with other structural components of cell wall such as xyloglucans or arabinoxylans

thus they possibly serve to anchor the pectic matrix in the cell wall also they increase hydration and water binding capacity and decrease inter chain association between pectin polymers, which is thought to be importance for modulation the porosity and passive diffusion **(Carpita & Gibeaut, 1993).** Fungi and plants make two types of galactan degrading enzymes include exo - β-1,3-galactanases and endo -β-1,6-galactanases **(Sakamoto, et al., 2007)**. Exo -β-1,3-galactanases(EC 3.2.1.145): degrade β-1,3-galactan backbones of arabino galactan and belong to glycoside hydrolase family (GH 43) and act on the non reducing ends of the substrate in an exo acting manner **(Ichinose, et al ., 2008)**. Endo -β-1,6-galactanases (EC 3.2.1.89): hydrolyze β-1,6galactan side chain of arabinogalactan and belong to glycoside hydrolase family (GH 53). Two types of arabinogalactans are present as side chains of pectins so for complete degradation of polysaccharide two enzymes must be founded **(Bonnin, et al., 1995)** . Galactanase can be used to hydrolyze galactans of coffee bean in the coffee bean production process **(Hashimoto & Fukumoto, 1969 and Godfrey, 1983).**Galactanase used in modifying the viscosity of plant cell wall also used for reducing the viscosity of feed, which contain galactan. Galactanase play an important role in the degradation or modification of plant cell wall **(Karr & Albersheim, 1970 and Basham & Bateman, 1975)**. The monosaccharides that are the building blocks of the pectin polymer and all have different food and nonfood applications as Production of textile fibers **(Silva, et al., 2005),** animal food additive and Juice production process**(EL-Tanash, et al., 2007)** , and the enzymatic release of these compounds is an important tool in industrial processes, **(deVries & Visser, 2001)**. Fungi and other different micro-organisms produce galactanase as Aspergillus niger **(Brillouet, et al., 1991)**, Fusarium oxysporium **(Sakamoto et al., 2007)**, Trichoderma viridi **(Kotake, et al., 2004)**, Streptomyces avermititis **(Ichinose**, **et al ., 2006),** Emericella nidulans **(Michalack, et al., 2012)** and Bacillus subtilis **(Nakano, et al ., 1990)**.

MATERIALS AND METHODS

Microorganisms:

The fungal cultures used in the present study were locally isolated by dilution plate method ,using Arabic gum as source of carbon in agar medium in soil samples and collected from Mansoura University garden according to the procedures adopted by **(Johnson, et al., 1960).** Fungal

isolates were subjected to full identification, using the most recent sophisticated facilities; an imaging analysis system using soft imaging GbH software (analysis pro ver.3.0) at the Regional Center for Mycology and Biotechnology (RCMB), AL-Azhar University, EGYPT. The stock cultures were maintained routinely on PDA slants. The freshly slant cultures were grown at 30°C and subsequently used for further work or stored at 4°C. The slants were sub- cultured routinely at intervals of 4-5 weeks.

Culture medium and cultivation:

The composition of the started basal medium was; 10gm Arabic gum as a natural source of galactan **(Ichinose, et al., 2008 and Reyes et al., 1992)** 2g $NaNO_3$ 2 g KH_2PO_4 , 0.3 g $CaCL_2$. $2H_2O$ and 0.3 g $MgSO_4.7H_2O$ and supplemented with1 g yeast extract. All of these components were dissolved in 1L of 0.1M sodium acetate buffer (pH 5.5) **(Araujo & Ward, 1990)**. The same culture medium was used for inoculua preparations. Erlenmeyer flasks (250 ml) each containing 50 ml of sterile medium was inoculated with 1ml spore suspension .The flasks were incubated at 30°C under shaking conditions (150 rpm) for 4 days after incubation the culture was filtered and the filtrate was centrifuged at 10000 rpm for 10 min to remove hyphae and spores. The clarified extract represented crude enzyme preparation of galactanase, this crude preparation of enzyme was used for enzyme activity assay.

Galactanase assay:

Galactanase activity was determined according to the method of **(Araujo & Ward, 1990) as follows** 0.5 ml of 1.0% (w/v) galactomannan, in 0.1 M acetate buffer (pH 5.8) was added to 0.5ml of the crude enzyme solution. The reaction mixture was incubated at 40°C for 15 min in a water bath. The reducing sugars released were measured as galactose by the method of (**Nelson, 1944 and Somogyi, 1952).** One unit of galactanase activity was defined as the amount of enzyme that releases one µmole of galactose per minute under assay conditions.

Optimization studies:

The effect of fermentation period (1-7days), initial culture pH value (3.5-8.0) ,incubation temperature (20-50°C), substrate level (Arabic gum 0.25-1.25 per 50 ml), carbon source (glucose ,galactose ,lactose, starch ,locust bean gum- and Arabic gum) and nitrogen source ($NaNO_3$-

NH_4cl-pepton-yeast extract-urea) were investigated to optimize the culture conditions to maximize galactanase production by Aspergillus fumigatus var. ellipticus and Aspergillus aculeatus. After incubation period in each experiment, the culture filtrate was separated and used as a source of galactanase**.**

RESULTS AND DISCUSSION

Screening of galactanase producing fungi:

Ten fungal isolates obtained from some local soil samples, were screened for their galactanase activity after 4.0 days incubation at 30°C on submerged culture of selective Arabic gum medium at pH 5.5.

Table (1): Screening for the most active galactanase producing fungi**.**

Fungal strain	Galactanase activity (U/ml)
Aspergillus aculeatus	**37.42 ± 1.213**
A. fumigatus var. ellipticus	**43.22 ± 4.104**
A.flavus	**23.99 ± 1.791**
Fusarium solani	**12.5 ± 1.445**
A.subolivaceus	**13.6 ± 1.213**
A. terreus	**27.41 ± 0.635**
Paecilomyces variotii	**11.71 ± 0.872**
Penicillium purpurogenum	**11.71 ± 1.219**
Trichoderma viridi	**5.68 ± 0.289**
Fusarium oxysporum	**16.71 ± 1.335**

From the result showed in Table (1), **it could be** indicated that Aspergillus fumigatus var. ellipticus and Aspergillus aculeatus are the most active galactanase producing fungi compared with other isolates. Therefore, these strains were used for further optimization studies. In this connecton, Talaromyces byssochlamydoides recorded galactanase activity of 0.36 , while U/ml and Talaromyces emersonii recorded 0.38 U/ml were the most galactanase producers (**Araujo and Ward, 1990).** This activity value is nearly similar to that obtained for galactanase yield from Aspergillus aculeatus.

Factors affecting galactanase production

Effect of different incubation periods

Fig. **(1)** showed that the maximum galactanase activity was recorded after 2.0 days incubation on rotating submerged culture containing Arabic gum (as galactan natural carbon source) .In this connection, **Araujo and Ward, (1990)** reported that 2.0 days was the optimum incubation period for galactanase activity under shaking conditions; However**, Bauer et al.,(1977)** reported that 14 to 20 days were needed for maximum galactanase activity under static culture conditions Also **Kotake et al., (2011)** reported that 20 days for galactanase production by winter mushroom Flammulina velutipes.

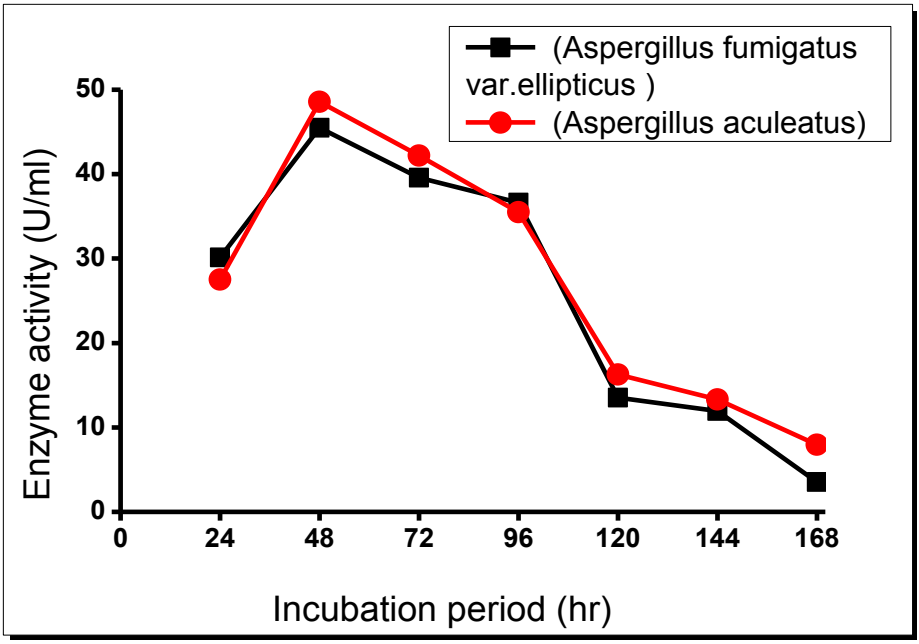


Fig. (1): Effect of different incubation periods on galactanase activity

Effect of different carbon source:

The effect of substitution of Arabic gum in the basal medium by Locust bean gum and other carbon sources namely glucose, galactose, starch and lactose) on an equal carbon basis was investigated. The results **(Fig 2)** indicated that arabic gum afforded maximal galactanase activity (45.496 U) by Aspergillus fumigatus var.ellipticus and (35.37U) by Aspergillus aculeatus . Lower values were recorded by locust bean gum or galactose as inducers for galactanase production for both organisms. **(Ichinose, et al., 2008; Kotake, et al., 2011 and Reyes, et al., 1992)** reported the same result by using Arabic gum as a carbon source for galactanase production by Streptomyces avermititis, winter mushroom Flammulina velutipes and Penicillium oxalicum; while **(Nakano, et al., 1990)** using soy bean as a carbon source for Bacillus subtilis. Also **(Bauer, et al., 1977)** using galactose as optimum carbon source for galactanase production by Sclerotinia sclerotiorum.

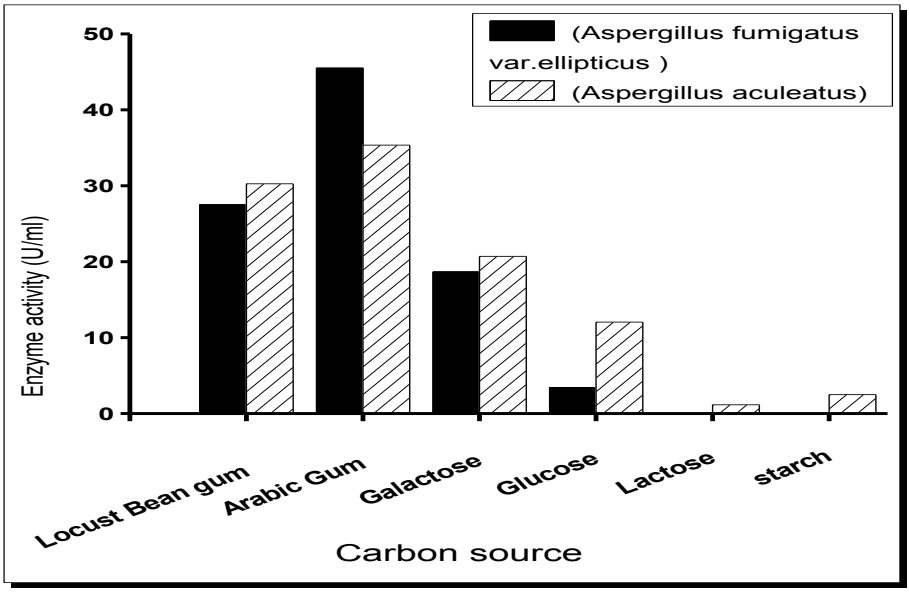


Fig. (2): effect of different carbon sources on galactanase activity

3-Effect of different nitrogen source:

On an equivalent nitrogen basis ,the nitrogen source in the basal medium was substituted by different nitrogen sources these including

organic-N (urea, peptone and yeast extract)and inorganic nitrogen sources ($NaNO_3$ and NH_4Cl). The result in Fig 3 showed that combination of sodium nitrate and yeast extract were the most effective for higher galactanase production by any of the two organisms and most suitable nitrogen source for Aspergillus fumigatus var.ellipticus activity was (55.84U) and for Aspergillus aculeatus was (47.99U). In this connection **(Araujo & Ward, 1990)** reported that $NaNO_3$ was the optimum nitrogen source for galactanase production by Aspergillus niger .

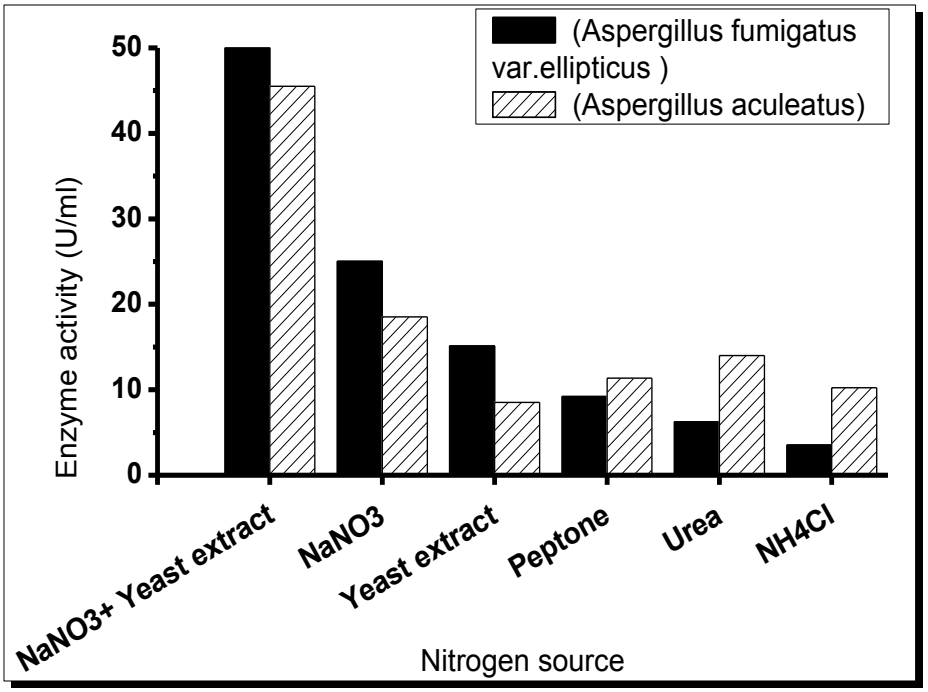


Fig (3): effect of different nitrogen sources on galactanase activity

4Effect of different Arabic gum concentration:

The effect of different Arabic gum as carbon source concentrations on galactanase production was investigated using different concentrations from (0.25-1.25g per 50ml). Maximum galactanase by Aspergillus fumigatus var. ellipticus was49.7U/ml and 42.08 by Aspergillus aculeatus at Arabic gum concentration of1% (w/v) for the two fungi (Fig .4) .The same amount was used by **(Araujo & Ward, 1990)** for galactanase production by Talaromyces byssochlamydoides Talaromyces emersonii have ever the optimum galactanase activitywas

achieved applying the same quantity of locust bean gum. 1.0%(w/v) Arabic gum Arabic was also used by **(Ichinose, et al., 2008)** for maximal production of galactanase by Streptomyces avermititis. On the other hand 2.0%(w/v) of Arabic gum was reported for the maximal galactanase production by winter mushroom Flammulina velutipes.

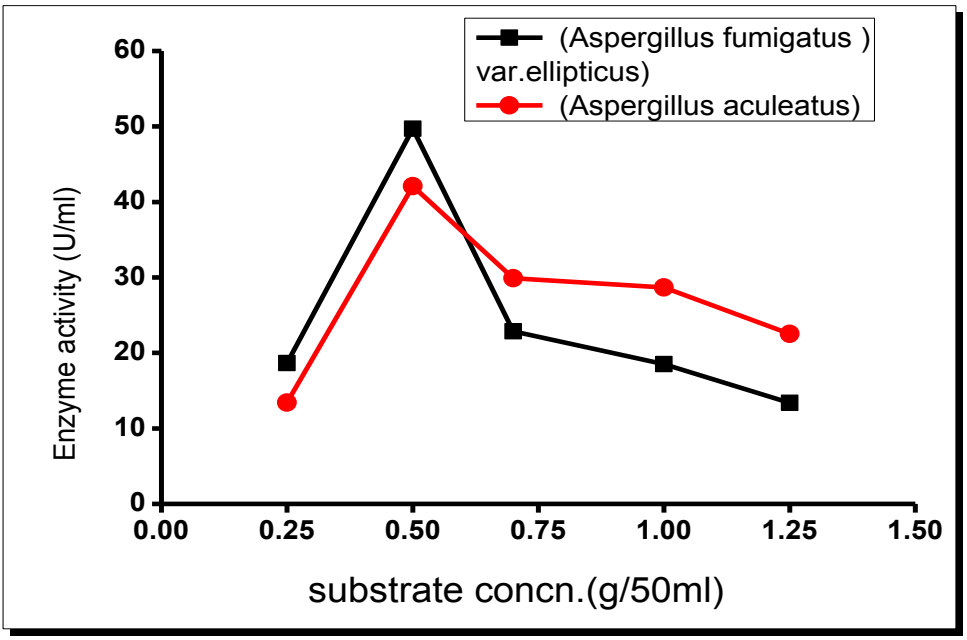


Fig (4): effect of different substrate concn. on galactanase activity

5-Effect of growth temperature:

The effect of different temperature (Fig .5) showed that 30°C was the optimum for galactanase production by thetwo tested fungal strains and the maximum activity recorded 70 U/ml by Aspergillus fumigatus var. ellipticus and 64.149 U/ml by Aspergillus aculeatus. While **(Araujo & Ward, 1990)** reported the optimum growth temprature of 50°C 50oC for Aspergillus niger and Talaromyces byssochlamydoides on the same connection **(Ichinose, et al., 2008)** used 37°C for Streptomyces avermititis.23°C was the optimum for galactanase production by Clostridium thermocellum **(Ichinose, et al ., 2006).**

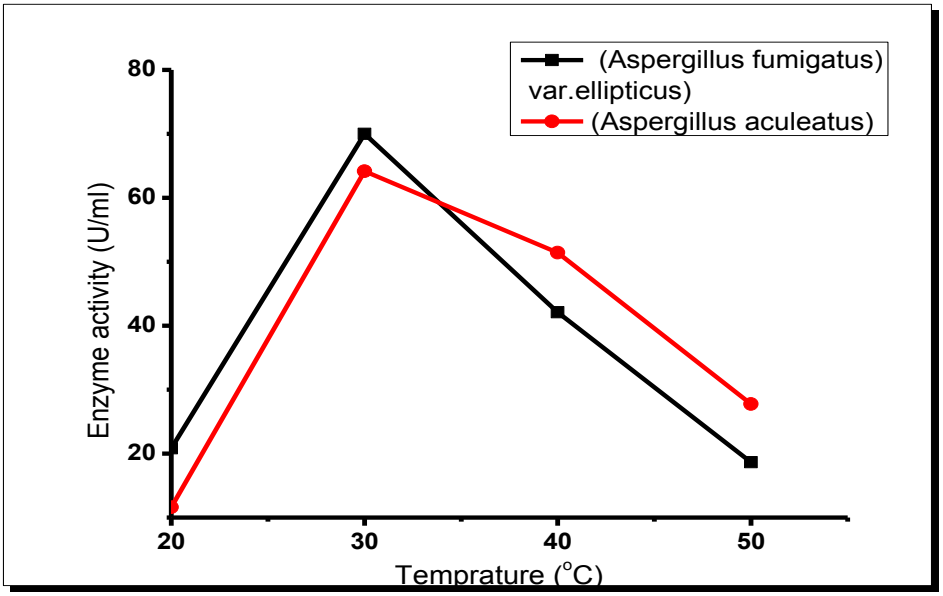


Fig. (5): effect of different temperature on galactanase activity

6-Effect of different initial pH values:

Optimization of galactanase production at different initial **pH values** for the two fungal strains Aspergillus fumigatus var. ellipticus and Aspergillus aculeatusare was investigated using all the optimum conditions previously mentioned. The results in Fig (6) showed that the initial pH had asignificant effect on the yield of galactanase production by the two fungal strains. Initial pH 6.5 was the optimum for the maximal galactanase production by each of Aspergillus fumigatus var. ellipticus (68.244) Aspergillus aculeatus (61.87 U) .On the same connection**, (Araujo & Ward, 1990)** reported that the initial pH of 6.4 was the optimum for Aspergillus niger, while 6.6 was the optimum for Talaromyces byssochlamydoides **,** 6.3 was the optimum for Talaromyces emersonii and 7.2 for Thermoascus aurantiacus

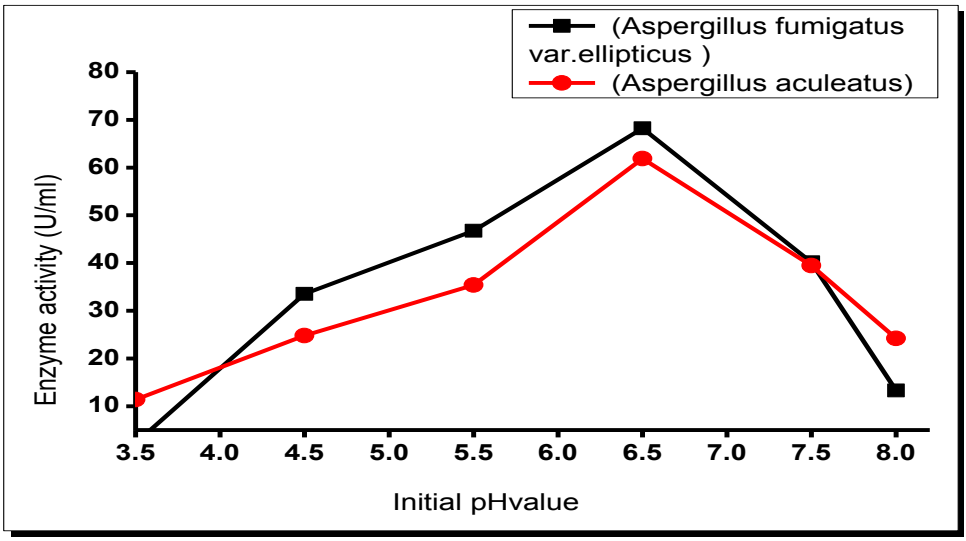
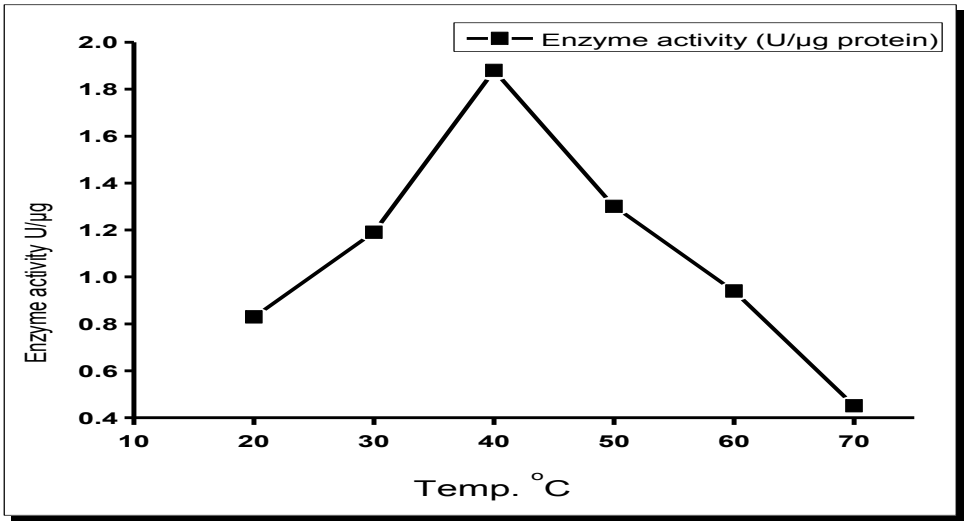


Fig. (6): effect of different initial pHvalues on galactanase activity

From the preceding results, the optimized culture medium consisted (gram / liter) 10.0 g Arabic gum, as a natural source of galactan**,** $NaNO_3$2.0 g , $KH_2PO_4$2.0 g , $CaCL_2$ $2H_2O$ 0.3 g , $MgSO_4.7H_2O$ 0.3 g, yeast extract 1.0 g. All of these components were dissolved in 1L of 0.1M sodium acetate buffer (pH 6.5). The optimum culture medium yielded 68.244 U/ml by Aspergillus fumigatus var. ellipticus and 61.87 U/ml by Aspergillus aculeatus .

Aspergillus fumigatus var.ellipticus Galactanase charactarization:
Effect of different temperatures on galactanase activity

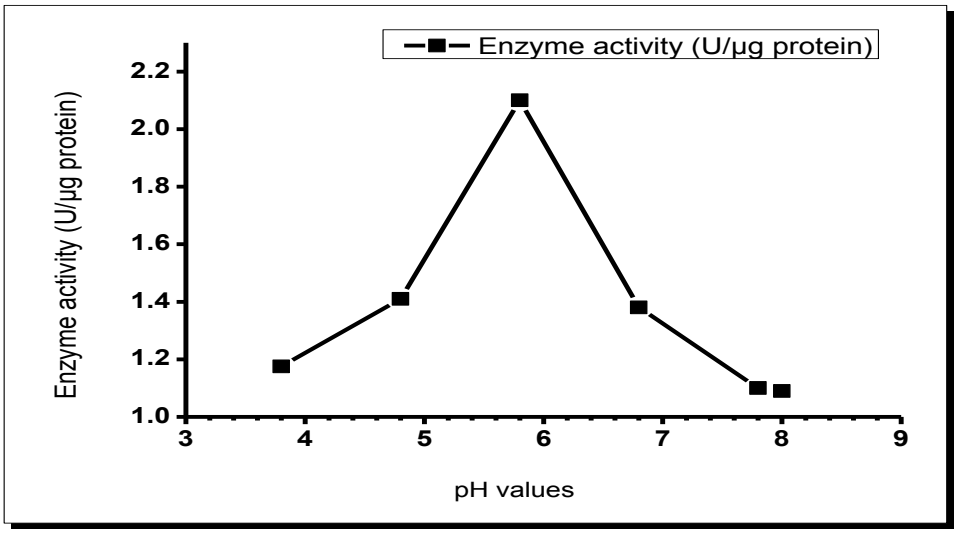
Results illustrated in Fig.(7) indicated that galactanase has the maximal activity at 40^oC . Similary, **(De vries, et al., 2001)** recorded optimum temperature 40^oC for maximal galactanase activity by Aspergillus niger.



Fig(7):.effect of different temperatures on galactanase activity

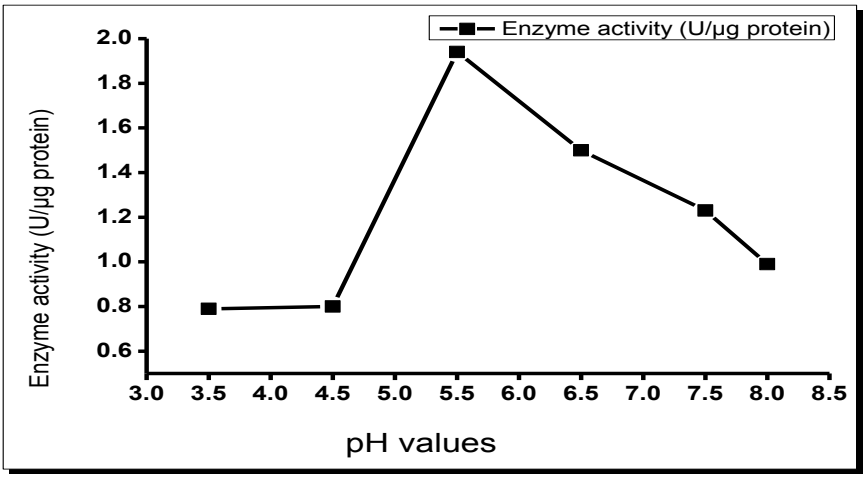
Effect of different pH values on galactanase activity:

The **Fig. (8)** Showed that pH levels are effective on galactanase activity. A. fumigatus var.elliptius galactanase exhibited the maximum activity at pH range (5.0- 7.0). The maximal galactanase activity obtained at pH 5.8 for A. fumigatus var.elliptius.While **(Sakamoto, et al ., 2007)** reported that pH5 has maximal galactanase activity by Fusarium oxysporum.



pH stability of galactanase :

galactanase activity was greatly affected by different pH values as shown in **Fig. (9)**. At lower pH values (pH 3.5- pH 4.5) and at higher pH values (pH 8.0), the enzyme activity was inactivated. More stable A. fumigatus var.ellipticus galactanase retained 88.44% of initial activity at pH 5.5.While **(Kotake, et al., 2011)** recorded that Flammulina velutipes galactanase was stable at pH range 4.0-6.0. Also **(Sakamoto, et al., 2007)** recorded that Fusarium oxysporium galactanase was stable at pH range 3.4-8.



Fig(9):pH stability of galactanas

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دراسات على انتاج انزيم الجلاكتانيز بواسطة بعض الفطريات

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قسم الأحياء – كلية العلوم – جامعة تبوك – المملكة العربية السعودية.
عبد الدايم ابو الفتوح شريف : قسم النبات –كلية العلوم– جامعة المنصوره– مصر.

يهدف هذا البحث الى دراسة انتاج انزيم الجلاجتانيز المحلل للجلاكتان .من بعض السلالات الفطريه المعزوله محليا.

تناولت الدراسة عزل وتعريف١٠ فطريات من التربه المحليه وبعد دراسة واختبار للنشاط الانزيمى تم اختيار الفطريات الاكثر نشاطا لانتاج الجلاكتانيز.

اثبتت النتايج ان الظروف المزرعيه المثلى لانتاج انزيم الجلاكتانيز تتلخص فى استخدام الصمغ العربى كمصدركربونى من الطبيعة للمزرعه لاحتوائة على نسبه عاليه من الجلاكتان واستخدام مخلوط مستخلص الخميره ونترات الصوديوم كمصدر نيتروجينى مع التحضين فى بيئة غذائية ذات رقم هيدروجينى٥.٥ وعند درجة حراره ٣٠°م و قد سجل انزيم الجلاكتانيز اعلى نشاط لكل من فطرتى

Aspergillus fumigatus
var ellipticus, Aspergillus aculeatus

من الدراسه ثبت ان الظروف المثلى لانتاج الانزيم للفطرين كما يلى :
مده التحضين ٤٨ ساعة و٣٠°م هى درجة حراره التحضين وذلك لانتاج الانزيم وايضا درجه pHالابتدائيه المثلى هى 6.5 وذلك لتحقيق اعلى انتاجيه لانزيم الجلاكتانيز باى من الفطرين سابقى الذكر.