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PRODUCTION AND CHARACTERIZATION OF AMYLASE FROM SOME WHITE-ROT FUNGI GROWN ON AGRICULTURAL WASTES

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ABSTRACT: The ligninolytic white-rot fungi *Trametes versicolor* R-105 (ATCC 11235) and *Phanerochaete chrysosporium* BKM-F-1767 (ATCC 24725) were investigated to produce the extracellular amylase enzyme. The two fungi were grown in liquid medium supplemented with wheat straw or sugarcane bagasse as a carbon source at a concentration of 1.0%. The changes in pH and soluble protein content were monitored in the culture filtrates. The optimum pH and temperature and thermostability of the enzyme were taken also into account. The results showed that the two fungi have the ability to produce the investigated enzyme using different substrates and the highest activity was obtained after 15 days of cultivation. *Trametes versicolor* was better than *Phanerochaete chrysosporium* in secretion of amylase and sugarcane bagasse was found to be the best inducer for this enzyme. The results indicated also that the pH values and soluble protein content in the culture filtrates were changed during the course of experiment. The optimum activity of amylase from the two fungi was detected at pH 5.0 and temperature 60 °C. The results showed also that after prolonged heating (5 h) at 60 °C, the amylase enzyme retained about 10% of the original activity. This enzyme could be classified as relatively thermostable for all practical purposes.

Key words: *Trametes versicolor, Phanerochaete chrysosporium,* Amylase, Agricultural wastes

INTRODUCTION

Lignocellulose biomass is continuously produced in great quantities, giving rise to disposal problems. Because of its chemical composition it could be an excellent substrate for biotechnological processes (Müller and Trösch, 1986). Lignocellulose waste material, can be pretreated chemically or physically to increase its biotechnological substrate value (Detroy *et al.*, 1981; Fan *et al.*, 1981).

White-rot fungi can be used for the biological pretreatment. They are the only microorganisms known for their ability to degrade lignin completely (Müller and Trösch, 1986). The most investigated fungus for lignin degradation is *Phanerochaete chrysosporium* (Keyser *et al.*, 1978; Tien and Kirk, 1983). Also, the fungus *Trametes versicolor*, degrades both lignin and carbohydrate components of wood with almost the same proportional rates (Rogalski *et al.*, 1993).

Lignocellulosic materials and agricultural waste materials which contain both cellulose and starch can be conveniently hydrolyzed to fermentable sugars by using a mixture of amylases and cellulases (Shambe and Ejembi, 1987).

Amylases are used extensively in various industries and the suitability of any one amylase to a particular process will depend on its specific characteristics such as the selection of thermostable amylases for use in the liquefaction of starch (Fogarty and Kelly, 1990). The microbial α-amylases for industrial processes are derived mainly from two genera, *Bacillus* and *Aspergillus* (Jensen and Olsen, 1992).

The commercial potential of amylases is enormous. The development of a cheaper and more stable fungal amylase capable of operating in a high temperature range is no doubt advantageous especially if the amylase-producing organism could be grown on inexpensive local carbon source (Uguru *et al.*, 1997). The starch processing industry requires the use of amylolytic enzymes at high temperatures. Thermostability is a feature of most of the enzymes sold for bulk industrial usage. Thermophilic organisms are therefore of special interest as a source of novel thermostable enzymes. Recent research with thermostable α-amylases has concentrated on the enzymes of thermophiles and extreme thermophiles (Bolton *et al.*, 1997). Thermoactive and thermostable amylases have wide

applications in various industrial processes. Saccharification processes are usually carried out at temperatures above 60°C in order to obtain higher substrate solubility and reduced microbial contamination (Odibo and Ulbrich-Hofmann, 2001).

The significance of the production of α-amylase depends upon the fact that *Phanerochaete chrysosporium* can utilize starch as co-substrate for lignin degradation. The ability of ligninase producers to secrete higher titres of amylases would help develop an economic biodelignification process using low-cost starchy materials as co-substrates (Dey *et al.*, 1991).

Generally, there is no enough data on the production of amylase from the white-rot fungi. Therefore, the aim of the present study was to investigate the ability of the ligninolytic white-rot fungi *Trametes versicolor* and *Phanerochaete chrysosporium* to produce amylase enzyme using wheat straw and sugarcane bagasse as carbon sources. These substrates as agricultural wastes rich in lignocellulose are very rarely utilized in most of the developing countries and consider as a source of pollution.

MATERIALS AND METHODS

Agricultural wastes

Wheat straw and sugarcane bagasse were mechanically milled and used as carbon sources.

Microorganisms

The two white-rot fungi *Trametes versicolor* R-105 (American Type Culture Collection, ATCC 11235) and *Phanerochaete chrysosporium* BKM-F-1767 (American Type Culture Collection, ATCC 24725) were grown on 2.0% (w/v) malt agar slants at 30 and 37 °C, respectively for up to 6 days for inoculum preparation.

Inoculum preparation

Inoculum was prepared by scraping 6 day-old culture with sterile-distilled water to obtain a standard concentration of the spore suspension (10^6-10^7 spores per ml); counting of spores was carried by the haemocytometer (Khalil *et al.*, 2002).

Production of amylase and culture conditions

Amylase production was studied in 250-ml Erlenmeyer flasks. Each flask contained 50 ml of culture medium and 1.0% (w/v)

wheat straw or sugarcane bagasse. The culture medium used was the same as described by Copa-Patiño *et al.* (1993), which contained (g/l): KH_2PO_4, 0.6; $MgSO_4.7H_2O$, 0.5; K_2HPO_4, 0.4; $CaCl_2.2H_2O$, 0.74; $NH_4H_2PO_4$, 2.32 and yeast extract, 1.0 and 7 ml of trace salts solution/l which contained (mg/100 ml): $CoCl.7H_2O$, 200; $FeSO_4.7H_2O$, 500; $MnSO_4.H_2O$, 160 and $ZnSO_4.7H_2O$, 140. The initial pH was adjusted to pH 5.0. The flasks were autoclaved at 120 °C for 20 min, then inoculated with 0.5 ml spore suspension (10^6-10^7 spores/ml) and incubated on a rotary shaker (200 rpm) at 30 and 37 °C for *Trametes versicolor* and *Phanerochaete chrysosporium*, respectively. The cultures were harvested in duplicate every 3 days. The cultural samples were centrifuged and the clear supernatants were used for assay of pH, soluble protein and enzyme activity.

Assays

Soluble protein in the culture filtrates was determined by the method of Lowry *et al.* (1951), using bovine serum albumin as a standard. Amylase activity was assayed by the addition of 0.5 ml enzyme to 0.5 ml soluble starch (1.0%, w/v) in 0.1 M acetate buffer, pH 5.0, and incbated for 30 min at 50°C. The reducing sugars produced were determined using the dinitrosalicylic acid method of Miller (1959). One unit of amylase activity was defined as the amount of enzyme that released 1 µmole of reducing sugars equivalent to glucose per minute under the assay conditions. All assays were done in triplicate.

Characterization of enzyme

The clear culture filtrate obtained after cultivation the two fungi for 15 days in liquid medium supplemented with 1.0% sugarcane bagasse was used as a source of enzyme. The effect of pH on the enzymatic activity was tested at pH from 3.8 to 5.8 using 0.1M sodium acetate buffer under the standard assay conditions as described before. The effect of temperature on the enzymatic activity was determined under the standard assay conditions in the range of temperatures from 35 to 75 °C at the optimum pH value. The thermostability of enzyme was determined by preincubation of the diluted enzyme solutions at 60 °C (in 0.1M sodium acetate buffer at the optimum pH value) in the absence of substrate. Aliquots were removed at different times between 0 and 5 h and immediately cooled.

The treated enzyme solutions were then assayed for residual enzymatic activity using standard assay conditions.

RESULTS AND DISCUSSION

Production of amylase

The ability of the ligninolytic fungi *Trametes versicolor* R-105 and *Phanerochaete chrysosporium* BKM-F-1767 to secrete extracellular amylase enzyme was investigated. This experiment was carried out using wheat straw or sugarcane bagasse as a carbon source. The change in amylase activity during the cultivation of the two fungi is illustrated in Fig. 1. Generally the maximum activities were detected after 15 days of cultivation in all cases and the highest activity was obtained when sugarcane bagasse was used. It is noticed also that *Trametes versicolor* was better than *Phanerochaete chrysosporium* in secretion of amylase enzyme. In case of *Trametes versicolor*, the maximum activities (0.691 and 1.2 µmol/ml/min) were found with the addition of wheat straw and sugarcane bagasse, respectively, whereas on using *Phanerochaete chrysosporium*, the maximum activities (0.334 and 0.533 µmol/ml/min) were detected with the addition of the two substrates, respectively. Generally, as concluded by Tuohy and Coughlan (1992), the phenomena of enzyme production reflect one or more of the following: differential timing of the expression of enzymatic components, differential inhibition by products of substrate hydrolysis or differential inactivation of components as a result of the change in the pH of the medium during cultivation.

Changes in pH values

The pattern of pH change during the cultivation of *Trametes versicolor* R-105 and *Phanerochaete chrysosporium* BKM-F-1767 is shown in Fig. 2. With the two substrates used, the pH of the culture filtrates dropped gradually from the original value (pH 5.0) after 3 days of cultivation. Such decrease in pH was accompanied with slowly increase during the whole period of cultivation. On using *Trametes versicolor*, the pH revealed increases and reached 5.0 and 5.05 after 12 and 15 days with wheat straw and sugarcane bagasse, respectively (Fig. 2A). In case of *Phanerochaete chrysosporium*, the pH revealed increases and reached 5.34 and 5.77 after 12 days with

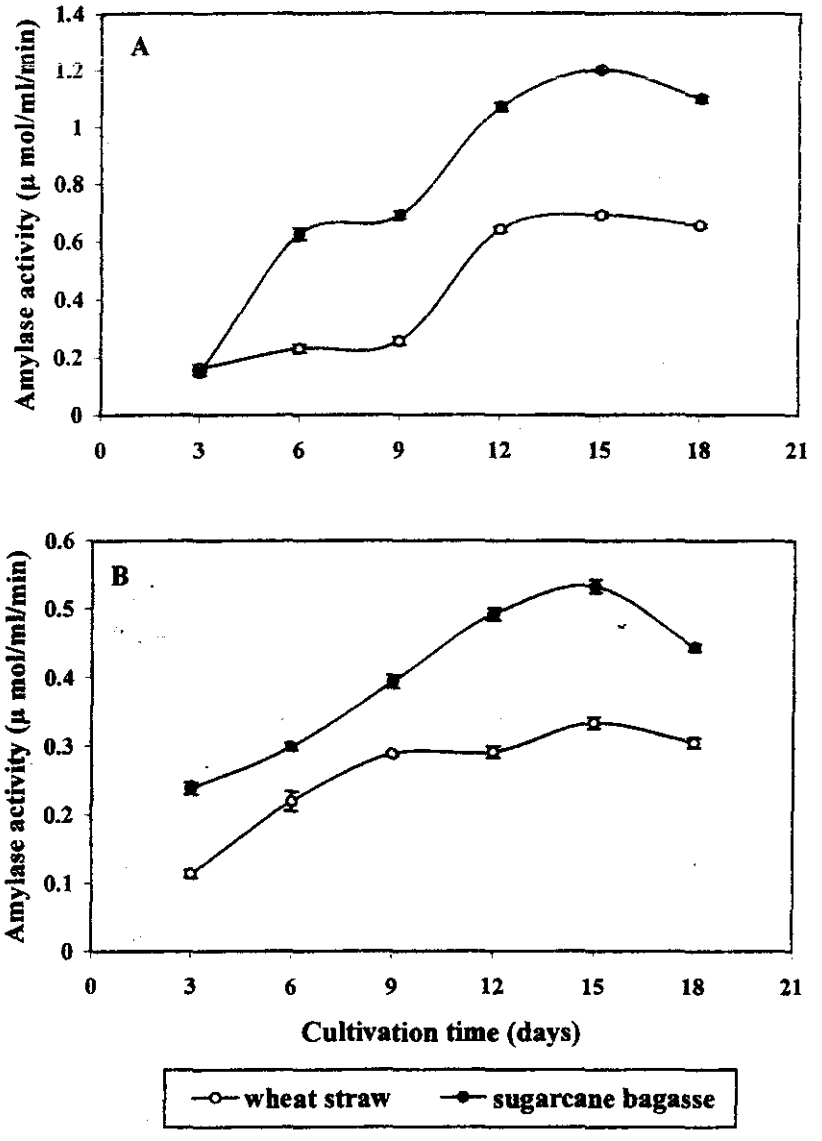

Fig.(1): Changes in the activity of extracellular amylase during the cultivation of *Trametes versicolor* R-105 (A) and *Phanerochaete chrysosporium* BKM-F-1767 (B) in liquid medium supplemented with 1.0% agricultural waste. Values are means± standard deviations.

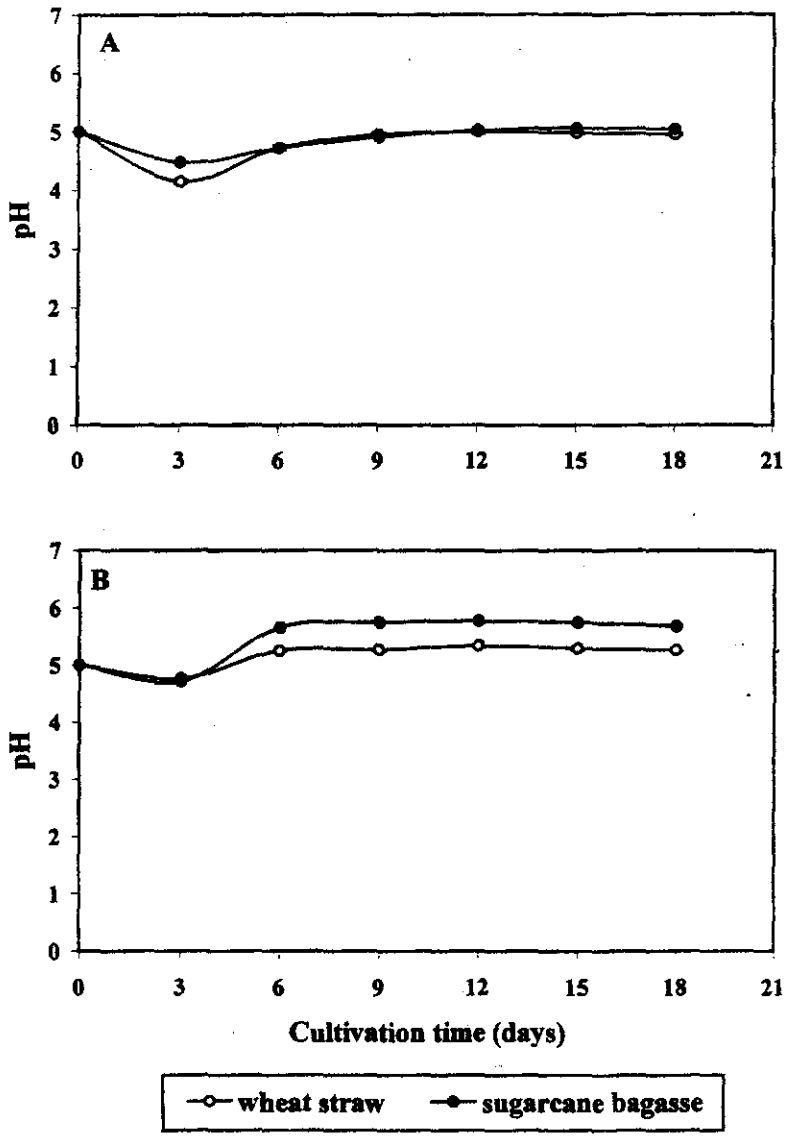

Fig.(2): Changes in pH values during the cultivation of *Trametes versicolor* R-105 (A) and *Phanerochaete chrysosporium* BKM-F-1767 (B) in liquid medium supplemented with 1.0% agricultural waste. Values are means± standard deviations.

wheat straw and sugarcane bagasse, respectively (Fig. 2B). The same trend was observed with *Trichoderma reesei* when grown on wheat straw (Acebal *et al.* 1986). The reason for the decrease in medium pH is probably due to the high consumption of carbohydrates and NH_4^+ ions during maximal cell growth as stated by Mandels and Andreotti (1978) and Blanch and Wilke (1983), or to the uptake of NH_4^+ ions as mentioned by Harchand and Singh (1997). Further, the decrease in the pH of medium might be attributed to the accumulation of oxalate during growth of white-rot fungi as indicated by Dutton *et al.* (1993).

Changes in soluble protein content

The pattern of change in soluble protein content of the culture filtrates is shown in Fig. 3. The maximum value of soluble protein content (0.672 mg/ml) was obtained after 12 days with the two fungi, especially when sugarcane bagasse was used as a carbon source. Generally, the soluble protein content gradually increased and reached the maximum after 12 days in all cases except in case of wheat straw with *Trametes versicolor*, the soluble protein content decreased after 6 days and then gradually increased and reached the maximum (0.386 mg/ml) after 15 days. The same trend was detected for *Phanerochaete chrysosporium* (Copa-Patiño *et al.*, 1993; Cancell *et al.*, 1993). Generally, the present investigation has shown that, the increase in amylase activity was associated with an increase in extracellular protein levels.

Characterization of amylase

Effect of pH on amylase activity

The pH profile of the amylase was determined by assaying the amylase activity at different pH values at 50°C. The optimum pH value for amylase activity was detected at pH 5.0 as shown in Fig. 4A. It is noticed that amylase enzyme remained highly active over a broad range of pH from pH 4.2 to 5.4 with maximum activity at pH 5.0. The same result was found with the amylase from the fungus *Thermomyces lanuginosus* (Jensen and Olsen, 1992; Odibo and Ulbrich-Hofmann, 2001). On the other hand, the result obtained was higher than that reported for amylase from *Trichoderma harzianum* (de Azevedo *et al.*, 2000) and lower than the results reported for amylase from other fungi (Shambe and Ejembi, 1987;

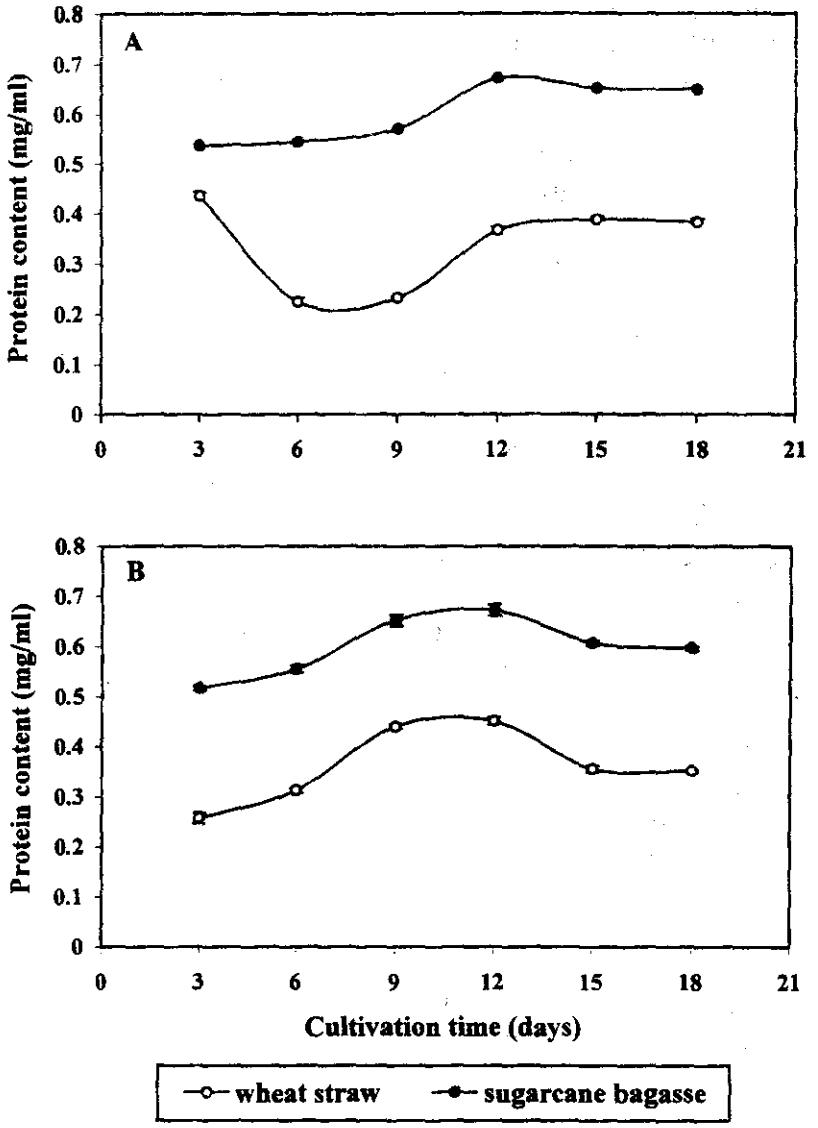

Fig.(3): Changes in soluble protein content during the cultivation of *Trametes versicolor* R-105 (A) and *Phanerochaete chrysosporium* BKM-F-1767 (B) in liquid medium supplemented with 1.0% agricultural waste. Values are means± standard deviations.

Uguru *et al.*, 1997) and bacteria (Ben Ali *et al.*, 1999; Hamilton *et al.*, 1999, Mamo and Gessesse, 1999). As mentioned by Hamilton *et al.* (1999), most bacterial amylases are optimally active at slightly acidic to near neutral pH.

Effect of temperature on amylase activity

The temperature profile of the amylase was determined by assaying the amylase activity at different temperatures at pH 5.0. The optimum temperature for the activity of amylase from the two fungi was 60 °C (Fig. 4B). This finding is in agreement with the data previously reported for amylase preparations obtained from other microorganisms such as *Trichoderma harzianum* (de Azevedo *et al.*, 2000), *Thermomyces lanuginosus* (Jensen and Olsen, 1992; Odibo and Ulbrich-Hofmann, 2001) and *Sacharomyces cerevisiae* (de Moraes *et al.*, 1999). The results obtained were lower than those reported for amylase preparations from other microorganisms such as *Aspergillus niger* (Uguru *et al.*, 1997) and *Bacillus* sp.(Ben Ali *et al.*, 1999; Hamilton *et al.*, 1999, Mamo and Gessesse, 1999).

Thermostability of amylase

The thermostability of amylase enzyme was determined by pre-incubation of the diluted enzyme solutions at 60 °C for different periods of time. The treated enzyme solutions were then assayed for the residual activity using standard assay conditions and expressed as percentage of the appropriate zero time (control).

As shown in Fig. 5, no difference was observed in the thermal stability of amylase from the two fungi. The results show that the amylase from these fungi is relatively resistant to thermal inactivation. This finding is in agreement with the data previously reported for amylase preparations from thermophilic microorganisms. At 60 °C, the invistigated enzyme retained about 50% of the original activity after 1 h and about 10% after 5 h. At the same temperature, the amylase from the fungus *Thermomyces lanuginosus* lost all activity after 30 min (Jensen and Olsen, 1992; Mishra and Maheshwari, 1996), whereas the amylase from *Thermomyces lanuginosus* F1 retained 71% activity after 1 h (Odibo and Ulbrich-Hofmann, 2001). At 65 °C, the amylase from *Bacillus* IMD434 retained 50% activity after 18 min (Hamilton *et al.*, 1999). At 80 °C,

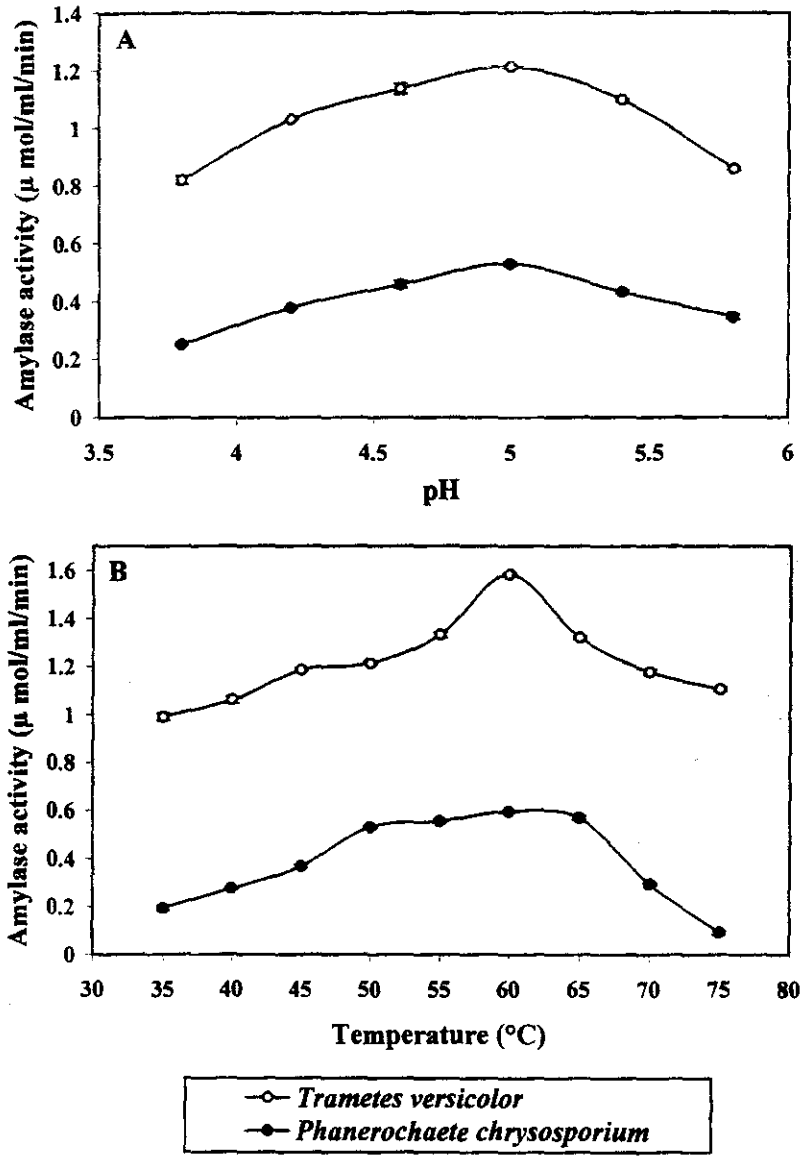

Fig.(4): Effect of pH (A) and temperature (B) on the activity of extracellular amylase of *Trametes versicolor* R-105 (A) and *Phanerochaete chrysosporium* BKM-F-1767 (B) grown in liquid medium supplemented with 1.0% sugarcane bagasse for 15 days. Values are means± standard deviations.

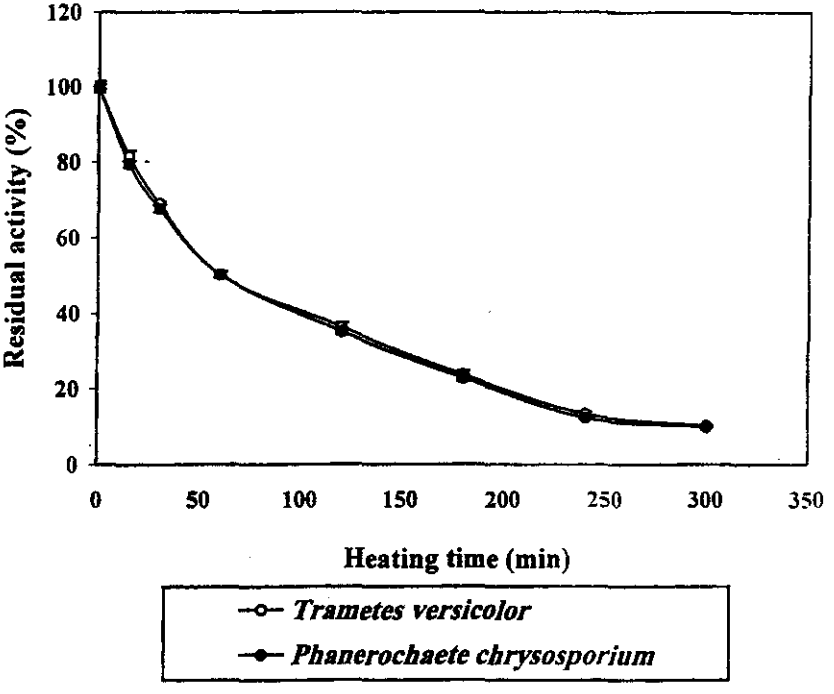

Fig.(5): Thermal stabilty of extracellular amylase of *Trametes versicolor* R-105 and *Phanerochaete chrysosporium* BKM-F-1767 grown in liquid medium supplemented with 1.0% sugarcane bagasse for 15 days. Values are means± standard deviations.

the amylase from *Bacillus* IMD434 retained 50% activity after 5 min (Hamilton *et al.*, 1999) and that from *Aspergillus niger* retained 50% activity after 50 min (Uguru *et al.*, 1997).

Generally, enzyme stability is one of the important factors for its recovery and reuse that contributes toward the economics of enzymatic hydrolysis (Deshpande *et al.*, 1988). Therefore, this enzyme could be classified as relatively thermostable for all practical purposes and the higher thermostability is a highly desirable characteristic for the industrial enzymes. In the bioconversion of lignocellulosic residues it could be desirable to have a thermophilic and thermostable enzymes system. By operating at elevated temperature, we could limit the incidence of contamination while the thermostable enzymes maintained their activity and could be recycled for addition to fresh substrate (Breuil *et al.*, 1986).

The ligninolytic white-rot fungi *Trametes versicolor* R-105 (ATCC 11235) and *Phanerochaete chrysosporium* BKM-F-1767 (ATCC 24725) which have been investigated for their ability to produce the cellulolytic and xylanolytic enzymes (data not shown) proved also to be potential candidates for the production of amylase enzyme involved in the bioconversion of agricultural wastes with yields and properties. These findings make these fungi worthy of further investigation for industrial applications.

REFERENCES

Acebal, C., M.P. Castillon, P. Estrada, I. Mata, E. Costa, J. Aguado and F. Jimenez. 1986. Enhanced cellulase production from *Trichoderma reesei* QM9414 on physically treated wheat straw. Appl. Microbiol. Biotechnol., 24: 218-223.

Ben Ali, M., M. Mezghani and S. Bejar. 1999. A thermostable α-amylase producing malto-hexaose from a new isolated *Bacillus* sp. US100: study of activity and molecular cloning of the corresponding gene. Enzyme Microb. Technol., 24: 584-589.

Blanch, H.W. and C.R.Wilke.1983. Sugars and chemicals from cellulose. Rev. Chem. Eng., 1: 71-119.

Bolton, D.J., C.T. Kelly and W.M. Fogarty. 1997. Purification and characterization of the α-amylase of *Bacillus flavothermus*. Enzyme Microb. Biotechnol., 20: 340-343.

Breuil, C., G. Wojtczak and J.N. Saddler. 1986. Production and localization of cellulases and β-glucosidase from the thermophilic fungus *Thielavia terrestris*. Biotechnol. Lett., 8: 673-676.

Cancell, A.M., A.B. Orth and M.Tien. 1993. Lignin and veratryl alcohol are not inducers of the ligninolytic system of *Phanerochaete chrysosporium*. Appl. Environ. Microbiol., 59: 2909-2913.

Copa-Patiño, J.L., Y.G. Kim and P. Broda. 1993. Production and initial characterisation of the xylan-degrading system of *Phanerochaete chrysosporium*. Appl. Microbiol. Biotechnol., 40: 69-76.

de Azevedo, A.M., J.L. De Marco and C.R. Felix. 2000. Characterization of an amylase produced by a *Trichoderma harzianum* isolate with antagonistic activity against *Crinipellis perniciosa*, the causal agent of witches'broom of cocoa. FEMS Microbiol Lett., 188: 171-175.

de Moraes, L.M.P., S.A. Filho and C.J. Ulhoa. 1999. Purification and some properties of an α-amylase glucoamylase fusion protein from *Sacharomyces cerevisiae*. World J. Microbiol. Biotechnol., 15: 561-564.

Deshpande,V., R. Seeta, C. Mishra and M. Rao. 1988. Stability and recovery of *Penicillium funiculosum* cellulase under use conditions. Appl. Biochem. Biotechnol., 19: 11-19.

Detroy, R.W., L.A. Lindenfelser, S. Sommer and W.L. Orton. 1981. Bioconversion of wheat straw to ethanol; chemical modification, enzymatic hydrolysis and fermentation. Biotechnol. Bioeng., 23: 1527-1535.

Dey, S., T.K. Maiti, N. Saha, R. Banerjee and B.C. Bhattacharyya. 1991. Extracellular protease and amylase activities in ligninase-producing liquid culture of *Phanerochaete chrysosporium*. Process Biochem., 26: 325-329.

Dutton, M.V., C.S. Evans, P.T. Atkey and D.A. Wood. 1993. Oxalate production by basidiomycetes, including the white-rot species *Coriolus versicolor* and *Phanerochaete chrysosporium*. Appl.Microbiol. Biotechnol., 39: 5-10.

Fan, L.T., M.M. Gharpuray and Y.-H. Lee. 1981. Evaluation of pretreatments for enzymatic conversion of agricultural residues. Biotechnol. Bioeng. symp., 11: 29-45.

Fogarty, W.M. and C.T. Kelly. 1990. In: *Microbial Enzymes Biotechnology*. London: Elsevier Science Publishers, pp.71-132.

Hamilton, L.M., C.T. Kelly and W.M. Fogarty. 1999. Purification and properties of the raw starch-digesting α-amylase of *Bacillus* sp.IMD 434. Biotechnol. Lett., 21: 111-115.

Harchand, R.K. and S. Singh. 1997. Extracellular cellulase system of a thermotolerant streptomycete: *Streptomyces albaduncus*. Acta Microbiol. Immunol. Hung., 44: 229-239.

Jensen, B. and J. Olsen. 1992. Physicochemical properties of a purified alpha-amylase from the thermophilic fungus *Thermomyces lanuginosus*. Enzyme Microb. Technol., 14: 112-116.

Keyser, P., T.K. Kirk and J.G. Zeikus. 1978. Ligninolytic enzyme system of *Phanerochaete chrysosporium*: synthesized in absence of lignin in response to nitrogen starvation. J. Bacteriol., 135: 790-797.

Khalil, A.I., A. Krakowiak and S. Russel. 2002. Production of extracellular cellulase and xylanase by the ligninolytic white-rot fungus *Trametes versicolor* grown on agricultural wastes. Annals Agric. Sci., 47: 161-173.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Mamo, G. and A. Gessesse. 1999. Purification and characterization of two raw starch-digesting thermostable α-amylases from a thermophilic *Bacillus*. Enzyme Microb. Technol., 25: 433-438.

Mandels, M. and R. Andreotti. 1978. Problems and challenges in the cellulose to cellulase fermentation. Process Biochem., 13: 6-31.

Miller, G. L. 1959. Use of dinitrosalicylic agent for determination of reducing sugars. Anal. Chem., 31: 426-428.

Mishra, R. and R. Maheshwari. 1996. Amylases of the thermophilic fungus *Thermomyces lanuginosus*: Their purification, properties, action on starch and response to heat. J. Biosci., 21: 653-672.

Müller, H.W. and W. Trösch. 1986. Screening of white-rot fungi for biological pretreatment of wheat straw for biogas production. Appl. Microbiol. Biotechnol., 24: 180-185.

Odibo, F.J.C. and R. Ulbrich-Hofmann. 2001. Thermostable α-amylase and glucomylase from *Thermomyces lanuginosus* F1. Acta Biotechnol., 21: 141-153.

Rogalski, J., A. Hatakka, M. Wojtas-Wasilewska and A. Leonowicz. 1993. Cellulolytic enzymes of the ligninolytic white-rot fungus *Phlebia radiata*. Acta Biotechnol., 13: 41-45.

Shambe, T. and O. Ejembi. 1987. Production of amylase and cellulase: degradation of starch and carboxymetylcellulose by extracellular enzymes from four fungal species. Enzyme Microb. Technol., 9: 308-312.

Tien, M. and T.K. Kirk. 1983. Lignin degrading enzyme from the hymenomycete *Phanerochaete chrysosporium*. Sci., 221: 661-663.

Tuohy, M.G. and M.P. Coughlan. 1992. Production of thermostable xylan-degrading enzymes by *Talaromyces emersonii*. Bioresour. Technol., 39: 131-137.

Ugua, G.C., J.A. Akinyanju and A. Sani. 1997. The use of yam peel for growth of locally isolated *Aspergillus niger* and amylase production. Enzyme Microb. Technol., 21: 48-51.

إنتاج وخواص إنزيم الأميلاز من بعض فطريات العفن الأبيض المنماة على مخلفات زراعية

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أجريت هذه الدراسة بغرض معرفة إمكانية إنتاج إنزيم الأميلاز من فطرى العفن الأبيض (ترامبتس فيرسيكولور R-105 (ATCC 11235 وفانيرو شيتا كريسوسبوريم BKM-F-1767 (ATCC 24725)). تم تنمية هذين الفطرين في بيئة سائلة مضاف إليها ١٪ مخلف زراعي (تبن قمح أو مصاصة قصب السكر) كمصدر للكربون . خلال هذه الدراسة تم معرفة التغيرات في كلا من الرقم الهيدروجيني والبروتين الذائب للمرشحات المزرعية. كما تم دراسة خواص هذا الإنزيم (الرقم الهيدروجيني ودرجة الحرارة المثاليين وكذلك الثبات الحراري). أظهرت النتائج قدرة هذين الفطرين على إنتاج إنزيم الأميلاز باستخدام الأنواع المختلفة من المخلفات الزراعية وأن مصاصة قصب السكر أعطت أفضل النتائج. كما أن الفطر ترامبتس فيرسيكولور كان الأفضل في إنتاج هذا الإنزيم. أشارت النتائج المتحصل عليها إلى أن الرقم الهيدروجيني وكذلك البروتين الذائب للمرشحات المز رعية حدث لهما تغير واضح أثناء إجراء التجربة وأن هناك علاقة موجبة بين زيادة البروتين الذائب وكذلك نشاط الإنزيم. دلت نتائج دراسة الخواص الإنزيمية إلى أن أعلي نشاط لإنزيم الأميلاز كان عند رقم هيدروجيني (٥,٠) ودرجة حرارة (٦٠° م). كذلك بعد تحضين الإنزيم على درجة حرارة (٦٠°م) لمدة ٥ ساعات إحتفظ الإنزيم بحوالي ١٠٪ من نشاطه الأصلي مما يدل على أن هذا الإنزيم له ثبات حراري. من النتائج المتحصل عليها يمكن استنتاج أن هذه الفطريات المحللة لمادة اللجنين لها القدرة أيضا على إنتاج إنزيم الأميلاز والذي يلعب دورا هاما في عملية التحول الحيوي للمخلفات الزراعية المسببة للتلوث البيئي وأن هذا الإنزيم يتحمل درجة حرارة مرتفعة مما يجعله مفيدا في التطبيقات الصناعية.