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Production and partial characterization of glucose oxidase and catalase from xerophytic strain of *Aspergillus niger*

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Six *Aspergillus niger* strains isolated from semi-desert of Coahuila State (Mexico) were screened for an extracellular catalase (CAT) and glucose oxidase (GOX) production using solid-state and submerged fermentations. The best enzymes producer, *A. niger* ASPN 1.1, was selected for enzymes production under submerged fermentation condition. The higher activity was observed at 24 and 48 h of submerged fermentation, respectively. The enzymes were partially purified to a yield of 98 and 48%, and fold 9.8 and 5, corresponding to specific activity of 589.60 and 0.60 U/mg, respectively for CAT and GOX, using ultrafiltration with 100 kDa filter. The enzymes showed high affinity for H_2O_2 and D-glucose with a K_m value of 80 and 4.24 mM, respectively. Both enzymes exhibited a greater catalytic activity at pH 6. Optimum temperature for glucose oxidation was 50°C, while for peroxide decomposition, it was 45°C. The enzymes showed a high thermostability at 50°C with a half-life time of 99 and 86 min, respectively, for GOX and CAT. These characteristics suggest the use of xerophytic *A. niger* strain as a potential producer for both enzymes, which have analytical and industrial application.

Key words: Catalase, glucose oxidase, xerophytic, *Aspergillus niger*.

INTRODUCTION

Xerophytic fungi grow in absence of free water due to use of water vapor from the air for growth. Fungi from semi-desert are characterized by this aptitude in addition to their ability to resist temperature changes of arid environment. The arid and semiarid regions of the world are recognized as one of the least explored niches occupied by fungi. It is well-known that xerophytic fungal strains are characterized by their adaptability to extreme conditions such as long drought periods, high mid-day temperatures, low night temperature, high osmotic pressures and changes in humidity level. Moreover, their ability to produce variety of enzymes with extraordinary

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Table 1. Morphological characteristics of selected fungi and their response to GOX and CAT qualitative tests.

Strain	Colonia	Vesicles	Hyphae	Phialides	Conidia	GOX test	CAT test
ASPN 1.1	Black and granular texture	Round	Septate hyaline	Primary densely covering vesicle	Dark brown, globular, smooth, radially	+	+
ASPN 3	Black and cottony texture	Round	Septate hyaline	Primary covering the entire vesicle and racemes	Round and radiated, hyaline	+	+
ASPN 4	Black and cottony texture	Round	No septate hyaline	Covering the entire vesicle	Globular, dark brown	+	+
ASPN 5	Brown and cottony texture	Round	No septate hyaline	Primary and secondary covering the entire vesicle	Globular, radial-shaped, dark brown	+	+
ASPN 7´	Dark brown, cottony texture, little sporulation	Round	Septate hyaline	Primary covering the entire vesicle	Globular, dark brown, with conidia exiting the conidiophore	+	+
ASPN 12	Black and granular texture	Round	No septate hyaline	Primary	Globular, dark brown	+	+

characteristics has been reported (Cruz-Hernández et al., 2005; Cruz-Hernández et al., 2006; Flores-Gallegos et al., 2012). In the present study, the strains of *Aspergillus niger* isolated from the Mexican semi-desert were screened for their ability to excrete extracellular enzymes catalase (CAT, H_2O_2: H_2O_2 oxidoreductase, EC 1.11.1.6) and glucose oxidase (GOX, β-D-glucose: oxygen-1 oxidoreductase, EC 1.1.3.4).

Both enzymes have various applications in industry, as well as in analytical chemistry (Tzonka et al., 2006; Wang et al., 2008; Singh and Verma, 2013). For example, they are applied as additives for food preservation, because they promote the elimination of residual oxygen after packaging. The fungus *A. niger* is a widely used source for obtaining GOX and CAT, since it has many biotechnological advantages, it is not pathogenic and can grow with a wide variety of nutrients (Schuster et al., 2002; Cruz-Hernández et al., 2006). This is one of the reasons to study this type of fungi. Furthermore, to our knowledge, the production of these enzymes by xerophytic fungi was not reported previously.

To characterize studied fungi, we applied traditional methods involving morphological and microscopical characterization, as well as rDNA identification for the strain with highest activities (Arnaud et al., 2012). The operational properties of enzymes were characterized using partially purified extracellular extract. Ultrafiltration with a semi-permeable membrane with pore sizes enough to permit movement of molecules smaller than 100 kDa and to prevent the passage of proteins with higher molecular weight was applied to this. This method used centrifugation to induce the movement of water and small molecules through the membrane under centrifugal force. This method is fast, allows separating one part of proteins, estimate molecular weight of enzymes, and results in concentration of the protein sample (Tauro et al., 2012).

The goals of the present study are: 1) to screen the collection of xerophytic *A. niger* strains for an extracellular catalase (CAT) and glucose oxidase (GOX) production; 2) partial purification of enzymes from one strain exhibiting greater enzymatic activities, and 3) to characterize some operational properties of both enzymes.

MATERIALS AND METHODS

Strains morphological characterization

Fungal strains used for the present study were obtained from Fungi Collection of Food Research Department, School of Chemistry, Autonomous University of Coahuila (Saltillo, Mexico). These cultures were originally isolated from soil or stems of semi-desert plants from semi-arid zone of Coahuila State (Mexico) and stored on potato dextrose agar (PDA) slants. The fungal strains were further transferred/sub-cultured by growing on freshly prepared PDA plates, as well as under slide culture conditions (Sarma et al., 2002). Morphological and microscopic characteristics described in Table 1 were noted to verify *A. niger* properties.

Screening for glucose oxidase and catalase production in solid-state fermentation

To select the fungi with glucose oxidase production, the fungi were grown on PDA medium and applied to solid diagnostic test, which allowed estimating the glucose oxidase activity by the presence of the brown zones of *o*-dianisidine (100 g/L) oxidation developed around the colonies in the presence of horseradish peroxidase type VI (15 U) (Fiedurek and Gromada, 2000).

Catalase activity was screened by applying a drop of 30% (v/v) H_2O_2 with a syringe to the edge of each colony. Catalase producing strains were selected due to appearance of oxygen bubbles (Kim et al., 1994).

Screening for catalase and glucose oxidase production in submerged fermentation

Promising *A. niger* strains were grown in submerged mode at 30°C and 320 rpm in the liquid medium (Fiedurek and Gromada, 2000), which contained (g/L): glucose, 80; peptone (type I, Sigma), 3; $CaCO_3$, 35; $(NH_4)_2HPO_4$, 0.388; K_2HPO_4, 0.188; and $MgSO_4 \cdot 7H_2O$, 0.156. This medium was inoculated to a density of 10^5 spores/mL. The enzymatic activities and protein concentration were monitored for 60 h after liquid aliquot filtration. Fungal mycelium grown for 60 h was collected by filtration, washed, dried at 90°C to a constant weight. The culture filtrate was used for the respective assays of enzymes partial purification and characterization.

Catalase was assayed by spectrophotometric measurement of the decomposition of H_2O_2, (Zeng et al., 2010). Sample (0.5 mL of culture medium) was added to 0.95 mL of H_2O_2 (0.05 M in phosphate buffer 0.02 M, pH 7). After stirring, the decrease in absorbance at 240 nm was measured for 3 min (Cary-50 UV/VIS Spectrometer). The initial rate of decomposition was determined after fitting the kinetic curves. The absorption coefficient at 240 nm for H_2O_2 was taken to be 40 M^{-1} cm^{-1}. One unit of catalase activity was defined as the amount of enzyme required to decompose 1.0 µmol of H_2O_2/min at 25°C.

Glucose oxidase activity was determined in aliquots of culture liquids by using a coupled *o*-dianisidine-peroxidase reaction (Singh and Verma, 2013). One unit (U) of enzyme activity was defined as the amount that produces 1 µmol H_2O_2/min at 30°C. The reaction mixture contained 0.6 mL of 0.01 M glucose, 0.33 mL of *o*-dianisidine (0.16 g/L), 0.12 mL of horseradish peroxidase type VI at 20 µg /mL (Sigma-Aldrich). All reactants were previously dissolved in 0.02 M phosphate buffer at pH 6. The reaction was initiated by addition of 0.12 mL of the extract containing the enzyme. The absorbance increase was detected continuously in Cintra-20 spectrophotometer at 436 nm for 105 s. The activity was calculated from the linear portion of the curve using a molar extinction coefficient of 8300 $M^{-1}min^{-1}$. Protein concentrations were estimated by Bradford method using bovine serum albumin as the standard.

Confirmation of fungal strain by rDNA identification

Fungal DNA, which was selected due to greater enzymatic activities, was isolated using the protocol previously reported by Barth and Gaillardin (1996). In particular, chopping of fungal mat (0.4 g on dry mass basis) was done using pestle and mortar with liquid nitrogen. Chopped material was transferred to 50 mL centrifuge tube containing 5 mL of extraction buffer TES (100 mM Tris-HCl of pH 8.0, 20 mM EDTA, and 0.8 % SDS) along with 2.5 mL of 5 M sodium acetate of pH 5.2 and 5 M NaCl. Centrifuge tube was placed at -20°C for 20 min. After centrifugation, done at 14800 rpm for 20 min at 4°C, supernatant was transferred to a new tube and an equal volume of isopropanol was added to the tube. Pellet DNA was obtained after 5 min centrifugation at the same conditions as earlier. Pellet of DNA was washed thrice using ethanol. After washing, the pellet was dissolved in 0.05 mL of 0.1X TE buffer (1 mM Tris-HCl, 0.1 mM EDTA, pH 8). Electrophoresis was carried out at 100 V in 1% agarose gel with 1xTAE buffer (40 mM Tris, 20 mM acetic acid and 1 mM EDTA, pH 8) and ethidium bromide. The marker Hyperladder I (0.002 mL of the marker and 0.003 mL of TE buffer, pH 8.0) was used (Hovda et al., 2007; Rojas et al., 2008; Moreno-Dávila et al., 2010). The concentration of extracted DNA was checked on NanoDrop spectrophotometer using 0.001 mL of sample.

PCR conditions and primers for amplification of 18S rDNA were used as reported by Melchers et al. (1994). The primers were nu-SSU-0817-5´ (foward) (5'- TTAGCATGGAATAATRRAATAGGA-3') and nu-SSU-1193-3´ (reverse) (5´- TCTGGACCTGGTGAGTTTCC -3´). The PCR mixture consisted of 200 mM Tris-HCl pH 8.4, 2.5 mM $MgCl_2$, 0.25 mM dNTPs, 0.4 µM of each primer, 0.2 µg of DNA and 1.0 U of Taq polymerase. The PCR system (Biometra®) was used for amplification. Initial denaturation, annealing and extension steps were performed at 95, 57 and 72°C, respectively. Analysis of the PCR products was performed by electrophoresis on 1.8% agarose gels using standard conditions and Hyperladder V marker.

The 18S rDNA product was extracted from agarose gel with the help of gel extraction kit (Fermentas). The PCR product was sent to Macrogen (USA) for sequencing. The alignment of the sequences was performed using the software BioEdit and taxonomic classification, as well as determination of the nearest neighbors by the NCBI database: *microbes*. Alignments (BLAST) were performed with each of the sequences obtained from the amplified 422 bp using the database "fungi-genomes" NCBI.

Partial enzymes purification

The enzymatic extracts obtained by submerged fermentation were filtered through Whatman No. 41 filter under vacuum. Then they were concentrated on Amicon Ultra-15 units (Millipore) containing the membrane for separation of 100 kDa proteins. The procedure was performed as follows: 15 mL of crude extract were placed in Amicon tube, which was centrifuged at 10,000 rpm, 4°C for 15 min. The extract obtained on the filter was separated to be applied in the subsequent assays. The protein concentration was detected by the Bradford method. The GOX and CAT activities were evaluated in all recovered samples as described above.

Partial enzymes characterization

Effect of pH on glucose oxidase and catalase activities was determined by assaying the enzymes as mentioned before with the difference that the activity was determined at different pH ranging from 1-8 using various buffer solutions as described by Tzonka et al. (2006). Temperatures ranging from 35-65°C, activation energy was determined from the Arrhenius plot. Thermal stability was evaluated applying activity assays, the enzymes pre-incubated at 50°C before activity measurements. To determine the kinetic parameters (V_{max} and K_m), the enzymatic reactions were carried out using different substrate (peroxide or glucose, respectively for catalase and glucose oxidase) at pH 7 and 6, respectively. The data was analyzed according to Lineweaver-Burk plot.

RESULTS

Different fungal strains were tested to select the fungi with characteristics of *A. niger*, as well as GOX and CAT production. The 6 strains of fungi that met the morphological characteristics of *A. niger* (Table 1) were selected due to the positive response on GOX and CAT qualitative tests.

Considering the evidence of GOX and CAT activity, submerged fermentation was carried out using selected strains. Kinetic data corresponding to the activities of both extracellular enzymes detected during this assay are shown in Figures 1 and 2. Both activities were detected from 12 h of fermentation, and were characterized by the presence of maximum values at the time different for each strain (Figures 1, 2 and Table 2).

The relation between catalase and glucose oxidase is significant for fungi due to hydrogen peroxide production

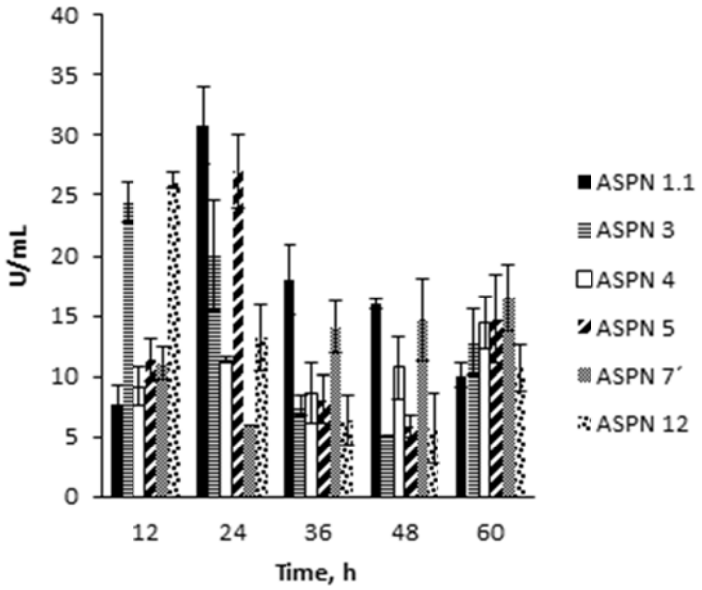

Figure 1. Glucose oxidase activity expressed by *Aspergillus niger* strains at different times of submerged fermentation.

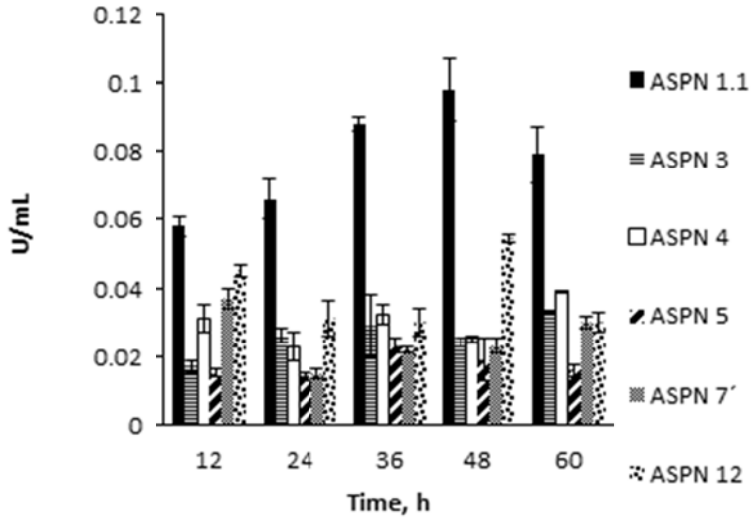

Figure 2. Catalase activity expressed by *Aspergillus niger* strains at different times of submerged fermentation.

Table 2. Comparison of enzyme extracts at the point of higher enzyme activity.

Strain	Time (h)	GOX volumetric activity (U/mL)	GOX specific activity (U/mg)	Time (h)	CAT volumetric activity (U/mL)	CAT specific activity (U/mg)
ASPN 1.1	48	0.10	0.8	24	30.83	560.5
ASPN 3	60	0.03	0.3	24	24.42	228.3
ASPN 4	60	0.04	0.6	60	14.46	241.0
ASPN 5	36	0.02	0.4	24	27.07	466.7
ASPN 7´	12	0.04	0.2	60	16.56	215.1
ASPN 12	48	0.05	0.4	60	26.24	430.2

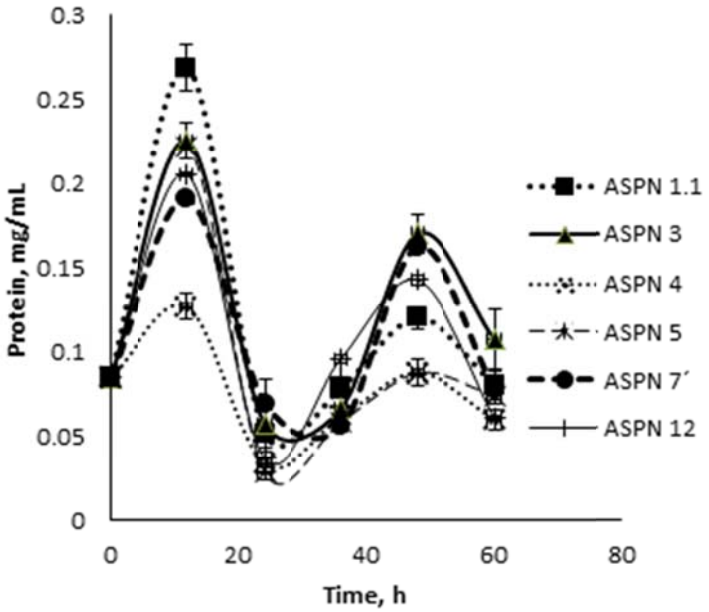

Figure 3. Protein concentration detected in culture medium during submerged fermentation of *A. niger* strains.

by GOX activity, the level of which is controlled by higher CAT activity (Singh and Verma, 2013). The CAT activity is considerably higher than GOX activity, even higher than that reported in some studies (Fiedurek and Gromada, 2000). However, in the case of three of six fungi (*A. niger* ASPN 1.1, ASPN 3 and ASPN 5), the maximum values of enzymatic activities were detected at different times: higher catalase activity was detected earlier than glucose oxidase (Figures 1, 2 and Table 2). Commonly, the maximum activities of both enzymes are detected at the same time as in the case of *A. niger* ASPN 4, or CAT appears later than GOX (Fiedurek and Gromada, 2000) as in the case of ASPN 7´and 12. This may be attributed to specific behavior of some xerophytic fungus *A. niger*, which can differ from common fungi in profile of enzyme excretion, as well as enzyme production.

The greatest GOX, as well as CAT activity was quantified in the case of *A. niger* ASPN1.1. In this case, the GOX maximum value of 0.1 U/mL was observed at 48 h, while CAT higher activity of 31 U/mL at 24 h of fermentation was observed.

For all studied strains, the change of proteins concentration was characterized by kinetic curves with two maximum values at 12 and 48 h. At 12 h, the higher concentration was detected for *A. niger* ASPN 1.1 strain, while at 48, for ASPN 5 and ASPN 7´strains (Figure 3). Using values of detected protein concentration, specific activity was calculated (Table 2). The comparison of characteristics of enzymes contained extracts obtained at the time corresponding to their maximum activity is shown in Table 2. The greater specific activities of GOX and CAT also were observed for strain ASPN 1.1.

Although the fermentation was performed using the same concentration of inoculum, quantity values of biomass recovered after 60 h fermentation were different. The strain ASPN 1.1 showed a similar level to the biomass quantized for strain ASPN 12, providing both higher detected values (Figure 4). Taking into account the higher values of CAT and GOX activities, the strain *A. niger* ASPN 1.1 was selected for subsequent assay, previously performing molecular confirmation of fungal species.

The identification of presumptive *A. niger* for strain ASPN 1.1 was performed by PCR using the primers (forward) (5'-TTAGCATGGAATAATRRAATAGGA-3') and (reverse) (5´- TCTGGACCTGGTGAGTTTCC-3´). The PCR products were visualized by electrophoresis and a 422 pb fragment was obtained (Figure 5A). The obtained sequence was compared to those reported at "Basic Local Alignment Search Tool" (BLAST, http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi/) (National Center for the Biotechnology Information, NCBI, USA) (Figure 5B). This analysis shows that the resulting fungus match the nucleotide sequence of *A. niger* (CBS 513.88 superconting An03, 18S ribosomal RNA) with an 99-100% identity.

Partial purification of crude extract was performed by means of ultrafiltration with 100 kDa filter to separate 99% of protein present in the sample. The enzymes were partially purified to a yield of 98 and 48%, and fold 9.8 and 5 corresponding to specific activity 589.60 and 0.60 U/mg, respectively for CAT and GOX (Table 3).

Glucose oxidase from *A. niger* was active within the pH

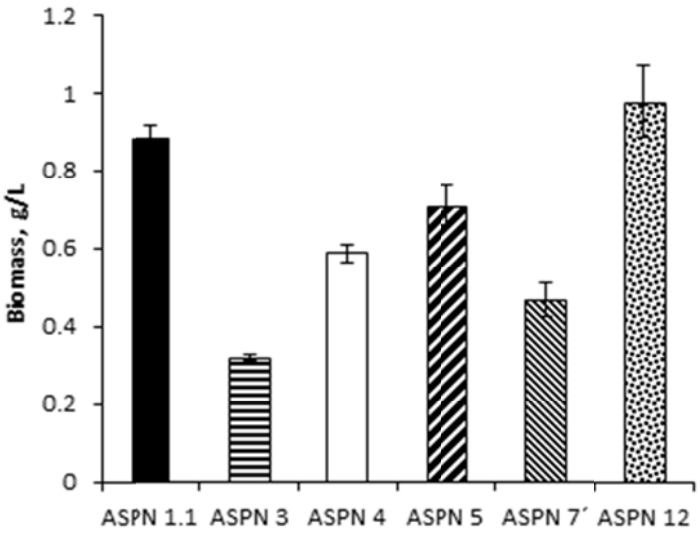

Figure 4. Biomass dry weight of *A. niger* strains after 60 h of submerged fermentation.

range of 1-7 and catalase of 1-8, while maximum activity was observed at pH 6.0 (Figure 6). The optimum temperature for glucose oxidase and catalase catalyzed reactions was found to be 50 and 45°C (Figure 7), and activation energies (E_a), calculated from initial part of obtained curves, were 32.33 and 73.5 kJ/mol, respectively.

Partially purified enzymes samples having a protein content of 0.48 mg/mL were used for the kinetic characterization (Figure 8). The K_m and V_{max} values obtained from Lineweaver-Burk plot for glucose oxidase were 4.24 mM and 3.2 M/min, and for catalase, 80 mM and 0.15 mM/min (Table 4).

Thermostability is the ability of enzyme to resist thermal treatment in substrate absence. GOX and CAT from xerophytic *A. niger* showed appreciable stability at 50°C (Figure 9). The linearization of kinetic curves in semi-logarithmic coordinates was applied to quantify inactivation constant and subsequently a half-life time, which were estimated as 0.007 and 0.008 1/min, as well as 99 and 87 min, respectively for GOX and CAT. Table 4 shows the data which compare between the enzyme from xerophytic *A. niger* and enzymes from some other sources.

DISCUSSION

Morphological characterization of studied fungi allowed the selection of *A. niger* strains. The conidia heads of all selected strains are characteristic to distinguish *A. niger* strains (Table 1). These heads are formed by conidiophores, vesicle, and a series of primary sterigma, followed by a second series of secondary sterigma of which the conidia sprout. The major morphological distinction of *A. niger* from other species of *Aspergillus* is the presence of carbon black or dark brown spores on biseriate phalides, which are arranged in a globule head radiating from a vesicle conidiophore.

A. niger is a filamentous fungus belonging to phylum Ascomycota. This fungus is commonly found in mesophilic environments such as soil, plants and enclosed air environments, but it is also capable of surviving in various environments: high or low temperatures, as well as humidity, etc. In the present work, xerophytic *A. niger* strains were studied.

A. niger fermentation is commonly accepted as safe by the United States Food and Drug Administration under the Federal Food, Drug and Cosmetic Act (Schuster et al., 2002) so the enzymes produced by *A. niger* can be used in food and medical industries without any objection. GOX and CAT are important enzymes due to a vast number of applications in various fields. The most important application of glucose oxidase is in diagnostics, as a part of colorimetric diagnostic kits for the determination of glucose in blood, serum or plasma. CAT is used in textile industry and environmental monitoring (Kim et al., 1994; Chun et al., 2008; Wang et al., 2008).

Submerged fermentation of selected fungi (Figures 1 and 2) led to obtain high activity levels for both extracellular enzymes. In the case of the *A. niger* strains ASPN 1.1, ASPN 3 and ASPN 5, the maximum activity of CAT was detected before the maximum activity of GOX, while for strains ASPN7´and ASPN12, was upside down, and in the case of *A. niger* ASPN 4, at the same time (Table 2). Production of more than one enzyme in one step suggestively increases the effectiveness and advances the process economy.

There are many reports on GOX and CAT production by fungal and bacterial strains (Federici et al., 1996;

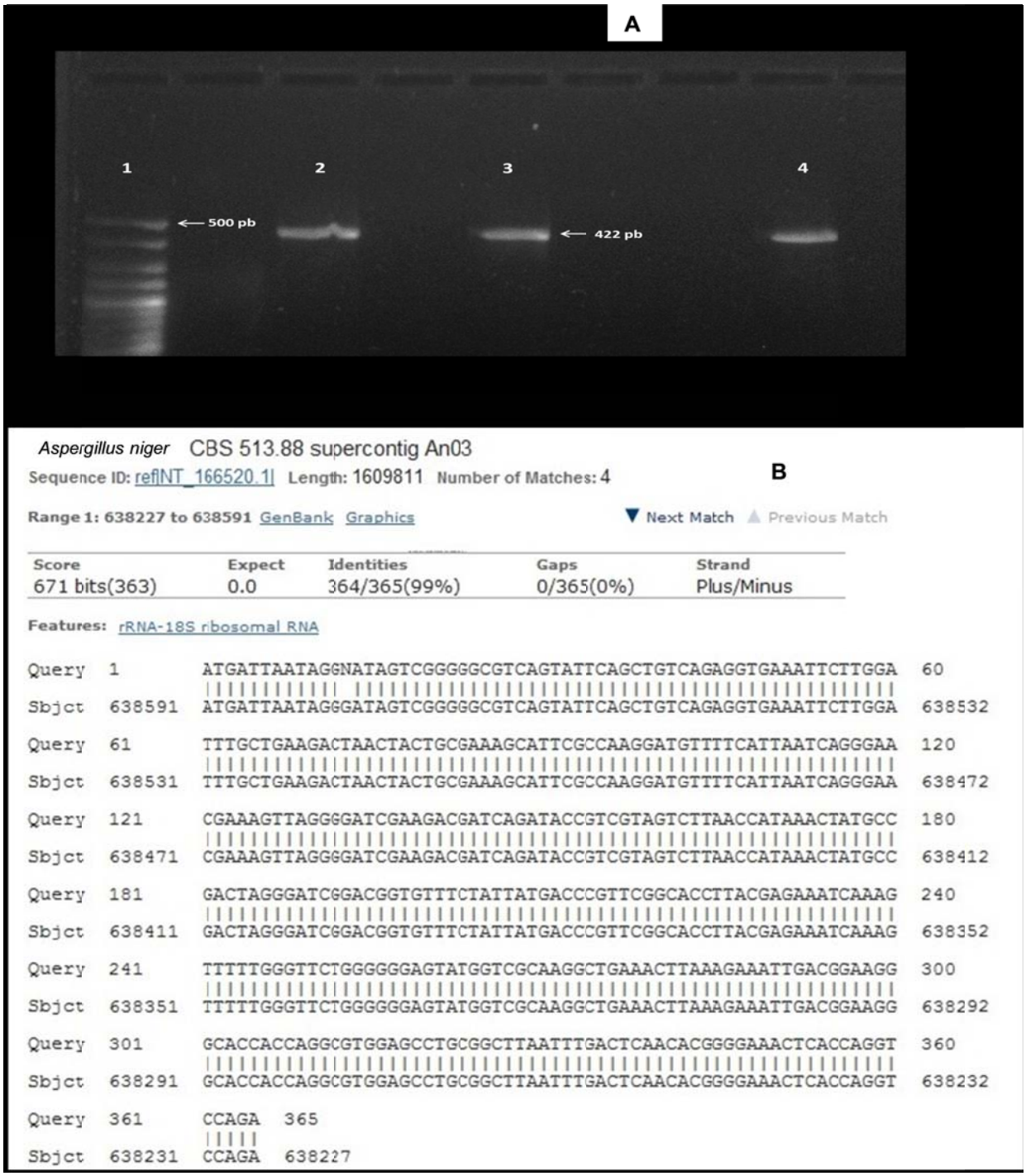

Figure 5. A) Agarose gel electrophoresis of PCR products amplified from DNA of *Aspergillus niger* ASPN 1.1: 1, molecular weight marker Hyperlader V; 2, 3, 4– PCR products from *A. niger* strain (repetitions). B) Sequence alignment of *A. niger* ASPN 1.1 (Query) with partial 18S rDNA sequence of *Aspergillus niger* (CBS 513.88 superconting An03) (Sbjct).

Fiedurek and Gromada, 2000; Wang et al., 2008), but few papers reported the simultaneous production of both enzymes, for example by submerged fermentation of *Penicillium variabile* P16 (Petruccioli et al., 1995), and with *A. niger* (Fiedurek and Gromada, 2000). The nature of the mechanisms responsible for the induction of enzymes synthesis has only been investigated in a few cases. For example, Fiedurek and Gromada (2000) reported that molecular oxygen increased expression of GOX and CAT probably at the transcriptional level, and

Table 3. Characteristics of partial GOX and CAT purification by ultrafiltration on 100 kDa filter (AMICON) assisted by centrifugation.

Fraction	GOX					CAT			
	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Fold	Total activity (U)	Specific activity (U/mg)	Yield (%)	Fold
Crude extract	0.6	4.8	0.12	100	1	288.8	60.16	100	1
Fraction recuperated on 100 kDa filter	0.29	0.48	0.60	48	5	283.0	589.60	98	9.8

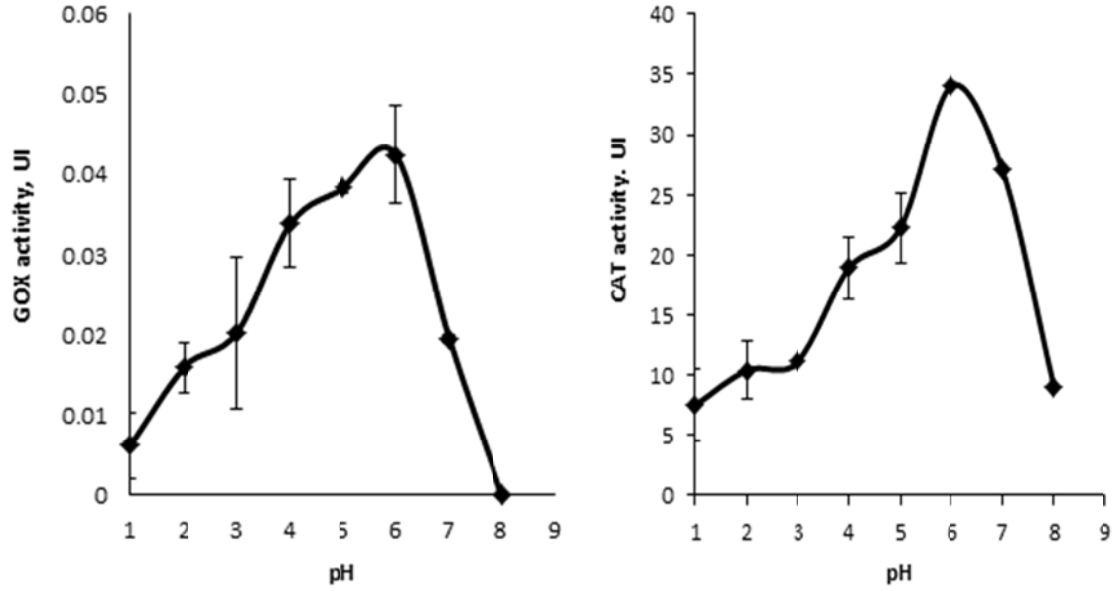

Figure 6. Effect of pH on activity of: left, - glucose oxidase; right, - catalase from xerophytic *Aspergillus niger.*

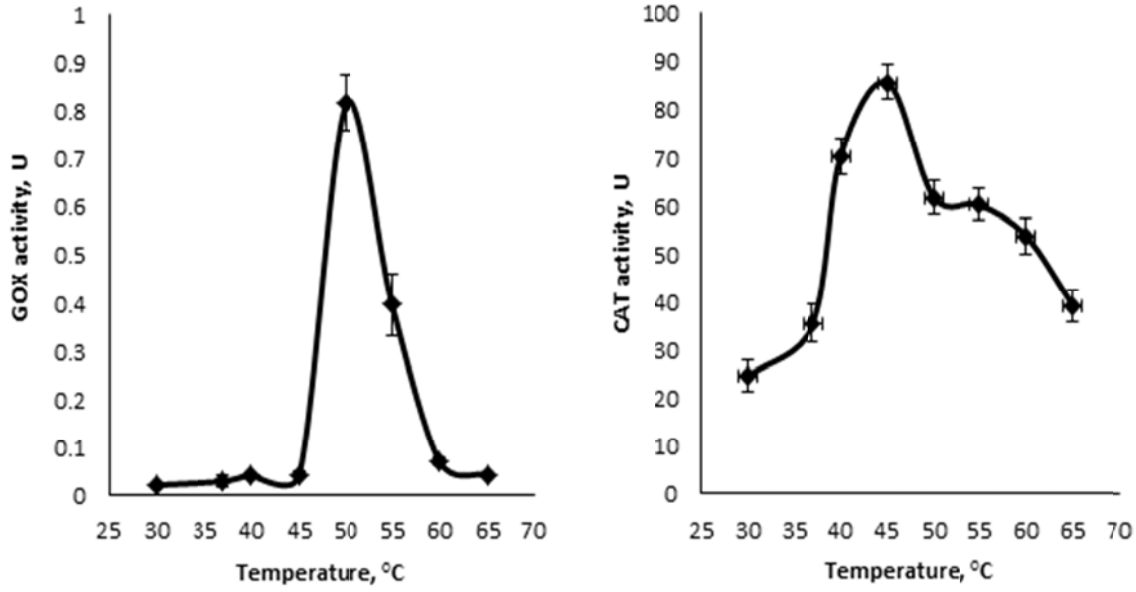

Figure 7. Effect of temperature on activity of: left, - glucose oxidase; right, - catalase from xerophytic *Aspergillus niger.*

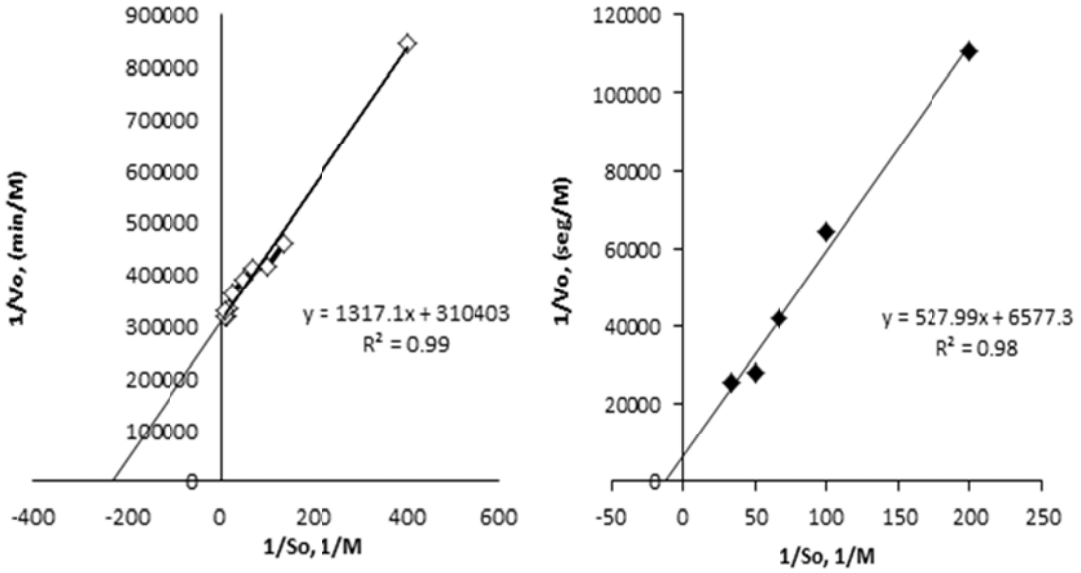

Figure 8. Linearization of Michaelis-Menten curve in Lineweaver- Burk coordinates: left, - for glucose oxidase; right, - for catalase.

Table 4. Comparison of various characteristics of partially purified glucose oxidase and catalase from xerophytic *A. niger* with other sources (the name of microorganism is noted when it is different from *Aspergillus* sp.)

Characteristics	GOX	GOX from other sources	CAT	CAT from other sources
Specific activity	0.6 U/mg	Obtained with ammonium sulfate at 0.75 U/mg (Singh and Verma, 2013)	589.6 U/mg	443.7 U/mg (Chandrashekar, 2011)
K_m	4.24 mM	10.3 mM (Garjonyte and Malinauskas, 2000) 25 mM (Bhatti et al., 2006) 2.56 mM (Zia et al., 2007) 5.7 mM (Zia et al., 2007) 10.5 mM H.N. (Bhatti and Saleem, 2009) 7.1 mM (Singh and Verma, 2013)	80.00 mM	599 mM (Switala and Leuwen, 2002) 127 mM (from *Proteus mirabilis*) (Lorentzen et al., 2006) 103.6 mM (from *Vibrio salmonicida*) (Lorentzen et al., 2006) 41.5 mM (from *Bacillus* sp. N2) (Wang et al., 2008) 29.7 mM (from *Serratia marcescens*) (Zeng et al., 2010) 10.5 mM (from *L. arborícola*) (Kapoor et al., 2013)
Optimum pH	6	5.5 (Bhatti et al., 2006; Zia et al., 2007) 5.4 (Bhatti and Saleem, 2009) 5.5-6 (Singh and Verma, 2013)	6	6 (Chandrashekar, 2011) 6.5 (Bayramoglu et al., 2011) 7-8 (from *L. arborícola*) (Kapoor et al., 2013)

that the metabolism of *A. niger* is changed from glycolysis to the pentose phosphate pathway after the addition of calcium carbonate. The tendency of higher levels of catalase activity in comparison with glucose oxidase, observed in the present study, was also reported previously (Fiedurek and Gromada, 2000).

The levels of activities detected in submerged fermentation were similar to those reported by Fiedurek and Gromada (2000) in the case of GOX, and considerably higher in the case of CAT. Moreover, the biomass generation (Figure 4) was significantly lower than in the case of *A. niger* mutants reported in literature

Table 4. Contd

Optimum temperature	50°C	40°C (Bhatti et al., 2006; Zia et al., 2007) 45°C (Bhatti and Saleem, 2009) 25-30°C (Singh and Verma, 2013)	45°C	20-50°C (from *Proteus mirabilis*) (Lorentzen et al., 2006) 30°C (from *Deinococcus radiodurans*) (Kobayashi et al., 2006) 0-10°C (from *Vibrio salmonicida*) (Lorentzen et al., 2006) 35°C (Senay et al., 2007) 25°C (from *Bacillus* sp.) (Wang et al., 2008) 20°C (from *Serratia marcescens*) (Zeng et al., 2010) 45°C (Bayramoglu et al., 2011) 45°C (Chandrashekar, 2011) 40-60°C (from *L. arborícola*) (Kapoor et al., 2013)
E_a	32.33 kJ/mol	15.46 kJ/mol (Zia et al., 2007) 44 kJ/mol (Ramos et al., 2011)	73.5 kJ/mol	11.28 kJ/mol (from *Scytalidium thermophilum*) (Kocabas et al., 2008)
k_{in} at 50°C **$T_{1/2}$**	0.007 min^{-1} 99 min	30 min (Bhatti et al., 2006) 0.012 min^{-1} and 58 min at 52°C (Zia et al., 2007) 0.015 min^{-1} and 46.2 min (Bhatti and Saleem, 2009) 60 min at 50°C (Singh and Verma, 2013)	0.008 min^{-1} 87 min	After 45 min at 60°C retained 20% (from *Klebsiella pneumonia*) (Goldberg and Hochman, 1989) After 5 min at 60°C had 0% of activity (from *Streptomyces coelicolor*) (Kim et al., 1994) After 15 min at 60°C had 0% of activity (from *Vibrio rumoiensis* S-1T) (Yumoto et al., 2000) After 5 min at 60°C retained 50% of activity (from *Vibrio salmonicida* LFI1238) (Lorentzen et al. 2006) After 50 min at 60°C retained 50% (from *Proteus mirabilis*) (Lorentzen et al., 2006) After 30 min at 60°C retained 30% (from *Deinococcus radiodurans*) (Kobayashi et al., 2006) After 15 min at 60°C retained 12% (from *Bacillus* sp.) (Wang et al., 2008)

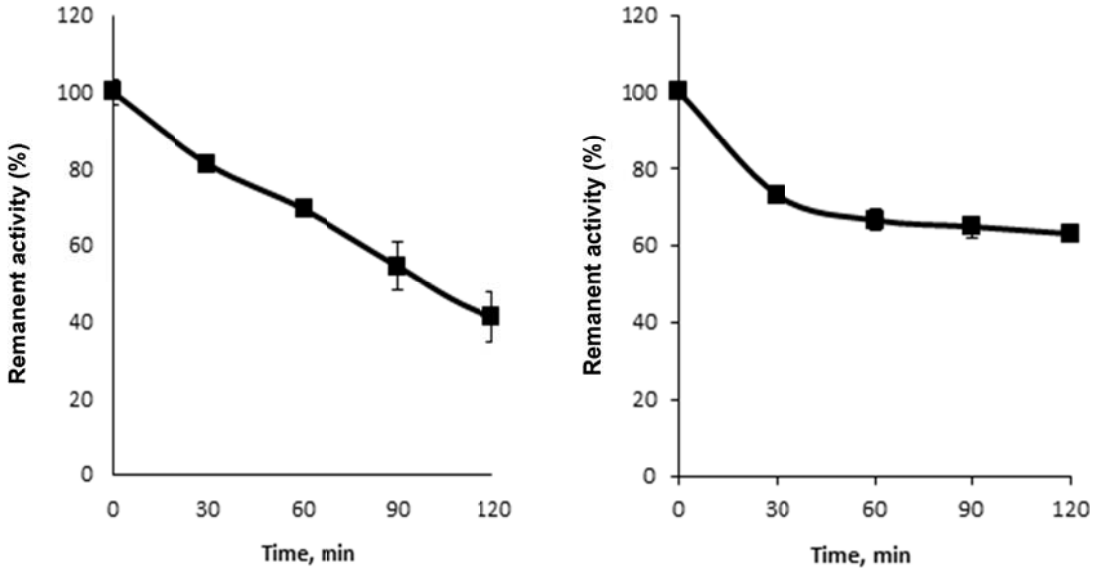

Figure 9. Kinetics of thermal denaturation of: left, - glucose oxidase; right, - catalase from xerophytic *Aspergillus niger* during enzyme pre-incubation at 50°C.

(Fiedurek and Gromada, 2000). However, these authors studied intracellular enzymes (Fiedurek and Gromada, 1997), while in the present study, the xerophytic *A. niger* strains, which produced extracellular GOX and CAT (Table 2), were investigated. The literature data (Chaouche et al., 2005) confirmed that in different fungal cultures, the excretion of catalase into the submerged culture began around 20 h after inoculation and increased as the time progressed, moreover the excretion was preceded by the intracellular catalase activity. GOX production from *A. niger*, for which excretion kinetics have been reported (Pluschkell et al., 1996), showed the presence of a signal peptide confirming that the glucose oxidase (GOD) is actively secreted in the culture medium. Chaouche et al. (2005) suggested that the intra- and extracellular catalase production was related neither to the fungal biomass nor to the size of pellet. However, it was demonstrated that this production may be directly related to the external layer of the pellet and precisely to the morphology of the hyphae in this region, and that secretion of proteins is primarily associated with the apical and subapical regions, called active region (Wongwicharn et al., 1999; Ramos et al., 2011).

As was mentioned above, the strain ASPN 1.1 was selected due to its greater activities levels in comparison with other studied strains. As compared to results obtained in the present study (Figures 1 to 4); in various previous reports, the *A. niger* strains produced extracellular GOX and CAT (Ojeda et al., 2011; Zoghbia et al., 2008) with lower activity, higher protein concentration and higher weight of generated biomass.

The *r*DNA identification (Figure 5) confirmed that the selected strain is *A. niger*. Genomic DNA sequence of *A. niger* strain CBS 513.88, its annotation and an initial gene expression study as well as genetic maps are described by Pel et al. (2007) and Arnaud et al. (2012).

The enzymes partial purification (Table 3) demonstrated that the molecular weight of GOX and CAT is higher than 100 kDa, because greatest part of both enzymes was concentrated in the filter with pores allowing separation of proteins with lower molecular weight. Various literature data report that molecular weight of GOX produced by fungi is around 160 kDa, while for CAT, is 210-280 kDa (Kirman and Gaetani, 1984; Singh and Verma, 2013).

The optimum pH of GOX (Figure 6) is slightly higher than that reported for other glucose oxidases from *A. niger*, while for CAT, is similar or slightly lower than the one reported for enzymes from different microbiological sources (Table 4). The effect of pH on enzyme activity is related to the ionization of essential active site amino acid residues, which participate in substrate binding and catalysis. Our results is consistent with those reported by Weibel and Bright (1971), who defined that GOX is working in the pH range of 4-7, and by Chandrashekar (2011), who demonstrated that pH optimum of catalase form *Aspergillus* sp. is equal to 6.

The plot (Figure 7) describes the effect of temperature on CAT and GOX activities; it is obvious that both enzymes had a single conformation up to transition temperature. The optimum temperatures are superior to the enzymes from various other sources (Table 4). This becomes more evident when comparing with bacterial catalase, as well as with various fungal GOX (Table 4). However, the E_a values are greater than that reported for enzymes from some sources that may be related to difference of enzymes structures.

Regarding properties of biotechnological relevance (Figure 8), the GOX of *A. niger* ASPN 1.1 exhibited a high affinity for D-glucose as it has low *K*m value for the substrate as compared to a high *K*m value of enzymes isolated from the *A. niger* reported earlier (Table 4). However, the K_m value quantified for CAT is higher in various cases than reported for enzymes from alternative sources (Table 4). Other important characteristic of studied enzymes is their high thermostability that is appreciated in lower values of inactivation constant and higher half-life time (Figure 9 and Table 4). The advantage that CAT from *A. niger* ASPN 1.1 has in comparison with enzymes from some bacterial sources (Table 4) is evident. High substrate affinity and specificity, in addition to its long-term stability at relatively high temperature, proved enzymes of *A. niger* ASPN 1.1 as a suitable biocatalyst for wide applications.

Thus, in the present study, different xerophytic *A. niger* strains were assayed for the presence of GOX and CAT activities. The strain with higher activity was selected to confirm its identification and to be used for enzymes partial purification and characterization. Partial purification was performed by means of ultrafiltration with 100 kDa filter. The enzymes showed high affinity for substrates, exhibited optimum catalytic activity at pH 6 and optimum temperature at 50 and 45°C, for GOX and CAT, respectively. Both enzymes showed a high thermal stability at 50°C having a half-life of nearly 90 min. These properties suggest the use of catalase and glucose oxidase from xerophytic *A. niger* strain for wide industrial, clinical and biochemical applications. However, further studies are required to optimize the fermentation process and obtain higher enzymatic activities.

Conflict of Interests

The authors have not declared any conflict of interest.

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