African Journal of Biotechnology Vol. 8 (22), pp. 6317-6326, 16 November, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Screening of culture condition for xylanase production by filamentous fungi

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Accepted 25 June, 2008

The objective of this research was to investigate xylanase production by filamentous fungi (*Trichoderma viride*) to determine the best cultivation conditions in the process, aiming toward optimization of enzyme production. The best temperature, as well as the best carbon source, for biomass production was determined through an automated turbidimetric method (Bioscreen-C). The enzyme activity of this fungus was separately evaluated in two solid substrates (wheat and soybean bran) and in Vogel medium, pure and by adding other carbon sources. Temperature effects, cultivation time, and spore concentrations were also tested. The best temperature and carbon source for enzyme and biomass production was 25°C and sorbitol, respectively. Maximum xylanase activity was achieved when the fungus was cultivated in wheat bran along with sorbitol (1%, w/v), using a spore concentration of 2 x 10^6 spores.mL^{-1}, pH 5.0, for 144 h cultivation. The study demonstrated not only the importance of the nature of the substrate in obtaining a system resistant to catabolic repression, but also the importance of the culture conditions for biosynthesis of this enzyme. *T. viride* showed a high potential for xylanase production under the conditions presented in these assays.

Key words: *Trichoderma viride*, xylanase activity, enzyme optimization.

INTRODUCTION

Xylanases catalyze xylan hydrolysis, the major hemicellulose component in plant cell walls. The xylan structure, however, can differ greatly depending on its origin (Huisman et al., 2000), but basically, xylan is a branched heteropolysaccharide constituting a backbone of β-1,4 linked xylopyranosyl units substituted with arabinosyl, glucuronyl and acetyl residues (Shallom and Shoham, 2003). The hydrolysis of the xylan backbone is accomplished by endoxylanases (EC 3.2.1.8) and β-xylosidases (EC 3.2.1.37) along with a variety of debranching enzymes, that is, α-L-arabinofuranosidases, α-glucuronidases and acetyl esterases (Collins et al., 2005).

Xylanase is one of the microbial enzymes that has aroused great interest recently due to its biotechnological potential in many industrial processes, for example, in xylitol and ethanol production (Beg et al., 2001), in the cellulose and paper industry (Wong et al., 1988), in the production of oligosaccharides (Pellerin et al., 1991), to obtain cellular proteins, liquid fuels and other chemical substances (Biely, 1985), in the food industry (Haltrich et al., 1996), and in poultry, pork, and caprine feeding (Pucci et al., 2003). Xylanases are extracellular enzymes produced by microorganisms such as bacteria (saprophytic and phytopathogenous), mycorrhizic fungi, and some yeasts. The enzyme is also found in protozoa, insects, crustaceans, snails, seaweed, and also seeds of plants during the germination phase in the soil (Wong et al., 1988).

Filamentous fungi are useful producer of xylanases from the industrial point of view. The reasons are many fold - they are non pathogenic, capable of producing high levels of extra cellular enzymes and they can be cultivated very easily (Kar et al., 2006). Nevertheless, microbial

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metabolic activity and enzyme production are extremely sensitive to environmental factors, that is, pH, culture time, incubation temperature (Haltrich et al., 1996), inoculate concentration (Fadel, 2000), and, principally, the substrate utilized (Lenartovicz et al., 2003). The suitable substrate chosen for microorganism's cultivation aiming the enzymatic biosynthesis depend on a series of factors which include cost and usage viability (Pandey et al., 2000). The use of wheat bran is an efficient medium for xylanase production (Lucena-Neto and Ferreira-Filho, 2004), on the other hand it can be an expensive carbon source for large scale usage, and which can represent problems for its industrial application (Virupakshi et al., 2005; Yang et al., 2006). Thus, soy flour was evaluated in the present work because it is a residue rich in hemicellulose and cellulose and can be generated in large scale as there are vast soy plantations areas in Brazil. Most enzyme manufacturers produce enzymes using submerged fermentation (SmF) techniques, however production of enzymes by solid-state fermentation (SSF) has potential advantages over submerged fermentation regarding to operation simplicity, high productivity fermentation, less favorable for growth of contaminants and concentrated product formation (Gupta et al., 2001). Thus, the aim of the present study was to evaluate the best cultivation conditions for xylanase production by several filamentous fungi.

MATERIALS AND METHODS

Microorganism

Filamentous fungi (*Penicillium implicatum*, *Penicillium commune*, *Penicillium verruculosum*, *Penicilium lividum*, *Penicilium brevicompactum*, *Penicilium raciborskii*, *Aspergillus fumigates*, *Aspergillus niger*, *Aspergillus aculeatus*, *Aspergillus flavus*, *Aspergillus tamari, Mucor circinelloides*, *Rhizopus microsporus*, *Trichoderma viride*, *Fusarium oxysporum*, *Cladosporium cladosporioides* and *Emericellopsis minima*) were isolated from a 10 - 15 cm depth in the soil of a "Caatinga" area, at Contendas do Sincorá National Forest in the micro-area of the homogeneous Chapada Diamantina, in Ituaçu, Bahia, Brazil, was used. The fungi identification was performed at Departamento de Micologia da Universidade Federal de Pernambuco, Brasil by Profª. Drª. Maria Auxiliadora Cavalcanti. Stock cultures were kept in malt extract agar, performing constant replications and maintainance at 4°C.

Effect of different carbon sources on filamentous fungi growth in a Bioscreen C automated system

The selection of the best carbon source for biomass growth was performed in a Labsystem Bioscreen C automated growth system (Simões and Tauk-Tornisielo, 2005). The culture medium containing 1% liquid malt extract was distributed in penicillin flasks and supplemented individually with a single carbon source (1%, w/v): lactose, sucrose, maltose, glucose, glycerol, sorbitol, fructose, xylose, or galactose. The flasks were sealed, labeled, and autoclaved at 121°C for 20 min. Afterwards, 360 µL of the cultivation medium containing the cited carbon sources were added to the cells of the micro-cultivation plates of Bioscreen C (Quadruplicates) and added individually for each carbon source tested 40 µL of 1 x 10^7 spores.mL^{-1} suspension of each evaluated fungus. In the control cells, 400 µL of each culture medium was used. The plates were placed in the Bioscreen C, using the following settings: 25°C, absorbance at 540 nm, reading intervals every 4 h, cell agitation 10 s before each reading, and a total experiment time of 60 h. The automatic readings were sent to a computer, where the growth curves were determined after the 60 h experiment for each carbon source. The same procedure was performed at 35°C.

Qualitative screening for detection of xylanase producer microorganisms

Tubes containing minimum solid Vogel medium along with xylan 1% (v/w) as the only carbon source were inoculated with mycelia and spores obtained from the fungal evaluated (triplicate) and incubated at 25°C for 5 days. The tubes that showed growth were considered positives (+) regarding to xylanase enzyme production.

Microorganism cultivation to determine xylanolytic activity in SSF (solid state fermentation) and SmF (submerged fermentation)

For SSF cultivation, the culture media used was wheat bran medium (MFT) (Yoshioka et al., 1981) and soybean bran medium (MSF). Erlenmeyer flasks (in triplicate), containing 5 g of wheat bran or 10 g of soybean bran (finely ground) were added 5 mL of distilled and the pH adjusted to 5.0 (triplicate). The media were homogenized and the flasks were covered and autoclaved at 121°C for 30 min, after that they were inoculated with 1 x 10^7 spores.mL^{-1} concentration of each evaluated fungus and incubated at 25 and 35°C for 120 h.

Microorganism cultivation to determine xylanolytic activity in SSF (solid state fermentation) and SmF (submerged fermentation) with the addition of other carbon sources

The same procedure cited above was performed, adding individually to each medium the best carbon source as determined by the Bioscreen C, xylan and carboxymethylcellulose (CMC) (1%, w/v). For SmF cultivation Erlenmeyer flasks (250 mL) were prepared with 25 mL of Vogel medium (Vogel, 1956), added individually the same carbon sources, as described above, and incubated in shaker at 125 rpm, at 25 and 35°C for 120 h.

Effect of temperature on enzymatic activity

Erlenmeyer flasks (in triplicate), containing wheat bran media with the addition of sorbitol (1%, w/v), pH 5.0, were inoculated with a solution of spores of *T. viride* in a concentration of 1 x 10^7 spores.mL^{-1}, and incubated at temperatures of 20, 25, 35, and 40°C for 120 h.

Effect of culturing time on enzymatic activity

Erlenmeyer flasks (in triplicate), containing wheat bran media with the addition of sorbitol (1%, w/v), were inoculated with a solution of spores of *T. viride* in a concentration of 1 x 10^7 spores.mL^{-1}, and incubated at 25°C for 24, 48, 72, 96, 120, 144, and 168 h.

Table 1. Determination of the best carbon source for biomass growth of filamentous fungi (Bioscreen-C) and qualitative screening for determination of xylanase activity.

Microorganism	Carbon source	Xylanase production
Penicillium implicatum	Lactose	(+)
Penicillium commune	Sucrose	(+)
Penicillium verruculosum	Glucose	(+)
Penicillium lividum	Sucrose	(+)
Penicillium brevicompactum	Glucose	(-)
Penicillium raciborskii	Glucose	(+)
Aspergillus fumigatus	Glucose	(-)
Aspergillus aculeatus	Maltose	(+)
Aspergillus niger	Maltose	(+)
Aspergillus flavus	Maltose	(+)
Aspergillus tamarii	Maltose	(+)
Mucor circinelloides	Maltose	(+)
Rhizopus microsporus	Sorbitol	(+)
Trichoderma viride	Sorbitol	(+)
Fusarium oxysporum	Glucose	(-)
Cladosporium cladosporioides	Maltose	(-)
Emericellopsis mínima	Galactose	(+)

(+)- Xylanase activity.

Effect of the concentration of the inoculate on enzymatic activity

Erlenmeyer flasks (in triplicate), containing wheat bran media with the addition of sorbitol (1%, w/v), pH 5.0, were inoculated with a solution of spores of *T. viride* in concentrations of 2 x 10^6, 1 x 10^7, 2 x 10^7, and 4 x 10^7 spores.mL^{-1}, and incubated at 25°C for 144 h.

Determination of the xylanolitic activity

After the incubation, to the flasks containing the solid media (MFT and MSF) were added 30 mL of sterilized distilled water. The cultures were homogenized, and the flasks were maintained at 4°C for 3 h, then the cultures were filtered under vacuum. The biomass obtained in Vogel medium was also filtered using the methodology mentioned above, and the filtrates were considered as crude enzymatic extract (Linko et al., 1978). The determination of xylanolitic activity was carried out at 50°C, using Xylan Birchwood (Sigma, U.S.: Bailey et al., 1992) 1% (w/v) in sodium acetate buffer 50 µmol (pH 5.0). The release of the reducing sugars was determined by the dinitrosalicylic acid method (ADNS) (Miller, 1959). One unit of xylanase activity (U) was defined as the amount of enzyme that produced reducing sugar equivalent to 1 µmol of xylose min^{-1} under the assay conditions.

Quantitative protein determination

Protein concentration was determined by Lowry's method (Lowry, 1951), using bovine serum albumin as the standard.

RESULTS AND DISCUSSION

Qualitative screening for detection of xylanase producer microorganisms

Qualitative analysis of xylanase production by the evaluated fungi through the negative or positive growth in medium containing xylan as the sole carbon source (Table 1) was valuable regarding to make a previous evaluation of the producer fungi and afterwards the discharge of the non producers. However, even the ones that did not show growth in this previous assay (*P. brevicompactum* and *A. fumigatus*) were then evaluated to prove the efficiency of this method, being that during the quantitative assays the results were the same (Table 2a).

Effect of different carbon sources on filamentous fungi growth in a Bioscreen C automated system

The best carbon sources for the growth of each evaluated fungus are shown on Table 1, with glucose and maltose being the two carbon sources that showed the best results. The use of maltose is cited for fungal bio-mass production for the growth of diverse fungi (Simões and Tauk-Tornisielo, 2006; Peixoto et al., 2003; Fadel,

Table 2a. Xylanolitic activity of filamentous fungi in SSF (MFT and MSF), pH 5.0, 25 and 35°C for 5 days.

Microorganism	Xylanase activity ($U.mL^{-1}$)			
	MFT		MSF	
	25°C	35°C	25°C	35°C
Penicillium implicatum	27,4	25,7	(-)	(-)
Penicillium commune	14,5	32,7	(-)	8,7
Penicillium verruculosum	5,4	1,4	(-)	(-)
Penicillium lividum	5,4	4,0	(-)	(-)
Penicillium brevicompactum	(-)	(-)	(-)	(-)
Penicillium raciborskii	12,6	1,7	(-)	(-)
Aspergillus fumigatus	(-)	(-)	(-)	(-)
Aspergillus aculeatus	18,9	16,3	(-)	(-)
Aspergillus niger	23,2	28,1	(-)	19,4
Aspergillus flavus	11,2	0,3	(-)	(-)
Aspergillus tamarii	10,8	1,4	(-)	(-)
Mucor circinelloides	16,9	18,9	(-)	(-)
Rhizopus microsporus	17,6	36,6	(-)	1,9
Trichoderma viride	93,5	34,1	(-)	(-)
Emericellopsis mínima	1,9	11,9	(-)	(-)

SSF = solid-state fermentation; MFT = Wheat bran medium; MSF = and soybean bran medium.
(-) No production.

Table 2b. Effect of different incubation periods on xylanase biosynthesis by *T. viride* cultivated in wheat bran increased with sorbitol 1% (v/w), pH 5.0, a 25ºC, 1 x 10^7esporos.mL^{-1}, for 168 h.

Incubation time (h)	Xylanase activity			$U.mL^{-1}.h^{-1}$
	$U.mL^{-1}$	$U.30\ mL^{-1}$	$U.g^{-1}$	
0	0	0	0	0
24	0,6	18,0	3,6	0,7
48	9,2	276,0	55,2	5,7
72	65,9	1977,0	395,4	27,4
96	112,2	3366,0	673,2	35,0
120	135,6	4068,0	813,6	33,9
144	139,8	4194,0	838,8	29,1
168	97,5	2925,0	585,0	17,4

2000), and glucose is one of the most used carbon sources for biomass growth because it is easily metabolized (Andrade et al., 2002). Lactose was found to be the best carbon source for the growth of *A. oryzae* (Ramachandran et al., 2004) and *T. reesei* Rut C-30 (Haltrich et al., 1996), sucrose was one of the sugars used in the growth of *M. musicola* (Montarroyos et al., 2007) and *T. harzianum* 1073 D3 (Seyis and Aksoz, 2004) and galactose was the second best carbon source for *U. esculenta* (Chung and Tzeng, 2004).

In the present work, *s*orbitol increased the growth of *T. viride* (Figure 1) and although there are not so many reports about the use of this source as the best for fungal biomass production, Rölla et al. (1981) and Ramón et al. (2007) used it in *S. mutans* and *Rhizopus oryzae* cultivation, respectively, being considered mainly as non-repressing carbon source for fermentation (Thorpe et al., 1999). The cultivation accomplished cultivation for biomass production through the turbidimetric Bioscreen C method showed in other research works, to produce the same results as the ones produced by traditional methods in Erlenmeyers (Horakova, 2003; Simões and Tauk-Tornisielo, 2005). It consists of an attractive method for kinetic parameters determination for biomass production or

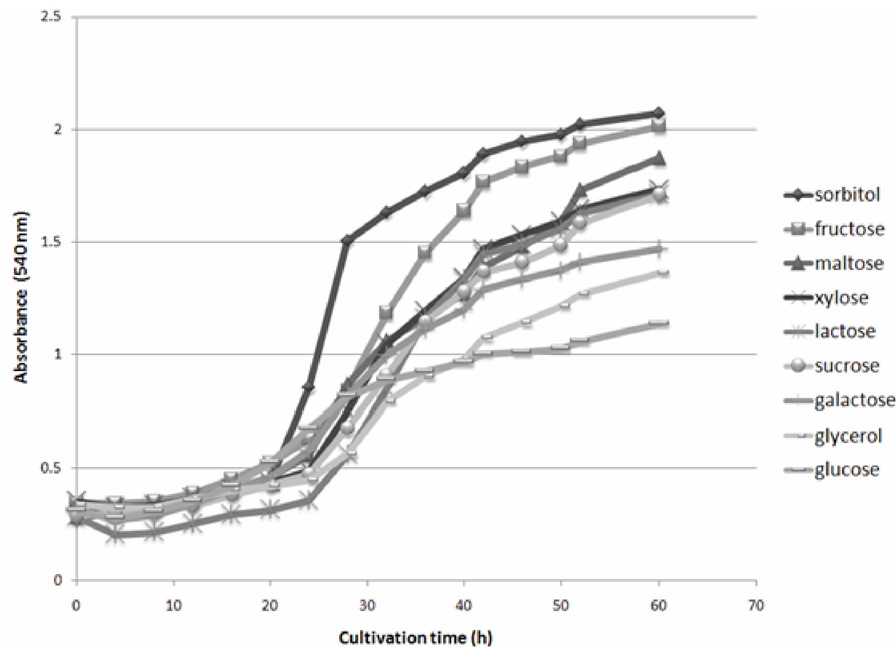

Figure 1. Effect of carbon source in biomass production of *T. viride* in an automated cultivation system (Bioscreen C), at 25°C for 60 h.

other assays of microbial activity, because the time of analysis and mainly costs of culture media, are extremely minimized.

Effect of the substrate on the biosynthesis of xylanase

The production of xylanase by filamentous fungi was higher in SSF, using wheat bran as carbon source, when compared to the results obtained with other substrates, mainly in SmF using Vogel medium, in which the fungus did not present enzymatic activity (Table 2a). A higher efficiency on enzymatic production SSF is described by several authors for various enzymes and microorganisms (Kamra and Satyanarayana, 2004; Sharma et al., 2005; da Silva et al., 2005). While in submerged fermentation (SmF), the fungus is exposed to hydrodynamic forces, in SSF, growth is restricted to the surface of the solid matrix. Another factor is that the use of solid systems (SSF) provides the fungus with an environment closer to its natural habitat (wood and decayed organic matter), which stimulates the fungi to produce more hemicellulolytic enzymes (da Silva et al., 2005).

Many authors report the advantages of using wheat bran as a substrate for xylanase production in SSF when compared to other solid wastes. De Souza et al. (2001) and Ferreira et al. (1999), cultivating *A. tamari* in wheat bran, obtained a higher enzymatic activity than in sugar cane bagasse and corn cob. Gawande and Kamat (2000) using *A. terreus* and Qinnghe et al. (2004) using *P. ostreaus*, tested the xylanase activity of these fungi in different substrates, and highest production was obtained with wheat bran, maybe because wheat bran is rich in proteins and hemicelluloses, which are used by microorganisms as energy and carbon sources, through specific enzymes such as xylanase and α-amylases (Adolph et al., 1996). According to de Souza et al. (2001), wheat bran, among other substrates used in solid systems, is the most efficient, because it does not perform catabolic repression in the enzymatic activity. Some authors suggest that the lack of catabolic repression is because the diffusion processes are slow due to the low water activity in the system (Ramesh and Lonsane, 1991). On the other hand, the use of soy bran as substrate was not efficient, as described by He et al. (2003) researching *Bacillus* sp. elastase. The use of this agroindustrial waste is reported in the literature as an alternative source of nitrogen, promoting good results (Bakir et al., 2001).

Effect of the addition of other carbon sources in the synthesis of xylanase

The production of enzymes is related to the type and concentration of the carbon source in the medium (Gawande and Kamat, 2000), and according to Hrmova et al. (1989), a substance that presents itself as an enzymatic inducer for one species can be an inhibitor of

Table 3. Xylanolitic activity of filamentous fungi in SSF (MFT) and SmF (Vogel medium), pH 5.0, a 25 and 35°C for 5 days, individually added with the best carbon sources determined by Bioscreen C, xylan and CMC (1%, w/v).

Microorganism	Carbon source	Xylanase activity ($U.mL^{-1}$)			
		MFT		Vogel medium	
		25°C	35°C	25°C	35°C
A. niger	Xylan	(-)	17,9	(-)	13,1
	CMC	(-)	17,9	(-)	1,7
	Maltose	(-)	17,8	(-)	0,2
P. commune	Xylan	(-)	21,7	(-)	25,0
	CMC	(-)	2,2	(-)	1,6
	Fructose	(-)	6,4	(-)	2,1
P. implicatum	Xylan	16,9	(-)	(-)	(-)
	CMC	3,6	(-)	(-)	(-)
	Lactose	19,5	(-)	(-)	(-)
R. microsporus	Xylan	(-)	11,4	(-)	32,7
	CMC	(-)	22,5	(-)	(-)
	Glycerol	(-)	(-)	(-)	(-)
T. viride	Xylan	143,0	(-)	21,7	(-)
	CMC	87,5	(-)	2,2	(-)
	Sorbitol	169,0	(-)	6,4	(-)

SSF = solid-state fermentation; MFT = Wheat bran medium; CMC = carboxymethylcellulose.
(-) No production.

the same activity in another species. Addition of glucose and xylose to the culture media containing another carbon source is known to affect negatively the enzymatic synthesis of many microorganisms (Bindu et al., 2006). This inhibition of xylanase synthesis could be interpreted in terms of catabolite repression likewise described for other enzymes (Kermnický and Biely, 1998). Catabolite repression refers to the repression of enzyme synthesis by glucose or other easily metabolized sugars. In general, the addition of other carbon sources to the substrate promoted a repressive effect on xylanolitic activity in most of the fungi studied (Table 3), except *T. viride*. When this fungus is cultivated on pure wheat bran medium, it produces 93.50 $U.mL^{-1}$ of xylanase (Table 2a); however, when sorbitol was added to the medium there was a significant improvement (169.01 $U.mL^{-1}$) (Table 3). Perhaps, this is due to sorbitol's low concentration in the medium itself, and being a more easily assimilated sugar favors the growth of the fungus and subsequent production of xylanase. Besides sorbitol addition to the substrate also promotes protection against thermal-inactivation (George et al., 2001; Lemos et al., 2006; Simpson et al., 1991). Sorbitol is also an inducer for sorbose production by *Gluconobacter oxydans* (Rosenberg et al., 2004) and Penicillin G Acylase by *Escherichia coli* (Liu et al., 2000).

The addition of xylan (1%, v/w) caused a less significant increase in the production of xylanase by *T. viride*, which was also found by Gawande and Kamat (2000) using *Aspergillus* sp, Ahmed et al. (2003) using *T. harzianum* and Rawashdeh et al. (2005) using *Streptomyces* sp. The addition of CMC caused a decrease in the production of this enzyme, which could be attributed to the production of CMCase which hydrolyzes the substrate cellobiose, leading to a repressive action on xylanase production (Ahmed et al., 2003).

There was no production of the enzyme in liquid Vogel medium without the addition of a carbon source by *T. viride*, demonstrating that this enzyme needs the presence of an inducer for its production. The addition of sorbitol to the Vogel medium did not have the same result demonstrated when sorbitol was added to wheat flour medium; showing that sorbitol by itself is not a good inducer. The addition of CMC to Vogel medium also did not induce a great production. The addition of xylan was more significant.

Table 4. Influence of different cultivation temperatures on xylanase activity by *T. viride* wheat bran medium increased with sorbitol 1% (v/w), pH 5.0, 1 x 10^7 sporos.mL^{-1}, for 120 h.

Temp. (ºC)	Xylanase activity				
	U.mL^{-1}	U.30 mL^{-1}	U.g^{-1}	U.mL.$^{-1}h^{-1}$	U.mg^{-1} Prot.
20	77.9	2338.5	467.7	19.5	10.8
25	93.5	2805.0	561.0	23.4	14.6
35	45.9	1378.5	275.7	11.5	12.4
40	33.0	989.4	197.8	8.2	6.20

Effect of temperature on enzymatic activity by *T. viride*

The growth of *T. viride* was evaluated by Bioscreen automated culturing where at 25°C the greatest production of biomass was produced. As the best temperature for the growth of fungus is not always the best for the production of enzymes (Khan and Omar, 2005) and the incubation temperature is a critical factor in enzymatic productivity (Seyis and Aksoz, 2003), in the present study, the production of xylanase by this fungus was tested at four different temperatures (20, 25, 35 and 40°C) (Table 4). The greatest enzymatic production occurred at a temperature of 25°C (93.5 U.mL^{-1}), with a production of 77.95 U.mL^{-1} at 20°C. For the cultures at 35 and 40ºC, enzymatic activity suffered a substantial decline (45.95 and 32.98 U.mL^{-1}, respectively). The best temperature for enzymatic production was, thus, the same temperature found to be optimum for growth of this fungus. The result is in accordance with that obtained by Sater and Said (2001) on *Trichoderma reesei*. Kheng and Omar (2005), studying *Aspergillus niger*, obtained optimum activity at a similar temperature to the optimum temperature for the strain growth. The large decrease in activity at very low or very high temperatures is due to the fact that at these temperatures the growth of the fungus is inhibited, causing a decrease in the synthesis of the enzymes. The best temperature range for the production of xylanase by fungi is usually from 20 to 30°C (Rahman et al., 2003).

Effect of cultivation time on enzymatic activity by *T. viride*

The present research has demonstrated that culturing time is a determining factor in the process of xylanase production by *T. viride* because production reaches the maximum in a relatively gradual manner and then falls abruptly. Activity in the first 24 h was 0.6408 U.mL^{-1}, reaching a maximum activity at 144 h culturing (139.82 U.mL^{-1}), and falling to 97.34 U.mL^{-1} during the following 24 h (Table 2b). Production effectively started on the second day, attaining its maximum on the sixth day, and suffered a rapid decline on day 7.

The optimum culturing time varied, but the majority of studies on the generation of xylanase by this genus, report similar data. The optimum production time obtained by Sater and Said (2001) with *T. harzianum* was on day 8, and Seyis and Aksoz (2005), with *T. harzianum*, obtained maximum activity on day 7.

The rapid decline after attaining the maximum might be due to the depletion of macro-and micronutrients in the fermentation medium with the lapse in time, which stressed the fungal physiology resulting in the inactivation of secretary machinery of the enzymes.

Effect of inoculum concentration on enzymatic activity by *T. viride*

Maximum activity on xylanase production by *T. viride* was obtained by using a concentration of 2 x 10^6 spores.mL^{-1} (234.56 U.mL^{-1}), with a decrease in production when using a concentration of 1 x 10^7 spores.mL^{-1} (220.80 U.mL^{-1}) and 2 x 10^7 spores.mL^{-1} (213.92 U.mL^{-1}). The decrease in production was drastic when the inoculum was in a concentration of 4 x 10^7 spores.mL^{-1} (93.50 U.mL^{-1}) (Table 5). Various studies point out that, over a certain inoculum concentration, a decline in enzymatic activity begins to occur. Seyis and Aksoz (2004), with *Trichoderma* sp., and Gawande and Kamat (2000), with *Aspergillus* sp., obtained the best xylanase activity using the concentrations of 1.5 x 10^6 spores.mL^{-1} and 1 x 10^6 spores.mL^{-1}, respectively.

According to Sikyta (1983) the spore concentration on fungal cultivation aiming enzyme production must be big enough to colonize all substrate particles. However, a very little spore concentration lead to a mycelium production, and therefore to a low efficiency in the assimilation of carbon source and nitrogen assimilation (Meyrath and Macintosh, 1963). On the other hand when the spore concentration is very high according to Brown and Zainudeen (1978) a decrease in the specific velocity of oxygen consumption can occur. In both cases the fungal metabolism is affected including the enzymatic activity. In general optimal spore concentration is between 10^6 and 10^7 spores.mL^{-1}; out of this range, a decrease in xylanase activity occurrs (Qinnghe et al., 2004).

Table 5. Effect of the different inocula concentrations on xylanase activity produced by *Trichoderma viride* cultivated in wheat bran medium increased with sorbitol 1% (v/w), pH 5.0, a 25ºC, for 144 h.

Inocula conc. spores.mL^{-1}	Xylanase activity				
	U.mL^{-1}	U.30. mL^{-1}	U.mL^{-1}.h^{-1}	U.g^{-1}	U. mg^{-1} protein
2 x 10^6	234.5	7035.0	48.8	1407.0	21.3
1 x 10^7	220.8	6624.0	46.0	1324.0	17.9
2 x 10^7	213.9	6417.0	44.5	1283.4	21.8
4 x 10^7	93.5	2805.0	19.5	561.00	14.6

Conclusion

Comparing the maximum activity obtained in this study by *T. viride* of 234.5 U.mL^{-1}, (1407.0 U.g^{-1}) with the ones obtained by Gutierrez-Correa and Tengerdy (1998) using *Trichoderma reesei* (1900 U.g^{-1}), Colina et al. (2003) with *T. reesei* (122 U.mL^{-1}), Haq et al. (2006) with *A. niger* (154.56 U.mL^{-1}), Merchant and Margaritis (1988) with *Thielavia terrestris* (18.8 U.mL^{-1}), Kheng and Omar (2005) with *A. Níger* (25.40 U.g^{-1}); Solórzano et al. (2008) using *Aspergillus awamori* (100 U.ml^{-1}), Katapodis et al. (2006) with *Sporotrichum thermophile* (56 U.mL^{-1}), Okafor et al. (2007) with *P. chrysogenum* (47 U.mL^{-1}) among others, it can be considered that *T. viride* is an excellent xylanase enzyme producer. The carbon source and cultivation temperature that induced more biomass also induced higher xylanase activity and the maximum production was obtained when the culture was performed in solid medium of wheat bran, with the addition of sorbitol (1%, v/w), at 25°C for 144 h, using a concentration of 2 x 10^6 spores.mL^{-1}. However, such results point out the neces-sity of a better understanding of production process, mainly regarding to induction and repression processes of the substrates used. Caatinga soil area showed to be an excellent biological ecosystem for future researches and for a better understanding of biodiversity, yet it has only been barely studied.

ACKNOWLEDGEMENTS

The authors acknowledge the support of the Centro de Estudos Ambientais (CEA) at UNESP, and of the Postgraduate Course in Biological Sciences, Applied Microbiology, at the Rio Claro, SP, Campus of UNESP.

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