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Screening and Production Study of Microbial Xylanase Producers from Brazilian Cerrado

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Abstract Hemicelluloses are polysaccharides of low molecular weight containing 100 to 200 glycosidic residues. In plants, the xylans or the hemicelluloses are situated between the lignin and the collection of cellulose fibers underneath. The xylan is the most common hemicellulosic polysaccharide in cell walls of land plants, comprising a backbone of xylose residues linked by β-1,4-glycosidic bonds. So, xylanolytic enzymes from microorganism have attracted a great deal of attention in the last decade, particularly because of their biotechnological characteristics in various industrial processes, related to food, feed, ethanol, pulp, and paper industries. A microbial screening of xylanase producer was carried out in Brazilian Cerrado area in Selviria city, Mato Grosso do Sul State, Brazil. About 50 bacterial strains and 15 fungal strains were isolated from soil sample at 35 °C. Between these isolated microorganisms, a bacterium *Lysinibacillus* sp. and a fungus *Neosartorya spinosa* as good xylanase producers were identified. Based on identification processes, *Lysinibacillus* sp. is a new species and the xylanase production by this bacterial genus was not reported yet. Similarly, it has not reported about xylanase production from *N. spinosa*.

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The bacterial strain P5B1 identified as *Lysinibacillus* sp. was cultivated on submerged fermentation using as substrate xylan, wheat bran, corn straw, corncob, and sugar cane bagasse. Corn straw and wheat bran show a good xylanase activity after 72 h of fermentation. A fungus identified as *N. spinosa* (strain P2D16) was cultivated on solid-state fermentation using as substrate source wheat bran, wheat bran plus sawdust, corn straw, corncob, cassava bran, and sugar cane bagasse. Wheat bran and corncobs show the better xylanase production after 72 h of fermentation. Both crude xylanases were characterized and a bacterial xylanase shows optimum pH for enzyme activity at 6.0, whereas a fungal xylanase has optimum pH at 5.0–5.5. They were stable in the pH range 5.0–10.0 and 5.5–8.5 for bacterial and fungal xylanase, respectively. The optimum temperatures were 55C and 60 °C for bacterial and fungal xylanase, respectively, and they were thermally stable up to 50 °C.

Keywords Microbial enzyme · Xylanase · Brazilian cerrado · *Lysinibacillus* sp. · *Neosartorya spinosa*

Introduction

Cellulose, hemicellulose, and lignin are the major components of plant cell walls. When combined, they form the support framework for plants and defense against the invasion of aggressors. Successfully invading or living on plant tissues, microorganisms have to synthesize a great number of different enzymes in order to hydrolyze cellulose or hemicellulose. Thus, effectively degrading the plant cell wall complex, many microorganisms develop an associated multiprotein complex, which contains cellulases, xylanases, and cellulose-binding factors, called cellulosome or xylosome. Hemicellulose belongs to a group of heterogeneous polysaccharides that are relatively easily hydrolyzed by acids to their monomer components consisting of xylose, mannose, glucose, galactose, arabinose, and small amounts of rhamnose. Xylan is the main carbohydrate found in hemicellulose. Its complete degradation requires the cooperative action of a variety of hydrolytic enzymes. Hemicellulases are frequently classified according to their action on distinct substrates, endo-1,4-β-xylanase (EC 3.2.1.8) generates oligosaccharides from the cleavage of xylan and xylan 1,4-β-xylosidase (EC 3.2.1.37) produces xylose from oligosaccharides [1–3].

Savannas are tropical and subtropical formations characterized by almost continuous grass layers, interrupted only by shrubs and trees in varying proportions, and in which the main growth patterns are closely associated with alternating wet and dry seasons. The Brazilian Cerrado is a savanna-like region that covers a large area of Brazil, roughly equivalent to half the size of European Union and it is located mainly in the Brazilian central plateau, under a seasonal climate of wet summers and dry winters. Despite its biological importance, the Cerrado biome has been the focus of very few studies about its biological diversity.

The industrial enzyme production is frequently limited by the costs of substrates for the cultivation of the producer microorganisms. The use of low-cost substrates, such as agricultural wastes, has been suggested as an alternative to reduce the production costs [4–6]. Agricultural and agro-industrial wastes, such as sugar cane bagasse (a fibrous residue of cane stalks leftover after the crushing and extraction of the juice from the sugar cane) [5], wheat bran, rice peel, corn straw, corncob, effluents from paper industry, fruit peels, and

seeds have increased as a result of industrialization, becoming a problem, regarding space for disposal, and causing environmental pollution. However, those residues represent an alternative source for the microbial growth aiming the production of biomass or enzymes. Hemicelluloses and celluloses represent more than 50% of the dry weight of agricultural residues [1, 7, 8]. They can be converted into soluble sugars either by acid or enzymatic hydrolysis. So, they can be used as a plentiful and cheap source of renewable energy in the world [1, 4, 5, 9].

A great number of bacteria and fungi were isolated from different places, and they present a good xylanase production [1, 8, 10]. On the other hand, some authors use agro-industrial waste for submerged fermentation processes for cultivating bacteria or fungi, such as *Bacillus circulans* AB16 [11], *Clostridium absonum* CFR-702 [12], *Aspergillus foetidus* MTCC4898 [13], *Trichosporon cutaneum* SL409 [14], *Alternaria* sp. ND-16 [15], *Chaetomium thermophile* NIBGE [16], *Aspergillus tamari* [17]. Other authors use solid-state fermentation in minor scale for preferentially cultivating fungi, such as *Thermoascus aurantiacus* [9, 18], *Trichoderma harzianum* [19], *Bacillus* sp. JB-99 [20].

So, in this work, some microorganisms that are capable to hydrolyze hemicellulolitic materials were studied. These microorganisms were isolated in the Brazilian Cerrado preserved area at the Experimental and Research Farm at Engineering Faculty at UNESP. For microorganisms screening, corn straw was used as carbon source in replacement of xylan and other agro-industrial wastes were used for xylanase production study.

Materials and Methods

Medium

For microbial screening, an alternative carbon source was used, corn straw, as a substitute for xylan, reducing price cost with screening medium. So, the screening medium used for alkaline bacterium isolation was reported by Damiano [21] with minimal modifications, containing 2.5 g L^{-1} of meat extract, 2.5 g L^{-1} of peptone 10.0 g L^{-1} of NaCl, 1.0 g L^{-1} of KH_2PO_4, 5.0 g L^{-1} of Na_2CO_3, (sterilized separately) and two corn straw strips (0.5×3.0 cm), pH 9.0. Isolation medium was prepared containing same ingredients and supplemented with 10.0 g L^{-1} of corn straw triturate and 15.0 g L^{-1} agar prepared in Petri dishes. The isolated colonies were maintained in maintenance medium composed by 5.0 g L^{-1} of Birchwood xylan, 2.5 g L^{-1} of meat extract, 2.5 g L^{-1} of peptone, 10.0 g L^{-1} of NaCl, 1.0 g L^{-1} of KH_2PO_4, and 5.0 g L^{-1} of Na_2CO_3 (sterilized separately), pH 9.0 at 4 °C in slant tube.

The screening medium used for fungi isolation was composed by 2.0 g L^{-1} of yeast extract, 2.0 g L^{-1} of peptone, 1.5 g L^{-1} of $(NH_4)_2$ SO_4, 2.0 g L^{-1} of KH_2PO_4, 0.3 g L^{-1} of urea, 0.03 g L^{-1} of $CaCl_2$, 0.2 g L^{-1} of $MgSO_4$ $7H_2O$, 5 g L^{-1} of $FeSO_4$ $7H_2O$, 2.0 mg L^{-1} of $CoCl_2$, 1.6 mg L^{-1} of $MnSO_4$ H_2O, 1.4 mg L^{-1} of $ZnSO_4$ $7H_2O$ and two corn straw strips (0.5 cm×3.0 cm), pH 5.0. In the isolation medium, 10.0 g L^{-1} of corn straw triturate, 1.5 g L^{-1} of agar, and 0.1 g L^{-1} of streptomycin (added separately) were added. The streptomycin was used for inhibiting bacterial growth. The isolated colonies were maintained in the same screening medium, containing 5.0 g L^{-1} of Birchwood xylan, instead of corn straw strips, and 15.0 g L^{-1} agar at 4 °C in slant tube.

Microorganism Isolation

About 1 g of shaded soils from wood and organic material in decomposition from the Cerrado area were added to flasks with 5-mL screening medium containing corn straw strip.

The samples were incubated at 35 °C for 24 h and then inoculated on Petri dishes containing isolation medium. The plates were incubated at 35 °C for a period of 144 h. Isolated colonies were transferred to tubes containing maintenance medium.

Microbial Identification

Morphological Characterization of Filamentous Fungus

Fungal morphology was characterized by colony observation with a stereoscope (Leica MZ6, Wetzlar, Germany) and by squash mounts stained with Lactophenol and Cotton Blue using a light microscope (Leica DM LS, Wetzlar, Germany). Based on these observations and following the methods and morphological criteria determined in the literature, the fungus was identified.

Molecular Characterization and Phylogenetic Analyses

Filamentous fungi were cultured on Saboraud Dextrose Broth (Difco), incubated at 28 °C for 5 days, and genomic DNA extraction was performed according to da Silva [22]. The bacterial strain was grown on Nutrient Agar (Difco), incubated at 28 °C for 24 h, and genomic DNA extraction was performed according to Pitcher [23]. ITS1-5,8S-ITS2 region of the filamentous fungi and 16S rRNA gene of the bacterial strain were amplified from genomic DNA by PCR using the following sets of primers, respectively: ITS 1 (5′ CCGTAGGTGAACCTGCGG 3′), ITS4 (5′ TCCTCCGCTTATTGATATGC 3′) [24], 27f (5′ AGA GTT TGA TCM TGG CTC AG 3′) [25], and 1401r (5′ CGG TGT GTA CAA GGC CCG GGA ACG 3′) [26]. PCR was performed in reaction mixtures containing 0.4 μM each primer, 0.2 μM dNTPs (GE Healthcare), 1.5 μM $MgCl_2$ (Invitrogen), 2.0 U *Taq* polymerase (Invitrogen), and 1.0 X·reaction buffer (Invitrogen) in a final volume of 25 uL for fungi (5–25 ng genomic DNA), and 50 μL for the bacterium (50–100 ng genomic DNA). The PCR amplifications were carried out using an initial denaturation step of 5 min at 95 °C, followed by 30 cycles of 1 min at 94 °C for denaturation, 1 min at 55 °C for annealing, and 3 min at 72 °C for extension, with a final extension cycle of 3 min at 72 °C in an Eppendorf thermal cycler.

Amplified products were purified using GFX PCR DNA and gel band purification kit (GE Healthcare) for subsequent sequencing using DYEnamic ET Dye Terminator Cycle Sequencing Kit for an automated MegaBace DNA Analysis System 1000 (GE Healthcare), in accordance with the manufacturer's instructions. Sequencing was performed according to Sette [27] for filamentous fungi and Vasconcellos [28] for the bacterial strain. The ITS1-5,8S-ITS2 and 16S rRNA sequences obtained from the isolates were assembled in a contig using the phred/Phrap/CONSED software. The identification of the microorganisms was achieved by comparing the contiguous rRNA gene sequences obtained with rRNA sequence data from reference and type strains available in the public database GenBank (http://www.ncbi.nlm.nih.gov) using the BLASTn routine. The sequences were aligned using CLUSTAL X program [29] and analysed with MEGA software version 4.0 [30]. The evolutionary distances were derived from the sequence-pair dissimilarities and calculated as implemented in MEGA using the DNA substitution model reported by Kimura [31]. The phylogenetic reconstruction was done using the neighbor-joining algorithm [32], with bootstrap values calculated from 1,000 replicate runs, using the routines included in MEGA software.

Enzyme Production

Xylanase production was studied on submerged fermentation for bacterial strains and solid-state fermentation for fungal strains. The microorganisms were cultivated using seven different types of substrates: corn straw, corncob, wheat bran, sugar cane bagasse, cassava bran, sawdust, and xylan. Wheat bran was obtained in local commerce, sugar cane bagasse was granted by a local alcohol industry, and the other substrates were obtained from the Experimental and Research Farm at Engineering Faculty at UNESP. All substrates, except sawdust, were washed and dried on circulation stove at 60 °C, removing residual sugar. In order to evaluate enzymatic production, samples were analyzed after 72 h.

Submerged Fermentation

Submerged fermentation (SmF) was used to analyze xylanase production from bacterial strains. The production medium was composed by 2.5 g L^{-1} of meat extract, 2.5 g L^{-1} of peptone, 10.0 g L^{-1} of NaCl, 1.0 g L^{-1} of KH_2PO_4, 5.0 g L^{-1} of Na_2CO_3 (sterilized separately), and 10.0 g L^{-1} of carbon source, pH 9.0. The carbon sources analyzed were: corn straw, corncob, xylan, wheat bran, and sugar cane bagasse.

The preinoculum was prepared using 125-mL Erlenmeyer flasks containing 20 mL of the production medium with xylan as carbon source and a microbial mass produced in an agar slant tube. Microbial growth was carried out using a rotary shaker at 35 °C and 150 cycles min^{-1} for 12 h. SmFs were carried out in 125-mL Erlenmeyer flasks containing 20 mL of production medium with different carbon sources studied. Shaken flasks were inoculated with 0.5–mL cellular suspension (10^7 cells mL^{-1}) obtained from a 12-h preinoculum. The Erlenmeyer flasks were incubated in a rotary shaker at 35 °C and 150 cycles min^{-1}, for 72 h. After fermentation, the volume of all flasks was centrifuged at 10,000×*g* at 5 °C for 10 min. The cell-free supernatant was utilized to determine enzymatic activities, as crude enzyme.

Solid-State Fermentation

Solid-state fermentation (SSF) was used to analyze xylanase production from fungal strains. The production medium was composed by different substrate sources, such as wheat bran, sawdust, corn straw, corncob, cassava bran, and sugar cane bagasse. SSF was carried out in 250-mL Erlenmeyer flasks containing 5.0 g of substrate that was moistened with 10 mL of mineral solution (1.5 g L^{-1} of $(NH_4)_2\ SO_4$, 2.0 g L^{-1} of KH_2PO_4, 0.3 g L^{-1} of urea, 0.03 g L^{-1} of $CaCl_2$, 0.2 g L^{-1} of $MgSO_4\ 7H_2O$), aiming an initial humidity content of 75%. A mixture of wheat bran and sawdust was prepared using 1:1 proportion. After sterilization, this solid material was inoculated. Fungal inoculum was prepared from Petri dish cultivation for 48 h. Five discs (0.5×0.5 cm) of Petri dish growth were used as inoculum and transferred to Erlenmeyer flask. A sterile glass stick was used to homogenize inoculum in substrate. Erlenmeyer flasks were incubated in an oven at 35 °C for 72 h. The microorganism growth was detected through visualization of the typical micelial growth. The crude enzyme was obtained by adding 40 mL of distilled water to the solid media. These solid media were gently cleaved using a glass stick and then shaken at 100 cycles min^{-1} for 1 h at room temperature. The suspension was filtered under vacuum using Whatman filter paper followed by centrifugation at 10,000×*g* at 5 °C for 15 min. The liquid supernatant was used as crude enzyme for determining enzymatic activities.

Physico-Chemical Characteristics

Physico-chemical properties of crude xylanase were determined on better activities obtained by bacterial and fungal strains. So, the optimum pH of both crude xylanases was measured using xylan 0.5% (*v*/*w*) at 50 °C in McIlvaine buffer 100 mM (pH 3.0–8.0) and in Glycine–NaOH buffer 100 mM (pH 8.0–10.5) for a 10-min reaction. Then, tests were carried out at the pH-producing maximum activity to find the optimal temperature. The optimum temperature of the crude xylanases was determined using xylan 0.5% (*v*/*w*) and incubating the reaction mixture in different temperatures ranging from 45 to 70 °C for 10 min on circulation bath water. The pH stability was determined incubating crude enzymes without substrate for 24 h at room temperature in the same buffer systems. The remaining xylanase activity was measured under standard conditions at optimal temperature. The thermostability was tested by incubating the crude enzymes without substrate at different temperatures from 45 up to 70 °C for 60 min in a circulation bath water. After incubation, aliquots were withdrawn and cooled on ice before measuring the residual activities under standard conditions at optimal pH and temperature.

Enzyme Assays

Xylanase was assayed in accordance with Damiano [21], by incubating 0.1 mL of crude enzyme, with 0.9 mL of a suspension containing 1% of Birchwood xylan (Sigma) in 100 mM acetate buffer at pH 6.0. After incubation at 50 °C for 10 min, the released reducing substances were assayed by 3,5-dinitrosalicylic acid method [33]. Controls were prepared with enzyme added after the 3,5-dinitrosalicylic acid reagent addition. One unit of xylanase activity was defined as the amount of enzyme required to release 1 μmol of xylose per minute under the above assay conditions, using a xylose standard curve.

Carboxymetilcelulase (CMCase) was measured determining the release of reducing sugars by the 3,5-dinitrosalicylic acid method [33]. The reaction mixture was prepared by incubating 0.1 mL of crude enzyme in 0.9 mL of substrate (0.5% of Carboxymetilcellulose (Sigma) on 100 mM acetate buffer, pH 6.0) at 50 °C for 10 min. Controls were prepared with enzyme added after the 3,5-dinitrosalicylic acid reagent addition. One unit of CMCase activity was expressed as the amount of enzyme required to release 1 μmol of glucose per minute under the above assay conditions.

Protein Assays

The protein content was determined according to Hartree–Lowry [34] using bovine serum albumin as the standard.

Results and Discussion

Microorganism Isolation

Fifty bacterial strains and 15 fungal strains of xylanase producers were isolated. Only five bacterial strains (P1D2b, P4B3, P5A2, P5B1, and P5B4) and four fungal strains (P1C4, P2B4, P2D17, and P2D19) show high xylanase activity and grew at 35 °C. These strains were isolated from the Cerrado soil in shady areas of the Experimental and Research Farm at UNESP, locate in Selviria city, Mato Grosso do Sul State-Brazil (20°20′04.38″S and 51°24′

Table 1 Xylanase activity and protein determination to bacterial and fungal strains isolated from Brazilian Cerrado, after 72 hour fermentation.

Strains	Xylanase activity (U mL^{-1})	Protein (mg mL^{-1})
Submerged fermentation with corn straw as carbon source		
P5B1	25.40	4.75
P5B4	13.44	5.97
P5A2	9.31	2.83
P4B3	8.50	2.70
P1D2b	6.36	3.51
Solid-state fermentation with wheat bran as carbon source		
P2D19	10.37	4.56
P2D17	7.35	3.18
P2B4	7.55	3.51
P1C4	7.92	4.37

36.04″W). Bacterial strain P5B1 (Table 1) and fungal strain P2D19 (Table 1) presented the highest xylanase activity and were selected for additional studies for enzymatic fermentation.

The xylanase production study was carried in submerged fermentation for bacterial strain P5B1 and in solid-state fermentation for fungal strain P2D19.

Microbial Identification

Only fungal strain P2D19 and bacterial strain P5B1 were identified by the Brazilian Collection of Microorganisms from Environment and Industry. The isolated fungal strain P2D19 that showed high xylanase activity was identified by conventional and molecular approaches. Data derived from BLAST (ITS-rDNA region) showed that isolated strain P2D19 had high sequence similarities (98%) with different species from the genus *Neosartorya*, including *N. spinosa*, *N. botucatensis*, and *N. fischeri*. However, in the phylogenetic tree (Fig. 1), the strain P2D19 showed to be related with *N. spinosa* and *N.*

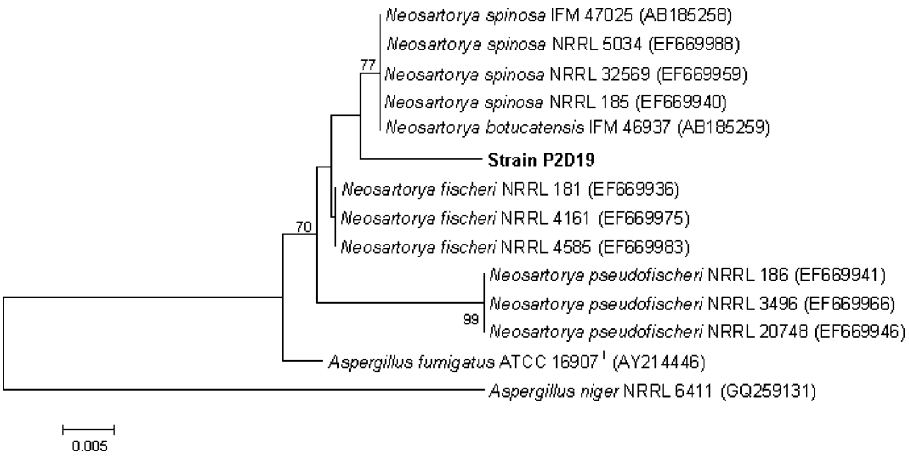

Fig. 1 Phylogenetic analysis of ITS1-5.8S-ITS2 sequence of fungal isolate P2D19 and related microorganisms. Bootstrap values (1,000 replicate runs, shown as percent) greater than 70% are listed. GenBank accession numbers are listed after species names

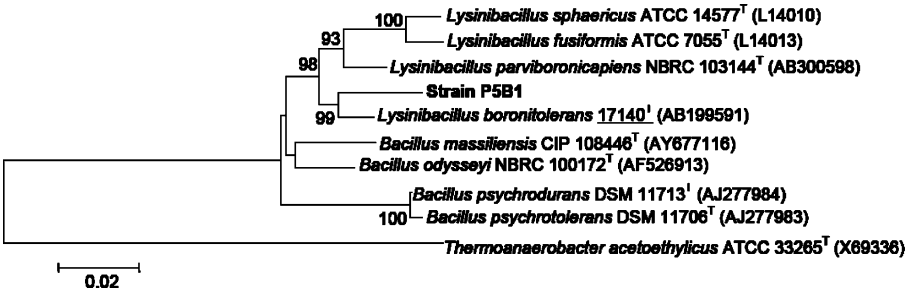

Fig. 2 Phylogenetic analysis of partial 16S rRNA gene sequence of bacterial isolate P5B1 and related microorganisms. Bootstrap values (1,000 replicate runs, shown as percent) greater than 70% are listed. GenBank accession numbers are listed after species names. *Thermoanaerobacter acetoethylicus* ATCC 33265^T was used as outgroup

botucatensis. Subsequent analyses of macro- and micro-morphological characteristics [35] corroborate the data from molecular and phylogenetic analyses allowing the identification of the strain P2D19 as *N. spinosa* (teleomorph of *Aspergillus spinosus*). This specie is a thermotolerant fungus commonly found in soil and in foods [35, 36].

Phylogenetic analysis based on the 16S rRNA gene molecular marker showed that the bacterial strain P5B1 belonged to the genus *Lysinibacillus*, being recovered in a tight cluster, supported by a high boostrap value (99%), with the species *Lysinibacillus boronitolerans* (Fig. 2). However, the sequence similarity at the 16S rRNA gene level between isolate P5B1 and its closest relatives, named *L. boronitolerans*, *L. fusiformis*, and *L. sphaericus*, was too low (94%) to allow the assignment of the isolate to any of these *Lysinibacillus* sp. Additionally, the low level of sequence similarity observed could be an indicative of a new *Lysinibacillus* sp.

Submerged Fermentation

Submerged fermentations were evaluated using five different media compositions. The studied carbon sources were xylan, corn straw, corncob, wheat bran, and sugar cane bagasse. For all studied media, there was biomass growth after 24 h of fermentation. The most significant xylanase production was shown on medium containing corn straw, followed by medium containing wheat bran and xylan as carbon source (Table 2). The high activity in this assay was 17.8 U mL^{-1} after 72 h of cultivation. This activity level is only minor and presented by other bacterial strains such as *Bacillus circulans* AB16 with xylanase activity of 55 U mL^{-1} when cultivate on rice straw [11] and *Clostridium absonum*

Table 2 Xylanase and CMCase activity of crude enzyme from *Lysinibacillus* sp. strain P5B1 obtained by submerged fermentation using different substrates, after 72-h fermentation.

Substrates	Xylanase activity (U mL^{-1})	CMCase activity (U mL^{-1})
Corm straw	17.8	0.65
Wheat bran	5.4	0.43
Xylan	5.0	058
Corncob	3.4	0.51
Sugar cane bagasse	1.6	0.62

CFR-702 that presents better activity when cultivated on corncob and corn stalk [12]. Until this moment, it had not found references that describe the xylanase production by *Lysinibacillus* sp. So, the analyses of this new bacterial strain should be carried out in detail. It is observed that *Lysinibacillus* sp. strain P5B1 hydrolyzes the hemicellulose fiber presenting good growth and good xylanase production using corn straw as carbon source. Actually, the corn straw substrate has a rich hemicellulose and cellulose composition in its dry matter [1].

Solid-State Fermentation

For the composition of culture media, the use of traditional commercial sources of carbon and nitrogen leads to high costs for industrial enzyme production [37, 38]. So, researches that investigate enzyme production using low-cost agro-industrial residues are important for analysis of commercial enzymes economically feasible use. In this case, commercial sources of carbon and nitrogen were replaced by low-cost agro-industrial residues [1, 4, 39]. Six different media compositions with solid substrates were analyzed for xylanase production from *N. spinosa* P2D19, (Table 3). All six analyzed media presented microbial growth, measured as protein content and micelial growth visualization. The media composed of wheat bran and corncob presented good xylanase production. The enzymatic activity was 15.10 U mL^{-1} for wheat bran and 8.50 U mL^{-1} for corncob after 72 h of fermentation. These results are similar to those obtained from *Aspergillus foetidus* which presents 29.0 U mL^{-1} on corncobs and 20.0 U mL^{-1} on wheat straw in submerged fermentation [13]. *Trichosporum cutaneum* [14], *Alternaria* sp. ND-16 [15], *Chaetomium thermophile* [16], and *Aspergillus tamarii* [17], when cultivated on submerged fermentation using wheat bran as carbon source, presented xylanase activity of 39.7 U mL^{-1}, 20.0 U mL^{-1}, 27.8 U mL^{-1}, and 54.32 U mL^{-1}, respectively. Better results of xylanase activity of 107 U mL^{-1}, 102 U mL⁻1, and 97 U mL^{-1} were obtained when *Thermoascus aurantiacus* was cultivated on solid-state fermentation using corncob, grass, and corn straw, respectively [9]. Solid-state fermentation is generally defined as the growth of micro-organisms on solid material in the absence or near absence of free water, which is physiologically more favorable to the cultivation of filamentous fungi [4, 5, 39].

The time course of enzyme production in solid-state fermentation from *N. spinosa* P2D19 was analyzed using wheat bran as carbon source. So, the xylanase activity and the CMCase activity were determined. The xylanase activity increased at 72 h of fermentation (20.6 U mL^{-1}) and a low CMCase activity (0.15 U mL^{-1}) was shown in this assay (Fig. 3). These results can indicate that the xylanase activity can be improved with changes into fermentation parameters.

Table 3 Xylanase and CMCase activity on crude enzyme from *N. spinosa* strain P2D19 obtained by solid-state fermentation using different substrates, after 72-h fermentation.

Substrates	Xylanase activity (U mL^{-1})	CMCase activity (U mL^{-1})
Wheat bran	15.1	3.60
Corncob	8.50	0.20
Cassava bran	3.45	0.09
Wheat bran+sawdust	2.10	0.11
Corm straw	0.95	0.16
Sugar cane bagasse	0.50	0.05

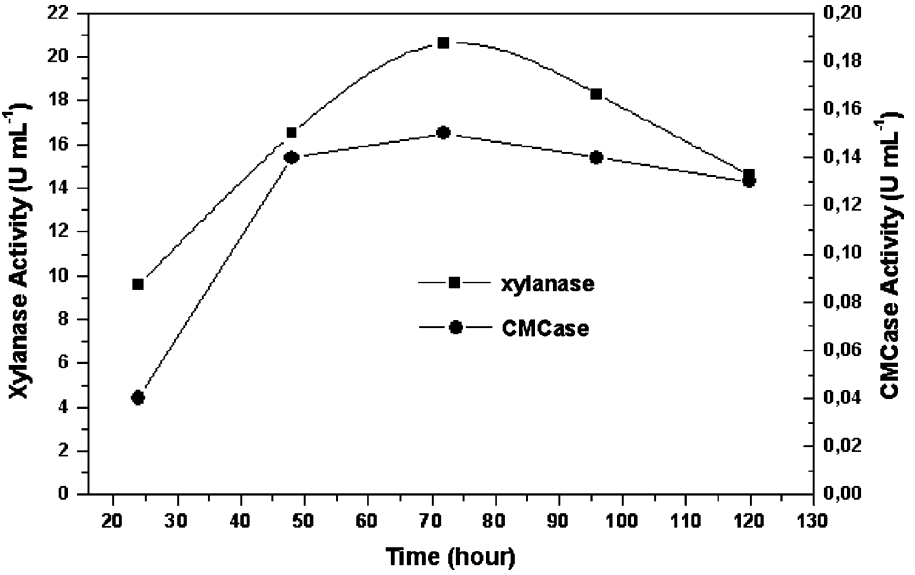

Fig. 3 Time course of xylanase and CMCase production from *Neosartorya spinosa* strain P2D19 at solid-state fermentation using wheat bran as substrate

Physico-Chemical Characteristics

Enzymatic characteristics were determined on crude xylanase produced by submerged fermentation using corn straw as carbon source for *Lysinibacillus* sp. P5B1. The optimal temperature and pH reaction of this xylanase were 60 °C and pH 6.0, as shown in Fig. 4a and b. This xylanase was inactive above 50 °C and 90% of your activity was maintained at 55 °C, as shown in Fig. 4a. Figure 4b shows that the enzyme lost stability below pH 4.5 and above pH 10.0. Because of these properties, activity and stability xylanase, at higher pH and temperature, it is expected to increase the enzyme suitability for application in the paper and pump industry [40].

Enzymatic properties were determined on crude xylanase produced by solid-state fermentation using wheat bran as carbon source for *N. spinosa* P2D19. The optimal temperature activity was 60 °C and this xylanase was stable until 55 °C after 1 h of heat (Fig. 5a). The optimal pH was pH 5.0–5.5, but 87% of activity was maintained on pH 7.5 (Fig. 5b). So, in optimal pH activity, it was seen at two peaks of pH. It may suggest that there are two proteins with distinct catalytic activities or that the same enzyme is capable of acting at different pH values. On the other hand, these two peaks of pH can be related to isoenzymes. Badhan [41], studying xylanase produced by *Myceliophthora* sp., found different xylanase isoforms using an isoelectric foccusing and these isoforms are dependent on the substrate type used on solid-state fermentation. More studies are necessary for accurate conclusions about this proposed work, because it is obtained of crude enzyme and purification assays were not realized yet. Based on performed analyses, this xylanase was stable between pH 5.5 to pH 8.5 after 24 h. (Fig. 5b).

Both xylanase studied, bacterial, and fungal strains, show similarity in physic and chemical characteristics. However, the fermentation conditions were different. So, some studies searching for better knowledge about these xylanases production and purification

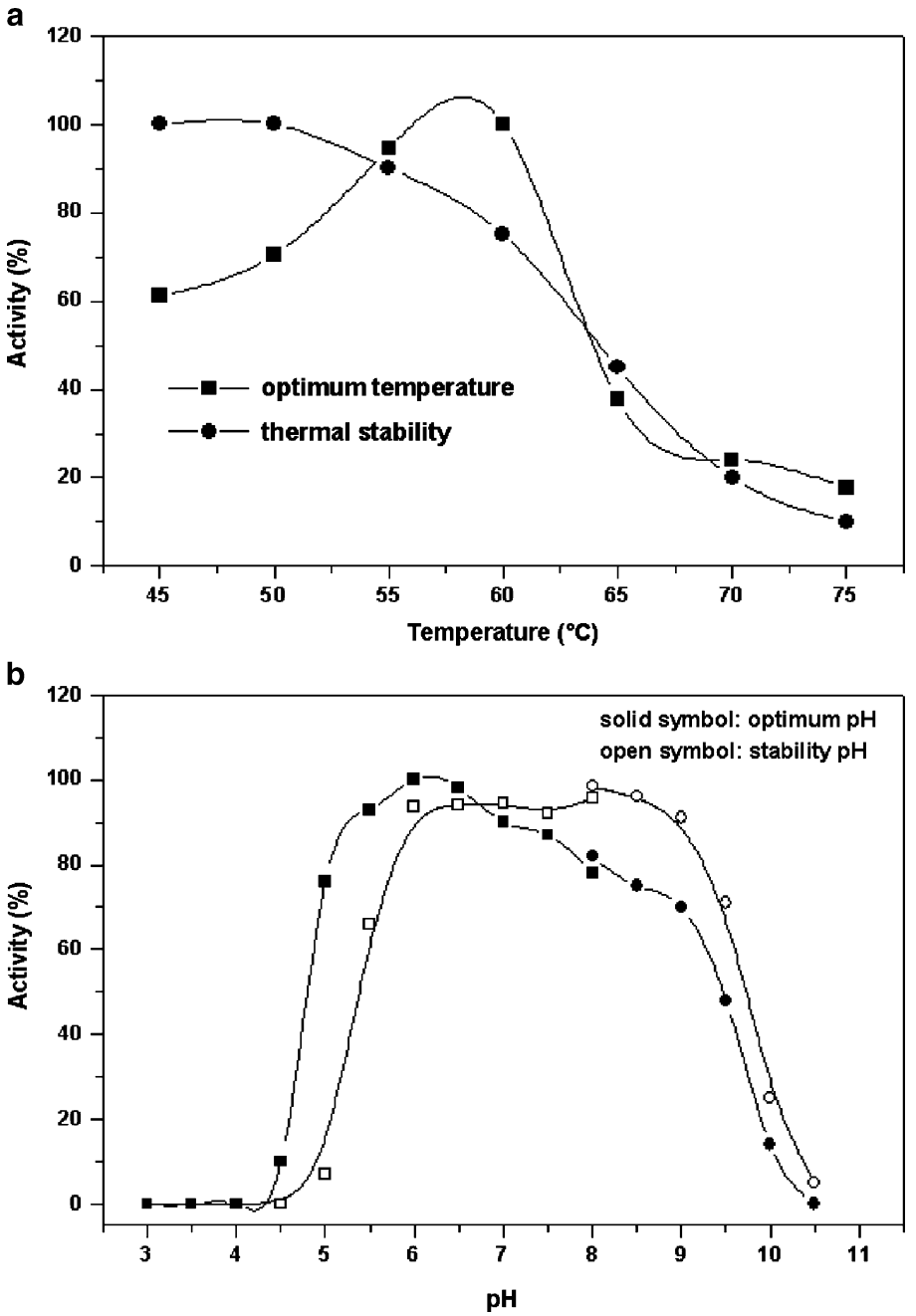

Fig. 4 Effect of temperature (**a**) and pH (**b**) on xylanase activity and xylanase stability from *Lysinibacillus* sp. strain P5B1. The buffers used were: McIlvaine (*filled square* pH 3.0–8.0) and glycine–NaOH (*filled circle* pH 8.0–10.5)

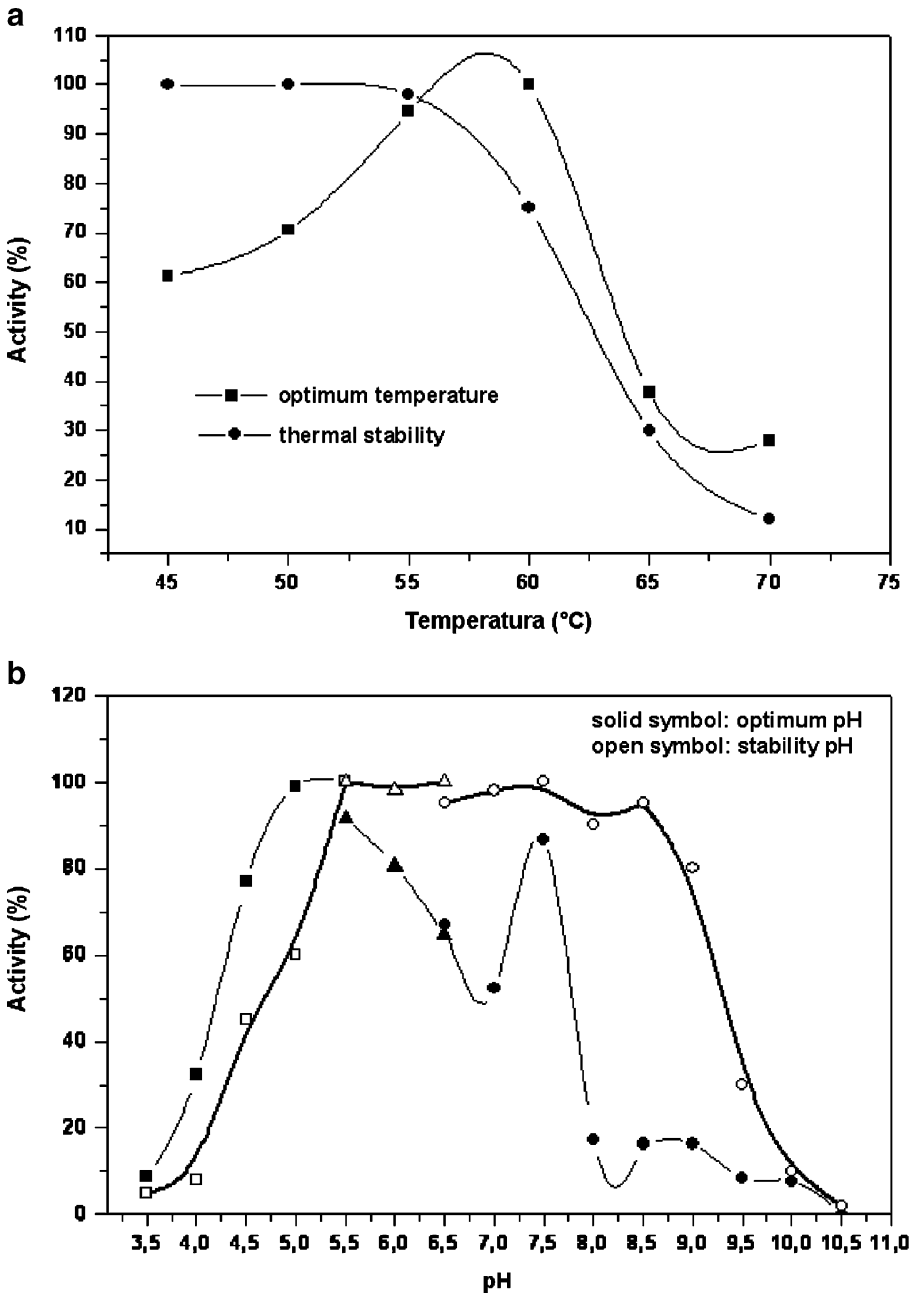

Fig. 5 Effect of pH (**a**) and temperature (**b**) on xylanase activity and xylanase stability from *Neosartorya spinosa* strain P2D19. The buffers used were: Acetate (*filled square* pH 3.5–5.5), MES (*filled triangle* pH 5.5–6.5) and Glycine–NaOH (*filled circle* pH 7.0–10.5)

of these xylanases are necessary to indicate the industry application potential of these enzymes.

Conclusion

Brazilian Cerrado has a high diversity of microorganisms. Bacterial and fungal strains xylanase were isolated and the xylanase production from *Lysinibacillus* sp. strain P5B1 and *N. spinosa* strain P2D19 were studied. In this case, the identification microorganism processes indicate that *Lysinibacillus* sp. strain P5B1 is a new bacterial species. For fungal species, it was not reported yet about xylanase production by *N. spinosa*. So, these are important contributions of the enzyme research. These results also demonstrate that it is very important microorganism screening studies in different environments such as the Brazilian Cerrado. Corn straw was used in screening and isolation media for xylanase producer microorganisms. *Lysinibacillus* sp. strain P5B1 shows a good xylanase activity in submerse fermentation using corn straw as carbon source. *N. spinosa* strain P2D19 shows a better xylanase production at solid-state fermentation using different substrates as a carbon source. Wheat bran and corncob were agro-industrial residues with better enzymatic activity than other analyzed residues. The xylanase activity was high at 72 h fermentation, and it was characterized. The optimum pH was 5.0–5.5 and 86% of residual activity was maintained on pH 7.5. Optimum temperature was determined at 60 °C. So, the fungus *N. spinosa* strain P2D19 and the bacterium *Lysinibacillus* sp. strain P5B1 could be considered as target microorganisms for enzymatic production studies.

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