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Amazonian Lignocellulosic Materials-V

Screening of Xylanolytic Fungi

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ABSTRACT

A plate-agar technique for fungal screening was applied to evaluate the xylanolytic activities of 18 *Penicillium janthinellum* and 10 *Aspergillus sydowi* species from the Amazon region. In order to compare these genera with those of other regions, one *Aspergillus sp.*, one *P. janthinellum*, and 12 unknown genera from the southern region of Chile were studied. From these fungi strain, *A. sydowi* (56 strain) (25.2 IU/mL), *P. janthinellum* (671 strain) (47.3 IU/mL) from Amazonia, *P. janthinellum* (X4Z2 strain) (9.5 IU/mL), and an *Aspergillus sp.* (X2M1 strain) (33.3 IU/mL) from the southern region of Chile were identified.

Index Entries: Xilanases; xylosidases; *Aspergillus, Penicillium,* Amazonia.

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Table 1
Enzyme Activities in Solid Medium from Different
A. sydowi Strains from Sawdust Pile from Manaus Area

	β-Xylanase		β-Xylosidase	
Strain	IU/mL	IUt/g[a]	IU/L	IUt/g[a]
29-C	12.8	301.8	39	0.95
56	25.2	855.2	56	1.89
131-C	16.4	495.5	44	1.32
199	25.0	658.9	39	1.02
178	21.9	651.7	36	1.07
234-B	15.1	414.7	46	1.26
287-D	21.4	629.4	50	1.47
531	11.5	374.1	38	1.23
570-F	23.1	446.2	54	1.04
661	25.0	587.7	47	1.10

[a]IUt/g is total units in 15 mL of buffer/g of dry wt of agar-mycellium.

INTRODUCTION

In previous screenings of fungi from the Amazon region, many interesting industrial enzymes were selected (*1–3*). Owing to the importance of xylanase in the pulp and paper industry, several new xylanases were produced commercially, e.g., Cartazyme and Pulpzyme (*4,5*). Recently, Asperzyme 2M1 was isolated from *Aspergillus sp.* (*6*), and Penjanzyme AA and AB from *Penicillium janthinellum* (87M-115 strain) (*7,8*) were tested on soft and hardwood Kraft pulps (*9*). Hence, it is important to select new fungi from different regions in which the possibility of finding xylanases with different characteristics is more likely (*10*). The aim of this work was to identify strains of different *Penicillium* and *Aspergillus* that are known to produce active, well-characterized xylanase complexes capable of attacking most lignocellulosic substances (*11–13*).

MATERIAL AND METHODS

Collection of Fungi

The fungi were collected from sawdust piles in Manaus, Amazonia, Brazil (Table 1; *Aspergillus sydowi* strains; Table 2, *Penicillium janthinellum* strains) and from the petroleum-producing region in Urucu, Amazonia (Table 3, *P. janthinellum* strains), and isolated by perfusion or dilution techniques as previously described (*3,14*). Nine samples of wood and vegetal soil in decomposition from the X and VII Regions in Chile were collected.

Table 2
Enzyme Activities of Different *P. janthinellum* Strains
Grown in Solid Media Containing Xylan from Sawdust Pile from Manaus Area

Strains[a]	β-Xylanase		β-Xylosidase		β-Glucosidase, IU/L
	IU/mL	IUt/g[b]	IU/L	IUt/g[b]	
011-B	18.7	958.9	13.0	0.67	—
012-B	13.9	1082.9	2.1	0.16	—
1683-D	2.0	131.1	0.6	0.04	—
1682-D	1.9	142.9	0.0	0.00	—
1681-D	2.7	252.6	0.0	0.00	—
132-D	20.8	1444.4	0.0	0.00	23.0
1321-D	19.5	1546.6	0.0	0.00	—
100-B	8.2	955.6	290.0	32.64	244.0
132-D	12.5	725.1	0.0	0.00	—
168-D	6.8	329.9	5.0	0.24	21.0
0.1-B	13.3	719.5	0.0	0.00	71.0
28	14.1	768.2	23.0	1.25	233.0

[a]Numbers with no additional letter mean perfusion method and with additional letter mean dilution method of isolation.
[b]Ut/g is total units in 15 mL of buffer/g of dry wt of agar-mycellium.

Table 3
Enzyme Activities in Solid and Liquid Culture
of *P. janthinellum* Strains from Sawdust Pile in Manaus Area

Strains	β-Xylanase		β-Xylosidase		β-Glucosidase	
	Liquid, IU/mL	Solid, IU/mL	Liquid, IU/L	Solid, IU/L	Liquid, IU/L	Solid, IU/L
011-B	31.0	18.7	56.0	13.0	29.0	50.0
012-B	15.0	13.9	34.0	2.0	70.0	34.0
1683-D	5.3	2.0	26.0	0.6	29.0	14.0
1682-D	32.7	1.9	31.0	0.0	43.0	29.0
1681-D	22.8	2.7	158.0	0.0	85.0	41.0
132-D	17.8	20.8	45.0	0.0	34.0	37.0
1321-D	34.7	19.4	0.0	0.0	4.0	0.0

Culture Medium

Collection from Amazonia

The isolation technique has been previously described (*14*), and the following media were used: Agar-Sabouraud, agar-malt-yeast extract and potato dextrose-agar.

Table 4
Xylanase Activities in Solid State from Different *P. janthinellum* Strains from Petroleum Well Area

	β-Xylanase			
Strains[a]	IU/mL	IUt, IU/mL × 15 mL	Agar-Mycellium mass, g	IUt/g[b]
11433-2	27.3	409.3	0.366	1121.6
11422-1-1	21.6	323.3	0.386	838.1
11422-1-2	17.8	267.3	0.316	845.4
11433-2-98	25.5	383.1	0.432	886.2
671-PET-1	47.3	709.7	0.398	1783.5
671-CY1	16.3	243.7	0.381	640.4

[a]Dilution method.
[b]Ut/g is total units in 15 mL of buffer/g of dry wt of agar-mycellium.

Collection from Chile

Ten grams of each soil sample were stirred for 1 h with 90 mL of sterile salt solution with 0.25 mg/mL of chloramphenicol. From this solution, aliquots of 0.1 mL were placed in a series of Petri plates with Sabouraud agar medium and glucose, and incubated at 28°C for 7 d. The spores obtained were transferred to other plates with Sabouraud medium and again incubated for 7 d. This process was repeated until the isolates were pure. The purified fungi were transferred to plates that contained agar-Vogel (*15*) solution with birchwood xylan (1%) as inductor.

Enzyme Production

A recent method for growth was followed (*1*).

Solid Medium

The isolated fungi were cultivated in plates containing 20 mL of culture medium: Agar (2%), Vogel medium (*15*) with 1% of birchwood xylan as the carbon source in 50 m*M* phosphate buffer, pH 6.0, at 28°C, for 4 d. After fungal growth, the plate was divided into two halves. One-half of the solid agar was cut off and extracted with 15 mL of 20 m*M* phosphate buffer, pH 6.0, or 15 mL of 50 m*M* acetate buffer, pH 4.8, and agitated at 150 rpm by 30 min. The heterogeneous solution was filtered, and the enzymatic activities were measured. The second half of the plate (agar-fungi) was dried to constant weight. Then the activity in these cases was described as: total units of activity by 15 mL of 20 m*M* buffer divided by the solid weight of dry agar-mycellium mass of a half plate (IU/mL × 15 mL/g of d.a.m. = IUt/g, *see* Table 4 as example). The same procedure was followed when other xylans as the carbon source were used.

Liquid Medium

Fifty milliliters of Vogel solution in the presence of 1% xylan as the carbon source and 50 m*M* phosphate buffer, pH 6.0, in 250-mL Erlenmeyer flasks were used. The growth phase was initiated by inoculation of 1 mL of spores in 10^7–10^8 U/mL. The incubation was carried out in an orbital shaker at 150 rpm at 28°C for 4 d.

Birch Xylan Solutions

The birch xylan solution was prepared by the standard method (*16*): 1 g birch xylan/80 mL 50 m*M* acetate buffer, pH 5.3, was heated to boiling, cooling with stirring, left over night, diluted to 100 mL with buffer, and then stored at 4°C.

Enzymatic Assays

The β-xylanase was determined by measuring the reducing sugars (*17*) by the standard method (*16*). The xylanase activity was measured as the xylose equivalents/min/mL (μmol/mL/min, IU).

β-xylosidase and β-glucosidase activities were determined by measuring the *p*-nitrophenol released by the enzyme from *p*-nitrophenyl-β-D-xylopyranoside and from *p*-nitrophenyl-β-D-glucopyranoside at 50°C with 50 m*M* acetate buffer, pH 5.0 (*18*). Cellulase activity was measured by a published method (*19*). Protease activity was measured with azocasein as the substrate. One unit of protease is the amount of enzyme that catalyzes the release of azo dye by changing 0.001 OD/min (*14*).

All the experiments were carried out in triplicate and the values on tables are the average ones (< 2% error was found in all the measurements).

RESULTS AND DISCUSSIONS

Table 1 summarizes the β-xylanase and β-xylosidase activities in a solid medium for the *A. sydowi* strains isolated from a sawdust pile. All of the fungi, except 29C and 531, demonstrated high xylanase activities. The *A. sydowi* 56 strain showed the highest xylanase activity, as well as the largest β-xylosidase activity. Cellulase and protease activities were absent or in a very low amounts (not shown), and in view of these results, the *A. sydowi* 56 strain appeared as the most convenient strain for xylanase application in biobleaching of Kraft pulps.

Since the *P. janthinellum* strain produced higher activities (*12*), the present screening was restricted to members of this species. Twelve different *P. janthinellum* strains were taken from a sawdust pile from the Manaus region (Table 2). The highest levels of xylanases were isolated from 132-D and 011-B. The *P. janthinellum* strains, however, showed lower

β-xylosidase activities than *Aspergillus*. The 100-B strain, which produces higher β-xylosidase than the others strains (290 IU/L) is interesting, but it was eliminated from further study because it also produced the same level of β-glucosidase (not shown). In cases where there was not a complete breakdown of xylan to xylose, another source of β-xylosidase, such as that from 100-B strain, could be added to complete xylan degradation.

Therefore, both *P. janthinellum* or *A. sydowi* are good xylanolytic fungi, but the former was more efficient in total units by grams of agar-mycellium than the *A. sydowi*. Although *A. sydowi* presented differences between strains (i.e., from 301.8 IUt/g d.a.m. to 855.2 IUt/g d.a.m.), the differences between the *P. janthinellum* strains were more significant (i.e., from 131.1 IUt/g d.a.m. to 1546.4 IUt/g d.a.m.).

Table 3 shows the comparison between solid and liquid cultures for some of the *P. janthinellum* strains. In control experiments with different culture, broth volumes in the solid plates did not change the actual value of xylanase activity. Also, the extraction efficiency was almost 100% in the first extraction with 15 mL of buffer and shaking. Then, it was possible to generalize in these cases. For *P. janthinellum* strains, the liquid medium was more convenient for enzyme production, except for the 132-D strain in which higher xylanase activity in solid than in the liquid culture was observed.

Table 4 shows that the same fungi species as in Table 2, isolated from petroleum oil fields in the Urucu area in Amazonia, exhibited different xylanase activity characteristics. The best strain from the oil field area was 671-PET-1 for xylanase production, and all strains, except 671-CY1, exhibited higher activities (IU/mL) than the *P. janthinellum* strains isolated from sawdust piles.

Since the *P. janthinellum* strains from the Amazon region were more efficient producers of xylanases, experiments were carried out in the southern region of Chile, where low temperatures were common at the time of collection (July to August), for purposes of comparing the potential of xylanase production of these genera (i.e., *Aspergillus* and *Penicillium*) in warm and cold regions.

Table 5 shows the enzymatic activities from fungi grown in the cold region of Chile. The most efficient *Aspergillus* strain was 2M1 and an unknown genera XM3. The *Aspergillus sp.* (2M1 strain), although not characterized as to which species it belongs, exhibited a higher activity than all the *Aspergillus* in Table 1. *P. janthinellum* (X4Z2 strain) from Chile exhibited lower activity than all those isolated from the petroleum area and the majority of those from sawdust piles from Amazon region.

With these strains, a comparison of solid and liquid cultures indicated that for *Aspergillus sp.* (X2M1 strain) for the unknown strain (CP1 strain), the liquid culture was more convenient than the solid medium (Table 6). The lack of a correlation in the enzymatic activities of solid and liquid cultures (Tables 3 and 6) is owing to the nature of the fungi and not to the technique used (*20*).

Table 5
Screening of Enzymatic Activities Carried Out on Isolated Fungi from Soil and Wood in Chile

Fungi	β-Xylanase[a]		β-Xylosidase	
	IU/mL	IUt/g[b]	IU/L	IUt/g[b]
P. janthinellum X4Z2	9.5	501	35.0	1.7
Aspergillus sp. X2M1	33.3	1891	11.0	0.5
Unknown genera				
X292	10.8	503	19.7	0.7
X3Z1	9.8	495	16.0	0.5
X2Z1	1.1	52	0.8	0.04
X1M2	5.0	121	24.9	1.3
XM3	35.0	1953	11.3	0.8
X1M1	5.5	292	101.0	5.5
C4Z3	1.4	76	3.0	0.2
CP1	16.1	896	3.9	0.2
CP2	12.3	790	35.7	2.2
CP3	3.6	52	6.7	0.1
S392	0.5	31	0.6	0.04
S3Z3	7.5	437	223.0	13.0

[a]Birch xylan as substrate.
[b]Ut/g is total units in 15 mL of buffer/g of dried agar-mycellium.

Table 6
Comparison of the Enzymatic Activities Found in the Solid and Liquid Medium

Solid culture agar plates	β-Xylanase,[a] IU/mL		β-Xylosidase IU/L		β-Glucosidase IU/L	
	Solid	Liquid	Solid	Liquid	Solid	Liquid
Aspergilus sp. (X2M1 strain)	33	127	11	13	33	26
Unknown strains:						
CP1	16	59	4	1	37	1
CP2	12	6	36	20	43	23

[a]Birch xylan as substrate.

In summary, a screening using the plate method (*20*) is perfectly applicable to a large number of genera and species of different fungi for a specific enzyme selection and for biodiversity studies in any kind of region. Since xylanases are essential for the degradation of xylans, the results of this selection suggest that some of the *A. sydowi*, notably the *A. sydowi* 56 strain, the *P. janthinellum* 1321-D strain from Amazonia, and *Aspergillus sp.* X2M1 strain from Chile may be of commercial interest as sources of extracellular xylanases and are suitable for strain improvement studies for the pulp biobleaching in the pulp and paper industry.

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