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Enzymes for second generation ethanol: exploring new strategies for the use of xylose

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Considering that the cost of cellulases and hemicellulases substantially contributes to the price of 2nd-generation ethanol, reducing the cost of the substances used as inducers of cellulose and xylose is essential to reducing the cost of these enzymes. *Penicillium echinulatum* is able to secrete most hemicellulolytic and cellulolytic enzymes. In this context, the aim of this work was to evaluate the use of xylose for the production of cellulases and xylanases by two mutant strains of *P. echinulatum* (9A02S1 and S1M29) and the parental 2HH. Xylose acts as an inducer for the production of xylanases and cellulases, especially endoglucanases. These data indicate the possibility of using the pentose xylose, which is not used by the majority of yeast for ethanol production, as a total or partial substitute in cellulose-based processes for the production of enzymes for enzymatic hydrolysis.

1. Introduction

Given the current energy demand, the possibility of using ethanol instead of gasoline in combustion engines is becoming attractive. This would have the added benefit of reducing the emission levels of greenhouse gases.[1,2] Second-generation ethanol is one of the most attractive alternative biofuels. However, the high cost of hydrolyzing lignocellulosic polysaccharides into fermentable sugars remains a major obstacle for cost-effective ethanol production.[3–6] Because the costs of lignocellulolytic enzymes, cellulases and xylanases contribute substantially to the price of 2nd-generation ethanol, cheaper sources of these enzymes are preferred.[3–5,7]

Cellulase is the generic name for a group of enzymes that catalyze the hydrolysis of cellulose and related cellooligosaccharides. The synergistic action of three types of enzymes, namely, endo-1-4-β-glucanase (EG, EC 3.2.1.4), cellobiohydrolase (CBH, EC 3.2.1.91) and β-glucosidase (BGL, EC 3.2.1.21), is vital for complete enzymatic hydrolysis.[2,8]

Xylanases (1,4-β-D-xylan xylanohydrolases, EC 3.2.1.8) are a group of enzymes produced by fungi, yeast and bacteria that are used in the production of animal feed and pulp as well as in food processing. These enzymes play an important role in the hydrolysis of xylan, a polysaccharide formed of xylose monomers and one of the major components of hemicellulose.[9]

Many filamentous fungi secrete cellulases that have applications in industrial processes.[10] Among the cellulase- and xylanase-producing microorganisms, *Penicillium echinulatum* shows excellent potential for the secretion of enzyme complexes that enable the efficient enzymatic conversion of biomass into ethanol. *P. echinulatum* mutants are able to secrete high cellulase titers in both submerged fermentation[11,12] and solid-state fermentation.[1] Moreover, the Filter Paper Activity (FPA) and β-glucosidases of *P. echinulatum* have relatively good thermal stability at 50 °C,[13] and the cellulase complex presents a ratio of FPA to β-glucosidase that favors more efficient hydrolysis of cellulose when compared to the cellulases of *Trichoderma reesei*.[8]

After hydrolysis of the lignocellulosic material, sugar fermentation is carried out.[14] Some of the bottlenecks in the production of 2nd-generation ethanol include the limitations of xylose fermentation and the high cost of cellulases and xylanases. Knowing that xylose is an inducer of the cellulase and xylanase genes in *Aspergillus niger*[15] and *T. reesei*,[16] this study evaluated the use of xylose for the production of cellulases and xylanases by two mutant strains of *P. echinulatum* (9A02S1 and S1M29) and the wild-type 2HH to utilize the xylose derived from the hydrolysis of hemicellulose present in lignocellulosic material, and reduce the production costs of the enzymes required for the production of 2nd-generation ethanol. It is possible to use xylose in association with cellulose sources to produce enzymes that contribute to the reduction of the cellulose concentration in media, which at high concentrations can cause rheological and oxygen-transfer problems.[17,18]

2. Methods

2.1 Microorganisms

The mutant *P. echinulatum* strains 9A02S1 (DSM 18942) and S1M29 were used for the production of cellulases and xylanases.

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These strains were obtained by exposing the wild-type *P. echinulatum* strain 2HH to ultraviolet (UV) light and hydrogen peroxide (H_2O_2).[11,12] The wild-type strain 2HH was also evaluated. These strains are stored in the culture collection of the Enzyme and Biomass Laboratory of the Institute of Biotechnology at the University of Caxias do Sul, Brazil. The cultures were grown on C-agar slants for up to 7 days at 28 °C until conidia were formed[12] and then stored at 4 °C until use.

2.2 Production of cellulases and xylanases

The production medium used as a basis for the remaining formulations had the following composition: 0.2% (w/v) peptone, 0.05% (w/v) Prodex® crude yeast extract, 1% (w/v) carbon source (xylose Sigma®, glucose Quimidrol® or cellulose Celuloflok 200®, Cotia, SP), 0.1% (v/v) Tween 80®, 0.002% (v/v) ciprofloxacin (Proflox®, EMS S/A), 5% (v/v) mineral solution prepared as described by Mandels and Reese,[19] and distilled water to a final volume of 100 mL.

Xylose was used to investigate the possibility of using this pentose to produce cellulases and xylanases. Glucose was used as a negative control since it is known to be a repressor source, and cellulose was used as a positive control since it is known to be an inducer source.

Erlenmeyer flasks (500 mL) containing 100 mL of production medium were inoculated with a spore suspension containing 1×10^7 spores and kept at 28 °C with reciprocal shaking at 180 rpm for 120 h. All experiments were performed in triplicate, and samples were collected once a day. The samples were refrigerated, and sodium azide was added to a final concentration of 0.02% (w/v).[11]

2.3 Cell growth

The growth of cultures containing soluble carbon sources was determined gravimetrically. In the samples containing cellulose, growth was estimated by measuring the *N*-acetyl-D-glucosamine levels. The fungal biomass was determined indirectly by enzymatic hydrolysis of the chitin in the cell wall. For this procedure, 1 mL of washed sample was kept at 40 °C for 24 h. After this, 3 mL of citrate buffer (pH 4.8, 0.05 M) was added, and the samples were treated with ultrasound for 60 min. Two milliliters of 30% Viscozyme® L was added, and the solution was incubated for 24 h at 45 °C. After this period, the samples were boiled for 10 min to deactivate the enzyme. The samples were cooled and centrifuged (4000 rpm, at 4 °C during 30 min).

For the determination of *N*-acetyl-D-glucosamine resulting from the hydrolysis of chitin, the methodology of Aidoo *et al.*[20] was used. One milliliter of acetylacetone solution (0.75 mL of acetylacetone brought to 25 mL with a solution of 1.25 N sodium carbonate) was added to tubes containing 1 mL of the hydrolyzed and centrifuged sample. Samples were boiled for 20 min. After cooling, 6 mL of ethanol and 1 mL of 4-dimethylaminobenzaldehyde (3.2 g in 20 mL of ethanol and 20 mL of HCl) were added. The tubes were incubated at 65 °C for 10 min, and the absorbance was read at 530 nm. The concentration of *N*-acetyl-D-glucosamine was determined from a standard curve constructed with 0.5 to 1 mg mL^{-1} *N*-acetyl-D-glucosamine and, to show the data in mycelial mass, correlation was made with a standard curve prepared with different concentrations of *P. echinulatum* mycelium grown in medium with glucose (source of soluble carbon) instead of cellulose (insoluble source).

2.4 Enzymatic assays

Enzyme activity was analyzed with Whatman no. 1 filter paper, as described by Mandels *et al.*[21] and Camassola and Dillon.[22] Endoglucanase activity was determined according to Ghose[23] using 2% carboxymethyl cellulose in 0.05 M sodium citrate buffer (pH 4.8). β-Glucosidase activity was measured using ρ-nitrophenyl-β-D-glucopyranoside.[24] The xylanase activities were determined according to Bailey *et al.*[25] The concentrations of reducing sugars were estimated using dinitrosalicylic acid according to Miller.[26] One unit of enzyme activity was defined as 1 μmol of reducing sugar released per min under the assay conditions.

2.5 Zymogram activity

SDS-polyacrylamide gel electrophoresis (PAGE) was performed as described by Laemmli.[27] The samples were prepared with the same enzymatic broth volume for each substrate. After electrophoresis on a 12% separating gel, the gel was stained with Coomassie Blue dye in an ethanol–acetic acid–water solution (5 : 1 : 4, by volume) for 20 min and destained in the same solution without dye. To determine endoglucanase activity, the polyacrylamide gels were prepared with 0.2% carboxymethyl cellulose, stained with 0.1% Congo red and developed with 1 M NaCl. To determine xylanase activity, the polyacrylamide gels were prepared with 0.1% oat spelt xylan and stained with the same procedure used for endoglucanases. Clear bands against a red background indicated the breakdown of carbohydrates.[28] For β-glucosidase activity, the polyacrylamide gels were treated with 0.2% esculin and developed with 1% ferric chloride.[28] The electrophoretic runs were performed at 200 V for approximately 1 h. To indirectly characterize the position of a band, not to determine the molecular mass, a solution of molecular weight markers (Bio-Rad, USA) was used. The position of a band was associated with the position of a marker and not with its molecular mass.

2.6 Statistical treatment

The results were subjected to analysis of variance with Tukey's *post hoc* test with $p < 0.05$ considered statistically significant using the Prism GraphPad program (GraphPad, San Diego, CA).

3. Results

To secrete xylanases and cellulases, filamentous fungi require an inducer substrate, such as xylan and/or cellulose. In this study, the potential for the induction of xylanases and cellulases was evaluated in *P. echinulatum* employing the xylose in comparison to cellulose, a known inducer and carbon source, and glucose, a carbon source known to repress cellulase and xylanase production.

3.1 Production of xylanases

Xylanase production was detected for all carbon sources evaluated, although the activity profiles were different for each carbon source and strain (Fig. 1A–C).

In the medium formulated with cellulose, the mutant strains had the highest xylanase activity, reaching values of 9.35 ± 0.09 IU mL^{-1} and 9.00 ± 1.07 IU mL^{-1} for 9A02S1 and S1M29, respectively. No xylanase activity was detected in the wild strain (Fig. 1A). These data were corroborated by activity gels (Fig. 2). Based on the zymograms, it is possible that some enzymes, including xylanases, were differentially expressed between the two mutant strains; for strain 9A02S1, there were bands with a molecular weight of approximately 20 kDa after 44 and 68 h. These bands were not detected for strain S1M29 (Fig. 2A).

In the medium supplemented with glucose, at 24 h, the 2HH wild-type strain showed activity of approximately 5 IU mL^{-1}. Similar activity in the mutant strains was found only after 44 h (Fig. 1B). After this time, no significant activity was detected in the zymograms for the mutant strains (Fig. 2B). When the carbon source was xylose (Fig. 1C), at 20 h, the wild-type strain had a xylanase activity of 16.62 ± 0.54 IU mL^{-1}, and the mutant S1M29 showed an activity of 14.36 ± 2.96 IU mL^{-1}, whereas the mutant 9A02S1 exhibited a lower value (6.26 ± 0.81 IU mL^{-1}). At 48 h, the activities decreased to less than 5 IU mL^{-1}. The zymograms verified the expression of xylanase for all evaluated strains, indicating that xylose and cellulose act as inducers of the production of xylanases (Fig. 2C).

Comparing the enzymatic activities with mycelial growth, there was a direct relationship between the growth and production of xylanases (Fig. 3). In Fig. 3, especially in Fig. 3B, it is possibly observed that wild-type parental strain showed large growth in comparison to the mutant strains. It is possible that these mutants produce more soluble proteins (Fig. 2G–I) than structural proteins.

The different expression levels of xylanases with different carbon sources in the cultivation media for the different tested strains originating from the same wild-type parental strain indicate that there is alteration in both transcription and translation in relation to the expression of xylanases in the tested strains.

3.2 Production of endoglucanases

The mutant strains showed peak endoglucanase activity in the medium with cellulose. Both strains showed peak activity of about 1.60 IU mL^{-1} at 92 h. In the parental 2HH strain, low endoglucanase activity was detected (Fig. 1D). However, higher expression was observed for strain S1M29 in the endoglucanase zymograms, including many bands of activity, suggesting that even though they occur in lower quantities, the endoglucanases of 9A02S1 are more active (Fig. 2D). Comparing the gels of strain 9A02S1 with those of wild-type 2HH, the bands were similar; however, the mutant showed greater intensity. In contrast, S1M29 exhibited the same bands in addition to bands with an approximate molecular mass of 50 kDa (Fig. 2D), indicating that

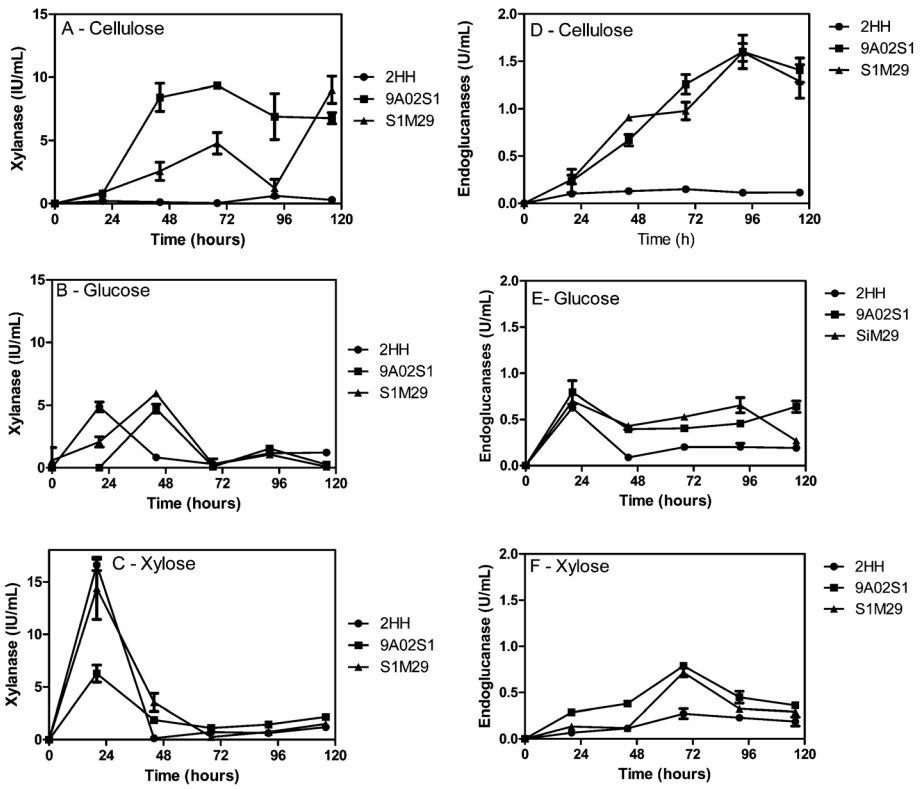

Fig. 1 Variations in xylanases and endoglucanases of different strains of *Penicillium echinulatum* in submerged cultivation in medium supplemented with cellulose, glucose and xylose.

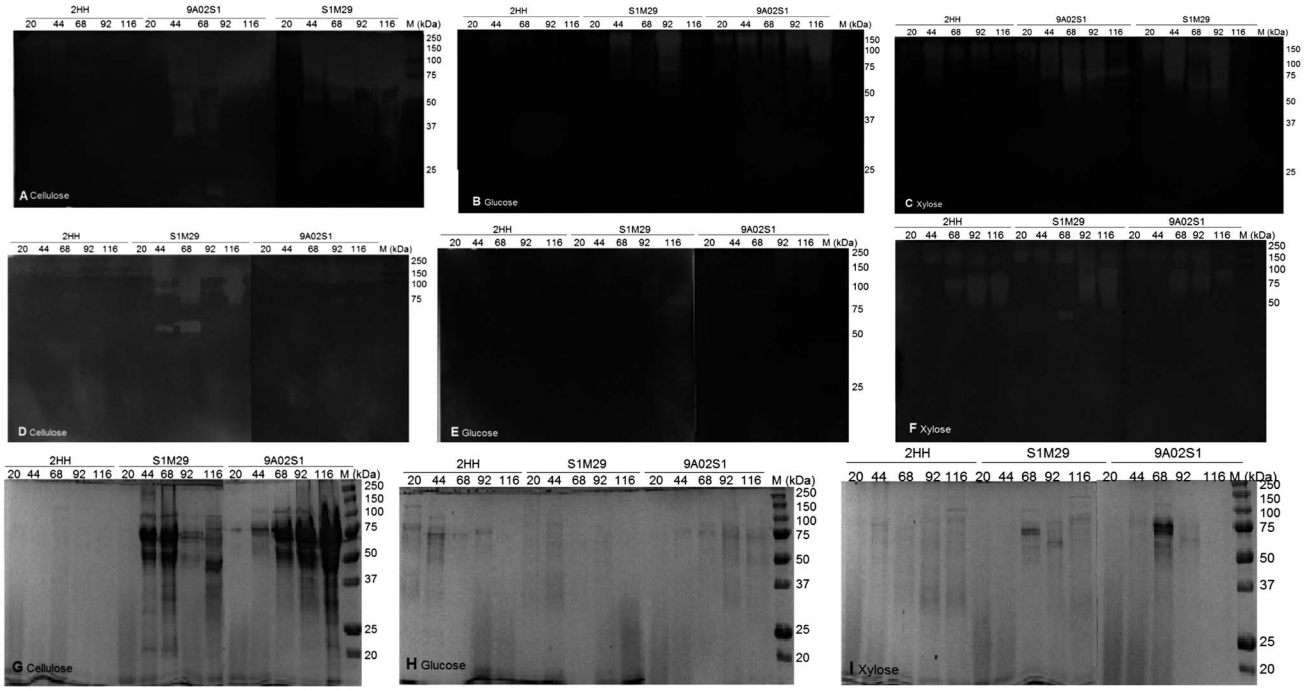

Fig. 2 Profiles of the xylanase (A–C) and endoglucanase (D–F) activities, and total protein (G–I) based on polyacrylamide gel electrophoresis of samples from submerged cultivation of different strains of *Penicillium echinulatum* using 1% of the noted carbon source. The numbers at the top of the each gel indicate the cultivation time in hours and the strain. M – molecular weight marker. The molecular weights of the markers are indicated on the right. The carbon source employed is indicated on each gel.

there are differences in post-translational processing in this strain compared to the wild type.

In culture medium supplemented with glucose, the highest endoglucanase activity of approximately 0.63 IU mL^{-1} was observed for the parental 2HH strain at 20 h, and the mutants 9A02S1 and S1M29 exhibited values of 0.79 $\pm$ 0.13 and 0.70 $\pm$ 0.02 IU mL^{-1}, respectively (Fig. 1E). In the zymogram, the bands are quite discrete; in the mutants, they are in the same positions throughout the experiment, while for the 2HH strains, these bands were only observed at 44 h (Fig. 2E). Bands were present in all strains in the presence of glucose, suggesting that the detected activities are constitutively expressed enzymes.

In medium supplemented with xylose, the parental 2HH showed low endoglucanase activities during all experiments. The mutant strains 9A02S1 and S1M29 showed the highest activities at 72 h with approximate values of 0.8 and 0.7 IU mL^{-1}, respectively (Fig. 1F). In the gel, activity was found for all bands in all strains at all times, including bands of different molecular weights from those observed in medium with cellulose and glucose (Fig. 2F). The bands had the greatest intensity at positions different from those observed in medium with cellulose and glucose, indicating that xylose acts as an inducer of the production of endoglucanases in *P. echinulatum* for both the mutant and wild-type strains evaluated.

Endoglucanase activity was correlated with growth in the presence of cellulose; activity was only detected at a certain level of biomass. The same correlation was not observed for xylose and glucose; the major activities in these media were detected in the presence of minor amounts of biomass (Fig. 3).

3.3 Production of filter paper activity (FPA)

In culture medium with cellulose, the mutant strains showed FPA at 44 h. The mutant S1M29 reached peak values of approximately 0.8 IU mL^{-1} at 116 h, and the mutant 9A02S1 peaked at 68 h at approximately 0.6 IU mL^{-1}. In the 2HH strain, FPA was not detected (Fig. 4A). These data can be related to the different profiles of protein production. Fig. 2G shows that the wild-type strain produced lower amounts of protein than the mutant strains.

In medium supplemented with glucose, the FPA was reduced in the first 20 h, with values near 0.2 IU mL^{-1} for all three strains (Fig. 4B). These data are attributed to reduced protein expression for all strains tested (Fig. 2H) because there is no need for the fungus to produce cellulases and xylanases in medium with a source of easily usable carbon such as glucose.

In medium formulated with xylose, the wild-type 2HH strain showed an FPA peak of 0.31 IU mL^{-1}, and strains 9A02S1 and S1M29 reached a peak of 0.20 IU mL^{-1} in the first 20 h. At 44 h, these values declined significantly in the parental 2HH to values of 0.06 IU mL^{-1}, and in the 9A02S1 and S1M29 mutants to values of 0.18 IU mL^{-1} and 0.14 IU mL^{-1}, respectively. After 68 h, the FPA was no longer significant (Fig. 4C). The total protein observed in the zymogram was higher in the medium formulated with glucose, especially at 44 h for the 2HH strain. However, the mutant strains exhibited a higher expression of proteins in the medium with glucose (Fig. 2I).

Comparing the FPA data for the three media evaluated, at 20 h, the activities of the three strains in the presence of glucose

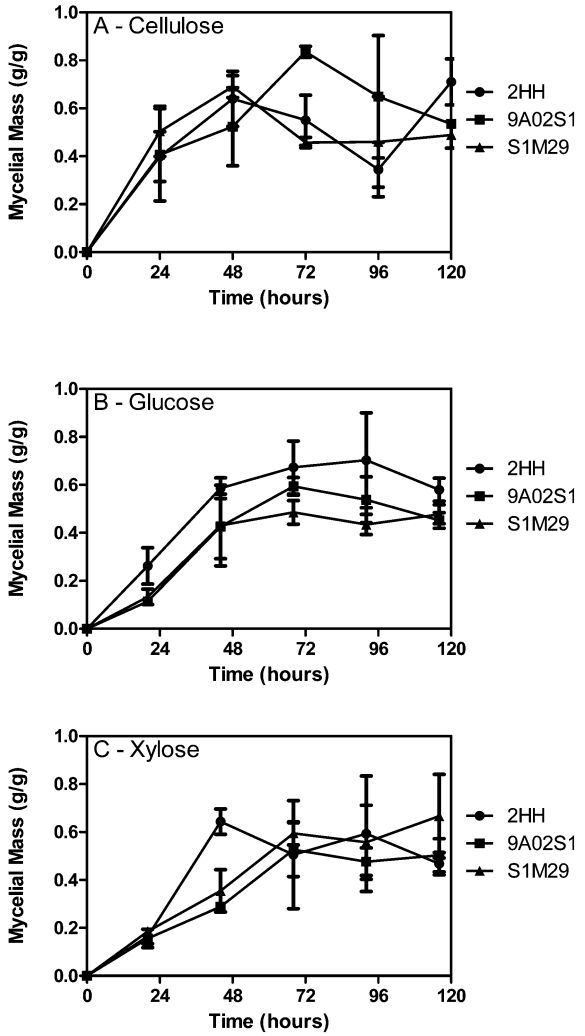

Fig. 3 Mycelial mass produced by different strains of *Penicillium echinulatum* in submerged cultures supplemented with cellulose (A), glucose (B) and xylose (C). The data are shown as grams of biomass per grams of carbon source.

and xylose were significantly higher than that obtained in the presence of cellulose (Fig. 4). Comparing only the media formulated with glucose and xylose, there was no significant difference in activity for the same strains with the same monosaccharides. As observed for xylanase and endoglucanase activity, the FPA data in the presence of cellulose are indicative of changes in the genome, transcriptome and proteome of the wild-type and mutant strains evaluated.

Although FPA activity was correlated with growth (Fig. 3), for xylose and glucose, the same relationship was not observed; the major activities in these media were detected in the presence of minor amounts of biomass (Fig. 3).

3.4 Production of β-glucosidases

In medium supplemented with cellulose, the mutants strains showed the highest β-glucosidase activity at 116 h, reaching approximately 0.13 and 0.36 IU mL^{-1} for 9A02S1 and S1M29, respectively, whereas the wild-type 2HH showed no significant activity (Fig. 4D). In the medium with glucose, the highest β-glucosidase activity was observed at 116 h. The values were 0.44 ± 0.05 (2HH), 0.36 ± 0.01 (S1M29) and 0.55 ± 0.02 (9A02S1) (Fig. 4E).

No β-glucosidase activity was detected before 44 h in the medium with xylose. At 116 h, activity was observed for the mutant S1M29, with values near 0.54 IU mL^{-1}. The wild-type and 9A02S1 mutant showed similar values of 0.13 IU mL^{-1} (Fig. 4F).

There was no correlation between β-glucosidase activity and growth in the medium supplemented with cellulose. Although the mycelial mass was constant, there was an increase in β-glucosidase activity (Fig. 3 and 4D–F). No bands of β-glucosidase activity were observed in the zymograms.

4. Discussion

The differences in production of cellulases and xylanases by different strains clearly indicate genetic differences among the strains resulting from the various stages of mutagenesis and protoplast fusion.[11,12]

P. echinulatum is an attractive producer of lignocellulolytic enzymes, and the production of the major extracellular enzymes (*i.e.*, cellulases)[1,8,11] is transcriptionally regulated and carbon-source dependent. Lignocellulolytic fungi secrete a complex arsenal of enzymes that synergistically deconstruct plant cell wall polysaccharides. The capacity of these enzyme cocktails to release usable sugars from non-food lignocellulosic material represents an opportunity for the development of a new generation of biofuels produced directly from plant biomass without extensive pre-treatment.[29]

The secretory behavior of this fungus depends strongly on the carbon source.[3] In fungi, lignocellulolytic enzyme production is tightly controlled at the transcriptional level by the competitive action of transcriptional activators and repressors.[30] In *Aspergillus nidulans*, *Hypocrea jecorina* and *Neurospora crassa*, the orthologous repressors CreA/Cre1 have been shown to block the transcription of genes associated with the utilization of alternate carbon sources, including cellulolytic and xylanolytic enzymes, when glucose is present.[31]

The utilization of xylose in the production medium as inductor of the production of enzyme or mycelial biomass is an important alternative use of this pentose because there are difficulties in obtaining a microorganism that can convert this sugar into ethanol. According to Witteveen *et al.*,[32] xylose, the main component of the xylan backbone, and other pentoses derived from branched chain xylans, such as arabinose, are assimilated by the pentose phosphate pathway. In *A. niger*, pentose catabolism includes a reduction and oxidation step leading to D-xylulose, which is converted to D-xylulose-5-phosphate and enters the pentose phosphate pathway. This information is supported by promoter analysis of genes with putative binding sites for XlnR and by the induction of their expression when *A. niger* grows on xylose.[33]

The data obtained in this study indicate that xylose acts as an inducer of xylanase and endoglucanase expression in *P. echinulatum*. These data are in agreement with data previously

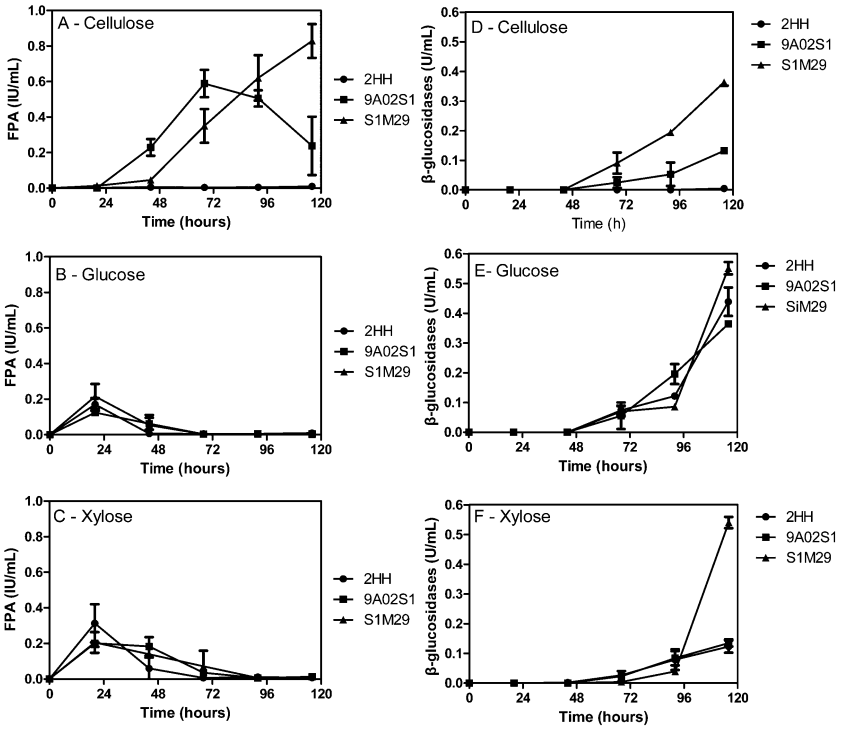

Fig. 4 Variations in the FPA and β-glucosidase activity of different strains of *Penicillium echinulatum* in submerged cultivation in medium supplemented with cellulose, glucose and xylose.

reported for *A. niger*. In *A. niger*, the expression of all major cellulases and hemicellulases is coregulated by the same inducer molecule (that is, D-xylose). The induction mechanisms in *T. reesei* are more diverse. At least four different inductor molecules (that is, D-xylose, xylobiose, sophorose and lactose) have been described, but none of them has the potential to trigger the expression of all of the primary cellulases and hemicellulases.[34] Herold *et al.*[16] observed that the *xyn3* gene is induced by D-xylose, in *T. reesei*.

In this study, glucose was found to repress the production of cellulases and xylanases; lower expression of these enzymes was verified in this medium. Similar results have been reported for other *Penicillium* and *Aspergillus* strains.[35,36] In *Aspergillus*, the protein CreA, which is also responsible for carbon catabolite repression, represses the expression of genes encoding cellulases and xylanases in the presence of glucose.[37] According to Chavez *et al.*,[35] a consensus sequence for CreA binding has been found in the promoters of cellulase and xylanase genes in *Penicillia*. However, looking at the β-glucosidase data, the results of this study indicate that xylose is either a repressor or an inductor of the expression of enzymes involved in lignocellulose degradation.

The results obtained for β-glucosidases in the *P. echinulatum* strains are different from those observed for three other *Penicillium* species (*P. pinophilum* IBT 4186, *P. persicinum* IBT 13226 and *P. brasilianum* IBT 20888). In these three species, Jorgensen *et al.*[36] observed low β-glucosidase activity in medium formulated with glucose. According to these authors, the regulation of the β-glucosidase genes either differs from the regulation of the endoglucanase genes, or the repression of these genes by glucose is less strong.

According to Zeilinger *et al.*,[38] cellulases and hemicellulases work synergistically to completely degrade biopolymeric substrates in *T. reesei*. In this particular breakdown process, these enzymes hydrolyze large compounds to form smaller, soluble oligo- and monosaccharides that either act directly as low-molecular-weight inducer substances (*e.g.*, xylobiose and xylose) or, according to Vaheri *et al.*,[39] are converted to their respective inducers (*e.g.*, sophorose) *via* the transglycosylation activity of some of these enzymes.

Xylose is known to be an inducer of xylanases in *T. reesei*, as was observed for *P. echinulatum*. Mach-Aigner *et al.*[40] demonstrated that the degree of xylanase-encoding gene induction strictly depends on the concentration of D-xylose in *T. reesei*. The optimal concentration was determined to be 0.5 to 1 mM for 3 h of cultivation. At higher concentrations of D-xylose, a reduced level of xylanase gene expression was observed. The authors also provide evidence that D-xylose concentration-dependent induction is antagonized by carbon catabolite repressor 1. This repressor mediates its influence on D-xylose indirectly, by reducing the expression of xylanase regulator 1, the main activator of most hydrolase-encoding genes.

Although enzyme production in *T. reesei* can be induced by a variety of carbohydrates, the range of technically applicable substrates is still limited because most carbon sources are too expensive for industrial fermentation.[41] Jun *et al.*[3] showed that both lactose and xylose can serve as an excellent growth substrate for *T. reesei*. Lactose induction resulted in higher

Table 1 Comparisons of enzyme production from different fungi grown on lignocellulosic materials[a]

Microorganism	Carbon source	Enzymatic activities (IU) and production time				Source
		FPA	Endoglucanase	β-Glucosidase	Xylanase	
P. echinulatum M29	Xylose	0.14 (44 h)	0.72 (68 h)	0.54 (116 h)	14.37 (20 h)	This work
P. echinulatum 9A02S1	Xylose	0.18 (44 h)	0.79 (68 h)	0.36 (116 h)	16.61 (20 h)	This work
Penicillium brasilianum	Xylose	0	≅0.1 (58 h)	0.45 (80 h)	0	36
Trichoderma reesei Rut C-30	AFEX™ pretreated corn stover	1.9 (144 h)	ND	ND	ND	42
T. reesei	Solka Floc	0.58 (168 h)	4.2 (168 h)	8.0 (168 h)	1985 (168 h)	43
T. reesei	Steam pretreated corn stover	0.52 (168 h)	3.8 (168 h)	4.7 (168 h)	1077 (168 h)	43
T. reesei	Steam pretreated spruce	0.45 (168 h)	3.7 (168 h)	8.8 (168 h)	203 (168 h)	43
T. reesei	Steam pretreated willow	0.56 (168 h)	5.4 (168 h)	5.8 (168 h)	868 (168 h)	43

[a] ND, not determined.

microbial biomass production and higher cellulose activity, whereas xylose induction produced more xylanase in the medium. The results of Jun *et al.*[3] suggest that enzyme production by *T. reesei* is carbon-source dependent, as was observed for *P. echinulatum* in this study.

Jun *et al.*[3] also showed that xylose could serve as a potent xylanase inducer in *T. reesei*. Two major xylanases (Xyn1 and Xyn2) were identified. *T. reesei* produced more xylanases in xylose-containing culture than in a lactose-containing culture. β-Xylosidase (Bxl1), which is required for the complete degradation of xylan to xylose, was also elevated in the xylose-containing culture (increased 3-fold).

It can be seen in Table 1 that the value for FPA and endoglucanase in this study is higher than those shown previously for *Penicillium brasilianum* in xylose, but when the values are compared with lignocellulosic material, the values obtained for xylose are low. But even so, the use of xylose as a carbon source for the production of cellulases and xylanases is promising, as few microorganisms are capable of converting xylose and ethanol, and the microorganisms that make this conversion have low yields.

5. Conclusion

This study showed that the secretory behavior of this fungus depends strongly on the carbon sources and the strain. Xylose acts as an inducer for the production of xylanases and cellulases, especially endoglucanases. These data indicate the possibility of using xylose, a pentose that is not used by the majority of yeast, in ethanol production as a complete or partial substitute for cellulose in the production of enzymes for enzymatic hydrolysis.

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