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Improved production of β-galactosidase and β-fructofuranosidase by fungi using alternative carbon sources

Diandra de Andrades[1], Vanessa Cristina Arfelli[1], Alesandra Oriente[1], Caroline Henn[2], Veridiana Araujo Alves da Costa Pereira[2], Rita de Cássia Garcia Simão[3], Jose Luis da Conceição Silva[3] and Marina Kimiko Kadowaki[3]*

[1]Centro de Ciências Biológicas e da Saúde – UNIOESTE - Rua: Universitária 2069 CEP:85814-110, Cascavel, Paraná, Brazil.
[2]Divisão de Reservatório – MARR.CD, Itaipu Binacional, Avenida Tancredo Neves, 6731, CEP 85866-900 – Foz do Iguaçu, Paraná, Brazil.
[3]Centro de Ciências Médicas e Farmacêuticas – UNIOESTE - Rua: Universitária 2069 CEP:85814-110, Cascavel, Paraná, Brazil.

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The influence of alternative carbon sources as inducers of β-galactosidase and β-fructofuranosidase by filamentous fungi (that are, *Aspergillus aculeatus, Chrysonilia sitophila, Gliocladium virens, Aspergillus fumigatus* and *Trichoderma longibrachiatum*), recently isolated from Brazil's Atlantic Forest biome has been investigated. The greatest levels of intracellular β-galactosidase activity were obtained using orange peel waste (56.31 U/mL) with *A. aculeatus*, rice straw (22.57 U/mL) with *G. virens*, sorghum straw (16.48 U/mL) with *C. sitophila*, and passion fruit peel with either *A. fumigatus* (17.26 U/mL) or *T. longibrachiatum* (17.53 U/mL). The most effective intracellular β-fructofuranosidase activity was obtained by *A. aculeatus* using trub (409.46 U/mL) or passion fruit peel (44.59 U/mL). Thus, alternative carbon sources, such as orange peel and trub, exhibit great potential as inducers for the production of these enzymes. Such fungal isolates from the Atlantic Forest of Paraná, Brazil are promising candidates for generating significant amounts of β-galactosidase and β-fructofuranosidase using abundant and inexpensive agro-industrial substrates.

Key words: Agro-industrial residue, Atlantic Forest, lactase, invertase, fungus.

INTRODUCTION

The use of agro-industrial wastes in bioconversion by microorganisms has been the subject of extensive research in the last year, especially with reference to the production of proteins, enzymes, organic acids and some secondary metabolites. As a developing country, Brazil continually undergoes extensive agricultural and industrial activity, and consequently annually generates an enormous amount of agro-industrial waste. In this

*Corresponding author. E-mail: marinakk@gmail.com, Tel: +55 45 3220-3292.

context, such biodegradable waste is recognized as a potential sustainable source for use in the production of various value-added products such as biofuel, animal feed, chemicals and enzymes (Saha and Ghosh, 2014). Thus, the exploitation of agro-industrial waste for microbial bioprocesses may be an answer in the search for alternative and inexpensive carbon sources. Some agro-industrial wastes such as cassava bagasse, sugarcane bagasse, sugar beet residues, sludge and coffee husks, the peel and pulp of citrus fruits and wheat meal, have been used as carbon sources, mainly in fermentation processes using microorganisms (Soccol and Vandenberghe, 2003). Hence, the selection of microorganisms capable of producing β-galactosidase and β-fructofuranosidase, using agro-industrial waste as a carbon source in fermentation processes, may aid in overcoming high enzyme production costs, particularly in relation to biotechnological applications.

β-D-fructofuranosidase (E.C. 3.2.1.26) or more commonly named of invertase, is an important enzyme in the food industry and is responsible for the irreversible hydrolysis of β1-2 sucrose to produce a mixture of equal amounts of glucose and fructose (Rustiguel et al., 2010). β-fructofuranosidase has been used extensively in the production of invert sugar syrups, mixtures of glucose and fructose, which are sweeter than sucrose due to the high degree of sweetness exhibited by fructose, because these sugars do not crystallize. This enzyme is widely used in processed foods, in lactic acid production, in the fermentation of cane sugar molasses, in calf feed production, in ethanol production and as food for honeybees (Ikram-ul-Haq et al., 2005; Rashad and Nooman, 2009). β-D-fructofuranosidase is also employed in the pharmaceutical industry in the form of digestive aid tablets and as powdered milk for infant food (Uma et al., 2012). β-fructofuranosidase is produced by various microorganisms, such as *Saccharomyces cerevisiae* (Andjelkovic et al., 2012), *Penicillium chrysogenum* (Nuero and Reyes, 2002), *Aspergillus ochraceus* (Guimarães et al., 2007) and *Aspergillus niger* (Madhan et al., 2010).

β-galactosidase (E.C. 3.2.1.23) also known as lactase, catalyzes the hydrolysis of lactose (Galβ1-4Glc) to produce glucose and galactose (Ansari and Satar, 2012). This enzyme is extensively used by the dairy industry in the hydrolysis of lactose in milk or products derived from whey. Furthermore, the enzyme is clinically important during the preparation of lactose-free milk and milk products for patients with lactose intolerance (Shaikh et al., 1999). In recent years, the transgalactosylation activity of β-galactosidase has also been exploited in the production of galactooligosaccharide (GOS) and other functional galactosylated products (Oliveira et al., 2011). In addition, a significant market for lactose-free milk and dairy products exists among ice cream and confectionery industries, to assist patients with lactose intolerance (Adam et al., 2005; Husain, 2010). β-galactosidase is commonly produced by animal cells, plants and microorganisms, including *A. niger, Aspergillus oryzae, Kluyveromyces fragilis* and *Kluyveromyces lactis* (Husain, 2010).

The use of sucrose and lactose are commonly cited for β-fructofuranosidase and β-galactosidase production by microorganisms, and, less often, unconventional sources such as from agricultural waste industries.

Therefore, the growth of microorganisms and subsequent enzyme production are strongly influenced by medium composition; the carbon source of medium is one of the most important components of culture media for fungal growth and enzyme production because it may regulate the biosynthesis of enzymes in microorganisms (Akcan, 2011). Thus, screening for appropriate carbon is one of the most critical step in the development of an efficient and economic bioprocess (Konsoula and Liakopoulou-Kyriakides, 2007; Akcan, 2011). In this context, the aim of the present study was to investigate the influence of agro-industrial waste as inducers of β-galactosidase and β-fructofuranosidase production by several fungal strains recently isolated from the Atlantic Forest biome in Paraná state, Brazil.

MATERIALS AND METHODS

Microorganisms

The filamentous fungi used in this study were isolated from samples of soil or decaying plants obtained from the Bela Vista Biological Refuge in Foz do Iguassu in the State of Paraná, Brazil. These fungal strains were taxonomically identified by mycology collection of the Federal University of Pernambuco, Brazil, as *Aspergillus aculeatus, Aspergillus fumigatus, Chrysonilia sitophila, Gliocladium virens* and *Trichoderma longibrachiatum*. The conidia of the strains were maintained on potato dextrose agar at 4°C for a month, and the stock culture was maintained in 10% glycerol solution at -80°C.

Culture conditions

Liquid medium for enzyme production consisted of Modified Czapek medium (g/L) with: yeast extract, 5; $(NH_4)_2SO_4$, 2.8; KH_2PO_4, 4; $MgSO_4 \cdot 7H_2O$, 0.9; and $CaCl_2 \cdot 2H_2O$, 0.9. Each culture was grown in 125 mL Erlenmeyer flasks containing 25 mL of sterile medium supplemented with 1% (w/v) dry matter agro-industrial waste (that are, banana peel, orange peel, passion fruit peel, walnut peel, pear peel, quinoa meal, soybean meal, rice straw, sorghum straw or trub). These alternative carbon sources were obtained from the market and industries of Cascavel, Paraná, Brazil. After a 1 mL inoculum (10^5 spores/mL) of mesophilic (that is, *A. aculeatus, C. sitophila* and *G. virens* were incubated at 28°C) or thermophilic fungus (*T. longibrachiatum and Aspergillus fumigatus* were grown at 42°C), fungi were grown in static conditions for five days. Each culture was vacuum filtered to obtain a cell-free filtrate of extracellular enzyme activity. Intracellular extracts were obtained from frozen mycelia (previously separated and washed with distilled water) after maceration in a porcelain mortar with twice their weight in glass beads (0.1 mm diameter), resuspended in distilled water and then centrifuged at 5000 × *g* for 5 min at 4°C. The filtrate was dialyzed overnight at 4°C against the buffer used for each enzymatic assay.

Enzymatic assay

β-fructofuranosidase activity was determined by analyzing reducing sugar that is released after incubation of the properly diluted enzyme with 0.2 M sucrose in 50 mM sodium acetate buffer (pH 4.0) at 60°C for 10 min. The amounts of reducing sugars were determined using dinitrosalicylic acid according to Miller (1959). One unit of enzyme activity was defined as the amount of enzyme capable of releasing 1 µmol of reducing sugar (glucose) per minute under the experimental conditions used.

The β-galactosidase activity assay was performed by mixing 100 µL of appropriately diluted enzyme with 500 µL of the synthetic substrate, β-D-galactopyranoside (ONPGal; 3 mM) dissolved in 50 mM sodium citrate buffer (pH 4.5), and incubating the mixture at 40°C for 10 min. The reaction was stopped by the addition of 2 mL of 0.2 M Na_2CO_3. Enzyme activity was determined from the amount of released o-nitrophenol, which was measured using a spectrophotometer at 410 nm. One unit of enzyme activity was defined as the amount of enzyme capable of releasing 1 µmol of o-nitrophenol per minute under the assay conditions used.

Statistical analysis

All experiments were performed in triplicate, and results are presented as mean ± standard deviation. Significant differences between the means of enzymatic activities were determined using an analysis of variance (ANOVA), followed by the Tukey test at a 5% level of significance ($P < 0.05$).

RESULTS AND DISCUSSION

Influence of agro-industrial waste on the production of fungal β-galactosidase

The use of agricultural residues as carbon sources in biotechnological processes can reduce the production costs of microbial fermentations. Thus, the search for both alternative carbon sources and fungal strains with the ability to produce appropriate enzymes is essential to obtain an economically viable fermentation process.

In this study, several alternative carbon sources were examined; among the agricultural residues tested, orange peel enhanced extracellular β-galactosidase production for most fungal strains tested (Table 1). The highest extracellular β-galactosidase activities were observed with orange peel by *G. virens* (8.47 U/mL), followed by pear peel (2.66 U/mL) by the same organism. Orange peel has also been reported to induce β-galactosidase by *A. niger, Aspergillus terreus, Aspergillus flavus, Penicillium brevicompactum* and *Fusarium oxysporum* (Vidya et al., 2014). However, an extracellular β-galactosidase (Bga1) from *Hypocrea jecorina* (*T. reesei*) was induced by D-galactose mediated by galactitol (Fekete et al., 2007). Raol and colleagues (2014) reported that in polysaccharides such as wheat bran, pectin and polygalacturonic acid, the β-galactosidase cleaves and releases galactose residues from the side-chains of galactan and it is suggested not to repress the biosynthesis of β-galactosidase. Thus, carbohydrates such as hemicellulose monomers or galactose found in these agro-residues may induce the production of β-galactosidase by fungi.

In this study, the induction of intracellular β-galactosidase by alternative carbon sources was higher than for the extracellular β-galactosidase produced by these fungal strains (Table 2). The highest value of intracellular β-galactosidase activity was obtained with the fungus *A. aculeatus* using orange peel waste (56.31 U/mL), rice straw (47.97 U/mL) and pear peel (44.45 U/mL); some other high values were obtained with rice straw and *G. virens* (22.57 U/mL), sorghum straw with *C. sitophila* (16.48 U/mL), and passion fruit peel with *A. fumigatus* (17.26 U/mL) or *T. longibrachiatum* (17.53 U/mL) (Table 2). To the best of our knowledge, this is the first report of β-galactosidase production by fungi of the genera *Gliocladium* and *Chrysonilia*. There are few reports of the use of agricultural waste as a carbon source for the production of β-galactosidase, but in one study, β-galactosidase from *Aspergillus tubengensis* GR-1 was produced using wheat bran (Raol et al., 2014).

However, there are many reports concerning β-galactosidase production induced with lactose or its derivatives, such as in the fungus *Trichoderma* sp. which reached 0.35 to 2.24 U/mL (Akinola et al., 2012); *Teratosphaeria acidotherma* AIU BGA-1 produced four intracellular β-D-galactosidases using lactose as a carbon source (Isobe et al., 2013). In this study, *T. longibrachiatum* produced significant amounts of β-galactosidase using alternative carbon sources, with enzymatic activities ranging from 1.46 to 17.53 U/mL. These results suggest that the fungi tested were capable of performing the bioconversion of these residues and of consequently providing monosaccharides such as arabinose, galactose and xylose; these, in turn, favored the induction and biosynthesis of β-galactosidase.

According to Fekete and colleagues (2002), the biosynthesis of β-galactosidase from *Aspergillus nidulans* was also induced by L-arabinose and hemicellulose monomers, and similar results were also observed in *A. niger* and *Penicillium canescens*. The reason for the induction of β-galactosidase by L-arabinose and D-xylose in *A. nidulans* was unknown, but according to the author, this induction was related to the concomitant release of D-galactose, L-arabinose and D-xylose from these sources of natural polysaccharide (Fekete et al., 2002).

β-fructofuranosidase production by fungi induced by agro-industrial wastes

The production of β-fructofuranosidase of these fungi was also examined in the presence of various agro-industrial wastes. Extracellular β-fructofuranosidase was induced by several carbon sources, such as soybean meal (9.48 U/mL), orange peel (4.09 U/mL) and passion fruit peel (4.07 U/mL) for *A. aculeatus,* whereas banana (4.64 U/mL) and pear peels (4.62 U/mL) favored the

Table 1. Production of extracellular β-galactosidase by filamentous fungi in liquid culture with agro-industrial residues as carbon sources.

Carbon source (1%)	*Aspergillus aculeatus*	*Aspergillus fumigatus*	*Chrysonilia sitophila*	*Gliocladium virens*	*Trichoderma longibrachiatum*
	β-galactosidase activity (U/mL)				
Banana peel	1.42 ± 0.08^{bc}	0.88 ± 0.10^{bc}	0.73 ± 0.04^{b}	1.63 ± 0.13^{cd}	1.12 ± 0.09^{b}
Pecan shell	0.27 ± 0.06^{e}	0.73 ± 0.07^{cd}	0.43 ± 0.18^{c}	0.81 ± 0.09^{f}	0.65 ± 0.08^{de}
Orange peel	1.66 ± 0.21^{ab}	2.12 ± 0.19^{a}	1.72 ± 0.18^{a}	8.47 ± 0.26^{a}	2.17 ± 0.06^{a}
Passion fruit peel	2.10 ± 0.26^{a}	1.17 ± 0.13^{b}	0.42 ± 0.14^{c}	1.98 ± 0.20^{c}	0.96 ± 0.12^{bc}
Pear peel	1.06 ± 0.14^{cd}	0.08 ± 0.02^{f}	0.33 ± 0.05^{cd}	2.66 ± 0.28^{b}	0.82 ± 0.06^{cd}
Quinoa meal	0.83 ± 0.10^{d}	0.47 ± 0.13^{de}	0.11 ± 0.02^{d}	1.59 ± 0.11^{cde}	0.21 ± 0.08^{g}
Rice straw	0.96 ± 0.09^{cd}	0.40 ± 0.05^{e}	0.14 ± 0.12^{cd}	1.95 ± 0.18^{c}	0.21 ± 0.04^{g}
Sorghum straw	0.93 ± 0.23^{cd}	0.62 ± 0.12^{cde}	0.40 ± 0.08^{cd}	1.45 ± 0.15^{de}	0.49 ± 0.11^{ef}
Soybean meal	1.39 ± 0.20^{bc}	0.67 ± 0.17^{cde}	0.26 ± 0.20^{cd}	0.92 ± 0.19^{f}	0.27 ± 0.12^{g}
Trub	0.88 ± 0.08^{d}	0.80 ± 0.21^{c}	0.10 ± 0.03^{d}	1.18 ± 0.10^{ef}	0.34 ± 0.08^{fg}

Values are mean ± SD of three replicates. Values followed by different letters in each column differ significantly according to the Tukey test ($P < 0.05$).

Table 2. Production of intracellular β-galactosidase by filamentous fungi in liquid culture with agro-industrial residues as carbon sources.

Carbon source (1%)	*Aspergillus aculeatus*	*Aspergillus fumigatus*	*Chrysonilia sitophila*	*Gliocladium virens*	*Trichoderma longibrachiatum*
	β-galactosidase activity (U/mL)				
Banana peel	7.58 ± 0.32^{g}	8.01 ± 0.10^{c}	7.37 ± 0.33^{de}	9.00 ± 0.13^{d}	2.58 ± 0.25^{d}
Pecan shell	0.56 ± 0.03^{h}	6.42 ± 0.27^{d}	1.29 ± 0.15^{g}	2.02 ± 0.10^{h}	6.35 ± 0.35^{c}
Orange peel	56.31 ± 0.56^{a}	12.44 ± 0.60^{b}	8.46 ± 0.30^{b}	18.51 ± 0.76^{c}	16.20 ± 0.59^{b}
Passion fruit peel	9.31 ± 0.13^{f}	17.26 ± 0.44^{a}	7.87 ± 0.22^{cd}	8.18 ± 0.15^{de}	17.53 ± 0.22^{a}
Pear peel	44.45 ± 0.34^{c}	1.88 ± 0.10^{ef}	7.65 ± 0.27^{cd}	6.49 ± 0.33^{f}	1.46 ± 0.23^{e}
Quinoa meal	18.88 ± 0.74^{e}	1.43 ± 0.12^{f}	7.60 ± 0.26^{cd}	9.29 ± 0.21^{d}	6.74 ± 0.19^{c}
Rice straw	47.97 ± 0.52^{b}	2.17 ± 0.12^{e}	8.01 ± 0.07^{bc}	22.57 ± 1.21^{a}	2.06 ± 0.14^{de}
Sorghum straw	20.43 ± 0.92^{d}	1.60 ± 0.19^{ef}	16.48 ± 0.23^{a}	7.54 ± 0.30^{ef}	2.22 ± 0.07^{d}
Soybean meal	19.80 ± 0.32^{de}	2.16 ± 0.12^{e}	6.55 ± 0.28^{f}	20.17 ± 0.49^{b}	6.98 ± 0.17^{c}
Trub	19.11 ± 0.54^{e}	1.71 ± 0.05^{ef}	6.83 ± 0.17^{ef}	4.84 ± 0.20^{g}	2.45 ± 0.21^{d}

Values are mean ± SD of three replicates. Values followed by different letters in each column differ significantly according to the Tukey test ($P < 0.05$).

production of β-frutofuranosidase by *A. fumigatus* (Table 3). Soybean meal also significantly induced extracellular β-fructofuranosidase by *C. sitophila* (3.98 U/mL). The use of alternative carbon sources by microorganisms as inducers of β-fructofuranosidase have been reported mainly for: sugar cane bagasse, cassava flour and corn cob (Alegre et al., 2009); food processing residues (Rashad and Nooman, 2009); and pineapple peels, sweet lime, pomegranate, orange and mosambi (Uma et al., 2012). The choice of carbon source for the production of β-fructofuranosidase is important for ensuring that this process is economical and feasible.

In contrast to *A. aculeatus* and *A. fumigatus*, *G. virens* and *T. longibrachiatum* exhibited low extracellular β-fructofuranosidase activity with the particular agro-industrial wastes used in this study (Table 3). The production of enzymes is influenced by both induction and catabolite repression. Thus, the low yield of β-fructofuranosidase, seen specifically with these fungi, may be due to the final products (glucose and fructose) released by polysaccharidases' action on waste, since the presence of these monomers does not favor the biosynthesis of β-fructofuranosidase due to a catabolite repression effect.

The production of intracellular β-fructofuranosidase was higher than for the extracellular enzyme in the presence of agro-industrial wastes used as carbon sources for all fungi in this study except *A. fumigatus* (Table 4).

Table 3. Production of extracellular β-fructofuranosidase by fungi in liquid culture with agro-industrial residues as carbon sources.

Carbon source (1%)	*Aspergillus aculeatus*	*Aspergillus fumigatus*	*Chrysonilia sitophila*	*Gliocladium virens*	*Trichoderma longibrachiatum*
	β-fructofuranosidase activity (U/mL)				
Banana peel	3.30 ± 0.26^{c}	4.64 ± 0.43^{a}	0.68 ± 0.17^{cd}	0.15 ± 0.04^{bcd}	0.28 ± 0.05^{b}
Nut shell	1.01 ± 0.18^{de}	3.80 ± 0.03^{b}	0.31 ± 0.04^{de}	0.15 ± 0.02^{bcd}	0.29 ± 0.0^{b}
Orange peel	4.09 ± 0.26^{b}	0.46 ± 0.10^{d}	0.23 ± 0.04^{de}	0.32 ± 0.14^{a}	0.42 ± 0.15^{b}
Passion fruit peel	4.07 ± 0.03^{b}	0.52 ± 0.13^{d}	1.79 ± 0.27^{b}	0.16 ± 0.02^{bcd}	2.86 ± 1.18^{a}
Pear peel	1.17 ± 0.03^{de}	4.62 ± 0.25^{a}	0.48 ± 0.09^{de}	0.13 ± 0.02^{bcd}	0.20 ± 0.03^{b}
Quinoa meal	0.87 ± 0.24^{e}	1.76 ± 0.09^{c}	1.03 ± 0.22^{c}	0.25 ± 0.03^{ab}	0.21 ± 0.03^{b}
Rice straw	0.21 ± 0.02^{f}	0.14 ± 0.02^{d}	0.22 ± 0.04^{de}	0.12 ± 0.03^{cd}	0.11 ± 0.02^{b}
Sorghum straw	1.41 ± 0.06^{d}	0.13 ± 0.02^{d}	0.10 ± 0.02^{e}	0.12 ± 0.02^{bcd}	0.23 ± 0.04^{b}
Soybean meal	9.48 ± 2.67^{a}	0.32 ± 0.05^{d}	3.98 ± 0.47^{a}	0.05 ± 0.0^{d}	0.03 ± 0.01^{b}
Trub	2.93 ± 0.10^{c}	3.78 ± 0.10^{b}	0.66 ± 0.17^{cd}	0.21 ± 0.04^{abc}	0.18 ± 0.04^{b}

Values are mean ± SD of three replicates. Values followed by different letters in each column differ significantly according to the Tukey test ($P < 0.05$).

Table 4. Production of intracellular β-fructofuranosidase by fungi in liquid culture with agro-industrial residues as carbon sources.

Carbon source (1%)	*Aspergillus aculeatus*	*Aspergillus fumigatus*	*Chrysonilia sitophila*	*Gliocladium virens*	*Trichoderma longibrachiatum*
	β-fructofuranosidase activity (U/mL)				
Banana peel	26.48 ± 1.32^{c}	2.94 ± 0.28^{b}	2.12 ± 0.81^{d}	0.13 ± 0.03^{d}	3.04 ± 0.23^{b}
Nut shell	4.75 ± 0.21^{e}	0.17 ± 0.05^{f}	0.21 ± 0.01^{d}	0.21 ± 0.03^{cd}	0.80 ± 0.10^{de}
Orange peel	23.49 ± 0.32^{cd}	3.20 ± 0.16^{b}	11.53 ± 0.34^{b}	0.93 ± 0.34^{bcd}	0.43 ± 0.01^{e}
Passion fruit peel	44.59 ± 2.90^{b}	2.39 ± 0.21^{c}	5.43 ± 0.16^{c}	0.95 ± 0.29^{bcd}	4.54 ± 0.39^{a}
Pear peel	11.47 ± 0.64^{de}	1.27 ± 0.07^{de}	0.27 ± 0.06^{d}	0.74 ± 0.28^{bcd}	1.13 ± 0.31^{cd}
Quinoa meal	40.60 ± 2.01^{b}	1.01 ± 0.08^{e}	27.91 ± 3.17^{a}	2.06 ± 0.44^{a}	1.44 ± 0.34^{c}
Rice straw	0.79 ± 0.10^{e}	0.25 ± 0.03^{f}	0.20 ± 0.01^{d}	0.16 ± 0.05^{cd}	0.62 ± 0.06^{de}
Sorghum straw	11.87 ± 2.02^{de}	0.18 ± 0.03^{f}	0.08 ± 0.02^{d}	0.34 ± 0.09^{bcd}	1.17 ± 0.06^{cd}
Soybean meal	19.08 ± 0.41^{cd}	1.65 ± 0.51^{d}	0.95 ± 0.03^{d}	1.06 ± 0.36^{b}	1.09 ± 0.20^{cd}
Trub	409.46 ± 17.79^{a}	4.00 ± 0.09^{a}	1.42 ± 0.45^{d}	0.97 ± 0.26^{bc}	4.14 ± 0.16^{a}

Values are mean ± SD of three replicates. Values followed by different letters in each column differ significantly according to the Tukey test ($P < 0.05$).

Enhanced production of intracellular β-fructofuranosidase by fungi was observed in *A. aculeatus* (409.46 U/mL) induced by trub (wastes obtained from brewing fermentation). Trub is commonly used as a supplement in animal feed because of its significant nutritional value, or discarded into the environment, and, thus, may be useful in microbial processes. Passion fruit peels were also effective in inducing intracellular β-fructofuranosidase by *A. aculeatus* (44.59 U/mL). Likewise, quinoa meal also significantly induced intracellular β-fructofuranosidase in *A. aculeatus* (40.60 U/mL) and *C. sitophila* (27.91 U/mL). On the other hand, carbohydrates such as sucrose or raffinose added to the medium induced large amounts of β-fructofuranosidase in *A. nidulans* (Vainstein and Peberd, 1991). Rubio and Navarro (2006) have also reported higher levels of β-fructofuranosidase obtained from *A. niger* using sucrose, turanose, raffinose and inulin; the authors suggested that carbohydrates that induce invertase synthesis have a β-link and a fructose located at the end of the molecule as a common feature in their structure. Nonetheless, the use of pure carbohydrates for the large-scale production of β-fructofuranosidase by fungi can become very expensive, and the choice of cheaper and renewable substrates, in the form of agro-industrial wastes may be advantageous. Agro-industrial residues are most commonly cited for the induction of xylanases, cellulases and pectinases by fungi. However, studies using lignocellulosic biomass sources for the induction of enzymes, such as β-galactosidase and β-fructofuranosidase, are still few in number; it is envisioned that the microbial process will become economically more feasible with time, while

retaining the potential to minimize the impact of industrial wastes on the environment.

Conclusion

In this study, screening for strong fungal producers of β-galactosidase and β-fructofuranosidase was performed using alternative carbon sources obtained from agro-industrial substrate. The highest β-galactosidase production was found as an intracellular enzyme produced by *A. aculeatus* and *G. virens* using orange peel, rice straw and pear peel. The most effective production of intracellular β-fructofuranosidase was obtained by *A. aculeatus* with trub (that is, brewing residue), passion fruit peel or quinoa meal as carbon sources, followed by the fungus *C. sitophila,* which also produced significant amounts of β-fructofuranosidase with quinoa meal. It was therefore concluded that these fungi can be exploited to generate significant amounts of β-galactosidase and β-fructofuranosidase using alternative agro-industrial substrates that are abundantly available, mainly in agricultural production regions. Thus, fungi isolated from the Atlantic Forest biome of Paraná, Brazil may be promising candidates for the production of β-galactosidase and β-fructofuranosidase for commercial use in the future. However, further studies on improved and optimized culture conditions will be needed to increase enzyme yield, particularly since the natural fungal sources of these enzymes have only relatively recently been isolated from nature and therefore their properties remain largely unknown.

Conflict of Interest

The authors do not have any financial or commercial conflicts of interest to declare.

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