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Use of agro-industrial wastes as substrates for α-amylase production by endophytic fungi isolated from *Piper hispidum* Sw

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ABSTRACT. Millions of tons of corn, pineapple, sugarcane and wheat are annually produced and their industrial processing generates large amounts of wastes. Current study evaluated the use of corncob (CC), pineapple peel (PP), sugarcane bagasse (SB) and wheat bran (WB) as substrates for α-amylase production by submerged cultures of nine endophytes from *Piper hispidum* Sw. Initially, fungi were grown on a starch medium and the cup plate assay showed that five strains were amylase-positive: *Bipolaris* sp. JF767001, *Marasmius cladophyllus* JF767003, *Phlebia* sp. JF766997, *Phyllosticta capitalensis* JF766988 and *Schizophyllum commune* JF766994, with enzymatic halos ranging between 6.30 ± 0.08 and 8.87 ± 0.68 mm in diameter. Further, the use of agro-industrial wastes was evaluated by the cup plate assay, where the highest halo diameters were obtained from fungi grown on PP or SB: up to 15.00 ± 0.16 mm (*Phlebia* sp.) and 14.80 ± 0.18 mm (*S. commune*), respectively. Submerged cultures with PP or SB showed the highest levels of α-amylase activity according to the starch-iodine assay, or rather, up to 4.14 ± 0.02 U mL^{-1} (*Bipolaris* sp.) and 4.09 ± 0.02 U mL^{-1} (*Phlebia* sp.), respectively. Results give an in-depth knowledge on tropical endophytes that might be α-amylase sources, and indicate the suitability of these agro-industrial wastes as substrates for fungal enzymatic production.

Keywords: amylolytic activity, endophytes, microbial enzymes, submerged fermentation.

Uso de resíduos agroindustriais para a produção de α-amilase por fungos endofíticos isolados de *Piper hispidum* Sw

RESUMO. Milhões de toneladas de milho, abacaxi, cana-de-açúcar e trigo são anualmente produzidas e seu processamento industrial gera grandes quantidades de resíduos. Este estudo avaliou o uso de sabugo de milho (SM), casca de abacaxi (CA), bagaço de cana-de-açúcar (BC) e farelo de trigo (FT) como substratos para a produção de amilase em culturas submersas de nove endófitos de *Piper hispidum* Sw. Inicialmente, os fungos foram crescidos em amido e o ensaio *cup plate* mostrou resultados positivos para cinco fungos: *Bipolaris* sp. JF767001, *Marasmius cladophyllus* JF767003, *Phlebia* sp. JF766997, *Phyllosticta capitalensis* JF766988 e *Schizophyllum commune* JF766994, com halos enzimáticos de 6,30 ± 0,08 a 8,87 ± 0,68 mm. Posteriormente, o uso dos resíduos foi avaliado pelo ensaio *cup plate*, em que os maiores halos foram obtidos para CA ou BC: até 15,00 ± 0,16 mm (*Phlebia* sp.) e 14,80 ± 0,18 mm (*S. commune*), respectivamente. As culturas submersas com CA ou BC também apresentaram os maiores níveis de α-amilase de acordo com a reação dextrinizante: até 4,14 ± 0,02 U mL^{-1} (*Bipolaris* sp.) e 4,09 ± 0,02 U mL^{-1} (*Phlebia* sp.), respectivamente. Os resultados expandem o conhecimento sobre endófitos de regiões tropicais que são fontes de α-amilase, indicando que os referidos resíduos são adequados para a produção enzimática de tais fungos.

Palavras-chave: atividade amilolítica, endófitos, enzimas microbianas, fermentação submersa.

Introduction

Every year, millions of tons of corn, pineapple, sugarcane and wheat are produced worldwide and large amounts of wastes are generated during the industrial processing of these agricultural products (Table 1). Several agro-industrial wastes are rich in sugars, minerals and proteins, providing low-cost raw materials which may be used in industrial processes (Raol, Raol, Prajapati, & Bhavsar, 2015) for the production of value-added compounds such as enzymes. The recycling of these wastes is also important to maintain equilibrium between environment and industries.

Amylases are ubiquitous enzymes found in prokaryotes, plants, animals, fungi and unicellular eukaryotes (Zaferanloo, Bhattacharjee, Ghorbani, Mahon, & Palombo, 2014). In particular, α-amylases (1,4 α-glucan glucanohydrolase, EC 3.2.1.1) from microorganisms are more stable than those from

plants or animals, and represent about 25-33% of the global enzyme market (Rajagopalan & Krishnan, 2008; Souza & Magalhães, 2010). These enzymes replace the chemical hydrolysis of starch in starch-processing industries and are also traditionally used for the preparation of oriental food. Advances in Biotechnology expanded their application to various fields such as food, beverages, detergents, textile and pharmaceutical industries (Annamalai, Thavasi, Vijayalakshmi, & Balasubramanian, 2011).

Table 1. Data retrieved from the literature on crop production (average for 2003-2013 period) and agro-industrial wastes generated during crop processing.

Crop	Top producer	Production average[1]	Waste	Waste generation
Corn	USA	~303.1 Mt	Cob	~180 kg ton^{-1} corn[2]
Pineapple	Brazil	~2.4 Mt	Peel	~35% of fresh fruit[3]
Sugarcane	Brazil	~594.4 Mt	Bagasse	~280 kg ton^{-1} sugarcane[4]
Wheat	China	~108.7 Mt	Bran	14-19% of wheat grain[5]

Mt = million tones. References: [1]Food and Agriculture Organization of the United Nations [FAO](2015); [2]Zhang et al. (2013); [3]Huang, Chow and Fang (2011); [4]Rezende et al.(2011); [5]Merali et al. (2015).

An α-amylase from *Aspergillus oryzae* was the first microbial enzyme manufactured for sale (Gupta, Gupta, Modi, & Yadava, 2008). Although the filamentous fungal strains have a remarkable capacity of secreting extracellular proteins, filamentous endophytes remain under-exploited as enzymatic sources (Corrêa et al., 2014). These fungi colonize intra- and inter-cellular plant tissues, establishing a harmonious relationship without causing any apparent damage to the host plant (Fouda, Hassan, Eid, & Ewais, 2015). The microorganism obtains nutrients and shelter, while the plant receives protection against pathogens, herbivores and insects (Giménez, Cabrera, Reina, & González-Coloma, 2007; Alvin, Miller, & Neilan, 2014). Hydrolytic enzymes degrade the plant´s cell wall, facilitating penetration into host tissues (Bischoff et al., 2009). Amylases also help endophytes to degrade the available starch when the plant reaches senescence (Sunitha, Devi & Srinivas, 2013).

The medicinal plant *Piper hispidum* Sw. (called 'cordoncillo' in Mexico and 'falso-jaborandi' in Brazil) harbors a diversity of endophytes (Orlandelli, Alberto, Rubin Filho, & Pamphile, 2012a), which include isolates that are sources of antimicrobial metabolites (Orlandelli, Alberto, Almeida, Azevedo, & Pamphile, 2012b) and exopolysaccharides (Orlandelli, Vasconcelos, Azevedo, Corradi da Silva, & Pamphile, 2016). Some are protease-producing strains and the increased activity in the presence of rice or soy flour suggests their potential to produce enzymes with agricultural residues (Orlandelli et al., 2015). Now, current study evaluated the suitability of four agro-industrial wastes (corncob, pineapple peel, sugarcane bagasse and wheat bran) as low-cost substrates for the α-amylase production by nine *P. hispidum* endophytes belonging to the genera *Bipolaris*, *Colletotrichum*, *Diaporthe*, *Phoma, Phyllosticta*, *Marasmius*, *Phlebia* and *Schizophyllum*. Data on amylase activity of these fungal genera are rare, and they had not been cultivated in identical conditions to that reported herein. Since microbial biosynthesis is affected by culture medium composition and cultivation conditions (Elisashvili, 2012), current study provides an in-depth knowledge on tropical endophytic strains that may be used as α-amylase sources.

Material and methods

Endophytic fungi

Nine endophytes were used: the ascomycetes *Bipolaris* sp. JF767001, *Colletotrichum* sp. JF766996, *Diaporthe* sp. JF766998, *Diaporthe* sp. JF767007, *Phoma herbarum* JF766995 and *Phyllosticta capitalensis* JF766988, and the basidiomycetes *Marasmius cladophyllus* JF767003, *Phlebia* sp. JF766997 and *Schizophyllum commune* JF766994.

Fungi were isolated from healthy leaves of the medicinal plant *P. hispidum* located in a forest remnant in southern Brazil (Orlandelli et al., 2012a), belonging to the fungal culture collection of the Laboratório de Biotecnologia Microbiana, Universidade Estadual de Maringá, Maringá, Paraná State, Brazil. Molecular identification was based on sequencing of ITS1-5.8S-ITS2 region of rDNA. Sequences were deposited in the GenBank database.

Agro-industrial wastes

Corncob (CC), pineapple peel (PP) and sugarcane bagasse (SB) were obtained from local vendors in Maringá, Paraná State, Brazil, as food and beverage production wastes. Materials were first washed in running tap water and subsequently in hot water to remove dirt and impurities. The washed substrates were dried in sunlight and blended into ~1-mm particles. The wheat bran (WB) was obtained from the local market and used without any further pre-treatment.

Submerged fermentation

All endophytes were previously grown in Petri dishes with Potato Dextrose Agar (PDA) medium (HiMedia Laboratories, Mumbai, MH, India), at 28 ± 2°C, for seven days. Further, three 6-mm mycelial plugs of each endophyte were transferred to

125-mL Erlenmeyer flasks containing 50 mL of Manachini's solution (Manachini, Fortina, & Parini, 1987) comprising 2 g L^{-1} KH_2PO_4,1 g $L^{-1}(NH_4)_2SO_4$, 0.1 g L^{-1}MgSO4.7H2O, 0.9 g $L^{-1}Na_2HPO_4.2H_2O$, 1 gL^{-1},1 g L^{-1} yeast extract; volume was completed to 1 L with distilled water. The following substrates (0.5% w/v) were added to the medium: commercial corn starch Maizena® (CS) for the initial screening of amylase-positive endophytes. Each agro-industrial waste (CC, PP, SB or WB) was used in comparison to CS. Negative control was the liquid medium incubated without fungal inoculation. The cultures were incubated in triplicate at 28 ± 2ºC on a rotary shaker at 140 rpm for 144h, then filtered with sterile gauze to separate the fungal mycelia and centrifuged at 5000 × *g* for 10 min. to separate other cellular debris. The cell-free filtrates (supernatants) were used as amylase sources.

Cup plate assay

The supernatants were inoculated (50 µL) on Petri dishes containing starch-agar medium (18 g agar, 10 g starch, 0.1 M citrate-phosphate buffer, pH 5.0, per liter) with the surface perforated for cup plates (6-mm in diameter). A commercial α-amylase from porcine pancreas (type VI-B, ≥ 10 units mg^{-1} solid), purchased from Sigma-Aldrich (St. Louis, MO, USA), was used as positive control. The experiment was performed in triplicate and dishes were incubated at 28 ± 2ºC. After 24h, dishes were flooded with iodine-iodide solution, or rather, 1% iodine alcoholic solution, 10% potassium iodide and distilled water 1v:1v:3v (Fuwa, 1954). Amylase activity was evaluated by the development of colorless halos on a blue background and measured in millimeters.

Determination of α-amylase activity

A modified version of the starch-iodine method (Fuwa, 1954; Xiao, Storms, & Tsang, 2006) was used. In test tubes, assay reactions were initiated by adding 80 µL of 500 mM sodium acetate buffer pH 6.0, 20 µL of filtrates and 100 µL of 0.5% w/v starch solution (HiMedia Laboratories, Mumbai, MH, India). After 20 min. at 50ºC, 200 µL of 1 M acetic acid were added to stop the enzymatic reaction, with a further addition of 200 µL of iodine-iodide solution (Fuwa, 1954). The volume of each tube was completed to 10 mL with distilled water. The assay was performed in triplicate and absorbance was measured in a Libra S60PC spectrophotometer (Biochrom, Cambourne, CBE, United Kingdom) at 660 nm. The standard curve was performed according to Xiao et al. (2006). One unit (U) of α-amylase was defined as the amount of enzyme capable of hydrolyzing 1 mg of starch per min in the assay reaction, calculated by the formula (1), proposed by Xiao et al. (2006):

$$U\ mL^{-1} = \frac{\left(\frac{A_{control} - A_{sample}}{A_{starch}}\right)/min.}{vol_{sample}} \quad (1)$$

where:

$A_{control}$ = absorbance for negative control;

A_{sample} = absorbance for starch digested with enzymatic sample;

A_{starch} = absorbance for 1 mg of starch derived from standard curve; min. = incubation time (20 min.);

vol_{sample} = volume of the enzymatic sample (cell-free filtrate) used in the assay (0.02 mL).

Statistical analyses

Cup plate and starch-iodine assays were analyzed by analysis of variance (ANOVA) and means were compared by Tukey test ($p < 0.05$) with SISVAR 5.3.

Results and discussion

Screening of amylase-positive endophytes

Fungal sources of amylase in current study were initially screened by the cup plate assay. Endophytes were grown for 144h under submerged cultures containing CS; filtrates were then used for the cup plate assay whereby the diameters of enzymatic halos were measured and compared to halos obtained in the positive control, which consisted of a commercial α-amylase from porcine pancreas. In this assay, halo formation indicated that the microbial strains produced enzymes that were able to hydrolyze the inducer substrate.

In the case of screening of amylase sources (Table 2), the two ascomycetes (*Bipolaris* sp. JF767001 and *P. capitalensis* JF766988) and three basidiomycetes tested (*M. cladophyllus* JF767003, *Phlebia* sp. JF766997 and *S. commune* JF766994) produced positive results. ANOVA showed statistically significant differences among the enzymatic halos ranging between 6.30 and 8.87 mm in diameter; highest rates were obtained for *Bipolaris* sp. JF767001 (8.87 ± 0.68 mm) and *M. cladophyllus* JF767003 (8.87 ± 0.12 mm).

The ascomycetes *Colletotrichum* sp. JF766996, *Diaporthe* sp. JF766998, *Diaporthe* sp. JF767007 and *P. herbarum* JF766995 had no amylase activity. In fact, other authors have also reported negative results for endophytes belonging to the genera *Colletotrichum* (Hegde, Ramesha, & Srinivas, 2011) and *Phoma* (Sunitha et al., 2013). On the other hand, Choi, Hodgkiss and Hyde (2005) reported amylase

production by *Diaporthe* (= *Phomopsis*) and *Colletotrichum* endophytes using a different culture condition. Since fungal metabolic activity is affected by nutritional and physical parameters, the *P. hispidum* endophytes should be further evaluated on their capacity of producing amylase with different cultivation condition.

Table 2. Screening of amylase-positive endophytes using commercial corn starch as carbon source for the submerged fermentation. Results of cup plate assay are given as mean±standard deviation.

Endophytic fungi / Controls	Halos (mm)
Bipolaris sp. JF767001	8.87 ± 0.68^{b}
*Marasmius cladophyllus*JF767003	8.87 ± 0.12^{b}
Phlebia sp. JF766997	7.63 ± 0.12^{c}
Schizophyllum commune JF766994	7.50 ± 0.22^{c}
Phyllosticta capitalensis JF766988	6.30 ± 0.08^{d}
Colletotrichum sp. JF766996	0.00 ± 0.00^{e}
Diaporthe sp. JF766998	0.00 ± 0.00^{e}
Diaporthe sp. JF767007	0.00 ± 0.00^{e}
Phoma herbarum JF766995	0.00 ± 0.00^{e}
Positive control	12.63 ± 0.20^{a}
Negative control	0.00 ± 0.00^{e}

Means of triplicates followed by different letters are significantly different by Tukey test ($p < 0.05$). Positive control: α-amylase from porcine pancreas (type VI-B, ≥ 10 units/mg solid; Sigma-Aldrich) used directly in the cup plate assay. Negative control: liquid medium incubated without fungal inoculation.

Use of agro-industrial wastes as substrates for enzymatic activity

After the initial screening, CS and agro-industrial wastes were compared by the cup plate and starch-iodine assays. Since among the amylases the α-amylase seems to be the most versatile enzyme due to its wide application spectrum (Li, Yang, Yang, Zhu, & Wang, 2012), it was chosen to be quantified by the starch-iodine method. Results of the cup plate assay (Table 3 and Figure 1) revealed that halo formation obtained for the five fungi indicated that all tested strains produced extracellular enzymes which hydrolyzed their respective substrates.

PP was the most suitable substrate for the amylase activity of *Bipolaris* sp. JF767001, where halos mean (14.93 ± 0.09 mm) was statistically superior to that for other substrates. An increased enzymatic activity was obtained when *M. cladophyllus* JF767003 was grown on substrates CC (11.73 ± 0.66 mm) or SB (11.73 ± 0.52 mm); while PP (15.00 ± 0.16 mm) and SB (14.26 ± 0.41 mm) caused statistically higher results detected for *Phlebia* sp. JF766997. The fungus *S. commune* JF766994 showed best results when SB (14.80 ± 0.18 mm) or PP (14.13 ± 0.47 mm) was used. Only *P. capitalensis* JF766988 showed similar production (between 5.53 ± 0.09 and 6.53 ± 0.25 mm) when cultivated with CS or agro-industrial wastes except CC (5.53 ± 0.09 mm). The latter was statistically less efficient than CS (6.33 ± 0.25 mm) as substrate for fungal fermentation.

Table 3. Effect of different carbon sources on enzymatic activity of endophytic fungi, evaluated by cup plate and starch-iodine assays. Results are given as means of triplicates ± standard deviation.

	Fungi	CS	Agro-industrial wastes		
			CC	PP	SB	WB
		Enzymatic halos (mm)				
Cup plate assay	BP	8.86 ± 0.68^{BCb}	5.93 ± 0.77^{Cc}	14.93 ± 0.09^{ABa}	6.60 ± 0.72^{Cc}	7.60 ± 0.16^{Cbc}
	MC	8.86 ± 0.34^{Bb}	11.73 ± 0.66^{Ba}	5.00 ± 0.00^{Ec}	11.73 ± 0.52^{Ba}	9.60 ± 0.43^{Bb}
	PH	7.53 ± 0.41^{CDd}	13.40 ± 0.16^{Ab}	15.00 ± 0.16^{Aa}	14.26 ± 0.41^{Aab}	10.13 ± 0.19^{Bc}
	SC	7.60 ± 0.49^{BCDc}	13.07 ± 0.50^{ABb}	14.13 ± 0.47^{Bab}	14.80 ± 0.18^{Aa}	12.87 ± 0.50^{Ab}
	PC	6.33 ± 0.25^{Da}	5.53 ± 0.09^{Cb}	6.07 ± 0.33^{Dab}	6.53 ± 0.19^{Ca}	6.20 ± 0.16^{Dab}
	C+	12.67 ± 0.30^{A}	12.67 ± 0.30^{AB}	12.67 ± 0.30^{C}	12.67 ± 0.30^{B}	12.67 ± 0.30^{A}
	C-	0.00 ± 0.00^{E}	0.00 ± 0.00^{D}	0.00 ± 0.00^{F}	0.00 ± 0.00^{D}	0.00 ± 0.00^{E}
		α-Amylase activity (U mL^{-1})				
Starch-iodine assay	BP	3.56 ± 0.10^{Ab}	2.67 ± 0.05^{Cd}	4.14 ± 0.02^{Aa}	3.12 ± 0.06^{Cc}	3.42 ± 0.11^{Cb}
	MC	3.61 ± 0.02^{Ac}	3.75 ± 0.02^{Bb}	2.55 ± 0.04^{Dd}	3.91 ± 0.02^{Ba}	3.60 ± 0.02^{Bc}
	PH	3.41 ± 0.05^{ABd}	3.90 ± 0.05^{ABb}	4.04 ± 0.02^{Aa}	4.09 ± 0.02^{Aa}	3.66 ± 0.02^{Bc}
	SC	3.33 ± 0.04^{Bc}	3.80 ± 0.02^{Ab}	3.87 ± 004^{Bb}	4.07 ± 0.00^{Aa}	3.85 ± 0.02^{Ab}
	PC	2.99 ± 0.05^{Ca}	2.53 ± 0.02^{Db}	2.91 ± 0.02^{Ca}	3.01 ± 0.06^{Ca}	2.92 ± 0.05^{Da}

Means followed by different upper-case letters (columns) or lower-case letters (rows) are significantly different, according to Tukey test ($p < 0.05$). BP = *Bipolaris* sp. JF767001; MC = *M. dadophyllus* JF767003; PH = *Phlebia* sp. JF766997; SC = *S. commune* JF766994; PC = *P. capitalensis* JF766988; CS = commercial corn starch; CC = corncob; PP = pineapple peel; SB = sugarcane bagasse; WB = wheat bran. C+ (positive control): α-amylase from porcine pancreas; C- (negative control): liquid medium incubated without fungal inoculation.

The starch-iodine assay (Table 3) confirmed PP as the most suitable substrate for *Bipolaris* sp. JF76700 fermentation (4.14 ± 0.02 U mL^{-1}), following decreasing order in α-amylase activity: PP > CS ≥ WB > SB > CC. Data on amylase from *Bipolaris* endophytes are rare. The activity (≥ 3.18 U mL^{-1}) described for different subpopulations of phytopathogenic *B. sorokiniana* strains was positively correlated with area under disease progress curve and lesion development (Chand, Kumar, Kushwaha, Shah, & Joshi, 2014). Since endophytes are latent pathogens, the two fungal groups may have a similar metabolism (Lana et al., 2011).

M. cladophyllus JF767003 showed the highest activity (3.91 ± 0.02 U mL^{-1}) when grown on SB (SB > CC > CS ≥ WB > PP). A similar result was observed for *S. commune* JF766994 when SB was used: 4.07 ± 0.00 U mL^{-1} (SB > PP ≥ WB ≥ CC > CS). In the case of *Phlebia* sp. JF766997, ANOVA revealed that SB (4.09 ± 0.02 U mL^{-1}) and PP (4.04 ± 0.02 U mL^{-1}) were equally efficient as substrates for α-amylase activity (SB ≥ PP > CC > WB > CS).

The literature reports scantily the enzymatic activity of basidiomycete genera, focusing on soil (and not endophytic) strains that produce enzymes

as laccase, peroxygenase and peroxidase (Arora & Gil, 2005; Schückel, Matura, & Van Pée, 2011; Järvinen, Taskila, Isomäki, & Ojamo, 2012; Yarman et al., 2012). It should be underscored that Arora and Gil (2005) demonstrated that SB was suitable for the production of laccase (8.38 ± 0.40 U mL^{-1}) but not for the peroxidases activity of *Phlebia floridensis*, whereas WB was effective for laccase production (5.90 ± 0.18 U mL^{-1}), albeit less efficient for lignin peroxidase (0.014 ± 0.002 U mL^{-1}) and manganese peroxidase (0.120 ± 0.02 U mL^{-1}) activity.

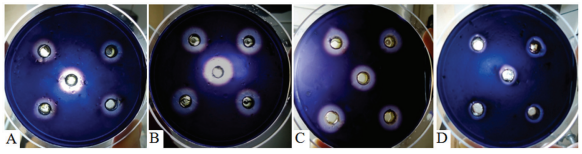

Figure 1. Cup plate assay showing amylase activity of *P. hispidum* endophytes: a) *M. cladophyllus* JF767003 grown on corncob (CC); b) *Phlebia* sp. JF766997 grown on pineapple peel (PP); c) *S. commune* JF766994 grown on sugarcane bagasse (SB); d) *Bipolaris* sp. JF767001 grown on wheat bran (WB).

Corroborating with the cup plate assay, the ascomycete *P. capitalensis* JF766988, showed statistically similar results (2.91 ± 0.02 to 3.01 ± 0.06 U mL^{-1}) when cultivated with all substrates, except CC (2.53 ± 0.02 U mL^{-1}). Rates were higher than those described by Romão, Spósito, Andreote, Azevedo and Araújo (2011) who investigated the amylase activity of endophytic (1.53 U mL^{-1}) and pathogenic (1.84 U mL^{-1}) strains of *Guignardia* (= *Phyllosticta*) strains isolated from citrus.

With the exception of *P. capitalensis* JF766988, higher levels of α-amylase were found in submerged culture filtrates with PP and/or SB. Cellulose is a highly stable polymer of glucose, and the glucose role in amylase production is still controversial: although starch and glucose are documented to support and repress the amylase activity, respectively (Rajagopalan & Krishnan, 2008), it stimulated the amylase activity of *Aspergillus flavus* and *Penicillium* sp. (Bhattacharya, Bhardwaj, Das, & Anand, 2011; Costa & Nahas, 2012). According to Bhattacharya et al. (2011), SB might be an efficient substrate for amylase activity due to the high moisture content in sugarcane fibers. Investigating the α-amylase synthesis of *Bacillus subtilis* KCC103, Rajagopalan and Krishnan (2008) reported lack of repression by glucose, where the strain was able to produce α-amylase using SB as substrate, at a level equivalent to that in starch medium. Thus, the above authors reported that the replacement of starch by SB was highly feasible to obtain low-cost microbial α-amylase.

In present-day Biotechnology, the demand for new and more potent sources of industrial enzymes is ever growing (Zaferanloo, Virkar, Mahon, & Palombo, 2013); therefore, the enzyme production by fungi represents a crucial sector of the fermentation industry (Göğüş et al., 2014). Brazilian researchers should further explore the potential of endophytes as enzymatic sources, as the country has a continental area with hundreds of plant species that harbor a highly diverse endophytic assemblage (Orlandelli et al., 2015). For the maintenance of symbiosis, endophytes secrete varieties of extracellular enzymes that contribute to the fungal colonization and growth; however, further quantitative assays for endophytic enzymes are required to understand the ecological role of these fungi. All these specific enzymes could be exploited under certain conditions (Wang & Dai, 2011). Although amylases from *Aspergillus* and *Bacillus* strains have been employed in the industry for a long time (Souza & Magalhães, 2010), endophytes have been recently described as new sources of novel and useful enzymes. Moreover, endophyte-derived amylolytic enzymes are being assessed to improve industrial processes for polysaccharides and protein biodegradation (Fouda et al., 2015). Although the initial investigation on α-amylase activity of *P. hispidum* endophytes has been done, further studies should be undertaken to improve enzymatic production.

Conclusion

Current investigation is the first report on α-amylase activity of *P. hispidum* endophytes and contributes on the reuse of agro-industrial wastes generated worldwide. In general, the two assays revealed that starchy substrates were less efficient than cellulose-rich substrates. Remarkable results were obtained for *Bipolaris* sp. JF767001 (4.14 ± 0.02 U mL^{-1}) cultivated with pineapple peel, and for *Phlebia* sp. JF766997 (4.09 ± 0.02 U mL^{-1}) and *S. commune* JF766994 (4.07 ± 0.00 U mL^{-1}) with sugarcane bagasse. Further investigations on the effect of cellulose on α-amylase synthesis should be undertaken to confirm the absence of repression on the enzymatic activity of the endophytes tested herein.

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