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Endophytic Actinomycetes as Potential Producers of Hemicellulases and Related Enzymes for Plant Biomass Degradation

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HIGHLIGHTS

- Selection of endophytic actinomycetes as producers of hemicellulases and related enzymes
- Actinomycetes strains present different glycohydrolases profiles
- The strains produce enzymes against a wide range of plant biomass substrates

Abstract: Tailor made enzymatic preparation must be design to hydrolyze efficiently plant biomass, once that each plant biomass possesses a distinct cell wall composition. Most of actinomycetes used for plant cell wall degradation are focused on the cellulases and xylanases production. However, a wide range of enzymes must be produced for an efficient degradation of lignocellulose materials. During the last decade several unusual environments were studied to obtain strains that produce glycohydrolases with innovator characteristics. In this context, the present work concerned the selection of endophytic actinomycetes as producers of hemicellulases and related enzymes with different enzymatic profiles, for use in the deconstruction of lignocellulosic biomass. A total of 45 Brazilian

actinomycetes previously isolated from plants (endophytics) and soil were prospected for hemicellulases and β-glucosidase production. Four strains highlighted for hemicellulase production (DR61, DR63, DR69 and DR66) and were selected for cultivation under other inductors substrates (xylan and pectin). All strains belong to *Streptomyces* genera and have their extracts tested for degradation of several hemicellulolytic substrates. The strains presented different glicohydrolyse enzymes profiles mainly for xylans and glucans that can be used for specific formulations of enzymes applied on the biomass deconstruction, principally on sugar cane bagasse.
Keywords: Endophytic actinomycetes; hemicellulases; accessory enzymes

INTRODUCTION

In biofuels area, the competition to obtain glycohydrolases, mainly cellulases, that hydrolyze efficiently plant biomass cell wall has been extensively studied. Over the years, it has become clear that tailor made enzymatic preparation must be design to hydrolyze efficiently plant biomass, once that each plant biomass possess a distinct cell wall composition [1].

In nature, the lignocellulose materials are decomposed by several microbial glycohydrolases that act on a synergist approach, once that these materials are a recalcitrant substrate. For this reason combinations of enzymes from different sources and types, helped to balance the ideal enzymatic proportion for an efficient hydrolysis. Combinations of cellulolytic enzymes with others glycohydrolases such as xylanases, pectinases, arabinofuranosidase and β-glucosidase are able to increase the hydrolysis yields in several plant biomass [2-5].

The screening of microorganisms is based on the capacity of bacteria and fungi to produce several compounds with industrial application, *e.g* enzymes. During the last decade several unusual environments were studied to obtain strains that produce glycohydrolases with different characteristics in plant biomass degradation. Delabona *et al.* [6] isolated fungi from Amazon forest that produce high activity cellulases and xylanase. Robledo *et al.* [7] isolated thermophilic fungal strains from maize silage capable to produce thermostable xylanase. Robl *et al.* [8] screened endophytic fungi strains with a wide range of hydrolytic enzyme profiles to lignocellulose deconstruction.

Endophytic microorganisms showed interest potential for the production of substances with industrial interest such as phytohormones [9], antibiotics [10, 11], antiprotozoal and antitumor molecules [12]. This organism also produce several enzymes such as proteases, amylases, phenol oxidases, lipases, laccases [13-15], and recently it has been shown their potential as lignocellulolytic enzyme producers, *e.g* cellulases and hemicellulases [4, 5, 8, 16].

Endophytic microorganisms are ubiquitous in a mutualistic relation [17]. These microorganisms are present within plant tissues and may be able to initiate plant material decomposition process before it becomes dominated by saprophytic species [18, 19]. The hydrolytic enzyme production by these microorganisms might be important for nutrition during the endophytic stage, but also to compete for substrate during saprophytic stage [8].

Most of the actinomycetes used for plant cell wall degradation are focused on the cellulases and xylanases production [20, 21]. Although a wide range of enzymes must be produced for an efficient degradation of lignocellulose materials in nature. In this context, the present work concerns the selection of endophytic actinomycetes as producers of hemicellulases and related enzymes with different enzymatic profiles, for use in the deconstruction of lignocellulosic biomass.

MATERIAL AND METHODS

Actinomycetes strains

Prospection of hemicellulase and related enzymes for plant biomass degradation was performed using an actinomycetes culture collection maintained at the Bioproducts Laboratory (ICB/USP). A total of 45 Brazilian actinomycetes were selected, isolated from *Citrus reticulate*, *Citrus sinensis*, *Theobroma cacao*, *Saccharum officinarum, Catharanthus roseus* and soil.

Agro-industrial waste materials

The liquor (HL) used was derived from the hydrothermal pretreatment of sugar cane bagasse and was obtained from Robl *et. al* [8] study. The sugar cane bagasse was obtained from a local mill (Usina Vale do Rosário, Orlândia, SP, Brazil)The hydrothermal pretreatment sugar cane bagasse process consisted of suspending an amount of bagasse (10% w/w, dry basis) in water and loading it into a laboratory-scale reactor (7.5 L total volume, Model 4554, Parr, USA). The temperature was raised from room temperature (25 ºC) to 190ºC, over a period of 1 h. After 10 minutes, the reactor was cooled to ambient temperature and the pentose-rich liquor (HL) was collected with the aid of a laboratory-scale screen filter (Nutsche filter, POPE Scientific, USA). The soybean bran (SB) was obtained from Agricola (São Carlos, Brazil) and was characterized by Rodriguez-Zuniga *et al.* [22].

Hemicellulolytic plate assay

The selection of hemicellulolytic strains was performed by cultivation on solid medium as described by Kasana *et al.* [23] containing 0.2% beechwood xylan (Sigma) or liquor 25% (v/v). The bacteria strains were first grown on Tryptic Soy Agar (TSA) for 7 days at 29 °C and then inoculated onto the test media and incubated for 72 h at 29°C. The pH was adjusted to 7.0. The hydrolysis halos were revealed by application of Congo Red (1%) for 15 minutes, followed by washing with 1 M NaCl for 10 minutes [23]. The hydrolysis rates were calculated by dividing the diameters of the hydrolysis halos by the diameters of the colony halos.

β-glucosidase plate assay

The strains were grown for 5 days in liquid medium [24] with carboxymethylcellulose (CMC, 1%) as sole carbon source, in 10 mL tubes (200 rpm, 29°C, pH 7.0). The biomass was separated by centrifugation, and the extract was subjected to an esculin gel diffusion assay (EGDA), as described by Saqib and Whitney [25], for 5 h at 37°C. The plate was then placed on ice, and measurement was made of the dark brown zone formed by the action of β-glucosidase on esculin.

Shake flask cultures

The composition of the main culture medium was used the medium described by Nascimento *et al.* [26] : 1 g/L de proteose peptone; 0.1% (v/v) de tween 80; $NaNO_3$ 1.2 g/L; KH_2PO_4 3.0 g/L; K_2HPO_4 6.0 g/L; $MgSO_4.7H_2O$ 0.2 g/L; $CaCl_2$ 0.05 g/L; $MnSO_4.7H_20$ 0.01 g/L; $ZnSO_4.7H_2O$ 0.001 g/L; 10 g/L carbon source. As carbon source, it was used 10 g/L of pretreated delignified sugar cane bagasse (DEB) plus SB, at a 3:1 ratio, once that has been show a potential composition for glycohydrolase liquid prospection [8]. DEB was prepared and characterized by Rocha *et al.* [27]. The previously selected strains were grown on TSA for 3 days at 29°C, after which one 0.5 cm diameter disc was removed from each colony edge, transferred to an Erlenmeyer flask containing 20 mL of medium, and incubated for 144 h at 29°C and 200 rpm. The best four strains were selected for growth using the same conditions and same medium described above, but with the carbon source changed to citrus pectin or beechwood xylan. Samples were removed daily for determination of enzyme activities as described below. The glycohydrolase profile was performed with the enzymatic extracts from the time point that showed the highest glycohydrolase activity over time of culture.

Enzymatic assays

Measurement of enzymatic activities (in International Units, IU) was performed using different substrates in order to determine global and single activities. Filter paper activity (FPase) was determined as described by Xiao *et al.* [28].

All the polysaccharides were purchased from Sigma Aldrich or Megazyme, and were assayed at 0.5% in a 10 minutes reaction. The polysaccharides used were: Beechwood xylan; Birchwood xylan; Rye arabinoxylan; Wheat arabinoxylan; Sugar beet arabinan; CMC; Barley β-glucan; Tamarind xyloglucan; Icelandic moss lichenan; Laminarin from *Laminaria digitata*; Chitosan from shrimp shells; Konjac glucomannan; Carob galactomannan; 1,4 β-mannan and citrus pectin. CMC was assayed in a 30 minutes reaction. The enzymatic activity was determined from the amount of reducing sugars released from the different polysaccharide substrates, using the DNS method [29] with glucose as standard. The activities of β-glucosidase, β-xylosidase, β-mannosidase, α-L-arabinofuranosidase, and cellobiohydrolase II were measured using the respective p-nitrophenol residues (pNP) (Sigma-Aldrich, USA). The assays employed 10 µL of culture supernatant and 90 µL of the respective pNP (0.5 mM, diluted in citrate buffer), and the mixtures were incubated for 10 min at 50°C. The reactions were stopped by adding 100 µL of 1 M Na_2CO_3, and the absorbance was measured at 400 nm using a Tecan Infinite® 200 instrument (Männedorf, Switzerland). All the assays utilized an epMotion® 5075 automated pipetting system (Eppendorf) and were performed at pH 7.0 with 50 mM phosphate buffer. One unit of glycohydrolases activity corresponds to 1 µmol of monosaccharide or pNP released per minute.

RESULTS

Plate screening

The plate screening assay tested 45 actinomycetes for hemicellulases screening on plate assay. Among them 15 strains were not able to grow on media with liquor (25% v/v) and only 23 grew and produced halos in the presence of this waste. All the bacterial strains were able to grow on media containing xylan and 25 hydrolyzed this polysaccharide (Table 1). The sugar cane hydrothermal pretreatment liquor showed the following composition (g/L): xylo-oligosaccharides (9.98), xylose (4.70), glucose (0.55), arabinose (0.77), cellobiose (0.0), furfural (1.05), hydroxymethylfurfural (0.18), acetic acid (1.47), formic acid (0.23), and total soluble lignin (3.15). Although this waste present a potential carbon source for hemicellulases screening producers microorganisms, 33% of the tested strains were inhibited by this substrate. To select actinomycetes that produce β-glucosidase the EGDA assay was performed. All the strains were able to grow on CMC as sole carborn source. However, when the enzymatic extracts were analyzed, only 11% actinomycetes were positive for β-glucosidase production (Table 1).

Table 1 - Results of the selection of actinomycetes strains using the sum of the hydrolysis ratios for liquor agar and xylan agar, and calculation of the average halos obtained in the esculin gel diffusion assay (EGDA)

Strain	Identification	Source	Hydrolysis ratio using liquor agar[b,c]	Hydrolysis ratio using xylan agar[b,c]	Ratio sum[b,c]	EGDA halo average (mm)[d,e]
DR59	*Streptomyces galileus*	Soil	4.80	3.37	8.17	-
DR60	*Streptomyces* sp.	*Theobroma cacao*	4.57	3.27	7.84	-
DR61	*Streptomyce globisporus*	*C. roseus*	4.22	3.21	7.43	-
DR62	*Streptomyces* sp.	*C. sinensis*	3.60	3.20	6.80	-
DR63	*Streptomyces* sp.	Unknown	2.36	4.27	6.63	-
DR64	*Streptomyces* sp.	*C. sinensis*	3.24	3.25	6.49	-
B6P4	*Streptomyces* sp.	*S. officinarum*	2.64	3.83	6.48	-
H4P4	*Streptomyces* sp.	*S. officinarum*	2.31	3.83	6.15	-
A10	*Streptomyces sampsonii*	*C. reticulata*	3.40	2.73	6.13	-
A82	*Streptomyces pseudogriseolus*	*S. officinarum*	3.67	2.31	5.97	-
A12,1(31)	*Streptomyces* sp.	*S. officinarum*	1.94	3.21	5.16	-
A25	*Streptomyces* sp.	*C. sinensis*	2.71	2.35	5.07	-
H4.3 / C7.3	*Streptomyces* sp.	*S. officinarum*	1.94	2.77	4.72	-
G1P1	*S. pseudogriseolus*	*S. officinarum*	1.88	2.77	4.66	-
A01	*Streptomyces* sp.	*C. reticulata*	2.00	2.56	4.56	-
DR71	*Streptomyces capoamus*	Unknown	2.00	2.40	4.40	-
DR69	*Streptomyces roseochromogenus*	*C. roseus*	1.50	2.80	4.30	12.00
DR66	*Streptomyces olindenses*	Soil	2.00	2.22	4.22	+
ATCC 31267	*Streptomyces avermitilis*	Solo	1.20	3.00	4.20	-
A07	*Nocardiopsis* sp.	*C. sinensis*	1.00	3.13	4.13	-
G10P4	*Streptomyces macrosporeus*	*S. officinarum*	1.40	2.10	3.50	-
A28	*Streptomyces* sp.	*C. sinensis*	2.44	1.00	3.44	-
A18	*Streptomyces* sp.	*C. sinensis*	0.00	3.25	3.25	-
A03	*Nocardiopsis* sp.	*C. reticulata*	0.00	3.00	3.00	-
A12P2	*Streptomyces* sp.	*S. officinarum*	0.00	2.93	2.93	-
DR67	*Streptomyces lividans*	Soil	0.00	2.86	2.86	-
DSM46458	*Streptomyces chartresuts*	Unknown	1.60	1.00	2.60	-
CCT2398	*Streptomyces rimosus*	Unknown	1.33	1.00	2.33	-
A04	*Nocardiopsis* sp.	*C. reticulata*	0.00	2.27	2.27	-
A30	*Streptomyces verne*	*C. sinensis*	0.00	2.05	2.05	-
A11P2	*S. macrosporeus*	*S. officinarum*	1.00	1.00	2.00	-
DR65	*Streptomyces* sp.	*C. sinensis*	1.00	1.00	2.00	+
H4.3 C7.3	*Streptomyces* sp.	*S. officinarum*	1.00	1.00	2.00	-
A08	*Streptomyces* sp.	*C. reticulata*	1.00	1.00	2.00	-
A09	*Streptomyces* sp.	*C. sinensis*	1.00	1.00	2.00	-
A11	*Nocardiopsis* sp.	*C. sinensis*	0.00	1.56	1.56	-
DR70	*Nocardiopsis* sp.	*C. sinensis*	0.00	1.55	1.55	14.50
DR68	*Nocardiopsis* sp.	*C. sinensis*	0.00	1.00	1.00	+
A16	*Nocardiopsis* sp.	*C. sinensis*	0.00	1.00	1.00	-
A23	*Streptomyces* sp.	*C. sinensis*	1.00	1.00	1.00	-
A32	*Streptomyces* sp.	*C. sinensis*	0.00	1.00	1.00	-
A3P1	*Streptomyces albus*	*S. officinarum*	0.00	1.00	1.00	-
A4P1	*Streptomyces pulveraceus*	*S. officinarum*	0.00	1.00	1.00	-
F7P4	*Streptomyces akiyoshiensis*	*S. officinarum*	0.00	1.00	1.00	-
H4P3	*Streptomyces tsukiyonensis*	*S. officinarum*	0.00	1.00	1.00	-

[b]0.00 = No growth; [c]1.00 = Growth and absence of hydrolysis halo; [d]+ = Positive unmeasured halo; [e]- = No halo.

Shake flask culturing

Twelve strains were chosen for shake flask cultivations selection. Eight strains were chosen based on the highest hydrolysis ratio sum and four based on EGDA activity. The DEB was composed of 77.89% cellulose, 7.09% hemicellulose, and 16.22% lignin. The SB consisted of 34% cellulose, 18.13% hemicellulose, 9.78% lignin, and 43.22% protein. The media prepared using these waste materials can provide a suitable ratio of cellulose and hemicellulose for the synthesis of glycohydrolases, as well as a good source of nitrogen. This composition could induce the production of cellulolytic and hemicellulytic enzymes in several endophytic fungi [8]. All actinomycetes strains presented grow on DEB+SB media at 29ºC, 200rpm, pH 7.0 and enzymatic activities are shown in Figure 1. Low titration of β-glucosidase, FPase, CMCase and pectinase were detected during 48 and 96 h of cultivations for all the strains. The low β-glucosidase production was already present on plate assay. Among 45 strains, 5 showed β-glucosidase activity and four strains highlighted to xylanase production, DR6, DR66 and DR69, at 48h, and DR63 at 96h.

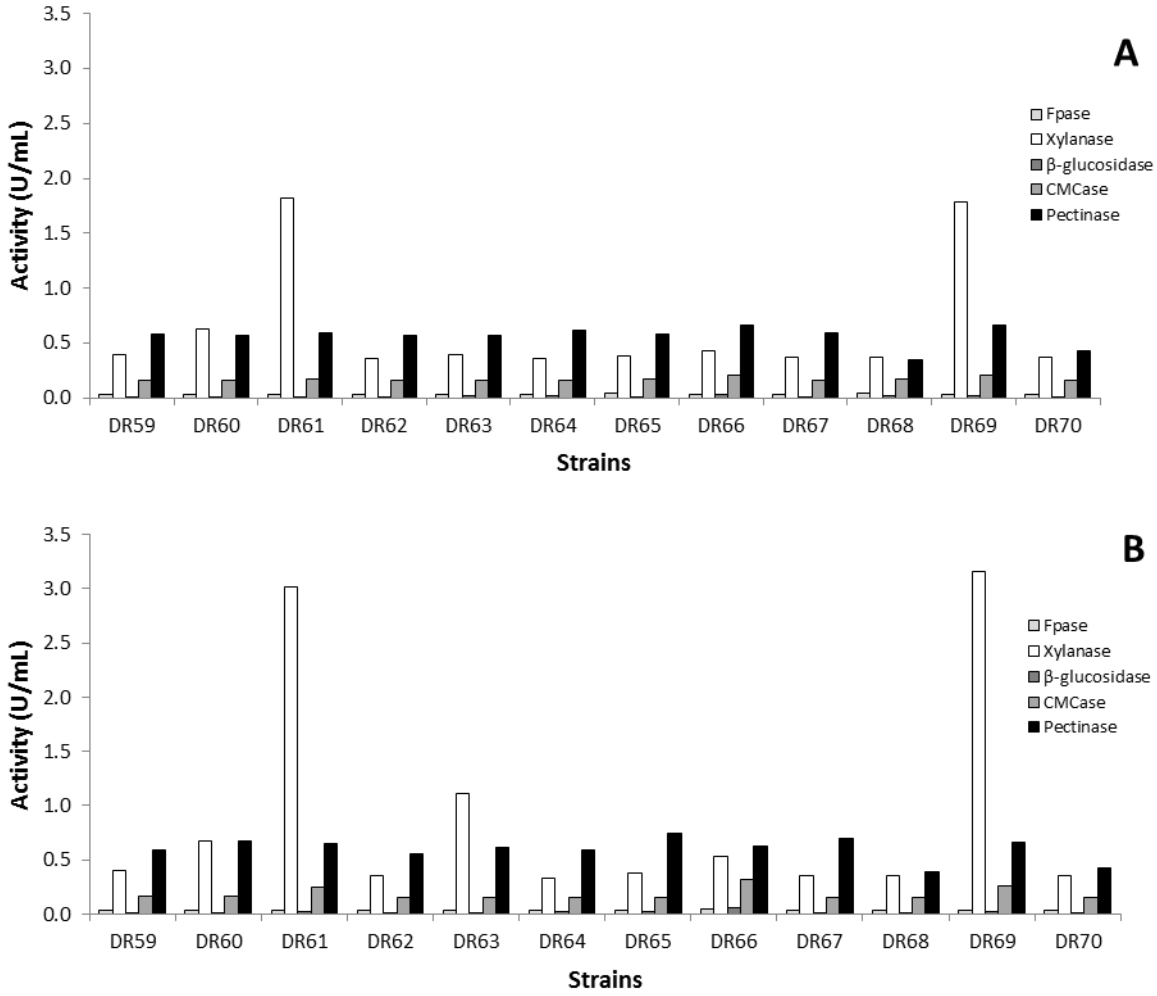

Figure 1 Enzymatic activities of actinomycetes pre-selected strains, grown in shake flasks with DEB+SB (3:1), after 48 h (A) and 96 h (B).

Glicohydrolyse profile

In accordance with previous results 4 strains that presented potential for xylanase production (DR61, DR63, DR69 and DR66), for this reason these strains were selected for cultivation under other inductors substrates (xylan and pectin). These strains belong to *Streptomyces* genera and have their extracts tested for degradation of several hemicellulolytic substrates (Table 2). It was used the extract from the time point that showed

the highest glycohidrolase activity over time of culture. Low production of cellulose was visualized for the four strains and corroborate with the selection performed. In this way, the absence of β-glucosidase and cellobiohydrolase activities may be correlated low cellulase production.

High levels of xylanase activity were detected for the 4 strains, although the strains DR63 (19.26 U/mL) and DR69 (14.68 U/mL) produced highest titrations. The xylanase production was associated with xylan presence in the media (Table 2) and on hydrolysis a higher hydrolysis against beechwood xylan was presented, probably due to the selection on xylan agar plates. Between branched xylan, rye arabionoxylan was more hydrolyzed for induced xylan enzymes extracts then wheat arabionoxylan.

Some strains, such as DR66 e DR69 were able to produce enzymes with activity to β-D-glucosil-(1→4)-β-D-glucose links from different substrates (β-glucan, lichenan and CMC), but low affinity on branched glucan (xyloglucan) was visualized. Xylan was also able to induce in the DR63 and DR61 the production of enzymes with activity to β-D-glucosil-(1→3)-β-D-glucose links from laminarin. Pectin presented the same effect in the DR69 strain.

All endophytic strains produced pectinases and its production presented no differences between xylan and pectin used as carbon source for cultivation. The strains DR66 was able to produced enzymes against glucomanan and DR63 produced hydrolytic activity against hetero/homo mannan. No expressive levels of α-arabinofuranosidase, β-xylosidase, β-manosidase and cellobiohydrolase II were presented under the conditions tested.

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Table 2 Glycohydrolases activities (U/mL) of six selected actinomycetes strains grown on pectin, xylan and DEB.

Strain	DR61			DR63			DR66			DR69		
Carbon source	**BED+FS**	**Pectin**	**Xylan**	**BED+FS**	**Pectin**	**Xylan**	**BED+FS**	**Pectin**	**Xylan**	**BED+FS**	**Pectin**	**Xylan**
Time (h)	144	96	144	144	144	144	144	96	144	96	144	144
Birchwood xylan	0.73	0.53	4.66	1.22	1.00	10.99	0.76	0.53	2.56	3.16	2.52	9.98
Beechwood xylan	1.51	0.89	8.60	1.96	1.20	19.26	1.33	0.66	4.36	3.48	1.82	14.68
Rye arabinoxylan	0.99	0.28	2.26	1.31	1.70	3.74	1.18	0.53	2.15	1.80	2.05	3.27
Wheat arabinoxylan	0.69	0.44	0.27	0.67	0.18	0.00	0.61	0.47	0.24	0.65	0.60	0.48
Arabinan	0.38	0.52	0.93	0.40	0.11	0.41	0.41	0.41	0.40	0.35	1.09	1.27
CMC	0.51	0.69	1.02	0.46	0.56	0.93	1.61	0.60	1.01	0.84	0.77	1.01
β-glucan	0.48	0.44	0.82	0.37	0.30	0.31	1.89	0.54	0.95	2.18	1.30	0.57
Xyloglucan	0.53	0.42	0.59	0.36	0.24	0.37	0.83	0.58	0.38	0.35	1.05	0.41
Lichenan	0.67	0.65	1.44	0.46	0.64	1.27	2.45	0.62	1.48	2.26	1.52	0.81
Laminarin	0.43	0.50	1.16	0.41	0.73	2.39	0.47	0.43	0.40	0.40	2.22	0.62
1,4 β-mannan	0.38	0.31	0.79	0.38	0.54	0.71	0.64	0.39	0.40	0.39	0.48	0.77
Glucomannan	0.36	0.66	0.57	0.49	1.10	0.44	1.03	0.61	0.78	0.65	0.87	0.93
Galactomannan	0.36	0.52	0.77	0.41	0.50	0.75	0.67	0.39	0.37	0.36	0.46	0.08
Pectin	0.68	0.64	0.91	0.69	0.82	1.01	0.75	0.72	0.74	0.66	0.74	0.98
pNP β-D-xylopyranoside	0.01	0.03	0.01	0.02	0.03	0.02	0.03	0.05	0.03	0.02	0.03	0.01
pNP β-D-mannopyranoside	0.05	0.04	0.02	0.02	0.03	0.03	0.04	0.04	0.02	0.02	0.04	0.01
pNP β-D-cellobioside	0.01	0.04	0.01	0.01	0.04	0.07	0.03	0.04	0.04	0.03	0.06	0.07
pNP α-L-arabinofuranoside	0.04	0.07	0.02	0.02	0.06	0.01	0.05	0.07	0.07	0.02	0.05	0.02
pNP β-D-glucopyranoside	0.01	0.00	0.01	0.01	0.02	0.02	0.08	0.00	0.03	0.02	0.02	0.03

DISCUSSION

Actinomycetes is an important class of bacteria with industrial interest. The strains used in this work were previously identified by sequencing of 16S DNAr region by Andrielli [30]. Among the 4 strains, only DR66 was isolated from soil and belongs to *Streptomyces olindensis* species. The strains DR60 and DR69 are endophytics of *C. roseus* and were identified as *Streptomyces globisporus* and *Streptomyces roseochromogenus*, respectively. Only the strain DR63 is from an unknown origin and identified as *Streptomyces* sp.. Even though *S. olindensis* is capable to produce the antitumor cosmomycin D [31], *S. globisporus* is known as a producer of N-Acetylmuramidase [32] and *S. roseochromogenus* of the antibiotic roseomycin [33], none of these species have been described as producer of plant biomass degradation enzymes.

The selection of hemicelullase producer actinomycetes based on plate and liquid were efficient once that it was possible to select strains with different hemicellulolytic profiles. Enzymatic profiles suggested that endophytic strains produced high hemicelullase titration then the soil strain (DR66) and that endophytic strains possess a similar pattern. This corroborate with Book *et al.* [34], that suggested that free-living soil *Streptomyces* have not evolved the capacity to rapidly utilize all the components of plant biomass degradation but phytopathogenic strains present CAZy gene content similar to cellulolytic strains which contributes to phytopathogenesis. Even though, endophytic strains does not causes plant diseases, these microorganisms may initiate plant material decomposition process before it becomes dominated by saprophytic species [18, 19].

The production of β-glucosidase is widespread in *Streptomyces* species [34, 35]. However, the screening for β-glucosidase production using de EDGA method lead for false positive selection, once that none of the strains were able to produce detectable amounts of β-glucosidase by pNP method. Robl et al. [8] used successfully this plate method for fungi β-glucosidase screening and the plate data corroborated with β-glucosidase liquid activity.

The formulation of enzymatic cocktails aiming an efficient plant biomass degradation must focus on plant cell wall composition. The mainly component that varies greatly between crops is hemicellulose and lignin. Sugar cane presented mainly xyloglucan and arabinoxylan closely associated with cellulose, whereas pectins, mixed-linkage-β-glucan (BG), and less branched xylans are strongly bound to cellulose [36]. For this reason this authors proposed an enzymatic hierarchical order to attacked sugar cane cell wall until naked cellulose fiber to become accessible to cellulases. In our work several enzymatic extracts presented potential on the sugar cane cell wall degradation. Bacterial extracts rich in lichenase, β-glucan, laminarin and pectinase would be useful in the first step to remove the matrix of pectin and β-glucan. Further extracts rich in endoxylanase activity would be necessary to degraded arabionoxylan. Finally, extracts rich in xyloglucanase would remove the xyloglucan that together with phenolic compounds involves microfibrils of cellulose into macrofibrils. Some enzymes activities were not detected and would be needed to a complete biomass deconstruction, such as α-arabinofuranosidase, β-galactosidase, feruloyl esterase. However, the addition of others microbial enzymatic extracts or recombinant enzymes could overcome this absence.

CONCLUSION

The present work demonstrated that it is possible to select endophytic strains that can produce glycohydrolases with activities against a wide range of plant biomass substrates. However, biochemical characterization of new reported glycohydrolases producer strains, as well as a bioprocess development of the selected strains in large scale, must be conducted to evaluate the enzyme applicability on the biomass deconstruction, principally on sugar cane bagasse.

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