Biological and Applied Sciences

Vol. 59: e16150475, January-December 2016
http://dx.doi.org/10.1590/1678-4324-2016150475
ISSN 1678-4324 Online Edition

BRAZILIAN ARCHIVES OF
BIOLOGY AND TECHNOLOGY

A N I N T E R N A T I O N A L J O U R N A L

Selection of Xilose-Fermenting Yeast Strains

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ABSTRACT

In Brazil, ethanol is obtained by fermentat of sugar cane juice using Saccharomyces cerevisiae. The cane juice extraction generates the bagasse that has been used for obtaining generation biofuel. However, the sugarcane bagasse has 30% pentose that cannot be fermented to ethanol by S. cerevisiae. Thus the aim of this study was to isolate a yeast able to ferment xylose to ethanol. Samples of cane juice and flowers were used for the isolation of 165 strains that were then screened for ethanol production using plate testing. Among them, the ethanol positive strains Wickerhamomyces anomalus, Schizosaccharomyces pombe and Starmerella meliponinorum were selected for a xylose fermentation assay, using a semi-synthetic and bagasse hydrolysate as must. S. meliponinorum and S. pombe produced 0.63 and 2.7 gL^{-1} of ethanol, respectively, from xylose in a semisynthetic medium. In the medium consisting of bagasse hydrolysate must, 0.67 and 1.1 gL^{-1} of ethanol were obtained from S. meliponinorum and S. pombe, respectively. All the yeasts produced xylitol from xylose in the semisynthetic medium and S. meliponinorum was that which produced the highest quantity (14.5 g L^{-1}).

Key words: xylose, ethanol, yeast, sugarcane bagasse.

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INTRODUCTION

In Brazil, ethanol is obtained by fermenting sugar cane juice and molasses (a residue from sugar making) using *Saccharomyces cerevisiae*. In the 2013/2014 season, the ethanol production reached 27 billion liters (Companhia Nacional de Abastecimento-CONAB, 2013) and the juice extraction resulted in 148 million tons of the sugar cane bagasse, a lignocellulosic feedstock that is used for burning to generat electricity. An alternative use for the bagasse is in secondary ethanol production. However, the saccharification of hemicellulose, that corresponds to 20 to 30% of the dry weight of bagasse, releases pentoses, mainly xylose and arabinose, which cannot be fermented to ethanol by *S. cerevisiae* (Jeffries; Jin, 2000). Currently, the xylose-assimilation pathway has been used to engineer *S. cerevisiae* with genes from xylose-assimilating yeasts (Steensel et al, 2014). However, maintaining these strains remain in the bioreactor over successive fermentation cycles is still a challenge.

The ethanol production system in Brazil is a fed-batch fermentation process with several cell recycling, requiring yeast with high viability over the month sin which the process occurs. Therefore, genetically modified yeast needs to be tolerant to industrial fermentation conditions, i.e high temperature, high ethanol content and high osmotic pressure and also the need to compete with other yeasts bioreactor contaminants (Basso et al, 2011). An alternative use of the pentoses would be the application of strains able to transform these sugar into economically attractive bio-products in a biorefineary system (Faria et al., 2014)

To obtain the fermentable sugar from the hydrolysis of bagasse, conventionally acidor alkali pre-treatments are applied that result in the release of inhibitor compounds of *S.cerevisiae* such as furfurals, weak acids and phenols (Palmqvist; Hahn-Hagerdal, 1999). Therefore, micro-organisms which could be used for producing value-added compounds from pentose from the bagasse hydrolysate should be tolerant to these compounds.

Thus, the goals of this work were to isolate yeasts able to ferment xylose to ethanol, using bagasse hydrolysate as substrate for fermentation and to evaluate the xylitol production.

MATERIAL AND METHODS

Sample collection and yeast isolation

For the isolation of yeasts from sugarcane juice, samples were collected from September to December 2011, at the Barraálcool plant, in Bugres/ State of Mato Grosso, Brazil (highway 246, km 3.5, 15º04'21" S, 57º10'52"W). The juice was extracted from sugarcane varieties RB 86-7515, 75-5113 RB, SP 83-5073, SP-8642, RB 92-8064. The yeast colonies were obtained by plating aliquots of serial dilutions of samples in a saline solution 0.85% w / v on culture medium composed of 2% peptone, 1% yeast extract, 2% xylose, 2% agar (YEPX) and 50 mg mL^{-1} ampicillin. The incubation was at 30 °C for 48 h and the 12 colonies per sample were taken at random and streaked to obtain pure cultures.

Samples of flowers were collected monthly from March to June 2012in São José do Rio Preto (20049'13"south latitude and49022'47" west longitude) and in Ilha Solteira, SP (20025'58" south latitude and 51020'33" west longitude). Approximately 500 mg of flowers samples were placed in tubes containing 5 mL of basal culture medium compoused by (g L^{-1}) 0.5 of $MgSO_4$; 1.0 of KH_2PO_4,0.1 of NaCl, 0.1 of $CaCl_2$; 2.0 $NH4)_2SO_4$), 10.0 of yeast extract, 0.0005 of H_3BO_3, 0.00004 of $CuSO_4$, 0.0001 of $FeCl_3$, 0.0004 of Na_2MoO_4, 0.0002 of $ZnSO_4$, 0.0004 of $MnSO_4$, 30.0 xylose and an initial pH of 3.5. Each sample inoculated in culture medium was incubated for 8 h. at 30ºC. After incubation the yeasts were streaked on YEPX and four to six colonies per sample were randomly selected and streaked again to obtain pure cultures. The yeasts were preserved in glycerolat -80ºC.

Yeast screening for ethanol production from xylose

The plate test for ethanol production was carried out according Grabek-Lejko et al. (2006). In this technique, pentose-fermenting yeasts are placed to grow in a solid culture medium containing D-xylose as the sole carbon source and the presence of ethanol is detected by growth of a reference yeast strain that does not grow on xylose but grows ethanol. The colonies of xylose fermenting yeast that presented a halo resulted from growth of ethanol-using yeast were selected as positive strains for ethanol production from xylose. For this experiment, an aliquot of 800 µL of a suspension of not-using xylose and ethanol-using *Candida*

silvae BR3-3BY was inoculated pour-plate using YNB medium with 2% of xylose. Then, the testing strains and a control ethanol positive strain *Candida shehatae* CG8 8BY were inoculated at points equidistant on the surface of the medium and incubated at 30ºC for 10 days. The *Candida silvae* and *Candida shehatae* were provided by Prof. Fernando C. Pagnocca, Laboratory of Microbiologia – CEIS/IB/UNESP, Rio Claro, SP, Brazil.

Yeast identification

The identification of isolated strains was made by sequences of the D1/D2 domains of the rDNA (Fell et al., 2000; Scorzetti et al., 2002) by Prof. Fernando C. Pagnocca, Laboratory of Microbiology – CEIS/IB/UNESP, Rio Claro, SP, Brazil.

Batch fermentation screening

To evaluate the potential of xylose fermentation, the strains were pre-cultivated in YEPD medium (1% yeast extract, 2% peptone and 2% glucose) for 24 h at 28 °C (for *Schizosaccharomyces pombe* was 32ºC). After 24 h of incubation the aliquots were centrifuged at 150 rpm and biomass was used for inoculate 60 mL of basal medium described above (pH 4.0) in concentration of 1.0 x 10^8 mL^{-1}. 125 mL Erlenmeyer flasks, adapted for alcoholic fermentation with a valve containing sodium metabisulphite solution at 1 $g.L^{-1}$ to ensure that no oxygen could get in, were used. The assays were done in duplicate. Every 24 h, a flask was taken, centrifuged and the supernatant used to quantify the remaining glucose or xylose, ethanol, xylitol, glycerol, acetic acid, and the biomass was analyzed for growth and cell viability. The strains that were positive for ethanol were used for fermentation of hydrolyzed sugarcane bagasse. To evaluate the ability of the strains to produce ethanol and / or xylitol from hemicellulose hydrolysates of cane bagasse, the same methodology used for fermentation using semisynthetic medium was followed, but with changes in the volume of the medium and agitation speeds. 15 ml of hydrolysate and an agitation speed of 100 rpm for *S. pombe* while for *S. meliponinorum* 150 rpm were used. The samples were analyzed at the beginning and after 96 hours of fermentation. The parameters evaluated in the fermentation were: consumption of xylose, arabinose and glucose, ethanol production, xylitol production, cell growth and viability.

Process efficiency

Ethanol and Xylitol Yield (Yp/s): The conversion factor, which express the ratio between the mass of ethanol produced and the mass of xylose consumed, in grams.

Ethanol and Xylitol Volumetric Productivity (Qp): The volumetric ethanol productivity express the ratio between the mass of ethanol produced ($g\ L^{-1}$) per hour.

Conversion factor of xylose in cell mass (Y x/s): This parameter express the ratio between the cell mass (g_{cel}) produced and the consumed xylose mass (g).

Conversion efficiency (η) of ethanol: This fermentation parameter, expressed as a percentage, is the ratio between the experimental yield (Y p/s) and the theoretical yield (Y_T) of 0.511 g g^{-1} of glucose and/or xylose consumed.

Conversion efficiency (η) of Xylitol: This fermentation parameter, expressed as a percentage, is the ratio of the experimental yield (Y p/s) to the theoretical yield (Y_T) of 0,905 g g^{-1} calculated by Barbosa et al (1988).

Sugarcane hydrolysate bagasse obtaining and fermentation

The sugarcane bagasse was provided by the sugar and alcohol plant Virgolino de OliveiraS/A,in, Jose Bonifacio, SP, Brazil. The material was washed, dried to a humidity of 8%, milled to3 mm thickness and subjected to acid hydrolysis according to methodology adapted from Pessoa Jr (1997). 3 g of bagasse submersed in 30 mL of 2% sulfuric acid solution (200 mg of sulfuric acid per gram of bagasse) were maintained at 121 ºC for 15 min and then, vacuum filtered to remove residual solid.

The hydrolysate was concentrated in a rotary evaporator at 90 ºC until one third of the initial volume and the pH, xylose, arabinose, glucose, furfural, 5-hydroxymethylfurfural and acetic acid concentrations were determined.

The detoxification was done according to the method described by Marton (2002). The initial pH of material (1.7) was raised to 7.0 with NaOH (solid) and immediately decreased to 2.5 with sulfuric acid (72 % v/v) and mixed with activated charcoal powder (Merck-1% m/v). After agitation at 200 rpm at 50 °C for 60 min., the material was centrifuged. The supernatant had the pH adjusted to 4.0, sterilized at 121 ºC for 30 min. and

supplemented with mineral nutrients described for basal medium to compose the fermentation must.

Analytical methods

To evaluate the dry cell biomass, samples from fermented medium were centrifuged at 10,000 x g for 15 min., the supernatant was discarded and the precipitated cells were dried at 60 °C until constant weight.

The cell viability was monitored by counting in a Neubauer chamber as described in Ceccato-Antonini (2010), using the staining with methylene blue method described by Lee et al. (1981).

Ethanol concentration was measured by gas chromatograph (HP 5890) with a FFAP capillary column (polyethylene glycol - 30 mx 0.22 mm x 0.3 microns) and a flame ionization detector, one split/splitless injector where the needle was split in the ratio1:20. Nitrogen was utilized as a carrier gas at 30 $mL.min^{-1}$. The temperature at both injector and detector was 250 °C.

The xylitol quantification was performed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). All samples were filtered (0.22 micron membrane) and injected (20 µL) in the HPAEC-PAD System (ICS, Dionex Corporation, USA) equipped with an automatic sampler AS40. The standard quadruple waveform was used with the following potential pulse and durations: E1 = 0.10V (t1 = 0.40s); E2 = -2.00V (t2 = 0.02 seconds); E3 = 0.60V (t3 = 0.01s); E4 = -0.10V (t4 = 0.06 s). An isocratic run was performed with 10 mM NaOH at a flow rate of $1mL.min^{-1}$ and 35°C.

The glycerol and acetic acid were determined by liquid chromatography (Agilent 1290 Infinity Technologies) under the following conditions: column Phenomenex Rezex ROA maintained at 50 ^{o}C; Refractive index detector RID; eluent water acidified with trifluoroacetic acid and a flow rate of 0.6 mL min^{-1}; volume of the injected sample 20 µl. The samples were centrifuged and then filtered through a Whatman 0.22 micron Teflon.

Glucose, xylose, arabinse and xylitol were quantified using an ICS 5000 Dionex HPAEC-PAD ionic chromatograph with anionic column CarboPac PA-1. Solvent A (deionized water); B (500 mM NaOH) and C (300 mM sodium acetate with 150 mM NaOH). The elution (1.0 mL min^{-1}) was performed from 0 to 11 min in isocratic mode with 6 % C; 94 % B, and then changed in gradient mode to 20 % B; 60 % C and 20% D at 26 min.

Total phenolic compounds were quantified using the Folin-Ciocalteu method (singleton; orthofer; lamuela-raventós, 1999).

The concentrations of furfural and hydroxymethyl furfural(HMF) were determined using an HPLC-UV -Vis 3000 UHPLC Dionex, P680 pump, column compartment at 300 ^{0}C with a flow of 0.8 ml min-1 and EC 250 /4.6 Nucleosil column 100-5 reversed phase CN. The isocratic mobile phase consisted of 90 % water and 10 % methanol, with detection at 276 nm using a Dionex UV D-340 V detector. The volume of the injected sample was 20 µl. The samples were pre-diluted and filtered in a filter (Whatman) with a pore size of 0.22 microns.

The humidity of the bagasse was determined using an OHAUS Moisture Analyzer, set in an agri business crusher TRAPP 400,with a standardized mesh size of 3 mm.

RESULTS AND DISCUSSION

Isolation and selection of ethanol-producing yeasts

There were 165 isolated strains of yeast and the plate assay showed that among all of them, only 4, namely, *S. pombe* BB.92, *Wickerhamomyces anomalus* FRP.04, *W. anomalus* BB.10 and *Starmerella meliponinorum* FRP.04 were able to ferment xylose to ethanol (Table 1; Figure 1). There is little in the literature about ethanol production from xylose by *S.pombe* and *W.anomalus* (Gong, 1983; Barbosa et al. 1988). No reports were found of ethanol production from xylose by *S.meliponinorum.*

These strains were used to evaluate the ethanol production from xylose in a liquid medium.

Production of ethanol from glucose and xylose in synthetic media

In batch fermentation in a flask, using xylose as the only carbon source, the strains *W. anomalus* BB.10 and *W. anomalus* FRP.04 produced 0.50 g L^{-1} of ethanol and *S. meliponinorum* FRP.09 and *S. pombe* BB.92 produced 0.63 and 2.7 g L^{-1},respectively. *W. anomalus* BB.10, *W. anomalus* FRP.04 and *S. meliponinorum* FRP.09 produced xylitol from xylose.

W. anomalus BB.10 showed higher growth in glucose than xylose with consumption of 100% of the glucose in 24h h of fermentation and the production of 30 g L^{-1} of ethanol (yield = 0.35 g g^{-}

^{1}) and 17 g L^{-1} of dry biomass. This strain assimilated only 55.3 % (38.7 g L^{-1}) of xylose in 96 h of fermentation with 7 g L^{-1} of dry biomass and 8.0 g L^{-1} of xylitol (yield 0.21 g g^{-1}).

The *S. pombe* BB.92 used the glucose efficiently for growth but produced a lower level of ethanol. This yeast presented a very low ability to use xylose with a tiny production of ethanol and a non-detectable level of xylitol.

The *W. anomalus* FRP.04 showed a profile similar to fermentative yeasts in oxygen-limited condition using glucose to produce ethanol with a low growth level and assimilated only 30% of the xylose and did not produce any xylitol.

S. meliponinorum FRP.09 assimilated 41% of the xylose of the medium and produced 14.5g L^{-1} of xylitol but produced a small quantity of ethanol. The acetic acid production by this strain was the highest among the evaluated yeasts. The fermentation profile in the medium with glucose was similar to the others.

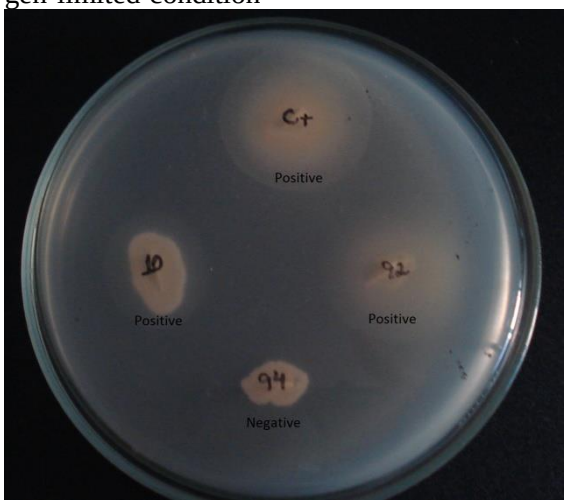


Figure 1 - Growth of ethanol-assimilating *Candida silvae* BR3-3BY as positive test for ethanol production from xylose.

Table 1 - Isolation of yeasts producing ethanol from xylose

Collect Place	Sample	Number of isolated	Positive for ethanol from xylose
Barra do Bugres/MT	Sugarcane juice	100	*Wickerhamomyces anomalus* BB.10 *Schizosaccharomyces pombe* BB.92
São José do Rio Preto/SP	Flowers	30	*Wickerhamomyces anomalus* FRP.04 *Starmerella meliponinorum* FRP.09
Ilha Solteira/SP	Flowers	35	None

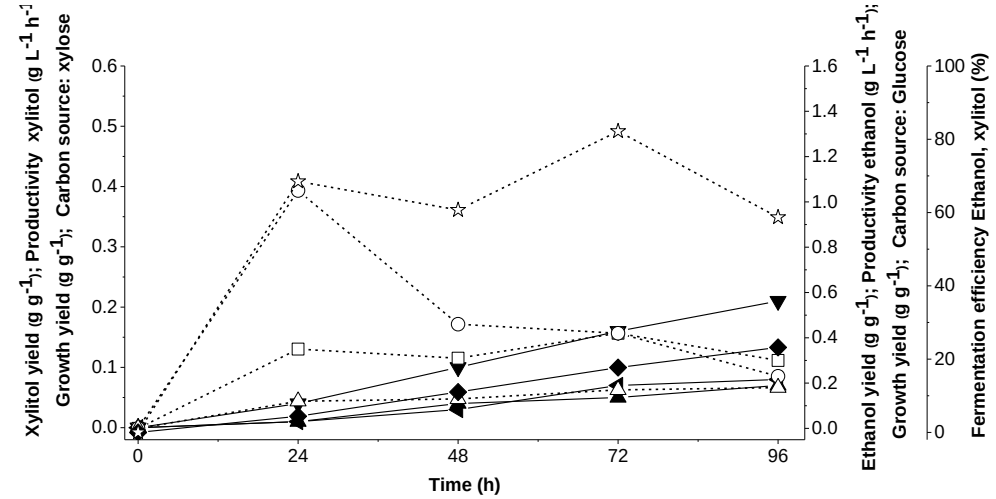
The highest xylitol yield was obtained with *S. meliponinorum* FRP.09 (Yp/s = 0.50 g g^{-1}) in 96 h (Figure 3d). This strain presented low growth and ethanol yield when xylose was the carbon source (Yp/s= 0.07 and 0.02 g g^{-1} respectively) with no significant difference then growth yield obtained from glucose. On the other hand, in media with glucose the ethanol yield and volumetric productivity were higher (- Yp/s = 0.47 g g^{-1} and Qp = 1.16 g g $^{-1}$ h^{-1} , in 24 h) (Figure 2d).

The highest production of ethanol from xylose was obtained with *S. pombe* BB.92. However, the

ethanol yield (Yp/s=0.55) and volumetric productivity (Qp) were lower (72h against 48h in glucose) (Figure 3b).These parameters (Y p/s = 0.10; Qp = 0.03) are low compared to those obtained with *Candida shehatea*, *Scheffersomyces stipitis* and *Fusarium oxysporum* that reached 0.45 g g^{-1} xylose (productivity> 0.17 g L^{-1} h^{-1}) according to Hahn-Hagerdal et al. (1994). Millati et al.(2004) and by Du Preez (1994) obtained Yp/s = 0.32 and 0.39 with *P. tannophilus* and *S. stiptis.* Stoichiometrically, the theoretical yield of ethanol is 0.511g g^{-1} of glucose or xylose consumed. However, in cultivation, even if all the sugar is consumed the yield is less than the theoretical since part of the sugar is used for cell growth and secondary metabolite production can be reduced to 90 -93% (Ingledew, 1987).

On the other hand, the ethanol yield obtained with *W. anomalus* FRP.04 and *S. meliponinorum* FRP.09 in the medium with glucose were high(0.47 g g^{-1}), which is a fermentative efficiency of 92%. Similar result (0.46 g g^{-1}) was reported by Lima et al. (2001).

A



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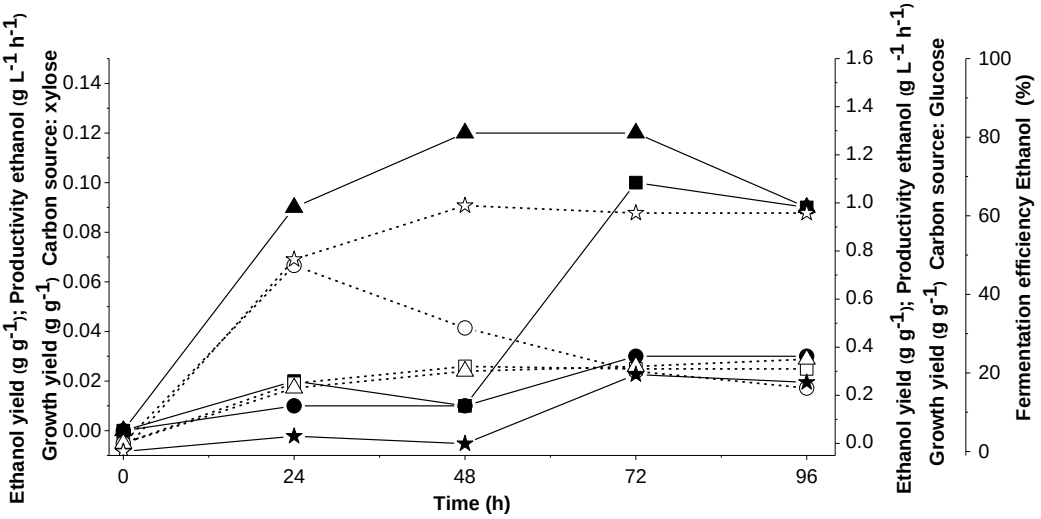

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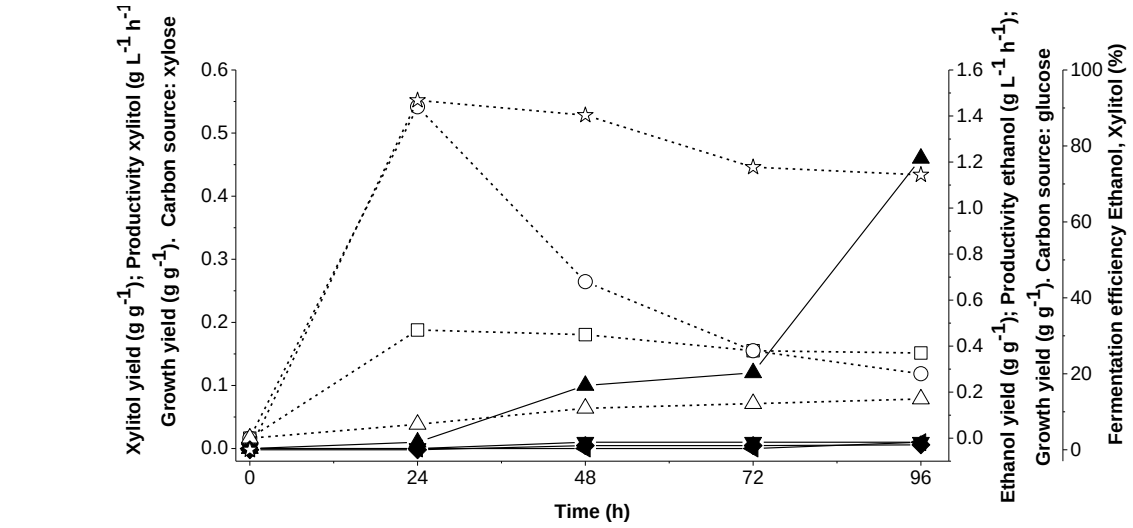

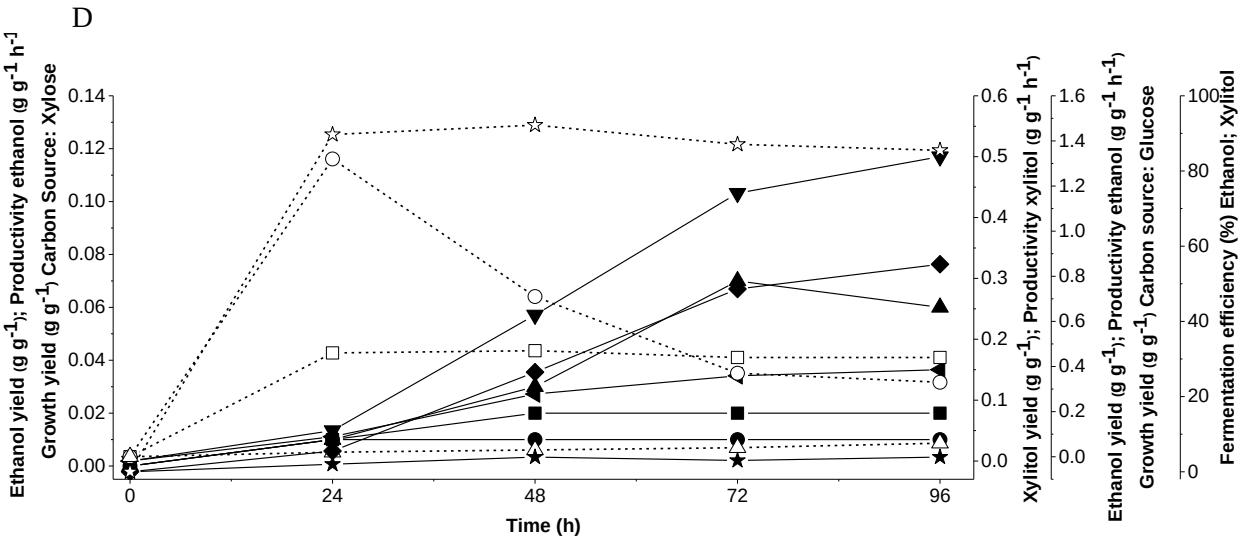

Figure 2 - Fermentation profile in medium with xylose and glucose . *W. anomalus* BB.10 (a), *S. pombe* BB.92 (b), *W. anomalus* FRP.04 (c) , *S. meliponinorum* FRP.09 (d). □ = Ethanol yield (glucose); ■= Ethanol yield (xylose); ○= Volumetric ethanol productivity (glucose) ●= Volumetric ethanol productivity (xylose); △ = Growth yield (glucose); ▲= Growth yield (xylose); ▼= Xylitol yield (xylose); ◄= Volumetric xylitol productivity (xylose); ☆= Ethanol fermentation efficiency (glucose); ★= Ethanol fermentation efficiency (xylose); ◆= xylitol fermentation efficiency (xylose).

Fermentation of the sugar cane hemicellulosic hydrolysate

The *S. meliponinorum* FRP.09 and *S. pombe* BB.92 were selected for hydrolysate fermentation assays based on their ability to assimilate xylose.

Table 3 shows the composition of the hydrolysate after acid hydrolysis, detoxification and concentration and the final must had been obtained. Figure 3 shows the visual aspect of the material. The acid hydrolysis resulted in eluted that, after three times concentration and detoxification, contained 55 g L^{-1} of pentoses and a very low glucose content (0.71 g L^{-1}) which is a good culture medium to assess the fermentation of xylose. The detoxification with activated charcoal resulted in clarification, decreasing the furfural (99 %), HMF (97 %) and phenols (89 %) concentrations but it removed only 31% of the acetic acid and a little of the sugar. The must obtained from the hydrolysate was used for

fermentation whose results of yeast growth and viability, sugar consumption, ethanol and xylitol production, yield of ethanol and xylitol (Y= p/s g g^{-1}), volumetric productivity (Qp = g $g^{-1}h^{-1}$), yield biomass (Y = x/s g g^{-1}) and conversion efficiency (Ƞ%) are shown in Table 2 and Figures 4.

The yeast growths were lower than those observed in a semi-synthetic medium but only a slight decrease in the cell viability was observed for *S pombe* (13%) after 96 h indicating a low toxicity effect of hydrolyzed (Table 2).

S meliponinorum consumed a higher quantity of xylose than *S pombe* but was not able to assimilate arabinose. The arabinose assimilation pathway of yeast is very similar to that of xylose, however, as observed in this study, arabinose consumption is much slower than that of xylose. Similar behavior was observed by Lima (2004) during fermentation of sugar cane bagasse hemicellulosic hydrolysate with *C. guilliermondii*, as well as synthetic medium by Felipe et al. (1995).

The highest production of xylitol in fermentation of the hydrolysate was presented by *S meliponinorum* A (0.78 g L^{-1}) after 96 h with a yield of 0.13 g g^{-1} and efficiency of 14.5%, (Figure 4) but it was smaller than that obtained in the semi-synthetic medium (Figure 2d).

The highest ethanol production (1.1 g L^{-1}) was reached in fermentation with *S. pombe* when the consumption was 6.6 g L^{-1} of sugar with a yield of 0.2 g of ethanol (g g^{-1}) resulting in a fermentative efficiency of 45.5%, higher than those observed in the semi-synthetic medium (Figure 2).

Roviero et al (2015), evaluating the production of ethanol by the yeast *Rhodotorula glutinis* J10 in sugarcane juice and sugar cane bagasse hemicellulosic hydrolysate, obtained 24.0 and 9.0 g L^{-1} of ethanol in the respective substrates. Qiang Yi (2014), using *S. stiptis* for fermentation of bagasse hydrolysate showed a production of 4.64 g L^{-1} of ethanol(a yield of 0.47 g g^{-1}).

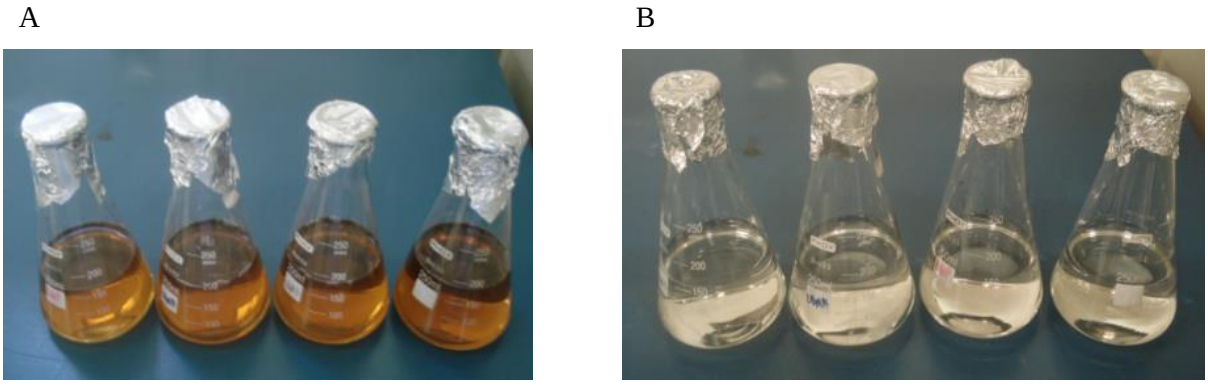

Figure 3- Visual aspect of bagasse hydrolysate before (a) and after detoxification (b)

Table 2 - Composition of hydrolysate bagasse (0.2 g H_2SO_4 per gram of dry bagasse) detoxified with activated charcoal powder (Merck-1% m/v). Final pH = 4.0

Compounds (g L^{-1})	Hydrolysate	Concentrated Hydrolysate (three times)	Must after detoxication and nutrient addition
Xylose	20.8 (±1.26)	65.5 (±0.99)	51.9 (±0.34)
Glucose	0.55 (±0.77)	1.67 (±0.35)	0.71 (±0.98)
Arabinose	1.09 (±0,218)	4.27 (±0,167)	3.18 (±0.15)
Furfural	0.08 (±0.0023)	0.23 (±0.002)	0.001 (±0.21)
Hydroxymethylfurfural	0.006 (±0.0001)	0.015 (±0.002)	0.0003 (±0.0001)
Total phenols	3.00 (±0.21)	7.80 (±0.49)	0.85 (±0.18)
Acetic acid	1.26 (±0.32)	2.97(±0.26)	1.85(±0.29)

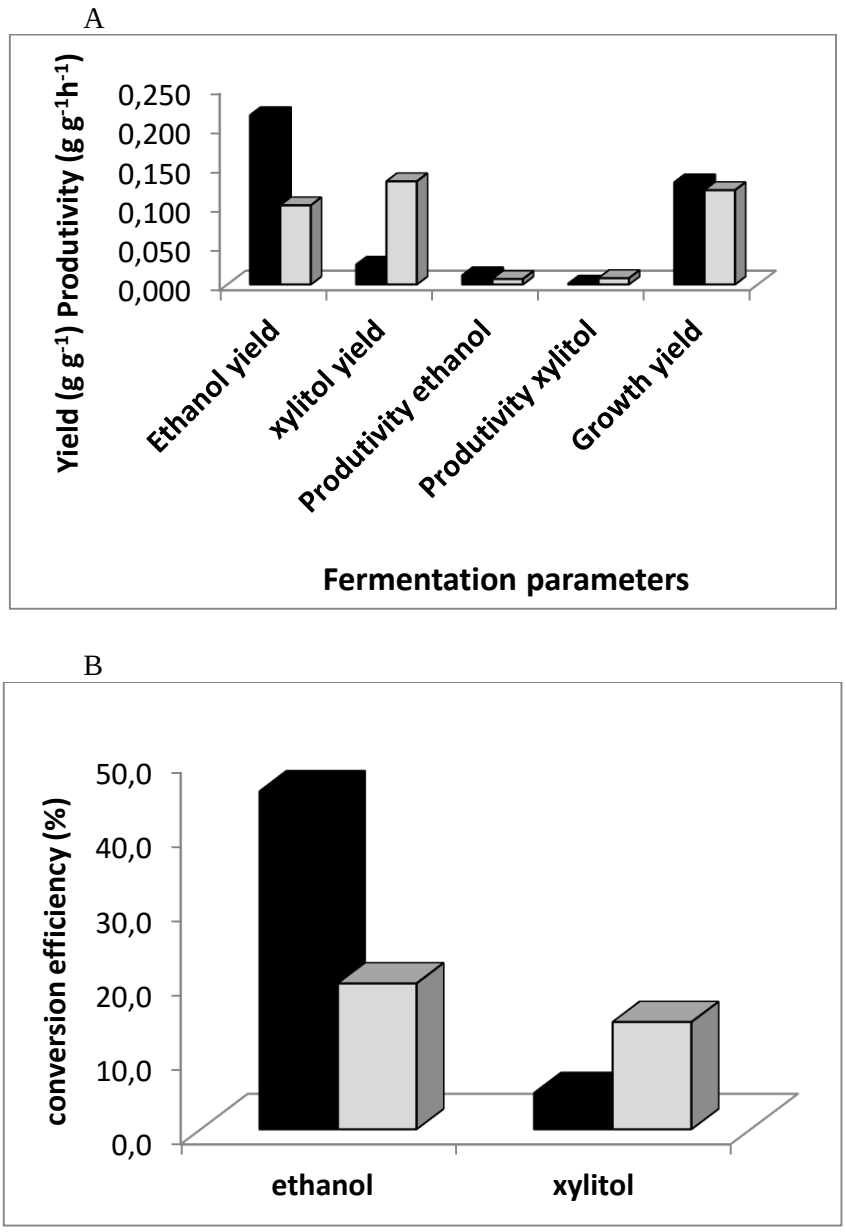

Figure 4 – Fermentation of the hydrolysate after 96 h of at 28 ºC and 150 rpm for *S. meliponinorum* and 32ºC and 100 rpm for *S. pombe*. a= Yield, volumetric productivity of ethanol and xylitol and growth yield; b= conversion efficiency. Black bar= *Schizosaccharomyces pombe* BB.92; gray bar=***S****tarmerella meliponinorum* FRP.09.

With the exception of the yeast *S. meliponinorum*, that showed no significant difference between growth in glucose and xylose, none of the other tested yeasts were able to grow in xylose with the same efficiency that in glucose, although an increase in biomass has been shown in the presence of the first sugar. The three genera showed to be different concerning its biomass production capacity and the production of ethanol and also xylitol from xylose.

The xylose assimilation pathway described for yeasts involves a xylose reductase (XR) dependent on NADPH or NADH (in general, with a preference for NADPH) which converts xylose to xylitol, and a xylitol dehydrogenase dependent of NAD + or NADP + (with a preference for NAD +) for the conversion of xylitol to xylulose, subsequently phosphory lated by xylulose kinase which is inserted in the glycolysis or pentose phosphate pathway. In most xylose fermenting

microorganisms, the conversion of xylose to xylulose implies the production of NADP + and NADH, that need to be regenerated for maintaining the redox balance. The regeneration of NADPH can be achieved in the oxidative step in the pentose phosphate pathway and NADH can be reoxidized through the respiratory chain or needs another electron acceptor. However, different yeasts have different redox balances and cofactor specificities. Yeasts with xylose reductase activities related to NADH and NADPH regenerate NAD + in the second xylose metabolism step. In this case, the main product is ethanol and there is no xylitol accumulation. In contrast, yeasts that ferment D-xylose only by the xylose reductase NADPH-dependent (with complete absence of xylose reductase connected to NADH) at the first step of D-xylose metabolism, accumulate xylitol (Skoog, 1988; Jeffries, Jin, 2004). On the other hand, any yeast demonstrating good xylose assimilation with biomass with little or no accumulation of ethanol and xylitol, such as *S.pombe* BB.92, deserves further investigation.

CONCLUSIONS

Ethanol was produced from xylose by the *Schizosaccharomyces pombe* and *Starmerella meliponinorum* strains in both a semisynthetic and a hydrolyzed medium but the performance in this was below the yield found for other yeasts reported in the literature. The four yeasts showed potential for ethanol production from glucose, especially the yeast strains *Starmerella meliponinorum* FRP.09 and *Wickerhamomyces anomalus* FRP.04, which showed a 92% glucose to ethanol conversion efficiency when compared to the theoretical yield. The Starmerella meliponinorum yeast has the potential to produce xylitol from xylose in a semisynthetic medium.

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Received: August 11, 2015;
Accepted: December 16, 2015.