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Research article

Xylitol production and furfural consumption by a wild type *Geotrichum* sp.

Ítalo Thiago Silveira Rocha Matos [a,*], Edson Júnior do Carmo [b], Enedina Nogueira de Assunção [b], Richardson Alves de Almeida [c], Verena Makarem Soares [a], Spartaco Astolfi Filho [a]

[a] Universidade Federal do Amazonas, Departamento de Genética, Av. Gal. Rodrigo Octávio, 3000, Campus Universitário, Setor Sul., Manaus, AM CEP 69.077-00, Brazil
[b] Universidade Federal do Amazonas, Centro de Apoio Multidisciplinar, Av. Gal. Rodrigo Octávio, 3000, Campus Universitário, Setor Sul., Manaus, AM CEP 69.077-00, Brazil
[c] Universidade Federal do Amazonas, Departamento de Química, Av. Gal. Rodrigo Octávio, 3000, Campus Universitário, Setor Norte, Manaus, AM CEP 69.077-00, Brazil

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ABSTRACT

Background: Xylitol is a five carbons polyol with promising medical applications. It can be obtained from chemical D-xylose reduction or by microbial fermentation of Sugarcane Bagasse Hemicellulosic Hydrolysate. For this last process, some microbial inhibitors, as furfural, constitute severe bottleneck. In this case, the use of strains able to produce xylitol simultaneously to furfural neutralization is an interesting alternative. A wild-type strain of *Geotrichum* sp. was detected with this ability, and its performance in xylitol production and furfural consumption was evaluated. Furthermore, were analyzed its degradation products.
Results: Geotrichum sp. produced xylitol from D-xylose fermentation with a yield of 0.44 $g \cdot g^{-1}$. Furfural was fully consumed in fermentation assay and when provided in the medium until concentration of 6 $g \cdot L^{-1}$. The furfural degradation product is not an identified molecule, presenting a molecular weight of 161 $g \cdot mol^{-1}$, an uncommon feature for the microbial metabolism of this product.
Conclusion: This strain presents most remarkable potential in performing furfural consumption simultaneous to xylitol production. Subsequent efforts must be employed to establish bioprocess to simultaneous detoxification and xylitol production by *Geotrichum* sp.

1. Introduction

Xylitol is a sugar-alcohol (polyol) of five carbons, produced from the reduction of D-xylose, being obtained by chemical synthesis or microbial fermentation [1]. This product is commonly described as a powerful sweetener, with flavor resembling to sucrose, but with 40% less calories of that sugar [2].

The medical applications of xylitol are related to the reduction of dental caries [3] and dry mouth sensation in patients with xerostomia [4], and prophylactic effects against obesity [5] and middle ear infection [6]. Its potential to induce apoptosis in some cell-cancers strains has been investigated [7].

Most of industrial xylitol production is performed by chemical dehydrogenation of purified D-xylose, but this process is too expensive and requires several proceedings to remove these by-products [8]. In this context, microbial fermentation is a profitable alternative for xylitol production.

Microbial fermentation is performed mainly by culturing yeasts and/or bacteria in hemicellulosic hydrolyzate derived from lignocellulosic waste. In addition to D-xylose and other sugars, the pre-treatment used for biomass hydrolysis releases some microbial inhibitors such as furfural, hydroxyl-methyl-furfural, phenolic compounds and acetic acid, being these the most important hindrance for xylitol production using fermentative process [9].

For inhibitor neutralization, some methods must be applied as electrodialysis, filtration, addition of activated charcoal and hydroxides. As all these detoxification techniques have some collateral effects, especially the reduction of sugar yield from hydrolysis, the biodetoxification, using microorganisms to consume the inhibitors, is an interesting alternative [10].

* Corresponding author.
E-mail address: italo_matos@ufam.edu.br (Í.T.S.R. Matos).
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A wild-type strain of *Geotrichum* sp. was isolated from xylophagous beetles gut and preliminary tests had identified that it is able to produce xylitol and perform furfural detoxification. The aim of this work was to characterize the fermentation of Sugarcane Bagasse Hemicellulosic Hydrolysate (SBHH) to xylitol production by *Geotrichum* sp. KP276644 and its capability to perform biodetoxification by furfural consumption.

2. Material and methods

2.1. Microorganism reactivation and pre-inoculum

Geotrichum sp. KP276644 was reactivated in Sabouraud Agar (glucose, 40 $g \cdot L^{-1}$; Peptone, 10 $g \cdot L^{-1}$ and Agar 20 $g \cdot L^{-1}$), at 28°C for 48 h. Pre-inoculum was prepared by culturing a loopful of reactivated yeast in YGX broth (yeast extract, 10 $g \cdot L^{-1}$; glucose 20 $g \cdot L^{-1}$ and xylose 20 $g \cdot L^{-1}$), at 28°C for 72 h and 120 rpm. This culture was centrifuged at 3500 × g, 4°C for 40 min, and the sediment was used as inoculum.

2.2. SBHH preparation and fermentation assay

For SBHH obtainment, the sugarcane bagasse was collected from Jayoro Agroindustrial Inc., at Presidente Figueiredo city, Amazonas State, Brazil (02°02′04″ S–60°01′33″ W). This material was washed for removing residual sucrose, dried until constant weight and mixed with sulfuric acid (3% v/v) in solid:liquid ratio of 1:5. After 24 h at room temperature, this mixture was autoclaved (121°C) for 40 min. It was cooled at room temperature and pressed (until 10 ton), being collected liquid phase and adjusting its pH for 5.0 by calcium hydroxide addition. After this, the SBHH was filtered in vacuum, and its chemical composition (Table 1) was evaluated.

Fermentation assay was performed in Erlenmeyer flasks containing SBHH supplemented with urea (1.25 $g \cdot L^{-1}$), and *Geotrichum* sp. KP276644 inoculated with initial cell concentration about 5.00 $g \cdot L^{-1}$. The flasks were incubated at 28°C and 120 rpm for 120 h. Xylose consumption and xylitol yield were calculated as described by Silva et al. [11].

2.3. Furfural consumption assay

The furfural consumption was evaluated by culturing *Geotrichum* sp. KP276644 in Erlenmeyer flasks (28°C, 120 rpm for 120 h) containing different concentrations of this substance, supplemented or not with glucose, according Table 2. An aliquot of each flask was collected each 24 h for measuring cell growth by optical density at 600 nm (OD_{600}) and furfural concentration by High Performance Liquid Chromatography (HPLC), as described forward.

2.4. Characterization of furfural metabolism products

To evaluate furfural metabolism products, *Geotrichum* sp. was cultivated in different culture media composed of yeast nitrogen base (without amino acids, 6.7 $g \cdot L^{-1}$), furfural (4 $g \cdot L^{-1}$) and glucose (40 $g \cdot L^{-1}$) or xylose (40 $g \cdot L^{-1}$) or a mixed carbon source glucose–xylose (20 $g \cdot L^{-1}$, each one). A control assay was performed using glucose (40 $g \cdot L^{-1}$) without addition of furfural. These flasks were incubated at 30°C and 120 rpm for 120 h. After this period, an aliquot was analyzed by direct injection in mass spectrometer.

Table 1
Sugarcane bagasse hemicellulosic hydrolysate composition.

Component	Concentration
Xylose	52.46 $g \cdot L^{-1}$
Arabinose	6.58 $g \cdot L^{-1}$
Galactose	1.82 $g \cdot L^{-1}$
Cellobiose	ND
Glucose	5.52 $g \cdot L^{-1}$
Acetic acid	1.93 $g \cdot L^{-1}$
Furfural	3.60 $g \cdot L^{-1}$
Hydroxy-methyl-furfural	0.92 $g \cdot L^{-1}$
Total phenolic compounds	461 $mg \cdot L^{-1}$

ND: not detected.

Table 2
Detailing of assays for furfural consumption characterization.

Assay	Glucose concentration ($g \cdot L^{-1}$)	Furfural concentration ($g \cdot L^{-1}$)
GF2	20.0	2.00
GF4	20.0	4.00
GF6	20.0	6.00
GF8	20.0	8.00
F2	–	2.00
F4	–	4.00
C	20.0	–

C: Control assay.

2.5. Analytical methods

Furfural, hydroxyl-methyl-furfural and total phenolic compounds concentrations were determined by HPLC using C18 column and UV detector (Shimadzu®). Furfural metabolism products were evaluated by direct injection in Mass Spectrometry (MS), using a TSQ Quantum Access detector (Thermo Scientific®). Xylose, arabinose, galactose, glucose, cellobiose, acetic acid and xylitol concentrations were determined by HPLC using Rezex Monosaccharides Pb^{2+} 8% column (Phenomenex®), refractive index detector.

2.6. Statistical analysis

As all assays were performed in triplicate, significant difference among them was analyzed by Kruskal–Wallis non-parametric statistical test (Statsoft 6.0; $\alpha = 0.05$).

3. Results and discussion

3.1. Fermentation assay

After 120 h of fermentation, SBHH analysis indicates 97.6% of D-xylose consumption and xylitol yield of 0.44 $g \cdot g^{-1}$. This value is similar to most of biological processes employed in xylitol production [2]. Ethanol was detected probably resulting from glucose and other hexose fermentation. This is evidenced because when xylose was provided as a sole carbon source to *Geotrichum* sp. KP276644, ethanol was not detected. Xylitol yield obtained here is similar to that presented by Cunha et al. [12] and greater than the one by Carvalho et al. [13], bothusing *Candida guilliermondii* to produce xylitol in SBHH. *Geotrichum* sp. reaches considerable xylitol yield demanding less nutritional

Table 3
Final concentration and percentage consumption rate of each compound in fermented SBHH.

Compound	Concentration	Consumption rate	Yield
Xylose	1.27 $g \cdot L^{-1}$ (±0.47)	97.60%	–
Arabinose	0.49 $g \cdot L^{-1}$ (±0.12)	92.55%	–
Galactose	0.15 $g \cdot L^{-1}$ (±0.03)	91.75%	–
Cellobiose	0.48 $g \cdot L^{-1}$ (±0.08)	–	–
Glucose	ND	100%	–
Acetic acid	0.53 $g \cdot L^{-1}$ (±0.19)	72.95%	–
Furfural	0.0345 $g \cdot L^{-1}$ (±0.03)	99.04%	–
Hydroxy-methyl-furfural	0.1415 $g \cdot L^{-1}$ (±0.14)	84.64%	–
Total phenolic compounds	440 $mg \cdot L^{-1}$ (±18)	4.55%	–
Xylitol	22.64 $g \cdot L^{-1}$ (±0.24)	–	0.44 $g \cdot g^{-1}$
Ethanol	0.29 $g \cdot L^{-1}$ (±0.07)	–	–

ND: Not detected.

supply, indicating that this strain is a promising xylitol producer. Full composition of fermented SBHH is summarized at Table 3.

Total phenolic compound's final concentration was not significantly different from initials, meaning low consumption of these inhibitors. Acetic acid concentration indicates the consumption of 72.95%, whereas furfural and hydroxyl-methyl-furfural were almost fully consumed by *Geotrichum* sp. KP276644. Furfural was not detected at fermented SBHH in two of the three assays, whereas 84.64% of hydroxyl-methyl-furfural was consumed in average (Table 3).

The consumption percentage of furfural here presented is greater than obtained by Zhang et al. [14] using *Enterobacter* sp. FDS8, Hou-Rui et al. [10] using *Issatchenkia orientalis* and *Issatchenkia occidentalis*, and Ran et al. [15] using *Amorphotheca resinae* ZN1. These results indicate that *Geotrichum* sp. KP276644, besides a promising xylitol producer, presents remarkable biotechnological potential in perform furfural consumption. By this way, this interesting capability is explored ahead.

Cellobiose, undetected in SBHH before fermentation assay, was, probably, produced as a compatible solute. According to Empadinhas and Costa [16], it is a common feature that cellobiose and other glucose dimers be produced by yeasts and bacteria as compatible solute in osmotic stress situations, but it remains unclear.

3.2. Furfural consumption assay

Geotrichum sp. KP276644 was unable to growing when furfural was the sole carbon source (F2 and F4 assays), occurring biomass loss in F4. Analyzing the OD_{600} of GF2, GF4 and GF6, cell growth of these assays have no significant difference to control assay ($p = 0.056$). The same was observed when GF8 was compared to F2 and F4 assays ($p = 0.069$). However, when the OD_{600} of GF8 was compared to GF2, GF4, GF6 and control, statistical analysis indicates a significant difference ($p = 0.018$), meaning that this furfural concentration (8 $g \cdot L^{-1}$) affects the cell growth of *Geotrichum* sp. KP276644. Growth curves of all assays are presented in Fig. 1.

Furfural was fully consumed in GF2, GF4 and GF6 assays, being this inhibitor not detected in these flasks after 96 h of culturing, indicating that this strain presents high applicability in biorefinery [17]. Maximum furfural consumption in GF8, F2 and F4 assays was 96,12%, 93% and 88.5%, respectively. Despite the reasonable difference between minimum and maximum consumption value, statistical analysis indicates that this is not significant ($p = 0.067$). The variation of furfural concentration along the culturing time is presented in Fig. 2.

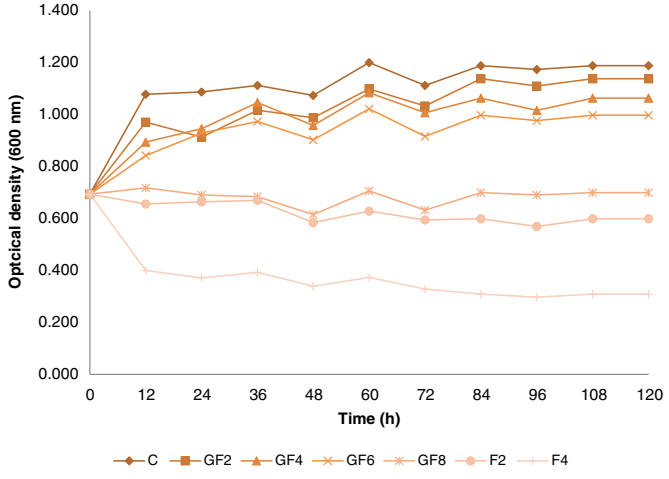

Fig. 1. Growth curves of *Geotrichum* sp. KP276644 in different concentration of furfural, supplemented or not with glucose.

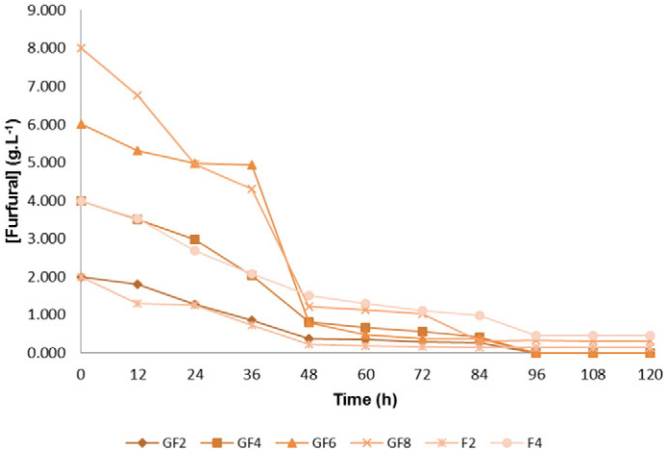

Fig. 2. Furfural concentrations along culturing time.

3.3. Characterization of furfural degradation products

Furfural, peak of 97 $g \cdot mol^{-1}$ in mass spectrograms, was fully consumed in assays that use glucose as sole carbon source, whereas a moderated consumption was observed when xylose was combined to glucose and not consumed when xylose was provided as a sole sugar source, as shown in Fig. 3.

This fact indicates that, in the chemically defined medium, xylose has inhibited the furfural metabolism in this strain of *Geotrichum* sp. This result differs from those obtained by Ran et al. [15], who had the furfural metabolism inhibited in glucose presence. Niel et al. [18] have described furfural consumption associated to glucose in *Lactobacillus reuteri*, but using low concentration of this inhibitor. These results point that high concentration of furfural was consumed in co-metabolism with glucose.

Comparison of spectrograms allows concluding that the most abundant product resulting from furfural metabolism is that with 161 $g \cdot mol^{-1}$. Among microorganisms, first step in furfural metabolism is its conversion to furoic acid (MW: 112 $g \cdot mol^{-1}$) or furfuryl alcohol (MW: 98 $g \cdot mol^{-1}$). These are metabolized in 2-oxoglutaric acid (MW: 146 $g \cdot mol^{-1}$), and that is consumed by Krebs cycle [19].

According to Kroes [20], in mammals such as human and rodents, furfural is excreted at urine as 2-furoylglycine (MW: 169 $g \cdot mol^{-1}$) or 2-furanacryloylglycine (MW: 195 $g \cdot mol^{-1}$). May occur some intermediary metabolites as furoic acid and 2-furanacrylic acid (MW: 138 $g \cdot mol^{-1}$).

These results lead to a conclusion that *Geotrichum* sp. KP276644 presents an uncommon product furfural metabolism, which is currently unknown. Other analytical methods will be demanded to elucidate its molecular structure.

4. Conclusion

Geotrichum sp. KP276644 is a wild-type strain able to produce xylitol by xylose fermentation with yield close to most of industrial used strains, demanding less nutritional supply.

Its most remarkable biotechnological potential concerns to furfural consumption, being able to consume this inhibitor in high concentration when compared to current literature.

Despite not being used as sole carbon source, this yeast has a high furfural tolerance, not occurring significant cell growth alteration until concentration 6 $g \cdot L^{-1}$ of this inhibitor.

Furfural is consumed in co-metabolism with glucose, resulting in an uncommon metabolite of 161 $g \cdot mol^{-1}$, currently unknown and demanding further studies.

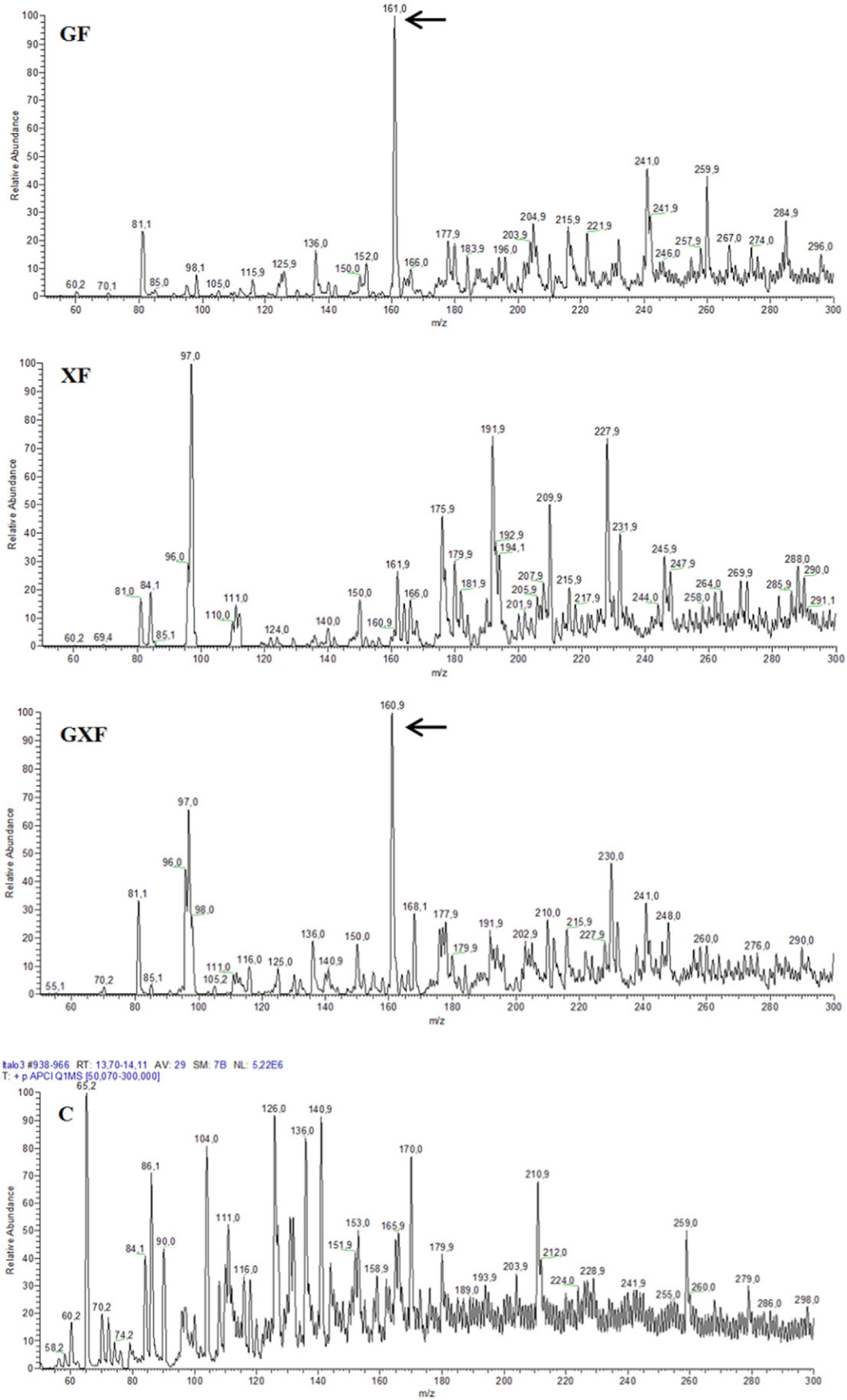

Fig. 3. Spectrograms of assays for characterization of furfural degradation product. (Legends: GF — Glucose and Furfural; XF — Xylose and Furfural; GXF — Glucose, Xylose and Furfural; C — Control. Black arrow indicates most abundant product of furfural metabolism).

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