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Industrial robust yeast isolates with great potential for fermentation of lignocellulosic biomass

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HIGHLIGHTS

- Industrial robust strains were screened for lignocellulosic ethanol production.
- Screen was conducted on a real hydrolysate from *Eucalyptus globulus* wood.
- The tolerance of the lignocellulose hydrolysate was highly variable among strains.
- A correlation between final ethanol titer and furfural detoxification was found.
- Distilleries showed to be a remarkable yeast source for lignocellulose fermentation.

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ABSTRACT

The search of robust microorganisms is essential to design sustainable processes of second generation bioethanol. Yeast strains isolated from industrial environments are generally recognised to present an increased stress tolerance but no specific information is available on their tolerance towards inhibitors that come from the pretreatment of lignocellulosic materials. In this work, a strategy for the selection of different yeasts using hydrothermal hydrolysate from *Eucalyptus globulus* wood, containing different concentrations of inhibitors, was developed. Ten *Saccharomyces cerevisiae* and four *Kluyveromyces marxianus* strains isolated from industrial environments and four laboratory background strains were evaluated. Interestingly, a correlation between final ethanol titer and percentage of furfural detoxification was observed. The results presented here highlight industrial distillery environments as a remarkable source of efficient yeast strains for lignocellulosic fermentation processes. Selected strains were able to resourcefully degrade furfural and HMF inhibitors, producing 0.8 g ethanol/Lh corresponding to 94% of the theoretical yield.

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1. Introduction

Lignocellulose raw materials derived from agricultural, industrial and forest sources can provide environmental, economic and strategic benefits, not competing with food production, when used as sustainable feedstock in a biorefinery context (Ruiz et al., 2013). An example of these feedstocks is *Eucalyptus globulus* wood residues, such as bark, cross-cut ends and wood chips resulted from kraft pulping processing, being that large amounts are currently being burned for electricity or heat production (Moshkelani et al., 2013). Therefore, a promising strategy for the valorisation of these residues could be its utilization as main feedstock for the production of bioethanol and other value-added products by incorporating a biorefinery unit in an operating paper industry (Mussatto et al., 2010; Phillips et al., 2013).

Bioethanol from lignocellulose materials or also called second generation bioethanol is obtained by following main steps: (i) pretreatment of lignocellulose biomass (ii) saccharification of cellulose and (iii) fermentation of glucose. The pretreatment is carried out to alter its recalcitrant structure (formed by hemicellulose, cellulose and lignin) and to improve the enzymatic accessibility towards cellulose. In this context, the hydrothermal treatment or autohydrolysis is an environmentally-friendly treatment that follows the biorefinery concept (Ruiz et al., 2013). The hydrothermal treatment allows obtaining a solid phase composed by cellulose

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and lignin and solubilising the hemicellulose fraction into hemicellulose-derived compounds (mainly oligo- and mono-saccharides) (Garrote et al., 2008). Nevertheless, with the hardness of pretreatment, some degradation products of both sugars and lignin are released in liquid hemicellulosic phase. These can be grouped around three main classes, weak acids, furans and phenolics compounds. While acetic acid, the most common weak acid derived from lignocellulosic hydrolysates, is formed by deacetylation of hemicelluloses, furan compounds, 2-furaldehyde (furfural) and 5-hydroxymethyl-2-furaldehyde (HMF), are formed by dehydration of pentoses and hexoses, respectively (Pereira et al., 2011a). These degradation compounds are considered potent inhibitors of yeast growth and induce a harsh effect on yeast machinery reducing the ethanol yield and productivity (Modig et al., 2008). The presence of inhibitor compounds generated during the treatment is one of the major challenges faced in commercial production of lignocellulosic bioethanol (Palmqvist and Hanhn-Hägerdal, 2000).

One approach to tackle the inhibitor challenge is by using natural robust yeast strains. Industrial isolates are known to be very robust, show higher fermentation capacity (Mussatto et al., 2010; Pereira et al., 2010) and stress tolerance that is developed in presence of stress factors related with harsh industrial processes such as: high sugar and ethanol concentrations, elevated temperatures, pH variations and presence of toxic compounds (Pereira et al., 2011b; Della-Bianca et al., 2013). The microflora of traditional and industrial fermentation processes constitutes a potential source of microbial natural isolates that exhibit at least some of the desired physiological background characteristics for lignocellulosic fermentation even if they have not been traditionally exposed to these particular inhibitors. Some strains of *Saccharomyces cerevisiae* isolated from Brazilian sugarcane-to-ethanol distilleries (“cachaça” and bio-ethanol plants) have shown high fermentation efficiency with prolonged persistence in the fermentation system (Basso et al., 2008; Pereira et al., 2010, 2011b, 2012). Also, a flocculating strain isolated from a Swedish second generation bioethanol plant showed high tolerance to ethanol, osmotic stress and inhibitor presence (Westman et al., 2012). On the other hand, the evaluation of industrial strains as *Kluyveromyces marxianus* can be interesting since these yeasts are able to work at elevated temperatures and ferment glucose and xylose (Fonseca et al., 2008), desirable properties for a cost-efficient process. Moreover, the environmental conditions of stress are related with the expression of flocculent character of some laboratory strains. This characteristic could be helpful for lignocellulosic ethanol production (Landaeta et al., 2013). Despite being potential candidates to overcome the stressful conditions imposed to yeast cells in lignocellulosic fermentation processes and thus to drive this technology further, the use and characterization of these isolates in lignocellulosic fermentations has not been reported.

In order to select a promising yeast strain for lignocellulosic fermentation we conducted in this study a screening comprising ten *S. cerevisiae* and four *K. marxianus* strains isolated from harsh industrial environments and four laboratory background strains. For a more dose-to-reality approach, the inhibitor tolerance and fermentation performance was evaluated using a real hydrolysate from hydrothermally pretreated *E. globulus* wood (containing inhibitory compounds).

2. Methods

2.1. Yeasts

The strains tested in this work included ten industrial *S. cerevisiae* strains: three isolated from Brazilian bio-ethanol production plants – PE-2, CAT-1, VR-1 (Basso et al., 2008); one flocculating yeast strain isolated from a Swedish second generation bio-ethanol plant – CCUG53310 (Purwadi et al., 2007); five belonging to the UFLA collection (Federal University of Lavras, Brazil) isolated from Brazilian “cachaça” fermentation processes – CA11, CA1162, CA1185, CA1187, CA155 (Pereira et al., 2010) and one industrial *S. cerevisiae* strain isolated from a beer plant (Portugal) – 1762 BELG. Four industrial *K. marxianus* strains isolated from “cocoa” fermentations (Brazil) – CH2-2, CH9-1, CH8-1 and CH1-1 (Pereira et al., 1999). The set of *S. cerevisiae* laboratory strains (routinely used in research laboratory) included CEN.PK 113-7D (Pereira et al., 2010), NRRL Y-265 (Hojo et al., 1999) and an adapted laboratory strain of the flocculating yeast NRRL Y-265-ADAPT (Landaeta et al., 2013). For comparative propose, the *K. marxianus* CBS 6556 laboratory background strain was also included (Ribeiro et al., 2007). Stock cultures were maintained on YPD [1% (w/v) yeast extract, 2% (w/v) bacto-peptone and 2% (w/v) glucose] agar plates at 4 °C.

2.2. Preparation of E. globulus wood (EGW) hydrothermal hydrolysate

The chips of *E. globulus* wood (kindly provided by pulp mill – ENCE, Pontevedra, Spain) were milled to pass an 8 mm screen, air-dried, homogenized and stored until use. The raw material was then assayed for composition (see Table 1) according to Romaní et al. (2010). The hydrothermal treatment was carried out following the procedure described by Romaní et al. (2010). Briefly, the EGW was mixed with water at a Liquid Solid Ratio (LSR) = 8 kg/kg in a 3.75 L stainless steel reactor (Parr Instruments Company, Moline, IL). The treatment was performed at 150 rpm and heated at desired maximal temperature (T_{max}) of 210 °C in non-isothermal conditions, following the standard heating temperature–time profile (Garrote et al., 2008). The operational conditions of treatment were chosen on the basis of a previous work in which total saccharides released upon pretreatment achieved the maximum value (93.8% of polysaccharide recovery) in the liquid and solid phases (Romaní et al., 2010). When the

Table 1
EGW characterization concerning raw material composition, solid yield and composition of solid pre-treated and hemicellulosic liquor phase (hydrolysate).

EGW composition	g/100 g raw material, oven dry basis
Glucan	44.7 ± 0.81
Xylan	16.01 ± 0.35
Arabinan	1.09 ± 0.05
Acetyl groups	2.96 ± 0.28
Klason lignin	27.7 ± 0.61
EGW pre-treatment*	g/100 g raw material, oven dry basis
SY (solid yield)	71.66
NVC (non-volatile compounds)	14.91
Solid phase analysis	g/100 g pre-treated solid
Glucan	59.26 ± 0.47
Xylan	1.95 ± 0.10
Arabinan	0
Acetil groups	0.29 ± 0.06
Klason lignin	33.60 ± 0.5
Liquid phase analysis	g/L hemicellulosic liquor
Glucose	0.64
Xylose	8.85
Arabinose	0.18
Acetic acid	3.11
HMF	0.33
Furfural	1.66
Glucooligosaccharides	1.15
Xylooligosaccharides	8.97
Arabinooligosaccharides	0
Acetyl groups	2.55
Phenolic compounds	2.01

* EGW pre-treatment: T_{max} = 210 °C or S_0 = 4.08.

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