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ORIGINAL ARTICLE

Xylan-hydrolyzing thermotolerant *Candida tropicalis* HNMA-1 for bioethanol production from sugarcane bagasse hydrolysate

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Abstract Sugarcane bagasse is one of the low-cost substrates used for bioethanol production. In order to solubilize sugars in hemicelluloses like xylan, a new thermotolerant isolate of *Candida tropicalis* HNMA-1 with xylan-hydrolyzing ability was identified and characterized. The strain showed relative tolerance to high temperature. Our results demonstrated 0.211 IU ml^{-1} xylanase activity at 40 °C compared to 0.236 IU ml^{-1} at 30 °C. The effect of high temperature on the growth and fermentation of xylose and sugarcane bagasse hydrolysate were also investigated. In both xylose or hydrolysate medium, increased growth was recorded at 40 °C. Meanwhile, the efficiency of ethanol fermentation was adversely affected by temperature since yields of 0.088 g g^{-1} and 0.076 g g^{-1} in the xylose medium, in addition to 0.090 g g^{-1} and 0.078 g g^{-1} in the hydrolysate medium were noticed at 30 °C and 40 °C, respectively. Inhibitory compounds in the hydrolysate medium demonstrated negative effects on fermentation and productivity, with maximum ethanol concentration attained after 48 h in the hydrolysate, as opposed to 24 h in the xylose medium. Our data show that the newly thermotolerant isolate, *C. tropicalis* HNMA-1, is able to efficiently ferment xylose and hydrolysate, and also has the capacity for application in ethanol production from hemicellulosic sources.

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Introduction

Lignocellulose as a waste product is an attractive feedstock for the production of ethanol, owing to its availability in large quantities and low-cost. Among the numerous agricultural residues, the cellulosic and hemi-cellulosic fractions of sugarcane bagasse can be hydrolyzed to sugars, which eventually could be fermented to ethanol (Cardona et al. 2010). It is essential to note that the hydrolysate composition is completely dependent on the method of pretreatment. Generally, sugarcane bagasse consists of cellulose 43.6%, hemicellulose 33.8%, lignin 18.1%, ash 2.3% and wax 0.8% on a dry weight basis (Sun et al. 2004). Hydrolysis of the sugarcane bagasse preparations using sulfuric acid and analyzing the hydrolysate using gas chromatography demonstrated that glucose is a predominant monosaccharide, comprising 92.8–96.8% of the total sugars. Xylose (at 1.7–4.7% w w^{-1}) and galactose (at 0.3–1.6% w w^{-1}) concentrations appear in the second and third place, and, finally, arabinose and mannose exhibited less than 1% (w w^{-1}) of total sugars in the hydrolysate (Sun et al. 2004). Hemi-celluloses are renewable low molecular weight heteropolysaccharides in which xylan constitutes the major component. Next to cellulose, xylan is the most abundant polysaccharide, and is made up of a backbone of xylose residues linked by β-1,4-glycosidic bonds (Alves et al. 2005; Collins et al. 2005). Cellulose and hemicellulose appear to be able to supply most of the global demand for renewable resources as raw materials (Zabed et al. 2016). This implies that all types of sugars present in cellulose and xylan must be converted efficiently to soluble sugars. Microorganisms living on plant residues produce a coordinate action of several hydrolytic

enzymes, such as xylanases, that are necessary for complete hydrolysis of xylan (Basaran et al. 2000; Talamantes et al. 2016). Production of saccharolytic enzymes, such as cellulases and hemicellulases, is a limiting step in the full hydrolysis of lignocellulosic polymer, which leads to the release of hexoses and pentoses (Kádár et al. 2004). Differences in the optimum temperature for xylanase activity and that of the fermenting organism can be a challenging factor, and optimization of these factors is undoubtedly an important step in developing a feasible process. On the one hand, it is crucial to optimize conditions and enhance saccharification yield prior to fermentation (Kádár et al. 2004), while on the other, the recalcitrant structure of the lignocellulosic substrate hinders the efficient activity of the enzymes. Pretreatment make the biomass more accessible to enzymatic saccharification. So, pretreatment of lignocellulosic substrates before fermentation is an alternative method that can potentially increase bioethanol production (Eijsink et al. 2008; Kim et al. 2011).

The cost of enzymatic hydrolysis is a limiting factor in industrial processes. As a result, considerable interest has focused on the use of low-cost substrates, such as waste materials of wood and agriculture. These raw materials have high potential to be degraded into useful residues aimed at developing inexpensive enzyme production technologies (Hahn-Hägerdal et al. 2007; Alves-Prado et al. 2010). Although xylanase producers are extensively distributed among bacteria and fungi, information on xylan hydrolyzing yeasts is limited (Polizeli et al. 2005; Bhadra et al. 2008). Thus, finding potent yeast strains for obtaining greater amount of enzymes that are particularly active in extreme conditions is required. Following sugarcane bagasse pretreatment for releasing fermentable sugars, if any xylooligosacharides remain in hydrolysate, xylanase activity of these isolates could disrupt the structure of the remaining oligosaccharides (Lee et al. 1986; Kulkarni et al. 1999).

Tolerance to high temperature is an essential feature in yeasts of interest for ethanol and xylanase production. This has several distinct advantages in industry, including energy saving as a result of the reduction in cooling costs, facilitation of downstream ethanol recovery, and avoidance of the contamination (Sree et al. 2000). In addition, it is not unrealistic to predict that by developing the yeasts capable of fermenting D-xylose (xylan monomer) to ethanol, the substitution of crude oil by this product could be possible (Kulkarni et al. 1999; Basaran et al. 2000). Potential uses of lignocellulosic biomass like sugarcane bagasse in bioethanol production has attracted great attention for the past few decades but its production on an industrial scale has not yet become feasible (Dias et al. 2009).

In the present study, we report the biological conversion of sugarcane bagasse to ethanol by a newly thermotolerant xylan-decomposing yeast. Xylan hydrolyzation is a feature that is found in most xylose-fermenting molds and yeasts (Lee et al. 1986; Morais et al. 2013). Growth rate and ethanol production were compared at 30 °C and 40 °C using xylose and sugarcane bagasse hydrolysate as fermentation medium. Considering the significance of fermentation of sugarcane bagasse hydrolysate to ethanol at higher temperature, the isolated yeast strain, *Candida tropicalis* HNMA-1, has high capacity for application in ethanol production from hemicellulosic sources.

Materials and methods

Isolation, purification and growth media

Soil samples were collected from natural environments such as deteriorating plant residues, agricultural soils, and soils in contact with fallen leaves from north of Iran. The medium for isolation and purification of yeasts contained the following: yeast extract (10 g), peptone (10 g), $MnSO_4 \cdot H_2O$ (0.01 g), $MgSO_4 \cdot 7H_2O$ (0.2 g), $FeSO_4 \cdot 7H_2O$ (0.01 g) and agar (20 g), in 1 L distilled water. Media were enriched by incorporating 20 g L^{-1} oat spelt xylan. In the isolation medium, 0.05 g L^{-1} chloramphenicol was added to inhibit the growth of bacteria. The pH was adjusted to 5.5 with 1 *N* HCl. Cultures were incubated at 30 °C for 48 h. The isolated strains were maintained in the same medium containing 10 g L^{-1} oat spelt xylan.

Xylanase production

The basal medium employed for enzyme production contained the following: yeast extract (10 g), KH_2PO_4 (2 g), $(NH_4)_2SO_4$ (1 g), $MgSO_4 \cdot 7H_2O$ (1 g), in 1 L distilled water (Basaran et al. 2000). Cells were grown in 20 mL medium in a 100 mL Erlenmeyer flask shaken at 150 rpm, and incubated at either 30 °C or 40 °C. All experiments were repeated three times. Inoculum culture was prepared by growing the active yeasts in 2% (w v^{-1}) xylan for 24 h. The same basal medium was utilized for enzyme production, which was inoculated with 2 mL inoculum supplied with 2% xylan (w v^{-1}), for 96 h. Cell density was measured turbidometrically at 600 nm. Fermentation broth samples were centrifuged at 5000 rpm at 4 °C for 10 min. Cell-free supernatant solutions were utilized for determining the enzyme activity.

Enzyme assay

The assay mixture comprised 0.5 ml 1% (w v^{-1}) xylan in acetate buffer, 50 mM, pH 5.5, to which 0.5 mL supernatant was added and kept at 45 °C for 60 min. The reaction was brought to a halt by the addition of 3,5-dinitrosalicylic acid (DNS) solution, followed by boiling for 10 min, thus the amount of reducing sugar was analyzed as xylose equivalent

using the DNS method (Miller 1959). Controls were prepared by adding supernatant after the addition of DNS reagent. One unit (U) of xylanase activity was determined as the amount of enzyme that liberated 1 μmol of xylose per minute at pH 5.5 and 45 °C (Basaran et al. 2000).

Yeast identification

Morphological and biochemical characterization

Yeasts morphology and standardized biochemical tests and molecular identification were applied for yeast identification. Yeasts morphology was characterized by colony observation with a stereoscope, and staining and visualization of the cells using a light microscope. Biochemical tests were conducted by the standard methods proposed by Van der Walt (White et al. 1990; Kurtzman et al. 2011).

Molecular characterization and phylogenetic analyses

Cells were grown in the YMB medium and harvested by centrifugation. The cells were washed twice with distilled water and were broken apart with a pipette tip, and ca. 0.5 mL 0.5-mm diameter glass beads. Afterwards, cells were suspended in 1 mL lysis buffer [200 mM Tris-HCl (pH 8.5), 250 mM NaCl, 25 mM EDTA (pH 8.0), and 0.5% sodium dodecyl sulfate]. The phenol-chloroform method was employed for extraction (White et al. 1990).

Sequences containing about 600–650 bp of the 26S rDNA were amplified by using universal primers: forward, NL-1 (5′-GCA TAT CAA TAA GCG GAG GAA AAG-3′) and reverse, NL-4 (5′-GGT CCG TGT TTC AAGACG G-3′) (White et al. 1990). The amplified product was then purified and sequenced (Collins et al. 2005). The identification of yeast was achieved by aligning and comparing 26S rRNA gene sequences in CBS database. Multiple alignments were performed using the CLUSTAL-X. Phylogenetic tree was constructed by neighbor-joining analysis (Saitou and Nei 1987) based on 26S rDNA D1/D2 region sequence data. Bootstrap analyses were performed on 1000 random samplings using the routines included in MEGA6 software (Kumar et al. 2001).

Sugarcane bagasse hydrolysate preparation

Sugarcane bagasse from Karoon Agro-Industry, Shoushtar, Khuzestan Province, Iran, was employed as raw material. The powdered biomass at a solid loading of 10% (w v^{-1}) was mixed with dilute sulfuric acid (0.7%, v v^{-1}) and pretreated in a BatchSynth® microwave (Milestone, Sorisole, Italy) at 162 °C with the residence time of 38 s. The pretreated slurry was cooled to 50 °C within 30 min and then immediately adjusted to pH 4.8 using 10 M NaOH. The liquid fraction was separated by filtration using Whatman No. 1 filter paper (Ahi et al. 2013). The filtrate containing reducing sugars was used as fermentable sugar for bioethanol production. Achieved bagasse hydrolysate samples were analyzed spectrophotometrically for measurement of furfural by measuring the absorbance of the samples at 277 nm (Martinez et al. 2000). Samples absorbance were compared to a freshly distilled furfural (185,914, Sigma-Aldrich), in concentrations between 0 and 6 mg L^{-1}, as standard curve. Total phenols in the samples was measured by the Folin-Ciocalteu method (Wrolstad 2001), against a standard curve made from 0 to 500 mg L^{-1} gallic acid (G7384, Sigma, St. Louis, MO) in ethanol.

Ethanol production

The fermentation performance of *C. tropicalis* was first evaluated in a synthetic medium containing 50 g L^{-1} pure xylose supplied by yeast extract 10 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 1 g L^{-1}, KH_2PO_4 2 g L^{-1} and $(NH_4)_2SO_4$ 1 g L^{-1}. Cells were grown in 20 mL medium in a 100 mL Erlenmeyer flask shaken at 150 rpm and incubated at 30 °C and 40 °C. Afterwards, 20 mL of pretreated hemicellulose hydrolysate (pH 5.5) was applied for fermentation. The fermentation media comprised sugarcane bagasse hydrolysate as fermentable sugars (34 g L^{-1}) and supplemented by yeast extract 10 g L^{-1}, KH_2PO_4 2 g L^{-1}, $MgSO_4 \cdot 7H_2O$ 1 g L^{-1} and $(NH_4)_2SO_4$ 1 g L^{-1}. Culture conditions were same as xylose fermentation. The hydrolysate and supplied ingredients were sterilized by autoclave, individually. Cell density was quantified by Neubauer® cell counting chamber. In order to determine the concentration of ethanol, the medium was centrifuged at 5000 *g* for 10 min followed by distillation of 5 mL centrifuged medium. The ethanol content of the distilled medium was quantified spectrophotometrically (potassium dichromate method), by using a standard curve prepared from absolute ethanol (Merck, Darmstadt, Germany) at 590 nm (Pilone 1984). For this purpose, 150 μl samples were added to 1.5 mL potassium dichromate reagent (0.1 M $K_2Cr_2O_7$ in 5 M H_2SO_4) and kept at 30 °C for development of the color, and the absorbance was read at 590 nm.

Measurement of hexose and pentose sugars

The amount of reducing sugar released from bagasse by acid hydrothermal treatment and total reducing sugars was measured by the DNS method. Total pentose was measured with the modified orcinol method by the addition of 3.0 mL iron solution (0.5 mL 10% $FeCl_3 \cdot 6H_2O$ solution in 100 mL 12 M HCl) and 0.2 mL orcinol solution (0.6 g orcinol dissolved in 10 mL methanol) to 1.5 mL sample, and heated in a boiling-water bath for 10 min, and the absorbance was read at 665 nm (Herbert et al. 1971; Standing et al. 1972). Glucose content of samples was determined by using a glucose oxidase-based enzymatic kit. Concentrations of reducing sugar released by

hydrolysis in the reaction medium were determined after the preparation of a calibration curve with xylose at different concentrations.

Results and discussion

Isolation of thermotolerant xylanase-hydrolyzing yeasts

One of the desirable yeast characteristics in the conversion of hemicelluloses such as xylan is hydrolysis of xylan and fermentation of the released sugar to ethanol (Den Haan and Van Zyl 2003; Collins et al. 2005). In order to obtain a strain with these properties, xylan-decomposing yeasts were screened in 50 samples. All of these xylanase-positive strains were tested by growing on broth medium in the presence of xylan as a carbon source. Among these strains, 17 isolates showed significant growth on xylan medium and were selected for further evaluation due to their capacity for bioethanol production. Another important characteristic of yeast for use in industry is tolerance to fluctuations in high temperature during fermentation and downstream processes (Edgardo et al. 2008; Antil et al. 2015). Thermotolerant strains with xylanase activity were selected on Petri dishes with xylan-containing medium by incubating them at 35 °C, 40 °C, 42 °C, 45 °C, 47 °C and 50 °C. Three isolates exhibited significant growth at temperatures up to 37 °C, and two could tolerate 42 °C thus indicating feeble growth. It is worth mentioning that isolate No. 8 was able to grow up to 47 °C. The effect of different temperature (30–50 °C) on the growth of six selected isolates is presented in Table 1.

Fermentation of xylose to ethanol

Saccharomyces cerevisiae—the predominant organism for industrial ethanol production—is unable to utilize xylose. Thus, the ability to ferment other sugars besides glucose, such as D-xylose, which is obtained from the hydrolysis of lignocellulosic biomass, is important in the fermentation process (Hande et al. 2013). All six thermotolerant xylan-hydrolyzing isolates were tested for ethanol production from xylose at 30 °C. Table 2 shows the fermentation results of these selected isolates.

The highest ethanol concentration and ethanol yield were obtained with isolates 14, 16 and 8, respectively. Isolates 6 and 8 demonstrated maximum specific sugar uptake rates of 0.41 g g^{-1} h^{-1} and 0.36 g g^{-1} h^{-1}, and volumetric ethanol productivities of 0.121 g g^{-1} h^{-1} and 0.126 g g^{-1} h^{-1}, respectively (Table 2). In addition to excellent xylose fermentation based on sugar uptake rate by isolates No. 5, 6 and 8 (Table 2), slight production of ethanol from xylose was also observed in other yeasts.

Table 1 Growth of six thermotolerant isolates at various temperatures after 48 h on xylan medium

Strain	Temperature °C							
	30	35	37	40	42	45	47	50
5	++[a]	++	+	−	−	−	−	−
6	++	++	+	+	−	−	−	−
8	++	++	++	++	++	+	+/−	−
10	++	++	++	+	+/−	−	−	−
14	++	++	+	−	−	−	−	−
16	++	++	++	+	+/−	−	−	−

[a] ++ Good growth, + Weak growth, +/− Feeble growth, − No growth

Xylanase activity

In the lignocellulose to ethanol conversion process, despite the existence of xylanase-producing yeast strains, it is crucial to hydrolyze the recalcitrant structure of lignocellulosic materials and thereby facilitate enzymatic hydrolysis of the polysaccharides (Antil et al. 2015). In the other words, complete degradation of the complex structure of the lignocellulosic materials requires the pretreatment and synergistic action of a range of different enzymes such as cellulases and xylanase. de Souza Moreira et al. (2013) reported the xylanase activity of *Aspergillus terreus* on the use of pretreated sugarcane bagasse as a carbon source followed by purification of two xylanases.

Xylanase activity was compared among six selected isolates at 30 °C. As illustrated in Fig. 1, the measurement of xylanase activity in selected strains revealed that the isolate 10 is almost similar to the isolate 14, with activities of 0.195 IU ml^{-1} and 0.193 IU ml^{-1}, respectively. According to the results, the best xylanase activity, 0.236 IU ml^{-1}, was represented by thermotolerant isolate 8 (Fig. 1). Isolate 8 showed higher xylanase activity than *Pichia stipitis* CBS 6054 (0.057 IU ml^{-1}), *Trichosporon cutaneum* CCY 30–5-4 (0.2 IU ml^{-1}) and *Pichia stipitis* CBS 5776 (0.107 IU ml^{-1}) as reported by Lee et al. (1986). In order to compare the production of xylanase at 30 °C and 40 °C, cultures of the isolate No. 8 were incubated at the stated temperatures and the enzyme produced was assayed. It was observed that 0.211 IU mL^{-1} enzyme was produced at 40 °C compared to 0.236 IU mL^{-1} at 30 °C.

Thus, here we introduce a thermotolerant yeast strain with xylanase activity at 40 °C. Highly thermostable xylanase from different microorganisms have been reported, for example, Fan et al. reported thermostable xylanase at 70 °C from *Malbranchea cinnamomea* (Fan et al. 2014), but in ethanol production processes, temperatures higher than 40 °C may not be suitable, due to higher evaporation and loss of the ethanol production (Abdel-Banat et al. 2010).

Table 2 Fermentation parameters for six positive xylanase strains at 30 °C

Parameter[a]	Isolate No.					
	5	6	8	10	14	16
Ethanol yield, Yp/s (g g $^{-1}$)	0.0514	0.0579	0.060	0.060	0.117	0.094
Biomass yield, Yx/s (g g $^{-1}$)	0.1142	0.1015	0.114	0.3007	0.1693	0.20066
Maximum ethanol conc. Pmax (g L^{-1})	2.5704	2.8972	3.024	3.0424	5.8923	4.7306
Maximum biomass conc. X_{max} (g L^{-1})	5.71	5.075	5.71	15.035	8.466	10.033
Volumetric ethanol productivity Qp (g L^{-1} h^{-1})	0.107	0.121	0.126	0.0633	0.123	0.098
Volumetric biomass productivity Qx (g L^{-1} h^{-1})	0.238	0.211	0.238	0.313	0.176	0.209
Specific sugar uptake rate qs (g g^{-1} h^{-1})	0.365	0.41	0.365	0.07	0.123	0.104
Specific ethanol productivity qp (g g^{-1} h^{-1})	0.0187	0.0237	0.0220	0.0042	0.0145	0.0098

[a] *X* Biomass concentration (g l^{-1}), *S* substrate concentration (g l^{-1}), *P* product concentration (g l^{-1}), P_{max} maximum ethanol concentration (g l^{-1}), X_{max} maximum biomass concentration (g l^{-1}), *Yx/s* biomass yield coefficient (g g^{-1}), *Yp/s* product yield coefficient (g g^{-1}), Qp volumetric ethanol productivity (g^{-1} h^{-1}), *Qx* volumetric biomass productivity (g L^{-1} h^{-1}), *qs* specific sugar uptake rate (g g^{-1} h^{-1}), *qp* specific ethanol productivity (g g^{-1} h^{-1}). Subscripts: *max* maximum, *p* product, *s* substrate, *x* biomass

Biochemical and molecular identification of a thermotolerant D-xylose-hydrolyzing yeast

We carried out biochemical analysis to identify isolate No. 8. The results are presented in Table 3. A phylogenetic study on isolate No. 8 based on D1/D2 26S rRNA gene sequences specified that the closest relative strain to our isolate is *Candida tropicalis* with 100% similarity. The sequenced gene was submitted to the NCBI database under GenBank accession number No. JQ991933. Figure 2 illustrates the phylogenetic tree of *Candida tropicalis* HNMA-1 and related species.

Fermentation of xylose and sugarcane bagasse hydrolysate by *C. tropicalis* HNMA-1

Pretreated hemicellulose hydrolysate was utilized for ethanol production. The total reducing sugars, glucose, pentose, furfural, and phenolic compounds of the sugarcane bagasse hydrolysate are presented in Table 4. Hydrolysis of hemicelluloses such as sugarcane bagasse, particularly xylan, occurs during dilute acid pretreatment because the cellulose structure is more acid-stable, while the structure of the hemicellulose is more easily hydrolyzed. During this process, some toxic compounds, such as furfural, hydroxymethyl furfural and phenolic compounds, may also be produced (Hendriks and Zeeman 2009; Talamantes et al. 2016). According to Jeffries and Kurtzman (1994), yeast strains with the ability to ferment hemicellulosic sugars will enhance the bioconversion of lignocellulosic biomass to biofuel and can act as sources of xylanases and as D-xylose-fermenting agents.

To investigate ethanol production in xylose-containing medium compared with sugarcane bagasse hydrolysate medium, *C. tropicalis* HNMA-1 was cultured in both conditions. The results are given in Table 5.

Ethanol production and sugar concentration resulting from hemicellulosic hydrolysate and in the xylose medium are shown in Fig. 3a. Most of the sugar was consumed after 24 h in the

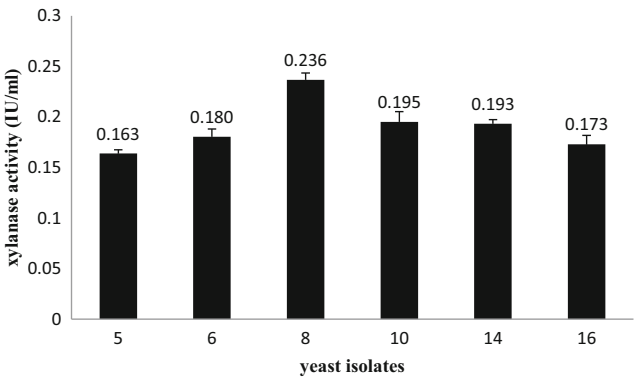

Fig. 1 Xylanase activity in different yeast isolates

Table 3 Biochemical and tolerance characteristics of *Candida tropicalis* HNMA-1

Fermentation test		Assimilation test	
Glucose	+	Glucose	+
Galactose	+	Galactose	+
Sucrose	+	Sucrose	+
Maltose	+	Maltose	+
Xylose	−	Xylose	+
Lactose	−		
Urease	−	Diazonium Blue B (DBB)	−
Temperature range for growth			
Growth at 4 °C	+		
Growth at 10 °C	+		
Growth at 37 °C	+		
Growth at 45 °C	+		
Growth at 10% NaCl +5% glucose	+		

+ Positive result or growth; − Negative result or no growth

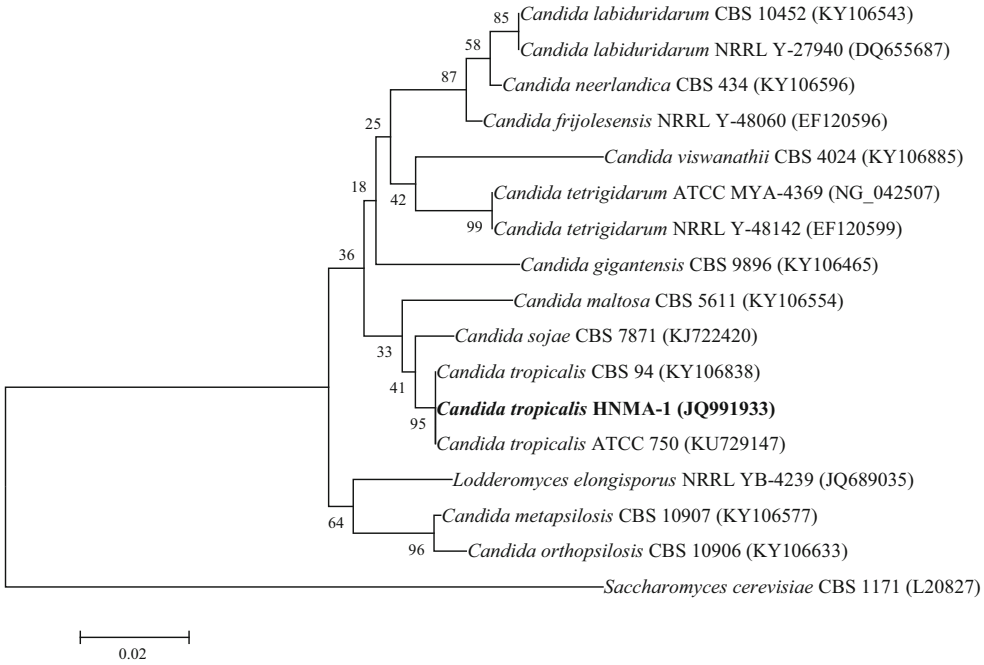

Fig. 2 Phylogenetic tree of *Candida tropicalis* HNMA-1 with bootstrap replications of 1000 using neighbor-joining algorithm

xylose medium and the highest ethanol concentration of 3.57 g L^{-1} and 0.088 g yield of ethanol g^{-1} xylose was achieved (Table 5). In the hydrolysate medium, most of the sugar was fermented after 48 h, when the ethanol concentration and yield reached about 2.83 g L^{-1} and 0.090 g of ethanol g^{-1} xylose, respectively (Table 5). The higher yield that was obtained in the hydrolysate medium compared to the xylose medium may be as a result of there being a lower initial amount of sugar in the former medium. Although xylose and other sugars in the hydrolysate are fermented simultaneously by the thermotolerant *C. tropicalis* HNMA-1, the ethanol yield and productivity are insufficient. The sugar was converted to ethanol with a productivity of 0.150 g L^{-1} h^{-1} and 0.059 g L^{-1} h^{-1} in the xylose and hydrolysate media, respectively (Table 5). Ethanol productivities of 0.12 g L^{-1} h^{-1} by *S. stipitis* NRRL Y-7124 on detoxified sugarcane bagasse acid hydrolysate (Milessi et al. 2013), 0.041 g L^{-1} h^{-1} by *Pichia* strain BY2 from non-detoxified sugarcane bagasse acid hydrolysate (Hande et al. 2013) and 0.04 g L^{-1} h^{-1} by *P. stipitis* DSM 3651 using the non-detoxified hydrolysate (Canilha et al. 2010) have been reported. In accordance with previous studies, the lower ethanol production in the hydrolysate medium may be due to the presence of inhibitory compounds, such as furfural, hydroxymethl furfural and phenols (Canilha et al. 2010). Kwon et al. (2011) reported that lignocellulosic inhibitory compounds not only prolonged the lag phase of cell growth but also decreased ethanol yield and production.

According to the results presented, xylose was consumed more rapidly than hydrolysate. The growth rate in hydrolysate was slightly lower than in xylose medium (Fig. 3C). Inhibitory compounds in hydrolysate may have influenced growth of the yeast during the initial phase of the incubation process (Canilha et al. 2012).

Table 4 Composition of sugarcane bagasse hydrolysate

	Total reducing sugar (g L^{-1})	Glucose (g L^{-1})	Pentose (g L^{-1})	Furfural (g L^{-1})	Phenolics (g L^{-1})
Sugarcane bagasse hydrolysate	34	2.93	27.72	0.83	1.017

Fermentation of xylose and sugarcane bagasse hydrolysate by *C. tropicalis* HNMA-1 at high temperature

We evaluated the effect of temperature on ethanol production by assaying the fermentation performance at 30 °C and 40 °C. Table 5 gives a summary of the ethanol yield, concentration and productivity at 30 °C and 40 °C.

Table 5 Fermentation parameters for ethanol production from xylose and sugarcane bagasse hydrolysate at 30 °C and 40 °C by *C. tropicalis* HNMA-1

Parameters		Xylose	Sugarcane bagasse hydrolysate
Ethanol yield Yp/s (g g^{-1})	30 °C	0.088	0.090
	40 °C	0.076	0.078
Maximum ethanol conc. Pmax (g L^{-1})	30 °C	3.57	2.83
	40 °C	3.39	2.5
Volumetric ethanol productivity Qp (g L^{-1} h^{-1})	30 °C	0.150	0.059
	40 °C	0.141	0.052

C. tropicallis HNMA-1 was able to grow better in both xylose and hydrolysate media at 40 °C. Furthermore, the positive effect of higher temperature was observed during growth in pure and lignocellulosic substrates. The capability of *C. tropicallis* HNMA-1 can be used in simultaneous saccharification and fermentation of lignocellulosic biomass where the process temperature is kept around 40 °C (Olofsson et al. 2008; Cardona et al. 2010). The lower growth observed in the hydrolysate medium compared to the xylose medium may be connected with the lower concentration of sugar in the hydrolysate medium. These results show that the isolate is a suitable yeast strain for growth at high temperatures. Also, we found better ethanol production from xylose at 30 °C than 40 °C, reaching yields of 0.088 g g^{-1} and 0.076 g g^{-1}, and maximum ethanol concentrations of 3.57 g L^{-1} and 3.39 g L^{-1}, respectively, in 24 h (Table 5). Abdel-Banat et al. (2010) reported that although their yeast isolate can grow at 45 °C, yield was poor at this temperature. In addition, high temperature will be a very severe stress condition during fuel ethanol fermentation (Basso et al. 2008). In hydrolysate medium at 30 °C and 40 °C, the fermentation process was completed in approximately 48 h as compared to 24 h in xylose medium, demonstrating lower ethanol productivity (Fig. 3a,b). The lower hydrolysate fermentation efficiency is possible as a result of the presence of inhibitory compounds. Consequently, higher temperature adversely affects ethanol production, since, at high temperature, more sugars are consumed. The current investigation also showed the negative effect of hydrolysate inhibitory compounds, which increase fermentation time (De Souza et al. 2012).

In some studies, lignocelluolosic sources (such as sugar cane bagasse, rice straw, wheat straw and sweet sorghum) as well as different approaches to saccharification, have been applied for bioethanol production (Anwar et al. 2014). The ethanol production yield in these studies is different. Various material sources and treatments are the main reason for different ethanol yield production. Here, we evaluated ethanol production by *C. tropicalis* HNMA-1 in sugarcane bagasse hydrolysate and xylose medium. Our results showed that the yield of ethanol production by this strain is 0.088 g g^{-1} and 0.090 g g^{-1} in xylose and sugarcane bagasse medium, respectively. In a similar study, the immobilized thermotolerant *Debaromyces hansenii* cells in Ca-alginate matrix produced ethanol with a yield of 0.46 g g^{-1} from hemicellulosic hydrolysate at 40 °C (Menon et al. 2010). Kuhad and colleagues used *Lantana camara* containing 61.1% (w w^{-1}) hemicellulose and the yields of ethanol production by *P. stipitis* and *S. cerevisiae* were 0.32 g

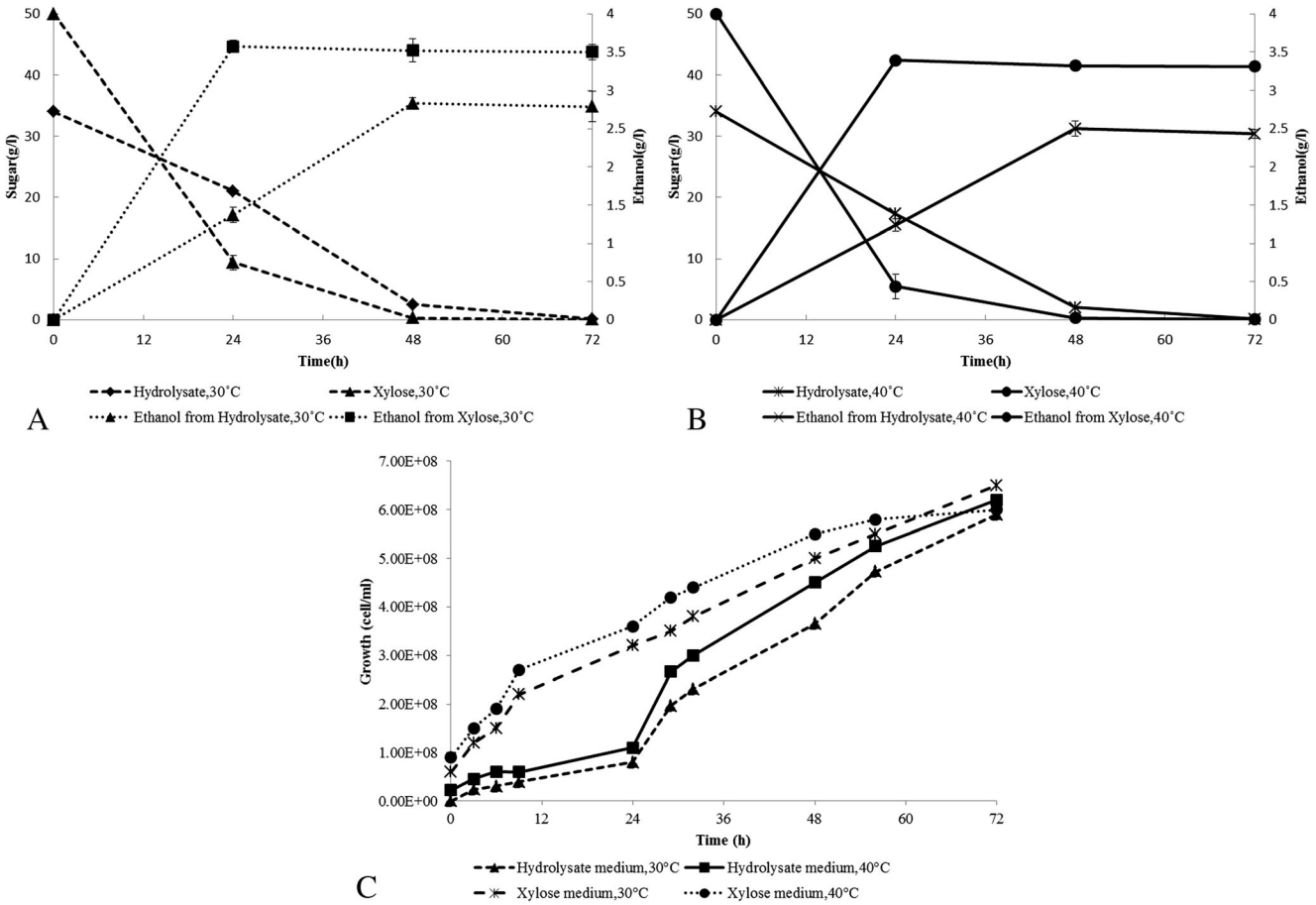

Fig. 3 Comparison of time courses of xylose and sugarcane bagasse hydrolysate consumption and ethanol production at **a** 30 °C, **b** 40 °C. **c** Yeast growth rate

g^{-1} and 0.48 g g^{-1}, respectively (Kuhad et al. 2010). Brar et al. (2016) reported a hybrid approach by using acid hydrolysate and enzymatic treatment of corncob to ethanol production. The yield of produced ethanol with co-culture of *S. cerevisiae* and *P. stipitis* was 3.42% (v v^{-1}). Reis et al. (2016) investigated the efficiency of ethanol production by yeast strain *Dekkera bruxellensis* from bagasse of sugarcane (SCB) and sweet sorghum (SSB), and the final yields were 0.42 g g^{-1} for SCB and 0.44 g g^{-1} for SSB. Meethit et al. (2016) reported the synergistic effect of co-culture of *S. cerevisiae* and *Candida shehatae* for consumption of SCB and rice straw (RS) and ethanol production. The results showed that continuous ethanol production yielded 0.38 g g^{-1} and 0.40 g g^{-1} from SCB and RS hydrolysate, respectively.

Conclusion

The current study describes comparative data from thermotolerant *C. tropicalis* HNMA-1 concerning its fermentation and growth performance at high temperature in pure and hemicellulosic media. Also, production of thermostable xylanase was shown in *C. tropicalis* HNMA-1 at 40 °C. Our results demonstrated the positive effect of higher temperatures on the growth in hemicellulosic hydrolysate and xylose media. In addition, the isolate indicated higher ethanol yield in both the xylose and hydrolysate medium at 30 °C as compared to 40 °C. The thermal stability of the new isolate revealed that ethanol fermentation by this strain is promising when lignocellulosic biomass is used. Based on the different stresses to which yeast cells are exposed, further studies aimed at the commercialization of lignocellulose hydrolysate fermentation with low cost and improved strains are necessary, and process optimization will be required.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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