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ORIGINAL PAPER

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Adaptive response of yeasts to furfural and 5-hydroxymethylfurfural and new chemical evidence for HMF conversion to 2,5-bis-hydroxymethylfuran

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Abstract Renewable lignocellulosic materials are attractive low-cost feedstocks for bioethanol production. Furfural and 5-hydroxymethylfurfural (HMF) are among the most potent inhibitory compounds generated from acid hydrolysis of lignocelluloses to simple sugars for fermentation. In *Saccharomyces cerevisiae* ATCC 211239 and NRRL Y-12632 and *Pichia stipitis* NRRL Y-7124, furfural and HMF inhibition were determined to be dose-dependent at concentrations from 10 to 120 mM. The yeast strains were more sensitive to inhibition by furfural than HMF at the same concentration, while combined treatment of furfural and HMF synergistically suppressed cell growth. A metabolite transformed from HMF by strain NRRL Y-12632 was isolated from the culture supernatant, and conclusively identified as 2,5-bis-hydroxymethylfuran, a previously postulated HMF alcohol, with a composition of $C_6H_8O_3$ and a molecular weight of 128. It is proposed that, in the presence of HMF, the yeast reduces the aldehyde group on the furan ring of HMF into an alcohol, in a similar manner as for furfural. The accumulation of this biotransformed metabolite may be less toxic to yeast cultures than HMF, as evidenced by the rapid yeast fermentation and growth rates associated with HMF conversion. The ability of yeasts to adapt to and transform furfural and HMF offers the potential for in situ detoxification of these inhibitors and suggests a genetic basis for further development of highly tolerant strains for biofuel production.

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Introduction

As interest in alternative energy sources increases, lignocellulosic materials have become attractive potential low-cost feedstocks for bioethanol production. For economic reasons, dilute acid hydrolysis is commonly used to prepare lignocelluloses for enzymatic saccharification and fermentation. However, numerous side-products are generated by this pretreatment, many of which inhibit microbial fermentation. Furfural and 5-hydroxymethylfurfural (HMF) are representative of the many inhibitory compounds from lignocellulosic hydrolysis [21]. During sugar degradation with acid treatment, furfural is mainly derived from pentose dehydration and HMF is formed from dehydration of hexoses [1, 7, 8]. These compounds damage microorganisms by reducing enzymatic and biological activities, breaking down DNA, inhibiting protein and RNA synthesis, and are considered among the most potent inhibitors [6, 10, 17]. Most yeasts, including industrial strains, are susceptible to various inhibitory compounds derived from acid hydrolysis pretreatment and especially susceptible to the presence of multiple inhibitors [9, 21]. To facilitate fermentation processes, additional treatments are often needed to remediate these inhibitory compounds, including physical, chemical or biochemical detoxification. However, these additional steps add cost and complexity to the process and generate additional waste products.

Genetically manipulated *Saccharomyces* strains have shown enhanced properties for ethanol fermentation through improved utilization of starch, lactose, and xylose, as well as enzyme production [2–4, 15]. Development of genetically engineered strains with greater inhibitor tolerance, especially to furans, is a promising alternative to including separate detoxification steps.

However, few yeast strains genetically improved for inhibitor tolerance are available. Development of such strains has been hindered by a lack of understanding of the basic mechanisms underlying stress tolerance in microorganisms. Based on a preliminary evaluation of the inhibitor tolerance of 15 ethanologenic yeasts in nine species, we selected two strains of *Saccharomyces cerevisiae* and one strain of *Pichia stipitis* for further study. The objective of this study was to evaluate chemical stress response to furfural and HMF of the selected yeast strains using cultures grown in defined medium under controlled conditions. Comparison and characterization of fermentation profiles of selected yeast strains will provide a basis for future studies on stress-tolerance mechanisms. Such knowledge can be applied to develop more stress-tolerant strains of yeast for bioethanol production.

Materials and methods

Yeast strains and chemicals

Pichia stipitis NRRL Y-7124 and *S. cerevisiae* NRRL Y-12632 were obtained from the Agricultural Research Service Culture Collection (Peoria, Ill., USA) and *S. cerevisiae* ATCC 211239 was obtained from the American Type Culture Collection (Manassas, Va., USA). Basic medium components were supplied by Difco (Detroit, Mich., USA). Amino acids, furfural, HMF, furfuryl alcohol, and all chemicals used were provided by Sigma-Aldrich (St. Louis, Mo., USA).

Culture maintenance, inoculum preparation, and culture conditions

Yeast strains were maintained on YM agar (3 g yeast extract, 3 g malt extract, 5 g peptone, 20 g agar in 1 l of distilled water) after their recovery from a lyophilized form. Stock cultures were kept at −80°C in YM broth amended with 30% glycerol. Cultures were routinely maintained on a synthetic complete medium consisting of 6.7 g yeast nitrogen base without amino acids and 20 g dextrose supplemented with 16 amino acids [19, 22]. The amino acid components were added into the medium aseptically at the final concentrations (per l) of 20 mg adenine sulfate, 20 mg uracil, 20 mg L-tryptophan, 20 mg L-histidine hydrochloride, 20 mg L-arginine hydrochloride, 20 mg L-methionine, 30 mg L-tyrosine, 30 mg L-leucine, 30 mg L-isoleucine, 30 mg L-lysine hydrochloride, 50 mg L-phenylalanine, 100 mg L-glutamic acid, 100 mg L-aspartic acid, 150 mg L-valine, 200 mg L-threonine, and 400 mg L-serine. When solid medium was prepared, agar was autoclaved and the sterilized components added afterward. A loopful of cells of each strain from the synthetic medium agar plate was transferred into a synthetic broth and incubated at 30°C with agitation and maintained in the broth prior to inoculum preparation. Cells were harvested by centrifugation 8–15 h after incubation and suspended in a fresh synthetic broth serving as the inoculum source. The initial OD at 620 nm of the inoculated medium for each culture was adjusted and recorded. Each strain was grown in a 15-ml test tube containing 3 ml of synthetic broth amended with inhibitory compounds in an incubator at 30°C with agitation at 220 rpm under micro-oxic conditions. Cell growth was monitored by measuring optical absorbance at 620 nm using a spectrophotometer and samples were taken periodically. Three replicated experiments were carried out for each strain and condition.

Inhibitory compound treatment

HMF was prepared in a 1.2 M stock by filter sterilization, and furfural was directly used to amend a medium. Broth media were supplemented with furfural and HMF individually at 10, 30, 60, and 120 mM, and in combination at 10, 30, and 60 mM, respectively. The purity of each compound was tested using HPLC. Control cultures were not supplemented with either inhibitor. Triplicate cultures were carried out for each treatment.

Sample collection and analysis

Cultures were monitored for cell growth during the fermentation. Glucose, HMF, furfural, and ethanol, as well as other fermentation products, were measured using a Waters HPLC equipped with a Fast Acid column (Bio-Rad Laboratories, Hercules, Calif., USA) and a refractive index detector. The column was maintained at 65°C, and samples were eluted with 5 mM H_2SO_4 at 0.6 ml/min. Furfural, HMF, furfuryl alcohol, and a previously postulated HMF alcohol were confirmed by analysis using reverse-phase HPLC equipped with a C18 column and a dual wavelength UV detector.

Isolation and identification of the biotransformed HMF metabolite

A 500-ml aliquot of medium from a completed biotransformation experiment containing an initial concentration of 30 mM HMF was reduced to dryness by lyophilization. The resulting material was resuspended in 50 ml of methanol, filtered, and evaporated to dryness on a rotoevaporator. The resulting solid material was resuspended in approximately 2 ml of water and filtered through a 0.45-μm nylon 66 filter (Alltech Associates, Deerfield, Ill., USA) for injection. The metabolite was isolated by preparative HPLC system.

The preparative HPLC system was from Shimadzu (Columbia, Md., USA) and consisted of two LC-8A pumps, an SCL-10A controller, an SPD10Avp photodiode array detector, and an SIL-10A autoinjector, all

running under Shimadzu Class VP software version 5.032. The column was a reverse-phase C-18 Licrosorb column, 250×10 mm, 5 μm (Phenominex, Torrance, Calif., USA). The solvents for the binary gradient were 0.5% acetic acid and methanol. The initial conditions on the column were 2% methanol, at a flow rate of 5 ml per min. The wavelength monitored was 220 nm. After 2 min at the initial conditions after the sample injection, the column was developed with a linear gradient to 34% methanol over 20 min. Three peaks were resolved and collected manually. This was repeated ten times using an injection volume of 50 μl. The resulting fractions were rotoevaporated to remove methanol and then lyophilized to dryness.

The molecular weight of the postulated HMF alcohol was obtained on a Hewlett Packard 6890 Series GC attached to a 5973 N MS detector (Agilent Technologies, Wilmington, Del., USA) running the Agilent MSD Chemstation software package (D.01.00 build 75). An HP-5MS 5% phenyl methylsiloxane (30 m× 0.25 mm×25 μm film thickness) column was applied with a typical helium gas flow rate of 38 ml/min. Initial oven temperature was set at 50°C. One microliter of diluted sample was injected in the splitless mode, and the column was developed at a constant flow rate at 5°/min to a final temperature of 315°C. The MS was set to collect mass scans between 50 and 550 AMUs.

Spectra of ^{1}H NMR and ^{13}C NMR were obtained on a Bruker Avance Ultrashield 500 MHz NMR spectrometer (Billerica, Mass., USA) equipped with a 5-mm inverse broadband Z-gradient probe (^{13}C NMR, 125 MHz; ^{1}H, 500 MHz) and running the ICON software package (version 3.5). The NMR spectra were recorded with the sample in deuterated water.

Results

Dose-dependent response of yeasts

In the individual treatments with the inhibitive compounds, all three strains examined grew in the synthetic medium amended with either furfural or HMF at 10 and 30 mM, respectively (Fig. 1a, b, d, e, g, and h). However, at 10 mM, *S. cerevisiae* ATCC 211239 showed a prolonged lag phase of 8 and 4 h for furfural- and HMF-treated cultures, respectively, compared with that of the control. Similarly, *P. stipitis* NRRL Y-7124 had a 4-h lag phase for furfural but only a limited lag time for HMF. Stain NRRL Y-12632 grew with a limited lag phase for furfural and cell growth of HMF-treated cultures slightly suppressed. For cultures growing in 30 mM inhibitor-treated media, this lag time extended to 24 and 16 h for strain ATCC 211239 for furfural and HMF, respectively. For strain NRRL Y-7421, the lag time extended to 24 and 8 h for furfural and HMF, respectively. Strain NRRL Y-12632 responded with a reduced growth rate, rather than complete suppression during the initial incubation period. No cell growth was observed when cultures were exposed to 60 mM furfural or HMF, except for strain ATCC 211239 at 60 mM HMF treatment (Fig. 1a, b, d, e, g, and h). It appeared that, at the same concentration, furfural was more inhibitory to cell growth than was HMF. No cell growth was observed for strains grown in the presence of 120 mM of either furfural or HMF (data not shown). For the combined treatment of the two inhibitors, cell growth was only recovered at 10 mM each of furfural and HMF, with a lag time for at least 8–16 h after incubation for strains ATCC 211239 and NRRL Y-7124, or a limited lag time for strain NRRL Y-12632 (Fig. 1c, f, and i). No substantial cell growth or recovery was observed at any higher concentrations of the combined inhibitors. The combined treatment of furfural and HMF seemed to act synergistically to suppress cell growth.

Fermentation profiles of HMF and furfural treatment

In parallel to the delayed cell growth, glucose utilization was also delayed for all yeast strains tested. Figure 2 showed a typical response of strain NRRL Y-12632 to 30 mM HMF treatment with delayed glucose consumption and ethanol production compared with the control. A similar trend for cell growth and glucose consumption was observed for inhibitor treatment at 10 mM, but the delay only lasted 4–8 h (data not shown). In contrast, such a lag stage persisted for more than 2 days at 60 mM HMF. Similar delayed glucose consumption was also observed for furfural treatment at 10 and 30 mM (data not shown). Once a culture was able to recover from the furfural or HMF treatment, the final ethanol yield showed a similar level with that of the control (data not shown for the furfural treatment). However, with a high dose of HMF, it took a week or longer to reach the maximum potential yield compared with 24 h for the control. In addition to ethanol and inhibitor converted compounds, other fermentation products, including acetic acid and formic acid, were also observed (data not shown).

Cell growth in cultures exposed to furfural and/or HMF coincided with the disappearance of these compounds from the culture. Concentrations of these inhibitors did not decrease linearly during the lag phase, but rather around the same time as cultures entered the exponential phase for mass growth. This trend was observed even when the lag time lasted for days under 60 mM, a high-dose treatment. As expected, increases in furfural alcohol concentration in a culture were correlated with decreasing concentrations of furfural. Similarly, in HMF treated cultures, a new peak appeared to increase in size at the same time the HMF peak decreased during the fermentation.

Biotransformation of HMF

The HMF-converted product was consistently observed at all HMF treatment concentrations but not in the

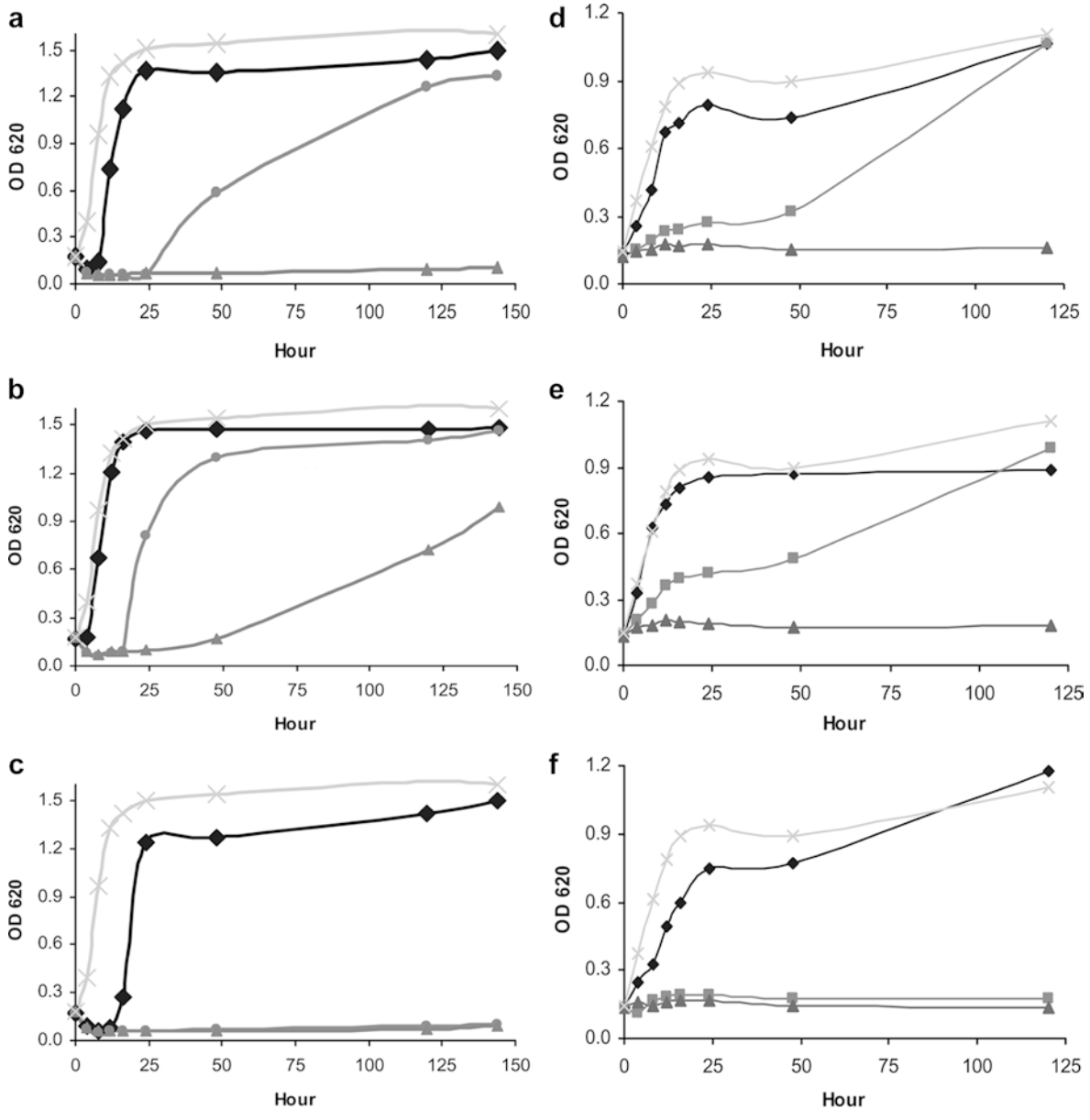

Fig. 1a–i Cell growth of *Saccharomyces cerevisiae* ATCC 211239 (**a**, **b**, **c**), *S. cerevisiae* NRRL Y-12632 (**d**, **e**, **f**), and *Pichia stipitis* NRRL Y-7124 (**g**, **h**, **i**) as measured at OD_{620} during growth in the presence or absence of furfural (**a**, **d**, **g**), HMF (**b**, **e**, **h**), or a combined treatment of furfural and HMF (**c**, **f**, **i**). Yeasts were cultured in a synthetic basal medium. Concentrations of furfural and HMF in the medium were 10 (◆), 30 (■), and 60 mM (▲) each. Neither inhibitor was added to the control (x)

control cultures. The accumulation of this HMF-associated compound in the medium appeared not to inhibit cell growth or final ethanol production. Once formed, the product was persistent throughout the fermentation. Analysis of fermentation-broth supernatant by reverse-phase HPLC with UV detection showed that HMF absorbed at 282 nm at 0 h (Fig. 3a). At 48 h, the end of the fermentation, HMF was no longer detected in the broth, but a new peak, the HMF-associated product, was detected at 222 nm (Fig. 3b). When samples were eluted on a Biorad Fast Acid HPLC column and measured with an RI detector, HMF had a retention time of 11.0 min and the conversion product a retention time of 6.2 min (data not shown).

The metabolite was purified from lyophilized medium by preparative HPLC. The pure material formed light crystals after drying by lyophilization. An aliquot was resuspended in methanol and analyzed by GC-MS. The resulting chromatogram showed a single peak with a molecular mass ion of 128, and major fragments of MW 111, 113, 97, 83, 81, 69, and 55. ^{1}H NMR δ (D_2O): 4.49 (2H, s, CH_2) and 6.28 (1H, s, CH). ^{13}C NMR δ (D_2O): 153.7 (C1, s); 109.1 (d); 55.9 (q). The ^{1}H NMR showed only the signals for the four identical aliphatic protons of the two methylene groups (δ 4.49) and the two identical protons of the furan ring (δ 6.28). The ^{13}C NMR showed the presence of the two aliphatic carbons of the methyl groups (δ 55.9), the two protonated carbons of

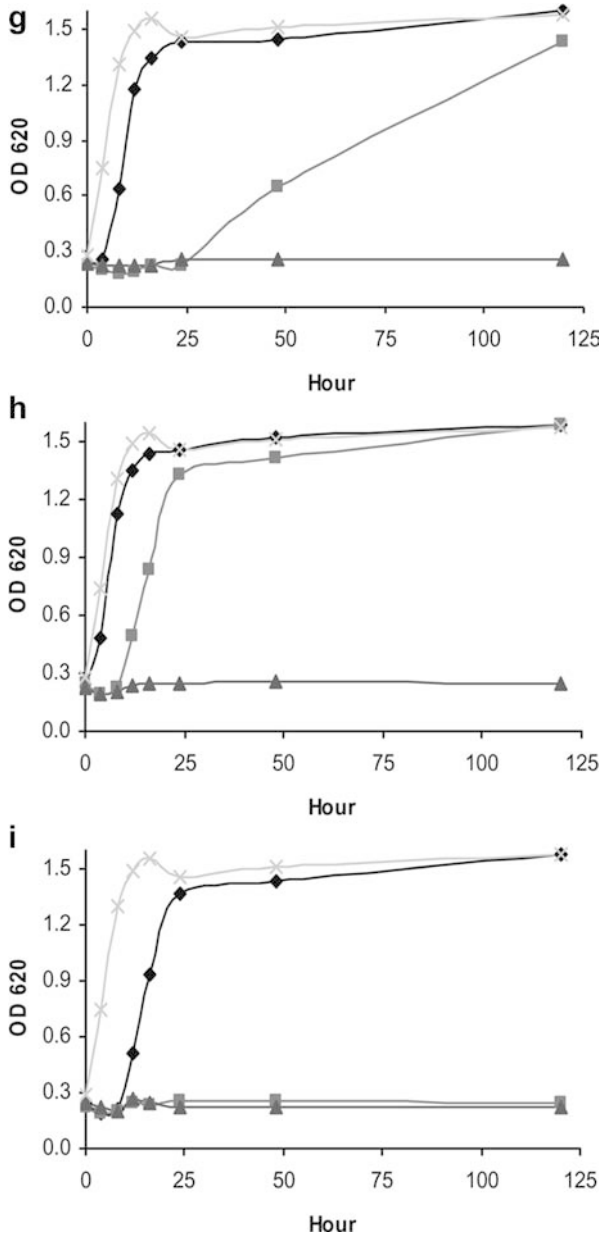

Fig. 1g–i (Contd.)

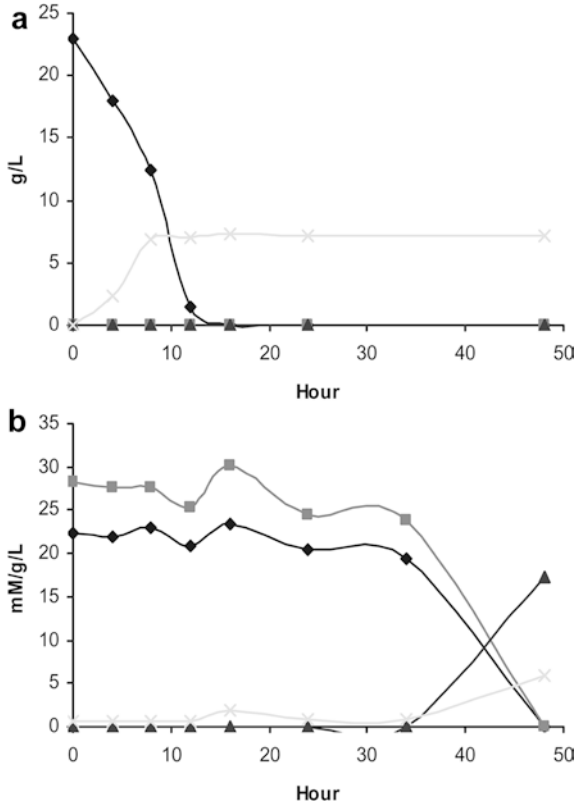

Fig. 2a, b Glucose consumption, ethanol production, and HMF transformation of *S. cerevisiae* NRRL Y-12632 in a complete synthetic medium in the presence of 30 mM HMF (**b**) compared with no inhibitor added (control, **a**). Concentrations for glucose (◆) and ethanol (x) were estimated in grams per liter, and HMF (■) and 2,5-bis-hydroxymethylfuran(bis-hmf) (▲), in mmol

the furan ring (δ 109.1), and the two unprotonated carbons of the furan ring (δ 153.7). The signals for the aldehyde proton and the asymmetric spectra of HMF were absent. The NMR spectra are consistent with that of a symmetrical molecule with a furan ring. The chemical structure was identified as a compound with a composition of $C_6H_8O_3$, corresponding to a structure of 2,5-bis-hydroxymethylfuran (furan-2,5-dimethanol) (Fig. 4).

Discussion

Two *S. cerevisiae* strains, ATCC 211239 and NRRL Y-12632, and one *P. stipitis* strain, NRRL Y-7124, were evaluated for their response to furfural and HMF at concentrations from 10 to 120 mM, and to combined treatment from 10 to 60 mM of each compound. Given a tolerable concentration, all strains were able to recover from a prolonged lag phase during the initial part of the incubation. The lag phase lasted from a few hours to several days depending upon the amount of inhibitor added to the cultures. Once cell growth recovered, cultures inoculated with all strains were able to consume glucose and thereafter produce ethanol. This demonstrated a clear dose-dependent inhibition of yeasts to furfural and HMF. The inhibition completely suppressed cell growth at 120 mM under the conditions of this study. When both inhibitors were applied in combination, cell growth was only recovered at 10 mM of each inhibitor, which indicated these inhibitors acted in a negative synergic fashion even at low concentrations. This negative synergy suggests that the inhibitors may act by different mechanisms or that yeast strains adapt differently to the presence of each.

Furfural-treated cultures tend to have a longer lag time than those treated with HMF given equal inhibition concentrations. Apparently, furfural was more suppressive to cell growth. Similar observations were reported previously [17, 21]. In yeast cultures treated with

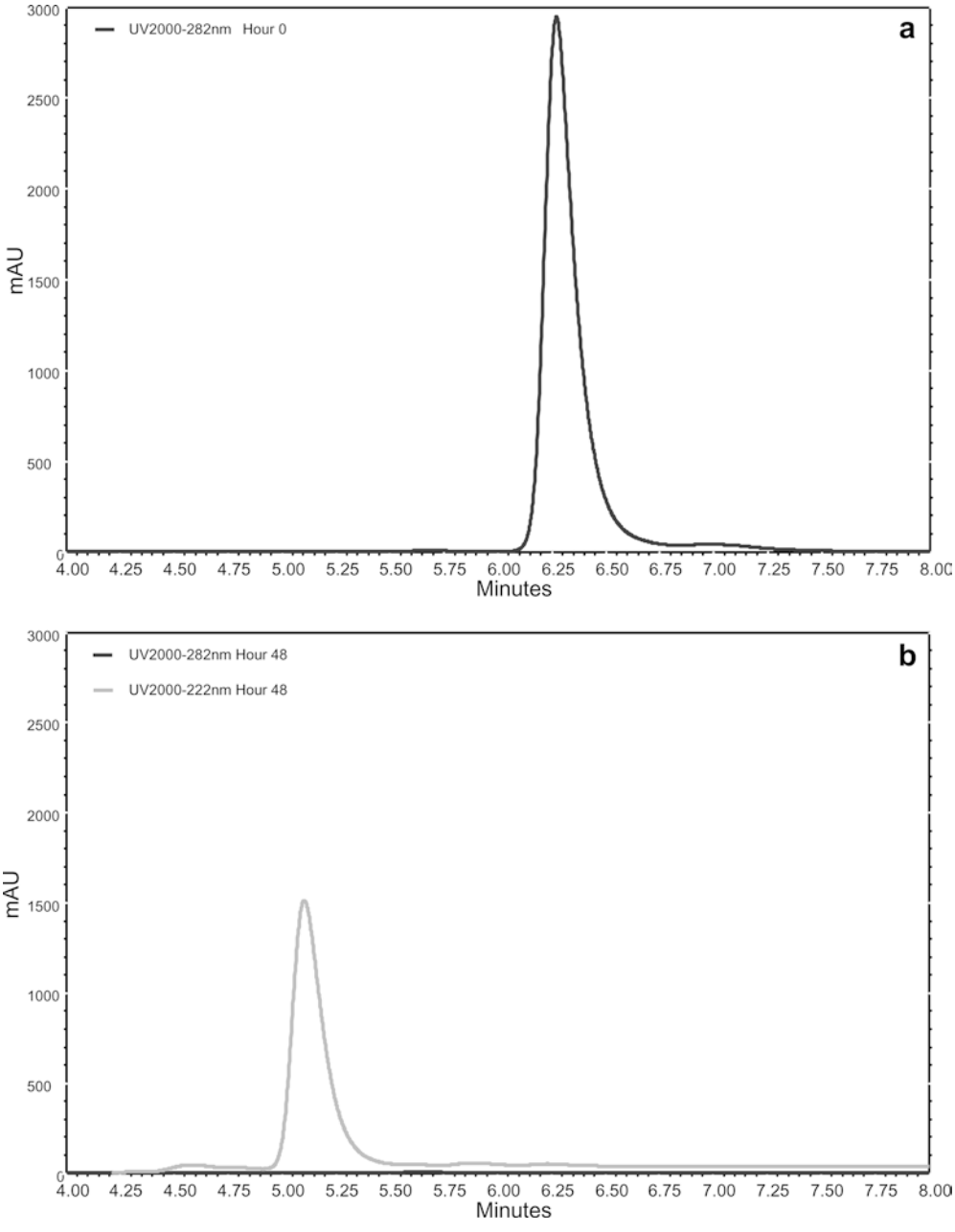

Fig. 3a, b Reverse-phase HPLC chromatogram of *S. cerevisiae* NRRL Y-12632 showing HMF detected by UV absorbance at 282 nm at time zero in a complete synthetic medium supplemented with 30 mM HMF (**a**), and 2,5-bis-hydroxymethylfuran at 222 nm and no HMF recovered at 282 nm 48 h after the incubation (**b**). No peaks were observed absorbing at 222 nm in the 0-h samples

furfural, furfural was converted into furfuryl alcohol, which did not significantly affect the final ethanol yield. These results were in agreement with previous observations concerning the effect of furfural on yeasts [16, 17, 21]. The furfuryl alcohol concentration formed from furfural by the yeast strains was proportional to their initial concentrations. Conversion of furfuryl to furfural alcohol has been well established since the first report three decades ago [1, 11, 12, 16, 21]. Our results demonstrated that this conversion is dose-dependent in yeasts.

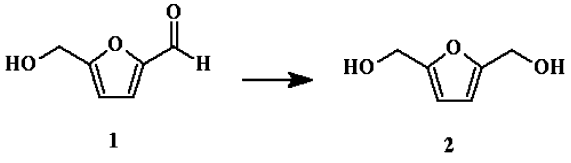

Fig. 4 A schematic diagram showing conversion of HMF (1) to 2,5-bis-hydroxymethylfuran (2) recovered from a yeast fermentation culture amended with HMF

In contrast to furfural, which is well studied, knowledge of HMF conversion is limited due to the lack of an internal control. Unlike furfuryl alcohol, a commercial source for HMF alcohol is not readily available. In this study, we isolated the yeast-based conversion product of HMF and identified it as 2,5-bis-hydroxymethylfuran (also termed furan-2,5-dimethanol). This is the first time that the chemical structure has been proven for the end product of HMF reduction by yeasts. Historically, HMF is believed to be converted into HMF alcohol

following a similar conversion route as that of furfural. Nemirovskii et al. [14] and Nemirovskii and Kostenko [13] demonstrated the conversion of furfural first to furfuryl alcohol and then to 2-furoic acid, and proposed a possible chemical pathway. They observed that HMF was also converted to another compound by yeast and interpreted it as HMF alcohol since HMF has a structure similar to furfural [8, 13, 14]. Subsequently, other studies assumed that the unidentified HPLC peak associated with HMF conversion was in fact HMF alcohol [21, 22]. However, this assumption was based on refractive index and no internal control was applied, nor was a structure identified [13, 14, 20]. Our study indicated that 2,5-bis-hydroxymethylfuran converted from the HMF increased with time and persisted in the culture broth at the end of the fermentation. Thus, it seemed unlikely that the alcohol was an intermediate in the pathway of an acid. Apparently, the HMF reduction to an alcohol was significant under the conditions of our study. We also observed acid production in some culture fermentation broths. The acid could be produced by an oxidation reaction from an aldehyde. In our study, it appeared that oxidation might not be significant. However, it is not clear whether oxidation during yeast fermentation is essential. Adding greater uncertainty to the current situation, Sanchez and Bautista [17] suggested different metabolic pathways for furfural and HMF based on their experiments. The mechanisms of furfural and HMF being involved in the fermentation pathway are not clear. The identity of the HMF conversion product is expected to assist with pathway analysis of fermentation in the presence of inhibitor.

The significance of our identification of the HMF conversion product in this study may not be limited to the analysis of yeast metabolism. Independent from yeast studies, Boopathy et al. [1] reported the ability of enteric bacteria to biotransform furfural and HMF. They confirmed the bacterial conversion of furfural to furfuryl alcohol. As observed for yeasts, these bacteria also converted HMF into an unidentified chemical compound, which they postulated as HMF alcohol. A further study by Boopthy et al. [1] showed that the unknown compound had a maximum UV absorbance of 222 nm; HMF had a maximum absorbance of 282 nm. Biotransformation of HMF by the bacteria was also observed. Based on the UV spectral change and HPLC analysis, the authors suggested that the transformed product from HMF was HMF alcohol. In this study, we also determined that the HMF-associated conversion product could be detected at 222 nm, but not at 282 nm, the maximum absorbance of HMF. Furthermore, we isolated and purified the compound, and proved the structure of this unknown using NMR. Our NMR data were similar to those reported previously [18]. Our chemical description and identification of 2,5-hydroxymethylfuran as a conversion product of HMF adds to the understanding of HMF metabolism and biotransformation by microorganisms. It appeared that, during yeast fermentation, the aldehyde was used as an electron acceptor in a reduction reaction. As observed for furfuryl alcohol in furfural conversion, the accumulated 2,5-bis-hydroxymethylfuran in the medium did not affect cell growth nor the final ethanol production. This is consistent with previous observations [7].

It is interesting to note that, after a prolonged lag phase for cell growth caused by HMF , glucose consumption and ethanol production were finally prompted to reach the maximum extent. This period of bioactivity was shorter than that for the control culture. It seemed that yeasts were able to adapt to the inhibitors at tolerable concentrations. Alcohol dehydrogenase was also observed to be induced by furfuryl alcohol [5]. It is possible that enzyme profiles are altered under the chemical activation and adaptation process triggered by 2,5-bis-hydroxymethylfuran converted from HMF after its introduction. However, the conversion relied on a nutrient supply, and neither furfural nor HMF could be used as sole carbon and energy source for microbial growth [1]. This bioconversion was not affected by either oxic or anoxic conditions [1, 21]. On a side note, we used a complete synthetic growth medium with a moderate amount of glucose in this study. Enriched media, including complex media or high glucose concentrations, influence the tolerance of yeast cells to these types of inhibitors [unpublished data; 21]. To simplify interpretation of the stress response data, we recommend the use of a defined medium, such as the complete synthetic medium.

As we observed in our previous screening and in the current study, yeast strains showed differences in inhibitor tolerance reflected by varied durations of the lag phases for cultures exposed to furfural and/or HMF. This variability in response suggests that some yeasts have more effective mechanisms to withstand these inhibitors than others. The alcohol form reduced from the aldehyde form appeared to not affect yeast fermentation. Furfural and HMF have been shown in vitro to inhibit important metabolic enzymes, including alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase [10]. The prolonged lag phase before the recovery of the cell growth suggests a major shift in the physiology of the cells adapting to chemical stress. Yeast strains can be further adapted to such an inhibitory stress. In fact, using the adaptation strategy, we have developed strains exhibiting a multiple-fold increase in tolerance to furfural, HMF, and other inhibitory compounds compared to their parental strains. Studies on the mechanisms of this dose-dependent tolerance are underway. A better understanding of the genetic mechanisms and biochemical pathways responsible for inhibitor response in yeast may allow the development of genetically engineered novel strains to withstand major inhibitors generated from biomass pretreatment. Combined with chemical engineering advances, these efforts could lead to in situ detoxification of inhibitors, including furfural and HMF, and thereafter to more efficient processes for converting lignocelluloses to ethanol.

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References

1. Boopathy R, Bokang H, Daniels L (1993) Biotransformation of furfural and 5-hydroxymethyl furfural by enteric bacteria. J Ind Microbiol 11:147–150
2. Hahn-Hagerdal B, Wahlbom CF, Gardony M, Van Zyl WH, Otero RRC, Jonsson LJ (2001) Metabolic engineering of *Saccharomyces cerevisiae* for xylose utilization. Adv Biochem Eng Biotechnol 73:53–84
3. Ho NWY, Chen Z, Brainard AP (1998) Genetically engineered *Saccharomyces* yeast capable of effective cofermentation of glucose and xylose. Appl Environ Microbiol 64:1852–1859
4. Jeffries TW, Shi N-Q (1999) Genetic engineering for improved fermentation by yeasts. Adv Biochem Eng Biotechnol 65:117–161
5. Kang SS, Okada H (1973) Alcohol dehydrogenase of *Cephalosporium* sp. induced by furfural alcohol. J Ferment Technol 51:118–124
6. Khan QA, Hadi SM (1994) Inactivation and repair of bacteriophage lambda by furfural. Biochem Mol Biol Int 32:379–385
7. Larsson S, Palmqvist E, Hahn-Hagerdal B, Tengborg C, Stenberg K, Zacchi G, Nilvebrant N-O (1999) The generation of inhibitors during dilute acid hydrolysis of softwood. Enzyme Microb Technol 24:151–159
8. Lewkowski J (2001) Synthesis, chemistry and applications of 5-hydroxymethylfurfural and its derivatives. Arkivoc 1:17–54
9. Martin C, Jonsson LJ (2003) Comparison of the resistance of industrial and laboratory strains of *Saccharomyces* and *Zygosaccharomyces* to lignocellulose-derived fermentation inhibitors. Enzyme Microb Technol 32:386–395
10. Modig T, Liden G, Taherzadeh MJ (2002) Inhibition effects of furfural on alcohol dehydrogenase, aldehyde dehydrogenase and pyruvate dehydrogenase. Biochem J 363:769–776
11. Morimoto S, Murakami M (1967) Studies on fermentation products from aldehyde by microorganisms: the fermentative production of furfural alcohol from furfural by yeasts (part I). J Ferment Technol 45:442–446
12. Morimoto S, Hirashima T, Ohashi M (1968) Studies on fermentation products from aldehyde by microorganisms: the fermentative production of furfural alcohol from furfural by yeasts (part II). J Ferment Technol 46:276–287
13. Nemirovskii VD, Kostenko VG (1991) Transformation of yeast growth inhibitors which occurs during biochemical processing of wood hydrolysates. Gidroliz Lesokhimm Prom-st 1:16–17
14. Nemirovskii VG, Gusarova LA, Rakhmilevich YaD, Sizov AI, Kostenko VG (1989) Furfural and hydroxymethylfurfural transformation route during culturing of nutrient yeasts. Biotekhnologiya 5:285–289
15. Ostergaard S, Olsson L, Nielsen J (2000) Metabolic engineering of *Saccharomyces cerevisiae*. Microbiol Mol Bio Rev 64:34–50
16. Palmqvist E, Almeida JS, Hahn-Hagerdal B (1999) Influence of furfural on anaerobic glycolytic kinetics of *Saccharomyces cerevisiae* in batch culture. Biotechnol Bioeng 62:447–454
17. Sanchez B, Bautista J (1988) Effects of furfural and 5-hydroxymethylfurfrual on the fermentation of *Saccharomyces cerevisiae* and biomass production from *Candida guilliermondii*. Enzyme Microb Technol 10:315–318
18. Schiavo V, Descotes G, Mentech J (1991) Catalytic hydrogenation of 5-hydroxymethylfurfural in aqueous medium. Bull Soc Chim Fr 128:704–711
19. Sherman F (2002) Getting started with yeast. In: Guthrie C, Fink GR (eds) Guide to yeast genetics and molecular biology. Academic, San Diego, pp 3–41
20. Taherzadeh MJ, Eklund R, Gustafsson L, Niklasson D, Liden G (1997) Characterization and fermentation of dilute-acid hydrolyzates from wood. Ind Eng Chem Res 36:4659–4665
21. Taherzadeh MJ, Gustafsson L, Niklasson C (2000) Physiological effects of 5-hydroxymethylfurfural on *Saccharomyces cerevisiae*. Appl Micorbiol Biotechnol 53:701–708
22. Wickerham LJ (1951) Taxonomy of Yeast. Tech Bull No 1029 US Dept Agric, Washington