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ORIGINAL ARTICLE

Harnessing *Paecilomyces variotii* to remove lignocellulose-derived inhibitors: potential evaluation, lignocellulosic biorefining route design, and transcriptome analysis

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Abstract

Discovery and identification of robust biodetoxification strain is crucial for the sustainable and efficient operation of lignocellulosic biorefining process. *Paecilomyces variotii* FN89, a recently isolated mesophilic filamentous fungi, was herein shown to be able to biodegrade lignocellulose-derived inhibitors including furfural (1.5 g/L), 5-hydroxymethylfurfural (4 g/L), acetic acid (4 g/L), hydroxybenzaldehyde (0.2 g/L), syringaldehyde (0.2 g/L), and vanillin (1.5 g/L) efficiently and completely. *P. variotii* FN89 was adapted to mixed inhibitors and relatively low dissolved oxygen conditions, which can detoxify both the highly viscosity hydrolysate and solid biomass with the well preserve of fermentable sugars and no addition of any nutrients. Two biorefinery chains involving biodetoxification process were thus established to cope with different forms of pretreated biomass for cellulosic lactic acid production. The cellulosic lactic acid titers were above 100 g/L from 25% (w/w) solids loading pretreated wheat straw. The global transcriptome analysis of *P. variotii* FN89 in the presence of mixed inhibitors suggested that the glycolysis pathway and pentose phosphate pathway were repressed while tricarboxylic acid cycle was enhanced, ensuring the complete degradation of the inhibitors-derived intermediates and efficient energy supply. This study provided a unique and practical biodetoxification strain for lignocellulosic biorefinery, as well as enriched the knowledge of the molecular basis of lignocellulose-derived inhibitors tolerance and carbohydrates metabolism of *P. variotii*.

Keywords Biodetoxification · *Paecilomyces variotii* · Lignocellulose · Lactic acid · Transcriptome analysis

Introduction

Lignocellulose biomass requires effective pretreatment to break up its compact lignin-carbohydrate-complex structure, thus facilitating the subsequent enzymatic hydrolysis to release the soluble reducing sugars [1]. However, the pretreatment process generates various toxins such as furfural, 5-hydroxymethylfurfura (HMF), acetic acid, syringaldehyde, vanillin, which inhibit the subsequent enzymatic hydrolysis and the activity of fermenting microorganisms [2]. Many detoxification methods, i.e. water-washing, evaporation, extraction, adsorption, oxidation, over-liming, and biological detoxification (biodetoxification) had been investigated to mitigate the negative effect of these inhibitors on enzymatic hydrolysis and fermentation [3]. Nevertheless, these detoxification methods have several drawbacks, e.g. significant solids/sugars loss, high energy input, and additional waste generation [4].

The biodetoxification method offers a superior alternative to conventional physical and chemical detoxification methods, as it features milder reaction conditions, lower energy input, greater efficiency and selectivity, and reduced toxic emissions [5]. Biodetoxification refers to the use of specific enzymes or microorganisms to degrade inhibitors

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in lignocellulose biomass [6]. Several microorganisms have been isolated and validated for biodetoxification, such as the fungus *Amorphotheca resinae* ZN1, which can degrade various phenolic aldehydes and weak acids inhibitors in solid lignocellulose biomass under adequate aerobic conditions [7]. The newly screened *Bordetella* sp. BTIITR was reported to degrade most of furfural, HMF and acetic acid in sugarcane bagasse hydrolysate [8]. The cellulase-producing strain *Trichoderma viride* R16 was found to degrade low concentration phenolic inhibitors in pretreated corncobs [9].

Although the use of various microorganisms for biodetoxification was widely reported, the current biodetoxification processes still suffer from some shortcomings due to the inherent metabolic characteristics of the biodetoxification strain, including: (i) additional nutrients requirement [10]; (ii) undesired byproduct generation [11]; (iii) simultaneous consumption of fermentable sugars [12]; (iv) low tolerance to high concentration of mixed inhibitors [13]; (v) incomplete biodegradation including residual of inhibitors, only conversion from phenolic aldehydes to corresponding hypotoxic alcohol compounds [14], as well as the degradation capacity of only a few inhibitors [6, 15]; high energy consumption of vigorous aeration due to the rigorous high oxygen demand of biodetoxification strain [7, 16].

Paecilomyces variotii has been shown to grow in various typical agro-industrial wastes with limited oxygen supply [17], and was also reported to biodegrade ricin and phenol [18, 19]. A recently isolated *P. variotii* FN89 strain preliminarily showed the potentials for the biodegradation of organic acid catalyst and several inhibitors from pretreated solid lignocellulose biomass [20]. In this study, a comprehensive evaluation of its potential to biodegrade high concentrations of lignocellulose-derived inhibitors was further conducted. Two different biorefinery chains were proposed and verified based on the cell growth profiling and metabolic properties of *P. variotii* FN89 in order to cope with different forms (slurry or solid particles) of pretreated biomass, and used for high-titer cellulosic lactic acid production from wheat straw. Global transcriptome analysis was performed in the presence of mixed inhibitors, with a view to mining the important information for in-depth understanding of the carbohydrate metabolism and inhibitors biodegradation pathway of *P. variotii* FN89.

Materials and methods

Microorganisms

The mesophilic *Paecilomyces variotii* FN89 (CGMCC No. 17665) was originally isolated from the pretreated corn stover, culture and stored on PDA plate [20]. The synthetic medium was used for biodetoxification evaluation in flasks and fermenter, which contained 5–40 g/L glucose, 0–15 g/L xylose, 1 g/L $(NH_4)_2SO_4$, 1 g/L yeast extract (YE), 2 g/L KH_2PO_4, 1 g/L $MgSO_4 \cdot 7H_2O$, 1 g/L $CaCl_2$, and mixed lignocellulose-derived inhibitors.

Pediococcus acidilactici ZY271 (CGMCC No. 13611), the lactic acid-producing strain was activated and cultured in simplified MRS medium. The cultivation procedure was according to the previous report [21]. The additional nutrients for lactic acid fermentation in lignocellulose hydrolysate contained 10 g/L peptone, 15 g/L yeast extract, 2 g/L $(NH_4)_2C_6H_6O_7$, and 0.25 g/L $MnSO_4 \cdot H_2O$.

Biomass, cellulase, and reagents

Wheat straw was harvested from Henan Province, China in fall 2022. The pre-handling of raw wheat straw for biorefining process was according to the previously described procedures [20]. Cellulase Cellic CTec 2.0 was obtained from Novozymes (China). The analytical-grade chemical reagents were sourced from Sinopharm Chemical Reagent (China).

Biodetoxification evaluation in flasks and fermenter

The spores of *P. variotii* FN89 on PDA plate were washed by 0.05% (w/w) Tween 80 solution, collected, and inoculated into 50 mL synthetic medium at the volume ratio of 1% (v/v) in 250 mL flask as the seed. The seed was cultured at 37 °C, 200 rpm for 24 h, then inoculated into 50 mL synthetic medium containing different single inhibitor (0–2 g/L furfural, HBA, syringaldehyde and vanillin; 0–8 g/L HMF and acetic acid) at 10% (v/v) volume ratio in 250 mL flask. The cultivation in flaks was conducted at 37 °C, 200 rpm for 48 h. The biodegradation of mixed inhibitors was also conducted in 3 L fermenter. The seed culture (100 mL) was inoculated at 10% (v/v) ratio into 1 L synthetic medium containing 5 g/L glucose, 15 g/L xylose, and mixed inhibitors (0.6 g/L furfural, 1 g/L HMF, 2 g/L acetic acid, 0.2 g/L hydroxybenzaldehyde (HBA), 0.2 g/L syringaldehyde, and 0.2 g/L vanillin). The biodetoxification in 3 L fermenter was conducted at 37 °C, 200 rpm, 0.5 or 1.0 vvm for 72 h.

Pretreatment, submerged liquid and solid-state biodetoxification, hydrolysis

Sulfuric acid was used as the catalyst for dilute acid pretreatment at a dosage of 4.0% (w/w) based on the dry weight of wheat straw [22]. Wheat straw and sulfuric acid solution were simultaneously loaded into a 20 L reactor with the solid/liquid ratio of 2:1 and total weight of 1800 g. The pretreatment was maintained at 175 °C for 5 min.

There are two technical processes for biodetoxification to degrade the inhibitors generated from the pretreatment in biomass. The biodetoxification didn't require the addition of any nutrients. The pretreated biomass in solid particle form can be directly biodetoxified (solid-state biodetoxification) by inoculating the spores of *P. variotii* FN89. *P. variotii* FN89 was firstly cultured on PDA plate for 4 days at 37 ◦C. The spores on PDA plate were washed by 0.05% (w/w) Tween 80 solution and then inoculated onto the pretreated wheat straw with the concentration of ~ 106 spores/g wheat straw, and statically maintained at 37 °C for 3 days as the biodetoxification seed. The seed was then inoculated onto the pretreated straw at 10% (w/w) mass ratio. The conditions for solid-state biodetoxification were set at 37 °C, 1.0 vvm for 84 h. The biodetoxified solid biomass were then hydrolyzed into slurry for lactic acid production. The pretreated biomass also can be firstly hydrolyzed into slurry and then used for submerged liquid biodetoxification by inoculating the liquid seed of *P. variotii* FN89 at 10% (v/w) inoculum ratio in 3 L fermenter. The enzymatic hydrolysis was conducted at 30% (w/w) solids loading in a 5 L bioreactor equipped with single helical stirring at 150 rpm, 50 °C for 12 h. The cellulase dosage was 4 mg of total proteins per gram of dry wheat straw matter for each case, and no further cellulase enzyme addition during each case of fermentation. The conditions for submerged liquid biodetoxification were set at 750 rpm, 37 °C, and 1.0 vvm. The biodetoxified hydrolysate was then sent for lactic acid production.

Cellulosic lactic acid fermentation

P. acidilactici ZY271 was cultured in 50 mL of MRS medium at 42 °C, 150 rpm for 12 h. Then transferred to 100 mL of fresh MRS medium at the inoculum ratio of 10% (v/v), and culture for another 12 h as the seed. The seed was inoculated into biodetoxified hydrolysate at the inoculum ratio of 10% (v/v) in 3 L fermenter. The fermentation was conducted at 42 °C, 200 rpm for 72 h. The fermentation pH was controlled at 5.5 by automatically adding 25% (w/v) calcium hydroxide slurry.

RNA extraction and transcriptomic sequencing

P. variotii FN89 was cultured in synthetic medium with and without adding mixed inhibitors at 37 °C, 200 rpm for 12 h. The mixed inhibitors included 0.6 g/L furfural, 1 g/L HMF, 2 g/L acetic acid, 0.2 g/L hydroxybenzaldehyde (HBA), 0.2 g/L syringaldehyde, and 0.2 g/L vanillin. Then the cells were collected for RNA extraction. The RNA extraction and RNA-seq library preparation were described in our previous study [23]. Total RNA was extracted by Trizol Reagen kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. RNA-Seq was performed by CapitalBio Technology Co., Ltd, Beijing, China, All the RNA samples were performed for RNA-seq library and direct-sequencing on Illumina platform. The random hexamer primers and reverse transcriptase were utilized for first-strand cDNA generation, and the second-strand cDNA was synthesized using DNA polymerase I. After that, RNA libraries of single and paired-end were constructed. The whole procedure followed Illumina's standard protocols.

Analytical methods

Glucose, xylose, lactic acid, acetic acid, HMF, and furfural were measured by HPLC method using Bio-Rad Aminex HPX-87 H column. HBA, syringaldehyde, and vanillin were measured using reverse-phase HPLC equipped with YMC-Pack ODS-A column [23]. For dry cell weight (DCW) determination, the fermentation broth was filtered, and the solid fraction was air-dried at 65 °C to constant weight. DNA/RNA concentrations were measured using Nano-300 analyzer (Allsheng Instrument Co., Ltd, Hangzhou, China).

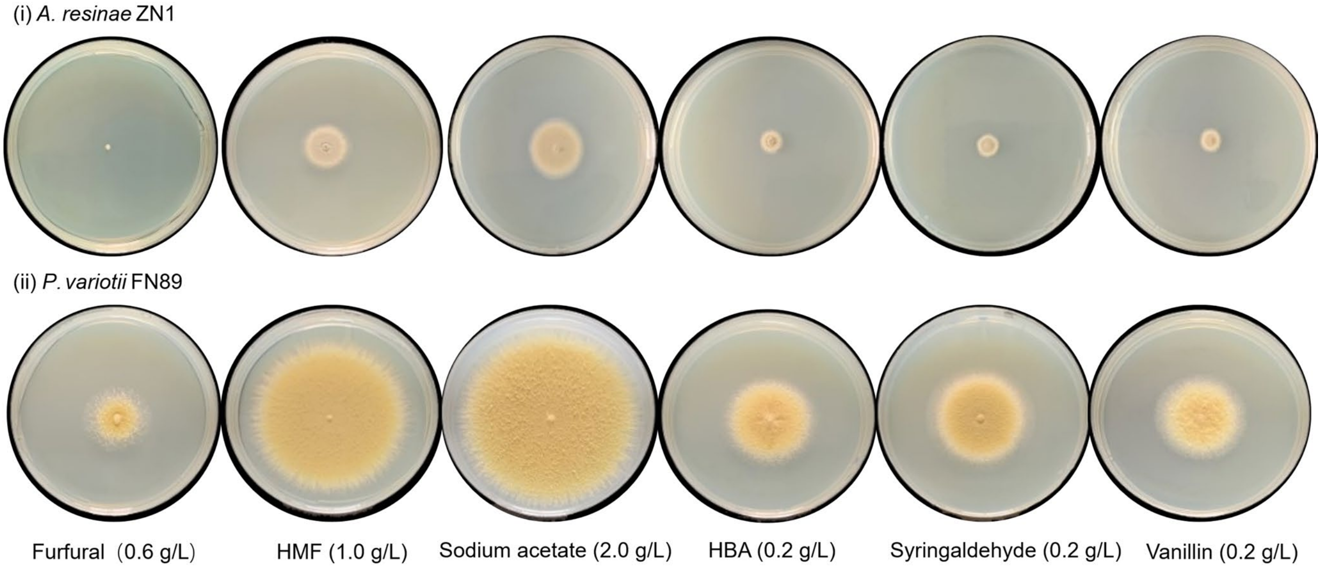
Results and discussions

Growth properties of *P. variotii* FN89

Six representative inhibitors derived from the pretreatment of lignocellulosic biomass, furfural, HMF, acetic acid, HBA, vanillin, and syringaldehyde were added to PDA plate, respectively. The strains of *P. variotii* FN89 and the previously discovered biodetoxification strain *A. resinae* ZN1 were cultured on these inhibitor-containing PDA plates and observed for colonial growth (Fig. 1a). The morphological size of the single colony starting from ~ 10^6 spores after 72 h of cultivation showed that *P. variotii* FN89 grew more vigorously compared to *A. resinae* ZN1 on the inhibitor-containing plates, and produced a large number of greyish yellow spores. The order of the inhibition by these lignocellulose-derived inhibitors on cell growth of *P. variotii* FN89 from strongest to weakest was furfural, HBA, vanillin, syringaldehyde, HMF and acetic acid.

The pretreated wheat straw was hydrolyzed at 10% solids loading (w/w), centrifuged to remove solid particles, and prepared to agar plates without addition of nutrients. The hydrolysate plate containing 30.2 g/L glucose, 15.5 g/L xylose, 0.2 g/L furfural, 0.3 g/L HMF, and 3.1 g/L acetic acid was inoculated with *P. variotii* FN89 and *A. resinae* ZN1 (Fig. 1b). The observation showed that the cell growth of *P. variotii* FN89 was still significantly better than that of *A. resinae* ZN1.

(a) The growth of *P. variotii* FN89 on PDA plate containing different inhibitors compared to *A. resinae* ZN1



(b) Hydrolysate plate (c) Growth temperature optimization of *P. variotii* FN89

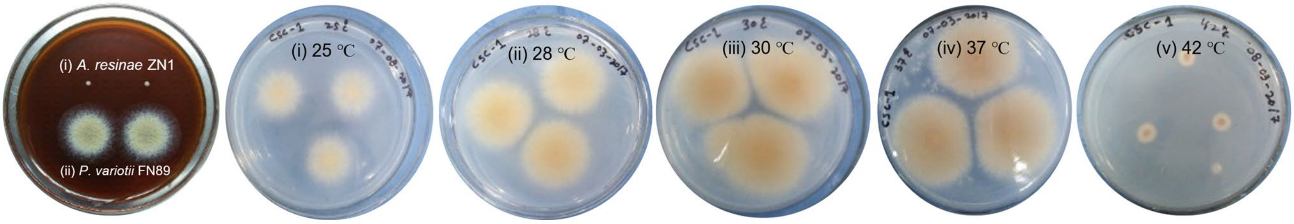


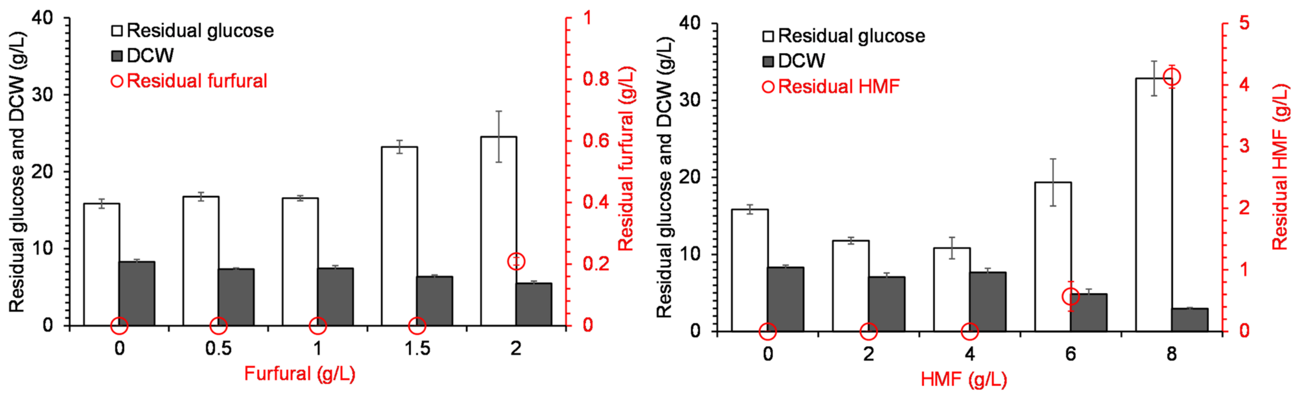
Fig. 1 The colonial morphology of strain *P. variotii* FN89. (**a**) The growth of *P. variotii* FN89 on PDA plate containing 0.6 g/L furfural, 1.0 g/L HMF, 2.0 g/L sodium acetate, 0.2 g/L HBA, 0.2 g/L syringaldehyde, or 0.2 g/L vanillin at 30 °C for 72 h. (i) The previously discovered biodetoxification strain *A. resinae* ZN1; (ii) *P. variotii* FN89. All the colony was started from ~ 10^6 spores. (**b**) The growth of *A. resinae* ZN1 and *P. variotii* FN89 on clarified wheat straw hydrolysate plate at 30 °C for 72 h. All the colony was started from ~ 10^6 spores. (i) *A. resinae* ZN1; (ii) *P. variotii* FN89. The wheat straw was pretreated, hydrolyzed at 10% solids loading (w/w), centrifuged, and prepared to agar plates. The clarified hydrolysate contained 30.2 g/L glucose, 15.5 g/L xylose, 0.2 g/L furfural, 0.3 g/L HMF, and 3.1 g/L acetic acid. (**c**) Growth temperature optimization of *P. variotii* FN89 on PDA plate for 48 h. (i) 25 °C; (ii) 28 °C; (iii) 30 °C; (iv) 37 °C; (v) 42 °C

The thermal tolerance of *P. variotii* FN89 was further investigated (Fig. 1c). The results showed that *P. variotii* FN89 was a mesophilic filamentous fungus that can tolerate temperature up to 42 °C, and its optimum growth temperature reached 37 °C. This growth property of *P. variotii* FN89 was consistent with previous report that *P. variotii* had the higher growth rate at 25 °C and 37 °C and formed powdery olive brown colonies [24]. The uneven heat transfer in large-scale bioreactor easily resulted in local higher temperature [25]. The favorable adaptability of *P. variotii* FN89 to high temperatures is advantageous for its application in practical production.

Potentials of *P. variotii* FN89 to degrade lignocellulose-derived inhibitors

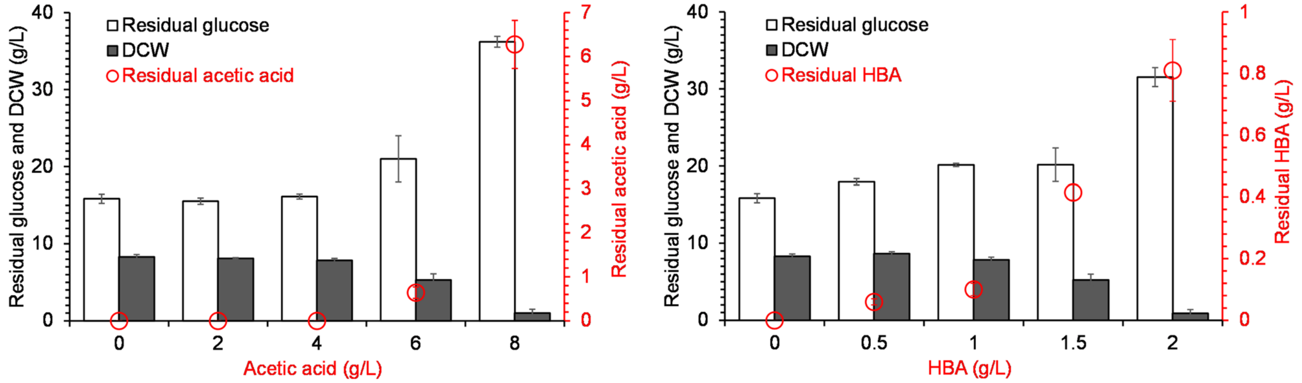
The ability of *P. variotii* FN89 to degrade lignocellulose-derived inhibitors was characterized in shake flasks. The inhibitors with different concentration gradients (0–2 g/L for furfural, HBA, syringaldehyde and vanillin; 0–8 g/L for HMF and acetic acid) were added to synthetic medium containing 40 g/L glucose. After inoculation of *P. variotii* FN89 and cultivation for 48 h, the residual sugar and inhibitors concentrations and dry cell mass (DCW) were determined (Fig. 2).

P. variotii FN89 exhibited better capacity to degrade acetic acid, HMF, furfural, and vanillin, with the inhibitor residuals occurring only when they were added at the concentration above 6 g/L, 6 g/L, 2 g/L, and 2 g/L (Fig. 2a, c and f). The addition of acetic acid decreased the initial pH of the synthetic medium, which exacerbated the negative effects. Except for syringaldehyde, both sugar metabolism and cell growth of *P. variotii* FN89 were inhibited with the increasing of concentrations of the added inhibitors, as indicated by the increase in residual glucose concentration and the decrease in DCW. *P. variotii* FN89 showed a relatively



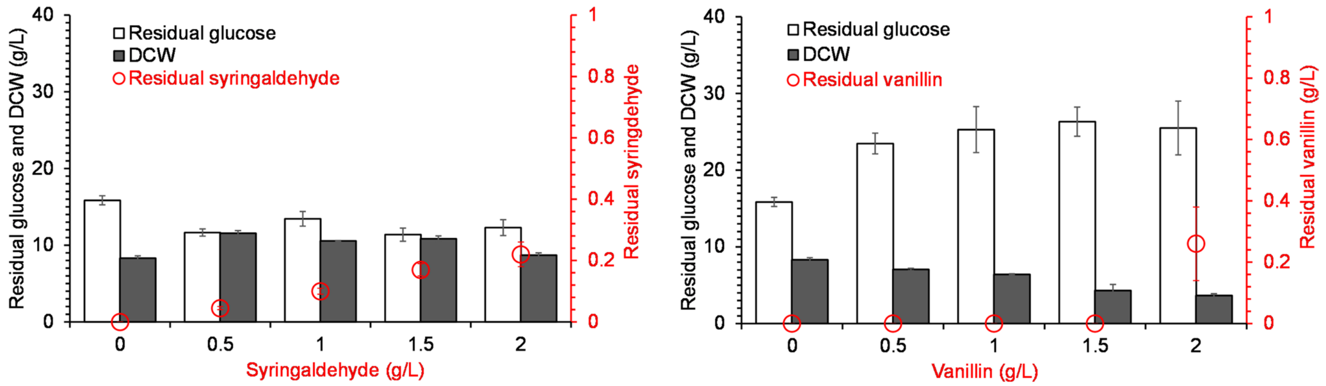
(a) Degradation of furfural and 40 g/L glucose

(b) Degradation of HMF and 40 g/L glucose



(c) Degradation of acetic acid and 40 g/L glucose

(d) Degradation of HBA and 40 g/L glucose



(e) Degradation of syringaldehyde and 40 g/L glucose

(f) Degradation of vanillin and 40 g/L glucose

Fig. 2 The degradation of different concentrations of inhibitors by *P. variotii* FN89 in shake flasks. (**a**) Furfural; (**b**) HMF; (**c**) Acetic acid; (**d**) HBA; (**e**) Syringaldehyde; (**f**) Vanillin. The inhibitors were added to sterilized synthetic medium separately. The addition of acetic acid led to the decrease in pH value. Conditions: inoculum 10% (v/v), 37 °C, 200 rpm, 48 h, natural pH (~4.6)

low capacity to degrade HBA, and only 59.5% of HBA was degraded after the addition of 2 g/L of HBA (Fig. 2d). The addition of 2 g/L of HBA reduced glucose consumption by 64.9% and DCW by 89.2%. In contrast, the sugar metabolism and cell growth of *P. variotii* FN89 were not significantly affected by the addition of 2 g/L of syringaldehyde (Fig. 2e).

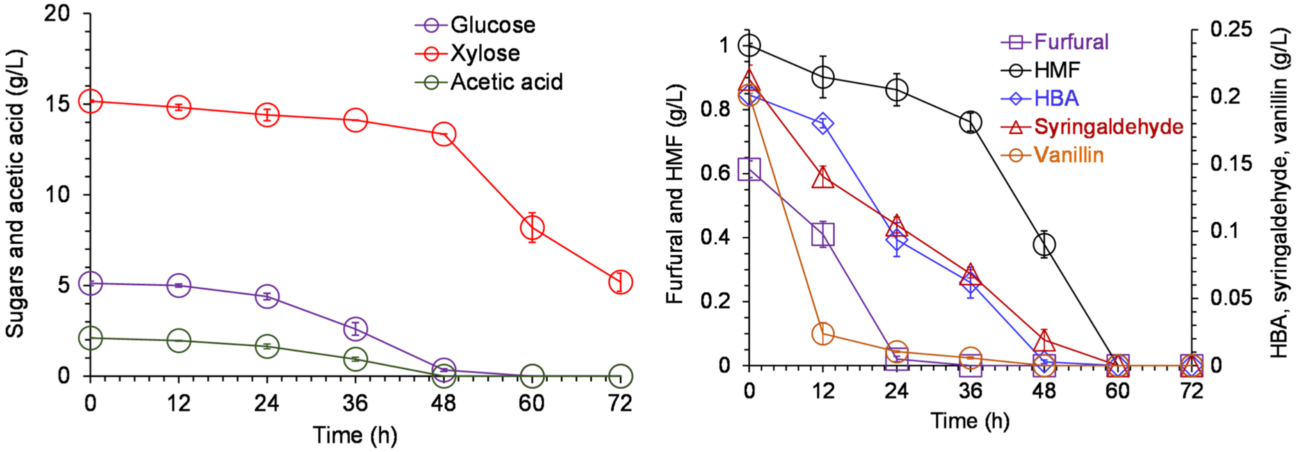
The above results indicated that *P. variotii* FN89 had a good ability to degraded major microbial growth inhibitors derived from lignocellulose. More importantly, *P. variotii* FN89 showed better ability to degrade furfural and HMF, which are the most toxic compounds to.

microorganisms among these inhibitors.

The biodetoxification capacity of *P. variotii* FN89 to degraded mixed inhibitors was further investigated in synthetic medium in 3 L fermenter (Fig. 3). Considering that the aeration rate is the key factor for limiting the biodetoxification rate whether in liquid or solid substrate [7, 16], the biodetoxification profiles of *P. variotii* FN89 were examined at airflow rates of 1.0 vvm and 0.5 vvm, respectively.

At the aeration rate of 1.0 vvm, *P. variotii* FN89 completely eliminated the six inhibitors within 48 h (Fig. 3a). Although the lower aeration rate reduced the biodetoxification rate, most of inhibitors were still removed within 72 h (except HBA) (Fig. 3b). An interesting phenomenon was observed that the consumption of fermentable sugars always lagged behind the degradation of inhibitors by *P. variotii* FN89. This phenomenon was even more pronounced at lower aeration rate (0.5 vvm). When 81.5% of furfural, 79.9% of HMF, 100% of acetic acid, 73.7% of HBA, 89.2% of syringaldehyde, and 97.8% of vanillin were degraded at 72 h, only 30.2% of glucose and 11.4% of xylose were consumed at 0.5 vvm(Fig. 3b). The possible reason was that the presence of these inhibitors suppressed the sugar metabolism of *P. variotii* FN89, which will be further verified and discussed in the subsequent section according to global transcriptome analysis. In previous reports, *Acinetobacter baylyi* was knocked out the gene for glucose dehydrogenase (*gcd*). The engineered strain showed to be incapable of consuming glucose, xylose, and arabinose, but rapid utilization of acetate, formate and HBA [15, 26]. This unique property of *P. variotii* FN89 can naturally avoid the sugars loss during the biodetoxification process without complex metabolic engineering operation.

(a) Sugars consumption and mixed inhibitors degradation in synthetic medium at 1.0 vvm



(b) Sugars consumption and mixed inhibitors degradation in synthetic medium at 0.5 vvm

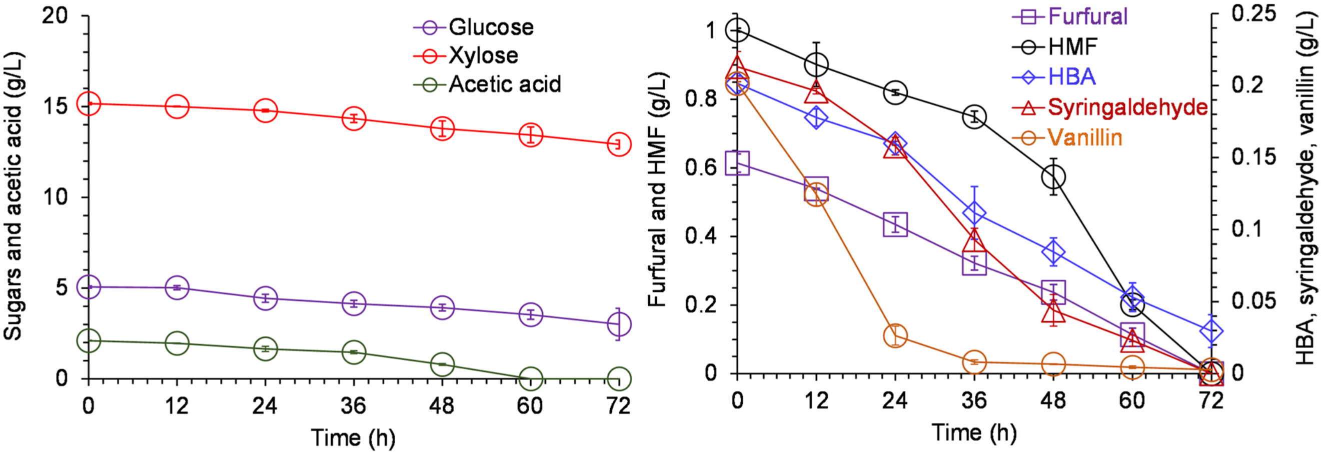


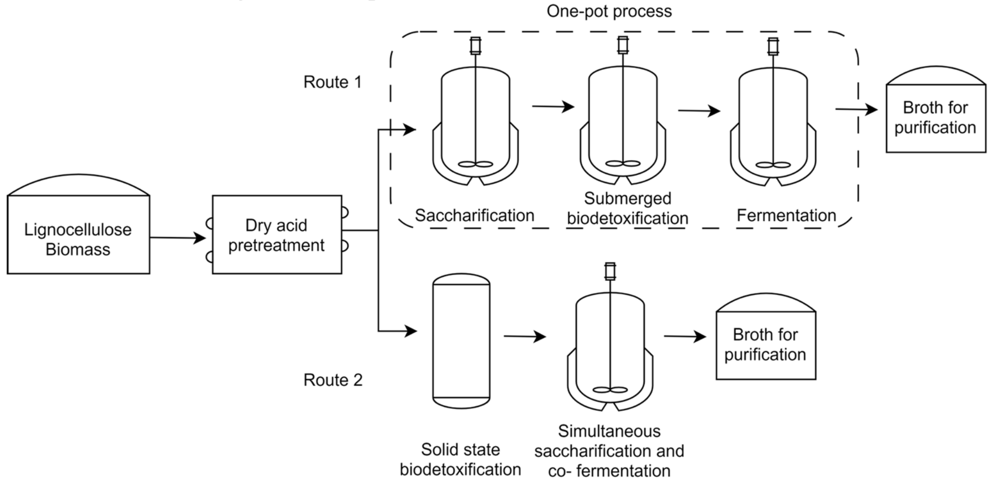
Fig. 3 The consumption of sugars and degradation of inhibitors by *P. variotii* FN89 in 3 L fermentor at different aerations (1 vvm or 0.5 vvm). Conditions: inoculum 10% (v/v), 200 rpm, 37 °C, 72 h, natural pH. All the inhibitors were added io medium after sterilization. The SM medium after adding inhibitors contained 15 g/L glucose, 5 g/L xylose, 0.6 g/L furfural, 1 g/L HMF, 2 g/L acetic acid, 0.2 g/L HBA, 0.2 g/L syringaldehyde, 0.2 g/L vanillin, and other nutrients

Biorefining route design with the core of biodetoxification by *P. variotii* FN89

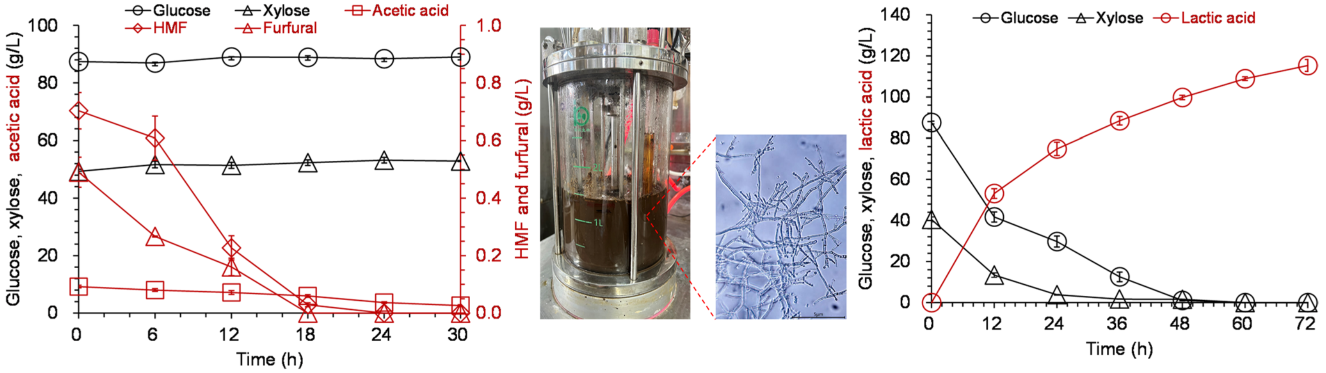
P. variotii FN89 showed excellent detoxification property on lignocellulose-derived inhibitors, two feasible overall biorefining routes were therefore designed based on the different forms of biomass (slurry or solid particles) and biodetoxification methods (submerged liquid and solid state) (Fig. 4a).

For route 1, the pretreated biomass was firstly enzymatically hydrolyzed to slurry at 25% (w/w) solids loading, followed by submerged liquid biodetoxification and fermentation in a one-pot process. All of furfural and HMF, and 70% of acetic acid were removed from the hydrolysate

(a) The possible routes for biorefinery chain using *P. variotii* FN89 as biodetoxification strain



(b) Submerged biodetoxification and lactic acid bioconversion



(c) Solid state biodetoxification and lactic acid bioconversion

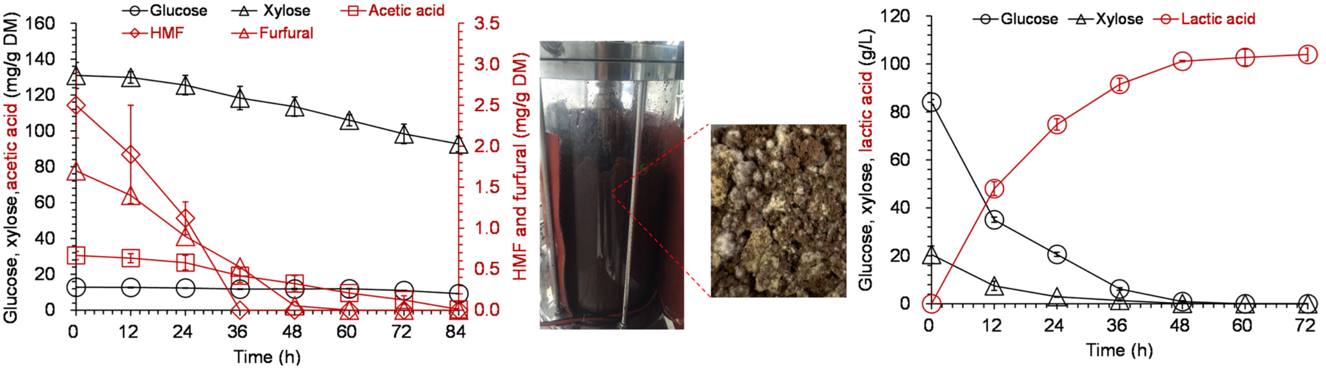


Fig. 4 Biorefining process for cellulosic chiral lactic acid production involving biodetoxification by *P. variotii* FN89. (**a**) Two possible routes for biorefinery chain using *P. variotii* FN89 as biodetoxification strain. (**b**) Submerged liquid biodetoxification and lactic acid bioconversion. Submerged liquid biodetoxification conditions: 25% (w/w) solids loading, 750 rpm, 37 °C, 1.0 vvm, natural pH. (**c**) Solid state biodetoxification and lactic acid bioconversion. Soli state biodetoxification conditions: 37 °C, natural pH, 1.0 vvm. Lactic acid fermentation conditions: 25% (w/w) solids loading, 42 °C, 200 rpm, pH 5.5

by *P. variotii* FN89 within 30 h, indicating that the biodetoxification strain *P. variotii* FN89 successfully overcame the obstacle of oxygen transfer limitation in the highly viscous hydrolysate. The residual acetic acid (2.6 ± 0.2 g/L) can be used as nutrient for lactic acid production by *P. acidilactic* ZY271. The concentrations of glucose and xylose showed a slight increasing trend during the submerged liquid biodetoxification, owing to the continuous hydrolysis of the biomass. The final cellulosic lactic acid titer reached 115.3 ± 2.9 g/L (Fig. 4b).

For route 2, the pretreated wheat straw was directly biodetoxified in the form of solid-state fermentation. The longer period was required for solid-state biodetoxification, which was 84 h. The prolonged solid-state biodetoxification inevitably resulted in 27.7% loss of free glucose and 29.2% loss of free xylose. The pretreated and biodetoxified wheat straw was then enzymatically hydrolyzed, and used for cellulosic lactic acid production by SSCF. The final cellulosic lactic acid titer reached 103.9 ± 2.7 g/L (Fig. 4c).

It is worth noting that although the solid-state biodetoxification is relatively simple and does not require vigorous agitation compared to the submerged liquid biodetoxification, it is not applicable to the large-scale lignocellulosic biorefinery process. The main shortcomings of current solid-state biodetoxification include (i) the time- and energy-consuming transport, loading, and emptying of the solid biomass [27]; (ii) difficulties of controlling the temperature and oxygen transfer in solid biomass, which would lead to the local over-temperature or oxygen shortage [28, 29]; (iii) the need to set up a huge bioreactor for the solid biomass with low bulk density (~380 kg/m^3) [30], resulting in significant investment in equipment costs; (iv) difficulty of determining the endpoint of solid-state biodetoxification resulting in the loss of a large amount of free fermentable sugars [31]. Although the lignocellulosic biorefinery involving either solid-state or submerged liquid biodetoxification enables the efficient production of biochemicals, the appropriate choice has to be made according to the actual production requirements.

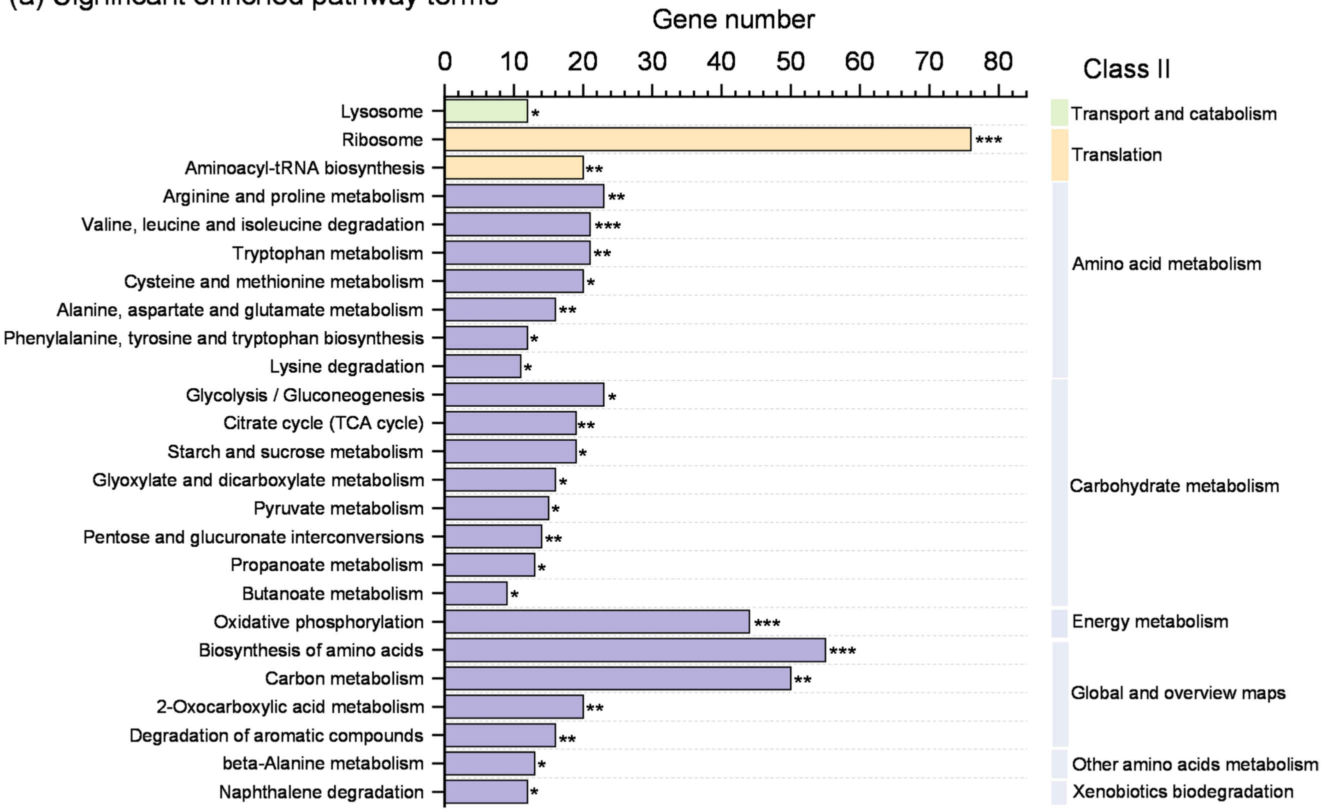
Global transcriptional profiling of *P. variotii* FN89 to degrade mixed inhibitors

A global transcriptional analysis of *P. variotii* FN89 was conducted with a view to elucidating its behavior in relation to inhibitors degradation and sugar metabolism. The transcriptome was sequenced on Illumina platform, resulting in over 278 million raw short reads from three parallel samples for each culture condition (with or without mixed inhibitors). After filtering out low-quality reads and those mapped to noncoding RNA, more than 97% of the sequences were deemed suitable for identification. Each sequence was annotated and analyzed for transcriptional profiling, with all samples showing a mapped ratio exceeding 86%. The reproducibility among biological replicates indicated high quality of the cDNA reads, with the correlation coefficients ranging from 0.91 to 1.00.

The genome of *P. variotii* CBS144490 HYG1 was selected as the reference genome, which is archived on the JGI website (https://mycocosm.jgi.doe.gov/mycocosm/home) [32]. A sequencing saturation analysis was conducted to assess the transcriptome coverage. RNA-seq alignment, along with de novo gene prediction, revealed that the sequence reads corresponded to all 9,230 coding genes, suggesting that the RNA sequencing for each unique gene sufficiently covers nearly all the identified transcripts in *P. variotii* cells.

In the presence of mixed inhibitors, genes with statistically significant up-regulation ($^*P < 0.05$, (log2 fold change > 2.0) in expression level were 115 in total, and genes with statistically significant down-regulation ($^*P < 0.05$, log2 fold change < -2.0) in expression level were 643 in total. The significantly differentially expressed genes were used for KEGG analysis and GO analysis (Fig. 5). KEGG analysis showed that top 25 enriched metabolic pathway mainly associated with cell metabolism (purple column), among which glycolysis/gluconeogenesis, citrate cycle (TCA cycle), starch and sucrose metabolism were significantly changed, suggesting that the addition of inhibitors influenced the sugar metabolic process of *P. variotii* FN89 (Fig. 5a). Expression changes in the pathway of degradation of aromatic compounds facilitated the degradation of lignin-derived aromatic inhibitors. Changes in the expression of oxidative phosphorylation indicated that *P. variotii* FN89 adjusted the intracellular energy supply in response to the presence of inhibitors. In addition, processes related to ribosome and amino acids metabolism also changed significantly, indicating that the presence of mixed inhibitors affected the intracellular protein synthesis (orange column). For *Saccharomyces cerevisiae*, the genes involved in ribosome biogenesis amino acid and derivative metabolic process were repressed after HMF treatment [33]. Bajwa et al. [34] also discovered that the expression levels of the genes related to ribosome biogenesis, proteins synthesis and transport, as well as carbohydrates metabolism in *S. cerevisiae* were decreased in response to lignocellulosic hydrolysate. A prokaryotic regulator IrrE from *Deinococcus radiodurans* was heterologously expressed in *S. cerevisiae* and the inhibitors tolerance of the engineered strain was improved [35]. Some sets of the genes related to carbohydrates metabolism, membrane proteins, transport proteins, and ribosome were up-regulated in engineered strain. Collectively, enhancing energy supply (carbohydrate metabolism) and protein

(a) Significant enriched pathway terms



(b) Gene ontology analysis

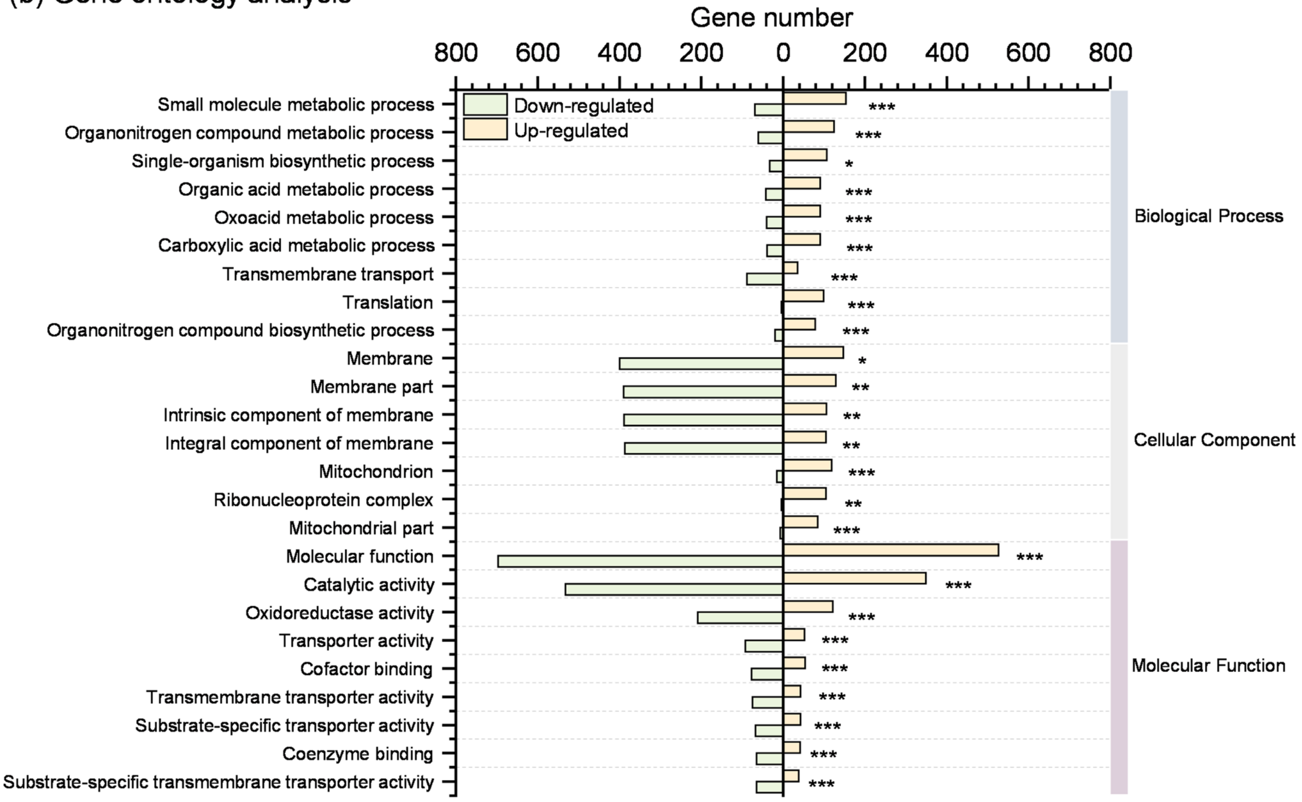


Fig. 5 Global transcriptional profiling of *P. variotii* FN89 in the synthetic medium containing mixed phenolic aldehyde inhibitors. (**a**) Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis; (**b**) Gene ontology (GO) analysis. *$P<0.05$, **$P<0.01$, ***$P<0.001$

synthesis (ribosome and amino acids biogenesis) seem to be necessary for strain to resist inhibitors damage.

GO analysis showed that the differentially expressed genes enriched in molecular function, catalytic activity, oxidoreductase activity, as well as the membrane component metabolism (Fig. 5b). Gu et al. [36] reported that *Saccharomyces cerevisiae* could spontaneously reduce cell volume and enhance cell wall thickness through the structural rearrangement of cell wall components, while also improve membrane integrity via the proliferation of the cytoplasmic membrane in response to phenolic acid stress. The changes in membrane component metabolism would contribute to the tolerance of *P. variotii* FN89 to mixed inhibitors.

For phenolic aldehyde inhibitors degradation in the previous reports, phenolic aldehyde inhibitors were reduced to the corresponding less toxic alcohols by alcohol dehydrogenase (ADH) or aldo-keto reductase (AKR/ARI) in irreversible reaction, and then low concentration phenolic aldehyde inhibitors were oxidized to the corresponding acids catalyzed by aldehyde dehydrogenase (ALDH) (Fig. 6a). These acids were converted to intermediates and finally entered TCA cycle for complete degradation [23].

A series of genes encoding ADH, AKR/ARI, and ALDH were identified in *P. variotii* FN89, and the changes in the expression level of these genes in the presence of inhibitors were showed in Fig. 6b and d. The expression levels of the genes Gene_4947, Gene_7217, and Gene_4418 encoding AKR/ARI (Fig. 6b), MSTRG. 5521 and Gene_2998 encoding ADH (Fig. 6c) were up-regulated by 10.3-114.2 folds, which may have a key role in the conversion of phenolic aldehyde inhibitors to less toxic alcohols. The changes in expression levels of ALDH were mild, with only the Gene_1910 up-regulated 4.2 folds (Fig. 6d). The results of the transcriptional analysis reconfirmed that *P. variotii* FN89 preferentially converts the aldehydes inhibitors into their corresponding alcohols initially, and subsequently converts these alcohols into acids for final degradation. The possible metabolic pathways for the ultimate degradation of inhibitors-derived intermediates into CO_2 and H_2O in *P. variotii* FN89 can refer to the known fungal biodetoxification mechanisms of *A. resinae* ZN1 [23], though its specific enzymatic profile remains to be characterized.

The changes in expression levels of the genes associated with central carbon metabolic pathway including glycolysis pathway, pentose phosphate pathway and TCA cycle in the presence of mixed inhibitors were presented in Fig. 7. The results suggested that most of the genes involved in glycolysis pathway and pentose phosphate pathway exhibited down-regulated expression (Fig. 7a and b), whereas the genes involved in TCA cycle showed up-regulation (Fig. 7c). The genes MSTRG. 3994 encoding isocitrate dehydrogenase ($NADP^+$ dependent) and Gene_5479 encoding succinyl-CoA synthetase in TCA cycle were up-regulated by 5.1 folds and 4.8 folds. The inhibition of glycolysis pathway and pentose phosphate pathway in the presence of inhibitors resulted in the decrease in the uptake and utilization of exogenous carbon source of *P. variotii* FN89. The activation of TCA cycle, however, ensured the complete degradation of the inhibitors-derived intermediates and efficient energy supply.

In summary, this study systematically investigated the biodetoxification capacity of *P. variotii* FN89 in solid and liquid biomass scenarios, demonstrating its robustness under mixed lignocellulosic biomass and low oxygen conditions. Additionally, this study conducted the first preliminary investigation into the mechanisms of degradation inhibitors through transcriptomic analysis in *P. variotii*, identifying a series of genes with significantly altered expression levels involved in inhibitor degradation. We emphasize that this study represents a preliminary investigation of *P. variotii* FN89's inhibitors degradation capabilities. While we provide plausible explanations based on prior fungal studies, the exact molecular mechanisms (e.g., key enzymes, gene regulation) will be the focus of our future work.

P. variotii is a common cosmopolitan mesophilic fungus, and have been isolated for the production of different enzymes and byproducts [17]. *P. variotii h*ave been rarely reported for biodetoxification of lignocellulosic biomass. We accidentally discovered contaminated colonies on acid pretreated biomass, which was isolated and identified as *P. variotii* [20]. The lignocellulosic biomass detoxified by *P. variotii* FN89 has been successfully applied for the production of cellulosic ethanol [20], chiral lactic acid [37], single cell proteins and microbial lipid [38]. Two parallel studies published in conjunction with this work include the use of adaptive evolution method to enhance its inhibitors tolerance, achieving the biodegradation of furfural residue containing high concentrations of acetic acid and HMF [39]; and multi-parameter monitoring of the submerged liquid biodetoxification process, with the pH peak identified as a visual biodetoxification endpoint to avoid complex detection and loss of fermentable sugars [31]. However, this biodetoxification method still faces some issues that need to be solved in large-scale application to improve the feasibility, including: (1) the fast switching between the biodetoxification process and fermentation process; (2) improving the thermostability to match the saccharification process; (3) the treatment of spore-containing exhaust gas; and (4) in-depth analysis of sugar metabolism, inhibitor metabolism and comprehensive analysis of metabolites.

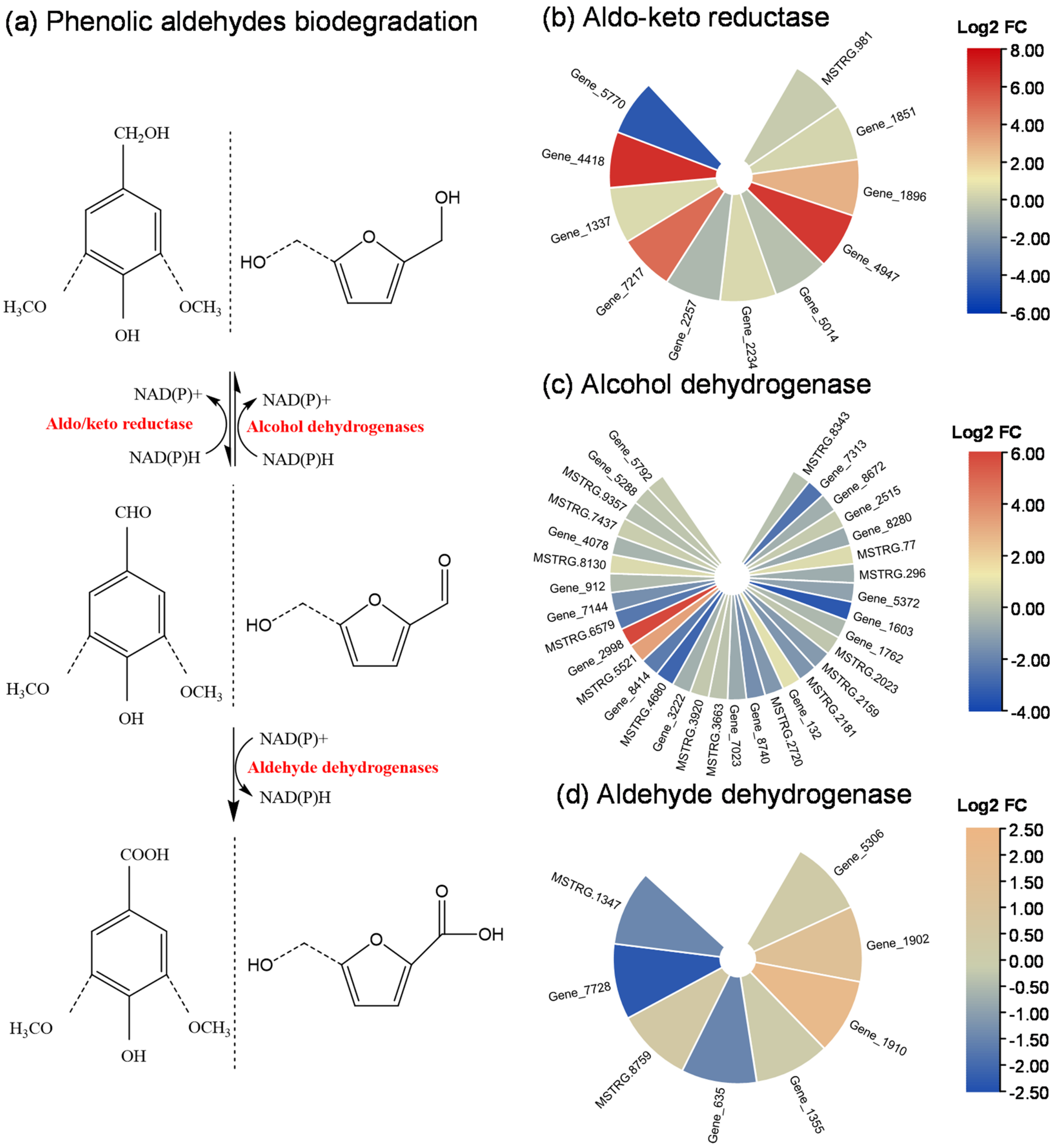
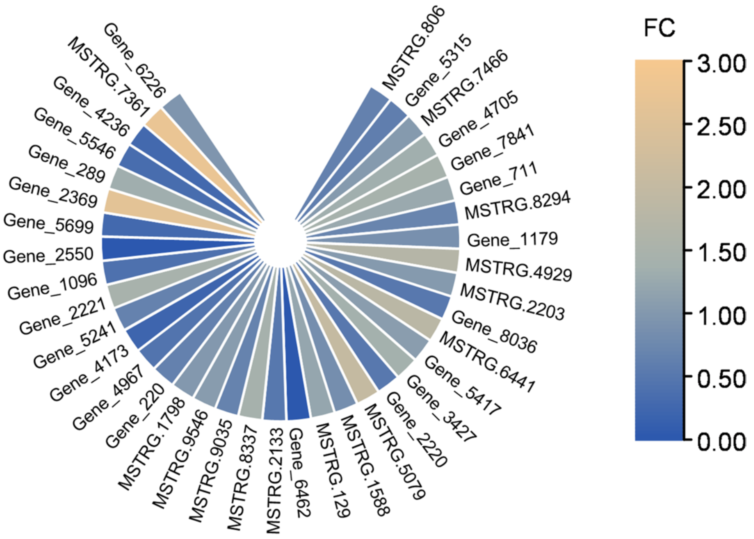
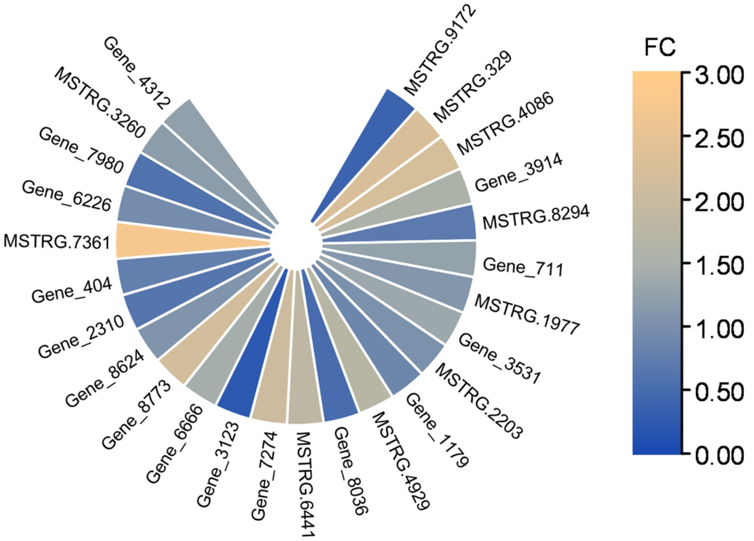
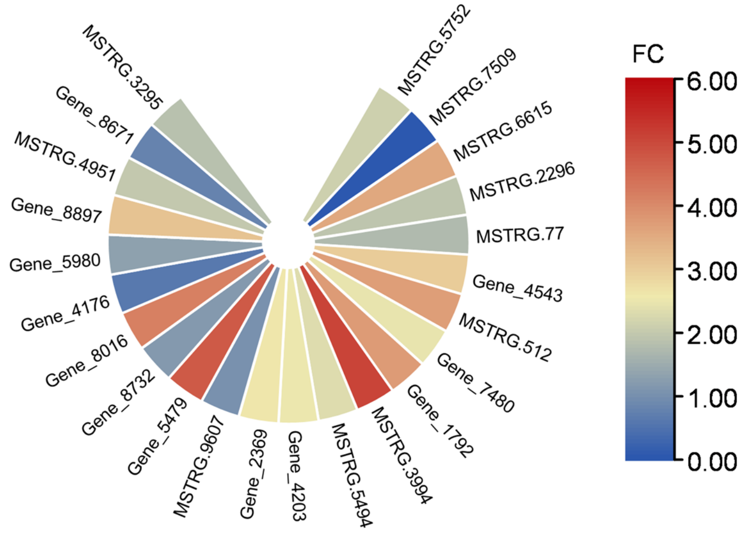


Fig. 6 Phenolic aldehydes biodegradation profile by *P. variotii* FN89. (**a**) Pathway; (**b**) the changes in gene expression levels related to aldo-keto reductase; (**c**) the changes in gene expression levels related to alcohol dehydrogenase; (**d**) the changes in gene expression levels related to aldehyde dehydrogenase. The Fig. 6b and d were drawn using the software Tbtools (version 2.008). FC, fold change. Log2 FC < 0 means the expression level was down-regulated

Fig. 7 The changes in gene expression related to sugars metabolism of *P. variotii* FN89 in the synthetic medium containing mixed phenolic aldehyde inhibitors. (**a**) Glycolysis pathway; (**b**) Pentose phosphate pathway (PPP); (**c**) Tricarboxylic acid cycle (TCA cycle). The Fig. 7a and c were drawn using the software Tbtools (version 2.008). FC, fold change. FC < 1 means the expression level was down-regulated


(a) Glycolysis pathway


(b) Pentose phosphate pathway


(c) TCA cycle

Conclusions

The isolated strain *P. variotii* FN89 showed strong ability to degrade high concentration lignocellulose-derived inhibitors. *P. variotii* FN89 effectively adapted to mixed inhibitors and relatively low dissolved oxygen conditions. Two biorefinery chains were established to cope with different forms of pretreated biomass for cellulosic lactic acid production owing to that *P. variotii* FN89 can efficiently detoxify both the highly viscosity hydrolysate and solid biomass. The biodegradation of inhibitors by *P. variotii* FN89 was prior to the consumption of fermentable sugars. The cellulosic lactic acid titers were above 100 g/L from 25% (w/w) solids loading pretreated wheat straw. The global transcriptome analysis further enriched the knowledge of the molecular basis of lignocellulose-derived inhibitors tolerance and carbohydrates metabolism of *P. variotii*.

Author contributions *BZ*: methodology, validation, investigation, visualization, funding acquisition, writing – original draft. *SW*: resources, investigation. *AC*: resources, investigation. *XY*: software, data curation, visualization. *JB*: supervision, project administration, funding acquisition, writing – reviewing & editing.

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Declarations

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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