Bioprocess and Biosystems Engineering (2021) 44:1875–1882
https://doi.org/10.1007/s00449-021-02567-x

RESEARCH PAPER

Transporter proteins in *Zymomonas mobilis* contribute to the tolerance of lignocellulose-derived phenolic aldehyde inhibitors

Xia Yi[1] · Ling Lin[1] · Jun Mei[1] · Wei Wang[2]

Received: 12 February 2021 / Accepted: 31 March 2021 / Published online: 10 April 2021

Abstract

Transporter proteins are of great importance for improving the tolerance of fermentation strains to lignocellulose-derived furans and phenolic inhibitors. Different from the documented transporter proteins responsible for the tolerance of furfural and 5-hydroxymethyl-furfural (HMF), transporters responsible for that of varied phenolic aldehyde inhibitors were less investigated and elucidated. Here, an interesting phenomenon was found that no phenolic alcohols were accumulated from phenolic aldehydes degradation in *Zymomonas mobilis*. A transcriptional profiling of transporter genes was established in *Z. mobilis* ZM4 under phenolic aldehydes stress using DNA microarray. Six transporter proteins were identified as the potential candidates responsible for the tolerance of phenolic aldehydes including ABC transporter (*ZMO0799* and *ZMO0800*), MFS transporter (*ZMO1288* and *ZMO1856*), and RND transporter (*ZMO0282* and *ZMO0798*). Furthermore, the analysis showed that the key transporters were significantly correlated with oxidoreductases and transcriptional regulators. This work would provide several important transporter genes serving as synthetic biology tools for improving the robustness of biorefinery strains.

Keywords *Zymomonas mobilis* ZM4 · Phenolic aldehyde inhibitors · Transporter proteins · Biorefinery

Introduction

Phenolic compounds derived from partial breakdown of lignin components, a heterogeneous aromatic polymer, inhibit fermenting strains and cellulase activities during fermentation by disrupting cell membrane and enzyme hydrophobic sites [1]. 4-Hydroxybenzaldehyde, syringaldehyde, and vanillin are the typical model phenolic compounds separately with *p*-hydroxyphenyl group (H), syringyl group (S), and guaiacyl group (G) according to methoxyl and functional groups [2]. The growing evidence had proved that phenolic aldehydes are the most toxic inhibitors in biofuel fermentation [3–5]. Tolerance and detoxification of phenolic inhibitors are the intractable barriers because of their poor water solubility and large numbers of derivatives. Expression of transporter genes is one of the practical methods to overcome the damage of toxic phenolic inhibitors [3, 6].

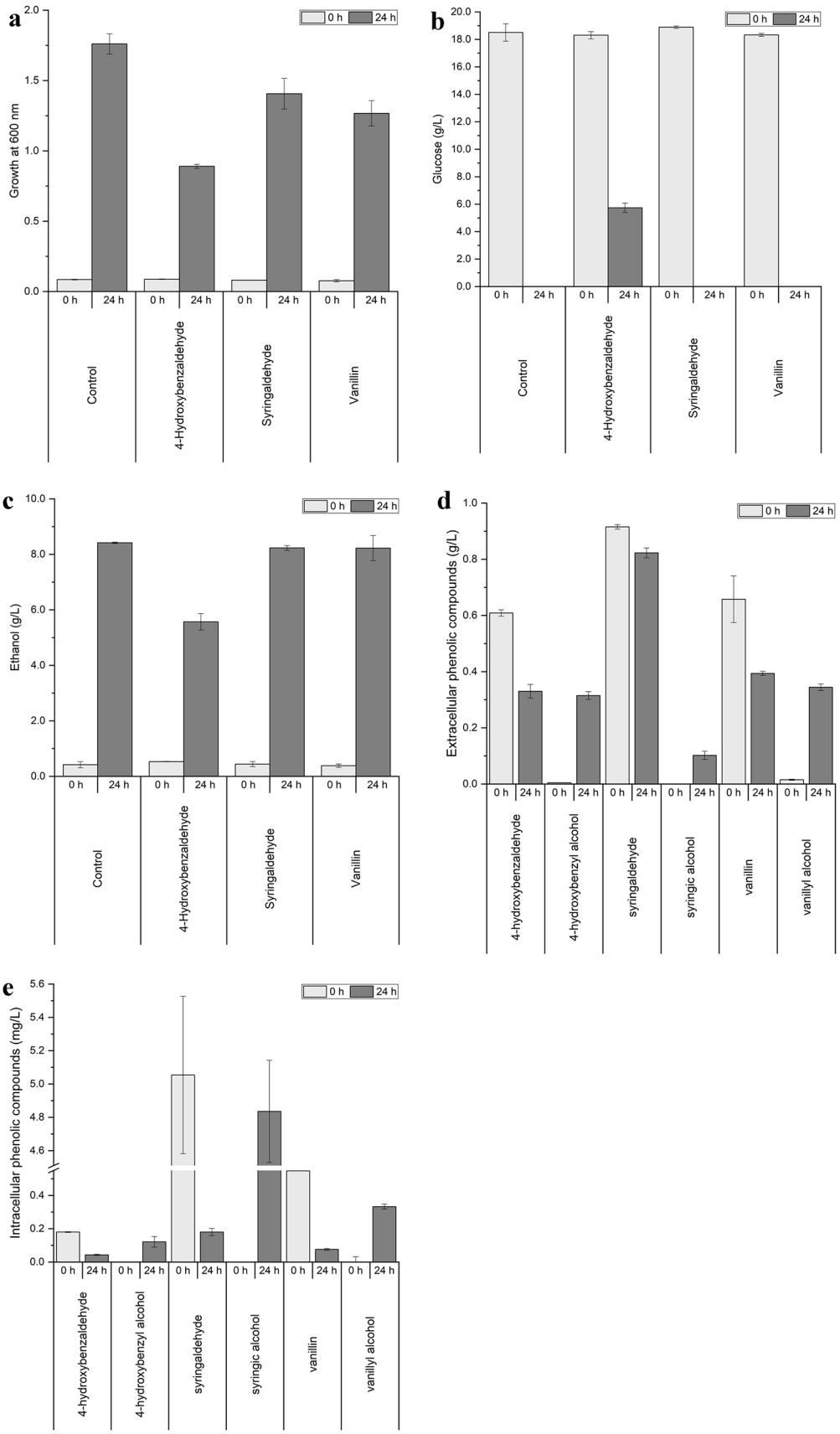
Efflux of toxic substances is an important way to enhance inhibitor tolerance of microbial organisms [7]. Bacterial multidrug resistance (MDR) efflux pump proteins include five active transporters, adenosine triphosphate (ATP)-binding cassette (ABC), major facilitator superfamily (MFS), multidrug and toxin extrusion (MATE) families, resistance-nodulation cell division (RND), and small multidrug resistance (SMR) [8]. Modifying microbial efflux systems is one way to reduce cytotoxicity of lignocellulose-derived inhibitors like furfural [9–11] and vanillin [6].

Ethanologenic bacterium *Zymomonas mobilis* ZM4 behaves high tolerance to the phenolic acids and is able to thrive in the presence of high concentrations of phenolic aldehydes and produce the corresponding phenolic alcohols [3, 12, 13]. Carbohydrate transporters in *Z. mobilis* have been applied for ethanol production and sugar transportation [14–17]. In the previous study, it confirmed that the overexpression of *ZMO1288* encoding a MFS transporter protein contributed to the increase of cell growth, glucose consumption, ethanol productivity, and phenolic aldehyde conversion in *Z. mobilis* ZM4 [3].

✉ Xia Yi
yixia@mail.ecust.edu.cn

[1] Jiangxi Provincial Key Laboratory of Systems Biomedicine, Jiujiang University, 17 Lufeng Road, Jiujiang 332000, China

[2] State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China


a
Growth at 600 nm
0 h
24 h
Control
4-Hydroxybenzaldehyde
Syringaldehyde
Vanillin
b
Glucose (g/L)
c
Ethanol (g/L)
d
Extracellular phenolic compounds (g/L)
4-hydroxybenzaldehyde
4-hydroxybenzyl alcohol
syringaldehyde
syringic alcohol
vanillin
vanillyl alcohol
e
Intracellular phenolic compounds (mg/L)

◂**Fig. 1** The extracellular and intracellular phenolic compounds for ethanol fermentation under the stress of phenolic aldehydes in *Z. mobilis* ZM4. **a** Cell growth measured at 600 nm; **b** glucose consumption; **c** ethanol production; **d** extracellular phenolic compounds; **e** intracellular phenolic compounds. Error bars indicate the standard deviations (SD) of three replicates

In this study, it showed no phenolic aldehydes and phenolic alcohols accumulated in the intracellular of *Z. mobilis*. Just as good as the bioinformatic data available for the genome of *Z. mobilis* ZM4, the reliable and more cost effective high-throughput sequencing technology DNA microarray was used to achieve the gene transcriptional profiling of the stress response to phenolic aldehydes. The comprehensive gene transcriptional landscapes of transporter proteins responsible for the tolerant mechanism of phenolic aldehydes were established in *Z. mobilis* ZM4 using DNA microarray. ABC transporter (*ZMO0799* and *ZMO0800*), MFS transporter (*ZMO1288* and *ZMO1856*), and RND transporter (*ZMO0282* and *ZMO0798*) were identified as the potential candidates for improving the tolerance of phenolic aldehydes in *Z. mobilis* ZM4. Meanwhile, the significant correlation was also constructed between the key transporter proteins with oxidoreductases and transcriptional regulators. This study would provide the important transporter genes serving as the synthetic tools for the robustness strengthening of biorefinery strains.

Materials and methods

Strain and medium

Z. mobilis ZM4 (ATCC 31821) was from ATCC (American Type Culture Collection, Manassas, VA, USA). Yeast extract was purchased from Oxoid, Hampshire, UK. Phenolic aldehydes, including 4-hydroxybenzaldehyde, syringaldehyde, vanillin, and the corresponding phenolic alcohols were from Sangon Biotech Co. Ltd., Shanghai, China. All the other analytical grade chemicals were from Sinopharm Chemical Reagents (Shanghai, China).

Inoculum preparation and cell disrupt

Z. mobilis ZM4 was cultured in Rich medium (RM) containing 20.0 g/L glucose, 10.0 g/L yeast extract, and 2.0 g/L KH_2PO_4 at 30 °C without shaking. The degradation assays of phenolic aldehydes were carried out with the middle inhibitory concentration of 5.0 mM 4-hydroxybenzaldehyde, syringaldehyde, and vanillin separately added in RM. To determine the transport of phenolic compounds, inocula of *Z. mobilis* ZM4 were grown in 100 mL RM medium in a 250 mL flask and harvested at 0 and 24 h of fermentation. Cell growth was determined at 600 nm.

The supernatants of *Z. mobilis* ZM4 culture were harvested after being centrifuged at 12,000 rpm for 1 min. The collected cells were lyophilized and stored at − 80 °C after being disrupted with three cycles of freezing and thawing. Prior to analysis, the freeze-dried samples were re-suspended and spin-filtered with 0.22 μm filter.

Data analysis of DNA microarray

DNA microarray data under the stress of phenolic aldehyde inhibitors in *Z. mobilis* ZM4 was performed in the previous study [3]. In this study, the transporter genes were analyzed according to the threshold of the differently expressed genes (DEGs) with two foldchange and significantly DEGs combining two foldchange with $p\_$ value < 0.05. It analyzed significance correlation of transporter genes following the threshold of | correlation | ≥ 0.95 (Pearson correlation coefficient) and $p\_$ value ≤ 0.05.

HPLC and GC/MS analysis

Phenolic aldehydes, including 4-hydroxybenzaldehyde, syringaldehyde, and vanillin, were determined at 35 °C using reverse-phase HPLC (SPD-20A, Shimadzu, Kyoto, Japan) with the YMC-Pack ODS-A column (Tokyo, Japan). Both 4-hydroxybenzaldehyde and syringaldehyde were measured at 270 nm at 1.0 mL/min using 30% acetonitrile solution as mobile phase, and vanillin was measured at 320 nm using 50% acetonitrile solution at 0.8 mL/min.

The same with the previous study [3], phenolic aldehydes and their intermediates in *Z. mobilis* ZM4 were determined using GC/MS. Samplings were at 0 and 24 h after inoculation. The intracellular and extracellular phenolic compounds dissolved in ethyl acetate and acetonitrile solution (2:1, v/v) were silylated with NO-bis-trimethylsilyl trifluoro-acetamide after being concentrated by rotary evaporator with vacuum system [18]. The supernatant was analyzed using Agilent 6890 GC/MS fitted from 80 °C for 4 min to 280 °C at 8 °C/min with a HP-5 MS column (30 m × 0.25 mm × 0.25 μm) (Agilent Technologies, Santa Clara, CA, USA). One microliter sample was used to perform splitless GC detection.

Results and discussion

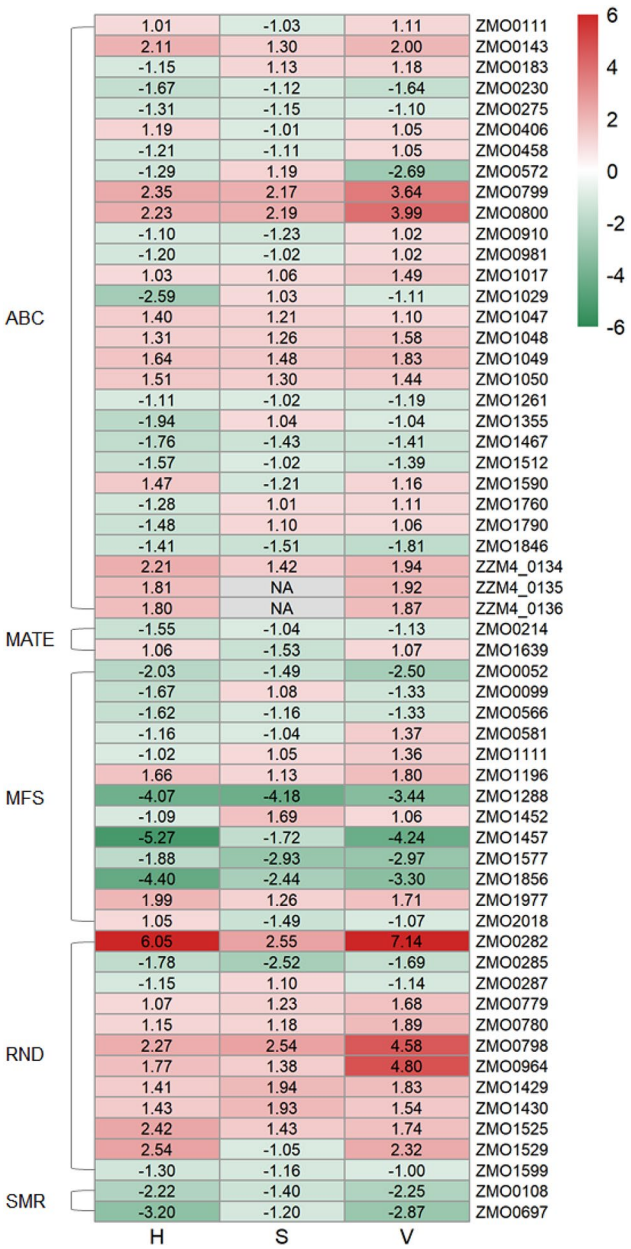
No phenolic compounds accumulated in the intracellular of *Z. mobilis* ZM4

Reduction and transport of biological process were the main molecular mechanism responsible for the degradation of the phenolic aldehydes to the less toxic phenolic

alcohols [3]. The phenolic aldehydes tolerance was investigated by assaying the accumulation of the three model phenolic aldehydes in the culture broth of *Z. mobilis* ZM4 (Fig. 1). Compared with the non-phenolic aldehydes-treated control, the cell growth of *Z. mobilis* ZM4 was brought down approximately 49.43, 20.10, and 28.03% under the stress of 4-hydroxybenzaldehyde, syringaldehyde, and vanillin, respectively (Fig. 1a). Except for the stress of 4-hydroxybenzaldehyde, *Z. mobilis* ZM4 completely consumed glucose at 24 h (Fig. 1b). Ethanol productivity was decreased from 0.35 to 0.23, 0.34, and 0.34 g/L/h when separately adding 4-hydroxybenzaldehyde, syringaldehyde, and vanillin (Fig. 1c). Degradation of phenolic aldehydes to the intermediates phenolic alcohols occurred in the extracellular rather than intracellular space of *Z. mobilis* ZM4 at 24 h (Fig. 1d–e). Furthermore, the decrease of phenolic aldehydes was almost equal to the increase of phenolic alcohols. The results indicated that less accumulation in the intracellular space of *Z. mobilis* ZM4 was beneficial for the tolerance of *Z. mobilis* ZM4 to the toxic microenvironment caused by phenolic compounds. The poor water solubility led to the low concentration of phenolic aldehyde inhibitors in extracellular environment and simply not easy to undertake by fermenting strains as the sole carbon source for cell growth on phenolic aldehydes. It is necessary to make the further adjustments (including metabolic engineering and synthetic biology) for phenolic aldehydes degradation in *Z. mobilis* [3].

Gene transcriptional profiling of transporter proteins under phenolic aldehydes stress

To determine the contribution of transporter proteins to the tolerance of phenolic aldehyde inhibitors in *Z. mobilis* ZM4, it analyzed the gene transcriptional profiling of transporter proteins in the genome and plasmid of *Z. mobilis* ZM4, including ABC, MATE, MFS, RND, and SMR. It showed that 15, 8, and 15 genes differentially expressed separately under the stress of 4-hydroxybenzaldehyde, syringaldehyde, and vanillin (Fig. 2). Except for MATE and SMR, other transporter genes covered the DEGs under the stress of the above three model phenolic aldehydes, for example *ZMO0799* and *ZMO0800* for ABC, *ZMO1288* and *ZMO1856* for MFS, *ZMO0282* and *ZMO0798* for RND. GO analysis also showed that the screened DEGs mostly enriched in protein localization, protein transport, and establishment of protein localization of biological process (Table 1). In summary, the transcriptional profiling suggested that the genes encoding transporter proteins were in response to the stress of phenolic aldehydes.



	H	S	V	
ABC	1.01	-1.03	1.11	ZMO0111
	2.11	1.30	2.00	ZMO0143
	-1.15	1.13	1.18	ZMO0183
	-1.67	-1.12	-1.64	ZMO0230
	-1.31	-1.15	-1.10	ZMO0275
	1.19	-1.01	1.05	ZMO0406
	-1.21	-1.11	1.05	ZMO0458
	-1.29	1.19	-2.69	ZMO0572
	2.35	2.17	3.64	ZMO0799
	2.23	2.19	3.99	ZMO0800
	-1.10	-1.23	1.02	ZMO0910
	-1.20	-1.02	1.02	ZMO0981
	1.03	1.06	1.49	ZMO1017
	-2.59	1.03	-1.11	ZMO1029
	1.40	1.21	1.10	ZMO1047
	1.31	1.26	1.58	ZMO1048
	1.64	1.48	1.83	ZMO1049
	1.51	1.30	1.44	ZMO1050
	-1.11	-1.02	-1.19	ZMO1261
	-1.94	1.04	-1.04	ZMO1355
	-1.76	-1.43	-1.41	ZMO1467
	-1.57	-1.02	-1.39	ZMO1512
	1.47	-1.21	1.16	ZMO1590
	-1.28	1.01	1.11	ZMO1760
	-1.48	1.10	1.06	ZMO1790
	-1.41	-1.51	-1.81	ZMO1846
	2.21	1.42	1.94	ZZM4_0134
	1.81	NA	1.92	ZZM4_0135
	1.80	NA	1.87	ZZM4_0136
MATE	-1.55	-1.04	-1.13	ZMO0214
	1.06	-1.53	1.07	ZMO1639
MFS	-2.03	-1.49	-2.50	ZMO0052
	-1.67	1.08	-1.33	ZMO0099
	-1.62	-1.16	-1.33	ZMO0566
	-1.16	-1.04	1.37	ZMO0581
	-1.02	1.05	1.36	ZMO1111
	1.66	1.13	1.80	ZMO1196
	-4.07	-4.18	-3.44	ZMO1288
	-1.09	1.69	1.06	ZMO1452
	-5.27	-1.72	-4.24	ZMO1457
	-1.88	-2.93	-2.97	ZMO1577
	-4.40	-2.44	-3.30	ZMO1856
	1.99	1.26	1.71	ZMO1977
	1.05	-1.49	-1.07	ZMO2018
RND	6.05	2.55	7.14	ZMO0282
	-1.78	-2.52	-1.69	ZMO0285
	-1.15	1.10	-1.14	ZMO0287
	1.07	1.23	1.68	ZMO0779
	1.15	1.18	1.89	ZMO0780
	2.27	2.54	4.58	ZMO0798
	1.77	1.38	4.80	ZMO0964
	1.41	1.94	1.83	ZMO1429
	1.43	1.93	1.54	ZMO1430
	2.42	1.43	1.74	ZMO1525
	2.54	-1.05	2.32	ZMO1529
	-1.30	-1.16	-1.00	ZMO1599
SMR	-2.22	-1.40	-2.25	ZMO0108
	-3.20	-1.20	-2.87	ZMO0697

Fig. 2 Transcriptional profiling of transporter genes in response to 4-hydroxybenzaldehyde (H), syringaldehyde (S), and vanillin (V), in *Z. mobilis* ZM4

ABC transporters for the tolerance of phenolic aldehydes

ABC transporters contain both uptake and efflux transport systems as the primary active membrane proteins translocating solutes across lipid bilayers [19]. There're totally 29 genes encoding ABC transporter proteins in the plasmid and genome of *Z. mobilis* ZM4. Among the genes, *ZMO0799* and *ZMO0800* were significantly differentially expressed in response to the stress of the three phenolic

Table 1 GO analysis of transporter proteins in *Z. mobilis* ZM4

GO	Genes
Protein localization	*ZMO0282*, *ZMO0287*, *ZMO0458*, *ZMO0779*, *ZMO1430*, *ZMO1529*
Protein transport	*ZMO0282*, *ZMO0287*, *ZMO0458*, *ZMO0779*, *ZMO1430*, *ZMO1529*
Establishment of protein localization	*ZMO0282*, *ZMO0287*, *ZMO0458*, *ZMO0779*, *ZMO1430*, *ZMO1529*
Protein secretion	*ZMO0282*, *ZMO0287*, *ZMO0779*, *ZMO1430*, *ZMO1529*
Secretion by cell	*ZMO0282*, *ZMO0287*, *ZMO0779*, *ZMO1430*, *ZMO1529*
Secretion	*ZMO0282*, *ZMO0287*, *ZMO0779*, *ZMO1430*, *ZMO1529*
Ion transport	*ZMO1047*, *ZMO1048*, *ZMO1049*, *ZMO1050*
Phosphate transport	*ZMO1047*, *ZMO1048*, *ZMO1049*, *ZMO1050*
Anion transport	*ZMO1047*, *ZMO1048*, *ZMO1049*, *ZMO1050*
Inorganic anion transport	*ZMO1047*, *ZMO1048*, *ZMO1049*, *ZMO1050*
Drug transport	*ZMO0214*, *ZMO1196*, *ZMO1639*, *ZMO1977*
Response to drug	*ZMO0214*, *ZMO1196*, *ZMO1639*, *ZMO1977*
Multidrug transport	*ZMO0214*, *ZMO1639*
Organic alcohol transport	*ZMO1196*, *ZMO1977*
Tetracycline transport	*ZMO1196*, *ZMO1977*
Antibiotic transport	*ZMO1196*, *ZMO1977*
Response to antibiotic	*ZMO1196*, *ZMO1977*
Transmembrane transport	*ZMO0214*, *ZMO1639*
Lipoprotein transport	*ZMO0458*

aldehydes. *ZMO0799* was differentially down-regulated by 2.35-, 3.64-, and 2.17-fold, under the stress of 4-hydroxybenzaldehyde, syringaldehyde, and vanillin, respectively, and *ZMO0800* was separately differentially up-regulated by 2.23-, 2.19-, and 3.99-fold. Both *ZMO0799* and *ZMO0800* were the members of the gene cluster *ZMO0797-ZMO0798-ZMO0799-ZMO0800-ZMO0801* in respect of phenolic aldehydes degradation according to the previous study [3]. ABC transporters prevented *Saccharomyces cerevisiae* from HMF damage by exporting excessive HMF [20]. The promotion by ABC transporter was also found for the production of biofuels or chemicals from cellulose in Gram positive strain *Ruminiclostridium cellulolyticum* [21]. Importantly, *ZMO0799* and *ZMO0800*, especially the gene cluster of *ZMO0799-ZMO0800*, would be an alternative for genetic engineering of lignocellulosic inhibitor tolerance for fermentation strains in biorefinery fields.

MFS transporters for the tolerance of phenolic aldehydes

MFS, the largest class of secondary active transporters, was found the interaction between protonation and the negative-inside membrane potential [22]. Among thirteen MFS genes in the genome of *Z. mobilis* ZM4, *ZMO1288* was significantly down-regulated by 4.07-, 4.18-, and 3.44-fold under the stress of 4-hydroxybenzaldehyde, syringaldehyde, and vanillin, respectively, and *ZMO1856* was separately down-regulated by 4.40, 2.44, and 3.30-fold. It should be noted that the overexpression of *ZMO1288* facilitated cell growth, glucose consumption, ethanol productivity, and the conversion of phenolic aldehydes in the previous study [3]. It predicted that the enhanced expression of *ZMO1288* would accelerate the transport of phenolic compounds out of intracellular space and no harm was for the intracellular activity of the *Z. mobilis* ZM4. Membrane transport protein was also involved with the enhancement of phenolic compounds tolerance in *Clostridium beijerinckii* NCIMB 8052 [23]. Indeed, the gene expression of MFS transporter proteins improved the metabolite production [24, 25]. Therefore, *ZMO1288* and *ZMO1856* encoding MFS transporters would be the candidates for the genetic strengthening of phenolic aldehydes tolerance in *Z. mobilis* ZM4.

RND transporters for the tolerance of phenolic aldehydes

RND transporters, the most efficient mechanism of solvent tolerance in Gram-negative bacteria expulsing compounds, are characterized to catalyze substrate efflux via the mechanism of a substrate/H^+ antiport [26]. In this study, among 12 genes (*ZMO0282*, *ZMO0285*, *ZMO0287*, *ZMO0779*, *ZMO0780*, *ZMO0798*, *ZMO0964*, *ZMO1429*, *ZMO1430*, *ZMO1525*, *ZMO1529*, and *ZMO1599*) encoding RND efflux pumps in the genome of *Z. mobilis* ZM4, *ZMO0282* was separately significantly differentially up-regulated by 6.05, 2.55, and 7.14 folds under the stress of 4-hydroxybenzaldehyde, syringaldehyde, and vanillin, respectively, and *ZMO0798* was separately up-regulated by 2.27, 2.54, and 4.58 folds (Fig. 2). It suggested that RND transporter genes

were in response to the stress of phenolic aldehydes. RND was important for the efficient production of ethanol and free fatty acid using genetically engineered cyanobacteria *Synechococcus elongatus* strain PCC 7942 and *Thermoanaerobacter* sp. X514 [27, 28]. RND would be a rational choice for the gene engineering of Gram-negative bacteria *Z. mobilis* ZM4 characteristically surrounded by the additional membrane layer, an outer membrane (OM) serving as the most important function of a selective permeation barrier [29, 30].

MATE transporters for the tolerance of phenolic aldehydes

MATE transporters conserved in biology and functioned in the efflux of cationic and lipophilic substances and xenobiotics with an electrochemical gradient of H^+ or Na^+ across the cell membrane [31]. In *Z. mobilis* ZM4, *ZMO0214* and *ZMO1639* encoding MATE were not differentially expressed under the stress of the three phenolic aldehydes. It showed that more DEGs of the other transporters, especially for ABC, MFS, and RND transporter, than MATE transporter. However, MATE transporter gene worked after co-expressed with biosynthetic gene cluster [32]. This suggested that transporter genes combining with functional genes would be a favorite for inhibitor tolerance enhancement in *Z. mobilis* ZM4.

SMR transporters for the tolerance of phenolic aldehydes

SMR family of membrane proteins, prominent for its rare dual-topology architecture, exhibited low substrate specificities and was resistant to the large hydrophobic and cationic molecules [33]. Two genes, *ZMO0108* and *ZMO0697*, encoding SMR in the genome of *Z. mobilis* ZM4, were just differentially down-regulated under the stress of the more toxic 4-hydroxybenzaldehyde and vanillin in *Z. mobilis* ZM4 (Fig. 2). The expression of the SMR pump potentially enhanced furfural tolerance in ethanologenic *Escherichia coli* [10]. In few, SMR would be the other synthetic tools for the increase of the lignocellulose-derived inhibitor tolerance in biorefinery field.

Significance correlation analysis of transporter proteins

To determine the expression network in *Z. mobilis* ZM4, the significance correlation between transporters and their corresponding targets was established (Fig. 3). Among the screened key transporters, ABC transporter (*ZMO0799* and *ZMO0800*), MFS transporter (*ZMO1288* and *ZMO1856*), and RND transporter (*ZMO0282* and *ZMO0798*) just

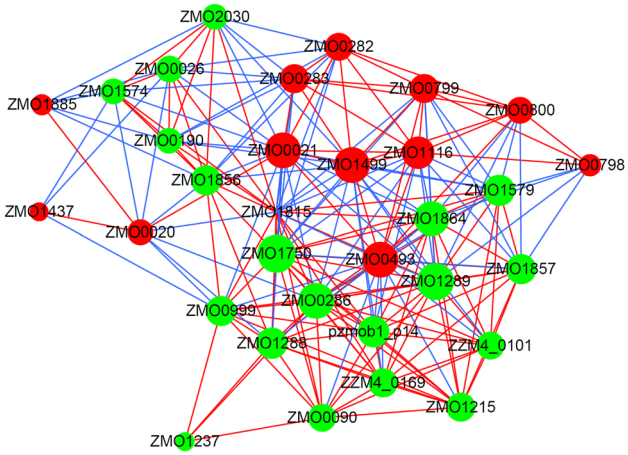


Fig. 3 Significant correlation analysis of transporter proteins under the stress of phenolic aldehydes in *Z. mobilis* ZM4. Red and blue line separately indicated the positive and negative correlation, and red and green circles were the up- and down-regulated genes, respectively. The transcriptional level of relating genes was listed in the previous study [3]

significantly connected with oxidoreductases (*ZMO0090*, *ZMO1116*, *ZMO1237*, and *ZMO1885*) and transcriptional regulators (*ZMO0190*, *ZMO1574*, *ZMO1857*, and *ZMO2030*) besides hypothetical proteins (*ZMO0020*, *ZMO0021*, *ZMO0286*, *ZMO1215*, *ZMO1750*, *ZZM4_0101*, and *ZZM4_0169*). The growing evidence of transporter proteins working as a trigger in transport and regulation to increase transport and stress tolerance had been established, such as transcriptional regulator with ABC transporter [6, 33–35], MFS transporter [37, 38], and RND [39, 40].

Specially, *ZMO1116* encoding NAD(P)-dependent oxidoreductase was one of the targets of RND transporter (*ZMO0798*) and ABC transporter (*ZMO0799* and *ZMO0800*), the members of the gene cluster *ZMO0797-ZMO0798-ZMO0799-ZMO0800-ZMO0801* [3]. The overexpression of *ZMO1116* raised the fermenting parameters including 4-hydroxybenzaldehyde conversion. *ZMO0282* for RND transporter was also tightly linked to *ZMO1116*. The establishment of the significant correlation between transporters and their targets would well elucidate the acceleration mechanism of ethanol fermentability under phenolic aldehydes stress in *Z. mobilis* ZM4 and provide synthetic tools for the genetic modification of bioethanol production.

Conclusion

Taken together, a globally detailed gene transcriptional profiling for transporter proteins in Gram-negative ethanologenic bacterium *Z. mobilis* ZM4 was presented under the stress of the model phenolic aldehydes

4-hydroxybenzaldehyde, syringaldehyde, and vanillin using DNA microarray. No phenolic aldehydes and phenolic alcohols were accumulated in the intracellular of *Z. mobilis* ZM4. Among five bacterial transporter proteins, ABC transporter (*ZMO0799* and *ZMO0800*), MFS transporter (*ZMO1288* and *ZMO1856*), and RND transporter (*ZMO0282* and *ZMO0798*) were identified as the potential candidates for improving phenolic aldehydes tolerance in *Z. mobilis* ZM4. Meanwhile, it showed that these key transporters were significantly correlated with oxidoreductases and transcriptional regulators. This work would provide the key transporter proteins as synthetic biology tools for the robustness enhancement of biorefinery strains.

Author contributions XY and WW designed the experiment and drafted the manuscript. XY, JM, and LL carried out the experiment. WW and XY were in charge of the overall project. All authors read and approved to publish the final manuscript.

Funding This research was supported by Natural Science Foundation of Jiangxi Province (20192BAB204002), Open Funding Project of the State Key Laboratory of Bioreactor Engineering, and Doctor Science Research Foundation of Jiujiang University (8879524).

Data availability The data and materials that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Informed consent All authors are aware of the content and agree with the submission.

References

1. Jing X, Zhang X, Bao J (2009) Inhibition performance of lignocellulose degradation products on industrial cellulase enzymes during cellulose hydrolysis. Appl Biochem Biotechnol 159:696–707
2. Klinke HB, Thomsen AB, Ahring BK (2004) Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl Microbiol Biotechnol 66:10–26
3. Yi X, Gu H, Gao Q, Liu ZL, Bao J (2015) Transcriptome analysis of *Zymomonas mobilis* ZM4 reveals mechanisms of tolerance and detoxification of phenolic aldehyde inhibitors from lignocellulose pretreatment. Biotechnol Biofuels 8:153
4. Gu H, Zhu Y, Peng Y, Liang X, Liu X, Shao L, Xu Y, Xu Z, Liu R, Li J (2019) Physiological mechanism of improved tolerance of *Saccharomyces cerevisiae* to lignin-derived phenolic acids in lignocellulosic ethanol fermentation by short-term adaptation. Biotechnol Biofuels 12:268
5. Luo H, Zheng P, Bilal M, Xie F, Zeng Q, Zhu C, Yang R, Wang Z (2020) Efficient bio-butanol production from lignocellulosic waste by elucidating the mechanisms of *Clostridium acetobutylicum* response to phenolic inhibitors. Sci Total Environ 710:136399
6. Wang X, Liang Z, Hou J, Shen Y, Bao X (2017) The absence of the transcription factor Yrr1p, identified from comparative genome profiling, increased vanillin tolerance due to enhancements of ABC transporters expressing, rRNA processing and ribosome biogenesis in *Saccharomyces cerevisiae*. Front Microbiol 8:367
7. Doshi R, Nguyen T, Chang G (2013) Transporter-mediated biofuel secretion. Proc Natl Acad Sci U S A 110(19):7642–7647
8. Kumar A, Schweizer HP (2005) Bacterial resistance to antibiotics: active efflux and reduced uptake. Adv Drug Deliv Rev 57(10):1486–1513
9. Geddes RD, Wang X, Yomano LP, Miller EN, Zheng HB, Shanmugam KT, Ingram LO (2014) Polyamine transporters and polyamines increase furfural tolerance during xylose fermentation with ethanologenic *Escherichia coli* strain LY180. Appl Environ Microbiol 80(19):5955–5964
10. Kurgan G, Panyon LA, Rodriguez-Sanchez Y, Pacheco E, Nieves LM, Mann R, Nielsen DR, Wang X (2019) Bioprospecting of native efflux pumps to enhance furfural tolerance in ethanologenic *Escherichia coli*. Appl Environ Microbiol 85(6):e02985-e3018
11. Jiménez-Bonilla P, Zhang J, Wang Y, Blersch D, de-Bashan LE, Guo L, Wang Y (2020) Enhancing the tolerance of *Clostridium saccharoperbutylacetonicum* to lignocellulosic-biomass-derived inhibitors for efficient biobutanol production by overexpressing efflux pumps genes from *Pseudomonas putida*. Bioresour Technol 312:123532
12. Franden MA, Pilath HM, Mohagheghi A, Pienkos PT, Zhang M (2013) Inhibition of growth of *Zymomonas mobilis* by model compounds found in lignocellulosic hydrolysates. Biotechnol Biofuels 6(1):99
13. Gu H, Zhang J, Bao J (2015) High tolerance and physiological mechanism of *Zymomonas mobilis* to phenolic inhibitors in ethanol fermentation of corncob residue. Biotechnol Bioeng 112(9):1770–1782
14. Dunn KL, Rao CV (2014) Expression of a xylose-specific transporter improves ethanol production by metabolically engineered *Zymomonas mobilis*. Appl Microbiol Biotechnol 98(15):6897–6905
15. Ren C, Chen T, Zhang J, Liang L, Lin Z (2009) An evolved xylose transporter from *Zymomonas mobilis* enhances sugar transport in *Escherichia coli*. Microb Cell Fact 8:66
16. Weisser P, Krämer R, Sahm H, Sprenger GA (1995) Functional expression of the glucose transporter of *Zymomonas mobilis* leads to restoration of glucose and fructose uptake in *Escherichia coli* mutants and provides evidence for its facilitator action. J Bacteriol 177(11):3351–3354
17. Zhang K, Shao H, Cao Q, He MX, Wu B, Feng H (2015) Transcriptional analysis of adaptation to high glucose concentrations in *Zymomonas mobilis*. Appl Microbiol Biotechnol 99(4):2009–2022
18. Klinke HB, Ahring BK, Schmidt AS, Thomsen AB (2002) Characterization of degradation products from alkaline wet oxidation of wheat straw. Bioresour Technol 82:15–26
19. Locher KP (2016) Mechanistic diversity in ATP-binding cassette (ABC) transporters. Nat Struct Mol Biol 23(6):487–493
20. Ma M, Liu ZL (2010) Comparative transcriptome profiling analyses during the lag phase uncover YAP1, PDR1, PDR3, RPN4, and HSF1 as key regulatory genes in genomic adaptation to the lignocellulose derived inhibitor HMF for *Saccharomyces cerevisiae*. BMC Genomics 11:660
21. Fosses A, Maté M, Franche N, Liu N, Denis Y, Borne R, de Philip P, Fierobe HP, Perret S (2017) A seven-gene cluster in *Ruminiclostridium cellulolyticum* is essential for signalization, uptake

and catabolism of the degradation products of cellulose hydrolysis. Biotechnol Biofuels 10:250
22. Zhang XC, ZhaoY HJ, Jiang D (2015) Energy coupling mechanisms of MFS transporters. Protein Sci 24(10):1560–1579
23. Liu J, Lin Q, Chai X, Luo Y, Guo T (2018) Enhanced phenolic compounds tolerance response of *Clostridium beijerinckii* NCIMB 8052 by inactivation of Cbei_3304. Microb Cell Fact 17(1):35
24. Chen Z, Huang J, Wu Y, Wu W, Zhang Y, Liu D (2017) Metabolic engineering of *Corynebacterium glutamicum* for the production of 3-hydroxypropionic acid from glucose and xylose. Metab Eng 39:151–158
25. Mori K, Niinuma K, Fujita M, Kamimura N, Masai E (2018) DdvK, a novel major facilitator superfamily transporter essential for 5,5'-Dehydrodivanillate uptake by Sphingobium sp. Strain SYK-6. Appl Environ Microbiol 84(20):e01314-e1318
26. Ramos JL, Duque E, Gallegos MT, Godoy P, Ramos-Gonzalez MI, Rojas A, Teran W, Segura A (2002) Mechanisms of solvent tolerance in gram-negative bacteria. Annu Rev Microbiol 56:743–768
27. Kato A, Takatani N, Use K, Uesaka K, Ikeda K, Chang Y, Kojima K, Aichi M, Ihara K, Nakahigashi K, Maeda SI, Omata T (2015) Identification of a cyanobacterial RND-type efflux system involved in export of free fatty acids. Plant Cell Physiol 56(12):2467–2477
28. Lin L, Ji Y, Tu Q, Huang RR, Teng L, Zeng XW, Song HH, Wang K, Zhou Q, Li YF, Cui Q, He ZL, Zhou JZ, Xu J (2013) Microevolution from shock to adaptation revealed strategies improving ethanol tolerance and production in *Thermoanaerobacter*. Biotechnol Biofuels 6(1):103
29. Segura A, Molina L, Fillet S, Krell T, Bernal P, Muñoz-Rojas J, Ramos JL (2012) Solvent tolerance in Gram-negative bacteria. Curr Opin Biotechnol 23(3):415–421
30. Nikaido H (2003) Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev 67(4):593–656
31. He GX, Kuroda T, Mima T, Morita Y, Mizushima T, Tsuchiya T (2004) An $H^{(+)}$-coupled multidrug efflux pump, PmpM, a member of the MATE family of transporters, from *Pseudomonas aeruginosa*. J Bacteriol 186(1):262–265
32. Darbani B, Motawia MS, Olsen CE, Nour-Eldin HH, Møller BL, Rook F (2016) The biosynthetic gene cluster for the cyanogenic glucoside dhurrin in *Sorghum bicolor* contains its co-expressed vacuolar MATE transporter. Sci Rep 6:37079
33. Rapp M, Granseth E, Seppälä S, von Heijne G (2006) Identification and evolution of dual-topology membrane proteins. Nat Struct Mol Biol 13(2):112–116
34. Poudyal B, Sauer K (2018) The ABC of biofilm drug tolerance: the MerR-like regulator BrlR Is an activator of ABC transport systems, with PA1874-77 contributing to the tolerance of *Pseudomonas aeruginosa* Biofilms to Tobramycin. J Antimicrob Chemother 62(2):e01981-e2017
35. Seaton K, Ahn SJ, Sagstetter AM, Burne RA (2011) A transcriptional regulator and ABC transporters link stress tolerance, (p)ppGpp, and genetic competence in Streptococcus mutans. J Bacteriol 193(4):862–874
36. Görke B (2012) Killing two birds with one stone: an ABC transporter regulates gene expression through sequestration of a transcriptional regulator at the membrane. Mol Microbiol 85(4):597–601
37. Huang YW, Hu RM, Chu FY, Lin HR, Yang TC (2013) Characterization of a major facilitator superfamily (MFS) tripartite efflux pump EmrCABsm from *Stenotrophomonas maltophilia*. J Antimicrob Chemother 68(11):2498–2505
38. Lin HC, Yu PL, Chen LH, Tsai HC, Chung KR (2018) A major facilitator superfamily transporter regulated by the stress-responsive transcription factor Yap1 is required for resistance to fungicides, xenobiotics, and oxidants and full virulence in *Alternaria alternata*. Front Microbiol 9:2229
39. Bador J, Neuwirth C, Grangier N, Muniz M, Germé L, Bonnet J, Pillay VG, Llanes C, de Curraize C, Amoureux L (2017) Role of AxyZ transcriptional regulator in overproduction of AxyXY-OprZ multidrug efflux system in *Achromobacter* Species mutants selected by Tobramycin. Antimicrob Agents Chemother (Bethesda) 61(8):e00290-e317
40. Cerminati S, Giri GF, Mendoza JI, Soncini FC, Checa SK (2017) The CpxR/CpxA system contributes to Salmonella gold-resistance by controlling the GolS-dependent gesABC transcription. Environ Microbiol 19(10):4035–4044