Overexpression of an oxidoreductase YghA confers tolerance against furfural in ethanologenic *Escherichia coli* strain SSK42

TABLE S1: List of plasmids and strains used in the study.

TABLE S2: Percent COV observed in first screen of *E. coli* BW25113 mutant strains at 3h and 6h of cultivation.

TABLE S3: The maximum specific ethanol productivity values of SSK42 (pTrcHisA) and SSK42 (pTrcHisA-*yghA*) strains at 5% and 10% xylose concentration

Figure S1: LD_{50} values of parent *E. coli* BW25113 strain towards furfural in media containing 0.2% (wt/vol) of either glucose (A) or xylose (B) as sole carbon source.

Figure S2: First screening of *E. coli* BW25113 derived mutant strains. Cultivation was performed in media containing 0.2% (wt/vol) xylose and 0.25 g L^{-1} furfural.

Figure S3: Second screening of selected *E. coli* BW25113 mutant strains in AM1 media containing 5% (wt/vol) xylose and 1 g L^{-1} furfural.

Figure S4: Transformation of *yghA* gene in *E. coli* BW25113Δ*yghA* genetic background.

Figure S5: Influence of carbon source in promoting biomass formation in *E. coli* SSK42 host with either pTrcHisA (dark gery) or with pTrcHisA-*yghA* (light grey).

Figure S6: Overexpression of YghA also leads to metabolism of 1 g L^{-1} 5-HMF from media containing 5% (wt/vol) xylose and 0.1 mM IPTG.

Figure S7: Influence of IPTG concentration on furfural metabolism in SSK42 harboring either pTrcHisA (dark grey) or pTrcHisA-*yghA* (light grey).

Figure S8: Furfural concentration in bioreactor containing 5% xylose (wt/vol) and 1 g L^{-1} furfural.

TABLE S1. List of plasmids and strains used in the study

Plasmid/Strain	Description	Source/Reference
Plasmid		
pTrcHisA	P_{trc} *bla* oriR *rrn*B *lacI*^{q} 6XHis	Invitrogen
pTrcHisA-*yghA*	*yghA* ORF cloned into pTrcHisA	This study
pCP20	*bla*, *flp*, temperature-conditional replicon	CGSC #7629
Strain		
SSK42	*E. coli* B P_{gapA}PDH *ΔldhΔfrdAΔpflB* undergone evolutionary adaptation.	Jilani SB, Venigalla SSK, Mattam AJ, Dev C, Yazdani SS. *J Ind Microbiol Biotechnol* 44:1375–1384 (2017)
BW25113 (pTrcHisA)	BW25113, Δ*yghA* :: FRT-kan-FRT - pTrcHisA	This study
BW25113 (pTrcHisA-*yghA*)	BW25113, Δ*yghA* :: FRT-kan-FRT - pTrcHisA-*yghA*	This study
SSK42 (pTrcHisA)	SSK42 harboring pTrcHisA	This study
SSK42 (pTrcHisA-*yghA*)	SSK42 harboring pTrcHisA-*yghA*	This study
BW25113	F_, DE(*araD-araB*)567,*lacZ*4787(del)::*rrnB*_3, LAM-, *rph*_1, DE(*rhaD-rhaB*) 568,*hsdR*514	CGSC#7636
ΔadhP	BW25113, *ΔadhP* :: FRT-kan-FRT	CGSC#9282
ΔaldB	BW25113, *ΔaldB* :: FRT-kan-FRT	CGSC#10626
ΔaldA	BW25113, *ΔaldA* :: FRT-kan-FRT	CGSC#9239
ΔastD	BW25113, *ΔastD* :: FRT-kan-FRT	CGSC#11289
ΔbetB	BW25113, *ΔbetB* :: FRT-kan-FRT	CGSC#8504
ΔdkgA	BW25113, *ΔdkgA* :: FRT-kan-FRT	CGSC#11427
ΔdkgB	BW25113, *ΔdkgB* :: FRT-kan-FRT	CGSC#12026
ΔeutG	BW25113, *ΔeutG* :: FRT-kan-FRT	CGSC#9943
ΔfeaB	BW25113, *ΔfeaB* :: FRT-kan-FRT	CGSC#9220
ΔfrmA	BW25113, *ΔfrmA* :: FRT-kan-FRT	CGSC#8536
ΔfucO	BW25113, *ΔfucO* :: FRT-kan-FRT	CGSC#11871
ΔgabD	BW25113, *ΔgabD* :: FRT-kan-FRT	CGSC#10077
ΔgldA	BW25113, *ΔgldA* :: FRT-kan-FRT	CGSC#11940
ΔhdhA	BW25113, *ΔhdhA* :: FRT-kan-FRT	CGSC#9372
ΔmaoC	BW25113, *ΔmaoC* :: FRT-kan-FRT	CGSC#9222
ΔmhpF	BW25113, *ΔmhpF* :: FRT-kan-FRT	CGSC#8532
Δpgi	BW25113, *Δpgi* :: FRT-kan-FRT	CGSC#10867
Δprr	BW25113, *Δprr* :: FRT-kan-FRT	CGSC#9259
ΔpuuC	BW25113, *ΔpuuC* :: FRT-kan-FRT	CGSC#11640
ΔrspB	BW25113, *ΔrspB* :: FRT-kan-FRT	CGSC#9342
Δsad	BW25113, *Δsad* :: FRT-kan-FRT	CGSC#11267

Δtas	BW25113, *Δtas* :: FRT-kan-FRT	CGSC#10189
Δusg	BW25113, *Δusg* ::FRT-kan-FRT	CGSC#9858
ΔucpA	BW25113, *ΔucpA* :: FRT-kan-FRT	CGSC#11361
ΔyahK	BW25113, *ΔyahK* :: FRT-kan-FRT	CGSC#8516
ΔyajO	BW25113, *ΔyajO* :: FRT-kan-FRT	CGSC#8578
ΔyagR	BW25113, *ΔyagR* :: FRT-kan-FRT	CGSC#8491
ΔyagS	BW25113, *ΔyagS* :: FRT-kan-FRT	CGSC#8492
ΔybdR	BW25113, *ΔybdR* :: FRT-kan-FRT	CGSC#8716
ΔycjQ	BW25113, *ΔycjQ* :: FRT-kan-FRT	CGSC#9171
ΔycjS	BW25113, *ΔycjS* :: FRT-kan-FRT	CGSC#9172
ΔyciK	BW25113, *ΔyciK* :: FRT-kan-FRT	CGSC#9138
ΔydfG	BW25113, *ΔydfG* :: FRT-kan-FRT	CGSC#9318
ΔydhE	BW25113, *ΔydhE* :: FRT-kan-FRT	CGSC#9408
ΔydjL	BW25113, *ΔydjL* :: FRT-kan-FRT	CGSC#9483
ΔyeaE	BW25113, *ΔyeaE* :: FRT-kan-FRT	CGSC#9486
ΔygbJ	BW25113, *ΔygbJ* :: FRT-kan-FRT	CGSC#10129
ΔygfF	BW25113, *ΔygfF* :: FRT-kan-FRT	CGSC#10226
ΔyghA	BW25113, *ΔyghA* :: FRT-kan-FRT	CGSC#10283
ΔyghZ	BW25113, *ΔyghZ* :: FRT-kan-FRT	CGSC#10282
ΔyggP	BW25113, *ΔyggP* :: FRT-kan-FRT	CGSC#11416
ΔygiB	BW25113, *ΔygiB* :: FRT-kan-FRT	CGSC#11658
ΔygjR	BW25113, *ΔygjR* :: FRT-kan-FRT	CGSC#10334
ΔyhdH	BW25113, *ΔyhdH* :: FRT-kan-FRT	CGSC#10438
ΔyhhX	BW25113, *ΔyhhX* :: FRT-kan-FRT	CGSC#10532
ΔyhiN	BW25113, *ΔyhiN* :: FRT-kan-FRT	CGSC#10562
ΔyiaY	BW25113, *ΔyiaY* :: FRT-kan-FRT	CGSC#11499
ΔyihU	BW25113, *ΔyihU* :: FRT-kan-FRT	CGSC#10784
ΔyjgB	BW25113, *ΔyjgB* :: FRT-kan-FRT	CGSC#11992
ΔykgE	BW25113, *ΔykgE* :: FRT-kan-FRT	CGSC#11999
ΔyohF	BW25113, *ΔyohF* :: FRT-kan-FRT	CGSC#9723
ΔyphC	BW25113, *ΔyphC* :: FRT-kan-FRT	CGSC#12007
ΔyqhD	BW25113, *ΔyqhD* :: FRT-kan-FRT	CGSC#10288

TABLE S2: Percent Coefficient of Variation (COV) observed in first screen of *E. coli* BW25113 mutant strains at 3 and 6 h of cultivation.*

	3h	6h
WT BW25113	**0**	**4**
yiaY	7	27
yjgB	1	6
feaB	13	35
eutG	3	7
ybdR	12	71
ycjQ	8	47
ycjS	**3**	**4**
rspB	3	35
ygfF	4	7
ucpA	6	14
ydjL	3	20
pgi	12	13
yghZ	14	8
ydfG	3	7
ygiB	1	14
hdhA	3	6
ygjR	5	15
ykgE	4	18
yhdH	4	8
yhhX	**5**	**5**
ydhE	7	16
tas	3	7
yeaE	1	10
aldB	2	7
maoC	8	27
yagR	2	15
gabD	1	13
prr	6	21
yagS	5	26
dkgB	3	19
dkgA	7	15
betB	**1**	**3**
sad	8	25
yhiN	6	15
yphC	**0**	**0**
yajO	6	12

frmA	1	13
yahK	1	15
yciK	0	6
yqhD	6	29
mhpF	0	9
adhP	9	26
aldA	5	10
fucO	8	9
yggP	12	26
ycjS	4	17
ygbJ	2	9
yohF	4	25
usg	2	10
gldA	4	14
yihU	3	14
astD	4	39
puuC	5	10
yghA	**3**	**5**

*Strains having COV equal to or less than 5 at both 3 h and 6 h time points were considered for further experiment and have been highlighted in green.

TABLE S3: The maximum specific ethanol productivity values of SSK42 (pTrcHisA) and SSK42 (pTrcHisA-*yghA*) strains at 5% and 10% xylose concentration

Strain	Furfural Concn ($g\ L^{-1}$)	5% xylose		10% xylose	
		Sp ethanol productivity ($g\ g^{-1}\ h^{-1}$)	Time period (h)	Sp ethanol productivity ($g\ g^{-1}\ h^{-1}$)	Time period (h)
SSK42 (pTrcHisA)	0	0.56	0-24	0.51	0-24
SSK42 (pTrcHisA-*yghA*)	0	0.41	0-24	0.51	0-24
SSK42 (pTrcHisA)	1	0.35	72-96	0.34	96-120
SSK42 (pTrcHisA-*yghA*)	1	0.4	24-48	0.39	48-72

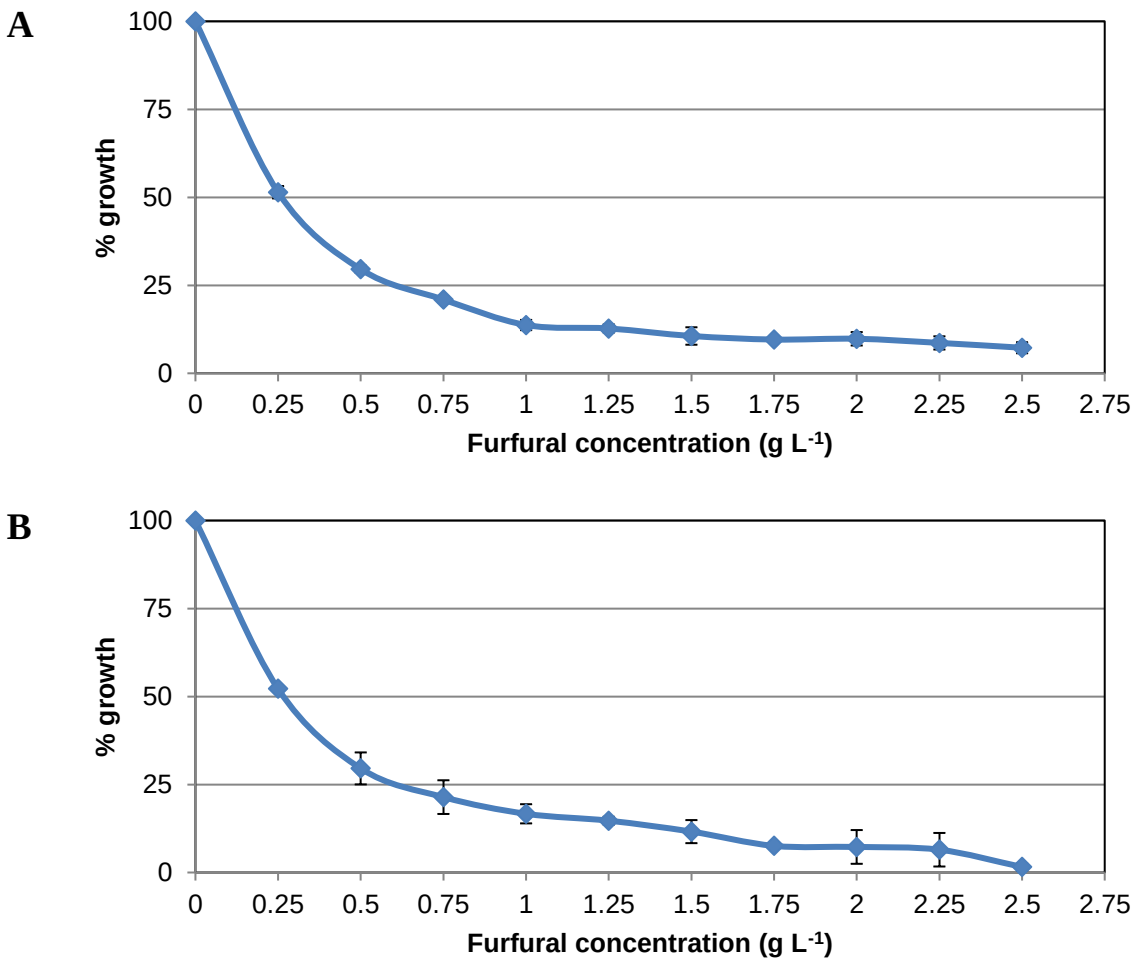

Figure S1: LD_{50} values of parent *E. coli* BW25113 strain towards furfural in media containing 0.2% (wt/vol) of either glucose (A) or xylose (B) as sole carbon source. The observations were recorded at 3 h and LD_{50} values of the BW25113 strain against furfural for either carbon source was determined to be 0.25 g L^{-1}. Values are average of n=2 independent experiments. Error bars represent SD of the mean.

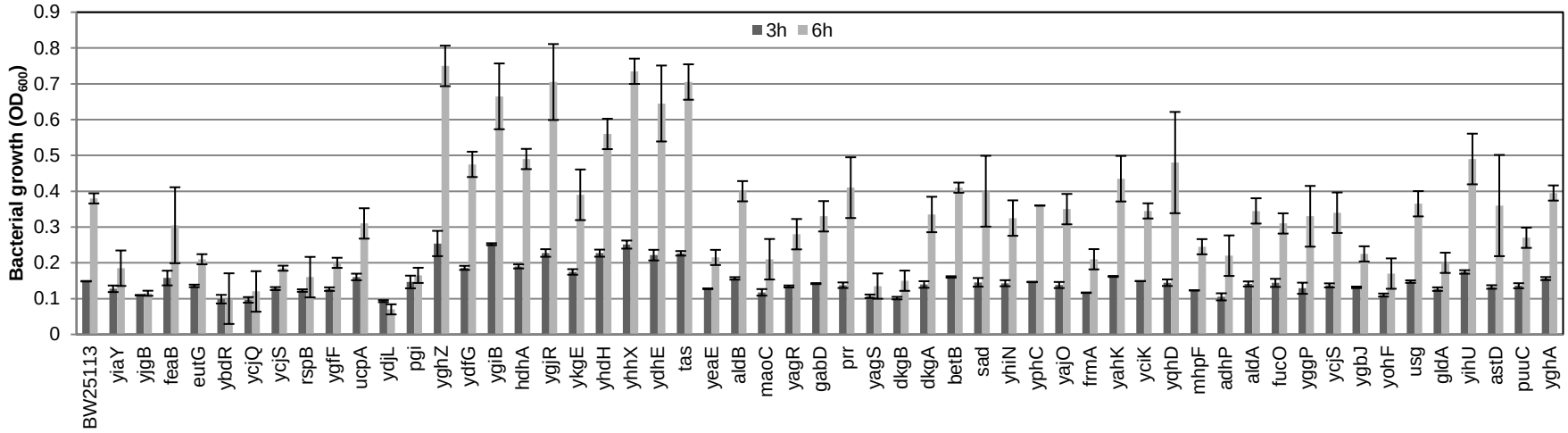

Figure S2: First screening of *E. coli* BW25113 derived mutant strains. Cultivation was performed in media containing 0.2% (wt/vol) xylose and 0.25 g L^{-1} furfural. Cultures were seeded at OD_{600}=0.1 and observations recorded at 3 h and 6 h.Values are average of at least n=2 independent experiments. Error bars represent SD of the mean.

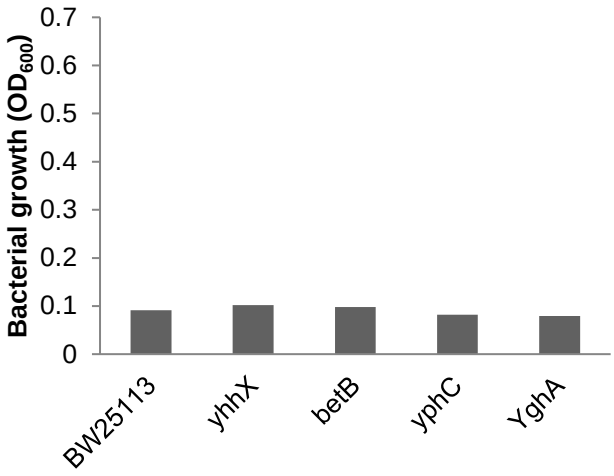

Figure S3: Second screening of selected *E. coli* BW25113 mutant strains in AM1 media containing 5% (wt/vol) xylose and 1 g L^{-1} furfural. Cultures were seeded at OD_{600}=0.1 and growth recorded at 48 h. Values represent outcome of one observation.

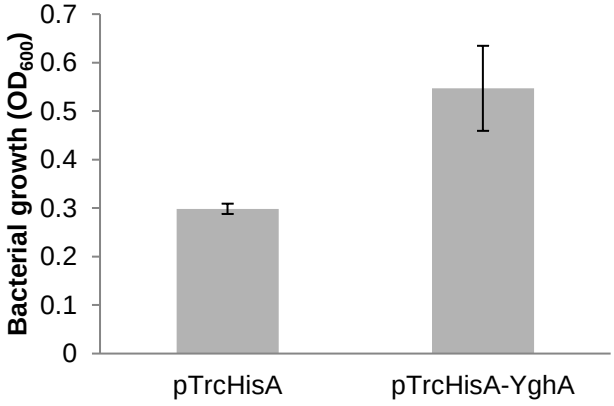

Figure S4: Transformation of *yghA* gene in *E. coli* BW25113Δ*yghA* genetic background. Strain harbored either empty plasmid as a control (pTrcHisA) or cloned *yghA* gene (pTrcHisA-*yghA*). Cultures were grown in media containing 5% (wt/vol) xylose and 0.5 g L^{-1} furfural. OD_{600} was recorded at 24 h. Values are average of n=2 independent experiments. Error bars represent SD of the mean.

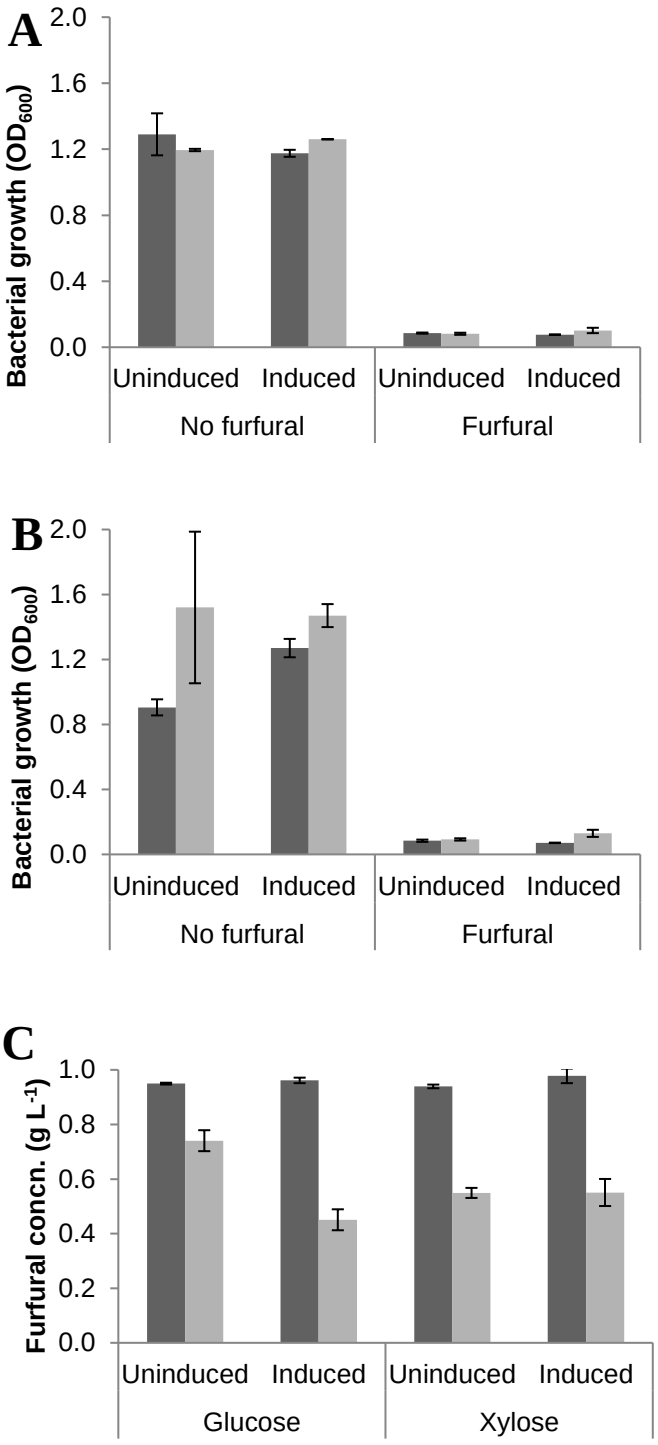

Figure S5: Influence of carbon source in promoting biomass formation in *E. coli* SSK42 host with either pTrcHisA (dark grey) or with pTrcHisA-*yghA* (light grey). Media consisted of 1 g L^{-1} furfural, 0.1 mM IPTG and either 5% (wt/vol) glucose (A) or xylose (B) as carbon source. OD_{600} was recorded at 48 h. (C) Furfural concentration was measured at 48 h of cultivation. Values are average of n=2 independent experiments. Error bars represent SD of the mean.

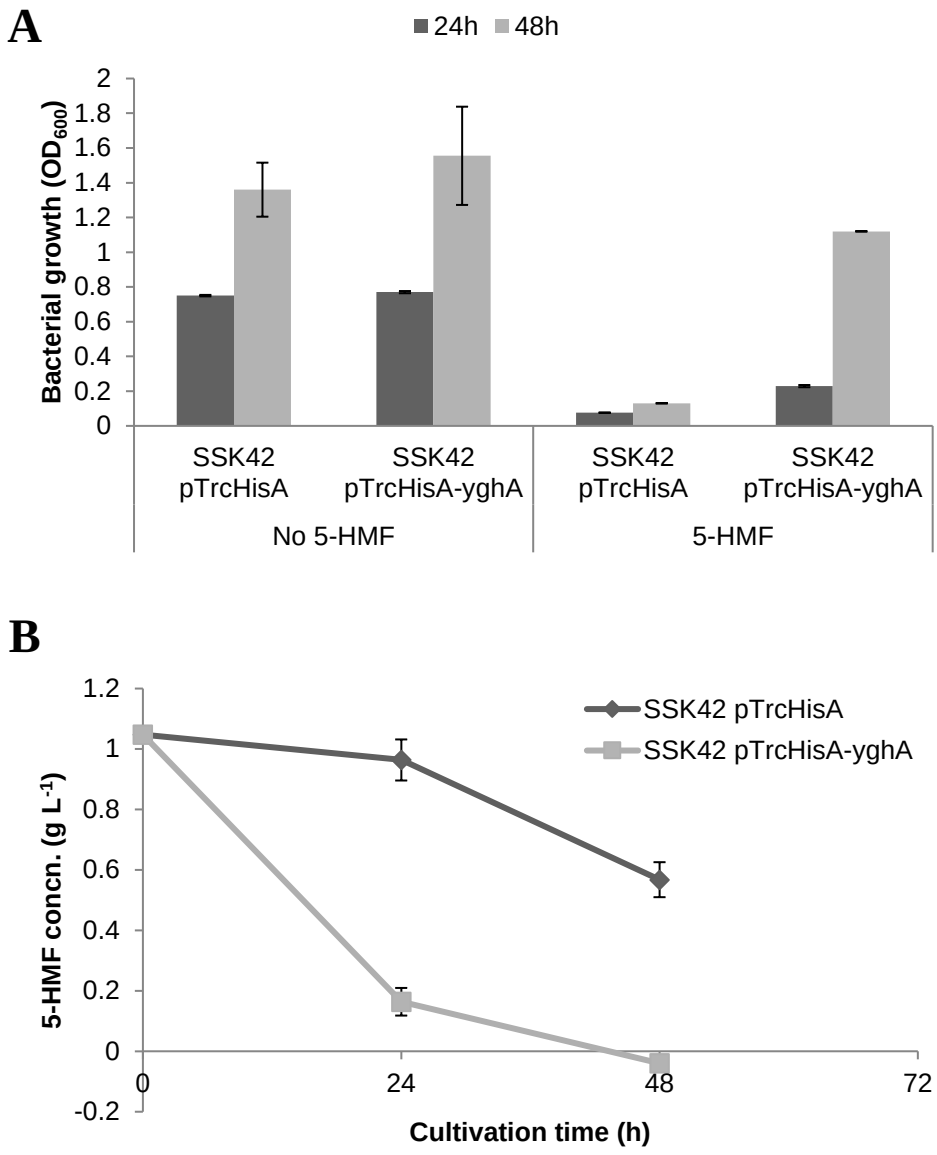

Figure S6: Overexpression of YghA also leads to metabolism of 1 g L^{-1} 5-HMF from media containing 5% (wt/vol) xylose and 0.1 mM IPTG. OD_{600} (A) and concentration of 5-HMF (B) was recorded at 24 h and 48 h. Values are average of n=2 independent experiments. Error bars represent SD of the mean.

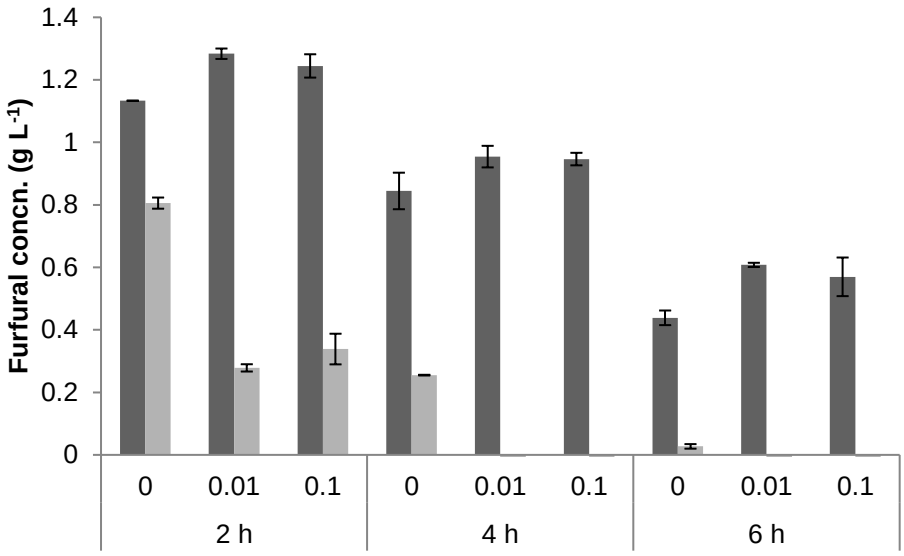

Figure S7: Influence of IPTG concentration on furfural metabolism in SSK42 harboring either pTrcHisA (dark grey) or pTrcHisA-*yghA* (light grey). Strains were seeded at OD_{600} of 2.0 in media containing 5% (wt/vol) xylose and 2.0 g L^{-1} furfural. Concentration of IPTG used was 0, 0.01 and 0.1 mM. Concentration of furfural was measured at 2, 4 and 6 h of growth. Values are average of n=2 independent experiments. Error bars represent SD of the mean.

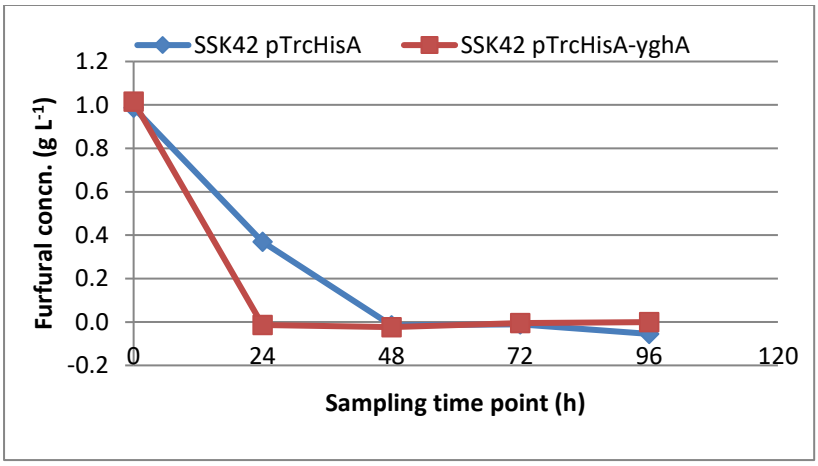

Figure S8: Furfural concentration in bioreactor containing 5% xylose (wt/vol) and 1 g L^{-1} furfural. Samples were analyzed immediately after extraction at indicated time points. Values represent outcome of one experimental observation.