

Metabolic engineering of *Escherichia coli* for the *de novo* stereospecific
biosynthesis of 1,2-propanediol through lactic acid

Wei Niu^{a*}, Levi Kramer^a, Joshua Mueller^a, Kun Liu^b, Jiantao Guo^{b*}

^a Department of Chemical & Biomolecular Engineering, University of Nebraska-Lincoln, Lincoln, Nebraska, 68588, United States.

^b Department of Chemistry, University of Nebraska-Lincoln, Lincoln, Nebraska, 68588, United States.

* To whom correspondence should be addressed: wniu2@unl.edu

Table of Contents

Table S1. Primers used in the study.	Page 3-4
Figure S1. Specific activity of 1,2-PDO pathway enzymes in fed-batch fermentations	Page 5
Table S2. Cell growth at the aerobic stage of fermentations.	Page 6
Figure S2. Glucose accumulation by strain $\Delta^8\text{Ildh}/2.094/2.096$ (280 rpm)	Page 7

Table S1. Primers used in the study.

Primers	Sequences (5' -> 3')
PflB-SacB-F	tcgaagtacgcagtaataaaaaatccacttaagaaggtaggtgttacatgtagtctgcaaatcctttatg
PflB-SacB-R	gagcctttattgtacgcttttactgtacgatttcagtcaaataattatgatctgatcctcaactcag
PflB-F-del	gatatcgcatgcggtaccatgtggctttccggcgagtatg
PflB-R-del-internal	aggtgttactaattagattgactgaaatc
PflB-F-del-internal	tctaattagtaacacctaccttctaag
PflB-R-del	gttgggtccagacaggtatg
FrdA-SacB-F	accctgaagtacgtggctgtgggataaaaaacaatctggaggaatgtcgtg tagtctgcaaatcctttatg
FrdA-SacB-R	cgactccgggttatagcgcaccacctcaatttcagggttttcatctca tgatctgatcctcaactcag
FrdA-F-del	gaaacgtgtctcaaacgggac
FrdA-R-del-internal	caggttttgacattcctccagattgttttatc
FrdA-F-del-internal	gaatgtcaaaaacctgaaaattgaggtggg
FrdA-R-internal	catacgtccttcttaccgtg
AldA-SacB-F	aacaatgtattcaccgaaaacaacatataaatcacaggagtcgccatg tagtctgcaaatcctttatg
AldA-SacB-R	ctctgacgcgcacaggcggaggaaaaaacctccgctcttctactcatta tgatctgatcctcaactcag
AldA-F-del	tatgactggggacaatcccgatg
AldA-R-del-internal	ttcactcagggcgactcctgtgatttatg
AldA-F-del-internal	agtcgccctgagtgaaagaggcggagggttttc
AldA-R-del	gcaccagtcactggtggatg
MgsA-SacB-F	taagtgttacagtaatctgtaggaaagtaactacggatgtacattatgtagtctgcaaatcctttatg
MgsA-SacB-R	ggcgagaaaaccgtaagaaacaggtggcggttgccacctgtgcaatatta tgatctgatcctcaactcag
MgsA-F-del	ctggtggtcagtttaataccag
MgsA-R-del-internal	ctgtgcaatataaatgtacatccgtagttaac
MgsA-F-del-internal	gtacatttaattgacacaggtggcaaacg
MgsA-R-del	ctaaacagcttaaccaatggagac
ArcA-SacB-F	cctttgtactcctgtttcgatttagttggcaatttaggtagcaaacatgtagtctgcaaatcctttatg
ArcA-SacB-R	aaaacggcgctaaaaagcgccgttttttgacgggtgtaagccgattatgatctgatcctcaactcag
ArcA-F-del	tcgttagtcaaccggaatcttc

ArcA-R-del-internal	taaagccgagtttgctacctaattgccaac
ArcA-F-del-internal	gtagcaaaactcggctttaccaccgtcaaaaaaac
ArcA-R-del	taggcaagccatttattgtgattag
IdhA-SacB-F	tatttttagtagctaaatgtgattcaacatcactggagaaagtcttatgtagctgcaaatacctttatg
IdhA-SacB-R	ggggattatctgaatcagctcccctggaatgcaggggagcggcaagattatgatctgatccttcaactcag
IdhA-UP-F	ctattttcctgccagtcagctc
IdhA-UP-R-Ildh	aagactttctccagtgatgttgaatc
Ildh-F	tcactggagaaagtcttatgaaggaatggatattatgtc
Ildh-R	caggggagcggcaagatcatttgcttgttttcagcaag
IdhA-DOWN-F-Ildh	tcttgccgctcccctgcattccag
IdhA-DOWN-R	gaaattgctgcgcgcccagtag

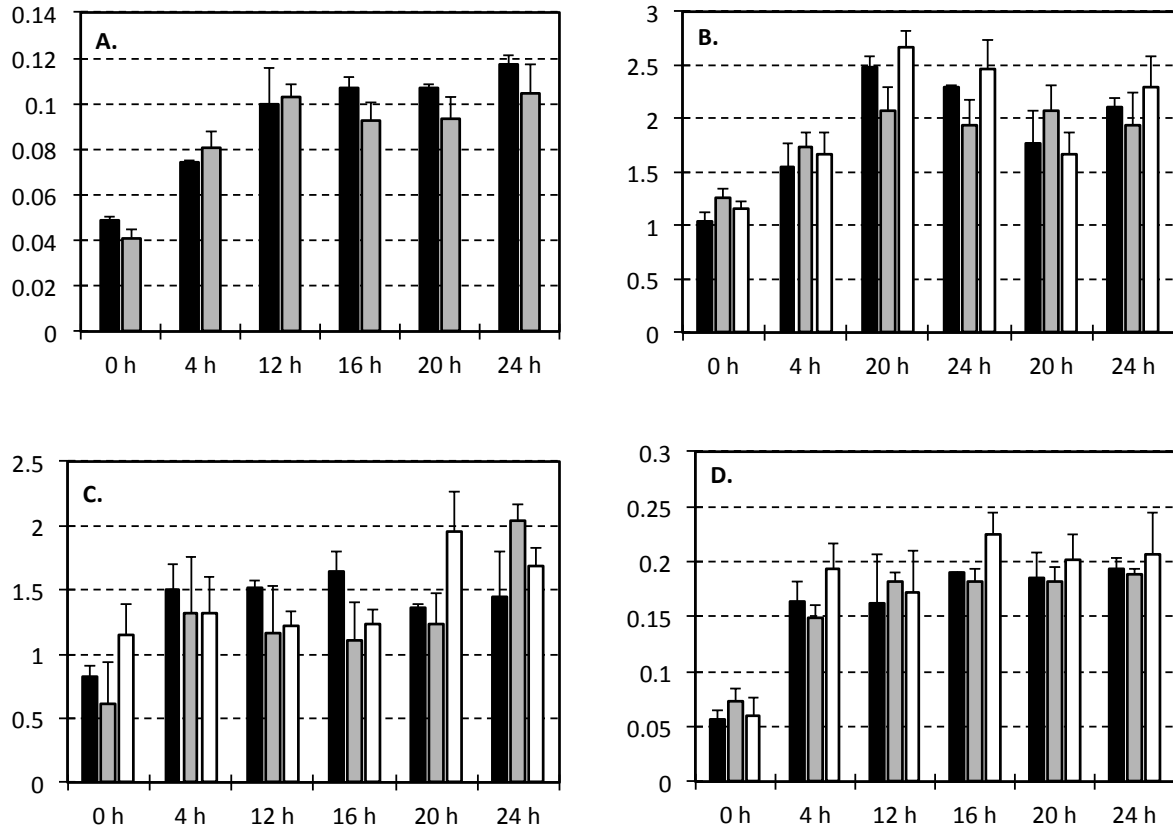


Figure S1. Specific activity of 1,2-PDO pathway enzymes in fed-batch fermentations. All numbers are reported as units of enzyme activity per mg of soluble cell lysate. A. Lactate dehydrogenase (LdhA). One unit of LdhA was defined as the oxidation of 1 μmol of NADH per min at 37 $^{\circ}\text{C}$; B. Lactoyl-CoA transferase (Pct). One unit of Pct was defined as the formation of 1 μmol of lactoyl-CoA per min at 37 $^{\circ}\text{C}$; C. Lactoyl-CoA reductase (PduP). One unit of PduP was defined as the oxidation of 1 μmol of NADH per min at 37 $^{\circ}\text{C}$; D, Lactaldehyde reductase (YahK). One unit of YahK was defined as the oxidation of 1 μmol of NADPH per min at 37 $^{\circ}\text{C}$. In each plot, filled bars in black, $\Delta^7/2.094/2.096$; filled bars in grey, $\Delta^8/2.094/2.096$; open bars, $\Delta^8 \text{ lldh}/2.094/2.096$.

Table S2. Cell growth at the aerobic stage of fermentations.

	<u>$\Delta^7/2.094/2.098$</u>	<u>$\Delta^8/2.094/2.098$</u>	<u>$\Delta^8\text{ldh}/2.094/2.098$</u>	<u>$\Delta^8/2.094/2.098$</u>	<u>$\Delta^8\text{ldh}/2.094/2.098$</u>
time ^a	560 rpm	560 rpm	560 rpm	280 rpm	280 rpm
-8 h	0.33±0.01	0.33±0.02	0.34±0.01	0.32±0.03	0.33±0.01
-2 h	7.1±0.5	6.8±0.7	7.4±0.4	7.1±0.9	7.1±0.4
-1 h	14.7±0.8	14.3±0.6	14.8±0.7	15.0±0.7	16.2±0.6
0 h	23.8±0.4	24.0±0.5	24.7±1.0	23.8±0.3	24.5±0.5

^at = -8h, time of inoculation; t = -1 h, time of induction with IPTG; t = 0 h, initiation of the micro-aerobic stage.

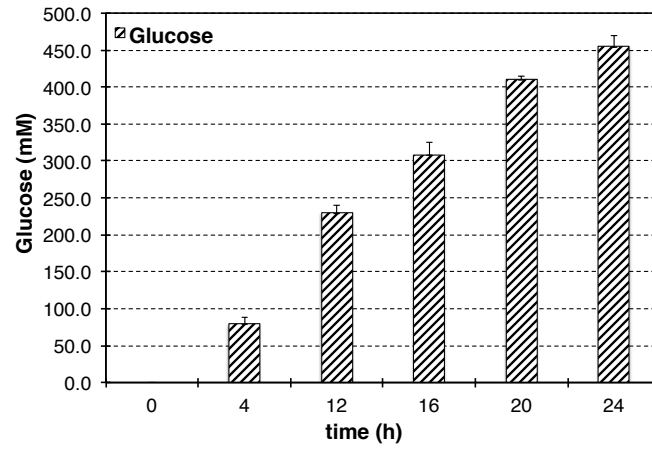


Figure S2. Glucose accumulation by strain Δ^8 Ildh/2.094/2.096 (280 rpm stirring rate).