

## Supplementary information (ESI)

**Table S1.** The primers and digestion sites correspond to the *E. coli* plasmids.

Plasmid	Primer	Restriction enzyme
pET32a(+)-ScFDH	F: 5'-cgcgccggatccATGTCGAAGGGAAAGGTTTGCTGGTT-3' R: 5'-aaatatcgccgcTTTCTTGTCCATAAGCTCTGGTGGCA-3'	BamHI NotI
pET32a(+)-TsFDH	F: 5'-cgcgccggatccATGGCGAAAATCCTGTGC-3' R: 5'-cccgaaaaagttACCCGCTTTTGAAATTGCGC-3'	BamHI HindIII
pET32a(+)-AtFDH	F: 5'-cgcgccggatccATGGCTATGCGTCAGGCGGC-3' R: 5'-cccgccgtcgacACGGTACTGCGGCGCC-3'	BamHI XbaI
pET32a(+)-MtFDH	F: 5'-cgcgccggatccATGGTTAAAGTTCTGGCGGTTCTG-3' R: 5'-cccgaaaaagttGCTGGTGTCAACTTCACGCTG-3'	BamHI HindIII
pACYCDuet-CA	F: 5'-cccgccggatccATGAGCCACCCTGGGGTTACGG-3' R: 5'-cccgaaaaagttTTTGGGAAACCACGAACCTGACGG-3'	EcoRI HindIII

**Table S2.** The primers and digestion sites correspond to the *S. cerevisiae* plasmids.

Plasmid	Primer	Restriction enzyme
pRS424-PGKp	F: 5'-tccgagctcggaACTGTAATTGCTTTAGTTGTG-3' R: 5'-cccgccgccaaTGTGTTATATTGTTGTAAGA-3'	SacI NotI
pRS424-PGKp-tsfdh	F: 5'-aaatatcgccgcATGGCTAAGATTTG-3' R: 5'-cccgccggatccTTAACCCGCTTTTGAAATTGCGC-3'	NotI BamHI
pRS424-PGKp-scfdh	F: 5'-aaatatcgccgcATGTCGAAGGGAAAGGTTTGCTGGTTCTTACG-3' R: 5'-acgcgcgtcgacTTATTTCTCTGTCCATAAGCTCTGGTGGCA-3'	NotI SalI
pRS424-PGKp-tsfdh-TDH3p	F: 5'-ccgcggggatccACAGTTTATTCCCTGGCATCC-3' R: 5'-ccgcgggaaattcTTTGTGTTATGTGTGTTATTGCG-3'	BamHI EcoRI
pRS424-PGKp-tsfdh-TDH3p-ca	F: 5'-ccgcgggaaattcATGTCCCATCATTGGGGTTACGG-3' R: 5'-gcggaaatcgacTTACTTTGGGAAACCTCTAACTTGTCTG-3'	EcoRI SalI
pRS424-PGKp-tsfdh-TDH3p-ca-TEF1p	F: 5'-acgcgtcgacCCACACACCAGCTTCAAA-3'	SalI
pRS424-PGKp-tsfdh-TDH3p-ca-TEF1p-Bica	R: 5'-GCAATCTAATCTAAGATGCAAATCACTAAC-3' R: 5'-ccgggtaccTTAACCCATTTCAGAAGATGGAG-3'	KpnI

**Table S3.** Overview of the kinetic values of NAD<sup>+</sup>-dependent FDHs that have CO<sub>2</sub> reduction capability.

ID	Organism	CO <sub>2</sub> reduction					Formate oxidation					Ref.	
		pH	K <sub>cat</sub> (s <sup>-1</sup> )	K <sub>m</sub> (mM)	K <sub>cat</sub> / K <sub>m</sub> (mM <sup>-1</sup> s <sup>-1</sup> )		pH	K <sub>cat</sub> (s <sup>-1</sup> )	K <sub>m</sub> (mM)	K <sub>cat</sub> / K <sub>m</sub> (mM <sup>-1</sup> s <sup>-1</sup> )			
					1)	2)				3)	4)		
CbFDH	<i>Candida boidinii</i>	5.5	0.015	31.28	0.0004	7	1.08	8.55	0.13	[1]			
CmFDH	<i>Candida methylica</i>	8	0.008	0.78	0.01	8	1.31	7.01	0.187	[2]			
MtFDH	<i>Myceliophthora thermophila</i>	6	0.76	1.2	0.63	10.5	0.32	7.2	0.04	[3]			
CtFDH	<i>Chaetomium thermophilum</i>	5	0.023	0.36	0.069	5	2.04	3.30	0.62	[2]			

TsFDH	<i>Thiobacillus</i> sp. KNK65MA	5.5	0.32	9.23	0.034	6.5	1.77	16.24	0.11	[1]
PoFDH	<i>Pseudomonas oxalaticus</i>	/	3	40	0.08	/	/	0.1	/	[4]
ScFDH	<i>Saccharomyces cerevisiae</i>	/	/	/	/	7	6.5	36	0.18	[5]

- (1) Choe, H.; Joo, J. C.; Cho, D. H.; Kim, M. H.; Lee, S. H.; Jung, K. D.; Kim, Y. H. Efficient CO<sub>2</sub>-Reducing Activity of NAD-Dependent Formate Dehydrogenase from *Thiobacillus* Sp. KNK65MA for Formate Production from CO<sub>2</sub> Gas. *PLoS ONE* **2014**, *9* (7), e103111. <https://doi.org/10.1371/journal.pone.0103111>.
- (2) Aslan, A. S.; Valjakka, J.; Ruupunen, J.; Yildirim, D.; Turner, N. J.; Turunen, O.; Binay, B. *Chaetomium Thermophilum* Formate Dehydrogenase Has High Activity in the Reduction of Hydrogen Carbonate (HCO<sub>3</sub><sup>-</sup>) to Formate. *Protein Eng. Des. Sel.* **2017**, *30* (1), 47–55. <https://doi.org/10.1093/protein/gzw062>.
- (3) Altas, N.; Aslan, A. S.; Karatas, E.; Chronopoulou, E.; Labrou, N. E.; Binay, B. Heterologous Production of Extreme Alkaline Thermostable NAD<sup>+</sup>-Dependent Formate Dehydrogenase with Wide-Range PH Activity from *Myceliophthora Thermophila*. *Process Biochemistry* **2017**, *61*, 110–118. <https://doi.org/10.1016/j.procbio.2017.06.017>.
- (4) Rusching, U.; Muller, U.; Willnow, P.; Hopner, T. CO<sub>2</sub> Reduction to Formate by NADH Catalysed by Formate Dehydrogenase from *Pseudomonas Oxalaticus*. *Eur J Biochem* **1976**, *70* (2), 325–330. <https://doi.org/10.1111/j.1432-1976.tb11021.x>.
- (5) Serov, A. E.; Popova, A. S.; Fedorchuk, V. V.; Tishkov, V. I. Engineering of Coenzyme Specificity of Formate Dehydrogenase from *Saccharomyces Cerevisiae*. *Biochemical Journal* **2002**, *367* (3), 841–847. <https://doi.org/10.1042/bj20020379>.

Table S4. Oxidative specific enzyme activity and reductive specific enzyme activity of the four FDHs at different pH.

FDHs	Formate oxidation [U/mg enzyme]				CO <sub>2</sub> reduction [U/mg enzyme]				
	pH	5	6	7	8	5	6	7	8
ScFDH		22.27±0.84	20.50±0.11	18.52±0.13	11.96±0.19	0.14±0.01	0.12±0.00	0.10±0.01	0.09±0.00
TsFDH		17.37±0.21	15.09±0.97	17.79±0.29	13.56±1.16	0.45±0.03	0.53±0.03	0.50±0.02	0.479±0.04
AtFDH		13.29±0.40	13.48±0.32	14.58±0.16	14.55±0.17	0.27±0.00	0.25±0.01	0.28±0.00	0.251±0.06
MtFDH		25.26±0.79	26.40±0.63	24.11±0.84	21.73±1.26	0.13±0.00	0.16±0.00	0.16±0.01	0.134±0.01

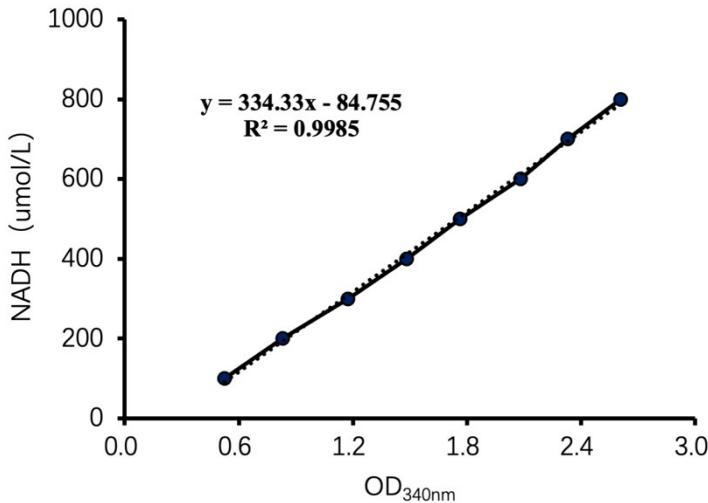


Figure S1 Standard curve of NADH.

← N-terminal loop →

TsFDH/1-401	1	MAKILCQLVYDDPVDGYFKYTDHYPGGQTLPTIKAIDFTPGQLLGGSVS <sub>GEL</sub>	58
AfFDH/1-384	1	MAMRQAAKATIRACSSSSS <sub>G</sub> YFARRQFNASSGDSKKIVGVFYKANEY	63
ScFDH/1-376	1	MSK <sub>G</sub> KVLLVLYEGGKH	31
CbFDH/1-364	1	MKVIVLVLVYDAKGKH	28
CnFDH/1-364	1	MKVIVLVLVYDAKGKH	28
CfFDH/1-370	1	MVKVLAVLVYDGGEH	29
MfFDH/1-367	1	MVKVLAVLVYDGGEH	29
TsFDH/1-401	59	GLRKYLEANGHTFVVTSDKDGP-DSVFEKELVDA <sub>D</sub> W <sub>V</sub> I <sub>S</sub> GPFWPAYLTPERIAKAKNL <sub>L</sub> ALTAGIGSDHVDLQ <sub>A</sub> ID- <sub>-</sub> RQITVAEV <sub>I</sub> YC <sub>N</sub>	147
AfFDH/1-384	64	GIRDWLES <sub>G</sub> HGYIVV <sub>V</sub> DDKEGGP-DCELEKHK <sub>I</sub> PDLHVL <sub>I</sub> STPF <sub>P</sub> AAVTTAEIKRAKAKNL <sub>L</sub> LTAGIGSDHVDLQ <sub>A</sub> AAA- <sub>-</sub> AGLTVT <sub>E</sub> V <sub>I</sub> GSN	152
ScFDH/1-376	32	GIRNFIEFGYELVTTIDKDPPE <sub>I</sub> STVDR <sub>E</sub> LDAE <sub>V</sub> I <sub>V</sub> TTPF <sub>P</sub> AAVTSRNNIAEAPN <sub>L</sub> LCVIA <sub>G</sub> VGSDHVDLQ <sub>A</sub> AAE- <sub>-</sub> RK <sub>I</sub> TT <sub>E</sub> V <sub>I</sub> GSN	121
CbFDH/1-364	29	GIANWLKDQGH <sub>E</sub> L <sub>I</sub> TSDKEGG-N <sub>S</sub> VLDQH <sub>I</sub> PDAD <sub>I</sub> TT <sub>P</sub> PF <sub>P</sub> AAVTSRNNIAEAPN <sub>L</sub> LCVIA <sub>G</sub> VGSDHVDLQ <sub>A</sub> AAE- <sub>-</sub> RK <sub>I</sub> TT <sub>E</sub> V <sub>I</sub> GSN	119
CnFDH/1-364	29	GIANWLKDQGH <sub>E</sub> L <sub>I</sub> TSDKEGG-N <sub>S</sub> VLDQH <sub>I</sub> PDAD <sub>I</sub> TT <sub>P</sub> PF <sub>P</sub> AAVTSRNNIAEAPN <sub>L</sub> LCVIA <sub>G</sub> VGSDHVDLQ <sub>A</sub> AAE- <sub>-</sub> RK <sub>I</sub> TT <sub>E</sub> V <sub>I</sub> GSN	119
CfFDH/1-370	30	GLRKWLEDQGH <sub>E</sub> L <sub>I</sub> TSDKEGP-G <sub>S</sub> TFDRE <sub>E</sub> LADE <sub>V</sub> I <sub>V</sub> TT <sub>P</sub> PF <sub>P</sub> GYLTAELARAAKEL <sub>L</sub> ATAGIGSDHVDLQ <sub>A</sub> AAE- <sub>-</sub> RNGGITVAEV <sub>I</sub> GSN	120
MfFDH/1-367	30	GLRKWLEDQGH <sub>E</sub> L <sub>I</sub> TSDKEGP-N <sub>S</sub> TFDREL <sub>V</sub> DAE <sub>I</sub> TT <sub>P</sub> PF <sub>P</sub> GYLTAELARAAKEL <sub>L</sub> ATAGIGSDHVDLQ <sub>A</sub> AAE- <sub>-</sub> RNGGITVAEV <sub>I</sub> GSN	120
TsFDH/1-401	148	SIISVAEHVVMMLG <sub>L</sub> GLVRNYI <sub>P</sub> SHDWARKGGVNIACDV <sub>E</sub> HSDYLEG <sub>M</sub> VG <sub>V</sub> AA <sub>G</sub> RIGL <sub>G</sub> LAGVRL <sub>L</sub> AP <sub>F</sub> D <sub>V</sub> <sub>E</sub> - <sub>-</sub> LH <sub>T</sub> YDRHRL <sub>P</sub> AEV <sub>E</sub> ELG <sub>G</sub>	235
AfFDH/1-384	153	VVSVTAEDLMR <sub>I</sub> L <sub>I</sub> LMRNFP <sub>P</sub> Q <sub>V</sub> YV <sub>V</sub> KG <sub>E</sub> NNVAG <sub>I</sub> AYRAYDLEG <sub>I</sub> IGT <sub>G</sub> AG <sub>G</sub> RC <sub>G</sub> GCN- <sub>-</sub> LH <sub>H</sub> DR <sub>L</sub> QMAPELE <sub>E</sub> ETG <sub>G</sub>	240
ScFDH/1-376	122	VVSVVAEHV'MA <sub>I</sub> ILVVL <sub>I</sub> LRN <sub>I</sub> INGGGHQ <sub>E</sub> AINGEWDIA <sub>G</sub> V <sub>A</sub> NEYDLE <sub>D</sub> ED <sub>I</sub> I <sub>I</sub> STVGAGRIGY <sub>G</sub> V <sub>E</sub> EL <sub>V</sub> AFNP <sub>I</sub> K <sub>L</sub> YYDYS <sub>G</sub> ELPAE <sub>A</sub> INRLNEAS	213
CbFDH/1-364	120	VVSVVAEHV'VMTMLV <sub>L</sub> LVRNF <sub>P</sub> PAHEQ <sub>I</sub> I <sub>I</sub> NHDWEVA <sub>A</sub> IA <sub>A</sub> RD <sub>D</sub> DE <sub>E</sub> GA <sub>I</sub> TA <sub>I</sub> AG <sub>G</sub> GRIGY <sub>G</sub> V <sub>E</sub> EL <sub>V</sub> VP <sub>N</sub> P <sub>E</sub> LLYYDYS <sub>G</sub> ALPRD <sub>A</sub> E <sub>E</sub> EKVG <sub>G</sub>	208
CnFDH/1-364	120	VVSVVAEHV'VMTMLV <sub>L</sub> LVRNF <sub>P</sub> PAHEQ <sub>I</sub> I <sub>I</sub> NHDWEVA <sub>A</sub> IA <sub>A</sub> RD <sub>D</sub> DE <sub>E</sub> GA <sub>I</sub> TA <sub>I</sub> AG <sub>G</sub> GRIGY <sub>G</sub> V <sub>E</sub> EL <sub>V</sub> VP <sub>N</sub> P <sub>E</sub> LLYYDYS <sub>G</sub> ALPR <sub>A</sub> E <sub>E</sub> EKVG <sub>G</sub>	208
CfFDH/1-370	121	VVSVVAEHV'VMTMLV <sub>L</sub> LVRNF <sub>P</sub> PAHEQ <sub>I</sub> I <sub>I</sub> NHDWEVA <sub>A</sub> IA <sub>A</sub> RD <sub>D</sub> DE <sub>E</sub> GA <sub>I</sub> TA <sub>I</sub> AG <sub>G</sub> GRIGY <sub>G</sub> V <sub>E</sub> EL <sub>V</sub> VP <sub>N</sub> P <sub>E</sub> LLYYDYS <sub>G</sub> ALPR <sub>A</sub> E <sub>E</sub> EKVG <sub>G</sub>	209
MfFDH/1-367	121	VVSVVAEHV'VMTMLV <sub>L</sub> LVRNF <sub>P</sub> PAHEQ <sub>I</sub> I <sub>I</sub> NHDWEVA <sub>A</sub> IA <sub>A</sub> RD <sub>D</sub> DE <sub>E</sub> GA <sub>I</sub> TA <sub>I</sub> AG <sub>G</sub> GRIGY <sub>G</sub> V <sub>E</sub> EL <sub>V</sub> VP <sub>N</sub> P <sub>E</sub> LLYYDYS <sub>G</sub> ALPR <sub>A</sub> E <sub>E</sub> EKVG <sub>G</sub>	209
TsFDH/1-401	236	- <sub>-</sub> LWVHDTREDMYPHCDVV <sub>I</sub> LNVP <sub>L</sub> PH <sub>E</sub> <sub>I</sub> EHMINDE <sub>I</sub> TKLKF <sub>G</sub> RAYIVNTARGKLADR <sub>I</sub> AI <sub>A</sub> RAIE <sub>I</sub> Q <sub>G</sub> LAGYAGDVW <sub>P</sub> Q <sub>P</sub> A <sub>P</sub> K	318
AfFDH/1-384	241	- <sub>-</sub> A <sub>F</sub> VEDLENLMP <sub>K</sub> CD <sub>V</sub> V <sub>I</sub> V <sub>I</sub> NMP <sub>L</sub> TEK <sub>I</sub> RGMF <sub>N</sub> KEL <sub>I</sub> G <sub>L</sub> KE <sub>G</sub> VL <sub>I</sub> V <sub>N</sub> NARGA <sub>I</sub> MA <sub>E</sub> AVVDA <sub>E</sub> VE <sub>E</sub> AV <sub>G</sub> GH <sub>I</sub> GGY <sub>E</sub> SDVW <sub>P</sub> Q <sub>P</sub> A <sub>P</sub> K	323
ScFDH/1-376	214	KLFN <sub>G</sub> R <sub>G</sub> D <sub>I</sub> V <sub>I</sub> Q <sub>V</sub> E <sub>K</sub> LED <sub>M</sub> V <sub>A</sub> Q <sub>D</sub> SV <sub>V</sub> I <sub>I</sub> NC <sub>L</sub> HKD <sub>I</sub> S <sub>R</sub> GLF <sub>N</sub> KL <sub>I</sub> B <sub>H</sub> MKD <sub>G</sub> A <sub>V</sub> L <sub>V</sub> NT <sub>A</sub> RG <sub>A</sub> IC <sub>V</sub> AE <sub>D</sub> V <sub>A</sub> <sub>E</sub> AV <sub>G</sub> GH <sub>I</sub> GG <sub>G</sub> YGDVW <sub>K</sub> Q <sub>P</sub> A <sub>P</sub> K	303
CbFDH/1-364	209	- <sub>-</sub> ARRV <sub>E</sub> EN <sub>I</sub> EELV <sub>A</sub> QAD <sub>I</sub> V <sub>V</sub> NA <sub>P</sub> L <sub>H</sub> AG <sub>T</sub> K <sub>G</sub> L <sub>I</sub> N <sub>E</sub> LL <sub>S</sub> RF <sub>K</sub> KG <sub>G</sub> AW <sub>V</sub> NT <sub>A</sub> RG <sub>A</sub> IC <sub>V</sub> AE <sub>D</sub> V <sub>A</sub> <sub>E</sub> AA <sub>A</sub> LE <sub>E</sub> Q <sub>G</sub> QLR <sub>G</sub> Y <sub>G</sub> GDVW <sub>P</sub> Q <sub>P</sub> A <sub>P</sub> K	291
CnFDH/1-364	209	- <sub>-</sub> ARRV <sub>E</sub> EN <sub>I</sub> EELV <sub>A</sub> QAD <sub>I</sub> V <sub>V</sub> NA <sub>P</sub> L <sub>H</sub> AG <sub>T</sub> K <sub>G</sub> L <sub>I</sub> N <sub>E</sub> LL <sub>S</sub> RF <sub>K</sub> KG <sub>G</sub> AW <sub>V</sub> NT <sub>A</sub> RG <sub>A</sub> IC <sub>V</sub> AE <sub>D</sub> V <sub>A</sub> <sub>E</sub> AA <sub>A</sub> LE <sub>E</sub> Q <sub>G</sub> QLR <sub>G</sub> Y <sub>G</sub> GDVW <sub>P</sub> Q <sub>P</sub> A <sub>P</sub> K	291
CfFDH/1-370	210	- <sub>-</sub> CRRVENLEEMLAQCDVV <sub>V</sub> I <sub>C</sub> NPL <sub>H</sub> EST <sub>R</sub> GLF <sub>N</sub> KD <sub>L</sub> I <sub>S</sub> SMK <sub>R</sub> G <sub>S</sub> W <sub>V</sub> NT <sub>A</sub> RG <sub>A</sub> IV <sub>V</sub> K <sub>E</sub> ED <sub>V</sub> A <sub>E</sub> AL <sub>R</sub> IGH <sub>R</sub> GY <sub>G</sub> GDVW <sub>P</sub> Q <sub>P</sub> A <sub>P</sub> K	292
MfFDH/1-367	210	- <sub>-</sub> CRVYLDEEMLGQCDVV <sub>V</sub> I <sub>C</sub> NPL <sub>H</sub> E <sub>K</sub> RG <sub>L</sub> ND <sub>L</sub> I <sub>A</sub> MK <sub>P</sub> G <sub>S</sub> W <sub>V</sub> NT <sub>A</sub> RG <sub>A</sub> IV <sub>V</sub> K <sub>E</sub> ED <sub>V</sub> A <sub>E</sub> AL <sub>R</sub> IGH <sub>R</sub> GY <sub>G</sub> GDVW <sub>P</sub> Q <sub>P</sub> A <sub>P</sub> K	292
TsFDH/1-401	319	DHPWRTIM <sub>P</sub> W <sub>-</sub> - <sub>-</sub> E <sub>G</sub> MTP <sub>H</sub> I <sub>S</sub> G <sub>T</sub> SL <sub>S</sub> QA <sub>A</sub> RYA <sub>A</sub> ACT <sub>T</sub> REILECFFEG <sub>E</sub> <sub>P</sub> <sub>I</sub> P <sub>I</sub> D <sub>E</sub> Y <sub>E</sub> L <sub>I</sub> V <sub>I</sub> Q <sub>G</sub> GA <sub>L</sub> AG <sub>T</sub> G <sub>A</sub> H <sub>S</sub> Y <sub>S</sub> K <sub>G</sub> N <sub>A</sub> T <sub>G</sub> <sub>G</sub> SEEAAKF <sub>K</sub> K <sub>G</sub>	401
AfFDH/1-384	324	DHPWRTIYMP <sub>N</sub> <sub>-</sub> - <sub>-</sub> QAMTP <sub>H</sub> TS <sub>G</sub> T <sub>I</sub> DAQ <sub>L</sub> Y <sub>A</sub> AG <sub>Z</sub> K <sub>D</sub> ML <sub>E</sub> R <sub>F</sub> FK <sub>G</sub> <sub>E</sub> -DF <sub>F</sub> TEN <sub>X</sub> IV <sub>K</sub> D <sub>G</sub> EL <sub>A</sub> PO <sub>W</sub> <sub>R</sub> <sub>-</sub> -	384
ScFDH/1-376	306	DHPWRTIM <sub>D</sub> <sub>M</sub> <sub>D</sub> KDHVG <sub>N</sub> AM <sub>V</sub> H <sub>I</sub> S <sub>G</sub> T <sub>S</sub> DAQ <sub>R</sub> Y <sub>A</sub> AG <sub>V</sub> KN <sub>I</sub> LN <sub>S</sub> Y <sub>F</sub> SK <sub>I</sub> F <sub>D</sub> Y <sub>P</sub> Q <sub>D</sub> <sub>I</sub> IV <sub>Q</sub> GS <sub>A</sub> TR <sub>A</sub> W <sub>G</sub> Q <sub>K</sub> <sub>K</sub>	376
CbFDH/1-364	292	DHPWRTDM <sub>N</sub> <sub>K</sub> Y <sub>G</sub> AG <sub>N</sub> AM <sub>T</sub> PH <sub>S</sub> GT <sub>T</sub> LD <sub>A</sub> Q <sub>T</sub> RY <sub>A</sub> EG <sub>T</sub> KN <sub>I</sub> LE <sub>S</sub> FT <sub>G</sub> K <sub>F</sub> D <sub>Y</sub> <sub>P</sub> Q <sub>D</sub> <sub>I</sub> LL <sub>N</sub> GE <sub>Y</sub> IT <sub>K</sub> AG <sub>K</sub> <sub>K</sub>	364
CnFDH/1-364	292	DHPWRTDM <sub>N</sub> <sub>K</sub> Y <sub>G</sub> AG <sub>N</sub> AM <sub>T</sub> PH <sub>S</sub> GT <sub>T</sub> LD <sub>A</sub> Q <sub>T</sub> RY <sub>A</sub> EG <sub>T</sub> KN <sub>I</sub> LE <sub>S</sub> FT <sub>G</sub> K <sub>F</sub> D <sub>Y</sub> <sub>P</sub> Q <sub>D</sub> <sub>I</sub> LL <sub>N</sub> GE <sub>Y</sub> IT <sub>K</sub> AG <sub>K</sub> <sub>K</sub>	364
CfFDH/1-370	293	DHVLRTAK <sub>N</sub> <sub>F</sub> GGGNAM <sub>V</sub> P <sub>H</sub> MS <sub>G</sub> T <sub>S</sub> DAQ <sub>R</sub> Y <sub>A</sub> EG <sub>V</sub> KR <sub>I</sub> LDS <sub>L</sub> Y <sub>S</sub> SG <sub>F</sub> D <sub>Y</sub> <sub>P</sub> ED <sub>L</sub> I <sub>V</sub> HOG <sub>K</sub> Y <sub>A</sub> TR <sub>A</sub> Y <sub>G</sub> Q <sub>R</sub> ED <sub>V</sub> K <sub>I</sub> <sub>P</sub> <sub>G</sub>	370
MfFDH/1-367	293	DHPLRTAK <sub>N</sub> <sub>F</sub> GGGNAM <sub>V</sub> P <sub>H</sub> MS <sub>G</sub> T <sub>S</sub> DAQ <sub>R</sub> Y <sub>A</sub> EG <sub>V</sub> KR <sub>I</sub> LES <sub>L</sub> Y <sub>S</sub> SG <sub>F</sub> D <sub>Y</sub> <sub>P</sub> ED <sub>L</sub> I <sub>V</sub> HOG <sub>K</sub> Y <sub>A</sub> TR <sub>A</sub> Y <sub>G</sub> Q <sub>R</sub> EV <sub>S</sub> TS <sub>-</sub>	367

← C-terminal loop →

Figure S2 Sequence comparison of FDHs

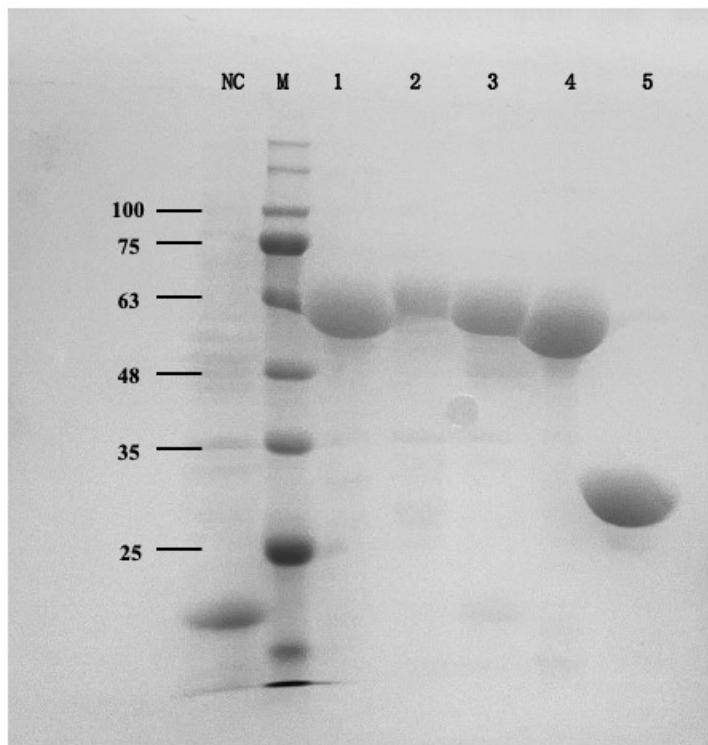


Figure S3. SDS-PAGE analysis of purified FDHs. Lane NC: negative control, Lane M: molecular mass marker (245kDa), 1: ScFDH (59.4 kDa), 2: TsFDH (62.2 kDa), 3: AtFDH (60.3 kDa), 4: MtFDH (58.4 kDa), 5: CA (28.6 kDa).

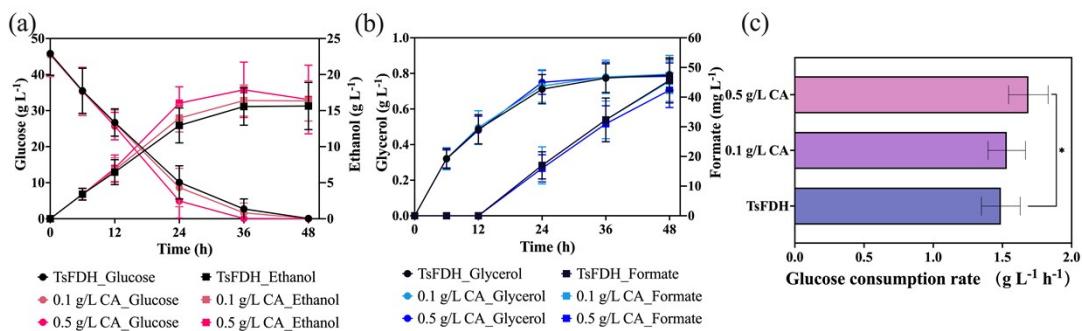


Figure S4. Fermentation properties of the *S. cerevisiae-tsfdh* (TsFDH) with different concentrations Carbonic Anhydrase (0, 0.1, 0.5 g L<sup>-1</sup> CA) was conducted at 30 °C and 180 rpm in SD media. (a) glucose and ethanol concentrations; (b) glycerol and formate concentrations; (c) glucose consumption rate. Carbonic anhydrase enzyme concentration measured by BSA method.

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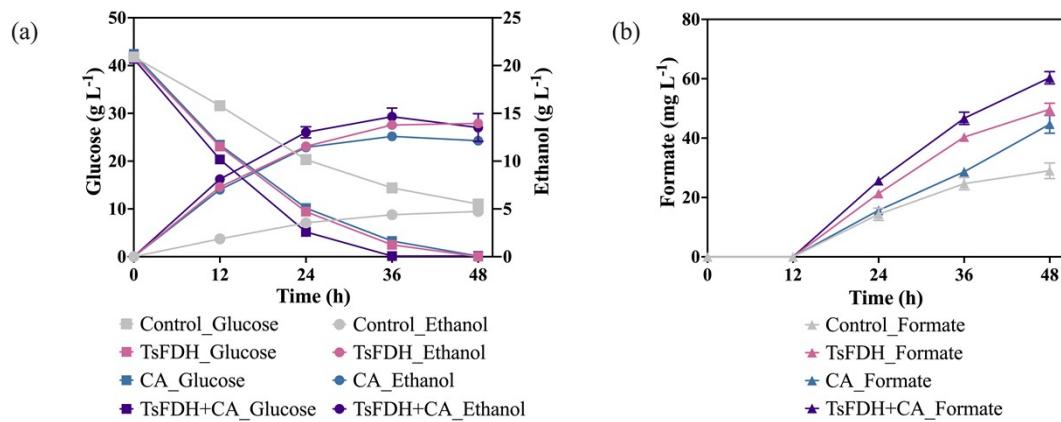


Figure S5. Fermentation properties of the *S. cerevisiae*-control, *S. cerevisiae-tsfdh* (TsFDH), *S. cerevisiae-tsfdh-ca* (CA) and *S. cerevisiae-tsfdh-ca-bica* (TsFDH + CA) were conducted at 30 °C and 180 rpm in SC media. (a) glucose and ethanol concentration; (b) formate concentration.



Figure S6. A tail gas analyzer measured the CO<sub>2</sub> released during the fermentation process in real-time.