Supplementary information (ESI)

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

Plasmid	Primer	Restriction enzyme
pET32a(+)-ScFDH	F: 5'-cgcgcgggatccATGTCGAAGGGAAAGGTTTTGCTGGTT-3'	*BamH*I
	R: 5'-aaatatgcggccgcTTTCTTCTGTCCATAAGCTCTGGTGGCA-3'	*Not*I
pET32a(+)-TsFDH	F: 5'-cgcgcgggatccATGGCGAAAATCCTGTGC-3'	*BamH*I
	R: 5'-cccgggaagcttACCCGCTTTTTTGAATTTTGCC-3'	*Hind*III
pET32a(+)-AtFDH	F: 5'-cgcgcgggatccATGGCTATGCGTCAGGCGGC-3'	*BamH*I
	R: 5'-ccgcggctcgagACGGTACTGCGGCGCC-3'	*Xho*I
pET32a(+)-MtFDH	F: 5'-cgcgcgggatccATGGTTAAAGTTCTGGCGGTTCTG-3'	*BamH*I
	R: 5'-cccgggaagcttGCTGGTGCTAACTTCACGCTG-3'	*Hind*III
pACYCDuet-CA	F: 5'-ccgcgggaattcATGAGCCACCACTGGGGTTACGG-3'	*EcoR*I
	R: 5'-cccgggaagcttTTTCGGGAAACCACGAACCTGACGG-3'	*Hind*III

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

Plasmid	Primer	Restriction enzyme
pRS424-PGKp	F: 5'-tccgagctcggaACTGTAATTGCTTTTAGTTGTG-3'	*Sac*I
	R: 5'-ccgcggccgcaaTGTTTTATATTTGTTGTAAAA-3'	*Not*I
pRS424-PGKp-tsfdh	F: 5'-aaatatgcggccgcATGGCTAAGATTTTG-3'	*Not*I
	R: 5'-cgcgcgggatccTTAACCCGCTTTTTTGAATTTTGCC-3'	*BamH*I
pRS424-PGKp-scfdh	F: 5'-aaatatgcggccgcATGTCGAAGGGAAAGGTTTTGCTGGTTCTTTACG-3'	*Not*I
	R: 5'-acgcgcgtcgacTTATTTCTTCTGTCCATAAGCTCTGGTGGCA-3'	*Sal*I
pRS424-PGKp-tsfdh-TDH3p	F: 5'-cgcgcgggatccACAGTTTATTCCTGGCATCC-3'	*BamH*I
	R: 5'-ccgcgggaattcTTTGTTTGTTTATGTGTGTTTATTCG-3'	*EcoR*I
pRS424-PGKp-tsfdh-TDH3p-ca	F: 5'-ccgcgggaattcATGTCCCATCATTGGGGTTACGG-3'	*EcoR*I
	R: 5'-gcggaagtcgacTTACTTTGGGAAACCTCTAACTTGTCTG-3'	*Sal*I
pRS424-PGKp-tsfdh-TDH3p-ca-TEF1p	F: 5'-acgcgtcgacCCACACACCATAGCTTCAAAA-3'	*Sal*I
	R: 5'-GTTAGTGATTTGCATCTTAGATTAGATTGC-3'	
pRS424-PGKp-tsfdh-TDH3p-ca-TEF1p-Bica	F: 5'-GCAATCTAATCTAAGATGCAAATCACTAAC-3'	
	R: 5'-cggggtaccTTAACCCATTTCAGAAGATGGAG-3'	*Kpn*I

Table S3. Overview of the kinetic values of NAD^+-dependent FDHs that have CO_2 reduction capability.

ID	Organism	CO_2 reduction				Formate oxidation				Ref.
		pH	K_{cat} (s^{-1})	K_m (mM)	K_{cat}/K_m ($mM^{-1} s^{-1}$)	pH	K_{cat} (s^{-1})	K_m (mM)	K_{cat}/K_m ($mM^{-1} s^{-1}$)	
CbFDH	*Candida boidinii*	5.5	0.015	31.28	0.0004	7	1.08	8.55	0.13	[1]
CmFDH	*Candida methylica*	8	0.008	0.78	0.01	8	1.31	7.01	0.187	[2]
MtFDH	*Myceliophthora thermophila*	6	0.76	1.2	0.63	10.5	0.32	7.2	0.04	[3]
CtFDH	*Chaetomium thermophulum*	5	0.023	0.36	0.069	5	2.04	3.30	0.62	[2]

TsFDH	*Thiobacillus* sp. KNK65MA	5.5	0.32	9.23	0.034	6.5	1.77	16.24	0.11	[1]
PoFDH	*Pseudomonas oxalaticus*	/	3	40	0.08	/	/	0.1	/	[4]
ScFDH	*Saccharomyces cerevisiae*	/	/	/	/	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_2-Reducing Activity of NAD-Dependent Formate Dehydrogenase from *Thiobacillus* Sp. KNK65MA for Formate Production from CO_2 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_3^-) to Formate. *Protein Eng. Des. Sel.* **2017**, *30* (1), 47–55. https://doi.org/10.1093/protein/gzw062.

(3) Altaş, N.; Aslan, A. S.; Karataş, E.; Chronopoulou, E.; Labrou, N. E.; Binay, B. Heterologous Production of Extreme Alkaline Thermostable NAD^+-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_2 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.1432-1033.1976.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_2 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

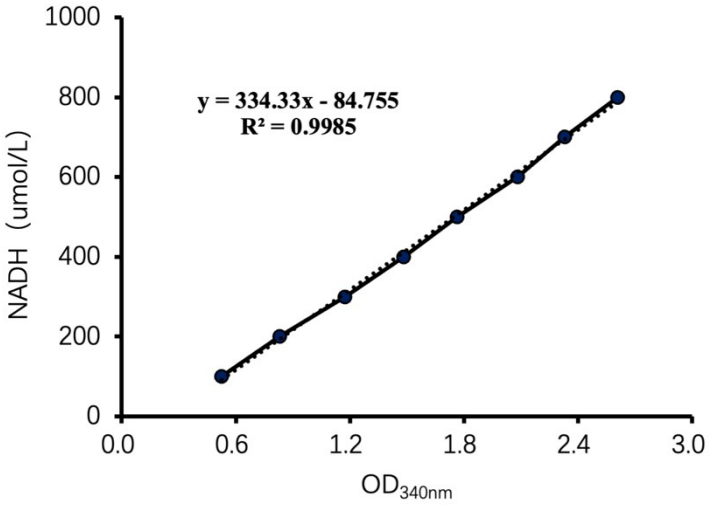

Figure S1 Standard curve of NADH.

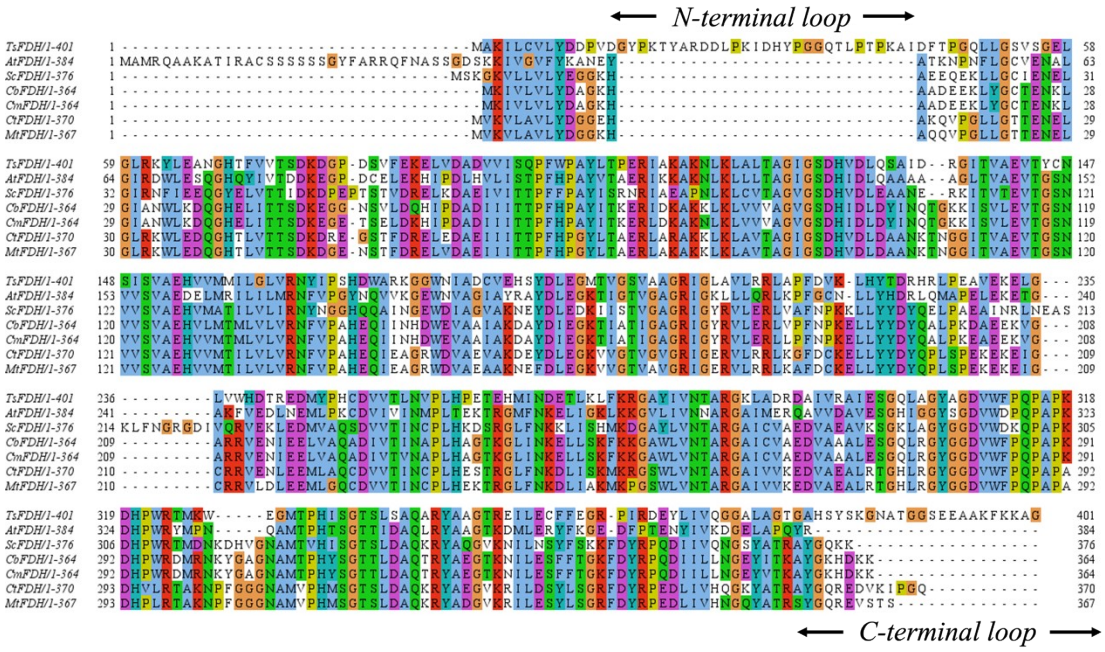

Figure S2 Sequence comparison of FDHs

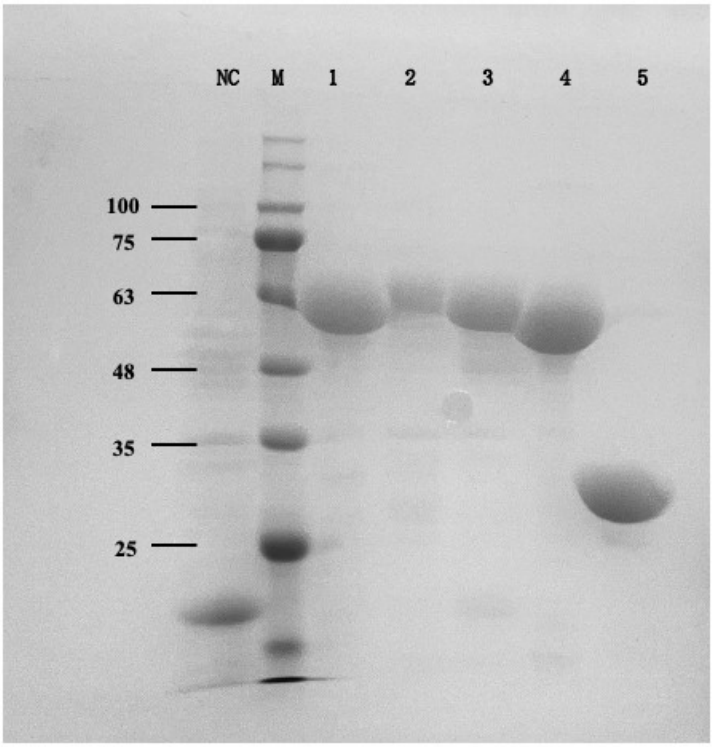

Figure S3. SDS-PAGE analysis of purified FDHs. Lane NC: negative control, Lane M: molecular mass marker (245kDa), 1: ScFDH (59.4 kDa), 2: TsFDH (62.2 kDa), 3: AtFDH (60.3 kDa), 4: MtFDH (58.4 kDa), 5: CA (28.6 kDa).

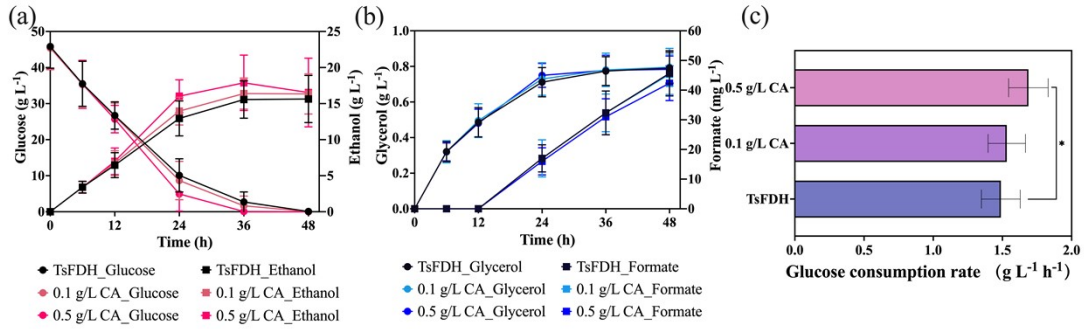

Figure S4. Fermentation properties of the *S. cerevisiae-tsfdh* (TsFDH) with different concentrations Carbonic Anhydrase (0, 0.1, 0.5 g L^{-1} CA) was conducted at 30 °C and 180 rpm in SD media. (a) glucose and ethanol concentrations; (b) glycerol and formate concentrations; (c) glucose consumption rate.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

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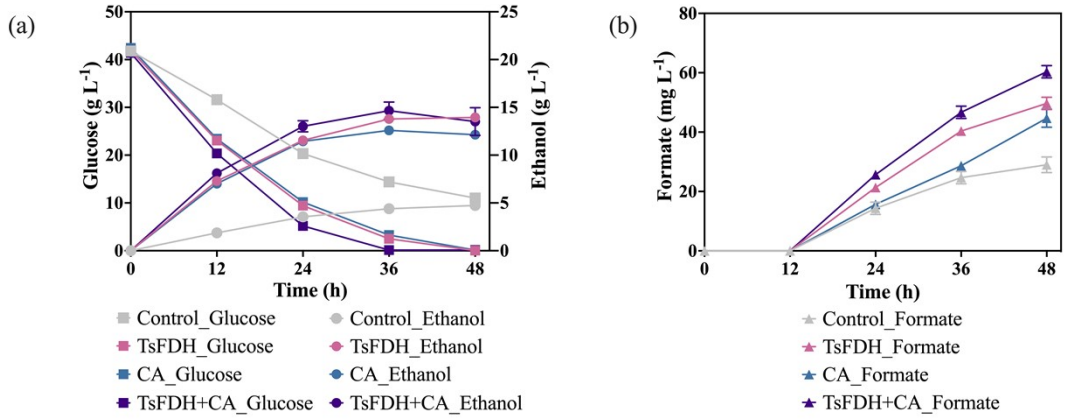

Figure S5. Fermentation properties of the *S. cerevisiae*-control, *S. cerevisiae-tsfdh* (TsFDH), *S. cerevisiae-tsfdh-ca* (CA) and *S. cerevisiae-tsfdh-ca-bica* (TsFDH + CA) were conducted at 30 °C and 180 rpm in SC media. (a) glucose and ethanol concentration; (b) formate concentration.

Figure S6. A tail gas analyzer measured the CO_2 released during the fermentation process in real-time.