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Low-Level Organic Solvents Improve Multienzyme Whole-Cell Catalytic Synthesis of Myricetin-7-*O*-glucuronide

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1. Tables

Table S1. The yields of myricetin-7-*O*-glucuronide (M7GA) and the solubilities of myricetin or M7GA obtained in the modified M9 reaction system containing different concentrations of organic solvents.

No.	Solvents	Reagents concentration (%, v/v)	M7GA yield (μg/mL)	Relative yield (%) [a]	Conversion (%)	Myricetin solubility (μg/mL)	Relative solubility (%) [b]	M7GA solubility (μg/mL)	Relative solubility (%) [c]
1	Control (M9)	—	71.4 ± 5.4	100	7.2	12.4 ± 1.3	100	678.5 ± 39.5	100
2	Acetone	2	934.7 ± 100.3	1309	94.6	33.5 ± 1.4	270	683.5 ± 45.3	101
3		5	664.8 ± 70.6	931	67.3	36.5 ± 6.4	295	717.3 ± 50.0	106
4		10	51.9 ± 17.9	73	5.3	287.7 ± 47.5	2320	856.6 ± 74.5	126
5	N, N–dimethylformamide (DMF)	2	671.2 ± 68.7	940	67.9	25.5 ± 1.5	206	735.4 ± 10.9	108
6		5	476.8 ± 36.2	668	48.3	117.1 ± 2.2	944	777.0 ± 12.9	115
7		10	223.3 ± 22.0	313	22.6	606.7 ± 10.6	4893	773.8 ± 24.6	114
9	Dimethyl sulfoxide (DMSO)	2	641.1 ± 5.2	898	64.9	27.9 ± 1.9	225	679.1 ± 25.5	100
10		5	497.6 ± 38.8	697	50.4	64.6 ± 10.8	521	781.6 ± 52.8	115
11		10	377.9 ± 51.7	529	38.2	326.9 ± 5.7	2636	815.6 ± 92.5	120
	Ethanol (EtOH)	2	970.4 ± 79.3	1359	98.2	14.4 ± 1.5	116	677.7 ± 29.8	100
13		5	709.0 ± 60.2	993	71.8	21.1 ± 0.6	170	690.2 ± 60.1	102
14		10	64.2 ± 11.1	90	6.5	70.1 ± 3.8	565	704.7 ± 52.9	104
16	Methanol (MeOH)	2	986.1 ± 19.1	1381	99.8	14.9 ± 0.6	120	712.2 ± 26.5	105
17		5	762.1 ± 88.5	1067	77.1	25.9 ± 6.1	209	752.1 ± 64.6	111
18		10	252.1 ± 25.5	353	25.5	34.2 ± 1.4	276	889.4 ± 91.1	131

[a] The yield of M7GA obtained in the reaction system containing organic solvents relative to the one obtained in the reaction system without organic solvents (control group).

[b] The solubility of myricetin obtained in the modified M9 medium containing organic solvents relative to the one obtained in the modified M9 medium (control).

[c] The solubility of M7GA obtained in the modified M9 medium containing organic solvents relative to the one obtained in the modified M9 medium (control).

Table S2. The proportion of the live and dead cells as determined by FCM.

Solvents	Live cells (%)	Dead cells (%)	Solvents	Live cells (%)	Dead cells (%)
Acetone (2%)	96.0	4.0	EtOH (2%)	97.7	2.3
Acetone (5%)	95.0	5.0	EtOH (5%)	96.2	3.8
Acetone (10%)	87.3	12.7	EtOH (10%)	90.5	9.5
Acetone (20%)	18.4	81.6	EtOH (20%)	2.6	97.4
DMF (2%)	95.7	4.3	MeOH (2%)	97.0	3.0
DMF (5%)	94.7	5.3	MeOH (5%)	97.3	2.7
DMF (10%)	95.0	5.0	MeOH (10%)	95.5	4.5
DMF (20%)	53.9	46.1	MeOH (20%)	26.4	73.6
DMSO (2%)	97.6	2.4	Control-BPGUT	97.7	2.3
DMSO (5%)	96.5	3.5	Control-wild	92.1	7.9
DMSO (10%)	96.7	3.3	Myricetin (2 mM)	91.3	8.7
DMSO (20%)	59.5	40.5	M7GA (2 mM)	92.8	7.2

Table S3. Concentrations of proteins released from *E. coli* BPGUT cells after being exposed to organic solvents for 6h.

Solvents	Extracellular protein concentration (μg/mL)	Relative concentration (%) [a]	Solvents	Extracellular protein concentration (μg/mL)	Relative concentration (%) [a]
Acetone (2%)	22.9 ± 4.7	114.9	EtOH (2%)	36.3 ± 7.2	182.3
Acetone (5%)	37.0 ± 5.2	186.1	EtOH (5%)	75.8 ± 9.7	380.8
Acetone (10%)	60.9 ± 11.0	305.9	EtOH (10%)	130.2 ± 14.9	654.1
Acetone (20%)	165.9 ± 19.5	833.8	EtOH (20%)	202.4 ± 17.9	1017.2
DMF (2%)	32.6 ± 5.6	163.6	MeOH (2%)	31.8 ± 2.6	159.9
DMF (5%)	34.0 ± 3.4	171.1	MeOH (5%)	35.5 ± 2.2	178.6
DMF (10%)	34.8 ± 5.2	174.8	MeOH (10%)	72.0 ± 11.0	362.0
DMF (20%)	72.0 ± 4.7	362.0	MeOH (20%)	119.7 ± 4.7	601.7
DMSO (2%)	26.6 ± 4.5	133.7	Control	19.9 ± 4.5	100.0
DMSO (5%)	26.6 ± 5.9	133.7			
DMSO (10%)	39.3 ± 2.6	197.3			
DMSO (20%)	63.1 ± 5.6	317.1			

[a] The content of extracellular proteins leaked from the organic solvents-treated cells relative to the one leaked from the untreated cells (control group).

Table S4. Concentrations of proteins released from *E. coli* BPGUT cells after being exposed to organic solvents for 12 h.

Solvents	Extracellular protein concentration (μg/mL)	Relative concentration (%) [a]	Solvents	Extracellular protein concentration (μg/mL)	Relative concentration (%) [a]
Acetone (2%)	55.6 ± 5.2	160.2	EtOH (2%)	59.1 ± 1.7	170.3
Acetone (5%)	81.0 ± 4.3	233.4	EtOH (5%)	68.3 ± 3.1	196.8
Acetone (10%)	148.7 ± 9.7	428.5	EtOH (10%)	157.7 ± 7.1	454.3
Acetone (20%)	188.0 ± 14.0	541.8	EtOH (20%)	195.5 ± 7.9	563.4
DMF (2%)	41.2 ± 0.9	118.6	MeOH (2%)	52.6 ± 3.1	151.6
DMF (5%)	51.6 ± 6.0	148.8	MeOH (5%)	58.1 ± 3.0	167.4
DMF (10%)	54.6 ± 9.9	157.4	MeOH (10%)	143.7 ± 11.3	414.2
DMF (20%)	88.0 ± 2.6	253.5	MeOH (20%)	161.1 ± 3.0	464.4
DMSO (2%)	34.2 ± 4.0	98.6	Control	34.7 ± 2.3	100.0
DMSO (5%)	43.2 ± 1.5	124.4			
DMSO (10%)	47.1 ± 6.0	135.9			
DMSO (20%)	72.5 ± 1.7	209.0			

[a] The content of extracellular proteins leaked from the organic solvents-treated cells relative to the one leaked from the untreated cells (control group).

2. Figures

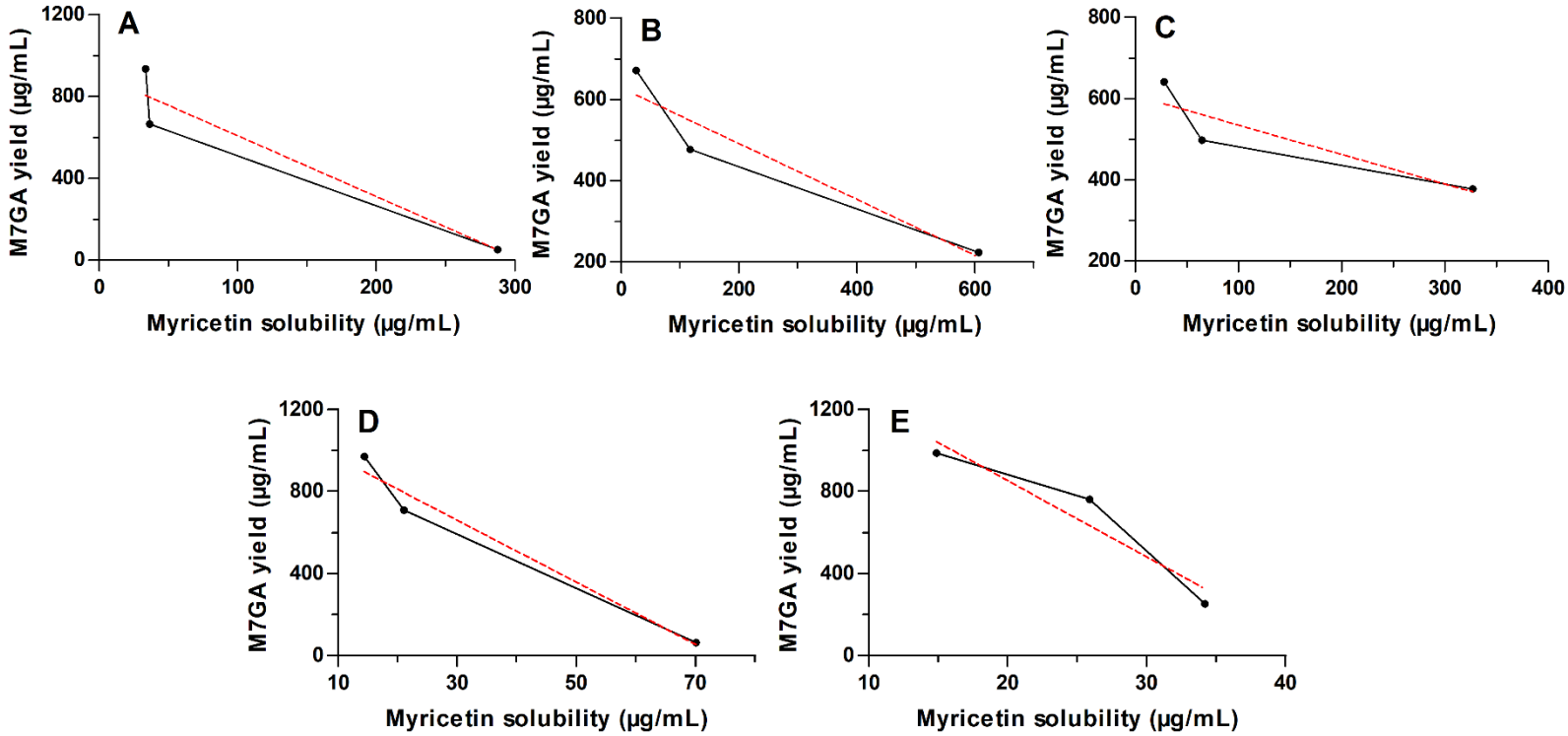

Figure S1. Correlations between the myricetin-7-*O*-glucuronide (M7GA) yield and the solubility of myricetin obtained in the modified M9 reaction system containing different concentrations of organic solvents. (A) Acetone; (B) DMF; (C) DMSO; (D) EtOH; (E) MeOH.

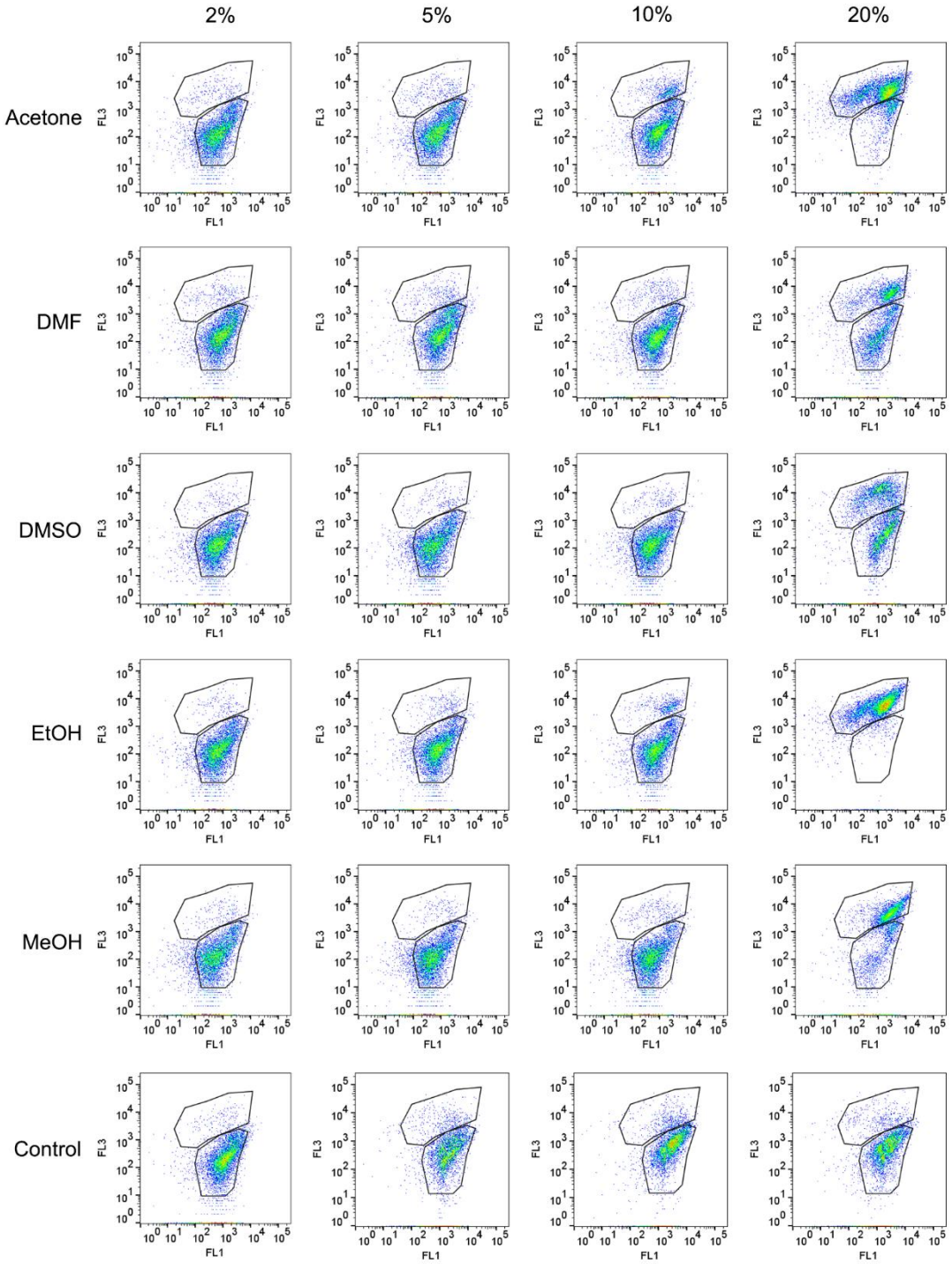

Figure S2. FCM images of *E. coli* BPUGT cells or wild type cells after being treated for 6 h at 30 °C in a modified M9 solution containing 2% (v/v), 5% (v/v), 10% (v/v) and 20% (v/v) acetone, DMSO, DMF, EtOH and MeOH, or myricetin (2 mM) and myricetin-7-*O*-glucuronide (2 mM), respectively.

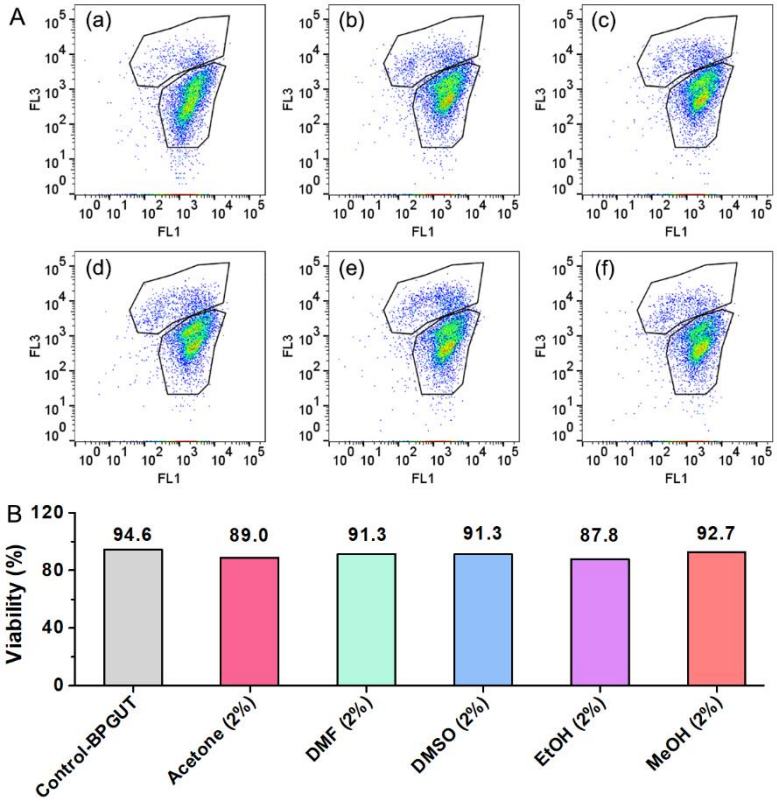

Figure S3. Viability of *E. coli* BPUGT cells after being treated for 12 h at 30 °C in a modified M9 solution containing 2% (v/v) organic solvents. (A) FCM images of untreated (a, control-BPGUT), acetone-treated (b), DMF-treated (c), DMSO-treated (d), EtOH-treated (e) and MeOH-treated (f) *E. coli* BPUGT cells; (B) The cell viability as determined by FCM.

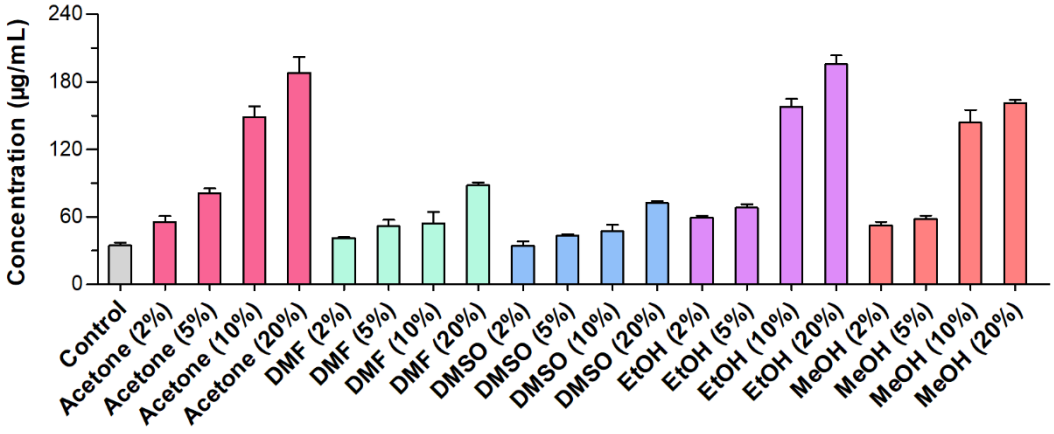

Figure S4. Concentrations of proteins released from *E. coli* BPGUT cells after the cells were exposed to 2% (v/v), 5% (v/v), 10% (v/v) and 20% (v/v) acetone, DMF, DMSO, EtOH, and MeOH for 12 h, respectively.

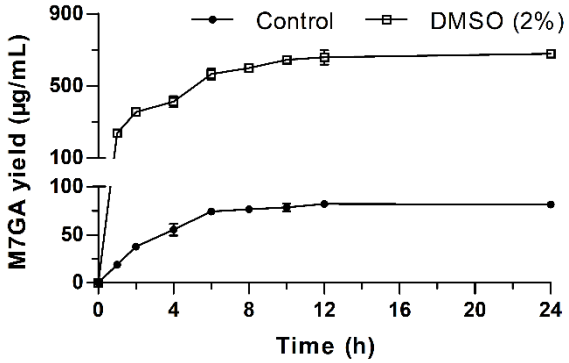

Figure S5. Time course for the production of myricetin-7-*O*-glucuronide (M7GA) obtained in a 100-mL shake-flask system containing 10 mL of whole-cell catalyst. Myricetin solid powder (6.36 mg) or stock solution (100 mM, dissolved in DMSO) was added to the above whole-cell biocatalyst system to form a working concentration of 2 mmol/L, which was then kept shaking at 30 °C for 24 h.