Supporting information

Contracted but effective: production of enantiopure 2,3-butanediol by thermophilic and GRAS *Bacillus licheniformis*

Yongsheng Ge [a, §], Kun Li [a, §], Lixiang Li [a, b], Chao Gao [a,*], Lijie Zhang [a], Cuiqing Ma [a, **], and Ping Xu [b]

[a] State Key Laboratory of Microbial Technology, Shandong University, Jinan 250100, China

[b] State Key Laboratory of Microbial Metabolism, Shanghai Jiao Tong University, Shanghai 200240, China

§ These authors contributed equally to this work.

**Corresponding author: Fax: +8653188369463.

*Corresponding author: Fax: +8653188364003.

E-mail addresses: macq@sdu.edu.cn (C. Ma), jieerbu@sdu.edu.cn (C. Gao).

Table S1 Primers used in this work

Primer [a]	Sequence (5'–3') [b]	Use
E*budC*-f	AAAGAATTCGTTAATTAAATACCATTCCGC	Amplification of *budC*
E*budC*-r	AAAGTCGACATGAGTAAAGTATCTGGAAA	Amplification of *budC*
Δ*budC*-L-f	AAACCATGGAATAAACGAGTTGACGGAAA	Amplification of left homologous arm of *budC*
Δ*budC*-L-r	GCAAAGCAATTGCGGTTAAATTGCATTAAAACGCTTATCC	Amplification of left homologous arm of *budC*
Δ*budC*-R-f	GGATAAGCGTTTTAATGCAATTTAACCGCAATTGCTTTGC	Amplification of right homologous arm of *budC*
Δ*budC*-R-r	TTTGGATCCTATGCTCGCGGTGTTCTAT	Amplification of right homologous arm of *budC*
E*gdh*-f	AAAGGATCCATGTCAAAATCAGTAAAATCAG	Amplification of *gdh*
E*gdh*-r	TTTAAGCTTTTAATCGTGATAAGATTCTGC	Amplification of *gdh*
Δ*gdh*-L-f	ATTTAGATCTAACAAGCCGCGTCATTCAAG	Amplification of left homologous arm of *gdh*
Δ*gdh*-L-r	ACTTGGCGCCATTCTTCTTCGACACATCGCAAATGATA	Amplification of left homologous arm of *gdh*
Δ*gdh*-R-f	TATCATTTGCGATGTGTCGAAGAAGAATGGCGCCAAGT	Amplification of right homologous arm of *gdh*
Δ*gdh*-R-r	GGAGTACCGTGGATCCGCTTTAAG	Amplification of right homologous arm of *gdh*

[a] "f" of primer name means the sense primer; "r" of primer name means antisense primers; "L" of primer name means the left homologous arm

of the target gene; “R” of primer name means means the right homologous arm of the target gene.

[b] Restriction sites are underlined.

Table S2 Microbial production of (2*R*,3*R*)-2,3-BD.

Strain	Concentration (g/L)	Yield (g/g)	Productivity (g/[L h])	Reference
Paenibacillus polymyxa	111.0	ND[a]	2.1	[1]
Escherichia coli	6.1	0.31	0.13	[2]
Clostridium acetobutylicum	1.98	0.45	0.01	[3]
E. coli	9.54	0.34	0.20	[4]
Saccharomyces cerevisiae	43.6	0.227	0.2	[5]
S. cerevisiae	100.0	0.35	0.33	[6]
Enterobacter cloacae	152.0	0.488	3.5	[7]
E. coli	115	0.42	1.44	[8]
Bacillus licheniformis	30.76	0.25	1.28	[9]
Klebsiella pneumonia	61	0.36	0.51	[10]
Serratia marcescens	89.81	0.35	1.91	[11]
Klebsiella oxytoca	106.7	0.40	3.1	[12]
B. licheniformis	123.7	0.565	2.95	This work

[a] ND, not determined.

Table S3 Microbial production of *meso*-2,3-BD.

Strain	Concentration (g/L)	Yield (g/g)	Productivity (g/[L h])	Reference
E. coli	4.2	0.42	0.17	[13]
E. coli	1.12	0.29	0.009	[14]
E. coli	14.1	0.321	0.2	[15]
E. coli	15.7	0.31	0.33	[16]
E. coli	13	0.415	0.36	[17]
E. coli	73.8	0.41	1.19	[18]
S. marcescens	152	0.463	2.67	[19]
E. coli	17.7	0.175	0.31	[20]
B. licheniformis	90.1	0.492	2.82	This work

Table S4 Scenarios of *meso*-2,3-BD production using *E. coli* BL21/pET-RABC and *B. licheniformis* MW3 (Δ*gdh*)

Description	*E. coli* BL21/pET-RABC[a]		*B. licheniformis* MW3 (Δ*gdh*)	
	Real	Theoretical	Real	Theoretical
Input bacteria (g)[b]	0.12		16.32	
Output bacteria (g)[b]	4.21		412.8	
Initial fermentation volume (L)	0.8		40	
Final fermentation volume (L)	0.8		40	
Input glucose (g)	151.2		7580	
Final glucose concentration (g/L)	9		6.5	
Output glucose in final volume (g)	7.2		260	
Input nutrients in final volume (g)	12.50		846	
Output nutrients in final volume (g)[c]	4.25[b]		287.64[b]	
Mass of AC (g/L)	9.34	0	3.6	0
Mass of AC in total volume (g)	7.47	0	144	0

Mass of *meso*-2,3-BD (g/L)	73.8	90[d]	90.1	91.5[d]
Mass of *meso*-2,3-BD in total volume (g)	59.04	72	3604	3660
E-factor[e]	0.392[f]	0.218[f]	0.306[f]	0.262[f]

[a] Xu et al. [18] for the fed-batch production of *meso*-2,3-BD using *E. coli* BL21/pET-RABC. [b] Dry cell weight was converted from optical density using the following equations: for *E. coli* BL21/pET-RABC, DCW (g/L) = 0.39× OD 620 nm ; for *B. licheniformis* MW3 (Δ*gdh*), DCW (g/L) = 0.48 × OD 620 nm .[c] For calculations the excess of nutrients was considered to be 34% like Matos et al.[21]. [d] Recalculated from 0.5 g *meso*-2,3-BD/g glucose. [e] E-factor=(mass of wastes)/(mass of products). [f] Calculated considering output of glucose, bacteria, nutrients and AC as a waste.

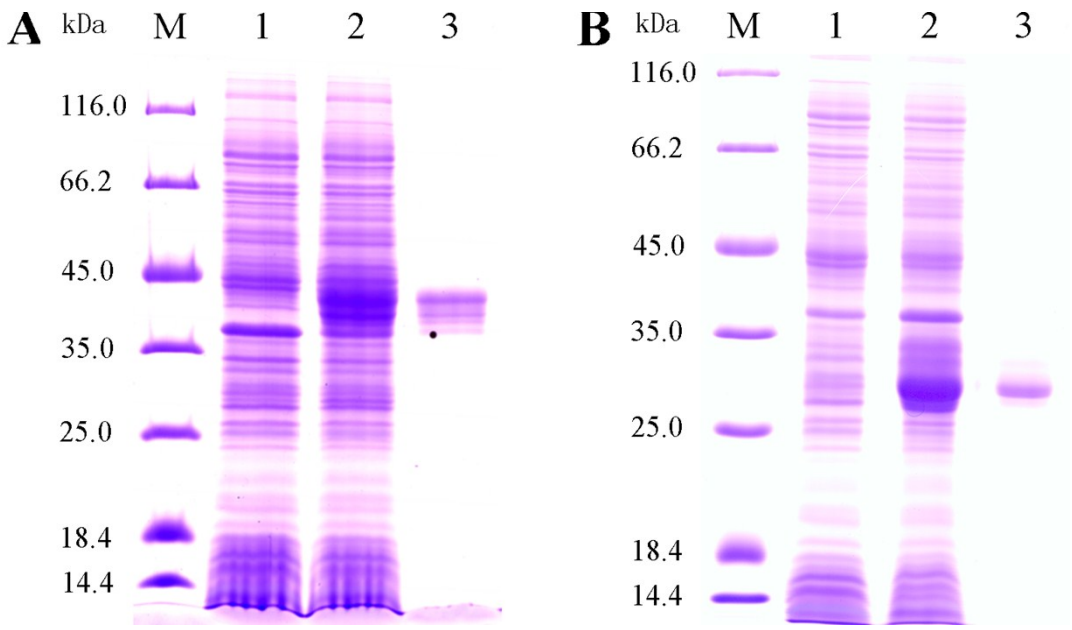

Fig. S1. SDS-PAGE of the purified 2*R,*3*R*-BDH and *meso*-BDH. A, SDS-PAGE analysis of the purified 2*R,*3*R*-BDH: lane M, marker; lane 1, crude extract of *E. coli* BL21 (DE3) (pET28a); lane 2, crude extract of *E. coli* BL21 (DE3) (pET28a-*gdh*); lane 3, purified 2*R,*3*R*-BDH. B, SDS-PAGE analysis of the purified *meso*-BDH: lane M, marker; lane 1, crude extract of *E. coli* BL21 (DE3) (pETDuet-1); lane 2, crude extract of *E. coli* BL21 (DE3) (pETDuet-*budC*); lane 3, purified *meso*-BDH.

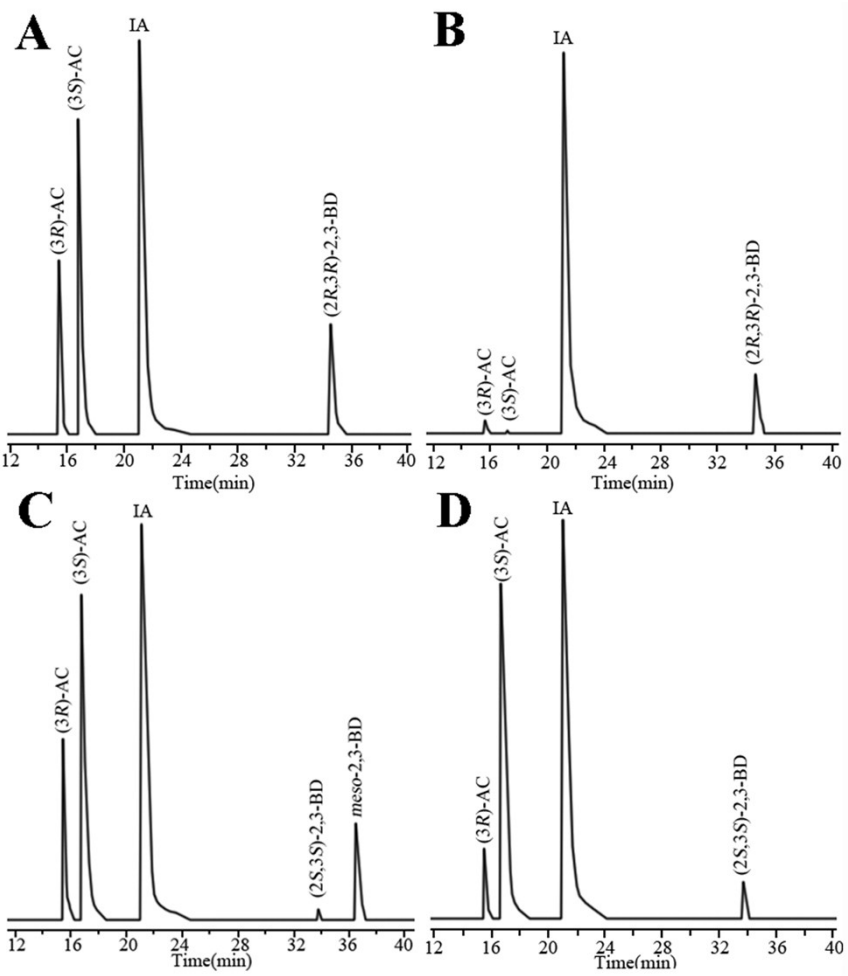

Fig. S2. GC analysis of the reaction products of 2*R,*3*R*-BDH (A, B) and *meso*-BDH (C, D). A, GC analysis of the 2*R,*3*R*-BDH catalyzed products of racemic AC with NADH as a cofactor. B, GC analysis of the 2*R,*3*R*-BDH catalyzed products of DA with NADH as a cofactor. C, GC analysis of the *meso*-BDH catalyzed products of racemic AC with NADH as a cofactor. D, GC analysis of the *meso*-BDH catalyzed products of DA with NADH as a cofactor. IA, Isoamyl alcohol was used as the internal standard.

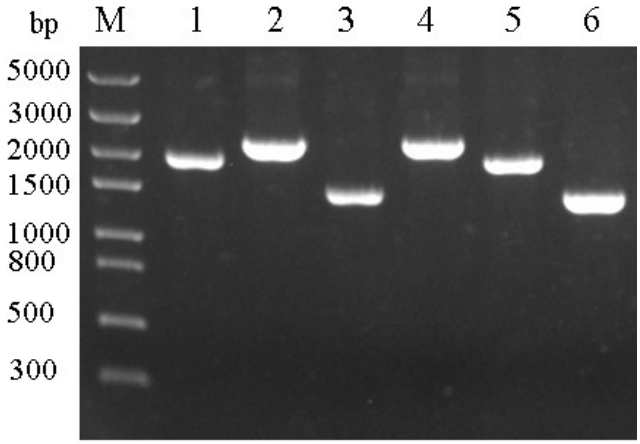

Fig. S3. PCR verification of recombinant strains. Lane M, marker; lane 1, fragment amplified from the genomic DNA of *B. licheniformis* MW3 using primers of Δ*budC*-L-f and Δ*budC*-R-r; lane 2, fragment amplified from the genomic DNA of *B. licheniformis* MW3 using primers of Δ*gdh*-L-f and Δ*gdh*-R-r; lane 3, fragment amplified from the genomic DNA of *B. licheniformis* MW3 (Δ*budC*) using primers of Δ*budC*-L-f and Δ*budC*-R-r; lane 4, fragment amplified from the genomic DNA of *B. licheniformis* MW3 (Δ*budC*) using primers of Δ*gdh*-L-f and Δ*gdh*-R-r; lane 5, fragment amplified from the genomic DNA of *B. licheniformis* MW3 (Δ*gdh*) using primers of Δ*budC*-L-f and Δ*budC*-R-r; lane 6, fragment amplified from the genomic DNA of *B. licheniformis* MW3 (Δ*gdh*) using primers of Δ*gdh*-L-f and Δ*gdh*-R-r.

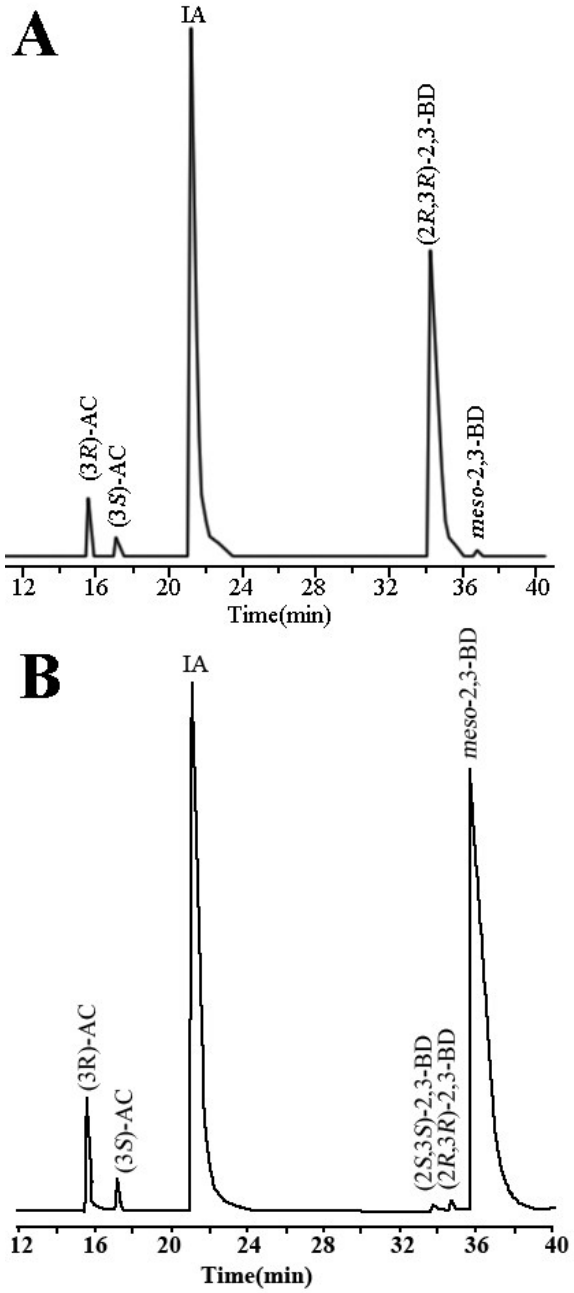

Fig. S4. GC analysis of the 2,3-BD produced by strain *B. licheniformis* MW3 (Δ*budC*) (A), *B. licheniformis* MW3 (Δ*gdh*) (B). IA, isoamyl alcohol was used as the internal standard.

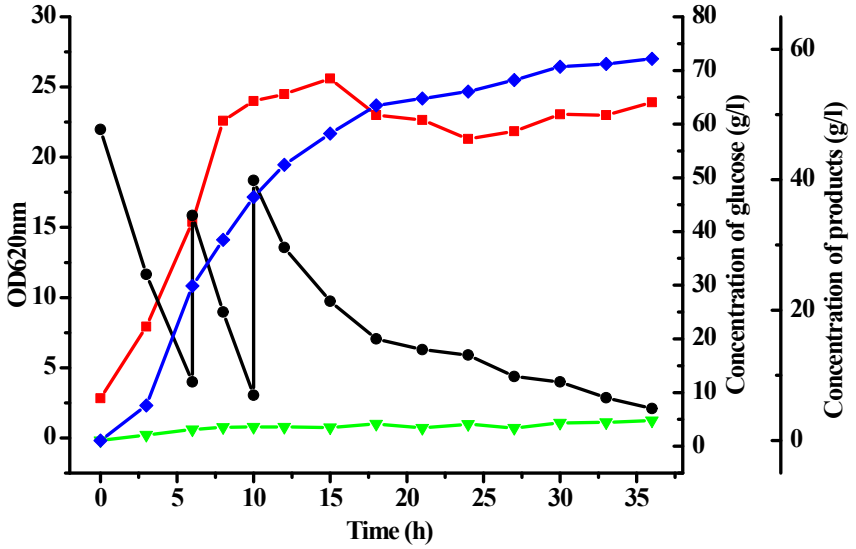

Fig. S5. Time-course of fed-batch fermentation by *B. licheniformis* MW3 (Δ*gdh*) in 1-L bioreactor. ●, Glucose; ■, OD620 nm;◆, 2,3-BD; ▼, AC.

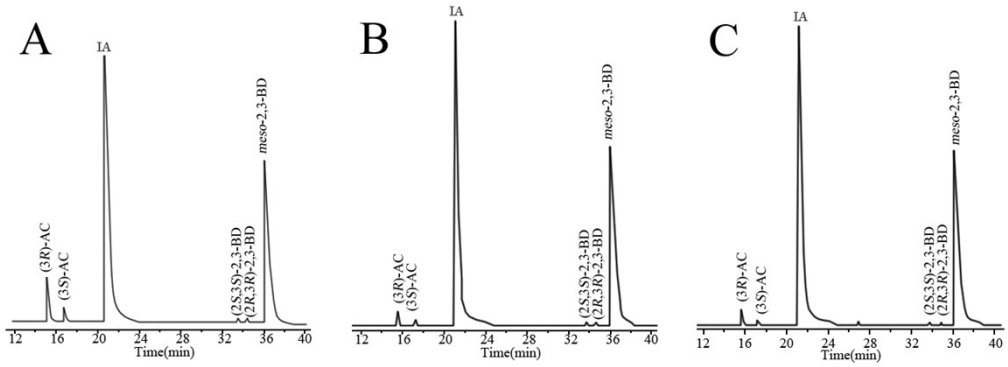

Fig. S6. GC analysis of the *meso*-2,3-BD produced by strain *B. licheniformis* MW3 (Δ*gdh*) in 1-L (A), 5-L (B), and 50-L (C) bioreactor. IA, Isoamyl alcohol was used as the internal standard.

References

1. T. Häßler, D. Schieder, R. Pfaller, M. Faulstich and V. Sieber, *Bioresour. Technol.*, 2012, **124**, 237–244.
2. Y. Yan, C. C. Lee and J. C. Liao, *Org. Biomol. Chem.*, 2009, **7**, 3914–3917.
3. M. A. Siemerink, W. Kuit, A. M. L. Contreras, G. Eggink, J. van der Oost and S. W. Kengen, *Appl. Environ. Microbiol.*, 2011, **77**, 2582–2588.
4. X. Shen, Y. Lin, R. Jain, Q. Yuan and Y. Yan, *J. Ind. Microbiol. Biotechnol.*, 2012, **39**, 1725–1729.
5. S. J. Kim, S. O. Seo, Y.-C. Park, Y. S. Jin and J. H. Seo, *J. Biotechnol.*, 2014, **192**, 376–382.
6. J. Lian, R. Chao and H. Zhao, *Metab. Eng.*, 2014, **23**, 92–99.
7. L. Li, K. Li, Y. Wang, C. Chen, Y. Xu, L. Zhang, B. Han, C. Gao, F. Tao and C. Ma, *Metab. Eng.*, 2015, **28**, 19–27
8. X. J. Ji, L. G. Liu, M. Q. Shen, Z. K. Nie, Y. J. Tong and H. Huang, *Biotechnol. Bioeng.*, 2015, **112**, 1056–1059.
9. G. Qi, Y. Kang, L. Li, A. Xiao, S. Zhang, Z. Wen, D. Xu and S. Chen, *Biotechnol. Biofuels*, 2014, **7**, 16.
10. S. Lee, B. Kim, J. Yang, D. Jeong, S. Park and J. Lee, *J. Biotechnol.*, 2015, **209**, 7–13.
11. F. Bai, L. Dai, J. Fan, N. Truong, B. Rao, L. Zhang and Y. Shen, *J. Ind. Microbiol. Biotechnol.*, 2015, **42**, 779–786.
12. J. M. Park, C. Rathnasingh and H. Song, *J. Ind. Microbiol. Biotechnol.*, 2015, **42**, 1–7.
13. H. D. Shin, S. H. Yoon, J. Wu, C. Rutter, S. W. Kim and R. R. Chen, *Bioresour. Technol.*, 2012, **118**, 367–373.

14. D. R. Nielsen, S. H. Yoon, C. J. Yuan and K. L. Prather, *Biotechnol. J.*, 2010, **5**, 274–284.
15. S. Mazumdar, J. Lee and M.-K. Oh, *Bioresour. Technol.*, 2013, **136**, 329–336.
16. S. Lee, B. Kim, K. Park, Y. Um and J. Lee, *Appl. Biochem. Biotechnol.*, 2012, **166**, 1801–1813.
17. Z. J. Li, J. Jian, X. X. Wei, X. W. Shen and G. Q. Chen, *Appl. Microbiol. Biotechnol.*, 2010, **87**, 2001–2009.
18. Y. Xu, H. Chu, C. Gao, F. Tao, Z. Zhou, K. Li, L. Li, C. Ma and P. Xu, *Metab. Eng.*, 2014, **23**, 22–33.
19. L. Zhang, J. A. Sun, Y. Hao, J. Zhu, J. Chu, D. Wei and Y. Shen, *J. Ind. Microbiol. Biotechnol.*, 2010, **37**, 857–862.
20. S. Ui, Y. Okajima, A. Mimura, H. Kanai and T. Kudo, *J. Ferment. Bioeng.*, 1997, **84**, 185–189.
21. C. T. Matos, L. Gouveia, A. R. C. Morais, A. Reis and R. Bogel-Lukasik, *Green Chem*., 2013, **15**, 2854−2864.