Supplementary information

A strategy to increase rebaudioside A content based on one-step bioconversion of Stevia extract to steviol

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Supporting Figures

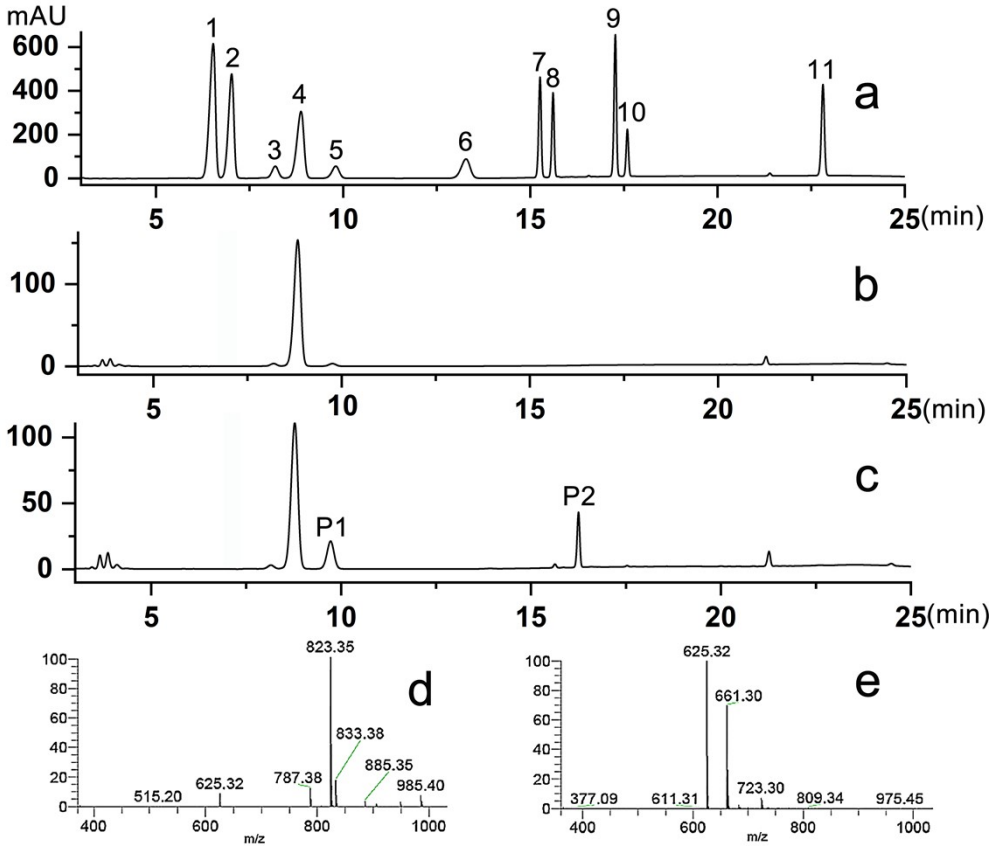

Fig. S1 HPLC and MS analysis of RC hydrolysis by SBGL3. (a): Standard: RA (1), ST (2), rebaudioside F (3), RC (4), dulcoside A (5), RU (6), RB (7), steviolbioside (8), steviol 19-glycoside (9), steviolmonoside (10), SV (11); (b): RC standard; (c): RC was catalyzed by SBGL3; (d) and (e): The MS analysis of P1 and P2 marked in (c).

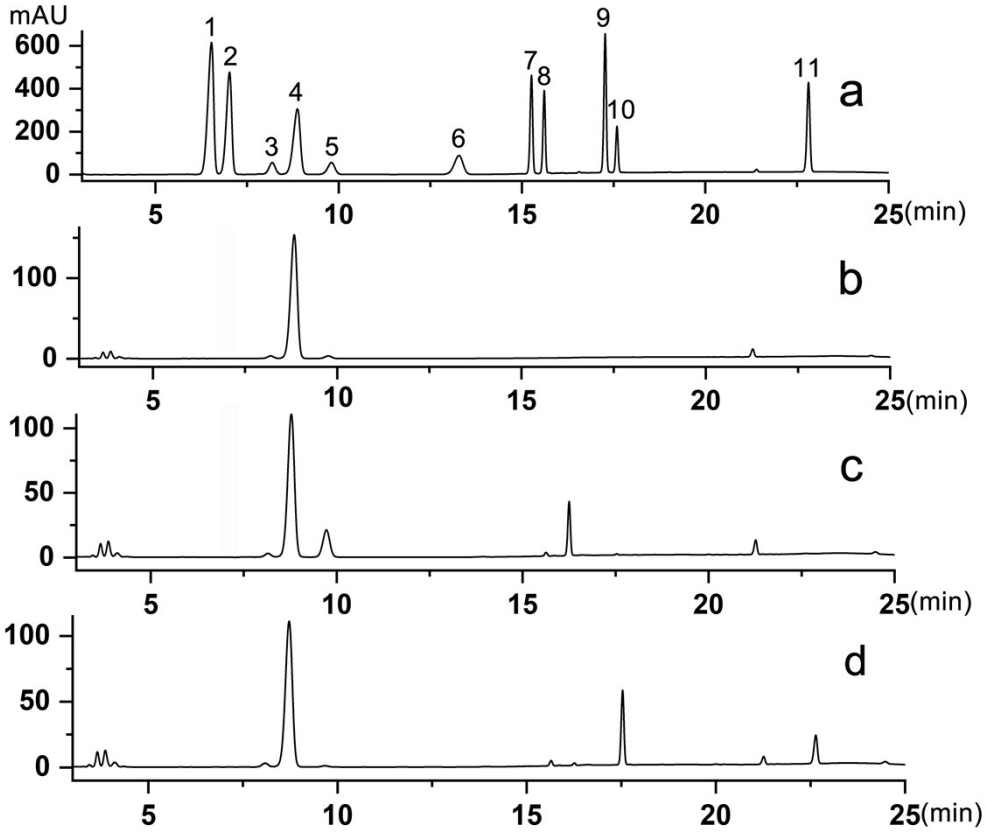

Fig. S2 HPLC analysis of RC co-hydrolysis by SBGL3 and SPRHA2. (a): Standard: RA (1), ST (2), rebaudioside F (3), RC (4), dulcoside A (5), RU (6), RB (7), steviolbioside (8), steviol 19-glycoside (9), steviolmonoside (10), SV (11); (b): RC standard; (c): RC was catalyzed by SBGL3; (d): RC was co-hydrolyzed by SBGL3 and SPRHA2.

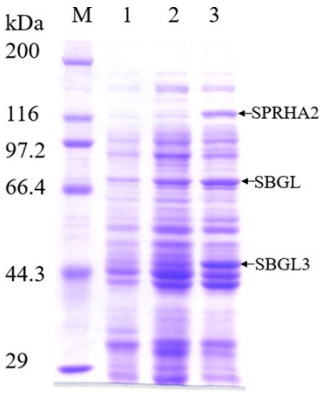

Fig. S3 SDS-PAGE analysis of crude enzyme extracts of *E.coli* strain E1 and E2. Lane M: Protein Marker; Lane 1: Induced cell lysate of *E.coli* JM109 with pSE380; Lane 2

and 3: Induced cell lysate of *E.coli* strain E1 and E2, respectively.

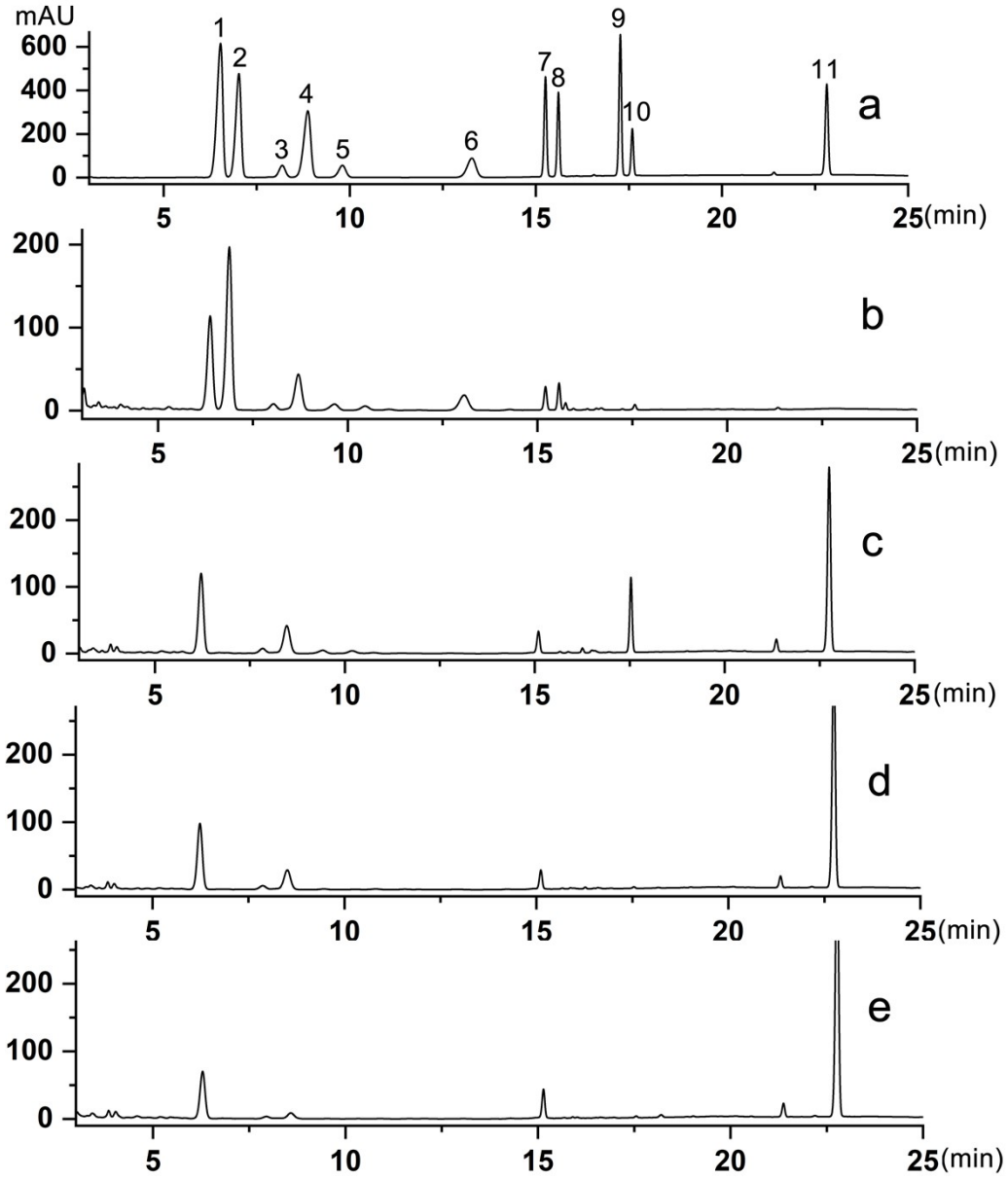

Fig. S4 HPLC analysis of Stevia extract hydrolysis by crude enzyme extract of *E.coli* strain E4. (a): Standard: RA (1), ST (2), rebaudioside F (3), RC (4), dulcoside A (5), RU (6), RB (7), steviolbioside (8), steviol 19-glycoside (9), steviolmonoside (10), SV (11); (b): Stevia extract; (c-e): The dosage of crude enzyme extract was 5, 15, 65 μL in the reactions, respectively.

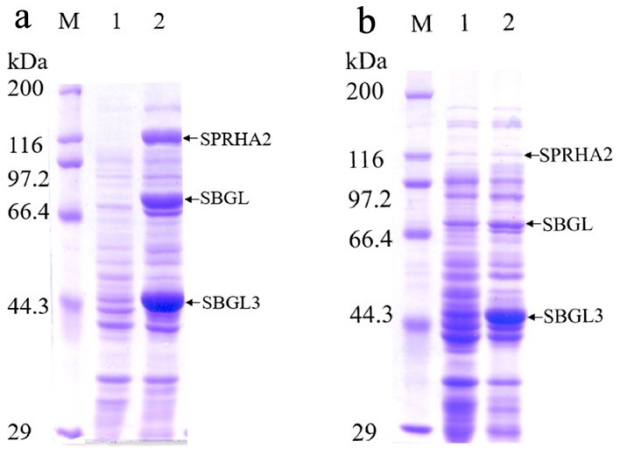

Fig. S5 SDS-PAGE analysis of crude enzyme extracts of *E.coli* strain E3 (a) and E4 (b). (a): Lane M: Protein Marker; Lane 1: Induced cell lysate of *E.coli* BL21(DE3) with pRSFDuet-1 and pSE380; Lane 2: Induced cell lysate of *E.coli* strain E3. (b): Lane M: Standard protein Marker; Lane 1: Induced cell lysate of *E.coli* BL21 (DE3) with pRSFDuet-1 and pSE380; Lane 2: Induced cell lysate of *E.coli* strain E4.

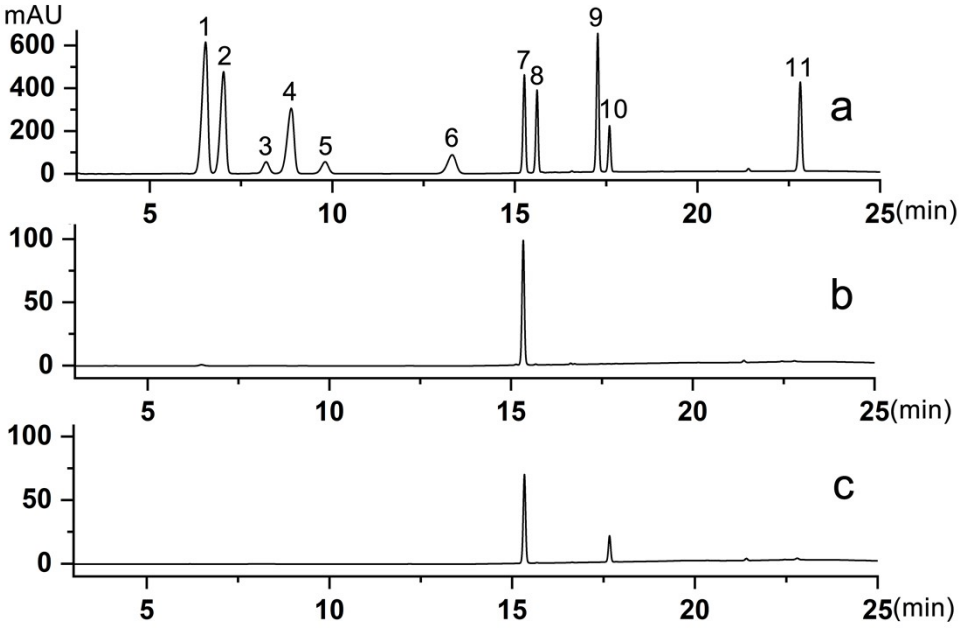

Fig. S6 HPLC analysis of RB hydrolysis by SBGL2. (a): Standard: RA (1), ST (2), rebaudioside F (3), RC (4), dulcoside A (5), RU (6), RB (7), steviolbioside (8), steviol 19-glycoside (9), steviolmonoside (10), SV (11); (b): RB standard; (c): RB was catalyzed by SBGL2.

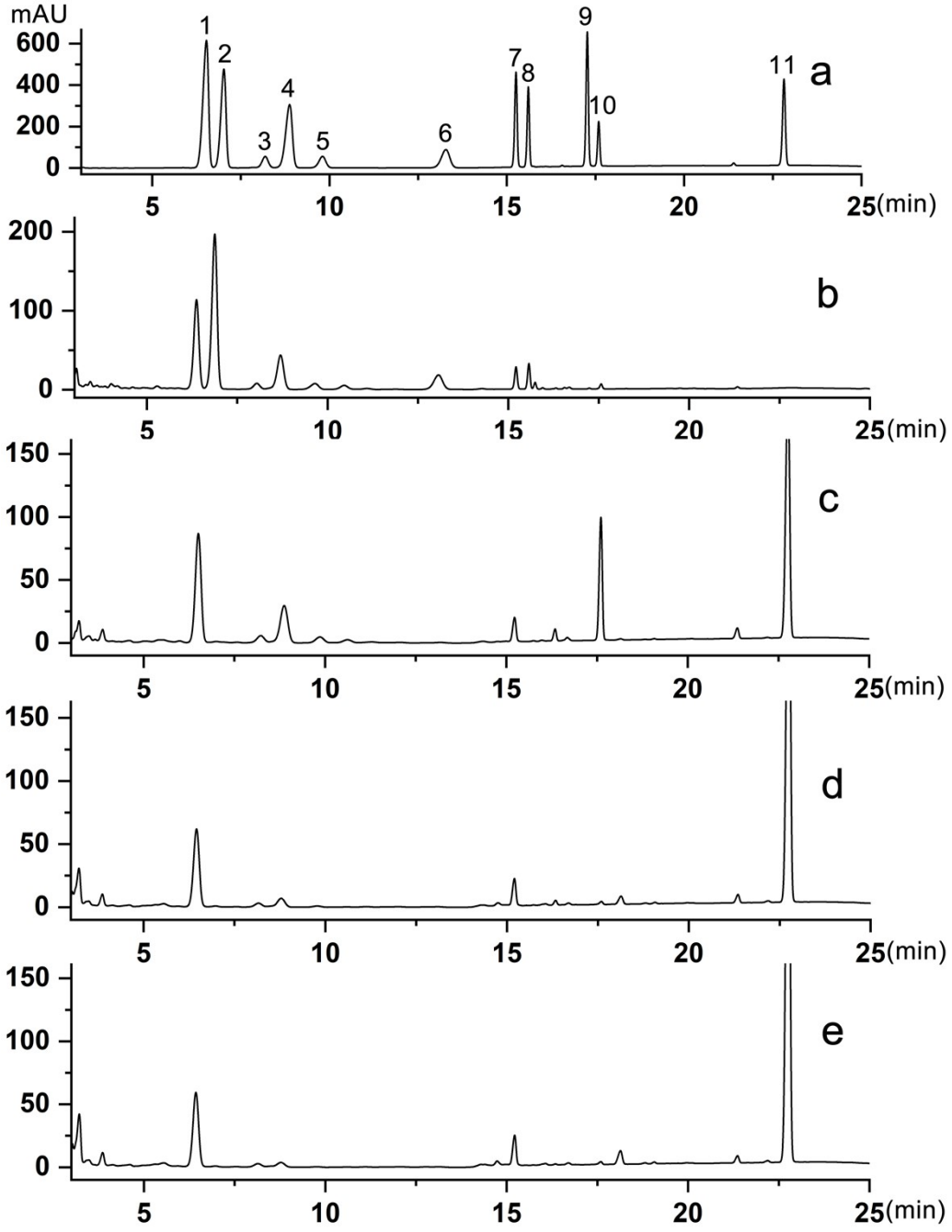

Fig. S7 HPLC analysis of Stevia extract hydrolysis by crude enzyme extract of *E.coli* strain E5. (a): standard: RA (1), ST (2), rebaudioside F (3), RC (4), dulcoside A (5), RU (6), RB (7), steviolbioside (8), steviol 19-glycoside (9), steviolmonoside (10), SV (11); b: Stevia extract; (c-e): The dosage of crude enzyme extract was 5, 35, 65 μL in the reactions, respectively.

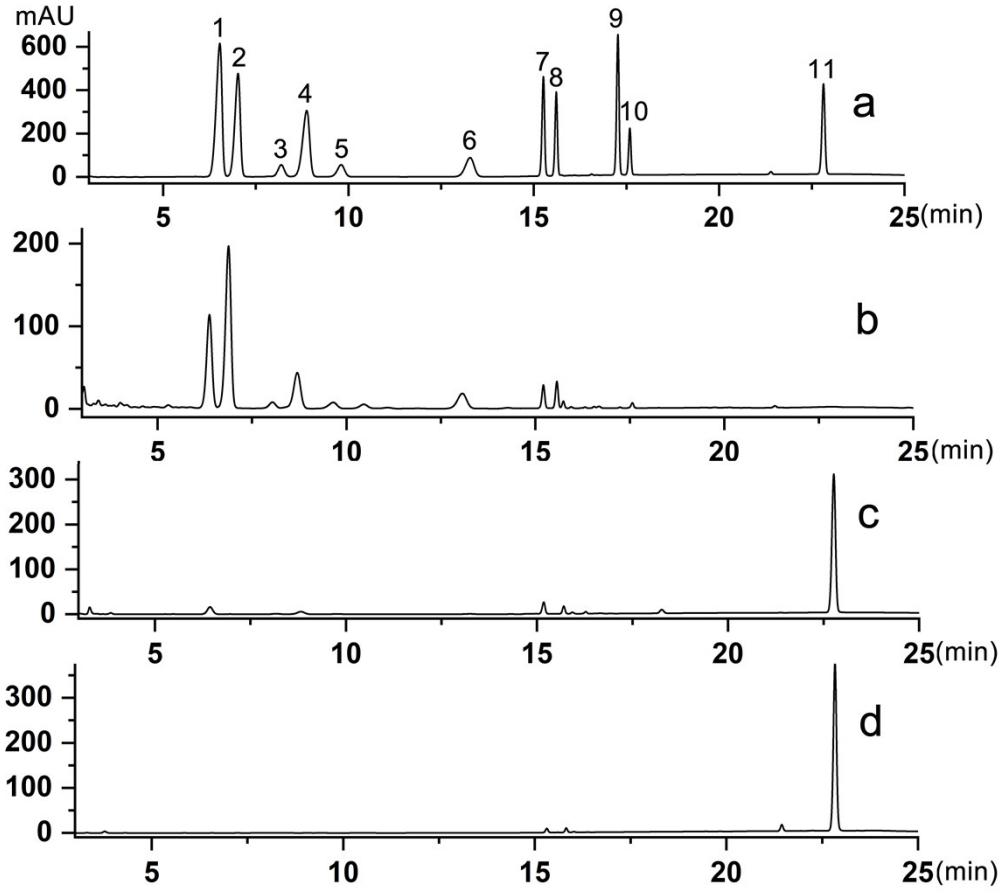

Fig. S8 HPLC analysis of 10 mL reaction system for whole-cell catalysis. (a): Standard: RA (1), ST (2), rebaudioside F (3), RC (4), dulcoside A (5), RU (6), RB (7), steviolbioside (8), steviol 19-glycoside (9), steviolmonoside (10), SV (11); (b): Stevia extract; (c): Precipitate in the reaction; (d): Purified SV.

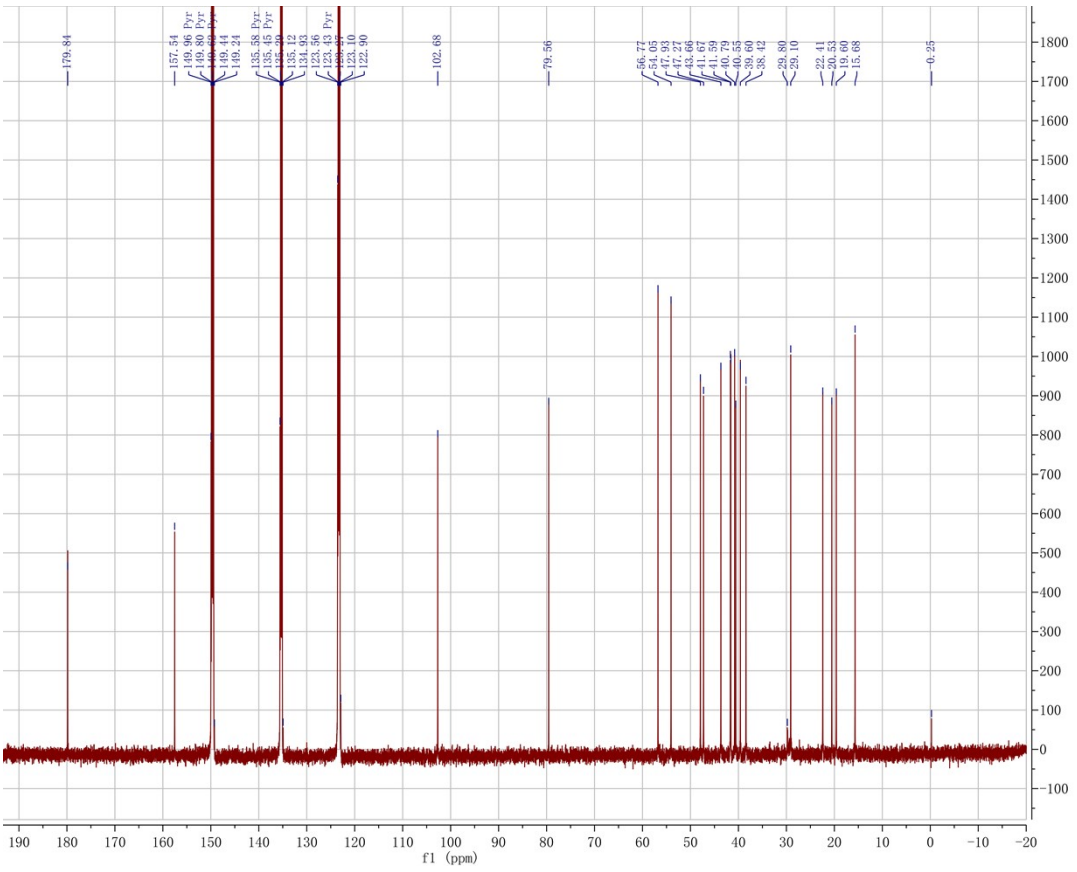

Fig. S9 NMR spectrum of isolated steviol. ^{13}C NMR (151 MHz, Pyridine-*d5*) δ 179.84 (C19, C), 157.54 (C16, C), 102.68 (C17, CH_2), 79.56 (C13, C), 56.77 (C5, CH), 54.05 (C14, CH_2), 47.93 (C9, CH), 47.27 (C4, CH_2), 43.66 (C8, C), 41.67 (C15, CH_2), 41.59 (C12, CH_2), 40.79 (C1, CH_2), 40.55 (C7, CH_2), 39.60 (C10, CH), 38.42 (C3, CH_2), 29.10 (C18, CH_3), 22.41(C6, CH_2), 20.53 (C11, CH_2), 19.60 (C2, CH_2), 15.68 (C20, CH_3).

Supporting table

Table S1 Primers in this study.

Primers	Sequence
02-F	5′-ATAGGATCCAAGGTTGCCAAGGCTGCCGCTGCG-3′
02-R	5′-GGTAAGCTTTCACACGCGATTGGCCCGCGCGATC-3′
03-F	5′-TCAAGATCTATCGATCGACGCAGCCTGGTCACCT-3′
03-R	5′-TCTAAGCTTTCACAGGCTGTTGCGCCGGGCGATG-3′
011-F	5′-GAACAAAAACTCATCTCAGAAGAGGATCTG-3′
011-R	5′-AGCTTCAGCCTCTCTTTTCTCGAGAGATAC-3′
012-F	5′-GTATCTCTCGAGAAAAGAGAGGCTGAAGCTATGCTGCTCCAGGCGG GTGCGCGGAAGCTG-3′
012-R	5′-CAGATCCTCTTCTGAGATGAGTTTTTGTTCTCAGGCCAGTTCGAAGC TGCCGGACACCCC-3′
021-F	5′-GTATCTCTCGAGAAAAGAGAGGCTGAAGCTATGCAGGAAGCCGGT GCCCCGCCGG-3′
021-R	5′-CAGATCCTCTTCTGAGATGAGTTTTTGTTCTCAATCCTTCCAGCTAC GCGCCGGC-3′
031-F	5′-GTATCTCTCGAGAAAAGAGAGGCTGAAGCTATGCAAGAAGCCGGT GCTCCTCCTGTTAA-3′
031-R	5′-CAGATCCTCTTCTGAGATGAGTTTTTGTTCTCAATCTTTCCATGATC TAGCTGGAAGTC-3′
0011-F	5′-GCTGGGCACGACCCGCTGACTCGAGAAGGAGATATACATATGCAG GAAGCCGGT-3′
0011-R	5′-CATCGGGTGCCGGCTCCATAGATCTTCTGTTTCCTGTGTGAAATTGT TATCCGCT-3′
0012-F	5′-GGAAGATCTATGGAGCCGGCACCCGATGCGGCCGCCA-3′
0012-R	5′-TTCTCGAGTCAGCGGGTCGTGCCCAGCGTGACCGGGCCA-3′
0021-F	5′-ATGGAGCCGGCACCCGATGCGGCCGCCA-3′
0021-R	5′-GCTCATTTCAGAATATTTGCCAGAACCG-3′
0022-F	5′-GCAAATATTCTGAAATGAGCCCCGGGTTGACAATTAATCATCCGGC TCGTA-3′
0022-R	5′-GCATCGGGTGCCGGCTCCATATGTATATCTCCTTAGATCTTCACAGG CTGTTGCGCCGGGCGATG-3′
0023-F	5′-GCAAATATTCTGAAATGAGCCCCGGGTCATAAAAAATTTATTTGCT TTGTGA-3′
0023-R	5′-GCATCGGGTGCCGGCTCCATATGTATATCTCCTTAGATCTTCACAGG CTGTTGCGCCGGGCGATG-3′
0031-F	5′-GGGGAATTCAATCGATCGACGCAGCCTGGTCACCT-3′
0031-R	5′-GGGAAGCTTTCACAGGCTGTTGCGCCGGGCGATG-3′
0041-F	5′-GGAAGATCTAGAGCCGGCACCCGATGCGGCCGCCA-3′
0041-R	5′-TCGAGTCAGCGGGTCGTGCCCAGCGTGACC-3′
0051-F	5′-GGAAGATCTAAAGGTTGCCAAGGCTGCCGCTGCGCCCAC-3′
0051-R	5′-TTCTCGAGTCACACGCGATTGGCCCGCGCGATCGCGCCCA-3′

Table S2 Plasmids and strains used in this study.

Plasmids	Backbone	Description	References
pSH1	pSE380	pTrc-*spbgl1*, pRR322, Amp$^+$	[1]
pSH2	pSE380	pTrc-*sbgl*, pRR322, Amp$^+$	[2]
pSH3	pQE30	pT5-*sprha2*, pRR322, Amp$^+$	This study
pSH4	pQE30	pT5-*sbgl2*, pRR322, Amp$^+$	
pSH5	pQE30	pT5-*sbgl3*, pRR322, Amp$^+$	
pSH6	pSE380	pTrc-*sbgl-spbgl1* , pRR322, Amp$^+$	
pSH7	pSH6	pTrc-*sbgl3-sprha2-sbgl* , pRR322, Amp$^+$	
pSH8	pSH7	pT5-*sbgl3-sprha2-sbgl* , pRR322, Amp$^+$	
pSH9	pRSFDuet-1	pT7-*sbgl3*, RSF, Kan$^+$	
pSH10	pSH9	pT7-*sbgl3*-T7-*sprha2*, RSF, Kan$^+$	
pSH11	pSH2	pTrc-*sprha2-sbgl*, pRR322, Amp$^+$	
pSH12	pSH9	pT7-*sbgl3*-T7-s*bgl2*, RSF, Kan$^+$	
Strains			
C1	*E. coli* JM109	JM109 carrying pSH1	This study
C2	*E. coli* JM109	JM109 carrying pSH2	
C3	*E. coli* JM109	JM109 carrying pSH3	
C4	*E. coli* JM109	JM109 carrying pSH4	
C5	*E. coli* JM109	JM109 carrying pSH5	
C6	*E. coli* JM109	JM109 carrying pSH6	
E1	*E. coli* JM109	JM109 carrying pSH7	
E2	*E. coli* JM109	JM109 carrying pSH8	
E3	*E. coli* BL21 (DE3)	BL21 (DE3) carrying pSH2 and pSH10	
E4	*E. coli* BL21 (DE3)	BL21 (DE3) carrying pSH9 and pSH11	
E5	*E. coli* BL21 (DE3)	BL21 (DE3) carrying pSH11 and pSH12	

The coding sequence of genes in this study.

>spbgl1

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