**Supplementary materials**

# Cofactor Self-Sufficient Whole-Cell Biocatalysts for the Relay-Race Synthesis of Shikimic Acid

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**Supplementary Materials and Methods**

*DHS fermentative broth preparation*

For preparation of DHS fermentative broth, fed-batch fermentation was conducted in a 5-L bioreactor (BIOTECH-5BG, Bxbio, China). A single colony was inoculated into a falcon tube containing 5 mL LB medium and cultured overnight at 37 ℃. The overnight seed was then inoculated into a 1 L shake flask containing 200 mL LB medium at a ratio of 1:100 and incubated at 37 ℃, 250 rpm for 10-12 h. After this, the seed culture was transferred into 1.8 L of fermentation medium at a 1:10 (v/v) inoculum: medium ratio and incubated at 37 ℃. The fermentation medium contained 7.5 g/L $K_2HPO_4 \cdot 3H_2O$, 2 g/L $MgSO_4 \cdot 7H_2O$, 1.6 g/L $(NH_4)_2SO_4$, 2 g/L citric acid monohydrate, 0.075 g/L $FeSO_4 \cdot 7H_2O$, 4.5 mg/L $MnSO_4 \cdot H_2O$, 20 mg/L $Na_2SO_4$, 6.4 mg/L $ZnSO_4 \cdot 7H_2O$, 4 mg/L $CoCl_2 \cdot 6H_2O$, 0.6 mg/L $CuSO_4 \cdot 5H_2O$. The agitation, air supplementation and feed rate were changed to maintain the dissolved oxygen (DO) concentration above 30% saturation. The pH was maintained at 6.8 using 25% (w/v) $NH_3 \cdot H_2O$. The DO-stat feeding strategy was employed to supply 60% (w/w) glucose to the fermenter. Samples were collected every 2 h to determine cell density ($OD_{600}$), residual glucose and DHS titer.

*SDS-PAGE analysis of protein expression*

SDS-PAGE was carried out to analyze the expression of different GDHs and AroE. For protein preparation, the *E. coli* BL21(DE3) cells were harvested by centrifugation at 5,000 g for 5 min at 4 ℃. Cell pellets were washed once with 15 mL PBS buffer and resuspended in 10 mL of the same buffer. The suspensions were sonicated for 10 min

(300 W, pulse 2 s, interval 3 s) with an ultrasonic homogenizer (SCIENTZ-II D, Ningbo Scientz Biotechnology, China) in an ice water bath, and then centrifuged at 8,000 g for 10 min at 4 ℃. After the cell precipitations were washed twice with PBS buffer, the supernatants and cell precipitations along with whole-cell lysates were analyzed by electrophoresis on an 12% (w/v) SDS-PAGE gel. The gels were stained with Coomassie blue R-250 solution.

## Supplementary Figures

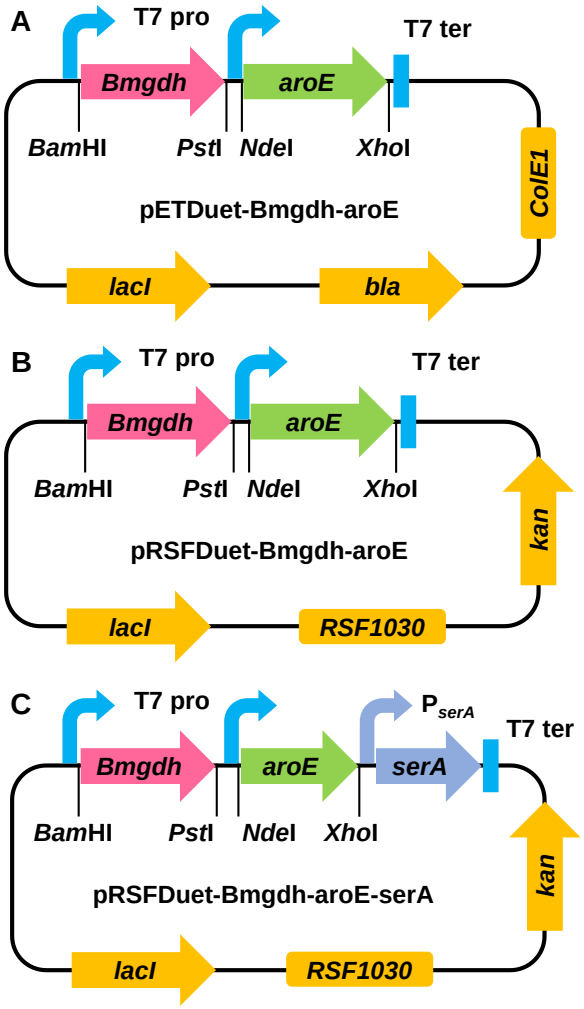


**Figure S1.** Simple diagrams of the plasmids for pETDuet-Bmgdh-aroE (**A**), pRSFDuet-Bmgdh-aroE (**B**) and pRSFDuet-aroE-Bmgdh-serA (**C**). If the *Bmgdh* in pETDuet-Bmgdh-aroE was replaced with *gdh* from other organisms, such as *Tagdh*, *Lsgdh*, *Bsgdh* or *Bagdh*, the corresponding plasmid can be obtained.

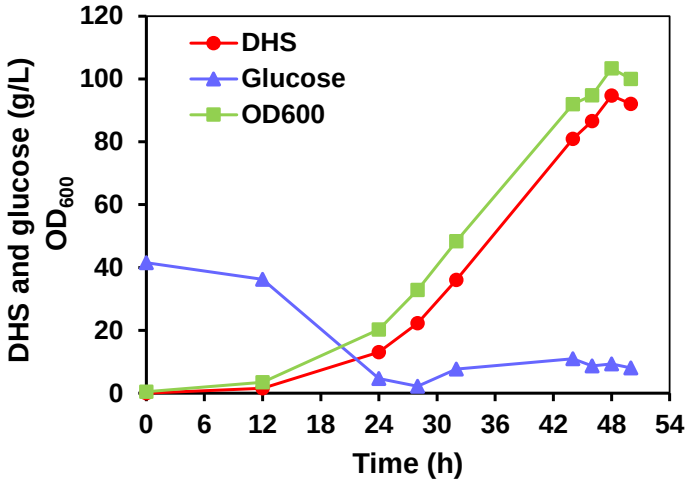


**Figure S2.** Fed-batch fermentation of DHS overproducing strain WJ060. Cell density ($OD_{600}$), DHS production and residual glucose concentration were measured during a fermentation period of 50 h.

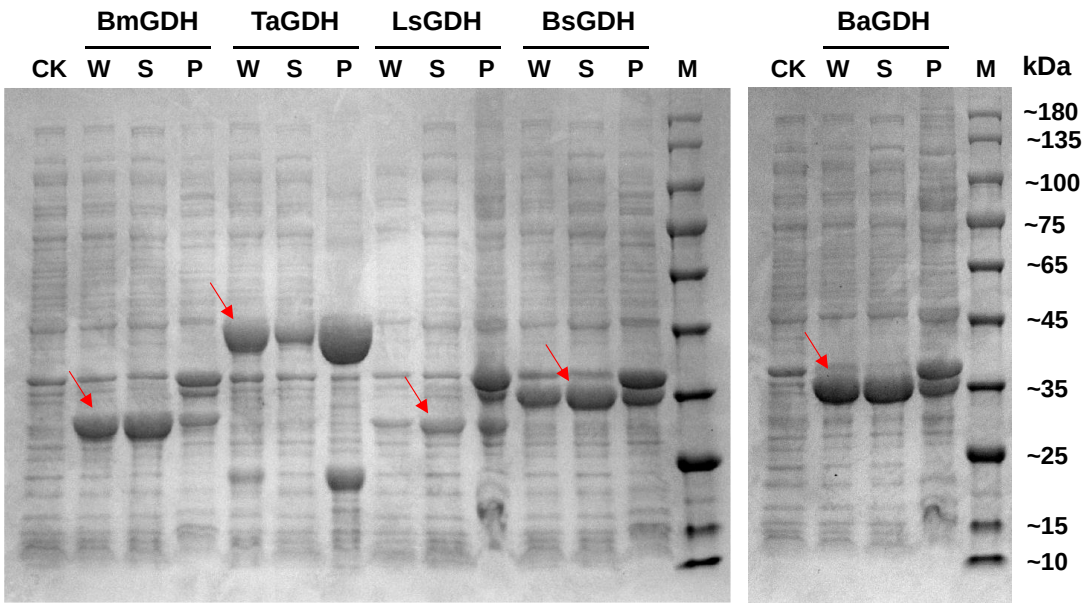


**Figure S3.** SDS-PAGE analysis of different GDH proteins. BL21(DE3) strain transformed with empty pETDuet-1 plasmid was used as the negative control (CK). Same volume (6 μL) of supernatant or cell precipitation was loaded on each lane. The GDH bands were labeled with red arrows. Note: W, whole-cell lysate; S, supernatant; P, precipitation; M, protein marker.

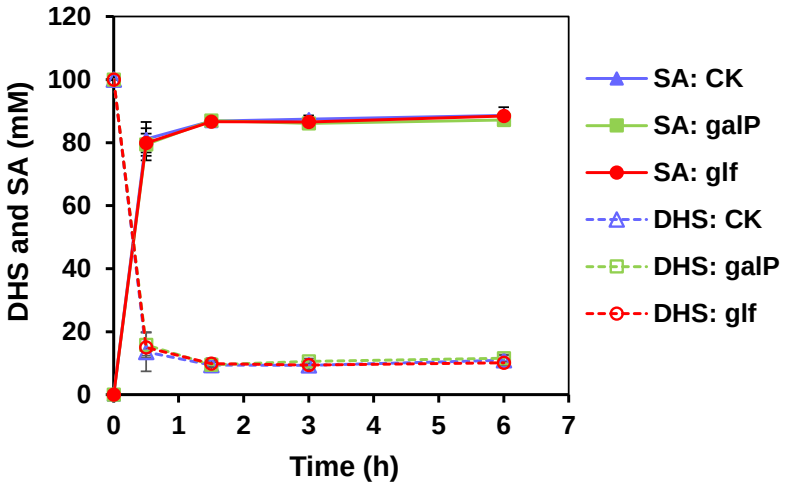


**Figure S4.** Effects of galactose permease GalP or glucose facilitator Glf on the catalytic efficiencies of whole-cell biocatalysts. PET strain transformed with empty pACYCDuet-1 plasmid was used as the negative control (CK). Reaction conditions: 100 mM sodium phosphate buffer (pH 7.0), 100 mM DHS, 150 mM glucose, 5 $OD_{600}$ whole-cells, 10 mL of total volume, 37 °C. Data are presented as the mean ± standard deviation of three independent experiments.

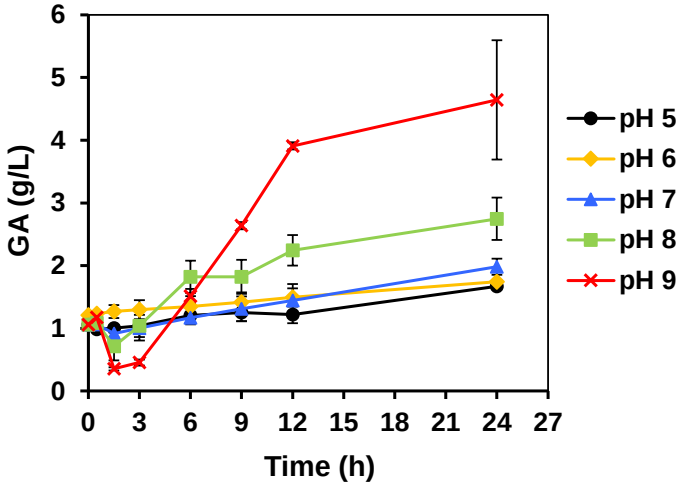


**Figure S5.** The effect of pH on bioconversion of DHS to GA by PET whole-cell biocatalyst. Reaction conditions: 100 mM sodium phosphate buffer (pH 5.0, 6.0, 7.0, 8.0 or 9.0), 100 mM DHS, 150 mM glucose, 2 $OD_{600}$ of PET whole-cells, 10 mL of total volume, 37 ℃. Data are presented as the mean ± standard deviation of three independent experiments.

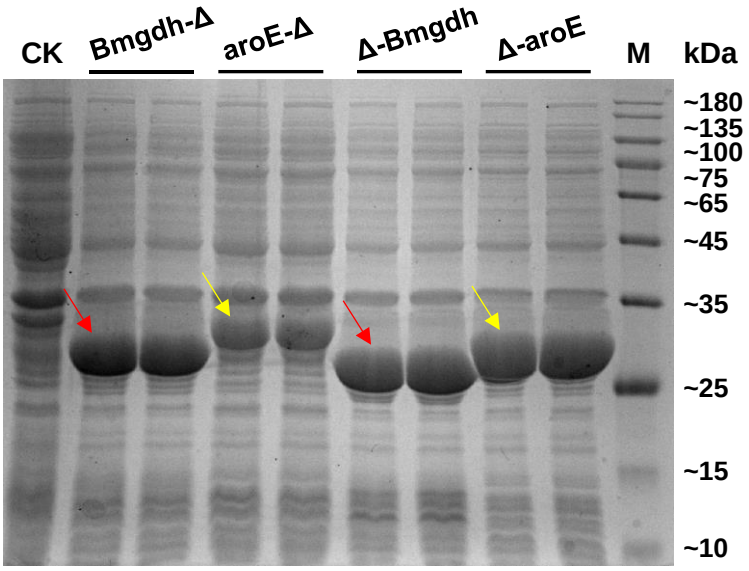


**Figure S6.** SDS-PAGE analysis of BmGDH and AroE proteins. BL21(DE3) strain transformed with empty pRSFDuet-1 plasmid was used as the negative control (CK). Same volume (8 μL) of whole-cell lysate was loaded on each lane. The BmGDH and AroE bands were labeled with red and yellow arrows, respectively. Note: M, protein marker.

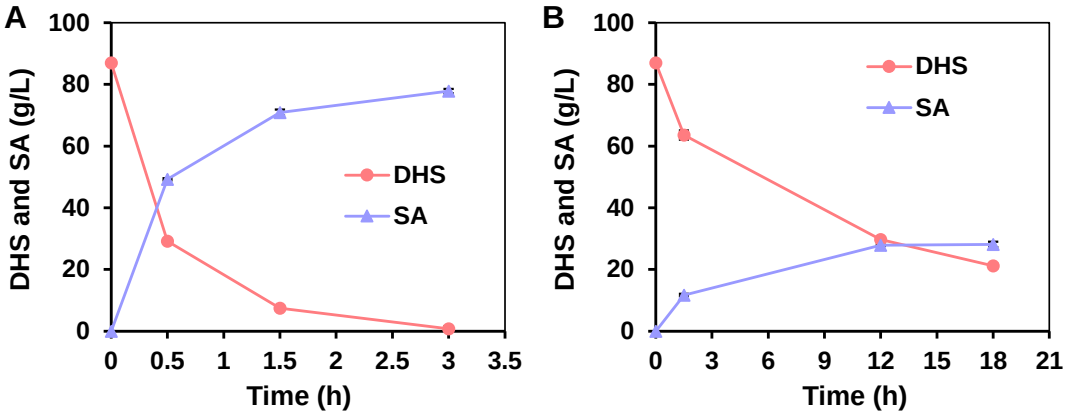


**Figure S7.** Bioconversion of DHS to SA by RSF-serA whole-cell biocatalyst. (**A**) Time courses of DHS and SA titers catalyzed by fresh RSF-serA whole-cell catalyst; (**B**) Time courses of DHS and SA titers catalyzed by reused RSF-serA whole-cell catalyst. Reaction conditions: 87 g/L DHS, 1.4 equivalent of glucose, 20 $OD_{600}$ of RSF-serA whole-cells, 1 L of total volume, 34 ℃, pH 7.0. Data are presented as the mean ± standard deviation of two independent experiments.

## Supplementary Tables

**Table S1.** Comparison of different catalytic methods for the production of SA.

| Methods | Enzymes or strains | Catalyst load | Substrates | Exogenous cofactor | Time (h) | Titers | Yields (mol/mol) | Ref. |
|---|---|---|---|---|---|---|---|---|
| Biocatalysis by two enzymatic systems | Quinate dehydrogenase and 3-dehydroquinate dehydratase | 25 g/L dried cells or 50 g/L dried membrane fraction | 52 mM quinate | None | 20 | 40 mM DHS | 77% | [16] |
| | Shikimate dehydrogenase and glucose dehydrogenase | 1 U/mL and 50 U/mL | 1.4 mM DHS | $NADP^+$ | 1.25 | 1.4 mM SA | 100% | |
| Biocatalysis by Immobilized enzyme | Quinate dehydrogenase and 3-dehydroquinate dehydratase | 20 g/L dried membrane fraction | 50 mM quinate | None | 36 | 50 mM DHS | 100% | [19] |
| | Shikimate dehydrogenase and glucose dehydrogenase | 0.2 U/mL and 10 U/mL | 20 mM DHS | $NADP^+$ | Data not shown | 20 mM SA | 100% | |
| Enzymatic biocatalysis | Shikimate dehydrogenase and phosphite dehydrogenase | 0.17 U/mL and 20 ug/mL | 8 mM DHS | $NADP^+$ | 1.5 | 8 mM SA | 100% | [21] |
| Whole-cell biocatalysis | *B. megaterium* MTCC 428 | 200 g/L wet whole cells | 5 mM quinate | None | 3 | 4.45 mM | 89% | [20] |
| Whole-cell biocatalysis | Engineered *C. glutamicum* strain SKM7 | 100 g/L wet whole cells | 286.3g/L glucose | None | 48 | 141g/L SA | 51.0% | [1] |
| Relay-race synthesis | Engineered *E. coli* strain WJ060 | 1:10 (v/v) inoculum: medium ratio | 60% (w/v) glucose | None | 48 | 88.6 g/L DHS | 33.0% | This study |
| | Shikimate dehydrogenase and glucose dehydrogenase | 20 $OD_{600}$ (~30 g/L wet whole cells) | 88.6 g/L DHS | None | 2 | 77.1 g/L SA | 98.4% | |

**Table S2.** The strains and plasmids used in this study.

| Name | Characteristics | Source |
|---|---|---|
| **Strains** | | |
| WJ060 | DSM 1576 P1-*aroE*$^{TTG}$ P2-*aroF*$^{fbr}$ Δ*tyrR* P4-*tktA* Δ*ptsI* P1-*galP* P4-*glk* P1-*pykF*$^{TTG}$ P1-*pykA*$^{TTG}$ P1-*pgi*$^{TTG}$ | Lab collection |
| DH5α | F$^{-}$φ80 *lacZ*ΔM15 Δ(*lacZYA-argF*) U169 *end*A1 *rec*A1 *hsd*R17(rk$^{-}$, mk$^{+}$) *sup*E44λ$^{-}$ *thi*$^{-}$1 *gyr*A96 *rel*A1 *pho*A | TransGen Biotech |
| BL21(DE3) | F$^{-}$ *ompT hsdS*$_B$ ($r_B^{-}$ $m_B^{-}$) *gal dcm* (DE3) | Novagen |
| BL21(DE3) Δ*serA* | BL21(DE3) Δ*serA* | This study |
| PET | BL21(DE3) pETDuet-Bmgdh-aroE | This study |
| RSF | BL21(DE3) pRSFDuet-Bmgdh-aroE | This study |
| RSF-serA | BL21(DE3) Δ*serA*, pRSFDuet-Bmgdh-aroE-serA | This study |
| **Plasmids** | | |
| pCas | *repA101(Ts) kan* P*cas-cas9* P*araB-Red lacIq* P*trc-sgRNA-pMB1* | [22] |
| pTargetF | *pMB1 aadA sgRNA-cadA* | [22] |
| pTargetF-serA | *pMB1 aadA sgRNA-serA* | This study |
| pETDuet-1 | *ColE1 bla lacI* | Novagen |
| pRSFDuet-1 | *RSF1030 kan lacI* | Novagen |
| pACYCDuet-1 | *p15A cat lacI* | Novagen |
| pETDuet-Bmgdh-Δ | *ColE1 bla lacI* P$_{T7}$-*Bmgdh* | This study |
| pETDuet-Tagdh-Δ | *ColE1 bla lacI* P$_{T7}$-*Tagdh* | This study |
| pETDuet-Lsgdh-Δ | *ColE1 bla lacI* P$_{T7}$-*Lsgdh* | This study |
| pETDuet-Bsgdh-Δ | *ColE1 bla lacI* P$_{T7}$-*Bsgdh* | This study |
| pETDuet-Bagdh-Δ | *ColE1 bla lacI* P$_{T7}$-*Bagdh* | This study |
| pETDuet-Δ-aroE | *ColE1 bla lacI* P$_{T7}$-*aroE* | This study |
| pETDuet-Bmgdh-aroE | *ColE1 bla lacI* P$_{T7}$-*Bmgdh* P$_{T7}$-*aroE* | This study |
| pETDuet-Tagdh-aroE | *ColE1 bla lacI* P$_{T7}$-*Tagdh* P$_{T7}$-*aroE* | This study |
| pETDuet-Lsgdh-aroE | *ColE1 bla lacI* P$_{T7}$-*Lsgdh* P$_{T7}$-*aroE* | This study |
| pETDuet-Bsgdh-aroE | *ColE1 bla lacI* P$_{T7}$-*Bsgdh* P$_{T7}$-*aroE* | This study |
| pETDuet-Bagdh-aroE | *ColE1 bla lacI* P$_{T7}$-*Bagdh* P$_{T7}$-*aroE* | This study |
| pACYCDuet-galP-Δ | *p15A cat lacI* P$_{T7}$-*galP* | This study |
| pACYCDuet-glf-Δ | *p15A cat lacI* P$_{T7}$-*glf* | This study |
| pRSFDuet-Bmgdh-Δ | *RSF1030 kan lacI* P$_{T7}$-*Bmgdh* | This study |
| pRSFDuet-Δ-Bmgdh | *RSF1030 kan lacI* P$_{T7}$-*Bmgdh* | This study |
| pRSFDuet-aroE-Δ | *RSF1030 kan lacI* P$_{T7}$-*aroE* | This study |
| pRSFDuet-Δ-aroE | *RSF1030 kan lacI* P$_{T7}$-*aroE* | This study |
| pRSFDuet-aroE-Bmgdh | *RSF1030 kan lacI* P$_{T7}$-*aroE* P$_{T7}$-*Bmgdh* | This study |
| pRSFDuet-Bmgdh-aroE | *RSF1030 kan lacI* P$_{T7}$-*Bmgdh* P$_{T7}$-*aroE* | This study |
| pRSFDuet-Bmgdh- | *RSF1030 kan lacI* P$_{T7}$-*Bmgdh* P$_{T7}$-*aroE serA* | This study |

aroE-serA

$^{TTG}$: The starting codon of the gene is replaced with TTG.

**Table S3.** Primers used in this study

| Primer name | Primer sequence (5′ to 3′) | Description |
|---|---|---|
| aroE-NdeI-F | GTCGTTGCATATGGAAACCTATGCTGTTTTTGG | Construction of *aroE* expression vector |
| aroE-XhoI-R | CAGTCTCGAGTTACGCGGACAATTCCTCCTG | |
| aroE-BamHI-F | CGCGGATCCGATGGAAACCTATGCTGTTTTTGG | |
| aroE-PstI-R | CAGTCTGCAGTTACGCGGACAATTCCTCCTG | |
| Bmgdh-NdeI-F | GTCGTTGCATATGTACAAGGATCTGGAAGG | Construction of *Bmgdh* expression vector |
| Bmgdh-XhoI-R | CAGTCTCGAGTTAGCCGCGGCCTGCCTG | |
| serA-HR-F | GGAGGAATTGTCCGCGTAACTCGAGCCTGGCT ATTGTCGATTGCTC | Construction of *serA* expression vector |
| serA-HR-R | GCAGCGGTTTCTTTACCAGATTAGTACAGCAGA CGGGCG | |
| serA-N20-F | CTGACTAGTTCTGTTGCGGAGCTGGTGATGTTT TAGAGCTAGAAATAGC | Deletion of *serA* with CRISPR method |
| pTargetF-R | ATGACTAGTATTATACCTAGGACTGAGC | |
| serA-D-1F | CCTGGCTATTGTCGATTGCTC | |
| serA-D-1R | TTACCCAATCCTGTCTTTTGAAATG | |
| serA-D-2F | TCAAAAGACAGGATTGGGTAATTCCCCTTCTCT GAAAATCAAC | |
| serA-D-2R | GTTACAGCCCCATGCTGCC | |