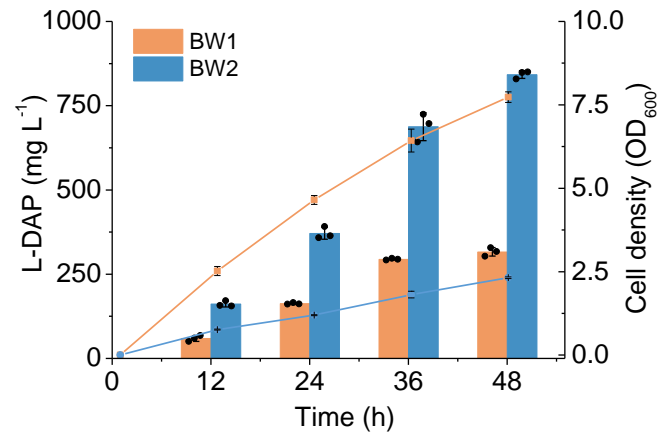


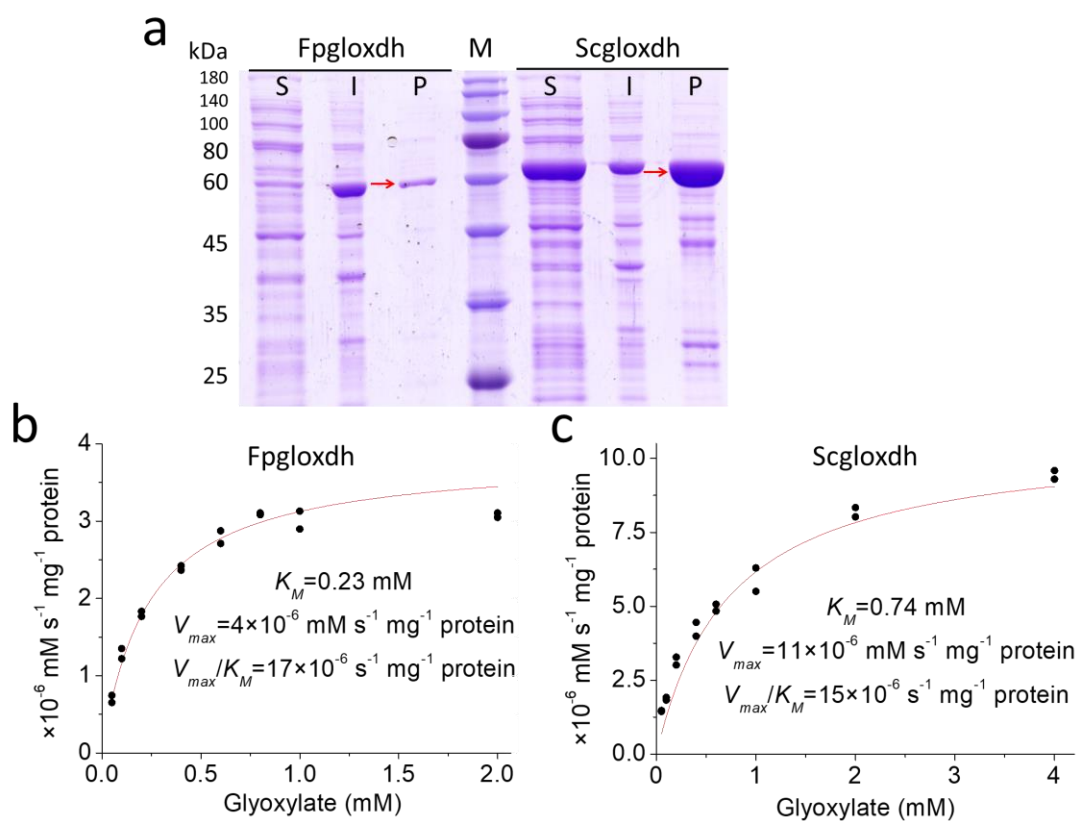
**Biosynthesis of plant hemostatic dencichine in *Escherichia coli***

Li *et al.*

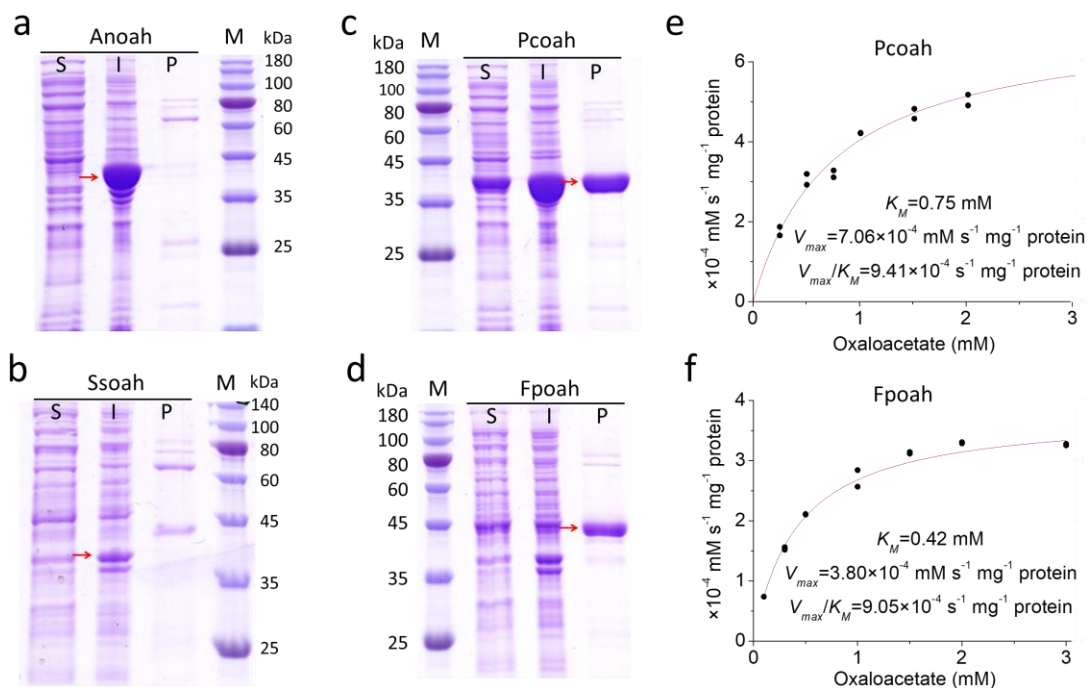


**Supplementary Figure 1. Production and optimization of *L*-DAP by strain BW1 and BW2.**

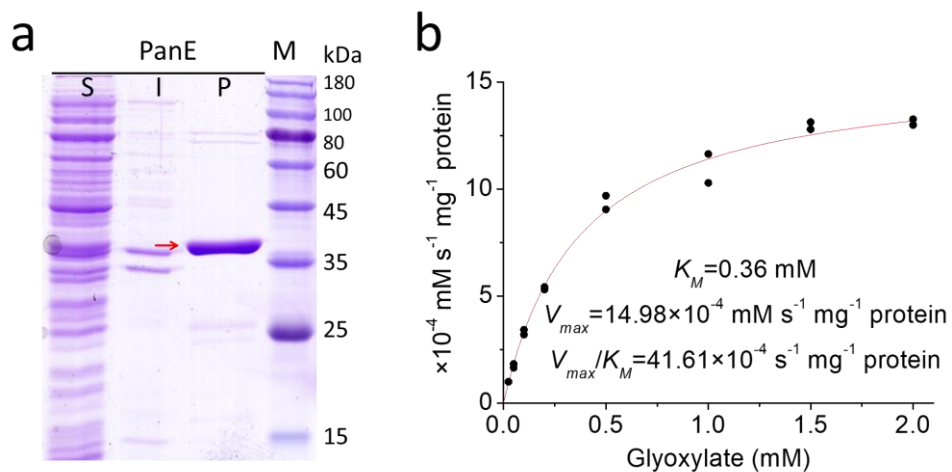
The bars indicate the titer of *L*-DAP and the lines indicate biomass at OD<sub>600</sub>. Data shown are mean ± SD (n=3 independent experiments). Source data are provided as a Source Data file.



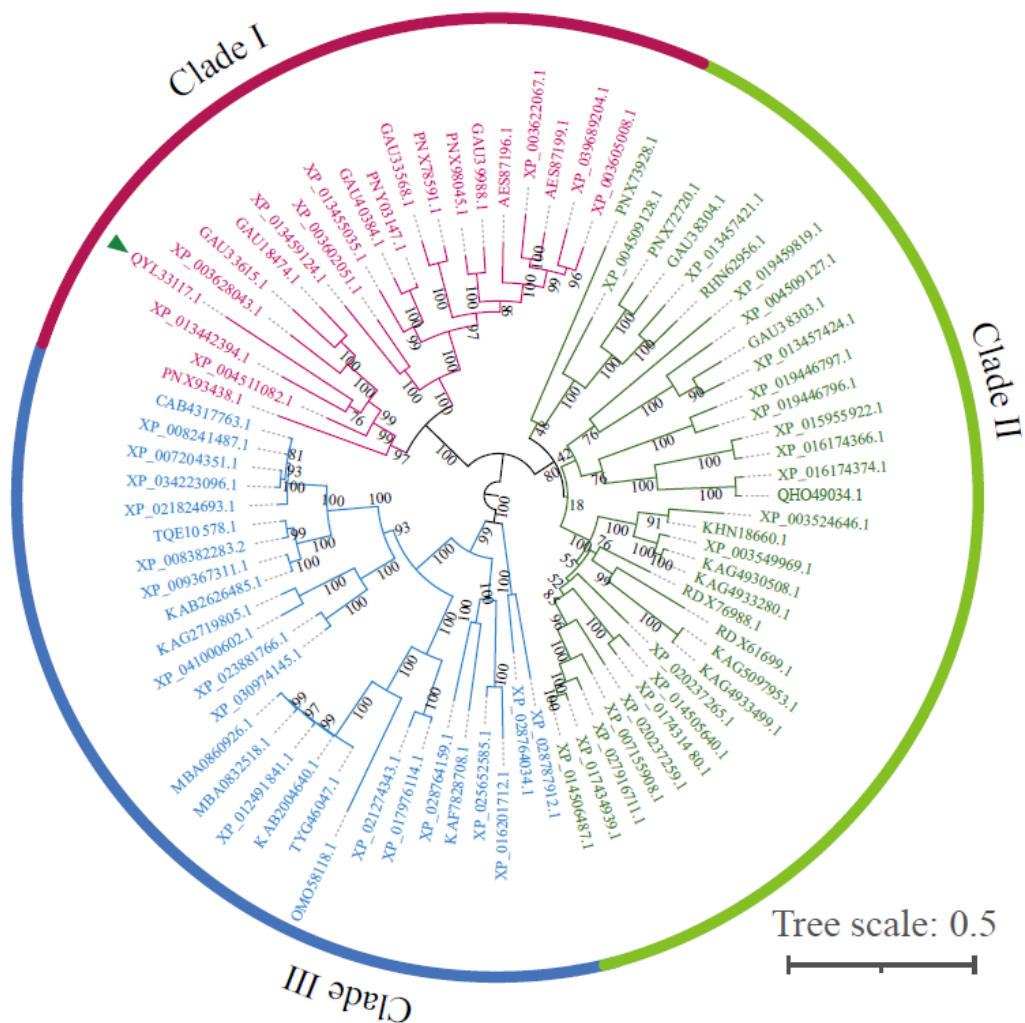
**Supplementary Figure 2. Biochemical analysis of Fpgloxhdh and Scgloxhdh.** **a** SDS-PAGE analysis of nickel-affinity purified codon-optimized His-Fpgloxhdh and wild-type His-Scgloxhdh. This experiment was repeated independently twice with similar results. **b, c** Kinetic analysis of Gloxhdh over a gradient of glyoxylate concentrations ( $n=2$ , data distribution is less than 7.84% in duplicate experiments). Enzyme activity was determined by measurement the production of cytochrome *c* reduced at 550 nm. M, marker II; S, soluble fraction; I, insoluble fraction; P, purified protein. Source data are provided as a Source Data file.



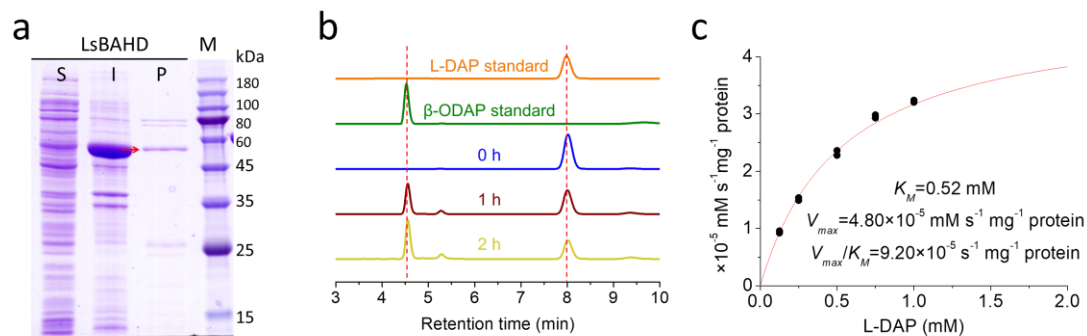
**Supplementary Figure 3. Biochemical analysis of Anoah, Ssoah, Pcoah and Fpoah.** **a, b, c, d** SDS-PAGE analysis of nickel-affinity purified Anoah, Ssoah, Pcoah and Fpoah. This experiment was repeated independently twice with similar results. **e, f** Kinetic analysis of Oah over a gradient of oxaloacetate concentrations ( $n=2$ , data distribution is less than 6.13% in duplicate experiments). Enzyme activity was determined by measurement the consumption of oxaloacetate at 255 nm. M, marker II; S, soluble fraction; I, insoluble fraction; P, purified protein. Source data are provided as a Source Data file.



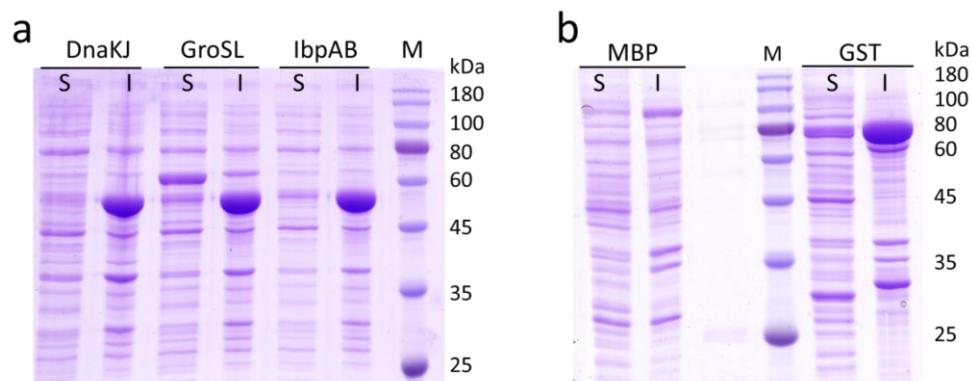
**Supplementary Figure 4. Biochemical analysis of PanE.** **a** SDS-PAGE analysis of nickel-affinity purified PanE. This experiment was repeated independently twice with similar results. **b** Kinetic analysis of PanE over a gradient of glyoxylate concentrations (n=2, data distribution is less than 6.16% in duplicate experiments). Enzyme activity was determined by measurement the production of NADPH at 340 nm. M, marker II; S, soluble fraction; I, insoluble fraction; P, purified protein. Source data are provided as a Source Data file.



**Supplementary Figure 5. Phylogenetic relationships of LsBAHD in *L. sativus* with sequences encoding highly homologous proteins.** Amino acid sequences were aligned and the Maximum Likelihood (ML) tree was built using MAFFT (version 7.407). The phylogeny was constructed with 1000 implementations of ultrafast bootstrap tests with IQ-TREE. The Model of substitution is JTT+I+G4 (Jones-Taylor-Thornton model, Has Invariant sites plus discrete Gamma model). The tree was further annotated by iTOL (Interactive Tree Of Life).

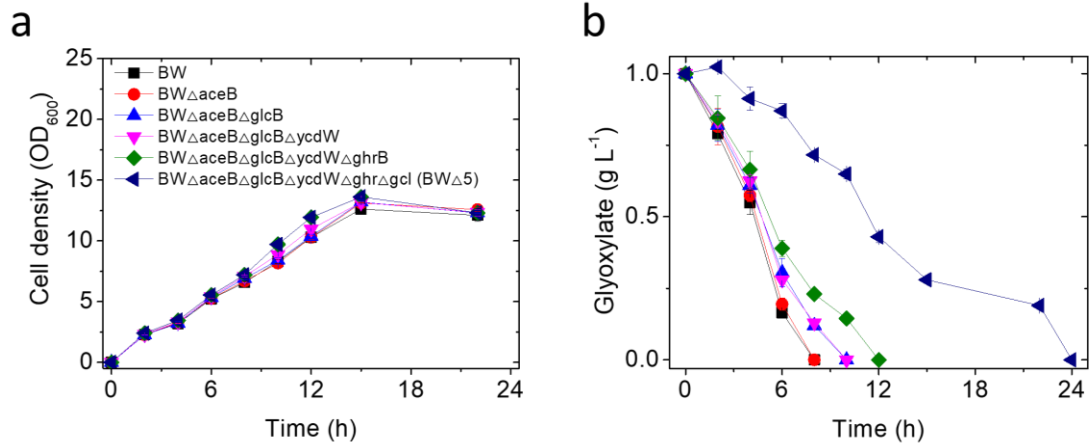


**Supplementary Figure 6. Biochemical analysis of LsBAHD.** **a** SDS-PAGE analysis of nickel-affinity purified LsBAHD. This experiment was repeated independently twice with similar results. **b** The *in vitro* assay substrates and products were monitored by HPLC analysis. A PanE-LsBAHD coupling assay was used due to the chemical oxalyl-CoA is not commercially available. From top to bottom are L-DAP standard,  $\beta$ -ODAP standard and the reaction at 0 h, 1 h and 2 h, respectively. **c** Kinetic analysis of LsBAHD over a gradient of L-DAP concentrations ( $n=2$ , data distribution is less than 1.97% in duplicate experiments). Enzyme activity was determined by measurement the production of NADPH at 340 nm. M, marker II; S, soluble fraction; I, insoluble fraction; P, purified protein. Source data are provided as a Source Data file.



**Supplementary Figure 7. Solubility optimization of LsBAHD by conventional methods.** Expression levels of **a** LsBAHD co-expressed with DnaKJ, GroSL and IbpAB and **b** fusion enzymes (MBP: maltose-binding protein-LsBAHD and GST: glutathione S-transferase) were identified through SDS-PAGE analysis. M, marker II; S, soluble fraction; I, insoluble fraction. This experiment was repeated independently twice with similar results. Source data are provided as a Source Data file.





**Supplementary Figure 8. Block the degradation pathways of glyoxylate. a** Cell growth with time. **b** Glyoxylate consumption with time. Data shown are mean  $\pm$  SD (n=3 independent experiments). Source data are provided as a Source Data file.

**Supplementary Table 1. Strains used in this study.**

<b>Strains</b>	<b>Description</b>	<b>Source</b>
<i>E. coli</i> BW25113	<i>rrnBT14 ΔlacZWJ16 hsdR514 ΔaraBADAH33 ΔrhaBADLD78</i>	Coli genome stock center
<i>E. coli</i> XL-1 Blue	<i>recA1 endA1gyrA96thi-1hsdR17supE44relA1lac</i>	Stratagene
<i>E. coli</i> BL21 Star (DE3)	F <sup>-</sup> <i>ompT hsdS<sub>B</sub> (r<sub>B</sub><sup>-</sup>m<sub>B</sub><sup>-</sup>) gal dcm (DE3)</i>	Invitrogen
BW1	BW25113, pZE-sbnAB	This study
BW2	BW25113Δ <i>serB</i> , pZE-sbnAB	This study
BW3	BW25113, pCS-LsBAHD-panE	This study
BW4	BW25113, pCS-LsBAHD-ScAAE-Scgloxdh	This study
BW5	BW25113, pCS-LsBAHD-ScAAE-Fpoah	This study
BW6	BW25113, pCS-LsBAHD*-panE	This study
BW7	BW25113, pCS-LsBAHD*-ScAAE-Scgloxdh	This study
BW8	BW25113, pCS-LsBAHD*-ScAAE-Fpoah	This study
BWΔ5	BW25113Δ <i>aceBAglcBAycdWΔghrBAgcl</i>	This study
BW9	BWΔ5, pCS-LsBAHD*-panE	This study
BW10	BWΔ5, pCS-LsBAHD*-ScAAE-Scgloxdh	This study
BW11	BWΔ5, pZE-sbnAB, pCS-LsBAHD*-panE	This study
BW12	BWΔ5, pZE-sbnAB, pCS-LsBAHD*-ScAAE-Scgloxdh	This study
BW13	BW25113, pZE-sbnAB, pCS-LsBAHD*-ScAAE-Fpoah	This study
BW14	BWΔ5, pZE-sbnAB, pCS-LsBAHD*-ScAAE-Fpoah-Scgloxdh	This study
BW15	BWΔ5Δ <i>serB</i> , pZE-sbnAB, pCS-LsBAHD*-ScAAE-Fpoah-Scgloxdh	This study
BW16	BWΔ5Δ <i>serB</i> , pZE-sbnAB, pCS-LsBAHD*-ScAAE-Fpoah-Scgloxdh, pSA-aceA	This study

**Supplementary Table 2. Plasmids used in this study.**

<b>Plasmids</b>	<b>Description*</b>	<b>Source</b>
pZE12-luc	P <sub>L</sub> lacO1, <i>colE</i> ori, <i>luc</i> , <i>Amp</i> <sup>R</sup>	Ref. 1
pCS27	P <sub>L</sub> lacO1, <i>p15A</i> ori, <i>Kan</i> <sup>R</sup>	Ref. 1
pSA74	P <sub>L</sub> lacO1, <i>pSC101</i> ori, <i>Cl</i> <sup>R</sup>	Ref. 1
pETDuet-1	PT7, <i>pBR322</i> ori, <i>Amp</i> <sup>R</sup>	Ref. 2
pMAL-c2x	Ptac, <i>pBR322</i> ori, MBP tag, <i>Amp</i> <sup>R</sup>	Ref. 3
pGEX-6p-1	Ptac, <i>pBR322</i> ori, GST tag, <i>Amp</i> <sup>R</sup>	Solarbio
pZE-sbnAB	pZE12-luc, <i>sbnA</i> and <i>sbnB</i> from <i>S. aureus</i>	This study
pET-Fpgloxdh	pETDuet-1, <i>Fpgloxdh</i> from <i>F. palustris</i>	This study
pET-Scgloxdh	pETDuet-1, <i>Scgloxdh</i> from <i>S. cerevisiae</i>	This study
pET-Anoah	pETDuet-1, <i>Anoah</i> from <i>A. niger</i>	This study
pET-Pcoah	pETDuet-1, <i>Pcoah</i> from <i>P. chrysogenum</i>	This study
pET-Fpoah	pETDuet-1, <i>Fpoah</i> from <i>F. palustris</i>	This study
pET-Ssoah	pETDuet-1, <i>Ssoah</i> from <i>S. sclerotiorum</i>	This study
pET-panE	pETDuet-1, <i>panE</i> from <i>M. extorquens</i>	This study
pET-LsBAHD	pETDuet-1, <i>Lsbahd</i> from <i>L. sativus</i>	This study
pCS-dnaKJ	pCS27, <i>dnaK</i> and <i>dnaJ</i> from <i>E. coli</i>	This study
pCS-groSL	pCS27, <i>groS</i> and <i>groL</i> from <i>E. coli</i>	This study
pCS-ibpAB	pCS27, <i>ibpA</i> and <i>ibpB</i> from <i>E. coli</i>	This study
pMAL-LsBAHD	pMAL-c2x, <i>Lsbahd</i>	This study
pGEX-LsBAHD	pGEX-6p-1, <i>Lsbahd</i>	This study
pET-LsBAHD*	pETDuet-1, <i>Lsbahd</i>	This study
pCS-LsBAHD-panE	pCS27, <i>Lsbahd</i> and <i>panE</i>	This study
pCS-LsBAHD-ScAAE-Scgloxdh	pCS27, <i>Lsbahd</i> , <i>ScAAE</i> and <i>Scgloxdh</i>	This study
pCS-LsBAHD-ScAAE-Fpoah	pCS27, <i>Lsbahd</i> , <i>ScAAE</i> and <i>Fpoah</i>	This study
pCS-LsBAHD*-panE	pCS27, <i>Lsbahd</i> * and <i>panE</i>	This study
pCS-LsBAHD*-ScAAE-Scgloxdh	pCS27, <i>Lsbahd</i> *, <i>ScAAE</i> and <i>Scgloxdh</i>	This study
pCS-LsBAHD*-ScAAE-Fpoah	pCS27, <i>Lsbahd</i> *, <i>ScAAE</i> and <i>Fpoah</i>	This study
pCS-LsBAHD*-ScAAE-Fpoah-Scgloxdh	pCS27, <i>Lsbahd</i> *, <i>ScAAE</i> , <i>Fpoah</i> and <i>Scgloxdh</i>	This study
pSA-aceA	pSA74, <i>aceA</i>	This study

\**Amp*<sup>R</sup>, ampicillin resistant; *Kan*<sup>R</sup>, kanamycin resistant; *Cl*<sup>R</sup>, chloramphenicol resistant.

### Supplementary references

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3. Chen Z, Li Y, Sun X, Yuan Q. Improvement of expression level of polysaccharide lyases with new tag GAPDH in *E. coli*. *J. Biotechnol.* **236**, 159-165 (2016).