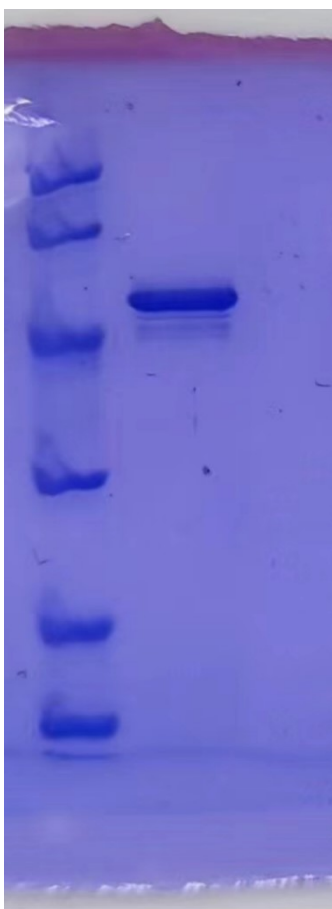
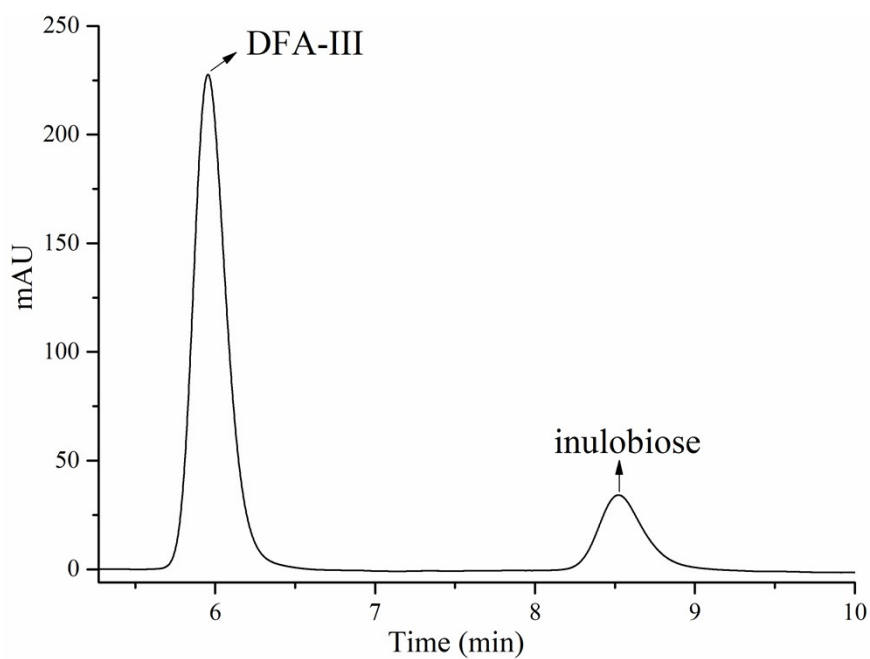


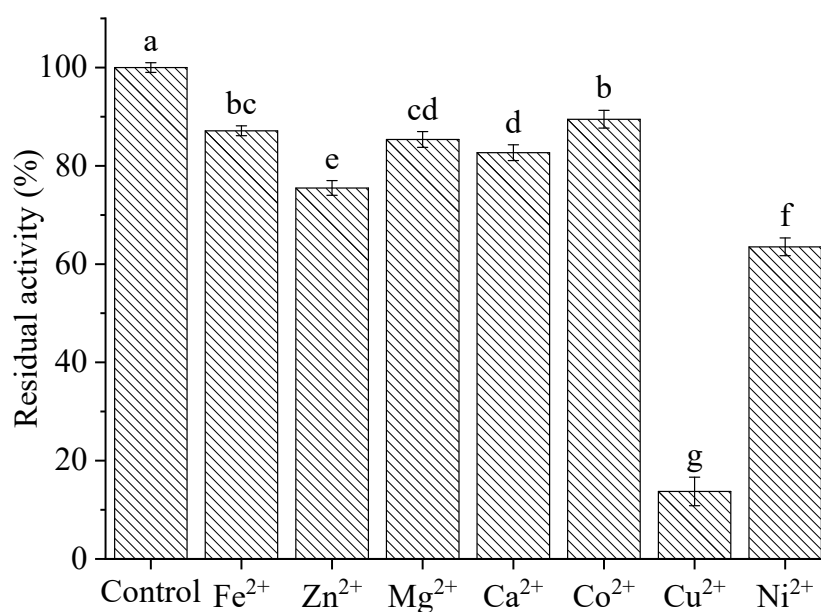
### Supporting information



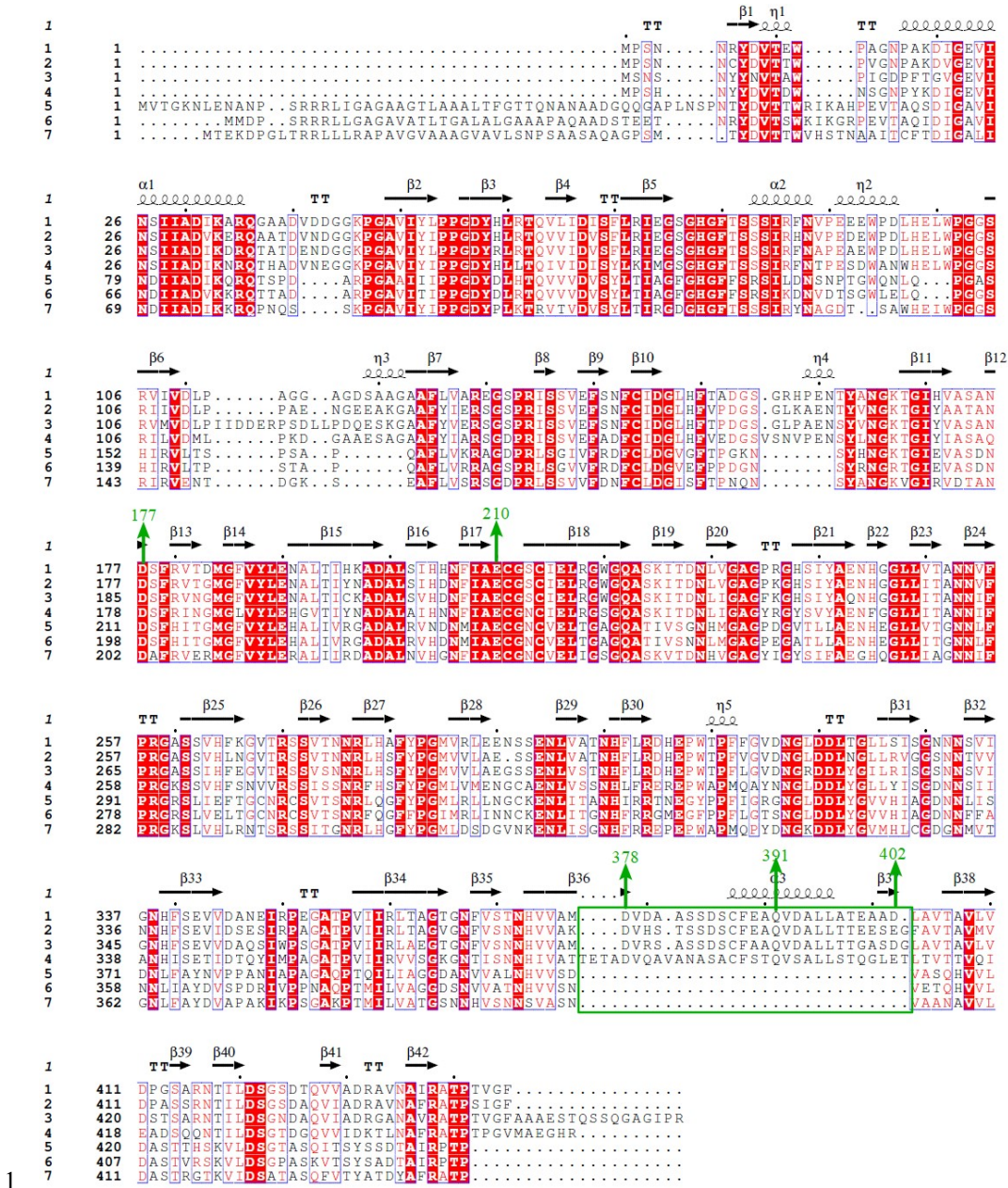
**Figure S1. SDS-PAGE of the purified enzyme (right lane) and protein marker (left lane).** The molecular weight of marker proteins from top to bottom were 97.2, 66.4, 44.3, 29.0, 20.1, and 14.3 kDa, respectively.



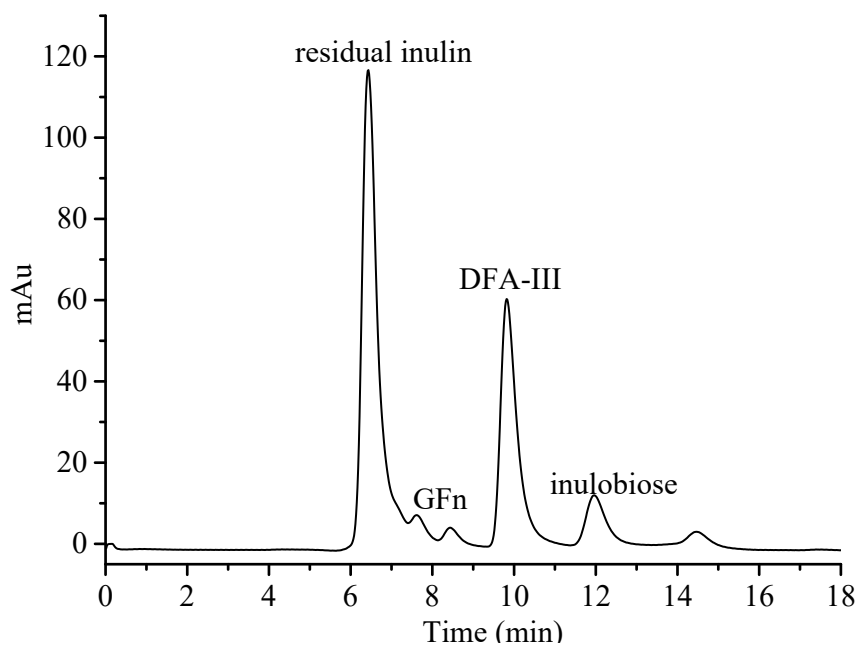
**Figure S2. High performance liquid chromatography analysis of reaction products.**



**Figure S3. Effect of metal ions on the activity of DgDFA-IIIase.** Each value is the mean of three replications  $\pm$  standard deviation. Lowercase letters (a-g) indicate significance ( $p < 0.05$ ) between groups. Statistical significances were carried out by ANOVA and Duncan's test.

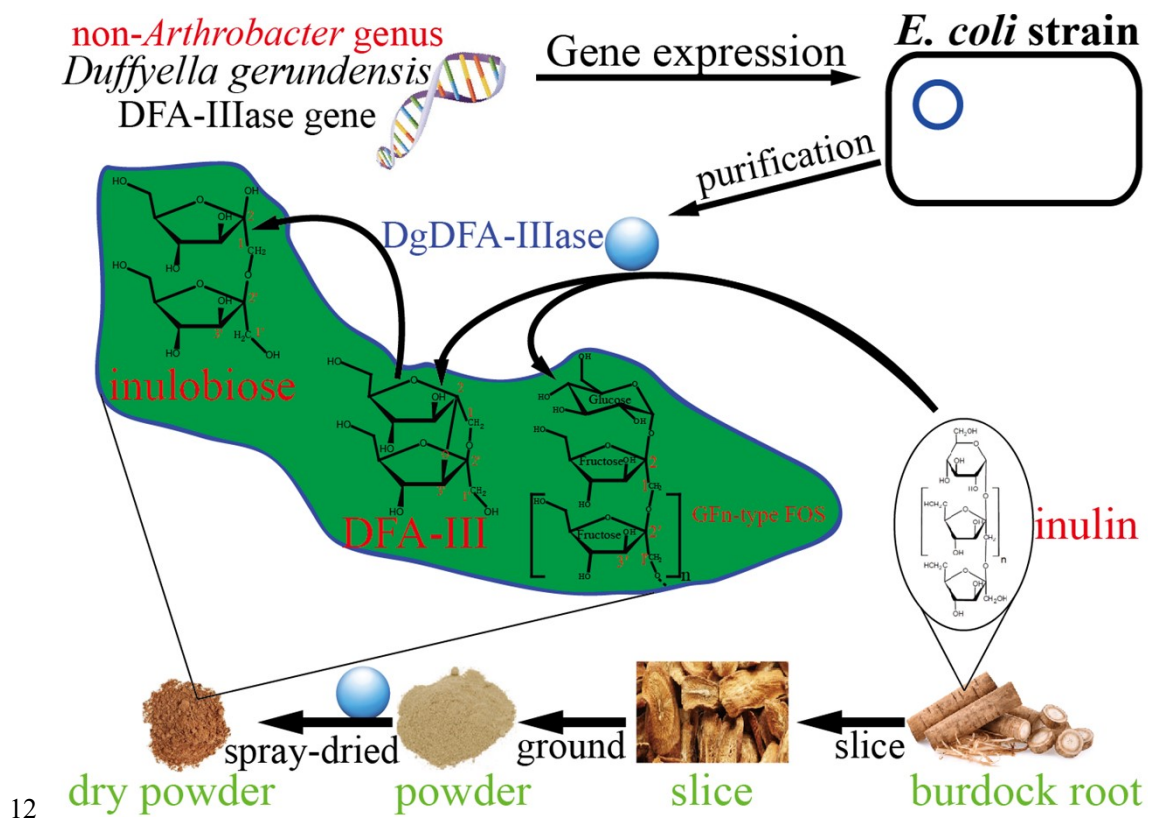


2 **Figure S4. Multiple sequence alignment of DFA-IIIases and IFTases.** 1 – 4  
3 represented AcDFA-IIIase (GenBank ID: ACL40859.1) <sup>1</sup>, *Arthrobacter* sp. H65-7  
4 DFA-IIIase (BAD06469) <sup>2</sup>, *A. aurescens* SK 8.001 DFA-IIIase (KR534324) <sup>3</sup>, and  
5 DgDFA-IIIase (QTO55438.1), respectively. 5 – 7 represented IFTase-III from *Bacillus*  
6 sp. *snu-7* (AAZ66341) <sup>4</sup>, *Arthrobacter* sp. H65-7 (BAA18967) <sup>5</sup>, and *Nonomuraea* sp.  
7 ID06-A0189 (BAN62836) <sup>6</sup>, respectively.



8

9 **Figure S5. Inulin catalyzed by DgDFA-IIIase.** GFn represented fructo-  
10 oligosaccharide including 1-kestose (GF<sub>2</sub>), nystose (GF<sub>3</sub>), and fructofuranosyl nystose  
11 (GF<sub>4</sub>).



## 19 Reference

- 20 1. S. Yu, H. Shen, Y. Cheng, Y. Zhu, X. Li and W. Mu, Structural and functional  
21 basis of difructose anhydride III hydrolase, which sequentially converts inulin  
22 using the same catalytic residue, *ACS Catal.*, 2018, **8**, 10683-10697.
- 23 2. K. Saito, Y. Sumita, Y. Nagasaka, F. Tomita and A. Yokota, Molecular cloning  
24 of the gene encoding the di-D-fructofuranose 1,2':2,3' dianhydride hydrolysis  
25 enzyme (DFA IIIase) from *Arthrobacter* sp H65-7, *J. Biosci. Bioeng.*, 2003, **95**,  
26 538-540.
- 27 3. S. Yu, X. Wang, T. Zhang, T. Stressler, L. Fischer, B. Jiang and W. Mu,  
28 Identification of a novel di-D-fructofuranose 1,2':2,3' dianhydride (DFA III)  
29 hydrolysis enzyme from *Arthrobacter aurescens* SK8.001, *PLoS One*, 2015, **10**,  
30 e0142640.
- 31 4. C. Kim, C. Hong, K. Kim, X. Wang, S. Kang and S. Kim, Cloning, expression,  
32 and characterization of *Bacillus* sp. snu-7 inulin fructotransferase, *J. Microbiol.*  
33 *Biotechnol.*, 2007, **17**, 37-43.
- 34 5. A. Yokota, S. Hirayama, K. Enomoto, Y. Miura, S. Takao and F. Tomita,  
35 Production of inulin fructotransferase (depolymerizing) by *Arthrobacter* sp.  
36 H65-7 and preparation of DFA III from inulin by the enzyme, *J. Ferment.*  
37 *Bioeng.*, 1991, **72**, 258-261.
- 38 6. S. Pudjiraharti, M. Ohtani, N. Takano, A. Abe, P. Lisdiyanti, M. Tanaka, T.  
39 Sone and K. Asano, *Nonomuraea* sp ID06-A0189 inulin fructotransferase (DFA  
40 III-forming): gene cloning, characterization and conservation among other

