Additional file

A novel strategy for efficient disaccharides synthesis from glucose by β-glucosidase

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**Table S1** The comparation of the production of laminaribiose and sophorose synthesized by *β*-glucosidase from different species

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**Fig. S1** The phylogenetic tree of *Tr*Cel1b and other GH1 family *β*-glucosidases. Sequences were obtained from the following accession number: *Tr*Cel1b (EGR49111.1), *Th*Bgl2 (5JBO), *Th*Bgl1 (5JBK), *Hi*BG (AII80277.1), *Nk*Bgl (BAB91145.1) and *Ca*BglA (JX030398.1)



**Fig. S2** The 3-D model of *Tr*Cel1b predicted by SWISS-MODEL (**A**) and I-TASSER (**B**) using *β*-glucosidase 6KHT as template

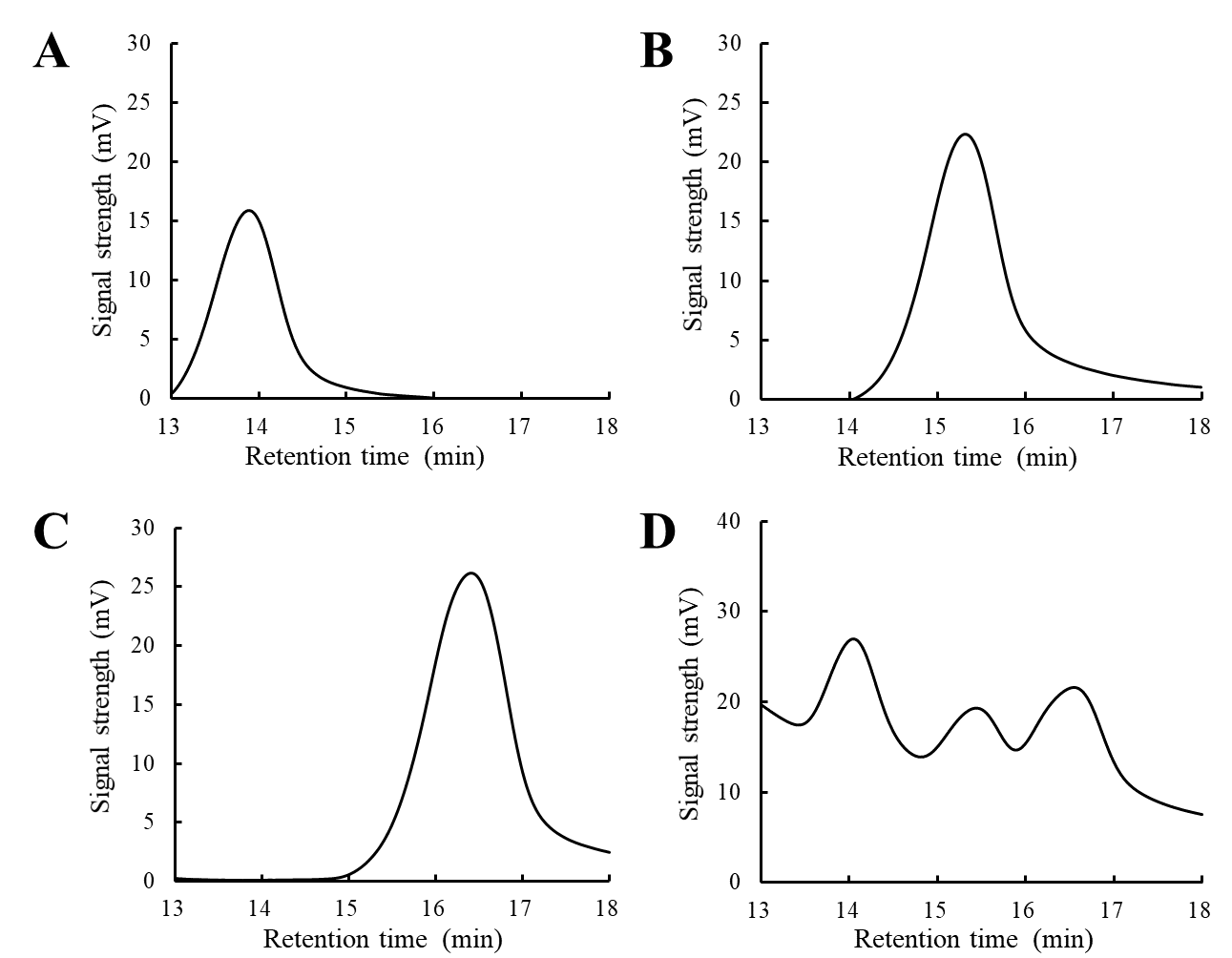
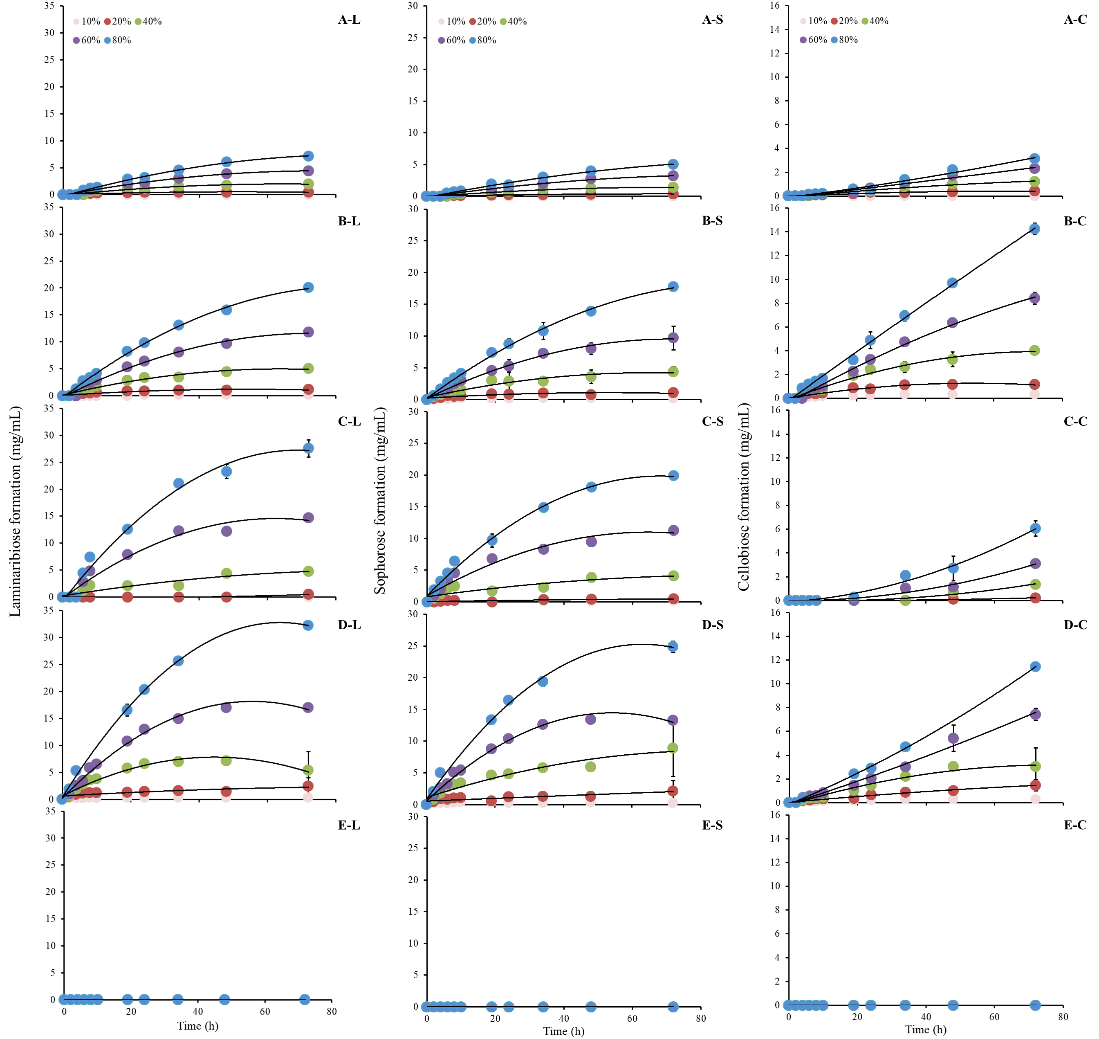
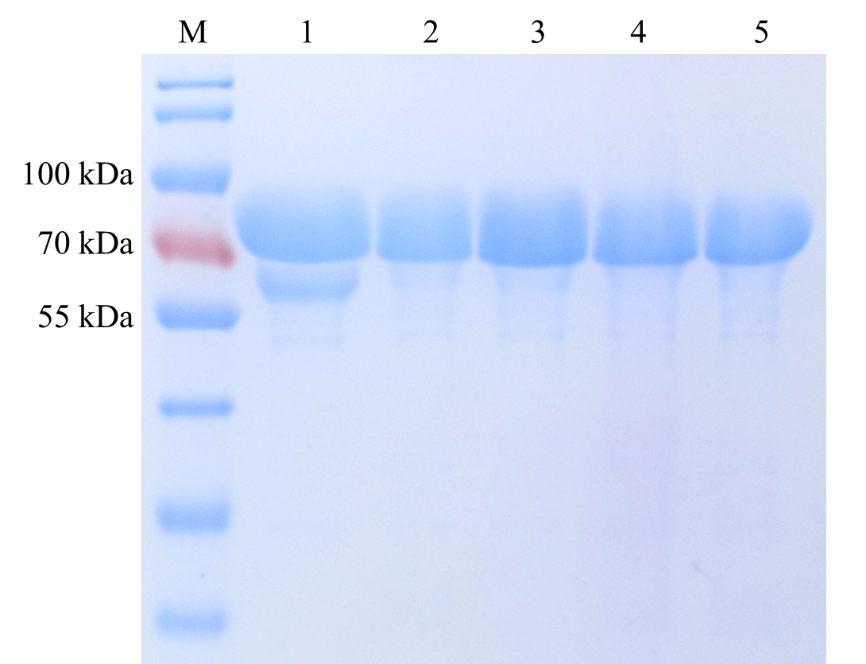


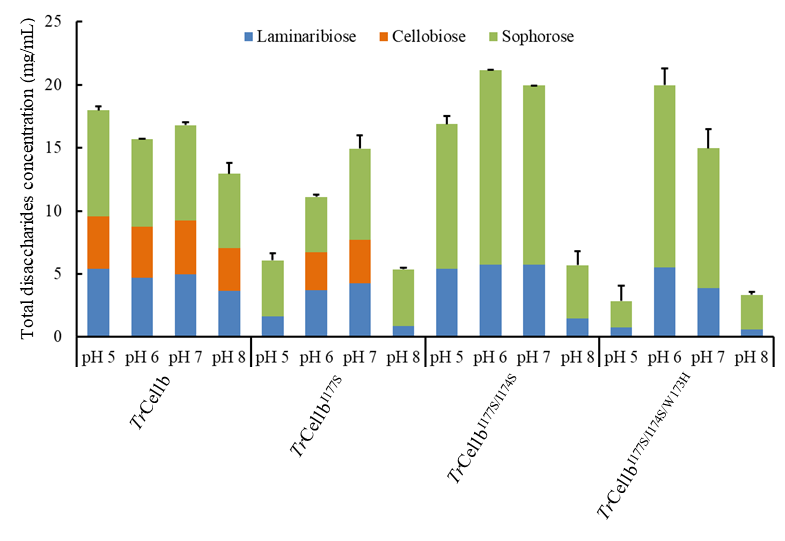
Fig. S3 Analysis of the disaccharides with HPLC. (A) Laminaribiose; (B) Cellobiose; (C) Sophorose; (D) Synthesized disaccharides by *Tr*Cel1bI177S/I174S/W173H



**Fig. S4** The disaccharides synthesis of *Tr*Cel1b and its variants under different concentrations of glucose. L, S, C represent laminaribiose (**L**), sophorose (**S**) and cellobiose (**C**); A, B, C, D, and E represent *Tr*Cel1b (**A**), *Tr*Cel1bI177S (**B**), *Tr*Cel1bI177S/I174S (**C**), *Tr*Cel1bI177S/I174S/W173H (**D**),and *Tr*Cel1bN240I (**E**). The reactions were carried out in 50 mM phosphate buffer at pH 7.4. *Tr*Cel1b and its variants were incubated with 10%, 20%, 40%, 60%, and 80% glucose as substrate. Reaction samples were taken at 0, 2, 4, 6, 8, 10, 19, 24, 34, 48, and 72 h and then were analyzed by HPLC (for detailed explanation, see experimental section)



**Fig. S5** SDS-PAGE analysis of purified *Tr*Cel1b and its variants. lane 1-5: purified *Tr*Cel1b, *Tr*Cel1bI177S, *Tr*Cel1bI177S/I174S; *Tr*Cel1bI177S/I174S/W173H; *Tr*Cel1bN240I with similar molecular masses of 74 kDa. M: Protein molecular weight marker

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**Fig. S6** Effects of pH value on disaccharides synthesis. Total disaccharides concentration represents the sum of production of laminaribiose, sophorose and cellobiose. The reactions were carried out with 40% glucose as substrate in 0.2 M sodium phosphate dibasic and 0.1 M citric acid buffer at 30 °C. Reaction samples were taken at 72 h and then were analyzed by HPLC



**Fig. S7** The interaction profiles between *Tr*Cel1b (**A**), *Tr*Cel1bI177SI174SW173H (**B**) or *Tr*Cel1bN240I (**C**) and cellobiose analyzed by Discovery Studio 4.5, respectively

**Table S1 The comparison of the production of laminaribiose and sophorose synthesized by β-glucosidase from different species**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Enzyme | Temperature  (oC) | Laminaribiose production  (mg/mL/mg enzyme) | Sophorose production  (mg/mL/mg enzyme) | Reference |
| *Tr*Cel1bI177S/I174S/W173H | 30 | 92.3 | 71.1 | This studya |
| *An*Bgl | 70 | 4.3 | 2.3 | M. V. Semenova, 2015b |
| *Pv*Bgl | 70 | 1.9 | 1.2 | M. V. Semenova, 2015b |
| *Tr*Bgl | 70 | 2.4 | 0.2 | M. V. Semenova, 2015b |
| *Corynascus* sp. Bgl | 70 | 2.7 | 3.8 | M. V. Semenova, 2015b |
| Almond Bgl | 55 | 6-9 | 6-9 | C. Ravet, 1993c |

a: the reactions were carried out at 30 oC for 72h using 800 g/L glucose as substrate.

b: the reactions were carried out at 70 oC for 48 h using 600 g/L glucose as substrate.

c: the reactions were carried out at 55 oC for 100 h using 900 g/L glucose as substrate.

**Table S2** **Primers used in this study**

|  |  |
| --- | --- |
| Name of Primer | Nucleotide Sequence |
| Cel1b-F | CCGGAATTCCCCGAGTCGCTAGCTCTG |
| Cel1b-R | CCCAAGCTTTGCCGCCACTTTAACCCTCTG |
| 177S-F | AGCTATGGATATGCCACCGGCAGCAACGC |
| 177S-R | GGCCTGAATCCAGGGTTCGTTGATGGTG |
| I177S/I174-F | AGTCAGGCCAGCTATGGATATGCCACCG |
| I177S/I174-R | CCAGGGTTCGTTGATGGTGATCCAGTTCT |
| I177S/I174/W173H-F | CACAGTCAGGCCAGCTATGGATATGCCACC |
| I177S/I174/W173H-R | GGGTTCGTTGATGGTGATCCAGTTCTGG |
| N240I-F | ATCGGCGACTACTATGAGCCCTGGGACAG |
| N240I-R | GAGCGAGATGCCGATCTGGCCCTTTTGC |