Supporting Material

Enzymatic synthesis of 6'-sialyllactose, a dominant sialylated human milk oligosaccharide, by a novel *exo*-α-sialidase from *Bacteroides fragilis* NCTC9343

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Running title: Synthesis of 6'-sialyllactose by an *exo*-α-sialidase

Table of Contents

Table S1 Sequence analysis of seven *exo*-α-sialidases

Enzyme source	Signal peptide [a] (Amino acid No.)	Transmembrane region [b] (Amino acid No.)
B. fragilis NCTC9343		
BfGH33A	1-22	5-27
BfGH33B	N	N
BfGH33C	1-21	N
C. perfringens ATCC13124		
CpGH33A	1-26	N
CpGH33B	N	N
CpGH33C	N	N
B. bifidum JCM1254		
SiaBb2	1-35	13-35, 807-829

[a] Signal peptide was predicted using online tools (http://www.cbs.dtu.dk/services/SignalP/).

[b] Transmembrane region was predicted using online tools (http://www.cbs.dtu.dk/services/TMHMM/)

N, no signal peptide or transmembrane region.

Table S2 Kinetic parameters of BfGH33C with sialylated substrates[a]

Substrate	V ($\mu mol \cdot min^{-1} \cdot mg^{-1}$)	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)
4MU-Neu5Ac	293.5±10.5	0.06±0.01	283.2	4857.3
Sialic acid dimer	341.6±9.3	0.75±0.07	329.6	438.0

a The data represent triplicate experiments (for details, see materials and methods).

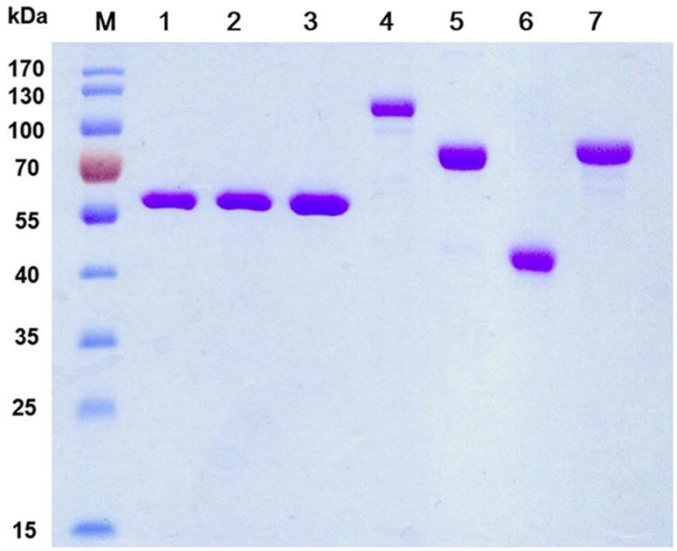

Fig. S1 SDS-PAGE analysis of the recombinant *exo*-α-sialidases purified from *E. coli*. Lane M, marker proteins; lanes 1, 2, 3, 4, 5, 6, and 7 were purified enzyme BfGH33A, BfGH33B, BfGH33C, CpGH33A, CpGH33B, CpGH33C and SiaBb2, respectively.

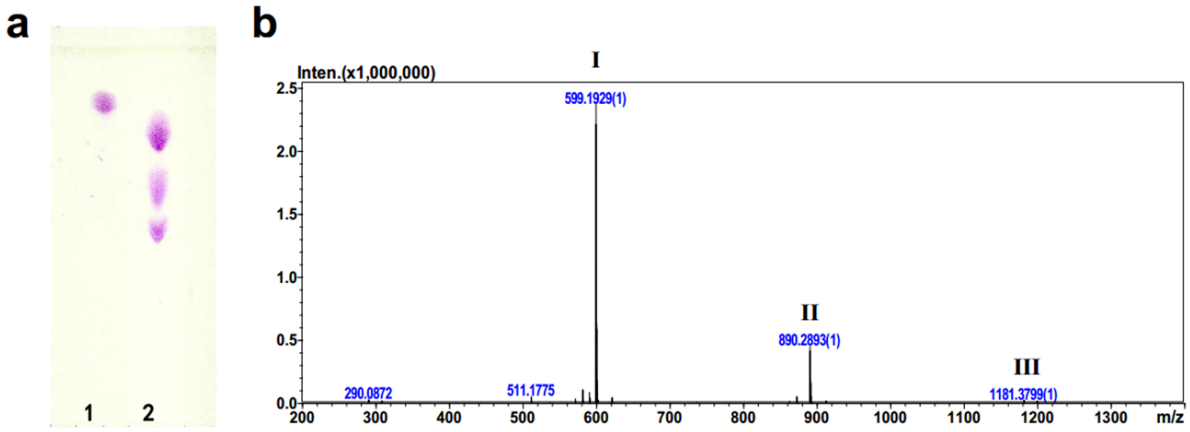

Fig. S2 TLC (**a**) and MS (**b**) analysis of the oligosialic acid prepared from colominic acid. **a** Lane 1, the standard sialic acid. Lane 2, the oligosialic acid prepared from colominic acid. **b** I, sialic acid dimer (M_r 600); II, sialic acid trimer (M_r 891); III, sialic acid tetramer (M_r 1182).

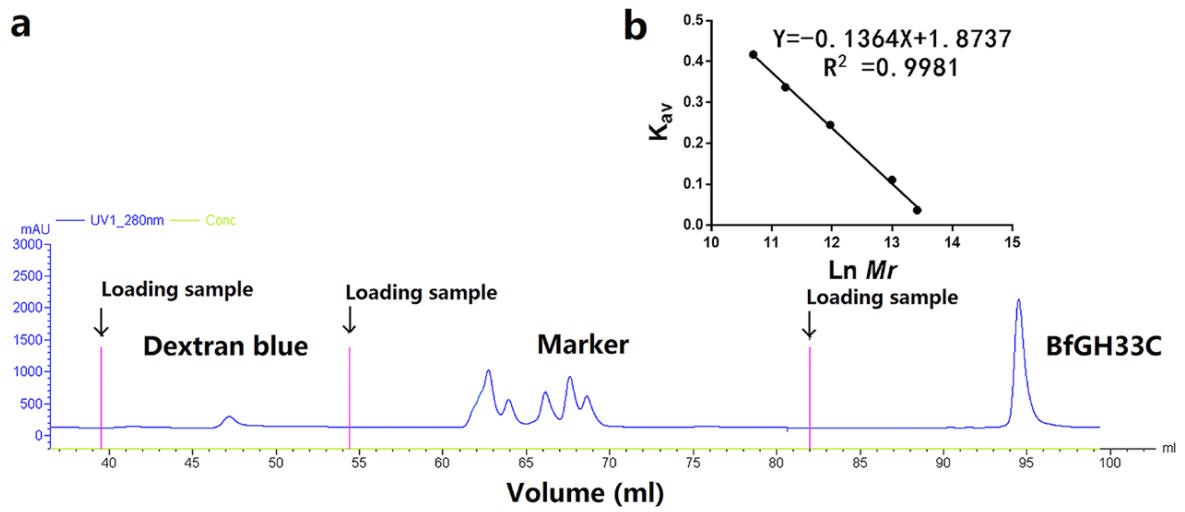

Fig. S3 Gel filtration chromatography analysis of recombinant BfGH33C. (**a**) The elution patterns of dextran blue, marker proteins thyroglobulin (669 kDa), ferritin (440 kDa), aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (44 kDa), and BfGH33C. The elution volumes of them were 7.7 ml, 8.3 ml, 9.5 ml, 11.7 ml, 13.2 ml, 14.5 ml, and 12.5 ml, respectively. The arrows represent the beginning of loading samples. (**b**) The marker standard curve based on the equation $K_{av}=(V_e-V_o)/(V_c-V_o)$. V_o and V_e represent the elution volumes of dextran blue and protein, respectively. V_c represents column volume (24 ml). The K_{av} of BfGH33C was calculated as 0.29, and its native molecular weight was about 113.6 kDa.

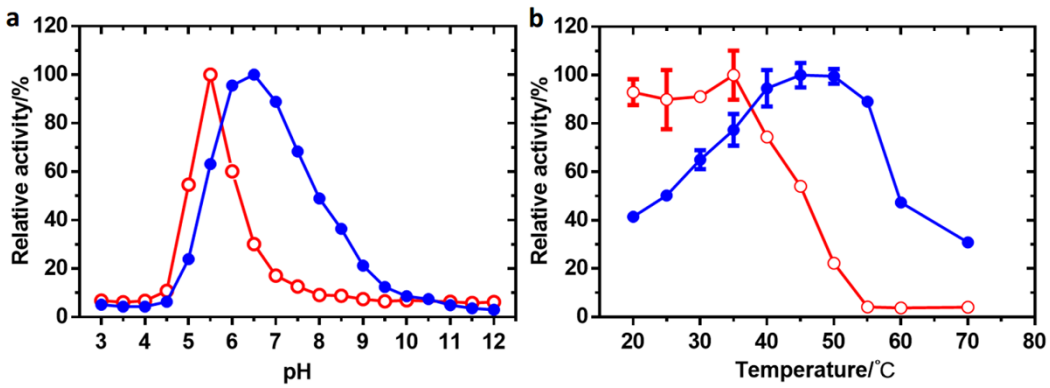

Fig. S4 Effects of pH (**a**) and temperature (**b**) on BfGH33C hydrolase activity (●) and stability (○). Data points represent the means ± SD of three replicates.

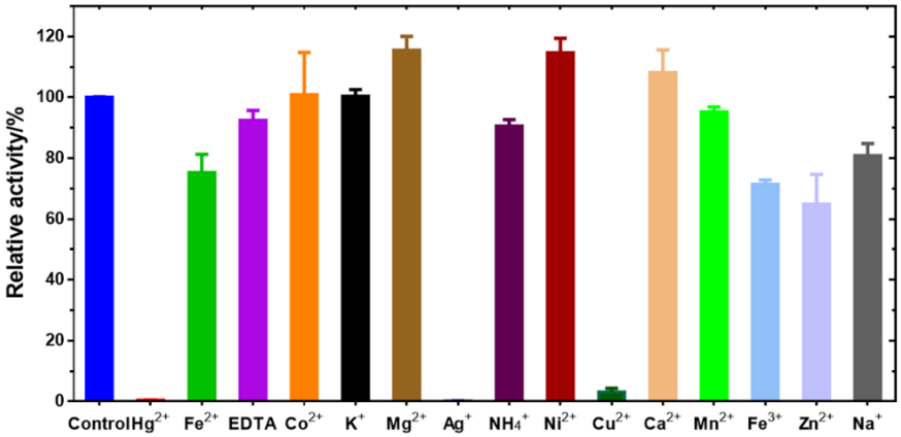

Fig. S5 Effect of metal ions and EDTA on the hydrolysis activity of BfGH33C. Data points represent the means ± SD of three replicates.

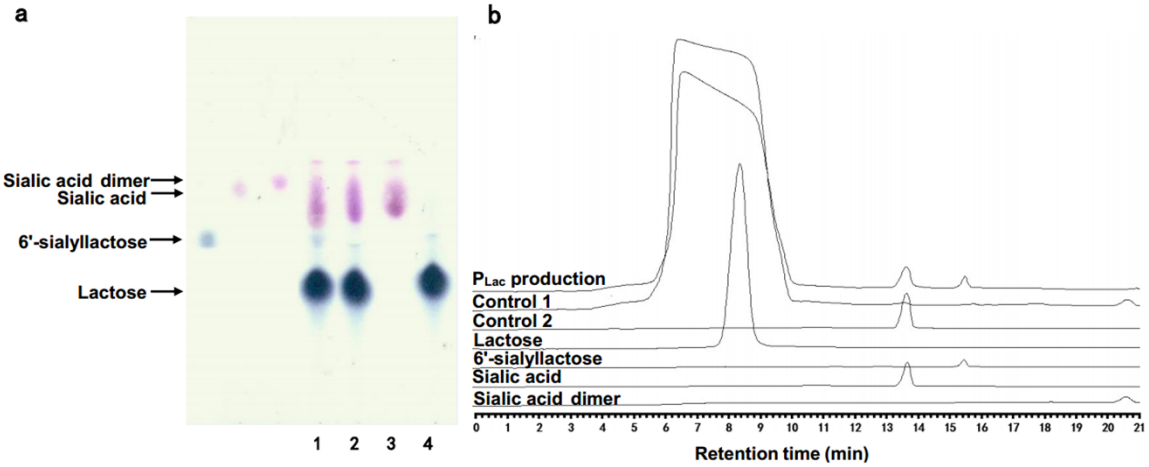

Fig. S6 Analysis of transglycosylation reaction mixture of BfGH33C by TLC (**a**) and HPAEC (**b**). **a** Lane 1, reaction containing sialic acid dimer, lactose and BfGH33C; Lane 2, control reaction containing sialic acid dimer, lactose and inactivated BfGH33C; Lane 3, control reaction containing sialic acid dimer and BfGH33C; Lane 4, control reaction containing lactose and BfGH33C. **b** P_{Lac} production, the same as lane 1 in (**a**); control 1, the same as lane 2 in (**a**); control 2, the same as lane 3 in (**a**).

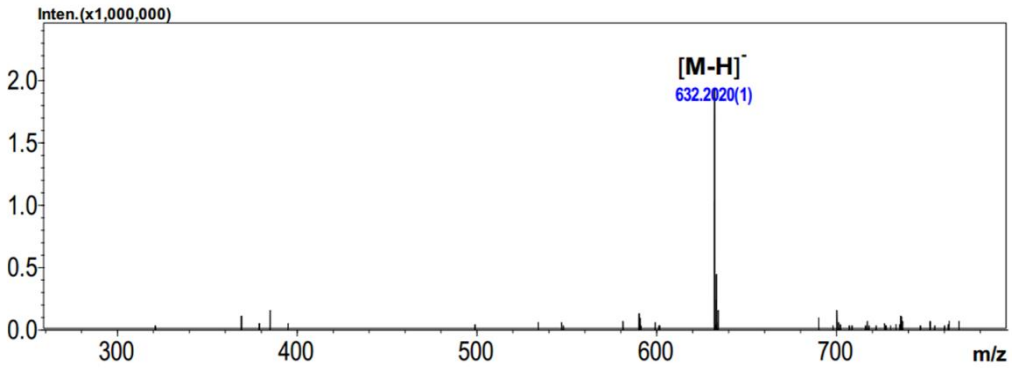

Fig. S7 ESI-MS spectra of P_{Lac} (M_r 633) produced by BfGH33C.

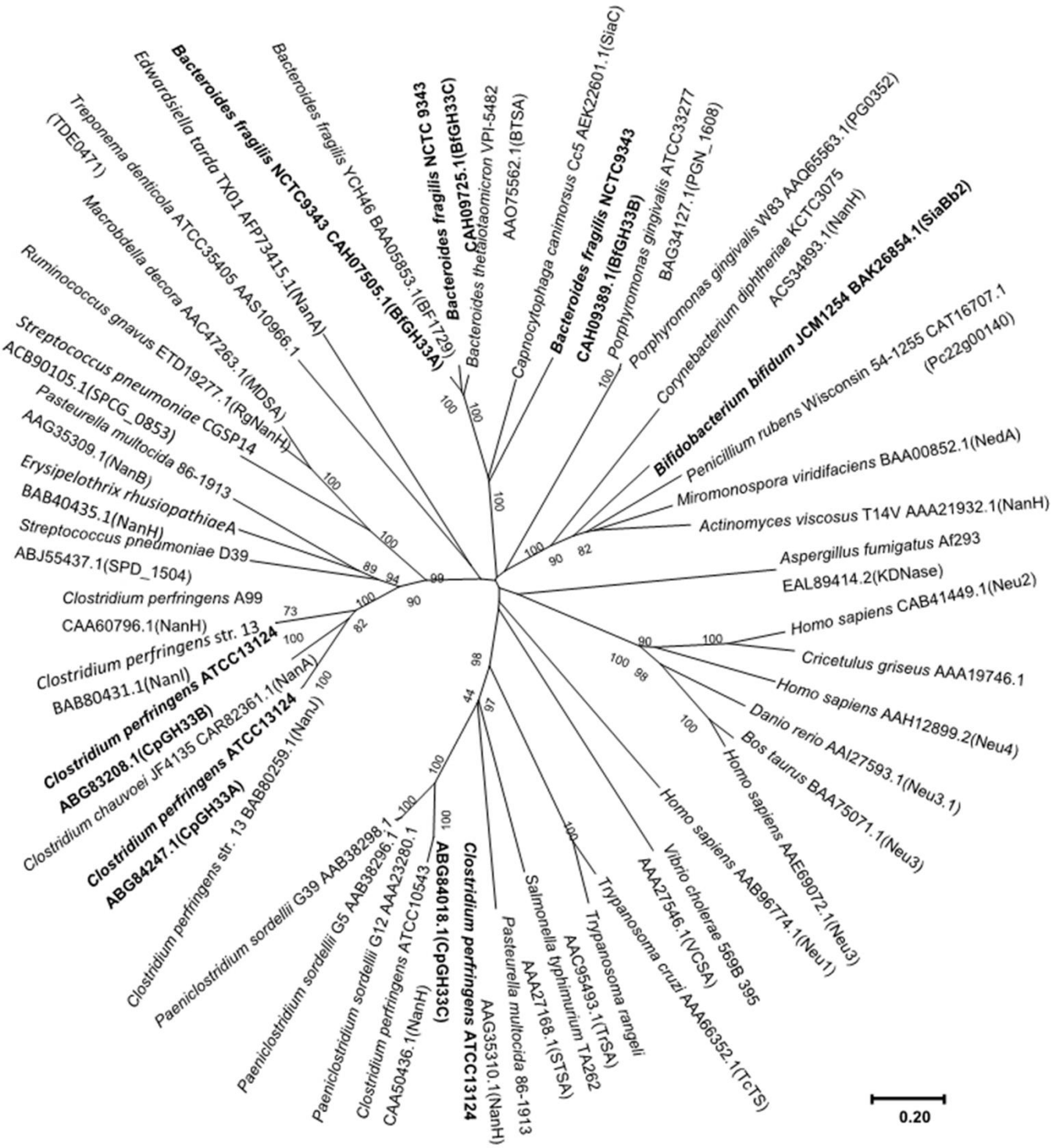

Fig. S8 Phylogenetic analysis of catalytic modules from 45 GH33 *exo*-α-sialidases. The domain analysis was predicted using online tools (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi/). The domain sequences of the *exo*-α-sialidases were firstly subjected to the multiple alignments by the ClustalX 2.1 program and then converted to a phylogenetic tree by the MEGA7 program. The sequences from the enzymes BfGH33A, BfGH33B, BfGH33C, CpGH33A, CpGH33B, CpGH33C and SiaBb2 are highlighted in bold.