

Supplementary Information

Mining novel starch-converting Glycoside Hydrolase 70 enzymes from the Nestlé Culture Collection genome database: The *Lactobacillus reuteri* NCC 2613 GtfB

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SUPPLEMENTARY TABLE

SUPPLEMENTARY FIGURES

Table S1. GenBank accession numbers of the family GH70 protein sequences used in the phylogenetic tree of Figure 1.

Accession number	Bacterium strain
AAC43483.1	<i>Streptococcus gordonii</i> str. Challis substr. CH1
BAA26114.1	<i>Streptococcus mutans</i>
BAC07265.1	<i>Streptococcus sobrinus</i> B13N
KY595679	<i>Lactobacillus delbrueckii</i> NCC 119
KY595675	<i>Lactobacillus delbrueckii</i> NCC 147
KY595681	<i>Lactobacillus delbrueckii</i> NCC 1579
KY595680	<i>Lactobacillus delbrueckii</i> NCC 164
KY595682	<i>Lactobacillus delbrueckii</i> NCC 167
KY595683	<i>Lactobacillus delbrueckii</i> NCC 169
KY595684	<i>Lactobacillus delbrueckii</i> NCC 188
KY595685	<i>Lactobacillus delbrueckii</i> NCC 2506
KY595688	<i>Lactobacillus delbrueckii</i> NCC 2510
KY595686	<i>Lactobacillus delbrueckii</i> NCC 2574
KY595687	<i>Lactobacillus delbrueckii</i> NCC 2595
KY595689	<i>Lactobacillus delbrueckii</i> NCC 2727
KY595690	<i>Lactobacillus delbrueckii</i> NCC 2812
KY595691	<i>Lactobacillus delbrueckii</i> NCC 3035
KY595692	<i>Lactobacillus delbrueckii</i> NCC 3077
KY595678	<i>Lactobacillus delbrueckii</i> NCC 32
KY595693	<i>Lactobacillus delbrueckii</i> NCC 39
KY595699	<i>Lactobacillus delbrueckii</i> NCC 56
KY595694	<i>Lactobacillus delbrueckii</i> NCC 590
KY595695	<i>Lactobacillus delbrueckii</i> NCC 601
KY595698	<i>Lactobacillus delbrueckii</i> NCC 603
KY595696	<i>Lactobacillus delbrueckii</i> NCC 610
KY595697	<i>Lactobacillus delbrueckii</i> NCC 612
KY595700	<i>Lactobacillus delbrueckii</i> NCC 618
KY595704	<i>Lactobacillus delbrueckii</i> NCC 621
KY595702	<i>Lactobacillus delbrueckii</i> NCC 627
KY595701	<i>Lactobacillus delbrueckii</i> NCC 636
KY595703	<i>Lactobacillus delbrueckii</i> NCC 637
KY595705	<i>Lactobacillus delbrueckii</i> NCC 648
KY595706	<i>Lactobacillus delbrueckii</i> NCC 653
KY595707	<i>Lactobacillus delbrueckii</i> NCC 657
KY595708	<i>Lactobacillus delbrueckii</i> NCC 667
KY595709	<i>Lactobacillus delbrueckii</i> NCC 675
KY595710	<i>Lactobacillus delbrueckii</i> NCC 687
KY595711	<i>Lactobacillus delbrueckii</i> NCC 696
KY595712	<i>Lactobacillus delbrueckii</i> NCC 706
KY595714	<i>Lactobacillus delbrueckii</i> NCC 707
KY595713	<i>Lactobacillus delbrueckii</i> NCC 710
KY595715	<i>Lactobacillus delbrueckii</i> NCC 716
KY595677	<i>Lactobacillus delbrueckii</i> NCC 71
KY595716	<i>Lactobacillus delbrueckii</i> NCC 723
KY595717	<i>Lactobacillus delbrueckii</i> NCC 731
KY595674	<i>Lactobacillus delbrueckii</i> NCC 749
KY595718	<i>Lactobacillus delbrueckii</i> NCC 800
KY595719	<i>Lactobacillus delbrueckii</i> NCC 801
KY595720	<i>Lactobacillus delbrueckii</i> NCC 828
KY595721	<i>Lactobacillus delbrueckii</i> NCC 829
KY595676	<i>Lactobacillus delbrueckii</i> NCC 82
KY595722	<i>Lactobacillus delbrueckii</i> NCC 853
KY595723	<i>Lactobacillus delbrueckii</i> NCC 861
KY595724	<i>Lactobacillus delbrueckii</i> NCC 865
KY595725	<i>Lactobacillus delbrueckii</i> NCC 875
KY595726	<i>Lactobacillus delbrueckii</i> NCC 895

KY595727	<i>Lactobacillus delbrueckii</i> NCC 902
KY595728	<i>Lactobacillus delbrueckii</i> NCC 924
KY595731	<i>Lactobacillus delbrueckii</i> NCC 946
KY595729	<i>Lactobacillus delbrueckii</i> NCC 960
KY595730	<i>Lactobacillus delbrueckii</i> NCC 968
AOR73699	<i>Lactobacillus fermentum</i> NCC 2970
KY595732	<i>Lactobacillus fermentum</i> NCC 3053
KY595733	<i>Lactobacillus fermentum</i> NCC 3057
KY595734	<i>Lactobacillus fermentum</i> NCC 3064
KY595737	<i>Lactobacillus fermentum</i> NCC 3065
KY595735	<i>Lactobacillus fermentum</i> NCC 515
KY595736	<i>Lactobacillus fermentum</i> NCC 528
KY595738	<i>Lactobacillus fermentum</i> NCC 729
KY595739	<i>Lactobacillus fermentum</i> NCC 743
KY595741	<i>Lactobacillus plantarum</i> NCC 1287
KY595740	<i>Lactobacillus plantarum</i> NCC 1323
KY595742	<i>Lactobacillus plantarum</i> NCC 1517
KY595743	<i>Lactobacillus plantarum</i> NCC 1690
KY595744	<i>Lactobacillus plantarum</i> NCC 1736
KY595745	<i>Lactobacillus plantarum</i> NCC 2502
KY595748	<i>Lactobacillus plantarum</i> NCC 79
KY595673	<i>Lactobacillus plantarum</i> NCC 81
KY595749	<i>Lactobacillus plantarum</i> NCC 84
KY595746	<i>Lactobacillus reuteri</i> NCC 1945
KY595747	<i>Lactobacillus reuteri</i> NCC 2592
KY595750	<i>Lactobacillus reuteri</i> NCC 2603
KY595751	<i>Lactobacillus reuteri</i> NCC 2613
KY595752	<i>Lactobacillus reuteri</i> NCC 2884
KY595753	<i>Lactobacillus reuteri</i> NCC 2885
KY595754	<i>Lactobacillus reuteri</i> NCC 3062
KY595756	<i>Lactobacillus reuteri</i> NCC 3072
KY595755	<i>Lactobacillus sanfranciscensis</i> NCC 2505
KY595757	<i>Lactobacillus sanfranciscensis</i> NCC 2512
KY595758	<i>Lactobacillus sanfranciscensis</i> NCC 2532
KY595759	<i>Lactobacillus sanfranciscensis</i> NCC 2550
KY595760	<i>Lactobacillus sanfranciscensis</i> NCC 2558
KY595761	<i>Lactobacillus sanfranciscensis</i> NCC 2572
KY595762	<i>Lactobacillus sanfranciscensis</i> NCC 2580
KY595763	<i>Lactobacillus sanfranciscensis</i> NCC 2602
KY595764	<i>Lactobacillus sanfranciscensis</i> NCC 2607
KY595765	<i>Lactobacillus sanfranciscensis</i> NCC 2615
KY595766	<i>Lactobacillus sanfranciscensis</i> NCC 2629
KY595767	<i>Lactobacillus sanfranciscensis</i> NCC 2673
KY595768	<i>Lactobacillus sanfranciscensis</i> NCC 2678
KY595769	<i>Lactobacillus sanfranciscensis</i> NCC 2681
KY595770	<i>Lactobacillus sanfranciscensis</i> NCC 2694
KY595771	<i>Lactobacillus sanfranciscensis</i> NCC 2731
KY595772	<i>Lactobacillus sanfranciscensis</i> NCC 2764
KY595773	<i>Lactobacillus sanfranciscensis</i> NCC 2769
KY595774	<i>Lactobacillus sanfranciscensis</i> NCC 463
KY595776	<i>Leuconostoc citreum</i> NCC 2514
KY595775	<i>Streptococcus thermophilus</i> NCC 2408
KY595777	<i>Streptococcus thermophilus</i> NCC 903
AAU08014.2	<i>Lactobacillus reuteri</i> 121
ACB62096.1	<i>Exiguobacterium sibiricum</i> (strain DSM 17290 / JCM 13490 / 255-15)
AJE22990.1	<i>Azotobacter chroococcum</i> NCIMB 8003
WP_052702730.1	<i>Paenibacillus beijingensis</i>
CCK33644.1	<i>Lactobacillus animalis</i>
CCK33643.1	<i>Lactobacillus curvatus</i>
AAU08008.1	<i>Lactobacillus fermentum</i>
AAU08006.1	<i>Lactobacillus parabuchneri</i>

AAU08015.1	<i>Lactobacillus reuteri</i>
AAU08001.1	<i>Lactobacillus reuteri</i>
AAU86923.1	<i>Lactobacillus reuteri</i>
AAU08004.1	<i>Lactobacillus reuteri</i>
ABP88726.1	<i>Lactobacillus reuteri</i>
AAU08011.1	<i>Lactobacillus sakei</i>
AIM52834.1	<i>Leuconostoc citreum</i>
ACY92456.2	<i>Leuconostoc citreum</i>
BAF96719.1	<i>Leuconostoc citreum</i>
ACA83218.1	<i>Leuconostoc citreum</i> (strain KM20)
AAB95453.1	<i>Leuconostoc mesenteroides</i>
ACT20911.1	<i>Leuconostoc lactis</i>
ABC75033.1	<i>Leuconostoc mesenteroides</i>
ABF85832.1	<i>Leuconostoc mesenteroides</i>
CAB76565.1	<i>Leuconostoc mesenteroides</i>
AAG38021.1	<i>Leuconostoc mesenteroides</i>
AAS79426.1	<i>Leuconostoc mesenteroides</i>
AFP53921.1	<i>Leuconostoc mesenteroides</i>
AAQ98615.2	<i>Leuconostoc mesenteroides</i>
AAG61158.1	<i>Leuconostoc mesenteroides</i>
CAB65910.2	<i>Leuconostoc mesenteroides</i>
AAN38835.1	<i>Leuconostoc mesenteroides</i>
AAD10952.1	<i>Leuconostoc mesenteroides</i>
BAA90527.1	<i>Leuconostoc mesenteroides</i>
BAF62338.1	<i>Streptococcus criceti</i>
AAA26898.1	<i>Streptococcus downei</i> (<i>Streptococcus sobrinus</i>)
AAC63063.1	<i>Streptococcus downei</i> (<i>Streptococcus sobrinus</i>)
AAN58619.1	<i>Streptococcus mutans</i> serotype c (strain ATCC 700610 / UA159)
AAN58705.1	<i>Streptococcus mutans</i> serotype c (strain ATCC 700610 / UA159)
AAN58706.1	<i>Streptococcus mutans</i> serotype c (strain ATCC 700610 / UA159)
BAA95201.1	<i>Streptococcus oralis</i>
BAF62337.1	<i>Streptococcus orisuis</i>
AAA26896.1	<i>Streptococcus salivarius</i>
CAA77898.1	<i>Streptococcus salivarius</i>
AAC41412.1	<i>Streptococcus salivarius</i>
AAC41413.1	<i>Streptococcus salivarius</i>
BAF43788.1	<i>Streptococcus sanguinis</i>
BAA14241.1	<i>Streptococcus downei</i> (<i>Streptococcus sobrinus</i>)
BAA02976.1	<i>Streptococcus sobrinus</i>
AAX76986.1	<i>Streptococcus sobrinus</i>
ACK38203.1	<i>Weissella cibaria</i>
ADB43097.3	<i>Weissella cibaria</i>
AKE50934.1	<i>Weissella confusa</i> (<i>Lactobacillus confusus</i>)
CCF30682.1	<i>Weissella confusa</i> LBAE C39-2
AHU88292.1	<i>Weissella confusa</i> (<i>Lactobacillus confusus</i>)
CDX66641.1	<i>Leuconostoc citreum</i> NRRL B-1299
CDX66896.1	<i>Leuconostoc citreum</i> NRRL B-1299
CDX66895.1	<i>Leuconostoc citreum</i> NRRL B-1299
CDX66820.1	<i>Leuconostoc citreum</i> NRRL B-1299
CDX67012.1	<i>Leuconostoc citreum</i> NRRL B-1299

FIG. S1. SDS-PAGE analysis of the recombinant *L. reuteri* NCC 2613GtfB protein purified from *E. coli* BL21 Star (DE3). Lane M, molecular mass standards; lanes 1 and 2, samples of cell free extract; lane 3, sample of the insoluble fraction after centrifugation of lysed cells; lane 4, pooled fractions after Ni-NTA agarose column chromatography. Bands corresponding to the GtfB protein are marked with an arrow.

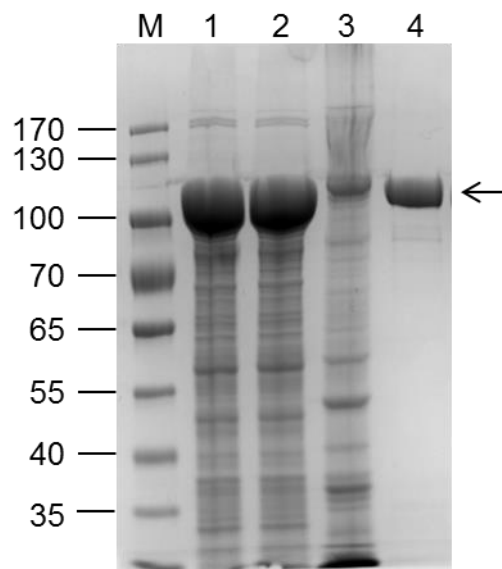


FIG. S2. 500-MHz 1D ^1H NMR spectrum, 2D ^1H - ^1H TOCSY spectra (mixing time 150 ms), and 2D ^{13}C - ^1H HSQC spectrum (D_2O , 298K) of the α -glucan generated by the *L. reuteri* NCC 2613 GtfB- ΔN enzyme, isolated by size-exclusion chromatography on a Biogel P2 column. The reaction products were obtained from 0.6% (w v $^{-1}$) amylose V, incubated with 40 $\mu\text{g ml}^{-1}$ of the *L. reuteri* NCC 2613 GtfB- ΔN enzyme for 24 h at 37°C and pH 5.5. Peaks for ($\alpha 1\rightarrow 4$) and ($\alpha 1\rightarrow 6$) anomeric signals have been indicated. Structural reporter peaks a: H-4 for 6-substituted Glcp, b: H-4 for terminal Glcp, c: for H-4 for 4-substituted Glcp, d: H-6a for 6-substituted Glcp and e: H-6b for 6-substituted Glcp.

