SUPPLEMENTAL MATERIAL

An extracellular cell-attached pullulanase confers branched α-glucan utilization in human gut probiotic *Lactobacillus acidophilus*

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Table S1. Predicted extracellular enzymes (SignalP (1)) of GH13 from available *Lactobacillus* genomes.

Genbank accession	Organism	GH13 subfamily[a]	SLAP domain[b]	Isolate origin
AAV43522	*L. acidophilus* NCFM	14	+	**Human gut**
AJP47013	*L. acidophilus* FSI4	14	+	Yogurt
AGK94861	*L. acidophilus* La-14	14	+	**Human gut**
ADZ06675	*L. amylovorus* 30SC	14	+	**Porcine gut**
ADQ58495	*L. amylovorus* GRL1112	14	+	**Porcine gut**
AEA31469	*L. amylovorus* GRL1118	14	+	**Porcine ileum**
AAC45781	*L. amylovorus* CIP 102989	28	-	Cattle (waste-corn fermentation)
AAD45245	*L. manihotivorans* LMG 18010T	28	-	Cassava
AAC45780	*L. plantarum* A6	28	-	Cassava
BAF93906	*L. plantarum* L137	14	-	Fermented food
AHX97726	*L. plantarum* S21	28	-	Fermented rice noodles

[a]Subfamily classification in the CAZy data base (www.CAZy.org).
[b]The occurrence of a surface layer association protein domain in the enzyme is denoted by "+"

Table S2. Comparison of kinetic parameters of pullulanases. Purple, GH13_13, i.e. enzymes mainly acting on β-limit dextrins; Blue, GH13_14, i.e. enzymes with activity on β-limit dextrins as well as the polymeric α-glucans amylopectin and glycogen; Orange, Unclassified/no protein sequence in CAZy. The enzyme from *L. acidophilus* NCFM displays the highest catalytic efficiency for any debranching enzyme owing to an unprecedented low K_m.

Organism	pH, temperature	K_m *mg ml*$^{-1}$	k_{cat} s^{-1}	k_{cat}/K_m *ml* s^{-1} *mg*$^{-1}$	Reference
PULLULAN					
Barley (*Hordeum vulgare*)	5.5, 37°C	0.081±0.003	61±13	753	(2)
Klebsiella pneumoniae,	5.0, 40°C	0.017	103.3	6076	(3)
Klebsiella pneumoniae	5.4, 37°C	0.617	116	188	(4)
Rice (*Oryza sativa* L. *japonica*)	6.0, 37°C	0.625	23.1	37.0	(5)
Spinach *(Spinacia oleracea)*	6.0, 37°C	0.78/0.70			(6, 7)
Anaerobranca gottschalkii	8, 60°C	0.75			(8)
Bacillus acidopullulyticus	5.0, 70°C	4.0			(9)
Bacillus deramificans	4.5, 60°C	0.70±0.02	1900.4±103.5	2712.9±121.6	(10)
Bacillus subtilis strain 168	5.4, 37°C	1.284	97	75.5	(4)
Exiguobacterium sp. SH3	7.0, 37°C	0.069			(11)
Fervidobacterium pennavorans	6.0, 80°C	0.4			(12)
***Lactobacillus acidophilus* NCFM**	**5.0, 37°C**	**0.05±0.004**	**518.4±10.5**	**10368**	**This study**
Paenibacillus barengoltzi	5.5, 50°C	2.94			(13)
Paenibacillus polymyxa Nws-pp2	6.0, 35°C	15.25			(14)
Bacillus cereus Nws-bc5	7.0, 40°C	0.45			(15)
Bacillus megaterium WW1210	6.5, 55°C	3.3±0.25			(16)
Bacillus naganoensis	4.5, 60°C	1.22±0.11	0.72±0.01	0.59	(17)
Bacillus sp. AN-7	6, 80°C	1.3			(18)
Bacillus sp. S-1	9.0, 50°C	7.92			(19)
Thermoanaerobacter thermohydrosulfuricus (Clostridium thermohydrosulfuricum)	6.0, 60°C	0.675	271	410	(20)
Exiguobacterium acetylium a1/YH5	6.0, 50°C	0.12±0.02			(21)
Lactococcus lactis IBB 500	4.5, 60°C	0.34±0.02			(22)
Oat (*Avena sativa*)	5.0, 30°C	0.17			(23)
Sorghum bicolor	5.0, 30°C	0.2			(24)
Sugar beet (*Beta vulgaris* var. *altissima*)	5.6, 37°C	0.31			(25)
Thermus caldophilus GK-24	7.0, 73°C	0.42			(26)
POTATO AMYLOPECTIN					
Barley (*Hordeum vulgare*)	5.5, 37°C	6.9±1.0	15.6±1.2	2.3	(27)
Klebsiella pneumoniae	5.5, 40°C	10.1	14.1		(3)
Rice (*Oryza sativa* L. *japonica*)	6.0, 37°C	1.538			(5)
Spinach (*Spinacia oleracea*)	6.0, 37°C	7			(6, 7)
***Lactobacillus acidophilus* NCFM**	**5.0, 37°C**	**0.37±0.041**	**24.9±0.7**	**67**	**This study**
Bacillus megaterium WW1210	6.5, 55°C	3.6±0.18			(16)
Bacillus sp. S-1	9.0, 50°C	1.63			(19)
Broad bean (*Vicia faba* L.)	30°C	1.2			(28)
Oat (*Avena sativa*)	5.0, 30°C	1.4			(23)
Sugar beet (*Beta vulgaris* var. *altissima*)	5.6, 37°C	4.55			(25)
AMYLOPECTIN β-LIMIT DEXTRIN					
Broad bean (*Vicia faba* L.)	30°C	1			(28)
Sorghum bicolor	5.0, 37°C	2.5			(24)
***Lactobacillus acidophilus* NCFM**	**5.0, 37°C**	**0.20±0.090**	**189±15.8**	**945**	**This study**

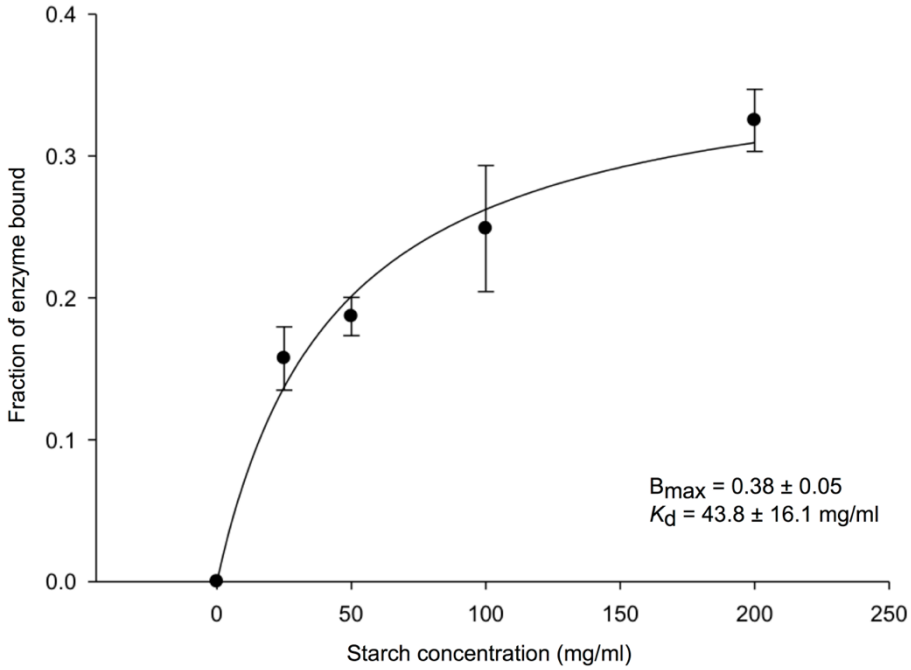

Fig. S1. Binding of *La*Pul13_14 to starch granules. The data is from four replicate experiments. The solid line is the fit of a one binding site model to the data, with the B_{max} and the K_d as the maximum binding capacity and dissociation constant, respectively.

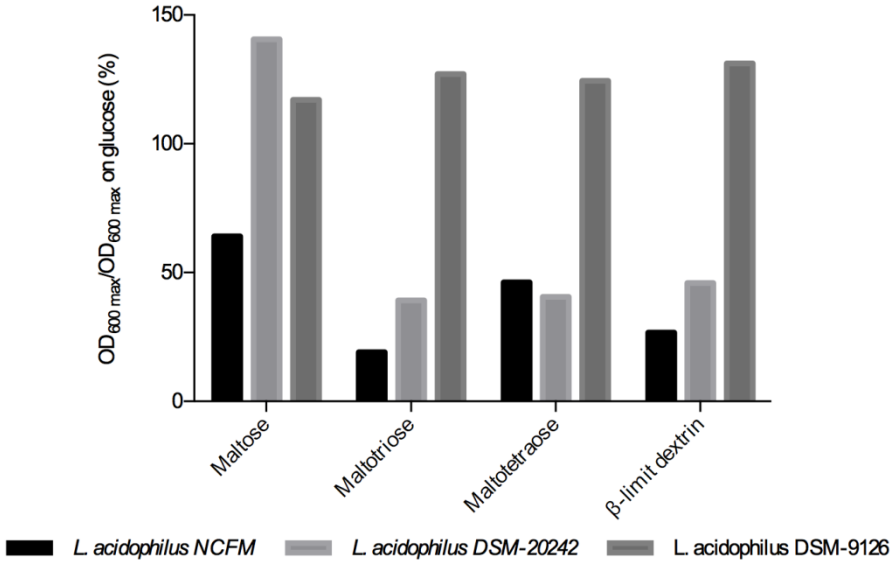

Fig. S2. Maximal growth of three *L. acidophilus* strains on maltooligosaccharides and β-limit dextrin relative to the maximal growth on glucose. *L. acidophilus* NCFM and *L. acidophilus* DSM-20242 have a comparable maltooligosaccharides utilization gene cluster, *i.e.* they have a transposase included in the cluster. The *L. acidophilus* DSM-9126 does not have a transposase in its maltooligosaccharides utilization gene cluster, and is likely to have an intact expression of the ATP-binding cassette transporter.

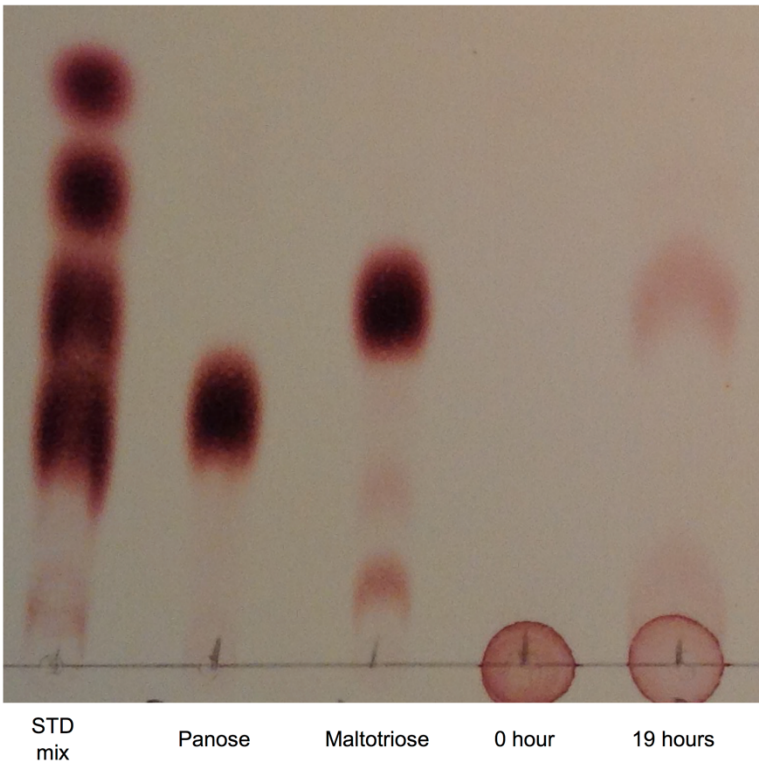

Fig. S3. Thin layer chromatography analysis of the degradation product from whole cell assay with pullulan. The standard mix (STD mix) consists of 20 mM of (mentioned from the top); glucose, maltose, maltotriose and panose. Furthermore, 20 mM panose and 20 mM maltotriose were spotted separately. The plate clearly shows the exclusive release of maltotriose after 19 h (first lane on the right side of the plate), confirming the pullulanase activity.

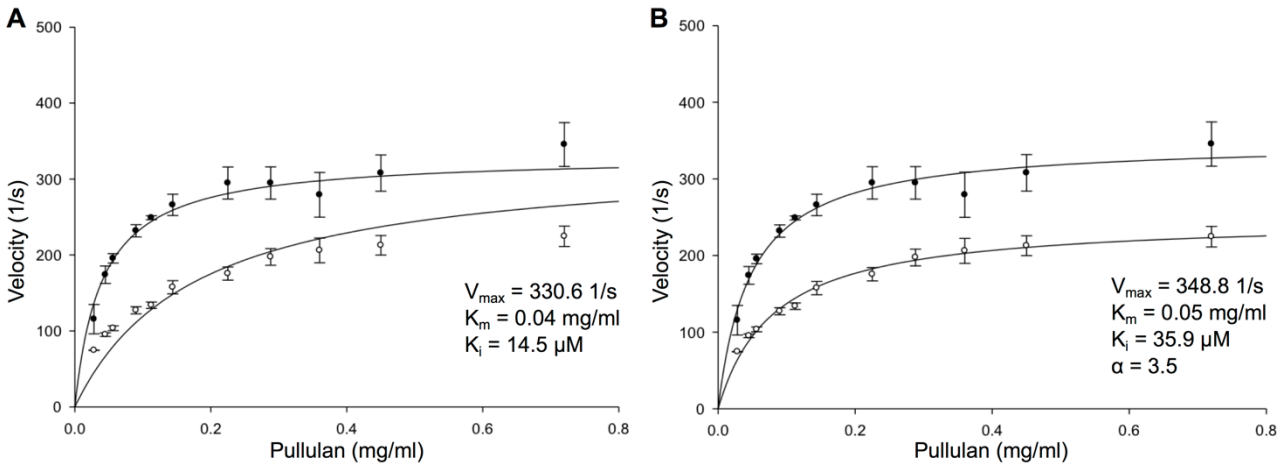

Fig. S4. Inhibition kinetics of *La*Pul13_14 on pullulan by β-cyclodextrin (50 µM). Two different inhibition models are shown. The solid and hollow circles represent the initial rates in the absence and the presence of β-cyclodextrin, respectively. The solid lines represent the fit to: (A) a competitive inhibition model and (B) mixed inhibition model. The competitive inhibition model results is systematic poor fits to the data, whereas the data is well modelled by a mixed inhibition model, which is the best model also as compared to a non-competitive inhibition model (not shown).

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