A novel maltotriose hydrolyzing thermo-acidophilic pullulan hydrolase type III from *Thermococcus kodakarensis*

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TABLE S1 Purification of recombinant TK-PUL produced in *E. coli* (8 g wet cell mass)

Purification Step	Total Protein (mg)	Total activity (U)	Specific activity (U/mg)	Yield (%)	Purification fold
Crude extract	930	5821.8	6.3	100	----
Heat treatment	253.75	4973.5	19.6	85.4	3.11
Ammonium sulfate precipitation	179.8	5753.6	32	98.8	5.07
Resource Q column	73.66	5193	70.5	89.2	11.19

TABLE S2 Comparison of TK-PUL activity in the presence of various reagents

Reagent	Concentration	Relative activity(%)
None	-	100
SDS	1 mM	7
Triton X-100	0.10%	110
	1%	116
Tween 20	0.10%	131
	1%	131
Iodoacetamide	10 mM	105
	20 mM	107
Ammonium sulfate	0.5 M	6
Urea	4 M	72
	6 M	44
Guanidine HCl	0.4 M	57
	1 M	19
	4M	0
$CaCl_2$	0.05 mM	102
	5 mM	103
$MgCl_2$	0.05 mM	99
	5 mM	95
$MnCl_2$	0.05 mM	102
	5 mM	104
$CoCl_2$	0.05 mM	107
	5 mM	94
$ZnCl_2$	0.05 mM	100
	5 mM	94
$NiCl_2.6H_2O$	0.05 mM	97
	5 mM	90
$CuCl_2$	0.05 mM	99
	5 mM	48
$FeSO_4.7H_2O$	0.05 mM	92
	5 mM	33

Properly diluted enzyme (1.7 U/mL, final concentration) was mixed with metal ions (either 50 μM or 5 mM, final concentrations) and incubated at 60 °C for 15 min. Samples were withdrawn and pullulanase activity was examined by DNS method.

TABLE S3 Effect of inhibitors on pullulanase and amylase activities of TK-PUL

Reagent	Pullulanase (%)	Amylase (%)
None	100	100
β-cyclodextrin (0.1 %)	75	73
p-chloromercuribenzoic acid (0.01 %)	83	78
N-Bromosuccinimide (0.01 %)	3	3

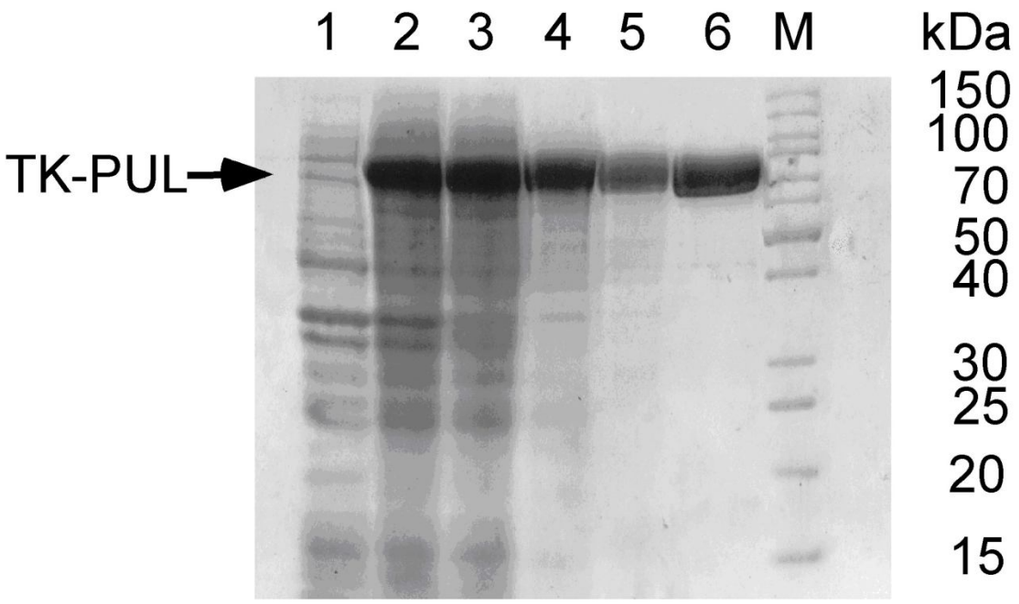

FIG S1 CBB stained SDS-PAGE demonstrating purification of recombinant *Tk*-PUL. Lane 1, total lysate of cells carrying pET-21a(+); Lane 2, total lysate of cells carrying pET-Pul; Lane 3, soluble fraction after sonication; Lane 4, supernatant after heat treatment; Lane 5, protein precipitated by ammonium sulfate; Lane 6, purified *Tk*-PUL after Resource Q anion exchange column; Lane 7, molecular mass marker (Page Ruler™ unstained protein ladder, Fermentas Life Sciences).

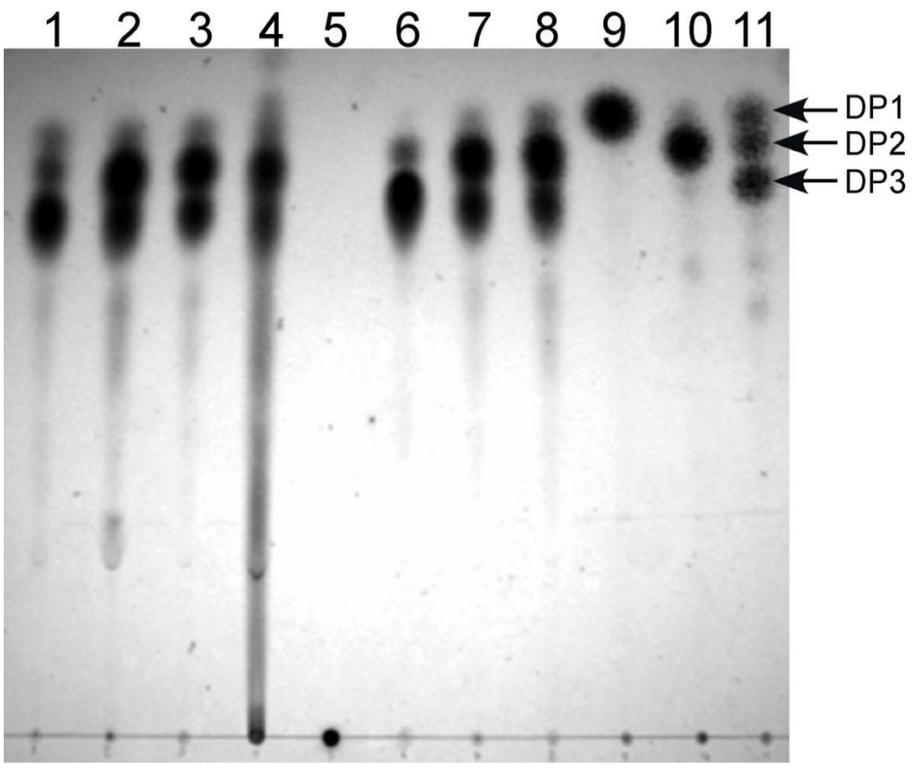

FIG S2 Thin layer chromatogram showing the reaction products obtained after 4 h incubation of purified recombinant *Tk*-PUL with various substrates at 0.25% (w/v) final concentration and 90 °C. Lane 1, pullulan; Lane 2, starch; Lane 3, glycogen; Lane 4, dextrin; Lane 5, dextran; Lane 6, α-cyclodextrin; Lane 7, β-cyclodextrin; Lane 8, γ-cyclodextrin; Lane 9, glucose; Lane 10, maltose; Lane 11, standards (glucose [DP1], maltose [DP2] and maltotriose [DP3]).

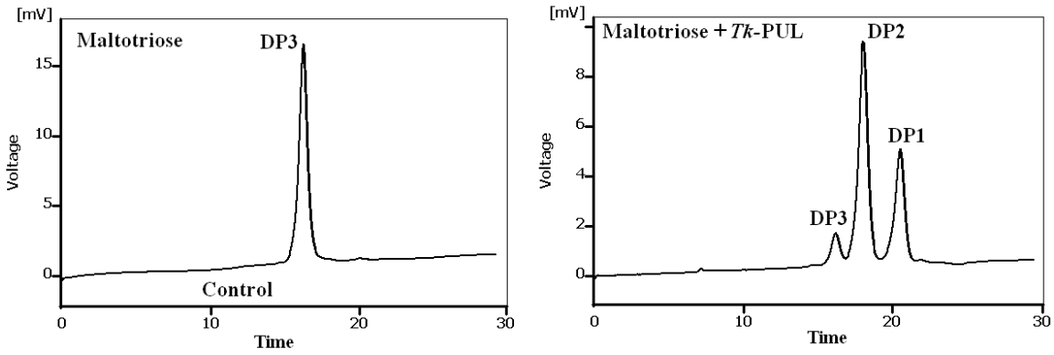

FIG S3 HPLC peaks showing hydrolysis of Maltotriose by the action of *Tk*-PUL. Purified *TK*-PUL (2.6 U ≈ 40 μg) was mixed in a total volume of 250 μL with maltotriose at 0.25% final concentration in 50 mM sodium citrate buffer pH 4.2 and after 16 h of incubation at 90 °C 20 μL sample was analyzed on Aminex HPX-42A column. DP stands for degree of polymerization.