SUPPLEMENTARY MATERIAL

Purification of the recombinant PttXET16A and PttXET16A N93S

An optimised method for the purification of the recombinant PttXET16A was developed, that consisted of two sequential cation-exchange chromatography steps. The purified enzyme was obtained in good overall yield, although a significant loss of activity was observed due to concentration of the enzyme by ultrafiltration. Although the exact cause for this loss is unknown, PttXET16A was observed to bind irreversibly on various surfaces, including those of Sephadex- and Sephacryl-based gel filtration matrices. The data reported in Table S1 represent the total protein amounts and the average activity values for each chromatographic step. Owing to the lower yield of the PttXET16A N93S mutant protein, a slightly modified version of the purification protocol was employed.

Table S1. Purification of recombinant PttXET16A and PttXET16A N93S. One unit of activity is defined as 1 nmol of XLLGol incorporated / min in the radioactive assay.

Step	Volume (mL)	Activity (U/mL)	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Yield (%)	Purification factor
PttXET16A:							
Culture supernatant	2650	1.1	2915	111.3	26.1	100	1
Concentration + buffer exchange	110	16.0	1764	52.8	33.4	61	1.3
SP Trisacryl	210	7.3	1542	46.2	33.4	53	1.3
RESOURCE S	27	52.0	1404	33.0	42.5	48	1.6
PttXET16A N93S:							
Culture supernatant	535	0.17	91	8.03	11.3	100	1
Concentration + dialysis	70	0.67	47	3.85	12.2	52	1.07
SP Trisacryl	10	0.76	7.6	0.22	34.5	4.2	1.83

Determination of the unfolding temperature for PttXET16A under various solvent conditions.

The unfolding temperature (T_m) for PttXET16A wild-type was determined in a series of buffers (Table S2). Also, the effect of different additives was investigated (Table S3 and S4).

Table S2: T_m values for PttXET16A in a series of buffers.*

Added buffer (final concentration 100mM)	T_m (°C)
H_2O	42.72
Potassium phosphate, pH 5	48.77
Potassium phosphate, pH 6	48.90
Potassium phosphate, pH 7	44.86
Sodium phosphate, pH 5.5	49.45
Sodium phosphate, pH 6.5	47.18
Sodium phosphate, pH 7.5	42.23
Sodium acetate, pH 4.5	42.93
Sodium acetate, pH 5.0	45.13
Sodium citrate, pH 4.7	48.50
Sodium citrate, pH 5.5	49.13
Sodium Cacodylate, pH 6.5	49.13
Ammonium acetate, pH 7.3	48.30
Imidazole, pH 8.0	40.02
Bicine, pH 8.5	37.80
Bicine, pH 9.0	34.72
MES, pH 5.8	49.95
MES, pH 6.2	49.58
MES, pH 6.5	48.94
HEPES, pH 7.0	47.14
HEPES, pH 8.0	40.65
TRIS, pH 7.5	44.46
TRIS, pH 8.0	41.55
TRIS, pH 8.5	37.99

* 7.5 µl of SYPRO® Orange protein gel stain (stock solution diluted 1000X), 5 µl of protein (12 µg in 100 mM sodium acetate pH 5.5 with 250 mM of NaCl) and 12.5 µl of buffer was added to each well.

Table S3. Influence of additives on the T_m value of PttXET16A.*

Final concentration of additive	T_m (°C)
No additive	50.22
10% glycerol	54.33
20% glycerol	55.79
500mM NaCl	45.51
250mM NaCl	47.20
500mM $CaCl_2$	31.32
250mM $CaCl_2$	38.78
500mM $MgCl_2$	35.35
250 mM $MgCl_2$	39.16
10% sucrose	52.57
20% sucrose	54.27
5mM XGO*	51.24
1 mM XGO*	50.65
500 μM XGO*	50.08
100 μM XGO*	49.83

* 7.5 μl of SYPRO® Orange protein gel stain (stock solution diluted 1000X), 5 μl of protein (12 μg in 100 mM sodium acetate pH 5.5 with 250 mM of NaCl) and 12.5 μl of additive was added to each well. The additives were dissolved in 100 mM sodium acetate, pH 5.5. XGO = Mixture of xyloglucan oligosaccharides

Table S4. Influence of NaCl on the T_m value of PttXET16A.*

Final concentration of NaCl (M)	T_m (°C)
0	51.6
0.2	48.1
0.4	45.8
0.6	44.1
0.8	43
1.0	41.9

***** 7.5 µl of SYPRO® Orange protein gel stain (stock solution diluted 1000X), 9 µl of protein (10 µg in 100 mM sodium acetate pH 5.5), 5 µl of additive (0-5M NaCl in H_2O) and 3.5 µl buffer (100mM ammonium acetate, pH 5.5) was added to each well.