Electronic Supplementary Material (ESI) for RSC Advances.

Supporting information

Table S1 Oligonucleotide primers used for site-directed mutagenesis of the appAM10

Names of primers	The sequences of primers (5′→3′)
appA(W46E)- Forward	GCCAGTCAAGCTGGGTGAGTTGACACCTAGAGG
appA (W46E)- Reverse	CCTCTAGGTGTCAACTCACCCAGCTTGACTGGC
appA(Q62W)- Forward	GTCACTACTGGAGACAGCGTCTTGTTGC
appA(Q62W)- Reverse	GCAACAAGACGCTGTCTCCAGTAGTGAC
appA(A73P, K75C)- Forward	GGATTGTTGCCCAAGTGTGGTTGTCCACAATC
appA(A73P, K75C)- Reverse	GATTGTGGACAACCACACTTGGGCAACAATCC
appA(S146E)- Forward	GCTATCTTGGAGAGAGCTGGAGGATC
appA(S146E)- Reverse	GATCCTCCAGCTCTCTCCAAGATAGC
appA(R159Y)- Forward	GACTTCACCGGTCACTACCAGACTGCCTTCAGAG
appA(R159Y)- Reverse	CTCTGAAGGCAGTCTGGTAGTGACCGGTGAAGTC
appA(N204C)- Forward	GAAGGTCTCCGCCGACTGCGTCTCTTTG
appA (N204C)- Reverse	CAAAGAGACGCAGTCGGCGGAGACCTTC
appA(Y255D)- Forward	CGCTCAATTCGACTTGCTGCAGAGAACTCC
appA(Y255D)- Reverse	GGAGTTCTCTGCAGCAAGTCGAATTGAGCG
appA(Q258N)- Forward	CGACTTGCTGAACAGAACTCCAGAGG
appA(Q258N)- Reverse	CCTCTGGAGTTCTGTTCAGCAAGTCG
appA(Q349N)- Forward	CTCTCAATGGATTAACGTTTCGTTGG
appA (Q349N)- Reverse	CCAACGAAACGTTAATCCATTGAGAG

Underlined primer sequences represent the mutated codon for the target amino acid residue.

Table S2 Protein-engineering examples of *Escherichia coli* phytase appA

Project goal	Expression system	Experimental strategies	Results	Reference
Improve thermostability and specific activity	*Escherichia coli* BL21(DE3) *Pichia pastoris* X33	Structure-based rational design, site-directed mutagenesis	V89T, N204A and S206A exhibited increase in catalytic activity. Three glycosylation sites (NQT74-76, NFS171-173, NGT282-284) were found beneficial in promoting thermostability.	[13]
Improve thermostability	*Escherichia coli* Origami(DE3)	Error-prone PCR, high-throughput screening	After treatment at 85°C for 5 min, the mutant I408L remained 51.3% residual activity and the thermostability of a 23.3% increase compared with wild-type.	[27]
Improve thermostability and gastric performance	*Escherichia coli* K-12	Gene site saturation mutagenesis (GSSM), high-throughput screening	The mutant Phy9X was a highly active phytase with no loss of activity after heating at 62 °C for 1 h and 27% of its initial activity after 10 min at 85 °C and a 3.5-fold enhancement in gastric stability.	[14]
Improve thermostability	*Escherichia coli* Origami(DE3)	Change salt bridges, site-directed mutagenesis	After being heated at 80°C for 10 min, the salt bridge addition mutant Q307D was 40.57% residual activity and showed 9.15% thermostability enhancement than the wild-type.	[28]
Improve thermostability	*Escherichia coli* Origami(DE3)	Multi-factors rational design strategy, site-directed mutagenesis	Three thermostable mutants (Q206E/I427L Y311K/I427L and Q206E/Y311K/I427L) were obtained. The mutant Y311K/I427L got 61.7 % residual activity after being heated at 80 °C for 10 min and catalytic efficiency (4.46 was compared to 2.37) improved more than the wild-type.	[29]

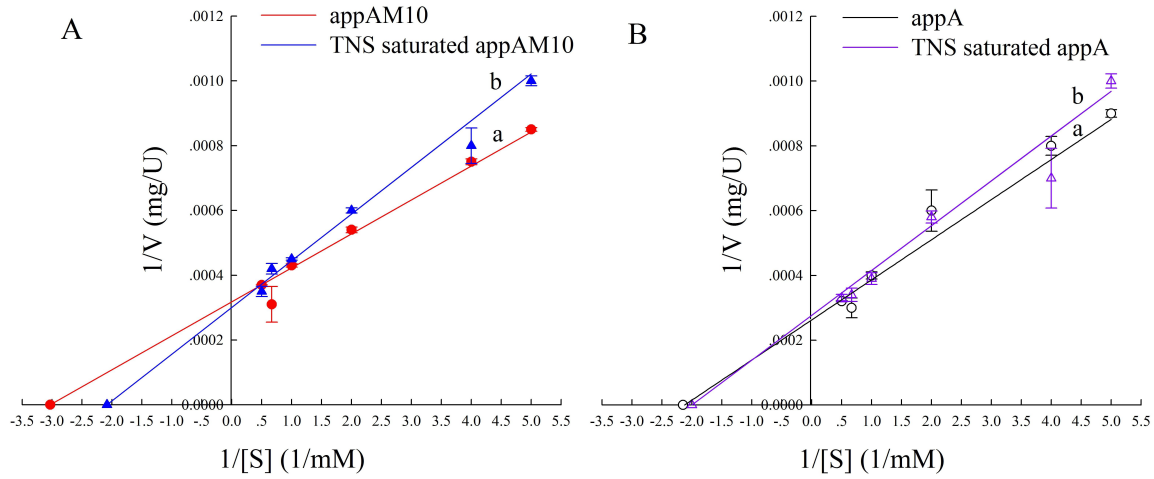

Fig. S1 (A) The plot of $\frac{1}{V}$ versus $\frac{1}{[S]}$ of appAM10 (a) and TNS saturated appAM10 (b). (B) The plot of $\frac{1}{V}$ versus $\frac{1}{[S]}$ of appA (a) and TNS saturated appA (b).