Biochemical Characterization of a Novel Alkaline-Tolerant Xaa-Pro Dipeptidase from *Aspergillus phoenicis*

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Table S1. Xaa-Pro dipeptidases that were characterized by molecular and/or biochemical techniques.

Enzyme	Organism	GenBank accession No.	Optimum pH and temperature	pH stability and thermostability	Monomer/Multimer (MW of subunits, or native enzymes) [a]	Inhibitor (cofactor) [b]	Substrate specificity [c]	Reference
Hyperthermophilic archaea								
PfPEPQ	*Pyrococcus furiosus* DSM 3638	P81535.1	pH 7.0, 100 °C	Half-life at 100 °C for native and recombinant enzymes is 4 h and 1 h, respectively	Homodimer (39.4 kDa, 100±10 kDa)	EDTA, Zn^{2+} (Co^{2+}, Mn^{2+})	Xaa-Pro (Xaa=Met, Leu, Val, Phe or Ala)	[25,35,50]
PhPROL	*P. horikoshii* OT3	BAA30249.1	pH 7.0, 100 °C	Half-life at 90 °C is 21.5 h	39.27 kDa, NA	Zn^{2+}(Co^{2+})	Met-Pro, Leu-Pro, and organophosphorus nerve agents	[34,51]
Eubacteria								
AhOPAA	*Alteromonas haloplanktis* ATCC 23821	P77814.1	pH 7.5, 40 °C		Monomer (50 kDa, NA)	DFP analog Nipafox, pCMB, NEM (Mn^{2+})	Xaa-Pro and OP compounds	[45]
AmOPAA	*A. macleodii* NCIMN1963	AEI26268.1			Dimer (50.6 kDa, 101.2 kDa)	cofactor: Mn^{2+}	Xaa-Pro and OP compounds	[9]
AsOPAA	*Alteromonas* sp. JD6.5	Q44238.3	pH 8.5, 50 °C	ND [a]	Monomer (60 kDa, 60 kDa)	pCMB, IAA, NEM, EGTA, Zn^{2+} (Mn^{2+})	DFP, NPMPP, NPEPP, paraoxon, and nerve agents such as sarin, soman, and O-cyclohexyl methylphosphonofluoridate; Xaa-Pro dipeptides such as Gly-Pro	[38,52,53]
AuOPA	*A. undina*ATCC		pH 8.0, 55 °C		Monomer (53	IAA, EGTA,	A wide range of nerve	[54]

A	29660				kDa, 53 kDa)	NEM, Ni^{2+} and Zn^{2+} (Mn^{2+})	agents and several chromogenic phosphinates	
DrXPD	*Deinococcus radiodurans* R1	WP_027479553.1			Dimer (45 kDa, NA)	cofactor: Mn^{2+}	Xaa-Pro	[43]
EcPEPQ	*Escherichia coli* BL21 (DE3)	P21165.2	pH 8.0, 60 °C	Incubation at 4-30 °C for 10 min (> 80%), incubation at pH 7.0-9.0 for 1 h (30 °C, > 60%)	Dimer	cofactor: Mn^{2+}	Xaa-Pro dipeptides, DFP, organophosphate diesters and triesters, and nerve agents GB (sarin), GD (soman), GF, and VX	[37,47,55]
LcXPD	*Lactobacillus casei* subsp. *casei* IFPL 731		pH 6.5-7.5, 55 °C	Retain 36% activity after incubation at 30°C for 30 min	Monomer (41 kDa, 41 kDa)	EDTA and 1,10-phenanthroline, DTT, ME, pHMB and IAA , Cu^{2+}, Zn^{2+} , Fe^{3+}(Mn^{2+}, Co^{2+})	Hydrolyze Xaa-Pro, Ala-Ala,and Ala-Phe except for Pro-Pro and Gly-Pro	[40]
LdPEPQ	*L. delbrueckii* subsp. *bulgaricus* CNRZ 397	CAA73815.1	pH 6.0, 50 °C		Homodimer (45 kDa, 68-70 kDa)	Bestatin, DTT, EDTA (Zn^{2+})	Hydrolyzes Xaa-Pro dipeptides except for Pro-Pro and Gly-Pro	[26]
LlXPDI	*Lactococcus lactis* subsp. *cremoris* AM2		8.3–9.0 (universal buffer)		NA, 42 kDa	NEM, benzamidine, bestatin, bacitracin, PMSF, pCMB, IAA, leupeptin	Xaa-Pro, Pro-Pro, Pro-Ala, Pro-Val, etc.	[56]
LlXPDII	*L. lactis*NRRL B-1821	ABW84230.1	pH 7.0, 40-50 °C	Incubation at 20-50 °C for 30 min (> 60%)	Dimer (40 kDa, 80 kDa)	cofactor: Zn^{2+}	Leu-Pro, Arg-Pro, Val-Pro, Phe-Pro, and Lys-Pro; does not hydrolyze Gly-Pro, Glu-Pro, Asp-Pro, Pro-Pro, Leu-Leu-Pro, and Leu-Val-Pro	[42]
MtXPD	*Mycobacterium*	P9WHS7.1	pH 7.5–8.0, 55	Completely lost its		Phosphate ion,	Hydrolyze only dipeptides,	[4]

	tuberculosis H37Rv		°C	activity at 50°C and 65°C in the absence and presence of 1 mM Mn^{2+}, respectively		cacodylate	but failed to cleave tripeptides or long chain peptides like bradykinin	
PlOPAA	*Pseudoalteromonas lipolytica* SCSIO04301	WP_036973676	pH 8.5, 55 °C	Incubation at pH 5.0-8.0 for 2 h (> 60%); half-lives at 55, 60 and 65 °C are 88.87, 78.77 and 33.49 min, respectively	Tetramer (53 kDa, 200 kDa)	EDTA, Ca^{2+}, Mg^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}(Mn^{2+})	Gly-Pro shows a preference for oxon-phosphoryl than thiono-phosphoryl, hydrolyzes dichlorvos, methyl-paraoxon, paraoxon, and profenofos	[13,39]
ScXPD	*Streptococcus cremoris* H61		pH 6.5-7.5, 40 °C	Quickly lose activity at temperatures above 40 °C	Monomer (NA, 43 kDa)	EDTA and 1,10-phenanthroline, pCMB, NEM, ME, Zn^{2+}, Cu^{2+}, Hg^{2+}, Fe^{2+} (Co^{2+})	Xaa-Pro, e.g., Leu-Pro	[41]
TsPROL	*Thermococcus sibiricus* MM 739	ACS89882.1			Homodimer (39 kDa, 70-75 kDa)	cofactor: Zn^{2+}		[57,58]
XcXPD	*Xanthomonas campestris* pv. *campestris* ATCC 33913	Q8P839	pH 7.5–8.0, 55 °C	Completely lost its activity at 60 °C and 80 °C in the absence and presence of 1 mM Mn^{2+}, respectively	Dimer	Phosphate ion, cacodylate (Mn^{2+})	Hydrolyze only dipeptides, but failed to cleave tripeptides or long chain peptides like bradykinin	[4,33]
XmXPD	*X. maltophilia*		pH 7.5, 35 °C	pH 6.0-8.5; half-life at 37 °C is 60 min	Dimer (51 kDa, 100 kDa)	pCMB, o-phenanthroline, Z-L-proline, and phenylacetyl-thio proline, Cu^{2+}, Zn^{2+}, Hg^{2+} (Mn^{2+})	Xaa-Pro, e.g., Leu-Pro	[59]

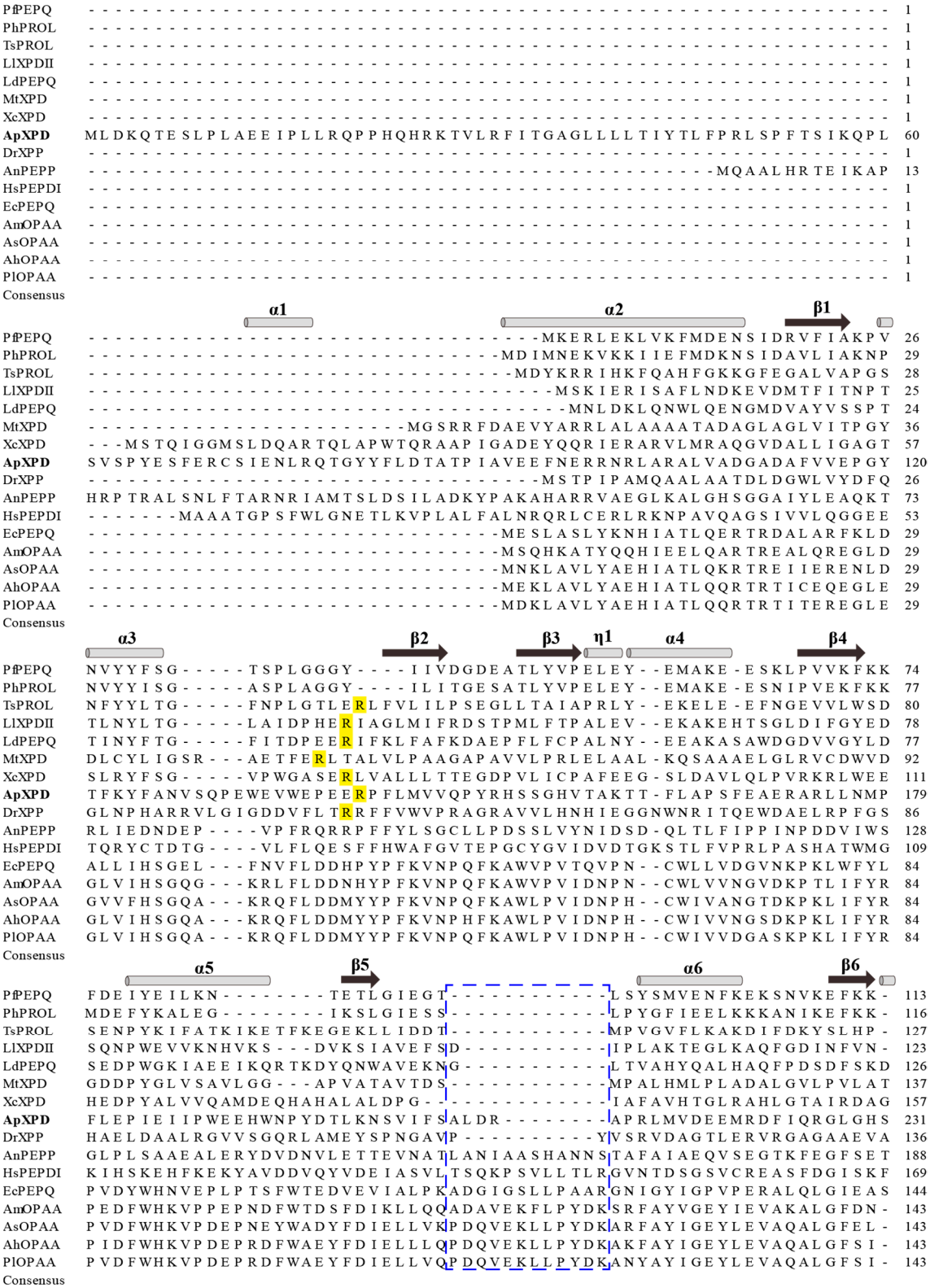
Eukarya								
AnPEPP	*Aspergillus nidulans* WG312	CAC39600.1	pH 7.0	Stable at 40°C for 60 min	Homodimer (58 kDa, 125 kDa)	cofactor: Mn^{2+}	Met-Pro, Ala-Pro, Phe-Pro, Leu-Pro, and Val-Pro; could not hydrolyze Pro-Ala, Ala-Ala, and Ala-Pro-Gly	[16]
HsPEPDI	*Homo sapiens*	AAA60064.1	pH 7.8, 50 °C	37 °C for at least 8 days	Dimer (54 kDa, NA) [c]	Cbz-Pro, guanidine hydrochloride (Mn^{2+})	Gly-Pro>Ala-Pro>Phe-Pro>Leu-Pro, DFP, sarin, soman, tabun, and cyclosarin	[34,46,60]

ND, not determined; NA, not available.

[a] Molecular weight of subunits and native enzymes were determined by SDS-PAGE and gel filtration, respectively;

[b]pCMB, *p*-chloromercuribenzoic acid; IAA, iodoacetic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid; pHMB, *p*-hydrocymercuribenzoic acid; NEM, *N*-ethylmaleimide; DTT, dithiothreitol; ME, *β*-mercaptoethanol

[c] DFP, diisopropylfluorophosphate; NPMPP, *p*-nitrophenylmethyl(phenyl)phosphinate; NPEPP, *p*-nitrophenylethyl(phenyl)phosphinate; GF, *O*-cyclohexylmethylphosphonofluoridate; VX, *S*-2(diisopropylamino)ethyl *O*-ethyl methylphosphonothioate; OP compounds, organophosphate compounds.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α2
β1
α3
β2
β3
η1
α4
β4
α5
β5
α6
β6
PfPEPQ
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TsPROL
LlXPDII
LdPEPQ
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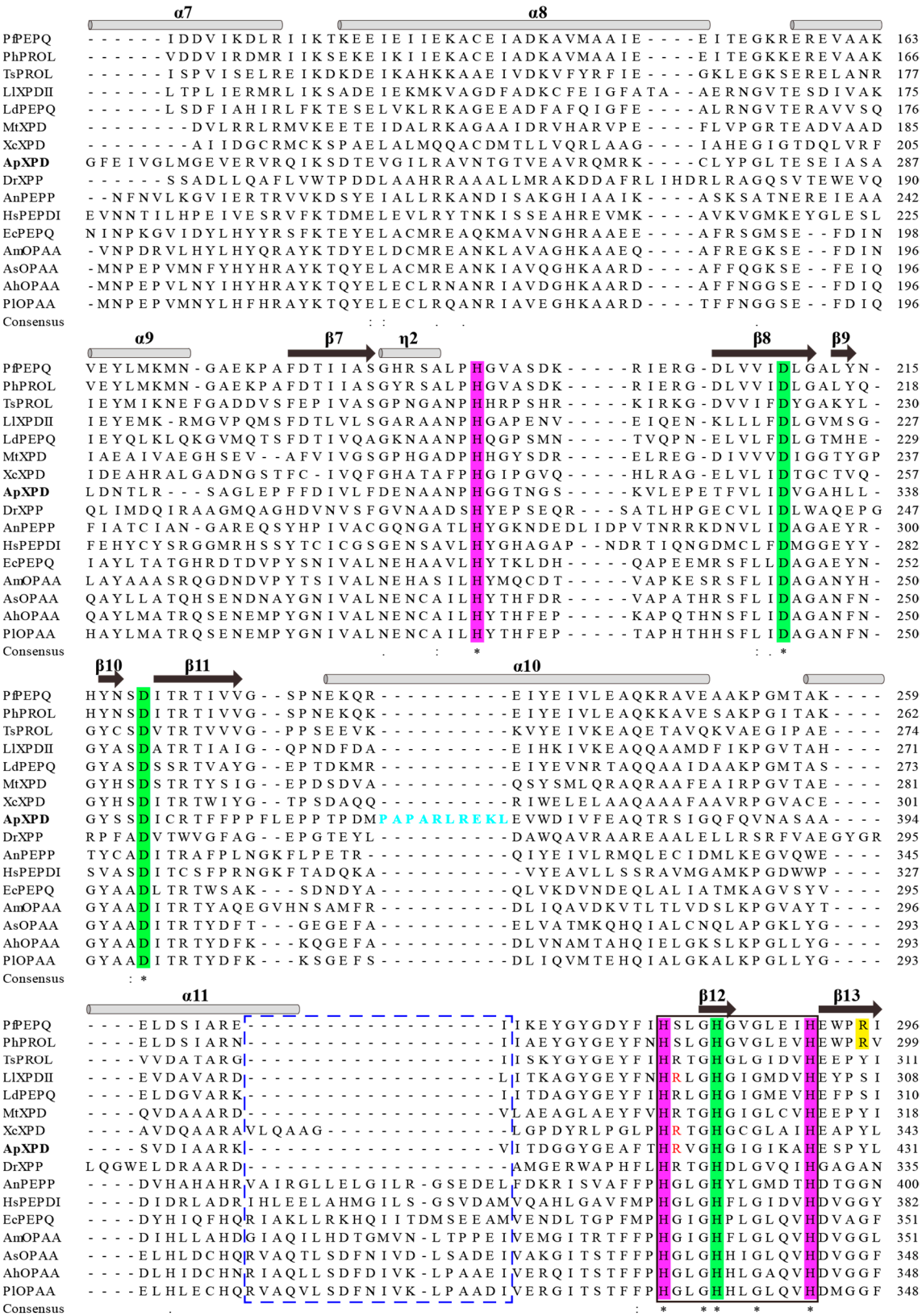
Consensus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β8
β9
β10
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β12
β13
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LdPEPQ
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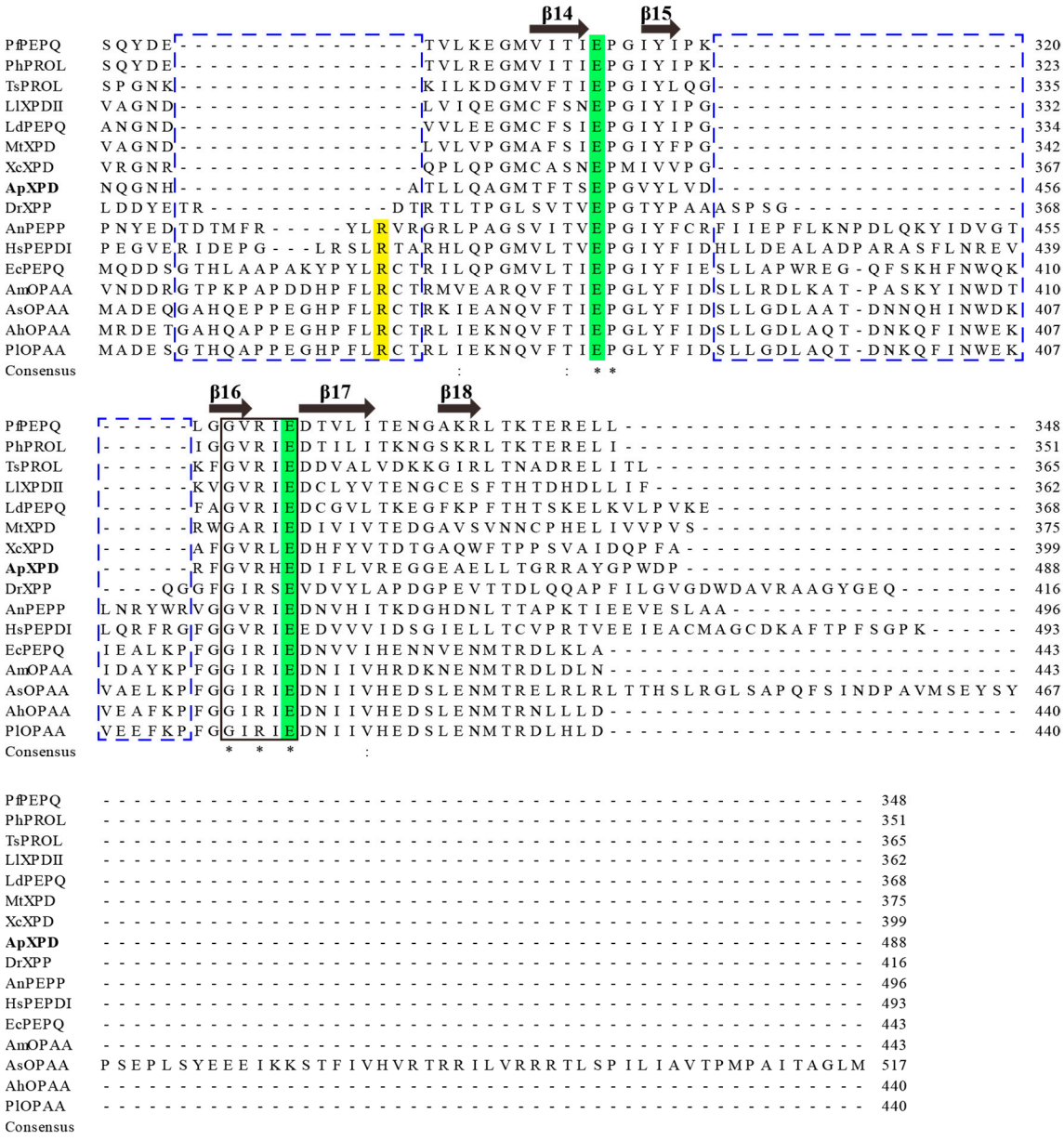

Figure S1. Multiple sequence alignment of ApXPD with all the characterized XPDs from various organisms. (*), the conserved amino acids; (:), the conservative replacement; (.), semiconservative replacement. Dashes indicate gaps to maximize alignment. The secondary structural elements of XcXPD are shown above the sequence. The strictly conserved motifs HXXGHXXGXXXH and GXRXE (X represents any amino acid) are boxed. Four regions with at least ten residues that appear in prolidases from subfamilies M24.003 and M24.007 but are absent in members of the unknown and M24.008 subfamilies are indicated by dashed rectangles. The putative catalytic triads, metal ion binding residues, and the specific arginine residues that mainly determine the substrate length are highlightedby purple, green, and yellow, respectively. Ten additional residues, PAPARLREKL in ApXPD, and the critical arginine residues involved in the formation of the allosteric site are shown in cyan and red, respectively.

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