QAPQGYELVYSELVS RPPAGFAPVFTENVS	RHG RHG	SRAL	SSFKYDDISMQVWREAQKQGALTPLGEKLGAEIE TDGADGELILALWDKAEAEGLLTARGREFGPEVR
RPPAGFAPVFTENVS TVPASYTLVYTELV2	RHG	SRSA	TDGADGELILALWDKAEAEGLLTARGREFGPEVR
TVPASYILVYTELVA	RHG	SRBA	TDGADGELILALWDKABAEGLLTARGREFGPEVR SSPKYDDISLKIWQAAKEQNALTPLGEHLGAEIE
RPPAGFAPVFTENVS TVPASYILVYTELVA	RHG RHG	SRSA SRAI	TDGADGELILALWDKAEAEGLLTARGREFGPEVR SSPKYDDISLKIWOAAKEONALTPLGEHLGAEIE
RPPAGFAPVFTENVS	RHG	SRSA	TDGADGELILALWDKAEAEGLLTARGREFGPEVR
QAPQGYELVYSELVS	RHG	SRAL	SSFKYDDISMQVWREAQKQGALTPLGEKLGAEIE
QAPAGEVEVETENVE	RIG	SICHA	SDREDGDLI LALWERARSEGQLIRIGERFGGDAE
OAPKGFVPVFTENVS	RHG	SRAA	SDREDGDLILALWEKARSEGOLTRTGERFGGDAE
APPTGYIPVYTESVA	RHG	SRAL	SSLKYDDLSLQLWEIAEAENALTPVGKEFGPALR
PAPAGPAPVITESVA	RnG	SKAL	SSEKIDSPIIČAMPČAKSPOAPLIPOČIPOLETČ
DADAGEADUYTESUA	DHC	CD AT	SERVICEI TOURIEONDERG
KAPEGYEPIYTESMA	RHG	SRGL	SSYKYDALLMKMAEAAERDGGFKSDAIKTEFMKNLN
SFPESCAIKQVHLLQ	RHG	SRNF	TGDDTATDVSSAQYIDIFQNKLLNGSI
PPSLDCRAIHMVSVI	RHG	TRYE	1"TKNVRKIAQLFDLVKSDSLRLA
DESTROPATION	DUC	DDVD	
LLEGTCTPVOLVALI	RHG	TRYE	TVKOIRKLROLHGLLOARGSRDGGASS
SIPDGCRVIHLNLVA	RHG	TRAF	TKKRIKELDRLAVRLEALLKEANQV
SVPDGCRAIHLNLVP	RHG	TRAF	TREATEBMDQLAIREDALLTDAKER
OUDDCOD ATUL NUM	DITC	mp ar	THEFT FUTCH AT A DEDUCE
RSVLAKELKFVTLVE	RHG	DRSF	IDTFPT
QSEPELKLESVVIVS	RHG	VRAF	TKATQLMQD
ALFORINDATIBOV	Rene	SRGI	ODINI DADIADIAQOARDAA I AGE VOEDVORDE INA VA
AT DODVHI AVTEON	PHO	SPOT	SSYKYDALLALMAOSAAFNNYAGEVSBEVGKEETNNVN
KAPKGYGPIYTESMA	RHG	SRGL	SSYKYDALLMRMAETAARDGGFKSEAIKAEFVKNLS

gi|213691183 (PhyIA) Bifidobacterium longum spp infantis gi 225352176 (PhypA) Bifidobacterium pseudocatenulatum gi|130735 (AppA) Escherichia coli gi|189613 (PAP) Homo sapiens gi|90655301 (LIALP1) Lilium longiflorum gi|18491908 Zea mays gi|37182994 (MINPP1) Homo sapiens gi|41055415 Danio rerio gi|3947867 Schizosaccharomyces pombe EDT45330 Bifidobacterium dentium CAQ02958 Clavibacter michiganensis BAH30792 Rhodococcus erythropolis YP_004806097.1 Streptomyces sp SirexAA-E GAA06490 Photobacterium leiognathi EFL21248 Streptomyces himastatinicus YP_001339041.1 Marinomonas sp. MWYL1 YP 004393571.1 Aeromonas veronii ZP_01233650.1 Photobacterium angustum YP_001809928.1 Burkholderia ambifaria ZP_04942680.1 Burkholderia cenocepacia YP 439843.1 Burkholderia thailandensis _ YP_004349319.1 Burkholderia gladioli

ZP_06711296.1 Streptomyces sp. e14 ZP_03450126.1 Burkholderia pseudomallei

ADGGTIAYQSSGEA	ATESGE	VFEKGFNEAS	G	GRLIGNVSAPTNE
KQGLRVSYQSSGEP	ATESGE	VFKLGFDQASI	N	GLLANAVVAPNNE
PQSGQVAIIADVDER	TRKTGE	AFAAGLAPDC	A	-ITVHTQADTSSE
YKHEQVYIRSTDVDR	TLMSAM	PNLAALFPPE	G	-VSIWNE
HPHIYSIRATQVPR	ASASAV	AFGIGLFSGR	G	-HLGP
HPDVYSIRATQVPR	ASASAV	AFGLGLLSGK	G	-KLGQ
YGRLRLITSSKHR	CMDSSA	AFLQGLWQHYI	H	-PGLP
LRANRIEFMTSSKHR	CVDSVK	AFQEGLHRLW	D	
VYDINTAAQER	VVDSAE	VFSYGMFGDDI	M	-Q
VDGGTISYQSSGEAR	ATESGEN	VFEKGFNAAS(G	GKLTDSVVKPTDE
AGSDQVTLESSGEAR	ATASGK	AFAEGLRKAD	P	-LLASHLP
AQQGTITLTSSGVDR	AVDSAE	VFAQGLTGAF	P	-AVAGDVA
RNSEQINVVNSGKDR	AVDSGTI	LFAEALAEGD	P	-SLKPLIT
ANQRHIVVEYSGKDR	ALASAN	AFTEGLTENN	Q	-DLADLLL
KTSEKIDVVSSGQGR	AVDSGN'	FAASLGDAD	P	-ALKPLIG
QNQQQIAFEYSGQDR	ARDSGL	AFIQGLENVN	P	-ALAPLIS
PHCIKVVTSGKDR	ANESAFT	FMESLKRDVI	NYVSDSAQCYLT	QDDPSKID
ENQRHVVVEYSGKDR	ALASAN	AFTEGLTENN	Q	-DLAGLLL
GGRQIVVVNSGQDR	AVDSST	FSAALVAAQ	P	-ALAAAIMLPAAF
GNRQVVVVNSGQD	AVDSST	FSAALVAAQ	P	-ALAPAITLPAAF
GAAGGAARRIVVVHSGQDR	AVDSSA	FSGALVAAR	P	-ALAPAITLPSAE
GNRQVVVVNSGQDR	AVDSST	FSAALVAAQ	P	-ALAPAITLPAAB
AGHEAVVVETSGVPR	AVASAE	AFTGGLVAGE	P	-GLTGLVE
GTTGGAARKIVVVHSGQDR	AVDSSA	FSGALVAAR	P	-ALAPAITSPSAI
*				

gi|213691183 (PhyIA) Bifidobacterium longum spp infantis gi|225352176 (PhypA) Bifidobacterium pseudocatenulatum gi|130735 (AppA) Escherichia coli gi|189613 (PAP) Homo sapiens gij90655301 (LIALP1) Lilium longiflorum gi|37182994 (MINPP1) Homo sapiens gi|41055415 Danio rerio gi|3947867 Schizosaccharomyces pombe EDT45330 Bifidobacterium dentium CAQ02958 Clavibacter michiganensis BAH30792 Rhodococcus erythropolis YP_004806097.1 Streptomyces sp SirexAA-E GAA06490 Photobacterium leiognathi EFL21248 Streptomyces himastatinicus YP_001339041.1 Marinomonas sp. MWYL1 YP_004393571.1 Aeromonas veronii ZP_01233650.1 Photobacterium angustum YP_001809928.1 Burkholderia ambifaria ZP_04942680.1 Burkholderia cenocepacia YP_439843.1 Burkholderia thailandensis YP_004349319.1 Burkholderia gladioli ZP_06711296.1 Streptomyces sp. e14 ZP_03450126.1 Burkholderia pseudomallei

gi|213691183 (PhylA) *Bific* gi|225352176 (PhypA) *Bific*

PhylA) Bifidobacterium longum spp infantis	DARVNG-GSTVATFRFAHAETMMPFAALLGLPGSTQQAPASTTDV
hypA) Bifidobacterium pseudocatenulatum	DTRERA-G-TAATFRFAHAETIIPFAALLKLPGSQQQASEL
gi 130735 (AppA) Escherichia coli	TPHPPQKQAYG-VTLPTSVLFIAGHDTNLANLGGALELNWTLPGQPDN
gi 189613 (PAP) Homo sapiens	KRATQIPSYKKLIMYSAHDTTVSGLQMALDV
gil90655301 (LIALP1) Lilium longiflorum	EQAIVANEENHKPGNFEKARLRFAHAETIVPFTCLLGLFLEGSEFEQIRAEQPLSLPP
gi 18491908 Zea mays	EEAIIAREENRADGTFEKARLRFA <mark>H</mark> AETVVPFSCLLGLFLEGPEIEKIQREEALDLPP
gi 37182994 (MINPP1) Homo sapiens	DKAVEQKQRSQPISSPVILQFGHAETLLPLLSLMGYFKDKEPLTAYNY
gi 41055415 Danio rerio	DNAAKDHRFGEVKKTATIQVGHGETLLPLLSLMGFFKDEKPLTSENF
gi 3947867 Schizosaccharomyces pombe	RKGVNNASDRKVFLAFTHDSQIIPVEAALGFFPDITPEHPLPTDK
EDT45330 Bifidobacterium dentium	DERVNG-GSTVATFRFAHAETMMPFAALLGLPGSTQQAPASTTDV
CAQ02958 Clavibacter michiganensis	DA-RLAG-STTAATFRFAHAETIIPFAALLGLPGSTQQVTPEAP
BAH30792 Rhodococcus erythropolis	QE-HTGDAAVPGADFRFAHAETIIPFAALLKLPGSTVSQAEGDL
YP_004806097.1 Streptomyces sp SirexAA-E	EA-KRAGTSTVGAELRFTHAEEIIPLAALMELPGSTKQVSPSRP
GAA06490 Photobacterium leiognathi	EK-IKQGNEQAALKVRFAHAETIMPFAANMQLKGSEEGVDPQTT
EFL21248 Streptomyces himastatinicus	EA-KRDGTSKLGAELRFTHAEEIIPLATLMRLPGSTKAVTTDGP
YP_001339041.1 Marinomonas sp. MWYL1	KG-VQDGTNTKAAKIRFAHAETIIPFAAQMQLKGSETGVSRDTG
YP_004393571.1 Aeromonas veronii	QRQVVEGEQSRRATLRFAHAEAIIPLAALMKLEGSRQGASPDQL
ZP_01233650.1 Photobacterium angustum	EK-IKQGNDQAALKVRFAHAETIMPFAANMQLKGSEEGVDPQTT
YP_001809928.1 Burkholderia ambifaria	DA-IARGDLTRAAKLRFTHAEIVIPFASIMNLKNVFVPTPQAQT
ZP_04942680.1 Burkholderia cenocepacia	DA-IARGDLTHAAKLRFTHAEIVIPFASIMNLKNVFVPTPQAQT
YP_439843.1 Burkholderia thailandensis	DA-IARGDLTNAAKLRFTHAEVVIPFASILKLKNVFAPVPQAQT
YP_004349319.1 Burkholderia gladioli	DA-IARGDLTHAAKLRFTHAEIVIPFASIMNLKNVFVPTPQAQT
ZP_06711296.1 Streptomyces sp. e14	EA-KADGTGGKGAVLRFTHAEEIEPLAALMGLPGSTEPAAPGRA
ZP_03450126.1 Burkholderia pseudomallei	DA-IARGDLTNAAKLRFTHAEVVIPFASILKLKDVFAPVPQAQT
	. * : . :

FIG. S1 Refined multiple sequence alignment of PhypA from B. pseudocatenulatum ATCC 27919, PhylA from B. longumspp Infantis ATCC 15697 and different phosphatases from the HAP family. Non-homologous regions were eliminated with GBlocks. Invariant conserved sequences in all members of the family and conserved sequences that differ between members are boxed.



FIG. S2 Phylogenetic analysis of phosphatases from different origins belonging to the branch 2 HAP group, including PhylA and PhypA from bifidobacteria. Amino acid sequences were aligned with the EBI ClustalW2 tool and sequence trimming was done with GBlocks. The phylogenetic trees were constructed with MEGA4 (Poisson correction model, pairwise deletion and 1000 replicates).



FIG. S3 (A) Schematic representation of the PhylA and PhypA phytases and the truncated 6X(His)-tagged version lacking the signal peptide (sp) and the hydrophobic C-terminal region (tm). The (+) symbol at the C-terminal part represent positively charged amino acids. Signature sequences of the different conserved motifs are also shown (HAPs RHGSRGL and HA domains and the C-terminal cell-wall anchoring domains (LAXTG)); (B) SDS-PAGE analysis of purified 6X(His) truncated phytases. Lane 1, 6x(His)⁴⁹PhypA⁶¹² from *B. pseudocatenulatum*; lane 2, 6x(His)³²PhylA⁵⁹⁹ from *B. longum* spp *infantis.* The calculated molecular weight of the truncated enzymes is 61.8 and 62.2 kDa, respectively. Attempts to express in *E. coli* the N-terminal deleted proteins carrying the C-terminal hydrophobic helices (6x(His)⁴⁹PhypA⁶³⁹ and 6x(His)³²PhylA⁶²³) which form part of the sortase-dependent cell wall anchoring motif, always rendered insoluble inclusion bodies.