

Supplementary Information

In Search of Monocot Phosphodiesterases: *Identification of a Calmodulin Stimulated Phosphodiesterase from *Brachypodium distachyon**

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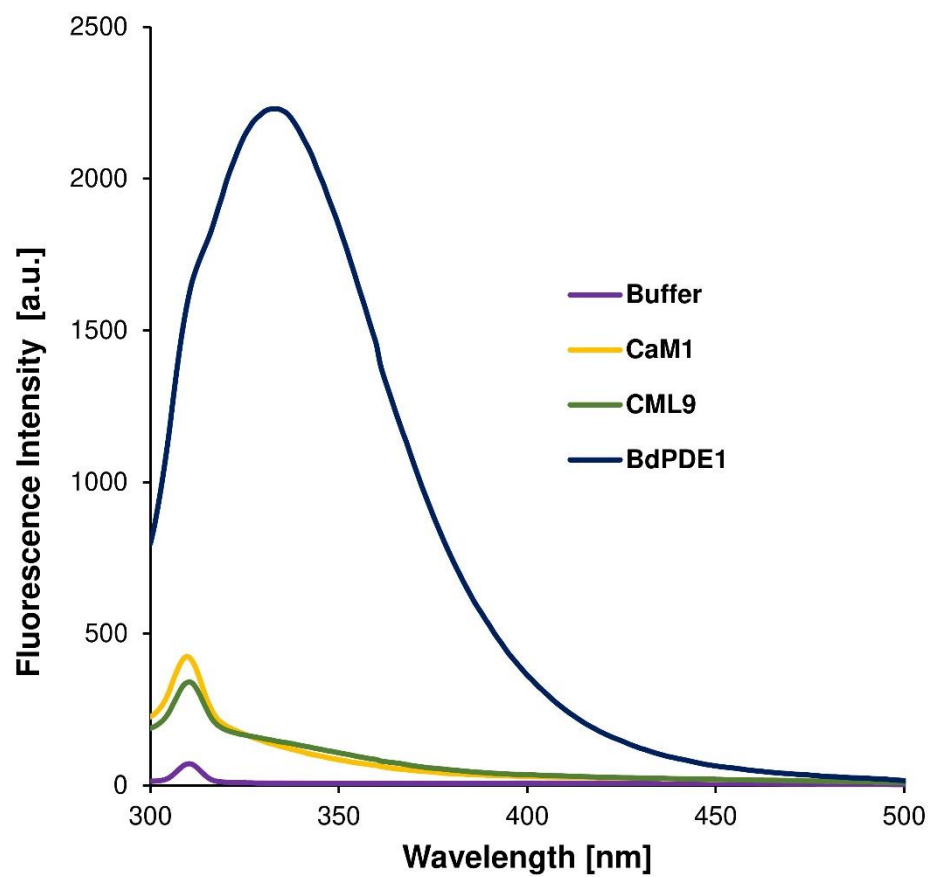
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Supplementary Information contains 2 figures, 1 table and supplementary methods

Supplementary Figure S1



Emission spectra of 0.5 μ M BDPDE1, 10 mM glutathione in 50 mM Tris-HCl (pH 9.0), 0.5 μ M CaM1 and 0.5 μ M CML9.

Supplementary Figure S2

A

AtCaM1	MADQLTDEQISEFKEAFSLFDKDGDCITTKELGTVMRSLGQNPTAEALQDMINEVDADG	60
BdCaM1	MADQLTDEQIAEFKEAFSLFDKDGDCITTKELGTVMRSLGQNPTAEALQDMINEVDADG	60
	*****:*****	
AtCaM1	NGTIDFPEFLNLMAKMKDSTDSEELKEAFRVFDKQNGFISAAELRHVMTNLGEKLTDE	120
BdCaM1	NGTIDFPEFLNLMARKMKDSTDSEELKEAFRVFDKQNGFISAAELRHVMTNLGEKLTDE	120
	*****:*****	
AtCaM1	EVEEMIREADVDDGQINYEYEFVKIMMAK	149
BdCaM1	EVDEMIREADVDDGQINYEYEFVKVMMMAK	149
	*****.*****	

B

AtCaM3	MADQLTDDQIAEFKEAFSLFDKDGDCITTKELGTVMRSLGQNPTAEALQDMINEVDADG	60
BdCaM3	MADQLTDDQIAEFKEAFSLFDKDGDCITTKELGTVMRSLGQNPTAEALQDMINEVDADG *****:*****	60
AtCaM3	NGTIDFPEFLNLMARKMKDTSSEELKEAFRVFDKQNGFISAAELRHVMTNLGEKLDE	120
BdCaM3	NGTIDFPEFLNLMARKMKDTSSEELKEAFRVFDKQNGFISAAELRHVMTNLGEKLDE *****	120
AtCaM3	EVDEMIKEADVDDGQINYEDEFVKVMMAK	149
BdCaM3	EVDEMVREADVDDGQINYEDEFVKVMMAK *****.*****	149

C

AtCaM7	MADQLTDDQISEFKEAFSLFDKDGDCITTKELGTVMRSLGQNPTAEALQDMINEVDADG	60
BdCaM7	MADQLTDDQIAEFKEAFSLFDKDGDCITTKELGTVMRSLGQNPTAEALQDMINEVDADG	60
	*****:*****	
AtCaM7	NGTIDFPEFLNLMARKMKDTSSEELKEAFRVFDKQNGFISAAELRHVMTNLGEKLTDE	120
BdCaM7	NGTIDFPEFLNLMARKMKDTSSEELKEAFRVFDKQNGFISAAELRHVMTNLGEKLTDE	120

AtCaM7	EVDEMIREADVDDGQINYEETFVKVMMAK	149
BdCaM7	EVDEMIREADVDDGQINYEETFVKVMMAK	149

D

AtCaM1	MADQLTDEQISEFKEAFSLFDKDGDCITTKELGTVMRSLGQNPTAEALQDMINEVDADG	60
AtCML9	MADAFTDEQIQEFYEAFCLIDKSDSGFITKEKLTKVMKSMGKNPKAEQLQQMMSDVIDFG	60
	*** :*****.* ** *.*:***.* **.:*: ***:*:*:**.:**:*:.* ** *	
AtCaM1	NGTIDFPEFLNLMAKKMKDSTDSEELKEAFRVFDKDGNGFISAAELRHVMTNLGEKLTDE	120
AtCML9	NGGTFDDFLYIMAQNTSQESASDELIEVFRVFDKDGGLISQLELGEGMKDMGMKITAEE	120
	** * * :** :***.:. :. :.:** *.*****:* :*:** ** . *. :. * * :* *	
AtCaM1	EVEEMIREADVDDGQGINYEYEFVKIMMAK--	149
AtCML9	EAEHVMVREADLDGDFLSFHEFSKMMIAASY	151
	..*.*****:.* ** *:*:*	

Sequence comparison of calmodulins derived from *A. thaliana* and *B. distachyon*. The CaM 1, CaM3 and CaM7 alignments are shown in figure 1A, B and C, respectively. D is the alignment of CaM1 and the calmodulin-like protein 9 (CML9) derived from *A. thaliana*.

Supplementary Methods

Specific primers used in site-directed mutagenesis

- *BDPDE1*^{L124E} (forward)

5' - GCGGACGCGGCGCGCGTGGGC -3'

- *BDPDE1*^{L124E} (reverse)

5' - GCCCACGCGCGCCGCGTCCGC -3'

- *BDPDE1*^{H155G} (forward)

5' - TAATCCCCGATGTCGCCCAGCAGAGCAGCCAG -3'

- *BDPDE1*^{H155G} (reverse)

5' - CTGGCTGCTCTGCTGGGCGACATCGGGGATTA -3'

- *BDPDE1*^{D156G} (forward)

5' - CCTTGGTATACTTGTAACCCCCGATGTCGTGCAGC-3'

- *BDPDE1*^{D156G} (reverse)

5' - GCTGCACGACATCGGGGGTTACAAGTATACCAAGG-3'

- *BDPDE1*^{Y237E} (forward)

5' - TTCTTGCTCCCTCCTTCTGTAAAACATCTTGCAACACCAATTGC-3'

- *BDPDE1*^{Y237E} (reverse)

5' - GCAATTGGTGTTGCAAGATGTTTTACAGAAGGAGGGAGCAAGAA-3'

- *BDPDE1*^{L52K} (forward)

5' - CGCTTCGTCGGACTCGAAGTTTACGATTCTCGATCTCCCTA-3'

- *BDPDE1*^{L52K} (reverse)

5' - TAGGGAGATCGAGAATCGTAAACTTCGAGTCCGACGAAGCG-3'

Supplementary Table S1

List of *B. distachyon* candidate PDEs

Search pattern [AYFW] H x [LEYFW] R x {20,40} [HRK] [DE] x {60,90} R x {3} [YFW]
 Dataset searched *Brachypodium distachyon* proteome ID UP000008810

Gene ID	Region	Hit pattern	Description
A0A0Q3H5X5	353-481	FHMWRMVSVNQSYGYGQQLRSIPSQPHCHLRF VDITGFYGGKQDQLELALHILRNAAMLEAMKID PKPSIAAEYQMQGPFFLDGYQVARDYVLRED KCNVLYIKHVPRKTIEAKFLSGSLWSKLRTLSY	F-box domain containing protein
I1I25	295-417	AHPERIQKL VKNYSQFVSFPIYTWQEKGTKEV EVDEDPAAAKTEGDGDEKQAKKKTCTVVEK YWDWELTNETQPIWLRNPREVSTEEYNEFYKK TFNEYLDPLASSHFTTEGEVEFRSILY	HATPase cdomain containing protein
I1H3Y3	127-237	FHKERYNKDVKEKERLKTSAIVLRGGENKEV RFK GKTEIKDLKV KADAITRLMERGYRVKCM MPAGNEEEDLGGPLSRLGLIQDVCIVESGPHL DSKHAYVIVRHVKF	IF3_N domain containing protein
I1ITQ2	526-630	AHRYRHLITNDQLFRPVLDPQGWHDHIGFDGI NFESSHELKRNISISSIAGQMRKDKEDMYIQSEC SVSYTDQRGYSMLGGMDMQTASKDLVCTVHG DAKYRNFPW	AIG1-type G domain containing protein
I1GWH8	158-285	AHQFRWLLSQVNYPKLGDLCLVIPCALTALD HWSPDVKEQGVVSFMHIAKNVKVTELSLYED AILDACCNYIPADDDLWYHIVEVSVLLLTCTQR SNPRSPWYDRMLSAMLGHLERQLNQKRRIW	Uncharacterized protein
A0A0Q3GZJ1	142-262	FHQLRSGTSSSVSSQDGDSNERSTDDETEKEEV NADNEHDEEGVKVVAWSADDQKNILKIGCLEI ERNQRLEMLIARHRVRKDVDRNLIDFGSGDSIP TVEELSKFNVQIPTVFAPRRNPF	Uncharacterized protein
I1H012	2-108	AHEWRRAAASASAAALDAAEDALFVDIAHEA PLSCQRQSQSI VGGTLYCILLAGYAGVAIAAPW IFVLIPEMTLPLLCSCNVLLIVTGIFQQYWVHQ VTKVRLQGY	Uncharacterized protein
A0A2K2DUJ7	168-289	AHRLRPPRRVTALVPLDALDAEKLYRRRFARD VEFFPTDIGAVLGNTLSLGTFLAVVVGAEADASK KFEWRGVEQFLASPPASWAVASLWDCGGVFR LEMRGASRARRALAAASRALDRAAKW	N-acetyltransferase domain protein
A0A0Q3GP66	245-353	AHEWRYVVENQCMLWEQRDGEGVVPPEGRV GRHERPVQVAGAGQLLPKPPKLPGGVQGGQLG AAAGHARRLP RRRREPMGELRRRRFLQKKRW RRRGGGHRQGQRRAGLW	PLCXc domain containing protein
A0A0Q3GWX6	103-215	AHSLRGFEAIDTAKAALELACPGVVSCADVLA FAARDASYILSVGNYPNIEFAMPGGRLDGRRS MAEDTLHGSLPSASANVTELVD AFQTKGLGVE DLVVLGAHSARCSFF	Peroxidase

A0A0Q3FAV4	121-233	AHRWRDAAAAAPSSCPASSTAAIGGGILPPTTA SLARRETPPPPPRPPTGASPPAPSTAAASFPSA WRRPCDGGSGEARVLRRQLRGQQAWSAPWTA TAPLPAVHSSRVYKY	Uncharacterized protein
I1IA86	215-329	AHVYRSRDFKTKWRAKHPLHSALTGMWECPD FFPVHEAGVQNGLETSQFAAKYVLKNSLDLTR YDYYTVGTYNKTDTRYVPDDANGDYRRLQY DYGNFYASKTFYDPAKQRRVLF	Uncharacterized protein
A0A0Q3FTD3	265-383	WHEERCVRASNITIDARMLEVSGLPCRPPPRDH SNDEAAAQAQQQLEFHNLSMCQPALCPNGED DVVYLLAREKYLHPKAWCLA VDMKNQGTLK HVAYIGNQGHFPCHRIFCLSRISKY	DUF1618 domain containing protein
A0A0Q3IC40	279-394	AHTWRAVSRARRLPPTTEETTYTVLVGCRDRVK GVPRDYAGNAVVRATARSTAGEILGHGLGWT ARLLNRAVASSGDEAAVRGFLGRWAREPRFA YLAGFWNPAMVVTGNSPRFDAF	Uncharacterized protein
I1J2V7	33-148	WHAERAASSAAQPASGTRSQYNVELESVNRFL DGILREKPARFTAENLREFTGGYAERVGSGGFG VVYRGRFPNGVAVAVKVLNGTLDRRAEEQFM AEVGTAGRTYHINLVRLYGF	Protein kinase domain protein
I1HF09	665-781	FHLRRGPLLSIVGHEDERSVLRNLFLNASFDL SLRMLAPRCIMHREGGTFEELPAYDLAMQSYS AVVLDHGTDFIWLGAELATKEGQSAAALAAC RTLAEELSELRFAPRILSF	Protein transport SEC23
I1GRD2	66-193	AHALRRPIEVLLYTRPVHGCGEEDLGLDALAV VSGSGDRDRRRRLASIPAMPHRCGPRPCCCLA SCDGLLLLGHGRGNFQPYASYLVCNPATRQW TQLPRVTAEFAAAGKRPGHGPARELFRESGF	F-box domain containing protein
I1HUJ6	662-778	AHTLRFEAYELPKPSLPTATSQTSISLPTDLVP VPEQSYKKDDHQMSRPQPSGNALSGEFGTKLR LDGVQKKWGRESYSSSSTPSSSTSSQQAANGST NSDGGGLVSSQARESSY	AP4 complex subunit epsilon
<u>A0A0Q3LY42</u>	121-237	AHALRVRDLALSAAEQGLSSPDRLLLIVELAAL LHDIGDYKYTKDNMEDMSIVERFLQDVGLEKG QREEIVSIITGMGFKNEVSGKAAEPTLEFAVVQ DADRLDAIGAIGVARCFTY	BdPDE1, HD domain containing protein
A0A0Q3GTT5	173-293	AHLFRSWATFLNRSIILTPEGDRTDKRGISAFNT WKDIIIPGNVDDSMVKPDARAVQPIPLTKRKYL ANFLGRAQKGAGRLKLVELAKQYPDKLESPEL KLSGPDKLGRIDYFKHLRNAKF	Exostosin domain containing protein
I1GZC4	244-351	FHPERGSSTADLADDSRFIEFYNLVFMQYNKK DDGSLEPLKQKNIDTGMGLERMARILQNVNPN YETDLIFPIIKKAASMALVSYSTADDTMKTNLK IIGDHMRVAVVY	Probable alanine tRNA ligase
I1HR05	55-164	AHYRRRCRIRAPCCNEIFDCRHCHNEAKNSIGI DTMRRHEIPRHEVQQQVICSLCGTEQEVRIQIC NCGVCMGKYFCEVCKLFDDDDVSKQYHCHGC GICRIGGRENFF	Uncharacterized protein

A0A2K2CIP6	381-510	WHVWRSQIHNNNNGGFDFAWLDAAFLFY DEEARLVVRVRDCLDMASLGAYQEVPLPW LDARPTKPASPGTPARALDALPATLDKTVRVA VARPRASRSAKEKEEQEEVVVVEGIQVSDCSRF VKF	Uncharacterized protein
A0A2K2D3H0	58-190	FHHFRALISFDSISSIKSQLENGSAKPNWTQSSPP RPRDHHDLRHSSVLRLLLLRRRRTARPHSRAPLP GVPDYPRRKFAFGCIRGTMLFMKKPFSLEPP KDLPKEKVFQIRFTKEIFLDYQEYVKRLNLY	Uncharacterized protein
I1IFT6	227-338	FHALRNTRGLPWPKEHEKKSDADLLDWLQAM FGFQTDSVSNQREHLILLANMHIRQISKPDQQ SKLDDGALDKVMKKLFFKNYKRWCKYLGRKSS LRLPTIQQEVQQRKLLY	1,3-beta- glucansynthase