

Additional file:1

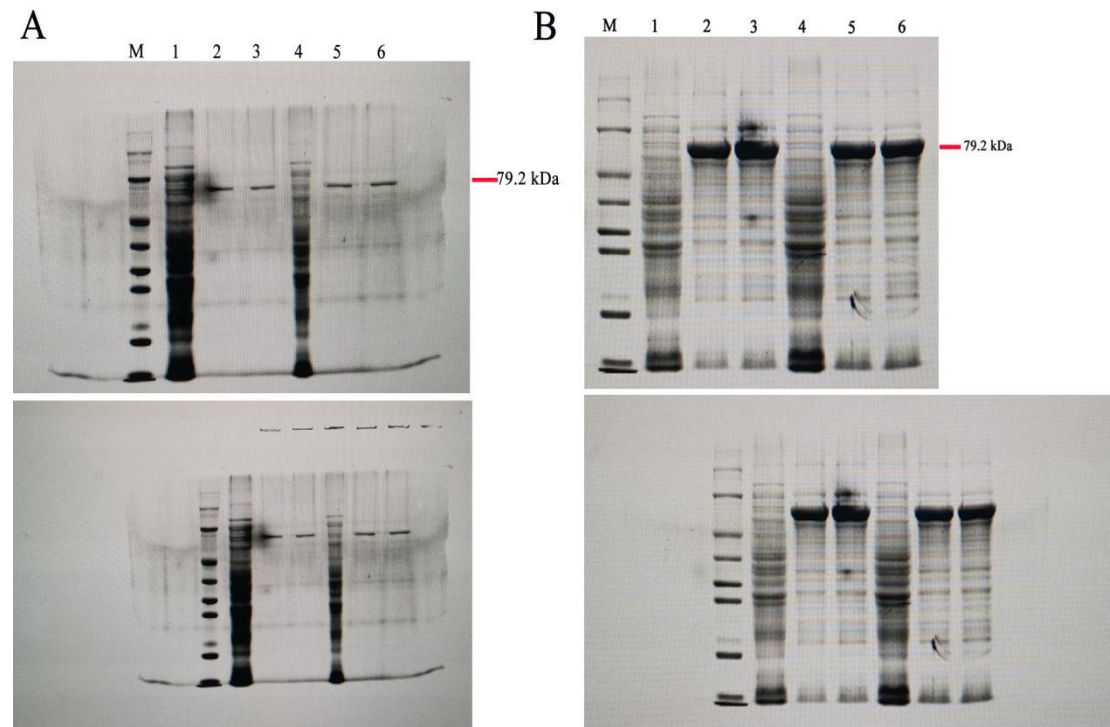


Figure. S1 Soluble analysis of recombinant pET-22b-PbPAL1/2

A: SDS-PAGE of protein induction (M: Mark, 1: a uninduced sample, 2-3 are samples induced by PbPAL1 proteins, 4: a uninduced sample, 5-6: are samples induced by PbPAL1 PbPAL2 proteins); B: is the purification of PbPAL1 and PbPAL2 proteins (M: Mark, 1: a uninduced sample, 2-3 are samples induced by PbPAL1 proteins, 4: a uninduced sample, 5-6: are samples induced by PbPAL1 PbPAL2 proteins)

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PIPAL      .....MKARDVQPTIIINKNGI..ISLEETIYDIAMKQKK...VEISTEITEL..ITYGREPLEEKLNSEVINGINTGEGGNAN..LVVFEFKISEHQCNLTIFLS  92
PcPAL2     EDFLYWGIAAEAMTGSLSLDEVKRMVAEYRKF.VVRLGGET..ITISCVAAISARDGSGVTVELSEARRAG.VKASSDWVMSMNGTDSNGVITGGFATSHRRTRKGGALQKELIRFINAGI 142
JrPAL      SDFLNWGLAAESLSGSHLDEVKRMVVEEYRKF.VVRLGGET..ITISCVAAIATHDAG.VKVELSEARRAG.VKASSDWVINSVNGTDSNGVITGGFATSHRRTRKGGALQKELIRFINSGI 132
JcPAL      RDFLSWGVAAESMKGSHLDEVKRMVVEEYRKF.LVRLGGET..ITVACVAGIASHDSG.VKVELSEARRAG.VKASSDWVMSMNGTDSNGVITGGFATSHRRTRKGGALQKELIRFINAGI 137
TpPAL      GDFLNWGAARESLTGSLSLDEVKRMVVEEYRKF.LVRLGGET..ITVACVAGIASHDSG.VKVELSEARRAG.VKASSDWVMSMNGTDSNGVITGGFATSHRRTRKGGALQKELIRFINAGI 136
AtPAL      ADFLNWGLAAEQMGSHLDEVKRMVVEEYRKF.VVRLGGET..ITISCVAAISTVGGG.VKVELSEARRAG.VKASSDWVMSMNGTDSNGVITGGFATSHRRTRKGGALQKELIRFINAGI 141
PvPAL      TDFLNWGLAAEQMGSHLDEVKRMVVEEYRKF.LVRLGGET..ITVACVAAVSGHDQG.VKVELSEARRAG.VKASSDWVMSMNGTDSNGVITGGFATSHRRTRKGGALQKELIRFINAGI 138
VvPAL      LAFPHNKKAAEQMGSHLDEVKRMVVEEYRKF.LVRLGGET..ITVACVIALARGDHV..RVALLCAVARGVTRSAWVAENISRGITDTGVTGGFATSHRRTRKGGALQKELIRFINAGI 157
PpHAL      .....MTETLTLPFGT..ITLACLRRAIHAAPVR...LQLDASAPAC..IDASVACVEQI IAEERTANGINTGGFLLASTRIAS.HLENLQRSVLVSHA  85
PpHAL      .....MNVTALNLIPGQ..ITLACLRRAIYQQPLT...LLDGSAPAC..IDASVACVEQI IAEERTANGINTGGFLLASTRIAS.HLENLQRSVLVSHA  87
RnHAL      EVVIEGVMSDFDFIPSCPEGVFLYSKYREPEKYIALDGS..ISTEDLVNLKGKHYK...IKLTISAEKK.VKQSEVIDSI IKERTVVGINTGGFLLASTRIAS.HLENLQRSVLVSHA 195
PbPAL1     KDFLNWGLAAEQMGSHLDEVKRMVVEEYRKF.VVRLGGET..ITISCVAAIATHDAG.VKVELSEARRAG.VKASSDWVMSMNGTDSNGVITGGFATSHRRTRKGGALQKELIRFINAGI 144
PbPAL2     KDFLNWFSIGEAQMGSHLDEVKRMVVEEYRKF.VVRLGGET..ITVGVASVSVRHV...VELCKSARAA..VEASSVWMDGVNSGKDINGINTGGFANSRRTRKGGALQKELIRFINAGI 135
Consensus  .....MKARDVQPTIIINKNGI..ISLEETIYDIAMKQKK...VEISTEITEL..ITYGREPLEEKLNSEVINGINTGEGGNAN..LVVFEFKISEHQCNLTIFLS  92
PIPAL      AGTG.....DYMSEPCIKASQFTMLLSVCKKSAIRP IVAQIVDHHNMDIVELVRYESVQASG..HPLSYIARAD..IGKVYYMGAEVDAEAIKRRAGLTPLS..LQAKEGL 199
PcPAL2     FNGSD.....NTLPHSATRAAMLVRINTILCOSSGIRFEILENITKFLNNITFCIPRGTITASG..HPLSYIAGL..IGRPNKAVGEGTGVILSPEEAFKLAGEGGFFELQPKRGL 256
JrPAL      FNGTSC....HTLPHSATRAAMLVRINTILCOSSGIRFEVMEIAKLNNVITFCIPRGTITASG..HPLSYIAGL..IGRPNKAVGEGNGESLDAKAFQLAGIDGGFFELQPKRGL 248
JcPAL      FNGTETC....HTLPHSATRAAMLVRINTILCOSSGIRFEILENITKFLNNITFCIPRGTITASG..HPLSYIAGL..IGRPNKAVGEGNGESLDAKAFRLAIDGGFFELQPKRGL 253
TpPAL      FNGTESN....CTLPHSATRAAMLVRINTILCOSSGIRFEILENITKFLNNITFCIPRGTITASG..HPLSYIAGL..IGRPNKAVGEGNGESLDAKAFRLAIDGGFFELQPKRGL 252
AtPAL      FNGTETC....HTLPHSATRAAMLVRINTILCOSSGIRFEILENITKFLNNITFCIPRGTITASG..HPLSYIAGL..IGRPNKAVGEGNGESLDAKAFRLAIDGGFFELQPKRGL 257
PvPAL      FNGTSS....HTLPHSATRAAMLVRINTILCOSSGIRFEILENITKFLNNITFCIPRGTITASG..HPLSYIAGL..IGRPNKAVGEGNGESLDAKAFRLAIDGGFFELQPKRGL 254
VvPAL      IG.....KETLPASFSAKAMLVRINTILCOSSGIRFEILENITKFLNNITFCIPRGTITASG..HPLSYIAGL..IGRPNKAVGEGNGESLDAKAFRLAIDGGFFELQPKRGL 267
PpHAL      AGTG.....APLDDLVLIMVLKINSLSRGSIGIRKVIDALIALVAEVYHIEHPSVQASG..HPLAHNSLV..IG..EGKAR.YKGCWLSATEALAVAGLEPL..TLAAKEGL 192
PpHAL      AGVG.....EPISDDLVLIMVLKINSLSRGSIGIRKVIDALIALVAEVYHIEHPSVQASG..HPLAHNSLV..IG..EGKAR.YKGCWLSATEALAVAGLEPL..TLAAKEGL 194
RnHAL      SGVG.....KPLSPERCMLLALRINVLAKSGISLETILKQVIEVNASCLSVVEKTVQASG..HPLSHLALC..IG..EGKAR.YKGCWLSATEALAVAGLEPL..TLAAKEGL 303
PbPAL1     FGSATES....HTLPHSATRAAMLVRINTILCOSSGIRFEILENITKFLNNITFCIPRGTITASG..HPLSYIAGL..IGRPNKAVGEGNGESLDAKAFRLAIDGGFFELQPKRGL 260
PbPAL2     FNGTEACQCHTLTLPHSATRAAMLVRINTILCOSSGIRFEILENITKFLNNITFCIPRGTITASG..HPLSYIAGL..IGRPNKAVGEGNGESLDAKAFRLAIDGGFFELQPKRGL 255
Consensus  g          g s          n          p g          asd l p          l g

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Figure. S2 Sequence alignment of PAL amino acids in different species

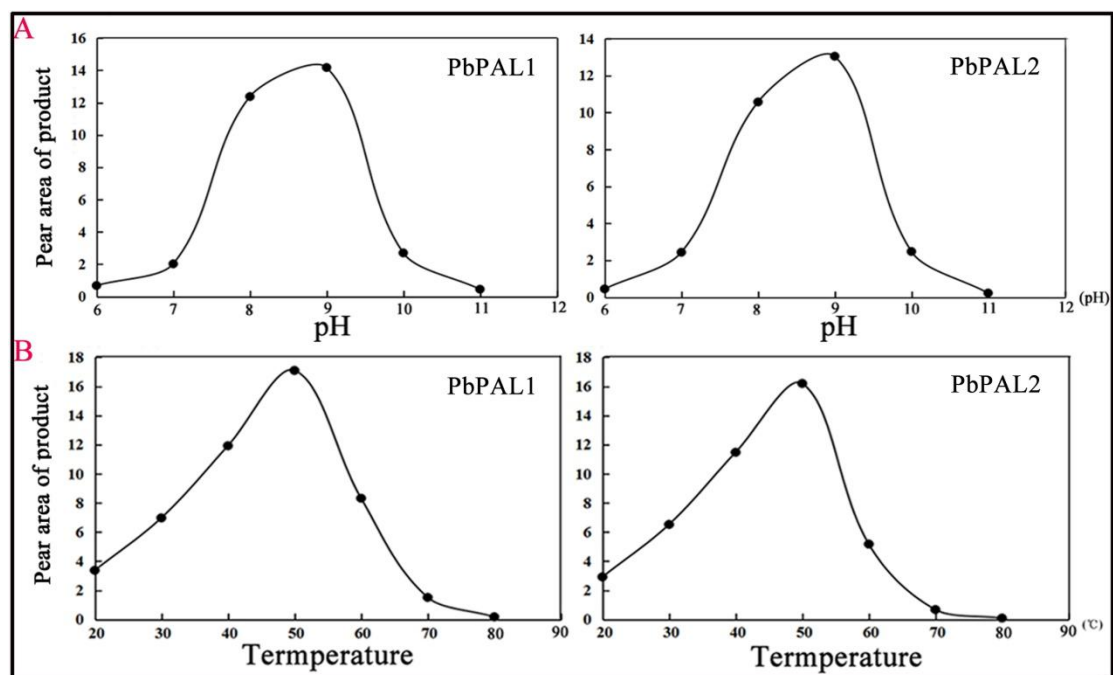


Figure. S3 The optimum pH and temperature of the two purified PAL proteins

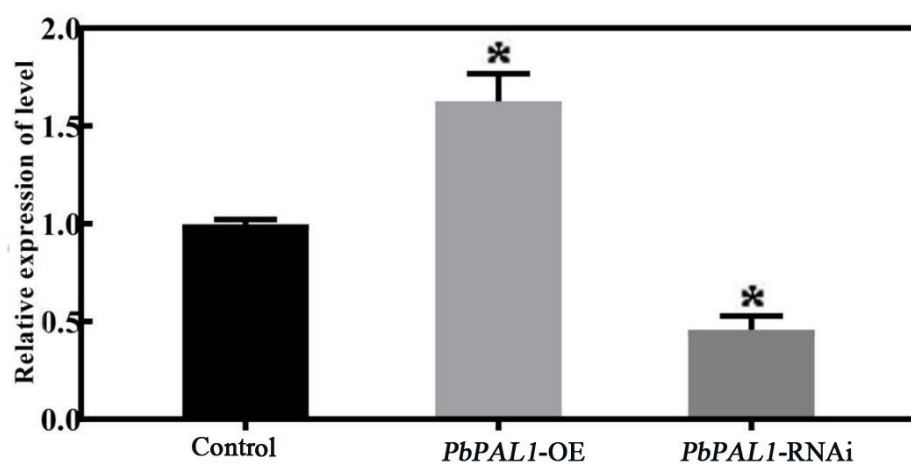


Figure. S4 Analysis of *PbPAL1* expression level in overexpression and RNAi pear fruit