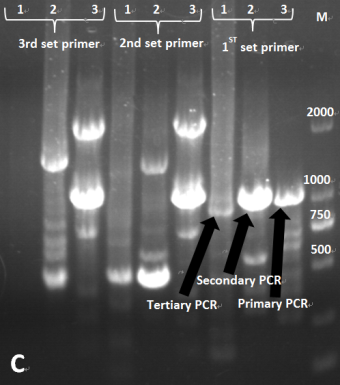
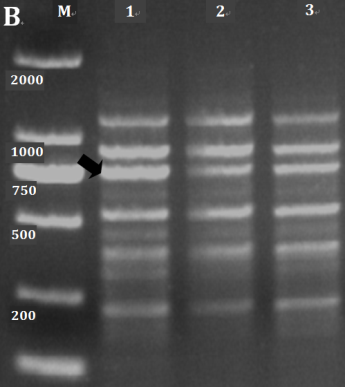
**Supplementary Table 1** Primers used in this study

|  |  |  |
| --- | --- | --- |
| **Purpose of primer** | **Primer sequence** | **Accession no. of gene** |
| Degenerated primers for fungal *ACCD gene* | PL3F 5′-GCCTACGGCGGNAAYAAR-3′  PL4R 5′-CTCGTAGACGGGGTCGGTDATRAANGC-3′ | KX762324 |
| Nested gene Specific primers (1st set) | PL1 5′-GAGTACCTCGTCCCACAAGC-3′  (Position of primer 22-41bp)  PL2 5′-ACAAGCCGTCGCCCAAG-3′  (Position of primer 35-52bp)  PL3 5′-AGCACTGGGTCGACTGGA-3′  (Position of primer 155-172bp) | - |
| Nested gene Specific primers (2nd set) | PL4 5′-CCATCGAGGCCATCAAATTT-3′  PL5 5′-TTTGGTGCCAGCACAG**AGG**C-3′  PL6 5′-AAGCCCAGGAGAGTCATCGG-3′ | - |
| qRT-PCR primers for fungal ITS as ref. | ITSP. LIQUIDAMBARIS-F 5′-GACGACATGGAGAAGATCTGGCAC-3′  ITSP. LIQUIDAMBARIS-R 5′- GTTGAACGTCTCGAAGACGATCTG-3′ | Yang et al. 2012 |
| qRT-PCR primers for rice *ACCD gene* | OsC2138-ACCD gene F 5′-TGGCGCAGAATGGATCTTGA-3′  OsC2138-ACCD gene R 5′-ACTGGAGATCCTGCAAGCTG-3′ | LOC\_Os02g53330 |
| qRT-PCR primers for rice *ACCD gene* | OsC1134-ACCD gene F 5′-CTGCGGCCACTGGTGTTA-3′  OsC1134-ACCD gene R 5′-TACCGCTCCAAGAGCTAACC-3′ | LOC\_Os01g50060 |
| qRT-PCR primers for rice ACO | OsC4H2130-aco F 5′-AGCATAGAGTGGTGGCGAAG-3′  OsC4H2130-aco R 5′-GGCGGGTTCTCATCAGACAA-3′ | LOC\_Os04g10350 |
| qRT-PCR primers for rice ACS | OsC3H1141-acs F 5′- CTCGTCCTACTTCCTGGGGT-3′  OsC3H1141-acs R 5′-GGGTTCTTCTCCAGCCACTC-3′ | M96672.1 |
| qRT-PCR primers for rice actin as ref. | Os-act F 5′-CAGCCACACTGTCCCCATCTA-3′  Os-act R 5′-AGCAAGGTCGAGACGAAGGA-3′ | Yang et al. 2012 |
| PCR primers for *nif*H | IGK3 (5′- CCCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCA CGGGCCGGCIWTHTAYGGIAARGGIGGIATHGGIAA-3′)  DVV R 3′**-**CTRCAICAIACRCCICCIAARCGITA-5′ | Gaby and Buckley 2012 |



**A**



**Supplementary Fig. 1** Confirmation test showing that *P. liquidambaris* express ACC deaminase**.** Qualitative assay of *P. liquidambaris* presenting ACC deaminase activity; (A) Agarose gel electrophoresis of the PCR-amplified ACC deaminase gene; (B) Amplification of *ACCD gene* flanking sequences from *P. liquidambaris* by nested-PCR using different nested gene specific and walker-adapter primers (C). Three pairs of PCR primers were used and the first pair amplified the *ACCD gene* as an ordinary PCR and the second pair of primers (**nested primers**) bind within the first PCR product and produces a second PCR product that is shorter than the first one and the corresponding secondary and tertiary products show the expected differential shift of 105 bp. Agarose gel electrophoresis of the primary (1), secondary (2), and tertiary (3) product of nested PCR-amplified ACC deaminase gene; M: 2-kb DNA ladder.

CGCAACAAGGTCCGCAAGCTGGAGTACCTCGTCC**CACAAGC**CGTCGCCCAAGGCGCAGACACGCTCGTCTCCATTGGCGGTGTCCAGTCCAACCACACCCGCGCCGTCACGGCCGTGGCCATCGCCAGCGGGCTGAAGGCGGCCACCGTGCAGGAGCACTGGGTCGACTGGACAGACCCTGGCTACGAGAAGGTCGGCAACCTGCAGCTGAGCAGGCTCATGGGTGGAGATGTCCACCTGGACCCGAGCGGCTTTGGCATTGAGCACAAGACCACCCTGGCGAATCTCACCAAGGACCTGGAGAGCAAGGGACAGAAGCCCTATTACATCCCTGCGGGCGCGAGTGATCACCCCTTCGGAGGGCTGGGCTTTGCAAGGTGGGCGTTTGAGGTGGAACAGCAAGAAGCGGAGCTTGGGGTCTTCTTCGACACAGTCATCGTGTGTGCCGTCACCGGAAGCACATTCGCCGGCATGATTGCTGGATTCAAATTGGCGCAGAAGAATGGCAGCAAGCCCAGGAGAGTCATCGGTATCGACGCCAGTGCCAAGGTGAAGCAGACTTTTGACCAGGTCTTGAGGATCGCCAAGTTCACCGCCGCAAAAATTGGCCTGTCGGAGGACGATATTACGGAGAAGGATGTCGAGCTGGTAGACAGGTACCATGCCGGAACTTATGGTATCCCGGATGAGCAGACCATCGAGGCCATCAAA**TTT**GGTGCCAGCACAGAGGCGTTCATCACCGACCCGG

PL6 (21)

PL5 (21)

PL4 (21)

PL3 (18)

PL2 (17)

PL1 (20)

**Supplementary Fig. 2** Specific primers along with genome walker primers used for nested-PCR. Nucleotide sequences of nested gene specific primers PL1, PL2, and PL3 were used to determine downstream flanking sequences. Primer PL1 and PL2 were designed to overlap (bold letters) with each other. On the other hand, PL2 and PL3 are positioned 103 bases apart from each other to facilitate the confirmation of product specificity by size comparison. Nucleotide sequences of nested gene specific primers PL4, PL5, and PL6 were used to determine upstream flanking sequences. Primer PL4 and PL5 were designed to overlap (bold letters) with each other. On the other hand, PL5 and PL6 are positioned 164 bases apart from each other to facilitate the confirmation of product specificity by size comparison. The sequence shown in this figure was determined by using degenerated primers.

|  |  |
| --- | --- |
| 1 1 76 26 151 51 226 76 301 101 376 126 451 151 526 176 601 201 676 226 751 251 826 276 901 301 976 326 1051 351 1126 376 | ATGGCGTTTGCGAGC ATTCCGCGCCATGAA CTGACCCTGGGCCCG AGCCCGATTCATGCG CTGCCGGAAATTAGC  M  A  F  A  S   I  P  R  H  E   L  T  L  G  P   S  P  I  H  A   L  P  E  I  S  GCGGCGCTGGGCGGC AAAGTGTGCATTTAT GCGAAACGCGAAGAT TGCAACAGCGCGCTG GCGTTTGGCGGCAAC  A  A  L  G  G   K  V  C  I  Y   A  K  R  E  D   C  N  S  A  L   A  F  G  G  N  AAAGTGCGCAAACTG GAATATCTGGTGCCG CAGGCGGTGGCGCAG GGCGCGGATACCCTG GTGAGCATTGGCGGC  K  V  R  K  L   E  Y  L  V  P   Q  A  V  A  Q   G  A  D  T  L   V  S  I  G  G  GTGCAGAGCAACCAT ACCCGCGCGGTGACC GCGGTGGCGATTGCG AGCGGCCTGAAAGCG GCGACCGTGCAGGAA  V  Q  S  N  H   T  R  A  V  T   A  V  A  I  A   S  G  L  K  A   A  T  V  Q  E  CATTGGGTGGATTGG ACCGATCCGGGCTAT GAAAAAGTGGGCAAC CTGCAGCTGAGCCGC CTGATGGGCGGCGAT  H  W  V  D  W   T  D  P  G  Y   E  K  V  G  N   L  Q  L  S  R   L  M  G  G  D  GTGCATCTGGATCCG AGCGGCTTTGGCATT GAACATAAAACCACC CTGGCGAACCTGACC AAAGATCTGGAAAGC  V  H  L  D  P   S  G  F  G  I   E  H  K  T  T   L  A  N  L  T   K  D  L  E  S  AAAGGCCAGAAACCG TATTATATTCCGGCG GGCGCGAGCGATCAT CCGTTTGGCGGCCTG GGCTTTGCGCGCTGG  K  G  Q  K  P   Y  Y  I  P  A   G  A  S  D  H   P  F  G  G  L   G  F  A  R  W  GCGTTTGAAGTGGAA CAGCAGGAAGCGGAA CTGGGCGTGTTTTTT GATACCGTGATTGTG TGCGCGGTGACCGGC  A  F  E  V  E   Q  Q  E  A  E   L  G  V  F  F   D  T  V  I  V   C  A  V  T  G  AGCACCTTTGCGGGC ATGATTGCGGGCTTT AAACTGGCGCAGAAA AACGGCAGCAAACCG CGCCGCGTGATTGGC  S  T  F  A  G   M  I  A  G  F   K  L  A  Q  K   N  G  S  K  P   R  R  V  I  G  ATTGATGCGAGCGCG AAAGTGAAACAGACC TTTGATCAGGTGCTG CGCATTGCGAAATTT ACCGCGGCGAAAATT  I  D  A  S  A   K  V  K  Q  T   F  D  Q  V  L   R  I  A  K  F   T  A  A  K  I  GGCCTGAGCGAAGAT GATATTACCGAAAAA GATGTGGAACTGGTG GATCGCTATCATGCG GGCACCTATGGCATT  G  L  S  E  D   D  I  T  E  K   D  V  E  L  V   D  R  Y  H  A   G  T  Y  G  I  CCGGATGAACAGACC ATTGAAGCGATTAAA TTTGGCGCGAGCACC GAAGCGTTTATTACC GATCCGGTGTATGAA  P  D  E  Q  T   I  E  A  I  K   F  G  A  S  T   E  A  F  I  T   D  P  V  Y  E  GGCAAAAGCCTGGCG GGCATGATGGATATG ATTAAAAAAGGCGAT ATTCCGGCGGGCAGC AACGTGCTGTATGCG  G  K  S  L  A   G  M  M  D  M   I  K  K  G  D   I  P  A  G  S   N  V  L  Y  A  CATCTGGGCGGCCAG CTGGCGCTGAACGCG TATAGCATGATTCAG GGCTGGAACCGCAAA ATGCCGACCTATAAC  H  L  G  G  Q   L  A  L  N  A   Y  S  M  I  Q   G  W  N  R  K   M  P  T  Y  N  CAGGTGAACTTTTAT GATAAAGTGCTGCTG GAAATGTTTTTTTAT CCGCGCTTTCAGCTG CTGAACGGCCTGATT  Q  V  N  F  Y   D  K  V  L  L   E  M  F  F  Y   P  R  F  Q  L   L  N  G  L  I  ACCGTGGCGGTGACC CTGCGCGCGAGC  T  V  A  V  T   L  R  A  S |

**Supplementary Fig. 3** The nucleotide (1152 bp) sequence of the *ACCD gene* gene and deduced and analyzed amino acid sequence (384 aa) of ACC deaminase generated using translator site <http://www.fr33.net/translator.php>. The amino acid residues are indicated by a single letter code. Two sequences in the rectangles were used to design degenerated primers for PCR amplification. The sequence shown in this figure was determined by direct sequencing of the secondary amplification product.

*Diaporthe ampelina* | MSTTTVTLPEPFASIPRSTLTFGPSPIQALPKISQALGGKVNVYAKREDV

*Valsa mali* | -MSTTVTLPEPFASIPRSTLTFGPSPIQSLPKISQALGGKVNVYAKREDV

*Phomopsis liquidambaris* | ---------MAFASIPRHELTLGPSPIHALPEISAALGGKVCIYAKREDC

*Pseudomonas sp*. EcB10 | ---------MNLKRFNRYPLTFGPTPISPLKRLSQELGGKVELYAKREDC

: : \* \*\*:\*\*:\*\* .\* .:\* \*\*\*\*\* :\*\*\*\*\*\*

*Diaporthe ampelina* | NSGLAYGGNKVRKLEYLVPQAISQGADTLVSIGGVQSNHTRAVTAVAVAS

*Valsa mali* | NSGLAFGGNKVRKLEYLVPEAVAEGCDTLVSIGGVQSNHTRAVTAVAVAS

*Phomopsis liquidambaris* | NSALAFGGNKVRKLEYLVPQAVAQGADTLVSIGGVQSNHTRAVTAVAIAS

*Pseudomonas sp*. EcB10 | NSGLAFGGNKTRKLEYLIPDALEQGCDTLVSIGGIQSNQTRQVAAVAAHL

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*Diaporthe ampelina* | GLKAATVQEHWVDWTDPGYEKVGNLQLSRLMGGDVHLDPSGFGIEHKTTL

*Valsa mali* | GLKAATVQEHWVDWTDPGYEKVGNLQLSRLMGGNVNLDPSGFGIEHKSTL

*Phomopsis liquidambaris* | GLKAATVQEHWVDWTDPGYEKVGNLQLSRLMGGDVHLDPSGFGIEHKTTL

*Pseudomonas sp*. EcB10 | GMKCVLVQENWVNYEDAVYDRVGNISMSRIMGADVRLADDGFDIGIRKSW

\*:\*.. \*\*\*:\*\*:: \*. \*::\*\*\*:.:\*\*:\*\*.:\*.\* .\*\*.\* :.:

*Diaporthe ampelina* | ADLTKDLESKGQKPYYIPAGASDHPFGGLGFARWAFEVEQQEAELGVFFD

*Valsa mali* | ANLTKDLESKGQKPYYIPAGASDHPLGGLGFARWAFEVEQQEKEMGVFFD

*Phomopsis liquidambaris* | ANLTKDLESKGQKPYYIPAGASDHPFGGLGFARWAFEVEQQEAELGVFFD

*Pseudomonas sp*. EcB10 | EEAMDNVRARGGKPYPIPAGCSEHPLGGLGFVGFAEEVRQQEQKLGFEFD

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*Diaporthe ampelina* | TVIVCAVTGSTFAGMIAGFKLAQKNGGKPRRVIGIDASAKVKQTFDQVLR

*Valsa mali* | TVLVCAVTGSTFAGMIAGFKLAQKQGSKPRRIIGIDASAKVKQTFDQVLR

*Phomopsis liquidambaris* | TVIVCAVTGSTFAGMIAGFKLAQKNGSKPRRVIGIDASAKVKQTFDQVLR

*Pseudomonas sp*. EcB10 | YIVVCSVTGSTQAGMIVGFAADGRN----KSVIGIDASAKPEKTAKQILR

:: \*\*:\*\*\*\*\*:\*\*\*\*.\*\* :: : :\*\*\*\*\*\*\*\* ::\* .\*:\*\*

*Diaporthe ampelina* | IAKFTAAKIGLSEDDITEKDVELVDRYHAGTYGIPDEQTIEAIKFGASTE

*Valsa mali* | IAKFTAAKIGLSEDDITEADVELVDRYHAGTYGIPDEQTIEAIKFGASTE

*Phomopsis liquidambaris* | IAKFTAAKIGLSEDDITEKDVELVDRYHAGTYGIPDEQTIEAIKFGASTE

*Pseudomonas sp*. EcB10 | IAEHTAELVDLG-REITDEDVVLDTRYAFPEYGLPNEGTLAAIRLCGRLE

\*\*:.\*\* :.\*. :\*\*: \*\* \* \*\* \*\*:\*:\* \*: \*\*:: . \*

*Diaporthe ampelina* | AFITDPVYEGKSLAGMMDMIKKGDIPGGSTVLYAHLGYVGFMGYVGYVDF

*Valsa mali* | AFITDPVYEGKSLAGMMDMIKKGEIPAGSNVLYAHLGGQLALNAYSTIP-

*Phomopsis liquidambaris* | AFITDPVYEGKSLAGMMDMIKKGDIPAGSNVLYAHLGGQLALNAYSMIQG

*Pseudomonas sp*. EcB10 | GVLTDPVYEGKSMHGMIDEVRRGEFPEGSKVLYAHLGGVPALYAYSFLFR

..:\*\*\*\*\*\*\*\*\*: \*\*:\* :::\*::\* \*\*.\*\*\*\*\*\*\* : . :

*Diaporthe ampelina* | FMGYILGYVG---------------------------------

*Valsa mali* | -------------------------------------------

*Phomopsis liquidambaris* | WNRKMPTYNQVNFYDKVLLEMFFYPRFQLLNGLITVAVTLRAS

*Pseudomonas sp*. EcB10 | EG-----------------------------------------

**Supplementary Fig. 4** Alignment of amino acid sequences of ACC deaminase proteinsfrom *Phomopsis liquidambari*, *Diaporthe ampelina* (94% similarity), *Valsa mali* (92% similarity),and *Pseudomonas* sp. EcB10(73% similarity) produced by ClustalW. Amino acid sequences were aligned by fitting at the PLP binding site. The conserved amino acid residues that distinguish ACCDs are marked with a box. Twelve residues that are 100% homologous at least in 4 bases with all sequences are underlined. Below the protein sequences is a key denoting conserved sequence (\*), conservative mutations (:), semi-conservative mutations (.), and non-conservative mutations ( ).