LEGENDS TO SUPPLEMENTARY FIGURES

Fig. S1. Amplification of cDNAs by the primers designed on the basis of the EST information. The cDNA fragments for *CURS* (A) and DCS (B) were amplified.

Fig. S2. Multiple alignment of the amino acid sequences of plant type III PKSs. The red box indicates the three amino acids forming the Cys-His-Asn catalytic triad that is essential for starter substrate loading and malonyl-CoA condensation. DCS, diketide-CoA synthase; CURS, curcumin synthase; MsCHS, a chalcone synthase from *Medicago sativa* (DNA accession no. AAA02824); RhBAS, a benzalacetone synthase from *Rheum palmatum* (AAK82824); WtPKS1, a polyketide synthase from *Wachendorfia thyrsiflora* (AAY727928).

Fig. S3. Expression of *DCS* (A) and *CURS* (B) in turmeric. The relative expression was quantified by qPCR and normalized to 18S rRNA.

Fig. S4. SDS-PAGE of purified DCS and CURS by Ni-NTA column. Lane 1, marker; lane 2, purified DCS and CURS.

Fig. S5. Analysis of the compound derived by hydrolysis of feruloyl-diketide-CoA and *trans*-5-(4-hydroxy-3-methoxyphenyl)-3-oxopent-4-enoic acid methyl ester. The alkaline hydrolysis of feruloyl-diketide-CoA [**3b**] (*A*) and *trans*-5-(4-hydroxy-3-methoxyphenyl)-3-oxopent-4-enoic acid methyl ester (*B*) resulted in the formation of the same compound, *trans*-5-(4-hydroxy-3-methoxyphenyl)-3-oxopent-4-enoic acid [**3c**]. *Trans*-5-(4-Hydroxy-3-methoxyphenyl)-3-oxopent-4-enoic acid [**3c**] is readily decarboxylated to yield dehydrozingerone [**3d**]. A methyl ester synthesized from *trans*-5-(4-hydroxy-3-methoxyphenyl)-3-oxopent-4-enoic acid, as a reference, was hydrolyzed by 1 M KOH just before LC-ESIMS analysis. The UV (*C*), MS (*E*), and MS/MS (*G*) spectra of the compound derived by hydrolysis of feruloyl-diketide-CoA are shown, together with the UV (*D*), MS (*F*), and MS/MS (*H*) spectra, of the hydrolysis product of the control methyl ester.

Fig. S6. HPLC analysis of the products after co-incubation of DCS and CURS in the presence of feruloyl-CoA [**3a**], *p*-coumaroyl-CoA [**2a**], and malonyl-CoA (*A*) and a similar HPLC analysis of an ethyl acetate extract of the rhizome of turmeric (*B*). Curcumin [**3e**], demethoxycurcumin [**4e**], and bisdemethoxycurcumin [**2e**] were detected in both analyses, although the abundance ratios of curcumin [**3e**] to demethoxycurcumin [**4e**] and bisdemethoxycurcumin [**2e**] were different.

Supplementary Table 1. Primers used for quantitative real time PCR

Gene	Primer sequence (5’ to 3’)
DCS (forward)	CAACAGCACGCCCCAGTCGA
DCS (reverse)	GTGCTGTTCATCCTGGACGAG
CURS (forward)	TCAGCTCATCCATCACGAAGTACAC
CURS (reverse)	CATCATTGACGCCATCGAAGC
18S rRNA (forward)	CCTTCCTCTAAATGATAAGGTTCAATGG
18S rRNA (reverse)	GATTGAATGGTCCGGTGAAGTGTT

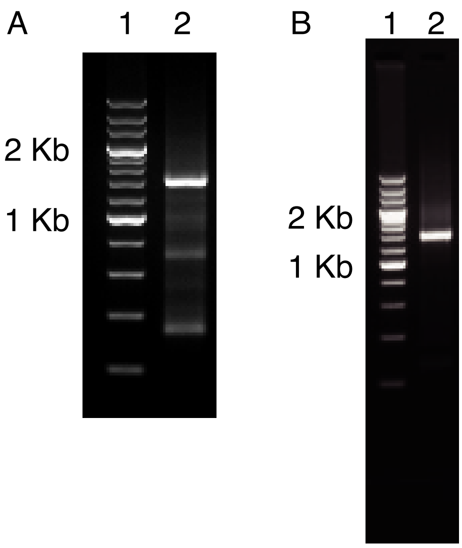

Fig. S1. Katsuyama et al.

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DCS        1 -----------MEANGYRITHS--ADGPATILAIGTANPTNVVDQNAYPDFYFRVTNSEY
CURS       1 ----------MANLHALRREQR--AQGPATIMAIGTATPPNLYEQSTFPDFYFRVTNSDD
MsCHS      1 ----------MVSVSEIRKAQR--AEGPATILAIGTANPANCVEQSTYPDFYFKITNSEH
RhBAS      1 -----------MATEEMKK--------LATVMAIGTANPPNCYYQADFPDFYFRVTNSDH
OsCUS      1 MAPTTTMGSALYPLGEMRRSQR--ADGLAAVLAIGTANPPNCVTQEEIPDFYFRVTNSDH
WtPKS1     1 ----------MASTEGIQAYRNNMAEGPATIMAIGTANPPNVVDASTFPDYYWRVTNSEH

DCS       48 LQ-ELKAKFRRICEKAAIRKRHLYLTEEILRENPSLLAPMAPSFDARQAIVVEAVPKLAK
CURS      49 KQ-ELKKKFRRMCEKTMVKKRYLHLTEEILKERPKLCSYKEASFDDRQDIVVEEIPRLAK
MsCHS     49 KT-ELKEKFQRMCDKSMIKRRYMYLTEEILKENPNVCEYMAPSLDARQDMVVVEVPRLGK
RhBAS     42 LI-NLKQKFKRLCENSRIEKRYLHVTEEILKENPNIAAYEATSLNVRHKMQVKGVAELGK
OsCUS     59 LT-ALKDKFKRICQEMGVQRRYLHHTEEMLSAHPEFVDRDAPSLDARLDIAADAVPELAA
WtPKS1    51 LSPEYRVKLKRICERSSIRKRHLVLTEQLLKENPTLTTYVDASYDERQSIVLDAVPKLAC

DCS      107 EAAEKAIKEWGRPKSDITHLVFCSASGIDMPGSDLQLLKLLGLPPSVNRVMLYNVGCHAG
CURS     108 EAAEKAIKEWGRPKSEITHLVFCSISGIDMPGADYRLATLLGLPLTVNRLMIYSQACHMG
MsCHS    108 EAAVKAIKEWGQPKSKITHLIVCTTSGVDMPGADYQLTKLLGLRPYVKRYMMYQQGCFAG
RhBAS    101 EAALKAIKEWGQPKSKITHLIVCCLAGVDMPGADYQLTKLLDLDPSVKRFMFYHLGCYAG
OsCUS    118 EAAKKAIAEWGRPAADITHLVVTTNSGAHVPGVDFRLVPLLGLRPSVRRTMLHLNGCFAG
WtPKS1   111 EAAAKAIKEWGRPKTDITHMVVCTGAGVDVPGVDYKMMNLLGLPPTVNRVMLYNVGCHAS

DCS      167 GTALRVAKDLAENNRGARVLAVCSEVTVLSYRGPHPAHIESLFVQALFGDGAAALVVGSD
CURS     168 AAMLRIAKDLAENNRGARVLVVACEITVLSFRGPNEGDFEALAGQAGFGDGAGAVVVGAD
MsCHS    168 GTVLRLAKDLAENNKGARVLVVCSEVTAVTFRGPSDTHLDSLVGQALFGDGAAALIVGSD
RhBAS    161 GTVLRLAKDIAENNKGARVLIVCSEMTTTCFRGPSETHLDSMIGQAILGDGAAAVIVGAD
OsCUS    178 CAALRLAKDLAENSRGARVLVVAAELTLMYFTGPDEGCFRTLLVQGLFGDGAAAVIVGAD
WtPKS1   171 GTVLRIAKDLAENNKGARVLVVSSEVSVMFFRGPAEGDVEILLGQALFGDGSAAIIVGAD

DCS      227 PVDGVERPIFEIASASQVMLPESAEAVGGHLREIGLTFHLKSQLPSIIASNIEQSLTTAC
CURS     228 PLEGIEKPIYEIAAAMQETVAESQGAVGGHLRAFGWTFYFLNQLPAIIADNLGRSLERAL
MsCHS    228 PVPEIEKPIFEMVWTAQTIAPDSEGAIDGHLREAGLTFHLLKDVPGIVSKNITKALVEAF
RhBAS    221 PDLTVERPIFELVSTAQTIVPESHGAIEGHLLESGLSFHLYKTVPTLISNNIKTCLSDAF
OsCUS    238 -ADDVERPLFEIVSAAQTIIPESDHALNMRFTERRLDGVLGRQVPGLIGDNVERCLLDMF
WtPKS1   231 PIEGVEKPIFQIFSASQMTLPEGEHLVAGHLRELGLTFHLKPQLPNTVSSNIHKPLKKAF

DCS      287 SPLGLSD----WNQLFWAVHPGGRAILDQVEARLGLEKDRLAATRHVLSEYGNMQSATVL
CURS     288 APLGVRE----WNDVFWVAHPGNWAIIDAIEAKLQLSPDKLSTARHVFTEYGNMQSATVY
MsCHS    288 EPLGISD----YNSIFWIAHPGGPAILDQVEQKLALKPEKMNATREVLSEYGNMSSACVL
RhBAS    281 TPLNISD----WNSLFWIAHPGGPAILDQVTAKVGLEKEKLKVTRQVLKDYGNMSSATVF
OsCUS    297 GPLLGGDGGGWNDLFWAVHPGSSTIMDQVDAALGLEPGKLAASRRVLSDYGNMSGATVI
WtPKS1   291 EPLNITD----WNSIFWIVHPGGRAILDQVQEKIGLEENKLDVSRYVLAENGNMMSASVF

DCS      343 FILDEMRNRSAAEGHATTGEGLDWGVLLGFGPGLSIETVVLHSCRLN-
CURS     344 FVMDELRKRSAVEGRSTTGDGLQWGVLLGFGPGLSIETVVLRSMPL--
MsCHS    344 FILDEMRKKSTQNGLKTTGEGLEWGVLFGFGPGLTIETVVLRSVAI--
RhBAS    337 FIMDEMRKKSLENGQATTGEGLEWGVLFGFGPGITVETVVLRSVPVIS
OsCUS    357 FALDELRRQRKE--AAAAGEWPELGVMMAFGPGMTVDAMLLHATSHVN
WtPKS1   347 FIMDEMRKRSAAQGCSTTGEGHEWGVLFGFGPGLSIETVVLHSVPLSI
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Fig. S2. Katsuyama et al.

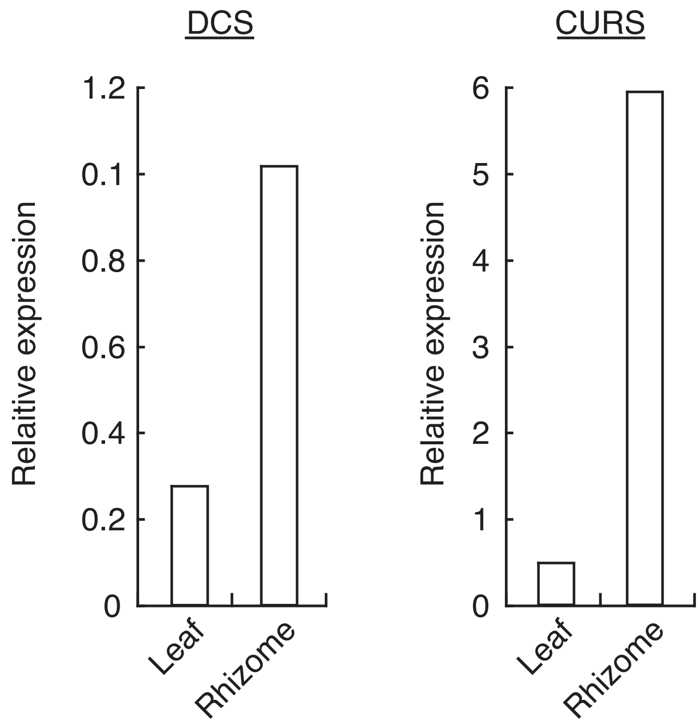

Fig. S3. Katsuyama et al.

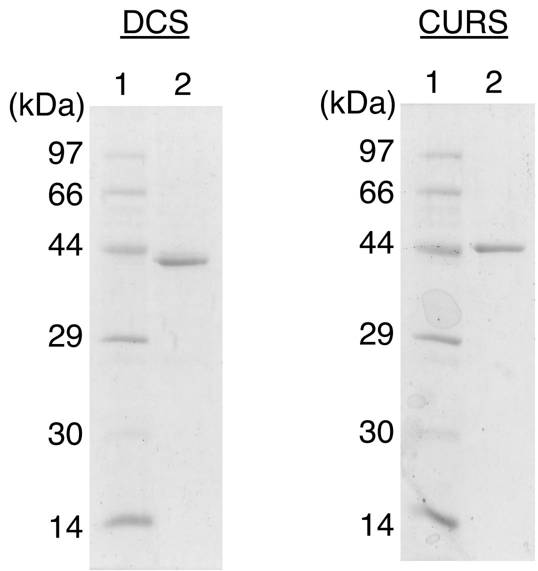

Fig. S4 Katsuyama et al.

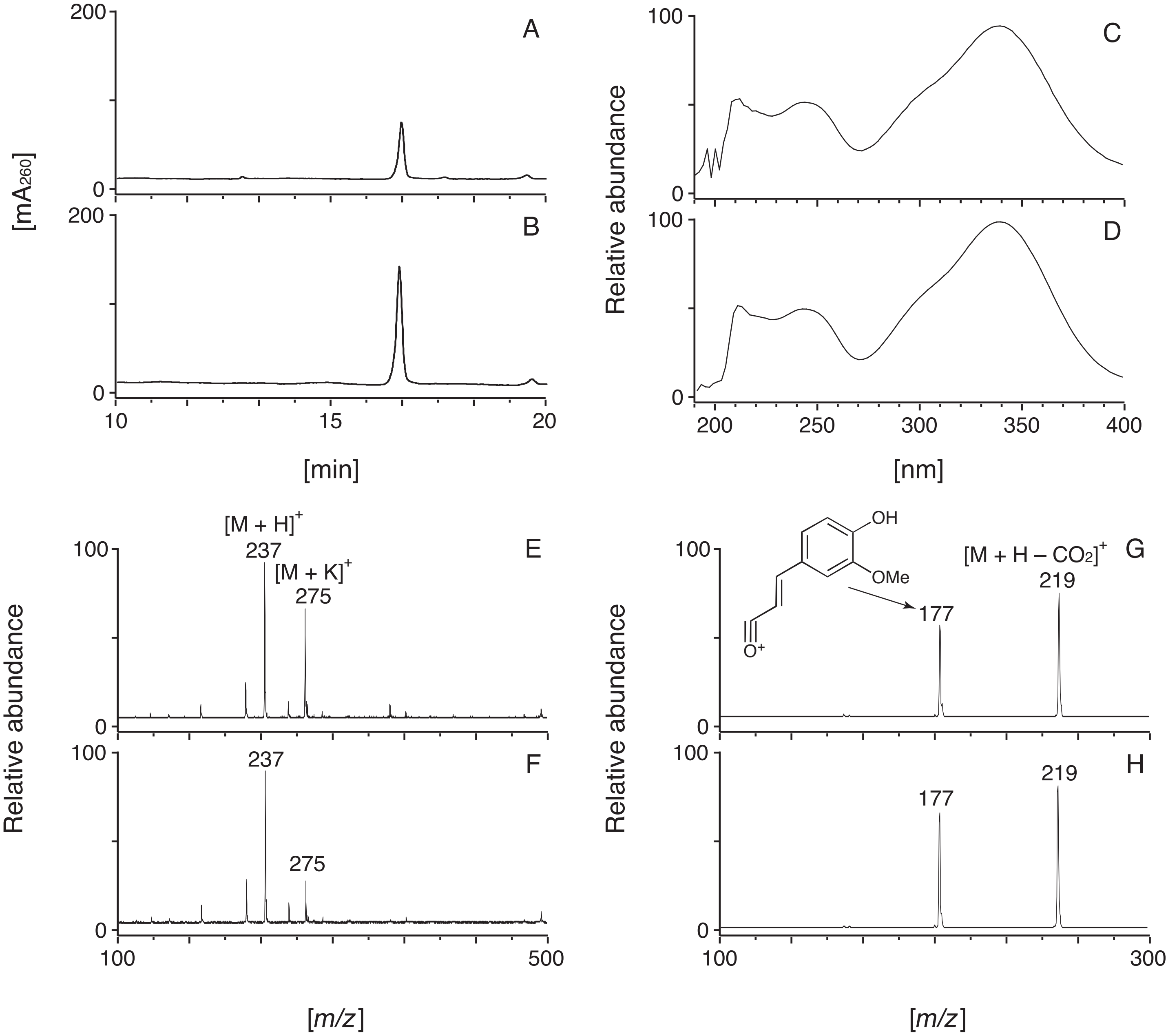

Fig. S5. Katsuyama et al.

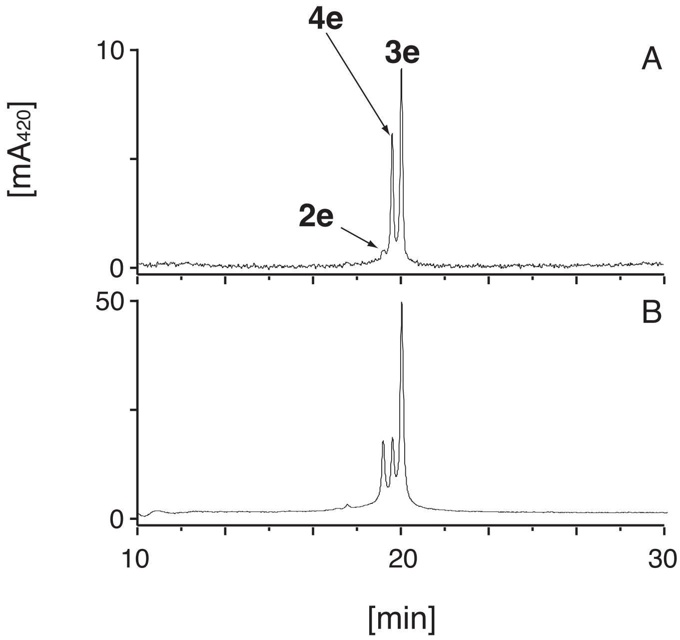

Fig. S6. Katsuyama et al.