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Molecular cloning and functional characterization of an *O*-methyltransferase catalyzing 4′-*O*-methylation of resveratrol in *Acorus calamus*

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Resveratrol and its methyl ethers, which belong to a class of natural polyphenol stilbenes, play important roles as biologically active compounds in plant defense as well as in human health. Although the biosynthetic pathway of resveratrol has been fully elucidated, the characterization of resveratrol-specific *O*-methyltransferases remains elusive. In this study, we used RNA-seq analysis to identify a putative aromatic *O*-methyltransferase gene, *AcOMT1*, in *Acorus calamus*. Recombinant AcOMT1 expressed in *Escherichia coli* showed high 4′-*O*-methylation activity toward resveratrol and its derivative, isorhapontigenin. We purified a reaction product enzymatically formed from resveratrol by AcOMT1 and confirmed it as 4′-*O*-methylresveratrol (deoxyrhapontigenin). Resveratrol and isorhapontigenin were the most preferred substrates with apparent K_m values of 1.8 μM and 4.2 μM, respectively. Recombinant AcOMT1 exhibited reduced activity toward other resveratrol derivatives, piceatannol, oxyresveratrol, and pinostilbene. In contrast, recombinant AcOMT1 exhibited no activity toward pterostilbene or pinosylvin. These results indicate that AcOMT1 showed high 4′-*O*-methylation activity toward stilbenes with non-methylated phloroglucinol rings.

[**Key words:** Stilbenes; *O*-Methyltransferase; Resveratrol; *Acorus calamus*; *S*-Adenosyl-L-methionine]

Stilbenes are a small class of phenolic compounds that have a C_6-C_2-C_6 core skeleton. The best-studied stilbene is resveratrol (3,5,4′-trihydroxystilbene), which is produced by plants and is well-known for its potential health benefits in humans. Resveratrol is thought to have the ability to prevent oxidative DNA damage by reactive oxygen species and could aid in the prevention of cardiovascular disease (1,2). In plants, resveratrol has various roles in stress responses to both biotic and abiotic stresses. Irradiation of plant tissues with UV light induces resveratrol biosynthesis, suggesting that these compounds are involved in the protection of plant cells from harmful UV radiation (3). In addition, resveratrol can act as an antioxidant and is also involved in plant defense, acting as a repellent against both herbivore attack and pathogenic infection (4). Resveratrol derivatives have been detected in many plant species including mulberry (*Morus alba*), grape (*Vitis vinifera*), peanut (*Arachis hypogaea* L.), sorghum (*Sorghum bicolor*), Scots pine (*Pinus sylvestris* L.), and various medicinal plants including *Acorus* (*Acorus gramineus*) (5).

Stilbenes including resveratrol and its derivatives are biosynthesized via the phenylpropanoid pathway starting from L-phenylalanine, sharing the biosynthetic pathways of both lignin and flavonoids (Fig. 1). In this pathway, phenylalanine is converted into *p*-coumaric acid by phenylalanine ammonia lyase (PAL) and cinnamic acid 4-hydroxylase (C4H). *p*-Coumaric acid is then activated to 4-coumaroyl-CoA using the 4-coumaroyl-CoA ligase (4CL). This 4-coumaroyl-CoA is condensed with three molecules of malonyl-CoA by stilbene synthase (STS), resulting in the formation of the resveratrol skeleton.

In several plants, resveratrol, which has three hydroxyl groups in two aromatic rings, is then methylated by *S*-adenosyl-L-methionine (SAM) dependent *O*-methyltransferases (OMTs). *O*-Methylations of resveratrol are considered to play an important role in facilitating its high bioactivity because the substitution of hydroxyl with methoxy groups increases the lipophilicity of resveratrol. In fact, analysis of relationships between chemical structure and bioactivity revealed that methylated resveratrols show increased cytotoxic and antitumor activities when compared to the non-methylated resveratrols (1,6). Thus, methylated resveratrols have become attractive target compounds for both bioproduction and metabolic engineering (7,8).

To date, many plant OMTs have been reported, and most of them have been found to be involved in the methylation of aromatic hydroxyl groups in compounds such as flavonoids (9), phenylpropanoids (10,11), and benzylisoquinoline alkaloids (12,13). However, only a few stilbene OMTs have been characterized. Grapevine (*V. vinifera*) resveratrol OMT (VvROMT) was shown to catalyze the transfer of methyl groups from SAM to hydroxyl group(s) at the C-3 or C-5 positions of resveratrol or pinostilbene (3,4′-dihydroxy-5-methoxystilbene) (14). Similarly, Scots pine (*P. sylvestris*) pinosylvin OMT (PsPMT2) has been revealed to mediate 3-*O*-methylation of substrate stilbenes (3,15). Additionally, *S. bicolor* OMT (SbOMT1) has been shown to catalyze the 4′-*O*-methylation of resveratrol (7,16). In *A. gramineus*, accumulation of resveratrol derivatives has been previously reported (5). Therefore,

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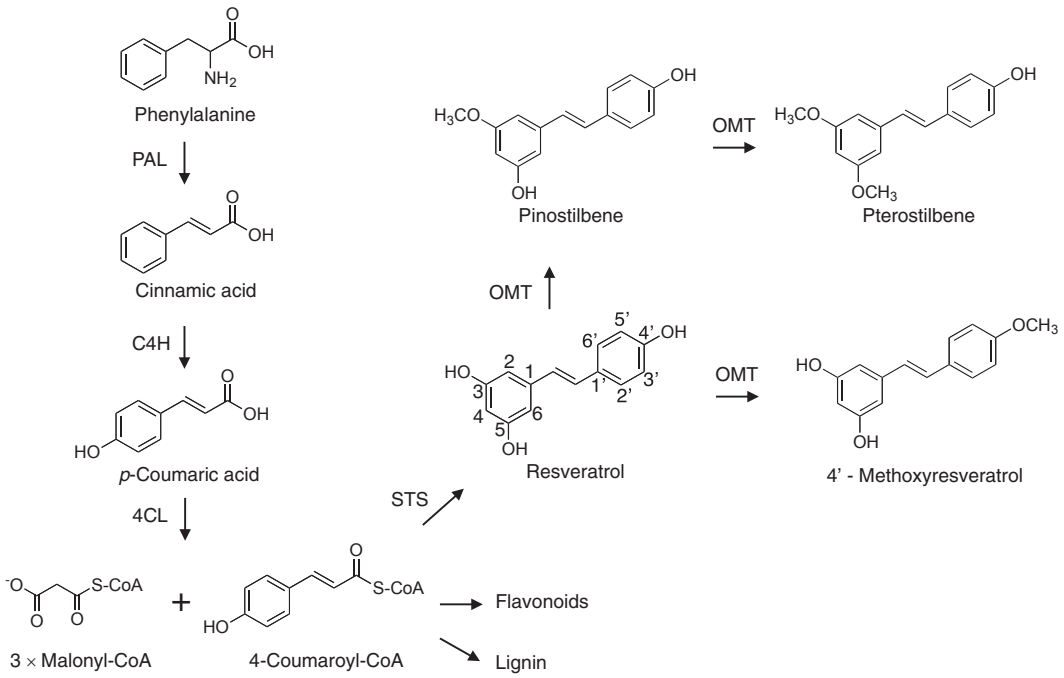

FIG. 1. Phenylpropanoid pathway leading to resveratrol derivatives.

it is considered that *Acorus calamus*, which belongs to the same genus as *A. gramineus*, contains resveratrol derivatives and would be a good plant resource to discover the biosynthetic enzymes that are involved in resveratrol formation. Given that methylated resveratrols have potential uses as dietary supplements with possible pharmacological applications (17–19), identifying additional resveratrol specific OMTs from plants is beneficial for the future construction of synthetic biological systems to produce methylated resveratrols.

Here, to identify candidate OMT sequences, we performed RNA-sequencing from the leaves of *A. calamus* and identified a resveratrol-specific OMT, AcOMT1. AcOMT1 is responsible for catalyzing the 4′-*O*-methylation of resveratrol and the formation of its 3′-methoxylated derivative, isorhapontigenin, and is highly specific for substrates that contain hydroxyl groups at the C4′ and C3 or C5 positions of the stilbene core skeleton.

MATERIALS AND METHODS

Plant materials and chemicals *A. calamus* specimens were purchased from a nursery (Tojaku Engei Co. Ltd., Kyoto, Japan) and grown at 25°C before their use in this study. Anol and chavicol were obtained from our laboratory's stock (20). All other chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA), Wako Pure Chemical Industries (Osaka, Japan), Tokyo Chemical Industry Co, Ltd. (Tokyo, Japan), or Nacalai Tesque Inc. (Kyoto, Japan), unless otherwise specified.

RNA-sequencing analysis Total RNA was extracted from the leaves as described previously (21). The RNA quality was evaluated using the BioAnalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). An aliquot (10 μg) of the total RNA was used to construct a cDNA library using the Illumina TruSeq Prep Kit v2 (Illumina, San Diego, CA, USA). The resulting cDNA library was sequenced using the HiSeq 1000 system (Illumina) with 100 bp paired-end reads. Reads were assembled using CLC Genomics Workbench version 5.5.2 (CLC Bio, Aarhus, Denmark) with a minimum contig length of 300 bp after adaptor sequences and low-quality reads were removed. For each sample, the reads were aligned to obtain reads per kilobase of exon per million mapped reads (RPKM).

To identify putative *A. calamus* OMT genes, a BLAST search with the tblastn program was performed using the protein sequence of ROMT (accession number FM178870) from *V. vinifera* as a protein query in the *A. calamus* database. Full-length sequences showing over 45% identity at amino acid levels with the query were used for further analysis.

RT-PCR analysis of AcOMT1 expression Total RNA was extracted from *A. calamus* leaves, rhizomes, and roots as described above and semi-quantitative RT-PCR was carried out for AcOMT1 in 15 μL reactions, using KOD FX Neo polymerase (Toyobo Co., Ltd., Osaka, Japan), according to the manufacturer's instructions. The PCR was performed under the following conditions: 94°C for 2 min; 25–33 cycles at 98°C for 10 s, 57°C for 30 s, and 68°C for 15 s; followed by 68°C for 5 min. The *A. calamus* tubulin gene (*AcTUB*) was also amplified to verify that approximately equal amounts of cDNA were used for each reaction.

Heterologous expression of AcOMT1 in *E. coli* The DNA fragments corresponding to the coding region of *AcOMT1* gene were amplified from the 1st strand cDNA prepared from *A. calamus* leaves with the gene-specific primers (Table S1) using KOD FX Neo polymerase (Toyobo Co., Ltd.) under the following conditions: 94°C for 2 min; 35 cycles at 98°C for 10 s, 56°C for 30 s, and 68°C for 1 min; followed by 68°C for 5 min. The amplified fragments were cloned into a pENTR/D-TOPO vector (ThermoFisher Scientific, Waltham, MA, USA). The resulting vector was then subcloned into the expression vector pHis9-GW by LR-reaction using LR clonase 2 (ThermoFisher Scientific) to produce a recombinant protein with a nine His-tag at the N-terminal (22). Finally, the plasmid was transformed into *E. coli* strain Rosetta 2(DE3) pLysS (Merck Millipore, Billerica, MA, USA). The recombinant AcOMT1 was expressed under the addition of isopropyl β-D-1-thiogalactopyranoside (0.5 mM) for 24 h at 16°C. The cells were collected and then resuspended with buffer A [50 mM Tris–HCl (pH 7.5) and 14.3 mM β-mercaptoethanol] containing 0.1 mM phenylmethylsulfonyl fluoride and 50 mg/L lysozyme. Following disruption of the cells by sonication for 30 × 3 s (Tomy Ultrasonic Disruptor UD-201, Tomy Seiko Co., Ltd., Tokyo, Japan), the soluble solution was fractionated by step-wise ammonium sulfate precipitation. Ammonium sulfate was added to 35% saturation and the solution was stirred at 4°C for 1 h. The insoluble materials were then removed by centrifugation at 10,000 × *g* for 10 min at 4°C. The resulting supernatant was stirred at 4°C for 1 h after addition of ammonium sulfate at 40% saturation and centrifuged at 10,000 × *g* for 10 min at 4°C. The un-precipitated fraction containing AcOMT1 activity was stirred in the presence of ammonium sulfate at 42.5% saturation at 4°C for 1 h. The precipitated AcOMT1 was dissolved in buffer A and then loaded onto a desalting column PD-10 (GE Healthcare UK Ltd., Buckinghamshire, UK).

Biochemical characterization of AcOMT1 activity To investigate the activity of AcOMT1 *in vitro*, the heterologously expressed and purified proteins were assayed against several putative substrates including resveratrol as follows: a reaction mixture (50 μL) was prepared with 0.5 mM substrate (dissolved in 1% methanol), 1.7 μM [Me-^{14}C]-SAM (58.0 mCi mmol^{-1}; Perkin–Elmer Co., Ltd, Yokohama, Japan), and purified enzymes (1 μg) in the reaction buffer [50 mM Tris–HCl (pH 7.0)]. The enzymatic reaction was initiated by adding a substrate, and the mixture was incubated at 25°C for 15 min. The reaction was terminated by the addition of 0.2 mL ethyl acetate. After centrifuging the samples for 1 min at 12,000 × *g*, the radioactivity of the organic phase was measured using a scintillation counter (AccuFLEX LSC-7200: Hitach Aloka Medical, Ltd., Tokyo, Japan), as previously described (10).

To investigate the effect of pH on enzymatic activity, 50 mM MES-KOH buffer (pH 5.0–6.5), 50 mM Na-phosphate buffer (pH 6.0–7.5), and 50 mM Tris–HCl buffer (pH 7.0–9.0) were used as the buffers and resveratrol was used as the substrate. Substrate specificity of AcOMT1 was calculated from the enzymatic activity using resveratrol or related phenolic compounds as a substrate. Three independent assays were conducted for each substrate, and the average activity was calculated. To identify the reaction products of AcOMT1, purified proteins were combined with 250 μM resveratrol, 500 μM unlabeled SAM, and 50 mM Tris–HCl (pH 7.0) in 5.0 mL reactions at 30°C for 5 h. The reaction products were extracted with ethyl acetate (1.6 mL), concentrated under N_2 gas, and analyzed using a GC-MS (QP-5050, Shimadzu Co., Kyoto, Japan) equipped with a DB-5MS column (30 m length × 0.25 mm diameter × 0.25 μm film thickness; Agilent Technologies, Santa Clara, CA, USA). The column temperature was programmed as follows: 80°C for 2 min, increasing by 10°C min^{-1} to 250°C, and held at 250°C for 10 min. Helium was used as the carrier gas at a pressure of 79.5 kPa. The GC-MS was operated in the electron ionization mode with an ionization energy of 70 eV, and the temperatures of the ion source and interface were 250°C with a continuous scan of *m/z* 60–600.

^{1}H NMR analysis of methylated resveratrol To identify the reaction products of AcOMT1 by ^{1}H NMR, crude *E. coli* lysate including AcOMT1 proteins was incubated with 250 μM resveratrol, 312 μM unlabeled SAM, and 50 mM Tris–HCl (pH 7.0) in 320 mL reactions at 30°C for 24 h. The reaction products were extracted with ethyl acetate (40 mL × 3), concentrated under N_2 gas, and fractionated by thin-layer chromatography (TLC) [TLC Silica gel 50 F_{256} (20 cm × 20 cm; Merck Millipore)]. The TLC plates were developed with a solvent of ethyl acetate/hexane (1/1, v/v). Then, the reaction products formed by AcOMT1 were recovered by scratching the surface of the TLC plate under UV light and subsequently extracting with ethyl acetate. This preparative TLC purification was repeated several times to obtain adequate amounts of the reaction products. The obtained products were combined, filtered through a DISMIC-03JP filter (Advantec Toyo, Tokyo, Japan) with 0.5 μm pore size, and evaporated. The evaporated residues were subjected to ^{1}H NMR analysis (JNM-LA400MK FT-NMR system; JEOL, Tokyo, Japan), and the signals are consistent with 4′-*O*-methylresveratrol (deoxyrhapontigenin) (23): δ_H (acetone-d_6) 3.80 (3H, s, methyl-H), 6.27–6.28 (1H, br d, *J* 4.0, ArH), 6.55–6.56 (2H, br d, *J* 4.0, ArH), 6.91–6.93 (2H, br d, *J* 8.0, ArH), 6.94 (1H, d, *J* 16, 8-H), 7.04 (1H, d, *J* 16, 7-H), and 7.49–7.51 (2H, br d, *J* 8.0, ArH), where chemical shifts and coupling constants (*J*) are given in δ and Hz, respectively.

Phylogenetic analysis of predicted proteins The deduced amino acid sequences of AcOMT1 and other related OMTs from different plant species were aligned using MAFFT v7.220 (24). MEGA6 software was used to construct the phylogenetic tree using a neighbor-joining method with 1000 bootstrap replicates (25).

The amino acid sequences of functionally characterized OMTs from other organisms are as follows: *Picea abies* caffeoyl-CoA OMT (CCoAOMT, CAK18782); *Ocimum basilicum* eugenol OMT (EOMT, AF435008); *O. basilicum* chavicol OMT (CVOMT, AF435007); *V. vinifera* ROMT (FM178870); *Rosa hybrida* orcinol OMT (OOMT1, AF502433); *R. hybrida* OOMT2 (AF502434); *Pisum sativum* (+)-6a-hydroxymaackiain 3-OMT (HM3OMT, AAC49856); *Glycyrrhiza echinata* 2-hydroxyisoflavanone 4′-OMT (HI4′OMT, AB091684); *Lotus japonicus* HI4′OMT (Q84KK4); *Pimpinella anisum* anol/isoeugenol OMT (AIMT, EU925389); *Oryza sativa* naringenin 7-OMT (NOMT, Q0IP69); *Clarkia breweri* (iso)eugenol OMT (IEMT1, U86760); *C. breweri* caffeic acid OMT (COMT, AF006009); *Malus domestica* COMT1 (DQ886018); *M. domestica* OMT1 (AB091684); *Rosa chinensis* OMT2 (AB086104); *Papaver somniferum* norcoclaurine 6-OMT (N6OMT, Q6WUC1); *Eschscholzia californica* N6OMT (BAM37634); *Coptis japonica* N6OMT (Q9LEL6); *Thalictrum flavum* subsp. *glaucum* N6OMT (AY610507); *P. sylvestris* pinosylvin OMT (PMT2, KX545296); *Medicago sativa* isoflavone 7-OMT (I7OMT, AAC49928); and *S. bicolor* SbOMT1 (EF189707).

RESULTS

Sequence and phylogenetic analysis of AcOMT1 We performed BLAST analysis of the RNA-seq database from *A. calamus* leaves using the amino acid sequence of a characterized resveratrol OMT as query. The RNA-seq analysis revealed a full-length OMT-like sequence, which was designated AcOMT1 (DDBJ accession number LC387636). The AcOMT1 protein sequence exhibited 50% identity to grapevine (*V. vinifera*) ROMT (accession number FM178870), which is involved in resveratrol methylation. AcOMT1 exhibited several conserved sequence motifs that could possibly function as SAM-binding domains (motifs A, B, C, J, K, and L in Fig. S1) (26,27). The evolutionary relationships to other plant OMTs that catalyze the formation of some specialized metabolites are illustrated in Fig. 2. This OMT family includes genes for caffeic acid, flavonoid, resveratrol, and alkaloid OMTs, as well as a number of genes related to volatile benzenoid/phenylpropanoid OMTs from several plant species. In the phylogenetic analysis presented in Fig. 2, AcOMT1 is closely related to plant class II OMTs, which can catalyze the methylation of phenolic compounds independently of divalent cations, and fall into distinct clades to caffeoyl-CoA 3-*O*-methyltransferase (CCoAOMT) belonging to plant class I OMTs. Together with the fact that the molecular mass of AcOMT1 is about 40 kDa, same as general class II OMTs, this result suggested that the isolated AcOMT1 is classified as a class II OMT.

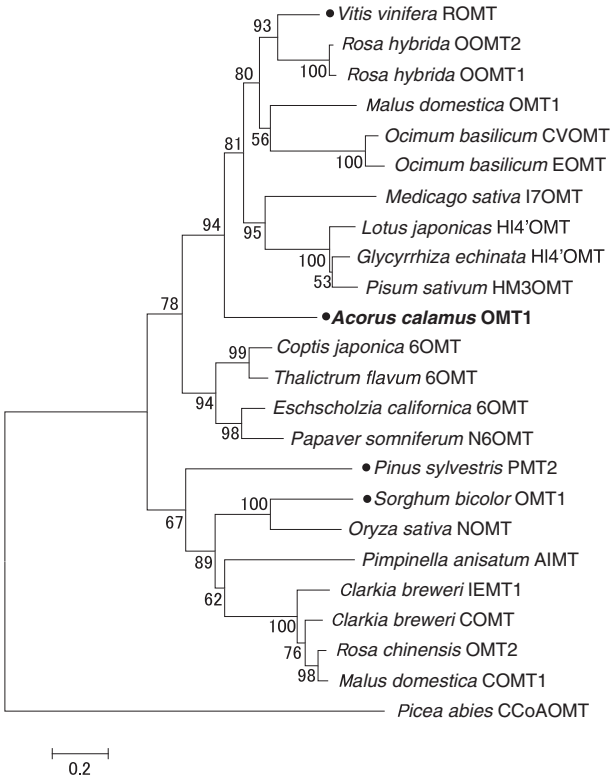

FIG. 2. Phylogenetic relationship of *Acorus calamus O*-methyltransferases (OMTs) with other related OMTs from different plant species. Phylogenetic analysis was performed using the neighbor-joining method in MEGA6 software. The scale bar represents 0.2 amino acid substitutions per site. *A. calamus* OMTs analyzed in this study are shown in bold. The proteins showing resveratrol OMT activities are indicated using closed circles.

Gene expression analysis of *AcOMT1* To examine the transcript expression levels of *AcOMT1* gene, total RNA was isolated from the leaves, rhizomes, and roots of *A. calamus* and then subjected to semi-quantitative RT-PCR analysis (Fig. 3). The fragment of the AcOMT1 cDNA (corresponding to bases 734–1032) was amplified using gene-specific primers (Table S1). *AcOMT1* transcripts primarily accumulated in the roots and leaves.

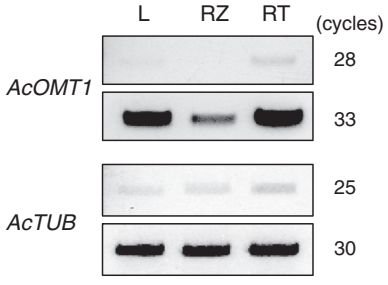

FIG. 3. Tissue-specific expression of *AcOMT1* gene. Relative expression levels of *AcOMT1* gene in the different tissues were examined by semi-quantitative RT-PCR. The different tissues, namely, leaves, rhizomes, and roots are shown as L, RZ, and RT, respectively.

Properties of recombinant AcOMT1 To determine the enzymatic activity of the putative AcOMT1 protein, the coding region of *AcOMT1* cDNA was amplified as described in Materials and methods. After confirming that full-length cDNA encoding *AcOMT1* gene exactly matches to the sequence of *AcOMT1* in the RNA-seq database, the amplified cDNA was subcloned into the *E. coli* expression vector and introduced into *E. coli* Rosetta 2(DE3) pLysS. The AcOMT1 was expressed as an N-terminal nona-His tagged protein, but was not eluted from nickel resin for unknown reasons during the affinity purification. Therefore, the AcOMT1 proteins were partially purified using ammonium sulfate precipitation. SDS-PAGE analysis revealed a protein band of approximately 38 kDa, corresponding to recombinant AcOMT1 (Fig. S2), which was used for an enzyme reaction with [Me-^{14}C]-SAM and a number of potential substrates (Fig. S3). The activity of AcOMT1 was the greatest toward isorhapontigenin, followed by resveratrol (Table 1) and does not show Mg^{2+} ion requirement like plant class I members. The activity of AcOMT1 with piceatannol, pinostilbene, naringenin, and anol was only 9 – 24% of the activity observed toward resveratrol. The enzyme also reacted with oxyresveratrol, isoeugenol, chavicol, eugenol, *p*-coumaric acid, and caffeic acid at lower activities. Pterostilbene, pinosylvin, olivetol, orcinol, or resorcinol did not serve as efficient substrates. Further characterization of AcOMT1 using resveratrol as a methyl acceptor substrate demonstrated that the enzyme was active in the pH range 6.0–7.5, with optimum activity at pH 7.0 in Tris–HCl buffer. AcOMT1 exhibited apparent K_m values of 1.8 ± 0.2 μM for resveratrol and 4.2 ± 0.2 μM for isorhapontigenin.

Verification of methylated products by GC-MS and ^{1}H NMR To identify the reaction products of the AcOMT1 protein with resveratrol in the presence of SAM, methylated resveratrol was analyzed using GC-MS (Fig. 4). The compound with an *m/z* of 242 corresponding to the molecular weight of mono-methylated resveratrol was observed as a new peak after the enzymatic reaction, the retention time of the product of AcOMT1 matched that of 4′-*O*-methylresveratrol, but not that of pinostilbene. To verify the methylated position of the reaction product, we purified it by preparative TLC and performed ^{1}H NMR analysis. ^{1}H NMR analysis supported a structure of 4′-*O*-methylresveratrol.

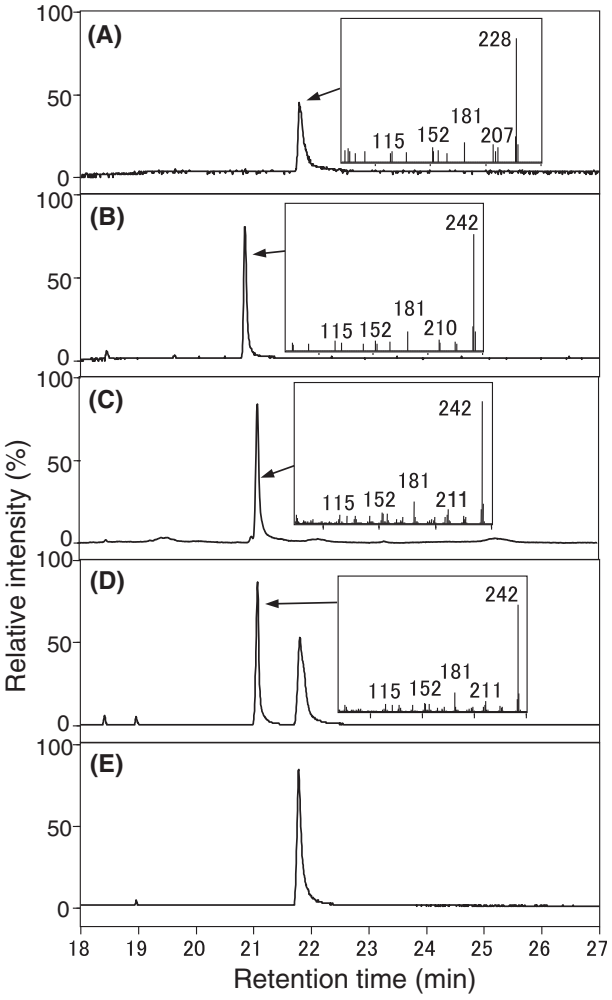

FIG. 4. Conversion of resveratrol to its methyl ethers by the AcOMT1. (A) Standard of resveratrol. (B) Standard of pinostilbene. (C) Standard of 4′-*O*-methylresveratrol. (D) Reaction product by AcOMT1 with *S*-adenosyl-L-methionine (SAM) and resveratrol. (E) Reaction product by heat-inactivated AcOMT1 with SAM and resveratrol.

DISCUSSION

Stilbenes including resveratrol and its derivatives have been the focus of many studies, primarily because of their potential health benefits and pharmacological applications (1,2,28). The distribution of stilbenes in the plant kingdom has been well studied; however, the OMT that biosynthesizes methylated resveratrols is characterized in only a limited number of plant species (3,7,14–16).

TABLE 1. Relative activity of AcOMT1 with various substrates.

Substrates	Relative activity (%)
Resveratrol	100.0
Isorhapontigenin	167.8 ± 9.4[a]
Piceatannol	23.8 ± 1.9
Oxyresveratrol	6.1 ± 1.3
Pinostilbene	9.1 ± 1.6
Pterostilbene	ND[b]
Pinosylvin	ND
Naringenin	18.5 ± 3.1
Anol	21.4 ± 0.3
Isoeugenol	1.4 ± 0.7
Chavicol	1.6 ± 0.4
Eugenol	1.0 ± 0.8
p-Coumaric acid	3.6 ± 1.0
Caffeic acid	1.6 ± 0.6
Olivetol	ND
Orcinol	ND
Resorcinol	ND

[a] Values indicate means ± standard error (SE; n = 3).
[b] ND, not detected.

In this study, we isolated and identified the OMT AcOMT1, which is predominantly expressed in the roots and leaves of *Acorus* plants (Fig. 3), and selectively catalyzes resveratrol conversion into 4′-*O*-methylresveratrol. We have also shown the significant activity of AcOMT1 toward isorhapontigenin and slight activities toward piceatannol, oxyresveratrol, and pinostilbene. Interestingly, AcOMT1 did not catalyze the addition of methyl groups to pinosylvin or pterostilbene (Table 1), suggesting that the presence of hydroxyl groups at both C-4′ and C-5 positions might be an important prerequisite for recognition by AcOMT1. The different positions of hydroxyl groups and the replacing of a hydroxyl group with a methoxy group may hamper methylation. These findings indicate that AcOMT1 might be a stilbene-specific OMT with a stringent substrate specificity. In addition, the fact that AcOMT1 was unable to methylate pinosylvin is consistent with the result of ^{1}H NMR analysis, which supports the formation of 4′-*O*-methylresveratrol.

The product specificity of AcOMT1 is similar to that of SbOMT1, which showed high activity for resveratrol and predominantly catalyzed 4′-*O*-methylation of resveratrol (7,16). However, it is likely that the substrate preferences between these two OMTs are different, as SbOMT1 also exhibited high activities for eugenol and orcinol monomethyl ether. Thus, we consider AcOMT1 to be a novel

class II OMT, one that is primarily involved in the formation of methylated resveratrols.

In the phylogenetic analysis, previously identified OMTs with similar substrate preferences were closely related to a complex clade containing proteins that were biochemically characterized as flavonoid, alkaloid, and caffeic acid OMTs (Fig. 2). For example, the OMTs involved in norcoclaurine biosynthesis from several plant species were grouped into the same clade. Similarly, several OMTs, such as ObEOMT, ObCVOMT, RhOOMTs, and MdOMT1, with volatile benzenoid/phenylpropanoid methylating activity, were closely related to each other. In contrast, the phylogenetic analysis revealed that AcOMT1 was located independently from any other clusters and was not closely related to the three known stilbene-specific OMTs. This suggests that the acquisition of resveratrol methylating proteins occurred independently in different plant lineages during plant evolution. Overall, our results provide new insights into the functional evolution of the plant OMT family. In addition, the identification of AcOMT1 will facilitate metabolic engineering efforts for bioproduction of ring-specific 4′-*O*-methylated resveratrol in plants or in heterologous expression systems.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbiosc.2018.10.011.

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