cDNA Cloning and Expression Analysis of Farnesyl Pyrophosphate Synthase from *Ornithogalum saundersiae*

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Farnesyl pyrophosphate synthase (FPPS, EC 2.5.1.10) catalyzes the consecutive head-to-tail condensations of isopentenyl diphosphate (IPP) with dimethylallyl diphosphate (DMAPP) to form farnesyl pyrophosphate (FPP), a key precursor of sesquiterpenoids, triterpenoids, sterols, and farnesylated proteins. Here we report the molecular cloning and functional identification of a new full-length cDNA encoding FPPS from *Ornithogalum saundersiae*, a potential medicinal plant that produces a promising antitumour sterol glycoside, OSW-1. An 1327 bp long unigene with an open reading frame of 1044 bp was retrieved from the transcriptome sequencing of *O. saundersiae*. The full-length *FPPS* cDNA, designated *OsaFPPS*, was isolated from *O. saundersiae* with gene-specific primers. The resultant *OsaFPPS* encodes a 347-amino acids protein with a calculated molecular mass of 40,085.6 Da, and a theoretical isoelectric point of 5.01. Phylogenetic tree analysis indicated that OsaFPPS belongs to the plant FPPS super-family. Expression of soluble OsaFPPS in *E. coli* was verified by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analysis. Functional analysis of the purified OsaFPPS protein was carried out using IPP and DMAPP as substrates, and the product was unambiguously determined by gas chromatography-mass spectrometry (GC-MS) analyses.

Key words: Farnesyl Pyrophosphate Synthase, *Ornithogalum saundersiae*, Sterols, OSW-1

Introduction

Farnesyl pyrophosphate synthase (FPPS, EC 2.5.1.10), an enzyme of the prenyltransferase family, catalyzes the head-to-tail condensation of dimethylallyl diphosphate (DMAPP) with two molecules of isopentenyl diphosphate (IPP) to form farnesyl pyrophosphate (FPP) (Fig. 1), a common precursor of sesquiterpenoids, triterpenoids, sterols, and farnesylated proteins (Dhar *et al.*, 2013; Jordao *et al.*, 2013; Lan *et al.*, 2013). Importantly, FPP acts as a substrate of squalene synthase which catalyzes the first committed step in the biosynthesis of sterols (Ginzberg *et al.*, 2012; Sun *et al.*, 2012). Therefore, molecular cloning and functional identification of FPPS is important for the characterization of the biosynthetic pathways of a large number of isoprenoids with varied structures and functions.

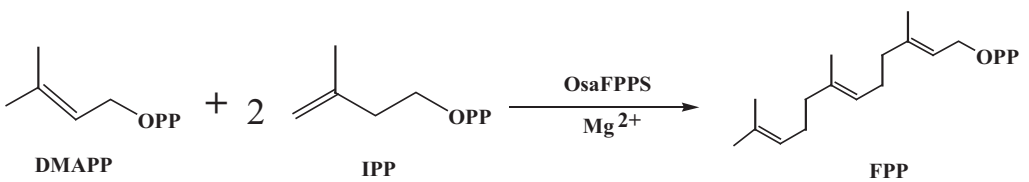

Fig. 1. Reaction catalyzed by FPPS: Condensation of two IPP molecules with DMAPP to give FPP.

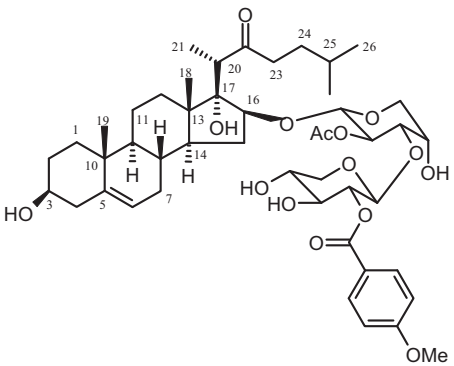

Fig. 2. Chemical structure of OSW-1.

A large number of *FPPS* genes has been cloned and characterized from plants (Xiang *et al.*, 2010; Cao *et al.*, 2012; Lan *et al.*, 2013), animals (Reilly *et al.*, 2002), fungi (Homann *et al.*, 1996; Fischer *et al.*, 2011), and bacteria (Lee *et al.*, 2005). FPPS is also an important regulatory enzyme in the biosynthesis of isoprenoid metabolites (Szkopinska and Plochocka, 2005; Kim *et al.*, 2010), and it has been identified as a molecular target for drug development (Nuttall *et al.*, 2012; Dhar *et al.*, 2013). Moreover, the *FPPS* gene has been used in the metabolic engineering of natural product production (Ro *et al.*, 2006; Kong *et al.*, 2009; Paddon *et al.*, 2013).

Ornithogalum saundersiae Baker (Asparagaceae) is a perennial plant native to Natal, Swaziland, and the eastern Transvaal (Kubo *et al.*, 1992; Mimaki *et al.*, 1997). A systematic survey of this plant's bioactive constituents with medicinal potential led to the isolation of OSW-1 (Fig. 2), a steroidal saponin exhibiting exceptionally potent cytotoxicity, with an IC_{50} value between 0.1 and 0.7 nM, against a variety of tumour cell lines and experimental animal tumours (Kubo *et al.*, 1992; Mimaki *et al.*, 1997). Therefore, OSW-1 is a promising lead compound for the development of novel antitumour drugs (Zhou *et al.*, 2005). Owing to its low content in the plant, on the one hand, and its laborious synthesis, on the other hand, investigations on the potential of OSW-1 as a drug have made little progress since the discovery in 1992 (Kubo *et al.*, 1992). It is necessary to search for an alternative method for the large-scale production of OSW-1. Therefore, a thorough understanding of its biosynthetic pathway and the characterization of the involved enzymes are important for the biological production of OSW-1 in a more economical way, such as metabolic pathway engineering and synthetic biology. OSW-1 is characterized by the attachment of an acylated disaccharide to the C-16 position of the sterol aglycone which is biosynthetically derived from FPP. As a starting point in the understanding of the enzymes of OSW-1 biosynthesis, we therefore decided to clone the *OsaFPPS* cDNA and to characterize OsaFPPS.

Experimental

Substrates, chemicals, and enzymes

The substrates IPP and DMAPP were obtained from Sigma-Aldrich (St. Louis, MO, USA). The In-Fusion® HD cloning kit and restriction enzymes were purchased from Takara Shuzo (Kyoto, Japan). KOD Plus Taq DNA polymerase was purchased from Toyobo (Osaka, Japan). All other fine chemicals were of analytical grade.

Strains and plasmids

The pEASY™-T1 vector was from TransGen (Beijing, China). The *E. coli* strains Trans1-T1 and *Transetta* (DE3) (TransGen) were used as a bacterial host for recombinant plasmid amplification and enzyme expression, respectively. The strains were grown in Luria-Bertani medium (LB) (10 g/L Bacto-tryptone, 5 g/L Bacto-yeast extract, 10 g/L NaCl) supplemented with appropriate antibiotics when required for selection.

The expression vector pET28a(+) was from Novagen (Madison, WI, USA) and was used for heterologous expression.

Plant material

O. saundersiae plants were grown under sterile conditions on 67-V medium (Veliky and Martin, 1970) at 22 °C and a 16-h light/8-h dark cycle. The bulbs of *O. saudersiae* were collected and used fresh or were frozen in liquid N_2 and stored at −80 °C for RNA isolation.

Transcriptome sequencing and analysis

RNA extraction and cDNA library construction were done as described in Kong *et al.* (2014). The resultant cDNA library was sequenced using Illumina HiSeq™ 2000. Short nucleotide reads obtained via Illumina sequencing were assembled by the Trinity software (http://www.trinity-software.com) to produce error-free, unique contiguous sequences (contigs). These contigs were ligated to obtain non-redundant

unigenes, which could not be extended on either end. Unigene sequences were aligned by Blast X to protein databases like NCBI nr, Swiss-Prot, KEGG, and COG (e value < 0.00001), and aligned by Blast N to nucleotide databases nt (e value < 0.00001), retrieving proteins with the highest sequence similarity with the given unigenes, along with their functional annotations. A candidate *FPPS* unigene, unigene 11705, was identified by sequence homology to known *FPPS* genes.

Bioinformatic analyses

The obtained *FPPS* candidate unigene was analysed using online bioinformatics tools from NCBI and ExPASy. Blast X was done at the NCBI server (http://blast.ncbi.nlm.nih.gov/Blast.cgi) using unigene 11705 as the searcher (McGinnis and Madden, 2004; Ye *et al.*, 2006; Johnson *et al.*, 2008). Open reading frame (ORF) finding was performed by the on-line program (http://www.ncbi.nlm.nih.gov/gorf/orfig.cgi) (Wheeler *et al.*, 2003). The amino acid sequence of the resultant ORF was deduced and analysed with the ProtParam tool (http://web.expasy.org/protparam/) (Sato *et al.*, 2011). The protein family of the deduced amino acid sequence was further predicted by Pfam (http://pfam.janelia.org/search) (Punta *et al.*, 2012; Finn *et al.*, 2014). The trans-membrane domains were predicted using TMpred software (http://www.ch.embnet.org/software/TMPRED_form.html) (Moller *et al.*, 2001). The signal peptide was analysed with the Signal P4.1 tool (http://www.cbs.dtu.dk/services/SignalP/) (Petersen *et al.*, 2011). The on-line tool Wolf PSORT (http://wolfpsort.org/) was used for subcellular location. Comparative modeling of the 3D structure of OsaFPPS was performed at ExPASy using SWISS-MODEL (http://swissmodel.expasy.org/).

Generation of the full-length OsaFPPS cDNA

RNA extraction was performed according to the standard protocol of RNeasy Plant Mini Kit (Qiagen, Hilden, Germany), and cDNA was synthesized using the ReverTra Ace Kit (Toyobo) according to the manufacturer's instruction. Amplification of *OsaFPPS* cDNA was performed by a nested polymerase chain reaction (PCR) method. The amplified product was inserted in the pEASY^{TM}-T1 vector to generate pEASY-*OsaFPPS* for sequencing.

The pEASY-*OsaFPPS* was used as a template for sub-cloning the full-length sequence of *OsaFPPS* with the primers F28aFPPS and R28aFPPS (Table I). The PCR product was fused to the linearized pET28a(+) vector digested with *Eco*RI and *Hin*dIII to yield pET28a-*OsaFPPS* by the in-fusion method (Evans *et al.*, 2007, 2009). The resultant plasmid pET28a-*OsaFPPS* was then transformed into the *E. coli* strain *Transetta* (DE3) to obtain the strain *E. coli*[pET28a-*OsaFPPS*] producing OsaFPPS as the His_6-tag fusion protein.

Table I. Primers used in cDNA amplification and plasmid construction.

Primer	Sequence (5′-3′)
Ffpps-1	5′-ccgagctccgatcggccatc-3′
Rfpps-1	5′-cagattggtgcttagtcgttct-3′
Ffpps-2	5′-atggcggattcgaacgggctgg-3′
Rfpps-2	5′-tcacttttgtctcttgtaaatc-3′
F28aFPPS	5′-tcgcggatccgaattcatggcggattcgaacgggctggacacg-3′
R28aFPPS	5′-gtgcggccgcaagctttcacttttgtctcttgtaaatcttgtgcaag-3′

Expression and immunoblot analysis of the recombinant OsaFPPS from E. coli

Production of OsaFPPS was induced by addition of isopropyl-β-D-thiogalactopyranoside (IPTG) at a final concentration of 0.4 mM at 20 °C for 6 h. Polyacrylamide gel electrophoresis (PAGE) was performed on a 12% sodium dodecyl sulfate (SDS) gel. Proteins were separated and transferred onto a polyvinylidine difluoride (PVDF) membrane (150 mA, 2.5 h, 4 °C). The membrane was incubated overnight at 4 °C with an anti-His antibody (Merck, Darmstadt, Germany), then hybridized for 2 h at 4 °C with peroxidase conjugated goat anti-rabbit IgG antibody (Rockland Immunochemicals, Gilbertsville, PA, USA) before being developed by ECL (enhanced chemiluminescence) detection reagents (Amersham, Little Chalfont, Buckinghamshire, UK).

Purification of expressed OsaFPPS from E. coli

The recombinant protein was purified on a HiTrap chelating HP column (GE Healthcare, Piscataway, NJ, USA), which was charged with nickel ions (Ni^{2+}) and eluted with increasing imidazole concentrations from 50 – 250 mM.

Activity assay and biochemical properties of OsaFPPS

The FPPS assay was performed in a final volume of 1 mL containing 200 μg purified OsaFPPS pro-

tein, 50 mM Tris-HCl (pH 7.6), 5 mM $MgCl_2$, 25 mM dithiothreitol (DTT), 50 μM IPP, and 50 μM DMAPP, at 30 °C for 5 h. Then the reaction was stopped by addition of 1 mL 200 mM Tris-HCl (pH 9.5), 10 units of calf intestine alkaline phosphatase, and 10 units of potato apyrase (New England Biolabs, Ipswich, MA, USA) at 30 °C for 1 h. After enzymatic hydrolysis, the resulting product was extracted into 2 mL *n*-hexane. The organic phase was concentrated to 200 μL in a helium stream and used for gas chromatography-mass spectrometry (GC-MS) measurements. GC-MS analysis was performed as previously described (Dhar *et al.*, 2013; Jordao *et al.*, 2013; Lan *et al.*, 2013) under the following conditions: Agilent 6890N-6975C GC-MS system (Santa Clara, CA, USA) equipped with an DB-5ms column (30 m $\times$ 0.25 μm $\times$ 0.25 mm); injected volume, 1 μL; injector temperature, 250 °C; flow rate, 1 mL/min; oven temperature, 80 °C for 2 min, 20 °C/min increase to 280 °C, and 280 °C for 3 min. The total ion and selected ion chromatogram revealed a molecular ion and selected ions of m/z 222, 204, and 93, respectively. As a control, the assay was performed with boiled purified OsaFPPS protein or crude extract from *E. coli* containing the empty vector.

Phylogenetic analysis

Sequences of OsaFPPS and other known FPPS retrieved from GenBank were aligned with CLUSTAL X 2.1. Subsequently, a phylogenetic tree was constructed using the neighbour-joining (NJ) method with MEGA 5.1 software. The reliability of the tree was measured by bootstrap analysis with 1000 replicates.

Results

Search for OsaFPPS by transcriptome analysis

OSW-1 is a cholestane saponin, featuring a novel 3β,16β,17α-trihydroxycholest-5-en-22-one aglycone with an acylated disaccharide attached to the 16-hydroxy group (Fig. 2). Biogenetic analysis revealed that there are at least five kinds of enzymes responsible for OSW-1 biosynthesis, including terpenoid backbone biosynthetic enzymes and sterol pathway enzymes (Kong *et al.*, 2014). A total of more than 40 enzymes are estimated to be involved in the biosynthesis of OSW-1. It will be very time-consuming to isolate and further functionally characterize all of these genes by conventional molecular biology techniques. Thus, it is particularly important to apply a high-throughput method, allowing for drastically faster and cheaper gene discovery, and leading towards a far more comprehensive view of the biosynthetic pathway of OSW-1. The advent of next-generation sequencing approaches, such as transcriptomic analysis, provides a platform which has been proven critical in accelerating the identification of a large number of related genes involved in secondary product synthesis. In the previous study, transcriptomic sequencing of *O. saundersiae* was performed with the aim of discovering genes involved in OSW-1 biosynthesis. A total of 210,733 contigs and 104,180 unigenes were acquired from transcriptome *de novo* assembly (Kong *et al.*, 2014). In the present investigation, these contigs and unigene sequences were used to search against protein databases like ICBN nr, Swiss-Prot, KEGG, and COG (*e* value < 0.00001) by Blast X. One unigene (11705) displaying high similarity with *FPPSs* was retrieved. This unigene was 1327 bp long and contained a full-length *FPPS* cDNA. Therefore, unigene 11705 was selected for further analysis and functional characterization.

Bioinformatic analysis of unigene 11705

Bioinformatic tools are instrumental in gene discovery and annotation. The NCBI on-line Blast X analysis revealed that unigene 11705 containes a sequence encoding a putative FPPS. As predicted by the ORF finder, the unigene containes a full-length *FPPS*, 1044 bp in length, with a 70-bp 5′ non-coding region and a 213-bp 3′ untranslated region. The full-length *FPPS* cDNA, designated *OsaFPPS*, encodes a 347-amino acids (aa) polypeptide with a theoretical molecular mass of 40,085.6 Da and an isoelectric point (pI) of 5.01. The instability index (II) was computed to be 39.51, which classifies the protein as stable.

Blast P in NCBI and multi-alignment analysis according to the CLUSTAL X algorithm indicated that the deduced polypeptide is 81 – 86 % identical to the FPPS of *Musa acuminata*, *Lilium longiflorum*, *Cymbidium goeringii*, *Dendrobium officinale*, *Chimonanthus praecox* (Xiang *et al.*, 2010), *Alisma plantago-aquatica* (Gu *et al.*, 2011), and *Alisma orientale* (Gu *et al.*, 2011) (Fig. 3). A total of five conserved regions were identified, including two aspartate-rich regions (DDXXD, D being Asp and X any amino acid) called FARM (98DDIMD102 in OsaFPPS) and SARM (237DDFLD241 in OsaFPPS) (Koyama *et al.*, 1996; Szkopinska and Plochocka, 2005; Dhar *et al.*, 2013). FARM and SARM motifs are in conserved regions II

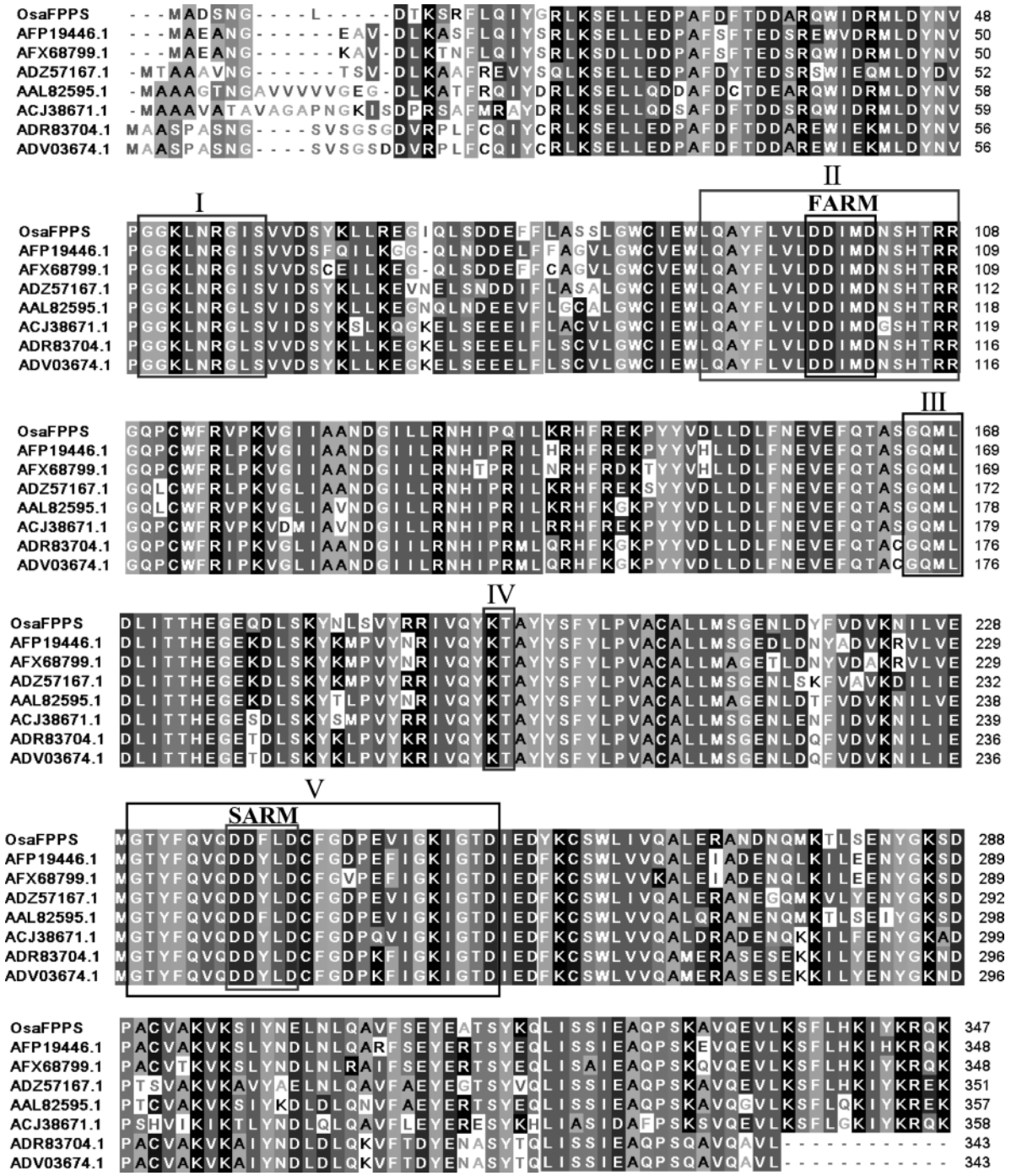

Fig. 3. Alignment of the deduced full-length amino acid sequences of known FPPSs with that of OsaFPPS. GenBank accession numbers are: *Musa acuminata* (AAL82595.1), *Lilium longiflorum* (ADZ57167.1), *Cymbidium goeringii* (AFP19446.1), *Dendrobium officinale* (AFX68799.1), *Chimonanthus praecox* (ACJ38671.1), *Alisma plantago-aquatica* (ADR83704.1), and *Alisma orientale* (ADV03674.1). The five conserved domains of prenyltransferases are boxed and numbered. The highly conserved aspartate-rich motifs [DDXX(XX)D] are present in domains II and V.

and V, respectively. The FARM motif is highly conserved in all known (*E*)-prenyltransferases and has been designated the chain length determination (CLD) region. In case of OsaFPPS, the 4th and 5th residues upstream from the FARM motif are the aromatic amino acids Phe and Tyr, which are supposed to be involved in the product chain length specificity of the enzyme. Moreover, another conserved sequence, designated FQ, was found upstream the SARM motif in region V, which is highly conserved in most FPPSs and is crucial for the enzyme activity. FQ amino acids are located in the substrate binding cavity and are responsible for keeping DMAPP or GPP (geranyl pyrophosphate) in the proper conformation necessary for the condensation with IPP (Koyama *et al.*, 1993; Szkopinska and Plochocka, 2005; Dhar *et al.*, 2013).

InterProscan on-line analysis predicted that the putative OsaFPPS protein has a polyprenyl synthase functional domain (aa 39 – 310), which is in accordance with the terpenoid synthase (aa 2 – 347) domain predicted by SWISS-MODEL, thus confirming that the putative OsaFPPS participates in terpenoid biosynthesis.

No trans-membrane domains and no signal peptide are predicted for the polypeptide, indicating that the polypeptide is a soluble protein that is not excreted. According to the Wolf PSORT algorithm, the putative protein is located in the cytoplasm, in agreement with the predicted absence of trans-membrane domains and a signal peptide.

The secondary structure of OsaFPPS predicted by SWISS-MODEL includes 72.33 % α-helices, 27.09 % random coil, and a minor extended β-strand (0.58 %).

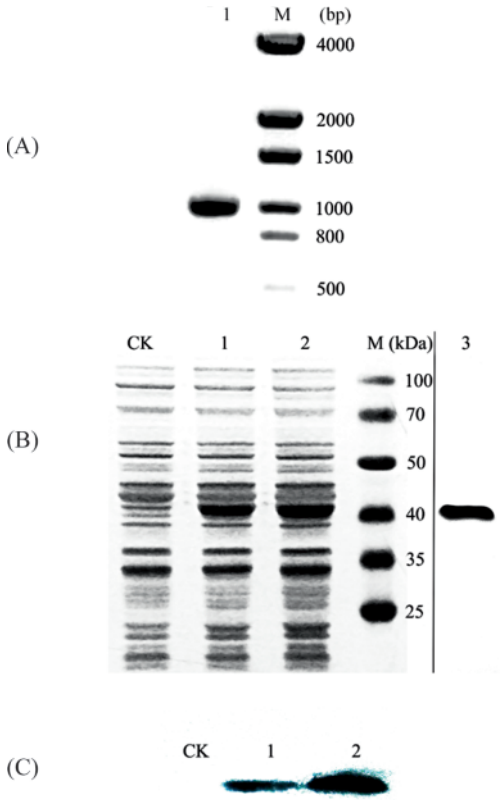

Fig. 4. (A) PCR analysis of the *OsaFPPS* cDNA. Lane 1, product of *OsaFPPS* cDNA amplification; lane M, DNA size marker. (B) SDS-PAGE analysis of proteins extracted from *E. coli*[pET28a-*OsaFPPS*]. Lane CK, uninduced; lanes 1 and 2, induced by IPTG; lane M, protein molecular marker; lane 3, purified His_6-OsaFPPS protein. (C) Western blot analysis of His_6-OsaFPPS protein. Lanes 1 and 2: Western blot of proteins from *E. coli*[pET28a-*OsaFPPS*]; lane CK, *E. coli*[pET-28a].

Cloning and analysis of the full-length cDNA encoding OsaFPPS

The assembled sequences were products of *de novo* assemblies, so they must be considered prone to error. To confirm that the sequence represents a true gene product, experimental verification was performed by designing gene-specific primers (Table I) for the *OsaFPPS* full-length sequence and verifying the identity of the amplified product by sequencing of the PCR products.

A full-length cDNA of 1044 bp was amplified using *O. saundersiae* cDNA as the template by nested PCR (Fig. 4A). Sequencing verified that the cDNA sequence was identical with that resulting from transcriptome sequencing. The *OsaFPPS* sequence was deposited in the GenBank database (accession number KF509889). Then the *OsaFPPS* cDNA was cloned into the *E. coli* vector pET28a(+) resulting in the heterologous plasmid pET28a-*OsaFPPS* by the in-fusion method.

Expression and purification of recombinant OsaFPPS

The recombinant expression vector pET28a-*OsaFPPS* was transformed into *E. coli*, strain *Transetta* (DE3), to obtain *E. coli*[pET28a-*OsaFPPS*] for heterologous expression. SDS-PAGE analysis indicated that the recombinant OsaFPPS polypeptide has a mass of approx. 40 kDa (Fig. 4B, lanes 1 and 2), in accordance with the molecular mass calculated from the amino acid sequence. In the Western blot

analysis, the 40-kDa band reacted positively with the His-Tag monoclonal antibody. No band, however, was observed with the negative control culture containing the pET28a(+) vector without an insert (Fig. 4C).

Following induction of transformed *E. coli* cells by IPTG, the recombinant OsaFPPS protein was purified by immobilized metal affinity chromatography (IMAC). A purified OsaFPPS protein with a mass of approximately 40 kDa by SDS-PAGE was obtained

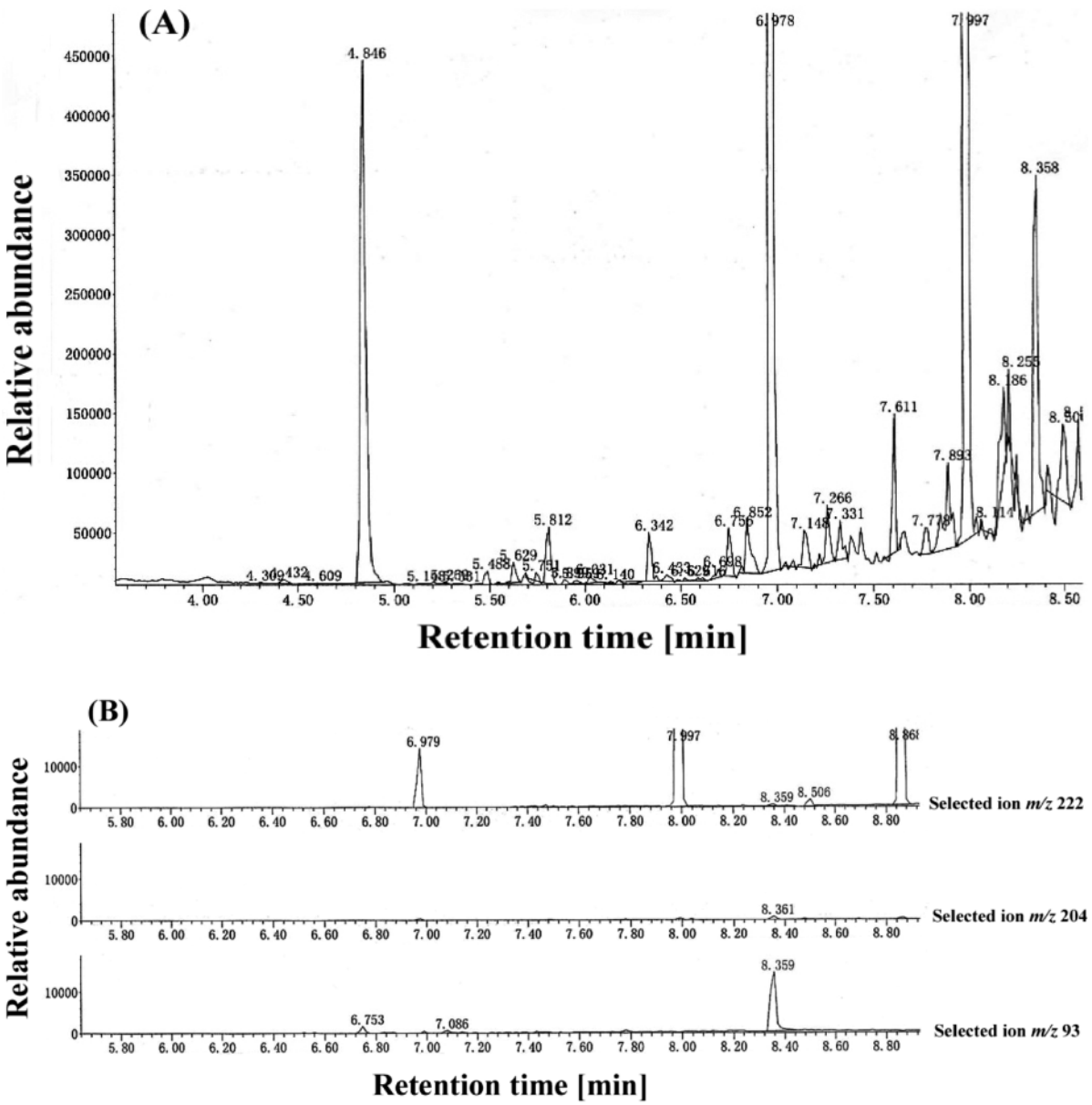

Fig. 5. GC-MS analysis of the product formed by purified recombinant OsaFPPS from IPP and DMAPP. (A) Total ion chromatogram of the dephosphorylated reaction products. (B) Select ion monitoring of the dephosphorylated reaction products. (C) Mass spectrum of the compound with a retention time of 8.359 min (farnesol). (D) Mass spectrum of authentic farnesol from the NIST library.

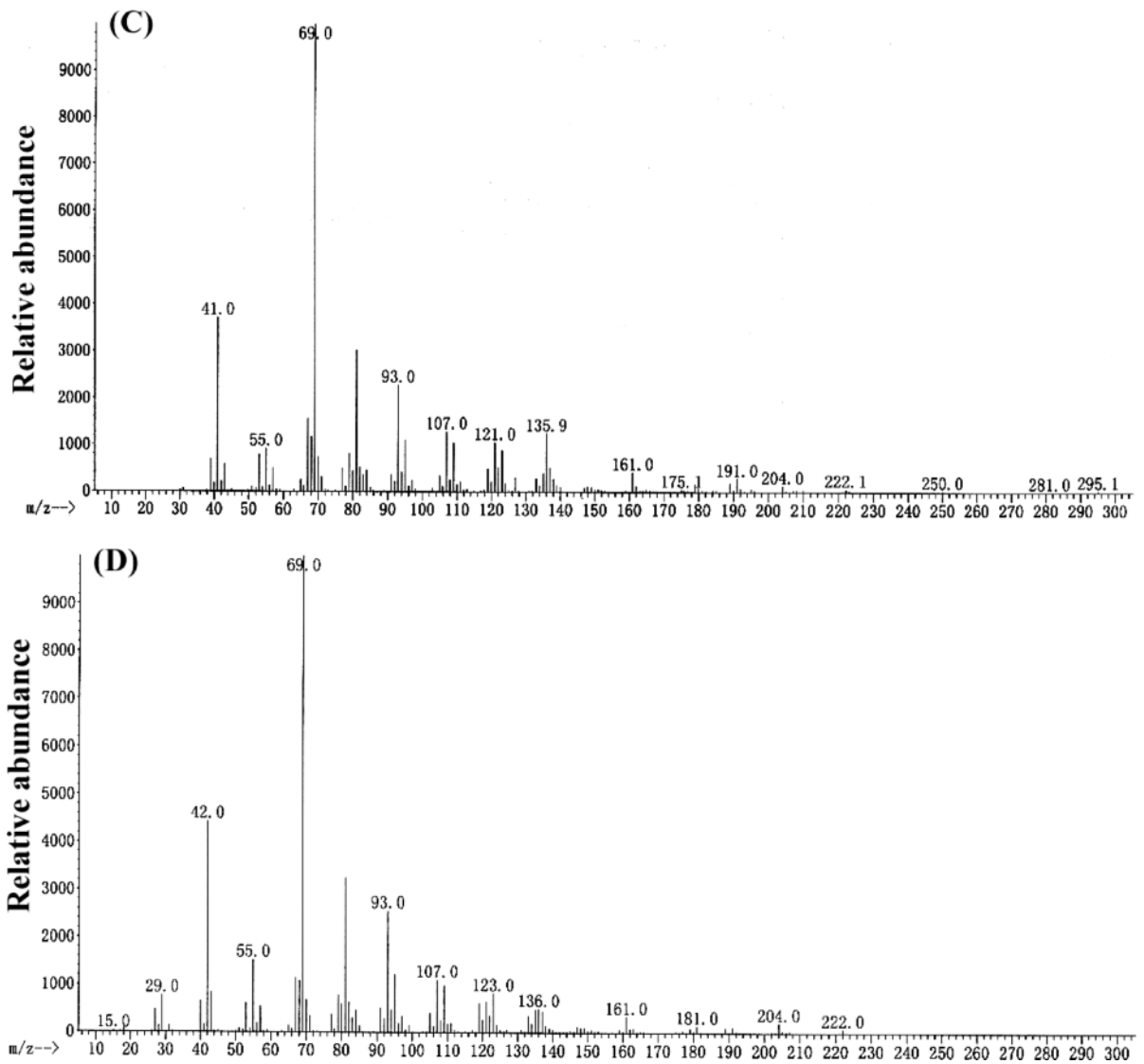

Fig. 5. Continued.

(Fig. 4B, lane 3). The concentrations of the purified proteins were determined as 0.651 mg/mL based on the standard concentration curve of bovine serum albumin (BSA) [$Y = 25.52X - 0.958$ ($R^2 = 0.991$), where Y refers to OD_{595} and X means the concentration of proteins (mg/mL)].

Functional characterization of recombinant OsaFPPS

The purified protein was assayed for FPPS activity. FPP was produced when the substrates DMAPP and IPP were both present in the reaction system containing purified OsaFPPS protein. FPP was determined via farnesol, which is formed from FPP by hydrolysis with apyrase (which cleaves the anhydride bond of FPP) and phosphatase (which cleaves the ester bond of FP). Analysis by GC-MS revealed that farnesol was present in the reaction system (Fig. 5). No farnesol, however, was detected, when extracts of *E. coli* cells with the empty vector were added, or with the boiled control. Thus, the coding region of the *OsaFPPS* cDNA encodes a functional FPPS.

Molecular phylogeny of OsaFPPS

A phylogenetic tree was constructed based on the deduced amino acid sequences of OsaFPPS and other FPPSs from different organisms, including eudicots (Chen *et al.*, 2000; Thabet *et al.*, 2011; Keim *et al.*, 2012), monocots (Sanmiya *et al.*, 1997; Cervantes-Cervantes *et al.*, 2006; Lan *et al.*, 2013), animals (Reilly *et al.*, 2002), and bacteria (Lee *et al.*, 2005) to investigate the evolutionary relationship of these homologous proteins. As shown in Fig. 6, the phylogenetic tree was grouped into five major clades, *viz.* eudi-

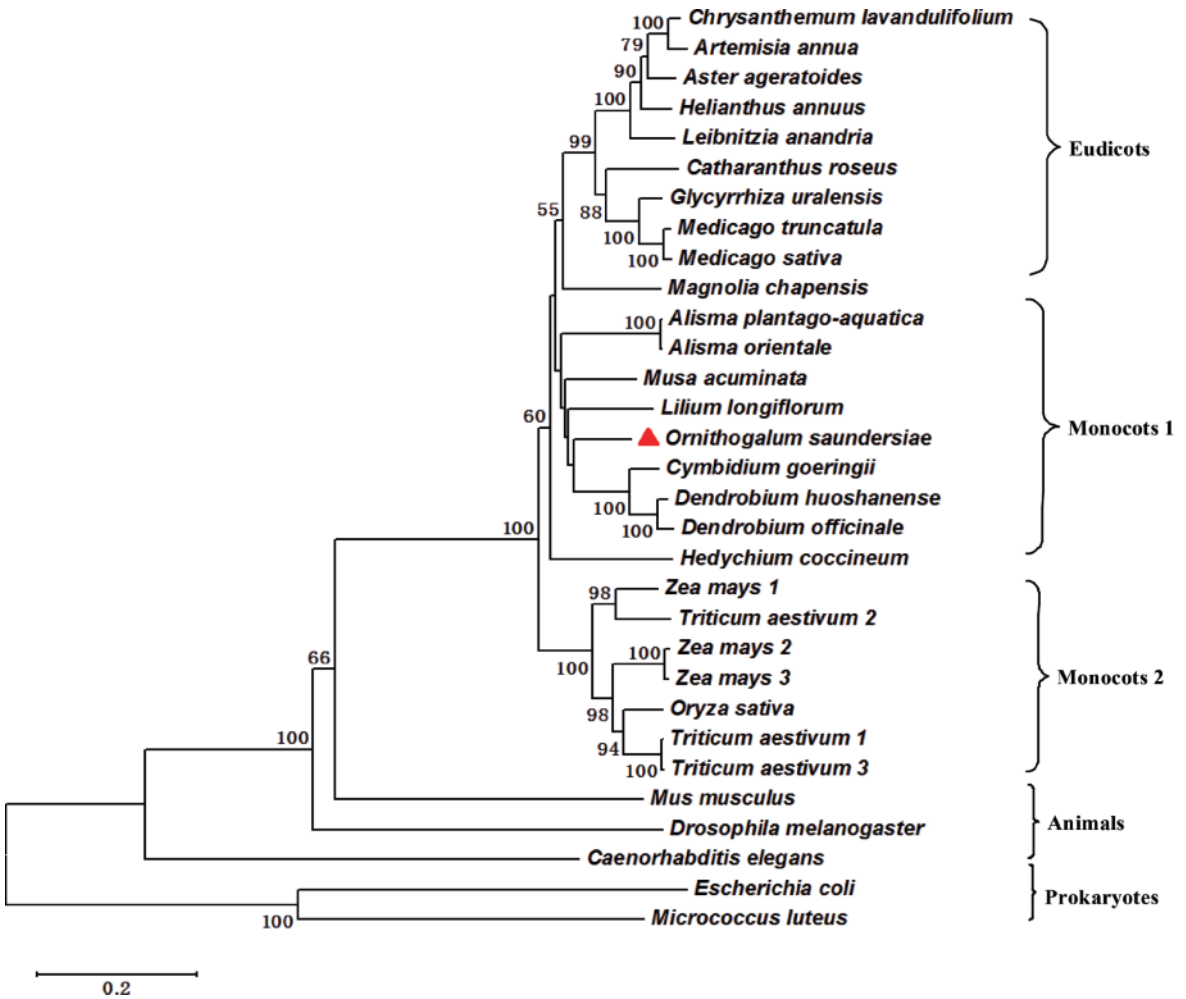

Fig. 6. Phylogenetic tree of FPPS from different organisms constructed by the neighbour-joining method on MEGA 5. GenBank accession numbers are: *Chrysanthemum lavandulifolium* (AFW98433.1), *Artemisia annua* (AAD17204.1), *Aster ageratoides* (AFW98436.1), *Helianthus annuus* (AFW98437.1), *Leibnitzia anandria* (AFW98445.1), *Catharanthus roseus* (ADO95193.1), *Glycyrrhiza uralensis* (ADE18770.1), *Medicago truncatula* (AES64578.1), *Medicago sativa* (ADC32809.1), *Magnolia chapensis* (ACS74708.1), *Alisma plantago-aquatica* (ADR83704.1), *Alisma orientale* (ADV03674.1), *Musa acuminata* (AF470318.1), *Lilium longiflorum* (ADZ57167.1), *Ornithogalum saundersiae* (KF509889.1), *Cymbidium goeringii* (AFP19446.1), *Dendrobium huoshanense* (AHC30884.1), *Dendrobium officinale* (AFX68799.1), *Hedychium coccineum* (AER12202.1), *Zea mays* 1 (AF330036.1), *Zea mays* 2 (ACG34051.1), *Zea mays* 3 (AFW83683.1), *Triticum aestivum* 1 (AFV51836.1), *Triticum aestivum* 2 (AGC11811.1), *Triticum aestivum* 3 (AFV51840.1), *Oryza sativa* ssp. *japonica* Group (BAA19856.1), *Mus musculus* (AAL09445.1), *Drosophila melanogaster* (CAA08919.1), *Caenorhabditis elegans* (CAB03221.2), *Escherichia coli* (BAA00599.1), *Micrococcus luteus* (BAA25265.1).

cots, monocots 1, monocots 2 (Poaceae), animals, and bacteria. As expected, OsaFPPS was clustered with FPPSs from monocot species of the order of Asparagales.

Discussion

Stimulated by the exceptionally potent antitumour activities of the saponin OSW-1, extensive efforts have been devoted to its total synthesis (Deng *et al.*, 1999; Yu and Jin, 2002; Xue *et al.*, 2008), as well as to investigations of structure-activity relationships (SAR) (Guan *et al.*, 2011; Maj *et al.*, 2011; Zheng *et al.*, 2011) and of its mode of action (Ying *et al.*, 2010; Garcia-Prieto *et al.*, 2013; Jin *et al.*, 2013). Our understanding of enzymes involved in OSW-1 biosynthesis, however, is inadequate (Kong *et al.*, 2014).

The transcriptome represents the universe of expressed genes within a cell at some particular state and time. Transcriptome sequencing is a high-throughput approach and can yield a vast amount of sequences in each run, far greater than that produced by traditional techniques. Transcriptome sequencing, therefore, can greatly accelerate the isolation of full-length cDNAs and genes. With the aim of speeding up the discovery of genes involved in OSW-1 biosynthesis, a profound transcriptome sequencing of *O. saundersiae* had previously been performed (Kong *et al.*, 2014). More than 40 full-length cDNAs, including *OsaFPPS*, related to OSW-1 biosynthesis were obtained without using methods of traditional molecular technology. As an important regulatory enzyme linking terpenoid precursor biosynthesis and the steroid pathway, OsaFPPS was selected for further functional identification in the present investigation. Moreover, in order to rapidly construct expression vectors for use in the heterologous expression of OsaFPPS, an in-fusion method based on the in-fusion enzyme (Evans *et al.*, 2007, 2009) was applied for plasmid construction, which can greatly improve the ligation efficiency of plasmid fragments and gene products. By combination of these biotechnologies, functional characterization of OsaFPPS was accomplished within a short period.

Acknowledgement

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