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Molecular cloning and sequence analysis of a *Terpene synthase* (*McTPS1*) gene in *Matricaria chamomilia*

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Abstract

A terpene synthase (TPS) gene (designated as *McTPS1*) cDNA was cloned from *Matricaria chamomilia* using a pair of specific primers. The cDNA fragment of *McTPS1* was 1719 bp and encoded a protein of 572 amino acids. The theoretical molecular weight and isoelectric point of the deduced McTPS1 protein are 67 kDa and 5.27, respectively. Multiple alignments showed the amino acid sequence of McTPS1 have extensive homology with those of TPS proteins from other plant. Phylogenetic tree analysis revealed that *McTPS1* had closer relationship with TPSs from Asteracae plants than from other plants. The molecular cloning and sequence analysis of *McTPS1* gene enabled us to further understand the role of *McTPS1* in the biosynthesis of α-bisabolol in *M. chamomilia*.

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Introduction

Matricaria chamomilia, a kind of annual or perennial herb, is with great exploitative value for its volatile oil (Jakoblev *et al*., 1979). Through the study of the active ingredients found the main component of volatile oil is chamazulene, the α-bisabolol and their oxides in *M. chamomilia* (Kumar *et al*., 2001; Raal *et al*., 2012). The α-bisabolol is a sesquiterpene, and the efficacy of anti-inflammatory, sterilization, heal ulcers, dissolve gallstones has been proved by pharmacology studies (Son *et al*., 2014). Moreover, the α-bisabolol has been used as an important cosmetic additive components due to its good skin effect (Bohlmann and Keeling, 2008; Peralta-Yahya *et al*., 2011). Therefore, the quality and value of chamomile herbs can significantly improved by increase the content of α-bisabolol in *M. chamomilia*. Terpenoids is a class of compound are composited with several isoprene. All terpenoids are derived from the five-carbon blocks isopentenyl pyrophosphate (IPP) and its isomer dimethylallyl pyrophosphate (DMAPP) (Andréa *et al*., 2003; Tholl, 2006).The α-bisabolol is an unsaturated teriary monocylic sesquiterpene compounds. In plants, two pathways for the synthesis of the isoprene building blocks are in operation (Kim *et al*., 2010): cytosolic mevalonate (MVA) pathway starting from 3 acetyl-CoA to finally yield IPP through catalyzed reaction by seven enzyme (Newman and Chappell, 1999), and the plastidial 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway simultaneously producing IPP and DMAPP from pyruvate and D-glyceraldehyde-3-phosphate (GA3P) through eight enzyme-catalyzed reactions (Christophe *et al*., 2009; Yu and Utsumi, 2009) (Fig. 1). Studies have demonstrated that steroids and sesquiterpene compound is synthesised by farnesyl pyrophosphate (FPP) through MVA pathway (Flugge and Gao, 2005). Monoterpenes, diterpenes and tetraterpenes are derived through the MEP pathway (Yu and Utsumi, 2009). The terpene synthase (TPS) is a key enzymes participate in the synthesis process of terpeniods (Bohlmann *et al*., 1998). The α-bisabolol as a sesquiterpene compound, which the precursor of the substrate from the MVA pathway intermediate FPP (Attia *et al*., 2012). So the molecular cloning and sequence analysis of *TPS* gene of chamomile is significant for increase the content of α-bisabolol in *M. chamomilia*.

The diversity of plant terpenes are mainly caused by the TPS species diversity and may have several TPS exist in a plant. As a key enzyme in the terpene biosynthesis, TPS has been widely studied. Up to now, *TPS* genes have been isolated from many plants, such as *Zostera marina* (Zhao *et al*., 2013), *Arabidopsis thaliana* (Guido *et al*., 2001), *Porphyra haitanensis* (Deng *et al*., 2013) and *Ginkgo biloba* (Parveen *et al*., 2013). In this report, we isolated *McTPS1* from chamomile and analyzed the structure of the sequence, aiming to provide the gene resource for increase the content of α-bisabolol in *M. Chamomilia* using genetic engineering. Through over expressing the key genes involved in α-bisabolol biosythesis and enrich the theory basis of molecular mechanism of α-bisabolol biosythesis.

Materials and methods

Plant material

M. chamomilia leaves were collected from botanical garden at Yangtze University, and immediately placed in a −80 °C freezer. Both the primers synthesis and DNA sequencing were performed by Shanghai Sangon Biotechnology Company, in China. Agarose Gel DNA purifieation Kit Ver.4.0, pMD18-T vector kit, AMV Reverse Transcriptase, dNTPs, RNasin and *Taq* DNA polymerase were purchased from Takara Company (Dalian, China).

RNA extraction and isolation of McTPS1

Total RNA was isolated from frozen plant tissues using the TaKaRa MiniBEST Plant RNA Extraction kit (Dalian, China). The specific primer McTPS1-s (5′-ATGTCAACTTTATCAGTTTCTACTCCTTCC-3′) and reverse primer McTPS1-a (5′-CTAGACAATCATAGGGTGAACGAAGAG-3′) were designed with the EST sequence of chamomile *TPS* gene. One-step reverse transcription PCR (RT-PCR) was performed using the one-step RT-PCR kit (Dalian TaKaRa, China) under the following conditions: 50 °C for 30 min and 94 °C for 3 min, followed by 32 cycles

of amplification at 94 °C for 1 min, 48 °C for 30 s, and 72 °C for 1 min; followed by an extension for 10 min at 72 °C.

The amplified products were analyzed by 1% gel electrophoresis and purified by a AxyPrep DNA Gel Extraction Kit (Flugge and Gao, 2005). The purified product was cloned into the pMD18-T vector, and then sequenced.

Bioinformatic analysis

Sequence assembly was performed with programs of DNAstar (http://www.dnastar.com). Protein and DNA homology searches were performed by using TBLASTN, TBLASTX, BLASTP and BLASTN programs (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple sequence alignment was performed with the software Vector NTI 11.5 program. Phylogenetic analysis of *McTPS1* from *M. chamomilia* and other TPSs from other plants were performed by using software CLUSTAL X 2 and MEGA 6with the neighbor-joining (NJ) method (Kumar *et al.*, 2004).

Results

Cloning of the cDNA of McTPS1

Using an RT-PCR method, a cDNA fragment encoding TPS, designated as *McTPS1*, was isolated and characterized. The length of *McTPS1* is 1719 bp with G/C content of 48.5%, encoding 572 amino acids (Fig.2). The nucleotide sequence of *McTPS1* had high similarity with *TPS* genes of other plants (Table 1).

Table 1. Nucleotide sequence of *McTPS1* similarity to the *TPS* genes of other plant species.

Species	GenBank Accession No.	Identity	E-value
Artemisia annua	GU294841	80%	0
Artemisia annua	GU294842	82%	7e-100
Solidago canadensis	AJ304452	69%	5e-127
Tanacetum parthenium	JE819849	66%	1e-90
Ageatina adenophora	FJ747651	69%	1e-59
Vitis vinifera	XM_002283034	65%	3e-42
Theobroma cacao	XM_007021053	68%	1e-34
Morus notabilis	XM_010093992	72%	2e-24
Azadirachta indica var.indica	KC631822	71%	9e-23
Populus euphratica	XM_010032444	70%	5e-20
Citrus sinensis	XM_006476842	69%	2e-19

The nucleotide sequence of *McTPS1* was 82%, 80%, 72%, 71%, 70%, 69%, 68%, 66%, 65% identical to *TPS* genes from *Artemisia annua*, *A. annua*, *Morus notabilis*, *Azadirachta indica var.indica*, *Populus euphratica*, *Solidago canadensis*, *Ageatina adenophora*, *Citrus sinensis*, *Theobroma cacao*, *Tanacetum parthenium*, and *Vitis vinifera*, respecitively, implying *McTPS1* was a member of *TPS* gene family. Furthermore, the homologous sequence of *TPS* gene among different species showed the *TPS* gene might keep a strong conservation during the molecular evolution.

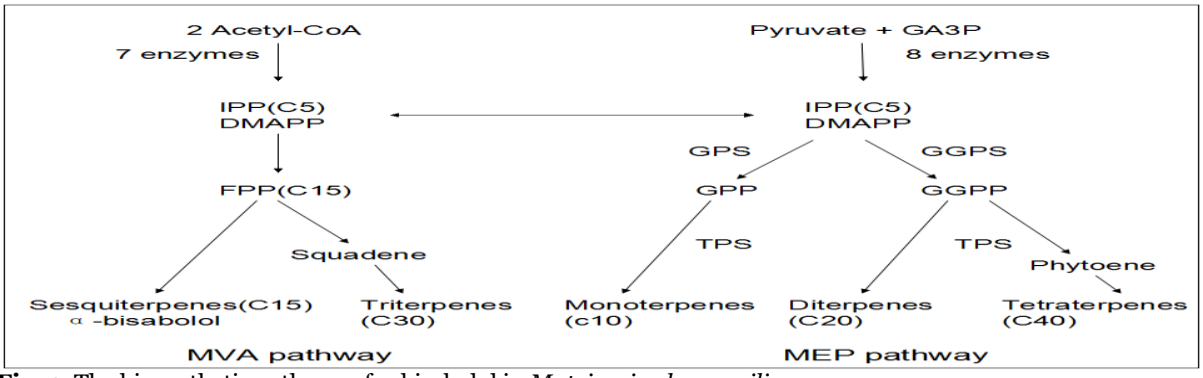

Fig. 1. The biosynthetic pathway of α-bisabolol in *Matricaria chamomilia*.

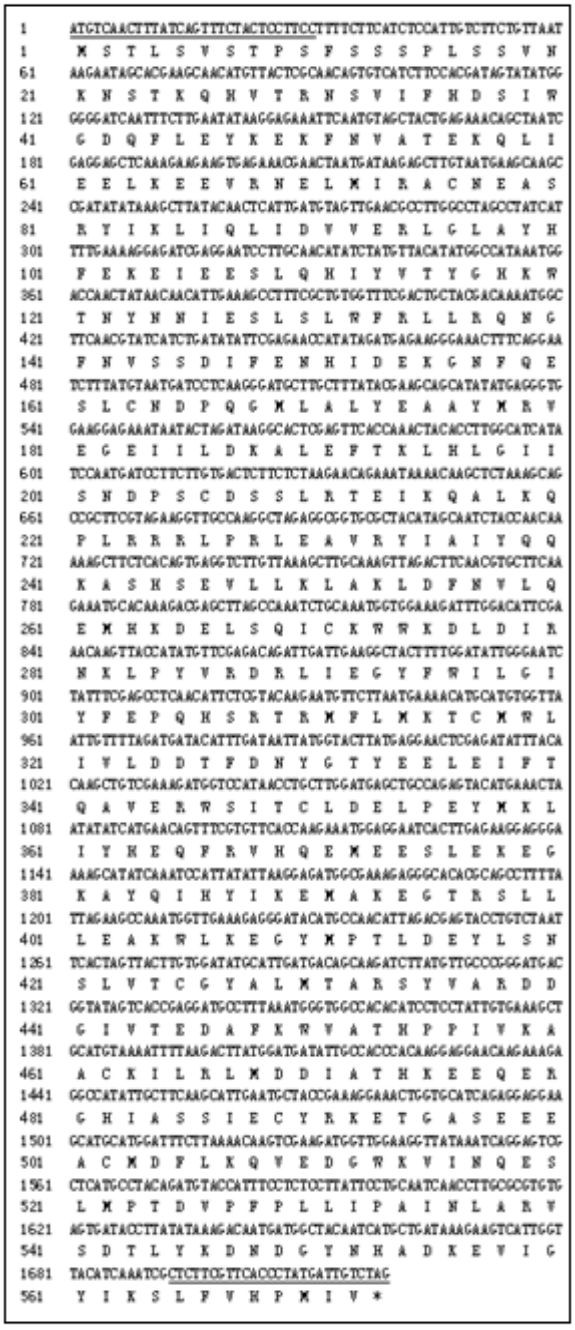

Fig. 2. Nucleotide sequence and deduced amino acid sequence of *McTPS1*. The primer sequences are underlined.

Characterization of the deduced McTPS1 protein

The theoretical molecular weight and pI of the deduced *McTPS1* were calculated as 67 kDa and 5.27, respectively, using software DNAMAN 6.0. A BLAST search of GeneBank and multi-alignment by Vector NTI showed that the deduced *McTPS1* polypeptide had high similarity with TPSs from other plant species (Fig.3). The amino acid sequence of *McTPS1* was 71%, 54%, 52%, 52%, 51% similarity to TPSs from *A. annua*, *S. canadensis*, *Achillea millefolium*, *A. absinthium*, *T. parthenium*, respectively. All of the data mentioned above indicate that *McTPS1* was a member of TPS family.

Molecular evolution analysis

To investigate the evolutionary relationships among *McTPS1* and other TPS proteins, a phylogenetic tree was constructed by using software Clustal X2 and MEGA6 with the neighbor-joining (NJ) method. As showed in Fig 4, the evolutionary tree was divided into two distinct categories. The results highlighted all plants derived from a common ancester in the evolution using TPS as outgroup, no matter whether they belonged to the xylophyta or herb plants. Secondly, TPS sequences from sevaral distinct branch-genus clusters. For instance, *M. chamomilia*, together with other Asteraceae species including *A. annua, S. canadensis*, *Ixeridium dentatu*m, *Mikamia micrantha*, *A. absinthium*, *A. millefolium* and *T. parthenium*, formed a cluster, implying they had a closer genetic relationship. In addtion, *Gossypium raimondii*, *G. hirsutum* and *G. arboreum* clustered together into Gossypium. *Jatropha curcas* and *Ricinus communis* clustered into Euphorbiaceae. Likewise, *Malus domestica*, *Fragaria vesca*, *Prunus mume* and *Pyrus* × *breschneideri* clustered into Rosaceae. Taken together, these result indicated that *McTPS1* shared a common evolutionary originals and the conserved sequences motifs with those of the Asteraceae specie TPS.

Discussion

A terpene synthase (*McTPS1*) gene cDNA was cloned from *M. chamomilia* in this study. The multiple sequensce alignment by using bioinformatics analysis software indicated that *McTPS1* had high identity with other *TPS* genes isolated from other plants. The homologous sequence of TPS gene among different species showed the TPS gene might keep a strong conservation during the molecular evolution. The conserved domain motif function further indicating *McTPS* might play important role in *α*-bisabolol

biosynthesis. Due to TPS as one of key enzymes in the synthesis pathway of *α*-bisabolol, an importent active compound, the present work on isolation and characteriation of McTPS1 could provied theoretical basis and gene resource for enhancement *α*-bisabolol by genetic engineering in *M. Chamomilia*.

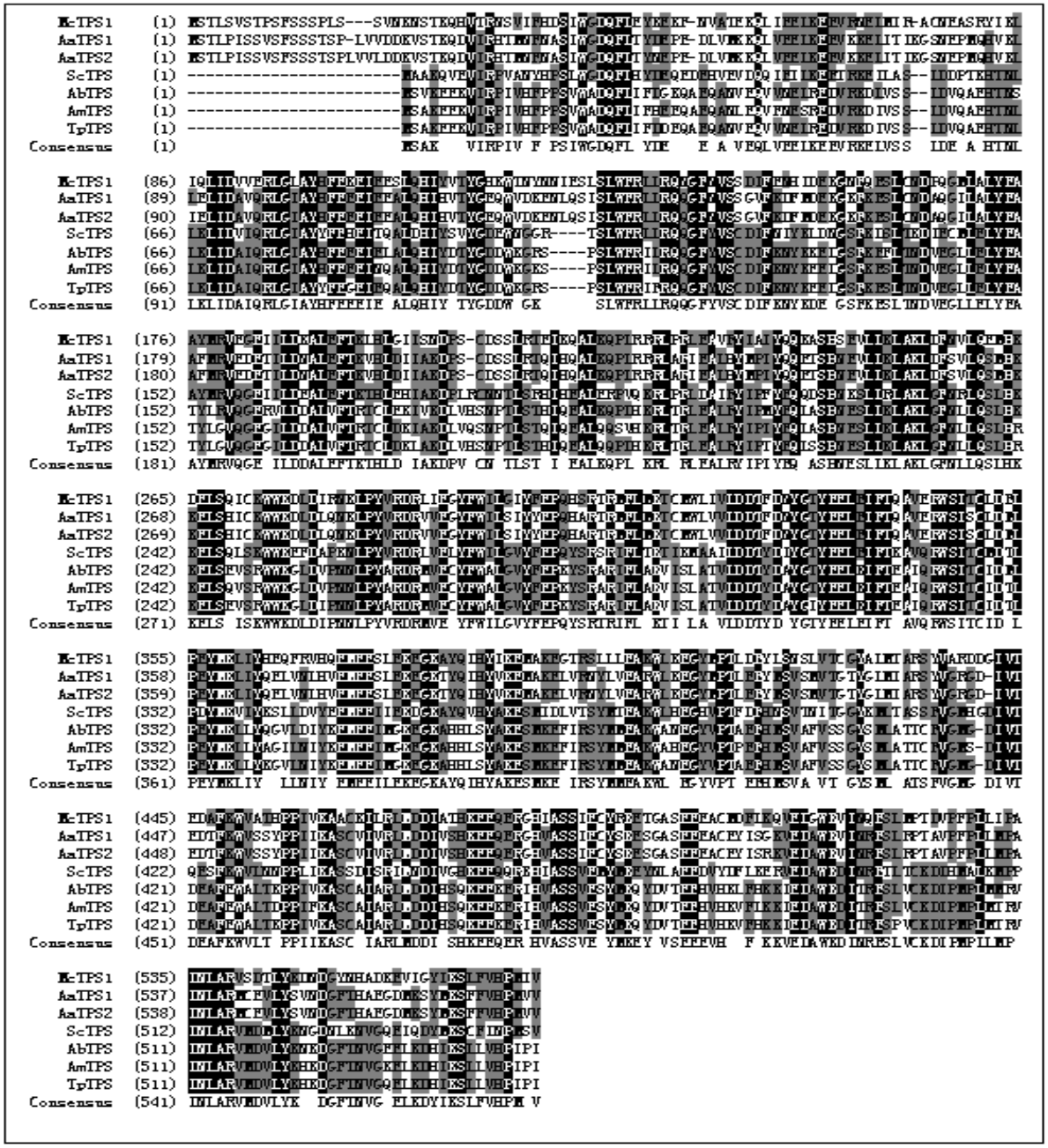

Fig. 3. Sequence multi-alignment of the deduced *McTPS1* protein with other TPS proteins. The specie and protein name and GenBank accession number are as following: *McTPS1*, *M.chamomilia*, AIG92846; *AaTPS2*, *Artemisia annua*, ADT64307; *AaTPS1*, *Artemisia annua*, ADT64306; *ScTPS*, *Solidago canadensis*, CAC36896; *AmTPS*, *Achillea millefolium,* AGZ84810; *AbTPS*, *Artemisia absinthium*, BAN81914; *TpTPS*, *Tanacetum parthenium*, AEH41845. Shaded in black are identical sequence. Shaded in gray are conservative sequence.

Up until now, the genomic analysis of *TPS* gene has been reported in many plants. A family of 40 terpenoid genes (*AtTPS*) was discovered by genome sequence analysis *Arabidopsis thaliana* (Aubourq *et al.*, 2002). A number of terpene synthases are also involved in biosynthesis of gingkolides and

bilobalides (Parveen *et al.*, 2013) in *G. Biloba*. The TPS gene from different species comprising sesquiterpene synthases with diverse catalytic activities (Sandra Irmisch *et al.*, 2012). The high homology of chamomile *TPS* gene from the Asteraceae showing that *McTPS1* is a key gene for synthesis of α-bisabolol in *M. Chamomilia*. In this report, Through over expression of *McTPS* gene in *M. Chamomilia* to confirm the regulation mechanism of α-bisabolol biosythesis. Further work need to isolate and function analysis other *TPS* genes from *M. Chamomilia*.

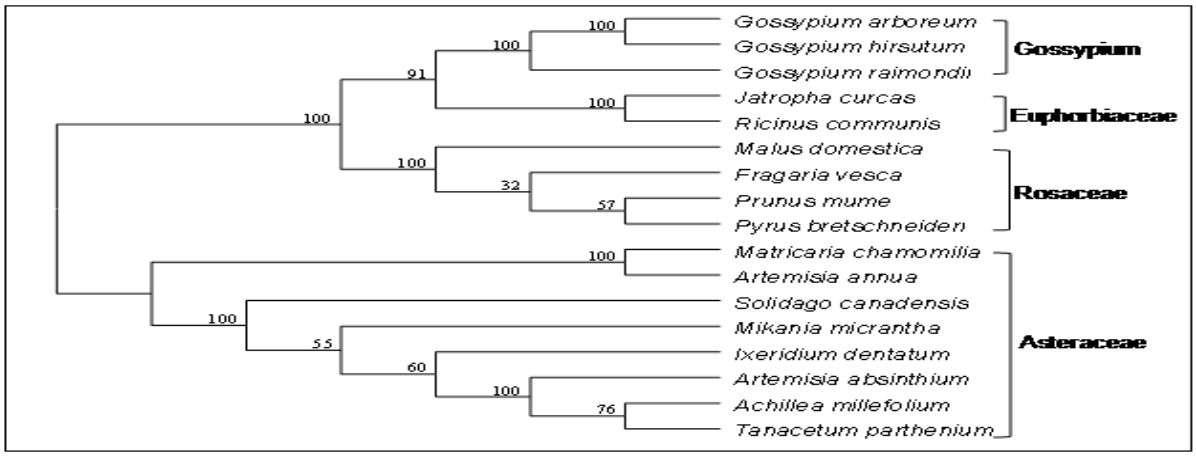

Fig. 4. The phylogenetic tree of terpene synthase including *McTPS1*. Phylogenetic analysis of *McTPS1* with other terpene synthase from other dicotyledon. Bootstrap values are expressed in percentages and placed at the nodes in the tree. The GenBank accession numbers of the TPS sequences and plant species are as following: *Gossypium arboreum* (KHG04103), *Gossypium hirsutum* (AFQ23183), *Gossypium raimondii* (KJB13541), *Jatropha curcas* (KDP36230), *Ricinus communis* (XP_002523635), *Malus domestica* (NP_001281061), *Fragaria vesca* (XP_004287071), *Prunus mume* (XP_008226803), *Pyrus* × *bretschneideri* (XP_009346480), *Matricaria chamomilla* (AIG92846), *Artemisia annua* (ADT64307), *Solidago canadensis* (CAC36896), *Mikania micrantha* (ACN67535), *Ixeridium dentatum* (AAX84550), *Artemisia absinthium* (BAN81914), *Achillea millefolium* (AGZ84810), *Tanacetum parthenium* (AEH41845).

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