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

Tingting Tao¹, Li Zhu¹, Xiaomeng Liu¹, Qiling Song¹, Feng Xu*, Jie Chang², Weiwei Zhang¹

¹Department of College of Horticulture and Gardening, Yangtze University, Jingzhou, China  
²Department of Hubei Collaborative Innovation Center of Targeted Antitumor Drug, Jingchu University of Technology, Jingmen, China

**Key words**: *Matricaria chamomilia*, McTPS1, Molecular cloning, Sequence analysis.

[http://dx.doi.org/10.12692/ijb/7.1.66-73](http://dx.doi.org/10.12692/ijb/7.1.66-73)  
Article published on July 14, 2015

**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 Asteraceae 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*.

*Corresponding Author*: Feng Xu* [xufeng198@126.com](mailto:xufeng198@126.com)
**Introduction**

*Matricaria chamomilla*, 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 chamazulenene, the α-bisabolol and their oxides in *M. chamomilla* (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. chamomilla*. Terpenoids is a class of compound are compositied 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 monocycic 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-glycaldehyde-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 farnesy l 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 terpenoids (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. chamomila*

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 biosynthesis and enrich the theory basis of molecular mechanism of α-bisabolol biosynthesis.

**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 purification 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′-ATGTCACCTTTATCGTTTTCTACTCCCTCC-3′) and reverse primer McTPS1-a (5′-CTAGACAATCTAGGGAACAGAAGAG-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 6 with 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.

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

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*, respectively, 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*. 
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 ancestor in the evolution using TPS as outgroup, no matter whether they belonged to the xyllophyta or herb plants. Secondly, TPS sequences from several distinct branch-genus clusters. For instance, M. chamomilia, together with other Asteraceae species including A. annua, S. canadensis, Xeridium dentatum, Mikamia micrantha, A. absinthium, A. millefolium and T. parthenium, formed a cluster, implying they had a closer genetic relationship. In addition, 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 origins and the conserved sequences motifs with those of the Asteraceae spece TPS.

Discussion

A terpene synthase (McTPS1) gene cDNA was cloned from M. chamomilia in this study. The multiple sequence 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 McTPS1 might play important role in α-bisabolol.
biosynthesis. Due to TPS as one of key enzymes in the synthesis pathway of α-bisabolol, an important active compound, the present work on isolation and characterization of McTPS1 could provide theoretical basis and gene resource for enhancement α-bisabolol by genetic engineering in *M. Chamomilla*.

**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. chamomilla*, 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 sequences.

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 biosynthesis. 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), Achillea millefolium (AGZ84810), Tanacetum parthenium (AEH41845).

Acknowledgments
This work was supported by the National Natural Science Foundation of China (31400603), the National Training Programs of Innovation and Entrepreneurship for Undergraduates (104892014043), the Natural Science Foundation of Hubei Province (2013CFA039).

References


Raal A, Orav A, Pussa T, Valner C. 2012. Content of essential oil, terpenoids and polyphenols in
commercial chamomile (Chamomilla recutita L. Rauschert) teas from different countries. Food Chemistry 131, 632–638. 
http://dx.doi.org/10.1016/j.foodchem.2011.09.042

http://dx.doi.org/10.1186/1471-2229-12-84

http://dx.doi.org/10.1042/BJ20140306

http://dx.doi.org/10.1016/j.pbi.2006.03.014

http://dx.doi.org/10.1007/s00018-009-0066-7

http://dx.doi.org/10.1016/j.gene.2013.09.016