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Original Research Article

Isolation and Sequence Analysis of a *Terpene Synthase* (*McTPS2*) Gene from *Matricaria chamomilla*

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Abstract

The present study cloned a *terpene synthase* gene (designated as *McTPS2*), which is one of the key genes involved in the biosynthesis of sesquiterpenoid α-bisabolol, the important pharmacological components in the plant *Matricaria chamomilla* L. A pair of specific primers was designed based on the transcriptome data of *M. chamomilia.* The *McTPS2* gene was amplified by one step RT-PCR. The cDNA sequence of *McTPS2* was used for further bioinformatic analysis. The cDNA fragment of *McTPS2* was 1641bp, which encoded 546 amino acids. The isoelectric point and molecular weight of *McTPS2* protein were 5.53 and 63.4 kDa, respectively. The multi-alignment analysis of protein sequence showed that *McTPS2* had high similar with the TPSs from other plants. The phylogenetic tree indicated that *McTPS2* had closer relationship with TPSs from Asteraceae plants than from other plants. The gene family of terpene synthase is one of the key regulators in terpenoid synthase of plants. The cloning and sequence analysis of *McTPS2* gene provided the basis for further study molecular mechanisms of α-bisabolol synthesis in *M. chamomilla*.

Keywords

Matricaria chamomilla

Molecular evolution

Sequence analysis

Terpene synthase

Introduction

Chamomile, a member of the daisy family, is native to Europe and western Asia. German chamomile, *Matricaria chamomilla*, is widely distributed in the countries of tropics and subtropics (Murti et al., 2012), but is now-a-days cultivated throughout the world. To evaluate the pharmaceutical value, analysis of essential oil of chamomile flower found that it has high presence of sesquiterpene derivatives (75-90%) including (E)-β-farnesene (4.9-8.1%), terpene alcohol (farnesol), chamazulene (2.3-10.9%), α-bisabolol (4.8-11.3%) (Lrmisch et al., 2012). The pharmaceutical active components of the flower oil are known for their anti-inflammatory, antisentic, antiphlogistic, and spasmoiytic properties (Su et al., 2015), and is the most commonly used in decorative cosmetics, fine fragrances, shampoos, and other toiletries as well as in non-cosmetic products such as household cleaners and detergents (Bhatia et al., 2008). Though more in-depth research on pharmacology principle, little is known about the genetic mechanism of the terpene synthase enzyme.

Given the complexity of the biosynthetic pathway of terpene compounds, the late cyclization and oxidation steps of the precursors of each class of terpenes are catalyzed by terpene synthase (TPS), the key enzymes are forming a simple or a complex mixture of reaction products of terpene metabolites (Degenhardt et al., 2009). TPS play a crucial role in synthesized of terpene, which make to change from the ubiquitous prenyl diphosphates, geranyl diphosphate (GPP), farnesyl diphosphate (FPP) and geranylgeranyldiposphate (GGPP) into the respective mono-, sesqui- and diterpene skeletons (Lrmisch et al., 2012).

The TPS contains a conserved motif associated with a shorter ion binding (DDxxD motif). TPS sequence similarities is determined by the relationship between species, not the enzyme substrate or product specificity (Chen et al., 2011). Thus, the complex terpene mixture of plants is often produced by only a limited multi-product of TPS enzymes. Cloning and characterization of the TPS gene from *M. chamomilla* is an important step to further study the sesquiterpenoids and their derivatives in *M. chamomilla*.

Even though the plants have multiple TPS, and TPS has become the largest and most in-depth study of terpene biosynthesis enzymes, for instance two sesquiterpene synthase gene were cloned in tobacco in 1992 (Degenhardt et al., 2009), and 200 monoterpene and sesquiterpene synthase gene from 40 kinds of plants had been cloned by researchers (Chen et al., 2011), little is known about the biosynthesis of its major constituents in *M. chamomilla*.

The sesquiterpenoid α-bisabolol is one of the important pharmacological components in *M. chamomilla*. TPS catalytic synthesise α-bisabolol in pathway of sesquiterpene synthetic metabolism. Therefore, according to the whole chamomile transcriptome sequence, one of the TPS genes designated as *McTPS2* was isolated. The sequence was analyzed by using bioinformatics software. The result provides a theoretical foundation for the research of α-bisabolol synthetic metabolism.

Materials and methods

Plant material and reagents

M. chamomilla were collected from Botanical garden of 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, Dalian, in China.

Molecular cloning of *McTPS2*

Total RNA was isolated from frozen plant tissues using the TaKaRa MiniBEST Plant RNA Extraction kit (Dalian). The specific primers *McTPS2*-F (5′-GACCGGATCCAACATGGTTTCGATGTTTGCCCAAC-3′) and *McTPS2*-R (5′-GGTCCTCGAGTCAGACACTTCCACGACTATGATCAC-3′) were designed with the EST sequence of *M. chamomilla* 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: protein denaturation at 94°C for 4 min followed by 30 cycles of 94°C for 30sec 59°C 1 min 72°C for 1min followed by extension of 59°C for 10 min. PCR reaction system: ddH_2O 16.75μl, buffer 2.5μl, $MgCl_2$ 2μl, primer (*McTPS2*-F) 1μl, primer (*McTPS2*-R) 1μl, DNTP 0.5μl, *Taq* polymerase 0.25μl, and cDNA 1μl.

Sequence and bioinformatic analysis

Sequence assembly was performed with programs from 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 *McTPS2* from *M. chamomilla* and other TPSs from other plants were performed by using software CLUSTAL X 2 and MEGA 6 with the neighbor-joining (NJ) method (Tamura et al., 2011).

Results and discussion

Cloning of the cDNA fragment of *McTPS2*

Using One-step reverse transcription PCR method, the full-length cDNA sequence of *McTPS2* gene was cloned. The full-length cDNA of *McTPS2* is 1641bp, coding by 546 amino acids (Fig. 1). BLASTN analysis on NCBI showed that the nucleotide sequence of *McTPS2* had high similarity with other TPS genes of reported plants (Table 1).

Fig. 1: **Nucleotide sequence and deduced amino acid sequence of *McTPS2***

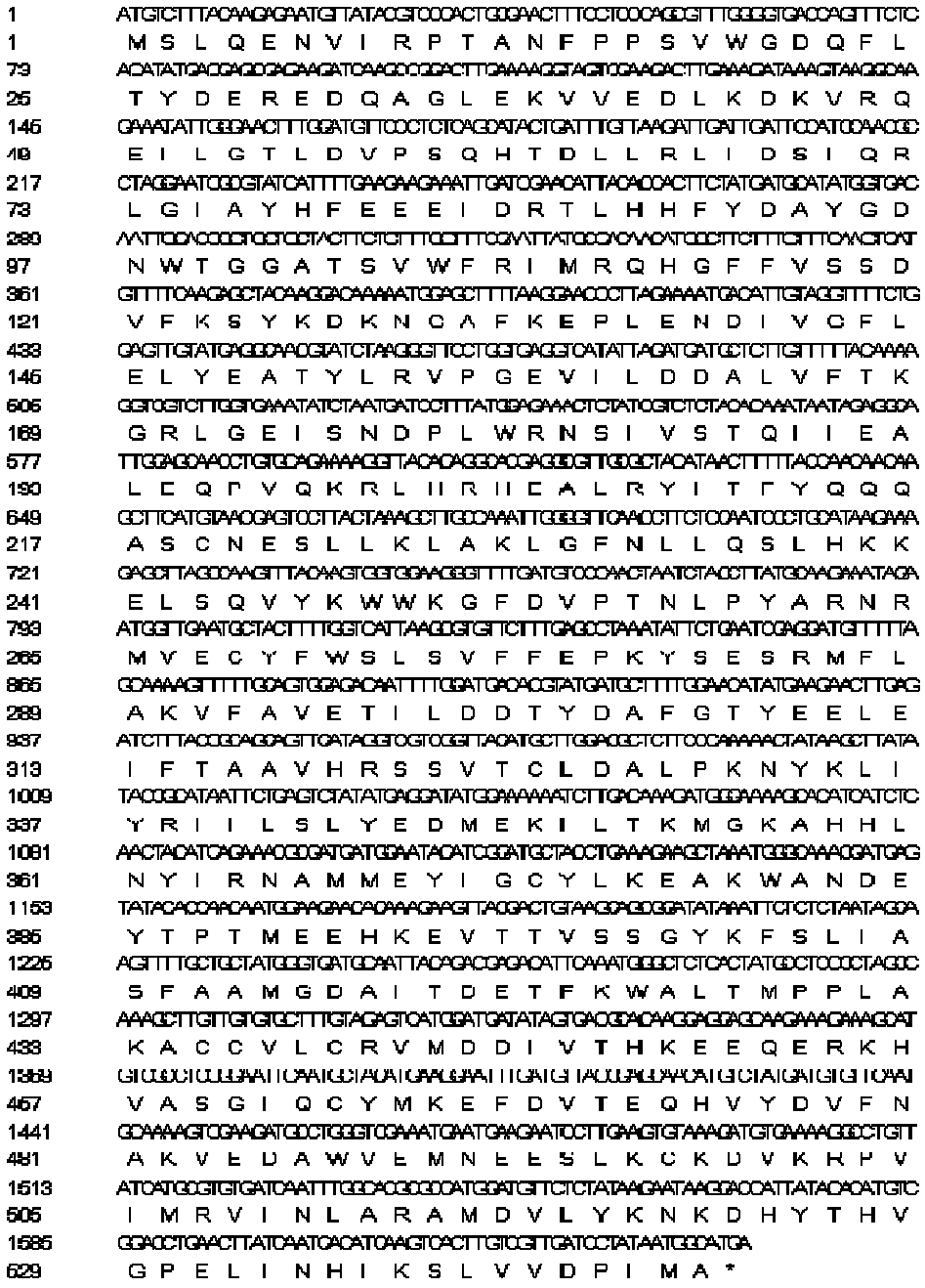
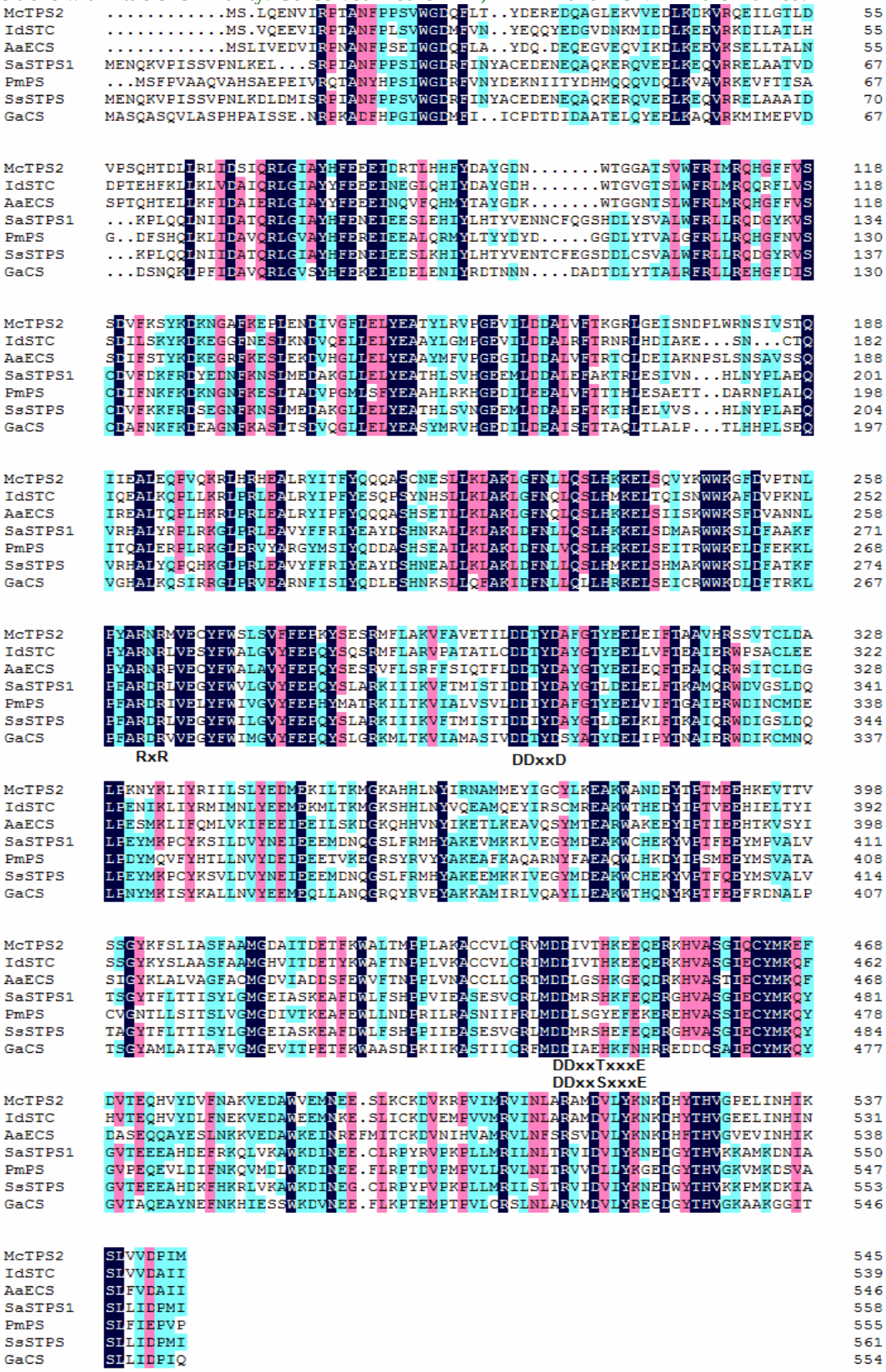


Table 1. Nucleotide sequence of *McTPS2* similarity to the TPS gene of other plant species.

Species	GenBank no.	Identity	E-value
Ixericaria dentatum	AAX84550.1	99%	1e-182
Artemisia annua	Q9LLR9.1	99%	2e-113
Santalum album	B5A435.1	98%	2e-167
Vitis vinifera	CBI20483.3	99%	9e-167
Prunus mume	XP_008226803.1	99%	6e-165
Santalum spicatum	E3W208.1	98%	1e-162
Gossypium arboreum	Q43714.1	98%	2e-158
Populus trichocarpa	XP_006374233.1	98%	8e-162

Fig. 2: Alignment of deduced amino acid sequence of *McTPS2* with terpene synthases from other plants. Highly conserved residues in the seven sequences are black; shaded in purple are amino acid positions with less of similarity; shaded in blue are amino acid positions with little of similarity. Conserved motifs RxR, DDxxD and NSE/DTE are marked.



The nucleotide sequence of *McTPS2* was 99%, 99%, 98%, 99%, 99%, 98%, 98%, and 98% identical to TPS genes from *Ixericaria dentatum*, *Artemisia annua*, *Santalum album*, *Vitis vinifera*, *Prunus mume*, *Santalum spicatum*, *Gossypium arboreum* and *Populus trichocarpa*, respectively, implying that the *McTPS2* was one 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 (Facchini and Chappell, 1992; Bohmann et al., 1998).

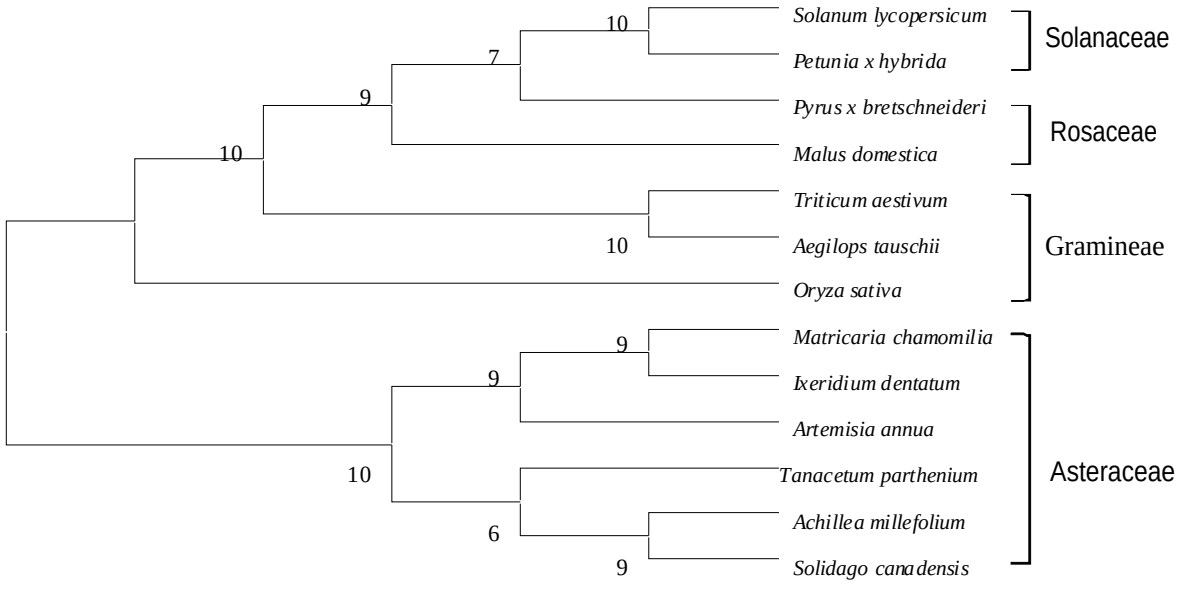
Characterization of the deduced *McTPS2* protein

The *McTPS2* protein contains 546 amino acids (Fig. 1). Using BLastx, and online website (http://web.expasy.org/ protparam/) for protein sequence analysis showed the isoelectric point and molecular weight of *McTPS2* protein are 5.53 and 63.4 kDa, respectively. A database search with BlastP (NCBI) and multialignment by Vector NTI 10.0 showed that the *McTPS3* protein had high similarity with TPSs form other plant species (Fig. 2). The amino acid sequence of *McTPS2* was 92%, 91%, 90%, 87%, 86%, and 85% similarity to TPSs, including IDSTC, AaECS, SaSTPS1, PmPS, SsSTPS, and GaCS, respectively. The strong conserved aspirate-rich DDxxD motif, which is involved in binding the divalent metal ion cofactor during the reaction mechanism (Bohmann et al., 1998), present in *McTPS2* protein. This aspartate rich region occurred as DDTYD motif shared by all plant TPS genotypes analyzed suggest that they share a common evolutionary origin. Another metal cofactor binding motif NSE/DTE, less well conserved in TPS, can be present in *McTPS2* as DDxxT/SxxxE motif. An additional conserved sequence observed in *McTPS2* was RxR motif which plays a role in the complexation of the diphosphate group after ionization of the substrate (Stark et al., 1997).

Sequence analysis of the structure of the system evolution

To investigate the evolutionary relationships among *McTPS2* and other TPS proteins, by using software Clustal X2 and MEGA6 with the neighbor-joining (NJ) method a phylogenetic tree was constructed. As can be seen from Fig. 3, and the evolutionary tree was divided into two distinct categories, xylophyta and herb. Firstly, the results highlighted all plants, no matter whether they belonged to the xylophyta or herb plants, derived from a common ancester in the evolution.

Fig. 3: Molecular evolutionary tree of *McTPS2* and other TPS proteins. Phylogenetic analysis of *McTPS2* with other terpene synthase from other plants. Bootstrap values are expressed in percentages and placed at the nodes in the tree. The *McTPS2* sequence was aligned with TPS sequence from *Solanum lycopersicum* (NP_001234879.1), *Petunia* x *hybrid* (ADO16162.1), *Pyrus* x *bretschneideri* (XP_009376725.1), *Malus domestica* (XP_008366020.1), *Triticum aestivum* (ACI16353.1), *Aegilops tauschii* (EMT23116.1), *Oryza sativa* (AEB53187.1), *Matricaria chamomilla* (I6R4V5.1). *Ixeridium dentatum* (AAX84550.1), *Artemisia annua* (CAB56499.1), *Tanacetum parthenium* (AEH41845.1), *Achillea millefolium* (AGZ84811.1), *Solidago Canadensis* (CAC36896.1).



Secondly, TPS sequences from several distinct branch-genus clusters, such as *M. chamomilla*, together with other *Asteraceae* species including *Ixeridium dentatum*, *Artemisia annua*, *Tanacetum parthenium*, *Achillea millefolium* and *Solidago canadensis* formed a cluster, suggest that *McTPS2* had a closer relationship with other TPSs from *Asteraceae* species. Meanwhile, *TPSs* from *Triticum aestivum*, *Aegilops tauschii* and *Oryza sativa* Indica Group, the Poaceae species, were grouped into a cluster in the tree. *Pyrus x bretschneideri* and *Malus domestica*, the Rosaceae species, were grouped into a cluster in the tree. *Solanum lycopersicum* and *Petunia* x *hybrid*, the Solanaceae species, were grouped into a cluster in the tree. *McTPS2* had the closest relationship with those of other *Asteraceae*, indicating that *McTPS2* shared a common evolutionary originals with the *Asteraceae* species and the conserved sequences motifs (Trapp and Croteau, 2001; Lrmisch et al., 2012).

Conclusion

In this work, a novel *McTPS2* cDNA was cloned and characterized from *M. chamomilla* for the first time. Multiple alignments showed that the deuced *McTPS2* was homologous with other known TPS proteins, and it contained conserved motif possessed by TPS protein family. Because the TPS might be a key enzyme in the synthesis of α-bisabolol, an important active compounds, the isolation and sequence analysis of *McTPS2* would not only enhance our understanding of the biosynthesis and regulation of terpene in *M. chamomilla*, but also provides a theoretical basis and genetic resources for improving the content of α-bisabolol.

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