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Cloning and Sequence Analysis of Mevalonate Kinase Gene (*CnMVK*) from *Chamaemelum nobile*

Xiangxiang Meng, Weiwei Zhang, Feng Xu*, Jiaping Yan, Xiaomeng Liu, Yong-ling Liao and Jie Chang

College of Horticulture and Gardening, Yangtze University, Jingzhou 434025, China

Corresponding author.

Abstract

Mevalonate kinase (MVK) is one of the key rate-limiting enzymes in mevalonic (MVA) pathway of terpenoid biosynthesis. To analyse the function of MVK gene in terpenoid biosynthesis in *Chamaemelum nobile*, the primers were designed according to the transcript unique sequence of *CnMVK* from the *C. nobile* transcriptome dataset. A MVK gene (designated as *CnMVK*, GenBank accession number KX894317) was cloned from *C. nobile* using RT-PCR method. The full-length cDNA of *CnMVK* gene is 1561bp and contains an open reading frame (ORF) of 1206bp, which encoding a 402 amino-acid protein. The theoretical molecular weight and pI of the *CnMVK* are 42.8kDa and 5.79, respectively. Multi-alignment comparison analysis showed the protein sequence of CnMVK had high similarity with MVK proteins from other plants. Furthermore, CnMVK has similar conserved domains, suggesting *CnMVK* is one of MVK family mumbers in *C. nobile*. The present study cloned, analyzed the sequence of *CnMVK* in *C. nobile* will provide a foundation for exploring the molecular mechanism of terpenoid biosynthesis in *C. nobile*.

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Keywords

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Introduction

Chamaemelum nobile is a herbaceous plant which belongs to Matricaria. The flowers of *C. nobile* rich in volatile aromatic oil, including α-bisabolol, α-bisabolol Alcohol Oxide A and B, chamazulene and flavonoids, the above components have anti-inflammatory, antibacterial, antioxidant and other effects (Carnat et al., 2004; Lemberkovics et al., 1998; Srivastava et al., 2010). Thus, *C. nobile* is widely used in pharmaceuticals, cosmetics and spices and other fields. However, the flowers of *C. nobile* only contains 1-2% of the volatile oil (Peña et al., 2006), which cannot meet the market demand. As the incidence of drug-resistant bacteria is increasing, the development of natural antimicrobial resources has become particularly important (Gundersen et al., 2015), thus, it has important significance to improve the content of terpenoids of *C. nobile* by genetic engineering.

Terpenoid is currently known as the largest class of plant secondary metabolites, they play an important role in the process of plant growth and development, and many special terpenoid also play an important function in plant cell signal transduction and stress-resistant physiological processes (Holstein and Hohl, 2004). It has been confirmed that the C5 universal precursor in the biosynthesis of natural terpene compounds is mainly

derived from mevalonate (MVA) pathway (Dewick, 2002). MVA pathway exists in the cytoplasm, with three acetyl Co A as a raw material through a series of enzymatic reaction to produce isopentenyl pyrophosphate (IPP), part of IPP isomerized to form dimethylallyl pyrophosphate (DMAPP). IPP and DMAPP to form geranyl-pyrophosphate (GPP) (Guo et al., 2012). GPP is combined with one IPP unit to produce farnesyl pyrophosphate (FPP), which is a precursor of sesquiterpene. FPP eventually forms sesquiterpene through heterogeneous, cyclization and complexation. MVA is currently considered to be the main pathway of sesquiterpene biosynthesis. Mevalonic kinase, phosphomevalonate kinase, and mevalonate-5-pyrophosphate decarboxylase are of the three consecutive ATP-dependent enzymes present in the enzymatic reaction of the mevalonate pathway, and the mevalonate kinase is the first of three consecutive ATP-dependent enzymes (Huang et al., 1999). It is responsible for the transfer of the phosphate group at the ATPγ position to the hydroxyl group at position 5 of mevalonate, which forms mevalonate-5-phosphate and is associated with the release of ADP and is one of the rate-limiting enzymes controlling the entire metabolic pathway (Chu and Ding, 2003). Related research (Guo et al., 2012; Huang et al., 2015; Wuyun et al., 2014) shows that the expression of MVK gene may directly or indirectly affect the content of terpenoids in medicinal plants.

The corresponding MVK gene has been cloned from *Arabidopsis thaliana* (Riou et al., 1994), *Salvia miltiorrhiza* (Ma et al., 2012), *Zea mays* (Alexandrov et al., 2009), *Catharanthus roseus* (Wuyun et al., 2014), *Eucommia ulmoides* (Simkin et al., 2011) and so on. However, the MVK gene has not been reported in *C. nobile* so far. In view of the fact that MVK is the key rate-limiting enzyme in terpenoids synthesis pathway, the *CnMVK* gene was cloned by RT-PCR and the bioinformatics analysis of the gene was carried out, and to provide a reliable theoretical basis for revealing the mechanism of *CnMVK* gene regulation on the biosynthesis of sesquiterpenoid compounds of *C. nobile*.

Materials and methods

Plant material and reagents

The leaves of *C. nobile* were collected from botanical garden at Yangtze University, China, and placed in a −80°C freezer immediately. In this experiment, both the primers synthesis and DNA sequencing were completed by Shanghai Sangon Biotechnology Company, in China. MiniBEST Plant RNA Extraction kit, PrimeScript™1st Strand cDNA Synthesis Kit, Agarose Gel DNA purification Kit Ver.4.0, dNTP, Taq DNA polymerase RNase, pMD18-T vector were purchased from Takara Company (Dalian, China).

Cloning of CnMVK

Total RNA was isolated from frozen leaves of *C. nobile* using MiniBEST Plant RNA Extraction kit. The extracted RNA was reverse transcribed into cDNA using PrimeScript™1st Strand cDNA Synthesis Kit. The specific primers as given below were designed based on the MVK unigene sequence of *C. nobile* transcriptome data.

CnMVK-Up (5′-CGGACTGAAGCCTCCAACAAC-3′)
CnMVK-Dn (5′-CGGCCCTTGAGATGGTCATATACTA-3′)

The MVK gene was amplified by reverse transcription-polymerase chain reaction (RT-PCR) method. The amplified products were detected by 1% gel electrophoresis and purified using Agarose Gel DNA purification Kit Ver.4.0. The purified product was cloned into the pMD18-T vector, then transformed into *Escherichia coli* DH5α. Positive clones were selected and sent to Shanghai Sangon Biotechnology Company for sequencing.

Bioinformatic analysis

CnMVK gene sequences were translated into amino acid sequences by using DNAMAN software. The open reading frame (ORF) of the *CnMVK* gene was predicted by using Vector NTI 11.5. Protein homology searches were performed by using the bioinformatics software on NCBI website (http://www.ncbi.nlm.nih.gov/BLAST/). Multiple sequence alignment was performed with the software Vector NTI11.5 program. Phylogenetic tree of MVK proteins was constructed with neighbor-joining method using Clustal X2.0 and MEGA5 (Larkin et al., 2007).

Results and discussion

Cloning and sequence analysis of *CnMVK*

A pair of specific primers was designed according to the MVK unigene sequence of *C. nobile* transcriptome data, the cDNA sequence of 1561bp was amplified by RT-PCR. The comparison analysis shows that the cDNA sequence is highly homologous to the MVK sequences

of other plants, it indicated that the cloned cDNA sequence was the MVK gene of *C. nobile*. The cDNA sequence was designated as *CnMVK* and GenBank accession number was KX894317. The full-length cDNA of *CnMVK* is 1561bp and contained a 1206 bp ORF which encoded 402 amino acids (Fig. 1).

```
1       TATGTCTCCAACAGTAACCTAAACTAACATAACGGACTGAAGCCTCCAACAACATTAATGGAAGTGGTATCAAGGGCTCCCGGAAAGATC
1                                                                M  E  V  V  S  R  A  P  G  K  I
91      ATACTCTCCGGTGAACATGCAGTCGTCCATGGATCCGCCGCCGTAGCTGCCGCCATCGACCTCTACACTTACGTCTCTCTTACGTTTCCT
31      I  L  S  G  E  H  A  V  V  H  G  S  A  A  V  A  A  A  I  D  L  Y  T  Y  V  S  L  T  F  P
181     CCACCTCCTCATAGCCCTGATACACTCATACTACACCTCAAAGACATTGAACTGGAAATTTCATGGCCAGTTAATAGACTTAAAGAAGCA
61      P  P  P  H  S  P  D  T  L  I  L  H  L  K  D  I  E  L  E  I  S  W  P  V  N  R  L  K  E  A
271     TTATCTTCAATGGTCAATGCTACTGCATCCTCACCAACGTCATGTTCTGCAGAGACCATTAAGATCATTACTACTCTAGTACTCGAAGAG
91      L  S  S  M  V  N  A  T  A  S  S  P  T  S  C  S  A  E  T  I  K  I  I  T  T  L  V  L  E  E
361     CATACAATATTGGAGTCAAAAACCGAAATTGCTGCAGCGGTTGTGGTTTTTCTCTGGTTATACACCTCTATACAAGGAAATAAACCAGCA
121     H  T  I  L  E  S  K  T  E  I  A  A  A  V  V  V  F  L  W  L  Y  T  S  I  Q  G  N  K  P  A
451     AGAGTAGTTGTTAGTTCAGAGCTTCCTTTGGGTTCAGGATTGGGGTCATCTGCAGCATACTGTGTTTCAATGTCTGGAGCGTTGCTTGCT
151     R  V  V  V  S  S  E  L  P  L  G  S  G  L  G  S  S  A  A  Y  C  V  S  M  S  G  A  L  L  A
541     TCGTCGGGTTCTTTGCATCTGGATTTCAACAGTGAGGATTGGCTATCACTTGGAGAAAAACAGCAGAAATTGGCTAATGAATGGGCGTTT
181     S  S  G  S  L  H  L  D  F  N  S  E  D  W  L  S  L  G  E  K  Q  Q  K  L  A  N  E  W  A  F
631     GAAGGTGAGAAGATTATTCATGGGAACCCATCTGGGATTGACAATACAGTTAGCACATTGGGTATGTCATGTTTCATAGTATACACTGGG
211     E  G  E  K  I  I  H  G  N  P  S  G  I  D  N  T  V  S  T  L  G  M  S  C  F  I  V  Y  T  G
721     AACTTGATAAAGCTACAATTAGGTGCTATGACATGCATCAAACCCAATATGCCACTCAAAATGCTAATTACTAACACGAAAGTTGGGAGA
241     N  L  I  K  L  Q  L  G  A  M  T  C  I  K  P  N  M  P  L  K  M  L  I  T  N  T  K  V  G  R
811     GACACAAAGGCACTAATCGCTCGTGTTTCAGAAAGAAGGAATAGACATCCAGATACCATGAAATCTGTGTTTGCAGCTGTTGACTATATC
271     D  T  K  A  L  I  A  R  V  S  E  R  R  N  R  H  P  D  T  M  K  S  V  F  A  A  V  D  Y  I
901     AGCAATGAACTCGCTTCTACAATCCAGTCCTCTTCTTCAAATGATCTTGCGGTAGTTGAGAAGGAAAAGAAAGTTGAAGAACTGATGGAA
301     S  N  E  L  A  S  T  I  Q  S  S  S  S  N  D  L  A  V  V  E  K  E  K  K  V  E  E  L  M  E
991     ATGAATCAAGGCTTGCTCCAATGCATGGGGGTTAGTCACGCGTCTATCGAAACAGTAATTAGCACAACACACAAGTATAAATTATCTTCC
331     M  N  Q  G  L  L  Q  C  M  G  V  S  H  A  S  I  E  T  V  I  S  T  T  H  K  Y  K  L  S  S
1081    AAATTGACTGGAGCTGGTGGCGGTGGATGTGTTATAACTCTATTACCAGCACTATTATCTGCCTCGGTTGTTGATGCAGTAACGGCTGAG
361     K  L  T  G  A  G  G  G  G  C  V  I  T  L  L  P  A  L  L  S  A  S  V  V  D  A  V  T  A  E
1171    CTTGAGCAGTGTGGATTCCAATCTTACATTGCAGGAATAGGTGGAAAAGGACTTGAAATTCGGTTTGGAGGAGTTCATGATATATTTAGT
391     L  E  Q  C  G  F  Q  S  Y  I  A  G  I  G  G  K  G  L  E  I  R  F  G  G  V  H  D  I  F  S
1261    ATATGACCATCTCAAGGGCCGGACCATCGTCAGTCTAGCGCAGATTGAAGTCAAGATCAATGAATCATCTTCCACATAAGCAGCGAAGGA
421     I  *
1351    GTACACGTACACTATTTTAAAGCATCAACGTTGTTAGTTTTTATCGTTATTCAGTTGACATTGGCGTTAGATCATTAGTCTCAACATTAT
1441    GTTCTTGTGTAACCATTTGAATTTCATGTTTTATAGATAAATTACGTTGCAAGAACATGTTCGCACCATAAAATTTTCCTAACATTTCAT
1531    CCGTTGCCCTCATACAACAAGAAAGAACACG
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Fig. 1: The Nucleotide sequence and deduced amino acid sequences of the full-length cDNA of *CnMVK*. The initial codon and the stop codon are highlighted in square box.

Characterization of CnMVK protein

ExPASy online (http://web.expasy.org/ compute_pi/) analysis results displayed that the theoretical molecular weight and isoelectric point (pI) of the CnMVK protein were 42.8 kDa and 5.79, respectively. The multiple sequence alignment of MVK protein sequences among different plants using software Vector NTI11.5 showed that CnMVK is highly homologous to the MVK protein sequences of other plants, further sequencing analysis showed that the protein sequence of CnMVK keep a strong conservation during the molecular evolution (Fig. 2), different plant MVK genes have similar conserved domains, indicating that each plant MVK gene has a similar enzymatic reaction (Chu and Li, 2003). As shown in Table 1, the protein sequence of CnMVK had high identity with other MVK proteins, it showed 68%, 67%, 67%, 66%, 66%, 66%, 65% and 65% similarity to MVK proteins from *Platycodon grandiflorus*, *Panax notoginseng*, *Nicotiana tomentosiformis*, *Sesamum indicum*, *Bacopa monnieri*, *Catharanthus roseus*, *Solanum pennellii* and *Olea europaea*, respectively, it was further verified that *CnMVK* is one of members of the MVK gene family of *C. nobile*.

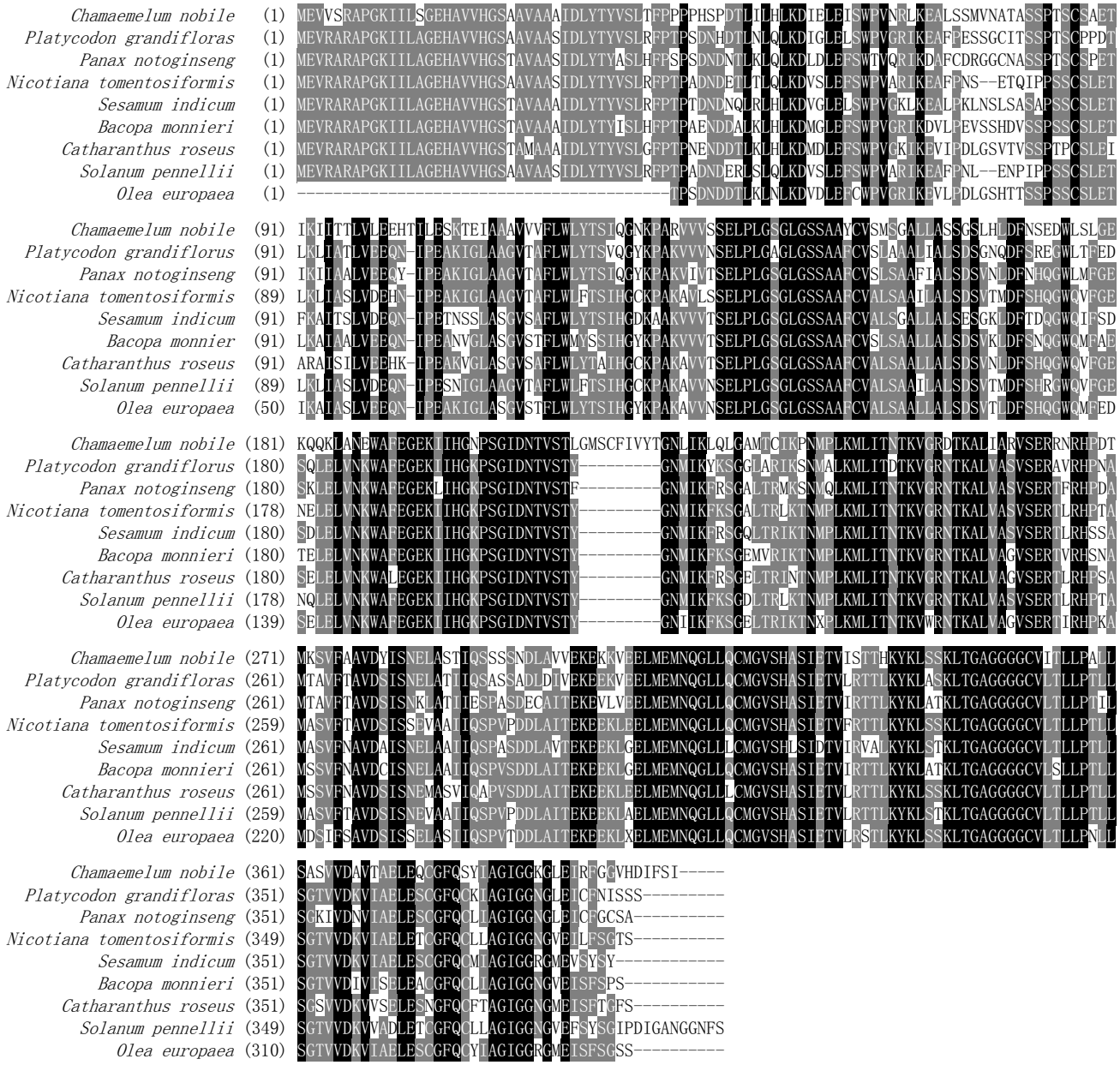

Fig. 2: Multiple sequence alignment of MVK proteins. The completely identical amino acids are indicated with white foreground and black background.

Table 1. Protein sequence of CnMVK similarity to MVKs of other plant species.

Species	Accession no. in GenBank	Identity/%	E-value
Platycodon grandiflorus	AGZ15315.1	68	2e-174
Panax notoginseng	AFN02124.1	67	1e-178
Nicotiana tomentosiformis	XP_009629945.1	67	3e-172
Sesamum indicum	XP_011092024.1	66	1e-179
Bacopa monnieri	AFJ93086.1	66	4e-171
Catharanthus roseus	ADR65111.1	66	3e-166
Solanum pennellii	XP_015062317.1	65	2e-179
Olea europaea	AFS28683.1	65	3e-150

Phylogenetic analysis of CnMVK

To investigate the evolutionary relationships among MVK proteins, a phylogenetic tree was constructed based on the deduced amino acid sequences of predicted CnMVK and MVK proteins from other plant species (Fig. 3). MVK phylogenetic tree is divided into two branches of monocotyledons and dicotyledons. *C. nobile* belonged to dicotyledons in the branch of dicotyledons and monocotyledons. *C. nobile* has the closest relationship with *Cynara cardunculus*, it is inferred that CnMVK has similar catalytic activity to MVK protein of *C. cardunculus*. *C. nobile* also has a genetic relationship with other dicotyledons and monocotyledons. This reflects the evolutionary conservation and evolutionary diversity of plant MVK genes, which is consistent with the morphological classification of plants.

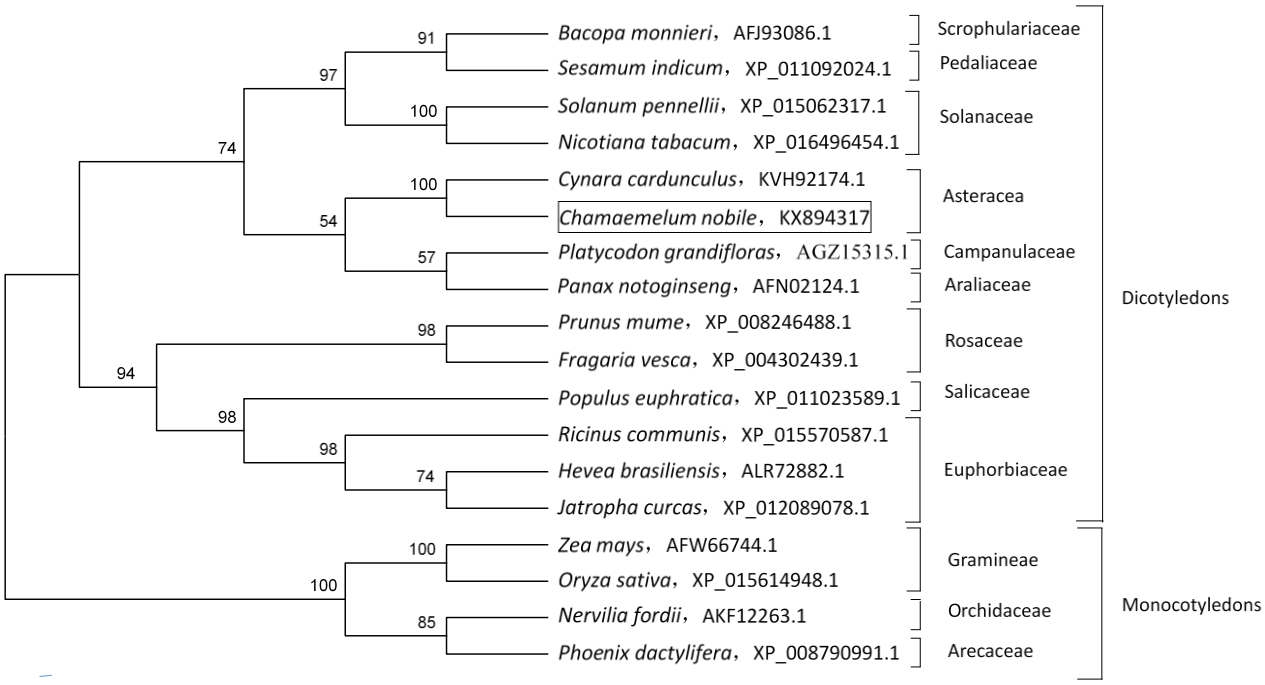

Fig. 3: Phylogenetic tree of MVK using Neighbor-Joining method. The number shown at each branch indicated the bootstrap values (%).

Conclusion

In this study, we have successfully isolated and cloned *CnMVK* gene from *C. nobile*, the full-length cDNA of the gene is 1561bp with an ORF 1206bp, encoding 402 amino acids. The multiple sequences alignment indicated that *CnMVK* had high identity with other MVK genes isolated from other plants. The phylogenetic analysis demonstrated that *CnMVK* keep a strong conservation during the molecular evolution and the evolutionary of plant MVK also keeps diversity. The study will provide evidence for the molecular function of *CnMVK* and lay the foundation for the further application of *CnMVK* in regulating the synthesis of terpenoid constituents.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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