Phytochemistry xxx (2017) 1–10

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

Bornyl-diphosphate synthase from *Lavandula angustifolia:* A major monoterpene synthase involved in essential oil quality

Yolande Despinasse [a, b, c], Sébastien Fiorucci [d], Serge Antonczak [d], Sandrine Moja [a, b, c], Aurélie Bony [a, b, c], Florence Nicolè [a, b, c], Sylvie Baudino [a, b, c], Jean-Louis Magnard [a, b, c], Frédéric Jullien [a, b, c, *]

[a] Université de Lyon, F-42023, Saint-Etienne, France
[b] Université de Saint-Etienne, Jean Monnet, F-42000, Saint-Etienne, France
[c] Laboratoire de Biotechnologies Végétales Appliquées aux Plantes Aromatiques et Médicinales, EA 3061, 23 Rue du Dr Michelon, F-42000, Saint-Etienne, France
[d] Institut de Chimie de Nice, UMR-CNRS 7272, Faculté des Sciences, Université de Nice-Sophia Antipolis, 06108 Nice Cedex 2, France

ARTICLE INFO

Article history:
Received 14 November 2016
Received in revised form
19 January 2017
Accepted 21 January 2017
Available online xxx

Keywords:
Lavandula angustifolia (*Lamiaceae*)
Terpene synthase
Transcript regulation
Camphor

ABSTRACT

Lavender essential oils (EOs) of higher quality are produced by a few *Lavandula angustifolia* cultivars and mainly used in the perfume industry. Undesirable compounds such as camphor and borneol are also synthesized by lavender leading to a depreciated EO. Here, we report the cloning of bornyl diphosphate synthase of lavender (*LaBPPS*), an enzyme that catalyzes the production of bornyl diphosphate (BPP) and then by-products such as borneol or camphor, from an EST library. Compared to the BPPS of *Salvia officinalis*, the functional characterization of LaBPPS showed several differences in amino acid sequence, and the distribution of catalyzed products. Molecular modeling of the enzyme's active site suggests that the carbocation intermediates are more stable in LaBPPS than in SoBPPS leading probably to a lower efficiency of LaBPPS to convert GPP into BPP. Quantitative RT-PCR performed from leaves and flowers at different development stages of *L. angustifolia* samples show a clear correlation between transcript level of *LaBPPS* and accumulation of borneol/camphor, suggesting that LaBPPS is mainly responsible of *in vivo* biosynthesis of borneol/camphor in fine lavender. A phylogenetic analysis of terpene synthases (TPS) pointed out the basal position of LaBPPS in the TPSb clade, suggesting that LaBPPS could be an ancestor of others lavender TPSb. Finally, borneol could be one of the first monoterpenes to be synthesized in the *Lavandula* subgenus. Knowledge gained from these experiments will facilitate future studies to improve the lavender oils through metabolic engineering or plant breeding.

Accession numbers: LaBPPS: KM015221.

1. Introduction

In 2015, cultivation of lavenders in the south of France extended over 20,000 ha leading to the production of respectively 1400 and 80 tons of lavandin (*Lavandula x intermedia*) and true lavender (*Lavandula. angustifolia subsp. angustifolia*) essential oils (EOs). Besides the fact that lavender is a touristic emblem of Provence, this agricultural activity has a major economic interest and represents more than half of the world production of lavender EO. The quality of lavender EO depends firstly on the amount of desirable major flavour compounds such as linalool and linalyl acetate that are characteristic terpenes of lavender scent, but also on the specific aromatic touch given by several minor compounds. Lavender EOs of higher quality are produced by a few *L. angustifolia* cultivars and mainly used in the perfume industry. Undesirable compounds such as camphor and borneol can also be produced by lavender leading to a depreciated EO; such terpenes are strongly produced in lavandin, a hybrid between *L. angustifolia* and *L. latifolia*, leading to a restricted use of their EOs in the soap industry. A genetic knowledge of camphor biosynthesis in lavender is therefore an important goal to develop new strategies leading to the production of lavender cultivars with improved EO depleted in camphor.

Monoterpenes and sesquiterpenes are the main components of lavender EO (Charles et al., 2002; Shellie et al., 2002) and are

* Corresponding author. Université de Lyon, F-42023, Saint-Etienne, France.
E-mail address: jullien@univ-st-etienne.fr (F. Jullien).

http://dx.doi.org/10.1016/j.phytochem.2017.01.015
0031-9422/

derived from the condensation of isopentenyl diphosphate (IPP) and its allylic isomer, dimethylallyl diphosphate (DMAPP). Condensation of one DMAPP and two IPP molecules catalyzed by farnesyl diphosphate synthase (FPPS) leads to the formation of FPP in the cytosol whereas the condensation of one DMAPP and one IPP catalyzed by geranyl diphosphate synthase (GPPS) leads to the formation of GPP in the plastid. GPP and FPP are substrates of terpene synthases (TPS) for the synthesis of mono- and sesquiterpenes respectively. Terpene metabolism in lavender has already been well depicted (Fig. 1), leading to the characterization of four monoterpene synthases (Demissie et al., 2011, 2012; Landmann et al., 2007) and five sesquiterpene synthases (Jullien et al., 2014; Lukman et al., 2013). Moreover a cis-prenyl diphosphate synthase involved in lavandulyl diphosphate synthesis, a precursor of lavandulol and lavandulyl acetate *in planta*, was also characterized (Demissie et al., 2013). All these enzymes are responsible for the biosynthesis of the major terpenes found in lavender EO, except for camphor and related compounds such as borneol and bornyl acetate.

Bornyl diphosphate synthase (BPPS), the first enzymatic step of camphor biosynthesis, has been cloned only once in *Salvia officinalis* (Wise et al., 1998). This unusual TPS is a multi product enzyme giving both several monoterpenes as minor compounds and bornyldiphosphate (BPP) as a major prenyldiphosphate (Fig. 2). BPP is further dephosphorylated, leading to the monoterpene borneol, then oxidized in camphor by a borneol dehydrogenase (Lukman et al., 2012). The homodimeric BPPS has raised notable attention and was the first monoterpene synthases (mTPS) to be crystallized (Whittington et al., 2002). This study revealed that water molecules firmly anchored in the active site could prematurely quench carbocationic substrates, leading to the synthesis of different monoterpenes. Recently, a computational enzymologic study of BPPS permitted to propose the formation of several possible carbocation intermediates in the active site (Fig. 2) leading to side-product monoterpenes and pointed out electrostatic factors steering favorably the formation of the bornyl cation further stabilized by the diphosphate moiety (Major and Weitman, 2012; Weitman and Major, 2010).

In this study, we characterized the BPPS of lavender as a new TPS involved in major terpene biosynthesis in *Lavandula* species. Molecular modeling of LaBPPS and SoBPPS was carried out to compare their active sites and explain the relationship between their structure and function, especially the differences in their products' distribution. Transcript levels from qRT-PCR and essential oil accumulation in *L. angustifolia* cv Diva were analyzed in leaves and flowers at different developmental stages to examine the regulation of this TPS.

2. Results and discussion

2.1. Sequence analysis

Partial sequences of *LaBPPS* were obtained from 12 sets of ESTs from a 454 cDNA library (Jullien et al., 2014). Amplification of 5′ and 3′ ends was carried out by RACE assays. Primers were then defined in both 5′ and 3′ UTR to amplify the *LaBPPS* in full length (Table S1).

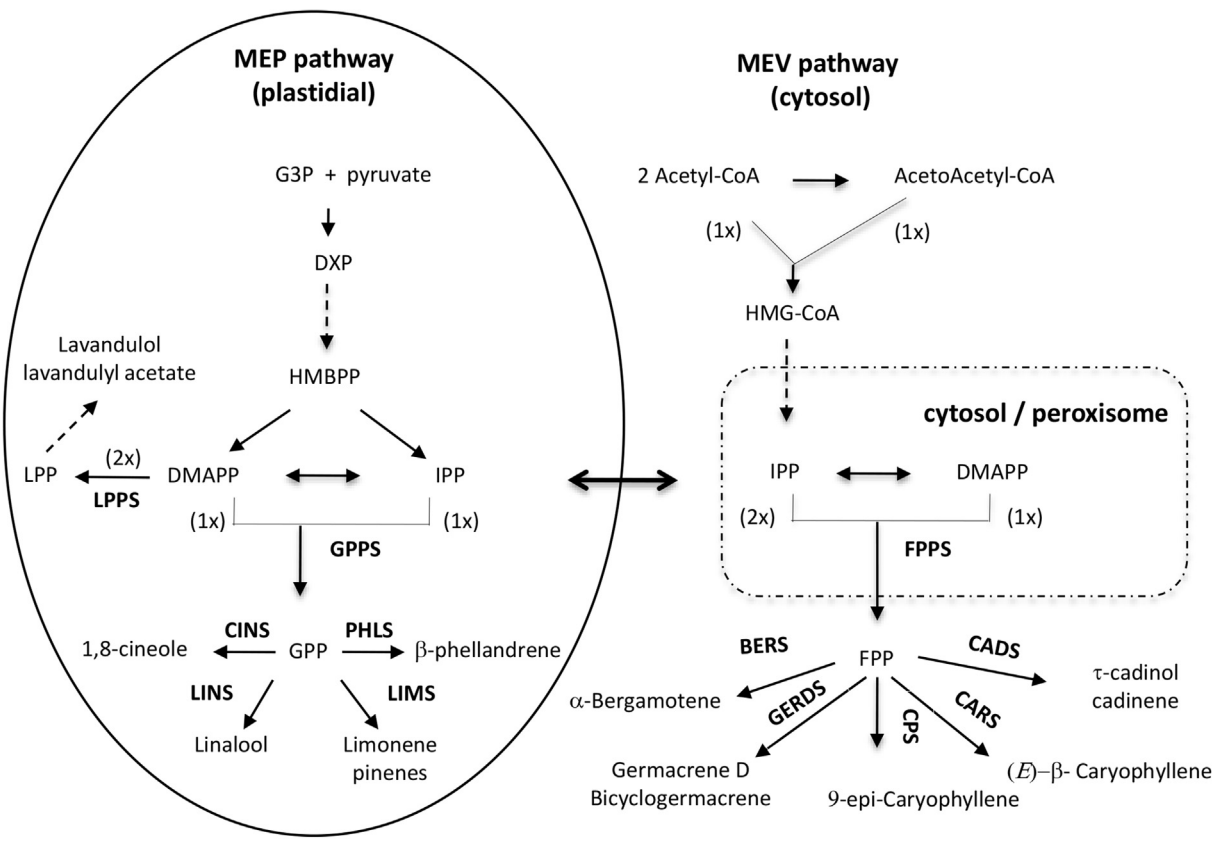

Fig. 1. Pathways of mono and sesquiterpene biosynthesis in *Lavandula angustifolia* and *L.* x *intermedia*. BERS, α-bergamotene synthase; CADS, cadinol synthase; CARS, β-caryophyllene synthase; CDP-ME, 4-(cytidine 5′-diphospho)-2-C-methyl-D-erythritol; CINS, cineole synthase; CPS, 9-epi-caryophyllene synthase; DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose 5-phosphate; FPP, farnesyl diphosphate; FPPS, FPP synthase; GERDS, germacrene D synthase; G3P, glyceraldehyde 3-phosphate; GPP, geranyl diphosphate; GPPS, GPP synthase; HMBPP, 1-hydroxy-2-methyl-2(*E*)-butenyl 4-diphosphate; IPP, isopentenyl diphosphate; LIMS, limonene synthase; LINS, linalool synthase; LPP, lavandulyl diphosphate; LPPS, LPP synthase; MEP, 2-C-methyl-D-erythritol 4 phosphate; MEV, mevalonate; PHLS, β-phellandrene synthase.

Please cite this article in press as: Despinasse, Y., et al., Bornyl-diphosphate synthase from *Lavandula angustifolia:* A major monoterpene synthase involved in essential oil quality, Phytochemistry (2017), http://dx.doi.org/10.1016/j.phytochem.2017.01.015