PS8: 2

Isolation and molecular cloning of γ-terpinene synthase gene from *Thymus caespititius*

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Thymus caespititius Brot., commonly known as 'tormentelo' or 'erva-úrsula', is a *Lamiaceae* aromatic specie endemic of the NW Iberian Peninsula, and of the Azores and Madeira archipelagos characterized for high essential oil chemical variability [1, 2]. As part of an ongoing effort to isolate genes involved in scent production on different chemotypes, genomic characterization of exon and intron numbers, sizes and placement, of a putative gene encoding a monoterpene synthase, γ-terpinene synthase (*TcTPS2*), was performed on chemically distinct *T. caespititius* accessions collected at Azorean islands and in the Mainland Portugal. In *Origanum vulgare TPS*2 is responsible for the first step of the 'cymyl'-pathway, giving rise to phenolic terpene isomers thymol and carvacrol and related compounds [3]. Being these terpenes the main components in two of the chemotypes of *T. caespititius*, the present work aims at showing the expression of *TcTPS*2 in *Thymus*. *T. caespititius* mRNA was isolated from aerial parts collected during the flowering stage and a homology based RT-PCR strategy was used to clone the *TcTPS2* gene. One cDNA clone (*TcTPS2*-D1) was chosen to perform the heterologous expression in *Escherichia coli* for further characterization. A BLASTP search on GenBank revealed 27 to 93% of similarity of the cloned Tc*TPS2* gene to other known terpene synthases genes from different members of other Lamiaceae species. Full-length His•Tag *TcTPS2*-D1 cDNA was ligated to the vector pET-29a(+) for protein expression. Recombinant TPS2 was detected in *E. coli* cultures by SDS-PAGE with the predicted molecular weight (67 kDa). The best soluble protein production was obtained for cultures induced with 0.2mM of isopropyl-1-thio-b-d-galactopyranoside (IPTG) for 19h at 20ºC in a rotary shaker. Scale-up protein production is in progress, and further purification as well as enzymatic assays will be performed. Herewith reported for the first time for the genus *Thymus*, these cloning and expression approaches will contribute to elucidate the function of these *TPS* genes.

1. Figueiredo A.C. et al. (2010) Nat. Prod. Commun. 5: 1465-1476, 2. Figueiredo A.C. et al. (2008). Cur. Pharm. Design 14: 3120-3140, 3. Crocoll C. et al. (2010). Plant Mol. Biol. 73: 587-603. *Acknowledgments: This study was partially funded by the Fundação para a Ciência e Tecnologia (FCT) under research contract PTDC/AGR-GPL/101334/2008.*