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Cloning and Characterization of a cDNA encoding Squalene Synthase from Panax ginseng C.A. Meyer

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Objectives

In order to metabolic enginneering of ginseng saponin, we have isolated and characterized a squalene synthase from a full-length cDNA library of Panax ginseng.

Materials and Methods

1. Material

Panax ginseng C.A. Meyer leaf cDNA library

2. Methods

A full-length cDNA clone of PSS was isolated from expressed sequence tags of ginseng leaf cDNA library. Full-length sequencing of a putative squalene synthase was performed by primer working with an automated DNA sequencer (ABI Prism 3700, Applied Biosystems, USA). The analysis of nucleotide and putative protein sequences was performed with ClustalW (http://www.ebi.ac.uk/ clustalw/) and the DNASIS program(Hitachi, Japan).

Results and Discussion

Squalene synthase (farnesyl-diphosphate farnesyltransferase) mediates the condensation of two molecules of farnesyl diphosphate to form an intermidiate, presqualene diphosphate (PSPP) and the subsequent rearrangement and reduction of PSPP, with NADPH as a hydride donor, to produce squalene.

We have isolated and characterized a full length cDNA clone encoding squalene synthase (AB115496, *PSS*) in *Panax ginseng*. Expressed sequence tag (EST) analysis was performed with a full length cDNA library constructed with ginseng leaf. One clone (DC05014g07) of 3,000 EST showed high homology with squalene synthase gene. We carried out full length sequencing with primer working using automatic sequencer (ABI3700). The cDNA clone was 1,476 bp long and carried an open reading frame of 415 amino acids giving a predicted molecular mass of 47 kDa. The *PSS* showed 100% identity at amino acid residues with previously reported ginseng squalene synthase (AB010148), but it was more long 42 bp at the 5' untranslated region. In comparison with currently described squalene synthase (*SS*) from other species, the deduced amino acids of *PSS* showed the highest identity with *Glycine max* (84.1%), followed by *Glycyrrhiza glabra* (82.6%), *Lotus japonicus* (72.4%). The *PSS* clone was clustered with *Nicotiana* tabacum and *SS* genes registered in plants were divided into two groups of monocotylendons and dicotylendons.

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