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Molecular cloning and tissue-specific expression of two cDNAs encoding polyketide synthases from *Hypericum perforatum*

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Summary

Two previously uncharacterized cDNAs encoding for polyketide synthases (PKSs), designated as *HpPKS1* and *HpPKS2*, were isolated from *Hypericum perforatum*. The full-length *HpPKS1* was 1573 bp containing an open reading frame (ORF) of 1161 bp encoding for a 386 amino acid protein. The full-length cDNA of *HpPKS2* was 1559 bp with an ORF of 1182 bp encoding for a 393 amino acid protein. The highly conserved catalytic amino acid residues common to plant-specific PKSs were preserved in both genes. *HpPKS1* and *HpPKS2* exhibited distinct tissue-specific expression patterns in *H. perforatum*. The *HpPKS1* expression was highest in flower buds and lowest in root tissues. The expression of *HpPKS2* was found to be high in flower buds and leaf margins and low in leaf interior parts, stems and roots. The expression of the *HpPKS1* was found to correlate with the concentrations of hyperforin and adhyperforin while the expression of *HpPKS2* showed correlation with the concentrations of hypericins and pseudohypericins in *H. perforatum* tissues.

Introduction

Polyketide synthases (PKSs) condensing various CoA-thioesters with malonyl-CoA biosynthesize a large variety of plant secondary metabolites, including flavonoids, stilbenes, benzophenones and benzalacetone derivatives (Schröder, 1997). Chalcone synthase (CHS) and stilbene synthase (STS) are the most investigated members of the plant-specific PKS family at both the biochemical

Abbreviations: BPS, benzophenone synthase; CHS, chalcone synthase; DAD, diode array detector; HPLC, high-performance liquid chromatography; ORF, open reading frame; PCR, polymerase chain reaction; PKS, polyketide synthase; RACE, rapid amplification of cDNA ends; rRNA, ribosomal RNA; STS, stilbene synthase; VPS, valerophenone synthase

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and the molecular levels. Recently, a growing number of functionally diverse plant-specific CHS-related PKSs have been characterized and cloned (Eckermann et al., 1998; Lukačin et al., 1999; Abe et al., 2001; Okada and Ito, 2001; Abe et al., 2004).

Despite the functional diversity, the amino acid sequences of the plant-specific PKS proteins share common features. Comparative analyses have revealed that these proteins contain approximately 400 amino acids with highly conserved amino acid residues in the active center. The catalytic triad, consisting of Cys164, His303 and Asn336 residues, is absolutely conserved in all plant-specific PKSs (Jez et al., 2002). The two ''gatekeeper'' phenylalanines are preserved in almost all of the plant-specific PKSs with a few exceptions (Abe et al., 2001; Jez et al., 2002). The PKS family proteins also exhibit some additional conserved amino acid residues that participate in shaping the geometry of the active site (Ferrer et al., 1999). All known plant-specific PKSs are homodimers with a subunit molecular mass of 40–45 kDa. The common features in amino acid sequences make possible the isolation of new genes coding for PKS family proteins by homology-based techniques, such as using degenerate oligonucleotide primers.

Hypericum perforatum L., St. John's wort, is a medicinal plant that is widely utilized for the treatment of mild to moderate depression (Müller, 2003). Extracts of this species have also been found to exhibit antitumoral (Hadjur et al., 1996; Schempp et al., 2002; Medina et al., 2006) and antibacterial activities (Schempp et al., 1999; Medina et al., 2006). The clinically demonstrated major bioactive compounds of *H. perforatum* are hypericins, pseudohypericins and acylphloroglucinols; more precisely, hyperforin and adhyperforin (Barnes et al., 2001). It is presumed that these compounds are biosynthesized via a polyketide pathway by PKSs (Adam et al., 2002; Bais et al., 2003; Klingauf et al., 2005), but genes encoding for PKSs responsible for their biosynthesis have not been isolated. Previously, three PKS family genes have been cloned from genus *Hypericum*. CHS and benzophenone synthase (BPS) have been cloned from cell cultures of *H. androsaemum* (Liu et al., 2003), and CHS has been cloned from *H. perforatum*.

In this study, we aimed to isolate PKS coding genes from *H. perforatum* by a homology-based method with degenerate primers. With this approach, we obtained two previously uncharacterized cDNAs from *H. perforatum*, designated as *HpPKS1* and *HpPKS2*, which both, based on sequence characterization, encode for PKS family proteins. The expression patterns of these genes in different *H. perforatum* tissues were analyzed by real-time polymerase chain reaction (PCR). The possible involvement of *HpPKS1* and *HpPKS2* in the biosynthesis of hypericins, pseudohypericins and acylphloroglucinols in *H. perforatum* is discussed.

Materials and methods

Plant material

H. perforatum roots, stems, leaves and flower buds were collected from the Botanical Gardens of the University of Oulu, Finland. The leaves were dissected into leaf margins containing dark glands and into leaf interior parts free of dark glands. Immediately after excision, all samples were frozen in liquid nitrogen and stored at −80 °C until used for gene expression analyses and determination of bioactive compounds.

Isolation of RNA and cDNA preparation

Total RNA was isolated from different tissues of *H. perforatum* according to Jaakola et al. (2001). The cDNA was synthesized from 3 μg of total RNA using SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA) with random primers according to the manufacturer's instructions. The cDNA was purified from contaminating genomic DNA by using the method described by Jaakola et al. (2004).

Isolation of core cDNA fragments of PKS

Degenerated oligonucleotide primers were designed based on the conserved amino acid sequences that have been found in the alignment of several previously isolated plant-specific PKS coding genes. The sequences of the degenerate primers were 5′-RGC MAT YRA DGA RTG GGG-3′ (forward primer) and 5′-TTY TCN GCN ADR TCC TTS G-3′ (reverse primer). PCR was performed with DyNazyme™ II DNA polymerase (Finnzymes, Espoo, Finland). The PCR conditions were 94 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, 50 °C for 2 min and 72 °C for 2 min, and final extension at 72 °C for 10 min. The PCR products were separated by electrophoresis on a 1% (w/v) ethidium bromide-stained agarose gel. The PCR fragment of the predicted size (205 bp) was excised from the agarose gel and purified using Montage® DNA Gel Extraction Kit (Millipore, Bedford, MA, USA). The purified PCR products were ligated into a pGEM-T Easy vector (Promega, Madison, WI, USA) and sequenced.

Obtaining full-length cDNAs of *HpPKS1* and *HpPKS2*

In order to obtain full-length cDNAs of *HpPKS1* and *HpPKS2* genes, 3′- and 5′-rapid amplification of cDNA ends (RACE) were performed using the SMART™ RACE cDNA Amplification Kit (Clontech, Palo Alto, CA, USA) according