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论文

金铁锁鲨烯合酶cDNA的克隆和功能鉴定

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摘要：

濒危药用植物金铁锁(*Psammosilene tunicoides* W.C.Wu et C.Y.Wu.)的有效成分三萜总皂苷有显著的药理活性。为克隆和鉴定金铁锁三萜皂苷生物合成途径中的关键酶基因——鲨烯合酶的全长cDNA，本研究采用同源兼并引物PCR和cDNA末端快速扩增(RACE)等方法克隆了其全长cDNA；结合大肠杆菌异源表达、体外酶促反应及针对产物化学结构的GC和GC-MS分析等方法鉴定了其功能。研究结果表明：金铁锁鲨烯合酶cDNA全长为1 663 bp，含有1 245 bp的开放阅读框(ORF)，编码414个氨基酸(计算分子质量为47.69 kD)，5′非编码区(UTR)和3′UTR分别为260 bp和158 bp，GenBank注册号为EF585250，与三七、人参和甘草的鲨烯合酶的氨基酸序列有较高的同源性，分别为83%、 82%和82%，而与裂变酵母、白色念珠菌和人的氨基酸序列的同源性分别只有35%、 39%和47%；表达产物具有催化两分子法呢烯焦磷酸连接成鲨烯的活性。本研究克隆和鉴定了金铁锁鲨烯合酶的全长cDNA，为金铁锁次生代谢工程研究提供了重要基础。

关键词： 金铁锁 三萜皂苷 RACE GC-MS 金铁锁鲨烯合酶

Cloning and characterization of cDNA encoding *Psammosilene tunicoides* squalene synthase

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Abstract:

The total triterpene saponins of *Psammosilene tunicoides* have significant pharmacologic activity. *Psammosilene tunicoides* squalene synthase (PSS) is a gateway enzyme to regulate the biosynthesis of total triterpene saponins extracted from the root of *Psammosilene tunicoides* which is an endangered species. In this paper, cDNA encoding of PSS was cloned by the degenerate primer PCR and rapid-amplification of cDNA ends (RACE). The full-length of cDNA of PSS is 1 663 bp, with an open reading frame (ORF) of 1 245 bp, encoding 414 amino acid polypeptide (calculated molecular mass, 47.69 kDa), 5′UTR (untranslated region) and 3′UTR are 260 bp and 158 bp, respectively. The deduced amino acid sequence of PSS has higher homology with the known squalene synthases of several species such as *Panax notoginseng* (83%), *Panax ginseng* (82%) and *Glycyrrhiza glabra* (82%) than that with *Schizosacharomyces pombe* (35%), *Candida albicans* (39%) and *Homo sapiens* (47%). The characterization of PSS was done by a series of methods, such as prokaryotic expression, the activity of enzyme *in vitro*, capillary gas chromatography (GC) and capillary gas chromatography mass spectrometry (GC-MS). The results showed that the cell-free extract of *E.coli* transformed with the recombinant plasmid can effectively convert farnesyl diphosphate into squalene *in vitro*. GenBank accession number is EF585250. Our research provided important base for the study of *Psammosilene tunicoides* secondary metabolism and metabolic engineering.

Keywords: triterpene saponins RACE GC-MS *Psammosilene tunicoides* squalene synthase *Psammosilene tunicoides*

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