ISOLATION AND CHARACTERIZATION OF cDNA CLONE CODING FOR LOTUS JAPONICUS NODULE SUCROSE SYNTHASE

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Key words: *Lotus japonicus*, clone, sucrose synthase (SuSy), root nodules.

Abstract: To study the expression of genes coding for enzymes involved in carbon metabolism, we were concerned with the isolation and characterization of cDNA clone coding for sucrose synthase. The nucleotide sequence of the cDNA clone encoding *Lotus japonicus* sucrose synthase was determined. The amino acid sequences of this clone were aligned with other homologous sucrose synthase sequences, derived from different organisms, in order to identify the conserved regions. The aim of the study was to describe the isolation and characterization of a cDNA clone encoding for a *Lotus japonicus* root nodule specific sucrose synthase, whose predicted amino acid sequence is conserved in plant kingdom.

INTRODUCTION

Bacteria belonging to the family Rhizobiaceae (*Rhizobium, Bradyrhizobium, Azorhizobium, Mesorhizobium and Sinorhizobium*) induce the formation of nitrogen fixing nodules on their leguminous hosts. This symbiotic interaction, which is governed by sequential signal exchange between rhizobia and their symbiotic partners, exhibits a high degree of specificity and a number of signal molecules involved in the initial stages of this specificity have been extensively studied. Nod factors are synthesized by the products of rhizobial nod genes, which are induced by plant-secreted molecules such as flavonoids (Hirsh, 1992; Long, 1996).

During the course of nodule initiation and development a number of plant genes, referred to as nodulin genes are induced (van Krammen, 1984; Handberg and Stougaard, 1992; Mylona et al., 1995). One of those, called late noduline is sucrose synthase (SuSy, E.C.2.4.1.13), which has an important role in nodule metabolism (Thummler and Verma, 1997, Verma, 1988; Verma and al, 1992). This enzyme play a key role in supplying energy for loading and unloading in phloem by providing substrate for respiration; is also involved in meeting the increased glycolytic demand during anaerobic and cold stress as well as in supplying UDP-glucose for cell wall biosynthesis (Chourey et. al., 1998; Nakai et al., 1999).

In the root nodule, there are some functions which have been attributed to SuSy: cleavage of sucrose is the initial step in the nodule carbohydrate metabolism, assimilate partitioning, osmoregulation, adaptation to cold and low oxygen levels response to wounding and infection (Winter and Huber, 2000).

An important method to obtain information on genes expressed in an organism is the systematic sequencing of cDNA clones, generally known as the **EST** (Expressed Sequence Tag) approach (Boguski, 1995; Claverie, 1995). It is the fastest way of gene discovery in eukaryotes. EST serves as markers for genes expressed by a certain cell type or tissue under specific environmental (culture) conditions, and are used for the discovery of full length cDNA or genomic clones, discovery of new genes, recognition of exon/intron boundaries and development of genetic maps. Furthermore, ESTs with no homology to known proteins may provide the first clues of new proteins (Baxevanis et al., 1998).

To make a catalogue of genes expressed in *L. japonicus* L. and understand biological processes specific to legume plants, large-scale EST analyses have been performed.

With the aim of understanding the genetic system related to legume-specific biological processes, recently, large numbers of expressed sequences tags (ESTs) from *Lotus japonicus* nodules have been deposited in public databases and analyzed by DNA arrays for transcriptome analysis. The sequence information and search results of these *Lotus japonicus* clones, generated in different studies, are available at the web sites: **http://www.kazusa.or.jp/en/plant/lotus/EST** and **http://www.agowa**. Similarity search was performed against the public EST database using the BLAST program. A BLAST score of more than 80 is generally regarded as a significant match (Pearson, 1997).

This enables identification of genes coding for enzymes involved in diverse metabolic pathways, whose expression is induced during nodule formation and functioning (Asamizu et al., 2000).

The aim of the study was to isolate and to characterize a cDNA clone coding for Lotus japonicus L. nodule sucrose synthase.

MATERIALS AND METHODS

Isolation of cDNA clones for *Lotus japonicus* L. specific sucrose synthase

For the isolation of *L. japonicus* clones homologues to GmSuSy, one αZAPII *L japonicus* cDNA library from 21-day-old nodules (kindly provided by Dr. J. Stougaard, University of Aarhus, Denmark) was screened by plaque hybridization using ^{32}P-dCTP- labelled insert of the GmSuSy cDNA clone as a probe. Hybridization was performed under low stringency conditions at 35ºC in the presence of 25% formamide. Plaque purification and *in vivo* excision of the pBlueScriptSK$^+$ phagemid from the positive αZAPII recombinant bacteriophages were performed according to the standard protocols (Stratagene, La Jolla, CA, U.S.A.). The nucleotide sequence of the cDNA clones giving positive hybridization signal was determined by the dideoxi chain termination method (Sanger et al., 1997, Sambrook et al., 1989).

Characterization of cDNA clone coding for sucrose synthase

The characterization of cDNA clone coding for sucrose synthase was done using the ExPASy Proteomics tools which translate a nucleotide sequence to a protein sequence (http://au.expasy.org/tools/dna.html).

RESULTS AND DISCUSSIONS

Isolation of cDNA clones for *Lotus japonicus* L. specific sucrose synthase

In order to isolate a cDNA clone coding for *L.japonicus* nodule sucrose synthase **(**designated **Lj SuSy)**, an αZAPII cDNA library prepared from 21-days-old nodules was screened under low stringency conditions using a previously published *Glycine max* L. (GmSuSy) cDNA clone as heterologous probe.

Characterization of cDNA clone coding for sucrose synthase

A nodule specific sucrose synthase cDNA clone contained an open reading frame (ORF) of 1202 base pairs encoding a polypeptide of 153 amino acids. The nucleotide sequences corresponding to the longest open reading frame (ORF) are shown in Fig. 1.

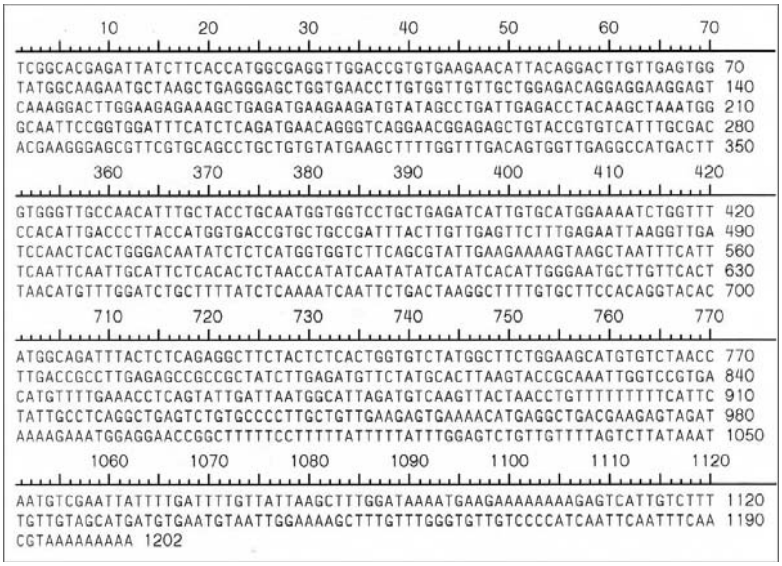

Figure 1: The nucleotide sequences of the cDNA clone encoding *Lotus japonicus* L. sucrose synthase (LjSuSy).

Most plant species contain at least two isoforms of sucrose synthase which usually have highly homologous amino acid sequences and similar biochemical properties. (Gross and Pharr, 1982; Buczynski et. al., 1993). By contrast, the regulation of their genes is markedly different and distinct developmental - and organ-specific expression patterns have been found (Chen et. al., 1989; Fu and Park, 1995; Sturm et al, 1999). Such an effort in regulating gene expression and the localization of an enzyme would suggest a crucial role in plant metabolism.

The complete nucleotide sequence of the cDNA clone coding for sucrose synthase (LjSuSy) was translated into amino acid sequence and compared with the published sequences in the GenBank database using the BLAST program at the ExPASy National Centre for Biotechnology Information website (http://www.ncbi.nlm.nih.gov/BLAST/).

The amino acid sequences of these clones were aligned with other homologous sucrose synthase sequences, derived from different organisms, in order to identify the conserved regions.

The multiple amino acid sequence alignment of LjSuSy with other known plant sucrose synthases expressed in sink tissues revealed that LjSuSy exhibits 97.4% and 96.7% similarity with *G. max* and *V. faba* nodule sucrose synthase respectively.

Low percentages of similarity were observed with sucrose synthase proteins isolated from *M. sativa* and *P. sativum* (96.1%). The lowest similarity was registered for the *A. thaliana* (88.2%) (Table 1).

Table 1. Comparative presentation of similarity of LjSuSy gene with other known plant SuSy genes expressed in sink tissues: *L. japonicus* SuSy, *V. faba* SuSy (P31926), *A. thaliana* SuSy (P49040), *G. max* SuSy (P13708), *M. sativa* SuSy (O65026), *P. sativum* SuSy (O81610). The analyze was made using the DNASTAR program, Clustal method with PAM250 residue weight table (Altschul et al., 1990).

	LjSuSy	VfSuSy	AtSuSy	GmSuSy	MsSuSy	PsSuSy
LjSuSy	***	**96,7**	**88,2**	**97,4**	**96,1**	**96,1**
VfSuSy		***	**87,8**	**95,0**	**97,6**	**99,0**
AtSuSy			***	**87,8**	**86,7**	**87,4**
GmSuSy				***	**93,8**	**95,0**
MsSuSy					***	**97,3**
PsSuSy						***

The dendrogramm shows that the sucrose synthase gene, isolated from nodules of *Lotus japonicus* L. is homologue with other known sucrose synthases identified from other eucariotic species, like *V. faba, A. thaliana, G. max, M. sativa* or *P. sativum*. It was observed that LjSuSy is phylogenetically closer to AtSuSy (Fig. 2).

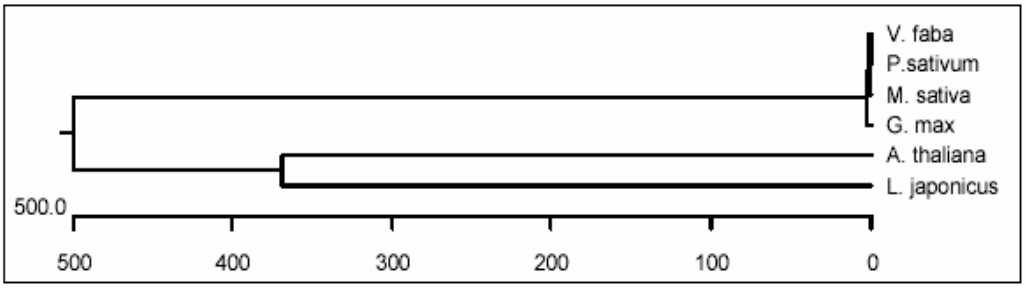

Figure 2. Phylogenetic distances among different cDNA clones coding for sucrose-synthase (SuSy) from various organisms (*L japonicus, A. thaliana, G. max, M. sativa, P. sativum, V. faba*). The dendrogram was constructed after a multiple sequence comparation following the Clustal method of the Lasergene program (DNASTAR) (Baxevanis and Ouellette, 1998).

CONCLUSIONS

As *Lotus japonicus* is one of the two model legumes used to study the function of genes involved in symbiosis, we set out to characterize the structure of the sucrose synthase isolated from *L. japonicus* as the first step towards elucidating their function.

The comparative analysis of amino acid sequence alignment of LjSuSy with other known plant sucrose synthases expressed in sink tissues demonstrated a high similarity expressed by a high level of sequence conservation.

The phylogenetic analysis showed that the sucrose synthase gene, isolated from nodules of *Lotus japonicus* L. is homologue with other known sucrose synthases identified from other eucariotic species.

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