Brazilian Journal of Microbiology (2009) 40:292-295
ISSN 1517-8382

PtSRR1, A PUTATIVE *PISOLITHUS TINCTORIUS* SYMBIOSIS RELATED RECEPTOR GENE IS EXPRESSED DURING THE FIRST HOURS OF MYCORRHIZAL INTERACTION WITH *CASTANEA SATIVA* ROOTS

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Submitted: May 20, 2008; Returned to authors for corrections: September 27, 2008; Approved: March 31, 2009.

ABSTRACT

PtSRR1 EST was previously identified in the first hours of *Pisolithus tinctorius* and *Castanea sativa* interaction. QRT-PCR confirmed *PtSRR1* early expression and *in silico* preliminary translated peptide analysis indicated a strong probability that *PtSRR1* be a transmembrane protein. These data stimulate the *PtSRR1* gene research during ectomycorrhiza formation.

Key words: ectomycorrhiza, symbiosis related genes/proteins, *Pisolithus tinctorius.*

The formation of ectomycorrhiza is a process governed by a complex biochemical and molecular interaction between the two partners before physical contact. Several stages of the ectomycorrhiza formation and maintenance processes from preinfection to the formation of the mantle and the Hartig net have been described, and it is obvious that changes in gene expression have to accompany the processes leading to symbiosis (6,8).

Studies evaluating the fungal transcript pattern during symbiosis formation have demonstrated that mycorrhization induces changes in the expression of genes normally expressed in the free organisms, without the participation of symbiosis specific genes (9). In this paper, we present a fungal cDNA EST representing a gene that is upregulated at 12 h of interaction between *P. tinctorius* and *C. sativa* (1). Its expression, the putative protein structure and its possible function in the symbiosis are discussed.

Biological material acquisition/maintenance and ectomicorrhizal induction is described by Baptista *et al.* (2007) (2). Micohhiza stimulated ("myc") and control mycelium (only in water) were harvested 12 h after contact, snap-frozen in liquid nitrogen and stored at -80ºC. A cDNA library of *P. tinctorius* was constructed from 6 µg of mRNA mix (control and "myc") using the SMART cDNA Library Construction Kit (BD Clontech, Palo Alto, CA, U.S.A) as presented by Acioli-Santos *et al.* (2008). For the quantification of the *PtSRR1* mRNAs, the reverse transcription of each target RNA (control RNA and "myc" at 6 h and 12 h of interaction) was carried out (7).

The cloned *PtSRR1* EST fragment is 432 bp long. An untranslated region is observed downstream from the putative open reading frame (Fig. 1). The *PtSRR1* sequence has 70% similarity to a sequence of *Pisolithus microcarpus* (CB010071), a fungus that forms ectomycorrhiza with *Eucalyptus*. The putative *PtSRR1* peptide has 48% similarity to a protein of the fungus *Schizophyllum commune* (AF335537) that is upregulated under low nitrogen conditions. The study of the *PtSRR1* expression using QRT-PCR allowed the confirmation of the up-regulation at 12 h of interaction, revealing positive transcription rates 1350 fold higher than the control. At 6 h of fungus-plant interaction, the relative values were close to one, suggesting that changes in the transcription levels may occur between 6 and 12 h of interaction.

In silico translation of *PtSRR1* nucleotide sequence resulted in a peptide fragment of 75 amino acids (8.2 kDa), without the

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1     AGT CGT CTG GGA CAC GAG TAC GCC CCT GCA CAA ATC TCA AAT TCA   45
1      S   R   L   G   H   E   Y   A   P   A   Q   I   S   N   S   15

46    GAG GGA CAG ATT TAT CTC GTC GTA AAC AAC CTC ATC GAT TTC GAC   90
16     E   G   Q   I   Y   L   V   V   N   N   L   I   D   F   D   30

91    TAC TTG TTG GCA AAT GAT TTC AAT ATT CTC GAT GGG AGT GTG ATG   135
31     Y   L   L   A   N   D   F   N   I   L   D   G   S   V   M   45

136   GTC ACA GTA CCG GAC GTG CCG ACT GGC ATT TAT GCC ATC GTC TTG   180
46     V   T   V   P   D   V   P   T   G   I   Y   A   I   V   L   60

181   TTT GGT GAT TCT GGT AAC TTT AGC CAG AAC TTC ACC ATC ATA GCG   225
61     F   G   D   S   G   N   F   S   Q   N   F   T   I   I   A   75

226   TGA TCC CAT CAC GTC CTT GCA ACT TTA TCT CTC TGA ACG ATT TCA   270
76     *

271   TGA ACA ATG ATG AAG GAC TTC TGT TTC GTT TAC CAC TCA GGA CTT   315

316   GGT TTC ATA CAT TAG GAC GAC AAA TAC AAT GCA TCC GGA ACA TTT   360

361   AGC AAT GGA CTT GTA ACC CCC TTT CGC ATT CTG CTG TAC GTA TAT   405

406   GGA CTA GGA TCC GGG ACC ATT CTA CTA   432
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Figure 1. The *PtSRR1* EST: nucleotide sequence (432 bp) and partial ORF (leters below the codons, totalling 75 aminoacids). The termination codon is assigned with an asterisc. The partial ORF was identified using MapDraw (Informatik Inc. USA) and represents the largest translation region for the sequence.

initial methionine. No cysteine residues were found in the PtSRR1 amino acid sequence. The analyses of the PtSRR1 peptide primary structure (http://ca.expasy.org/cgi-bin/prosite) enabled the identification of four post-translational modification sites as follows: two N-glycosylation sites with high probability of occurrence between the residues 66 to 69 (NFSQ) and 70 to 73 (NFTI), and two Casein Kinase II phosphorylation sites, in the positions 13 to 16 (SNSE) and 47 to 50 (TVPD), respectively (Fig. 2a). No usual protein domais were identified. Secondary *PtSRR1* structure analisys showed abundance of beta-structures (Fig. 2a). No helix was detected. The peptide shows a well-defined transmembrane region, despite the low probability suggested by its analysis (http://www.predictprotein.org). It was not possible to obtain a PtSRR1 three-dimensional model based on homology modeling (http://www.swissmodel.expasy.org/SWISS-MODEL.html) (Fig. 2b).

The expression of several genes at 6 h of interaction between *Laccaria bicolor* and *Pinus resinosa* has been reported (4,5). However, most of the differentially expressed fungal genes were observed in later stages of symbiotic development, especially after two or more days of interaction (3,6,9), which is corroborated by the 12 h *PtSRR1* transcription. Therefore, the high relative expression of *PtSRR1* at 12 h favours its investigation. QRT-PCR data confirmed the cDNA microarrays analysis of the fungal *PtSRR1* and its high relative transcription at 12 h of ectomycorrhizal interaction. Transcription of this gene does not occur until 6 h of contact, suggesting that this period between 6 and 12 h can be critical for its expression.

The *PtSRR1* gene is that probably triggered by the low availability of nitrogen that could function as an “indicator” of host root proximity. *PtSRR1* homologue peptide (AF335537) was identified in *Schizophyllum commune*. This homologue peptide presents high expression when the mycelium is growing under low nitrogen availability conditions. Further physiological studies and the acquisition of the complete ORF of this gene are necessary for functionality tests in the symbiosis. These results would allow to understand the real function of the *PtSRR1* protein.

As the *PtSRR1* amino acid sequence is not complete and the three-dimensional protein structure is not known, any conclusion about the role of this protein is premature. However, considering its secondary structure prediction, the *PtSRR1*

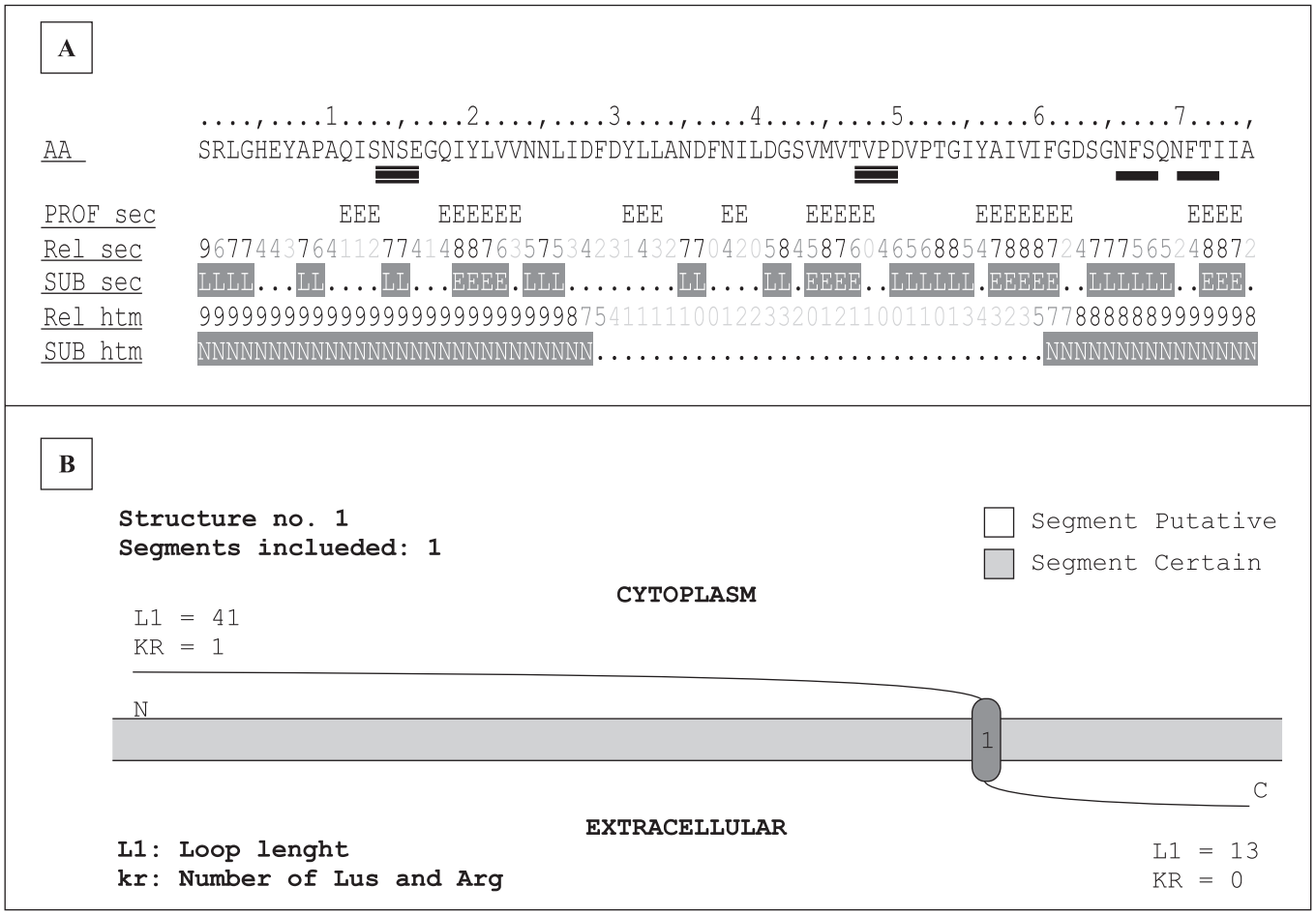

Figure 2. *PtSRR1 in silico* analysis. A) The sequence of the 75 amino acid residues. AA= amino acid sequence. Triple and single lines below the amino acid sequence indicate the phosphorylation and glycosylation sites, respectively. PROF_sec = PROF predicted secondary structure: H = helix, E = extended (sheet), blank = other (loop), Rel_sec = reliability index for PROFsec prediction (0 = low to 9 = high), SUB_sec = subset of the PROFsec prediction. For this subset the following symbols are used: L is loop, E is extended (sheet), and "." means that no prediction is made for this residue as the reliability is Rel < 5, Rel_htm = reliability index for PHDhtm (not shown) prediction (0 = low to 9 = high), SUB_htm = subset of the PHDhtm prediction (not shown), N is non-membrane region, "." means that no prediction is made for this residue as the reliability is Rel < 7. B) The putative transmembrane region of *PtSRR1*. Note the cytoplasmic region larger than extracellular segment of the protein.

seems to be a transmembrane protein with an intracellular segment containing at least one phosphorylation accessible site and an extracellular region containing two glycosylation sites. Thus, there is a possibility that the *PtSRR1* acts as membrane receptor/extra-intracellular signal-transducer element through sites of glycosylation and phosphorylation, or be a secreted protein. However, *in silico* data obtained using the truncated *PtSRR1* amino acid sequence would differ from the full-length amino acid sequence. These data strongly stimulate the research of *PtSRR1* gene role in the ectomycorrhizal process as a potential marker/regulator of the early stages of symbiotic interaction.

ACKNOWLEDGEMENTS

This work was partially supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior/MCT-Brazil) as a Ph.D. scholarship and grant to the first author.

RESUMO

PtSRR1, um possível receptor simbiose-regulado de *Pisolithus tinctorius* é expresso nas primeiras horas de interação ectomicorrízica com raízes de *Castanea sativa*

PtSRR1 foi isolado preliminarmente de *P. tinctorius* nas primeiras horas da interação com raízes de *C. sativa*. Análises de QRT-PCR confirmaram sua expressão positiva (12 h) e seu peptídeo putativo indicou forte possibilidade para proteína transmembranar. Estes dados estimulam o estudo do *PtSRR1* durante a formação de ectomicorrizas.

Palavras-chave: ectomicorriza, genes/proteínas simbiose-regulados, *Pisolithus tinctorius.*

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