Cloning, DNA sequencing and molecular modelling of an anti-fungal laticifer protein

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A laticifer protein from *Calotropis procera* (*CpOsm*) was recently shown to exhibit toxic activity against different phytopathogens. The mechanism underlying toxicity includes membrane damage of spores and hyphae, drastically reducing spore germination and mycelial growth. The present study aimed at cloning, sequencing and molecular modeling of *CpOsm*. A partial cDNA sequence was synthesized and amplified by RT-PCR from total RNA purified from *C. procera* leaves. Amplification was carried out using degenerate primers, designed according to the N-terminal amino acid sequence of the protein and close related sequences, retrieved from GenBank. Amplified *CpOsm* cDNA was inserted into the pGEM-T Easy vector and the complete nucleotide sequence of the insert was determined in a MegaBACE 1000 automatic sequencer. Homology-based molecular models of the *CpOsm* 3D structure were generated with the program *Modeller*. The best model was validated after analysis with *Whatchek* and *Verify3D*, and the structure visualized with *PyMOL*. The cloned cDNA fragment contained an open reading frame of 612 bp, encoding a protein with 204 amino acid residues. Searches on public protein databases using BLASTp revealed high sequence identity (70-83%) of *CpOsm* to thaumatin-like proteins (TLPs). Plant TLPs are pathogenesis-related (PR) proteins classified in the family 5 (PR5), which includes zeamatin and osmotin, for example. The primary structure of *CpOsm* also possess 16 cysteine residues which are conserved in most TLPs. The *CpOsm* DNA coding sequence has been cloned into a prokaryotic expression vector and the characterization of the recombinant protein is underway.

Word Keys: *Calotropis procera*, osmotin, sequencing.
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