

SUGAR-CANE DEFENSINS SD3, SD4 AND SD6: ACTIVITY AND STRUCTURAL FEATURES

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Plants defensins are small proteins of approximately 5 kDa, usually presenting an alpha/beta motif stabilized by cysteines. All known members of this family present a conserved three-dimensional folding, however, showing distinct activities against fungus and/or bacteria, and also inhibiting alpha-amylase or protease. We selected for cloning, purification and structural characterization, three members of defensin family named Sd3, Sd4 and Sd6, identified in the EST library of sugarcane. The Sd3 and Sd6 proteins were subcloned and purified in high degree of purity. Different methodologies were tested for re-folding these defensins that were expressed into inclusion bodies. We are able to successfully refold Sd3 and functional analysis was carried out against fungus and bacteria. The Sd3 defensin was active against *Neurospora crassa* and *Fusarium solani* but no effect was observed for any bacteria strain tested. Sd3 structure was monitored by ¹H NMR and CD spectroscopy. We observe large chemical shift dispersion in NMR proton spectrum and a minimal peak at 208 nm in the CD spectrum, indicating the presence of beta-sheet secondary structure. We intend to use the same approach for Sd4, focusing on its 3D structural determination.

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