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CLONING AND EXPRESSION OF THE *SCEXLX1* GENE FROM *SCHIZOPHYLLUM COMMUNE*.

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Introduction. Expansins are non-enzymatic proteins that induce extensibility and stress relaxation of plant cell walls, acting as loosening agents (1, 2). Also, they are implicated in cell enlargement and other developmental events requiring cell wall loosening, such as fruit softening, seed germination and organ abscission (3).
In this work, we found, cloned and expressed a novel expansin protein (ScExlx1) from the Basidiomycete fungus *Schizophylum commune*. This protein showed the canonical features of plant expansins. A novel property of ScExlx1 is its capacity to enhance reducing sugars (N-acetyl glucosamine) liberation from pretreated chitin and further added with chitinase, which has not been reported for any expansin or expansin-like protein.

Methods. Total RNA was extracted by the Trizol method. First-strand cDNA synthesis was performed using the RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Scientific). The ScExlx1 cDNA was further sequenced using the pJET primers pJET 1. 2.
The *ScExlx1* cDNA cloned into pJET vector was digested with *KpnI* and *XbaI* and purified with GeneJET Gel extraction kit (Thermo Scientific). In parallel, pPICZαA was digested using the same restriction enzymes, and *ScExlx1* was ligated at the corresponding sites into pPICZαA in frame with both the yeast α-secretion factor and C-terminal His6 tag encoding sequences.
The purified protein was incubated with cotton fibers, Avicel and chitin in order to determine the effect produced by this novel over these polysaccharides.

Results. Cotton fibers incubated with ScExlx1 protein exhibited the "bubble" effect produced by other expansin-related proteins from fungi (Fig. 1a and 1b). Moreover, Avicel particle size was reduced after tratment with ScExlx1 (Fig. 1c and 1d). Additionally, ScExlx1 enhanced the chitinolytic activity of a chitinase from *Streptomyces griseus* after incubating chitin from shrimp shells with ScExlx1 protein. Interestingly, this fact suggests a novel target for fungal expansins other than plant cell wall polysaccharides.

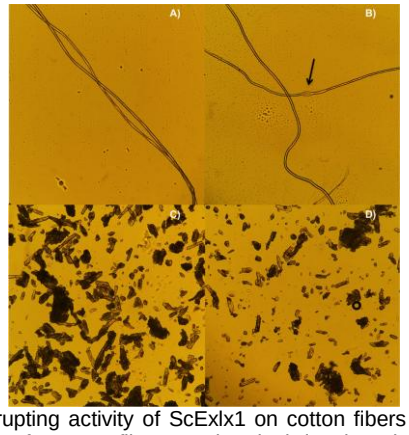

Fig. 1. Disrupting activity of ScExlx1 on cotton fibers and avicel. Light microscopy of cotton fibers and avicel incubated with ScExlx1 or proteins from mock supernatant for 72 h at 25ºC. A) Proteins from mock supernatant acting on cotton fibers. B) "Bubble" effect on cotton fibers generated by ScExlx1. C) Avicel incubated with proteins from mock supernatant. D) Reduction in avicel size particle mediated by ScExlx1.

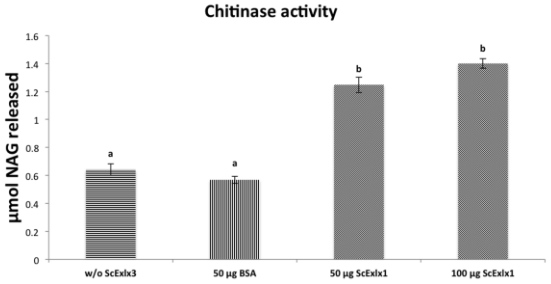

Fig. 2. ScExlx1 is a chitin active protein that enhances chitin hydrolysis. Chitin from shrimp shells (5 mg) was incubated with 50 and 100 µg of ScExlx1, 50 µg of BSA, or sodium phosphate buffer 100 mM pH 7 for 24 h at 25 ºC. After incubation, temperature was increased to 37ºC and chitinase from *S. griseus* (0.25 U) was added.

Conclusions. The ScExlx1 protein from *S. commune* was able to act and modify the cellulose fibers and chitin polysaccharide, which could suggest a novel target for fungal expansins.

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