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[P03-393] Biochemical and Functional Characterization of a Novel GH18 Family Chitinase, *Debaryomyces hansenii* Cts3 with Antifungal Activity

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Chitinases hydrolyze β−1,4-N-acetylglucosamine linkages in chitin, thereby disrupting fungal cell walls and arthropod exoskeletons. These enzymatic properties make them promising biocontrol agents in food preservation and agriculture. In this study, we identified three glycoside hydrolase family 18 (GH18) chitinases DhCts1, DhCts2, and DhCts3 through *in silico* analysis of the whole-genome sequence of *Debaryomyces hansenii* KD2, an isolate from traditional Korean soybean sauce.

Phylogenetic analysis of GH18 endochitinases classified DhCts1 into subfamily B with a shallow substrate cleft, while DhCts2 and DhCts3 into subfamily A with a deep substrate binding cleft. Notably, DhCts3 exhibits a multidomain architecture in contrast to DhCts2 consisting solely of the GH18 catalytic core. DhCts3 contains additionally a secretion signal sequence, a lysin motif (LysM) domain, which is commonly found in extracellular carbohydrate-binding proteins, and a chitin-binding domain (ChBD). For biochemical and functional characterization, DhCts3 was expressed as a secretory protein in *Pichia pastoris,* purified by His-tag affinity chromatography, and confirmed for its chitin-binding activity using chitin beads. PNGase F treatment of the purified DhCts3 protein indicated *N*-glycosylation at the predicted single site.

The endochitinase activity of DhCts3 was indicated in the fluorescence assay using 4-methylumbelliferyl β-D-N,N′,N″-triacetylchitotrioside as substrate, and its chitin hydrolytic activity was validated by thin-layer chromatography (TLC) and High-performance liquid chromatography (HPLC) using chitin oligomer $(GlcNAc)_n$ and colloidal chitin polymer as substrates. Its chitinase activity exhibited an optimal temperature of 40 ℃ with broad pH range. Intriguingly, the TLC and HPLC profiles of colloidal chitin degradation revealed the predominant release of $(GlcNAc)_2$, along with minor amounts of monomeric *N*-acetylglucosamine, indicating that DhCts3 primarily functions as an endochitinase while also displaying partial *N*-acetyl-β-D-glucosaminidase activity. Furthermore, antifungal activity against foodborne fungi, *Aspergillus flavus*, *Botrytis cinerea, Fusarium oxyporum* and *Penicillium digitatum*, was evaluated by monitoring growth inhibition of fungal colonies on plates over time.

Overall, our results presented DhCts3 as a novel chitinase with distinctive domain structures and support its potential as a biocontrol agent for mitigating foodborne fungal contamination. Future studies will focus on assessing the contributions of ChBD and LysM domains to the antifungal activity of DhCts3.