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Eco-Friendly Yeast-Derived Chitinase for Antifungal Use Via Fungal Cell Wall Disruption

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INTRODUCTION & AIM

Fungal pathogens severely impact agriculture and public health, while chemical fungicides cause resistance and environmental pollution. Chitinases, enzymes that hydrolyze chitin in fungal cell walls, represent eco-friendly biocontrol agents but their use is limited by low yield and costly purification. To overcome these barriers, a GRAS yeast (*Saccharomyces cerevisiae* Y2805) was engineered to secrete *Trichoderma atroviride* chitinase (Tch36) using a rice α-amylase signal peptide. This system allows direct use of culture filtrates without purification, aiming to evaluate broad-spectrum antifungal activity and cell wall disruption effects.

METHOD

The *tch36* gene was cloned from *T. atroviride* and fused with the rice α-amylase signal peptide under a constitutive GPD promoter in pYEGPD-TER vector. Full-length and mature (signal-truncated) constructs were transformed into *S. cerevisiae*, and expression was confirmed by qRT-PCR. Chitinase activity was measured using colloidal chitin and DNS assays, while antifungal activity was tested against nine plant and three animal pathogenic fungi. Culture filtrates were analyzed by microscopy to assess hyphal inhibition, spore germination, and *Aspergillus nidulans* protoplast formation.

RESULTS & DISCUSSION

The mature-form strain (TYETch-M) showed 1.5–2-fold higher *tch36* expression and up to six-fold higher chitinase activity than the full-length construct (Fig. 3–5). Modified media containing glycerol and chitin enhanced enzyme secretion and activity. Culture filtrates strongly inhibited fungal growth (>50 %) in *Fusarium graminearum*, *Rhizoctonia solani*, and *Botrytis cinerea* (Fig. 6), and reduced *Aspergillus* spore viability and biomass by up to 30 % (Fig. 7). Microscopy revealed shortened germ tubes, irregular hyphae, and disrupted cell wall surfaces (Fig. 8). Additionally, the filtrate promoted *Aspergillus nidulans* protoplast formation 2–5× more efficiently than controls, confirming enzymatic wall degradation (Fig. 9). These results demonstrate that the yeast-secreted Tch36 functions as an active antifungal enzyme capable of direct fungal cell wall lysis.

Expression Strategy & Vector Design

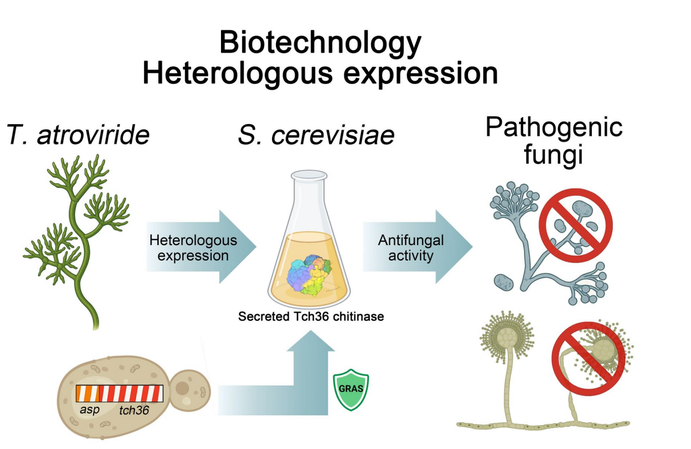

Figure 1. Schematic overview of the yeast secretion system expressing *T. atroviride* chitinase (Tch36), showing heterologous expression in *S. cerevisiae* and antifungal action via fungal cell wall degradation.

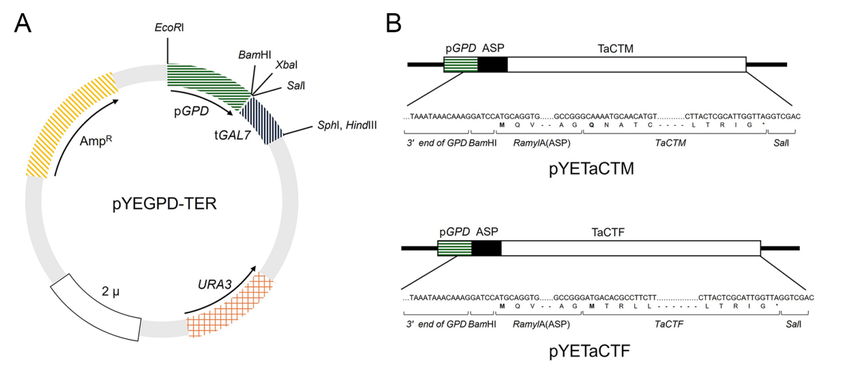

Figure 2. Schematic of yeast expression vector for Tch36 production. (A) Map of pYEGPD-TER vector. (B) Recombinant construct containing GPD promoter, Amy1A signal peptide, and tch36 gene under the GAL7 terminator.

Gene Expression & Enzyme Activity

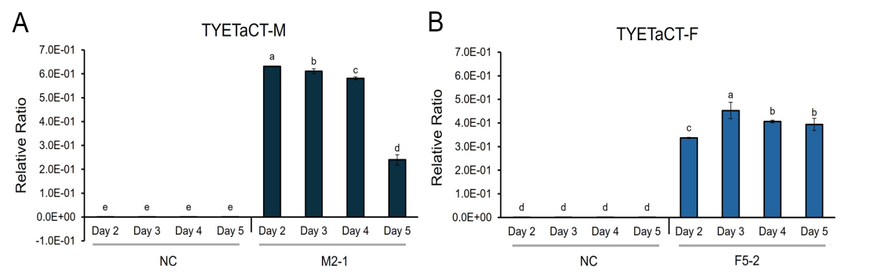

Figure 3. Temporal expression of tch36 in recombinant *S. cerevisiae*. (A) TYETch-M (M2-1) and (B) TYETch-F (F5-2) showed stable expression over 3–5 days, normalized to GPD. $p < 0.05$.

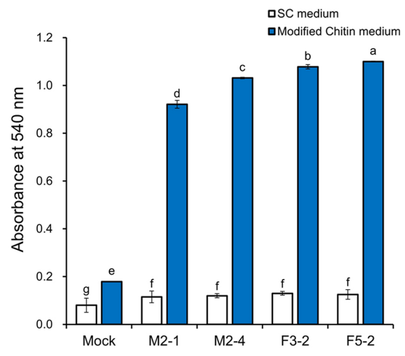

Figure 4. Effect of chitinase activity in modified chitin media. Recombinant *S. cerevisiae* strains (TYETaCT-M and TYETaCT-F) and the mock strain (NC) were cultured in SC(–Ura) and chitin media for 4 days. Blue and white bars indicate SC(–Ura) and chitin media, respectively.

Antifulgal Spectrum Analysis

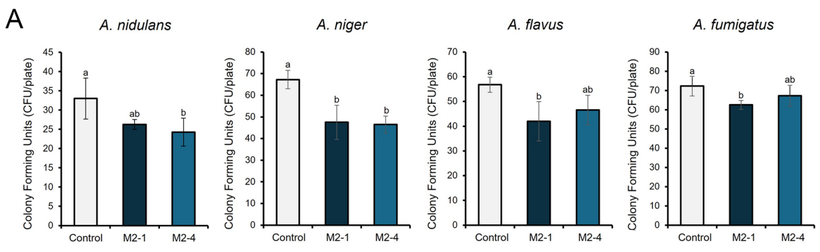

Figure 7. Antifungal activity against animal pathogenic fungi. (A) CFU counts of *A. niger*, *A. flavus*, *A. fumigatus* on PDA. (B) Biomass reduction in PDB after treatment with recombinant culture filtrates (TYETch-M2-4). $p < 0.05$.

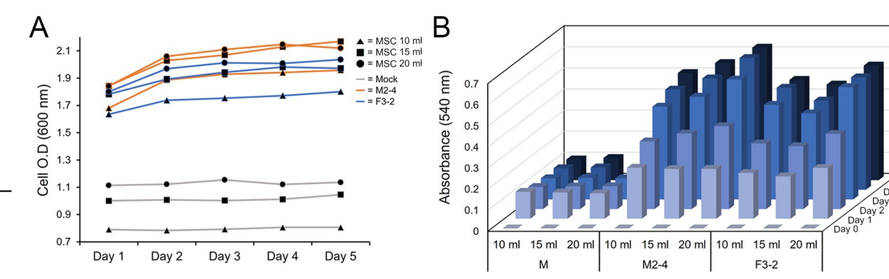

Figure 5. Growth and chitinase activity of recombinant *S. cerevisiae* under different colloidal chitin concentrations. (A) Cell growth (OD_{600}) over 5 days. (B) Extracellular chitinase activity measured daily; both transformants showed concentration-dependent increases.

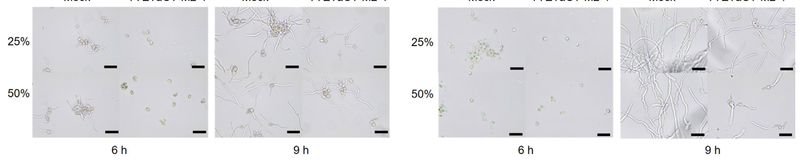

Figure 8. Antifungal effects of recombinant chitinase on early hyphal development. (A) Light microscopy of conidial germination and hyphal elongation of *B. cinerea* and *A. niger* in media containing 25 % or 50 % filtrate. (B) Quantitative hyphal length measurement after 9 h, showing dose-dependent inhibition.

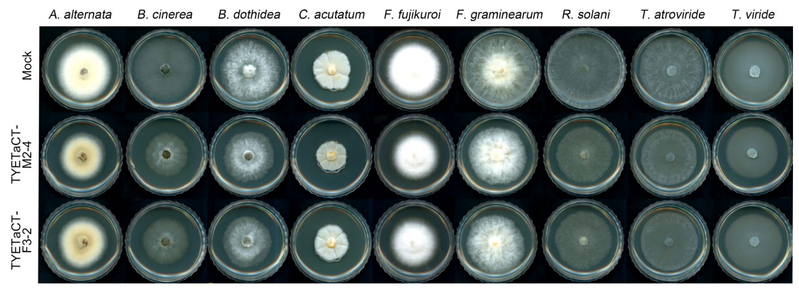

Figure 6. Antifungal activity of recombinant chitinase-containing culture filtrates against nine phytopathogenic fungi. Representative colony morphologies on PDA plates after 48 h.

CONCLUSION

- The yeast system secreted active *T. atroviride* chitinase without purification and inhibited diverse fungal pathogens.
- The mature construct with the rice signal peptide enhanced secretion and bioactivity.
- This platform offers a sustainable, low-cost route for antifungal biocontrol applications.

FUTURE WORK / REFERENCES

- Future studies will test enzyme stability, formulation, and large-scale production. Co-expression of multiple hydrolases will broaden antifungal efficacy.
- Long-term storage and eco-safety assessments will guide field application.
- Fisher *et al.*, 2020; Deng *et al.*, 2019; Song *et al.*, 2018; Wang *et al.*, 2020.