

Antimicrobial effect of a recombinant chitinase against different strains of bacteria and fungi

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Abstract: In this study, chitinase A from *Bacillus licheniformis* DSM8785 expressed in *Pichia pastoris* KM71H was subjected to several tests to determine its antimicrobial effect against bacteria that do not have chitin in their cell walls: *Escherichia coli*, *Staphylococcus aureus*, and fungi, which have chitin in their cell walls: *Saccharomyces cerevisiae*, *Candida albicans*.

Introduction

Chitinases are glycoside hydrolases, enzymes that cleave the glycosidic bonds in chitin. Chitin is the structural polysaccharide in the exoskeleton of crustaceans, insects, and fungal cell walls. Chitinases are found in many organisms, such as bacteria, fungi, plants, and mammals, and play a role in cell wall plasticity, in the division of mother and daughter cells at the bud neck during vegetative growth in yeasts, in anti-infective defense and repair responses in plants and mammals. The defensive role of chitinases involves their action in organisms infected with pathogens that have chitin in their cell wall structure.



Fig. 1. Organisms that contain chitin. (A) crabs, (B) shrimps, (C) lobsters, (D) scaly fish, (E) insects, (F) fungi.

Material and method

Different concentrations of ChiA (0.00; 0.007; 0.037; 0.075; 0.187; 0.375; 0.560 and 0.750 mg/mL) were added in Luria Bertani without antibiotic, or Yeast-Peptone-Dextrose culture media and 10 μ L of pre-culture from each strain (*Escherichia coli* and *Staphylococcus aureus*, *Saccharomyces cerevisiae* EBY100 and *Candida albicans*) were added to a microtiter plate. The optical density at 620 nm of the cells was read at time 0 (control) and after 24 h incubation at 37°C, 100 rpm. The optical density values on the graph were determined by making the difference between the density values at 24 h incubation and those at time 0.

Results and discussions

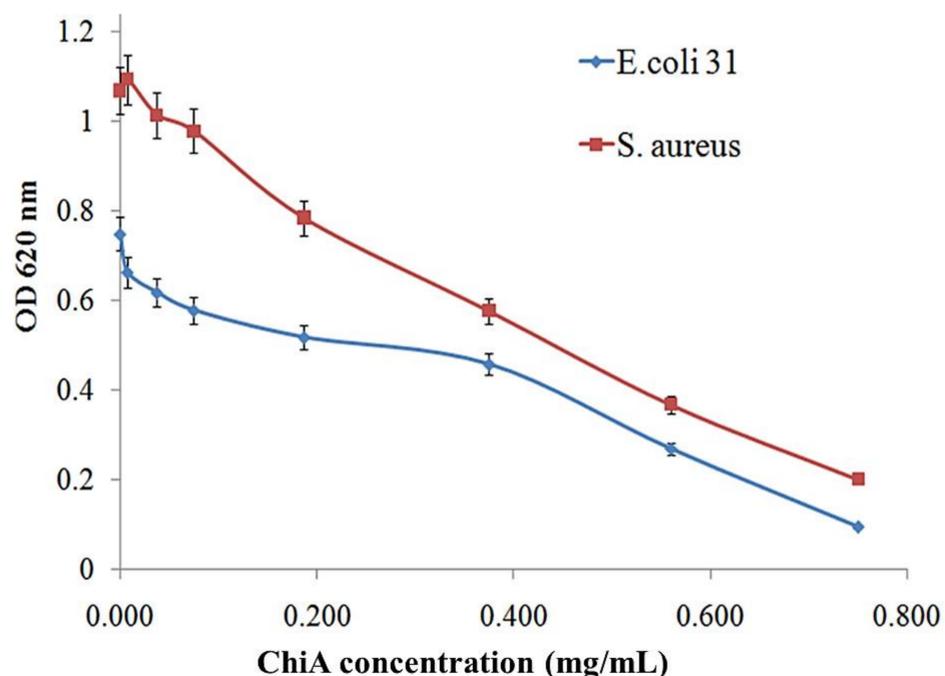


Fig. 2. Antimicrobial effect of recombinant ChiA produced in *P. pastoris* KM71H against *Escherichia coli* and *Staphylococcus aureus* bacterial strains.

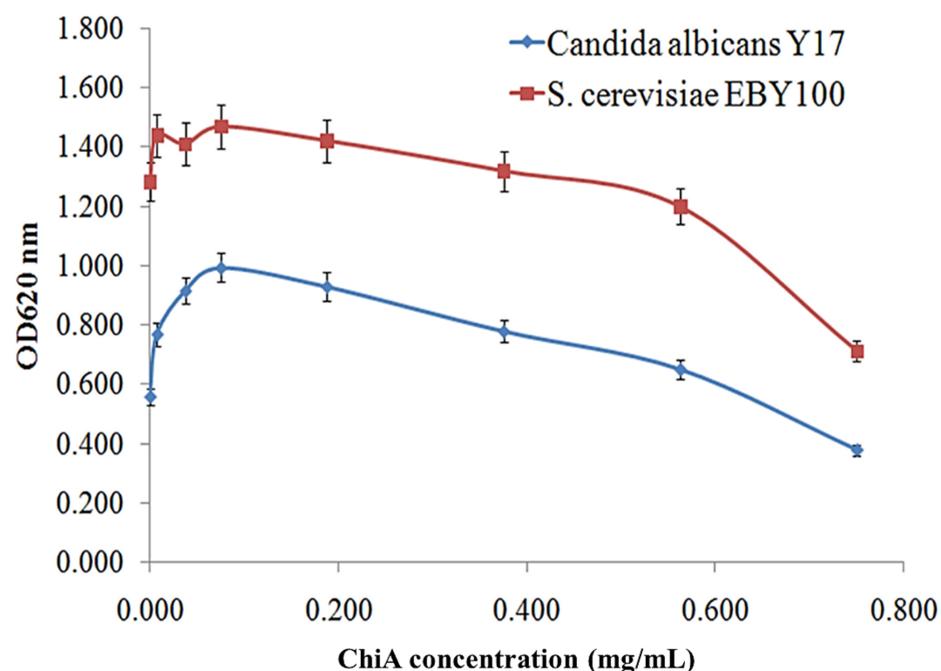


Fig. 3. Antifungal effect of recombinant ChiA produced in *P. pastoris* KM71H against *Saccharomyces cerevisiae* EBY100 and *Candida albicans* yeast strains.

Conclusions

The results showed that recombinant chitinase A inhibited bacterial growth starting at a concentration of 0.2 mg/mL. At the same concentration of the enzyme, fungal strains were unaffected, their growth being inhibited starting at a concentration of 0.8 mg/mL. Therefore, chitinase A can be considered an antimicrobial and antifungal enzyme with an impact on some pathogenic strains and could also be regarded as a potential antibiotic in the future.

References: Gheorghita Menghiu, Vasile Ostafe, Radivoje Prodanovic, Rainer Fischer, Raluca Ostafe, A high-throughput screening system based on fluorescence-activated cell sorting for the directed evolution of chitinase A, International Journal of Molecular Sciences, 2021, 22(6): p. 3041.
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