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Introduction

Many plants produce inhibitors of proteolytic enzymes for protection from parasitic microorganisms. However the attacking enzymes themselves are poorly investigated. The aim of the present study was to isolate and characterize proteolytic enzymes from mold samples grown on bread and tea.

Conclusions

Proteins from mold grown on bread and tea were isolated using method of affinity chromatography. The proteolytic activity of proteins was investigated by spectrophotometry. The enzyme from bread mold had $k_{cat}/k_m = 71.4 \text{ 1}/(\text{sec} \cdot \text{g}_{\text{protein}})$. The enzyme from tea mold had $k_{cat}/k_m = 1.1 \text{ 1}/(\text{sec} \cdot \text{g}_{\text{protein}})$. The molar weight of proteins was investigated by electrophoresis.

Results of work

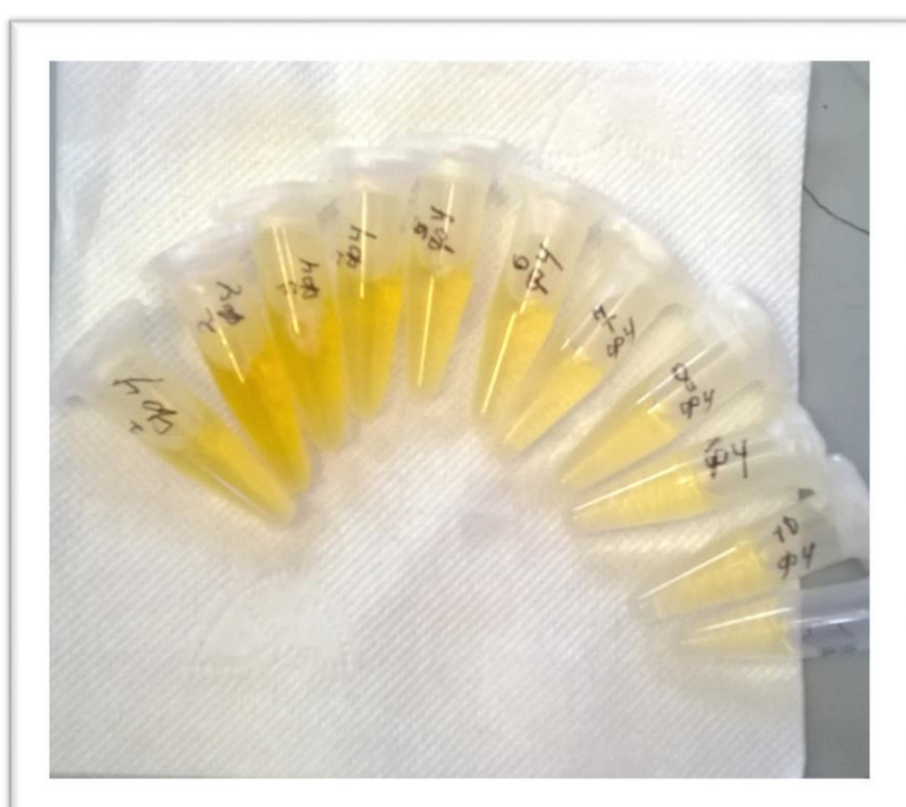
Preparation of mold extract

- The mold homogenization
- The proteins extraction by 0.1% NaCl solution
- Filtering through the filter paper
- Filtering through the 0.22 μm pore membrane
- Centrifugation for removal cell remnants
- Filtering through the 0.22 μm pore membrane
- Protein were concentrated by centrifugal filter units Amicon 3 kDa

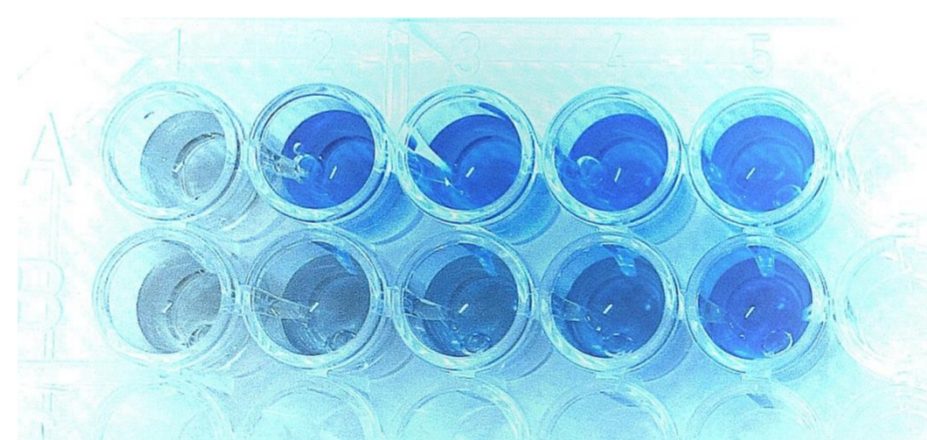
Preparation of chromatography column

- Washing and swelling resin in 1mM HCl during the night at $T = +4^\circ\text{C}$
- Pasting the resin into the column
- Washing with water, 5 volumes of column
- Washing with PBS, 5 volumes of column
- Incubation of resin with CTI (1mg/1ml) during the night at $T = +4^\circ\text{C}$
- Incubation of resin with glycine (0.2M) during 1.5h at room temperature
- Washing away unreacted ligand with PBS and Acetate buffer

Affinity chromatography

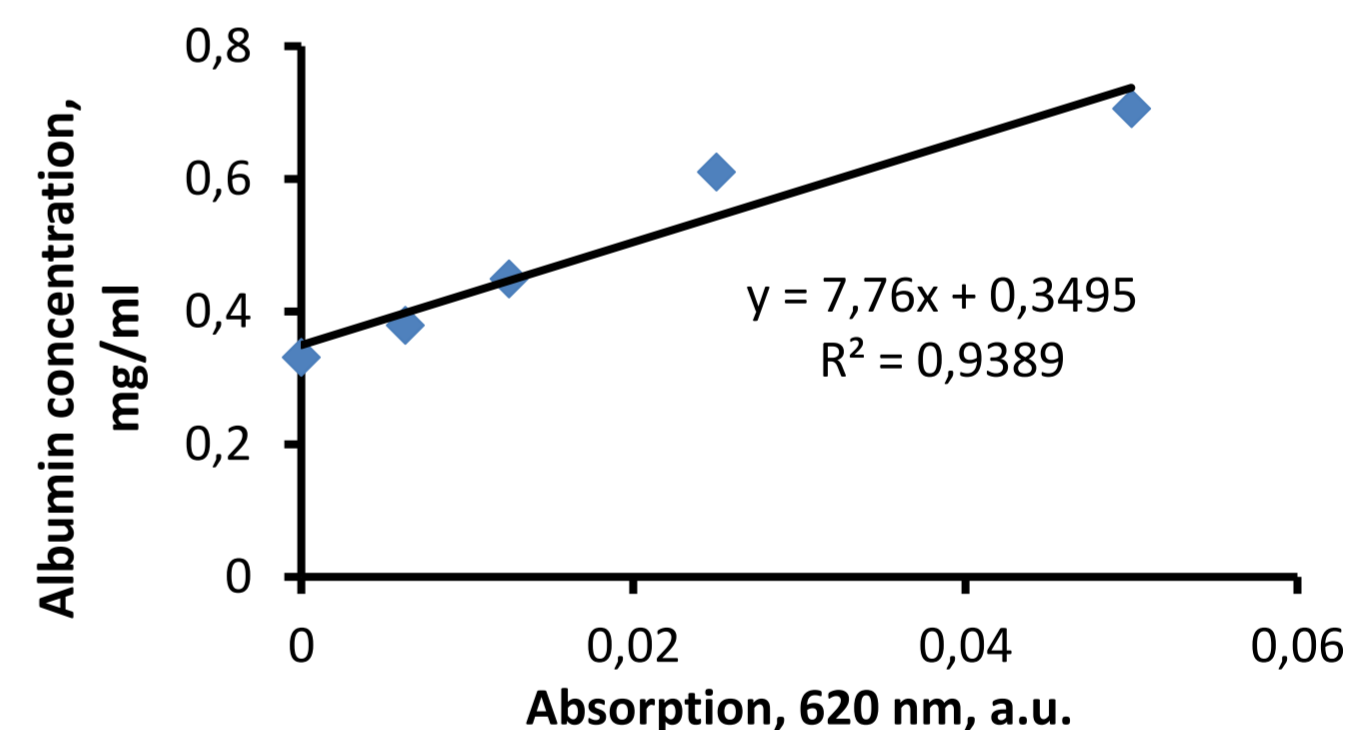


Measurement of protein concentrations using Bradford and Bicinchoninic Acid methods



Bread mold

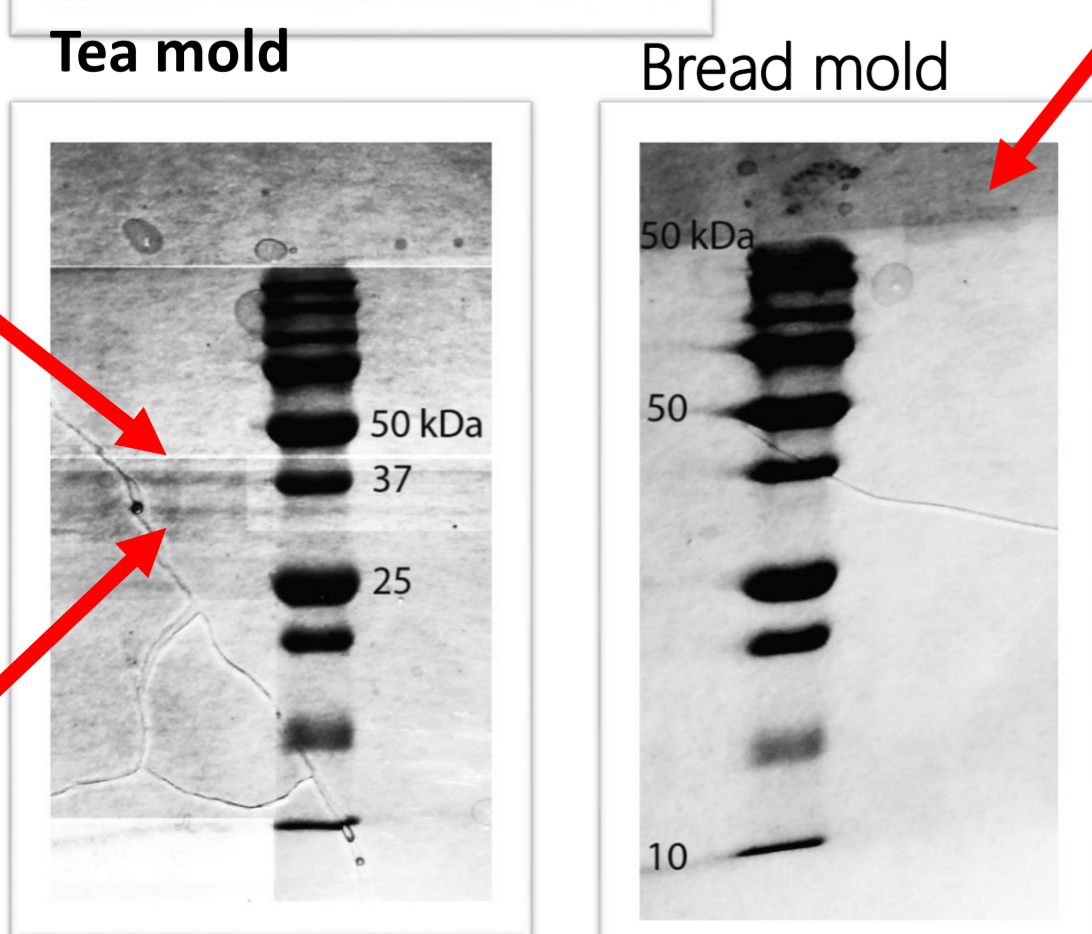
Nº sample	1	2
C, mg/ml	0.04	0.02



Tea mold

Nº sample	1	2	3	4	5	6	7	8	9	10	11
C, mg/ml	0.18	0.21	0.16	0.15	0.07	0.18	0.03	0	0	0.004	0.01

Electrophoresis



Determination of proteolytic activity using spectrophotometry

