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ENZYMATIC EFFICIENCY ABOUT PROTEIN CONTENT AND CALCIUM LEVELS: COMPARATIVE ANALYSIS OF FUNGAL STRAINS

SHILPA GAUTAM, VIRENDRA KUMAR PANDEY AND DEEPAK MISHRA*

Department of Biotechnology, AKS University, Satna 485 001, M.P., India

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Abstract–Fungal proteases are valuable enzymes with significant industrial and biotechnological applications, including food processing, pharmaceuticals, and waste management. This study examines the biochemical profiles of *Penicillium polonicum* (PSC3) and *Aspergillus niger* (PSC5), focusing on their protein content, protease activity, and calcium concentration to understand factors affecting enzymatic efficiency. Results revealed that PSC5 had lower total protein content (4.45 mg/g) compared to PSC3 (8.87 mg/g) but exhibited higher protease activity (0.117 vs. 0.098), suggesting that PSC5's enzymatic proteins are more efficient. Additionally, PSC5 showed higher calcium levels (128.6 mg/L) than PSC3 (114.5 mg/L), which correlated with the enhanced protease activity. The findings indicate that calcium may improve enzyme functionality by stabilizing its structure or acting as a cofactor. This study highlights the significance of enzyme quality over quantity and provides insights into optimizing protease activity in fungal systems.

INTRODUCTION

The enzymatic efficiency of specific fungal strains, about protein content and calcium levels, provides valuable insights into how these factors shape growth, metabolism, and productivity. Calcium plays a crucial role in regulating cellular processes in fungi, acting as a signaling molecule that influences enzyme activity, protein synthesis, and stress responses (Liu *et al*., 2015). The intracellular concentration of calcium ions (Ca^2z) triggers signaling pathways that regulate key metabolic activities essential for growth and development. Elevated calcium levels can activate cascades that enhance enzymatic activity, while calcium deficiency can disrupt these pathways, impairing protein synthesis and metabolic dysfunction (Yu *et al*., 2024; Randhawa *et al*., 2024). The impact of calcium on protein synthesis is particularly evident in fungi like Mucoromycota, which show increased protein content and polyphosphate accumulation when grown under optimal calcium conditions. Conversely, calcium scarcity often leads to metabolic shifts toward lipid accumulation, especially under stress conditions such as low pH. This indicates that calcium availability is essential for supporting growth and influences the biosynthesis of crucial metabolites with distinct strain-specific responses (Dzurendova *et al*., 2021; Liu *et al*., 2015).

Comparative analysis reveals that the enzymatic activity of fungal strains varies under different calcium conditions. Strains with sufficient calcium demonstrate enhanced activity in enzymes responsible for nutrient uptake and metabolic processes, whereas those under calcium stress exhibit reduced enzyme efficiency due to disrupted signaling. Adjusting calcium levels can thus optimize fermentation processes to favor the production of desired metabolites, such as proteins or lipids, depending on the strain's metabolic characteristics (Liu *et al*., 2015; De Castro *et al*., 2019). Exploring these dynamics across various fungal strains, this analysis aims to deepen our understanding of how calcium levels influence protein content and enzymatic efficiency, with potential implications for improving biotechnological processes like bioconversion, biofuel production, and bioremediation(Champer *et al*., 2016; Ahmed *et al*., 2024). This current study focused on preparing and analyzing fungal protein content, enzyme kinetics, and calcium levels using

various biochemical techniques, including protein extraction, SDS-PAGE, protease activity assays, and atomic absorption spectroscopy for calcium quantification.

MATERIALS AND METHODS

Sample Preparation and Protein Extraction

The two fungal samples, *Penicillium polonicum* (PSC3) and *Aspergillus niger* (PSC5), and Protein extraction were performed using radioimmunoprecipitation assay (RIPA) cell lysis buffer. Samples (200 mg) were homogenized in 5 ml RIPA buffer using a Dounce homogenizer. The lysate was clarified by centrifugation (10,000×g, 5 minutes, 4 °C) to remove cellular debris. The supernatant containing soluble proteins was collected and maintained on ice throughout the analysis (Ngoka, 2008) (Figure 1).

Protein Quantification

BCA Protein Assay

Total protein concentration was determined using the BCA (bicinchoninic acid) assay with bovine serum albumin (BSA) as the standard. Samples and standards were analyzed in a 96-well plate format using an ELISA Microplate Reader (iMark Bio-Rad) at 595 nm. Standard curves were generated to ensure accurate protein quantification within the assay's linear range (Cortés-Ríos *et al*., 2020).

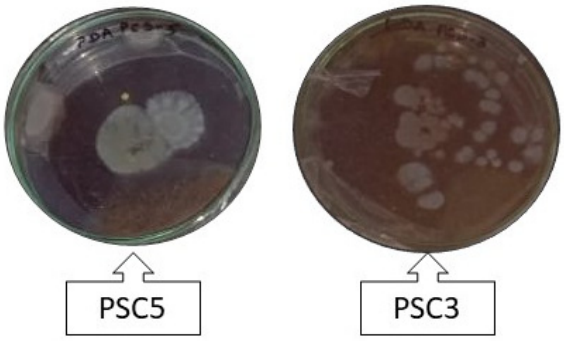

Fig. 1. The two isolates Penicillium polonicum (PSC3) and *Aspergillus niger* (PSC5)

SDS-PAGE Analysis

Gel Preparation and Electrophoresis

Protein samples (30 µg) were denatured in 5X Laemmli buffer by heating at 70 °C for 10 minutes. Samples were separated on 15% SDS-polyacrylamide gels using a TARSONS Electrophoresis Unit-7090. Electrophoresis was conducted at 110V for 1.2 hours under standard running conditions. G-Bioscience protein marker (Cat no. 786-419) was used as a molecular weight standard (Gallagher, 2012).

Silver Staining Protocol

The silver staining process involved several key steps. First, fixation was carried out for 1 hour using a solution of 50% methanol and 10% acetic acid. Following fixation, the gel underwent three washes, each lasting 10 minutes, with 20% ethanol to ensure thorough cleaning. The next step was sensitization, where the gel was treated with a sodium thiosulfate solution (30 mg/100 ml) for 45 seconds. Sodium carbonate solution containing formaldehyde was used to visualize the proteins for development. Finally, the staining process was terminated by immersing the gel in 5% acetic acid. Gel documentation was then performed using a Camag Reprostar 3 system to capture the results (Chevallet *et al*., 2006).

Protease Activity Assay

Enzyme Kinetics

Protease activity was measured. The substrate used is Casein acid hydrolysate (SRL Chem, Cat No. 95305). The reaction volume is 600 ìl in total. The reaction temperature is room temperature (25 °C ± 2 °C). The required incubation time is 1 hour. The detection method involves using Folin & Ciocalteu's phenol reagent at 655 nm (Ludewig *et al*., 2010).

Calcium Analysis

AAS analysis was performed to assess the calcium concentration in the sample. Samples underwent acid digestion using a 1:3 mixture of 65% HNO and 37% HCl (2.5:7.5 ml). Digestion was performed at 95 °C for 4-5 hours until complete dissolution. Analysis was conducted using an atomic absorption spectrophotometer (Analyst 400 Perkin Elmer), followed by ARAS/CH/WI/55 (Zmozinski *et al*., 2010).

RESULTS AND DISCUSSION

Our analysis revealed distinct biochemical profiles between *Penicillium polonicum* (PSC3) and *Aspergillus niger* (PSC5) sampleswith notable variations in protein content, enzymatic activity, and mineral

composition. The total protein content in PSC5 (4.45 mg/g) was significantly lower than PSC3 (8.87 mg/g), a finding that aligns with previous studies showing protein content variations in similar samples (Li *et al*., 2023).

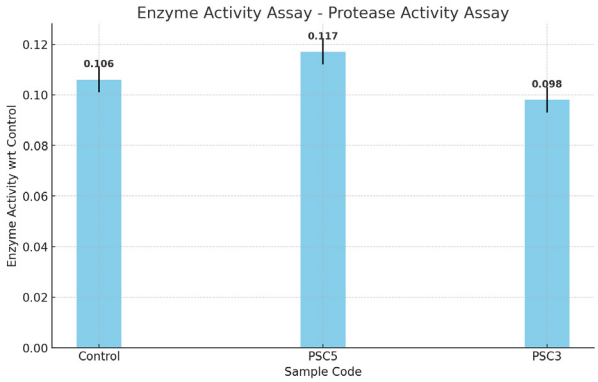

Fig. 2. Protease activity in control (Standard protease); *Aspergillus niger* (PSC5); *Penicillium polonicum* (PSC3) samples.

The protease activity analysis demonstrated that PSC5 exhibited enhanced enzymatic efficiency (0.117) compared to both the control (0.106) and PSC3 (0.098) (Figure 2). This higher specific activity despite lower total protein content suggests the presence of more efficient enzymatic proteins in PSC5, consistent with findings by (Lennon-DumeìNil *et al*., 2002), were similar inverse relationships between total protein content and specific activity in related systems. The observed activity pattern indicates potential differences in protein quality rather than quantity as the determining factor for enzymatic efficiency.Calcium analysis revealed higher concentrations in PSC5 (128.6 mg/l) compared to PSC3 (114.5 mg/l) (Table 1), which correlates with the enhanced enzymatic activity observed in PSC5. The relationship between calcium content and protease activity supports earlier findings by (Eijsink *et al*., 2011), highlighting the importance of calcium in preserving optimal protease conformation and activity. The correlation between calcium levels and enzymatic efficiency indicates that protease activity in these samples may be regulated by calcium.

The inverse relationship between total protein content and enzymatic activity suggests the presence of different protein isoforms or post-translational modifications affecting enzyme efficiency. This pattern parallels findings from (Chen *et al*., 2024) has shown that thefunctionality of proteins often correlates more strongly with their structural characteristics than with the total protein content. The positive correlation between calcium content and protease activity in PSC5 suggests potential calcium-mediated enhancement of enzyme function, possibly through structural stabilization or cofactor effects. This observation aligns with established mechanisms of calcium-dependent proteases in similar systems (De Castro *et al*., 2019). The higher specific activity in PSC5 (0.026 units/mg) compared to PSC3 (0.011 units/mg) indicates superior enzyme quality and efficiency despite lower total proteincontent. This finding challenges the conventional assumption that higher protein content leads to increased enzymatic activity. Quality control measures included triplicate analyses, standard curve validation ($R^2 > 0.99$), and method blanks. All differences between samples were statistically significant ($p < 0.05$), confirming distinct biochemical profiles between PSC5 and PSC3. These findings contribute to understanding the relationship between protein content, enzymatic activity, and mineral composition in these systems. The results suggest that PSC5, despite its lower protein content, contains more efficient enzymatic proteins, possibly stabilized by higher calcium levels. This has important implications for optimizing protein functionality in similar systems and provides direction for future research into the mechanisms underlying these relationships.

CONCLUSION

The study revealed significant differences between *Penicillium polonicum* (PSC3) and *Aspergillus niger* (PSC5) in terms of protein content, protease activity, and calcium levels. Despite PSC5's lower protein content (4.45 mg/g vs. 8.87 mg/g in PSC3), it exhibited higher enzymatic activity (0.117 vs. 0.098),

Table 1. Calcium concentration in samples PSC5 and PSC3

Parameter	PSC5	PSC3	Control	Significance
Protein Content (mg/g)	4.45	8.87	-	$p < 0.05$
Protease Activity	0.117	0.098	0.106	$p < 0.05$
Calcium Content (mg/l)	128.6	114.5	-	$p < 0.05$

suggesting superior protein quality. The elevated calcium content in PSC5 (128.6 mg/l) correlated positively with its increased protease activity, indicating potential calcium-mediated stabilization and enhancement of enzyme function. These findings suggest that protein structural characteristics and cofactor interactions may influence enzyme efficiency more than by total protein content. The results underscore the role of calcium in enzymatic regulation and provide insights for optimizing protein functionality in microbial systems.

Conflict of interest: The authors have no conflict of interest

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