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Enzymes of *Aspergillus japonicus* and *Aspergillus carbonarius* harbouring decayed banana: A comparative profiling

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Abstract
The study explores the extracellular enzyme profiles of two fungi—*Aspergillus japonicus* and *Aspergillus carbonarius*—recovered from decayed banana peel. The strains demonstrated the ability to secrete hydrolytic enzymes such as pectinase, cellulase, lipase, catalase, protease, and L-asparaginase in plate assays. Enzymes were assessed qualitatively through halo zone formation and quantitatively under submerged fermentation conditions. The experiments revealed distinct enzyme patterns between the two species. *A. japonicus* exhibited elevated pectinase, protease, and L-asparaginase activity, whereas *A. carbonarius* showed enhanced levels of lipases and cellulases. These differences point to species-specific metabolic potentials, suggesting targeted applications in biotechnological applications such as biomass conversion, pharmaceutical enzyme production, and food processing.

Keywords: *Aspergillus japonicus, Aspergillus carbonarius*, enzyme profiling, plate assay, submerged fermentation

Introduction
The genus *Aspergillus* is renowned for its diverse enzyme capabilities, which have significant industrial applications, including in the food, pharmaceutical and biofuel industries. Among the species within this genus, *Aspergillus japonicus* and *Aspergillus carbonarius* have been identified as potential sources of valuable enzymes. However, there is a lack of comprehensive studies comparing the enzyme profiles of these two species.

Previous research has primarily focused on the enzyme activities of single strain of *Aspergillus* species, but comparative studies are limited. The studies for instance, have shown that *A. niger* is a prolific producer of pectinase and cellulase, which are crucial for the degradation of plant materials. Similarly, *A. oryzae* has been known for its protease production, which has a vital role for the food industry. However, the enzyme capabilities of *A. japonicus* and *A. carbonarius* remain underexplored, particularly in terms of their potential for industrial applications.

This study aims to fill this gap by providing a detailed comparison of the enzyme profiles of *Aspergillus japonicus* and *Aspergillus carbonarius*. By understanding the specific enzyme activities of these species, we can assess their potential for various biotechnological applications. This research is significant as it provides new insights into the enzyme capabilities of these fungi, which could lead to the development of more efficient and sustainable industrial processes.

Materials and Methods
All chemicals used were of analytical grade. The fungal strains *Aspergillus japonicas (GenBank: PV549632.1)* and *Aspergillus carbonarius (GenBank: PV549633.1)* were isolated from decaying banana peels and identified based on morphological, microscopic and molecular characteristics. Preliminary screening of enzymes was conducted using substrate-specific agar media tailored to each enzyme type. The formation of halo zones around the colonies served as a qualitative indicator of extracellular enzyme secretion. Quantitative assessments of enzymes were undertaken via submerged fermentation.

Primary screening of fungal enzymes by the Plate assay

Catalase

Catalase was assayed based on the reaction that converts H_2O_2 into water and oxygen by the method of Bailey and Scott (1994). A 3mm disc of actively growing fungal mat was introduced into an aqueous substrate (3% H_2O_2). The appearance of bubbles around the fungal mat disc indicated positive catalase activity.

Cellulase

The cellulase was determined with Congo red test. A 1% carboxymethylcellulose (CMC) agar media with streptomycin was inoculated with the fungus and incubated at 28°C for three days. The presence of cellulolytic enzyme was confirmed by the formation of a bluish-black complex around the fungal mat.

Lipase

The lipase was assessed following the method of Haba *et al*. (2000) [15]. The reaction mixture contained; Tween 80 (1% W/V) as substrate and peptone (2% W/V), sodium chloride (1% W/V), calcium chloride (2% W/V), and agar (2% W/V) in Petri plate. The pH of the medium was adjusted to 6.0. A 3mm disc of actively growing fungus was placed in the center of the Petri plate and incubated for 72 hours.

Amylase

Amylase was assayed according to method of Bahailu Asrat *et al*. (2018) [3]. Briefly, the fungus was inoculated on 1% starch agar medium and placed at 28 °C. After 3 days of growth the Petri plate was flooded with iodine solution to detect the enzyme.

Urease

The urease activity was determined by Finegold and Baron method (1986) [12]. Fungal discs measuring 3 mm in diameter were placed on the petri plates containing 1% urea with 0.001% phenol red and incubated at 28°C for 72 hours. The change in media colour from pale yellow to deep pink indicates urease activity.

Pectinase

Fungus was inoculated on pectin (1%) - agar medium and incubated at 28º C for 3 days. The plates were flooded with 0.1% Congo red solution for primary screening of enzyme. The pectinolytic fungi produced a zone of clearance around the fungal colony.

Invertase

The invertase activity of the fugus was determined using sucrose (1%) - agar media. A 3mm disc of fungal mat was aseptically placed on media and incubated at 28º C for three days to test for enzyme.

Xylanase

The detection of xylanase was achieved on xylan (1% W/V) media containing NaCl (1% W/V), yeast extract (0.5% W/V), and agar (2%). The fungal hypha was streaked onto the media in a zigzag pattern and incubated at 28°C for 72 hours.

Protease

Skim milk agar medium is utilized to screen for protease production by fungi (Ayob & Simarani, 2016) [4]. A 3mm disc of pure culture isolates was inoculated onto the skim milk agar plates and incubated at 28°C for three days. The appearance of a clear zone in the medium around the colony indicates protease activity.

L-Asparaginase

A 3mm disc of fungal colony was plated on modified Czapek-Dox (MCD) agar plates (Mahajan *et al*., 2013) [26] with L-Asparagine as a sole nitrogen source and incubated at room temperature for 72 h. Fungal strains showing the change in colour from yellow to pink were recorded as positive activity.

Quantitative enzyme assays

The enzymes that exhibited a maximum zone of hydrolysis were further subjected to quantitative analysis.

Lipase Assay

Lipase activity was measured using a modified titrimetric protocol adapted from Borkar *et al*. The reaction mixture consisted of 10 mL of olive oil containing Tween-80 (1% v/v), 4 mL of sodium phosphate buffer (0.1 M, pH 7.0), 500 µL of calcium chloride (2%), and 1 mL of enzyme extract. The mixture was incubated at 37 °C for 20 minutes in a water bath with intermittent shaking every 5 minutes. The reaction was terminated by adding 20 mL of an acetone: ethanol mixture (1:1, v/v). Free fatty acids released were quantified by titrating with 0.1 N NaOH using phenolphthalein as an indicator.

Calculation

$$\text{Lipase activity (U/mL/min)} = \frac{(V_{Test} - V_{Control}) \times \text{Normality of NaOH} \times 100}{\text{Incubation time (min)}}$$

One unit (U) of lipase activity is defined as the amount of enzyme that releases 1 µmol of fatty acid per minute under the assay conditions.

Pectinase Assay

Fungi were cultured in pectinase screening broth medium (PSBM) at pH 4.5, 30 °C for 72 hours. Briefly, the PSBM medium was composed of (w/v); $(NH_4)_2HPO_4$ (0.14%), KH_2PO_4 (0.2%), K_2HPO_4 (0.6%), $MgSO_4$ (0.01%), and citrus pectin (1%). After incubation, cultures were centrifuged at 8000 rpm for 20 minutes, and the supernatant was used as crude enzyme. An aliquot of 0.1 mL of enzyme was added to 5 mL of pectin (0.1%) and incubated at 50 °C for 30 minutes. The reaction was arrested by adding 1 mL of DNS reagent, followed by boiling the mixture for 10 minutes. Absorbance was read at 540 nm using a spectrophotometer, with appropriate blanks.

One unit of pectinase activity is the amount of enzyme that releases 1 µmol of galacturonic acid per hour under the assay conditions.

Cellulase Assay

Cellulase production was evaluated using Cellulase Screening Broth Medium (CSBM) under static incubation for 72 hours at 30 ° C, pH 5. CSBM composition (w/v): $(NH_4)_2HPO_4$ (0.14%), KH_2PO_4 (0.2%), K_2HPO_4 (0.6%), $MgSO_4$ (0.01%) and carboxymethyl cellulose (1% CMC). Post-incubation, the culture was centrifuged at 8000 rpm for 20 minutes. For activity estimation, 0.1 mL of crude enzyme

was incubated with 5 mL of CMC (0.1%) at 50 °C for 30 minutes. DNS reagent was then added to stop the reaction, followed by boiling for 10 minutes. Absorbance was recorded at 540 nm.

One unit of cellulase activity corresponds to the enzyme amount required to release 1 µmol of glucose per minute from CMC under assay conditions.

Protease Assay

Fungal cultures were grown in casein (1%) broth for 72 hours at 30 ° C. After incubation, the culture was centrifuged at 8000 × g for 20 minutes to obtain the crude enzyme extract. A mixture containing 0.5-1.0 mL of enzyme and 0.05 M phosphate buffer was pre-incubated at 50 °C for 10 minutes. The reaction was initiated by adding 1 mL of casein (1%) solution. After 2 hours of incubation, 2 mL of trichloroacetic acid (10% TCA) was added to terminate the reaction. The mixture was filtered (Whatman No. 1), and residual protein was quantified via Lowry's method.

Protease activity is expressed as µmol of tyrosine equivalents released per hour per mg of protein under assay conditions.

L-Asparaginase Assay

L-asparaginase activity was determined by the direct Nesslerization method described by Castric *et al*. (1972) [7]. The cultures were grown in MCD medium supplemented with L-asparagine (1%) as the sole nitrogen source at 30 ° C for 72 hours. After centrifugation at 8000 × g for 20 minutes, the crude enzyme extract was used for analysis. The assay reaction mixture (1.5 mL total volume) contained 0.5 mL of L-asparagine (1%), 0.5 mL of phosphate buffer (50 mM, pH 8.0), and 0.5-1.0 mL of enzyme. After 30 minutes of incubation at 37 °C, 0.1 mL of TCA (20%) was added to stop the reaction. The mixture was centrifuged at 600 × g for 5 minutes, and the supernatant was diluted to 6 mL with distilled water. Then, 1 mL of Nessler's reagent was added, and the absorbance was measured at 480 nm after 10 minutes.

A standard curve was constructed using known concentrations of ammonium sulphate to calculate the amount of ammonia released.

Enzyme activity is expressed as µmol of ammonia produced per minute, and specific activity is reported as µmol NH_3/min/mg of protein.

Protein estimation

The protein content of the enzyme was determined according to Lowry's method (Lowry *et al*., 1951) [25].

Results and Discussion

Fungal identification (morphological/microscopic)

The fungal isolates obtained from decomposed banana peels were morphologically identified as *Aspergillus japonicus* and *Aspergillus carbonarius*. Colonies of *A. japonicus* exhibited compact, dark greenish-black growth with radial furrows, while *A. carbonarius* developed black, granular conidia with a cottony white margin on PDA (Fig. 1). Microscopic examination at 40× magnification revealed globose conidial heads and biseriate phialides in *A. carbonarius*, whereas *A. japonicus* displayed radiating conidial chains and smooth conidiophores (Fig. 2). These features corroborated earlier morphological keys for species-level identification within the genus *Aspergillus*.

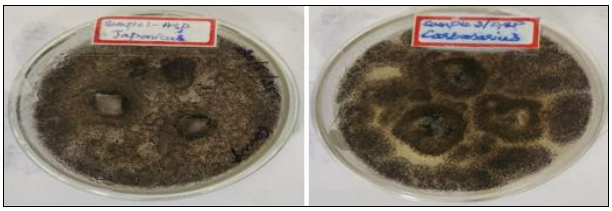

Fig 1: Fungal strains isolated from rotten banana peel grown on PDA

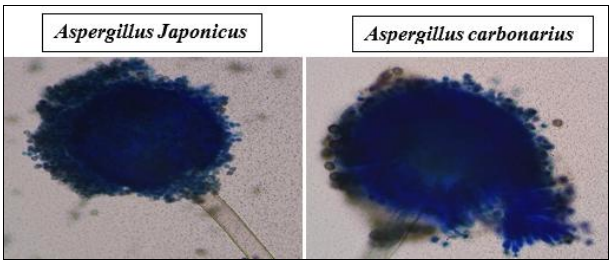

Fig 2: Micrograph of *Aspergillus japonicus* and *Aspergillus carbonarius* (40x)

Qualitative Enzyme Assay (Plate screening)

Plate assays revealed that both fungal species secreted multiple extracellular enzymes (Fig. 3, Table 1). Clear hydrolysis zones indicated active enzyme production; *A. japonicus* showed strong (+++) activity for pectinase, protease, and L-asparaginase, while moderate (++) activity was observed for cellulase and catalase. *A. carbonarius* demonstrated high (+++) activity for lipase, cellulase, and pectinase, and moderate (++) activity for protease, L-asparaginase, and catalase. Neither strain showed activity for amylase, urease, invertase, or xylanase under the tested conditions.

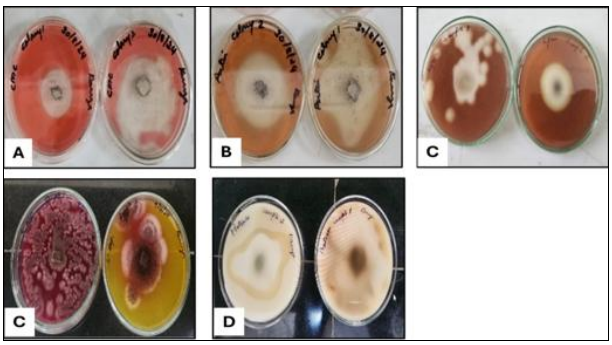

Fig 3: Image showing positive results for extracellular enzyme activity. (A) Cellulase; (B) pectinase; (C) lipase; (D) L-asparaginase; (E) protease

Table 1: Enzyme profile of *Aspergillus japonicus* and *Aspergillus carbonarius*

Strain	Amylase	Lipase	Cellulase	Catalase	Pectinase	Urease	Protease	L-Asparaginase	Invertase	Xylanase
Aspergillus japonicus	-	++	++	++	+++	-	+++	+++	-	-
Aspergillus carbonarius	-	+++	+++	++	+++	-	++	++	-	-
No activity '-', Poor activity '+', Moderate activity '++', High activity '+++'										

These patterns highlight species-specific enzymatic profiles that may reflect adaptation to substrate banana peels being rich in polysaccharides and lipids. The high pectinase and protease activity in *A. japonicus* aligns with reports of *Aspergillus* spp. thriving in fruit-rich substrates (Kc *et al*., 2020) [22]. The robust lipase activity in *A. carbonarius* reflects its capability to degrade lipid-rich matter, making it promising for biodiesel and detergent industries (Gopinath *et al*., 2013) [13].

Furthermore, combining qualitative screening with submerged fermentation provides a robust evaluation strategy for selecting fungal candidates based on both enzymatic breadth and productivity.

Quantitative Enzyme Assays (Submerged Fermentation)

To complement the qualitative findings, submerged fermentation was conducted to assess enzyme yield and productivity in standardized conditions. Quantitative results (Table 2, Fig. 4) revealed species-specific enzymatic dominance:

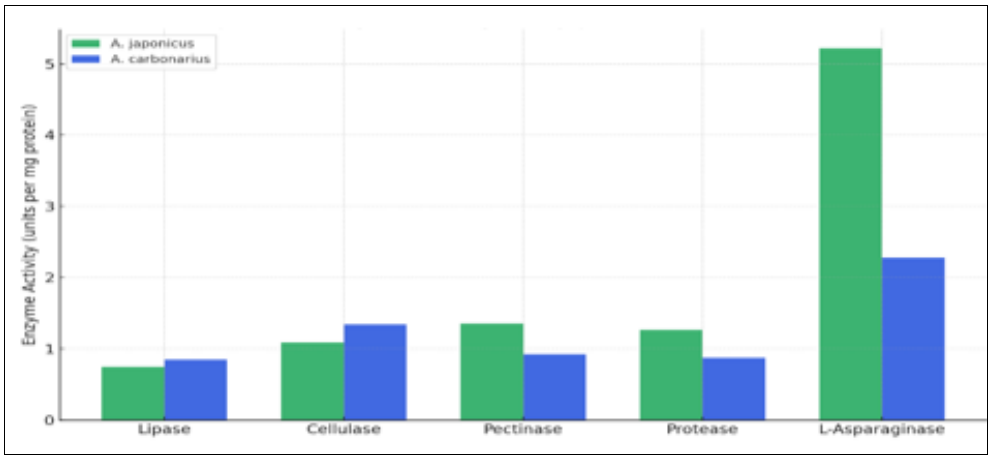

Fig 4: Comparative enzyme activity between *A. japonicus* and *A. carbonarius*

Lipase and Cellulase

A. carbonarius exhibited higher lipase and cellulase activity than *A. japonicus*, making it a strong candidate for use in biodiesel production (via transesterification reactions) and lignocellulose degradation for biofuel and pulp industries. Similar results were noted by Hasan *et al*. (2006) [17] and Kuhad *et al*. (2011) [23].

Pectinase

A. japonicus showed greater pectinase production, aligning with industrial requirements for fruit juice clarification and textile processing. Pectinase activity was significantly higher than in *A. carbonarius*, suggesting superior de-pectinization ability (Patil & Dayanand, 2006) [31].

Protease

The pronounced proteolytic activity in *A. japonicus* supports its application in protein hydrolysis for food and detergent industries, as also observed in *A. oryzae* and *A. niger* (Ayob & Simarani, 2016) [4].

L-Asparaginase

A. japonicus displayed nearly 2.3 times higher L-asparaginase activity than *A. carbonarius*, highlighting its biopharmaceutical potential, particularly in leukemia treatment. This finding resonates with studies on *A. niger* and *A. tamarii* strains for therapeutic enzyme production (Honfoga *et al*., 2025; Doriya & Kumar, 2016) [19, 10].

Table 2: Determination of enzyme activity

Enzymes	*Aspergillus japonicus*	*Aspergillus carbonarius*
*Lipase	0.75	0.85
#Cellulase	1.09	1.34
#Pectinase	1.36	0.93
#Protease	1.27	0.87
#L-Asparaginase	5.22	2.28
Enzyme Acitvity is expressesed as *U/ml/min/mg protein or #µmoles/ml/min/mg protein.		

Conclusion

This study elucidates the differential enzyme capabilities of *Aspergillus japonicus* and *Aspergillus carbonarius*, underscoring their industrial relevance and environmental sustainability. The distinct enzyme profiles suggest that these fungal strains can be harnessed for targeted applications, enhancing efficiency in sectors ranging from food processing to biofuel production. By leveraging their enzyme strengths, industries can reduce dependency on chemical processes, thus minimizing environmental footprints. Future research should prioritize the optimization

of fermentation parameters to boost enzyme yields and explore novel applications in emerging fields such as bioremediation and waste valorization. Continued exploration of fungal enzymology holds promise for advancing eco-friendly and economically viable industrial solutions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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