Microbiology Research International
Vol. 13(3), pp. 47-51, September 2025
ISSN: 2354-2128
Full Length Research Paper

Assessment of lipase and protease activities of aquatic fungi isolated from Yankari Warm Springs, Bauchi State, Nigeria

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Accepted 30 August, 2025

ABSTRACT

Microbial enzymes are essential in industrial bioprocesses, with applications in textiles, pharmaceuticals, food processing, and biofuels. This study investigated extracellular lipase- and protease-producing fungi isolated from Yankari Warm Springs, Bauchi State, Nigeria. Five dominant fungal species were selected for evaluation of their enzymatic activities. Isolates were obtained using baiting techniques with sterilized cannabis seeds in sterile water and identified morphologically and microscopically. Enzyme activities were quantified over five days using spectrophotometric assays. Significant ($p < 0.05$) variations in enzyme production were observed among species and across incubation periods. For lipase activity, *Aphanomyces astaci* exhibited early and consistently high activity (270.10 ± 0.04 µmol/min on Day 4), while *Penicillium chrysogenum* and *Saprolegnia parasitica* recorded the highest activities (282.89 ± 0.04 and 290.58 ± 0.01 µmol/min, respectively). Activity declined on Day 5, with some species showing negative values. Protease activity also varied: *P. chrysogenum* peaked on Day 1 (43.55 ± 0.08 µmol/min) but decreased thereafter, whereas *Leptolegnia caudata* and *A. astaci* recorded maximum activity on Day 3 (565.07 ± 0.39 and 477.35 ± 0.24 µmol/min, respectively). *S. parasitica* demonstrated sustained and significant protease production. These results underscore the high enzymatic potential of fungi from thermophilic aquatic ecosystems, particularly for industrial processes requiring thermostable enzymes. Fungi isolated from Yankari Warm Springs thus represent promising candidates for applications in biodegradation, waste treatment, and industrial biotechnology.

Keywords: Aquatic fungi, lipase activity, protease activity, enzyme assay, Yankari Warm Springs, fungal isolates, biotechnological potential.

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INTRODUCTION

Aquatic fungi are increasingly recognized as key components of microbial diversity in extreme environments, including geothermal springs, where they play significant ecological roles and present opportunities for biotechnological applications. Thermophilic and thermotolerant species thrive in high-temperature aquatic systems by producing extracellular enzymes such as cellulases, amylases, proteases, and lipases, which enable efficient degradation of organic matter (Maheshwari et al., 2000). Among these enzymes, lipases and proteases are of particular interest due to their broad industrial applications in pharmaceuticals, food processing, detergents, and biofuels (Singh et al., 2021). The extracellular secretion of these enzymes makes aquatic fungi promising candidates for sustainable and cost-effective enzyme production.

Lipases (triacylglycerol acyl hydrolases; EC 3.1.1.3) catalyze the hydrolysis of triglycerides into glycerol and free fatty acids, while proteases (EC 3.4) break down proteins into peptides and amino acids. Both enzyme classes are highly valued in industrial biotechnology for their catalytic versatility, substrate specificity, stability in organic solvents, and functionality across a wide range of pH and temperatures (Javed et al., 2018; Gupta et al., 2015). Thermophilic fungi from geothermal environments, such as hot springs, are especially attractive due to the inherent thermostability of their enzymes, which allows them to function effectively at elevated temperatures, reduce contamination risks, and enhance reaction efficiency (Ortega-Villar et al., 2024).

While bacterial enzyme producers from geothermal springs have been extensively studied, aquatic fungi - particularly from African geothermal systems—remain underexplored (Salano et al., 2017). The warm springs of Yankari Game Reserve, Nigeria, are characterized by unique temperature, pH, and mineral profiles that support diverse microbial communities with potential for high enzymatic output. Despite this ecological and biotechnological potential, little is known about the fungal diversity and enzymatic capacities of this environment.

This study aims to isolate aquatic fungi from Yankari Warm Springs and evaluate their extracellular lipase and protease activities. Given that microbial-derived lipases exhibit unique physicochemical and biological properties that enhance their role as biocatalysts, they represent effective alternatives to conventional organic methods for the selective transformation of complex molecules in various industries (Ali et al., 2023).

MATERIALS AND METHODS

Study area description

This study was conducted using samples collected from Yankari Warm Springs, located within the Yankari Game Reserve in Bauchi State, northeastern Nigeria. The warm spring, locally known as the Wikki Warm Spring, is a natural geothermal feature with an average temperature of 39–41 °C throughout the year (Obaje et al., 2015). The spring's constant flow, slightly alkaline pH, and moderate to high mineral content create a stable and nutrient-rich environment that supports thermotolerant and thermophilic microorganisms. These conditions are particularly favorable for fungi, as continuous water movement ensures oxygen availability, the alkaline pH promotes enzyme activity, and mineral content provides essential nutrients for metabolism and survival. The surrounding vegetation consists of savannah woodland, and the site remains largely undisturbed, with minimal anthropogenic influence (Osborne et al., 2018).

Sample collection

Water and sediment samples were collected from three different points along the warm spring using sterile 500 mL glass bottles for water and sterile spatulas with labeled polyethylene bags for sediments. Samples were transported on ice at 4 °C to preserve microbial integrity and processed within 24 hours of collection. For fungal enrichment, sediment samples were allowed to settle, and the supernatant was used for initial inoculation (Ghilamicael et al., 2017).

Isolation of aquatic fungi

Aquatic fungi were isolated using a baiting method with sterilized *Cannabis sativa* seeds, chosen for their nutrient-rich, oily composition that promotes fungal colonization and growth (Bello et al., 2025; Walk et al., 2018). A 20 mL water–sediment mixture was dispensed into sterile Petri dishes, and 4–6 surface-sterilized seeds were added. Seeds were sterilized by immersion in 70% ethanol for 1 minute, followed by five rinses in sterile distilled water. To inhibit bacterial growth, streptomycin sulfate (50 mg/L) was added to each dish. Petri dishes were incubated at 28–30 °C for 5–7 days, with fungal growth monitored daily under a stereomicroscope. Colonized seeds were aseptically transferred to malt extract agar (MEA) plates supplemented with streptomycin sulfate (50 mg/L) and incubated at 30 °C for 3–5 days. Emerging fungal colonies were repeatedly subcultured to obtain pure isolates.

Morphological and microscopic identification

Fungal isolates were identified based on macroscopic features such as colony morphology, pigmentation, texture, and growth rate on MEA plates. Microscopic characterization was performed by staining fungal mycelia with lactophenol cotton blue, followed by mounting on slides for observation under a light microscope at 1000× magnification with an oil immersion lens. Morphological traits including septation, spore arrangement, and hyphal structure were compared with standard mycological references, particularly Illustrated Genera of Imperfect Fungi (Barnett and Hunter, 1998).

Quantitative enzyme assays

Lipase and protease activities of selected fungal isolates were quantified in submerged fermentation. For lipase activity, isolates were cultivated in basal broth containing olive oil (lipid substrate) and peptone (nitrogen source) at 40 °C for 5 days. Culture supernatants were obtained by centrifugation and assayed using p-nitrophenyl palmitate (pNPP) dissolved in Tris–HCl buffer, with activity measured spectrophotometrically at 410 nm (Winkler and Stuckmann, 1979).

For protease activity, isolates were grown in casein-containing medium to induce enzyme production. Culture supernatants were analyzed using a modified Lowry method, where protease activity was determined by measuring tyrosine released from casein hydrolysis at 280 nm. The reaction mixture contained casein substrate incubated with enzyme, and the reaction was terminated with trichloroacetic acid (TCA) before color development with the Lowry reagent. One unit of protease activity was defined as the amount of enzyme required to release 1 µmol of tyrosine equivalent per minute under assay conditions (Winkler and Stuckmann, 1979).

Data analysis

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Clear zone diameters from enzyme screening were analyzed using one-way analysis of variance (ANOVA) to evaluate significant differences among isolates. Statistical analyses were conducted with GraphPad Prism version 8.1, with significance set at $p \leq 0.05$.

RESULTS

Lipase activities (µmol/min) of aquatic fungi isolated

Lipase activities of aquatic fungi isolated from Wikki Warm Spring varied significantly across species and incubation days ($p < 0.0001$; Table 1). On Day 1, *Aphanomyces astaci* exhibited the highest lipase activity (35.75 ± 0.07 µmol/min), followed by *Achlya bisexualis* (29.39 ± 0.24 µmol/min), while *Leptolegnia caudata* showed the lowest (17.53 ± 0.05 µmol/min).

Lipase activity generally increased until Day 4, with *Saprolegnia parasitica* peaking at 290.58 ± 0.01 µmol/min and *Penicillium chrysogenum* at 282.89 ± 0.04 µmol/min on Day 3. *A. astaci* maintained consistently high activity from Day 2 (41.20 ± 0.04 µmol/min) through Day 4 (270.10 ± 0.04 µmol/min), suggesting robust enzymatic potential.

However, most species exhibited negative lipase activity values on Day 5, including *A. bisexualis* (-8.85 ± 0.05 µmol/min) and *P. chrysogenum* (-8.64 ± 0.01 µmol/min). This decline may indicate enzyme degradation or metabolic inhibition due to nutrient depletion or toxic by-product accumulation. *L. caudata* was the exception, retaining slight positive activity (0.74 ± 0.01 µmol/min) on Day 5.

Protease activities (µmol/min) of aquatic fungi isolated

Protease activities also varied significantly across incubation days and fungal species ($p < 0.0001$; Table 2). On Day 1, *P. chrysogenum* exhibited the highest protease activity (43.55 ± 0.08 µmol/min), followed by *A. astaci* (35.83 ± 0.06 µmol/min), with *S. parasitica* recording the lowest (25.07 ± 0.72 µmol/min).

By Day 3, *L. caudata* demonstrated the highest protease activity (565.07 ± 0.39 µmol/min), followed by *S. parasitica* (492.13 ± 0.32 µmol/min) and *A. astaci* (477.35 ± 0.24 µmol/min). In contrast, *P. chrysogenum* peaked on Day 2 (252.37 ± 0.02 µmol/min) but declined thereafter.

Notably, *S. parasitica* maintained elevated activity through Day 5 (233.29 ± 0.39 µmol/min), highlighting its potential role in late-stage organic matter degradation. *L. caudata* and *A. astaci* also sustained considerable protease activity on Day 5 (152.57 ± 0.50 µmol/min and 176.52 ± 0.16 µmol/min, respectively).

Table 1. The lipase activities (µmole/minute) of the aquatic fungi isolated from Yankari Warm Springs.

Organisms	Day 1	Day 2	Day 3	Day 4	Day 5
Achlya bisexualis	$29.39{\pm}0.24^{b}$	$36.58{\pm}0.15^{e}$	$127.05{\pm}0.03^{e}$	$252.67{\pm}0.29^{d}$	$-8.85{\pm}0.05^{b}$
Aphanomyces astaci	$35.75{\pm}0.07^{a}$	$41.20{\pm}0.04^{d}$	$276.60{\pm}0.06^{b}$	$270.10{\pm}0.04^{c}$	$-7.17{\pm}0.01^{bc}$
Saprolegnia parasitica	$28.85{\pm}0.10^{c}$	$45.69{\pm}0.04^{c}$	$184.32{\pm}0.12^{d}$	$290.58{\pm}0.01^{a}$	$-5.10{\pm}0.02^{c}$
Leptolegnia caudata	$17.53{\pm}0.05^{d}$	$128.49{\pm}0.1^{b}$	$243.49{\pm}0.14^{c}$	$288.07{\pm}2.58^{b}$	$0.74{\pm}0.01^{a}$
Penicillium chrysogenum	$25.46{\pm}0.21^{c}$	$252.44{\pm}0.1^{a}$	$282.89{\pm}0.04^{a}$	$251.84{\pm}0.11^{d}$	$-8.64{\pm}0.01^{b}$
L. S. D	1.50				
P-value	<0.0001				

At P≤0.05, there was a significant difference in the lipase activities (µmole/minute) of the Aquatic Fungi Isolated from Yankari Warm Springs. Values are presented as mean±standard error of means. Ranking was done across the organisms and values with the same superscript are not significant.

Table 2. The Protease activities (µmole/minute) of the aquatic fungi isolated from Yankari Warm Springs.

Organisms	Day 1	Day 2	Day 3	Day 4	Day 5
Achlya bisexualis	$29.32{\pm}0.09^{d}$	$36.49{\pm}0.03^{e}$	$391.31{\pm}0.30^{d}$	$251.52{\pm}0.34^{d}$	$87.24{\pm}0.23^{b}$
Aphanomyces astaci	$35.83{\pm}0.06^{b}$	$41.18{\pm}0.01^{d}$	$477.35{\pm}0.24^{c}$	$341.09{\pm}0.28^{a}$	$176.52{\pm}0.16^{b}$
Saprolegnia parasitica	$25.07{\pm}0.72^{b}$	$45.70{\pm}0.02^{c}$	$492.13{\pm}0.32^{b}$	$290.94{\pm}0.34^{b}$	$233.29{\pm}0.39^{c}$
Leptolegni acaudata	$33.63{\pm}0.71^{c}$	$128.58{\pm}0.62^{b}$	$565.07{\pm}0.39^{c}$	$285.69{\pm}0.56^{c}$	$152.57{\pm}0.50^{a}$
Penicillium chrysogenum	$43.55{\pm}0.08^{a}$	$252.37{\pm}0.02^{a}$	$367.31{\pm}0.11^{e}$	$251.75{\pm}0.13^{d}$	$84.49{\pm}0.12^{e}$
L.S. D	0.98				
P-value	<0.0001				

At P≤0.05 there was a significant difference in the protease activities (µmole/minute) of the Aquatic Fungi Isolated from Yankari Warm Springs. Values are presented as mean±standard error of means. Ranking was done across the Organisms and values with the same super script are not significant.

DISCUSSION

The observed variations in lipase activity among aquatic fungi isolated from Yankari Warm Springs are consistent with previous reports on fungal enzyme dynamics under aquatic and thermophilic conditions. These results suggest that the fungi possess diverse adaptive strategies and enzymatic potentials that shape their ecological functions and possible biotechnological applications. The early and sustained lipase activity observed in *Aphanomyces astaci* aligns with findings by Bärlocher and Kendrick (1974), who reported that certain aquatic fungi rapidly secrete enzymes in nutrient-rich environments. In contrast, the delayed activity peaks of *Saprolegnia parasitica* and *Penicillium chrysogenum* are consistent with the observations of Gopinath et al. (2005a), who noted that lipase production in filamentous fungi is often strain-dependent and influenced by factors such as substrate availability and incubation time.

The decline in lipase activity by Day 5, including the negative values recorded in most isolates, may indicate enzyme instability, catabolic repression, or assay interference. Sharma et al. (2001) similarly reported that prolonged incubation in submerged cultures often leads to lipase degradation, either through proteolytic activity or unfavorable pH shifts. Interestingly, *Leptolegnia caudata* retained measurable activity on Day 5, highlighting its resilience under nutrient or thermal stress. This observation mirrors the findings of Raghukumar (2000), who described certain aquatic fungi as stress-tolerant organisms with adaptive metabolic strategies in extreme environments. Such stability suggests that *L. caudata* could serve as a reliable source of lipase for industrial processes requiring thermostable enzymes.

Protease activities also revealed dynamic, species-specific, and time-dependent profiles. The initial high protease activity in *P. chrysogenum* on Day 1 suggests rapid enzyme induction, likely driven by early nutrient availability. Similar trends were reported by Gopinath et al. (2005b), who observed elevated early protease production in filamentous fungi under favorable growth conditions. The subsequent decline in activity is consistent with nutrient depletion or repression mechanisms, as reported by Anitha and Palanivelu (2013), who found downregulation of protease production under nutrient-limiting conditions.

In contrast, *L. caudata* exhibited delayed but robust protease activity, peaking on Day 3. This suggests a strong late-phase enzymatic response, which could be advantageous for industrial applications requiring sustained enzyme output. This pattern is consistent with Panda et al. (2017), who noted that fungi from thermophilic environments often display delayed maximal enzyme production. Similarly, *S. parasitica* and *A. astaci* sustained high protease activity throughout the incubation period, peaking on Day 3 (492.13 and 477.35 µmol/min, respectively). These results highlight their significant ecological roles in protein degradation in aquatic ecosystems and their potential for biotechnological applications.

CONCLUSION

This study demonstrates that aquatic fungi isolated from Yankari Warm Springs exhibit significant variation in lipase and protease activities across species and incubation periods. *Saprolegnia parasitica* and *Leptolegnia caudata* emerged as prominent producers of both enzymes, with *L. caudata* showing exceptional stability in lipase and protease output. Such stability is advantageous for industrial applications that require consistent enzyme performance under variable conditions.

These findings highlight the potential of aquatic fungi from thermophilic environments as valuable sources of industrial enzymes with applications in bioremediation, waste management, and biotechnology. Further research is recommended to purify, characterize, and optimize enzyme production, as well as to evaluate their performance under large-scale industrial conditions.

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Citation: Auwalu L, Ali AD, Amienyo CO, **2025**. Assessment of lipase and protease activities of aquatic fungi isolated from Yankari Warm Springs, Bauchi State, Nigeria. Microbiol Res Int, 13(3): 47-51.