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Full Length Article

Optimization of Fermentative Parameters and *In Vitro* Safety Evaluation of Amylase Produced by *Mucor flavus*

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Abstract

Fungal species are a valuable resource for starch-based industries, particularly for amylase production. This study evaluated the amylolytic potential of *Mucor flavus* under submerged fermentation using agro-industrial substrates and evaluated the safety profile of its crude enzyme extract. The fungal isolates were identified by macroscopic and microscopic characters including brownish fluffy growth with no pigment on reverse side and coenocytic hyphae with sporangium. Molecular identification *via* PCR produced an amplicon of 290 bps on agarose gel. Screening on starch agar revealed *M. flavus* as the most potent amylolytic strain, demonstrating the largest zone of hydrolysis. Subsequently, amylase production was optimized under different growth conditions in submerged fermentation, including temperature (22, 28 and 37°C), pH (4.5, 6 and 7) and different natural substrates (maize flour, wheat bran, and rice husk) at varying concentrations (1, 3 and 5%) using one variable at a time. The effect of these parameters was assessed by measuring amylase activity (IU/mL/min) using the dinitrosalicylic acid (DNS) method. The amylolytic potential of *M. flavus* varied significantly with the change in temperature, pH, substrate and substrate concentration. The maximum amylase activity of 87.90 ± 1.30 IU was achieved at pH 6 and 3% concentration of rice husk followed by wheat bran, which yielded the maximum amylase activity (24.35 IU) at 22°C, pH 7.5 and 5% concentration. In case of maize flour, the maximum amylase activity was 7.33 IU at pH 4.5, 22°C and a substrate concentration of 3%. A linear increase in enzyme yield was recorded up to 3% concentration of maize flour and rice husk, beyond which a decline was observed. The ideal conditions include a temperature of 37˚C, a pH of 6, and rice husk as the most effective substrate. Statistical analysis revealed the significant variations among fermentation parameters ($P > 0.05$). A safety assessment using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay revealed a direct link between fungal supernatant concentration and reduced cell survival. The cell survival percentage dropped below 50% at a concentration of ≥ 31.25 μL/mL. Ultimately, these findings highlight the potential of M. flavus as a potential candidate for starch-based industries due to its rapid growth and exceptional enzyme productivity using cost-effective agro-industrial substrates.

Keywords: *Mucor flavus*; Amylase activity; PCR; Dinitrosalicylic acid; MTT assay; Submerged fermentation

Introduction

Enzymes play a critical role in decreasing energy of activation and speeding up several cellular activities that are essential for life without producing irreversible changes. These are found in every living organism, including animals, plants, and microorganisms. Several enzymes including amylases, lipases, proteases, cellulases, pectinases, xylanases and oxidoreductases have substantial application in various industries (Alves *et al.* 2002; Yadav *et al.* 2024). Amylases are glycoside hydrolytic industrial enzymes that can degrade starch into sugar, making it valuable for utilization in industries that require starch in their production processes (Ahmad *et al.* 2020). Amylases are one of the most indispensable enzymes (Farooq *et al.* 2021) contributing 25–30% to enzyme market worldwide (Sharif et al. 2023). These are widely used in the production of renewable energy, saccharification of starches, liquefication of starches, baking, paper industry, textile industry, pharmaceutical industry and in livestock to pretreat feed of animals. Amylases are broadly classified into three categories namely α-amylase, β-amylase, and glucoamylase

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(Sethi *et al.* 2016; Xia *et al.* 2021). Amylases can be acquired from prokaryotes and eukaryotes including plants, animals, yeast, mold, and bacterial cells (Zaferanloo *et al.* 2014; Gopinath *et al.* 2017). Among microorganisms, amylase production by fungi at industrial level is favorable because of higher yield, reduced isolation requirements, and effective catalysis with vital stability under harsh conditions (El-Gendi *et al.* 2021). These are also preferred because of the presence of mycelia, which allow the easy extraction of enzyme from fungal biomass (Lübeck and Lübeck 2022). Fungal enzymes now account for more than 50% of the overall enzymes market (El-Gendi *et al.* 2021).

Fungal species are well-known candidates for the enzyme industry. Data has been reported on starch hydrolyzing fungal species including *Aspergillus* and *Penicillium*. However, a limited amount of data is available on starch hydrolyzing Mucor spp. to the best of our knowledge. In contrast to other fungi, Mucor has a fast growth rate *i.e*., 15 mm/day at 20°C, 4 mm/day at 0°C (Danilova *et al.* 2024). Amylase production from *Penicillium* was recorded after 6–18 days of incubation (Dar *et al.* 2015). Similarly, the incubation period of *Aspergillus* to produce enzyme is about 8–10 days (Goto *et al.* 1998). However, Amylase production by *Mucor* spp. is amazingly fast. Alves *et al.* (2002) reported that 84% of *Mucor* spp. were able to produce amylases. Mohapatra *et al.* (1998) obtained a new type of amylase from *Mucor* spp. and *Spirastrella* spp. (a marine sponge).

Evaluating various fungal isolates and optimizing growth conditions are crucial for maximizing amylase production (Behailu and Abebe 2018). These growth conditions include temperature, pH, source of carbon and source of nitrogen. Different natural materials are being assessed as suitable substrates for amylase production by fungi include rice husk, wheat bran, sugarcane bagasse, vegetable waste, banana peels and date waste (Sethi *et al.* 2016; Sahu *et al.* 2024). *Mucor,* a saprophytic fungus, is widely used in the food industry and fermentation process, helps bioremediation with plants and cellulolytic activity for plant degradation in soil (Khalid *et al.* 2006; Zhu *et al.* 2015). One of the environmental factors that affect growth and metabolites production of *Mucor* is its inherent potential. The selected species of *Mucor* shows different enzyme activity from previous data due to difference in fermentation conditions used in this experiment. These conditions are pH, temperature, dissolved oxygen tension (DOT) and substrate concentration as used by previous researchers (Sundström 2007).

Mucor has a fast growth rate (Morin-Sardin *et al.* 2017) and it is one of the generally regarded as safe (GRAS) organisms (Gadre *et al.* 2003). To date limited data are available on starch hydrolysis potential of *Mucor* species. It is hypothesized that *M. flavus* could be a good candidate for amylase production. Moreover, optimization of fermentation parameters may enhance the amylase production by the *M. flavus.* The produced enzyme is expected to be safe. The *M. flavus* can be a potential source for industrial amylase. In present study Indigenous *M. flavus* was evaluated for *in vitro* amylolytic activity and fermentation parameters were optimized for amylase production on laboratory scale and the produced amylase was used for safety testing. This is the first comprehensive evaluation of *M. flavus* for amylase production using agro-industrial residues under submerged fermentation conditions.

Materials and Methods

Identification and culture of *Mucor* species

The fungal isolates used in this study were identified as *Mucor flavus* (Kingdom: Fungi, Phylum: Mucormycota, Class: Mucormycetes, Order: Mucorales, Family: Mucoraceae, Genus: *Mucor*, and species: *M. flavus*). It was previously isolated from soil of livestock farms and evaluated for amylase production. Different fermentation parameters (temperature, pH and substrates) were evaluated for their effect on starch hydrolyzing potential of indigenous *Mucor* spp.

The *M. flavus* (05) was procured from Mycology Laboratory, Institute of Microbiology, University of Veterinary and Animal Sciences Lahore, Pakistan. It was inoculated on Sabouraud Dextrose Agar (SDA) followed by incubation at 25°C for 3 days. Macroscopic features from obverse and reverse side of plate were observed with unaided eye. Microscopic structures were recorded by slide culture method (agar drop method). A small drop of molten SDA was placed on a sterile microscopic glass slide. A small number of spores from pure culture were transferred with inoculating needles and covered by cover slip. Slides were placed at 25°C in humid incubators and observed on daily basis at 100 and 400X under light microscope (Riddle 1950).

Molecular characterization of *Mucor* species

Mucor spp. was characterized by targeting internal transcribed (ITS) regions using polymerase chain reaction (PCR) with the modified method of Lau *et al.* (2007). Primer sequences are ITSI; 5'-TCCGTAGGTGAACCTGCGG-3', ITS2; 5'-GCTGCGTTCTTCATCGATGC-3. DNA was extracted from 24 h old mycelia using commercially available genome extraction kit (Gene JET Genomic DNA Purification Kit Thermo Scientific). PCR was carried out with 25 μL reaction (Extracted DNA 10 μL, Master mix 12.5 μL, Primers/each 0.4 μL and 1.7 μL nuclease free water). PCR conditions for amplification were initial denaturation 94°C/10 min followed by 35 cycles of denaturation 94°C/15 sec, annealing 55°C/30 sec and extension 72°C/30 sec. The final extension cycle was run at 72°C/7 min. After the completion of PCR, amplicons were loaded into 2% agarose gel and electrophoresis was conducted. The gel was observed in UV transilluminator.

Screening for non-toxigenic fungi

Non-toxigenic potential of *M. flavus* was confirmed by thin layer chromatography (TLC). Purified fungal culture was inoculated into Sabouraud dextrose broth (SDB) followed incubation at 25°C for 15 days in dark. After incubation, the broth culture was autoclaved at 121°C for 15 min under 15 lb/inch^2. Following homogenization, 12.5 g of fungal culture was mixed with extractants. These were 5 mL of chloroform, 5 mL of methanol and 2.5 g of sodium chloride. This mixture was incubated at 37°C for 30 min with constant shaking. This mixture was sieved by muslin cloth and then filtered by Whatman filter paper. This filtrate was evaporated at room temperature. Crystals obtained were crushed to fine powder and dissolved in 1 mL of chloroform. The presence or absence of mycotoxins was determined by TLC. Extracted toxins (20 μL) were spotted on silica coated TLC plate and placed in chromatographic tank having mobile phase of chloroform (95 mL) and acetone (5 mL). TLC was allowed to run. After the completion of process, results were observed in wooden lamp under UV light at 365 nm wavelength (Sana 2019).

Screening for starch hydrolysis

Preliminary screening for amylase production was carried out by inoculating *Mucor* spp. on starch agar (Difco^{TM}) by spot culture technique and placed at 25°C for 3 days. Starch agar having fungal colony were flooded with iodine solution. Colony showed zone of starch hydrolysis indicated for amylase production (Hamilton *et al.* 1999). The highest producer species was selected for optimization experiments.

Effect of fermentation parameters

The parameters evaluated for amylase production by *Mucor* spp. in submerged fermentation were temperature, pH, substrate, and substrate concentrations. One variable at a time (OVAT) experimental design was used in current study. Each variable was tested while maintaining all other factors constant (Stergiou *et al.* 2012; Ekedegba *et al.* 2022). All experiments were replicated three times (n = 3). Data were recorded as mean ± standard deviation and statistical significance was recorded by one-way analysis of variance with 5% level of significance.

Preparation of inoculum: A standard inoculum of *Mucor* spp. (10^6 spores /mL) was used in fermentation experiments. Spores were counted using Neubauer chamber as described by Arias *et al*. (2013). Spores from pure culture were transferred to sterile normal saline (0.85% NaCl). A uniform suspension was prepared and 10 μL suspension was poured into Neubauer chamber. Spores were counted in chamber boxes at 400X magnification under microscope and adjusted to 10^6 spores /mL. One mL of this suspension was used for inoculation.

Effect of pH: Inorganic broth 100 mL (g/L: $NaNO_3$ 1; K_2HPO_4 1; $MgSO_4.7H_2O$ 0.5 g; $FeSO_4$ 0.01) was prepared in 250 mL Erlenmeyer flasks having 1% substrate (maize flour, wheat bran and rice husk). Three different pH values (4.5, 6 and 7.5) were adjusted for each substrate. One mL of standard spore suspension was inoculated, and all flasks were incubated at 37˚C for seven days (Sunitha *et al.* 2012).

Effect of temperature: To evaluate the effect of temperature spore suspension were inoculated into inorganic broth having constant pH (6) and 1% of each substrate (maize flour, wheat bran and rice husk). Flasks were incubated at three different temperatures 22, 28 and 37°C for a period of seven days (Balkan *et al.* 2011).

Effect of substrate concentration: These experiments were conducted at constant pH (6) and temperature with varying concentrations of three different substrates (1, 3 and 5%). All flasks were placed at 37°C for seven days (Huitron *et al.* 2007).

Quantification of amylase

After each fermentation experiment, every flask was processed to obtain crude enzyme by sieving followed by centrifugation. Amylase was quantified by enzyme activity assay using dinitrosalicylic acid (DNS) method. One mL of crude enzyme, 2 mL of phosphate buffer (pH 6) and 2 mL of starch solution (1%) were mixed in glass test tube and placed at 37°C for 30 min. Following incubation 3 mL of DNS reagent was added and tubes were placed at boiling temperature until color changed. A tube without crude enzyme having 3 mL DNS reagent and 7 mL distilled water was used as blank. Absorbance was recorded at 540 nm. A standard curve of glucose between different glucose concentrations and absorbance was used to quantify enzyme units. One enzyme unit (IU/mL/min) was equal to μmol of reducing sugar released from substrate under assay conditions (Miller 1959).

Safety profile of *M. flavus*

The cytotoxicity of *M. flavus* broth was evaluated using BHK-21 cell line by MTT (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium) assay as described by Majoumouo *et al.* (2020). Monolayers of Vero cells were cultured into 96 wells cell culture plate in M-199 medium. The crude enzyme source (cell free supernatant) was then serially diluted using 100 μL as initial volume. All dilutions were added to cultured cell lines in 96-wells cell culture plate followed by incubation at 37°C temperature with CO_2 (5%) for 48 h. Following the incubation, 50 μL of MTT solution was added into each well, the cell culture plate was re-incubated for 3 h under same conditions. After the incubation period, the medium was discarded and 100 μL of dimethyl sulfoxide (DMSO) was poured into each well. The plate was then gently shaken. The plates were gently spun for 10 min at room temperature to dissolve the precipitate.

After that, the optical density was measured at 570 nm and cell survival percentage was calculated using the following formula:

$$\text{Cell servival percentage} = \left(\frac{\text{ODt} - \text{ODn}}{\text{ODc} - \text{ODn}}\right) \times 100$$

Where, ODt (test), ODc (control) and ODn (negative) denote the average optical densities measured at a wavelength of 570 nm for all treatment groups.

Statistical analysis

A one variable at a time (OVAT) was used to find the effect of fermentation parameters on amylase production by *M. flavus*. Results were analyzed using statistical package for social sciences (SPSS version 20) and expressed as mean ± SD. One-way analysis of variance (ANOVA) was applied with level of significance 0.5 followed by Duncan's multiple range tests (Singh *et al.* 2014).

Results

Identified culture of *M. flavus* was used for starch hydrolysis and production of amylase in submerged fermentation under different physical and chemical parameters. Prior to fermentation experiment, macroscopic and microscopic features were observed for identification of *Mucor* spp. It showed brownish color colony with fluffy texture on SDA; there was no pigmentation on reverse side of plate. Microscopy by slide culture revealed coenocyte hyaline hyphae at 400X magnification (Fig. 1). Sporangiospores were observed in closed vesicles called sporangium. Absence of rhizoids was recorded under sporangiophore. The zone of starch hydrolysis on starch agar by *Mucor* spp. indicated the potential for amylase production.

Amylase activities (IU) were calculated from crude enzymes obtained under different fermentation conditions. The variation in pH of medium keeping other variables constant influenced the quantity of amylases produced. The highest activity (IU) of amylase observed was 4.95 at pH 4.5 using maize flour as substrate. In the case of the wheat bran, the optimum pH recorder was 7.5, which gave 7.11 IU. Similarly, rice husk produced 6.05 IU at pH 7.5 when inoculated by *Mucor* spp. However, 0.11 IU was the lowest production of amylase produced using maize flour at pH 6 (Fig. 2). The temperatures of 22°C were optimum for *Mucor* sp. using maize flour and wheat bran as substrate with activity units of 7.33 and 24.35. However, in the case of rice husk higher yield (4.24 IU) was recorded at 28°C. The IU of amylases at different temperatures is indicated in (Fig. 3).

The highest quantity of amylases (87.90 ± 1.30 IU) was found at pH 6 and 3% concentration of rice husk. The lowest yield was 0.11 ± 0.01 at pH 6, 37°C using maize flour as substrate. Amylase production increased with an increase in concentration from 1–5% in the case of wheat bran as substrate. However, for maize flour and rice husks a

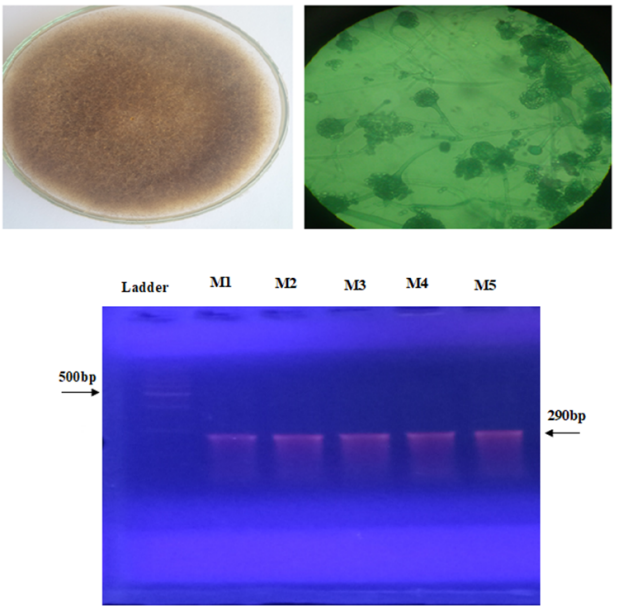

Fig. 1: Identification of *M. flavus*
(**a**): Macroscopic characters of *M. flavus;* (**b**): Microscopic examination of *M. flavus;* (**c**): Amplicons (290 bps) on agarose gel

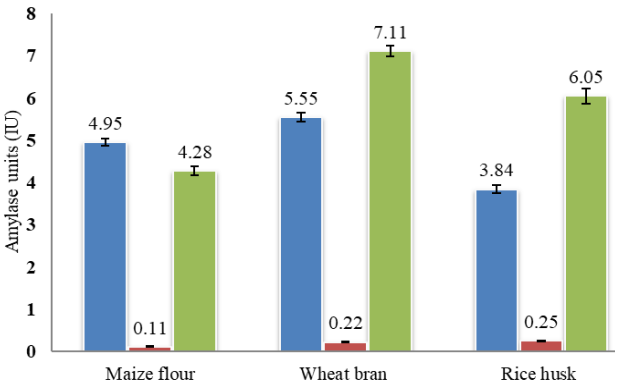

Fig. 2: Amylase activity of *M. flavus* at different pH

linear relation between enzyme units and substrate concentration was observed up to 3% after which reverse relation was found (Fig. 4). Statistically significant results were obtained ($P \leq 0.05$) showed variations among enzyme units at different culture parameters.

According to the results of safety profile, less than 50% cell survival was detected at and below 31.25 μL/mL while 15.625 μL/mL showed 75.22% cell survival, which was above the cut-off value. Overall, an inverse relation was detected between concentration of amylase and cell survival percentage (Fig. 5).

Discussion

M. flavus is an underexplored fungus for amylase production. Its faster growth and enzyme production rates as compared to *Aspergillus* and *Penicillium* make it

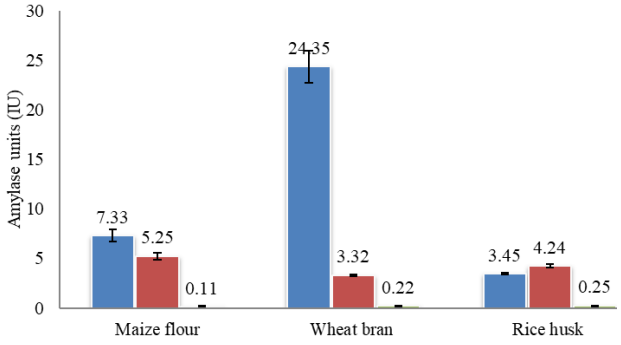

Fig. 3: Amylase activity of *M. flavus* at different temperatures

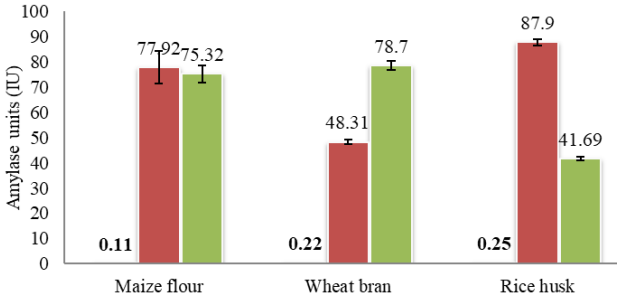

Fig. 4: Amylase activity of *M. flavus* with varying concentrations of different substrates

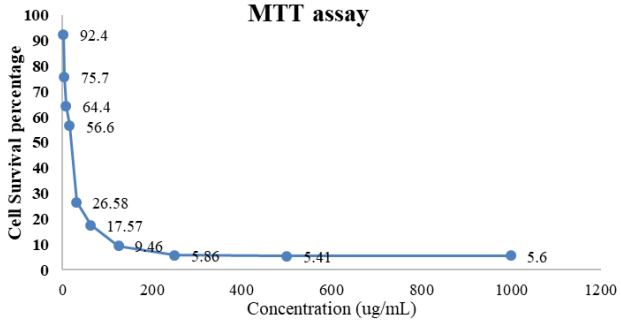

Fig. 5: Safety profile of cell free supernatant of *M. flavus* using MTT assay

an ideal candidate for industrial enzyme production. In this research, *M. flavus* was cultivated, and amylase was purified and evaluated for optimal activity. Previously, Hawar (2022) evaluated various *Rhizopus* and *Mucor* spp. for extracellular enzymes production including proteases, pectate lyase, ureases, ribonuclease polygalacturonase and amylases. Species from both genera were able to produce amylases in culture medium. In another study, Petruccioli and Federici (1992) evaluated 73 fungal species for extracellular enzymes. The species *Malassezia furfur, Saccharomycopsis malanga, M. racemosus* and *M. ramannianus* proved to be a source of amylase, phosphatase, polygalacturonase and urease. Ogundero (1979) found optimal temperature (45–50) and pH (6–7) activities of amylases harvested from cultivation of *M. pusillus* and some other fungal species. Previous studies indicated the amylase production potential of *Mucor* spp. with which, the findings of present study are substantiated for amylase produced from indigenous *M. flavus* isolate.

M. flavus is a saprophytic fungus which commonly found on decaying plant material, animal dung and in environmental reservoirs such as air, water, and soil (Walther *et al.* 2019). It is a ubiquitous fungus, with at least 74 reported strains globally (Schoch *et al.* 2020). *M. flavus* is recognized as a psychrophilic organism capable of growing within temperature range of –2 to 25°C (Danilova *et al.* 2024). Morphologically, this species is blackish brown with no pigmentation on the reverse side of culture media. *M. flavus* species have tall, branched sporangiophores and mid-sized sporangia with diameters over 80 μm but less than 200 μm (Walther *et al.* 2013). The mature sporangium has bulb shaped extracellular vesicle attached to the tip of sporangiophore. These are also known for egg-shaped columella (Hassan and Voigt 2019). It belongs to the order Mucorales which is famous for causing mucormycoses that can be life threatening if left untreated. However, the current study indicates that this species has a favorable safety profile at lower concentration suggesting its potential application in industrial settings.

Several factors affect the production of amylases by microorganisms including temperature, pH, phosphate, carbon and nitrogen source, metal ions, agitation, and metal ions. Although, a few studies have been reported on optimization of cultural conditions for amylase producing *Mucor* or related species. However, the amylase production by *M. flavus* is comparable with the well-known amylase producer. Temperature is a critical physical parameter that directly affects metabolism of microorganisms. In contrast to the present study's optimal temperature, Mohapatra *et al.* (1998) found the highest amylase yield by *Mucor* spp. at temperature 60°C. In another study, Varalakshmi *et al.* (2009) revealed that 22°C temperature as optimum for amylase production by *A. niger,* which also differs from current findings. Similarly, Divakaran *et al.* (2011) reported *Bacillus* spp. as amylase producer strains. The highest amylase activity was reported to be 3.64 IU at 37°C temperature. The results contrast with the present study. These variations highlight that the optimum temperature for amylase production varies significantly across the microbial taxa, which can be attributed to unique genetic trait of organism. Additionally, environmental origin, culture conditions, fermentation method affects the amylase activity. The current study is a valuable addition in understanding the less explored *M. flavus* which produce amylase under mesophilic conditions. In their study, Mohapatra *et al. (*1998) found amylases producing *Mucor* spp. and the highest amylase production was recorded at an optimal pH of 5.0. In contrast, Varalakshmi *et al.* (2009) study reported pH 7.5 as optimum conditions for amylase production by *A. niger* opposite to results of present study. However, Singh *et al.* (2014) determined pH 6.0, incubation

period of 6 as optimal conditions for amylase production of *A. fumigatus* as amylase producing strain, which is similar to results of present study. The similarity in findings may be due to metabolic traits which are comparable *M. flavus* and *Aspergillus* spp. Both fungi prefer mild acidic conditions and have common enzyme regulation.

Amylase production varies depending upon the substrate used. In an optimization study, Sana (2019) evaluated amylase production by *A. flavus* against different substrates such as maize sorghum, wheat bran and rice husk. The highest amylase production was recorded at higher concentration of substrates, corroborating the results of present study in which different amylases units were produced by *Mucor* spp. using different substrates at different concentrations. Similarly, Divakaran *et al.* (2011) produced different amylase units using different substrates for *Bacillus* spp. as amylase producer strains. The highest amylase activity was found to be 3.64 IU, suggesting that pure substrate (soluble starch) produce better results as compared to starch from agricultural sources. The results are in contrast with the findings of present study, which indicate that rice husk is the best substrate for amylase yield by *M. flavus*. The effect of various carbon sources was evaluated by Silva *et al.* (2005) on amylase production by *Rhizomucor* spp. belonging to zygomycetes. These were cassava pulp and cassava processing waste, corn bran and corn processing waste and soluble starch. Among these cassava pulp proved to be a good carbon source for amylase production by *Rhizomucor* spp. (Prongjit *et al.* 2022). Interestingly, cocultivation approach was used by Vinogradova and Kushnir (2003) and an increased hydrolytic activity was observed by *Mucor* spp. with *Schizophyllum commune*. Quantitatively, a significantly higher enzyme yield was reported by other fungal species. Like, Figueira and Hirooka (2000) reported *Fusarium moniliforme* produced 42.32 IU and *A. flavus* 4745.54 IU of amylase units. However, Singh *et al.* (2014) reported 341.7 IU of amylases produced by *A. fumigatus* under optimized conditions. The contrast result may be due to microorganism's differences of enzyme pathways. Substrate preferences also vary by species like purified starch is favorable for *Bacillus* and *M. flavus* may prefers complex substrate. In contrast to the above findings, in current study *Mucor* spp. produced 87.90 IU of amylases under selected conditions. Although comparatively lower than some previously reported results, it is still important due to the limited prior data on this species, its enzymatic potential and ability to produce under mesophilic conditions.

According to earlier research, fungal crude extracts include a number of metabolites with a range of biological activities (Majoumouo *et al.* 2020). Our study revealed that there is an inverse relationship between concentration and cell survival percentage, since as the concentration of the substance decreases the survival rate of the cells increases. This suggests that the substance may be toxic to the cells at higher concentrations, but less harmful at lower concentrations. However, 50% cell survival was cut-off value, and it was achieved at 31.25 μL/mL concentration of cell free supernatant of fungal culture. This concentration dependent decrease in cell survival correlates with another study, which discovered that the fungal extract had strong anticancer efficacy against human lung carcinoma (A549), with hepatocellular carcinoma coming in second (Kalaba *et al.* 2022). This concentration dependent cytotoxicity of crude extract of *M. flavus* may pose cytotoxic risk at higher concentration. It suggests further purification of amylase for potential applications.

Conclusion

Mucor spp. has amylase production potential and may be employed for amylase production to meet the industrial demands of amylases in starch-based industries. The study identified *M. flavus* as a promising candidate for amylase production in starch-based industries. The optimal fermentative parameters for amylase production by *M. flavus* were 37°C temperature, pH: 6 and rice husk is performing a better substrate than maize flour and wheat bran. The highest amylase activity achieved under these conditions was 87.90 ± 1.30 IU/mL/min. The safety profile assessment using MTT assay showed an inverse relationship between fungal supernatant concentration and cell survival percentage, indicating potential cytotoxicity at higher concentrations. *M. flavus* demonstrates potential as an efficient amylase producer for starch-based industries due to its fast growth rate and high amylase activity under optimized conditions. However, further research may be needed to address potential safety concerns indicated by the MTT assay results.

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Author Contributions

SS planned and designed the experiments. LN conducted the experiments. SG prepared the manuscript and MS format the manuscript.

Conflict of Interest

Authors have no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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