Comparative Study on the Morphological and Growth Characteristics of *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus oryzae*

Xingjian Duo

University of Nottingham, Nottingham, The United Kingdom
Email: dxingjian1918@163.com

***Abstract*—*Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus oryzae* are common filamentous fungi found in food, agriculture, and the environment. They play important roles in industrial production, food safety, and fermentation. Previous studies have mainly focused on metabolism, toxin production, or industrial applications of individual fungal species. In contrast, systematic comparative studies of the morphology and growth characteristics of these three molds remain limited. In this study, three standard strains were grown on PDA medium and evaluated using colony observation, microscopic examination, measurement of growth extent, and temperature adaptability tests. We compared their colony shape, microscopic structures, growth extent, and temperature adaptability. The results showed differences in colony shape, hyphal structure, and reproductive structures among the three species. These features are useful for fungal identification. *Rhizopus oryzae* grew the fastest, followed by *Aspergillus flavus* and *Aspergillus niger*. Among the three temperatures tested, all three fungi grew best at 28 °C. However, *Aspergillus niger* showed more tolerance to higher temperatures, *Rhizopus oryzae* showed better growth at lower temperatures, and *Aspergillus flavus* showed balanced adaptability to the environment. This study aimed to offer practical support for fungal identification and microbiology teaching.**

***Keywords*—*Aspergillus niger*, *Aspergillus flavus*, *Rhizopus oryzae*, morphology, growth characteristics**

I. Introduction

Filamentous fungi are important heterotrophic microorganisms that are widely distributed in soil, air, agricultural products, and other organic environments. They are important in nutrient cycling and are closely associated with food safety, industrial fermentation, and agricultural disease control. Among them, *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus oryzae* are three representative mold species with distinct biological characteristics and ecological functions. This makes them important in both industrial applications and food safety [1].

Aspergillus niger is widely used in industrial fermentation because it produces large amounts of extracellular enzymes and organic acids, such as citric acid, pectinase, and amylase [2]. In contrast, *Aspergillus flavus* is a foodborne fungus that produces aflatoxins. It contaminates cereals, nuts, and other agricultural products, posing a serious threat to food safety and human health [3, 4]. *Rhizopus oryzae* is commonly used in the production of traditional fermented foods, including rice wine and fermented soybean products. However, it can also cause spoilage of fruits, vegetables, and stored grains under humid conditions [5].

This study compared their colony morphology, microscopic structures, growth extent, and temperature adaptability using PDA culture, microscopic observation, colony coverage observation, and temperature adaptability tests. The findings provide useful information for fungal identification, food spoilage control, microbiology teaching, and the utilization of beneficial fungi [6].

II. Materials and Methods

A. Experimental Strains

The standard strains of *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus oryzae* used in this experiment were all kept in the Microbiology Laboratory Culture Collection of Henan University of Technology. All three fungi were kept under the same conditions to ensure comparability. Before the experiment, all strains were purified and reactivated on PDA plates to remove contamination from other microorganisms.

B. Reagents and Culture Medium

This study used fresh potatoes, glucose, agar powder, sterile saline, and 75% ethanol. Potato Dextrose Agar (PDA) medium was prepared using 200 g potatoes, 20 g glucose, 20 g agar, and 1000 mL distilled water. The medium was sterilized at 121°C for 20 min, cooled to approximately 50–55°C, poured into sterile Petri dishes, and allowed to solidify at room temperature before use.

C. Experimental Instruments

The main equipment included a laminar flow cabinet, an autoclave, a constant-temperature incubator, a light microscope, an analytical balance, micropipettes, inoculating loops, sterile Petri dishes, microscope slides,

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cover slips, and an alcohol lamp. All glassware and instruments were sterilized before use. Aseptic techniques were followed throughout the experiment to minimize contamination.

D. Experimental Design

A single-factor controlled experimental design was used in this study. Fungal species, inoculation method, and incubation temperature were the experimental variables. Five experiments were conducted, including colony morphology observation, microscopic structural observation, growth comparison, temperature adaptability testing, and comparison of colony coverage under different inoculation methods.

1) Strain inoculation and culture

Two inoculation methods were used: spore suspension inoculation and mycelial plug inoculation. For spore suspension inoculation, spores of each fungal strain were suspended in sterile saline and mixed well. After dilution, 0.1 mL of each suspension was spread evenly onto PDA plates. For mycelial plug inoculation, fresh colonies were cultured until actively growing hyphae developed at the colony margin. Agar plugs of equal size were cut from the colony edge with a sterile cork borer and placed at the center of fresh PDA plates with the mycelial side facing down. All inoculated plates were incubated at 28°C in the dark for 3 days.

2) Observation of colony morphology

Colony morphology was observed on days 1, 2, and 3 after inoculation. Colony color, texture, density, margin features, reverse pigmentation, and colony expansion were recorded and photographed for comparison.

3) Microscopic structural observation

Fresh hyphae and reproductive structures were taken from the colony margin and placed in sterile saline on microscope slides. After covering with a cover slip, the samples were examined under a light microscope. Hyphal structure, reproductive structures, and spore morphology were observed for species identification.

4) Growth comparison

Colony expansion was recorded by visual observation from day 1 to day 3 after inoculation. The colony coverage of the three fungal species was then compared.

5) Temperature adaptability test

The three fungal species were incubated at 20°C, 28°C, and 35°C under the same culture conditions. Colony growth was recorded by visual observation after 3 days to evaluate the effects of temperature on fungal growth and to find the best growth temperature and temperature tolerance of each species among these three temperatures [7].

6) Effect of inoculation method on colony coverage

Colony Coverage from spore suspension inoculation and mycelial plug inoculation was compared under the same incubation conditions. Colony development was recorded daily to find the influence of inoculation method on fungal growth.

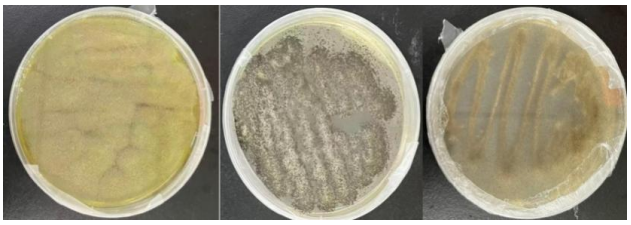
E. Data Processing and Analysis

Growth data from all experiments were collected and compared to examine fungal growth patterns. Colony morphology, microscopic structures, colony coverage, temperature response, and inoculation methods were analyzed together.

III. Results

A. Comparison of Colony Morphology

After 3 days of incubation at 28°C, the three mold species showed different colony shapes. This allowed early identification based on their visible features (Fig. 1). *Aspergillus niger* initially formed white, fluffy aerial hyphae. Black conidia slowly developed during incubation, so the colony color changed from white to black. Mature colonies were dense and powdery with smooth margins, and the reverse side remained pale yellow without obvious pigment diffusion. *Aspergillus flavus* produced white colonies during the early stage of growth. As conidia developed, the colony gradually turned yellow-green to olive-green, and the color progressively darkened as the colony matured. The colonies were loose in texture, spread faster than *Aspergillus niger*, and had irregular margins. The reverse side appeared light yellow. *Rhizopus oryzae* exhibited the fastest colony expansion among the three species. Colonies were initially white, soft, and cotton-like, with rapidly spreading hyphae and covering most of the PDA plate within two days. After three days, the colonies gradually became light grey-brown as they aged. The colony surface stayed soft and fluffy without a powdery appearance, and no obvious pigmentation was observed on the reverse side.

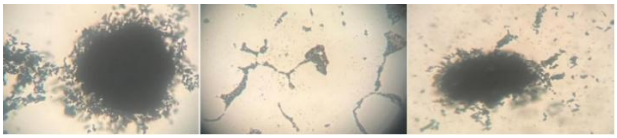


(a) (b) (c)

Fig. 1. Macroscopic colony morphology of (a) *Aspergillus flavus*, (b) *Aspergillus niger*, and (c) *Rhizopus oryzae* after 3 days of incubation.

B. Comparison of Microscopic Structural Characteristics

Microscopic observation revealed clear structural differences among the three mold species (Fig. 2).



(a) (b) (c)

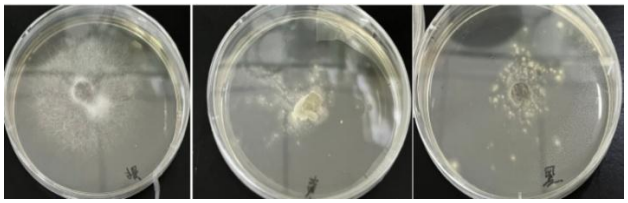
Fig. 2. Micrographs of (a) *Aspergillus niger*, (b) *Rhizopus oryzae*, and (c) *Aspergillus flavus* from left to right in sequence.

Aspergillus niger produced dense, nearly spherical

black conidial heads with many conidia. *Rhizopus oryzae* did not produce spherical conidial heads. Instead, it showed broad, non-septate stolons together with scattered sporangiospores. *Aspergillus flavus* formed smaller, loosely arranged grey-green to olive conidial heads, with conidia spread around the conidial head.

C. Comparison of Colony Expansion Characteristics

Growth monitoring over 3 days showed clear differences in colony expansion among the three mold species (Fig. 3). Based on visual observation of colony expansion during incubation, *Rhizopus oryzae* showed faster colony spreading than *Aspergillus flavus* and *Aspergillus niger* under the tested conditions. *Rhizopus oryzae* formed visible colonies after 1 day and almost covered the PDA plate by day 2, showing the fastest colony expansion. *Aspergillus flavus* grew rapidly during the early stage of incubation, but its growth slowed down as the colonies matured. *Aspergillus niger* showed the slowest and most stable growth throughout the experiment, and its colonies matured later than the other two species.



(a) (b) (c)

Fig. 3. Growth status of (a) *Rhizopus oryzae*, (b) *Aspergillus flavus* and (c) *Aspergillus niger* after 1 day of incubation at 28 °C (left to right).

D. Effect of Temperature on Mold Growth

Temperature had a clear effect on the growth of all three fungal species (Fig. 4). At 20°C, growth was reduced in all three species. *Rhizopus oryzae* grew the best at this low temperature. *Aspergillus niger* and *Aspergillus flavus* grew more slowly. They also formed smaller colonies. At 28°C, all three species grew strongly. Their hyphae developed well. Their colonies showed typical shape features. This was the best growth temperature for all three. At 35°C, *Aspergillus niger* kept growing well. This showed it had strong heat tolerance. *Aspergillus flavus* showed slightly reduced growth. Its colonies were less dense than at 28°C. *Rhizopus oryzae* was affected the most. Its hyphae did not develop well. Its colony expansion was limited.

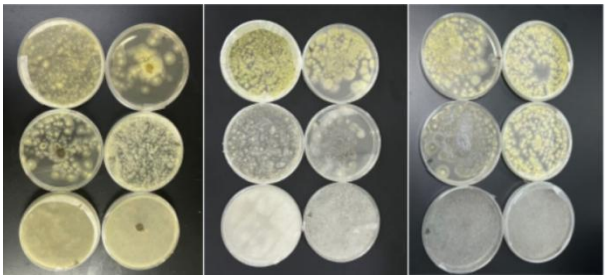
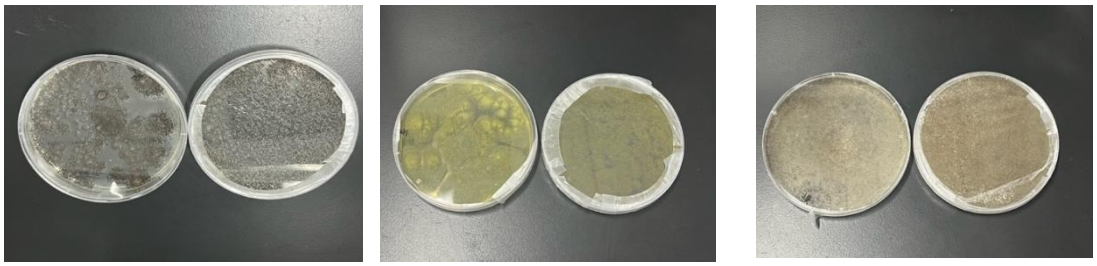


Fig. 4. Growth of the three fungal strains after 2 days of incubation at 20°C, 28°C, and 35°C (left to right), arranged from top to bottom as *Aspergillus flavus*, *Aspergillus niger*, and *Rhizopus oryzae*.

E. Effect of Inoculation Method on Growth Rate

The inoculation method affected the growth speed of all three mold species (Fig. 5). Under the same culture conditions, colonies grown from spore suspensions expanded faster than those grown from mycelial plugs. The scattered spores in the suspension germinated at the same time from many points, so that the colonies could expand quickly in all directions. However, mycelial plug inoculation started growth from a single point, so colony expansion was slower in the early stage of incubation.



(a) (b) (c)

Fig. 5. Growth status of (a) *Aspergillus niger*, (b) *Aspergillus flavus*, and (c) *Rhizopus oryzae* (left to right) inoculated by mycelial plug (left) and spore suspension (right) at the same incubation time.

IV. Discussion

The present study showed clear differences among *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus oryzae*. These differences appeared in colony morphology, microscopic structures, growth rate, temperature response, and inoculation effects. These differences should not be read only as variations in growth performance under the test conditions. They also reflected species-specific biological traits, developmental stages, environmental adaptation, and inoculation strategies.

The morphological differences among the three fungi match earlier taxonomic descriptions of Aspergillus and Rhizopus species. In this study, *Aspergillus niger* formed dense black conidial heads. *Aspergillus flavus* produced yellow-green colonies with smaller conidial structures. *Rhizopus oryzae* showed fast-expanding cotton-like colonies with sporangiospores. These differences mainly come from their genetic background and reproductive structures. Aspergillus species reproduce mainly through

conidia formed on special conidiophores. Rhizopus species produce sporangiospores inside sporangia. This leads to different colony textures and microscopic appearances [8]. However, colony morphology can also change as the culture ages. For example, pigment production and sporulation become more obvious as a colony matures. Therefore, differences in observation time may also affect comparisons among species.

The colony coverage results showed that Rhizopus species expanded faster than *Aspergillus flavus* and *Aspergillus niger* on PDA medium. This result matches reports describing Rhizopus species as fast-growing filamentous fungi. These fungi have a strong ability to colonize nutrient-rich substrates during fermentation and food spoilage. *Aspergillus niger*, on the other hand, showed slower but more stable colony development. This difference may reflect different ecological strategies. Rhizopus allocates resources primarily to rapid hyphal extension and substrate occupation. Aspergillus species often develop more complex sporulation systems and secondary metabolic capacities instead. Therefore, the growth rate measured on PDA does not necessarily reflect overall ecological competitiveness. Past studies have also shown that fungal growth rate can change depending on nutrient composition, strain origin, and experimental conditions [9].

Temperature affected the growth of all three fungi. All species grew best at 28°C in this experiment. In contrast, their responses at 20°C and 35°C were different. *Aspergillus niger* maintained fairly strong growth at 35°C. *Rhizopus oryzae* showed better growth at 20°C but was more inhibited at higher temperatures. These differences may relate to the natural habitats and physiological adaptation of each species. These differences may result from strain variation, growth stage, medium composition, and the specific temperature range tested.

The inoculation method also shows that experimental design can affect growth differences. Spore suspension inoculation led to faster colony establishment than mycelial plug inoculation. This happened because spores were spread over multiple sites. They could germinate simultaneously. Inoculum type and density affect early colony development and growth measurements. Comparisons of fungal growth should carefully control inoculation methods. This helps separate true species-level differences from experimental effects.

This study has several limitations. Only temperature was investigated as an environmental factor, whereas other variables such as pH, humidity, and nutrient availability were not examined [10]. In addition, small variations in inoculation density and manual measurements may have introduced minor experimental errors.

V. Conclusion

This study compared the shape and growth features of *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus oryzae*. It used PDA culture, microscopic observation, colony coverage observation, temperature adaptability tests, and different inoculation methods. The three fungal species showed clear differences in colony morphology, microscopic structures, colony coverage, and temperature adaptability. *Rhizopus oryzae* showed the fastest growth, followed by *Aspergillus flavus* and *Aspergillus niger*. All three species grew best at 28°C among the three different temperatures tested. *Aspergillus niger* showed more tolerance to high temperatures. *Rhizopus oryzae* adapted better to lower temperatures. *Aspergillus flavus* showed broad temperature adaptability. Spore suspension inoculation also led to faster colony development than mycelial plug inoculation under the same culture conditions. Growth comparisons were based on visual observation rather than statistical analysis. Therefore, the results are preliminary. These findings provide useful information for fungal identification, microbiology teaching, and food spoilage control.

Conflict of Interest

The authors declare no conflict of interest.

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