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General patterns of *Aspergillus spp.* biofilm development dynamics

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Abstract. During the development of local and systemic infections of humans and animals, phytopathology, degradation of natural and synthetic polymers, residential and industrial structures, the adaptive potential of pathogens is inhibited by QS molecules that block the synthesis of intercellular communication inducers. The aim of the study was to analyze the dynamics of changes in morphofunctional parameters during the initiation, development, and dispersion of Aspergillus spp. fungi. To study the phenotypic characteristics and take into account the morphometric parameters of biofilms, microorganisms were cultivated at 35 ± 2.0 °C for 168 ± 1h. Cultures of *A. flavus*, *A. fumigatus*, and *A. niger* microorganisms are fast-growing; depending on the composition of the nutrient media and the cultivation time, they differed both in the volume of aerial mycelium and in the staining of the reverse side. During the implementation of intercellular communication processes at the early stages of development, adhesion of conidia to the substrate surface was initiated. Due to the apical growth of germinal tubules, filiform structures — hyphae — subsequently differentiated. As cultivation time increased, areas of significant hyphal consolidation emerged, and conidiogenic structures differentiated. General patterns

of heterogeneous biofilm development during the representation of QS signaling molecules are mediated by growth cyclicity and population adaptation to environmental factors. Understanding the mechanisms of initiation, persistence, and dispersion during adventitious sporulation and filamentation of micromycetes will facilitate the optimization of long-term retrospective mycological studies, the development of effective methods for biofilm eradication, and the rationalization of the use of fungal biosynthetic activity in biotechnology and bioremediation.

Keywords: microscopic fungi, intercellular communication, adhesive properties of microorganisms, adventitious sporulation, filamentation, dispersion, conidiogenesis, eradication of biofilms

Authors contribution: Conceptualization — E.M. Lenchenko; methodology, validation — N.P. Sachivkina; data processing — E.M. Lenchenko, D.A. Bannikova; writing — revision and editing — E.M. Lenchenko, M.I. Shopinskaya. All authors have read the final version of the manuscript and approved it.

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Introduction

Given a statistically significant trend of increasing incidence of socially significant mycoses mediated by excessive growth and adventitious sporulation in tissues, the dominance of the etiological significance of *Aspergillus spp*. fungi has been established [1–3]. The duration of mycological studies in the differentiation of chronic phthisiatric processes reaches from 46 to 110 days, which is associated with the use of antifungal therapy [4–6]. Azole-resistant strains are classified as the first category of critical priority for research in accordance with the WHO Fungal Priority Pathogens List[1] [7, 8]. Territorial rankings of the representative sample make it possible to predict a further increase in resistance to drugs and disinfectants in isolates identified from pathological material of mammals, birds, reptiles, amphibians, fish, and bees [9–15]. Contamination of food products and feed with fungal metabolites at concentrations hazardous to human and animal health can reach 40.0 to 100.0% [16–19]. During the development of local and systemic infections in humans and animals, phytopathology, as well as degradation of natural and synthetic polymers, residential and industrial structures, the morphogenesis of micromycetes is initiated by the presentation of Quorum Sensing (QS) signaling molecules [20–23]. To optimize long-term and retrospective schemes of mycological research, to develop effective drugs that reduce the adhesive properties of microorganisms, block synthesis, or destroy the polymer matrix, studies of the mechanisms of initiation, development, formation, and dispersal of biofilms are of relevance and scientific novelty.

The aim of the study was to analyze the dynamics of changes in morphofunctional parameters during the initiation, development, and dispersal of *Aspergillus spp*. fungi.

[1] WHO fungal priority pathogens list to guide research, development and public health action. Geneva: World Health Organization; 2022. Available at: https://www.who.int/publications/i/item/9789240060241 (accessed on: 20.01.2026).

Materials and methods

Strains. The reference strains (ATCC) used in the experiments were *Aspergillus flavus* 16883, *Aspergillus fumigatus* 1028, and *Aspergillus niger* 16404[2].

Nutrient media. The following media were used for cultivation: meat-peptone broth (MPB), meat-peptone agar (MPA), corn agar; Czapek agar with yeast extract (Obolensk, Federal Budgetary Institution of Science "State Scientific Center for Applied Microbiology and Biotechnology", Russia); chocolate agar (BioMedia, Russia); Sabouraud Fluid Medium and Sabouraud Dextrose Agar with Chloramphenicol & Cycloheximide (Condalab, Spain).

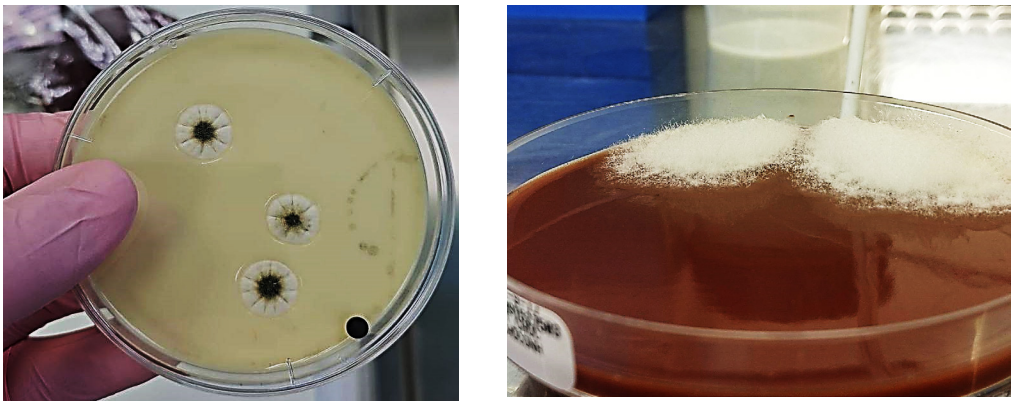
The microorganisms were cultivated at 35 ± 2.0 °C for 168 ± 1 h. Phenotypic characteristics were studied using standard mycological methods. For morphometric studies, samples were fixed with 30.0% NaOH solution for 30 min; stained with aqueous gentian violet solution 1:2000 and by Gram staining (BioVitrum, Russia). Crushed drop slides were made from the fungal mycelium by lightly touching the surface of the microbial colony with dissecting needles. The mycelial hyphae were carefully separated using dissecting needles, then a drop of glycerol was applied and covered with a coverslip[3]. To preserve the natural architecture of the biofilms, the preparations were fixed: in vapors of 25.0% glutaraldehyde solution for 8 h; and in 1.0% aqueous osmium tetroxide solution for 4 h. Dehydration of the samples was performed with increasing concentrations of ethanol (30, 50, 96, and 100°) [24]. Optical density of the samples was assessed using a photometric analyzer Immunochem-2100 (HTI, USA) at OD_{580}. Morphometric parameters were evaluated using a Trinocular Unico optical microscope (Unico, USA).

The results were processed using statistical analysis with Student's test. Differences were considered significant at $p \leq 0.05$.

Results and Discussion

When cultivated in liquid and solid media at 35 ± 2.0 °C for 168 ± 1 h, the studied microorganisms *A. flavus*, *A. fumigatus*, and *A. niger* exhibited characteristic growth, which proved to be highly informative. The microbial cultures were fast-growing: the growth of micromycetes was observed on the surface of liquid media in test tubes and at the periphery of Petri dishes. This is attributed to the aerobic properties of the fungi. Depending on the composition of the nutrient media and the cultivation time, the micromycetes differed both in the volume of aerial mycelium — ranging from extremely scant to abundant — and in the pigmentation of the reverse side of the colonies (reverse): from barely visible to intensely colored pinkish-brown tones. The central part of the colony is very dense and more intensely colored, while the peripheral part is thinner, and a pale pigment diffusing into the culture medium was observed (Fig. 1).

[2] ATCC. The Global Bioresource Center. Available at: https://www.lgcstandards-atcc.org (accessed on: 05.05.2023).
[3] Sutton D, Fothergill A, Rinaldi M. Identifier of pathogenic and opportunistic fungi. Moscow: Mir; 2001.



a *b*

Fig. 1. Cultures of microorganisms *A. flavus* (35 ± 2.0) °C, 48 ± 1 h:
a — Sabouraud's medium; *b* — chocolate agar

Source: compiled by E.M. Lenchenko, N.P. Sachivkina, D.A. Bannikova.

Cultures of *A. fumigatus* were smoky, grayish green, with a light yellow reverse. The colony texture ranged from woolly to cottony with some granularity. At the apical poles of the finest thread-like structures, rounded grain-like formations of light gray or intense brown color were observed. Cultures of *A. flavus* formed woolly green or white fluffy cottony colonies, diffusing into the depth of the medium (2.0–3.0 mm). Initially light green, yellow-green, or lemon in color, they became dark green, dark yellow-green, or brown-green in the center as sporulation progressed. The reverse of the colonies was white cream. Sclerotia composed of tightly interwoven mycelial hyphae were white; they became cream-colored with increasing cultivation time and turned brown after 168 h. Exudate was typically observed, the drops of which were initially colorless but gradually acquired a light brown color. Differential characteristics of the colonies included a dark green center and radial furrows, with a thin openwork (lace-like) periphery. Cultures of *A. niger* initially formed fluffy colonies, then granular colonies; the reverse was colorless or yellowish. With sporulation, the mycelial color varied from dark brown to black.

At 35 ± 2 °C for 168 ± 1 h under static cultivation conditions, depending on the stage of the cell cycle, general patterns of biofilm development were observed in the studied fungi. The dynamics of heterogeneous population development were mediated by adhesion, synthesis of extracellular molecules, proliferation, coaggregation, and differentiation of mycelial structures depending on the stage of the cell cycle. The implementation of intercellular communication processes was initiated by adhesion of conidia to the substrate surface (in our studies, glass). Due to the apical growth of germ tubes, thread-like structures — hyphae — differentiated. As cultivation time increased, areas of significant hyphal consolidation emerged, and conidiogenic structures differentiated. The hyphae of the studied fungal species were septate and colorless.

Conidiophores of *A. flavus* were coarsely roughened, colorless, 400.0–800.0 ± 0.8 μm in length; vesicles were rounded, 20.0–45.0 ± 0.7 μm, the surface of which was almost

completely covered with phialides, 8.0–12.0 ± 0.6 μm, arranged on metulae. Conidia were smooth, rounded, d = 3.0–6.0 ± 0.5 μm.

Conidiophores of *A. fumigatus* were smooth, colorless, 100.0–300.0 ± 0.4 μm in length; vesicles were dome-shaped, 20.0–30.0 ± 0.3 μm, with densely arranged phialides, 5.0–10.0 ± 0.6 μm. Conidia were smooth or finely roughened, rounded, d = 2.0–3.5 ± 0.8 μm.

Conidiophores of *A. niger* were smooth, colorless, dark near the vesicle, 300.0–400.0 ± 0.2 μm in length. Vesicles were spherical, 30.0–75.0 ± 0.5 μm; the surface was covered with phialides, 8.0–12.0 ± 0.6 μm, arranged on metulae. Conidia were roughened, rounded, d = 4.0–5.0 ± 0.5 μm (Fig. 2).

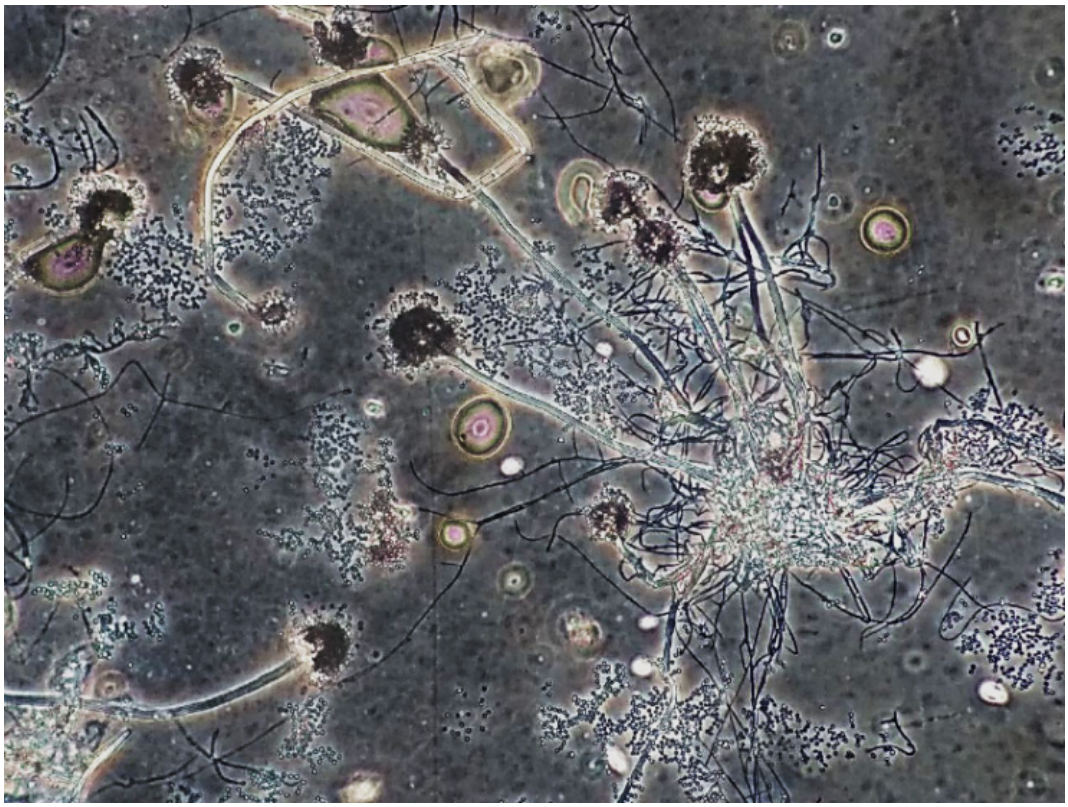


Fig. 2. Morphology of *A. niger* microorganisms, cultivated on MPA at 35 ± 2 °C for 168 ± 1 h. Fixation with 30.0% NaOH solution for 30 min: approx. ×10, vol. ×40, H604 Trinocular Unico, USA

Source: performed by E.M. Lenchenko, N.P. Sachivkina, D.A. Bannikova.

Population immobilization of biofilms is a genetically and phenotypically determined form of intercellular communication — quorum sensing — mediated by the characteristics of growth cycles and population adaptation to ecological and anthropogenic factors [5, 25, 26]. In the development and rotation of new drugs and disinfectants, broad-spectrum anti-adhesive composite preparations are recognized

as promising [11, 24, 27, 28]. The adaptive potential of a heterogeneous multicellular population is inhibited by QS molecules that block the synthesis of intercellular communication inducers, thereby reducing adhesion and, consequently, the degree of microbial contamination [24, 29, 30].

Conclusion

The present study has revealed fundamental aspects of the formation and development of biofilms in representatives of the genus *Aspergillus* under optimal temperature conditions of 35 ± 2 °C for 168 ± 1 hours. During the experiment, the ability of fungi for self-organization was demonstrated, manifested in the formation of spatially ordered multicellular structures. Each stage of biofilm development — from primary adhesion to final dispersal — demonstrates remarkable population coordination and adaptability. The complex dynamics of population immobilization, involving a successive sequence of phases — proliferation, coaggregation, filamentation, and other key closely interrelated processes — deserve special attention.

The obtained results open new horizons in understanding the mechanisms of micromycete vital activity and can be utilized in several promising areas: improving the methodology of long-term mycological studies; developing innovative strategies to combat persistent biofilms; optimizing biotechnological processes using fungi; and advancing bioremediation methods.

The study of the mechanisms of formation and functioning of fungal biofilms represents a significant contribution to modern mycology. Understanding these processes not only expands fundamental knowledge about fungal vital activity but also opens practical prospects for addressing pressing problems in medicine, veterinary science, and biotechnology.

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Общие закономерности динамики развития биопленок грибов *Aspergillus spp.*

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Аннотация. При развитии локальной и системной инфекции человека и животных, фитопатологии, деградации природных и синтетических полимеров, жилых и производственных конструкций адаптивный потенциал патогенов ингибируется QS молекулами, блокирующими синтез индукторов межклеточной

коммуникации. Цель исследования — анализ динамики изменений морфофункциональных показателей при инициации, развитии и дисперсии грибов *Aspergillus spp.* Для исследования фенотипических признаков и учета морфометрических показателей биопленок микроорганизмы культивировали при 35 ± 2,0 °C в течение 168 ± 1 ч. Культуры микроорганизмов *A. flavus*, *A. fumigatus*, *A. niger* — быстрорастущие, в зависимости от состава питательных сред и времени культивирования различались как по объему воздушного мицелия, так и окрашиванию обратной стороны. *При р*еализации процессов межклеточной коммуникации на ранних этапах развития инициировалась адгезия конидий к поверхности субстрата. За счет апикального роста гермативных трубочек впоследствии дифференцировались нитевидные структуры — гифы. По мере увеличения времени культивирования выявлялись участки значительной консоллидации гифов, дифференцировались конидиогенные структуры. Общие закономерности развития гетерогенных биопленок при репрезентации сигнальных молекул QS опосредованы особенностями цикличности роста и адаптацией популяции к действию факторов среды обитания. Раскрытие механизмов ***и***нициации, персистенции, дисперсии при адвентивной споруляции и филаментации микромицет будет способствовать оптимизации схемы длительных ретроспективных микологических исследований, разработке эффективных способов эррадикации биопленок, а также рационализации применения биосинтетической активности грибов в биотехнологии и биоремидиации.

Ключевые слова: микроскопические грибы, межклеточная коммуникация, адгезивные свойства микроорганизмов, адвентивная споруляция, филламентация, дисперсия, конидиогенез, эррадикация биопленок

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