Introduction

The form, the size and the color of a fungal colony are the features which can characterize genera and species of fungi. Besides the individual properties of fungal cultures (such as the existence of circadian rhythms, the ability to produce metabolites - growth inhibitors - antibiotics, organic acids, exoenzymes, etc.) the environmental parameters (nutrient concentration, temperature, light availability, etc.) also have a significant influence on the colony form [4,12,20,21].

On the other hand the process of a certain colony type formation can be considered as a phenomenon of biological self-organization, since the fungal system undergoes a number of spontaneous changes which include increase and/or complication of the system elements, modification of the system functioning conditions, etc. In the terms of complex systems approach we treat the fungal system as having a discrete number of macroscopic stable states on the colony level. The variety of these states is determined by a morphological potential of a certain fungal species. Transitions between states are managed by external control parameters (environmental factors). The process of pattern formation in fungi can be therefore regarded as an adaptation of the colony during its development to the changing environment due to the collective interactions of the system elements with each other and with environment as well as due to the existence of feedback between fungal system state and control parameters.

In this paper we describe the main types of stationary dissipative structures, which can arise in colonies of mycelial fungi. We investigate conditions required in order for patterns to appear and explain how changes in fungal environment influence the morphology of the colony, or, in other words, how the system interacts with its environment. On the basis of experimental data obtained we have developed a mathematical model for description of observed non-linear phenomena in colonies of mycelial fungi. By means of the model and experimental results we estimate the value of metabolite diffusion coefficient, which was shown to be one of the main parameters defining the colony shape.

Materials and methods

Experiments were carried out on mycelial fungi *Ulocladium chartarum*, *Ulocladium botrytis*, *Ulocladium consortiale*, *Alternaria alternata*, *Penicillium chrysogenum* (Deuteromycotina, Hyphomycetes: Hyphales). The given species were cultivated at 25º C and 8º C on standard and modified nutrient media in Petri dishes (d = 9 cm). We varied glucose concentration (0 – 3%) and volume of nutrient medium in a Petri dish (5 – 20 ml). Fungal growth patterns and their sizes were registered by a digital camera Casio-QV100. For confirmation of the mycelial property to produce metabolites (growth inhibitors) a pH-sensitive indicator was added to the nutrient medium, which caused the changes of medium color according to its pH. The spatial dynamics of metabolite distribution was estimated by means of vertical photometry on the device "Chicken" (Institute of Analytical Instrumentation, Russia). The optical density of medium is proportional to the metabolite concentration (λ = 610 nm) which allowed us to evaluate average radial distribution of metabolites around the colony and hence the values of metabolite diffusion coefficient.

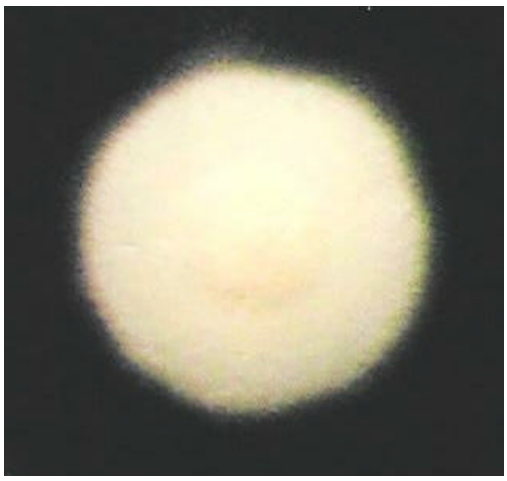
Results and discussion

The colony of mycelial fungi is a uniform multicellular structure developing radially by growth and branching of mycelium. The colony is able to consume substrate (carbon source, i.e. glucose) and to produce diffusible metabolites which can suppress fungal development. The final stage of fungal morphogenesis is the formation of spores.

Different growth patterns can arise in colonies of mycelial fungi while they are cultivated on solid agar media of various thickness and nutrient content. These macroscopic spatiotemporal patterns were classified into four main types: concentric rings (zones), "sparse lawn" and "dense lawn" (continuous mycelial growth), ramified (fractal-like) structures (Fig.1). The experimental data obtained enable us to construct a two-dimensional morphological diagram in which a characteristic colony form corresponds to each pair of given parameters (Fig.2) (in our case, we varied two parameters - nutrient concentration and volume of nutrient medium, i.e. medium thickness).

When fungi are grown on optimal nutrient media (glucose 1-3%, agar 2%, volume of medium in a Petri dish 5-20 ml), hyphal branching is maximal and the colony represents a continuous surface of well-developed mycelium ("dense lawn"). Variations of mycelium or spore density are not visible on the colony surface. On thick poor nutrient media (glucose < 0.1%, agar 2%, 15-20 ml of medium in a Petri dish) hyphal branching is minimal and the colony grows in the form of a weakly-developed lawn ("sparse lawn"). Cultivation of fungi on thin poor media (glucose 0-0.05%, agar 2%, 5-10 ml of medium in a Petri dish) can result in the emergence of fractal-like structures. There is a limited range of substrate concentrations (glucose 0.1-0.5%, agar 2%, medium volume 5-10 ml) in which zone formation occurs. Microscopic examination of concentric rings formed in colonies of *Hyphomycetes* has shown them to be regions of high mycelium or spore density intermitted by ones of lower density. We have found out that zone formation in fungal colonies can be effected by external synchronizing stimuli, for example, by alteration of temperature and light incubation conditions. In particular, the decrease of cultivation temperature causes the formation of distinctly-shaped concentric rings, and visible light can stimulate the emergence of synchronous wave structures.

a



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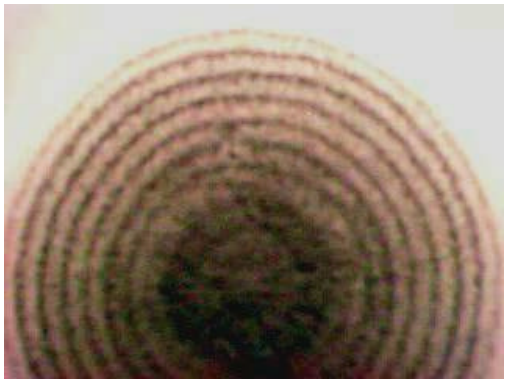

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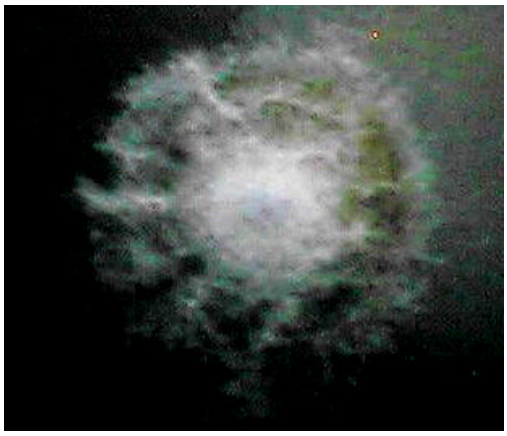

Fig.1. Different growth patterns which can develop in colonies of mycelial fungi:
a – dense lawn, b – concentric rings, c – fractal-like structures

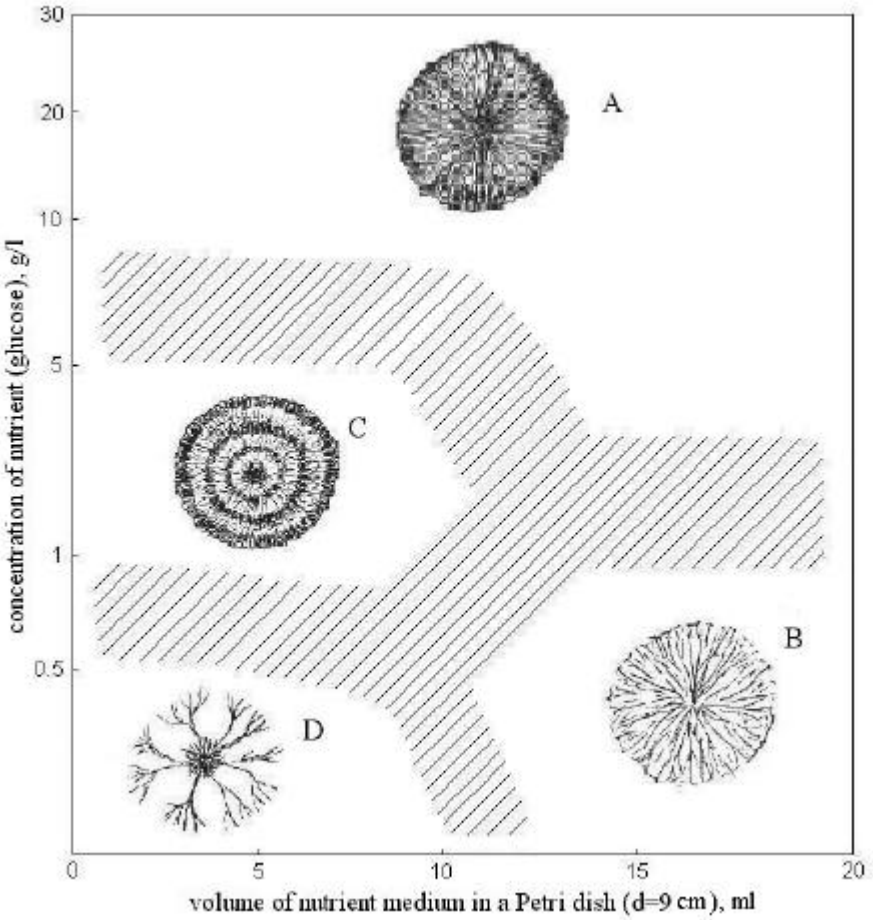

Fig.2. Morphological diagram of fungal colony patterns:
A – dense lawn, B – sparse lawn, C – concentric rings, D – fractal-like structures

We have proposed a general mechanism of fungal pattern formation, which is based on two simultaneous processes: consumption of substrate (s) (activator) by mycelium and suppression of mycelial growth by diffusible metabolites (m) (growth inhibitors). At the beginning of fungal colony development metabolite concentration is zero. As the colony grows metabolite concentration increases and substrate concentration decreases due to its consumption by mycelium. It is assumed also that there is a threshold $(s/m)_t$ at which growth of mycelium stops. In case of "lawn" formation (both, "sparse lawn" and "dense lawn" types) a certain ratio of substrate and metabolite concentrations $s/m > (s/m)_t$ is maintained so these patterns are relatively stable. In the range of concentrations suitable for zone formation spatial alternation of $s/m > (s/m)_t$, $s/m < (s/m)_t$ is established since diffusion coefficient of metabolite exceeds diffusion coefficient of substrate and the gradient of metabolite concentration is large enough. In other words during radial growth of the colony ratios of substrate and metabolite concentrations are broken alternately, which result in periodic changes of fungi growth modes. The essential factors, which are supposed to be responsible for the emergence of fractal-like fungal patterns, are diffusion of nutrients (in this case diffusion coefficient of substrate exceeds diffusion coefficient of metabolite) and unequal accessibility of nutrients to separate hyphae. Thus the conditions are created when several actively growing branches are always in a maximal nutrient gradient and inhibit competing branches development.

A mathematical model has been developed for description of observed non-linear phenomena [8], which is a system of reaction-diffusion-type equations [1,24] and takes into account the models of spatiotemporal order generation, for example [9,13,18,23]. The system of differential equations describes spatiotemporal distribution of mycelium (ξ), spore (χ), substrate (s) and metabolite (m) concentrations:

$$\frac{\partial m}{\partial \tau} = \alpha \xi^{2} U_1(s,\xi) + D_m \Delta_\rho m. \qquad (1)$$

$$\frac{\partial s}{\partial \tau} = -\gamma \xi K(s) + D_s \Delta_\rho s, \qquad K(s) = \frac{s}{s+1}. \qquad (2)$$

$$\frac{\partial \xi}{\partial \tau} = \lambda \xi (1 - \varepsilon \xi) K(s) U_2(m), \qquad U_2(m) = 1[-m(\tau - \tau^0) + \mu^0]. \qquad (3)$$

$$\chi(\tau) = \sigma \xi (\tau - \tau^*) U_3(s,m). \qquad (4)$$

The model contains non-dimensional parameters which characterize such quantities as radial growth rate of mycelium (v), specific growth rate of mycelium concentration (λ), substrate consumption rate (γ), scaling ratio of metabolite production rate (α), diffusion coefficients of metabolite (D_m) and substrate (D_s), initial mycelium concentration (ξ^0), maximal mycelium concentration (resource concentration) (ε^{-1}), initial substrate concentration (s^0), threshold of metabolite concentration* (μ^0) at which growth of mycelium stops, time of mycelium response delay on presence of metabolites (τ^0), etc. Trying different values of the above-mentioned parameters it is possible to model the main ways of fungal colony development and to determine parameters which have the strongest influence on pattern formation processes.

In ***computational experiments*** we have demonstrated that proposed model is able to describe properly the emergence of such macroscopic patterns as "concentric rings", "dense lawn" and "sparse lawn". The analysis of computer simulation results has shown α, μ^0, D_m and τ^0 to be among the most important factors influencing the distribution of mycelium concentration. Examples of fungi growth simulation are presented on Fig. 3 and Fig. 4.

* The values of μ^0 can differ depending on cultivation conditions and individual properties of fungal species.

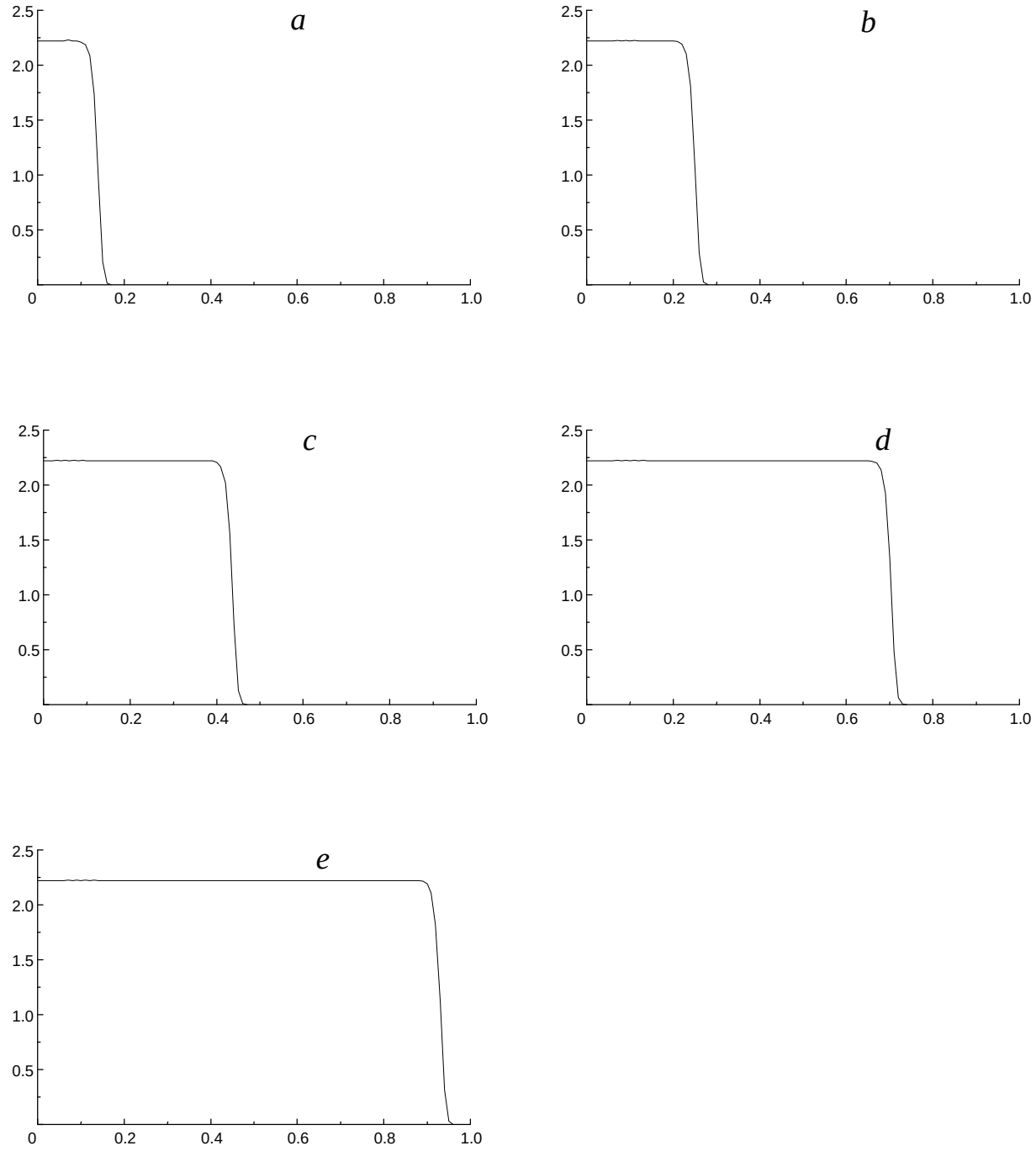

Fig.3. Dynamics of fungal colony growth on optimal nutrient media bringing to the development of "lawn" (x-axis is relative radius, y-axis is relative mycelium density). Parameters of the model: $v = 1.2$; $\rho^0 = 0.05$; $\xi^0 = 0.1$; $\lambda = 5$; $\varepsilon = 0.45$; $s^0 = 10$; $D_s = 1.5 \cdot 10^{-5}$; $\gamma = 1$; $D_m = 0.003$; $\alpha = 0.6$; $\mu^0 = 0.5$; $\tau^0 = 0.50$; $\eta = 0.80$. Time: *a* - $\tau = 4.2$; *b* - $\tau = 8.3$; *c* - $\tau = 16.7$; *d* - $\tau = 25.0$; *e* - $\tau = 33.3$.

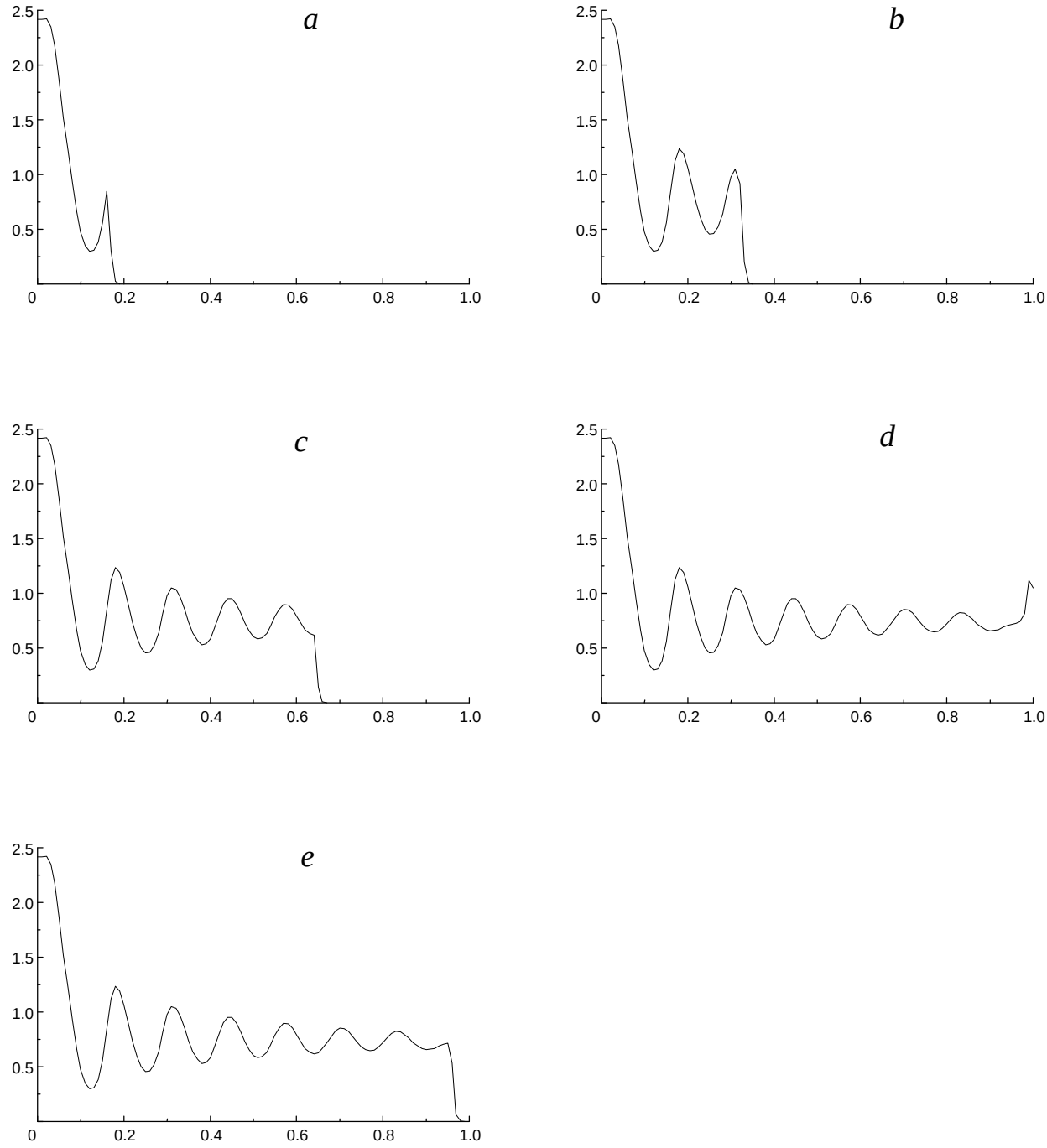

Fig.4. Dynamics of fungal colony development resulting in concentric rings formation (x-axis is relative radius, y-axis is relative mycelium density). Parameters of the model: $v = 1.2$; $\rho^0 = 0.03$; $\xi^0 = 0.1$; $\lambda = 7$; $\varepsilon = 0.40$; $s^0 = 10$; $D_s = 5.0 \cdot 10^{-5}$; $\gamma = 1$; $D_m = 0.001$; $\alpha = 0.5$; $\mu^0 = 0.1$; $\tau^0 = 0.50$; $\eta = 0.90$. Time: *a* - $\tau = 4.2$; *b* - $\tau = 8.3$; *c* - $\tau = 16.7$; *d* - $\tau = 25.0$; *e* - $\tau = 33.3$.

As it was mentioned above metabolite diffusion coefficient is one of the main parameters defining the colony morphology. The values of the given coefficient have been estimated by means of the model and experimental results.

We have proposed the so-called one-dimensional model of metabolite production and distribution, which assumes that a dominating direction of metabolite diffusion is its radial distribution in substrate due to a relatively thin layer of medium. The value of metabolite concentration can be represented as follows [22]:

$$y(r,t) = \sum_{i=0}^{\infty} C_i J_0(m_i r / r_{\max}) \exp(-m_i^2 / r_{\max}^2 D_m t). \quad (5)$$

Due to the boundary conditions, which correspond to a case of impermeable external walls:

$$J_1(m_i) = 0. \quad (6)$$

Besides, $C_i \sim J_0^{-2}(m_i)$.

Here J_0, J_1 - cylindrical Bessel functions of the orders 0 and 1, r – a radial coordinate, $r_{\max}$ - a radial coordinate, which corresponds to the external wall of a Petri dish, t – time.

The first roots of the equation (6) are [22]:

$$m_0 = 0;\ m_1 = 3{,}83;\ m_2 = 7{,}02.$$

Since all measurements were carried out at the same time, then

$$y(r_i, t) = y(r_i) = y_i,\ (i = 1, 2, 3).$$

Having chosen the first three items (the largest ones) from equation (5) it is possible to estimate the expression

$$\exp(-(m_2^2 - m_1^2) / r_{\max}^2 D_m t)$$

and respectively the value of D_m on the basis of ratio

$$(y_2 - y_1) / (y_3 - y_1).$$

The absolute values of D_m (Table 1) approximately correspond to a case of low molecular compounds diffusion in a liquid medium. The results of calculations presented in Table 1 demonstrate also a relatively weak influence of cultivation temperature on the value of metabolite diffusion coefficient. This fact allows us to give a possible explanation for the experimentally observed phenomenon of zone patterning stimulation due to the decrease of cultivation temperature. The point is that radial growth rate of mycelium considerably depends on temperature conditions. In particular, the decrease of temperature to 6-8 °C causes 2-4 times mycelium growth rate reduction. Thus, when the colony is grown at low temperatures v decreases while the value of D_m changes insignificantly, therefore metabolite concentration at the colony growth front can easily reach its threshold μ^0 which results in concentric rings formation or makes them more distinct.

Apparently, in case of “lawn” formation relative values of D_m are rather low and metabolite concentration doesn’t reach the threshold; consequently the colony develops evenly.

TABLE 1.

The values of metabolite diffusion coefficient and radial growth rate of mycelium at different cultivation temperatures (for *U. chartarum*)

Cultivation temperature T, ^{0}C	Time of cultivation t, days	Radial growth rate of mycelium v, mm per day	Metabolite diffusion coefficient $D_m \cdot 10^{-6}$, cm^2/s
8	14	0.75 ± 0.15	0.40 ± 0.10
25	8	2.05 ± 0.35	0.38 ± 0.11

Conclusion

It should be noted that there is a number of alternative approaches and models for description of fungal colonies patterning [3,10,14-17,19]. For example, concentric rings formation in *Neurospora crassa* is explained as a result of circadian clock functioning [5,10,19]. Other models also take into account the effect of toxic metabolites accumulation, which play an important role as inhibitors for cell division [14,15]. Anyway, fungal colonies patterning deserves consideration as an interesting example of biological self-organization since fungi are extremely labile and possess a potential for a variety of growth forms development. In turn, the understanding of general features and mechanisms of spatiotemporal self-organization in fungal colonies, as well as in colonies of bacteria [2,6,7,11] and other microorganisms, which are able to generate order on a macroscopic level displaying collective behavior, may help us to reveal the main laws of morphogenesis of multicellular tissues, organs and organisms.

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