Coordinated Development of Yeast Colonies: Quantitative Modeling of Diffusion-Limited Growth – Part 2

By *T. Walther**, *H. Reinsch*, *K. Ostermann*, *A. Deutsch*, and *T. Bley*

A mathematical model was developed which described the growth of yeast colonies based on the assumptions that (*i*) these populations were built up of single cells whose proliferation was (*ii*) exclusively controlled by nutrient availability in the environment. The model was of a hybrid cellular automaton type and described discrete cells residing on a one-dimensional lattice as well as on continuously distributed nutrients. Experimental results and numerical calculations were compared to elucidate under which cultivation conditions the diffusion-limited growth (DLG) was the major construction principle in yeast colonies. Simulations were scaled to the growth of *Yarrowia lipolytica* and *Candida boidinii* colonies under carbon and nitrogen limitation. They showed that nutrient-controlled growth of the individual cells resulted in DLG of the population. Quantitative predictions for the spatio-temporal development of the cell-density profile inside a growing yeast mycelium were compared to the growth characteristics of the model yeast mycelia. Only for the carbon-limited growth of *C. boidinii* colonies on glucose as the limiting nutrient resource did the DLG model reproduce the cell-density profile estimated at the end of the cultivation. Under all other cultivation conditions, strong discrepancies between calculations and experimental results were evident precluding DLG as the ruling regulatory mechanism. Thus, whether or not the development of a yeast population could be described by a DLG scenario, was strongly dependent on the particular cultivation conditions and the applied yeast species. In those cases for which the DLG hypothesis failed to explain the observed growth patterns, the underlying assumptions, i.e., the complete absence of nutrient translocation between the individual cells inside the yeast mycelia as well as the exclusively nutrient-controlled proliferation of the cells, have to be reevaluated. The presented study demonstrated how the mathematical analysis of growth processes in yeast populations could assist the experimental identification of potential regulatory mechanisms.

1 Introduction

Filamentous fungi are microorganisms of an extremely high economic relevance since they are employed in a large number of biochemical production processes. These processes can be roughly classified into liquid culture (submerse) cultivations, and in cultivations on substrates that lack a free aqueous phase, i.e., solid-state fermentations. While in submerse cultivations the homogeneous distribution of all state variables is ensured by an adequate mixing of the culture broth, solid-state fermentations are characterized by slow transport processes through the growth substrate and a heterogeneous distribution of biomass and nutrients in the system. Although submerse cultivations of fungi offer an optimum process control, solid-state fermentations recently gained increasing interest since it was found that final product concentrations are often higher than in submerse cultivations which may avoid an elaborative downstream processing of the products formed [1]. It was speculated that these higher productivities are caused by a more natural lifestyle of the fungi on the solid substrates since fungi are excellently adapted to the growth and survival in terrestrial ecosystems but are hardly found in aqueous environments.

Considering that the cultivation of fungi in solid-state fermentations may become more and more economical, surprisingly little is known about the mycelial life style of these organisms and their adaptation to heterogeneous environments. Investigations have often been carried out in rather simple model systems, where fungi are cultivated on agar plates [2] or in a chessboard-like system of agar tiles containing different nutrient concentrations [3]. It has been shown that the cell density of the mycelium increases with higher nutrient concentration, and that fungi are capable of translocating nutrients inside the hyphal network to compensate for limitations in parts of the mycelium that grow in a low-nutrient environment [4]. However, besides this rather qualitative description of phenomena observed during mycelial development, scientists are far from the establishment of a theoretical framework that quantitatively describes the interplay of mechanisms which coordinate fungal growth on the population level.

This lack of understanding is due to the complexity of fungal development: Mycelial growth is controlled on different system scales. On the one hand, it is determined by interactions of individual hyphae with their microscopic environment. On the other hand, it is influenced by long-range interactions on the population scale, i.e., by nutrient translocation inside the mycelium or by the formation of nutrient gradients in the growth substrate [2, 3]. The emerging behavior arising from the interplay of these different processes is

[*] T. Walther (author to whom correspondence should be addressed, e-mail: th.walther@mailbox.tu-dresden.de), H. Reinsch, T. Bley, Technische Universität Dresden, Institut für Lebenmitteltechnik und Bioverfahrenstechnik, D-01062 Dresden, Germany; K. Ostermann, Technische Universität Dresden, Institut für Genetik, 01062 Dresden, Germany; A. Deutsch, Technische Universität Dresden, Zentrum für Hochleistungsrechnen, D-01062 Dresden, Germany.

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hard to grasp without the abstracting power of mathematical models. As it was shown in simulations of bacterial colony development or for the growth of fungal mycelia, respectively, sophisticated and highly-organized patterns may be formed by the microorganisms as a result of rather simple construction principles [5, 6]. With the help of mathematical models it is possible to resolve complex experimental observations down to the underlying regulatory mechanisms.

In the search for regulatory mechanisms active during the growth of fungal mycelia, a quantitative model has been presented that is capable of reproducing the experimentally determined mycelial growth pattern of *Rhizoctonia solani* on glucose at the early stages of the cultivation [2]: The model describes mycelial growth based on the uptake of glucose, which is converted into new hyphal elements *via* a not further characterized intermediate compound that is referred to as an internal nutrient resource. While external glucose is assumed to freely diffuse through the agar substrate, in this model the internal nutrient resource was redistributed inside the mycelium by active and passive transport mechanisms. Furthermore, microscopic properties of fungal growth were incorporated, e.g., by restricting biomass growth to the active hyphal tips. Certainly, this approach represents a drastic improvement when compared to earlier published qualitative models [7–11]. However, even though the experimentally determined morphology of the cell-density profile could be quantitatively reproduced, model predictions have to be interpreted with extreme care.

The behavior of the model is mainly determined by the effect of a hypothetical internal nutrient resource which has been neither quantified nor chemically characterized. Thus, an experimental proof for the validity of underlying model assumptions and parameter estimations is still lacking. Obviously, model verification is a demanding experimental problem, since neither the chemical nature of the internal nutrient resource is yet known, nor are there monitoring methods at hand that facilitate the spatially resolved estimation of different compounds inside fungal mycelia. Because of the described difficulties it has been recently suggested by the authors to study mycelial development on a level of lower complexity, i.e., without considering the nutrient translocation through the hyphal network [12, 13].

In this context, yeast colonies can serve as a valuable model system: Colony patterns of dimorphic yeasts are macroscopically similar to fungal mycelia [12, 14]. However, in a first approximation the filamentous structures may be considered as chains of autonomous pseudohyphal cells which restrict the long-range nutrient translocation through the mycelium. Such an approach enables us to view mycelia as populations of single cells, which significantly simplifies the mathematical and experimental description of colony development. Since pseudohyphal cells are often hard to distinguish from the hyphal cell type, the assumption of the complete absence of nutrient translocation within yeast colonies might appear questionable [15]. However, if the mathematical model which describes colony development based on the proliferation of single cells is carefully defined, the comparison between model predictions and experimental results certainly helps to identify growth conditions where this assumption would be appropriate, or where nutrient translocation and/or other factors (i.e., the action of potential messengers) have to be considered.

In the present study, yeast colony development is described based on the proliferation of autonomously replicating cells, whereby the proliferation of these cells is only determined by nutrient availability in the growth substrate. Since the yeast cells are regarded as individuals, a cellular automaton model [16] has been chosen for the mathematical description of the growth process. The model describes discrete cells and continuously distributed nutrients. It has been adopted to the growth of *Yarrowia lipolytica* and *Candida boidinii* colonies under carbon and nitrogen limitation, respectively. Quantitative model predictions, particularly, the final colony diameter and the final cell-density profile, have been compared with experimental results to identify the nutrient-controlled growth of single cells as the ruling construction principle in the model yeasts colonies.

2 Model Definition

The growth of microorganisms strongly depends on nutrient availability in their environment. In the absence of important nutrients, cells stop to proliferate and undergo a transition to a stationary state [17]. To take the requirement of nutrients for cell growth into account, a mathematical model was developed that describes the growth of yeast colonies based on the assumption of exclusively nutrient-controlled proliferation.

The model incorporates the proliferation of cells, the consumption and diffusion of nutrient, and the transition of individual cells from an initially proliferating state to a stationary state. Proliferating cells and stationary cells reside on the same one-dimensional lattice *L(0,...i,...n)*, with $L(0)$ being the inoculation site and $L(n)$ representing the edge of the growth field[1)]. The state $s(i, t)$ of each lattice node i at time t is defined by the number of proliferating ($n_{C,p}$) and stationary cells ($n_{C,s}$), and the concentration of nutrient (c_N), respectively, which varies between the initial concentration ($c_{N,0}$) and zero.

$$s(i,t) = (n_{C,p}, n_{C,s}, c_N) \tag{1}$$

$$c_N \in [0, c_{N,0}] \tag{2}$$

$$n_{C,p}, n_{C,s} \in \{0, 1, 2, ...\} \tag{3}$$

1) List of symbols at the end of the paper.

2.1 Growth Dynamics

Initially, inoculated or newborn cells are in the proliferating state and possess the ability to give birth to a daughter cell. When cells divide, newborn cells grow in size at a constant rate. After they have reached a critical cell size, mature cells enter a new replication cycle, now themselves giving birth to a new cell. In the automaton model, cell divisions were assumed to occur at discrete intervals (Δt_p) representing the replication time of a cell.

For the distribution of newborn cells on the lattice, two different scenarios were tested regarding their influence on colony development. In a first approach, undirected cell growth was assumed. Daughter cells originating from proliferating cells at node (i) were placed randomly at the positions (i), (i–1), and (i+1). Accordingly, this scenario is referred to as random budding. Alternatively, the directed growth of the cells away from the inoculation site was implemented by placing cells born at node (i) exclusively to position (i+1). This behavior corresponds to the budding pattern of pseudohyphal cells, which solely form new buds at the pole distal to the mother cell's birth end. Therefore, the algorithm is referred to as distal budding.

Proliferating cells may change their state and become stationary. These cells are still viable but have lost their ability to form new cells. In the present model, the state transition was assumed to be controlled by nutrient availability. Proliferating cells ceased growth and entered the stationary state when the local nutrient concentration dropped to zero (Eq. 4). The state transition was assumed to be irreversible.

$$s_C = \begin{cases} \text{proliferating} & \text{if} \quad c_N(i,t) > 0 \\ \text{stationary} & \text{else} \end{cases} \tag{4}$$

2.2 Nutrient Balance

To maintain viability, all cells take up nutrient with rate $r_{N,main}$,

$$r_{N,main} = m_C \cdot R \tag{5}$$

where m_C is the mass of a single cell, and R represents the mass-specific maintenance requirement. Additionally, proliferating cells take up nutrient with rate $r_{N,prol}$ and transformed it into new biomass, i.e. into a new daughter cell. $r_{N,prol}$ was calculated from the cell mass (m_C) that was generated within the replication interval (Δt_p) (Eq. (6)). The yield (Y) describes the efficiency in which a particular nutrient is transformed into biomass.

$$r_{N,prol} = \frac{m_C}{\Delta t_p \cdot Y} \tag{6}$$

Nutrient was balanced by solving the reaction-diffusion equation

$$\frac{\partial c_N(x,t)}{\partial t} = D \cdot \frac{\partial^2 c_N(x,t)}{\partial x^2} - r_{N,con}(x,t) \tag{7}$$

where $r_{N,con}(x,t)$ is the local nutrient consumption rate. In the discretized form, the nutrient consumption rate at lattice node (i) is defined by

$$r_{N,con}(i,t) = \min[r'_{N,con}(i,t), c_N(i,t) \cdot \Delta t_p{}^{-1}] \tag{8}$$

Here

$$r'_{N,con}(i,t) = [r_{N,prol} \cdot n_{C,p}(i,t) + r_{N,main} \cdot (n_{C,p}(i,t) + n_{C,s}(i,t))] \cdot V_{node}{}^{-1} \tag{9}$$

is the maximum uptake rate as derived from equations (5) and (6), and $c_N(i,t) \times \Delta t^{-1}$ represents the uptake rate as limited by the locally available nutrient. V_{node} denotes the volume that is assigned to each lattice node. In order to account for the limited nutrient reservoir in the agar substrate, boundary conditions were chosen according to

$$\left[\frac{\partial c_N(i,t)}{\partial x}\right]_{i=0,i=n} = 0 \tag{10}$$

2.3 Parameterization of the Model

2.3.1 Experimental Setup and Simulation Dynamics

The automaton model describes the growth of unit cells which corresponded to pseudohyphal cells. Thus, the average length of the pseudohyphal cells determined in the experimental investigations ([12], see Tab. 1) was assigned to the length of a unit cell (l_C). The distance between two lattice nodes was chosen according to the unit-cell size. From the length of the growth-field moiety ($l = 26$ mm) ([12], see Fig. 1) and the cell length (l_C), the lattice size (n) was determined.

$$n = \left[\frac{l}{l_C}\right] \tag{11}$$

For the correct balancing of nutrient in the growth substrate, the agar height ($h = 0.9$ mm), calculated from the amount of agar filled into the Petri dish (2 mL), and the tile width ($w = 1$ cm) (see [12], Fig. 1) were incorporated into the model by assigning a volume (V_{node}) to each lattice node.

$$V_{node} = h \cdot w \cdot l_C \tag{12}$$

From the diffusion constant in agar (D_{agar}) and the distance between two lattice nodes (l_C) the time step (Δt) for the discretization of the continuous time scale (t) was calculated.

$$\Delta t = \frac{0.5 \cdot l_C^2}{D_{agar}} \tag{13}$$

Table 1. Biochemical and kinetic parameters for the growth of *Y. lipolytica* and *C. boidinii* used in the simulations of the colony development.

Nutrient	Replication time Δt_p [h]	Yield Y [g /g]	Spec. maintenance Requirement* R [g/(g × h)]	Cell length l_C [µm]	Cell diameter d_C [µm]
Carbon limitation					
Glucose	1.4 (1.8)**	0.33 (0.42)	0.01	30 (19)	2.3 (2.7)
Nitrogen limitation					
Ammonium	1.7 (2.1)	7.2 (5.2)	0	27 (24)	3.0 (2.7)

* The values were calculated according to [22].
** The numbers in parentheses indicate parameters used in the simulations for the growth of *C. boidinii*. The parameters were estimated in [12]. In all simulations a wet biomass density of $\rho_C = 1.1$ g/mL [23] was assumed.

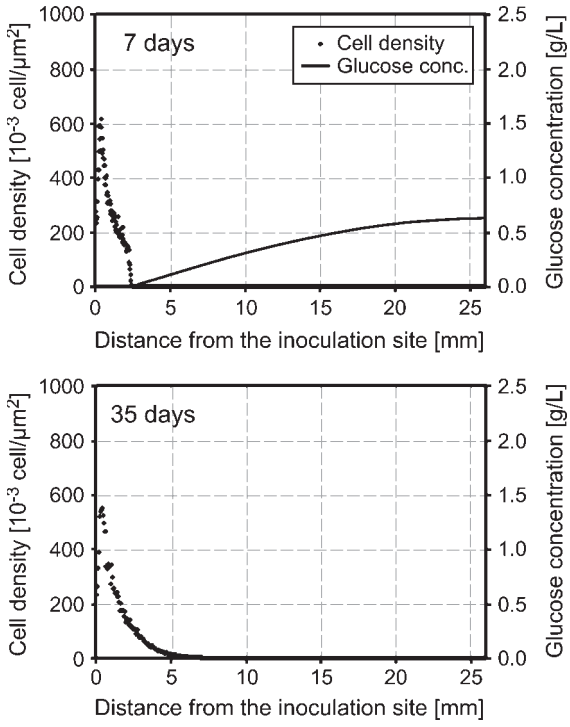

Figure 1. Simulated development of the cell-density profile under the assumption of DLG. Simulations describe the growth of a carbon-limited *Y. lipolytica* colony growing on glucose as the sole carbon source. Initial glucose concentration: 2 g/L, random budding, tile width: 1 cm. The figures show the average of 10 replicate runs.

The replication time (Δt_p) was estimated from the extension rate (v) of the colony diameter and the unit-cell length (l_C).

$$\Delta t_p = \frac{2 \cdot l_C}{v} \tag{14}$$

Accordingly, the cell array was updated in intervals of (t_p). Values estimated for Δt_p and v during the growth of the yeasts on different nutrients [12] are listed in Tab. 1.

2.3.2 Diffusion Constants of the Nutrients in Agar

According to Nicholson [18], the diffusion constant (D_0) of a small molecule in water can be corrected for the diffusion in agar by

$$D_{agar} = D_0 \cdot (1 - 0.023 \cdot \omega) \tag{15}$$

where ω is the weight percentage of agar. For glucose a diffusion constant of $D_0 = 6.7 \times 10^{-10}$ m^2/s was determined in water [19]. Despite an extensive literature review no estimates of diffusion constants for ammonium ions in water were found. However, the diffusion constants of small molecules are mainly dependent on their molecular weight and charge. Thus, the diffusion constant of sodium ions ($M_{Na+} = 23$ g/mol [20]) in water ($D_0 = 13 \times 10^{-10}$ m^2/s [21]) provided a realistic estimate for the diffusion rate of ammonium ions ($M_{NH4+} = 18$ g/mol [20]). From the diffusion constants in water (D_0), the diffusion constants in 2 % [w/v] agar (D_{agar}) were calculated according to Eq. (15) and used in the simulations (Tab. 2).

2.3.3 Cell Growth

The mass-specific nutrient-uptake rate to meet maintenance requirements (R) is mainly dependent on the cultivation temperature and only to a small extent on the particular carbon source or microorganism [22]. Therefore, the value of $R = 0.01$ g/(g × h) [22] was chosen for the simulation of the

Table 2. Diffusion constants applied in the simulations*.

Nutrient	D_0 [m^2/s]	D_{agar} [m^2/s]	Reference for D_0
Glucose	6.7×10^{-10}	6.4×10^{-10}	[19]
Ammonium	13.0×10^{-10}**	12.4×10^{-10}	[21]**

* The diffusion constants in water (D_0) were corrected for diffusion in agar (D_{agar}) using Eq. (15).
** The diffusion constant for ammonium ions in water was estimated from the diffusion constant of sodium ions in water.

carbon-limited growth of *Y. lipolytica* and *C. boidinii* on glucose. Nitrogen sources do not serve to provide energy. Thus, under nitrogen-limiting conditions the maintenance term did not contribute to the balance of the limiting nutrient, i.e. $R = 0$.

The mass of a single cell (m_C) was estimated using Eq. (16)

$$m_C = V_C \cdot \rho_C \cdot \mathrm{DW} \tag{16}$$

and

$$V_C = \frac{\pi}{4} \cdot d_C^2 \cdot l_C \tag{17}$$

where V_C is the experimentally determined volume of a pseudohyphal cell, ρ_C is the density of the *wet* biomass and DW represents the dry weight fraction of the total cell mass. A *wet* biomass density of $\rho_C = 1.1$ g/cm^3 was reported by Datar and Rosen [23] and used in the simulations. Depending on the vacuolization of the cells and the incorporation of storage materials, the dry weight fraction (DW) may vary greatly. In cultivations of fungi, values between 5 % [24] and 35 % [25, 26] were estimated. In the present study, the parameter DW was not determined experimentally but assigned to fit simulated results to data derived from the growth experiments.

The yield (Y) describes the efficiency of the transformation of a particular substrate into cellular mass. The yield coefficients of *Y. lipolytica* and *C. boidinii* on different nutrient sources were estimated in shake flask experiments [12] and are presented in Tab. 1.

3 Results and Discussion

The availability of nutrients represents the basis for microbial growth. This fundamental requirement was incorporated into a mathematical model which described yeast colony development based on the exclusively nutrient-controlled proliferation of single cells. The model was *scaled* for the growth of *C. boidinii* and *Y. lipolytica* on different nutrient resources. All parameters were in the physiologically relevant range and the limiting nutrient was balanced for a defined reservoir.

3.1 Characteristic Predictions

In Fig. 1 the simulated development of the cell-density profile in a glucose-limited *Y. lipolytica* colony is shown. Since yeast colonies were symmetrical in shape, simulations only account for one colony moiety ($x = 0$ mm: inoculation site; $x = 26$ mm: edge of the growth field). Rapid exponential cell growth at the early stages of colony development resulted in the accumulation of high cell numbers close to the inoculation site and caused the nutrient concentration to drop to zero underneath the colony. (The drop in cell density at the inoculation site was an artifact arising from the symmetry break in the simulations.) The nutrient concentration gradient spanned from the colony boundary to the edge of the growth field. In this phase, cell proliferation was determined by the amount of nutrient that diffused towards the colony. In the further course of the cultivation, the number of cells formed per time interval decreased resulting in the formation of a cell-density profile which declined monotonically from the inoculation site to the colony boundary. These simulations nicely illustrate how nutrient-controlled growth of individual cells resulted in the diffusion-limited growth of the population.

Furthermore, the predicted final colony diameter remained small when compared to the size of the growth field (see Fig. 1). This behavior was found to be insensitive to variations of the initial nutrient concentration in the growth substrate, the mass of a single cell (DW), comparatively large variations in the extension rate of the colony, and the diffusion speed of the nutrient, respectively [13]. Thus, the morphology of the cell-density profile and the final colony diameter provided robust criteria to identify a diffusion-limited growth mechanism in yeast populations.

3.2 Discussion of the Model Structure

In the presented DLG model, the replication interval (Δt_p) was defined to be independent of the local nutrient concentration. This definition was motivated by the observation of constant colony extension rates at nutrient concentrations that were changed over several orders of magnitude (see Figs. 2–5 and Tab. 2) [12]). This behavior may correspond to a constant extension rate which is inherent in fila-

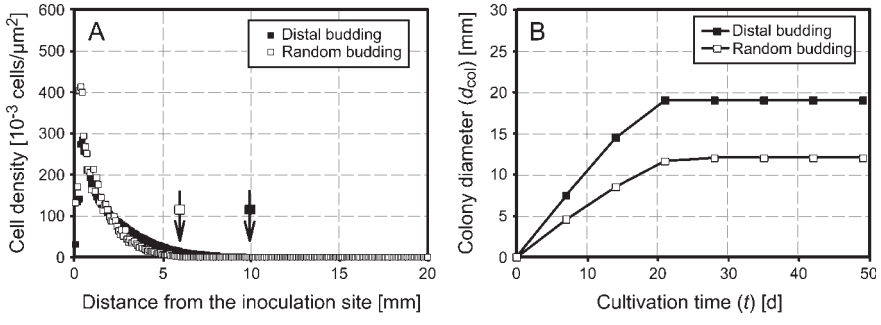

Figure 2. Influence of the assumed budding pattern on the simulated diffusion-limited development of *Y. lipolytica* colonies growing on glucose as the sole carbon source. (A) Cell–density profiles after 35 days of cultivation. (B) Colony diameter *vs.* cultivation time. Initial glucose concentration: 2 g/L, tile width: 1 cm. The arrows indicate the outer edge of the profiles. For further details see text.

mentous cells. Alternatively, nutrient uptake by the cells and transport limitation due to the slow diffusion of the nutrients may cause the food concentration at the colony boundary to drop to a value which is fairly constant throughout the cultivation period. Thus, the chosen definition of Δt_p represented an observation and does not explain its origin. However, the model correctly describes the consequences of such behavior by ensuring mass conservation.

An important discrepancy to the published reaction-diffusion models is the definition of the nutrient-uptake kinetics. In a number of qualitative models, nutrient uptake was assumed to be proportional to the square of the local biomass concentration [7–11], allowing for the complete uptake of nutrient even by a comparatively small cell number. In the present study, as well as in very recent quantitative models of fungal development [2, 3], a correct linear relationship (see Eq. (9)) was chosen yielding a characteristically declining cell density as a result of simulations.

Furthermore, the model in principle allowed different budding regimes to be incorporated. Distal budding could be implemented by placing newborn cells originating from node (i) exclusively to the position (i+1), while during random budding daughter cells were placed to the positions (i–1), (i), and (i+1) with equal probability. The model, however, did not explain the potential origin of such behavior. In the distal budding scenario, the growth towards higher nutrient concentrations, or the messenger-induced growth away from high cell densities (quorum sensing) could not be distinguished. In Fig. 2 the model predictions arising from different budding patterns are compared for the glucose-limited development of a *Y. lipolytica* colony.

When distal budding was assumed, the polarized growth of the cells away from the inoculation site resulted in a significantly higher colony extension rate, a larger final diameter, and a somewhat shallower shape of the cell-density profile (see Fig. 2). Thus, when the replication interval (Δt_p) of the cells was known, e.g., from the observation of replication cycles of individual cells by time-lapse photography, the comparison of simulated and experimentally determined colony extension rates may provide some indication of the biased placement of newborn cells. Using this strategy, a chemotactic force in colonies of motile bacteria was identified in [5], by elaborating that populations extend at a higher rate than expected for a solely random movement of the

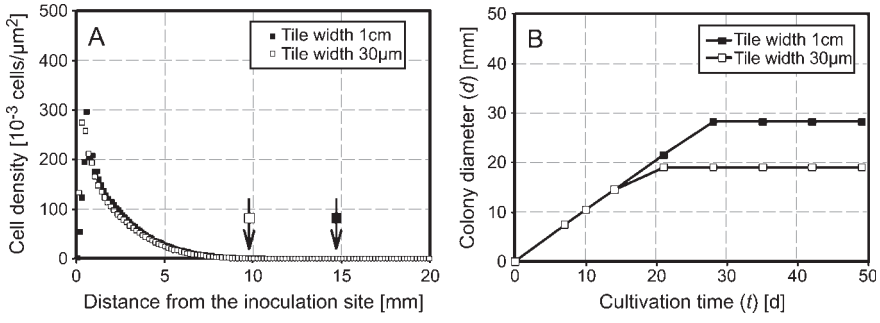

Figure 3. Influence of the tile width (w) on the simulated diffusion-limited development of *Y. lipolytica* colonies growing on glucose. (A) Cell-density profiles after 35 days of cultivation. (B) Colony diameter *vs.* cultivation time. Initial glucose concentration: 2 g/L, distal budding. The arrows indicate the outer edge of the profiles. For further details see text.

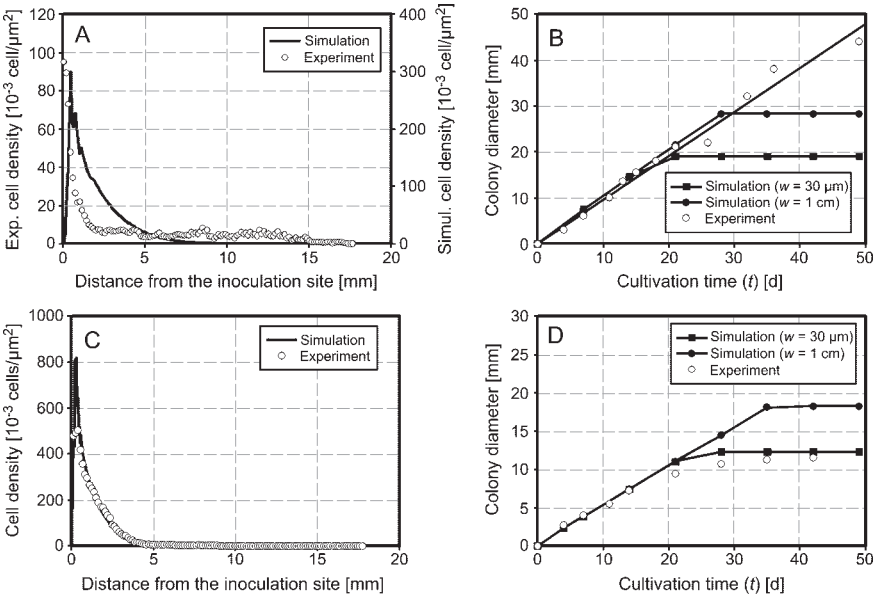

Figure 4. Comparison of experimentally determined and simulated (A, C) cell-density profiles after 35 days of cultivation, and (B, D) colony diameter extension of carbon-limited *Y. lipolytica* (A, B) and *C. boidinii* (C, D) colonies growing on glucose as the sole carbon source. Initial glucose concentration: 2 g/L (C-G-2 medium [12]). The simulations are based on the assumption of DLG and show the results for a distal budding regime (DW = 0.2). For further details of the parameterization see text.

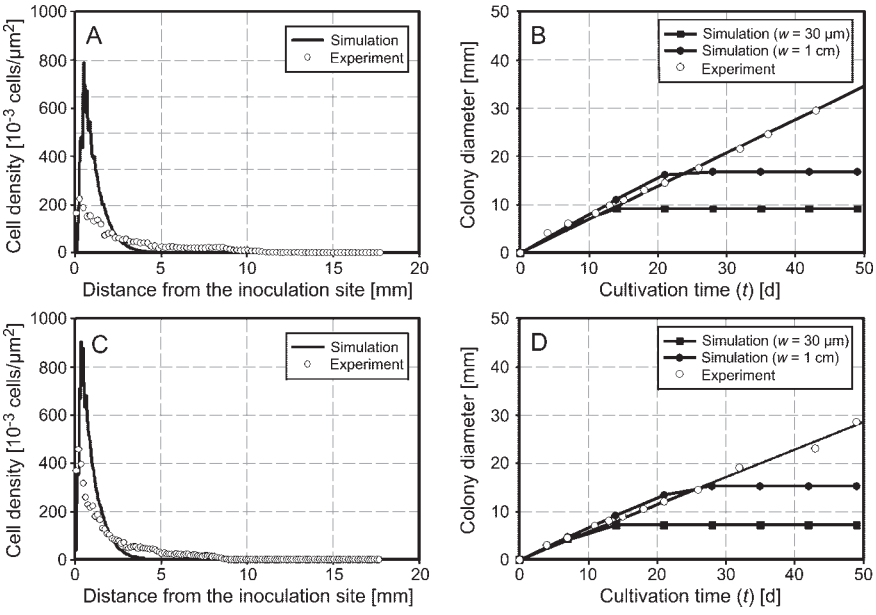

Figure 5. Comparison of experimentally determined and simulated (A, C) cell-density profiles after 35 days of cultivation, and (B, D) colony diameter extension of nitrogen-limited *Y. lipolytica* (A, B) and *C. boidinii* (C, D) colonies growing on ammonium sulfate as the sole nitrogen source. Initial ammonium sulfate concentration: 0.05 g/L (N-A-0.05 medium [12]). The simulations are based on the assumption of DLG and show the results for a distal budding regime (DW = 0.05). For further details of the parameterization see text.

cells. During the growth of (pseudo)hyphal yeast cells, daughter cells were exclusively formed at the pole distal to the mother cell's birth end.

In the growth experiments, the orientation of pseudohyphal cells deviated only slightly from the longitudinal colony axis, and no cells growing towards the inoculation site were observed. Therefore, the replication interval was calculated from the extension rate of the colony diameter and the length of a unit (pseudohyphal) cell (Eq. (14)). As a consequence, the *observation* of directed growth of individual cells (distal budding) was then incorported into the simulation routine, i.e., the colony extension rate predicted under these conditions is equal to the experimentally observed one. However, considering

(i) the strong drop in the colony extension rate when random budding is assumed, and

(ii) the observed polarized growth in the colonies,

the presence of a mechanism which biases the placement of newborn cells towards higher nutrient concentrations or away from high cell densities was likely. In any case, as the model was parameterized, only simulations of colony development incorporating the distal budding regime should be compared with experimental results.

3.3 Discussion of the Model Parameterization

The model was scaled for the dimensions of the experimental setup. Furthermore, stoichiometric as well as kinetic parameters were either directly measured or derived from literature data. Thus, all biochemical parameters that describe the growth of the cells were in a realistic range. The parameters referred to measures commonly used in the quantitative description of biomass growth. Therefore, the quality of the parameter estimations can be easily evaluated by their comparison to recognized data provided in the biochemical literature. Since the correct parameterization of the diffusion process was crucial for the predictions of the model, the influence of variations in the diffusion constant on model predictions, as well as the quality of the estimation of the diffusion constant in agar were particularly discussed in [13]. It was shown that the correction of D_0 for the diffusion in agar (D_{agar}) using Eq. (15) provided adequate estimates for the transport velocity of nutrients in the growth substrate.

An important scaling effect was identified by variations of the tile width (w). While the predicted final cell-density profiles and the total accumulation of cells were insensitive to changes of this parameter, the final colony diameter varied markedly (see Fig. 3). In simulations of the diffusion-limited growth of *Y. lipolytica* colonies on glucose it was shown, that a 55 % higher colony diameter was calculated for a tile width of 1 cm when compared to the predictions for a tile width of 30 µm.

The reason for this behavior lies in the model structure: In the one-dimensional model, the nutrient content of a discretized agar-tile is concentrated at one point. Thus, when a large tile width is chosen, even at extremely small concentrations the nutrient content of a tile may be high enough to fuel the proliferation of a cell, while in a small tile this reservoir may already be depleted. Thus, when the simulated final colony extension is compared with experimental results, simulations have to be carefully interpreted with respect to this parameter. For a further refinement of predictions for the final diameter of the populations, the nutrient transport perpendicular to the longitudinal colony axis has to be considered, i.e. two-dimensional models have to be applied.

3.4 Comparison of Simulations and Experimental Results

In order to identify DLG as the ruling construction principle in yeast populations, simulations were compared with experimental results obtained in cultivations of *C. boidinii* and *Y. lipolytica* on solid agar substrates. Colonies of the model yeasts were grown under conditions of carbon and nitrogen limitation. Glucose and ammonium sulfate served as the limiting nutrient sources. The experimental conditions were described in [12]. The models were scaled for each combination of cultivation conditions and yeast using the parameters listed in Tabs. 1 and 2, as well as the dimensions of the experimental setup [12, Fig. 1]. In all simulations, distal budding was assumed. In order to consider the uncertainties in the predictions of the final colony diameter which arise from the application of the one-dimensional model, the time course of colony-diameter extension was compared with simulations using a tile width (w) of 30 μm or 1 cm.

As can be seen in Figs. 4 and 5, only the experimental results of the glucose-limited development of *C. boidinii* colonies matched the simulations of diffusion-limited colony growth (see Figs. 4C and D). Under these conditions, a monotonically declining cell-density profile was formed and the colony diameter remained small compared to the size of the growth field. For a tile width of 30 μm, the model exactly predicted the experimentally determined final colony extension of approximately 12 mm. Therefore, DLG is suggested by the authors to be the major regulatory mechanism in *C. boidinii* colonies growing on glucose as the limiting nutrient source.

Under all other cultivation conditions, both yeasts extended until the edge of the growth field was reached, clearly exhibiting a growth characteristic which was different from the one expected for DLG (see Figs. 4B and 5B, D). Furthermore, the morphology of the cell-density profiles cannot be reproduced by the simulations (see Figs. 4A and 5A, C). Thus, for the carbon and nitrogen-limited growth of *Y. lipolytica*, as well as for the nitrogen-limited development of *C. boidinii* colonies, the hypothesis of DLG has to be rejected, i.e., under these conditions an important potential regulatory mechanism could be ruled out.

This result presented the opportunity to look for alternative explanations: Inspired by earlier studies on bacterial colonies [5, 27], the possibility of whether a messenger-mediated quorum-sensing mechanism could facilitate the evolution of a constant cell-density profile – observed during the glucose-limited growth of *Y. lipolytica* colonies – was explored (Fig. 4 A) [13]. A model was developed wherein a messenger was assumed to be emitted by all cells. This messenger inhibited proliferation once its local concentration exceeded a threshold. According to the model, proliferation could be truncated even though nutrient might still be available. Indeed, it was possible to reproduce the morphology of the profile assuming that the messenger was extremely volatile or unstable [13].

However, although a number of volatile messengers are known to influence the growth of yeast cells [28–31], the suggested mechanism clearly represents only one fundamental possibility out of a large number of explanatory concepts. Bearing in mind that the description of yeast colonies as a population of single cells could be an oversimplification since pseudohyphal cells are often hard to distinguish from true hyphae [15], the question arises whether the incorporation of nutrient translocation between the cells could explain the observed behavior. Furthermore, the estimation of cell-density profiles only ones during the cultivation did not provide the information as to how a particular state was reached, e.g., it did not capture potential cell-decay processes within the colonies. Thus, a continuous monitoring of the spatio-temporal development of the cell-density profile is essential to provide a larger basis of experimental data. In this context, the non-invasive monitoring technique of cell-density distributions inside fungal mycelia presented in [12] represents a valuable experimental tool to provide the necessary data. Further experimental investigations applying this technique are being conducted.

In summary, the presented model described the development of yeast mycelia based on the assumptions that
(i) well-separated single cells
(ii) proliferate exclusively by nutrient-control.

Simulations predicted the spatio-temporal development of the cell-density profile and, particularly, the final diameter of the populations.

The estimation of the local nutrient uptake from the generated cell mass and the experimentally determined yield on the particular nutrient represented the basis for an accurate nutrient balancing in the growth field, which facilitated a quantitative modeling of the growth process. From the comparison of simulations with experimental results that were obtained in cultivations of the model yeasts, *C. boidinii* and *Y. lipolytica*, it was possible to identify DLG as the ruling regulatory mechanisms in glucose-limited *C. boidinii* colonies.

Under all other cultivation conditions tested, both yeasts exhibited a growth characteristic that could not be reproduced by the DLG simulations. On the basis of these results, it is important to investigate, whether the fundamental assumptions (populations of single cells, nutrient controlled proliferation) had to be revised, or whether additional regulatory mechanisms were present in yeast mycelia, which facilitated the generation of the observed growth patterns. In either case, the presented study demonstrated how the mathematical analysis of growth processes in yeast populations could assist the experimental identification of potential regulatory mechanisms.

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Symbols used

c_N	[g//L]	nutrient concentration
$c_{N,0}$	[g/L]	nutrient concentration
D_0	[m^2/s]	diffusion constant in water
D_{agar}	[m^2/s]	diffusion constant in agar
d_C	[μm]	cell diameter
d_{col}	[mm]	colony diameter
DW	[–]	dry weight fraction of wet biomass
h	[μm]	height of the growth field
i	[–]	index of a lattice node
l	[μm]	length of the growth field
l_C	[μm]	length of a cell
n	[–]	number of lattice nodes
$n_{C,p}$	[–]	number of proliferating unit cells
$n_{C,s}$	[–]	number of stationary unit cells
$r_{N,con}$	[g/(L h)]	total nutrient consumption rate
$r_{N,main}$	[g/h]	nutrient uptake rate per cell due to maintenance
$r_{N,prol}$	[g/h]	nutrient uptake rate per cell due to proliferation
R	[g/(g H)]	specific nutrient uptake rate due to maintenance
s	[–]	state of a lattice node
s_C	[–]	state of a cell
t	[h]	cultivation time
Δt	[h]	time step for the discretization of the continuous time scale
Δt_p	[h]	replication interval (generation time)
V	[cm^3]	volume of the standard growth field
V_C	[μm^3]	volume of a cell
V_{node}	[L]	volume assigned to one lattice node
w	[μm]	standard width of the growth field
x	[mm]	spatial coordinate
Y	[g/g]	biomass yield on the nutrient
ρ_C	[g/cm]	density of wet biomass
ω	[% (w/v)]	agar concentration

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