Measurement and Simulation of the Morphological Development of Filamentous Microorganisms

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Growth of *Streptomyces tendae* was investigated in submerged culture. Images of several mycelia were analyzed by means of an image-processing system. The studies revealed that tip growth angles and branching outgrowth angles could be regarded as normally distributed. Based on these results, a random model for directional growth of hyphal tips as well as directional growth of branches is proposed. This model shows curved elongation of hyphal tips, so that the morphological development of a mycelium up to the formation of a pellet is predicted, similar to that observed in nature.
Key words: directional growth • modeling • morphology • pellet formation

INTRODUCTION

Mathematical models for growth and branching of filamentous fungi or bacteria have been postulated by several investigators.[3,4,6] Some properties predicted by these models, such as exponential increase in total length and number of branches, have been well or approximately confirmed by experimental results. However, simulations of mycelial growth assumed that hyphal growth takes place in a plane with tips growing in straight lines and the formation of branches right-angled or in a certain angle to the parent hyphae. Usually, random events were neglected. Therefore, a realistic picture of the development of a mycelium, which can lead to the formation of a pellet in liquid medium or a colony on a surface culture, could not be shown.

Until recently, no experimental studies on tip growth and branching directions of mycelial microorganisms have been made. In this article, we present some results of tip growth and branching directions of hyphal tips. Based on these results, a random model for tip growth as well as branching directions was developed, so that the geometry of a mycelium can be reproduced by simulations.

MATERIALS AND METHODS

Organism and Growth Medium

Streptomyces tendae Tü 901, which produces a group of antibiotics called nikkomycins, was used throughout this study. Spores were suspended in 4 mL autoclaved medium (D-glucose, 30 g/L; yeast extract powder, 5 g/L; neutralized soya peptone, 5 g/L). The suspension was thoroughly shaken in a vortex to separate spore aggregates.

Microscopical Investigations

Two milliliters of spore suspension were filled into a temperature controlled growth chamber.[5] The growth chamber was mounted on a scanning table of an inverse microscope (Zeiss, IM 35). To analyze the structures of the mycelia, a monochrome TV camera with a chalnikon tube (Bosch) connected to an image-processing system (IPS, Kontron) was attached to the microscope.

Morphological development of several mycelia that emerged from spores were observed at 31°C. From this, the initially observed specific growth rate[7] of an individual hypha μ_m, the finally observed linear extension rate of an individual hypha α_m, and the specific growth rate μ of a complete mycelium were determined. When a mycelium became too complex, the analysis was stopped. To calculate tip growth and branching directions, the hyphae of the final image were dissected into sections with the same length (l_e = 4.4 μm). The analysis was done only for mycelia growing in a plane. In contrast to growth in a three-dimensional space, one angle—the tip growth angle or the branching angle—is sufficient to characterize growth direction of a hypha or a branch in a plane. Tip growth angles and branching angles were calculated as follows:

1. The tip growth angle ($\Delta\gamma_{g,i}$), the angle between two neighboring sections $P_{i-1}P_i$ and P_iP_{i+1}, was calculated from the positions of the sections [Fig. 1(a)]. For a straight growth this angle is zero.

2. A branch B_k grew out of the branching point P_k of the two parent sections $P_{k-1}P_k$ and P_kP_{k+1} [Fig. 1(b)]. The two angles $\gamma_{b1,k}$ and $\gamma_{b2,k}$ of the branch to both parent sections were calculated. At the branching point, the average direction $(\overline{P_{k-1}P_{k+1}})$ of these two parent sections was drawn. Then, the axis perpendicular to this average direction was determined. Finally, the branching angle ($\Delta\gamma_{b,k}$), i.e., the angle of the branch to this perpendicular axis, was calculated by

$$\Delta\gamma_{b,k} = \frac{\gamma_{b1,k} - \gamma_{b2,k}}{2}.$$

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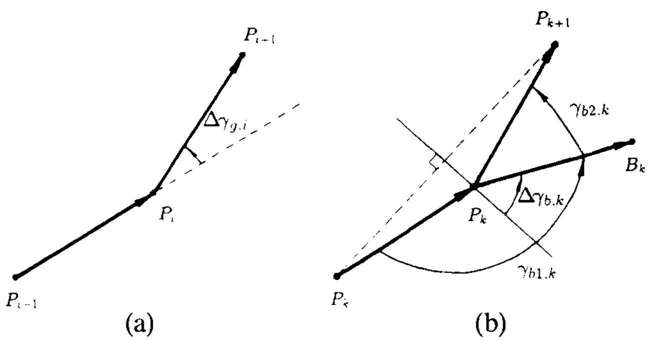


Figure 1. Schematic representation of (a) tip growth angles and (b) branching angles. The arrows indicate the directions of the tips.

EXPERIMENTAL RESULTS

The frequency distribution of tip growth angles $\Delta\gamma_{g,i}$ for 110 hyphae with a total of 1440 sections is shown in Figure 2. These angles were well described by a normal distribution with a mean value $\overline{\Delta\gamma_g}$ of 0.2°, which is practically zero; the standard deviation $\sigma_{\Delta\gamma_g}$ was 10.1°. Figure 3 shows the frequency distribution of branching angles $\Delta\gamma_{b,k}$ for 86 branches. These branching angles were also normally distributed with a mean value of 1.2°, which can approximately be taken as zero. The standard deviation $\sigma_{\Delta\gamma_b}$ amounted to 29.1°. From these results it follows that, in the average, hyphae grow in a straight direction, and branches are formed perpendicular to their parent hyphae.

MATHEMATICAL DESCRIPTION OF CURVED GROWTH

To describe the curved elongation of a hypha by a numerical simulation, a model is suggested that considers discrete sections of a hypha with constant length l_e. It is assumed that hyphae grow section-wise at a rate conforming to the hyphal extension rate α. Spherical coordinates (ϕ, ψ) are used to characterize the orientation of an individual section in a three-dimensional reference space. To keep the presentation simple, the spherical coordinates will not be shown in the following figures.

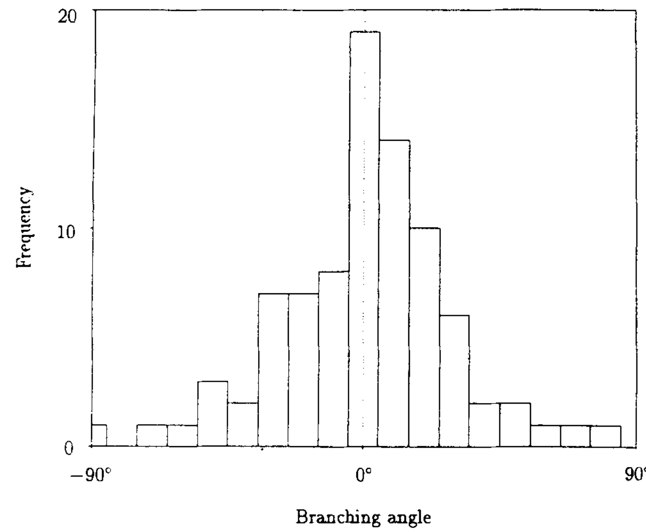


Figure 3. Frequency distribution of branching angles for 86 branches.

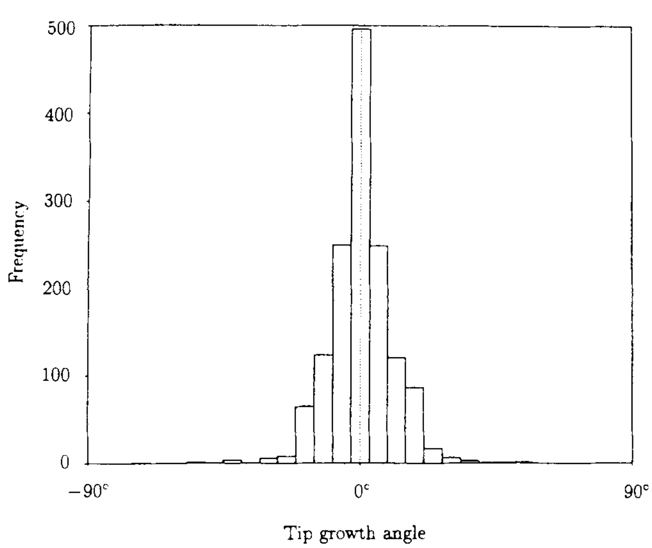


Figure 2. Frequency distribution of tip growth angles for 1440 sections.

It is assumed that, during early mycelial growth, concentration gradients in the mycelia can be ignored. Hyphal growth is not oriented in any preferred direction. Furthermore, it should be pointed out that the measurements in the growth chamber were only possible as long as hyphal growth took place in the plane that was determined by the experimental setup. However, this plane can be seen as a random plane with respect to the orientation of the spore and the resulting mycelium. It is reasonable to assume, therefore, that this plane represents all planes. The measured growth and branching angles are now valid for all planes in the case of three-dimensional growth.

In the following work, we will show that the orientation of a new section of a growing hypha can be calculated based on the orientation of the previous section and the statistical data obtained above. Accordingly, the orientation of the first section of a new branch can be determined by the orientation of the parent sections and the respective statistical data.

Tip Growth Directions

A hypha has elongated from point P_{i-1} to point P_i by a length of l_e (Fig. 4). The growth direction of this section $(\phi_{g,i}, \psi_{g,i})$ is given. The hypha is now elongating from point P_i to point P_{i+1} with the same length. The points P_{i-1}, P_i and P_{i+1} set up a plane. It is assumed that the angle between the two sections in the plane corresponds to the experimentally determined growth angle $\Delta\gamma_{g,i}$. For a constant $\Delta\gamma_{g,i}$, the new tip P_{i+1} is located on a ring that is perpendicular to the section $P_{i-1}P_i$. The distance from any point on the ring to P_i is l_e. A point on the ring can be determined by a new random quantity, angle $\theta_{g,i}$ (Fig. 4). Because hyphae grow uniformly in any direc-

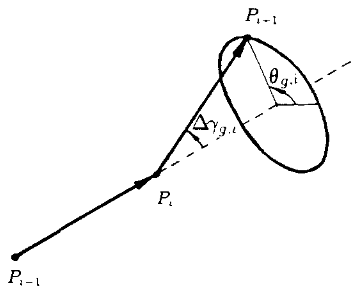


Figure 4. Schematic representation of growth direction of a hyphal tip in a three-dimensional culture. The arrows indicate the direction of the tip.

tion, the angle $\theta_{g,i}$ is assumed to be uniformly distributed in the interval $[0, 2\pi)$.

For given angles $\Delta\gamma_{g,i}$ and $\theta_{g,i}$, the point P_{i+1} is fixed. Therefore, the growth direction of the new section P_iP_{i+1} $(\phi_{g,i+1}, \psi_{g,i+1})$ can be determined by the direction of the section $P_{i+1}P_i$ $(\phi_{g,i}, \psi_{g,i})$ and the angles $\Delta\gamma_{g,i}$ and $\theta_{g,i}$. Thus, the growth direction of a tip can propagate section-wise with the two random angles, which will be generated in the simulation in every section anew by respective random generators.

Branching Directions

A branch is growing out of the branching point (P_k) of the parent sections $P_{k-1}P_k$ and P_kP_{k+1} (Fig. 5) according to an appropriate branching kinetic.[7] It will reach position B_k, and the first section P_kB_k of the branch will be formed with length l_e. In order to determine the position of point B_k, it is only necessary to find out the branching direction $(\phi_{b,k}, \psi_{b,k})$. Similar to the evaluation in the experiment, the average direction $\overline{P_{k-1}P_{k+1}}$ $((\phi_{g,k-1} + \phi_{g,k+1})/2, (\psi_{g,k-1} + \psi_{g,k+1})/2)$ of the two parent sections for the branching point is drawn. Instead of a perpendicular axis as in the experiment, a plane that is perpendicular to the average direction $\overline{P_{k-1}P_{k+1}}$ is used. The plane includes the branching point. The angle of the branch to this perpendicular plane is assumed to be normally distributed. In the three-dimensional case, the locus of the branch tip is again on a ring with a distance of l_e to the branching point. The section from any point on the ring to the branching point makes up an angle $\Delta\gamma_{b,k}$ to the perpendicular plane. The position of the branch tip B_k can be located

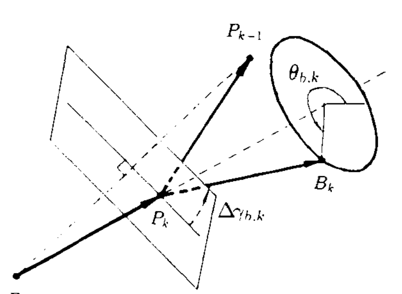


Figure 5. Schematic representation of branching direction of a branch in a three-dimensional culture. The arrows indicate the directions of the tips.

at any point on the ring. Similar to the tip growth direction, an angle $\theta_{b,k}$ is introduced to describe a point on the ring. $\theta_{b,k}$ is uniformly distributed in the interval $[0, 2\pi)$. Using the average direction of the two parent sections and the two random angles $\Delta\gamma_{g,i}$ and $\theta_{b,k}$, the branching direction $(\phi_{b,k}, \psi_{b,k})$ of the branch can be determined.

SIMULATION

To simulate hyphal growth, the model for tip growth and branching directions was incorporated in a model describing the kinetics for growth and branching.[7] The kinetic parameters used in our simulation were determined from an analysis of sequential mircoscopical pictures over periods of 6 to 7 h as above: The linear extension rate of a hyphal tip α_m was 28.3 μm/h; the specific growth rate of a hyphal tip μ_m and the specific growth rate of a complete mycelium μ amounted to 1.09 1/h and 0.82 1/h, respectively. According to our results, growth angles were regarded as normally distributed with a mean value of 0° and a standard deviation of 10.1° (l_e = 4.4 μm), while branching angles were also normally distributed with a mean value of 0° and a standard deviation of 29.1°. In the simulation, as mentioned above, hyphae grow section-wise. For each section or each branch, the random quantities ($\Delta\gamma_{g,i}$ and $\theta_{g,i}$ or $\Delta\gamma_{b,k}$ and $\theta_{b,k}$) are newly generated by random number generators of the computer.

Figure 6 shows the simulated morphological development of a mycelium that emerged from a single spore. Note that only a two-dimensional projection of the three-dimensional simulation is shown. The simulation starts with 3.6 μm long germ tube at time zero. About 2.5 h later, the first branch is formed [(Fig. 6(a)]. The mycelium becomes more compact during the simulation [Fig. (6b–d)]. Fourteen hours after starting simulation,

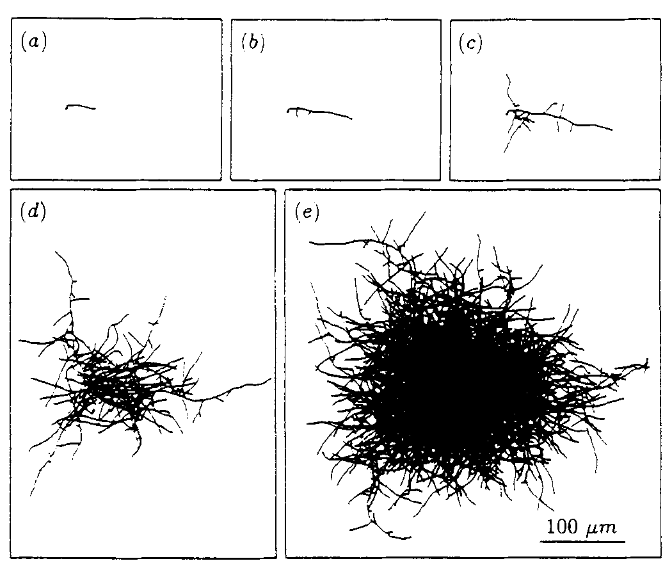
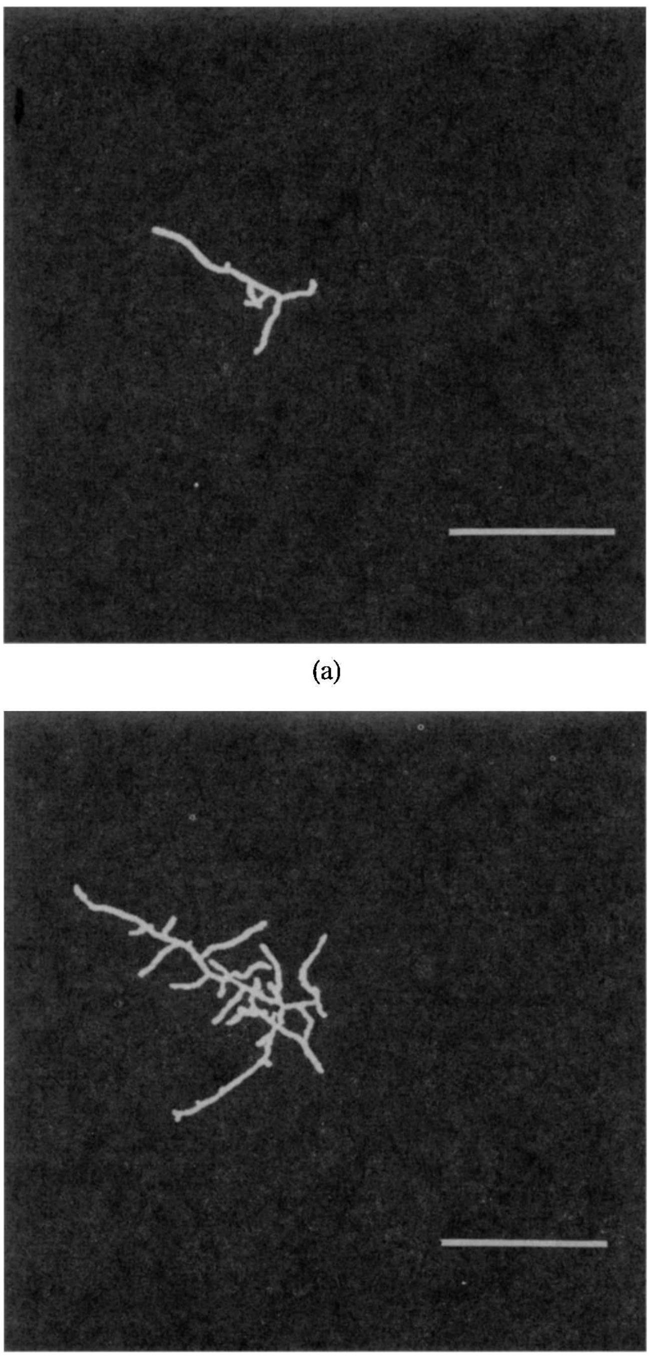


Figure 6. Simulated morphological development of the growth of a mycelium emerging from a spore: (a) t = 2.5 h, (b) t = 4 h, (c) t = 6 h, (d) t = 10 h, and (e) t = 14 h.

the mycelium is so compact that it can be regarded as a pellet [(Fig. 6(e)]. In order to give an impression of the true morphological development of a mycelium in liquid medium, photographs obtained in experiments are shown in Figure 7. The two photographs were taken 4 and 6 h after germination.

DISCUSSION

In our experiments, tip growth and branching angles of early mycelial growth have been shown to be normally distributed. In contrast to simulations of growing mycelia neglecting these random events,[3,4,6] the simulation of three-dimensional growth is able to give an impression of morphology of a developing mycelium that is very close to nature. In appearance, the simulation results shown in Figure 6 correspond well with experimental observations (Fig. 7). It could be shown that the formation of a pellet is possible by growth of a mycelium emerging from a single spore. Naturally, this model can be adapted to describe hyphal growth on a surface culture that will lead to the formation of a colony.



(a)

(b)

Figure 7. Observed morphological development of the growth of a mycelium emerging from a spore: (a) $t = 4$ h and (b) $t = 6$ h. Scale bar = 100 μm.

The simulation is based on the assumption that, during early mycelial growth, all hyphae were supplied with uniformly distributed substances. In this case, no concentration gradients of substances occur in the mycelia, and it can be assumed that hyphal tips can grow uniformly in any direction. This is true for the early mycelial growth and for a certain period after the formation of a pellet. When the center of a pellet becomes limited by diffusion of substrate or oxygen, this model must be modified.[1,2] However, the simulation shows that the model is able to describe the complexity of mycelial development for a long period of growth, much longer than the process can be completely analyzed by microscopical techniques. Additionally, systematic studies on the dependence of morphology on kinetics and morphological parameters can be carried out to gain more insight into the complex growth behavior of filamentous microorganisms.

NOMENCLATURE

l_e	length of a hyphal section
B	the first section point of a branch
P	section point of a hypha

Indices

b	branching
g	tip growth
i	number of a section used for tip growth
k	number of a section used for branching

Greek symbols

α_m	linear extension rate of a hypha
μ	specific growth rate of a complete mycelium
μ_m	specific growth rate of a hypha
ϕ	polar angle in spherical coordinates
ψ	cone angle in spherical coordinates
γ_{b1}	angle between a branch and one of the parent sections
γ_{b2}	angle between a branch and the other parent section
$\Delta\gamma_b$	branching angle
$\Delta\gamma_g$	tip growth angle
$\sigma_{\Delta\gamma_g}$	standard deviations of tip growth angles
$\sigma_{\Delta\gamma_b}$	standard deviations of branching angles
θ_b	angle for uniform branching directions
θ_g	angle for uniform growth directions

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