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Growth and Colony Patterning of Filamentous Fungi

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Abstract. A computer model of the fungal colony that grew consuming limited amount of nutrient with three control parameters, growth rate, and nutrient level and diffusion, was constructed to make a basic morphology diagram of mycelial colony. To apply the diagram with those parameters to the mycelial growth of *Aspergillus nidulans* wild and mutant strains, the relation between the colony expansion and the nutrient level was measured. Differences in the colony patterns of these strains at low nutrient level were attributed to the change in the above relation that caused a shift of the corresponding patterns in the diagram.

1. Introduction

The shapes of fungal colonies exhibit striking diversity depending on the substrate conditions as well as on the fungal species. Although the shapes and the surface textures of colonies provide useful information to determine the species or to monitor the state of growth, colony patterning looks to be highly sensitive to the environmental factors. However, there might be underlying basic rules of pattern selection common for any species.

Obviously, the nutrient level in the substrate is the main factor for the hyphal production. In addition, nutrient diffusion will be a significant factor, since it affects the nutrient flux into the colonized area and the distribution of the location in which uptake of nutrient occurs intensely. As for the internal parameter of mycelium, the growth rate of hyphae, or the rate of nutrient utilization, will largely contribute to determine where in the colony the hyphal production occurs. In this letter, these three control parameters are considered independent with each other, in order to make general morphology diagram of the mycelial colonies by using a model.

This letter explores the colony morphology of wild and mutant strains of *Aspergillus nidulans* based on a morphology diagram that was made by a colony model. To apply the morphology diagram to the real colony patterns, the manner of colony expansion was

examined for *A. nidulans*. It is suggested that the relationship between the nutrient level in the substrate and the growth rate of the mycelium is important to relate the real patterns with the morphology diagram.

2. Materials and Methods

The strains used in this study were *A. nidulans* wild type strain A4 (the index of strain in FGSC) and a mutant strain A583 supplied by the Fungal Genetic Stock Center (FGSC), Kansas City, USA. Spores of the strains were adsorbed to the silica particles for storage (TABOR and TABOR, 1970). The peptone agar media containing K_2HPO_4 (0.05%), KH_2PO_4 (0.05%), with Difco Neopeptone 0.01, 0.05, 0.1, 0.5, and 1%, and with Difco Bacto-agar 0.5% were prepared, and 20 ml of sterile medium was poured into 9-cm Petri plates. Silica particles containing spores were directly put on the medium for inoculation, and the strains were cultivated at 24°C. The plates were placed in closed vinyl bags to reduce drying of the media. The colonies were photographed with a 35 mm camera with oblique illumination from below.

3. Model

The colony patterns were developed on a 2-dimensional square lattice of 100×200 lattice constant. Each lattice point is supplied with N_i nutrient particles initially for storage. Each hyphal unit can occupy one lattice point. Seed hyphae are placed initially in a line of 100 lattice constants. A hyphal unit is chosen at random, and if the nutrient storage remains in the lattice point that the hyphal unit occupies, it absorbs one nutrient particle.

When a hyphal unit absorbs 3 nutrient particles, it creates one new hyphal unit at a randomly chosen unoccupied neighboring site and consumes the stored nutrient. Up to two daughter hyphal units are created by the parent one in the same mycelial plane to proceed dichotomous branching. When the neighboring sites in the same mycelial plane are already occupied, a higher or a lower plane is randomly chosen and the same process is repeated for the new plane. In case that no vacant site remains for a hyphal unit to create its daughter hyphae, it accumulates up to 4 nutrient particles and ceases absorbing.

For the nutrient diffusion, any one of the lattice sites is chosen at random. When the chosen site has at least one nutrient particle, a particle is released to walk at random for R_s steps. In the simulation, one sequence of random walk is regarded as the time unit. Since the trajectory length R_s is proportional to the diffusion coefficient in one unit time, let us regard the step length R_s as the diffusion coefficient of nutrient particles.

The growth rate G_r is defined as the frequency of nutrient uptake for unit time. When the random walk of nutrient was repeated n times before one nutrient uptake, G_r is equal to $1/n$. In case that nutrient uptake occurs m times per one nutrient walk, G_r is m.

4. Results and Discussion

Examples of model colonies at a fixed R_s are shown in Fig. 1. Splitting and ramified shapes appear with decreasing nutrient and growth rate. At high nutrient level with low growth rate, the colony forms thick layers due to the high nutrient influx. Under this condition, the growth is reaction-limited since the growth rate is the limiting factor for colony expansion. At low nutrient level with high growth rate, the colony is enlarged with thin mycelial layers. The nutrient influx or the nutrient level is the limiting factor. Since the nutrient particle diffuses into the colonized area during random walk without being trapped by the hyphae, hyphae are created homogeneously inside the colonized area. Thus, a typical branched shape that is generally seen under diffusion-limited growth does not appear.

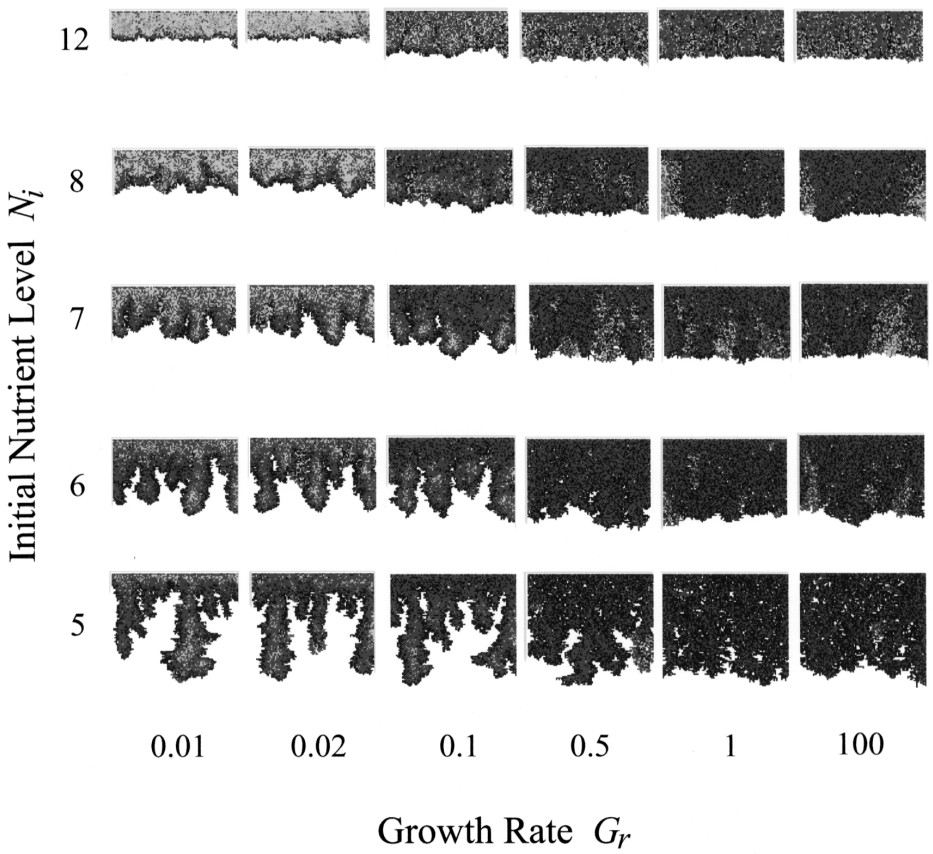

Fig. 1. Morphology diagram of model colonies. Model colonies were grown up to 10000 hyphal units with the fixed nutrient diffusion R_s of 50 steps. Initial nutrient level indicates the number of nutrient particles initially stored in each lattice site. Light color indicates higher mycelial layers.

Figure 2 shows the photographs of *A. nidulans* wild type strain colonies cultivated for 10 days and the mutant strain colonies cultivated for 40 days. Although the wild type strain forms circular colonies under all conditions tested, the size of the colony is slightly larger at lower nutrient levels. There appeared roughening in the colony interface at relatively high nutrient levels. At high nutrient level, hyphae were produced densely inside the colony. However, the colonies ceased to grow with large area of the medium space left unoccupied. This is thought to be due to the accumulation of inhibitory metabolite inside the colonized area. In the previous study on *Aspergillus oryzae* (MATSUURA, 1998), the germination rate at the neighboring sites of the growing colony was found reduced with time. This showed the decaying condition of the medium around the colony. Oppositely, colonies at low nutrient levels expand to cover almost entire medium surface with far less hyphal density.

The growth of mutant strain was remarkably lowered at low nutrient levels, and

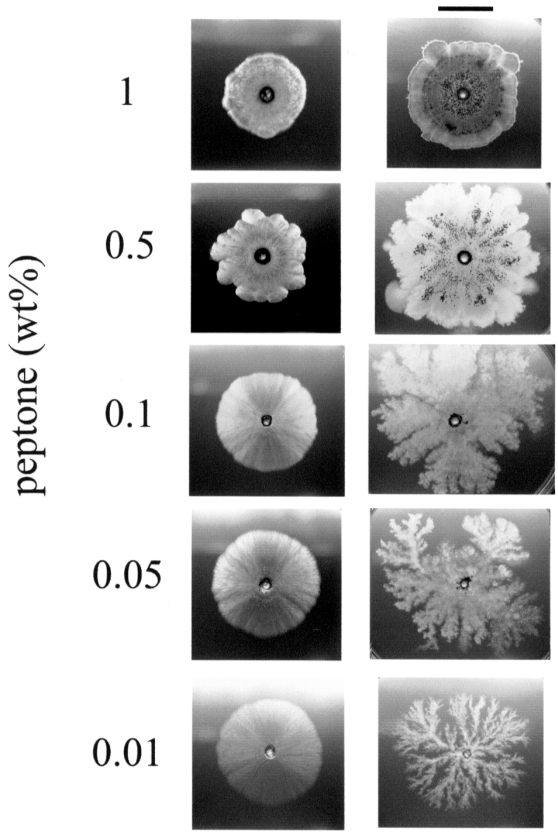

Fig. 2. Colonies of *Aspergillus nidulans* wild and mutant strains. Left row shows the colonies of a wild type strain at various nutrient levels cultivated for 10 days, and right row shows those of a mutant strain cultivated for 40 days. The bar indicates 2 cm.

the colonies exhibited roughened or ramified morphologies at these conditions. With increasing nutrient or agar content, colony shapes became similar to the compact morphology of the wild type strain.

To relate the growth rate of colonies with the nutrient levels, changes of colony sizes estimated from the photograph of colonies were plotted against cultivation days in Figs. 3a and 3b. The colony expansion of the wild strain was found lower as the initial nutrient level was raised. The colonies at high nutrient levels almost stopped expansion within 30 days, while those at low nutrient levels expanded to cover the entire medium surface.

The growth of mutant strain was quite similar to the wild strain at high nutrient levels. However, at low nutrient, the hyphal growth was found suppressed for the first 20 days. After this period, the colony began to expand forming complex shapes.

Let us now apply the model morphology diagram to the real colony patterns. In Fig. 4, the growth rate-nutrient level relations for wild and mutant strains were schematically drown on the morphology diagram of model colony. For the wild strain, the growth is diffusion-limited or nutrient level-limited at low nutrient level since the growth rate is maintained higher. As the nutrient level is raised, the growth rate is lowered probably due to the accumulation of inhibitory metabolites, and then the colony morphology approaches the roughening area in the diagram. Then, further raise of nutrient level brings about reaction-limited colony formation with lowered growth.

For the mutant strain, the growth rate-nutrient level relation is changed into lower growth at low nutrient levels as compared with the wild type strain. Thus, the colony morphology moves toward the ramified region at low nutrient levels as indicated in the figure.

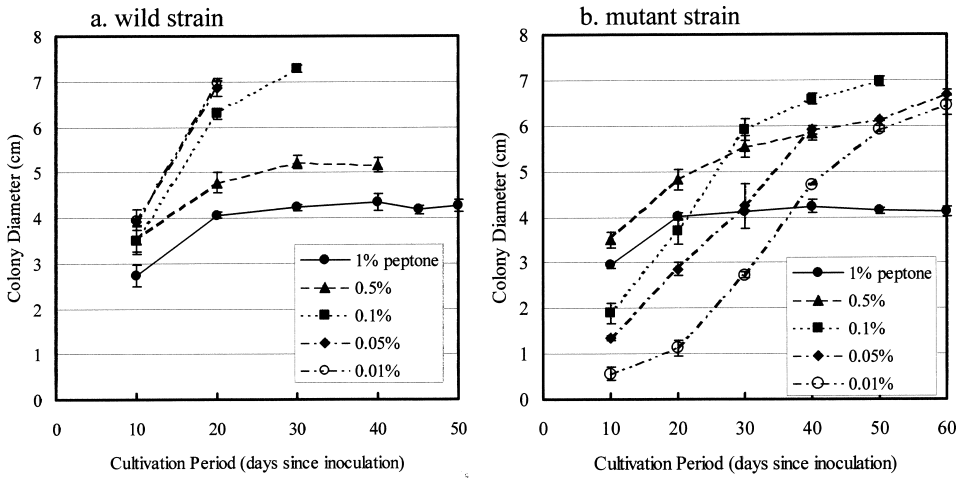

Fig. 3. Colony expansion of *Aspergillus nidulans* wild and mutant strains. Change of colony diameters were plotted against days since inoculation for a, the wild type strain; and b, the mutant strain. Colony diameters were calculated by approximating the colonized area as a circle.

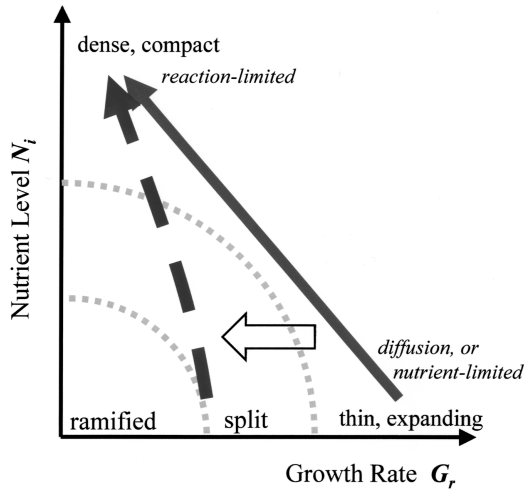

Fig. 4. Morphology diagram of model colony and its application to the real colony patterning. Large arrows schematically indicate the relation between nutrient level and growth rate of the strains. Solid arrow shows the relation for the wild type strain, and broken arrow indicates the mutant strain. Vacant arrow means a morphological transition of colonies at low nutrient levels from wild type to mutant.

5. Concluding Remarks

In this letter, a colony model with three independent control parameters, growth rate, nutrient level, and nutrient diffusion, was proposed to make a basic morphology diagram of mycelial colony. Then, the relation between the colony growth rate and the initial nutrient level was considered for the real fungal strains to apply the diagram. The difference in the colony patterns of wild and mutant strains at low nutrient levels was understood as caused by a change in the above relation.

Measurement of the growth rate-nutrient level relation for the fungal strains is expected useful to understand the diversity in the colony patterning under the change of other environmental factors, such as the stiffness of the substrate. As a future problem, the change of growth rate in the growth processes will have to be considered both in the model and in the measurement of real colonies, though the growth rate was kept constant in the model simulation of this study.

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