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Random Growth of Fungal Colony Model on Diffusive and Non-Diffusive Media

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Abstract. Colonies of filamentous fungi exhibit variety of shapes under various nutrient levels. To understand the generation of various colony patterns, a colony model with single- and multi-mycelial layers that develops consuming limited amount of nutrient is constructed. The external parameters of this model are the initial nutrient level and the diffusion coefficient of nutrient, and the internal parameters are the hyphal growth rate and the nutrient storage level required for hyphal growth. The development of front roughness of the multi-layer colony is found suppressed at high nutrient levels. The nutrient diffusion brings about variation of colony morphology for multi-layer colonies. At very low nutrient levels, ramified colonies with thick branches develop through high nutrient influx.

1. Introduction

Colony patterning of microorganisms has been found to exhibit a striking variety. Particularly, morphological development of bacterial colonies under various environmental conditions has attracted much attention, and extensive challenges have been made to clarify the selection of patterns based on non-linear physics (COHEN *et al*., 1999). There found various significant characteristics that emerged under the change of environmental conditions, such as swarming of populated cells, fixation of cells, secretion of extracelluar materials that enhanced motility of cells, dormation and activation of cells.

Colony formation of filamentous fungi is known to have roles for living strategies, such as the fixation of substrate and the warfare against other microorganisms. The main role of colonization is intensive uptake of nutrient substance and the efficient production of propagules under patchy environment. The growth of fungal colony proceeds simply by extension and branching of filamentous cells called hyphae.

Colony shapes of a fungus *Aspergillus oryzae* formed on soft agar media were found changed from “compact” to “ramified” as the nutrient level was decreased (MATSUURA, 1998). Common tendencies can be seen in the other strains. Figure 1 shows the photographs of a mutant strain of *Aspergillus nidulans* A378 (supplied by the Fungal Genetic Stock

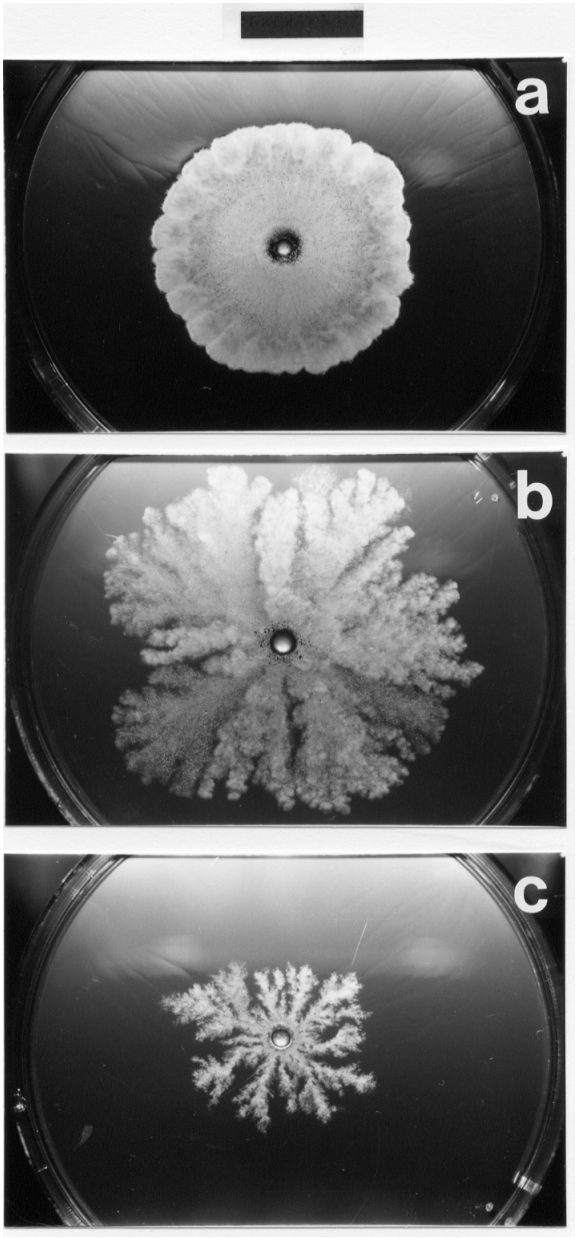

Fig. 1. Colonies of *Aspergillus nidulans* mutant strain A378. Colonies were cultivated on 0.5% agar media at 24°C for 40 days. Peptone (as a nutrient) level is: a, 1%; b, 0.1%; c, 0.01%. The bar indicates 2 cm.

Center, Kansas City, USA) cultivated on the soft agar media of 0.5% agar at various nutrient levels. At high nutrient level, the strain forms thick colony with smooth interface. As the nutrient level was lowered, colonies exhibited complex shapes on the surface of diffusive media.

The medium around the growing colony was found deteriorated as the colony expanded on it. Then, inhomogeneity in the colony interface appeared, and characteristic features of colonies developed. Thus, the self-inhibitory effect may not be ignorable, beside the effect of decreasing nutrient content. J. M. LÓPEZ and JENSEN (1998) presented a model of fungal colony growth that had an inhibitory effect of growth by sedimentation of waste products (LÓPEZ and JENSEN, 1998). The model exhibited a roughening transition of colony interface in the growth process due to the inhibitory effect.

On the other hand, a multi-layered branching model that developed consuming limited amount of resource was presented by MATSUURA (1998), and it was suggested that the balance between hyphal production and nutrient influx affected the morphological selection between compact and ramified shapes.

Targets of this study are to clarify the effects of nutrient level and diffusion on the colony formation based on model colonies. For simplicity, the growth rate and the diffusion coefficient are kept constant in the colony growth processes, and no explicit inhibitory effect on the hyphal production is introduced. Particularly, the growth of front roughness under no nutrient diffusion is analyzed to clarify the effect of nutrient consumption.

2. Model

The filamentous fungi consist of highly branched filaments called hypha. The branching of hypha is in many cases dichotomous. One whole system of hyphae is called mycelium. The hyphal growth is generally most active on the surface of the nutritive substrate. Hyphal extension is generally seen to be straightforward to the outer space on the surface of the substrate. The term “colony” used in this study expresses an assembly of mycelia growing as a group.

2.1. Single-layer no-diffusion model

The colony models in this study are performed on a 2-dimensional square lattice that is employed as the substrate for the model mycelium. As illustrated in Fig. 2a, the model colony consists of hyphal units that represent the unit cells of hyphae. The lattice sites define the positions of both the hyphal units and nutrient particles. The nutrient particles are stored at every lattice site. Each hyphal unit occupies one single lattice point. Each lattice point is supplied with N_i nutrient particles as the initial nutrient levels. Seed hyphal units are placed in a line.

For nutrient absorption, any one of hyphal units is chosen at random. When the lattice site that the chosen unit occupies has at least one nutrient particle, the hyphal unit absorbs one nutrient particle and stores it inside. For the non-consumption model, the number of nutrient particles stored in each lattice site is kept constant, so that the nutrient consumption does not occur in the medium during the process of nutrient uptake by the hyphal units.

When the number of nutrient particles stored within a hyphal unit reaches the amount n_r required for hyphal growth (n_r is called “nutrient requirement”, hereafter), the hyphal

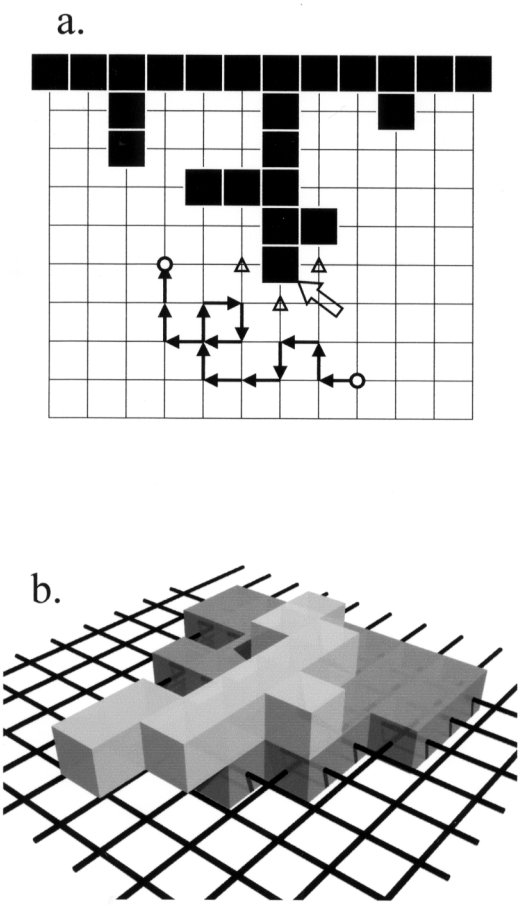

Fig. 2. Schematic illustration of colony model. a) hyphal units created on the 2D lattice space and the nutrient random walk. Filled squares represent hyphal units. Each lattice point is supplied with N_i nutrient particles initially. The large arrow indicates a hyphal unit whose nutrient storage reached the amount of nutrient requirement for growth n_r, and small vacant triangles indicate the lattice sites where the hyphal unit can create a daughter hyphal unit. For nutrient diffusion models, the nutrient particles are released to walk at random. Small circle represents a nutrient particle, and the sequence of small arrows shows the random walk trajectory of the particle on the lattice space. b) an illustration of multi-layer mycelium model created on the lattice space. The dark colored cubes show hyphal units that form the original mycelial layer on the 2D lattice sites. The light colored cubes show hyphal units created on the original layer, and they form the upper mycelial layer. The hyphal units in the upper layer keep creating their daughter units at their neighboring positions in the same layer as far as the unoccupied positions exist.

unit creates new one at a randomly chosen unoccupied neighboring site. Here, for efficiency, the backward site (i.e., the site nearest to the line of seed hyphae) is excluded for the hyphal creation. On the hyphal creation, the parent hypha consumes the stored nutrient for both consumption and non-consumption models. Every hyphal unit can create at most two daughter units, corresponding with the dichotomous branching of real hyphal

growth.

Since the consumption of stored nutrient occurs for any models, capability of hyphal growth decreases transiently just after the creation of daughter cell. This leads to a smoothing effect in the colony front formation. In case there remains no vacant site around a hyphal unit, the unit absorbs and stores up to $1 + n_r$ nutrient particles. In real mycelium, such inactive hyphal units are called storage compartments.

2.2. Multi-layer non-diffusion model

For multi-layer colony model, multi-layer hyphal planes are formed on the 2-dimensional lattice space of the substrate as illustrated in Fig. 2b, although the hyphal growth persists in the same plane as far as the vacant space remains around the growing hyphal units. When the chosen hyphal unit has stored the nutrient requirement n_r and the neighboring sites in the same mycelial plane have been already occupied, the upper or lower positions of the surrounding hyphal units are searched for the unoccupied positions. Then, one of the upper or lower positions (except for backward positions from the position of parent hyphal unit) is randomly chosen to create one daughter hyphal unit. This daughter hyphal unit continues to create new hyphal units in the same layer (upper or lower of the original mycelial plane) as far as the unoccupied positions exist. The resultant colony consists of multi-layer mycelial planes.

2.3. Nutrient diffusion

For nutrient diffusion, nutrient particles are released to make uniform random walk on the 2D-lattice space. First, any one of the lattice sites is randomly chosen. When the chosen site has at least one nutrient particle, a particle is released to move to one of the 4 neighboring sites. The particle repeats the motion R_s times in one sequence of random walk. R_s is then the step length of the random walk trajectory. In the simulation, one sequence of random walk is defined as the unit time of simulation. Thus, the diffusion coefficient of nutrient particles is proportional to the step length R_s of random walk in one unit time. Then, let us define the value of the diffusion coefficient of nutrient particles by the step length R_s.

The growth rate G_r is defined by the number of nutrient uptakes per unit time. When the nutrient random walks are repeated n times before one nutrient uptake, G_r is equal to $1/n$.

3. Results and Discussions

3.1. Growth of front roughness at various nutrient requirement n_r

Single-layer non-diffusion model provides basic cases. Hyphal formation inside the colony does not affect the growth of colony front. After hyphal units fill the interior of the single-layer colony, further nutrient uptake does not occur in the colonized area. Then, the dependence of front growth on the initial nutrient levels does not exist. Still, the parameter n_r introduces an averaging effect on the growth of front sites.

Front roughness is measured by the mean square deviation, $\sigma(L, N)$, of the distance from the line of seed hyphae to the colony front. $\sigma(L, N)$ is a function of hyphal mass N, and of the width of the lattice space L, which is also equal to the length of the line of seed

hyphae. The mean height h of the colony is defined by the mean distance from the seed line to the colony front, and h was found proportional to the hyphal mass N for non-diffusion models.

Generally, for self-affine surface growth, $\sigma(L, N) \sim N^{\beta}$ holds for early growth stage where $h << L$. In the random growth models in which the growth occurs only at the front positions, it is known that σ-value saturates with increasing h due to the limitation of L for the generation of large wavelength roughening (VICSEK, 1992). As the growth proceeds, roughness of growth front develops, and, gradually, lager wavelength roughening appears. In this growth stage, $\sigma(L, N)$ grows up with N. In the saturated range, the value of $\sigma(L, N)$ becomes a function of the scale of lattice width L. Then, if the values of $\sigma(L, N)$ are compared for clusters of various L, $\sigma(L, N) \sim L^{\alpha}$ holds for sufficiently large colony, $h >> L$.

The exponents α and β are called the roughness exponent and the growth exponent, respectively, hereafter. For 2-dimensional self-affine growth, a scaling relationship $\alpha + \alpha/\beta = 2$ is known to be well satisfied (VICSEK, 1992). This relation has been also found satisfied by a roughening transition model with the inhibitory effect by the toxic metabolite shown by LÓPEZ and JENSEN (1998).

Figure 3a shows the growth of front roughness $\sigma(L, N)$ for single-layer non-consumption model with various nutrient requirement n_r. The size of seed line L was $L = 100$. In sufficiently large N region, saturation of front roughness $\sigma(L, N)$ is observed for small n_r cases. The growth exponent β in the early stages is around 0.28 for all cases of n_r tested. However, the value of front roughness $\sigma(L, N)$ is found lowered for higher n_r models.

Also, as seen in Fig. 3b, the same tendency is found for the growth of single-layer consumption model at various nutrient levels N_i. There are little dependences of front roughness upon the initial nutrient levels N_i. Further, for the model with high n_r, development of front roughness $\sigma(L, N)$ is gradually enhanced as the hyphal mass N increases. This tendency is rather opposite to the normal self-affine growth where $\sigma(L, N)$ gradually saturates.

3.2. Growth of multi-layer model

Figure 4 shows a comparison of the growth of $\sigma(L, N)$ at various nutrient levels N_i for single- and multi-layer models with $L = 100$. For single-layer model, the front roughness is independent upon the nutrient levels, and the growth exponent β is $\beta = 0.28 \pm 0.02$ both for consumption and non-consumption cases. However, for multi-layer consumption model, the development of front roughness $\sigma(L, N)$ is remarkably reduced with increasing nutrient levels N_i. In this sense, multi-layer structure is important to consider the real colony formation.

In multi-layer model, hyphal creation continues to occur inside the colony until the nutrient inside is exhausted, so that the extension of colony is slow as seen in Fig. 6. Retardation of development of front roughness might also be related with the slow extension of colonies.

3.3. Roughness exponent α

Figure 5 shows the plots of $\sigma(L, N)$ for various lattice widths L, where N is sufficiently large and n_r is small. In these conditions, value of $\sigma(L, N)$ is saturated. For multi-layer

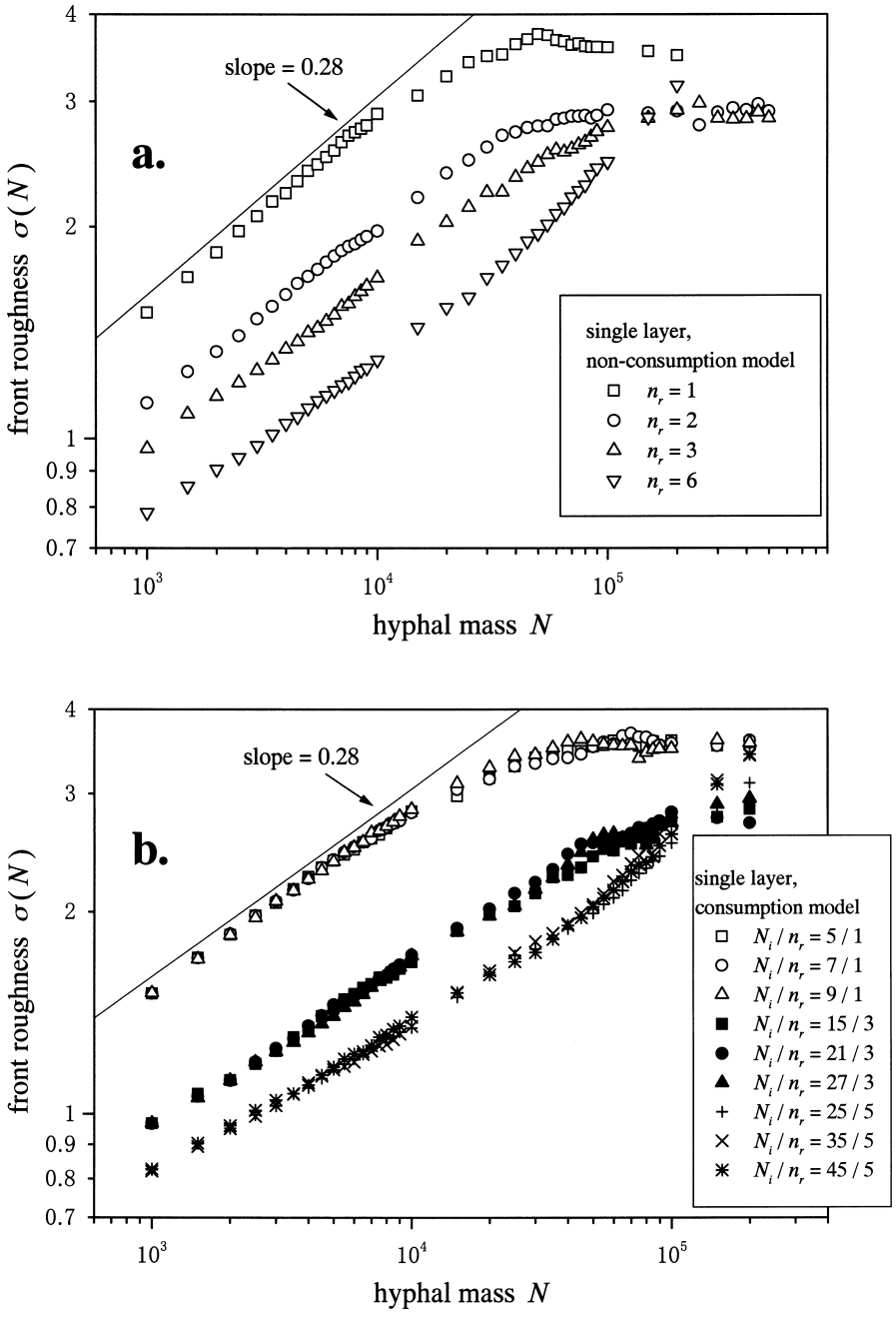

Fig. 3. Growth of front roughness of single layer colonies at various nutrient requirement n_r. a) non-consumption models; b) consumption models at various nutrient levels N_i. Colonies were formed on the lattice space of 100×2100 lattice unit. Plotted points represent values averaged over 200 or 400 realizations. The solid lines indicate $\beta = 0.28$.

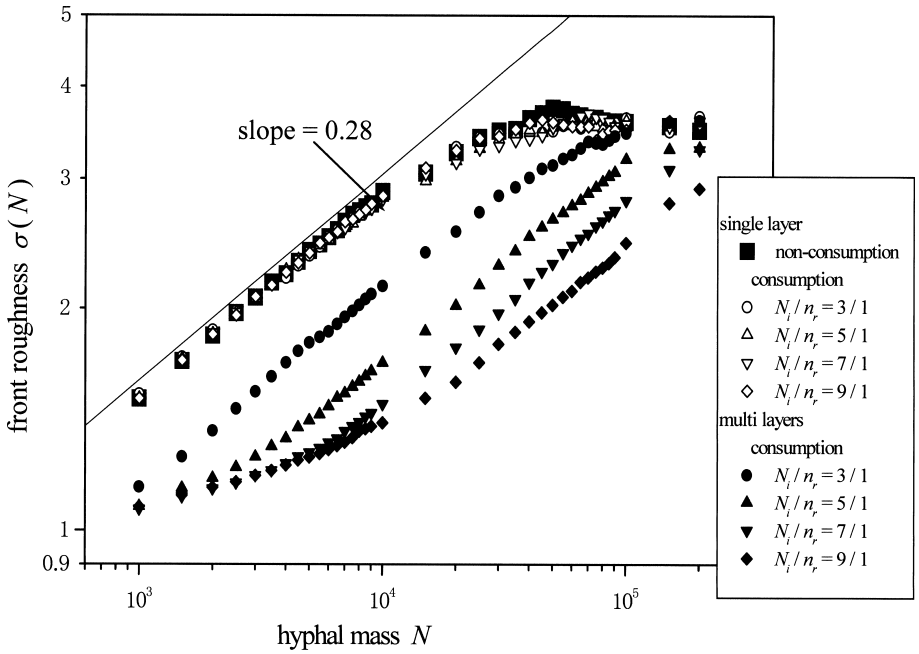

Fig. 4. Growth of front roughness of single- and multi-layer colonies at various nutrient levels. The nutrient requirement was fixed at 1. Colonies were developed on the lattice space of 100×2100 lattice unit. Plotted points represent values averaged over 200 or 400 realizations. The solid line indicates $\beta = 0.28$.

model, only the cases at very low nutrient levels are shown, since the saturation of $\sigma(L, N)$ was not found at high nutrient levels in the range of hyphal mass N tested. At low nutrient, the number of layers does not increase due to nutrient exhaustion inside colonized area, and the colony structure does not significantly deviate from single-layer model.

The values of roughness exponent α for single- and multi-layer models at low nutrient are $\alpha = 0.48 \pm 0.03$. This value is a little lower than 0.5, which is the case for non-correlated random interfaces. Probably a surface effect is brought about by the nutrient exhaustion in the parent hyphal unit at the time of hyphal creation. The set of the growth exponent $\beta = 0.28 \pm 0.02$ and the roughness exponent $\alpha = 0.48 \pm 0.03$ for the single layer model roughly satisfies the scaling relation of $\alpha + \alpha/\beta = 2$.

3.4. Effect of nutrient diffusion

Figure 6 shows the colonies grown at various nutrient levels N_i with various step sizes of nutrient random walk R_s for the fixed growth rate of $G_r = 1$. $R_s = 0$ means the multi-layer non-diffusion cases discussed above, and the colony did not develop in the range $N_i < 6$. Under the existence of nutrient diffusion, colony growth continues even in the range $N_i < 6$ because of the nutrient influx.

With small R_s, nutrients outside penetrate mostly within the interfacial area of colonies, so that rather uniform branching is formed in the interfacial area even in the low

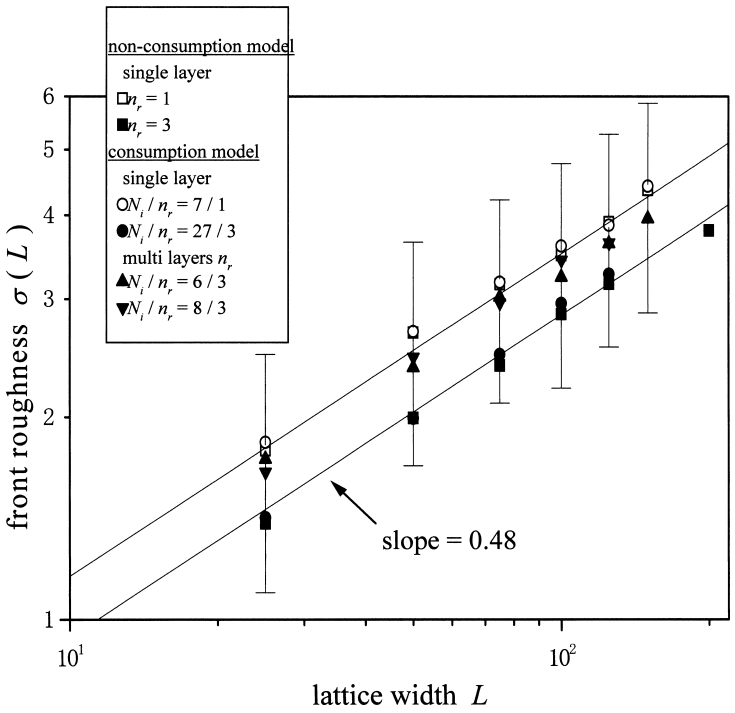

Fig. 5. Front roughness at saturation vs. lattice width. Error bars indicate the standard deviations for 400 realizations of non-consumption single-layer model with n_r = 1. Plotted points represent averaged values over 200 or 400 realizations. For multi-layer model, colonies were developed under low nutrient conditions, in which saturation of front roughness was clearly found. The solid lines indicate α = 0.48.

nutrient range of N_i < 6. With increasing R_s, diffusing nutrients reach deep into the colonized area to maintain the hyphal production inside the area, and the growth inside becomes more reaction-limited. The resultant colonies become small and condensed due to high nutrient influx. The nutrient diffusion seems to enhance the growth of the advanced portions in the colony front. Thus, the diffusion of nutrient into the colonized area leads to complexity in the colony patterning particularly at low nutrient conditions.

4. Concluding Remarks

In this study, a simplified colony growth model was presented to consider the effect of nutrient level and nutrient diffusion for the development of complex colony shapes. As the nutrient requirement n_r is raised, the roughness of colony front was found lowered. For multi-layer mycelial colonies, the development of front roughness was found significantly suppressed as the nutrient level in the substrate was raised.

The variety of patterns developed under the effect of nutrient diffusion at low nutrient levels. With low nutrient diffusion, uniformly ramified colonies appeared at very low nutrient levels. As the diffusion coefficient was raised, hyphal creation inside the colony

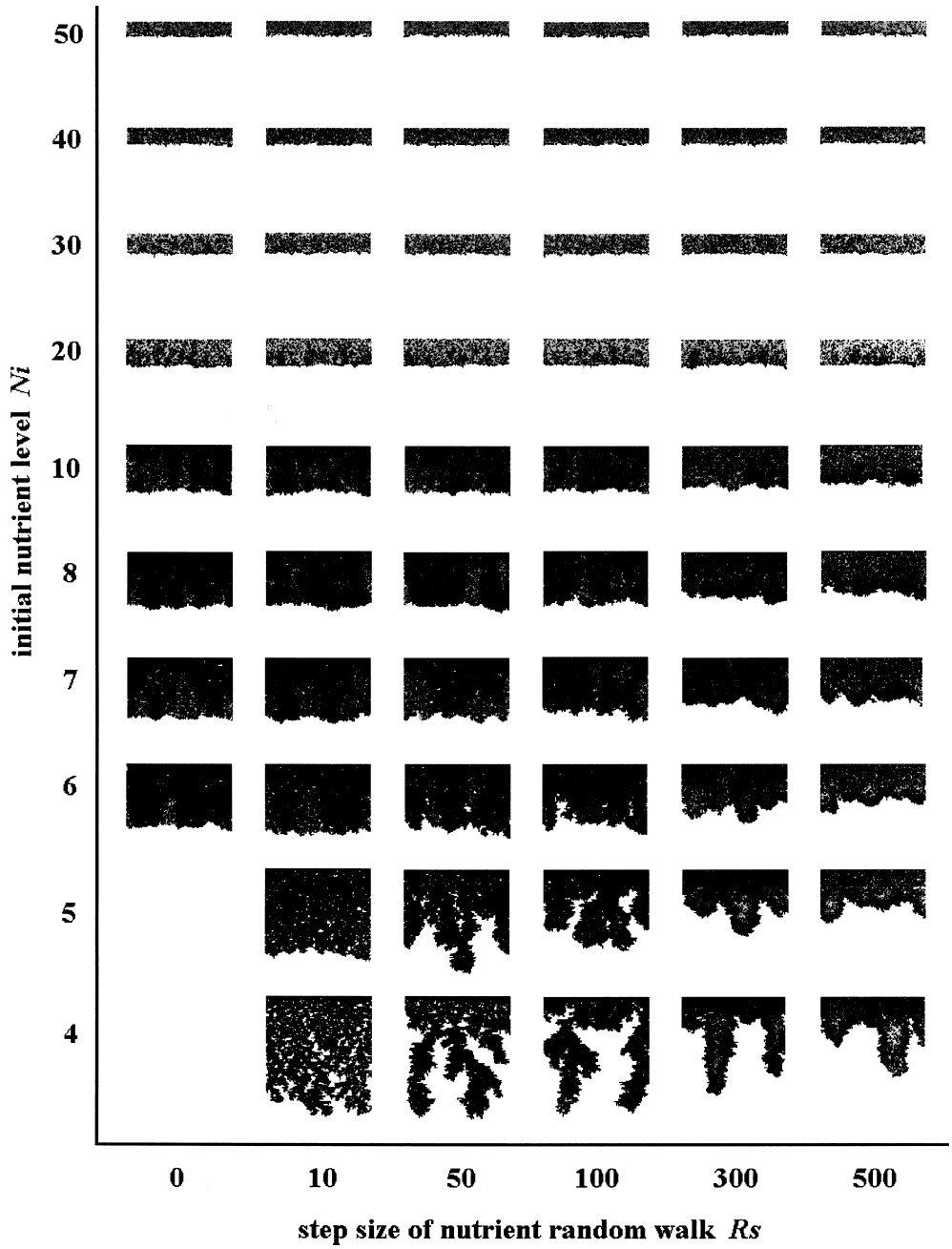

Fig. 6. Model colonies at various nutrient levels and nutrient diffusion. Number of hyphal units included in each colony is 1×10^4. The lattice width is 100. The growth rate G_r is fixed at $G_r = 1$. Light colors represent higher mycelial layers.

became frequent and densely aggregated inhomogeneous colony developed.

Although these results agree with the experimental observations, dependence of patterning on the hyphal growth rate has to be considered. Further, in the real colonies, sedimentation of inhibitory metabolites or cell aging is thought to bring about decay of hyphal growth rate in the growth process. As a future problem, the change of the growth rate should be considered as a factor for the further pattern development, in addition to the effect of nutrient diffusion.

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