Colony patterning of *Aspergillus oryzae* on agar media

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To clarify the effects of nutrient concentration and diffusion on the pattern formation of fungal colonies, the colony patterning of *Aspergillus oryzae* at various nutrient and agar levels was studied experimentally and was summarized in a colony morphology diagram. Roles of the nutrient content and the relaxation of nutrient distribution on the colony patterning were discussed based on a computer model of the mycelial growth. The colony morphology changed from compact to ramified as the nutrient and agar levels were lowered. No clear boundary was found between these two morphologies. The deterioration of substrate around the growing colony was detected when the morphic switching from homogeneous into splitting patterns emerged in the growth of ramified colonies. In the mycelial growth model, dense compact colonies developed at low growth rates and high nutrient influx into the colonized area. Under low nutrient levels, splitting colonies appeared at high growth rates as compared with the nutrient influx.

Key Words——colony model; colony morphology; morphology diagram; nutrient diffusion.

The profiles, colors, and textures of fungal colonies show astonishing diversity depending on the species and environmental conditions. However, we can expect there to be an underlying universality in patterning, since hyphal growth is mainly subject to the physical processes of environmental factors.

In particular, the diffusion of nutrient substances toward the mycelium must be one of the main factors influencing hyphal growth at the microscopic level and the colony pattern formation at the macroscopic level. Further, neighboring hyphal systems in the colony are externally connected through competitive nutrient uptake. The dominant hyphal systems fan out branches into fresh space to absorb nutrients predominantly for further hyphal production, so that the growth of neighboring systems is suppressed under low nutrient conditions.

The fungal colony shows striking similarities to such non-living random growth phenomena as diffusion-limited aggregation, Eden growth, and deposition. (Vicsek, 1992). Understanding of the fundamental nature of patterning will contribute to the analysis of the diversity in growth manner and, especially, to a better understanding of the positive nature of hyphal growth.

This paper explores colony patterning of a single strain of *Aspergillus oryzae* on Czapek-Dox agar media at various glucose and agar levels. First, the interactive nature of colony growth that led to asymmetrical growth from circular inoculation was described. Second, deterioration of the substrate in the colonization process was considered, based on a spore germination experiment. Third, colony morphology of the strain was presented under two environmental variables: nutrient concentration and agar concentration. Changes of these variables induced transformation of colony patterns from compact to ramified shapes.

Fourth, the diversity in the colony patterning was considered based on a computer model of mycelium that developed on a 2-dimensional square lattice by consuming a limited amount of diffusing nutrient. Construction of a simple model was a useful approach to extract the essential characteristics of the growth systems. It was suggested that two parameters, namely, the nutrient level and the frequency ratio of the nutrient absorption of hyphae to the nutrient diffusion in the substrate, were important for the pattern generation. The latter parameter characterized the growth rate of mycelium under a given rate of nutrient influx into the colonized area. The overall morphology was controlled by these parameters. To conclude, the positive nature of colony patterning and its switching for spatial exploration were suggested.

Materials and Methods

The strain used in this study was *A. oryzae* IFO 5239 (supplied by the Institute for Fermentation Osaka, Japan). It was cultivated at 24°C on Czapek-Dox agar medium containing $NaNO_3$ (0.3%), K_2HPO_4 (0.1%), $MgSO_4 \cdot 7H_2O$ (0.05%), KCl (0.05%), $FeSO_4 \cdot 7H_2O$ (0.001%), with the glucose content ranging from 0.01 to 5%. The stiffness of the medium was controlled by the content of Difco Bacto-agar ranging from 0.15 to 5%. Line inoculation experiments were performed in 9-cm Petri plates containing 20 ml of sterile medium, and circular inoculation experiments in 15-cm plates containing 75 ml of medium. Spores of the strain were collected directly from the colonies cultivated for about 3 wk on malt extract-agar medium containing Difco Malt-extract (2%), Difco Neopeptone (0.1%), glucose (2%) and Difco Bacto-agar (1.5%). They were washed and rinsed three times. Plates were inoculated by drawing lines on the

medium surface with a sterile string dipped in spore suspension of 10^6 conidia/ml.

The spatial distribution of hyphal systems under competitive growth was well exhibited when spores were inoculated circularly on a nutrient-poor medium. Spore suspension of *A. oryzae* was inoculated along circles of various diam from 6 to 13 cm on the Czapek-Dox medium with 0.01% glucose and 0.3% agar in 15-cm Petri plates.

For normal cultivation, spores were inoculated along a line on the medium surface to develop a band-shaped colony. A line of spore suspension was drawn with sterile string along a ruler consisting of a thin plate of sterile glass inserted into the agar medium. The glass plate was 65 or 55 mm in length in most cases, and it was removed after inoculation. For soft media with 0.15% agar, the spore suspension was inoculated without the glass ruler since the substrate could not support the ruler. 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.

Deterioration of the substrate due to colony growth was detected indirectly by measuring the spore germination rate at fixed sites on the medium surface. First, line-inoculated colonies were pre-cultivated overnight, or for 2, 3, 4, or 5 d on media with 0.01% glucose at various agar levels. Second, drops of spore suspension containing 10^3 spores (per drop) were inoculated at 1 cm and 4 cm from the original inoculation line. The test spores were collected from the whole area of colonies cultivated on the malt extract-agar medium with 1% glucose for about 3 wk. Third, after overnight incubation, the numbers of germinated spores and total number of spores were counted at each site under an optical microscope. The criterion of germination was the presence of a germ tube larger than the spore diam.

The morphic response of the ramifying colony in the early stages of cultivation was characterized by a change in the distribution of the active growth sites in the colony front. To demonstrate the localization of active sites, a colony was cultivated under 0.02% glucose and 0.4% agar. Then an agar bar containing 2% glucose was placed parallel to the growing colony at a distance less than 1 cm from the colony front. During overnight cultivation, the colony absorbs nutrient diffusing from the agar bar, and the front hyphae that retain the capacity to grow further are re-excited and fan out new branches. This early growth response visualizes the distribution of most active growth sites.

Model

The computer model of the mycelium was based on a 2-dimensional square lattice of $1,000 \times 300$ lattice constant. The hyphal unit was represented by a bar of 3 lattice constant that occupied the lattice space. Two opposite ends of the hyphal unit were distinguished as the base and the apex. The whole 2D lattice area was divided into small square compartments of 10×10 lattice con-

stant that initially contained an equivalent amount of nutrient particles. These are called storage compartments hereafter. Hyphal growth proceeds with the consumption of the nutrient particles stored within the storage compartments.

In order to introduce a relaxation mechanism of the nutrient distribution, nutrient particles were allowed to diffuse from one storage compartment to another without being captured by the hyphae. First, a lattice site was chosen randomly, and if the storage compartment that included the chosen site had at least one nutrient particle, a particle was released to move at random on the lattice. The total number of steps for one sequence of random motion was determined by a random natural number less than 300. Then, if the nutrient stock of the storage compartment at which the particle finally arrived was less than that of the compartment where the particle was released, one nutrient particle was allocated from the initial compartment to the final one.

The frequency ratio of the nutrient uptake of the hyphal unit to the random walk of nutrient is an important parameter. It affects the balance between the nutrient influx and exhaustion inside the colonized space. (At present, the term 'colonized' is not precisely defined. It roughly indicates the area that the colony covers, even when the colony ramifies and leaves a large vacant space inside.) The relative growth rate was defined as the number of nutrient uptake processes per nutrient launching, in order to relate the growth rate with the nutrient relaxation in the medium.

For the hyphal growth, any one of the hyphal units on the lattice space was randomly chosen. Then, the hypha was supplied with one nutrient particle from the storage compartment in which the chosen hypha lay, as far as the compartment had stock nutrients.

When a hypha accumulated 5 nutrient particles, it created one new branch as far as vacant space remained around it. No intersection was allowed for the hyphal texture in one lattice plane. At any time of branch creation, the parent hypha consumed 5 nutrient particles from the storage in the hypha.

A hyphal unit was allowed to make at most 3 daughter branches from its apex on one 2D lattice plane. Further, to let the hyphae branch out 3-dimensionally, multi-layered lattice planes were introduced. When the neighboring lattice sites were fully occupied, the hypha chose a higher or a lower plane of lattice at random, and it tried to find a vacant direction to create a branch. Thus, a hypha can make $3 + 4 \times 2$ branches at most. The multi-layered lattice planes simply define the locations of hyphae, and share the same storage compartments.

The maximum nutrient storage of a hypha was 6 nutrient particles. After accumulating 6 particles, the hypha ceased absorbing. In this model, hyphal growth was limited by both overcrowding and the exhaustion of nutrient in its storage compartment of the medium.

Results and Discussion

1. Change of growth polarity in the circular colony Formation of the hyphal systems under the competitive growth mode was well exhibited when spores were inoculated circularly as shown in Figs. 1a–f. Under conditions of 0.01% glucose and 0.3% agar, *A. oryzae* strain formed ramified colonies, in which branched sub-colonies appeared after a homogeneous growth period.

In the present experiments, the inoculation diam d ranged from 13 to 6 cm in the 15-cm Petri plates. At $d=d_c=10$ cm, the areas of the inner circle and of the outer concentric domain were equivalent. For the inoculation circle of $d>d_c$, the inner domain was larger in area than the outside. Correspondingly, the branch colonies extended toward the center of the circle as seen in Figs. 1a, b. Conversely for $d<d_c$, the branch colonies were predominantly directed toward the edge of the plate in the larger outer space. A few branches extending toward the center were found to cease growing.

At the critical radius of $d=d_c$, the colonies extended both to the center and to the edge of the plate as seen in Fig. 1c. Thus, the switching of the polarity occurred at this critical condition. Further, hyphae appeared to be produced more to the outer side than inside the inoculation circle.

Let us consider a "free volume" in which each branch colony extends. When a branch colony maintains a free volume widening to the outside, it will keep widening its branch systems toward the fresh space, since the front hyphae can receive nutrients diffusing into the free volume from the wider area. However, when the colony extends from the inoculation circle to the center, the shape of the free volume of a branch colony may taper due to the converging extension of neighboring branch colonies. Thus, the sub-colonies competitively interact with each other through nutrient uptake and metabolite accumulation, and, the whole colony appears to be avoiding overcrowding of sub-colonies.

Several branched colonies were observed to initiate from the early homogeneous growth phase at a site opposite a polarized colony. Thus, the deterioration of substrate around the inoculation circle is thought to exceed the condition for creation of a dominant branch colony, so that the active portions of colonies cannot escape from the battlefield. The colonies have less possibility of
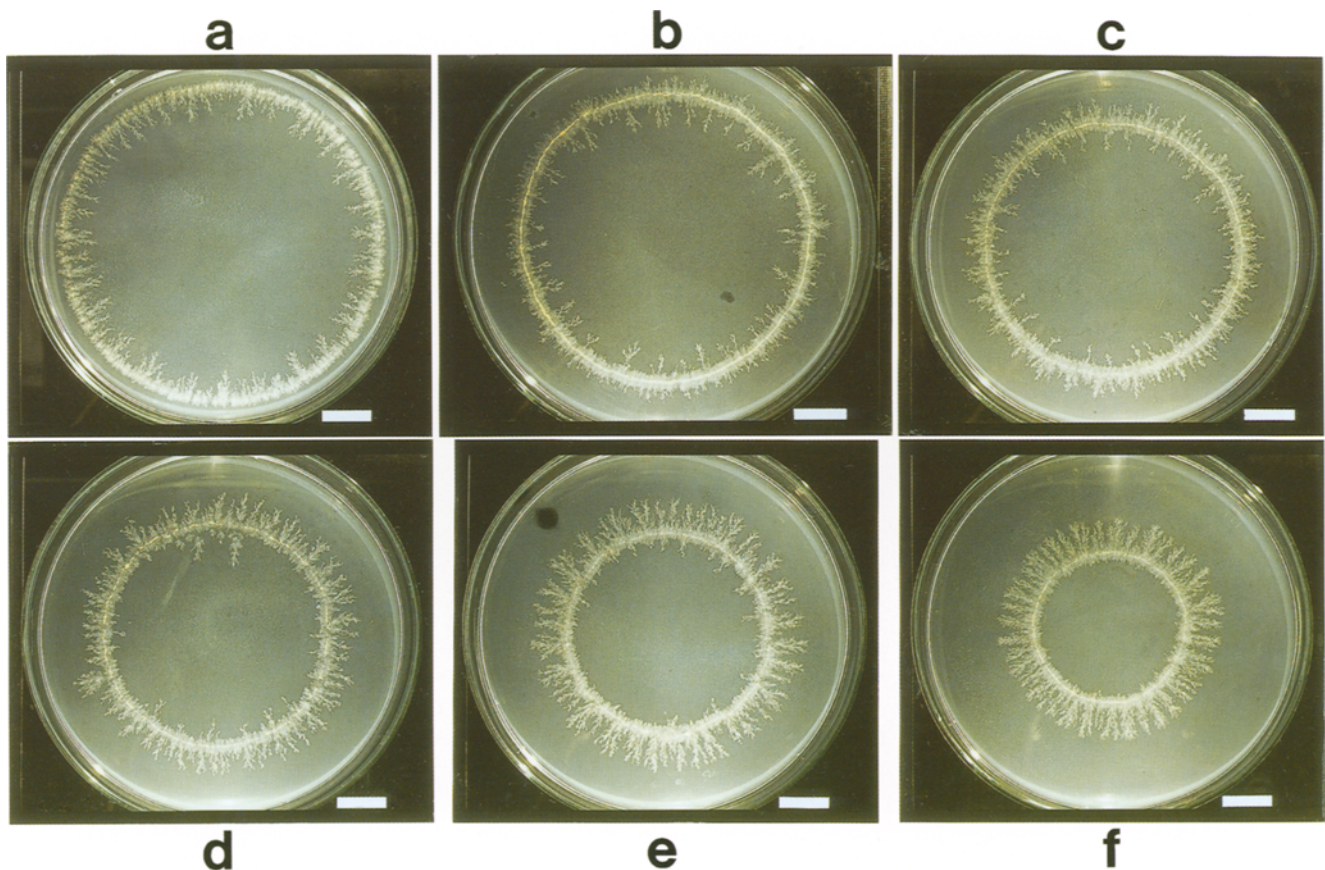

Fig. 1. *Aspergillus oryzae* colonies developed from circularly inoculated spores.
Radii of the inoculation circles are: a, 13 cm; b, 11 cm; c, 10 cm; d, 9 cm; e, 8 cm; and f, 6 cm. In c, the areas of the inner circle and of the outer concentric domain are almost equivalent. The strain was cultivated in 15-cm Petri plates at 24°C. The cultivation periods are 22 d for e and 20 d for the others. The medium was Czapek-Dox with 0.01% glucose and 0.3% agar. The white bars indicate 2 cm.

getting nutrient in the case of convergent growth.

The conditions for demonstrating the above phenomena may be quite specific to the strains. However, these reveal the underlying scenario that takes place in the course of gradual environmental change led by the competitive colony growth. Proliferation changes the environmental conditions, and the manners of response change accordingly.

2. Temporal change of substrate condition Colony growth induces the deterioration of substrate in the space explored by the growing colony. To detect the effect of colony growth on the surrounding substrate, the germination rate was measured at two fixed positions of

the plate in which a linear colony was cultivated. The test spores were inoculated after various cultivation periods of the growing colony. Figures 2a–d show the changes of overnight germination rates at the test sites 1 cm and 4 cm from the inoculation line of the growing colony.

The germination rate at 1 cm dropped rapidly within 5 d of colony expansion at each agar level. Within this period, the colonies did not reach 1 cm from the inoculation line. The germination rates at 4 cm did not show significant decrease in this time period. Thus, the colony affected its neighboring region from the early homogeneous growth stage.
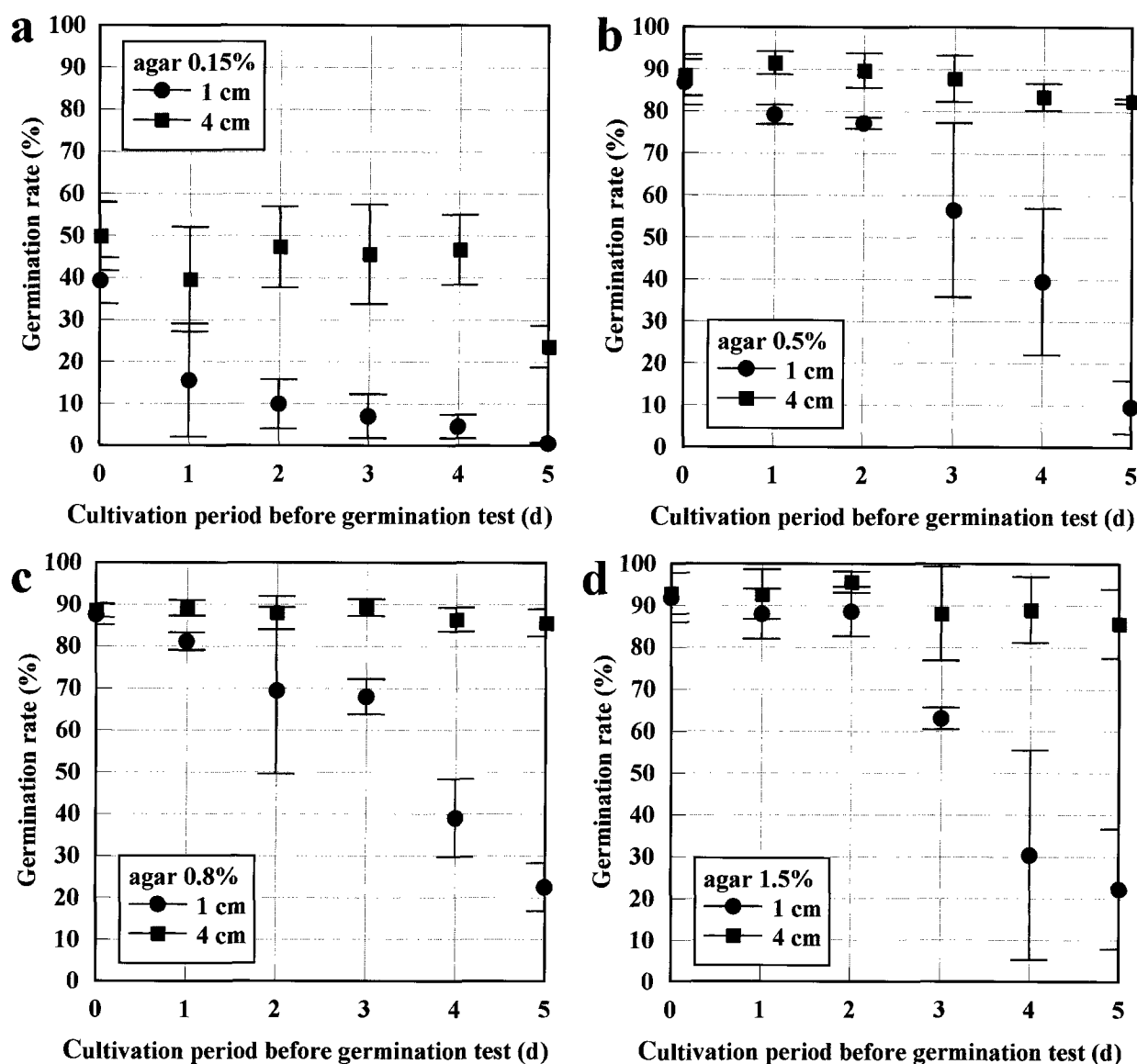

Fig. 2. Overnight germination rates at fixed positions of 1 cm and 4 cm from the inoculation line of the growing colony. The germination tests were carried out on the media with 0.01% glucose and various agar levels: a, 0.15%; b, 0.5%; c, 0.8%; and d, 1.5%. The horizontal axes show the periods of pre-cultivation of band-shaped colonies. After the pre-cultivation, test spores were inoculated at 1 cm (●) and 4 cm (■) from the inoculation line of pre-cultivated colonies. Error bars indicate the standard deviations of 4 or 6 samples.

At 0.15% agar, the medium was fluid-like, and test spores were suspended near the surface of the medium. Spores did not sink into the medium when the agar content was higher than 0.8%.

Figure 3 shows the overnight germination rates at various agar levels with no glucose and no any other colony cultivated previously. Here, test spores were inoculated at two sites in each plate with the distance 3 cm, to compare the germination rates with those in the presence of a growing colony. The germination rate decreased with decreasing agar content. Further, in the range of agar content less than 1%, the germination rates were far smaller than those for the media with 0.01% glucose at the sites of 4 cm from the colonies shown in Fig. 2. This indicates that the nutrient uptake of the growing colony leads to the decrease of germination rate near the colony front.

The above results suggest that the colony explores the space that has already deteriorated due to the growth of the colony itself. In this situation, a few strong hyphal systems branch out and occupy the front area to form ramified colonies in the long cultivation period.

3. Colony morphology of *Aspergillus oryzae* Although colony features are peculiar to the fungal species, they are highly dependent upon the medium conditions. Sensitivity to the environment will characterize the nature of strains. The variation of patterning processes under various conditions will determine the growth strategy of the strain. Below, two questions are addressed: How do colony shapes change with respect to the substrate conditions? Does any phase transition-like discontinuity ap-

pear in the morphology diagram?

The initial glucose concentration was taken as the first parameter in the morphology diagram. The second
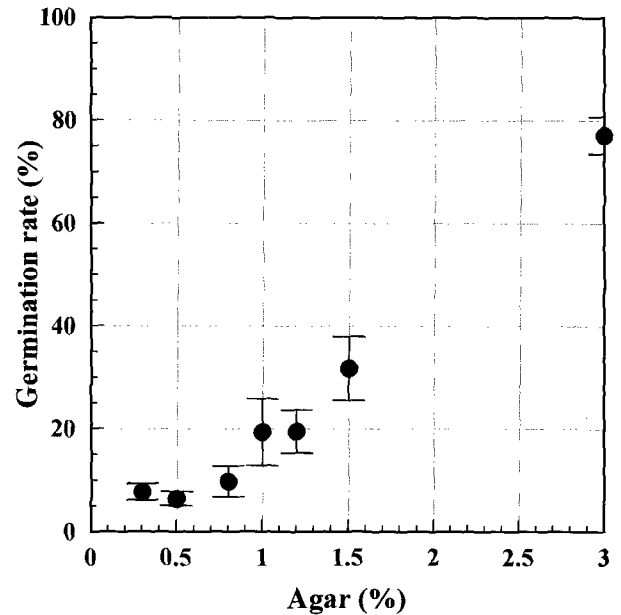

Fig. 3. Overnight germination rates on media with various agar levels and no glucose.
Test spores were inoculated on fresh glucose-free media at the same positions as the cases shown in Fig. 2. Error bars indicate the standard deviations of from 7 to 24 samples.
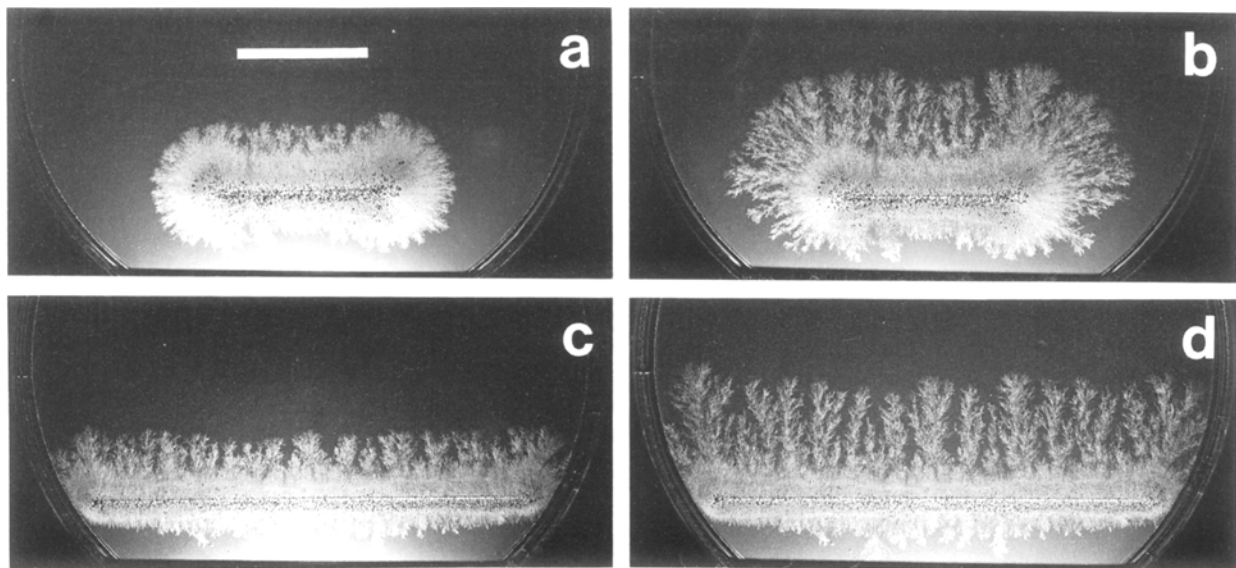

Fig. 4. Edge effect of line inoculation on colony formation.
Aspergillus oryzae colonies cultivated on the Czapek-Dox medium with 0.01% glucose and 0.5% agar at 24°C. Short colonies a and b are those developed from 24 mm inoculation lines, and long colonies c and d are from 65 mm lines. Photos a and c were taken at 10 d after inoculation; photos b and d show the same colonies after another 10 d. The bar in a indicates the scale of 2 cm. As the colonies from short line inocula developed, colony branches from both sides of the line extended radially and dominated the branches in the middle region. Dominance of edge branches was not apparent in the long inoculation line, since the space for growth of edge branches was nearly the same as the space for middle branches.

parameter was the agar concentration, which controlled the substrate stiffness. Low agar content induced a decrease in the germination rate, as seen in Fig. 3. The colony expansion rate was restricted on the soft media (Matsuura and Miyazima, 1993). Changing these two environmental variables was expected to reveal the underlying growth manners and strategies.

An edge effect of the line inoculation method was noted. Hyphae developing from the ends of an inoculation line usually extended faster and fanned out more densely than those from the middle of the line. Figure 4a–d show a comparison of colonies from the inoculation lines of 24 and 65 mm in length. A distinct edge effect was seen in the short line case. Hyphae at the edge branched out sideways more than those in the middle of the colony band. The edge branches appeared to interfere with the growth of branch colonies in the middle. This indicates that the pattern reflects a strong competitive interaction between neighboring colonies.

Figure 5 summarizes the colony morphology of the test strain, and Fig. 6 shows the photographs of real colonies in the region where colonies show interesting patterns. A compact morphology was evident in the high nutrient range, and a ramified morphology in the range of low nutrient and low substrate stiffness. Between these extremes could be seen a splitting morphology, in which colonies split or changed their shape from uniform to ramified as they colonized the space. About 140 positions were examined in the diagram. Many of the test positions were within the range of glucose content less than 0.1% and agar less than 0.8%. In this range, many of the patterns were intermediate between uniform and split (or ramified).
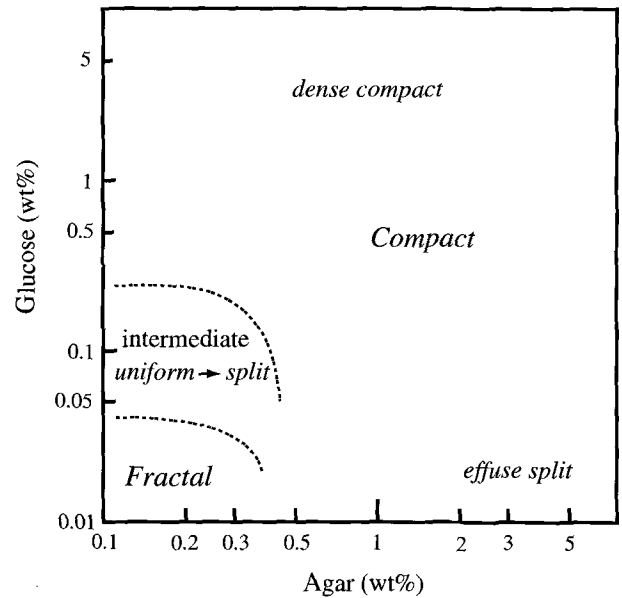

Fig. 5. Morphology diagram of *A. oryzae* strain at various glucose and agar concentrations.

Colony shapes were observed on the Czapek-Dox medium with various glucose and agar contents. No clear boundaries were found between compact and fractal shapes. In the intermediate morphology region, the morphic switching with time from uniform to splitting colony was distinctive. Photos of real colonies are shown in Fig. 6 as examples of morphologies.
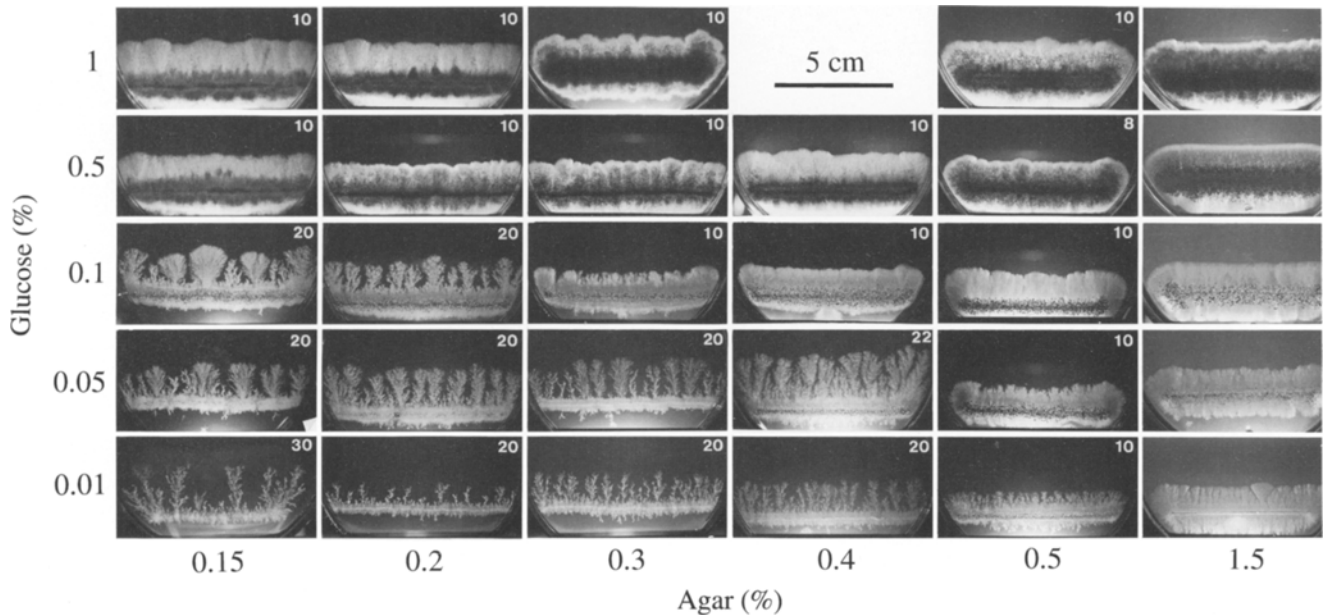

Fig. 6. Photographs of *A. oryzae* colonies from compact to fractal morphologies.
Numbers in the photos indicate the cultivation periods. The length of the inoculation line varies, but was mostly 65 or 55 mm. Free-hand inoculations are also included.

These morphological regions showed no clear boundaries. The change from one region to the other seemed to be almost continuous. Rather, from time to time, sample-to-sample deviations often appeared in the splitting morphology region, together with the morphic switching in the branch patterns. These morphological regions are discussed below.

3.1 Compact morphology Compact morphology indicates that the colony maintains a continuous (nonsplitting) growth front. A wide range of nutrient concentration allows hyphal systems of this strain to fan out uniformly. The excess nutrient storage and the high production of metabolites are thought to induce frequent sporulation (Cooke and Whipps, 1993).

A whole colony consists of arrayed sub-colonies that are trying to expand their territory. On a solid substrate with 1.5% agar, the colony front changed its shape depending upon the initial nutrient level (Matsuura and Miyazima, 1994). At 0.01% glucose, the colony showed a nearly splitting morphology, i.e., the sub-colonies growing side by side appeared to avoid overlapping at their interfaces.

With increasing nutrient level up to 0.5%, splitting in the colony front disappeared. Here, it may be possible to consider the state of colony formation to be well-balanced. First, a sufficient amount of nutrient sustains the extension of interfacial hyphae. Second, however, the production of vegetative hyphae is not yet sufficient to induce an inhibition effect, and the growth mode remains uniform. In this balanced state at 0.5% glucose and 1.5% agar, the colony front remained extremely flat in shape until the colony covered half the medium.

At nutrient levels higher than 0.5%, colonies are characterized by the rich production of propagules and aerial hyphae, and the roughening of colony fronts. The diffusion of nutrient into the colonized area also enhances the production of interior hyphae. Excessive nutrient is utilized to produce dense propagules and dominant hyphal systems at the colony front.

3.2 Ramified morphology Ramification of sub-colonies was distinctive at low nutrient (≤ 0.05 wt% glucose) and low agar levels (≤ 0.5wt% agar). At higher nutrient or higher agar conditions, sub-colonies spread wider, and colonies became thick. However, there appeared to be no clear divide between the formation of the ramified colony and that of the compact one in the colony diagram. Basically, such a discrete switch might be quite difficult to identify, since the colony growth in the plate is a far from being an equilibrium process. The morphic change of individual colony with time should be noticed, rather than with the initial conditions.

Figure 7 shows the reactivation of the colony front by addition of the nutrient source. In the photographs, the inoculation lines lie in the middle of the band-shaped colony, where the hyphae spread uniformly. Colonies in the same rows are identical.
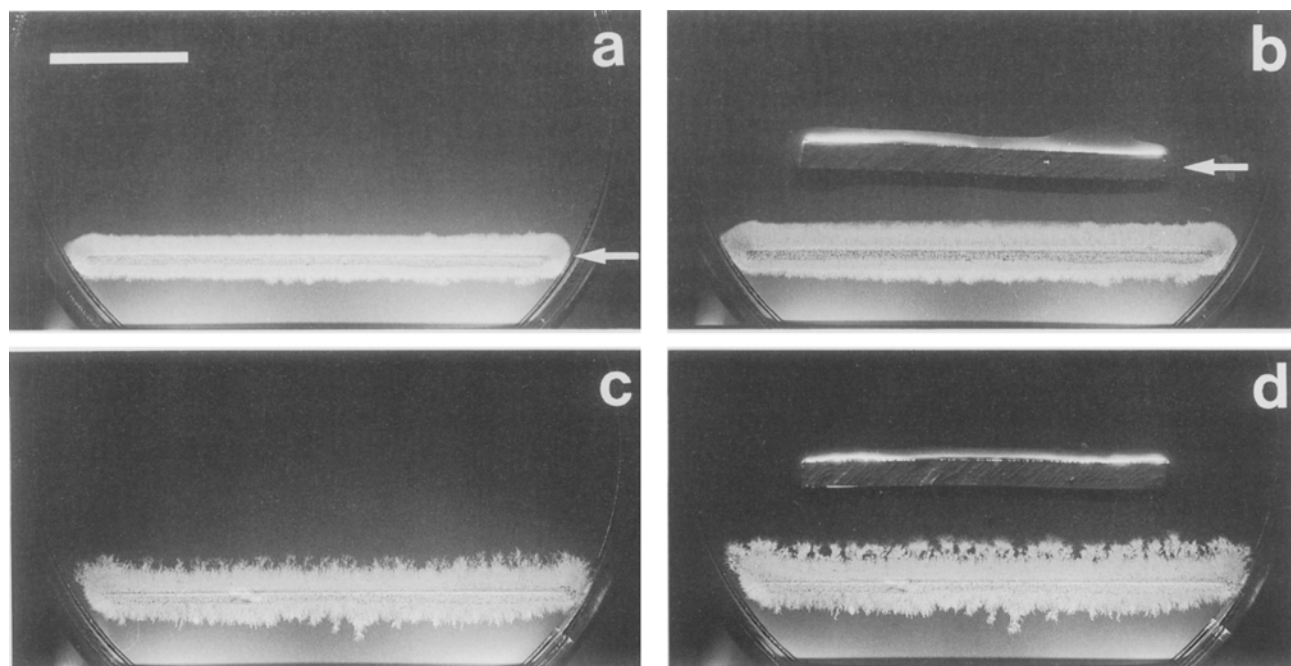

Fig. 7. Localization of active growth sites at the colony front.
   Colonies were cultivated on the Czapek-Dox agar medium with 0.02 wt% glucose and 0.4 wt% agar. Photographs at left show the colonies cultivated for 3 d for a, 6 d for c. The arrow in a indicates the inoculation line where the thin glass ruler was inserted. After taking the photographs at left, agar bars containing 2 wt% glucose were placed parallel to the linear colonies at a distance less than 1 cm (indicated by an arrow in b). Photographs at right show the colonies cultivated for 24 h after the addition of agar bars. The colonies a and b, and c and d are the same colonies, respectively. In d, colony branches extended into the fresh space and formed thick hyphae at the apical portions.

Mycelial growth was quite uniform within the first 3 d. As shown in Fig. 7b, the hyphae were uniformly created in the front line after addition of the nutrient source. This shows that the growth potential was sustained uniformly in the front hyphae. As seen in Fig. 2, the germination rate near the colony front remained high in this period. Growth in this period can be called uniform.

At around 6 d, a few dominant hyphae appeared at the front as seen in Fig. 7c. After addition of the nutrient block, potential growth sites were localized at the apical portions, where the hyphae were created densely as shown in Fig. 7d. Up to this stage, conflict among dominant hyphal systems was still not effective.

Then, dominant sub-colonies fanned out toward the fresh space. Less dominant ones ceased advancing. By fanning out at the peripheral area, hyphae occupied the "free space" earlier than others. As the nutrient around the colony is consumed, a few active hyphal systems absorb and utilize the limited resource exclusively. Growth in this stage can be called the splitting mode.

As ramification emerged, dominant sub-colonies proceeded to enlarge their territory. Gradually, selection of growth sites occurred under the deteriorating conditions. A few surviving branches were singled out, and the whole shape of the individual sub-colony became linear.

Production of exploratory branches that can escape from plundered territory might need the pre-exploitation and energy storage at some level. Also, if a hyphal system fails to create exploratory branches under the deteriorated environment, it sometimes has to abandon further advancement.

3.3 Intermediate morphology Between the compact and ramified morphology regions, there lay a broad intermediate morphology. It was found that the uniform growth mode switched into the splitting mode at a certain stage. The uniform colony region became larger as the agar concentration was raised. On the stiff substrate with more than 1.5% agar, the hyphae maintained effusing growth, forming a uniform colony. On the other hand, many of the splitting colonies were dense and aggregated.

While the hyphal systems in the uniform mode showed similar morphology, the splitting colonies were seen to have a variety of types ranging from dense ramified to sparse effusing. As the depletion of resource proceeds around the colony, the latent competition among hyphal systems turns into a creative competition for exploratory colony growth. This creative competition induces morphic switching or change of the growth scenario into positive adaptation to the deteriorated environment.

4. Colony model Figures 8 and 9 show the model colonies grown from seed hyphae placed at the top of lattice space. The colony growth process ends with total exhaustion of nutrient. Those colonies shown in the figures are the final ones after nutrient exhaustion. The main parameters discussed in this study are the initial amount of nutrient and the relative growth rate. The lat-

ter value indicates the frequency ratio of the nutrient uptake by the hyphae to the nutrient launch in the lattice space. It characterizes the growth rate at a given condition of nutrient relaxation in the substrate. This growth rate is called RGR hereafter.

Figure 8 shows the colonies developed under a high nutrient level at various RGR. Although the colonies were compact, their shapes and extensions depended strongly on the RGR values. As RGR was decreased, the colony became compact and exhibited a flat front. Since the nutrient particles flowed into the colonized area frequently, the hyphal units were created densely inside the colony.

The 3-dimensional structure also depended on RGR. The thickness of the colony was defined by the number of mycelial layers. At low RGR, the thickest areas appeared in the basal zone of the colony (i.e., near the start line where the seed hyphae were located). Then, as RGR was raised, the thick regions shifted from the basal zone to the peripheral zone. These results indicate that the profile of colonies strongly reflects the colony expansion rate. When the hyphal extension is slow, and the nutrient outside keeps diffusing deep into the colonized area, the interior hyphae are able to continue to proliferate. As the growth rate increases, the hyphae in the peripheral zone capture the nutrient influx and form thick layers.

As RGR was raised further, the colony rapidly explored the medium space with low hyphal density, and exploited the colonized area until the exploiting hyphae depleted the resource remaining or supplied afterward.

Figure 9 shows the colonies under a low nutrient level. At low RGR, the colony again exhibited compact shapes. This may correspond to a highly protective mode of colony formation under poor resource conditions. At high RGR, the colony became localized from the early growth stage, and only a few sub-colonies explored outer space. During the development of this ramified type colony, the flow of nutrient into the colonized area was quite small and the nutrient was always depleted from the area. The hyphae that could actively proliferate were restricted to the apical region of the branched sub-colonies.

The total amount of hyphae created increased with RGR as shown in Fig. 10. Conversely, the amount of nutrient stored within mycelium decreased at higher RGR values as shown in Fig. 11. As the hyphae grew at lower rate in a constant diffusion field, the colony stored more nutrient with less hyphal production. Further, the distribution of nutrient stocks within the colony was also interesting. At low RGR, most of the hyphae had maximum nutrient storage except for those at the edge of the colony. Thus, the compact morphology appeared to be related with the less production with high storage. At high RGR, the interfacial hyphae mainly proliferated and explored the fresh space as a result. In this case, the stock distribution was rather inhomogeneous, and the amount of nutrient storage of the individual hyphae did not reach its maximum in most cases.

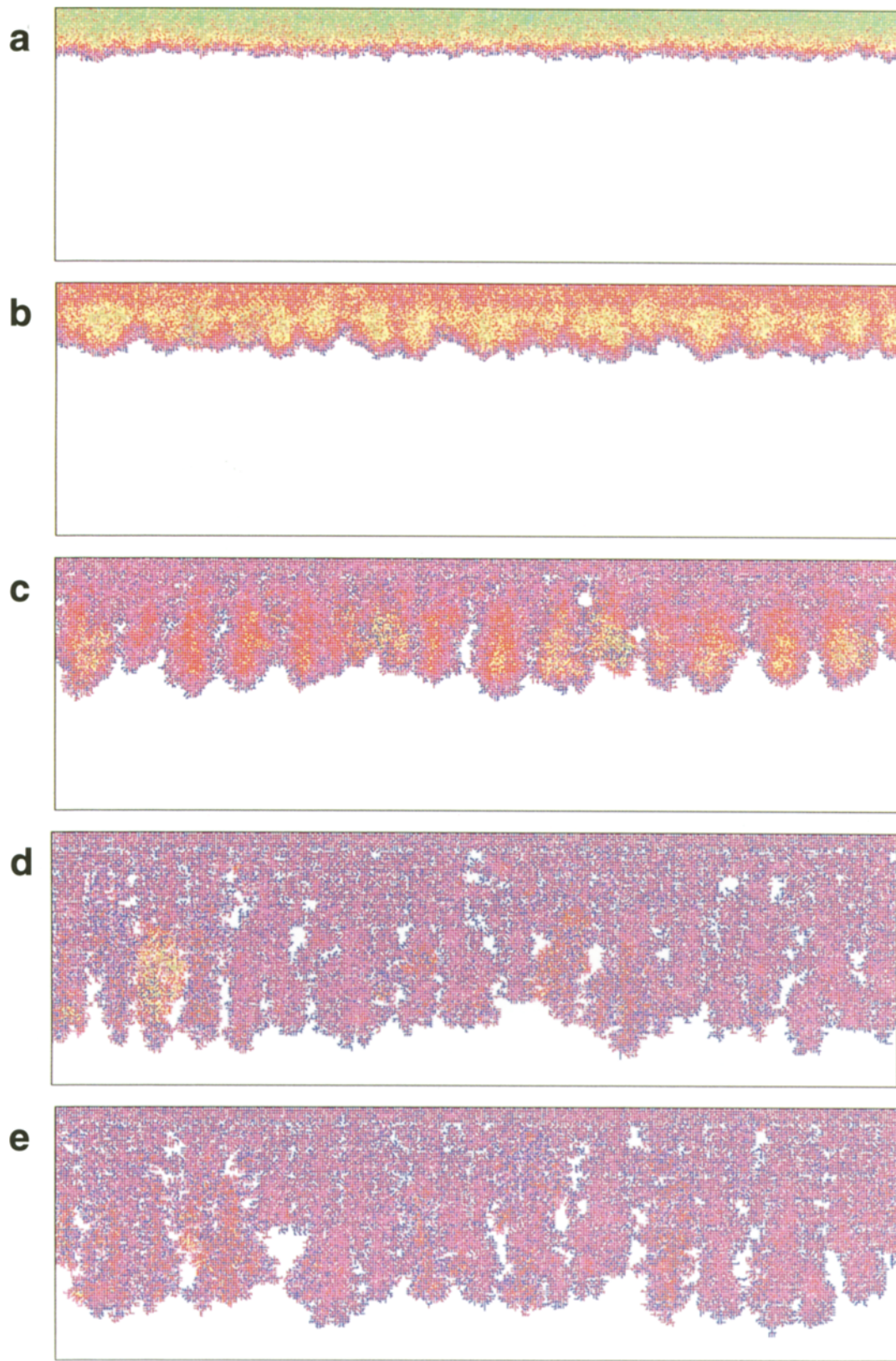Let us compare the models with the real colonies

Fig. 8. Model colonies generated at the initial nutrient level of 6×10^5.
The values of relative growth rate are: a, 0.02; b, 0.05; c, 0.2; d, 1; and e, 10. Colors identify the planes of mycelial layers. The horizontal length of the colonies is 1,000 lattice constant.
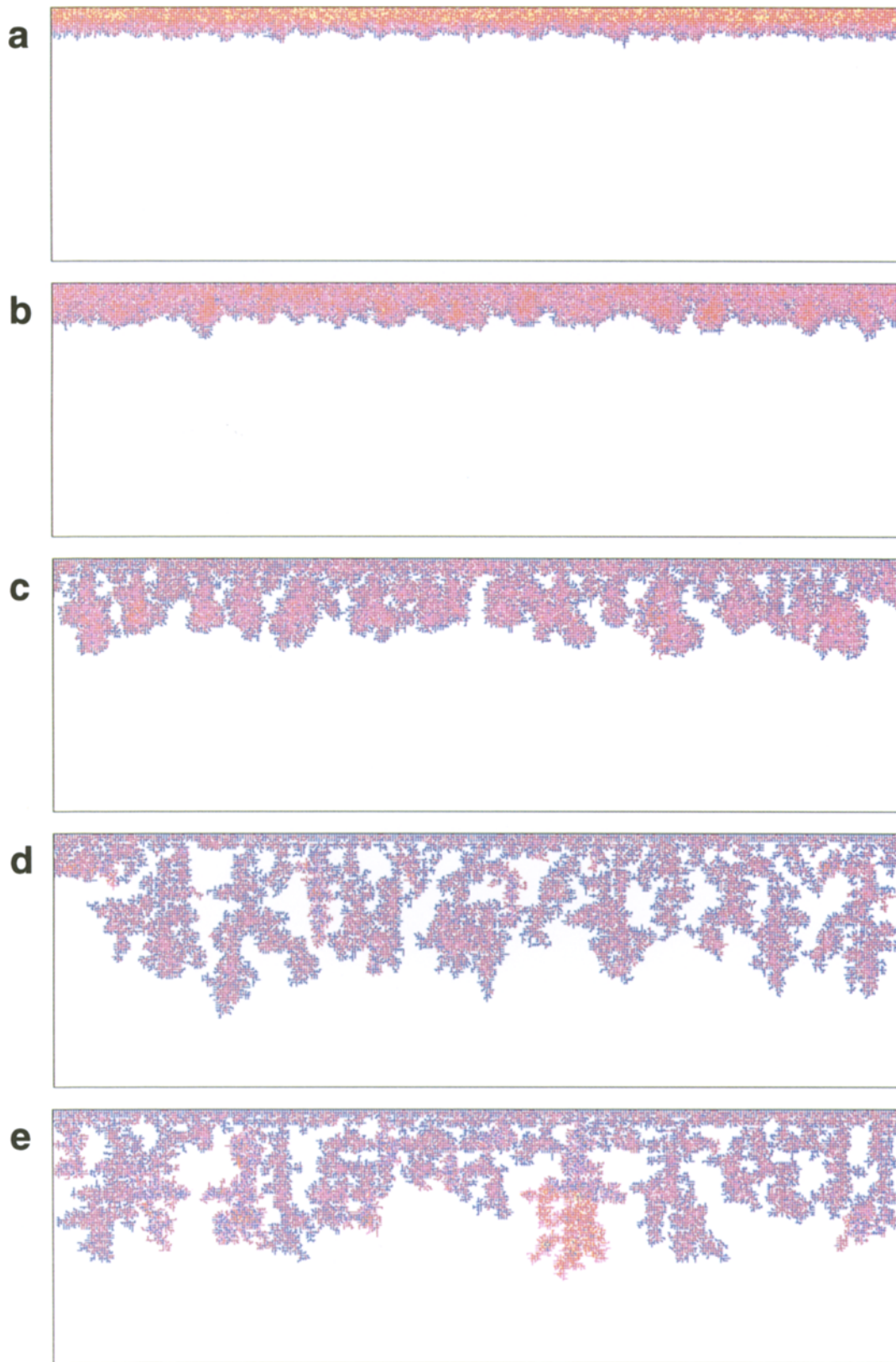
Fig. 9.   Model colonies generated at the initial nutrient level of 2×10^5.
   The values of relative growth rate are: a, 0.02; b, 0.05; c, 0.2; d, 1; and e, 10.   Colors identify the planes of mycelial layers in the same way as in Fig. 8.
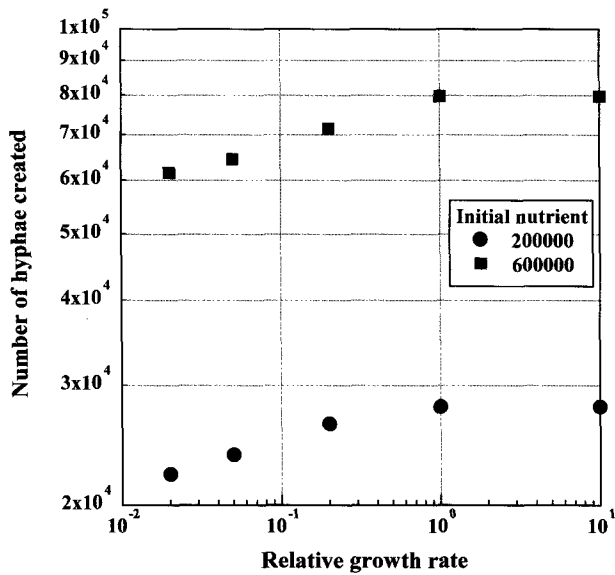
Fig. 10. Total number of hyphal units created until nutrient exhaustion.

The number of hyphae are plotted for each relative growth rate (RGR). The initial nutrient levels are 2×10^5 (●) and 6×10^5 (■). Plotted points are the averaged values over 100 trials. The range of the standard deviations is far smaller than the size of plot marks. The hyphal production increases with RGR, as the colony morphology changes from dense compact at low RGR to open (or effusing) at high RGR.
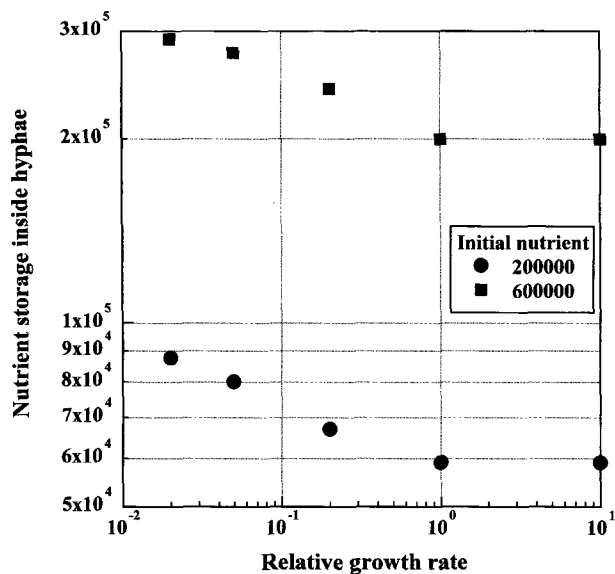

Fig. 11. Total amount of nutrient stored within model colonies until the depletion of nutrient in the substrate.

The amount of stored nutrient is plotted against relative growth rate. The initial amounts of nutrient particles in the substrate are 2×10^5 (●) and 6×10^5 (■). The nutrient storage decreased as the relative growth rate was raised. The dense compact colony stored larger amount of nutrient.

shown in Fig. 6. A thick region appeared in the older area of the compact colonies grown at high nutrient levels. This corresponds with the low RGR model with high nutrient levels. Many of the colonies of intermediate morphology showed the dense hyphae in their peripheral regions. The higher RGR model shows the thick area near the periphery. This implies that the space exploration is preferred and a high hyphal growth rate is sustained under relatively adverse conditions. The ramified colonies of this strain at low nutrient levels and low agar levels indicate positive exploratory growth, similar to model colonies at high RGR with low nutrient. The model suggests that this strain prefers to adopt high growth rates under severe environmental conditions.

In order to construct more realistic models, it will be necessary to consider the nutrient allocation within hyphal systems and the inactivation of hyphal reproduction due to senescence. The mechanisms of nutrient storage and the way nutrient is reused in an adverse environment are of great importance.

5. Concluding remarks It was found that the competitive growth of sub-colonies led to the asymmetric development of whole colony. Colony growth may induce substrate deterioration from the early stage of growth. Within this period, the homogeneous growth mode switches into splitting growth mode at low nutrient levels with low agar concentrations. The colony patterning has been summarized in a morphology diagram. To understand the morphic changes from compact to ramified, a simple colony model was constructed with the parameters of the initial nutrient level and the relative growth rate. These two parameters were found to control the morphic change of the colonies.

Colony patterning was found to change with time depending on the changing conditions of the environment. Various patterns are stimulated as a result of changes in environmental factors. Generally, if the colony front faces a deteriorated substrate, it gives up uniform spreading, which might be costly. Instead, the branch systems that are efficient in exploring for fresh resources develop and dominate the interfacial area. Also, when the colony expands at extremely low speed, the diffusive influx of nutrient becomes a limiting factor for the hyphal production. Then, the growth probabilities of front hyphae remain uniform provided the nutrient influx is uniform, and the competition among hyphal systems is less apparent.

This study focused the role of diffusing nutrient related with the rate of hyphal production. This simple aspect will be useful to understand overall tendency of colony patterning. The combination of the innate growth regulation and the diffusion properties will determine the common tendency in patterning. However, the living mycelium is not a simple passive system toward external phenomena. In particular, under adverse conditions, the colonies show various intriguing responses in their pattern formations. Detailed features of branch patterns are thought to represent a rich variety of space exploration strategies.

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