Enhancement of L(+)-Lactic Acid Production Using Mycelial Flocs of *Rhizopus oryzae*

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Abstract: L(+)-Lactic acid production was enhanced in the culture of *Rhizopus oryzae* using mycelial flocs formed by addition of 3 g/L mineral support and 5 ppm polyethylene oxide. By addition of the mineral support, an electrostatic repulsion between mycelia increased by 3.5-fold compared to that of mycelia, which allowed a dispersed growth of *R. oryzae* in the early growth phase. In conventional culture the morphology of *R. oryzae* is that of a pellet-like cake, however, when support and polyethylene oxide are added to the culture, the morphology of *R. oryzae* takes on a cotton-like appearance. The formation of these cotton-like mycelial flocs was induced by the addition of 5 ppm polyethylene oxide into a 14 h culture containing the mineral support before the formation of the conventional pellet morphology. The cotton-like flocs were also formed in cultures grown in a fermentor. This morphology allowed effective mass transfer inside the flocs and effective fluidity of culture broth in the reactor. L(+)-Lactic acid concentration produced by mycelial flocs in fermentor, with the support and polyethylene oxide, was 103.6 g/L with the yield of 0.86 using 120 g/L of glucose as the substrate for this cultures without both, the concentration was 65.2 g/L. It demonstrates that cotton-like mycelial flocs are the optimal morphology in the culture of *R. oryzae*. *Biotechnol Bioeng* **55**: 461–470, 1997.
Keywords: mycelial flocs; mineral support; polyethylene oxide; L(+)-lactic acid; *Rhizopus oryzae*

INTRODUCTION

Many important industrial processes utilize submerged fermentation of molds. The products of these fermentations are of high economic value, e.g., antibiotics, alcohols, and organic acids. Variation of the fungal morphology encountered in these processes is widespread within and between species. Morphologies from discrete filaments of hyphae to pellets of highly entangled hyphal mass are found.

In bioreactors, the mycelial growth typical of filamentous fungi results in the formation of pellets or in highly viscous mycelial suspension (Gerin et al., 1993). The pellets are composed of highly interwoven hyphae and are generally spherical in shape. Such pellets are subject to mass transfer limitations which produce solute gradients through the spheres. Mycelium at the center of the pellet become nutrient limited as the pellet increases in size; growth is eventually continued in a shell of limited thickness at the surface of the pellet (Pirt, 1967). In cases of filaments and pellets, mass transfer limitations are commonly encountered and growth of the mycelium on the impeller or on the electrodes hampers optimal control of the process.

There has been much progress in the study of mold molecular biology in recent years but the advancements in culture techniques of mold has proceeded at a slow pace. The reason for this is that it is difficult to control the morphology of the mold which will lead to a high reactor performance. Phenomenon of flocculation may be one of methods to culture mycelia (Mill, 1964). The other method may be a dispersion of mycelia in the culture broth. We reported that the mineral support dispersed mycelial pellets in the culture broth and emulsified soybean oil used as a carbon source in *Streptomyces* culture, which made possible to enhance cephamycin C production (Ichida et al., 1996). The mineral support is a bundle of fibers, 30–50 μm in length and 0.2 μm in diameter which have lots of rectangular tunnels of 10×3.6 Å. Silica dioxide and magnesium oxide constituted approximately 53% and 23%, respectively, of the total components. The chemical formula for the support is $(OH_2)_4(OH)_4Mg_8Si_{12}O_{30}6 \sim 8H_2O$. Average particle size and porosity of the support was 16 meshes and 70% respectively. The support enhanced an emulsion of soybean oil-water in the culture broth and dispersion of mycelia pellets, which led to improve both oil consumption and antibiotic production. The dispersed growth of *Streptomyces* is particularly difficult to achieve in conventional culture methods using chemically defined or a complicate media.

In this study, we tried to control the mold morphology in a manner suitable for culture on a reactor scale. To investigate this phenomenon, *Rhizopus oryzae,* a potential L(+)-lactic acid producer, was chosen as a model strain. The morphology of the *R. oryzae* was character-

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ized by the formation of big pellet-like cake using conventional culture methods such as a shake flask or reactor. The L(+)-lactic acid produced by the *R. oryzae* can be polymerized to form polylactic acid, a polymer used in the manufacture of new biodegradable plastics (Vert et al., 1992). It could play an increasing role in industry in the near future because it is environmentally friendly (Cooke, 1990), and could be considered as a substitute for plastics manufactured from petroleum derivatives. However, little resarch about the morphology of *R. oryzae* has been reported, despite its industrial importance. This might be due to problems in controlling morphology that would prevent it from being suitable in cultivation. This article reports on the suitable morphology for production of L(+)-lactic acid by *R. oryzae,* and the effects of mineral support and flocculant on the formation of morphology.

MATERIALS AND METHODS

Strain and Medium

The microorganism used was *Rhizopus oryzae* NRRL 395 (Dr. J. L. Swezey, Northern Regional Research Center, USDA, Peoria, Illinois). This mold is known to directly ferment starch to L(+)-lactic acid (Hang, 1989). Cultures were maintained and spores produced on potato dextrose agar slant. The composition (g/L) of fermentation medium used in this study consisted of 120 glucose, 3.02 $(NH_4)_2SO_4$, 0.25 $MgSO_4 \cdot 7H_2O$, 0.04 $ZnSO_4 \cdot 7H_2O$, 0.15 KH_2PO_4. To avoid pH decrease due to the lactic acid production in the reactor, concentrated $CaCO_3$ solution was added intermittently.

Cultivations

Spore solution of 10^7 spores/mL was inoculated into 500 mL Erlenmeyer flasks containing 100 mL of the culture broth in flask cultivation. All flasks were incubated at 30°C shaker with an agitation rate of 150 rpm for 60 h. After 24 h cultivation, 4 g $CaCO_3$ was added to each flask.

For the production of lactic acid, 10% of the spore solution of 10^7 spores/mL was inoculated into a 3 L jar-fermentor (MDL 300, Marubishi Bioeng. Co., Tokyo) containing 1.5 L medium. The culture temperature was maintained at 35°C throughout the experiments. In order to maintain dissolved oxygen concentration at 3–5 ppm, aeration rates were varied from 0.5 to 1.0 vvm at fixed agitation rate of 200 rpm. After culture time of 24 h, to avoid pH decrease, 150 mL of 40% $CaCO_3$ solution was added to the jar-fermentor. The cultivation in the jar-fermentor was carred out for 64 h.

Preparation of Mineral Support

Mineral support, Aid-Plus (ML-50D, Mizusawa Chemical CO., Niigata, Japan) was used in this study. Before use, in order to avoid an interruption of mineral ions contained in the support on the *R. oryzae* culture, the support was rinsed with a 0.1 *M* phosphate buffer (pH 7.0). After rinsing, the support was suspended with distilled water until pH of the water decreased to 7.0. After preparation, the rinsed support was dried at 60°C for 24 h and a defined amount of the support was added to the medium before being autoclaved.

Morphological Control with Mineral Support and Flocculant

Morphological change was investigated by adding the mineral support and flocculant in the culture. The additive effect of the mineral support was tested with addition of various concentrations from 0 to 10 g/L at the initial culture. To investigate the effect of flocculants on morphology, four different kinds of flocculants such as sodium tripolyphosphate, sodium hexametaphosphate, polyethylene oxide, and sodium pyrophosphate were tested. The tested concentrations were varied from 0 to 10 ppm. The optimal concentration was added to the culture at a time before the formation of the mycelial cake, 12–14 h culture. All culture times were 64 h.

Zeta Potential

Zeta potentials of the support, mycelium, and mycelial pellet containing the support and mycelium were measured using Laser Zee Meter (Model 501, Penkem, Inc., Bedford Hills, NY). Samples were taken and diluted with various pH-buffers and measured. Its measuring principle is similar to that of an electrophoresis. When a constant direct current is applying, the particle moves to the other side of its electric charge. It is because a particle in a solution charges with electricity. The movement (V) is represented as follows:

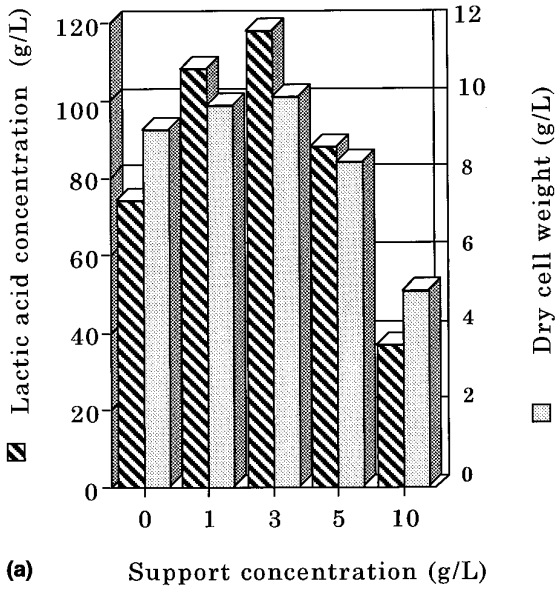
$$V = \epsilon \cdot E \cdot \zeta/(4\pi \cdot \eta) \quad (1)$$

where E, ϵ, ζ, and η denote an electric field strength, dielectric constant, zeta potential, and viscosity, respectively. Therefore, the zeta potential is calculated as follows:

$$\zeta = V \cdot \eta \cdot 4\pi/(\epsilon \cdot E) \quad (2)$$

Analytical Methods

Cell concentration was represented as dry cell weight. Samples were filtered through a weighed ashless filter paper (Whatman No. 4) which were washed with water and dried to constant weight at 80°C. The total biomass was calculated from the weight measurements. The biomass including the support was also determined in a similar way. Preweighed support weight was subtracted



from the weight of biomass and support, and then the biomass concentration was determined.

The concentration of lactic acid produced was measured based on the Baker-Summerson method (Baker and Summerson, 1941). A diluted sample was added to 1 mL of 20% $CuSO_4$ solution, and water was added to a total volume of 10 mL. Approximately 1 g of $Ca(OH)_2$ was added, and shaken vigorously. After sitting at room temperature for 30 min, the mixture was centrifuged. The 1 mL supernatant was transferred to a test tube containing 0.05 mL of 3% $CuSO_4$ solution, and the tube was chilled in ice. Five milliliters of concentrated H_2SO_4 was added slowly, while the contents of the tube was mixed. The tubes were then placed in a boiling water bath for 5 min and cooled to room temperature. After two drops of the *p*-hydroxydiphenyl solution were added to the test tube, the tubes were placed at 30°C for 30 min. Colorimetric readings were taken at 560 nm and lactate concentration was calculated using a calibration curve.

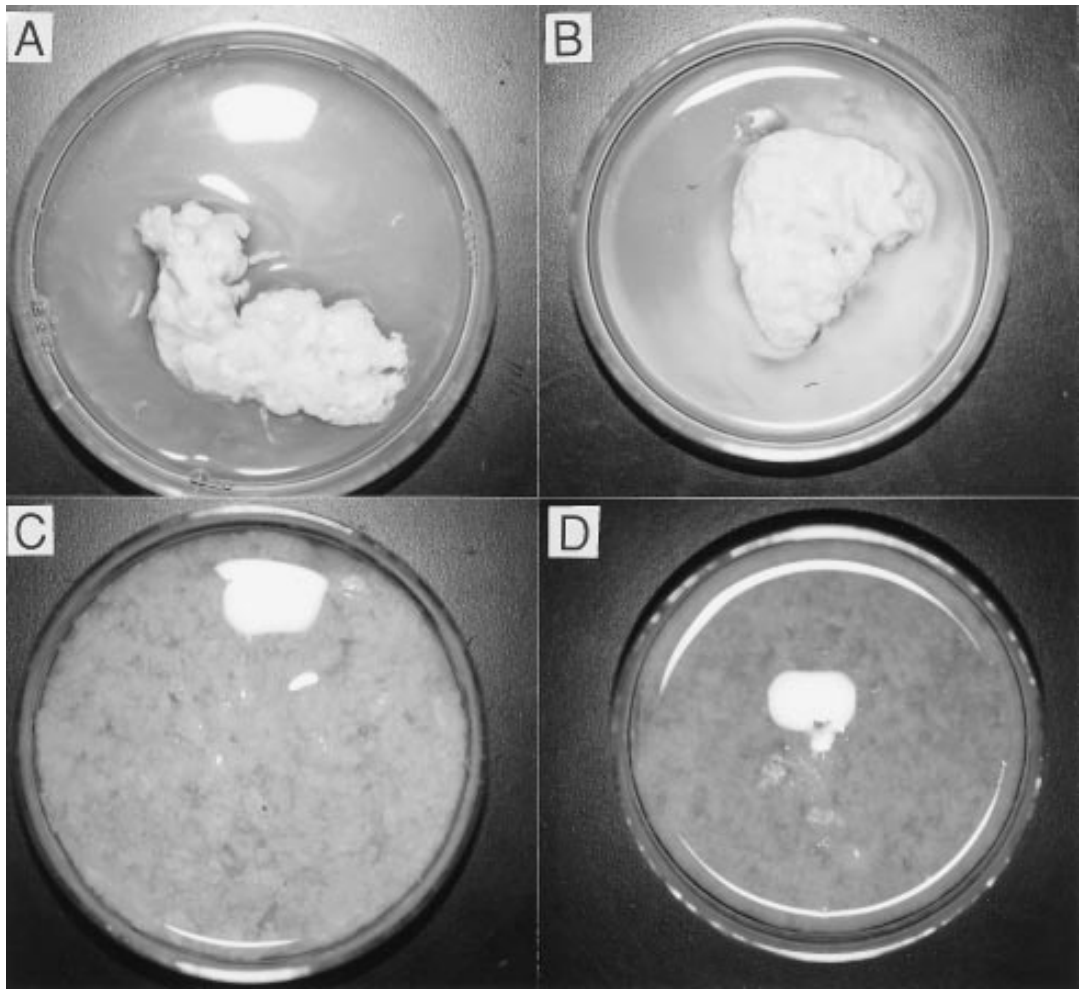


Figure 1. (a) Effect of support concentration on the production of lactic acid and mycelia, and (b) their morphological changes among cultures. The support was added at the beginning of the culture. The added concentration in Figure 1b were 0 (A), 1 (B), 3 (C), and 10 g/L (D).

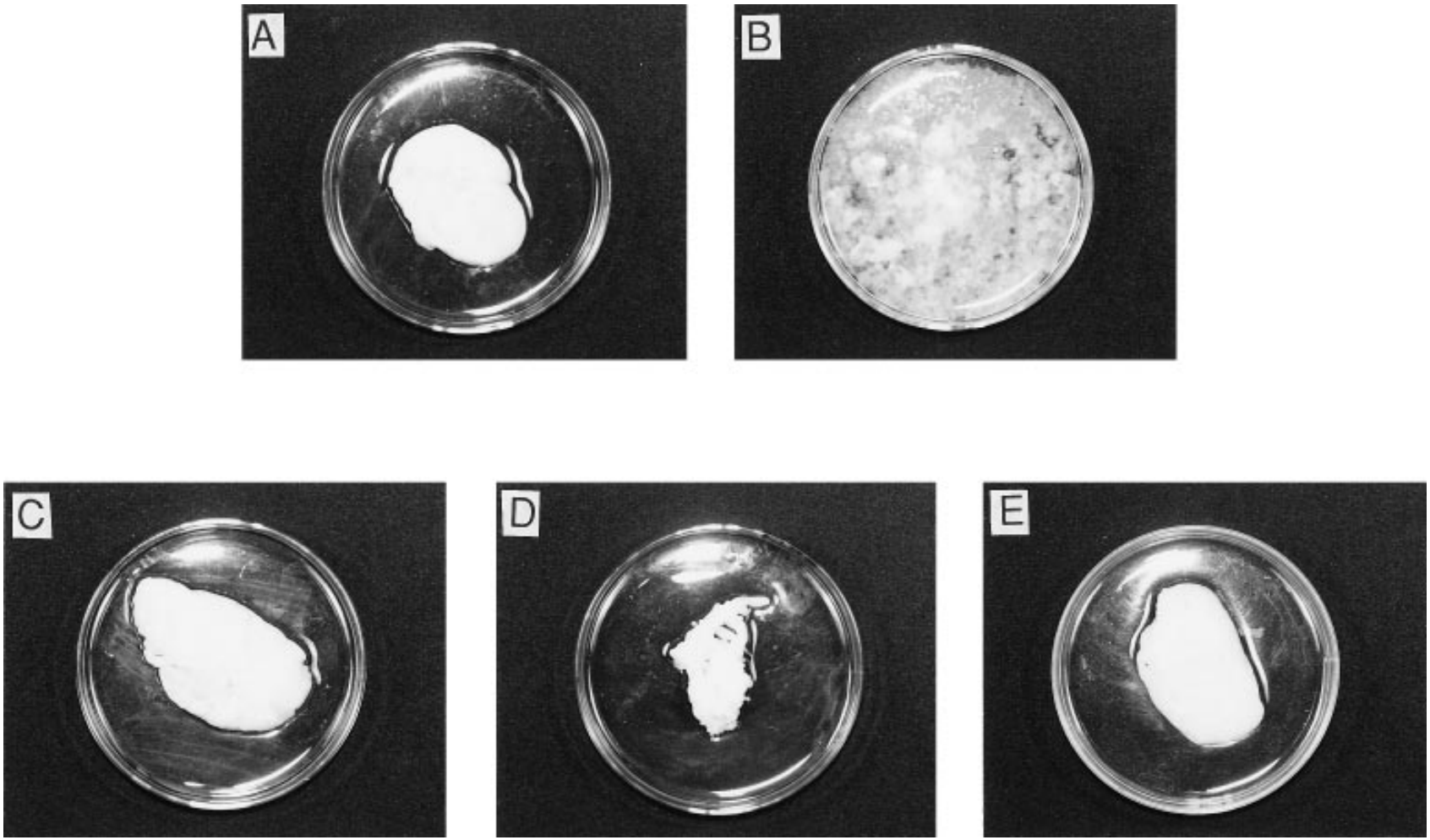


Figure 2. Influence of flocculants on the mycelial morphology among the cultures. For investigation of the mycelial morphology, various flocculants were added to the culture containing the support. At 60 h culture, mycelial morphologies were photographed. (A) was the control culture. Flocculants added were PEO (B), sodium hexametaphosphate (C), sodium pyrophosphate (D), and sodium tripolyphosphate (E).

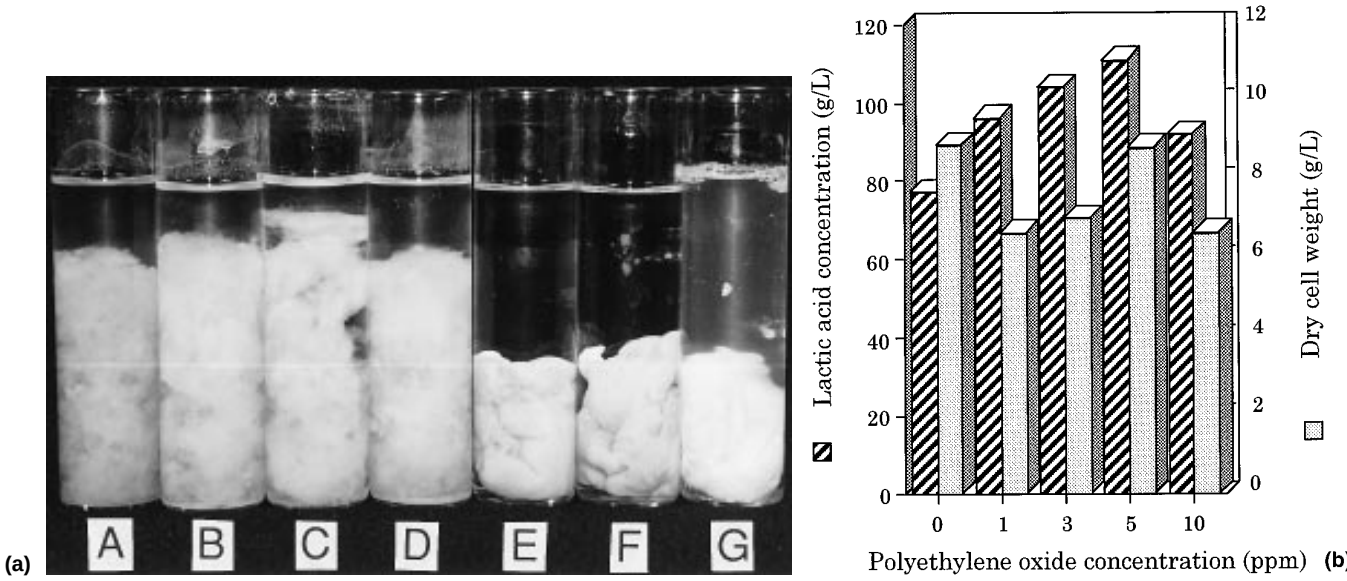


Figure 3. (a) Effect of PEO concentrations on flocs formation, and (b) the production of lactic acid and mycelia in the case of addition of 5 ppm PEO. Various concentrations of PEO were added into the 24 h culture and then mixed for 10 sec. Then tubes were placed for 20 min in room temperature, and were photographed (a). Concentrations added were 1 ppm (A), 3 ppm (B), 5 ppm (C), and 10 ppm (D), respectively. (E) denotes culture without the PEO and the support. (F) and (G) denote cultures with the support only and with the PEO only, respectively.

Glucose in the filtrate was measured as reducing sugar using the ferricyanide method (Dubios et al., 1956).

RESULTS

Effect of Mineral Support on Lactic Acid Production in Cultivation of *R. oryzae*

The support concentration was varied from 0 to 10 g/L in the flask culture; concentrations of lactic acid and dry cell weight are shown in Figure 1a. The concentration of lactic acid produced increased with the increase in the support concentration to 3 g/L. In the case of 3 g/L of the support, the maximum lactic acid concentration was 117.9 g/L, which was 1.6-fold to that without the support. In the case of concentrations higher than 3 g/L, the lactic acid production and cell growth were drastically decreased. This might be due to an increase in ion concentration of magnesium and hydroxyl, and viscosity due to the addition of the support (data not shown). When the support was added at concentrations less than 1 g/L, the mycelia formed one big cake (A and B in Fig. 1b), while in the case of the addition of the support at more than 3 g/L, mycelia were well dispersed and shown as a hyphal growth in the culture (C and D in Fig. 1b). At 5 and 10 g/L support, even if the mycelia were dispersed in the culture, mycelial growth was inhibited, resulting in low production of lactic acid.

Effect of Flocculant on Floc Formation in the Culture

When the support was added to the culture, the mycelia were shown as filamentous growth, but in the late growth phase, the morphology of dispersed mycelia containing supports changed to a big cake (shown later). To make small flocs of the mycelia, four kinds of flocculants were tested. After 5 ppm of flocculant was added to each tube containing the 3 g/L support and mixed for 10 sec, the tubes were then placed for 2 min at room temperature. Only the support in the tube containing polyethylene oxide (PEO) clearly formed a precipitate, indicating the PEO is the most effective flocculant for use with this mineral support (data not shown).

Figure 2 shows the mycelial morphology when each of these four flocculants were separately added to 12 h cultures containing 3 g/L of support. In the case of addition of the PEO (B in Fig. 2), small flocs were formed which did not coaggregate. In other cases, the mycelia clung to each other and formed a big pellet-like cake. This shows that PEO is the best flocculant for the formation of small flocs in cultures of *R. oryzae*.

When various concentrations of PEO were added into the culture containing the support at the culture time of 14 h, mycelial sedimentation properties was observed. When the broth of the 60 h culture was placed for 20 min in room temperature, the properties were different among the cultures (Fig. 3a). In the cases without the PEO and the support (E in Fig. 3a), with the support without PEO (F in Fig. 3a), and with the PEO without the support (G in Fig. 3a), mycelial morphology was that of a big cake which precipitated quickly. The addition of PEO at more than 1 ppm caused the formation of mycelial flocs containing the support (A to D in Fig. 3a). This might lead to hindering the formation of a cake or a large pellet. When 5 ppm PEO was added, the mycelial flocs were maintained without sedimentation.

Effect of PEO concentration on the lactic acid production is shown in Figure 3b. When the concentrations from 0 to 10 ppm were added into the culture containing the support, the highest lactic acid concentration was 110.7 g/L at 5 ppm of PEO. When the concentration increased, the lactic acid concentration increased until 5 ppm, and then decreased. If the flocculant effect is too strong, strong polymer bonding may occur, which may hinder substrate difusion inside the flocs and extension of mycelia. This indicates that lactic acid production was decreased with the addition of 10 ppm PEO.

Effect of Zeta Potential on Mycelial Morphology

Mycelial morphology and zeta potentials of the support and mycelium are closely connected. The zeta potential is varied from an ionic environment. Figure 4 shows the zeta potentials when mycelium, support, and support attached to the mycelia were placed at various pH buffers. When pH transferred from acidic pH to alkali region the zeta potential increased, leading to increase electrokinetic force among support particles. The zeta potentials of the mycelium and the support were −30 and −52 mV at pH 7, respectively. However, when mycelia contained supports, the electrokinetic force increased to −105.1 mV which was 2- and 3.5-fold more compared to that of the support and the mycelium, respectively. This caused the mycelia to be dispersed in the culture medium at the initial cultivation time when pH was at 6–7. However, as the mycelia grew and produced lactic acid in the medium, the electrokinetic force decreased with the decrease in pH of the culture judging from Figure 4. This caused the mycelia to twist around impellers or baffles, and to form big cakes which had the appearance of snowballs (later shown in Fig. 7).

When 5 ppm of the PEO was added to the support, mycelia, or support and mycelia, their zeta potentials were decreased to 55% compared to those without PEO as shown in Figure 4. In the case of support and mycelia added PEO, the zeta potential was −36 mV, which was one third of that without PEO. The reason for the decrease is supposedly due to the formation of mycelial flocs. The support and mycelia might be inserted into the polymer of the PEO chain, leading to form cotton-like flocs (see Figs. 2 and 3a).

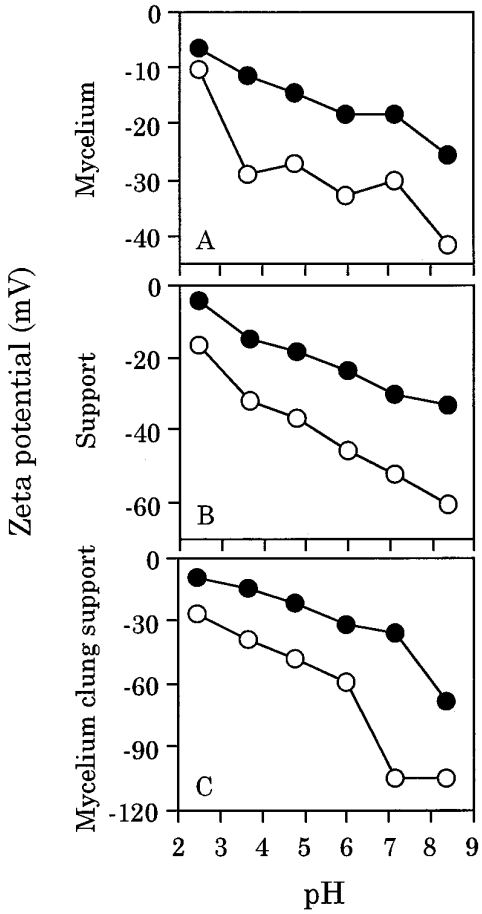


Figure 4. Change of zeta potential of mycelium (A), support (B), and mycelium and support (C) by addition of 5 ppm PEO. Closed symbols denoted the zeta potential obtained in the case of addition of PEO.

Mycelial FLoc Formation in a Jar-Fermentor

To investigate the formation of mycelial flocs in the jar-fermentor, successive micrographs of spores were photographed and shown in Figure 5. When 3 g/L of the support was added to the culture, a germ tube emerged at the 7 h culture (B in Fig. 5) and elongated as a mycelium at the 11 h culture (C in Fig. 5). At the 13 h culture, the elongated mycelia began to form small pellets (D in Fig. 5). However, the small pellets formed flocs by addition of PEO at 14 h culture (E in Fig. 5), and then three or four flocs were linked by mycelia which led to the formation of cotton-like flocs. This morphology of the cotton-like flocs was not changed throughout the cultivation.

The optimal time of PEO addition to the culture containing the mineral support of 3 g/L was determined by the use of an image analysis to ascertain the time before the formation of mycelial pellets. The mycelia showed logarithmic elongation until the culture time of 12 h, and after 12 h, mycelial length was nearly constant (data not shown). Since the mycelia began to entangle each other after the culture time of 12 h, the apparent mycelial length might be constant. In order to investigate the morphological difference between PEO addition times, 5 mg/L of PEO was added at the different culture times of 0, 13, 24 h. In the case of 0 h, mycelial flocs were not formed, but large pellet-like cakes were formed. In the case of 24 h, since large mycelial cakes were formed already, PEO did not affect the formation of mycelial flocs. On the other hand, when PEO was added at 13 h culture time, all mycelia formed small mycelial flocs. Therefore, the optimum addition time

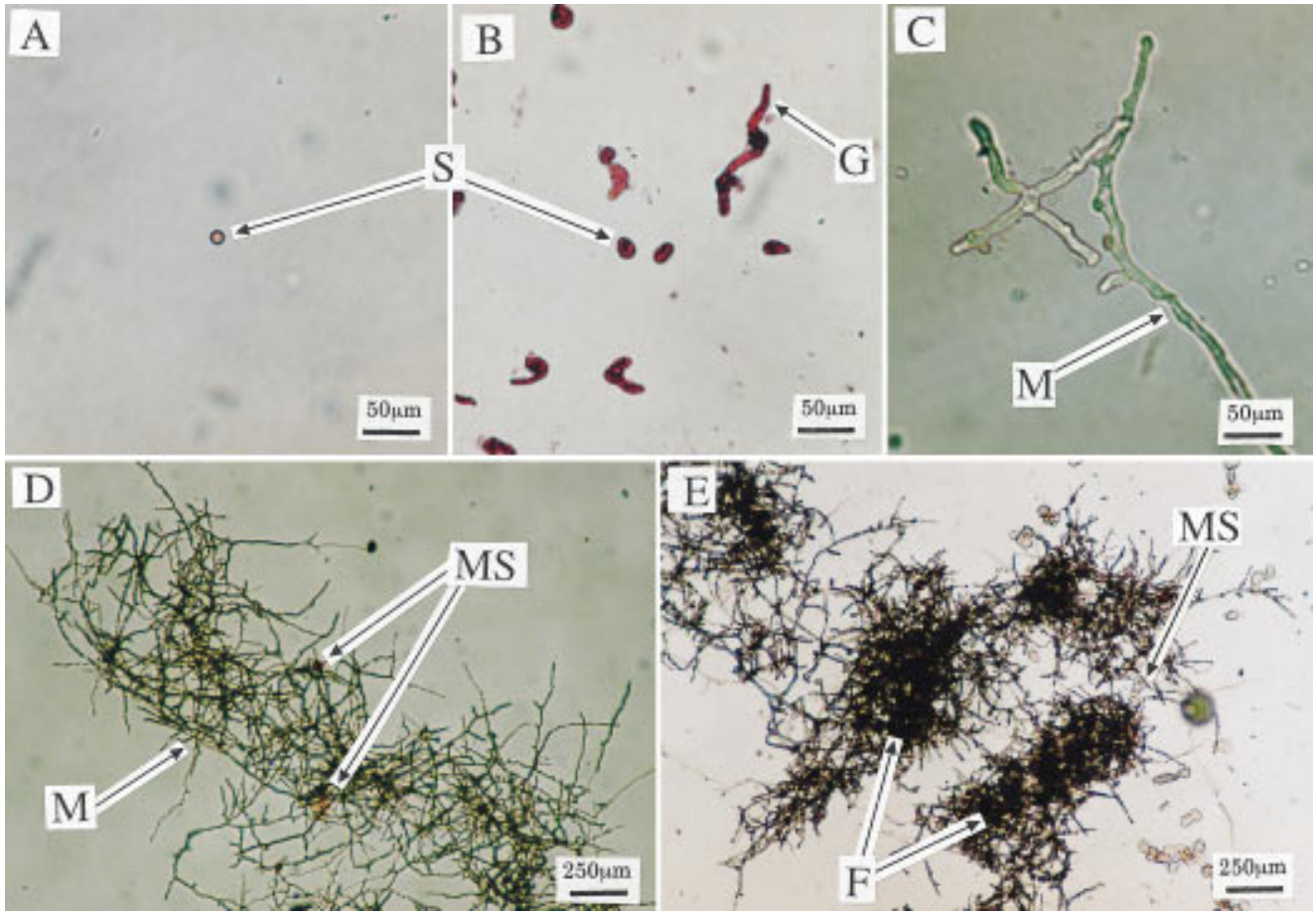


Figure 5. Spore germination and formations of small pellet and flocs. Spores were inoculated in the jar-fermentor (A) and began to germinate at 7 h (B). Germinated mycelia were elongated at 11 h (C), and elongated mycelia began to entangle at 13 h, and formed a small pellet (D). When the PEO was added to the culture at 14 h, two or three small pellets were gathered and then flocs were formed at 16 h (E). S, G, M, MS, and F denote spore, germ tube, mycelium, mineral support, and flocs respectively.

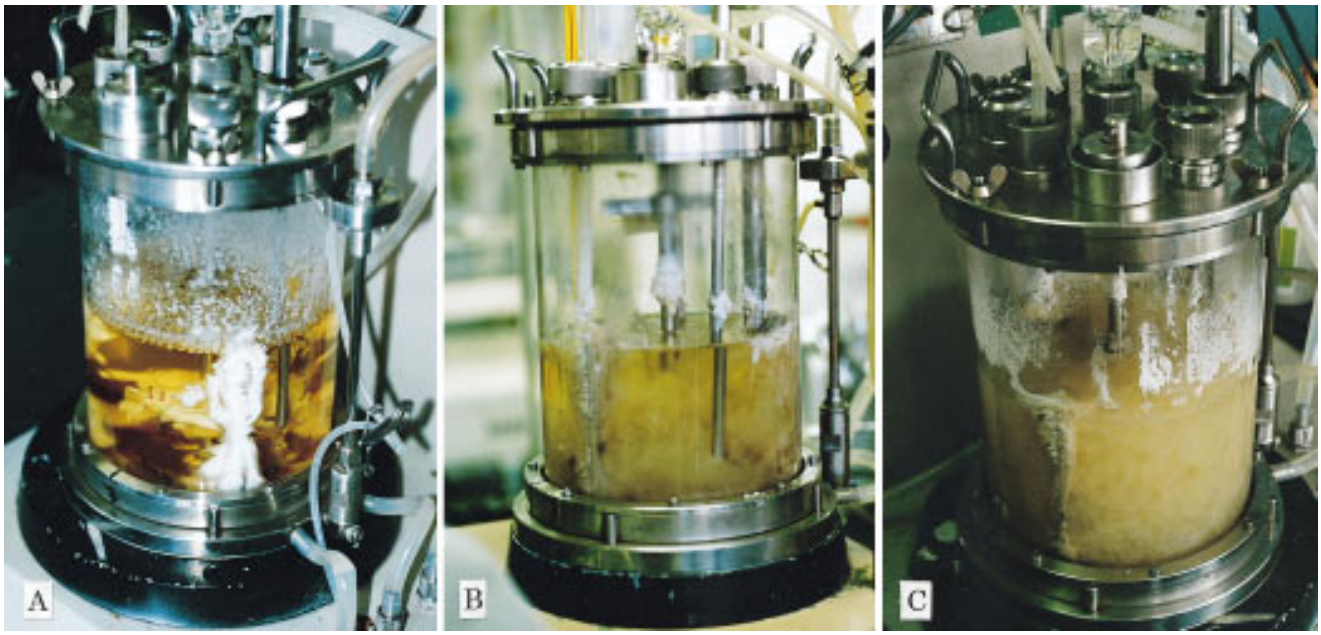


Figure 7. Morphological changes among the cultures of *R. oryzae* in the jar-fermentor. The cultures were carried out by mycelium only (A), mycelium and support without PEO (B), and mycelium and support with addition of PEO (C).

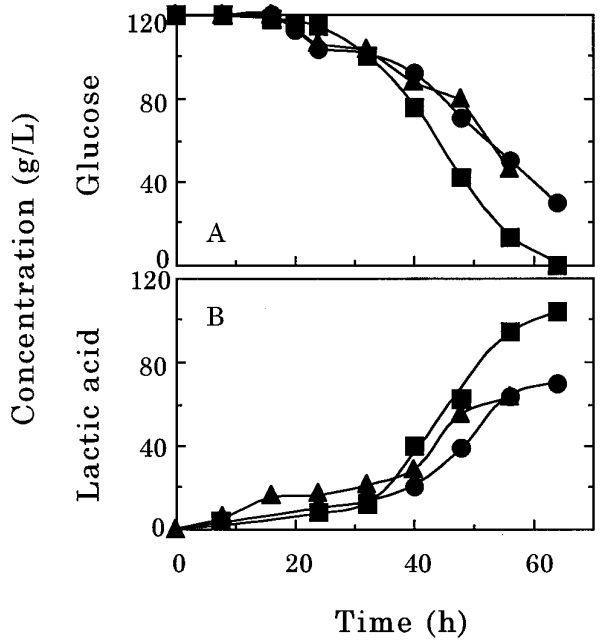


Figure 6. Concentrations of glucose (A), and lactic acid (B) in batch culture of *R. oryzae* in the jar-fermentor. In the case of PEO addition, the addition time was 13 h. The cultures were carried out by mycelium and support with addition of PEO (■), mycelium and support without PEO (▲), and mycelium only (●).

was determined at the time of late logarithmic elongation.

Jar-Fermentor Culture with the Addition of the Mineral Support and Polyethylene Oxide

When 3 g/L of the support or 3 g/L of the support and 5 ppm of PEO were added into the culture, the lactic acid production and glucose consumption were compared to those without both (Fig. 6). The rates of lactic acid production and glucose consumption were slow until 30 h. In the culture to which the support and flocculant were added, the rates increased drastically after 30 h. The final concentrations of lactic acid and residual glucose were 103.6 and 0 g/L, respectively. In the case without the support and PEO, the concentrations were 65.2 and 27 g/L, respectively. In the case of addition of the support, the lactic acid concentration at the 56 h culture was 63.7 g/L which was similar to that of the control. Dissolved oxygen concentration was maintained at more than 25% throughout the cultures.

Appearances among cultures are shown in Figure 7. In the case of the control culture, mycelia clung to the baffles or impellers of the jar-fermentor and formed a big cake (A in Fig. 7), while in the case with addition of the support, the mycelia were suspended in the culture, but also clung to the baffles or impellers in the late growth phase (B in Fig. 7). However, in the case of adding both the support and flocculant, most of the mycelia were suspended in the culture broth as small flocs (C in Fig. 7). This means that the support and PEO were effective on the dispersion of mycelia and the floc formation, leading to a improvement of the lactic acid production.

DISCUSSION

To cultivate fungi with a high production rate, it is important to control mycelial morphology. Fungal growth was characterized as a hyphal growth which resulted in formation of pellet or filaments. Much research has been done using various supports for the pellet formation (Keshavarz et al., 1990). It is useful to cultivate and to operate mycelia in large scale

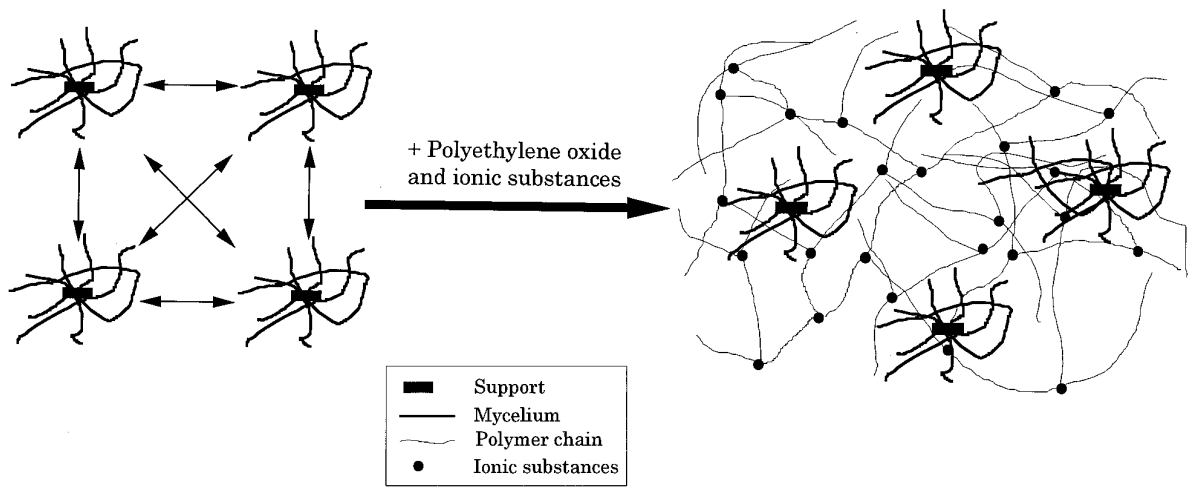


Figure 8. Hypothesis of formation of the cotton-like flocs among the mycelia, supports, and PEO. The small pellets containing support and mycelium were separated from each other due to the electrokinetic force of the support immobilized mycelia. When PEO was added to the culture, polymeric flocs were formed, which caused the pellets to be inserted into the flocs. The morphology, cotton-like flocs, are assumed to be formed from a bundle of flocs due to sterical and mechanical interactions of the mycelium and the matrix of the support and PEO.

cultivation, because of its easy handling and low viscosity. However, there is a diffusive limitation inside of the pellet, which leads to a decrease in the production rate. On the other hand, when mycelia grows as filaments, it is difficult to cultivate on a reactor scale. This is because of high viscosity, blocking of aeration nozzles, twisting around impellers or baffles, and sometimes the formation of big cakes.

To overcome those difficulties in fungal cultures, we tried to culture the *R. oryzae* with both a high production rate and an easy reactor operation. One of the methods is to use a mineral support that has the property of dispersion of the mycelia.

When the support was added to the culture, mycelia did not form big cakes, but grew in the state of dispersed culture. This is one method to avoid a cake formation. To maintain the dispersed condition in the culture by providing electrokinetic force between mycelium, the support should be added to the culture with the elongation of mycelia. This method is not promising because of increased costs, decreases in the lactic acid production, and the inhibition of mycelial growth.

Another method is to stabilize the ionic strength in the culture broth by using a flocculant which is widely used in water treatment processes. The flocculant selected was PEO (see Figs. 2 and 3a). The reason the support and the flocculant aggregated is that the hydroxyl group on the support surface and the oxygen of the PEO is assumed to form hydrogen bonds. This explained the decrease in zeta potential of the support from -52 to -30 mV.

Another possible explanation is that when cations are placed on the diluted PEO solution, the small mycelial pellets may be linked by salt bridges formed by cation atoms, leading to form mycelial flocs. Zhang et al. (1996) reported that the addition of cations could change the morphology of aggregates of amphiphilic block copolymers in dilute solutions. They obtained spherical, rodlike, univesicular or lamella aggregates, and a large compound vesicle morphology from a single block copolymer. Therefore, formation of flocs was possible becaue the broth contained cations, such as magnesium ion and other trace ions.

In the case of mycelia with the support added the mycelia were dispersed in the initial culture, but after 2 d culture they began to form a pellet or a cake (see B in Fig. 7). It may be because of insufficient supply of the support maintaining to sustain the dispersion of mycelia. PEO should be added to the culture while the mycelia and the support are still dispersed, about 12–14 h, in order to maintain the dispersion of mycelia. When PEO is added to the dispersed mycelia, the mycelia and polymers begin to form a small floc in the culture as shown in Figure 8. The mycelia twisted around the support are hypothesized to be spaced out from each other due to their sterical and mechanical interactions between mycelium and the matrix consisting of the support and PEO. The resulting morphology was cotton-like flocs that lead to a high production rate of lactic acid in the culture using bioreactors.

L(+)-lactic acid salts are used in the pharmaceutical industry and its polymer, polylactic acid (PLA), is attracting the attention of people because of environmental problems. In recent years, many researchers have carried out research on L(+)-lactic acid production in a reactor scale as shown in Table I. Soccol

Table I. Summary of lactic acid production by *Rhizopus oryzae*.

Culture method	Carbon source	Max. concentration of lactic acid (g/L)	Production rate (g/L/h)	Max. yield	Reference
Solid fermentation	Glucose	137	1.4	0.76	Soccol et al., (1994)
Immobilized on					
Calcium alginate	Glucose	62.4	2.6[a]	0.72	Hang et al., (1989)
Sodium alginate	Glucose	73.0	1.6	0.65	Hamamci et al., (1994)
Polymer support	Glucose	50.0	0.7	0.71	Tamada et al., (1992)
Submerged fermentation	Agricultural commodities	440 g/kg rice	4.6 g/kg rice/h	0.57	Yu and Hang, (1989)
	Agricultural commodities	320 g/kg cassava	3.3 g/kg cassava/h	0.74	Yu and Hang, (1989)
	Glucose	65.2	1.1	0.54	This work
Flocs formation in jar-fermentor	Glucose	103.6	1.7	0.86	This work

[a]Data cited from repeated batch culture.

et al. (1994) investigated solid fermentation techniques that had been used for several centuries, for the production of L(+)-lactic acid. They obtained 137 g/L of L(+)-lactic acid with the yield of 0.76 at the culture time of 96 h. Alginate salts were used as the support for immobilization of *R. oryzae* and tested the stability (Hamamci and Ryu, 1994) in repeated batch culture and in fluidized-bed tapered-column bioreactor (Gerin et al., 1993).

Tamada et al. (1992) tested the feasibility of polymer support to immobilize *R. oryzae*. Yu and Hang (1989) reported that agricultural commodities could ferment L(+)-lactic acid by *R. oryzae*. All the noted research was done by conventional culture methods. Production rate of lactic acid increased using an immobilized *R. oryzae,* but the maximum lactic acid concentration was lower than those of solid or submerged fermentations. This might be due to a difficulty in morphological problems. The *R. oryzae* was characterized by hyphal growth, formation of a big pellet-like cake, and twisting of hyphae in impellers and baffles of reactor. Therefore, it is difficult to obtain a high production rate with high yield in a reactor scale.

The cotton-like mycelial flocs and dispersed mycelia that we achieved were effective on the production of L(+)-lactic acid with high yield, because of an effective mass transfer inside the flocs. In jar-fermentor the L(+)-lactic acid concentration produced was higher than 100 g/L at 60 h culture with a yield of 0.86. This was 1.7-fold higher than that of the conventional batch culture. When a mycelium extends as a mycelial growth, mechanical agitation damages the mycelial growth. This technique will be applied also on an air-lift bioreactor in which mixing is by aeration. The mineral support was useful for the dispersion of mycelia, while the flocculant was useful for the formation of flocs. These cotton-like mycelial flocs will be useful to culture molds in a reactor scale. However, further investigation will be required to show the effects of this technique on the economics of lactic acid production and product recovery including disposal of support, scale-up, and problems in product recovery.

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