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Relationship between morphology and viscosity of the main culture broth of *Cephalosporium acremonium* M25

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Abstract

In this study, the relationship between morphology and viscosity of the main culture broth of *Cephalosporium acremonium* M25 was investigated in a 2.5 L bioreactor. The differentiation of *C. acremonium* M25 showed a complex pattern during the main culture. The morphological changes of *C. acremonium* M25 were related to the rheological properties of the culture broth and it was well agreed with the power law model. As a result of rheology study, it was found that rheological properties of the main culture broths of *C. acremonium* M25 in bioreactor were closely related to morphological changes. Also, fractal dimension fairly predicted morphological and rheological changes in the main culture broth.

Keywords : *Cephalosporium acremonium*, fractal analysis, morphology, rheology

1. Introduction

Cephalosporin C (CPC) fermentation by *Cephalosporium acremonium* is characterized by morphological differentiation and repression by glucose (Vicik *et al.*, 1990). The morphological differentiation of *C. acremonium* has been examined by several studies. In a submerged culture, *C. acremonium* shows various morphological cell types and the maximum rate of CPC production coincides with the differentiation of filamentous hyphae into wide, highly swollen and metabolically active hyphal fragments (Cruz *et al.*, 1999; Kim *et al.*, 2003).

Fungal morphology is an important parameter which influences the physical properties of the fermentation broth. The rheological behavior is closely related to the morphology and biomass concentration (Sinha *et al.*, 2001). The broth rheology determines the transport phenomena in bioreactors that is the key to improve yield of the desired product (Riley *et al.*, 2000). It is also necessary to identify the most productive morphology of the organism related to the desired product. In our previous work (Lim *et al.*, 2002), it was proved that the morphological differentiation of *C. acremonium* M25 in seed culture was closely related to the rheological properties of the culture broth. Considering CPC production, it is necessary to investigate the relationship between morphology and the rheological properties of the main culture broth of *C. acremonium* M25 in a bioreactor.

Fractals or self-similarities, have been introduced to describe natural phenomena. In a microbiological system, fractals were employed to describe growth patterns and morphology (Ryoo, 1999). However, most studies focused on the correlation between fractal dimensions and branching complexity. To be a useful tool in the morphology study of microorganisms, fractal analysis should be able to appropriately characterize the correlation between morphological changes and the physiological function of microorganisms that have more complex differentiation pattern.

In this study, the complex mycelial morphology of *C. acremonium* M25 in 2.5 L of main culture was analyzed using fractal dimension. And the relationship between fractal dimension and the rheological properties of the culture broth was investigated.

2. Materials and methods

2.1. Strain

In our previous work (Lee *et al.*, 2001a), *C. acremonium* ATCC 20339 was mutagenized by irradiation under U.V. and a mutant of *C. acremonium*, strain M25, was finally selected by an agar-diffusion method. This strain was used in this study.

2.2. Media and culture conditions

The basal seed medium consisted of 2.5% (w/v) sucrose,

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1.0% (w/v) glucose, 2.5% (v/v) corn steep liquor and 0.4% (w/v) $(NH_4)_2SO_4$. To improve the morphological differentiation, 3.0% (w/v) soy bean meal, 1.0% (w/v) cotton seed flour and 0.5% (w/v) $CaCO_3$ were added to the basal seed medium (Lee *et al.*, 2001b). The main medium consisted of 1.95% (w/v) glucose, 5% (v/v) corn steep liquor, 0.8% (w/v) $(NH_4)_2SO_4$, 0.3% (w/v) KH_2PO_4, 0.5% (w/v) K_2HPO_4, 0.5% (w/v) DL-methionine and 0.4% (v/v) trace element solution (Lim *et al.*, 2002). Sugars and $(NH_4)_2SO_4$ were sterilized separately from other components. pH was adjusted to 7.0 with 1N NaOH prior to sterilization. $CaCO_3$ was added after pH adjustment. The fermentation in the stirred-tank fermenter (2.5 L) was carried out at 27°C and 500 rpm. The operating volume was 1.5 L and the air flow rate was 1.0 vvm.

2.3. Image analysis for cell morphology

The cell morphology was studied on photomicrographs with optical microscopy (Samwonscientific Ind. Co. Ltd., Korea) connected with Image Pro 3.0 software (Media Cybernetics, Silver Spring, MD, USA). Morphological factors such as hyphal length, number of tips and number of arthrospores were measured manually or automatically after sorting and classifying by the image analyzing process. Each sample was diluted fivefold and the average values for hyphal length, number of tips and arthrospores were calculated from approximately 100 observations.

2.4. Determination of fractal dimension

The fractal dimension of the cell morphology was determined by a box counting method, derived from the method used by Obert *et al.* (1990). The binary image of mycelium was edited to remove foreign particles and correct optical errors. When the mycelium was covered by a grid of equal side length (L), the number of boxes (N) overlapped by a mycelium could be counted. The number of boxes overlapped by the mycelium image grew as the side length (L) of the box was increased. For well-defined fractal subjects, the following equation should be satisfied.

$$N(L) = \alpha L^{D} \tag{1}$$

Where α and D are proportionality constant and the fractal value of the subject, respectively. Equation (1) is expressed in logarithmic form.

$$\text{Log } N(L) = \text{D} \log L + \log \alpha \tag{2}$$

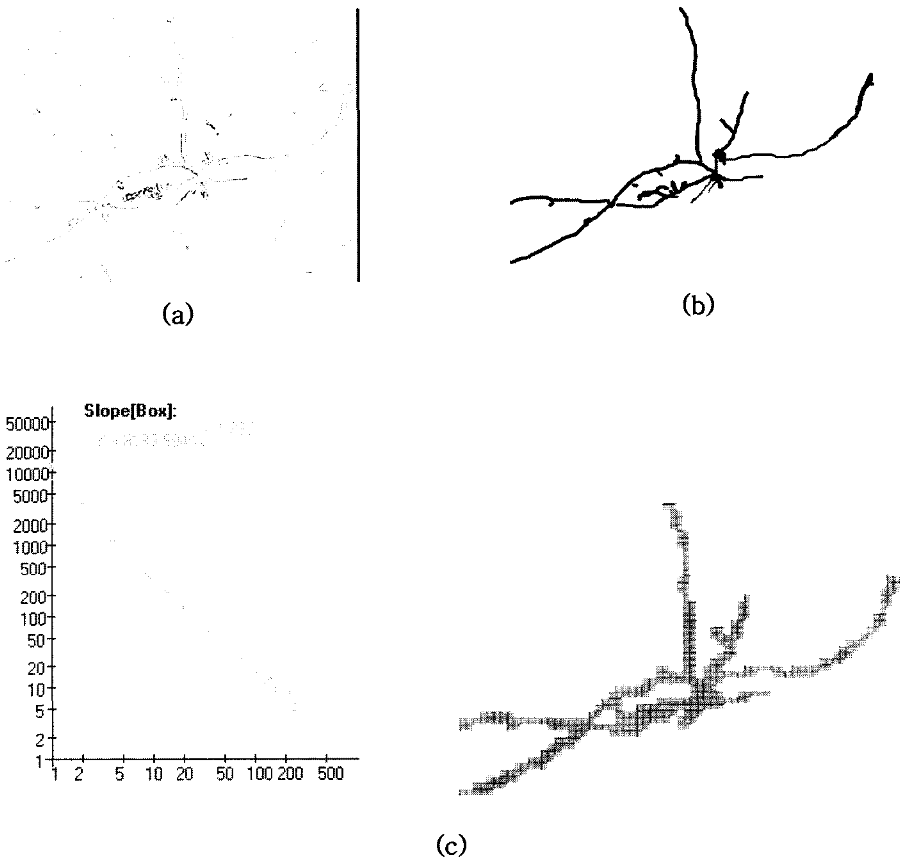

Fig. 1. Schematic diagram of the determination of fractal dimension using the box counting method. (a) image from optical microscopy, (b) image after processing and (c) fractal dimension determination in calculator.

For a well-defined fractal subject, the logarithmic number of overlapped boxes is linear to the logarithmic value of the side length of box with slope of fractal D. The fractal Dimension calculator (Ver. 1.1, Bar-Ilan University, Israel) automatically counts the number of overlapped boxes, and calculate fractal dimension through linear regression. More than 30 images were processed and the averages of fractal dimensions were derived at various culture times. The schematic diagram of the determination of fractal dimension is shown in Fig. 1.

2.5. Analysis of viscosity models

ARES (TA instrument, USA) was used to determine the shear stresses (τ) at different shear rates ($\dot{\gamma}$). The relationships were interpreted in terms of the different viscosity model: Power law; $\tau = \tau_0 \cdot \dot{\gamma}^n$, the Bingham plastic model; $\tau = \tau_0 + n\dot{\gamma}$, and the Herschel-Bulkley model; $\tau = \tau_0 + (K \cdot \dot{\gamma}^n)$.

3. Results and discussion

3.1. CPC production and morphological changes of *C. acremonium* M25 in 2.5 L of the main culture

Fig. 2 shows the time courses of CPC production by *C. acremonium* M25 in 2.5 L of the main culture. Cell mass increased rapidly to 24 g/L during 2 days and was maintained. When the increase of the cell mass stopped, CPC production increased significantly from 0.12 g/L at 2 days to 0.52 g/L at 5 days. pH decreased from 7.0 to 5.12 at 3 days as the cell mass increased and then increased gradually to 6.02 at the end of culture. Typical morphological changes over 7 days in the main culture are shown in Fig. 3. In the early stage of the culture, there were many filamentous hyphae and they differentiated into swollen hyphal fragments from 3 days. In the 4th and 5th day of culture broth, some filamentous hyphae and a large number of swollen hyphal fragments were observed. These hyphal

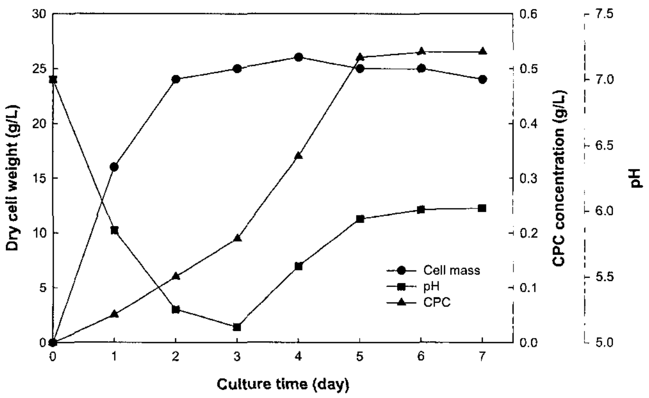

Fig. 2. Time courses of CPC production by *C. acremonium* M25 in 2.5 L main culture at 500 rpm. Culture was carried out at 27°C with an air flow rate of 1.0 vvm.

fragments differentiated into arthrospores at 7 days. To investigate the morphological changes quantitatively, the changes of the morphological factors were determined by the image analysis system (Fig. 4). As differentiation proceeded, mean hyphal length decreased from 460 μm to 32.5 μm. Mean hyphal thickness increased from 4 μm to 8.03 μm as filamentous hyphae differentiated into swollen hyphal fragments and then decreased to 6.58 μm as arthrospores were made from swollen hyphal fragments. The changes of the number of tips showed a similar tendency to that of mean hyphal length. The number of tips decreased from 5.1×10^2/ml to 0.6×10^2/ml. The number of arthrospores increased to 3.8×10^2/ml at 6 days and then decreased to 3.5×10^2/ml.

3.2. Main culture broth rheology of *C. acremonium* M25 in 2.5 L bioreactor

In our previous work (Lim *et al.*, 2002), it was concluded that the Herschel-Bulkley equation was in excellent agreement with the rheological properties of seed culture broth. Generally, in the main culture, cell growth and metabolite production show different pattern from seed culture because medium and culture conditions are changed. Considering CPC production in industrial scale, rheological behavior of main culture broth in bioreactor has significant importance. To compare rheological behavior between seed and main culture broths, the viscosity models were applied to the main culture broth of *C. acremonium* M25 in a 2.5 L bioreactor. As a result, the power law confirmed well with the results of the main culture broth. In the main culture, as cell growth and differentiation proceeded, the viscosity of culture broth increased till the 6th day and then it decreased on 7th day when most of the swollen hyphal fragments differentiated into arthrospores (Fig. 5). Compared to cell growth, it was found that broth viscosity was affected by not only cell mass but the morphology of *C. acremonium* M25. From the power law model, the consistency index (K) and the flow behavior index (n) were calculated. Fig. 6 shows the changes of each index value in the main culture of *C. acremonium* M25. The consistency index increased during 6 days of culture and then decreased rapidly on the 7th day. The flow behavior index decreased significantly over 6 days and then increased a little on the 7th day.

3.3. Relationship between fractal dimension and the rheological properties of the main culture of *C. acremonium* M25

To investigate the relation between the morphological changes and the rheological properties of main culture broth more quantitatively, fractal analysis was employed. Fig. 7 shows the variance of fractal dimension in the main culture broth of *C. acremonium* M25 in a 2.5 L bioreactor. During the main culture, fractal dimension increased from

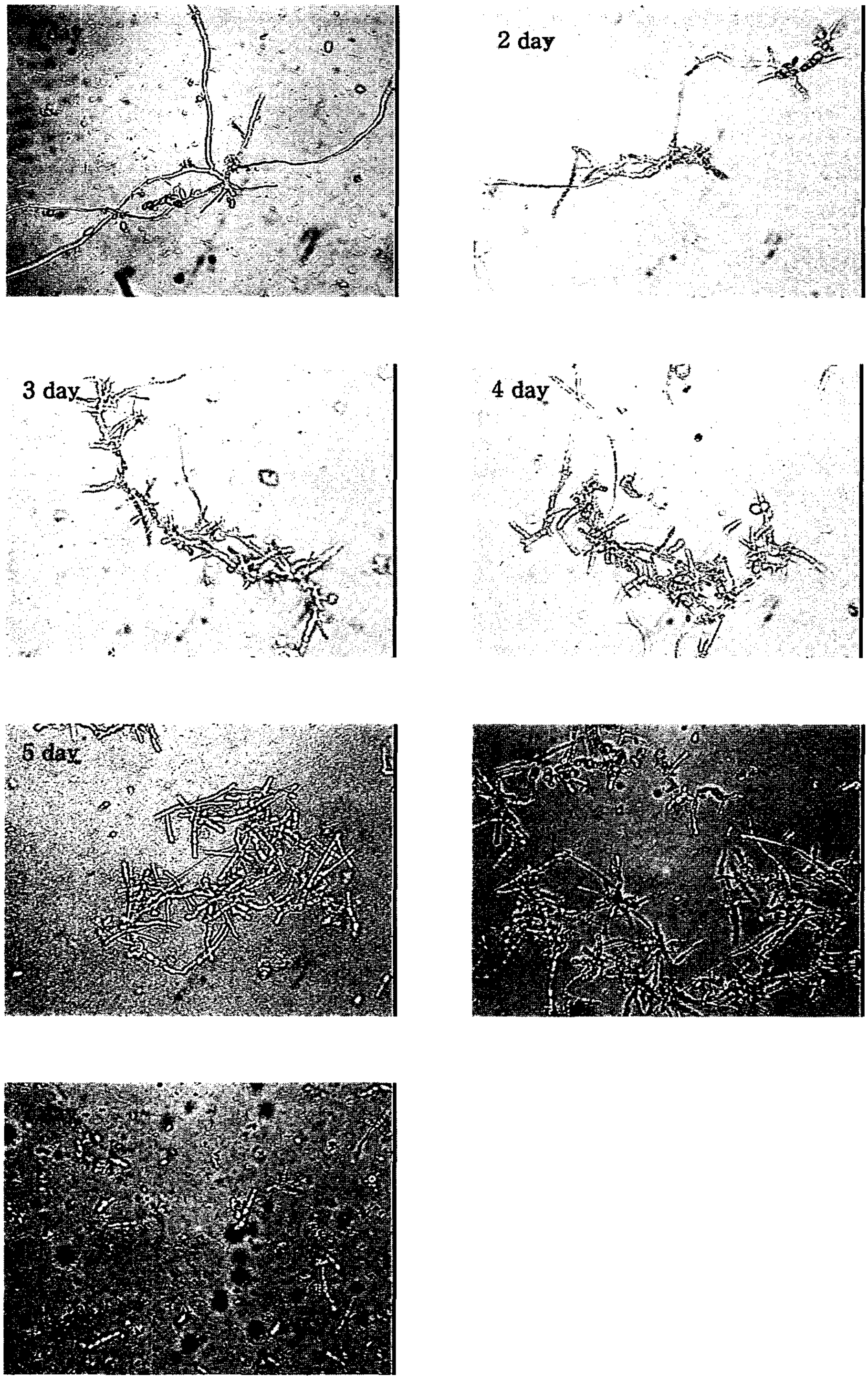

Fig. 3. Typical morphological changes in 2.5 L main culture of *C. acremonium* M25.

1.27 at 1 day to 1.57 at 6^{th} day and then decreased rapidly to 1.15 at 7^{th} day. The increase of fractal dimension shows that there are many complex morphological forms in the culture broth. Actually, most of filamentous hyphae differentiate into complex swollen hyphal fragments in this period (Fig. 3). Thereafter, swollen hyphal fragments mostly differentiated to arthrospores at 7^{th} day and fractal dimension decreased significantly. These changes of fractal dimension showed a similar pattern to that of the consistency index. Consistency index increased gradually during 5 days when filamentous hyphae dominated culture broth. Then it increased rapidly to 13.9 $mPas^n$ at 6^{th} day when most of filamentous hyphae differentiated into swollen hyphal fragments and significantly decreased to 5.3

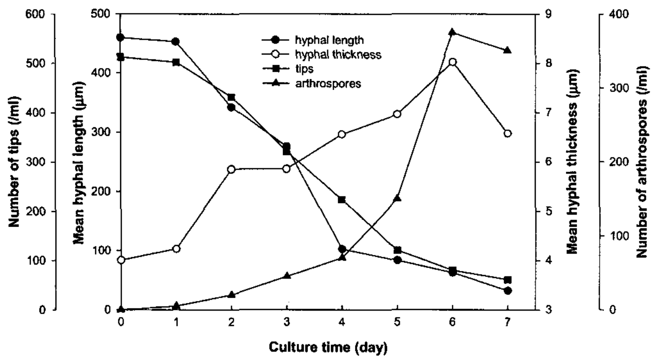

Fig. 4. Time courses of morphological factors of *C. acremonium* M25 in 2.5 L main culture.

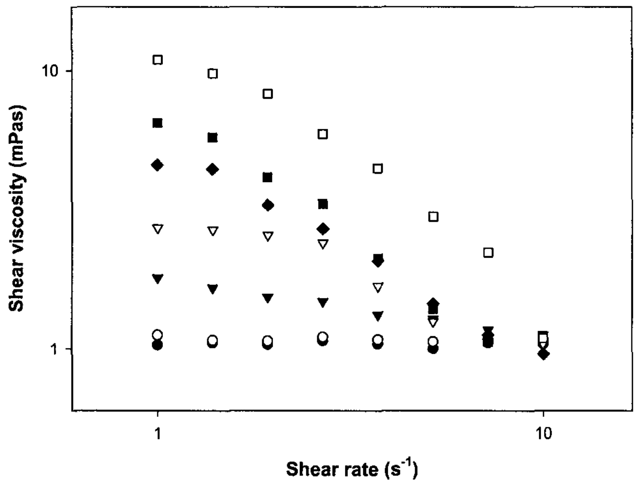

Fig. 5. Shear viscosity vs. shear rate plots made by the power law equation for the flow behavior of the main culture broth at different culture times: (●) 1 day, (○) 2 days, (▼) 3 days, (▽) 4 days, (■) 5 days, (□) 6 days, (◆) 7 days.

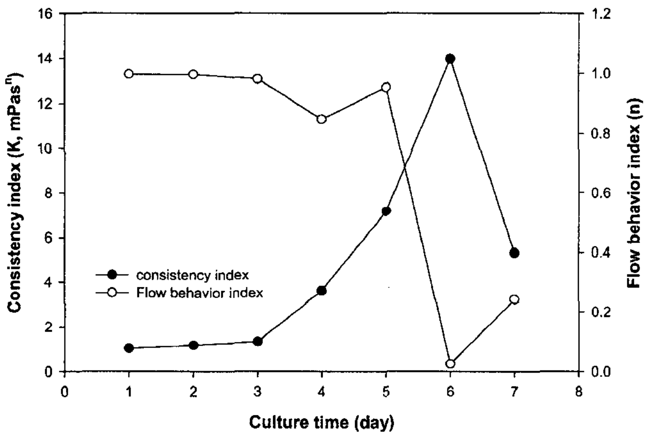

Fig. 6. Changes in the flow behavior index and the consistency index of the main culture broth of *C. acremonium* M25.

mPas^n at 7th day as swollen hyphal fragments differentiated to arthrospores. The change of flow behavior index was inversely related to that of consistency index. The large variation of the consistency index and the flow behavior index over 5 − 7 days is closely related to the morphological changes, and the changes of fractal dimension predicted this pattern well.

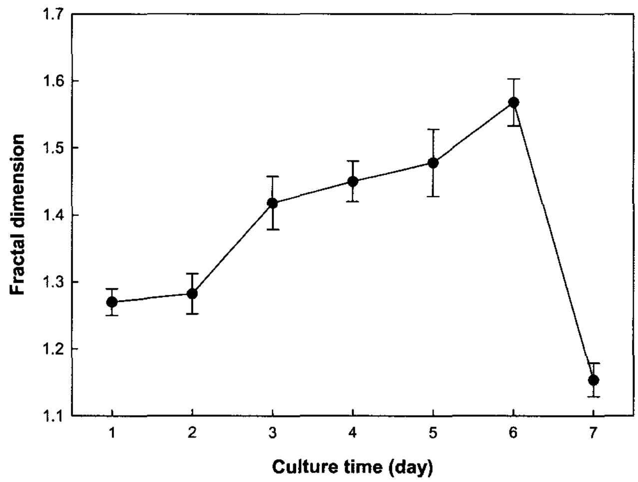

Fig. 7. Changes in fractal dimension of *C. acremonium* M25 in 2.5 L of the main culture.

4. Conclusion

In this study, the relationship between morphology and viscosity of the main culture broth of *C. acremonium* M25 in a 2.5 L bioreactor was determined by fractal analysis. In the early stage of the culture, there were many filamentous hyphae and then they differentiate into swollen hyphal fragments from 3 days. A large number of swollen hyphal fragments were observed in the fourth and fifth days of the culture broth. The CPC production increased significantly in this period. After 6 days, most of the swollen hyphal fragments differentiated into arthrospores. These morphological changes of *C. acremonium* M25 affected the rheological properties of the culture broth and it confirmed well with the power law equation. Also, the changes of fractal dimension fairly predicted the morphological changes and the rheological properties of *C. acremonium* M25 in 2.5 L of the main culture. Overall, rheological properties of the main culture broths of *C. acremonium* M25 in bioreactor showed different pattern from that of seed culture as a result of different morphological changes suggesting possibility of application to industrial process. Also, morphological and rheological changes in the main culture broth were fairly characterized by fractal dimension.

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