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Effect of mycelial morphology on bioreactor performance and avermectin production of *Streptomyces avermitilis* in submerged cultivations

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Abstract

Factors affecting the morphology of *Streptomyces avermitilis* and avermectin production in submerged cultivation, including nitrogen sources, inoculum level and DO (dissolved oxygen) tension in the broth were investigated in a 50-L bioreactor. It was found that a combination of soybean meal and yeast meal as nitrogen sources and 4.3% inoculum led to pellet formation, and the pellet morphology facilitated to maintain DO $> 20\%$ in the early stage of fermentation. With the aid of image analysis tools, area and density of pellets in different batches were calculated. Results show that higher dissolved oxygen tension was favorable for pellet formation and avermectin production.

Keywords: Avermectin; Image; Morphology; Pellet; *Streptomyces avermitilis*

1. Introduction

Growth and secondary metabolite production from filamentous microorganisms such as actinomyces and fungus in complex medium is poorly understood. The physiological state and morphology differentiation of such microorganisms appeared to be linked with perturbation of the experimental parameters in submerged cultivations. Furthermore, it has been reported that in many cases productivity correlated tightly with morphology (Atkinson and Daoud, 1976; Braun and Vecht-Lifshitz, 1991; Hermersdörfer *et al.*, 1987; Metz and Kossen, 1977; Steven and Bushell, 1996; Whitaker and Long, 1973). Also, in the cultures of many *Streptomyces* species, no secondary metabolite production has been found in cultures whose micromorphologies appeared fragmented (Roth *et al.*, 1982; Shomura *et al.*, 1979).

To investigate the relationship between mycelial morphology and bioreactor performance, many researchers have characterized or quantified the mycelial morphology under different operational conditions with the help of various image analysis tools. The reported operational conditions included agitation conditions (Justen *et al.*, 1996), inoculum levels (Nielsen *et al.*, 1995; O'Cleirigh *et al.*, 2003), pH, Nitrogen source in the medium (Choi *et al.*, 2000; Treskatis *et al.*, 1997), and dissolved oxygen tension in the broth (Du *et al.*, 2003).

The morphological differentiation of *Streptomyces avermitilis*, which produces avermectin in submerged cultivations, was strongly affected by many experimental parameters in our study, and it was observed that the difference in mycelial morphology correlated tightly with the avermectin productivity. To the best of our knowledge, little information about this has been reported in literature. In the present study, the effects of nitrogen sources in the medium, inoculum levels, and dissolved oxygen tension on the morphology of *S. avermitilis* cultivated in a 50-L bioreactor were investigated in detail. Pellets in different cultivations were quantified with the help of an image analysis tool developed by our laboratory.

2. Experiment

2.1. Materials and methods

2.1.1. Strain and media

An industrial strain of *S. avermitilis* (Biok Biology Co., Ltd., Huzhou, Zhejiang, P.R. China) was employed throughout this study.

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Table 1
Conditions and productivity in the Four Batch Cultivations of avermectin fermentation

	Nitrogen source	Inoculum amount (%)	Agitation(rpm)	DO	Productivity (μg/mL/h)
B1	Yeast extract	8.6	0–490	Fell to zero after 45 h	0
B2	Yeast meal	8.6	0–310	>20%	8.0
B3	Yeast meal	4.3	0–310	>20%	13.2
B4	Yeast meal	4.3	0–310 (stop at 15–20 h)	Fell to zero from 15 h to 25 h	11.5

The strain was maintained as spore stocks on agar slants at 4 °C. Spores were scalped from the agar surface and inoculated with 30 mL seed medium in a 250-mL Erlenmeyer flask. The seed medium contained (g/L in tap water) starch 3; corn steep liquor 1; yeast extract 1; amylase 3 U/g starch. After 48 h of incubation at 28 °C on a shaker operating at 220 rpm, about 3 mL of the seed culture was used to inoculate 10 L of seed medium in a 15-L bioreactor. Exponentially grown culture was used as inoculum for the 50-L bioreactor with 35 L working volume of complex medium containing (g/L in tap water) starch 12; soybean meal 3.5; yeast extract or yeast meal 0.8; KH_2PO_4 0.5.

2.1.2. Cultivation condition

All cultivations were performed in a 50-L fermenter (Guoqiang Bioengineering Equipment Co. Ltd., Shanghai, China) equipped with three stirrers: a six-bladed flexual-blade turbine at the bottom and two screw propellers in the upper space. The ratio of blade height to impeller diameter at the bottom was 1.1:1. Temperature was maintained at 28 °C, the head space pressure was 1.5 bars, and maximum air flow rate was 1.4 vvm.

The DO concentration was measured by a sterilizable Mettler Toledo electrode. Data acquisition and processing were carried out with Bioradar Ver. 2.0 to process the final computation and give the course parameters every time.

An overview of the cultivation conditions for four batches of experiments is shown in Table 1. The experimental parameters differed mainly in the nitrogen source, inoculum amount, and agitation rate and DO concentration in the broth. pH was not controlled throughout all of the fermentations.

2.2. Analytical methods

Microbial growth was estimated by the dry cell weight (DCW) of 10 mL culture broth after centrifugation at 2800 × *g* for 10 min. Residual sugar was analyzed by Fehling titration, the samples being completely hydrolyzed by HCl before Fehling titration (Chinese National Standard for Sugar Analysis GB/T 6194-86). NH_2–N was measured by the formaldehyde method. For avermectin extraction, 1 mL fermentation broth was extracted with 4 mL acetone and vortexed for 3 min and laid aside for 10 min. The supernatant was diluted with methanol and injected into an Agilent HPLC equipped with a C-18 column (Hypersil ODS, 5 μm, 250 mm × 4.0 mm). The running conditions were: methanol/water (90:10) as the mobile phase, flow rate set at 1 mL/min, column temperature set at 25 °C, and UV detection set at 246 nm. The quantities of avermectin were calculated using an authentic sample of avermectin B1a and B1b fraction as the standard.

2.3. Mycelial pellet measurement

About 0.05 mL aliquot of culture broth was taken at different culture times during batch fermentation, and was stained by a Safranin stain solution. Images of *S. avermitilis* were captured by BX51 microscopy (OLYMPUS Ltd., Japan) and a DEMO-16 CCD-camera; the captured image was analyzed by FerAnaForAv software which was developed by our laboratory. In the analysis system, five variables were used to characterize the morphology of *S. avermitilis* in cultivations. They were area of mask image (A_t), total area of pellet (A_o), area

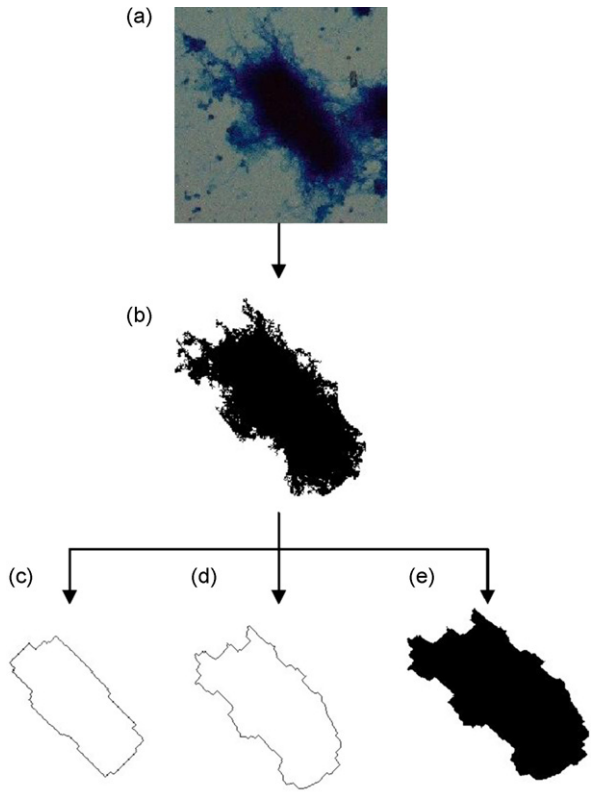

Fig. 1. Sequence of image analysis. After image acquisition, (a) the original image is segmented to define objects and is changed to (b) binary images, then the binary images are used to measure the object parameters. The parameters obtained include (c) core figure of pellet, (d) masked figure of pellet and (e) total figure of pellet.