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Short Communication

Nuclear distribution of conidia derived from a multinuclear autopolyploid strain of *Trichoderma reesei* QM 6a (ATCC13631)

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ABSTRACT

When the mycelial mat of *Trichoderma reesei* QM 6a was treated with the mitotic arrester colchicine for 30 days, various sizes of autopolyploid nuclei were produced in the mycelial mat. Such mycelia were named multinuclear autopolyploids. As the conidium of this fungus is mononucleate, only an autopolyploid nucleus was present in each conidium. Thus, it was concluded that genetically stable autopolyploid strains can be selected easily from among the conidia in this fungus.

KEYWORDS: *Trichoderma*, colchicine, cellulase, cellulose, polyploidy.

1. INTRODUCTION

The author has previously reported that the filter paper-degrading ability and proliferation characteristics of the cellulolytic fungus, *Trichoderma reesei*, could be enhanced by autopolyploidization using the mitotic arrester, colchicine [1]. In this study, the mycelia of this fungus were treated with colchicine for a month in order to form multinuclear autopolyploid mycelia and observation of the nuclear distribution in the conidia derived from such mycelia was carried out.

2. MATERIALS AND METHODS

2.1. Microorganism and media

A cellulolytic fungus, *Trichoderma reesei* QM 6a (ATCC13631) was used in this study. A piece of the mycelial mat (3 mm x 3 mm) of this fungus was placed on a potato dextrose agar (PDA) plate and incubated at 26 °C for 10 days followed by preservation at 4 °C. For colchicine treatment, 25 ml of Mandels' medium containing colchicine (Wako) 0.1%, peptone (Difco) 0.5%, and glucose (Wako) 1.0% was added to a 50 ml Erlenmeyer flask. Mandels' medium was composed of $(NH_4)_2SO_4$: 1.4 g, KH_2PO_4: 2.0 g, urea: 0.3 g, $CaCl_2$: 0.3 g, $MgSO_4 \cdot 7H_2O$: 0.3 g, $FeSO_4 \cdot 7H_2O$: 0.005 g, $MnSO_4 \cdot H_2O$: 0.0016 g, $ZnSO_4 \cdot H_2O$: 0.0014 g, $CoCl_2$: 0.0020 g, and distilled water: 1000 ml at pH 6.0 [2]. For conidial formation, PDA medium was used.

2.2. Colchicine treatment

A small piece of the mycelial mat grown on the PDA medium (20 mm x 10 mm) was added into the Mandels' medium for colchicine treatment and incubated at 26 °C.

2.3. Conidial formation

A small piece of the mycelial mat treated with colchicine (3 mm x 3 mm) was placed onto the PDA plate and incubated at 26 °C for 14 days in order to generate conidia.

2.4. Nuclear staining

The mycelial mat was treated with diluted Giemsa solution (Sigma) for 20 minutes and photomicrographs were taken after washing with distilled water. The conidia were fixed on a slide glass using a gas burner and treated with 18% HCl at 50 °C for 15 minutes in a deep glass plate (90 mm in diameter and 60 mm in depth) using a hot plate (Nissin NHP-M30N, Japan).

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After washing with distilled water, photomicrographs were taken. A microscope (Olympus BH-2, Japan) and a camera (Shimadzu Moticam 1000, Japan) were used to take the photomicrographs.

3. RESULTS

3.1. Nuclear staining of the mycelial mat treated with colchicine

A piece of the original mycelial mat grown on a PDA plate (20 mm x 10 mm) was added into the medium for colchicine treatment followed by incubation at 26 °C for 30 days. When the treated mycelia were stained with Giemsa solution, various globular structures were visible, as shown in Fig. 1b. The diameters of the globular structures were larger than that of the original nucleus, as shown in Fig. 1a, and were stained reddish purple. Those globular structures were therefore regarded to be autopolyploid nuclei. Such mycelia were named multinuclear autopolyploids.

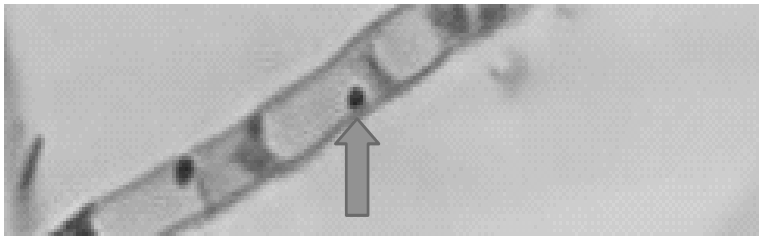
3.2. Nuclear staining of the conidia derived from the colchicine-treated mycelial mat

A piece of the colchicine-treated mycelial mat (3 mm x 3 mm) was put on a PDA plate and incubated at 26 °C for 14 days in order to form conidia. The conidia were treated with HCl at 50 °C for 15 minutes and photomicrographed after washing with distilled water. It appeared that an autopolyploid nucleus was present in each conidium, as shown in Fig. 2b.

The diameter of each nucleus was larger than that of the original nuclei, as shown in Fig. 2a. Various sizes of autopolyploid nuclei still existed in the mycelial mat, as shown in Fig. 3.

DISCUSSION

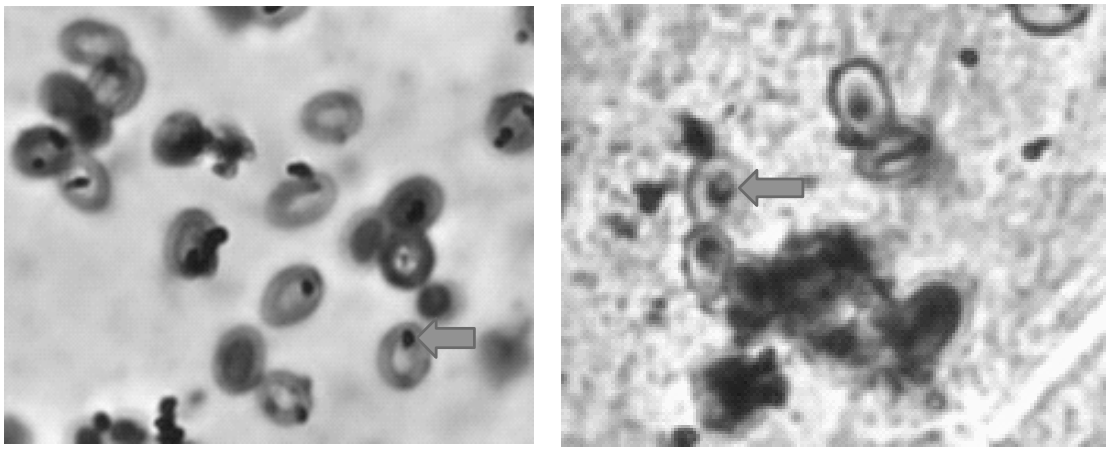
The initial question was why only a single autopolyploid nucleus is present in each conidium. It was suspected that this phenomenon occurred because the conidia of this fungus are mononucleate [3]. Next, it was discussed whether or not this phenomenon is useful. The fungus *Trichoderma reesei* is widely used for the industrial production of cellulase which is used for bioethanol production from cellulose [4, 5]. But, one of the bottlenecks with respect to the production of biofuel is the cost of cellulase [6]. Therefore, various studies have been attempted with the purpose of enhancing the cellulase productivity of this fungus [7]. The author also reported that a mycelial mat containing autopolyploid nuclei could enhance the degrading ability of microcrystalline cellulose in *Trichoderma reesei* [8]. The conidia described in this report will make the selection of a genetically



a

b

Fig. 1. a. The mycelium of *Trichoderma reesei* QM 6a after nuclear staining. The arrow indicates an original nucleus. **b.** The mycelium of the colchicines-treated strain after nuclear staining.



a **b**

Fig. 2. a. The original conidia after nuclear staining. The arrow indicates an original nucleus. **b.** The conidia derived from the colchicine-treated strain after nuclear staining. The arrow indicates an autopolyploid nucleus.

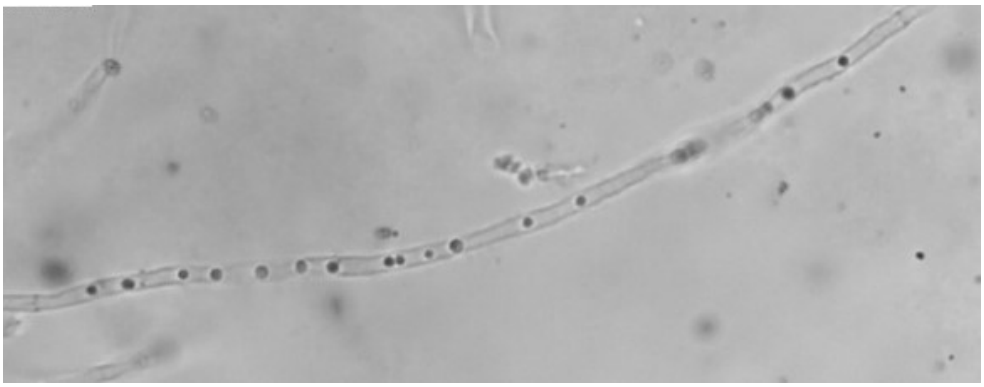


Fig. 3. The nuclear staining of the mycelium derived from the colchicine-treated strain after incubation for 14 days at 26 °C.

stable autopolyploid strain of this fungus easier. So, it was concluded that this technique is useful for enhancing the cellulase productivity of this fungus.

CONCLUSION

From the above point of view, it was concluded that a new breeding technique could be developed for *Trichoderma reesei* strain.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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