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ENHANCEMENT OF MICROCRYSTALLINE CELLULOSE DEGRADINGABILITY IN *Lentinula edodes* BY AUTOPOLYPLOIDIZATION AT REDUCED TEMPERATURE

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ABSTRACT

This study aimed to enhance the degrading ability of microcrystalline cellulose in the mycelia of the mushroom *Lentinula edodes*. The mycelia were incubated in an autopolyploidization medium containing colchicine for more than 1 week at low temperature. After incubation, many larger nuclei (autopolyploid nuclei) were observed in the mycelia.Mycelial mats were then incubated on agar medium containing 0.5% (w/v) microcrystalline cellulose for 2 weeks at 26 °C, followed by incubation at 50 °C. Under these conditions, almost all of the microcrystalline cellulose in the agar medium was degraded by the colchicine-treated strain, and the white agar plate turned transparent.

This phenomenon was not observed in the original strain.Similarly, *Trichoderma reesei* QM 9414 strain produced no transparent zone under the same incubation conditions.It appears that the degrading ability of microcrystalline cellulose can be enhanced in *L. edodes* by autopolyploidization at reduced temperatures.

1. INTRODUCTION

Lentinula edodes is an edible mushroom that is widely cultivated in East Asia.Known as Shiitake, this mushroom is a good source of vitamin D, eritadenine, and dietary fiber [1-3]. Previously, the author has developed techniques to induce autopolyploid nuclei in *L. edodes* and a cellulolytic fungus, *Trichoderma reesei*, which is widely used in industrial cellulase production. Stable autopolyploid nuclei formed in the Shiitake strain under autopolyploidization conditions at reduced temperature [4]. In *T. reesei* QM 9414, transparent zones appeared in agar plates containing 1.0% (w/v) microcrystalline

cellulose under the same incubation conditions [5].The original *T. reesei* QM 9414 strain (lacking autopolyploid nuclei) was unable to produce transparent zones on these agar plates.In the previous study, autopolyploidization was shown to enhance the microcrystalline cellulose degrading ability of *T. reesei* QM 9414 at lower incubation temperatures.However, Shiitake strain possesses cellulose and lignin degrading ability, indicating a more versatile application potential than is possible for *T. reesei*[6, 7]. In this study, the author seeks to increase the microcrystalline cellulose degrading ability ofShiitake strain more, incubated at reduced temperature.

2. MATERIALS AND METHOD

2.1 Microorganisms and media

The model strains *Lentinula edodes* (IFO 30724) and *Trichoderma reesei* QM 9414 (IFO31329) were used in the study. The strains were incubated on Potato dextrose agar (PDA) medium at 26 °C and preserved at 4 °C. The autopolyploidization medium for *L. edodes* comprised Czapek medium (2.0 g/l $NaNO_3$, 1.0g/l K_2HPO_4, 0.5 g/l $MgSO_4•7H_2O$, 0.5 g/l KCl, 0.01 g/l $FeSO_4•7H_2O$, and 1000ml distilled water (pH 6.0)) supplemented with 0.25 g glucose, 0.13 g bactopeptone (BD), and 0.025 g colchicine (Wako). The supplements were added to 25 ml Czapek medium in a 50 ml Erlenmeyer flask. The medium for testing the microcrystalline cellulose degrading ability of *L. edodes* comprised 2.0 g or 4.0 g microcrystalline cellulose (Merck), 4.0 g malt extract, 0.8 g yeast extract, and 6.0 g agar (BD). The ingredients were added to 400 ml distilled water in a 1000 ml Erlenmeyer flask (pH 6.0). The medium for testing the cellulose degrading ability of *T. reesei* was the same, except that 2.0 g microcrystalline cellulose was added instead of4.0 g in Mandels's medium [8].

2.2 Autopolyploidization at reduced temperature

To enable colony growth, a mycelial mat (2.0 mm × 2.0 mm) was placed on the PDA plate and incubated for 10 days at 26 °C.Following incubation, mycelial mats (2.0 cm × 2.0 cm) were cut from the colony, added to the autopolyploidization medium and incubated statically for 8 weeks at 15 °C.

2.3 Nuclear staining

To stain the nuclei, a mycelial mat (4.0 mm × 4.0 mm) was saturated with Giemsa solution for 10 min on a glass slide. After staining, the mycelial mat was rinsed with distilled water and photographed under the microscope (Olympus BH-2) and Moticam 1000 (Shimadzu).A mycelial mat (4.0 mm × 4.0 mm) was also stained with DAPI (4.6’-

diamidino-2-phenylindol) solution (Sigma) on a glass slide for fluorescence microscopy observation (Olympus BX-50). It was confirmed that the structure stained with Giemsa solution was also stained with DAPI solution.

2.4 Estimation of microcrystalline cellulose degrading ability

To estimate the microcrystalline cellulose degrading ability of *L. edodes* and *T. reesei*, a mycelial mat (2.0 mm × 2.0 mm) of each strain was incubated on the cellulose-supplemented medium for 14 days at 26 °C. Following cellulose degradation, the plate was heated at 50 °C for 72h in an incubator (LAB-LINE 120).A transparent zone was observed on the heated plate.

3. RESULTS

3. 1 Autopolyploidization at reduced temperature and microcrystalline cellulose degrading ability

Mycelial mats of the original and colchicine-treated strains of *L. edodes* were placed on the medium supplemented with 0.5% (w/v) microcrystalline cellulose, and incubated for 2 weeks at 26 °C followed by incubation for 72 h at 50 °C.As shown in Fig. 1, the original strain could not degrade microcrystalline cellulose, and no transparent zone was observed on the plate.The colchicine-treated strain, on the other hand, degraded almost all of the microcrystalline cellulose in its vicinity.

Next, a mycelial mat of *T. reesei* QM 9414 was placed on the medium supplemented with 0.5% (w/v) microcrystalline cellulose and incubated under the same conditions as for *L. edodes* (i.e. 2 weeks at 26 °C followed by incubation for 72 h at 50 °C). As shown in Fig. 2, this strain produced no transparent zone, indicating that it could not degrade cellulose.Thus, the microcrystalline cellulose degrading ability of the colchicine-treated *L. edodes* strain is demonstrably higher than that of *T. reesei* QM 9414. The above cellulose-degradation experiment was repeated on medium supplemented with 1.0% (w/v) microcrystalline cellulose.As before, no transparent zone appeared in the plate containing the original strain, whereas almost all of the microcrystalline cellulose was degraded in the plate containing the colchicine-treated strain (see Fig. 3).

3. 2 Nuclear staining of the mycelia

As shown in Fig. 4, the mycelia of the colchicine-treated strain contained many enlarged nuclei. Since these larger nuclei were also stained with DAPI solution, they were assumed as autopolyploid nuclei (data not shown). Next, the nuclear diameters of more than 100 nuclei were measured using a

digital caliper on enlarged photomicrographs, and the results are plotted as histograms in Fig. 5.

As shown in that figure, the mycelia of the colchicine-treated strain contained a higher proportion of larger nuclei than those of the original strain.

4. DISCUSSION

It appears that *L. edodes* can form stable autopolyploid nuclei under reduced temperature conditions. When the mycelia of *L. edodes* were incubated in colchicine solution at its optimal growth temperature (nearly 26 °C), autopolyploid nuclei were transiently formed, but collapsed into micronuclei [9]. This phenomenon could be related to nuclear division.Under lower-temperature colchicine treatment, stable formation of autopolyploid nuclei became possible, likely because nuclear division is slowed at lower incubation temperatures. It was also found that the colchicine-treated strain degraded 0.5% – 1.0% (w/v) microcrystalline cellulose, to form a transparent zone after heating.When the mycelial mat of *L. edodes* was incubated on the plate containing microcrystalline cellulose, a colony developed on the plate and accumulated enzymes associated with microcrystalline cellulose degradation in the agar medium.Because the optimum temperature of the cellulose degradation enzymes in *L. edodes* is 50 °C, when the plates are incubated at that temperature, the enzymes begin to degrade microcrystalline cellulose in the agar medium. A transparent zone will emerge only if sufficient enzyme is produced.Therefore, it was concluded that the colchicine-treated strain produces more microcrystalline cellulose degradation enzymes than the original strain. Furthermore, the degrading ability of microcrystalline cellulose can be enhanced by increasing the number of autopolyploid nuclei in *L. edodes* mycelia.

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Figures.

Fig. 1 Degradation of 0.5% (w/v) microcrystalline cellulose by *L. edodes* strains.

Left: Original strain **Right:** Colchicine-treated strain Mycelial mats were incubated on the microcrystalline plates for 2 weeks at 26 °C followed by incubation for 72 h at 50 °C. Bar indicates 1.0 cm.

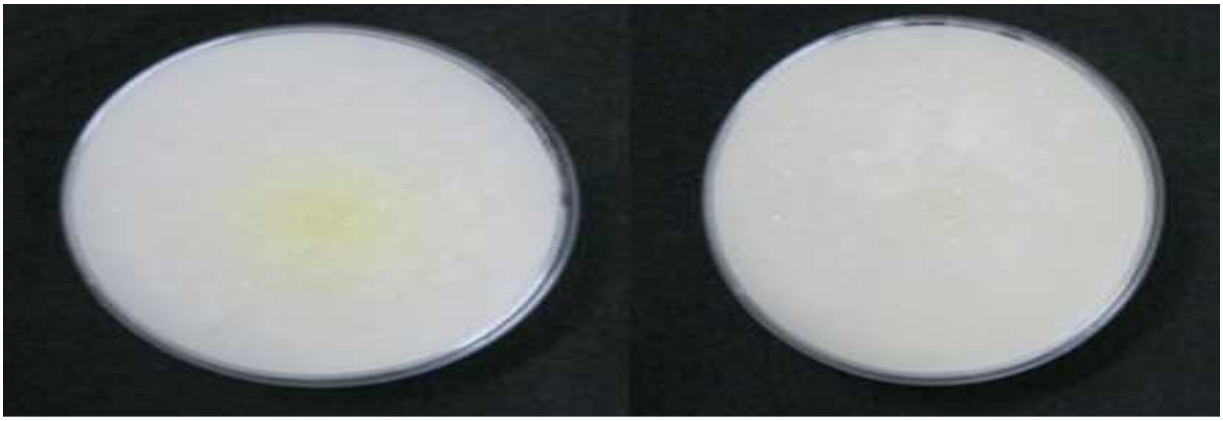

Fig. 2 Degradation of 0.5% (w/v) microcrystalline cellulose in the *T. reesei* QM 9414 strain.
Left: Before heating **Right:** After heating for 72h at 50 °C
Mycelial mats were incubated on the 0.5% (w/v) microcrystalline plates for 2 weeks at 26 °C followed by heating for 72 h at 50 °C. Bar indicates 1.0 cm

Fig. 3 Degradation of 1.0% (w/v) microcrystalline cellulose in *L. edodes* strains.
Left: Original strain **Right:** Colchicine-treated strain
Mycelial mats were incubated on the microcrystalline plates for 2 weeks at 26 °C followed by incubation for 72h at 50 °C. Bar indicates 1.0 cm.

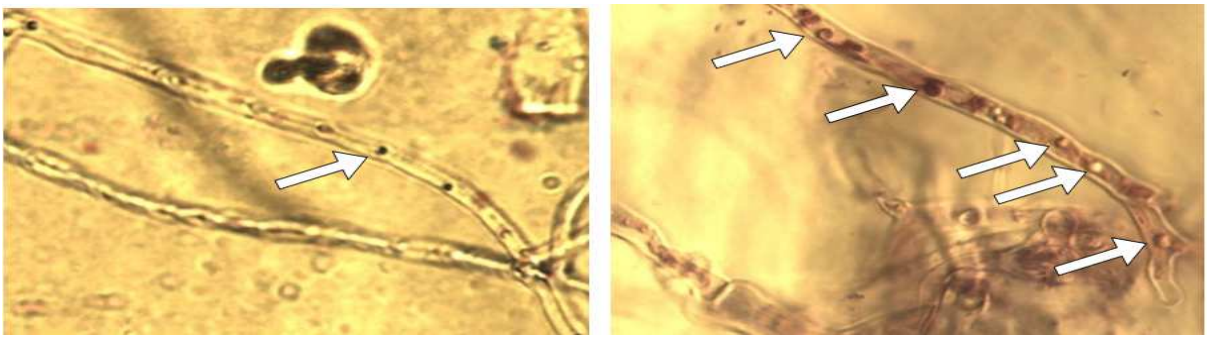

Fig. 4 Nuclear staining in the mycelia.
Left: Original strain **Right:** Colchicine-treated strain
Nuclear staining was carried out using Giemsa solution. Nuclei are indicated by arrows

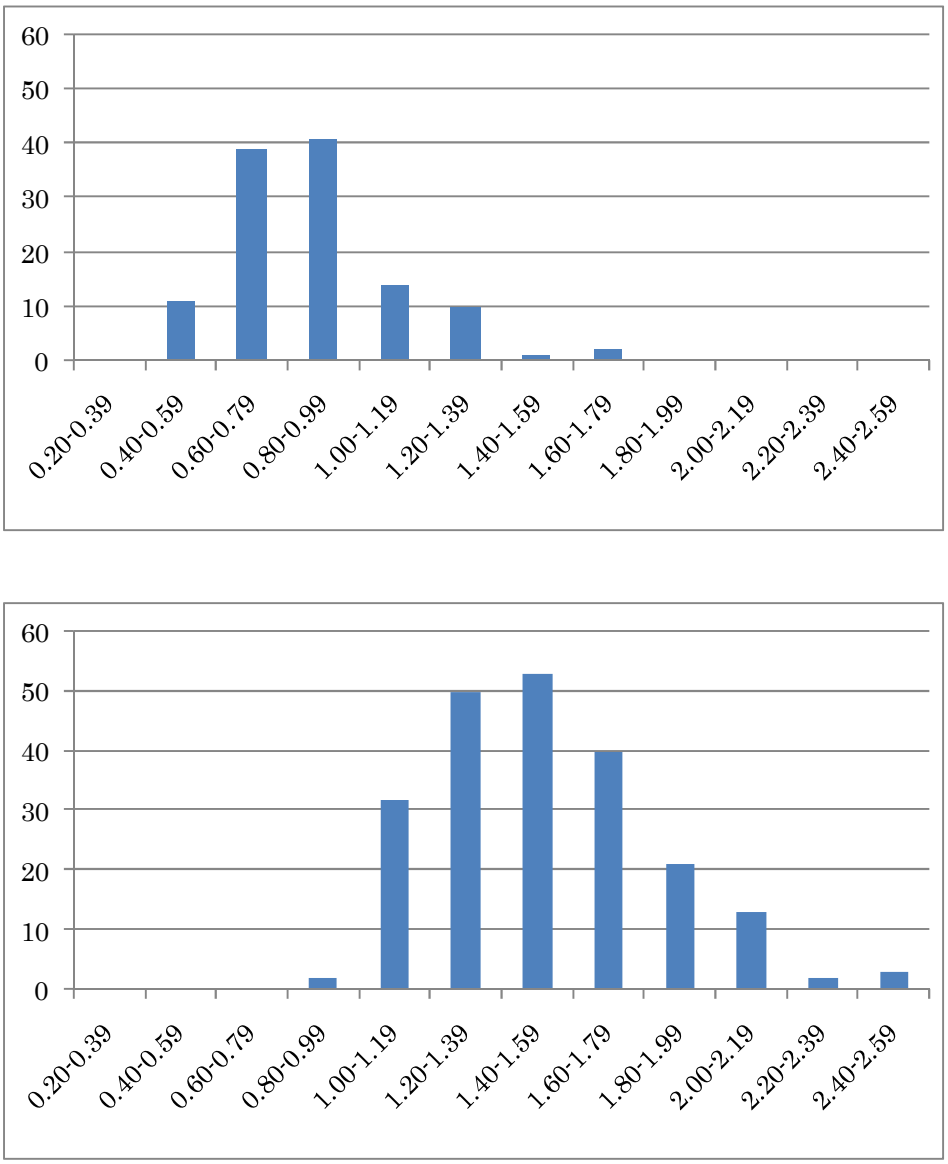

Fig. 5 Comparison of the distribution of nuclear diameter.

Upper : Before colchicine treatment

Bottom : After colchicine treatment Nuclear diameters (μm) of more than 100 nuclei were measured by a digital caliper on enlarged photomicrographs. The size distributions are plotted as histograms.