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Biobleaching of Hardwood Kraft Pulp with Cellulase-Deficient Mutant from Hyper Ligninolytic Fungus IZU-154

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ABSTRACT

Protoplasts of the monokaryotic strain from hyperligninolytic fungus IZU-154 were treated with UV irradiation, and the regenerants were screened for their inability to degrade Walseth cellulose (WC) and carboxymetylcellulose (CMC) on an agar plate. From 2700 regenerants, cellulase-deficient and noncellulase mutants were isolated and designated Cel-139 and Cel-145, respectively. Cel-139 showed 15% Avicelase, 60% CMCase, and 10% β-glucosidase activities; however, it retained the same level of lignin degrading capability and manganese peroxidase (MnP) production when compared with wild-type IZU-154. On the other hand, Cel-145 showed deterioration in lignin-degrading capability, despite a substantial level of MnP production. Furthermore, biobleaching of hardwood kraft pulp (HWKP) by wild-type IZU-154 and Cel-139 in the solid-state fermentation system, without supplemental nutrients, were investigated. After 5 d of treatments with both fungi, pulp brightness increased from 33 to 65% ISO brightness, and Kappa number decreased from 13.9 to 6.0. However, yield loss of biobleached pulp was 20% lower with Cel-139 than with wild-type IZU-154.

Index Entries: White rot fungus; lignin degradation; mutant; cellulase; biobleaching.

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INTRODUCTION

Multistage bleaching processes consisting of chlorination and alkaline extraction stages are conventionally used in the bleaching of kraft pulp. However, there have been growing environmental concerns, because the effluents from such bleaching processes contain chlorinated organic substances, including toxic, mutagenic, and carcinogenic polychlorinated dioxins, dibenzofulans, and phenols *(1)*.

Biobleaching with white rot fungi, including *Trametes versicolor* and IZU-154, which can selectively degrade lignin in wood *(2)*, has been studied to establish nonchlorine or chlorine-free bleaching processes *(3–5)*. However, the delignification rate in the biobleaching process is rather slow compared with chemical bleaching, and the cellulose in the pulp is also degraded by these fungal treatments. To overcome these drawbacks, therefore, direct application of ligninolytic enzymes, such as lignin peroxidase (LiP) *(6)*, manganese peroxidase (MnP) *(7)*, and laccase *(8)*, has been attempted. However, so far, application of these enzymes has not been practicable, since the cost of enzymes is still high. On the other hand, cellulolytic mutants of white rot fungus were isolated for selective lignin degradation *(9)*. Furthermore, the biobleaching of pulp by several cellulase-deficient and noncellulose mutants from *Phanerochaete chrysosporium* has been attempted, but a significant increase in brightness was not obtained by these mutants under the aerobic, agitated, liquid conditions *(10)*.

Previously, one of the authors showed that IZU-154 has a higher potential for delignification and brightening of hardwood kraft pulp (HWKP) than *T. versicolor* and *P. chrysosporium (4)*. This study isolated cellulase-deficient and noncellulase mutants from mycelial protoplasts of IZU-154, and applied these mutants in biobleaching studies of HWKP. The authors report here on the performance of delignification and brightening of HWKP by these mutants. One of the cellulase-deficient mutants showed a higher pulp yield than wild-type IZU-154, whereas the same levels of brightening and delignification were obtained in the solid-state fermentation system without supplemental nutrients.

MATERIALS AND METHODS

Microorganisms and Medium Conditions

The fungi used were hyperligninolytic fungus IZU-154, IZU-154-22, which is a monokaryotic strain derived from IZU-154 *(11)*, and *T. versicolor* IFO-6482. Potato dextrose agar (PDA) (Difco, Detroit, MI) was used for maintaining the vegetative culture of strains. A CSL-glucose medium con-

taining 1.3% corn-steep liquor, 1.8% glucose, at pH 4.5, was the grown medium of the inoculum mycelia for MnP production and biobleaching of HWKP. A low-nitrogen (N) medium *(12)* for MnP production consisted of 1.0% glucose as the carbon (C) source, 1.2 m*M* ammonium tartrate as the nitrogen source, 20 m*M* tartrate (pH 4.5) as the buffer, and 0.2 m*M* $MnSO_4$ as the inducer of MnP. A nutrient-salt (NS) medium *(9)* with 2% Avicel as the C source was employed for cellulase production. The NS medium contained 4 g/L $(NH_4)H_2PO_4$, 2 g/L K_2HPO_4, 5.0 mg/L $ZnSO_4 \cdot 7H_2O$, 1.0 mg/L $MnSO_4 \cdot 4H_2O$, 1.0 mg/L $CuSO_4 \cdot 5H_2O$, 5.0 mg/L ferric citrate, 0.1 mg/L thiamine, 20.0 mg/L $CaCl_2$, 0.89 g/L $MgSO_4 \cdot 7H_2O$. The last two ingredients were dissolved separately.

Mutagenesis and Screening of Cellulolytic Mutants

Mutagenesis was done by UV irradiation on the mycelial protoplasts of IZU-154-22, as described previously *(11,13)*. On the basis of lethal curve, the treatment time was adjusted to give 1% survival. Single colonies were isolated on PDA from regenerants of mutagenized protoplasts. Each strain was subcultured onto an NS agar plate overlaid with membrane filter (0.45 μm pore size, Durapore filter, Millipore, Bedford, MA). These media contained either 0.25% Walseth cellulose (WC) or 0.5% and carboxymetylcellulose (CMC) as the sole C source. After incubation at 30°C for 3–5 d, degradation of WC and CMC were checked, respectively, by degradation clear zone and Congo red staining *(14)*. After that, non-WC-degrading mutants were subcultured on an NS agar plate containing 0.5% xylan as the sole C source. Xylanase activity was also checked by degradation clear zone after 5 d incubation at 30°C.

Biobleaching of HWKP by IZU-154 and its Mutant

Biobleaching in the solid-state fermentation system without supplemental nutrients was performed. Mycelia grown on a PDA plate at 30°C for 5 d were cut off (1 × 1 cm) with a sterile knife into small pieces, and inoculated into 50 mL of CSL-glucose medium in a 300-mL Erlenmeyer flask. Cultivation was carried out at 30°C for 3 d, with rotary shaking. After that, mycelial pellets were separated from the medium, and were fragmented with 50 mL sterile water for 30 s. To determine inoculum volume, the dry wt of 5 mL mycelial suspension was measured with tarred glass filter GS25 (Advantec, Tokyo, Japan). The drying condition was at 120°C for 1 h.

Ten g of HWKP (brightness 33% ISO brightness, Kappa number 13.9) were sterilized, together with 10 mL water, in a 300-mL Erlenmeyer flask. The pulp, to which total volume of 30 mL water and the mycelial fragment solution (about 3 mL of mycelial suspension; 60 mg dry wt) were added, was incubated statically at 30°C.

Determination of Pulp Brightness, Kappa Number, and Yield

Pulp brightness and Kappa number were determined as described previously *(16)*. The pulp yield was determined by the dry wt of biobleached pulp. After incubation with fungi, the whole pulp sample in an Erlenmeyer flask was washed with 2000 mL deionized water, and a pulp sheet was prepared on a tarred filter paper with a Buchner funnel (diameter, 140 mm). The dry wt was measured after overnight drying at 105°C.

Treatment of Wood by IZU-154 and Its Mutant

One gram of extractive-free beech (*Fagus* sp.) wood powder (60–80 mesh) and 2.5 mL distilled water were added to a 50-mL Erlenmeyer flask. A disk (5-mm diameter) of mycelial mat, cultured at 30°C for 5 d on PDA, was inoculated and incubated at 30°C for 2 wk. Klason lignin content, acid-soluble lignin content, and dry wt were determined as previously described *(2)*.

Enzyme Productions

Cultivation for cellulase production was carried out as follows: Mycelial pellets, cultured with 50 mL CSL-glucose medium in a 300-mL Erlenmeyer flask at 30°C for 3 d, were homogenized. After that, 0.6% (dry wt/v) fragmented mycelial suspension was inoculated into 4 mL NS medium in a test tube. Cultivation was carried out at 30°C for 6 d, with reciprocal shaking. Cultivation for MnP production was carried out as described previously *(13)*.

Enzyme Assays

Cellulase activity was measured by the reducing sugar formed from each substrate, Avicel for Avicelase, CMC for CMCase, and salicin for β-glucosidase, as described previously *(14)*. All cellulase activities were shown as international units (IU). IU means the amount of enzyme producing 1 μmol of reducing sugar/min.

MnP activity was measured by 2,6-dimethoxyphenol (2,6-DMP) oxidation. One katal of MnP activity is defined as the amount of MnP that oxidizes 1 mol of 2,6-DMP/s at 30°C *(12)*.

Detection of CMCase in Isoelectric Focusing Gel

CMCase in isoelectric focusing gel was detected by CMC containing thin-layer gel overlay methods *(15)*. Following electrophoresis, gels were rinsed with 0.1 *M* acetate buffer (pH 5.0). After that, the gels were overlaid with thin film (approx 1 mm) of 1% agarose in 0.1 *M* acetate buffer (pH 5.0)

containing 0.5% CMC, and incubated at 37°C for 12 h. The thin agarose films were prepared on Gelbond (Pharmacia Biotech, Uppsala, Sweden). The activity was detected by Congo-red staining.

RESULTS

Mutant Production

From the 2700 UV irradiated single-protoplast-derived mycelial colonies, 11 mutants, unable to produce a clear zone on an NS agar plate containing WC within 5 d incubation, were isolated as non-WC-degrading mutants; wild-type IZU-154 produced the clear zone within 3 d. The HWKP was incubated with each mutant in the solid-state fermentation system at 30°C for 5 d. As shown in Table 1, a substantial level of brightness increase was obtained by four mutants having xylanase activity. However, the non-CMC-degrading mutant, Cel-145, had a lower potential for brightening than the other three mutants. Among these mutants, Cel-139 showed almost the same level of brightening as wild-type IZU-154, and a higher pulp yield than wild-type IZU-154, indicating that Cel-139 can degrade lignin more selectively than cellulose and hemicellulose in the pulp, compared with wild-type IZU-154. This was confirmed with the data summarized in Table 2. In this experiment, the beech wood powder was incubated with Cel-139, Cel-145, wild-type IZU-154, or *T. versicolor* at 30°C for 2 wk. After incubation, the Klason lignin and holocellulose contents of the wood powder were determined. Two strains, Cel-139 and IZU-154, had over 30% Klason lignin losses, which are greater than the 14.2% shown by the typical ligninolytic fungus *T. versicolor.* The Klason lignin and holocellulose losses by Cel-139 were 11.3 and 30.9% lower than those by wild-type IZU-154, respectively. These results indicate that the deterioration in cellulolytic activity of Cel-139 is much greater than that in ligninolytic activity, and that Cel-139 can degrade lignin more selectively than holocellulose, compared with wild-type IZU-154; Cel-145 degraded negligible amounts of Klason lignin and hollocellulose. Cel-139, together with other isolates, had a lower radial growth rate, which was about 75% of that of the wild-type IZU-154, and formed a thin mycelial mat on PDA (data not shown).

Production of Cellulolytic and Ligninolytic Enzymes by Isolates

Cellulolytic enzymes production of Cel-139, Cel-145, and wild-type IZU-154 were investigated. The 3 and 6 d culture fluids of NS medium were prepared as enzyme solutions. The concentrations of protein were measured with the protein assay kit (Bio-Rad, Richmond, CA). The concentrations of 3 and 6 d samples were about 60 μg/mL and 70 μg/mL,

Table 1
Phenotype of Selected Cellulolytic Mutants of IZU-154

Isolate no. (strain)	CMC[a] degrading	Xylan[b] degrading	Pulp bleaching	
			Brightness increase (ISO point)	Pulp yield (%)
4-1-80	+	−	−	N.D.[c]
4-17-133	++	+++	28.0	93.7
5-18-26	+	−	−	N.D.
5-18-32	+	−	−	N.D.
5-22-5	++	+	30.1	94.6
5-22-139 (Cel-139)	+	+	33.4	95.7
6-1-13	+	−	−	N.D.
6-1-24	+	−	−	N.D.
6-1-31	+	−	−	N.D.
6-1-37	+	−	−	N.D.
10-1-145 (Cel-145)	−	+	14.7	95.5
Wild-type (IZU-154)	++	++	32.5	94.4

[a] CMC degrading activity was determined from the Congo red staining halo on NS agar plate containing 0.5% CMC after 5 d incubation.
−, no reaction; +, 0–20 mm; ++, 20–40 mm; +++, 40–60 mm
[b] Xylan degrading activity was determined from the diameters of clear zone on NS agar plate containing 0.5% xylan after 5 d incubation.
−, no reaction; +, 0–20 mm, ++, 20–40 mm; +++, 40–60 mm
[c] N.D., not determined.

Table 2
Degradation of Beech Wood Powder by Isolates

Strain	Lignin content (%)[a,b]			Weight (%)[a,b] loss	Klason lignin loss (%)	Holocellulose loss (%)
	Klason lignin	Acid soluble lignin	Holocellulose[a,b] content (%)			
IZU-154	13.5	4.5	69.8	12.2	36.6	6.8
Cel-139	14.4	4.6	71.4	9.6	32.4	4.7
Cel-145	21.0	4.1	74.5	0.4	1.4	0.5
T. versicolor	18.3	3.8	67.7	10.1	14.2	9.5
Control[c]	21.3	3.8	74.9	–	–	–

[a] Data are the mean of three replicates.
[b] Based on untreated beech wood powder.
[c] Untreated beech wood powder.

Table 3
Production of Cellulolytic Enzymes by Cel-139 and Wild-Type IZU-154

Strain	Cultivation period (d)	Protein[a] concentration (mg/mL)	Cellulolytic activity (IU/mL)[a]		
			Avicelase	CMCase	β-glucosidase
IZU-154	3	61.5 ± 5.7	0.8 ± 0.5	6.4 ± 1.3	0.7 ± 0.2
	6	67.4 ± 5.5	8.5 ± 1.2	21.4 ± 1.9	8.8 ± 1.3
Cel-139	3	59.0 ± 5.1	0.6 ± 0.3	5.6 ± 1.5	0.4 ± 0.2
	6	72.7 ± 3.4	1.2 ± 0.3	13.0 ± 1.8	3.1 ± 0.5

[a] Data are the mean of three replicates.

respectively, in both Cel-139 and wild-type IZU-154. As can be seen in Table 3, wild-type IZU-154 showed a higher level of cellulolytic activities than those of Cel-139. Wild-type IZU-154 produced the following cellulases after 6 d of cultivation: 8.5 IU/mL Avicelase, 21.4 IU/mL CMCase, and 8.8 IU/mL β-glucosidase. On the other hand, Cel-139 showed 15% Avicelase, 60% CMCase, and 10% β-glucosidase activities, compared to wild-type IZU-154. Cel-145 showed negligible growth in the same culture conditions. Therefore, extracellular protein and cellulolytic activities were undetected.

To investigate how cellulolytic activities deteriorated in Cel-139, isozymes of CMCase were compared with those of wild-type IZU-154. The 6-d samples for cellulase assay (Table 3) were concentrated by ultrafiltration (Centricon-10 unit, Amicon, Bedford, MA), and 30 μg protein of each sample were separated onto an isoelectric focusing gel, and then CMCase activity was detected by thin-layer CMC agar overlay methods *(14)*. At least three active bands were observed (pI 3.5, 4.5, and 5.2) in wild-type IZU-154; only two bands, pI 4.5 and 5.2, were still observed, but others were undetected, in Cel-139 (Fig. 1).

Wild-type IZU-154 can produce MnP, but not detectable LiP and laccase, in ligninolytic conditions *(12)*. Furthermore, several recent studies have demonstrated that MnP plays an important role in delignification during the biobleaching of HWKP by white rot fungi *(7,16–18)*. Therefore, the authors investigated the MnP production of Cel-139, Cel-145, and wild-type IZU-154 (Fig. 2). In wild-type IZU-154, MnP activity was first detected after 1 d, and rose to maximum activity 5.2 nkat/mL after 2 d, and then declined after 3 d. The profile of MnP production by Cel-139 was very similar to that by wild-type IZU-154. On the other hand, Cel-145 produced a 50% level of MnP, compared to wild-type IZU-154.

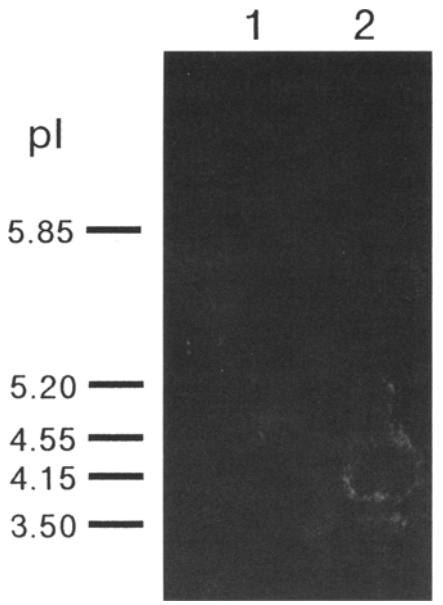

Fig. 1. CMCase isozymes on isoelectric focusing gel electrophoresis. Concentrated supernatants were separated on an isoelectric focusing gel Ampholine PAG plate (Pharmacia, Uppsala, Sweden) with a pH range of 3.0–7.0. Lane 1, Cel-139; Lane 2, wild-type IZU-154.

Biobleaching of HWKP by Cel-139

Biobleaching of HWKP by Cel-139 and wild-type IZU-154 were performed in a solid-state fermentation system without supplemental nutrients, and the changes in brightness, pulp yield, and Kappa number, which exhibits lignin contents in pulp, were examined. Figure 3 shows the pulp brightness increase during the treatment with both strains for 5 d of incubation. Marked brightness increases were obtained by Cel-139 and wild-type IZU-154 after 2 d of incubation, and the brightness increased by over 20 and 30 ISO points after 3 d and 5 d of fungal treatments, respectively. Figure 4A shows the relationship between pulp yield and pulp brightness increase, and Fig. 4B shows the relationship between pulp yield and Kappa number reduction during the treatments with Cel-139 and wild-type IZU-154. At a similar brightness and Kappa number, the pulp yield was higher with Cel-139 than with IZU-154. Therefore, it is concluded that Cel-139 selectively degrades residual lignin, rather than carbohydrate, in the pulp.

It is well known that culture conditions are critical to pulp brightness increase and pulp yield in biobleaching of kraft pulp *(7,19,20)*. The authors here investigated the effect of inoculum size on the biobleaching by wild-type IZU-154 and Cel-139. The brightness increased as the inoculum size increased, ranging from 0.15 to 0.6% (wt/pulp wt), and the brightness lev-

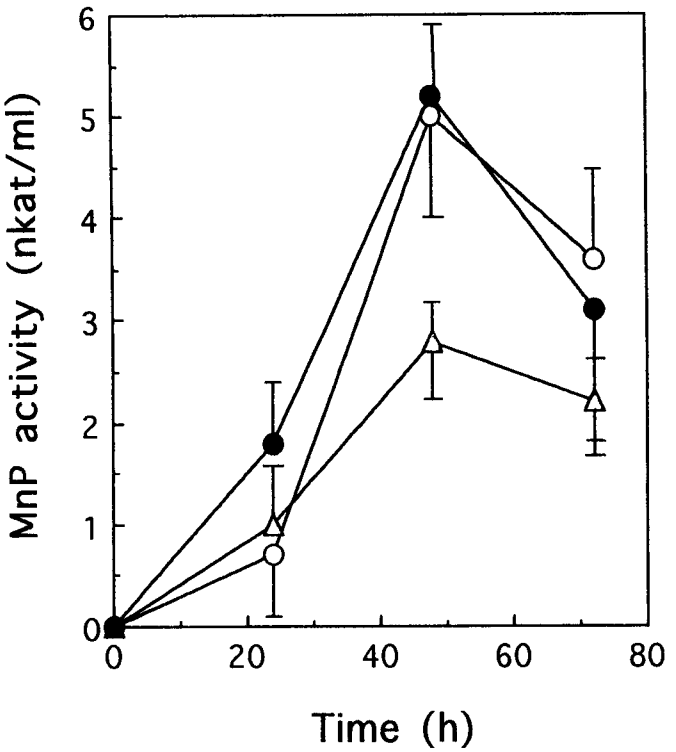

Fig. 2. Time-course of MnP activities of Cel-139, Cel-145, and wild-type IZU-154. ○, Cel-139; △, Cel-145; ●, wild-type IZU-154. Each point shows the mean of three replicates.

eled off at over 0.6% (wt/pulp wt) with both strains. Cel-139 showed a higher brightness increase than wild-type IZU-154 in the treatments under 0.3% (wt/pulp wt) inoculum size (Fig. 5A). In addition, Cel-139 showed a higher pulp yield than wild-type IZU-154 in the range between 0.15 and 1.2%, but pulp yield decreased as the inoculum size increased (Fig. 5B).

DISCUSSION

It is well known that white rot fungi can delignify and brighten unbleached kraft pulp (UKP); they also simultaneously degrade a portion of cellulose. Therefore, the pulp-yield loss and reduction in strength properties are brought about by their treatment. To overcome these drawbacks, noncellulase strains from *P. chrysosporium* have been isolated and evaluated in the biobleaching of HWKP *(10)*. However, these strains could not significantly brighten HWKP. The alternative solution is to control culture conditions, especially nutrient conditions, which are critical not only for a pulp yield, but also for a brightness increase in fungal treatments of UKP

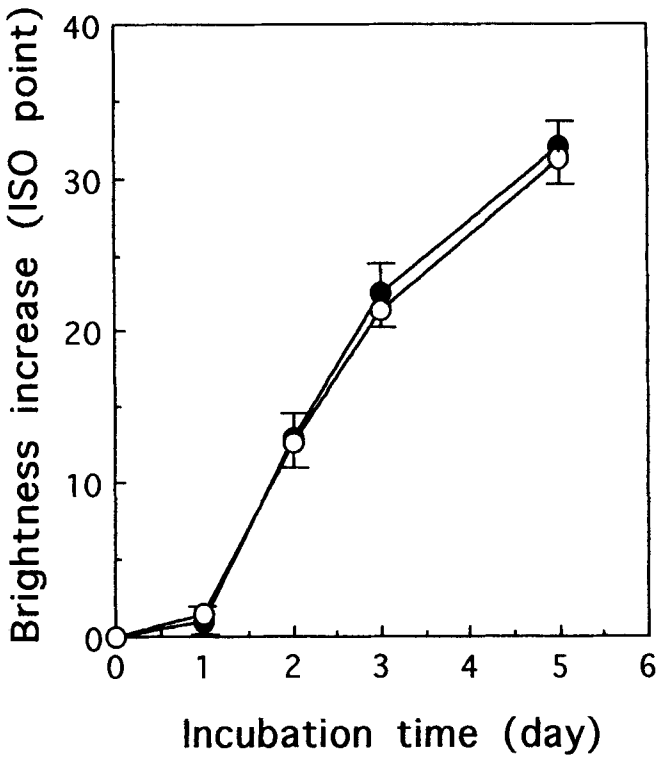

Fig. 3. Brightness increase of kraft pulp during biobleaching with Cel-139 and wild-type IZU-154. ○, Cel-139; ●, wild-type IZU-154. Each point shows the mean of three replicates.

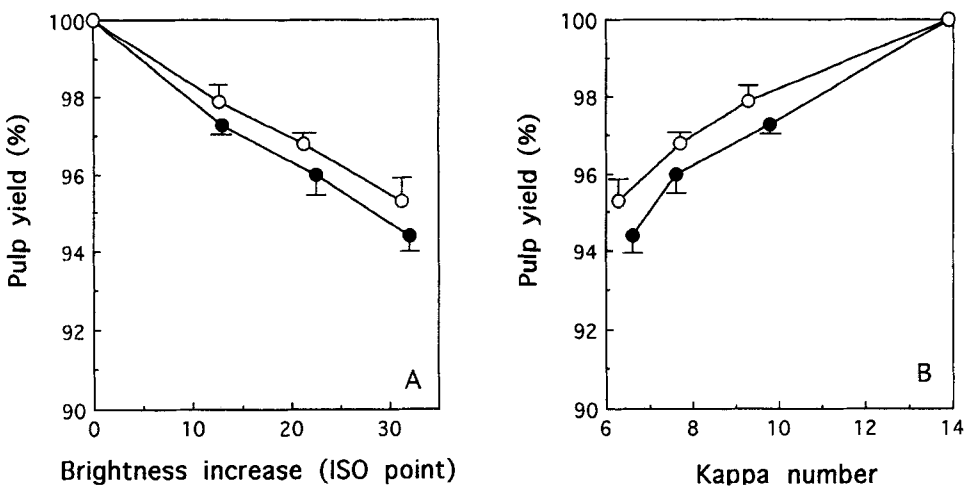

Fig. 4. The relationships between brightness increase, Kappa number, and pulp yield during the biobleaching by Cel-139 and wild-type IZU-154. **(A)** Relationship between brightness increase and pulp yield. **(B)** Relationship between Kappa number and pulp yield. ○, Cel-139; ●, wild-type IZU-154. Each point shows the mean of three replicates.

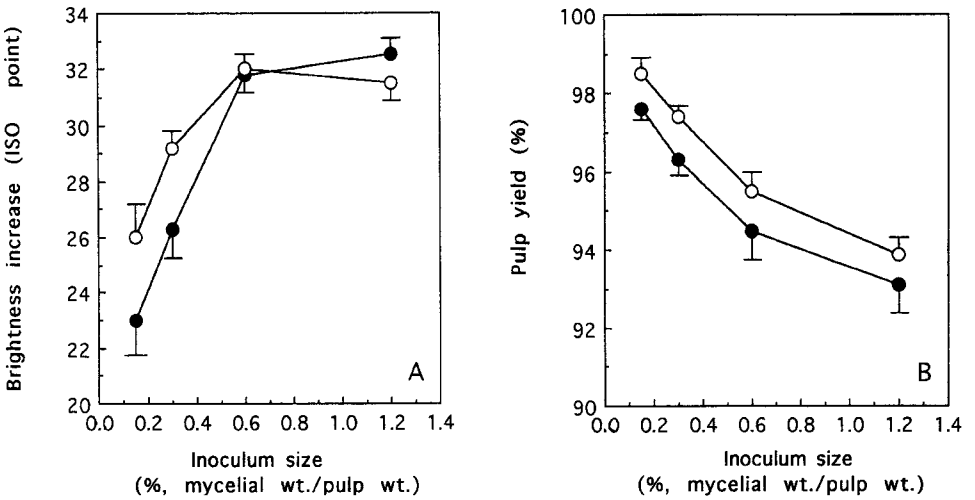

Fig. 5. The effect of inoculum size on brightness increase and pulp yield during the biobleaching by Cel-139 and wild-type IZU-154. Biobleaching was performed by 5-d treatment. **(A)** Effect of inoculum size on brightness increase. **(B)** Effect of inoculum size on pulp yield. ○, Cel-139; ●, wild-type IZU-154. Each point shows the mean of three replicates.

by white rot fungi *(7,19)*. Based on this concept, Kirk and Yang *(20)* supplemented 33% glucose (wt/pulp wt) to accelerate fungal growth and lignin degradation, and to suppress cellulose degradation in the biobleaching of softwood kraft pulp (SWKP) by *P. chrysosporium*. On the other hand, IZU-154, which was isolated in this laboratory *(2)*, showed a superior capability of brightening SWKP *(21)* and HWKP *(4)*, compared to typical white rot fungi such as *P. chrysosporium* and *T. versicolor*. Remarkably, IZU-154 can brighten HWKP over 30 ISO points for a 5-d treatment, without supplemental nutrients *(4)*. Therefore, in attempts to utilize IZU-154 on a more practical level, the authors intended to isolate a mutant that lacks cellulolytic activity, but not ligninolytic activity.

Eleven mutants were initially isolated as non-WC-degrading mutants. As shown in Table 1, the seven nonxylan-degrading mutants were incapable of brightening HWKP, but the other four xylan-degrading-positive mutants showed over 14 ISO points of brightness increase in the 5-d treatment of HWKP. Furthermore, in these xylan-degrading-positive mutants, the strain, which maintained some CMC-degrading activity, showed potential for brightening and delignifying HWKP when compared to the non-CMC-degrading strain, such as Cel-145. It has previously been demonstrated that lignin degradation by white rot fungi is an energy-requiring process, and that the energy required to degrade the lignin must be derived from more easily accessible energy sources, such as the wood

polysaccharides and low mol-wt sugars (22). Therefore, it might be possible that a significant brightness increase could be obtained with xylan-degrading-positive (xylanase-positive) and CMC-degrading-positive (endoglucanase-positive) mutants, such as Cel-139, which could gain the necessary energy for efficient lignin degradation.

At the same level of brightness increase and Kappa number reduction during the fungal treatments of HWKP, Cel-139 exhibited a higher pulp yield than wild-type IZU-154, even at any inoculum size (Figs. 4 and 5). This result suggests that Cel-139 degrades the carbohydrate portion of HWKP less than wild-type IZU-154. This was supported by the result that productions of cellulolytic enzymes by Cel-139 were much lower than those by wild-type IZU-154 (Table 3). Among the cellulolytic enzyme activity of Cel-139, Avicelase and β-glucosidase activities were decreased to 15 and 10%, respectively, compared to those of wild-type IZU-154, under similar protein secretion. These reductions of cellulolytic activity were more significant than that of CMCase, which decreased to 60% in the same experiment. This result suggests that exoglucanase and β-glucosidase activities decrease more specifically than the endoglucanase in the mutant.

Klason lignin loss after the fungal treatment of beech wood powder with Cel-139 was 11% lower than that with wild-type IZU-154 (Table 2). However, brightness increase and Kappa number decrease during the treatments of HWKP with both strains were almost the same (Fig. 4). Furthermore, Cel-145 shows substantial level pulp-brightening activity, in spite of the incapability of lignin degradation of beech wood powder (Tables 1 and 2). At least a part of this difference in delignification response between wood powder and HWKP is explained by the following reasons. First, the different contents of lignin in beech wood powder and HWKP were 24 and 3%, respectively, in this study. Therefore, the difference in lignin degradation activity of both strains might have been undetected in the case of biobleaching, because of the lower lignin content of HWKP. The second reason is the different state of lignin–carbohydrate complex in these objects. In HWKP, most of the lignin had been removed through the kraft pulping process, resulting in the structure of HWKP fiber becoming more porous. Therefore, hypha of fungi can easily penetrate into the pulp fiber and gain access to residual lignin. On the other hand, because wood has a rigid structure and its lignin is surrounded by wood carbohydrates, fungi cannot easily gain access to lignin in wood without cellulose degradation. Furthermore, lignin is degraded along with the degradation of its surrounding carbohydrates. It is possible that lower cellulose degradation activity of Cel-139 suppresses the simultaneous lignin degradation activity in beech wood degradation.

It has been widely reported that MnP plays an important role in the biobleaching of HWKP by white rot fungi (7,16–18). IZU-154 produces MnP

as a major extracellular protein and negligible LiP and laccase in a supernatant of ligninolytic liquid culture *(12)*. This result indicates that lignin degradation by IZU-154 is caused mainly by MnP. Cel-139 and IZU-154 showed the same level of MnP production (Fig. 2); therefore, no difference in brightening and delignification by either strain was observed in the biobleaching study (Figs. 3 and 4). Shown here is the isolation of a high pulp yield biobleaching strain, Cel-139, from IZU-154 by isolating cellulase-deficient mutants. On the other hand, pulp yields were decreased corresponding to the fungal treatment periods and inoculum size by both wild-type IZU-154 and the mutant (Figs. 4 and 5). From these results, hyper MnP producing mutants, which could brighten pulp during a shorter incubation period, or by a lower inoculum size, would be expected for an efficient biobleaching process. The authors have already isolated hyper MnP-producing mutant, IZU-882, from wild-type IZU-154 *(13)*; therefore, the isolation of cellulase-deficient mutants from IZU-882 should be the subject of future studies, to establish an industry-applicable biobleaching process that gives pulp both a higher brightness and a higher yield.

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