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Decolorization of kraft pulp bleaching effluent by a newly isolated fungus, *Geotrichum candidum* Dec 1

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Abstract Kraft pulp bleaching effluent supplemented with glucose was decolorized by a newly isolated fungus *Geotrichum candidum* Dec 1 (Dec 1) that showed a wide decolorizing spectrum to synthetic dyes. When the glucose concentration in the effluent was 30 g/l, the color removal and the reduction of absorbable organic halogens were 78% and 43% after 7 days culture, respectively. The average molecular weight of colored substances measured by gel filtration chromatography was lowered to less than 3000 from 5600 after 7 days culture. As the contribution of extracellular enzymes such as peroxidase (DyP), manganese peroxidase, and laccase to the decolorization of the kraft pulp bleaching effluent was small, Dec 1 appears to have a different mechanism of decolorizing kraft pulp bleaching effluent when compared with enzymes used to decolorize synthetic dyes.

Key words Decolorization · Kraft pulp bleaching effluent · *Geotrichum candidum* Dec 1

Introduction

Kraft pulp bleaching effluent containing some colored substances and chlorinated organic compounds is discarded as wastewater. These waste compounds are thought to cause environmental pollution[1,2] because of their poorly biodegradable and toxic properties. Many methods have been developed to remove these wastes (e.g., lime coagulation, rapid land infiltration, membrane processes, polymeric adsorbents[3]), but these processes have problems, such as high energy costs and generation of hazardous by-products. Microbial treatments to degrade waste compounds have also been reported. One of the most thoroughly studied microorganism is a white rot fungus, *Phanerochaete chrysosporium*, which has been shown to decolorize a kraft pulp bleaching effluent and dyestuff waste.[2,4–12] *Pleurotus ostreatus, Coriolus versicolor*, and *Streptomyces* spp. have also shown decolorizing activity of the effluent.[6,13–17] Although the microbial method saves energy cost when removing those wastes, it is difficult to apply the organisms for practical use because of their characteristics; they are plant pathogens, have limited substrate specificity, or have a slow growth rate. Therefore, developing a novel microorganism is urged.

From this viewpoint, we screened a novel decolorizing fungus, *Geotrichum candidum* Dec 1, which has decolorized 30 synthetic dyes and molasses.[18,19] We found that this strain secretes some enzymes, including a unique and novel peroxidase that degrades those dyes,[20,21] as well as manganese peroxidase. Moreover, this strain is not a pathogen, has wide degradative capacity for many substrates, and a faster growth rate than other white rot fungi.[18] In this work, we note that Dec 1 decolorizes the kraft pulp bleaching effluent effectively and speculate on its decolorizing mechanism.

Materials and methods

Strain and preparation

Geotrichum candidum Dec 1 was isolated in our laboratory.[18] Potato dextrose (PD) medium was prepared by the following method. Commercial potato (50 g) was cut to blocks around 1 cm^3 and boiled in 1 l of water for 30 min. Then the potato was mashed and the suspension filtered using filter paper and vacuum aspiration. Glucose (20 g) was added to the obtained filtrate. This solution was made up to 1 l with distilled water and adjusted to pH 5.5. A 10-μl aliquot of 25% glycerol stock suspension of Dec 1 spore was inoculated on a PD agar plate (90 mm ϕ) and incubated for 10 days at 30°C. The mycelia on three plates were collected and suspended in 10 ml of sterilized water with a vortex

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mixer. The suspension was filtered with gauze, and the filtrate was obtained as a fresh spore suspension.

Lignin decolorizing assay

A 10-μl aliquot of 25% glycerol stock suspension of Dec 1 spore was inoculated on a GY agar plate (glucose 30 g/l, yeast extract 3 g/l, K_2HPO_4 1 g/l, ammonium tartrate 0.5 g/l, $MgSO_4 \cdot 7H_2O$ 0.5 g/l, and agar 15 g/l) containing commercial lignin 1 g/l (Kanto Chemical, Tokyo, Japan). It was then incubated for 2 weeks at 30°C.

Effect of pH on the kraft pulp bleaching effluent

The effluent at the alkaline extraction stage of hard wood pulp (E-effluent) provided by Oji Paper Co. was used as the kraft pulp bleaching effluent. This effluent (2 ml) and 1 ml of 25 mM citrate buffer (pH 3.2, 4.5, or 5.0) were mixed, and the supernatant was obtained by centrifugation (12000 rpm for 5 min). Absorbance of the supernatant at 465 nm against distilled water was measured using a spectrophotometer (Shimadzu, UV-2200).

Culture method

The E-effluent was adjusted to pH 5.0 with 2N HCl and centrifuged to remove insoluble particles (6000 rpm, 60 min). Glucose (0.75, 1.5, 3.0 or 4.5 g), ammonium tartrate (0.075 g), KH_2PO_4 (0.15 g), $MgSO_4 \cdot 7H_2O$ (0.075 g), and yeast extract (0.45 g) were added to 150 ml of the E-effluent in 500-ml shake flasks and sterilized (121°C, 20 min). Dec 1 was inoculated into the flask and cultured with a reciprocal shaker (120 spm) at 30°C for 10 days. As a control, the decolorization without cells was tested in 150 ml of sterilized E-effluent mixture containing GY medium (glucose 30 g/l, ammonium tartrate 0.5 g/l, KH_2PO_4 1 g/l, $MgSO_4 \cdot 7H_2O$ 0.5 g/l, yeast extract 3 g/l) at pH 5.0, 4.0, and 3.0 under the same conditions.

Analytical methods

Color removal

The color of the effluent was determined by the standard method of the Canadian Pulp and Paper Association.[22] The absorbance at 465 nm against distilled water was measured using a spectrophotometer (Shimadzu, UV-2200). The absorbance values were then transformed into color units (CU) according to the following formula.

$$CU = 500 \times A_2/A_1$$

where A_1 corresponds to the A_{465} of a 500-CU platinum-cobalt standard solution (0.132); and A_2 is the absorbance of the effluent sample. The color removal (%) was defined as the ratio of CU of the culture supernatant to that of the initial medium. The average initial color unit of the E-effluent was 4200.

Color adsorption to cells

To investigate if color removal depends on the adsorption to the cells, colored substances were extracted with alkaline solution from the cells of the culture that showed maximum color removal during a culture period. After 3, 4, and 9 days of culture in the case of glucose concentrations of 5, 10, and 30 g/l, respectively, the CU of the culture supernatant was determined by the method described above. The obtained cells were agitated with 30 ml of 2N NaOH for 5 min and then centrifuged (12000 rpm for 5 min). The absorbance (465 nm) of the supernatant was measured, and its value was converted against the initial volume of the medium. The CU was then estimated to be the amount of colored substances adsorbed to the cells.

Absorbable organic halogens

Absorbable organic halogens (AOX) of E-effluent was measured with the AOX analyzer (TOX-10; Mitsubishi-Kasei, Tokyo, Japan). The initial AOX of the E-effluent was 34.2 mg/l.

Gel filtration of E-effluent

To estimate the distribution of the molecular weight of the colored substances in the E-effluent, Sephadex G-75 (Amersham-Pharmacia, Tokyo, Japan) gel filtration column chromatography (2.6 × 110 cm), of which the fraction range was 3000 to 80000 as globular protein, was performed. The elution solvent was 0.75 M NaCl, and the flow rate was 15 ml/h. The colored substances were detected at 280 nm absorbance. Trypsin (MW 24000), aprotinin (MW 6512), and vitamin B (MW 1355) were used as the standard molecular size markers. With this chromatography, substances with a molecular weight lower than 3000 are eluted together.

Enzyme assay

DyP assay. DyP is a novel peroxidase from *G. candidum* Dec 1 already reported by us.[20] Reactive blue 5 (RB5; ε_{600} = 8800 $M^{-1}cm^{-1}$), a representative anthraquinone dye, was used as the substrate. The substrate solution consists of 100 ppm (final) of RB5 in 25 mM citrate buffer pH 3.2. The 0.6 ml of culture supernatant, prepared by centrifugation (12000 rpm, 5 min), was mixed with the substrate solution, and then H_2O_2 was added at a final concentration of 0.2 mM. The total volume of the enzyme reaction was adjusted to 3 ml. The enzyme activity was calculated from the decrease in absorbance at 600 nm (A_{600}). One unit of enzyme activity was defined as the amount of the enzyme that decolorized 1 μmol of RB5 at 30°C for 1 min.

Manganese peroxidase assay. Guaiacol was used as the substrate for the manganese peroxidase (MnP) assay.[10] The total volume of the enzyme reaction was adjusted to 3 ml. The 0.6 ml of culture supernatant of Dec 1, guaiacol, and $MnSO_4$ at a final concentration of 0.1 mM were mixed

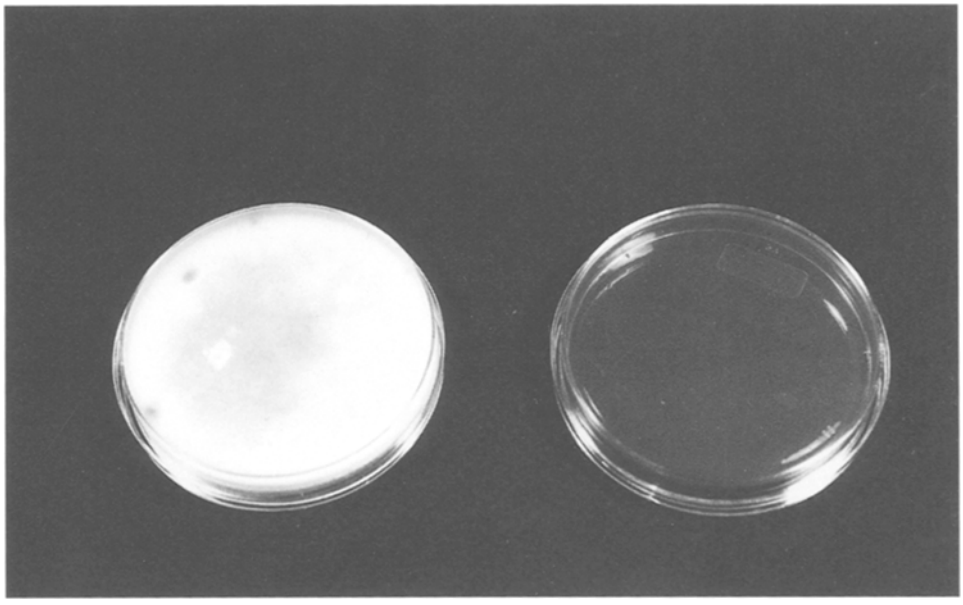

Fig. 1. Decolorization of commercial lignin by Dec 1. Left, lignin-containing plate (1 g/l) after 2 weeks of incubation with Dec 1. Right, lignin-containing plate (1 g/l) after 2 weeks of incubation with no inoculation

and adjusted to 2.99 ml of 0.1 M sodium tartrate (pH 5.0); then H_2O_2 at a final concentration of 0.1 mM was added. The enzyme activity, calculated from the increase in absorbance at 465 nm (A_{465}) as the molecular extinction coefficient (ε_{465}) of the reaction product, was $12000\,M^{-1}cm^{-1}$. One unit of enzyme activity was defined as the amount of enzyme that reacted with 1 μmol of guaiacol at 30°C for 1 min.

Lignin peroxidase assay. Veratryl alcohol was used as the substrate for the lignin peroxidase assay.[10] The total volume of the enzyme reaction was adjusted to 3 ml. The 0.6 ml of culture supernatant of Dec 1 and veratryl alcohol at a final concentration of 2 mM were mixed and adjusted with 2.97 ml of 50 mM tartrate (pH 2.5); then H_2O_2 at a final concentration of 0.4 mM was added. The enzyme activity, calculated from the increase in absorbance at 310 nm (A_{310}) as the molecular extinction coefficient (ε_{310}) of the reaction product, was $9300\,M^{-1}cm^{-1}$. One unit of enzyme activity was defined as the amount of the enzyme that reacted with 1 μmol of veratryl alcohol at 30°C for 1 min.

Laccase assay. 2,2′-Azino-di-(3-ethylbenzthiazoline)-6′-sulfonate (ABTS) was used as the substrate for the laccase (Lac) assay.[23] The total volume of the enzyme reaction was adjusted to 3 ml. Them 0.6 ml of the culture supernatant of Dec 1 and 2.99 ml of 0.1 mM sodium tartrate (pH 5.0) were mixed, and ABTS at a final concentration of 0.5 mM was added. The enzyme activity, calculated from the increase in absorbance at 420 nm (A_{420}) as the molecular extinction coefficient (ε_{420}) of the reaction product, was $36000\,M^{-1}cm^{-1}$. One unit of the enzyme activity was defined as the amount of the enzyme that reacted with 1 μmol of ABTS at 30°C for 1 min.

Results

Decolorization of lignin by Dec 1

The lignin-containing plate was decolorized by Dec 1 after 2 weeks in culture, as shown in Fig. 1.

Effect of pH on E-effluent

When the pH of the effluent was adjusted to 3.2, 4.5, or 5.0, the absorbances at 465 nm against distilled water were 0.474, 0.494, and 0.512, respectively. The faint decrease of the absorbance at an acidic pH resulted in only partial precipitation of colored substances. Therefore, it was clear that the pH difference did not drastically affect the decolorization of the effluent.

Decolorization of E-effluent

The time courses for color removal and the pH of E-effluent at various glucose concentrations are shown in Fig. 2. The residual glucose concentrations are shown in Fig. 3. The maximum color removal was 78% after 5 days of culture in the E-effluent containing glucose 20 g/l. The maximum color removal was also 78% after 6 days of culture in the E-effluent containing glucose 30 g/l. Color removal was 47% after 3 days of culture when the E-effluent contained glucose 5 g/l but decreased to 0 after 4 days. Color removal declined after glucose was completely consumed in all cases, but the pH increased when color removal decreased. On the other hand, when Dec 1 was not inoculated into the sterilized E-effluent mixture, color removal was 15% at most, as shown in Table 1.

Color adsorption to cells

The relation between color removal and color adsorption is summarized in Table 2. When the glucose concentration was 5 g/l, 100% of the color removal was due to color adsorption to the cells: On the other hand, when it was 10 or 30 g/l, the ratio of decomposed colored substances for color removal increased to 62% and 90%, respectively.

Reduction of AOX

Reduction of AOX during culture is shown in Fig. 4. The reduction of AOX and color removal gradually

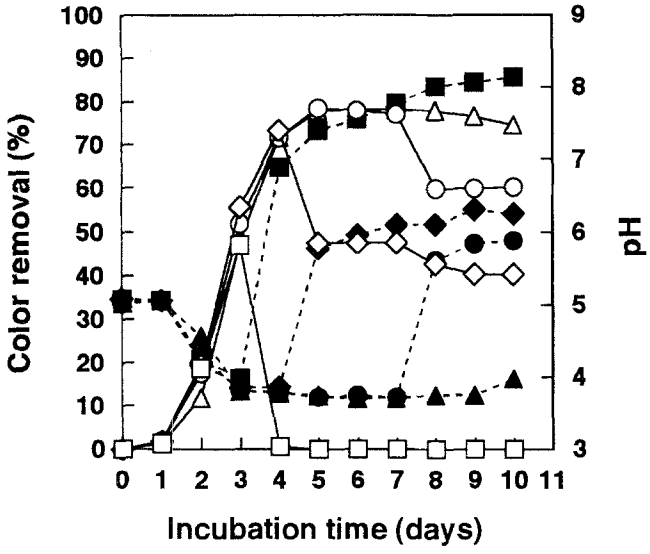

Fig. 2. Time courses of color removal and pH changes in the kraft pulp bleaching effluent supplemented by various glucose concentrations. Open and closed symbols represent color removal and pH, respectively. *Squares*, 5 g glucose/l; *diamonds*, 10 g/l; *circles*, 20 g/l; *triangles*, 30 g/l

Table 1. Color removal of noninoculated sterilized E-effluent mixture

	Color removal (%)		
Day	At pH 3.0	At pH 4.0	At pH 5.0
0	0	0	0
3	10	4.0	1.0
7	15	4.5	1.5
10	14	4.3	1.6

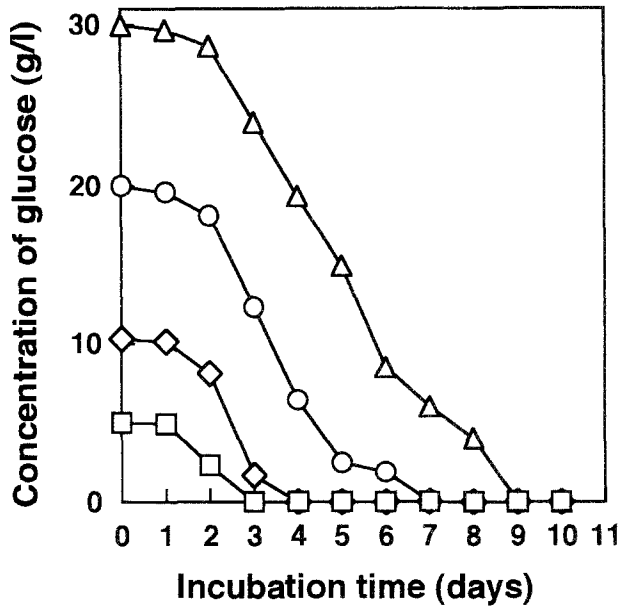

Fig. 3. Time course of residual glucose in the kraft pulp bleaching effluent supplemented by various glucose concentrations: *Squares*, 5 g/l; *diamonds*, 10 g/l; *circles*, 20 g/l; *triangles*, 30 g/l

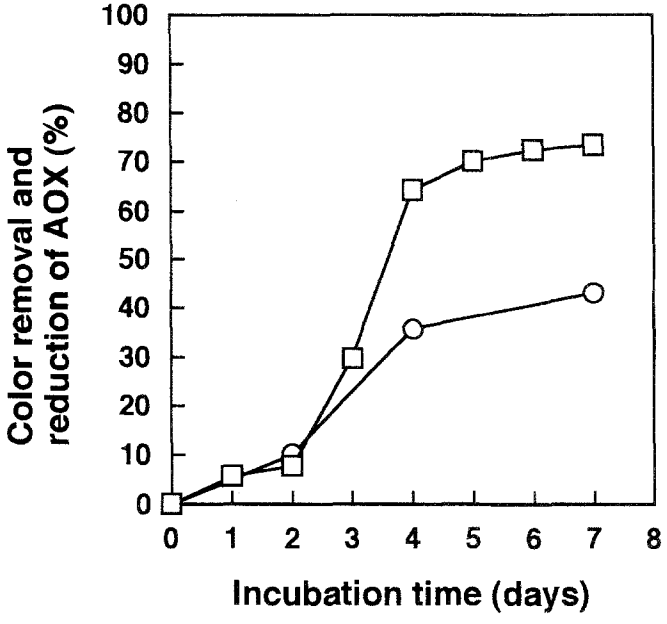

Fig. 4. Time courses of color removal and absorbable organic halogens (*AOX*) of the kraft pulp bleaching effluent supplemented by glucose 30 g/l. *Squares*, color removal; *circles*, AOX

Table 2. Relation between color removal and decomposed colored substances at various glucose concentrations

Glucose conc. of medium (g/l)	(A) Initial CU	(B) CU of culture supernatant[a]	Color removal (%) (A − B)/A × 100	(D) CU after alkaline-treated[b]	Ratio of decomposed colored substances[c] (%) (A − B − D)/(A − B) × 100
5	4110	2310	44	1960	0
10	4200	1230	71	1140	62
30	4410	1140	74	320	90

CU, color unit

[a] Culture times of 5, 10, and 30 g of glucose medium per liter correspond to 3, 4, and 9 days, respectively

[b] This value represents the amounts of adsorbed but not decomposed colored substances

[c] Minus value is treated as 0

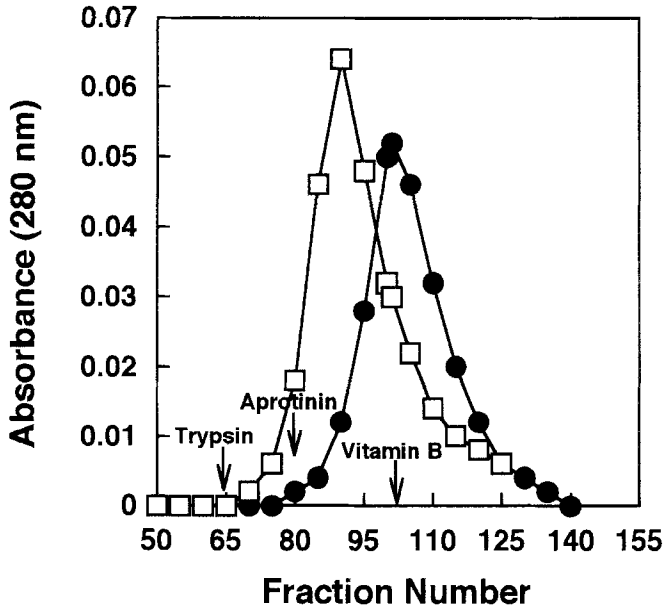

Fig. 5. Sephadex G-75 column (2.6 × 110cm) chromatography. *Squares*, original kraft pulp bleaching effluent; *circles*, kraft pulp bleaching effluent treated with Dec 1 for 7 days. The elution points of trypsin (MW 24000), aprotinin (MW 6512), and vitamin B (1355) are indicated by *arrows*

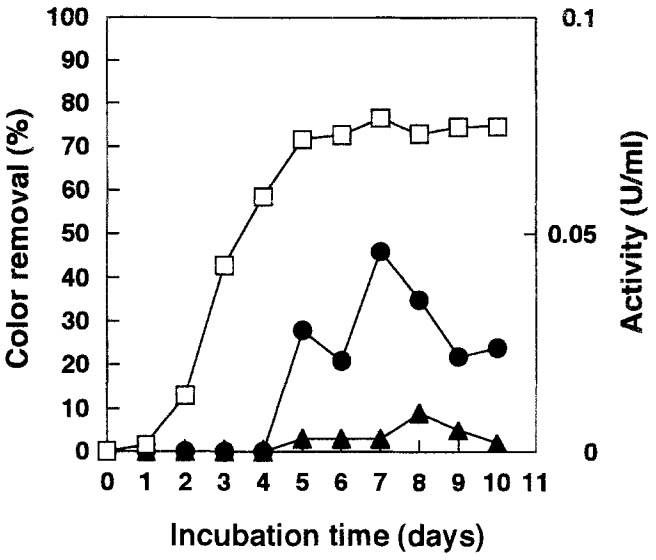

Fig. 6. Time courses of color removal and enzyme activities of kraft pulp bleaching effluent supplemented with glucose 30g/l. *Squares*, color removal; *circles*, DyP activity; *triangles*, manganese peroxidase activity

increased, achieving 43% and 78% after 7 days of culture, respectively.

Gel filtration column chromatography of E-effluent

The molecular weight of the main colored substances of the E-effluent was calculated from the elution peak, as shown in Fig. 5. and was estimated to be 5600. After 7 days the molecular weight of the colored substances apparently decreased and was estimated to be less than 3000 because its elution point was the same as that of vitamin B, as shown in Fig. 5.

Enzyme activity and color removal

The time courses of color removal and enzyme activities of the culture supernatant are shown in Fig. 6. Color removal started at 1 day and increased rapidly up to 5 days. DyP activity appeared after 5 days. On the other hand, the activity of MnP was weak compared with that of DyP. The activities of LiP and Lac were not observed.

Discussion

The main colored substances of general kraft pulp bleaching effluent were lignin and its related compounds.[24] Decolorizing activity by Dec 1 toward a commercial lignin on plate assay is obvious, as shown in Fig. 1. This result indicates that the decolorization is not due solely to adsorption of the colored substances to the Dec 1 cells.

Dec 1 decolorized the kraft pulp bleaching effluent when glucose was supplemented, as shown in Fig. 2. No color removal and growth were observed without glucose (data not shown). When the glucose concentrations were 10, 20, and 30g/l, color removal reached more than 70% after 4 days in culture and the pH decreased. As the pH decrease in acidic conditions did not affect decolorization of the effluent, the color removal was associated with Dec 1 activity. At a glucose concentration of 30g/l color removal maintained a high level (>70%) after 4 days of culture but decreased and the pH increased after complete consumption of glucose at other glucose concentrations. The pH increase suggests that by-products such as organic acids, which were converted from glucose by Dec 1, were further consumed by Dec 1 at 5, 10, and 20g/l. The decreased color removal in accordance with the pH increase strongly suggested that the adsorbed colored substances left the cells. This desorption was supported by results showing that the colored substances were eluted from the cells at low glucose concentrations, as summarized in Table 2.

Although the mechanism is unknown, color removal by Dec 1 is thought to proceed through two steps. The first step is color adsorption to the cells. Actually, the color of the cell pellet was observed to be enhanced gradually up to 3 to 4 days of culture. The second step is decomposition of the adsorbed colored substances. Probably the second step is induced by some secondary metabolites. If this is true, the shortage of glucose for the growth of Dec 1 results in insufficient production of those secondary metabolites. This hypothesis is not inconsistent with the data showing that the time course of color removal at a lower glucose concentration revealed more significantly reduced color removal after complete consumption of glucose, as shown in Figs. 2 and 3.

We estimated the molecular weight of the main colored substances of the E-effluent by gel filtration chromatography to be 5600. This value is not when high compared with other natural polymers such as polysaccharides and proteins. Therefore, the main structures of the colored substances were thought to be various derivatives from the partial degraded lignin-related compounds. The molecular weight of the E-effluent treated with Dec 1 became less than 3000, suggesting that Dec 1 degraded large molecular compounds, such as lignin-related compounds, to smaller compounds.

The decrease in AOX is the most important factor when evaluating the degree of waste cleanup. The main AOXs in the effluent were thought to be aromatic compounds (e.g., lignin and its derivatives) containing halogen. The decreasing AOX content was reported to be 48%–65% and 23%–32% by an activated sludge method and an aerobic lagoon method, respectively.[25,26] Lee et al.[26] reported that the decreasing contents of AOX were 40% and 25% using strain KS-62 (genus and species unknown) and *C. versicolor*, respectively. Dec 1 showed 43% AOX, which suggested that Dec 1 decolorized several aromatic halogens generating from lignin and chlorine during the pulp bleaching process.

So far, many researchers have reported enzymes related to lignin degradation and decolorization of kraft pulp bleaching effluent. LiP and MnP are well known representatives of lignin-degrading enzymes.[27,28] Gold and coworkers reported that MnP was more effective than LiP for decolorizing kraft pulp bleaching effluent.[29] However, the purified LiP showed no decolorizing activity.[10] Other researchers have reported that MnPs of white rot fungi played an essential role in decolorizing of the kraft pulp bleaching effluent.[26,30] When the purified MnP from *P. chrysosporium* was used to decolorize the effluent in vitro, the decolorizing ratio was only 25%, although it achieved 75% in vivo.[10] This finding suggests that extracellular enzyme(s) other MnP or intracellular enzymes are of importance to the decolorization process. One of those important enzymes was reported to be laccase. The decolorization of kraft pulp bleaching effluent using *Trametes versicolor* especially depended on laccase.[31] Therefore, so far MnP and laccase have been thought to play an important role in decolorization.

In this study, it is unclear what enzymes are associated with decolorization. Whereas Dec 1 showed more than 70% color removal of the E-effluent, MnP activity was low and LiP and laccase activities were not observed. As Dec 1 has lignin-decolorizing activity, as shown in Fig. 1, it is possible that the decolorizing system of Dec 1 is different from that of other known fungi such as *P. chrysosporium* and *T. versicolor*, or the role of the intracellular enzymes is significant. Moreover, DyP, which is involved in decolorizing synthetic dyes, is not thought to be a primary enzyme for decolorizing the effluent because its expression timing was delayed from the decolorizing timing of E-effluent by Dec 1, as shown in Fig. 6.

Dec 1 exhibited decolorizing ability for both synthetic dyes and natural-colored substances such as lignin-related compounds. These characteristics are considered to be advantageous for developing a practical method to clean up wastewater, including various artificial and natural pollutants.

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References

1. Prouty AL (1990) Bench-scale development and evaluation of a fungal bioreactor for color removal from bleach effluents. Appl Microbiol Biotechnol 32:490–493
2. Smeds A, Holmbom B, Pettersson C (1994) Chemical stability of chlorinated components in pulp bleaching liquors. Chemosphere 28:881–895
3. Eilbeck WV, Mattock G (1987) Chemical and adsorptive precipitation and flocculation. In: Horwood E (ed) Chemical processes in waste water treatment. Wiley, New York, pp 232–293
4. Cripps C, Bumpus JS, Aust SD (1990) Biodegradation of azo and heterocyclic dyes by *Phanerochaete chrysosporium*. Appl Environ Microbiol 56:1114–1118
5. Glenn JK, Gold MH (1983) Decolorization of several polymeric dyes by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl Environ Microbiol 45:1741–1747
6. Paszczynski A, Pasti MB, Goszczynski S, Crawford DL, Crawford RL (1991) New approach to improve degradation of recalcitrant azo dyes by *Streptomyces* spp. and *Phanerochaete chrysosporium*. Enzyme Microb Technol 13:378–384
7. Spadaro JT, Gold MH, Renganathan V (1992) Degradation of azo dyes by the lignin-degrading fungus *Phanerochaete chrysosporium*. Appl Environ Microbiol 58:2397–2401
8. Fukuzumi T, Nishida A, Aoshima K, Minami K (1977) Decolorization of kraft waste liquor with white rot fungi I. Mokuzai Gakkaishi 23:290–298
9. Yin CF, Joyce TW, Chang HM (1989) Role of glucose in fungal decolorization of wood pulp bleaching effluents. J Biotechnol 10:77–84
10. Jaspers CJ, Jimenez G, Penninckx MJ (1994) Evidence for a role of manganese peroxidase in the decolorization of kraft pulp bleach plant effluent by *Phanerochaete chrysosporium*: effects of initial culture conditions on enzyme production. J Biotechnol 37:229–234
11. Pellinen J, Joyce TW, Chang HM (1988) Dechlorination of high-molecular-weight chlorolignin by the white-rot fungus *Phanerochaete chrysosporium*. TAPPI J 71:191–194
12. Jaspers CJ, Penninckx MJ (1996) Adsorption effects in the decolorization of a kraft bleach plant effluent by *Phanerochaete chrysosporium*. Biotechnol Lett 18:1257–1260
13. Platt MW, Hadar Y, Chet I (1985) The decolorization of the polymeric dye poly-blue (polyvinalamine sulfonate-anthraquinone) by lignin degrading fungi. Appl Microbiol Biotechnol 21:394–396
14. Livernoche D, Jurasek L, Desrochers M, Dorica J (1983) Removal of color from kraft mill wastewaters with cultures of white-rot fungi and with immobilized mycelium of *Coriolus versicolor*. Biotechnol Bioeng 25:2055–2065
15. Watanabe Y, Sugi R, Tanaka Y, Hayashida S (1982) Enzymatic decolorization of melanoidin by *Coriolus* sp. no.20. Agric Biol Chem 46:1623–1630
16. Archibald F, Paice MG, Jurasek L (1990) Decolorization of kraft bleachery effluent chromospores by *Coriolus versicolor*. Enzyme Microb Technol 12:846–853
17. Pasti MB, Crawford DL (1991) Relationship between the abilities of streptomycetes to decolorize three anthron type dyes and to degrade lignocellulose. Can J Microbiol 37:902–907
18. Kim SJ, Ishikawa K, Hirai M, Shoda M (1995) Characteristics of a newly isolated fungus, *Geotrichum candidum* Dec 1, which decolorizes various dyes. J Ferment Bioeng 79:601–607
19. Kim SJ, Shoda M (1999) Decolorization of molasses and a dye by a newly isolated strain of the fungus *Geotrichum candidum* Dec 1. Biotechnol Bioeng 62:114–119

20. Kim SJ, Shoda M (1999) Purification and characterization of a novel peroxidase from *Geotrichum candidum* Dec 1 involved in decolorization of dyes. Appl Environ Microbiol 65:1029–1035
21. Sugano Y, Nakano R, Sasaki K, Shoda M (2000) Efficient heterologous expression in *Aspergillus oryzae* of a unique dye-decolorizing peroxidase, DyP, of *Geotrichum candidum* Dec 1. Appl Environ Microbiol 66:1754–1758
22. Nagarathnamma R, Pratima B (1999) Decolorization of extraction-stage effluent from chlorine bleaching of kraft pulp by *Rhizopus oryzae*. Appl Environ Microbiol 65:1078–1082
23. Bourbonnais R, Paice MG (1990) Oxidation of non-phenolic substrates: an expanded role for laccase in lignin biodegradation. FEBS Lett 267:99–102
24. Pfister K, Sjöström E (1979) Characterization of spent bleaching liquors. Paperi Puu 10:619–622
25. Gerkov M, Priha M, Talka E, Valttila O (1988) Chlorinated organic compounds in effluents treatment at kraft mills. TAPPI J 71:175–184
26. Lee SH, Kondo R, Sakai K (1994) Treatment of kraft bleaching effluents by lignin degrading fungi. III. Treatment by newly found fungus KS-62 without additional nutrients. Mokuzai Gakkaishi 40:612–619
27. Michel FC JR, Dass SB, Grulke EA, Reddy CA (1991) Role of manganese peroxidases and lignin peroxidase of *Phanerochaete chrysosporium* in the decolorization of kraft bleach plant effluent. Appl Environ Microbiol 57:2368–2375
28. Momohara I, Matsumoto Y, Ishizu Y, Chang HM (1989) Decolorization mechanism of spent bleaching liquor by *Phanerochaete chrysosporium*. In Proceedings of the 5th ISPWC, pp 373–376
29. Gold MH, Wriishi H, Valli K (1989) Extracellular peroxidases involved in lignin degradation by the white rot basidiomycete *Phanerochaete chrysosporium*. In: Whitaker JR, Sonnet PE (eds) Biocatalysis in agricultural biotechnology. America Chemical Society, Washington, DC, pp 127–136
30. Lacker R, Srebotnik E, Messner K (1991) Oxidative degradation of high molecular weight chlorolignin by manganese peroxidase of *Phanerochaete chrysosporium*. Biochem Biophys Res Commun 178:1092–1098
31. Archibald F, Roy B (1992) Production of manganic chelates by laccase from the lignin-degrading fungus *Trametes* (*Coriolus*) *versicolor*. Appl Environ Microbiol 58:1496–1499