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Cr(VI) reduction from contaminated soils by *Aspergillus* sp. N2 and *Penicillium* sp. N3 isolated from chromium deposits

Tsubasa Fukuda, Yasuhiro Ishino, Akane Ogawa, Kadzuyo Tsutsumi, and Hiroshi Morita*

Graduate School of Environmental Engineering, The University of Kitakyushu, Wakamatsu-ku, Kitakyushu 808–0135, Japan

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***Aspergillus* sp. N2 and *Penicillium* sp. N3 are chromate-resistant filamentous fungi that were isolated from Cr(VI) contaminated soil based on their ability to decrease hexavalent chromium levels in the growth medium. After 120 h of growth in a medium containing 50 ppm Cr(VI) at near neutral pH, *Aspergillus* sp. N2 reduced the Cr(VI) concentration by about 75%. *Penicillium* sp. N3 was able to reduce the Cr(VI) concentration by only 35%. However, *Penicillium* sp. N3 reduced the Cr(VI) concentration in the medium by 93% under acidic conditions. Interestingly, the presence of Cu(II) enhanced the Cr(VI) reducing ability of *Aspergillus* sp. N2 and *Penicillium* sp. N3 at near neutral pH. *Aspergillus* sp. N2 and *Penicillium* sp. N3 reduced the Cr(VI) concentration in the growth medium to a virtually undetectable level within 120 h. For both *Aspergillus* sp. N2 and *Penicillium* sp. N3, mycelial seed cultures were more efficient at Cr(VI) reduction than conidium seed cultures. The mechanisms of Cr(VI) reduction in *Aspergillus* sp. N2 and *Penicillium* sp. N3 were enzymatic reduction and sorption to mycelia. Enzymatic activity contributed significantly to Cr(VI) reduction. *Aspergillus* sp. N2 and *Penicillium* sp. N3 reduced the levels of Cr(VI) in polluted soil samples, suggesting that these strains might be useful for cleaning up chromium-contaminated sites.**

Key Words——*Aspergillus* sp.; bioremediation; chromate-resistant fungi; Cr(VI) removal; *Penicillium* sp.; polluted soils

Introduction

Chromium (Cr), a widespread environmental pollutant, is released from various industries including tanneries, metal cleaning and processing plants, and chromium plating, wood processing, and alloy formation facilities (Bartlett and James, 1988; Taylor et al., 1990). In developing as well as underdeveloped countries, industrial effluents are released directly or indirectly into natural water resources, mostly without proper treatment, posing a major threat to the environment (Fruchter, 2002).

Among the different forms of chromium, hexavalent chromium (Cr(VI)) is the most toxic and carcinogenic due to its high solubility in water, rapid permeability through biological membranes, and subsequent interaction with intracellular proteins and nucleic acids (Kamaludeen et al., 2003; Ortegel et al., 2002; Reeves et al., 1983). Heavy metals in general cannot be biologically transformed to more or less toxic products and, hence, persist in the environment indefinitely. Although the reduction of Cr(VI) causes chromate toxicity, further reduction leads to the formation of stable, less

* Address reprint requests to: Dr. Hiroshi Morita, Graduate School of Environmental Engineering, The University of Kitakyushu, 1–1 Hibikino, Wakamatsu-ku, Kitakyushu 808–0135, Japan.

Tel: +81–93–695–3289 Fax: +81–93–695–3381

E-mail: morita01@hibikino.ne.jp

soluble, and less toxic Cr(III) (Biedermann and Landolph, 1990). Reduction of potentially toxic Cr(VI) to Cr(III) is therefore a useful process for remediation of Cr(VI)-affected environments (Michel et al., 2001). Microbial viability is essential for biotransformation as reduction reactions are enzyme-mediated. Metal ions are generally converted into an insoluble form by specific enzyme-mediated reactions and as a result are removed from the aqueous phase (Park et al., 2000). There have been reports of the use of live microbial systems for remediation of contaminated soils and water (Cervantes and Silver, 1992). Higher fungi, yeast, bacteria, seaweed and plant dark materials are abundantly available in nature and can be used as low cost biosorbents (Cervantes et al., 2001; Shrivastava and Thakur, 2003). The use of microbial cells as biosorbents for heavy metals is a potential alternative to conventional methods that are used to decontaminate liquid wastes. Several bacteria possess chromate reductase activity, converting Cr(VI) to Cr(III), which is significantly less toxic and less soluble, and thus reduction by these enzymes affords a means of chromate bioremediation (Gadd and White, 1993). In this context, the detoxification of Cr by naturally-occurring microorganisms provides a viable option to protect the environment from chromium toxicity. Moreover, the continuous exposure of microbial populations in polluted environments to heavy metals selects resistant strains (Margesin and Schinner, 1996).

The ability of some microorganisms to interact with different forms of chromium makes them attractive in the context of environmental biotechnology. In this sense, the use of microbial biomass for the removal of Cr from industrial wastewater and polluted water has already been recognized (Cervantes et al., 2001; Pillichshammer et al., 1995). The ability of some microorganisms to both tolerate and reduce Cr(VI) enables their application in biotechnological processes focusing on detoxification of hexavalent Cr. Cr resistance has been described in bacteria and fungi isolated from Cr-contaminated environments. Chromate-resistant fungal strains were obtained in some cases by mutagenesis of laboratory strains (Cervantes et al., 2001). Yeast strains isolated from Cr-contaminated environments include those from the genera *Candida* (Baldi et al., 1990; Pepi and Baldi, 1992) and to a minor extent *Rhodosporidium* (Pepi and Baldi, 1992). In these yeasts, the general mechanism of chromate resistance is related to limited ion uptake rather than to chemical reduction of the toxic species (Baldi et al., 1990; Pepi and Baldi, 1992). However, other yeasts such as *Candida utilis* (Muter et al., 2001) and *Candida maltosa* (Ramirez-Ramirez et al., 2004) showed some ability to reduce Cr(VI) and also the capability to accumulate Cr in the biomass. Recent reports have also examined Cr(III) and/or Cr(VI) uptake and accumulation by different yeasts (Bingol et al., 2004; Kaszycki et al., 2004) and filamentous fungi (Dias et al., 2002; Vala et al., 2004).

Aspergillus sp. N2 and *Penicillium* sp. N3 were isolated from chromium deposits. Both strains were able to grow in the presence of up to 1,000 ppm of Cr(VI). *Aspergillus* sp. N2 could remove Cr(VI) (～50 ppm) completely from liquid medium after 240 h. *Penicillium* sp. N3 was efficient at removal of Cr(VI) (～50 ppm) under acidic conditions (Fukuda et al., unpublished). Then we examined chromate bioremediation using conidium seed cultures of these strains. In this paper, we investigated the ability of mycelial seed cultures of *Aspergillus* sp. N2 and *Penicillium* sp. N3 to remove Cr(VI) from liquid medium and contaminated soil.

Materials and Methods

Microorganisms. The filamentous fungi used in this study were *Aspergillus* sp. N2 and *Penicillium* sp. N3, which were isolated from chromium deposits in Fukuoka, Japan. These strains were identified based on their morphological structures such as color, diameter of the mycelia, and microscopic observation of the spore formation (Kirk et al., 2001). Both strains were able to grow in the presence of up to 1,000 ppm of Cr(VI). These strains were maintained on PG solid medium. PG medium consisted of 5 g bacto-peptone, 2 g glucose, and 15 g agar in 1.0 L deionized water. The pH value of the medium was adjusted to 6.0 with 6 M NaOH and 6 M HCl. The stock cultures were transferred to fresh PG slants and pre-cultured at 30°C for 1 week prior to use in fermentations.

Cr(VI) reduction experiments in liquid media. Mycelial biomass was obtained as described below. *Aspergillus* sp. N2 and *Penicillium* sp. N3 from a mature slant were suspended in sterilized water. Fungal spore concentrations were adjusted to approximately 1×10^7 spores/ml. In the case of inoculating spores, these spore suspensions were used directly in Cr(VI) reduction experiments. To generate mycelial cultures, PG liquid medium, which contained the same components

described above except agar, was used. The medium was autoclaved at 120°C for 20 min. After sterilization, 100 ml of liquid medium in a 300 ml conical flask was inoculated with 1 ml of the spore suspension and incubated at 30°C for 3 days in a 60 rpm shaking incubator. After incubation, these mycelia were collected by filtration and washed with sterile water.

The harvested mycelium was transferred to 100 ml of fresh PG liquid medium. After sterilization, a solution of chromium oxide was added aseptically to a final Cr(VI) concentration of 60 ppm. The dry mycelial weight (DMW) of each fungus was approximately 0.5 g/L. At various times during the course of incubation, aliquots were removed and centrifuged at 4,200×*g* for 20 min. The Cr(VI) concentrations in the supernatant fluid were determined.

The influence of pH on Cr(VI) reduction was studied by varying the pH of the reaction mixture from 3 to 6. These experiments were carried out with an initial Cr(VI) concentration of 60 ppm.

The influence of Cu(II) on Cr(VI) reduction was investigated. Stock solutions containing 50 ppm Cu(II) as copper sulfate were prepared in distilled water, filter-sterilized and diluted in PG liquid medium. These experiments were carried out with an initial Cr(VI) concentration of 60 ppm.

Batch sorption experiments. A stock solution containing Cr(VI) (50 ppm) was prepared by dissolving analytical grade $K_2Cr_2O_7$ in sterile PG medium, and the pH of the solution was adjusted to 3.0 or 6.0 using 0.1 M HCl or NaOH. Fifty milligrams of biomass was autoclaved at 120°C for 20 min and added to 100 ml of 60 ppm Cr(VI) solution in 300 ml Erlenmeyer flasks. The samples were incubated at 30°C for 48 h in a 60 rpm shaking incubator. Chromium adsorption to the fungal biomass was evaluated by measuring the amount of total Cr remaining in the supernatant.

Cr(VI) reduction experiment in contaminated soils. Contaminated soil samples were collected from a chromium-contaminated site located at Fukuoka, Japan. Soil samples were manually collected and transported to the laboratory in airtight polythene containers. The pH of the soil was 6.5.

Soluble contaminants were washed from the soil and the resulting aqueous fluid was used for laboratory-scale bioremediation experiments. Ten grams of contaminated soil was washed with 90 ml of distilled water for 24 h on a 100 rpm shaker. The pH of the distilled water was adjusted to 8.0 with 6 M NaOH and 6 M HCl. Mycelial biomass, obtained as described in the section on Cr(VI) reduction experiments in liquid media, was transferred to 10 ml of fresh PG liquid medium, and 100 ml of the contaminated fluid was added. The DMW of each fungus was approximately 0.5 g/L in these samples. At various times during the course of the incubation, aliquots were removed and centrifuged at 4,200×*g* for 20 min. The Cr(VI) concentrations in the supernatant fluid were determined.

Growth assessment of microorganisms. DMW was used as a measure of growth and was determined by drying cells at 105°C for 24 h in an oven. DMW was defined as mycelium weight per one liter of liquid culture medium.

Cr (VI) and total Cr measurements. Concentrations of Cr(VI) were determined by the diphenylcarbazide method (Lowe et al., 2003), and total Cr was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, PerkinElmer Japan Co., Ltd.). Mycelia were removed from the fermentation broth by filtration through filter paper and centrifugation at 4,200×*g* for 20 min. The supernatant fluid was filtered through a 0.45-μm-pore-size membrane filter (Toyo Roshi Kaisha, Ltd.). Five milliliters of culture supernatant was aseptically removed and transferred to a glass test tube. The sample was acidified with 1 ml of 10% H_2SO_4. The sample was then treated with 1 ml of a 10 mg/ml diphenylcarbazide solution in acetone, and distilled water was added to a total volume of 50 ml. Cr(VI) reacts with diphenylcarbazide to form a bright pink color, the intensity of which is directly proportional to the Cr(VI) concentration. This solution was shaken for 30 s, and after 5 min the absorbance at 540 nm was read on a UV-vis spectrophotometer (Amersham Pharmacia Biotech Co., Ltd.). In addition, this solution was analyzed by ICP-AES. Cr(VI) concentrations were estimated from standard curves generated with known amounts of Cr(VI) using potassium chromate in the growth medium.

Results and Discussion

We examined the abilities of the *Aspergillus* sp. N2 and *Penicillium* sp. N3 strains in liquid culture medium containing Cr(VI) at a concentration of 50 ppm. Figure 1A shows the results when the medium pH was near neutral (pH 6.0). After 120 h of incubation, *Aspergillus* sp. N2 reduced the Cr(VI) concentration in the medium by 74%. *Penicillium* sp. N3 was able to reduce the

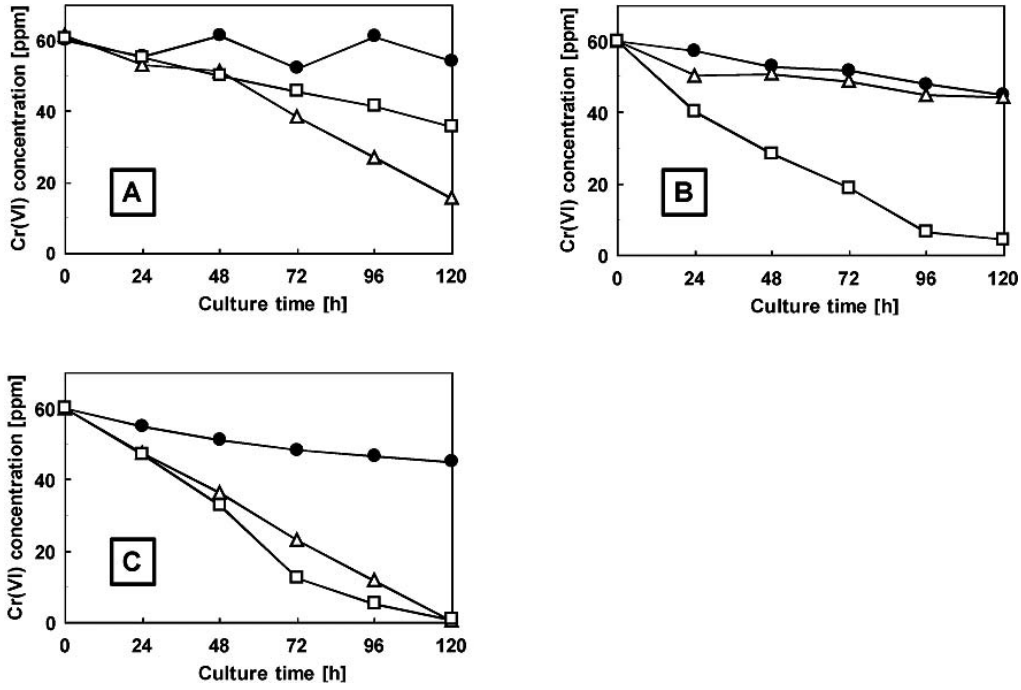

Fig. 1. Time courses of the decrease in the Cr(VI) concentration in the growth medium of the mycelial seed cultures initiated in PG medium supplemented with 60 ppm of Cr(VI) (A) at pH 6.0, (B) at pH 3.0, and (C) added Cu(II) (50 ppm) at pH 6.0.

Symbols represent: ●, blank; △, *Aspergillus* sp. N2; and □, *Penicillium* sp. N3. The data shown in the figure are the average values of three experiments.

Cr(VI) concentration in the growth medium by about 35%. In the *Aspergillus* sp. N2 culture, the yellow color of the soluble Cr(VI) turned into a slightly turbid brownish color (results not shown). In contrast, when grown in strongly acidic medium (pH 3.0), *Penicillium* sp. N3 was more efficient at reducing the Cr(VI) concentration than at neutral pH (Fig. 1B). Under acidic conditions, *Penicillium* sp. N3 reduced the Cr(VI) concentration in the medium by 93%, while *Aspergillus* sp. N2 was able to reduce the Cr(VI) concentration by only 20%. However, the Cr(VI) concentration also decreased by about 20% in the uninoculated control culture.

Cr(VI) reduction is influenced by many environmental factors, including the concentrations of other metal ions. It is known that copper is commonly present in Cr(VI)-contaminated wastewater and soil (Zafer et al., 2007; Zayed and Terry, 2003). Thus, we next investigated the influence of coexisting Cu(II) ions on the Cr(VI)-reducing properties of *Aspergillus* sp. N2 and *Penicillium* sp. N3. This experiment was performed using culture medium containing 60 ppm of Cr(VI) as the base growth medium to which Cu(II) was added to a final concentration of 50 ppm. Figure 1C shows a time course of the decrease in the Cr(VI) concentration in the presence of Cu(II) at neutral pH. Cu(II) affected the ability of *Aspergillus* sp. N2 and *Penicillium* sp. N3 to reduce the Cr(VI) concentration. Both *Aspergillus* sp. N2 and *Penicillium* sp. N3 decreased the Cr(VI) concentration from its initial concentration of 50 ppm to almost undetected levels after 120 h incubation.

There are two mechanisms by which chromate could be reduced. First, chromate could be reduced to a less toxic lower oxidation state by an enzymatic reaction. Results described here showed a direct positive correlation between Cr(VI) reduction and pH. Wang et al. (1990) reported that reduction of chromate to a lower oxidation state by an *Enterobacter* strain occurred between pH 6.5−8.5 and the reduction reaction was strongly inhibited at pH 5 and pH 9, while at pH 9.0 *Achromobacter* sp. completely reduced the Cr(VI) (Ma et al., 2007). However, since Cr(VI) reduction is enzyme-mediated, pH changes may affect the enzyme ionization rate and the protein conformation, and consequently may affect enzyme activity. A coexisting ion could also affect the enzyme activity. At neutral pH, the presence of Cu(II) affected the Cr(VI)-reducing abilities of these strains. Cu(II) enhanced the Cr(VI) reducing ability of *Penicillium* sp. N3 under acidic condi-

Table 1. Chromium sorption by *Aspergillus* sp. N2 and *Penicillium* sp. N3.

Strain	Cr sorption[a] [mg/g-dry mycelial weight]	
	pH 6.0	pH 3.0
Aspergillus sp. N2	4.8	10.3
Penicillium sp. N3	4.1	12.3

[a] The biomass was autoclaved at 120°C for 20 min and was added to sterilized PG medium containing 60 ppm Cr(VI) solution. The samples were kept at 30°C for 48 h in a 60 rpm shaking incubator. The chromium adsorptivity of biomass was evaluated by measuring the amount of total Cr remaining.

tions, causing a drop in the Cr(VI) concentration in the culture medium from its initial concentration of 60 ppm to almost undetectable levels after 120 h. This result is similar to that observed in *Bacillus* sp. expressing a Cr(VI) reducing enzyme, in that the enzyme activity was enhanced by Cu(II) and Ni(II) and inhibited by Hg(II) (Elsngovan et al., 2006). Currently, we do not know whether the fungal strains used in this study express any Cr(VI) reducing enzyme(s). Further studies are necessary to extend our understanding of the effects of coexisting ions on the Cr(VI)-reducing activity of the strains reported in this study.

Biosorption is the second mechanism by which the chromate concentration could be reduced. Biosorption of chromium to the fungal mycelium was determined by the batch method after 48 h (Table 1). Under neutral pH conditions (pH 6.0), chromium sorption by *Aspergillus* sp. N2 and *Penicillium* sp. N3 was 4.8 and 4.1 mg/g dry weight of mycelium. In contrast, under acidic conditions (pH 3.0), chromium sorption by *Aspergillus* sp. N2 and *Penicillium* sp. N3 was 10.3 and 12.3 mg/g dry weight of mycelium. Therefore, under acid conditions biosorption of chromium by both *Aspergillus* sp. N2 and *Penicillium* sp. N3 was higher than at neutral pH.

The fungal cell wall can be regarded as a mosaic of different functional groups that could form coordination complexes with metals. Zafar et al. (2007) reported the biosorption ability of *Aspergillus* strains isolated from wastewater-treated soil. At 6 mM initial concentration of Cr (pH 4.5), the Cr adsorption value of *Aspergillus* sp. 2 (1.56 mg/g) exceeded the Cr adsorption value (1.20 mg/g) of the less tolerant strain *Aspergillus* sp. 1. Some *Penicillium* strains were also reported to adsorb chromium (Zafar et al., 2007). For example, *Penicillium purpurogenum* has the capacity to adsorb 36.5 mg of Cr(VI) ions per g of fungal biomass (Say et al., 2004). Thus, the chromium removal abilities of these strains are equal or better than those of the other reported strains.

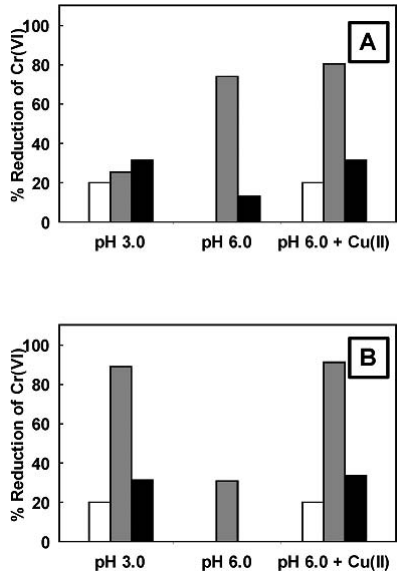

Fig. 2. Effect of inoculating fungi in different growth states on the Cr(VI)-reducing properties of (A) *Aspergillus* sp. N2 and (B) *Penicillium* sp. N3.

These strains were grown in PG medium containing 60 ppm Cr(VI) at 30°C for 98 h. White bars, % reduction of Cr(VI) concentration in uninoculated medium. Gray bars, % reduction in the Cr(VI) concentration in cultures inoculated with conidium seed cultures. Black bars, % reduction in the Cr(VI) concentration in cultures inoculated with mycelial seed cultures. The data shown in the figure are the average values of two experiments.

Production of various fungal products is influenced by the fungal state (mycelia or spores) at the time of inoculation (Stanbury and Whitaker, 1984). The chromium removal efficiencies of *Aspergillus* sp. N2 and *Penicillium* sp. N3 inoculated at different growth states (mycelia or conidia) after a 98 h incubation were compared. In the case of *Aspergillus* sp. N2, the mycelial seed culture was more efficient at Cr(VI) reduction than the sporal seed culture at neutral pH. However, at acidic pH, the Cr(VI) reduction rates for both inoculation conditions were approximately equivalent (Fig. 2A). In the case of *Penicillium* sp. N3, the mycelia were

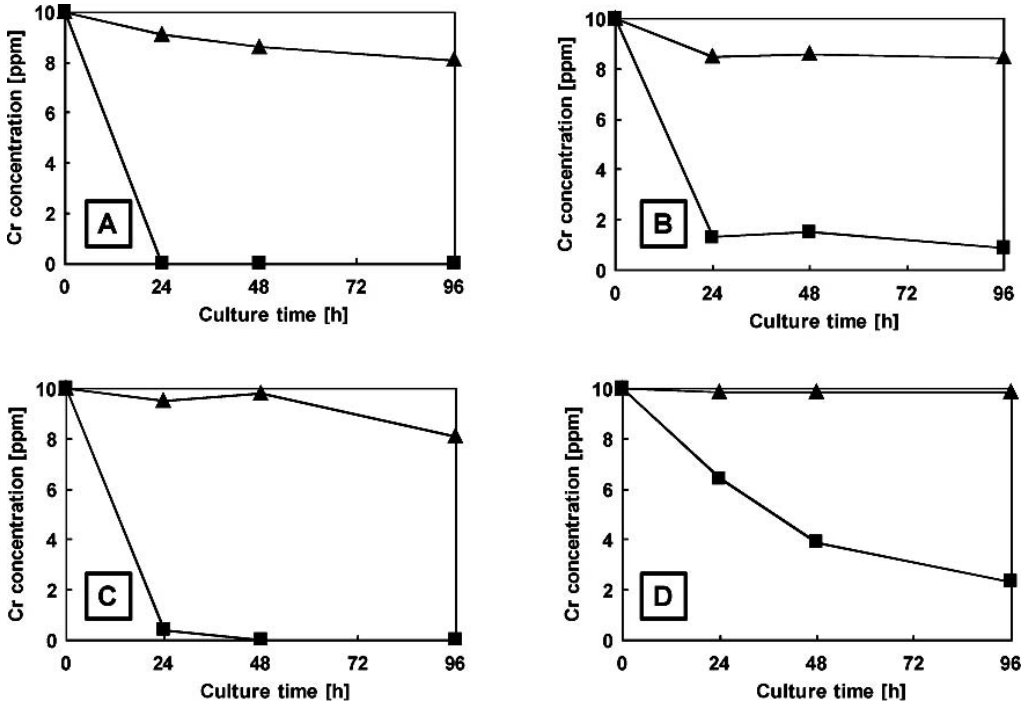

Fig. 3. Time courses of the decrease in Cr(VI) and total Cr in the growth medium of (A) mycelial seed cultures of *Aspergillus* sp. N2, (B) conidium seed cultures of *Aspergillus* sp. N2, (C) mycelial seed cultures of *Penicillium* sp. N3, and (D) conidium seed cultures of *Penicillium* sp. N3 initiated in PG medium supplemented with 10 ppm of Cr(VI) at near neutral pH (pH 6.0).

Symbols represent: ▲, total Cr; and ■, Cr(VI). The data shown in the figure are the average values of three experiments.

more efficient at Cr(VI) reduction than the organisms inoculated in the sporal state under both pH conditions (Fig. 2B). However, after 96 h of growth in liquid medium, both *Aspergillus* sp. N2 and *Penicillium* sp. N3 that were inoculated as conidium seed cultures were in logarithmic growth phase. *Aspergillus* sp. N2 and *Penicillium* sp. N3 in the mycelial seed cultures were in stationary phase at 240 h incubation, and these strains reduced the Cr(VI) concentrations by 96.1%, and 19.0% at neutral pH, respectively. Under these growth conditions, the DMWs of *Aspergillus* sp. N2 and *Penicillium* sp. N3 were 0.5 g/L, which is equal to the amounts in the mycelial seed cultures (data not shown). During actual effluent treatment (ex. activated sludge process), seed cultures of mycelia were used for bioremediation (Grazer and Nikaido, 1995). These results suggested that these strains have the potential for use in bioremediation.

Cr(VI) is mobile in the environment because of its high solubility in water (Zayed and Terry, 2003). Thus, polluted soils with low concentrations of chromium are widespread in the environment. We investigated the abilities of *Aspergillus* sp. N2 and *Penicillium* sp. N3 to lower the concentration of Cr(VI) (initial concentration 10 ppm) in the culture medium. Figure 3A shows a time course of the decrease in Cr(VI) and total Cr by the mycelial seed culture of *Aspergillus* sp. N2. After 24 h of incubation, the Cr(VI) was completely removed. In contrast, *Aspergillus* sp. N2 was able to reduce the total Cr concentration by only 20% after 96 h of incubation. Figure 3B shows a time course of the decrease in Cr(VI) and total Cr by the sporal seed culture of *Aspergillus* sp. N2. After 24 h of incubation, the Cr(VI) concentration decreased by 95%. In contrast, *Aspergillus* sp. N2 was able to reduce the total Cr concentration by only 15%. Under these conditions, the DMW of *Aspergillus* sp. N2 was 0.3 g/L after 24 h incubation, and subsequently *Aspergillus* sp. N2 was in stationary phase. Figure 3C shows a time course of the decrease in Cr(VI) and total Cr by the mycelial seed culture of *Penicillium* sp. N3. After 48 h of incubation, the Cr(VI) concentration decreased by 100%. In contrast, *Penicillium* sp. N3 was able to reduce the total Cr concentration by only 20% at 96 h incubation. Figure 3D shows a time course of the decrease in Cr(VI) and total Cr by the conidium seed culture of *Penicillium* sp. N3.

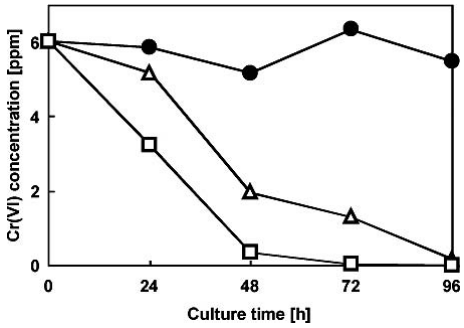

Fig. 4. Time course of the changes in Cr(VI) levels in samples from contaminated soil using water phase bioremediation.

Symbols represent: ●, blank; △, *Aspergillus* sp. N2; and □, *Penicillium* sp. N3. The data shown in the figure are the average values of three experiments.

After 24 h of incubation, the Cr(VI) concentration decreased by 80%. In contrast, *Penicillium* sp. N3 was able to reduce the total Cr concentration by only 2%. Under these growth conditions, the DMW of *Penicillium* sp. N3 was 0.5 g/L at 48 h incubation and subsequently *Penicillium* sp. N3 was in the stationary phase. Thus, these results suggested that *Aspergillus* sp. N2 and *Penicillium* sp. N3 would be useful for bioremediation of low concentration chromium contamination, and Cr(VI) reduction of both *Aspergillus* sp. N2 and *Penicillium* sp. N3 strains was due to enzymatic activity.

We adapted a water-phase bioremediation assay to explore possible usefulness of *Aspergillus* sp. N2 and *Penicillium* sp. N3 for eliminating Cr(VI) from contaminated soil containing approximately 60 mg Cr(VI)/g soil. Cr(VI) from the contaminated soil was removed indirectly by *Aspergillus* sp. N2 and *Penicillium* sp. N3. For this purpose the Cr(VI) was first leached out of the contaminated soil by shaking with distilled water at pH 8.0 for 24 h. We observed that the pH of the water used for leaching determined the rate of chromate extraction from the contaminated soil, and the optimal pH was 8.0 (data not shown). At this pH, 24 h of shaking with water extracted all of the chromium present in the contaminated soil. The mycelial biomass, obtained from a 3-day culture of the fungi in PG medium without added Cr(VI), was then mixed with the chromium-leached water, and fresh PG liquid medium (9 : 1), and the biomass mixture was incubated further at 30°C. The pH of this biomass mixture was approximately 7.5, and the Cr(VI) concentration was approximately 6 ppm. Figure 4 shows the time-dependent decrease in the Cr(VI) concentration by *Aspergillus* sp. N2 and *Penicillium* sp. N3. As shown, each strain was able to decrease the Cr(VI) concentration significantly until 48 h (67.2% for *Aspergillus* sp. N2 and 95.0% for *Penicillium* sp. N3). After 72 h incubation, *Penicillium* sp. N3 reduced the Cr(VI) concentration in the mixture by almost 100%. In addition, *Aspergillus* sp. N2 decreased the Cr(VI) concentration from its initial concentration of 6 ppm to nearly undetected levels after 96 h incubation. In the absence of any added fungal strain (control blank), the Cr(VI) concentration in the mixture did not decrease. Total Cr reduction did not change for different culture conditions (data not shown).

Aspergillus sp. N2 and *Penicillium* sp. N3 were able to decrease the initial Cr(VI) concentrations of the contaminated soils. While Cr(III) compounds are very stable in soils, Cr(VI) is very unstable and is easily mobilized in both acidic and alkaline soils (Zayed and Terry, 2003). Since Cr(VI) could be easily extracted from the soil into aqueous solution, we employed water phase bioremediation to determine the effectiveness of the isolated strains in removing Cr(VI) from the contaminated soils. In addition, we adopted seed cultures for these assays because the inoculated strains must grow preferentially. Our results clearly show that the fungal strains *Aspergillus* sp. N2 and *Penicillium* sp. N3 succeeded in reducing the Cr(VI) concentration in non-sterilized soils.

The chromium removal abilities of *Aspergillus* sp. N2 and *Penicillium* sp. N3 are equal or better than those of other reported strains, for example *Pseudomonas* CBR5 (Mclean and Beveridge, 2001), and *Candida maltose* RR1 (Ramirez-Ramirez et al., 2004). In particular, *Penicillium* sp. N3 was superior to the other strains because it has the capacity for efficient chromium reduction under acidic conditions. Most other Cr(VI) reduction studies were carried out at neutral pH (Acevedo-Aguilar et al., 2006; Fude et al., 1994; Laxman and More, 2002; Mclean and Beveridge, 2001; Ramirez-Ramirez et al., 2004). *Aspergillus niger* also has the ability to reduce and adsorb Cr(VI) (Srivastava and Thakur, 2006). When the initial concentration of Cr(VI) was 50 ppm, *Aspergillus niger* mycelium removed 8.9 mg of chromium/g dry weight of mycelium in 7 days. In the present study, *Aspergillus* sp. N2 and *Penicillium* sp. N3 removed 74.7 mg/g, and 41.4 mg/g, Cr(VI), respectively (pH 6.0, 120 h). These results suggest the potential applicability of *Aspergillus* sp. N2 and *Penicillium* sp. N3 for the remediation of Cr(VI) from con-

taminated soil in fields.

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