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Short communication

Iso-catalase Profiles of *Deinococcus* spp.

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The obligate aerobic *Deinococcus* are highly resistant against lethal effect of UV-and ionizing-radiation. Only five mesophilic *Deinococcus* species, *i. e. D. radiodurans*, *D. radiophilus*, *D. proteolyticus*, *D. radiopugnans*, and *D. grandis* are known. Since an indispensable role of catalase has been suggested in protecting cells against oxidative stress and UV radiation, Deinococcal catalase activity of each species and electrophoretic profiles of catalases were investigated on gel. Total catalase activity was varied among the species in the aerobically grown culture at stationary phase. The occurrence of multiple forms of catalases with different molecular weights in four species of *Deinococcus* and of a single catalase in *D. radiopugnans* suggests that each species shows the unique catalase profiles on gel. Some Deinococcal catalases also exhibit peroxidase activity. Since *Deinococcus* spp. are less-distinct to each other in their morphology, biochemical and physiological properties, the catalase profiles on PAGE would be useful in identifying the species of *Deinococcus*.

Keywords: *Deinococcus*, Electrophoretic profiles, Iso-catalases, UV resistance.

Introduction

The reactive oxygen intermediates, such as superoxide (O_2^{-1}), peroxide (O_2^{-2}), hydrogen peroxide (H_2O_2), hydroxyl (OH·)- and hydroperoxyl (HOO·)-radical are produced during aerobic respiration of cells (Halliwell and Gutteridge, 1999). These reactive oxidants could be also generated by a variety of environmental sources; *e. g.* ionizing radiations (X-ray, γ-ray), UV light, and redox cycling drugs (Hassan and Fridovich, 1979; Vile and Tyrrell, 1993; Jurkiewicz and Buettner, 1994). These oxidants cause chemical modification of the cellular components, nucleic acids, proteins, and lipids, resulting in a number of metabolic diseases, aging process, mutagenesis, carcinogenesis, and cell death (Halliwell and Gutteridge, 1999). However, cells operate several defense mechanisms against reactive oxygen intermediates by either scavenging the offending oxidants or repairing the damaged cellular components. The removal of reactive oxidants can be achieved by the action of scavenging enzymes such as superoxide dismutase (SOD), catalase, peroxidase, and glutathione reductase, and by a number of antioxidants occurring in cells (Cha and Kim, 1996; 1999; Halliwell and Gutteridge, 1999). Repairing enzymes such as DNA exonuclase III, endonuclase IV, proteolytic enzymes, and phospholipase A_2 are involved in restoration of damaged cellular components (Demple and Harrison, 1994; Gutman, *et al.*, 1994; Agostini *et al.*, 1996). Among the scavenging enzymes of reactive oxygen spp., hydroperoxidase, a multienzyme consisted of catalase and peroxidase is one of well-known scavengers.

The Genus *Deinococcus* is strictly aerobic and known to be extraordinarily resistant to UV- and ionizing-radiation (Murray, 1986; Mattimorre and Schellhorn, 1994). *Deinococcus* species have some peculiarities including thick cell wall contained L-ornithine, outer membrane-like structure, and orange-colored pigment due to membranous carotenoid, (Murray, 1986; Muller and Engel, 1996). However, the most peculiar feature of Genus *Deinococcus* is its extreme resistance to UV- and ionizing-ray. Although the mechanism of Deinococcal UV resistance has been studied with regard to the repairing the damaged DNA (Gutman *et al.*, 1994; Carroll *et al.*, 1996; Battista, 1997; Bauche and Laval, 1999), the roles of the antioxidant scavenging system were less investigated. However, studies on scavenging enzymes of the reactive oxidants in relation to the UV resistance have been recently undertaking in *Deinococcus radiophilus*. *D. radiophilus* is reported to possess three iso-catalases, *viz*, catalase-1, catalase-2, catalase-3, the latter two catalases are bifunctional enzymes, having both catalase and peroxidase activities (Lee and Lee, 1995). Enzymatic properties of these bifunctional catalases were quite different from each other (Lee and Lee, 1995; Oh and Lee, 1998). Not only this, *D. radiophilus* bifunctional catalases show dissimilar response toward oxidative stress and UV radiation with different cellular localization (Yun and Lee, 2000). Except *D.*

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radiodurans (Wang and Schellhorn, 1995) and *D. radiophilus* (Lee and Lee, 1995; Oh and Lee, 1998), the catalase of other *Deinococcus* species was not revealed yet. Here, we report the catalase profiles of the mesophilic *Deinococcus* spp.

Materials and Methods

Bacterial strains and culture condition *Deinococcus radiodurans* ATCC 13939, *Deinococcus radiophilus* ATCC 27603, *Deinococcus grandis* ATCC 43672, *Deinococcus proteolyticus* ATCC 35074, and *Deinococcus radiopugnans* ATCC 19172 were cultured in TYGM medium containing 1% tryptone, 0.5% yeast extract, 0.2% glucose, and 0.2% methionine (Oh and Lee, 1998). The incubation of the bacteria was carried out at 30°C for 3-6 days with continuous shaking (150 rpm). Bacterial growth was monitored at OD_{600} (DU-65 Spectrophotometer, Beckman, Fullerton, USA).

Regents Chemicals such as Coomassie Brilliant Blue G-250, D-glucose, DL-methionine, 3% hydrogen peroxide solution, horse radish peroxidase, diaminobenzidine, ammonium persulfate, acrylamide, bis-acrylamide, guaiacol, Tris-HCl and others were purchased from either Sigma Chemical Co., USA or Junsei Chem. Co., Japan. Most of the medium constituents were bought from Difco Lab.

Assay of catalase/peroxidase activity Catalase activity in sonic extract of cells was measured by following the decrease in absorbance at 240 nm caused by the disappearance of H_2O_2 in potassium phosphate buffer (pH7.0) at 25°C (Beer and Sizer, 1951; Oh and Lee, 1998). The enzyme activity decomposing 1 μmol of H_2O_2 per min was defined as one unit ($\varepsilon = 43.6\ M^{-1}cm^{-1}$). Peroxidase activity was determined by monitoring the increase in absorbance at 470 nm due to guaiacol oxidation. The standard reaction mixture for the assay of guaiacol peroxidase activity was composed of 100 mM $NaHCO_3$-NaOH buffer, pH 9.0, 30 mM guaiacol, 100 mM H_2O_2, and an appropriate amount of the enzyme preparation in a total volume of 1.0 ml. The amount of enzyme activity increasing OD_{470}= 1 per min. was defined as one unit (Bergmeyer, 1974). Protein quantitation was made by Bradford method (1976).

Gel electrophoresis and activity staining of catalase/peroxidase on gels Proteins in cell lysate prepared by ultrasonic disruption were resolved on 8.5% gel by PAGE in Tris-glycine buffer (Hedrick and Smith, 1968). Catalase bands resolved on native gel were visualized by incubating the gels in 5 mM H_2O_2, followed by freshly prepared mixture of 2% ferric chloride and 2% potassium ferric cyanide by the method of Wayne and Diaz (1986). Peroxidase activity band on gel was visualized in 50 mM potassium phosphate buffer, pH 7.0 containing diaminobenzidine of 0.5 mg/ml and 5 mM hydrogen peroxide. Diaminobenzidine was used as an electron donor for the peroxidatic activity. Peroxidase band on gel was appeared in dark brown color (Clariborne and Fridovich, 1979).

Molecular weight determination The molecular weights of catalases were determined by PAGE. Bovine serum albumin (66,000, monomer; 132,000, dimer), Jack bean urease (272,000, trimer; 545,000, hexamer) and apoferritin (699,000) (Sigma Chemical Co.) were used as size markers (Hedrick and Smith, 1968).

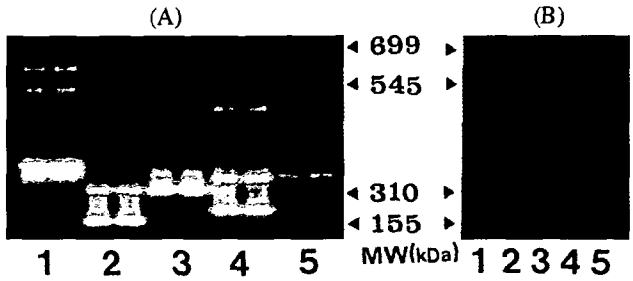

Fig. 1. Electrophoretic profiles of catalase/peroxidase of *Deinococcus* spp. Each well was loaded with 10 μg of protein in cell-free extracts of *Deinococcus* cultures at stationary phase. Activity staining of catalase (A) and peroxidase (B) on 8.5% polyacrylamide gel was performed as described in Materials and Methods. 1. *D. radiodurans*, 2. *D. radiophilus*, 3. *D. grandis*, 4. *D. proteolyticus*, 5. *D. radiopugnans*.

Results and Discussion

Catalase/peroxidase profiles of *Deinococcus* spp. on gel Resolution of proteins in cell lysates made with *Deinococcus* cultures at stationary phase revealed the multiple forms of catalase with different electrophoretic mobilities. It was observed that only one catalase band in *D. radiopugnans*, two bands in *D. grandis*, three bands in *D. radiodurans* and in *D. radiophilus*, and four catalase bands in *D. proteolyticus* were shown (Fig. 1A). Multiplicity of catalase is not rarely found in prokaryotes (Loewen and Switala, 1987; Goldberg and Hochman, 1989; Klotz and Hutcheson, 1992; Kim *et al.*, 1992). There are two classes of catalase; one class is monofunctional catalase exhibiting only catalatic activity, whereas the other is bifunctional enzyme showing both catalatic-peroxidatic activities (Loewen *et al.*, 1985). Some Deinococcal catalases also show peroxidase activity as shown in Fig. 1B. Multiple catalase-peroxidase bands with different electrophoretic mobilities were observed; two bands in either *D. radiophilus* or *D. proteolyticus*, and all of three catalases in *D. radiodurans* showed peroxidase activity. *D. grandis* possessed one of each monofunctional catalase and bifuctional catalase-peroxidase. The molecular weights of the catalases of *Deinococcus* spp. as estimated by PAGE varied from approximately 650 kDa to 155 kDa. The smallest (155 kDa) and the largest (650 kDa) catalases were occurred in *D. radiophilus* and *D. proteolyticus*, respectively. Two kinds of bifunctional catalase-peroxidase of *D. radiophilus* have been purified, having molecular weights of 310 kDa and 155 kDa (Lee and Lee, 1995; Oh and Lee, 1998). The catalase-peroxidase with 155 kDa seems to be smaller catalase-peroxidase compared with those found in some other bacteria

Table 1. Comparison of catalase/peroxidase occurred in *Deinococcus* spp.

Bacteria	Catalase band	Catalase activity	Peroxidase activity	Remark
D. radiodurans	1*	+**	+	bifunctional catalase
	2	+	+	bifunctional catalase
	3	+++	++	bifunctional catalase
D. radiophilus	1	+	-	monofunctional catalase
	2	++	+	bifunctional catalase
	3	+++	+	bifunctional catalase
D. grandis	1	+	-	monofunctional catalase
	2	+++	++	bifunctional catalase
D. proteolyticus	1	+	-	monofunctional catalase
	2	+	-	monofunctional catalase
	3	+++	+	bifunctional catalase
	4	+++	++	bifunctional catalase
D. radiopugnans	1	++	+	bifunctional catalase

*Numbering of catalase bands by electromobilities.
**+++, ++, +; intensity of enzyme activity bands, -; no activity band detected.

(Lee and Lee, 1995). Catalase/peroxidase profiles of each *Deinococcus* species were given on Table 1.

Catalase/peroxidase activity of *Deinococcus* spp. Both catalase and peroxidase are the indispensable enzymes for aerobic creatures because of their roles in scavenging the reactive oxygen intermediates. Environmental factors, particularly oxidative stress influences the level of these enzymes in biospecies along with the innate nature of cells (Loewen *et al.*, 1985; Farr and Kogoma, 1991; Yun and Lee, 2000). When catalase/peroxidase activities of each *Deinococcus* species at stationary phase were measured, these enzyme activities varied among the Deinococcal species, the highest catalase activity with *D. radiophilus*. Catalase activity of *D. radiophilus* was ca. 3-fold higher than that of *D. radiopugnans*, which showed the lowest activity (Fig. 2A). The highest peroxidase activity was obtained with *D. radiodurans*, whereas the lowest one was observed with *D. grandis* as depicted in Fig. 2B. The former was 6-fold higher above the latter. However, it is not yet confirmed a direct correlation between catalase activity level of *Deinococcus* species and their resistance to UV radiation and to oxidative stress.

The genus *Deinococcus* are very peculiar organisms showing extreme resistance to ionizing radiation and UV radiation (Murray, 1986). Recent study on phylogenetic diversity as determined by 16S ribosomal DNA sequence comparison confirms existence of five species of mesophilic *Deinococcus* (Rainey *et al.*, 1997). Among these, four species of *Deinococcus* are gram-positive cocci and one species is gram-negative rod. Since gram- positive *Deinococcus* species are very similar to each other in their morphology and biochemical-physiological properties, it is laborious to distinguish them from one to another, particularly when they are cross-contaminated. If a universally occurring cellular

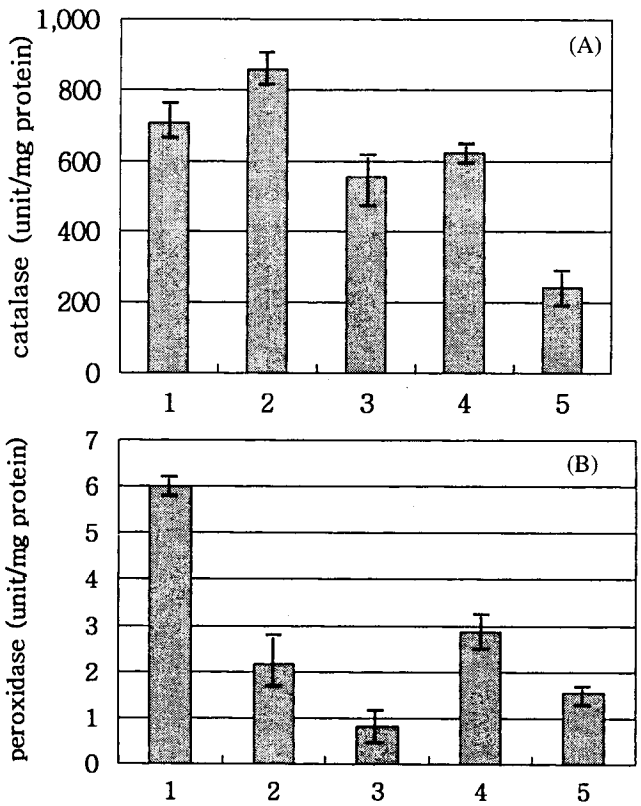

Fig. 2. Catalase/peroxidase activity of *Deinococcus* spp. at stationary phase. Catalase/peroxidase activities of cell cultures were assayed as described in Materials and Methods. 1. *D. radiodurans*, 2. *D. radiophilus*, 3. *D. grandis*, 4. *D. proteolyticus*, 5. *D. radiopugnans*.

constituent in the genus *Dinococcus* shows species-speicific physicochemical properties, this substance would be a very valuable marker in identifying Deinococcal species from each other. Such instances were made in the identification of *Leishmania* spp. by multiple isozyme analysis (Kreutzer *et al.*, 1983) and differentiation of *Saccaromyces paradoxus* by allozyme analysis (Naumov *et al.*, 1997). Our study on catalases occurred in *Deinococcus* species revealed that each species shows the unique electrophoretic profiles of catalase/peroxidase; a variety in catalase profiles with respect of number of catalase possessed, nature of catalase (either bifunctional or monofunctional enzyme), and their molecular sizes. With the exception of *D. radiopugnans*, each *Deinococcus* species possesses multiple forms of catalases. Therefore, we suggest that electrophoretic profiles of iso-catalase would be of useful in distinguishing each species of the genus *Deinococcus*.

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