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NOTE

Superoxide Dismutase Profiles in the Mesophilic *Deinococcus* Species

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Electrophoretic resolution of superoxide dismutase (SOD) from the highly UV-resistant bacteria, *Deinococcus* species revealed multiple forms of superoxide dismutases (SODs) in *D. radiodurans*, *D. grandis*, and *D. proteolyticus*, as judged from electrophoretic properties and metal cofactors. A single SOD occurred in both *D. radiophilus* and *D. radiopugnans*. Deinococcal SODs were either MnSOD, FeSOD or cambialistic Mn/FeSOD. The unique SOD profile of each mesophilic *Deinococcus* species, multiplicity and metal cofactors, would be valuable in identifying *Deinococcus* species.

Key words: *Deinococcus* spp., UV-resistant, multiplicity of SODs, electrophoretic profiles

Superoxide (O_2^-) generated in cells during aerobic respiration and by a variety of environmental factors, such as UV, ionizing radiation (X-ray, γ-ray, cosmic ray), cigarette smoking, and some redox cycling drugs are causing various cellular damage (9). However, most cells are able to protect themselves to a certain extent from the toxicity of superoxide ions by superoxide dismutases (SODs; EC 1.15.1.1) which dismutate superoxide anions into hydrogen peroxide and molecular oxygen. Regardless of their sources, all SODs are metalloenzymes, MnSOD, FeSOD, and Cu/Zn SOD. In addition, an occurrence of NiSOD in *Streptomyces* spp was also reported (29). Metal cofactors in active sites of SODs are different, depending upon cell types. Cu/Zn SOD occurs mainly in cytosols of animal cells and MnSODs are in both procaryotes and mitochondria, while FeSODs are found in cytosols of procaryotes, but also in primitive eucaryotes and in some green plants (9, 12).

An obligately aerobic bacterium, *Deinococcus* species have some peculiarities, such as a thick cell wall of several distinct layers including an outer membrane, membrane-bound carotenoid pigment and presence of ornithine in muramic acid cross bridges. The most peculiar feature of *Deinococcus* species is their extreme resistance to UV- and ionizing-radiation (2, 3, 6, 21, 23, 24). Although the unusual radio-resistance of *Deinococcus* might be attributed to their morphological characteristics, one can easily assume that operation of efficient scavenging systems against reactive oxygen species (ROS) generated by radiation along with the repairing of damaged cellular components mediated by toxic oxidants would contribute more to Deinococccal radiation resistance. Although the entire genome analysis has been recently reviewed (14, 19) and extensive studies on UV resistance of *Deinococcus* with regard to the repairing genes for damaged DNA were made (1, 4, 6, 8, 11, 22), lesser attention has been paid to the ROS scavenging enzymes in *Deinococcus*. Therefore, we have undertaken studies on key enzymes in the scavenging toxic oxidants, SODs and hydroperoxidases in UV resistant *Deinococcus*. *Deinococcus radiophilus* possesses three isoforms of hydroperoxidases removing peroxide radicals. Two of them are bifunctional catalase-peroxidases and each of the bifunctional enzymes responds in different ways toward UV and oxidative stress (17, 26, 30). We also described the presence of multiple forms of hydroperoxidases showing dissimilar electrophoretic properties and different functionality in the five mesophilic *Deinococcus* species (28). Each species has unique profiles of hydroperoxidases, which might be useful as an enzyme marker in species identification. In this paper, profiles of SODs occurring in five species of mesophilic *Deinococcus* are reported.

Deinococcus radiodurans ATCC 13939, *D. radiophilus* ATCC 27603, *D. grandis* ATCC 43672, *D. proteolyticus*

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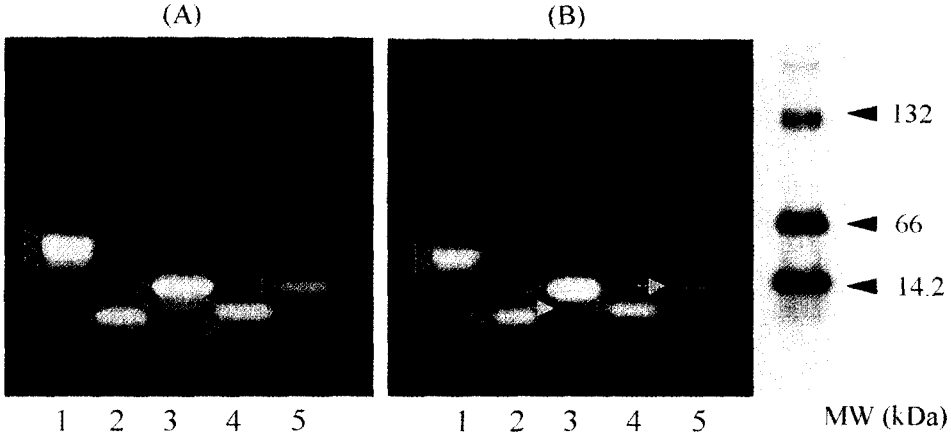

Fig. 1. Electrophoretic profiles of SOD of *Deinococcus* spp. Each well was loaded with 50 μg of protein in *Deinococcus* cell-free extracts obtained from the stationary culture. Activity staining of SOD on gel (10%) prior to H_2O_2 treatment (A) or after H_2O_2 treatment (B). Treatment of H_2O_2 caused inactivation of FeSOD. KCN treatment prior to activity staining caused no change of Deinococcal SOD profiles (Data not shown). This indicates that Deinococcal SODs are not CuZnSOD. See details in Methods (5, 7, 15). 1. *D. radiodurans* 2. *D. radiophilus* 3. *D. grandis* 4. *D. proteolyticus* 5. *D. radiopugnans*. Symbols (▷, ➡) for SOD activity bands. (➡) for SOD band whose intensity was reduced after H_2O_2 treatment.

ATCC 35074, and *D. radiopugnans* ATCC 19172 were cultured in TYGM medium (1% tryptone, 0.5% yeast extract, 0.2% glucose, and 0.2% L-methionine) at 30°C with continuous aeration at 150 rpm until the cultures reached the stationary growth phase (24, 28, 30). Proteins in cell-free extract prepared with cells in the stationary phase by ultrasonic disruption (28, 30) were resolved by 10.0% nondenaturing polyacrylamide gel electrophoresis (PAGE) in Tris-glycine buffer (9, 13). Size markers for gel electrophoresis were bovine serum albumin (132 kDa, 66 kDa) and bovine milk α-lactalbumin (14.2 kDa). Visualization of SOD bands resolved on polyacrylamide gel was made with the activity staining method (5) modified by Chou and Tan (7). The gels were soaked in 490 μM NBT for 20 min, then in a solution containing equal volumes of 28 mM TEMED, 28 μM riboflavin and 36 mM potassium phosphate buffer (pH 7.8) for 15 min. Then the gels were illuminated with a fluorescent lamp for 5-15 min to visualize white bands of SOD activity on the blue background. Distinction between FeSOD, MnSOD, and CuZnSOD was made by soaking the gels for 60 min at room temperature in a solution of either 20 mM H_2O_2 or 5 mM KCN and 50 mM potassium phosphate (pH 7.8) prior to activity staining for SOD. Hydrogen peroxide treatment causes selective inactivation of either FeSOD or CuZnSOD (16). KCN treatment selectively inhibits CuZnSOD. As seen in Fig. 1A, electrophoretic mobilities and the number of SODs from each *Deinococcus* species seemed to be quite different. Multiple SODs (2-3 SOD bands) were observed in three of the mesophilic *Deinococcus*, except *D. radiophilus* and *D. radiopugnans*. When SOD activity staining on gel was performed after a selective inactivation of FeSOD by H_2O_2 treatment, some of SOD bands shown in Fig. 1A disappeared (Fig. 1B). KCN-treatment of gels prior to SOD activity staining did not cause any change of SOD profiles on the gel (data not shown). These observations suggested that the metal ion cofactors at the active site of *Deinococcal* SODs are either Mn(II) or Fe(II) (Table 1). The majority of *Deinococcal* SODs showing rapid migration seemed to be rather peculiar proteins compared with other known SODs, either smaller in size or lower in their pIs.

SODs are indispensible to aerobic cells surviving superoxide toxicity and the level of SODs are influenced by oxidative stress along with the innate nature of the cells. We measured the SOD activity in cell-free extracts made with cells in the stationary growth phase by pyrogallol autooxidation (20), in which the NBT reduction by superoxide radicals generated photochemically from riboflavin

Table 1. Superoxide dismutases occurring in the mesophilic *Deinococcus* spp

Bacteria	SOD band	SOD activity		Remark
		without H_2O_2 treatment	with H_2O_2 treatment	
D. radiodurans	1[a]	+++[b]	+++	MnSOD
	2	+++	+++	MnSOD
	3	++	-	FeSOD
D. radiophilus	1	+++	+++	MnSOD
D. grandis	1	+++	+++	MnSOD
	2	++	+	Mn/FeSOD
D. proteolyticus	1	+++	+++	MnSOD
	2	+	-	FeSOD
D. radiopugnans	1	++	+	Mn/FeSOD

[a]Numbering of SOD bands on gel, from top to bottom.
[b]+++, ++, +; intensity of enzyme activity bands, -; no activity band detected.

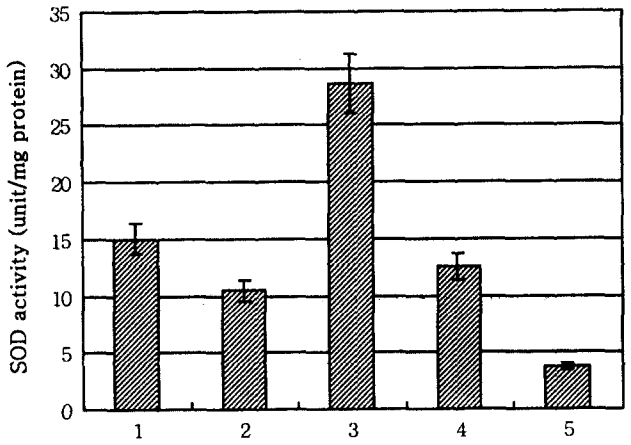

Fig. 2. SOD activity of *Deinococcus* spp. at the stationary phase. SOD activities of the cultured cell were assayed by the method of Marklund and Marklund (20). 1. *D. radiodurans* 2. *D. radiophilus* 3. *D. grandis* 4. *D. proteolyticus* 5. *D. radiopugnans*. Values are the means of three independent experiments. Error bars show the standard error.

was measured in the presence of SOD enzyme. One unit of SOD was defined as the amount of enzyme causing 50% inhibition of the pyrogallol autooxidation rate at 420 nm (20). Protein concentration was determined by the method of Lowry *et al* (18). As depicted in Fig. 2, the total SOD activity in the stationary culture of *Deinococcus* varied among species. The highest activity was obtained with *D. grandis*, whereas the lowest activity was in *D. radiopugnans*. The former was several times higher than that of the latter. *D. grandis*, which shows the highest level of SODs is the only gram-negative rod in *Deinococcus* genus. Although *D. radiodurans* is believed to be the most resistant species to UV among the coccus forms of mesophilic *Deinococcus* (3), a direct correlation between the SOD activity level of *Deinococcus* species and their resistance to UV radiation is not confirmed yet. Thus, it would be worthwhile to investigate whether SOD activity levels of *Deinococcus* species directly correlate with their resistance to UV and oxidative stress. A recent study on phylogenetic diversity as determined by 16S ribosomal DNA sequence comparison confirms the existence of five species of mesophilic *Deinococcus* (27). Among these, four species are gram-positive cocci and one species is gram-negative rod. Since the 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 another, particularly when they are cross-contaminated. If a universally occurring cellular constituent in the genus *Dinococcus* shows species-specific physicochemical properties, this substance would be a very valuable marker in distinguishing Deinococcal species from each other. Such instances were made in the identification of *Leishmania* spp. by multiple isozyme analysis (15) and differentiation of *Saccharomyces paradoxus* by allozyme analysis (25). Our study on SODs occurred in *Deinococcus* species revealed that each species shows unique electrophoretic SOD profiles with respect to the number of SOD owned, metal ion cofactors, and their molecular sizes. Therefore, we suggest that electrophoretic profiles of iso-SODs would be valuable in identifying each species of the genus *Deinococcus*, along with their iso-catalase profiles (28).

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