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Manganese(II) Induces Cell Division and Increases in Superoxide Dismutase and Catalase Activities in an Aging Deinococcal Culture

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Addition of Mn(II) at 2.5 μM or higher to stationary-phase cultures of *Deinococcus radiodurans* IR was found to trigger at least three rounds of cell division. This Mn(II)-induced cell division (Mn-CD) did not occur when the culture was in the exponential or death phase. The Mn-CD effect produced daughter cells proportionally reduced in size, pigmentation, and radioresistance but proportionally increased in activity and amount of the oxygen toxicity defense enzymes superoxide dismutase and catalase. In addition, the concentration of an Mn-CD-induced protein was found to remain high throughout the entire Mn-CD phase. It was also found that an untreated culture exhibited a growth curve characterized by a very rapid exponential-stationary transition and that cells which had just reached the early stationary phase were synchronous. Our results suggest the presence of an Mn(II)-sensitive mechanism for controlling cell division. The Mn-CD effect appears to be specific to the cation Mn(II) and the radioresistant bacteria, deinococci.

Bacteria of the genus *Deinococcus*, formerly a part of *Micrococcus* (27), are known for their extremely high radiation resistance (22). These bacteria also have unusual cell wall amino acids (38), polar lipids (10), and surface layers (4). Extensive investigations mainly on radiation-sensitive mutants have attributed the extreme radioresistance to effective DNA repair (13, 22–24, 26). The chemical requirements for optimal growth and for the expression of high radioresistance in deinococci remain largely unknown, partly because of the lack of appropriate chemically defined medium (19, 32). There is limited information on the effects of the divalent cation Mn(II). It has been reported that *Deinococcus radiodurans* can accumulate manganese in large amounts (18), and Mn(II) was found to be required for the activity of a DNA repair enzyme (14) in this bacterium and was implicated in its high radioresistance (37).

Recently, we observed that addition of Mn(II) to the medium unexpectedly caused a deinococcal culture in the stationary phase to be less resistant to γ irradiation. Further experiments revealed that the addition of Mn(II) resulted in a ninefold increase in maximum cell number. The growth curves showed that this increase in number was due to the ability of Mn(II) to trigger new rounds of cell division in a culture that was about to enter its stationary phase. In this report, we describe and discuss this Mn(II)-induced cell division (Mn-CD) in detail.

(Portions of this work have been presented elsewhere [F. I. Chou and S. T. Tan, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, I-136, p. 240]. This research was conducted by F. I. Chou in partial fulfillment of the requirements for the Ph.D. degree from National Tsing Hua University.)

MATERIALS AND METHODS

Strains, media, and growth conditions. *D. radiodurans* IR, formerly *Micrococcus* isolate C-7, has been characterized (34–36; S. T. Tan, S. T. Wang, S. C. Lu, F. I. Chou, F. M. Chang, C. I. Liu, D. L. Yung, and S. L. Tsai, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, I-106, p. 235). Cultures of *D. radiodurans* R1, *Deinococcus radiophilus*, *Deinococcus proteolyticus*, and *Deinococcus radiopugnans* were kind gifts from R. G. E. Murray, University of Western Ontario, Canada. *Micrococcus luteus*, *Micrococcus roseus*, *Bacillus subtilis*, *Escherichia coli* K-12, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, and *Saccharomyces cerevisiae* were from the Culture Collection and Research Center, Taiwan. TGY medium was prepared as described by Anderson et al. (1). Other media were purchased from Difco Laboratories. Deinococcal strains were propagated at 32°C, with shaking at 160 rpm, in plate count broth (PCB) containing 5 g of yeast extract, 10 g of tryptone, and 2 g of dextrose per liter. Other organisms were similarly cultivated but at their optimal temperatures.

Determination of cell number and cell mass. Plate count agar was used in cell enumeration by the standard plate count method as previously described (33). Counts expressed as CFU were determined after incubation for 2 to 3 days at the temperature used for the broth culture. To estimate the dry weight of a culture, cells harvested from a 30-ml culture were washed once with deionized water, dried at 80°C for 17 h, and weighed after equilibration at room temperature for 30 min.

Chemicals and enzymes. Diaminobenzidine and horseradish peroxidase were purchased from Sigma Chemical Co.; salts, buffers, and organic compounds, of at least reagent grade, were purchased from E. Merck AG. $MnSO_4 \cdot H_2O$ was used to prepare the Mn(II) solution.

Cell morphology. Cells were safranin stained and observed by phase-contrast microscopy.

Preparation of cell extract and determination of protein concentration. Cells harvested by centrifugation (7,000 × *g*, 4°C, 10 min) were washed twice and suspended in 50 mM potassium phosphate (pH 7.0). Cells were disrupted with a sonicator (Heat Systems Ultrasonic Inc.) at 75-W output for a total of 6 min. During sonication, cells were cooled on ice, and a 1-min interval was allowed between each 2 min of sonication. The cell homogenate was clarified by centrifugation (10,000 × *g*, 4°C, 30 min), and the cell extract was stored at 4°C before use. Protein concentration was determined by the method of Bradford (7), with use of the Bio-Rad protein

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dye reagent; bovine serum albumin was the calibrating standard.

Gel electrophoresis. Nondenaturing polyacrylamide gel electrophoresis was performed by the procedure of Davis (11). Cell extract was loaded on a 7.6% polyacrylamide gel, and electrophoresis was run at 4°C with a constant current of 30 mA per slab until the tracking dye (bromophenol blue) reached the bottom of the gel. Gel was stained with Coomassie blue R-250 for protein visualization or negative stained for enzyme activity as described below.

Enzyme activity assays. Superoxide dismutase (SOD) activity in the cell extract was assayed by the method of Beauchamp and Fridovich (5). In their method, photochemically generated superoxide radicals react with nitroblue tetrazolium (NBT), producing insoluble purple-blue formazan. Since SOD can compete with NBT in scavenging superoxide radical, inhibition of the color formation can be used to quantitate SOD activity in a sample. In using this method, we found that deinococcal cell extract contained an NBT-reactive substance (see reference 28 for chemical properties of NBT) that interfered with the assay by yielding a water-soluble magenta-colored product. To circumvent this problem, each sample was pretreated by ammonium sulfate fractionation. Ammonium sulfate (solid) was slowly added to the cell extract, making a final concentration equivalent to 65% saturation. This mixture was stirred at room temperature for 90 min and then centrifuged at 12,000 × *g* for 10 min to precipitate the interfering material. The supernatant was dialyzed to remove all ammonium sulfate and Mn(II) before assay for SOD. The reaction mixture contained 56 μM NBT, 10 mM methionine, 1.17 μM riboflavin, 20 μM sodium cyanide, 50 mM potassium phosphate (pH 7.8), and sample solution in a total volume of 5.0 ml. The reaction mixture was illuminated at ambient temperature with a 15-W fluorescent lamp; the color formation due to NBT reduction was quantitated by determination of A_{560}. Inhibition of this reaction by SOD in the sample was determined by plotting A_{560} against the quantity of each sample. Under the test conditions, 1 U of SOD was defined as that amount causing 50% inhibition of the rate of NBT reduction.

SOD isozymes on a nondenaturing polyacrylamide gel were stained for visualization essentially as described by Beauchamp and Fridovich (5), but a lower level of NBT (0.49 mM) was used to obtain sharper activity banding. The iron-containing SOD (FeSOD) band was distinguished from the MnSOD band by soaking the electrophoresed gel for 60 min at room temperature in a solution containing 20 mM H_2O_2, 1.0 mM potassium cyanide, 0.1 mM EDTA, and 50 mM potassium phosphate (pH 7.8) before staining for SOD activity. This treatment selectively inactivates FeSOD (3).

Catalase activity in free solution was assayed by monitoring the decomposition of hydrogen peroxide (decrease in A_{240}) by the method of Beers and Sizer (6). Catalase was visualized on a native gel by the method of Gregory and Fridovich (16). The electrophoresed gel was soaked for 40 min in a solution containing 50 mM potassium phosphate (pH 7.0), 0.125 mg of diaminobenzidine per ml, and 2 μg of horseradish peroxidase per ml. The gel was then rinsed twice with water and finally bathed in 20 mM H_2O_2 (prepared with 50 mM potassium phosphate [pH 7.0]) for developing activity bands.

Radiation sensitivity. Cells were washed and suspended in 50 mM potassium phosphate (pH 7.0). A 10-ml cell suspension (ca. 10^6 CFU/ml) placed in a glass petri dish with stirring was UV (254 nm) irradiated with a germicidal lamp (General Electric G15T8) at a dose rate of 4.6 J m^{-2} s^{-1} as determined with a radiometer (model UVX-25 sensor; UVX Inc.). Cells were γ irradiated with ^{60}Co at a dose rate of 0.5 Mrad/h. Irradiation was at ambient temperature; survivors were enumerated by plate count.

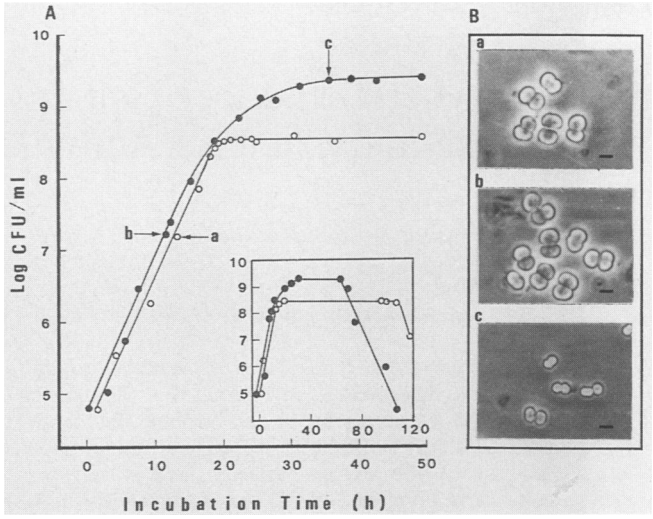

FIG. 1. (A) Growth of *D. radiodurans* IR in PCB with (●) or without (○) addition of 100 μM Mn(II) to the medium. The inset shows complete growth curves illustrating the faster decay of the Mn(II)-treated culture. (B) Phase-contrast light micrographs of cells examined at times a, b, and c (indicated by arrows in panel A), showing Mn(II)-induced changes in cell size. a, Nontreated cells in the exponential phase; b, treated cells in the exponential phase; c, treated cells in the Mn-CD phase. Bars = 2 μm.

RESULTS

Effect of Mn(II) addition on growth of *D. radiodurans* IR in PCB. The growth curves of strain IR cultivated in PCB with and without Mn(II) addition are shown in Fig. 1. By sampling at short intervals for cell enumeration, we noted that without Mn(II) addition there was a very abrupt transition from exponential to stationary phase. During this rapid transition, the cells were amenable to synchronization; when cells that had just reached the early stationary phase were put into fresh medium, they grew and divided together for at least two cycles (Fig. 2). With Mn(II) addition (100 μM) in PCB, the growth curve changed; there was a longer transition to stationary phase (Fig. 1A) and a shorter duration of that phase (Fig. 1A, inset). The two curves, however, overlapped in their exponential phases. Hence, the Mn-CD effect did not occur in an exponentially growing culture but rather at the end of exponential growth. The addition of Mn(II) stimulated growth and produced a maximum cell number of 2.7 × 10^9 CFU/ml, a higher value than usual for cultures of *D. radiodurans*. The growth, however, appeared to be exhausting, since the treated culture aged and lost viability much faster than did the control (Fig. 1A, inset). Mn-CD produced much smaller daughter cells (Fig. 1B), although they continued to display a tetracoccal-diplococcal form. Thus, cells produced after three rounds of Mn-CD had diameters only half that of the normal cells (Fig. 1B, panel c versus panel a or b). Since cells undergoing Mn-CD presumably divided under conditions of nutrient depletion, the cell doubling time became longer as Mn-CD proceeded. The exponential-phase doubling time was about 67 min, whereas it took about 2, 4, and 6 h, respectively, for the first, second,

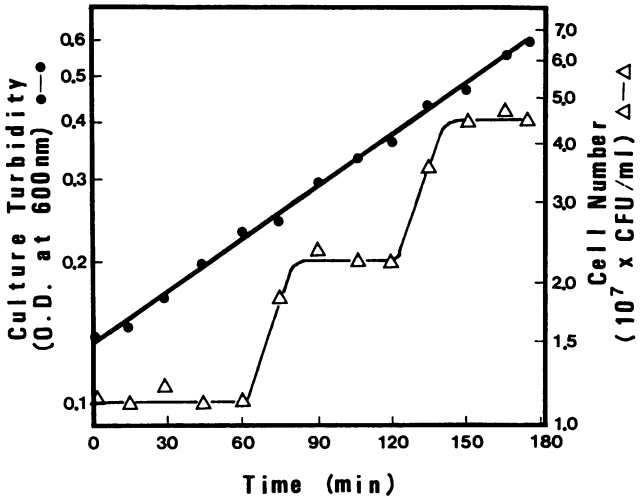

FIG. 2. Growth pattern showing cell synchronization. A 0.5-ml sample of the early-stationary-phase culture of *D. radiodurans* IR was put into 9 ml of fresh PCB. At intervals, cultures were sampled for cell number (△) by plate count and turbidity (●) by determination of optical density (O.D.) at 600 nm.

and third rounds of Mn-CD to occur (Fig. 1A). Adverse conditions, such as nutrient depletion or escalated oxidative stress, in an aging culture probably play roles in the Mn-CD effect. Mn-CD could occur when a culture was already in stationary phase, but viable cells in the final death phase were no longer Mn-CD responsive (Fig. 3). Mn-CD was also demonstrated with three other media: tryptic soy broth, nutrient broth, and TGY (data not shown).

Effect of Mn(II) dose on Mn-CD in *D. radiodurans* IR. Mn(II) addition (100 μM) at various growth stages (except the death phase) resulted in the same level of maximum cell number production (2.7×10^9 CFU/ml), which was nine times that for the untreated control (3.0×10^8 CFU/ml) (Fig. 1A and 3). To further study the dose effect in Mn-CD, a concentrated solution of $MnSO_4 \cdot H_2O$ in deionized water

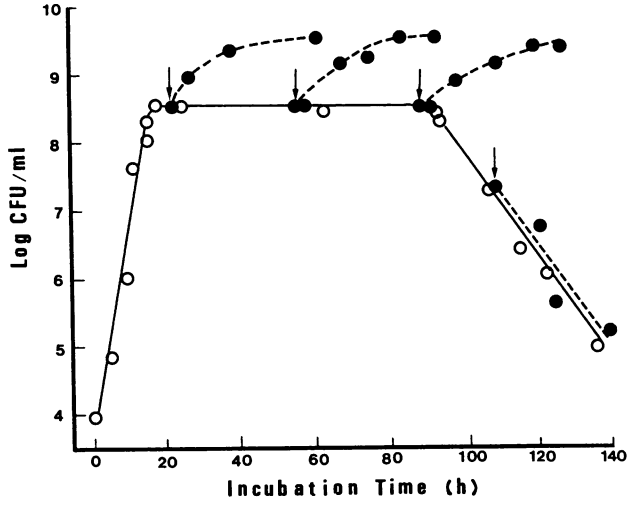

FIG. 3. Mn(II)-induced cell division of *D. radiodurans* IR cells pregrown in PCB to various stages of the stationary phase. The negative result for death-phase culture is also indicated. Arrows indicate the times of Mn(II) addition (100 μM).

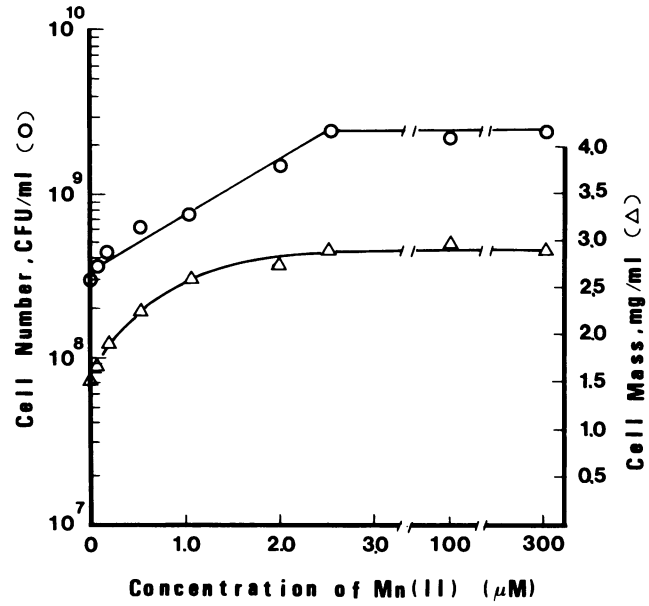

FIG. 4. Dose-response curves for the Mn-CD effect in *D. radiodurans* IR cells. A culture was grown to its own stationary phase (about 5 to 7 h after the exponential-stationary transition) and then sampled for determination of cell number (○) and cell mass (△).

was added to PCB to make Mn(II) concentrations ranging from 0 to 300 μM. The dose-response curves (Fig. 4) showed that addition of Mn(II) at a concentration as low as 0.1 μM resulted in a detectable Mn-CD effect, including increase in cell number and mass production. Maximum expression of these two growth parameters was similarly dose dependent in the range from 0 to 2.5 μM; from 2.5 to 300 μM, the values were constant. Mn-CD also occurred at an Mn(II) concentration as high as 1,000 μM, but cells thus grown aggregated intensively, making cell counting difficult. We also noted that in contrast to the marked cell number increase (ninefold), the Mn-CD culture was only 2.1-fold higher than the control in maximum cell mass production.

Specificity of the Mn-CD effect. The Mn-CD effect appeared to be specific to Mn(II) (sulfate and chloride salts worked similarly), since substitution of Mn(II) with Fe(II), Co(II), Ni(II), Cu(II), Zn(II), or Mo(V) (all tested at 10 μM) failed to trigger the same growth stimulation effect in a PCB-grown IR culture (data not shown). Moreover, Mn-CD appeared to be a novel property of deinococcal cells, since the results for all *Deinococcus* species tested were positive; there was no response in two *Micrococcus* species (*M. luteus* and *M. roseus*) (Table 1), *B. subtilis*, *E. coli* K-12, *S. aureus*, *S. typhimurium*, *P. aeruginosa*, and *S. cerevisiae*. In *Deinococcus* strains, cell numbers increased by factors of 3 to 10, but cell mass increased much less. The negative results for the other microorganisms are not shown except for species of *Micrococcus*, to which genus deinococcal strains formerly belonged.

Radiation sensitivity and pigmentation of Mn-CD cells. Mn(II)-treated and control IR cells at different growth stages were compared with respect to UV and γ-ray sensitivity (Fig. 5). Untreated cells (in both exponential and early stationary phases) and Mn(II)-treated cells in exponential phase were comparable in UV resistance, but cells in the Mn-CD phase became much more sensitive. The increase in sensitivity was more pronounced for cultures that had undergone more rounds of Mn-CD. Likewise, IR cells gradu-

TABLE 1. Increase in maximum cell number and cell mass production in *D. radiodurans* IR as a result of the Mn-CD effect[a]

Organism	Cell no. (10^8 CFU/ml)		Cell mass (mg/ml)		Mn-CD effect
	−Mn	+Mn	−Mn	+Mn	
Deinococcus spp.					
D. radiodurans IR	3.0	27	1.4	2.9	+
D. radiodurans R1	2.0	21	1.5	2.8	+
D. radiophilus	5.8	28	1.6	3.0	+
D. proteolyticus	11	35	1.8	4.3	+
D. radiopugnans	14	41	1.8	4.2	+
Micrococcus spp.[b]					
M. luteus	12	13	4.3	4.3	−
M. roseus	4.1	4.5	2.9	2.9	−

[a] A culture in stationary phase (about 5 to 7 h after the exponential-stationary transition) was sampled as the control (−Mn) for each strain. Then Mn(II) was added at 100 μM, and the cultivation continued. The Mn(II)-treated culture was sampled when maximum growth was reached (+Mn). Data are means of three replications.
[b] Included for comparison.

TABLE 2. Increase in SOD and catalase activities of *D. radiodurans* IR cells as a result of the Mn-CD effect

Bacterium and growth stage[a]	Sp act (U/mg of protein)[b]	
	SOD	Catalase
D. radiodurans IR		
Mn(II) untreated		
Exponential	32	590
Stationary	19	710
Mn(II) added		
Exponential	32	590
Mn-CD stage I	33	690
Mn-CD stage II	67	810
Mn-CD stage III	100	1,010
Mn-CD stage IV	94	1,200
E. coli K-12, stationary[c]	5	20
B. subtilis, stationary[c]	8	290

[a] Growth stages are described in the legend to Fig. 5.
[b] Data are means of three replications.
[c] Included for comparison.

ally declined in γ-ray resistance during Mn-CD. For both UV and γ radiations, the shoulder dose decreased with time of Mn-CD, suggesting that elimination of the high radioresistance might have been due to the loss of a DNA repair mechanism (25). In addition to the decrease in radiation resistance, continued Mn-CD resulted in gradual loss of red pigment, as determined by visual inspection and by visible-light absorption spectra of lipid extracts (data not shown).

Comparison of SOD and catalase activities of the Mn(II)-treated and control cultures. Strain IR is an absolutely aerobic bacterium; it fails to grow under anaerobic conditions (Forma Scientific anaerobic system). *Deinococcus* strains require vigorous shaking for optimal growth. In a shaken culture of an aerobe, the exponential-stationary transition in the growth curve possibly results from unfavorable redox-active conditions developed in an aging medium (9). Since Mn-CD occurred in an originally stationary-phase culture, it was thought that IR cells undergoing Mn-CD might require a high antioxidation capacity. To test this, IR cells at various growth stages were sampled for activities of the oxygen toxicity defense enzymes SOD and catalase. Mn-CD cells were, as expected, higher in activities of both enzymes than the Mn(II)-treated exponential and untreated cells, and these enzyme activities increased during the course of Mn-CD (Table 2). For example, when cells that had undergone three rounds of Mn-CD (Mn-CD stage IV) were compared with the untreated cells in the stationary

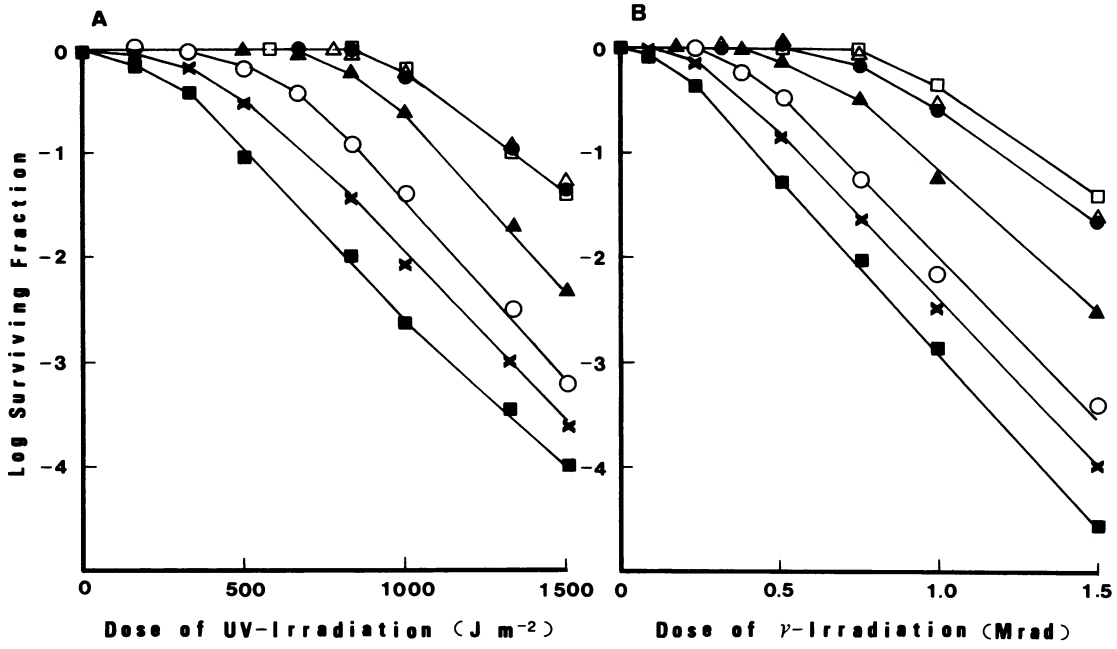

FIG. 5. Increase in UV (A) and γ-ray (B) sensitivity in *D. radiodurans* IR as a result of the Mn-CD effect. Symbols: ●, untreated cells in exponential phase; □, untreated cells in early stationary phase (about 1 to 2 h after the exponential-stationary transition); △, Mn(II)-treated exponential-phase cells; ▲, ○, ×, and ■, Mn-CD stages I, II, III, and IV, respectively. Mn-CD stages I, II, III, and IV were at 2, 5, 8, and 11 h after Mn(II) addition to an early-stationary-phase culture (ca. 2.2×10^8 CFU/ml) and were 4.0, 6.1, 8.0, and 18×10^8 CFU/ml in cell density, respectively. Data are means of at least two replications.

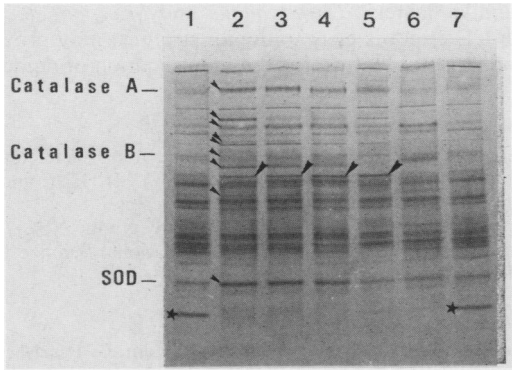

FIG. 6. Native gel electropherograms showing the modification in protein compositions of *D. radiodurans* IR cells as a result of the Mn-CD effect. Lanes: 1, Mn(II)-treated late-exponential-phase cells; 2 to 5, cells sampled in the early (lane 5), middle (lanes 4 and 3) and late (lane 2) Mn-CD stages; 6 and 7, early-stationary-phase and late-exponential-phase cells without Mn(II) treatment, respectively. Protein was loaded at 100 μg per well. Arrowheads between lane 1 and 2 point to bands that appeared to increase with time of Mn-CD. Stars indicate bands that were present in significant amounts only in exponential phase. Bands marked with large arrowheads were specifically present in markedly high amounts in all tested Mn-CD samples.

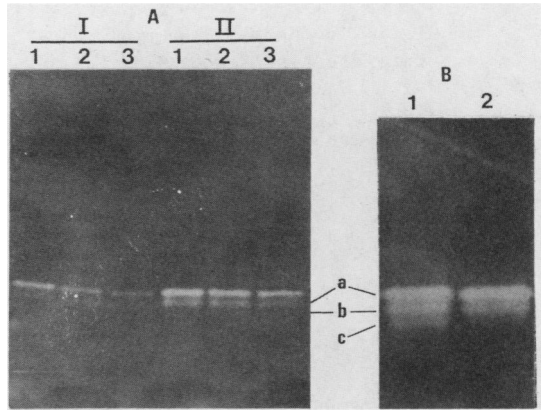

FIG. 7. Electropherograms showing the Mn-CD-induced increase in SOD activity in *D. radiodurans* IR. (A) A 1.1-μg (set I) or 4.4-μg (set II) amount of protein was loaded in each well. The light loadings produced sharper comparison among different treatments. Lanes 1, cells at Mn-CD stage III (see legend to Fig. 5); 2 and 3, untreated cells in the late exponential and the early stationary phases, respectively. (B) A 200-μg amount of protein was loaded in each well. This heavy loading allowed maximum detection of SOD isozymes (however, it saturated the activity staining of MnSOD). Lanes 1, untreated cells in the late exponential phase; 2, cells at Mn-CD stage III. a, MnSOD; b, hybrid (Mn-Fe)SOD; c, FeSOD.

phase, the increase was about 5-fold for SOD and 1.7-fold for catalase, suggesting that SOD, catalase, or both might play significant roles in the mechanism of Mn-CD.

Mn-CD-induced change in the native gel protein profile and activity banding of SOD and catalase. Figure 6 shows the protein profiles of the Mn(II)-treated and untreated cultures. The Mn(II)-treated culture in exponential phase (lane 1) was essentially identical in protein profile to the untreated culture in the same growth phase (lane 7). The pattern changed when the untreated culture shifted from the exponential (lane 7) to the stationary (lane 6) phase. When the culture went through the early (lane 5), middle (lanes 4 and 3), and late (lane 2) Mn-CD stages, there was a change in a set of proteins. Certain bands (marked with arrowheads between lanes 1 and 2) appeared to increase with time of Mn-CD. Some of them appear totally absent in exponential and early Mn-CD stages. Others were present in insignificant amounts in exponential or stationary phase. There were also high-mobility bands (marked with stars in Fig. 6) that were present in significant amounts only in exponential phase. In addition, a protein (large arrowheads in Fig. 6) was found in markedly high amounts in all tested Mn-CD samples (lanes 2 to 5) but was absent or present in minute amounts in untreated cells (lanes 6 and 7) and treated exponential-phase cells (lane 1). This protein appeared to be induced early in the Mn-CD phase and remained constant in concentration (on the basis of total soluble proteins) during the course of Mn-CD.

To further confirm the finding that the activities of SOD and catalase increased during the course of Mn-CD (Table 2), the proteins on the native gel were also subjected to activity assays for SOD and catalase. Duplicate gels were run, one for activity banding and the other for protein banding. Thus, SOD and catalase were positioned on the protein profile shown in Fig. 6. The increase in SOD activity due to Mn-CD could be demonstrated more clearly by loading a smaller amount of protein on the gel. When 1.1 μg of protein was loaded in each well, Mn-CD cells (Fig. 7AI, lane 1) were significantly higher in SOD activity than the untreated cells (lanes 2 and 3). When 4.4 μg of protein was loaded in each well (Fig. 7AII), the difference in SOD activity between Mn-CD (lane 1) and untreated exponential cells (lane 2) was less apparent; too much enzyme overloaded the reaction and reduced the ability of the test to discriminate between a positive and a negative result. Exponential-phase cells (Fig. 7AI and II, lanes 2) were higher than the stationary cells (lanes 3) in SOD activity. To detect the possible presence of SOD isozymes, a large amount (200 μg) of protein was loaded on the gel. Three SOD activity bands of *D. radiodurans* IR were resolved (Fig. 7B, lane 1). By a discrimination test (see Materials and Methods), the lower band was identified as FeSOD. During Mn-CD, activities of MnSOD and hybrid (Mn-Fe)SOD increased, whereas FeSOD activity decreased (lane 2). Both the amount (Fig. 6) and activity (Fig. 7) of SOD were increased during Mn-CD. Addition of Mn(II) caused no change in SOD and catalase levels in exponential-phase cells (Fig. 6).

As with SOD, both the specific activity (Table 2) and amount (Fig. 6) of catalase increased during Mn-CD. Catalases in IR cells were present in two forms, designated catalase A and catalase B. Figure 8 shows the activity banding of these two isozymes and the modification of each of them as a result of the Mn-CD effect (400 μg of protein was loaded in each well to optimize detection). Both the activity and the amount of these two catalases were comparable between Mn(II)-untreated cells in the exponential (lane 1) and the stationary (lane 3) phase, although catalase B was in a slightly different position on the gel in these phases. It appeared that catalase B was more responsive to the Mn-CD effect than was catalase A (lane 2).

DISCUSSION

We report several interesting observations of growth properties of *D. radiodurans* IR. (i) During growth in rich medium, the transition between exponential and stationary

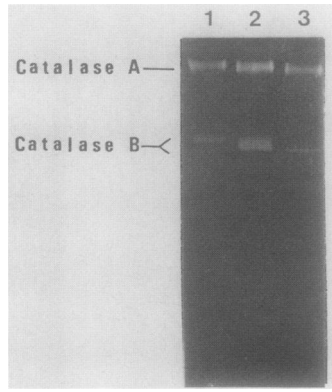

FIG. 8. Electropherograms showing catalase isozymes and the Mn-CD-induced increase in catalase activity in *D. radiodurans* IR. Lanes: 1 and 3, untreated cells in the late exponential and early stationary phases, respectively; 2, cells at Mn-CD stage III (see legend to Fig. 5).

phases is extremely sharp. This appears to be the result of a coordinated inhibition of cell division in all members of the population. (ii) Divalent manganese ions disrupt the inhibition of cell division during the stationary phase and induce at least three rounds of cell division, which are reductive. This Mn(II) effect may inactivate or bypass the arrest signal. (iii) Mn-CD is associated with several physiological changes, including a shortened stationary phase, an increased sensitivity to radiation-induced damage, and an increase in SOD and catalase activities.

Being specific to Mn(II), the Mn-CD effect must in some way depend on the function of Mn(II) in the cells. *D. radiodurans* R1 is able to accumulate a large amount of Mn(II), which in vivo is associated with the chromosome (18). In cells of this high-G+C-content organism (27), Mn(II) has been reported to (i) have preferential interaction with the guanine moiety in nucleosides, dinucleoside monophosphates, and DNA (2) and (ii) alter the interaction between DNA and proteins (12). Therefore, it is possible that Mn-CD involves specific binding among Mn, protein, and DNA.

SOD, a free-radical scavenger (20), has been reported to make procaryotic (21, 31) and eucaryotic (30) cells less susceptible to lethal damage by ionizing radiation. Although *D. radiodurans* IR (this report) and strains of other known *Deinococcus* spp. (our unpublished data) are extremely high in SOD activity, there is no direct evidence that the high radioresistance of *Deinococcus* spp. is due to SOD. The observed simultaneous increase in SOD activity and in radiation sensitivity in Mn-CD cells presents a problem that is worth further investigation.

Mn-CD is somewhat similar to starvation-induced cell division (15, 17, 29) in that daughter cells are reduced in size, although the Mn-CD size reduction is considerably greater than that of the same species subjected to starvation (data not shown). However, Mn-CD and starvation-induced cell division are different; the addition of Mn(II) to the IR culture undergoing starvation-induced cell division still led to production of the Mn-CD effect (data not shown).

Deinococci are gram-positive, aerobic, nonsporeforming, tetracoccal bacteria. Deinococci were formerly included in the genus *Micrococcus* (8) but on biochemical and genetic grounds have recently been classified in one genus, *Deinococcus*, in the family *Deinococcaceae* (27). The Mn-CD effect appears to be a trait specific to *Deinococcus* and another distinguishing feature between the two morphologically similar genera, *Deinococcus* and *Micrococcus*. Since the Mn-CD effect is easy to demonstrate, it may provide an additional useful feature for the identification of deinococci.

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LITERATURE CITED

1. **Anderson, A. W., H. C. Nordan, R. F. Cain, G. Parrish, and D. Duggan.** 1956. Studies on a radio-resistant micrococcus. I. Isolation, morphology, cultural characteristics, and resistance to gamma radiation. Food Technol. **10**:575–578.
2. **Anderson, J. A., G. P. P. Kuntz, H. H. Evans, and T. J. Swift.** 1971. Preferential interaction of manganous ions with the guanine moiety in nucleosides, dinucleoside monophosphates, and deoxyribonucleic acid. Biochemistry **10**:4368–4374.
3. **Asada, K., K. Yoshikawa, M. Takahashi, Y. Maeda, and K. Enmanji.** 1975. Superoxide dismutases from a blue-green alga, *Plectonema boryanum*. J. Biol. Chem. **250**:2801–2807.
4. **Baumeister, W., M. Barth, R. Hegerl, R. Guckenberger, M. Hahn, and W. O. Saxton.** 1986. Three-dimensional structure of the regular surface layer (HPI layer) of *Deinococcus radiodurans*. J. Mol. Biol. **187**:241–253.
5. **Beauchamp, C., and I. Fridovich.** 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. **44**:276–287.
6. **Beers, R. F., Jr., and I. W. Sizer.** 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J. Biol. Chem. **195**:133–140.
7. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
8. **Brooks, B. W., R. G. E. Murray, J. L. Johnson, E. Stackebrandt, C. R. Woese, and G. E. Fox.** 1980. Red-pigmented micrococci: a basis for taxonomy. Int. J. Syst. Bacteriol. **30**:627–646.
9. **Cohn, J. P.** 1987. The molecular biology of aging. BioScience **37**:99–102.
10. **Counsell, T. J., and R. G. E. Murray.** 1986. Polar lipid profiles of the genus *Deinococcus*. Int. J. Syst. Bacteriol. **36**:202–206.
11. **Davis, B. J.** 1964. Disc electrophoresis II: method and application to human serum proteins. Ann. N.Y. Acad. Sci. **121**: 404–427.
12. **Eisinger, J., F. Fawaz-Estrup, and R. G. Shulman.** 1965. Binding of Mn^{2+} to nucleic acids. J. Chem. Phys. **42**:43–53.
13. **Evans, D. M., and B. E. B. Moseley.** 1983. Roles of the *uvsC*, *uvsD*, *uvsE*, and *mtcA* genes in the two pyrimidine dimer excision repair pathways of *Deinococcus radiodurans*. J. Bacteriol. **156**:576–583.
14. **Evans, D. M., and B. E. B. Moseley.** 1985. Identification and initial characterisation of a pyrimidine dimer UV endonuclease (UV endonuclease β) from *Deinococcus radiodurans*; a DNA-repair enzyme that requires manganese ions. Mutat. Res. **145**: 119–128.
15. **Goodwin, B. C.** 1969. Synchronization of *Escherichia coli* in a chemostat by periodic phosphate feeding. Eur. J. Biochem. **10**:511–514.
16. **Gregory, E. M., and I. Fridovich.** 1974. Visualization of catalase on acrylamide gels. Anal. Biochem. **58**:57–62.
17. **Grossman, N., and E. Z. Ron.** 1989. Apparent minimal size required for cell division in *Escherichia coli*. J. Bacteriol. **171**:80–82.
18. **Leibowitz, P. J., L. S. Schwartzberg, and A. K. Bruce.** 1976. The *in vivo* association of manganese with the chromosome of *Micrococcus radiodurans*. Photochem. Photobiol. **23**:45–50.
19. **Little, J. G., and P. C. Hanawalt.** 1973. Thymineless death and ultraviolet sensitivity in *Micrococcus radiodurans*. J. Bacteriol.

113:233–240.

20. **McCord, J. M., and I. Fridovich.** 1970. The utility of superoxide dismutase in studying free radical reactions. II. The mechanism of the mediation of cytochrome c reduction by a variety of electron carriers. J. Biol. Chem. **245**:1374–1377.
21. **Misra, H. P., and I. Fridovich.** 1976. Superoxide dismutase and the oxygen enhancement of radiation lethality. Arch. Biochem. Biophys. **176**:577–581.
22. **Moseley, B. E. B.** 1983. Photobiology and radiobiology of *Micrococcus* (*Deinococcus*) *radiodurans*. Photochem. Photobiol. Rev. **7**:223–275.
23. **Moseley, B. E. B., and H. J. R. Copland.** 1975. Isolation and properties of a recombination-deficient mutant of *Micrococcus radiodurans*. J. Bacteriol. **121**:422–428.
24. **Moseley, B. E. B., and D. M. Evans.** 1983. Isolation and properties of strains of *Micrococcus* (*Deinococcus*) *radiodurans* unable to excise ultraviolet light-induced pyrimidine dimers from DNA: evidence for two excision pathways. J. Gen. Microbiol. **129**:2437–2445.
25. **Moseley, B. E. B., and H. Laser.** 1956. Similarity of repair of ionizing and ultra-violet radiation damage in *Micrococcus radiodurans*. Nature (London) **24**:373–375.
26. **Moseley, B. E. B., A. Mattingly, and H. J. R. Copland.** 1972. Sensitization to radiation by loss of recombination ability in a temperature-sensitive DNA mutant of *Micrococcus radiodurans* held at its restrictive temperature. J. Gen. Microbiol. **72**:329–338.
27. **Murray, R. G. E.** 1986. Family II. *Deinococcaceae*, p. 1035–1043. *In* P. H. A. Sneath, N. S. Mair, M. E. Sharpe, and J. G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams & Wilkins Co., Baltimore.
28. **Nineham, A. W.** 1955. The chemistry of formazans and tetrazolium salts. Chem. Rev. **55**:355–483.
29. **Novitsky, J. A., and R. Y. Morita.** 1976. Morphological characterization of small cells resulting from nutrient starvation of a psychrophilic marine vibrio. Appl. Environ. Microbiol. **32**: 616–622.
30. **Petkau, A., W. S. Chelack, and S. D. Pleskach.** 1976. Protection of post-irradiated mice by superoxide dismutase. Int. J. Radiat. Biol. **29**:297–299.
31. **Samuni, A., and G. Czapski.** 1978. Radiation-induced damage in *Escherichia coli* B: the effect of superoxide radicals and molecular oxygen. Radiat. Res. **76**:624–632.
32. **Shapiro, A., D. DiLello, M. C. Loudis, D. E. Keller, and S. H. Hutner.** 1977. Minimal requirements in defined media for improved growth of some radio-resistant pink tetracocci. Appl. Environ. Microbiol. **33**:1129–1133.
33. **Tan, S. T., and R. B. Maxcy.** 1982. Inactivation and injury of a hemolytic radiation-resistant micrococcus isolated from chicken meat. J. Food Sci. **47**:1345–1349, 1353.
34. **Tan, S. T., and R. B. Maxcy.** 1986. Simple method to demonstrate radiation-inducible radiation resistance in microbial cells. Appl. Environ. Microbiol. **51**:88–90.
35. **Tan, S. T., R. B. Maxcy, and T. L. Thompson.** 1983. Paper replication method for isolation of radiation-sensitive mutants. Appl. Environ. Microbiol. **46**:233–236.
36. **Welch, A. B., and R. B. Maxcy.** 1979. Characteristics of some radiation-resistant hemolytic micrococci isolated from chicken. J. Food Sci. **44**:673–675.
37. **Wierowski, J. V., and A. K. Bruce.** 1980. Modification of radiation resistance by manganese in *Micrococcus radiodurans*. Radiat. Res. **83**:384.
38. **Work, E.** 1964. Amino-acids of walls of *Micrococcus radiodurans*. Nature (London) **201**:1107–1109.