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Proteomic analysis of the gene DR1709 mutant in *Deinococcus radiodurans*

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Abstract DR1709 is a predicted Mn^{2+} transporter in *Deinococcus radiodurans*(*D.radiodurans*). The mensuration method to evaluate protein viability with two-dimensional electrophoresis in *D.radiodurans* and the mutants was established in this study. The results showed that after DR1709 was disrupted, the expressions of DR1120 (acetokinase), DR1691 (heat shock protein), DR1485 (putative lipase), DR2095 (putative c-type cytochrome) and other three hypothetical proteins (DR0124, DR0047 and DR2474) were repressed. However the expression of DR1794 (putative nosX) was induced. Phenomena above suggested that the increased radiation-sensitivity of the mutant cells may be attributed to not only the protection of gene DR1709, but also the proteins' different expressions between the wild type and the mutant might also play important roles in protecting *D.radiodurans* from irradiation. Although DR2095 was a homologue of c-type cytochrome, it has no realitic functions.

Key words *Deinococcus radiodurans*, DR1709, Proteomics

1 Introduction

Deinococcus radiodurans (*D. radiodurans*) shows extreme resistance to the lethal and mutagenic effects of ionizing radiations, and UV or other electromagnetic waves as well, which cause physical and chemical damage to DNA[1-3]. Different mechanisms of the resistances of the microorganism against the deleterious effects have been suggested[4-8]. Recently, it was found that the radiation resistance was determined by the level of oxidative protein damage induced during the irradiation, and Mn^{2+} ions could protect protein against oxidative stress in *D. radiodurans*[9]. But the mechanism is yet to be clarified.

DR1709 was a predicted Mn^{2+} transporter gene in *D. radiodurans*. With the disrupted DR 1709 gene, the mutant cells had a much less survival rate than wild type, when treated with radiation[10]. However, it was not sure whether the other genes assigned their roles to this less survival rate. In this paper, we report the proteomic analysis in an attempt to solve these problems. The results showed that nine genes were closely related with DR1709 at least. Their possible roles contributing to the irradiation in *D. radiodurans* were also discussed.

2 Materials and methods

2.1 Bacterial strain and growth conditions

D.radiodurans strain R1 was a generous gift from Institute of Nuclear-Agricultural Sciences, Zhejiang University. The DR1709 mutant strain was constructed at our laboratory. The *D.radiodurans* R1 and the mutant cells were grown at 31℃ in TGY broth (0.5% bacto tryptone, 0.1% glucose, and 0.3% bacto yeast extract)[11,12].

2.2 Reagents and apparatus

Protean IEF device and the second dimensional electrophoresis apparatus, ReadyStrip IPG Strip, Bio-Lyte Ampholyte, tributylphosphine, urea and CHAPS were from Bio-Rad (Hercules, CA, USA). The other chemicals of molecular biology grade were

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obtained from Sigma Chemical. Lysis buffer A (9 mol/L urea, 4% mass concentration CHAPS, 1% mass concentration DTT, 0.5% CA and a cocktail of protease inhibitor mixture), Lysis buffer B (40 mmol/L Tris-HCl, 7 mol/L urea, 2 mol/L thiourea, 4% CHAPS, 40 mmol/L DTT) and rehydration buffer (8 mol/L urea, 2% CHAPS, 0.5% IPG buffer, 0.002% bromphenol blue) were prepared according to ReadyStrip IPG Strip Instruction Manual (Catalog# 163-2099, Bio-Rad). The protein inhibitors mixture (35 μg/mL PMSF, 0.3 mg/mL EDTA, 0.7 μg/mL pepstatin, 0.5 μg/mL leupeptin) was made to prevent proteins from being degraded.

2.3 Extraction of soluble proteins

Cells were collected by centrifugation after culturing for 48 h. The pellets were suspended in phosphate buffer (1 mol/L, pH =7.0), and the suspension was mixed with four times the volume of Lysis buffer A in a 50-mL tube with a cocktail of protease inhibitor mixture. The cells in the mixture were sonicated at 0ºC by half-time intermittent sonication (Branson, Sonifier 450) for 1 h. The homogenates were centrifuged at 12000×g for 20 min at 4ºC. The supernatants were collected into a 1.5-mL microtube as a soluble protein fraction. Acetone of four volumes was mixed with the supernatants, so as to avoid carotenoids. The mixture was centrifuged at 32000×g for 20 min at 4ºC. Sedimentated protein was suspended with four volumes of Lysis buffer A. Total protein concentration was measured according to the modified Bradford[13]. The soluble proteins were stored at −70ºC for two-dimensional gel electrophoresis.

2.4 Two-dimensional gels electrophoresis

The procedure of the 2-D gel electrophoresis was carried out according to the modified standard method[14] . For each channel, 350 μg of purified sample was diluted with rehydration buffer (8 mol/L urea, 2% CHAPS, 0.5% IPG buffer, 0.002% bromphenol blue) and loaded onto the IPG strip (24 cm, pH 4–7; Amersham Biosciences, USA) and the isoelectric focusing was performed (50 V, 12 h; 200 V, 2 h; 500 V, 1 h; 1500 V, 30 min; 8000V, 1 h; 10000 V, 6 h.). The strips were treated with equilibration buffer I (6 mol/L urea, 30% glycerol, 2% SDS, 50 mmol/L Tris-HCl, 1% DTT, 0.002% bromphenol blue) and equilibration buffer II (6 mol/L urea, 30% glycerol, 2% SDS, 50 mmol/L Tris-HCl, 4% iodoacetamide, 0.002 % bromphenol blue), respectively. The second dimension was performed in 12.5% SDS-PAGE gels (5 W/gel, 30 min; 60 W/gel, 10 h). The gels were stained with a modified Neuhoff's colloidal Coomassie Blue G-250 stain[15,16].

2.5 Protein spots identification

The protein samples were in-gel digested and identified according to the standard method[17]. The work of MALDI-TOF-TOF MS and peptide mass fingerprinting was mainly done by Tianjin Biochip Corp.

3 Results and discussion

3.1 Proteins expressed differently between the mutant and the wild type

D.radiodurans is the most radiation-resistant organism described[18]. The recent research showed that Mn^{2+} ions could protect protein against oxidative stress in *D. radiodurans*[19]. DR1709 is a predicted Mn^{2+} transporter in *D.radiodurans*. After DR1709 was disrupted, the mutant had much less survival rate than the wild type when treated with UV irradiation and H_2O_2[10]. Using two-dimensional electrophoresis, the differences between the wild type and the mutant at the protein level were analyzed. The results showed that the expressions of one acetokinase gene (DR1120), one heat shock protein (DR1691), one putative lipase gene (DR1485), one putative c-type cytochrome (DR2095) and other three hypothetical proteins (DR0124, DR0047 and DR2474) in mutant cells were lower than those in the wild type. But the expression of one putative nosX protein (DR1794) was a reverse (Fig.1 and Table 1).

3.2 DR2095 might not serve as c-type cytochromes

C-type cytochromes are proteins that are essential for the life of virtually all organisms. Superoxide radicals arise from the autoxidation of respiratory dehydrogenases, where adventitious transfer of electrons from reduced flavins (FADH2) associated with c-type cytochromes. It was thought that the total

intracellular titer of cytochromes was a marker for the proclivity of cells to generate oxidative stress[20]. After DR1709 was disrupted, the expression of the putative c-type cytochrome was repressed (Fig.1), which might cause less oxidative stress and higher survival rate. However, the survival rate of the mutant was much less than that of the wild type. Therefore, DR2095 might not serve as c-type cytochromes.

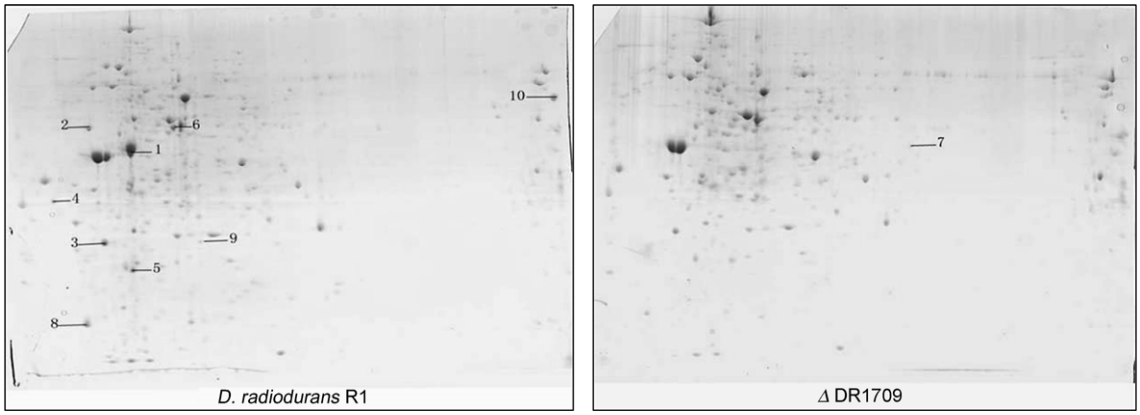

Fig.1 2-DE comass stained protein patterns of *D. radiodurans* R1 and the mutant ⊿DR1709.

Table 1 Proteins identified by MALDI-TOF-TOF MS analysis and peptide mass fingerprinting

No.	Locus name	Putative identification	Protein length	pI
1	DR_1485	Lipase, putative	323	5.05
2	DR_1120	Kinase, acetokinase family	394	4.88
3	DR_1691	Heat shock protein-related protein	166	4.53
4	DR_2095	c-type cytochrome, putative	210	4.33
5	DR_0124	Hypothetical protein	94	5.02
6	DR_1252	Conserved hypothetical protein	405	5.40
7	DR_1794	NosX protein, putative	330	7.02
8	DR_0047	Hypothetical protein	65	4.96
9	DR_2474	Hypothetical protein	165	5.91
10	DR_1709	Integral membrane protein, NRAMP family	436	9.76

Proteins playing important roles in protecting *D.radiodurans* from irradiation

Acetokinase catalyzes the virtually irreversible synthesis of adenosine triphosphate from acetyl phosphate and adenosine diphosphate. When the bacterium recovered from irradiation, energy was necessary. In the mutant cells, the expression of acetokinase (DR1120) reduced (Fig.1), where less adenosine triphosphate was produced and more cells died. These indicated that after disruption of the DR1709 gene, the increased radiation-sensitivity of the mutant cells may not be attributed to DR1709 only. Besides the genes described above, one putative lipase gene, one nosX protein and other three hypothetical proteins may also be involved in the process of protecting proteins from being oxidized (Fig.1). The details,however, shall be found in further studies. The proteins expressed differently between the wild type and the mutant might also play important roles in protecting *D.radiodurans* from irradiation.

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