

Profile of expressed proteins of γ -irradiated foodborne pathogen *Escherichia coli* O157:H7 analysed by capillary electrophoresis

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ABSTRACT

The aim of this study was to evaluate the effects of gamma irradiation treatment at different doses on expressed proteins in *Escherichia coli* (*E. coli*) O157:H7. Bacterial samples were gamma irradiated at doses of 0.4 and 1.3 kGy to create damaged cells and to kill cells, respectively. The bacterial proteins were extracted and analyzed using a ProteomeLab sodium dodecyl sulfate gel molecular weight (MW) analysis kit and performed on a capillary electrophoresis system (ProteomeLab™ PA 800). The major peaks were calculated and expressed as equivalent MW and corrected peak areas. One protein named as bacteriophage N4 adsorption protein B with a MW of 85.2 kilodalton (kDa) was determined and its expression level was not significantly changed ($p \geq 0.05$) at different doses of irradiation treatment. Seven proteins consisting of rhamnulokinase, glycerol kinase, hypothetical protein Z2695, preprotein translocase subunit SecD, DNA polymerase III subunits gamma and tau, choline transport protein-BetT and primosome assembly protein-PriA with the respective MW of 54.0, 56.8, 60.7, 66.5, 71.0, 75.7 and 81.5 kDa were statistically changed in their expressed level ($p < 0.05$) at different doses of irradiation treatment. The changes in expressed level of proteins could be related to the sensitivity or resistance of *E. coli* to gamma irradiation treatment.

Keywords: Capillary electrophoresis, *Escherichia coli* O157:H7, protein expression, gamma irradiation.

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INTRODUCTION

Enterohemorrhagic *Escherichia coli* (*E. coli*) O157:H7 is a foodborne pathogen that causes enteric infections such as hemorrhagic colitis. This bacterium can also produce a Shiga toxin into the intestine and the toxin can act on sensitive cells in the kidneys, brain and other organs and cause haemolytic uremic syndrome (HUS) (Ferens and Hovde, 2011). The symptoms of HUS are hemolytic anemia, acute renal failure and thrombocytopenia (Banatvala et al., 2001).

In order to prevent foodborne illness caused by foodborne pathogens (for example, *E. coli* O157:H7), irradiation of food is one of the most effective processes to eliminate them from food products (Monk et al., 1995). Irradiation is considered as a cold pasteurization

treatment and therefore, irradiation does not cause any significant loss of nutrients and sensory quality of food. Moreover, irradiation utilizes a source of ionizing radiation that passes through food and causes death of harmful bacteria as well as other organisms without increasing food temperature (Wood and Bruhn, 2000). To date, it is still not clearly confirmed how radiation causes bacterial death, but many theories implied DNA and/or protein damage. The ionizing radiation acts on the DNA of target organisms, causing swellings and breakings along the chain. The damage in DNA could be large, making it difficult or impossible to be repaired and therefore, finally causing the death of irradiated organisms (Daly, 2009). In the classical model of radiation toxicity, DNA is the

molecule which is mostly affected by ionizing radiation. However, recent data have shown that survival of bacteria after irradiation would be more related to the quantity of damages occurred in proteins than the quantity of damages occurred in the DNA during irradiation (Daly, 2009).

In a previous study, Caillet et al. (2008) treated *E. coli* by irradiation at different doses and analysed expression level of heat shock proteins (HSP) such as GroEL, DnaK and GroES by Western blot, the authors found that the expression of HSP in *E. coli* O157:H7 was induced by irradiation. However, it should be emphasized that besides the changes in the expression level of HSP in irradiated *E. coli*, it is not known if there are any changes in the expression level of other proteins. It is expected that the changes in expression level of other proteins in *E. coli* after irradiation treatment at different doses may give us an idea about their functions in bacterial survival. Thus, analysis of expressed proteins in irradiated *E. coli* cells is necessary.

Different techniques such as dimensional (2-D) gel electrophoresis, high performance liquid chromatography (HPLC)-mass spectrophotometry (MS), capillary electrophoresis (CE), CE-MS, etc. are currently utilized for analysis of proteins and peptides (Zhou et al., 2002; Gharbi et al., 2002; Peng et al., 2003; Bachmann et al., 2010; Trudeau et al., 2012). For example, Peng et al. (2003) used liquid chromatography (LC) coupled with tandem mass spectrophotometry (LC-MS/MS) to analyze yeast proteome. They found 26,815 spectra from 162,000 collected MS/MS spectra matched to yeast peptides. Among 26,815 matched yeast peptides, 7,537 were unique peptides. In another study, Zhou et al. (2002) used two dimensional differential in-gel electrophoresis (2D-DIGE) and HPLC/MS/MS to quantify the differences in protein expression between laser capture microdissection-procured esophageal carcinoma cells and normal epithelial cells. The authors identified 1,038 protein spots in cancer cell lysates and 1,088 protein spots in normal cell lysates. Among detected proteins, 58 spots were up-regulated by more than 3-fold and 107 were down-regulated by more than 3-fold in cancer cells. Among up-regulated proteins in cancer cells, a tumor rejection antigen (gp96) was identified by HPLC/MS/MS and this protein could become a cancer-specific protein marker (Zhou et al., 2002). CE is often utilized for proteomic analysis due to its advantages such as short analysis time and minimum consumption of both reagents and samples (Huck and Bonn, 2008). CE combines with MS resulted into a powerful tool for the analysis of complex samples of proteins and peptides (Bachmann et al., 2010). For the MS analysis in proteomics, matrix-assisted laser desorption/ionization (MALDI) or electrospray ionization (ESI) is often used (Bachmann et al., 2010).

In a recent research on utilization of CE for analysis of protein profiles of two foodborne pathogens *Listeria monocytogenes* and *Staphylococcus aureus* which were

irradiated at different doses, we found that there were some significant changes in several proteins which do not belong to HSP (Trudeau et al., 2012). For example, in *L. monocytogenes*, the protein with a molecular weight (MW) of 85.4 kiloDalton (kDa) is the enzyme formate acetyltransferase and its expression level was increased at a dose of 0.4 kGy (dose that creates damaged cells) while the expressed level of other protein with a MW of 70.2 kDa (part of the acyltransferase family proteins) was decreased at a lethal dose (Trudeau et al., 2012). Or in case of *S. aureus*, it was found that the expression of 50 S ribosomal protein (MW of 16.3 kDa) was significantly decreased at the low dose of irradiation treatment while the expression of transcriptional regulator (MW of 17.7 kDa) was significantly increased at all irradiated doses of treatment as compared to the control (Trudeau et al., 2012). This research confirmed the hypothesis that irradiation treatment could cause changes in some expressed proteins in foodborne pathogens and these proteins may contribute to their sensitivity or resistance to irradiation treatment (Trudeau et al., 2012).

Thus, the objective of this study was to analyse the profile of protein expression in *E. coli* O157:H7 cells treated at different doses of γ -irradiation using capillary electrophoresis. The irradiation treatments consisted of a control (0 kGy), a dose to create damaged cells (0.4 kGy) and a lethal dose (1.3 kGy). The research confirmed that capillary electrophoresis is a useful method to separate and analyse the expressed proteins that may be related to the resistance or tolerance of foodborne pathogen to γ -irradiation treatment.

MATERIALS AND METHODS

Bacterial strain and growth conditions

Escherichia coli O157:H7 strain EDL933 (Institut National de la Recherche Scientifique - Institut Armand Frappier, Laval, QC, Canada) was sub-cultured in tryptic soy broth (TSB; Becton Dickinson, Sparks, MD, USA) at 37°C for 24 h from the stock cultures maintained at -80°C in TBS containing 20% glycerol. One millilitre of each culture was incubated through two successive incubations of 24 h at 37°C in 9 ml of TSB to obtain approximately 10^9 colony forming unit (CFU)/ml. The cultures were centrifuged at 1,300 g for 15 min using a Sorvall RC5C centrifuge (Du Pont Company, Wilmington, DE, USA). The obtained cell pellet was washed with sterilized NaCl 0.85% weight/volume (w/v) and then resuspended in TSB (500 ml) and incubated at 37°C for 24 h. After the last incubation of 3 h to obtain exponential growth phase, bacterial cultures were irradiated at different doses.

Irradiation treatment

Bacterial cultures of *E. coli* O157:H7 were irradiated at the Canadian Irradiation Centre (Laval, QC, Canada) at doses of 0.36 to 0.44 kGy (mean, 0.40 kGy) to create damaged cells and 1.26 to 1.34 kGy (mean, 1.30 kGy) to kill cells (Caillet et al., 2005). An UC-15A irradiator (MDS Nordion International Inc., Kanata, ON, Canada) equipped with a ^{60}Co source was used to deliver radiation at a dose rate of 18.27 kGy. This irradiator was certified by

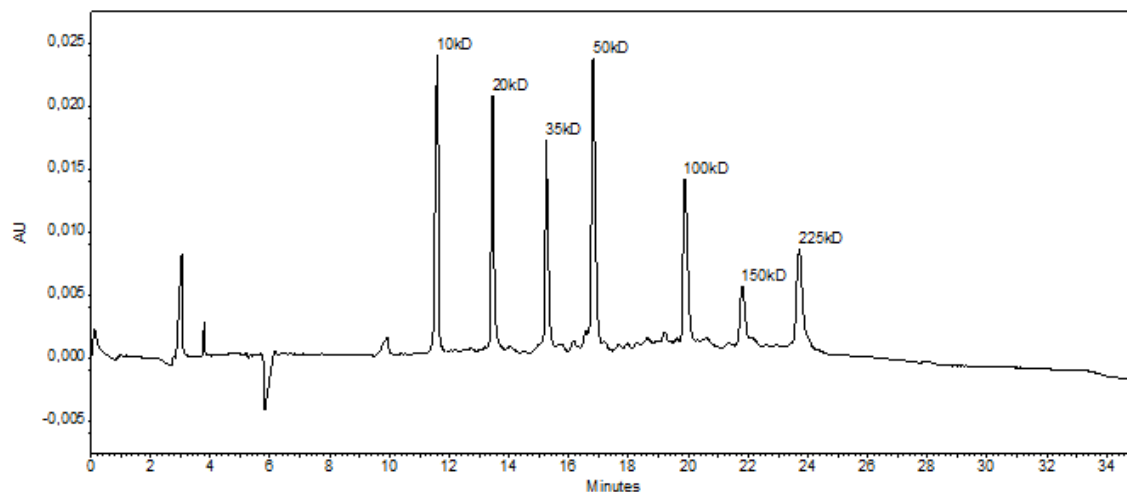


Figure 1. Electrophoretic profile of standard protein sizing. Experimental conditions: Bare-fused silica capillary, 30.2 cm (20 cm to the detection window) \times 50 μ m i.d.; temperature, 25°C; applied voltage, 15 kV; electrokinetic injection at 5.0 kV \times 20s. Samples were diluted at 1 mg/ml with SDS sample buffer. AU is absorbance unit at the wavelength of 220 nm.

the National Institute of Standards and Technology (Gaithersburg, MD, USA), and the dose rate was established using a correction for decay of source. Amber Perspex 3042D (Atomic Energy Research Establishment, Harwell, Oxfordshire, UK) was used to validate the dose distribution. The radiation treatment was carried out at room temperature ($20 \pm 1^\circ\text{C}$).

Protein extraction

Immediately after irradiation, 500 ml of each bacterial culture was quickly chilled in an ice/ethanol bath until the temperature dropped below 10°C. Cells were harvested by centrifugation for 15 min at 8670 g at 4°C and pellet (bacterial biomass) was washed with 10 ml of sterilized NaCl 0.85% (w/v). The pellet was then resuspended in 5 ml of lysis buffer [50 mM Tris-HCl (pH 7.5); 0.1 mM NaCl; 0.5 mM phenylmethanesulfonyl fluoride; 1 mg/ml iodoacetamide] and divided into micro-tubes containing glass beads (0.2 mm). Bacterial cell walls were then broken using a cell disrupter (FastPREP, model FP 120, Qbiogene Inc., Carlsbad, CA, USA) operating at maximum speed for 60 s. The broken cell suspension was centrifuged at 2,000 g for 10 min to remove the glass beads and unbroken cells. The supernatant was collected and desalted by filtration using Amicon Ultra-4 Centrifugal Units (Millipore, Billerica, MA, USA) as per instructions of the manufacturer.

Protein quantification

Concentration of proteins extracted from the bacterial cells was quantified using Bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL, USA) and procedure was followed as per the manufacturer's specifications. The absorbance was read at 562 nm using a DMS 100S spectrophotometer (Varian Canada Inc., Mississauga, ON, Canada). Protein concentration of samples was calculated based on a standard curve of bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO, USA).

Capillary electrophoresis

Protein profile of samples was analyzed using a ProteomeLab

SDS-Gel MW Analysis Kit and performed on a CE system of ProteomeLab™ PA 800 (Beckman Coulter Inc., Fullerton, CA, USA). Detail procedure for protein separation and analysis by the CE was mentioned in Trudeau et al. (2012). Briefly, a bare-fused silica capillary (30 cm total length, 50 μ m internal diameter), and a commercial sieving polymer (sodium dodecyl sulfate-SDS) for separation of proteins were used in this study. Protein samples were resuspended in the sodium dodecyl sulfate (SDS) sample buffer (1% SDS, 100 mM Tris-HCl pH 9.0) to obtain a final concentration of 1 mg/ml. Electrokinetic injection (5.0 kV, 20 s), voltage (15 kV) and temperature (25°C) and an internal standard protein of 10 kDa were applied for the CE separation of protein samples. Ultra-violet (UV) absorption was monitored by a diode array detector at 220 nm and at 350 nm (as a reference channel). A scan from 190 to 400 nm was also collected. In CE, electrophoretic mobility (μ) of analyte was used instead of relative migration time. Detail method for calculation the mobility of analyte was mentioned in Trudeau et al. (2012).

In this study, the wavelength of 220 nm was applied for detection of proteins as suggested by other authors (Dolnik, 1999; Huck and Bonn, 2008). A CE analysis of proteins with known MW (SDS protein sizing standard, 10 to 225 kDa, Beckman Coulter Inc.) was conducted and an electropherogram was recorded (Figure 1). Based on this electropherogram, a calibration curve (Coefficient of determination $R^2 = 0.998$) of electrophoretic mobility against the MW of known protein size was established (Figure 2). The relative MW of unknown protein was calculated based on the calibration curve.

Protein determination

As the genome of *E. coli* O157:H7 EDL933 has been sequenced and is available on [ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Perna et al., 2001](ftp://ftp.ncbi.nih.gov/genbank/genomes/Bacteria/Perna_et_al.,_2001), *in silico* studies were done to determine expressed proteins in *E. coli* based on the MW of detected peaks in the electropherograms recorded by CE.

Statistical analysis

Student's *t* test was utilized and differences between means were

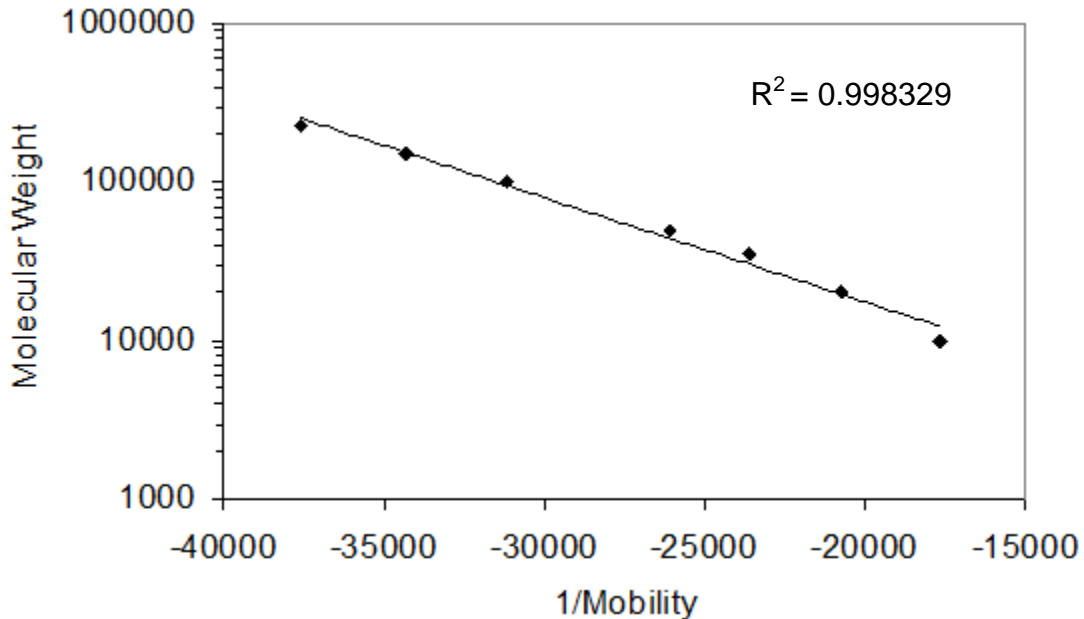


Figure 2. Calibration curve of SDS-MW Size Standards plotting the molecular weight towards mobility values.

considered significant at $P < 0.05$. Stat-Packets Statistical Analysis software (SPSS Base 18.0, SPSS Inc., Chigaco, Il, USA) was used for the analysis. In this study, three different samples were prepared on three different occasions and irradiated at different time. Each sample was injected three times.

RESULTS

Profiles of protein expression in *E. coli* cells irradiated at different doses are presented in Table 1. In this table, the major peaks were calculated and expressed as equivalent MW and corrected peak areas (%). For the non-irradiated bacterial cells (the control), 27 major peaks were detected (Table 1 and Figure 3). Irradiation at a dose of 0.4 kGy (to create damaged cells) has caused a significant increase ($P < 0.05$) in the peak areas (%) that represent proteins with MW of 28.6 kDa (from 2.17 to 2.38%) and 66.5 kDa (from 1.71 to 2.56%). This treatment also caused a significant decrease in the peak areas (%) that correspond to proteins with a MW of 13.7 kDa (from 1.36 to 0.58 %), 15.3 kDa (from 3.33 to 2.86%), 26.9 kDa (from 5.55 to 4.23%), 30.7 kDa (from 2.87 to 2.32%) and 34.5 kDa (from 4.70 to 4.40%) (Table 1 and Figure 3).

More changes in the expression level of several proteins in irradiated *E. coli* cells at the lethal dose (1.3 kGy) were recognized. A significant decrease in expression level of proteins with a MW of 15.3, 17.2, 18.5, 22.1, 26.9, 30.7, 32.8, 34.5, 40.0, 43.3, 54.0, 75.7 and 87.3 kDa was recorded (Table 1). Furthermore, the expression level of proteins with a MW of 22.8, 24.7, 36.7, 56.8, 60.7, 66.5, 71.0 and 81.5 kDa was increased

significantly as compared to the control without treatment (Figure 3 and Table 1).

DISCUSSION

In a complete genome analysis of *Escherichia coli* O157:H7 strain EDL933, 5298 putative proteins encoded in its genome were reported (Perna et al., 2001). However, based on the identity of the MW in the CE analysis of irradiated *E. coli* in our study, there were only seven expressed proteins that could be determined. In fact, it is obvious that the whole proteome of *E. coli* is not expressed at all times. Moreover, during the extraction, only cytosolic proteins were extracted expectedly. Furthermore, other possible reasons could be: (1) there might have interactions of proteins with the capillary wall which may lead to low sample recovery/detection (Bachmann et al., 2010); (2) resolution of CE might not be able to separate proteins that have the same molecular weight; (3) CE detection limit might not be able to detect weakly expressed proteins because the volume of sample injection in this study was a maximum of 25 μ l at the protein concentration of 1 mg/ml. It should be mentioned that in *E. coli* O157:H7 strain EDL933, many proteins with approximate same MW can be expected (Perna et al., 2001) but only some MW are represented for unique proteins. In this study, only unique proteins at identified MW that occurred in *E. coli* are presented and discussed.

It can be observed that at different doses of irradiation treatment, there is one protein (MW of 85.2 kDa) which

Table 1. Profiles of protein expression by *E. coli* at different doses of γ -irradiation treatment.

Molecular weight (kDa)	Peak number in figure 3	Corrected area of protein (%) ¹		
		0 kGy (Control)	0.40 kGy	1.30 kGy
13.7	1	1.36 ± 0.20	0.58 ± 0.20	ND
15.3	2	3.33 ± 0.20	2.86 ± 0.08	2.56 ± 0.40
17.2	3	2.15 ± 0.20	1.83 ± 0.20	1.19 ± 0.10
18.5	4	4.63 ± 0.20	4.38 ± 0.20	3.05 ± 0.04
19.4	5	1.76 ± 0.20	1.57 ± 0.10	1.76 ± 0.20
21.1	6	0.54 ± 0.20	0.80 ± 0.03	ND
22.1	7	1.93 ± 0.20	2.10 ± 0.20	1.21 ± 0.04
22.8	8	0.98 ± 0.20	1.03 ± 0.09	1.62 ± 0.08
24.7	9	2.15 ± 0.10	2.14 ± 0.30	6.19 ± 0.02
26.9	10	5.55 ± 0.20	4.23 ± 0.30	2.17 ± 0.01
28.6	11	2.17 ± 0.30	2.38 ± 0.20	1.83 ± 0.20
30.7	12	2.87 ± 0.20	2.32 ± 0.20	2.28 ± 0.20
32.8	13	8.06 ± 0.10	7.31 ± 0.10	7.58 ± 0.06
34.5	14	4.70 ± 0.08	4.40 ± 0.20	2.09 ± 0.08
36.7	15	2.26 ± 0.20	2.23 ± 0.10	2.95 ± 0.20
40.0	16	10.25 ± 0.20	10.26 ± 0.30	8.43 ± 0.09
43.3	17	5.07 ± 0.20	4.82 ± 0.50	3.86 ± 0.20
48.6	18	9.28 ± 0.20	9.80 ± 0.40	9.61 ± 0.30
54.0	19	3.41 ± 0.20	3.17 ± 0.30	2.24 ± 0.08
56.8	20	1.53 ± 0.10	1.45 ± 0.10	2.13 ± 0.08
60.7	21	3.06 ± 0.06	2.94 ± 0.10	3.84 ± 0.50
66.5	22	1.71 ± 0.40	2.56 ± 0.20	3.18 ± 0.30
71.0	23	2.04 ± 0.20	2.36 ± 0.20	2.86 ± 0.20
75.7	24	7.87 ± 0.20	7.53 ± 0.20	7.14 ± 0.30
81.5	25	1.21 ± 0.10	1.44 ± 0.20	1.82 ± 0.30
85.2	26	1.78 ± 0.10	1.85 ± 0.20	1.93 ± 0.30
87.3	27	4.87 ± 0.20	4.58 ± 0.30	3.49 ± 0.40

Values in bold differs statistically from the non-irradiated protein by *t*-test with $P < 0.05$. ¹ The values of corrected area presented are the mean \pm standard deviation of samples prepared in triplicate and each injected three times. 0 kGy, control without radiation; 0.4 kGy, damaged cells; 1.3 kGy, lethal. ND is non-determined.

its expression level was not significantly changed (Table 1). This protein is a bacteriophage N4 adsorption protein B encoded by *nfrB* gene (Perna et al., 2001). The product of the *nfrB* gene may be required for irreversible adsorption and injection of the phage genome (Kiino and Rothman-Denes, 1989). It is noteworthy to point out that there is no change in expression level of this protein that participates in the infection by the bacteriophage N4, so it implies that this protein may have another yet undefined role in *E. coli* O157:H7.

It can be observed that the peak areas of seven proteins were statistically changed after irradiation treatment (Table 1). The smallest one is a 54.0 kDa protein and its expression was decreased at the lethal dose. This protein is a rhamnokinase, an enzyme participating in the degradation of L-rhamnose which is a ubiquitous sugar molecule (Grueninger and Schulz, 2006). More precisely, the enzyme catalyzes the phosphorylation of L-rhamnulose, and then the product,

L-rhamnulose-1-phosphate, is subsequently split by aldolase into L-lactaldehyde and dihydroxyacetone phosphate. Because the expression of this protein was only decreased at the lethal dose, it could indicate that this enzyme is essential for bacterial survival after irradiation.

The second protein is a glycerol kinase, a 56.8 kDa protein. Its expression was increased only at the lethal dose. This enzyme is an important element in a signal transduction pathway that couples expression of genes required for glycerol metabolism to the relative availability of glucose and glycerol (Liu et al., 1994). However, this protein does not seem to play a key role in bacterial survival because its expression only found to be increased at the lethal dose.

The third protein is a hypothetical protein Z2695 of 60.7 kDa and was only increased at the lethal dose. It is an uncharacterized protein conserved in bacteria and its function is unknown (Perna et al., 2001).

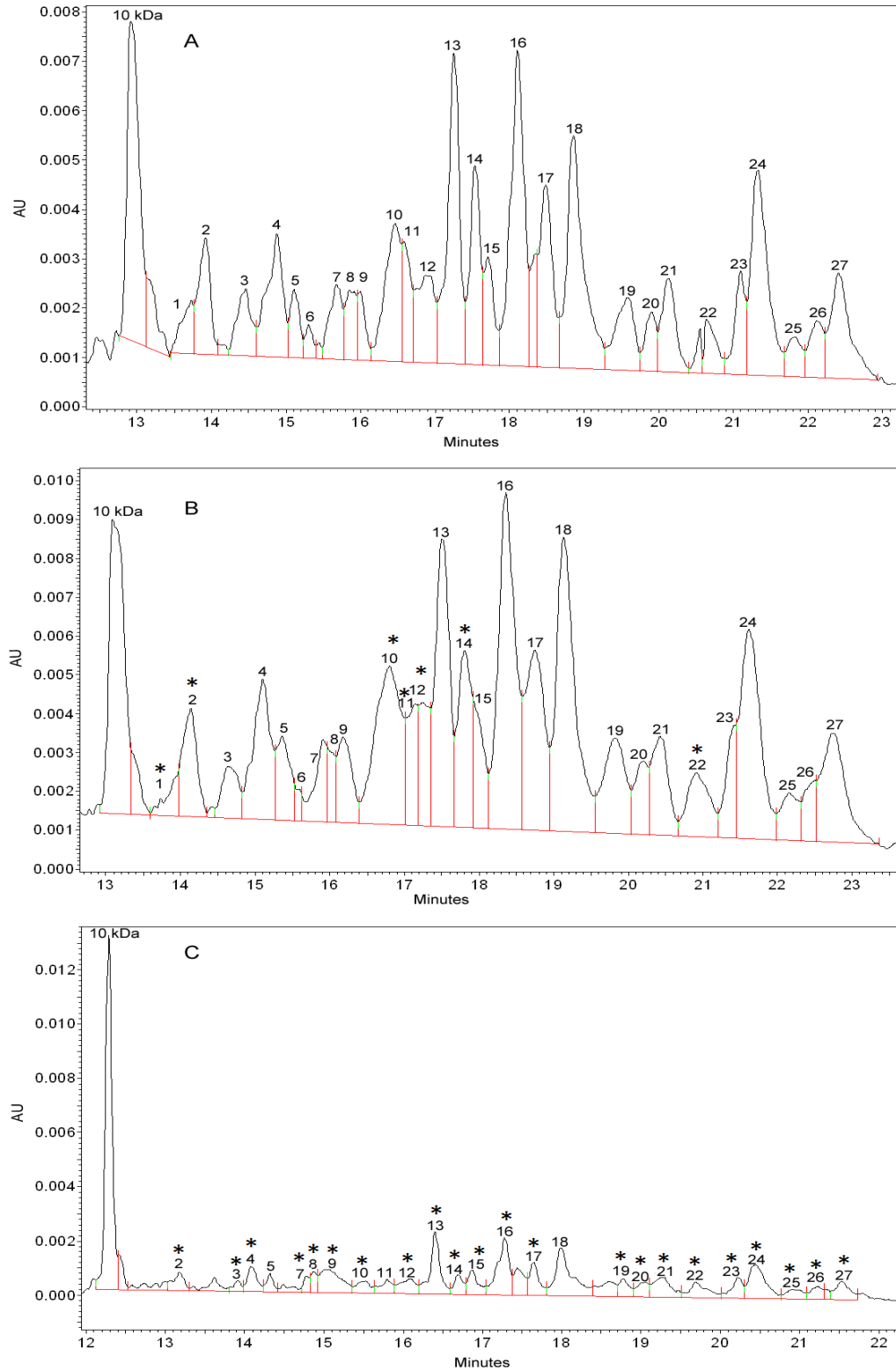


Figure 3. Electrophoretic profiles obtained for *E. coli* O157:H7 with (A) non-irradiated treatment; (B) irradiated treatment at 0.4 kGy to create cells damaged; and (C) irradiated treatment at 1.3 kGy to kill cells. The same number above the peak indicates the same protein occurred in (A) non-irradiated treatment; (B) irradiated treatment at 0.4 kGy to create damaged cells; and (C) irradiated at 1.3 kGy to kill cells. The vertical line in red solid indicates the area for each peak. AU is absorbance unit at the wavelength of 220 nm. * mean that corrected area differs statistically between irradiated and non-irradiated proteins by a *t*-test with $P < 0.05$.

The fourth protein is a 66.5 kDa protein, it is a preprotein translocase subunit SecD and its function is to transport large molecules (Perna et al., 2001). SecD is a part of the pre-protein secretory system and when it is complexed with proteins SecF and YajC, SecDFyajC stimulates the proton motive force-driven protein translocation (Nouwen and Driessen, 2005). Because the expression of this protein was increased in both irradiation doses (0.4 and 1.3 kGy) and the increase level at 1.3 kGy was higher than that of 0.4 kGy, it is expected that this protein might play an important function in the survival of *E. coli*.

The fifth protein is a 71.0 kDa protein, it is a DNA polymerase III subunits gamma-tau and its expression was only increased at the lethal dose. Its function is to catalyze the DNA-template-directed extension of the 3'-end of a DNA strand (Perna et al., 2001).

The sixth protein is a 75.7 kDa protein; it is a choline transport protein, BetT. Because its expression was only decreased at the lethal dose, it could be an important protein for the survival of the bacterium.

The last protein is a largest protein with the MW of 81.5 kDa. It is a primosome assembly protein, PriA (Perna et al., 2001). When this protein binds forked DNA, it starts the assembly of the protein complex responsible for creating RNA primers on single stranded DNA during DNA replication (Tanaka et al., 2007). This protein does not seem to play an essential role for bacterial survival because its expression was only increased at the lethal dose.

Thus, the obtained results on protein expression in irradiated *E. coli* O157:H7 in this study are important. It demonstrated that irradiation treatment at different doses could significantly change the expression levels of seven proteins as discussed above. It should be emphasized here that the functions of these expressed proteins are not related to known HSP such as DnaK, GroES, GroEL, etc. which are normally expressed in *E. coli* under heat treatment. Moreover, in this study, the changes in the HSP of *E. coli* could not be detected. It is possible that their expression levels were lower than the detection limit of CE. In this study, CE was used for separation of proteins and protein identification was mainly based on MW. In future research, other methods such as CE-MS, 2D-DIGE in combination with MS, Western blot should be used to further confirm the obtained results in this study. Moreover, knock out of genes encoding for proteins explored in this study should be conducted to confirm their roles in bacterial survival during irradiation.

CONCLUSIONS

The profiles of protein expression in *E. coli* at different doses of irradiation treatment were studied. The expression level of one protein, bacteriophage N4 adsorption protein B (MW of 85.2 kDa), was not significantly changed at different doses of irradiation

treatment. The expression levels of seven proteins (MW of 54.0, 56.8, 60.7, 66.5, 71.0, 75.7 and 81.5 kDa) of *E. coli* were statistically changed at different doses of irradiation treatment. These proteins are rhamnulokinase, glycerol kinase, hypothetical protein Z2695, preprotein translocase subunit SecD, DNA polymerase III subunits gamma and tau, choline transport protein-BetT and primosome assembly protein-PriA. The functions of these expressed proteins are not related to known HSP such as DnaK, GroES, GroEL, etc. The research further confirmed that capillary electrophoresis is a useful method to separate and analyse the expressed proteins that may be related to the resistance or tolerance of foodborne pathogen to γ -irradiation treatment.

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REFERENCES

- Bachmann S, Vallant R, Bakry R, Huck CW, Corradini D, Bonn GK, 2010. CE coupled to MALDI with novel covalently coated capillaries. *Electrophoresis*, 31:618-629.
- Banatvala N, Griffin PM, Greene KD, Barrett TJ, Bibb WF, Green JH, Wells JG, 2001. The United States national prospective hemolytic uremic syndrome study: microbiologic, serologic, clinical, and epidemiologic findings. *J Infect Dis*, 183:1063-1070.
- Caillet S, Millette M, Dussault D, Shareck F, Lacroix M, 2008. Effect of gamma radiation on heat shock protein expression of four foodborne pathogens. *J Appl Microbiol*, 105:1384-1391.
- Caillet S, Shareck F, Lacroix M, 2005. Effect of gamma radiation and oregano essential oil on murein and ATP concentration of *Escherichia coli* O157:H7. *J Food Prot*, 68: 2571-2579.
- Daly MJ, 2009. A new perspective on radiation resistance based on *Deinococcus radiodurans*. *Nat Rev Microbiol*, 7:237-245.
- Dolnik V, 1999. Recent developments in capillary zone electrophoresis of proteins. *Electrophoresis*, 20:3106-3115.
- Ferens WA, Hovde CJ, 2011. *Escherichia coli* O157:H7: animal reservoir and sources of human infection. *Foodborne Pathogen Dis*, 8:465-487.
- Gharbi S, Gaffney P, Yang A, Zvelebil MJ, Cramer R, Waterfield MD, Timms JF, 2002. Evaluation of two-dimensional differential gel electrophoresis for proteomic expression analysis of a model breast cancer cell system. *Mol Cell Proteomics*, 1:91-98.
- Grueninger D, Schulz GE, 2006. Structure and reaction mechanism of L-rhamnulose kinase from *Escherichia coli*. *J Mol Biol*, 359:787-797.
- Huck CW, Bonn GK, 2008. Analysis of proteins by capillary electrophoresis. *Methods Mol Biol*, 384:507-540.
- Kiino DR, Rothman-Denes LB. 1989. Genetic analysis of bacteriophage N4 adsorption. *J Bacteriol*, 171:4595-4602.
- Liu WZ, Faber R, Feese M, Remington SJ, Pettigrew DW, 1994. *Escherichia coli* glycerol kinase: role of a tetramer interface in regulation by fructose 1,6-bisphosphate and phosphotransferase system regulatory protein IIIglc. *Biochemistry*, 33:10120-10126.
- Monk JD, Beuchat LR, Doyle MP, 1995. Irradiation inactivation of foodborne microorganisms. *J Food Prot*, 58:197-208.
- Nouwen N, Driessen AJ, 2005. Inactivation of protein translocation by cold-sensitive mutations in the yajC-secDF operon. *J Bacteriol*, 187:6852-6855.

- Peng J, Elias JE, Thoreen CC, Licklider LJ, Gygi SP, 2003. Evaluation of multidimensional chromatography coupled with tandem mass spectrophotometry (LC/LC-MS/MS) for large-scale protein analysis: The yeast proteome. *J Proteome Res*, 2:43-50.
- Perna NT, Plunkett III G, Burland V, Mau B, Glasner JD, Rose DJ, Mayhew GF, Evans PS, Gregor J, Kirkpatrick HA, Posfai G, Hackett J, Klink S, Boutin A, Shao Y, Miller L, Grotbeck EJ, Davis NW, Lim A, Dimalanta ET, Potamousis KD, Apodaca J, Anantharaman TS, Lin J, Yen G, Schwartz DC, Welch RA, Blattner FR, 2001. Genome sequence of enterohaemorrhagic *Escherichia coli* O157:H7. *Nature*, 409: 529-533.
- Tanaka T, Mizukoshi T, Sasaki K, Kohda D, Masai H, 2007. *Escherichia coli* PriA protein, two modes of DNA binding and activation of ATP hydrolysis. *J Biol Chem*, 282:19917-19927.
- Trudeau K, Vu KD, Shareck F, Lacroix M, 2012. Capillary electrophoresis separation of protein composition of gamma-irradiated food pathogens *Listeria monocytogenes* and *Staphylococcus aureus*. *PLoS One*, 7: e32488.
- Wood OB, Bruhn CM, 2000. Position of the American Dietetic Association: food irradiation. *J Am Diet Assoc*, 100: 246-253.
- Zhou G, Li H, DeCamp D, Chen S, Shu H, Gong Y, Flaig M, Gillespie JW, Hu N, Taylor PR, Emmert-Buck MR, Liotta LA, Petricoin EF, Zhao Y, 2002. 2D Differential in-gel electrophoresis for the identification of esophageal scans cell cancer-specific protein markers. *Mol Cell Proteomics*, 1:117-123.