Microbiology Research International
Vol. 3(3), pp. 51-55, September 2015
ISSN: 2354-2128
Full Length Research Paper

Influence of gamma irradiation on expressed heat shock proteins of *Pantoea agglomerans*, a yellow pigmented phytopathogen

Dominic Dussault[1], Khanh Dang Vu[1], Stephane Caillet[1], Canh Le Tien[2] and Monique Lacroix[1]*

[1]Canadian Irradiation Centre and Research Laboratories in Sciences Applied to Food, INRS-Institut Armand-Frappier, Laval, Quebec, Canada.
[2]Département de Chimie and Centre Pharmaqam, Université du Québec à Montréal, Montréal, Québec, Canada H3C 3P8.

Accepted 24 August, 2015

ABSTRACT

The aim of this study was to evaluate the effect of γ-radiations on the heat shock proteins (Hsps) content of strains of *Enterobacteriacea* and *Pantoea agglomerans*. Radiation treatments (1 and 3.5 kGy) were performed to highlight the radiotolerance. Total proteins were extracted after irradiation treatments and heat shock proteins (Hsp) of interest (GroEL, GroES and DnaK) were visualized and quantified using Western blot analysis. It was found that GroEL synthesis was increased significantly ($P \leq 0.05$) for both strains while the DnaK seemed to be down regulated for the strain from ATCC 49174. Basal level of GroEL proteins was much higher in the strain isolated from carrots. The obtained results indicated the modification effects of γ-radiations on the Hsps at cellular level.

Keywords: *Pantoea agglomerans*, γ-irradiation, heat shock proteins, radiotolerance, western blot.

*Corresponding author. E-mail: monique.lacroix@iaf.inrs.ca. Tel: 1-450-687-5010 #4489. Fax: 1-450-686-5501.

INTRODUCTION

Gamma irradiation of food has been widely applied to ensure microbial safety and to extend shelf life of food while avoiding effects associated with thermal processing (Manas and Pagan, 2005). Lacroix and Lafortune (2004) has observed a resistant bacterium to γ-irradiation in a probable viable but non-culturable (VBNC) state and was identified as *Pantoea agglomerans* RL1. This bacterium is an *Enterobacteriaceae* like *Escherichia coli* but is up to 5 times more radiotolerant as compared to *E. coli* (Le-Tien et al. 2006).

In a previous research on the effect of γ-radiation on the carotenoid content of the two strains of *P. agglomerans* (ATCC 49174 and RL1), it was found that the strain ATCC 49174 irradiated at 1 kGy produced 4.3 times more carotenoids than the control, whereas carotenoid synthesis was increased by 2.9-fold in the strain RL 1 isolated from irradiated carrot (Dussault et al., 2008). However, it was found that there was no significant difference in the D_{10} values (dose required to inhibit 90% the growth of bacterial population) of two strains. Thus, the increase in carotenoid production was influenced by γ-irradiation but did not modify the tolerance of two strains to radiation (Dussault et al., 2008).

Further, the two strains of *P. agglomerans* (ATCC 49174 and RL1) were used to evaluate the effects of irradiation on membrane fatty acid (FA) and muropeptides of the bacterial cell walls. It was found that there was a significant ($P \leq 05$) modification of the membrane FA composition and the muropeptides of the bacterial walls when treated by irradiation. Thus, it was concluded that the irradiation had an effect on the bacterial membrane which could play an important role on the cellular response and ability to survive in a harsh environment (Dussault et al., 2009). These obtained results are important; however, further research on other possible mechanisms in which this bacterium can highly resist against the stress caused by γ-irradiation should be conducted.

Recently, it has been found protein oxidation by ionizing radiation could be one of the main causes of the

inability for irradiated microorganisms to recover and repair damage after such a stress (Daly et al., 2007). Environmental stresses such as heat shock, cold shock, osmotic shock, starvation, amino acid analogs, antibiotics, heavy metals, ultraviolet radiation, gamma radiation and alcohols produce a cellular response when the microorganisms are exposed to these conditions and it is known as heat shock response (Kvint et al., 2003). Exposure to an increasing heat will begin with an augmentation of the membrane fluidity, proteins denaturation and degradation and will finally lead to cell death (Aguilar and de Mendoza, 2006).

The response to heat shock has been associated with a high production level of proteins known as heat shock proteins (Hsps). Higher level of specific protein during heat shock response is due to the fact that an increasing concentration of the σ^{32} subunit of the RNA polymerase (RNAp) will enable the polymerase to attach to precise part of the DNA encoding for hsps genes (Wade et al., 2006). The classical Hsps are the molecular chaperones (e.g. DnaK, GroEL, GroES and their cohorts). These proteins play a role in protein folding, assembly, repair and prevention of aggregation under stress and non-stress conditions. The chaperones act with proteases to maintain quality control of cellular proteins (Gottesman et al., 1997; Marchenko et al., 2006).

It is hypothesized that the induction of Hsps synthesis after ionization radiations treatments might play a significant role in the radioresistance and in the recovery of bacteria during the VBNC state. Therefore, the objective of this study was to evaluate the expressed levels of three Hsps such as GroEL, GroES and DnaK in *P. agglomerans* ATCC 49174 and RL1 when they were treated by two radiation doses.

MATERIALS AND METHODS

Microorganisms and growth conditions

P. agglomerans ATCC 49174 (American Type Culture Collection, Manassas, VA, USA) and *P. agglomerans* RL1, previously isolated from irradiated carrots at 7 kGy (Lacroix and Lafortune, 2004) were used in this study (Dussault et al., 2008, 2009). These two strains were subcultured (1.0%, v/v) in tryptic soy broth (TSB, Difco Laboratories, Detroit, USA) at 30°C for 24 h from the stock culture maintained at −80°C in TSB containing glycerol (20%, w/v). Prior to the experiment, 1 ml of culture was incubated through one cycle of 24 h at 30°C in TSB to obtain a working culture containing approximately 10^9 CFU ml^{-1}. The bacterial culture was centrifuged at 2,000 × g for 15 min at 4°C and washed with NaCl (0.85 %, w/v) and then resuspended in 1 L of TSB and incubated for 24 h before irradiation treatments (Dussault et al., 2008, 2009).

Irradiation treatments

The bacterial cultures (50 ml) were irradiated with a mean dose of 1 kGy to create damaged cells and with a mean dose of 3.5 kGy to kill cells (Dussault et al., 2008). A UC-15A irradiator (MDS Nordion international Inc., Kanata, Ontario, Canada) equipped with a ^{60}Cobalt source was used to deliver radiations at a mean rate of 16.2 kGy h^{-1}. This irradiator was certified by the National Institute of Standards and Technology (Gaithersburg, Md.), and the dose rate was established using a correction for decays of source. Amber Oersoex 3042D (Atomic Energy Research Establishment, Harwell, Oxfordshire, UK) was used to validate the doses distributions. The radiation treatment was carried out at the Canadian Irradiation Centre (Laval, Quebec, Canada) at room temperature (20°C) (Dussault et al., 2008, 2009).

Extraction of proteins

Immediately after irradiation, 50 ml of each bacterial culture were quickly chilled in an ice/ethanol bath until the temperature dropped below 10°C. Cells were harvested by centrifugation for 15 min at 2000 × g at 4°C and the resulting pellet was washed in 25 ml NaCl (0.85%, w/v) at the same temperature. The bacterial pellets were then resuspended in 5 ml of buffer containing protease inhibitor (50 mmol L^{-1} Tris-HCl (pH 7.5), 0.1 mol L^{-1} NaCl, 1 mmol L^{-1} phenylmethanesulfonyl fluoride (PMSF), 1 mg ml^{-1} iodoacetamide) and cell were broken using a cell disrupter (FastPREP, model FP 120, Qbiogene Inc., Carlbad, CA, USA) containing glass beads (0.2 mm) and regulated at speed 6 for 30 s at 4°C. The suspension was centrifuged at 5000 × g for 5 min to remove the glass beads and the unbroken cells. Then, the resulting supernatant containing the proteins was harvested and stored at -20°C (Trudeau et al., 2013).

Proteins dosage

To determine the concentration of protein extract, the protein quantification was done using the bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, Ill, USA) according to the manufacturer's specifications. Absorbance was read at 562 nm with a DMS 100S spectrophotometer (Varian Canada Inc., Mississauga, ON, Canada). A standard curve (25 to 2000 µg ml^{-1}) of bovine serum albumin (Pierce) was established and used for quantification of protein concentration of the samples.

SDS PAGE and western blotting

An aliquot of the suspension (1 mg of protein ml^{-1}) was diluted with sample buffer 2x (62.5 mmol l^{-1} Tris-HCl, SDS (2%, w/v), glycerol (31.5%, w/v), bromophenol blue (0.01%, w/v), 7.15 mmol l^{-1} β-mercaptoethanol) to obtain 5 µg of total protein $well^{-1}$ for the GroEL detection and and 10 µg of total protein $well^{-1}$ for the GroES and DnaK detection. Proteins contained in the bacterial cell extracts were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) with 10% acrylamide/bis gel using the Novex® Sharp™ Pre-stained (Invitrogen Canada Inc., Burlington, ON, Canada) with molecular size standard (MultiMark Multi-Colored Standard, Invitrogen Inc.). The migration of the gels was carried out at 200 V during 45 min in a Mini-Protean® Tetra cell (Bio-Rad Laboratories Ltd., Mississauga, ON, Canada) and the coloration of the gel was done using Coomassie blue R-250. After electrophoresis, the proteins extract were transferred onto a nitrocellulose membrane Hybond™-ECL™ (Amersham Biosciences, Oakville, ON, Canada), and processed for immunoblotting in a Mini Trans-Blot® electrophoretic transfer cell (Bio-Rad) at 20 V overnight. The membrane was blocked with 3% skim milk for 1 h at room temperature. Blots were then incubated with either GroEL rabbit polyclonal antibody (Sigma-Aldrich, Oakville, ON, Canada) conjugated with peroxydase, DnaK mouse monoclonal antibody (Stressgen, Victoria, BC, Canada) or GroES rabbit polyclonal antibody (Stressgen) rose against corresponding proteins produced by *E. coli*. For DnaK and GroES proteins, immunocomplexes were incubated with sheep anti-mouse

peroxidase and donkey anti-rabbit peroxidase (Stressgen), respectively. Immunocomplexes were then visualized with SuperSignal® West Pico chemiluminescent substrate (Pierce). The films (Konica Minolta, Mississauga, ON, Canada) are then exposed on the membrane in a darkroom and developed with a mini-med/90 X-ray film processor (AFP Imaging Corporation, Elmsford, NY) .The resulting bands were analysed and quantified with a Alpha Imager^{TM} IS-3400 (Alpha Innotech Corporation, San Leandro, CA) using the standard curves. Results were expressed as µg of Hsps per mg of total proteins. A standard curve ranging from 5 to 80 ng well^{-1} was established for GroEL, DnaK and GroES (Caillet et al., 2008).

Statistical analysis

The experiments were conducted in duplicate. For each replicate, three samples were analyzed. An analysis of variance and Duncan's multiple-range tests were employed to analyze statistically all results. Differences between means were considered significant at $P \leq 0.05$. Stat-Packets Statistical Analysis software (SPSS Base 10.0, SPSS, Inc., Chicago, Ill.) was used for the analysis.

RESULTS

The image of western blots of standard protein GroEL at different concentration (5 to 40 µg/µl) is shown in Figure 1. Moreover, the blots of expressed protein GroEL by *P. agglomerans* ATCC 49174 and RL 1 at different irradiation treatment doses (0, 1 and 3.5 kGy) are also shown in Figure 1. The expressed levels (µg/mg total protein) of three Hsps: GroEL, GroES and Dnk are presented in Figure 2.

In case of *P. agglomerans* ATCC 49174, the expressed GroEL was increased significantly ($P \leq 0.05$) from 0.22 (µg/mg total protein) in the control without irradiation treatment to 2.64 (µg/mg total protein) and 0.74 (µg/mg total protein) in the irradiation treatments by 1.0 and 3.5 kGy, respectively (Figure 2). In case of *P. agglomerans* RL1, it can be observed that the basal expression level of GroEL (1.94 µg/mg total protein) at the control without irradiation treatment was 8.8 times higher than that of expressed GroEL (0.22 µg/mg total protein) in *P. agglomerans* ATCC 49174. The expressed GroEL in *P. agglomerans* RL1 at irradiation treatments of 1.0 and 3.5 kGy were 3.66 and 5.76 µg/mg total protein, respectively, which are significantly higher ($P \leq 0.05$) that that of the control (1.94 µg/mg total protein). It is interesting to find that the expressed level of GroEL in *P. agglomerans* ATCC 49174 at 3.5 kGy treatment was less than that of 1.0 kGy treatment while it was opposite in *P. agglomerans* RL1 (Figure 2).

The expressed GroES in *P. agglomerans* ATCC 49174 was increased significantly ($P \leq 0.05$) from 0.83 (µg/mg total protein) in the control without irradiation treatment to 3.86 (µg/mg total protein) in the irradiation treatment of 1 kGy. However, at the irradiation dose of 3.5 kGy, the expressed level of this protein was insignificantly ($P > 0.05$) as compared to the control without irradiation (Figure 2). It can be observed that the basal expressed level of GroES (3.5 µg/mg total protein) in *P. agglomerans* RL1 was significantly ($P \leq 0.05$) higher than that of expressed GroES (0.83 µg/mg total protein) in *P. agglomerans* ATCC 49174. Further, it is interesting to find that there were no significant differences ($P > 0.05$) in the expressed levels of GroES in *P. agglomerans* RL1 at all doses of irradiation treatments (Figure 2).

It is of interest to mention that the basal expressed level of DnaK (4.78 µg/mg total protein) in *P. agglomerans* ATCC 49174 was significantly ($P \leq 0.05$) higher than that of DnaK (2.83 µg/mg total protein) in *P. agglomerans* RL1. Moreover, this protein was decreased to 4.42 (µg/mg total protein) and 2.73 (µg/mg total protein) when *P. agglomerans* ATCC 49174 was irradiated at 1.0 and 3.5 kGy, respectively. However, this protein was not significantly ($P > 0.05$) changed in *P. agglomerans* RL1 for the control and the irradiated treatments (1.0 and 3.5 kGy).

DISCUSSION

In a previous research on the effects of irradiation on the Hsps (GroEL, DnaK and GroES) in two Gram-negative bacteria (*Escherichia coli* and *Salmonella* serotype Typhimurium) and two Gram-positive bacteria (*Staphylococcus aureus* and *Listeria monocytogenes*), the authors found that GroEL was strongly induced by gamma rays in a dose dependent manner in these pathogens. In addition, it was found that *E. coli* exposed to gamma radiation showed a significantly induction of DnaK and GroES proteins when compared with non-irradiated bacteria, whereas a slight induction of GroES and an inhibition of DnaK were observed in *S.* Typhimurium (Caillet et al., 2008).

Thus, it can be concluded that irradiation treatments influenced significantly the quantity of Hsps in the bacterial cytoplasm. It is known that to recover from the negative effects of some environmental stresses, bacteria use greater numbers of chaperones proteins to rectify and correctly fold their cellular proteins (Wade et al. 2006). A study on eukaryotic cells has shown that ionizing irradiation induce Hsps, in particular Hsp70 (Calini et al., 2003). As compared to our results, Hsp synthesis was also increased after exposition to γ radiations.

In this study, both strains of *P. agglomerans* did not show the same response to the radiations exposure. The level of induction of the GroEL synthesis in the strain isolated from the irradiated carrots RL1 were lower than the ATCC strain and this could be explained by the fact that the basal level was much lower in the ATCC strain. Moreover, in the ATCC 49174 strain, the DnaK chaperone system was down regulated as compared to the RL1 strain that did not show any modification as compared to the non-irradiated control.

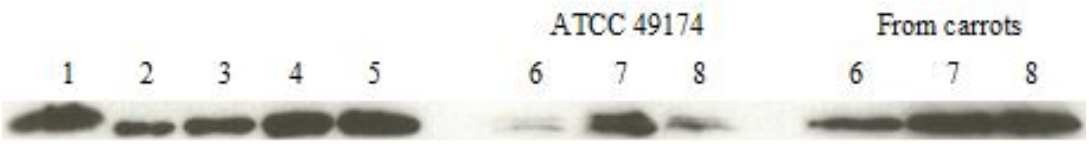

Figure 1. Western blot analysis of GroEL in *P. agglomerans* ATCC 49174 and *P. agglomerans* RL1 at different γ-irradiation treatments (non-irradiated, 1.0 and 3.5 kGy). 1, Ladder; 2, GroEL 5 µg ml^{-1}; 3, GroEL 10 µg ml^{-1}; 4, GroEL 20 µg ml^{-1}; 5, GroEL 40 µg ml^{-1}; 6, non-irradiated; 7, 1 kGy; 8, 3.5 kGy.

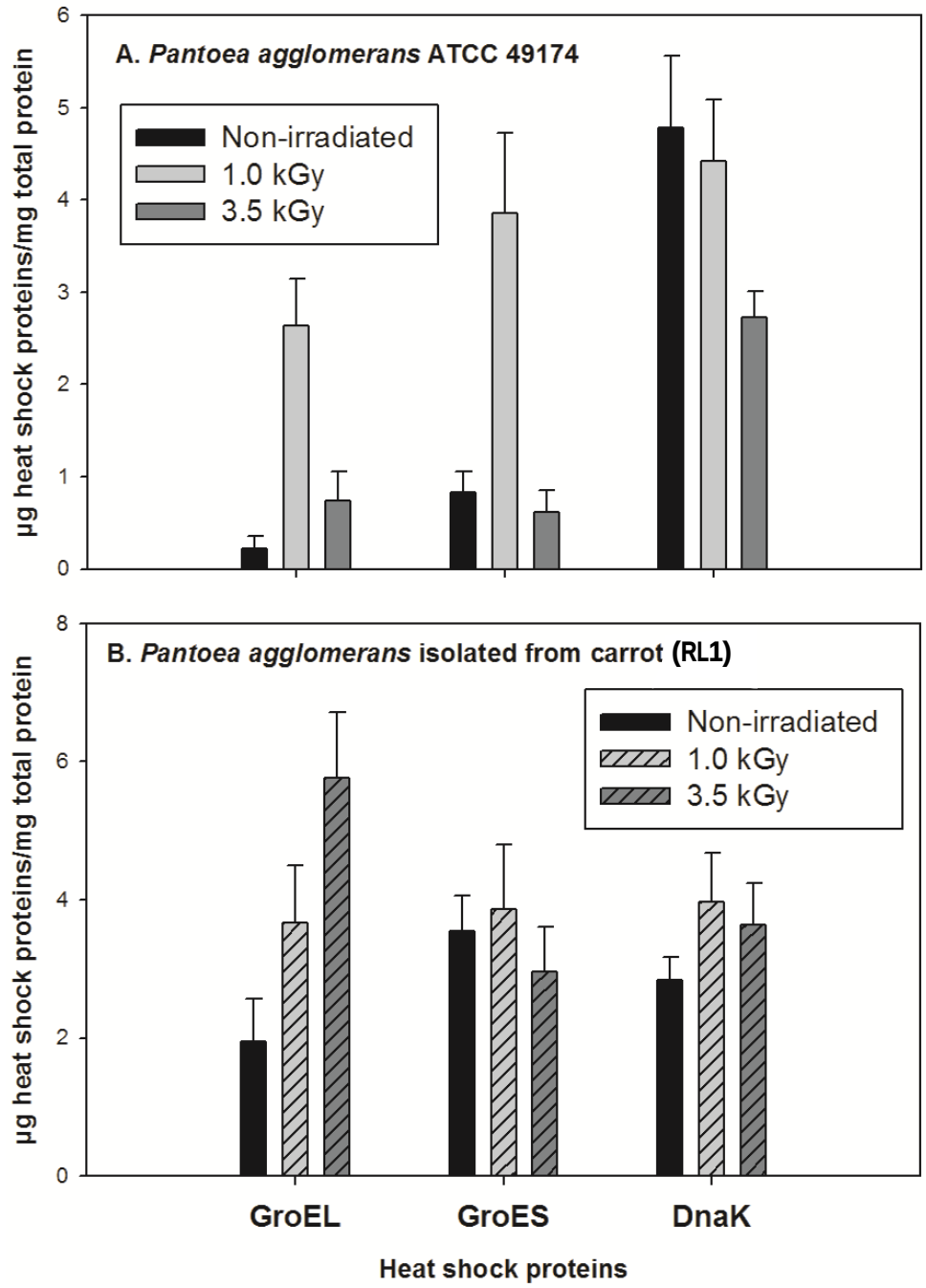

Figure 2. Concentration of GroEL, GroES and DnaK expressed in (A) *P. agglomerans* ATCC 49174 and (B) *P. agglomerans* RL1 at different γ-irradiation treatments (non-irradiated, 1.0 and 3.5 kGy).

The lack of induction of the DnaK mechanism as compared to the GroEL chaperone system could be explained by the fact that Hsp60 folding system completely traps the proteins in a closed container. The Hsp70 system only attaches to a part of the protein and could be exposing critical part of the proteins to the oxidative species damaging the cell components and therefore giving no advantage toward radioresistance (Lin and Rye, 2006).

The ability allowing an increase of the Hsps at 3.5 kGy for the strain *P. agglomerans* RL1 as compared to the ATCC strain could be the means of protection which are necessary for the cell survival at higher irradiation doses. Also the higher basal level of proteins synthesized by the strain RL1 could provide a better protection at the moment that reactive oxygen species (ROS) generated by the irradiation treatment.

Recently, Trudeau et al. (2014) quantified the expression levels of seven genes (*clpB, dnaK, groES, grpE, htpG, htpX, ibpB*) encoding heat shock proteins (HSPs) in *E. coli* O157:H7 strain EDL933 that was irradiated at different doses by real-time PCR. The authors found that all genes were downregulated during post-irradiation time from 0 to 120 min when *E. coli* was irradiated at 0.4 kGy. When *E. coli* was irradiated at 1.3 kGy, the genes *groES*, *grpE* and *ibpB* were more upregulated than that at 0.4 kGy. The authors hypothesized that irradiation of *E. coli* at 1.3 kGy might cause more stress to bacteria and therefore, higher gene expression to synthesize more heat shock proteins for cell damage reparation (Treaudeau et al., 2014). Thus, further research on quantification of expression level of genes encoding different Hsps in both *P. agglomerans* strains (ATCC 49174 and RL1) under irradiation treatment using real time PCR should be conducted to understand more details on the mechanism of Hsps expression in these strains.

CONCLUSIONS

There were significant effects of γ-irradiation on the expressed level of heat shock proteins in both *P. agglomerans* strains (ATCC 49174 and RL1). GroEL synthesis was increased significantly in both strains while the DnaK seemed to be down regulated in the strain ATCC 49174. Basal expression level of GroEL proteins was much higher in the strain RL1 compared to ATCC 49174 strain. The higher expression of Hsps in *P. agglomerans* RL1 strain at 3.5 kGy compared to the ATCC 49174 strain could be the means of protection which are necessary for the cell survival at higher irradiation dose. Moreover, a higher basal level of Hsps expression in the strain RL1 could provide a better protection at the moment that reactive oxygen species (ROS) generated by the irradiation treatment.

ACKNOWLEDGMENT

This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). We thank MDS Nordion for the irradiation operations.

REFERENCES

Aguilar PS, **de Mendoza** D, **2006**. Control of fatty acid desaturation: a mechanism conserved from bacteria to humans. Mol Microbiol, 62:1507-1514.

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.

Calini V, Urani C, Camatini M, **2003**. Overexpression of HSP70 is induced by ionizing radiation in C3H 10T1/2 cells and protects from DNA damage. Toxicol In Vitro, 17:561-566.

Daly MJ, Gaidamakova EK, Matrosova VY, Vasilenko A, Zhai M, Leapman RD, Lai B, Ravel B, Li SM, Kemner KM, Fredrickson JK, **2007**. Protein oxidation implicated as the primary determinant of bacterial radioresistance. PLoS Biol, 5: e92.

Dussault D, Caillet S, Le Tien C, Lacroix M, **2008**. Carotenoids' influence on radiotolerance of Pantoea agglomerans, a plant pathogen. Lett Appl Microbiol, 47:208-213.

Dussault D, Caillet S, Le Tien C, Lacroix M, **2009**. Effect of γ-irradiation on membrane fatty acids and peptidoglycan's muropeptides of Pantoea agglomerans, a plant pathogen. J Appl Microbiol, 106:1033-1040.

Gottesman S, Wickner S, Maurizi MR, **1997**. Protein quality control: triage by chaperones and proteases. Genes Develop, 11 815-823.

Kvint K, Nachin L, Diez A, Nystrom T, **2003**. The bacterial universal stress protein: function and regulation. Curr Opinion Microbiol, 6:140-145.

Lacroix M, **Lafortune** R, **2004**. Combined effects of gamma irradiation and modified atmosphere packaging on bacterial resistance in grated carrots (Daucus carata). Radiat Physics Chem, 71:77-80.

Le-Tien C, Lafortune R, Shareck F and Lacroix M, **2006**. DNA analysis of a radiotolerant bacterium Pantoea agglomerans by FT-IR scpectroscopy. Talanta, 71:1969-1975.

Lin Z, **Rye** HS, **2006**. GroEL-mediated protein folding: making the impossible, possible. Crit Rev Biochem Mol Biol, 41:211-239.

Manas P, **Pagan** R, **2005**. Microbial inactivation by new technologies of food preservation. J Appl Microbiol, 98:1387-1399.

Marchenko NY, Marchenkov VV, Kaysheva AL, Kashparov IA, Kotova NV, Kaliman PA, Semisotnov GV, **2006**. Affinity chromatography of GroEL chaperonin based on denatured proteins: Role of electrostatic interactions in regulation of GroEL affinity for protein substrates. Biochemisty, 71:1357-1364.

Trudeau K, Vu KD, Déziel E, Shareck F, Lacroix M, **2014**. Effect of γ-irradiation on gene expression of heat shock proteins in the foodborne pathogen *Escherichia coli* O157:H7. Int J Radiat Biol, 90: 268-273.

Trudeau K, Vu KD, Shareck F, Lacroix M, **2013**. Profile of expressed proteins of γ-irradiated foodborne pathogen Escherichia coli O157:H7 analysed by capillary electrophoresis. Microbiol Res Int, 1:40-47.

Wade JT, Roa DC, Grainger DC, Hurd D, Busby SJ, Struhl K, Nudler E, **2006**. Extensive functional overlap between sigma factors in *Escherichia coli*. Nat Struct Mol Biol, 13:806-814.

Citation: Dussault D, Vu KD, Caillet S, Tien CL, Lacroix M, 2015. Influence of gamma irradiations on expressed heat shock proteins of Pantoea agglomerans, a yellow pigmented phytopathogen. Microbiol Res Int, 3(3): 51-55.