Corvinus University of Budapest
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UNDERSTANDING STRESS ADAPTIVE RESPONSE IN *LISTERIA MONOCYTOGENES*

Theses

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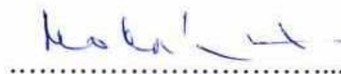

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INTRODUCTION

Thousands of people around the world die each year from pathogen and toxin contaminated foods. Pathogen contamination can occur at all points from the proverbial “farm to the fork”. Pathogens can be transmitted between humans, animals, the environment, and foods through air, water, soil, and also via contaminated or improperly cleaned equipment. The food industry is utilizing and exploring a variety of “hurdles” to prevent or eliminate pathogens from foods and extend the shelf-life of foods. These include reduction or elevation of temperature, modifying the gas composition within packages, use of ionizing radiation, reduction of water activity, pH reduction, oxygen removal, and use of bacteriocins and preservatives. A majority of these techniques are used alone, or in combination to inhibit pathogens and preserve foods to ensure their shelf-life. The response of food-borne bacterial pathogens to stresses caused by these hurdle technologies or “stressors” is a concern. Some stress responses can result in enhanced survival, enhanced virulence, and even cross protection against multiple stressors.

Heat treatment is still one of the most commonly used food preservation techniques. Sub-lethal heat stress (heat shock) or prior exposure to temperatures above optimal growth temperatures can make the organism to become more resistant to subsequent heat treatment which, under normal conditions would have been lethal. This stress response is termed induced thermo-tolerance. The practical importance of thermo-tolerance to food processors is for food products that are heated to temperatures below 65°C. Thermo-tolerance can become a concern for meat products kept on warming trays before a final heating or reheating step, or when there is an interruption in the cooking cycle during food processing (due to equipment failure).

Listeria monocytogenes is ubiquitous and can be found in soil, water, and on food processing equipments. The heat shock response and increased thermo-tolerance in *L. monocytogenes* is of particular concern to the Ready–To-Eat (RTE) food industry because this pathogen has a variety of genetically encoded survival mechanisms to withstand environmental stressors such as heat, cold, salt, and acidic conditions. More importantly, this pathogen has a very high case-fatality rate.

AIMS

The overall focus of my dissertation research was to better understand the stress adaptive response in *L. monocytogenes* during sub-lethal temperature exposure. As part of this study, I also included an investigation of the applicability of combining low-dose irradiation with modified atmosphere packaging to control *L. monocytogenes* in fresh produce. The underlying hypothesis of my research was that *Listeria monocytogenes* elicits unique physiological, genomic, and proteomic responses as part of its overall stress adaptation in response to sub-lethal temperature stress. The specific objectives of my research were,

1. To evaluate the applicability of combining low-dose γ irradiation with Modified Atmosphere Packaging (MAP) to control *L. monocytogenes* in packaged fresh produce
2. To understand the physiological responses of *L. monocytogenes* to sub-lethal temperature stress conditions
3. To understand the transcriptomic response of *L. monocytogenes* to sub-lethal temperature stress conditions
4. To understand the proteomic response of *L. monocytogenes* to sub-lethal temperature stress conditions.

MATERIALS AND METHODS

Microorganisms: Two strains of *Listeria monocytogenes* were employed in the studies. The *L. monocytogenes* 4ab strain (No. 10), an avirulent strain (originally isolated from meat) was used in the first part of my work. Specifically, this strain was used in the γ irradiation studies and the temperature tolerance studies. The *L. monocytogenes* virulent strain, ATCC 43256, was used in the studies performed at Texas A&M University in the United States. Specifically, this strain was used in understanding the transcriptomic, proteomic, and physiological responses of the pathogen when exposed to 60°C heat treatment with and without prior exposure to sub-lethal temperature conditions. This virulent strain was also used in determining the Viable But Non-Culturable (VBNC) state of the pathogen during such temperature treatments.

Fresh-cut Produce and Modified Atmosphere Packaging (MAP) Conditions: Fresh alfalfa and radish sprouts were obtained from a "Bio" shop in Budapest. Alfalfa and radish sprouts (5 g) were placed in CombiTherm80 bags, and flushed with 2 different gas mixtures namely Gas Mixture #1 and Gas Mixture #2. The composition of Gas Mixture #1 was oxygen-carbon dioxide-nitrogen (2%-4%-94%) while that of Gas Mixture #2 was 3-5 % oxygen and 10-15% carbon dioxide balanced with nitrogen.

Irradiation Conditions: Irradiation experiments were performed at room temperature to defined target doses (1 kGy and 2 kGy) using a NORATOM Co^{60} gamma irradiator at the Institute for Radiobiology, Budapest. The dose rate during the γ irradiation was 6.47 kGy/h. After irradiation, the samples were stored refrigerated at 5 °C.

D-value Determination: Overnight cultures were harvested by centrifugation and washed twice with phosphate buffer. The washed cell suspension was inoculated into Tryptic Soy Broth (TSB). The TSB medium was used as the test matrix. Portions of the culture were transferred into Eppendorf tubes. The samples were placed in a thermostatically controlled circulating water bath and exposed to 55°C (for 10, 20, 30, 40, 50, and 60 min), 60°C (for 3, 6, 9, 12, 15, and 18 min) and 65°C (for 1, 2, 3, 4, 5, and 6 min). The D-value of the strain at 55°C, 60°C, and 65°C was calculated.

Identification of Enhanced Heat Resistance: The time-temperature combinations used for sub-lethal heat stress was 46°C (for 30 and 60 min), 48°C (for 30 and 60 min), and 50°C (for 30 and 60 min). Based on the D-value, 60°C heat treatment was chosen for identifying the enhanced heat resistance. Portions of the washed culture were placed in TSB and exposed to the sub-lethal heat stress conditions mentioned above. After exposure to the sub-lethal heat stress, the samples were immediately transferred to a 60°C water bath and exposed to 60°C for 3, 6, 9, 12, and 15 minutes. The heat treated samples were immediately placed in an ice-bath prior and serially diluted in a peptone-NaCl (0.85 %) buffer. The diluted samples were spread plated on Tryptic Soy Agar (TSA) and on TSA plates supplemented with 5% NaCl. The aliquots were plated on the TSA+NaCl since previous studies have shown that sub-lethally heat injured cells were sensitive to NaCl. The difference in counts obtained on TSA and TSA+NaCl provided information on the percentage of cells that were heat-injured.

Viable but Non-Culturable State (VBNC) of *Listeria monocytogenes*: The virulent *L. monocytogenes* strain (ATCC 43256) was used in this study. The culture was washed and prepared as described earlier. The washed cells were re-suspended in Luria Bertani broth (LB). Portions of the washed culture were placed in microfuge tubes for the different heat treatments. The heat treatments were performed in a calibrated water-bath. Three experimental heat treatments were employed. The samples were centrifuged, washed and re-suspended in sterile water. Viability was quantified microscopically and using a fluorometer. Portions of the washed bacterial cell suspension from the test samples were placed in a 96-well microplate. The commercially available fluorescence-based LIVE/DEAD BacLight™ dye was used to determine viability. For fluorescence spectroscopy, the two-stain solution was freshly prepared. The staining solution was added to 96-well plates containing the heat-treated samples, and the samples incubated in the dark for 15 minutes. After incubation, the samples were measured at two wavelengths (A_{535} and A_{635}) using a microplate-based fluorometer.

The green/red fluorescence ratios ($R_{G/R}$) (A_{535}/A_{635}) were calculated for each experimental sample. For the direct microscopic examination, the samples were mixed with the two-stain dye mixture and incubated. The samples were analyzed using fluorescence microscopy. The images were captured using a high resolution CCD camera. The percentage of viable cells in each sample was calculated using image analysis. The ratio of green cells (live) and red cells (dead) were determined on the number of green pixels and red pixels. Green and red pixels above pixel intensities of 100 denoted live and dead cells respectively.

Microarray Analysis: The virulent *L. monocytogenes* strain (ATCC 43256) was used in these studies. Microarray analysis was performed to identify the differentially expressed genes during heat stress by comparing the transcriptome of *L. monocytogenes* under varying experimental temperature conditions. The four different experimental conditions namely: (i) 37°C (control), (ii) heat shock at 60°C (for 0 minute), (iii) prolonged heat shock at 60°C for 9 min, and (iv) thermo-tolerance inducing treatment at 48°C for 30 minutes followed by exposure to 60°C for 9 min were performed in a calibrated water-bath.

RNA Extraction, cDNA Synthesis, Hybridization and Data Analysis: Total microbial RNA was extracted from the samples after the applied heat treatments using a commercial RNA extraction kit. The standard operating protocols of The Institute of Genomic Research (TIGR, USA) were followed with slight modifications for cDNA synthesis, labeling, and hybridization. Total RNA was used to synthesize cDNA using a random primer for reverse

transcription. Purified cDNAs from the experimental samples were each labeled with Cy-3 mono-Reactive Dye and Cy-5 mono-Reactive Dye. The labeled cDNA from the treatment and control samples were used to hybridize *L. monocytogenes* genome microarrays (cDNA arrays with 2846 open reading frames each, with 4 replicate spots per ORF). Hybridization was performed overnight at 42°C and the slides were washed and scanned at 532 nm (Cy3 channel) and 635 nm (Cy5 channel). The data from four to five individual experiments (4 to 5 slides per experiment including dye swap) were initially filtered for spot quality (signal uniformity, signal to background ratio, threshold intensity). The array data were normalized and their statistical significance was evaluated. To identify genes differentially expressed between different treatment groups, a Student's *t*-test was performed and the FDR (False Discovery Rate) was calculated. Genes with FDR<0.05 were considered as differentially expressed between the experimental sample and the control sample.

<u>Proteomic Analysis</u>: The virulent *L. monocytogenes* strain (ATCC 43256) was used in these studies. The four heat treatments were performed as described earlier. The protein fractions were extracted from each sample using a commercial kit in combination with sonication and an ultrasonic cell disruptor. The proteins were then purified and concentrations were quantified. For the 1st dimensional electrophoresis, the isoelectric focusing of 7-cm and 11-cm IPG strips was conducted at a linear voltage gradient with 24,000 final V-h and 56,000 final V-h, respectively. The 2nd dimensional electrophoresis was performed using 10% SDS–polyacrylamide gel electrophoresis (PAGE) gel. The protein spots were visualized using commercial staining kits and the gels were used for spot excisions.

Data Analysis: The gels were scanned using commercially available software and the raw images were analyzed using the commercial software. To analyze the proteome under thermo-tolerance and heat shock conditions, the protein expression pattern observed at 37°C was compared against the other treatments namely: (i) 60°C for 0 minute, (ii) the 60°C for 9 min treatment, and (iii) the 48°C for 30 min followed by 60°C for 9 min treatment. Only those spots with spot intensities exhibiting ±1.5-fold change difference were picked for MALDI-TOF identification.

MALDI-TOF Identification: The protein spots of interest were manually excised and digested with trypsin. The digested samples were spotted onto matrix-assisted laser desorption/ionization (MALDI) targets. The mass spectrometry (MS) data for each gel spot was acquired using the reflection detector and 20 tandem MS spectra per spot were acquired. All MS and MS/MS data were queried against the Swiss-Prot protein sequence database

using commercial software. To verify the reproducibility of the MALDI-MS data, 10 protein spots were re-analyzed.

RESULTS

Effect of MAP and γ Irradiation on Shelf-life and Survival and Growth of *L. monocytogenes*: The total aerobic plate count (TPC) of MAP radish and alfalfa sprouts (in Gas Mixture #1 and Gas Mixture #2) was high starting at about 10^9 CFU/g at the beginning of the study. Majority of the bacteria belonged to the *Enterobacteriaceae* family. The numbers of LAB was two log cycles higher on radish sprouts in Gas Mixture #1. During 10 days of storage at 5°C, (due to elevated CO_2 content within the packages) the LAB were able to increase by one log unit, while number of *Enterobacteriaceae* remained steady. The Gas Mixture #1 appeared to be more effective at reducing the numbers of microorganisms. The composition of the Gas Mixture #2 did not appear to reduce the initial level of microorganisms. Neither were there increases in the population numbers. Gamma (γ) irradiation of MAP sprouts with 1 and 2 kGy reduced the numbers of both the total aerobic bacteria and *Enterobacteriaceae.* The D_{10}-value for the avirulent *L. monocytogenes* strain 4ab was 0.58 kGy under Gas Mixture #1, and the D_{10}-value was 0.45 kGy under Gas Mixture #2 conditions. Irradiation appeared to be less effective on the test strain in case of MAP samples (Gas Mixture #1) as compared to air packaged sprout samples (D_{10}=0.46 kGy). The *L. monocytogenes* strain multiplied after MAP irradiation with 1 kGy and during refrigerated storage. The numbers increased by 2 log units.

Enhanced Heat Resistance: The D-values of *L. monocytogenes* 4ab at 55°C, 60°C, and 65°C were calculated to be 15.19 min (R^2=0.93), 3.03 min (R^2=0.98) and 1.29 min (R^2=0.947), respectively. Exposing the strain to two different durations (30 min and 60 min) of sub-lethal temperatures enhanced the survival of this strain at 60°C. The D_{60}-value after 30 min exposure at 46°C was 5.24 min as compared to 16.18 min after exposure to 60 min at 46°C. Similarly, the D_{60}-value after 30 min and 60 min exposure at 48°C was 6.72 min and 14.83 min. However, the D_{60}-value was 13.88 min and 11.16 min after 30 min and 60 min exposure at 50°C, respectively. The enhanced heat resistance of this *L. monocytogenes* strain was particularly evident when the cells were exposed to 30 min at 46°C, 48°C and 50°C. The D_{60}-value increased from 5.24 minutes to 13.88 minutes. Exposure to 60 minutes at the sub-lethal temperature overall appeared to retard bacterial survival. As compared to the D_{60}-value when the cells were pre-exposed for 60 min at 46°C, the D_{60}-value decreased to 11.16 min

after pre-exposure to 50°C for 60 minutes. Exposure to sub-lethal temperatures caused heat injury in this *L. monocytogenes* strain (based on the TSA and TSA+NaCl plate counts after 30 min and 60 min of sub-lethal pre-exposure at 46°C, 48°C and 50°C). There were significant differences in the bacterial numbers obtained on the TSA plates and TSA plates that were amended with NaCl, suggesting heat injury. Plating onto TSA supplemented with 5% NaCl was also performed after direct 60°C heat treatment. However, there was no difference between the numbers obtained on TSA as compared to TSA+NaCl plates in samples from this treatment.

VBNC State During Heat Stress in *L. monocytogenes*: The D-value of *L. monocytogenes* ATCC 43256 strain at 55°C, 60°C and 65°C was 17.39 min (R^2=0.95), 3.74 min (R^2=0.96), 3.15 min (R^2=0.89), respectively. Pre-exposure to sub-lethal temperature of 48°C for 30 min increased the D-values at 60°C. The D_{60}-value (based only on the linear portion of the curve) changed from 3.74 minutes to 4.55 minutes. *L. monocytogenes* (ATCC 43256) when exposed to 60°C resulted in >2 log (~ 99%) reduction of culturable cells at the end of 9 minutes. When the cells were pre-exposed to 48°C for 30 minutes prior to 60°C heat treatment for 9 minutes, there was <2 log (99%) reduction. However, when these same samples were analyzed for % viability using the commercially available LIVE/DEAD BacLight™ staining reagents, the results were significantly different. Based on the fluorescence microplate read-out, there was no change in % viability even after exposure to 9 minutes at 60°C. The % viability remained at 100%. Based on the direct microscopic examination as well, there was only minimal reduction in viability. When the cells were exposed to 60°C for 9 minutes there was approximately only 1% reduction in viability based on the LIVE/DEAD BacLight™ viability assay. When the cells were pre-exposed to 48°C for 30 minutes prior to 60°C exposure for 9 minutes, the viability was reduced by only 0.9%.

Differential Expression of Genes During Heat Stress in *L. monocytogenes*: When the cells were exposed to: (i) 60°C heat shock conditions, 91 out of 6347 genes (~1.4%) were differentially expressed, 55 genes were up-regulated and 36 genes were down-regulated, (ii) 60°C for 9 minutes (prolonged heat shock), 80 out of 6347 (1.2%) were differentially expressed ($p \leq 0.05$) as compared to 37°C, 20 genes were up-regulated and 60 genes were down-regulated, (iii) thermo-tolerance inducing conditions (48°C for 30 minutes prior to 60°C for 9 minutes), 71 genes (1.1%) were differentially expressed, 17 genes were up-regulated and 54 genes were down-regulated. A majority (60%) of the differentially expressed genes were up-regulated at 60°C for 0 minute as compared to being down-

regulated when the cells were exposed to prolonged heat exposure and thermo-tolerance inducing conditions (75% and 76%, respectively). Only 10 genes were commonly expressed across the 3 different temperature treatments. There was only one gene, (coding for a hypothetical protein with an unknown function) which was commonly expressed between the prolonged heat exposure (60°C for 9 min) condition and the thermo-tolerance inducing (48°C 30 min followed by 60°C for 9 min) condition. However, this gene was up-regulated when exposed to sub-lethal temperature prior to heat exposure as compared to a down regulation when exposed to 60°C for 9 minutes directly. All the other 9 genes had similar expression patterns between the heat shock (60°C for 0 minute) and prolonged heat exposure condition.

Differential Expression of Protein During Heat Stress in *L. monocytogenes*: A high stringency cut-off was used to ensure that the protein identifications were accurate. Only those proteins that provided a CI (Confidence Interval) of >95% was assigned an identity. Proteins with a CI<95% were deemed "not identified". Out of 47 proteins that were differentially expressed (>±1.5-fold difference) under heat shock, prolonged heat shock and thermo-tolerance inducing conditions, 24 proteins could not be identified by the techniques employed in this study. Eighteen proteins were differentially expressed (6 proteins up-regulated and 12 proteins down-regulated) when cells were exposed to 60°C for 0 minute as compared to 37°C. Out of the 18 proteins that were differentially expressed, 6 proteins were unidentified. One of the proteins, Chaperonin GroEL (a heat shock protein) was down-regulated by as much as 4-fold. When the cells were exposed to 60°C for 9 minutes as compared to 37°C, 21 proteins (12 were up-regulated and 9 were down-regulated) were differentially expressed. One of the unidentified proteins (MW of 60.9) was up-regulated by as much as 6.8-fold. Under thermo-tolerance-inducing conditions, out of the 20 proteins were differentially expressed, 10 proteins were up-regulated and 10 were down-regulated as compared to proteins expressed at 37°C. One unidentified protein (MW 29.2) was up-regulated by as much as 12-fold. There was only one protein (MW of 50) that was up-regulated across all the three temperature treatments. No proteins were commonly expressed in cells exposed to heat shock at 60°C for 0 minute as compared to prolonged heat shock (60°C for 9 min). There was only one protein, tagatose 1,6-diphosphate aldolase (involved in carbohydrate transport and metabolism) that was up-regulated during both thermo-tolerance and heat shock. Eight different proteins (ranging in MW between 19 kDa and 62.5 kDa) were commonly expressed at both the thermo-tolerance inducing, and the prolonged heat stress conditions. However, none of these proteins were identifiable.

NEW SCIENTIFIC RESULTS

1. Combination of Modified Atmosphere Packaging (MAP) with 2 kGy of (γ) gamma irradiation reduces the natural bioburden of fresh alfalfa and radish sprouts to low levels, and improves microbiological safety and shelf-life. The D_{10}-value of *L. monocytogenes* 4ab strain on alfalfa sprouts was found to be between 0.46 kGy (when packaged in a gas mixture containing 3-5% oxygen, 10-15% carbon dioxide and balanced with nitrogen) and 0.58 kGy when packaged in an oxygen-carbon dioxide-nitrogen (2%-4%-94%) gas mixture.
2. Enhanced heat resistance was demonstrated in two *L. monocytogenes* strains (4ab and ATCC 43256) when they were exposed to sub-lethal heat conditions. The heat resistance of the strains was higher when exposed to 48°C for 30 minutes followed by 60°C treatment conditions as compared to a direct 60°C heat treatment. The D-value increased from 3.03 min to 6.72 min in case of *L. monocytogenes* 4ab, and from 3.74 minutes to 4.55 minutes in *L. monocytogenes* ATCC 43256.
3. This is the first report demonstrating that *L. monocytogenes* differentially expresses genes when exposed to sub-lethal heat conditions as compared to a direct heat stress. When the transcriptome of *L. monocytogenes* under optimal temperature (37°C) was used as base-line, heat shock (60°C for 0 minute) caused the up-regulation of 55 genes. Twenty (20) genes were up-regulated at prolonged heat shock (60°C for 9 minutes) conditions, and 17 genes were up-regulated under thermo-tolerance inducing (48°C for 30 minutes prior to exposure to 60°C for 9 minutes) conditions.
4. This study has demonstrated for the first time that sub-lethal temperature (48°C for 30 min) induces the over expression of unique heat stress-related proteins in *L. monocytogenes* ATCC 43256. Eighteen different proteins were differentially expressed at 60°C for 0 minute, 21 proteins were differentially expressed when the cells were exposed to 60°C for 9 minutes, and 20 proteins were differentially expressed when cells were initially exposed to 48°C for 30 minutes prior to 60°C for 9 minutes.
5. My dissertation has demonstrated that *L. monocytogenes* ATCC 43256 is capable of entering into a viable but non-culturable (VBNC) condition when exposed to sub-lethal temperature conditions. When the cells were exposed to 48°C for 30 minutes prior to

exposure for 9 minutes at 60°C, only 1% was viable (per culture methods) as compared to greater than 99% viability (based on a microscopic viability assay).

SUGGESTIONS

1. Further studies are necessary to optimize MAP conditions packaged fresh produce to prevent the potential growth of surviving pathogens such as *L. monocytogenes* during storage.
2. It is recommended that culture media for enumeration of *L. monocytogenes* in heat processed foods be carefully chosen to avoid obtaining misleadingly low counts arising from VBNC state microorganisms.
3. My results suggest that the current culture-plate based detection methods could be significantly underestimating the actual numbers of viable *Listeria* spp. in heat processed foods. More research needed to better understand the VBNC state of *L. monocytogenes* in foods and develop appropriate detection tools to detect cells in the VBNC state.
4. Additional research is needed to better understand the functional genomics and proteomics of *L. monocytogenes* so that different food processing techniques can be attributed to specific changes in pathogen function, virulence, or phenotype. A better understanding can help in designing effective hurdle technologies.
5. Predictive modeling to link microarray and proteomic studies should be pursued. Such as a systems biology based approach will greatly help improve our understanding of the ecology of *L. monocytogenes* during food processing.

LIST OF PUBLICATION

Journals

In journals with impact factor

Senses-Ergul, S., Ágoston, R., Belák, Á., Deák, T. (2006): Characterisation of some yeasts isolated from foods by traditional and molecular tests. *International Journal of Food Microbiology* 108, 120-124. (IF/2006: 2.608)

Friedrich, L., Siró, I., Dalmadi, I., Horváth, K., Ágoston, R. and Balla, Cs. (2007): Influence of various preservatives on the quality of minced beef under modified atmosphere at chilled storage. *Meat Science* 79 (2), 332-343. (accepted 13 October 2007) (Impact factor 2007: 2.006)

Ágoston, R., Soni, K. A., McElhany, K., Cepeda, M. L., Zuckerman, U., Tzipori, S., Mohácsi-Farkas, Cs., Pillai, S. D. (2009): Rapid concentration of *Bacillus* and *Clostridium* spores from large volumes of milk, using continuous flow centrifugation. *Journal of Food Protection* 72 (3), 666-668. (accepted 9 October 2008) (Impact factor 2008: 1.264)

Ágoston, R., Soni, K., Jesudhasan, P. R., Russell, W. K., Mohácsi-Farkas, Cs. and Pillai, S. D. (2009): Differential expression of proteins in *Listeria monocytogenes* under thermo tolerance-inducing, heat shock, and prolonged heat shock conditions. *Foodborne Pathogens and Disease*, In press (accepted 21 May 2009) (Impact factor 2008: 2.914)

Ágoston, R., Mohácsi-Farkas, Cs. and Pillai, S. D.: Exposure to sub-lethal temperatures induces enhanced heat resistance in *Listeria monocytogenes. Acta Alimentaria*, In press (accepted 25 May 2009) (Impact factor 2007: 0.398)

In journals without impact factor (articles in Hungarian)

Csóka M., Szabó S. A., Varga L., Ágoston R. és Mohácsiné Farkas Cs. (2007): Hosszú ideig tárolt, házi készítésű aszalványok vizsgálata. *Élelmiszervizsgálati közlemények, Élelmiszerminőség-Élelmiszerbiztonság*, LIII, 2, p. 79.; ISSN 0422-9576

Beczner J., Ágoston R., Cserhalmi Zs., Batáné Vidács I., Szekér K. (2008): *Alicyclobacillus acidoterrestris* II., A kezelések hatása a baktériumra. *Ásványvíz Üditőital Gyümölcslé Alkoholmentes Italok* IX (3), pp. 52-56.

Szabó S. A., Tolnay P., Mohácsiné Farkas Cs., Ágoston R. (2008): A CHIO-WOLF Magyarország Kft. burgonyachips termékeinek vizsgálata 7. rész. Csomagolás, jelölés és a mikrobiológiai jellemzők vizsgálata. *Élelmezési ipar*, 62. évf., 9. sz., pp. 273-275.

Conference proceedings

Hungarian (abstract)

Ágoston R., Beczner J., Cserhalmi Zs. (2004): Egyedi és kombinált kezelések hatása *Alicyclobacillus acidoterrestris* spórákra és vegetatív sejtekre. Keszthely, 2004. okt 7-9., A Magyar Mikrobiológiai Társaság 2004. évi Nagygyűlése és X. Fermentációs Kollokvium Előadás kivonatok, p. 2.

Ágoston R., Mohácsiné Farkas Cs., Kiskó G., Polyákné Fehér K. (2005): Lucernacsíra mikrobiológiai biztonságának növelése kombinált kezeléssel. Lippay János-Ormos Imre-Vas Károly Tudományos Ülésszak, 2005. október 19-20., Élelmiszertudományi Kar, Budapesti Corvinus Egyetem, Budapest, Összefoglalók, pp. 138-139.; ISBN 963 503 342 7

Ágoston R., Mohácsiné Farkas Cs. (2006): Hőstressz hatása *Listeria monocytogenes* 4ab hőpusztulására. Debrecen, 2006 március 29-31., EOQ MNB XV. Élelmiszer Minőségellenőrzési Tudományos Konferencia Konferenciakiadvány, p. 178.; ISBN: 963 229 636 2

Ágoston R., Mohácsiné Farkas Cs. (2007): Hőstressz *Listeria monocytogenes* 4 ab-re gyakorolt hatásának vizsgálata táplevesben és tejben. Lippay János-Ormos Imre-Vas Károly Tudományos Ülésszak, 2007. November 7-8., Élelmiszertudományi Kar, Budapesti Corvinus Egyetem, Budapest Összefoglalók, pp. 38-39.; ISBN 978 963 06 3350 5

Mohácsiné Farkas Cs., Farkas J., Ágoston R., Dalmadi I. (2008): DSC alkalmazása mikrobiológiai vizsgálatokban. EOQ MNB XVI. Élelmiszer minőségellenőrzési Tudományos Konferencia Kiadványa, Tihany 2008 ápr. 24-25., p. 289.

International Conferences (Abstract)

Beczner, J., Cserhalmi, Zs., Ágoston, R., Vidács, I., Szekér, K. (2004): Effect of combined treatments on spores of *Bacillus cereus* and *Alicyclobacillus acidoterrestris.* CEFood Congress, Budapest, April 26-28., 2004, Programme and Book of abstracts, p. 241.

Ágoston, R**.**, Mohácsi-Farkas, Cs., Kiskó, G., Dalmadi, I. (2005): Effects of combined treatments of MAP and irradiation on alfalfa sprouts. Abstracts of the 1st Central European Forum for Microbiology (CEFORM) and Annual Meeting of the Hungarian Society for Microbiology, Keszthely, October 26-28., 2005, Acta Microbiologica et Immunologica Hungarica 52, p. 2; ISSN 1217-8950

Ágoston, R., Mohácsi-Farkas, Cs., Dalmadi, I. (2006): Effect of high hidrostatic pressure (HHP) stress on the survival of *Listeria monocytogenes.* Keszthely, October 18-20., 2006, Abstracts of the Annual Meeting of the Hungarian Society for Microbiology, Acta Microbiologica et Immunologica Hungarica 53, 3, p. 238.; ISSN 1217-8950

Ágoston, R., Mohácsi-Farkas, Cs. (2006): Stress adaptive response of *Listeria monocytogenes* 4ab after heat and HHP treatment. Food Micro 2006, The 20th International ICFMH Symposium, Bologna, Italy, August 29-Sept 2., 2006., Abstracts, p. 113.

Ágoston, R., Mohácsi-Farkas, Cs. (2007): Modelling the effect of heat stress on survival of *Listeria monocytogenes* 4ab in tryptic soy broth and milk. Abstracts of the 15th International Congress of the Hungarian Society for Microbiology, Budapest, July 18-20., 2007, Acta Microbiologica et Immunologica Hungarica 54 (Suppl), p. 1.; ISSN 1217-8950

Ágoston, R., Soni, K., McElhany, K., Cepeda, M. L., Zuckerman, U., Tzipori, S. and Pillai, S. D. (2007): Continuous flow centrifugation (CFC) technology for pre-analytical sample processing to separate and concentrate bio-threat agents from large volumes of milk. Branch of the American Society for Microbiology, Annual Fall Meeting, Huntsville, Texas, November 15-17., 2007, p. 23.

Balla, Cs., Friedrich, L., Zeke, I., Horváth, K., Farkas, Cs., Ágoston, R. (2008): Temperature monitoring of refrigerated display cabinets in supermarkets in time and space with thermocamera and RFID technology. Proceedings of Cold Chain-Management, 3rd International Workshop, Bonn, June 2-3., 2008, pp. 298-303.

Ágoston, R., Soni, K., Jesudhasan, P., Russell, B., Mohácsi-Farkas, Cs., Pillai, S. D. (2009): Proteomic analysis of the differential expression of proteins in *Listeria monocytogenes* during 48°C heat shock and 60°C temperature exposure. The 21st International ICFMH Symposium, "Evolving microbial food quality and safety", Aberdeen, Scotland, Programme and Abstract book, Sept 1-4., 2008, p. 153.

Books, Book chapters, Textbooks

English

Mohácsi-Farkas, Cs., Farkas, J., Andrássy, É., Polyák-Fehér, K., Brückner, A., Kiskó, G., Ágoston, R. (2006): Improving the microbiological safety of some fresh pre-cut and pre-packaged chilled produce by low-dose gamma irradiation. In: Use of Irradiation to Ensure the Hygienic Quality of Fresh, Pre-Cut Fruits and Vegetables and Other Minimally Processed Food of Plant Origin; pp. 130-169., *IAEA TECDOC Series No. 1530,* ISBN 92-0-114006-1