Inactivation of *Cronobacter malonaticus* cells and inhibition of its biofilm formation exposed to hydrogen peroxide stress

Yingwang Ye,^{*†1,2} Maofeng Zhang,^{*2} Rui Jiao,^{*2} Na Ling,[†] Xiyan Zhang,^{*} Liaowang Tong,^{*} Haiyang Zeng,[†] Jumei Zhang,[†] and Qingping Wu^{†1}

^{*}School of Food Science and Engineering, Hefei University of Technology, Hefei 230009, China

[†]State Key Laboratory of Applied Microbiology Southern China, Guangdong Provincial Key Laboratory of Microbiology Culture Collection and Application, Guangdong Institute of Microbiology, Guangzhou 510070, China

ABSTRACT

Presence of *Cronobacter malonaticus* in powdered infant formula (PIF) poses a high risk to infant and public health. *Cronobacter malonaticus* has been widely distributed in food and food processing environments, and the true origin of *C. malonaticus* in PIF is poorly understood. Control and prevention of *C. malonaticus* is necessary for achieving microbial safety of PIF. However, little information about decontamination of *C. malonaticus* is available. In this study, effects of hydrogen peroxide on inactivation and morphological changes of *C. malonaticus* cells were determined. Furthermore, inhibitory effects of H₂O₂ on biofilm formation in *C. malonaticus* were also performed. Results indicated that H₂O₂ could completely inactivate *C. malonaticus* in sterile water with 0.06% H₂O₂ for 25 min, 0.08% H₂O₂ for 15 min, and 0.10% for 10 min, respectively, whereas the survival rates of *C. malonaticus* in tryptic soy broth medium significantly increased with the same treatment time and concentration of H₂O₂. In addition, morphological changes of *C. malonaticus* cells, including cell shrinkage, disruption of cells, cell intercession, and leakage of intercellular material in sterile water after H₂O₂ treatment, were more predominant than those in tryptic soy broth. Finally, significant reduction in biofilm formation by H₂O₂ was found using crystal violet staining, scanning electron microscopy, and confocal laser scanning microscopy detection compared with control samples. This is the first report to determine the effects of H₂O₂ on *C. malonaticus* cells and biofilm formation. The findings provided valuable information for practical application of H₂O₂ for decontamination of *C. malonaticus* in dairy processing.

Key words: hydrogen peroxide, *Cronobacter malonaticus*, biofilm formation

INTRODUCTION

Cronobacter species are considered to be occasional food-borne pathogens involved in neonatal meningitis, necrotizing enterocolitis, and sepsis (Beck-Sague et al., 1994; Badger et al., 1999; Blasig et al., 2001), and also isolated from various samples including food-processing plants, food, water, soil, and clinical samples (Gurtler et al., 2005; Ye et al., 2014). Genomic analysis indicated that *Cronobacter sakazakii*, *Cronobacter malonaticus*, and *Cronobacter turicensis* were associated with severe infections (Kucerova et al., 2010). The true origin of *C. malonaticus* in powdered infant formula is poorly understood, although *Cronobacter* (formerly known as *Enterobacter sakazakii*) strains in powdered infant formula might originate from food-processing environments, as described by Mullane et al. (2007).

Cross-contamination or persistent contamination of food-borne pathogens in food samples have been determined to be from bacterial biofilms on surfaces of food engineering plants and equipment (Srey et al., 2013; Cappitelli et al., 2014). In recent years, biofilm-forming abilities on surfaces in *Cronobacter* strains have been studied (Lee et al., 2012; Jung et al., 2013; Ye et al., 2015), and a capsule biofilm was considered to contribute to survival of *Cronobacter* strains under environmental stresses (Iversen and Forsythe, 2003). Therefore, controlling food-borne *C. malonaticus* strains and biofilm formation is extremely important to newborn and public health.

Hydrogen peroxide is a well-studied sanitizer for inactivate food-borne pathogens (Guan et al., 2013; Back et al., 2014) with bacteriostatic and bactericidal activity (Olmez and Kretzschmar, 2009). In addition, hydrogen peroxide is regarded as a safe disinfecting agent due to no harmful residue after treatment, and inactivation of H₂O₂ solutions on *Escherichia coli* O157, *Listeria monocytogenes*, and *Salmonella* have been studied (Sapers

Received July 9, 2017.

Accepted August 21, 2017.

¹Corresponding authors: yeyw04@mails.gucas.ac.cn and wuqp203@163.com

²These authors contributed to the manuscript equally.

et al., 2000; Huang and Chen, 2011; Choi et al., 2012; Guan et al., 2013; Back et al., 2014; Jiang et al., 2017). However, little work has focused on the effects of hydrogen peroxide on inactivation *C. malonaticus* cells and inhibition of biofilm formation.

In the present study, the effects of hydrogen peroxide on the survival and morphological injury of *C. malonaticus* cells were determined using plate counting method and scanning electron microscopy. Furthermore, inhibitory roles of hydrogen peroxide on biofilms formation in *C. malonaticus* were also performed using crystal violet staining (CVS), scanning electron microscopy, and confocal laser scanning microscopy (CLSM).

MATERIALS AND METHODS

Strains and Preparation of Hydrogen Peroxide

Cronobacter malonaticus GDMCC-11 is isolated from aquatic food samples and comes from Guangdong Microbiology Culture Center (GDMCC). Hydrogen peroxide (30%, Sinopharm Chemical Reagent Co. Ltd., Shanghai, China) were added into sterile tryptic soy broth (TSB; Huankai, Guangzhou, China) for preparation of 0 (control, vol/vol), 0.02, 0.04, 0.06, 0.08, and 0.10% H_2O_2 .

Inactivity of *C. malonaticus* Cells by Hydrogen Peroxide

Cronobacter malonaticus was inoculated into TSB for incubation at 37°C for 14 to 16 h. The *C. sakazakii* with 10^6 cfu/mL was then transferred into sterile water and TSB containing 0 (control, vol/vol), 0.02, 0.04%, 0.06, 0.08, and 0.10% H_2O_2 for incubation of 10, 20, and 30 min. Finally, the survival of *C. malonaticus* was counted using plating method and each experiment was done in triplicate.

Morphological Changes of *C. malonaticus* by H_2O_2 using Scanning Electron Microscopy

To determine the morphological changes of H_2O_2 treatment, 2 set of experiments were designed. In one set of experiments, *C. malonaticus* cells were inoculated into sterile TSB at 37°C, and then the enrichment culture (100 μ L, optical density (OD) at 600 nm = 0.5) was transferred into 5 mL of sterile TSB broth or sterile water containing 0, 0.02, 0.04, 0.06, 0.08, and 0.10% H_2O_2 for 15 min of treatment at 37°C. In another set of experiments, the enrichment culture (100 μ L, OD at 600 nm = 0.5) was transferred into 5 mL of sterile TSB broth or sterile water containing 0.6% H_2O_2 for treatment of about 5, 10, 15, 20, 25, and 30 min. The

cultures were then harvested, and pellets cells were used for gradient dehydration with ethanol described by Wang et al. (2013) with little modification. Finally, the treated cells by H_2O_2 were subject to scanning electron microscopy analysis (S8020, Hitachi, Tokyo, Japan).

Effects of H_2O_2 on Biofilm Formation in *C. malonaticus*

For CVS detection, *C. malonaticus* was inoculated into 5 mL of TSB and grown for 12 to 14 h at 37°C with constant shaking. Fifty microliters of culture (OD at 600 nm = 0.5) were inoculated into 96-well polystyrene plates containing 250 μ L of sterile Luria-Bertani broth with 0, 0.02, 0.04, 0.06, 0.08, and 0.10% H_2O_2 , and incubated at 37°C for 24, 48, and 72 h. The plates were rinsed 3 times with deionized water, and the adherent

Figure 1. Inactivation of *Cronobacter malonaticus* cells exposed to hydrogen peroxide with different concentrations in sterile water (A) and tryptic soy broth (TSB) medium (B). Survival rates: means $\pm$ SD.

bacteria cells were stained with 1% crystal violet for 30 min. After being rinsed 3 times with deionized water, the crystal violet was liberated by 30% acetic acid following a 10-min incubation. The OD values of each well were measured at 590 nm.

For biofilm formation detection by scanning electron microscopy, *C. malonaticus* was inoculated in sterile TSB broth at 37°C for 12 to 14 h, and 0.05 mL of the cultures were transferred to 24-wells plates (Baiyan, Shanghai, China) containing 5 mL of fresh TSB with 0 (control), 0.02, 0.04, and 0.06% H₂O₂. To test biofilm formation on glass, glass coverslips (Jingan, Shanghai, China) were immersed in TSB broth and then inoculated with *C. malonaticus*. The coverslips were incubated inside 24-well plates at 37°C for 24, 48, and 72 h, after which time bacterial biofilm formation was observed. During this biofilm-formation period, at 24 h-intervals old culture medium was replaced with fresh TSB. The glass coverslips with different incubation times were rinsed in PBS and fixed with 2.5% glutaraldehyde overnight at 4°C. Postfixation was carried out using 1% osmium tetroxide for 2 h before dehydration in an ethanol series (50, 70, 80, 90, and 100%; 30 min for each concentration). The dehydrated biofilms were coated with a thin layer of gold and examined under a

Hitachi SU1510 scanning electron microscope using an accelerating voltage of 5 kV.

To better visualize the biofilm architecture, *C. malonaticus* was inoculated in TSB at 37°C for overnight and 0.05 mL of the cultures were transferred to 24-well plates (Baiyan) containing 5 mL of sterile TSB with 0 (control), 0.02, 0.04, and 0.06% H₂O₂. To test biofilm formation on glass, glass coverslips (Jingan) were immersed in TSB broth and then inoculated with *C. malonaticus*. The coverslips were incubated inside 24-well plates at 37°C for 24, 48, and 72 h, after which time bacterial biofilm formation on glass slips was stained with LIVE/DEAD BacLight bacterial viability Kit (Invitrogen, Carlsbad, CA) and were subsequently observed by CLSM (Zeiss, Berlin, Germany).

RESULTS AND DISCUSSION

As one of the important species within the genus of *Cronobacter*, the control and prevention of *C. malonaticus* due to cross- or persistent-contamination is important for achieving microbial safety of powdered infant formula. In our study, with increasing amounts of H₂O₂ in sterile water, the survival rates of *C. malonaticus* cells significantly decreased ($P < 0.01$), as shown in

Figure 2. Morphological changes of *Cronobacter malonaticus* cells exposed to hydrogen peroxide with different concentrations in sterile water for 15 min. A = Control group (0% H₂O₂); B = 0.02% H₂O₂; C = 0.04% H₂O₂; D = 0.06% H₂O₂; E = 0.08% H₂O₂; F = 0.10% H₂O₂.

Figure 3. Morphological changes of *Cronobacter malonaticus* cells exposed to hydrogen peroxide with different concentrations in tryptic soy broth (TSB) medium for 15 min. A = Control group (0% H_2O_2); B = 0.02% H_2O_2 ; C = 0.04% H_2O_2 ; D = 0.06% H_2O_2 ; E = 0.08% H_2O_2 ; F = 0.10% H_2O_2 .

Figure 4. Morphological changes of *Cronobacter malonaticus* cells exposed to hydrogen peroxide with 0.06% H_2O_2 in sterile water. A = 5 min; B = 10 min; C = 15 min; D = 20 min; E = 25 min; F = 30 min.

Figure 1A. In addition, survival rates of *C. malonaticus* cells in sterile water with 0.02 and 0.04% H₂O₂ after 15 min of treatment tended to stabilize, and *C. malonaticus* cells were completely killed in sterile water with 0.06, 0.08, and 0.10% H₂O₂ for 25, 15, and 10 min, respectively. In Figure 1B, *C. malonaticus* in TSB medium was completely inactivated after treatment of 0.08% H₂O₂ for 25 min and 0.10% H₂O₂ for 20 min. In addition, H₂O₂ treatment at 0.06% could not inactivate *C. malonaticus* cells within 30 min, suggesting the TSB medium could significantly contribute ($P < 0.01$) to the increasing survival rates of *C. malonaticus* cells under the same concentration of H₂O₂ and treatment time. Consequently, nutrients played a protective role on *C. malonaticus* cells under treatment of H₂O₂, and organic contents in processing environments should be taken into consideration during control and prevention of *C. malonaticus*.

Aerosolized hydrogen peroxide-based sanitizer were used to reduce the levels of *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* Typhimurium on stainless steel surfaces, and the 3 pathogens were not detected after 60 min with 0.25% H₂O₂ (Choi et al., 2012). Abadias et al. (2011) found that a hydrogen peroxide-based sanitizer at a concentration of 20 mL/L for 5 min caused about 4 log reductions of *E. coli* O157:H7

and *Listeria* spp. Yeon et al. (2006) demonstrated that an aerosolized hydrogen peroxide-based sanitizer (1,100 mg/L) resulted in reductions of 3.5, 3.4, 3.0, and 2.8 log cfu/100 cm² of *E. coli*, *Staphylococcus aureus*, *Salmonella* Typhimurium, and *L. monocytogenes* in or on stainless steel, respectively. The aerosolized hydrogen peroxide-based sanitizer at 22.0% for 1 h was effective and resulted in an approximately 5 to 9 log reduction in *Salmonella* Typhimurium (Lee et al., 2007). Vaporized 10% hydrogen peroxide treatment for 10 min decreased levels of *Salmonella* Typhimurium, *E. coli* O157:H7, and *L. monocytogenes* on lettuce by 3.12, 3.15, and 2.95 log₁₀ cfu/g, respectively (Back et al., 2014). Treatment of lettuce with 2% H₂O₂ at 50°C for 2 min decreased *Escherichia coli* O157:H7 by 4 log (Lin et al., 2002); however, the effects of these sanitizers on inactivation of *E. coli* O157:H7 inoculated onto baby spinach are very limited (Lee and Baek, 2008). Treatment with 3% H₂O₂ and a combination of H₂O₂ and UV resulted in 0.77 and 0.87 log cfu/g reductions of *E. coli* O157:H7 inoculated on mushrooms (Guan et al., 2013). Jiang et al. (2017) demonstrated that efficacy of aerosolized hydrogen peroxide depended on type of inoculated bacteria, location of bacteria, and type of produce items, and aerosolized hydrogen peroxide could potentially be used to sanitize fresh fruits and vegetables.

Figure 5. Morphological changes of *Cronobacter malonaticus* cells exposed to hydrogen peroxide with 0.06% H₂O₂ in tryptic soy broth (TSB) medium. A = 5 min; B = 10 min; C = 15 min; D = 20 min; E = 25 min; F = 30 min.

Figure 6. Biofilm formation of *Cronobacter malonaticus* cells exposed to hydrogen peroxide. A = Quantitative analysis (mean $\pm$ SD) of biofilm formation using crystal violet staining; OD = optical density. B = Detection of biofilm formation under different H₂O₂ concentrations using scanning electron microscopy. C = Detection of biofilm formation under different H₂O₂ concentrations using confocal laser scanning microscopy. Color version available online. *Continued.*

Figure 6 (Continued). Biofilm formation of *Cronobacter malonaticus* cells exposed to hydrogen peroxide. A = Quantitative analysis (mean $\pm$ SD) of biofilm formation using crystal violet staining. OD = optical density. B = Detection of biofilm formation under different H_2O_2 concentrations using scanning electron microscopy. C = Detection of biofilm formation under different H_2O_2 concentrations using confocal laser scanning microscopy. Color version available online.

From Figure 2, morphological changes of *C. malonaticus* cells were also observed after treatment of H_2O_2 in sterile water. After treatment with 0.02 and 0.04%

H_2O_2 for 15 min, slight or weak cell shrinkage of *C. malonaticus* was observed, and significant injuries of *C. malonaticus*, including cell shrinkage, disruption of cell,

and leakage of intracellular material, were observed under treatment with 0.06, 0.08, and 0.10% H₂O₂. After treatment with H₂O₂ in TSB medium, injuries of *C. malonaticus* cells were slightly reduced compared with those in sterile water showed in Figure 3. Furthermore, with extension of treatment time by 0.06% H₂O₂ in sterile water (Figure 4) and TSB medium (Figure 5), injuries of *C. malonaticus*, such as cell shrinkage, cell disruption, and cell intercession, clearly increased. In addition, morphological injuries of cells were more predominant than those in TSB medium, which further confirmed protective roles of medium on *C. malonaticus* cells under H₂O₂ stress. In *E. coli*, exposure to low or high H₂O₂ concentrations resulted in morphological changes including cell filamentation, cell volume, and cell membrane damage, followed by loss of intercellular material (Brandi et al., 1989). In contrast, obvious filamentation of *C. malonaticus* cells was not observed using scanning electron microscopy detection in our study.

A hydrogen peroxide-based sanitizer at concentrations of 3 and 5% for 5 min led to reduction of the viable cells of >5 log cfu/mL on biofilms of *Staphylococcus epidermidis* (Presterl et al., 2007). The attachment of food-borne pathogens to biotic and abiotic surfaces, followed by biofilm formation, might be the critical factor for persistent-contamination of microorganism in food samples (Choi et al., 2012). In our study, the inhibitory effects of H₂O₂ on biofilm formation in *C. malonaticus* were studied for the first time. The highest biomass in *C. malonaticus* was formed after 48 h of incubation at 37°C by CVS in Figure 6A. The biofilm formation was significantly decreased with increasing concentration of H₂O₂ in medium, and we noted significant inhibition of biofilm formation of *C. malonaticus* in TSB containing 0.02, 0.04, and 0.06% H₂O₂ compared with control samples by the CVS method. In addition, *C. malonaticus* could hardly form biofilms in the TSB containing 0.08 and 0.10% H₂O₂.

Furthermore, biofilm formation of *C. malonaticus* in TSB medium with 0, 0.02, 0.04, and 0.06% H₂O₂ was detected using scanning electron microscopy in Figure 6B. We found that biofilm formation of *C. malonaticus* was significantly inhibited with increasing concentrations of H₂O₂, and incompact biofilms were observed in TSB medium with 0.06% H₂O₂. To date, little research has focused on control of biofilm formation in *C. malonaticus*. Our previous study indicated that D-Tryptophan significantly inhibits formation of mature biofilm of *C. malonaticus* and contributes to disassembly of existing biofilms (Li et al., 2015). After 72 h of incubation time, the percentage of dead cells (red or yellow) increased in all samples using CLSM detection. From Figure 6C, compared with control group, the amount of viable

cells and dead or injured cells increased. In addition, incompact structure of biofilm after treatment of H₂O₂ was also confirmed by CLSM; however, 0.06% H₂O₂ could not completely inhibit the biofilm formation and inactivate *C. malonaticus* cell in TSB medium, suggesting a combination of 2 or more sanitizers is required for removal of *C. malonaticus* strain and biofilms.

In summary, treatment of hydrogen peroxide with 0.08 and 0.10% H₂O₂ in sterile water and TSB within 25 min could completely inactivate *C. malonaticus* cells. In addition, medium played a protective role for *C. malonaticus* cells under H₂O₂ stress. With the increasing of H₂O₂ concentrations and treatment time, injuries of *C. malonaticus* cells were more predominant compared with the control groups. Furthermore, significant inhibition of biofilm formation in *C. malonaticus* by hydrogen peroxide was observed compared with the control groups for the first time. In addition, inhibitory effects of hydrogen peroxide on biofilm formation were also confirmed through detection of structural changes by scanning electron microscopy and CLSM. To achieve microbial safety of powdered infant formula from contamination with *C. malonaticus*, a combination of 2 or more sanitizers is recommended.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial support of the National Natural Science Foundation of China (31671951), the Anhui provincial Grand Project special of Science and Technology (15czz03109), Science and Technology Planning Project of Guangdong Province China (2016A050502033) Project of Science and Technology of Guangzhou, Guangdong Province, China (201604020036), and State Key Laboratory of Applied Microbiology Southern China Open Foundations, Guangzhou, China (SKLAM004-2015).

REFERENCES

- Abadias, M., I. Alegre, J. Usall, R. Torres, and I. Vinas. 2011. Evaluation of alternative sanitizers to chlorine disinfection for reducing foodborne pathogens in fresh cut apple. *Postharvest Biol. Technol.* 59:289–297.
- Back, K. H., J. W. Ha, and D. H. Kang. 2014. Effect of hydrogen peroxide vapor treatment for inactivating *Salmonella* Typhimurium, *Escherichia coli* O157:H7 and *Listeria monocytogenes* on organic fresh lettuce. *Food Contr.* 44:78–85.
- Badger, J. L., M. F. Stins, and K. S. Kim. 1999. *Citrobacter freundii* invades and replicates in human brain microvascular endothelial cells. *Infect. Immun.* 67:4208–4215.
- Beck-Sague, C. M., P. Azimi, S. Fonseca, R. Baltimore, D. Powell, L. Bland, M. Ardino, S. McAllister, R. Huberman, and R. Sinkowitz. 1994. Bloodstream infections in neonatal intensive care unit patients: Results of a multicenter study. *Pediatr. Infect. Dis. J.* 13:1110–1116.
- Blasig, I. E., H. Giese, M. L. Schroeter, A. Sporberr, D. I. Uteperger, I. B. Buchwalow, K. Neubert, G. Schonfelder, D. Freyer,

- I. Schimke, W. E. Siems, M. Paul, R. F. Haseloff, and R. Blasig. 2001. NO and oxyradical metabolism in new cell lines of rat brain capillary endothelial cells forming the blood-brain barrier. *Microvasc. Res.* 62:114–127.
- Brandi, G., M. Fiorani, C. Pierotti, A. Albano, F. Cattabeni, and O. Cantoni. 1989. Morphological changes in *Escherichia coli* cells exposed to low or high concentrations of hydrogen peroxide. *Microbiol. Immunol.* 33:991–1000.
- Cappitelli, F., A. Polo, and F. Villa. 2014. Biofilm formation in food processing environments is still poorly understood and controlled. *Food Eng. Rev.* 6:29–42.
- Choi, N. Y., S. Y. Baek, J. H. Yoon, M. R. Choi, D. H. Kang, and S. Y. Lee. 2012. Efficacy of aerosolized hydrogen peroxide-based sanitizer on the reduction of pathogenic bacteria on a stainless steel surface. *Food Contr.* 27:57–63.
- Guan, W., X. Fan, and R. Yan. 2013. Effect of combination of ultraviolet light and hydrogen peroxide on inactivation of *Escherichia coli* O157:H7, native microbial loads, and quality of button mushrooms. *Food Contr.* 34:554–559.
- Gurtler, J. B., J. L. Kornacki, and L. R. Beuchat. 2005. *Enterobacter sakazakii*: A coliform of increased concern to infant health. *Int. J. Food Microbiol.* 104:1–34.
- Huang, Y., and H. Chen. 2011. Effect of organic acids, hydrogen peroxide and mild heat on inactivation of *Escherichia coli* O157: H7 on baby spinach. *Food Contr.* 22:1178–1183.
- Iversen, C., and S. Forsythe. 2003. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. *Trends Food Sci. Technol.* 14:443–454.
- Jiang, Y., K. Sokorai, G. Pyrgiotakis, P. Demokritou, X. H. Li, S. Mukhopadhyay, T. Jin, and X. T. Fan. 2017. Cold plasma-activated hydrogen peroxide aerosol inactivates *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Listeria innocua* and maintains quality of grape tomato, spinach and cantaloupe. *Int. J. Food Microbiol.* 249:53–60.
- Jung, J.-H., N.-Y. Choi, and S.-Y. Lee. 2013. Biofilm formation and exopolysaccharide (EPS) production by *Cronobacter sakazakii* depending on environmental conditions. *Food Microbiol.* 34:70–80.
- Kucerova, E., S. W. Clifton, X. Q. Xia, F. Long, S. Porwollik, L. Fulton, C. Fronick, P. Minx, K. Kyung, W. Warren, R. Fulton, D. Feng, A. Wollam, N. Shah, V. Bhonagiri, W. E. Nash, K. Hallworth-Pepin, R. K. Wilson, M. McClelland, and S. J. Forsythe. 2010. Genome sequence of *Cronobacter sakazakii* BAA-894 and comparative genomic hybridization analysis with other *Cronobacter* species. *PLoS One* 5:e9556.
- Lee, S. Y., and S. Y. Baek. 2008. Effect of chemical sanitizer combined with modified atmosphere packaging on inhibiting *Escherichia coli* O157:H7 in commercial spinach. *Food Microbiol.* 25:582–587.
- Lee, S. Y., J. H. Jung, H. H. Jin, Y. H. Kim, and S. W. Oh. 2007. Inhibitory effect of aerosolized commercial sanitizers against food-borne pathogens. *J. Food Hygiene Saf.* 22:235–242.
- Lee, Y.-D., J.-H. Park, and H. Chang. 2012. Detection, antibiotic susceptibility and biofilm formation of *Cronobacter* spp. from various foods in Korea. *Food Contr.* 24:225–230.
- Li, H., Y. W. Ye, N. Ling, Q. P. Wu, and J. M. Zhang. 2015. Inhibitory effects of D-tryptophan on biofilm development by the foodborne *Cronobacter sakazakii*. *Int. Dairy J.* 49:125–129.
- Lin, C. M., S. S. Moon, M. P. Doyle, and K. M. McWatters. 2002. Inactivation of *Escherichia coli* O157:H7, *Salmonella enterica* serotype Enteritidis, and *Listeria monocytogenes* on lettuce by hydrogen peroxide and lactic acid and by hydrogen peroxide with mild heat. *J. Food Prot.* 65:1215–1220.
- Mullane, N. R., P. Whyte, P. G. Wall, T. Quinn, and S. Fanning. 2007. Application of pulsed-field gel electrophoresis to characterize and trace the prevalence of *Enterobacter sakazakii* in an infant formula processing facility. *Int. J. Food Microbiol.* 116:73–81.
- Olmez, H., and U. Kretzschmar. 2009. Potential alternative disinfection methods for organic fresh-cut industry for minimizing water consumption and environmental impact. *LWT Food Sci. Technol.* 42:686–693.
- Presterl, E., M. Suchomel, M. Eder, S. Reichmann, A. Lassnigg, W. Graninger, and M. Rotter. 2007. Effects of alcohols, povidone-iodine and hydrogen peroxide on biofilms of *Staphylococcus epidermidis*. *J. Antimicrob. Chemother.* 60:417–420.
- Sapers, G. M., R. L. Miller, M. Jantschke, and A. M. Matrazzo. 2000. Factors limiting the efficacy of hydrogen peroxide washes for decontamination of apples containing *Escherichia coli*. *J. Food Sci.* 65:529–532.
- Srey, S., I. K. Jahid, and S.-D. Ha. 2013. Biofilm formation in food industries: A food safety concern. *Food Contr.* 31:572–585.
- Wang, C. Y., C. P. Hsu, H. W. Huang, and B. B. Yang. 2013. The relationship between inactivation and morphological damage of *Salmonella enterica* treated by high hydrostatic pressure. *Food Res. Int.* 54:1482–1487.
- Ye, Y. W., H. Li, Q. P. Wu, J. M. Zhang, and Y. D. Lu. 2014. The *Cronobacter* sp. in milk and dairy products: Detection and typing. *Int. J. Dairy Technol.* 67:167–175.
- Ye, Y. W., N. Ling, R. Jiao, Q. P. Wu, Y. J. Han, and J. N. Gao. 2015. Effects of culture conditions on the biofilm formation of *Cronobacter sakazakii* strains and distribution of genes involved in biofilm formation. *LWT Food Sci. Technol.* 62:1–6.
- Yeon, J. H., I. J. Kim, K. H. Park, B. K. Park, H. K. Park, D. W. Park, Y. S. Kim, H. I. Kim, D. H. Jeon, Y. J. Lee, and S. D. Ha. 2006. Treatment and effect of sanitizers and disinfections in animal food manufacturing plant. *Korean J. Food Sci. Technol.* 38:599–603.