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Effects of Lactic Acid and Chitosan on the Survival of *V. parahaemolyticus* in Mussel Samples

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Abstract: Lactic acid and chitosan were evaluated for their effects on the growth and survival of *V. parahaemolyticus* on mussel meat. Mussel (*Mytilus* sp.) samples were collected from the Samsun region on the middle Black Sea coast of Turkey. Each shelled mussel was decontaminated by immersion in 4% formalin for 3 min. The decontaminated mussel was dipped in TSB broth containing between 8.25 and 7.60 log CFU g^{-1} of *V. parahaemolyticus* and left for 30 min at room temperature 25°C to allow attachment. Initial counts of *V. parahaemolyticus* in mussel meat immediately after dipping in TSB broth were in the range of 5.38-4.03 log CFU g^{-1}. Each inoculated mussel (25°C) was dipped in 0.5, 1, 1.5 or 2% of lactic acid (v/v) or 0.05, 0.1, 0.25 or 0.5% of chitosan (v/v) for 5, 15, 30 or 60 min. Initial counts of *V. parahaemolyticus* in mussel meat decreased following treatment with lactic acid for 5 min by 1.90, 2.13, 2.27 and 2.78 log CFU g^{-1}, respectively and following treatment with chitosan for 5 min by 1.33, 1.41, 1.56 and >2.03 log CFU g^{-1}, respectively. Growth of *V. parahaemolyticus* on mussels was completely inhibited after being dipped in 1.5-2% lactic acid for 15 min and 0.5% chitosan for 5 min.

Key words: Decontamination, chitosan, lactic acid, mussel, halpphilic marine bacterium

INTRODUCTION

Vibrio parahaemolyticus, a halophilic marine bacterium is a worldwide cause of food-borne gastroenteritis (Janda *et al*., 1988). Mussels are filter feeding bivalve molluscs that can concentrate bacteria in contaminated water. *V. parahaemolyticus* can be a risk factor, when these products are eaten raw or slightly cooked. It was first identified as a foodborne pathogen in Japan in 1950 (Fujino *et al*., 1953) and in 1977, *V. parahaemolyticus* caused the largest reported outbreak associated with eating raw oysters involving 209 persons in North America (CDC, 1998). *V. parahaemolyticus* has been successfully isolated by some researchers from fish (Dileep *et al*., 2003), shrimp (Robert-Pillot *et al*., 2004), mussels (Di Pinto *et al*., 2008; Martinez-Urtaza *et al*., 2008; Terzi *et al*., 2009) and oysters (Bej *et al*., 1999; Lee *et al*., 2008).

Many methods have been developed to prolong the shelf life of fishery products, such as washing, storage at low temperatures, cold shock, freezing, ultraviolet irradiation, salt treatment and decontamination using chitosan, chlorine, organic acids, ozone and chloroform (Chythanya *et al*., 2002; Manousaridis *et al*., 2005; Chaiyakosa *et al*., 2007; Masniyom and Benjama, 2007).

Lactic acid is one of the most widely used among the organic acids for decontamination of meat. Lactic acid inhibits the growth of microorganisms such as gram negative species of the families *Enterobacteriaceae* and *Pseudomonadaceae* (Anang *et al*., 2007).

The other decontaminant that is used in seafood to reduce the bacterial count is chitosan. Chitosan is a b-1, 4 linked N-acetyl-d-glucosamine polymer. It is derived by deacetylation of chitin, a major component of the shells of crustacea such as crab, shrimp and crawfish (Arvanitoyannis *et al*., 1998; No *et al*., 2002). Chitosan has been shown to have antibacterial activities against the gram-positive bacteria (*Listeria monocytogenes*, *Bacillus megaterium*, *B. cereus*, *Staphylococcus aureus*, *Lactobacillus plantarum*, *L. brevis*, *L. Bulgaris*) and gram negative bacteria (*E. coli, Pseudomonas fluorescens, Salmonella typhymurium, Vibrio parahaemolyticus*) (Chen *et al*., 2002; Liu *et al*., 2004; No *et al*., 2002). The antimicrobial activity of chitosan is reported to be dependent on its degree of deacetylation, molecular weight, concentration and viscosity (Mohy *et al*., 2008; No *et al*., 2002; Jeon *et al*., 2001).

The objective of this study was to investigate the effect of chitosan and lactic acid on the numbers of *V. parahaemolyticus* in mussels.

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MATERIALS AND METHODS

Bacterial strains: *V. parahaemolyticus* ATCC 17802 was grown in Tryptic Soy Broth (TSB, Merck, Darmstadt, Germany) (with 2% NaCl) at 37°C for 24 h and then adjusted to 1.6×10^8 CFU mL^{-1} with tube dilution methods.

Mussel samples: A total of 200 mussel (*Mytilus* sp.) samples were collected from the Samsun region on the middle Black Sea coast of Turkey, in September 2009. All samples were transported to the laboratory in containers with ice bags for analysis within a few hours. The samples were washed in sterile distilled water and disinfected with alcohol and then opened aseptically.

Artificial contamination of mussel samples with *V. parahaemolyticus*: Twenty five grams of shelled mussel were decontaminated by immersion in 4% formalin for 3 min and washed twice with 1% NaCl to remove any remaining formalin. The decontaminated mussel was dipped in 100 mL Tryptic Soy Broth (TSB, Merck, Darmstadt, Germany) containing 1.6×10^8 CFU mL^{-1} of *V. parahaemolyticus* and left for 30 min at room temperature (25°C) to allow attachment.

Decontamination with lactic acid or chitosan: Each contaminated mussel was dipped in 100 mL of either a 0.5, 1, 1.5 or 2% solution of lactic acid (v/v) (Merck, Darmstadt, Germany) or low molecular weight chitosan (v/v) (Aldrich-44.886-9, Milwaukee, WI, USA) (MW = 150 kDa, 75-85% deacetylation, viscosity 20-200 cps), at a concentration of 0.05, 0.1, 0.25 or 0.5% for 5, 15, 30 or 60 min. Each inoculated mussel was also dipped in 100 mL of sterile distilled water (2% NaCl) as a control. After dipping, the mussel samples were examined for *V. parahaemolyticus*.

Microbiological analysis: To determine the *V. parahaemolyticus* count, 25 g mussel samples were transferred aseptically to a stomacher bag containing 225 mL of alkali peptone water (APW; 1% tryptone peptone, 2% NaCl, pH 8.6) and homogenized for 2 min using a stomacher (Interscience-BagMixer 400, St. Nom., France). For microbial enumeration 0.1 mL samples of serial dilutions (1:10, diluent, alkali peptone water with 2% NaCl) of mussel homogenates were spread on the surface of thiosulphate citrate bile salt sucrose agar (TCBS; Merck, Darmstadt, Germany) and the TCBS plates were incubated at 37°C for 24 h. The formation of colonies that are round (2-3 mm diameter) and green on TCBS was considered positive for *V. parahaemolyticus* and microbial counts were expressed as CFU mL^{-1} (ISO 8914, 1990).

Statistical analysis: Statistical analysis was carried out using the Statistical Package for Social Science (SPSS 10.0 for Windows, SPSS Inc., Chicago, IL). All experiments were replicated three times. The results were evaluated by using one-way ANOVA and the level of significance was set at $p<0.05$.

RESULTS AND DISCUSSION

Table 1 shows the *V. parahaemolyticus* counts on mussel meat treated with different concentrations of lactic acid and chitosan. The initial count of *V. parahaemolyticus* on mussel meat after inoculation ranged from 5.38-4.03 logCFU g^{-1}. *V. parahaemolyticus* counts on mussels dipped in 0.5-2% lactic acid for 5 and 15 min were significantly lower ($p<0.05$). Morover, the growth of *V. parahaemolyticus* on mussels was completely inhibited after dipping in 0.5-1% lactic acid for 30 min and 1.5-2% lactic acid for 15 min.

The most widely used chemical decontaminants in the meat industry are organic acids (Belk, 2001). The antimicrobial activity of lactic acid has been demonstrated in a number of foods including chicken, meat and seafood (Ramirez *et al*., 2001; Masniyom and Benjama, 2007; Lin *et al*., 2005; Anang *et al*., 2007; McMullin and Steele, 2007). Masniyom and Benjamae (2007) found

Table 1: *V. parahaemolyticus* counts on mussel meat dipped in different concentrations of chitosan and lactic acid

No. of *V. parahaemolyticus* in TSB broth (logCFU g^{-1}	No. of *V. parahaemolyticus* on mussels (logCFU g^{-1})	Concentration	No. of viable cells (logCFU g^{-1}) Duration of decontamination (min) 5	15	30	60
Lactic acid						
8.25	5.38	0.5%	3.47^{a}	3.00^{a}	ND	ND
		1%	3.25^{ab}	2.00^{b}	ND	ND
		1.5%	3.11^{b}	ND	ND	ND
		2%	2.60^{c}	ND	ND	ND
Chitosan						
7.60	4.03	0.05%	2.70^{a}	ND	ND	ND
		0.1%	2.62^{ab}	ND	ND	ND
		0.25%	2.47^{b}	ND	ND	ND
		0.5%	ND	ND	ND	ND

Means in the same row followed by different letters are significantly different ($p<0.05$), $^{a-c}$Show number of *V. parahaemolyticus* differences duration of decontamination ($p<0.05$), Tukey test, ND: Not Detected (detection limit<log2.0)

Table 2: Log reduction in *V. parahaemolyticus* inoculated onto mussel meat dipped in different concentrations of chitosan and lactic acid

	Log CFU g reduction^{-1} *V. parahaemolyticus* duration of decontamination (min)			
Dipping solution	5	15	30	60
Lactic acid (%)				
0.5	1.91	2.38	>3.38	>3.38
1	2.13	3.38	>3.38	>3.38
1.5	2.27	>3.38	>3.38	>3.38
2	2.78	>3.38	>3.38	>3.38
Chitosan (%)				
0.05	1.33	>2.03	>2.03	>2.03
0.1	1.41	>2.03	>2.03	>2.03
0.25	1.56	>2.03	>2.03	>2.03
0.5	>2.03	>2.03	>2.03	>2.03

that mesophilic bacterial counts in green mussels declined from 3.63-2.36 logCFU g^{-1}, when treated with 0.2 M lactic acid. Sorrells *et al.* (1989) reported that *V. parahaemolyticus* was more acid tolerant than other food borne pathogens. In the present study, *V. parahaemolyticus* counts in mussels declined from 5.38 -3.47 logCFU g^{-1}, when treated with 0.5% lactic acid for 5 min. Moreover, when treated with 2% lactic acid for 5 min *V. parahaemolyticus* counts declined from 5.38-2.60 logCFU g^{-1}. The results indicate that the inhibition of *V. parahaemolyticus* is related to the concentration of lactic acid and a dipping time period. We observed that *V. parahaemolyticus* counts declined and even it inhibited completely, when increasing the concentration and also extended the dipping time of lactic acid. Table 2 presents the log reduction in numbers of *V. parahaemolyticus* on mussel meat dipped in different concentrations of chitosan and lactic acid. The maximum decrease in the initial population of *V. parahaemolyticus* on mussel meat treated with lactic acid ranged between 2.00 and >3.38 logCFU g^{-1}, from initial counts of 5.38 logCFU g^{-1}. The antimicrobial effects of the use of lactic acid have been reported in other studies, with a reduction of 1-2 log (Ransom *et al*., 2003).

Xiong *et al.* (1998) observed a 2.2 log CFU cm^{-2} decrease in Salmonella typhimurium following the use of thorough spraying of 1-2% lactic acid solution on chicken skin. In another study, Ramirez *et al.* (2001) reported a 1.6 logCFU cm^{-2} reduction in *Escherichia coli* after spraying lamb meat with 2% lactic acid for 9 sec. Ransom *et al.* (2003) reported a 3.3 log CFU cm^{-2} decrease in *Escherichia coli* 0157:H7 after dipping beef carcasses in 2% lactic acid solution. Anang *et al.* (2007) found a decrease in the initial population of *L. monocytogenes*, *S. enteritidis* and *E. coli* O157:H7 of 1.97, 1.71 and 2.59 logCFU g^{-1}, respectively, after dipping chicken breast in 0.5, 1, 1.5 and 2% lactic acid for 10, 20 and 30. The antibacterial effects of chitosan depend on its molecular weight, viscosity and the type of bacterium. It has been reported that chitosan generally shows stronger antibacterial effects against gram positive bacteria than gram negative bacteria (No *et al*., 2002; Jeon *et al*., 2001). Liu *et al.* (2006) reported that the antibacterial effect of low MW chitosan is higher than that of high MW samples. In this study, we used low MW chitosan (150 kDa) with 75-85% deacetylation. The activity of chitosan against *Vibrio* sp. has been reported by other researchers (Chaiyakosa *et al*., 2007; Wang and Chen, 2005). In a study conducted by No *et al.* (2002), different chitosans with a MW in the range of 28-1671 kDa were used to determine the effect of chitosan MW on the inhibition of *V. parahaemolyticus*. They found that *V. parahaemolyticus* counts declined from 6.64-3.29 logCFU g^{-1}, when treated with 470 kDa MW chitosan. They observed a 2.56 logCFU g^{-1} reduction in *V. parahaemolyticus*.

In the present study, *V. parahaemolyticus* counts declined from 4.03-2.70 logCFU g^{-1}, when treated with 150 kDa MW chitosan at a concentration of 0.05% for 5 min. The reduction in *V. parahaemolyticus* was 1.33 logCFU g^{-1}.

On the other hand *V. parahaemolyticus* was not detected after treatment with chitosan at a concentration of 0.05% and contact time of 15 min. Chaiyakosa *et al.* (2007) reported that in artificially inoculated shrimp, a >90% reduction in *V. parahaemolyticus* was observed, when the contact time was increased to 30 min and the concentration of chitosan was 1000 ppm (0.1%) and 2000 ppm (0.2%). Similary, in the present study, we found a >90% reduction in *V. parahaemolyticus*, when 0.05-0.5% chitosan was used for 15 min.

CONCLUSION

This study has demonstrated that *V. parahaemolyticus* counts decline by between 1.91 and >3.38 logCFU g^{-1} after dipping in 0.5-2% lactic acid and between 1.33 and >2.03 logCFU g^{-1} after dipping in 0.05-0.5% chitosan. The decontamination of mussel meat by lactic acid and chitosan significantly reduced *V. parahaemolyticus* counts.

REFERENCES

Anang, D.M., G. Rusul, J. Bakar and F.H. Ling, 2007. Effects of lactic acid and lauricidin on the survival of *Listeria monocytogenes Salmonella enteritidis* and *Escherichia coli* O157: H7 in chicken breast stored at 4°C. Food Control, 18: 961-969.

Arvanitoyannis, I.S., A. Nakayama and S. Aiba, 1998. Chitosan and gelatin based edible films: State diagrams, mechanical and permeation properties. Carbohydrate Polym., 37: 371-382.

Bej, A.K., D.P. Patterson, C.W. Brasher, M.C. Vickery, D.D. Jones and C.A. Kaysner, 1999. Detection of total and hemolysin-producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of *tl*, *tdh* and *trh*. J. Microbiol. Methods, 36: 215-225.

Belk, K.E., 2001. Beef decontamination technologies. Beef facts centennial, CO, National Cattlemen's Beef Association, Research and Technical Services. http://www.beefresearch.org/CMDocs/BeefResearch/ACFFC.pdf.

CDC, 1998. Outbreak of *Vibrio parahaemolyticus* infections associated with eating raw oysters-Pacific Northwest, 1997. MMWR Morb. Mortal. Wkly Rep., 47: 457-462.

Chaiyakosa, S., W. Charernjiratragul, K. Umsakul and V. Vuddhakul, 2007. Comparing the efficiency of chitosan with chlorine for reducing *Vibrio parahaemolyticus* in shrimp. Food Control, 18: 1031-1035.

Chen, Y.M., Y.C. Chung, L.W. Wang, K.T. Chen and S.Y. Li, 2002. Antibacterial properties of chitosan in waterborne pathogen. J. Environ. Sci. Health A., 37: 1379-1390.

Chythanya, R., I. Karunasagar and I. Karunasagar, 2002. Inhibition of shrimp pathogenic vibrios by a marine *Pseudomonas* I-2 strain. Aquaculture, 208: 1-10.

Di Pinto, A., G. Ciccarese, R. de Corato, L. Novello and V. Terio, 2008. Detection of pathogenic *Vibrio parahaemolyticus* in Southern Italian shellfish. Food Control, 19: 1037-1041.

Dileep, V., H.S. Kumar, Y. Kumar, M. Nishibuchi, I. Karunasagar and I. Karunasagar, 2003. Application of polymerase chain reaction for detection of *Vibrio parahaemolyticus* associated with tropical seafoods and coastal environment. Lett. Appl. Microbiol., 36: 423-427.

Fujino, T., Y. Okuno, D. Nakada, A. Aoyama, K. Fukai, T. Mukai and T. Uebo, 1953. On the bacteriological examination of Shirasu food poisoning. Med. J. Osaka. Univ., 4: 299-304.

ISO 8914, 1990. Microbiology-General Guidance on Methods for the Detection of *Vibrio parahaemolyticus*. ISO, Geneva, Switzerland.

Janda, J.M., C. Powers, R.G. Bryant and S.L. Abbott, 1988. Current perspectives on the epidemiology and pathogenesis of clinically significant *Vibrio* sp. Clin. Microbiol. Rev., 1: 245-267.

Jeon, Y.J., P.J. Park and S.K. Kim, 2001. Antimicrobial effect of chitooligosaccharides produced by bioreactor. Carbohydr. Polymers, 44: 71-76.

Lee, J.K., D.W. Jung, S.Y. Eom, S.W. Oh, Y. Kim, H.S. Kwak and Y.H. Kim, 2008. Occurrence of *Vibrio parahaemolyticus* in oysters from Korean retail outlets. Food Control, 19: 990-994.

Lin, Y.T., R.G. Labbe and K. Shetty, 2005. Inhibition of *Vibrio parahaemolyticus* in seafood systems using oregano and cranberry phytochemical synergies and lactic acid. Innov. Food Sci. Emerg., 6: 453-458.

Liu, H., Y. Du, X. Wang and L. Sun, 2004. Chitosan kills bacteria through cell membrane damage. Int. J. Food Microbiol., 95: 147-155.

Liu, N., X.G. Chen, H.J. Park, C.G. Liu, C.S. Liu, X.H. Meng and L.J. Yu, 2006. Effect of MW and concentration of chitosan on antibacterial activity of *Escherichia coli*. Carbohydr. Polymers, 64: 60-65.

Manousaridis, G., A. Nerantzaki, E.K. Paleologos, A. Tsiotsias, I.N. Savvaidis and M.G. Kontominas, 2005. Effect of ozone on microbial, chemical and sensory attributes of shucked mussels. Food Microbiol., 22: 1-9.

Martinez-Urtaza, J., A. Lozano-Leon, J. Varela-Pet, J. Trinanes, Y. Pazos and O. Garcia-Martin, 2008. Environmental determinants of the occurrence and distribution of *Vibrio parahaemolyticus* in the Rias of Galicia, Spain. Applied Environ. Microbiol., 74: 265-274.

Masniyom, P. and O. Benjama, 2007. Effect of lactic, acetic and citric acids on quality changes of refrigerated green mussel, *Perna viridis* (Linnaeus, 1758). Songklanakarin J. Sci. Technol., 29: 1123-1134.

McMullin, D.Q. and F.M. Steele, 2007. The examination of surface contamination on beef carcasses during slaughter and aging in a small-scale meat packaging operation equipped with an organic acid carcass washer. J. Anim. Vet. Adv., 6: 927-931.

Mohy, E.M.S., E.A. Soliman, A.I. Hashem and T.M. Tamer, 2008. Antibacterial activity of chitosan chemically modified with new technique. Trends Biomater. Artificial Organs, 22: 125-137.

No, H.K., N.Y. Park, S.H. Lee and S.P. Meyers, 2002. Antibacterial activity of chitosans and chitosan oligomers with different molecular weights. Int. J. Food Microbiol., 74: 65-72.

Ramirez, A.J., G.R. Acuff, L.M. Lucia and J.W. Savell, 2001. Lactic acid and trisodium phosphate treatment of lamb breast to reduce bacterial contamination. J. Food Prot., 64: 1439-1441.

Ransom, J.R., K.E. Belk, J.N. Sofos, J.D. Stopforth, J.A. Scanga and G.C. Smith, 2003. Comparison of intervention technologies for reducing *Escherichia coli* O157: H7 on beef cuts and trimmings. Food Prot. Trends, 23: 24-34.

Robert-Pillot, A., A. Guénolé, J. Lesne, R. Delesmont, J.M. Fournier and M.L. Quilici, 2004. Occurrence of the *tdh* and *trh* genes in *Vibrio parahaemolyticus* isolates from waters and raw shellfish collected in two French coastal areas and from seafood imported into France. Int. J. Food Microbiol., 91: 319-325.

Sorrells, K.M., D.C. Enigl and J.R. Hatfield, 1989. Effect of pH, acidulant, time and temperature on the growth and survival of *Listeria monocytogenes*. J. Food Prot., 5: 571-573.

Terzi, G., O. Büyüktanir and N. Yurdusev, 2009. Detection of the *tdh* and *trh* genes in *Vibrio parahaemolyticus* isolates in fish and mussels from Middle Black Sea Coast of Turkey. Lett. Applied Microbiol., 49: 757-763.

Wang, S.H. and J.C. Chen, 2005. The protective effect of chitin and chitosan against *Vibrio alginolyticus* in white shrimp *Litopenaeus vannamei*. Fish Shellfish Immun., 19: 191-204.

Xiong, H., L. Yanbin, M.F. Slavik and J.T. Walker, 1998. Spraying chicken kin with selected chemicals to reduce attached *Salmonella*. J. Food Prot., 61: 272-275.