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Mathematical modeling of growth of *Salmonella* spp. and spoilage microorganisms in raw oysters☆

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ABSTRACT

The main objective of this study was to develop the primary and secondary models to describe the growth kinetics of *Salmonella* as well as background microorganisms in raw, shucked oysters. Samples, inoculated with a cocktail of two *Salmonella* serotypes, *S.* Typhimurium (CICC22956) and *S.* Enteritidis (CICC21482), were incubated at 4, 8, 12, 16, 20, 25, 30, 33, 37, 40, and 43 °C. Growth of *Salmonella* was observed at all temperatures, except at 4 °C. The background microorganisms grew at all temperatures. All growth curves clearly exhibited lag, exponential and stationary phases, and were analyzed using the Huang growth model. Three secondary models (Ratkowsky square-root, Huang square-root, and Cardinal parameter models) were compared for evaluating the effect of temperature on bacterial growth rates. Data analysis was performed using IPMP 2013, a free predictive microbiology software tool developed by the USDA ARS.

The Cardinal parameters model underestimated the specific rates of the microorganisms at low temperatures. The Huang square-root model was more suitable than the Ratkowsky square-root model for describing the effect of temperature on growth of *Salmonella*, while the Ratkowsky square-root model, on the other hand, was more suitable for background microorganisms. For both *Salmonella* and background microorganisms, the logarithms of the lag phase were expressed as linear functions of the logarithms of specific growth rates. The results of this study can be used by the food retailers and regulatory agencies to estimate the microbial shelf-life of raw, shucked oysters.

1. Introduction

Oyster, a bivalve mollusk, is known for its delicacy and nutrient contents that are rich in zinc, vitamin A, and vitamin B_{12}, but low in food energy. Oysters can be consumed as raw, smoked, canned products or used in a variety of cocktails. In many parts of the world, some consumers, particularly from coastal regions, prefer to eat fresh oysters. In upscale restaurants, raw oysters are often consumed with a home-made Mignonette sauce by seafood lovers. Oyster is an important marine biological resource and consumed worldwide. As the largest farmed shellfish, more than 4 million tons of oysters are consumed annually worldwide. Just in the United States, the demand for oysters is over 69 million pounds each year, and half of which are supplied as raw oysters (FDA, 2005; NOAA, 2008).

While popular for fresh consumption, raw oysters may contain harmful bacteria. *Vibrio parahaemolyticus*, a major marine microorganism native to estuarine waters throughout the world, is a foodborne pathogen frequently found in seafood. Common in Asia and the United States, *Vibrio paraheomolyticus* is recognized as an agent that causes diarrheal disease around the world. *Salmonella* spp. is also a major enteric pathogen that has been found in aquatic and marine environments (Catalao Dionisio et al., 2000; Hatha & Lakshmanaperumalsamy, 1997; Heinitz, Ruble, Wagner, & Tatini, 2000; Martinez-Urtaza, Saco, Hernandez-Cordova, et al., 2003; Martinez-Urtaza, Liebana, Garcia-Migura, Perez-Pineiro, & Saco, 2004a; Martinez-Urtaza, Saco, Novoa, et al., 2004b; Venkateswaran et al., 1989). As with many typical filter-feeders, oysters may concentrate many human pathogens in the tissues from the surrounding waters, many of which may be contaminated with *Salmonella* and other pathogens (Burkhardt and Calci, 2000; Butt,

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Aldridge, & Sanders, 2004; Correa, Toso, Albarnaz, Simoes, & Barardi, 2006; Lofety, Hassanein, Fagr, Abdel-Gawad, El-Taweel, & Bassem, 2011; Martunez-Urtaza et al., 2003). Heinitz et al. (2000) reported a 1.2% prevalence of *Salmonella* in the U.S. domestic shellfish. Martinexz-Urtaza et al. (2003) reported an overall incidence of 1.8% of *Salmonella* spp. in a total of 2980 shellfish samples, with a higher incidence in mussels and oysters due to their distinct growing habitat. Among the samples tested positive for *Salmonella* spp., eighteen percent (18%) were identified as *S.* Typhimurium. Brands et al. (2005) reported that 7.4% of the oysters were tested positive with *Salmonella* among the samples collected from the U.S. West, East, and Gulf coasts in the summer and winter of 2002–2003. A 2007 survey of live oysters in the U.S. suggested 8.6% (by PCR method) and 1.5% (culture confirmed) prevalence of *Salmonella* spp. (Adak, Meakins, Yip, Lopman, & O'Brien, 2005). Higher prevalence of *Salmonella* was found in warmer weather than in winter weather (Brands et al., 2005). Collins, Rehnstam-Holm, and Hernroth (2008) reported that *S.* Typhimurium was found in 100% and 30% of the edible marine bivalve samples during the rainy and dry seasons, respectively. A more recent study reported that *Salmonella* was isolated from 10% of seafood samples (including oysters), and *S.* Typhimurium was among the many *Salmonella* isolates identified (Bakr, Hazzah, & Abaza, 2011).

Salmonella, a Gram-negative bacterium, is a major cause of foodborne infections around the world. In Southeast Asia and America, *Salmonella* is a common cause of gastrointestinal diseases and usually causes about 1.4 million cases of infections even death each year (Brands et al., 2005; Ponce, Khana, Cheng, West, & Cerniglia, 2008). *Salmonella* is also the most commonly isolated pathogen in seafood and it can easily contaminate molluscan shellfish in marine environment (Heinitz et al., 2000; Kumar, Surendran, & Thampuran, 2009). Incidents of *Salmonella* contaminations in oysters and mussels have been reported in USA (CSPI, 2009), India (Hatha et al., 1997), Thailand (Utrarachkij, Intalapaporn, Kumkrong, & Kittikul, 2006), Hong Kong (Yam et al., 1999), and Spain (Martinez-Urtaza, Liebana, et al., 2004a). Around the world, *Salmonella* continues to be a significant public health hazard (Sanchez-Vargas, Abu-El-Haija, & Gomez-Duarte, 2011).

While *V. parahaemolyticus* is by far a more important pathogen associated with raw shelf fish, including oysters, the contamination and risk of *Salmonella* in raw oysters should not be ignored. Recently, a kinetic study was conducted to evaluate the effect of temperature on growth of *V. parahaemolyticus* and *Vibrio vulnuficus* in flounder, salmon sashimi, and oyster meat (Kim, Lee, Hwang, & Yoon, 2012). However, no study on growth kinetics of *Salmonella* in raw oyster meat exists in the scientific literature. Therefore, the objective of this study was to investigate the growth kinetics of *Salmonella* in oysters and develop mathematical models to predict the bacterial growth. Additionally, this study also collected and analyzed the kinetic data of native background microorganisms. The kinetic models developed from this study can be used by the food industry to estimate the microbial shelf-life and conduct risk assessments of raw oysters for fresh market.

2. Materials and methods

2.1. Bacterial cultures and preparation

Two strains of freeze-dried *Salmonella*, *S.* Typhimurium (CICC22956) and *S.* Enteritidis (CICC21482), were obtained from Beijing Beina Chuanglian Biotechnology Research Institute (Beijing, China). Each strain of *Salmonella* was revived by inoculating into 10 ml Brain Heart Infusion broth (BHI broth, Qingdao Hope Biol-Technology Co., Ltd., Qingdao, China) and incubating at 37 °C for 24 h on an orbital shaker (120 rpm). The bacterial cultures were harvested after 3 consecutive transfers. After the cultures of *S.* Typhimurium and *S.* Enteritidis were activated and stabilized, they were induced to resist rifampicin (rif, Shanghai Shenggong Biological Engineering Co., Ltd., Shanghai, China) at 100 mg/L by gradually transferring the cultures to BHI broth containing 0, 25, 50, 75, 100 mg/L of rifampicin. Once the antibiotic resistance was induced, the growth of rif-resistant and natural strains of *Salmonella* inoculated to oysters was compared. No difference in the growth patterns was observed (Fig. 1). Therefore, the rif-resistant *Salmonella* cultures were used in further studies.

Thereafter, a rif-resistant working culture was prepared by plating each *Salmonella* strain onto Tryptic Soy agar (TSA, Qingdao Hope Biol-Technology Co., Ltd.) plates containing 100 mg/L of rifampicin (TSA/R). The working cultures were maintained on TSA/R plates, stored at 8 °C, and propagated once a month.

Fresh inocula were prepared before the experiment by inoculating *S.* Typhimurium and *S.* Enteritidis from TSA/R plates into 10 ml BHI broth containing 100 mg/L rifampicin, and incubated on an orbital shaker (120 rpm) at 37 °C for 24 h. The bacterial cultures were individually centrifuged (2900 × g, 10 min), washed with 0.1% peptone water (PW) twice, and then re-suspended in 5 ml PW. Equal volumes of each culture were combined in a sterile test tube to form a cocktail of the two *Salmonella* serotypes. Serial dilutions were made from the bacterial cocktail to obtain a properly diluted culture for inoculating shucked oysters.

2.2. Sample preparation and inoculation

Fresh oysters (*Ostrea rivularis*) were purchased from a local aquatic products market for every experiment. Oysters similar in size were selected and washed to remove sea mud on the shells. Each oyster was shucked to remove the flesh using a sterile surgical knife under an aseptic condition. The oysters (~25 g) were separated from the shells and were placed into individual sterile filter bags. The samples were randomly divided into two groups, labeled as the experimental group and the control group, respectively. For the experimental group, each sample was inoculated with 0.1 ml of appropriately diluted bacterial cocktail. The initial inoculum level of *Salmonella* was 10^2–10^3 colony-forming unit per g (CFU/g) of oyster flesh in the experimental group. Each sample bag was hand-shaken for about 1 min to disperse the bacterial culture in the

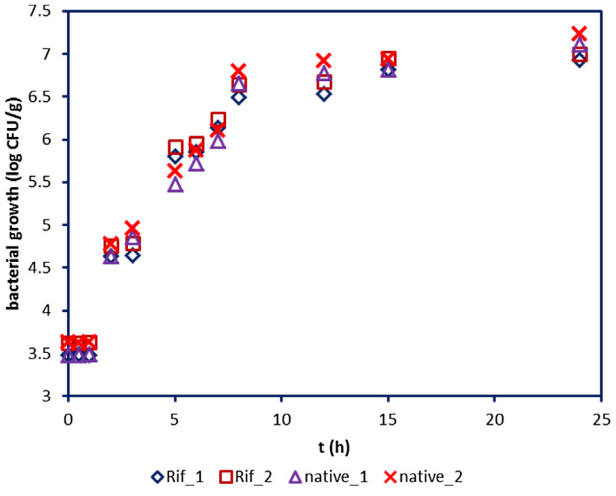

Fig. 1. Comparison of growth curves of native and rifampicin-resistant strains of *Salmonella* in fresh oyster flesh incubated at 37 °C (two replicates). Rif: raw bacterial counts of rifampicin-resistant strains of *Salmonella*; native: raw bacterial counts of *Salmonella*.