Hindawi
Journal of Food Quality
Volume 2018, Article ID 2060915, 6 pages
https://doi.org/10.1155/2018/2060915

Research Article

Temperature Effect Study on Growth and Survival of Pathogenic *Vibrio parahaemolyticus* in Jinjiang Oyster *(Crassostrea rivularis)* with Rapid Count Method

Yuan Wang,[1,2] Hai-yan Zhang,[3] Essy Kouadio Fodjo,[4] Cong Kong,[1] Run-Run Gu,[1] Feng Han,[1] and Xiao-Sheng Shen[1,3]

[1]*Key Laboratory of East China Sea Fishery Resources Exploitation, Ministry of Agriculture and East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China*
[2]*Key Laboratory of Control of Quality and Safety for Aquatic Products, Ministry of Agriculture and East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China*
[3]*Key Laboratory of Oceanic and Polar Fisheries, Ministry of Agriculture and East China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shanghai 200090, China*
[4]*Laboratory of Physical Chemistry, Université Felix Houphouet-Boigny, 22 BP 582, Abidjan 22, Côte d'Ivoire*

Correspondence should be addressed to Cong Kong; kongcong@gmail.com and Xiao-Sheng Shen; shenxs@ecsf.ac.cn

Received 10 September 2017; Revised 4 February 2018; Accepted 26 February 2018; Published 1 April 2018

Academic Editor: Haibo Luo

The growth of *Vibrio parahaemolyticus (V. parahaemolyticus)* in oysters during postharvest storage increases the possibility of its infection in humans. In this work, to investigate the growth or survival profiles in different media, pathogenic *V. parahaemolyticus* in APW, Jinjiang oyster (JO, *Crassostrea rivularis*) slurry, and live JO were studied under different temperatures. All the strain populations were counted through our double-layer agar plate (DLAP) method. In APW, the pathogenic *V. parahaemolyticus* showed continuous growth under 15, 25, and 35°C, while a decline in behavior was displayed under 5°C. The similar survival trend of pathogenic *V. parahaemolyticus* in JO slurry and live JO was observed under 5, 25, and 35°C, except the delayed growth or decline profile compared to APW. Under 15°C, they displayed decline and growth profile in JO slurry and live JO, respectively. These results indicate the different sensitivity of pathogenic *V. parahaemolyticus* in these matrices to temperature variation. Furthermore, nonpathogenic *V. parahaemolyticus* displayed little difference in survival profiles when inoculated in live JO under corresponding temperatures. The results indicate that inhibition or promotion effect could be regulated under different storage temperature for both pathogenic and nonpathogenic strains. Besides, the DLAP method showed the obvious quickness and efficiency during the bacteria count.

1. Introduction

Vibrio parahaemolyticus (V. parahaemolyticus) is one of the leading causes of acute gastroenteritis associated with exposure of raw or undercooked seafood in the diet. *V. parahaemolyticus* exists in a variety of seafood including raw, frozen, and even cooked products through posthandling contamination [1]. This human pathogen is usually a great concern in Asian countries, such as China, Vietnam, and Japan [2, 3]. *V. parahaemolyticus* could have a rapid generation times from 12 to 18 min in seafood [4]. Its populations in oysters could increase 10- to 100-fold in 10 h when left at ambient temperatures [5]. *V. parahaemolyticus* can grow at temperature of 8°C, which is within the range of household and retail refrigeration [6].

To reduce the risk of *V. parahaemolyticus* infection in raw or undercooked oysters, several procedures such as chilling [7], freezing [8], depuration [9], high hydrostatic pressure processing [10–12], and irradiation [13] have been conducted. However, these processes require either a significant amount of initial investment or operation costs, and oysters are often killed during processing. A cost-effective storage process

to limit the population of *V. parahaemolyticus* in seafood without significant adverse effects remains to be investigated. The regulation of temperature after postharvest process is a preferred strategy in terms of the easy operation and low cost.

Jinjiang oyster (JO, *Crassostrea rivularis*), commonly cultivated in the intertidal zones in south China, has been traditionally harvested and cooked as a food source for hundred years [14]. Following the introduction of western eating habits, Chinese diet contains increasing proportion of raw or undercooked JO. Although these eating ways are much more delicious and nutritious, there is a high risk of infections for individuals due to the possible existence of pathogenic *V. parahaemolyticus*. Surveys on some coastal cities in China have shown over 50% presence of *V. parahaemolyticus* in raw JO, regardless of their pathogenicity.

Although effects of temperatures on *V. parahaemolyticus* in oysters have been studied extensively, most of the studies so far have focused on Pacific oysters *(Crassostrea gigas)* [15], the Eastern oysters *(Crassostrea virginica)* [16], and Zhe oysters *(Crassostrea plicatula)* [5]. Little is known about the growth of pathogenic *V. parahaemolyticus* in JO during different storage temperatures. The pathogenic *V. parahaemolyticus* typically carry the thermostable directed hemolysin *(tdh)* gene or *tdh*-related hemolysin*(trh)* gene or both genes simultaneously [17, 18]. For the safety consumption, it makes sense to investigate whether there is some difference between the growth profile of pathogenic and nonpathogenic *V. parahaemolyticus* in oysters, so as to intentionally inhibit their growth with minimum control. Thus, studies on pathogenic *V. parahaemolyticus* in Alkaline Peptone Water (APW), JO slurry, and live JO were carried out under different temperature. Furthermore, survival of nonpathogenic *V. parahaemolyticus* in live JO was compared with pathogenic *V. parahaemolyticus* under different temperatures. In our previous study, double-layer agar plate (DLAP) was developed and verified in the 1-step procedure screening and counting of nonpathogenic/pathogenic *V. parahaemolyticus* in culture broth and different seafood samples [19]. The entire counting process was carried out through our DLAP method to demonstrate its efficacy and quickness in various *V. parahaemolyticus* count related study.

2. Materials and Methods

2.1. Bacteria Strains. Five local *V. parahaemolyticus* strains (two tdh+/trh+ strains: one was from clam and the other one was from oyster; three tdh−/trh+ strains: two were from razor fish, and one was from scallop) and three local strains without these virulence genes were isolated from commercial shellfish (all from oysters) in our laboratory. Two standard pathogenic *V. parahaemolyticus* strains (ATCC33847 tdh+/trh−, ATCC17802 tdh−/trh+) were kindly provided by Yong Zhao, Department of Microbiology, Faculty of Food Science and Technology, Shanghai Ocean University, Shanghai, China. The virulence genes *tdh* and *trh* in these strains were confirmed by PCR and could appropriately be utilized to produce the models. Cultures with these confirmed nonpathogenic or pathogenic strains were stored at −80°C in modified tryptone soy broth (mTSB) (Land Bridge Technology Co., Ltd., Beijing, China) supplemented with 3% NaCl and adjusted to pH 8.0 ± 0.2) with addition of 25% (v/v) glycerol (National Pharmaceutical Group Corporation, Shanghai, China). Frozen strains were reactivated in TSB with 1% yeast extract (Oxoid, LP0021, Nobleryder Co., Ltd., Beijing, China) and transferred to tryptone soy agar (TSA) (Land Bridge Technology Co., Ltd., Beijing, China) with overnight incubation. The multiple pathogenic or nonpathogenic strains of *V. parahaemolyticus* were obtained through the equal volume mixture of these strains, which were ready to use for inoculation.

2.2. Sample Preparation for Storage of V. parahaemolyticus Contaminated Cultures. For the cultures tested, 3.6 mL of sterile Alkaline Peptone Water (APW, Land Bridge Technology Co., Ltd., Beijing, China) with 3% NaCl was prepared in 5 mL Eppendorf tube. Each tube was then aseptically inoculated with 0.4 ml of diluted cultures of pathogenic or nonpathogenic *V. parahaemolyticus* to reach an initial population of ca. 10^3 cfu/mL and ca. 10^5 cfu/mL. The tubes were then immediately incubated at 5°C (10^5 cfu/mL) and 15, 25, and 35°C (10^3 cfu/mL) without agitation.

JO samples were purchased from the local fisheries wholesale market, Shanghai, China, between August and October 2014. Following the acquisition, the oysters were placed in cool box with gel packs and transported within 2 hours to our laboratory. For the tested oyster slurry, live oysters were immediately made into a slurry in a blender (Hanuo, JJ-2, Shanghai, China) after being transported to the laboratory. Then the slurry was sterilized at 450 KMPa, 20°C, by ultrahigh pressure sterilizer (Huatai Senmiao Co., Ltd., Tianjin, China) for 25 min. The inoculation method for JO slurry is carried out in the same way as described in APW method. For the live JO test, oysters were washed with tap water to remove excess mud on the shells and placed in artificial seawater (ASW; salinity: 20‰) at room temperature (approximately 20°C) for 6 hours to allow the oysters to regain viability before being inoculated with nonpathogenic or pathogenic *V. parahaemolyticus*. The oysters were exposed in ASW containing pathogenic or nonpathogenic *V. parahaemolyticus* (ca. 10^6 cfu/mL and ca. 10^7 cfu/mL, resp.) to be inoculated at 20°C for 10 hours to produce a contamination level of ca. 10^3 and ca. 10^5 cfu/g. Inoculated oysters were packed in sterile mesh bags and stored at various temperatures (the group of 10^3 cfu/g was stored at 15, 25, and 35°C; the group of 10^5 cfu/g was stored at 5°C). DLAP method was used to detect any nonpathogenic/pathogenic *V. parahaemolyticus* present in the oyster's samples before the contamination of *V. parahaemolyticus*, and none of them was found positive. A total of 300 oysters were used in these experiments.

It should be noted that the uninoculated samples for APW and JO slurry which were sterilized and live JO, which were confirmed to be nonpathogenic and pathogenic *V. parahaemolyticus* negative before inoculation, were run alongside the above samples to avoid the unexpected pollution from the environment. Negative results were obtained for all these samples.

2.3. Bacteriological Analysis in Different Cultures. Populations of Pathogenic or nonpathogenic *V. parahaemolyticus* in various cultures were analyzed through our previously developed double-layer agar plate (DLAP) method [19]. DLAP was prepared by pouring ten mL of sterile Bio-Chrome Vibrio medium (BCVM, Chromogenic medium for *V. parahaemolyticus*) into a sterile Petri dish (90 × 15 mm) and left to cool down and solidify at room temperature in a biological safety cabinet (MSC-Advantage, Thermo, Germany). After being autoclaved and tempered to about 50°C, an equal volume of TSA containing 3% NaCl was then covered on the BCVM layer and allowed to solidify again. All DLAPs were prepared on test days and used within two hours after preparation.

The APW cultures were decimally diluted in sterile 3% NaCl solution to appropriate dilution gradient and spread onto a DLAP. For the analysis of nonpathogenic/pathogenic *V. parahaemolyticus* in the slurries and live oyster, slurries, meat, or liquor of shucked oysters was placed in a sterile filter stomacher bag (Hanuo, Shanghai, China) with tenfold APW (3% NaCl, pH 8.5 ± 0.2). And then the sample was stomached (Colworth Stomacher 400; A. J. Seward, London, United Kingdom) for 2 min. Then 100 mL of crude filtrate was prepared into appropriate dilution gradient and spread onto DLAP and incubated at 37°C for 20 hours. Colony forming units (cfu) were counted manually to calculate the density of viable cells in the samples (cfu/g). All the analysis results were obtained through the average of three replicates.

3. Results and Discussion

In our studies, the DLAP method was applied for detecting nonpathogenic/pathogenic *V. parahaemolyticus* in APW, JO slurry, and JO samples. The selective and differential characteristic of this method has been tested in our previous report [19]. Compared with the commonly used MPN method, our DLAP method does not need enrichment of nonpathogenic/pathogenic *V. parahaemolyticus* and subsequent biochemical tests for confirmation as required by MPN method. Therefore, the test cycle of DLAP method was quite shorter than that of MPN by about 24 hours. Furthermore, DLAP method has been demonstrated to be as sensitive and precise as the MPN method in previous studies. As a result, DLAP has been successfully applied in detecting nonpathogenic/pathogenic *V. parahaemolyticus* in different samples, which indicate the feasibility in various *V. parahaemolyticus* count related studies with this straightforward and fast procedure.

Profile of the population development is shown in Figure 1. For pathogenic *V. parahaemolyticus* in APW, the inoculated pathogenic *V. parahaemolyticus* could grow at 15, 25, and 35°C and slowly decline at 5°C. For the live JO test, the analysis was continued until oyster shells visibly gaped. Pathogenic *V. parahaemolyticus* were artificially accumulated in live JO, with the contamination rise to 7.51, 7.90, and 5.11 log cfu/g in population, after 60 and 100 hours when stored at 35°C, 25°C, and 15°C. Strains multiplication was apparently slower than that in APW, respectively. For pathogenic strains inoculated in JO slurry, their population growed similarly to strains from a live JO at 35°C, and even slowed growth at 25°C. However, when kept in 15°C, the strains almost remained stable at 3.13 log cfu/g with a tiny reduction in population over 140 hours.

In other related studies, the Pacific oysters *(Crassostrea gigas)* were collected seasonally from regions historically associated with *V. parahaemolyticus* infections and stored over a range of temperatures used in industry and consumer practices [15]. *V. parahaemolyticus* is more likely to be present in market oysters rather than harvested oysters [20, 21]. What we built is a simulation for the pathogenic *V. parahaemolyticus* in market JO. In addition, the previous report showed that *V. parahaemolyticus* multiplied rapidly in live JO held at 26°C after harvest, while decreased during refrigeration storage at 3°C [22]. As temperature abuse during postharvest handling and storage may increase the risk of infection due to the consumption of raw or undercooked oysters, so we inoculated pathogenic *V. parahaemolyticus* strains into APW, JO slurry and live JO, and as well as nonpathogenic *V. parahaemolyticus* in live JO, to compare their growth profile.

The fast growth of pathogenic *V. parahaemolyticus* in APW, JO slurry, and live JO at 35 and 25°C indicate the active state of strains, implying the high risk of infection and easy spoilage in very short time. Less growth rate of the strains in JO slurry and live JO than in APW may be ascribed to some inhibitor agents present in JO strains and live JO or to that APW provide more appropriate nutrition than the food media. In general, the different sensitivity of pathogenic *V. parahaemolyticus* to temperature variation is caused by the different conditions of nutrition in these media. The fact that pathogenic *V. parahaemolyticus* slowly grew at 15°C in live JO but was inactivated in JO slurry demonstrates that the live JO is easier to be contaminated than JO slurry. The live JO may provide continuous active factors that stopped when ground into slurry and the homogenization process released inhibitory compounds or degradative enzymes to the matrix, which could account for the different growth profile between the two media. The different maximum population density of inoculated strains is also caused by the equilibrium of growth and death with varying temperature.

To investigate whether there are differences between the growth of pathogenic and nonpathogenic strains of *V. parahaemolyticus*. The inoculation of both pathogenic and nonpathogenic strains was conducted in live JO, respectively, at different temperatures. Figure 2 displays the growth development of these inoculated strains. It is noticed that the nonpathogenic *V. parahaemolyticus* shows similar growth or survival behavior to the pathogenic ones, when they were kept at the same temperatures, respectively. They could reach roughly the equal population when staying at stationary phase and follow the consistent growth rate during the exponential phase at 35, 25, and 15°C. Furthermore, they decline to similar amount after storage at 5°C with almost identical drop behavior, from the same initial inoculation amount, which demonstrates that their growth is not affected by toxin related genes at the temperature. From the population development of nonpathogenic/pathogenic *V. parahaemolyticus* in different media at 5°C, it can be deduced that fewer strains may survive over around 200 hours' storage.

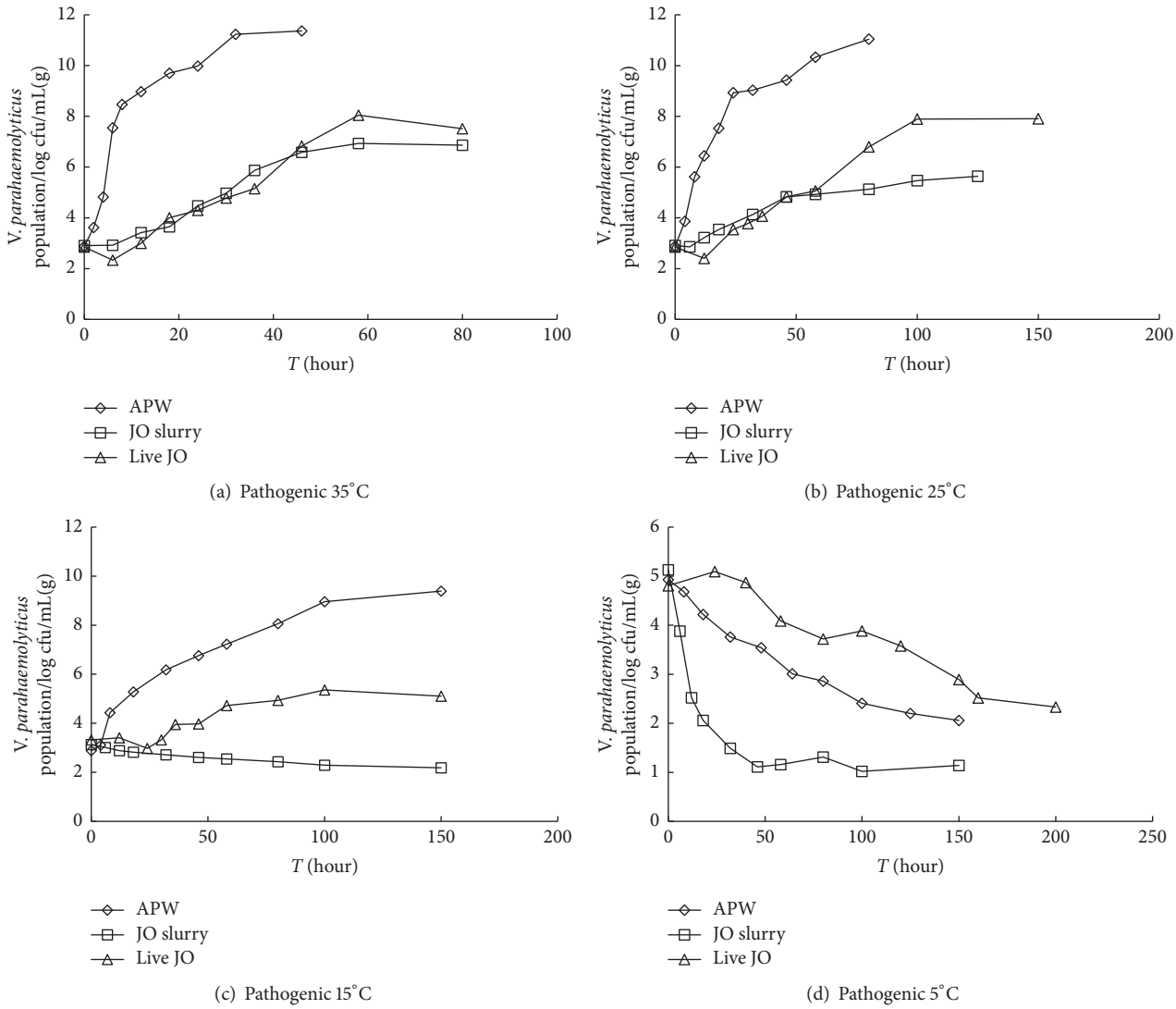

Figure 1: Comparison of growth profile of the multiple strains of pathogenic *V. parahaemolyticus* in APW, JO slurry, and JO at (a) 35°C, (b) 25°C, (c) 15°C, or (d) 5°C obtained through DLAP method.

However, even the low-temperature inhibition effect on the growth and survival of pathogenic and nonpathogenic *V. parahaemolyticus* was observed and confirmed by experiment; it does not mean the safe consumption of JO could be guaranteed, as there may be other microorganisms' contamination. Furthermore, even the initial high contamination can decline into much less population, toxic proteins in the live or dead residues in the food are likely to be the potential risk factor, which is recommended to be denatured and inactivated with a thermal process. Anyway, it could be deduced from the study that less risk of *V. parahaemolyticus* could be realized when JO samples are verified to be noncontaminated by these strains and stored at lower temperature continuously.

4. Conclusions

In conclusion, the DLAP method was demonstrated to be successfully applied in detecting nonpathogenic/pathogenic *V. parahaemolyticus* in different samples. The results of growth and survival trends in APW, JO slurry, and live JO indicate the different sensitivity of pathogenic *V. parahaemolyticus* in these matrices to temperature variation. Furthermore, nonpathogenic *V. parahaemolyticus* displayed little difference in growth and survival profiles when inoculated in live JO oysters at corresponding temperatures. Results indicate that inhibition or promotion effect could be regulated under different storage temperature for both pathogenic and nonpathogenic strains. However, results do not mean that the safe consumption of JO could be guaranteed, as there may be other microorganisms contamination or some toxic proteins are in the live or dead residues in the food, which are likely to be the potential risk factor. The least risk of pathogenic *V. parahaemolyticus* could be maintained when JO samples were verified to be noncontaminated by these strains and continuously stored at lower temperature. Our successful practice in the application of DLAP method

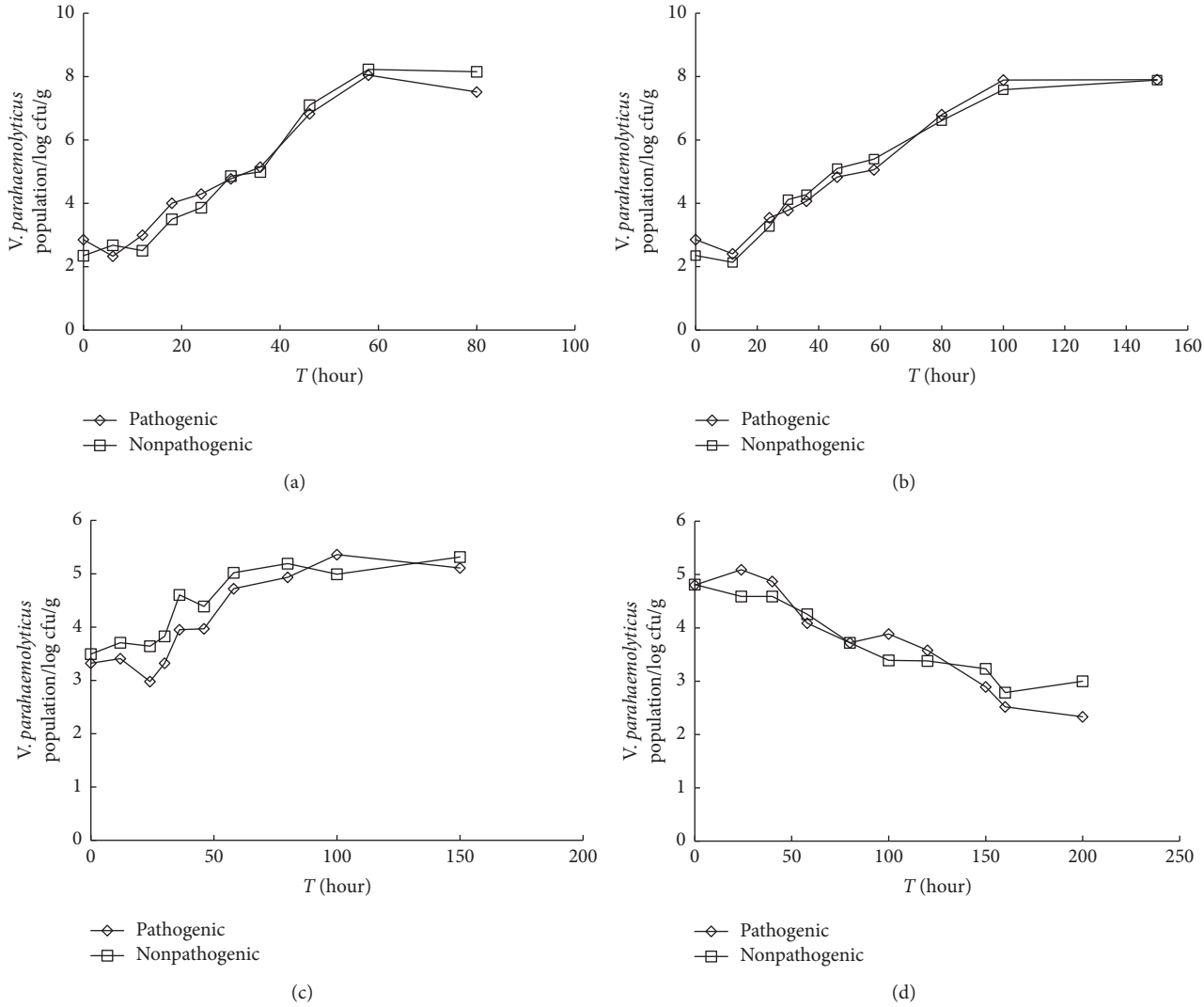

Figure 2: The observed growth of multiple strains of pathogenic and multiple strains of nonpathogenic *V. parahaemolyticus* in live JO stored at (a) 35°C, (b) 25°C, (c) 15°C, or (d) 5°C obtained through DLAP method.

indicates its feasibility in various nonpathogenic/pathogenic *V. parahaemolyticus* count related studies with this simple and fast procedure.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

This project was supported by special research fund for the national nonprofit institutes (East China Sea Fisheries Research Institute) (no. 2014T05; no. 2011M13), the Yangfan project (14YF1408100) from Science and Technology Commission of Shanghai Municipality, China, and Natural Science Foundation of China (31471672). Essy Kouadio Fodjo acknowledges financial support from Third World Academy of Science (TWAS) under Grant no. 16-510 RG/CHE/AF/AC_G–FR3240293301. The authors also thank Shanghai Ocean University for the kindly providing standard *V. parahaemolyticus* strains.

References

[1] Y. C. Su and C. Liu, "Vibrio parahaemolyticus: a concern of seafood safety," *Food Microbiology*, vol. 24, no. 6, pp. 549–558, 2007.

[2] S. Wang, H. Duan, W. Zhang, and J.-W. Li, "Analysis of bacterial foodborne disease outbreaks in China between 1994 and 2005," *FEMS Immunology & Medical Microbiology*, vol. 51, no. 1, pp. 8–13, 2007.

[3] M. Yingkajorn, N. Sermwitayawong, P. Palittapongarnpimp et al., "Vibrio parahaemolyticus and Its Specific Bacteriophages as an Indicator in Cockles (Anadara granosa) for the Risk of V. parahaemolyticus Infection in Southern Thailand," *Microbial Ecology*, vol. 67, no. 4, pp. 849–856, 2014.

[4] P. Vasudevan, P. Marek, S. Daigle, T. Hoagland, and K. S. Venkitanarayanan, "Effect of chilling and freezing on survival of Vibrio parahaemolyticus on fish fillets," *Journal of Food Safety*, vol. 22, no. 4, pp. 209–217, 2002.

[5] X. Shen, Y. Cai, C. Liu, W. Liu, Y. Hui, and Y.-C. Su, "Effect of temperature on uptake and survival of Vibrio parahaemolyticus in oysters (Crassostrea plicatula)," *International Journal of Food Microbiology*, vol. 136, no. 1, pp. 129–132, 2009.

[6] W. Thomson and C. Thacker, "Effect of Temperature on Vibrio parahaemolyticus in Oysters at Refrigerator and Deep Freeze Temperatures," *Canadian Institute of Food Science and Technology Journal*, vol. 6, no. 3, pp. 156–158, 1973.

[7] J. M. Muntada-Garriga, J. J. Rodriguez-Jerez, E. I. Lopez-Sabater, and M. T. Mora-Ventura, "Effect of chill and freezing temperatures on survival of Vibrio parahaemolyticus inoculated in homogenates of oyster meat," *Letters in Applied Microbiology*, vol. 20, no. 4, pp. 225–227, 1995.

[8] C. Vanderzant and R. Nickelson, "Survival of Vibrio parahaemolyticus in shrimp tissue under various environmental conditions.," *Journal of Applied Microbiology*, vol. 23, no. 1, pp. 34–37, 1972.

[9] M. J. Chae, D. Cheney, and Y.-C. Su, "Temperature effects on the depuration of vibrio parahaemolyticus and vibrio vulnificus from the american oyster (Crassostrea virginica)," *Journal of Food Science*, vol. 74, no. 2, pp. M62–M66, 2009.

[10] S. Phuvasate and Y.-C. Su, "Efficacy of low-temperature high hydrostatic pressure processing in inactivating Vibrio parahaemolyticus in culture suspension and oyster homogenate," *International Journal of Food Microbiology*, vol. 196, pp. 11–15, 2015.

[11] C.-Y. Wang, H.-W. Huang, C.-P. Hsu, Y.-T. Shyu, and B. B. Yang, "Inactivation and morphological damage of Vibrio parahaemolyticus treated with high hydrostatic pressure," *Food Control*, vol. 32, no. 2, pp. 348–353, 2013.

[12] M. Ye, Y. Huang, J. B. Gurtler, B. A. Niemira, J. E. Sites, and H. Chen, "Effects of pre- or post-processing storage conditions on high-hydrostatic pressure inactivation of Vibrio parahaemolyticus and V. vulnificus in oysters," *International Journal of Food Microbiology*, vol. 163, no. 2-3, pp. 146–152, 2013.

[13] X. Hu, P. Mallikarjunan, J. Koo, L. S. Andrews, and M. L. Jahncke, "Comparison of Kinetic Models To Describe High Pressure and Gamma Irradiation Used To Inactivate Vibrio vulnificus and Vibrio parahaemolyticus Prepared in Buffer Solution and in Whole Oysters," *Journal of Food Protection*, vol. 68, no. 2, pp. 292–295, 2005.

[14] H. Wang, X. Guo, G. Zhang, and F. Zhang, "Classification of jinjiang oysters Crassostrea rivularis (Gould, 1861) from China, based on morphology and phylogenetic analysis," *Aquaculture*, vol. 242, no. 1-4, pp. 137–155, 2004.

[15] C. Liu, J. Lu, and Y.-C. Su, "Effects of flash freezing, followed by frozen storage, on reducing Vibrio parahaemolyticus in pacific raw oysters (Crassostrea gigas)," *Journal of Food Protection*, vol. 72, no. 1, pp. 174–177, 2009.

[16] F. Chu and J. La Peyre, "Perkinsus marinus susceptibility and defense-related activities in eastern oysters Crassostrea virginica. temperature effects," *Diseases of Aquatic Organisms*, vol. 16, pp. 223–234, 1993.

[17] A. K. Bej, D. P. Patterson, C. W. Brasher, M. C. L. Vickery, D. D. Jones, and C. A. Kaysner, "Detection of total and hemolysin-producing Vibrio parahaemolyticus in shellfish using multiplex PCR amplification of tl, tdh and trh," *Journal of Microbiological Methods*, vol. 36, no. 3, pp. 215–225, 1999.

[18] A. M. Zimmerman, A. DePaola, J. C. Bowers et al., "Variability of total and pathogenic vibrio parahaemolyticus densities in Northern Gulf of Mexico water and oysters," *Applied and Environmental Microbiology*, vol. 73, no. 23, pp. 7589–7596, 2007.

[19] Y. Wang, X.-S. Shen, R. R. Gu, Y. F. Shi, and L. L. Tian, "Application of a rapid method for detecting vibrio parahaemolyticus in seafood," *Journal of Food Safety*, vol. 35, no. 1, pp. 26–31, 2015.

[20] B. A. Froelich, M. Ayrapetyan, P. Fowler, J. D. Oliver, and R. T. Noble, "Development of a matrix tool for the prediction of Vibrio species in oysters harvested from North Carolina," *Applied and Environmental Microbiology*, vol. 81, no. 3, pp. 1111–1119, 2015.

[21] S. Fu, J. Shen, Y. Liu, and K. Chen, "A predictive model of Vibrio cholerae for combined temperature and organic nutrient in aquatic environments," *Journal of Applied Microbiology*, vol. 114, no. 2, pp. 574–585, 2013.

[22] M. Ye, Y. Huang, and H. Chen, "Inactivation of Vibrio parahaemolyticus and Vibrio vulnificus in oysters by high-hydrostatic pressure and mild heat," *Food Microbiology*, vol. 32, no. 1, pp. 179–184, 2012.