Effect of food matrix type on growth characteristics and hemolysin production of *Vibrio alginolyticus*

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Abstract

Background: *Vibrio alginolyticus* is an important seafood-borne pathogen. There is increasing evidence that *V. alginolyticus* can also contaminate non-seafood by cross contamination and thereby cause food poisoning in humans. The growth and hemolysin production of *V. alginolyticus* at 30 °C in briny tilapia, shrimp, scallop, oyster, pork, chicken, freshwater fish and egg fried rice were investigated. Bacterial counts were enumerated by plate counting. Hemolysin production was evaluated by blood agar and hemolytic titer tests.

Results: Based on the goodness of fit primary model statistics (R^2 , MSE, BF, AF), the modified Gompertz model was a better fit to *V. alginolyticus* growth in foods than the logistic model. Growth kinetic parameters of *V. alginolyticus* displayed a higher μ_{max} and shorter λ in briny tilapia > shrimp > freshwater fish > egg fried rice > scallop > oyster > chicken > pork. It was notable that the *V. alginolyticus* counts were similar at the stationary phase, with no significant growth behavior difference between raw and cooked foods. However, higher thermostable direct hemolysin activity and hemolytic titer were observed in briny tilapia > egg fried rice > shrimp > freshwater fish > chicken > scallop > oyster > pork.

Conclusion: *V. alginolyticus* growth was good in all food matrix types tested. Contrary to current belief, *V. alginolyticus* displayed a higher hemolytic activity in some non-seafoods (freshwater fish, egg fried rice and chicken) than in scallop or oyster. This is the first report of growth and toxicity of *V. alginolyticus* in different food matrices and confirmation that some non-seafood contaminated with *V. alginolyticus* can be even more pathogenic. This study will enhance the awareness of non-seafood safety and improve the *V. alginolyticus* risk assessment accuracy.

Background

Vibrio alginolyticus, a Gram-negative, halophilic bacterium, is one of about a dozen of *Vibrio* species that cause significant morbidity and mortality in humans especially in those with low gastric acidity, immunodeficiency and liver disease [1, 2, 3]. Cholera and other *Vibrio* illness surveillance system data analyses have shown a dramatic increase in the incidence of *V. alginolyticus* infection, the third most common vibriosis related disease [4]. People come into contact with this bacterium via contaminated water (85 %) or seafood (15 %) [5]. Although foodborne transmission is rare, several cases of food poisoning have been reported in China, Japan and America [6, 7].

Foodborne illnesses caused by *V. alginolyticus* is most common during warmer (≥ 30 °C) summer and early autumn periods. Gastroenteritis is the most common syndrome with symptoms including fever, nausea, watery diarrhea and abdominal cramps [8, 9]. The bacterial growth and hemolysin production of *V. alginolyticus* contribute to its pathogenicity, causing invasive tissue damage and cytotoxicity, similar to the pathogenesis of other *Vibrio* species [10, 11]. So, if the growth characteristics and hemolysin production of *V. alginolyticus* in foods could be identified, it would help to develop measures to prevent this bacterium multiplying and producing hemolysin, thereby reducing pathogen concentration in foods and the occurrence of *V. alginolyticus* related food poisoning outbreaks.

V. alginolyticus is widely distributed in nature and has been frequently isolated from a variety of seafood including raw and processed briny tilapia, shrimp, oyster, scallop and food processing environments [12]. The wet environment in restaurant food processing is conducive to *V. alginolyticus* growth. Therefore, food processing gloves and cooking utensils are potential carriers of *V. alginolyticus* through contact with contaminated seafood surfaces and transfer of the organism to different types of food matrices, especially raw/cooked non-seafood. There is growing evidence that *V. alginolyticus* can also contaminate pork, poultry, egg and their products such as egg fried rice by cross-

contamination [13, 14, 15, 16]. Thus, there is a need to study the growth characteristics and hemolysin production of *V. alginolyticus* in seafood and non-seafood so that the risk posed by different food matrices could be accurately assessed.

V. alginolyticus was initially recognized as a biological type of *V. parahaemolyticus* but later characterized as an independent species [17]. Many studies have described the growth and hemolysin production of *V. parahaemolyticus* in various food matrices [18, 19] but not of *V. alginolyticus*. To date, most of the *V. alginolyticus* studies have focused on its prevalence [20], rapid detection [21] and pathogenic mechanisms [22]. To our knowledge, growth and hemolysin production by *V. alginolyticus* in foods including non-seafood has not been reported.

To better assess the risk of *V. alginolyticus* in seafood and non-seafood, a clear understanding of the growth characteristics and hemolysin production in varied food matrices is essential. In this study, we quantified the growth of pathogenic *V. alginolyticus* in four seafood (briny tilapia, shrimp, scallop, oyster), three non-seafood (pork, chicken, and freshwater fish) and three cooked food (pork, chicken, egg-fried-rice) types at 30 °C, then the data was used to establish the best growth model by comparing different mathematical equations incorporating with bacterial growth. The thermostable direct hemolysin (TDH) and total hemolytic activity were examined by using blood plate and hemolytic titers.

Results

Growth characteristics analysis of *V. alginolyticus* in different food types

The modified Gompertz and logistic models were applied to predict *V. alginolyticus* counts and to evaluate the model suitability to study the growth pattern in seafood and non-seafood matrices. Performance statistics of these two primary growth models are shown in Table 1, the R^2 values was > 0.98 and MSE value > 0.104 lg CFU/mL in each of the two models. Besides, nine data sets of each food matrix were determined to compare observed values with model predictive values, and the bias factor (BF) and accuracy factor (AF) values calculated by Eqs. (4) and (5) to assess the performance of the two growth models. As seen in Table 1, all the BF and AF values of the modified Gompertz model was within the limits of $1.0 \leq BF \leq AF \leq 1.1$ but not with the logistic model.

All growth curves were of sigmoidal shape with the initial concentrations smoothly changing to the exponential phase and stabilizing at the stationary phase (Fig. 1, Table 2). The growth kinetic parameters (Table 3) showed that the *V. alginolyticus* HY9901 strain initially inoculated at a concentration of 3.24 ± 0.24 lg CFU/g results in a maximum cell number of $\sim 7.98 \pm 0.53$ lg CFU/g, regardless of the of food matrix type. The maximum specific growth rate ($\mu_{\max}$) of the HY9901 strain changed with food matrix type and varied between 0.76 and 1.62 h^{-1} and the lag time (λ) varied between 2.27 and 3.43. *V. alginolyticus* exhibited higher $\mu_{\max}$ values and lower λ values in briny tilapia $>$ shrimp $>$ freshwater fish $>$ egg fried rice $>$ scallop $>$ oyster $>$ chicken $>$ pork. The $\mu_{\max}$ values and λ values of *V. alginolyticus* grown in raw pork and raw chicken were similar to those in cooked pork and cooked chicken.

Hemolytic activity

TDH activity

The TDH activity of *V. alginolyticus* HY9901 in seafood and non-seafood matrices by measuring the hemolytic circle diameter. The hemolytic zone diameter of briny tilapia was significantly higher ($p < 0.05$) than in freshwater fish $>$ shrimp $>$ chicken $>$ egg fried rice $>$ scallop $>$ oyster $>$ pork matrices' filtrates (Fig. 2). Besides, the TDH activity of *V.*

alginolyticus in scallop, oyster and pork was lower than in the LB medium and there were no significant differences in TDH activity of *V. alginolyticus* in fresh and cooked chicken and pork.

Hemolytic titer

Following incubation for 24 h, the hemolytic titer of *V. alginolyticus* in briny tilapia and egg fried rice was >1200 U, significantly higher ($p < 0.05$) than in shrimp > freshwater fish > chicken > scallop > oyster > pork. This result was consistent with the TDH activity of *V. alginolyticus* in above foods (Fig. 2). The hemolytic titer of *V. alginolyticus* in most food matrices was higher than ($p < 0.05$) in scallop, oyster and pork matrices in the LB medium indicating that scallop, oyster and pork may not be conducive for hemolytic activity of *V. alginolyticus*. There was no significant difference in hemolytic titer of *V. alginolyticus* in fresh and cooked pork and chicken ($p > 0.05$) (Fig. 3).

Discussion

The incidence of food poisoning caused by *V. alginolyticus* was positively correlated with temperature and global warming has increased it [23, 24]. Risk assessment investigations of *V. alginolyticus* in food matrices during warm temperature seasons are rare in the literature. Specifically, the comparison of growth characteristics of *V. alginolyticus* on seafood and non-seafood and the relative risk posed to consumers has not been reported. In order to explore the growth behavior and hemolysins production of *V. alginolyticus* in different food matrices during summer and early autumn seasons, the behavior of pathogenic *V. alginolyticus* HY9901 in terms of changes in cell counts and hemolytic activity in briny tilapia, shrimp, scallop, oyster, freshwater fish, pork, chicken and egg fried rice during incubation time at constant 30 °C were examined.

Results of goodness-of-fit primary model (Table 1) showed that the Gompertz model and logistic model met the defined criteria [25]. Compared with the logistic model, the Gompertz model showed a good statistical fit to the observed data and its R^2 values were closer to 1. The MSE values were also low within the precision of microbial enumeration indicating that the modified Gompertz model was a better fit to *V. alginolyticus* growth in foods than the logistic model. Besides, the BF and AF values were calculated and also used to assess the performance of the two growth models. The indices bias and accuracy provide an objective indication of model performance. In general, a BF value < 1 is considered unacceptable [26]. However, the BF does not indicate the average accuracy of estimates because under and over predictions tend to cancel out [27]. Therefore, AF should be calculated, which is the sum of the absolute difference between predicted and observed, and describes the overall model error. The higher the AF value, lower is the accuracy of the estimate [28]. As shown in Table 1, the average AF of the modified Gompertz and logistic models were 1.03 and 1.15, respectively. Results suggest that the predictions were almost identical with observations in the modified Gompertz model, and the predicted curves accurately describing the growth of *V. alginolyticus* in different food matrices at 30 °C. Ma and co-workers [29] showed similar results with the modified Gompertz model far more accurate than the logistic model in fitting *Vibrio* growth curves in shrimp and often been used to describe bacterial growth of different foods [30].

V. alginolyticus could grow well in all food matrix types tested and has a similar cell counts at the stationary phase (Fig. 1). Compared with the growth of other pathogenic organisms in seafood and meat such as *Listeria monocytogenes* [31], *Clostridium botulinum* [32] and *Salmonella* [33], *V. alginolyticus* exhibited a similar growth capacity. This means that it can survive in our daily food and rapidly proliferate when conditions such as temperature, salinity and also pH become favorable. According to Alam and co-workers [34], the ideal way to inactivate *Vibrios* in food products is to lower the storage temperature to < 8 °C which would impede the growth of pathogenic and non-pathogenic bacteria and thereby reduce the risk of pathogenic infections.

V. alginolyticus exhibited higher $\mu_{\max}$ values and lower λ values in briny tilapia > shrimp > freshwater fish > egg fried rice > scallop > oyster > chicken > pork (Table 3). *V. alginolyticus* is ubiquitous in brackish marine waters and in aquatic species. Its intrinsic characteristics have allowed it to adapt easily for growth in aquatic matrices but less in other meat types such as pork and chicken. The slow growth of *V. alginolyticus* in some non-aquatic foods may be due to the lack of water activity and different pHs, both of which are not suited for pathogenic *V. alginolyticus* growth in the initial 2 h. However, if egg fried rice is contaminated by other raw seafood, *V. alginolyticus* can grow quickly in < 2 h and thus increase the risk of human infection.

It is worth noting, the $\mu_{\max}$ values and λ values of *V. alginolyticus* grown in raw pork and raw chicken were similar to those in cooked state (Table 3) which means that the undercooked and cooked state of food has no bearing on the *V. alginolyticus* growth characteristics.

TDH controls a variety of biological activities including hemolytic activity, and cyto-, entero- and lethal- toxicities [35, 36]. TDH activity is the direct cause of the Kanagawa phenomenon in the Wagatsuma agar medium and it has been considered a major virulence determinant of the *Vibrio* species [37]. Results showed that the hemolytic zone diameter of briny tilapia was significantly higher ($p < 0.05$) than other matrices' filtrates (Fig. 2) which means that briny tilapia matrix can promote *V. alginolyticus* to produce more TDH than in other food matrices. Several studies [38, 39] have shown that *V. alginolyticus* induced serious damage to different fish types. The pathogenic *V. alginolyticus* strain HY9901 used in this study was isolated from diseased fish. We believe that the briny tilapia matrix was most beneficial to the growth (fast growth as shown in Fig. 1) and TDH production of *V. alginolyticus* and hence people who consume briny tilapia contaminated by *V. alginolyticus* are more likely to experience a serious food infection.

Interestingly, TDH activity of *V. alginolyticus* in scallop and oyster was not as high as in freshwater fish, chicken and egg fried rice and is most frequently isolated from shellfish [40]. It appears that the scallop and oyster matrices cannot provide sufficient nutrients required for TDH production in contrast to some non-seafoods and hence some non-seafood cross-contaminated by *V. alginolyticus* may pose a higher risk to humans. Therefore, we suggest inclusion of non-seafood products also in *V. alginolyticus* risk assessment.

The TDH activity of *V. alginolyticus* in scallop, oyster and pork was lower than in the LB medium which means that these food matrices are not ideal for *V. alginolyticus* to produce TDH as shown by the growth behavior profiles (Fig. 1). Besides, there were no significant differences in TDH activity of *V. alginolyticus* in fresh and cooked chicken and pork. Hence we believe that the TDH-producing capability of *V. alginolyticus* is similar in both raw and cooked foods.

Hemolytic titer reflects the total hemolytic activity and the degree of harm caused by *V. alginolyticus* extracellular products [41]. These extracellular products possess strong phospholipase and/or membrane pore-forming capacity and are cytotoxic to erythrocytes and many other cell types [42]. Results showed that the hemolytic titer of *V. alginolyticus* in briny tilapia and egg fried rice was significantly higher ($p < 0.05$) than in shrimp > freshwater fish > chicken > scallop > oyster > pork. It's consistent with the regular of TDH activity of *V. alginolyticus* in above foods (Fig. 2) and suggests that the briny tilapia and egg fried rice are more conducive than shrimp, chicken, scallop, oyster and pork for *V. alginolyticus* to produce hemolysis. Because some components of extracellular products are heat-stable even after 10 min at 100 °C [43], cooking at a lower temperature while it may kill *V. alginolyticus* bacteria, it only partly breaks down virulence factors in food products and hence the residual virulence factors may be harmful to human health. This means that when people consume unrefrigerated briny tilapia, egg fried rice, shrimp or freshwater fish contaminated by *V. alginolyticus* and not heated to at least 100 °C, food poisoning may occur. Seafood products have long been regarded as the only carrier of *V. alginolyticus* and have been the focus for its

toxic effects with less attention paid to non-seafood products. Our research suggests that if some non-seafood (freshwater fish, egg fried rice and chicken) are cross-contaminated by *V. alginolyticus*, these may also pose a higher risk than some seafood (e.g., scallop, oyster). Therefore, it is necessary to pay attention to non-seafood products also when risk assessment of *V. alginolyticus* is performed.

It is apparent that *V. alginolyticus* associated hemolytic titers in different food matrices are determined by the natural components in each food type. We hypothesize that intrinsic factors in egg fried rice promote *V. alginolyticus* to produce a concentration of the hemolytic factor, related to the thermolabile hemolysin (TLH). Jia and co-workers [44] have shown that the TLH gene from *V. alginolyticus* shared 94 % identity with the lecithin-dependent hemolysin of *V. parahaemolyticus* which can damage the cell membranes including that of flounder red blood cells. Thus, we deduce that the lecithin in egg fried rice stimulates the *V. alginolyticus* TLH gene resulting in an increase in the hemolytic activity. In the human *Vibrio* infections reported in more recent years, the growth characteristics of *V. alginolyticus* in non-seafood has been least studied. As shown in our study, an important finding is that *V. alginolyticus* can grow and show intense virulence in some non-seafood. Wang and co-workers [45, 46] reported a higher hemolytic activity of *V. parahaemolyticus* in egg fried rice than in shrimp or freshwater fish. It appears that *V. alginolyticus* and *V. parahaemolyticus* might possess the same virulence and pathogenic toxicity mechanisms. However, further studies are required to determine toxin-producing mechanism(s) and which factor(s) have the most influence on the production of hemolytic products on different foods.

Conclusions

This study investigated the growth of pathogenic *V. alginolyticus* in briny tilapia, shrimp, scallop, oyster, freshwater fish, pork, chicken and egg fried rice at 30 °C. Overall, the modified Gompertz model well fitted the growth characteristics of *V. alginolyticus* in seafood and non-seafood matrices. *V. alginolyticus* grew faster in briny tilapia, shrimp and egg-fried-rice with larger $\mu_{\max}$ values and shorter λ values but all food matrices had a similar cell density of *V. alginolyticus* at the stable growth phase. Higher TDH activity and hemolytic titers were observed in briny tilapia > egg fried rice > shrimp > freshwater fish > chicken > scallop > oyster > pork. Previous studies initially classified *V. alginolyticus* as a biological type of *V. parahaemolyticus*. So, the focus has been on the growth and pathogenicity of *V. parahaemolyticus* in different seafood and not on the growth behavior and hemolysins production of *V. alginolyticus* in seafood matrices. In addition, the growth in non-seafood products and potential cross contamination was completely ignored. Thus, we may have over the years underestimated the toxicity risk of *V. alginolyticus* in seafood and non-seafood matrices. In this study, we report on the growth characteristics and hemolytic activity of *V. alginolyticus* on different food matrices for a more accurate approach to human risk assessment. Because prepared foods may be exposed to a wide temperature range, future research on the growth and hemolytic activity at different temperatures would further improve risk assessment for *V. alginolyticus*.

Methods

Bacterial preparation

Vibrio alginolyticus strain HY9901 [11] (*tdh* gene positive) was originally isolated from spoiled *Lutjanus erythropterus* which cause food poisoning. This strain was kindly provided by the College of Fisheries, Guangdong Ocean University (Zhanjiang, China). The strain was confirmed using PCR by amplification of a hypervariable region of the 16S rDNA gene and preserved in Tryptone Soya Broth (Huangkai, Guangzhou, China) supplemented with 2 % NaCl and 20 % glycerol at - 80 °C. The strain was selected on thiosulfate citrate bile salt sucrose (TCBS) agar and

grown with agitation at 30 °C for 24 h in Luria-Bertani (LB) medium (Beijing Land Bridge Technology Co., Beijing, China) supplemented with 2 % (w/v) NaCl. The bacterial cells were centrifuged at 2,500 g for 5 min and re-suspended in LB medium. The bacterial concentration was confirmed by plate count and adjusted to 10⁵ CFU/mL prior to inoculation of different food matrices.

Food matrices preparation and bacterial inoculation

Four raw seafood [shrimp (*Litopenaeus vannamei*), briny tilapia (*Oreochromis mossambicus*), scallop (*Argopecten irradians*), oyster (*Crassostrea gigas*)] and three raw non-seafood [pork, chicken, freshwater fish (*Ctenopharyngodon idellus*)] types were obtained from a local supermarket in Zhanjiang, China, and stored at -20 °C. The meat (muscle) from these animals/fish was used in the study. For cooked foods, egg-fried-rice was prepared by mixing 50 g of egg with 50 g of boiled rice and cooked at 85 °C for 10 min, and the thawed pork and chicken separately added to boiling water and left for 20 min according to the method of Xie and co-workers [47]. Then the cooked egg-fried-rice, pork and chicken were transferred into a biosafety hood and left to cool to room temperature before subsequent treatment.

The number of each food matrix used in the study was eleven (n=11). Test portions, 10 ± 1 g each of raw briny fish, shrimp, scallop, oyster, pork, chicken and freshwater fish, were separately soaked in sterile water containing 100 ppm chlorine at 15 °C, gently shaken for 5 min and washed 10 times with sterile water to inactivate the native bacteria [48]. Salt at 2 % was added to all sterilized raw and cooked food matrices and transferred to sterile Erlenmeyer flasks. Next, each sample was inoculated with 1 ml of *V. alginolyticus* and mixed thoroughly in a vortex mixer (XW-80A, Qilinbei, Haimen, China) for 10 min to ensure uniform distribution of ~10⁴ CFU/g *V. alginolyticus* in the samples.

Experimental procedure

Construction of the primary growth predictive model

The inoculated samples were stored at 30 ± 0.1 °C in isothermal temperature incubators. Samples were taken at 0, 2, 4, 6, 9, 12, 18, 24, and 36 h for the growth characteristic study. At each time point, 10 g of each food matrix was mixed with 90 mL of sterile 0.85 % physiological saline and vortexed (XW-80A, Qilinbei, Haimen, China) for 2 min. The homogenates were diluted (10×) in sterile 0.85 % physiological saline and 100 µL homogenate was plated onto thiosulfate citrate bile salts sucrose agar (TCBS, Beijing Land Bridge Technology Co., Beijing, China) in triplicate and incubated at 30 °C for 18-20 h. Colony forming units (CFU) were counted manually to determine the density of viable cells in each sample (CFU/g). The plate counting test was repeated three times.

Model fitting

The experimental data obtained at different time points at 30 °C and conditions often used to describe the bacterial growth curves of foods were fitted to a modified Gompertz model [Equation 1] and a logistic model using Origin Pro. 9.0. The best fitting model was used to describe the growth characteristics of *V. alginolyticus* on different food matrices.

$$N_t = N_0 + A \times \exp \left\{ - \exp \left[\frac{\mu_{\max} \times \exp}{A} (\lambda \cdot t) \right] + 1 \right\} \quad (1)$$

where N_t :cell density at a particular storage time (lg CFU/g); N_0 : initial microbial cell density (lg CFU/g); λ : lag time (h); $\mu_{\max}$: maximum specific growth rate (h^{-1}).

Evaluation of model performance

In order to evaluate the goodness-of-fit of the modified Gompertz and logistic models, coefficient of determination (R^2), bias factor (BF), accuracy factor (AF) and the mean square error (MSE) were calculated. Goodness-of-fit of primary model was evaluated using the adjusted R^2 . The MSE was used to evaluate the difference between the growth data estimated by the model with that measured experimentally, with the MSE values approaching zero indicating a closer fit of the data for the model. Besides, validation experiments were carried out to evaluate the models by AF and BF. AF indicates the spread of the results around the predicted values. BF measures the relative average deviation of the predicted and observed *V. alginolyticus* growth. In the study, the predictions exceeding observed data and < 10% on average in terms of lg (CFU/g), were considered to be accurate. That is, $1.0 < \text{BF} < \text{AF} < 1.1$ was defined as a satisfactory limit. R^2 , MSE, BF and AF were defined by the following equations 2-5 [49, 50, 51, 52, 53].

$$R^2 = \frac{\sum (\log N_{\text{predicted}} - \overline{\log N_{\text{observed}}})^2}{\sum (\log N_{\text{predicted}} - \log N_{\text{observed}})^2 - \sum (\log N_{\text{predicted}} - \overline{\log N_{\text{observed}}})^2} \quad (2)$$

$$\text{MSE} = \frac{\sum (\mu_{\text{observed}} - \mu_{\text{predicted}})^2}{n - m} \quad (3)$$

$$\text{BF} = 10 \frac{\sum \lg\left(\frac{N_{\text{predicted}}}{N_{\text{observed}}}\right)}{n} \quad (4)$$

$$\text{AF} = 10 \frac{\sum \left| \lg\left(\frac{N_{\text{predicted}}}{N_{\text{observed}}}\right) \right|}{n} \quad (5)$$

In the above equations, $N_{\text{predicted}}$ is the predicted bacterial number, N_{observed} the observed bacterial number (lg CFU/g), μ_{observed} the observed specific growth rate, $\mu_{\text{predicted}}$ the predicted specific growth rate (h^{-1}), n the number of observations and m the number of parameters of the model.

Hemolysin measurement

All food samples were incubated at 30 °C for 24 h [54, 55] following which 10 g of each food matrix was separately washed with 10 mL of sterile 0.01 M phosphate buffered saline solution (PBS, pH 7.2) and the solution centrifuged (Thermo Lynx 6000, Thermo Scientific, Waltham, MA) at 12,000 rpm for 20 min at 4 °C. The supernatants were filtered (0.22 μm , Millipore, Billerica, MA) and stored at -20 °C until use [56, 57, 58]. The blank control of the different food matrices were subjected to the same procedure except they were not inoculated with *V. alginolyticus*. The *V. alginolyticus* cultured in LB medium was used as the control group.

TDH activity test

Kanagawa phenomenon was tested as previously described by Takeda [59] with slight modifications. The test plate consisted of 5 % rabbit red blood cells (RBCs), 5 µg/mL chloramphenicol and 0.1 % arabinose in Wagatsuma agar medium (Beijing Land Bridge Technology Co., Beijing, China). Once the blood agar solidified, holes were punched in the agar plate. Then, 50 µL of the sample was added to each hole and cultured overnight at 37 °C. The diameter of the β-hemolysin zone around each well was measured, an indicator of a positive reaction for TDH activity.

Hemolytic titer test

Total hemolytic products in the food samples were measured using a hemolytic titer assay [60]. Briefly, rabbit RBCs were extracted by centrifugation of blood (3,500 rpm for 10 min, Thermo Lynx 6000) three times (washing with PBS each time) and diluted to 2 % with PBS. The filtrate (100 µL) of each food matrix was mixed with 100 µL of PBS (0.01 M, pH 7.2) followed by serial 2-fold dilution up to 1: 4096 in a volume of 250 µL/well in a 96-well U-bottom microtiter plate. Twenty-five microliters of 2 % RBCs was added to each diluted solution in wells and incubated for 1 h at 37 °C, then RBCs were resuspended and the plates transferred to 4 °C for 2 h. The dilution that gave 50 % hemolysis was counted. The reciprocal value of this dilution indicated the hemolytic titer in the undiluted sample. The sample are defined as the filtrates of briny tilapia, shrimp, scallop, oyster, freshwater fish, raw pork, raw chicken, cooked pork, cooked chicken and egg fried rice, which were contaminated by *V. alginolyticus*. The rabbit used in this study was obtained from the animal center of Guangdong Province.

Statistical analysis

Results are expressed as mean ± standard error. The differences between groups were determined using the LSD test in SPSS software, version 19.0 (SPSS). A p value of < 0.05 was considered as statistically significant.

Declarations

Abbreviations

Not applicable.

Ethics approval and consent to participate

The animal work (rabbit) presented in this study was approved by the Animal Care and Welfare Committee of Guangdong Ocean University (SYXK 2019-0204).

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RW participated in the project conception, carried out all the experimental work, analyzed and interpreted the data and wrote the manuscript. XH and QD, the corresponding authors, designed, funded and supervised the entire project. YD, ZF, LS, YW and RG contributed to the design and interpretation of experimental results as well as editing and revising the manuscript. All authors have read and approved the final manuscript.

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Tables

Table 1 Modified Gompertz and Logistic models predicting the growth of *V. alginolyticus* in different food matrices.

Statistical indices	Model	Food matrix									
		Briny tilapia	Shrimp	Scallop	Oyster	Freshwater fish	Fresh pork	Fresh chicken	Cooked pork	Cooked chicken	Egg fried rice
R ²	Gompertz	0.998	0.995	0.993	0.994	0.999	0.993	0.995	0.998	0.999	0.994
	Logistic	0.982	0.981	0.984	0.985	0.990	0.988	0.981	0.991	0.985	0.989
MSE	Gompertz	0.0104	0.0145	0.0307	0.0272	0.0343	0.0284	0.0605	0.0202	0.0396	0.0672
	Logistic	0.0256	0.0207	0.0328	0.0313	0.0254	0.0327	0.0454	0.0219	0.0228	0.0661
BF	Gompertz	1.00	1.01	1.01	1.02	1.03	1.01	1.01	1.03	1.04	1.02
	Logistic	0.95	0.94	0.96	0.98	0.93	0.99	0.97	0.92	0.91	0.92
AF	Gompertz	1.02	1.02	1.01	1.03	1.04	1.03	1.05	1.03	1.04	1.03
	Logistic	1.16	1.15	1.16	1.07	1.13	1.14	1.15	1.15	1.14	1.16

Note: R² (coefficient of determination), MSE (the mean square error), BF (bias factor) and AF (accuracy factor).

Table 2 Modified Gompertz fitting model equations for *V. alginolyticus* growth in different food matrices

Food matrix	Curve fitting equation
Briny tilapia	$\text{Log } N_t = 3.38 + 4.73 \cdot \exp\{-\exp[-0.21(t-6.55)]\}$
Shrimp	$\text{Log } N_t = 3.49 + 4.42 \cdot \exp\{-\exp[-0.20(t-7.85)]\}$
Scallop	$\text{Log } N_t = 3.27 + 4.32 \cdot \exp\{-\exp[-0.22(t-7.68)]\}$
Oyster	$\text{Log } N_t = 3.28 + 4.57 \cdot \exp\{-\exp[-0.22(t-7.86)]\}$
Freshwater fish	$\text{Log } N_t = 3.27 + 4.88 \cdot \exp\{-\exp[-0.22(t-6.86)]\}$
Fresh pork	$\text{Log } N_t = 3.21 + 5.53 \cdot \exp\{-\exp[-0.16(t-9.01)]\}$
Fresh chicken	$\text{Log } N_t = 3.13 + 5.19 \cdot \exp\{-\exp[-0.19(t-7.92)]\}$
Cooked pork	$\text{Log } N_t = 3.12 + 5.39 \cdot \exp\{-\exp[-0.21(t-8.34)]\}$
Cooked chicken	$\text{Log } N_t = 3.15 + 4.63 \cdot \exp\{-\exp[-0.22(t-8.05)]\}$
Egg fried rice	$\text{Log } N_t = 3.14 + 5.56 \cdot \exp\{-\exp[-0.17(t-7.61)]\}$

Table 3 Modified Gompertz model fitted to characterise *V. alginolyticus* growth curves in different food matrices.

Food matrix	Growth characteristics			
	Lag time λ (h)	Maximum growth rate $\mu_{\max}$ (log CFU·g ⁻¹ h ⁻¹)	Primary quantity N_0 (log CFU/g)	Maximum quantity $N_{\max}$ (log CFU/g)
Briny tilapia	2.27	1.62	3.38	8.51
Shrimp	2.46	1.47	3.49	8.10
Scallop	3.02	1.32	3.27	7.59
Oyster	3.11	1.24	3.28	7.85
Freshwater fish	2.54	1.40	3.27	8.15
Fresh pork	3.41	0.77	3.21	7.45
Fresh chicken	3.26	0.89	3.13	7.63
Cooked pork	3.43	0.76	3.12	7.44
Cooked chicken	3.27	0.87	3.15	7.50
Egg fried rice	2.63	1.35	3.14	8.07

Figures

Figure 1

Growth curves of *V. alginolyticus* HY9901 in seafood (A) and non-seafood (B) at 30 °C.

Figure 2

Hemolytic zone diameter of *V. alginolyticus* HY9901 in different food matrix filtrates. Means $\pm$ SE with different lowercase letters are significantly different ($p < 0.05$) among different food matrices. LB medium refers to liquid broth medium.

Figure 3

Hemolytic titers of *V. alginolyticus* HY9901 in different food matrix filtrates. Means $\pm$ SE with different lowercase letters are significantly different ($p < 0.05$) between different food matrix types. LB medium refers to liquid broth medium.