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Growth variability of selected *Vibrio parahaemolyticus* strains isolated from seafood

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ABSTRACT

The aim of this study was to quantify the growth and assess the variability of *V. parahaemolyticus* strains isolated from seafood. A total of 35 *V. parahaemolyticus* strains were assessed, and their maximum specific growth rate (μ_{max}) was estimated by the Time-to-Detection Method by regression analysis using the Generalized Reduced Gradient algorithm. The highest μ_{max} (h^{-1}) value was 2.33 for *V. parahaemolyticus* isolated from Atlantic salmon, followed by 2.30 for Mediterranean horse mackerel and European seabass, 2.26 for Mediterranean mussels, 2.20 for veined rapa whelk, 1.88 for the pandemic strain O3:K6, 1.57 for oysters, 1.43 for bluefish, and 1.29 for Gilthead bream. This study provides useful information for the quantitative characterisation of *V. parahaemolyticus* growth, which can be a main input for microbial exposure assessments.

Keywords: *Vibrio parahaemolyticus*, Seafood, Specific growth rate

Introduction

Vibrio parahaemolyticus is a Gram-negative and halophilic bacterium which causes seafood-borne gastroenteritis worldwide (Narayanan et al., 2020). It is a normal habitant in marine and estuary environments. Hence, *V. parahaemolyticus* dwells freely in the water body, attached to the surface or parasite in the gastrointestinal tract of hydrobionts (Tan et al., 2020). The prevalence of *V. parahaemolyticus* varies significantly between geographical regions or different climatic conditions (Ma et al., 2023). However, this pathogen is usually higher in warmer months (Ndraha & Hsiao, 2021). *V. parahaemolyticus* can be more prevalent in fish, shrimps, oysters, mussels, clams, scallops, and squid (Vu et al., 2022; Wang et al., 2022). Seafood is contaminated with *V. parahaemolyticus* because of improper handling, lack of hygiene and refrigeration, and cross-contamination (Stratev et al., 2023). The pathogen can accumulate in hydrobionts, but it could be at higher levels in shellfish because of their filter-feeding behavior. The main pathogenic factors of *V. parahaemolyticus* are thermostable direct hemolysin (tdh) and thermostable direct-related hemolysin genes (trh) (Flynn et al., 2019). *V. parahaemolyticus*-associated gastroenteritis is due to ingesting raw or undercooked seafood. They are seasonally dependent because 67% of the gastroenteritis appear in August and September (Mok et al., 2021). The main clinical symptoms are diarrhoea, abdominal cramps, nausea, vomiting, and fever (Mai et al., 2022). The first outbreak of *V. parahaemolyticus* gastroenteritis was reported in Japan in 1950 after consuming contaminated fish. More outbreaks of contaminated seafood consumption have been reported in the United States, China, Taiwan, Spain, Italy, Chile, and Peru (Odeyemi, 2016).

Maximum specific growth rate (μ_{max}) is considered a universal indicator, relating kinetic information to food-borne pathogens' proliferation. Mathematical models based on μ_{max} allow predicting the behaviour of bacteria in different conditions while having a quantitative assessment at a population level. The maximum specific growth rate is a crucial parameter for developing predictive models which show the practical meaning of strain variability and provide key information for quantitative risk assessment (McMeekin, 1997).

Considering the scarce information and importance of μ_{max} for microbial exposure assessments of *V. parahaemolyticus*, we designed this study to fill these gaps and provide deeper knowledge.

Materials and Methods

Strains Used

In total, 35 *V. parahaemolyticus* strains previously isolated from Mediterranean mussel (*Mytilus galloprovincialis*) (M) (n=12), veined rapa whelk (*Rapana venosa*) (R) (n=7), Mediterranean horse mackerel (*Trachurus mediterraneus*) (SF) (n=5), oysters (*Ostreidae*) (OST) (n=3), Gilthead bream (*Sparus aurata*) (CP) (n=3), Atlantic salmon (*Salmo salar*) (SAL) (n=2), bluefish (*Pomatomus saltatrix*) (CH) (n=1), and European seabass (*Dicentrarchus labrax*) (LAV) (n=1) were used in this study (Stratev et al., 2023). The pandemic strain *V. parahaemolyticus* O3:K6 provided by the National Bank for Industrial Microorganisms and Cell Cultures (Sofia, Bulgaria) was also used as a reference strain.

Preparation of Inoculum

All strains were kept in CASO broth (HiMedia, India) supplemented with glycerin in a fridge at –20°C. After defrosting, each strain was streaked onto Zobell Marine Agar (HiMedia, India) and incubated overnight at 37°C. After that, a single colony was inoculated in alkaline saline peptone water (HiMedia, India) with 2% NaCl and pH 8.6 and incubated at 37°C for 24h to achieve an enriched broth culture of at least log 7 CFU/mL. The enriched broth was centrifuged at 6450 rcf for 5 min. Moreover, decanted, the cell pellet was washed twice, and the bacterial suspension was recovered in alkaline saline peptone water (HiMedia, India).

Determination of Maximum Specific Growth Rate (μ_{max})

A standard 96-well flat-bottom microplates were inoculated with 2-fold serial diluted bacterial cultures, and the optical density was measured every 30 min for 10 hours at 630 nm (Microplate Reader Rayto RT-2100C, China). The method of Cuppers & Smelt (1993) and Membre et al. (2002) was applied for computing the μ_{max} by regression analysis using the Generalized Reduced Gradient algorithm (Excel solver). Each isolate was assessed in triplicate, and the mean values of μ_{max} were calculated using the following basic formula:

$$Mean\ value = \frac{a + b + c}{3}$$

where **a** is the value of μ_{max} from the first assessment, **b** is the value of μ_{max} from the second assessment, and **c** is the value of μ_{max} from the third assessment.

Statistical Analysis

GraphPad Prism (ver. 8.0.1) was used for statistical data processing. Two-way ANOVA with Tukey's multiple comparisons test was performed to show significant differences in the specific growth rate between the investigated strains. The results are presented as mean values. The statistical significance was determined at $p<0.05$.

Results and Discussion

The mean μ_{max} (h^{-1}) of *V. parahaemolyticus* ranged from 0.73 to 2.26 for Mediterranean mussels, 1.63 to 2.20 for veined rapa whelk, 1.67 to 2.30 for Mediterranean horse mackerel, 1.19 to 1.57 for oysters, 0.99 to 1.29 for Gilthead bream, 2.01 to 2.33 for Atlantic salmon, while it was 1.43 for bluefish, 2.30 for European seabass, and 1.88 for the pandemic strain O3:K6. The strain with the highest growth was isolated from Atlantic salmon, i.e. SAL9 – 2.33, and the slowest grower was isolated from Mediterranean mussels, i.e. M5 – 0.73. There was a significant difference ($p<0.05$) in the growth characteristics between the investigated strains from Mediterranean mussels (Figure 1), Mediterranean horse mackerel (Figure 2), Gilthead bream (Figure 3), and between the strains isolated from oysters (Figure 4). No significant difference ($p>0.05$) in the growth characteristics between the strains from veined rapa whelk was found.

V. parahaemolyticus has been reported to be a major seafood-borne pathogen in Asia and the USA responsible for severe infections (Wang et al., 2020a). In China, 322 *V. parahaemolyticus*-associated gastroenteritis outbreaks were recorded, resulting in 9041 illnesses and 3948 hospitalisations between 2003 and 2008 (Wu et al., 2014), while vibrions cause 80000 illnesses and 100 deaths in the United States each year (Hanna et al., 2022). From the above, it is evident that *V. parahaemolyticus* is the most common pathogen in seafood, and the development of a predictive model has market importance for providing safe aquatic products (Wang et al., 2020b). Quantitative risk assessment can be applied to develop effective and efficient risk-based food safety programs. It comprises hazard identification, dose-response assessment, exposure assessment, and risk characterisation (Potter & Brudney, 1994). The exposure assessment step includes determining a few indicators, including the maximum specific growth rate or briefly μ_{max} (Hu et al., 2017). In this study, we determined the μ_{max} of 35 *V. parahaemolyticus* strains using a turbidimetric assay. This method is reliable for estimating bacterial growth under various conditions (Cuppers & Smelt, 1993). It is also rapid, non-destructive, inexpensive, and easily automated (Dalgaard & Koutsoumanis, 2001). Lianou & Koutsoumanis (2011) found higher intra-specific variability of μ_{max} among *S. enterica* strains compared to that observed among the different replicates of one strain. Our results align with this finding as we computed a high range of μ_{max} values, between 0.73 and 2.33, in the investigated strains. Whiting and Golden (2002) stated that this point is important for properly interpreting experimental results because some food microbiologists incorrectly assume that strain-to-strain variation is equal. It is not necessary to be estimated. Moreover, research data generated in this study should be useful in strain selection for food safety challenge tests, assessing the effect of hurdles, and the development of quantitative risk assessment models (Lianou & Koutsoumanis, 2013). Shi et al. (2021) calculated μ_{max} of 18 *V. parahaemolyticus* strains isolated from shrimps by the modified Gompertz model and found values ranging from 0.16 to 0.64 in 2-fold dilution broth culture. Similarly, Wang et al. (2020b) also applied the modified Gompertz model for the μ_{max} calculation of 27 *V. parahaemolyticus* strains isolated from shrimps, and the values ranged from 0.45 to 1.00. At 37°C, Liu et al. (2016) found that μ_{max} ranged from 0.03 to 0.24 at 0.5% NaCl, from 0.02 to 0.44 at 3% NaCl, from 0.01 to 0.26 at 5% NaCl, from 0 to 0.15 at 7% NaCl, and from 0 to 0.12 at 9% NaCl among 50 *V. parahaemolyticus* strains isolated from shrimps. When these results were compared with those of our strains, the higher μ_{max} estimates were evident.

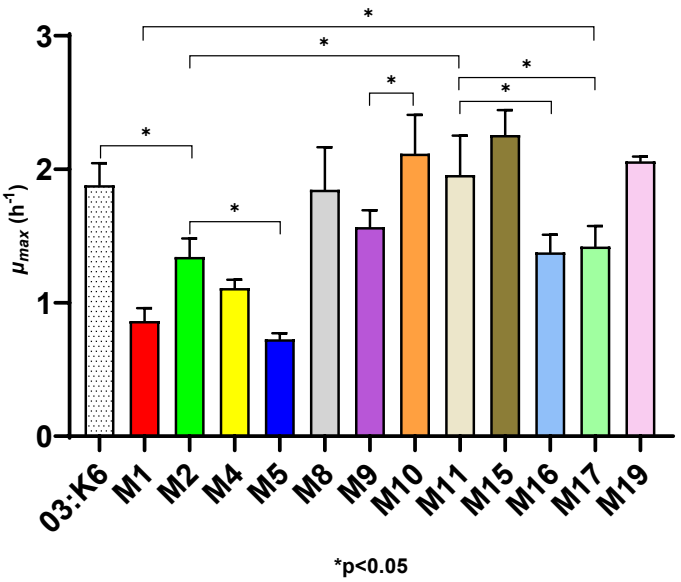

Figure 1. Significant differences between *V. parahaemolyticus* strains from Mediterranean mussels

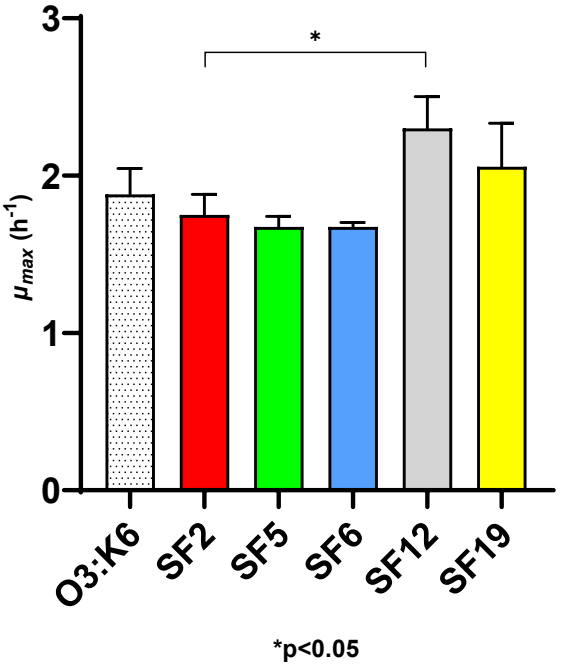

Figure 2. Significant differences between *V. parahaemolyticus* strains from Mediterranean horse mackerel

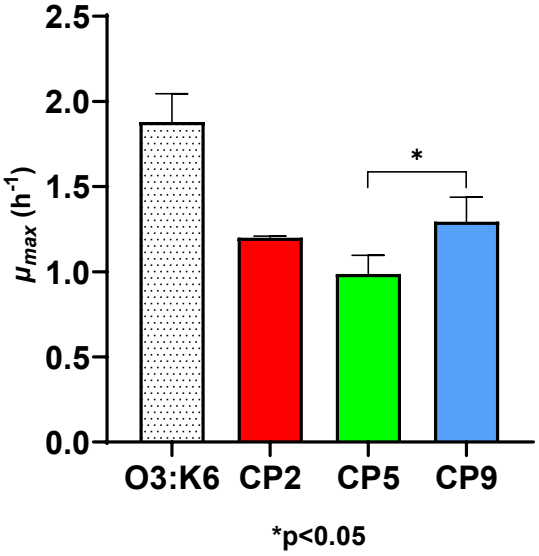

Figure 3. Significant differences between *V. parahaemolyticus* strains from Gilthead bream

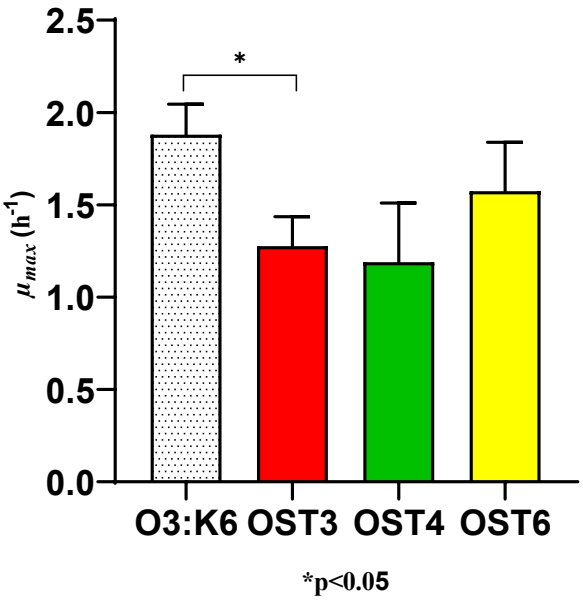

Figure 4. Significant differences between *V. parahaemolyticus* strains from oysters

Conclusion

The results showed that the highest μ_{max} value was for *V. parahaemolyticus* isolated from Atlantic salmon, followed by the values for Mediterranean horse mackerel, European seabass, Mediterranean mussels, veined rapa whelk, oysters, bluefish, and Gilthead bream. This study provides useful information for the quantitative characterisation of *V. parahaemolyticus* growth, which can be a main input for microbial exposure assessments as part of risk analysis of food-borne pathogens.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: This study does not require an ethics committee or special permission.

Data availability: Data will be made available on request.

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Disclosure: -

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