



Efficacy of commercial peroxyacetic acid on *Vibrio parahaemolyticus* planktonic cells and biofilms on stainless steel and Greenshell™ mussel (*Perna canaliculus*) surfaces

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ABSTRACT

The potential of using commercial peroxyacetic acid (PAA) for *Vibrio parahaemolyticus* sanitization was evaluated. Commercial PAA of 0.005 % (v/v, PAA: 2.24 mg/L, hydrogen peroxide: 11.79 mg/L) resulted in a planktonic cell reduction of >7.00 log₁₀ CFU/mL when initial *V. parahaemolyticus* cells averaged 7.64 log₁₀ CFU/mL. For cells on stainless steel coupons, treatment of 0.02 % PAA (v/v, PAA: 8.96 mg/L, hydrogen peroxide: 47.16 mg/L) achieved >5.00 log₁₀ CFU/cm² reductions in biofilm cells for eight strains but not for the two strongest biofilm formers. PAA of 0.05 % (v/v, PAA: 22.39 mg/L, hydrogen peroxide: 117.91 mg/L) was required to inactivate >5.00 log₁₀ CFU/cm² biofilm cells from mussel shell surfaces. The detection of PAA residues after biofilm treatment demonstrated that higher biofilm production resulted in higher PAA residues ($p < 0.05$), suggesting biofilm is acting as a barrier interfering with PAA diffusing into the matrices. Based on the comparative analysis of genomes, robust biofilm formation and metabolic heterogeneity within niches might have contributed to the variations in PAA resistance of *V. parahaemolyticus* biofilms.

1. Introduction

Vibrio parahaemolyticus is a Gram-negative marine-oriented microorganism, widely distributed in seafood such as shellfish, shrimp, and fish. It is a major seafood-borne pathogen, infecting humans via the consumption of raw or undercooked seafood. There are on average 34,664 *V. parahaemolyticus* infections each year in the USA, while 4116 cases have been reported due to cholera and other *Vibrio* illness reported in the Surveillance (COVIS) System during 2010 and 2018 (CDC, 2021; FAO, 2021). In China, they experienced an average of 523.5 reported cases each year during 2010 and 2020 (FAO, 2021). Large scale outbreaks have been reported in South America and Europe, including Chile (Gonzalez-Escalona et al., 2005), Peru (Martinez-Urtaza et al., 2008), and Spain (Baker-Austin et al., 2010). In recent years, *V. parahaemolyticus* outbreaks have been regularly reported in countries with little or no prior incidence including France, Canada, Australia and New Zealand, representing a global increase in *V. parahaemolyticus* as a

food safety concern (FAO, 2021).

The bacteria existing as planktonic cells, attach to surfaces and present in the form of biofilm, which is more resistant to environmental stress, including disinfectant treatments, and posing threats to public health. Although novel alternative control strategies have been studied, chemical sanitisers remain the most common method for controlling pathogens in food manufacture as they are cost effective and easy to use. It is critical to evaluate the effectiveness of each disinfectant on biofilm cells in order to understand the potential risks in the food industry.

V. parahaemolyticus biofilms form on biotic surfaces (e.g., copepods, oysters, clams, fish, shrimps, and mussels) and abiotic surfaces (stainless steel, polystyrene, glass, etc.) in food processing equipment and packaging materials. Biofilms show a higher resistance than their planktonic counterparts (Ashrafudoulla, Mizan, Ha, et al., 2020). Chlorine, one of the most common disinfectants, cannot effectively kill *V. parahaemolyticus* biofilm cells on biotic/abiotic surfaces at recommended concentrations. Rosa et al. (2018) examined the efficacy of

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sodium hypochlorite in reducing the bacterial population of *V. parahaemolyticus* in mature biofilms formed on stainless steel, glass, and polystyrene; they discovered that a 10-min treatment with sodium hypochlorite (20 ppm free available chlorine) reduced cell populations by 3.0 log₁₀ colony forming units (CFU)/cm² from 5.5 log₁₀ CFU/cm². Roy et al. (2021) showed that 5-min treatment with 12 % w/v sodium hypochlorite containing 6 % free chlorine equivalent at concentrations of 50, 100, 200, and 300 ppm, reduced *V. parahaemolyticus* biofilm cells by 0.54 to 2.59 and 0.64 to 2.32 log₁₀ CFU/cm² on shrimp and crab surfaces, respectively, from initial concentrations of 6.87 and 7.37 log₁₀ CFU/cm². Additionally, the authors of the current study found that inactivating strong biofilm-forming strains of *V. parahaemolyticus* on stainless steel coupons required free available chlorine concentrations of over 1000 ppm (Wang et al., 2023b). Therefore, the investigation of other chemical disinfectant alternatives is required.

Peroxyacetic or peracetic acid (PAA) has been proposed as a green alternative to sodium hypochlorite because it does not produce disinfection by-products (DBPs) and imparts no taste or odour (Wang et al., 2020). Sodium hypochlorite dissociates into hypochlorite ions in water, while PAA exists in aqueous equilibrium as acetic acid and hydrogen peroxide, producing further oxidative effects (Sharma et al., 2016). Several PAA commercial formulations are available, for instance, SaniDate® 5.0, VigorOx® 15 F&V, BioSide® HS-15 %, and Tsunami™ 100. PAA is approved for use as a sanitizer in the United States on food contact surfaces (Code of Federal Regulations 21 Part 178.1010) and for direct food contact with meat, poultry and seafood (Code of Federal Regulations 21 Part 173.310) at maximum concentrations of 80 and 110 ppm, respectively. In 2007, the U.S. Food and Drug Administration certified PAA for use as disinfectants in ice and wash water during the commercial preparation of fish and seafood, with a maximum allowed presence of PAA not exceeding 190 ppm (Food Contact Substance Notification FCN 000699). Since 2009, PAA has been legislatively restricted by Food Standards Australia New Zealand, with a limit of 0.7 mg/kg PAA allowed in food products (Australia New Zealand Food Standards F2009C00360). Followingly, the European Food Safety Agency (EFSA) accepts the use of PAA on poultry meat as effective against *Escherichia coli*, *Salmonella* and *Campylobacter* spp., with potential for use in seafood environments (EFSA, 2014).

Although PAA has the potential to control microorganisms in the seafood industry, there remain gaps. The objective of this study was to investigate the efficacy of PAA in reducing *V. parahaemolyticus* as planktonic cells and as biofilms grown on Greenshell™ mussels and food contact surfaces. Understanding the action of PAA on *V. parahaemolyticus* that may occur in the seafood processing environment will assist in the development of effective hygiene strategies.

2. Material & Methods

2.1. *V. parahaemolyticus* isolates

Ten *V. parahaemolyticus* strains were chosen for this investigation (Table 1). Seven of them were isolated from shellfish (mussels and

oysters) by Plant and Food Research Ltd. (Cruz et al., 2015), two were provided by the Institute of Environmental Science and Research Ltd., as pathogens, and the *V. parahaemolyticus* reference strain RIMD2210633 was kindly offered by Dr. Tetsuya Iida from Research Institute for Microbial Diseases in Osaka, Japan. According to the previous study (Wang et al., 2023b), two strains, PFR30J09 and PFR34B02 were proven to be strong biofilm formers and least susceptible to chlorine treatment, whereas PFR21C03 and PFR37D08 were weak biofilm forming strains. Isolates from -80 °C bead storage (Protect™, Thermo Fisher Scientific, USA) were recovered by incubating in 3 % NaCl tryptic soy broth (TSB; Difco™, Becton, Dickson and Company, France) with shaking at 37 °C, 120 rpm. The cells were then centrifuged (8425 ×g, 5 min) to obtain a cell pellet, that was washed and suspended in sterile phosphate buffered saline (PBS) for further use.

2.2. Food contact surface inoculation and biofilm formation

Stainless steel coupons (10 mm², 1 mm thick, 304 grade with a 2B finish; Advanced Sheetmetals, New Zealand) were prepared by soaking in 99.5 % acetone for 12 h, rinsing with distilled water, followed by immersion in alkaline detergent 1 % NaOH (w/v, pH ~13.0; Merck KGaA, Darmstadt, Germany) for 1 h and rinsing again with distilled water. The coupons were cleaned using an ultrasonic cleaner (DT52; BANDELIN, Berlin, Germany) for 60 min, rinsed with distilled water, left to dry and sterilized by dry cycle autoclaving at 121 °C for 15 min. Coupons were freshly prepared for each set of experiments.

A coupon was placed in each well of a 24-well plate (FALCON®, Corning Incorporated, Durham, USA), containing 1 mL of 3 % NaCl TSB with *V. parahaemolyticus* inoculum at a concentration of ~4.00 log₁₀ CFU/mL. The 24-well plates were placed in a static incubator for 6 h at 25 °C for biofilm growth.

2.3. Mussel shell surface inoculation and biofilm formation

Mussel shell surfaces were prepared according to a previous study with minor modifications (Fletcher and Statham, 1988). Briefly, the mussel shell surfaces were freshly cut into regular pieces (1.5 cm × 4 cm) using a disc grinder, washed with sodium carbonate (w/v, 2 %; Lab-Serv™, Thermo Fisher Scientific, USA) to remove grime, slime and naturally formed biofilms, and then immersed in 70 % ethanol to sanitize for 1 h. The mussel shell surfaces were thoroughly washed with sterile saline (0.85 % NaCl) to remove residual ethanol. This treatment was applied for each set of experiments.

Each shell surface was placed upright in a 20 mL sterilized flask containing 5 mL of 3 % NaCl TSB with *V. parahaemolyticus* inoculum at a concentration of ~4.00 log₁₀ CFU/mL (Determination of absorbance based on modelled linear relationship between absorbance of 595 nm and CFU plate counting). The flasks were placed in a static incubator for 6 h at 25 °C for biofilm growth.

Table 1

Vibrio parahaemolyticus strains examined in this study.

No.	Strain	Source	Pathogenicity	Collection date	Country	Reference
1	PFR21C03	Pacific oyster	Non-pathogenic	16/02/2009	New Zealand	(Cruz et al., 2015)
2	PFR24B07	Greenshell™ mussel	Non-pathogenic	2/03/2010	New Zealand	
3	PFR29A04	Pacific oyster	Non-pathogenic	22/11/2010	New Zealand	
4	PFR30G02	Pacific oyster	Non-pathogenic	8/03/2011	New Zealand	
5	PFR30J09	Pacific oyster	Non-pathogenic	21/03/2011	New Zealand	
6	PFR34B02	Pacific oyster	Non-pathogenic	27/03/2012	New Zealand	
7	PFR37C06	Pacific oyster	Non-pathogenic	17/01/2013	New Zealand	
8	PFR37D08	Clinical	Pathogenic	1/01/2013	New Zealand	
9	PFR37E03	Clinical NZRM 3391	Pathogenic	1/01/1975	New Zealand	
10	RIMD2210633	Clinical	Pathogenic	1996	Japan	(Makino et al., 2003)

2.4. Preparing PAA solutions and measuring their concentrations

Commercial PAA (ECOLAB, Hamilton, New Zealand), which contains hydrogen peroxide (10–30 %, CAS-No. 7722-84-1), acetic acid (5–10 %, CAS-No. 64-19-7) and peracetic acid (1–5 %, CAS-No. 79-21-0), was used in this study. The recommended dilution ranges from 0.2 % to 2.0 %. PAA solution were prepared and used within 20 min.

The actual PAA and H₂O₂ concentrations in the commercial preparation were determined using iodometric titrations. For PAA, hydrogen peroxide was first degraded with catalyse (Terminox®, Novozymes A/S, Denmark) followed by titration with sodium thiosulfate. Specifically, a 10 µL PAA sample was diluted with 10 mL of distilled water and kept at 4 °C. After adding 10 mL of Buffer solution A (5.014 g of Na₂HPO₄·12H₂O, ScharLab®, Spain; 4.627 g of KH₂PO₄, Merck, Germany; 0.061 g of EDTA in 1000 mL H₂O) at pH 5.5, the sample was vortexed for 60s. Next, 15 mL of 12 N sulphuric acid (97 %, J.T. Baker®, Avantor, UK) and 15 mL of 166 g/L potassium iodide (Merck, Darmstadt, Germany) solution were added, and the solution was kept in the dark for 20 min. The solution was titrated with 1 % w/v sodium thiosulfate (AnalaR®, BDH, UK) using starch as an indicator, titrating until the blue colour disappeared. The consumption of sodium thiosulfate solution corresponds to the concentration of PAA according to the following equation.

$$C_{PAA} = \frac{N_{Na_2SO_3} \times V_{Na_2SO_3} \times EW_{PAA} \times 1000}{V_{PAA}}$$

where C_{PAA} is the PAA concentration in commercial products (mg/L); $N_{Na_2SO_3}$ is the normality of the thiosulfate solution; $V_{Na_2SO_3}$ is the titration volume of sodium thiosulfate solution required (Jonas et al.); EW_{PAA} is the PAA equivalent weight; and V_{PAA} is the volume of commercial PAA diluted solution (Jonas et al., 2010).

To determine the H₂O₂ concentrations, 10 mL of 10 % chilled sulphuric acid was added to 10 µL of the PAA sample and then diluted with 10 mL of distilled water. Next, 3 drops of 0.025 mol/L Ferroin solution were added to the mixture and titrated using a 0.1 mol/L Ce⁴⁺ sulfate (UNIVAR®, Ajax Finechem, Australia) solution until the colour turned from orange to blue.

The determination of residual PAA and H₂O₂ was based on the *N,N*-diethyl-*p*-phenylenediamine sulfate salt (DPD; BDH, UK) photometric method, as reported by Liu et al. (2015) with minor modifications. PAA (100 µL) was mixed with 50 µL of Buffer solution B (30.25 g of Na₂HPO₄·12H₂O; 23 g of KH₂PO₄; 0.01 g of NaCl; and 0.5 g of KI, UNIVAR®, Ajax Finechem, Australia in 1000 mL of H₂O), and then 50 µL of DPD solution (1.6 g of DPD; 200 µL of 97 % H₂SO₄; and 0.02 g of EDTA in 100 mL of H₂O) was added. The absorption at 550 nm was measured after 30 s using a spectrophotometer (SpectrostarNano, BMG Labtech, Ortenberg, Germany). To measure residual H₂O₂, the same procedure was used, but Buffer solution C, with 5 mg of peroxidase from horseradish, was applied instead of Buffer solution B to determine total peroxide. The absorption at 550 nm was measured after 30 s using a spectrophotometer.

2.5. Disinfectant treatment

To determine the susceptibility of planktonic *V. parahaemolyticus* cells to PAA, sterilized 96-well polystyrene plates (FALCON®, Corning Incorporated, Durham, USA) were loaded with a cell suspension (~8.00 log₁₀ CFU/mL, 100 µL) in saline (0.85 %), along with 100 µL of PAA solution. After a 5-min exposure, the solution was neutralised with 50 µL of 1 % sodium thiosulfate. To test the susceptibility of biofilms, stainless steel coupons or mussel shell surfaces containing pre-formed biofilm were placed into 1 mL of PAA solution. After 5 min, they were transferred to 1 % sodium thiosulfate to neutralise the disinfectant. Each experiment included positive and negative controls.

2.6. Detachment of biofilm populations

The number of viable cells in the biofilm was examined using a glass bead vortex mixing method (Hayrapetyan et al., 2015), with minor modifications. Coupons/surfaces with cultured biofilm cells were gently washed using sterile distilled water to remove planktonic cells and aseptically transferred to bottles with sterile glass beads (D = 3–5 mm; Sigma-Aldrich®, Merck, Darmstadt, Germany) and 0.1 % buffered peptone water which includes 1 % NaCl (Difco™, Becton, Dickson and Company, France), followed by 1 min of vortex mixing to detach biofilm cells from the surfaces. Serial 10-fold dilutions of the solution containing detached biofilm cells were prepared in 0.1 % buffered peptone water and spread-plated on 3 % NaCl tryptic soy agar (TSA; Difco™, Becton, Dickson and Company, France) plates, and then incubated at 37 °C for 18 h.

2.7. Identification of functional genes for strong biofilm formation

The Centre for Environment, Fisheries, and Aquaculture Science (CEFAS) did the DNA extraction, library construction and whole genome sequencing of *V. parahaemolyticus* using MiSeq with a coverage of 40–120× (Baker-Austin et al., 2020). Clean reads were used for de novo assembly and annotation via the Bactopia pipeline using Velvet and SPADES as the assembler (Petit 3rd and Read, 2020). The quality of assembled contigs were assessed using QUAST and CheckM (Gurevich et al., 2013; Parks et al., 2015). The draft genomes were submitted to NCBI GenBank under the BioProject PRJNA808748.

The amino acid sequences of all *V. parahaemolyticus* candidates were analysed using Roary version 3.13.0 with MAFFT as the alignment tool. Comparative analysis of these genomes was presented using a Flower plot. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was conducted using BlastKOALA (<https://www.kegg.jp/blastkoala/>). The variances in KEGG pathways across strong biofilm-forming strains (PFR30J09 and PFR34B02) and the reference strain (RIMD2210633) of *V. parahaemolyticus* were visualized using ggplot2 (version 3.4.0).

2.8. Data analysis

Viable colony counts of cells were enumerated and transformed as log₁₀ CFU/mL or log₁₀ CFU/cm². The mean and standard deviation (SD) for *V. parahaemolyticus* cell counting was based on three biological replicates and three technical replicates. One-way analysis of variance (ANOVA) with a *t*-test (*p* < 0.05) indicated significance of the results. Principle component analysis (PCA) was used to compare biofilm cell resistance against commercial PAA, the analysis was conducted using XLSTAT-Premium software (version 19.3).

3. Results and discussion

3.1. Efficacy of PAA against planktonic *V. parahaemolyticus* cells

Fig. 1 displays the effect of PAA on planktonic *V. parahaemolyticus*. The control cells of planktonic *V. parahaemolyticus* ranged from 7.27 log₁₀ CFU/mL to 7.84 log₁₀ CFU/mL. The concentrations of PAA ranged from 5 to 50 ppm. A concentration of 5 ppm produced a <2.00 log₁₀ CFU/mL reduction. At 15 ppm, there was an average cell decrease of 2.70 ± 0.40 (mean ± SD) log₁₀ CFU/mL. PAA of 35 ppm resulted in an average cell reduction of 4.76 ± 0.38 log₁₀ CFU/mL. At 50 ppm PAA, there were no viable cells detected, indicating a cell reduction of >7.00 log₁₀ CFU/mL (with a 1.00 log₁₀ CFU/mL detection limit, Fig. 1).

3.2. Efficacy of PAA against *V. parahaemolyticus* on stainless steel coupons

Fig. 2 illustrates the effectiveness of PAA in reducing

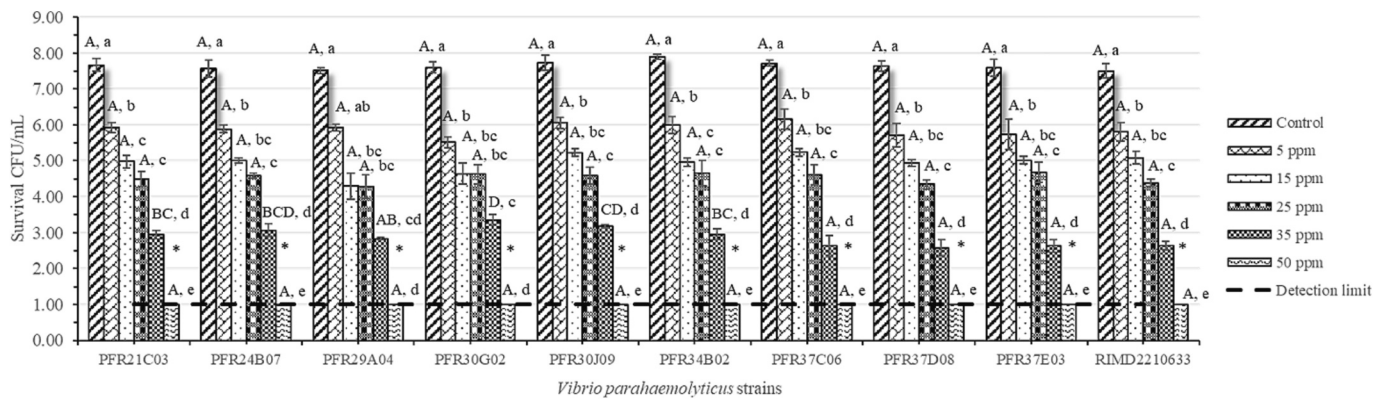


Fig. 1. The efficacy of peroxyacetic acid (PAA) for the inactivation of planktonic *V. parahaemolyticus* cells. The detection limit was 1.00 log₁₀ colony forming units (CFU)/mL. * indicates no detectable cells after exposure of *V. parahaemolyticus* to 50 ppm PAA for 5 min. The means followed by different letters are significantly different ($p < 0.05$), the upper-case letters presented significant difference within species, the lower-case letters presented significant difference after treatment using PAA of different concentrations within the strain.

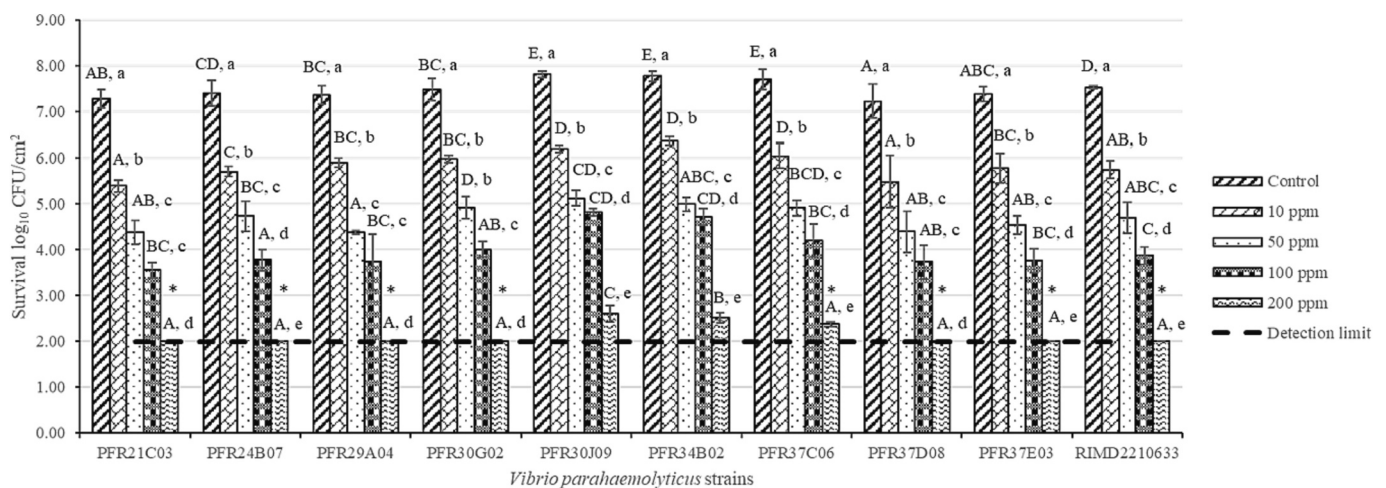


Fig. 2. The efficacy of PAA for the inactivation of *V. parahaemolyticus* on stainless steel coupons. The detection limit was 2.00 log₁₀ colony forming units (CFU)/cm². The means followed by different letters are significantly different ($p < 0.05$), the upper-case letters presented significant difference within species, the lower-case letters presented significant difference after treatment using PAA of different concentrations within the strain.

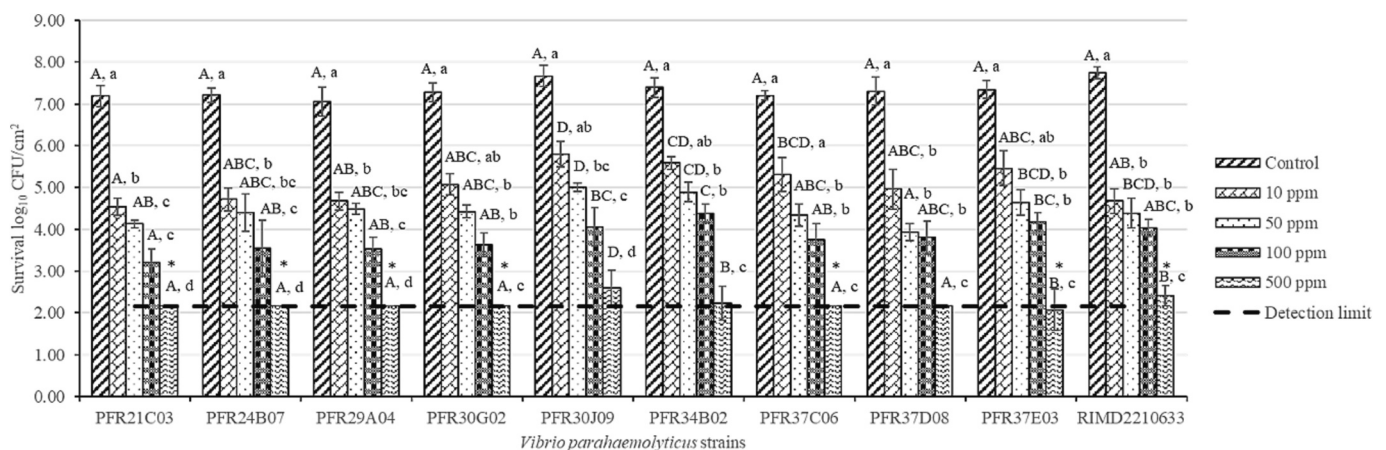


Fig. 3. The efficacy of PAA for the inactivation of *V. parahaemolyticus* on mussel shell surfaces. The detection limit was 2.17 log₁₀ colony forming units (CFU)/cm². The means followed by different letters are significantly different ($p < 0.05$), the upper-case letters presented significant difference within species, the lower-case letters presented significant difference after treatment using PAA of different concentrations within the strain.

V. parahaemolyticus biofilms grown on stainless steel surfaces. *V. parahaemolyticus* biofilm cells averaged $7.50 \pm 0.21 \log_{10}$ CFU/cm² in the untreated control. PAA of 10 ppm, produced log reductions ranging from 1.86 to 2.06 \log_{10} CFU/cm². The average cell numbers decreased by $2.80 \pm 0.52 \log_{10}$ CFU/cm² after treatment with 50 ppm PAA. High biofilm cell reduction was recorded for 100 ppm PAA treatment, averaging of $3.19 \pm 0.44 \log_{10}$ CFU/cm². Cell reductions after PAA treatment using 200 ppm, were over $5.00 \log_{10}$ CFU/cm² (undetectable) for most strains. However, the two strongest biofilm formers (PFR30J09 and PFR34B02) still showed average cell numbers of 2.60 and 2.52 \log_{10} CFU/cm², respectively, indicating that concentrations exceeding 200 ppm of PAA are required to inactivate their biofilm communities.

3.3. Efficacy of PAA against *V. parahaemolyticus* on mussel shell surfaces

Fig. 3 shows the effect of PAA on *V. parahaemolyticus* biofilm cells formed on mussel shell surfaces. The average cell number of biofilm formed on mussel shell surfaces was $7.34 \pm 0.21 \log_{10}$ CFU/cm². The removal of viable cells was 1.42 ± 0.29 , 2.18 ± 0.32 and $2.54 \pm 0.32 \log_{10}$ CFU/cm² after PAA treatment with concentrations of 10, 50 and 100 ppm, respectively. Higher PAA concentrations were required to inactivate *V. parahaemolyticus* biofilms on mussel shell than on stainless steel surfaces; 500 ppm of PAA was required to inactivate $>5.00 \log_{10}$ CFU/cm² biofilm cells from mussel shell surfaces whereas 200 ppm was required to achieve a similar reduction on stainless steel coupons. Four biofilm formers (PFR30J09, PFR34B02, PFR37D08 and RIMD2210633) were still detected after treatment with 500 ppm of PAA. The pathogenic strains RIMD2210633 and PFR37E03 showed greater biofilm community viability on mussel surfaces rather than on stainless steel coupons, suggesting that additional protection may be associated with pathogenic *V. parahaemolyticus* biofilm cells.

3.4. PAA sanitizer residues following biofilm treatment

The original concentrations of peracetic acid and hydrogen peroxide in commercial PAA were titrated as 44.78 ± 6.01 g/L and 235.82 ± 6.37 g/L, respectively. Via absorbance screen of diluted commercial PAA, the linear relationship between peracetic acid concentration (x axis) and OD₅₅₀ (y axis) was $y = 0.0373x + 0.0694$ ($R^2 = 0.9436$), the one between H₂O₂ (x axis) and OD₅₅₀ (y axis) was $y = 0.1032x + 0.2655$ ($R^2 = 0.998$). The residues of peracetic acid and hydrogen peroxide in different dilutions of commercial PAA following biofilm treatment are listed in Tables 2 and 3. The residue concentrations were higher for the PAA treatment of robust biofilm communities of PFR30J09 and PFR34B02, indicating less diffusion into these biofilm matrices and lower effectiveness of the sanitizers.

Table 2
Residues after treating biofilms formed on stainless steel coupons.

PAA dilution	PAA residue (mg/L)*				H ₂ O ₂ residue (mg/L)*			
	200 ppm	100 ppm	50 ppm	10 ppm	200 ppm	100 ppm	50 ppm	10 ppm
PFR21C03	1.88 ^{AB} , a ± 0.16	1.77 ^A , a ± 0.17	1.29 ^{AB} , b ± 0.16	0.25 ^{ABC} , c ± 0.16	18.27 ^{BC} , a ± 0.37	17.9 ^A , b ± 0.61	6.79 ^A , c ± 0.08	1.7 ^A , d ± 0.08
PFR24B07	2 ^{AB} , a ± 0.17	1.8 ^A , ab ± 0.18	1.61 ^B , b ± 0.35	0.16 ^{ABC} , c ± 0.17	21.6 ^{BC} , a ± 0.62	18.93 ^A , b ± 0.49	6.47 ^{AB} , c ± 0.34	1.66 ^{AB} , d ± 0.07
PFR29A03	2.75 ^C , a ± 0.44	2.35 ^B , ab ± 0.28	2.01 ^C , b ± 0.2	0.09 ^{ABC} , c ± 0.15	21.51 ^D , a ± 0.61	18.74 ^C , b ± 0.12	6.52 ^{AB} , c ± 0.23	1.48 ^{ABC} , d ± 0.06
PFR30G02	3.29 ^D , a ± 0.17	2.8 ^C , b ± 0.25	2.07 ^C , c ± 0.16	0.3 ^{BC} , d ± 0.15	21.1 ^{CD} , a ± 1.9	18.49 ^A , a ± 1.17	6.13 ^{BC} , b ± 0.07	1.46 ^{BCD} , d ± 0.17
PFR30J09	4.16 ^E , a ± 0.33	3.36 ^D , b ± 0.3	2.48 ^D , c ± 0.22	0.4 ^D , d ± 0.16	23.85 ^{AB} , a ± 0.87	19.22 ^B , b ± 4.53	6.12 ^{BC} , c ± 0.15	1.22 ^{EF} , d ± 0.05
PFR34B02	5.4 ^F , a ± 0.21	5.33 ^E , a ± 0.16	3.04 ^E , b ± 0.4	0.71 ^E , c ± 0.14	24.49 ^A , a ± 0.98	19.09 ^A , b ± 0.17	6.44 ^{AB} , c ± 0.18	1.48 ^{ABC} , d ± 0.05
PFR37C06	2.03 ^{AB} , a ± 0.18	1.62 ^A , b ± 0.15	1.23 ^{AB} , c ± 0.15	0.38 ^C , d ± 0.19	19.46 ^{CD} , a ± 0.77	17.27 ^B , b ± 0.15	5.23 ^D , c ± 0.2	1.5 ^{ABC} , d ± 0.06
PFR37D08	1.79 ^A , a ± 0.13	1.63 ^A , a ± 0.15	0.99 ^A , b ± 0.14	0 ^A , c ± 0.14	18.83 ^{CD} , a ± 3.02	17.82 ^A , a ± 1.18	5.99 ^{BC} , b ± 0.4	1.06 ^F , d ± 0.25
PFR37E03	2.21 ^B , a ± 0.16	1.71 ^A , b ± 0.14	1.23 ^{AB} , c ± 0.14	0.13 ^{ABC} , d ± 0.16	17.41 ^D , b ± 0.96	18.09 ^A , a ± 0.84	5.78 ^{CD} , b ± 0.32	1.36 ^{CDE} , d ± 0.06
RIMD2210633	2.11 ^{AB} , a ± 0.16	1.53 ^A , b ± 0.22	1.15 ^A , c ± 0.19	0.03 ^{AB} , d ± 0.21	17.33 ^D , a ± 2.07	16.55 ^{AB} , a ± 2.59	6.45 ^{AB} , b ± 0.63	1.24 ^{DEF} , d ± 0.14

* Within the same examined strains, means not followed by the same letter are significantly different ($p < 0.05$). The upper-case letters presented significant difference within species, the lower-case letters presented significant difference after treatment using PAA of different concentrations within the strain.

3.5. Identification of functional genes for robust biofilm formation

PCA analysis revealed that PFR30J09 and PFR34B02 had distinct biofilm communities in terms of PAA resistance compared to the other strains (Fig. 4a). This is in accordance with the results from a previous study, that these two strains are strong biofilm forming strains of *V. parahaemolyticus*. According to comparative analysis of the genomes in Fig. 4b, there are 3854 core genes shared by these ten *V. parahaemolyticus* candidate strains, with PFR30J09 having 778 shell genes and 282 unique genes, while PFR34B02 had 765 shell genes and 239 unique genes, the reference strain RIMD2210633 had 481 shell genes and 240 unique genes. KEGG analysis was used to compare functional variances based on the shell genes and unique genes in *V. parahaemolyticus*. Fig. 4c and Supplemental Table 1 show that PFR30J09 and PFR34B02 had more genes in certain functional pathways, such as metabolic pathways, microbial metabolism in diverse environments, degradation of aromatic compounds, pentose and glucuronate interconversions, amino sugar and nucleotide sugar metabolism, fructose and mannose metabolism, benzoate degradation, xylene degradation, dioxin degradation and mismatch repair. RIMD2210633, being the pathogenetic strain, had more gene counts in the pathway of polyketide sugar unit biosynthesis, bacterial secretion system and flagellar assembly.

4. Discussion and conclusion

In this PAA trial, PAA of 50 ppm resulted in a planktonic cell reduction of $>7.00 \log_{10}$ CFU/mL when initial *V. parahaemolyticus* cells averaged $7.64 \log_{10}$ CFU/mL. Wong et al. (2018) reported PAA at a concentration above 5 ppm (65.75 μ M) was bactericidal to the wild-type *V. parahaemolyticus* strain, KX-V231, and using 5, 7.5 or 15 ppm of PAA resulted in the killing of about 1.00, 2.00 or 4.50 \log_{10} CFU/mL of planktonic cells, respectively. However, the exposure time of PAA in this study was only 5 min, which is much shorter than the one-hour exposure time reported by Wong et al. (2018). This may explain why different effective PAA concentrations for killing planktonic *V. parahaemolyticus* strains were reported between the two studies. Little is known about PAA treatment to *V. parahaemolyticus* biofilm communities. In this study, the difference in sensitivity between planktonic and biofilm cells to PAA treatments demonstrated biofilm matrices lowered the cell susceptibility to the sanitization. Moreover, higher PAA concentrations were required to remove *V. parahaemolyticus* biofilm cells on biotic mussel shell surfaces compared to abiotic stainless steel in this study, with 500 ppm required to inactivate $>5.00 \log_{10}$ CFU/cm² biofilm cells on mussel shell surfaces and 200 ppm required for a similar log reduction on stainless steel coupons. This may be due to the chitin/other nutrient sources in the mussel shell, the pits, edges, and the activation of genes responsible for promoting distinctive biofilm formation in seafood environments.

Table 3
Residues after treating biofilms formed on Greenshell™ mussel shell surfaces.

PAA dilution	PAA residue (mg/L)*				H ₂ O ₂ residue (mg/L)*			
	500 ppm	100 ppm	50 ppm	10 ppm	500 ppm	100 ppm	50 ppm	10 ppm
PFR21C03	5.62 ^A , a ± 0.55	2.13 ^A , a ± 1.21	1.61 ^A , a ± 2.32	0.46 ^A , b ± 0.87	47.51 ^{AB} , a ± 2.76	24.3 ^C , b ± 0.86	15.52 ^{AB} , c ± 0.34	1.49 ^A , d ± 0.04
PFR24B07	5.86 ^D , a ± 0.13	2.35 ^D , b ± 0.07	1.1 ^{CD} , b ± 0.14	0.82 ^C , c ± 0.02	51.37 ^{AB} , a ± 3.73	25.13 ^C , b ± 0.74	16.01 ^A , c ± 0.49	1.49 ^A , d ± 0.04
PFR29A03	7.37 ^D , a ± 0.09	3.19 ^{DE} , b ± 0.03	0.92 ^{CD} , c ± 0.02	0.55 ^C , d ± 0.04	51.13 ^{AB} , a ± 2.81	24.54 ^C , b ± 0.83	15.36 ^B , c ± 0.2	1 ^B , d ± 0.02
PFR30G02	8.45 ^B , a ± 1	3.73 ^{DE} , b ± 0.07	2.85 ^B , b ± 0.48	0.73 ^A , a ± 3.74	51.72 ^{AB} , a ± 3.64	29.54 ^B , b ± 0.3	11.88 ^C , c ± 0.15	1.63 ^C , d ± 0.04
PFR30J09	10.18 ^C , a ± 0.44	4.4 ^B , b ± 0.24	3.35 ^B , c ± 0.22	0.85 ^B , c ± 0.16	53.63 ^{AB} , a ± 1.51	29.79 ^{AB} , b ± 0.53	11.72 ^C , c ± 0.15	1.72 ^C , d ± 0.04
PFR34B02	12.52 ^D , a ± 0.17	6.86 ^C , b ± 0.17	4.02 ^{BC} , b ± 0.1	1.22 ^{BC} , c ± 0.09	51.38 ^{AB} , a ± 4.45	30.84 ^A , b ± 0.28	12.03 ^C , c ± 0.12	1.88 ^D , d ± 0.03
PFR37C06	5.92 ^E , a ± 0.11	2.31 ^G , b ± 0.08	1.84 ^D , b ± 0.1	0.82 ^C , b ± 0.15	46.88 ^B , a ± 3.36	17.92 ^{DE} , b ± 0.3	11.53 ^C , c ± 0.4	0.9 ^E , d ± 0.05
PFR37D08	5.44 ^{EF} , a ± 0.07	2.33 ^{FG} , b ± 0.05	1.56 ^D , b ± 0.18	0.35 ^C , b ± 0.08	46.3 ^B , a ± 2.03	18.89 ^D , b ± 0.73	11.69 ^C , b ± 0.19	1.06 ^B , d ± 0.03
PFR37E03	6.29 ^E , a ± 0.07	2.42 ^{DE} , b ± 0.11	1.85 ^{CD} , b ± 0.29	0.52 ^C , c ± 0.11	48.6 ^{AB} , a ± 0.8	17.71 ^E , b ± 0.6	10.69 ^D , b ± 0.08	0.58 ^F , d ± 0.1
RIMD2210633	6.08 ^F , a ± 0.41	2.2 ^{FG} , b ± 0.1	1.75 ^D , c ± 0.08	0.41 ^C , d ± 0.1	46.62 ^B , a ± 3.99	15.69 ^F , b ± 0.54	6.68 ^E , b ± 0.3	0.26 ^G , d ± 0.09

* Within the same examined strains, means not followed by the same letter are significantly different (*p* < 0.05). The upper-case letters presented significant difference within species, the lower-case letters presented significant difference after treatment using PAA of different concentrations within the strain.

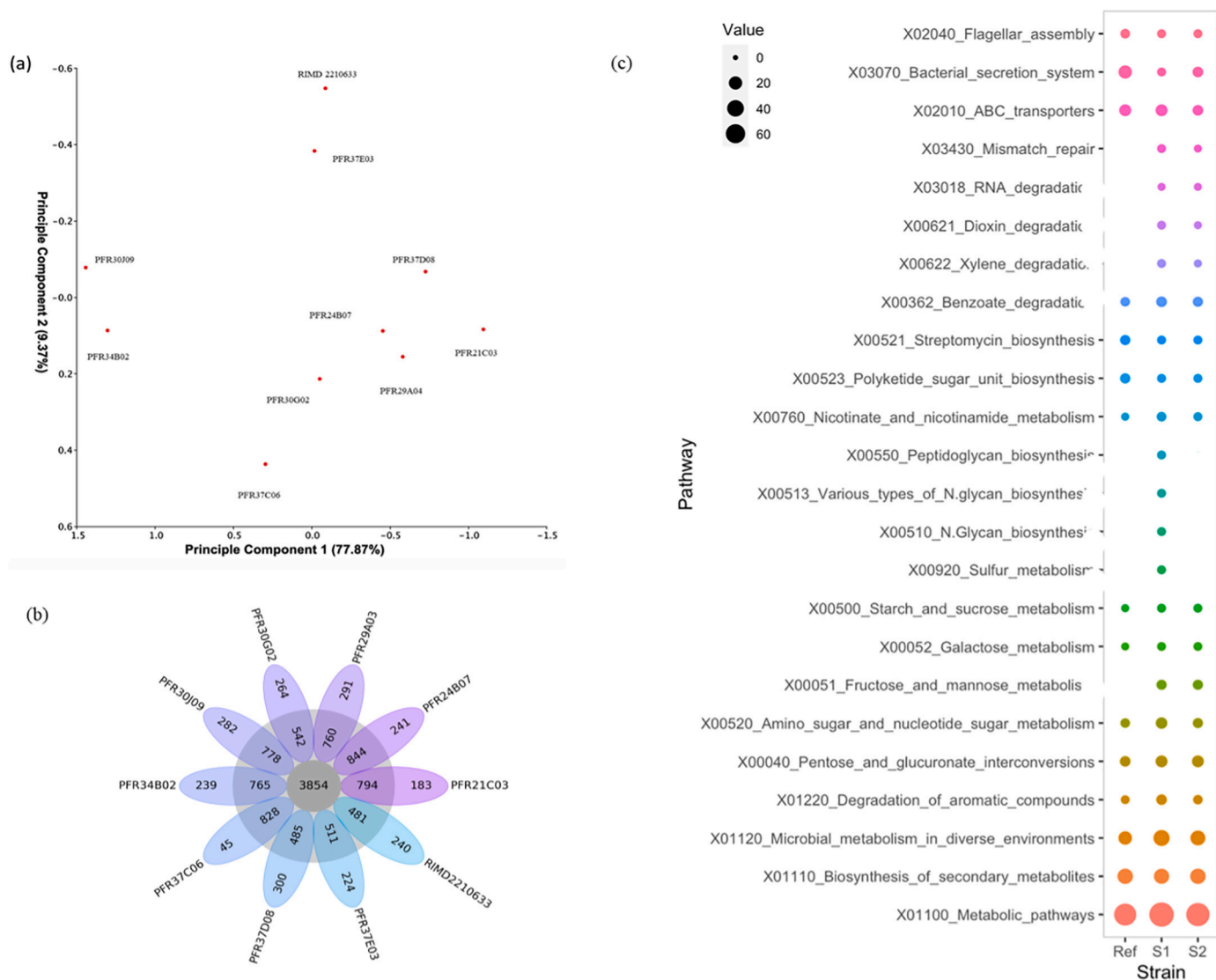


Fig. 4. Distinctive patterns of PAA resistance of *V. parahaemolyticus* biofilm cells and determination of the functional genes in these substrate-dependant resistance pathogens. (a) PCA analysis results of PAA resistance in various *V. parahaemolyticus* biofilm cells. (b) Pangenome analysis of *V. parahaemolyticus* candidate strains. (c) KEGG annotation for shell and unique genes from strong biofilm forming strains (S1: PFR30J09, S2: PFR34B02) and reference strain RIMD2210633, respectively. Multiple colours represent different KEGG pathways, round shape size represents gene count values.

Microorganisms of different species are known to vary in their sensitivity to sanitisers. Wang et al. (2020) reported that *Mycobacterium pschrotoletrans* was reduced to undetectable levels (<1.70 log₁₀ CFU/

mL) with 20 ppm PAA treatment for 5 min, while 40 ppm for 1 min inactivated the cells to undetectable levels. In another study, 80 ppm of PAA exposure for 5 min was reported to inactivate *E. coli* O157:H7

(Rodgers et al., 2004). PAA at 90 ppm is the minimum inhibitory concentration for planktonic *Salmonella* Thompson (Nahar et al., 2022). Melchior et al. (2007) indicated inactivation of cells in a biofilm depends on the sensitivity of each strain in the biofilm. For *Listeria monocytogenes* biofilms formed on polystyrene and stainless steel, PAA of 2000 ppm reduced the biofilm cells by 2.80 log₁₀ CFU/cm² and 3.50 log₁₀ CFU/cm², respectively (Poimenidou et al., 2016). The use of a wide collection of strains for the assessment of the bactericidal activity of disinfectants seems to be necessary to ensure the optimal concentration is used, and the precise concentrations of PAA and H₂O₂ should be detailed.

PAA has shown potential for use in seafood or other meat related environments. For example, Thi et al. (2015) reported that 50 ppm of PAA (240 s exposure) decreased *E. coli* levels below the detection level (<1.00 log₁₀ CFU/g) on *Pangasius* fillets. Wang et al. (2020) reported that 80 ppm PAA on saury (*Cololabis saira*) surfaces, for 1 min led to a 0.50 log₁₀ CFU/cm² reduction in *M. psychrotolerans*, while a 5 min exposure time decreased the biofilm by 2.23 log₁₀ CFU/cm². PAA treatment also has the potential to extend shelf life of stored fish at 4 °C (Wang et al., 2020). Similar results have been reported in poultry: the treatment of beef with 200 ppm PAA delayed the onset of spoilage by 7, 21, and 54 days at 4, 2, and -1 °C, respectively (Yang et al., 2021).

PAA has generally been found to be a more effective disinfectant than sodium hypochlorite against planktonic cells of various microorganisms (Vázquez-Sánchez et al., 2014). However, Alasri et al. (1992) investigated the biocidal activity of some disinfectants against *E. coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and found that chlorine (sodium hypochlorite) was more effective than PAA, even when the latter was considered in combination with H₂O₂. Comparing the sodium hypochlorite effective concentration in inactivating *V. parahaemolyticus* biofilm cells with that reported in a previous study, PAA at 200 ppm was more effective in reducing cells to non-detectable levels for 80 % of strains from stainless steel coupons whereas sodium hypochlorite could not achieve this result (Wang et al., 2023b). In a similar research reported on biofilms of *E. coli*, *Salmonella Typhimurium* and *L. monocytogenes*, exposure to 100 ppm of sodium hypochlorite and PAA reduced biofilms cells by 0.50 to 3.63 and 2.83 to 5.78 log₁₀ CFU/coupon (5 cm × 2 cm), respectively (Park et al., 2012).

Oxidizing disinfectants kill pathogens by oxidizing the cell wall and causing lysis, or by diffusing through the cell wall and oxidizing the intracellular material (United States Environmental Protection Agency EPA 832-F-12-030). PAA treatment of *M. psychrotolerans* cells was found to cause damage to the cell membrane and cell surface without damaging chromosomal DNA and protein profiles, suggesting a mechanism involving oxidative damage (Wang et al., 2020). Although the mechanisms of planktonic *V. parahaemolyticus* resistance to PAA are not fully understood, it is believed that the accumulation of reactive oxygen species and the presence of catalase genes *kate1* and *kate2* contribute to resistance (Wong et al., 2018). In this study, the resistance of robust biofilm matrices and physiological heterogeneity across strains may have contributed to variances in resistance. The identified variations in metabolic pathways within robust and weak biofilm-forming strains could explain mechanisms induced by sanitization in *V. parahaemolyticus*. Related results have been reported in recent studies. Wang et al. (2023a) identified that chlorine sanitization induced *E. coli* on pea sprouts into a VBNC state with differentiated metabolic pathways and metabolite contents (culturable counterparts as the control). The metabolic pathways of amino acid, organic acids, sugars, alcohol and nucleotide derivatives contributed to the dormancy and stronger resistance against sanitizers. Electrolyzed water acts as a sanitizer containing high oxidative and chlorine components. Electrolyzed water (4 mg/L free available chlorine) in addition with 50 °C heat treatment induced a reduction of *E. coli* by 2.31 log₁₀ CFU/mL. Liu et al. (2020), through metabolomic analysis, demonstrated discriminative metabolic pathways of amino acid metabolism, nucleotide synthesis as well as lipid biosynthesis resulting in cell adaptation and stress response against the electrolyzed water and mild heat treatment in *E. coli* O157:H7. Another

study showed the antimicrobial mechanisms of chlorine against *E. coli* biofilms (formed on stainless steel and high-density polyethylene surfaces) based on metabolomic investigation. The altered pathways were associated with amino acid metabolism, energy metabolism, and anti-chlorine metabolism in response to oxidative and osmotic stressors (Lin et al., 2022).

In accordance with published research, this study pointed out associations between metabolism of biofilm cells and sanitizer resistance. However, how metabolite patterns vary with sanitization treatment time is not clear. Inactivation kinetics is a predictable approach to determine the efficacy of sanitizers over time on biofilm cells. Zhao et al. (2022) reported the inactivation kinetics against electrolysed water combined with ultrasound treatment. A modified Weibull model (R²: 0.81–0.97; RMSE: 0.04–0.71) was in a good fit, providing detailed information during the decontamination process. This study also screened metabolite profiles of *E. coli* biofilm cells, showing that the ultrasound treatment disrupted nucleotide metabolism and the electrolyzed water suppressed pathways of nucleotide biosynthesis, amino acid biosynthesis and energy-associated metabolism, suggesting a decreasing presence of nucleotide-related compounds (e.g., uridine, ATP, ADP) and most carbohydrates.

The present study revealed mechanisms of sanitization on *V. parahaemolyticus* biofilm cells based on whole genome sequencing. Integrated omics may provide more detailed information to understand antimicrobial and antibiofilm activities of various sanitizers (Lin et al., 2023; Liu et al., 2023; Mao et al., 2022). A combination of transcriptomics, proteomics, and metabolomics will help support the verification of results from whole genome sequencing, particularly demonstrating how sanitization influences phenotypic expression. Further exploration is needed to understand how these genes cooperate in *V. parahaemolyticus* and how they are quantitatively regulated.

5. Conclusion

This study evaluated the efficacy of using commercial PAA to sanitize *V. parahaemolyticus* planktonic cells and biofilm cells formed on stainless steel and Greenshell™ mussel shell surfaces, mimicking the scenarios in the seafood industry. PAA of 50 ppm resulted in total inactivation (>7.00 log₁₀ CFU/mL) of planktonic cells. PAA of 200 ppm reduced >5.00 log₁₀ CFU/cm² biofilm cells from stainless steel surfaces for 80 % of *V. parahaemolyticus* strains. However, strong biofilm-forming strains showed decreased efficacy, indicating that biofilm matrices interfere with PAA diffusion and highlighting the need to screen for persistent *V. parahaemolyticus* in the seafood industry. PAA of 500 ppm was required to inactivate >5.00 log₁₀ CFU/cm² biofilm cells from mussel shell surfaces, indicating lower efficacy on biotic surfaces. Overall, PAA was found effective in reducing the burden of food safety and public health risks associated with *V. parahaemolyticus* contamination.

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CRedit authorship contribution statement

Dan Wang: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft, review & editing; Steve Flint: Project administration, Resources, Supervision, Conceptualization, Methodology, Writing – review & editing. Graham Fletcher: Resources, Conceptualization, Methodology, Supervision, Writing – review & editing. Jon Palmer, Dragana Gagic & Stephen On: Conceptualization, Methodology, Supervision, Writing – review & editing.

- 0.05 % commercial PAA was required to inactivate biofilm cells from mussel shell surfaces, whereas 0.02 % PAA for biofilm cells removal from stainless steel surfaces.

- Robust biofilm formation and metabolic heterogeneity within niches might have contributed to the variations in PAA resistance of *V. parahaemolyticus* biofilms.

Declaration of competing interest

The authors declare no competing financial interests or personal relationships.

Data availability

Data will be made available on request.

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References

- Alasri, A., Roques, C., Michel, G., Cabassud, C., Aptel, P., 1992. Bactericidal properties of peracetic acid and hydrogen peroxide, alone and in combination, and chlorine and formaldehyde against bacterial water strains. *Can. J. Microbiol.* 38, 635–642.
- Baker-Austin, C., Stockley, L., Rangdale, R., Martinez-Urtaza, J., 2010. Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: a European perspective. *Environ. Microbiol. Rep.* 2, 7–18.
- Australia New Zealand Food Standards F2009C00360. Processing aids. Retrieved from <https://www.legislation.gov.au/Details/F2009C00360/>.
- Baker-Austin, C., Jenkins, C., Dadzie, J., Mestanza, O., Delgado, E., Powell, A., Bean, T., Martinez-Urtaza, J., 2020. Genomic epidemiology of domestic and travel-associated *Vibrio parahaemolyticus* infections in the UK, 2008–2018. *Food Control* 115, 107244.
- CDC, 2021. The Cholera and Other *Vibrio* Illness Surveillance (COVIS) System. US Department of Health and Human Services.
- Code of Federal Regulations 21 Part 173.310. Secondary direct food additives permitted in food for human consumption: boiler water additives. Retrieved from <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/cfrsearch.cfm?fr=173.310>.
- Code of Federal Regulations 21 Part 178.1010. Substances utilized to control the growth of microorganisms: sanitizing solutions. Retrieved from <https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?FR=178.1010>.
- Cruz, C.D., Hedderley, D., Fletcher, G.C., 2015. Long-term study of *Vibrio parahaemolyticus* prevalence and distribution in New Zealand shellfish. *Appl. Environ. Microbiol.* 81, 2320–2327.
- EFSA, 2014. Scientific opinion on the evaluation of the safety and efficacy of peroxyacetic acid solutions for reduction of pathogens on poultry carcasses and meat. *EFSA J.* 12, 3599.
- FAO, 2021. Advances in science and risk assessment tools for *Vibrio parahaemolyticus* and *V. vulnificus* associated with seafood: meeting report. Retrieved from <https://www.who.int/publications/i/item/9789240024878>.
- Fletcher, G.C., Statham, J.A., 1988. Shelf-life of sterile yellow-eyed mullet (*Aldrichetta forsteri*) at 4 °C. *J. Food Sci.* 53, 1030–1035.
- Food Contact Substance Notification FCN 000699. 190 ppm PAA in water and ice for seafood. Retrieved from <http://vm.cfsan.fda.gov/~dms/opa-fcn.html>.
- Gonzalez-Escalona, N., Cachicas, V., Acevedo, C., Rioseco, M.L., Vergara, J.A., Cabello, F., Romero, J., Espejo, R.T., 2005. *Vibrio parahaemolyticus* diarrhea, Chile, 1998 and 2004. *Emerg. Infect. Dis.* 11, 129–131.
- Gurevich, A., Saveliev, V., Vyahhi, N., Tesler, G., 2013. QUASt: quality assessment tool for genome assemblies. *Bioinformatics* 29, 1072–1075.
- Hayrapetyan, H., Muller, L., Tempelars, M., Abee, T., Groot, M.N., 2015. Comparative analysis of biofilm formation by *Bacillus cereus* reference strains and undomesticated food isolates and the effect of free iron. *Int. J. Food Microbiol.* 200, 72–79.
- Jonas, K., Edwards, A.N., Ahmad, I., Romeo, T., Römling, U., Melefors, O., 2010. Complex regulatory network encompassing the Csr, c-di-GMP and motility systems of *Salmonella* Typhimurium. *Environ. Microbiol.* 12, 524–540.
- Lin, Z., Chen, T., Zhou, L., Yang, H., 2022. Effect of chlorine sanitizer on metabolic responses of *Escherichia coli* biofilms “big six” during cross-contamination from abiotic surface to sponge cake. *Food Res. Int.* 157, 111361.
- Lin, Z., Wang, G., Zhang, K., Jiang, S., Li, S., Yang, H., 2023. Metabolomics investigation of global responses of *Cronobacter sakazakii* against common sanitizing in infant formula processing environments. *Food Res. Int.* 172, 113162.
- Liu, D., Straus, D.L., Pedersen, L.F., Meinelt, T., 2015. Comparison of the toxicity of Wofasteril peracetic acid formulations E400, E250, and Lspez to *Daphnia magna*, with emphasis on the effect of hydrogen peroxide. *N. Am. J. Aquac.* 77, 128–135.
- Liu, J., Zhao, H., Yin, Z., Dong, H., Chu, X., Meng, X., Li, Y., Ding, X., 2023. Application and prospect of metabolomics-related technologies in food inspection. *Food Res. Int.* 171, 113071.
- Liu, Q., Chen, L., Laserna, A.K.C., He, Y., Feng, X., Yang, H., 2020. Synergistic action of electrolyzed water and mild heat for enhanced microbial inactivation of *Escherichia coli* O157:H7 revealed by metabolomics analysis. *Food Control* 110, 107026.
- Makino, K., Oshima, K., Kurokawa, K., Yokoyama, K., Uda, T., Tagomori, K., Iijima, Y., Najima, M., Nakano, M., Yamashita, A., Kubota, Y., Kimura, S., Yasunaga, T., Honda, T., Shinagawa, H., Hattori, M., Iida, T., 2003. Genome sequence of *Vibrio parahaemolyticus*: a pathogenic mechanism distinct from that of *V. cholerae*. *Lancet* 361, 743–749.
- Mao, X., Xia, L., Yang, L., You, Y., Luo, P., Li, Y., Wu, Y., Jiang, G., 2022. Data mining of natural hazard biomarkers and metabolites with integrated metabolomic tools. *J. Hazard. Mater.* 427, 127912.
- Martinez-Urtaza, J., Huapaya, B., Gavilan, R.G., Blanco-Abad, V., Ansedo-Bermejo, J., Cadarso-Suarez, C., Figueiras, A., Trinanes, J., 2008. Emergence of Asiatic *Vibrio* diseases in South America in phase with El Niño. *Epidemiology* 19, 829–837.
- Melchior, M., Fink-Gremmels, J., Gaastra, W., 2007. Extended antimicrobial susceptibility assay for *Staphylococcus aureus* isolates from bovine mastitis growing in biofilms. *Vet. Microbiol.* 125, 141–149.
- Nahar, S., Jeong, H.L., Cho, A.J., Park, J.-H., Han, S., Kim, Y., Park, S.-H., Ha, S.-D., 2022. Efficacy of ficin and peroxyacetic acid against *Salmonella enterica* serovar Thompson biofilm on plastic, eggshell, and chicken skin. *Food Microbiol.* 104, 103997.
- Park, S.-H., Cheon, H.-L., Park, K.-H., Chung, M.-S., Choi, S.H., Ryu, S., Kang, D.-H., 2012. Inactivation of biofilm cells of foodborne pathogen by aerosolized sanitizers. *Int. J. Food Microbiol.* 154, 130–134.
- Parks, D.H., Imelfort, M., Skennerton, C.T., Hugenholtz, P., Tyson, G.W., 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res.* 25, 1043–1055.
- Petit 3rd, R.A., Read, T.D., 2020. Bactopia: a flexible pipeline for complete analysis of bacterial genomes. *mSystems* 5, e00190-00120.
- Poimenidou, S.V., Chrysadakou, M., Tzakoniati, A., Bikouli, V.C., Nychas, G.-J., Skandamis, P.N., 2016. Variability of *Listeria monocytogenes* strains in biofilm formation on stainless steel and polystyrene materials and resistance to peracetic acid and quaternary ammonium compounds. *Int. J. Food Microbiol.* 237, 164–171.
- Rodgers, S.L., Cash, J.N., Siddiq, M., Ryser, E.T., 2004. A comparison of different chemical sanitizers for inactivating *Escherichia coli* O157: H7 and *Listeria monocytogenes* in solution and on apples, lettuce, strawberries, and cantaloupe. *J. Food Prot.* 67, 721–731.
- Rosa, J.V., Conceição, N.V., Conceição, R.C.S., Timm, C., 2018. Biofilm formation by *Vibrio parahaemolyticus* on different surfaces and its resistance to sodium hypochlorite. *Cien. Rur.* 48.
- Roy, P.K., Mizan, M.F.R., Hossain, M.I., Han, N., Nahar, S., Ashrafudoulla, M., Toushik, S. H., Shim, W.B., Kim, Y.M., Ha, S.D., 2021. Elimination of *Vibrio parahaemolyticus* biofilms on crab and shrimp surfaces using ultraviolet C irradiation coupled with sodium hypochlorite and slightly acidic electrolyzed water. *Food Control* 128, 108179.
- Sharma, V.K., Johnson, N., Cizmas, L., McDonald, T.J., Kim, H., 2016. A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. *Chemosphere* 150, 702–714.
- Thi, A.N.T., Sampers, I., Van Haute, S., Samapundo, S., Nguyen, B.L., Heyndrickx, M., Devlieghere, F., 2015. Decontamination of *Pangasius* fish (*Pangasius hypophthalmus*) with chlorine or peracetic acid in the laboratory and in a Vietnamese processing company. *Int. J. Food Microbiol.* 208, 93–101.
- Vázquez-Sánchez, D., Cabo, M.L., Ibusquiza, P.S., Rodríguez-Herrera, J.J., 2014. Biofilm-forming ability and resistance to industrial disinfectants of *Staphylococcus aureus* isolated from fishery products. *Food Control* 39, 8–16.
- Wang, D., Yamaki, S., Kawai, Y., Yamazaki, K., 2020. Sanitizing efficacy and antimicrobial mechanism of peracetic acid against histamine-producing bacterium, *Morganella psychrotolerans*. *LWT* 126, 109263.
- Wang, D., Fletcher, G.C., On, S.L.W., Palmer, J.S., Gagic, D., Flint, S.H., 2023b. Biofilm formation, sodium hypochlorite susceptibility and genetic diversity of *Vibrio parahaemolyticus*. *Int. J. Food Microbiol.* 385, 110011.
- Wang, Y., Chen, Z., Zhao, F., Yang, H., 2023a. Metabolome shifts triggered by chlorine sanitisation induce *Escherichia coli* on fresh produce into the viable but nonculturable state. *Food Res. Int.* 171, 113084.
- Wong, H.-C., Liao, R., Hsu, P., Tang, C.-T., 2018. Molecular response of *Vibrio parahaemolyticus* to the sanitizer peracetic acid. *Int. J. Food Microbiol.* 286, 139–147.
- Yang, X., Wang, H., Hrycauk, S., Klassen, M.D., Ercolini, D., 2021. Effects of peroxyacetic acid spray and storage temperature on the microbiota and sensory properties of vacuum-packed subprimal cuts of meat. *Appl. Environ. Microbiol.* 87, e03143-03120.
- Zhao, L., Poh, C.N., Wu, J., Zhao, X., He, Y., Yang, H., 2022. Effects of electrolysed water combined with ultrasound on inactivation kinetics and metabolite profiles of *Escherichia coli* biofilms on food contact surface. *Innovative Food Sci. Emerg. Technol.* 76, 102917.
- United States Environmental Protection Agency EPA 832-F-12-030, Alternative disinfection methods fact sheet: peracetic acid. Retrieved from https://www.epa.gov/sites/default/files/2019-08/documents/disinfection_paa_fact_sheet_2012.pdf.