Sanitizing efficacy and antimicrobial mechanism of peracetic acid against histamine-producing bacterium, *Morganella psychrotolerans*

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Abstract

In this study, we aimed to investigate the sanitizing effects of peracetic acid (PAA), and the underlying mechanism of its antimicrobial action against *Morganella psychrotolerans*. The exposure of *M. psychrotolerans* to 20 ppm PAA for 5 min led to its decrease below the detection level, indicating that PAA has a significant antibacterial effect against *M. psychrotolerans* in *vitro*. Moreover, the viable counts of *M. psychrotolerans* on saury (*Cololabis saira*) surface were showed a reduction of 1.40 and 2.23 log CFU/cm^2 upon treatment with 80 ppm PAA for 1 and 5 min, respectively. Further, PAA treatment delayed the growth of *M. psychrotolerans* on saury surface during storage at 4°C. Next, the antimicrobial mechanism of PAA against *M. psychrotolerans* cells was investigated, and the damage to the cell membrane and cell surface upon PAA treatment was observed using scanning electron microscopy (SEM) and epifluorescence microscopy. The chromosomal DNA and the protein profiles after PAA treatment were also analyzed. Form our results, we hypothesized that the bactericidal effect of PAA treatment was mainly attributed to damage the bacterial cell membrane. These results indicate that PAA may be an efficient disinfectant against *M. psychrotolerans* and has applications in seafood processing and storage.

Keywords

Morganella psychrotolerans; disinfection; peracetic acid; disinfectant effect; seafood safety; antimicrobial mechanism

1. Introduction

The consumption of seafood has increased considerably worldwide, over the past few decades, because of its rich nutritional composition (Seves *et al.*, 2016). However, histamine food poisoning is a major concern due to seafood consumption. This kind of food poisoning is mainly caused by the ingestion of seafood containing high levels of histamine (Bjornsdottir-Butler, McCarthy, Dunlap, & Benner, 2016). Histamine is generated from free histidine by the action of the enzyme bacterial decarboxylase. The psychrotrophic histamine-producing bacteria are of particular concern in the seafood industry. They can cause outbreaks of histamine poisoning at low temperatures (Emborg, 2006; Morii & Kasama, 2004). *M. psychrotolerans*, a pathogenic psychrotolerant histamine-producing bacterium, belongs to the family *Enterobacteriaceae* and is able to grow and produce toxic levels of histamine (Emborg, 2006; Emborg & Dalgaard, 2006). In previous studies, the link between the cases of histamine poisoning and seafood contaminated with *M. psychrotolerans* has been reported (Dalgaard, Emborg, Kjølby, Sørensen, & Ballin, 2008; Emborg & Dalgaard, 2006; Emborg, Laursen, & Dalgaard, 2005). In our previous studies, we revealed the presence of *M. psychrotolerans* in retail seafood distributed in Japan (Kato *et al*., 2017) and also showed that *M. psychrotolerans* had high histamine-producing abilities (Wang, Yamaki, Kawai, & Yamazaki, 2020). Therefore, controlling *M. psychrotolerans* populations in seafood is a major challenge faced by the seafood industry.

In general, sanitizers can generally disinfect foodborne pathogens effectively and affordably from the seafood surfaces, attributing to their high prevalence within the food industry (Shen, Luo, Nou, Wang, & Millner, 2013; Rahman, Jin, & Oh, 2011). Sodium hypochlorite (SH) is an effective disinfectant and is widely used to reduce microbial counts. However, it has been shown that the improper use of chlorine can produce carcinogenic by-products and residues, such as chloroform and bromodichloromethane (Gil, Selma, López-Gálvez, & Allende, 2009; Reckhow, Singer, & Malcolm, 1990). Therefore, it is necessary to find safer antimicrobial agents for food sanitization. Peracetic acid (PAA) is a well-known sanitizer generally used for processing water, fruits, and vegetables (Leggett *et al*., 2016; Ho, Luzuriaga, Rodde, Tang, & Phan, 2011; Hilgren & Salverda, 2000; Lambert, Johnston, & Simons, 1999; Rudd & Hopkinson, 1989). PAA by-products are safer than those produced during SH treatment (Mora, Veijalainen, & Heinonen-Tanski, 2018; Dell'Erba, Falsanisi, Liberti, Notarnicola, & Santoro, 2007). An earlier report shown that 15 ppm PAA treatment resulted in the elimination of *Vibrio parahaemolyticus* cultured in Luria-Bertani-3 % NaCl culture broth (Wong, Liao, Hsu, & Tang, 2018). Sheng, Shen, & Zhu (2020) reported that 80 ppm PAA treatment for 2 min resulted in a 1.8 log CFU/apple reduction in *Enterococcus faecium*. Moreover, 50 ppm PAA treatment for 240 s was shown to reduce the count of *Escherichia coli* below the detectable level (< 1 log CFU/g) on *pangasius* fillets (Thi *et al*., 2015). However, there are limited

74 scientific reports on the sanitizing effects and antimicrobial mechanisms of PAA on
75 histamine-producing bacteria.
76 Therefore, in the present study, we evaluated the sanitization effect of PAA against
77 *M. psychrotolerans* both in *vitro* and on saury (*Cololabis saira*) surface. The effect of
78 PAA treatment on cell morphology, cell membranes, cellular protein, and
79 chromosomal DNA was investigated to better understand the disinfection mechanism
80 of PAA. Our study provides useful insight on the use of PAA as a disinfectant for
81 seafood to prevent *M. psychrotolerans*-related histamine food poisoning.
82

83 **2. Materials and methods**

84 *2.1. Cell culture conditions*

85 *M. psychrotolerans* JCM 16473^{T} was cultured in tryptic soy broth (TSB, BD,
86 Franklin Lakes, NJ) at 25 °C for 24 h. The cell cultures (approximately 10^{9} CFU/mL)
87 were centrifuged at 6,000 × *g* for 10 min at 4 °C. The harvested cell pellets were
88 washed twice with phosphate-buffered saline (0.01 M PBS, pH 7.2) and subjected to
89 PAA treatments as described below.
90

91 *2.2. Evaluation of antibacterial activity of PAA*

92 The minimum inhibitory concentration (MIC) and minimum bactericidal
93 concentration (MBC) were assessed using a 96-well microplate. The washed bacterial
94 cells were resuspended in 2-fold TSB at the inoculum concentration of approximately

95 5.2×10^5 CFU/mL. In each plate, 0.1 mL of 2-fold TSB inoculated with *M.*
96 *psychrotolerans* were mixed with 0.1 mL of PAA (Tec P-10, Adeka Clean Aid Co.
97 Ltd. Tokyo, Japan), and incubated at 25 °C for 48 h. The MICs were determined by
98 evaluating the lowest concentration of PAA with no bacterial growth observed by the
99 visual turbidity (Chotigarpa *et al*., 2018). The MBCs were determined by spreading
100 the cells on tryptic soy agar (TSA, BD, Franklin Lakes, NJ) plates and incubating at
101 25 °C for 48 h after the inactivation step. Briefly, the 0.1 mL of the culture broth
102 without the microorganism growth was inactivated using 0.9 ml of the inactivation
103 buffer (Lectin 10 g/L, Polyoxyethylene (20) sorbitan monooleate 30 g/L, L-histidine 1
104 g/L, Tryptone 1 g/L, $Na_2S_2O_3{\cdot}5H_2O$ 20 g/L) and then inoculated the cells and plated
105 on the TSA plate. The MBCs were determined by the lowest concentration of PAA
106 that completely inhibited the *M. psychrotolerans* colony growth (Meireles *et al*.,
107 2015).
108 Next, the bacterial disinfection with PAA treatment was tested in *vitro.* The washed
109 bacterial cells were resuspended in PBS at the inoculum size of approximately 10^9
110 CFU/mL, and 9 mL of the bacterial suspension was mixed with 1 mL PAA (10, 20,
111 40, and 80 ppm). Sterile deionized water was used as the control. After treatment for
112 1, 3, or 5 min, 0.1 mL of treated suspension was immediately mixed with 0.9 mL of
113 inactivation buffer. The neutralized samples were serially diluted (1:10) with PBS
114 (pH 7.2) and 0.2 mL from each dilution was spread on agar plates consisting of TSA
115 supplemented with 0.2 % sodium pyruvate. The *M. psychrotolerans* survivor colonies

were counted after incubation at 25 °C for 72 h.

2.3. Analyses of the disinfectant effect of PAA on saury surfaces

Fresh sauries placed on ice were purchased from a local supermarket in Japan and immediately transported to the laboratory and stored at -70 °C. The whole saury was initially placed in tap water for 30-40 min until it was completely thawed and was then washed three times with 9-fold distilled water. Next, the saury was submerged in the *M. psychrotolerans* culture suspension (1:9 w/v, about 10^9 CFU/mL) for 5 min. Then, the inoculated sauries were air-dried in a biological safety cabinet for 12 min (initial inoculum size: approximately 10^6 CFU/cm^2). Next, the samples were dipped into 9-fold (w/v) of 80 ppm PAA or 100 ppm SH (free available chlorine concentration; chlorine concentration assayed using the method regulated by the Ministry of Health, Labour and Welfare, 318, Japan) solutions for 1 or 5 min, respectively. Sterile deionized water was as control. Thereafter, the saury samples were immediately dipped into the deionized water (1:9, w/v), to remove the excess sanitizer. The saury surfaces were then swabbed with a 10 mL phosphate-buffered saline to perform the attached swab test (Kanto Chemical, Co., Inc, Tokyo, Japan).

For monitoring the growth of *M. psychrotolerans* on saury surfaces during storage, the guts and gills were removed from the sauries firstly removed after thawing and then washed several times with tap water. Thereafter, the sauries were washed three times using sterile deionized water, again. Washed sauries were inoculated with the

bacterial suspension. The inoculated sauries were dipped in 80 ppm PAA or 100ppm SH solutions for 5 min, and then were placed into a stomacher bag (Seward Ltd., Worthing, U.K.). The samples were stored at 4 °C. The *M. psychrotolerans* cells were counted at different time points by swabbing the surfaces. *M. psychrotolerans* cells were counted suing the spreading method based on (Niven, Jeferry, & Corlett, 1981), based on growing the cells on Niven's agar supplemented with 0.2% sodium pyruvate at 25 ℃ for 72 h.

2.4. Scanning Electron Microscopy (SEM) observation

The morphology of *M. psychrotolerans* cells, that were treated with PAA, was observed with a scanning electron microscope (SEM). The harvested cells were centrifuged (3,000 × *g,* 5 min, 4 °C) and washed three times with 0.2 M phosphate buffer (PB, pH 7.4). The cells were then fixed with 2% glutaraldehyde–PB (2 h, 25°C), washed, resuspended in the same PB, and placed on a Sempore (φ0.6 μm, JEOL, Tokyo, Japan) as a 100 μL droplet. After washing three times with PB, the samples were dehydrated in a graded ethanol solution (50% - 100%). The cells were washed with t-butyl alcohol (Wako Pure Chemicals Industries, Osaka, Japan) for 30 min and lyophilized. Finally, samples were then sputtered with Pt-Pd and observed using SEM (JSM-6010LA, JEOL, Tokyo, Japan).

2.5. Investigation of cell membrane permeability using fluorescent staining

158 Following disinfectant treatment, the cells were stained, using Bacteria Live/Dead
159 Staining Kit (Promokine, PromoCell GmbH, Heidelberg, Germany). The DMAO
160 (green fluorescence) and EtD-III (red fluorescence) dyes were mixed with 0.85%
161 NaCl to obtain the dye mixture. Next, 1 µL mixture dye was added to 100 µL cell
162 suspension, followed by dark incubation for 15 min at room temperature. Then, 5 µL
163 of the stained bacterial cells were placed on a glass slide and immediately visualized
164 using the epifluorescence microscope (Olympus BX 51-34, Melville, USA).
165 Fluorescence images were processed using the Image J software package (Schneider,
166 Rasband, & Eliceiri, 2012).
167
168 *2.6. Chromosomal DNA examination by agarose gel electrophoresis*
169 The bacterial DNA was extracted from the PAA treated and control groups
170 described in section 2.2, using the Genomic DNA Extraction Kit (Macherey-Nagel
171 GmbH, Germany), according to the manufacturer's instructions. Extracted DNA was
172 separated by agarose gel electrophoresis (1% agarose). After ethidium bromide
173 staining, the agarose gel was digitalized, and the DNA bands were visualized using
174 the UV Transilluminator STAGE-1000 (AMZ, Inc., Japan).
175
176 *2.7. SDS-PAGE*
177 After disinfection, the bacterial cells were centrifuged to obtain the cell pellets. The
178 pellets were washed and resuspended in PBS. The bacterial cells were disrupted with

179 the Zirconia Beads Kit (Zircon prep mini, Nippon Genetics Co.Ltd., Tokyo, Japan)
180 and the Bead Beater (2,500 rpm, 30 min, CD-1000, EYELA, Tokyo, Japan). This was
181 followed by centrifugation at 6,000 × *g* for 10 min at 4 °C and the supernatants were
182 collected. The protein concentrations of the supernatants were determined according
183 to the Bradford method (Bradford, 1976). Loading samples were prepared according
184 to the method described by Wang, Chang, Yang, & Cui, (2015). The supernatant (80
185 μL, protein concentration of approximately 1.2 mg/mL) were mixed with the loading
186 buffer (20 μL), boiled for 5 min, cooled on ice, and centrifuged at 4,000 × *g* for 5 min
187 at 4°C. Finally, 20 μL sample solutions were resolved by SDS-PAGE, and the gel was
188 stained with Coomassie Brilliant R250 until the bands were clearly visible.
189
190 *2.8. Statistical analysis*
191 All experiments were performed in triplicate and the data was presented as the
192 mean ± standard deviation. The data was analyzed by ANOVA and the least
193 significant difference (LSD) was calculated for comparison of means. Statistical
194 significance was evaluated based on ≤ 0.05. All statistical analysis was performed
195 using RStudio Desktop (RStudio Desktop, Inc., Boston, MA).
196
197 **3. Results and discussion**
198 *3.1. Sanitizing effect on PAA against* M. psychrotolerans *in* vitro

199 The MIC and MBC of PAA against *M. psychrotolerans* were 7.5 ppm and 12 ppm,
200 respectively. Bridier, Briandet, Thomas, & Dubois-Brissonnet, (2011) have reported
201 the MBC of PAA against *E. coil* PHL628 to be 7.4 ppm. Moreover, our results
202 showed 10 ppm PAA treatment for 5 min significantly reduced the bacterial cell count
203 from 8.92 to 2.00 log CFU/mL ($p \leq 0.05$). And, 20 ppm PAA treatment for 5 min
204 reduced the populations of *M. psychrotolerans* to undetectable levels (< 1.7 log
205 CFU/mL) (Table 1). Additionally, the antibacterial efficiency of PAA treatment
206 increased with an increased its concentration. The counts of *M. psychrotolerans* also
207 decreased to the undetectable levels upon 40 ppm PAA treatment for 1 min. In a
208 previous study reported that the 80 ppm PAA treatment for 5 min was lethal for *E.*
209 *coli* O157:H7 (Rodgers, Cash, Siddiq, & Ryser, 2004).
210
211 *3.2. Sanitizing effect of PAA against* M. psychrotolerans *on saury surface*
212 The antimicrobial effects of PAA and SH on *M. psychrotolerans* present on the
213 saury surfaces are shown in Figure 1. The control group showed a 0.5 log CFU/cm^2
214 reduction of *M. psychrotolerans*. Treatments with 80 ppm PAA or 100 ppm SH for 1
215 min, showed 1.4 and 1.27 log CFU/cm^2 reductions, respectively. An increase in
216 washing time improved the reduction in the *M. psychrotolerans* counts from the saury
217 surfaces. Viable cell counts were reduced by 2.23 and 1.99 log CFU/cm^2 upon
218 washing with 80 ppm PAA and 100 ppm SH for 5 min, respectively (Fig 1A). These
219 results indicate that PAA as well as SH exhibits effective antimicrobial activity

220 against *M. psychrotolerans* on saury surface.

221 In addition, during the storage of saury samples at 4 °C, the viable counts of *M.*
222 *psychrotolerans* in the control sample reached to 7.11 log CFU/cm^2 after 3 days, but
223 the viable counts of *M. psychrotolerans* in PAA or SH treated samples reached to the
224 same level (6.8 log CFU/cm^2) after 5 days (Fig. 1B). In our previous study, we
225 observed a large amount of histamine accumulation by *M. psychrotolerans* in broth,
226 when the viable count of *M. psychrotolerans* reached 10^7 CFU/ml (Wang, Yamaki,
227 Kawai & Yamazaki, 2020; Kato *et al*., 2017). Therefore, our results suggested that
228 treatment with PAA was similar to SH during the fish washing process could extend
229 the shelf life of stored fish.

230 In a previous study, it has been reported that soaking tomatoes and lettuce in 30
231 ppm PAA solution for 10 min significantly reduced the *E. coli* counts by 4.39 and
232 2.33 log CFU/g, respectively (Keeratipibul *et al*., 2011). In this study, we reported the
233 similar reduction in *M. psychrotolerans* counts on saury surfaces in response to 80
234 ppm PAA and 100 ppm SH treatments (Fig. 1). The 50-100 ppm SH solution is
235 conventionally used to disinfect fish fillets (Thi *et al*., 2013). Park *et al*. (2012)
236 reported that PAA can potentially replace SH, as it was shown to be more effective at
237 an equivalent concentration against *E. coli* O157:H7, *Salmonella* Typhimurium, and
238 *Listeria monocytogenes*. Lee and Huang (2019) also demonstrated that PAA treatment
239 generated much less disinfection-related by-products than SH treatment. Based on
240 earlier studies and our results, we propose that PAA can be a better sanitizer for

241 seafood processing than SH.

242

243 *3.3. SEM analysis*

244 The sanitizing effect was determined by assessing the impact of PAA on cells
245 morphology using SEM, The *M. psychrotolerans* cells exhibited a smooth and intact
246 surface morphology in control groups (Fig. 2A). After 20 ppm PAA treatment for 5
247 min, the surface of *M. psychrotolerans* cells showed no clear differences compared to
248 the control group (Fig. 2B). However, after a 5 min treatment with 80 ppm PAA,
249 some bacterial cells became corrugated (Fig. 2C). This indicates that PAA treatment
250 impacts the outer membrane of *M. psychrotolerans* cells and that the level of damage
251 might depend on PAA concentration. Some studies revealed that the PAA treatment
252 generates cell surface irregularities and partial grooves, with the damage becoming
253 increasingly apparent as the sanitizer concentration is increased (Chino *et al*., 2017;
254 Ujimine *et al*., 2017; Park *et al*., 2013; Mustapha and Liewen, 1989). We did not
255 observe any grave damage to *M. psychrotolerans* cell morphology after 20 ppm PAA
256 treatment. Additionally, it has also been reported that the effectiveness of PAA
257 treatment on bacterial cell morphology was significantly low below the concentration
258 of 60 mg/L (Zhang *et al*., 2019).

259

260 *3.4. Evaluation of cell membrane integrity after sanitizing treatment*

261 Bacterial cytoplasmic membrane permeability was evaluated by staining with

262 Bacterial Live/Dead Staining Kit. The kit provides two stains, DMAO and EtD-III.
263 DMAO can stain both, live and dead bacteria, with intact and damaged cell
264 membranes, while EtD-III can stain only dead bacteria with damaged cell membranes
265 (Kaprelyants and Kell, 1992). *M. psychrotolerans* cells treated with water displayed
266 green fluorescence, and the cells from the sanitizer-treated group displayed red
267 fluorescence (Fig. 3). This indicates that cells lose their membrane integrity upon
268 PAA treatment. However, the level of damage was unclear by staining the bacterial
269 cells with this staining kit. Changes in bacterial cell membrane permeability and
270 integrity might be attributed to various factors, such as inactivation of enzymes and
271 alterations in membrane potential (Joux and Lebaron, 2000). SYTO 9 and PI staining
272 of *Staphylococcus aureus* SA1 and *L. monocytogenes* cells, after 5000 ppm PAA
273 treatment for 15 s also revealed cell membrane damage (Lee *et al*., 2016). Zhang *et al*.
274 (2018) reported that PAA disrupts the cell membrane integrity of inactivate microbes.
275 These results were correspondent with our observations in figures 3.
276
277 *3.5. Analyses of chromosomal DNA through agarose gel electrophoresis*
278 Changes in bacterial chromosomal DNA upon PAA treatment were analyzed
279 through agarose gel electrophoresis (Fig. 4). The DNA extracted from the control and
280 the test group treated with PAA displayed a bright single band on the agarose gel.
281 Additionally, an increase in PAA concentration showed no particular difference in the
282 DNA band profiles. This result suggests that PAA kills *M. psychrotolerans* cells

283 without damaging the bacterial DNA. This finding confirms with the results of
284 previous study that PAA exerted antimicrobial effects on *E. coil* TOP10 independent
285 of affecting the plasmid DNA (Zhang *et al*., 2019). Leggett *et al.* (2015) also reported
286 no damage to the spore's DNA following PAA treatment. The PAA disinfection
287 mechanism might differ from SH, that damages the bacterial DNA as a disinfectant
288 (Fukuzaki, 2006). Ujimine *et al*. (2017) demonstrated that 3.1 ppm SH treatment
289 damaged the molecular DNA in *S. aureus* cells and the DNA was undetectable on the
290 agarose gel. However, there might be variations related to the differences in sanitizer
291 efficacy, target microorganism, sanitizer concentration and treatment time (Zoellner *et*
292 *al*., 2018). Overall, our observations suggest that the bactericidal activity of PAA is
293 not related to the damage to the bacterial chromosomal DNA.
294
295 *3.6. SDS-PAGE analysis to investigate the bacterial proteins profiles*
296 The cell protein profiles of *M. psychrotolerans* after PAA treatment are shown in
297 Figure 5. The protein profile of *M. psychrotolerans* cells exhibited no major
298 difference between the PAA-treated and control cells. However, there were some
299 protein bands that appeared to be slightly faint with increased PAA concentration. In
300 some studies, the mechanisms of PAA action is reported to include, degradation of
301 proteins during treatment (Kerkaert *et al*., 2011), enzyme oxidation and inactivation
302 (Fraser, *et al*., 1985) and impairment of lipoprotein cytoplasmic membrane
303 permeability (Kitis, 2004). In this study, we demonstrated that PAA destroyed the cell

304 membrane integrity (Fig. 3), and did not affect the bacterial DNA integrity in the
305 treated cells (Fig. 4). Therefore, the reduced amount of proteins after treating with
306 PAA on the gel could be because of the damaged cell membrane proteins (Nakayama
307 *et al*., 2013). Also, depending on previous investigations, the difference in the cell
308 protein profiles after PAA treatment might be attributed to alterations in some
309 metabolic or protein synthesis pathways upon PAA treatment (Du, Liu, Cao, Zhao, &
310 Huang, 2018; Liu *et al*., 2018). Hence, further scientific studies are required to
311 elucidate the underlying mechanism of PAA disinfectant function.
312

313 **4. Conclusion**

314 To the best of our knowledge, this is the first report to investigate the sanitizing
315 efficacy of PAA against *M. psychrotolerans*, a histamine-producer responsible for
316 seafood poisoning. We revealed that PAA could significantly reduction of *M.*
317 *psychrotolerans,* both in *vitro* and on saury surfaces. And we also demonstrated that
318 the antimicrobial mechanism of PAA was mainly related to damage the cell
319 membranes. Our study provides evidence on the disinfectant efficiency of PAA and
320 its potential application to improve seafood safety.
321

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Conflict of Interest

None declared.

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554

555 TABLE AND FIGURE LEGENDS

556

557 Table 1. Survival of *M. psychrotolerans* under treatment with PAA in *vitro.*

558

559 Fig. 1. (A) Reduction in bacteria cell counts of *M. psychrotolerans* on saury surfaces,
560 after washing with sanitizers for 1 or 5 min; (B) Effect of different sanitizer
561 treatments against *M. psychrotolerans* on saury surfaces during storage at 4 °C. White
562 bars, black bars, and gray bars represent control, 80 ppm PAA and 100 ppm SH,
563 respectively. Different lowercase letters are significantly different within same
564 treatment time group ($p<0.05$).

565

566 Fig. 2. Scanning electron microscopic (SEM) observations of *M. psychrotolerans*
567 undergoing PAA and sodium hypochlorite (SH) treatments. A- Control; B- 20 ppm
568 PAA treated for 5 min; C- 80 ppm PAA treated for 5 min.

569

570 Fig. 3. Epifluorescence microscopic images of *M. psychrotolerans* cells, stained with
571 Live/Dead Kit after PAA treatments. A- Control; B- 20 ppm PAA treated 5 min; C- 80
572 ppm PAA treated 5 min.

573

574 Fig. 4. Chromosomal DNA of *M. psychrotolerans* after PAA treatment. M- Marker;

575 C- Control; P1- 20 ppm PAA treated 5 min; P2- 80 ppm PAA treated 5 min.

576

577 Fig. 5. SDS-PAGE profiles of protein in *M. psychrotolerans* cell proteins after PAA

578 treatment. M- Marker; C- Control; P1- 20 ppm PAA treated 5 min; P2- 80 ppm PAA

579 treated 5 min.

Table. 1 Wang et al.

Treatment	Treatment time (min)			
	0	1	3	5
Control	8.92 ± 0.04	8.93 ± 0.04[a]	8.92 ± 0.03[a]	8.90 ± 0.03[a]
10 ppm	8.92 ± 0.04	4.59 ± 0.25[b]	2.07 ± 0.22[b]	2.00 ± 0.05[b]
20 ppm	8.92 ± 0.04	1.87 ± 0.18[c]	1.80 ± 0.17[b]	ND
40 ppm	8.92 ± 0.04	ND	ND	ND
80 ppm	8.92 ± 0.04	ND	ND	ND

[1]Different lowercase letters are significantly different within the same treatment time group ($p < 0.05$).

[2]ND:Below detection level: < 1.7 log CFU/mL.

Fig. 1 Wang et al.

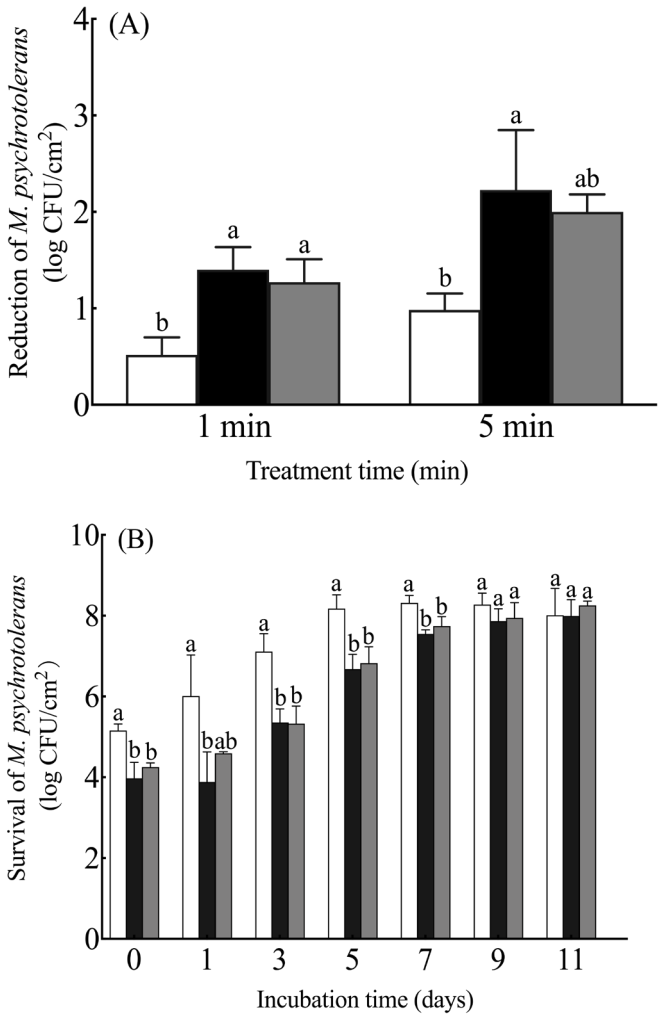

Fig. 2 Wang et al.

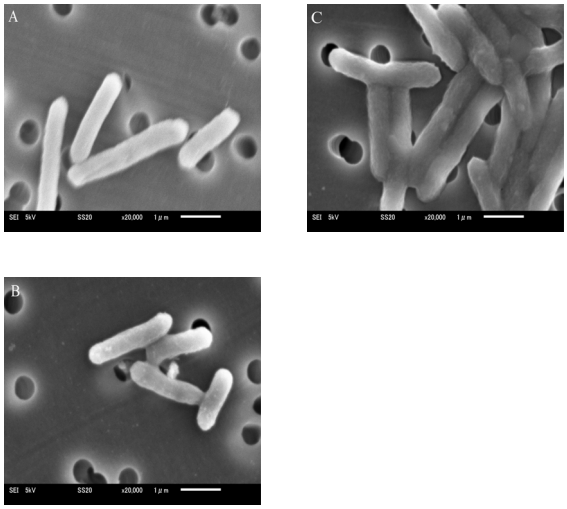

Fig. 3 Wang et al.

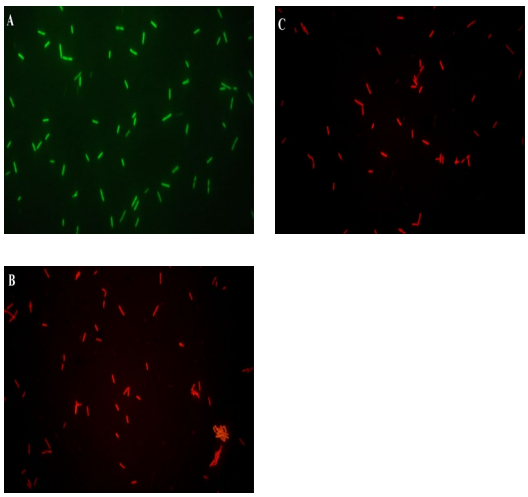

Fig. 4 Wang et al.

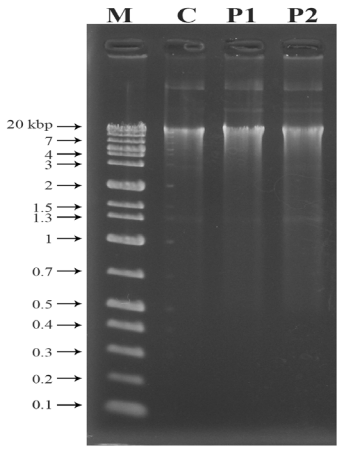

Fig. 5 Wang et al.