APPLICATION OF FRAGMENTED EXTRACELLULAR SELF-DNA (esDNA) CONCEPT AS AN ALTERNATIVE PROPHYLACTIC APPROACH AGAINST *Vibrio parahaemolyticus* AND *Vibrio harveyi* INFECTION IN BRINE SHRIMP ARTEMIA

Abstract

The purpose of this study was to investigate species specific inhibitory effects of esDNA isolated from two conspecific organisms: *Vibrio parahaemolyticus* (VP) and *Vibrio harveyi* (VH), and to assess the functional role of esDNA to enhance the survival rate of *Artemia* sp. In an *in vitro* study, nine doses of Extracellular self-DNA of *Vibrio parahaemolyticus* (esDNAVP) and *Vibrio harveyi* (esDNAVH) were used as the target for the challenge test with the conspecific bacteria. In an *in vivo* study, the protective effect of esDNA was then tested in nauplii of the brine shrimp *Artemia* at various priming times and concentrations of esDNA under gnotobiotic conditions prior to challenge with VP and VH at the concentration of 5×10^5 CFU mL^{-1}. The results from *in vitro* study showed that the use of esDNAVP at levels of 24.02 and 48.05 ng μl^{-1} and esDNAVH at concentrations of 13.33 and 26.67 ng μl^{-1} were able to inhibit the growth of the conspecific species when added to the culture medium at the concentration level of 5×10^5 CFU mL^{-1}. The results from *in vivo* study showed that the use of 24.02; 48.05 and 72.07 ng μl^{-1} of esDNAVP as well as the use of 13.33; 26.67 and 40.00 ng μl^{-1} of esDNAVH inhibited the growth of VP and VH and enhanced the survival rate of *Artemia* sp compared to the control treatment ($P<0.05$). Taken together, we confirmed that esDNA obtained from the extraction and random fragmentation from esDNAVP and esDNAVH, produces a species-specific inhibitory effect on

the same species and can serve as a potential alternative strategy for disease control to deliver the functionality of esDNA to the fish and shrimp.

Key words: esDNA, *Vibrio parahaemolyticus, Vibrio harveyi, Artemia, larviculture*

Introduction

Disease outbreaks are being increasingly reported as a major constraint to the sustainability of aquaculture production, resulting in significant mortality and economic losses annually to the industry worldwide (Flegel, 2019; Leung and Bates, 2013; Novriadi, 2016) . Among the groups of pathogenic microorganisms, bacterial diseases, especially in the group of *Vibrio*, are become the major problem in ensuring the production sustainability (de Souza Valente and Wan, 2021; Sanches-Fernandes et al., 2022). In the shrimp industry, infection of *Vibrio* spp have been causing great economic losses (Ahmmed et al., 2019; Boopathi et al., 2023). The culture environment, along with the complexity of organic waste that has accumulated during the production period, is an excellent source to support the growth of bacteria, which then the ingestion or drinking process become the main routes for the entry of these pathogens to the aquatic organisms (Defoirdt et al., 2011).

The rapid development of aquaculture in recent decades requires increasing supply of fingerlings as one of the most critical factors for commercial success of the industry (Gephart et al., 2020). However, there are two bottlenecks in larviculture industry: disease outbreaks (Borges et al., 2021; Xue et al., 2022) and proper feed at the early larvae stage when larvae deplete yolk reserve and need to shift the feeding process from endogenous to exogenous system (Pan et al.,

46 2022). Therefore, the combination of diseases control and viable phytoplankton and zooplankton
47 is important, not only to provide more bio-available nutrients, but also to trigger higher responses
48 to the pathogens (Kandathil Radhakrishnan et al., 2020; Pan et al., 2022). Among the live feed,
49 *Artemia* sp is one of important species and extensively used in second stage larviculture
50 production system due to the (1) durable cyst and can be harvested at different time points for
51 larval feeding; (2) size suitability, and (3) as a vector to deliver required nutrients or medicine
52 through their non-selective filter feeder properties (Eryalcin, 2018; Pan et al., 2022). In addition,
53 the infiltration of anti-microbial substances can also help to reduce the presence of infectious
54 pathogens that can also cause massive mortalities in *Artemia* cultures (Rahman et al., 2022).

55 To overcome the diseases outbreaks, traditional treatment, such as the use of disinfectants
56 and antibiotics, have become the common method to kill or inhibit the bacterial growth (Defoirdt
57 et al., 2007). However, the use of antibiotics will only stimulate the development of bacterial
58 resistance in the surrounding environment and allergy to humans due to the presence of residual
59 antibiotics in commercialized of aquaculture products (Chen et al., 2020; Defoirdt et al., 2007;
60 Subasinghe, 1997), alternative approaches are urgently needed. Several prophylactic approaches
61 directed towards vibriosis has been developed and applied in aquaculture, including:
62 Immunostimulation (Apines-Amar and Amar, 2015; Mehana et al., 2015), vaccination (Ji et al.,
63 2020; Silvaraj et al., 2020); probiotics (El-Saadony et al., 2021) and quorum sensing to inhibit
64 the virulence factors of bacteria (Defoirdt, 2019; Girard, 2019). However, today's concern has
65 emerged that we are entering the development of modern technique to inhibit the growth of
66 conspecific mechanisms by using fragmented extracellular self DNA (esDNA) mechanisms
67 (Lanzotti et al., 2022; Mazzoleni et al., 2015; Zhou et al., 2023). This concept is based on the
68 recent findings in which DNA that normally exist in the living cells can be released into the

69 environment of damaged or infected cells in the extracellular space and then degraded into
70 fragments in a variable size (De Lorenzo et al., 2018; Wu et al., 2013; Zhou et al., 2023). Several
71 studies mentioned that the fragmented esDNA (i.e. DNA originating from conspecifics) had
72 species-specific inhibitory effects, trigger the generation of reactive oxygen species, and play an
73 active role in cell defense actions and microbial biofilm formations (Barbero et al., 2016; Duran-
74 Flores and Heil, 2018; Mazzoleni et al., 2015; Monticolo et al., 2020; Vega-Muñoz et al., 2018).
75 The involvement of esDNA in signaling, self-recognition and species-specific inhibitory growth
76 effects of conspecific individuals has been discussed widely in relation to plants (Bhat and Ryu,
77 2016; Cartenì et al., 2016; Mazzoleni et al., 2015). However, the functional roles of esDNA to
78 the aquatic organisms are still poorly known. Therefore, the specific aims of this research were
79 to investigate species specific inhibitory effects of esDNA isolated from two conspecific
80 organisms: *Vibrio parahaemolyticus* and *Vibrio harveyi*, and to assess the functional role of
81 esDNA to enhance the survival rate of *Artemia* sp after exposed with several doses of esDNA
82 through the growth inhibition of the conspecific bacteria that are widely known as pathogen to
83 *Artemia* sp.

84

85 **2. Material and Methods**

86 **2.1 Conspecific bacteria (*Vibrio parahaemolyticus* and *Vibrio harveyi*) – extracellular self**
87 **DNA (esDNA) *in vitro* co-culturing assays**

88 **2.1.1 Bacterial culture**

89 Isolates of the bacterial strain *Vibrio parahaemolyticus* and *Vibrio harveyi* BT1H, which
90 was obtained from Department of Fisheries, Faculty of Agriculture, Universitas Gadjah Mada,
91 were used for the *in-vitro* and *in-vivo* inactivity experiments. Working culture were maintained

92 on marine 2216E agar (MA; Difco), with sub culturing every 1 to 2 wk. In subsequent
93 experiment, the isolates was grown in Zobell's medium, prepared with 5 g L^{-1} peptone
94 bacterioligical (HiMedia; India) and 1 g L^{-1} yeast bacterioligical (Oxoid; UK) for 24 h at 28 °C.
95 The bacterial densities were determined spectrophotometrically at an optical density of 625 nm.
96
97
98 **2.1.2 DNA extraction**
99 DNA extraction of *V. parahaemolyticus* and *V. harveyi* BT1H was performed manually
100 using TNES (Tris NaCl EDTA) and PCIAA (Phenol Chloroform Isoamyl Alcohol) solutions.
101 Bacterial cells of 50 mg was placed in 400 µl of buffer (10 mM Tris-HCl, 125 mM NaCl, 10 mM
102 EDTA, 0.5% SDS) and 3 µl of proteinase K (3 mg/ml) was added and homogenized and then
103 incubated at 37 °C for 2.5 hours, then inverted for 15 minutes and centrifuged for 6 min at
104 10,000 rpm and top aqueous layer was recovered. 400 µl of Phenol: Chlorophorm: Isoamyl
105 Alcohol (25:24:1) was added to the microcentrifuge tube containing the mixture which was then
106 inverted for 15 minutes. The microcentrifuge tube was then centrifuged for 6 min at 10,000 rpm
107 and top aqueous layer was recovered. The DNA was added 1/10 5 M NaCl and twice the volume
108 of absolute ethanol from the supernatant before being stored at 4 °C for 24 hours. The DNA was
109 washed with 70% ethanol, air dried and stored in 100 µl Tris EDTA (TE) solution and 3 µl
110 RNAse free water.
111
112 **2.1.3 DNA Electrophoresis**
113 The quality of DNA isolated from *V. parahaemolyticus* and *V. harveyi* BT1H was
114 evaluated by gel electrophoresis. The DNA solution was visualized in 1% agarose gel using 0.75

115 µL florosafe (1st BASE, Singapore), by direct comparison with a standard marker (50bp DNA
116 ladder, Thermo Fisher Scientific, USA). The electrophoresis product was documented using gel
117 documentation system (Advanced Mupid-Exu, Japan) and visualized using a UV illuminator
118 (Vilber lourmart, France).
119
120
121 **2.1.4 DNA Sonication**
122 A US-300T sonicator (Nissei, Japan) was used to fragment DNA from sequences up to
123 100 bp in length. Per the manufacturer's recommendation, sonication process was carried out
124 indirectly using microtube containing the extracted DNA. The sonication was carried out in ten
125 stages, where one stage was carried out for three minutes and rests for 30 seconds before moving
126 on to the next stage. The fragmented esDNA was then electrophoresed again to determine the
127 length of the fragments in the DNA. Prior to the challenge test, nano drop (Thermo Fisher
128 Scientific, USA) were used to quantify the number of the fragmented of esDNA.
129
130 **2.1.5 Assessment of conspecific bacteria (*V. parahaemolyticus* and *V. harveyi*) towards
131 self-DNA**
132 Extracellular self-DNA of *V. parahaemolyticus* (esDNAVP) and *V. harveyi* (esDNAVH)
133 were used as the target for the challenge test with the conspecific bacteria. Nine doses of
134 esDNAVP: 0; 0.37; 0.75; 1.50; 3.00; 6.01; 12.01; 24.02 and 48.05 ng μL^{-1} together with nine
135 doses of esDNAVH: 0; 0.21; 0.42; 0.84; 1.67; 3.34; 6.67; 13.34; and 26.67 ng μL^{-1} were used
136 with three replicates for every treatment dose (**Table 1).** A 100 µL mixture solution of Zobell
137 medium and different dose of s-DNAVP or s-DNAVH was added into each hole in the

138 microplate and then challenged with 10 μL or 10^5 CFU mL^{-1} of the conspecific bacteria. The
139 growth of conspecific bacteria after adding the different dose solution of esDNAVP or
140 esDNAVH for 24 h were observed using the Elisa microplate reader (Diatek DR-200bc; China)
141 at a wavelength of 550 nm.
142

143 2.2 Conspecific bacteria (*V. parahaemolyticus* and *V. harveyi*) – extracellular self DNA 144 (esDNA) *in vivo* co-culturing assays with *Artemia* sp

145 Based on the *in-vitro* test, the significant dose of esDNAVP and esDNAVH that was able
146 to inhibit the growth of the bacteria was selected for further study with *Artemia* sp. In addition to
147 the significant dose, control (without any esDNA); 50% lower and 50% higher from the
148 significant dose were also used for each treatment of esDNAVP and esDNAVH to provide better
149 understanding on the growth of the conspecific bacteria and survival rate of *Artemia* sp as the
150 consequence of the growth of VP and VH that are widely known as the pathogen for *Artemia*.
151 The axenic brine shrimp *Artemia* sp (instar II) were immersed with selected dose of esDNAVP
152 and esDNAVH for three different times, namely 6 h; 12h, and 24h. Then, as much as 10 mL of
153 *V. harveyi* or *V. parahaemolyticus* at a density of 5×10^5 CFU mL^{-1} was added to the container
154 containing 100 mL of sterile seawater, increasing concentration level of esDNAVP and
155 esDNAVH, and 60 individuals of instar II *Artemia* sp. Observation on the growth of VP and VH
156 within the body of the *Artemia* sp was carried out by using Total Plate Count (TPC) after 24 h of
157 post treatment for each immersion time period. In addition, visual observation was performed to
158 count the survival rate (%) of *Artemia* sp after exposed to VP and VH as follows:

159 $$SR\,(\%) = \frac{final\ number\ of\ Artemia\ sp}{initial\ number\ of\ Artemia\ sp} \times 100$$

160 2.3 Statistical analysis

161 Total numbers of bacteria from *in-vitro* and *in-vivo* test as well as the survival rate of
162 *Artemia* sp were analyzed using regression and one-way analysis of variance (ANOVA) to
163 determine significant differences among treatments followed by Tukey's multiple comparison
164 tests to determine the difference between treatment means among the treatments. All statistical
165 analyses were conducted using the SAS system (V9.4. SAS Institute, Cary, NC, USA).

166 **3. Results**

167 **3.1 Screening for *in-vitro* inhibition activity**

168 Growth inhibition activity toward two species of pathogenic Vibrios (*V.*
169 *parahaemolyticus and V. harveyi*) was exhibited by using different doses of conspecific self-
170 DNA (s-DNAVP and s-DNAVH). The growth of *V. parahaemolyticus and V. harveyi* were
171 significantly decreased as the doses of self-DNA were added to the culturing medium increases
172 ($P<0.05$). Statistically, the growth of *V. parahaemolyticus* was significantly lower with the use
173 of 48.05 ng μL^{-1} compared to the control. In addition, the use of 26.67 ng μL^{-1} of *V. harveyi*
174 was able to significantly lowering the growth of *V. harveyi* (Table 2).

175

176 **3.2 Growth and survival testing of conspecific bacteria within *Artemia* immersed with**
177 **self-DNA**

178 The growth of VP and VH within *Artemia* sp enriched with several doses of conspecific
179 esDNA at different immersion time period as well as the survival rate (%) of *Artemia* were
180 evaluated. Instar II of *Artemia* sp were treated with esDNA *Vibrio parahaemolyticus* for 0; 6; 12;
181 and 24 h. Untreated *Artemia* sp were used as control (Table 3 and 4). For the untreated group,
182 growth of VP and VH were higher compared to the growth VP and VH in the group of *Artemia*
183 treated with both esDNAVP and esDNAVH. The higher the concentration levels of the esDNA

184 the lower the growth number of VP and VH within the *Artemia* sp (Table 3) ($P< 0.05$).
185 Immersion time also plays a significant role reducing the growth number of pathogen. In the
186 group of *Artemia* treated with 48.05 and 72.07 ng μl^{-1} of esDNAVP showed that the 12 h and 24
187 h exposure time generate the lowest growth of VP compared to the 6 h exposure time.
188 Meanwhile in the group of esDNA VH, the use of 13.33; 26.67; and 40.00 ng μl^{-1} of esDNAVH
189 were able to suppress the growth of VH compared to the 6 h exposure time period.

190 For the survival rate, the untreated group of *Artemia* has the lowest survival rate (%) after
191 challenged with VP and VH at the concentration level of 10^5 CFU mL^{-1} compared to the group of
192 *Artemia* treated with esDNA (Table 4; $P<0.05$). For the challenged with VP, after 24 h post
193 immersion at different immersion time, the survival rate of *Artemia* sp were 49.50 ± 4.31; 48.00
194 ± 5.76; and 49.50 ± 5.39 % after immersed in distilled water for 6; 12 and 24 h, respectively.
195 Meanwhile, using esDNAVP with the concentration of 24.02 ng μl^{-1}, the survival rate of *Artemia*
196 sp were 47.67 ± 4.53; 50.83 ± 2.97; and 61.17 ± 2.73 % after immersed with esDNA for 6; 12
197 and 24 h, respectively. Immersion with 48.05 ng μl^{-1} generated survival rate at the range of 56.33
198 ± 4.43; 74.50 ± 2.84

199 Among the treated group, immersion time with both esDNAVP and esDNAVH also
200 played significant impact to enhance the survival rate of *Artemia* sp during the challenge period.
201 In general, as the treatment dose and exposure time of esDNA to *Artemia* sp increases, the
202 survival rate of *Artemia* also increases when challenged with VP and VH at dose of 10^5 CFU
203 mL^{-1}. In the group of esDNAVP, the immersion of *Artemia* sp with 48.05 and 72.07 ng μl^{-1} of
204 esDNAVP for 12 and 24 h provide better protection against VP and significantly enhance the
205 survival of *Artemia* sp compared with 6 h exposure period. Moreover, the use of lowest
206 concentration of esDNAVP in this study (24.02 ng μl^{-1}), required longer exposure time since

207 there is no significant difference in the survival rate of *Artemia* sp between 12 h and 6 h exposure
208 time. Similar trends also observed in the survival rate of *Artemia* in the group of VH. The use of
209 medium and highest treatment dose of esDNAVH (26.67 and 40.00 ng μl^{-1}) provide better
210 survival rate of *Artemia* sp compared to lower dose of esDNAVH (13.33 ng μl^{-1}) in all exposure
211 time (P<0.05).
212

213 **4. Discussion**

214 The first report provided by Mazzolini et al. (2015a,b) showing that the exposure to
215 fragmented extracellular self DNA (esDNA) triggers the inhibitory effects on the conspecifics
216 organisms, while the treatment with extracellular non-self DNA did not show similar effect.
217 Moreover, exposures to the esDNA limited the cell permeability and play an active role in cell
218 defense actions as well as the microbial biofilm formation (Chiusano et al., 2021; Monticolo et
219 al., 2020). The current study shows that exposure to esDNA can inhibit the growth of the
220 conspecific microorganisms, namely: *V. parahaemolyticus* (VP) and *V. harveyi* (VH) and
221 considered as a species-dependent manner. In parallel, remarkable differences were also detected
222 between the different times of exposure to the growth of VP and VH within *Artemia* in each
223 treatment group as well as the survival of *Artemia* during the observation period.

224 The significantly different growth of VP and VH after exposure with esDNAVP and
225 esDNAVH for 24 h depended on the concentration level of the esDNA (Table 2). In line with our
226 studies, Palomba et al. (2022) demonstrating that the use of 30 ng μL^{-1} esDNA provide the
227 highest growth inhibition of *Nannochloropsis gaditana* compared with 3 and 10 ng μL^{-1} of
228 esDNA. The presence of esDNA has been demonstrated to be sensed in animals by receptors
229 located in various cellular compartments, such as the nucleus, endosomes and cytoplasms

230 (Hemmi et al., 2000; Palomba et al., 2022; Szczesny et al., 2018; Wang et al., 2019). Specifically
231 in plants, it was proposed by Mazzoleni et al. (2015) that the growth inhibition ability could be
232 the result of a mechanism resembling the process of interference based on sequence specific
233 recognition of small-sized nucleotide molecules. The amounts of extracellular nucleotides has
234 many functions; including the ability to induce the activation of innate immunity and possibly
235 suppress the cell growth (Sawa et al., 2021).

236 In aquaculture production systems, brine shrimp *Artemia* is the most important live feed
237 organism to satisfy the requirements of most diversified groups of aquatic organisms during the
238 early life cycle stages (Albano et al., 2021; Kumar and Babu, 2015; Léger et al., 1987). In
239 addition, *Artemia* also serves as an important animal model to study about host-microbial
240 interactions and to understand the link between diet and immunity as an impact of nutritional
241 input (Rojas-García et al., 2008). Since there are so many diseases outbreaks in larviculture of
242 fish and shrimp (Faruk and Anka, 2017), the use of live feed *Artemia* as the non-selective filter
243 feeders organisms allows this animal to behave as a vector for delivering esDNA to the fish and
244 shrimp. In this research, different treatment doses show a similar pattern with low number of
245 conspecific microorganisms: VP and VH within *Artemia* sp after the brine shrimp treated with
246 esDNA for 6; 12 and 24 h. In general, as the treatment doses and immersion period increases, the
247 growth suppression of the conspecific organisms also increases. According to Samant et al.
248 (2008) the scarcity of nucleotides precursors, but not other nutrients, is the key limitation for the
249 growth of bacteria. Strong evidence demonstrated by the study from Samant et al. (2008) where
250 the inactivation of nucleotide biosynthesis genes in another gram-negative pathogen,*Salmonella*
251 *enterica*, and in the gram-positive pathogen *Bacillus anthracis*, prevented their growth in human
252 serum. In addition, Hannon (2002) mentioned that the uptake of random fragments by the living

253 organisms could produce inhibition of cell functionalities at multiple levels either blocking the
254 transfer of genetic information from DNA to proteins, based on the well-known interference
255 exerted by small-sized nucleotide molecules through sequence-specific recognition (Ecker and
256 Davis, 1986) or by affecting the stability of the genome (Gruenert et al., 2003).

257 The current study also showed that the inhibitory effect of esDNA could enhance the
258 survival rate of *Artemia* sp after enriched with esDNA for three different immersion period of
259 time: 6, 12 and 24 h, prior to challenge with VP and VH. The percentage survival (%) of *Artemia*
260 sp increases with the increasing dose treatment of esDNA and immersion period. In general, all
261 treatment doses provide similar responses at 24 h observation post immersion with higher
262 survival (%) occurred at 12 and 24 h immersion period compared to 6 h immersion period with
263 esDNA.

264 Invertebrates lack the complexity of the adaptive immune system compared to vertebrates
265 and only solely on innate immunity as their primary defense mechanisms (Figueras et al., 2021;
266 Kulkarni et al., 2021). The production of a given antimicrobial agent is amplified by regulation
267 of transcription and there is normally no memory (Ali and Abd El Halim, 2020; Roy et al.,
268 2022). However, their amazing diversity, abundance and success story in immune system
269 evolution argue for a highly efficient defence system against various pathogens (Pope et al.,
270 2011). Study from Cerenius and Söderhäll (2021) showing evidence that the acquired (specific)
271 immunity might be present in invertebrates. This immunity was obtained by previous contact
272 with pathogens or biological polymers from microbiological organisms (Hauton and Smith,
273 2007). This mechanism has been known as "immune priming" to set it apart from the "memory"
274 in vertebrates (Schmid- Hempel, 2005). In this study, priming was defined as an activity to
275 stimulate the immunological response after expose the *Artemia* for 6, 12 and 24 h immersion

276 period with the esDNA. Based on the situation, the patent from Mazzoleni (2019) that report a
277 new unexpected function role of DNA, after extraction and random fragmentation, to produces a
278 species-specific inhibitory effects could explain the lower mortality rate in *Artemia* obtained in
279 this study after priming with esDNA against VP and VH.

280

281 **5. Conclusion**

282 These results suggest the possible use of esDNA isolated from two conspecific
283 organisms: *Vibrio parahaemolyticus* and *Vibrio harveyi* for biological control of conspecific
284 organisms both in vitro and in vivo assays. This approach could become an alternative approach
285 to the use of antibiotics for more sustainable aquaculture production system. Taken together, the
286 results of this study lead us to propose the inclusion level of 24.02 to 72.07 ng μl^{-1} of esDNAVP
287 as well as the inclusion of 13.33 to 40.00 ng μl^{-1} of esDNAVH provide an optimum pathway to
288 inhibits the growth and induce defense mechanisms through the immune priming system. Further
289 is study needed to confirm the optimum dose of esDNA by applying higher inclusion level of
290 esDNAVP and esDNAVH and the ability of esDNA to inhibit the growth of conspecific
291 organisms in a commercial larviculture or even to aquaculture production system.

292

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298 the product and does not imply its approval to the exclusion of other products that may also be
299 suitable.

300 **Ethical Approval:** All procedures and handling process in the present study were approved by
301 the recommendations in the Guide for the Use of experimental Animals of the Jakarta Technical
302 University of Fisheries

303 **Data Availability Statement:** The data that support the findings of this study are available from
304 the corresponding author, [RN], upon reasonable request.

305

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Table 1. The research design for the dose of in-vitro analysis to reveal the efficacy of extracellular self-DNA (esDNA) against the conspecific bacteria.

No	*Vibrio parahaemolyticus* (VP)		*Vibrio harveyi* (VH)	
	Treatment Code	**esDNAVP (ng μl^{-1})**	**Treatment Code**	**esDNAVH (ng μl^{-1})**
1.	VP-0	0.00	VH-0	0.00
2.	VP-1	0.37	VH-1	0.21
3.	VP-2	0.75	VH-2	0.42
4.	VP-3	1.50	VH-3	0.84
5.	VP-4	3.00	VH-4	1.67
6.	VP-5	6.01	VH-5	3.34
7.	VP-6	12.01	VH-6	6.67
8.	VP-7	24.02	VH-7	13.34
9.	VP-8	48.05	VH-8	26.67

Table 2. The growth number of conspecific bacteria after added to the different doses solution of esDNA at the concentration level of 10^5 CFU mL^{-1}. Values represent the mean of three replicates. Results in the same columns with different superscript letter are significantly different (*P<0.05*) based on analysis of variance followed by the Tukey's multiple comparison test.

No	esDNAVP (ng μl^{-1})	Growth of VP (10^9 CFU mL^{-1})	esDNAVH (ng μl^{-1})	Growth of VH (10^9 CFU mL^{-1})
1.	0.00	1.0797 ± 0.0159^{b}	0.00	7.8608 ± 0.1984^{b}
2.	0.37	1.0596 ± 0.0024^{b}	0.21	7.7428 ± 0.1405^{b}
3.	0.75	1.0503 ± 0.0035^{b}	0.42	7.7056 ± 0.1056^{b}
4.	1.50	1.0580 ± 0.0137^{b}	0.84	7.6292 ± 0.0632^{b}
5.	3.00	1.0604 ± 0.0019^{b}	1.67	7.4808 ± 0.0467^{b}
6.	6.01	1.0606 ± 0.0061^{b}	3.34	7.4876 ± 0.1653^{b}
7.	12.01	1.0624 ± 0.0022^{b}	6.67	6.8744 ± 0.0612^{ab}
8.	24.02	1.0318 ± 0.0475^{ab}	13.34	6.7892 ± 1.0429^{ab}
9.	48.05	0.7606 ± 0.0193^{a}	26.67	5.4868 ± 1.0965^{a}

Note: VP = *Vibrio parahaemolyticus;* VH = *Vibrio harveyi*

Table 3. The growth number of conspecific bacteria (initial addition number of 10^5 CFU mL^{-1}) to the solution consist with *Artemia* sp and different doses of extracellular self-DNA (esDNA). Values represent the mean of forty replicates. Results in the same columns with different superscript letter are significantly different (*P<0.05*) based on analysis of variance followed by the Tukey's multiple comparison test.

No	esDNAVP (ng μl^{-1})	Immersion time with esDNAVP	Growth of VP (10^2 CFU mL^{-1})	esDNAVH (ng μl^{-1})	Immersion time with esDNAVH	Growth of VH (10^2 CFU mL^{-1})
1.	0.00	06:00	3.68 ± 0.47^{e}	0.00	06:00	5.27 ± 0.37^{f}
		12:00	4.43 ± 0.41^{f}		12:00	6.11 ± 0.32^{g}
		24:00	3.98 ± 0.22^{e}		24:00	6.02 ± 0.37^{g}
2	24.02	06:00	3.85 ± 0.23^{e}	13.33	06:00	5.09 ± 0.20^{f}
		12:00	3.31 ± 0.20^{cd}		12:00	3.50 ± 0.27^{d}
		24:00	3.67 ± 0.16^{de}		24:00	3.45 ± 0.08^{d}
3	48.05	06:00	3.03 ± 0.15^{c}	26.67	06:00	4.02 ± 0.23^{e}
		12:00	1.72 ± 0.21^{b}		12:00	1.30 ± 0.22^{b}
		24:00	1.65 ± 0.12^{b}		24:00	1.49 ± 0.14^{b}
4	72.07	06:00	1.42 ± 0.10^{b}	40.00	06:00	2.83 ± 0.19^{c}
		12:00	0.99 ± 0.18^{a}		12:00	0.24 ± 0.10^{a}
		24:00	0.87 ± 0.13^{a}		24:00	0.17 ± 0.06^{a}

Note: VP = *Vibrio parahaemolyticus;* VH = *Vibrio harveyi*

Table 4. The survival rate of *Artemia* sp enriched by esDNA at different immersion time after exposed with the VP and VH at the concentration level of 10^5 CFU mL^{-1}. Values represent the mean of ten replicates. Results in the same columns with different superscript letter are significantly different (*P<0.05*) based on analysis of variance followed by the Tukey's multiple comparison test.

No	esDNAVP (ng μl^{-1})	Immersion time with esDNAVP	Survival rate of Artemia (%)	esDNAVH (ng μl^{-1})	Immersion time with esDNAVP	Survival rate of Artemia (%)
1.	0.00	06:00	49.50 ± 4.31[a]	0.00	06:00	42.83 ± 3.60[a]
		12:00	48.00 ± 5.76[a]		12:00	45.67 ± 2.96[a]
		24:00	49.50 ± 5.39[a]		24:00	44.50 ± 5.39[a]
2	24.02	06:00	47.67 ± 4.53[a]	13.33	06:00	55.50 ± 3.24[b]
		12:00	50.83 ± 2.97[ab]		12:00	59.33 ± 3.06[bc]
		24:00	61.17 ± 2.73[c]		24:00	61.83 ± 3.28[c]
3	48.05	06:00	56.33 ± 4.43[bc]	26.67	06:00	63.00 ± 3.12[c]
		12:00	74.50 ± 2.84[d]		12:00	73.50 ± 3.88[d]
		24:00	77.33 ± 3.44[d]		24:00	77.67 ± 3.26[d]
4	72.07	06:00	79.50 ± 3.85[d]	40.00	06:00	78.50 ± 2.28[d]
		12:00	87.50 ± 4.98[e]		12:00	85.00 ± 3.04[e]
		24:00	88.00 ± 1.72[e]		24:00	87.50 ± 2.26[e]

Note: VP = *Vibrio parahaemolyticus;* VH = *Vibrio harveyi*