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RESEARCH ARTICLE

PROTECTIVE EFFECT OF SHORT CHAIN FATTY ACIDS ON GNOTOBIOTIC *ARTEMIA FRANCISCANA* NAUPLII AGAINST *VIBRIO HARVEYII*

***[1]Sivagnanavelmurugan, M., [2]Palavesam, A., [1]Arul, V. and [2]Immanuel, G.**

[1]Department of Biotechnology, School of Life Sciences, Pondicherry University, Pondicherry, India- 605014
[2]Marine biotechnology Division, Centre for Marine Science and Technology, Manonmaniam Sundaranar University, Rajakkamangalam – 629 502, Kanyakumari District, Tamilnadu, India

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ABSTRACT

The present study was carried out to investigate the protective effect of short chain fatty acids (acetic, propionic and butyric acids) on gnotobiotic *Artemia franciscana* nauplii against the shrimp pathogen *Vibrio harveyii*. The antibacterial effect of different concentrations (1, 10 and 100mM) of individual short chain fatty acids against *V. harveyii* was determined by bacterial growth study at pH 6. At 100mM concentration of all the tested short chain fatty acids, the pathogen growth was completely inhibited. The pH (5-7) dependent growth inhibition of 20mM concentration of short chain fatty acids against *V. harveyii* was determined by bacterial growth study. At pH 5, the pathogen growth was highly inhibited in all the tested short chain fatty acids. The *Artemia* nauplii (instar II) gnotobiotically reared in 20 mM short chain fatty acids supplemented medium were challenged with *V. harveyi*, and the mortality was recorded at an interval of 6 h up to 60 h. In this study, the reduction in mortality of *Artemia* nauplii was 36.95, 30.05 and 24.15%, respectively in acetic acid, propionic acid and bytyric acid. The present results provide justification for short chain fatty acids as alternative antibiotic in aquaculture against shrimp pathogen *V. harveyii*.

Key words: Acetic acid; propionic acid; butyric acid; Gnotobiotic; Artemia franciscana; Vibrio harveyii.

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INTRODUCTION

Mass production of juvenile stages of aquatic organisms and their live preys for aquaculture obliges the use of intensive culture systems, which often leads to high mortalities caused mostly by pathogenic or opportunistic bacteria (Bachere, 2003). The pathogenic or opportunistic strains such as *V. alginolyticus* (Lee *et al.,* 1996), *V. damsela* (Song *et al.,* 1993), *V. harveyi* (Liu *et al.,* 1996), *V. parahaemolyticus* (Sung *et al.,* 2001) and *V. vulnificus* (Song and Sung, 1990) cause traumatic losses in aquaculture system. Among these, *V. harveyi* is a ubiquitous, bioluminescent bacterium causing life-threatening vibriosis in marine vertebrates and invertebrates, leading to severe losses in the aquaculture industry especially in shrimp industries (Austin and Zhang, 2006). Many shrimp farmers use antibiotics for the treatment of Vibriosis (Moriarty, 1999). However, this practice promotes the selection and dissemination of antibiotic-resistant bacteria in the target organism and throughout the environment (Hameed and Balasubramanian, 2000).

***Corresponding author:* Sivagnanavelmurugan, M.,**
Department of Biotechnology, Scholl of Life Sciences, Pondicherry University, Pondicherry. India- 605014.

For this reason, a variety of alternative strategies such as the use of probiotic bacteria (Irianto and Austin, 2002; Immanuel *et al.,* 2007), antimicrobial plant extracts (Immanuel *et al.,* 2004), gossy pol (Yildrim-Aksoy *et al.,* 2004), antimicrobial peptides (Kjuul *et al.,* 1999; Sarmasik and Chen, 2003), medium chain fatty acid - caprylic acid (Immanuel *et al.,* 2010) and short chain fatty acids (Immanuel *et al.,* 2011) have been explored for the usage of alternative antibiotics in aquaculture. In order to study the effects of microorganisms more accurately, a model system was employed using the brine shrimp, *Artemia franciscana*, as a test organism (Marques *et al.,* 2005), because, it can be cultured easily in gnotobiotic condition (Austin *et al.,* 2005). *Artemia* nauplii are widely recognized as the best natural, long-term storable live feed for marine finfish and crustacean hatcheries (Lavens and Sorgeloos, 1996). This is due to the fact that *Artemia* possess some unique features like small size, easy for ingestion (Leger *et al.,* 1986), high nutritional value (Browne and Bowen, 1991), high tolerance to various culture environments (Leger *et al.,* 1987), short generation time (Reeve, 1963; Persoone and Sorgeloos, 1980), etc. The brine shrimp *Artemia* has been recently shown to be instrumental in the development of a

gnotobiotic test system for studying the effect of food composition on survival and growth in the presence or absence of a challenge test (Marques *et al.,* 2004; 2005). Marques *et al.* (2006) have established a gnotobiotic system, where larvae are hatched, grown in axenic conditions and exposed to known bacteria has facilitated research on host microbe interactions, particularly as they relate to *Artemia* larvae. Some investigations reported that short-chain fatty acids inhibit the growth of yeast and enterobacteria such as *Salmonella typhimurium*, *Escherichia coli* and *Shigella flexneri* (Bergeim, 1940; Wolin, 1969; Cherrington *et al.,* 1991; Bearson *et al.,* 1997; Sun *et al.,* 1998; Van Immerseel *et al.,* 2003). Short-chain fatty acids have also been reported for the protection of *A. franciscana* nauplii against the shrimp pathogens such as, *V. cambelli* and *V. parahaemolyticus* (Defoirdt *et al.,* 2006; Immanuel *et al.,* 2011). Defoirdt *et al.* (2007) reported that the well known bacterial storage compound poly-β-hydroxybutyrate (PHB), a polymer of the short-chain fatty acid β-hydroxybutyrate, also protected *Artemia* nauplii from the virulent *V. campbellii* strain. Considering the importance of the above, the present study was carried out to investigate the protective effect of selected short chain fatty acids such as acetic, propionic and butyric acids on gnotobiotic *Artemia franciscana* naupii against *V. harveyii*.

MATERIALS AND METHODS

Effect of short chain fatty acids on growth of *V. harveyii*

Short chain fatty acids such as acetic, propionic and butyric acids (Himedia, Mumbai, India) were individually dissolved in Luria Bertani (LB) broth at different concentrations (1.0, 10.0 and 100.0 mM) and also a positive control (LB broth without short chain fatty acids) were maintained and their pH was adjusted to 6.0 by using digital pH meter. The media were individually filter sterilized and the sterile media were inoculated with *V. harveyii* (CMB-57) culture (diluted to an OD_{600} of 1) individually at the rate of 20µl/ml medium. Simultaneously, triplicates were maintained for each concentration and control group. Then the inoculated media were incubated at 28^0C for 5 h and the growth of the pathogen was measured by taking the optical density (OD) at 600 nm at an every one hour interval using an UV Spectrophotometer (Techcomp-8500; Hong Kong).

pH Dependent growth inhibitory effect of short chain fatty acids against *V. harveyii*

20 mM of individual short chain fatty acids in LB broth medium were prepared. Subsequently, a control (LB broth without short chain fatty acids) was also maintained. The pH of individual group of fatty acid was adjusted to 5, 6 and 7, respectively. The media were filter sterilized and inoculated with *V. harveyii* culture individually @ 20µl/ml medium. Then the inoculated media were incubated at 28 ºC for 5 h and the OD (600 nm) was measured at an every one hour interval.

Gnotobiotic Artemia nauplii culture

Experiments were carried with brine shrimp *Artemia franciscana* (Great Salt Lake, USA). Bacteria free cyst and nauplii obtained through decapsulation technique (Sorgeloos *et al.,* 1986). During decapsulation of cyst 0.22 µm filtered aeration was provided. All the works were done under a laminar hood and all the necessary tools involved in the experiment were previously sterilized through autoclaving at 120 °C for 20 min. Decapsulated cysts were washed carefully using filtered and sterilized seawater (autoclaved at 120°C for 20min) and resuspended in 50ml capacity screw cap tubes containing 30 ml of filtered and sterilized seawater and hatched for 24 h on a rotor (4 min) at 28 °C with constant illumination (approx. 2000 lux).

In vivo challenge test

In vivo challenge test was performed by the method described by Defoirdt *et al.* (2005) and Immanuel *et al.* (2010; 2011). After hatching, a group of 25 nauplii (instar II) of *A. franciscana* were transferred to sterile falcon tubes containing filtered and sterilized seawater with 20 mM of individual short chain fatty acids. A positive control was also maintained without short chain fatty acid. The pH of the experimental media was adjusted to 7.0. The *Artemia* nauplii were fed with an autoclaved suspension of *Aeromonas hydrophila* (LVS3) strain at approximate cell density of 10^7 $CFUmL^{-1}$ daily. Before feeding, the strain was grown overnight on marine agar (Himedia, Mumbai) suspended in filtered seawater, autoclaved and added to *Artemia* culture. Then the falcon tubes were inoculated with 10^5 $CFUmL^{-1}$ of *V. harveyii*, individually. Simultaneously, a negative control group was maintained without short chain fatty acid and pathogen. The falcon tubes were incubated on a rotor at 28 ºC. The survival rate of *Artemia* was measured for 60 h interval. The survival percentage was determined every 6h for each treatment. For this purpose, the number of live *Artemia* nauplii was registered before feeding or adding bacteria by counting with the naked eye while exposing each transparent falcon tube to an incandescent light without opening the tube to maintain the gnotobiotic environment (Soltanian *et al.,* 2007). Simultaneously, triplicates were maintained in each group. The Cumulative Mortality Index (CMI) of *Artemia* nauplii was calculated by summing the mortality counts noted at each time interval (Immanuel *et al.,* 2001; 2004; 2007; 2010; 2011).

$$CMI = Dx_1 + Dx_2 + Dx_3 \ldots\ldots\ldots\ldots Dx_n$$

Where, D is the number of dead individuals at the time, x_1, x_2, x_3 …… x_n.

Statistical analysis

The data obtained in the present study were expressed as Mean ± SD and were analyzed using student't' test at 5 % significant level. Further a multiple comparison test (SNK) was conducted to compare the significant differences among the parameters using computer software Statistica 6.0 (Statosoft, UK).

RESULTS

Effect of short chain fatty acids on growth of *V. harveyii*

The initial experiment was performed to determine the effect of different concentrations (1.0, 10.0 and 100mM) of short chain fatty acids on the growth of pathogen *V. harveyii* (Fig.1). In control group, the growth of the pathogen was prolonged till the end of the experiment. The pathogen growth was completely inhibited at highest concentration (100 mM) of all short chain fatty acids, but in 1mM and 10mM concentrations, the growth of the pathogens was not completely inhibited.

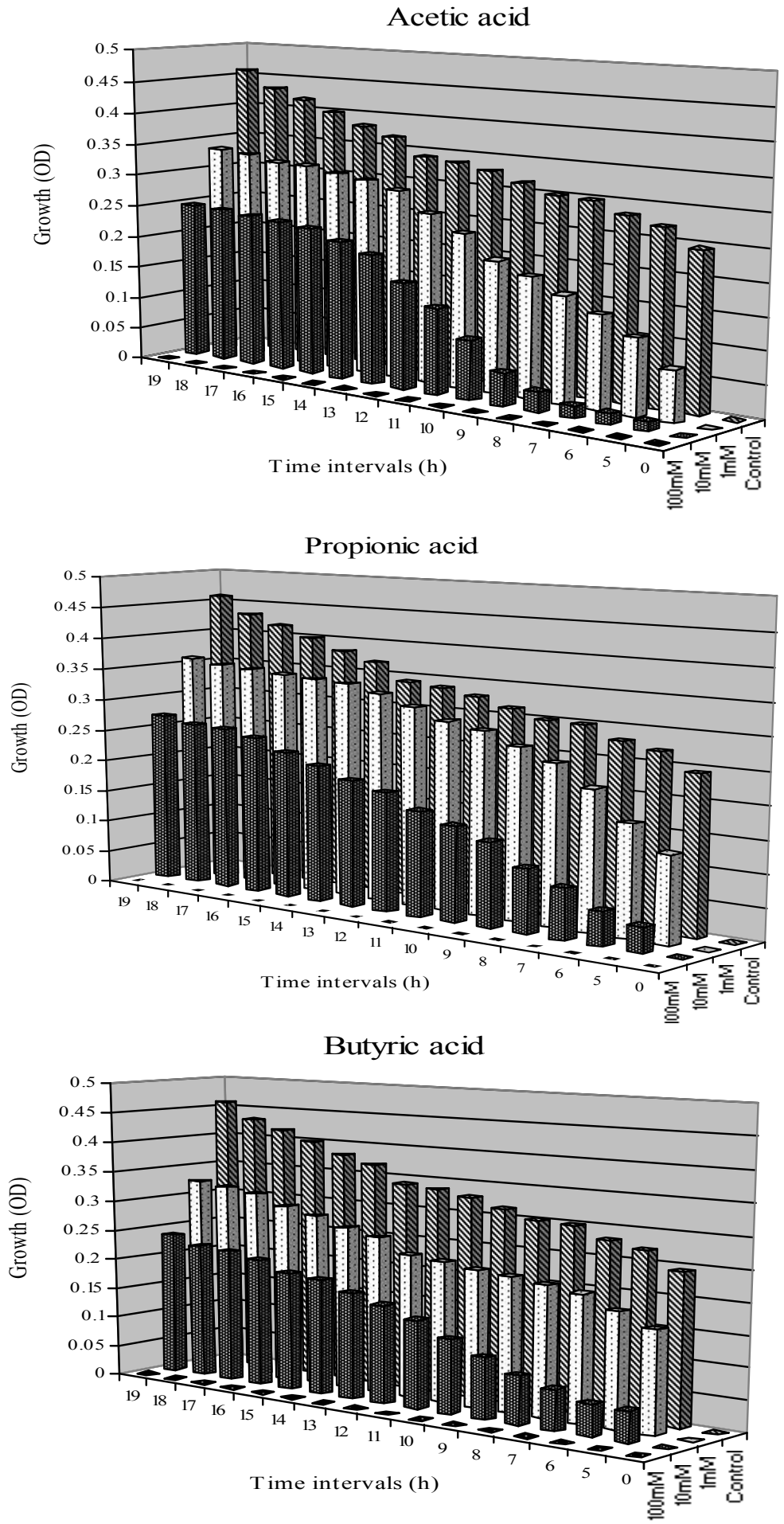

Figure 1. Effect of different concentrations (1, 10 and 100mM) of individual short chain fatty acids on growth of *V. harveyii* at different time intervals

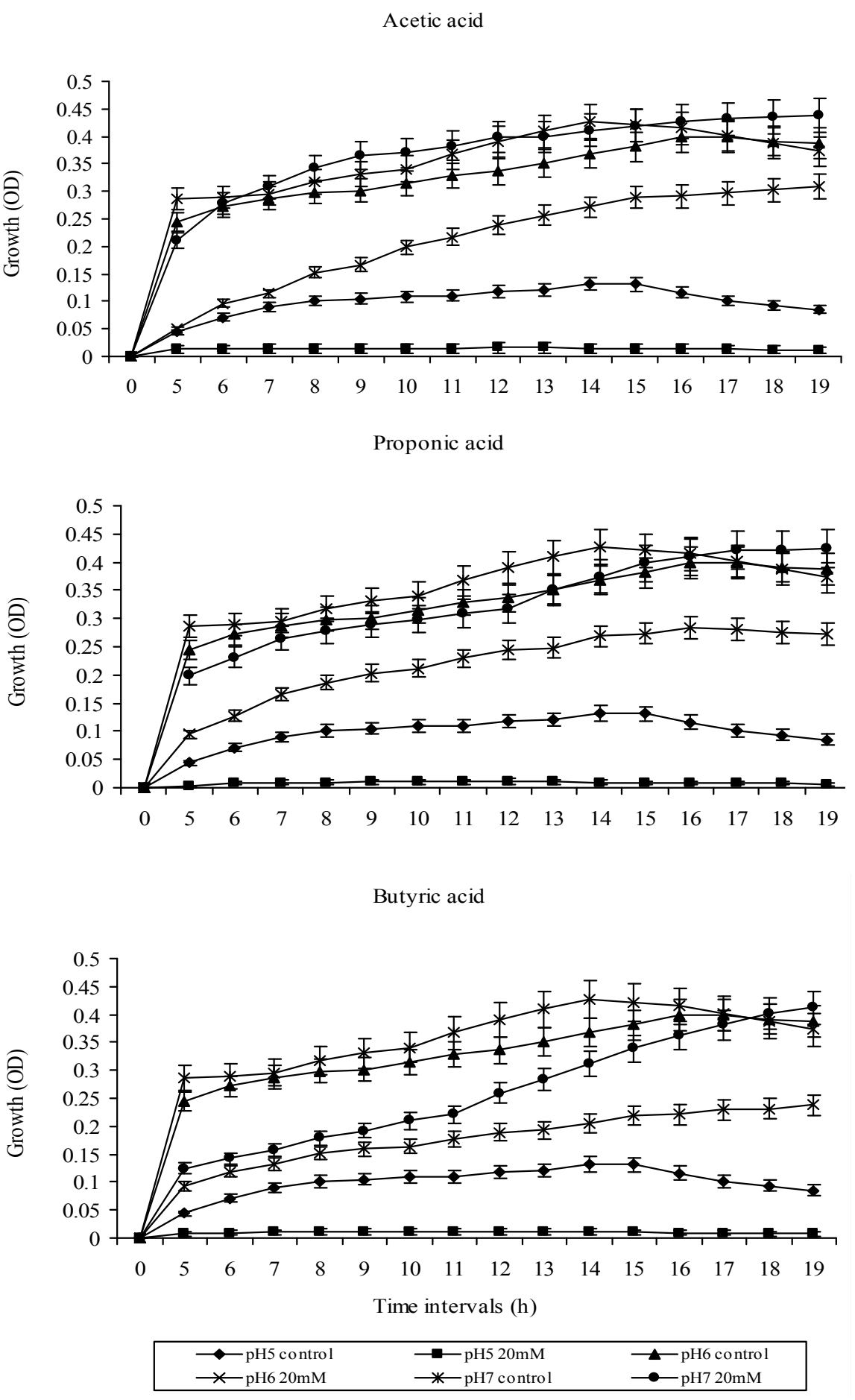

Figure 2. Effect of 20mM concentration of individual short chain fatty acids on growth of *V. harveyii* at different pH (5 – 7) in different time intervals

Table 1. Cumulative percentage (%) mortality of *A. franciscana* nauplii challenged with *V. harveyii* in individual short chain fatty acids (20mM) supplemented culture media at pH7 during different time intervals

Samples	Cumulative percentage (%) mortality in different time (h) intervals									
	6	12	18	24	30	36	42	48	54	60
Positive control	21.26 ± 0.82	31.62 ± 1.32	48.30 ± 1.24	53.44 ± 1.12	63.80 ± 1.83	68.36 ± 1.54	73.76 ± 1.46	78.28 ± 2.02	91.65 ± 1.92	100± 0.00[a]
Negative Control	0 ± 0.0	0 ± 0.0	1.90 ± 0.47	3.30 ± 0.26	5.72 ± 0.64	6.85 ± 0.42	9.30 ± 0.72	12.40 ± 0.98	15.26 ± 0.92	17.46 ± 1.02[b]
Acetic acid	8.30 ± 0.92	16.60 ± 0.96	25.20 ± 1.12	31.25 ± 1.64	36.46 ± 1.36	41.15 ± 2.08	48.30 ± 2.56	53.56 ± 1.84	57.30 ± 1.38	65.65 ± 2.82[c]
Propionic acid	13.30 ± 1.02	21.65 ± 1.26	28.30 ± 1.92	33.86 ± 1.86	38.20 ± 1.54	43.42 ± 1.36	53.28 ± 1.46	58.86 ± 1.92	66.68 ± 2.02	72.30 ± 3.05[d]
Butyric acid	16.65 ± 1.16	23.30 ± 1.84	31.56 ± 1.36	36.35 ± 1.92	41.52 ± 2.01	48.20 ± 1.53	58.32 ± 1.78	65.24 ± 1.96	71.65 ± 2.76	76.45 ± 3.36[e]

Each value is the mean ± SD of triplicate analysis. Within each column, means with the different superscript letters are statistically significant (t-test; P< 0.05) and subsequent *post hoc* multiple comparison with the SNK test.

Table 2. Cumulative Mortality Index (CMI) and reduction in mortality (%) over control value of *A. franciscana* nauplii challenged with *V. harveyii* in 20mM concentration of individual short chain fatty acids supplemented culture media

Samples	CMI	Reduction in mortality (%)
Positive control	24838.38 ± 263.36[a]	0.0 ± 0.0
Negative control	3389.04 ± 72.24[b]	86.35 ± 1.04
Acetic acid	15660.48 ± 126.28[c]	36.95 ± 0.186
Propionic acid	17372.52 ± 144.68[d]	30.05 ± 0.121
Butyric acid	18837.84 ± 176.18[e]	24.15 ± 0.098

Each value is the mean ± SD of triplicate analysis. Within each column, means with the different superscript letters are statistically significant (t-test; P< 0.05) and subsequent *post hoc* multiple comparison with the SNK test.

pH dependent growth inhibitory activity of short chain fatty acids against *V. harveyii*

20 mM concentration of short chain fatty acids were tested against *V. harveyii* at various pH (5, 6 and 7) (Fig.2). At pH5, growth of the pathogen was very less. But when the pH level increased to 6 and 7, the growth of the pathogen was increased.

In vivo challenge test

The cumulative percentage mortality of *Artemia franciscana* (Great Salt Lake) nauplii after challenged with *V. harveyii* is given in Table 1. *A. franciscana* nauplii were succumbed to death from 6th h during challenge test. The mortality of control group (without short chain fatty acid) was 21.26%. At the same time, the mortality percentage of short chain fatty acids supplemented groups was 8.30, 13.30 and 16.65%, respectively in acetic, propionic and butyric acids. Similarly, the negative control group showed no mortality during 6h of experimental period. The mortality percentage of all the tested groups of *A. franciscana* nauplii was increased with increasing time duration. Within 60h, 100 % mortality was observed in control group. But in the experimental groups, the mortality of 20mM acetic, propionic and butyric acids supplemented groups showed 65.65, 72.30 and 76.45%, respectively. Only 17.46% mortality was observed in negative control group at the end of the experiment.

CMI and reduction in mortality (%)

The CMI for the positive control group of *A. franciscana* nauplii challenged with *V. harveyii* was 24838.38, which was reduced 36.95% in 20mM acetic acid, 30.05% in 20mM propionic acid and 24.15% in 20mM butyric acid. Similarly, the reduction in mortality of negative control group was 86.35% (Table 2).

DISCUSSION

An initial experiment was carried out to determine the effect of different concentrations (1, 10 and 100mM) of short chain fatty acids (acetic, propionic and butyric acids) on the growth inhibitory activity of *V. harveyii* at pH 6. At the highest concentration (100mM), all the tested short chain fatty acids completely inhibited the growth of the *V. harveyii*, but in the lowest concentrations (1 and 10mM), the pathogen growth was not completely inhibited. Similarly, Immanuel *et al.* (2011) have reported that the effect of different concentrations (1, 10 and 100mM) of short chain fatty acids on the growth of *V. parahaemolyticus* at pH 6. They pointed out that the growth of *V. parahaemolyticus* was completely inhibited at 100mM concentration of all tested short chain fatty acids, but at 1 and 10mM of all the tested short chain fatty acids, the *V. parahaemolyticus* growth was not completely inhibited.

Immanuel *et al.* (2010) also investigated the effect of different concentrations (1, 10 and 100mM) of medium chain fatty acid-caprylic acid on the growth of *V. harveyii* and *V. parahaemolyticus* at pH 6. They suggested that the growth of both the pathogens was completely inhibited at higher concentration (100 mM) of caprylic acid within 5 h. But in 1 and 10 mM concentrations of caprylic acid, the growth of the pathogens was not completely inhibited. Defoirdt *et al.* (2006) have studied the effect of different concentrations (1, 10 and 100mM) of short chain fatty acids (formic, acetic, propionic, butyric and valeric acids) on the inhibition of the growth of *V. campbelli* at pH 6. They reported that, all the tested short chain fatty acids completely inhibited the growth of *V. campbelli* at a concentration of 100mM, whereas there was no effect on the growth of the pathogen at 1 and 10mM concentrations. Similarly, Wolin (1969) reported a partial inhibition of the growth of *E. coli* by approximately 10 mM of short chain fatty acids such as propionic, acetic and butyric acids at pH 6. Van Immerseel *et al.* (2003) have also reported that the growth of *S. enterica* sub sp. *enterica* was completely inhibited by 100 mM short chain fatty acids like formic, acetic and propionic acids at pH 6. Another experiment was carried out to determine the pH dependent growth inhibitory activity of short chain fatty acids against *V. harveyii*.

The growth inhibitory effect of short chain fatty acids was decreased with increasing the pH. At lowest pH (pH5), the growth of the pathogen was inhibited. But at the highest pH (6 and 7), there was no inhibition observed. Similarly, Immanuel *et al.* (2011) have reported that the effect of different pH (5-7) on the growth inhibition of 20mM short chain fatty acids and observed the growth of the pathogen was inhibited at pH5, but there was no inhibition observed in pH 6 and 7. Immanuel *et al.* (2010) have also observed the pH variation (5-7) on the growth inhibitory effect of 10 mM concentration of caprylic acid against *V. harveyii* and *V. parahaemolyticus*. They pointed out that, at pH 5, the growth of both pathogens was highly inhibited (65–80%) than at pH 6 and 7 (43–46%). Defoirdt *et al.* (2006) reported the pH dependent growth inhibitory effect of 20mM short chain fatty acids (formic, acetic, propionic, butyric and valeric acids) on *V. campbelli*. They confirmed that, at pH 5, the growth was completely inhibited, whereas at pH 6, the growth was delayed and at pH 7, no inhibition was observed. The pH dependent effect was also reported for the inhibition of the growth of *E. coli* and *S. enterica* sub sp. *enterica* by short chain fatty acids (Wolin, 1969; Mc Han and Shotts, 1993; Van Immerseel *et al.,* 2003). The pH dependent effect can be explained by the fact that the fatty acids can pass the cell membrane only in their undissociated form, which is more dominant at lower pH (Sun *et al.,* 1998). In the present study, the *in vivo* challenge study was conducted to determine the protective effect of 20mM short chain fatty acids on *Artemia* nauplii from the *V. harveyii*.

The mortality of *Artemia* nauplii was reduced to 36.95% in 20mM acetic acid, 30.05% in 20mM propionic acid and 24.15% in 20mM butyric acid. Similarly, Immanuel *et al.* (2011) have investigated the protective effect of 20mM short chain fatty acids on *Artemia* nauplii against *V. parahaemolyticus* and the reduction in mortality was 38.5, 30.7 and 25.3%, respectively in acetic acid, propionic acid and butyric acid. Immanuel *et al.* (2010) have also reported that the reduction in mortality of *A. franciscana* nauplii reared in 10 mM concentration of caprylic acid supplemented medium and challenged with *V. harveyii* and *V. parahaemolyticus*. They suggested that the reduction in mortality of *Artemia* nauplii was 20.61 and 16.30% in *V. parahaemolyticus* and *V. harveyi* challenged groups, respectively. Defoirdt *et al.* (2006) have also recorded the percentage survival of *Artemia* nauplii supplemented with 20mM of short chain fatty acids and challenged against *V. campbelli*. Their result showed that the percentage survival of short chain fatty acids supplemented group of *A. franciscana* nauplii had 45, 48, 48, 42 and 47% in the respective groups of 20mM of formic, acetic, propionic, butyric and valeric acids.

Wang and Johnson (1992) suggested that the short chain fatty acids exhibit stronger antibacterial activity than long chain fatty acids because the antibacterial property of fatty acids decreased with increasing chain length. Galbraith *et al.* (1971) noted that fatty acids must be in solution and remain sufficiently lipophilic to enable adsorption to bacterial cell surface for antibacterial activity. However, the exact mechanism of action of short chain fatty acids on bacteria is not known, numerous hypothesis have been suggested to explain the general mode of antimicrobial activity of free fatty acids. One hypothesis proposed that the undissociated form of short chain fatty acids diffuse in to bacterial cells, dissociate within the protoplasm and reduce intracellular pH (Sun *et al.,* 1998). The lower intracellular pH can lead to inactivate the intracellular enzymes and inhibit the aminoacid transport (Viegas and Sa-Correia, 1991; Freese *et al.,* 1973). The potential for developing bacterial resistance to these molecules is relatively negligible, because the bacteria killed by multiple mechanism of short chain fatty acids. Bergsson *et al.* (1999) revealed that free fatty acids exert their antimicrobial effect on membranes of bacteria, thus the chances of emergence of bacterial resistance are rare.

Conclusion

In conclusion, the antibacterial effect of short chain fatty acids on gnotobiotic *Artemia franciscana* nauplii against *V.harveyii* could be attributed to its short chain length. The present study showed that the application of short chain fatty acids is an effective alternative method for the treatment of luminescent Vibrios in aquaculture. This alternative method could lead to greater ecological and economic sustainability of the aquaculture industry, minimizing the amount of antibiotics entering in to the environment.

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REFERENCES

Austin, B. and Zhang, XH. 2006. *Vibrio harveyi*: a significant pathogen of marine vertebrates and invertebrates. *Letters in Applied Microbiology,* 43: 119-124.

Austin, B., Austin, D,, Southerland, R., Thompson, F. and Swings, J. 2005. Pathogenicity of vibrios to rainbow trout (*Oncothynchus mykiss*, Walbaum) and *Artemia* nauplii. *Environmental Microbiology,* 7: 1488-1495.

Bachere, E. 2003. Anti-infectious immune effectors in marine invertebrates: potential tools for disease control in larviculture. *Aquaculture,* 227: 427-438.

Bearson, S., Bearson, B. and Foster, JW. 1997. Acid stress responses in enterobacteria. *FEMS Microbiology Letter,* 147: 173–180.

Bergheim, O. 1940. Toxicity of intestinal volatile fatty acids for yeast and *E. coli*. *Journal of Infectious Diseases,* 66: 222–234.

Bergsson, G., Steingrimsson, O. and Thormar, H. 1999. *In vitro* susceptibilities of *Neisseria gonorrhoeae* to fatty acids and monoglyceridses. *Antimicrobial Agents Chemotherapy,* 43: 2790–2792.

Browne, RA. and Bowen, S, 1991. Taxonomy and population genetics of *Artemia*. In: Browne RA, Sorgeloos P, Trotman CNA (eds) *Artemia* biology, CRS Press, Boca Raton, pp 221–235

Cherrington, CA., Hinton, M., Pearson, GR. and Chopra, L. 1991. Short chain organic acids at pH 5 kill *Escherichia coli* and *Salmonella* sp. without causing membrane perturbation. *Journal of Applied Bacteriology,* 70: 161–165.

Defoirdt, T., Bossier, P. and Verstraete, W. 2005. The impact of mutations in the quoreum sensing systems of *Aeromonas hydrophilla*, *Vibrio anguillarum* and *V. harveyi* on their virulence towards gnotobiotically cultured *Artemia franciscana*. *Environmental Microbiology,* 7: 1239–1247.

Defoirdt, T., Halet, D., Sorgeloos, P., Bossier, P. and Verstraete, W. 2006. Short chain fatty acids protect the growth of gnotobiotic *Artemia franciscana* from pathogenic *Vibrio campbelli*. *Aquaculture,* 261: 804–808.

Defoirdt, T., Halet, D., Vervaeren, H., Boon, N., Van de Wiele, T. Bossier, P. and Verstraete, W, 2007. The bacterial storage compound poly-β-hydroxybutyrate protects *Artemia franciscana* from pathogenic *Vibrio campbellii*. *Environmental Microbiology,* 9: 445–452.

Freese, E., Sheu, CW. and Galliers, E. 1973. Functions of lipophilic acids as antimicrobial food additives. *Nature,* 241: 321–325.

Galbraith, H., Miller, TB., Paton, AM. and Thompson, JK. 1971. Antibacterial activity of long chain fatty acids and the reversal with calcium, magnesium, ergocalciferol and cholesterol. *Journal of Applied Bacteriology,* 34: 803–813.

Hameed, A. and Balasubramanian, G, 2000. Antibiotic resistance in bacteria isolated from *Artemia* nauplii and efficacy of formaldehyde to control bacterial load. *Aquaculture,* 183: 195–205.

Immanuel, G., Citarasu, T., Sivaram, V., Michael Babu, M. and Palavesam, A. 2007. Delivery of HUFA, probionts and biomedicine through bioencapsulated *Artemia* as a means to enhance the growth and survival and reduce the pathogenicity in shrimp *Penaeus monodon* postlarvae. *Aquaculture International,* 15: 137–152.

Immanuel, G., Peter Marian, M. and Palavesam, A, 2001. Effect of feeding lipid enriched *Artemia* nauplii on survival, growth, tissue fatty acids and stress resistance of postlarvae *Penaeus indicus*. *Journal of Asian Fisheries Science,* 14(4): 377–388.

Immanuel, G., Sivagnanavelmurugan, M. and Palavesam, A. 2011. Antibacterial effect of short-chain fatty acids on gnotobiotic *Artemia franciscana* nauplii against *Vibrio parahaemolyticus*. *Aquaculture Research,* (doi:10.1111/ j.1365-2109.2011.02857.x).

Immanuel, G., Sivagnanavelmurugan, M. and Palavesam, A. 2010. Antibacterial effect of medium-chain fatty acid: caprylic acid on gnotobiotic *Artemia franciscana* nauplii against shrimp pathogens *Vibrio harveyi* and *V. parahaemolyticus*. *Aquaculture International,* 19: 91-101.

Immanuel, G., Vincy Bai, VC., Sivaram, V., Palavasem, A. and Peter Marian, M, 2004. Effect of butanolic extracts from terrestrial herbs and seaweeds on the survival, growth and pathogen (*Vibrio parahaemolyticus*) load on shrimp *Penaeus indicus* juveniles. *Aquaculture,* 236: 53–65.

Irianto, A. and Austin, B. 2002. Probiotics in aquaculture. *Journal of Fish Diseases,* 25: 633–642

Kjuul, AK., Bullesbach, EE., Espelid, S., Dunham, R., Jorgensen, TO., Warr, GW. and Styrrold, OB, 1999. Effects of cecropin peptides on bacteria pathogenic to fish. *Journal of Fish Diseases,* 22: 387–394.

Lavens, P. and Sorgeloos, P, 1996. Manual on the production and use of live food for aquaculture. FAO, Fisheries Technical Paper 361, 295 pp

Lee, KK., Yu, SR., Chen, FR., Yang, TZ. and Liu, PC. 1996. Virulence of *Vibrio alginolyticus* isolated from diseased tiger prawn *Penaeus monodon*. *Current Microbiology,* 32: 229–231.

Leger, P., Bengtson, D., Sorgeloos, P., Simpson, KL. and Beck AD. 1987. The nutritional value of Artemia-a review. In: Sorgeloos P, Bengston DA, Decleir W, Jaspers E (eds) *Artemia* Research and its applications, vol 3. Universa Press, Wetteren, pp 352–372.

Leger, P., Bengtson, DA., Simpson, KL. and Sorgeloos, P, 1986. The use and nutritional value of *Artemia* as a food source. *Oceanography and Marine Biology Annual Review,* 24: 521–623

Liu, PC., Lee, KK., Yii, KC., Kou, GH. and Chen, SN. 1996. Isolation of *Vibrio harveyi* from diseased prawn *Penaeus japonicus*. *Current Microbiology,* 33: 129–132.

Marques, A., Ollevier, F., Verstrate, W., Sorgeloos, P. and Bossier, P, 2006. Gnotobiotically grown aquatic animals; opportunities to investigate host microbe interactions. *Journal of Applied Microbiology,* 100: 903–918.

Marques, A., Dhont, J., Sorgeloos, P. and Bossier, P. 2004b. Evaluation of different yeast cell wall mutants and microalgae strains as feed for gnotobiotically-grown brine shrimp *Artemia franciscana*. *Journal of Experimental Marine Biology and Ecology,* 312 (1): 115–136.

Marques, A., Dinh, T., Ioakeimidis, C., Huys, G., Swings, J., Verstraete, W., Dhont, J., Sorgeloos, P. and Bossier, P, 2005. Effects of bacteria on *Artemia franciscana* cultured in different gnotobiotic environments. *Applied Environmental Microbiology,* 71: 4307–4317

Marques, A., Francis, JM., Dhont, J., Bossier, P. and Sorgeloos, P. 2004a. Influence of yeast quality on performance of gnotobiotically grown *Artemia*. *Journal of Experimental Marine Biology and Ecology,* 310: 247–264.

Mc Han, F. N and Shotts, EB, 1993. Effect of short chain fatty acids on the growth of *Salmonella typhimurium* in an in vitro system. *Avian Diseases,* 37: 396–398.

Moriarty, DJW, 1999. Disease control in shrimp aquaculture with probiotic bacteria. In: Bell, C.R., Brylinsky, M., Johnson-Green, P. (Eds.), Microbial Biosystems: New Frontiers. Proceedings of the 8th International Symposium on Microbial Ecology. Atlantic Canada Society for Microbial Ecology, Halifax, Canada.

Persoone, G. and Sorgeloos, P, 1980. General aspect of the ecology and biogeography of Artemia. In: Persoone G, Sorgeloos P, Roels O, Jasper E (eds) The brine shrimp *Artemia* ecology, culturing, use in aquaculture. Universa Press, Wettern, pp 3–24.

Reeve, MR. 1963. The filter feeding of Artemia II. In suspension of various particles. *Journal of Experimental Biology,* 40: 207–214.

Sarmasik, A. and Chen, TT. 2003. Bacteriocidal activity cells (CHSE-214): application in controlling fish bacterial pathogens. *Aquaculture,* 20: 183–194.

Soltanian, S., Francois, JM., Dhont, J., Arnouts, S., Sorgeloos, P. and Bossier, P, 2007. Enhanced disease resistance in *Artemia* by application of commercial β-glucans sources and chitin in a gnotobiotic *Artemia* challenge test. *Fish and Shellfish Immunology,* 23: 1304–1314.

Song, YL. and Sung, HH. 1990. Enhancement of growth in tiger shrimp (*Penaeus monodon*) by bacteria prepared from *Vibrio vulnificus*. *Bullet in European Association of Fish Pathology,* 10: 98–99.

Song, YL., Cheng, W. and Wang, CH. 1993. Isolation and characterization of *Vibrio damsela* infectious for cultured shrimp in Taiwan. *Journal of Invertebrate Pathology,* 61: 24–31.

Sorgeloos, P., Lavens, P., Leger, P., Tackaert, W. and Versichele, D. 1986. Manual for the culture and use of brine shrimp *Artemia* in aquaculture. *Artemia* reference centre. Faculty of Agriculture, State University of Ghent, Belgium.

Sun, CQ., Connor, CJO., Turner, SJ., Lewis, GD., Stanley, RA. and Robertson, AM, 1998. The effect of pH on the bacterial growth by physiological concentrations of butyric acid. Implications for neonates fed on suckled milk. *Chemical and Biological Interaction,* 113: 117–131.

Sung, HH., Hsu, SF., Chen, CK., Ting, YY. and Chao, WL. 2001. Relationships between disease outbreak in cultured tiger shrimp (*Penaeus monodon*) and the composition of *Vibrio* communities in pond water and shrimp hepatopancreas during cultivation. *Aquaculture,* 192: 101–110.

Van Immerseel, F., De Buck, J., Pasmans, F., Velge, P., Bottreau, E., Fievez, V., Haesebrouck, F. and Ducatelle, R, 2003. Invasion of *Salmonella enteritis* in avian intestinal epithelial cells *in vitro* is influenced by short chain fatty acids. *International Journal of Food Microbiology,* 85: 237–248.

Viegas, CA. and Sa-Correia, I. 1991. Activation of plasma membrane ATPase of *Saccharomyces cerevisiae* by octanoic acid. *Journal of General Microbiology,* 137: 645–651.

Wang, LL. and Johnson, EA, 1992. Inhibition of *Listeria monocytogens* by fatty acids and monoglycerides. *Applied Environmental Microbiology,* 58: 624–629.

Wolin, MJ. 1969. Volatile fatty acids and the inhibition of *Escherichia coli* growth by rumen fluid. *Journal of Applied Microbiology,* 17: 83–87.

Yildrim-Aksoy, M., Lim, C., Dowd, MK., Wan, PJ., Klesius PH. and Shoemaker, C. 2004. *In vitro* inhibitory effect of gossypol from gossypol-acetic acid and (-)-isomers of gossypol on the growth of *Edwardsiella ictaluri*. *Journal of Applied Microbiology,* 97: 87–92.

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