 doi:10.20944/preprints202104.0144.v1

Article

Biofilm inhibition and antimicrobial properties of silver ion-exchanged zeolite A against *Vibrio spp* marine pathogens

Zarina Amin[1], Nur Ariffah Waly[2], Sazmal Effendi Arshad[2*]

[1] Biotechnology Research Institute, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia; zamin@ums.edu.my
[2] Faculty of Science & Natural Resources, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia; arifahwaly@gmail.com
* Correspondence: sazmal@ums.edu.my; Tel.: +60178180618

This study highlights a potential application of ion-exchanged Zeolite A against marine microbial pathogens and their biofilms.

Abstract: A challenging problem in the aquaculture industry is bacterial disease outbreaks which results in the global reduction of fish supply and foodborne outbreaks. Biofilms in marine pathogens protect against antimicrobial treatment and host immune defense. Zeolites are minerals of volcanic origin made from crystalline aluminosilicates which are useful in agriculture and in environmental management. In this study, silver ion-exchanged zeolite A of four concentrations; 0.25M (AgZ1), 0.50M (AgZ2), 1.00M (AgZ3)and 1.50M (AgZ4) were investigated for biofilm inhibition and antimicrobial properties against two predominant marine pathogens *V. campbelli* and *V. parahemolyticus* by employing the Minimum Inhibitory Concentration(MIC), Crystal Violet Biofilm Quantification assays as well as Scanning Electron Microscopy. In the first instance, all zeolite samples AgZ1-AgZ4 showed antimicrobial activity for both pathogens. For *V. campbellii* AgZ4 exhibited the highest MIC at 125.00 µg/ml while for *V. parahaemolyticus* the highest MIC was observed for AgZ3 at 62.50 µg/ml. At sublethal concentration, biofilm inhibition of *V. campbelli* and *V. parahemolyticus* by AgZ4 were observed at 60.2% and 77.3% inhibition respectively. Scanning electron microscopy exhibited profound structural alteration of the biofilm matrix by AgZ4. This is the first known study that highlights the potential application of ion-exchanged Zeolite A against marine pathogens and their biofilms.

Keywords: Biofilms, Zeolite A, *Vibrio sp*, Antimicrobials

1. Introduction

Aquaculture farming has been the fastest growing food producing sector in the last few decades and an important industry in many developing countries. However, the industry currently faces a threatening challenge due to the bacterial disease outbreaks resulting in high mortality rates in the aquaculture population (1,2). This is in part due to extensive use of antibiotics in fish farms leading to antimicrobial resistance in fish pathogens (3-5). Vibriosis is an important bacterial disease in wild and farmed marine fishes which results in severe economic loss of more than USD 1 billion (6). In addition, bacteria from seafood sources have been associated with foodborne outbreaks (7). Biofilms are self-assembled communities of microorganisms embedded in a self-developed polymeric matrix to a biotic or abiotic surface and comprise exopolysaccharides, proteins, and extracellular DNA (eDNA) (8). Biofilms shield bacteria from external disturbances for improved resilience to

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 April 2021 doi:10.20944/preprints202104.0144.v1

the environmental conditions as well as enhancing virulence. They primarily confer protection against antimicrobial agents, disinfectants and host defense mechanisms, making them more difficult and expensive to treat (9). The prevalent challenge has necessitated efforts on new antibacterial materials that can effectively inhibit the growth of the resistance aquatic pathogens while minimizing negative impacts on human, animal health and to the environment. Recently, several studies on natural therapeutics from plants and immunostimulants (10-12), and by alternative inorganic material have been explored (13-14), including the application of zeolites as antimicrobial agents (15-17).

This study aimed to investigate antimicrobial and antibiofilm applications of silver ion-exchanged zeolite A against marine bacterial pathogens *V. parahaemolyticus* and *V. campbelli*. Study findings strongly indicated antimicrobial and antibiofilm characteristics of the silver ion-exchanged zeolite A against the bacterial pathogens, with the highest MIC levels observed for AgZ4 (1.50M) for *V. campbelli* and AgZ3 (1.00M) for *V. parahaemolyticus*. Scanning electron microscopy exhibited profound breakages in the biofilm structures of both marine pathogens when grown in media added with 1.50M silver ion-exchanged zeolite A (AgZ4). Taken together, this study highlights the potential application of ion-exchanged zeolite A against marine pathogens and their biofilms.

2. Materials and Methods

Liquid Ion Exchange of Zeolite A

The initial synthesis of zeolite A from bentonite clay comprised the activation of precursor bentonite clay by thermochemical treatments in HCl, addition of alkaline activators, ageing and crystallization processes as well as characterization by X-Ray diffraction (XRD) and Scanning Electron Microscopy (SEM) analysis (data published elsewhere).

For liquid ion exchange, the integration of silver (Ag) ion into zeolite A was performed by using the liquid ion exchange method as described by Demirci *et al.,* (17) with slight modifications.. Briefly, 1 g zeolite A was added into 10 ml of four $AgNO_3$ concentrations: 0.25 M, 0.50 M, 1.00 M and 1.50 M (denoted AgZ1, AgZ2, AgZ3 and AgZ4 respectively) and mixed at 150 rpm for 3 days. The obtained zeolite A were then vacuum filtered, washed with deionized water and dried at 80°C overnight.

In order for confirm the capacity of ion exchange in the AgZ, ICP-OES analysis was carried out. Briefly, 200 mg of AgZ1, AgZ2, AgZ3 and AgZ4 was added into separate tubes containing 14 ml of acid mixture, aqua regia (40% HNO_3 + 60% HCl) and left overnight at room temperature. 4 ml of aqua regia solution was then added into the tubes and mixed for 30 minutes at 80°C before being filtered and diluted prior the ICP-OES analysis**.**

Minimum Inhibition Concentration (MIC) Assay of Ag-exchanged zeolite A

The Minimal Inhibitory Concentration (MIC) assay of Ag-exchanged zeolite A against *V. parahaemolyticus* and *V. campbellii* was carried out as described (18). Briefly, frozen stocks of *V. campbellii* and *V. parahaemolyticus* were grown on Nutrient Agar (NA) supplemented with 2% of NaCl and incubated overnight at 37°C. Overnight cultures were inoculated into Tryptone Soy Broth (TSB) and absorbances were adjusted accordingly to an initial starting OD_{600} of 0.05. As seen in Table 1, samples AgZ1, AgZ2, AgZ3 and AgZ4 of respective AgZ concentrations were then added.

Table 1 Concentrations of Silver Ion Zeolites used for the Minimum Inhibitory Concentration Assay.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 April 2021 doi:10.20944/preprints202104.0144.v1

Metal-Ion Zeolite/Concentration(M)	0.25 M	0.50 M	1.00 M	1.50 M
Silver (AgZ)	AgZ1	AgZ2	AgZ3	AgZ4

For each bacterial species, doubling dilutions up to 1/512 of AgZ1, AgZ2, AgZ3 and AgZ4 were firstly carried out. 500 µl of liquid inoculum (OD_{600} adjusted to 0.05) was then added and incubated in a shaking 37°C incubator for 16 hours. 100 µl of the culture was then plated and spread on to Nutrient Agar (NA) plates supplemented with 2% NaCl and further incubated overnight at 37°C with appropriate controls. The minimum inhibitory concentration (MIC) of AgZ1, AgZ2, AgZ3 and AgZ4 was determined by the lowest concentration of samples that did not exhibit bacterial growth.

Quantitation of Bacterial Biofilm Density by Crystal Violet Assay

The biofilm density of AgZ treated biofilm were quantitated by the crystal violet assay using the methods described by O'Toole (19) with modifications. Briefly, cultures of *V. campbellii* and *V. parahaemolyticus* were incubated overnight at 37°C in Tryptone Soy Broth (TSB) supplemented with 2% NaCl.

For the biofilm assay, the sublethal concentration obtained from the MIC assay from was selected as the starting assay concentration. Briefly, doubling dilutions of the sample were performed in a 96 well microtiter plate (Corning) containing Tryptone Soy Broth supplemented with 2% NaCl. 50 µl of the overnight cultures (with OD_{600} adjusted to 0.05) were then inoculated into each well and was then incubated at 37°C for 72 hours. Following incubation, wells were washed three times with distilled H_2O, desiccated at 50°C , stained with 1% crystal violet for 30 min and added with 95% ethanol. The absorbance of solubilized dye was then determined at 570nm (Shimadzu Spectramax).

Scanning Electron Microscopy (SEM) of *V. campbellii* and *V. parahaemolyticus* biofilms

The cell morphologies of *V. campbellii* and *V. parahaemolyticus* biofilms grown in TSB media and in TSB with AgZ4 was analyzed by SEM. Briefly, 5 ml overnight liquid cultures of *V. campbellii* and *V. parahaemolyticus* were cell fixated according to protocol by Gomes and Mergulhão (20) with modifications. The biofilm samples were then fixed in 5% glutaraldehyde prepared in 0.1 M PBS pH 7.2 at 4°C for 12 hours. Following the fixation process, the samples were dehydrated by introduction into a series of ethanol solution of varying concentration gradients (35%, 50%, 75%, 95%, and 2 x 100%). The dehydrated samples were then immersed in HMDS for 10 minutes. Upon dehydration the samples were dried overnight and then sputter–coated with platinum before being analyzed by the SEM (Hitachi SEM, S 3400N).

3. Results

The incorporation of silver ion (Ag) into the zeolite A framework (AgZ) was determined by introducing the zeolite sample to four different concentrations of Ag solution AgZ1, AgZ2, AgZ3 and AgZ4. During this process, the potassium and sodium ions that exist in the zeolite framework was exchanged by metal ions. ICP-OES analysis of AgZ1, AgZ2, AgZ3 and AgZ4 are as shown in Table 2.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 April 2021 doi:10.20944/preprints202104.0144.v1

Table 2 Silver ion content (mg/g) of zeolite of samples

Metal Ion/Concentration	0.25 M (1)	0.50 M (2)	1.00 M (3)	1.50 M (4)
Silver (AgZ)	80.949 ± 0.389	160.041 ± 1.328	177.481 ± 1.483	190.511 ± 1.989

As seen in Table 2, The highest silver ion content was observed for AgZ4 at 190.511 ± 1.989 mg/g, followed by the lowest concentration for AgZ1 at 80.949 ± 0.389 mg/g.

Minimum Inhibitory Concentration (MIC) of *V. campbellii* and *V. parahaemolyticus* in AgZ

The MIC values for each zeolite sample against *V. campbellii* and *V. parahaemolyticus* are as summarized in Table 3 below.

Table 3 Minimum Inhibitory Concentration (MIC) of silver ion loaded zeolite A against *V. campbellii* and *V. parahaemolyticus*

Bacterial Pathogens	MICs value of four different Silver Ion Loaded Zeolite A (mg/ml)			
	AgZ1	**AgZ2**	**AgZ3**	**AgZ4**
V. campbellii	2.0000	0.5000	0.2500	0.1250
V.parahaemolyticus	0.5000	0.1250	0.0625	0.0625

As seen in Table 3, both *V. campbellii* and *V. parahaemolyticus* showed susceptibility to all four AgZ1-AgZ4 concentrations. For *V. campbellii*, the highest MIC was observed in AgZ4 at 0.1250 mg/ml. For *V. parahaemolyticus*, the highest MIC was observed in AgZ3 at 0.0625 mg/ml. The lowest MIC for both *V. campbellii* and *V. parahaemolyticus* was observed in AgZ1 at 2.0 mg/ml and 0.5 mg/ml respectively.

Quantitation of Bacterial Biofilm Density by Crystal Violet Assay

The AgZ4 MIC concentration for both pathogens were further selected for biofilm inhibition crystal violet assay. Figure 1 demonstrated biofilm inhibition percentages of ion-exchanged zeolite in comparison with the untreated control. As shown in Figure 1, at 570nm absorbance, biofilm formation of AgZ4 against *V. campbellii* and *V. parahaemolyticus* exhibited up to 60.2% and 77.3% inhibition respectively.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 April 2021 doi:10.20944/preprints202104.0144.v1

Figure 1. Biofilm Inhibition of *V. campbellii* and *V. parahaemolyticus* by 1.50M AgZ4 assessed by CV staining.

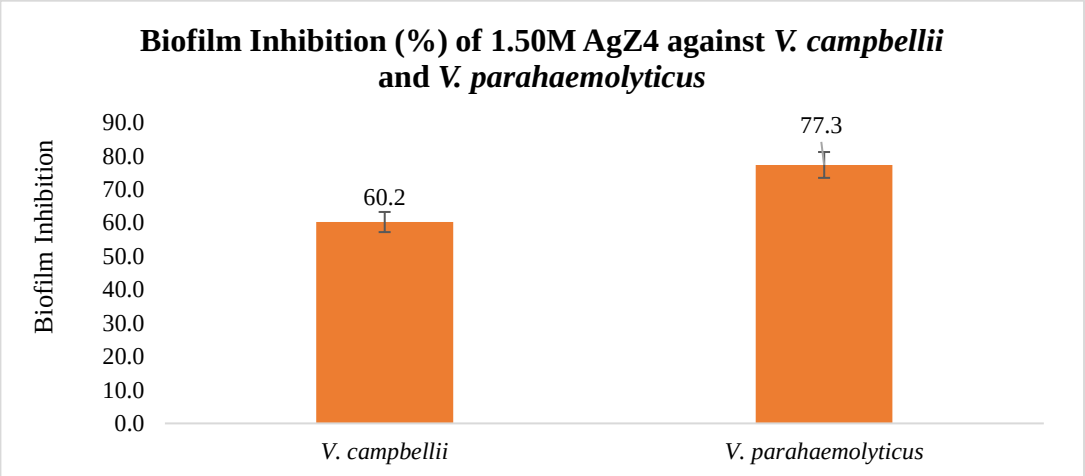

Figure 1. Biofilm Inhibition of *V. campbellii* and *V. parahaemolyticus* by 1.50M AgZ4 assessed by CV staining. Biofilm inhibition of *V. campbellii* and *V. parahaemolyticus* isolates after 24 h growth with 1.50M AgZ4, was assayed by CV staining$_{A570}$.

Scanning Electron Microscopy of *V. campbellii* and *V. parahaemolyticus* in AgZ4

Figure 2(a) and 3(a) represent the SEM images of untreated samples of *V. campbellii* and *V. parahaemolyticus* respectively , while Figure 2(b) and 3(b) represent the SEM images of *V. campbellii* and *V. parahaemolyticus* after growth in media culture treated with 1.50M AgZ4.

Figure 2 (a) shows clustering and aggregation of *V. campbellii* covered with a network of extracellular matrix (ECM) as a typical representation of intact bioflims. In contrast, Figure 2 (b) showed markedly reduced ECM and aggregation of bacteria.

Figure 2. SEM Analysis of untreated *V. campbellii* biofilm formation (a), and treated with AgZ4 (b).

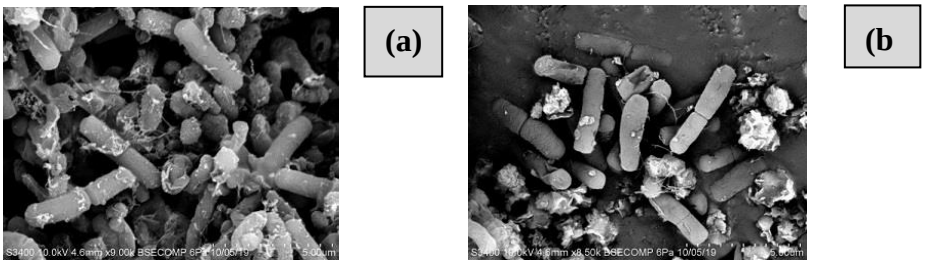

Figure 2. SEM Analysis showing (a) untreated *V. campbellii* biofilm bacteria embedded in extracellular matrix biofilm and **(b)** *V. campbellii* treated with AgZ4 where profound loss of biofilm matrix is seen.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 April 2021 doi:10.20944/preprints202104.0144.v1

Similarly, Figure 3 (a) below showed clustering and aggregation of untreated *V. parahaemolyticus* covered with a fine network of extracellullar matrix (ECM) In contrast, as seen in Figure 3 (b) *V. parahaemolyticus* exposure to AgZ4 showed a markedly reduced ECM and cell clustering.

Figure 3. SEM Analysis of untreated *V. parahaemolyticus* biofilm formation (a), and treated with AgZ (b).

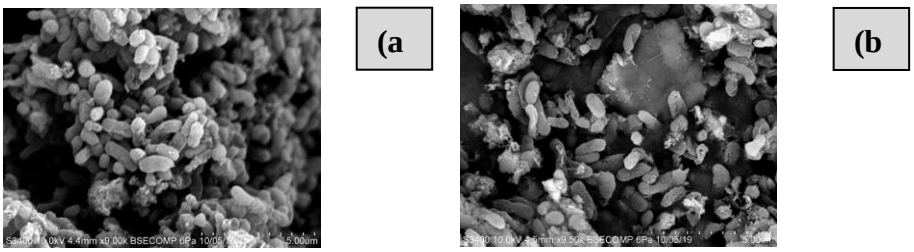

Figure 3: SEM Analysis showing (a) untreated *V. parahaemolyticus* biofilm bacteria embedded in extracellullar matrix biofilm and **(b)** *V. parahaemolyticus* treated with AgZ4 where profound loss of biofilm matrix is seen.

4. Discussion

V. parahemolyticus is a prevalent food-poisoning bacterium associated with seafood consumption, typically causing self-limiting gastroenteritis and commonly found in temperate and tropical marine and coastal waters globally (21). Vibriosis is a systemic bacterial infection in farmed and wild marine fishes, which is considered to be a profoundly significant problem due to intensive economic losses in aquaculture industry worldwide (22). *V. campbelli* is as an emerging marine pathogen recently associated with diseased farm shrimps (23). The development of biofilms in marine bacteria further complicate bacterial disease management in aquaculture as the extracellular matrix in biofilms confer protection against antimicrobial substances (24). Zeolites have been found to be useful in various applications in aquaculture especially in the purification of hatchery tanks (25). This study aimed to investigate antimicrobial and antibiofilm applications of silver ion-exchanged zeolite A against marine bacterial pathogens *V. parahaemolyticus* and *V. campbelli*. The applicability of the bentonite-based silver ion loaded zeolite A for samples AgZ1, AgZ2, AgZ3 and AgZ4 as antibacterial agents was evaluated against *V. campbellii* and *V. parahaemolyticus*.

The initial ICP-OES analysis of AgZ1, AgZ2, AgZ3 and AgZ4 revealed the successful incorporation of Ag ion into the zeolite samples to be typically in direct correlation with the increase in Ag ion concentration introduced. The highest Ag ion concentration was observed in sample AgZ4 (1.50M) at 190.511 $\pm$ 1.989 mg/g. At 0.50M, the Ag ion concentration of AgZ2 was double that of AgZ1 at 160.041 $\pm$ 1.328 mg/g compared to 80.949 $\pm$ 0.389 mg/g. Nonetheless doubling concentration increases observed for AgZ2 was not indicated in AgZ3 and AgZ4. A possible explanation for this is saturation of available sites in AgZ3 and AgZ4 for the ion exchange to occur.

In general, Minimum Inhibitory Concentration (MIC) evaluation against *V. campbellii* and *V. parahaemolyticus* firstly revealed that the incorporation of Ag ions into the zeolites

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 5 April 2021 doi:10.20944/preprints202104.0144.v1

samples AgZ1, AgZ2, AgZ3 and AgZ4 was successful in inhibiting bacterial growth. Silver (Ag) has long been established in various studies as an effective antibacterial agent. Several studies have also demonstrated the efficiency of Ag exchanged zeolite against may microbial pathogens (26-27) and concur with the findings of this study. Additionally, a typically inverse relationship between sample concentration and MIC for AgZ1, AgZ2, AgZ3 and AgZ4 was also observed. As seen in Table 3, a lower MIC which signifies stronger antibacterial activity, was indicated for each bacterial type as the concentration of metal ion in the zeolite increased. Therefore, the higher the Ag ion loading in the zeolite, the lower the concentration of ion-loaded zeolite A was needed to inhibit the growth of the bacteria. There are many studies into the mechanism of microbial killing by metal ion. A possible mechanism involves the ability of the metal ion to attach to the bacteria membrane through electrostatic interaction and drastically alter the integrity of the bacterial membrane. Consequently, it promotes the formation of reactive oxygen species, (ROS) which will induce the oxidative stress to the bacteria cell resulting the oxidation of cellular component, DNA damage, mitochondria damage and disruption of the cell membrane which lead to the death of the bacteria (28).

MIC studies also revealed that between the two bacterial species, *V. parahaemolyticus* indicated higher susceptibility against Ag ion compared to *V. campbellii* as lower MICs were observed across AgZ1-AgZ4. Under antibiotic pressure, bacterial phenotypes such as susceptibility, resistance, tolerance and persistence differ from one bacteria to the other. In a review by Li *et al* (29), the efficacy of antimicrobials are influenced by many factors including bacterial status, host factors and antimicrobial concentrations.

A typical feature of bacterial biofilms is the extracellular matrix which provides protection and structure to the cell population within it (30). The CV assay is a useful tool for rapid and simple assessment of biofilm formation differences between bacteria, as it stains the extracellular matrix as well as the aggregated bacterial cells (O' Toole, 2011). The CV biofilm assays of *V. campbelli* and *V. parahaemolyticus* isolates grown in AgZ4 overnight showed biofilm growth were effectively inhibited in the presence of AgZ4. However, *V. parahaemolyticus* biofilms were indicated to be more susceptible against AgZ4 with a percentage inhibition of 77.3% compared to *V. campbelli* at 60.2%. This concurred with the MIC assays which also showed a higher susceptibility of *V. parahaemolyticus* when compared against *V. campbelli* and demonstrated significant attenuation of biofilm formation against *V. campbellii* and *V. parahaemolyticus*.

The ability of Ag ion-exchanged zeolite AgZ4 to disrupt biofilm development for *V. campbellii* and *V. parahaemolyticus* was further supported by SEM analysis. As seen in Figures 2 and 3, the exposure of AgZ4 to both *V. campbellii* and *V. parahaemolyticus* displayed significant structural alteration of biofilm phenotypes when compared to bacterial isolates grown in the absence of AgZ4 including profound loss of the biofilm extracellular matrix (ECM) as well as markedly reduced cell aggregation. While SEM analysis of untreated isolates showed tight aggregation of cells held together by ECM, isolates exposed to AgZ4 showed higher numbers of singular isolates with lessened clustering. Breakages on the extracellular matrices of biofilms will result in increased susceptibility of bacteria against antibacterial agents and chemicals (31). Several studies have demonstrated biofilm inhibition by various antibiofilm agents including plant species (31-32). Despite much literature on antibiofilm activities of bacteria by zeolite, the mechanism of toxicity of AgZ against biofilms of *V. campbellii* and *V. parahaemolyticus* are still poorly understood.

 doi:10.20944/preprints202104.0144.v1

Therefore future studies on the probability of modification of gene expression in the *Vibrio* polysaccharide (VPS) and matrix protein biosynthesis are recommended to further inform on genes or protein that are significantly affected by metal loaded zeolites.

5. Conclusions

Taken together, the results of this study strongly indicate the strong antibacterial and antibiofilm potentials of Ag-ion exchanged zeolite A which can be applied in the aquaculture industry to combat against infectious pathogens in particular *V. campbellii* and *V. parahaemolyticus*.

Author Contributions: All authors contributed equally to the research work and preparation of this manuscript.. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Universiti Malaysia Sabah, grant number GUG0111-1/2017 . The APC was funded by Universiti Malaysia Sabah.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Ortega, C., Mùzquiz, J., Docando, J., Planas, E. and Alonso, J. (1995). Ecopathology in aquaculture: risk factors in infections disease outbreak. *Vet. Res.*, 26(1), 57-62.

2. Pridgeon, J. W., and Klesius, P. H. (2012). Major bacterial diseases in aquaculture and their vaccine development. *Anim. Sci. Rev*, *7*, 1-16.

3. Miranda, C.D., Godoy, F.A. and Lee, M.R. (*2018*) Current Status of the Use of Antibiotics and the Antimicrobial Resistance in the Chilean Salmon Farms. *Front. Microbiol,* 9 (1284), 1-14.

4. Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environ. Microbial.*, *8*(7), 1137-1144.

5. Cabello, F. C., Godfrey, H. P., Tomova, A., Ivanova, L., Dölz, H. et al. (2013). Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ. Microbial.*, *15*(7), 1917-1942.

6.Zorriehzahra, M.J. and Banaederakshan, R. (2015) Early Mortality Syndrome (EMS) as new Emerging Threat in Shrimp Industry. *Adv Anim Vet Sci*, 3 (2), 64-72.

7. Letchumanan, V., Yin, W.F., Lee, L.H and Chan, K.G (2015). *V. parahaemolyticus* : A review on the pathogenesis, prevalence and advance molecular identification techniques. *Front. Microbiol* 9:2513.

8. Donlan, R.M. (2002). Biofilms : Microbial Life on Surfaces. *Emerg Infect Dis, 8(9),* 881-890

9. Costerton, J. W., Stewart, P. S., and Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science*, *284*(5418), 1318-1322.

10. Costa, E. M., Silva, S., Tavaria, F. K., and Pintado, M. M. (2013). Study of the effects of chitosan upon *Streptococcus* mutans adherence and biofilm formation. *Anaerobe*, *20*, 27-31.

11. Chakrabarti, R. and Vasudeva, R. (2006). *Achyrantes aspera* stimulates the immunity and enhances the antigen clearance in *Catlacatla*. *Int. J. Immunopharmaco.*, 6, 782-790.

12. Albert, V., and Ransangan, J. (2013). Antibacterial potential of plant crude extracts against Gram negative fish bacterial pathogens. *Int. J. Res. Pharm. Biosci*, *3*(2), 21-27.

13. Mu, H., Tang, J., Liu, Q., Sun, C., Wang, T. et al. (2016). Potent antibacterial nanoparticles against biofilm and intracellular bacteria. *Sci. Rep.*, *6*(1): 1-9.

14. Sakai, M. (1999). Current research status of fish immunostimulants. *Aquac.*, *172*(1-2), 63-92. Thill, A. Zeynos, O., Spalla, O., Chauvat, F., Rose, J. et al. (2006). Cytotoxicity of CeO_2 nanoparticles for *Escherichia coli.* Physico-chemical insight of the cytotoxicity mechanism. *Environ. Sci. Technol.*, 40, 6151–6156.

15. Krishnani, K. K., Zhang, Y., Xiong, L., Yan, Y., Boopathy, R. et al. (2012). Bactericidal and ammonia removal activity of silver ion exchanged zeolite. *Bioresour. Technol*, 117, 86–91.

16. Belaabed, R., Elabed, S., Addaou, A., Laajab, A., Rodriguez, M. A. et al. (2016). Synthesis of LTA zeolite for bacterial adhesion. *Ceramica y Vidrio,* 55, 152-158.

17. Demirci, S., Ustaoğlu, Z., Yılmazer, G. A., Sahin, F., and Baç, N. (2014). Antimicrobial properties of zeolite-X and zeolite-A ion exchanged with silver, copper, and zinc against a broad range of microorganisms. *Appl. Biochem. Biotech.*, *172*(3), 1652-1662.

18. Zhang, Y., Zhong, S., Zhang, M. and Lin, Y. (2009). Antibacterial activity of silver-loaded zeolite A prepared by a fastmicrowave loading method. *J. Mater. Sci.*, 44(2), 457-462.

19. O'Toole, G.A. (2011). Microtiter Dish Biofilm Formation Assay, *J. Vis Exp*., 47: 2437.

20. Gomes, L. C., and Mergulhão, F. J. (2017). SEM analysis of surface impact on biofilm antibiotic treatment. *Scanning*, *2017*.

21. Nair, G.B., Ramamurthy, T., Bhattachrya, S.K., Dutta, B., Takeda, Y. and Sack, D.A. (2007). Global dissemination of *V. parahaemolyticus serotype* O3:K6 and its serovariants. *Clin. Microbiol. Rev.* 20, 39-48.

22. Mohamad, N., Amal, M.N.A., Md Yasin, I.S., Saad, M.Z., Nasruddin, N.S., Al-saari, N., Mino, S. and Sawabe, T. (2019). Vibriosis in cultured marine fishes: A review. *Aquaculture.* 512 (734289) 1-17.

23. Haldar, S., Chatterjee, S., Sugimoto, N., Das, S., Chowdury, N., Hinenoya, A., Asakura, M. and Yamasaki, S. (2011). Identification of *V. campbelli* isolated from diseased farm shrimps from South India and establishment of its pathogenic potential in an *Artemia* model. *Microbiology.* 157, 179-188.

24. Mizan, M. F. R., Jahid, I. K., Kim, M., Lee, K. H., Kim, T. J. et al. (2016). Variability in biofilm formation correlates with

 doi:10.20944/preprints202104.0144.v1

hydrophobicity and quorum sensing among *Vibrio parahaemolyticus* isolates from food contact surfaces and the distribution of the genes involved in biofilm formation. *Biofouling*, *32*(4), 497-509.

25. Breck, D. W. (1974). Structure, chemistry and use. *Zeolite Molecular Sieves. Wiley, New York*.

26. Leung, Y. H., Xu, X., Ma, A. P., Liu, F., Ng, A. M. et al. (2016). Toxicity of ZnO and TiO_2 to Escherichia coli cells. *Sci. Rep.*, *6*, 35243.

27. Magana, S. M., Quintana, P., Aguilar, D. H., Toledo, J. A., Angeles-Chavez, C. et al. (2008). Antibacterial activity of montmorillonites modified with silver. *J. Mol. Catal. A Chem.*, *281*(12), 192-199.

28. Nel, A. E., Mȁdler, L., Velegol, D., Xia, T., Hoek, E. M. V. et al. (2009). Understanding biophysicochemical interactions at the nano-bio interface. *Nat. Mater.*, 8, 543-557.

29. Li, J., Xie, S., Ahmed, S., Wang, F., Gu, Y., Zhang, C., Chai, X., Wu, Y., Cai, J. and Cheng, G. (2017). Antimicrobial Activity and Resistance : Influencing Factors. *Front. Pharmacol,* 8, 1-13.

30. Maric, S. and Vranes, J. (2007). Characteristics and significance of microbial biofilm formation. *Period Bilogor,* 109, 115-21.

31. Das, A., Das, M. C., Sandhu, P., Das, N., Tribedi, P. et al. (2017). Antibiofilm activity of *Parkia javanica* against *Pseudomonas aeruginosa:* a study with fruit extract. *RSC advances*, *7*(9), 5497- 5513.

32. Chakrabarti, R. and Vasudeva, R. (2006). *Achyrantes aspera* stimulates the immunity and enhances the antigen clearance in *Catlacatla*. *Int. J. Immunopharmaco.,* 6, 782-790.

33. Kumaran, N. S., Bragadeeswaran, S., and Meenakshi, V. K. (2011). Evaluation of antibacterial activity of crude extracts of ascidian Didemnum psammathodes Sluiter, 1895 against isolated human and fish pathogens. *Asian Pac. J. Trop. Biomed.*, *1*(1), S90 S99.