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# THE EFFECT OF *Bacillus* sp. ADDITION ON THE VIRULENCE OF *Vibrio parahemolyticus* TOWARD AXENIC CULTURE OF *Artemia franciscana*

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**ABSTRACT**

Research on the benefits of *Bacillus* sp. in aquaculture has been done a lot. Currently, the aquaculture industry, especially shrimp farming, is facing AHPND disease, which is known to be caused by *Vibrio parahaemolyticus*. This study aims to see the effect of *Bacillus* sp. against virulence of *V. parahaemolyticus* in vitro using caseinase production as an indicator and survival of *Artemia franciscana* cultured axenically when challenged with *V. parahaemolyticus*. In in vitro tests, cultures of *V. parahaemolyticus* were grown with and without the addition of *Bacillus* sp. on Luria Bertani (LB) liquid media, and then the caseinase test was carried out using skim milk (SM) agar as a growth medium. In the challenge test, 10 axenic cultured *Artemia* sp. were distributed in falcon tubes containing 10 ml of sterile seawater. Challenge test treatments consisted of treatment A as control of *Artemia* culture without the addition of bacteria, treatment B *Artemia* culture adding by $10^6$ CFU/ml *V. parahaemolyticus*, treatment C *Artemia* culture with the addition of 106 CFU/ml Bacillus sp., and treatment D Artemia culture with the addition of $10^6$ CFU/ml *V. parahaemolyticus* and $10^6$ CFU/ml *Bacillus* sp. All treatments were done in triplicates. The results showed that the addition of *Bacillus* sp. was able to reduce the caseinase production of *V. parahemolyticus* up to 29% indicated by the decrease of the clearing zone diameter formed on SM agar. Furthermore, the addition of *Bacillus* sp. in treatment D was able to significantly increasing ($P<0.05$) of *Artemia*'s survival when challenged with *V. parahamolyticus*. This indicates that *Bacillus* sp. has a potential as a probiotic candidate to prevent disease caused by *V. parahaemolyticus.*

## INTRODUCTION

Virulence is the degree of ability of a pathogen to cause disease. The virulence potential of bacteria is influenced by several factors, namely toxin production, enzymes, host resistance and speed of reproduction (Pelcxar et al., 1986), examples of bacteria that have virulence potential are bacteria of the Vibrionaceae group. V. parahaemolyticus belongs to the Vibrionaceae group which is one of the main pathogens at the shrimp hatchery level (Chatterjee and Haldar, 2012) with a high virulence level.

Common symptoms that appear in vibriosis in shrimp, such as reddish body color, anorexia, weakness in movement, swimming to the side. The condition of the hepatopancreas that has experienced shrinkage and destruction cannot function normally. This causes the shrimp to become weak and eventually die (Rozik, 2014). Virulence of pathogenic Vibrio is not only due to its ability to synthesize exotoxins but is also influenced by the conditions of the host factors which include the type of species, age and condition of the species (Kurniawan et al., 2014).

Efforts to suppress vibriosis in shrimp are generally often carried out with the application of disinfectants and antibiotics. However, continuous administration of antibiotics can cause bacterial resistance and cause residues that are harmful to living things and the environment (Labreuche, 2012). In addition to antibiotics, there is another solution, namely the use of probiotics that can improve the immune system (Watson et al., 2008). Probiotics are additional microbes that can increase the nutritional value of feed and can improve host response to disease and improve environmental quality (Verschuere et al., 2000). Microbes or microorganisms that have the potential as probiotic candidates generally come from the gram-positive group of bacteria such as Lactobacillus, Bifidobacterium, and Bacillus sp., (Flores 2011).

This study aims to test the effectiveness of Bacillus sp. against V. parahaemolyticus virulence in Artemia axenic culture. So far, research on probiotic protection testing on Vibrio sp. has been published by Wijayanti et al., 2018 about the analysis of the vannamei shrimp challenge test given the probiotic Bacillus and Diem et al., 2021 about the reduction of AHPND disease by Bacillus subtilis.

## METHODOLOGY

This research was conducted on a laboratory scale at the Fisheries Science Laboratory, Faculty of Marine Affairs and Fisheries, Udayana University. This research was carried out from September 2021 to December 2021. This study was carried out in vitro by conducting a caseinase test and in vivo by conducting a challenge test on artemia cultured axenically.
The tools that will be used in this research are petri dishes, loop needle, bunsen, laminar, glass, micropipette, spectrophotometer, falcon tube, rotor, aerator, lamp, incubator, microscope, counter and for the material to be used in this research is LB media. agar/broth, skim milk agar, sterile seawater media, 30% NaCL, 50% NaOCL, 200 mg artemia cyst, pathogen (Vibrio parahaemolyticus) and Bacillus sp. (both are isolates from the FKP Unud fisheries lab collection)

In in-vitro tests, cultures of V. parahaemolyticus were grown without and with the addition of Bacillus sp. on Luria Bertani (LB) liquid media, and then the caseinase test was carried out using skim milk (SM) agar as a growing medium. In the challenge test, 10 Artemia sp. axenically cultured were distributed in falcone tubes containing 10 ml of sterile seawater. Challenge test treatments consisted of treatment A as control of Artemia culture without the addition of bacteria, treatment B with Artemia culture adding 106 CFU/ml V. parahaemolyticus, treatment C Artemia culture with addition of 106 CFU/ml Bacillus sp. and treatment D culture of Artemia with the addition of 106 CFU/ml V. parahaemolyticus and 106

CFU/ml Bacillus sp. All treatments were carried out with 3 replications so that the resulting data were statistically valid. The data obtained were processed by ANOVA test using the SPSS (Statistical Product and Services Solution) version 23 application.

## RESULT

The results of the caseinase test using SMA (Skim Milk Agar) media showed the proteolytic activity of the bacterial isolate Vibrio parahaemolyticus with an indication of the formation of a clear zone around the bacterial colony (Shuva et al., 2015). The results of the caseinase test can be seen in Figure 1.

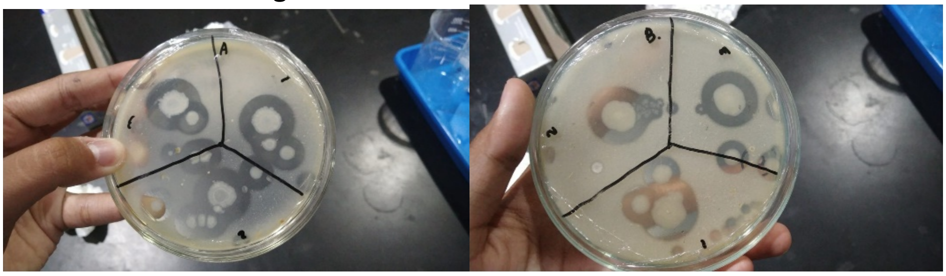


Figure 1. Diameter of the clear zone of caseinase test results on Vibrio Parahaemolyticus by treatment (a) without the addition of Bacillus sp, and (b) with the addition of Bacillus sp.

Figure 1 shows the clear zone formed in the culture of V. parahaemolyticus without the addition of Bacillus sp. (Treatment A) had a larger diameter when compared to the culture of V. parahaemolyticus added with Bacillus sp. (Treatment B).

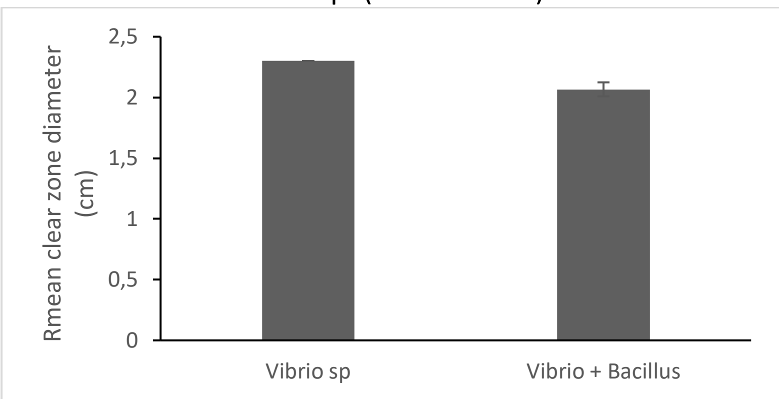


Figure 2. Average caseinase production of V. parahaemolyticus without and with the addition of Bacillus sp.

The results of the caseinase test in treatment A and treatment B showed that the addition of Bacillus sp. in treatment B affected the production of caseinase enzymes in V. parahaemolyticus. The results of the ANOVA test also showed the addition of Bacillus sp. significant effect ($P<0.05$) on the diameter of the clear zone which is an indication of the production of caseinase enzymes in V. parahaemolyticus.

Furthermore, the in-vivo test results on Artemia franciscana grown using the axenic method showed that the addition of Bacillus sp. also had a significant impact ($P<0.05$) in increasing the survival of Artemia which was challenged with the pathogenic bacterium V. parahaemolyticus (Figure 3).

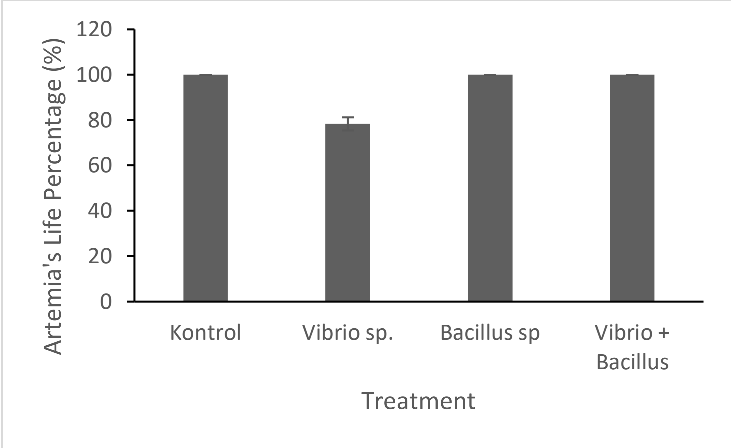


Figure 3. Percentage of survival of Artemia franciscana cultured axenically when tested against V. parahamolyticus without and with the addition of Bacillus sp.

Figure 3. shows that treatment A (control) resulted in an average survival rate of A. franciscana of 100 ± 0%. In treatment B where Artemia was added with the pathogenic bacterium V. parahaemolyticus 106 CFU/ml, the SR value was 78.3%. Furthermore, in treatment C where Artemia was added with 106 CFU/ml Bacillus sp. resulting in a 100% survival value. and in treatment D where Artemia was added 106 CFU/ml V. parahamolyticus. and 106 CFU/ml Bacillus sp., resulting in a 100% survival rate.

The results of the above challenge of A. franciscana with V. parahaemolyticus showed that Bacillus sp. affect the survival of Artemia challenged with V. parahaemolyticus. The results of the ANOVA test showed that the addition of Bacillus sp. significant effect ($P<0.05$) on Artemia's survival.

## DISCUSSION

The results of this study indicate that the bacteria Bacillus sp. was able to reduce caseinase production in V. parahaemolitycus up to 0.3 cm from the clear zone formed by the caseinase enzyme produced by V. parahaemolyticus. This is thought to be caused by the ability of Bacillus which is able to produce 1,3-glucanase, cyanide, chitinase, antibiotics and can dissolve phosphate which is known to suppress the growth of harmful microbes accordingly (Husen, 2003; Frederiksen et al., 2013). This statement is also in line with the research conducted by Sugita et al., 1998, by testing the effectiveness of antimicrobial enzymes from Bacillus subtillis against the growth of Vibrio fulnificus bacteria.

In the Artemia axenic challenge, the addition of Bacillus sp. significant effect ($P<0.05$) on the survival rate of Artemia when challenged with V. parahaemolyticus. In treatment A, Artemia's survival reached 100%. This could be presumably due to the absence of other microorganisms that would interfere with the growth of Artemia. Wedemeyer (1996) stated that pathogen-free culture conditions can reduce the risk of death of cultured organisms including crustaceans. In treatment C, the survival rate of Artemia also reached 100%, this is presumably due to the addition of Bacillus sp. which is a gram-positive bacterium that does not have pathogenic properties and has the potential as a probiotic agent in aquaculture, so that it can increase immunity from Artemia (Lazado and Caipang (2014)). .

In treatment B, the survival rate of artemia was the lowest because it was suspected that infection with V. parahaemolyticus was suspected. According to Chatterjee and Haldar, 2012, V. parahaemolyticus which belongs to the Vibrionaceae group of bacteria is one of the main pathogens in shrimp culture. In treatment D, the addition of Bacillus sp. affect the survival rate up to 100% presumably because Bacillus sp. able to inhibit the virulence factors produced by V. parahemolyticus, one of which is the production of the caseinase enzyme which has also been tested in this study. Nguyen et al., (2014) and Sahoo et al., (2014) describe several antibacterial compounds from the gram-positive group of waters including Bacillus sp. has broad-spectrum inhibitory activity and can be used to treat pathogens in aquaculture. Based on the results of this study, Bacillus sp. has the potential as one of the probiotic candidates that is needed to reduce the use of antibiotics in the cultivation area.

## CONCLUSION

The conclusion of this research is Bacillus sp. was able to reduce the production of caseinase enzymes from V. parahaemolyticus to 0.3 cm by measuring the formation of the clear zone as an indicator, Bacillus sp. also able to increase the survival of Artemia up to 100% when challenged with V. parahaemolyticus. These results indicate that the isolate Bacillus sp. used in this study has the potential as a probiotic candidate to realize an environmentally friendly and sustainable cultivation system.

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## REFERENCES

Chatterjeem.S., S., & Haldar. (2012). Vibrio related diseases in aquaculture and development of rapid and accurate identification methods. *J. Marine Sci Res Dev*, 1–7.

Flores, G. (2011). Microbial biogeography of public restroom surfacesNo Title. *Plos One*, *6*(11).

Husen, E. (2003). No TitleScreening of soil bacteria for plant growth promotion activities in vitro. *Indonesian Journal of Agricultural Science*.

Kurniawan., K., A., T., & .Kadriah, I. A. K. (2014). Uji Patogenitas dan Gambaran Histologi Hepatopankreas Infeksi Bakteri Vibrio Patogen Secara Penyuntikan. *Prosiding Seminar Nasional Tahunan XI Hasil Perikanan Dan Kelautan*.

Lazado, C.C., C. (2014). *NoAtlantic Cod in The Dynamic Probiotics Research in Aquaculture Title*.

Nguyen, P. (2014). *No TitleEvaluation of the antiprion activity of 6-aminophenanthridines and related heterocycles*.

Rozik, M. (2014). Pengaruh Imunostimulan OMP terhadap Histopatologi Hepatopankreas Udang Windu (Peneus monodon fabricus) pasca Uji Tantang dengan Vibrio harveyiNo Title. *Journal of Tropical Fisheries*, *10*(1).

Shuva. B, S., B., I. M. M. A. M., Md., H. &, & Hossain., A. (2015). No TitleProtease Producing Bacteria and Activity in Gut of Tiger Shrimp (Penaeus monodon). *Journal of Fisheries and Aquatic Science*.

Sugita, H. Y. Hirose, N. Matsuo, and Y. D. (1998). No TitleProduction of the Antibacterial Substance by Bacillus sp strain NM 12, an Intenstinal bacterium of Japanese Coastal Fish. *J. Aquaculture*.

Verschuere, L., Rombaut, G., Sorgeloos, P., & Verstraete, W. (2000). No TitleProbiotic Bacteria as Biological Control Agents in Aquaculture. *Microbiology and Molecular Biology Reviews*, *64*(4), 655–671.

Wedemeyer. (1996). *No TitleGrowth and Ecology of Fish Populations*.

Wijayanti. Arlin, Nandya, D., Uun, F., Esti, H., Wardiyanto., & Wardiyanto. (2018). No Title Analisis Uji Tantang Udang Vaname (Litopenaeus vannamei) Yang Diberbakteri Probiotik Bacillus Sp. D2.2 Dan Ekstrak Ubi Jalar Sebagai Sinbiotik. *Biospecies*, *11*(2), 63–71.