Alginate oligosaccharide/polysaccharide and lactic acid bacteria (Lactobacillus bulgaricus FNCC–0041 & Streptococcus thermophilus FNCC–0040) as immunostimulants against pathogenic Vibrio spp. using

by Yudiati Et Al.

Submission date: 16-May-2022 10:34AM (UTC+0700)
Submission ID: 1837162927
File name: Lamp._V_C-30.pdf (1.45M)
Word count: 4058
Character count: 21543

Alginate oligosaccharide/polysaccharide and lactic acid bacteria (*Lactobacillus bulgaricus* FNCC–0041 & *Streptococcus thermophilus* FNCC–0040*)* as immunostimulants against pathogenic *Vibrio* spp. using Artemia bio model

To cite this article: Ervia Yudiati *et al* 2021 *IOP Conf. Ser.: Earth Environ. Sci.* **919** 012060

Alginate oligosaccharide/polysaccharide and lactic acid bacteria (*Lactobacillus bulgaricus* FNCC–0041 & *Streptococcus thermophilus* FNCC–0040) as immunostimulants against pathogenic *Vibrio* spp. using Artemia bio model

Ervia Yudiati[1,2], D P Wijayanti[1], N Azhar[1], A I Chairunnisa[1], S Sedjati[1] and Z Arifin[2]

[1]Department of Marine Science, Faculty of Fisheries and Marine Science, Diponegoro University, Semarang, Central Java, 50275, Indonesia
[2]Main of Brackishwater Aquaculture Development Centre, Jepara, Central Java, 59418, Indonesia

*Corresponding author e-mail: eyudiati@gmail.com; erviayudiati@lecturer.undip.ac.id

Abstract. Alginate polysaccharide/oligosaccharide (APS/AOS) has been proven as a good immunostimulant. FNCC–0041 & FNCC–0040 are lactic acid bacteria (LAB) producing exopolysaccharides. Vibrio bacteria are pathogenic for aquatic cultivans. This study determines the performance of APS/AOS in combination with LAB as immunostimulants. These were conducted in Artemia challenge test assay against three species of *Vibrio* spp., namely *Vibrio parahaemolyticus* (Vp), *V. harveyi* (Vh), and *V. vulnificus* (Vv). The treatments were prepared by Factorial Design with two factors (APS/AOS doses and *Vibrio* spp.) and replicated three times. The APS/AOS concentration was 0, 300, 600, 900 ppm. There were 8 levels of vibrio challenges, namely non-Vibrio, Vp, Vh, Vv, Vp-Vh, Vh-Vp, Vv-Vp, and Vp-Vh-Vv. LAB and APS/ AOS were fermented with seawater encapsulated by newly hatched Artemia's nauplii for one hour. Ten nauplii were taken out and challenged with 10^8 cells/mL Vibrio. Its survival rate (SR) was counted every six hours until reached 100% mortality. Results show that SR of all nauplii Artemia bio encapsulated treatments was higher than control ($p<0.05$). The best survival rate was reached from 400 ppm AOS. It has appeared that there is a synergically positive effect among the bio encapsulated AOS and LAB to accelerate the Artemia's immune system.

1. Introduction

Sargassum spp., the brown macroalgae, is a non-commercial alga that spread all over the Indonesian coast. At the peak season, the mass of *Sargassum* spp. is plentiful and unused. Our previous study has shown that *Sargassum siliquosum* from the southern coast of Java is rich in alginate (40.34%) [1]. Alginate is a linear polysaccharide composed of C5 epimer β-D-mannuronate (M) and α-L-guluronate (G). Our previous study had been revealed that alginate enabled to arise the shrimp immune system [2, 3]. According to [4], the oligosaccharides tend to have the higher biological activity compared to its'

polymer chain. Alginate oligosaccharides are composed of around 5 to 25 monosaccharides [5]. Polysaccharides are depolymerized by cleavage of the glycosidic bonds to produce AOS [6]. This seems correlated to the molecular weight and the presence of new functional groups [7].

Vibrios are γ-proteobacteria, gram-negative and pathogenic bacteria that exhibiting preferences for warm tropical waters [8]. *Vibrio harveyi, Vibrio vulnificus, Vibrio parahaemolyticus* are the causative agents of the most severe diseases of estuarine and marine aquaculture systems [9], potential pathogens for humans [8], and pathogenic in medicine as well as animal husbandry [10]. There were some clinical signs in shrimp and fish culture, which are slow growth, skin necrosis. For humans, the symptoms of gastroenteritis from undercooked or raw seafood consumption are shown [8]. We found that Vibrios originally from Indonesian shrimp pond were resistant to beta-lactam type of 5 antibiotics (Ampicillin, Co-Amoxiclav, Amoxicillin, Azithromycin, and Ciprofloxacin HCL). In [9], have also reported the antimicrobial resistance of these three *Vibrio* spp.

Lactic acid bacteria are characterized by gram-positive, facultative anaerobes non-spore-forming, catalase-negative, and lacking cytochromes [11]. LAB produces exopolysaccharides (EPS) [12], and due to their health-improving effects, they have been exploited as probiotics since the 1930s [13]. EPS have been claimed to possess beneficial in health [12]. Besides controlling the host's well-being and encourage the immune system, LAB was also keeping the inflammation control, potentially [14, 15]. According [16], stated that *Lactobacillus pentosus* could improve the growth rate, immune modulator, bacterial diversity in the gut, and *Vibrio* pathogens of *Litopenaeus vannamei* (Crustacea).

AOS served as prebiotics for LAB in humans as well as an animal [17-19]. Various dietary supplements of prebiotics and/or probiotics have been applied to boost the immune system by nutrient absorption and health regulation in *Litopenaeus vannamei* gut [20, 21]. Prebiotics can serve the nutrition for the LAB, and so, therefore, the immune system will arise [22]. The first step screening of AOS and LAB can be applied in Artemia. According to [23], *Artemia* sp. is an important bio model to investigate the interaction between the microbe's host (gnotobiotics). Artemia is a perfect organism to study probiotics activity and pathogenic bacteria [24]. Furthermore, Artemia is a nonselective filter feeder, so, therefore can be used as bio encapsulation from enriched media to enhance that finally improve its survival rate [25].

The application of probiotics (*Bacillus* spp.) and prebiotics (mannan oligosaccharides) for encapsulated Artemia, improved growth, larval survival, intestinal microbial communities of lobster larvae (*Homarus gammarus*) [26]. The updated information concerning the assessment of probiotics *Lactobacillus bulgaricus* FNCC–0041 & *Streptococcus thermophilus* FNCC–0040 application in combination with alginate polysaccharide (APS) and alginate oligosaccharide (AOS) to combat *Vibrio parahaemolyticus*, *V. vulnificus*, and *V. harveyi* is still very limited and need to be done. In this research, we pointed to ascertain the immunity screening of Artemia nauplii Instar II by determining its' survival rate. APS and AOS in combination with LAB and without lab were compared, and the best alginate concentration was defined.

2. Materials and methods

2.1. Alginate Polysaccharide (APS) extraction and Alginate Oligosaccharide (AOS) Production

Sargassum spp. was collected from Teluk Awur Coast, Jepara, Central Java. The fresh Sargassum was then rinsed with tap water, dried up, and grounded into a powder. The extraction of alginate was done by methods with Na_2CO_3 maceration, KCl, and ethanol absolute precipitation. The AOS production was prepared by similar technics in [7] using thermal heating.

2.2. *Lactobacillus bulgaricus* FNCC–004, *Streptococcus thermophilus* FNCC–0040, and *Vibrio* spp. isolates

FNCC–0041 and FNCC–0040 were obtained from the Center of Food Study, Laboratory of Microbiology, Gadjah Mada University, Yogyakarta. FNCC–0041 and FNCC–0040 were refreshed and recultured to this experiment in the Laboratory of Biology. *Vibrio vulnificus*, *Vibrio*

parahaemolyticus, and Vibrio harveyi as pathogenic bacteria were bought from Brackishwater Aquaculture Development Centre, Jepara, and refreshed at the similar Biology Laboratory, Department of Marine Science, Diponegoro University, Semarang.

2.3. Preparation of Artemia nauplii and LAB Fermentation

Glasswares, medium, and materials were sterilized using an autoclave. All the plastic materials sterilization was done by spraying with 70% alcohol and UV light exposure. Entirely trials were conducted in Laminar Air Flow (LAF) [27]. Cysts of Artemia (Supreme Plus®, Golden West Artemia) were purchased. One gram of Artemia cysts was then weighed out and then continue by soaking into 1000 mL seawater for hatching. This condition was kept in strong aeration for approximately one night [28]. On the following day, the newly Instar II hatched Artemia were collected and ready to be treated.

Vibrio spp. was cultured from one ose of *Vibrio* spp. isolate in Nutrient Agar/NA (Merck, USA) to Nutrient Broth/NB (Merck, USA). These bacteria were then scaled up cultured at 100 mL liquid media for 18 hrs to get the concentration of 0.5 McFarland [29]. While one ose of FNCC–0041 and FNCC–0040 from de Man, Rogosa and Shape Agar/MRS Agar was suspended in 100 mL Nutrient Broth/NB (Merck, USA) media and continued to incubation for 24 hrs at 37°C [30].

The APS and AOS concentration were arranged at 0, 400, 600, and 800 ppm by adding 0.006, 0.012, and 0.018 g Alginate and diluted in 20 mL FNCC–0041 and FNCC–0040 and 180 mL seawater. This fermentation of LAB was done in 72 hrs, kept stirring at 150 rpm, in room temperature [31-33].

2.4. Screening Immunity and Challenge Test of Artemia

These experiments were designed with a Factorial Design (two factors) and two sets of experiments. One set of experiments was applied to two LAB with alginate polysaccharides (APS) and oligosaccharides. Another experiment set was applied Non-LAB with alginate polysaccharides (APS) and oligosaccharides (AOS). The experiment was designed by two factors. The first factor was *Vibrio* spp. bacteria. We had applied 8 levels of vibrio challenges, namely Non-*Vibrio* spp. (without *Vibrio* spp.) as control, *Vibrio vulnificus* (Vv), *Vibrio harveyi* (Vh), *Vibrio parahaemolyticus* (Vp), the combination of *Vibrio vulnificus* and *Vibrio parahaemolyticus* (Vv-Vh), *Vibrio parahaemolyticus* and *Vibrio vulnificus* (Vp-Vv), *Vibrio harveyi* and *Vibrio parahaemolyticus* (Vh-Vp) and the combination of all three *Vibrio* spp: *Vibrio parahaemolyticus, Vibrio vulnificus and Vibrio harveyi* (Vp-Vv-Vh). The second factor was APS/AOS concentration, i.e. 0, 400, 600, and 800 ppm as described before. Each treatment was operated in three replications.

As the LAB fermentation was completed, the 180 mL of seawater and 20 mL of LAB were admixed and diluted with AOS at the different serial concentrations (400, 600, and 800 ppm). All the hatched from one gram Artemia nauplii were put into this mixed solution for one hour [34]. On the other side, we prepared some vials to run into trials. To apply one single species of *Vibrio* spp., we measured 9 mL seawater and one mL single *Vibrio* species and poured them into each vial. For two combinations of *Vibrio* spp (Vp-Vv; Vv-Vh, Vh-Vp) treatments, we measured 8 mL seawater and 2 mL of *Vibrio* spp. We were also mixed 3mL of Vibrio species combination (Vp-Vv-Vh) and 7 mL of seawater as the seventh treatment. After mixing those fermentors with each AOS treatment, ten Instar II nauplii Artemia were put into the vial and challenged with *Vibrio* spp. for immunity screening. The challenge test was administered until 100% mortality was achieved. The Artemia mortality was recorded every 6 hrs [35].

2.5. Statistical Analysis

Before this, all the data were checked for normality and homogeneity. To assess the differences and interaction among treatments, data were analyzed using two-way ANOVA (T-test, 95% significant level). The analysis was assisted by Ms. Excel 2007 and SPSS version 2016 software. The differences between treatments were then continued analyzed using Dunnet T3.

3. Results and Discussion

3.1. Artemia Survival Rate of Alginate Polysaccharide (APS) without LAB at different Hour Post Infection (HPI)

The survival rate of Instar II encapsulated Artemia with different APS concentrations every six hours HPI is presented in Figure 1. The survival rate at 24 HPI is depicted in Figure 2.

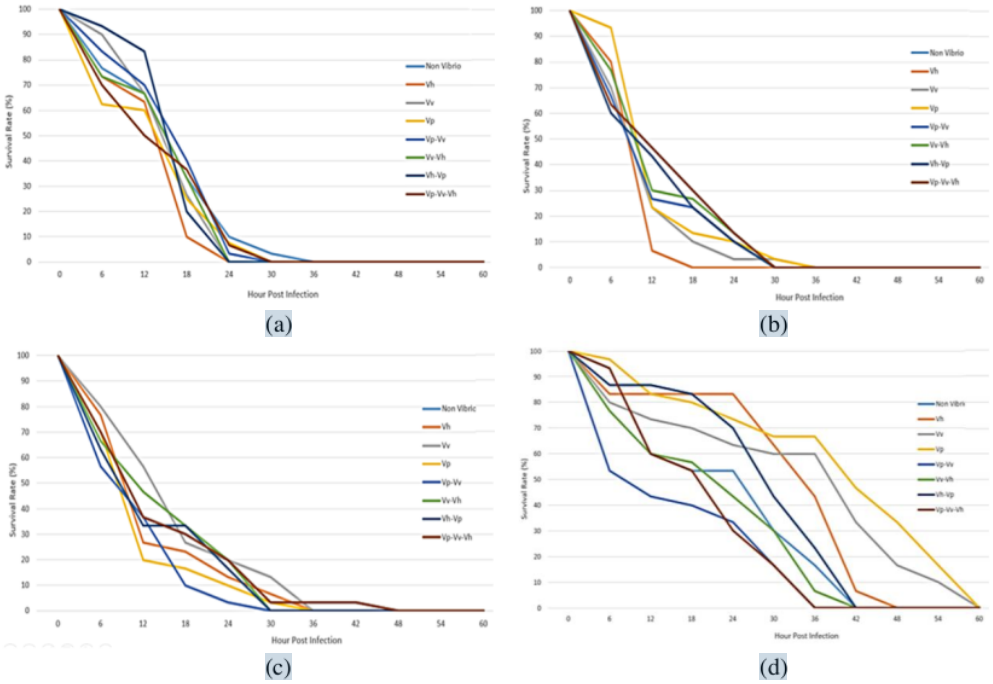

Figure 1. The survival rate of Artemia encapsulated at different concentrations of APS 0 ppm (a), 400 ppm (b), 600 ppm (c), and 900 ppm (d) without LAB after *Vibrio* spp. challenged.

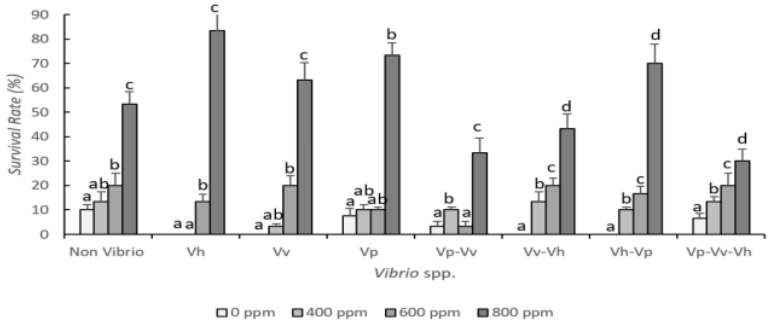

Figure 2. The survival rate of Artemia encapsulated at different concentrations of APS without LAB after *Vibrio* spp. challenged at 24 hrs post-infection.

Figure 1 shows that without any LAB, all the Artemia nauplii encapsulated with APS at 0, 400, 600, and 800 ppm were 100% died at different HPI (36, 36, 48, and 40 hrs), respectively. This indicates that a higher concentration of APS gave the higher protection to Vibrios challenged, even though, at 800 ppm, the HPI was slightly lower. On the other hand, 800 pp concentration of APS enables to reach the maximum protection in all challenged Vibrio treatments whether single, two combinations as well as three Vibrios combinations. It was ensuring that this concentration gave enough power for protecting the nauplii. This postulated that the activity of APS was in dose dependant manner [3].

3.2. Artemia Survival Rate of Alginate Oligosaccharide (AOS) without LAB at different Hour Post Infection (HPI)

The survival rate of Instar II encapsulated Artemia at different AOS concentrations every six hours HPI is presented in Figure 3 and at 24 HPI is in Figure 4.

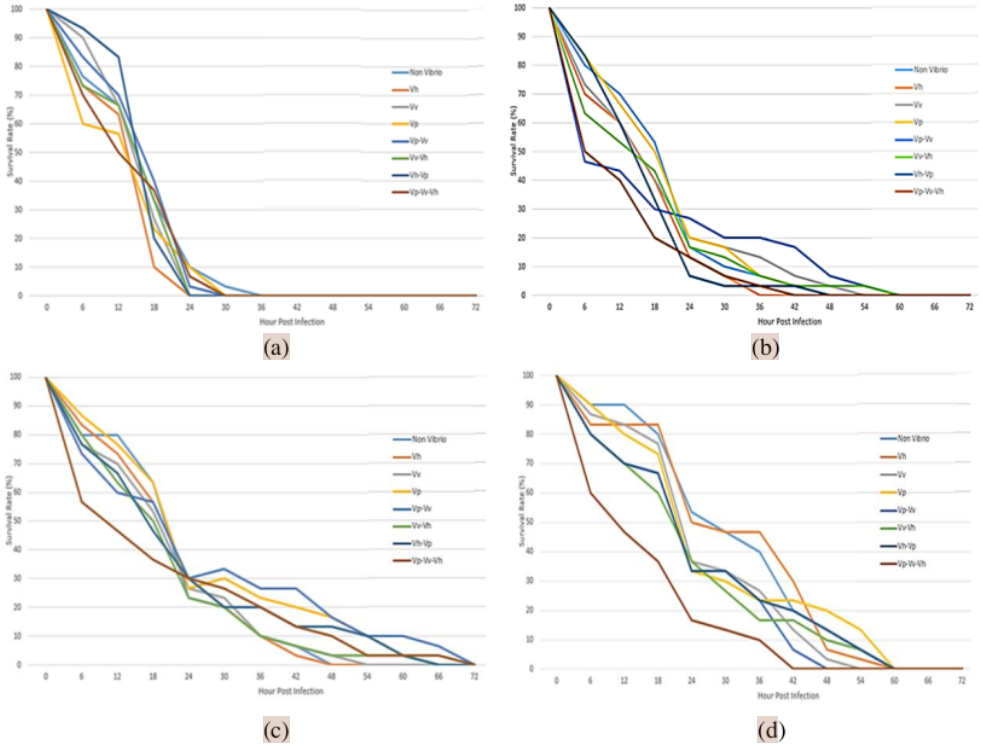

Figure 3. The survival rate of Artemia encapsulated at different concentrations of AOS 0 ppm (a), 400 ppm (b), 600 ppm (c), and 900 ppm (d) without LAB after *Vibrio* spp. challenged.

Figure 3 shows that without any LAB, all the Artemia nauplii encapsulated with APS at 0, 400, 600, and 800 ppm were 100% died at different HPI (36, 60, 72, and 60 hrs), respectively. These results show that AOS is more effective rather than APS. According to [4], marine polysaccharide degrading enzymes, particularly remarkable as those catalyze the cleavage of glycosidic bonds in polysaccharide macromolecules and generate oligosaccharides with low degrees of polymerization. Figure 4 is interesting since it has shown that the AOS is capable of managing the challenge of three combination Vibrios even at minimal concentration.

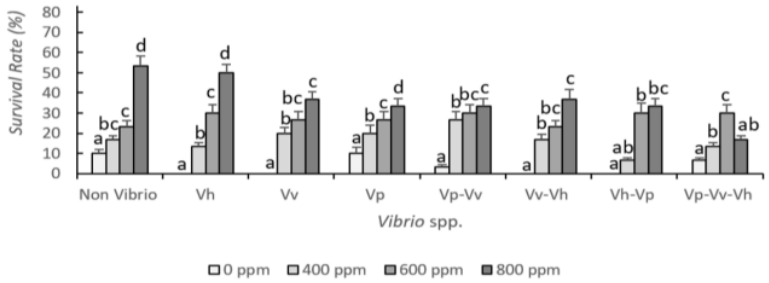

Figure 4. The survival rate of Artemia encapsulated at different concentrations of AOS without LAB after *Vibrio* spp. challenged at 24 hrs post-infection.

Figure 4 expressed that APS at minimum 400 ppm concentration with LAB encapsulation gave good results in combating the single and doubled Vibrios combination. Even though at the Vibrios combination, 600 ppm APS would be adequate, this is related to the dose-dependent manner [3].

3.3. Artemia Survival Rate of Alginate Polysaccharide (APS) and LAB at different Hour Post Infection (HPI)

The survival rate of Instar II encapsulated Artemia at different APS concentrations and LAB every six hours HPI is presented in Figure 5 and at 24 HPI is in Figure 6.

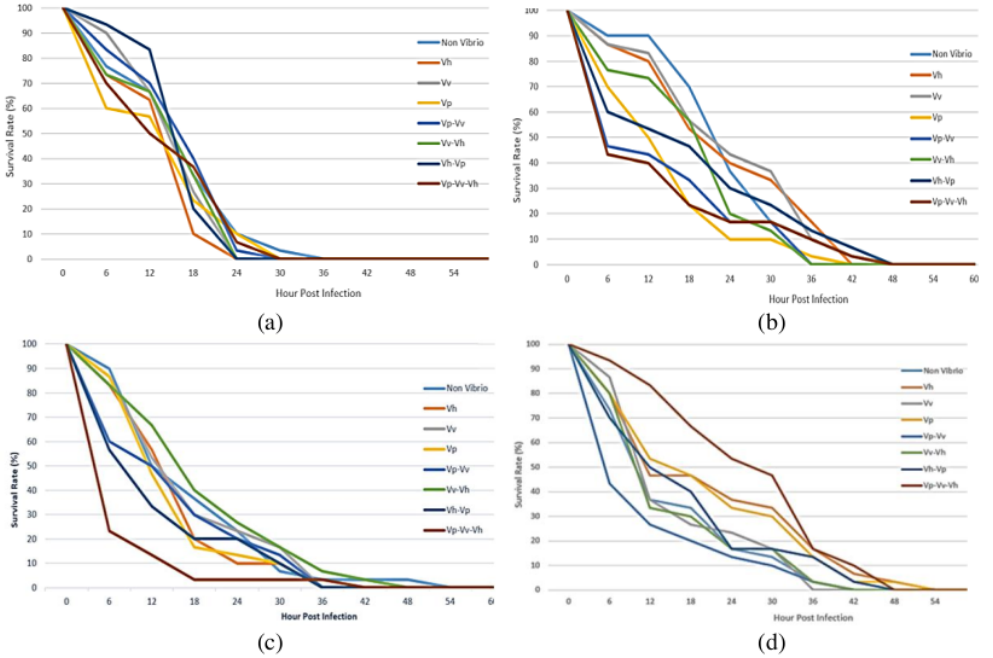

Figure 5. The survival rate of Artemia encapsulated at different concentrations of APS 0 ppm (a), 400 ppm (b), 600 ppm (c), and 900 ppm (d) with LAB after *Vibrio* spp. challenged.

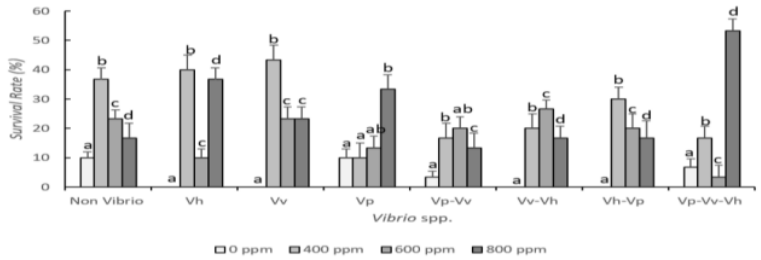

Figure 6. The survival rate of Artemia encapsulated at different concentrations of APS with LAB after *Vibrio* spp. challenged at 24 hrs post-infection.

Figure 5 shows that all the Artemia nauplii encapsulated with LAB and APS at 0, 400, 600, and 800 ppm were 100% died at different HPI (36, 48, 54, and 54 hrs), respectively. This indicates that LAB and its EPS [12] enhanced and prolonged the survival of Artemia compared to non LAB immersion. LAB mainly induced metabolic, cell to cell signaling-related protein upregulation and cell adhesion. These also provided bacterial adhesion and colonization in the midgut to increase the immune system [16].

3.4. Artemia Survival Rate of Alginate Oligosaccharide (AOS) and LAB at different Hour Post Infection (HPI)

The survival rate of Instar II encapsulated Artemia at different AOS concentrations and LAB every six hours HPI is presented in Figure 7 and at 24 HPI is in Figure 8.

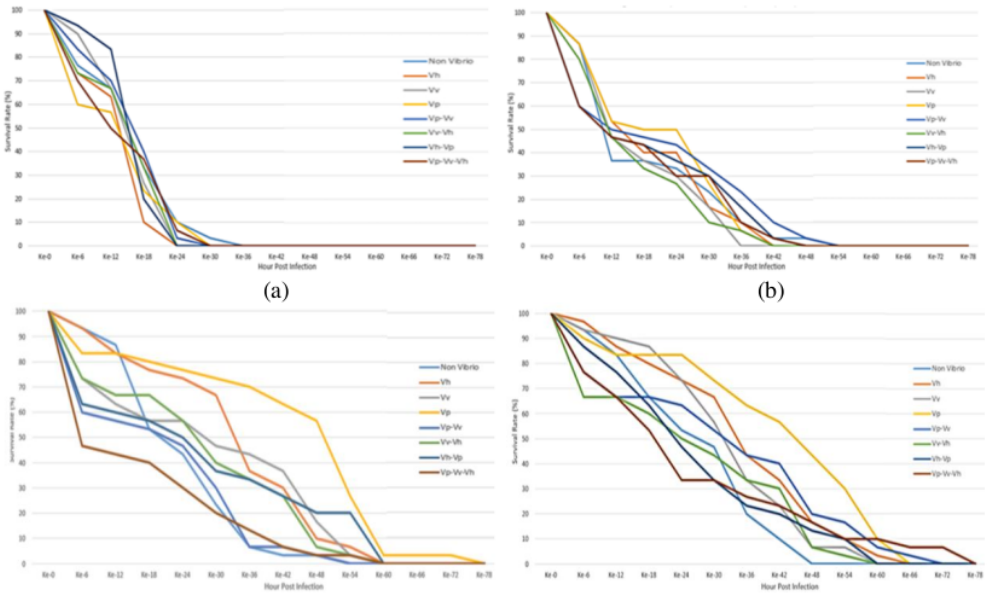

Figure 7. The survival rate of Artemia encapsulated at different concentrations of AOS 0 ppb (a), 400 ppm (b), 600 ppm (c), and 900 ppm (d) with LAB after *Vibrio* spp. challenged.

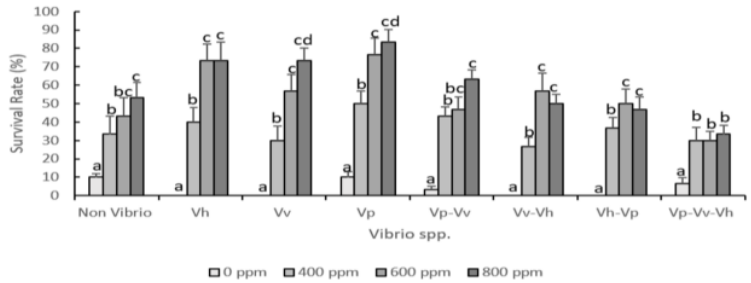

Figure 8. The survival rate of Artemia encapsulated at different concentrations of AOS with LAB after *Vibrio* spp. challenged at 24 hrs post-infection.

Compare to other treatments, which are non LAB APS and AOS, with LAB APS and AOS, the bio encapsulated Artemia nauplii with LAB and AOS in these treatments gave the best results (Figure 7 and 8). The survival rate of Artemia nauplii reached 70-80% at 600 ppm AOS concentration. At three Vibrios combination challenges, the minimum concentration of AOS completed with LAB EPS secretion was adequate to fight the pathogenic Vibrio.

Based on Figures 1, 3, 5, and 7, it is shown that with and without any LAB immersed but encapsulated with APS or AOS, and all the Artemia were 100% died at longer HPI according to higher APS and AOS concentration. This indicates that a higher concentration of APS gave the higher protection to Vibrios challenged approving that alginate act as immunostimulants in agrees to our previous research [2, 3] regarding shrimp (*L. vannamei*). AOS was also given stress protection in Zebrafish (*Danio rerio*) [36]. Results in Figure 2,4,6,8 show that control (0 ppm APS and AOS) in all treatments reached lower effect ($p<0.05$) when compared to treated Artemia (with and without LAB immersion). This has proven that alginate, whether it is poly or oligosaccharide, enabled to gave more immune booster by promoting cell proliferation, increasing immune parameters including Phenol Oxidase and Super-oxide Dismutase as well as related-immune gene expression [2, 3, 37].

Generally, the results conveyed that the application of AOS is more effective in comparison to APS. Our AOS has low molucular weight ie. 217.5 KDa [7]. Similar result in [38] has also noticed that AOS possesses more attractive biological activity and this is due to the fact of its low molecular weight (MW).

By comparing Figure 1,2,3,4 (non LAB) with Figure 5,6,7,8, (with LAB), it was seen that the immersion of LAB resulted in higher survival of Artemia nauplii significantly ($p<0.05$). *Lactobacillus bulgaricus* & *Streptococcus thermophilus* secreted exopolysaccharides [12] (Daba, 2021). *L. pentosus*, a probiotic, managed to increase the immune response, disease resistance, Du g bacterial diversity, and growth performance [16]. Furthermore according [23], *L. pentosus* can selectively adhere to mucosal surfaces of Artemia nauplii and produce cell-bound biosurfactants, displacing the pathogenic strains. Moreover, LAB act as probiotics, and alginate serve as prebiotics [17] which work synergically to boost up the nauplii Artemia immune system.

4. Conclusion

The bio encapsulation at 400 ppb concentration of alginate oligosaccharide in combination with *Lactobacillus bulgaricus* FNCC–0041 and *Streptococcus thermophilus* FNCC–0040 were synergically enhanced the survival rate of Artemia's nauplii. It has been approved that there is a synergically affirmative, positive impact in alginate oligosaccharide and lactic acid bacteria secretion to develop the Artemia immune system.

Acknowledgments
This research was fully supported by the Indonesian Government, Brackishwater Aquaculture Development Centre, and Ministry of Research and Higher Education (contract no: 187-64/UN7.6.1/PP/2021).

References

[1] Yudiati E and Isnansetyo A 2017 *IJMR* **22** 7-14
[2] Yudiati E, Isnansetyo A, Murwantoko, Ayuningtyas, Triyanto and Handayani C R 2016 *Fish Shellfish Immunol*. **54** 46-53
[3] Yudiati E, Isnansetyo A, Murwantoko, Triyanto and Handayani C R 2019 *Mar. Biotechnol*. **21** 503-14
[4] Sun H, Gao L, Xue C and Mao X 2020 *Compr. Rev. Food Sci. Food Saf*. **19** 2767-96
[5] Addina S, Subaryono S and Sukarno S 2020 *Jurnal Pascapanen dan Bioteknologi Kelautan dan Perikanan* **15** 47-61
[6] Kelishomi Z H, Goliaei B, Mahdavi H, Nikoofar A, Rahimi M, Moosavi-Movahedi A A, Mamashli F and Bigdeli B 2016 *Food Chem*. **196** 897-902
[7] Yudiati E, Pringgenies D, Djunaedi A, Arifin Z and Sudaryono A 2018 *Aquacultura Indonesiana* **19** 1-27
[8] Padovan A, Siboni N, Kaestli M, King W L, Seymour J R and Gibb K 2021 *Mar. Environ. Res*. **169** 105405
[9] Sony M, Sumithra T, Anusree V, Amala P, Reshma K, Alex S and Sanil N 2021 *Aquaculture* **539** 736608
[10] Li F, Tian F, Li J, Li L, Qiao H, Dong Y, Ma F, Zhu S and Tong Y 2021 *Virus Res*. **302** 198481
[11] Mathur S and Singh R 2005 *Int. J. Food. Microbiol*. **105** 281-95
[12] Daba G M, Elnahas M O and Elkhateeb W A 2021 *Int. J. Biol. Macromol*. **173** 79-89
[13] Borges S, Silva J and Teixeira P 2014 *Arch. Gynecol. Obstet*. **289** 479-89
[14] Wasilewska E, Zlotkowska D and Wroblewska B 2019 *J. Dairy Sci*. **102** 37-53
[15] Wasilewska E and Wroblewska B 2018 *Postepy Hig. Med. Dosw*. **72** 159–74
[16] Du Y, Wang M, Wang B, Liu M, Jiang K and Wang L 2019 *Fish Shellfish Immunol*. **92** 119-24
[17] Afni F S, Purwaningsih S, Nurilmala M and Peranginangin R 2017 *Jurnal Pengolahan Hasil Perikanan Indonesia* **20** 109-22
[18] Liu J, Yang S, Li X, Yan Q, Reaney M J and Jiang Z 2019 *Compr. Rev. Food Sci. Food Saf*. **18** 1859-81
[19] Yudiati E, Subagiyo S and Djarod M S R 2020 *Jurnal Kelautan Tropis* **23** 234-8
[20] Li E, Xu C, Wang X, Wang S, Zhao Q, Zhang M, Qin J G and Chen L 2018 *Reviews in Fisheries Science & Aquaculture* **26** 381-99
[21] Zhang C, Li M, Rauf A, Khalil A A, Shan Z, Chen C, Rengasamy K R and Wan C 2021 *Crit. Rev. Food Sci. Nutr*. **13** 1-27
[22] Aich N, Ahmed N and Paul A 2018 *Int. J. Curr. Microbiol. Appl. Sci*. **7** 26-41
[23] Garcés M E, Sequeiros C and Olivera N L 2015 *Dis Aquat Organ*. **113** 41-50
[24] Marques A, Dinh T, Ioakeimidis C, Huys G, Swings J, Verstraete W, Dhont J, Sorgeloos P and Bossier P, 2005 *Appl Environ Microbiol*. **71** 4307-17
[25] Patra S and Mohamed K 2003 *Aquacult. Int*. **11** 505-14
[26] Daniels N A, MacKinnon L, Bishop R, Altekruse S, Ray B, Hammond R M,

Thompson S, Wilson S, Bean N H, Griffin P M and Slutsker L 2000 *J Infect Dis*. **181** 1661-66
[27] Guridi A, Sevillano E, de la Fuente I, Mateo E, Eraso E and Quindós G 2019 *Int. J. Environ. Res. Public Health* **16** 4747
[28] Rudtanatip T, Boonsri B, Praiboon J and Wongprasert K 2019 *Fish Shellfish Immunol*. **94** 90-8
[29] Andrews J M 2001 *J Antimicrob Chemother*. **48** 5-16
[30] Yudiati E, Sedjati S, Susanto A, Azhar N and Alghazeer R 2021 *Jurnal Kelautan Tropis* **24** 25-33
[31] Niccolai A, Shannon E, Abu-Ghannam N, Biondi N, Rodolfi L and Tredici M R 2019 *J. Appl. Phycol*. **31** 1077-83
[32] Bhowmik D, Dubey J and Mehra S 2009 *World Journal of Dairy Food Sciences* **4** 160-3
[33] de Caire G Z, Parada J L, Zaccaro M C and de Cano M M S, 2000 *World Journal of Microbiology Biotechnology* **16** 563-5
[34] Hidayati J R 2019 *Thesis*. **Unpublished**
[35] Immanuel G, Sivagnanavelmurugan M and Palavesam A 2011 *Aquacult. Int*. **19** 91-101
[36] Yudiati E, Ginzel F I, Hidayati J R, Rizfa M S, Azhar N, Djarod M S R, Heriyati E and Alghazeer R, 2020 *Indonesian Journal of Marine Sciences* **25** 7-14
[37] Xing M, Cao Q, Wang Y, Xiao H, Zhao J, Zhang Q, Ji A and Song S, 2020 *Mar. Drugs* **18** 144-69
[38] Zhang C, Wang W, Zhao X, Wang H, Yin H, 2020 *Carbohydr. Res*. **494** 108056

Alginate oligosaccharide/polysaccharide and lactic acid bacteria (Lactobacillus bulgaricus FNCC–0041 & Streptococcus thermophilus FNCC–0040) as immunostimulants against pathogenic Vibrio spp. using

ORIGINALITY REPORT

15%	13%	14%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to University of Queensland Student Paper	4%
2	repository.bsi.ac.id Internet Source	2%
3	S Mellisa, Fitria, I Hasri, N Nurfadillah, I I Arisa. "The effectiveness of feeding artemia enriched with vitamin c on the growth performance and survival of Lemeduk fish larvae (Barbonymus schwanenfeldii)", IOP Conference Series: Earth and Environmental Science, 2021 Publication	1%
4	ijcmas.com Internet Source	1%
5	E Yudiati, M S R Djarod, D Pringgenies, E S Susilo. "Accelerating The Physilogical Properties of Sodium Alginate Paste by Thermal Method and Microwave Irradiation",	1%

	IOP Conference Series: Earth and Environmental Science, 2019 Publication	
6	repository.ung.ac.id Internet Source	1 %
7	E Yudiati, G W Santosa, M R Tontowi, S Sedjati, E Supriyantini, M Khakimah. " Optimization of alginate alkaline extraction technology from and its antioxidant properties ", IOP Conference Series: Earth and Environmental Science, 2018 Publication	<1 %
8	www.sid.ir Internet Source	<1 %
9	www.ncbi.nlm.nih.gov Internet Source	<1 %
10	www.science.gov Internet Source	<1 %
11	biblio.ugent.be Internet Source	<1 %
12	Yang Du, Han Fang, Xuqing Shao, Mei Liu, Keyong Jiang, Mengqiang Wang, Baojie Wang, Lei Wang. "Exploration of the influence of surface proteins on the probiotic activity of Lactobacillus pentosus HC-2 in the Litopenaeus vannamei midgut via label-free	<1 %

quantitative proteomic analysis", Fish & Shellfish Immunology, 2019
Publication

13	eprints.undip.ac.id Internet Source	<1 %
14	ir.unimas.my Internet Source	<1 %
15	Reshmi Ghuglot, William Titus, Ananya S. Agnihotri, V. Krishnakumar, Ganesan Krishnamoorthy, Nidhin Marimuthu. "Stable copper nanoparticles as potential antibacterial agent against aquaculture pathogens and human fibroblast cell viability", Biocatalysis and Agricultural Biotechnology, 2021 Publication	<1 %
16	William Leonard, Pangzhen Zhang, Danyang Ying, Benu Adhikari, Zhongxiang Fang. "Fermentation transforms the phenolic profiles and bioactivities of plant-based foods", Biotechnology Advances, 2021 Publication	<1 %
17	www.mdpi.com Internet Source	<1 %
18	Ervia Yudiati, Subagiyo, Nuril Azhar. "Antimicrobial susceptibility and minimum inhibition concentration of Vibrio	<1 %

parahaemolyticus, Vibrio vulnificus and Vibrio harveyi isolated from a white shrimp (Litopenaeus vannamei) pond", IOP Conference Series: Earth and Environmental Science, 2021
Publication

19	docplayer.net Internet Source	<1 %
20	eprints.lincoln.ac.uk Internet Source	<1 %
21	iopscience.iop.org Internet Source	<1 %
22	M E Sawitri, M C Padaga, A Manab. "Physicochemical and Microbiological Characteristics of Fermented Synbiotic Milk Enriched with Inulin at Room Temperature as Functional Drinking Products", IOP Conference Series: Earth and Environmental Science, 2020 Publication	<1 %
23	ejournal2.undip.ac.id Internet Source	<1 %

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		

Alginate oligosaccharide/polysaccharide and lactic acid bacteria (Lactobacillus bulgaricus FNCC–0041 & Streptococcus thermophilus FNCC–0040) as immunostimulants against pathogenic Vibrio spp. using

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11