RINGKASAN

HAMSAH. Kinerja Pertumbuhan, Respons Imun dan Resistansi Larva Udang Vaname yang Diberi Pseudoalteromonas piscicida dan Mannanoligosakarida melalui Bioenkapsulasi Artemia sp. Dibimbing oleh WIDANARNI, ALIMUDDIN, MUNTI YUHANA, dan MUHAMMAD ZAIRIN JUNIOR.

Udang vaname (Litopenaeus vannamei) merupakan salah satu komoditas ekspor unggulan Indonesia di sektor perikanan budidaya. Perkembangan produksi udang vaname harus didukung oleh ketersediaan benih udang yang berkualitas baik dalam jumlah dan waktu yang tepat. Namun demikian, serangan penyakit masih menjadi salah satu kendala utama dalam usaha pembenihan udang vaname, yang menyebabkan rendahnya kelangsungan hidup dan pertumbuhan larva udang. Salah satu jenis penyakit yang menyerang udang vaname adalah vibriosis yang disebabkan oleh bakteri Vibrio harveyi, yang dapat menyebabkan kematian pada seluruh stadia udang, mulai dari stadia nauplius, zoea, mysis dan pascalarva sampai pada udang dewasa di tambak pembesaran.

Aplikasi probiotik, prebiotik, dan sinbiotik (kombinasi probiotik dengan prebiotik) merupakan salah satu alternatif pengendalian penyakit yang ramah lingkungan karena dapat meningkatkan pertumbuhan, respons imun, dan resistansi udang terhadap serangan penyakit.

Tujuan dari penelitian ini adalah untuk mengevaluasi kinerja pertumbuhan, respons imun dan resistansi larva udang vaname yang diberi probiotik Pseudoalteromonas piscicida, prebiotik mannanoligosakarida (MOS), dan gabungan keduanya (sinbiotik) melalui bioenkapsulasi Artemia sp. terhadap infeksi bakteri patogen Vibrio harveyi. Secara garis besar untuk menjawab tujuan

tersebut, penelitian dibagi dalam empat tahap, yaitu: 1. Evaluasi potensi bakteri P. piscicida (1Ub) sebagai probiotik dan kemampuannya memanfaatkan prebiotik MOS; 2. Populasi bakteri dan kandungan nutrisi Artemia sp. hasil bioenkapsulasi dengan probiotik P. piscicida (1Ub), prebiotik MOS, dan sinbiotik; 3. Kinerja pertumbuhan dan sintasan larva udang vaname yang diberi probiotik P. piscicida (1Ub), prebiotik MOS, dan sinbiotik melalui bioenkapsulasi Artemia sp; 4. Respons imun dan resistansi larva udang vaname yang diberi probiotik P. piscicida (1Ub), prebiotik MOS, dan sinbiotik melalui bioenkapsulasi Artemia sp. Penelitian tahap pertama bertujuan untuk mengevaluasi potensi bakteri P. piscicida (1Ub) sebagai probiotik dan kemampuannya dalam memanfaatkan prebiotik mannanoligosakarida (MOS). Hasil uji pertumbuhan bakteri berdasarkan densitas optikal (OD) dan jumlah koloni bakteri (TPC) diperoleh pertumbuhan maksimal bakteri P. piscicida (1Ub) dicapai setelah 18 jam inkubasi dengan kepadatan rata-rata bakteri sebanyak 109 CFU/mL. Bakteri P. piscicida 1Ub juga menunjukkan toleransi yang baik dan mampu bertahan hidup dalam kondisi asam (pH 4) dan basa (pH 8.5) selama periode pengamatan. Hasil uji penempelan menunjukkan bakteri P. piscicida 1Ub memiliki kemampuan menempel dan membentuk biofilm pada permukaan lempeng stainless steel dengan kepadatan bakteri sebesar 7.91 log CFU/cm2. Bakteri P. piscicida 1Ub mampu menghasilkan beberapa enzim eksogenus, seperti protease (0.031±0.030 U/mL/menit), lipase (0.198±0.058 U/mL/menit), amilase (0.008±0.003 U/mL/menit), dan mananase (0.019±0.004 U/mL/menit) serta berpotensi menghambat bakteri patogen Vibrio harveyi. Bakteri P. piscicida 1Ub juga mampu memanfaatkan mannanoligosakarida sebagai sumber nutrisi untuk pertumbuhan yang ditunjukkan dengan kepadatan bakteri P. piscicida 1Ub yang

tumbuh pada media SWC-cair yang ditambahkan 0.2 g MOS selama masa inkubasi 16-20 jam berkisar 11.2–11.8 log CFU/mL, sedangkan yang tumbuh pada media SWC-cair tanpa penambahan MOS hanya berkisar 9.2–9.5 log CFU/mL.

Penelitian tahap kedua bertujuan mengevaluasi populasi bakteri dan kandungan nutrisi Artemia sp. hasil bioenkapsulsi dengan probiotik P. piscicida (1Ub), prebiotik MOS, dan gabungan keduanya (sinbiotik). Bioenkapsulasi Artemia sp. dilakukan pada stadia instar 2 menggunakan wadah masing-masing bervolume 1 liter air laut dengan kepadatan Artemia sp. sebanyak 100 individu/mL dan dilengkapi dengan jaringan aerasi. Bioenkapsulasi dilakukan dengan cara pada setiap media pemeliharaan Artemia sp. ditambahkan masingmasing bakteri P. piscicida (1Ub) konsentrasi 106 CFU/mL untuk perlakuan probiotik, 12 mg/L MOS untuk perlakuan prebiotik, dan kombinasi bakteri P. piscicida (1Ub) konsentrasi 106 CFU/mL dengan 12 mg/L MOS untuk perlakuan sinbiotik. Bioenkapsulasi Artemia sp. dilakukan selama 4 jam, selanjutnya dilakukan pengukuran parameter meliputi populasi bakteri dalam tubuh Artemia sp. dan kandungan nutrisi Artemia sp. hasil bioenkapsulasi. Populasi bakteri dalam tubuh Artemia sp. yang diberi probiotik (8.0 log CFU/0.1 g Artemia), prebiotik (7.7 log CFU/0.1 g Artemia), dan sinbiotik (8.6 log CFU/0.1 g Artemia) lebih tinggi dibanding kontrol (6.8 log CFU/0.1 g Artemia). Probiotik P. piscicida 1Ub juga mampu hidup dan berkolonisasi dalam tubuh Artemia sp. masingmasing sebanyak 6.9 log CFU/0.1 g Artemia pada perlakuan sinbiotik dan sebanyak 6.4 log CFU/0.1 g Artemia pada perlakuan probiotik. Bioenkapsulasi Artemia sp. dengan probiotik P. piscicida 1Ub, prebiotik MOS, dan sinbiotik (kombinasi P. piscicida 1Ub dan MOS) juga mampu meningkatkan nilai nutrisi

Artemia sp. terutama kadar protein, kadar lemak, dan profil asam lemak.

Penelitian tahap ketiga bertujuan mengevaluasi kinerja pertumbuhan, aktivitas enzim, populasi bakteri, dan sintasan larva udang vaname yang diberi probiotik P. piscicida (1Ub), prebiotik MOS, dan gabungan keduanya (sinbiotik) melalui bioenkapsulasi Artemia sp. Larva udang vaname dipelihara dalam akuarium berisi air laut sebanyak 10 L dengan kepadatan 20 ekor/L dan dilengkapi jaringan aerasi. Prosedur bioenkapsulasi Artemia sp. dilakukan sama seperti pada penelitian tahap kedua. Artemia sp. hasil bioenkapsulasi selanjutnya diberikan ke larva udang vaname (Mysis 3 sampai pascalarva 12) sebanyak 8-10 individu/larva setiap kali pemberian dengan frekuensi pemberian lima kali sehari. Hasil penelitian menunjukkan pemberian probiotik, prebiotik, dan sinbiotik memberikan pengaruh nyata ($P<0.05$) terhadap daily growth rate (DGR), panjang mutlak dan tingkat kelangsungan hidup, namun terhadap rasio RNA/DNA hanya pemberian probiotik dan sinbiotik yang berpengaruh nyata ($P<0.05$). Nilai DGR, panjang mutlak, rasio RNA/DNA, dan SR tertinggi ($P<0.05$) diperoleh pada perlakuan sinbiotik. Aktivitas enzim larva udang vaname pada perlakuan sinbiotik juga lebih tinggi ($P<0.05$) dibandingkan kontrol dan perlakuan lainnya. Demikian pula total bakteri dan total probiotik 1Ub dalam tubuh larva udang vaname yang diberi sinbiotik lebih tinggi ($P<0.05$) dibandingkan perlakuan lainnya dan kontrol yaitu masing-masing sebanyak 6.7 x 107 CFU/0.1 g larva dan 4.75 x 106 CFU/0.1 g larva.

Penelitian tahap keempat bertujuan mengevaluasi respons imun dan resistansi larva udang vaname yang diberi probiotik P. piscicida (1Ub), prebiotik MOS, dan sinbiotik melalui bioenkapsulasi Artemia sp. serta diinfeksi V. harveyi. Masing-masing sebanyak 30 ekor PL13 udang vaname yang sebelumnya telah

diberi perlakuan probiotik, prebiotik, dan sinbiotik dipelihara dalam wadah bervolume 1 liter air laut steril dan dilengkapi sistem aerasi. Selanjutnya dilakukan uji tantang dengan cara menambahkan bakteri patogen V. harveyi MR5339 konsentrasi 3 x 107 CFU/mL dalam masing-masing wadah pemeliharaan postlarva udang vaname pada perlakuan probiotik, prebiotik, sinbiotik, dan kontrol (+). Sementara untuk perlakuan kontrol (-) ditambahkan 30 mL media SWC-cair. Uji tantang dilakukan selama 5 hari. Hasil penelitian menunjukkan nilai total hemosit count (THC) dan aktivitas phenoloxidase (PO) larva udang vaname yang diberi sinbiotik lebih tinggi ($P<0.05$) dibandingkan perlakuan lainnya, baik sebelum maupun setelah uji tantang. Aktivitas respiratory burst (RB) larva udang vaname yang diberi sinbiotik sebelum dan setelah uji tantang berbeda nyata ($P<0.05$) dengan kontrol, namun tidak berbeda nyata ($P>0.05$) dengan yang diberi probiotik dan prebiotik. Peningkatan imunitas larva udang vaname yang diberi probiotik, prebiotik, dan sinbiotik juga terlihat dari hasil pengukuran ekspresi gen imun larva udang. Ekspresi gen serine protein (SP) larva udang yang diberi sinbiotik menunjukkan nilai paling tinggi ($P<0.05$) dibandingkan perlakuan lainnya. Ekspresi gen peroxinectin (PE) larva udang yang diberi sinbiotik dan probiotik tidak berbeda nyata ($P>0.05$), namun keduanya menunjukkan nilai paling tinggi ($P<0.05$) dibandingkan perlakuan prebiotik dan kontrol. Ekspresi gen lipopolysaccharide and β-1,3-glucan-binding protein (LGBP) larva udang vaname yang diberi sinbiotik juga lebih tinggi ($P<0.05$) dibandingkan kontrol dan perlakuan lainnya. Pascauji tantang, SR larva udang vaname yang diberi probiotik, prebiotik, dan sinbiotik lebih tinggi (81.7 - 90.0%) dibandingkan kontrol positif (68.3%) atau terjadi peningkatan SR sebesar 19 - 32%.

Berdasarkan hasil dari seluruh tahap penelitian ini, dapat disimpulkan bahwa probiotik P. piscicida 1Ub, prebiotik MOS, dan gabungan keduanya (sinbiotik) mampu meningkatkan kinerja pertumbuhan, respons imun dan resistansi larva udang vaname terhadap infeksi bakteri patogen V. harveyi dengan hasil terbaik diperoleh pada pemberian sinbiotik.
Kata kunci: P. piscicida 1Ub, MOS, sinbiotik, larva udang vaname, V. harveyi

SUMMARY

HAMSAH. Growth Performance, Immune Response and Resistance of Pacific White Shrimp Larvae Administered Pseudoalteromonas piscicida and Mannanoligosaccharide through Artemia sp. Bio-encapsulation. Supervised by WIDANARNI, ALIMUDDIN, MUNTI YUHANA, and MUHAMMAD ZAIRIN JUNIOR.

Pacific white shrimp (Litopenaeus vannamei) is one of Indonesia's major export commodities in aquaculture sector. The development on the production of Pacific white shrimp has to be supported by the availability of the good quality shrimp larvae in the right quantity and time. Nevertheless, disease outbreaks still become one of the main obstacles in Pacific white shrimp hatcheries, causing a low survival and growth of the shrimp larvae. One of diseases that attacks Pacific white shrimp is vibriosis caused by Vibrio harveyi, which can cause mortality in all shrimp stadia, from nauplius, zoea, mysis and post-larva to adult shrimp in grow out ponds.

The application of probiotics, prebiotics, and synbiotics is one of environmentally friendly alternative disease controls because they can improved the growth, increase immune responses and disease resistance of shrimps

The objective of this study was to evaluate growth performance, immune response and resistance of Pacific white shrimp larvae administered probiotic Pseudoalteromonas piscicida, prebiotic mannanoligosaccharide, and a combination of the two (synbiotic) through Artemia sp. bio-encapsulation against the infection of Vibrio harveyi. To answer that objective, the study was divided

into four stages, that were: 1. The evaluation of the potency of P. piscicida (1Ub) as probiotic and its ability to utilize prebiotic MOS; 2. The bacterial population and the nutritional content of Artemia sp. encapsulated with probiotic P. piscicida (1Ub), prebiotic MOS, and synbiotic; 3. The growth performance and the survival of Pacific white shrimp larvae administered probiotic P. piscicida (1Ub), prebiotic MOS, and synbiotic through Artemia sp. bio-encapsulation; 4. The immune response and the resistance of Pacific white shrimp larvae administered probiotic P. piscicida (1Ub), prebiotic MOS, and synbiotic through Artemia sp. bioencapsulation. The first stage experiment aimed to evaluate the potency of P. piscicida (1Ub) as probiotic and its ability in utilizing prebiotic mannanoligosaccharide (MOS). The result of the bacterial growth based on optical density (OD) and the number of bacterial colonies (TPC) was obtained the maximum growth of P. piscicida (1Ub) reached after 18 hours of incubation with an average bacterial density of 109 CFU/mL. P. piscicida 1Ub also demonstrated a good tolerance and was able to survive in acid (pH 4) and alkaline (pH 8.5) conditions during the observation period. The result of the attachment test indicated that P. piscicida 1Ub had an attachment ability and formed biofilm on a stainless steel plate surface with a bacterial density of 7.91 log CFU/cm2. P. piscicida 1Ub was able to secrete several exogeneous enzymes, such as protease (0.031±0.030 U/mL/minute), lipase (0.198±0.058 U/mL/minute), amylase (0.008±0.003 U/mL/minute), and mannanase (0.019±0.004 U/mL/minute) and potentially inhibited Vibrio harveyi. P. piscicida 1Ub was also able to utilize mannanoligosaccharide as a nutrition source for its growth indicated by the density of P. piscicida 1Ub that grew in SWC-broth added 0.2 g MOS during an incubation period of 16-20 hours ranged 11.2-11.8 log CFU/mL, while that growing in SWC-broth without the addition of

MOS only ranged 9.2-9.5 log CFU/mL.

The second stage experiment aimed to evaluate the bacterial population and the nutritional content of Artemia sp. encapsulated with probiotic P. piscicida (1Ub), prebiotic MOS, and a combination of the two (synbiotic). Artemia sp. bioencapsulation was conducted on instar 2 stadia using containers with a volume of 1 liter sea water with an Artemia sp. density in each container of 100 individuals/mL and equipped with an aeration system. Bio-encapsulation was performed by adding P. piscicida (1Ub) at a concentration of 106 CFU/mL for probiotic treatment, 12 mg/L MOS for prebiotic treatment, and a combination of P. piscicida (1Ub) at a concentration of 106 CFU/mL and 12 mg/L MOS for synbiotic treatment in each rearing medium. Artemia sp. bio-encapsulation was conducted for 4 hours, then conducted the measurements of parameters including the bacterial population in the Artemia sp. body and the nutritional content of the bio-encapsulated Artemia sp. The bacterial populations in the Artemia sp. body administered probiotic (8.0 log CFU/0.1 g Artemia), prebiotic (7.7 log CFU/0.1 g Artemia), and synbiotic (8.6 log CFU/0.1 g Artemia) were higher compared to control (6.8 log CFU/0.1 g Artemia). P. piscicida 1Ub was also able to live and colonize in the Artemia sp. body at values of 6.9 log CFU/0.1 g Artemia in synbiotic treatment and 6.4 log CFU/0.1 g Artemia in probiotic treatment. Artemia sp. bio-encapsulation with probiotic P. piscicida 1Ub, prebiotic MOS, and synbiotic (a combination of P. piscicida 1Ub and MOS) was also able to increase the nutritional value of Artemia sp. especially protein level, fat level, and fatty acid profile.

The third stage experiment aimed to evaluate growth performance, enzymes activities, bacterial population, and survival of Pacific white shrimp administered

probiotic P. piscicida (1Ub), prebiotic MOS, and a combination of the two (synbiotic) through Artemia sp. bio-encapsulation. Pacific white shrimps were reared in aquariums filling 10 L sea water with a stocking density of 20 individuals/L and equipped with an aeration system. Artemia sp. bioencapsulation procedure was performed as same as on the second stage experiment. The bio-encapsulated Artemia sp. were then administered to Pacific white shrimp larvae (Mysis 3 to post-larvae 12) at a range of 8-10 individuals/larvae every administration with a frequency of five times a day. The experimental results demonstrated that the administration of probiotic, prebiotic, and synbiotic gave significant effects ($P<0.05$) on daily growth rate (DGR), absolute length growth and survival, but on RNA/DNA ratio only the administration of probiotic and synbiotic significantly affected ($P<0.05$). The highest DGR, absolute length, RNA/DNA ratio, and SR values ($P<0.05$) were obtained in synbiotic treatment. Enzymes activities of Pacific white shrimp larvae on synbiotic treatment were also higher ($P<0.05$) compared to control and other treatments. Similarly, total bacteria and total probiotic P. piscicida 1Ub in the Pacific white shrimp larvae body administered synbiotic were higher ($P<0.05$) compared to other treatments and control that were 6.7 x 107 CFU/larvae and 4.75 x 106 CFU/larvae, respectively.

The fourth stage experiment aimed to evaluate immune response, genes expressions related to immunity, and resistance of Pacific white shrimp larvae administered probiotic P. piscicida (1Ub), prebiotic MOS, and synbiotic through Artemia sp. bio-encapsulation and infected by V. harveyi. The PL13 Pacific white shrimps at a number of 30 individuals in each treatment that were previously treated by probiotic, prebiotic, and synbiotic were reared in containers with a

volume of 1 liter sterile sea water and equipped with an aeration system. Therefore, the challenge test was performed by adding V. harveyi MR5339 at a concentration of 3 x 107 CFU/mL into each rearing container of Pacific white shrimp post-larvae in probiotic, prebiotic, synbiotic, and control (+) treatments. For control (-) treatment, it was added 30 mL SWC-broth medium. The challenge test was conducted for 5 days. Experimental results demonstrated that total hemocyte count (THC) and phenoloxydase (PO) activity values of Pacific white shrimp larvae administered synbiotic were higher ($P<0.05$) compared to other treatments, both before and after the challenge test. The respiratory burst (RB) activities of Pacific white shrimp larvae administered synbiotic before and after the challenge test were significantly different ($P<0.05$) from control, but it was not significantly different ($P>0.05$) than those administered probiotic and prebiotic. The increasing of the immunity of Pacific white shrimp larvae administered probiotic, prebiotic, and synbiotic were indicated from results of immune genes expressions measurements of the shrimp larvae. The serine protein (SP) gene expression of the shrimp larvae administered synbiotic demonstrated the highest value ($P<0.05$) compared to other treatments. The peroxinectin (PE) gene expressions of the shrimp larvae administered synbiotic and probiotic were not significantly different ($P>0.05$), but those showed higher values ($P<0.05$) compared to prebiotic and control treatments. The lipopolysaccharide and β-1,3-glucan-binding protein (LGBP) gene expression of Pacific white shrimp larvae administered synbiotic was also higher ($P<0.05$) compared to control and other treatments. On post-challenge test period, Pacific white shrimp SR administered probiotic, prebiotic, and synbiotic were higher (81.7-90.0%) compared to positive control (68.3%) or there was an increase in SR about 19-32%.

Based on results of all stages in this study, it could be concluded that probiotic P. piscicida 1Ub, prebiotic MOS, and a combination of the two (synbiotic) could improve growth performance, immune response and resistance of Pacific white shrimp against the infection of V. harveyi with the best results obtained in the administration of synbiotic.

Keywords: P. piscicida 1Ub, MOS, synbiotic, Pacific white shrimp larvae, V. harveyi