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Full Length Research Paper

Effect of beneficial bacteria on larval culture of Pacific whiteleg shrimp, *Litopenaeus vannamei*

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Beneficial microorganisms isolated from the gut of adult shrimp, *Litopenaeus vannamei* and adult Cortez oyster, *Crassostrea corteziensis* was tested to determine larval survival of whiteleg shrimp *L. vannamei*. The isolates were characterized through tests for hemolytic and antagonism to select potential beneficial strains. Strains YC5-8, Y02-1, YC5-1 and YPD10-4 were selected from *in vitro* assays and evaluated in larval cultures at 1 × 10^5 CFU/ml. *Pseudomonas aeruginosa* (strain YC5-8) and *Burkholderia cepacia* (strain Y02-1) were selected and two subsequent bioassays were performed to evaluate these two strains on shrimp larvae. At the first bioassay, two concentrations of the strains were used: 1 × 10^4 and 1 × 10^6 CFU/ml. During the second trial, two mixed concentrations (1:1) and an antibiotic were tested: 1 × 10^4 CFU/ml; 1 × 10^5 CFU/ml; oxytetracycline at 4 mg/l. A significant increase in larval survival following treatment with YC5-8 and YO2-1 at 1 × 10^4 and 1 × 10^6 CFU/ml was registered. Shrimp larvae exposed to mixed strains and oxytetracycline also had significantly higher survival than the control group.

Key words: *Pseudomonas* sp., *Burkholderia* sp., *Litopenaeus vannamei*, larvae, probiotics.

INTRODUCTION

Pacific whiteleg shrimp (*Litopenaeus vannamei*) is widely cultivated in Central and South America (Burge et al., 2007). In hatcheries, rigorous cleaning and sanitation are required. Careful control of water temperature, salinity, pH, optimization of stocking densities and balanced nutrition are also very important to improve survival (Inglis, 1996). However, the shrimp industry suffers from repeated appearance of diseases that affect the sustainability of aquaculture (Jory, 1998). In shrimp larviculture, diseases are mainly caused by *Vibrio* species: *Vibrio anguillarum*, *Vibrio parahaemolyticus*, *Vibrio alginolyticus*, *Vibrio harveyi*, *Vibrio cambelli* and *Vibrio penaeicida* (Garriques and Arevalo, 1995; Rattanama et al., 2009). Treatment of shrimp suspected of being infected with *Vibrio* is mainly based on the use of antibiotics and chemotherapeutics, but the susceptibility of *Vibrio* to antibiotics varies widely, even among strains of the same species of microorganisms (Soto-Rodríguez et al., 2008). Antibiotics have been used in Latin America and Southeast Asia, where there are few restrictions on these products (Gomez-Gil et al., 2000). Antibiotics commonly used to avoid major outbreaks of pathogens in

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aquaculture are furazolidone, chloramphenicol, streptomycin, erythromycin, kanamycin, oxytetracycline, neomycin and oxolinic acid (Benbroock, 2002). As resistant strains becoming more prevalent and difficult to treat, alternative methods of controlling the microbial environment are investigated (Zokaeifar et al., 2012a). One of the methods gaining recognition for controlling pathogens within the aquaculture industry is the use of beneficial microorganisms (Irianto and Austin, 2002). The use of probiotics to manipulate the microbiota in larval cultivation have become increasingly popular (Gomez-Gil et al., 2000; Vine et al., 2006; Silva et al., 2011; Nimrat et al., 2011, 2012), and are gaining interest as an environmentally safe alternative to antibiotics and chemotherapeutics (Song et al., 1997).

Probiotics have been described as live microorganism food supplement that improve the microbial balance of the host intestinal flora (Vine et al., 2006) and also provide health benefit to the host (Crittenden et al., 2005; Vieira et al., 2007; Kongnum and Hongpattarakere, 2012). Probiotics might be useful in controlling microbial infections through competition with harmful microorganisms, production of inhibitory compounds, or by stimulition of the immune system of the host (Bachère, 2003; Zokaeifar et al., 2012b). Beneficial effects of probiotics include growth, survival and feed efficiencies (Venkat et al., 2004). Probiotics used in aquaculture studies include Gram positive and negative bacteria, bacteriophages, yeast and unicellular algae (Irianto and Austin, 2002). *Bacillus* spp. used as probiotics in aquaculture (Decamp et al., 2008) includes *Lactobacillus* spp., *Vibrio* spp., and *Pseudomonas* spp. (Kesarkodi-Watson et al., 2008). *Bacillus* constitutes a diverse group of rod-shaped, Gram-positive bacteria characterized by their ability to produce spores (Ge et al., 2004; Ninawe and Selvin, 2009). They possess adhesion abilities, produce antimicrobial peptides and provide immuno-stimulation (Coffman and Britigan, 1990; Britigan et al., 1992; Mashburn-Warren et al., 2009). The aim of this study was to evaluate the effect of benefic bacteria, isolated from the gut of two marine invertebrates of commercial importance, on *L. vannamei* larvae.

MATERIALS AND METHODS

Bacterial isolates from shrimp and oyster

Bacterial strains were isolated from the intestinal tract of 10 live adult shrimp (*L. vannamei*) and from 10 live adult oysters (*C. corteziensis*). The isolation of benefic microorganisms from marine invertebrates is usually able to grow at high temperatures and salinities. The samples were homogenized and serially diluted, plated on yeast, peptone, dextrose agar (YPD broth, #2216, Difco: Becton, Dickenson and Company, Franklin Lakes, NJ) and incubated at 30°C for 24 to 48 h. Colonies of single and rod-shaped were selected and re-streaked onto marine agar to obtain pure cultures after incubation for 24 h at 30°C. Of these, 44 strains were selected and stored at -85°C in YPD broth containing 2.5% (w/v) NaCl and 15% (v/v) glycerol until used in the experiments.

Hemolytic activity

To select the non pathogenic strains, the hemolytic test was performed (Chin et al., 2000). To measure hemolytic activity, 44 rod bacteria were inoculated by streaking on plates containing blood-based agar (#211728, Difco) supplemented with 5% (w/v) human sterile blood and 3% (w/v) NaCl. Plates were incubated at 30°C for 24 h (Koneman et al., 2001). The hemolytic activity was expressed as follows: α-hemolysis (slight destruction of erythrocytes with a green zone around the bacterial colonies); β-hemolysis (hemolysin that causes a clean zone of hemolysis around the bacterial colonies); and γ-hemolysis (without any change in the agar around the bacterial colonies).

Pathogenic strains

Pathogenic bacterial strains *Vibrio alginolyticus* CAIM 57 and *V. parahaemolyticus* CAIM 170 were obtained from the Colección de Microorganismos de Importancia (CAIM, www.ciad.mx/caim). Strains were maintained in trypticase soy broth (#236950, Difco) containing 3% (w/v) NaCl and 15% (v/v) of glycerol at -80°C until used.

Antagonism test

The 44 potential probiotic strains and two pathogenic strains previously cryopreserved at -80°C were thawed. Each bacterial strain was cultured in 10 ml of YPD at 30°C for 24 h. Samples were centrifuged at 5000 ×*g* for 10 min; each pellet was re-suspended in a sterile saline solution containing 3% w/v NaCl. Density of bacteria was measured by spectrophotometry (DU 640, Beckman Coulter, Brea, CA) at 600 nm. Optical density was adjusted to 1.0 to obtain a final density of 1×10^9 cells/ml; this inoculum was serially diluted to a density of 1×10^6 cells/ml for *in vitro* antagonism tests (Dopazo et al., 1988). For this test, 10 µl of each suspension of bacteria were blotted on the surface of trypticase soy agar (TSA) + NaCl medium (TSA containing 3% w/v) NaCl (S-7653, Sigma, St. Louis, MO) and then incubated for 24 h at 37°C. The plates were then placed in a closed chamber and exposed to chloroform vapors for 45 min to kill the bacteria. Each plate was covered with 6 ml TSA + NaCl medium containing a 0.1 ml suspension of either *V. alginolyticus* CAIM 57 or *V. parahaemolyticus* CAIM 170. Plates were examined after incubation at 30°C for 24 h. Strains showing an inhibition zone at least 5 mm were considered positive for the test, strains producing smaller inhibition zones were considered as sensitive without total inhibition. Control plates to test the potential effect of the chloroform on the growth of the target bacteria were also included.

Salinity tolerance of the isolated strains

The selected strains were exposed to saline stress at different concentrations of NaCl: 0, 1, 3, 6, 8 and 10% (Lightner, 1996).

Survival of shrimp larvae cultivated with probiotics

The first experiment determined the effect of four probiotic strains selected from *in vitro* assays (YC5-8, YPD10-4 and YC5-1 from shrimp and YOI2-1 from oyster). Groups of 225 nauplii/l were inoculated daily after exchange of water with one isolate at a final density of 1×10^5 CFU/ml (Guo et al., 2009). A control group consisted of shrimp not exposed to probiotics. The treated groups were cultured in triplicate. The experiment started at nauplii V stage and finished when the shrimp reached the postlarvae-1 stage; final

Table 1. Test for antagonism of potential probiotic isolates against pathogenic *Vibrio* strains, hemolytic activity, and salinity tolerance.

Train	Inhibition zone (mm)		Salinity (%)						Hemolytic activity	Metabolism
	V. alginolyticus CAIM 57	*V. parahaemolyticus* CAIM 170	0	1	3	6	8	10	Erythrocytes	
YOI2-1	13	19	+	+	+	+			α	Facultative
YC5-8	21	9	+	+	+	+			NR	Facultative
YC5-1	10	9	+	+	+	+	+	+	β	Facultative
YPD10-4	5.5	20	+	+	+				β	Facultative

α, β = hemolytic; NR = negative response to the test.

survival was recorded. The second experiment included the strains YC5-8 and YOI2-1 at two densities in the tanks: 1×10^4 and 1×10^6 CFU/ml. The control group was maintained without probiotics. The treatments were performed in triplicate, starting at nauplii V (225 nauplii/l). Survival was recorded every 24 h until the end of the experiment, when larvae reached the postlarvae-1 stage. The third experiment consisted of Mix-1 (YC5-8 and YOI2-1; 1:1 w/w) at 1×10^4 CFU/ml and Mix-2 (YC5-8 and YOI2-1; 1:1 proportion w/w) at 1×10^5 CFU/ml, oxytetracycline (#05750, Sigma) at 4 mg/l, and a control group. Every tank contained 225 nauplii/l, the treatments were performed in triplicate. Survival was recorded every 24 h until the end of the experiment, when larvae reached the postlarvae-1 stage.

Identification of the probiotics strains

Bacterial strains were previously identified by the BIOLOG identification system (Campa-Córdova et al., 2011). This method is based on the exchange of electrons generated during respiration, and tests the ability of a microorganism to oxidize a panel of 95 different carbon sources. The BIOLOG MicroPlates were read between 24 and 72 h following inoculation with a pre-grown isolate.

Statistical analysis

One-way ANOVA was assessed for significant differences in survival (%) among treatments. Survival data were arcsine transformed. Where significant ANOVA differences were found, Tukey's HSD test was used to identify the nature of these differences at $P < 0.05$. Statistical analyses were performed using software (Statistica 6.0, StatSoft, Tulsa, OK).

RESULTS

Bacterial isolates and selected strains

We isolated 44 morphologically different colonies from *L. vannamei* and *C. corteziensis*; strains with cytotoxic activity and low tolerance to salinity were discarded. From antagonism tests against *V. alginolyticus* and *V. parahaemolyticus*, four strains were selected (Table 1), three from shrimp (YC5-8, YPD10-4 and YC5-1) and one from oyster (YOI2-1).

Larval survival cultured with probiotics

Figure 1 shows the survival of shrimp larvae exposed to YC5-8, YPD10-4, YC5-1 and YOI2-1 at a single concentration of 1×10^5 CFU/ml. The larvae exposed daily to YOI2-1, YPD10-4 and YC5-8 had significantly ($P < 0.05$) higher survival than the control group. Larvae exposed to YOI2-1 had the highest survival (30.35%), followed by YPD10-4 (27.8%), YC5-8 (20.32%), control (8.94 %) and YC5-1 (8.14%). Although, the treatment YPD10-4 had higher survival than the control, the larvae showed physical deformities. Thus, the treatment was discarded for subsequent assays.

Effect of probiotic density on survival

Larvae inoculated with YOI2-1 at a density of 1×10^4 and 1×10^6 CFU/ml induced significantly ($P < 0.05$) higher survival than the control (Figure 2). Shrimp larvae cultivated with YC5-8 at two different densities (1×10^4 and 1×10^6 CFU/ml) had significantly ($P < 0.05$) better survival than the control group (Figure 3). The results did not show significant ($P > 0.05$) survival between both doses (Figures 2 and 3).

Effect of mixed probiotics on the larval survival

The results of the third bioassay are shown in Figure 4. MIX-1 (1×10^4 CFU/ml), MIX-2 (1×10^5 CFU/ml) and oxytetracycline (4 mg/l) had significantly higher survival ($P < 0.05$) than the control group. The highest survival occurred with MIX-2 (50.15%), followed by MIX-1 (49.8%), and oxytetracycline (41.03%). Survival in the control was 20%. No significant ($P > 0.05$) differences between MIX-1 and MIX-2 were observed (Figure 4).

The selected strains YC5-8 (isolated from shrimp gut) and YOI2-1(isolated from oyster gut) were identified as *Burkholderia cepacia* and *Pseudomonas aeruginosa*, respectively.

DISCUSSION

In intensive aquatic systems, disease control plays a key role, where intimate relationships between bacteria and host occur. Fish and shellfish cultivated under poor

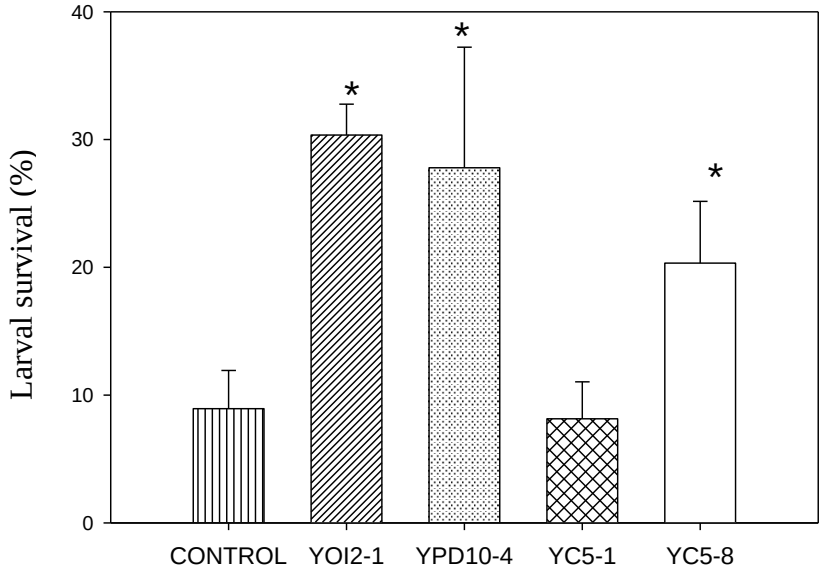

Figure 1. Larval survival of *L. vannamei* cultured during 9-day trial. Treatments were: (1) Control; (2) YOI2-1; (3) YPD10-4; (4) YC5-1; (5) YC5-8. Data are expressed as mean ± SD. ∗ = Significantly different from control. Concentration of each isolates 1×10^5 CFU/ml.

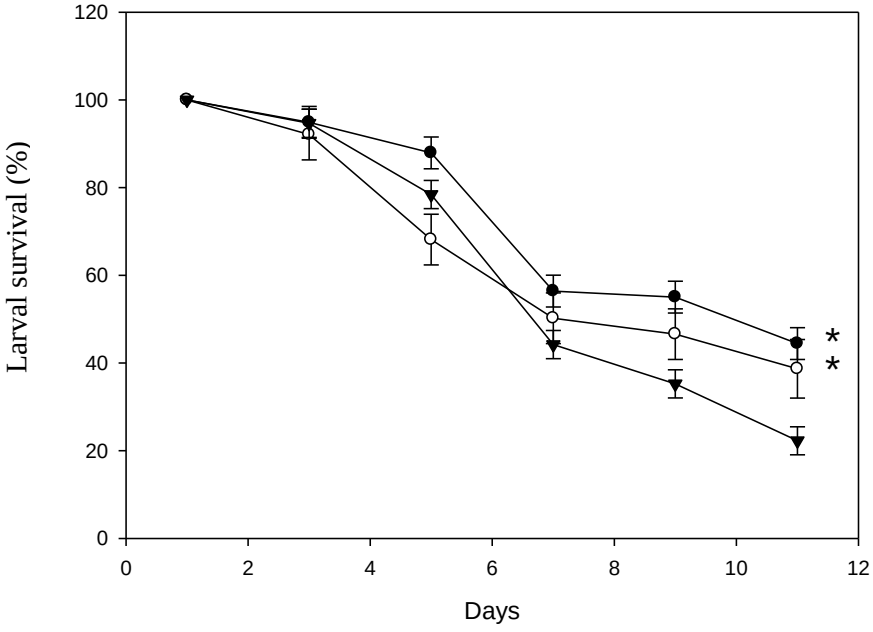

Figure 2. Larval survival of *L. vannamei* exposed to the strain YOI2-1 during 11-day trial at different concentrations: (1) Control (▼); (2) Y0I2-1 = concentration at 1×10^4 CFU/ml (●); and (3) YOI2-1 concentration at 1×10^6 CFU/ml (○). Data are expressed as mean ± SD. * = Significantly different from control.

environmental conditions become highly susceptible to bacterial and viral pathogens (Liu, 1990; Gibson et al., 1998; Luna-González et al., 2002).

The prophylactic use of probiotics in aquaculture prevents diseases (Riquelme et al., 1997; Das et al., 2006) and provides higher survival of shrimp larvae (Silva et al., 2011). Interest in marine probiotic bacteria is increasing for aquaculture purposes as an alternative to antibiotics (Lemos et al., 1985; Riquelme et al., 1997). Using bacteria isolated from the gut of local shrimp and

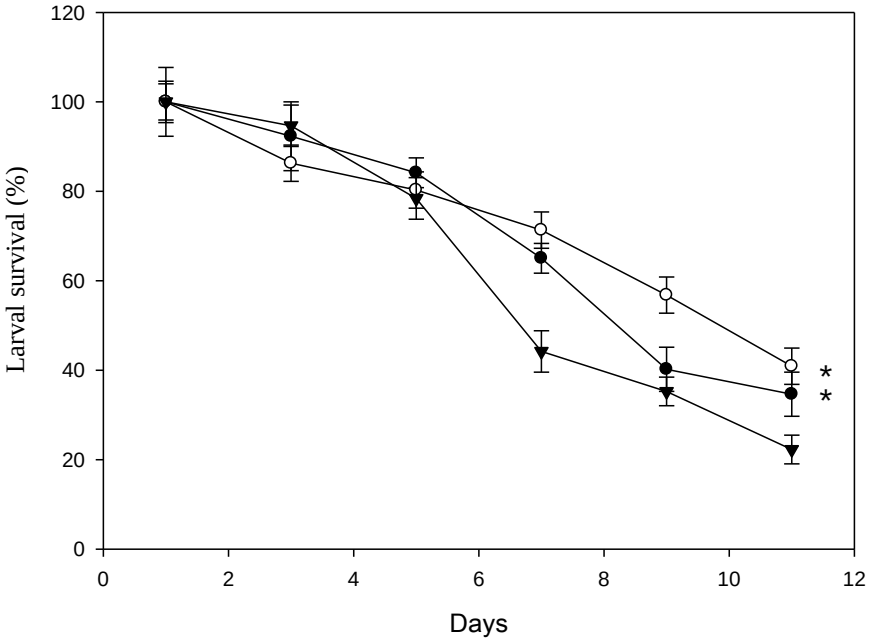

Figure 3. Larval survival of *L. vannamei* exposed to the strain YC5-8 at different concentrations: (1) Control (▼); (2) 1 × 10^4 CFU mL^{-1} (●); (3) 1 × 10^6 CFU/ml (○). Data are expressed as mean ± SD. * = Significantly different from control.

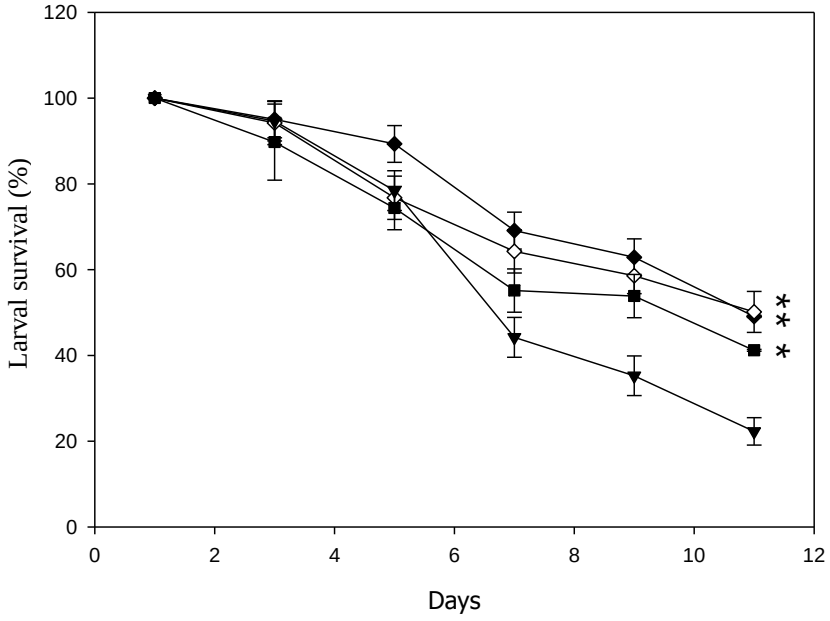

Figure 4. Larval survival of *L. vannamei* exposed to mixed strains. Treatment were: (1) Control (▼); (2) Mix 1 (YC5-8 + YOI2-1; 1:1 ratio, concentration of 1 ×10^4 CFU/ ml) (♦); (3) Mix 2 (YC5-8 + YOI2-1; 1:1 ratio, concentration of 1 × 10^5 CFU/ml) (◊); (4) oxytetracycline (OTX) at 4 mg/l (■). Data are expressed as mean ± SD. * = Significantly different from control.

oysters is an accepted strategy because the bacteria are adapted to the conditions in the intestinal tract of the marine host, offering better benefits than probiotics isolated from terrestrial animals. In this study, live probiotics applied by immersion in growing tanks at a daily single concentration at 1 × 10^5 CFU/ml increased survival

of *L. vannamei* larvae. Zhou et al. (2009) reports a beneficial effect in *L. vannamei* larvae after application of *Bacillus coagulants* strain SC8168. Ziaei-Nejad et al. (2006) recommend *Bacillus* spp. applied directly to the water or via *Artemia* spp. to improve survival of *Farfantepenaeus indicus* larvae.

Vibrio spp. and *Pseudomonas* spp. are commonly found in the intestinal microbiota of crustaceans (Moriarty, 1999). *Pseudomonas* spp. were studied as biological control agents against pathogens (Smith and Davey, 1993; Gram et al., 1999). The production of siderophore by *Pseudomonas* would be one of the important inhibiting agents of pathogens (Vijayan et al., 2006), and considering the higher protein levels reported in the *Pseudomonas* sp. (Hai et al., 2009), the bacterial biomass per se would serve as single cell protein as well. Vijayan et al. (2006) reported that in the beginning of the shrimp larval culture, *Pseudomonas* spp. dominates members of the family Vibrionaceae. Therefore, if we could introduce probiotic isolates that could exclude the vibrios from the culture system, it should be possible to improve larval survival. Moriarty (1998) and Verschuere et al. (2000), reported that *Bacillus* spp. are able to compete with other bacteria, such as *Vibrio*, for nutrients and space, but may also exclude other bacteria by producing antibiotics to increase their proportion in the gut flora of shrimp. In our study, the strongest antagonic activity against *V. alginolyticus* and *V. parahaemolyticus* was provided by the bacilli *P. aeruginosa* (YOI2-1) and *B. cepacia* (YC5-8). Torrento and Torres (1996) reported specific inhibition of *V. harveyi* by *P. aeruginosa*. Das et al. (2006), reported antagonic effect of *Pseudomonas* spp. against the fish pathogen *Aeromonas hydrophila*.

Some studies used *Pseudomonas* spp. as probiotics. Gram et al. (1999) observed *in vitro* inhibition of *V. anguillarum* by *P. fluorescens* and lower mortality in probiotic-treated rainbow trout *Oncorhynchus mykiss*. Riquelme et al. (1997, 2001) used *Pseudomonas* sp. strain 11 to induce resistance to bacterial infections and improve survival in larval cultivation of the South American *Argopecten purpuratus*. In our study, the increased larval survival shown by the exposure to the mixed strains (YOI2-1 and YC5-8 at 1×10^4 or 1×10^5 CFU/ml), may be caused by a synergic effect (Ruiz-Ponte et al., 1999; Irianto and Austin, 2002; Balcazar et al., 2003). Vine et al. (2006) recommend doses of probiotics from 1×10^4 to 1×10^6 CFU/ml in larvae cultivation tanks. Douillet and Langdon (1994) used bacteria strain CA2 at 1×10^5 CFU/ml to increase growth of *Crassostrea gigas* larvae. Peeters and Rodríguez (1999) used at 1×10^5 CFU/ml to avoid colonization of pathogen bacteria during cultivation of a probiotic bacterium of larval *L. vannamei*.

Prophylactic and therapeutic use of antibiotics has been used in commercial hatcheries for decades (Gatesoupe, 1989), but this appears to have led to resistance by pathogenic bacteria (Sahul Hameed et al., 2003). Oxytetracycline is a common antibiotic used in hatcheries to prevent vibriosis (Uno et al., 2010), but induces bacterial resistance after ten days of use (Abraham et al., 1997; Tendencia and de la Peña, 2001). In our study, the daily use of 4 mg/l of oxytetracycline during 11 days, showed a significant ($P < 0.05$) higher larval survival than control group; however, the larvae treated with oxytetracycline did not increase significantly ($P > 0.05$) the survival than larvae treated with probiotics. Thus, the results of this study showed that the prophylactic use of benefic microorganisms in larval culture of *L. vannamei*, may be an alternative treatment than the antibiotics.

For desired results, probiotics isolates used in aquaculture should not be pathogenic to the host and should support health (Moriarty, 1999). *P. aeruginosa* and *B. cepacia* isolates did not cause harmful effects on shrimp larvae at doses from 1×10^4 to 1×10^6 CFU/ml. However, larvae treated with the isolate YPD10-4 induced deformities and isolate YC5-1 did not improve survival. *In vitro* tests showed β-hemolysis for strains YPD10-4 and YC5-1 that caused lysis of shrimp hemocytes by bacterial hemolysins.

Conclusion

Survival of shrimp larvae was enhanced by probiotic strains YC5-8 and YO2-1 at doses of 1×10^4, 1×10^5 or 1×10^6 CFU/ml. Shrimp larvae exposed to MIX-1 (1×10^4 CFU/ml), or MIX-2 (1×10^5 CFU/ml), had higher survival than the control group.

This study showed the beneficial effect of *P. aeruginosa* (strain YOI2-1) and *B. cepacia* (strain YC5-8) during larval cultivation of *L. vannamei*.

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REFERENCES

Abraham TJ, Palaniappan R, Dhevendaran K (1997). Epibiotic infestation of luminous bacteria in penaeid shrimp *Penaeus indicus* (H. Milne Edwards) larvae. Indian J. Mar. Sci. 26:209-211.

Bachère E (2003). Anti-infectious immune effectors in marine invertebrates: potential tools for disease control in larviculture. Aquaculture 227:427–438.

Balcazar JL (2003). Evaluation of probiotic bacterial strains in *Litopenaeus vannamei*. Final report. National Center for Marine and Aquaculture Research, Guayaquil, Ecuador.

Benbroock CM (2002). Antibiotic drug use in U.S. aquaculture: *Information available on the World Wide Web,* http://www. iatp.org.

Britigan BE, Roeder TL, Rasmussen GT, Shasby DM, McCormick M, Cox CD (1992). Interaction of the *Pseudomonas aeruginosa* secretory products pyocyanin and pyochelin generates hydroxyl radical and causes synergistic damage to endothelial cells implications for *Pseudomonas* associated tissue injury. J. Clin. Invest. 90:2187−2196.

Burge EJ, Madigan DJ, Burnett LE, Burnett KG (2007). Lysozyme gene expression by hemocytes of Pacific white shrimp, *Litopenaeus vannamei*, after injection with *Vibrio*. Fish Shellfish Immunol. 22:327–339.

Campa-Córdova AI, Luna-González A, Mazón-Suástegui JM, Aguirre-Guzmán G, Ascencio F, González-Ocampo HA (2011). Effect of probiotic bacteria on survival and growth of Cortez oyster larvae *Crassostrea corteziensis* (Bivalvia:Ostreidae). Int. J. Trop. Biol. 59(1):183-191.

Chin-I C, Wen-Yu L, Chung-Zen S (2000). Use of prawn blood agar hemolysis to screen for bacteria pathogenic to cultured tiger prawns *Penaeus monodon*. Dis. Aquat. Organ. 43:153–157.

CoffmanTJ, Cox CD, Edeker BL, Britigan BE (1990). Possible role of bacterial siderophores in inflammation. Iron bound to the *Pseudomonas* siderophore pyochelin can function as a hydroxyl radical catalyst. J. Clin. Invest. 86:1030−1037.

Crittenden R, Bird AR, Gopal P, Henriksson A, Lee YK, Playne MJ (2005). Probiotic research in Australia, New Zealand and the Asia-Pacific Region. Curr. Pharm. Des. 11:37–53.

Das KD, Surya KS, Biswa RS, Satyanarayana S, Phalguni P, Bibhudendu KM (2006). Antagonistic activity of cellular components of *Pseudomonas* species againts *Aeromonas hydrophila*. Aquaculture 253:17–24.

Decamp O, Moriarty DJW, Lavens P (2008). Probiotics for shrimp larviculture: review of field data from Asia and Latin America. Aquac. Res. 39:334–338.

Dopazo C, Lemos M, Lodeiros C, Barja J, Toranzo A (1988). Inhibitory activity of antibiotic-producing bacteria against fish pathogens. J. Appl. Bacteriol. 65:97–101.

Douillet PA, Langdon CJ (1994). Use of a probiotic for the culture of larvae of the Pacific oyster (*Crassostrea gigas*, Thunberg). Aquaculture 119:25–40.

Garriques D, Arevalo G (1995). An evaluation of the production and use of a live bacterial isolate to manipulate the microbial flora in the commercial production of *Penaeus vannamei* postlarvae in Ecuador. 53-59 pp. In: Browdy CL, Hopkins JS (eds.), Swimming through troubled water. Proceedings of the Special Session on Shrimp Farming, Aquaculture 1995. W. Aquac. Soc. Baton Rouge, LA.

Gatesoupe FJ (1989). The effect of bacterial additives on the production rate and dietary value of rotifers as food for Japanese flounder, *Paralichthys olivaceus*. Aquaculture 83:39–44.

Ge Y, Huang Y, Wang S, Zhang X, Xu Y (2004). Phenazine-1-carboxylic acid is negatively regulated and pyoluteorin positively regulated by gac A in *Pseudomonas* sp. M18. FEMS Microbiol. 237:41–47.

Gibson LF, Woodworth J, George AM (1998). Probiotic activity of *Aeromonas media* on the Pacific oyster, *Crassostrea gigas*, when challenged with *Vibrio tubiashii*. Aquaculture 169(1-2):111–120.

Gram L, Melchiorsen J, Spanggard B, Huber I, Nielsen TF (1999). Inhibition of *Vibrio anguillarum* by *Pseudomonas fluorescens* AH2, a possible probiotic treatment of fish. Appl. Environ. Microbiol. 65:969–973.

Gómez-Gil B, Roque A, Turnbull JF (2000). The use and selection of probiotic bacteria for use in the culture of larval aquatic organisms. Aquaculture. 191:259–270.

Guo JJ, Liu KF, Cheng SH, Chang CI, Lay JJ, Hsu YO, Yang JY, Chen TI (2009). Selection of probiotic bacteria for use in shrimp larviculture. Aquac. Res. 40:609–618.

Hai NV, Buller N, Fotedar R (2009). Effects of probiotics (*Pseudomonas synxantha*, and *Pseudomonas aeruginosa*) on the growth, survival and immune parameters of juvenile western kings prawns (*Penaeus latisulcatus* Kishinouye, 1896). Aquac. Res. 40:590-602.

Inglis V (1996). Antibacterial Chemotherapy in Aquaculture: Review of Practice, Associated Risks and Need for Action. Institute of Aquaculture, University of Stirling, Stirling, Scotland.

Irianto A, Austin B (2002). Probiotics in Aquaculture. J. Fish Dis. 25:633–642.

Jory DE (1998). Use of probiotics in penaeid shrimp growout. Aquac. Magaz. 24(1):62-67.

Kesarcodi-Watson AH, Kaspar MJ, Lategan L, Gibson (2008). Probiotics in aquaculture: The need, principles and mechanisms of action and screening processes. Aquaculture 274:1–14.

Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC (2001). Diagnóstico Microbiológico. Editorial Médica Panamericana. México City. 1432 pp.

Kongnum K, Hongpattarakere T (2012). Effect of *Lactobacillus plantarum* isolated from digestive tract of wild shrimp on growth and survival of White shrimp (*Litopenaeus vannamei*) challenged with *Vibrio harveyi*. Fish Shellfish Immunol. 32:170–177.

Lemos M, Toranzo A, Barja J (1985). Antibiotic activity of epiphytic bacteria isolated from intertidal sea-weeds. Microb. Ecol. 11:149–163.

Lightner DV (1996). A handbook of pathology and diagnostic procedures for diseases of penaeid shrimp. World Aquac. Soc. Louisiana State University, Baton Rouge, LA.

Liu CI (1990). The diseases of cultured *Penaeus monodon* with emphasis on recent discoveries in Taiwan. In Kou GH, Wakabayashi H, Liao IC, Chen SN, Lo CF (eds.). Fish Dis. Proc. R. Jpn. Symp. Taipei, Taiwan. 180-201.

Luna-González A, Maeda-Martínez A, Ascencio-Valle F (2002). Comparative susceptibility of veliger larvae of four bivalve mollusks to a *Vibrio alginolyticus* strain. Dis. Aquat. Organ. 49:221–226.

Mashburn-Warren L, Howe J, Brandenburg K, Whiteley M (2009). Structural requirements of the *Pseudomonas* quinolone signal for membrane vesicle stimulation. J. Bacteriol. 191:3411–3414.

Moriarty DJW (1998). Control of luminous *Vibrio* species in penaeid aquaculture ponds. Aquaculture, 164:351–358.

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

Nimrat S, Boonthai T, Vuthiphandchai V (2011). Effects of probiotic forms, compositions of and mode of probiotic administration on rearing of Pacific white shrimp (*Litopenaeus vannamei*) larvae and postlarvae. Anim. Feed Sci. Tech., 169: 244–258.

Nimrat S, Suksawat S, Boonhtai T, Vuthiphandchai V (2012). Potential Bacillus probiotics enhance bacterial numbers, water quality and growth during early development of white shrimp (*Litopenaeus vannamei*). Vet. Microbiol. 159(3-4):443-450.

Ninawe AS, Selvin J (2009). Probiotics in shrimp aquaculture: avenues and challenges. Crit. Rev. Microbiol. 35:43-66.

Peeters M, Rodríguez J (1999). Problemas bacterianos en la industria camaronera Ecuatoriana, prácticas de manejo y alternativas de control. El Mundo Acuícola 5:13–18.

Rattanama P, Srinitiwarawong K, Thompson JR, Pomwised R, Supamattaya K, Vuddhakul V (2009). Shrimp pathogenicity, hemolysis, and the presence of hemolysin and TTSS genes in *Vibrio harveyi* isolated from Thailand. Dis. Aquat. Organ. 86:113–122.

Riquelme C, Araya R, Vergara N, Rojas A, Guaita M, Candia M (1997). Potencial probiotic strains in culture of the Chilean scallop *Argopecten purpuratus* (Lamarck, 1819). Aquaculture 154:17–26.

Riquelme C, Jonquera M, Rojas A, Avendaño R, Reyes N (2001). Addition of inhibitor-producing bacteria to mass culture of *Argopecten purpuratus* (Lamarck, 1819). Aquaculture, 192: 111–119.

Ruiz-Ponte C, Samain JF, Sanchez JL, Nicolas JL (1999). The benefit of a *Roseobacter* species on the survival of scallop larvae. Mar. Biotechnol. 1:52–59.

Sahul Hameed AS, Rahaman KH, Alagan A, Yoganandhan K (2003). Antibiotic resistance in bacteria isolated from hatchery-reared larvae and postlarvae of *Macrobrachium rosenbergii*. Aquaculture 217:39–48.

Silva EF, Soares MA, Calazans NF, Vogeley JL, do Valle BC, Soares R, Peixoto S (2011). Effect of probiotic (*Bacillus* spp.) addition during larvae and postlarvae culture of the white shrimp *Litopenaeus vannamei*. Aquac. Res. 42:1–9.

Smith P, Davey S (1993). Evidence for the competitive exclusion of *Aeromonas salmonicida* from fish with stress-inducible furunculosis

by a fluorescent pseudomonas. J. Fish Dis. 16:521–524.
Song YL, Liu JJ, Chan LC, Sung HH (1997). Glucan induced disease resistance in tiger shrimp (*Penaeus monodon*). Dev. Biol. Stand. 90:413–421.
Soto-Rodríguez S, Gómez-Gil B, Lozano R (2008). MICs de antibióticos de *Vibrio* spp. aislados del *L. vannamei* cultivado en México. Panorama Acuícola 14(1):52–57.
Tendencia EA, de la Peña LD (2001). Antibiotic resistance of bacteria from shrimp ponds. Aquaculture 195:193–204.
Torrento M, Torres J (1996). *In vitro* inhibition of *Vibrio harveyi* by *Pseudomonas* sp. isolated from aquatic environment. University of the Philippines Visayas. J. Nat. Sci. 1:130–138.
Uno K, Chaweepack T, Ruangpan L (2010). Pharmacokinetics and bioavailability of oxytetracyline in vannamei shrimp (*Penaeus vannamei*) and the effect of processing on the residues in muscle and shell. Aquacult. Int. 18:1003–1015.
Venkat HK, Shau NP, Jain KJ (2004). Effect on feeding *Lactobacillus*-based probiotics on the gut microflora, growth and survival of postlarvae of *Macrobrachium rosenbergii* (de Man). Aquac. Res. 35:501–507.
Verschuere L, Rombaut G, Sorgeloos P, Verstraete W (2000). Probiotic bacteria as biological control agents in aquaculture. Microbiol. Mol. Biol. Rev. 64:655–671.
Vieira FN, Pedrotti FS, Neto CCB, Mouriño JLP, Beltrame E, Martins ML (2007). Lactic-acid bacteria increase the survival of marine shrimp, *Litopenaeus vannamei*, after infection with *Vibrio harveyi*. Braz J. Oceanography 55:251–255.
Vijayan KK, Singh ISB, Jayaprakash NS, Alavandi SV, Pai SS, Preetha R, Rajan JJS, Santiago TC (2006). A brackishwater isolate of Pseudomonas PS-102, a potential antagonistic bacterium against pathogenic vibrios in penaeid and non-penaeid rearing systems. Aquaculture 251:192-200.
Vine NG, Leukes WD, Kaiser H (2006). Probiotics in marine larviculture. FEMS Microbiol. Rev. 30:404–427.
Zhou X, Wang Y, Li W (2009). Effect of probiotic on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities. Aquaculture 287:349–353.
Ziaei-Nejad S, Rezaei MH, Takami GA, Lovett DL, Mirvaghefi A, Shakouri M (2006). The effect of *Bacillus* spp. bacteria used as probiotics on digestive enzyme activity, survival and growth in the Indian white shrimp *Fenneropenaeus indicus*. Aquaculture 252:516–524.
Zokaeifar H, Balcázar JL, Kamarudin MS, Sijam K, Arshad A, Saad CR (2012a). Selection and identification of non-pathogenic bacteria isolated from fermented pickles with antagonic properties against two shrimp pathogens. J. antibiot. 65:289-294.
Zokaeifar H, Balcázar JL, Saad CR, Kamarudin MS, Sijam K, Arshad A, Nejat N (2012b). Effect of Bacillus subtilis on the growth performance, digestive enzymes, immune gene expression, and disease resistance of white shrimp, *Litopenaeus vannamei*. Fish Shellfish Immunol. 33:683-689.