Aquaculture Reports 11 (2018) 24–30

Contents lists available at ScienceDirect

Aquaculture Reports

journal homepage: www.elsevier.com/locate/aqrep

Effect of isolated bacteria and microalgae on the biofloc characteristics in the Pacific white shrimp culture

Juan M. Pacheco-Vega[a,*], Marco A. Cadena-Roa[b], Jesús A. Leyva-Flores[b], O. Iram Zavala-Leal[a], Elizabeth Pérez-Bravo[b], Javier M.J. Ruiz-Velazco[a]

[a] Escuela Nacional de Ingeniería Pesquera, Universidad Autónoma de Nayarit (UAN), Bahía de Matanchén Km. 12, Carretera a los Cocos, 63740 San Blas, Nayarit, Mexico

[b] Universidad Autónoma de Baja California Sur (UABCS), Unidad Pichilingue, Apartado Postal 19-B, 23080 La Paz, Baja California Sur, Mexico

ARTICLE INFO

Keywords:
Induced biofloc
B. plantarum
L. vannamei
Microalgae

ABSTRACT

At present, the Pacific white shrimp *Litopenaeus vannamei* Boone, 1931, is one of the species with the greatest potential to be reared under biofloc Technology (BFT). Although BFT presents promising culture methods, some of these require improvements in efficiency by the use of native microalgae and bacteria (BFNO) as an alternative to commercial organism probiotics (BFCO). This experiment was developed using a culture system for the shrimp *L. vannamei* to a stocking density of 400 in./m^3 for 44 days. *Schizochytrium* sp. and recently isolated *Latobacillus plantarum* (class: Bacilli), a probiotic strain (used as experimental biofloc), were incorporated to geomembrane tanks holding 28 m^3 seawater to evaluate if these presented bioregulating effects influencing water quality and growth performance of *L. vannamei*. These microorganisms were compared with the BFCO. Results showed that both promoted bioflocs maintained optimal concentrations of total ammonium, nitrites, and nitrates for *L. vannamei* cultures. However, the BFCO required to be supplied every 10 days, whereas the experimental biofloc only required an initial stocking and maintenance of sodium carbonate levels > 100 mg L^{-1} and pH > 7.5. Additionally, the load of *Vibrio* spp. in experimental biofloc was less than that observed for BFCO. Furthermore, the results of growth performance did not demonstrate differences between both treatments. Therefore, it is suggested that *Schizochytrium* sp. and the bacterial strain of *L. plantarum* should be used as initiator and basic constituents of the biofloc-based culture systems for *L. vannamei*, maintaining good water quality and avoiding periodical probiotic supplementation in cultures.

1. Introduction

In recent decades, several studies have focused on the design and development of a series of alternative production systems for the culture of diverse aquatic organisms. Such systems have the objective of reducing the use of water and space while simultaneously allowing increased stocking densities (Ray et al., 2011). One interesting example of this type of systems is denominated the biofloc technologic system - BFT, which consists in a water management that includes fostering the development of microbial flocs (bio-flocculated aggregates). Biofloc growth requires the addition of substrates that present high carbon:-nitrogen (C:N) ratio, little or null water replacement, and high oxygenation rates (Avnimelech, 1999; Emerenciano et al., 2013a). Formulated diets can be supplied to the system, but these must have low levels of crude protein as the supplementary contribution of biofloc is significant. External sources of carbon to promote bacterial growth in these culture systems come from molasses (sugar cane), rice, and wheat brans, among others.

Results from intensive and biofloc systems obtained in recent years (Monroy-Dosta et al., 2013; Schveitzer et al., 2013; Jiménez-Montealegre et al., 2015) indicate that biofilms, particularly autotrophic microorganisms and heterotrophic bacteria, tend to be important elements for water quality. Likewise, these can act as a source of protein that enhances shrimp growth, even replacing the use of *Artemia* nauplii for bioflocs to feed *L. vannamei* (Becerra-Dorame et al., 2011). In addition, the production of white shrimp in zero water exchange systems was achieved by a balance of bioflocs and their essential microminerals (Becerra-Dorame et al., 2011).

The overall dynamic of BFT results from ecological relationships (commensalism, competition, and predation, among others) that represent a trophic micronetwork comprised of rotifers, ciliates, heterotrophic bacteria, and microalgae (Collazos-Lasso and Arias-Castellanos,

Abbreviations: BFT, Biofloc technology; BFNO, Biofloc by inoculation of native organisms; BFCO, Biofloc by inoculation commercial organisms

* Corresponding author.
E-mail address: pachecovjm@yahoo.com.mx (J.M. Pacheco-Vega).

https://doi.org/10.1016/j.aqrep.2018.05.003
Received 25 April 2017; Received in revised form 22 May 2018; Accepted 23 May 2018

2015). The latter two groups tend to be the most abundant within the biofloc community. Nevertheless, the chemical structure of extracellular products of microalgae and bacteria are largely unknown and can act as growth inhibitors or stimulators in the trophic network depending on the species (Ribalet et al., 2008).

Some microalgae are highly nutritive and thus effectively used for live food enrichment in larviculture operations of several aquaculture species (Farhadian et al., 2008). Similarly, different species of bacteria are also nutrient-rich sources and have been previously included in aquaculture diets (Gamboa-Delgado and Marquez-Reyes, 2016); given their metabolic diversity, some bacteria have proved to be useful as water quality bioregulators in aquaculture systems. The microalgae–bacteria interaction is a process denominated by primary symbiosis, which has been the result of thousands of millions of years of microalgal–bacterial coevolution (Natrah et al., 2014). This association (microalgae–bacteria) has been evaluated to have an effective result for the control of *Vibrio harveyi* and other *Vibrio* spp. pathogens (Lio-Po et al., 2005; Decamp et al., 2008).

Diverse opportunistic bacteria are able to grow within these systems, among which the genus *Vibrio* spp. is prominent. Representatives of this genus are causing agents of the early mortality syndrome (EMS) or acute hepatopancreatic necrosis syndrome (AHPNS) found in the Pacific white shrimp *L. vannamei* (Leaño and Mohan, 2012). In an attempt to control these pathogens, current tendencies are oriented toward the search for probiotic bacteria that, in addition to their water quality bioregulatory functions, represent a useful tool in tackling these diseases and allow to avoid the use of drugs in shrimp cultures. At present, diverse microalgae and bacteria can be found in the market; however, the origin of several of the species remains unknown. The latter has led researchers to the pursuit of native microbiota found in *L. vannamei* culture areas. These microorganisms are expected to present greater efficiency (growth rate and removal of organic waste) in BFT due to their physiological adaptations to thrive under these particular culture conditions. Therefore, the objective of the present study was to evaluate the effect of a microalgal strain, native to the Gulf of California and a probiotic bacterial strain, isolated from the digestive tract of shrimp on water quality and production parameters for shrimp grown in outdoor biofloc. This culture system was compared with another biofloc system using commercial probiotic bacteria.

2. Materials and methods

2.1. Biological material and experimental design

This study was carried out from May to August 2015 in the Unidad Pichilingue at Universidad Autónoma de Baja California Sur, Mexico. The experiment was conducted in triplicate (n = 6) in 40,000 L high-density polypropylene tanks filled up to an operative volume of 28,000 L, respectively. Units were individually equipped with aeration systems. For the development of microbiota whit inoculation of native organisms (BFNO), bacteria and microalgae were added 8 days before the beginning of the experiment. Stocking consisted in inoculating *Schizochytrium* sp. (microalgae) and *Lactobacillus plantarum* (Lactic-Acid Bacteria, key T19). The microalgal strain was isolated from Bahia de La Paz in the Mexican state of Baja California Sur (located in the Gulf of California), whereas the probiotic bacteria were isolated from the digestive tract of shrimps. A symbiotic relationship was previously determined and evaluated between both species (Hernández Castro and Pacheco-Vega, 2015). *Schizochytrium* sp. cultures were scaled up using F/2 medium (Guillard, 1975). Probiotic bacteria were cultured in Man-Rogosa-Sharpe medium (MRS; Difco) broth and, from a volume of > 500 mL, MRS medium was replaced by modified F/2: micronutrient F/2 (Guillard, 1975- Proline), sugarcane molasses (2.5 g L^{-1}), and sodium citrate (1.17 g L^{-1}). Each tank was incubated with 800 L of microalgal culture and 100 L of probiotic bacterial culture at a density of 20×10^3 cells mL^{-1} and 1×10^8 colony-forming units (CFU) mL^{-1}, respectively. Micronutrients of the F (Guillard and Ryther, 1962) medium equivalent to F/14 (medium nutrient concentration) and sugarcane molasses 17 g/m^3 ratio (as carbon source) were added every other day. Regarding the second treatment, the microbiota stared whit commercial organisms (BFCO) bacterol-shrimp composed of 14 species of bacteria: *Bacillus* spp., *Lactobacillus* spp., *Streptococcus* spp. as well as nitrifying and photosynthetic bacteria, was reactivated following the manufacturer's guidelines by adding 25 mg/m^3 and resupplying every 10 days on average during the shrimp culture. The system underwent a maturation process of 8 days before shrimp stocking.

2.2. Shrimp stocking and tank management

Shrimp seeds of *L. vannamei* for stocking were obtained from Acuacultura Mahr Hatchery, Baja California Sur, Mexico. The post-larvae were acclimatized in two 13,000-L tanks for 3 weeks before stocking the experimental tanks. Shrimps weighing 420 ± 0.10 mg were stocked at a density of 400 shrimp/m^3, which was maintained until the end of the experiment. Shrimp were fed eight times a day (every 3 h) with a 35% crude protein commercial diet (Nutrimentos Acuicolas Azteca®, Guadalajara, Jalisco, Mexico; 35% protein) at a rate of 10% of the estimated standing biomass at the beginning, and reduced to 6% toward the end of the experiment. The tank was fully covered by a shade cloth to reach an approximate sunlight reduction of 80%. Each tank was fertilized once a day using sugarcane molasses as carbon source, in accordance with Avnimelech (1999). The balance between carbon and nitrogen (C:N) was maintained at a ratio of 15:1. Shrimp (individual mean) weight (100 shrimp per tank) was monitored on a weekly basis to determine shrimp growth, the amount of food and organic carbon provided was adjusted according to Avnimelech (1999).

2.3. Assessment of water quality parameters

Water temperature and oxygen content were measured every 3 h in each tank using an oxymeter (YSI 55). The following were evaluated every third day: nonionized ammonium, nitrite, nitrate, and sodium carbonate with a spectrophotometer (YSI 9500) and pH using a potentiometer (HANNA, Hi 98127). Measurements were taken from one randomly chosen tank per treatment every day. Sodium carbonate (Na_2CO_3) was applied to maintain alkalinity levels between 100 and 150 mg L^{-1} and pH of > 7.5 in the experimental units. Ten percent of the water was exchanged every 10 days to maintain salinity levels at < 40 and to compensate for losses due to evaporation.

2.4. Bromatological analysis of biofloc particles

Biofloc samples were sequentially collected from the tanks: at the beginning of the experiment, on day 22, and at the end of the experiment. Samples were collected by passing tank water through 30-μm-size mesh and washed with distilled water to remove excess soluble salts. The concentrated biofloc samples from each tank were dried in an oven at 85 °C until constant weight and then stored at 5 °C. Samples were analyzed for protein, carbohydrate, and lipid contents, according to the standard methods proposed by Lowry et al. (1951), DuBois et al. (1956), and Bligh and Dyer (1959) and modified by Chiaverini (1972), respectively. For ash content determination, 1 g of ground biofloc was burnt in a muffle furnace at 480 °C for 24 h. The residue or ash content was weighed, and its percentage was calculated.

2.5. Microbiological evaluation

Biotic communities of lactic acid bacteria (LAB) and *Vibrio* spp. were evaluated every 4 days. Water samples were separately taken and plated on agar MRS (for lactic-acid bacteria) and thiosulfate citrate bile salts sucrose agar (TCBS, for *Vibrio* spp.) and incubated at 30 °C for 24 h. After this period, the colonies were counted to estimate the