Aquaculture 489 (2018) 85–95

Contents lists available at ScienceDirect

Aquaculture

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

Administration of purple nonsulfur bacteria as single cell protein by mixing with shrimp feed to enhance growth, immune response and survival in white shrimp (*Litopenaeus vannamei*) cultivation

Supaporn Chumpol[a], Duangporn Kantachote[a,*], Teruhiko Nitoda[b], Hiroshi Kanzaki[b]

[a] Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat Yai 90112, Thailand
[b] Graduate School of Environmental and Life Science, Okayama University, 1-1-1 Tsushima-Naka, Kita-Ku, Okayama 700-8530, Japan

ARTICLE INFO

Keywords:
Growth performance
Immune response
Purple nonsulfur bacteria
Shrimp
Single cell protein
Water quality

ABSTRACT

Single cell protein (SCP) is an alternative way to increase nutrients for animal consumption; and purple nonsulfur bacteria (PNSB) should be considered as SCP due to their rich sources of protein, vitamins and photopigments. Hence, the aim of this study was to investigate the potential of promising PNSB to be used as SCP by mixing with commercial shrimp feed for white shrimp cultivation starting from postlarval until early juvenile stages for 60 days. PNSB strains, *Rhodobacter sphaeroides* SS15 and *Afifella marina* STW181 were selected, based on high amounts of biologically complete protein and photopigments, to use as SCP at a ratio of 1:1; and their lyophilized cells at 1, 3 and 5% (w/w) were mixed well with commercial shrimp feed to obtain modified shrimp feed recipes; Diet 1, Diet 2 and Diet 3, respectively. Levels of NH_4^+, NO_2^-, NO_3^- and COD in rearing water from Diet 2 and 3 sets were significantly higher than control set. However, Diet 1 set showed the lowest levels of these water parameters among modified diet sets as no significant difference levels of NO_3^- and COD between Diet 1 and control sets. Shrimp growth performance on the basis of relative gain rate and other growth parameters found that Diet 1 set was much better than sets of Diet 2, Diet 3 and control with the lowest found in the control set. In addition, the maximum shrimp survival was observed in Diet 1 set (85%) although no significant difference among them as 80% in control set. No significant difference was found among control and all modified shrimp diets for total hemocyte count in shrimp; however, significant increases of superoxide dismutase activity found in sets of Diet 1, while phenoloxidase activity found in Diet 2 and Diet 3. The results of hepatopancreas (HP) histopathology analysis also showed a good condition of HP as healthy shrimp. PNSB biomass as SCP at optimal level has the potential to be an effective source of a novel protein in shrimp feed to enhance shrimp growth and also to increase shrimp survival as more nutritious with no effect on water quality.

1. Introduction

Aquaculture is one of the important sources of food, nutrition, and livelihoods for hundreds of million people around the world. Among animal species cultured in aquaculture, shrimp production is second ranked of the most-traded production in these decades; whereas fishery production ranks first. Asia's shrimp farms show fast growing for supporting people demand, although they face with a decline in output because of shrimp diseases (FAO, 2016). Recently, productions of shrimp broodstock and shrimp larvae are characterized by the universal use of a combination of live and/ or processed natural feed items, including marine polychaetes, hermit crabs, Artemia biomass, edible insects, oysters, and formulated dry (FAO, 2013; Tacon, 2017). Despite the widespread use of live and fresh unprocessed shrimp feeds, but there are many diseases that can transmit to shrimp through the use of raw materials such as hermit crabs present a risk factor for white spot syndrome virus (WSSV) through vertical virus transmission to shrimp cultivation because hermit crab is a common natural host of WSSV (Chang et al., 2012). For the risk of shrimp diseases from the current use of live feeds, it is clear that the commercial shrimp feed manufacturing industry must step up to produce nutritionally complete formulated feeds for the entire shrimp production cycle, from first feeding larvae to maturing broodstock as replacement for the use of live feeds (Tacon, 2017).

Shrimp feed consists of protein, lipid and carbohydrate as a major component with a minor component of vitamin, mineral and water; and shrimp requires 35–55% protein in their feed (Goddard, 1996). Shrimp growth in each stage requires high protein diet although at different

* Corresponding author.
E-mail addresses: duangporn.k@psu.ac.th (D. Kantachote), nitoda@cc.okayama-u.ac.jp (T. Nitoda), hkanzaki@cc.okayama-u.ac.jp (H. Kanzaki).

https://doi.org/10.1016/j.aquaculture.2018.02.009
Received 27 September 2017; Received in revised form 26 January 2018; Accepted 4 February 2018
Available online 06 February 2018
0044-8486/

concentrations such as 48% for a juvenile stage and only 32% for a sub-adult stage of *Litopenaeus vannamei* (Kureshy and Davis, 2002). The low protein content in shrimp feed causes low growth rate and weight loss because protein in muscle is used as no sufficient for protein in feed; while high protein content, shrimp has an excessive protein that is used for supplying energy and eliminating nitrogen in a form of ammonia/ ammonium ion in rearing water (Goddard, 1996). The high amount of biologically complete protein is required in shrimp feed which leads to high cost as its source normally originate from fish meal protein rather than plant protein; thereby, exploring sources of protein for producing inexpensive shrimp feed with high efficiency to enhance shrimp growth has been extensively studied.

According to above information, microbes should be considered to use as a protein source in order to replace the sources of common protein in shrimp feed as their high protein consists of essential amino acids (EAA) including vitamins. Single cell protein (SCP) is the protein produced from biomass, originating from many species of micro-organisms, including algae, fungi and bacteria (Ravinda, 2000). Among microbes, bacteria are the most attractive for use as a source of SCP because they rapidly grow in various substrates such as by-products from agro-industry and wastewaters. Purple nonsulfur bacteria (PNSB) can be utilized as SCP because they are nutritious and non-toxic to host (Azad et al., 2002). Their cells are not only high in biologically complete protein but also in photopigments and vitamins (Kantachote et al., 2005; Kornochalert et al., 2014; Chumpol et al., 2017a). As versatile organisms, PNSB are able to grow in a variety of growth modes such as photoautotroph/ photoheterotroph under anaerobic/ microaerobic-light conditions and heterotroph under aerobic-dark conditions (Kornochalert et al., 2014); so it would be possible to produce mass biomass from PNSB for using as SCP with low cost.

Water quality during shrimp cultivation is a very important factor to concern for a design diet formulation which approaches to balance feed utilization and feed wastage for healthy shrimp and also an environmentally friendly maintenance (Cho et al., 1994). It is well recognized that health status of shrimp depends on rearing water (Zhang et al., 2016; Chumpol et al., 2017a, 2017b) as inappropriate water quality directly causes low growth rate, stressful and weak shrimp that stimulate pathogenic infection and high mortality (Goddard, 1996). Shrimps are invertebrate animals that depend on innate immune responses to combat invading microbes and lack of an adaptive immune system of no lymphocytes and functional immunoglobulin (Rowley and Powell, 2007). Major immune reactions of shrimp take place in hemolymph as several immune molecules are produced and stored in the granules of hemocyte before releasing into the hemolymph such as phenoloxidase (PO), antimicrobial peptides (AMPs) and superoxide dismutase (SOD) (Tassankajon et al., 2013). It would be great to induce strong innate defense mechanisms for resistance to pathogens on the basis of immunostimulants by bacterial preparation in the dietary feed (Wang et al., 2017). Therefore, this study aimed to investigate the possibility of promising PNSB to be used as SCP by mixing with commercial shrimp feed at different levels and their effects on water quality, growth performance and immunity of white shrimp (*L. vannamei*) cultivation.

2. Materials and methods

2.1. Bacterial strains used and culture preparation

To investigate PNSB for considering as a potential SCP, PNSB used in this study included *Rhodobacter sphaeroides* strains; SS15, S3 W10, TKW17 and *Afifella marina* STW181. Regarding our previous studies, they were isolated from shrimp ponds in the south of Thailand and were proved for their probiotic PNSB (Chumpol et al., 2017a, 2017b). In this study, these PNSB were tested as a source of protein for expecting a novel shrimp feed to provide another choice for using promising PNSB for shrimp cultivation. Each PNSB strain was separately cultured in duran bottles containing 500 mL of basic isolation medium (BIM + 1.5% NaCl) for *R. sphaeroides* and glutamate acetate medium (GA + 2% NaCl) for *A. marina* (Chumpol et al., 2017a) and incubated under microaerobic-light conditions with tungsten light at 3500 lx for 48 h. Each culture broth was centrifuged at 10418 ×*g* for 10 min (Sorval, RC 5C plus, USA) to obtain a cell pellet and then washed three times with 0.85% normal saline solution (NSS), and finally lyophilized to obtain dried cells (lyophilized cells) for the use as SCP in this study.

2.2. Proximate analysis of PNSB and shrimp feeds

Pure culture of each PNSB strain was determined its proximate analysis including crude protein, crude lipid, crude fiber, moisture and ash using standard methods (AOAC, 2010). Crude protein was analyzed using Kjeldahl method; while crude fat was analyzed by the Soxhlet extraction method. Fiber analyzer (Anikom^{200}, USA) was used to analyze crude fiber. Moisture content was analyzed by drying at 135 ± 2 °C; and ash was analyzed by burning at 550–600 °C. Carbohydrate content was calculated by deduction from a total 100% of nutrient contents (protein + fat + moisture + ash). The different shrimp feeds, commercial shrimp feed (TNT 4, Charoen Pokphand Foods) as a control and a mixture of control with different amounts of PNSB addition, were investigated for their proximate analysis according to the methods as previously described. Among 3 strains of *R. sphaeroides* only one strain that showed maximal protein content was selected for the analysis of its amino acid profile. However, *A. marina* STW181 was also selected to analyze the amino acid composition as it might have a different amino acid profile from *R. sphaeroides*.

2.3. Amino acid profiles of selected PNSB

Each PNSB was grown as previously mentioned in Section 2.1. The amino acid analysis was carried out by using 0.2 g of lyophilized cells to digest by adding 5 mL 6 N HCl and incubated at 110 °C for 22–24 h under vacuum. The hydrolyzed sample was adjusted to 10 mL by 0.1 N HCl and filtered through Whatman No. 40, and finally diluted with sodium citrate buffer (0.07 M, pH 2.2). A 20 μL of sample solution was injected to HPLC-LC-10ADvp (model LC-20A Series, Shimadzu, Japan, Shim-pack ISC-07/S 1504 Na) by following the conditions according to Kantachote et al. (2016).

2.4. Pigment analysis of PNSB

PNSB were cultured under microaerobic-light conditions for 48 h to obtain cell pellets as previously described in Section 2.1. Each cell pellet collected from 10 mL of culture broth was washed twice with distilled water to remove residual medium and it was used for bacteriochlorophyll *a* (Bchl *a*) extraction using 1 mL acetone/methanol solvent (7:2, v/v). The mixture was left for 1 h at room temperature in dark; and Bchl *a* was measured by UV–visible spectrophotometer (Genesys 10S, Thermo Scientific) at a wavelength of 771 nm. The amount of Bchl *a* was calculated according to the following formula; Bchl *a* content (mg L^{-1}) = (ADV_1 / $76V_2$) where A: the absorbance of Bchl *a* in diluted extract solution at 771 nm, D: the dilution ratio, V_1: the volume of extracted solvent added, V_2: the volume of PNSB culture broth (Zhou et al., 2014). The determination of extracted carotenoids was also investigated by acetone solvent as acetone was added in a tube and ultrasonic power was set at 390 W for 6 min to break cells, and the tube was kept in a water bath, 20 °C for 10 min. The mixture was centrifuged at 10,418 ×*g* for 10 min; and the supernatant was used to determine the content of total carotenoids using UV–visible spectrophotometer at 480 nm with the formula as follows. Carotenoids yield (mg L^{-1}) = ADV_1 / $0.16V_2$, where A: the absorbance of diluted extract solution at 480 nm, D: the dilution ratio, V_1: the volume of acetone added, 0.16 is extinction coefficient of carotenoids, and V_2: the volume of PNSB culture broth (Zhang and Hu, 2015).