

Supplementation of fish processing wastes in an enriched culture media promotes growth, biomass, protein and carotenoid production of *Rhodobacter sphaeroides*

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ABSTRACT

This study describes the development of an enriched culture media for growth, biomass and carotenoid production of the photosynthetic bacteria *Rhodobacter sphaeroides* PSB1 through supplementation of fish processing wastes in the Acetate Yeast Extract (AYE) medium. Proximate compositions of the fish processing wastes were determined prior to supplementation. Cultures were incubated and analyzed for growth, biomass and carotenoid production after 14 days. Results showed that addition of fish viscera yielded significantly higher ($P < 0.05$) growth ($2.75 \times 10^5 \text{ ml}^{-1}$ cells), protein content ($71.37 \mu\text{g mL}^{-1}$) and carotenoid production (22.11 mg L^{-1}) than supplementation of fish frames and control which may be attributed to the high protein and lipid content of the viscera. Biomass production was however higher in cultures supplemented with fish frames (22.67 g L^{-1}) which showed higher ash content than the viscera. Findings of this study showed that fish processing wastes may be used as supplemental nutrient source to promote the growth, biomass, protein and carotenoid production of *R. sphaeroides*, an industrially-important photosynthetic bacterium.

KEYWORDS:

Photosynthetic bacteria, *Rhodobacter sphaeroides*, fish processing wastes, biomass, carotenoid

INTRODUCTION

Photosynthetic bacteria are Gram-negative prokaryotes that use light as energy source which is converted into chemical energy by chlorophyll or bacteriochlorophyll syntheses. They are mostly found in freshwater, saltwater, aquaculture and wastewater ponds, lakes, lagoons, marine coastal sediments, moist soils and paddy fields (Banerjee et al. 2000). These phototrophs are widely applied in bioremediation and biodegradation, production of biofuels, cosmetics or natural medicines since these bacteria are able to take in carbon dioxide, regulate photosynthetic nitrogen and they can be grown on different wastes (Choi et al. 2002; Paronyan and Gasparyan, 2009; Amezaga et al. 2014; Kis et al. 2015). This group of microorganisms include oxygenic and anoxygenic phototrophic bacteria which have various species containing several types of (bacterio) chlorophylls and different carotenoids that are

commonly used in industry as food coloring agents, additives and precursors of vitamins, astaxanthin and β -carotene (Barredo et al. 2017).

Rhodobacter sphaeroides, a facultative phototrophic bacteria contains abundant amount of proteins, vitamins and amino acids that are essential for fish feeding thus production of its biomass is desirable (Kuo et al. 2012; Prachanurak et al. 2014). Biomass of *R. sphaeroides* SS15 and *Afifella marina* STW181 as Single Cell Protein (SCP) at 1% inclusion has shown potential as alternative protein source in shrimp feed as it enhanced growth and increased the survival (Chumpol et al. 2018). In another study, it was shown that these bacteria contain antivibrio compounds inhibiting various shrimp pathogenic *Vibrio* spp. under the conditions of shrimp farming (Chumpol et al. 2019). *R. sphaeroides* was also reported to contain antimicrobial agents, fatty acids like pantothenic acid and biopolymers and their bacterial cells have physiologically active substances that are used as probiotics acting as a disease-

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prevention agent that is widely used in aquaculture production (Kuo et al. 2012; Zhou et al. 2015). The strain *R. sphaeroides* P47 produced 75 µg/g dry cells of Vitamin B₁₂ which has been used to treat anemia and neuritis, and as an eye lotion. It also produced about 10-100 mg/L of porphyrin that is used as medicine for liver diseases, cancer diagnosis and cancer treatment Sasaki et al. (2005).

Different studies have demonstrated the use of *R. sphaeroides* in bioremediation of Lead-contaminated soil (Li et al. 2016), in degradation of organophosphorus pesticides in soil (Wu et al. 2019), and in the reduction of phosphorus content from the mud sediment of an oyster farm (Takeno et al. 1999). Furthermore, *R. sphaeroides* are also known to produce carotenoids that can be used as feed supplement. A carotenoid product, Lycogen™ from *R. sphaeroides* mutant strain WL-APD911 supplemented at 1%, improved the growth performance of red tilapia (*Oreochromis mossambicus* x *Oreochromis niloticus*) by significantly increasing muscle weight, weight gain, specific growth rate (SGR) and feed conversion ratio (FCR) (Chiu and Liu, 2014).

One advantage of utilizing *R. sphaeroides* for various industries is that they can grow directly in high organic load wastewater. A study has indicated that a strain of PSB grown in sago-starch effluent diluted in basal mineral (BM) medium produced high cell dry weight after eight days of incubation (Getha et al. 1998). Rice straw broth was also shown as an appropriate isolating medium than the commercial Glutamate-Acetate (GA) medium for purple nonsulfur bacteria as it was found to have more nutrients and is cheaper (Nunkaew et al. 2012). Moreover, previous studies also showed that the use of agro-industrial wastes are considered best substrates to improve the bacterial growth, biomass and production of microbial enzymes since they are rich in carbon and nitrogen sources.

There are published studies that utilized processing wastes or by-products in optimizing the growth of photosynthetic bacteria (Azad et al. 2003; Paronyan and Gasparyan, 2009; Kamble, 2016). Fish processing by-products accounting for the 25% of fish production (Rebah and Miled, 2013) can be a potential candidate as culture media supplement for photosynthetic bacteria. Solid fish wastes containing skin, bones, heads, entrails, liver, gonads and muscle tissues are rich sources of lipids, minerals and proteins.

In a study conducted by Caigoy et al. (2016), addition of fish processing wastes to Basic I media showed promising results in improving the cell growth, biomass and protein production of photosynthetic bacteria. In the present study, fish processing wastes were characterized in terms of its proximate composition and added to Acetate Yeast Extract (AYE) media where PSB growth was faster based on a preliminary study and is cheaper than the Basic I media which contains expensive growth factor and trace element solutions. The wastes served as supplemental nutrient source for growing the facultative phototroph *R. sphaeroides*. The objective is to find a low cost nutrient source to improve the growth, biomass, protein and carotenoid production of this PSB *R. sphaeroides* strain that may significant to various fields such as in agriculture and aquaculture in terms of feed supplement for animals, agents of pollution removal, and for other industrial applications.

MATERIALS AND METHODS

Photosynthetic Bacterial Strain

Rhodobacter sphaeroides PSB1 used in this study was isolated from fish processing wastes by Caigoy et al. (2016). The strain belonging to Class Proteobacteria was identified using biochemical tests used by Del Socorro et al. 2013 including gelatin liquefaction and citrate, glucose, starch, mannitol and sorbitol utilization tests and 16s rRNA gene analysis. Specifically, it is a Gram-negative, purple non-sulfur bacterium (PNSB) classified under the Class Alphaproteobacteria and Family Rhodobacteraceae.

Fish processing waste preparation

Wastes from processing of the red mangrove snapper *Lutjanus argentimaculatus* was obtained from the Miagao Public Market right after they were removed from the fresh fish during cleaning. The collected wastes were then placed in ice and transported to the Fish Processing Laboratory of the Institute of Fish Processing Technology, Miagao, Iloilo. They were then sorted according to different parts such as heads, tails, fins and viscera. Heads, tails, and fins hereon referred to as fish frames, were combined together for single preparation, while the viscera were processed separately. The wastes were converted into powders using the method of Ellouz et al. (2001) with some modifications. Briefly, samples were cooked, minced, and dried at 80°C for about 24-

48 h. The dried fish waste preparation was ground using a hammer mill (Ika-Werk) and the fine powders were sieved with a nominal mesh aperture of 180 microns. The sifted powders from the two groups of fish processing wastes were stored in sealed plastic bags for proximate analysis and for supplementation into the PSB culture medium.

Media preparation

The base culture medium used was Acetate-Yeast Extract (AYE) composed of K_2HPO_4 (1.0 g L⁻¹), $MgSO_4$ (0.2 g L⁻¹), $CaCl_2$ (0.02 g L⁻¹), $Na_2S_2O_3$ (0.10 g L⁻¹), Na-Acetate (2.2 g L⁻¹), Yeast Extract (4.0 g L⁻¹) and was added with fish processing wastes at 20g L⁻¹. It was prepared in one liter distilled water, pH adjusted within 6.6-7.0, sterilized using autoclave at 121°C (15 psi) for 15 min. and then stored at ambient temperature (26-31°C) until use.

Proximate composition of fish processing wastes

The method for proximate analysis was based on standard procedures found in the manual of the Association of Official Analytical Chemists (AOAC 1990). The moisture content was determined by oven method which involved drying the samples at 102 ± 3 °C until constant weight. Ash content was determined by furnace method through heating the dried samples at 500 ± 40°C and incinerated until they are free from black carbon particles. Total nitrogen content was determined using Kjeldahl method wherein samples were digested with concentrated H₂SO₄ in the presence of Hg to convert all the N present to (NH₄)₂SO₄. The samples were then added by excess NaOH to liberate NH₃ from the digest and steam distillation of NH₃ produced into saturated boric acid. Subsequently, the liberated NH₃ was determined by titration to the endpoint with standard HCl. Estimated crude protein was obtained by multiplying the total nitrogen content by 6.25. The lipid content of dried samples was analyzed by Soxhlet (Mishra 2017) extraction method using petroleum ether.

Culture and growth condition of R. sphaeroides PSB1

The starter culture was obtained in a sterile 50-mL glass tube containing the AYE medium, aseptically inoculated with colonies and incubated under blue led for 24 hours. Sterile AYE broth (250 mL) were added with 2.0% (w/v) viscera part or fish frame powders and were placed in scintillation vials. Exactly 500 µL of 24 h culture with an initial density of approx. 5.1 x 10³ cells mL⁻¹ was inoculated to each vial and were anaerobically incubated under continuous

illumination for 14 days at 37°C under LED blue bulbs (3.53 V, 0.28 A, 470 nm) (Kuo et al. 2012). AYE broth without the nutrient source served as the control. The treatments were set-up in three replicates. After the 14-day incubation, cell growth, biomass, carotenoid and soluble protein content were determined.

Determination of cell counts

Cell counts in all treatments containing 2% of fish processing wastes were counted microscopically by the use of haemocytometer. In each treatment, 1 mL of the culture was added to Eppendorf tube and stained with 100 µL methylene blue for 3 min. Then, the total amount of cells in four squares was counted using the light compound microscope. The average of the number of cells was computed and expressed as cells per milliliter (cells mL⁻¹) using the formula (Mather and Roberts, 1998).

Determination of biomass production

Measurement of PSB biomass was determined using conventional oven method based on the study of Li and Mira de Orduña (2010). Samples of 5 mL wet biomass each from the treatments were added to pre-dried (105°C in oven, overnight) and pre-weighed aluminum pans and then dried overnight in convection oven at 105°C. Constant weights of the dried samples were obtained by repeated drying, cooling in the dessicator and weighing. Biomass were expressed as dry weight per liter (g L⁻¹) of PSB culture.

Determination of soluble protein content

The protein content in PSB culture grown in the different media were quantified using the Lowry method (Dulekgurgen 2004). In this method, 0.5 mL of the 14-day culture was transferred into a 10-mL glass tube and added with 0.7 mL Lowry reagent. Then the mixtures were cooled in room temperature for 20 min. About 0.1 mL of the diluted Folin Reagent was added to each tube. The mixtures were incubated for 30 min under the same condition. Thereafter, 1.3 mL of the solution was transferred into glass cuvettes and the absorbance was determined at 750 nm using UV-Vis spectrophotometer (Cary 60 UV-Vis). Protein content was calculated based on the standard curve using Bovine Serum Albumin as standard.

Protein production attributed to PSB from the different treatments were determined by subtracting the initial soluble protein content of AYE media with supplemental nutrient sources from the final soluble protein content after 14 days of incubation.

Determination of carotenoid content

Carotenoid production in the different treatments were determined using the method of Gu et al. (2008). The collected (40 mL) wet biomass from the cultures were placed in 50-mL tube and centrifuged at 10,000 rpm for 20 min. The collected residue was washed twice with distilled water, placed in beakers and freeze-dried (Labconco) for 48 h at -50°C and <1 Pa pressure. Carotenoid was extracted through HCl-assisted extraction procedure with some modifications. The dried biomass was soaked into 3 mol L^{-1} HCl solution at 28°C , shake at 100 rpm for 20 min using a vortex mixer, transferred to micro centrifuge tubes and centrifuged at 10,000 rpm for 10 min. The supernatant was then removed and acetone was added. The extraction was performed by shaking at 100 rpm for 20 min at 28°C . The extracts were then transferred to fresh tubes and centrifuged to obtain the supernatant (10,000 rpm, 10 min). Thereafter, absorbance was determined using a UV-Vis spectrophotometer at 480 nm after dilution. Total carotenoid (TC) yield was calculated using the following equation: where TC represents the total carotenoid yield (mg L^{-1} culture liquid), A is the absorbance value at 480 nm, D is the dilution ratio, V_1 is the acetone's volume added, 0.16 indicates the carotenoid's extinction coefficient and V_2 is the volume of the culture (Deming et al. 2006).

Statistical analysis

Statistical analyses used in the study were Levene's test (Test for Homogeneity) to verify the assumption of equal variances and one-way Analysis of Variance (ANOVA) to determine significant differences among treatments in proximate composition of fish processing wastes, PSB biomass, soluble protein

content and cell growth. Duncan's Multiple Range Test was used for statistical significance. All analyses were set at 95% confidence level.

RESULTS

Proximate composition of fish processing wastes

The proximate composition of the fish processing wastes used for enriched media supplementation are shown in Table 1. The dried fish viscera and fish frames powder have moisture content of 6.52% and 6.95%, respectively. Ash content in fish frames of about 44.61%, was significantly higher ($P<0.05$) than fish viscera which was only 4.83%. Both the lipid (19.04%) and crude protein content (60.04%) in fish viscera were significantly higher ($P<0.05$) than those found in fish frames.

Growth and biomass production

Fig. 1 shows the cell count obtained in the cultures of photosynthetic bacteria grown on media supplemented with different fish processing wastes. Results showed that supplementation of fish viscera and fish frames produced higher cell counts of 2.75×10^5 cells ml^{-1} and 2.14×10^5 cells ml^{-1} , respectively than the control. No statistical difference ($P>0.05$) was found between the two waste-supplemented treatments. Cultures grown in media with fish viscera however showed significantly higher ($P<0.05$) cell density than in the control (1.37×10^5 cells ml^{-1}).

Biomass production of PSB from enriched culture media supplemented with different fish processing wastes are shown in Fig. 2. The culture media supplemented with fish frames (head, tails, and fins) and fish viscera produced significantly higher ($P<0.05$) amount of PSB biomass of 22.67 g L^{-1} and

Table 1. Proximate composition of the fish processing waste powders used as supplemental nutrient source.

Supplemental nutrient source	Proximate composition (%) [*]			
	Moisture	Ash	Lipid	Crude protein
Fish viscera	6.52±0.08 ^a	4.83±0.61 ^a	19.04±0.13 ^b	60.04±2.46 ^b
Fish frames	6.95±0.10 ^a	44.61±0.43 ^b	4.64±0.14 ^a	40.20±0.76 ^a

^{*}Proximate composition (%) ± SD; Different letters indicate values are significantly different ($p<0.05$) for each nutrient source [T-test].

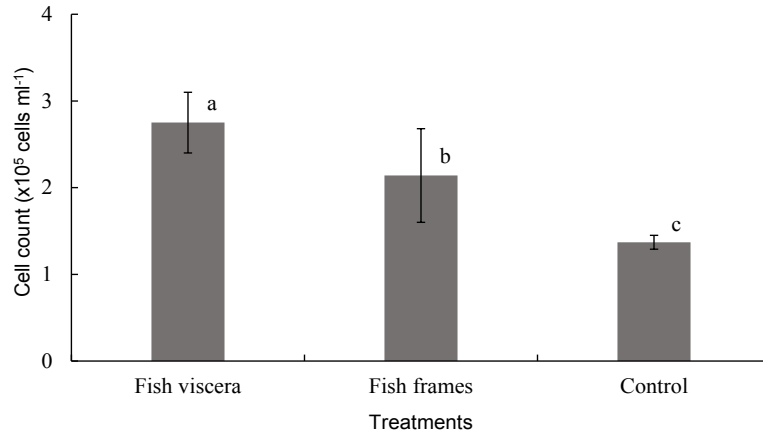


Figure 1. Cell count (\pm SD) of *R. sphaeroides* grown in AYE media with and without supplementation of fish processing wastes after 14 days. Different letters indicate values are significantly different ($p < 0.05$) for each nutrient source [Duncan's Multiple Range Test].

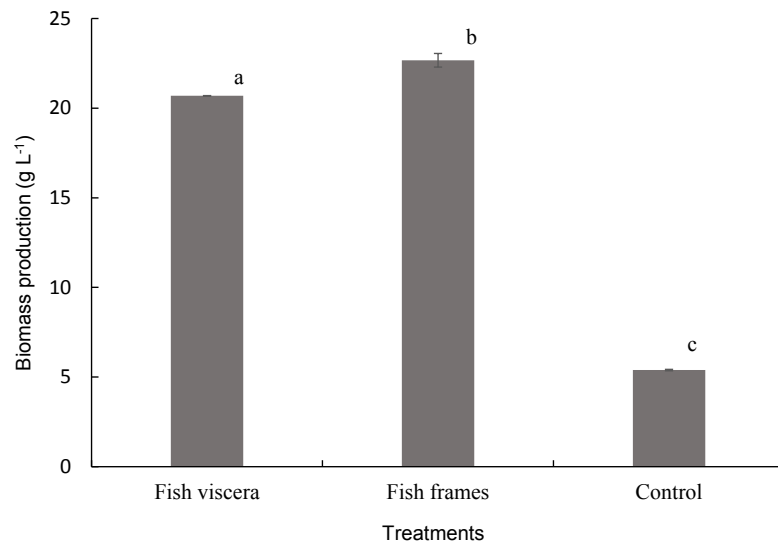


Figure 2. Biomass production (\pm SD) of *R. sphaeroides* grown in AYE media with and without supplementation of fish processing wastes after 14 days. Different letters indicate values are significantly different ($p < 0.05$) for each nutrient source [Duncan's Multiple Range Test].

20.69 g L⁻¹, respectively, compared to the culture media without supplementation.

Soluble protein production

Results of protein content determination (Fig. 3) showed that the supplementation of fish processing wastes enhanced the protein production of the PSB. The supplementation of fish viscera produced significantly highest protein (71.37 μ g ml⁻¹) ($P < 0.05$) compared to the cultures supplemented with fish

frames (10.87 μ g ml⁻¹) and without supplementation (7.88 μ g ml⁻¹).

Carotenoid production

Determination of carotenoid content of PSB cultures revealed that carotenoid was only produced in the presence of fish viscera and fish frames in the AYE media at 22.11 mg L⁻¹ and 2.32 mg L⁻¹, respectively (Table 2). Carotenoid was not detected in the control after the 14-day culture may be due

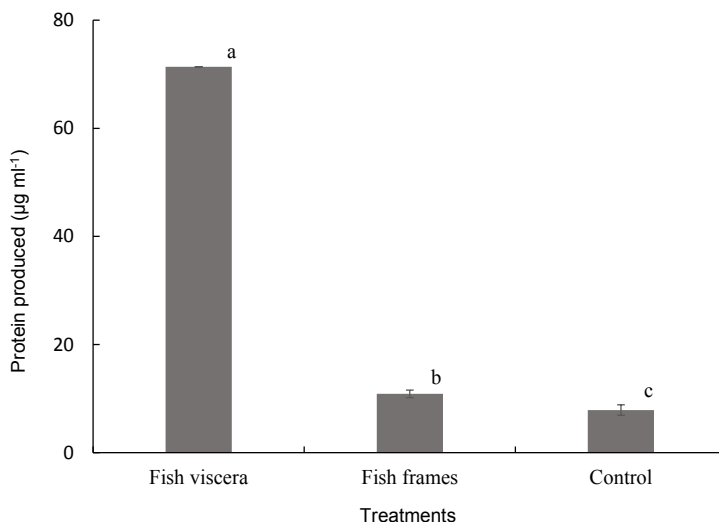


Figure 3. Soluble protein production (\pm SD) of *R. sphaeroides* grown in AYE media with and without supplementation of fish processing wastes after 14 days. Different letters indicate values are significantly different ($p < 0.05$) for each nutrient source [Duncan's Multiple Range Test]; n.d.-not detected.

Table 2. Carotenoid production of *R. sphaeroides* grown in AYE media with and without supplementation of fish processing wastes after 14 days.

Supplemental nutrient source	Carotenoid production (mg L ⁻¹)*
Fish viscera	22.11 \pm 7.69 ^b
Fish frames	2.32 \pm 0.99 ^a
None (Control)	n.d.

*Carotenoid produced \pm SD; Different letters indicate values are significantly different ($p < 0.05$) for each nutrient source [T-test]; n.d.-not detected

to possible inhibiting factor of some components present in the medium. Other studies indicated that AYE media enriched with soil samples only showed presence of carotenoids at the peaks of 475 nm and 525 nm (Montano et al. 2009) and results observed in isolated bacteria grown in this media exhibited an orange-color photosynthetic pigment which denoted the presence of carotenoids (Del Socorro et al. 2013). On the other hand, several studies have shown that carotenoid production in basal medium were observed to be 3.02 $\mu\text{g mL}^{-1}$ (Bhosale and Bernstein, 2004) and 12.438 mg/L (Chen et al. 2006). Supplementation of fish viscera produced significantly higher ($P < 0.05$) amount of carotenoid compared to the culture media supplemented with fish frames.

DISCUSSION

In the present study, *R. sphaeroides* were grown photoheterotrophically wherein cultures incubated under anaerobic/light condition appeared dark brown and reddish-brown after 14 days of incubation. This result is similar to the findings of Getha et al. (1998), wherein the PSB cultures also appeared reddish-brown.

AYE medium contains several nutrients and minerals that could enhance the growth of the PSB as it grows best in medium containing different carbon sources such as acetate, pyruvate, malate, glucose, soluble starch and citrate (Del Socorro et al. 2013). The suitability of using AYE medium for growing PSB

can be mainly attributed to acetate acting as the major carbon source along with glutamate and yeast extract as supplemental carbon and nitrogen sources. It is common to PSB to metabolize acetate because it is a major product from anaerobic digestion and an essential source of carbon for Purple Nonsulfur Bacteria (PNSB) (Nunkaew et al. 2012). Potassium, magnesium and sodium thiosulfate in the medium are necessary for the formation of lipids and nucleic acids, for bacteriochlorophyll synthesis and for the photosynthetic system (Heyes and Hunter, 2009).

In the present study, development of enriched culture media through supplementation of fish processing wastes in AYE medium enhanced the growth and metabolite production in *Rhodobacter sphaeroides* PSB1. High cell counts obtained from cultures containing fish viscera may be due to the high protein content of the powdered viscera, providing the bacteria with additional carbon and nitrogen sources for growth and reproduction (Ghaly et al. 2013). Addition of fish frames also resulted in high cell count, possibly due to the high mineral content which may have provided inorganic nutrients for the growth of the bacteria. Magnesium and sodium which can usually be found abundant in fish bones are important for the production of bacteriochlorophyll and photosynthetic pigments. Presence of these elements in a culture medium has also been shown to improve the growth of the PSB *Rhodospseudomonas palustris* strain B1 cultured in sago effluent medium where significantly higher cell dry weight of 0.84 g L⁻¹ was obtained compared to those grown in basal mineral (BM) medium only (Getha et al. 1998). Similarly, wastewater supplemented with cuttlefish wastes containing high protein enhanced the growth of *Bacillus cereus* BG1 (Rebah and Miled, 2013). The study of Vazquez et al. (2008) also showed that peptones from cartilagenous fish produced the high biomass which lead to high specific rate of $\mu_{mn} = 8.73 \text{ h}^{-1}$ in *Pediococcus acidilactici*. Fish processing wastes including both fish head and viscera also served as the best substrates for the protease synthesis by *Bacillus subtilis* (Ellouz et al. 2001).

Results of the present study showed that biomass production of *R. sphaeroides* in medium containing fish frames produced was the highest among the treatments. Fish bones comprising a vital 10-15% the total fish biomass are rich in calcium and phosphorus, collagen proteins and some carbohydrates and lipids, which are essential for bacterial growth (Toppe et al. 2007). In this study, proximate composition revealed

that fish frames have 44.61% ash suggesting high mineral content which may consist of calcium, phosphorus and hydroxyapatite. Calcium helps in the formation of "enzoskeleton" which significantly plays a role in cell division in bacteria (Onoda et al. 2000) while phosphorus was shown to promote the biomass of bacteria and phytoplankton in a lake with high nutrient level (Kisand et al. 2001). Ellouz et al. (2001) showed that fish substrates containing higher bone content with more minerals and less protein enhanced the biomass production of *B. subtilis*.

Results of the present study also showed that the medium containing fish viscera, which has higher crude protein and lipid than the fish frames, produced significantly higher ($P < 0.05$) biomass than the control. This result is in agreement with a previous study showing that residual waters such as in poultry slaughterhouse containing high amounts of organic materials such as lipids, proteins and suspended solids produced higher PSB biomass after 72 h compared to those grown in treated wastewater medium and Pfenning's synthetic liquid medium (Ponsano et al. 2007).

R. sphaeroides PSB 1 grown in medium containing fish viscera resulted in significantly highest protein content ($P < 0.05$) in this study. Previous studies have also shown that fish by-products including viscera and heads can be used as microbial growth media, for biomass and enzyme production. Culture media with combined viscera and heads allowed the cultivation of *Pseudomonas aeruginosa* and *Bacillus subtilis* in an acceptable level (Rebah and Miled, 2013). The high protein content in fish peptone became an excellent nitrogen source for the growth of *Lactobacillus sakei* (Aspmo et al. 2005). In a study by Prachanurak et al. (2014) *Rhodobacter palustris* and *R. capsulatus* showed high protein production when cultured in a medium added with wastewater. Soluble protein content in PSB culture supplemented with fish frames was not high mainly due to the low protein content of the powdered bones.

Supplementation of fish processing wastes also enhanced carotenoid production of *R. sphaeroides* in the present study. Carotenoid is an active component and auxiliary light-harvesting pigment in PSB when there is a weak exposure to light and functions as photo protector when exposed to high light intensity (Zhou et al. 2014). It is a known antioxidant used for variety of purposes such as food and pharmaceutical applications. It is therefore worthwhile to look at

this industrial potential of the PSB. In this study, carotenoid production in medium supplemented with fish viscera showed the highest carotenoid yield. The high protein content of fish viscera could also possibly explain the increased carotenoid production since it has been shown that sucrose and glucose which are products of protein metabolism has contributed to the carotenoid production of *Rhodotorula* spp. (Chandi and Gill, 2011; Mata-Gomez et al. 2014). In cultures with fish frames, lower carotenoid production of PSB was observed. It is possible that too much influx of minerals can inhibit the production of carotenoid. Bhosale and Bernstein (2004) reported that several inorganic salts like Zn^{2+} and Mn^{2+} inhibit hydroxylation process, growth and carotenoid production of *Flavobacterium*. Furthermore, in the study of Hui et al. 2015, the different carbon sources present in the medium affects the growth and production of carotenoids and bacteriochlorophyll. It was believed that these photosynthetic molecules might undergone degradation or differentiation throughout the incubation process. In addition, sodium carbonate was reported that few only use it as a nutrient source and it had never enhanced a carotenoid production (Bhosale and Bernstein, 2004). A study also shown that the carotenoid production of these bacteria is influenced by the composition of the medium and also by the speed, aeration and temperature. Likewise, nitrogen and carbon sources, inorganic salts, metal ions and chemical agents produce in either higher or lower amount of carotenoids (Cardoso et al. 2017).

Rhodobacter sphaeroides is an essential photosynthetic bacteria in terms of economic, agricultural, industrial and environmental purposes (Getha et al. 1998; Madukasi et al. 2011; Kim et al. 2013; Caigoy et al. 2016). It possesses capacity for various metabolic processes such as photosynthesis, lithotrophy, and aerobic and anaerobic respiration. Thus, it is logical that this species can be grown abundantly in different waste products or in medium that are cheap but contain excessive amounts of nutrients. This study showed that the developed enriched culture media supplemented with fish processing wastes enhanced its biomass and carotenoid production. PSB culture medium supplemented with fish viscera produced the highest carotenoid production and cell growth, while supplementation of fish frames resulted in the highest biomass. Thus this study has demonstrated that fish processing by-products may be used as a cheaper and effective supplemental nutrient sources for the

growth of industrially-important microorganisms such as PSB.

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LITERATURE CITED

- Amezaga JM, Amtmann A, Biggs CA, Bond T, Gandy CJ, Honsbein A, Karunakaran E, Laton L, Madsen MA, Minas K, Templeton MR. 2014. Biodesalination: A Case Study for Applications of Photosynthetic Bacteria in Water Treatment. *Plant Physiology*. 164: 1661-1676.
- Aspmo SI, Horn SJ, Eijsink VGH. 2005. Hydrolysates from Atlantic cod (*Gadus morhua* L.) viscera as components of microbial growth media. *Process Biochemistry*. 40: 3714-3722.
- Azad SA, Vikineswary S, Chong VC, Ramachandran KB. 2003. *Rhodovulum sulphidophilum* in the treatment and utilization of sardine processing wastewater. *Lett. Appl. Microb*. 38: 13-18.
- Banerjee S, Azad SA, Vikineswary S, Selvaraj OS, Mukherjee TK. 2000. Phototrophic Bacteria as Fish Feed Supplement. *Asian-Aus J. Anim. Sci*. 13 (7): 991-994.
- Barredo JL, Garcia-Estrada C, Kosalkova K, Barreiro C. 2017. Biosynthesis of Astaxanthin as a Main Carotenoid in the Heterobasidiomycetous Yeast *Xanthophyllomyces dendrorhous*. *Journal of Fungi*. 3 (44): 1-17.
- Bhosale P, Bernstein P. 2004. b-Carotene production by *Flavobacterium multivorum* in the presence of inorganic salts and urea. *J. Ind Microbiol Biotechnol*. 31: 565-571.
- Bunch AW. 1994. High Cell Density Growth of Micro-organisms. *Biotechnology and Genetic Engineering Reviews*. 12: 535-562.
- Caigoy JC, Nuñal S, Berco R. 2016. Isolation and identification of photosynthetic bacteria (PSB) from fish processing waste water and its biomass production using supplemental nutrient sources. *Phil J of Nat Sci*. 21 (1): 37-47.

- Cardoso L, Kanno K, Karp S. 2017. Microbial production of carotenoids- A review. African Journal of Biotechnology. 16 (4): 139-146.
- Chandi G, Gill B. 2011. Production and characterization of microbial carotenoids as an alternative to synthetic colors: A review. International Journal of Food Properties. 14: 503-513.
- Chen D, Han H, Gu Z. 2006. Application of statistical methodology to the optimization of fermentative medium for carotenoids production by *Rhodobacter sphaeroides*. Process Biochemistry. 41: 1773-1778.
- Chiu K-H, Liu W-S. 2014. Dietary administration of the extract of *Rhodobacter sphaeroides* WL-APD911 enhances the growth performance and innate immune responses of seawater red tilapia (*Oreochromis mossambicus* x *Oreochromis niloticus*). Aquaculture. 418-419: 32-38.
- Choi, Han-Pil, Hyun-Jun K, Ho-Chan S, Ha-Ching S. 2002. Isolation and Identification of Photosynthetic Bacterium Useful for Wastewater Treatment. J. Microbiol. Biotechnol. 12 (4): 643-648.
- Chumpol S, Kantachote D, Nitoda T, Kanzaki H. 2018. 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. Aquaculture. 489: 85-95.
- Chumpol S, Kantachote D, Rattanachuy P, Torpee S, Nitoda T, Kanzaki H. 2019. Optimization of culture conditions for production of antiviral compounds from probiotic purple nonsulfur bacteria against acute hepatopancreatic necrosis disease- causing *Vibrio parahaemolyticus* and *Vibrio* spp. Aquaculture. 505: 72-83.
- Del Socorro MML, Mehid JB, Ladion WLB, Teves F. 2013. Purple Nonsulfur Bacteria (PNSB) Isolated from Aquatic Sediments and Rice Paddy in Iligan City, Philippines. J Multidisciplinary Studies. 1 (1).
- Dulekgurgen, E. 2004. Proteins (Lowry) Protocol. UIUC. 1-5.
- Ellouz Y, Bayouh A, Kammoun S, Gharsallah N, Nasri M. 2001. Production of protease by *Bacillus subtilis* grown on sardinelle heads and viscera flour. Bioresource Technology. 80: 49-51.
- Getha K, Vikineswary S, Chong V. 1998. Isolation and growth of the phototrophic bacterium *Rhodospseudomonas palustris* strain B1 in sago-starch-processing wastewater. World Journal of Microbiology & Biotechnology. 14: 505-511.
- Ghaly AE, Ramakrishnan VV, Brooks MS, Budge SM, Dave D. 2013. Fish Processing Wastes as a Potential Source of Proteins, Amino Acids and Oils: A Critical Review. J Microb Biochem Technol. 5 (4): 107-129.
- Gu Z, Chen D, Han Y, Chen Z, Gu F. 2008. Optimization of carotenoid extraction from *Rhodobacter sphaeroides*. LWT. 41: 1082-1088.
- Helrich K, editor. 1990. Official Methods of Analysis of the Association of Official Analytical Chemists. Arlington, Virginia: Association of Official Analytical Chemists, Inc.
- Heyes D, Hunter C. 2009. Biosynthesis of Chlorophyll and Bacteriochlorophyll. In Warren MJ and Smith AG, editors. Tetrapyrroles: Birth, Life and Death. Landes Bioscience and Springer Science+Business Media. pp. 235-249.
- Hui CJ, Prihastyanti MNU, Brotosudarmo THP. 2015. Preliminary Evaluation of the Pigments Content from *Rhodobacter sphaeroides* at Stages during Photosynthetic Growth. Procedia Chemistry. 14: 101-107.
- Kamble KD. 2016. Cultivation of purple phototrophic bacteria using agricultural waste media. Int. Res. J. Pharma. Biosciences. 3(5): 20-27.
- Kim DH, Lee JH, Hwang Y, Kang S, Kim MS. 2013. Continuous cultivation of photosynthetic bacteria for fatty acids production. Bioresource Technology. 148: 277- 282.
- Kis M, Gábor S, Emese A, Rázga Z, Maróti P. 2015. Purple non-sulfur photosynthetic bacteria monitor environmental stresses. Journal of Photochemistry and Photobiology B: Biology. 151: 110-117.
- Kisand V, Tuvikene L, Noges T. 2001. Role of phosphorus and nitrogen for bacteria and phytoplankton development in a large shallow lake. Hydrobiologia. 457: 187-197.
- Kuo F, Chien Y, Chen C. 2012. Effects of light sources on growth and carotenoid of photosynthetic bacteria *Rhodospseudomonas palustris*. Bioresource Technology. 113: 315-318.
- Li E, Mira de Orduña R. 2010. A rapid method for the

- determination of microbial biomass by dry weight using a moisture analyser with an infrared heating source and an analytical balance. *Letters in Applied Microbiology*. 50: 283-288.
- Li X, Peng W, Jia Y, Lu L, Fan W. 2016. Bioremediation of lead contaminated soil with *R. sphaeroides*. *Chemosphere*. 156: 228-235.
- Madukasi EI, Chunhua H, Zhang G. 2011. Isolation and application of a wild strain photosynthetic bacterium to environmental waste management. *Int. J. Environ. Sci. Tech.* 8(3): 513-522.
- Mata-Gomez L, Montañez JC, Mendez-Zavala A, Aguilar CN. 2014. Biotechnological production of carotenoids by yeasts: an overview. *Microbial Cell Factories* 1-11.
- Mather J, Roberts P. 1998. *Introduction to Cell and Tissue Culture: Theory and Technique*. New York. Plenum Press.
- Mishra, A. 2017. Soxhlet Extraction Method- Estimation Of Fat In Food. *Discover Food Tech* [Internet]. [cited 2018 August 15]. Available from <http://discoverfoodtech.com/soxhlet-extraction-method/>.
- Montano G, Chan J, Jarabelo R, Pastor A, Dela Cruz T. 2009. Isolation and Characterization of Purple Nonsulfur Bacteria (PNSB) from a Rice Paddy Soil in Bulacan, Philippines. *Philippine Journal of Systematic Biology*. 57-67.
- Nunkaew T, Kantachote D, Nitoda T, Kanzaki H. 2012. The use of rice straw broth as an appropriate medium to isolate nonsulfur bacteria from paddy fields. *Electronic Journal of Biotechnology*. 1-12.
- Onoda T, Enokizono H, Oshima A, Freestone P, Norris V. 2000. Effect of Calcium and Calcium Chelators on Growth and Morphology of *Escherichia coli* L-Form NC-7. *Journal of Bacteriology*. 1419-1422.
- Paronyan AK, Gasparyan AV. 2009. Production of Biomass of Photosynthetic Bacteria on Base of Stock-Breeding and Poultry Wastes. *Journal of Armenia*. 1 (61).
- Ponsano E, Paulino C, Pinto M. 2007. Phototropic growth of *Rubrivivax gelatinosus* in poultry slaughterhouse wastewater. *Bioresource Technology*. 99: 3836-3842.
- Prachanurak P, Chiemchaisri C, Chiemchaisri W, Yamamoto K. 2014. Biomass production from fermented starch wastewater in photo-bioreactor with internal overflow recirculation. *Bioresource Technology*. 165: 129-136.
- Rebah F, Miled N. 2013. Fish processing wastes for microbial enzyme production: a review. *Biotechnol.* 3: 255-265.
- Sasaki K, Masanori W, Suda Y, Ishizuka A, Noparatnaraporn N. 2005. Applications of Photosynthetic Bacteria for Medical Fields. *Journal of Bioscience and Bioengineering*. 100 (5): 481-488.
- Takeo K, Sasaki K, Watanabe M, Kaneyasu T, Nishio N. 1999. Removal of phosphorus from oyster farm mud sediment using a photosynthetic bacterium, *Rhodobacter sphaeroides* IL106. *J. Biosci Bioeng.* 88 (4): 410-415.
- Toppe J, Albrektsen S, Hope B, Aksnes A. 2007. Chemical composition, mineral content and amino acid and profiles in bones from various fish species. *Comparative Biochemistry and Physiology*. 146: 395-401.
- Vazquez JA, Docasal SF, Prieto MA, Gonzalez MP, Murado MA. 2008. Growth and metabolic feature of lactic acid bacteria in media with hydrolysed fish viscera. An approach to bio-silage of fishing by-products. *Bioresource Technology*. 6246-6257.
- Wu P, Zhang Y, Chen Z, Wang Y, Zhu F, Cao B, Wu Y, Li N. The organophosphorus pesticides in soil was degraded by *Rhodobacter sphaeroides* after wastewater treatment. *Biochemical Engineering Journal*. 141: 247-251.
- Zhou Q, Zhang P, Zhang G. 2014. Biomass and carotenoid production in photosynthetic bacteria wastewater treatment: Effect of light intensity. *Bioresource Technology*. 171: 330-335.
- Zhou Q, Zhang P, Zhang G, Peng M. 2015. Biomass and pigments production in photosynthetic bacteria wastewater treatment; Effects of photoperiod. *Bioresource Technology*. 190: 196-200.
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