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Inclusion of Purple Non-sulfur Bacterial Biomass in Formulated Feed to Promote Growth, Feed Conversion Ratio and Survival of Asian Seabass *Lates calcarifer* Juveniles

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ABSTRACT

Phototrophic bacterium, *Rhodovulum* sp. (purple non-sulfur bacteria, PNSB) was isolated from the seabass broodstock tank at the Hatchery of Universiti Malaysia Sabah and mass-cultured in synthetic media 112 under anaerobic light condition. The dry cell of bacterial biomass was included in the commercial formulated seabass feed by mixing the fresh biomass with the ground pellet, re-pelleted and air-dried. The *Rhodovulum* sp. biomass was added at 0.3, 0.6 and 0.9% in the diets. The control diet was prepared without addition of bacterial biomass. Experimental diets were fed to triplicate groups of seabass juveniles twice a day at apparent satiation level over a 12-week feeding trial. Even though there were no significant differences detected in the growth performance, feed conversion ratio and survival rate of fish fed the experimental diets, there were trends of increased growth, improved survival rate and better feed conversion ratio when fish fed with 0.3% bacterial biomass. At the end of feeding trial, weight gain and specific growth rate of fish in this group were 419 and 1.87 %/d, respectively, compared to 373 and 1.82 %/d, respectively, in the control group. The best Feed Conversion Ratio (FCR; 1.95) and survival rate (86.7%) were also obtained in fish fed 0.3% bacterial biomass. Apparently, increasing level of bacterial biomass above 0.3% did not give any significant benefit to growth, feed conversion ratio and survival of seabass juveniles. This study suggested the potential of PNSB *Rhodovulum* sp. at lower inclusion level to promote growth, FCR and survival of Asian seabass juveniles.

Key words: Purple non-sulfur bacteria, *Rhodovulum* sp., formulated feed, seabass, *Lates calcarifer*

INTRODUCTION

There is increasing interest in the use of probiotics in aquaculture industry in response to the challenging environment. In general, the use of these beneficial bacteria is mainly designated for improvement of aquatic environment quality (Soundarapandian and Sankar, 2008; Subasinghe *et al*., 2003). However, in recent years, a few studies reported the use of a diverse variety of probiotics as supplements to improve nutritive value of aquaculture feeds (El-Haroun *et al*., 2006; El-Dakar *et al*., 2007; Rengpipat *et al*., 2008; Merrifield *et al*., 2010a, b). Nevertheless, the application of probiotics in aquaculture is normally hindered by the increased production cost due the expensive price of commercial probiotics. Fortunately, there exists the potential bacterial candidate such as phototrophic bacteria which is also known as purple non-

sulfur bacteria and widely distributed in nature. Purple non-sulfur bacteria (PNSB) is reported to contain high quality protein, carotenoids and highly digestible cell wall (Kobayashi and Kobayashi, 1995), making them a promising growth enhancer for aquaculture. PNSB was also reported to be able to improve water quality (Kim *et al*., 2004; Tadesse *et al*., 2003). However, limited information is available on the full potential of PNSB as a feed additive and investigation on the effects of PNSB in fish diets deserved an investigation to support this rapidly growing aquaculture industry. In the present study, Asian seabass, *Lates calcarifer* was selected as a target species due to its stable demand in the market and good price. Growth performance, survival, feed conversion ratio and body proximate composition of Asia seabass juveniles were evaluated after feeding them with feeds supplemented with PNSB biomass.

MATERIALS AND METHODS

Production of PNSB biomass: The strain of phototrophic bacterium *Rhodovulum* sp. was isolated locally from the seabass broodstock tank at the hatchery of Borneo Marine Research Institute, Universiti Malaysia Sabah. The synthetic 112 media was used for the inoculum preparation as well as for the mass production of PNSB biomass (Ronald, 1996). A 5% inoculum (v/v) of *Rhodovulum* sp. was inoculated into a 2-L bottle contained sterilized 112 media. The bottles were incubated under 2500 lux light intensity at a temperature of 30±2°C under anaerobic condition for 48 h. The bacterial cultures were centrifuged to obtain biomass. The harvested fresh biomass of *Rhodovulum* sp. was used in the diet preparation.

Fish feed preparation: The commercial seabass sinking feed (Cargill Feed Sdn. Bhd., Malaysia) was used in this experiment. The feed were ground into powder using a grinder and PNSB biomass was included at 0.3, 0.6 and 0.9% of the diets. A commercial feed binder, carboxymethyl cellulose (1%) was also added in this mixture to bind all ingredients. Water was then pour into the mixture and mixed well to form dough. The dough was screw-pressed through a 3 m die and the strands of feeds were air-dried overnight. The control diet was without the addition of PNSB biomass. The experimental diets were refrigerated until used.

Feeding trial: A total of 12 cylindrical cages (61 cm depth and 43 cm diameter) were randomly placed in a 15-tons circular polyethylene tank with flow-through system and continuously aerated. Temperature, salinity, pH and dissolved oxygen were measured every day and maintained at 27.9±1.1°C, 32.6±1.8 ppt and 7.4±1.6 and 6.5±0.4 mg L^{-1}, respectively. Fish were acclimatized to the experimental cages for two weeks and fed a commercial seabass diet before the start of the experiment.

The triplicate fish groups (15 individual per cage) of initial body weight 6.5±0.5 g were fed experimental feed at apparent satiation level by hand twice daily. The total amount of given feed was recorded every day. Fish was individually weighed and total length was recorded at the beginning and end of feeding trial. Bulk weight of the fish was recorded once a week. The feeding trial was conducted for 12 weeks.

Proximate composition analysis: Experimental feeds and whole-body fish were subjected to proximate analysis using standard methods. Experimental feed and fish carcasses were blended, oven-dried and ground into powder before proximate analysis. Moisture, crude protein, crude lipid, crude fiber and crude ash were determined using methods described by AOAC (1990).

Calculation and statistical analysis: Growth performance, Feed Conversion Ratio (FCR), survival and condition factor were calculated as follows:

- % weight gain = percentage of initial body weight at the end of 10 weeks
- Feed conversion ratio (FCR) = dry feed consumed (kg)/wet weight gain (kg)
- Survival rate = [final number of fish/initial number of fish]×100
- Condition factor (CF) = [fish weight/(total length)^3]×100

One-way ANOVA was used to compare growth performance, FCR, survival, condition factor and whole-body proximate composition. Homogeneity of variances was tested with Levene's test and multiple comparisons among treatments were performed with a Tukey HSD *post hoc* test. Significance level was set at $p<0.05$. Statistical package SPSS v.11.0 for Windows was used for all statistical analyses.

RESULTS AND DISCUSSION

Analysed proximate composition of experimental feeds is presented in Table 1. There was no significant different ($p>0.05$) detected in the proximate composition of the experimental feeds. Moisture, crude ash, crude lipid and crude protein ranged from 12.34-12.47%, 11.13-12.03%, 7.50-8.12% and 38.12-39.18%, respectively. These values correspond to the proximate composition provided by the feed manufacturer (moisture 11%, crude ash 11%, crude lipid 8.0% and crude protein 45%). At the end of feeding trial, fish fed diet with 0.3% PNSB biomass obtained the highest weight gain (419%), followed by the control feed (373%), feeds with 0.9% (363.9%) and 0.6% (363.3%) *Rhodovulum* sp. biomass. Specific growth rates were also following the weight gain trend with values ranged from 1.46 (0.6% PNSB) to 1.87 (0.3% PNSB). The best FCR was also obtained in feed with 0.3% PNSB (1.95) (Table 2). The FCRs in other feeds ranged from 2.04-2.15. In term of survival rate, fish fed control feed resulted in the lowest survival rate with 73.3%. Survival rates in other treatments were 80-86.7%. Similarly, condition factor was not affected

Table 1: Analysed proximate composition of experimental feed on % dry matter basis

	PNSB supplementation (%)			
Feed	Control	0.3	0.6	0.9
Moisture	12.0±0.3	11.1±0.3	11.2±0.5	11.6±0.4
Crude ash	12.3±0.1	12.3±0.1	12.5±0.1	12.4±0.2
Crude lipid	7.9±0.2	8.1±0.3	8.14±0.1	7.5±0.6
Crude protein	38.8±1.1	38.2±0.2	38.1±0.1	39.2±0.8

Table 2: Growth performance and feed conversion ratio of experimental fish at the end of feeding trial

	PNSB supplementation (%)			
Feed	Control	0.3	0.6	0.9
Final weight (g)	31.10±0.2	32.30±0.2	30.30±0.2	31.10±0.2
Weight gain (%)	373.10±0.1	419.10±0.1	363.30±0.1	363.90±0.1
SGR (%/d)	1.82±0.3	1.87±0.2	1.79±0.1	1.82±0.1
FCR	2.15±0.7	1.95±0.03	2.07±0.3	2.04±0.5
Condition factor	1.60±0.1	1.94±0.1	1.50±0.2	1.60±0.1

SGR: Specific growth rate, FCR: Feed conversion ratio

Table 3: Whole-body proximate composition of experimental fish at the end of feeding trial on % dry matter basis

	PNSB supplementation (%)			
Feed	Control	0.3	0.6	0.9
Moisture	66.1±0.2	68.2±0.2	67.9±0.2	68.8±0.8
Crude ash	3.7±0.4	4.1±0.3	3.5±0.2	3.3±0.2
Crude lipid	10.0±0.2	9.4±0.3	9.4±0.3	9.5±0.2
Crude protein	16.9±0.4	16.1±0.3	16.1±0.2	15.6±0.3

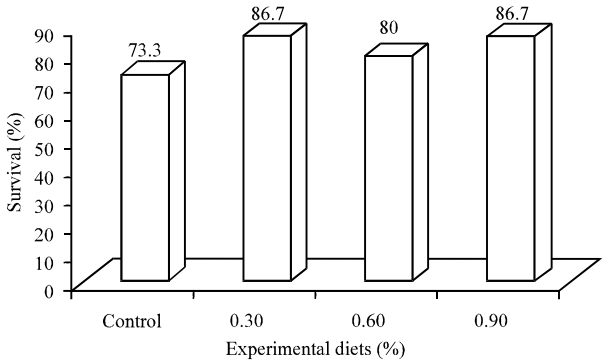

Fig. 1: Survival rate (%) of fish fed experimental feed after 12 week feeding trial

by the dietary treatments (Fig. 1). Whole-body proximate composition of fish is shown in Table 3. Inclusion of PNSB in the feed of seabass juveniles did not significantly ($p>0.05$) affect the body proximate composition.

In general, the growth of juvenile seabass in the present study is similar to the growth obtained in our separate study on the use of alternative oil in seabass feeds (Shapawi *et al*., 2011). Even though there were no significant differences detected in the growth performance, FCR and survival rate, the fish fed 0.3% PNSB consistently giving better values in all mentioned parameters. It is interesting to note that higher PNSB inclusion (0.6 and 0.9%) has resulted in poorer fish performance than in control feed and 0.3% PNSB. This finding suggested that high level of PNSB biomass in fish feed might not give much benefit to fish growth. This is in agreement with a finding by Azad *et al*. (2002) which reported a growth benefit of phototrophic bacteria in juvenile tiger prawn (*Penaeus monodon*) when included at only 0.1% of the feed. However, inclusion of probiotic in the feeds might have some influence in the survival of the target fish. In the present study, the survival rate of fish fed the control feed was about 10% lower than the fish fed feed with PNSB. This can be considered a significant contribution in lowering the cost of feed production since only little amount of PNSB biomass is required to promote the growth performance and survival of cultured fish.

Little is known about the potential of PNSB as a formulated feed supplement in cultured fish. Several studies reported the positive effects of other species of probiotics on growth performance of cultured aquatic animals when included in the feeds such as in Asian seabass (Rengpipat *et al*., 2008), tilapia (El-Haroun *et al*., 2006; Chantharasophon *et al*., 2011), African catfish (Abdelhamid *et al*., 2009) rabbit fish (El-Dakar *et al*., 2007), rainbow trout (Merrifield *et al*., 2010a, b), giant freshwater prawn (Deeseenthum *et al*., 2007) and penaeid shrimp (Fernandez *et al*., 2011). Merrifield *et al*. (2010b) was also reported a similar finding to the present study in term of whole-body proximate composition, where no significant different was observed

as a result of probiotic inclusion in the feeds. In contrast, Bagheri *et al.* (2008) reported that rainbow trout fed feed supplemented with probiotics resulted in higher protein and reduced lipid levels in the carcass.

In general, the findings from the present study suggested that *Rhodovulum* sp. might be able to promote growth, FCR and survival of cultured fish when included at low level of below 0.4%. Further research on the lower inclusion level (<0.3% of the diets) of PNSB deserved an investigation.

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REFERENCES

AOAC, 1990. Official Methods of Analysis of AOAC International. 15th Edn., AOAC, Arlington, Virginia, USA.

Abdelhamid, A.M., A.I. Mehrim, M.I. El-Barbary, S.M. Ibrahim and A.I. Abd El-Wahab, 2009. Evaluation of a New Egyptian probiotic by African catfish fingerlings. J. Environ. Sci. Technol., 2: 133-145.

Azad, S.A., V.C. Chong and S. Vikineswary, 2002. Phototrophic bacteria as feed supplement for rearing *Penaeus monodon* larvae. J. World Aqua. Soc., 33: 158-168.

Bagheri, T., S.A. Hedayati, V. Yavari, M. Alizade and A. Farzanfar, 2008. Growth, survival and gut microbial load of rainbow trout (*Onchorhynchus mykiss*) fry given diet supplemented with probiotic during the two months of first feeding. Turk. J. Fish. Aquatic. Sci., 8: 43-48.

Chantharasophon, K., T. Warong, P. Mapatsa and V. Leelavatcharamas, 2011. High potential probiotic *Bacillus* species from gastro-intestinal tract of Nile Tilapia (*Oreochromis niloticus*). Biotechnology, 10: 498-505.

Deeseenthum, S., V. Leelavatcharamas and J.D. Brooks, 2007. Effect of feeding *Bacillus* sp. as probiotic bacteria on growth of giant freshwater prawn (*Macrobrachium rosenbergii* de Man). Pak. J. Biol. Sci., 10: 1481-1485.

El-Dakar, A.Y., S.M. Shalaby and I.P. Saoud, 2007. Assessing the use of a dietary probiotic/prebiotic as an enhancer of spinefoot rabbitfish *Siganus rivulatus* survival and growth. Aquac. Nut., 13: 407-412.

El-Haroun, E.R., A.M.A.S. Goda and M.A.K. Chowdhury, 2006. Effect of dietary probiotic Biogen® supplementation as a growth promoter on growth performance and feed utilization of Nile tilapia *Oreochromis niloticus* (L.). Aquac. Res., 37: 1473-1480.

Fernandez, R., M. Sridhar and N. Sridhar, 2011. Effect of lactic acid bacteria administered orally on growth performance of *Penaeus indicus* (H. Milne Edwards) Juveniles. Res. J. Microbiol., 6: 466-479.

Kim, M.K., K.M. Choi, C.R. Yin, K.Y. Lee, W.T. Im, J.H. Lim and S.T. Lee, 2004. Odorous swine wastewater treatment by purple non-sulfur bacteria, *Rhodopseudomonas palustris*, isolated from eutrophicated ponds. Biotechnol. Lett., 26: 819-822.

Kobayashi, M. and M. Kobayashi, 1995. Waste Remediation and Treatment Using Anoxygenic Photosynthetic Bacteria. In: Anoxygenic Photosynthetic Bacteria, Blakenship, R.E., M.T. Madigan and C.E. Bauer (Eds.). Kluwer Academic Publishers, Netherlands, pp: 1269-1282.

Merrifield, D.L., A. Dimitroglou, G. Bradley, R.T.M. Baker and S.J. Davies, 2010a. Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) I. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria. Aquacult. Nutr., 16: 504-510.

Merrifield, D.L., G. Bradley, R.T.M. Baker and S.J. Davies, 2010b. Probiotic applications for rainbow trout (*Oncorhynchus mykiss* Walbaum) II. Effects on growth performance, feed utilization, intestinal microbiota and related health criteria postantibiotic treatment. Aquacult. Nutr., 16: 496-503.

Rengpipat, S., T. Rueangruklikhit and S. Piyatiratitivorakul, 2008. Evaluations of lactic acid bacteria as probiotics for juvenile seabass *Lates calcarifer*. Aquacult. Res., 39: 134-143.

Ronald, M.A., 1996. Handbook of Microbial Media. 4th Edn., CRC Press, USA.

Shapawi, R., M.A.M. Zain and S. Senoo, 2011. Palm oil based-diet enhances growth performance of Asian seabass (*Lates calcarifer*, Centropomidae) juveniles. Asian Fish. Sci., 24: 218-223.

Soundarapandian, P. and S. Sankar, 2008. Effects of probiotics on the survival and production of black tiger shrimp *Penaeus monodon* (Fabricius). Int. J. Zool. Res., 4: 35-41.

Subasinghe, R.P., D. Curry, S.E. McGladdery and D. Bartley, 2003. Recent Technological Innovations in Aquaculture. In: Review of the State of World Aquaculture, FAO (Ed.). FAO Fisheries Department, Rome, Italy, pp: 59-74.

Tadesse, I., S.A. Isoaho, F.B. Green and J.A. Puhakka, 2003. Removal of organics and nutrients from tannery effluent by advanced integrated Wastewater Pond systems technology. Waste Sci. Technol., 48: 307-314.