HAYATI Journal of Biosciences June 2010
Vol. 17 No. 2, p 79-84
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.17.2.79

Effect of *Paracoccus* sp. and their Genetically Modified on Skin Coloration of Red Sea Bream

AGUS KURNIA[1]*, SHUICHI SATOH[2], SATOSHI HANZAWA[3]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Science, Haluoleo University, Kendari 93232, Indonesia
[2]Laboratory of Fish Nutrition, Department of Marine Bioscience, Tokyo University of Marine Science and Technology, Konan, Minato-ku, Tokyo 108-8477, Japan
[3]Tokyo Research Laboratory, Tosoh Corporation, Ayase, Kanagawa 252-1123, Japan

Received October 21, 2008/Accepted March 22, 2010

Astaxanthin (Asx) content in the skin of red sea bream was observed in a feeding trial with two Asx sources: marine bacteria (*Paracoccus* sp.) and Asx concentrated marine bacteria modified genetically (GM marine bacteria). Four semi purified diets were prepared and contained two levels of marine bacteria (15 and 30 mg Asx/kg), 30 mg Asx/kg of GM marine bacteria and control diet without additional Asx. The diets were fed to fourteen fish of red sea bream (initial weight 128.5 g) which were randomly distributed in each 60-l glass tank. Asx content in the skin of fish fed on non-GM marine bacteria diets were higher than that of fish fed on GM marine bacteria. Additionally, Asx accumulation in the skin of fish fed on diet of non-GM marine bacteria containing 15 mg Asx/kg diet was higher than those fish fed on diet supplemented with 30 mg Asx/kg of GM marine bacteria. The results showed that by adding the supplement of Asx derived from marine bacteria (*Paracoccus* sp.) to the red sea bream diets might enhance the skin coloration of red sea bream and the other carotenoids contained in marine bacteria might also enhance the coloration.

Key words: skin coloration, marine bacteria, genetically modified marine bacteria, red sea bream

INTRODUCTION

Despite the more optimistic still forecast, a certain increase in marine finfish markets during the next years dependson Japan fish farm on two species most popular in this region, yellow tail and red sea bream. Red sea bream, a species has been found to be suitable candidate cultured for aquaculture diversification for a high market price in Japan. This species has highly market price because of the reddish color of their skin. However, several authors have found that this species loses its natural skin coloration under culture conditions. Thus, whereas wild specimens exhibit a red pink silver color, under captivity the red sea bream skin turn dark grey (Kentouri *et al*. 1995; Cejas *et al*. 2003).

Consumers perceive that the redder skin of red sea bream is equated tofresher, better flavor, higher quality, and higher price fish. Commonly when fish were introduced to fish farm, the skin color changed to be pale or colorless after a few time of rearing. The colorless skins of fish lead people to reject the fish. Maintaining the natural skin pigmentation is of great importance from a commercial point of view, being directly associated with acceptance or rejection by the consumers (Shahidi *et al*. 1998) and the product market price. To solve this problem, therefore, the Asx (astaxanthin) as a coloration agent should be supplied into the diet.

*Corresponding author. Phone/Fax: +62-401-3193782, E-mail: fatmi_70@yahoo.com

Skin and muscle coloration are provided by carotenoid inclusion in fish diets. For red sea bream, carotenoids are included in commercial diets to maintain good skin coloration (Katayama *et al*. 1965; Tanaka *et al*. 1976; Nakazoe *et al*. 1984).

Carotenoids are natural lipid-soluble pigments produced primarily within bacteria, algae, and plants. These pigments are responsible for the wide variety of colors seen in nature. Astaxanthin (3,3′-dihydroxy-â,â-carotene-4,4′-dione) is an abundant carotenoid found in marine animals, including salmonids and crustaceans (Miki *et al*. 1982; Wade *et al*. 2005). Bjerkeng (2000) suggested that fish skin coloration was affected by Asx source, dosage level, duration of feeding and dietary composition Astaxanthin has been isolated from various sources, including the heterobasidiomycetous yeast *Phaffia rhodozyma* (Johnson *et al*. 1978), the green alga *Haematococcus pluvialis* (Bubrick 1991), the gram-positive bacterium *Brevibacterium linens* (Krubasik & Sandmann 2000), and the marine bacterium *Paracoccus haeundaensis* (Lee *et al*. 2004). Many carotenoid biosynthesis genes have been cloned and characterized from the various organisms producing Asx, and the functions of the gene products have been determined (Pasamontes *et al*. 1997; Harker *et al*. 1998; Krubasik & Sandmann 2000). Extensive studies have been conducted on the general aspects of the chemical structures, physical and biochemical properties, biosynthetic and molecular genetics, and biotechnological applications of carotenoids (Sandmann 2001; Johnson 2003). The pigments were

produced by *Escherichia coli* with the clone gene cluster (Lee & Kim 2006).

One of the Asx source is marine bacteria (*Paracoccus* sp.) the results of previous study demonstrated that marine bacteria (*Paracoccus* sp.) was better for skin coloration of red sea bream than synthesized Asx (Kurnia *et al*. 2007). In addition, recently, it had been discovered that Asx production could be enhanced by modifying the Asx gene ofthe bacteria. The GM marine bacteria was isolated and produced by Tosoh Corporation, but it was not commercial yet and still will be developed as a potential Asx source. Therefore, the general aim of this study was to observed the effect of Asx and other carotenoids on skin coloration of red sea bream, *Pagrus major*, by using Asx concentrated marine bacteria (after gene modification) and different level of marine bacteria.

MATERIALS AND METHODS

Fish, Experimental Conditions, and Feeding. The experiment was conducted at Fish Nutrition Laboratory of Tokyo University of Marine Science and Technology. Red sea bream, *Pagrus major,* were obtained from Seiho Suisan Co. Ltd. (Mie, Japan) and fed on commercial diet. Duplicate groups of red sea bream were fed on four different diets in total 8 tanks for 12 weeks. A total of fourteen fish (initial weight 128.5 g) were randomly distributed in each well-aerated 60-l glass tanks. The feeding trial was conducted in re-circulated artificial seawater (Sea Life®, Tokyo, Japan) system at a flow rate of 700-800 ml/min; salinity, 29$^{o}/_{oo}$. The water renewal rate in the system was 50% carried out weekly. Important water quality parameters such as temperature, pH, salinity were monitored daily and dissolved oxygen was measured fortnightly. All the observed parameters were to be within the acceptable limit for fish culture. The temperature ranged between 18-23 ^{o}C. The fish were hand-fed each diet daily three times a day until near satiation. Tank bottoms were siphoned as needed, and mortalities were recorded daily.

Prior to the beginning of the experiment, fish were fed on a common carp diet without Asx. All the fish groups were fed by hand three times a day (8.00, 13.00, and 17.00) till satisfied.

Experimental Diets and Asx Sources. Four different experimental pelleted diets containing 42% protein, 13% lipid, and 9% ash (dry matter basis) were evaluated in duplicate groups. A control diet was without Asx; two diets were supplemented with marine bacteria (*Paracoccus* sp.) to contain 15 and 30 mg Asx/kg and the last one was supplemented with genetically modified marine bacteria at a level of 30 mg Asx/kg. The ingredient and biochemical analysis of the diets are shown in Table 1 and 2.

The Asx contents in marine bacteria (MB) and genetically modified marine bacteria (GMB) were 4500 and 3300 mg/kg, respectively. All the Asx sources, MB and GM of marine bacteria (*Paracoccus* sp.), were produced and received from Tokyo Research Laboratory, Tosoh Corporation.

Table 1. Formulation of the experimental diet of red sea bream

Ingredients (%)	Diets[1]			
	C	GMB	MB-15	MB-30
Jack mackerel meal	45	45	45	45
Soybean meal	10	10	10	10
Wheat flour	21.5	21.5	21.5	21.5
Pregelatinized starch	5	5	5	5
Pollock liver oil	5	5	5	5
Soybean oil	3.5	3.5	3.5	3.5
Mineral mix.[2]	1	1	1	1
Vitamin mix.[3]	3	3	3	3
Choline chloride	0.5	0.5	0.5	0.5
Vitamin E (50%)	0.1	0.1	0.1	0.1
Cellulose	5.4	4.37	4.49	4.49
Gene modified bacteria(GMB)	0	0.60	0	0
Marine bacteria (MB)	0	0	0.46	0.91

[1]Diets were recognized by their Asx source: C = Control diet; GMB = genetically modified marine bacteria; MB = Marine bacteria. [2]P-free mineral mixture (g/100 g) contains: NaCl, 5.0; $MgSO_4 \cdot 7H_20$, 74.5; $FeC_6HO_7 \cdot 7H_2O$, 12.5; Trace element mix., 5.0; Cellulose, 3.0. Trace element mix (mg/g) contains: $ZnSO_4 \cdot 7H_2O$, 353; $MnSO_4 \cdot 5H_2O$, 162; $CuSO_4 \cdot 5H_2O$, 31; $AlCl_3 \cdot 6H_2O$,10; $CoCl \cdot 6H_2O$, 1; KIO_3, 3; Cellulose, 440. [3]Vitamin premix (mg/100 g) contains: Vitamin B1, 6.0; Vitamin B2, 10.0; Vitamin B6. 4.0; Vitamin B12, 0.01; Vitamin C, 500.0; Niacin, 40.0; Ca-pantothenate, 10.0; Inositol, 200.0; Biotin, 0.6; Folic acid, 1.5; p-amino benzoic acid, 5.0; Vitamin K3, 5.0; Vitamin A, 4000.0IU; Vitamin D3, 4000.0IU.

Table 2. Results of proximate analysis and Asx content in the experimental diet

	Diets[1]			
	C	GMB	MB-15	MB-30
Moisture (% dry weight)	4.02	2.69	3.40	4.43
Crude protein (%DW)	42.80	42.90	42.80	43.20
Crude lipid (% DW)	12.90	13.80	13.10	13.80
Ash (%DW)	9.71	9.71	9.79	9.76
Total carotenoids (mg/kg)	5.13	54.50	80.40	136
Astaxanthin (mg/kg)	0	33.70	15.00	33.10

[1]Diets were recognized by their Asx source: C = Control diet; GMB = genetically modified marine bacteria; MB = non GM marine bacteria.

Diets were pelleted by using the laboratory pelletizer (AEZ12M, Hiraga-Seikakusho, Kobe, Japan), dried with a vacuum freeze-drier (RLE-206, Kyowa Vacuum Tech., Saitama, Japan). All feeds were stored in plastic bags for the duration of the study. The feed were filled in the plastic bags and stored in a freezer (4 ^{o}C) to protect them from light and oxidation of nutrients and carotenoids content.

Sample Collection and Chemical Analyses. Biochemical analysis of feed was conducted in triplicate. The diets were analyzed for proximate composition as described by Watanabe (1988). In the first day of experiment, five fish were blotted dry, weighed individually and dissected to measure the initial weight, total carotene and Asx content in the skin of the fish. The fish were sampled to assess growth response and determine the feed utilization every three weeks. Five fish from each tank were randomly selected for taking the skin and muscle. The skin was removed by cutting the body skin except head and tail, while muscle were minced by a centrifugal mill (Retsch ZM 100, Germany) fitted with a 0.25 mm screen. These samples were collected and kept at -20 ^{o}C until

analysis. Total carotenoids content in diets and skin were determined by spectrophotometer after extraction with acetone. For carotenoid extraction, sample was weighted and 60 ml acetone and some sodium sulphate anhydrous were added. The mixture was ground and filtered through glass microfibre filters (GF/A, Whatman paper) and rinsed with chloroform to increase the boiling point of the mixture. After mixing and phase separation between diethyl ether and water in separatory funnel, the upper layer was taken and placed in a round bottle to allow evaporation in a rotary evaporator at 35 °C. The extract was concentrated and dissolved in benzene. Total carotenoids concentration was calculated from the absorbance of the benzene solution according to the method of McBeth (1972). The absorbance was measured by spectrophotometer (Shimadzu, Inc. Kyoto Japan), at wavelength of 460 and 480 nm for yellow and red carotenoids, respectively. Total carotenoids were quantified by using an equation as follow:

$$\text{Total carotemoid (mg/100 g sample)} = \frac{\text{ABS}}{E^{1\%}_{1\ cm}} \times \frac{\text{Dilution volume (ml)}}{\text{Sample weight (g)}} \times 1000$$

ABS: optical density, E: extinction coefficient value = 1900 (red carotenoid in benzene) = 2500 (yellow carotenoid in benzene)

Asx content of the diet, skin and muscle was determined by HPLC. The sample extract which still diluted with benzene was re-evaporated in an evaporator at 35 °C and then dissolved in 1 ml with n- hexane and 20 µl was used for injected into HPLC (Shimadzu, LC-10 AD). This system consisted of a 110 x 4.6 mm Lichosorb SI-60 (GL Sciences Inc.), with temperature of 35 °C, using 20% acetone in 80% n-hexane as a mobile phase and a flow rate of 1 ml/min. The retention times and peak areas of Asx were compared with those obtained from standard Asx (F. Hoffman-La Roche AG, Switzerland).

Statistical Analysis. Means and standard deviations were calculated for all fish for each parameter measured. All data were tested for normality and homogeneity of variance. Differences among groups were determined by one way ANOVA. When appropriate, means were compared by Duncan's multiple range test. Statistical significance was tested at a 0.05 probability level.

RESULTS

At the end of experiment, there were no significant (P = 1.256) differences between fish fed on the different diets in final mean weight (overall mean, 199 g), specific growth rate (overall mean, 0.55 g) and feed gain ratio (overall mean, 2.21) (Table 3).

The total carotenoids content of the skin of fish fed on the different experimental diets are shown in Figure 1. The Asx in the diet significantly (P = 0.943) affected total carotenoids in skin of the fish.

Initially, the mean of skin pigment content was 7.62 mg/kg of total carotenoids and 3.32 mg/kg of Asx content. After 12 weeks of rearing, total carotenoids content in the skin ranged from 5.62 to 27.2 mg/kg. Whereas, the fish fed on control diet decreased in total carotenoids in their skin when compared to the initial fish. Between fish fed on diet containing Asx sources, total carotenoids in the skin of red sea bream fed on marine bacteria (*Paracoccus* sp.) was higher than those fed on GM bacteria. Final mean Asx content in the skin ranged from 1.95 to 7.20 mg/kg (Figure 2).

Skin Asx content were significantly (P = 5.375) lower in the fish fed on control diet than those fed on the diet with Asx sources. After 12 weeks of experiment the skin Asx content in fish fed on marine bacteria (*Paracoccus* sp.) was higher (P = 2.247) than those fish fed onGM bacteria.

Table 3. Effect of feeding Asx supplements on growth and feed utilization parameters after 12 weeks of experiment

	Diets			
	C	GMB	MB-15	MB-30
Initial (g)	129 ± 0.7[1]	129 ± 1.8	128 ± 1.3	127 ± 1.5
Final weight (g)	193 ± 20	216 ± 45	191 ± 6.6	186 ± 30
Feed intake (g)	145 ± 2.4	156 ± 23	151 ± 12	130 ± 21
Growth (g)[2]	64.1 ± 20[a]	87.4 ± 47[b]	63.6 ± 5.3[a]	58.8 ± 28[a]
SGR (g)[3]	0.51 ± 0.1[b]	0.63 ± 0.2[c]	0.57 ± 0.0[b]	0.45 ± 0.2[a]
FGR (g)[4]	2.41 ± 0.7	2.01 ± 0.8	2.40 ± 0.4	2.40 ± 0.8

[1]Values are mean ± S.D of three groups per treatment. [2]Growth (g) = Final weight – initial weight. [3]SGR: specific growth rate = 100 x (ln final weight – ln initial weight)/no. of days. [4] FGR: feed gain ratio= feed intake (g)/weight gain.

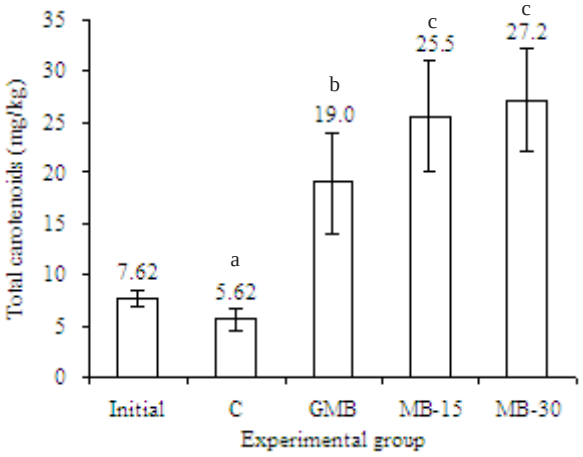

Figure 1. Total carotenoids content in the skin of red sea bream after 12 week of rearing.

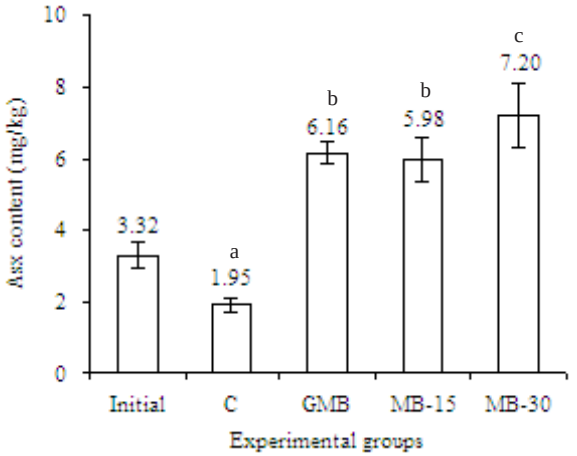

Figure 2. Asx content in the skin of red sea bream after 12 week of rearing.

DISCUSSION

The red skin colour of red sea bream is an important quality criterion for consumer acceptance (Shahidi *et al*. 1998). It is a result of the deposition of 4(4′)-ketocarotenoids such as astaxanthin (3,3′-dihydroxy-â,â-carotene-4,4′-dione) and canthaxanthin (â,â-carotene-4,4′-dione) in the skin. These carotenoids must be provided in the diet because all animals, including sea bream fishes are unable to biosynthesize them de novo. Carotenoids are, however, poorly utilised by the fish, and the muscle retention of astaxanthin in Atlantic salmon is usually less than 12% (Bjerkeng *et al*. 1999a,b; 2000). One reason for this is the poor absorption of carotenoids from the gut. When precautions are taken to avoid breakdown of carotenoids in the faeces, the apparent digestibility coefficient (ADC) is around 0.5 (Bjerkeng & Berge 2000). Diet composition and water temperature affect the digestibility of carotenoids (Torrissen *et al*. 1989; Storebakken & No 1992; Ytrestøyl *et al*. 2005), and it is hypothesized here that ration level may influence the digestibility as well. The absorption from the gut is believed to occur by passive diffusion mechanisms and involves several steps from breakdown of the food matrix, solubilization of carotenoids into mixed bile salt micelles, movement across the unstirred water layer adjacent to the microvilli, uptake by the enterocyte and incorporation into chylomicrons (Furr & Clark 1997). However, more recent evidence on selective uptake of E/Z-isomers from the intestine (Bjerkeng *et al*. 1997) and Caco-2 cell model systems (During & Harrison 2005) indicate a facilitated uptake from the gut. The proximal intestine is the major site of carotenoid absorption (Torrissen *et al*. 1990; White *et al*. 2002). The carotenoid uptake from the gut is a rather slow process, and peak plasma levels are observed 18-30 h after intake of a single dose in salmonid fishes (Gobantes *et al*. 1997; Maltby *et al*. 2003).

In the present study, growth and feed efficiency of red sea bream were not affected by the addition of Asx into their diets. Our results relating to the study of growth are similar to the conclusions published by Foss *et al*. (1984) in trials with rainbow trout weighting 0.35 kg, found no differences in growth and mortality when testing Asx and canthaxanthin. In contrast, some studies have reported that Asx supplement could enhance the fish growth (Bonyaratpalin & Unpraset 1989; Christiansen *et al*. 1995).

Overall results on this study showed that the various dietary Asx supplements given increased the total carotenoids content in skin of fish when compared to the total carotenoid content of the initial and control fish groups. In the group of fish fed on diet supplemented with Asx, lower efficacy Asx in the skin of fish fed on Asx concentrated marine bacteria diet was observed than that of fish fed on marine bacteria. It might be due to Asx concentrated marine bacteria contain predominantly adonixanthin around 47% of the total carotenoids and it was also often more than Asx (Yokoyama *et al*. 1994). Some of studies reported that Asx was superior as a pigmenting agent compared to other carotenoids (Katayama *et al*. 1965; Kalinowski 2005). Nakazoe *et al*. (1984) conducted an experiment with red sea bream (*Chrysophrys major*) and concluded that red sea bream fed on diets supplemented with β-carotene and cantaxanthin diets resulted in a decrease in the carotenoid level, while fish fed on diets supplemented with zeaxanthin, lutein, free Asx, or Asx ester diets resulted in a increase of carotenoids level. In other experiment it was also reported that when fish were fed on a total of 21.4 mg of zeaxanthin, 21.4 mg of lutein, and 18.5 mg of free Asx total carotenoid became 482.1, 256.2, and 235.5 mg respectively. However, when only 9.68 mg of Asx ester was given, the total carotenoid accumulation was 542 mg. Therefore, Tanaka *et al*. (1976) suggested that it is necessary to provide a source of dietary Asx in order to yield the reddish coloration of cultured red sea bream. Furthermore, between marine bacteria and GM bacteria, total carotenoids in fish fed on diet containing marine bacteria was higher than those fed on GM bacteria diet. Marine bacteria (*Paracoccus* sp.) also contained 4-keto-zeaxanthin, presumably as an intermediate of Asx biosynthesis (Yokoyama *et al*. 1994). It might enhance the Asx accumulation in the skin of red sea bream fed on marine bacteria diet more than that of fish fed on Asx concentrated marine bacteria diet.

This experiment showed that fish fed on marine bacteriacontaining 30 mg Asx/kg diet was higher in its skin Asx content than that fed on the bacteria containing 15 mg Asx/kg diet. It suggests that Asx absorption is affected by the Asx content in the diet. Meyers (1994) reported that Asx absorption also depended on the content and the form of Asx, free or diester. Australian snapper fed on the diet containing 72 mg Asx/kg were significantly more reddish than fish fed on the diet with 36 mg Asx/kg after three weeks feeding, but the skin redness was similar in both groups of fish after 6 and 9 weeks (Booth *et al*. 2004). The other study with *Pagrus pagrus* observed that skin coloration can be modified from a dark grey to a red pink silver color by supplementing the diet with 40 mg of Asx/kg diet contained in shrimp shell meal (Kalinowksi *et al*. 2005).

The highly skin coloration in the fish fed on diet supplemented with marine bacteria might be related to the highest total carotenoids content in the diet. It means that not only astaxanthin contained in the marine bacteria but the other carotenoids also affected the skin coloration of red sea bream. Hirschberg (1997) observed that the carotenoids in *Paracoccus* sp. contain 87% of free adonixanthin, only 8% of free Asx and the rest are various other ketocarotenoids (such as lycopene, β-carotene, echinenone, -cryptoxanthin, canthaxanthin, adonirubin, cis-adonixanthin, and zeaxanthin). It was also supported by the results that the Asx content on fish fed on marine bacteria containing 15 mg Asx/kg diet was higher than that feed on Asx concentrated marine bacteria containing 30 mg Asx/kg diet. It suggest that marine bacteria (*Paracoccus* sp.) was better as a pigment agent than GM-marine bacteria (*Paracoccus* sp.).

Overall results of this study revealed that inclusion the marine bacteria (*Paracoccus* sp.) into the diet can be recommended to enhance the skin coloration of red sea bream.

REFERENCES

Bjerkeng B, Følling M, Lagocki S, Storebakken T, Olli JJ, Alsted A. 1997. Bioavailability of all-E-astaxanthin and Zisomers of astaxanthin in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 157:63-82.

Bjerkeng B, Hamre K, Hatlen B, Wathne E. 1999a. Astaxanthin deposition in fillets of Atlantic salmon *Salmo salar* L. fed two dietary levels of astaxanthin in combination with three levels of á- tocopheryl acetate. *Aquac Res* 30:637-646.

Bjerkeng B, Hatlen B, Wathne E. 1999b. Deposition of astaxanthin in fillets of Atlantic salmon *(Salmo salar*) fed diets with herring, capelin, sandeel, or Peruvian high PUFA oils. *Aquaculture* 180:307-319.

Bjerkeng B, Hatlen B, Wathne E. 2000. Corrigendum to Deposition of astaxanthin in fillets of Atlantic salmon (*Salmo salar*) fed diets with herring, capelin, sandeel, or Peruvian high PUFA oils. *Aquaculture* 189:389-390.

Bjerkeng B, Berge GM. 2000. Apparent digestibility coefficients and accumulation of astaxanthin E/Z isomers in Atlantic salmon (*Salmo salar* L.) and Atlantic halibut (Hippoglossus hippoglossus L.). *Comp Biochem Physiol* 127:423-432.

Bonyaratpalin M, Unpraset N. 1989. Effects of pigments from different sources on colour changes and growth of red *Oreochromis niloticus*. *Aquaculture* 79:375-380.

Booth M, Warner-Smith R, Allan G, Glencross B. 2004. Effect of dietary astaxanthin source and light manipulation on the skin color of Australian snapper *Pagrus auratus* (Bloch and Schneider, 1801). *Aquac Res* 35:458-464.

Bubrick P. 1991. Production of astaxanthin from *Haematococcus*. *Bioresour Technol* 38:237-239.

Cejas JR, Almansa E, Tejera N, Jerez S, Bolannos A, Lorenzo A. 2003. Effect of dietary supplementation with shrimp on skin pigmentation and lipid composition of red porgy (*Pagrus pagrus*) alevins. *Aquaculture* 218:457-469.

Christiansen R, Lie O, Torrisen OJ. 1995. Growth and survival of Atlantic salmon, *Salmo salar* L., fed different dietary levels of astaxanthin. First-feeding fry. *Aquaculture Nutrition* 1:189-198.

During A, Harrison EH. 2005. An *in vitro* model to study the intestinal absorption of carotenoids. *Food Res Int* 38:1001-1008.

Foss P. Storebakken T, Schiedt K, Liaaen-Jensen S, Austreng E, Streiff K. 1984. Pigmentation of rainbow trout with the individual optical isomers of astaxanthin in comparison with canthaxanthin. *Aquaculture* 41:213-226.

Furr HC, Clark RM. 1997. Intestinal absorption and tissue distribution of carotenoids. *Nutr Biochem* 8:364-377.

Gobantes I, Choubert G, Laurentie M, Milicua J-CG, Gomez R. 1997. Astaxanthin and canthaxanthin kinetics after ingestion of individual doses by immature rainbow trout Oncorhynchus mykiss. *J Agric Food Chem* 45:454-458.

Harker M, Hirschberg J. 1997. Biosynthesis of ketocarotenoids in transgenic cyanobacteria expressing the algal gene for beta-C-4-oxygenase, crtO. *FEBS Lett* 404:129-134.

Harker M, Hirschberg J, Oren A. 1998. *Paracoccus marcusii* sp. nov., an orange gram-negative coccus. *Int J Syst Bacteriol* 48:543-548.

Hirschberg J. 1997. Production of Carotenoids (Including the Ketocarotenoids Astaxanthin and Adonixanthin) using a Novel Carotenoid-Secreting Bacterial Species. Science Letter Hebrew university of Yerusallem. p 1-5.

Johnson EA. 2003. *Phaffia rhodozyma*: colorful odyssey. *Int Microbiol* 6:169-174.

Johnson EA, Villa TG, Lewis MJ, Phaff HJ. 1978. Simple method for the isolation of astaxanthin from the basidiomycetous yeast *Phaffia rhodozyma*. *Appl Environ Microbiol* 35:1155-1159.

Kalinowski CT, Robaina LE, Fernandez-Palacios H, Schuchardt D, Izquierdo MS. 2005. Effect of different carotenoid sources and their dietary levels on red porgy, *Pagrus pagrus*, growth and skin color. *Aquaculture* 244:223-231.

Katayama T, Ikeda N, Harada K. 1965. Carotenoids in sea breams, *Chrysophrys major* and *schlegel*. *Bull Jpn Soc Sci Fish* 31:947-952.

Kentouri M, Pavlides M, Papandroulakis N, Divanach P. 1995. Network on Technology of Aquaculture in the Mediterranean_TECAM. Nicosia_Cyprus. p 65-78.

Krubasik P, Sandmann G. 2000. A carotenogenic gene cluster from *Brevibacterium linens* with novel lycopene cyclase genes involved in the synthesis of aromatic carotenoids. *Mol Gen Genet* 263:423-432.

Kurnia A, Satoh S, Hanzawa S. 2007. Effect of different astaxanthin sources on skin pigmentation of red sea bream. *Aquaculture Sci* 55:441-447.

Lee JH, Kim YS. 2006. Cloning and characterization of the astaxanthin biosynthesis gene cluster from the marine bacterium *Paracoccus haeundaensis*. *J Gene* 370:86-95.

Lee JH, Kim YS, Choi TJ, Lee WJ, Kim YT. 2004. *Paracoccus haeundaensis* sp. nov., a gram-negative, halophilic, astaxanthin-producing bacterium. *Int J Syst Evol Microbiol* 54:1699-1702.

Maltby JB, Albright LJ, Kennedy CJ, Higgs DA. 2003. Effect of route of administration and carrier on bioavailability and kinetics of astaxanthin in Atlantic salmon *Salmo salar* L. *Aquac Res* 34:829-838.

McBeth JW. 1972. Carotenoids from nudibranchs. *Comp Biochem Physiol* 41B:55-68.

Meyers SP. 1994. Developments in world aquaculture, formulations, and the role of carotenoids. *Pure Appl Chem* 66:1069-1076.

Miki W, Yamaguchi K, Konosu S. 1982. Comparison of carotenoids in the ovaries of marine fish and shellfish. *Comp Biochem Physiol* 71:7-11.

Nakazoe J, Ishii S, Kamimoto M, Takeuchi M. 1984. Effects of supplemental carotenoid pigments on the carotenoid accumulation in young sea bream, *Chrysophrys major*. *Bull Tokai Reg Fish Res Lab* 113:29-41.

Pasamontes L, Hug D, Tessier M, Hohmann HP, Schierle J, van Loon AP. 1997. Isolation and characterization of the carotenoid biosynthesis genes of *Flavobacterium* sp. strain R1534. *Gene* 185:35-41.

Sandmann G. 2001. Carotenoid biosynthesis and biotechnological application. Arch. *Biochem Biophys* 385:4-12.

Shahidi F, Metusalach A, Brown JA. 1998. Carotenoid pigments in sea foods and aquaculture. *Crit Rev Food Sci Nutr* 38:1-6.

Storebakken T, Goswami UC. 1996. Plasma carotenoid concentration indicates the availability of dietary astaxanthin for Atlantic salmon, *Salmo salar*. *Aquaculture* 146:147-153.

Storebakken T, No HK. 1992. Pigmentation of rainbow trout. *Aquaculture* 100:209-229.

Tanaka Y, Katayama T, Simpson KL, Chichester CO. 1976. The carotenoids in marine red fish and the metabolism of the carotenoids in Sea Bream, *Chrysophrys major Temminch* and *Schegel*. *Nippon Suisan Gakkaishi* 42:1177-1182.

Torrissen OJ, Hardy RW, Shearer KD, Scott TM, Stone FE. 1990. Effects of dietary canthaxanthin and lipid level on apparent digestibility coefficients for canthaxanthin in rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 88:351-362.

Torrissen OJ, Hardy RW, Shearer KD. 1989. Pigmentation of salmonids. Carotenoid deposition and metabolism. *Rev Aquatic Sci* 1:209-225.

Yokoyama A, Izumida H, Miki W. 1994. Production of astaxanthin and 4-Ketozeaxanthin by the Marine Bacterium, *Agrobacterium aurantiacum*. *Biosci Biotech Biochem* 58:1842-1844.

Ytrestøyl T, Struksnæs G, Koppe W, Bjerkeng B. 2005. Effects of temperature and feed intake on digestibility and metabolism of astaxanthin in Atlantic salmon (*Salmo salar*). *Comp Biochem Physiol* 142:445-455.

Wade N, Goulter KC, Wilson KJ, Hall MR, Degnan BM. 2005. Esterified astaxanthin levels in lobster epithelia correlate with shell colour intensity: potential role in crustacean shell colour formation. *Comp Biochem Physiol B Biochem Mol Biol* 141:307-313.

Watanabe T. 1988. Fish Nutrition and Mariculture. Japan International Cooperation Agency, Yokosuaka. 1988. p 233.

White DA, Page GI, Swaile J, Moody AJ, Davies SJ. 2002. Effect of esterification on the absorption of astaxanthin in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Aquac Res* 33:343-350.