Food Sci. Biotechnol. Vol. 18, No. 2, pp. 533 ~ 536 (2009)

Food Science and Biotechnology

RESEARCH NOTE

Establishment of Withdrawal Time of Erythromycin for the Cultured Black Rockfish (*Sebastes schlegeli*) after Oral Administration

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Abstract Withdrawal time of erythromycin in the cultured black rockfish (*Sebastes schlegeli*) was investigated to provide regulatory authority and fishery industry with full information needed to secure food safety of the fish treated with erythromycin. Medication was carried out using the experimental diet containing erythromycin of which concentration was 200 mg/kg diet and its daily dose was about 40 mg/kg body weight. The withdrawal time needed to reduce antibiotic contents around 10.0 mg/kg accumulated during 9 days medication below 0.2 mg/kg was identified about 10 days. The antibiotic with 13.7 mg/kg of fish on the 9th days of medication was completely depleted after 30 days from stop of medication.

Key words: erythromycin, liquid chromatography tandem mass spectrometry, black rockfish, withdrawal time

Introduction

Erythromycin has been used to prevent and treat for streptococosis (1) in olive flounder (*Paralichthys olivaceus*) and black rockfish (*Sebastes schlegeli*) that occupied more than 80% of total cultured fish production in Korea (2). At 2004, the total used amount of erythromycin was reported as 7,266 kg in Korea and ranked the second place followed to oxytetracycline (3).

Erythromycin belongs to macroride group antibiotics and is produced by *Saccharopolyspora erythraea* known as *Streptomyces erythraeus* (4). Erythromycin shows a bactericidal activity to Gram-positive cocci (*Staphylococci, Strepotococci*), Gram-positive bacilli (*Bacillus anthracis, Corynebacterium* sp., *Clostridium* sp.) and Gram-negative bacilli. But most Enterobacteriaceae (*Pseudomonas* sp. and *Escherichia coli*) has resistance against erythromycin.

At the present time, erythromycin being used for aquaculture has been supplied by thiocianate style in Korea and permitted for olive flounder, black rockfish, red sea bream (*Pagrus major*), yellow tail (*Seriola quinqueradiata*), eel (*Anguilla japonica*), rainbow trout (*Oncorhynchus mykiss*), sweetfish (*Plecoglossus altivelis*), tilapia (*Oreochromis niloticus*), and fish eggs (5). Erythromycin shows good effect for Streptococosis and it has been also used to prevent and treat bacterial enteritis such as Streptococosis and Edwardsiellosis in the cultured fish (1).

In EU committee, 0.2 mg/kg of muscle has been established as permission level of erythromycin for most food products (6). But someone urge that the permission level of erythromycin (0.2 mg/kg of muscle) should be changed to ‘not detected’ (7) because most EU countries do not allow the use of erythromycin for food production. In the USA, erythromycin has been managed as ‘not approved drug’ for aquaculture (8). In Japan, the permission level of erythromycin was established as 0.2 mg/kg of muscle for salmonidae, anguillidae, crustacean, shellfish, and other fishes, and 0.06 mg/kg for perciformes according to the positive system (9).

In Korea, erythromycin also has been used for stockbreeding and its permission level is 0.1 mg/kg for beef, pork, and chicken, and 0.125 mg/kg for turkey (10, 11). In case of seafood products, while it has been permitted the use for aquatic drug, its permission level is not established yet.

The Guidance of Aquatic Drug Use issued by National Fisheries Research and Development Institute (NFRDI) of Korea, they recommend the withdrawal time of erythromycin for black rockfish as 30 days (5). However they do not mention about the permission level adopted to establish withdrawal time in this guidance.

In this study, a recommended withdrawal time of erythromycin for black rockfish was investigated for satisfaction of 2 kinds of permission level, the one is 0.2 mg/kg adopted by EU and Japan, and the other is ‘not detected’ as a hypothetic permission level to secure safety of seafood products.

Materials and Methods

Fish species The black rockfish used for the experiment were purchased from a fish farm located in Tongyeong, Gyeongnam, Korea in April, 2007. The fish were 2 years

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Received October 18, 2007; Revised December 18, 2008;
Accepted December 19, 2008

old and their average body weight was about 150 g. The fish had no identified fish disease externally. Two-hundred-fifty fish were taken into separated 2 round type fish tanks with name of A and B (φ 2.5 m, 4 kL) installed in the Aquaculture Environment Institute of the NFRDI, respectively. Before the experimental medication, the fish were habituated at the same tanks for 1 week.

Medication of erythromycin For the establishment of withdrawal time of erythromycin for black rockfish, the medication and reduction of erythromycin in black rockfish were checked at the same time in each fish tank, A and B, from April 15 to May 25. Medication for the sample fish were carried out by oral administration using extruded pellet (EP) including erythromycin.

The experimental diet for the medication was prepared with 1 kg of the commercial EP diet and 0.2 g of commercial erythromycin (20% erythromycin in the drug). The medication dose of erythromycin was controlled to about 40 mg/kg body weight from daily dose of fish meal with 0.5% of body weight.

Sampling Three to 5 fish had been sampled from A and B tank at fixed time every day, respectively, during medication. After expiration of medication, diets without erythromycin were supplied and 5 fish had been sampled at scheduled time interval for 30 days. The sampled fish were eviscerated and filleted. The filleted meat was supplied for erythromycin analysis.

Erythromycin analysis Erythromycin analysis was performed according to the method developed by Park *et al.* (12). Erythromycin in the fish meat was extracted with 0.2% meta-phosphoric acid (Sigma-Aldrich, St. Louis, MO, USA) and methanol (Merck Co, Darmstadt, Germany). The concentration of erythromycin in the extract was analyzed using liquid chromatography tandem mass spectrometry (LC/MS/MS, Quattro Premier XE; Waters, Milford, MA, USA) system. Erythromycin standard was purchased from Sigma-Aldrich. Calibration curve was made based on the chromatogram that were produced from 3 times injection of same concentration ranged 10-0.005 μg/mL of erythromycin.

To detect the erythromycin standard and the erythromycin in the sample extract, the tandem mass spectrometry system equipped with ultra performance liquid chromatography (UPLC) system (Acquity; Waters) and UPLC exclusive column (Acquity UPLC™ BEH C_{18}, 1.7 μm, 2.1 mm i.d. ×100 mm; Waters) were used. The LC/MS/MS operation condition for erythromycin detection was shown in the Table 1. The target mass of the erythromycin was selected as parent ion *m/z* 734.3 and daughter ion *m/z* 576.3, 158.1.

Establishment of withdrawal time of erythromycin for black rockfish A permission level or tolerance limit of erythromycin in fish products was not established yet in Korea. In this study, therefore, the withdrawal time of erythromycin was calculated for reference of 0.2 mg/kg of fish meat that is a permission level in EU Committee and Japan, and a hypothetical permission level, 'not detected'.

Table 1. Operation conditions of LC/MS/MS for detection of erythromycin

MS/MS conditions				
Ionization	ESI, positive			
MRM transition (*m/z*)	734→576.2			
	734→157.9			
Cone voltage (V)	36			
Collision energy (eV)	28, 20			
Desolvation gas flow (L/hr)	800			
UPLC conditions				
Column	Acquity UPLC BEH C_{18} 1.7 μm			
	Time (min)	A (%)	B (%)	
Mobile phase[1)] A: 0.1% formic acid in DW B: 0.1% formic acid in ACN	Initial	90	10	
	1.0	90	10	
	5.0	10	90	
	5.1	90	10	
	6.0	90	10	
Flow rate	0.3 mL/min			
Oven temperature	50°C			
Injection volume	5 μL			

1)DW, distilled water; ACN, acetonitrile.

Results and Discussion

Erythromycin accumulation and discharge in the cultured black rockfish Average body weight and total length of the sampled fish of tank A were 155.5±39.7 g and 21.4±2.1 cm, respectively. Average water temperature in the tank during the experiment was 13.6±1.2. Erythromycin concentration in the fish muscle was increasing continuously during medication periods. The erythromycin concentration in the fish muscle had kept around 10.0 mg/kg as maximum value of each sampling day from the 4th to 8th day of medication and the maximum value of the erythromycin concentration increased to 12.0 mg/kg at the 9th day (Table 2).

The individual difference of the erythromycin concentration in the fish muscle sampled at the same day was significant and the maximum difference was about 12.0 mg/kg. The erythromycin concentration in the fish muscle was rapidly decreased after stop of medication and the erythromycin concentration was decreased from 12.0 mg/kg at the 9th day of medication to 0.05 mg/kg on the 5th day after stop of medication. Although the erythromycin concentration was decreased slowly after the 5th day of stop of medication, the erythromycin in the fish muscle was completely depleted on the 30th day after stop of medication (Fig. 1).

In this experiment, to get objectivity, the tank B was also prepared with the same operation condition as the tank A. Average body weight and total length of the sampled fish in the tank B were 156.7±37.5 g and 21.5±1.9 cm, respectively. And average water temperature in the tank during the experiment was 13.6±1.2.

The erythromycin accumulation trend in the tank B was a little bit different from the tank A. The accumulated

Table 2. Accumulation of erythromycin in the cultured black rockfish in the fish tank A and B

Time (day)	Concentration (mg/kg)											
	No. 1		No. 2		No. 3		No. 4		No. 5		Average	
	A	B	A	B	A	B	A	B	A	B	A	B
Before feeding	ND[1)]	ND	ND	ND	ND	ND	-	-	-	-	ND	ND
Feeding 1-d	0.488	0.025	0.009	0.090	0.018	0.354	-	-	-	-	0.171±0.274	0.129±0.195
Feeding 2-d	0.008	0.008	2.700	0.009	6.763	0.404	-	-	-	-	3.157±3.400	0.140±0.228
Feeding 3-d	0.146	0.008	6.891	0.026	4.750	1.300	-	-	-	-	3.929±3.447	2.633±0.814
Feeding 4-d	0.054	0.126	8.766	0.044	8.000	0.044	-	-	-	-	5.606±4.824	0.071±0.047
Feeding 5-d	0.027	7.019	1.351	0.785	9.696	0.062	-	-	-	-	3.691±5.243	2.622±3.825
Feeding 6-d	9.200	2.945	9.700	2.868	9.100	3.000	-	-	-	-	9.533±0.289	2.938±0.066
Feeding 7-d	0.178	1.700	5.223	3.000	10.014	3.200	-	-	-	-	5.138±4.919	0.305±0.187
Feeding 8-d	9.308	0.144	0.150	6.018	3.544	1.326	7.993	-	-	-	5.249±4.199	2.496±3.107
Feeding 9-d	10.371	13.700	12.000	5.877	1.546	13.000	1.155	3.700	0.018	8.350	5.018±5.687	8.925±4.368

[1)]Not detected.

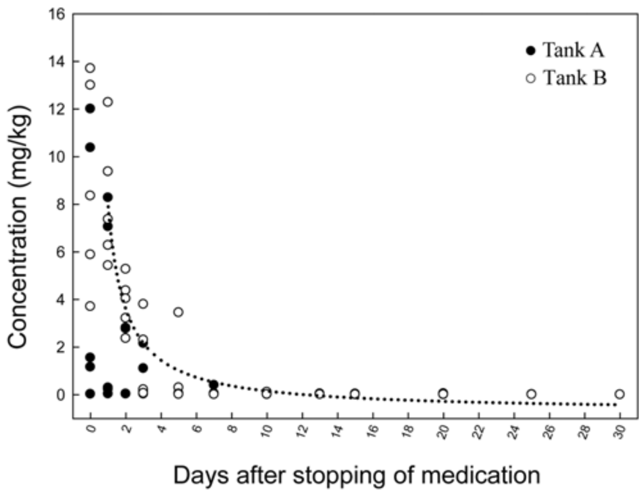

Fig. 1. Changes of erythromycin concentration in the cultured black rockfish after oral administration (40 mg/kg body weight) in the fish tank A and B.

erythromycin concentration in the tank B after 4 days medication was near 0.071±0.047 mg/kg and very lower than that in the tank A with the erythromycin concentration of 5.606±4.824 mg/kg (Table 2).

After 5 days of medication, the erythromycin concentration was rapidly increased and reached at 13.7 mg/kg on the 9th day of the medication. The individual difference was similar to the tank A. After 9 days medication, the maximum erythromycin concentration was 13.7 mg/kg and the minimum value was 3.7 mg/kg in fish muscle. The individual difference was estimated due to individual feeding difference.

The erythromycin depletion trend after stop of medication in the tank B was similar to tank A. From the 1st day of stop of medication, the erythromycin concentration in the fish muscle was sharply decreased and on the 2nd day, the concentration decreased to 5.27 mg/kg. On the 3rd day, the erythromycin was decreased below 0.1 mg/kg in some fish muscle. On the 7th day of stop of medication, the erythromycin in the all samples was decreased below 0.1 mg/kg. From the 8th day, the erythromycin concentration was decreased slowly and the erythromycin was completely depleted from all samples on the 30th day of stop of medication (Fig. 1).

Establishment of withdrawal time of erythromycin for the cultured black rockfish From above results, the erythromycin concentration in the fish muscle was continuously increased during medication with 9 days. However, the erythromycin concentration was rapidly decreased at early time of stop of medication from the 1st day to the 5th day and decreased below 0.2 mg/kg on the 10th day of stop of medication. And then the erythromycin was decreased slowly and was completely depleted in the all samples on the 30th day of stop of medication. From the results of this study, the proper withdrawal time of erythromycin for black rockfish will be 10 days if we adopt the permission level in EU and Japan (0.2 mg/kg of fish products) and the proper withdrawal time will be 30 days if we establish the permission level of erythromycin as 'not detected', the proper withdrawal time will be 30 days.

The erythromycin was shown very specific characteristics such as rapid accumulation during medication and very rapid depletion after stop of medication. These characteristics may be very profitable to secure food safety of antibiotic treated cultured fish rather than other antibiotics such as oxytetracycline or spiramycin (13).

Esposito *et al.* (7) reported the proper withdrawal time of erythromycin for 0.2 mg/kg that is the permission level in EU and Japan for rainbow trout was 22 days when they were medicated as 100 mg/kg of trout body weight/day for 21 days by oral administration.

Moffit and Schreck (14) reported the withdrawal time of erythromycin for 'not detected' in Chinook salmon (*Oncorhynchus tshawytscha*) was 10 days when they were medicated same as Esposito *et al.* (7). Fairgrieve *et al.* (15) reported the withdrawal time of erythromycin for 'not detected' in Chinook salmon was 28 days when they were medicated same as Esposito *et al.* (7).

These results including results of this study suggest that a withdrawal time of erythromycin could be different by fish species, medication strength, and period.

However, the withdrawal time of erythromycin for the cultured black rockfish suggested in this paper can be applied to actual industry case to secure food safety of the erythromycin treated cultured fish product because the medication strength and period applied in this study were higher and longer than those in actual application of this antibiotic in the aquaculture industry in Korea.

Acknowledgments

This research was supported by grant from Brain Korea 21 Program of the Ministry of Education Science and Technology.

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