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A prebiotic effect of *Ecklonia cava* on the growth and mortality of olive flounder infected with pathogenic bacteria

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ABSTRACT

Olive flounder (*Paralichthys olivaceus*), also known as the Japanese flounder in Japan, is one of the most important commercial marine finfish species cultured in Korea and Japan. The purpose of this study was to evaluate how a species of brown algae (*Ecklonia cava, E. cava*) affects the growth rate of olive flounder and its immune response to pathogenic bacteria. First, the experimental fish were divided into four groups: the control group was fed the diet containing only 1.0% *Lactobacillus plantarum* (*L. plantarum*), group I was fed 1.0% *L. plantarum* and 1.0% *E. cava* (EC), group II was fed 1.0% *L. plantarum* and 0.1% ethanol extract of EC (EE), and group III was fed 1.0% *L. plantarum* and 0.5% EE. The diets fed to the fish twice a day for 16 weeks. The results indicated that supplementation with 1.0% EC and 0.1% EE improved the growth and body weight of olive flounder, and decreased its mortality. This diet, however, did not significantly affect the biochemical profiles of the experimental flounder. The supplementation of 1.0% EC also enhanced the innate immune response of the fish, as evidenced by the high respiratory burst, and increased serum lysozyme and myeloperoxidase activity. The addition of 1.0% EC and either 0.1% or 0.5% EE also decreased the accumulative mortality of olive flounder infected by pathogenic bacteria (*Edwardsiella tarda, Streptococcus iniae, and Vibrio harveyi*). Overall, these results suggest that *E. cava* can act as a prebiotic by improving the innate immune response in fish infected with pathogenic bacteria as increased the growth of the probiotic.

1. Introduction

During the last 15 years, cultured fish production has more than doubled, and marine aquaculture industries in South Korea now produce a number of fish species [1–4]. In particular, the olive flounder is one of the most important commercial marine aquaculture species in Korea, and improvements to culturing techniques have increased its production [5]. Recently, however, outbreaks of infectious disease caused by viruses, bacteria, and parasites in aquaculture facilities have inflicted severe damage to fish production in Southeast Asia, which in turn has become a major economic problem [2]. Bacteria have been identified as being responsible for the majority of economic losses [6]. In cultured olive flounder, the most common pathogenic bacteria are *Edwardsiella tarda (E. tarda), Streptococcus* sp. and *Vibrio* sp. [7]. *E. tarda* affects both freshwater and marine fish, causing septicemia, skin lesions, and diseases of the muscles and internal organs, including the liver, kidney, and spleen [8]. *Streptococcus* sp. infection in fish is considered as a re-emerging disease affecting a variety of wild and cultured fish. These infections are actually a complex of diseases caused by different genera and species, and affect the central nervous system, causing suppurative exophthalmia and meningoencephalitis [6]. Pathogenic *Vibrio* sp., the etiological agent of classical vibriosis, is widely distributed, and it causes hemorrhagic septicemia in a variety of warm- and cold-water fish species of economic importance [6]. Researchers have studied the treatment of bacterial diseases using antibiotics and chemotherapeutics [9–12]. However, antibiotics use in aquaculture may be detrimental to the environment and human health, and it causes the development and transfer of resistance to other aquatic bacteria [13], fish pathogens, and human pathogens, and the accumulation of residual antibiotics in aquaculture products [13–17].

Probiotics, especially lactic acid bacteria (LAB), have been widely employed to protect fish against infectious diseases [18], including

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edwardsiellosis, furunculosis, and vibriosis [19–21]. In addition, LAB probiotics are safe to use in fish that will be consumed and they also protect against harmful pathogens by inhibiting the growth of *Aeromonas salmonicida*, *Vibrio anguillarum*, and *Flavobacterium psychrophilum*, as shown in rainbow trout [19,21]. For these reasons, the use of probiotics as a strategy to control fish pathogens is increasing [22–25]. Interestingly, prebiotics can enhance the growth of probiotics and induce their production of bioactive secondary metabolites. Therefore, probiotics and prebiotics can be useful in the study of how such components can limit bacterial disease in olive flounder.

Recently, the biological roles of *Ecklonia cava (E. cava), a brown seaweed contains* polyphenol and polysaccharide compounds as major components have been reported [26,27]. Especially, LBA-fermented *E. cava* increased the protein contents in comparison with the non-fermented *E. cava* [26,28]. However, until now, there is no report about effect of *E. cava* as a prebiotic in cultured olive flounder.

Therefore, the present study investigated the use of *E. cava* as a prebiotic in cultured olive flounder and evaluated its effect on the immunological response of fish infected with pathogenic bacteria.

2. Materials and methods

2.1. Preparation of experimental diets containing E. cava and L. plantarum

E. cava was collected along the coast of Jeju Island in South Korea during the period from March to May 2011 and identified by Dr. Lee (Jeju National University, South Korea, voucher specimen: Jeju-C-47). It was washed with fresh water, freeze-dried, and then pulverized into powder with a grinder. A previous study indicated the effect of *E. cava* polysaccharide on the growth of probiotics was similar to that of the original *E. cava* powder [26]. Therefore, the original *E. cava* powder (EC) was used in this study. To prepare the ethanol extract of *E. cava* (EE), which contains rich polyphenols with antibacterial effects, the powder (20 kg) was homogenized in 2 L of 100% ethanol. After 24 h, the EE was evaporated, freeze-dried, and then kept at −20 °C for later experiments. The experimental diets were prepared by adding 0.1% EE, 0.5% EE, or 1.0% EC to the soft extruded pellets (Daebong susan, Jeju, Korea, Table 1). In addition, 1.0% of *Lactobacillus plantarum* (*L. plantarum*) was added to all of the diets as a probiotic. The experimental diets were fed to all fish twice a day for 16 weeks.

2.2. Preparation of cultured olive flounder fish for the field trial

Olive flounder (mean body weights 300–350 g) were purchased from a private hatchery (Geumdeung, Jeju Island, South Korea) and acclimated for one week. The field trial was performed in Manhae susan (Pyoseon-ri, Pyoeonmyeon, Jejudo, South Korea) for 16 weeks (February 2013 to May 2013). Cylindrical polypropylene (PP) tanks (8 m in diameter and 1.5 m in height) were used to house the fish. Each tank was stocked with 1800 olive flounders, and the water was changed 10–15 times a day before samples were taken during the feeding trial. Seawater temperature (16–18 °C), dissolved O content (8.23–9.37 mg/L), salinity (27.68–31.36 ppt), and pH (7.5–8.7) were maintained in their respective ranges during the experimental feeding period. During the 16-week trial, the fish were fed their respective diets (as described in Section 2.1.1.) twice a day at 06:00 h and 17:00 h.

The experimental fish were divided into four groups: the control group was fed the diet containing only 1.0% *L. plantarum*, group I was fed 1.0% *L. plantarum* and 1.0% EC, group II was fed 1.0% *L. plantarum* and 0.1% EE, and group III was fed 1.0% *L. plantarum* and 0.5% EE.

2.3. Measurement of body weight and mortality in olive flounder

During the experimental period, the body weights and mortality of the olive flounder were recorded every 4 weeks for 16 weeks.

2.4. Analysis of biological indices in the serum of olive flounder

To investigate the effects of EC and EE on the biochemical blood profiles of the experimental olive flounder, blood samples were collected from the fish by a heparin-coated needle at 0 and 16 weeks of the experimental period. Individual fish were sampled only once to prevent multiple bleeds and stress from affecting the assays. The blood was allowed to clot or 30 min and then kept at 4 °C for 3 h. Then, the clotted samples were centrifuged at 3000 rpm for 10 min at 4 °C and the serum was collected and used to analyze total protein (TP), triglycerides (TRIG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), glucose (GLU), phosphorus (PHOS), cholesterol (CHOL), and hematocrit (HT) with biochemical analysis kits (STANBIO, TX, USA) and an automatic analyzer (Boehringer Mannheim, Mannheim, Germany).

2.5. Effects of EC and EE on the innate immunity of olive flounder

Normally, lysozyme can either act as an innate opsonin, or have lytic activity. To evaluate the effects of EC and EE on the innate immunity of olive flounder, we investigated respiratory burst activity by measuring neutrophils, lysozyme function, and myeloperoxidase (MPO) activity with slight modifications of previously suggested methods [1,29]. This testing was done using previously collected serum samples at 0 and 16 weeks into the experimental period.

2.6. Polymerase chain reaction analysis

To determine whether the olive flounder was successfully infected with the three administered bacteria (*E. tarda, Streptococcus iniae (S. iniae) and Vibrio harveyi (V. harveyi)*), samples of kidney and liver tissue from fish injected with the bacteria were subjected to polymerase chain reaction (PCR) analysis. The cDNA purified from the kidney and liver samples was used with the primers of the three bacteria (Bioneer, Daejeon, South Korea), shown in Table 2. Then PCR analysis was performed for 40 cycles with a 5 min denaturing step at 94 °C, a 1 min annealing step at

Table 1
The composition of the experimental diets in all the groups.

Experimental groups	Soft extruded pellets						EC (%)	EE (%)	*L. plantarum (%)*
	CP	CF	Ca	Ash	Fi	P			
Control group	37	10	1.5	17	3.0	2.7	–	–	1.0
EC 1.0%-fed group	37	10	1.5	17	3.0	2.7	1.0	–	1.0
EE 0.1%-fed group	37	10	1.5	17	3.0	2.7	–	0.1	1.0
EE 0.5%-fed group	37	10	1.5	17	3.0	2.7	–	0.5	1.0