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Distinct immune tones are established by *Lactococcus lactis* BFE920 and *Lactobacillus plantarum* FGL0001 in the gut of olive flounder (*Paralichthys olivaceus*)

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ABSTRACT

The immune tone is defined as an immunological state during which the readiness for immune response is potentiated. The establishment of immune tone in the gut of olive flounder (*Paralichthys olivaceus*) was investigated by feeding *Lactococcus lactis* BFE920 (LL) or *Lactobacillus plantarum* FGL0001 (LP). LL-fed flounder showed significantly increased levels of regulatory genes (*FOXP3*, *IL-10*, and *TGF-β1*), *CD18*, and *CD83* in the gut. In contrast, LP feeding drastically increased proinflammatory genes (*T-bet*, *IL-1β*, and *IFN-γ*) and *CD18*. This indicates that LL and LP establish different types of local immune tones in the gut through differential activation of innate immune cells: LL activates both macrophages and dendritic cells while LP activates macrophages only. Both of the immune tones required at least a total of 6 probiotic feeds during 72 h for a stable establishment. Once established, the type of immune tone remained steady even up to 30 days (a total of 60 feeds) probiotics feeding. The LL-induced regulatory immune tone enhanced the level of *occludin*, a tight junction molecule, significantly more than that observed with the proinflammatory immune tone established by LP feeding. Consequently, LL-fed fish showed considerably lower gut permeability than that of the LP-fed group. Furthermore, when orally challenged by *Edwardsiella tarda*, LL-fed flounder survived at a significantly higher rate than LP-fed fish. The data clearly demonstrate that individual probiotics establish distinct types of immune tone in the fish gut, which in turn influences the immunological status as well as the physiology of the gut. Selection of proper probiotics may be essential for optimal effects in aquaculture farming.

1. Introduction

The gut is an important mucosal organ that is exposed to various antigens all the time, such as food-driven antigens and microbes. The innate and the adaptive immune systems localized in this organ respond not only to foreign antigens but also to intrinsic microorganisms in the gut of mammals [1–4] as well as fish [5–8]. This gut microbiota refers to the collection of microbial populations that inhabit the gut, including bacteria, fungi, parasites, and viruses. In humans, more than 100 trillion microorganisms, mostly bacteria, colonize in the distal intestine. Recent findings have revealed the importance of the modulatory effects of the gut microbiota on the host's immune system [9–13]. This immunological modulation can result in either health by enhancing immunity, or disease via dysregulation of homeostasis. Innate immune cells, such as macrophages and dendritic cells (DCs), respond to gut microbiota differentially depending upon the types of microbes. The immune cells react to commensal microbiota with weak immune responses, in contrast, they act on pathogens with strong inflammatory immune responses. The immune responses initiated by innate immune cells in the gut are further extended into the modulation of the whole immune system by influencing the adaptive immune cells [10,11,13].

Innate immune cells recognize microbes through interactions between pattern recognition receptors (PRRs) expressed on the immune cells and microbe-associated molecular patterns (MAMPs) expressed by microorganisms. The MAMPs are structural elements of microorganisms, such as lipopolysaccharide (LPS), peptidoglycan,

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Table 1
Primers used for qRT-PCR. Abbreviations: OF, olive flounder; F, forward; R, reverse. Gene Bank accessions are noted for target genes according to each primer set. mRNA sequence information of genes noted in this study are provided in supplementary materials.

Primers	Sequences (5′–3′)	Target genes	Accessions
OF-β-actin-RT-F	TGCAGAAGGAGATCACAGCC	β-actin (reference gene)	HQ386788.1
OF-β-actin-RT-R	ACTCCTGCTTGCTGATCCAC		
OF-CD4-1-RT-F	AGTGAGCACGGACAATGGAG	CD4-1	AB716323.1
OF-CD4-1-RT-R	CACGATGACTGAAGCGATGC		
OF-CD4-2-RT-F	GGGTCTCTCACAAGCACACA	CD4-2	AB716324.1
OF-CD4-2-RT-R	CCCTTCCACTCAACAGGGTG		
OF-CD8a-RT-F	AGCCTGTGTTTGTAAGGGCA	CD8α chain	AB082958.1
OF-CD8a-RT-R	AGGAGAAGGAGAAGGCCACA		
OF-T-bet-RT-F	TGTGGGAAAGCAGAGGGCAA	T-bet (TBX21)	KR822591.1
OF-T-bet-RT-R	ATCCAGTGGGCTCCGGTGTT		
OF-GATA3-RT-F	GCCCACCATCACCCCATC	GATA3	This study
OF-GATA3-RT-R	GCCCTCTGAACAGGACCTTG		
OF-FOXP3-RT-F	CACACTGGGTTGAGGACAGG	FOXP3	This study
OF-FOXP3-RT-R	AGCCATGCTGGGATAACTCC		
OF-RORγ-RT-F	TAACCTGGACATGCTGGGTGG	RORγ	This study
OF-RORγ-RT-R	AATGCCGAGGTTGCCATCTACA		
OF-IL1b-RT-F	ACTTCTTCCTGTCATGCCAG	IL-1β subunit	AB720982.1
OF-IL1b-RT-R	CCGCTGTCCTGCTTGTAGAA		
OF-IL10-RT-F	TTTCAAAAGCCCGTTTGCGT	IL-10	KF025663.1
OF-IL10-RT-R	TTGGTTTCCTCCGTCACTCC		
OF-IL17-RT-F	AGGCGGCCAATCTAAACACT	IL-17A/F	This study
OF-IL17-RT-R	CAGTGGTCTGACGTTTGTGG		
OF-TGF-β-RT-F	CAGCGAACACGAGCCAAACAC	TGF-β1	This study
OF-TGF-β-RT-R	TGTTCTGAGGGATGGACATGGTG		
OF-IFN-γ-RT-F	CAGCTGCCGAACACGACTCC	IFN-γ	AB435093.1
OF-IFN-γ-RT-R	TCCGTCCTGACGCTGACCTC		
OF-CD18-RT-F	TGGGAGGAAATGGTTCGTGT	CD18	KR998307.1
OF-CD18-RT-R	AGCTGTTGGCACCACATACA		
OF-CD83-RT-F	CGGCACGACGACATACTACT	CD83	KR998303.1
OF-CD83-RT-R	ATCCCTGTTCTGCTCTCCCA		
OF-OCLN-RT-F	TCTTTGCTCTGAAGACCCGC	Occludin	This study
OF-OCLN-RT-R	ATTGTTCACCCATGCCTCCA		

teichoic acids, flagellin, and DNA. The MAMP receptors, namely PRRs, are also diverse and are localized on the membrane as well as in the cytosol of innate immune cells [10,14]. Individual microorganisms can induce distinct immune responses due to differences in the interactions between MAMPs and PRRs, and their downstream signaling pathways. Therefore, symbiosis or dysbiosis of gut microbiota can maintain or improve health, or cause diseases. Extensive studies demonstrate that probiotics can modify the symbiosis of the gut microbiota and improve health [9,15–17]. Probiotics have been defined as “live microorganisms which when administered in adequate amounts confer a health benefit on the consumer” [18]. Probiotic treatment in humans is now widely accepted as a promising therapeutic and prophylactic strategy for certain diseases, such as inflammatory bowel diseases.

Probiotics have also been proved to be beneficial to fish. These findings are well reviewed by Nayak [19], Lazado and Caipang [20], and Akhter et al. [21]. Probiotics feeding enhances fish health through improvement of innate immunity, such as serum complement system, lysozyme activity, phagocytosis activity, and respiratory burst activity [19]. In addition, probiotics modulate the cytokines released by innate and adaptive immune cells in the fish [20,21]. However, the mechanism underlying the probiotics-mediated immune modulation is poorly understood not only in the fish system but also in the mammalian system. Previously, it was reported that *Lactococcus lactis* (*Lc. Lactis*) BFE920 (LL) and *Lactobacillus plantarum* (*Lb. plantarum*) FGL0001 (LP) showed probiotics effects when fed to olive flounder (*Paralichthys olivaceus*) individually or in combination [22,23]. Interestingly, however, LL and LP induced different types of immune modulation. We, therefore, attempted to investigate the different types of immune modulation induced by two different probiotics, namely LL and LP. These probiotics established distinct types of immunological potentiation, defined as immune tone, in the gut in a time-dependent manner. Therefore, we also studied the physiological effect of the immune tone on the gut.

Our data strongly suggested that LL and LP establish distinct types of immune tones in the gut of olive flounder, an inflammatory immune tone and a regulatory immune tone. Both types of immune tones stabilized when the probiotics were fed twice a day for three days. Once established, the immune tone remained the same even with up to 30 days feeding. Under the regulatory immune tone, the integrity of the gut significantly improved by inducing the expression of the gut *occludin*, a tight junction molecule, which indicates that the immune tone influences not only the immune readiness for reacting to pathogens but also the physiology of the gut.

2. Materials and methods

2.1. Experimental animals

Olive flounder weighing 42.7 ± 1.61 g were separated into three groups containing 40 fish per group as follows: Control (Ctrl), *Lc. lactis* BFE920 (LL)-fed group, and *Lb. plantarum* FGL0001 (LP)-fed group. Flounder were maintained in a 300 L tank at 17 °C with a closed circulation system. Olive flounder were kindly provided by the GyeongSangbuk-Do Fisheries Technology Center, Pohang, Korea.

2.2. Probiotics feeding and sampling

Bacterial culture, maintenance, and feed preparation were performed as described elsewhere [23]. Briefly, each of LL and LP probiotics (10^7 CFU/g) were adsorbed onto commercial extruded