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Immunotherapies Targeting Fish Mucosal Immunity – Current Knowledge and Future Perspectives

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In recent years, studies on the mucosal immunity in fish species have shown much progress. Although there are some organs such as skin, gills, and gut are directly associated with the mucosal immunity of fish species, this mini review emphasizes the general knowledge on the role and production figures of skin mucus and factors affecting the secretion of skin mucus of fish species. As the skin mucus of fish species is the first defense line for protection against invading microorganisms such as pathogens (bacteria, virus), parasites, etc., the information for understanding the roles of the skin mucus is very important. Furthermore, the information in the review will shed light on the development of high quality aquafeeds for the sustainable aquaculture field as well.

Keywords: skin mucus, immunostimulant, functional feed, aquaculture, immunity

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GENERAL INTRODUCTION

In fish mucosal immunity, the innate components of the immune system are the first barrier by which the host fish are protected from the attack of microorganisms. Gut, skin, and gills in fish species are the major mucosal surfaces and immune barriers. The mucus is one of the most important components for fish mucosal immunity. In adaptive immunity, on the other hand, there are many important components such as immunoglobulins (Igs), B and T lymphocytes, etc. [see the review by Gomez et al. (1)]. In this review, recent knowledge and findings on fish skin mucus are focused.

The presence and importance of innate immune parameters in the epidermal mucus on fish is well documented in the past (2, 3). It has been found that the epidermal layer of the skin is important because it secretes the mucus in addition to providing physical protection. The skin mucus plays a key role as the first defense line for protection in aquatic animals (2–5), and fish epidermal mucus contains numerous innate immune components such as glycoproteins, lysozyme, complement proteins, lectins, C-reactive proteins, flavoenzymes, proteolytic enzymes, and antimicrobial peptides as well as immunoglobulins (3, 6–9).

The external constituent of this barrier is a mucous gel that forms a layer of adherent mucus covering the epithelial cells (10) and is secreted by various epidermal or epithelial mucus cells (7). The fish skin mucus is mainly composed of water and glycoproteins (11), containing a large amount of high molecular weight oligosaccharides, namely mucins (12, 13). Mucin is one of the most important components in fish mucus. Previously, it was found that there are two structurally separate groups of mucins such as large secreted gel-forming mucins and membrane-bound forms (14). Recently, Perez-Sanchez et al. (15) challenged in the study of gilthead sea bream, *Sparus aurata*, to clarify mucin gene family, the tissue-specific expression pattern of mucins, and to determine whether mucins were altered by nutritional conditions and parasite infections. They identified six sequences, such as intestinal mucin (I-Muc), mucin2 (Muc2), mucin2-like (Muc2-like), mucin13

(Muc13), mucin18 (Muc18), and mucin 19 (Muc19), in which I-Muc, Muc13, and Muc18 are the members of membrane-bound-mucins, and Muc2, Muc2-like, and Muc19 are of secreted gel-forming mucins. Furthermore, the mucin gene expression pattern was tissue specific with a relatively low expression level in skin, gills, and stomach, respectively, but Muc18 is a major mucin in fish skin (15). According to the study of Van der Marel et al. (16) on carp, the mucin5B was mostly expressed in the skin, and its expression was up-regulated when β-glucan was administered.

It has been demonstrated that fish skin mucus is involved in the respiration, osmoregulation, reproduction, locomotion, defense against microbial infections, disease resistance and protection, etc. (7, 17). Fish skin mucus is continuously produced, and the structure and/or functions prevent the pathogen adherence to the underlying tissues and provide a medium in which antibacterial mechanisms may act (8). In a study on salmonids, Roberts and Powell (18) found that Atlantic salmon and brown trout responded to amoebic gill disease with a whole-body mucus, but rainbow trout did only with a gill mucus.

Recently, comparative studies on fish mucus for both freshwater and marine species were reported (8, 9). Five freshwater species such as mrigal, rohu, catla, rita, and spotted snakehead were used, and the components in mucus, namely lysozyme, proteases, phosphatases, esterase, and sialic acid were investigated in the study (8). The study indicated that enzyme activity depended on the species and/or habitat environment. For example, the values were high in mrigal and snakehead, but low in rohu and catla, which are relatively distributed in clean water. On the other hand, the levels of all factors measured in rita was found to be low. Serine and metalloproteases were the major mucus proteases in all fish species in the study. In marine species, five species such as gilthead seabream, European sea bass, shi drum, common dentex, and dusky grouper were investigated (9). The study showed that skin mucus contains *N*-acetylneuraminic acid, glucose, *N*-acetyl-glucosamine, *N*-acetyl-galactosamine, galactose, and fucose residues. It was also indicated that although IgM and lysozyme activity were very similar among species tested, protease, anti-protease, alkaline phosphatase, esterase, and peroxidase activities varied depending on the fish species tested. The mucus of gilthead seabream revealed high bactericidal effects against pathogenic bacteria not for the non-pathogenic ones.

To characterize the mechanisms of mucus as the first-line defense against pathogens, the study of Guardiola et al. (19) demonstrated that gilthead seabream skin mucus contained lower contents of IgM, similar level of lysozyme alkaline phosphatase and proteases, and higher level of esterase, peroxidase and antiprotease activities than those in serum. And the skin mucus revealed stronger bactericidal activity than the serum activity. Furthermore, a recent study on Atlantic salmon against salmon louse indicated difference in skin immune responses between the selected families (20) although increased mucus secretion by the Atlantic salmon when infected by salmon lice has been indicated (21). The study of Holm et al. (20) suggested that the ability to resistance against salmon lice depends on avoiding immunosuppression and not as much on the physical tissue barrier functions, but they assumed that increased mucous secretion by the Atlantic salmon might be important for parasite survival as nutritive elements for developing lice.

FACTORS AFFECT THE MUCUS PRODUCTION IN FISH

To produce healthy cultured aquatic species is one of the most important and not so easy tasks for fish farmers with the sustainable operation. To achieve this, it is needed to promote the immunological responses of aquatic species under culture conditions. Therefore, it would be very important to know the role and efficient action of fish skin mucus. In recent years, there were several studies on the effects of functional substances and micronutrients with measuring mucus production and status.

Those like probiotic bacteria, oligosaccharide, β-glucan, etc. have been tested on mucus production (22–29). Epidermal mucus was enhanced by intake of lactic acid bacteria in Atlantic salmon (22) and mannan oligosaccharide in sea bass (24). Rodriguez-Estrada et al. (25) showed that the mucus production increased in rainbow trout-fed diet-containing inactivated *Enterococcus faecalis* or mannan oligosaccharide or a combination of both. Hoseinifar et al. (26) indicated in a study on freshwater swordtail that *Lactobacillus acidophilus* as feed supplement was effective on enhancing antibacterial activity of skin mucus, and the skin mucus protein level and alkaline phosphatase activity were also higher in *Lactobacillus* fed groups. They suggested that the recommended inclusion level was 6×10^8 CFU/g. Recently, feeding trials were conducted to determine the effects of heat-killed *Lactobacillus plantarum* (HK-LP), β-glucan, and inactivated *Pediococcus pentosaceus* on immunological responses as well as growth performances of marine fish (27–29). Mucus secretion of red sea bream fed with all diet-containing HK-LP was higher than that fed with a HK-LP-free diet, and the value from a diet with 1000 ppm HK-LP concentration was significantly higher than that from a HK-LP-free diet (27). Dawood et al. (28) demonstrated that mucus secretion of red sea bream was significantly affected by either HK-LP or β-glucan, but they did not affect mucus bactericidal activity (**Table 1**). Relative amount of mucus of red sea ream fed with a diet containing 1000 ppm HK-LP together with 1000 ppm β-glucan was significantly higher than that with the basal diet. It was also found in their study that the mucus lysozyme

TABLE 1 | Mucus status of red sea bream fed diets containing different levels of heat-killed *Lactobacillus plantarum* (HK-LP) and β-glucan (BG).*

HK-LP (ppm)	BG (ppm)	LA(unit/ml)	BA (10^5 CFU/ml)	Total amounts (relative value)
0	0	31.6^a	5.71	1.00^a
250	0	37.0^{ab}	5.69	1.07^{ab}
500	0	37.9^b	5.34	1.02^{ab}
1000	0	38.7^b	6.67	1.12^{ab}
250	1000	42.3^b	6.19	1.08^{ab}
500	1000	42.8^b	7.47	1.10^{ab}
1000	1000	40.5^b	6.70	1.15^b

**Within a column, values with different letters are significantly different ($P < 0.05$). LA, lysozyme activity; BA, bactericidal activity.*

activity significantly increased with increased HK-LP levels without β-glucan supplement while with β-glucan it did not change.

In a *Pediococcus* study, Dawood et al. (29) found that the lysozyme activity in mucus of red sea bream was affected by the concentrations of inactivated *Pediococcus pentosaceus*, and the lysozyme activity was significantly higher in fish fed with a diet containing 1.6×10^{12} concentration than in fish fed with the basal diet (**Table 2**). Furthermore, mucus was significantly more secreted in *Pediococcus pentosaceus*-fed groups.

Other than probiotic bacteria, some micronutrients like vitamins have been found to be effective for mucus secretion. When red sea bream was fed with a diet containing 325 ppm vitamin C, lysozyme activity of skin mucus seemed to increase compared to that fed a vitamin C-free diet (30). Ren et al. (31) indicated in a study on Japanese eel that fish fed with diets containing 762 ppm vitamin C showed significantly higher lysozyme activity and bactericidal activity of mucus than fish fed with a diet with 32 ppm vitamin C. Furthermore, the mucus bactericidal activity was further enhanced with supplementation of dietary lactoferrin. Furthermore, it was found in a study on Caspian roach that dietary vitamin C significantly elevated skin mucus alkaline phosphatase, protein levels, and antimicrobial activity compared to a vitamin C-free group (32). As a functional supplement, lactoferrin has been tested to improve the health status of aquatic animals. Yokoyama et al. (33) demonstrated that mucus secretion significantly increased in spotted grouper when fed with diets containing lactoferrin from 400 to 1200 ppm compared to that of fish fed with a lactoferrin-free diet and concluded that oral lactoferrin administration could be an effective method to improve natural barriers of finfish.

TABLE 2 | Mucus status of red sea bream fed with diets containing different concentrations of inactivated *Pediococcus pentosaceus.**

Concentration (cells/g)	LA (unit/ml)	Total amounts (relative value)
0	32.5[a]	1.00[a]
1.6×10^{10}	37.1[ab]	1.41[b]
1.6×10^{11}	34.6[a]	1.42[b]
1.6×10^{12}	51.3[b]	1.40[b]
3.2×10^{12}	40.8[ab]	1.51[b]

**Within a column, values with different letters are significantly different (P < 0.05).*
LA, lysozyme activity.

In conclusion, since the skin mucus plays a key role as the first-defense line for protection of aquatic animals, controlled skin mucus secretion is very important for them to improve the survival, particularly for aquacultured species, leading to the fact that important components in mucus also increase. Although several dietary materials induce the increase of mucus secretion in aquatic animals, the effects against parasites are still under investigation. On the other hand, when aquatic animals are under stress conditions, the mucus secretion will also increase. Thus, the difference of mucus production between normal and stressed conditions should be carefully investigated.

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