

# A Review On Composition Of Epidermal Mucus, A Key Factor In Fish Health

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## Abstract:

Innate immune components found in fish epidermal mucus are released by goblet cells, which serve as a barrier between the fish and its local habitat and offer the first line of defense against many harmful bacteria. Microbe trapping and sloughing is one of mucus' primary roles. Innate immunity is provided by a variety of substances found in mucus, including lysozymes, lectins, proteases, and antimicrobial peptides (AMPs). A range of pathogens were susceptible to the antibacterial action of the AMPs released by epidermal mucus cells. Furthermore, mucosal lysozyme was observed to have strong bacteriolytic activity, whereas several proteases discovered in fish skin mucus have the ability to eliminate infections by either cleaving their protein or by inducing an immune response. Lectins are another type of mucosal agglutinin that have a variety of functions in innate immunity, including complement activation and opsonization. Fish's epidermal mucus, which is shown to be relatively temperature independent, offers an inbuilt, quick-acting, non-specific defense. The current review's objective is to give a thorough understanding of the many elements of epidermal mucus, such as AMPs, proteases, and lysozymes, as well as how they work against infections.

Keywords : AMP, Mucus, Immunity, Lysozyme, Proteases

## Introduction

Fish farms that have large fish densities are vulnerable to illnesses brought on by a variety of viruses. Fish skin mucus serves as the first line of physical defense against viruses, among its many unique and intricate defense mechanisms against these pathogenic diseases (Wang et al., 2011). The mucus on fish skin acts as a steady chemical or physical barrier to keep viruses out. Fish mucus is a slimy, slippery coating that covers the epithelial surfaces of the fish. It can also be described as a viscous colloid known as mucins that contains water, proteins, and antibacterial enzymes. It performs two crucial functions for the innate immune system. First of all, it inhibits the adhesion of pathogens, the stable colonization of potentially infectious bacteria, and the invasion of parasites by generating constantly and being shed periodically (Arasu et al., 2013). Second, lysozyme, immunoglobulin, complement proteins, lectins, C-reactive protein (CRP), proteolytic enzymes, transferring, alkaline phosphatase (ALP), and numerous other antibacterial proteins and peptides are among the many components of innate immunity that it contains (Arockiaraj et al., 2012, 2014; Arasu et al., 2013). There are many harmful organisms in the water environment. Fish and other aquatic creatures are unaware of how

vulnerable they are to the invasion of these diseases (Dash et al., 2008). As a result, fish skin mucus is important since it acts as their first line of defense and is continuous throughout their bodies, including the fins. Numerous processes are facilitated by mucus, such as immunity to disease, protection, respiration, ionic and osmotic regulation, reproduction, excretion, communication, feeding, and nest construction (Ingram, 1980). The immune system of the skin mucosa, its various components, and their possible function in innate immunity are the main topics of this paper. To gain a better understanding of the antibacterial qualities of skin mucus and to help create antimicrobial drugs for therapeutic purposes, the antimicrobial role of skin mucus has also been explored.

## Analysis

Fish's skin mucosa is a vital barrier that protects them from both biotic and abiotic elements in their environment. There are two components to the mucosa: humoral and cellular. The extracellular chemicals found in skin mucus make up the humoral part, whereas the cellular portion is made up of the mucous membrane and the connective tissue that supports it.

(2011) Salinas et al. Fish skin mucosa is made up of a variety of substances, including lipids, proteins, carbohydrates, and metabolites (Zaccone et al., 2001).

Fish mucus contains a variety of vital proteins and enzymes that have been identified as being important to fish innate immunity, including proteases, antimicrobial peptides (AMP), lectins, lysozyme, immunoglobulin, complement proteins, CRP, transferrins, ALP, and numerous other antibacterial proteins and peptides (Shoemaker et al., 2005; Swain et al., 2007). High molecular weight glycoproteins called mucins are found in mucus and provide it viscoelastic and rheological characteristics. Although neutral glycoproteins can be found in fish mucus, sulfated or carboxylated monosaccharides, such as silic acid, frequently cause them to become acidic. According to Rose and Voynow (2006), repeated areas rich in threonine, serine, and proline are commonly found in mucins. Additionally, there aren't many carbohydrates in fish mucus. Some protective roles have been proposed, despite the fact that their functions are not clearly defined (Esteban, 2012). It has been observed that various saturated fatty acids (SFA), monosaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA) are present in fish skin mucus. Stearic and palmitic acids are the SFA found in mucus. Oleic acid is a monosaturated fatty acid. Alpha-linolenic, moroctic, and linoleic acids are examples of polyunsaturated fatty acids. It is thought that these fatty acids are crucial for pathogen protection (Balasubramanian and Gunasekaran, 2015). Azelaic acid, N-acetylneuraminic acid, N-acetylglucosamine, and hydroxyisocaproic acid are among the few metabolites with antibacterial qualities that have been found in fish skin mucus (Ekman et al., 2015). The makeup of mucus differs throughout fish species. Numerous endogenous (such as sex, developmental stage) and exogenous (such as stress, hyperosmolarity, pH, and infections) variables affect the mucus cells and the composition of the mucus (Ellis, 2001). There are times when fish specimens have a lot of proteins on their mucus, particularly when they are scared or hurt. These fish's epidermis secretes a gel-like substance that sticks to the skin for several days, even when they swim at different speeds (Esteban, 2012).

Fish epidermis contains three types of mucus-secreting cells: goblet cells, sacciform cells, and club cells. On the epidermal surfaces of all fish, but especially on the gill surfaces, are plenty of goblet cells that secrete mucus. Mucus granules produced by these cells rupture to discharge their contents. Sialylated, sulfated, or neutral glycoproteins are

seen in goblet cells (Shephard, 1993). Mucus is produced when goblet cell secretions combine with those of other secretory cells that have been discovered. These include acidophilic granular cells, often known as serous goblet cells, and sacciform cells. The latter generate basic proteins as opposed to glycoproteins (Zaccone et al., 2001). Sacciform cells' secretions provide a defensive and regulating function in addition to being crinotoxic and repulsive, similar to the granular glands of amphibians. Greater amounts of protein and fewer amounts of carbohydrates are present in the secretions from club cells (Fasulo et al., 1993; Zaccone et al., 2001).

### **Skin mucus components' function in innate immunity**

Because of its components, which include proteases, AMPs, lysozyme, and CRP, mucus appears to play a vital role in providing fish with effective separation between their internal and external environments, abrasion resistance, and primary defense against several aquatic infections. These are covered in more detail down below.

Fish mucus contains a range of proteases, which play a vital part in the innate immune processes. Proteases are a class of enzymes whose catalytic purpose is to hydrolyze peptide bonds of proteins (Ingram, 1980). Pathogens and other foreign compounds are frequently broken down by these proteases.

Depending on the chemical groups involved in catalysis, the proteases can be roughly categorized into four classes: metalloproteases, aspartic proteases, cysteine proteases, and serine proteases (Hartley, 1960). According to reports, serine protease makes up more than 25% of the complement system and is one of the main mucus proteases in a variety of fish species, including *Cirrhinus mrigala*, *Labeo rohita*, *Catla catla*, *Rita rita*, and *Channa punctata* (Nonaka and Miyazawa, 2002; Nigam et al., 2012). Fish mucus from rainbow trout, coho and Atlantic salmon, Japanese eel, and Salles et al., 2007; Subramanian et al., 2007; Aranishi et al., 1998; Morrissey, 1998; Cho et al., 2000a; Firth et al., 2000; Fast et al., 2012) has been found to contain proteases such as trypsin (serine protease), cathepsin B and L (cysteine proteases), and metalloproteases. It has also been shown that proteases in skin mucus contribute to fish's innate defenses against infections (Ingram, 1980). The skin's proteases either directly attack a pathogen or indirectly stop it from invading by changing the quality of mucus, which causes the pathogens to peel off the body's surface (Aranishi et al., 1998). Proteases are also thought to

stimulate and improve the Psciene system's synthesis of innate immunity components such as complement, immunoglobulins, and AMPs (Aranishi, 1999). Fish skin mucus has been shown to include a number of proteases with diverse functions. For example, cathepsin D helps catfish skin mucosa produce parasin I, a strong antimicrobial peptide, from histone H2A. Procathepsin D and a metalloprotease are rendered inactive by cathepsin D, and procathepsin D is then cleaved to produce active cathepsin D. In order to create AMPs like parasin I, active cathepsin D cleaves the Ser19-Arg20 link of histone H2A (Cho et al., 2002a). Firth et al. (2000) reported the production of a serine protease resembling trypsin in the skin mucus of Atlantic salmon, *Salmo salar*, in response to infection by the salmon louse, *Lepeophtheirus salmonis*. Aranishi and Nakane (1997) revealed the presence of L-like proteases, cathepsin B, and aminopeptidase in the dorsal surface of the European eel (*A. anguilla*) and the epidermal cell layer of the Japanese eel (*Anguilla japonica*). High bacteriolytic activity was demonstrated by cathepsins B and L against fish pathogens *Flavobacterium columnare*, *Edwardsiella tarda*, and *L. anguillarum* (Aranishi, 1999; Aranishi, 2000). Five Indian carps were used for the analysis, and the results showed that *C. punctata* and *C. mrigala* had significant protease activity whereas *L. rohita* and *C. catla* had low activity (Nigam et al., 2012). Of the three principal carp species found in India, *C. mrigala*, *C. catla*, and *L. rohita*, the protease activity of the latter's epidermal mucus was shown to be the greatest in another study (Dash et al., 2014).

Antimicrobial peptides are present in a variety of taxa, including microorganisms, plants, and animal species. They are becoming more widely acknowledged as an essential component of the host defense system (Fernandes et al., 2004; Kennedy et al., 2009). Since fish rely more on their innate immune system than do mammals, it is also more significant in fish (Hancock, 1997; Hancock and Scott, 2000). All of the major classes of peptides, including defensins, cathelicidins, hepcidins, histone-derived peptides, and a fish-specific class of the cecropin family termed piscidins, are expressed in fish, which makes them an excellent source of these AMPs (Valero et al., 2013). Fish and human pathogens are also killed by the fish peptides, demonstrating broad-spectrum antibiotic action (Das et al., 2013). Their genes are extremely responsive to innate immunostimulatory chemicals and microorganisms, and they can also be immunomodulatory (Masso-Silva et al., 2014). Broad range cationic molecules with low

molecular weight peptides (size <10 kDa; length 12-50 amino acids) with a net positive charge due to an excess of basic lysine and arginine residues over acidic residues are commonly referred to as antimicrobial peptides. They fold into three-dimensional amphiphilic structures due to the presence of disulphide bridges or contact with membranes. Nonetheless, reports of some anionic AMP forms have also been made (Vizioli and Salzet, 2002). The amphipathic  $\alpha$ -helical structures of antimicrobial peptides are similar to those of a wider group of naturally occurring short polypeptides, which can interact strongly and permeate phospholipid membranes (Rakers et al., 2013). The ability of AMPs to act without high specificity or memory is one of its advantages. These can also be mass stored, produced at low metabolic cost, and made easily accessible upon infection. These kinds of compounds are ideal for interacting with the hydrophilic head groups and hydrophobic cores of negatively charged bacterial membranes.

According to Dash et al. (2011), lysozyme plays a crucial role in the innate immune system and mediates defense against pathogenic invasion. It is a leucocytic-derived mucolytic enzyme. All lysozymes have the same ability to hydrolyze  $\beta$ -(1,4)-glycosidic linkages between the peptidoglycan of bacterial cell walls' alternating N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) residues. This leads to rapid cell lysis in an environment with low osmotic pressure. Apart from bacteria, it has also been observed that lysozyme inhibits viruses (Lee-Huang et al., 1999), parasites (Leon-Sicairos et al., 2006), and fungi (Wu et al., 1999) even if their envelopes do not contain the usual peptidoglycan. Additionally, the enzyme targets muramic acid-containing structures, hydrolyzes glycol chitin, and has a limited ability to degrade chitin, a significant constituent of fungal cell walls and some invertebrate exoskeletons (Wu et al., 1999). Additionally, lysozyme stimulates polymorphonuclear leucocytes and macrophages, which directly or indirectly stimulates phagocytosis through an opsonic action. Due to its hydrolytic activity, alkaline phosphatase, a lysosomal enzyme found in fish epidermal skin mucus, has been demonstrated to have antibacterial properties (Dash et al., 2011; Dash et al., 2014; Guardiola et al., 2014). The fish's level of ALP rose in response to stress, parasite infection, skin regeneration, and the early phases of wound healing (Rai and Mittal, 1983; Iger and Abraham, 1990, 1997; Ross et al., 2000). Unless the fish were moved from freshwater to sea water, ALP in the skin mucus of rainbow trout,

coho salmon, and Atlantic salmon was not found (Fast et al., 2002). Numerous stressors, including acidity, heat elevation, contaminated water, and distilled water, have been shown in studies to increase the quantity of alkaline phosphatase-positive Rodlet cells in rainbow trout skin (Iger and Abraham, 1997). Rodlets' non-specific defense mechanisms are aided by the presence of ALP at their perimeter and peroxidase activity in their centers.

## Prospects for the future and conclusion

Understanding the mucosal immune response of fish mucus has advanced significantly in the last few years, which has benefited the expanding aquaculture market globally. The need to create new antimicrobial medications to combat illnesses has drawn more focus to the antibacterial properties of fish mucus and how they might be used to treat illnesses brought on by a range of viruses. To do so, a deeper comprehension of the mucosal innate immune system is required.

stop and manage infectious fish illnesses. Fish epidermal skin mucus contains a variety of significant bactericidal compounds that may be used as a source for innovative antibacterial components in aquaculture procedures. Fish with varying structural profiles generate antimicrobial peptides that may be utilized to create new therapeutic agents that can combat infections that are resistant to drugs.

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