Review Article

Epidermal mucus, a major determinant in fish health: a review

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Summary

Fish epidermal mucus contains innate immune components, secreted by globlet cells that provide the primary defence against different pathogenic microbes and act as a barrier between fish and its immediate niche. The major function of mucus includes entrapment and sloughing of microbes. The mucus also contains many factors such as antimicrobial peptides (AMPs), lysozymes, lectins, proteases, etc that provide innate immunity. The AMPs secreted by epidermal mucus cells displayed antimicrobial activity against a variety of pathogens. Besides, mucosal lysozyme was found to produce significant bacteriolytic action whereas different proteases found in skin mucus of fish can kill the pathogens by cleaving its protein or by activating immunological mechanisms. Lectins are also mucosal agglutinins that play a diverse role in innate immunity like opsonization, activation of complement, etc. Epidermal mucus in fish thus provides an innate and fast acting protection which is non-specific and is found to be relatively temperature independent. The aim of the present review is to provide a broad overview of the different components of epidermal mucus including AMPs, proteases, lysozymes as well as their mode of action on pathogens.

Key words: Antimicrobial peptides, Innate immunity, Lysozyme, Mucus, Proteases

Introduction

The aquaculture industries with high densities of fishes are susceptible to diseases caused by many pathogens. Fish possess numerous distinct and complex defense mechanisms to protect themselves from these pathogenic infections amongst which fish skin mucus acts as the first line of physical defense against pathogens (Wang *et al.*, 2011). The fish skin mucus provides a stable physical or chemical barrier against the invading pathogens. In fish, the epithelial surfaces are covered by a slimy, slippery layer called the mucus. It can also be defined as a viscous colloid containing the antibacterial enzymes, proteins and water, etc known as mucins. It serves as an important component of innate immune mechanism in two ways. Firstly, by producing continuously and being sloughed off regularly, it prevents the adherence of pathogens and stable colonization of potential infectious microbes and invasion of parasites (Arasu *et al.*, 2013). Secondly, it contains a number of factors of innate immunity like proteins and enzymes such as lysozyme, immunoglobulin, complement proteins, lectins, C-reactive protein (CRP), proteolytic enzymes, transferring, alkaline phosphatise (ALP) and various other antibacterial proteins and peptides, etc (Arockiaraj *et al.*, 2012, 2014; Arasu *et al.*, 2013).

The aquatic environment is rich in pathogenic organisms and the aquatic animals including fish are obliviously prone to the invasion of these pathogens (Dash *et al.*, 2008). Therefore, the skin mucus in fish plays a significant role as it provides the first line of defence and is continuous with the linings of all body openings covering the fins also. The mucus has a wide range of functions including disease resistance, protection, as well as respiration, ionic and osmotic regulation, reproduction, excretion, communication, feeding and nest building (Ingram, 1980).

This review focuses on the immune mechanism of skin mucosa and their different components along with their potential role in innate immunity. Further, the antimicrobial role of the skin mucus has been discussed to provide a better understanding on the antimicrobial properties of skin mucus which could be useful in development of antimicrobial agents for therapeutic applications.

Components of fish epidermal mucus

The skin mucosa of fish is an essential barrier and serves as a protection against the surrounding environment with biotic and abiotic factors. The mucosa consists of a cellular and a humoral part. The cellular part consists of the mucous membrane and its underlying connective tissue and humoral part consists of the extracellular molecules present in the skin mucus

(Salinas *et al*., 2011). The skin mucosa of fish has different components such as proteins, carbohydrates, lipids, metabolites (Zaccone *et al*., 2001).

Many important proteins and enzymes such as proteases, antimicrobial peptides (AMP), lectins, lysozyme, immunoglobulin, complement proteins, CRP, transferrins, ALP and various other antibacterial proteins and peptides have been characterized in fish mucus which plays a significant role in innate immunity of fishes (Shoemaker *et al*., 2005; Swain *et al*., 2007). The mucins present in mucus are high molecular weight, glycoproteins that impart viscoelastic and rheological properties to the mucus. Neutral glycoproteins may be found in the fish mucus but are often made acidic by sialic acid (a carboxylated monosaccharide) or sulfated monosaccharides. Mucins typically possess repetitive regions rich in threonine, serine and proline (Rose and Voynow, 2006). The fish mucus also contains few carbohydrate components. Although their functions are not well defined, some protective roles have been suggested (Esteban, 2012). The skin mucus of fish is reported to contain different saturated fatty acids (SFA), monosaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The SFA in the mucus are palmitic acid and stearic acids. Monosaturated fatty acid is the oleic acid. Polyunsaturated fatty acids are linoleic, alpha linolenic and moroctic acid. These fatty acids are believed to play an important role of defense against pathogens (Balasubramanian and Gunasekaran, 2015). The fish skin mucus also reported to contain few metabolites having antibacterial properties such as azelaic acid, N-acetylneuraminic acid and N-acetylglucosamine, and hydroxyisocaproic acid (Ekman *et al*., 2015). Mucus composition varies amongst different fish species. The mucus cells and the composition of the mucus are influenced by various endogenous (e.g. sex, developmental stage) and exogenous factors (e.g. stress, hyperosmolarity, pH and infections) (Ellis, 2001). In some occasions, especially when fish specimens are frightened or injured a high amount of proteins are present on mucus. The epidermis of such fish secretes a gel-like material which adheres to the skin even when they swim at varying speeds and for several days (Esteban, 2012).

There are three mucus secreting cells in the epidermis of fish namely goblet cells, sacciform cells and club cells. Mucus secreting globlet cells are abundant on all fish epidermal surfaces and particularly on gill surfaces. These cells produce mucus granules which burst to release their contents. Goblet cells contain sialylated, sulfated or neutral glycoproteins (Shephard, 1993). Other secretory cells have also been identified whose secretions mix with the goblet cell secretions to give rise to mucus. These include sacciform cells and acidophilic granular cells (orserous goblet cells) wherein the latter produce basic proteins rather than glycoproteins (Zaccone *et al*., 2001). Sacciform cells may be analogous to granular glands of amphibians which produce crinotoxic and repellent substances but secretions of these cells also have a protective and regulatory role. The club cell secretes have larger proteinaceous and lesser carbohydrate components (Fasulo *et al*., 1993; Zaccone *et al*., 2001).

Role of skin mucus components in innate immunity

The major function of the mucus appears to provide the effective separation between internal and external environment of fish, abrasion resistance and primary defense against the numerous aquatic pathogens by virtue of its components which include proteases, AMPs, lysozyme, CRP. These are discussed in detail below.

Proteases

Protease refers to a group of enzymes whose catalytic function is to hydrolyze peptide bonds of proteins and mucus of fish contains a variety of proteases which have a significant role in the innate immune mechanisms (Ingram, 1980). These proteases are often responsible for degrading pathogens and other foreign substances.

The proteases are broadly classified into four categories such as serine proteases, cysteine proteases, aspartic proteases and metalloproteases depending upon the chemical groups responsible for catalysis (Hartley, 1960). Serine protease is reported as one of the major mucus proteases in several fish species such as *Cirrhinus mrigala*, *Labeo rohita*, *Catla catla*, *Rita rita* and *Channa punctata* and comprises more than 25% of the complement system (Nonaka and Miyazawa, 2002; Nigam *et al*., 2012). Proteases such as trypsin (serine protease), cathepsin B and L (cysteine proteases), cathepsin D (aspartic protease), and metalloproteases have been identified in fish mucus of Rainbow trout, coho and Atlantic salmon, Japanese eel (Aranishi and Nakane, 1997; Aranishi *et al*., 1998; Morrissey, 1998; Cho *et al*., 2000a; Firth *et al*., 2000; Salles *et al*., 2007; Subramanian *et al*., 2007; Fast *et al*., 2012). Proteases in skin mucus are also reported for their role in the natural resistance of fish against pathogens (Ingram, 1980). The proteases in skin directly act on a pathogen or may indirectly prevent pathogen invasion by modifying mucus consistency to increase the sloughing of mucus and removing pathogens from the body surface (Aranishi *et al*., 1998). Furthermore, proteases are also believed to activate and enhance the production of innate immune components like complement, immunoglobulins or AMPs in Psciene system (Aranishi, 1999). Several proteases have been characterized in the skin mucus of fishes that display various activities, for e.g. cathepsin D participates in the production of parasin I, a potent antimicrobial peptide from histone H2A in catfish skin mucosa. Cathepsin D inactivates proenzyme procathepsin D and a metalloprotease, which cleaves procathepsin D to generate active cathepsin D. The activated cathepsin D in turn cleaves the Ser19-Arg20 bond of histone H2A to produce AMPs like parasin I (Cho *et al*., 2002a). Similarly, expression of a typsin like serine protease in the skin mucus of Atlantic salmon, *Salmo salar*, in response to the infection with salmon louse, *Lepeophtheirus salmonis* was demonstrated by Firth *et al*. (2000). Aranishi and Nakane (1997) demonstrated the presence of aminopeptidase, cathepsin B and L-like proteases in the epidermal cell layer of Japanese eel (*Anguilla japonica*) as well as from dorsal surface of European eel (*A. anguilla*). Cathepsins B and L exhibited high bacteriolytic activity against the fish pathogens *Edwardsiella tarda*, *Flavobacterium columnare* and *L. Anguillarum* (Aranishi, 1999; Aranishi, 2000). The analysis of skin mucus of five Indian carps demonstrated high protease activity in *C. punctata* and *C. mrigala* and low activity in *L. rohita* and *C. catla* (Nigam *et al*., 2012). In another study, the protease activity of epidermal mucus of *L. rohita* was reported to be highest amongst the three Indian major carps species, i.e. *C. mrigala*, *C. catla* and *L. rohita* (Dash *et al*., 2014). Proteases isolated from skin mucus of different fish have been summarized in Table 1.

Lectins

The terms agglutinin, phytoagglutinin, hemagglutinin and lectin are interchangeably used to express the naturally existing proteins/glycoproteins with multiple binding sites capable of agglutinating cells or precipitating glycoconjugates (Denis *et al*., 2003). The diversity of these lectins has expanded their definition to include any protein containing a non-catalytic carbohydrate-recognition domain (CRD). One of the chief events in innate immune defense include the pattern-based recognition of microbial targets as "non-self" by host lectins and related proteins and their subsequent destruction by complement and/or phagocytic cells (Matsushita *et al*., 2004). These can recognize the non-self cells and enveloped viruses by the means of the carbohydrates present on their surface and then target them for destruction. They may recognize a specific site in sugar or whole sugar or a sequence of sugar and their glycosidic linkages such as glycoproteins and glycolipids or in bacterial polysaccharides on the cell surface glycoconjugates namely (Mercy *et al*., 1993).

Different types of lectins have been reported in epidermal mucus of fishes. Tsutsui *et al*. (2011) reported the presence of a new type of skin mucus lectin in cat fish *Silurus asotus* which displayed Ca^{+2} dependent mannose binding activity. A mannose binding lectin (MBL) that binds to pathogens was reported in Atlantic cod mucus (Rajan *et al*., 2011). Galectins of different forms were also demonstrated to have antibacterial activity (Stowell *et al*., 2014; Cha *et al*., 2015). Nattectin, a C-type lectin binding to galactose, was also reported in Atlantic salmon affected by amoebic gill disease (Valdenegro-Vega *et al*., 2014). Fructose binding lectin was reported in sea bass mucus (Cordero *et al*., 2015). Lectins are probably present in the skin mucus of many additional species, because the mucus of many fishes induces hemagglutination, a property typical of lectins (Suzuki, 1995). The sugar specificities, molecular weights and metal requirements of some fish mucus lectins have been summarized in Table 2.

The Japanese eel has two different types of lectin, AJL-1 and AJL-2. AJL-1 is a galectin, which is characterized by its specific binding to β-galactoside and is homologous to the lectins from Conger eel *C.*

Table 1: Proteases isolated from fish skin mucus

Proteases	Species	Function	References
Cathepsin D	Mucus surface of cat fish	Regulate production of parasin I	Cho *et al*. (2002a)
Serine proteases	Epidermal cell layer of Japanese eel/dorsal surface of European eel	Bacteriolytic activity against fish pathogen	Aranishi (1999); Aranishi (2000)
Amino proteases	Epidermal cell layer of Japanese eel/dorsal surface of European eel	Bacteriolytic activity against fish pathogen	Aranishi (1999); Aranishi (2000)
Amino peptidase	Epidermal cell layer of Japanese eel/dorsal surface of European eel	Bacteriolytic activity against fish pathogen	Aranishi (1999); Aranishi (2000)
Cathepsin B	Epidermal cell layer of Japanese eel/dorsal surface of European eel	Bacteriolytic activity against fish pathogen	Aranishi (1999); Aranishi (2000)
L-like proteases	Epidermal cell layer of Japanese eel/dorsal surface of European eel	Bacteriolytic activity against fish pathogen	Aranishi (1999); Aranishi (2000)
Metello proteases	Epidermal cell layer of Japanese eel/dorsal surface of European eel	Bacteriolytic activity against fish pathogen	Aranishi (1999); Aranishi (2000)
Trypsin like proteases	Rainbow trout/Atlantic salmon	Bacteriolytic activity against the Gram-negative fish pathogen/act against salmon louse	Firth *et al*. (2000)

Table 2: Sugar specificities, molecular weights and metal requirements of fish mucus lectins

Species	Erythrocytes	Sugar specificity	MW (kDa) native (subunit)	Metal requirement	References
Eptatretus stoutii	Human O				Spitzer *et al*. (1976)
Misgurnus anguillicaudatus	Human A, B; rabbit	Lactose, D-Man, D-GlcNac, NeuNac	>300	s-type	Kamiya *et al*. (1995)
Tachysurus australis	Human A, B, O; rabbit		>200		DiConza (1970)
Arias thallasinus	Human A, B, O; rabbit	D-Gal, lactose	200	s-type	Al Hassen *et al*. (1986)
Plecoglossus altivelis	Rabbit	D-Gal, L-Rha, D-Fuc, D-man			Itami *et al*. (1992)
Lophopsena maculata	Rabbit	NeuNac	68	s-type	Kamiya and Shimizu (1980)
Repomucenus richardsonii	Rabbit	D-Gal, lactose	48	s-type	Shiomi *et al*. (1990)

myriaster. In contrast, AJL-2 is a unique lectin, having a highly conserved sequence of C-type lectins but displaying Ca^{2+} independent activity (Tsutsui *et al*., 2003). Puff lectin, a mannose specific lectin purified from the skin mucus of the puffer fish, is the third type of lectin (Tsutsui *et al*., 2003). Puff lectin showed no sequence similarity with any known animal lectins but, surprisingly, shares sequence homology with MBLs of monocotyledonous plants. The fourth type of lectin was found in the pony fish which exhibits homology with rhamnose-binding lectins. Carbohydrates which are specific to this type of lectins are galactose, lactose, fucose, melibiose and rhamnose (Takashima and Hibiya, 1995). A lactose-specific lectin (pentraxin) of molecular mass 25 kDa was purified from the skin mucus of a cartilaginous fish *Raja kenojei* (Tsutsui *et al*., 2009). All these observations about lectins in fish mucus suggest that they actively participate in the self-defence system by acting on the external body surface.

Antimicrobial peptides

Antimicrobial peptides are increasingly recognized as a critical factor in host defence mechanism and found in organisms ranging from microbes, plants to animal species (Fernandes *et al*., 2004; Kennedy *et al*., 2009). It also plays an important role in fish when compared with mammals as fish rely more on their innate immune system (Hancock, 1997; Hancock and Scott, 2000). Fish are a great source of these AMPs and they express all of the major classes of peptides like defensins, cathelicidins, hepcidins, histone-derived peptides, and a fish-specific class of the cecropin family, called piscidins (Valero *et al*., 2013). The fish peptides exhibit broad-spectrum antimicrobial activity by killing both fish and human pathogens (Das *et al*., 2013). They can also be immunomodulatory and their genes are highly responsive to microbes and innate immuno-stimulatory molecules (Masso-Silva *et al*., 2014).

Antimicrobial peptides are generally defined as broad spectrum cationic molecule having low molecular weight peptides (size <10 kDa; length 12-50 amino acids) with a net positive charge because of the presence of excess basic lysine and arginine residues over acidic residues. They fold, owing to the presence of disulphide bridges or contact with membranes, into three dimensional amphiphilic structures. However, some anionic forms of AMPs have also been reported (Vizioli and Salzet, 2002). Antimicrobial peptides belong to a larger group of naturally occurring short polypeptides and share similar amphipathic α-helical structures that can interact strongly and permeate phospholipid membranes (Rakers *et al*., 2013). The advantage of the AMPs is that they can function without either high specificity or memory. Also these are synthesized at low metabolic costs, capable of mass storage and readily available after infection. Such molecules are well suited for interacting with bacterial membranes having negatively charged and hydrophilic head groups and hydrophobic cores. Antimicrobial peptides adopt several mechanisms for their activities: some appears to associate on the membrane surface and displace the outer bilayer (Shai, 2002) and others have shown to span the bilayer and associate to form the channel depolarizing the target cells. Few peptides have been proposed to cross the bilayer and interact with the microbial DNA similar to that of histones in eukaryotic DNA, disrupting the transcription and/or replication. Antimicrobial peptides can interact with bacterial, fungal or protozoan cells and many recognize all three of them. There is also evidence regarding the role of peptides in stimulation of other immune processes and tissue repair through their action as signalling molecules in host response (Hancock and Scott, 2000; Shai, 2002). A few of the important AMPs found in fish are described below:

Pardaxin

Pardaxin is 33 residues in length and has a helix-hinge-helix structure similar to cecropin and mellitin. This peptide shows characteristic pore forming properties in the metazoan membranes and also displays shark repellent properties. This AMP is highly effective against Gram +ve and Gram -ve bacteria. In fish, it was first reported from the skin mucus of Moses sole, *Pardachirus marmoratus* (Oren and Shai, 1986).

Pleurocidins and Moronecidins

Pluerocidin, a 25 residue cationic peptide was reported in winter flounder (*Plueronectes americanus*) and other white eyed flounders. Southern blot analysis of genomic DNA also revealed the presence of multigene families of pluerocidin related genes in Atlantic halibut (*Hippoglossus hippoglossus*), Yellow tail flounder (*Pleuronectes ferruginea*) and American plaice (*Hippoglossoides platessoides*) (Douglas *et al*., 2001). A 22 residue α-helix AMP, Monocidin is also found in skin and gill of Hybrid striped bass (*Morone chrysops* × *Morone saxatilis*). Like Paradaxin, monocidins showed wide spectrum activity against the Gram +ve and Gram -ve bacteria, fungi and yeast. Pluerocidins may provide pattern recognition with direct destruction and in many cases is likely to save the fish the cost of a more involved acute-phase response (Lauth *et al*., 2002).

Ribosomal peptides

A protein (6.7 kDa) isolated from the skin of Rainbow trout (*Oncorhynchus mykiss*) is effective against Gram +ve bacteria is found to be similar to the ribosomal protein S30 isolated from various species (Fernandes and Smith, 2002). Similarly, three 60S ribosomal proteins, L40, L36A, and L35 were identified from the skin of Atlantic cod which also reported to have antimicrobial activities (Bergsson *et al*., 2005).

Derivatives of histones

Certain AMPs in fishes are believed to be derived from various histones. Hipposin of Atlantic halibut (*H. hippoglossus*) and parasin I of Amur catfish (*Parasilurus asotus*), are identified to be the N-terminal fragments of H2A (Cho *et al*., 2002b). Hipposin, a 51 residue long cationic peptide effective against a large number of

Gram-positive as well as negative bacteria is found in the skin mucus of the healthy halibut (Birkemo *et al*., 2003). A 19 residue AMP, parasin I was discovered in the mucus of the injured fish. It was found to be quite similar to Buforin I, a H2A derived peptide in toads (Park *et al*., 1996). It was active against various Gram +ve and Gram -ve bacteria and fungi as well. Cleavage of large sized protein like cathepsin-D is believed to give rise to these AMPs. This protease is activated by matrix metalloprotease-2 whose production is increased during the immune response or tissue repair (Cho *et al*., 2002a). A 30-residue N-terminally acetylated peptide derived from the N-terminal part of histone H1 in skin mucus of Atlantic salmon (*S. salar*) (Luders *et al*., 2005) is termed as salmon antimicrobial peptide [SAMP H1] and found to be active against both Gram-negative and Gram-positive bacteria.

Amongst the cysteine-rich AMPs, cathelicidins, defensins, and liver-expressed antimicrobial peptides (LEAPs) are mostly found in teleosts (Silphaduang and Noga, 2001). Nevertheless, putative cathelicidins have also been reported in fish like Rainbow trout (Smith and Fernandes, 2009), Atlantic salmon (Chang *et al*., 2006), Arctic char (*Salvelinus alpines*), Atlantic cod and brook trout (*Salvelinus fontinalis*) (Maier *et al*., 2008). Furthermore, genes coding for cathelicidin has also been reported for jawless fish, like Atlantic hagfish (*Myxine glutinosa*) (Uzzell *et al*., 2003).

Defensins from teleost were identified by different molecular methodologies (Matsuzaki *et al*., 1999; Uzzell *et al*., 2003; Roussel and Delmotte, 2004; Zou *et al*., 2007; Maier *et al*., 2008; Casadei, 2009). The genomic data on the defensin indicate resemblance with the β-defensins of birds and mammals (Zhao *et al*., 2009). Falco *et al*. (2008) demonstrated antiviral activity against viral haemorrhagic septicaemia rhabdovirus (VHSV). Recently, three novel β-defensins from Rainbow trout were cloned that constitutively expressed that further increased during bacterial and simulated viral challenges (Zou *et al*., 2007). In another study, a β-defensin-like gene from the olive flounder has been identified and expressed in larval fish, one day after hatching (Nam *et al*., 2010).

Other cysteine-rich AMPs found in fish are the LEAPs (Park *et al*., 2001). The LEAP family include different peptides such as hepcidins isolated from winter flounder, turbot, and red sea bream, Sal-1 and Sal-2 from Atlantic salmon, JF-1 and JF-2 from Japanese flounder, and LEAP-2 from catfish and trout (Smith and Fernandes, 2009). Antimicrobial peptides isolated from fish skin mucus with their structure and specificity have been summarized in Table 3.

Lysozyme

Lysozyme is an important component of the innate immune system and mediates protection against pathogenic invasion (Dash *et al*., 2011). It is a mucolytic enzyme of leucocytic origin. The common feature of lysozyme is their ability to hydrolyse β-(1,4)-glycosidic bonds between the alternating N-acetylmuramic acid (NAM) and N-acetyl glucosamine (NAG) residues of peptidoglycan of bacterial cell wall resulting in rapid cell lysis in a hypo-osmotic environment. In addition to bacteria, lysozyme has also been reported to inhibit viruses (Lee-Huang *et al*., 1999), parasites (Leon-Sicairos *et al*., 2006) and fungi (Wu *et al*., 1999) despite the absence of typical peptidoglycan in their envelopes. The enzyme also attacks structures containing muramic acid, hydrolyses glycol chitin and has a restricted degrading effect on chitin, which is a major component of the cell walls of fungi and the exoskeletons of certain invertebrates (Wu *et al*., 1999). Lysozyme also promotes phagocytosis directly by activating polymorphonuclear leucocytes and macrophages or indirectly by an opsoninic effect.

Broadly, lysozymes are classified into five major types such as (i) chicken-type lysozyme (c-type) that includes stomach lysozyme and Ca^{+2} binding lysozyme,

Table 3: A few important antimicrobial peptides from fish skin mucus with their structure and specificity

Peptide	Species	Structure	Specificity	References
Pardaxin	Moses sole	Cationic, amphipathic, á-helix	Gram-positive and Gram-negative bacteria	Shai (1994)
Hipposin	Atlantic halibut	Histone H2A N-terminal fragment	Gram-positive and Gram-negative bacteria	Park *et al*. (1996)
Parasin I	Amur catfish	Histone H2A N-terminal fragment	Gram-positive and Gram-negative bacteria, fungi	Park *et al*. (1996)
Pluerocidin	Winter flounder	Cationic, amphipathic, á-helix	Gram-positive and Gram-negative bacteria	Douglas *et al*. (2001)
Piscidins	*Gadus morhua*	Cationic, amphipathic, á-helix	Bacteria	Fernandes *et al*. (2010); Ruangsri *et al*. (2012)
Epinecidins	*Epinephelus coioides*	Cationic, amphipathic, á-helix	Bacteria, virus	Yin *et al*. (2006); Peng *et al*. (2010)
Gaduscidins	Atlantic cod	Cationic, amphipathic, á-helix	Bacteria, virus	Browne *et al*. (2011)
Cathelicidins	Atlantic salmon	Cationic, amphipathic, á-helix	Bacteria	Chang *et al*. (2006); Bridle *et al*. (2011)
Grammistins	*Grammistes sexllineatus*	Cationic, amphipathic, á-helix	Bacteria	Shiomi *et al*. (2000); Sugiyama *et al*. (2005)
Oncorhyncin III	Rainbow trout	Cleavage product of the non-histone chromosomal protein H6	Gram-positive and Gram-negative bacteria	Douglas *et al*. (2001)
Moronecidins	Hybrid striped bass	Cationic, amphipathic, á-helix	Gram-positive and Gram-negative bacteria, fungi, yeast	Lauth *et al*. (2002)
SAMP H1	Atlantic salmon	Proline-rich histone H1 N-terminal peptide fragment	Gram-positive and Gram-negative bacteria	Luders *et al*. (2005)

(ii) goose-type lysozyme (g-type), (iii) plant-type lysozyme, (iv) bacterial lysozyme, and (v) T4 phage lysozyme (phage-type). However, in fishes only c- and g-type lysozyme have been reported (Beintema and Terwisscha van Scheltinga, 1996; Fastrez, 1996; Irwin *et al*., 1996; Prager and Jolles, 1996; Qasba and Kumar, 1997). The lysozyme response has been found to be variable in its potency depending on the species and the tissue location (Qasba and Kumar, 1997). It was reported that increase in mucus lysozyme activity in Rainbow trout and Atlantic salmon when infected with sea lice in the earlier days of infection that decreased further (Fast *et al*., 2002). However, lysozyme activities did not increase in coho salmon in earlier days, but increased significantly in the later stage of post infection. It appears that the lysozyme response in fish may be induced very rapidly and is not only related to bacterial presence but also to other alarm situations such as stress. Thus, lysozyme in fish would be involved in the overall alarm response, acting as an acute-phase protein. The estimation of lysozyme may be of diagnostic value to determine the disease status of fish.

Other innate immunity components in fish mucus

There are also a few important components of fish epidermal mucus which play an important role in innate immunity of fishes such as C-reactive proteins (CRPs), transferrin, ALP, complement proteins, etc. CRPs belong to a family of multifunctional proteins (pentraxins) and are capable of binding various ligands in a Ca^{2+} dependent binding affinity. These are characterized by their cyclic pentameric structure and high sequence similarity (Gewurz *et al*., 1995). The main ligand for CRP includes the phosphoryl choline moiety of pneumococcal C-polysachcharides (CPS) and phospholipids. C-reactive protein takes part in innate immune defense through activation of the complement pathways and plays an important role in the recognition and clearance of apoptotic cells (Nauta *et al*., 2003). It can also act as opsonin and can cause the precipitation and agglutination of macromolecules or microorganisms with surface phophorylcholine. Also, increased levels of CRP were found in the serum and mucus of *Tilapia mossambica* after induced physical injury which suggested its role in early detection of diseases (Ramos and Smith, 1978). Pentraxin-like molecules have been isolated from a number of teleost fish species including Atlantic salmon (*S. salar*), common wolfish (*Anarhichas lupus*), cod (*Gadus morhua*), halibut (*H. hippoglossus*) and Indian carp (*Catla catla*) (Lund and Olafsen, 2003).

Transferrin, an iron binding glycoprotein plays a significant role in transportation of iron between the sites of absorption, storage and utilization in vertebrates (Putnam, 1975). All organisms including microbes and parasites require iron for growth and it is considered to be an essential element in the establishment of infection (Sussman, 1974). Hence, transferrin plays an important role in innate defense mechanism of fish by binding iron and reducing its availability to the invading pathogens by chelating iron. It helps in countering the growth of pathogens until immune system can respond. The range of molecular weights of transferrins occurring in fishes is 70-80 kDa (Alexander and Ingram, 1992). Its presence as a non-specific immunity component has been reported in skin mucus of olive flounder (*Paralichthys olivaceus*) (Palaksha *et al*., 2008). Increased levels of cleaved transferrin fragments were detected after infection with sea lice (*L. salmonis*) in channel catfish (Ourth *et al*., 1991) and Atlantic salmon (Easy and Ross, 2009) as part of the fish's immune response to sea lice infection. Further, presence of transferrin was also reported in sea bass and sea bream (Cordero *et al*., 2015; Sanahuja and Ibarz, 2015).

Alkaline phosphatase, a lysosomal enzyme present in epidermal skin mucus of fish has been shown to act as an antibacterial agent because of its hydrolytic activity (Dash *et al*., 2011; Dash *et al*., 2014; Guardiola *et al*., 2014). The level of ALP increased in the fish during skin regeneration, in the initial stages of wound healing, stress and parasitic infection (Rai and Mittal, 1983; Iger and Abraham, 1990, 1997; Ross *et al*., 2000). In the case of Rainbow trout, coho salmon and Atlantic salmon ALP was undetected in the skin mucus unless the fish were transferred from freshwater to marine water (Fast *et al*., 2002). Studies have demonstrated several stressors such as acidity, thermal elevation, polluted water and distilled water caused increases in the number of alkaline phosphatase-positive Rodlet cells in the skin of Rainbow trout (Iger and Abraham, 1997). Rodlets contain ALP at their periphery and peroxidase activity at their cores which contributes to the non-specific defense mechanisms of skin of fish.

Complement system constitutes another important component of innate response in mucus. It contains a group of protein and non-protein components that play a major role in both innate and adaptive immunity. It contains approximately 35 plasma and membrane-bound proteins that mediate a chain reaction of proteolysis which results in the elimination of invading microorganisms (Boshra *et al*., 2006). Important complement proteins like C3, C7 and C1q have been reported in skin mucosa of Atlantic halibut (*H. hippoglossus*), grass carp (*Ctenopharyngodon idella*) and Siberian sturgeon (*Acipenser baerii*) respectively (Magnadottir *et al*., 2005; Shen *et al*., 2012; Fan *et al*., 2015).

Future perspectives and conclusion

In the past few years, great progress has been made in understanding the mucosal immune response of epidermal fish mucus which has benefitted the growing aquaculture industry worldwide. The need to develop alternative antimicrobial drugs to counter diseases has given rise to increased attention to the antimicrobial components of the fish mucus and the ways in which they can be harnessed against diseases caused by a variety of pathogens. For that, a better understanding of the mucosal innate immune system is needed in order to prevent and control infectious diseases in fishes. Epidermal skin mucus of fish possesses many important bactericidal substances which can be a potential source of novel antibacterial components in aquaculture practices. Antimicrobial peptides secreted by different fishes with structural variations can be used for development of novel therapeutic agents to treat drug resistant pathogens.

Conflict of interest

The authors confirm that there is no conflict of interest.

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