Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi
Menba Journal of Fisheries Faculty
ISSN 2147-2254 | e-ISSN: 2667-8659

Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2020; 6(2): 100-107 Derleme Makale/Review Article

A Review on Antibacterial Effects of Fish Skin Mucus and Fish Lectins

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Article Info

Received: 17/11/2020
Accepted: 09/12/2020

Keywords:
Antibacterial
Disease
Fish skin mucus
Lectin
in vivo

Abstract

The skin of many fish species is covered with a layer of mucus that acts as a barrier between the fish and the environment. Mucus, which plays an important role in disease resistance, contains lectins along with many other components. Lectins are proteins, that are naturally produced by many organisms, with carbohydrate recognition and binding properties. Lectins take part in intercellular communication, pathogen-host relationship, and immune response by binding to specific carbohydrates on cell surfaces. In innate immune response, lectins increase opsonization by binding to pathogenic microorganisms. Moreover, many *in vitro* studies, as well as a few *in vivo* studies have shown that certain lectins can exhibit bactericidal effects without needing the presence of phagocytes. These effects occur by agglutination of bacteria, preventing biofilm formation, or disrupting the cell wall structure. The antibacterial effects of fish mucus and fish lectins have been reported in many studies. In this review, some of these studies are summarized and the potential use of mucus and/or lectins as *in vivo* antimicrobial agents is evaluated.

Balık Mukusu ve Balık Lektinlerinin Antibakteriyel Etkileri Üzerine Bir Derleme

Makale Bilgisi

Alınış Tarihi: 17/11/2020
Kabul Tarihi: 09/12/2020

Anahtar Kelimeler:
Antibakteriyel
Balık mukusu
Hastalık
Lektin
in vivo

Öz

Birçok balık türünün derisi, balığın çevre ile arasında bir bariyer görevi gören bir mukus tabakası ile kaplıdır. Hastalık direncinde önemli bir rol oynayan mukus, birçok bileşenin yanı sıra lektin proteinlerini de içermektedir. Lektinler, doğal olarak birçok organizma tarafından üretilen karbonhidrat tanıma ve bağlanma özellikleri olan proteinlerdir. Lektinler; hücre yüzeylerindeki spesifik karbonhidratlara bağlanarak hücreler arası iletişim, patojen-konak ilişkisi ve bağışıklık yanıt gibi birçok işlevde rol oynar. Doğal bağışıklık yanıtta patojen mikroorganizmalara bağlanarak opsonizasyonu arttırır. Dahası, bazı lektinlerin birçok *in vitro* çalışmada, hatta birkaç *in vivo* çalışmada, fagositlere ihtiyaç duymadan da bakterisidal etki gösterebildiği ortaya konulmuştur. Bu etki; bakterinin aglütinasyonu, biyofilm oluşumunun engellenmesi veya hücre duvarı yapısının bozulması sayesinde gerçekleşir. Balık mukusunun ve balık lektinlerinin antibakteriyel etkileri birçok çalışmada bildirilmiştir. Bu derlemede, bu çalışmalardan bahsedilmiş ve mukus ve/veya lektinlerin *in vivo* antibakteriyel ajanlar olarak kullanılma potansiyeli değerlendirilmiştir.

Atıf bilgisi/Cite as: Taştan, Y. & Sönmez, A. Y., (2020). A review on antibacterial effects of fish skin mucus and fish lectins. Menba Kastamonu University Faculty of Fisheries Journal, 6(2), 100-107.

INTRODUCTION

Due to the constantly increasing demand for animal protein, aquaculture practices are intensified. Fishes grown in intense aquaculture systems are susceptible to diseases (Dash et al., 2018). Although there are various methods to prevent or combat diseases, these methods are often considered to possess a risk for the health of both the fish and the environment (Bilen et al., 2020). Therefore, to fight against diseases caused by microorganisms, recent studies have focused on natural antimicrobial agents (Nguafack et al., 2020; Palanikani et al., 2020; Elbeshti et al., 2020).

Several studies investigated antimicrobial effects of fish skin mucus (Momoh et al., 2014; Wei et al., 2010; Kuppulakshmi et al., 2008; Fuochi et al., 2017; Del Rosario et al., 2012; Lirio et al., 2019). The findings of these studies are promising and researchers often conclude that mucus shows antimicrobial properties. However, many bioactive compounds in mucus contribute to this effect. One of them is the lectin. Lectins are carbohydrate binding proteins that are synthesized by various organisms. It was suggested that they can be used as antimicrobial agents (Coelho et al., 2018). Lectins play an important role in the immunity of fish and are reported to be present in many organs and tissues such as the gill, skin, liver, kidney, intestine, and blood of fishes (Elumalai et al. 2019). Furthermore, lectins can be isolated from fish skin mucus. In this review, we will narrate the possible utilization of fish mucus and fish lectins to treat or prevent fish diseases.

Fish Skin Mucus

Fish skin mucus is a material that plays important role in reproduction, swimming, excretion, communication, disease resistance, nutrition, ionic and osmotic regulation, and nesting processes (Dash et al., 2018). Mucus has a slippery structure due to the presence of gel-forming macromolecules and high-water content (Sheppard, 1994). The macromolecules that form a gel in many vertebrates, including fish, are glycoproteins (mucins) (Asakawa, 1970; Fletcher et al., 1976). The glycoproteins present in fish mucus are similar in structure to mucins of mammalians (Harris and Hunt, 1973; Alexander and Ingram, 1992). It has been demonstrated that fish mucus contains other components, but since it is not possible to obtain skin mucus without stressing the fish, it is not known whether these components occur naturally in fish as in mammals or are produced as a result of stress, injury or disease (Sheppard, 1994; Palaksha et al., 2008; Guardiola et al., 2014). Again, there are discussions about to what extent these components affect the physical and chemical properties of mucus. Especially glycosaminoglycans have been found in fish mucus. This component is also seen in mammalian mucus. Other known components are proteolytic enzymes, pheromones, lipids, calmodulin, crinotoxins, lectins, complements, immunoglobulins, and lysozyme (Sheppard, 1994; Reverter et al., 2018). These components play a wide range of biological roles (Reverter et al., 2018). As listed in Table 1, various studies have shown that fish skin mucus exhibits antimicrobial properties.

Table 1. Certain studies revealing the *in vitro* antibacterial effect of fish mucus

Fish species	Mucus extraction method	Bacteria	Reference
Dormitator latifrons	Direct use from skin	*Bacillus sp.* *Vibrio harveyi* *Vibrio anguillarum* *Vibrio vulnificus*	Del Rosario et al. (2012)
Dasyatis pastinaca	Crude extract	*Escherichia coli* *Pseudomonas aeruginosa* *Klebsiella pneumoniae*	Fuochi et al. (2017)
Oreochromis niloticus *Clarias batrachus* *Channa striata*	Acidic extraction	*Enterococcus faecalis* *Staphylococcus aureus* *Micrococcus luteus* *Klebsiella pneumoniae* *Pseudomonas aeruginosa* *Aeromonas hydrophila* *Escherichia coli* *Serratia marcescens*	Lirio et al. (2019)
Channa striata	Acidic extraction Aqueous extract Crude extract	*Bacillus subtilis* *Pseudomonas aeruginosa* *Klebsiella pneumoniae* *Aeromonas hydrophila*	Wei et al. (2010)
Channa punctatus *Cirrhinus mrigala*	Direct use from skin	*Escherichia coli* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Lactobacillus vulgaris* *Proteus mirabilis* *Pseudomonas aeruginosa* *Salmonella paratyphi* *Salmonella typhi* *Staphylococcus aureus* *Vibrio cholera*	Kuppulakshmi et al. (2008)

Many studies have examined *in vivo* effects of fish mucus. However, to date, there is no study on fish. Analgesic, anti-inflammatory, and therapeutic effects of mucus (fillet extracts in some cases) were investigated in mice, rats, and pregnant women (Cetin et al., 2016; Hitit et al., 2020; Ab Wahab et al., 2015; Michelle et al., 2004; Suhartono et al., 2013). As mentioned above, these effects may occur due to diverse compounds present in the mucus. Lectin is one of the most interested among these compounds since it can bind to specific cell surfaces and is broadly used in biomedical research such as cancer detection, blood typing, antimicrobial studies, etc. (Hashim et al., 2017; Dan et al., 2015).

Lectins

Glycan-recognizing proteins are classified into two groups: sulfated glycosaminoglycan (SGAG) binding proteins (evolved by convergent evolution) and lectins [mostly containing an evolutionarily conserved carbohydrate-recognition domain (CRD)]. The "lectin" term comes from the Latin word "Legere" which means "to select". Phylogenetically, lectins are very ancient and they have the ability to recognize and bind to complex carbohydrates of glycoconjugates. Lectins were discovered in plants more than 100 years ago. Today, it is known to exist in many organisms such as bacteria, viruses, plants, and animals (Gupta, 2012).

It is thought that the first definition of lectin was made in 1888 by Peter Hermann Stillmark in his doctoral dissertation. Stillmark isolated an extremely toxic hemagglutinin, ricin, from the seeds of the castor bean plant (*Ricinus communis*). Although animal hemagglutinins have been known for a long time in both invertebrates and lower vertebrates, merely three of them (horseshoe crab, snail, and eel) were successfully isolated and characterized until the mid-1970s. The first animal lectin specific to a sugar (L-fucose) was obtained from eel by Watkins and Morgan (1952). Since the early '80s, the number of lectins purified from animals has increased rapidly with the recombinant techniques becoming widespread (Gupta, 2012).

Today, lectins are known as a heterogeneous group of non-immune system-derived carbohydrate-binding proteins that agglutinate cells and/or precipitate glycoconjugates without affecting their covalent bonds (Goldstein et al., 1980). By this definition, it is understood that lectin molecules have two or more carbohydrate-binding sites to enable crosslinking between the cell and a sugar-containing macromolecule. In recent years, with the realization that lectin acts as a cell recognition agent in biological systems, studies on lectins have intensified (Sharon, 2006). Although lectins are considered not to be produced by the immune system, the agglutination ability of lectins is similar to that of antibodies. Alongside being able to differentiate between different monosaccharides, lectins can also selectively bind to oligosaccharides by detecting minute variations in complex carbohydrate structures. They also diverge from carbohydrate modifying enzymes since they do not perform glycosyltransferase or glucosidase reactions. Lectins are present in all plant-based foods (Goldstein et al., 1980). Plant lectins are suggested to have serious and harmful effects as a result of raw consumption in both food and feed (Liener, 1986; Gupta, 2012).

Role of Lectins in Fish Immunity

Fish have a highly complex immune system divided into two categories: innate and adaptive immunity (Medzhitov and Janeway, 2000). Essentially, all jawed vertebrates including bony fish and cartilaginous fish, have developed an adaptive immune system containing memory cells (Hsu and Criscitiello, 2006; Hinds and Litman, 1986; Solem and Stenvik, 2006). On the other hand, the innate immune system relies on the cooperation of lymphocytes and humoral proteins to eradicate pathogens without immunological memory. In order to differentiate a self and a non-self molecule, the innate immune system needs the recognition of the "conserved pathogen-associated molecular patterns" (PAMPs) that are present on the surface of many microbes (Medzhitov and Janeway, 2000). This recognition process takes place through "pattern recognition receptors" (PRRs). When these receptors encounter PAMPs, they elicit the signal that causes the release of inflammatory mediators (Fujita, 2002; Nonaka and Azumi, 1999; Sekine et al., 2001; Kania et al., 2010).

Other innate immune molecules constitute the complement system. These molecules trigger a series of proteolysis processes that allow the assembly of protein complexes belonging to the immune system, leading to the destruction of the pathogen. The complement system exists in many organisms (Fujita, 2002; Nonaka and Azumi, 1999; Sekine et al., 2001). Evolutionarily oldest known components of this system are mannose-binding lectin-associated serine proteases (MASP), factor B, and C3 (Smith et al., 1996; Smith et al., 1998; Ji et al., 1997; Skjoedt et al., 2009). In most higher vertebrates, the complement system can be activated in three different ways: classical, alternative, and lectin activation pathway. The lectin activation pathway is initiated by the binding of ficolins or mannose-binding lectins (MBL) to PAMPs on the microorganism surface (Holmskov et al., 2003). After binding, the proenzymes of MBL-associated serine proteases (MASP1-3) cleavage into the C4 scavenging complex. As in the classical way, this situation causes the conversion of C4bC2a to C3, which leads to C3 accumulation on the surface of the microbe. This process increases opsonization through C3 receptors on the surface of phagocytes or leads to the assembly of the membrane attack complex (C5-C9) (Vorup-Jensen et al., 2000; Kania et al., 2010).

Lectins as Antibacterial Agents

The resistance of microorganisms to antimicrobial agents makes it difficult to treat and prevent infections. Recently, many studies have focused on natural components to treat or prevent diseases. Lectins are potent and natural antimicrobials that can bind to carbohydrates on microbial surfaces (Coelho et al., 2018). Carbohydrate recognition domain (CRD) binds specifically to carbohydrate molecules expressed on the pathogen, increasing opsonization and phagocytosis; subsequently accelerating the elimination of bacteria (da Silva et al., 2012). Another antibacterial effect of lectin occurs when it interacts with bacterial cell wall components, affecting the pore-forming ability of bacteria and damaging cell permeability. Its antifungal activity on the other hand is related to the binding property of lectin to chitin, causing the cessation of fatty acid synthesis in the cell wall of the fungus during growth or division, which eventually leads to the loss of integrity of the cell wall (Coelho et al., 2018). Lectins are also reported to show antiviral and anti-cancer activity (Zhou and Sun, 2015; Gondim et al., 2017). Lectins are generally classified according to their carbohydrate binding specificity, biological function, cellular location, and cation dependence (Russell et al., 2008).

Various studies have shown immunomodulatory action and antimicrobial activity of certain lectins from various animals and plants (Ferreira et al., 2011; Zhang et al., 2018). Moreover, many investigations have been conducted on the antibacterial properties of fish lectins (Table 2).

Table 2. Effects of fish lectins on certain bacteria species

Lectin type	Fish isolated	Bacteria	Effect	Reference
C-type	*Etroplus suratensis*	*Aeromonas hydrophila* *Vibrio parahaemolyticus*	Agglutination and interfering with biofilm formation	Rubeena et al. (2019)
C-type	*Cynoglossus semilaevis*	*Escherichia coli* *Vibrio anguillarum* *Vibrio parahaemolyticus* *Pseudomonas aeruginosa* *Streptococcus agalactiae*	Antimicrobial activity	Huang et al. (2019)
C-type	*Oreochromis niloticus*	*Streptococcus agalactiae* *Aeromonas hydrophila*	Agglutination and binding activity	Mu et al. (2017)
C-type	*Salmo salar*	*Vibrio anguillarum* *Aeromonas salmonicida*	Binding activity	Ewart et al. (1999)
GANL (Family not specified)	*Aristichthys nobilis*	*Vibrio harveyi*	Agglutination	Pan et al. (2010)
A peptide (QP13) from a Lily type lectin	*Channa striata*	*Vibrio harveyi* *Escherichia coli* *Bacillus mycoides* *Micrococcus luteus* *Salmonella enterica* *Staphylococcus aureus*	Antimicrobial activity	Arasu et al. (2017)
ComaSeL (Serum lectin)	*Colossoma macropomum*	*Edwardsiella tarda* *Aeromonas hydrophila* *Aeromonas sobria*	Antimicrobial activity	Maciel Carvalho et al. (2012)
C-type	*Anguilla japonica*	*Escherichia coli*	Agglutination	Tasumi et al. (2002)

To date, only a few studies have examined the *in vivo* effects of fish lectins. Zhou and Sun (2015) characterized a C-type lectin (CsCTL1) from tongue sole (*Cynoglossus semilaevis*) and studied its antimicrobial effects. After characterization, they produced recombinant lectin (rCsCTL1) and administered the fish with *Vibrio anguillarum*, megalocytivirus, and lectin. Researchers found out that rCsCTL1 exhibited both antibacterial and antiviral effects. In a similar work, Sun et al. (2016) investigated the antibacterial activities of three novel B-type mannose-specific lectins of tongue sole (*Cynoglossus semilaevis*). They first cloned CsBML1, CsBML2, and CsBML3 protein genes from the fish and then produced recombinant versions. According to the results of their preliminary bacterial binding and agglutination assays, they administered the fish with *Vibrio harveyi* and recombinant lectins. It was concluded that two of the lectins examined reduced the bacterial infection significantly.

In both studies above, researchers administered the lectin by intraperitoneal injection as a single dose at the same time with bacteria or virus. We think there is a need for more studies to be conducted on fish where different administration routes are applied with different doses and duration to either prevent or treat the disease. Agglutinating, binding, and bactericidal effects of lectins suggest that lectins may be used for this purpose. In addition, by opsonizing pathogenic microorganisms, lectins can also enhance phagocytic activity when administered.

CONCLUSION

There are many studies on fish mucus, and mostly antimicrobial effects were observed in these studies. Of course, lectins and many other bioactive components in the content of mucus play a role in this effect. Using the lectin alone will give more reliable results scientifically. However, this process is very costly as it requires protein isolation. On the other hand, mucus can be obtained in large quantities from captured fish or can be procured from farmed fish without harming them. The use of mucus will therefore be more economical, yet one can argue that the scientific explanation of the effect will be nearly impossible. As a result, we conclude that both substances have the potential to be used as antibacterial agents and in-depth *in vivo* effects of them should be investigated.

COMPLIANCE WITH ETHICAL STANDARDS

Author contributions

AYS designed the conception. YT conducted the literature review. Both authors contributed equally to writing of the manuscript.

Conflict of interest

Authors declare that they have no conflict of interest.

Animal welfare statement

No animals were used in this study.

Human rights statement

Official approval is not required for this type of study.

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