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Fish pathogen bacteria: Adhesion, parameters influencing virulence and interaction with host cells

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ABSTRACT

Wild fisheries are declining due to over-fishing, climate change, pollution and marine habitat destructions among other factors, and, concomitantly, aquaculture is increasing significantly around the world. Fish infections caused by pathogenic bacteria are quite common in aquaculture, although their seriousness depends on the season. Drug-supplemented feeds are often used to keep farmed fish free from the diseases caused by such bacteria. However, given that bacteria can survive well in aquatic environments independently of their hosts, bacterial diseases have become major impediments to aquaculture development. On the other hand, the indiscriminate uses of antimicrobial agents has led to resistant strains and the need to switch to other antibiotics, although it seems that an integrated approach that considers not only the pathogen but also the host and the environment will be the most effective method in the long-term to improve aquatic animal health. This review covers the mechanisms of bacterial pathogenicity and details the foundations underlying the interactions occurring between pathogenic bacteria and the fish host in the aquatic environment, as well as the factors that influence virulence. Understanding and linking the different phenomena that occur from adhesion to colonization of the host will offer novel and useful means to help design suitable therapeutic strategies for disease prevention and treatment.

1. Introduction

There is an increasing worldwide demand for seafood due to the awareness of fish as an important protein source for a growing population. Wild fisheries are currently in a state of decline because of overfishing, climate change, pollution and marine habitat destruction, among other factors. However, aquaculture is rising all around the world. Du to commercial and production reasons, farmed fish are held in much more crowded conditions than those existing in the wild, with the consequence that farmed fish are more stressed and hypersensitive than savage ones. Previous studies agreed that the response of fish to stress conditions depends on the stressor (temperature, crowding, hypoxia, the presence of heavy metals, etc.), and also on the characteristics of the fish themselves (e.g. fish species, age or sex) [1,2]. Such stressful conditions could enhance the spread of pathogenic bacteria and cause serious outbreaks of disease. Indeed, the diseases caused by bacterial infections and the low survival rate of the fish represent significant challenges to fish farmers [3]. Given that bacteria can survive well in aquatic environment independently of their hosts, bacterial diseases have become major impediments to aquaculture [4].

Both prophylactic and therapeutic treatments use drug supplemented feeds to keep farmed fish free of diseases [5]. Unfortunately, the indiscriminate use of antimicrobial agents has led to the development of resistant strains and the search for alternatives to antibiotics [6]. Diseases caused by antibiotic-resistant bacteria are difficult to treat, and there are only a few new antimicrobial compounds in the drug development pipeline.

Likewise, antibiotics may reduce larval growth and inhibit the defence mechanisms of the fish larvae [7]. Besides sensitization reactions and other undesirable side effects on fish, it has been demonstrated that between 60% and 73% of antibiotics and related chemical drugs administered to farmed fish are excreted with the faeces [8]. In our point of view, a strategy that combines the pathogen, the host and the environment characteristics will be the most effective method in the long-term to improve aquatic animal health.

In the aquaculture industry, when fish diseases are diagnosed, the

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treatments starts with a global identification of the pathogen and, subsequently, with the administration of antibiotics in water or mixed with feed pellets in an attempt to eradicate the pathogen. Unfortunately, these administration methods usually neglect the adhesive capacity of bacteria and their ability to arrange themselves as a biofilm [9]. New studies on fish pathogenic bacteria are required in order to understand: (i) their adhesion mechanisms and factors influencing the same in aquatic environment, (ii) the interaction mechanism established between pathogen and host cells and (iii) the factors involved in virulence, colonization and fish disease. The adhesion of bacteria to mucus seems to be a prerequisite for successful infection, so that more works focusing on adhesins (which can be considered primary virulence factors), exopolymers and flagella, which play an important role in establishing the initial interaction with mucosal surfaces or cells, are needed to understand the initial steps of mucosal infection as well as those involved in bacterial virulence. Concomitantly, new studies attempt to better understand the physico-chemical properties of skin mucus and immune components (constitutive or induced upon infection), this will help in the design of appropriate preventive strategies in order to offer better protection in fish against invading pathogens. Furthermore, more studies are needed to understand the interaction of pathogen and fish cells under different environmental conditions (temperature, salinity).

Complicated local signaling arrangements are present in the fish skin but intracellular signaling pathways induced by pathogen are still less studied. Further studies are necessary to help us understand these immune reactions in order to develop new practices that will improve the health and welfare of farmed fish [10]. To the best of our knowledge, this is the first review focuses on the interaction between bacteria and fish cells, with emphasis on the importance of the link between adhesion capacity, interactions occurring in biofilm, the parameters influencing bacterial virulence, the role of biofilm in pathogenicity and the susceptibility of pathogenic bacteria to antibiotics. The well understanding of the link between all these parameters could prevent important fish diseases that cause serious economic lost in aquaculture.

2. Adhesion of pathogenic bacteria and biofilm formation

The survival, multiplication and pathogenicity of bacteria require the existence of both pathogen and fish as well as the successful invasion of the host fish by the pathogen. It was reported long ago that adhesion is a prerequisite for successful infection. A deep study of the mechanisms implicated in the adhesion mechanism could intensely contribute on protecting fish from diseases. Although the number of fish pathogens identified is relatively limited compared to other pathogens, most studies have focused on treatment strategies essentially, by antibiotics. However, information is lacking concerning the adhesion of pathogens to the skin and gut mucus of fish.

2.1. Adhesion of pathogenic bacteria

Adhesion is an important factor in bacterial pathogenicity because it promotes the delivery of bacterial toxins and precedes penetration into target cells by the microorganism. Specific adherence is mediated through compounds on the surface of the bacteria, which bind via rigid stereo-chemical bonds to particular molecules on the support to which it is adhering [11].

Any study of pathogen bacteria adhesion to the host fish should starts with a clear understanding of the mechanisms and the factors involved in the attachment of bacteria to the fish. The initial attachment of bacterial cells to a surface (e.g. mucus) involves many factors, including hydrophobicity, surface charge, surface roughness, surface micro-topography and water flow, components of the surface, pH of the milieu and viscosity, among others [12]. The adhesion process of bacteria to surface and fish mucus, the different forces involved, the successive steps and the specific adhesion receptors that facilitate strong adhesion was discussed in detail in our previous review [10]. In the present review, we focus on the adhesion mechanism in relation with pathogenicity and as a factor that might influence virulence. For the pathogenic bacteria, it seems that a natural selection process is operating, whereby the most adhesive and resistant bacteria survive to induce disease. Many studies have indicated that external fish mucus has a protective role and evolved robust mechanisms that can trap and immobilize pathogens before they can contact epithelial surfaces. Indeed, the mucus layer is impermeable to most bacteria and many pathogens (reviewed by Refs. [13] and [10]). However, other studies have suggested that fish mucus could act as a nutrient source for the pathogenic bacteria, and that the microorganisms could persist within the aquatic environments, using mucus of reservoir fish hosts similar to other fish pathogens [11,14]. Regardless of whether the mucus in itself serves as a nutrient or whether it serves as a substance to collect nutrients, it represents a microenvironment that harbors pathogenic bacteria. Being near to each other, these bacteria will multiply and aggregates. Although the exact aggregation capacity, aggregation kinetic and adhesion kinetic will depend on the bacteria, the mucus represents a substratum that provides cohesive forces [15]. Biochemical interactions between the bacteria and mucus, cells or tissues may be enabled by a hydrated matrix of exopolymers, capsular material including exopolysaccharides (EPSs) and others that provides a buffer against sudden changes in the adjacent osmotic environment [16,17]. Such a stable environment may aid in the localization of secreted exoenzymes, which are essential for the adhesion mechanism and the sequestration of nutrients [17,18].

Some bacterial surface structures such as, adhesin, flagella, pili and fimbriae, extend from the bacterial surface to mediate attachment, while attachment receptors bind to specific ligands on the surface. Bacterial adhesin mediates binding to the epithelial extracellular matrix by recognizing specific carbohydrate structures [19]. Bacterial exoproducts, such as outer membrane protein or extracellular polysaccharides, may be involved in adhesion, and there is also evidence supporting the involvement of lipopolysaccharides [20,21]. These polysaccharides may mediate carbohydrate-carbohydrate interactions that are important for adhesion.

Once adhesion occurs, the bacteria can induce the expression of other virulence genes and cause the activation of host cell signaling pathways [10,22,23]. Thus, organisms may undergo specific molecular changes to become pathogenic or establish biofilms. Furthermore, several studies have reported that the flagellum is involved in directing the adhesion mechanism. For example, *Pseudomonas* strain was observed to attach to hydrophilic surfaces via flagella, while assuming random orientations on hydrophobic surfaces [24,25]. Experiments with the pathogens *P. aeruginosa* [25] and *Aeromonas hydrophila* [26] demonstrated the important role of flagella in establishing the initial interaction with mucosal surfaces or cells. It was reported that flagellar rotation increases the attachment rates of *Escherichia coli* bacteria to glass [27], although it could not be ascertained whether the bacteria were adhering by their cell bodies or by their flagella.

Morisaki et al. [28] studied the attachment of *Vibrio alginolyticus*, *Vibrio alcaligenes* and *Alteromonas* spp., and concluded that the attachment of these bacteria depended on the electrostatic energy interaction between the polymers existing on the bacterial cell surface and the polymers that exist on the attachment surface. Hence, the role of high ionic conditions such as sea water in the bacterial attachment process was confirmed.

2.2. Mechanism of biofilm formation

After achieving the adhesion stage, the bacteria transform from planktonic to sessile mode of growth. The pathogenic bacteria start their proliferation, aggregate and invade the surface to form a biofilm. Although the biofilm formation is a highly complex process, but it could be abridged in the formation of micro-colony followed by a three