Probiotics in fish aquaculture: The cure against parasitic diseases?

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Abstract

Fish is an attractive source of protein, especially in regions where agricultural practises are limited. Aquaculture has emerged as a promising enterprise to keep up with the increasing demand for high-quality protein-rich food in an ever-growing human world population. With increasing intensiveness of the practise, stress levels in animals are also increasing. High levels of stress can lead to high susceptibility to parasitic diseases (diseases caused or transmitted by parasites) such as Ich/White Spot Disease caused by *Ichthyphtirius multifiliis*, Dactylogyrosis caused by gill fluke (*Dactylogyrus* sp.) and saprolegniasis caused by parasitic heterokonts including the genus *Saprolegnia* sp. Parasitic diseases have been notoriously challenging to treat because of the different parasite life stages and similarity to the host organism. In recent years, probiotics have taken the stage as an eco-friendly alternative to conventional disease treatments as they can boost fish health and growth substantially without harming the host. While there has been extensive research conducted on probiotics use against bacterial and viral infections, only few studies have proposed a probiotic treatment against parasitic diseases. In this essay, I want to discuss whether probiotics could be applied to parasitic diseases in the same manner as they can be applied to bacterial infections. I will introduce common parasitic diseases and their mode of infection together with an introduction to the most important probiotics used in aquaculture today. I will highlight the different beneficial effects that probiotics have shown in studies against bacterial diseases and evaluate whether they might have a similar effect on parasitic diseases. Finally, I will argue whether some defence mechanisms against parasitic diseases, either direct or indirect, would play a more important role than other.

Content

Introduction

Aquaculture holds great potential to support the food supply to an ever-expanding human population. As an enterprise, aquaculture has grown substantially over time alongside an increasing intensiveness of the practise. In order to grow more fish in a short time, densities of cultured fish held in cages or tanks are notoriously high, inducing high levels of stress in the fish caused by a decrease in water quality and hypoxia (reviewed in Oliva-Teles 2012). Increased stress causes a decreased robustness of the immune system to pathogens, causing various kinds of diseases (Oliva-Teles 2012, Uribe et al. 2011).
Consequently, fish farms suffer great losses of stock and, therefore, revenue due to high mortality rates and reduced growth of infected fish. Typical losses per affected cultured species are in the millions of dollars annually (reviewed in Bondad-Reantaso et al. 2005).

Traditional treatments of fish diseases in aquaculture included toxic chemicals and synthetically manufacture antimicrobials, many of which led to numerous strains of bacteria and parasites resistant to veterinary medicines, especially antibiotics ("The Antibiotic Resistance Crisis", Ventola 2015). Together with a rising demand of an eco-friendlier aquaculture with little impact on the environment, the use of probiotics to counter fish infections has gained great interest in the industry. Probiotics offer an alternative way to increase disease resistance and higher feed conversion rates often leading to elevated growth rates of aquatic organisms including fish, While the excitement about an eco-friendlier disease treatment alternative has sparked many studies on probiotics to tackle bacterial and viral infections, parasitic diseases have been widely overlooked with only few studies assessing possible probiotics strains to treat parasitic diseases. Moreover, mechanisms underlying increased parasitic disease resistance are still largely unknown. In this essay, I want to explore characteristics of parasitic infections as opposed bacterial infections. I will first highlight some of the most common parasitic diseases in cultured freshwater and marine fishes, explain their life cycles and modes of infection. Furthermore, I will present the different proposed mechanisms and beneficial effects probiotics against bacterial and viral infections have shown in the host. Based on these mechanisms and the characteristics of parasites, I will discuss possible methodology to combine probiotics with parasitic diseases in some fish host species and speculate whether probiotics could be applied successfully to parasitic diseases in fishes. In other words: Can probiotics be applied to tackle parasitic diseases in commercially cultured fish species?

Common parasitic diseases in aquaculture

Parasitic diseases are infectious diseases caused or transmitted by parasites. Although members from taxa such as viruses, bacteria and fungi can act as parasites in an ecological sense (i.e. living on or in another organism, the host, resulting in some harm), the term "parasitic disease" is usually reserved to eukaryotic species such as protozoans and helminths that exhibit a parasitic lifestyle. In this essay, the term "parasite" exclusively encompasses eukaryotic parasites.
Parasites can affect all kinds of cultured organisms, such as crustaceans, fish and shellfish. In this essay, I will mainly focus on parasitic diseases affecting cultured freshwater and marine fishes.

In a recent review on fish disease management in India, Mishra et al. (2017) estimated that that parasitic diseases made up the majority of all fish diseases in freshwater aquaculture in India with an estimated 45%, while bacterial and viral diseases together only made up 30%. As a third major cause of losses, the authors mention alterations in water quality with 24%. Substantial changes in water quality can favour infections of parasites as I will discuss later in this essay. According to Mishra et al. 2017, parasitic diseases account for substantial losses in revenue for fish farms. However, the majority of these losses in parasitic diseases is not only due to increased mortality, but rather due to reduced growth of infected fish and thus, decreased value (Mishra et al. 2017). The reason for this is that parasites normally do not kill their host as it would disrupt their lifecycle and, therefore, lead to their death as well. Nonetheless, some fish parasites, especially in monogenetic parasites (i.e. single host parasites), can induce high mortalities in their hosts. Fish parasites cover a wide range of taxa (reviewed in Paperna 1991). Some of the most important parasites in fishes include ectoparasitic protozoa, most notably *Ichthyopthirius multifiliis*, and Myxosporea, a subclass of microscopic parasites belonging to the phylum Cnidaria. Furthermore, larger parasites such as Monogenea (notably the genera *Dactylogyrus* & *Gyrodactylus* sp.), Nematoda and Trematoda are prevalent. The largest parasites of cultured fishes belong to different classes in the subphylum crustacea, most notably the genus *Argulus spp.*, known as fish lice, the most common and widespread parasitic crustaceans (Walker et al. 2011, Mishra et al. 2017).

In this essay, I will highlight the lifecycles of three parasites: *Ichthyopthirius multifiliis*, the genus *Dactylogyrus* sp., and a genus of common parasitic fungi-like pathogens called *Saprolegnia* sp.

The first parasite, *I. multifiliis*, is the largest known protozoan ectoparasite of freshwater fish (Francis-Floyd and Reed 1991). The size of one adult *I. multifiliis* cell can measure up to 1.0mm in diameter which is around 30x bigger than an average human skin cell and 500x bigger than the average bacterial cell (Anderson 2019).It is the causative agent of the infamous White Spot Disease or Ich which can lead to unusually high mortality rates of around 100% in infected fish populations if untreated. It should not be confused with the White Spot Syndrom in shrimps which is caused by the White Spot Syndrom Virus that is responsible for an estimated $8bn loss (possibly twice as much) in revenue since its emergence in the 1990s (Lightner et al. 2012).

It is a very unspecific parasite and parasitizes a wide range of both ornamental fish (i.e. fish held in aquaria) and commercially cultured fish. The organism is obligate parasitic which means it cannot survive without fish present. While some parasites have very complex life cycles, often including multiple hosts, the life cycle of *I. multifiliis* is fairly simple (Noga 2010, Durborow, Mitchell and Crosby 1998). Fish are the only host the organism parasitizes. This is a common characteristic of parasitic diseases in aquaculture because of a lack of other hosts present (monoculture). Furthermore, as mentioned above, high densities of hosts favour directly-transmitted parasites greatly and facilitate rapid spreading of the disease in aquaculture.

The infectious stage infects the skin or gills of their victims and feeds within a nodule formed in the skin or gill epithelium (trophont stage; active protozoan stage). The organism grows in size and then detaches from the epithelium while entering the tomont stage swimming in the water. In this stage, the organism produces many hundred young infectious theronts within the cell in a process called binary fission rather than replication and cell division. These young stages then infect new hosts and the cycle repeats itself. Time to complete the life cycle varies greatly as is the case in many parasitic organisms (Barber et al. 2016). An important factor hereby is water temperature. *I. multifiliis* shows a wide range of temperature tolerance (1°C to over 30°C), but the parasites life cycle is greatly affected by it (Durborow, Mitchell and Crosby 1998). The organism approximately needs four days to complete its life cycle at temperatures above 24°C, while it needs more than five weeks to complete the cycle at temperatures lower than 7°C.

The second parasite I want to highlight is *Dactylogyrus* sp., a genus of monogenetic trematodes (Monogenea). *Dactylogyrus* is commonly known as gill fluke, because it mainly targets the gills of freshwater fishes and causes Dactylogyrosis (also referred to as gill fluke) that manifests in a destruction of gill filaments and white cysts on top of the gills (Mishra et al. 2017). This genus is closely related to the genus *Gyrodactylus* which mainly infects the skin (skin fluke). Diagnosis is easily done by examining infected gill tissue under a microscope, because the parasite measures around 0.1 to 0.3 millimetres. They anchor themselves to the gills via two posterior anchoring hooks (Banner 2014).
Similar to *I. multifiliis*, species of monogeneans are characterized by only parasitizing one host during their life cycle. Hermaphroditic oviparous adults lay their eggs out into the water while they are attached to a host. These eggs hatch and develop into a so-called onchomiricidium (Moeller and Robert 2009). Once hatches, the parasite needs to find a new host within six to eight hours otherwise it will die. If the parasite is successful then its life cycle will start anew.
Exact mortality rates of *Dactylogyrus* sp. are not known, although there have been some reports in the past that heavy infestations by the parasite caused mass mortality in carp fry (Paperna 1991).

Finally, the last parasitic genus I will highlight is *Saprolegnia* sp.
Saprolegnia sp. (often called water moulds) belongs to the class Oomycota within the phylum Heterokontophyta. Oomycetes are protists that were once thought to belong to the kingdom of fungi because of their filamentous growth and feeding off of decaying tissue and organisms (Sleigh 1989). However, their cell wall, which contains a mixture of cellulose-like compounds and glycan, is quite distinct from the fungi cell wall which is mainly made up of chitin. The name “oomycota” refers to the large and round storage structure of the female gametes called oogonia. *Saprolegnia* has a diploid life cycle and reproduces both sexually and asexually. Starting as a spore, *Saprolegnia* releases zoospores that encysts and releases new zoospores in an asexual reproductive process called polyplanetism (Hohnk 1933). This process repeats itself until the parasite finds a suitable substrate to attach on. Sexual reproduction starts once *Saprolegnia* has attached to its host by producing male and female gametangium, antheridia and oogonium. They unite and fuse via fertilization tubes to produce the zygote called oospore.
Saprolegnia are rather opportunistic than active parasites and parasitize a wide range of species (Bruno and Wood 1994). Some *Saprolegnia* species are parasitic on aquatic invertebrate such as nematodes, arthropods and rotifers. Other *Saprolegnia* species parasitize diatoms. And finally, there are *Saprolegnia* species which parasitize fishes.
Other than their life cycle, parasitic diseases are fairly distinct from bacterial (and viral)

diseases. Some of the characteristics of parasitic diseases in aquaculture are compared to bacterial diseases in the next paragraph.

Bacterial vs. parasitic infections

To put parasitic diseases in perspective, I will briefly explain the differences of them compared to bacterial diseases in fish species. Note that viral diseases have their own characteristics, but for the sake of this essay, I will not go into this matter.

First, there is a phylogenetic difference between the two. All bacteria lack a nucleus and are therefore classified as prokaryotes while all parasites possess a nucleus which classifies them as eukaryotes (Alberts et al. 2002). Apart from the lack of a nucleus, bacteria have plasma membrane together with a cell wall while the eukaryotic cells of parasites only possess a plasma membrane. Because prokaryotes are almost exclusively unicellular, there is an apparent size difference compared to parasites which can be unicellular and multicellular. Bacterial cell sizes are usually in micro- to millimetre range while parasite sizes can range from a few micrometres (protozoa) to several centimetres (parasitic crustaceans) (Mishra et al. 2017).

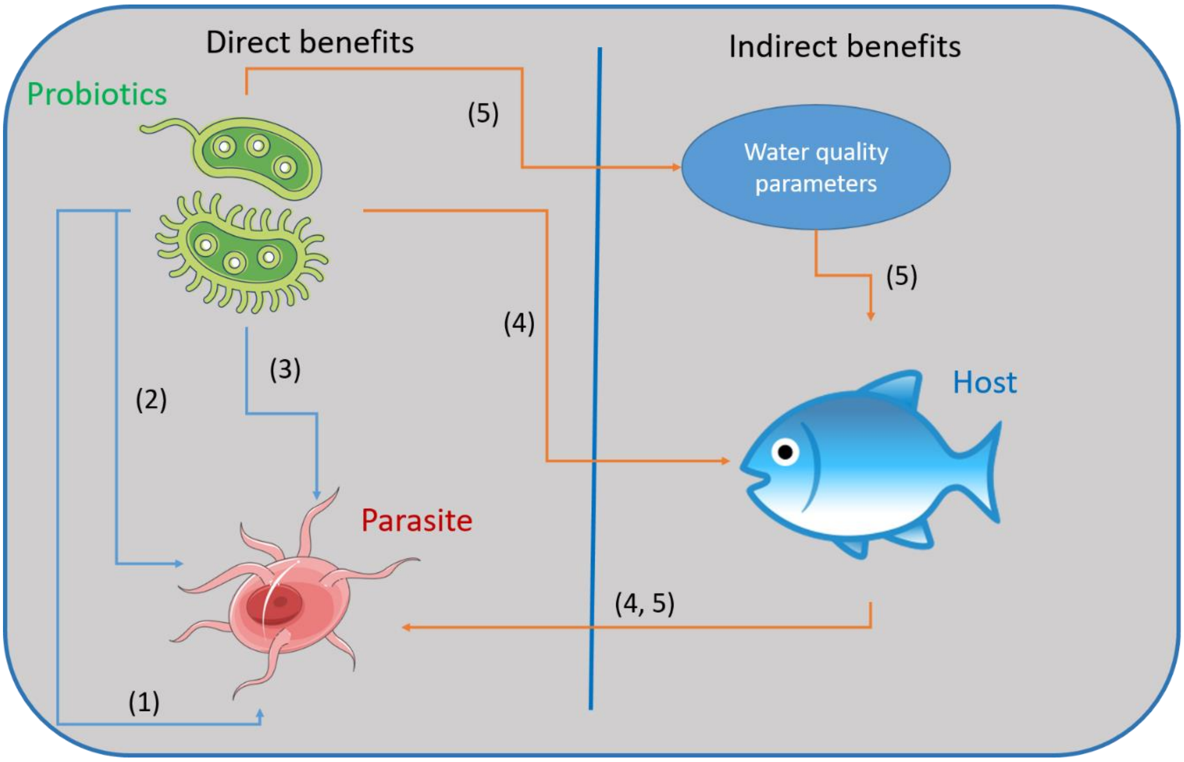
Furthermore, most bacteria are harmless and some are beneficial to their host (e.g. the probiotics discussed in this essay) (Alberts et al. 2002). Parasites on the other hand always have negative health effects for their host and feed off of tissue and blood without returning the favour.

Regarding immune response in the host bacterial and parasitic infections are known to activate several pathway of the innate and adaptive immune system in fishes (Ellis 1999, Alvarez-Pellitero 2008) Parasitic diseases tend to activate the immune system locally in the infected tissue, while the host mounts a more general immune response when challenged with a bacterial pathogens, but this is not to taken as a general different. Mucosal immunity plays an important role in defending ectoparasites, while systemic immunity is more important against bacterial intruders. Moreover, production of antimicrobials will be more specifically tailored to the type of pathogen the host faces including peptides with anti-parasitic or antibacterial properties (e.g. Colorni et al. 2008, Cuesta et al. 2008).

Finally, there are key differences in treatment against bacterial and parasitic infections. Because of the bacterial cell wall and other cellular structure that unique to bacteria, antibiotics that target these structure specifically have a high chance of abolishing a bacterial infection without harming the host organism (if it is an eukaryotic host) (Kohanski, Dwyer and Collins 2010). For the same reason antibiotics are effective against bacteria, fungicides are effective against fungal diseases, although some fungal infections can appear to be of parasitic nature.

Parasitic diseases, however, are harder to treat because of the high degree of similarity between the parasite and the host organism (Alberts et al. 2002). Treatments often involve practices and substances that are not only toxic to the parasite, but toxic to the host as well. One example would be the treatment of White Spot Disease caused by the protozoan parasite *Ichthyopthirius multifiliis* introduced in last paragraph: The most effective treatment would be either a bath in low-salinity water or chemical treatment with formalin and malachite green or even with chelated copper and copper sulfate (Andrews et al. 2010). All these substances are toxic to fish (and humans) as well and have to be introduced at high enough concentrations to kill the parasite but low enough concentrations not to kill the fish.

Another challenge of parasitic disease treatment are the different life cycle stages of parasites



Schematic Fig.1. Different proposed beneficial mechanisms probiotics can have on host health and parasitic disease resistance in aquaculture. Direct effects of the probiotics on the parasite are shown as blue pathways: (1) Competitive exclusion of the parasite either due to competition for adhesion sites on the host epithelium or competition for nutrients, (2) production of antimicrobials or (3) production of organic acids which lower the pH of the water medium.
Indirect effects of the probiotics on the parasite are shown as orange pathways: (4) Enhancing either innate or adaptive immune response in the host when challenged with a parasite and (5) improving rear water quality parameters such as dissolved oxygen (O_2), nitrite (NO_2^-), ammonia (NH_3) and sulfide (S^{2-}) which ultimately leads to reduced stress levels in the host and to a more robust immune response, too. Hence why (4, 5) are shown as a common arrow at the end.

(Alberts et al. 2002). In the case of *I. multifiliis*, for example, chemical treatment with formalin or malachite green is only effective against the free-swimming theront stage of the parasite (Matthews 2005, Andrews et al. 2010).
Some anti-parasitic drugs have been used in aquaculture before. For example, eugenol (a member of the allylbenzen class extracted from essential oils of certain plants) possess certain antihelmintic properties against monogenean infection in tropical fish (de Lima Bojink et al. 2015). However, some of the anti-parasitic drug treatments have led to the rise of resistant parasitic strains as well (Aaen et al. 2015).
Considering these major limitations to parasitic disease treatment, it would be highly beneficial to isolate probiotic strains of bacteria which inhibit the growth of the parasitic intruders, but do not harm to host organism.

Role of probiotics in aquaculture

The term “probiotics” has been introduced by Parker (1974). He described probiotics as any organism or substance that contribute the balance of the intestinal microflora. Because of confusion what “substances” are specifically, Fuller (1989) redefined “probiotics” as “a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance”. The current definition of the Food and Agricultural Organisation (FAO) and the World Health Organisation (WHO) picks up the emphasis on live cells in probiotics by defining “probiotics” as “live microorganisms that when administered in adequate amounts confer a health benefit on the host” (FAO and WHO 2001). As it emerged that, in aquaculture, dead cells or even just parts of cells can act in the same manner as live cells, another revised definition was proposed. According to Merrifield et al. (2010a), “a probiotic organism can be regarded as a live, dead or component of a microbial cell, which is administered via the feed or to the rearing water, benefiting the host by improving disease resistance, health status, growth performance, feed utilization, stress response or general vigour, which is achieved at least in part via improving the hosts microbial balance or the microbial balance of the ambient environment.”

The use of probiotics in aquaculture, specifically, was proposed by Gatesoupe (1999).

One of the main drivers that accelerated research in the field of probiotics is the rapidly growing number of resistant bacterial strains against synthetic antibiotics (De et al. 2014, Ventola 2015). Together with a demand for eco-friendlier aquaculture and implementation of strict regulation of antibiotics use in aquaculture, research in the field of probiotics has been of great interest over the past two decades.

This has led to a wide range of studies on various probiotic strains exhibiting different beneficial effects to their hosts when this one is challenged with a pathogen (De et al. 2014, Hoseinifar et al. 2018). The five most commonly proposed pathways are shown in Fig.1. For easier comparison, I will indicate with a number [e.g. (1)] which pathway I am talking about in the section below.

Probiotics have the ability to benefit the host by modulating mucosal and systemic immunity (4), and improving the balance of gut microbiota (Villamil et al. 2002). Probiotics possess conserved microbe-associated molecular patterns (MAMPs) that are recognized by certain pattern recognition receptors (PRRs). Activation of these PRRs induced a signal cascade leading to upregulation of effector molecules such as cytokines and chemokines, therefore upregulating overall immune system activity (Remus et al. 2012, Bron et al. 2012). This can lead to several enhanced immunological reactions such as heightened respiratory burst activity of phagocytes (Diaz-Rosales et al. 2009), increased levels of humoral components of the innate immune response like serum protein and immunoglobins (Sayed et al. 2011) and higher levels of cellular components (e.g. granulocytes, macrophages and leukocytes) (Nayak et al. 2007, Kumar et al. 2008). Consequently, higher innate immune activity coincided with higher survival and improved fish health (e.g. Dias-Rosales et al. 2009, Korkea-aho et al. 2012). Apart from immunomodulation, multiple other beneficial effects of probiotics to the host were found (De et al. 2014, Hoseinifar et al. 2018). Competition for adhesion sites (1) was one of the proposed beneficial mechanisms. In order for an infection to occur the pathogen needs to establish itself first by adhesion to the fish epithelium (Mahdhi et al. 2012, Merrifield et al. 2010b). Because there is limited space available to adhere on the epithelium, there is competition for adhesion sites and the pathogen cannot establish itself if the epithelium is already colonized by probiotics (= competitive exclusion). Moreover, studies suggest that probiotics produce antimicrobial substances (2) which further reduce establishment of a pathogen. Substances include antibacterial (Ringo et al. 2010, 2012), antiviral (Lakshmi et al. 2013) and antifungal substances (e.g. Lategan et al 2004), although only few studies suggested the latter.

In the digestive tract, probiotics can improve feed conversion rate of the host and break down indigestible organic matter (Mohapatra et al. 2012), increase feed digestibility through elevated activity of digestive enzymes such as proteases, amylases and lyases (Ringo et al.

1995, Boyd and Gross 1998, Balcazar et al. 2008). Probiotics have also been found to increase overall host health by producing nutrients such as fatty acids and vitamins, some of which the host cannot synthesize himself (Bonnet et al. 2010).

Some of the most commonly administered probiotics in aquaculture include lactic acid bacteria (LABs) and *Bacillus* sp. (De et al. 2014, Hoseinifar et al. 2018).

Up until now, research has been focussed mainly on bacterial and viral infections, presumably because of complete loss of stock due to high relative mortality compared to parasitic diseases (De et al. 2014, Mishra et al. 2017). Moreover, bacterial diseases were more prevalent in some regions (e.g. Faruk et al. 2004).

Parasitic diseases, however, have received little attention in probiotics research. I will highlight the studies conducted on parasitic disease resistance in aquaculture using probiotics in the next paragraph.

Treating parasitic diseases with probiotics

As of the writing of this essay, only a handful of studies have addressed the beneficial effects probiotics on host health against parasitic pathogens with the majority targeting fungi-like parasitic diseases such as *Saprolegnia* sp.

Pieters et al. (2008) were the first to demonstrate the beneficial effects of probiotics on fish health when challenged with a eukaryotic parasite. They investigated the effects of dietary administration of *Aeromonas sobria* GC2 and *Bronchotrix thermosphacta* BA211 to rainbow trout (*Oncorhynchus mykiss*) at different concentrations over 14 days. Rainbow trout is one of many fish species which gets parasitized by the previously introduced *Ichthyophthirius multifiliis*, one of the most common protozoan ectoparasites in fishes. The GC2 treatment above protected rainbow trout from *I. multifiliis* regardless of concentrations they used and the mortality plummeted from 98% to 0%. However, the second probiotic, *Brochothrix thermosphacta*, administered orally in high concentrations, did not protect rainbow trout from the highly infectious ectoparasite.

Furthermore, Pieters et al. 2008 assessed the changes in innate immune response after feeding the two probiotics. This revealed that GC2 enhanced phagocytic activity (4) while BA211 fed fishes exhibited higher respiratory burst activity (4). It has to be emphasized that the study was only duplicated and that more replicates would manifest the potentially beneficial effects of GC2 further.

Nurhajati et al. (2012), on the other hand, challenged the parasitic heterokont *Saprolegnia parasitica* A3 with the probiotic *Lactobacillus plantarum* FNCC 226 (a LAB strain) in catfish *in vivo* and *in vitro*. The authors state that growth of *Saprolegnia* in vivo and in vitro was significantly reduced with higher concentrations of administered *L. plantarum* FNCC 226. While the authors did not investigate which mechanism might be responsible for the impediment of *S. parasitica* growth, they proposed that either a decrease of the medium pH (3) to 4.0 or the production of a specific anti-parasitic compound ((2), although they use the term fungicide) by *L. plantarum* FNCC 226 might be the cause of this result.

Heikkinen et al. 2013 reported that several probiotics strains enhanced immune activity again saprolegniasis (a collective disease term used for diseases caused by *Saprolegnia*, *Achlya* and *Aphanomyces*) (Das et al. 2014). The study found that utilization of probiotics strains inhibited saprolegniasis through immunostimulatory effects (4) and siderophore production (1). Siderophores are small compounds produced and secreted by bacteria, fungi and plants (De et al. 2014). They have high affinity towards ferric Fe^{3+} in the environment and scavenge for this metal. Iron is usually present in extremely low concentrations in biological systems, especially in aquatic systems, which makes it a major growth limiting factor (Norman et al. 2014). Because of low bioavailable iron and its importance in metabolic processes (including bacterial and fungal infections), competition for Fe^{3+} among species is high. In extremely low-iron environments, this leads to the production of said siderophores by probiotics (Korhea-aho et al 2012). Through this mechanism, probiotics starve pathogens of iron and, therefore, limit their growth.

While all three studies clearly showed certain impeding effects of administered probiotics towards fish parasites, the mechanisms behind the impediment remain largely obscure. Through what kinds of mechanisms could probiotics act against parasitic pathogens in fish aquaculture?

I will discuss possible and previously proposed modes of action of probiotics which potentially could play a role as growth inhibitors towards parasites in the next paragraph.

Future prospects of probiotics against parasitic diseases

We have seen that probiotics have a wide range of benefits to hosts. The benefits can roughly be categorized into five major pathways that are either directly or indirectly beneficial to the host (Fig.1). I categorized three proposed mechanisms as direct: (1) Competitive exclusion of the parasite through competition for nutrients or adhesion sites, (2) production of a variety antimicrobials targeting different pathogens and (3) lowering medium pH through the production and secretion of organic acids.

Furthermore, I categorized two proposed mechanisms as indirect: (4) probiotics that enhance innate and adaptive immune response of the fish host via MAMPs and PRRs and (5) water probiotics that have the ability to modulate and balance water quality parameters as described above which themselves can enhance immunity as well (reviewed thoroughly in De et al. 2014).

We have also seen that fish parasites infect their hosts based on a variety of factors (Paperna 1991). Infections spread rapidly when fish densities are high and water quality is low. Moreover, generation time of parasites can vary greatly with temperature (see example above).

The majority of common fish parasites in aquaculture are ectoparasites rather than endoparasites with some exceptions (Mishra et al. 2017). Most of them target the fish skin or gills and not the intestine, the target site of many bacterial pathogens (De et al. 2014). In order for competition for adhesion sites (1) to occur, the probiotic needs to be able to proliferate on the fish skin and gills. I would recommend to isolate probiotic strains directly from the parasite target sites. Another reason why competition for adhesion sites might not be an important defence mechanism is the apparent size difference between probiotics and most parasites. Parasites are usually an order of magnitude larger than probiotic bacteria (Anderson 2019). Because of bigger size, parasites might be more competitive for adhesion sites which makes it less likely to be a mechanism of parasite resistance, but it might be important against parasitic protozoans such as *Ichthyophthirius multifiliis*.

Although competition for adhesion site might be less likely to occur, competition for nutrients (1) has been proposed as a mode of action against smaller parasitic pathogens like *Saprolegnia* sp. (Nurhajati et al. 2012). Especially the production of siderophores in low-iron environments can have great impact on the growth of fungal pathogens, the authors proposed.

To investigate all the proposed mechanisms, there is no universal probiotics administration method, because it largely depends on the research question. To investigate immunostimulation (4), for example, it might be better to administer the probiotics as feed additives, because we can exclude other possible mechanisms like competitive exclusion (1). Immunostimulatory effects have been reported in numerous probiotics studies (e.g. the previously highlighted study by Pieters et al. 2008). The most prominent class of probiotics which exhibit immonustimulation in their host are lactic acid bacteria (LABs).

Although fish possess a very basal immune system and rely more on the innate immune response compared to mammals, it is still a complex system and thus not easy to study due to the fact that multiple players and pathways can have an effect on pathogen inhibition (Uribe et al. 2011).

Because fungi-like parasitic pathogens are distinct from eukaryotic pathogens, they possess certain unique cellular structures (e.g. cell wall) which could potentially be a target of antithelminths (sometimes referred to as fungicides by some authors) produced by probiotics without harming the host organism (2). In fact, production of fungicides has been proposed as a possible mechanism in lactic acid bacteria (Livia 1998). Furthermore, lactic acid bacteria caused impediment of fungal growth by lowering the pH of the medium (3) to 4.0 (Livia 1998). In line with these results, Klaenhammer (1993) reported that lactic acid bacteria have the ability to produce organic acid to lower pH value down to 5.3-3.0. This can have a dramatic effect on microorganism growth (Moat and Foster 1995).

Probiotics producing specific anti-parasitic compounds (2) have not been reported up until the writing of this essay, but lowering the pH might be act as an anti-parasitic mechanism as well. I was not able to find any studies on skin pH and infection rates in fishes, but did find a study on water pH and parasite infection.

Garcia et al. (2011) investigated whether different water pH levels have an effect on *Ichthyophthirius multifiliis* infection and cumulative mortality of silver catfish fingerlings (*Rhamdia quelen*). They found that *R. quelen* held at water pH of 5.0 showed lower numbers of *I. multifiliis* trophonts per fish and a 40% reduce cumulative mortality after 16 days. This treatment is obviously not ideal, because it can affect the fish health as well and cannot be applied to fish sensitive to pH change in water.

Lastly, because probiotics can be administered in various manners, Mortiarty (1998) suggested to add probiotics to rear water to improve the water quality (5). To investigate competitive exclusion mechanisms (1), I would suggest this administration method as well. Another reason why I suggest this administration method is that probiotics have been found to improve water quality parameters through the process of bioaugmentation (Ashraf 2000, Venkateswara 2007). Bioaugmentation is defined as the degradation or removal of water pollutants, especially organic matter and nutrients by adding probiotics to a medium, usually water bodies (De et al. 2014). This technique can remediate water quality parameters such as dissolved oxygen (O_2, to counter hypoxia in the culture), nitrite (NO_2^-), ammonia (NH_3) and sulphide (S^{2-}) (Nzila 2016). Good water quality reduces stress levels in fishes and, therefore, might improve overall performance of the immune system and reduce infection rates of opportunistically parasitic pathogens. Parasites, on the contrary, show reduced infection rates when water quality is kept at constant high levels. Therefore, water probiotics might act as a preventive measure against parasitic infections.

Concluding remarks

The euphoria and great progress in the field of probiotics has concentrated on bacterial and viral diseases in fish aquaculture. Parasitic diseases (including fungal diseases) have been overlooked throughout the years, although they can be accountable for huge losses in fish stock and, therefore, revenue with impacts in the millions of dollars annually. Parasitic diseases propose several different challenges: their life cycles, their size compared to administered probiotics and their site of infection.

I conclude that probiotics hold great potential against parasitic diseases, because the repertoire and modes of action of probiotics can be very diverse. Therefore, I urge to continue the research in this direction. Moreover parasites have different weaknesses which probiotics can exploit. Production of antithelminths might be the most effective mode of action against certain parasitic diseases. Enhanced activity of the innate immune system of the fish and the quality of the rear water might be the two most effective mechanisms to fight higher eukaryotic parasites such as parasitic cnidarians and parasitic crustaceans. The selection of probiotics to test should be made on the basis of their proposed mode of action, and the type and mode of action of the parasite-of-interest.
However, we should not forget that the best treatment remains to be: Prevention.

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