Trends in Microbiology

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Opinion

Biological Approaches for Disease Control in Aquaculture: Advantages, Limitations and Challenges

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Although aquaculture activity has experienced a great development over the past three decades, infectious diseases have become a limiting factor for further intensification. Because the use of antibiotics has led to the widespread emergence of antibiotic resistance, the search for alternative environmentally friendly approaches is urgently needed. This Opinion paper offers an update on the successes and challenges of biological approaches for bacterial disease prevention and control in aquaculture. Although most of these approaches are still in research and development stages, some of them have shown promising results in field trials. Therefore, a better understanding of the mechanisms of action of these approaches will help to maximise their beneficial properties.

Highlights

Growing global concerns about antibiotic resistance have prompted the search for environmentally friendly approaches for preventing and controlling diseases in aquaculture.

Probiotics, prebiotics, their combination (synbiotics), nonviable bacterial or metabolic byproducts derived from probiotic bacteria (postbiotics), plant-derived natural compounds (phytobiotics), bacteriophages, and quorum sensing interference could be potential alternatives to the use of antibiotics in aquaculture.

Biological approaches used as preventive or therapeutic measures are needed when vaccination is not feasible in juvenile fish or farmed crustaceans and molluscs.

The Importance of Developing and Implementing Biological Control Strategies in Aquaculture Systems

Aquaculture has become an increasingly important food source worldwide. According to the Food and Agriculture Organisation (FAO) of the United Nations, global aquaculture production has grown from 31.1% in 2004 to 44.1% of the total production of 73.8 million tonnes of fish produced in 2014 [1]. However, this growth has been accompanied by the emergence, or re-emergence, of several infectious diseases. Given that aquaculture usually requires large-scale production facilities, high-density animal populations provide ideal conditions for the emergence and spread of infections, thereby causing severe economic losses. Environmental deterioration can also contribute to the prevalence of infections in aquaculture, particularly because diseases result from disturbed pathogen–host–environment interactions (Figure 1). Although antibiotics have been commonly used as prophylactic and therapeutic agents, the selective pressure created by their extensive use in animals and humans has contributed to the selection, persistence, and spread of antibiotic-resistant bacteria [2]. A recent UK government report has estimated that 700 000 people annually are dying due to infections caused by antibiotic-resistant bacteria, and this number may rise to 10 million deaths annually by 2050 if steps are not taken (http://amr-review.org). The use of antibiotics in aquaculture depends on local regulations, which may vary widely. In Europe, North America, and Japan, regulations on their use are strict, and only a few substances are licensed [3]. However, developing countries contribute to 90% of the world aquaculture production, with many of them lacking specific regulations [4]. For instance, a recent report from Oceana has shown that antibiotic use in Chilean salmon farming was ~900 g/ton of harvested biomass, whereas 0.17 g/ton was used in Norway (http://oceana.org/). Vaccination represents an alternative control strategy for infectious diseases; however, its efficacy is often limited or ineffective when applied to juvenile fish because they are not fully immunocompetent. Vaccination is also not feasible for farmed crustaceans and molluscs because they do not possess the capacity to develop long-term

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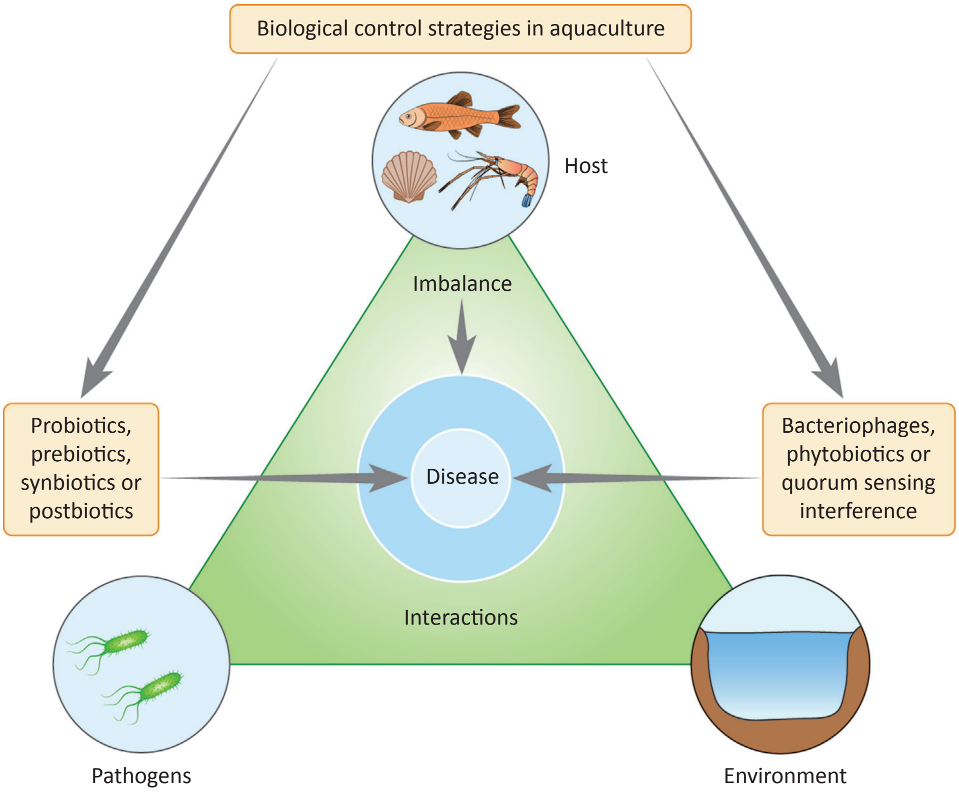

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Figure 1. Disturbance of Pathogen–Host–Environment Interactions Leads to Disease. Appropriate strategies should therefore be established to restore a disturbed microbiota to its normal beneficial composition. Applications of probiotics, prebiotics, synbiotics, postbiotics, bacteriophages, or phytobiotics may provide protection by creating a hostile environment for pathogens through several mechanisms, such as production of antimicrobial compounds, competition for available space and nutrients, inhibition of virulence gene expression, disruption of quorum sensing, or immunomodulatory properties. These features make them suitable agents for therapeutic applications in aquaculture. This figure has been adapted from Defoirdt *et al.* [62].

acquired immunity [5]. In addition to the low effectiveness of vaccines in early stages of development, and the lack of a true adaptive immune response in some species, there is a limited number of vaccines with marketing authorisation in aquaculture due to the complicated process before commercialisation. Given this, several biological control strategies have been proposed to promote the health and welfare of farmed species [6,7] (Box 1). In this Opinion paper we provide an update on the successes and challenges of these biological approaches for the prevention and/or control of infectious diseases in aquaculture.

Applications of Probiotics, Prebiotics, Synbiotics, or Postbiotics

Comparative analyses between animals exposed and unexposed to microorganisms have revealed that the **microbiota** (see Glossary) is substantially involved in a wide range of host functions [8,9]. Evidence obtained from these studies suggests that the intestinal microbiota provides both nutritional benefit and protection against pathogens and contributes to the development and differentiation of immune responses, which has resulted in the promotion of its manipulation through the use of **probiotics** [10]. Based on their mechanisms of action, probiotics can create a hostile environment for pathogens by the production of antimicrobial compounds, competition for available space and nutrients, inhibition of virulence gene

Glossary

Bacteriophages: viruses that infect bacterial cells.

Microbiota: all commensal, symbiotic, and pathogenic microorganisms sharing a defined niche (e.g., intestinal ecosystem).

Phytobiotics: plant-derived natural bioactive compounds which are added to the diet to improve nutrition and health in farm animals and humans [59].

Postbiotics: nonviable bacterial products or metabolic byproducts from probiotic microorganisms that have biological activity in the host [60].

Prebiotics: fermented ingredients that selectively stimulate the growth and/or activity in the gastrointestinal microbiota that confers benefits upon host well being and health [21].

Probiotics: formulations of live microorganisms which, when administered in adequate amounts, confer a health benefit on the host [61].

Synbiotics: combination of probiotics and prebiotics, which can result in additive or synergistic effects [23].

Box 1. Why Do We Need New Approaches for Disease Prevention and Control In Aquaculture?

Although aquaculture has experienced a remarkable growth and expansion during recent years, infectious diseases are a limiting factor and, in some cases, causing severe economic losses. We therefore need new strategies to prevent and control diseases in aquatic species, especially due to the following issues:

- Limited effectiveness of vaccines in early stages when the immune response is not fully developed.
- Medicated feed used as a preventive measure could contribute to an increase in antibiotic resistance.

Several environmentally friendly approaches for preventing and controlling diseases have been proposed; however, there are some obstacles that must be overcome before their potential use, such as:

- Difficulty in performing field trials for products/substances with a promising result under experimental conditions.
- Limited marketing authorisation in aquaculture.
- Lack of registered products for different aquatic species.

expression, or disruption of quorum sensing [11]. The antimicrobial effects of probiotics can be related to the production of antibiotics, bacteriocins, fatty acids, hydrogen peroxide, lytic enzymes, or organic acids. In particular, bacteriocins are ribosomally synthesized antimicrobial peptides produced by bacteria that have bactericidal or bacteriostatic effects on closely related bacterial strains [12]. These peptides have a number of properties that make them ideal candidates for disease control, although some studies indicate that bacteriocinogenic bacteria may harbour antibiotic-resistance genes [13].

It has also been suggested that bacteriocins play an important role as signalling peptides, communicating with other bacteria via quorum sensing (a chemical way that bacterial cells use to interact with each other and coordinate certain behaviours, such as biofilm formation), and with cells of the host immune system [14]. Nisin is one of the bacteriocins currently approved as a food preservative in over 80 countries, including the European Union and the USA [15]. A recent study demonstrated that the administration of *Lactococcus lactis* subsp. *cremoris* WA2-67 conferred protection against *Lactococcus garvieae* in rainbow trout (*Oncorhynchus mykiss*), and nisin Z production played a relevant role in this protection [16].

Likewise, among the beneficial effects attributed to probiotic bacteria, their capacity to interact with the host immune system is now recognised as a key mechanism of action protecting fish and shellfish against infections, and this is supported by an increasing number of *in vitro* and *in vivo* studies [11,17]. Probiotic bacteria can modulate the production of pro- and anti-inflammatory cytokines, which are crucial chemical messengers involved in the regulation, activation, growth, and differentiation of immune cells. For instance, dietary administration of *Lactobacillus plantarum* subsp. *plantarum* was not only able to upregulate interleukin-8 (IL-8) expression in the intestine, and stimulate the expression of several cytokines in the head kidney of rainbow trout, but was also effective in conferring protection against *Lactococcus garvieae* infection [18]. Dietary administration of *Lactococcus lactis* and *Lb. plantarum* also revealed an upregulation of cytokine gene expression in the intestine of olive flounder (*Paralichthys olivaceus*), as well as an increased resistance to *Streptococcus iniae* infection [19]. Similar results have also been observed in crustaceans, where dietary administration of *Bacillus subtilis* strains resulted in an upregulated expression of immune-related genes and an increased disease resistance of white shrimp, *Litopenaeus vannamei* [20].

Prebiotic are nondigestible food ingredients that have a beneficial effect through their selective metabolism in the gastrointestinal tract, and which allow specific changes in the composition of the microbiota [21]. Considering that **prebiotics** can promote the colonization and growth of beneficial bacteria, such as probiotics, within the intestinal ecosystem, their use may potentially reduce the number of pathogenic bacteria by competing for the same glycoconjugates on the surface of epithelial cells and improving the production of mucus, short-chain fatty acids, and cytokines [22]. Among them, mannan-oligosaccharides, fructo-oligosaccharides, short-chain fructo-oligosaccharides, inulin, chitosan oligosaccharide, galacto-oligosaccharides, arabino-xylo-oligosaccharides, and isomalto-oligosaccharides have shown promising results in aquaculture [23]. A recent study demonstrated that dietary administration of low-molecular-weight sodium alginate conferred beneficial effects in tilapia (*Oreochromis niloticus*), such as better growth performance, immune response, and resistance to *Streptococcus agalactiae* [24]. Dietary administration of *Astragalus* polysaccharides and chitooligosaccharides, alone or combined, also resulted in an increased immune response and disease resistance in juvenile largemouth bass (*Micropterus salmoides*) [25].

Altogether, these studies suggest that probiotics and prebiotics may be an ideal alternative to antibiotics in aquaculture. However, probiotics are not exempt from acquiring antibiotic resistance, and the long-term effect of adding high numbers of live bacteria to aquaculture systems has been questioned because those bacteria may also carry high levels of antibiotic resistance genes [3]. Potential adverse effects of horizontal gene transfer should therefore be taken into consideration. A better understanding of the intestinal microbial community under both homeostasis and disease states will permit the development of rationally designed approaches (such as optimal doses and intake durations). Such knowledge will permit the use of metabolic by-products (**postbiotics**), as well as the development and optimization of synergistic combinations (**synbiotics**) as viable strategies for therapy and prevention (Table 1). In fact, inactivated probiotic preparations or postbiotics appear as an interesting alternative to live probiotics. Dietary administration of four inactivated probiotic strains conferred protection against furunculosis in rainbow trout [26]. A recent study demonstrated that dietary supplementation of heat-killed *Lb. plantarum* and β-glucan had a significant interaction on growth performance, digestibility, and immune response in juvenile red sea bream [27]. Likewise, supplementation with synbiotics has shown promising results on growth performance and

Table 1. Biological Control Strategies with Their Primary Advantages and Limitations

Strategy	Advantages	Limitations	Refs
Probiotics, prebiotics, synbiotics, and postbiotics	Improve growth performance and health	Limited protection with some pathogens	[17–20,22, 24–29,63]
	Initiate and modulate immune responses	Variable synergistic effects	
	Prevent pathogen colonization and infection	Marketing authorisation is complex	
Phage therapy	Target specific, whereby avoiding damage to host microbiota	Potential for transfer of virulence and/or antibiotic-resistance genes	[31–39]
	Phage cocktails can reduce resistance development and be more effective than single phages	Potential for resistance development	
Phytobiotics	Antimicrobial, antiparasitic, anti-inflammatory, and antioxidative activities	Some constituents are unstable, e.g., they are photo- and thermo-labile	[41–44]
	Increase host survival	Interactions with host microbiota are unknown	
Quorum sensing interference	Represses biofilm formation and virulence factor production	Dose–response effects are unknown	[53–55]
	Increase host survival	Practical applications are still in progress	

survival rate. For instance, dietary administration of *Pediococcus acidilactici* and galacto-oligosaccharides was reported to synergistically increase immune response and disease resistance in rainbow trout fingerlings compared to when both were given individually [28]. Similar results were observed in Nile tilapia, when the synbiotic diet containing kefir and low-molecular-weight sodium alginate was administered [29].

Treatment with Bacteriophages

Since their discovery in the early 20th century, **bacteriophages** (phages) were recognised to have great potential for treating bacterial infections, an enthusiasm that was discouraged soon after the discovery of antibiotics [30]. However, the increasing prevalence of antibiotic-resistant bacteria has renewed interest in their use as antimicrobial agents to control pathogens through an environmentally friendly alternative [31]. Phages may be grouped into two categories by their life cycle: lysogenic (temperate) phages and lytic phages. The latter have the ability to rapidly lyse infected bacteria, and the capacity to increase their number during infection, which makes them potential biological control agents [32]. Moreover, phages are typically highly specific for their bacterial targets at the species or strain level, thereby minimising adverse effects on commensal bacteria. These properties offer an advantage for controlling specific pathogens because of their selective elimination without affecting the normal microbiota [33].

Use of *Aeromonas* phages pAh1-C and pAh6-C, either via oral administration or intraperitoneal injection, exerted noticeable protective effects – such as reduced mortality rates – in cyprinid loach (*Misgurnus anguillicaudatus*) exposed to *Aeromonas hydrophila* infection, with no side-effects during or after treatment [34]. Similar results were observed when *Aeromonas* phage AS-A was administered to juvenile Senegalese sole (*Solea senegalensis*), in which no mortality was observed in those exposed to *Aeromonas salmonicida* infection [35]. Although host specificity of phages can be considered as a disadvantage for phage therapy, this could be overcome by applying cocktails of phages. A comparative study demonstrated that phage cocktails are more efficient than a single phage in controlling the growth of *Vibrio parahaemolyticus* [36]. Likewise, administration of phage cocktails resulted in an increased survival rate in tiger shrimp (*Penaeus monodon*) larvae exposed to *Vibrio harveyi* infection [37]. Despite their antimicrobial potential, some important concerns remain about the use of phages in aquaculture (Table 1). Bacteria can develop resistance to phages through a variety of mechanisms, including blocking phage adsorption, inhibiting the injection of phage genomes, restriction–modification systems, and abortive infection systems [38]. Temperate phages can also transfer antibiotic resistance and virulence determinants from the phage to the host bacterium, although even obligate lytic phages harbour genes of unknown function that could result in undesired gene transfer [39]. Moreover, a recent study has demonstrated that phages can promote horizontal gene transfer by transformation [40]. These concerns should be taken into account in further studies; therefore, the use of purified phage components (e.g., lysins) could be considered to avoid these possible risks.

The Use of Plant Extracts (Phytobiotics)

Plant extracts, also known as **phytobiotics**, have been relatively recently exploited in aquaculture (Table 1), particularly for their antimicrobial, anti-inflammatory, antioxidative, and anti-parasitic activities [41–44]. Previous studies have demonstrated that essential oils and their major constituents – such as thymol and carvacrol – are active against *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella* spp. but are less effective against *Pseudomonas* spp. due to the formation of exopolysaccharides that increase resistance to these compounds [45,46]. Although the mechanism of action depends on their chemical composition, most essential oils have a higher bactericidal effect on Gram-positive bacteria than on Gram-negative bacteria, particularly due to differences in the cell membrane composition [46]. For instance, a study demonstrated that administration of a commercial feed additive containing essential oils (such as carvacrol, thymol, anethole, and limonene) confers protection against *A. salmonicida* infection in rainbow trout [43]. Dietary administration of oregano (*Lippia berlandieri* Schauer) or neem (*Azadirachta indica*) extracts also showed higher survival rates in white shrimp postlarvae exposed to *Vibrio parahaemolyticus* infection than in the control group [44]. It should be noted that azadirachtin is the main bioactive compound of neem, whereas thymol and carvacrol are the two major compounds in the essential oil obtained from oregano, which are of special interest due to their antioxidant and antimicrobial properties [47]. A recent study also demonstrated that the growth, feed intake, lysozyme, and mean corpuscular haemoglobin content of channel catfish (*Ictalurus punctatus*), reared at a low temperature, were enhanced by the addition of flaxseed oil to the diet compared with the unsupplemented diet [48]. Moreover, a comparative study showed that dietary administration of papaya (*Carica papaya*) extract can significantly promote growth and delay gonadal maturation in both male and female tilapia, while camphor (*Cinnamomum camphora*) extract was the most effective for controlling *Streptococcus agalactiae* infection [49]. Some essential oils and their major constituents have also shown anti-quorum sensing activity. Among them, cinnamaldehyde is one of the most studied essential oil components. For instance, the ability of 3,4-dichloro-cinnamaldehyde to decrease quorum sensing-regulated virulence of *Vibrio* species has been demonstrated using a nematode model [50]. It is well known that several *Vibrio* species are opportunistic pathogens of fish, shrimp, oysters, and other shellfish, whereby these essential oil components may be an interesting biological control strategy for aquaculture.

Quorum Sensing Interference

Quorum sensing (QS) is a mechanism of microbial cell-to-cell communication that regulates gene expression in response to population density to coordinate collective behaviours, such as virulence factor production, biofilm formation, and bioluminescence [51]. QS systems in bacteria have been generally divided into at least three classes: (i) LuxI/LuxR-type QS in Gram-negative bacteria, (ii) oligopeptide-two-component-type QS in Gram-positive bacteria; and (iii) *luxS*-encoded autoinducer 2 (AI-2) QS in both Gram-negative and Gram-positive bacteria [52]. The discovery of such mechanisms has led to identification and characterization of compounds or enzymes that quench QS, called QS interference. Some studies have suggested that QS interference represents a promising therapeutic approach (Table 1), and it could be considered a potential strategy for preventing disease in aquaculture systems [53–55]. In fact, several plants, algae, and bacteria produce compounds that mimic QS signals of many bacteria, interfering with bacterial QS and its controlled activities. Purified limonoids, particularly isolimonic acid and ichangin, have shown the ability to interfere with *V. harveyi* cell–cell signalling and biofilm formation by modulating the expression of the response regulator *luxO* [56]. Interestingly, addition of *Bacillus* sp. NFMI-C – which inactivates *N*-hydroxybutanoyl-l-homoserine lactone – to the rearing water improved survival of giant river prawn (*Macrobrachium rosenbergii*) larvae when challenged with pathogenic *Vibrio campbellii* [57]. Although QS interference provides a promising alternative to attenuating pathogenicity, bacteria can evolve resistance to QS inhibitors. However, it has been suggested that the chances of developing resistance to QS inhibitors are smaller than those to conventional antibiotics [55,58].

Concluding Remarks

Antibiotic resistance is an increasingly serious threat to global public health that requires action at different levels. Aquaculture activity is not exempt from these threats, as antibiotic agents have been widely used to protect fish and shellfish against diseases. Appropriate strategies should therefore be established to mitigate the emergence and spread of antibiotic resistance.

Outstanding Questions

Are there potential risks associated with the use of these biological control approaches?

What doses of these biological control approaches should be used, and for how long?

How do probiotics and prebiotics work together?

Why do some probiotic strains – even those belonging to the same species – show beneficial effects while others do not?

Can we get similar results with the use of postbiotics versus probiotics if the same strain is applied?

Is it really necessary to give a multi-strain preparation to get the most benefit?

This Opinion paper focuses on alternative biological strategies for sustainable aquaculture production. Probiotics, prebiotics, synbiotics, postbiotics, phytobiotics, bacteriophages, and QS interference may be considered environmentally friendly strategies for preventing and controlling diseases in aquaculture. Although most of these strategies have shown promising results, additional studies, particularly at field scale, are needed to select the most adequate strategy on the basis of its mechanism of action (see Outstanding Questions). A better understanding of how fish and shellfish immune systems generally respond to certain microbiota components (e.g., probiotics, postbiotics, etc.) will provide a basis for targeting manipulation of the microbial composition, which could be used to design adequate strategies for disease prevention and treatment. Recent advances in high-throughput sequencing technologies, such as metagenomics and metatranscriptomics, may help to reach these goals.

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References

1. FAO (2016) *The State of World Fisheries and Aquaculture 2016*: *Contributing to Food Security and Nutrition for All*, FAO. http://www.fao.org/3/a-i5555e.pdf
2. Cabello, F.C. *et al.* (2013) Antimicrobial use in aquaculture re-examined: its relevance to antimicrobial resistance and to animal and human health. *Environ. Microbiol.* 15, 1917–1942
3. Watts, J.E.M. *et al.* (2017) The rising tide of antimicrobial resistance in aquaculture: sources, sinks and solutions. *Mar. Drugs* 15, 158
4. Chuah, L.O. *et al.* (2016) Antibiotic application and emergence of multiple antibiotic resistance (MAR) in global catfish aquaculture. *Curr. Environ. Health Rep.* 3, 118–127
5. Falaise, C. *et al.* (2016) Antimicrobial compounds from eukaryotic microalgae against human pathogens and diseases in aquaculture. *Mar. Drugs* 14, 159
6. Akhter, N. *et al.* (2015) Probiotics and prebiotics associated with aquaculture: a review. *Fish Shellfish Immunol.* 45, 733–741
7. Hai, N.V. (2015) The use of probiotics in aquaculture. *J. Appl. Microbiol.* 119, 917–935
8. Rawls, J.F. *et al.* (2004) Gnotobiotic zebrafish reveal evolutionarily conserved responses to the gut microbiota. *Proc. Natl. Acad. Sci. U. S. A.* 101, 4596–4601
9. Galindo-Villegas, J. *et al.* (2012) Regulation of immunity and disease resistance by commensal microbes and chromatin modifications during zebrafish development. *Proc. Natl. Acad. Sci. U. S. A.* 109, E2605–E2614
10. Pérez, T. *et al.* (2010) Host–microbiota interactions within the fish intestinal ecosystem. *Mucosal Immunol.* 3, 355–360
11. Lauzon, H.L. *et al.* (2014) Probiotic applications in cold water fish species. In *Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics* (Merrifield, D. and Ringø, E., eds), pp. 223–252, John Wiley
12. Klaenhammer, T.R. (1993) Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol. Rev.* 12, 39–85
13. Imperial, I.C. and Ibana, J.A. (2016) Addressing the antibiotic resistance problem with probiotics: reducing the risk of its double-edged sword effect. *Front. Microbiol.* 7, 1983
14. Ventura, M. *et al.* (2015) Bifidobacteria of the human gut: our special friends. In *Diet-Microbe Interactions in the Gut: Effects on Human Health and Disease* (Tuohy, K. and Del Rio, D., eds), pp. 41–51, Elsevier
15. Gálvez, A. *et al.* (2012) Bacteriocins. In *Decontamination of Fresh and Minimally Processed Produce* (Gómez-López, V.M., ed.), pp. 317–332, John Wiley
16. Araújo, C. *et al.* (2015) Nisin Z production by *Lactococcus lactis* subsp. *cremoris* WA2-67 of aquatic origin as a defense mechanism to protect rainbow trout (*Oncorhynchus mykiss*, Walbaum) against *Lactococcus garvieae*. *Mar. Biotechnol.* 17, 820–830
17. Lazado, C.C. and Caipang, C.M. (2014) Mucosal immunity and probiotics in fish. *Fish Shellfish Immunol.* 39, 78–89
18. Pérez-Sánchez, T. (2011) Expression of immune-related genes in rainbow trout (*Oncorhynchus mykiss*) induced by probiotic bacteria during *Lactococcus garvieae* infection. *Fish Shellfish Immunol.* 31, 196–201
19. Beck, B.R. *et al.* (2015) The effects of combined dietary probiotics *Lactococcus lactis* BFE920 and *Lactobacillus plantarum* FGL0001 on innate immunity and disease resistance in olive flounder (*Paralichthys olivaceus*). *Fish Shellfish Immunol.* 42, 177–183
20. Zokaeifar, H. *et al.* (2012) Effects of *Bacillus subtilis* on the growth performance, digestive enzymes, immune gene expression and disease resistance of white shrimp, *Litopenaeus vannamei*. *Fish Shellfish Immunol.* 33, 683–689
21. Gibson, G.R. *et al.* (2004) Dietary modulation of the human colonic microbiota: updating the concept of prebiotics. *Nutr. Res. Rev.* 17, 259–275
22. Hoseinifar, S.H. *et al.* (2017) Prebiotics and synbiotics. In *Diagnosis and Control of Diseases of Fish and Shellfish* (Austin, B. and Newaj-Fyzul, A., eds), pp. 185–188, John Wiley
23. Huynh, T.G. *et al.* (2017) Current applications, selection, and possible mechanisms of actions of synbiotics in improving the growth and health status in aquaculture: a review. *Fish Shellfish Immunol.* 64, 367–382
24. Van Doan, H. *et al.* (2016) Effects of low molecular weight sodium alginate on growth performance, immunity, and disease resistance of tilapia, *Oreochromis niloticus*. *Fish Shellfish Immunol.* 55, 186–194
25. Lin, S.M. *et al.* (2017) Effects of *Astragalus* polysaccharides (APS) and chitooligosaccharides (COS) on growth, immune response and disease resistance of juvenile largemouth bass, *Micropterus salmoides*. *Fish Shellfish Immunol.* 70, 40–47
26. Irianto, A. and Austin, B. (2003) Use of dead probiotic cells to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). *J. Fish Dis.* 26, 59–62
27. Dawood, M.A. *et al.* (2015) Interaction effects of dietary supplementation of heat-killed *Lactobacillus plantarum* and β-glucan on growth performance, digestibility and immune response of juvenile red sea bream, *Pagrus major*. *Fish Shellfish Immunol.* 45, 33–42

28. Hoseinifar, S.H. *et al.* (2015) Modulation of innate immune response, mucosal parameters and disease resistance in rainbow trout (*Oncorhynchus mykiss*) upon synbiotic feeding. *Fish Shellfish Immunol.* 45, 27–32
29. Van Doan, H. *et al.* (2017) The effects of dietary kefir and low molecular weight sodium alginate on serum immune parameters, resistance against *Streptococcus agalactiae* and growth performance in Nile tilapia (*Oreochromis niloticus*). *Fish Shellfish Immunol.* 62, 139–146
30. Pires, D.P. *et al.* (2016) Genetically engineered phages: a review of advances over the last decade. *Microbiol. Mol. Biol. Rev.* 80, 523–543
31. Quiroz-Guzmán, E. *et al.* (2018) Bacteriophage cocktails as an environmentally-friendly approach to prevent *Vibrio parahaemolyticus* and *Vibrio harveyi* infections in brine shrimp (*Artemia franciscana*) production. *Aquaculture* 492, 273–279
32. Duckworth, D.H. and Gulig, P.A. (2002) Bacteriophages: potential treatment for bacterial infections. *BioDrugs* 16, 57–62
33. Letchumanan, V. *et al.* (2016) Insights into bacteriophage application in controlling *Vibrio* species. *Front. Microbiol.* 7, 1114
34. Jun, J.W. *et al.* (2013) Protective effects of the *Aeromonas* phages pAh1-C and pAh6-C against mass mortality of the cyprinid loach (*Misgurnus anguillicaudatus*) caused by *Aeromonas hydrophila*. *Aquaculture* 416–417, 289–295
35. Silva, Y.J. *et al.* (2016) Biological control of *Aeromonas salmonicida* infection in juvenile Senegalese sole (*Solea senegalensis*) with Phage AS-A. *Aquaculture* 450, 225–233
36. Mateus, L. *et al.* (2014) Efficiency of phage cocktails in the inactivation of *Vibrio* in aquaculture. *Aquaculture* 424–425, 167–173
37. Stalin, N. and Srinivasan, P. (2017) Efficacy of potential phage cocktails against *Vibrio harveyi* and closely related *Vibrio* species isolated from shrimp aquaculture environment in the south east coast of India. *Vet. Microbiol.* 207, 83–96
38. Labrie, S.J. *et al.* (2010) Bacteriophage resistance mechanisms. *Nat. Rev. Microbiol.* 8, 317–327
39. Allen, H.K. *et al.* (2013) Treatment, promotion, commotion: antibiotic alternatives in food-producing animals. *Trends Microbiol.* 21, 114–119
40. Keen, E.C. *et al.* (2017) Novel ‘superspreader’ bacteriophages promote horizontal gene transfer by transformation. *mBio* 8, e02115-16
41. Na-Phatthalung, P. *et al.* (2018) Immunomodulatory effects of *Rhodomyrtus tomentosa* leaf extract and its derivative compound, rhodomyrtone, on head kidney macrophages of rainbow trout (*Oncorhynchus mykiss*). *Fish Physiol. Biochem.* 44, 543–555
42. Zhang, Q. *et al.* (2013) Evaluation of an antiparasitic compound extracted from *Galla chinensis* against fish parasite *Ichthyophthirius multifiliis*. *Vet. Parasitol.* 198, 45–53
43. Menanteau-Ledouble, S. *et al.* (2015) Effect of a phytogenic feed additive on the susceptibility of *Onchorhynchus mykiss* to *Aeromonas salmonicida*. *Dis. Aquat. Organ.* 115, 57–66
44. Morales-Covarrubias, M.S. *et al.* (2016) Evaluation of medicinal plants and colloidal silver efficiency against *Vibrio parahaemolyticus* infection in *Litopenaeus vannamei* cultured at low salinity. *Dis. Aquat. Organ.* 122, 57–65
45. Burt, S. (2004) Essential oils: their antibacterial properties and potential applications in foods – a review. *Int. J. Food Microbiol.* 94, 223–253
46. Nazzaro, F. *et al.* (2013) Effect of essential oils on pathogenic bacteria. *Pharmaceuticals* 6, 1451–1474
47. Dunford, N.T. and Silva Vazquez, R. (2005) Effect of water stress on plant growth and thymol and carvacrol concentrations in Mexican oregano grown under controlled conditions. *J. Appl. Hortic.* 7, 20–22
48. Thompson, M. *et al.* (2015) A dietary dairy/yeast prebiotic and flaxseed oil enhance growth, hematological and immunological parameters in channel catfish at a suboptimal temperature (15 °C). *Animal* 9, 1113–1119
49. Kareem, Z.H. *et al.* (2016) Effects of some dietary crude plant extracts on the growth and gonadal maturity of Nile tilapia (*Oreochromis niloticus*) and their resistance to *Streptococcus agalactiae* infection. *Fish. Physiol. Biochem.* 42, 757–769
50. Brackman, G. *et al.* (2011) Structure-activity relationship of cinnamaldehyde analogs as inhibitors of AI-2 based quorum sensing and their effect on virulence of *Vibrio* spp. *PLoS One* 6, e16084
51. Rutherford, S.T. *et al.* (2011) AphA and LuxR/HapR reciprocally control quorum sensing in vibrios. *Genes. Dev.* 25, 397–408
52. Li, Y.H. and Tian, X. (2012) Quorum sensing and bacterial social interactions in biofilms. *Sensors* 12, 2519–2538
53. Zhao, J. *et al.* (2014) Mechanisms of quorum sensing and strategies for quorum sensing disruption in aquaculture pathogens. *J. Fish Dis.* 38, 771–786
54. Defoirdt, T. *et al.* (2011) Alternatives to antibiotics for the control of bacterial disease in aquaculture. *Curr. Opin. Microbiol.* 14, 251–258
55. Kalia, V.C. (2013) Quorum sensing inhibitors: an overview. *Biotechnol. Adv.* 31, 224–245
56. Vikram, A. *et al.* (2011) Citrus limonoids interfere with *Vibrio harveyi* cell–cell signalling and biofilm formation by modulating the response regulator *LuxO*. *Microbiology* 157, 99–110
57. Pande, G.S. *et al.* (2015) Isolation of AHL-degrading bacteria from micro-algal cultures and their impact on algal growth and on virulence of *Vibrio campbellii* to prawn larvae. *Appl. Microbiol. Biotechnol.* 99, 10805–10813
58. Defoirdt, T. *et al.* (2010) Can bacteria evolve resistance to quorum sensing disruption? *PLoS Pathog.* 6, e1000989
59. Cheng, G. *et al.* (2014) Antibiotic alternatives: the substitution of antibiotics in animal husbandry? *Front. Microbiol.* 5, 217
60. Patel, R.M. and Denning, P.W. (2013) Therapeutic use of prebiotics, probiotics, and postbiotics to prevent necrotizing enterocolitis: what is the current evidence? *Clin. Perinatol.* 40, 11–25
61. Reid, G. *et al.* (2003) New scientific paradigms for probiotics and prebiotics. *J. Clin. Gastroenterol.* 37, 105–118
62. Defoirdt, T. *et al.* (2007) Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example. *Trends Biotechnol.* 25, 472–479
63. Hoseinifar, S.H. *et al.* (2017) *In vitro* selection of a synbiotic and *in vivo* evaluation on intestinal microbiota, performance and physiological response of rainbow trout (*Oncorhynchus mykiss*) fingerlings. *Aquac. Nutr.* 23, 111–118