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PHYTOBIOTICS AND THEIR ANTIBACTERIAL ACTIVITY AGAINST MAJOR FISH PATHOGENS. A REVIEW

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ABSTRACT

This review focuses on the characteristic of major fish bacteria, antimicrobial resistance and antibiotic substitution in aquaculture. Nowadays aquaculture represents the fastest growing industry leading to the use of intensive and semi-intensive methods resulting in outbreaks of bacterial diseases. Antibiotics are used to treat and prevent these bacterial diseases, which can cause an increase in resistance. Many antibiotics applied in aquaculture were originally used in human medicine and many countries do not have strict limits for their application. Annually, more than 700 000 people worldwide die from bacterial infections caused by bacteria resistant to antibiotics and even though European countries have strict rules for the use of antibiotics, the death rate resulting from resistance represents more than 33 000 people per year. One of the options how to resolve this problem is the replacement of antibiotics with bioactive compounds of plant origin that exhibit strong inhibitory activity against pathogenic bacteria.

Key words: antibiotic resistance; aquaculture; fish bacteria; phytobiotics

INTRODUCTION

Aquaculture is currently the most developing branch compared to other branches of the food industry. Based on the Food and Agriculture Organization [25], the global production of aquaculture products reached approximately 43 million tons in 2016 and about 61 million tons in 2020. This was due to the rapidly growing human population, which needs to be supplied with complete nutrients and aquaculture products are a good source of these [67]. They contain high-quality protein and omega-3 fatty acids, such as docosahexaenoic acid (DHA) that plays an important role in optimum brain development and neurodevelopment, and eicosapentaenoic acid (EPA) that has beneficial impact on cardiovascular health. Aquaculture products are rich in minerals like selenium, iron, zinc, calcium, potassium, phosphorus and also in vitamin A and D and some B vitamins [79]. Another advantage is related to the beneficial effect of aquaculture on global warming due to reduced hunting of wild fish and reduced marine pollution [67]. Development in fish farming technologies also contributed to the rapid growth of aquaculture [74]. On the other hand, the rapid increase has several disadvantages such as the use of intensive and semi-intensive farming methods, which involve higher concentration of

fish in a small water volume that is related to higher stress and a higher risk of bacterial diseases [67]. The bacteria most common in aquaculture are Gram-negative bacteria such as *Aeromonas salmonicida* [43], *Aeromonas hydrophila* [46], *Vibrio anguillarum* [27], *Vibrio alginolyticus* [38], *Edwardsiella tarda* [44], *Flavobacterium psychrophilum* [8] and *Yersinia ruckeri* [86]. Of the Gram-positive bacteria, the most common are *Staphylococcus* spp. and *Streptococcus* spp. [60]. All these bacteria can cause serious diseases, survive in aquatic environment without the host, increase mortality and cause great economic losses. These are the reasons why bacterial diseases have become the major obstacles to aquaculture. Antibiotics are used to prevent (prophylactic purpose) and treat (therapeutic purpose) these bacterial diseases but their increased use results in developing antibiotic resistance. Fish bodies absorb only 20–30% of the received antibiotics and the rest ends in the environment because fish are not able to metabolize them efficiently [70]. This is the reason why in aquaculture the fish, other aquatic animals and aquatic environment form reservoirs of resistant bacteria and the resistance is expanded by mutations in bacterial DNA or by horizontal gene transfer including conjugation with other bacteria, transduction with bacteriophage and transformation of free DNA [63, 68]. Since resistance to antibiotics is becoming more widespread every year, it is important to look for new methods to replace them. One of the perspective candidates are bioactive compounds of natural origin, which can be found in plants, animals, microorganisms and marine organisms. Bioactive compounds of plant origin include alkaloids, saponins, polyphenols, terpenes, essential oils and flavonoids [89] while polysaccharides, polyunsaturated fatty acids, coenzyme Q10, choline and peptides from milk protein belong to those typical for animals [88]. Many of the listed compounds are characterized by an anti-inflammatory, antioxidant, antitumor, and mainly by antimicrobial activities, however more studies are needed to improve their stability, obtain more knowledge about their mechanism of action, and about their impact on the environment, host microbiota and their stability in the feed [51].

THE MAIN BACTERIAL AGENTS IN AQUACULTURE

Aeromonas salmonicida

Aeromonas salmonicida is a Gram-negative bacterium also known as the oldest fish pathogen [43]. It is found in fresh and salt water where it causes disease in salmonids and non-salmonid fish, such as minnow, goldfish, carp, perch and catfish. The main clinical symptom is furunculosis, which is shown in Figure 1 and it also manifests as ulcerative dermatitis and granulomatous dermatitis [5]. The virulence factors of *A. salmonicida* are the A-layer, extracellular molecules as proteases, lipases, and type III

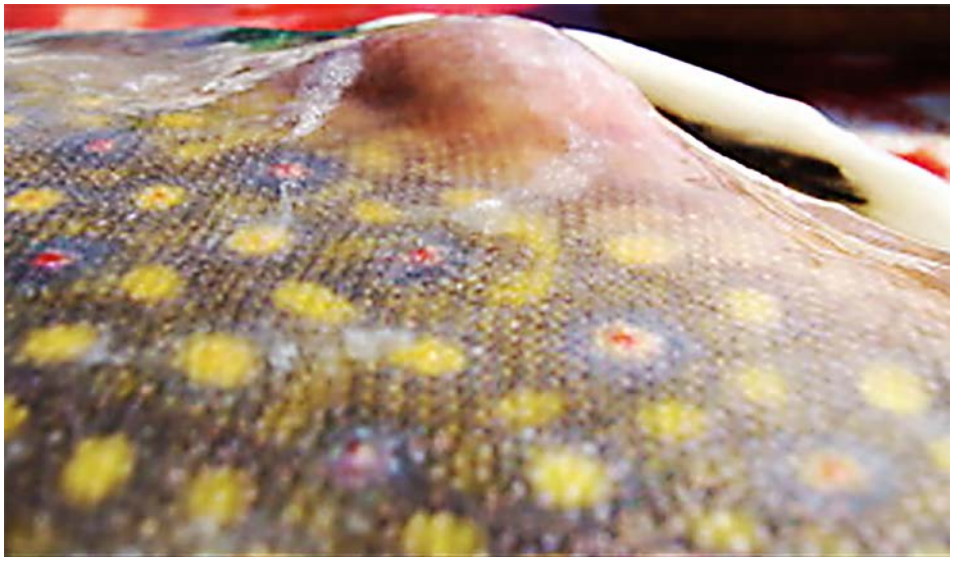


Fig. 1. Clinical manifestation of furunculosis [18]

secretion system [18]. The A-layer is also known as the S-layer and it occurs on the surface of the cell. It provides protection against the specific immune system and resistance to cytotoxicity of macrophage [54]. The extracellular molecules include serine protease, which causes muscle liquefaction leading to furunculosis or lipase, which participates in the formation of bleeding boils (Fig. 1) [18]. The type III secretion system enables the translocation of effector proteins, causing inhibition of phagocytosis and induction of apoptosis from the bacterial cytoplasm into the host cell [54].

Aeromonas hydrophila

Aeromonas hydrophila is characterized as a Gram-negative bacterium common in fresh water. It has been isolated from catfish, bass, carp and can be found also in marine fish species [17]. This bacterium is the causative agents of motile *Aeromonas septicaemia* known as tail and fin rot that is clinically manifested by haemorrhage, anaemia, ascetic fluid, abdominal distension and ulcerations [2]. The virulence factors include O-antigen that has several roles such as protection and adhesion against the host immune system, and cytotoxic enterotoxin that has the ability to destroy tissue culture cell lines and cause lysis of red blood cells. The virulence factors also include haemolysin, enzymes such as proteases, amylases, lipases and type III secretion system [14, 72, 80].

Vibrio anguillarum

Vibrio anguillarum, also known as *Listonella anguillarum*, is a Gram-negative bacterium causing big economy losses in marine, fresh and brackish water fish such as salmon, sea bass, cod and rainbow trout. Infection by *V. anguillarum* leads to haemorrhagic septicaemia called vibriosis that manifests with red spots, swollen and dark skin lesions, ulceration and haemorrhages of skin and fin [27]. Virulence factors of *V. anguillarum* include motility and chemotaxis, an iron uptake system, haemolysins and zinc metalloprotease that degrades the tissue in the host [30, 31].

Edwardsiella tarda

Edwardsiella tarda is characterized as a Gram-negative intracellular bacterium that can survive in an environment with 0–4% sodium chloride. It infects marine and fresh water fish worldwide and results in a systemic disease called edwardiellosis that manifests as exophtalmus, petechial haemorrhages on the skin and fin, rectal hernia, swelling of the abdominal surface and ascites [55]. The most important virulence factors of *E. tarda* are the type III and the type VI secretion systems. The type III secretion system allows to replicate intracellularly in phagocytes and evade phagocytosis and the type VI secretion system is only recently discovered secretion system, which contributes to bacterial virulence [37]. Other virulence factors include fimbrial adhesion-like protein, siderophore and exoenzymes, such as protease and catalase and outer membrane proteins (OMPs) that play a key role in the integrity and selective permeability of bacterial membranes [42, 83, 87].

Flavobacterium psychrophilum

Flavobacterium psychrophilum is a Gram-negative bacterium that is able to survive outside the fish in fresh water for a few months to several years [8]. This pathogen is characteristic for cold water fish species such as trout and salmonids and the infection leads into the cold water disease and rainbow trout fry syndrome (RTFS). The cold water disease in salmonids is manifested with erosion lesions on the caudal fin, necrosis and ulcerations of the skin on the lower jaw. Increasing pigmentation leads into the “black tail” and RTFS manifests with anaemia, haemorrhagic and protruding anus and skin erosions located mainly behind the dorsal fin or on the lateral side of the body [10, 41, 76]. Virulence factors consist of the type IX secretion system that is able to secrete proteins to the cell surface and beyond, cell surface adhesins, soluble and cell-associated peptidases, nucleases and more hydrolytic enzymes [7]. Another virulence factor is the gliding motility that allows movement of the cells over surfaces without the help of pili or flagellum and also include cytolytic toxins and proteases [23, 58].

Yersinia ruckeri

Yersinia ruckeri is a Gram-negative bacterium causing yersiniosis and enteric red-mouth disease mainly in salmonids and rainbow trout. The characteristic clinical symptoms of *Y. ruckeri* include haemorrhages on the surface of the body, redness at the base of the fins, along the lateral line and also in the head area [81]. The virulence factors include extra-cellular products, which have cytolytic and haemolytic activity, metalloprotease that causes degrada-

tion of fibronectin, actin and myosin [34]. Another virulence factors of *Y. ruckeri* are the type III secretion system, the type IV secretion system that is used to transport macromolecules across the bacterial cell membrane and the iron acquisition system which is involved in colonization and invasion of host tissues [81, 86].

ANTIMICROBIAL RESISTANCE

Antimicrobial resistance (AMR) is a characteristic trait of bacteria, viruses, parasites and fungi. The resistant agents have the ability to grow and spread when an antimicrobial medicine is present even though these medicines are normally effective against them [26]. There are several types of antimicrobial agents such as antibiotics, chemotherapeutics, food preservatives and disinfectants that are used against different microorganisms. Antimicrobial resistance is the main problem of healthcare organizations because the increasing use of antibiotics leads to the emergence of resistance [1]. Different origins of antibiotic resistance have been distinguished, such as natural, which is divided into intrinsic and induced resistance and then adaptive and acquired resistance. Intrinsic resistance is defined as a trait that is generally shared within a bacterial species and is always expressed. On other hand, induced resistance is normally occurring in the bacteria but is expressed only if there is some exposure to an antibiotic. Adaptive resistance is caused and induced by a specific environmental signal such as pH, stress or concentrations of ions. Acquired resistance develops in originally sensitive bacteria in two different ways. The first develops through mutation and the next by obtaining new genetic material from an exogenous source. It is also known as a horizontal gene transfer that includes transduction, transformation and conjugation [11, 16]. Aquatic environment is a well-suited reservoir of antibiotic resistance because water is a favourable environment for horizontal gene transfer and has a high infiltration capacity, which allows the contamination of clean water with unsanitary water containing antimicrobial compounds [6]. The ability of transfer of resistant pathogens from aquaculture to natural aquatic environment causes an increase in the resistance of fish, other aquatic animals and humans to antimicrobials that results in infections and failure of treatment [51]. In addition, many antibiotics used in aquaculture are also used in human medicine which promotes the risk of development of resistance in humans [60]. The antibiotics most frequently used in aquaculture worldwide, including the USA and European and Asian countries, are described in Table 1.

While European countries and USA have strict regulations for the use of antibiotics in aquaculture, in other countries like China; the limits for their use are poor. Studies showed a high rate of antimicrobial residues in various countries such as India, Indonesia, Bangladesh, Iran, Japan and Nigeria [12, 51]. Globally, approximately 10 259 tons of antimicrobials were used in aquaculture in 2017 and their consumption is expected to increase by 33% to around 13 600 tons in 2030 [71]. Because of the increasing antibiotic resistance in animal and human environments, it is necessary to look for new replacements to antibiotics. Annually, more than 700 000 human deaths worldwide, more than 35 000 deaths in the USA, 33 000 deaths in Europe and 58 000 deaths in India have been attributed to the increasing resistance of disease agents, and by 2050 around 10 million of deaths annually on account of antimicrobial resistance are expected [65, 85].

Table 1. The antibiotics most frequently used in aquaculture

Antibiotics			
Worldwide [57]	**European countries** [39, 67]	**Asian countries** [39]	**USA** [29]
Ampicillin	Erythromycin	Amoxicillin	Florfenicol
Erythromycin	Florfenicol	Chlortetracycline	Oxytetracycline
Florfenicol	Oxytetracycline	Florfenicol	Chloramphenicol
Streptomycin	–	Neomycin / Oxytetracycline	Erythromycin

ALTERNATIVES TO ANTIBIOTICS

New alternatives to antibiotics need to be discovered because of their negative impact on the environments, animals and humans. One of the promising alternatives are plants and their bioactive compounds known as phytobiotics, due to their antimicrobial, antioxidative, anti-inflammatory, antiparasitic and appetite stimulating effect [59]. Bioactive compounds represent secondary metabolites of the plant and due to their structural diversity and efficiency could be used as a drug candidate. The antibacterial activity of phytobiotics manifest with different mechanisms such as bacterial cell wall disruption, nucleic acid translation, transcription blockage, lysozyme and complement activity enhance but the efficiency depends on the target bacteria, phytobiotic and its dozen. Phytobiotics represent compounds such as flavonoids, tannins, alkaloids, terpenes, saponins and phenolic compounds. Their presence in the plant depends on the climate, the type of soil, the state of maturity of the plant and on the part of used plant [33, 48].

Terpenes

Terpenes represent the largest group of secondary metabolites, which show important functions in nature such as anti-inflammatory, antibacterial, antiviral and antiseptic function. Depending on the number of carbon atoms they can be divided into monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), tetraterpenes (C40) and poly-terpenes (C>40). They occur in different parts of the plants, such as the flowers and fruits and they can be commonly found in tea, thyme, Spanish sage, cannabis and citrus fruits [15, 48].

Alkaloids

Alkaloids are widely spread compounds produced by plants, bacteria, fungi and animals. These compounds contain nitrogen atoms in their chemical structure. Only 20 % of plant species contains alkaloids and are known for their variety of functions. They have analgesic, anticancer, antiseptic, antibacterial, antifungal and antiviral functions [13, 66]. Plants belonging to the families Amaryllidaceae, Berberidaceae, Liliaceae, Leguminaceae, Papaveraceae, Ranunculaceae, and Solanaceae contain a large amount of alkaloids [9].

Phenolic compounds

Phenolic compounds have typical structure formed in an aromatic ring with one or more hydroxyl groups. They commonly occur in bark, seeds, leaves and flowers [32]. They can be divided into five subgroups: i.e., coumarins, flavonoids, phenolic acids, lignins and tannins. The major subgroup of phenolic compounds represents flavonoids which acts as antioxidants and also have anti-inflammatory, anti-mutagenic and anti-carcinogenic effects [53]. Coumarins are known for their influence on the central nervous system, antioxidant, antibacterial effect and for their role in cancer prevention [35]. Phenolic acids have a wide spectrum of actions: such as antioxidant, anti-inflammatory, anticancer, antimicrobial or immunoregulatory [62]. Another subgroup represents lignins, which protect polysaccharides in cell wall from microbial degradation. [82]. The last one, tannins, have anticancer, antioxidant, antimutagenic function [19].

Saponins

Saponins are important secondary metabolites that are characterized with antibacterial, anti-tumor, antioxidative, anti-inflammatory, antidiabetic and neuro-protective function. Depending upon the chemical structures they are divided into two classes: i.e., triterpenoid and steroidal saponins. Their high concentrations can be found in plants like quinoa, spinach, oat, beans, peas and citrus [49].

The main bioactive compounds and properties of some plants usable in aquaculture are described in Table 2.

Hayatgheib et al. [28] investigated the effect of phytobiotics against *Aeromonas salmonicida* subsp. *salmonicida* isolated from rainbow trout and discovered the effectiveness of the following herbs: *Cinnamomum zeylanicum; Origanum vulgare; Thymus vulgaris*. The main bioactive compounds, which showed suitable inhibitory effect belongs to the phenolic compounds (eugenol, carvacrol, thymol) and to the aldehydes (cinnamaldehyde). Also study involving *Thymus vulgaris* confirmed a strong inhibitory effect against *Vibrio anguillarum*. Major components with antibacterial effect represent phenolic compounds (thymol, carvacrol) and monoterpenes (p-Cymene) [47]. The studies conducted by Musa et al. and Nya et al. showed benefits of *Allium sativum* toward inhibition of *Staphylococcus aureus, Aeromonas hydrophila* and *Edwardsiella tarda*. Allicin represent the compound with antibacterial activity that *Allium sativum* contain [45,

Table 2. List of bioactive compounds of plant origin used in aquaculture

Plant scientific name	Common Name	Biological active compounds	Properties	References
Allium sativum	Garlic	Saponins, phenolic compounds, polysaccharides and organic sulphides	Antibacterial, anti-inflammatory, antifungal	[33, 36, 73]
Cinnamomum verum	True cinnamon tree	Phenolic and terpenoids compounds, tannins	Antibacterial, anticancer, anti-inflammatory	[4, 64, 84]
Cymbopogon citratus	Lemongrass	Flavonoids, phenolic compounds, terpenoids	Antibacterial, antifungal	[20, 52]
Eupatorium odoratum	Siam weed	Flavonoids, phenolic compounds	Antibacterial, antiviral	[33, 69]
Ocimum sanctum	Tulsi	Flavonoids, tannins, saponins	Antibacterial, antifungal	[40, 56]
Origanum onites	Greek oregano	Terpenoids, flavonoids, phenolic compounds	Antibacterial, antiviral, anti-inflammatory	[20, 75, 78]
Origanum vulgare	Oregano	Phenol compounds, flavonoids	Antibacterial, antifungal	[20, 61]
Thymus vulgaris	Thyme	Phenolic compounds	Antibacterial, anti-inflammatory, antiviral, immune stimulation	[3, 22]
Zingiber officinalis	Ginger	Flavonoids, phenolic compounds saponins, tannins	Antibacterial, antiviral, antifungal, anti-inflammatory	[24, 77]

50]. De Rezende et al. [21] focused on Nile tilapia's dietary supplementation with a blend of phytobiotics contained in *Lippia sidoides, Thymus vulgaris* and *Thymus zygis* with the phenol compounds (thymol) as their major component. Studies have shown showed improvement in tilapia's resistance against *A. hydrophila*, better biochemical responses and benefits for the immune system.

CONCLUSIONS

This review describes the necessity of dealing with antibiotic resistance as it represents a major obstacle in maintaining animal and human health. Due to the rapid development of the aquaculture industry, there is an increased incidence of bacterial diseases requiring application of antibiotics for preventive and treatment purposes. However, their application is not regulated appropriately in some countries, especially in Asia, and results in increase resistance of disease agents to antimicrobials. A suitable substitute for antibiotics could be phytobiotics especially due to their antibacterial effect but also other benefits such as: antiviral, anti-inflammatory and antifungal properties. In order to successfully replace antibiotics with phytobiotics, studies need to be performed to obtain more knowledge about their mechanisms of action, impact on the environment and the host microbiota and their stability in feed.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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