IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

BACTERIAL COLD-WATER DISEASE, AN EMERGING FLAVOBACTERIAL DISEASE OF FISHES: A REVIEW

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Abstract

Bacterial cold-water disease caused by *Flavobacterium psychrophilum* is a major threat to both cultured and wild fish populations globally. The disease often results in high mortality with survivors suffering from long-term effects such as stunted growth and bodily deformities. The pathogenesis involves complex interactions between the bacteria and host fish, with environmental factors like poor water quality aggravating the infections. While traditional antibiotic-based therapeutics are becoming seemingly less effective due to rapid development of resistance, alternative approaches, including probiotics, immunostimulants, and vaccine development, show promise. Therefore, prevention strategies focus on improved hatchery management, stress reduction, and enhanced biosecurity measures need to be the rule. Continued research is essential to develop effective diagnostics, treatments, and preventive measures to mitigate the impact of these diseases on aquaculture.

Keywords: Bacteria, cold water, pathogenic, rainbow trout

Introduction

Flavobacterial diseases, firstly documented by in 1922, have become significant threats to both wild and cultured fish populations. Three bacteria within the Flavobacteriaceae were the etiological agents of these diseases: *Flavobacterium psychrophilum (FP)*, the cause of bacterial cold-water disease (BCWD) and rainbow trout fry syndrome (RTFS) (Bernardet & Nakagawa, 2006; Starliper, 2011); *Flavobacterium columnare*, of columnaris disease (Shotts & Starliper, 1999) and *Flavobacterium branchiophilum*, associated with bacterial gill disease (Shotts & Starliper, 1999). Few more *Flavobacterium* species were also found in diseased fish *F. johnsoniae*, *F. succinicans*, *F. hydatis*. Flavobacteriosis is a grave disease and can result in higher mortality surpassing 70% in affected fish populations and the survivors never attain normal weight and the body confirmation, spinal deformities and stunted growth are often the rule. Subacute and chronic infections contribute to ongoing mortality and significant economic losses.

Characteristics of *Flavobacterium* species

The *Flavobacterium* are Gm-ve rod shaped un-flagellated bacteria of 0.3–0.5 µm diameter and 1.0 to 40.0 µm length, either non-motile or show gliding motility. Their gliding motion is particularly effective in environments with low nutrient levels and high moisture. The presence of non-diffusible, non-fluorescent pigments like flexirubin and carotenoids producing a pale to bright yellow color to the colonies are the characteristics of *Flavobacterium*. Most species are mesophilic, having optimal growth at temperatures of 20 to 30°C, while psychrophilic species prefer cooler conditions of 15 and 20°C. Some species are found to grow at ~37°C (*F. granuli*, *F. columnare*, *F. suncheonse*, *F. succinicans*) or even at 40–45°C (*F. defluvii*, *F. indicum*, *F. croceum*). Therefore, *Flavobacteria* were reported from diverse environments, such as freshwater streams, lakes, and sediments (Qu *et al.*, 2009; Lee *et al.*, 2010), glaciers and arctic ice (Xu *et al.*, 2011; Dong *et al.*, 2012), freshwater shrimp ponds (Sheu *et al.*, 2011), seawater and marine sediments (Fu *et al.*, 2011; Yoon *et al.*, 2011) and marine algae (Miyashita *et al.*, 2010).

Most *Flavobacterium* species grow on nutrient agar and trypticase soy-agar (TSA) without the need for additional growth factors, however certain pathogenic members such as *F. psychrophilum*, *F. columnare* and *F. branchiophilum* do not grow on TSA. While some species can oxidize carbohydrates, almost all exhibit strong proteolytic activity. The isolation and identification of these bacteria can be challenging due to their need for specific low-nutrient media and a relatively long incubation period (at least a week) to form visible colonies, making the process labor-intensive (Bernardet & Bowman, 2015).

***Flavobacterium* species as fish pathogens**

Flavobacteria are the major fish pathogens globally, *Flavobacterium psychrophilum*, *F. branchiophilum*, and *F. columnare* being responsinle for spiking losses in cultured and wild fishes. *FP* is the etiological agent of bacterial coldwater disease (BCWD) and rainbow trout fry syndrome (RTFS) (Starliper, 2011), *F. branchiophilum* is the culprit of bacterial gill disease (Shotts & Starliper, 1999) while *F. columnare* results in columnaris disease (Shotts & Starliper, 1999). Among these three bacteria, *FP* drags out the global concern due to its broader habitat range and widespread distribution (Table 1) and also due to its close link to intensive salmonid aquaculture.

Table 1. Geographical distribution of *Flavobacterium psychrophilum* in different fish species

Fish	Country	Reported from	Source
Ayu fish	Japan	Kidney, Ovay, Lower jaw, Gill, Ceolomic fluid, Skin lesion, Egg	Nagata *et al.* (2024)
Rainbow trout	Japan	Kidney, Gill,	
Coho salmon		Kidney, Peduncle	
Common carp/Koi		Skin lesion, Gill	
Eel	Scotland, UK	Eggs	Soares *et al.* (2019)
Rainbow trout		Eyed Egg	Donati *et al.* (2021)
Rainbow trout		Fry	Nilsen, *et al.* (2011)
Rainbow trout		Unfertilized Eggs	Vatsos *et al.* (2001)
Atlantic salmon		Fry	Macchia *et al.* (2022)
Rainbow trout		Milt	Madsen *et al.* (2005)
Tench	Germany	Liver, Spleen	Nicolas *et al.* (2008)
Brown trout	France	Kidney	Nagata *et al.* (2013)
Chum salmon	Japan	Ovarian fluid, kidney, milt	Misaka & Suzuki (2007); Norhita (2023)
Masu salmon	Japan	Ovarian fluid, kidney, milt	Norhita (2023)

Epidemiology

Flavobacterium psychrophilum (FP) infections were previously known as peduncle disease or low-temperature disease (Barnes & Brown, 2011). Other terms for the disease include fin rot disease, rainbow trout fry mortality syndrome, rainbow trout fry syndrome, bacterial disease of cold water, and bacterial coldwater disease (Ekman *et al.*, 2003; LaFrentz & Cain, 2004; Lumsden *et al.*, 2006; Leon *et al.*, 2009). The infection is referred to as Bacterial Coldwater Disease (BCWD) in North America while it is known as rainbow trout fry syndrome (Ekman, 2008) in Europe.

FP infections is cosmopolitan in distribution (Toranzo, 2004; Nicolas *et al.*, 2008), being common in North America (Hesami *et al.*, 2008), Europe (Nilsen *et al.*, 2011), Australia (Schmidtke & Carson, 1995), Asia (Lee & Heo, 1998), and Turkey (Gultepe & Tanrikul, 2006; Kum *et al.*, 2008). Juvenile rainbow trout and coho salmon are particularly vulnerable to BCWD (Chen *et al.*, 2008), though infections have been reported in various salmonids of different sizes, both anadromous and non-anadromous (Madetejo *et al.*, 2011; Nagai & Nakai, 2011). Additionally, *FP* has been detected in species such as *Anguilla japonica*, *Anguilla anguilla*, *Cyprinus carpio*, *Carassius carassius*, *Tinca tinca*, *Plecoglossus altivelis*, *Zaco platypus*, perch (*Perca fluviatilis*), and *Rutilus rutilus* (Izumi *et al.*, 2003; Madetoja *et al.*, 2002).

Pathogenesis

FP can survive in fresh water environment for several months (Michel *et al.*, 2003; Vatsos *et al.*, 2003) which act as a source of infection. Madeoja *et al.* (2000) reported that it may also be transmitted from infected live and dead fish shedding 10×10^3- 10×10^7 bacteria/hour. The bacteria invade through lesions in the fish skin, with abrasions significantly increasing its ability to infect (Decostere *et al.*, 2000; Miwa & Nakayasu, 2005), multiply there and get distributed to the other predilection sites of the infections. *FP* is found to have higher affinity to regions like the lower jaw, fins, and caudal peduncle (Kondo *et al.*, 2002; Martinez *et al.*, 2004). Ecto-endo-parasitic loads, and poor water quality may further amplify the risk of infection (Busch *et al.*, 2003; Nematollahi *et al.*, 2003).

Vertical transmission is possible but Madsen & Dalsgaard (2008) did not agree to this and suggested that the bacterium might be attached to the egg membrane rather (Madsen & Dalsgaard, 2008). The above statement is valid as Cipriano (2005) and Madsen *et al.* (2005) reported *FP* on the eggs surface, in milt, and in ovarian fluid. The literature had shown the bacteria can enter eggs when they are immersed in a suspension of *FP* (Kumagai, 2005). Once inside the fish, *FP* secretes protease, that aids in its spread through the connective tissue and musculature, leading to open ulcers and internal lesions (Miwa & Nakayasu, 2005; Secades *et al.*, 2001). The bacteria also suppress the nonspecific humoral immunity of fish as viable bacteria were observed and reported in spleen phagocytes (Siwicki *et al.*, 2004; Nematollahi *et al.*, 2005). As like other animals, a potent immune response against *FP* requires both non-specific immune modulations and specific antibodies (LaFrentz *et al.*, 2003).

FP has three main serotypes and multiple specific genetic lineages, with considerable variation and varying virulence among strains (Izumi *et al.*, 2003; Soule *et al.*, 2005). Some strains' virulence may be specific to certain fish species (Nagai & Nakai, 2011). Chakroun *et al.* (1998) suggested that *FP* originated in North America and spread to Asia, while a European strain spread to Australia and then back to Europe. However, Nicolas *et al.* (2008) found no evidence that North America was the original source and noted significant strain diversity in Europe, particularly in wild, non-salmonids.

Clinical symptoms and Diagnosis

The erosion of tissue, particularly around the caudal peduncle or fin, is a hallmark of BCWD. Martínez *et al.* (2004) observed that early signs of infection include whitish material along the fin margin, followed by necrosis. Even without visible fin erosion, other clinical signs of BCWD include jaw ulcerations, pale or necrotic gills, increased mucus, pigmentation (leading to "black tail"), lethargy, blindness, anemia, enlarged spleen, intestinal inflammation, and abnormal swimming behavior (Leon *et al.*, 2009; Ryce & Zale, 2004). Larger fish tend to show more classic necrotic lesions, while histological signs are consistent across *FP* infections (Ekman, 2008; Duchaud *et al.*, 2007). Nervous symptoms like spiral swimming may persist long after the initial infection (Madsen *et al.*, 2001). Histology reveals necrosis in most internal organs (Ekman & Norrgren, 2003; Decostere *et al.*, 2001), with *FP* strongly associated with phagocytes in kidney and spleen (Wiklund & Dalsgaard, 2002). The spleen shows significant damage, including hemosiderosis, hemorrhages, necrosis, and a high presence of bacteria (Decostere *et al.*, 2001).

Mortality rates due to BCWD vary widely. The highest reported mortality is 90% in rainbow trout (Nilsen *et al.*, 2011). 85% mortality in steelhead and up to 70% in rainbow trout have also been noted (Kum *et al.*, 2008). In contrast, Denmark reported an average of 34% mortality, the UK 10-30%, and Turkey 20% (Gultepe & Tanrikul, 2006). Coho salmon fry experience up to 50% mortality, with 5–30% in slightly larger fingerlings. Cutthroat trout and lake trout have mortality rates ranging from 25% to 45% (Ryce & Zale, 2004). Variations in mortality are likely influenced by factors such as water temperature, which is crucial for the severity of the disease (Noga, 2000). While BCWD typically occurs at 4–10°C, it is most severe at 15°C (Groff & LaPatra, 2001). However, 16–21°C temperature was found the most appropriate factor for FP outbreaks in Japan (Asakawa *et al.*, 2000). Bacterial genetics and specific virulence are another critical factor, with different strains showing varying levels of virulence to fish immune responses (Stenholm *et al.*, 2008; Johnson *et al.*, 2008).

Bacterial culture and Pathogen identification

Davis (1946) first identified bacterial rods in rainbow trout (*Oncorhynchus mykiss)* from caudal peduncle lesions scrapings. Similar bacteria were isolated from coho salmon (*O. kisutch)* lesions and kidneys (Borg, 1948) and reported to be non-spore former rods, having Gm-ve staining reaction with gliding motility. The bacteria do not grow or grow sluggishly above 25°C, was initially named *Cytophaga psychrophila*, but later recognized as *Flavobacterium psychrophilum* (Bernardet, 2001).

FP is a slender, flexible, gram-negative rod (Madetoja *et al.*, 2011) with size of 0.75 µm diameter and 1.5-7.5 µm length. It is strictly aerobic, displaying variable colony morphology, and forms bright yellow colonies on cytophaga agar (Toranzo, 2004). *FP* reacts weakly on chemical tests but is highly proteolytic, capable of hydrolyzing casein, digesting albumin, and producing catalase and oxidase (Møller *et al.*, 2005; Nematollahi *et al.*, 2005). It does not hydrolyze starch, decompose cellulose, or produce hydrogen sulfide. Growth in NaCl varies, with some strains inhibited at 2.0%, while others show no growth above 0.5%.

Culturing *FP* can be challenging due to its fastidious nature (Nematollahi *et al.*, 2005; Álvarez & Guijarro, 2007). Various media, including cytophaga agar and tryptone-yeast extract-salts agar, have been used, with some success in isolating the bacterium (Madetoja *et al.*, 2002; Cepeda *et al.*, 2004). Improved culture results have been achieved with the addition of activated charcoal to the media (Álvarez & Guijarro, 2007). Diagnostic methods for *FP* have advanced, with immunofluorescence, ELISA, and PCR being used for rapid identification and detection (Lindstrom *et al.*, 2009; Álvarez *et al.*, 2004). PCR, including nested PCR, has proven particularly useful in detecting the bacterium in samples where traditional culturing methods fail (Crumlish *et al.*, 2007; Izumi *et al.*, 2005). Recent developments include a loop-mediated amplification assay (LAMP) for quick detection (Fujiwara-Nagata *et al.*, 2009).

Treatment

Antibiotics are the primary treatment of BCWD outbreaks. Nifurpirinol is effective, but it's not approved for food fish in the US and most countries due to its carcinogenic properties. Sulfonamides are also effective but similarly restricted. Oxytetracycline (OTC), Amoxicillin and Oxolinic acid have been widely used globally to control BCWD (Bruun *et al.*, 2000; Lumsden *et al.*, 2006; LaFrentz & Cain, 2004), however, development of resistance against these drugs are quite rapid (Antaya, 2008; Bruun *et al.*, 2003). This is why the use of Florfenicol, recently approved antibiotic, is gaining faith among the aquaculturists (Hadidi *et al.*, 2008). Antibiotic resistance in *FP* is a significant challenge. Initially, there were no OTC-resistant strains (Bruun *et al.*, 2000), but by 2000, there was 100% resistance to Oxolinic acid in Denmark, making OTC more common. By 1998, 60-75% of *FP* from Danish trout farms were OTC resistant, and Amoxicillin resistance was also noted (Brunn et al., 2000). By 2003, OTC use was rare due to resistance (Bruun *et al.*, 2003), and Florfenicol resistance was reported (Kum *et al.*, 2008). The bacteria's ability to form resistant biofilms contributes to this issue, leading to recurrent infections (Sundell & Wiklund, 2011).

Alternative treatments approaches involve non-antibiotics chemicals against FP. A hydrogen peroxide bath followed by Florfenicol medicated diets was used (Gultepe & Tanrikul, 2006) with promising results in Turkey. Similarly, Potassium permanganate treatments have also been used to reduce bacterial load and expedite the removal of infected fish (LaFrentz & Cain, 2004; Madetoja *et al.*, 2002). Salt baths were also listed as a treatment for BCWD (Groff & LaPatra, 2001; LaFrentz & Cain, 2004).

Despite significant efforts, no viable commercial vaccine for BCWD exists. Live attenuated strain vaccines show potential (LaFrentz *et al.*, 2008). Álvarez *et al.* (2008) achieved significant disease resistance with attenuated live bacteria injections. Formalin-killed *FP* injections also improved survival (Kundo *et al.*, 2003). Bath vaccination with heat-inactivated *FP* has shown success, but must occur at least 50 days post-hatch. Immunity has also been induced through non-attenuated *FP* bath treatments (Lorenzen *et al.*, 2010), and including *FP* in polyvalent vaccines showed promise (Nikoskelainen *et al.*, 2007). Phage therapy is also under investigation, along with the use of probiotic bacteria (Stenholm *et al.*, 2008; Kim *et al.*, 2010; Ström-Bestor *et al.*, 2011).

Prevention

Egg disinfection with iodophor is commonly recommended for BCWD prevention (Groff & LaPatra, 2001; LaFrentz & Cain, 2004), but *FP* shows high iodine tolerance, surviving treatments of at least 100 mg/l for 30 minutes (Kumagai, 2005). The limited effectiveness of current disinfection protocols is evident in the global spread of virulent strains (Kumagai, 2005). Cipriano (2005) found that standard and even triplicate iodophor treatments fail to eliminate the bacteria within eggs. Non-chemical methods, like UV disinfection, require high doses of 126 or 256 mWs/cm² to be effective tried.

Minimizing stress during handling is crucial in preventing BCWD outbreaks, as these factors cause immunosuppression and create clicking environment for the bacterial invasion through skin lesions (LaFrentz & Cain, 2004; Decostere *et al.*, 2000; Miwa *et al.*, 2005). Ryce & Zale (2004) observed that BCWD outbreaks often occurred three weeks after fish handling. Reducing stocking and rearing densities might help to reduce the risks of infection (Taylor, 2004).

High-quality diets may help prevent BCWD. A study showed that rainbow trout fed diets high in oxidized lipids had higher mortality rates after a *FP* challenge compared to those on control diets (Daskalov *et al.*, 2000). Poor water quality is another primary contributor to BCWD outbreaks. Maintaining optimal water quality is recommended (Groff & LaPatra, 2001; Taylor, 2004), with reduced organic loads and nitrite concentrations potentially decreasing *FP* infectivity (Garcia *et al.*, 2000; Nematollahi *et al.*, 2003). Using pathogen-free water supplies is suggested; interestingly elevating water temperatures may also help prevent infections (Groff & LaPatra, 2001; Chen *et al.*, 2008).

Bloodstock screening is another preventative measure (Groff & LaPatra, 2001). Lindstrom *et al.* (2009) recommended ELISA and FAT for broodstock selection, though Lumsden *et al.* (2006) questioned its practicality in contaminated environments. Genetic selection for specific resistance in brood fish has shown promising results, with moderate heritability of BCWD resistance in rainbow trout (Henryon *et al.*, 2005; Silverstein *et al.*, 2009). Finally, removing dead fish, thus reducing the reservoirs for *FP*, is highly recommended (Lumsden *et al.*, 2006). Avoiding the introduction of wild or novel fish into existing stocks, maintaining infected stocks downstream in production systems, and routine equipment sanitation are also suggested preventative measures.

Conclusion

FP pose a significant threat to both wild and cultured fish population causing high mortality rates in hatchery-reared fish globally, despite extensive containment effort against it. Future research should focus on developing smart and accurate diagnostic tests to allow for the rapid implementation of interventions and therapeutics. Traditional therapeutic approaches, primarily antibiotic-based, have been increasingly challenged by the rapid development of resistance, necessitating the exploration of alternative approaches, specifically an exemplary deployment of probiotics and immunostimulants use plan and the development of effective vaccines.

Further research into dietary interventions is necessary, particularly in enhancing current formulations to reduce BCWD susceptibility. The impact of novel dietary ingredients, such as replacing fish meal with plant-

based proteins, should also be assessed. Continued efforts in breeding BCWD-resistant bloodstock are essential. Additionally, controlled studies on hatchery management techniques aimed at reducing stress and minimizing infection entry points should be prioritized. Although some progress has been made in biologicals development and alternative therapies, the absence of a commercially viable vaccine and the persistence of antibiotic resistance highlight the ongoing challenges in managing BCWD in aquaculture.

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