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Chlorella in aquaculture: challenges, opportunities, and disease prevention for sustainable development

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Abstract

Aquaculture is the farming of aquatic organisms like fish, crustaceans, mollusks, and aquatic plants, which has become a crucial source of protein and income. However, bacterial infections pose a significant challenge to the aquaculture industry and traditional treatments, such as antibiotics and chemicals, have limitations and environmental concerns. Disease prevention and control measures, such as the use of probiotics, vaccines, and biosecurity measures, are essential for the sustainable development of the aquaculture industry. Further research is also needed to develop more effective and sustainable strategies for the prevention and control of bacterial fish pathogens in aquaculture, where alternative treatments such as herbal extracts, essential oils, and probiotics require further investigation for efficacy and safety. Microalgae, particularly Chlorella, have potential applications in various industries such as biofuels, pharmaceuticals, and wastewater treatment. However, their large-scale production and commercialization face challenges. Safety of Chlorella to fish is a crucial issue that requires careful evaluation, with hematology being an essential tool to assess its effects on fish health and physiology. Studies show that Chlorella is safe for fish and does not have adverse effects on growth, survival, or immune system function. Chlorella is a safe and sustainable option for aquaculture, free from harmful chemicals and antibiotics. The Green Water System utilizes Chlorella as a natural filter and nutrient recycler, improving water quality and providing a well-balanced diet for aquatic animals. This eco-friendly approach also enhances fish immune systems, growth rates, and survival rates. The scientometric review shows significant research activity, with Chang JS being a prominent author and People's R China and the Chinese Academy of Sciences leading in contributions. The use of Chlorella shows promise as an alternative treatment for bacterial fish pathogens in aquaculture due to its antibacterial properties, safety, and sustainability. However, challenges such as cost-effectiveness and standardization need to be addressed for successful implementation in the aquaculture industry.

Keywords Aquaculture · Bacterial pathogens · Chlorella · Treatment · Antibacterial

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Introduction

Aquaculture is a growing industry, with global production increasing by an average of 5.8% per year between 2000 and 2018 (FAO 2020). In 2018, aquaculture production reached 114.5 million tonnes, with a value of US$ 263.6 billion. Asia is the largest producer of farmed fish, accounting for 89% of global production, followed by Africa (4.1%) and Latin America and the Caribbean (3.2%) (FAO 2020). The major species produced by aquaculture include carp, tilapia, salmon, shrimp, and mollusks such as mussels and oysters.

As the demand for fish production increases, aquaculture has evolved into a more intensive system. However, like any animal production industry, aquaculture faces numerous complex constraints and challenges. Among these challenges, infectious diseases stand out as a significant concern, leading to billions of dollars in annual losses (Assefa and Abunna 2018). Bacterial pathogens, in particular, pose a major threat, causing high fish mortalities and resulting in substantial economic losses (Hassan et al. 2020). These infectious diseases not only impact the aquaculture industry but also have broader implications for food security and the sustainability of fish production. Effective management strategies and preventive measures are crucial to mitigate the impact of bacterial diseases and ensure the continued growth and success of the aquaculture sector.

The use of antibiotics to control bacterial infections in fish farming poses significant constraints on the aquaculture sector. Antibiotics not only pose a threat to fish due to potential drug residues but also contribute to the development of antibiotic-resistant pathogens, posing risks to humans and the environment (El-Habashi et al. 2019; Desbois et al. 2021). In the search for more environmentally friendly alternatives to decrease the reliance on chemical medication in fish farming, microalgae, particularly Chlorella, have emerged as a promising solution. Chlorella supplementation has shown numerous beneficial effects in aquaculture, such as growth promotion in various fish species like carp and tilapia, immune stimulation in rainbow trout, and enhancement of reproductive performance in yellowtail cichlid (Alagawany et al. 2021). Commercially, *Chlorella vulgaris*, with its good biomolecular composition, is widely used in aquaculture (Ahmad et al. 2020). While studies have investigated Chlorella's role as a growth promoter and its protective effects against water toxicity from harmful chemicals (Tejido-Nuñez et al. 2019), there is relatively limited research on its potential antibacterial activity and immune-enhancing properties to prevent and control bacterial diseases in aquaculture.

The Green Water System, incorporating Chlorella, is a prominent and sustainable approach in aquaculture management. Chlorella, cultured within the water, acts as a natural filter and nutrient recycler, actively absorbing excess nutrients like nitrogen compounds to maintain water quality and prevent harmful substance accumulation (Bosma and Tendencia 2014). This integration offers multiple advantages, including enhanced water clarity, facilitating better observation and monitoring of fish behavior and health, leading to improved well-being and growth rates. Chlorella's nutrient recycling capabilities also reduce the need for frequent water changes, promoting water conservation and creating a stable and balanced aquatic environment. Its resilience to environmental fluctuations ensures system stability, supporting long-term sustainability in aquaculture operations (Yang et al. 2017). From an environmental perspective, Chlorella reduces waste and chemical dependency, minimizing water pollution and fostering a cleaner and healthier aquatic ecosystem. The Green Water System with Chlorella aligns with sustainable practices, contributing to the conservation of aquatic resources and ecological preservation (Ru et al. 2020).

Aquaculture and bacterial fish pathogens

Aquaculture is the farming of aquatic organisms such as fish, crustaceans, mollusks, and aquatic plants. This practice has become an important source of protein and income for many countries, particularly in Asia and Latin America. However, with the intensification of aquaculture practices, the occurrence of diseases has become a major challenge for the industry. Bacterial infections are among the most common and devastating diseases affecting farmed fish, causing significant economic losses. In this paper, we will provide an overview of aquaculture and the bacterial fish pathogens that pose a threat to the industry with recommended control measures.

One of the challenges facing the aquaculture industry is the occurrence of diseases, which can cause significant economic losses. Bacterial infections are among the most common and devastating diseases affecting farmed fish. The major bacterial fish pathogens include *Aeromonas hydrophila*, *Edwardsiella tarda*, *Flavobacterium columnare*, *Photobacterium damselae* subsp. *piscicida*, and *Streptococcus agalactiae* (Austin and Austin 2016). These pathogens can cause a range of diseases, such as hemorrhagic septicemia, enteric septicemia, columnaris disease, and streptococcal infections, which can lead to mortality rates of up to 100%.

Bacterial fish pathogens can be transmitted through several routes, including water, feed, equipment, and personnel. The intensity of aquaculture practices, such as high stocking densities, use of antibiotics, and poor water quality, can create favorable conditions for the growth and transmission of these pathogens (Austin and Austin 2016; Stephens 2016). Therefore, disease prevention and control measures are essential for the sustainable development of the aquaculture industry.

Several strategies can be employed to prevent and control bacterial fish pathogens, including the use of probiotics, vaccines, and biosecurity measures. Probiotics are beneficial bacteria that can improve the gut health of fish, enhancing their immune response against pathogens. Vaccines are another effective way to prevent bacterial infections, as they stimulate the production of specific antibodies against the pathogen. Biosecurity measures, such as disinfection of equipment and personnel and quarantine of new fish stocks, can also prevent the introduction and spread of bacterial pathogens (Austin and Austin 2016; Cabello et al. 2016; Nayak 2010).

Traditional treatments for bacterial fish pathogens: limitations and environmental concerns

Bacterial infections are a major challenge in aquaculture, causing significant economic losses to the industry. Traditional treatments for bacterial fish pathogens, such as antibiotics and chemicals, have been widely used to prevent and control these infections. However, these treatments have limitations and environmental concerns that need to be addressed. In this paper, we will provide an overview of the traditional treatments for bacterial fish pathogens and the limitations and environmental concerns associated with these treatments.

Antibiotics have been extensively used in aquaculture to prevent and control bacterial infections. However, the overuse and misuse of antibiotics have led to the emergence and spread of antibiotic-resistant bacteria, posing a significant threat to human and animal health (Cabello et al. 2013). Moreover, the use of antibiotics in aquaculture can also result in the accumulation of antibiotic residues in the aquatic environment, which can have negative impacts on non-target organisms and the ecosystem (Qin et al. 2023; Okeke et al. 2022). Therefore, there is a need to reduce the use of antibiotics in aquaculture and adopt alternative treatments.

Chemicals, such as copper sulfate and formalin, have also been used to control bacterial fish pathogens. However, these chemicals can have toxic effects on fish and other aquatic organisms and can also accumulate in the environment (de Andrade Waldemarin et al. 2012). Moreover, the repeated use of chemicals can lead to the development of resistance in bacterial pathogens, reducing the effectiveness of these treatments (Austin and Austin 2016).

Herbal extracts and essential oils have been proposed as alternative treatments for bacterial fish pathogens. These natural compounds have antimicrobial properties and are considered safe and environmentally friendly (Fei 2020). However, the efficacy of these treatments varies depending on the type and concentration of the extract or oil, as well as the bacterial strain (Mandalakis et al. 2021). Moreover, the use of herbal extracts and essential oils can have unintended consequences, such as the disruption of the natural microbial community in the aquatic environment (Ferraz et al. 2022).

Probiotics, which are beneficial bacteria that can improve the gut health of fish and enhance their immune response against pathogens, have also been proposed as an alternative treatment for bacterial fish pathogens (El-Saadony et al. 2021; Wang et al. 2021). Probiotics have been shown to be effective in preventing and controlling bacterial infections in some fish species (Nayak 2010). However, the efficacy of probiotics varies depending on the fish species, the bacterial strain, and the dose and duration of treatment (El-Saadony et al. 2021).

Microalgae

Microalgae are photosynthetic microorganisms that are widely distributed in various aquatic environments, such as freshwater, marine, and brackish water. These microorganisms are considered as potential sources of biofuels, pharmaceuticals, and other valuable products due to their high growth rates, photosynthetic efficiency, and ability to synthesize various compounds (Pulz and Gross 2004; Arenas et al. 2017).

Microalgae comprise a diverse group of organisms with varying characteristics such as size, shape, color, and structure, but share a common feature of having a photosynthetic system that captures carbon dioxide and incorporates it into their central metabolism. Their photosynthetic system is highly similar to that in plants, but microalgae have a simpler whole organism organization level, primarily as unicellular species.

Based on their pigment type, cell structure, and life cycle, microalgae are classified into nine categories, namely, *Chlorophyta*, *Chlorarachniophyta*, *Cryptophyta*, *Dinophyta*, *Euglenophyta*, *Glaucophyta*, *Haptophyta*, *Heterokontophyta*, *and Rhodophyta*, and two prokaryotic divisions, *Cyanophyta and Prochlorophyta* (Heraud et al. 2006; Neofotis et al. 2016; Singh and Saxena 2015).

Microalgae have attracted considerable attention as a sustainable source of biofuels, such as biodiesel, ethanol, and hydrogen. These microorganisms can produce high amounts of lipids, which can be converted into biodiesel through transesterification. In addition, microalgae can also produce ethanol through fermentation of their carbohydrates. Furthermore, microalgae are capable of producing hydrogen through a process called photobiological water splitting, which involves the use of solar energy to split water into hydrogen and oxygen (Spolaore et al. 2006; Shuba and Kifle 2018).

Besides biofuels, microalgae also possess several potential applications in the pharmaceutical industry. These microorganisms can synthesize various bioactive compounds, such as carotenoids, polyunsaturated fatty acids, and polysaccharides, which possess various health benefits. For instance, carotenoids, such as astaxanthin and β-carotene, have antioxidant and anti-inflammatory properties and are used in the prevention and treatment of several diseases, such as cancer, cardiovascular diseases, and age-related macular degeneration. Moreover, microalgae-derived polysaccharides, such as carrageenan and agar, have applications in the food and cosmetic industries due to their gelling and thickening properties (Pulz and Gross 2004; Arenas et al. 2017).

Microalgae also have potential applications in wastewater treatment and carbon capture. These microorganisms can remove nutrients, such as nitrogen and phosphorus, from wastewater through a process called bioremediation. In addition, microalgae can capture carbon dioxide from the atmosphere and convert it into organic compounds through photosynthesis, which can help in mitigating the effects of climate change (Biris-Dorhoi et al. 2016).

One particular genus of microalgae is Chlorella, which is known for its high nutritional value, especially in terms of protein and lipid content. Chlorella has been studied for its potential as a source of food and feed, as well as for its applications in biofuels and wastewater treatment. Some studies have also suggested that Chlorella may have beneficial effects on human health due to its antioxidant and anti-inflammatory properties (Saini et al. 2021; Bannu et al. 2019; Gorgich et al. 2021). Chlorella has been also shown to possess antibacterial properties against various fish pathogens (Little et al. 2021; Nagarajan et al. 2021).

According to Arenas et al. (2017) and Shuba and Kifle (2018), the utilization of microalgae for various applications holds great promise. However, achieving large-scale production remains challenging due to factors like high production costs, low biomass yields, and contamination issues. To overcome these obstacles, extensive research is needed to optimize microalgae growth conditions, develop cost-effective cultivation systems, and enhance downstream processing of their products.

Chlorella

Chlorella, a freshwater microalga, has been proposed as an attractive alternative for treating bacterial fish pathogens due to its antibacterial properties, safety, and sustainability.

Nature and type

Chlorella is a genus of unicellular, freshwater green algae that belongs to the family Chlorellaceae, which is one of several families within the order Chlorellales. Over 30 species of Chlorella have been identified to date based on morphological and molecular characteristics. Chlorella cells are small and spherical, with a diameter ranging from 2 to 10 μm, and have a single cup-shaped chloroplast that contains chlorophyll a and b, as well as various pigments such as carotenoids and phycobiliproteins. The cell wall of Chlorella is composed of cellulose and other polysaccharides, which is an important characteristic that distinguishes Chlorella from other green algae and is responsible for its resistance to environmental stresses, such as high salt concentrations and low pH levels (Fu et al. 2019).

Chlorella species are differentiated based on their DNA sequences, with the 18S rRNA gene commonly used for species identification and phylogenetic analysis. Phylogenetic analysis has revealed several clades or groups of Chlorella based on molecular data, including *C. vulgaris*, *C. sorokiniana*, *C. pyrenoidosa*, *C. ellipsoidea*, *C. minutissima*, *and C. protothecoides* (Vello et al. 2014).

Chlorella species have potential applications in various industries, including food, feed, biofuels, and pharmaceuticals, due to their high growth rates, photosynthetic efficiency, and ability to accumulate various compounds (Liu and Chen 2014). Chlorella cells are rich in proteins, essential amino acids, vitamins, and minerals, making them a potential source of food and feed for humans and animals (Liu and Chen 2014). Chlorella cells can also accumulate high amounts of lipids, which can be converted into biodiesel through transesterification (Liu and Chen 2014). Chlorella also produces various bioactive compounds, such as carotenoids, phycobiliproteins, and polysaccharides, which possess various health benefits and have applications in the pharmaceutical and cosmetic industries (Koyande et al. 2019).

Culture and propagation of chlorella

Culture techniques

Chlorella can be cultured in various types of media, including freshwater, seawater, and artificial media. The choice of media depends on the desired application and the characteristics of the Chlorella strain being used. For example, freshwater Chlorella strains grow well in media containing nitrates and phosphates, while marine Chlorella strains require a high concentration of sodium and magnesium (Chia et al. 2013).

Chlorella can be cultured in open or closed systems. Open systems, such as raceways and ponds, are simple and cost-effective but are vulnerable to contamination from other microorganisms and require a large land area. Closed systems, such as photobioreactors and fermenters, offer better control over the culture conditions, minimize contamination, and have higher productivity but are more expensive to construct and maintain (Rodolfi et al. 2009).

Chlorella sp. is a type of microalgae that has great potential for use in various industries, including food, nutraceuticals, biofuels, and wastewater treatment. Iriani et al. (2021) optimized the growth of Chlorella sp. using Bean Sprouts Extract Media (BSEM) with continuous light and obtained the highest cell density and specific growth rate. Rahardini et al. (2018) also investigated the effect of inorganic fertilizer composition on the growth of Chlorella sp. and found that the fertilizer composition of 3:3:1 provided the highest growth rate and population density. Mtaki et al. (2021) demonstrated that *Chlorella vulgaris* could be cultivated using aquaculture wastewater (AWW) supplemented with NPK fertilizer as a low-cost nutrient source, resulting in good growth and high vitamin and mineral content compared to expensive media.

Li et al. (2023) investigated the effects of tetracycline (TC) and metronidazole (MTZ) on the growth and cell morphology of *Chlorella pyrenoidosa* and found that TC was more toxic to the algae than MTZ, and the combined toxicity of TC and MTZ was synergistic. Elbasuni et al. (2022) found that *Chlorella vulgaris* (CLV) supplementation reduced aflatoxin-induced hepatic damage, oxidative stress, and inflammation, improved the nutritional value of meat, and significantly reduced aflatoxin residues. Jahromi et al. (2022) found that *Chlorella vulgaris* cultivated with citrus peel fatty acids had increased biomass, lipid content, and nutritional quality, suggesting that citrus peel waste can be used as an inexpensive and nutritious nutrient source for Chlorella biomass production. Lele et al. (2022) investigated the toxicity of phenanthrene on *Chlorella vulgaris* and Skeletonema costatum and found that both species were affected at an environmentally relevant level, but different tolerance levels were detected. Toumi et al. (2022) obtained two oil fractions from microalgae *Chlorella sorokiniana* for use in the food, biofuels, and nutraceutical industries, including a DHA-EPA-rich fraction and a non-urea complexed fraction that was rich in polyunsaturated fatty acids. These studies highlight the potential of Chlorella sp. as a versatile microorganism with various applications in different fields.

Shigeki et al. (2018) investigated the effects of different sterilization and disinfection treatments on three microalgae species and found that high-temperature high-pressure treatment enhanced the digestibility of *Nannochloropsis oculata* by Artemia nauplii and rotifers. Ip (2005) suggested that *Chlorella zofingiensis* can be a potential source of natural astaxanthin. Wu et al. (2022) aimed to reduce the cost of carbon sources in the heterotrophic culture of *Chlorella vulgaris* by using sweet sorghum extract and its enzymatic hydrolysate and found that it is a low-cost alternative for the growth of *Chlorella vulgaris*. Paes et al. (2016) evaluated the effects of nitrogen starvation on the growth and chemical composition of Chlorella sp. and *Nannochloropsis oculata* and found that *Nannochloropsis oculata* is more promising for lipid-rich uses such as biodiesel production. Ratomski (2021) studied the effects of nutrient access on the growth and development of *Chlorella vulgaris* and the amount of lipids stored in its cells for biodiesel production and found that the microalgae efficiently used nutrients in aquaculture wastewater. Kanza Erdawati et al. (2020) investigated the effectiveness of chitosan nanoemulsion as a bioflocculant for harvesting Chlorella sp. biomass and found that chitosan nanoemulsion is an efficient bioflocculant for harvesting Chlorella biomass.

Overall, the studies highlight the potential of microalgae as a source of nutrients and valuable products such as astaxanthin and biodiesel. They also demonstrate the importance of cultivation conditions and treatment methods in enhancing the quality and digestibility of microalgae biomass. The findings of these studies can inform the development of sustainable and cost-effective microalgae cultivation practices for various applications, including aquaculture, biofuel production, and pharmaceuticals.

Propagation techniques

Chlorella can be propagated by two main methods: asexual reproduction and sexual reproduction. Asexual reproduction is the most common method and involves the division of the mother cell into daughter cells through mitosis. This method produces genetically identical cells and is used for mass production of Chlorella. Sexual reproduction involves the fusion of two gametes to form a zygote, which undergoes meiosis to produce genetically diverse offspring. This method is used for creating new Chlorella strains with desired characteristics (Liu and Chen 2014).

Factors affecting growth and productivity of Chlorella

Chlorella is a genus of unicellular, freshwater green algae that has gained significant interest due to its potential applications in various industries, including food, feed, biofuels, and pharmaceuticals (Liu and Chen 2014). However, the growth and productivity of Chlorella are affected by various factors, including environmental conditions, nutrient availability, and light intensity.

Environmental conditions, such as temperature, pH, and salinity, play a crucial role in the growth and productivity of Chlorella. The optimal temperature for Chlorella growth ranges from 25 to 35 °C, with higher temperatures leading to decreased growth rates and lower productivity (Vu et al. 2010). Similarly, Chlorella requires a pH range of 6.5 to 8.5 for optimal growth, with higher or lower pH levels leading to decreased growth rates and lower productivity (Vu et al. 2010). Salinity also affects Chlorella growth, with optimal growth occurring at low to moderate salinities (less than 2% NaCl) (Zhuang et al. 2018).

Nutrient availability is another critical factor that affects Chlorella growth and productivity. Chlorella requires a range of essential nutrients, including carbon, nitrogen, phosphorus, and trace elements, for optimal growth (Zhuang et al. 2018). Carbon dioxide (CO_2) is the primary carbon source for Chlorella, and increasing CO_2 levels can significantly enhance Chlorella growth and productivity (Chen et al. 2017). Nitrogen is required for protein synthesis, and insufficient nitrogen levels can lead to decreased growth rates and lower productivity (Chen et al. 2017). Phosphorus is essential for energy transfer and cell division, and insufficient phosphorus levels can also lead to decreased growth rates and lower productivity (Chen et al. 2017). Trace elements, such as iron, zinc, and manganese, are required for various enzymatic reactions and can limit Chlorella growth and productivity if they are deficient (Zhuang et al. 2018).

Light intensity is another critical factor that affects Chlorella growth and productivity. Chlorella requires sufficient light for photosynthesis, and inadequate light levels can lead to decreased growth rates and lower productivity (Chen et al. 2017). However, excessive light levels can also lead to photoinhibition and damage to the photosynthetic apparatus, resulting in decreased growth rates and lower productivity (Chen et al. 2017). The optimal light intensity for Chlorella growth ranges from 50 to 150 μmol photons m-2 s-1, depending on the strain and environmental conditions (Zhuang et al. 2018).

In conclusion, various factors, including environmental conditions, nutrient availability, and light intensity, play a crucial role in the growth and productivity of Chlorella. Optimization of these factors is essential to achieve maximum growth and productivity for various applications, including food, feed, biofuels, and pharmaceuticals.

Antibacterial mechanisms of Chlorella and its bioactive compounds

An overview of Chlorella as a potential treatment for bacterial fish pathogens with its advantages and limitations is reported in Table 1.

Antibacterial properties of Chlorella

Chlorella has been shown to possess antibacterial properties against various fish pathogens, including *Aeromonas hydrophila*, *Vibrio anguillarum*, and *Pseudomonas aeruginosa* (Little et al. 2021; Nagarajan et al. 2021). The antibacterial activity of Chlorella is attributed to its bioactive compounds, such as polysaccharides, proteins, and lipids, which have been shown to inhibit bacterial growth and biofilm formation (Hussein et al. 2018; Cavallo et al. 2013), (Table 2).

Table 1 Antibacterial mechanisms of Chlorella and its bioactive compounds

Mechanism	Bioactive compound	References
Disrupting cell membrane	Chlorella extract, Chlorella polysaccharides, *Chlorella vulgaris* extract, chlorophyll, chlorophyllin	Abdel-Karim et al. 2019; Adesiyun et al. 2007; Krüger et al. 2019
Inhibiting bacterial enzymes	*Chlorella vulgaris* extract, chlorophyllin, chlorophyll	Adesiyun et al. 2007; Krüger et al. 2019
Producing reactive oxygen species	Chlorella extract, chlorophyllin, chlorophyll	Abdel-Karim et al. 2019; Krüger et al. 2019
Quorum sensing inhibition	Chlorella extract	Bahi et al. 2023

Safety and sustainability of Chlorella

Hematology

Hematology is an essential tool to assess fish health and evaluate the effects of dietary interventions on fish physiology. Several studies have investigated the effects of Chlorella on fish hematology parameters, including red blood cell count, hematocrit, hemoglobin concentration, and white blood cell count. A study by Xu et al. (2014) evaluated the effects of dietary Chlorella on the hematology of juvenile gibel carp (*Carassius auratus gibelio*) and found no significant differences in red blood cell count, hematocrit, or hemoglobin concentration between the control and Chlorella-fed groups. However, the white blood cell count was significantly higher in the Chlorella-fed group, indicating a potential immune-stimulatory effect of Chlorella.

Another study by Kang et al. (2013) evaluated the effects of dietary Chlorella on the hematology of rainbow trout (*Oncorhynchus mykiss*) and found no significant differences in red blood cell count, hematocrit, or hemoglobin concentration between the control and Chlorella-fed groups. However, the white blood cell count was significantly lower in the Chlorella-fed group, indicating a potential immunosuppressive effect of Chlorella.

Survival

Several studies have investigated the effect of chlorella on fish survival, and the results have been promising. In a study conducted by Shi et al. (2017), it was found that supplementation of chlorella in the diet of juvenile grass carp did not have any significant effect on fish survival. Similarly, in another study by Mahmoud et al. (2020), it was observed that the inclusion of chlorella in the diet of Nile tilapia did not affect fish survival.

The positive impact of chlorella on fish survival can be attributed to its nutritional value. Chlorella is a rich source of protein, amino acids, vitamins, and minerals, which are essential for fish growth and survival (Ahmad et al. 2020). Additionally, chlorella contains various bioactive compounds, such as polysaccharides, phycobiliproteins, and carotenoids, which have been shown to have immunomodulatory and antioxidant properties (Panahi et al. 2016). These compounds may contribute to improving the overall health and survival of fish. Nik et al. (2023) investigated the growth and survival rate of Angelfish larvae fed with various types of enriched Artemia (*Tetraselmis* sp., *Chlorella* sp., and mixed diet;

Table 2 List of studies on the antibacterial properties of Chlorella

Chlorella species	Bacteria tested	References
C. vulgaris	*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa*	Ferreira et al. 2021
C. vulgaris	*Listeria monocytogenes*	Zielinski et al. 2020
C. vulgaris	*Streptococcus mutans*	Jafari et al. 2018
C. vulgaris	*Salmonella enterica, Listeria monocytogenes*	Adesiyun et al. 2007
C. vulgaris	*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Klebsiella pneumonia*	Schuelter et al. 2019
C. vulgaris	*Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Bacillus cereus*	Oussalah et al. 2007
C. vulgaris	*Vibrio harveyi, Streptococcus iniae*	Bahi et al. 2023
C. pyrenoidosa	*Vibrio anguillarum, Vibrio harveyi*	Jang et al. 2018

Tetraselmis sp. + *Chlorella* sp.). The experiment was conducted for 35 days, and it was found that the mixed diet resulted in the highest growth and survival rate compared to other enrichments.

Virulence

The safety of Chlorella to fish through virulence is an important concern when considering its potential use as a feed supplement. Some microalgae species, including certain strains of Chlorella, have been reported to produce toxins that could harm fish health and survival (Kang et al. 2015). Therefore, it is crucial to evaluate the virulence of Chlorella before incorporating it into fish diets.

Several studies have investigated the virulence of Chlorella to fish, and the results have been largely reassuring. In a study by Badwy et al. (2008), Nile tilapia fed with diets containing up to 15% Chlorella powder showed no significant differences in growth rate, feed utilization, or survival compared to the control group. Similarly, in a study by Zahran et al. (2019), Nile tilapia fed with diets containing up to 20% Chlorella showed no adverse effects on survival or growth performance.

Another study by Raji et al. (2018) evaluated the virulence of Chlorella on the immune system of African catfish. The results showed that feeding African catfish with diets containing Chlorella resulted in increased phagocytic activity and respiratory burst activity, indicating a positive effect on the fish's immune system.

Overall, the available evidence suggests that Chlorella is safe for fish and can be used as a feed supplement without adverse effects on growth, survival, or immune system function. However, it is essential to use high-quality Chlorella products and to ensure that the feed is properly formulated to avoid any potential adverse effects on fish health and productivity (Mustafa et al. 2021) (Table 3).

Table 3 Safety and sustainability of Chlorella

Aspect	Results	References
Acute toxicity	No mortality observed in mice at a dose of 5 g/kg body weight	Shigeoka et al. 1988
Sub-chronic toxicity	No adverse effects observed in rats fed with Chlorella for 90 days	Kapoor et al. 2022
Allergenicity	No allergenic potential observed in vitro and in vivo studies	Mišurcová et al. 2012
Heavy metal contamination	Chlorella can accumulate heavy metals, but levels are typically low and safe for human consumption	Li et al. 2021
Sustainability	Chlorella cultivation is sustainable due to its high productivity, efficient use of resources, and ability to grow in various environments	Tredici 2010; Xu et al. 2009

Limitations of Chlorella

Although Chlorella shows promise as an alternative treatment for bacterial fish pathogens, there are some limitations that need to be addressed (Table 4). One of the limitations is the variability in the antibacterial activity of Chlorella depending on the strain, growth conditions, and extraction methods (Hussein et al. 2018; Cavallo et al. 2013). Therefore, further research is needed to identify the most effective Chlorella strains and optimize the growth and extraction conditions for antibacterial activity.

Another limitation of Chlorella is the potential impact of its use on the aquatic environment. The release of Chlorella biomass and metabolites into the environment may have unintended consequences, such as the eutrophication of water bodies and the alteration of microbial communities (Andreotti et al. 2020). Therefore, the environmental impact of Chlorella-based treatments needs to be carefully evaluated and monitored.

Chlorella shows promise as an attractive alternative for treating bacterial fish pathogens due to its antibacterial properties, safety, and sustainability. However, further research is needed to optimize the growth and extraction conditions for antibacterial activity and to evaluate the environmental impact of Chlorella-based treatments. Overall, Chlorella represents a promising option for the prevention and control of bacterial fish pathogens in aquaculture.

In vitro and in vivo studies

Chlorella and fish nutrition

This summary pertains to various studies on the use of Chlorella sp. and mixed diets in fish nutrition. Nik et al. (2023) found that a mixed diet of Artemia enriched with Tetraselmis sp. and Chlorella sp. resulted in the highest growth and survival rate of Angelfish larvae, while Manganang et al. (2020) showed that feeding algae biofuel-waste to fish had similar effects on fish growth as commercial fish feed. Al-Faiz et al. (2022) suggested that using a mixture of artificial and live food after four weeks of age can reduce production costs and provide better growth and survival rates of *Luciobarbus xanthopterus* larvae. Enyidi (2017) found that *Chlorella vulgaris* is a viable alternative to fishmeal in the diet of African catfish.

Table 4 Limitations of Chlorella

Aspect	Description	References
High cost of production	Chlorella cultivation can be expensive due to the need for controlled environments, sterilization, and specialized equipment	Spolaore et al. 2006; Rajkumar et al. 2014
Difficulty in large-scale production	Scaling up Chlorella cultivation can be challenging due to issues such as contamination, variability in growth rates, and the need for specialized infrastructure	Xu et al. 2009
Limited digestibility	Chlorella's cell wall is resistant to digestion, limiting its bioavailability	Merchant et al. 2002; Panahi et al. 2016
Heavy metal accumulation	Chlorella can accumulate heavy metals from its environment, posing a risk of contamination	Zeraatkar et al. 2016
Undesirable taste and odor	Some consumers find Chlorella's taste and odor unpleasant, which can limit its use in food and beverage applications	Nakano et al. 2007

Yuslan et al. (2021) demonstrated that Chlorella sp. enriched copepods had the highest specific growth rate and survival rate of Betta splendens larvae. Pradhan et al. (2023) showed that Chlorella supplementation improved the non-specific immunity parameters, growth performance, and disease resistance of Labeo rohita fingerlings. Kalayda and Dementiev (2019) suggested that power plants can help to create favorable conditions for fish farming. Seong et al. (2021) found that a mixed microalgae diet of *Nannochloropsis* meal and *Schizochytrium* meal along with Chlorella meal resulted in the highest growth rate of red sea bream. Simanjuntak (2020) investigated the effects of supplementing *Chlorella vulgaris* into gourami feed and fasting frequency on gourami body composition. These studies indicate that Chlorella sp. and mixed diets are a sustainable and viable alternative to commercial fish feed for the aquaculture industry, and power plants can be used to create favorable conditions for fish farming.

In vitro studies of Chlorella against bacterial fish pathogens

Several in vitro studies have investigated the antibacterial activity of Chlorella against fish pathogens. One study found that *Chlorella vulgaris* extracts had significant antibacterial activity against *Aeromonas hydrophila* and *Vibrio harveyi* (He et al. 2018). Another study demonstrated that the crude extract of *Chlorella pyrenoidosa* had strong inhibitory effects against *Streptococcus iniae* and *Edwardsiella tarda* (Velichkova et al. 2018). In addition, Chlorella extracts have been shown to inhibit biofilm formation of various fish pathogens, including *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Rendueles et al. 2013), (Table 5).

In vivo studies of Chlorella against bacterial fish pathogens

Several in vivo studies have also investigated the efficacy of Chlorella against bacterial fish pathogens. One study demonstrated that feeding Chlorella to juvenile tilapia infected with *Aeromonas hydrophila* improved their survival rate and reduced bacterial loads (Aly et al.

Table 5 Studies of Chlorella against bacterial fish pathogens

Chlorella	Fish pathogen	Reference
C. vulgaris	*Aeromonas hydrophila*	Velichkova et al. 2019; Raji et al. 2019
C. vulgaris	*Aeromonas salmonicida*	Vu et al. 2010
C. vulgaris	*Vibrio harveyi*	Yuniarti et al. 2023; Jusidin et al. 2022
C. vulgaris	*Vibrio alginolyticus*	Sharifah and Eguchi 2012; Amaro et al. 2011
C. vulgaris	*Streptococcus iniae*	Yuniarti et al. 2023; Imran Bashir et al. 2018
C. vulgaris	*Streptococcus agalactiae*	Yuniarti et al. 2023; Velichkova et al. 2018
C. vulgaris	*Edwardsiella tarda*	Rusmawanto et al. 2019

2022). In addition, a recent study explored the antibacterial activity and potential efficacy of Chlorella vulgaris dietary supplements in enhancing the immune response, and disease resistance of *Labeo rohita* fingerlings against *Aeromonas hydrophila* infection. The findings from their study indicate that the most favorable dietary Chlorella supplementation-tation level lies between 0.5 to 1.0 g Kg(-1) of the diet. This supplementation range has shown to stimulate immunity and offer protection to L. rohita against *A. hydrophila* infection (Pradhan et al. 2023) (Table 5).

Mechanisms of action of Chlorella against bacterial fish pathogens

The antibacterial activity of Chlorella is attributed to its bioactive compounds, including polysaccharides, proteins, and lipids (Hussein et al. 2018; Cavallo et al. 2013). These compounds have been shown to inhibit bacterial growth and biofilm formation by interfering with quorum sensing, cell signaling, and gene expression (Cavallo et al. 2013; Little et al. 2021).

In vitro and in vivo studies have demonstrated the potential of Chlorella as a treatment for bacterial fish pathogens in aquaculture. The antibacterial activity of Chlorella is attributed to its bioactive compounds, including polysaccharides, proteins, and lipids, which have been shown to inhibit bacterial growth and biofilm formation. However, further research is needed to optimize the growth and extraction conditions of Chlorella and to evaluate the environmental impact of Chlorella-based treatments. Overall, Chlorella represents a promising option for the prevention and control of bacterial fish pathogens in aquaculture (Table 6).

Chlorella and Green Water System

The Green Water System is an aquaculture management approach that utilizes Chlorella, a type of algae, to create a closed-loop aquatic ecosystem. Chlorella plays a central role as a natural filter and nutrient recycler, utilizing excess nutrients from the water to maintain water quality (Bosma and Tendencia 2014). Chlorella's remarkable nutritional value, rich in essential nutrients, makes it an excellent supplement for aquatic animals, supporting their health, growth, and immune systems (Muys et al. 2019; Enyidi 2017). Incorporating Chlorella into the system fosters a well-balanced and sustainable environment, benefiting both the fish and the overall aquatic ecosystem (Muys et al. 2019).

Table 6 Mechanisms of action of Chlorella against bacterial fish pathogens

Chlorella type/species	Pathogen	Mechanism of action	References
C. vulgaris	*Vibrio parahaemolyticus*	Inhibition of quorum sensing system	Torabfam and Yüce 2020
C. vulgaris	*Streptococcus iniae*	Downregulation of virulence genes, inhibition of biofilm formation, and disruption of cell membrane integrity	Velichkova et al. 2018
C. vulgaris	*Aeromonas hydrophila*	Downregulation of virulence genes, inhibition of biofilm formation, and disruption of cell membrane integrity	Velichkova et al. 2018
C. vulgaris	*Streptococcus agalactiae*	Downregulation of virulence genes, inhibition of biofilm formation, and disruption of cell membrane integrity	Velichkova et al. 2018
C. vulgaris	*Vibrio harveyi*	Downregulation of virulence genes, inhibition of biofilm formation, and disruption of cell membrane integrity	Velichkova et al. 2018
C. vulgaris	*Vibrio parahaemolyticus*	Modulation of immune response and increase in antioxidant activity	Huang et al. 2023
C. vulgaris	*Vibrio alginolyticus*	Inhibition of quorum sensing system, downregulation of virulence genes, and disruption of cell membrane integrity	Sharifah and Eguchi 2012
C. vulgaris	*Streptococcus iniae*	Inhibition of quorum sensing system, downregulation of virulence genes, and disruption of cell membrane integrity	Yuniarti et al. 2023
C. vulgaris	*Streptococcus agalactiae*	Inhibition of quorum sensing system, downregulation of virulence genes, and disruption of cell membrane integrity	Yuniarti et al. 2023
C. vulgaris	*Vibrio harveyi*	Inhibition of quorum sensing system, downregulation of virulence genes, and disruption of cell membrane integrity	Yuniarti et al. 2023

The introduction of Chlorella into the Green Water System brings benefits such as improved water clarity, better observation of fish behavior, and enhanced oxygenation (Sinha et al. 2016; Wang et al. 2022). Chlorella's ability to absorb nutrients reduces excess nitrogen and other compounds, resulting in a cleaner and clearer aquatic environment (Sinha et al. 2016). Moreover, Chlorella's nutrient recycling capabilities decrease the need for frequent water changes, saving time and effort while promoting water conservation (Campos et al. 2022; Chithambaran et al. 2017).

The Green Water System with Chlorella positively impacts fish health and vitality, as Chlorella's nutrient-rich composition supports their overall well-being (Sharifah and Eguchi 2012). The algae's nutritional value, with high-quality proteins and essential vitamins and minerals, boosts fish growth rates and enhances their immune systems, leading to healthier populations (Mathew et al. 2021; Galal et al. 2018). This aspect is particularly beneficial for commercial aquaculture operations (Enyidi 2017).

The successful implementation of the Green Water System with Chlorella requires diligent monitoring and management. Beginners may face a learning curve in finding the right balance of nutrients and algae growth (Enyidi 2017). Vigilant observation and regular monitoring of water parameters and Chlorella's growth rate are crucial for maintaining a stable and healthy aquatic environment (Bosma and Tendencia 2014). By controlling nutrient levels and fine-tuning the system, aquaculturists can promote a cost-effective and sustainable approach to aquaculture (Muys et al. 2019).

Chlorella as a sustainable solution for the aquaculture industry

Strain selection is an important factor in determining the efficacy of Chlorella in treating bacterial fish pathogens. Different strains of Chlorella have varying levels of antibacterial activity and bioactive compounds, which can affect their ability to inhibit bacterial growth and biofilm formation. For example, Das and Pradhan (2010) found that *Chlorella vulgaris* extracts had significant antibacterial activity against *Aeromonas hydrophila* and *Vibrio harveyi*, while Pradhan et al. (2023) demonstrated that the crude extract of *Chlorella*

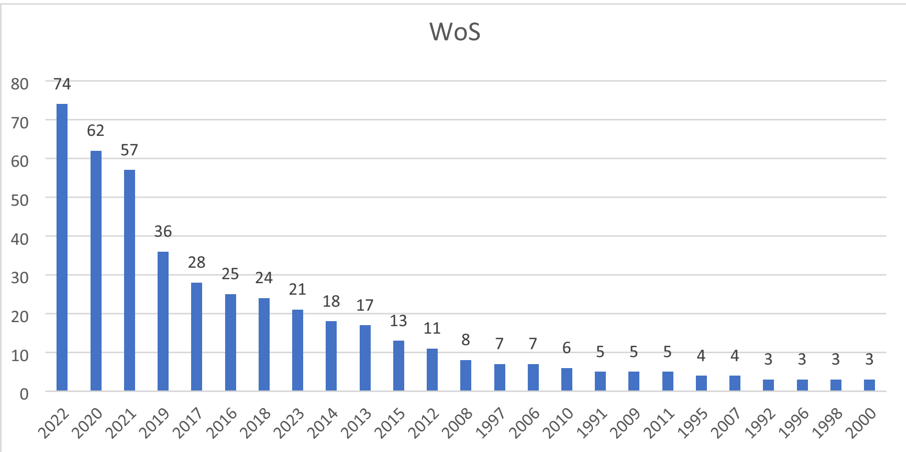

Fig. 1 Number of publications per year about aquaculture and Chlorella (search title, abstract, and keywords) in the Web of Science (WoS)

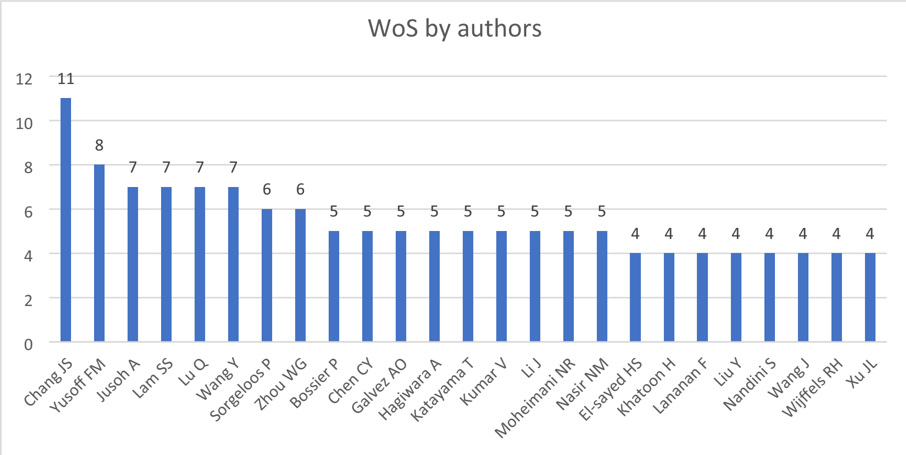

Fig. 2 Top authors that had published about aquaculture and Chlorella (search title, abstract, and keywords) in the Web of Science (WoS)

pyrenoidosa had strong inhibitory effects against *Streptococcus iniae* and *Edwardsiella tarda*. Therefore, selecting the appropriate strain of Chlorella is critical in ensuring its efficacy as a treatment for bacterial fish pathogens.

Growth conditions also play a significant role in determining the efficacy of Chlorella as a treatment for bacterial fish pathogens. The composition of the growth medium can influence the production of bioactive compounds and the antibacterial activity of Chlorella. Martinez and Orus (1991) demonstrated that Chlorella grown in a medium supplemented with glucose had higher levels of bioactive compounds and stronger antibacterial activity against *Vibrio parahaemolyticus* than Chlorella grown in a medium without glucose. In addition, the growth temperature and pH of Chlorella can also affect its antibacterial activity.

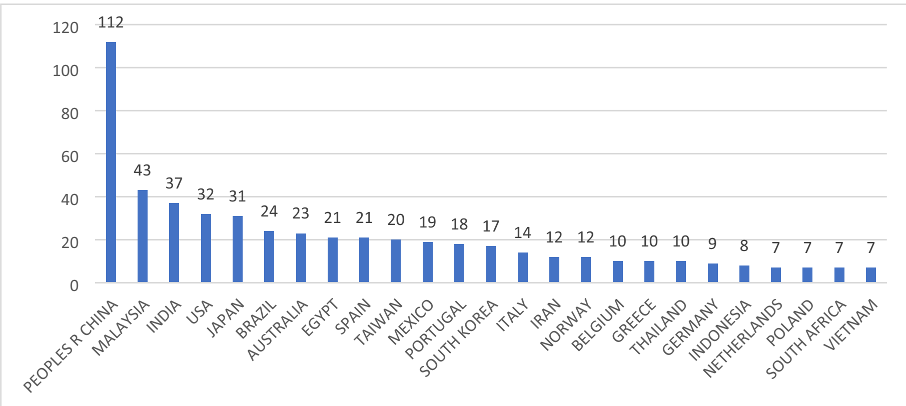

Fig. 3 Number of papers published by country about aquaculture and Chlorella (search title, abstract, and keywords) in the Web of Science (WoS)

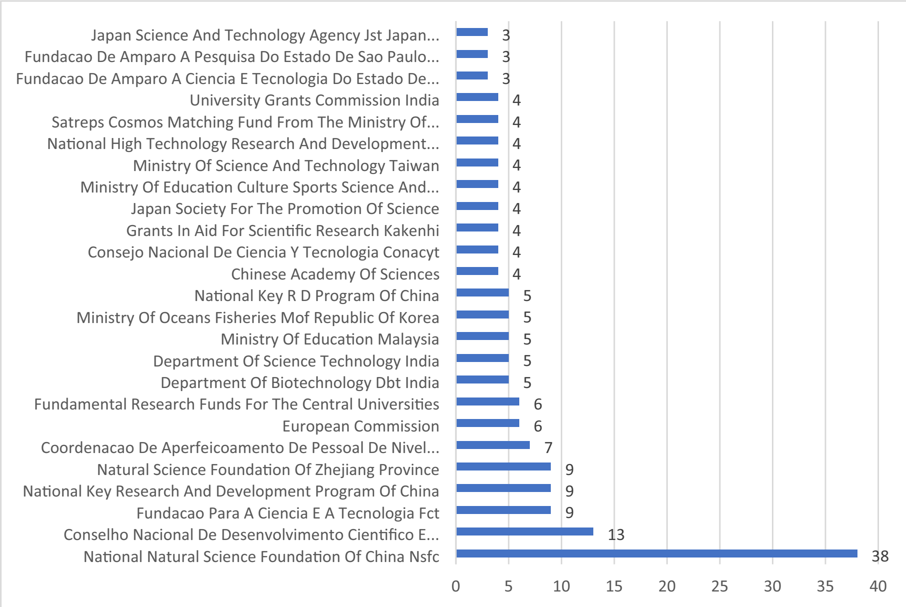

Fig. 4 Top funding agencies that had funded work about aquaculture and Chlorella (search title, abstract, and keywords) in the Web of Science (WoS)

The administration route of Chlorella-based treatments can also affect their efficacy in treating bacterial fish pathogens. Chlorella can be administered orally or through immersion, and the efficacy of each administration route can vary depending on the fish species and the bacterial pathogen. El-Habashi et al. (2019) demonstrated that feeding Chlorella to juvenile tilapia infected with *Aeromonas hydrophila* improved their survival rate and reduced bacterial loads. In contrast, Li et al. (2023) showed that immersion treatment with Chlorella extract was more effective than oral administration in reducing bacterial loads and improving the immune response in grass carp infected with *Aeromonas hydrophila*.

Future perspectives and challenges in the use of Chlorella for bacterial fish pathogen control

Chlorella, a freshwater microalga, has been proposed as a sustainable alternative for the control of bacterial fish pathogens in the aquaculture industry due to its antibacterial properties, safety, and environmental friendliness. As discussed in previous literature, Chlorella's efficacy as a treatment for bacterial fish pathogens can be influenced by various factors such as strain selection, growth conditions, extraction methods, and administration routes. However, future perspectives and challenges in the use of Chlorella for bacterial fish pathogen control remain to be addressed.

One potential future perspective is the use of Chlorella-based probiotics as a preventive measure against bacterial infections in aquaculture. Probiotics are live microorganisms that confer a health benefit on the host and have been shown to improve disease resistance

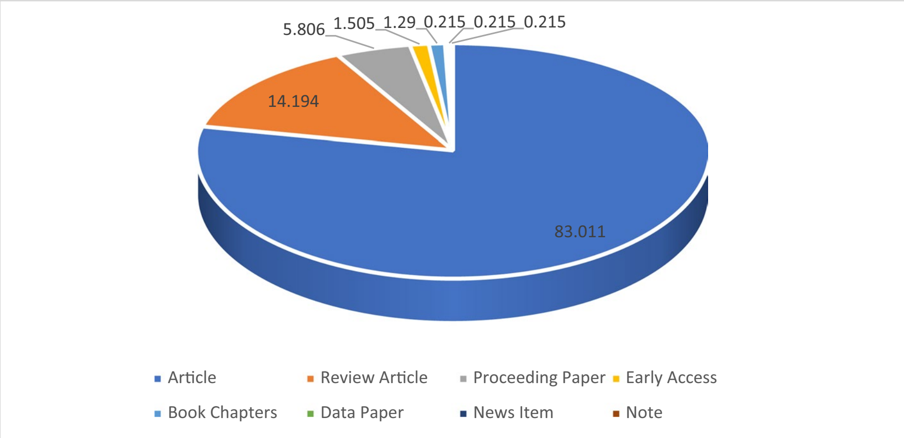

Fig. 5 Percentage of publication by document type about aquaculture and Chlorella (search title, abstract, and keywords) in the Web of Science (WoS)

and growth performance in fish. Chlorella has been demonstrated to possess probiotic properties, such as the ability to improve the immune response and gut microbiota of fish. For example, Huang et al. (2023) found that feeding Nile tilapia with Chlorella sp. improved their immune response and gut microbiota composition. Therefore, the use of Chlorella-based probiotics may offer a sustainable and effective solution for preventing bacterial infections in aquaculture.

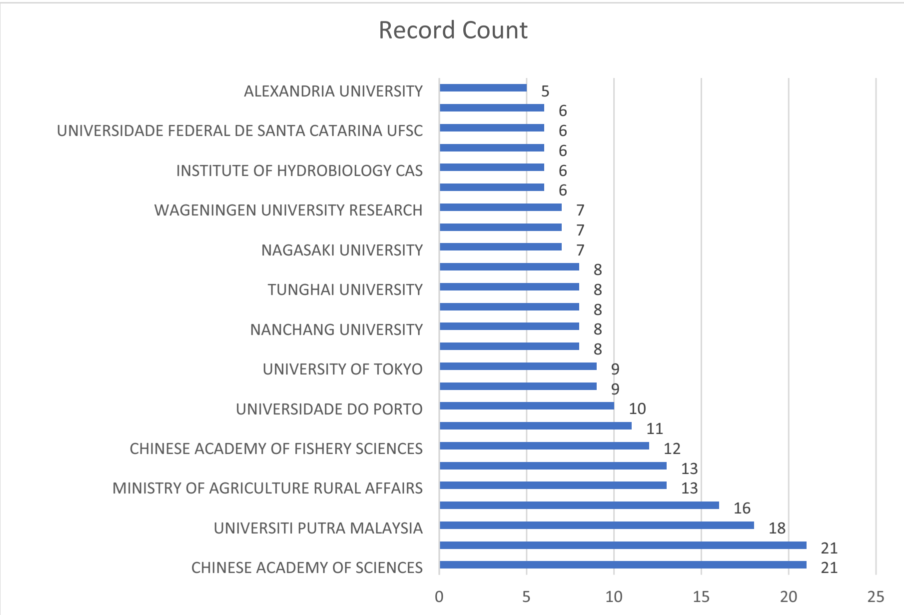

Fig. 6 Top affiliations that had published work about aquaculture and Chlorella (search title, abstract, and keywords) in the Web of Science (WoS)

However, several challenges need to be addressed for the successful implementation of Chlorella-based treatments for bacterial fish pathogen control. One major challenge is the cost-effectiveness of Chlorella production at a commercial scale. Although Chlorella is a fast-growing microalga, the high cost of production and extraction may limit its feasibility for large-scale applications. Therefore, the development of cost-effective and scalable Chlorella production methods is necessary to ensure its economic viability for the aquaculture industry.

Another challenge is the standardization of Chlorella-based treatments. The variation in antibacterial activity and bioactive compounds among different Chlorella strains, growth conditions, and extraction methods may hinder the development of standardized Chlorella-based treatments. Therefore, the establishment of standardized protocols for Chlorella production, extraction, and administration is essential to ensure consistent and reliable antibacterial activity.

Also, the use of Chlorella as a sustainable solution for bacterial fish pathogen control in aquaculture shows great potential. Future perspectives such as the use of Chlorella-based probiotics and combination with other natural antimicrobials may enhance its efficacy and sustainability. However, challenges such as cost-effectiveness and standardization need to be addressed for successful implementation in the aquaculture industry.

Aquaculture and Chlorella: a scientometric analysis (1991–10 May 2023)

The scientometric method was employed to analyze research articles published in the field of aquaculture and Chlorella between 1991 and 10 May 2023. This method was selected because of the ample availability of bibliographic databases Web of Science, as noted by Bar-Ilan (2008) and Adriaanse and Rensleigh (2013).

Database search

To mine data, the Web of Science (WoS) core collection database by Clarivate Analytics was chosen as the primary sources. These databases are widely used for review articles and are known for their comprehensive coverage of various areas of knowledge, as noted by Aryadoust and Ang (2021). Additionally, WoS provides country, scientist, paper, categories, document type, and funding agency rankings for research. The search method used here follows the protocol established by Azzeri et al. (2020) and Aryadoust et al. (2019). The "TS" field was selected to include the manuscript title, abstract, keywords, and Keywords Plus in the search, as recommended by Bar-Ilan (2008) and Adriaanse and Rensleigh (2013). The search was conducted in WoS using common keywords (search code) for aquaculture biosecurity, as identified by Dong et al. (2012) and Du et al. (2014). The Boolean operator "and" was employed to capture all specified terms related to aquaculture biosecurity. Based on the systematic review study by Barrett et al. (2019) on the impacts of aquaculture on wildlife, the following search code was used: TS = (aquaculture) and (Chlorella).

To ensure the scope of the search was limited to relevant publications, the WOS databases were searched for publications between 1991 and 10 May 2013. It is worth noting that abstracts of publications published before 1991 were not available in either database. The review included original research articles, commentaries, short communications, books and book chapters, protocol papers, and theoretical papers.

The goal of this section of the review was to summarize the literature available on aquaculture biosecurity using the scientometric method for the period between 1991 and 10 May 2023. The results showed that as of May 10, 2023, there is a total of 463 publications with a total citation count of 13,408, while the H-index were retrieved 55. The highest number of publications on aquaculture and Chlorella was recorded in the year 2022 with 74 publications. The author with the most publications on aquaculture and Chlorella was found to be Chang JS. Furthermore, the study indicates that the People's R China and the Chinese Academy of Sciences are the leading country and organization, respectively (Figs. 1, 2, 3, 4, 5, and 6).

Conclusion

Aquaculture is a rapidly growing industry that provides a valuable source of protein and income for many countries. However, bacterial fish pathogens pose a significant threat to the sustainability of the industry, causing economic losses and endangering food security. Disease prevention and control measures, such as the use of probiotics, vaccines, and biosecurity measures, are essential for the sustainable development of the aquaculture industry. However, further research is needed to develop more effective, safe, and sustainable strategies for the prevention and control of bacterial fish pathogens in aquaculture.

Traditional treatments for bacterial fish pathogens, such as antibiotics and chemicals, have limitations and environmental concerns that need to be addressed. Alternative treatments, such as herbal extracts, essential oils, and probiotics, have been proposed as safer and more environmentally friendly options. However, further research is needed to determine the efficacy and safety of these treatments and to develop more sustainable and effective strategies for the prevention and control of bacterial fish pathogens in aquaculture.

Microalgae are versatile microorganisms that possess various potential applications in different industries, such as biofuels, pharmaceuticals, and wastewater treatment. However, further research is needed to overcome the challenges associated with their large-scale production and commercialization.

Chlorella is a diverse group of unicellular, freshwater green algae that is classified based on morphological and molecular characteristics. Chlorella cells are small and spherical, with a single cup-shaped chloroplast and a cell wall composed of cellulose and other polysaccharides. Chlorella species have potential applications in various industries, including food, feed, biofuels, and pharmaceuticals, due to their high growth rates, photosynthetic efficiency, and ability to accumulate various compounds.

The safety of Chlorella to fish is a complex issue that requires careful evaluation of its effects on fish health and physiology. Hematology is an essential tool for assessing the effects of dietary interventions on fish physiology and evaluating the safety of Chlorella to fish. Further studies are needed to investigate the long-term effects of dietary Chlorella on fish health and the potential risks associated with its use in aquaculture. The available literature suggests that Chlorella is safe for fish, and its inclusion in fish diets does not have any adverse effect on fish survival. However, further studies are required to investigate the long-term effects of chlorella supplementation on fish health and growth.

The Green Water System, with Chlorella at its core, is an innovative and eco-friendly approach to aquaculture management. Chlorella serves as a natural filter and nutrient recycler, maintaining water quality by absorbing excess nutrients. Its exceptional nutritional value ensures a well-rounded diet for aquatic animals, enhancing their immune systems, growth rates, and survival rates. The system's benefits extend to improved water clarity, easier observation of fish behavior, and a stable aquatic environment. Diligent monitoring and attentive management are essential for successful implementation, and the system's cost-effectiveness and resilience further contribute to its sustainability. Ultimately, the Green Water System with Chlorella promotes a balanced and thriving aquatic habitat while minimizing environmental impact.

In this scientometric review of research articles published in the field of aquaculture and Chlorella between 1991 and 10th May 2023, the Web of Science (WoS) core collection database was used as the primary source for data mining. The search followed a protocol established by previous studies and employed common keywords related to aquaculture and Chlorella. A total of 463 publications were retrieved with a total citation count of 13,408 and an H-index of 55. The year 2022 had the highest number of publications with 74 articles. The most prolific author in this field was Chang JS, while the People's R China and the Chinese Academy of Sciences emerged as the leading country and organization, respectively. Overall, the review aimed to summarize the available literature on aquaculture and Chlorella using the scientometric method.

Chlorella shows promise as an eco-friendly remedy for bacterial infections that impair aquaculture production. Its antibacterial properties, safety, and sustainability make it an attractive alternative to traditional treatments, but its successful implementation in the aquaculture industry requires careful evaluation, optimization, and standardization. Further research is necessary to develop effective and sustainable strategies for the prevention and control of bacterial fish pathogens in aquaculture, and Chlorella-based treatments require further investigation for efficacy, safety, and environmental impact.

Recommendations

To successfully implement Chlorella-based treatments in aquaculture, several recommendations must be considered.

Firstly, the safety of Chlorella to fish is a crucial issue that requires careful evaluation. Hematology can be an essential tool to assess its effects on fish health and physiology. Although studies have shown that Chlorella is safe for fish and does not have adverse effects on growth, survival, or immune system function, further investigation is needed to evaluate its long-term effects on fish health and growth.

Secondly, optimizing growth and extraction conditions for antibacterial activity is necessary for effective Chlorella-based treatments. Factors such as light intensity, temperature, and nutrient availability can affect Chlorella growth and antibacterial activity, and their optimization can enhance the efficacy of Chlorella-based treatments.

Thirdly, the environmental impact of Chlorella-based treatments must be evaluated. Although Chlorella is considered environmentally friendly due to its ability to sequester carbon dioxide and other pollutants, large-scale production and application in aquaculture may have unintended consequences. Therefore, studies on the potential effects of Chlorella on the aquatic ecosystem are necessary to ensure its sustainability and minimize its impact.

Finally, cost-effectiveness and standardization are crucial for successful implementation of Chlorella-based treatments in the aquaculture industry. Although Chlorella is a natural and sustainable alternative to traditional treatments, its commercialization and large-scale production face several challenges that require further research and innovation.

Author contribution The study's conception and design involved the contributions of all authors. Mohamed Fathi and Salah Aly were responsible for material preparation, data collection, and analysis. Mohamed Fathi and Noha ElBanna wrote the initial draft of the manuscript, while all authors provided feedback on earlier versions. The final manuscript was reviewed and approved by all authors.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval This research study was conducted in an ethical and responsible manner, in full compliance with all relevant codes of experimentation and legislation. All data collected during the study was handled and analyzed with utmost care and integrity. The authors affirm that this research study was conducted with the highest level of ethical standards and in accordance with the ethical principles of research.

Competing interests The authors declare no competing interests.

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