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REVIEW ARTICLE

Incorporation of Transgenic Microalgae Harbouring Vaccines into Feed to Improve the Efficacy of Oral Vaccination: A Review

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Abstract The aquaculture industry has been rapidly progressive and is contributing to economic growth in many countries worldwide. However, one of the factors that is affecting the aquaculture industry are diseases caused by pathogens such as bacteria, viruses, fungi, and parasites. The utilization of antibiotics has been one of the measures taken in controlling fish diseases. However, continuous treatment with antibiotics for a long period of time will lead to the development of antibiotic-resistant pathogens which can cause harm to the well-being of humans, animals, and the environment as well. An alternative method in combating fish diseases is through vaccination. Vaccination for fish is commonly done by injection and is known to be very effective in protecting fishes from various ailments. However, this method is labour intensive, costly, stressful to the fish and not suitable for juvenile fish. Therefore, other methods are necessary to be performed for vaccinating the fish. This review aims to demonstrate effective methods of delivering vaccine to the fish using transgenic microalgae, specifically via oral vaccination. This review article summarizes the challenges faced in the aquaculture industry which could be mitigated via improved vaccination procedures. In addition to that, the potentials of incorporating transgenic microalgae whole cells into fish feed formulation as an alternative method of disease control is also outlined.

Keywords: Aquaculture, Diseases, Feed Formulation, Oral Vaccination, Transgenic microalgae

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Introduction

Global aquaculture production has been reported to be increasing dramatically since the year 1994. In 2020, the production of aquaculture had reached almost 122.6 million tonnes which comprises 57.5 million tonnes of finfish, 17.7 million tonnes of mollusc mainly bivalve, 11.2 million tonnes of crustaceans and 525 000 tonnes of aquatic invertebrates and 537 000 tonnes of other semi-aquatic species [1]. Hence, the total sales of the aquaculture production reported in the year 2020 were estimated to be around USD 281.5 billion [1]. Based on the reports, China is the major producer of aquaculture which contributing 35 percent of global fish production in the year 2022 [1]. Following China, India, Indonesia, Viet Nam, and Peru are also known as major producers for aquaculture production contributing about 58 percent production of aquaculture in the world [1]. Large production of aquaculture is vital in ensuring sufficient food supply for the continuous growing population in all parts of the world. Food scarcity is a concerning global issue as the damage and problems it entails would be distressing. It has been reported by the United Nations that in 2050, the population will increase from 7.6 billion people to 9.8 billion people whereby 50% of this growth is anticipated to be among the less developed countries [2]. The increase of population will create further damage especially to the poor communities that are already facing malnutrition due to the lack of food supply. For preventing food scarcity from happening, a sustainable of food supply need to be implemented especially in aquaculture production. However, maintaining greater aquaculture production over the year can be challenging due to several factors such as diseases, climatic factors, management practices and many more.

Due to the nature of the fish farm being high in density and having a limitation towards the usage of clean water, the fish are more susceptible to the bacterial, viral, and fungal pathogens [3]. In other words, the fishes in a fish farm have a high possibility to be infected and unrevivable. The common pathogens and parasites that cause fish diseases are *Aeromonas, Vibrio, Edwardsiella*,*Streptococccus, Saprolegnia, Ichthyophonus Birnavirus* and *Lymphocystivirus*.[4,5,6,7,8].The use of antibiotics has been widely implemented for controlling the bacterial diseases however, the formation of the an antibiotic-resistant bacteria that is proven to be harmful for humans, animals and the environment creates a problem. Additionally, the utilization of the antibiotic can be only used for treating the infectious diseases caused by the bacteria but not for the viral and fungal diseases. Therefore, other alternatives are required for treating bacterial, viral and fungal diseases.

Vaccination of the aquaculture species is another strategy for treating bacterial and viral diseases. However, the commercial vaccine that has been used is only limited to certain aquaculture species. Most commercial vaccines are made of inactivated bacterial pathogens, and it has been performed on various types of fish. Recently, another technology has been introduced for developing the vaccine carrier for aquatic animals which is by genetically modifying microalgae [9,10]. This invention has been known to be a promising technology that could enhance the efficacy of the oral vaccine for controlling the outbreak of diseases. However, studies on using microalgae as a vaccine delivery system for vaccinating fish via oral route are limited. This review discusses the overview technology for oral vaccination especially in using transgenic microalgae which can become a promising application for development of the oral vaccine in aquaculture. The challenges faced in the aquaculture industry are also being summarized so the challenge could be mitigated via vaccination procedure.

Vaccination

Vaccination has the potential to control and prevent the outbreak of diseases by introducing an antigen to the animal. The antigen is known as a component contained in the vaccine that helps in stimulating an innate or adaptive immune response of the fish. Vaccination has become a preferred method for controlling the outbreak of fish disease compared to antibiotics. This is because a long-term and large amount of usage of antibiotics will lead to the development of antibiotic-resistant bacteria [11]. Also, a higher chance of horizontal gene transfer between the bacteria species can occur which will cause negative impacts on humans, animals, and the environment [11]. Due to these harmful consequences, vaccination is considered as a more efficient and safer procedure to be performed in controlling diseases, especially in aquaculture sectors. Safety concerns regarding the presence of antibiotic residues in fish have become one of the factors to start considering in reducing the usage of antibiotics for disease treatment. Other efficiencies and the safety of the vaccines are also summarized in Table 1 [12,13,14,15].

Table 1. A comparison between efficiency and safety of vaccines and antibiotics in aquaculture sectors

	Vaccines	Antibiotics
Infection	As preventative measure prior to the infection	As a treatment after infection
Disease control	The efficiency depends on immune response of the animal	The efficiency depends on the duration of using the therapeutics agent
Effect on environment	Safer, no development of bacterial resistance	Highly potential for development of antibiotic-bacterial resistance
Treatment availability	Able to control disease caused by bacterial and viral pathogen	Not able to treat viral disease
Food safety	No issues regarding food safety	Presence of the antibiotic residues in food products
Prophylactics effect	Long-lasting	Not long-lasting after treatments
Quality of the products	Good quality of the fish products	Poor quality fish products

In previous years, many commercial vaccines were used for the prevention of fish diseases in aquaculture industries. More than 26 commercial vaccines are available for a certain type of fish as a precaution to control the outbreak of diseases [15]. Some of the commercial vaccines has been approved by the United States Department of Agriculture (USDA) and are safe to be used in aquaculture industries [9]. Most of the commercial vaccines are made of inactivated bacterial pathogens and are delivered via intraperitoneal injection to a variety of fishes such as Atlantic salmon (*Salmon salar*), rainbow trout (*Oncorhynchus mykiss*), channel catfish (*Ictalurus punctatus*), European seabass (*Dicentrarchus labrax*), tilapia (*Oreochromis niloticus*), and Atlantic cod (*Gadus morhua*) [16]. Several commercially licensed vaccines for controlling infectious diseases, especially the vaccine for Atlantic salmon has already been made available in the United Kingdom, Chile, Norway, and the United States of America [17]. Infectious pancreatic necrosis, furunculosis, and enteric redmouth disease (ERM) are the common diseases that infects the Atlantic salmon, therefore many farmers to have taken precautions and vaccinated their fish.

Challenges in Vaccination of Fish

Even though vaccination has become one of the potential ways for protecting fish from disease outbreaks, there are some challenges in fish vaccination that need to be pointed out. Vaccines usually being administered via different routes such as oral, injection and immersion as shown in Figure 1. However, most commercially available vaccines nowadays are being administered via intraperitoneal and intramuscular injections to the fish. It has also been reported that delivering vaccines by injection seems to be the most effective technique as a higher survival rate of the fish was recorded in previous studies compared to the other two methods [18]. However, this administration technique is costly, time-consuming, creates a stressful environment for the fish, and is labour-intensive [19]. In some cases, the injection route is not suitable to be applied for vaccinating the fish at an early age due to their small size. Thus, the efficiency of the other route needs to be explored for vaccine administration to be more practical for vaccinating small fish at a large scale. Many studies related to the performance of oral vaccination and immersion are still being discovered by many researchers. Among the three main routes for delivering the vaccine, oral vaccination is more practical and is less stressful for the fish. Nevertheless, the oral vaccine might not be as efficient as the injected vaccine due to the antigen is not capable of inducing the immune response of the fish. This is because antigen degradation might occur as the antigen enters through the oral route of the host [19].

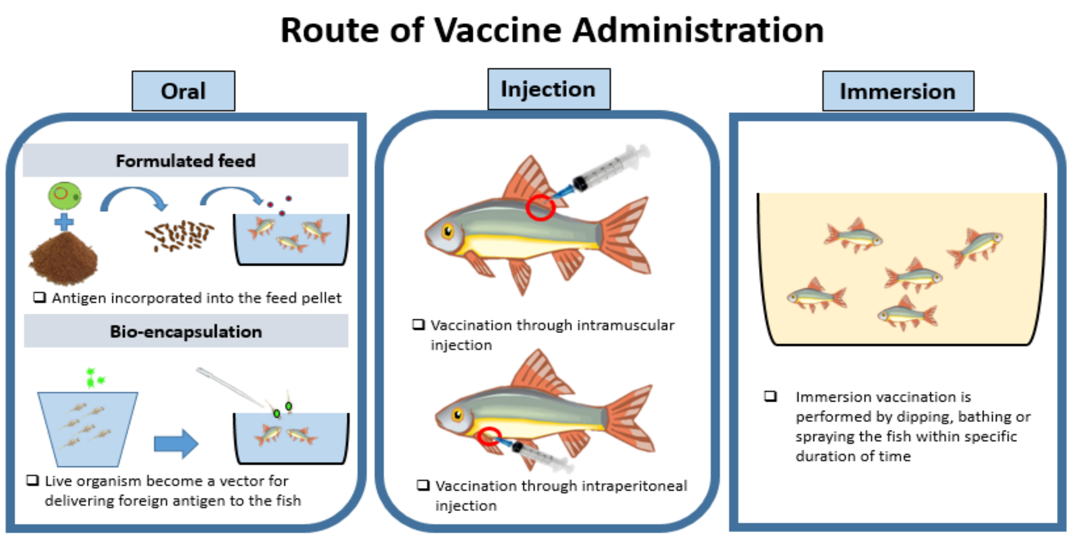

Figure 1. Routes of vaccine administration to the fish.

Oral Vaccination of Fish

Oral vaccination is known as the suitable method for vaccinating fish especially for mass vaccination of farmed fish. By vaccinating the fish, this action will protect fish from getting infected by the virulent bacteria, as the vaccine will introduce the pathogenic antigen to the fish that are capable to stimulate the immune response of the fish. Many advantages can be obtained by performing oral vaccination such as a reduction in cost, time, and labour services [20]. Other than that, vaccinating small fish and many fish can be done at one time. This application does not require any services for delivering the vaccines as the farmers can do it by themselves. Besides, this administration method causes no stress to the fish and any physical damage on the fish body can be avoided as they uptake the vaccine in a natural way.

Despite these advantages, this approach also has its limitation as the delivered vaccine must pass through the gastrointestinal conditions in the animal's gut. The antigens of the vaccines are more likely to be degraded in acidic condition [21]. Thus, for performing oral vaccination, it is significant to formulate a vaccine that protects the antigen from being degraded in the stomach. The efficacy of the vaccine depends on the native antigens, the process of preparing the vaccine, and the dose of the vaccine given to the animals [18]. All these factors need to be analysed first before initiating the treatment process.

Fish Feed

Fish feed is significantly useful in aquaculture sectors, and it has a major contribution to fish health, growth, reproduction, and immunity. The fish feed can be divided into groups which are formulated feed and live feed. The formulated feed can also be classified into different groups according to their moisture content. Normally, wet food is prepared by mixing the raw fish with a small amount of dry material (DM) with the DM of the formulated feed below 30%. Meanwhile, for moist and dry feed, their DM is identified to be around 60% and 90% respectively [22]. For producing the dry fish feed, an extruder is used. The ingredients of the fish feed can be modified if it is needed. Much research has been using the feed pellet as a medium for delivering any potential protein such as growth hormone or vaccine that can enhance the growth or the health of the fish. For vaccination purposes, formulated fish feed is one of the most suitable methods for delivering antigenic protein into the fish gut. While administering the vaccine, the fish should be avoided from stress conditions. The efficiency of the vaccine will be low if the fish is under stress conditions and there is a possibility that the formulated fish feed is not eaten by the fish. To avoid the fish from being stressed, the density of the fish in the area should not be too high, sufficient nutrition is provided, water quality is good, parasite infections are controlled and there is proper management of the fish health [18].

Bioencapsulation

In aquaculture, bio-encapsulation is a term where the live organism incorporates various nutritional or beneficial products which then form a live capsule [23]. Generally, the live organisms are fed with various products that are significant for enhancing the growth, health, and quality of the aquatic animal. Thus, this alternative has become a potential application where the live organism becomes a vector for delivering the essential nutrients or components to the other larval stages of animal [24]. Two live organisms are extensively being used as a vector which are brine shrimp (*Artemia nauplii*) and rotifer (*Brachionus plicatilis*). Brine shrimp (*Artemia nauplii*) is widely used in the aquaculture sectors as it is suitable for feeding fish, has a high level of nutrition and is easy to handle. In many research studies, *Artemia nauplii* has been utilized for bio encapsulating drugs, potential growth proteins, probiotics, and antigenic proteins. One study by Rudtanatip et al. [25] reported that sulfated galactans conjugated with fluorescein isothiocyanate (FITC) bio encapsulated in *Artemia* were fed to the shrimp resulting in higher-level expression of the immune gene in the larvae shrimp.

Types of Vaccines Available

Killed Pathogen

Killed vaccines are typically prepared by killing the virulent microorganisms and they are used as antigens for inducing the immune response of living organisms. This type of vaccine is easily prepared, has no virulence issues, is cheaper, and more stable in storage [26]. Most of the commercial vaccines available today are made of killed or inactivated living organisms, and it has been officially approved to be used in aquaculture sectors for controlling the outbreak of diseases. The vaccines are prepared through physical, chemical, or radiation processes without destroying the antigenic properties of the microbes [15,27]. The killed vaccine is also found to be much safer as the cells have lost the ability to replicate in the host body which lowers the risk of disease induction. Although the vaccine is safe, the killed or inactivated vaccine may induce short-term immunity and a weak induction of the fish immune response. Thus, the efficiency of the vaccine to induce the immunity of the fish can be increased by adding the adjuvants as a vaccine carrier. One study by Matsumoto et al. [28] reported the addition of the adjuvant to the killed vaccine increased their effectiveness in inducing protective immunity of the amberjack *Seriola dumerili*. They found the addition of the recombinant IL-12 adjuvant to conventional formalin-killed *Nocardia seriolae* by intraperitoneal injection (IP) challenged with *N. seriolae* resulted in 88.3% survival of amberjack *Seriola dumerili.* Meanwhile, the relative percentage of survival for the fish that was vaccinated without the adjuvant was found to be 0 which indicated that the rIL-12 was effective as adjuvant. Besides, Firdaus-Nawi et al. [29] used incomplete Freund's adjuvant (20% v/v) in the formalin killed *Streptococcus agalactiae* vaccine where the adjuvant-vaccinated fish were challenged by intraperitoneal injection with *S. agalactiae* resulting in a 100% survival rate of the red tilapia compared to the non-adjuvant vaccinated fish which only resulted in 50% survival rate.

Attenuated Vaccines

Live-attenuated vaccines are being prepared without killing viruses or bacteria. However, they are being attenuated to lose their virulence by undergoing repeated laboratory passage, physical and chemical attenuation. This type of vaccine can stimulate greater immune responses as they have the potential to proliferate in the host body inducing innate and adaptive immune responses [15,30]. The efficiency of the live-attenuated vaccines has also been proven in many research studies as they can induce cellular, mucosal, and humoral immunity of the organisms [26]. Thus, this live-attenuated vaccine has the potential to induce a greater adaptive immune response in fish compared to the other vaccines such as the killed vaccines or subunit vaccines [31,32]. Normally, this vaccine carries the antigenic component that is commonly expressed by the pathogen in the host body which becomes the reason why the vaccine is highly efficient. The mimicking of the natural exposure of the pathogen infection, causes the stimulated immune response by the live-attenuated vaccine to be nearly the same as that induced in the normal infection. When the pathogen is continuously replicated in the host body, the animal develops cellular memory of the infection which then leads to strong and long-lasting immunity within the host [15,27]. Other than that, the usage of the adjuvant when administering the live-attenuated vaccine to the animal is not required as it is potentially effective in inducing the immune system of the animal in single or small doses. The live-attenuated vaccine usually being administered by oral and immersion routes. A study by Laith et al. [33] reported the addition of the live-attenuated vaccine into the feed could provide strong protective immunity due to the elevation of the antibody IgM levels and lysosomal activity in the host body. According to the study, weakening the *S. agalactiae* by chemical agent Acriflavine dye (LAV1) and serial passages of bacteria on broth media (LAV2) are proven effective as the survival rate of the *Oreochromis niloticus* (tilapia) in both methods are 81.58% and 65.79% respectively. Ye et al. [34] utilized live-attenuated *Pseudomonas plecoglossicida* strain ΔtssD-1 for protecting the large yellow croaker (*Larimichthys crocea*) by administering the vaccine via immersion route resulted in a higher-level expression of IgM antibody, CD8α and MHCIα, and the survival rate of the fish was at 86.3%. The vaccine was considered safe as no other clinical symptoms or death were recorded when the fish were vaccinated at a higher dose (1.8×10^6 CFU per fish) of ΔtssD-1.

Subunit Vaccines

Subunit vaccines are one of the advanced biotechnology developments that have the potential to fight against various pathogens. In aquaculture, this vaccine has been utilized and explored for its potential to prevent disease outbreaks. Subunit vaccines consist of only purified antigens for inducing the immune response of the host [35]. The vaccine does not cause any harm to the host since it cannot proliferate in the host body. However, in some cases, this vaccine may have lower efficacy in protecting the animal compared to the killed or live-attenuated vaccine due to limited exposure of the antigenic component on the host body [15]. This limitation leads to a weaker induction of the hosts' immune response. Other possibilities for improving the effectiveness of the vaccine are by applying the adjuvant when administering the vaccine to the host and performing several booster immunizations for inducing a long-lasting immune response. For producing the subunit vaccine, different alternatives have already been discovered. The production of the antigenic protein can be expressed by various recombinant vectors, or the antigenic component can be isolated and purified from the pathogen. For designing the recombinant protein, it is significant to have the specific gene sequences of the antigenic component that can be recognized by the host immune system [14]. Technically, the plasmid that carries the sequence is inserted into the prokaryotic or eukaryotic cells. The antigenic protein then being expressed by the cells and harvested by fermentation process under controlled laboratory conditions [14]. Various potential cells can express the foreign gene such as bacteria, yeast, cell culture, insect cells, microalgae, and transgenic plants [36]. *Escherichia coli* is the most common expression system being utilized for encoding the specific gene sequence for producing the antigenic protein. Shahin et al. [37] reported two different types of cells for preparing the subunit vaccines which were *E. coli* expressing GroEL protein and *Thalassiosira pseudonana* expressing IgIC for protecting tilapia from piscine francisellosis, a highly infectious granulomatous disease. Based on the study, they found that the IgIC-Montanide group had the highest relative percentage of survival, higher-level production of antibodies and lower bacterial load in the spleen of the challenged tilapia fish. Another study related to the usage of recombinant protein was also reported by Atujona et al. [38]. The study demonstrated that the administration of the recombinant VirB11 protein via intraperitoneal injection, in which the gene was cloned from the *Vibrio harveyi*, resulted in greater immune protection in orange-spotted grouper against vibriosis. The study found that the vaccinated orange-spotted grouper produced higher antibody level and their relative percentage rate for both vaccinated and unvaccinated grouper were observed at 90% and 13% respectively.

Microalgae as A Vaccine Delivery System

Microalgae are unicellular organisms that are typically found in freshwater and marine ecosystem. They are small in size ranging between 2 µm to 5 µm. Due to their small size, these organisms tend to be food sources for small aquatic animals such as larvae finfish, rotifers, and crustaceans [39]. As microalgae contain a lot of nutritional values, this has encouraged many researchers to study other potential applications of microalgae for improving the quality of aquaculture production. Several microalgae species are used commercially such as *Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema,* and *Thalassiosira* [39,40]. Biochemical substances that are commonly being produced by microalgae are polyunsaturated fatty acids (PUFAs), lipids, enzymes, vitamins, sterols, minerals, and pigments [41]. Some of the bioactive compounds from microalgae also have potent antioxidant activity that is useful in the pharmaceutical sector especially for cancer therapy [42]. Carotenoid is one of the important chemical compounds that are being synthesized by microalgae that can exhibit antioxidant properties in response to the oxidative stress [43]. In the aquaculture sector, microalgae are commonly added to the feed for adding more nutritional values. Sarker et al. [44] found the replacement of the fish oil with *Schizochytrium* sp., a marine microalga in fish feed aids in improving weight gain and fatty acid deposition in juvenile Nile tilapia (*Oreochromis niloticus*). All the above studies show that the incorporation of the microalgae into the feed has the potential to improve the quality of aquaculture products.

Other than that, recent studies discovered that microalgae managed to provide a stable platform for expressing a foreign gene [9,46]. This approach creates a potential development of many therapeutics proteins that is responsible for sustaining aquaculture animal health. Generally, bacteria are the common organisms that act as a cell factory for producing recombinant protein, especially in industrial sectors [45]. However, the discovery of transgenic microalgae seems to be more beneficial compared to bacteria as their production is much safer, lower-cost, and solar-fuelled. Different methods were performed by many researchers for producing transgenic microalgae namely enzyme-mediated transformation, electroporation, *Agrobacterium tumefacient*, glass beads, silicon carbide whiskers, and microprojectile bombardment [45]. Also, various types of microalgae species were explored for their ability to express foreign protein. A study showed by Michelet et al. [46] where the *Chlamydomonas reinhardtii* was successfully transformed by introducing two antigenic proteins of *Aeromonas salmonicida*, AcrV and VapA in the chloroplast using different promoters. The introduced genes were successfully expressed in the microalgae. A study carried out by Abidin et al. [9] looked at the efficiency of different methods for transforming microalgae and the electroporation method was found to be the most efficient method. According to the study, the foreign gene was successfully inserted into *Nannochloropsis* sp. and it was verified via the polymerase chain reaction (PCR) and gene sequencing. The stability of the transgene has also been performed by Abidin et al. [10] where the stability of the *Nannochloropsis* harbouring outer membrane protein kinase (*OmpK*) gene fragment was evaluated over different subculturings. Based on the study, the *OmpK* gene was successfully amplified and was shown to be expressed up until the fifth subculturing of the transgenic lines. All of the research above indicates that microalgae have the potential to produce recombinant proteins in which will be beneficial for delivering vaccine via the oral route to aquatic animals.

Oral Vaccines of Transgenic Microalgae

As microalgae is commonly known as living feed, it is generally being fed to aquatic animals for an additional nutritional value. In many previous studies, microalgae were incorporated into the feed, and it was proven to bring benefits in improving the growth and the quality of aquaculture products. Thus, this approach can also be applied by incorporating transgenic microalgae harbouring vaccine into the feed as shown in figure 2. In figure 3, the developed transgenic microalgae bio-encapsulated in zooplankton or brine shrimp has also been explored by researchers for delivering vaccine to aquatic animals. Additionally, it should be made known that, the utilization of microalgae for vaccination can avoid the degradation of the antigen when delivered to the fish gut. The cell wall and the cell membrane of the microalgae will become a protective layer making the microalgae to be a potential vaccine carrier [45]. A study conducted by Kwon et al. [47] showed that GFP genes were successfully inserted into the chloroplast of *Chlamydomonas reinhardtii,* and they were orally administered to the fish. Based on the result, the GFP genes were able to be detected in the blood and the intestines of the fish which indicates the expressed proteins were fully protected from degradation in the gut by the microalgae. Utilizing the microalgae as a vaccine delivery system, making itself as a feed providing nutrient with a potential to stimulate the immune response of the aquatic animals.

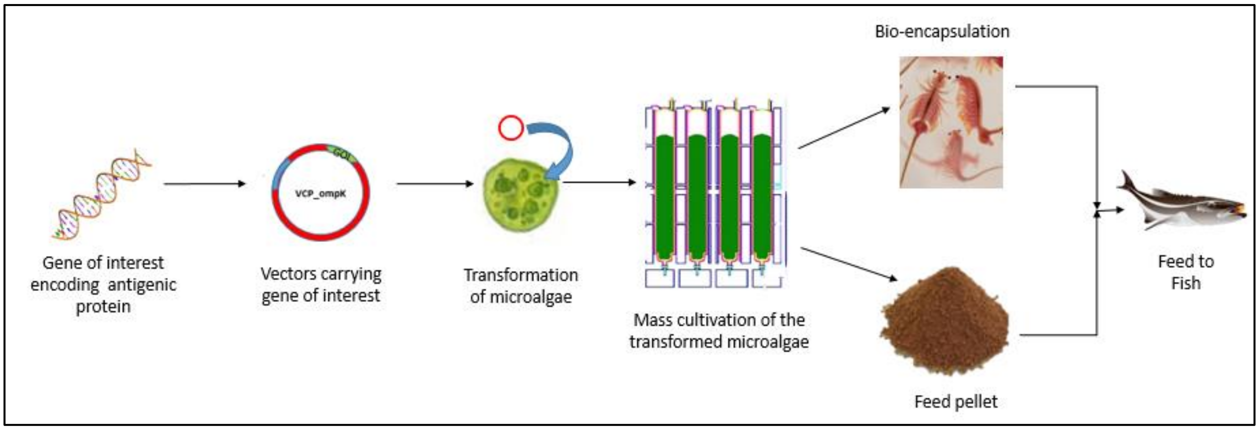

Figure 2. Oral vaccination of fish by using transgenic microalgae-formulated feed

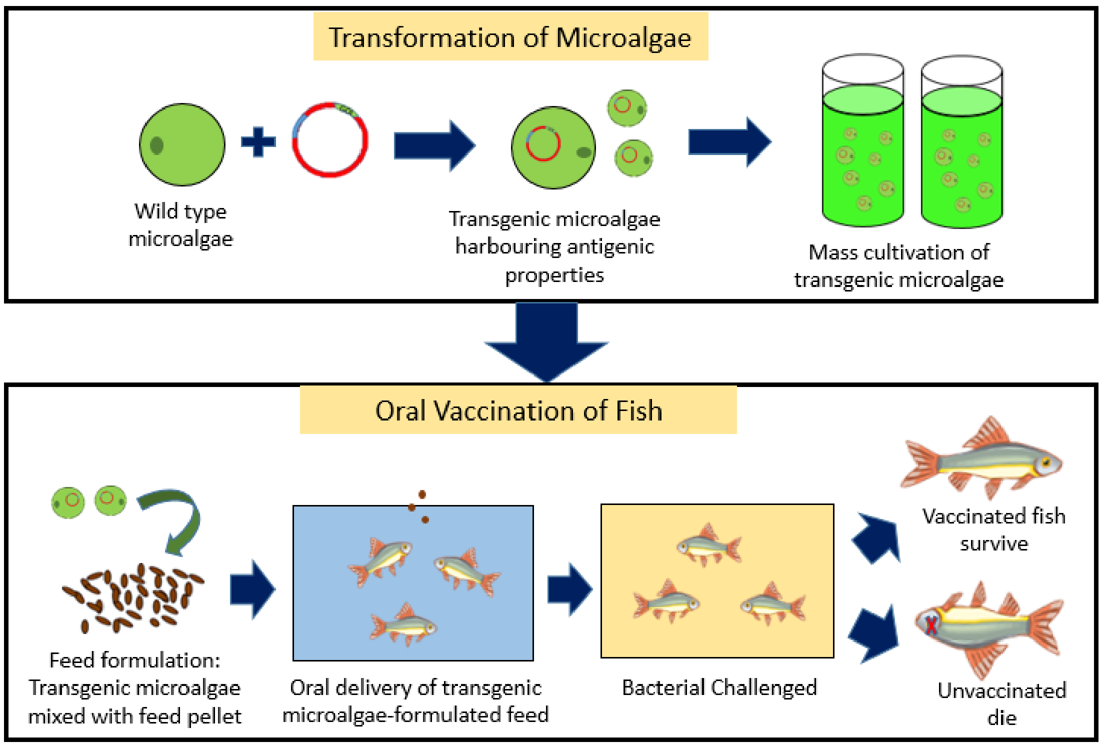

Figure 3. Transgenic microalgae being incorporated into the feed and bio-encapsulated with *Artemia*.

Many studies related to oral vaccination using transgenic microalgae to control various diseases in aquatic animals have been explored. One study reported by He et al. [48] showed that *Chlorella* sp. was successfully transformed by integrating two different genes, *Scy* and *PC-Heps* genes into the microalga. In this study, the fused genes were successfully expressed and capable of protecting the black sea bream (*Sparus microcephalus*) and hybrid grouper (*Epinephelus fuscoguttatus* (♀) × *Epinephelus lanceolatus* (♂)) from *Aeromonas hydrophila* infection via oral vaccination. The result showed that the survival rate of the vaccinated black sea bream and the vaccinated hybrid grouper after being infected by *A. hydrophila* are 80% and 55% respectively which are much higher than the control group. Other than that, Kiataramgul et al. [15] developed transgenic *Chlamydomonas reinhardtii* harbouring the white spot syndrome virus (WSSV) VP28 viral envelope protein. The transgenic microalga was incorporated into the feed and was administered orally to the shrimp which resulted in an 87% survival rate of the shrimp after being challenged with WSSV. Besides, Charoonnart et al. [49] successfully transformed the chloroplast of the *Chlamydomonas reindhartii* for expressing the antiviral dsRNA. The transformed microalga was then incorporated into shrimp feed and the relative percentage survival of the shrimps fed with the dsRNA expressed algal cells was observed to be 50% at 8 day-post infections, whereas only a

15.9% survival rate was observed in the control group. Based on the RT-PCR analysis, the result indicated the infection rate in the vaccinated shrimp is much lower than in the control groups. Feng et al. [50] have successfully expressed the heterologous expression of VP28 in *Dunaliella salina* and cyanobacteria *Anabaena* sp. The transgenic microalga was then incorporated into the commercial feed, and the results showed that the survival rate of the vaccinated crayfish is 59%. Meanwhile, the survival rate of the control group is 0%, and this shows that the VP28 protein expressed in the microalga has the potential to fight against the white spot syndrome virus (WSSV) disease.

All the above research studies discuss the administration route of vaccines by incorporating transgenic microalgae into the feed. The studies demonstrated the potential of the recombinant protein expressed that the genetically modified microalgae can protect aquatic animals from various infectious diseases even though they are administered orally. Also, the survival rate of different aquatic animals shows that the oral delivery of transgenic microalgae plays an important role as a potential approach for improving the efficiency of oral vaccination. Meanwhile, research studies on preparing oral vaccines using brine shrimp (*Artemia nauplii*) or phytoplankton are still lacking. Thus, more future work needs to be explored in preparing and delivering transgenic microalgae via oral vaccination to the aquatic animals.

Challenges in Fish Oral Vaccination using Transgenic Microalgae

Developing stable transgenic microalgae is significant especially if they are being utilized for producing in high value biochemical compounds in the industrial sector. The production of stable transgenic microalgae and cultivating them on a large scale is quite challenging. The development of robust transgenic microalgae has led to improvements in photosynthetic efficiency, the productivity of the sustainable compound and the development of new useful products. The method for transforming microalgae needs to be established by the biotechnologist to produce robust transgenic lines. Biotechnologists have used various techniques for transforming the microalgae by targeting three main genomes in the cells such as the nucleus, chloroplast, and mitochondrial genome [51,52]. Generally, most enzymes that correlated to the secondary metabolism are coded in the nuclear genome and only certain enzymes are coded in the chloroplast [53]. Thus, these genomes can be engineered for obtaining any desired products that can be useful for synthesizing biochemical compounds. Even though various approaches have been discovered for delivering foreign DNA to microalgae, there is no assurance that every transformed microalga can grow well. Many factors can affect transgene stability and microalgae are also known as species-specific which its molecular genetic mechanism can function differently in each species. Thus, new advanced molecular biological tools and genetic engineering technology must be upgraded for obtaining more stable transformed microalgae.

Other than that, the biomass production of the transgenic microalgae can also be challenging as improper management of the transgenic microalgae production can cause harm to the animals, the environment as well as humans. The engineered microalgae also behave differently from the wild type microalgae which becomes one of the reasons for protecting the transgenic microalgae from contaminating the wild type microalgae, and it can also affect other phytoplankton community in the water [54]. The presence of the altered gene microalgae in nature has the potential to cause interbreeding, competition between the natural species and the occurrence of horizontal gene transfers of the recombinant gene to other microorganisms which increases the risks to the ecosystem impacts [55]. All these issues become the reasons why open cultivation is not a suitable method for biomass production of transgenic microalgae. However, in industrial sectors, many producers prefer to cultivate the transgenic microalgae in an open pond instead of cultivating the organism in an enclosed bioreactor system [54]. This implementation can cause many potential problems as the release of the transgenic organism into the surrounding ecosystem is inevitable. Besides, the utilization of transgenic microalgae in the production of feed and food industry sectors need to be examined carefully. Thus, proper regulation and enforcement for ensuring the biosecurity of production of the transgenic microalgae is needed. The regulations and the policies can be followed by all researchers or producers that have the intention of developing and making use of the engineered microalgae on a large scale.

Conclusion

The aquaculture industry is growing rapidly and increasing in demands. However, it is currently facing many obstacles and challenges for sustainable aquaculture production. The outbreak of diseases can severely harm the aquaculture industry and cause billion-dollar losses. Additionally concerned, if there is no action taken for controlling the diseases, food scarcity is more likely to occur due to the large population in the world. Therefore, the initiative for taking control measures and planning the prevention methods are recommended as the strategies will help to sustain the economic value in aquaculture sectors. In the development of vaccines for aquatic animals, various technologies have been invented. These alternatives have been proven to be much safer and quite efficient. Many researchers put a major interest in exploring the potential of performing the oral vaccination as this method is much more practical and suitable for vaccination on large scale and smaller size of aquatic animals. The invention of preparing the oral vaccine by incorporating the transformed microalgae into the feed also has been discovered and explored. Additionally, the advanced application of utilizing the transgenic microalgae as a vaccine led to other alternatives for preparing a potential oral vaccine that can control the disease. However, despite the current progress and advancements, more research is still needed for establishing this technology and discovering other applications for preparing effective oral vaccines so that it can be readily adopted and practiced in the aquaculture industry sectors.

Conflicts of Interest

The authors declare no conflict of interest.

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