



Applications of Marine Bacteria in the Aquaculture Industry for Improving Water Quality and Treating Microbial Attack

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ABSTRACT

Marine bacteria are one of the most important sources of novel enzymes (such as lipase, chitinase, etc.) in the domain of industry. The importance of biofilm-forming bacteria for heavy metal bioremediation has been previously described in many studies. Some bacterial processes are included in heavy metal metabolism and detoxification. Because the expenses of the gas and oil industries, aquaculture, desalination plants, maritime transport, and other industries are so high, numerous solutions for preventing biofilm formation and cleaning contaminated surfaces have been developed. Biological filters might be a viable option for producing high water quality while conserving water in a recirculation aquaculture system. In a recirculating aquaculture system, the biofilters can remove nitrate, nitrite, and ammonia from wastewater, particularly through denitrification and nitrification processes, which are the objects of biological wastewater treatment. Probiotics are increasingly being utilized as a supplement in aquaculture to improve the quality and safety of protein output. Probiotics have been applied to rearing water, complicating the concept by using terminology, such as biocontrol, bioremediation and bioaugmentation. They can be utilized in aquaculture in a variety of ways, including direct immersion in water, feeding, or injection, and they can be singularly utilized or in combination. The use of bacterial aggregated or kept together in a medium with particle organic matter is known as bioflocculation technology, which improved water quality and boosted aquaculture growth. On the other hand, the production of nanoparticles is an important tool for inhibiting biofouling, and it is used in aquaculture to maintain and preserve water quality and reduce economic losses.

INTRODUCTION

The α -Proteobacteria are the most common category of microorganisms in the marine environment, as they are more acclimated to the changing conditions of marine environments. Marine bacteria are thought to represent new sources of a vast array of active biological compounds. Over the last few decades, the research and discovery of new drugs derived from marine bacteria have played a significant role. However, marine bacteria can cause serious problems, including biofouling and bacterial fish illnesses (bacterial pathogenicity). Bacteria, like all living organisms, possess defensive strategies and immune systems. Antibiotics, bacteriocins, lysozymes, siderophores, proteases, and/or hydrogen peroxide, as well as

the change of pH values are antagonistic agents, and they serve as defensive mechanisms (**Oren&Garrity, 2021**).

Marine bacteria are one of the most important sources of novel enzymes in industry. Lipase, chitinase, protease, α -amylase, cellulases, agarase, and galactosidase are some of these enzymes. Industrial enzymes are produced by *Enterobacter* sp., *Pseudomonas* sp., *Pseudoalteromonas* sp., *Aeromonas* sp., *Klebsiella* sp., *Moraxella* sp., *Bacillus* sp., *Flavobacterium* sp., *Vibrio* sp., *Streptomyces* sp., *Psychrobacter* sp., *Listonella* sp. and *Marinobacter* sp. The group of Gram-positive Actinobacteria has produced several bioactive compounds including antibiotics, while Gram-negative bacteria have received less attention. Actinomycetes are the most important source of bioactive natural compounds in general. Bacteriocins are efficient proteins, and antimicrobial peptides can be found in almost all bacterial species examined thus far. Researchers are most concerned about bacterial bacteriocins because of their potential as antibiotics and probiotics in marine aquaculture and the seafood sector (**Ghalet al., 2013**).

Biological filters might be a viable option for producing high water quality while also conserving water in a recirculation aquaculture system. In a recirculating aquaculture system, various types of biofilters have been used. These biofilters include fluidised bed filters, trickling filters, revolving filters, bead filters and submerged filters. Heterotrophic microorganisms and nitrifying bacteria make up the biofilter. They can remove nitrate, nitrite, and ammonia from wastewater, particularly through denitrification and nitrification processes, which are the object of biological wastewater treatment (**Chaudhary et al., 2003**).

Probiotics is a term that refers to bacteria that help other organisms thrive by promoting their health. In place of antibiotics and chemotherapy, probiotics are increasingly being used as a supplement in aquaculture to improve the quality and safety of protein output. Many researches have reported using probiotics in rearing water, complicating the concept by using terminology like biocontrol, bioremediation, and bioaugmentation (**Nisar et al., 2022**). Recent studies have demonstrated that fish pathogenic bacteria in live feed can be controlled by probiotics, and that the mortality of infected fish larvae can be significantly reduced by probiotic bacteria. However, the successful management of the aquaculture microbiota is currently hampered by our lack of knowledge on relevant microbial interactions and the overall ecology of these systems (**Bentzon-Tilia et al., 2016**).

Biofloc technology is mainly based on the principle of waste nutrients recycling, in particular nitrogen, into microbial biomass that can be used *in situ* by the cultured animals or harvested and processed into feed ingredients (**Kuhn et al., 2010**). Heterotrophic microbiota are stimulated to grow by steering the C/N ratio in water through the modification of the carbohydrate content in the feed or by the addition of an external carbon source in the water so that the bacteria can assimilate the waste ammonium for new biomass production. Hence, ammonium/ammonia can be maintained at a low and non-toxic concentration so that water replacement is no longer required. Biofloc technology enhances production and productivity through its contribution to supply good quality fish juveniles; the latter is

one of the most important inputs in the production. In addition, it contributes to improving fish production (Bossier and Ekasari, 2017).

Nanoparticle synthesis is an important tool for the inhibition of biofouling and is applied in the aquaculture field to maintain water quality and reduce the economic loss. Both intracellular and extracellular synthesis mechanisms of nanoparticles depend mainly on oxido-reductase enzymes. Nanoparticle creation is mostly dependent on oxido-reductase enzymes, both intracellularly and extracellularly (Negm *et al.*, 2018).

The goal of this review was to focus on the bioactivity of marine bacteria and their generated metabolites as relevant solutions for aquaculture problems, especially microbial pathogenicity and poor water quality, using a variety of methods such as biological filters, probiotics, the generation of biofloc and nanoparticles.

1. Valuable applications of marine bacteria for aquaculture treatment

1.1. Biological filters

Areerachakul (2018) explained that, a biofilter can be a useful tool for generating high-quality water and conserving water for recirculation aquaculture systems. A variety of biofilters have been used in a recirculating aquaculture system. Each biofilter has benefits and disadvantages that should be considered when designing a fish farm. Submerged filters, trickling filters, rotating filters, bead filters and fluidised bed filters are examples of common types, as illustrated in Table (1) and Fig. (1).

Table 1. Comparison among different biofilter types for aquaculture (Areerachakul, 2018)

Filter type	Advantages	Disadvantages
Submerged	High loading rate and high velocity Fixed media low head loss Easy to operate Low capital cost	Media can be clogged Aeration required Potential “dead zone” Large volumes Frequent backwashing
Trickling	No aeration required Low head loss Easy to operate Low capital cost	Sloughing bacteria High media cost High pumping cost
Rotating Filter	No aeration required Fixed media Low head loss Self cleaning	Limited surface area High maintenance cost High operating cost
Bead	Compact High efficiency Solid removal Short detention time High efficiency quality	Skilled operation High maintenance Expensive media High energy consumption Aeration required
Fluidised bed	Compact Low media cost	Skilled operation High energy consumption High maintenance

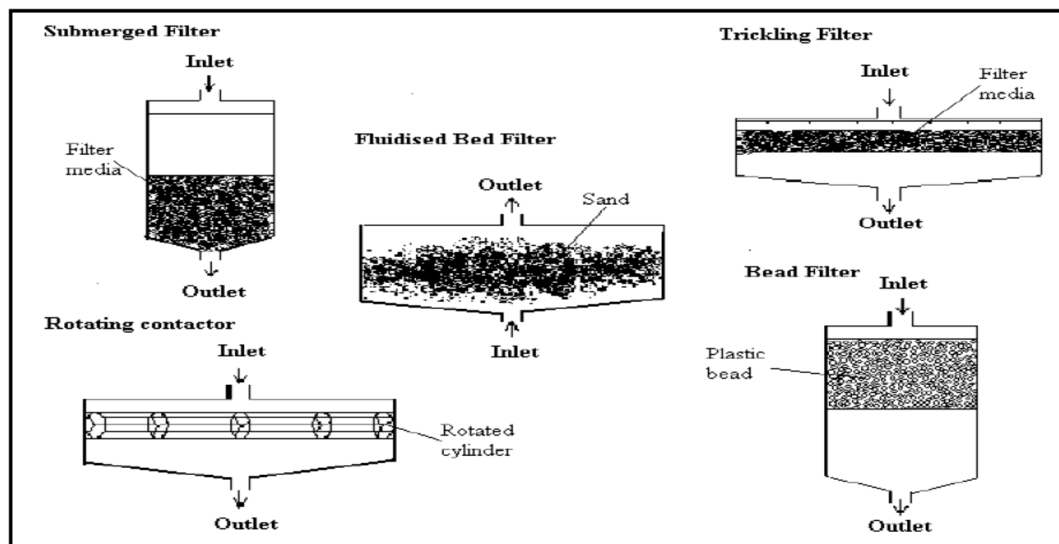


Fig.1.Main types of biofilters in aquaculture recirculation(Areerachakul, 2018)

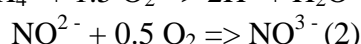
The use of biofilters is common in closed aquaculture systems such as recirculating aquaculture systems (RAS). Many designs are used, with different benefits and drawbacks; however, the function is the same which is reducing water exchanges by converting ammonia to nitrate. Ammonia (NH_4^+ and NH_3) originates from the brachial excretion from the gills of aquatic animals and the decomposition of organic matter. As ammonia-N is highly toxic, it is converted to a less toxic form of nitrite (by *Nitrosomonas* sp.) and then to an even less toxic form of nitrate (by *Nitrobacter* sp.). This "nitrification" process requires oxygen (aerobic conditions) without which the biofilter can crash. Furthermore, as this nitrification cycle produces H^+ , the pH can decrease which necessitates the use of buffers such as lime(Chaudhary *et al.*, 2003).

As a result, the target of biological wastewater treatment is to remove ammonia, nitrite, and nitrate from wastewater, particularly through nitrification and denitrification (Fdz-Polanco *et al.*, 2000). Heterotrophic bacteria convert complex organic nitrogen molecules to simpler organic compounds, which are then transformed to ammonia (the direct inorganic result). Ammonia will be oxidised by several autotrophic bacterial genera. Two bacteria are involved in the conversion of ammonia to nitrite and then to nitrate. Some bacteria, especially from the *Nitrosomonas* genus, perform the first step, oxidizing ammonia (NH_4) to nitrite (NO_2). Bacteria of the genus *Nitrobacter* perform the second step, oxidizing nitrite (NO_2) to nitrate (NO_3). Heterotrophic bacteria rely on organic substances as energy sources, whereas autotrophic bacteria rely on inorganic compounds. Heterotrophic bacteria are increasing considerably more than *Nitrosomonas* and *Nitrobacter*. As a result, trying to combine the conversion of organic compounds and inorganic forms of nitrogen in the same biological filter causes heterotrophic and autotrophic bacteria to compete for growing space (Wheaton *et al.*, 1994).

Furthermore, the presence of organic material will disrupt the biofilter's nitrification rate (Zhu & Chen, 2001). Heterotrophic bacterial growth is stimulated by easily biodegradable organic matter in wastewater, and these bacteria

subsequently compete with autotrophic bacteria in the film-making process, oxygen utilization, nutrient uptake and space utilization. In biological filters, there is a substantial correlation between the C/N ratio and heterotrophic abundance (**Ling & Chen, 2005**). Increases in the C/N ratio lowered nitrification effectiveness, suggesting that fast-growing heterotrophs in the biofilm's upper layers limited oxygen availability and ammonia diffusion to the deeper levels, where the slow-growing nitrifiers were most likely found. Furthermore, heterotrophic populations produce large amounts of bacterial biomass, which can clog filters and impair nitrification capability. The heterotrophic layer, on the other hand, could benefit nitrification by sheltering nitrifiers from detachment and grazing (**Michaud *et al.*, 2006**).

When a synthetic medium is mixotrophic (i.e. comprises both ammonia and organic materials) in an experimental case, the biofilm segregates into separate layers (**Hagopian & Riley, 1998**). When a series of biofilters are utilized, bacterial competition is reduced (**Hargrove *et al.*, 1996**). Only anaerobic circumstances allow for denitrification from NO_3 to NO_2 (nitrate to nitrite), NO_3 to N_2O or N_2 (nitrate to dinitrogen oxide or nitrogen gas, respectively), and N_2O to N_2 . However, in a well-aerated filter, this reaction is not predicted. Equations 1 and 2 show basic chemistry conversion happening in a nitrification filter:



Reactions 1 and 2 released energy for *Nitrosomonas* and *Nitrobacter*. They use this energy to complete their life cycle. These reactions require oxygen and generate hydrogen ions (reducing pH) as well as nitrite as an intermediate product (**Wheaton *et al.*, 1994**). However, Fig. (2) explains a side of these reactions and pathways.

1.1.1 Factors affecting biofilter performance

The pH level. The optimum pH range for nitrification is 6-9, although the optimum range for the specific filter is narrower. Biofilter bacteria, which are vital in the decomposition of waste products, are ineffective over a large pH range. Biofilter bacteria thrive in a pH range of 7 to 8. In fact, the activity of the NH_3 -oxidizers was not inhibited by pH 6. The pH adaptation of the bacterial species in the filter determines the specific range. The pH is usually kept in the lower optimal range because the percentage of unionised ammonia increases as the pH rises. Ammonia stress in nitrification can be reduced by lowering pH to the lower optimal limit (**Chen *et al.*, 2005**).

Oxygen. When oxygen levels are insufficient for nitrification bacteria to function, the rate of nitrification slows down; yet, some bacteria may remove both ammonia and nitrite simultaneously under anoxic conditions (**Sliekers *et al.*, 2002**). Some variables such as temperature, organic concentration in the filter feed water and bacteria biomass play a role in limiting oxygen concentration. Filters contain both heterotrophs and nitrifiers, thus the filter that receives culture water must have a large percentage of heterotrophic bacteria in the first phase since the nitrogen cycle begins with the

conversion of an organic component to ammonia (Van Gorder, 1994). The population of *Nitrosomonas* will then grow in the filter, and they will remove ammonia. *Nitrobacter* will then grow simultaneously with the increase in nitrite in the biofilter. The order of bacterial strains in the filter showed that heterotrophic bacteria use oxygen first, followed by *Nitrosomonas*, and finally *Nitrobacter*. Adjusting oxygen concentrations at least 2mg/L in wastewater nitrification filters and aquaculture operations will be safer. As a result, aquacultural nitrification filters are frequently limited by ammonia or nitrite rather than oxygen. In this situation, rising oxygen levels will have a minor effect on filter performance until oxygen levels reach the limiting factor (Chaudhary *et al.*, 2003). In any case, Figs.(3,4) present a biological filter with a ball and an aeration system for the biological filter, respectively.

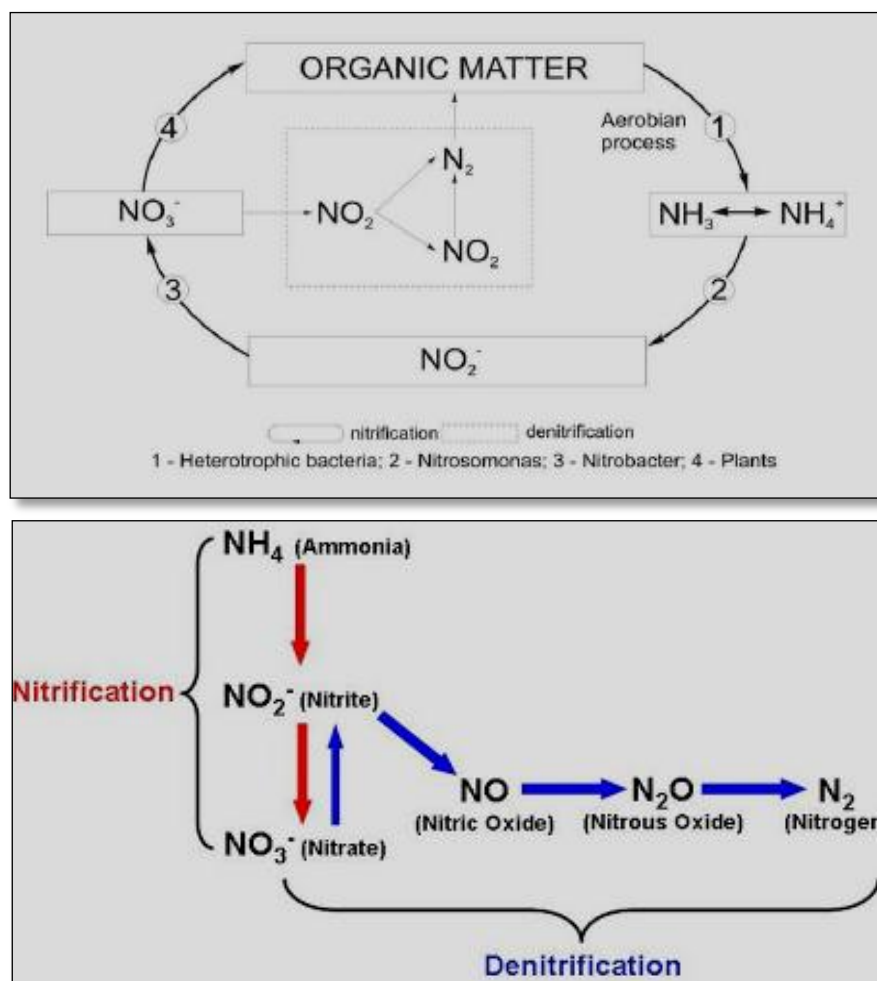


Figure (2) Nitrification and denitrification processes taking place during the biological filtration process of water (E3S Web of Conferences, 2021).

Fig.3. Biological filter with a ball (Lazaet *al.*, 2021)



Fig.4. Aeration system of the biological filter (Lazaet *al.*, 2021)



Temperature. There are a variety of viewpoints on the precise effect of nitrification. These differing viewpoints indicated that nitrification bacteria can adapt to a wide range of temperature conditions, including those found in filters. Temperature had little effect on the nitrification rates of fixed-film filters, with temperatures ranging from 14 to 27°C when dissolved oxygen was limited. During saturation, dissolved oxygen concentration decreases with increasing temperature; the temperature effect on the nitrification rate diminishes as biofilm bacteria development improves (Zhu & Chen, 2002).

Types of filter media. Filter media represent a solid material placed within the filter that provides bacteria with a surface area to thrive on. Biofilters require an adequate medium that can create an environment conducive to the growth of autotrophic nitrifying bacteria. Sand and stone, as well as plastic media are among many forms of media used in nitrification systems. The selection of media types is usually based on some factors (Ridha & Cruz, 2001).

Ammonia/nitrite concentration. The nitrification filter process will become lethal if the ammonia and/or nitrite concentrations are too high. Compared to the inhibitory concentration for *Nitrobacter* (10-150 mg/L vs. 0.1-1.0 mg/L), unionised ammonia inhibits *Nitrosomonas* at a greater concentration. Nitrite-nitrogen concentrations in a recirculating system with a mature biofilter should not exceed 10 mg/L for lengthy periods of time, and in most situations should remain below 1 mg/L (Losordo *et al.*, 1998). Furthermore, rather than filtering nitrification in municipal wastewater, nitrification aquaculture focuses on reducing ammonia and nitrite concentrations. The

presence of ammonia and/or nitrite in an aquaculture nitrification filter might indicate the rate of nitrification(**Chaudhary et al., 2003**).

1.2.Probiotics in aquaculture field

Aquaculture's global expansion needs the introduction of new biotechnologies and innovations to boost production costs, especially in places where fish are in short supply.FAO (2014) stated that,aquaculture productsare a very valuable source of essential protein and micronutrients needed for global balanced nutrition and overall health. With a predicted annual population growth rate of 1.6%, a probable food supply shortage, particularly in protein sources, is foreseen.To meet the global need for fish protein, aquaculture is one of the most stable and cost-effective options for long-term fish supply (**Eyo&Akanse, 2018**). However, an environmentally sustainable aquaculture production route is required. The use of probiotics as a supplement in aquaculture is gaining popularity to improve the quality and safety of protein production without using antibiotics or chemotherapy.Probiotic supplementation is an example of biotechnology that has beneficial impacts on various aspects of aquaculture. Probiotics is a term that refers to bacteria supporting the health of other organisms (**Balcázaret al., 2006**).

Probiotics are live microorganisms providing health benefits to the host when administered in appropriate proportions, according to the World Health Organization (**FAO/WHO, 2002**).The use of probiotics in the rearing water was described by **Lauzon et al. (2014)**, complicating the definition process by including terminology like biocontrol, bioremediation and bioaugmentation.The term "biocontrol agent" was recommended in the studies of**Maeda et al. (1997)** and **Moriarty (1997)** to describe antagonistic microorganisms to aquatic pathogenic microbes that do not exist in the host's gastrointestinal system. The term "bioremediation or bioaugmentation" on the other hand, is the utilization of aquatic microorganisms to improve waterquality by breaking down contaminants or wastes. **Merrifield et al. (2010)** proposed,according to these explanations, a classic and broader definition of probiotics suggested for use in aquaculture as 'any microbial cell introduced via rearing water or diet that benefits the host, farmer, or consumers of fish, which is realized, partially at least, by enhancing the microbial balance of the host`.

Furthermore, they fulfil some of the recently expanded criteria for being suited probionts in aquaculture(**Merrifield et al. 2010**), such as the ability to colonize intestinal mucus and exhibit resistance to low pH and bile salts. These traits imply a focus on the gastrointestinal tract however, in fish larvae the gastrointestinal tract does not represent an isolated microbiome as such, but the entire larva-associated microbiota is rather an extension of the microbiota of the surrounding environment. Probiotics that exert their probiotic effect in association with gills, skin, feed, water or abiotic surfaces are likely equally, or more relevant in larviculture.

Candidate probiotics that could potentially assert their biological control effects in the extended larviculture environment include species of the genus *Bacillus*, which have been shown to reduce the expression of virulence factors in *Aeromonas hydrophila* through quorum-sensing inhibition, increasing the survival of

fish in challenge trials as well (Chu *et al.*, 2014). *Bacillus* strains have also been shown to exhibit probiotic effects in this setting, i.e. in brine shrimp cultures (Niu *et al.*, 2014). Members of the *Roseobacter* clade have shown great potential as antagonists of fish pathogens in the marine larviculture environment including live feed cultures (Grothkjær *et al.*, 2016) and larvae of turbot and cod (D'Alvise *et al.*, 2016).

Their probiotic effect does not probably rely on quorum-sensing inhibition, but rather on the production of one or more antimicrobial compounds including tropodithietic acid (TDA), indigoidine, tryptanthrin and dicyclic peptides (Bentzon-Tilia & Gram, 2016). Furthermore, the roseobacters seem to be indigenous to the larvi- and aquaculture setting with numerous strains isolated from these environments (Grothkjær *et al.*, 2016). This suggests that the roseobacters can establish themselves in the systems, and that they do not have a negative impact on the fish larvae directly. One of the challenges in the implementation of these probiotics is; however, assessing the impact of the probiotics on the system as such, which remains unknown. The mode of action of TDA was recently shown to be disruption of the proton motive force (Wilson *et al.*, 2016). This explains earlier reports, which noted that TDA is effective against a broad range of Gram-positive and Gram-negative bacteria (Porsby *et al.*, 2011). Whether the application of TDA-producing roseobacters can have undesirable effects on the aquaculture microbiota has to be investigated in comparative studies of microbial community composition and function. An additional concern is how fish pathogens will interact and evolve with added probiotics over time (Bentzon-Tilia & Gram, 2016).

1.2.1 Characteristics of promising probiotics

There are several characteristics for the most potent probiotics. They are very essential as they should be able to have a positive impact on the host animal, such as disease resistance or improved growth; they should not be harmful or pathogenic; they must exist as a significant number of viable cells; they must be able to metabolise and survive in the gut, as well as withstanding organic acids and low pH; they must be stable and capable of long-term viability in both field and storage circumstances (Michael *et al.*, 2014).

1.2.2. Mode of action of probiotics in aquaculture

Many researches have been interested in the physiological, growth-promoting and immunological responses of fish after being exposed to probiotic supplements (Akinbowale *et al.*, 2006). In aquaculture, there are numerous ways to achieve probiotics (Verschuere *et al.*, 2000), comprising competition and changing pathogen enzymes to exclude pathogenic bacteria, improving water quality and immune response and feeding the host.

1.2.3. Criteria for probiotic selection

In the study of Merrifield *et al.* (2010), the authors defined two types of criteria that are used to select probiotics for use in aquaculture, which are namely favorable and essential criteria. In essential criteria, probiotics must be able to withstand bile salts and low pH. Regarding the host species, aquatic organisms and

human consumers, they must not be pathogenic. Antibiotic resistance genes encoded by plasmids must be absent. While in favorable criteria, the probiotics should be native to the host or the rearing environment. They should also be capable of colonizing the epithelial surface of the intestine and able of attaching to and/or growing within the intestinal mucus. They must be safe to be used as a feed additive as well as possessing favorable growth characteristics (e.g., quick doubling time, short lag time, and/or growth at host rearing temperatures). The probiotics should produce vital extracellular digestive enzymes, such as cellulase and chitinase to easily use cellulose or chitin-rich components if they are incorporated into the diet. Finally, probiotics should show potent antagonistic activity. On the other side, there are four types of probiotics, which are presented in Table (2) (Annam, 2010) and (Table 3).

Table 2. The diverse types of probiotics (Rao, 2010)

Type of probiotics	Description
Non-viable probiotics	These are dead.
Freeze-dried probiotics	These will die rapidly upon leaving refrigeration.
Fermentation probiotics	These are produced through dermentation.
Viable probiotics	These are live with guaranteed number of organisms, have a protocol for counting and being very stable and efficacious.

1.2.4. Administrative methods

Probiotics can be employed in aquaculture in a variety of ways, including direct immersion in water, feeding and injection (Michael *et al.*, 2014), and they can be used singularly or in combination (Hai, 2015). Fig. (5) shows the different routes for probiotic administration.

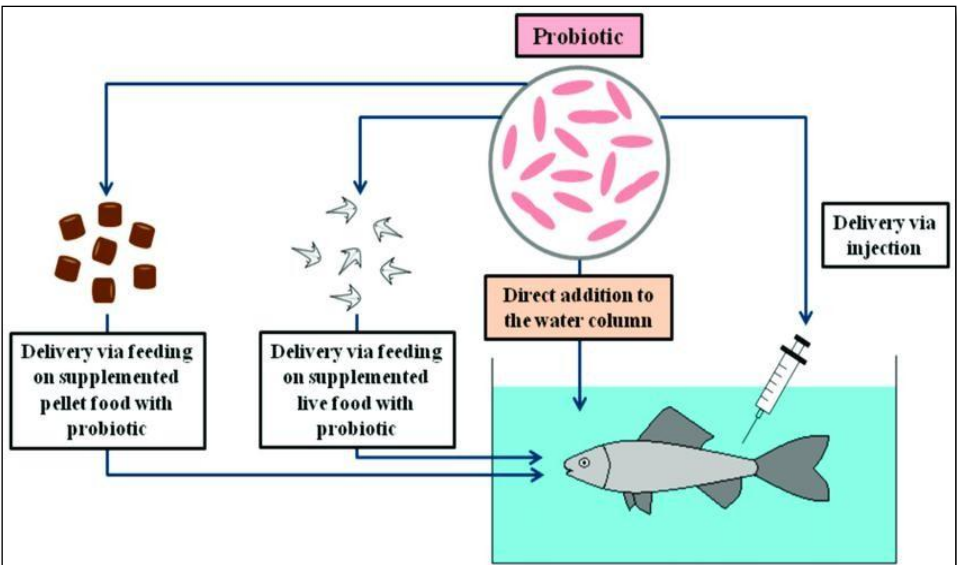


Fig. 5. Different routes for administration of probiotic (Jahangiri & Esteban, 2018).

Feed additives, water additives and injection. Integration into the feed (92.8%), direct integration in live food (1.6%) and water (4.8%) are the most common methods for managing probiotics (Melo *et al.*, 2020). Some bacterial species can be taken by mouth, as additive to feeding pellets, or encapsulated into live feedstock (Das *et al.*, 2017). In aquaculture, probiotics, which include extracted materials and bacterial strains, are often used as feed additives (Tuanet *et al.*, 2013).

Feed additives containing the probiotic *Lactibacillus plantarum* CR1T5 improved non-specific immunity and growth performance in black eared catfish (Silarudeet *et al.*, 2019). The supplementation of probiotic *Bifidobacterium* strains enhanced rainbow trout fry development and nutrients uptake (Sahandiet *et al.*, 2018). Probiotics can also be used as water additives (Gupta *et al.*, 2016). In the sea bass, for example, probiotic *Vibrio lentus* was given at a concentration of 10^6 CFU/ml, resulting in changes in gene expression and immunological response (Schaeckel *et al.*, 2017). Furthermore, injections of the probiotic *Enterobacter* sp. strain C6-6 via intraperitoneal and intramuscular routes boosted immunity in rainbow trout (Laptraet *et al.*, 2014) (Fig.5).

Single /or combination usage of probiotics. Probiotics can be taken solely or incorporated with other supplements (Shifat, 2018). Many aquaculture studies have focused on the use of single probiotics, but a combination of probiotics is more beneficial since it has greater sensitivity to

pathogens and activity against a wider range of aquaculture animals (Pannuet *et al.*, 2014). When administered in the feed of catfish *Clarias* sp., a blend of *Pediococcus pentosaceus* E2211 and *Bacillus megaterium* PTB 1.4 produced better results than solo probiotics (Hamkaet *et al.*, 2020). However, in the Nile tilapia (*Oerochromis niloticus*), the mixture of *Bacillus velezensis* H3.1 and *Lactobacillus plantarum* N11 enhanced a greater percentage of survival (58.33%) than the single probiotics (Doan *et al.*, 2018). The fish species *Catla catla* showed more immunity, nutrient retention, and growth performance when plant extract *Mentha piperita* and probiotic *Bacillus coagulans* were used in combination, compared to a single treatment (Bhatnagar & Saluja, 2019).

Dosage quantity. The appropriate dosage of the probiotic should be supplemented for maximum efficacy. The physiological status of the fish species as well as the probiotic species, all play a role in determining appropriate probiotic levels (Hai, 2015). The growth of rainbow trout was improved when they were dosed with probiotic *Bifidobacterium* strains at a concentration of 1×10^7 CFU/g (Sahandiet *et al.*, 2018). Dosing is crucial when it comes to adding beneficial nutrients to diets. Recently, Chowdhury *et al.* (2020) reported that, by adding more than 0.2 % of dietary commercial probiotics to pabda catfish (*Ompok pabda*), weight gain was reduced. This study found that higher concentrations may not be able to preserve the entire physiology of fish and may cause disruptions in lipid and carbohydrate metabolism.

1.2.5. Vital roles of probiotics in aquaculture

Improving feed utilization. Probiotics have been shown in many studies to affect enzymes, and thus improving feed consumption. *Lactobacillus pentosus* was added to the meal of white shrimp *Litopenaeus vannamei*, which improved feed utilization (Zheng & Wang, 2016). Heat-killed *Lactobacillus plantarum* added at 1000, 100, or 50 mg/kg for 12 weeks increased the activities of protease, lipase and amylase in the Nile tilapia (Dawood *et al.*, 2019). Furthermore, the addition of *Lactobacillus plantarum* at concentrations of 10^9 , 10^8 and 10^7 CFU/gm improved the activity of alkaline phosphatase, amylase, lipase and protease in crayfish, *Astacus leptodactylus* (Valipouret *et al.*, 2019).

Enhancement of immune response. Probiotics can help aquaculture species improve a variety of immunological parameters. Probiotics can help to prevent pathogen infection by boosting the host's immune system and stimulating cellular immunity (Hamkaet *et al.*, 2020). The presence of *Bacillus velezensis* and *Lactobacillus plantarum* in the host increased innate immune variables, viz., serum peroxidase activity, serum lysozyme, skin mucus lysozyme, respiratory burst and phagocytosis in the Nile tilapia (Doan *et al.*, 2018). The addition of *Bacillus pumilus* to the juvenile golden pompano, *Trachinotus ovatus*, increased total protein and lysozyme activity in the fish (Liu *et al.*, 2019).

After 15 days of feeding, dietary addition of *Lactobacillus plantarum* at 10^8 and 10^9 CFU/g considerably enhanced complement activity, while it increased dramatically the respiratory burst activity and lysozyme activity in black eared catfish, *Pangasius larnaudii* after 30 and 45 days of feeding, respectively (Silarudeeet *et al.*, 2019). *Bacillus subtilis* was added to the diet of white shrimp, *Litopenaeus vannamei*, at doses of 10^9 and 10^7 CFU/kg, which increased immunity through phagocytic activity (Kewacharoen & Srisapoome, 2019). It was shown that incorporation of *Pediococcus pentosaceus* in the meal of the common carp increased antibacterial activity, hematocrit, white blood cells, and red blood cells, as well as lysozyme, protease, and total serum antibody levels (Ahmadifaret *et al.*, 2019).

Improvement of water quality. Probiotics' direct decomposition or uptake of organic materials in the water ameliorates water quality. Probiotics can decompose the residual food ingredients, such as prawn or fish excreta, as well as other organic materials to nitrate, phosphate and CO_2 , thus boosting the nutrition cycle and preserving water quality (Annam, 2010). After adding mixed *Bacillus* probiotics to the white shrimp rearing water, considerable improvements in water quality, comprising levels of nitrite, pH, and ammonia were observed (Nimratet *et al.*, 2012). Supplementing shrimp culture rearing water with probiotic *Bacillus subtilis* at a concentration of 10^3 - 10^5 CFU/ml considerably reduced total ammonia and ameliorated water quality (Kewacharoen & Srisapoome, 2019). Furthermore, adding probiotics *Pediococcus acidilactici* and *Bacillus cereus* (10^6 CFU/ml) to pond water lowered efficiently biological oxygen demand, ammonia and nitrate levels (Khademzadeet *et al.*, 2020).

Stress tolerance.Supplementation of probiotic improves the stress tolerance of aquaculture species. *Lactobacillus plantarum* supplementation, when compared to the control meal, boosted fish tolerance to ammonia and induced a lower increase in cortisol (a stress hormone) (Nguyen *et al.*, 2019).The addition of 0.2% of commercial probiotics to the diet of pabda catfish resulted in remarkable tolerance to high saline water (Chowdhury *et al.*, 2020).

Growth promoter.One of the expected concerns is the direct influence of bacterial probiotics on fish growth performance through the provision of nutrients or a direct improvement in nutrition intake (Joel *et al.*, 2020). The production of vitamins, improvement of gut shape, as well as the increase in digestive enzymes and the breakdown of complex components may all contribute to improving the growth performance (Doan *et al.*, 2018). *Bacillus subtilis* added to the meal at 10^9 and 10^7 CFU/kg for five weeks increased effectively the growth of Pacific white shrimp (Kewacharoen&Srisapoom, 2019). Moreover, the addition of *Pediococcus acidilactici* and *Enterococcus faecalis* in the *Scylla paramamosain*'s diet of the mud crab increased specific growth rate and weight gain significantly (Yang *et al.*, 2019).Furthermore, feeding zebra fish (*Danio rerio*)with *P.acidilactici* increased their growth performance, without affecting their appetite (Ahmadifaret *et al.*, 2020).

It is worthy to mention that, probiotics are widely used in finfish aquaculture to stimulate growth.The addition of *B.pumilus*to the diet of *Trachinotus ovatus*, a juvenile golden pompano, enhanced weight gain and specific growth rate (Liu *et al.*, 2019).The inclusion of *B. circulans* PB7 in the meals promoted *Catla catla*'s growth (Bandyopadhyay& Mohapatra, 2009). While, the addition of *P.pentosaceus* and *B. megaterium*, either together or separately, improved the growth of catfish *Clarias* sp. (Hamkaet *et al.*, 2020). On the other hand, the supplementation of *P.pentosaceus* increased grass carp growth rates by changing the gut microbiome (Ahmadifaret *et al.*, 2019). The growth of the Nile tilapia, *Oreochromis niloticus*, was enhanced after four weeks of supplementation with 10^8 CFU/gm *Lactobacillus plantarum*(Zhait *et al.*, 2017).Recently, Chowdhury *et al.* (2020) elucidated that, when dietary probiotics were supplied, higher feed consumption and growth performance of *Ompok pabda* were detected as a consequence of increased digestive enzyme activity.

Increase of disease resistance.One major constraint in fish farming is the proliferation of pathogenic microorganisms and subsequent disease outbreaks. Outbreaks can have dire economic consequences for individual fish farmers, and in some cases, entire subsectors may be limited by the spread of infectious diseases (Verschuere *et al.*, 2000). Bacterial fish pathogens are in general considered the most important infectious microbes in aquaculture, and the industry goes to great lengths to reduce the number of pathogenic bacteria in their facilities. Besides the application of disinfectants and biocides, antimicrobials may be applied to treat infected fish, and unfortunately they are sometimes used as a prophylactic measure (Cabello, 2006).

Probiotic bacteria have the potential to generate chemical compounds that have a bacteriostatic or bactericidal effect on pathogenic bacteria in the host intestine.

For example, *Bacillus* sp. produced polymyxin and bacitracin, which increased disease resistance (Cruz *et al.*, 2012).

The use of the probiotic *Enterobacter* sp. enhanced the protection of rainbow trout, *Oncorhynchus mykiss*, against *Flavobacterium psychrophilum* (LaPtraet *et al.*, 2014). *Aeromonas veronii* and *F. sasangense*, two isolated gut autochthonous probiotic bacteria, improved disease resistance in common carp against *A. hydrophila* (Chi *et al.*, 2013). The supplementation of 10^7 cells/gm *L. garvieae* in the diet of the Nile tilapia for ten days increased resistance against *Staphylococcus aureus* pathogen (Abdelfatah & Mahboub, 2018). On the other hand, *Cromileptes altivelis* was supplemented with 10^6 , 10^8 , and 10^{10} CFU/g of *L. lactis* to increase resistance against *Vibrio harveyi* (Sun *et al.*, 2018). It was found that, supplementing rainbow trout with *Enterococcus faecalis* for thirty days increased resistance against *L. garvieae* pathogen (Banoset *et al.*, 2018). Antibacterial activity of the probiotic *P. pentosaceus* was also observed against *Edwardsiella tarda*, *A. sobria*, *A. veroni* and *A. hydrophila*, which are all common fish pathogenic bacteria.

1.3. Biofloc technology

The species *Nitrospira*, *Sphingomonas*, *Nitrosomonas*, *Acinetobacter*, *Nitrobacter*, *Bacillus*, *Pseudomonas*, *Rhodopseudomonas*, *Micrococcus* and *Cellulomonas* are among the heterotrophic beneficial microbial communities found in certain biofloc. In the biofloc system, these bacteria act as potential bioremediation agents, improving water quality and growth performance (Adel *et al.*, 2017) (Table 3 & Fig. 6).

In biofloc systems, the accumulation of particulate and dissolved organic matter is a common occurrence; nevertheless, high levels of heterotrophic bacteria effectively reduce the organic nitrogen and carbon levels in the system. As possible bioremediators, these heterotrophic bacteria generate a variety of metabolic enzymes that help in the safe removal of pollutants, either by transforming them to less hazardous or via direct annihilation (Dash & Das, 2012). For instance, Manan *et al.* (2017) conducted an experiment to assess the role of aggregating biofloc in the bioremediation procedure, which involved organic matter degradation and decomposition. The findings revealed that, heterotrophic bacteria belonging to the *Aeromonas* (*A. salmonicida* and *A. hydrophila*) and *Pseudomonas* (*P. aeruginosa*) families uptook the bottom organic matter of shrimp cultivation biofloc tanks.

Furthermore, after being converted through chemical processes, these bottom wastes aid in the production of high protein flocs that are used by cultivated shrimp (Manan *et al.*, 2017). It is crucial to note that, *Aeromonas* and *Pseudomonas* sp. may be pathogenic to shrimp species (Zhou *et al.*, 2019). Hence, the pathogenicity of these bacterial strains must be checked before considering them as beneficial microbes. Additionally, limited studies proposed combining bacteria (*P. stutzeri* LZ301/*Nitrobacter*/*B. subtilis*) with yeast (*Candida tropicalis* HH8) or microalgae (*Schizochytrium* sp.) to reduce total ammonium nitrogen, nitrite and nitrate concentrations, which help to preserve optimal water quality (Gao *et al.*, 2019).

Table 3. *Bacillus* species reported to be used in the process of natural bioremediation, disease tolerance, immunity and growth of aquaculture animals (adopted from **Kumar, 2021**)

Bacterial species	Target system	Bioremediation process	Immune response	Antimicrobial activity	Reference
✓ <i>Bacillus pumilus</i>	Biofloc water	Total ammonia nitrogen (TAN) concentration (-) after the 7 th week in common carp culture system	Lysozyme, respiratory burst and myeloperoxidase activity (+)	Survival against <i>Aeromonas hydrophila</i> challenge (+)	Dash <i>et al.</i> (2018)
✓ <i>Bacillus</i> sp.	Pond wastewater	Ammonium, nitrite and nitrate (-) in 4 days period	-	-	Naderi Samani <i>et al.</i> (2016)
✓ <i>Bacillus vietnamensis</i>	Pond wastewater	Total ammonia nitrogen (TAN) and nitrite concentration (-) in 5 days	-	-	Muthukrishnan <i>et al.</i> (2015)
✓ <i>Bacillus</i> sp. mixture	Shrimp culture water	Total ammonia nitrogen, nitrite and nitrate level (-) in 8 week- <i>Litopenaeus vannamei</i> culture	Expression of prophenoloxidase (proPO), peroxinectin (PE), lipopolysaccharide- and b-1,3-glucan-binding protein (LGBP) and serine protein (SP) (+)	Survival (80%) (C) as compared to control (40%) against <i>Vibrio harveyi</i> infection	Zokaie <i>et al.</i> (2014)
✓ <i>Bacillus amyloliquefaciens</i>	Sewage water	Total ammonia nitrogen (TAN) 93% (-) within 24 h	-	-	Yu <i>et al.</i> (2012)
✓ <i>Bacillus subtilis</i> , ✓ <i>Bacillus mycoides</i> ✓ <i>Bacillus licheniformis</i>	Recirculatory system water	Ammonium, nitrite, nitrate and phosphate levels (-) in recirculation tanks	-	<i>In vitro</i> antimicrobial activity against <i>Aeromonas hydrophila</i> (+)	Lalloo <i>et al.</i> (2007)
✓ <i>Bacillus subtilis</i> ✓ <i>Bacillus megaterium</i>	Recirculatory system water	Total ammonia nitrogen and chemical oxygen demand (COD) (-) in red parrot fish recirculation tanks	-	-	Chen and Chen (2001)

Kurniawan *et al.* (2020) also used 16S rDNA sequencing to evaluate the diversity and abundance of biofloc producing bacteria in river waters. The previous authors noticed that, seven bacterial phyla comprising *Proteobacteria*, *Cyanobacteria*, *Verrucomicrobia*, *Actinobacteria*, *Bacteroidetes*, *Chloroflex*, and *Planctomycetes* and 14 bacterial genera, including *Streptococcus*, *Staphylococcus*, *Bacillus*, *Neisseria*, *Lactococcus*, *Rhodococcus*, *Kocuria*, *Pseudomonas*, *Nitrospira*, *Rhodobacter*, *Sphingomonas*, *Burkholderia*, and *Acinetobacter* were capable of forming biofloc.

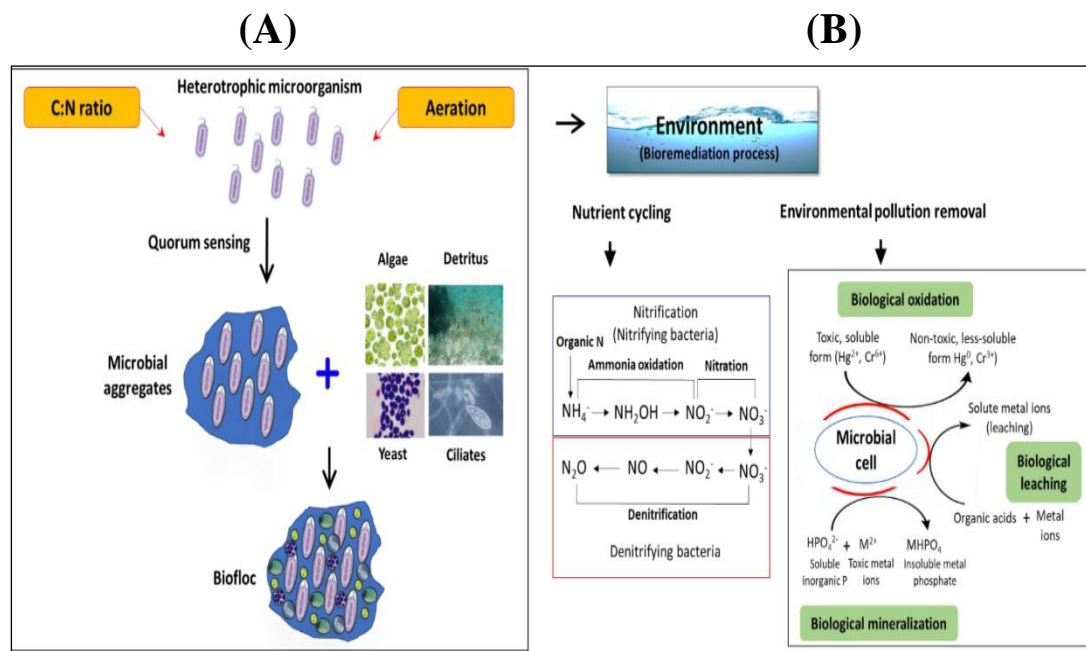


Fig.6. Schematic overview on the possible role of the biofloc microbiome. Where (A) refers to the development of a biofloc system, while (B) refers to the potential role of the biofloc system in the bioremediation process (Kumar *et al.*, 2021).

1.4. Microbial production of nanoparticles

The formation of nanoparticles by microbes from metals, metal oxides, or metalloids, both intracellular and extracellular, has been thoroughly described in literature (Patil & Chandrasekaran, 2020). The extracellular process incorporates microbial enzymes and proteins, bacterial or fungal cell wall constituents, or organic compounds present in the culture media, which reduce metal ions for NPs formation. Whereas, the intracellular process begins with metal ions being electrostatically attracted to carboxyl groups in the microbial cell wall, resulting in metal ions passing into the cells and being reduced by intracellular proteins and cofactors to form NPs (Siddiqi *et al.*, 2018). Microbial resistance strategies for cellular detoxification include biochemical pathways involving microorganism-mediated nanoparticle production. Enzymatic reduction and/or precipitation of inorganic and toxic ions in the form of nanostructures affect the solubility of these ions. Extracellular and intracellular bio-catalytic synthesis methods have been hypothesized, including oxido-reductase enzymes (e.g., NADH-dependent nitrate reductase, NADPH-dependent sulfite reductase, flavoprotein subunit, and cysteine desulfhydrase) and cellular transporters which play a major role (Grasso *et al.*, 2019).

Within microorganisms, nano-dimension materials are biosynthesized by linking target ions from the surroundings and transforming these hazardous metal ions into the matching element metal via cellular enzymes. Nanoparticles can be categorised as intracellular or extracellular depending on where they are synthesized.

In the intracellular process, ions are transported inside the microbial cell and combined with enzymes to produce nanoparticles. While, in the extracellular process,

metal ions are trapped on the cell surface, and reduced ions are produced in the presence of enzymes (Li *et al.*, 2011).

Compared to physical and chemical approaches, the use of microbial enzymes/proteins as potential reducing agents for synthesizing NPs has expanded quickly. It's a rapid, ecologically friendly, and cost-effective solution. Fungi and bacteria are preferred among biogenic sources not only for their potential to produce a higher amount of reductase enzyme to transform the ionic forms into their nano forms but also for the ease with which they can cultivate and control the size and morphology of the synthesised NPs, lowering the cost of large-scale manufacture. The NPs can effectively limit bacterial growth by penetrating through the exopolysaccharides of a biofilm matrix. The NPs enter the biofilm and disrupt the quorum-sensing gene cascades, inhibiting biofilm formation by interfering with cell-to-cell communication. Diagrammatically, Fig(7) illustrates the bioreduction of metal, metalloid and nonmetal ions to nanoparticles by microbial enzymes (Lahiri *et al.*, 2021).

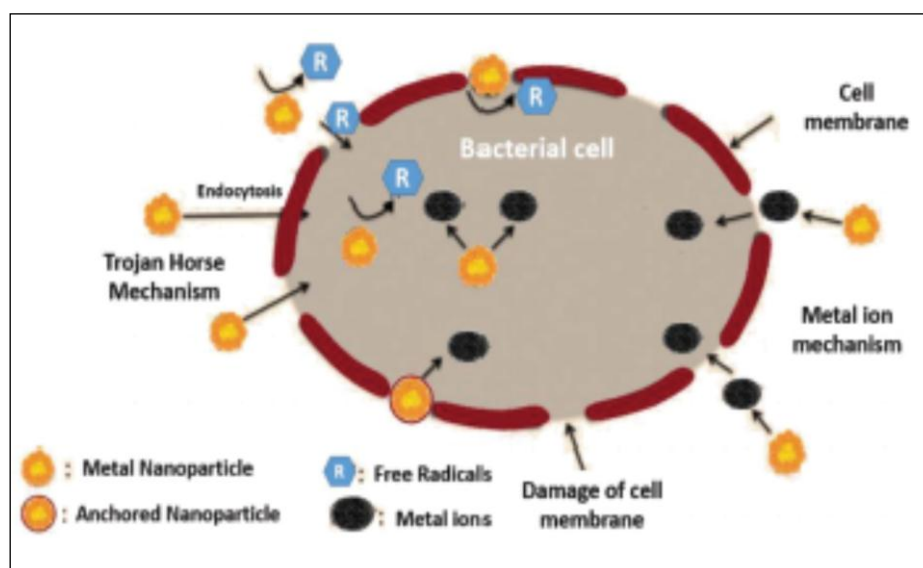


Fig.7. Microbial enzymes in bio-reduction of metal, metalloid and nonmetal ions to nanoparticles (Lahiri *et al.*, 2021)

Furthermore, nanotechnology has a wide range of applications in aquaculture. Various nanotechnology-based methods are now being used to augment aquaculture productivity, effectiveness and sustainability. Recent attempts have been undertaken in the domain of health administration and dietary complements with nutraceuticals to improve the growth of fish and shellfish (Fajardo *et al.*, 2022). Fish immune systems, for instance, have been boosted with DNA-nano vaccines. Iron nanoparticles, on the other hand, can help fish grow faster (Mohammadi & Tukmechi, 2015).

Nanotechnology also includes the improvement of fish packaging techniques, and the enhancement of quality in terms of flavor, texture, odor, appearance and taste, as well as improving fish nutrients absorption. Moreover, nanotechnology is applied to improve bioavailability including functional compounds (Fajardo *et al.*,

2022). Amazingly, **Kolinkoet al. (2014)** concluded that a recombinant approach will enable the sustainable production of tai-lored biogenic nanoparticles in non-native biotechnologically important hosts. Additionally, they suggested that this is a step towards the endogenous magnetization of different organisms using synthetic biology(Fig.8).

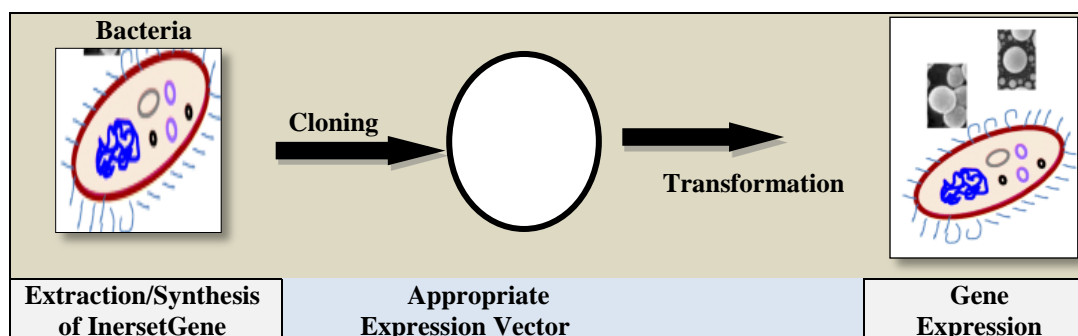


Fig.8.Cloningsystem for biological synthesis of MNPs(adopted from**D'Alviseet al., 2013**).

CONCLUDING REMARKS

From the previous information, it is possible to draw some general conclusions regarding the characteristics of the marine bacteria and their bioactivity and pathogenicity, as follows:

1. Marine bacteria have attracted the attention of both scientists and industrialists because of their uniqueness and the characteristic features besides their ability to adapt to environmental patterns, as well as their capability to produce bioactive substances, such as enzymes, antibiotics, bacteriocins, siderophores, nanoparticles, etc.
2. Marine bacteria have a significant role in the bioremediation of pollutants (e.g. dyes, heavy metals and hydrocarbons, etc.).
3. Moreover, they play a vital role in the marine biogeochemical cycles (carbon, nitrogen and sulfur).
4. Marine bacteria are applied as probiotics for increasing disease resistance in fish and as biological filters in aquaculture treatment via biofloc formation.
5. Despite the gain of these benefits, some marine bacteria have several harmful effects, such as the formation of bacterial biofilm, biofouling and bacterial pathogenicity.

RECOMMENDATIONS

Taking into consideration the advances in this field, we believe that more financial support should be provided by universities, research institutes and the academy of scientific research as well as funds organized by the responsible ministry.

In addition, much more research should be carried out on superior isolates to increase production and reduce the cost of bacterial bioactive compounds and probiotics. Moreover, the research should be focused much more on the production media from

inexpensive sources such as agro-industrial wastes. Further studies should be carried out on different types of marine extremophilic bacteria- because of their superior characteristics- for applicable purposes, especially in the bioremediation sector. Indeed, we need to succeed in such manner, to do in-depth investigations on how the microbial ecology of aquaculture systems works, and we need to identify key processes and key interactions between relevant organisms in these settings over the coming years. Finally, the production of different bioactive compounds and probiotics should be done by the molecular engineering with interest in modern science such as bio-informatics and glycomics.

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