APPLICATIONS OF POLYSACCHARIDE, POLYPHENOLS & PHAGES (3Ps) FORTIFIED FISH FEED IN AQUACULTURE

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Introduction

The demand for food is constantly increasing worldwide due to population growth. In this context, aquaculture food industry is a sustainable and fast-growing food-producing sector in the world.

India's fishery sector has been growing steadily and production is increasing every year. Due to the fast depletion of resources of wild fish species in marine ecosystems and to meet the growing demand, Government of India seeks to promote aquaculture through the "Blue Revolution" program, to cultivate the targeted commercial fishes in mainland ponds and water bodies. There is a high demand for such farmed fish feeds.

Recent studies suggested that the majority of fish feeds are supplemented with fishes caught from natural sources and these marine captures again leads to further exploitation of marine natural resources. The balanced feed that contains carbohydrate, protein, lipids, minerals and vitamins are very vital for growth and well-being of fish. There are several types of fish feed available commercially. Due to advancement of feed technology, tailor-made feeds for both aquariums and aquacultures are available. At present, selection of right feed at an affordable price is a challenge for commercial farmers. Medicated fish food is often used by the farmer to combat infection and bacterial outbreak in aquaculture. However, use of antibiotics in fish feed are not much encouraged due to emergence of antimicrobial resistance and also due to regulatory restrictions.

Chitosan is a natural polysaccharide polymer, which is endowed with potential biological benefits in fish and animals. It is eco-friendly and recently got lots of attention to use in fish feed because it meets environmental requirements and provides sustainability to aquaculture. Chitosan plays a significant role in feed additives due to its low side effects, improves growth, provides immunity and also acts as antimicrobial agents (Abdel-Ghanyand Salem 2020). Polyphenols obtained from natural sources are endowed with potential with antioxidant and anti-inflammatory activities. Polyphenols such as flavonoids, phenolic acids, lignans and stilbenes are widely used as food additives in fish feed to improve the health status and production of fish in terrestrial fish farms (Ahmadifar et al., 2021). Polyphenolic compounds like caftaric acid, chlorogenic acid, cynarin, echinacoside and cichoric acid are present in plant parts. For example, purple coneflower that have been found as immune-modulator and antioxidant agent of fishes.

Vibriosis causes endemic bacterial infections and outbreaks in marine aquaculture farms, which may enter into fish through water and contaminated feed. To combat the microbial pressure in aquaculture, the bacteriophages are widely used as natural antimicrobial agents. Due to increase in antimicrobial resistance among the fish pathogens, there is an increasing trend in use of bacteriophages for multiple applications inaquaculture. Polysaccharide like chitosan, polyphenols and bacteriophages has shown individually as a promising candidate for providing health benefits and growth of fish. There is a vast potential scope for biological applications and health benefits using a combination of these 3Ps such as Polysaccharide, Polyphenols and Phages in aquatic organisms.

Source of chitosan and its applications

Chitin is a large, structural polysaccharide made from chains of modified glucose. Chitin (Fig. 1) is abundantly present in the exoskeletons of insects, cell walls of fungi, invertebrates and fish.

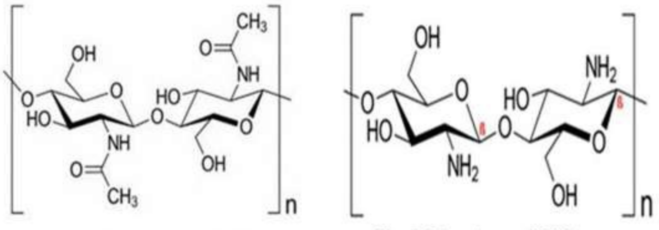
Fig. 1 Structure of Chitin   Fig. 2 Structure of Chitosan

Chitosan (Fig. 2) is obtained by deacetylation of chitin (Fig. 3), which is composed of β-(1-4)-linked d-glucosamine and N-acetyl-d-glucosamine randomly distributed within the polymer. The cationic nature of chitosan is rather special, as the majority of polysaccharides are usually either neutral or negatively charged in an acidic environment. Chitosan has been recognized as versatile biomaterials for their unique properties such as biocompatibility, biodegradability, non-toxicity and low allergenicity (Kumar et al., 2004). However, the application of chitosan depends on the degree of deacetylation, molecular weight and characteristics like appearance and turbidity of polymer in aqueous solution (Nwe et al., 2014). Several studies have been carried out for biomedical applications of chitosan includingpharmaceuticals industry, tissue engineering, drug delivery, antitumor and antimicrobial effects (Cheung et al.,2015).

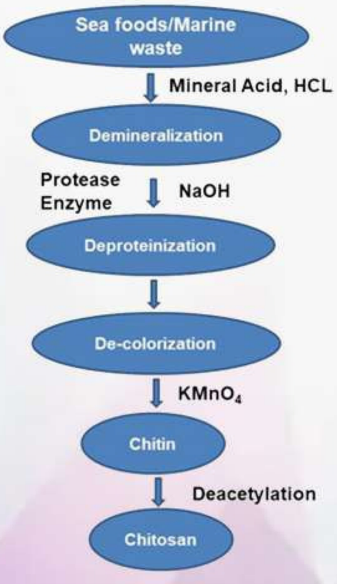

Fig. 3. Purification of chitosan from biological sources

Water Treatment

Chitosan is an excellent biomaterial for adsorption of organic and inorganic pollutant in the water treatment systems. Presence of multiple functional groups enables the molecule to interact with chemically active substance such as dyes, metals and organic micropollutants. To increase the adsorption properties of chitosan, functional groups may be modified by cross linking and grafting with active molecules or with elements. Chitosan cross-linked with epichlorohydrin, ethylene glycol diglycidyl ether, glutaraldehyde and tripolyphosphate have shown to improve the physical properties and also dye adsorption properties (Kyzas et al.,2015). Recently, chitosan-oxalic acid-biochar composite proved for adsorption of synthetic azo-dye (Doondani et al.,2022). Chitosan structure modified with carboxylic acid functional (-COOH) group improved the solubility and acquire tendency to chelate the heavy metals in addition to other functional groups such as amino and hydroxyl (-NH2 and –OH) functional groups (Boamah et al.,2015).

Aquaculture

In aquaculture, chitosan renders several functions like coagulant, adsorbent, or bactericide.It has been used to improve the water quality of aquaculture wastewater by adsorption of suspended solids, organic compounds, NH3, PO43-, and pathogens. The treatment efficiency improved based on the degree of deacetylation and acidic pH (Chung et al., 2006). The nanocomposite forms of chitosan are used for food preservation purposes and diagnosis of fish diseases (Ahmed et al.,2019). In fish models, chitosan has shown to have manyphysiological functions such as growth promoting,antioxidant, antimicrobial effect and immunostimulant effect. The antioxidant activity of chitosan was attributed due to radical scavenge ability, presence of hydroxyl moiety and metal chelation properties. This activitywas directly proportional to the concentration of chitosan. The dietary supplementation of chitosan alone or with vitamin C increased the efficiency of antioxidant properties of fish exposed to heavy metals like cadmium, chloride and also involved in the regulation of enzymes like alanine aminotransferase, creatine phosphokinaseand catalase (Banaee et al.,2015). The optimal concentration of chitosan in the fish feed was estimated between 1 and 2 g /kg for fish growth.

However, the concentration above 4 g/kg feed attributed to heavy development of intestinal microvilli that leads to blockage food mobility and fish growth depression and death (Zaki et al.,2015). In contrast to this observation, a basal diet supplemented with chitosan upto 20g/kg diet was fed to Asian seabass for 60 days challenged with Vibrio anguillarum and showed highest haematological and innate immune parameters compared to control group. In addition,10 g/kg diet was found effective concentration for prophylactic purpose against marine vibrio infections (Ranjan et al., 2012). Chitosan has been used as a candidate for delivery of nucleic acid, vitamin C and hormones. Delivery of these molecules into several organism such as Litopenaeus vannamei, Labeo rohita, Solea senegalensis and Lates calcarifer have been reported (Bhoopathy et al., 2021). There are several research works on successful delivery of chitosan-based therapeutics and nucleic acid into fish and aquaculture. Dietary RNA has been successfully prepared and used in rohu (Ferosekhan et al., 2014) and nanoparticles of chitosan encapsulated with inactive particles of the viral haemorrhagic septicaemia virus (VHSV) tested against Olive flounder (Paralichythys olivaceus) (Kole et al., 2019). Chitosan coated membrane vesicles from intracellular fish pathogen Piscirickettsia salmonis was injected into zebrafish that provided immunogenic and increased survival against pathogen challenge (Tandberg et al., 2018). Chitosan is a versatile biomaterial and widely used many fields such as food industry, photography, wastewater treatment, aquaculture and chemical industry. However, low solubility in neutral and alkaline solution poses to limit the use of this compound abundantly. Chemical modification or functionalization of chitosan with other biologically active materials enhanced the physic-chemical and functional properties of this compound for different applications.

Applications of Polyphenols in aquaculture

Polyphenols are plant-derived compounds endowed with biological and potential health benefits. Polyphenols are considered a viable alternative to synthetic chemicals like antimicrobial compounds to improve fish health status and to enhance the fish quality, productivity and food safety. Polyphenols are extensively investigated in aquaculture as functionalfeed additives in the form of polyphenols and polyphenols rich additives. Polyphenolic compounds such as flavonoids, phenolic acids, lignans and stilbenes are known to provide health benefits on the overall performances and immunity of fish; thereby improving the health status and production of fish farms. These compounds are expected to act on the pathways of antioxidant, pro-oxidant activities, regulation of gene expression and different immune parameters (Ahmadifar et al., 2021). The purple coneflower [Echinacea purpurea (L.) Moench.] were added to fish feed as adjuvant therapy for the prevention of fish diseases. The most active compounds of E. purpurea have been reported to contain polyphenols such as caftaric acid, chlorogenic acid, cynarin, echinacoside and cichoric acid (Oniszczuk et al., 2019). Plant extracts of polyphenols are known as tea phenols. It has been used to enhance and preserve the yellow croaker (Pseudosciaena crocea), which is known for important commerce and aquaculture particularly in Chinese cuisine.

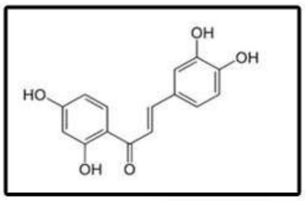

Fig. 4. Structure of Butein

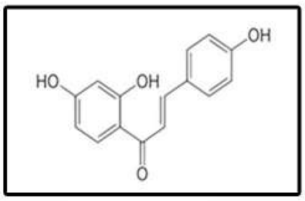

Fig. 5. Structure of Isoliquiritigenin

Phenolic plant extract has shown to interact with lipidperoxy or lipidoxy free radicals obtained from lipid oxidation and further stop breakdown of lipids. Hence inclusion of polyphenolic compounds in fish meat products would enhance the shelf life of fish food products (Ali et al., 2019). Myofibrillar proteins (MP) are most abundantly present in fish, which is likely to hydrolyse during storage. The polyphenols like chlorogenic acid and quercetin were investigated for the binding affinity with this protein and shown that chlorogenic acid bind to MP by Van der Waals forces and hydrogen bonds; quercetin binds to MPs by electrostatic interactions with tryptophan and tyrosine residues there by prevent degradation of protein (Xie et al., 2020). The polyphenoic compounds like butein (Fig. 4) and Isoliquiritigenin (ILT) (Fig. 5) are endowed with potential biological and pharmacological activities but very poorly soluble in water. Polyphenolic compounds are expected to promote the growth of symbiotic commensal bacteria and also ease the stress of the host as an antioxidant thereby increasing the health benefits of the host when consumed.

Among the polyphenolic compounds, Resveratrol has been extensively investigated in in-vitro and in animal models and found to increase lifespan of yeast, roundworms and fish (Cherniack et al., 2010). Butein and ILT have structural similarity with resveratrol but yet not investigated its health benefits in fish. These polyphenolic compounds containing plant extracts were tested against fish pathogens and proven effective. However, many polyphenolic compounds have low oral bioavailability, which limits the application of polyphenols in nutraceuticals. Food-grade delivery carriers encapsulation; and delivery of polyphenolic compounds could resolve poor water solubility and low bioavailability of polyphenols for various practical applications.

Interaction of polyphenols and polysaccharides

Naturally, polysaccharides and polyphenols coexist in many plant-based food products. Interactions of this polyphenol–polysaccharide affect physicochemical, functional and physiological properties, such as digestibility, bioavailability and stability of foods (Guo et al.,2022). The presence of multiple functional groups in polysaccharide makes them ideal for conjugation. The conjugations of polysaccharide with polyphenols are primarily to increase the solubilisation and control release of hydrophobic moieties.

Several approaches are employed for conjugation of polysaccharides with polyphenols such as esterification, free radical grafting, etc., these methods are selected based on the structural nature of conjugants. However, the integrity of the compound must not be affected. A polysaccharide like chitosan was conjugated with small molecule drugs, natural compounds, proteins/peptides, nucleic acids, etc., Introduction of these active molecule impart better biological activities like antimicrobial and antioxidant and also improve solubility. The accepted mechanism action of antimicrobial action of chitosan conjugated polyphenols is electrostatic interactions between the protonated amino group of the chitosan molecule and the anionic surface of the pathogen under acidic conditions (Qin et al., 2020).

Bacteriophages

Bacteriophages or phages are bacterial viruses that can invade bacterial cells and cause cell death by disruption of bacterial metabolism. Phages are isolated from environmental water samples or from host pathogens (Fig. 4). Before the discovery of bacteria, bacteriophages were widely used as antimicrobial agents. In order to combat the microbial pressure in aquaculture, the bacteriophages are used as natural antimicrobial agents. Due to increase in antimicrobial resistance among the fish pathogens, there is an increasing trend in use of bacteriophages for multiple applications in aquaculture.

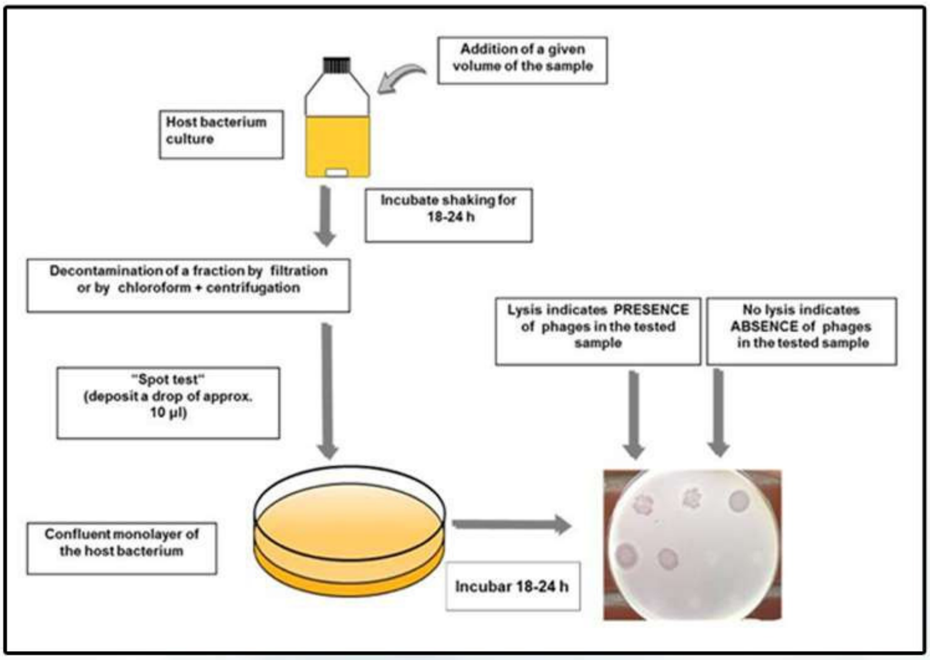

Fig. 6. Isolation of phage from marine water or sewage sample (Courtesy: Jebri et al., 2017)

The bacteriophages may be used as natural, genetically engineered phages and also phages derived from lethal genes and with other cargoes are used as antimicrobial agents (Nair et al., 2022). Phage based approaches have gained importance for antimicrobial treatment or as prophylactic measures due to its sustainable alternative to chemical antimicrobials. Recently, live artemia-mediated phage delivery methods were found promising tools for phage-based therapy against pathogenic bacteria to prevent aquatic diseases (Nikapitiya et al., 2020).

Phages can be administered through oral feed pellets sprayed with concentration range between 1-2 ×108 PFU/g and other routes of administration such bath or injections. The concentration and route of administration needs to be selected based on phage titer value against specific bacteria killing ability (Donati et al.,2021). The successful phage therapy treatment in aquaculture depends on the optimization of phage delivery methods and use of appropriate phage to control bacterial infections (Kunttu et al.,2021). The better bio-control was achieved by mixing therapeutic phages into cocktails of different virus types. In clinical and aquaculture, infrequently detected with appearance of MDR (multidrug-resistant), XDR (extensively drug-resistant) and PDR (pan drug-resistant) bacteria that leads to failure in the infecting the control. In these cases, the bacteriophages have become one the best hope for the future treatment of resistant bacteria that do not respond to available antimicrobial agents. However, for the commercial application of phage based therapies in aquaculture require more experimental evidence and field trials.

Application of 3Ps (Polysaccharide, Polyphenols and Phages) in Aquaculture

Polysaccharide like chitosan, polyphenols and bacteriophages has shown individually a promising candidate for providing health benefits and growth to fish. Chitosan itself acts as a carrier polymer molecule for delivery of a variety of compounds into the target of the host including polyphenols and bacteriophages. Chitosan functionalized with polyphenols are expected to improve the solubility of these compounds and expected to provide antioxidant, improve health benefits by overall wellbeing and when these are combined with phage are expected to provide better infection control. Chitosan is a biocompatible and mucoadhesive polymer. Due to the mucoadhesive property of chitosan, polyphenol and phages enter into the fish through gill, skin and into the intestine through the feeds. All these three agents are antimicrobial by themselves and when combined together, these may have synergistic effects, thereby providing better infection control of fish. Polyphenols are expected to provide stress relief and also modify the gut microbiome.

Conclusion

Based on the available data, it is obvious that chitosan (polysaccharide), polyphenols and bacteriophages have great potential for health benefits in fish. These three components were tested individually or in combination of two against the health benefits of fish. It was found that Chitosan conjugated polyphenol particular, butein and ILT with phage cocktail will have most desirable properties for healthy growth of fish, supports free from infection, stress and also expected to modify the gut microbiota. More investigations are required on these 3Ps with reference to the health benefits for fish when administered together through the food, particularly relating to infection control and analysis of gut microbiome.

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