FAITT

Biopigments and their application in the aquaculture industry

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Introduction

Numerous synthetic colourants have been created in recent centuries. They are utilised for a wide variety of purposes in the cosmetics, food, pharmaceutical, and textile sectors, due to their lower costs, easier manufacture, desirable flavours and better colouring qualities.

However, due to their toxicity and the production of harmful effluents while manufacturing, their market prominence subsequently declined (Manikprabhu and Lingappa, 2013).

Contrary to this, biopigments, which are made naturally by living organisms, are non-toxic and have increasing market demand than synthetic colourants. According to Sen et al. (2019), consumer demand for natural food colours will progressively rise by 7 percent yearly. As a result, natural colourants are predicted to generate $1620 million in revenue by 2023, representing a 5.4 percent CAGR from $1180 million in 2017. (https://www. marketresearchfuture.com/reports/).

Moreover, the international market for riboflavin is anticipated to grow by 4.8 percent over the next five years, with estimates ranging from $7790 to $10,300 million during 2019-2024 (https://www.fiormarkets.com/report/global-vitamin-b2-riboflavin-market-growth-2019-2024-374891.html). The global carotenoid market is anticipated to reach $2000 million by 2026.

Natural pigments

Natural colours are primarily derived from living organisms. E.g. Anthocyanins from fruits and vegetables (such as the skins of black grapes, black carrots, and red cabbage), annatto from achiote seeds, betanins from red beets, carmines from the blood of cochineal beetles, capsanthin from red peppers, and lycopene from tomatoes, blue pigment phycocyanins from algae Spirulina, Chlorophylls from green plants such as nettles, asparagus, avocado, broccoli, brussels sprouts, cabbage, celery, cucumber, green beans, green onion, kiwi fruit, lettuce, okra, spinach, zucchini, spinach, grass, parsley, Curcumin from turmeric, carotenoids from palm trees, and yellow pigment xanthones from plant families like Bonnetiaceae, Clusiaceae, and Podostemaceae (Henry, 1996; Park et al, 2018; Simpson and Klomklao, 2012).

Nonetheless, animal/plant pigments have limitations due to unsteady/seasonal availability, deforestation concerns, and the production of unstable and insoluble compounds. Microalgae, on the other hand, is considered an important source of pigments, and a source of natural nutrients in the aquaculture industry (Natrah et al, 2007; Park et al, 2018; Koller et al 2014). Microalgae are used as live feeds, supplements for water quality, bioremediation, growth promoters, antioxidants and animal colour enhancers (Khatoon et al, 2007; Begum et al, 2016). Microalgae are a source of several polyunsaturated fatty acids such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) and omega-3 fatty acids, and also do have several pigments such as chlorophylls, carotenoids, phycobiliproteins and phenolic compounds which enhances the functional, nutritional, therapeutic values (Natrah et al, 2007; Park et al, 2018; Koller et al 2014).

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The cultivation of microalgae is becoming more and more essential due to its sustainable use in fish and shellfish aquaculture and other industries.

Natural Pigments such as astaxanthin and β-carotene are products used in aquaculture for their immune-enhancing functions such as antioxidants, immune enhancers, anti-inflammatory agents and vitamin precursors. Hence, the cultivation of natural microalgae is gaining an advantage globally because of its sustainability and eco-friendly production. The antioxidants astaxanthin, β carotene, lutein, lycopene, and canthaxanthin are the most significant carotenoids obtained from microalgae (Gong & Bassi 2016). Only a few numbers of microalgae have been used in industries. Genera like Chlorella, Spirulina, Dunaliella, Muriellopsis and *Haematococcus* is widely used (Sanchez et al. 2008; Lin et al. 2015; Wang et al. 2018a).

Haematococcus pluvialis is rich in xanthophylls and is regarded as the best natural source of astaxanthin. *Dunaliella salina* is used as an industrial source of β-carotene (Levi 2001; Sanchez et al. 2008; Jeffrey & Egeland 2019).Although microalgae production is eco-friendly, the extraction process will be costlier for biomass or bio-compound production (Yusoff et al. 2019) and needs large-scale harvesting methods like centrifugation, fractionation, flocculation and coagulation, filtration and ultrasonic separation.

Microbes such as fungi and bacteria are also reliable alternate sources of biopigments due to their rapid growth, and production of stable and high product yields through cost-effective strategies (Narsing et al, 2017).

Several microbes are used for the production of pigments in various industries. Many vibrantly coloured pigments, including anthraquinones, carotenoids, dihydroxy naphthalenes, flavins, indigo pigments, naphthoquinones, phenazines, melanins, monascins, violaceins, etc., are produced by microorganisms from different biological niches (Fig 1).

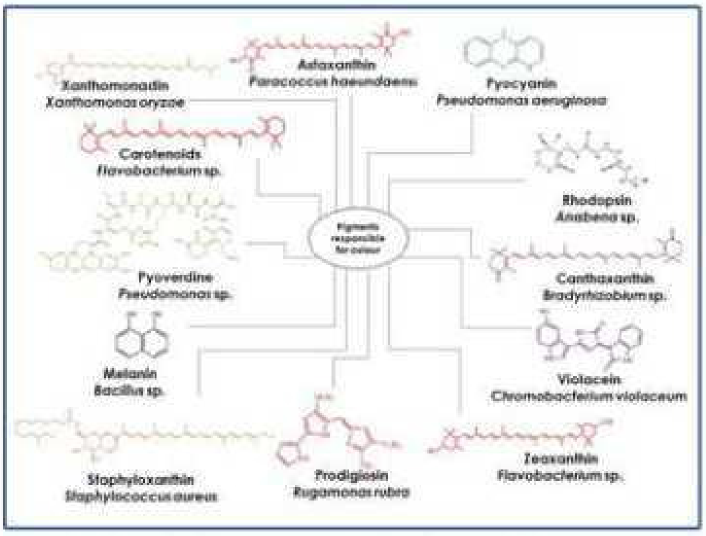

Fig. 1. Biopigments and their bacterial source.

Benefits of biopigments

It has been noted that biopigments made by microbes in reaction to environmental factors frequently have positive effects on human health. *Serratia* and other gram-negative bacteria produce the versatile red bio pigment prodigiosin. According to reports, prodigiosin has cytotoxic, anti-inflammatory, antibacterial, antiprotozoal, and antifungal activities. The pharmacological characteristics of Monascus pigments, such as their anti-cancer, anti-mutagenic, anti-obesity, and antibacterial activities, are also well documented. Additionally, biopigments made by yeasts have been described as having antioxidant, anti-carcinogenic, and immune response-enhancing properties (Demain and Martens, 2017; Panesar et al., 2015). Melanin is another biopigment that is typically present in microbes, plants, and animals and has qualities that include immunogenic, antibacterial, anti-inflammatory, and anti-oxidant (El-Naggar and El-Ewasy, 2017; Rosas et al., 2000).

Pigment Name	Properties
Astaxanthin	Anti-stress
Canthaxanthin	
Carotenoids	Antibiotic, Antioxidant, Cytotoxic activity
Melanin	Photoprotection, Antioxidant, Anticancer
Zeaxanthin	
Indigoidine	Antioxidant, Signaling, Antibiotic
Prodigiosin	Biocontrol, Antibiotic, Algaecidal, Anti-inflammatory, Anticancer, Antimalarial, Antidiabetic, Immune system modulator
Staphyloxanthin, Zeaxanthin	
Violacein	Antibiotic, Antiparasitic, Antiviral, Antitumoral Anticancer
Pyocyanin	Virulence factor, Iron uptake, Cytotoxic activity Antibacterial activity
Pyoverdine	Bioluminescence, Virulence factor, Iron uptake
Rhodopsins	Active transport
Xanthomonadin	

Table 1: Various functions and properties of biopigments

After that, strains can be cultured in a fermenter/bioreactor to obtain pigments at the commercial scale. Photobioreactors are often used for the cultivation of algae for the production of algal pigments.

Genetic engineering approaches have been used to improve pigment production in native strains such as site-directed mutagenesis, or random mutation. Besides, heterologous hosts such as model bacteria or yeast have also been tried to produce pigment after transporting the genes or operons from pigmented strains. This method resolves the issues such as variable yield, safety, or difficulty to cultivate strains (Orlandi et al., 2020). Efforts have been taken, in particular for the biosynthesis of astaxanthin, owing to its high commercial demand in various industries including livestock, fish feed, cosmetics and nutraceuticals (Gong et al., 2020). Genes involved in the manufacture of carotenoids have been characterised in *Hematococcus pluvialis*, *Chlorella zofingiensis*, and *Chlamydomonas reinhardtii* (Gao, Honzatko, and Peters 2012; Kathiresan et al. 2015).

Fig. 2. Outdoor photobioreactor for the cultivation of *Haematococcus pluvialis* for the production of astaxanthin (source: https://www.scientistlive.com/content/high-quality-microalgae-products)functions and properties of biopigments

Strategies to obtain biopigments

Microbial biopigment production has two basic approaches: either, discovers new pigmented microbes, and optimizes the productivity; or, enhances the production of pigment from already proven strain by strain improvement and process development (Venil et al., 2020). As mentioned earlier, numerous microbes have been isolated producing diverse pigments, shown to have diverse applications. However, the yield of the pigment depends on environmental conditions. Hence, optimization is required to increase the production of pigment in the native-producing strains in laboratory settings.

Carotenoid biosynthesis involves multi-copy genes, which makes the route specialised and challenging but also adaptive. Evolutionary studies showed that the majority of the important genes involved in carotenogenesis in algae were derived from cyanobacteria (Shanshan et al. 2018). The genes involved in carotenogenesis are coordinated to create a specific type of carotenoid depending on environmental conditions. PSY enzyme which is a crucial regulatory enzyme in carotenoid biosynthesis is encoded by the PSY gene in algae, cyanobacteria, and plants and the crtB gene in bacteria. These enzymes' potential genes, once identified, can be inserted into the right hosts to produce particular bio-pigments.

The pigments produced by organisms are mostly intracellular, e.g. carotenoids by yeast. Hence, cell disruption and pigment extraction are the two main processes in recovering intracellular bio pigments. The effectiveness of this process depends on the processing method including the protection of pigment from high temperatures, and organic solvents (Nigam and Luke, 2016). Cell disruption can be done mechanically or through a chemical process. Chemical extraction has been proven to yield higher concentrations (Park et al., 2007). Different extraction methods are known to produce biopigments with unique profiles (Mapari et al., 2005). It is important to note that intracellular biopigment extraction remains difficult because there aren't any standardised, environmentally friendly methods for doing so (Park et al., 2007). The usage of optimum solvent also depends on the information on pigment molecular properties. For E.g. carotenoids are solubilized in various non-polar solvents because they are held in lipid vesicles (Park et al., 2007). Differently, prodigiosin is a pigment that has a higher hydrophobicity than several carotenoids and may be easily removed using acetone (Sun et al., 2015).

If the pigments are produced extracellularly, cell disintegration is not required, e.g. Monascus pigments. The supernatant is used and generally processed using ethanol (Nimnoi and Lumyong, 2011).

Potential applications in aquaculture

Under some rearing circumstances, aquatic species' vibrant and natural colouration may fade (Pavlidis et al., 2006). The vibrant reds and yellows found in fish skin, as well as the orange and green hues of the eggs and muscle, are all a result of fat-soluble bio-pigments called carotenoids (Kop & Durmaz, 2008). These substances are not necessary nutrients, but they can help organisms develop (Dall, 1995; Petit et al., 1997), survive (Wyban et al., 1997), maintain a healthy immune system, reproduce (Linan Cabello et al., 2003), have antioxidant action (Meyers & Latscha, 1997), and withstand stress (Chien et al., 2003; Guerin et al., 2003).

Salmonids consume astaxanthin, a crucial micronutrient with important biological properties. These include improving the biological status of the organism as a whole and the stability of cell membranes, as well as enhancing fish health and immunity by reducing free radicals and boosting resistance to environmental stressors (Latscha, 1989). Astaxanthin also can produce vitamin A and shield the body from the harmful effects of UV light (Higuera-Ciapara et al., 2006). The pigment also inhibits the oxidation of unsaturated fatty acids; astaxanthin antioxidant activity has been compared to beta-carotene and alpha-tocopherol and is 10 and 100 times more potent, respectively (Hussein et al., 2006; Rao et al., 2014).

In reality, astaxanthin is what gives rainbow trout their natural colour, but in a fish-rearing setting, astaxanthin or canthaxanthin must be added to the fish diet (Craik & Harvey, 1986). The pigmentation of fish depends on the storage and retention of pigment in muscle, which varies from 3 to 18 percent for astaxanthin, in addition to the dietary component (Choubert et al., 2009). To increase the protection of astaxanthin in the feed, several techniques have been developed, including nanoencapsulation using emulsions, liposomes, and polymer nanoparticles (Rao et al., 2014; Raposo et al., 2015).

According to the findings of a study by Besharat et al. (2021), adding nanoliposome-coated astaxanthin to rainbow trout diets at doses up to 75 mg/kg can improve growth metrics. Fish growth was positively impacted by the presence of nanoliposome-coated astaxanthin, as shown by the fact that the mean total length and weight of the fish rose significantly with this dose and differed significantly from the control diet.

Microalgae are one of the most chosen microorganisms for biotechnology and applied processes due to their high photosynthetic efficiency, rapid growth rate, and capacity to collect a wide variety of bioactive chemicals (Guedes et al. 2011; Hayes et al. 2018). The extraction of these compounds from microalgae has resulted in biotechnological innovations that have helped the food, pharmaceutical, cosmetic, nutraceutical, and aquaculture industries (Pulz & Gross 2004; Richmond 2004; Shah et al. 2016). More than 30 000 species have been reported, which means that many unique products could potentially be commercially exploited from a huge number of existing species (Lorenz & Cysewski 2000; Leon et al. 2003; Ip et al. 2004; Sanchez et al. 2008; Hayes et al. 2018). More than 7000 of these involve green algae, which are found in many habitats (Shah et al. 2016).

These microalgae are being used as additives for poultry, crustacean, and fish feeds because they provide vibrant colours in egg yolks, skin, and fatty tissues due to their pigmenting properties (Levi 2001; Sanchez et al. 2008), free radical scavenging capacity (Shah et al. 2016), and also,they have shown to improve growth and reduce mortality in larval development (Muller-Feil). So that, these pigments are in strong demand (Ignacio et al, 2019).

Other uses

Apart from aquaculture use, bio-pigments are used as colourants in the food, textile, cosmetic, and pharmaceutical industries, and also used in different functional applications like antioxidant, anticancer, and antibacterial characteristics. (Gilda Mariano-Silva, Salvador Sa'nchez-Mun oza 2020).

Aqua - commercial products

There are several bio pigment products esp. astaxanthin which is used in the hatchery sectors of shrimp larval development. Astaxanthin is derived from either synthesis or natural production or krill extract oil. The products are formulated in different methods such as conjugated with starch or any other carbon source or in the form of oils or microencapsulated powder. Several companies are coming up with different formulations as feed additives or direct feed supplements. The pigmentation and animal immunity are enhanced by the use of astaxanthin. Few countries are adding astaxanthin to their feed formulations itself to enhance the immunity of the animal.

Amaze asta

Amaze Asta is one such product which was derived naturally from *H. pluvialis* and is 10% pigment conjugated with corn starch which is 100% water soluble. The product can be used as a feed supplement and also can be used for post-larvae packing. It acts as a perfect stress reliever and antioxidant.

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