

PIGMENT DYNAMICS FROM MARINE COMMUNITIES: AN UPDATED COMPREHENSIVE REVIEW

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Abstract: Marine water is the source of highly diverse group of microbial communities. These microbial communities are possessing the immense natural repository of diversified secondary metabolites bioactive compounds. One such group of such molecules are microbial pigments. Microbial pigments are been produced by almost all bacterial communities ranging from archeal, eubacterial, cyanobacterial, fungal and algal communities. The production of these pigments by microbes appears to mediate the quorum sensing mechanism and exhibits vast array of activities ranging from anticarcinogenic, antibacterial and immunomodulatory. This review focuses on how naturally available microbial pigment can be better than synthetic ones. The intensity of the pigment obtained from the microbes can easily be altered by altering the growth condition. This helps in exploring a wide variety of colours which can be helpful in various industrial application. Synthetic chemicals in the form of pigments are causing numerous problems and eventually there will be a time to eradicate its use and, in that situation, the extracellular microbial pigment will play a vital role. Therefore, it is highly pivotal to unravel the hidden diversity of marine microorganisms virtually present in every nook and cranny of our biosphere.

Keyword- Marine, pigments, Bacteria, Enviornments, Microorganisms.

1. INTRODUCTION

Approximately 70% of earth's crust is sheltered with water most of that is marine. Marine environment is filled with beneficial and harmful microorganisms, they include bacteria, viruses, archaea, protists, and fungi. Different pigments have different colours, this colours have number of beneficial properties like antibacterial, anticarcinogenic, antioxidant, antitumor, antiviral, photodynamic therapy, photoprotection etc. Microbial pigments are used in a wide variety of industries, including food, dairy, printing, textiles, and pharmaceuticals. Bacterial species possess the function or the feature of pigmentation. Colour display of organism occurs due to light absorbing compounds of the pigments. The bacterial domain produces a wide variety pigments in the present scenario and the survival of organism depends on these features, which are pigments. In industrial processes it has been seen that xanthomonadin is able to protect *Xanthomonas oryzae* from damage, which is caused because of light and thus can be chemically treated [Pawar et al., 2015]. Undiscovered world of science represents a wide variety of microorganisms which in future will be able to possess new discoveries.it has also been reported that the number of microorganisms discovered every year will keep on increasing in the upcoming decades and also widen the field of marine research [Soliev et al., 2011] .it is difficult to deal with fatal diseases as the current treatment measures are inefficient but in near features the new discovery of marine world would help overcoming this problem with the discovery of new drugs, human learned the method to isolate medicinal drugs and also their application to defeat poisonous substances for non-medicinal purposes. According to estimates, 50% of commonly used medicines to treat human disease come from natural sources, the bulk of which are derived from terrestrial species thus eventually became one of the promising pharmaceutical approach [Pawar et al., 2015]. Moreover, Marine bacterial communities have a tremendous potential for developing a large variety of bioactive molecules, including pigment molecules and microbial culture media. Several marine gram-negative and gram-positive bacteria tend to produce a variety of pigments, and microbes appear to mediate the quorum sensing process by producing these pigments [Grossart et al., 2009]. Many marine bacterial pigments have been shown to have biological activities such as immunosuppressive, antibacterial, and anticarcinogenic properties, which

has increased demand for novel eco-friendly natural products such as bacterial pigments for biomedical and industrial applications, terrestrial counterparts have lesser diversity in-comparison to that of aquatic organism with the new discoveries of marine environment, the mysteries related to an aquatic flora and fauna will be solved. This makes marine environment became attractive research subject for the researchers [Reichenbach et al., 1980]. Pigmentation is common among bacteria, and carotenoids, flexirubin, xanthomonadine, and prodigiosin are among the pigments found in marine heterotrophic bacteria [Kim et al., 2007]. Carotenoids are the most common and abundant pigment group, produced by a diverse range of organisms ranging from non-phototrophic prokaryotes to higher plants, with over 700 different structures known to date. The biosynthesis of the carbon skeleton is based on condensation of isoprenyl units, and they appear yellow, orange, or red. The carbon backbone can be modified in a number of ways (oxidation, hydroxylation, etc.) to create a wide range of structures. The synthetic dyes have been found to be unsafe to human health. Bacteria, fungi, yeast etc., are common and rich in the production of pigments. Among this, the yeast is easily grown unicellularly [Zhao et al., 2019].

1.1. Source of Marine Microbial Pigments

Natural pigments have been derived from microorganisms and animals found in the sea. Marine algae play an important role in the manufacture of pigments in factories, and they also have biological applications such as antioxidant, anticarcinogenic, antimutagenic, antiangiogenic, photoprotective, anti-obesity, anti-inflammatory, neuroprotective, and so on. Red, green, and brown algae are classified as Chlorophyceae, Rhodophyceae, and Phaeophyceae, respectively, in the marine setting [Manivasagan et al., 2018]. Chlorophylls, carotenoids (carotenes and xanthophylls), and phycobilins are the three main photosynthetic pigments present in algae. Chlorophylls derived from thylakoid membranes using organic solvents including acetone, methanol, or DMSO are fat soluble molecules. Water soluble phycobilins and peridins are derived from algal tissues after chlorophyll extraction using an organic solvent. The source of various marine microbial pigments is listed in (Table: 1).

1.1.1. Glaukothalin; (Blue pigment)-The source of this pigment from the marine *Rheinheimera* spp. The two γ -Proteobacteria strains produce a diffusible blue pigment, HP1 and HP9 of the genus *Rheinheimera*. This pigment was isolated from German Wadden sea and from the Øresund, Denmark, respectively. The name of this blue pigment is glaukothalin, because of its blue colour and its marine origin (glaukos, gr = blue. Thalatta, gr = sea). MB2216 agar plate is used for the isolation of the blue pigment [Grossart et al., 2009].

1.1.2. *Micrococcus luteus*; (Yellow Pigment), saprotrophic bacterium that belongs to the family Micrococcaceae. The normal microflora of mammalian skin was mainly found in soil, dust, water and air. It gives the yellow pigment and it shows anti-bacterial and anti-fungal properties. The water sample collected from different parts of Marve beach and Aksa beach, Malad. Isolation of this yellow pigment is carried out on Luria Bertani media. The zone of inhibition for *S. aureus* can be seen in yellow pigmented colonies. Agar well diffusion method is used to detect the antimicrobial activity of pigments. To evaluate the antimicrobial activity they were used five pathogens against the extracted pigment (*Escherichia coli*, *Staphylococcus aureus*, *Candida*), they find the zone of inhibition. The sensitivity of the yellow pigment was tested by performing antifungal test, this can also be done by well-diffusion method [Minal et al., 2018].

1.1.3. Novel Yellow Pigment; this pigment is isolated from the marine bacterium *Pseudoalteromonas tunicata*. It is the new member of the tambjamine class of compounds. *Pseudoalteromonas* species produce some active biologically compounds that have the activity against common fouling organisms. By a combination of 1D and 2D-NMR spectroscopy and high-resolution mass spectrometry data was used to help achieve the structural identification. This is the first reported isolation of a tambjamine with an unsaturated alkyl chain from nature isolated from sponges and bryozoans [Franks et al., 2005].

1.1.4. Pigmented Bacteria From Malaysian Marine Environment; There are fifty-five marine pigmented bacteria were isolated from the sponges, seawater, mangrove sediment, sea cucumber and mussel from different coastal areas of Malaysia. Disc diffusion method is used for the detection of antimicrobial activity of the marine pigmented bacteria against the pathogenic bacteria. From these fifty-five pigmented bacteria of Malaysia, exhibited 41% of orange colony, 31% yellow, 11% red, 11% pink, 4% blue-green while 2% red and/or violet in colony [Jafarzade et al., 2013].

1.1.5. Pigmented Bacteria Associated with Marine Macroalgae from Antarctica; it is the Gram-positive strains were isolated from the microalgae collected in the King George Island, South Shetland Islands. They study about the diversity and antimicrobial activity of pigmented bacteria. Total 31 marine pigmented bacteria were isolated. 18 phylotypes were defined on the basis of 16S rRNA gene sequencing similarity $\geq 99\%$, which were clustered into 11 genera of Actinobacteria (*Agrococcus*, *Arthrobacter*, *Brachybacterium*, *Citricoccus*, *Kocuria*, *Labeledella*, *Microbacterium*, *Micrococcus*, *Rhodococcus*, *Salinibacterium* and *Sanguibacter*) and one genus of the Firmicutes (*Staphylococcus*). It was found that 5 isolates displayed antimicrobial activity against a set of macroalgae-associated bacteria [Leiva et al., 2015].

Table 1: Marine microbial pigment and their source and function.

Pigment	Source	Function	References
Glaukothalin (blue pigment)	<i>Rheinheimera</i> spp	Antibacterial	[Grossart et al., 2009]
Yellow pigment	<i>Micrococcus luteus</i>	Antibacterial, Antifungal	[Minal et al., 2018]
Novel yellow pigment	<i>Pseudoalteromonas tunicate</i> .	Antibacterial	[Franks et al., 2005]
The colony is 41% orange, 31% yellow, 11% red, 11% pink, 4% blue-green, and 2% red and/or violet.	<i>Vibrio parahaemolyticus</i> , <i>Staphylococcus aureus</i> , <i>Escherichiacoli</i> , <i>Pseudomonas aeruginosa</i> and <i>Candida albicans</i> <i>Bacillus subtilis</i> <i>Aeromonas hydrophila</i> , and <i>Aspergillus fumigatus</i>	Antimicrobial activity	[Jafarzade et al., 2013]
Pigmented bacteria associated with Marine Microalgae from Antarctica	<i>Kocuria palustris</i> , <i>Janibacter anophelis</i> , <i>Algibacterectus</i> , <i>Citricoccus zhacaiensis</i> and <i>Zobellia laminariae</i> .	Antimicrobial agent	[Leiva et al., 2015]

1.2. Classification of Microbial Pigments

The ease gene modification and short life cycle makes the bacterial pigment product more efficient over fungi. However, the research and production of bacterial pigment is at developing stage and thus requires intensive research and development approach to make it available in market [Narsing et al., 2017]. Pigment-producing bacteria can be found in a wide variety of ecological niches. Organic/inorganic and natural/synthetic pigments are the two types of pigments. The structural affinities and natural occurrence of biological pigments may be used to identify them. The following are some examples of naturally occurring pigments:

1.3. Types Bacterial Pigments

1.3.1. Carotenoids

The bacteria which are capable of producing such pigment are ubiquitous in the environment. Carotenoids were first isolated by Heinrich Wilhelm Ferdinand Wackenroder [Wilhelm et al., 2020]. Carotenoids are classified in to xanthophylls and carotenes. [Omayma et al., 2013] Carotenoids are tetraterpenoids and about 600 known carotenoids are present. Among the various variety of carotenoids, alpha and beta-are of major importance.

1.3.2. Melanin

All the living forms comprises melanin which suggest its evolutionary importance. *Magnaporthe grisea*, *Paracoccidioides brasiliensis*, *Colletotrichum lagenarium*, *Sporothrix schenckii*, *Cryptococcus neoformans*, *Aspergillus fumigates* [Langfelder et al., 2003] and *Vibrio cholerae*, *Shewanella colwelliana*, *Alteromonas nigrifaciens* have all been reported to produce melanin. [Soliev et al., 2011], Melanins are indolic polymers that contain eumelanins, allomelanins and pheomelanins.

1.3.3. Prodigiosin

Serratia marcescens, a red-colored bacteria, was the first to develop prodigiosin. [Dale et al., 1987]. Prodigiosin production has been identified in *Vibrio gazogenes*, *Vibrio psychroerythrous*, *Pseudomonas magnesorubra*, *Alteromonas rubra*, *Rugamonas rubra*, and *Streptovercillium rubrircetuli*, in addition to *Serratia marcescens*. Microbes that produce prodigiosin are common and isolated from marine ecosystems. [Darshan et al., 2015].

1.3.4. Violacein

Violacein is a violet pigment that was first discovered in the gram-negative bacteria *Chromobacterium violaceum* in Brazil and the Amazon River. Violacein has been reported from various organisms including *Chromobacterium violaceum*. [Yang et al., 2007].

1.3.5. Riboflavin

Riboflavin is a water-soluble pigment that has a strong yellowish-green fluorescence. Alexander Wynter was the first to isolate this pigment, and Kuhn and Weygand confirmed its structure. They proposed that riboflavin is made up of two distinct parts: a ribose sugar unit and a three-ring flavin structure known as lumichrome. Humans are needed to be supplemented with the vitamin riboflavin. Riboflavin also serves as a coenzyme for flavin mononucleotide and flavin adenine dinucleotide, which cause a non-enzymatic redox reaction by acting as dehydrogenating hydrogen carriers in the ATP production transport system. [Kutsal et al., 1989].

1.3.6. Pyocyanin

Pseudomonas aeruginosa blue pigment, pyocyanin [Hassan et al., 1980]. Pyocyanin has antibacterial and antifungal properties, so it's also used as a bicontrol agent. It's made up of two N-methyl-1-hydroxyphenazine subunits. [Norman et al., 2004]. However, the pyocyanin synthesized specific genes that function effectively, operons 1 and 2 of *phzRABCDEF*, is regulated by quinolone, which is produce by these genes [Mavrodi et al., 2001]. There are commonly used different types of pigments listed in ("Table: 2").

Table 2: commonly used pigments and activity.

Pigment	Activity	Strains of bacteria	References
Astaxanthin	Antioxidation	<i>Agrobacterium aurantiacum</i>	[Misawa et al., 1995]
Melanins	Protection from UV irradiation	<i>Shewanella colwelliana</i> <i>Vibrio cholera</i> <i>Cellulophaga</i>	[Kotob et al., 1995] [Ruzafa et al., 1995] [Kahng et al., 2009]
Prodigiosin	Antibacterial; Anticancer; Algicidal	<i>Hahella chejuensis</i> , <i>Pseudoalteromonas rubra</i>	[Gerber et al., 1979] [Kim et al., 2007]
Violacein	Antibiotic; Antiprotozoan;	<i>Pseudoalteromonas tunicata</i>	[Yada et al., 2008] [Hakvag et al., 2009]
Pyocyanin	Antibacterial	<i>Pseudomonas aeruginosa</i>	[Saha et al., 2008]
Benzoquinon	Anticancer	<i>Fusarium sp</i>	[Zheng et al., 2017]
Anthraquinone	Antioxidant	<i>Stemphylium lycopersici</i>	[Lif et al., 2017]
Blue Pigment	Heavy metal detection	<i>Vogesella indigofera</i>	[Gu et al., 2021]

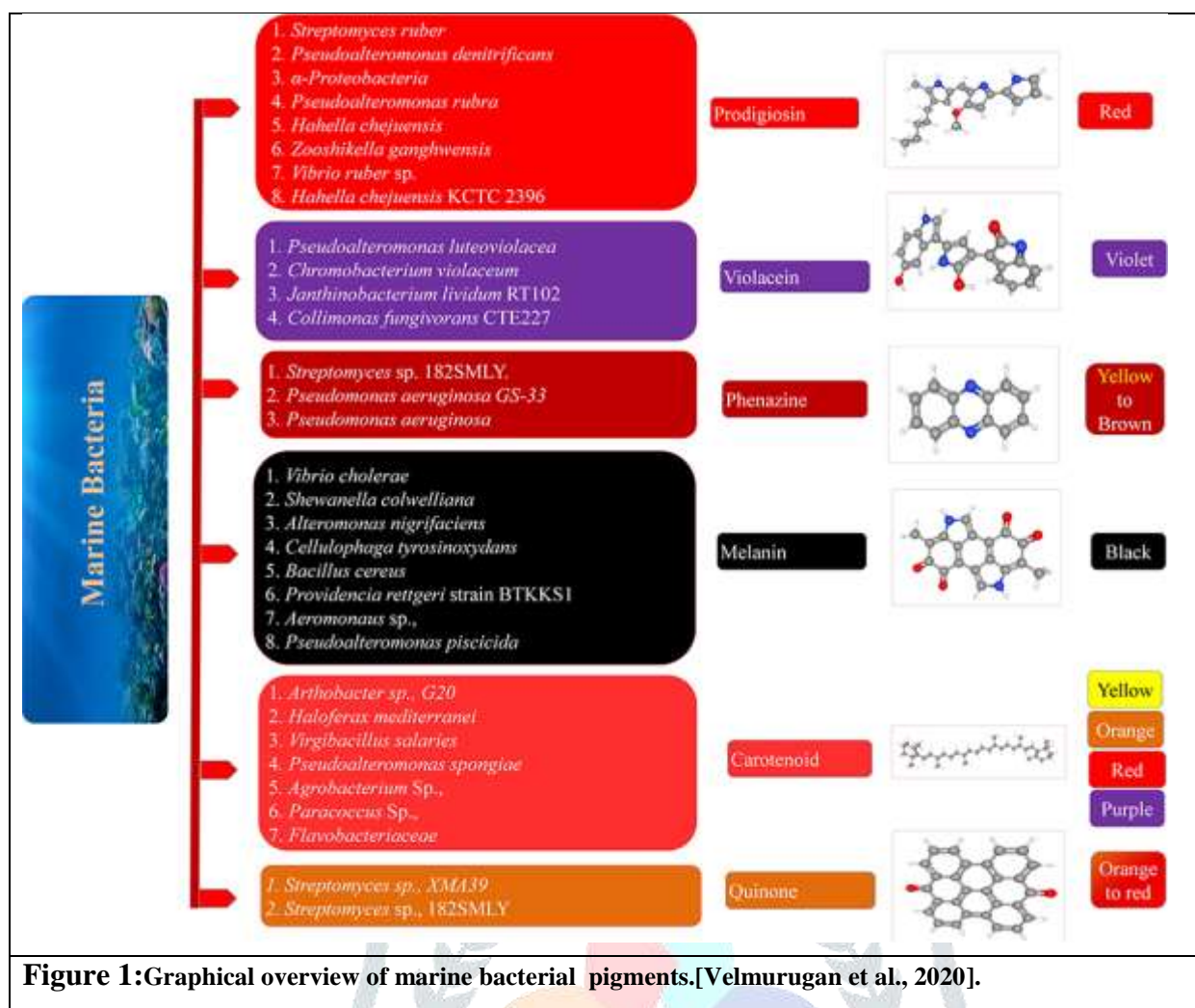


Figure 1: Graphical overview of marine bacterial pigments. [Velmurugan et al., 2020].

1.4. Physiological and biochemical properties of microbial pigments

1.4.1. Algal Pigments

Chlorophylls; mainly derived from *Chlorella* spp. It is a photosynthetic green pigment. Chlorophyll has been discovered to have anti-mutagenic effects as a food colourant. This is achieved by increasing the production of carcinogen detoxifying enzymes, which reduces the risk of cancer. Chlorophyll a and b are the two primary forms of chlorophyll. Which can be slightly differentiated because of their composition of side chain that is for chlorophyll a it is CH₃ and for chlorophyll b it is CHO.

1.4.2. β -Carotene; the halophilic green algae *Dunaliella salina* is used to produce β -carotene. This pigment is commonly used as a yellow-orange food colourant. Because of its high Vitamin A content, D. Solina popularised it as a nutraceutical additive. [Alam et al., 2018].

1.4.3. Phycocyanin; this blue pigment is made from blue green algae (*cyanophyta*). The main source of this pigment is *Spirulina platensis*. *Spirulina* grows best at a temperature of 27°C and in an alkaline pH range of 7.2 to 9.0.

2. APPLICATION OF MICROBIAL PIGMENTS

The pigment has been thoroughly researched in the last decade and its use can be traced back to the middle ages [Venil et al., 2014]. By using low-cost and sufficient growth mediums in industrial production of bacterial pigments, the cost and applicability of the pigments can be reduced. Natural pigments were commonly used in the trade until the discovery of synthetic pigments for colouring natural fibres (wool, silk, and cotton), leather, and fur. It also used to color, cosmetic product, ink production, water-cooling and paints.

Since Perkin's invention of synthetic dyes in 1856, a multitude of convenient and inexpensive synthetic pigments have become available. Natural dyes have become less common as synthetic pigments have become more affordable. Recently new pigment is being produced by a variety of microorganisms. Low temperature, high pressure, lack of light, and salinity characterise the marine environment. Pigment formation has been recorded in *Penicillium* [Dhale et al., 2009], *Aspergillus* [He et al., 2012], *Eurotium* [Smetanina et al., 2007], and *Trichoderma* [Blaszcecyk et al., 2014]. The pigments produced by terrestrial fungi are very similar to those produced by marine fungi. *Microsporium sp* produce the yellow pigment which happens to be derived from marine fungus [Li et al., 2006]. Fungus of the marine environment are found to be associated with algae and coral reef. Following are the advantages of bacterial pigments.

- It enables wide strain selection and simple propagation.
- Extremely versatile and highly productive in comparison with other sources.
- In comparison to any other chemical process, fermentation is much faster and more efficient.
- Manipulation of gene is easy.
- Continuous operation of bioreactor due to fast and simple culturing technique.
- Industrial needs are fulfilled by structural complexity.
- Makes the process cost low as the technique used for extraction of pigment is liquid-liquid extraction technique.
- Bulk production can be done using cheap substrates.
- Highly attractive to the field of science due to the broad ranging activities.

2.1. Pigments in Textile Industry

In the textile industry, approximately 1.3 million tonnes of dye precursors are used as synthetic dye. Around 1.3 million tonnes of synthetic dyes and dye precursors are used in the textile industry. Anthocyanin is one of the pigments that can be used as a natural dye [Priti et al., 2020]. *Serratia sp.* produces BTWJ8, a red pigment. *Serratia sp.* pigment development is influenced by a variety of bioprocess parameters. BTWJ8 is an abbreviation for maximum pigment production was achieved using a mineral salts tryptone sucrose (MSTS) medium. The pigment has been adopted by all textile firms, and sample evaluations revealed that the pigment has dyeing properties. The pigment is lost from the material after wash in soap solution at room temperature (28 °C) and at 40°C, according to wash efficiency studies with textile materials treated with pigment. At the incubation temperatures, pigment loss from comparable textile materials treated with thiourea as mordant was found to be less. As a result, it can be concluded that thiourea is an effective mordant for treating dyed textile materials. [Jissa et., 2011] The list contains dyes that are widely used in the textile industry (Table: 3).

Table 3: Pigments used as dye in textile industry.

Name of dye	Source	Application	References
Prodigiosin	<i>vibrio spp</i>	Wool, acrylics and silk	[Chidambaram et al., 2009]
Red prodigiosin	<i>Serratiamarcescens</i>	Acrylics, polyester, polyester microfiber, silk and cotton	[Usman et al., 2017]
Violacein (violet pigment)	<i>Chromobacterium violaceum</i>	Pure cotton, pure silk, pure rayon, acrylic, cotton, silk stain and polyester	[Chidambaram et al., 2009]
Pigment from	<i>Fusarium oxosporum</i> , <i>Trichoderma</i>	Cellulosic fibers	[Chidambaram et al., 2009]

	<i>virideand Alternaria spp</i>		
Red pigment	<i>Talaromyces verruculosus</i>	Antimicrobial(dye textile)	[Chadni et al., 2017]
Anthraquinone	<i>Fusarium oxysporum</i>	Dyeing of wool fabrics	[Nagia et al., 2007]

2.2. Role in food industries

The important goal in food industry is the attractive appearance of food. Due to the harmful health effects of artificial colour colouring, food processing facilities are turning to natural food colours. The pigment plays a significant role in the food industry because of the simple down-streaming process and production. Bacterial pigments play a significant role due to their ease of processing and down-streaming [Sen et al., 2019]. *Monascus*, *Rhodotorula*, *Bacillus*, *Achromobacter*, *Yarrowia*, and *Phaffia* are examples of microorganisms that contain a large number of pigments. Microbial pigments such as astaxanthin and carotenoids are common. Carotenoids are red and yellow pigments that are commonly used as food or feed supplements as well as antioxidants [Miura et al., 1998]. Phycocyanin is non-toxic and has no cancer-causing properties. The colouring of fermented milk goods, ice creams, chewing gum, soft drinks, alcoholic drinks, cookies, sweet cake decoration, and milk shakes are all examples of phycocyanin's uses in food [Flowerlet et al., 2017]. Many pigments are useful in the food industry; some of them are mentioned in the table below (Table: 4).

Table 4: Pigments used in the Food industries.

Pigment	Color	Microorganism	References
Astaxanthin	Pink-red	<i>Haematococcus pluvialis</i>	[Reyes et al., 1996], [Terao et al., 1989], [Guerin et al., 2003]
β -Carotene	Orange	<i>Dunaliella salina</i>	[Dufosse et al., 2005], [Kobayashi et al., 1993]
Lutein	Yellow	<i>Chlorella</i> and some other microalgae	[Dufosse et al., 2005], [Chen et al., 2016]
Phycoerythrin	Red	<i>Porphyridium cruentum</i> and many other microalgae and , <i>Cyanobacteria</i>	[Dufosse et al., 2005]
Phycocyanin	Blue-green	<i>Pseudomonas</i> spp.	[Baronet al., 1981]
Prodigiosin	Red	<i>Serratia marcescens</i>	[Deorukhkar et al., 2007]
Violacein	Purple	<i>Pseudomonas</i> spp.	[Duran et al., 2012], [Matz et al., 2004]
Canathaxanthin	Orange, pink	<i>Monascus</i> spp.	[Dufosse et al., 2018], [Mathews et al., 1982]
Riboflavin	Yellow	<i>Ashbya gossypii</i>	[Dufosse et al., 2018]
Carotenoids	Red	<i>Streptomyces</i>	[Dharmaraj et al., 2009]

2.3. Cosmetic and Cosmeceutical Pigments

Skin care is the most diverse segment of the industry. The use of natural ingredients will have a huge effect on the global cosmetics industry in the coming year. Surfactants, cleaning agents, emulsifying agents, thickening agents, stabilising agents, moisturising agents, gelling agents, and drying agents are all used by cosmetic companies. These natural pigments are safe for the consumers and does not affecting the body structure and functions. They are nontoxic, non-carcinogenic, and have no negative health implications. Natural pigments may also have other advantages, such as high antioxidant activity. Solid antioxidants and dyes can be made from marine pigments. Macroalgae are useful in a variety of industries: phycocyanin, a

blue pigment used in eyeliners, is one of the colourants. Cyanobacteria and the glaucophyte *Cyanophora paradoxa* are the primary sources of this material. Red–pink pigments such as - and R-phycoerythrin (R-PE) are used in lipsticks, eyeliners, and other cosmetics [Serive et al., 2018]. The most widely used pigments in the cosmetics industry are listed in the (Table 5).

Table 5: Pigments used in cosmetics

Bioactive Compounds	Microalgae/Cyanobacteria	Uses in Cosmetics	References
β -carotenes	<i>Dunaliella salina</i>	Antioxidant	[Chidambara et al., 2005]
Astaxanthin	<i>Haematococcus pluvialis</i>	Antioxidant Sunscreen protection	[Focsan et al., 2017], [Kobayashi et al., 2000]
Phycocyanobilin phycoerythrobilin	<i>Spirulina</i> <i>Porphyridium</i>	Antioxidant Pigment for eye-liner and lipsticks	[Mourelle et al., 2017]
β -Cryptoxanthin	<i>Dunaliella salina</i>	Anti-inflammatory Promote Hyaluronan synthesis	[Heydarizadeh et al., 2013],
Phycocyanin	<i>Porphyridium cruentum</i> <i>Spirulina platensis</i>	Eye-shadows	[Mourelle et al., 2017], [Balasubramaniam et al., 2021]

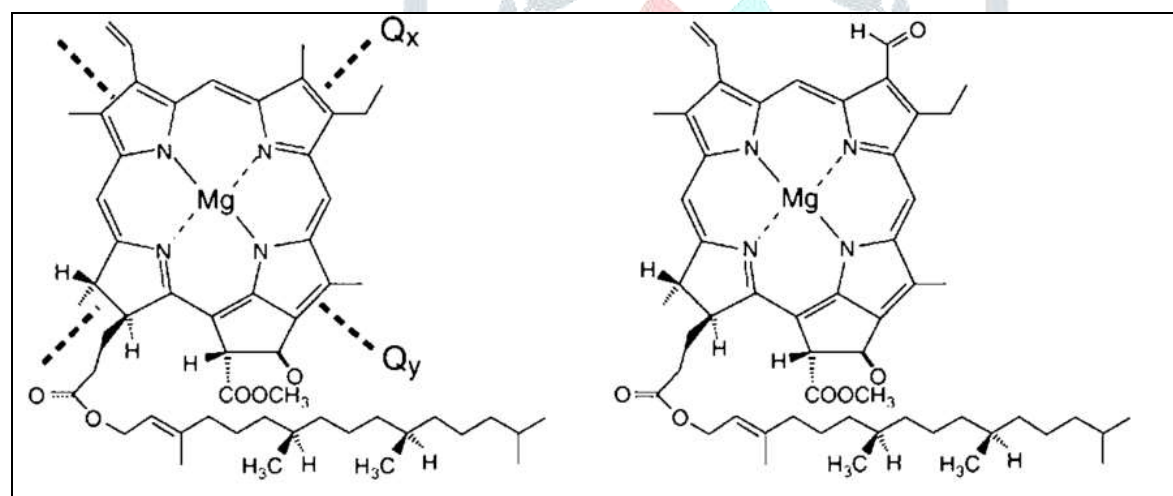


Figure 2: Chlorophyll a and b chemical structures.[Chen et al., 2011]

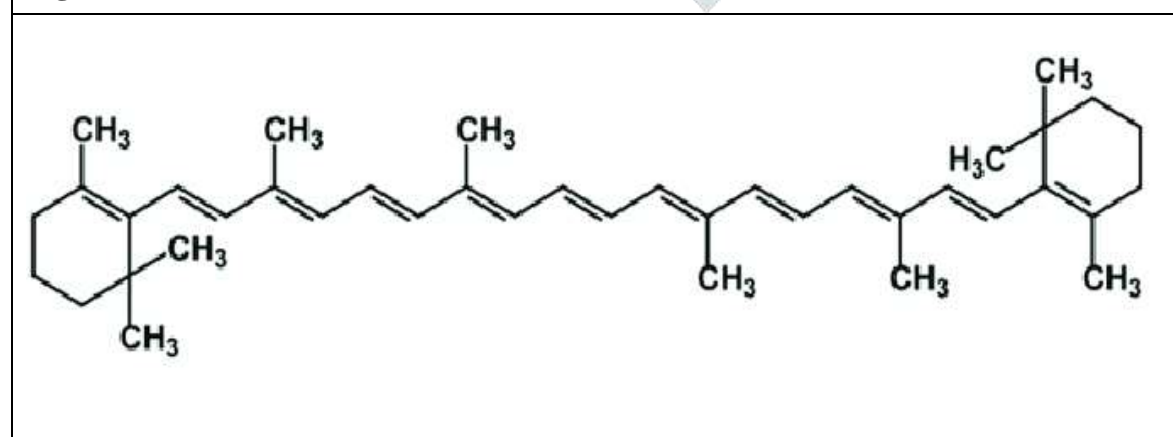


Figure 3: Carotene's chemical structure.[Shankaranarayanan et al., 2018]

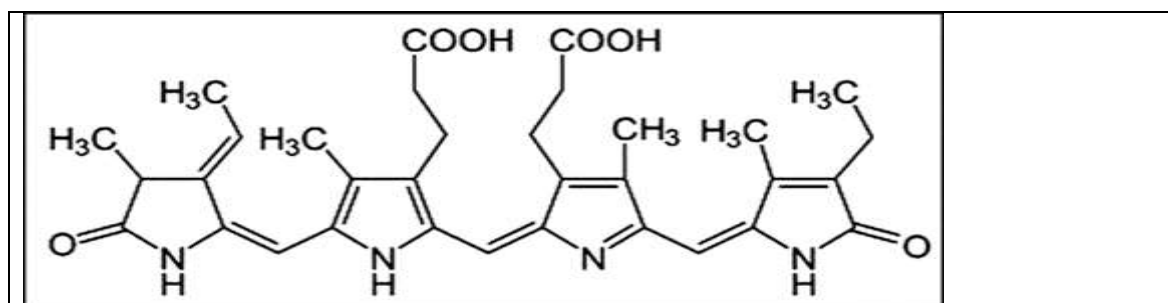


Figure 4: Chemical structure of Phycoerythrin.[Hosseini et al., 2013]

3. CONCLUSION

Natural pigments compounds originate from microbial source like bacteria, fungi and microalgae, in the case of synthetic pigments they cause human health issues. Microbial pigments are used in the textile industry, as well as antioxidants, food colourants, bio-indicators, anticancer agents, and antimicrobial agents. Apart from being used as a food colourant, flavouring agent, and dyeing agent, microbial pigments are also used in clinical therapy to reduce blood cholesterol, inflammation, and anti-diabetic activity etc. On many bench, natural pigment proves to be the way better than the artificial colour. Microbes will dominate the future, and it is urgently needed for sustainable growth, with natural pigments derived from marine bodies playing a significant role. Since water bodies cover 70% of the earth's surface, new findings would be abundant.

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