



Microbial Pigments: Sources and Applications in the Marine Environment

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ARTICLE INFO

Article History:

Received: Nov. 1, 2021

Accepted: Dec. 3, 2021

Online: Jan. 12, 2022

Keywords:

Microbial pigments,
marine sources,
applications

ABSTRACT

Pigments are colored metabolites secreted by different microorganisms. The uses of microbial pigments have amplified many folds due to numerous benefits over the synthetic pigments and are generally preferred due to the simplicity of culturing and extraction of pigments, cost-effective, safety for humans, thus having a wide range of applications in pharmaceutical, textile, cosmetics and food industries. Marine species represent a noteworthy sector of the oceanic population, which significantly produces biologically active moieties with different industrial and medical applications. Owing to the remarkable importance of microbial pigments in the marine ecosystem, the current review sheds light on marine microbial sources of pigments together with some applications in the marine environment. It also encompasses the need for prolonging the survey on novel extreme habitats and highlighting the future potential, targeting to contribute to the global economy.

INTRODUCTION

1. Introduction

Pigments are crucial compounds for several industries and can be used as color intensifiers, antioxidants, additives, etc. Pigments come in a wide diversity of colors; some of them are water soluble. Traditional sources of colorants including natural products such as anthraquinones and flavonoids are produced by animals and plants. For example, a deep red anthraquinone and carminic acid produced by insects are now used as a pigment in food colors, crimson ink, paints and cosmetics. Although synthetic colors are well-designed and are mostly used in the world marketplaces (42%) (Okafor *et al.*, 2016; Rajapaksha *et al.*, 2017), they have various side effects (e.g., teratogenic, cancer, etc.) (Okafor *et al.*, 2016). In addition, some of them are non-biodegradable such as textile dyes, producing problems to humans and aquatic organisms (Saini, 2017; Berradi *et al.*, 2019; Lellis *et al.*, 2019).

Now a world trend targeting the usage of biodegradable and eco-friendly natural colorants is increasing day by day. Therefore, investigators are trying to discover alternative biological, physical, and chemical techniques instead of the synthetic colors to evade the harmful effects on the environmental and public health (**Roy, 2018; Jamee *et al.*, 2019**).

Accordingly, microbial pigments have attracted an excessive request to improve textile grade, natural pigments, food grade and drug grade. The high request of natural microbial pigments are due to the talented limitless properties, including high manufacture of required pigments quantity, being eco-friendly, having no side effects, biodegradability, easiness of cultivation, less cost-effectiveness, stability, adaptability to various environments, optimization, genetic engineering. Further pigment properties worth mentioning, including their essential applications in several features, such as biomedical evolutionary, ecological, industrial and agriculture investigations (**Dufossé, 2020; Venil *et al.*, 2020**).

Several microorganisms are well-known to produce a an extensive diversity of pigments with inestimable industrial and biological activities (**Ramesh *et al.*, 2019; Nawaz *et al.*, 2021**). Absence of information on isolation sources of microbial pigments and their bioprospecting methods would make investigators make trials in research of natural microbial pigments. Constructed on the available review of the literature (**Ramesh *et al.*, 2019; Venil *et al.*, 2020**), current investigators are searching for novel strains, producing pigments such as chlorophylls, phycoerythrin and phycobiliproteins (**Joye & Kostka, 2020; Sigwart *et al.*, 2021**).

Spectral pigments have been determined from microbial and higher sources for different uses. However, the ecological significance and the biotechnological applications of nonspectral pigments in nature are not well investigated. Thus, investigations on these pigments require further research (**Chatragadda & Dufossé, 2021**).

The reserve of pigments, rate of production, price, effectiveness, durability, transport, sustainability, palatability, regulatory, jurisdictional, consumers' demand, and approval are the chief basics required for applications in marketable applications (**Ramesh *et al.*, 2019**).

2. Microbial pigments

Microbial pigments exert numerous roles in the survival and construction of the ecosystem. Significantly, pigments produced by fungi, bacteria, microalgae, and yeast compromise an extensive assembly of dyes, colorants and food applications. The demand for natural pigments is more than synthetic pigments due to the side effects of synthetic pigments on human healthiness. Consequently, further research regarding microbial

pigments is required to find prospective industrial applications (Chatragadda & Dufossé, 2021). Fig. (1) represents different types of microbial pigments.

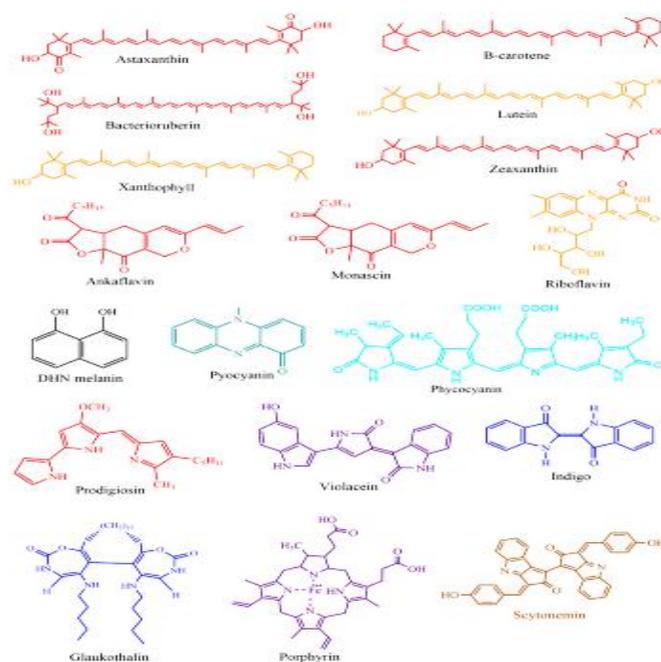


Fig. 1. Chemical structures of microbial pigments (Chatragadda & Dufossé, 2021)

3. Habitats and ecology of microorganisms producing pigments

A superfluity of research has described the isolation microorganisms producing pigments such as fungi, yeast and bacteria from terrestrial and marine environments. They are spread in several ecological area, from tropical to Polar Regions and from deep-sea to aerial districts. Microbes from divergent ecological areas are assumed to endure extreme conditions by the production of pigments. Some pigment producers, such as yeast, bacteria, and fungi that exist in terrestrial areas go into shore areas through the disposal of domestic sewages and hospitals, thus acclimatizing to coastal environment (Ramesh *et al.*, 2019).

Studies indicate that bacteria producing pigments could be classified into two groups; namely, true marine producers from mostly marine derivation and adaptive producers devised from terrestrial origin, which can live and multiply in aquatic ecosystem (Fig. 2). Regardless of the mutual existence in terrestrial area, marine producers gained more consideration owing to the diverse pigments production. Marine microbial pigments are considered as novel chromogenic compounds for industrial and biotechnological applications. The incidence of pigmented microbes in aquatic environment differ in terms of nutritional and geographical circumstances. For instance,

the diversity of pigmented heterotrophic bacteria is fewer in abundance comparing to the vast variety of heterotrophic bacteria in the marine area. The distribution and abundance may diverge reliant on seasonal deviation, accessibility of nutrients and sampling sites. High occurrence of bacteria producing pigments is noticed in ice cores (Zhang *et al.*, 2008), glaciers (Miteva *et al.*, 2004), salt lakes (Khanafari *et al.*, 2009), air–water interfaces (Hermansson *et al.*, 1987), underlying waters (Agogu e *et al.*, 2005) (sea surface microlayer), deep sea hydrothermal vents (Yurkov *et al.*, 1999), and hot springs (e.g., *Thermus*). Moreover, different pigmented populations have been gained from lava caves (Hathaway *et al.*, 2014).

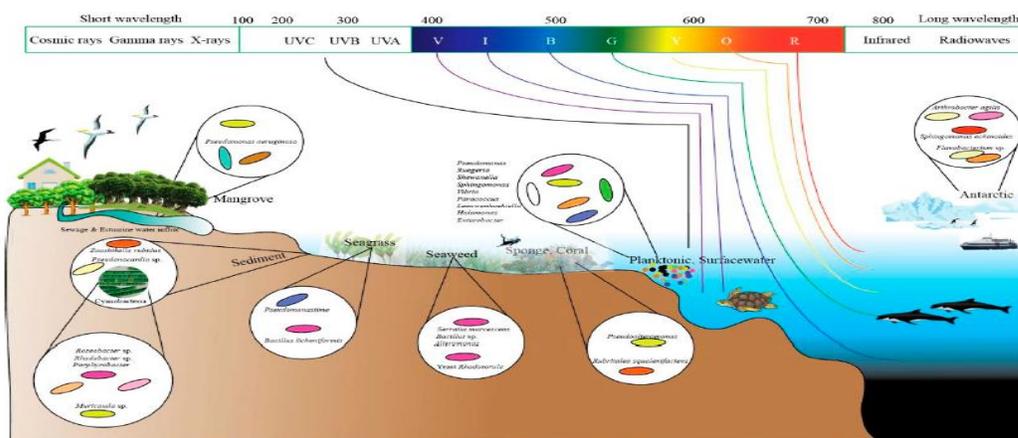


Fig. 2. Distribution of marine pigment producing microorganisms in different places (Ramesh *et al.*, 2019)

4. Microbial sources of pigments

Different research studies have been conducted to substitute synthetic pigments with natural pigments due to the richness of nature with pigment producing organisms. In 2011, total sales of natural colors amounted to a rate of \$600 millions. Microorganisms are acknowledged as a prospective source for production of biopigment due to their advantages over plants regarding cost efficiency, stability, availability, yield, labor and easiness in processing (Joshi *et al.* 2003). Varieties of bio-pigments, such as melanins, violancein, carotenoids, monascins, flavins and quinines are produced by microorganisms (Duffose, 2006).

Microbial cultivation can be achieved through submerged and solid fermentation, depending on industrial organic waste/ natural raw material. The majority of the microbial pigments possess not only the function as coloring agents in various cosmetics and food processing, but also antimicrobial, anti-inflammatory, anticancer and antioxidant applications (Venil & Lakshmanaperumalsamy, 2009). Moreover, there is a vast use for coloring agents in textile, paper and printing, plastic and paint industries.

The low microbial proportion that can be cultured and grow under laboratory conditions is one of the obstacles for the characterization of microbial pigments. In fact, more than 99% of marine microbes have not been effectively cultured under lab conditions (**Jiao *et al.*, 2021**). Almost 107 kinds of bacteria were gained from 1 gram of sediment, among which only 5% of the microorganisms were capable to grow in the lab (**Sun *et al.*, 2019**).

This phenomenon is determined as the “great plate count anomaly” (**Staley & Konopka, 1985**), which defines the differences between the cell number from the normal environment forming colonies on agar plates and that is countable using microscopes. Appropriately, in marine ecosystems, only 0.01 to 0.1% of marine bacterial cells yield colonies using standard plating techniques (**Connon & Giovannoni, 2002**). The second challenge is realizing an acceptable amount of the favorite metabolites to permit identification of bacterial pigments and their bioactivity (**Joint *et al.*, 2010**). Another challenge in this investigation relates to genome mining. As the number of the available genomes is increasing, genome mining is a suitable challenging way to identify new natural products and to confirm data (**Albarano *et al.*, 2020**).

4.1. Bacteria

The most extracted pigments from marine bacterial isolates in the past 5 years are shown in Table (1). At least seventeen kinds have been effectively isolated from their natural habitats, including sediments and seawater in association with marine invertebrates. It has been widely reviewed that marine sediment contains unexploited prospective for novel bacteria producing bioactive metabolites compared to seawater (**Bech *et al.*, 2020**). It was reported that sediments and seawater comprise a similar number of distinct bacterial species with a mean \pm standard deviation (SD) of 1452 ± 74 species for sediments and 1378 ± 61 species for seawater (**Bech *et al.*, 2020**). Symbiosis between microorganisms and invertebrates as hosts is mutual in marine environments and relies on chemical defense. It is a proficient policy for survival (**Flórez *et al.*, 2015**) leading to production of variability of biochemical traits due to coevolution with their specific hosts, making them a rich source of metabolites, particularly pigments with commercial interesting bioactivities (**Zhang *et al.*, 2015**; **Blockley *et al.*, 2017**; **Torregrosa-crespo *et al.*, 2018**).

Carotenoids “as a group of the new compounds that have been categorized over the past 5 years” are yellow to orange-red pigments, generally containing a 40-carbon skeleton composed of 8-isoprene units. Seven uncommon compounds include C50 and C45 carotenoids. By 2017, more than 250 carotenoids of marine source were recognized (**Galasso *et al.*, 2017**), and the inimitable compositions existing in marine microorganisms have encouraged the usage of carotenoids as a chemical mark for quick chemotaxonomic profiling.

Table 1. Recent bio-pigments extracted from marine bacterial isolates determined in the last five years (Brotosudarmo *et al.*, 2020)

Pigment	Chemical Formula	λ_{max} (nm)	Molecular ion (m/z)	Marine bacterial species	Therapeutic application
Carotenoid group					
Decaprenoxanthin	C ₅₀ H ₇₂ O ₂	417, 442, 471	705.6 [M+H] ⁺	<i>Arthrobacter</i> sp. P40	Antioxidant
Decaprenoxanthin monoglucoside	C ₅₆ H ₈₂ O ₇	417, 442, 471	867.6 [M+H] ⁺	<i>Arthrobacter</i> sp. P40	Antioxidant
Decaprenoxanthin diglucoside	C ₆₂ H ₉₂ O ₁₂	417, 442, 471	1029.6 [M+H] ⁺	<i>Arthrobacter</i> sp. P40	Antioxidant
2 ⁰ -Hydroxy-astaxanthin	C ₄₀ H ₅₂ O ₅	478	613 [M+H] ⁺	<i>Brevundimonas</i> sp. strain N-5	Antioxidant
2,2 ⁰ -Dihydroxy-astaxanthin	C ₄₀ H ₅₂ O ₆	478	629.0 [M+H] ⁺	<i>Brevundimonas scallop</i> <i>Brevundimonas</i> sp. strain N-5	Antioxidant
Dehydroflexixanthin	C ₄₀ H ₅₂ O ₃	-	581 [M+H] ⁺	<i>Rhodopirellula rubra</i> LF2T	Antioxidant
2 ⁰ -Isopentenyldehydrodicroxanthin	C ₄₅ H ₆₄ O ₂	470, 500	637 [M+H] ⁺	<i>Rhodopirellula rubra</i> LF2T	Antioxidant
Saproxanthin	C ₄₀ H ₅₆ O ₂	444, 470, 5	590 [M+Na] ⁺	<i>Rhodopirellula rubra</i> LF2T <i>Rubinisphaera brasiliensis</i> Gr7	Antioxidant
Zeaxanthin-sulfate	C ₄₀ H ₅₅ SO ₅ Na	427, 453, 481	648.5 [M+Na] ⁺	<i>Erythrobacter flavus</i> KJ5	Antioxidant
Quinone group					
Bisantraquinone 1	C ₃₂ H ₂₅ O ₉	229, 261, 287, 361, 415	553.15 [M+H] ⁺	<i>Ecteinascidia turbinada</i>	Antibacterial Anticancer
Fridamycins H	C ₂₅ H ₂₆ O ₁₁	231, 253, 293	503.15 [M+H] ⁺	<i>Actinokineospora spheciospongiae</i> sp. nov.	Antitrypanosomal
Fridamycins I	C ₃₂ H ₃₃ NO ₁₀ Na	232, 253, 294	614 [M+H] ⁺	<i>Actinokineospora spheciospongiae</i> sp. nov.	Antitrypanosomal
N-acetyl-N demethyl-mayamycin	C ₂₇ H ₂₅ NO ₈	328, 443	514.15 [M+Na]	<i>Streptomyces</i> sp. 182SMLY	Antibacterial
Mersaquinone	C ₁₉ H ₁₂ O ₆	218, 277, 308, 350, 480, 515, 550	337.07 [M+H] ⁺	<i>Streptomyces</i> sp.	Antibacterial
Streptoanthra quinone A	C ₂₈ H ₂₂ O ₈	220, 330, 445	509.12 [M+Na] ⁺	<i>Streptomyces</i> sp. 182SMLY	Antibacterial
Prodigiosin group					
Prodigiosin	C ₂₀ H ₂₅ N ₃ O	537	324.4 [M+H] ⁺	<i>Pseudomonas rubra</i> strain PS1 and SB14 <i>Zooshikella</i> sp. <i>Streptomyces</i> sp.	Antibacterial Anticancer Antiinflammatory
Phenazine group					
Phenazine-1-carboxylic acid	C ₁₃ H ₈ N ₂ O ₂	252, 365, 354	247.05 [M+Na] ⁺	<i>Pseudomonas aeruginosa</i> strain PA31x	Antibacterial Antifungal
Pyocyanin	C ₁₃ H ₁₁ N ₂ O	201, 238, 318, 710, 886	211 [M] ⁺	<i>Pseudomonas aeruginosa</i>	Antibacterial
Violacein group					
Violacein	C ₂₀ H ₁₃ N ₃ O ₃	585	344.12 [M+H] ⁺	<i>Janthinobacterium lividum</i> <i>Chromobacterium violaceum</i> <i>Pseudoalteromonas byunsanensis</i>	Antimicrobial Anticancer

Newly recognized marine bacteria that yield high levels of astaxanthin and its derivatives, such as 20-hydroxyastaxanthin and 2,20-dihydroxyastaxanthin, have also been studied (Asker, 2017; Liu *et al.*, 2020). The distinctive structure of astaxanthin, which contains both hydroxyls and a keto group, shows a vital role in neutralizing reactive oxygen species (ROS) (Brotosudarmo *et al.*, 2020). Newly described bioactive pigments from the quinone group have also been described, such as fridamycins, fridamycins H and I from *Actinokineospora spheciospongiae* sp. nov., beside 3 known pigments (actinosporins C, D and G) (Tawfike *et al.*, 2019).

Mersaquinone, a new tetracene derivative known for its antibacterial activity towards methicillin-resistant *Staphylococcus aureus*, was positively reported and characterized (Kim *et al.*, 2020). In addition, 2 new polycyclic anthraquinones, streptoanthraquinone A and N-acetyl-N-demethyl-mayamycin, existing in *Streptomyces* sp. 182SMLY were gained from a sediment sample at a 3.6 m depth (Liang *et al.*, 2016). Although prodiginines were originally recognized from the terrestrial bacterium *Serrantia marcescens*, these pigments were later gained from a number of bacteria in diverse marine habitats, for example *Pseudomonas rubra* strains PS1 and SB14, which were isolated from seawater (Setiyono *et al.*, 2020). Furthermore, they were gained from *Zooshikella* sp. and *Streptomyces* sp. in sediment (Ramesh *et al.*, 2020). Phenazine pigments, including pyocyanin and phenazine-1-carboxylic acid were successfully characterized from *Pseudomonas aeruginosa* isolated from sediment (Zhang *et al.*, 2017; DeBritto *et al.*, 2020). Fig. (3) represents different classes of pigments produced by marine bacteria. The search for novel pigments produced by marine bacteria during 1975-2021 is illustrated in Fig. (4).

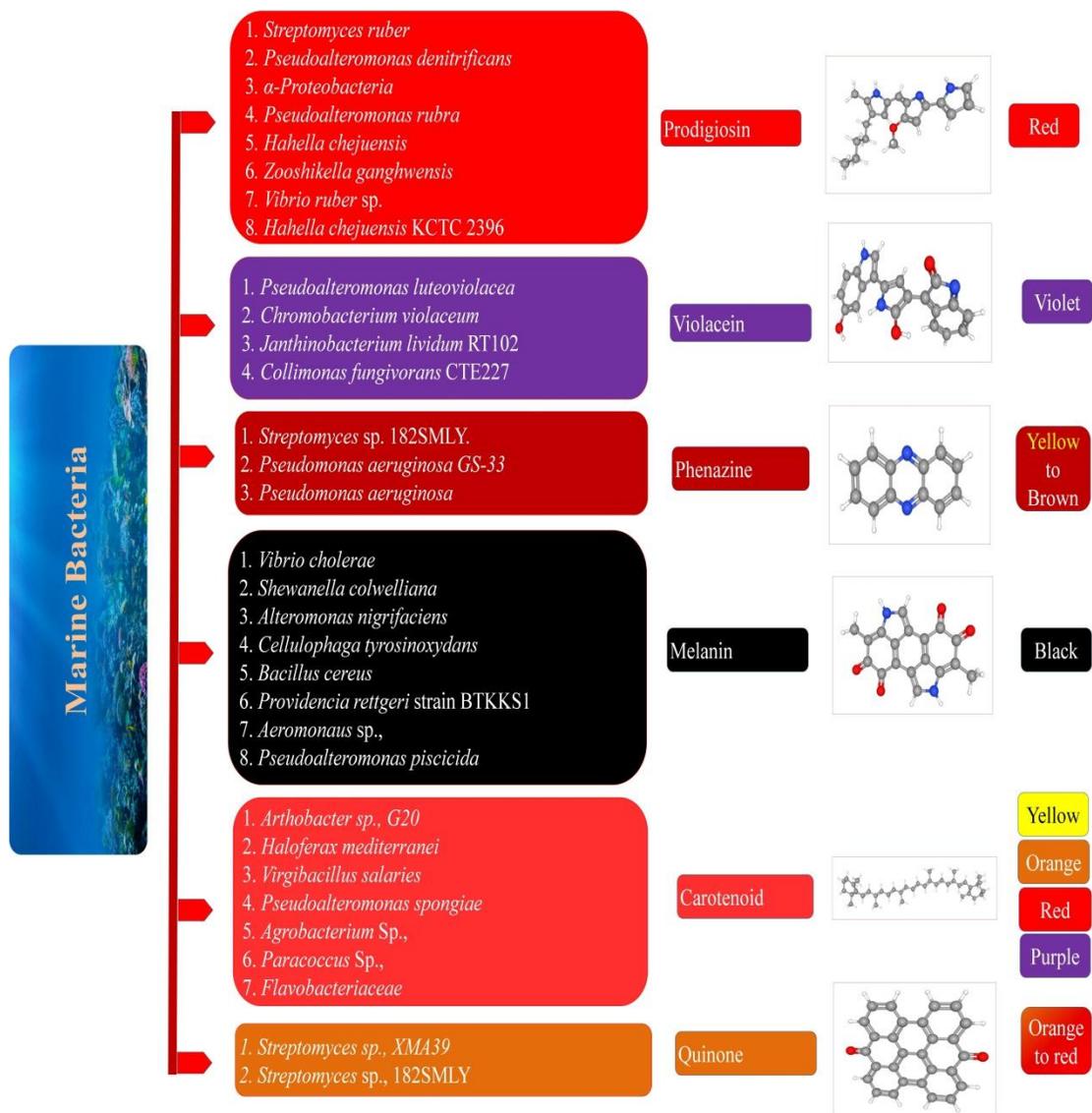


Fig.3. Kinds of marine bacterial pigments (Velmurugan *et al.*, 2020)

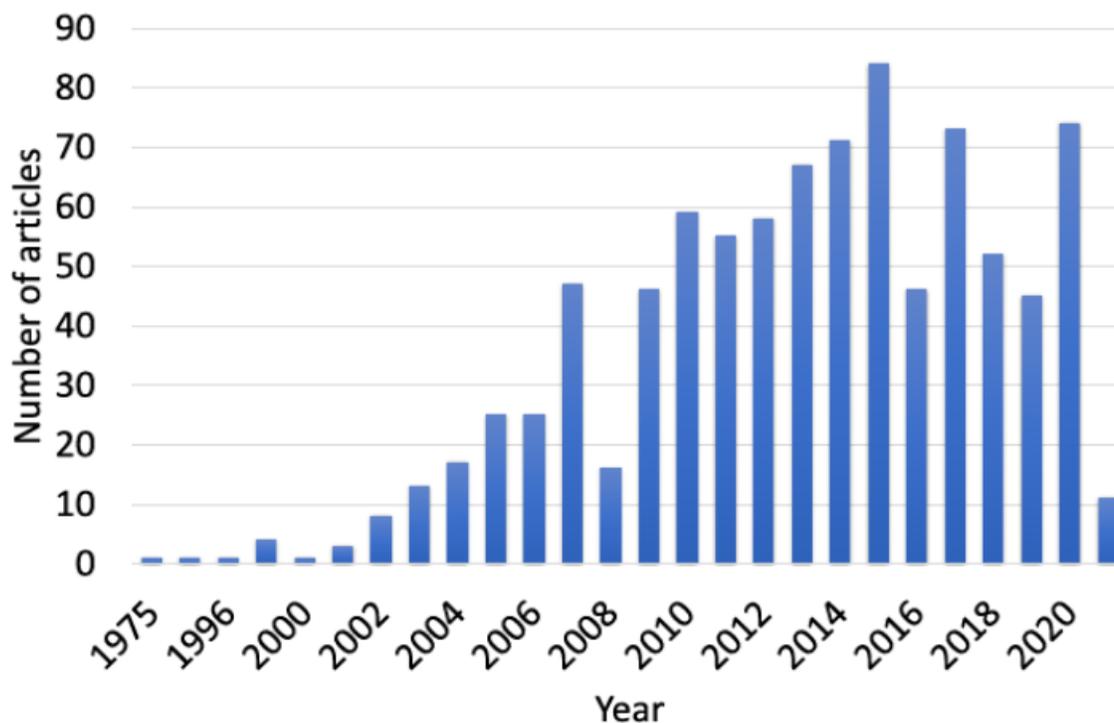


Fig. 4. Novel bacterial pigments produced from 1975 to 2021 (Velmurugan *et al.*, 2020).

4.2. Actinobacteria

Actinobacteria is the energetic genera in the excretion of secondary metabolites with unique compounds that can exhibit effectively vital traits in industrial applications. Sometimes production of pigments by actinobacteria has an imperative role in characterizing them. They are extensively used as coloring agents in printing industry, dyeing industry, textile industry and food industry (Chakraborty *et al.*, 2015).

Melanins represent the most mutual pigmented compounds produced by actinobacteria with colors ranging from black, brown to olive. In addition, they are produced by carotenoids with colors ranging from red, yellow, and pink to violet and from actinorhodin-related blue pigments (Rao *et al.*, 2017). Carotenoids group exhibits antioxidant capabilities and is used as a natural colorant in food, cosmetics, textile industries and pharmaceutical products (Parmar & Singh, 2018).

However, extraction of pigment from actinobacteria is not much experienced due to the limited fermentation methods. In some cases, the capability of the microorganism to yield pigment is either extremely improved or completely degraded due to the culturing techniques, the improper nutrition or other unknown explanations. Therefore, it

may be expected that incidence of strains which produces pigments with stability deserves study (Chakraborty *et al.*, 2015).

4.3. Fungi

Marine derived fungi have high diversity, and they exist in different habitats (Fig. 5). Their unique production of secondary components helps them survive in extreme environmental conditions, such as high pressure, high or low temperature, salinity and absence of light. These conditions lead to create progressive extremophiles capable of yielding certain exclusive compounds (Coker, 2016; Duarte *et al.*, 2019). Fungal strains from marine location exemplify a key basis of novel pigments and could be objective zones for marketable research.

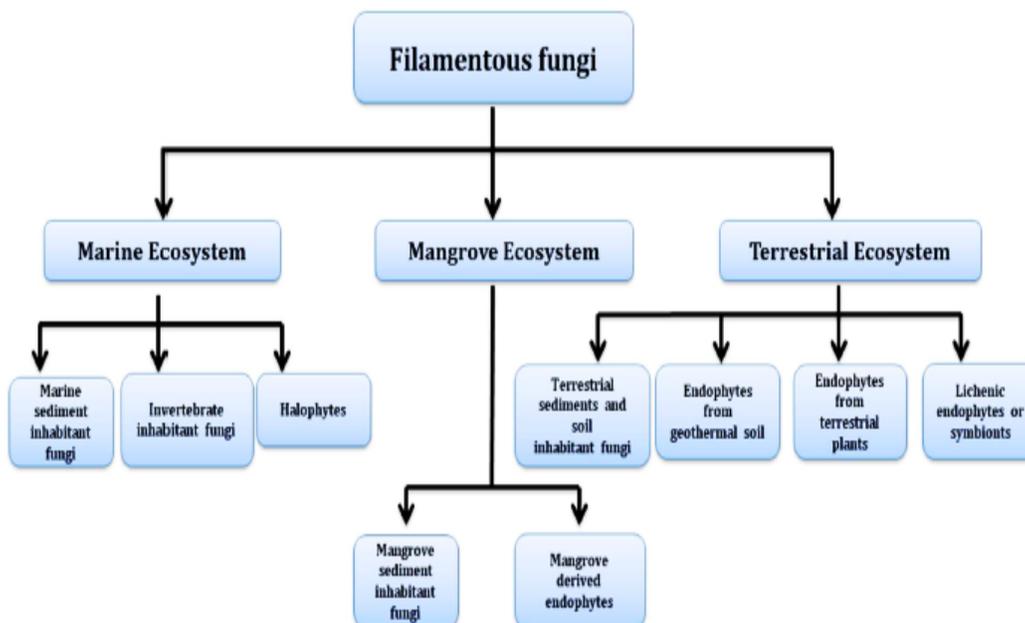


Fig.5. Several ecosystems forming a source of pigmented fungi (Kalra *et al.*, 2020)

Mycologists have been interested in fungal pigments production since the 19th century regarding them as a reservoir for the manufacture of food grade pigments. Fungal species yield a varied group of pigments from numerous groups such as anthraquinones, melanins, azaphilones, hydroxyanthraquinones, oxopolyene, carotenoids, naphthoquinone and quinones (Fig. 6) (Xie *et al.*, 2016; Chuyen & Eun, 2017; Pombeiro-Sponchiado *et al.*, 2017).

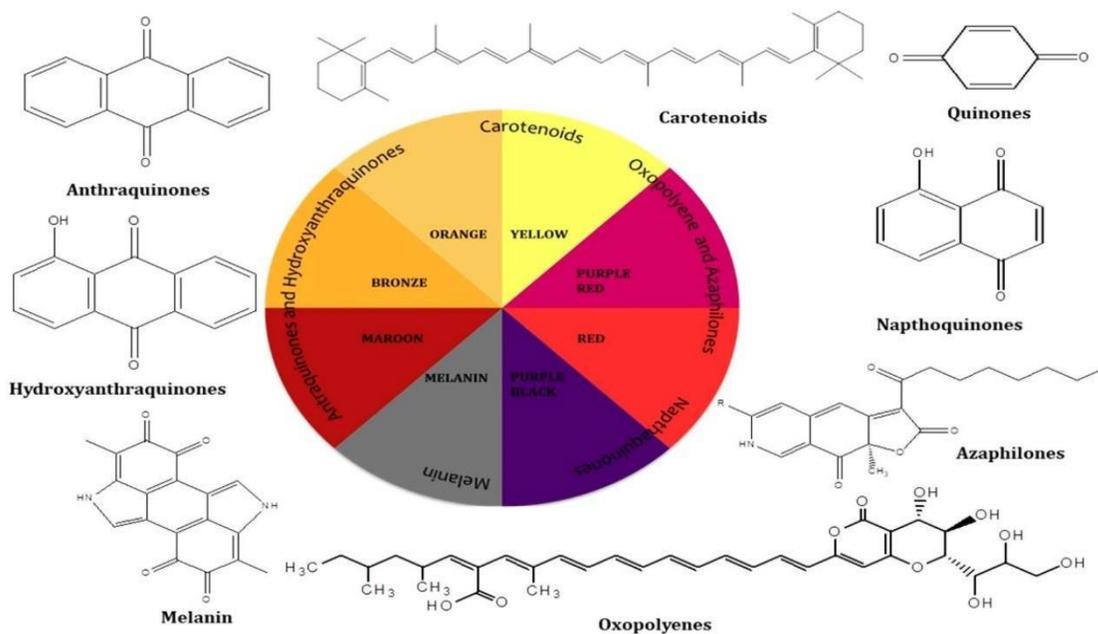


Fig. 6. Different kinds of colors representing different kinds of compounds (Kalra *et al.*, 2020)

The first natural red pigment Arpink red™ pigment (Natural red™) was produced from the soil fungus *Penicillium oxalicum* var. *armeniaca* CCM 8242 (Caro *et al.*, 2017). Several produced pigments are derivatives of polypeptides produced mainly by *Monascus* spp and *Neurospora* spp. and are classified as the ascomycetous fungi. Terpenoids, polyphenols, and carotenoids are other groups of polyketide based structure.

Several types of the produced natural pigments have a series of pharmacological applications, and they aid fungi in several biological actions, as for instance carotenoids (Gmoser *et al.*, 2017). They prevent the harmful effects of photooxidation and melanins and protect against environmental stress (Dufosse *et al.*, 2014), in addition to flavins which help as enzyme cofactors (Rao *et al.*, 2017). Although pigments produced by fungi have gained diverse biological activities, the factors that regulate the production and physiological roles are mostly unstudied (Sen *et al.*, 2019).

i. Invertebrate inhabitant fungi

Sessile and non-sessile invertebrates including squirts, sponges, and corals that are found in the aquatic ecosystem with brilliant colors, resulting from symbiotic microorganism and photosynthetic pigments found inside the invertebrates. Xu *et al.* (2008) examined fungi from tropical coral reefs to assess the degree of their bioactive metabolites. The previous authors isolated two novel yellow pigments from the *Aspergillus* sp. in the coral reef of Manado, Indonesia (Xu *et al.*, 2008). Additionally, *Eurotium cristatum* (ECE) isolated from the marine sponge *Mycale* sp. secreted a yellow

colored pigment known as 2-(2', 3-epoxy-1',3'-heptadienyl)-6-hydroxy- 5-(3-methyl-2-butenyl) benzaldehyde and 1,8-dihydroxy-6-methoxy-3-methyl-9,10-anthracenedione (physcion) (Almeida *et al.*, 2010).

In another research, an array of orange/red pigments was produced by *Talaromyces albobiverticillius* (B and C) (Venkatachalam *et al.*, 2019). These species belong mostly to the *Talaromyces*, *Penicillium* and *Aspergillus*, genera (Fouillaud *et al.*, 2017). They are important genera for industry. Moreover, *Alternaria* is recognized for the bioactive compounds such as anthraquinone derivatives, which represent commonly pigmented molecules with extensive array of bioactivities. Zheng *et al.* (2012) isolated several anthraquinone and hydroanthraquinones from the fungal isolate *Alternaria* sp. ZJ-2008003 existing in soft coral of South China Sea. Some of the isolated molecules, with yellow, red, and pink colors, form an additional indication of the wide range of colors attainable from invertebrates.

ii. Halophytes

Halophilic extremophiles are microbes that can grow and survive in regions of high salt (NaCl) content. They have excessive implication in biotechnological uses because of their capability to produce plentiful quantities of extracellular compounds (Ali *et al.*, 2014) from which melanins represent a group of these pigmented compounds, making a vital point in several cosmetic and pharmaceutical uses. The halophilic *Phaeotheca triangularis* and *Trimmatostroma salinum* gained from the halophiles area of Adriatic Sea was detected as a melanin producer (Kogej *et al.*, 2004). On the other hand, black yeast *Hortaea werneckii* forms a diffusible dark pigment on top of the growing potato dextrose agar. The produced pigment has an excessive important activity against *Vibrio parahaemolyticus* and *Salmonella typhi* (Rani *et al.*, 2013).

Halotolerant fungal species comprise some quinone compounds with bright colors, among which Variecolorquinones A and B were recorded being secreted by *Aspergillus variegator* B-17 (Wang *et al.*, 2007). Production of colors in the blue range made halophytes exhibit promising importance to food industry.

iii. Mangrove ecosystem

Mangrove ecosystems provide an extraordinary diverse environment, offering distinctive things of both terrestrial and marine ecosystems in one ecosystem. However, tidal habitat, high temperature, extreme stress and the fluctuating saline, surroundings of the mangrove validate a restricted number of kinds to survive in such intimidating environment (Kathiresan & Bingham, 2001). Consequently, the species inhabiting this environment represent an extremely rich area for substantial active and novel metabolites.

Nevertheless, limited studies have addressed mangrove fungi despite of their prospective secretion of a range of pigments (Zhang *et al.*, 2012).

iv. Lichens and endolichenic fungi

Lichens have significant rank and have become the emphasis of substantial medicinal concerns because of the incidence of distinctive compounds (Calcott *et al.*, 2018). Lichens represent a group of microorganisms with a close symbiotic association between the algal partner and fungal partner (DePriest, 2004). In this relationship, the fungal partner shields the algal partner from antagonistic geoclimatic effects. The production of pigmented molecules is a way of fungal partner to protect algal partner from ultraviolet radiation (Nguyen *et al.*, 2013). Although the slow rate of lichen growth in nature is a main obstacle to gain the produced pigments for commercial manipulation, modern studies have designated gaining pigments from cultivated fungi which opened novel trails for marketing commercial usages of pigments from lichens (Calcott *et al.*, 2018). Miyagawa *et al.* (1994) extracted two novel pigments, known as graphenone and graphisquinone, from the fungi of *Graphis scripta* and *Graphis desquamescens*. Some types of mycobionts have been detected in lichen forming new compounds under extreme circumstances such as osmotic hassle in the lab, which are not formed in the normal conditions (Kalra *et al.*, 2020).

A pigment includes a yellow pigment, tryptethelamide A and a novel dark violet red pigment 5'-hydroxytryptethelone along with three known dark violet-red pigments (–)-tryptethelone, (+)-tryptethelone and (+)-8-hydroxy-7-methoxytryptethelone isolated from the mycobiont of lichen *Tryptethelium eluteriae* (Basnet *et al.*, 2019). Endolichenic fungi were isolated from the thallus of lichen and attracted the attention for their ability to yield a series of compounds comprising pigments. A modern study on the separation of active compounds from the endolichenic fungus *Talaromyces funiculosus* generated 3 molecules including ravenelin, which is a yellow colored homogeneous powder that also retain respectable antimicrobial action, thus making it valuable for food and pharmaceutical divisions (Padhi *et al.*, 2019).

4.4. Marine yeast

Among the microorganisms, yeasts are conventionally used in food fermentation due to their less toxicity in nature compared to other microorganisms, in addition to their wide spread in almost all parts of the aquatic environment (Fell, 2001). Marine yeast form a respectable source of pigments, among which the most important recorded genera are *Rhoasporium*, *Candida*, *Metchnikowia*, *Cryptococcus*, *Kluyveromyces*, *Rhodotorula* and *Toruiopsis* (Hoog *et al.*, 2001). Metabolites produced by marine yeast are more stable in different temperature and pH. Morphologically, thirty different marine yeast species were isolated from twelve different samples collected from six different

saltern areas of Kelambakkam, India and were named as AMBY130 and AMBY101. Among them, only three strains were able to produce pigments (**Muthezhilan *et al.*, 2014**). **Ghosh (2011)** isolated yeast strains from West Bengal, India and were identified as *Candida ipomoeae*, *Rhodototula mucilaginosa*, *Candida famata*, *Kodamaea anthophila*, *Debaryomyces hansensii*, *Candida succiphila* and *Pichia lachancei*.

Methanol extract of the pigment produced by marine yeast *Rhodotorula* sp. (Amby109) exhibited antibacterial activity against different human pathogens, such as *Enterococcus faecalis*, *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *V. parahaemolyticus* and *Vibrio cholera* (**Muthezhilan *et al.*, 2014**). Additionally, different authors have studied the production of carotenoids by some yeast species such as *Rhodotorula* spp., which are broadly dispersed in nature and are capable of synthesizing specific types of carotenoids, such as torularhodin, β -carotene and torulene (**Perrier *et al.*, 1995**; **Tinoi *et al.*, 2005**).

4.5. Microalgae

Microalgae comprise 16 classes and 72,500 species. The largest groups are Cyanobacteria (Cyanophyceae), golden algae (Chrysophyceae), diatoms (Bacillariophyceae) and green algae (Chlorophyceae); green algae and diatoms are the most commonly used for biotechnological applications (**Guiry, 2012**).

Microalgae represent tremendous bases of molecules that are beneficial for health, such as pigments, lipids, proteins, vitamins, polyunsaturated fatty acids, polysaccharides, and minerals awarding pronounced prospectives in different markets. The universal concern to discover microalgae-created products has been growing due to their high-abundance, especially in the aquatic environment in addition to the value of their biological compounds (**Christaki *et al.*, 2015**; **Khanra *et al.*, 2018**).

Fig. (7) displays the evolution of publication number per year along the last ten years in the field of microalgal pigments. It is possible to detect an increase in the publication number in the periods from 2009 to 2013, 2014 to 2016, and from 2017 to 2019. While, in the periods from 2013–2014 and from 2016–2017, the number of publications was conserved. The results showed an 80% increase in the publication number concerning pigments from microalgae in the period from 2009 to 2019, which confirms the improved attentiveness in the area of pigments produced by microalgae.

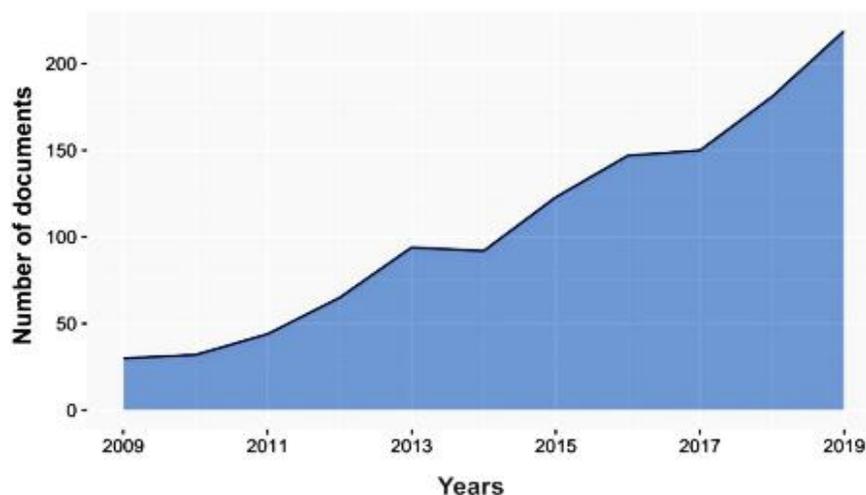


Fig. 7. The number of publications recorded from 2009 to 2019 in the area of pigments produced by microalgae (Silva *et al.*, 2020)

Among the varied range of compounds produced by microalgae, natural pigments are significant groups to be explored due to their health benefits (anti-inflammatory, anticancer and antioxidant) substituting artificial colorants (Rodrigues *et al.*, 2015). Three groups of pigments exist in microalgae; namely, chlorophylls, phycobiliproteins and carotenoids (Christaki *et al.*, 2015; D'Alessandro *et al.*, 2016).

Chlorella sp. and *Spirulina* sp. are mostly used as food and feed additives due to their high lipid content, proteins, minerals and vitamins. They are the most abundant in the microalgae market used as pigment sources, such as carotenoids and phycobiliproteins. *Haematococcus pluvialis* and *Dunaliella salina* are used at industries to gain carotenoids, especially astaxanthin which exhibit antioxidant activity and β -carotene (vitamin A precursor), respectively (Da Silva Vaz *et al.*, 2016).

For phycocyanin pigment produced by *Spirulina* according to a report published by the Future Market, the worldwide market was valued at USD 112.3 million in 2018. By the end of 2025, it is expected to exceed USD 232.9 million. The increasing awareness in phycocyanin pigment is noticeable through the recently approved European project SPIRALG (Horizon 2020) (www.spiralg.eu). Now a Givaudan business (the major flavor and fragrances producer) announced the production of phycocyanin through a new extraction line (Silva *et al.*, 2020).

Phycocyanin synthesized by *Spirulina* has been used in the cosmetic/food industries, specifically in beverages (e.g., Bloo Tonic®, B-blue *Spirulina* drink, and M&Ms® chocolates). Phycocyanin can also be used either as a colorant or food supplement due to its efficient benefits. In this context, AlgoSource

(www.algosource.com), a French company, produces phycocyanin as a liquid extract (Spirulysat®) that is sold in phials (10 mL). Another company under the name of Spira (www.spirain.com) yields phycocyanin in the form of powder (Electric Sky®) to be used for health benefits (food supplement) and coloring applications (Silva *et al.*, 2020).

5. Applications of biopigments in the marine environment

5.1. Application in aquaculture

Aquaculture is developed in the coastal area of the world. Now, ~50% of the fish production in the world for human feeding and the non-fish production are based on aquaculture (FAO, 2016). Egypt, India, China, Bangladesh, and Vietnam are the main fish manufacturers. It is important to regulate the cycle of fish reproduction and manage the capability of feeding them during part/ whole of the breeding cycle. The value of the diet is critical, owing to its effect on the organoleptic value and the time of breeding. Thus, studies focused on the increase of growth and health of farmed species. As a result of the deficiency of diversity of food under breeding conditions, addition of pigments to the diets is particularly significant. In this respect, canthaxanthin and asthaxanthin are used in feeding salmonid (Serive & Bach, 2018), making the final product more simply marketed.

In the Western countries, consumers are ready to pay more and find their demanded food in return. In this perspective, the use of asthaxanthin to color prawns reared in aquaculture was reported. In aquaculture, the high density of rearing of larval stage causes great rate of mortality and oxidative stress. To overcome this, farmers rear in green waters to improve larval survival. The effect is not completely understood, but the composition of pigment might have a role. Larvae have phototaxis, which is critical for their survival, and this can be improved using carotenoid supplements such as astaxanthin. Zeaxanthin and Lutein might have an essential role in this physiological function. Astaxanthin supplements can promote the number of eggs during laying with respect to the striped jack fish (*Pseudocaranx dentex*). β -Carotene can be used in diet of prawn before harvesting (175 ppm during 5–6 weeks for *Penaeus monodon*). The action of carotenoids is not restricted to pigmentation, but it can improve growth, tolerance to salinity and ammonia as well. Phycobiliproteins and chlorophylls can be implemented on diets when living prey is used as a feed of cultivated species (Boonyaratpalin *et al.*, 2001).

5.2. Antifouling agents

Billions of dollars are spent every year for antifouling agents to control activities of fouling on diverse matters existing in the marine environment. Biofouling on ships can promote frictional resistance causing a rise in fuel consumption and other resultant environmental problems. Heavy metal-based antifouling agents cause severe environmental complications, which further obligate the requisite for “eco-friendly”

antifoulants. Scientists have also displayed the use of pigments- based antifouling agents, for example, prodigiosin gained from *Serratia* sp. showed antifouling activity towards marine *Gallionella* sp. and *Alteromonas* sp. that are known as fouling bacterial species. It also repressed the adhesion of *Cyanobacterium* sp. on the glass surface (Priya *et al.*, 2013). Similarly, the pigment polymelanin made by the marine bacterium *P. lipolytica* sp. inhibited metamorphosis and reduced the settlement of invertebrate larvae (Zheng *et al.*, 2017), thereafter demonstrating the action of marine pigments as prospective antifoulants.

5.3. Absorption of heavy metals

The extracted melanin from bacteria has also been described for the treatment of heavy metals. Manirethan *et al.* (2018, 2020) used the extracted melanin pigment from *P. stutzeri* for heavy metals absorption of lead, copper, mercury, arsenic and chromium, where the absorption may be due to the presence of the COOH, phenolic OH and NH groups, and the maximum efficiency was absorbed in mildly acidic to neutral range of pH. The efficiency of melanin in removing the arsenic by chemisorption was proved, and melanin displayed a thermostability up to 120°C and reached a peak of absorption of arsenic at pH 4–6. Impregnated melanin was oxidized to arsenate and finally removed the Cu and Fe by using HCl for the recycling process of melanin. Then, it was re-soaked with fresh Fe and Cu elements for absorption. This revealed 99% efficiency to 4 absorption and desorption cycles.

Batch adsorption studies showed that 280 mg/g of lead and 169 mg/g of copper were adsorbed by immobilized melanin-alginate beads. The metal ion adsorption capacity of the melanin-alginate beads was relatively significant compared to alginate beads (Sajjan *et al.*, 2013).

6. Pathogenicity of pigments producing microbes

In spite of the various acknowledged applications of pigments, the studies suggest that some bacterial producers of pigments are evolved in humans and in aquaculture farms as pathogens. *Chromobacterium violaceum* producing Violacein cause infections in adults and children (Jitmuang, 2008). *Serratia marcescens* produces Prodigiosin, which infects invertebrates, vertebrate, humans and insects (Grimont & Grimont, 2006; Petersen & Tisa, 2013). Strains of *C. violaceum* and *S. marcescens* are opportunistic pathogenic bacteria to humans (Mahlen, 2011; Sharmin & Kamal, 2019). Nonetheless, no reports are detected about the contribution of prodigiosin and violacein in virulence function. Notably, prodigiosin pigment did not cause a virulence role in entomopathogenic *S. marcescens* (Zhou *et al.*, 2016). Yet, few pigments such as bacterial melanins (Nosanchuk & Casadevall, 2003) and pyoverdines (Kang *et al.*, 2019) regulated virulence function.

Fungi such as *Monascus* and *Fusarium* produce red pigment known as mycotoxins linked to pathogenicity (Wang *et al.*, 2005). Thus, investigators are searching for species of fungi that do not yield toxins, and it was suggested that determining the

pathogenicity (hemolytic activity) of the produced pigment would aid avoiding toxicities and mortality (Mapari *et al.*, 2009).

7.Future perspective

Some limitations such as lower stability, high cost needed for large scale production, variations in operational parameters, less percentage regarding annual production, specifications concerning microorganisms capable of producing different colors, and technological limitations cause delay in the advancement of the industrial production and the extraction of pigments in a pure form. Future researches should be carried out particularly to find the easiest way for gathering microbial pigments to enhance their applications in industry. Correspondingly, there is a requirement to regard several operational factors that may lead to a disparity due to the alteration and progress of new process by using low cost agrowaste as substrate in the future to recognize high production of pigments. Research on numerous technologies that could decrease the cost and increase the yields of pigment production on large scale should be conducted in the future.

Extending the survey on the undiscovered samples from novel environments belonging to marine ecosystem would surely provide talented results in finding new metabolites from promising microbes. Numerous pigment producing microbes have been stated till now, while their bioactivities ought to be assessed. Therefore, creating records from novel microorganisms is of great significance in understanding the biological actions of pigments. The survey of pigment chemodiversity is still prevalent in biotechnologies, agronomy, aquaculture and oceanography. Pigments may receive a renewal awareness for the discovery of drugs and other promising applications in the coming decades.

8.Conclusion

Nature is a gorgeous source of pigmented microbes, such as bacteria, actinobacteria, fungi and yeasts. Natural pigmented compounds became more demandable and valuable over synthetic pigments. Marine ecosystem has been a focused poin for the investigation of novel natural pigmented compounds with an extensive array of bioactivities owing to massive marine resources, which comprise numerous novel pigment producing microbes. Remarkably, microbial pigments represent attractive field of study in life sciences due to the innumerable applications in different activities.

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