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INTRODUCTION OF NATURAL PIGMENTS FROM MICROORGANISMS

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1.1. INTRODUCTION

Pigments are widely used in a variety of industries. In the food industry, one of the most important goals is to develop foods that have an attractive flavor and appearance. Artificial food coloring using synthetic dyes can make foods more appealing and desirable. However, the safety of these dyes has been questioned. Recent research has linked synthetic food dyes to a number of potential health problems, such as cancer in animals and attention-deficit disorder in children (Potera 2010). Synthetic colorants are criticized for having these problems, and consumers are showing more and more interest in products that do not include artificial coloring agents. Therefore, various natural sources of food-grade colorants are in high demand. The textile industry also uses millions of tons of dyes, pigments, and dye precursors every year, and almost all of them are manufactured synthetically (Chequer *et al.* 2013). Synthetic dyes have serious limitations in that their production involves the use of toxic chemicals and can generate hazardous wastes, which is unfriendly to the environment and to human health (Khan *et al.* 2013).

Biological pigments are substances from biological sources that have a particular color, corresponding to their structure. They are found in plants, animals,

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and microbial organisms. Natural pigments have been long studied, but they are receiving increasing attention from industry because of the potential health and environmental concerns around synthetic dyes. Biological pigments from microbial cells are termed "microbial pigments." In addition to their function as colorants, some microbial pigments are also used to promote human health, providing key nutrients or compounds required by the body. Some also have particular biological activities, such as anti-inflammatory, antibiotic, anticancer, and immunosuppressive properties (Soliev *et al.* 2011). Microbial pigments with fluorescence are used in laboratories to label antibodies (Mahmoudian *et al.* 2010). Some pigments can also be used to indicate the progress of specific reactions or to track pH changes through changes in their color (Venil *et al.* 2014). A large number of pigments are produced by various species of bacteria, yeasts, fungi, and algae, with colors including brown, black, red, orange, yellow, green, blue, and purple, and structures such as carotenoids, anthraquinones, flavonoids, and tetrapirroles. Different biosynthetic enzymes are involved in the biosynthesis of microbial pigments. For example, carotenoids are typically synthesized by terpene synthases, flavonoids are assembled by polyketide synthases (PKSs), and indigoidine – a bacterial blue pigment – is synthesized by a nonribosomal peptide synthetase. Microbial pigments are used for different purposes depending on their color property and biological function. This chapter covers a variety of microbial pigments from eukaryotic and prokaryotic sources and discusses their properties and applications.

1.2. MICROBIAL PIGMENTS FROM EUKARYOTIC SOURCES

The cells of eukaryotes such as plants, animals, and fungi contain a nucleus and other organelles. Eukaryotic microorganisms produce a lot of different pigments. Some representative pigments from these organisms are described in this section, categorized according to their source: algae, fungi, and yeasts.

1.2.1. Pigments from Algae

Algae produce a variety of pigments. The most commonly used in the industry is the carotenoid β-carotene (Figure 1.1). Carotenoids belong to the family of tetraterpenoids and are found in the chloroplasts and chromoplasts of plants, algae, fungi, and some bacteria (Asker *et al.* 2007). They are yellow, orange, and red pigments that can be used for coloration. β-carotene is a red-orange nonpolar pigment that can be obtained from *Dunaliella salina*, a kind of marine green microalga. The production of β-carotene in *D. salina* is affected by high salinity, temperature, and light intensity. A high β-carotene content in *D. salina* can help it protect itself from intense light and osmotic pressure in the ocean (Oren 2005). β-carotene is well known for its antioxidant activity and for its use as food supplement (Stargrove *et al.* 2008). It is commercially produced across the world, due to its widespread use (Oren 2005). The first company to manufacture and sell natural β-carotene, Betatene Ltd., was established in 1985 (Nelis and Deleenheer 1991). Production of β-carotene from

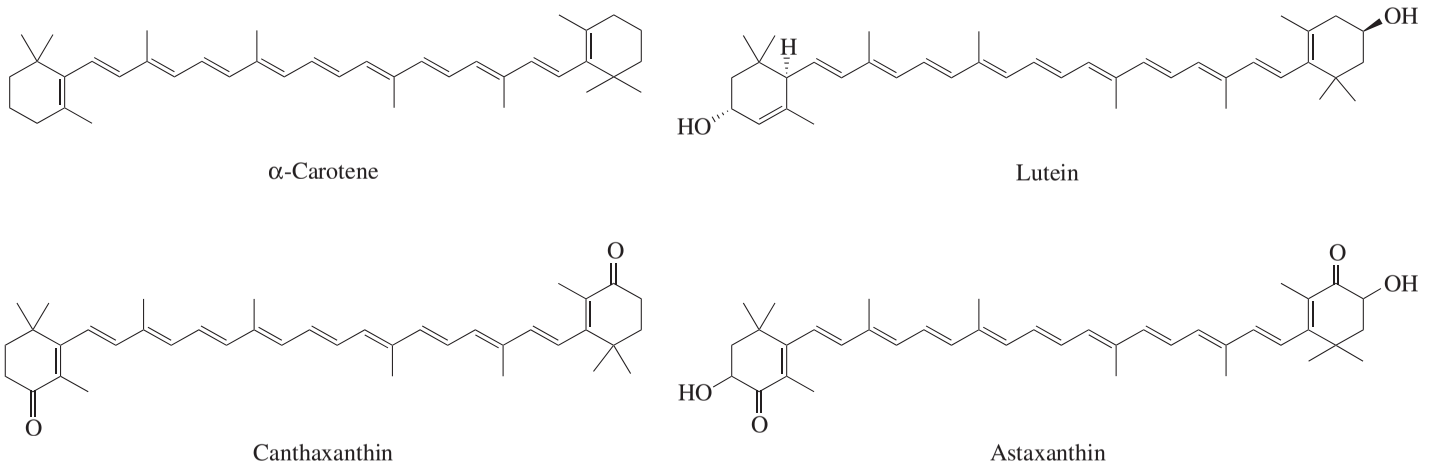


Figure 1.1. Structures of four representative carotenoids: β-carotene, lutein, canthaxanthin, and astaxanthin.

D. salina is often seen in large open ponds located in or near salt lakes in Australia, the United States, and China.

Besides β-carotene, many other carotenoids are produced by microalgae. For example, lutein (Figure 1.1) is obtained from different green algae, such as *Chlorella*, *Chlorococcum*, *Chlamydomonas*, and *Spongiococcum*. Lutein is a red-orange pigment that is generally insoluble in water. For some time, it was widely used in chicken feeds to improve the color of broiler chicken skin and egg yolks (Philip *et al.* 1976). In the human body, lutein is concentrated in the macula. Some research has revealed that lutein protects eyes against oxidation (Berendschot *et al.* 2000; Malinow *et al.* 1980). Canthaxanthin (Figure 1.1), a dark red food coloring agent, is another example of a cartenoid produced by algae. *Dictyococcus cinnabarinus* was reported to produce it canthaxanthin in 1970. The final concentration of cellular canthaxanthin in this organism is 1.0–1.2 mg/g (Tuttobelll and Ranciag 1970). Astaxanthin (Figure 1.1) is a red terpene that is biosynthesized by *Haematococcus pluviais* with up to 2% dry weight quantity (Nonomura 1990). This compound is a food coloring agent approved by the US Food and Drug Administration (FDA).

Algae produce many other microbial pigments, including water-soluble green chlorophyll, blue phycocyanins, and red phycoerythrins, from *Rhodophta*, *Cyanophta*, and *Cryptophyta*, respectively (Telford *et al.* 2001). *Halobacterium* spp. have been found to be responsible for the red color in the Great Salt Lake, Dead Sea, and Lake Magadi (Oren 2005).

1.2.2. Pigments from Fungi

Fungi comprise a diverse group of eukaryotic organisms, including yeasts, molds, and mushrooms. Some fungi are known to produce color compounds with particular biological properties. Many fungal pigments possess ecological functions varying from providing protection against environmental stress to preventing photo-oxidation. Some pigments, such as flavins, can even act as cofactors in enzyme catalysis (Mapari *et al.* 2010).

Riboflavin (vitamin B2) is a yellow food colorant that is approved for use in many countries. It is also used in the clinic to treat neonatal jaundice (Bailey *et al.* 1997) and it has been reported to prevent migraine (Sandor *et al.* 2000). Its structure is shown in Figure 1.2. Many molds can be used to produce riboflavin through fermentation (Jacobson and Wasileski 1994; Santos *et al.* 2005; Stahmann *et al.* 2000). *Ashbya gossypi* has been widely used in the production of riboflavin, as it provides a high yield and good genetic stability. Its final riboflavin level can reach 15 g/L (Broder and Koehler 1980).

A variety of color compounds have been discovered from fungi. The same genus may produce different pigments. This is exemplified by *Monascus*. *Monascus* can be classified into four different species: *M. pilosus*, *M. purpureus*, *M. ruberand* and *M. froridanus*. Different *Monascus* species produce many different industrially important pigments with three colors: red, orange, and yellowish. For example, *M. purpureus* 192F produces the yellow pigments monascin and ankaflavin, the orange pigment rubropunctatin, and the red pigment monascorubramine (Figure 1.2).

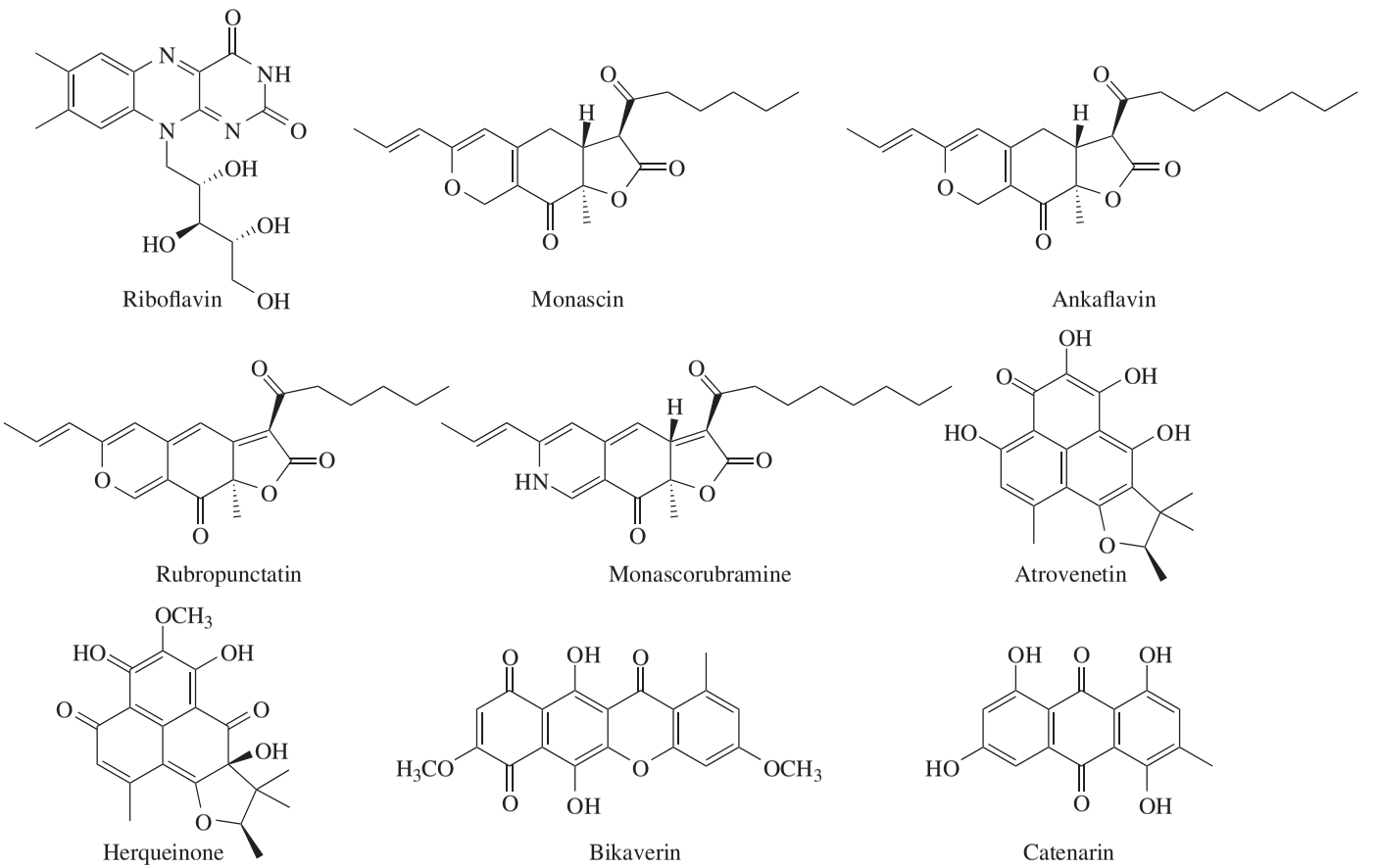


Figure 1.2. Nine representative fungal pigments: riboflavin, monascin, ankaflavin, rubropunctatin, monascorubramine, atrovenetin, herqueinone, bikaverin, and catenarin.

Monascorubramine is the major product. The pH and nitrogen source in the fermentation broth affect the composition and yield of the pigments. Supplementation of *Monascus* pigments as a coloring agent into food can provide novel flavors (Chen and Johns 1993). These fungal metabolites have also shown interesting biological activities. For example, monascin and ankaflavin are natural 5′ adenosine monophosphate-activated protein kinase (AMPK) activators and have shown hypolipidemic and anti-inflammatory activities (Hsu *et al.* 2013, 2014). The two compounds have been found to improve memory and learning ability in amyloid β-protein intracerebroventricular-infused rat by suppressing Alzheimer's disease risk factors (Lee *et al.* 2015). Anticancer, antiatherosclerotic, antiallergic, antioxidant, and antidiabetic properties have also been reported (Hsu and Pan 2014; Hsu *et al.* 2011, 2012, 2014; Lee *et al.* 2012).

While the most common method of pigment production from microbes on an industrial scale is submerged fermentation, an immobilized culture system or solid-state fermentation system can be used for *Monascus* fermentation, with rice cassava, corn, and oat as the substrates. Under this system, the carbon source, nitrogen source, pH, and temperature can be easily controlled during production (Chen and Johns 1993; Tuli *et al.* 2015). Blue light has also shown various effects on pigment production in *Monascus* (Chen *et al.* 2016; Wang *et al.* 2015).

Bikaverin (Figure 1.2) is a red pigment that comes from fungi such as *Fusarium* and *Gibberella* (Chelkowski *et al.* 1992; Zhan *et al.* 2007). It represents a medicinally relevant compound, having been found to possess strong antimicrobial activity against certain protozoa and fungi, as well as promising anticancer activity (Deshmukh *et al.* 2014; Zhan *et al.* 2007). It is a polyketide compound that is assembled by a nonreducing type I PKS from ten units of malonyl-CoA. Its production has been extensively studied. During production from *Gibberella fujikuro*, its production medium was determined by a fractional factorial design and tested in a fluidized bioreactor, with the pigment found to be produced at 6.83 g/L (Escamilla-Silva *et al.* 2001).

Atrovenetin and herqueinone (Figure 1.2) are two structurally related pigments from filamentous fungi such as *Penicillium herquei* (Narasimhachari and Ramaswami 1966; Narasimhachari and Vining 1963) and *Penicillium atrovenetum* (Neill and Raistrick 1957). These compounds belong to the family of polyketides. Atrovenetin is purified as yellow-orange plates. It is a deoxyherqueinone-type phenalenone that has characteristic color reactions. It is orange in sodium hydroxide, yellow in concentrated sulfuric acid (with an intense yellow-green fluorescence), and red-brown in ethanolic ferric chloride. It has shown potent antioxidant activity and can stabilize vegetable oils such as soybean, rapeseed, and palm oils (Ishikawa and Sada 1991; Ishikawa *et al.* 1991). Herqueinone is a red pigment from *P. herquei*. Recently, the herqueinone biosynthetic gene cluster was identified from the genome of *P. herquei*. A nonreducing PKS in this gene cluster named PhnA synthesizes the heptaketide backbone and cyclizes it into the angular, hemiketal-containing naphtho-γ-pyroneprephenalenone (Gao *et al.* 2016), which is subjected to additional tailoring to form herqueinone.

The aforementioned pigments are just the tip of the iceberg of microbial pigments that can be produced from fungi. Fungal pigments exhibit rich chemical and structural diversity, with different colors. *Emericella* represents another good example of the diversity of fungal pigments: epurpurins A–C can be isolated from *Emericella purpurea*, falconensins A–H from *Emericella falconensis*, and falconensones A1 and B2 from *Emericella fructiculosa* (Mapari *et al.* 2005; Ogasawara and Kawai 1997). Anthraquinone (octaketide) pigments such as catenarin (Figure 1.2), parietin, macrosporin, chrysophanol, cynodontin, helminthosporin, tritisporin, and erythroglaucin are polyketide compounds produced by *Eurotium* spp., *Fusarium oxysporum*, *Curvularia lunata*, *Dermocybe sanguinea*, *Penicillium* sp., and *Drechslera* spp. (Gessler *et al.* 2013; Zhan *et al.* 2004). Catenarin is a red compound that has been isolated from a variety of fungi, including *Pyrenophora tritici-repentis* (Wakulinski *et al.* 2003), *Ventilago leiocarpa* (Lin *et al.* 2001), *Talaromyces stipitatus* (van Eijk 1973), and marine sponge-associated fungus *Eurotium cristatum* (Lin *et al.* 2001). It is phytotoxic and has been proposed to cause the red smudge symptom and contribute to tan spot, an important foliar disease of wheat caused by *P. tritici-repentis* (Bouras and Strelkov 2008). Catenarin has been found to inhibit the growth of fungi accompanying *P. tritici-repentis* during the saprophytic phase of development, with *Epicoccum nigrum* as the most sensitive species (Wakulinski *et al.* 2003). A recent study showed that catenarin can prevent type 1 diabetes in non-obese diabetic mice via inhibition of leukocyte migration involving the MEK6/p38 and MEK7/JNK pathways (Shen *et al.* 2012). This pigment has also shown *in vitro* inhibition of DNA-dependent RNA polymerase from *Escherichia coli* (Anke *et al.* 1980).

Besides the structural diversity, fungal pigments demonstrate a wide range of applications in industry and in the clinic, and their use is thus not limited to coloring agents. While anthraquinone from *D. sanguinea* and other pigments from *Trichoderma* spp. are widely involved in the wool and silk fiber industry, a red anthraquinone isolated from *Penicillium oxalicum* has been reported to have anticancer effects when used in food supplements (Sardaryan 2002). Some pigments mentioned in the algae section, such as β-carotene, astaxanthin, and canthaxanthin, can be produced by some fungi as well. Given the huge reservoir of fungi and their complex metabolic networks, it is expected that more and more pigments will be discovered from them in the future.

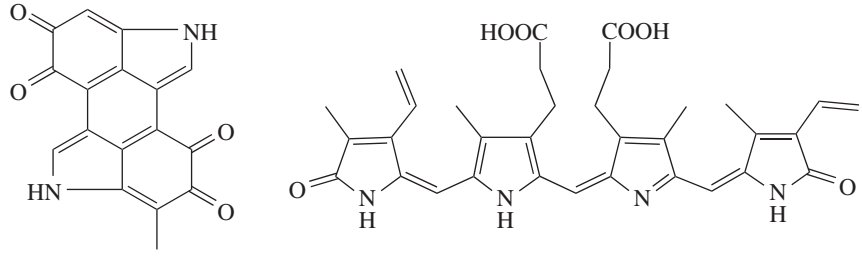
1.2.3. Pigments from Yeasts

Yeasts are a good source of microbial pigments. Different yeast strains, such as *Rhodotorula glutinis*, *Cryptococus* sp., *Phaffia rhodozyma*, and *Yarrowia lipolytica*, are able to produce different microbial pigments (Buzzini 2001). *R. glutinis* is a good example of why the biotech industry is so interested in yeasts, as it can make a number of different high-value pigments, such as β-carotene, torulene, and torularhodin (Latha and Jeevaratnam 2010). Researchers have engineered the production of total carotenoids from this strain by ultraviolet (UV)-B radiation mutation, because the low production rate of the wild type limited its industrial application (Moline *et al.* 2012). *R. glutinis* is also rich in vitamins and fat, and its

extract has thus been used in feeds to enrich their nutrition and to protect against fungal contamination (Buzzini 2001).

Another specific yeast worth mentioning here is the basidiomycetous *P. rhodozyma*, also known as "colorful odyssey." *P. rhodozyma* was first isolated in the 1960s. Researchers first became interested in this pink yeast because of its ability to biosynthesize the economically important pigment astaxanthin. An efficient method for the isolation of this pigment from *P. rhodozyma* has been established (Johnson *et al.* 1978). In fact, it has been found that the production of astaxanthinin in *P. rhodozyma* protects the strain against reactive oxygen species (ROS) (Johnson 2003).

In addition to the previously mentioned microbial pigments, yeasts can biosynthesize other kinds of pigment as well. Melanin has been reported to be produced by *Saccharomyces neoformans* var. *nigricans* (Vinarov *et al.* 2003). "Melanin" (Figure 1.3) and "melanin-like pigment" are broad terms for the black pigments observed in various organisms, including yeasts and bacteria. The biosynthesis of melanin results from the oxidation of tyrosine. This group of pigments can efficiently dissipate UV radiation. Therefore, melanin is used to protect against UV radiation and reduce the risk of skin cancer (Brenner and Hearing 2008). Another yeast species, *Y. lipolytica*, has been reported to produce a brown microbial pigment from tyrosine. Based on the production of this pigment in *Y. lipolytica*, Carreira *et al.* (2001) were able to reveal the mechanism of pigment production from tyrosine in a yeast species. Biliverdin (Figure 1.3) is a green tetrapyrrolic bile pigment found in human and non-human animals. This compound has shown promising antimutagenic and antioxidant properties. It is generated from heme by heme oxygenase. It can be further converted to bilirubin by biliverdin reductase. Microorganisms, including yeasts, are known to produce this pigment as well. For example, it has been reported that *Candida lipolytica* produces biliverdin with glucose or hexadecane as the carbon source (Finogenova and Glazunova 1969). The gene responsible for the biosynthesis of biliverdin has been discovered in yeast. Though biliverdin's production yield is low in yeast, bioengineers have successfully cloned, optimized, and expressed it in engineered *E. coli* (Chen *et al.* 2012), which represents a scalable and more efficient production method.



Melanin Biliverdin

Figure 1.3. Structures of melanin and biliverdin.

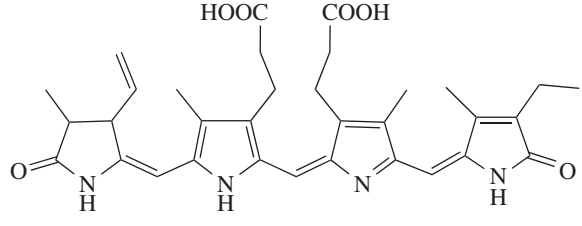
1.3. NATURAL PIGMENTS FROM PROKARYOTES

Prokaryotes are structurally simpler and have fewer metabolic pathways than eukaryotes. However, they are also known to produce a variety of metabolites with different colors. Pigments from cyanobacteria and other bacteria are discussed in this section.

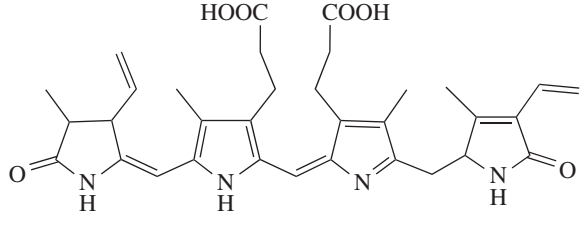
1.3.1. Natural Pigments from Cyanobacteria

Cyanobacteria are a diverse and ubiquitous group of prokaryotes that were formerly called blue-green algae. Unlike other algae, cyanobacteria are unicellular organisms and lack a nucleus and other membrane-bound organelles. Thus, they belong to prokaryotes, and have some features similar to those of common bacteria.

Many cyanobacteria produce light-absorbing pigments such as chlorophylls, carotenoids, and phycobiliproteins. Separation of cyanobacterial pigments by chromatography has been reported (Merzlyak *et al.* 1983). Most photosynthetic pigments bind to specific proteins in cyanobacteria to form complexes. Phycocyanin (blue), allophycocyanin (red), and phycoerythrin (red) are representative phycobiliproteins from cyanobacteria such as *Oscillatoria redekei*. Phycocyanobilin (Figure 1.4) is a blue phycobillin that is present in allophycocyanin and phycocyanin, while phycoerythrobilin (Figure 1.4) is a red phycobillin from phycoerythrin. These water-soluble pigment–protein complexes possess a variety of pharmacological properties. For example, phycocyanin is known to have antioxidant, anti-inflammatory, hepatoprotective, and neuroprotective activities (Rajagopal *et al.* 1997b). Phycocyanin can be used as a natural dye and food additive, and has applications in the nutraceutical



Phycocyanbilin



Phycoerythrobilin

Figure 1.4. Structures of phycocyanbilin and phycoerythrobilin.

and pharmaceutical industries. It has also been proposed that phycocyanin may act as a nitrogen reserve that can be reused during nitrogen starvation (Allen and Smith 1969).

Scytonemin (Figure 1.5) is an extracellular pigment produced by various sheathed cyanobacteria, such as *Scytonema myochrous*, *Calothrix* sp., and *Lyngbya aestuarii* (Dillon and Castenholz 2003). It has a yellow-brown color. Scytonemin becomes green and red in oxidized and reduced states, respectively. This pigment is an effective, photostable UV shield in prokaryotes (Rastogi *et al.* 2013, 2015). Though it was discovered in 1849, its structure was not characterized until 1993. This compound contains novel indolic and phenolic subunits (Proteau *et al.* 1993). Its biosynthesis in *Lyngbya aestuarii* has been studied. Three enzymes, ScyA, ScyB, and ScyC, are involved in the biosynthetic pathway that converts L-tryptophan and *p*-hydroxyphenylpyruvic acid into scytonemin (Figure 1.5) (Balskus *et al.* 2011). In addition to its UV-blocking activity, scytonemin has also shown anti-inflammatory, anticancer, antiproliferative, and antioxidant activities. Thus, it has found applications in sunscreen and as a therapeutic agent. In addition, scytonemin can be used as a biosignature in searching for life on Mars and other planets (Mishra *et al.* 2015).

1.3.2. Natural Pigments from Bacteria

The pigments produced by bacteria are usually light-absorbing compounds. They are responsible for the colors displayed by the organisms that produce them (Rajagopal *et al.* 1997b). As an alternative to the synthetic pigments used in various industries (food, drinks, cosmetics, textiles, pharmaceuticals), bacterial pigments provide a promising avenue for various applications, because of their significantly better biodegradability, safety profile, health benefits, and compatibility with the environment.

Bacteria produce a variety of carotenoids. The ketocarotenoid pigments astaxanthin and canthaxanthin, described in Section 1.2.1, are widely distributed in nature. Astaxanthin, a red ketocarotenoid, exhibits health-promoting activities such as antioxidant and anti-inflammatory effects. A unique astaxanthin-producing bacterium (strain TDMA-17T) belonging to the family *Sphingomonadaceae* has been isolated (Asker *et al.* 2012a). Photosynthetic bacteria have also been reported to produce carotenoids. *Bradyrhizobium* sp. strain ORS278 can produce a higher quantity of canthaxanthin, and the pigment represents 85% of its total carotenoid content (Hannibal *et al.* 2000). Humans and animals must obtain carotenoids through their diet as they lack the ability to synthesize carotenoids (Sacchi 2013). Carotenoids are added to animal feed to improve the color of chicken skin, egg yolks, and salmon (Rajput *et al.* 2012). β-carotene and zeaxanthin (Figure 1.6), which belong to the carotene family, are produced by many bacteria, including *Flavobacterium* sp. and *Paracoccus xanthinifaciens* (Berry *et al.* 2003). Zeaxanthin, with a yellow color, is a promising nutraceutical with many applications in the feed, food, and pharmaceutical industries due to its powerful antioxidant property. Dalal Asker isolated two effective zeaxanthin-producing bacteria, strains TDMA-5T and -16T, from the families of *Sphingobacteriaceae* and *Sphingomonadaceae*, respectively (Asker *et al.* 2012b).

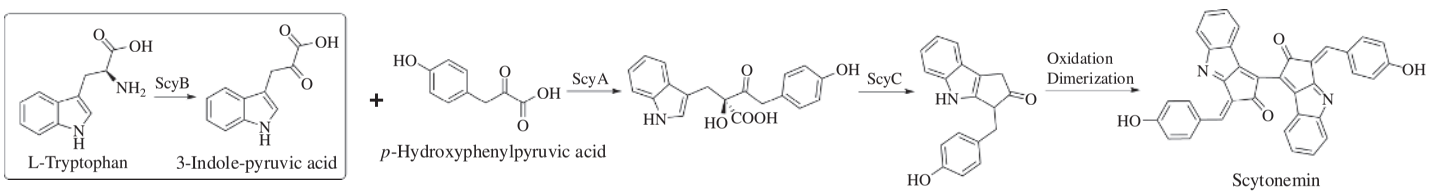


Figure 1.5. Biosynthetic pathway of scytonemin.

These carotene pigments are essential to maintaining the yellow color of the retinal macula, which gives them the ability to act as a sunblock on certain parts of the retina.

The phytopathogenic genus *Xanthomonas* produces a group of carotenoid-like pigments called xanthomonadins. These yellow, water-insoluble pigments are brominated aryl-polyenes associated exclusively with the outer membrane of the bacterial cell wall. Studies have shown that xanthomonadins are associated with the protection of the producing strains against photobiological damage (Jenkins and Starr 1982; Poplawsky *et al.* 2000; Rajagopal *et al.* 1997a). The structure of xanthomonadin I (Andrewes *et al.* 1976) is shown in Figure 1.6.

The bright-red pigment prodigiosin (Figure 1.6) is a tripyrrole. It was first characterized from *Serratia marcescens* and has been shown to be localized in extracellular and cell-associated vesicles and in intracellular granules (Kobayashi and Ichikawa 1991). A wide variety of bacteria can produce prodigiosin-related metabolites, and *S. marcescens* is a major producer of prodigiosin (Furstner 2003). Prodigiosin has been found to provide significant protection against UV stress in *Vibrio* sp. DSM 14379 (Boric *et al.* 2011). Immunosuppressive and anticancer activities have been reported for different prodigiosin analogs and synthetic indole derivatives (Montaner and Perez-Tomas 2003; Pandey *et al.* 2007). Prodigiosin has also been reported to be an active component in preventing and treating diabetes mellitus, and it has some applications in this regard (Hwanmook *et al.* 2003). Prodigiosin shows a red color, which means it can be used to dye many fibers, including wool, nylon, acrylics, and silk (Alihosseini *et al.* 2008). Ahmad *et al.* (2012) tested prodigiosin for its dyeing efficiency in a number of different fabrics (pure cotton, pure silk, pure rayon, jacquard rayon, acrylic, cotton, silk satin, and polyester). The results suggest that it could be used to dye acrylic. They also evaluated the potential of prodigiosin in coloring candles, paper, and soap and to be used as ink. Translucent candles showed a more intense coloration than fluted varieties. Prodigiosin-dyed paper became substantially reduced in color upon exposure to both sunlight and fluorescent light (Ahmad *et al.* 2012).

Violacein (Figure 1.6) is a natural pigment with striking purple hues. It is produced by diverse genera of bacterial strains, including *Collimonas* and *Duganella*. It has strong antibacterial effects due to its function as a toxin guarding against diverse potential bacterial predators, which makes it a promising drug candidate against *Staphylococcus aureus* and other Gram-positive pathogens. It has also shown activities against various cancer cells (Choi *et al.* 2015). Because it is easy to visualize, production of violacein by *C. violaceum* has become a useful indicator of quorum-sensing molecules and their inhibitors (Burt *et al.* 2014). The production of violacein by *Duganella* sp. B2 has been studied. The concentrations of potassium nitrate, L-tryptophan, and beef extract, the volume in the flask, and the pH showed significant effects on the production yield. The yield of violacein by *Duganella* sp. B2 reached 1.62 g/L under optimal conditions (Wang *et al.* 2009).

Melanin is a negatively charged, high-molecular-weight polymer with a black, brown, or gray color. It is synthesized from polymerized phenolic and/or indolic compounds and is usually used in sunblock to protect the skin against UV radiation. It can be found in many bacteria, including *Cryptococcus neoformans* and *Burkholderia*

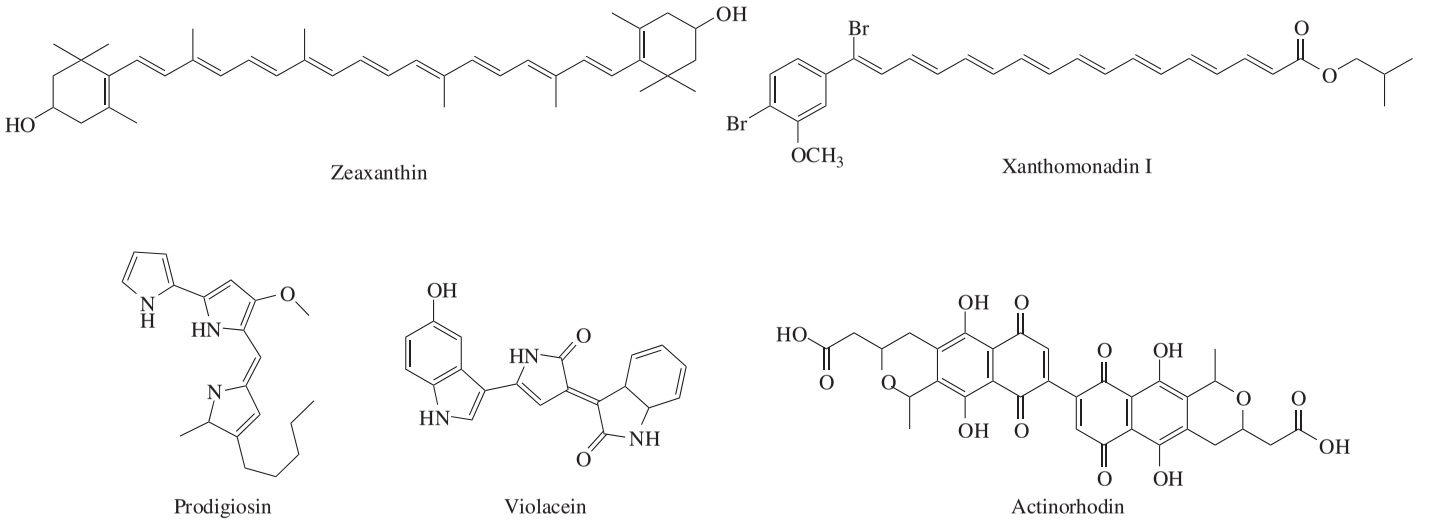


Figure 1.6. Structures of five bacterial pigments: zeaxanthin, xanthomonadin I, prodigiosin, violacein, and actinorhodin.

cepacia (Nosanchuk and Casadevall 2006). Microbes that can produce melanin show a metal-chelating ability (McLean *et al.* 1998). In addition, melanin shows significant antioxidant activity (Plonka and Grabacka 2006).

Actinorhodin (Figure 1.6) is a benzoisochromanequinone polyketide antibiotic produced from *Streptomyces coelicolor* (Magnolo *et al.* 1991). It belongs to a class of aromatic polyketides synthesized by type II PKSs (Manikprabhu and Lingappa 2013). It can be used as a pH indicator, turning red below pH 8.5 and blue above.

Indigo (Figure 1.7a) is a widely used natural dye originally from plants such as *Indigofera*. Since the natural source for indigo is limited, chemical synthesis has become the most economic method of producing this dye. However, chemical synthesis requires harsh conditions and the use of a strong base, which is environmentally unfriendly. Indigoidine (Figure 1.7b) is a water-insoluble blue pigment that was first isolated from phytopathogenic *Erwinia* as a powerful radical scavenger that enables phytopathogens to tolerate oxidative stress, organic peroxides, and superoxides during the plant defense response due to its structure of carbon–carbon double bonds conjugated with a carbonyl group. This bacterial pigment shows a bright blue color similar to that of indigo. Several different strains are reported to produce it. Indigoidine is assembled from two units of L-glutamine by a nonribosomal peptide synthetase (e.g. IndC from *Erwinia chrysanthemi* and *Streptomyces aureofaciens* CCM 3239, BpsA from *Streptomyces lavendulae* and Sc-indC from *Streptomyces chromofuscus* ATCC 49982) (Figure 1.7b). Recently, an indigoidine biosynthetic gene cluster was located in the genome of *S. chromofuscus* ATCC 49982. The gene cluster

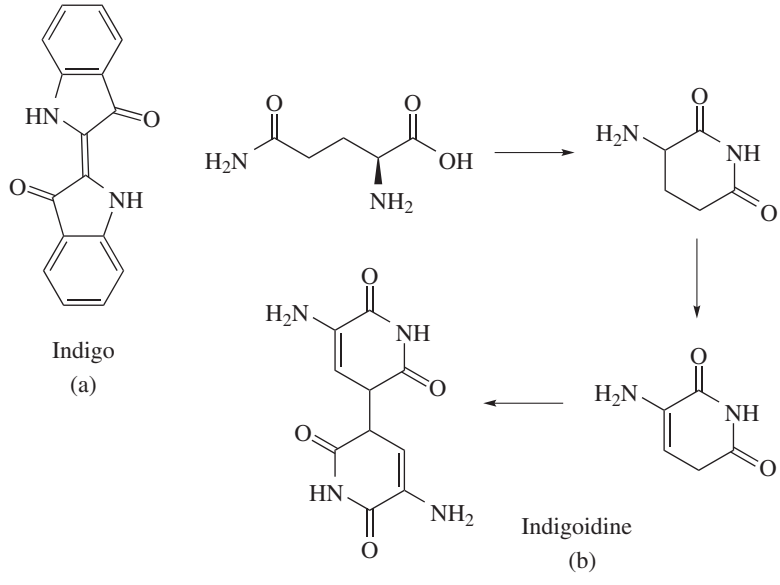


Figure 1.7. Indigo (from plants) and indigoidine (from bacteria). (a) Structure of indigo. (b) Biosynthetic pathway of indigoidine.

is silent and consists of five open reading frames, called *orf1, Sc-indC, Sc-indA, Sc-indB*, and *orf2*. Sc-IndC was functionally characterized as an indigoidine synthase through heterologous expression of the enzyme in both *Streptomyces coelicolor* CH999 and *E. coli* BAP1. The titer of indigoidine in *E. coli* BAP1 was reported to be 2.78 g/L under optimized conditions. Its production was dramatically increased (by 41.4%/3.93 g/L) when Sc-IndB was co-expressed with it in *E. coli* BAP1 (Yu *et al.* 2013). In order to further improve production, a glutamine synthetase gene was amplified from *E. coli* and co-expressed with *Sc-indC* and *Sc-indB* in *E. coli* BAP1. At 2.5 mM $(NH_4)_2HPO_4$, the titer can reach 7.08 ± 0.11 g/L (Xu *et al.* 2015). This provides a green, efficient production process for this promising blue dye.

Flaviolin is a dark yellow-brown compound from bacteria. It is synthesized through a type III polyketide biosynthetic pathway (Figure 1.8). Sequencing of the genome of *Streptomyces toxytricini* NRRL 15443 revealed a type III polyketide

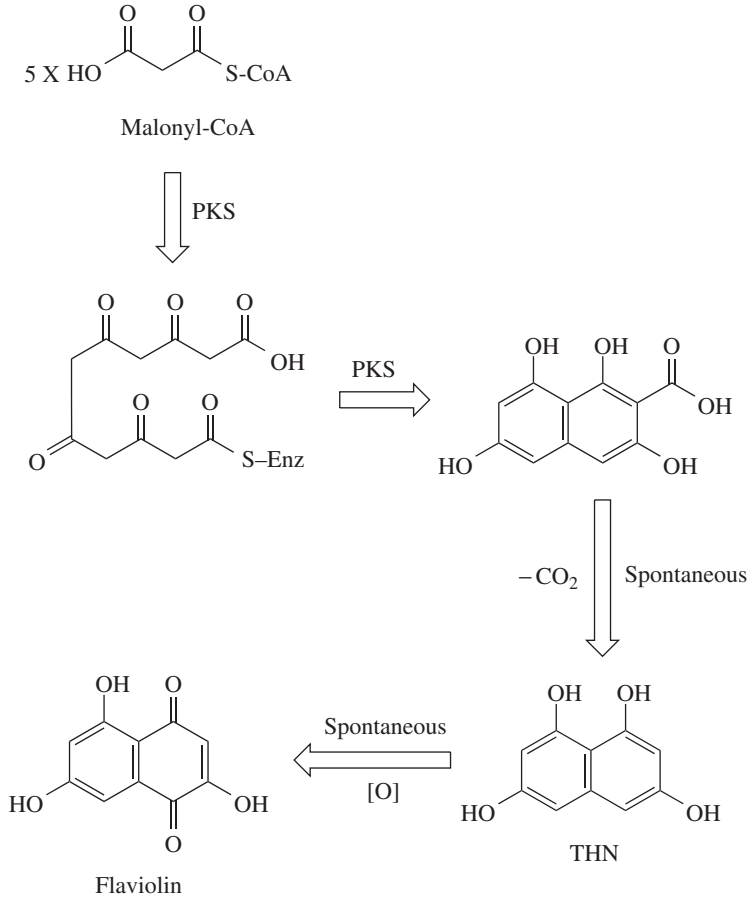


Figure 1.8. Biosynthetic pathway of flaviolin.

biosynthetic gene cluster, which includes *stts* (type III PKS), *stmo* (monooxygenase), and two cytochrome P450 genes, *stp450-1* and *stp450-2*. StTS is a type III polyketide synthase that is homologous to RppA, a 1,3,6,8-tetrahydroxynaphthalene (THN) synthase from *Streptomyces griseus* (Funa *et al.* 1999). When it was overexpressed in *E. coli* BL21(DE3), flaviolin was produced. StTS utilizes five units of malonyl-CoA to synthesize THN, which can be oxidized by StMO or air to generate flaviolin. UV irradiation test showed that expression of StTS in *E. coli* BL21(DE3) provides strong protection of the cells against UV radiation.

1.4. CONCLUSION

Microorganisms produce a variety of pigments – many more than have been discussed in this chapter. The structures and functions of some of these microbial pigments are well established, but many others still remain to be solved. It is important to discover and identify more pigments and understand their physical, chemical, and biological properties, in order to use them in industry. In comparison to pigments from other sources, such as animals and plants, the production of microbial pigments can be easily scaled up. The recent development of recombinant technology, synthetic biology, and metabolic engineering will further facilitate cost-effective production of microbial pigments for industrial applications.

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