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REVIEW ARTICLE

A review on Natural Dye: Gift from bacteria

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Abstract: Numerous pigments have been isolated from a variety of microorganisms including bacteria, fungi and protozoa. Bacterial pigments are water soluble or insoluble; water soluble pigments are diffused in the growth medium. Chemically, bacterial pigments are pyrrole, phenazine, carotenoid, xanthophylls and quinine or quinone derivatives. Structural information on many of these compounds like quinone which are amphiphilic in nature is an indicative of a strong membrane association potential. So as Food colorants may be classified into synthetic, nature-identical, inorganic, and natural colorants. Most often, the colorants are extracted from plant material, but other sources such as insects, algae, cyanobacteria, and fungi are used as well. A Halophylic bacterium has also been reported of producing different pigments. Synthesis of polymeric pigments is also been extensively studied now a days. The molecular genetic studies of pigment synthesis present vital scope for scaling up industrial importance of useful pigmented bacteria. The main objective of this study is to use pigments from locally isolated coloured bacteria as natural dyes to replace the existing synthetic dye.

Key words: Carotenoid; Colorants; Dye; Pigment; Gram-positive bacteria; Gram-negative bacteria

Introduction

A pigment is a material that changes the color of reflected or transmitted light as the result of wavelength-selective absorption. This physical process differs from fluorescence, phosphorescence, and other forms of luminescence, in which a material emits light. Pigments are used for coloring paint, ink, plastic, fabric, cosmetics, food and other materials. The worldwide market for inorganic, organic and special pigments had a total volume of around 7.4 million tons in 2006. For this various necessary of pigment, they are formed by different chemical methods. But the synthetic dyes have toxic or carcinogenic effects. So to eradicate this problem we must use natural dyes which are produced large amount from different microorganisms. Natural dyes/colorants derived from flora and fauna are believed to be safe because of non-toxic, non-carcinogenic and biodegradable in nature. As the present trend throughout the world is shifting towards the use of eco-friendly and biodegradable commodities, the demand for natural dyes is increasing day by day. Amongst the source of natural pigments are ores, insects, plants and microorganisms. Lately, the potential of using microbial pigments as natural colourants is being investigated. Microbes such as bacteria are one of the most likely sources of new pigments because they have the potential of being exploited using existing culture techniques.

The pigments of higher organisms, animal, plant and fungal, may be less accessible to exploitation because of the structural complexity of the pigment-bearing tissue or because the pigment is formed only at critical points of development within a complex life cycle. The natural pigment has various significance. Pigments confer antibacterial and heavy metal resistance. Carotenoids protect pigmented cells against ultraviolet radiation, and therefore many pigmented microorganisms inhabit the air; in photoautotrophic bacteria, carotenoids take part in photosynthesis. Some pigments possess antibiotic properties. The main purpose of this is to use the natural pigment instead of synthetic dyes. Here, we have given a brief overlook on the pigment forming microorganisms like *Rhodospirillum rubrum*, *Chromobacterium violaceum*, *Chlorobium tepidum*.

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Pigment forming microorganisms

There are different types of microorganisms which can produce the pigments. Mainly the bacteria, fungi & algae produced the coloured pigments. By this process we can got the natural colours and dyes which is more preferable instead the synthetic dye or pigments. Natural pigments are highly resistant to the effects of light and air.

Pigment forming bacteria

Bacteria are pigmented or colored. Pigmented bacteria are also known as chromo bacteria. Bacterial pigments are water soluble or insoluble; water soluble pigments are diffused in the growth medium. Chemically, bacterial pigments are pyrrole, phenazine, carotenoid, xanthophylls and quinine or quinone derivatives. The pigment molecules are synthesized in cell wall or periplasmic space. We can visualize pigmentation in bacteria in specific growth medium or by staining bacterial cells with a dye to observe under microscope. It has been proved that only aerobic

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and facultative aerobic bacteria are pigmented because, molecular oxygen is essential for pigmentation. Therefore, anaerobic bacteria are non-pigmented. Pigment synthesis is also dependent on light, pH, temperature and media constituents like indicator dyes. They display all the colours from rainbow including light or dark tinges and unusual colours like black, white, brown, golden, silver and fluorescent green, yellow or blue.

Table 1: Pigment forming microorganisms

Purple	*Spirillium rubum*
Violet	*Chrombacterium violacein*
Indigo	*Janthinobacterium violacein*
Blue	*Streptomyces coelicolor*
Green	*Chlorobium tepidum*
Yellow	*Xanthomonas campestris*
Orange	*Sarcina aurentiaca*
Red	*Serratia marcescens*
Brown	*Rhizobium etli*
Black	*Prevotela melaninogenica*
Golden	*Staphylococcus aureus*
Silver	*Actinomyces sp*
White	*Staphylococcus epidermidis*
Cream	*Proteus vulgaris*
Pink	*Micrococcus roseus*
Maroon	*Rugamonas rubra*
Fluorescent blue/ green	*Pseudomonas aeruginosa*

Rhodospirillum rubrum

Scientific classification:

KINGDOM: Bacteria,
PHYLUM: Proteobacteria,
CLASS: Alpha proteobacteria,
ORDER: Rhodospirillales,
Family: Rhodospirillaceae,
Genus: *Rhodospirilum*,
Species: rubrum

Rhodospirillum rubrum (*R. rubrum*) is a Gram-negative, purple-coloured Proteobacterium, with a size of 800 to 1000 nanometres. It is a facultative anaerobe; it can therefore use alcoholic fermentation under low oxygen conditions or use aerobic respiration in aerobic conditions. Under aerobic growth photosynthesis is genetically suppressed and *R. rubrum* is then colorless (Fig.1). After the exhaustion of oxygen, *R. rubrum* immediately starts the production of photosynthesis apparatus including membrane proteins, bacteriochlorophylls and carotenoids, i.e. the bacterium becomes photosynthesis active. *R. rubrum* is also a nitrogen fixing bacterium, i.e., it can express and regulate nitrogenase, a protein complex that can catalyse the conversion of atmospheric dinitrogen into ammonia Selao TT & Nordlund, (2008). Due to this important property, *R. rubrum* has been the test subject of many different groups, so as to understand the complex regulatory schemes required for this reaction to occur Wolfe *et al.*, (2007). It was in *R. rubrum* that, for the first time, post-translational regulation of nitrogenase was demonstrated. Nitrogenase is modified by an ADP-ribosylation in the arginine residue 101 Pope *et al.*, (1985) in response to the so-called "switch-off" effectors – glutamine or ammonia - and darkness Neilson and Nordlund, (1975).

R. rubrum has several potential uses in biotechnology:

- Quantitative accumulation of PHB (poly-hydroxy-butric-acid) precursors in the cell for the production of biological plastic
- Production of biological hydrogen fuel
- Model system for studying the conversion from light energy to chemical energy and regulatory pathways of the nitrogen fixation system

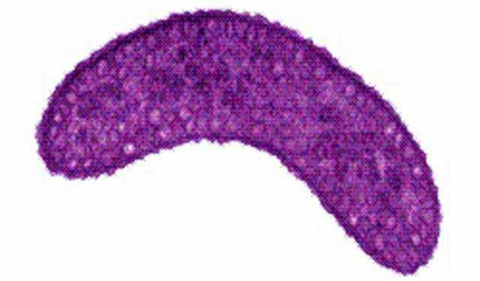

Figure 1: *Rhodospirillum rubrum*

Chromobacterium violaceu

Scientific classification:

KINGDOM: Bacteria,
PHYLUM: Proteobacteria,
CLASS: Betaproteobacteria,
ORDER: Neisseriales,
FAMILY: Neisseriaceae,
GENUS: *Chromobacterium*,
SPECIES: *violaceum*

Chromobacterium violaceum is a Gram-negative, facultative anaerobic, non-sporing coccobacillus. It is part of the normal flora of water and soil of tropical and sub-tropical regions of the world (Fig.2). It produces a natural antibiotic called **violacein**, which may be useful for the treatment of colon and other cancers Kodach *et al.*, (2006). It grows readily on nutrient agar, producing distinctive smooth low convex colonies with a dark violet metallic sheen (due to violacein production). Its full genome was published in 2003 (Brazilian National Genome Project Consortium 2003). It has the ability to break down tarballs Itah *et al.*, (2005). Violacein is a violet pigment extracted from the gram-negative bacterium *Chromobacterium violaceum*. It presents bactericidal, tumoricidal, trypanocidal, and antileishmanial activities.

C. violaceum produces a number of natural antibiotics:

- Aztreonam is a monobactam antibiotic that is active against gram-negative aerobic bacteria including *Pseudomonas aeruginosa*. It is marketed as

Azactam.

- Violacein is active against amoebae and trypanosomes;
- Aerocyanidine is active against Gram-positive organisms;
- Aerocavin is active against Gram-positive and Gram-negative organisms.

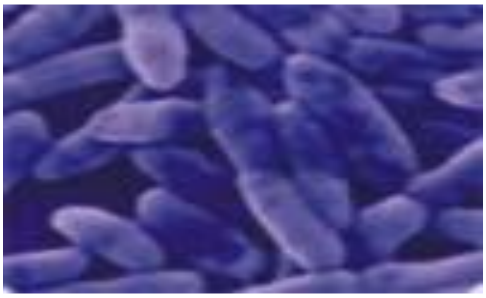

Figure 2: *Chromobacterium violaceum*

Chlorobium tepidum

Scientific classification:

KINGDOM: Bacteria,
PHYLUM: Chlorobi,
CLASS: Chlorobia,
ORDER: Chlorobiales,
FAMILY: Chlorobiaceae,
GENUS: *Chlorobaculum*,
SPECIES: *tepidum*

Chlorobium species exhibit a dark green color; in a Winogradsky column, the green layer often observed is composed of *Chlorobium*. This genus lives in strictly anaerobic conditions below the surface of a body of water, commonly the anaerobic zone of a eutrophic lake Prescott *et al.*, (2005). *Chlorobium aggregatum* is a species which exists in a symbiotic relationship with colorless, nonphotosynthetic bacteria (Fig.3). This species looks like a bundle of green bacteria, attached to a central rod-like cell which can move around with a flagellum. The green, outer bacteria use light to oxidize sulfide into sulfate. The inner cell, which is not able to perform photosynthesis, reduces the sulfate into sulfide. These bacteria divide in unison, giving the structure a multicellular appearance which is highly unusual in bacteria Postgate and John, (1994).

Chlorosomes are the main light harvesting complexes of green photosynthetic bacteria. The complete *C. tepidum* genome, which consists of 2.15 megabases (Mb), was sequenced and published in 2002 Eisen *et al.*, (2002). It synthesizes chlorophyll *a* and bacteriochlorophylls (BChls) *a* and *c*, of which the model organism has been used to elucidate the biosynthesis of BChl *c* (Frigaard *etal.* 2006). Several of its carotenoid metabolic pathways (including a novel lycopene (cyclase) have similar counterparts in cyanobacteria Frigaard *et al.*, (2004).

This suggests that the lamellar model is universal among green sulfur bacteria. In contrast to green-colored *Chl. tepidum*, chlorosomes from the brown-colored species often contain domains of lamellar aggregates that may help them to survive in extremely low light conditions. We suggest that carotenoids are localized between the lamellar planes and drive lamellar assembly by augmenting hydrophobic interactions.

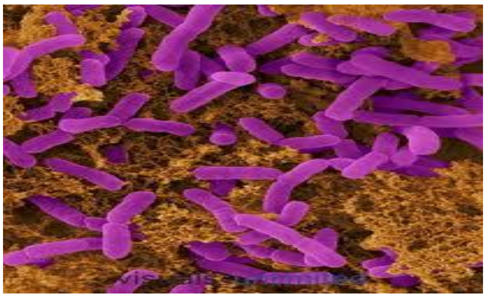

Figure 3: *Chlorobium tepidum*

Significance:

Pigments confer antibacterial and heavy metal resistance. Pathogenic staphylococci are multidrug resistant because of their pigment which acts as barrier for antibiotics acting on cell wall and plasma membrane. Bacteria showing heavy metal resistance are usually pigmented as they have been exploited for remediation of soil and water polluted by heavy metals like arsenic, copper, cadmium, mercury and nickel. Pigmented bacteria have also been used as biosensors to detect environmental pollution like oil spills or pesticide and heavy metal recalcitrance.

Many important applications of bacterial pigments are enlisted:

In pathogenesis:

Resistance to phagocytosis
Heat resistance and acid stability
Unpalatability to protozoa
In vitro antibody formation enhancers
Antitumor properties

Industrial applications:

In paint formulations
Alternatives to color additives of plant origin
In textile dyeing
Food colorants
Source of vitamin A
In therapeutics
Indicators of oil spill
Biosensors and markers of water, soil and air pollution.

Taxonomical significance:

Pigments are important characteristics of particular genus and are very helpful in the identification and classification of microorganisms. The best example of pigmentation is Xanthomonas spp. All the species of Xanthomonas produce yellow colored pigments known as xanthomonadins. Taxonomically, xanthomonadin synthesis is an important trait because they have similar chromatographic and absorption spectra which form the basis of classification of xanthomonads. The molecular genetic studies of pigment synthesis present vital scope for scaling up industrial importance of useful pigmented bacteria.

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