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Microbial Production and Applications of Pigments

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Abstract - *The word pigment of Latin origin means a colored matter; now elaborated to all the coloured materials of/in fruits, vegetables, flowers, textile, paper etc.. From the middle ages, the coloured plant and vegetable were processed to obtain food colourants. The "pigment" as a word has been now associated with all the organic and natural colouring materials of animals and plants origin. The definition also includes organic compounds isolated from cells and their modified structure. The natural pigments include carotenoids, flavonoids (anthocyanin's) and some tetrapyrroles (chlorophylls, phycobiliproteins). The first industrial production of astaxanthin was reported from Phaffia rhodozyma and Haematococcus pluvial is and other organisms. In the recent times researchers have reported and optimized the factors improving the production of pigments by rent microbes. The methods for their extraction and application as colourants in various fields, like food industries, pharmaceuticals, etc., have also been standardized.*

Key Words: **Carotenoids, Flavonoids, Microbial colourants, Pigments, Industrial Scale,**

1. INTRODUCTION

Before the turn of 19th century, nature was the only sources of color available and widely used and traded, providing a major source of wealth creation around the globe. Since the availability of synthetic dyes, many easily available and cost effective artificial pigments are now available with Azo dyes as most frequently used synthetic dyes on the industrial scale. However, environmental toxicity and mutagenicity of these compound ds have serious health issues (Singh *et al*., 2022). These adversities of the synthetic dyes saw revival in applications of natural dyes having better biodegradability and have a higher compatibility with the environment. Lately, the natural color production potential of microbes and its successive use as natural colorants is actively investigated (Cerdá-Olmedo, 2010). Hence, work on the production of pigments and other compounds from microbes should be intensified (Carvalho *et al*., 2019, Chander 2021) especially in finding cheap and suitable growth medium which may decrease the cost and increase its applicability for industrial production (Abed *et al.,* 2008).

The word pigment has been derived from Latin denoting a colored matter but it was later extended to indicate all colored objects. In the start of the middle ages, the word was also used to describe the diverse plant and vegetable extracts, especially those used as food colorants (An *et al*., 2001, Wang, 2002). The pigment is still used in this sense in the biological terminology such as the colored matter present in animals and plants, occurring in the granules inside the cells as deposit on tissues or suspended in body fluids (Ernst, 2002, Nigam and Luke, 2016). It also includes organic compounds isolated from cells and their modified structure.

Some of more important natural pigments are the carotenoids, flavonoids (anthocyanin's) and some tetrapyrroles (chlorophylls, phycobiliproteins). Other groups are less important than betalains and quinones (Verdoes, 2003, Sen *et al*., 2019). The carotenoids molecules are made up of isoprenoids subunits and the most important used as colorant and base of the alpha and beta carotene which in turn basis form biosynthesis of vitamin A, and some xanthophylls as astaxanthin (Sardaryan, 2004). The pigment more used in the industry is the beta-carotene which is obtained from some microalgae and cyanobacteria. This carotenoid, a red pigment has a great commercial value as raw material/feed for pharmaceutical feed and aquaculture industries. This pigment is mainly obtained from *Phaffia rhodozyma* (Vazquez, 2001, Dominiguez *et al*., 2007) and *Haematococcus pluvial* is and other organisms. It has also been employed as fluorescent marker in certain immunological assays (Johnson *et al.,* 2003). A few recent studies have reported the optimization of physico-chemical growth conditions and other growth affecting factors that improve the microbial production of pigments and their higher extraction from fermentation media Flores-Cotera, 2001). Thus, microbial pigments are the colorants obtained from microorganisms that are used in various fields, like food industries, pharmaceuticals, etc.

2. STEPS INVOLVED IN PRODUCTION OF MICROBIAL PIGMENTS

2.1 Isolation of Pigment-Producing Microbes and Screening of the Colored Metabolic

Microbes synthesize pigments as part of their metabolic cycles which inturn have important role in their life cycle. Some microbes like bacteria (cyanobacteria) require pigments like phycobilin for their photosynthesis. Other example for pigment producer microbes include

Serratia marcescens (produces prodigiosin), *Streptomyces coelicolor* (synthesize prodigiosin and actinorhodin), *Chromobacterium violaceum* (produce violacein) and *Thialkalivibrio versutus*. They can be isolated and purified from various ecosystems namely water bodies, soil, plant, insects etc. A variety of nutrient mediums are available which can be used to screen bacteria with desired characters. However, cost limitations of using synthetic medium restricts it's use in screening for low economy processes. However, cheap agro- residues can be used for microbial isolation hence achieving the higher purification causing the cost cutting of pigments. The further purification and characterization of these pigments is required for evaluation of physico-chemical characteristics using various biochemical and biophysical techniques namely TLC, UV–vis Spectroscopy, FTIR, FPLC and Gel Permeation Chromatography.

2.2 Growth Medium

Nutrient broth and Nutrient agar were used as growth medium. The authors sterilized the growth media by wet-heating at 121º C for 15 min. The agar was allowed to solidify and further kept at incubation for 25°C for 4 h to ensure that it is free from contamination.

2.3 Location and Techniques of Sampling

Liquid and soil samples used by authors were obtained aseptically from the brackish water Aquaculture Research Centre (BARC), Johor and one oil refinery facility in Port Dickson, Negeri. A total of 16 soil samples, consisting of a mixture of clay and sand, were collected in the vicinity of the wastewater treatment pond of the oil refinery while at the Brackish water Aquaculture Research Centre, solid samples were taken from area near the shrimp pond and the liquid samples, from the effluent fraction of various tanks namely fish rearing, rotifer breeding, fish breeding and organic waste collector. The sampling were done according to Standard Methods of American Public Health Association (APHA) standards. Pre-sterilized 250 mL Schott bottles were filled with liquid and soil samples and sample air space (for liquid samples), about 2.5 cm, were left to facilitate mixing, aeration and thermal expansion normally encountered during handling and transportation. The bottles were placed inside an iced-box polystyrene container during the 2–3 h transportation journey to the laboratory (to minimize indigenous microbial activity and preserve original speciation of chemicals present).

2.4 Cultivation and Isolation of Cultures

Each of the liquid samples (2.5 mL) was aseptically transferred into a series of 250 mL conical flasks containing 22.5 mL NB medium followed by incubation at 30 ºC, 200 rpm for 24 h (Certomat-B, B-Braun). One loopful of bacterial cultures was then transferred onto NA plates and incubated at 30 ºC for 24 h (Memmert, USA). Serial sub-culturing was carried out until single bacterial colonies were obtained. Similar experimental procedures were repeated for the soil samples using one gram of soil sample as inoculant. Single bacterial colonies were identified via the 16S rRNA gene sequencing analysis carried out by Vivantis Technology Sdn. Bhd., Malaysia.

The sample taken form BARC, Johar formed 45 bacterial colonies from solid and while Negeri Semblian sample cold form only32 colonies. liquid samples from the BARC, Johor (45 colonies) and oil refinery wastewater treatment plant at Port Dickson, Negeri Sembilan (32 colonies). The culture number S8b of Johar sample was further chosen for further studies based on its yellow-orange coloration in nutrient agar and nutrient agar and nutrient broth media. While only 8 colonies obtained for oil recovery centre sampl., Port Dickson, 8 were colored. Of these, S1a culture undergoes color change gradually from grey to dark violet during the incubation period was chosen for further study.

2.5 Characterization of Microorganisms

The authors characterized the bacteria by using 16S rRNA sequence analysis. From the analysis, the pigment-producing bacteria were identified as follows; yellow–orange as *Chryseobacterium* sp. violet pigment as *Chromobacterium violaceum.* This bacterium and *Serratia marcescens,* produced red pigment. *C. violaceum*, belongs to family Rhizobiaceae and is Gram-negative bacteria, is a soil/water inhabiting microcosmic saprophyte found in tropic and subtropical zones. It forms slightly convex, non-gelatinous, regular to irregular and non-pigmented colonies on solid growth media. However, authors have reported *C. violaceum* to be producing "violacein", a violet pigment. Violacein has pronounced bio-activity and shows anti-leishimanial, anti-viral, antitumoral and anti- tubercules activities. This bacteria also has a great role in industrial microbiology as producer microbe of cyanide, solubilizer/bioleacher of gold ore, chitinolytic enzymes producer, and involved in the synthesis of bioplastics. It has a great potential in environmental detoxification process in microbial treatment processes.

The members of Genera – *Flavobacterium* were relocated in new Genera – *Chryseobacterium*. Now the new genera include *Chryseobacterium balustinum, C. gleum, C. indologenes, C. indoltheticum, C. meningosepticum* and *C. scophthalmum, C. defluvii, C. joost, C. miricola , C. daecheongense* , and *C. taichungense*. *Members of this genera* produce translucent, shiny, smooth edged colonies on agar medium. However, upon prolonged incubation the colony forming units (CFU) merged to form a single, fused large colony. On NA, it can produce a bright yellow non diffusible, non-fluorescent flexi Rubin pigment. It was also reported to have an ability to produce heat stable metalloproteases and protein deamidating enzymes.

2.6 Maintenance of Stock Culture

The authors of present work used LB-glycerol solution for long-term storage (months to years) of the nutrient agar (NA) plate and incubated at 30^0C for 24 hrs. The bacterial CFUs were then sub-cultured on LB-glycerol medium and stored in a deep freezer maintained at -20ºC. LB-glycerol medium contained tryptone (10 g), yeast extract (5 g) and NaCl (10 g) dissolved in 1 L distilled water (pH=7.0). The medium was sterilized before use. The bacterial stock cultures were prepared by transferring 2 mL of active culture (from NA plates) into a series of 5 mL screw caped vials containing 2 mL of glycerol, 25% (v/v) followed by storage at -20 ºC prior to use. Another medium used for the maintenance of bacterial stock cultures was 0.1% (w/v) peptone water, which was used specifically for *C. violaceum*. This was due to the inability to revive its growth in broth medium upon storage for 14 days in LB-glycerol. The medium containing peptic digest of animal tissue rich in tryptophan can preserve the microbes for several years at low temperature storage. During the biosynthesis of violacein tryptophan is required as a precursor further its an essential raw material for development of pigment in *C. violaceum*. The absence of tryptophan/cofactors and accessory proteins may also disturb the normal host cell metabolism and hindering catalytic activity of various biosynthetic enzymes.

2.7 The Fluorescent Pigment of *Pseudomonas fluorescens*: Production

The studies have suggested that availability of specific type of carbon source (organic or inorganic) may also affect the microbial potential to synthesize pigments. Author have reported that *Pseudomonas fluorescens* produces yellow-green, fluorescent pigment only when grown in an iron-deficient media and the pigment production potential was independent of the presence or absence of the organic carbon source. The pigment was synthesized as a complex with Fe^{3+} ions and was down-stream processed in same form. *P. fluorescens* synthesized only one form of fluorescent pigment; however, its alkaline affinity may lead to the generation of several isomeric degradation products. The authors further consolidated the biochemical properties of this pure pigment spectroscopically, showing its molecular mass (1500 ± 75) and stability constant for Fe^{3+} (of the order of 10^{32}). Hence the "desferri-siderophore" characteristic of this fluorescent pigment was confirmed.

3. TEQNIQUES USED FOR CHARACTERISATION OF MICROBIAL PIGMENTS

3.1. Modular Spectral System for Imaging Characterization of Pigments in Biological Systems

Spectral imaging is a technique in which spectral information (i.e., the spectrum of light that is scattered from, transmitted through, or emitted by an object) is acquired at every location in an image. Since the spectral information reflects the object's identity, status, and/or composition, combining it with spatial information (i.e., the size, shape, and location of the object) enhances our ability to unravel and understand possible links between the spatial organization and functional relationships for constituents of a system (Polerecki *et al*., 2008).

These attributes have endorsed the role of spectral imaging in different areas of basic and applied research, finding their scale-up to industrial applications.

3.2 Hardware components and measurement configurations

The use of modular spectral imaging (MOSI) system has been reported by the authors. It was constructed using a combination of commercially available and self-made components by the authors. They used a fire-wire hyper spectral (HS) imaging system (Pika II; Resonon, USA), it performed spectral detection in 640nm discrete bands with the same bandwidth (~0.7 nm) in wavelength spectrum, ranging from 460 to 913 nm. The spectra were recorded with 480 pixels line of view (LOV) or view field. By scanning the LOV over the sample at a uniform velocity, it was possible to perform 2-D spectral imaging. Thus, each pixel in the HS data set, referred to as the "hypercube," had a spectral dimension in addition to two spatial dimensions.

1, HS camera;

2, motorized linear stage;

3, LEDs with collimating optics and short-pass excitation filters;

4, LED power supply;

5, holder for the long-pass emission filter;

6, substrate for the sample;

7, microscope;

8, motor for microscope table movement. (Inset) Orientation and angular divergence (not to scale) of the LOV imaged by the camera

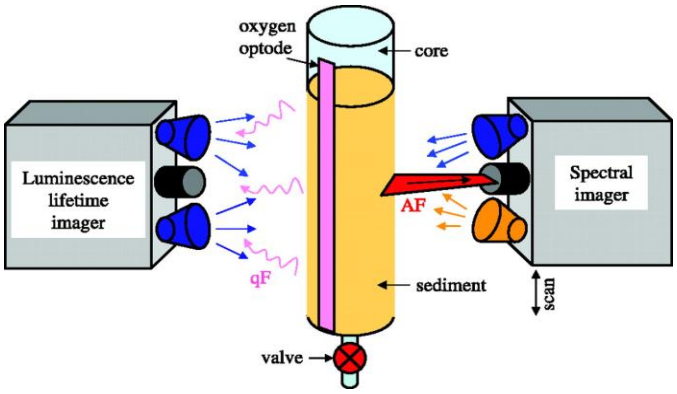

Fig.1.MOSI system showing the arrangement of the components

For imaging in vide range of spectral reflectance and transmission, a set of light-emitting diodes (LEDs), in combination with either a halogen bulb or fiber optic light guide (halogen bulb KL-2500; Schott) was used for sample illumination in the visible to near-infrared range (visible light, 400 to 700 nm; near-infrared light, 700 to 1,000 nm) . For imaging of auto fluorescence, high-power LEDs (Lumileds, United States) that emitted blue, green, and amber light and were equipped with 5° collimating optics (LXHL-NX05-5; Lumileds) were used as narrow-band excitation sources. Additionally, a set of low-pass optical filter placed in front of the LEDs and a complementary high-pass optical filter placed in front of the HS camera was used to remove the longer-wavelength LED emission and thus allowed sensitive detection of auto fluorescence at wavelengths higher than the cutoff wavelength (λ_c). LEDs were powered by a self-made power supply that provided direct current in the range from 1 to 999 mA. Alternatively, a mercury lamp (HBO 50; Zeiss) and a combination of low-pass and high-pass optical filters were used when auto fluorescence was imaged with an epi fluorescence microscope.

3.3 Measurement procedure

The spectral scanning was done under conditions excluding ambient light so as to avoid variable illumination. It was necessary during auto fluorescence imaging because of low intensity of the fluorescence in comparison with the ambient light levels. The assembled system can be operated in any desired mode (macro, meso, microscopic) for scanning of spectral reflectance, transmission, or auto fluorescence. It working involved the following steps. First, an appropriate light source was selected for beaming the sample and moved in order to cover a spatial feature with distinct spectral properties by LOV. The LOV was continuously displayed, and the frame rate, gain, and shutter duration for the HS camera were adjusted interactively to ensure that the brightest possible image which was not saturated was obtained. After this, the LOV was focused, either by refocusing the objective lens or, preferably, by slowly moving the sample toward or away from the objective lens while observing the level of sharpness of the spatial feature seen in the LOV. After focusing, with a final check for signal oversaturation, the sample was scanned using a uniform velocity of the LOV and image was acquired at a frame rate of 3.75 frames per second, which was used in our measurements, the typical velocities were 1 to 2, 10 to 20, and 50 to 200 µm s^{-1} during the micro-, meso-, and macroscopic scans, respectively. Depending on the velocity and the scan range, measurement took from several tens of seconds to a few minutes, which resulted in a 50- to 200-MB hypercube. For auto fluorescence scanning, the sample was beamed with the excitation light for a few minutes prior to scanning in order to ensure that the variable fluorescence of pigments associated with reaction centers of the photosynthetic apparatus had reached a steady state (Govindjee *et al*., 2004) and thus would not play a role when the extent of fluorescence in different parts of the same sample were compared.

3.4 Identification of pigments in living cells by microscopic spectral imaging

The application of the MOSI system involved microscopic spectral imaging of cells. A sample sets was prepared by pipetting a small drop of cyanobacterial and algal cells grown in a culture onto microscope slides. The microbes used were

(i) *Leptolyngbya* sp. strain PCC 8103, Red filamentous organism
(ii) The green filamentous *Leptolyngbya*- M1C10, and
(iii) The yellowish green spherical *Rhabdoderma*-related morpho type M7R1.

The authors purchased first organism from the Pasteur collection of cyanobacteria (Paris), and the other two organisms were isolated from microbial mats on intertidal flats in the Arabian Gulf (Abu Dhabi, United Arab Emirates) (Abed, *et al.,* 2008). In the present study, all organisms were maintained at 25°C with a light-dark cycle consisting of 12 h of light and 12 h of darkness (light intensity, 25 µmol photons m^{-2} s^{-1}) in ASN-III B_{12} medium. The oval-shaped green algae used (species not determined) originated from Riou Mort (France) and were grown at 15°C with a light-dark cycle consisting of 12 h of light and 12 h of darkness (light intensity, 100 µmol photons m^{-2} s^{-1}) in modified Bold's basal medium. The diatoms used (*Cylindrotheca closterium* CCMP1855; United States) were grown at 15°C with a light-dark cycle consisting of 12 h of light and 12 h of darkness (light intensity, ~100 µmol photons m^{-2} s^{-1}) in f/2 medium with additional silicate. Transmission and auto fluorescence spectra for the samples were obtained immediately after sample preparation, using an Axiophot microscope and a ×40 or ×100 objective lens. The spectra were averaged for 20 to 50 pixels associated with a single cell and used to determine the cellular pigment

content. For tested samples, scan were observed at absorption maxima of the major pigments in the cells. The intensity of the auto fluorescence peak were used to visualize the cells. The spectra were averaged for 20 to 30 pixels for one cell or filament and used to determine the cellular pigment content.

The transmission and auto fluorescence spectra for single cells were clearly resolved when the Axiophot microscope was used. The spectra obtained for different pixels for the same cell or for different cells of the same species in the same physiological state were very similar, and thus only the average spectra are discussed below. The transmission and emission spectra of the cyanobacteria (morphotypes M1C10 and M7R1) were found to be similar.

The flat zones in transmission spectrum around 675 and 460 nm indicated the presence of *Chlorophyll a*, whereas phycocyanin (PC) peak was a observed at 630 nm. The emission spectra had a pronounced peak at 653 nm and a shoulder around 685 nm, which were due to PC and Chl a, respectively.

In cyanobacteria (*Leptolyngbya* sp. strain PCC 8103) phycoerythrin (PE) was the main accessory pigment as shown by a peak around 574 nm. Chl. and PC were also detected in the filaments, as shown by the valleys around 678 and 630 nm, respectively. The data suggested that the red cyanobacteria had a much lower PC/Chl. a ratio than the green cyanobacteria. The presence of PE and PC in *Leptolyngbya* sp. strain PCC 8103 was also confirmed by emission spectral peaks at 588 and 650 nm under blue and green light, respectively. Furthermore, when blue light was used, a shoulder in the spectrum due to Chl. A was also observed. The major pigments detected in the green algal cells were Chl. A and Chl. b, as indicated by the valleys in the transmission spectra around 460 and 675 nm and around 652 nm, respectively. The presence of Chl A was confirmed by the emission peak at 685 nm when either blue or green excitation was used.

4. TEXTILE AND OTHER COLORANTS

The use of textile colorants known as “dyes” is not dealt with very hard safety requirements as food colorants. However, there are many more bacterial pigments used as dyes in textiles, paper and leather industries other non- than in food. Ahmad et al. describes the extraction of yellow-orange, red, and purple pigments from *Chryseobacterium* sp., *Serratia marceseus,* and *Chromobacterium violaceum,* respectively. The red (prodigiosin) and purple (violacein) were then used as colorants on different fabrics (acrylic, spun, silk, viscose, cotton, polyester, and polyester microfiber), and their colorfastness (tendency to cling onto fabric when washed, exposed to light or perspiration) on the fabrics was recorded. Both pigments showed high colorfastness when stained on to acrylic fiber, silk, and polyester microfiber, while displaying average colorfastness to cotton and polyester.

Furthermore, both pigments displayed a high staining capability on all the fabrics, implying that a minimum amount of pigment was required to stain the fabrics. These tests show the ability of specific pigments to be employed effectively as dyes/colorants for textiles. As new safe and effective natural colorants are discovered, they can replace the potentially harmful synthetic dyes that are currently used on products such as denim and yarn.

5. FLUORESCENCE-BASED INDICATORS

Bacterial pigments with fluorescence are used in laboratories to label antibodies and also indicate the progress of specific reactions. A key example of this is phycoerythrin, an accessory pigment to chlorophyll in photosynthetic bacteria. It is essential because it captures light energy and then transfers it to the chlorophyll reaction center.

The pigment named phycoerythrin has been applied as an indicator {fluorescent} to analyze the effect of free peroxy radicals on living celss and tissues. As phycoerythrin has strong affinity for free radicals, researchers can make an assay that shows the change in the concentration of pigment over time. Initially, the pigment will appear on the assay as an area of fluorescent glow. As peroxy radicals are added to the pigment, dark spots appear where radicals have reacted with the pigment, causing the overall fluorescence emission to decrease over time. This assay can then be used to predict the rate of peroxy radical scavenging in human plasma. A specific type of Phycoerthyrin, known as R Phycoerthyrin, may be used to fluorescently label antibodies. Mahmoudian *et al*., reported that R-Phycoerthyrin was bound to Ig G (an antibody) by SPDP, a linker protein. Fluorescently-labeled antibodies can be exposed to a pathogen such as *E. histolytica*, and consequently stain the pathogen as the labeled antibodies bind to it.

6. HUMAN HEALTH

Interestingly, one major industrial application of bacterial pigments has nothing to do with the pigments’ visual properties. Some bacterial pigments are used to promote human health, providing key nutrients and compounds that are needed by the body.

The β-carotene represent an important category of colored compounds known as carotenes. These pigments induce many useful metabolites which has beneficial effects in different organ systems in man. Apart from vegetables, different bacteria also synthesize β-carotene and astaxanthin (a xanthophyll). The two pigments are required for normal functioning of human eye, as the yellow pigment of retinal macula for blocking exposure of sensitive parts of eye

to sunlight. Thus improving the health of the human eyes. Furthermore, common bacteria pigments such as prodigiosin (red color), carotene, and xanthophylls have carcinogenesis-prevention roles, as these pigments have anti-oxidative, anti-free radical and apoptosis-inducing activity. Melanin, a common pigment that creates the black, brown, and grey colors in many bacteria, has also shown significant antioxidant activity. Furthermore, melanin is used in sunblock to protect the skin from UV radiation.

7. APPLICATIONS

7.1 Applications of microbial pigments in paints

7.1.1 *Sources of pigments*

Conventional sources of pigments range from inorganic metals and metal oxides to organic molecules. Paleolithic humans knew the art of making pigments from ores namely red ochre, which gets its color from hematite (Fe_2O_3), yellow ochre ($Fe_2O_3{\cdot}H_2O$), and green malachite ($Cu_2Co_3(OH)_2$) and suspending these in their own saliva as a binder. These 30K year old pigments have been used byu the artists till date. Additionally use of some inorganic pigments including titanium white (TiO_2), cobalt blue ($CuO + Al_2O_3$), Egyptian blue ($CuO{\bullet}CaO{\bullet}SiO_2$), cadmium red (CdSe), and black oxide of iron (FeO) is quite common. . The source of delocalized electrons in these pigments is the metal–ligand complex.

Traditional sources of organic pigments and dyes include natural products such as flavinoids and anthraquinones produced by plants and animals. Dapson (2007) reported a deep red anthraquinonic pigment of insect's origin, now successfully used as a coloring agent in paints, ink, cosmetics, and food pigmentation. From the onset of the 20th century, due to high demand and lower availability the natural pigments have been seldom used for their scale-up or industrial applications. The synthetic colors namely phthalocyanines (blue to green), arylides (yellow to greenish/ reddish-yellow), and quinacridones, (orange to violet) have totlally replaced their organic counterparts (Lomax *et al.,* 2006). Advances in chemical synthesis has enabled high scale production while cutting the overall process cost drastically, hence making these colors a best choice for industrial applications.

The bacterial genus of *Streptomyces* has been reported to be producing important antimicrobial compounds such as streptomycin and tetracycline (Keiser *et al.,* 2000). They also produce many intensely pigmented molecules that can be isolated in a pure form including "polyketides" the colored pigments that have absorption maxima in visible region of light spectrum. *Streptomyces coelicolor* synthesized an ultramarine blue pigment in submerged fermentation called actinorhodin (Leathers, 2004).

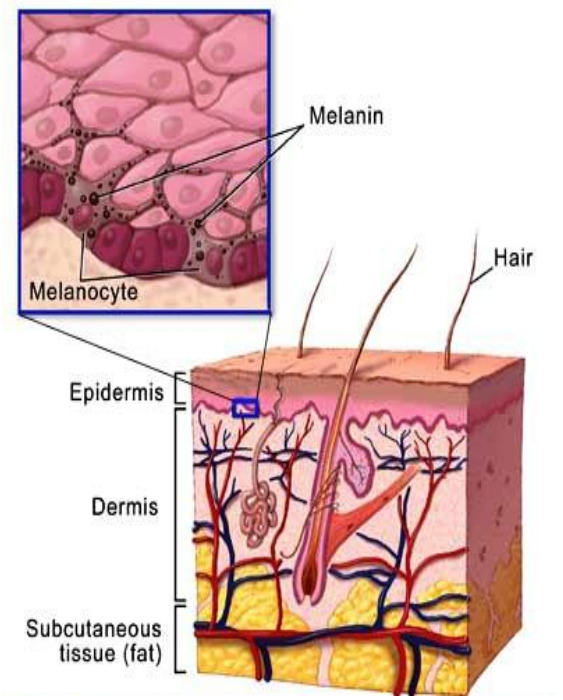

Fig.3. Location of melanin in human skin layer

Every *Streptomyces* strain has capability to produce colored pigments falling in various zones of visible light spectrum, that determines the strain's characteristic leached color saturation and contrast. Some pigments, such as actinorhodin, change color in a pH-dependent manner (Keiser *et al.,* 2000), whereas others maintain their characteristic color over a range of pH.

8. RESEARCH APPLICATION

8.1 Natural pigments

There is increasing demand for naturally-derived food grade pigments. The majority of existing pigments are plant extracts subject to variability and seasonal supply. The CMI has the ability to select and isolate novel pigments from microbial sources, offering a more reliable and scalable technology. This places the CMI in a strong position to respond to increasing market demand for natural colors over their synthetic counterparts.

Dr. Silas Villas-Bôas' research group has screened hundreds of New Zealand pigment-producing fungi, bacteria and algae, resulting in successful isolation of a variety of yellow, red, orange, pink and blue pigments. These have been tested for water solubility and heat, light and pH.

8.2 Dairy products

Dr. Susan Turner's research group is using molecular biological methods to characterize the microbial communities and interactions present in milk. Dr. Turner's

group also aims to create a 'wiring' diagram to help understand how the microbes in cheese products and processes behave. It is widely assumed that all the microbes present in cheese are introduced with the starter culture, but they actually come from the milk environment and are thus present from the start.

8.3 Modelling lipid metabolism in *Lactococcus*

Lipid molecules are important mediators of central cellular processes, but the metabolic pathways involving lipids are poorly understood (Dufosse, 2005). Manipulation of microbial lipid metabolism may result in improved food fermentation processes, but this requires better knowledge of the pathways involved. The lactic acid bacterium *Lactococcus lactis* is commonly used in seed inmoculum for cheese production in New Zealand, and can produce "functional" lipids with health benefits. Dr. Silas Villas-Bôas' research group is constructing a genome-scale mathematical model of *Lactococcus* metabolism. Identification of the genes, enzymes and pathways involved in fatty acid metabolism will provide a basis for improvement of *Lactococcus* fermentation processes, with the aim of enhancing production of desirable functional lipids in dairy products.

8.4 Food processing

Professor Mohammed Faid is a chemical engineer whose research expertise includes the development of innovative food processing methods, especially food sterilization and pasteurization techniques. He is well-known nationally and internationally for his work in non-thermal inactivation of microorganisms, including spores, by using high pressure, pulsed electric field and UV processing.

9. LARGE SCALE PRODUCTIONS OF DESIGNER BACTERIAL PIGMENTS

The small scale production of pigment are not sufficient for industrial applications. Therefore, the utility of a pigment is dictated not only by its inherent properties but also by the ability to produce it in sufficient quantities. This scale may face several associated challenges to biological production and isolation from *Streptomyces* bacteria,. However, studies involving technical advancement to overcome these challenges has already been initiated, which provides a potential route for reintroducing bio-pigments to a cost-sensitive world. For example, the design of continuously stirred bioreactor (CSTR) has given the solution to problems of aseptic process operation, stirring/agitation, aeration, maintenance of pH and temperature etc. during *Streptomyces* submerged fermentations. These findings of fermentation technology has already facilitated the microbial growth and productions in pharma, animal health, food, and agricultural applications. Most important of all is to scale-up microbial fermentations to highest level so that maximum production of pigments can be achieved in minimum incubation periods while minimizing production and purification costs.

The genetic engineering has an important role to play in cloning of various genes involved in biosynthesis of pigments. The underlying recombinant -DNA technology can further help in hyperproducing these pigments in prokaryotic hosts (Hannibal 2000). A workgroup at at Amgen, Inc. has achieved success in hybridizing a widely used non-pathogenic strain of *Escherichia coli* to hyperproduce indigo dye (wood plant derivative) in fermentation tanks (Iturriaga, 2005).

The Biotechnologists can also modify the biosynthetic pathways to transform a pigment's molecular structure and consequently its color (Jones, 2004). In one such process, *Streptomyces coelicolor*, producing actinorhodin (blue pigment), can be genetically engineered to hyper-produce a kalafungin (a bright yellow colored polyketide). Alternatively, actinorhodin generation can also modified genetically to synthesize orange anthraquinones.

The downstream processing or isolation of these pigments and further purification to analytical or food – grade is also a great challenge. Broadly speaking, *Streptomyces* produce two classes pigments—intracellular (those remain inside the bacterial mycelia) and intercellular (secreted into the fermentation medium). The recovery of intracellular pigments require extra steps of disrupting the mycelia and further using available separation techniques to isolate the pigment (Fang, 2002), adding to the cost involved and making product costlier. In such cases, the use of large quantities of organic solvents such as ethyl acetate for product recovery has adverse environmental impacts

Last but not least, to be useful in paint, a pigment must have acceptable stability when exposed to environmental stresses, especially UV light. It starts rapid free-radical reactions in paints causing separation of its constituents ultimately lead to their degradation. These degradation product if released untreated in effluent, have proved to be highly toxic and mutagenic in nature (Chander, 2020). Hence use of UV absorbers (benzotriazole) and free-radical scavengers became utmost important in the paint industry. Whereas their effectiveness in conjunction with bio- pigments remains to be studied, as these processes may also enhance the application of *Streptomyces* pigments in paint industry.

10. CONCLUSION

The colors are beauty of nature and the microbes involved in pigment production add up to this attraction. The microbes namely fungi, and bacteria

(thermoactinomycetes) needed to be screened for their carotenoids, melanins, flavins, quinones, monascins, violacein , indigo and several other pigments production potential. The various aspects like acceptability in the market, regulatory approval, and the size of the capital investment involved in microbial pigment production industry will decide the future of enhanced use of biological pigments in various coloring processes. However, some reservations about use of natural pigments has been removed by the scientific data and public awareness drives awakening masses. The biosafety, toxicity studies regarding use of these products done by the regulatory agencies have increased the demand of natural pigments in public. Hence public perception of biotechnology-derived products should also be taken into account.

Nowadays some fermentative food grade pigments are available in the market and the successful marketing of algae-derived or vegetable-extracted pigments, food color as well as a nutritional supplement, highlights the presence and importance of growing markets where consumers are paying a premium for natural or organic products.

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