

PRODUCTION OF FOOD GRADE PIGMENTS FROM MICROBIAL SOURCES

Abstract

For long time, the utilization of manufactured synthetic colorants has been highly questionable because of its negative evaluation. The best substitute for this would be the natural food colorants from sources such as plants, animals and microorganism's, which have much positive health benefits. For biotechnological production of colorants when compared with other sources, microorganisms are more suitable due to ease of availability, rapid culturing and more probability of genetic manipulation. Food-grade colours like quinones, carotenoids, microorganisms produce flavins, melanins and more precisely monascins, violacein, indigo, etc. The food industry's output of components obtained from fermentation is growing steadily each year, but manufacture of food-grade pigments from microbial sources is still in developmental stage. To name a few, *Monascus* pigments, β carotene from *Blakeslea trispora*, Riboflavin from *Ashbya gossypii*, *Phaffia rhodozyma*, Arpink red from *Penicillium oxalicum*, Astaxanthin from *Xanthophyllomyces dendrorhous*, lycopene from *Erwinia uredovora* and *Fusarium sporotrichioides*. Additionally, some bacteria, including *Serratia* sp. and *Streptomyces* sp., create large quantities of carotenoids.. Numerous factors such as pH, temperature, carbon sources, minerals, nitrogen sources, moisture content, types of fermentation and aeration rate can affect the production of food grade pigments effectively. Aside from the optimal production strategies of the food grade pigments, they got lots of health benefits like can act as antibiotics, antioxidant activity, anticancer agents, anti-proliferative effects and immune-modulators. So natural bio-colorant like food grade pigments from microbial sources has great demand and still more research probability can be expanded to develop more bio-products.

Keywords: Food Grade Pigments, Microbial Carotenoids, Antioxidants

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I. INTRODUCTION

The food manufacturing and processing sectors place a high value on colour, which enhances the sensory qualities of food. It indicates the food's nutritional content, safety, aesthetic value, and freshness, and it has an immediate effect on the market price of the coloured food product (Sen et al., 2019). Color is the primary feature of food that determines its consumer appeal. Bio colorants are substances that come from living organisms like plants, animals, and bacteria. Natural color chemistry cannot fail to fascinate and intrigue, and it has become the most important component of any commodity. The current consumer preference for naturally derived colorants is primarily due to their healthfulness and high quality. Furthermore, synthetic colorants have an unpleasant taste, are harmful to humans because they cause allergic, and intolerance reactions (Rymbai et al., 2011). Four types of food colors (1) Natural, (2) Nature-identical, (3) Synthetic, (4) Inorganic. Natural color pigments made by moderation of resources from living organisms, Nature-identical colors are natural and synthetic pigments both exist in nature. Synthetic colors are artificial pigments that are not also present in nature, Sample of inorganic colors gold and silver (Aberoumand, 2011). The Natural Food Colorants Association proposed including food colorants in the EU definition of natural flavour. The lack of agreement on how consumers use the term makes it unlikely that a universal definition of natural colour will be adopted any time soon (Wrolstad & Culver, 2012). Food colour influences physiological and psychological beliefs and attitudes, which are influenced by knowledge, tradition, studies, and environment. (Deanna & Minich, 2013). Tradition has long accepted several of the "natural" food colorants that are today legal. It has been suggested that natural colorants of comparable variable quality and alien origin might not be acceptable if they were introduced into commercial food manufacturing today (Mapari et al., 2005). For one or more of the following causes to the food: To replace colour in food that is produced by an unknown technique, to improve colour already existing, to colour otherwise achromic food, and to provide nutrients (Lakshmi., 2014)

For the past several years, it has been challenging to create new colourants for the food business since they must be compatible with a meal's flavour, safety, and nutritional content while also having a negligible effect on the price of the final product. The main sources of natural colourant include plants, insects, mineral ores, and microbiological sources. Because of their simplicity in scalability and potential for cheaper manufacturing costs, microbial colourants are favoured. Lower production costs, larger yields, simpler extraction, less expensive raw materials, no seasonal changes, and strain modification procedures are just a few benefits of using microbial fermentation to produce natural pigments (Sen et al., 2019). A variety of colour pigments have been reported to be produced by microorganisms. As a result, they have emerged as a potential source of natural colorants for food, cosmetics, and pharmaceuticals. Color pigments are secondary metabolites that are produced primarily from a bacterial, algal, or fungal culture, which has completed its growth phase. The amount of colour pigments produced is directly proportional to the amount of biomass accumulated. Some bacteria have been used as colorants or food additives in traditional Asian foods. Several bacterial pigments have been reported to be used as food colourant. While considering about the safety aspects, certain fungus are well exploited for their secondary metabolites production and their application as natural food colorants (Marlia Singgih & Elin Julianti, 2015).

II. FOOD COLOURANTS

The US FDA states that “Food additive subject to FDA Premarket approval is any material logically anticipates to become an element of food”. The latest research says that there are listed in the database of food additives in the US FDA are presently nearly 2500 kinds of food additives being used worldwide and about 3000 components. It also says that nearly 200,000 colorants of food additive are used annually; highlighting the true, that Western nutrition consists of about 75% of processed food. The average annual food additive consumption of each person is estimated to be 3.6–4.5 kg (Poonam et al., 2013). Foods with high nutritional values, flavours, aromas, and textures cannot be consumed unless colour is given to make them seem appetising. Customers loved around the world by colorful food products and food dishes. Food additives are categorized as artificial colors, antimicrobial agents, artificial flavors and thickening, chelating agents, antioxidants, flavor enhancers, and stabilizing agents (Pandey & Upadhyay, 2012).

Colouring agents were used in applications such as bakery products, beverages, confectionery, milk, dairy, and dairy-like products, meat and meat products and other food products (Luzardo et al., 2021).

1. History of Food Colourants: Color has been a significant criterion for products such as textiles, cosmetics, food and other items to be acceptable and it was practiced in Europe during the Bronze Age. Dating from 2600 BC, The first known written account of the usage of natural dyes was found in China. Dyeing known in the Indian subcontinent even during the Indus Valley period (2500 BC) and was substantiate in the ruins of Mohenjodaro and Harappa civilization (3500 BC) by findings of colored cloth garments and traces of madder dye. Mummies were discovered wrapped in colored cloth in Egypt. Alizarin, a madder-derived pigment, was found in King Tutankhamen's burial in Egypt, according to chemical red cloth research. Aztec and Mayan societies in Central and North America employed cochineal colouring. Dyes such as woad, madder, weld; Brazil woad, indigo and a dark reddish-purple have been known by the 4th century AD. The name of Brazil came from the woad discovered there. Even before 2500 BC, Henna was used, while the Bible mentions saffron. Japan's use of natural bio-colorants in food is recognized in the Nara era (8th century) shosoin text, which includes references to coloring soybean and adzuki-bean cakes (Aberoumand, 2011). The study of colour was intensified in the late 19th century, according to Aberoumand and Mortensen, in order to comprehend:

- The phenomenon for plant and animal survival,
- The connection between theories of evolution and colour; and
- Comparative physiology's giving function (Rymbai et al., 2011).

2. Classification of Food Colourants: Food colorants categories(Figures:1)

- Synthetic
- Nature-identical
- Inorganic
- Natural colorants.

- **Synthetic Colourants and its Limitations:** Synthetic food colorants are often water-soluble chemicals that are produced in the factories and can be used in foods directly. Ponceau 4R, Carmoisine, Erythrosine, Tartrazine, and sunset Yellow FCF are some of the artificial food colorings. Artificial food dyes have a detrimental effect on people's health. (Sadar, P et al., 2017). The majority of the chemicals used to create synthetic food colourants include an azo group and are derived from coal tar. These colours can be divided into groups that are allowed and those that are not. Synthetic food colourants have been linked to several significant health problems, including low haemoglobin levels, allergic responses, cancer, mutations, irritability, restlessness, and disturbed sleep, according to numerous research. They can also have negative effects on the kidney, liver and intestine, as well as cause ear infections, asthma, and eczema in children and cause them to become hyperactive. Additionally, it is unsafe to utilize authorized synthetic colorants carelessly (Dilrukshi et al., 2019). Due to their consistency, stability, and affordability, synthetic dyes are utilized extensively for coloring purposes (El-Wahab & Moram., 2013).
- **Natural Food Colourants:** Natural colorants are typically taken from fruits, vegetables, seeds, roots, and microbes; due to their biological origin, they are frequently referred to as "bio colors". Plant pigments are typically regarded as safe due to their natural prevalence in edible plants. Numerous vivid pigments are produced by nature, some of which have commercial use for colouring food. Microorganisms produce .Numerous colours, such as carotenoids, flavins, monascins, chlorophyll, quinines, prodigioson, and violacin. Consequently, they constitute a potential source of naturally occurring pigments with commercial potential for use in a variety of sectors, including food, feed, cosmetics, medicines, and nutraceuticals (Shatila et al., 2013).

Because of multiple health gains, greater customer requirements on natural ingredients boost the use of natural colorants rather than synthetic food colorants (Dufosse, 2006). Superiority of natural colourant:

- Greater variety of colors
- High mass-to-coloration ratio
- Improved stability
- Little difference from batch to batch
- Pure and chemically outlined
- Reasonably affordable (Damant, 2011).

However, the number of natural pigments that can be used in human foods is extremely restricted, and it is difficult to get new sources approved because the U.S. Food and Drug Administration (FDA) views the pigments as additives. As a result, the pigment industry is subject to tight rules (Vargas et al., 2000).

- **Sources Of Biocolourants /Biological Pigments:** Natural bio colorants can be sourced from plants, animals, and microbes (Figures:2). Due to their knowledge of appropriate cultural practise and processing, plants and microorganisms are more suitable for biotechnological production of such colorants. Pepper, red beet, grapes, and saffron are examples of natural colorants derived from plants (Chattopadhyay et

al., 2008). Micro algae are preferred over various types of plants and organic sources for the production of a variety of bio pigments due to the following qualities: (1) They can be grown on non-arable land, avoiding competition with agricultural lands; (2) They can grow and flourish throughout the year in a variety of conditions, increasing their harvest potential (Sain et al., 2020). When evaluating possible toxicity, it is essential to correctly identify and characterize the source of the bio colorant because harmful chemicals are concentrated in specific taxa. Different taxa also have their own distinctive pigments, whether they be fungus or plants. According to the developmental stage and harvesting period, the composition and quantities of bio colorants vary in plant parts such as stems, leaves, flowers, roots, bark, berries, and cones (Räisänen et al., 2020).

3. Plant Pigments: Four major families of plant pigments are formed based on a shared structural and metabolic basis. In addition to these major pigment classes, there exist a wide variety of pigments that have a restricted taxonomic distribution and are frequently not well understood. (Swami et al., 2020)

- **Chlorophylls:** Chlorophyll is composed of a porphin ring, a symmetrical cyclic tetrapyrrole with a phytol connection and a center-positioned magnesium ion. All terrestrial crops, green plants and green algae have two types of this particular pigment, Chlorophyll a and Chlorophyll b, while red algae have only Chlorophyll a (Sigurdson et al., 2017)
- **Carotenoids:** Carotenoids, an important family of lipid-soluble pigments with colours ranging from yellow to orange to red, are present in a wide range of creatures, including higher bacteria, plants, fungi, yeast, birds, and insects. Lycopene, beta-carotene, and lutein are examples of acyclic, monocyclic, and dicyclic carotenoids, respectively (Langi et al., 2018).
- **Anthocyanins:** These are water-soluble flavonoid pigments that look like pH-based red to blue. Commonly consumed fruits and vegetables include six main aglycones (anthocyanidins), which vary in hydroxylation and methoxylation levels. Due to their poor stability, anthocyanins have had difficulty being used as natural food pigments; their colour is susceptible to oxygen, heat, light, and pH conditions, which restricts their use in various food products (Ovando et al., 2009).
- **Betalains:** Betalains, which are frequently found in red beetroot and cactus pear, are water-soluble pigments that are responsible for the colours of plants in the order Caryophyllales. Among the many N-heterocyclic compounds are betalains, the red and yellow pigment ammonium derivatives of betalamic acid. They are divided into two major categories: red-violet betacyanins, which are the condensation products of betalamic acid with cyclo-Dopa, and yellow-orange betaxanthins, which are the condensation products of betalamic acid and amines.

Limitations: Although plant pigments are emerging solutions, they suffer from several bottle necks, e.g.; abundant orange-yellow pigment such as curcumin (from *Curcuma longa* plant rhizome) must be debittered to prevent odor and strong taste. The majority of natural colours used in the food business are plant extracts, which have a number of

drawbacks including light, heat, or pH instability, limited water solubility, and seasonal unavailability. Therefore, pigments such as anthocyanins, chlorophyll, and betanine impose certain constraints such as being pH-dependent, oxygen-sensitive, heat-sensitive, and photo-oxidant (Tibor et al., 2007).

4. Animal Pigments: Animals can display colourful colorants thanks to a variety of pigment classes, such as the red, orange, and yellow carotenoids. However, it is unknown whether other chemical pigments in animals have similar physiological advantages. Other important groups of animal pigments, including melanins, pterins, porphyrins, psittacofulvins, and flavonoids, have been discovered to also exhibit antioxidant action in living systems. Due to the immunomodulatory effects of several pigment-based animal colour decorations, which they may be able to correctly represent in the health state of (McGraw, 2005).

- **Melanins:** The most prevalent biological colours in nature are melanins. The growth of life on Earth depends on the existence of melanins in both the integument and interior structures since melanization is the basic physiological reaction of animals. Because orthodiphenols are oxidised to orthoquinones to accomplish polymerization of the subunits that form the big pigment molecules, melanin formation (also known as melanogenesis) in melanocytes should be seen as an oxidative process. Some of the genes that regulate melanogenesis are also involved in cellular antioxidant responses; however, pleiotropic effects rather than melanins' direct antioxidant activity would serve as a mediator between melanins and these responses. (Galván & Jorge., 2016).
- **Pterin:** Pterin pigments are UV-fluorescent nitrogen-rich compounds that animals synthesise from basic purine (e.g. guanine) precursors (Steffen & McGraw 2007). Adenine and guanine are two examples of the family of important nucleotides known as purines that are catabolized by-products of pterin pigments, a class of nitrogenous, heterocyclic compounds. Many insects, fish, amphibians, and reptiles employ red, orange, and yellow pigments in their sexual colour displays. Examples include orange sulphur butterflies (*Colias eurytheme*), guppies (*Poecilia reticulata*), and green anoles (*Anolis* sp.). The vibrant red, orange, and yellow eyes of birds like starlings, blackbirds, owls, and pigeons have also been found to contain pterins (McGraw., 2005).
- **Porphyrins:** Porphyrins are natural pigments found in a wide range of organisms that can bind to proteins such as cytochromes and haemoglobin. Their major structural features can be traced back up to 1.1 billion years. Macrocyclic tetrapyrrole derivatives known as porphyrins are widely distributed in nature. They may be changed by a number of substituents, such as extra rings or ring opening, and are commonly complexed with a metal ion that is present in the middle of the ring system. This allows for a wide range of functionalities (Tahoun et al., 2021).
- **Flavonoids:** Flavonoids function as beneficial antioxidants found in plant diets. There is a massive literature on flavonoids' positive effects for human health in veggies, fruits, and plant extracts. Instead of a structural event that is typical of such colours, blue butterflies from the family Lycaenidae develop blue and UV hues on their wings

from flavonoids. One of the main groups of colourants in plants are flavonoids, which give flowers, fruits, and berries their vibrant hues. Flavonoids also include the anthocyanins, flavonols, flavones, and flavanones (Solovchenko et al., 2019).

- **Psittacofulvins:** Ornithologists have known for more than a century that parrots use a unique class of pigments to colour their feathers red, orange, and yellow. However, it wasn't until recently that the biological nature of these substances was clarified and it was discovered that the red pigments in the scarlet macaws' (*Ara macao*) plumage are a group of endogenously generated linear polyenes (Masello et al., 2008).

III. MICROBES AS SOURCES OF BIOCOLOURANTS

Microorganisms play an important role as food coloring agent for the reason that they are most potent living things, which control life and death on Earth. All the foods we eat contain microorganisms, which are also used as a source of food in the form of single cell proteins and food supplements as amino acids, vitamins, pigments, organic acids, and enzymes. Microorganisms are also responsible for the formation of some food products through the process of fermentation (Malik et al., 2012). Microorganisms are acknowledged as a viable source for the synthesis of bio-pigments due to their benefits over plants in terms of availability, stability, cost effectiveness, labour, yield, and simplicity of downstream processing. Numerous bio pigments, including carotenoids, melanins, flavins, quinines, monascins, and violacein, have been produced by microorganisms. Microorganisms can be grown via solid state and submerged fermentation on natural source materials or industrial organic waste. In addition to being colouring agents in the food processing and cosmetics sectors, many microbial pigments include anti-cancer, antioxidant, anti-inflammatory, and anti-microbial characteristics. (Tuli et al., 2015).

To create natural colours that are suitable for use in food, textiles, and medications, microbial pigments are in high demand. Microbial pigments are in high demand due to their endless potential resources, high pigment production, year-round harvestability, ease of cultivation, adaptability to various environments, genetic engineering, lack of negative side effects, eco-friendliness, and biodegradability, as well as their essential uses in a variety of fields including ecological, evolutionary, biomedical, agricultural, and industrial studies. (Chatragadda & Dufossé 2021).

In comparison to synthetic and inorganic pigments, microbial pigments have several benefits. Microbes can develop quickly and easily in regardless of the weather. Microbial pigments come in a variety of colorants. Because their comparatively long gene strands can be easily changed, microorganisms can be genetically modified. In the large-scale manufacturing of natural pigments and colorants, microbes are also more versatile and productive than higher forms of life (Kumar et al., 2015). Microbial pigments are a significant alternative that can eventually compete with synthetic dyes, despite the fact that they are not commonly used in colorant formulations. Numerous microorganisms, such as bacteria, fungus, and microalgae, create pigments. Despite the lack of a clear classification for all the colorants that bacteria can produce naturally, *in vitro* and *in vivo* research suggests that some of these compounds may be useful for the treatment or prevention of degenerative disorders. In the inexpensive culture medium, microbes can develop quickly and easily, independent of weather conditions. There are microbial colors available in various shades. These colors are

environmentally friendly and biodegradable. They also have countless clinical properties such as *anticancer*, *antioxidant*, *anti-proliferative*, *diabetes mellitus therapy*, *immunosuppressive* and so on. Therefore, the manufacturing of microbial pigments is now one of the emerging study areas to show its potential for different industrial applications (Kumar et al., 2015). The majority of bacterial pigment production is currently in the research and development stage. The production of pigments by microorganisms and microalgae is widespread in nature. In nature, microorganisms that produce pigment are fairly common and have a wide range of colours. Microbiological pigments come in a variety of hues. These pigments are biodegradable and environmentally friendly. Because of their unique chemical makeup and the presence of certain chromophores, pigments vary widely. *Blakeslea trispora*, *Streptomyces chrestomyceticus*, *Flavobacterium sp.*, *Phycomyces blakesleeanus*, *Phaffia rhodozyma*, and *Rhodotorula sp.* are a few examples of microorganisms that produce pigments (Dharmaraj & Dhevendaran., 2009; Vikas Bhat et al., 2013).

On several food pathogens and bacteria that cause food spoiling, microbial pigments have demonstrated an antimicrobial effect. The growing body of evidence supporting the benefits of carotenoids for human health as well as the advancement of some areas of agriculture, particularly aquaculture and poultry, have contributed to the major increase in interest in carotenoids. Many customers are probably unaware of the unusual origins of some of the already legal "natural" pigments, and fungus are reportedly one such potent microorganisms producing food grade pigments (Babitha et al., 2007).

A uncountable bacteria, molds, yeasts and algae produce pigments. The following requirements should be met by suitable species:

- Being able to utilize a variety of C and N sources;
- Must be capable of withstanding changes in growth circumstances, temperature, mineral concentration, and pH;
- Must be non-toxic and non-pathogenic
- Suitable color yield;
- Should be quickly divisible from the cell mass.

1. Fungal Pigments: Filamentous fungi known create a fantastic variety of colorants. The past report of fungal production pigment as *Monascus* for ang-kak. Carotenes production record of fungal species more than 200 species. At a variety of pH levels, several fungi colours remain stable. Latest research has widely discussed the fascination with *marine* species as sources of novel chemicals, such as new colorants. A number of marine-derived endophytic fungi such as *Eurotium rubrum*, *Haloroselli*, *Hortaea*, *Phaeotheca*, and *Trimmatostroma* have been reported for pigment production. However most fungi have been found to produce stable, non-toxic colorants, the development of pigments produced from fermentation requires a significant upfront expenditure in terms of medium components. The synthesis of carotene by microbes is the greatest illustration. Compared to the cost of producing -carotene synthetically, which costs US\$500/kg, microbial production costs about US\$1000/kg.

- **Bacterial Pigments:** In comparison to fungus, using bacteria for pigment manufacturing has a number of advantages, including a shorter life cycle and ease of genetic manipulation. Numerous biological niches, including soil, rhizospheric soil,

dry sand, fresh water, and marine samples, are home to pigment-producing bacteria. They have been found in places with low and high temperatures, can survive in salty environments, and can exist as endophytes. It is now possible to alter the bacteria to create the desired colour thanks to current innovations in genetic engineering. Blue-pigment-producing *Streptomyces coelicolor* can be genetically altered to create bright yellow (*kalafungin*), orange, or yellow-red pigments (*anthraquinones*) (Narsing Rao et al., 2017).

- **Yeast Pigments:** *Rhodotorula*, *Yarrowia lipolytica*, *Cryptococcus sp.*, and *Phaffia rhodozyma* are only a few yeasts that are good sources of microbial colours. Red pigment astaxanthin is typically found in animals but is quite infrequent in microbes like *P. rhodozyma*. The cultural environment has a significant impact on the production of carotenoid from *P. rhodozyma*. The ability to use urea is found in *Basidiomycetes* yeasts but is less frequent in *Ascomycetous* yeasts (V K Joshi et al 2003). Moreover, several reports also mention the effective production of pigments by *R. graminis*, *R. mucilaginosa*, and *R. gracilis*. These red yeasts mostly produce torulene and torularhodin, with trace amounts of beta-carotene (Zoz et al., 2015).

In comparison to the price of generating synthetic or natural pigments, efforts have been made to lower the cost of producing colours generated from microorganisms. By isolating new or better microorganisms, as well as by enhancing the processes, innovations will advance the economy of pigment manufacturing. Therefore, research on microbial bacterial pigments should be emphasised, especially in finding affordable and suitable growing media that can reduce costs and increase their suitability for industrial manufacturing (Gomez et al., 2014).

2. **Advantages of Using Microbes for Biotechnological Production of Food Grade Pigments:** Due to the stability of the pigments generated and the accessibility of cultivation technology, the microbial pigments are of excellent concern. There are many benefits for microbial pigments over artificial and inorganic colorants. It is possible to isolate, extract, characterize and purify microbial strains generating pigments from various environmental sources such as plants, animals, water, and soil (Sen et al., 2019). The results of recent studies demonstrate the enormous potential of microbial pigments in the food industry as well as the possibility of discovering novel strains from various underutilised sources, including agro-industrial waste, marine fungi, filamentous fungi, etc. Microbial pigments give food colour, but they also have well-known anti-inflammatory, anti-cancer, immune-regulating, anti-inflammatory, antiproliferative, and immunosuppressive properties (Sen et al., 2019). Pigments of β -carotene, Arpink red, riboflavin, lycopene and Monascus are the most widely used food grade pigments. Many presently approved natural food coloring products have a number of disadvantages, including a reliance on raw material supply and pigment extraction variants. The use of fungal pigments in the beverage, food, pharmaceutical, cosmetic, textile, and painting industries is then discussed, along with its limitations and future prospects (Vendruscolo et al., 2016).

Significance of Microbial Pigments as Natural Colorants: The most common species on Earth, microorganisms control life and death on this planet. All the ingredients we use are affected by microorganisms, which also produce some food items through the

fermentation process and can be used as a food source for single-cell proteins and a dietary supplement for organic acids, amino acids, vitamins, pigments, and enzymes. (Rajendra Singh et al 2017). Thus, microbial pigments are a great substitute. Since microorganisms are known to produce a wide range of pigments, they provide a viable source of food coloring. Several of the most significant natural pigments are carotenoids, flavonoids, tetrapyrroles, and several xanthophylls, including astaxanthin. The most typical colour used in sectors is beta-carotene, which is generated from certain microalgae and cyanobacteria. A very important red pigment derived from the algae *Phaffia rhodozoa* and *Haematococcus pluvialis*, astaxanthin is employed in the feed, pharmaceutical, and aquaculture industries. (Vikas bhat et al., 2013). *Monascus* species, *Paecilomyces* species, *Serratia* species, *Cordyceps* species, *Streptomyces* species, yellow-red and blue compounds produced by *Penicillium herquei*, *Penicillium atrovirens*, *Rhodotorula* species, *Sarcina* species, *Cryptococcus* species, *Monascus purpureus* species, *Phaffia rhodozyma* species, *Bacillus* species, *Achrom.*

In order to manufacture colours for use in food, the food industry has grown more interested in utilising microbial technology. Additionally, it can help allay public apprehension regarding the harmful effects of artificial colour additives on human health. Additionally, using natural colourants will benefit both human health and biodiversity preservation since harmful chemicals that would otherwise be released into the atmosphere due to the production of synthetic colourants might be avoided. These natural colours are found in kid-friendly meals, breakfast cereals, sauces, pastas, processed cheese, fruit-flavored drinks, vitamin-fortified milk products, and some energy drinks. Thus, natural colours can fulfil the twin need for aesthetically pleasing colours and probiotic health benefits in food items, in addition to being environmentally sustainable. (Rymbai et al., 2011).

IV. PRODUCTION OF FOOD GRADE PIGMENTS FROM MICROBIAL SOURCE

- **Monascus:** Traditional oriental dishes contain monascus pigments as a natural colouring ingredient. These are obtained by solid state fermentation from *M. purpureus* cultured on steamed rice. Food colorant of carotene produced by *Dunaliella salina* algae. Chemical alterations involving interactions between the natural pigments and, amino acids, nucleic acids, proteins, lactic acid, amino alcohols, chitosan, etc. were made to improve the quality (water solubility) of *Monascus* colorants (Vendruscolo et al., 2016).
- **Rhodotorula:** The *Rhodotorula* species are purely aerobic yeasts with peculiar metabolic characteristics, such as the capacity to synthesise glycogen during the exponential growth phase and significant quantities of lipids and carotenoid pigments during the stationary growth phase. Species of *Rhodotorula* can be found in animals and the natural world. *Rhodotorula* can be found in milk and cheese products, as well as the phyllosphere (leaf surfaces), soil, and air. (Almanza et al., 2014).
- **Phaffia Rhodozyma:** *Rhodotorula* is a common yeast found in the environment, including soil, lakes, air, , milk, fruit juice, and ocean water. *Rhodotorula* species, which are classified as Basidiomycota, colonise humans, plants, and other mammals.

Rhodotorula produces pink to red colonies and unicellular blastoconidia devoid of pseudohyphae and hyphae (Wirth & Goldani 2012).

- **Bacillus Subtilis:** *B. subtilis* creates pigment during sporulation. Spizizen's salt, glucose, 27.7, L-tryptophan, 0.25, L-tyrosine, 0.25, L-histidine, 0.055, and MnSO₄, 0.67 mM were the ingredients that worked best as a liquid medium to produce color. Before being injected with bacteria, each of the salt, amino acid, and glucose components was individually autoclaved.
- **Aspergillus Oryzae:** An orange-red pigment from the anthraquinone family is produced by *A. oryzae* var. *effusus* (Joshi., 2003). By genetically modifying *Aspergillus oryzae*'s non-ribosomal peptide biosynthesis pathway to overexpress the indigoidine synthase gene, this fungus was used as a platform cell factory to produce the blue pigment indigoidine. (Panchanawaporn et al., 2022).

1. **Preparation of Inoculums:** The NA slant's pure bacterial culture was transferred to 100 ml of pre-sterilized nutrient broth, where it was shaken for 24 hours at 30 °C. Inoculums were created using 1% of the aforementioned cell suspension and are depicted in Flow Chart 1.
2. **Types of Food Grade Pigments Produced by Microorganisms:** Last few years, microorganisms have been used to produce uncountable molecules that are as diverse as antibiotics, anticancer, antioxidants, enzymes, vitamins, textured agents, etc. Additionally, businesses are becoming more and more interested in producing various pigments. In nature, microorganisms (yeasts, bacteria and Fungi,) that are high in color and pigment are frequent. Microorganisms produce a wide range of colours, some of which are particularly important as food-grade pigments. These pigments include melanins, flavins, carotenoids, prodigiosins quinones, specifically violacein, indigo and monascins,

V. CLASSIFICATION OF MICROBIAL PIGMENTS

Colorants produced by bacteria are prevalent referred to as bio pigments as similar to their secondary metabolism. These bio pigments are generally used both artificial and marketable. Biological pigments can be divided into groups according to their structural similarities and geographic distribution. The following are some instances of naturally occurring microbial pigments:

1. **Carotenoids:** *Heinrich Wilhelm Ferdinand Wackenroder* was the first to discover carotenoids. There are a variety of microorganisms that produce carotenoids, including, *Rhodobacter sphaeroides*, *Mucor circinelloides*, *Dunaliella sp.*, *Blakeslea trispora*, *Rhodotorula mucilaginosa*, *Sphingomonas sp.*, *Phycomyces blakesleeanus*, , *Fusarium sporotrichioides* and *Flavobacterium multivorum*. Microorganisms that make carotenoids have been found in slattern crystallizer ponds, caves, coastal settings, and soil. (Narsing Rao et al., 2017).
2. **Riboflavin:** Numerous microorganisms create the yellow pigment known as water-soluble vitamin B₂. It is utilized in sauces, fruit drinks, morning cereals, infant foods,

dairy products, and energy beverages. Through commercially viable biotechnological processes, the traditional chemical manufacture of riboflavin is currently being restored by filamentous fungi like *Candida famata*, bacteria like *Bacillus subtilis*, and ascomycetes like *Ashbya gossypii*, (Averianova et al., 2020).

3. **Beta-Carotene:** A reddish-orange-tinted organic colorant that is mostly derived from the algae *Dunaliella salina*, which is more in beta-carotene. The fermentation of *Blakeslea trispora* produces a pigment that is an appropriate colouring agent and is comparable to pigments made chemically. Its hues range from red to yellow and a variety of culinary products use it. (Veni et al., 2009).
4. **Canthaxanthin:** Canthaxanthin is a fat-soluble carotenoid with an orange to deep pink hue and is a potent antioxidant. A trans-carotenoid pigment was discovered from *Bradyrhizobium Sepp* and is used as a food coloring in many different foods, including salmon and poultry feed. (Kirti et al., 2014).
5. **Astaxanthin:** Astaxanthin (AX) is a ketocarotenoid that plays an important role as a pigment. It gives flamingos, crustaceans, and salmonid fish their pink to orange color. Some bacteria, fungi, and microalgae are the primary producers of astaxanthin, which accumulates the pigment intracellularly for its photoprotective role against excessive light (Júlio Cesar de Carvalho et al., 2022).
6. **Prodigiosin:** Prodigiosin, a bright red pigment produced by *Serratia* organisms, is one of the most visible pigments in the microbial world. Prodigiosin's chemical structure is still being researched, but it has been identified as a tri-pyrrolymethene. The rather rapid production of a flashy red pigment, which escaped the notice of men before they understood the nature of microbial growth, can now be explained in terms of prodigiosin production. Prodigiosin pigments have piqued the interest of organic chemists and pharmacologists, and they may one day be used to treat infectious diseases such as malaria, as well as immunosuppressive agents (Veni et al., 2020).
7. **Phycocyanin:** Cyanobacteria that possess chlorophyll A create the blue pigment known as phycocyanin. *Spirulina* and *Aphanizomenon flos-aquae* both produce phycocyanin, a natural colouring ingredient called "Lina Blue" that is utilised in the food and beverage sector. It may also be found in desserts and ice cream (Eriksen., 2008).
8. **Violacein:** Gram-negative bacteria like *Chromobacterium violaceum* produce the pigment violacein. Due to its distinctive physiological and biological activities, as well as its interactions with different antibiotics, it has attracted a lot of attention. Numerous microorganisms, in addition to *C. violaceum*, are known to produce violacein, including *Duganella* sp., *Pseudoalteromonas* sp., *Iodobacter* sp., and *Massilia* sp (Park et al., 2021).
9. **Melanin:** Natural pigments called melanins can be found in many microorganisms, plants, and animals. They are extensively utilised in a variety of products, including eyeglasses, cosmetics, food, sunscreen lotions, and medications.
10. **Lycopene:** The bright red carotenoid pigment that is abundant in tomatoes and consumed there. It has been extracted from microorganisms including *Blakeslea trispora*, *Fusarium*,

and Sporotrichioides, and has the potential to diminish chronic illnesses like coronary heart disease and certain cancers. It is used to color meat in countries like Australia, New Zealand and USA. (Sen et al., 2019)

VI. LARGE SCALE INDUSTRIAL PRODUCTION OF FOOD GRADE PIGMENTS

Finding an economical and acceptable growing medium is of particular importance for bacterial pigments in order to lower costs and expand their suitability for industrial manufacture. Fermentation is fundamentally quicker and extra efficient than other chemical processes, making it more suitable for industrial manufacture. Because microorganisms can easily alter their relatively long strands of DNA, they are amenable to genetic modification. Genetic engineering can thereby improve the synthesis of microbial pigments in geometric ratios as compared to chemical scaling techniques. In the commercial synthesis of natural pigments and colourants, microbes are more prolific and flexible than larger living organisms. Genetic engineering has enhanced the fermentation process, and additional research into non-toxic microbial pigment might result in enormous advances in microbial pigment economics. Any microbial pigment created biotechnologically (for instance, by fermentation) depends on customer acceptability, regulatory approval, and the funding needed to bring the product to market. A few years previously, some questioned the value of marketing fermenting-derived food grade pigments due to the significant capital investment demands for fermentation equipment and the pricey and time-consuming hazard assessments required by regulatory organizations. Microbial colourants, for instance, are already used in the seafood company to enhance the salmon's pink hue. It's also possible that some natural food dyes will be used commercially as antioxidants. The FDA has approved and deemed safe a number of fermented food grade pigments, including, Arpink Red from *Penicillium oxalicum*, Ashbygossypii riboflavin, *Blakeslea trispora trispora* carotene, monascus pigments, and astaxanthin from *Xanthophyllomyces dendrorhous* which are currently on the market. The successful marketing of microbial pigments as a food colouring and dietary supplement serves as an example of the significance of niche markets with active customers (Rana et al 2021).

VII. NOVEL PRACTICES OF MICROBIAL PIGMENT PRODUCTION

A pigment-producing bacterial strain was altered to overproduce pigment, changing its hue and structure. It has been genetically modified in *Streptomyces coelicolor*, a bacterium that generates the blue pigment actinorhodin, to generate the brilliant yellow polyketide kalafungin, which is utilised to generate pigments related to anthraquinones. The development of cell factories to effectively create pigments employed the biosynthetic route from recognised pigment producers. Targets for pigment synthesis by genetically engineered bacteria include the red (lycopene), orange (-carotene), and purple (violacein) pigment-producing bacteria that significantly contribute to pigment creation (Venil et al., 2020).

As the colours produced by natural-type strains are frequently insufficient in quantity and necessitate longer fermentation times, the approach is normally unproductive and strain improvement is crucial. Popular mutagens include, ethyl methyl sulfonate (EMS), 1-methyl-3-nitro-1-nitrosoguanidine (NTG) and ultraviolet (UV) can enhance strains and multiply pigment output by a ratio of several hundred (Sen et al., 2019).

VIII. FACTORS AFFECTING MICROBIAL PRODUCTION OF FOOD GRADE PIGMENTS

- 1. Temperature:** Incubation temperature is the primary variable that depends on the microorganism type. *Monascus* sp. development. While *Pseudomonas* sp. needs 35–36°C for pigment formation and synthesis, requires 25–28°C for pigment manufacturing (Joshi et al., 2003).
- 2. pH:** Compared to bacteria, moulds and yeasts can thrive at lower pH levels, and gram-negative bacteria are more sensitive to low pH than their Gram-positive counterparts. Microorganisms can be categorised as follows according to their pH ranges: I) pH levels between 5 and 8 are ideal for neutrophilic growth. II) At a pH lower than 5.5, acidophiles thrive. III) At a pH greater than 8.5, alkaliphiles thrive (Hamad, 2012).
- 3. Carbon Source:** The mycelial growth of microorganisms that create color is influenced by the type of carbon source, such as lactose, galactose, glucose, fructose and maltose. For growth and the production of pigments, glucose and its oligosaccharides are preferable carbon sources (Joshi et al., 2003).
- 4. Fermentation:** Modern fermentation methods have made it simple to produce and isolate colour pigments. Both submerged and solid substrate fermentation can be used to make microbial pigments. During solid substrate fermentation (SSF), microbial biomass is grown on the surface of a solid substrate (Tuli et al., 2015).
- 5. Minerals:** In the creation of pigments, minerals are crucial. In a liquid medium, Zinc (2 10⁻³ M and 3 10⁻³ M) stopped the growth, but this was not seen in a solid medium.
- 6. Nitrogen Source:** The most effective ingredients for making *Monascus* pigment are ammonium chloride, ammonium nitrate, and glutamate. The least effective nitrogen source is potassium nitrate, while glutamate has proven to be excellent for the production of pigments (Joshi et al., 2003).
- 7. Moisture Content:** Simply put, a product's moisture content is its water content. It affects a substance's weight, density, viscosity, conductivity, and other physical characteristics. The two main techniques for determining moisture content are Karl Fischer titration and loss on drying (Mermelstein, 2009).
- 8. Aeration Rate:** The bed of rice is continuously aerated by splitting with humidified air (95-97% percentage moisture) when the *Monascus* colors are produced from the solid-state fermentation. Forced aeration rates larger than 0.5 L min⁻¹ reduce the formation of biomass and colorants because water is lost from the bed. At forced aeration rates of 0.05 to 0.2 L min⁻¹, the greatest pigment concentrations may be reached (Kumar et al., 2015).

IX. APPLICATIONS OF MICROBIAL PIGMENTS

Microbial pigments have tremendous applications in food, textile, pharmaceuticals, cosmetics, Nutraceuticals and medicinal fields. In addition, it has many clinical properties as immunosuppression, antioxidant, anticancer, antiproliferative, and treatment for diabetes

mellitus. Due to their functions as immunosuppressive, anticancer, anti-aging, and antioxidative agents, carotenoids, prodigiosin, astaxanthin, and violacein have found use in the medical field among the many microbial pigments. (Guerin et al., 2003; Williamson et al., 2007; Raj et al., 2009). Red bio-pigments generated by certain *Serratia* species (*Serratia marcescens*) as a typical secondary metabolite, fungus *Monascus* sp and actinomycetes demonstrate antimicrobial activity (Mekhael & Yousif, 2009) and have a powerful capacity to create antitumor medicine (Tomas et al., 2003). Table 1 describes about the different microbial pigments structures and its applications.

1. Applications in Food Industry: Beta carotene from the European *Blakeslea trispora* fungus or Asian *Monascus* pigments are two examples of colours produced by fermentation that are currently used in the food sector. In addition to their nutritional and medicinal advantages, antibiotics and antioxidants also give many colours a healthy look. (Venil & Lakshmanaperumalsamy, 2009). For instance, the organoleptic features of food products are improve by *Monascus* red pigments, usually manufactured as MFR (*Monascus* Fermented Rice). These pigments contain monocolin, increasing HDL cholesterol and reducing LDL cholesterol. Microbiological pigments exist and are being researched for the future.

- **β -Carotene Production:** Pro-vitamin of yellowish carotenoid pigment called as (β -Carotene A). It activity as antioxidant and has potential positive effects product certain diseases. Following microbes are mainly using for β -Carotene production.
 - **Dunaliella Salina:** A microalgae that produces beta-carotene called *Dunaliella Salina* is renowned for its exceptional capacity to store essential beta-carotene. It examines what influences the downstream processes, culture system, and culture conditions that result in the manufacture of beta-carotene from *Dunaliella salina*. Studies show that utilizing an airborne photo-bioreactor culture system improves the mechanism of production (Pourkarimi et al., 2020).
 - **Blakeslea Trispora:** Certain strains of this mold produce an excessive amount of -carotene. B. There are two categories of *Trispora* strains: (+) match type and (-) match type. (-) Carotene is produced by strains derived from mating ratios above two different types of mating. Two industries currently produce B. *Trispora* fungal -carotene, one in Leone, Spain, and the other in Russia (Papadaki et al., 2021).
 - **Mucorcircinelloides:** A zygomycete fungus that accumulates beta-carotene is called *Mucor circinelloides*, and It is one of the model organisms used to study how fungus produce carotenes. The -carotene ketolase gene (*crtW*) of the marine bacterium *Paracoccus* sp. N81106 was fused with fungal promoter and terminator regions and integrated into the *M. circinelloides* genome to produce stable canthaxanthin-producing strains (Papp et al., 2013).
 - **Phycomyces Blakesleeanus:** *Phycomyces* is largely used in the production of many compounds, including -carotene. They demonstrate improved carotenogenic capacity when grown on sturdy substrates or in liquid media.
- **Arpink Redtm Production:** The species *Penicillium oxalicum* is the source of the red pigment that it has drawn from the soil. It has anthraquinone class chromophores in it.

The Codex Alimentarius Commission recommended using the red pigment Arpink Red in a variety of food products.

- **Riboflavin (Vitamin B2) Production:** Many countries legally used yellow food colorant. Due of its unique affinity, it is primarily used for products made from grains. Riboflavin's applications are somewhat limited by its unpleasant taste and indistinct odour. Microorganisms frequently produce riboflavin through fermentation. There are three types of riboflavin fermentation: weak over producer, mild over producer, and vigorous over producer. Due to its higher production and greater genetic stability, *Ashbya gossypi* fermentation is preferred; riboflavin concentrations above 15 g/L have been seen (Venil et al., 2013).

- ***Monascus* Pigments Production:** It is specifically a member of the Monascaceae family and the Ascomycetes group. The *Monascus* genus may be divide into 4 species: *M. Froridanus*, *M. pilosus*, *M. Ruber* and *M. purpureus*, the large part of which are isolated strains from conventional oriental cuisine. Red red rice, MFR (Monascus Fermented Rice), benikoji (Japanese), hung-chu, hongqu, zhitai (Chinese), angkak, red leaven, red mould (USA), and Yeast Rice (RYR), are some of the more well-known names for this fungus product. Industrial pigments are manufacture in large quantities, and there are mainly three types of industrial pigments: red dyes, orange dyes, and yellowish dyes (Mapari et al., 2005). A prospective source of pigment is the red mould, *Monascus purpureus*, which historically used to make red rice. It is simple to tell the two species apart using the ascospores of *M. purpureus*, which have a diameter of 5 microns or are ovoid (65 microns) and seem spherical. The *Monascus* fungus-producing organisms that create angkak have the capacity to convert starchy substrates into a variety of substances including ethanol, prescription antibiotics, antihypertensives enzymatic agents, fats, flavorings that cause flocculation, ketone bodies, organic acids, colors, and micronutrients. Consequently, the addition of a particular flavour to food made possible by the employment of *Monascus* pigment as a colouring agent (Agboyibor et al., 2018).

- **Lycopene Production:** A terpene from the C40 family of terpenoids, lycopene is a deep red carotenoid. Ripe red fruits and vegetables in particular have a high prevalence of it. Lycopene has been shown to reduce the risk of cardiovascular disease, prostate cancer, and other cancers. one of the most often used carotenoids in pharmaceutical goods. Currently, the main source of lycopene used in commercial products is tomatoes. It is the longest carotenoid known and is an unsaturated red open-chain beta-carotene isomer. Psi-carotene, commonly known as lycopene, is water-insoluble and sensitive to heat and oxidation. According to a study, lycopene cis-isomers are more stable and have higher antioxidant capacity than lycopene all-trans (Lei Li et al., 2020). Lycopene can be found in beverages, dairy products, surimi, confectionary, soups, dietary bars, breakfast cereals, pastas, chips, sauces, candies, dips, and spreads. Reports on the production of food-grade pigments (carotenoids) produced during fermentation with feed ingredients and supplemented with decorative fish, *Xiphophorus helleri*, led to an improvement in fish pigmentation. The sea sponge *Callyspongia diffusa* yielded the white series strain of *Streptomyces* lycopene (AQBWWS1) (Dharmaraj et al., 2011).

2. Applications in Pharmaceutical Industry: The pharmaceutical sector incorporates several microbial pigments into its goods. Significant pharmacological and therapeutic potential exists for many of the bacterium's colored secondary metabolites. It shows that more different eubacterial species, including *Vibrio psychroerythrus*, *S. marcescens*, *Pseudomonas magnesorubra*, and others, have cytotoxic action. A tri-pyrrole called prodigiosin is the subject of numerous investigations to treat conditions including cancer, leukaemia, diabetes mellitus, etc. Antibiotic, anti-cancer, anti-proliferative, and immunosuppressive chemicals can all be made from these colours.

- **Antioxidant Properties of Pigments Synthesized from Microorganisms:** Carotenoids are pigments of natural origin that appear to play a key role in the human diet due to their potential benefits as pro-vitamins, antioxidants, or tumor-fighting agents and inhibiting substances. When membranes are under stress, torulerhodin's significant antioxidant activity aids in keeping them stable. Because they are precursors to hormones and vitamin A and have antioxidant and anti-aging properties, these carotenoids are advantageous. They might also strengthen the immune system and prevent some cancers. A distinct carotenoid with carboxylic acid, torulerhodin exhibits significant antioxidant activity. The most potent natural antioxidant, lycopene, is a tetraterpene with equal sides made up eight units of isoprene. *Phycomyces* and *Blakeslea* fungus have the potential to produce lycopene (Konuray & Erginkaya, 2018).
- **Anticancer Properties:** One of the deadliest illnesses a person can have is cancer. Numerous microbial pigments have anticancer properties (Rao et al., 2017) (Table 1).
- **Antifungal Properties:** According to various investigations, violacein from *Chromobacterium* sp. also possesses antifungal activities, as do prodiginine colorants (cycloprodigiosin and prodigiosin) isolated from the Indonesian marine bacteria *P. rubra* sp. The fungus *Aspergillus flavus* sp., *Rhizoctonia solani* sp., *Fusarium oxysporum* sp., *Penicillium expansum* sp., and *Fusarium oxysporum* sp. were among those that violacein inhibited. Additionally, studies have demonstrated that the pure antifungal compound violacein, derived from *Chromobacterium* sp., has an antifungal activity similar to that of basting and amphotericin B, pointing out the possible use of marine-derived bpBPs as potent antifungal agents over presently accessible manufactured antifungal properties compounds. (Ali Nawaz et al., 2020).
- **Anti Bacterial Properties:** Bacterially generated pigments, which are widely utilised in eastern nations, have been the subject of significant investigation in recent decades due to their potential for uses. Because of this, the quantity of chemicals recovered from bacteria is increasing more quickly than from other sources. Anthocyanins participate in many biological processes that benefit health and lower the risk of diseases including cancer, inflammation, and immunological response. Gram-negative bacteria that make violacein include *Janthinobacterium lividum*, *Ps. sp. 710P1*, *Ps. sp. 520P1*, *Pseudoalteromonas luteoviolacea*, and *Chromobacterium violaceum* (Venil et al., 2013).
- **Antileishmanial Properties:** A protozoan called *Leishmania* causes the fatal and disfiguring disease leishmaniasis. This disease affects more than 12 million people

worldwide. Only Leon et al. reported that bacterial pigments had antileishmanial activity. They claimed that violacein, a substance, had strong antileishmanial properties (Numan et al., 2018).

- **Antiviral Properties:** It has been reported that phenazine compounds produced by *Pseudomonas* and *Streptomyces* species exhibit promising antiviral activities. Violacein showed a high level of antiviral activity against the simian rotavirus SA II, poliovirus, and herpes simplex virus. Quinone substances with antiviral properties include benzoquinones, naphthoquinones, and anthraquinones.
 - **Anti-HIV Properties:** Chemicals derived from pigmented *Phoma* species have shown to inhibit the HIV integrase enzyme. Additionally, studies were started in vitro to determine how violacein affected lymphoma linked to AIDS (Ramesh et al., 2019).
- 3. Other Applications:** The rice carbohydrate used in the metabolism of *Monascus ruber*, which is used in the dairy industry to prepare flavor-infused milk, results in pigment as a secondary metabolite 60. Red, orange, and yellow pigments are produced when rice goes through solid state fermentation. The waste from the textile industry is significant and primarily made up of synthetic dyes. These synthetic dyes are employed in industries due to their straightforward and low-cost production, durability in temperature and light conditions, and sophisticated colors that cover the whole color spectrum (Kanchan et al., 2017).

X. CONCLUSION

Microorganisms are the most flexible biotechnological instruments because they can create a wide variety of compounds, such as, pigments enzymes, antibiotics, and organic acids. To date, the Latest research has shown some microbes have potential as an alternative to natural colors. According to numerous recent researches, pigments derived from microbes are clearly preferable to synthetic pigments and pigments derived from plants because of their stability, availability due to no seasonal variations, cost-effectiveness, high yield through strain enhancement, and straightforward downstream processing for extraction. It has been noted that the market for food colorants is growing at a rate of 10-15% annually. A study by Leather Head Food International (LFI) projects that by 2015 and beyond, the global market for food-grade pigment will have grown by 10% to reach \$1.6 billion. The United States accepts about 30 food additives, 6 of which are derived from microorganisms, while the European Union has authorized 43 colorants as food additives. The government's understanding of precautions for the environment and people is also up to date. Consumers have developed an aversion to the use of synthetic food coloring, so natural food coloring is in high demand, opening up potential study opportunities to investigate new techniques that could eventually lead to the development of food-grade pigments derived from microorganisms.

XI. ACKNOWLEDGMENT

Conflict of Interest: None

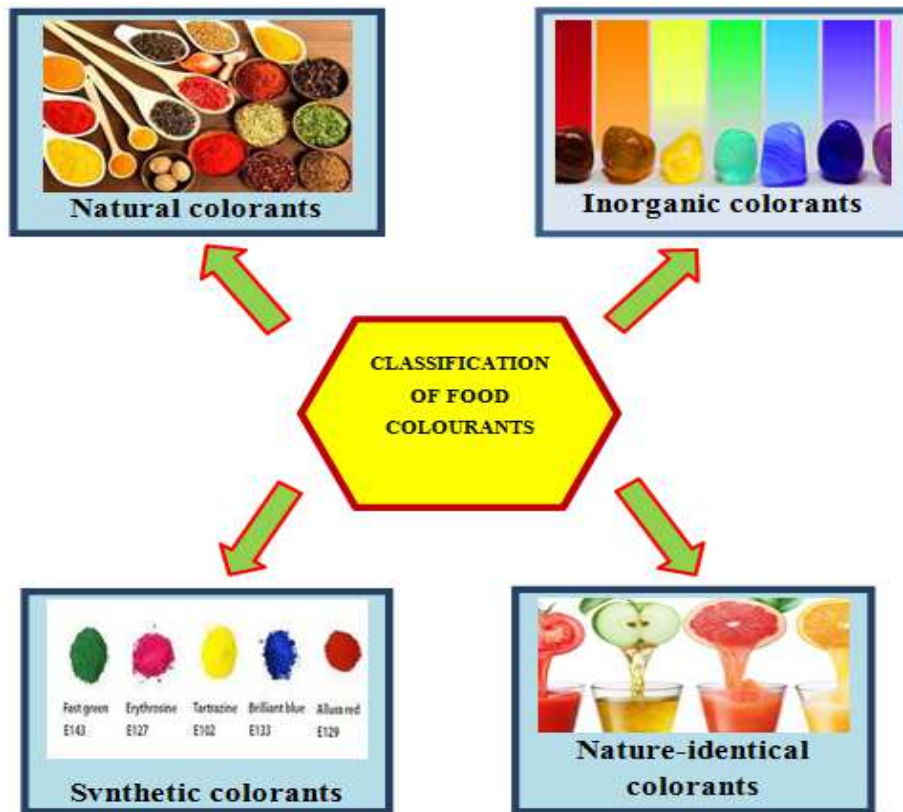


Figure 1: Classification of Food Colorants

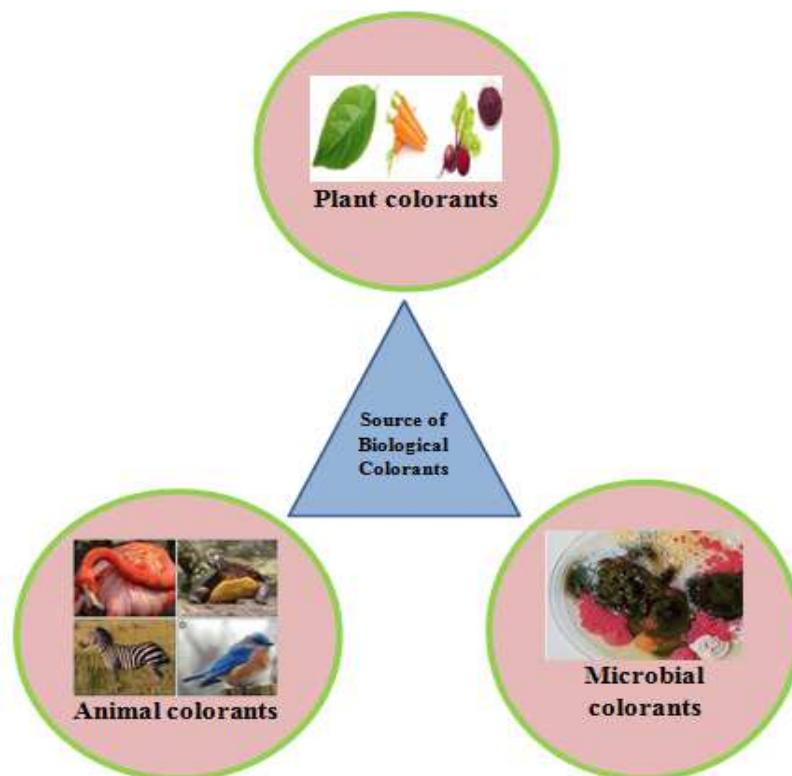
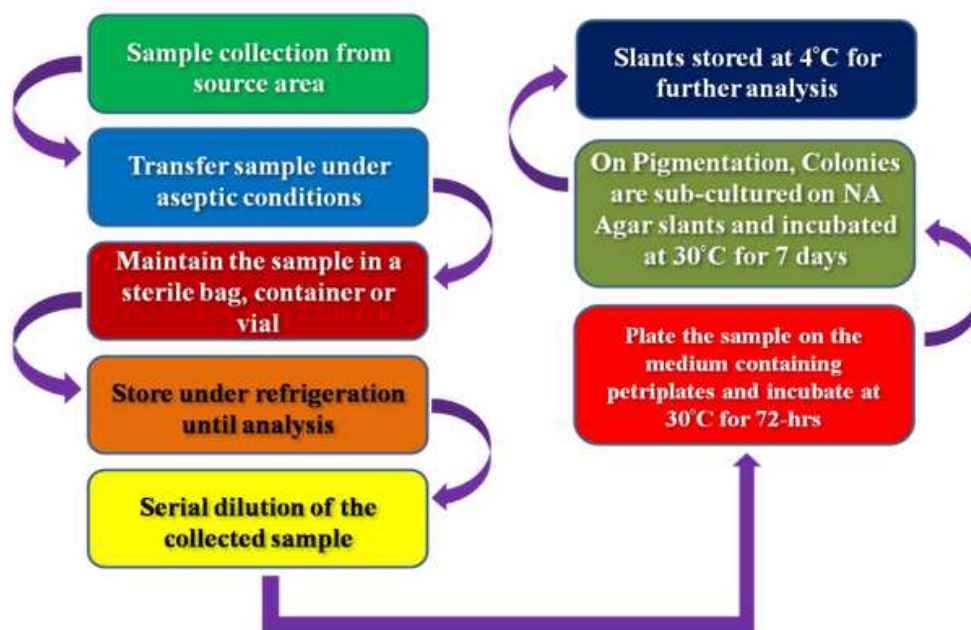


Figure 2: Source of Biological colorants

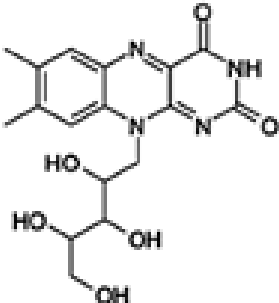
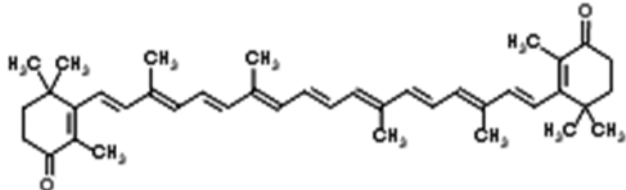
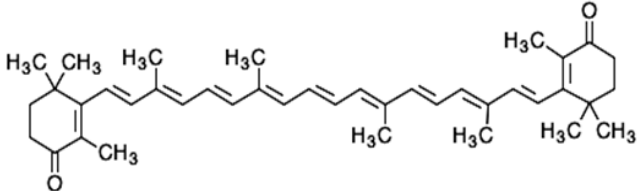


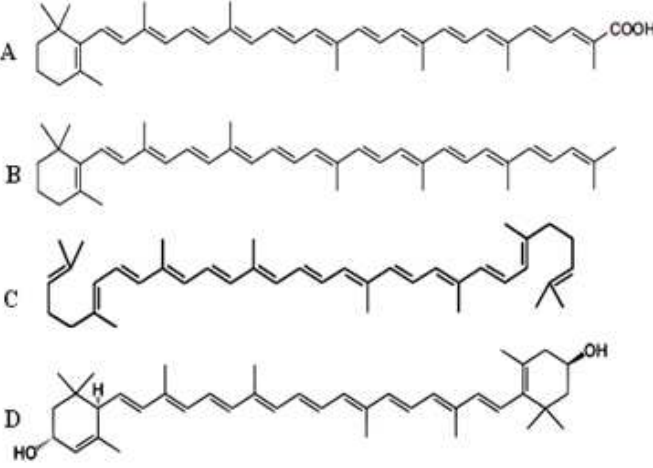
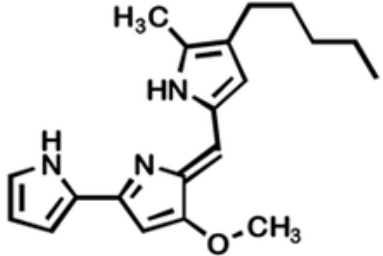
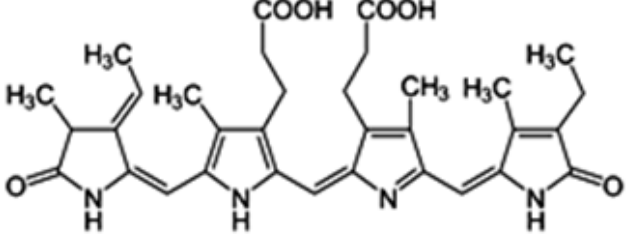
Flow Chart 1: Preparation of inoculum for microbial pigment production before fermentation

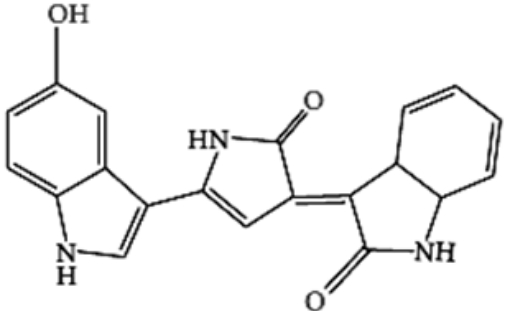
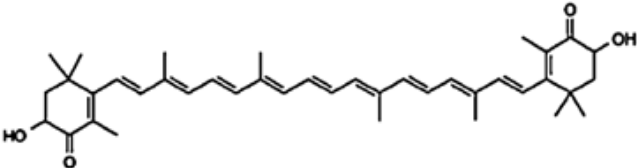
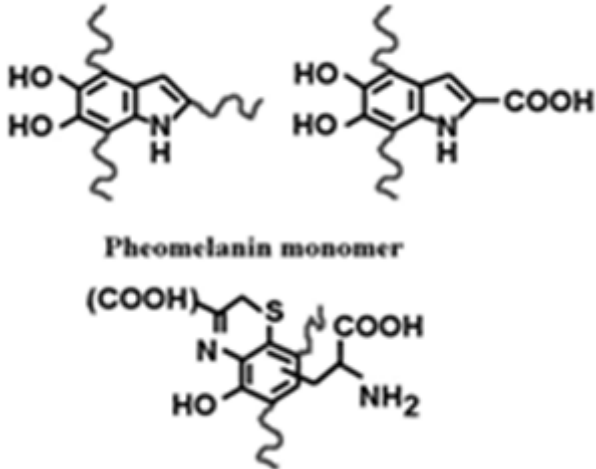
Table 1: Microbial pigments application of Anticancer properties

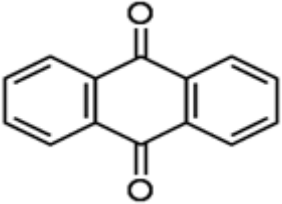
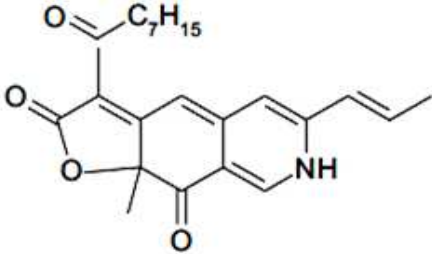
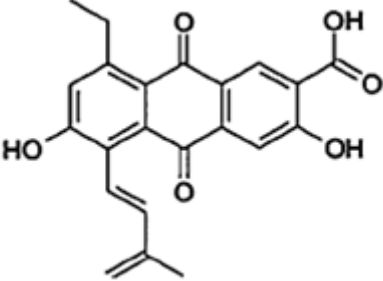
S. No	Microbial Pigments	Type of Cancer
1	Prodigiosin from <i>Pseudoalteromonas</i> sp	Cytotoxicity against U937 leukemia cells
2	Melanin from <i>Streptomyces glaucescens</i> NEAE-H	Skin cancer cell line
3	Antraquinone from <i>Alternaria</i> sp. ZJ9-6B	Human breast cancer cell lines
4	<i>Monascus</i> , such as monascin	Mouse skin carcinogenesis
5	Ankaflavin	Cancerous mammalian cell lines
6	Monaphilone B and Monaphilone A	Human laryngeal carcinoma cell lines

Table 2: Structure of Various Microbial Pigments and its Applications

Si No.	Microbial pigments used in food industry	Structure	Applications
1	Riboflavin	 <p>The structure shows the isoalloxazine ring system of riboflavin, which is a fused bicyclic heterocyclic ring system consisting of a benzene ring fused to a pyrimidine ring, which is further fused to an imidazole ring. The ribityl side chain is attached to the 10-position of the imidazole ring.</p>	<p>Baby meals, morning cereals, pastas, and vitamin-enriched meal replacement items all include riboflavin. Due to riboflavin's weak solubility in water, it is challenging to incorporate it into liquid goods; for this reason, riboflavin-5'-phosphate, a more soluble version of riboflavin, is needed. Another contemporary food colouring agent with widespread therapeutic application is riboflavin.</p>
2	β -Carotene	 <p>The structure shows the long polyene chain of beta-carotene, consisting of 11 conjugated double bonds and two non-conjugated double bonds. The chain is terminated by two beta-ionone rings, each with a methyl group and a ketone group.</p>	<p>Reduces the risk of breast cancer and is used to treat a variety of illnesses, such as erythropoietic protoporphyria.</p>
3	Canthaxanthin	 <p>The structure shows the long polyene chain of canthaxanthin, consisting of 11 conjugated double bonds and two non-conjugated double bonds. The chain is terminated by two beta-ionone rings, each with a methyl group and a ketone group.</p>	<p>Food, beverage, and medicinal preparations using colours</p>

4	<p>Carotenoids</p> <p>a. Torularhodin</p> <p>b. Torulene</p> <p>c. Lycopene</p> <p>d. Lutein</p>		<p>The antioxidant properties of torularhodin (acidic) and torulene (hydrocarbon) contribute to the stabilisation of membranes under stress. Because they are precursors to hormones and vitamin A and have antioxidant and anti-aging properties, these carotenoids are advantageous. They could also strengthen the immune system and prevent some cancers.</p> <p>Lycopene is a valuable antioxidant that is finding new uses as a dietary supplement and a component in cosmetic goods.</p> <p>3) Age-related macular degeneration may be prevented or treated using lutein, an antioxidant that has attracted growing interest.</p>
5	<p>Prodigiosin</p>		<p>Anticancer, immunosuppressant, antifungal, algicidal; dyeing (textile, candles, paper, ink)</p>
6	<p>Phycocyanin</p>		<p>One of the main pigment components of spirulina, phycocyanin, is utilised as a nutritional supplement and has anti-inflammatory and antioxidant qualities.</p>

7	Violacein		Pharmaceutical (antioxidant, immunomodulatory, antitumoral, antiparasitic activities); dyeing (textiles), cosmetics (lotion)
8	Astaxanthin		Used as feed supplement both for fish and shellfish.
9	<p>Melanin</p> <ol style="list-style-type: none"> Eumelanin Pheomelanin Allomelanin 	<p>Black melanin Brown melanin</p>  <p>Pheomelanin monomer</p>	It possesses antioxidant, antiphagocytic and blocks antimicrobials.

10	Anthocyanin	 <p>The structure shows a central carbon atom double-bonded to two oxygen atoms and single-bonded to two benzene rings.</p>	<p>They are in charge of giving many fruits, vegetables, grains of rice, and flowers their red, purple, and blue hues. They aren't utilised in medications or cosmetics. They are utilised in alcoholic drinks, fruit fillings, snacks, dairy goods, and confections.</p>
11	Monascorubramine	 <p>The structure is a complex polycyclic molecule with a central ring system, a lactone ring, a methyl group, a carbonyl group, and a long alkyl chain labeled C₇H₁₅.</p>	<p>The pigment is a secondary metabolite generated mostly in cell-bound form during <i>Monascus</i> fermentation. It is a component of processed meats, seafood, tomato ketchup, and other items.</p>
12	Arpink red	 <p>The structure is a complex polycyclic molecule with a central ring system, a carbonyl group, a methyl group, a hydroxyl group, and a long alkyl chain.</p>	<p>Arpink Red makes assertions about the anti-cancer properties of anthraquinone derivatives and their uses in the food and pharmaceutical industries.</p>

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