PRODUCTION OF FOOD GRADE PIGMENTS FROM MICROBIAL SOURCES

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Abstract

For long time, the utilization of manufactured synthetic colorants has been highly questionable on account 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. Microorganisms synthesize food grade pigments such as carotenoids, melanins, flavins, quinones, and more specifically monascins, violacein, indigo, etc. Every year, productions of fermentation-derived ingredients in the food industry are expanding constantly, but manufacture of food-grade pigments from microbial sources is still in developmental stage. To name a few, *Monascus* pigments, Astaxanthin from *Xanthophyllomyces dendrorhous*, *Phaffia rhodozyma*, Arpink red from *Penicillium oxalicum*, Riboflavin from *Ashbya gossypii*, βeta carotene from *Blakeslea trispora*, lycopene from *Erwinia uredovora* and Fusarium sporotrichioides. Furthermore, there are number of microorganisms like *Serratia* sp. and *Streptomyces* sp. produces carotenoids in great sums. Numerous factors such as pH, temperature, carbon sources, minerals, nitrogen sources, moisture content, types of fermentation and aeration rate can impact 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

1. INTRODUCTION

Color plays an important role in the food production and processing industries which contributes to the sensory attributes of food. It represents the freshness, nutritional, safety, and aesthetic value of food, and has a direct impact on the market value of the coloured food product (Tanuka Sen et al.,2019).Color is the primary feature of food that determines its consumer appeal. Bio colorants are agents that are derived from biological sources like plants, animals and microbes. Natural colour 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 and are harmful to humans because they cause allergenic and intolerance reactions (H. Rymbai1 , R.R. Sharma2 , Manish Srivastav et al. 2011). Four type of food colors (1) Natural , (2) Nature-identical ,(3) Synthetic , (4) Inorganic. Natural colors pigment made by moderation of resources from living organism, Nature-identical colors are nature and synthetic pigments both exist in nature. Synthetic colors are artificial pigment that are not also present in nature, Sample of inorganic colors gold and silver (Ali Aberoumand et al., 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 (Ronald E et al.,).Colour of foods produce physiological and psychological belief and attitudes that are grow by expertise, tradition, education and setting (Deanna M. Minich et al,.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 (Sameer AS Mapari et al.,). For one or more of the following causes to the food:

- To replace colour in the food, that is unknown process,
- To enhance color of the food before present,
- To color otherwise achromic food, and
- To supplement food with nutrients (Chaitanya Lakshmi G et al.,2014)

In the last few years, Developing new colourant for the food industry is difficult because colorants must be compatible with a food's flavour, safety, and nutritional value, while also having a minimal impact on the product's price.Plants, insects, mineral ores, and microbial sources are the primary sources of natural colourant. Microbial colorants are preferred because of their ease of scalability and potential lower production costs. Microbial fermentation for natural pigment production has several advantages, including lower production costs, higher yields, easier extraction, lower-cost raw materials, no seasonal variations, and strain improvement techniques to increase natural pigment production(Tanuka 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 the well exploited for their secondary metabolites production and their application as a natural food colorants (Marlia Singgih and Elin Julianti et al., 2015).

2. FOOD COLOURANTS

The US FDA states that" Food additive subject to FDA Premarket approval is any material logically anticipates to become a 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). Food items high in nutrients, flavor, aroma and texture cannot be eaten unless the color is added and make it appear pleasantly. Customers loved around the world by colourful food products and food dishes . Food additives are categorized as

- antimicrobial agents,
- antioxidants,
- artificial colors,
- artificial flavors and
- flavor enhancers,

- chelating agents
- thickening
- stabilizing agents (R. M. Pandey and S. K. Upadhyay et al,. 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 (Ivan Luzardo-Ocampo et al,. 2021).

2.1.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, China discovered the oldest written record of the use of natural dyes. Dyeing was known in the Indian subcontinent even during the Indus Valley period (2500 BC) and was substantiated 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. Chemical red fabric experiments discovered in King Tutankhamen's grave in Egypt showed alizarin, a pigment obtained from madder, was present. The cochineal dye was used by Central and North America's Aztec and Mayan cultures. 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 (Aberoumandet al., 2011). The study of colour was intensified in the late 19th century, according to Aberoumand15 and Mortensen2, 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 (H. Rymbai et al., 2011).

2.2. CLASSIFICATION OF FOOD COLOURANTS

Food colorants categories :

1. Synthetic
2. Nature-identical
3. Inorganic
4. Natural colorants.

2.2.1. 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. The artificial food colorants include Ponceau 4R, Carmoisine, Erythrosine, Tartrazine, and sunset Yellow FCF.The artificial food colorants had a negative impact on people's health (Prithviraj Sadar et al., 2017).Chemicals used to make synthetic food colorants come from coal tar derivatives, and the majority of them have an azo group. These hues can be separated into authorized and non-permitted categories.Numerous studies have shown that synthetic food colorants are a major cause of food intoxication and can cause serious health issues like low haemoglobin levels, allergic reactions, mutations, cancers, irritability, restlessness, and sleep disturbances. They can also have negative effects on the liver, kidney, 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 (P. G. T. Dilrukshi et al., 2019). Due to their consistency, stability, and affordability, synthetic dyes are utilized extensively for colouring purposes (Hanan Mohamed Fathy Abd El-Wahab et al., 2012).

2.2.2. 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. (Kakali Roy et al., 2004).Numerous colours, such as carotenoids, flavins, monascins, chlorophyll, quinines, prodigioson, and violacin, are produced by microorganisms.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 neutraceuticals (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 et al., 2006). Superiority of natural colourant:

a) Greater variety of colors

b) High mass-to-coloration ratio

c) Improved stability

d) Little difference from batch to batch

e) Pure and chemically outlined

f) Reasonably affordable (A. P. Damant et al., 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 (Delgado-Vargas et al., 2000).

2.2.3. SOURCES OF BIOCOLOURANTS /BIOLOGICAL PIGMENTS

Natural bio colorants can be sourced from plants, animals, and microbes. 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 (Pritam 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 (Dinesh Kumar Sain et al., 2019). 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 (Riikka Räisänen et al 2020).

2.3. 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. (Shrikant Baslingappa Swami et al., 2020)

CHLOROPHYLLS: A porphin ring, a symmetrical cyclic tetrapyrrole, with a phytol attachment and a centred magnesium ion, is the building block of chlorophyll.. 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.(Gregory T. Sigurdson et al., 2017)

CAROTENOIDS: With hues ranging from yellow to orange to red, carotenoids are a significant class of lipid-soluble pigments that are found in a broad variety of organisms, including higher plants, bacteria, fungus, yeast, birds, and insects. Acyclic, monocyclic, or dicyclic carotenoids include lycopene, beta-carotene, and lutein, respectively (Panagiota 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 light, heat, oxygen, and pH conditions, which restricts their use in various food products (Araceli Castañeda-Ovando et.,2003).

BETALAINS: Betalains are water soluble and responsible for the colors of plants of order Caryophyllales

and are commonly found in red beet and cactus pear. The red and yellow pigment ammonium derivatives of betalamic acid are known as betalains and are among the numerous N-heterocyclic chemicals. They consist of two main groups: yellow-orange betaxanthins, which are the condensation products of betalamic acid with amines, and red-violet betacyanins, which are the condensation products of betalamic acid and cyclo-Dopa (Gregory T. Sigurdson et al., 2017)

2.3.1. 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. Natural colors used in the food industry are mostly plant extracts with several disadvantages such as light, heat or negative pH instability, low water solubility and year-round non-availability. 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).

2.4. 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. Several researchers have noted that there are other significant categories of animal pigments, such as melanins, pterins, porphyrins, psittacofulvins, and flavonoids, also demonstrate antioxidant activity in living systems. Because many pigment-based colour ornaments in animals have an immunomodulatory effect and they may be able to accurately reflect in the health status (Kevin J.McGraw et al., 2005).

Melanins: Melanins are the most abundant biological pigments in nature.Because melanization is the primary physiological response of animals, the presence of melanins in both the integument and internal structures is critical for the development of life on Earth.Melanin synthesis (i.e., melanogenesis) in melanocytes should be considered an oxidative process because it consists primarily of the oxidation of orthodiphenols to orthoquinones to achieve polymerization of the subunits that form the large pigment molecules.Some of the genes that control melanogenesis are also involved in cellular antioxidant responses, but a link between melanins and these responses would be mediated by pleiotropic effects rather than by melanins' direct antioxidant activity (Ismael Galván et al., 2015).

Pterin: Pterin pigments are UV-fluorescent nitrogen-rich compounds that animals synthesise from basic purine (e.g. guanine) precursors (John E. Steffen et al., 2006).Pterin pigments are a class of nitrogenous, heterocyclic chemicals that are catabolized by-products of purines, the class of essential nucleotides that make up DNA and RNA and include adenine and guanine. They are red, orange, and yellow pigments that many insects, fish, amphibians, and reptiles use in their sexual colour displays, including 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 (Kevin J.McGraw et al., 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. Porphyrins are macrocyclic tetrapyrrole derivatives found in abundance in nature. They are frequently complexed with a metal ion located in the centre of the ring system and can be modified by a variety of substituents, including additional rings, or by ring opening, resulting in a wide range of functions (Mariam 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 (Alexei Solovchenko et al., 2019).

Psittacofulvins: Ornithologists have known for more than a century that parrots use an 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 (Juan F. Masello ey al.,2008).

3. MICROBES AS SOURCES OF BIOCOLOURANTS

Microorganisms plays a 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 pigments, amino acids, vitamins, organic acids, and enzymes. Microorganisms are also responsible for the formation of some food products through the process of fermentation *(Kamla Malik et al., 2012)*. As a result of their advantages over plants in terms of availability, stability, cost effectiveness, labour, yield, and ease of downstream processing, microorganisms are recognised as a promising source for the synthesis of bio-pigments. Microorganisms have been used to manufacture a variety of bio pigments, including carotenoids, melanins, flavins, quinines, monascins, and violancein. Solid state and submerged fermentation on natural raw materials or industrial organic waste can be used to cultivate microorganisms. Numerous microbial pigments include anti-cancer, antioxidant, anti-inflammatory, and anti-microbial properties in addition to serving as colouring ingredients in the food processing and cosmetics industries *(Hardeep S. Tuli et al., 2014).*

Microbial pigments are attracting great demand to develop food grade, textile grade, and drug grade natural pigments. The reasons for high demand for microbial pigments are their promising unlimited resources, high production of required quantity of pigments,, easy cultivation and can be harvested throughout the year, adaptability to various environments, optimization, genetic engineering, no side effects, eco-friendly, biodegradable, and indispensable applications in multidisciplinary aspects such as ecological, evolutionary, biomedical, agriculture, and industrial studies *(Ramesh Chatragadda et al., 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 (Abhishek 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 colourant 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 *(Maria Elisa Paillìè et al 2020)*.

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 *antioxidant, anticancer, anti-proliferative, immunosuppressive, diabetes mellitus therapy*, 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 (*Abhishek Kumaret 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 et al., 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:

1) Being able to utilize a variety of C and N sources;

2) Must be capable of withstanding changes in pH, temperature, mineral concentration, and growth circumstances;

3) suitable colour yield;

4) Must be non-toxic and non-pathogenic;

5) Should be quickly divisible from the cell mass.

a) **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 then 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.

b) **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) (Manik Prabhu Narsing Rao et al 2017).*

c) **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*(Mata-Gomez et al., 2014).*

3.1. 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 soil, water, plants and animals *(Tanuka Sen1 et al 2019)*. Recent study findings show the enormous potential of microbial pigments in the food industry and the chance of finding novel strains from different unexplored sources such as agro-industrial waste, marine fungi and filamentous fungi etc. Not only do microbial pigments add color to food, they also have popular *antioxidant, antimicrobial, anticancer, immunoregulation, anti-inflammatory, antiproliferative* and *immunosuppressive* characteristics, etc *(Tanuka Sen1 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 *(Yanis Caro et al., 2016).*

3.1.1. SIGNIFICANCE OF MICROBIAL PIGMENTS AS NATURAL COLORANTS

Microorganisms are the most prevalent species in existence and determine on this planet's lives and death. Microorganisms are connected to all the ingredients we consume and are responsible for the creation of certain food products through the fermentation method and can also be used as a food source in the form of single cell proteins and food supplements in the form of pigments, amino acids, vitamins, organic acids and enzymes (Rajendra Singh et al 2017). The microbial pigments are thus excellent alternative. It is known that microorganisms generate a variety of pigments; therefore they are a promising source of food colorants. Carotenoids, flavonoids, tetrapyrroles and some xanthophylls astaxanthin are some of the most important natural pigments. Beta-carotene, which is derived from some microalgae and cyanobacteria, is the most common pigment used in sectors. Astaxanthin from Phaffia rohodozoa and Haematococcus Pluviais is a commercially valuable red pigment used in the feed, pharmaceutical and aquaculture sectors (Vikas bhat et al., 2013). Microorganisms which have the capacity to produce pigments in high yields include species of *Monascus,* *Paecilomyces, Serratia, Cordyceps, Streptomyces* and yellow-red and blue compounds produced by *Penicillium herquei, Penicillium atrovenetum*, *Rhodotorula, Sarcina, Cryptococcus, Monascus purpureus, Phaffia rhodozyma, Bacillus* sp., *Achromobacter* sp., and *Yarrowia* sp., also produce a large number of pigments (Flombaum *et al*., 2013).

As a result, the food industry has become more and more interested in using microbial technology to create colors for food use. It can also assist overcome the increasing public concern about the adverse effects of adding synthetic colors to food products on health. In addition, natural colorants will not only be useful to human health, but will also be an advantage in preserving biodiversity as damaging chemicals produced into the atmosphere could be stopped while creating synthetic colorants. These natural dyes are used in child foods, cereals for breakfast, sauces, pastas, processed cheese, fruit beverages, milk products enriched with vitamins, and some energy drinks. Natural colors can thus serve the dual need for visually attractive colors and probiotic health advantages in food products in addition to being eco-friendly (Rymbai et al., 2011).

4. 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 proteins, amino acids, nucleic acids, laccaic acid, chitosan, amino alcohols, etc. were made to improve the quality (water solubility) of Monascus colorants *(Francielo Vendruscolo et al 2015)*.

Rhodotorula: Rhodotorula species are strictly aerobic yeasts with unusual metabolic properties such as the ability to produce glycogen during the exponential growth phase and large amounts of lipids and carotenoid pigments during the stationary growth phase.Rhodotorula species are found in both the natural environment and in mammals. Rhodotorula can be found in the air, soil, and the phyllosphere (leaf surfaces), as well as milk and cheese products *(Ayerim Herna´ndez-Almanza et al., 2013)*.

Phaffia rhodozyma: Rhodotorula is a common yeast found in the environment, including air, soil, lakes, ocean water, milk, and fruit juice. Rhodotorula species, which are classified as Basidiomycota, colonise plants, humans, and other mammals. Rhodotorula produces pink to red colonies and unicellular blastoconidia devoid of pseudohyphae and hyphae *(Fernanda Wirth and Luciano Z. Goldani et al., 2012).*

Bacillus subtilis: On sporulation, B. subtilis produces pigment. Spizizen's salt, glucose, 27.7, L-tryptophan, 0.25, L-tyrosine, 0.25, L-histidine, 0.055, and MnS04, 0.67 mM made up the liquid medium for the best pigment synthesis. Before being inoculated with microorganisms, the salt, amino acid, and glucose were autoclaved individually.

Aspergillus oryzae: An orange-red pigment from the anthraquinone family is produced by A. oryzae var. effuses(*V K Joshi et al., 2003).*By genetically modifying its non-ribosomal peptide synthetase system to overexpress the indigoidine synthetase gene, Aspergillus oryzae was used as a platform cell factory to produce the blue pigment indigoidine *(Sarocha Panchanawaporn, Chanikul Chutrakul et al., 2022).*

4.1. 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.

4.2. TYPES OF FOOD GRADE PIGMENTS PRODUCED BY MICROORGANISMS

Last few years, microorganisms have been used to produce uncountable molecules that are as differ as antibiotics, anticancer, antioxidants, enzymes, vitamins, textured agents, etc. Additionally, businesses are becoming more and more interested in producing various pigments. In nature, microorganisms (Fungi, yeasts, and bacteria) that are high in color and pigment are frequent. Microorganisms generate variety of pigments, which as a food grade pigment is of particular importance like carotenoids, melanins, flavins, quinones, prodigiosins, in particular monascins, violacein and indigo.

5. CLASSIFICATION OF MICROBIAL PIGMENTS

Pigments produced by bacteria are prevalent referred to as bio pigments as similar 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:

5.1.1 Carotenoids

Heinrich Wilhelm Ferdinand Wackenroder was the first to discover carotenoids. There are a variety of microorganisms that produce carotenoids, including *Flavobacterium multivorum, Rhodobacter sphaeroides, Rhodotorula mucilaginosa, Sphingomonas sp., Dunaliella sp., Blakeslea trispora, Phycomyces blakesleeanus, Mucor circinelloides, Fusarium sporotrichioide.* Microorganisms that produce carotenoids have been isolated from soil, cave, marine, and slattern crystallizer pond environments *(Manik Prabhu Narsing Rao1 et al., 2017).*

5.1.2 Riboflavin

Numerous microorganisms create the yellow pigment known as water-soluble vitamin B2. It is utilized in sauces, fruit drinks, morning cereals, infant foods, diary products, and energy beverages.Through commercially viable biotechnological processes, the traditional chemical manufacture of riboflavin is currently being replaced by ascomycetes like Ashbya gossypii, filamentous fungus like Candida famata, and bacteria like *Bacillus subtili*s *(Liudmila A. Averianova et al., 2020).*

5.1.3 Beta-carotene

An organic pigment with a reddish-orange tint that is mostly obtained from the beta-carotene-rich algae *Dunaliella salina*. A suitable coloring agent, production of β- through fermentation of Blakeslea trispora results in a pigment equivalent to pigments produced through a chemical procedure. Its color ranges from red to yellow and it is utilized in a wide range of food products *(Chidambaram Kulandaisamy Venie et al., 2020).*

5.1.4 Canthaxanthin

A fat soluble carotenoid with an orange to deep pink color, canthaxanthin is a powerful antioxidant. It is a trans-carotenoid pigment that was isolated from Bradyrhizobium Sepp and is used as a food colorant in a variety of meals, including salmon and poultry feed *(Kushwaha Kirti et al 2014).*

5.1.5 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).*

5.1.6 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-pyrrylmethene. 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 as immunosuppressive agents *(Chidambaram Kulandaisamy Veni et al 2009).*

5.1.7 . Phycocyanin

Cyanobacteria that possess chlorophyll A create the blue pigment known as phytocyanin. 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 *(Niels T. Eriksen et al., 2008).*

5.1.8 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 *(HyunA Park et al., 2021).*

5.1.9 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.

5.1.10 Lycopene

A bright red carotenoid pigment that is extensively prevalent and eaten in tomatoes. It has the ability to lessen chronic diseases including certain malignancies and coronary heart disease and has been isolated from microorganisms like Fusarium, Sporotrichioides, and Blakeslea trispora. In nations like the USA, Australia, and New Zealand, it is used to colour meat (*Tanuka Sen et al 2019)*

6. LARGE SCALE INDUSTRIAL PRODUCTION OF FOOD GRADE PIGMENTS

In particular, the bacterial pigments should be concerned with finding an economic and suitable growth medium to low costs and improve their applicability for industrial manufacturing. Compared to other chemical processes, fermentation is intrinsically speedier and more effective, making it more advantageous for industrial manufacturing. Microorganisms can easily be genetically modified because of how easily they can modify their relatively large strands of genes. Therefore, compared to chemical scaling methods, genetic engineering can enhance the production of microbial pigments in geometric ratios. Microbes are more adaptable and productive than larger living forms in the industrial production of natural pigments and colorants. The fermentation process has been improved by genetic engineering, and further study of non-toxic microbial pigment could lead to quantum leaps in microbial pigment economies. Any microbial pigment produced bio technologically (for example, by fermentation) depends on consumer acceptance, regulatory permission, and the amount of cash required to commercialize the product. Several years ago, some questioned the value of marketing fermentation-derived food grade pigments due to the high capital expenditure requirements for fermentation equipment and the pricey and time-consuming toxicity assessments required by regulatory agencies. In the fish industry, for example, microbial colorants are already in use to improve the pink color of farmed salmon. Additionally, it is anticipated that some natural food colorings may be employed commercially as antioxidants. Monascus pigments, astaxanthin from Xanthophyllomyces dendrorhous, Arpink Red from *Penicillium oxalicum, Ashbyagossypii riboflavin, and Blakeslea trispora trispora carotene* are among the pigments of fermented food grade that are currently available on the market and have been deemed safe and approved by the FDA. The successful promotion of microbial pigments as a food colour and a dietary supplement illustrates the existence and significance of niche markets with active consumer participation *(Babita Rana et al 2021)*.

7. NOVEL PRACTICES OF MICROBIAL PIGMENT PRODUCTION

A bacterial strain that produces pigment was modified to overproduce pigment, altering its colour and structure. Streptomyces coelicolor, a bacteria that produces the blue pigment actinorhodin, has been genetically altered to produce the bright yellow polyketide kalafungin, which is used to make pigments similar to anthraquinones. The biosynthetic pathway from known pigment producers has been used to develop the cell factories to efficiently produce pigments.Red (lycopene), orange (-carotene), and purple (violacein) pigment producing bacteria that make a significant contribution to pigment production are the targets for pigment production through genetically modified *(Chidambaram Kulandaisamy Venil et al., 2020)*.

Because the pigments produced by wild type strains are frequently too few in number and require longer fermentation times, the process is often unprofitable and strain development is crucial. Common mutagens like 1-methyl-3-nitro-1-nitrosoguanidine (NTG), ethyl methyl sulfonate (EMS), and ultraviolet (UV) can improve strains and increase pigment production by a factor of several hundred *(Tanuka Sen et al., 2019)*.

8. 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. Requires 25-28 C for pigment manufacturing, while *Pseudomonas* sp. needs 35-36 C for development and manufacturing of pigments (*V K 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 neutrophilial growth. II) At a pH lower than 5.5, acidophiles thrive. III) At a pH greater than 8.5, alkaliphiles thrive *(Siddig Hussein Hamad et al., 2012)*.

3. Carbon source

The type of carbon source, such as glucose, fructose, maltose, lactose, galactose, etc., has an impact on the mycelial growth of microorganisms that produce pigment. For growth and the production of pigments, glucose and its oligosaccharides are preferable carbon sources *(V K 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. The cultivation of microbial biomass takes place on the surface of a solid substrate during solid substrate fermentation (SSF) *(Hardeep S. Tuli et al., 2014)*.

5. Minerals

In the creation of pigments, minerals are crucial. In a liquid medium, Zn (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*(V K 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 *(Neil H. Mermelstein et al., 2009).*

8. Aeration rate

The bed of rice is continuously aerated by sparging with humidified air (95–97% relative humidity) when the Monascus pigments are made from the solid state fermentation. Due to water loss from the bed, forced aeration rates greater than 0.5 L min-1 decrease the production of pigments and biomass. The highest pigment concentrations can be attained at forced aeration rates of 0.05 to 0.2 L min-1 *(Abhishek Kumar et al., 2015).*

9. APPLICATIONS OF MICROBIAL PIGMENTS

Microbial pigments have tremendous applications in food, textile, pharmaceuticals, cosmetics, Nutraceuticles 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, actinomycetes and fungus *Monascus* sp demonstrate antimicrobial activity (Mekhael and Yousif, 2009) and have a powerful capacity to create antitumor drugs (Perez-Tomas et al., 2003). Table 1 describes about the different microbial pigments structures and its applications.

9.1 APPLICATIONS IN FOOD INDUSTRY

Some fermentation-derived colours, including beta carotene from the European Blakeslea trispora fungus or Asian Monascus pigments, are now utilised in the food industry. Antibiotics and antioxidants provide additional nutritional and therapeutic benefits, and they give different hues a healthy appearance *(Venil and Lakshmanaperumalsamy et al., 2009).* For instance, the organoleptic features of food products are improved by *Monascus* red pigments, usually manufactured as MFR (Monascus Fermented Rice). These pigments contain monocolin, reducing LDL cholesterol and increasing HDL cholesterol. There are microbial pigments that have potential for the future and are being researched.

9.1.1. β -Carotene production

Pro-vitamin of yellowish carotenoid pigment is called β-Carotene A). It activity as antioxidant and has potential positive effects product certain diseases. Following microbes are mainly using for β –Carotene production:

1. Dunaliella salina

A microalgae that produces beta-carotene called *Dunaliella Salina* is renowned for its exceptional capacity to store essential beta-carotene.It is examined what influences the culture system, culture conditions, and downstream processes that result in the production of beta-carotene from *Dunaliella salina*. Studies demonstrate that using an airborne photo-bioreactor culture system enhances the production mechanism *(SaraPourkarimi et al., 2020).*

2. ***Blakeslea trispora***:

Some of the strains of this mould produce excessive quantities 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 *(Eugenia Papadaki et al .,2021).*

3. ***Mucorcircinelloides***:

A zygomycete fungus that accumulates beta-carotene is called Mucor circinelloides, and it serves as one of the model organisms used to research the synthesis of carotenes in fungi. In order to create stable canthaxanthin-producing strains, the -carotene ketolase gene (crtW) of the marine bacterium Paracoccus sp. N81106 was fused with fungal promoter and terminator areas and incorporated into the M. circinelloides genome (Tamás Papp et al 2012).

4. ***Phycomyces blakesleeanus:***

The manufacture of numerous chemicals, including -carotene, uses Phycomyces primarily. When grown on robust substrates or in liquid media, they exhibit increased carotenogenic capacity.

9.1.2 Arpink Red^{tm} Production

The species Penicillium oxalicum is the source of the red pigment that it has drawn from the soil. It contains chromophores of the anthraquinone class. The red pigment Arpink Red was suggested to be used in various food products by the Codex Alimentarius Commission.

9.1.3 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).

9.1.4 *Monascus* pigments production

It belongs to the Ascomycetes group and especially to the Monascaceae family. You can divide the *Monascus* genus into four species: *M. pilosus, M. purpureus, M. Ruber, M. Froridanus*, the bulk of which are separated strains from traditional oriental food. The popular names of this fungal product are Red Yeast Rice (RYR), red rice, angkak, red leaven, benikoji (Japanese), hung-chu, hongqu, zhitai (Chinese), red mold (USA) and MFR (Monascus fermented rice). Many industrial pigments are produced and these pigments are primarily of three kinds, i.e. red dyes, orange dyes and yellowish dyes (Mapari, et al., 2005). The red mould, Monascus purpureus, which is historically used to prepare red rice, is a promising source of pigment. The ascospores of M. purpureus, which have a diameter of 5 microns or are moderately ovoid (65 microns) and seem spherical, can be used to distinguish the two species easily. Monascus fungus, the organisms that make angkak, may convert starchy substrates into a variety of metabolites, including alcohols, antibiotics, antihypertensives, enzymes, fatty acids, flavourings that cause flocculation, ketones, organic acids, colours, and vitamins. As a result, the use of Monascus pigment as a colouring factor in food gave the products the added benefit of a distinctive flavour *(Clement Agboyibor et al., 2018).*

9.1.5 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 & Zhen Liu 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. *Streptomyces* strain lycopene (AQBWWS1) of the white series was isolated from the marine sponge *Callyspongia diffusa (Selvakumar Dharmaraj et al., 2009).*

9.2. APPLICATIONS IN PHARMACEUTICAL INDUSTRY

The pharmaceutical sector incorporates several microbial pigments into their goods. Many of the pigmented secondary metabolites of the bacterium have significant pharmacological and therapeutic potential. It shows that many different eubacterial species, including *Vibrio psychroerythrus, S. marcescens, Pseudomonas magnesiorubra,* 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.

9.2.1. 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 symmetrical tetraterpene made up of 8 isoprene units. Phycomyces and Blakeslea fungi have the potential to produce lycopene *(Gozde Konuray et al., 2018)*.

9.2.2. Anticancer properties

One of the deadliest illnesses a person can have is cancer. Numerous microbial pigments have anticancer properties *(Manik Prabhu Narsing Rao et al., 2017)*

S.NO	Microbial Pigments	Type of cancer

1	prodigiosin from *Pseudoalteromonas* sp	cytotoxicity against U937 leukemia cells
2	Melanin from *Streptomyces glaucescens* NEAE-H	skin cancer cell line
3	anthraquinone from *Alternaria* sp. ZJ9-6B	human breast cancer cell lines
4	*Monascus*, such as monascin	mouse skin carcinogenesis
5	ankaflavin	human cancer cell lines
6	monaphilone A and monaphilone B	human laryngeal carcinoma cell lines

9.2.3.Antifungal properties

According to several studies, prodiginine pigments (prodigiosin and cycloprodigiosin) extracted from the Indonesian marine bacterium P. rubra sp. have antagonistic activity toward Candida albicans sp., and violacein from Chromobacterium sp. also has antifungal properties. Other fungi that were inhibited by violacein included Aspergillus flavus sp., Rhizoctonia solani sp., Fusarium oxysporum sp., Penicillium expansum sp., and Fusarium oxysporum sp. Additionally, studies have revealed that violacein, which is derived from a pure Chromobacterium sp., exhibits antifungal activity comparable to that of bavistin and amphotericin B, highlighting the potential of marine-derived bpBPs as effective antifungal agents over existing synthetic antifungal compounds *(Ali Nawaz et al.,2020).*

9.2.4.Anti bacterial properties

Due to their potential for applications, bacteria-produced pigments, which are used traditionally in eastern countries, have been the focus of intense research in recent decades.As a result, compared to other sources, the number of compounds isolated from bacteria is growing faster. Anthocyanins are involved in a variety of biological processes that have a positive impact on health and reduce the risk of cancer, inflammation, and immune response.The Gram-negative bacteria Chromobacterium violaceum, Janthinobacterium lividum, Pseudoalteromonas luteoviolacea, Ps. sp. 520P1 and Ps. sp. 710P1 are among those that produce violacein *(Chidambaram Kulandaisamy Venil et al., 2013).*

9.2.5.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 *(Muhammad Numan et al., 2018)*.

9.2.6 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.

9.2.7.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 lumphoma linked to AIDS *(Chatragadda Ramesh et al., 2019).*

9.3. 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. Due to their simple and inexpensive synthesis, stability in light and temperature, and advanced colours that cover the entire colour spectrum, these synthetic dyes are used in industries*(Kanchan Heer et al., 2017).*

10. CONCLUSION

The most adaptable biotechnological tools are microorganisms, which can produce a wide range of molecules, including enzymes, antibiotics, organic acids, and pigments. Recent research has indicated that microorganisms hold promise as a source for natural colours. According to numerous latest 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 colourants 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 authorised 43 colourants 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 colouring, so natural food colouring 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.

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Conflict of Interest

None

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Captions for Tables

1. Table 1: Structure of various microbial pigments and its applications

Captions for Flow Chart

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

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

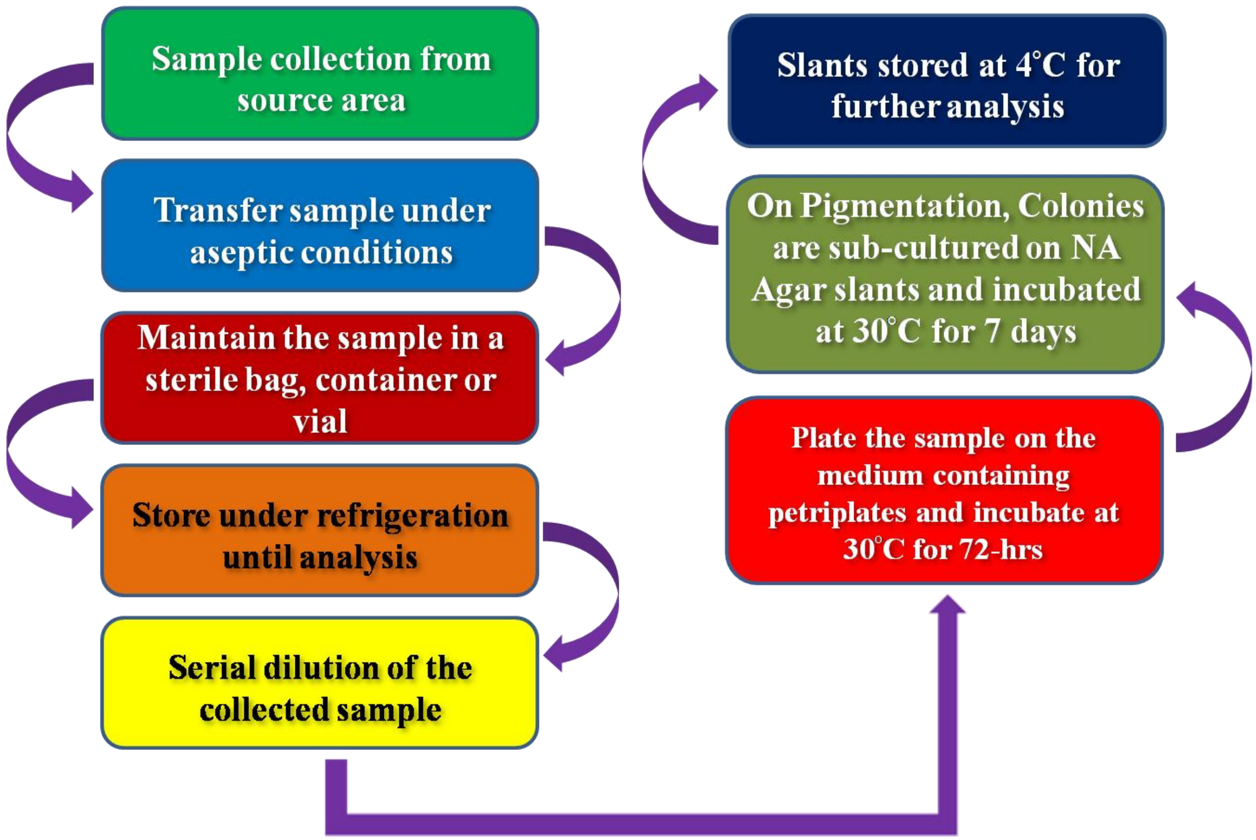
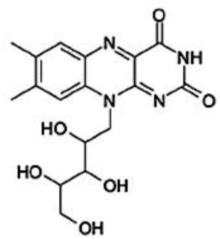
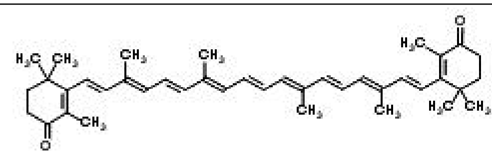
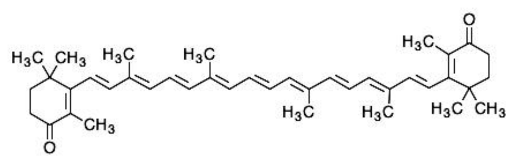
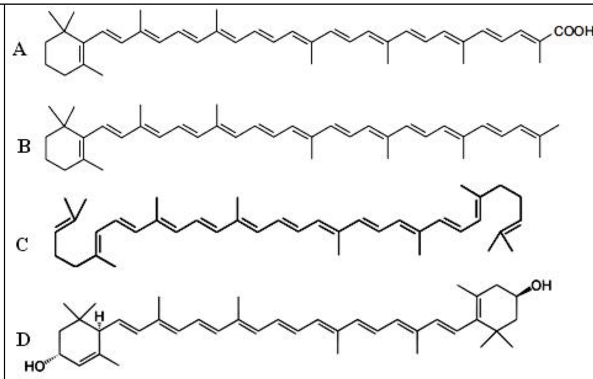
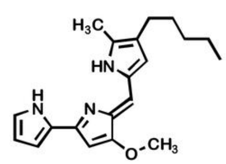
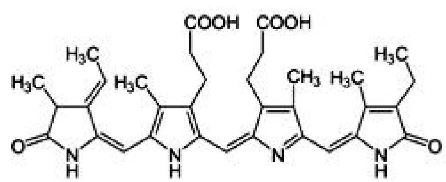
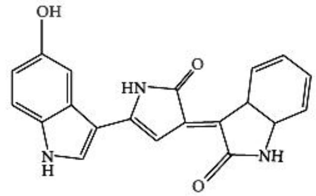
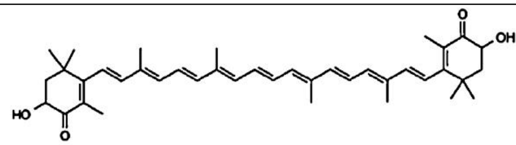
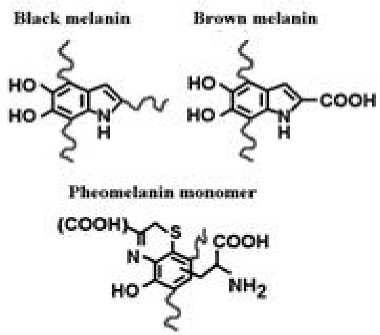
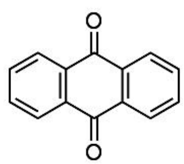
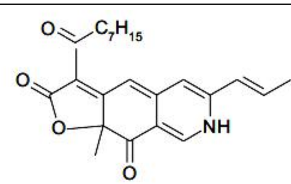
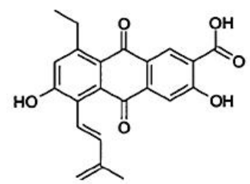


Table 1: Structure of various microbial pigments and its applications

Si No.	Microbial pigments used in food industry	Structure	Applications
1	Riboflavin		Riboflavin is added to baby foods, breakfast cereals, pastas and vitamin-enriched meal replacement products. It is difficult to incorporate riboflavin into liquid products because it has poor solubility in water, hence the requirement for riboflavin-5'-phosphate, a more soluble form of riboflavin. Riboflavin is also one of the food coloring agents used recently and has vast pharmaceutical usage
2	β-Carotene		Used to treat various disorders such as erythropoietic protoporphyria, reduces the risk of breast cancer
3	Canthaxanthin		Colorant in food, beverage and pharmaceutical preparations

4	Carotenoids a. Torularhodin b. Torulene c. Lycopene d. Lutein		1) Torularhodin (acidic) and Torulene (hydrocarbon) shows a considerable antioxidant activity that helps in the stabilization of membranes under stress conditions. These carotenoids are beneficial because they are precursors of vitamin A and hormones and they have anti-aging and antioxidant capacity. They may also prevent certain types of cancer, and enhance the immune system. 2) Lycopene is emerging as a valued antioxidant, with many new applications as a nutritional supplement and an active ingredient in cosmetic products. 3) Lutein is an antioxidant that has gathered increasing attention due to its potential role in preventing or ameliorating age-related macular degeneration.
5	Prodigiosin		Anticancer, immunosuppressant, antifungal, algicidal; dyeing (textile, candles, paper, ink)
6	Phycocyanin		Phycocyanin is one of the major pigment constituents of Spirulina, used as a dietary supplement; possess antioxidant properties and anti-inflammatory activities.

7	Violacein		Pharmaceutical (antioxidant, immunomodulatory, antitumoral, antiparasitic activities); dyeing (textiles), cosmetics (lotion)
8	Astaxanthin		Used as feed supplement both for fish and shellfish.
9	Melanin a. Eumelanin b. Pheomelanin c. Allomelanin		It possesses antioxidant, antiphagocytic and blocks antimicrobials.
10	Anthocyanin		They are responsible for the red, purple, and blue colors of many fruits, vegetables, cereal grains, and flowers. They are not used in drugs and cosmetics. They are used in beverages, fruit fillings, snacks, dairy products and confectionery.
11	Monascorubramine		The pigment is secondary metabolite of *Monascus* fermentation and produced mainly in cell bound state. It is used in processed meats products, marine products, tomato ketchup etc.

12	Arpink red		Arpink Red with claims of anti-cancer effects of the anthraquinone derivatives and applications within the food and pharmaceutical fields.