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Food additives: production of microbial pigments and their antioxidant properties

Poonam Singh Nigam and Jasmine Sharon Luke

Synthetic food additives pose the risk of hazardous effects and toxicity to the consumers whereas the application of natural pigments as food additives is safer and in demand worldwide. Food industry worldwide is dependent on colors to make food appealing to the consumers and to add variety to the food types. Therefore, a need exists to explore the novel strains of microorganisms and suitable strategies for commercial production of microbial pigments, to meet the high demand of microbial pigments as food additives. Traditional methods of microbial isolation, development and extraction are now replaced with novel techniques and strategies through biotechnology; with the advent of fermentation technology and genetic engineering techniques. Current review describes the immense potential of microbes being used as food colorants and we also present an insight on novel strains and production methods which can be explored further for application in food industry.

Address
School of Biomedical Science, University of Ulster, Coleraine BT52 1SA, UK

Corresponding author: Nigam, Poonam Singh (p.singh@ulster.ac.uk)

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Introduction

US FDA states that "any substance that is reasonably expected to become a component of food is a food additive that is subject to premarket approval by FDA". Microorganisms and plants produce certain substances which exhibit different colors due to selective color absorption; these substances are termed as pigments. These pigments are extracted and utilized in pharmaceutical industry, textile and dyeing industry, food & dairy industry and cosmetics industry [1••,2,3••,4,5••,6••,7]. Naturally occurring pigments like isoprenoids, alkaloids and flavonoids, have been used for fragrance, flavor and color in various food types since prehistoric times [1••,8,9,10]. According to various studies conducted recently it is well evident that pigments extracted from microbes are beneficial over synthetic pigments and pigments that are extracted from plants owing to their stability, availability due to no seasonal variations, cost-effectiveness, high yield through strain improvement [5••,6••,11] and smooth downstream processing for extraction [12]. Also, there exists a recent trend of public awareness on adopting environmental friendly and human safety measures [13]. Consumers have developed aversion toward application of synthetic food colorants; therefore natural food colorants are in huge demand [14,15].

In past various research studies have been conducted to explore the existence of diverse coloring pigments in plants and microbes [4,11,16]. Microbial strains producing pigments can be isolated, extracted, characterized and purified from different environmental sources like — soil, water, plants and animals [17,18]. Recent research studies indicate the immense potential of microbial pigments in the food industry and possibility of discovering novel strains from various unexplored sources like agro industrial waste, marine fungi, and filamentous fungi etc. [19,20•,21,22].

Recent review study states that currently there are almost 2500 types of food additives being used globally and US FDA lists around 3000 ingredients in the food additive database [23]. It also states that almost 200 thousand tones of food additive are being used per year; which highlights the fact that western diet consists of approximately 75% of the processed food. Each person's average annual intake of food additives is estimated to be 3.6–4.5 kg [23].

Food items that are rich in nutrients, flavor, aroma and texture cannot be consumed unless the color exhibited makes its appearance relishing. Worldwide consumers are fond of colorful food products and food dishes [3••,24]. Microbial pigments not only add color to food, they also have populous medicinal properties like antioxidant, antimicrobial, anticancer, immunoregulation, anti-inflammatory, antiproliferative, and immunosuppressive etc. [1••,3••,25]. Most commonly used food grade pigments are β-carotene, arpink red, riboflavin, lycopene and Monascus pigments [1••,3••,12].

The production of many currently authorized natural food colorants has a number of disadvantages, including a dependence on the supply of raw materials and variations in pigment extraction. Currently, fermentative large scale production of natural food colorants is feasible in the global market [69]. Maximization of the pigment yield while minimizing the production costs has been the attention of current techniques applied to manufacture microbial pigments at large-scale. It has also been reported that process optimization techniques have deployed statistical experimental designs and response surface analysis along with limited use of artificial intelligence like genetic algorithms [52]. Potential of renewable sources like fruits, vegetables, lichens, and marine life etc. for novel commercial food colorants is questionable with respect to raw material's availability and high investments which are recurring. Commercial production of microbial pigments for application in food industry has been attained as a result of variety of techniques combined together, namely — fermentation techniques (solid or submerged state), chemical modifications, production using agro-industrial wastes, genetic modification techniques etc. [56,71••,82]. Previous research studies demonstrate that commercial production of microbial pigments remains to be in the research and development stage [3••].

Current literature review article elucidates the current scenario of microbial pigments as food colorants and underlines the importance of investigating large scale production strategies for microbial pigments, novel strains of microbes producing colored pigments and techniques aiding in high yield-extraction of colored pigments using microorganisms.

Why microbial pigments as food additive?

Consumers' ability to differentiate between the benefits of microbial pigments and hazardous effects of synthetic pigments has greatly boosted the application of microbial pigments as food additive. According to current trends consumers' tendency to interpret utilization of synthetic pigments as mere contaminants has been augmented [26]. Robust development and advances in technology and genetic engineering techniques have enabled food industry to produce microbial pigments [27–30]; this has led to increase in demand of natural food additives. In current times public's interest in synthetically extracted pigments has decreased owing to their toxicity, oncogenicity and teratogenic properties whereas microbial sources of pigments have gained consideration as safe alternatives [1••,16,23]. Precursors used in the production process of synthetic pigment have many carcinogenic hazardous effect on the workers as well as the waste produced by the production process is harmful, environment-unfriendly and non-biodegradable [3••]. Worldwide interest is in the process of development for the production of pigment from natural sources [4].

Recent review states that studies conducted to observe the effects of consumption of large amount of synthetic food additives by humans leads to gastrointestinal problems, respiratory disorders, dermatologic issues, and neurologic adverse reactions [31,32]. There exists extensive research evidence which demonstrates the association between hypersensitivity, childhood hyperactivity, physical and mental disorders with food additive intolerance [33,34]. Synthetic pigments like amaranth, erythrosine and tartrazine are potentially toxic to human lymphocytes cells *in vitro* and they have the ability to bind directly with DNA [35].

Pigments extracted from plants have drawbacks such as instability against light, heat or adverse pH, low water solubility and are often non-availability throughout the year [12,3••]. The pigment production from microorganisms is efficient and beneficial process as compared to chemical synthesis of pigments [3••,8,36]. Likewise, extraction of pigments from microbes is not dependent on weather conditions, which gives ability to get several color shades while being cultures grown on cheap substrates [1••,11] and these colors are biodegradable and environment-friendly. Microbes can be grown easily and fast in the inexpensive culture medium and are independent of weather conditions. Microbial pigments possess numerous clinical properties like antioxidant, anticancer, antiproliferative, immunosuppressive, treatment of diabetes mellitus etc. [1••,9,25]. Role of food colorant as additive in food industry is very important not only for the consumers but also for the manufacturers; colors and variety in the food leads to acceptability of the food product amongst the consumers which further leads to greater demand in market and hence earns large amount of profits [37,38].

Food colorants and global market

Food colorant industry market has been noted to grow at the rate of 10–15% annually. A research report published by Leatherhead Food International (LFI) (www.leatherheadfood.com) states that by 2015 the global market for food grade pigment is expected to rise by 10% to be $1.6 billion USD [70•]. As per the current legislation by European Union 43 colorants are approved and permitted as food additives and approximately 30 additives are approved in the United States; 6 colorants amongst these 30 additives are of microbial origin as listed in Table 1. However, in both the regions EU and USA listed color additives are pigments extracted from natural sources mainly plants, fruits, vegetables, animal and microorganisms [39]. In addition to the food colorants there are 19 approved commercial food additives extracted from microbial source and being used globally (Table 2 lists the approved food additives) (*http://www.fda.gov*).

Microbial pigments production technologies and challenges

Algae, fungi and various types of bacteria have been used as source for commercial production of microbial pigments to be used as food additives and colorants [2,8,10,15,40,55]. Extraction of colored pigments plays pivotal role in

Table 1

List of food grade colorants extracted commercially from microbes — authorized and currently available in the current global market.

Serial No.	Color/shade	Pigment E-Number*	Natural colorant (microbial source)	Chemical category
1.	Yellow	E101 (iii)	**Riboflavin** (from *Bacillus subtilis*) other sources: *Ashbya gossypii*, *Candida guilliermondii*, *Clostridium acetobutylicum* and *Debaryomyces subglbosus*	Flavin
2.	Orange-Yellow	E160a (ii)	**β-Carotene** (from *Blakeslea trispora*)	Carotenoid
3.	Orange-Yellow	E160a (iv)	**β-Carotene** (from *Dunaliella salina*) other sources: *Dunaliella bardawil*	Carotenoid
4.	Yellow to red	E160d (iii)	**Lycopene** (from *Blakeslea trispora*)	Carotenoid
5.	Yellow to red	E-161j	**Astaxanthin** (from *Haematococcus pluvialis*) other sources: *Haematococcus lacustris*, *Xanthophylomyes dendrorhous*	Carotenoid
6.	Orange, Red	E161g	**Canthaxanthin** (from *Haematococcus lacustris*) other sources: *Bradyrhizobium* sp.	Carotenoid

Adapted from Caro *et al.* [70•].

production at industrial level [70•]. Several research studies have demonstrated that an ideal microbe producing pigment is the one which has the ability to utilize wide range of carbon and nitrogen sources and is tolerant to temperature pH and minerals and it must give sufficient color yield [1••,3••]. These kinds of pigmented microbes can be isolated from different sources in the environment which are then cultured and purified [6••]. Since synthetic growth media are expensive to culture these microbes, a recent review states that the use of agro-industrial residues [19] would be a profitable means of reducing the cost. Currently advance techniques adopted for color extraction are (but not limited to) high hydrostatic pressure (HHP) and pulse electric field (PEF), sonication assisted extraction, gamma irradiation enzymatic extraction and membrane technology [5••]. It has been observed that in comparison to the scaling up methods of chemists, microbial pigment production can be increased through the technique of genetic engineering [41–43]. It has been noted that major factors affecting microbial pigment production are temperature, pH, carbon source, type of fermentation, minerals, nitrogen source, moisture content and aeration rate [3••,11]. The microorganism used

Table 2

Food additives derived from microorganisms listed in 21 CFR 172 and 173 of US-FDA.

Serial No.	Regulation in 21 CFR	Ingredient
1.	§172.155	**Natamycin** derived from *Streptomyces natalensis* and *Streptomyces chattanoogensis*
2.	§172.325	**Bakers yeast protein** derived from *Saccharomyces cerevisiae*
3.	§172.590	**Yeast-malt sprout extract**, derived from *Saccharomyces cerevisiae*, *Saccharomyces fragilis*, *Candida utilis*
4.	§172.620	**Carrageenan**, a hydrocolloid extracted from the following members of the families *Gigartinaceae* and *Soliericeae* of the class *Rodophyceae* (red seaweed): *Chondrus crispus*, *Chondrus ocellatus*, *Eucheuma cottonii*, *Eucheuma spinosum*, *Gigartina acicularis*, *Gigartina pistillata*, *Gigartina radula*, *Gigartina stellata*
5.	§172.655	**Furcelleran,** the refined hydrocolloid extracted from *Furcellaria fastigiata* of the class *Rodophyceae* (red seaweed)
6.	§172.695	**Xanthan Gum** derived from *Xanthomonas campestris*
7.	§172.725	**Gibberellic acid** derived by fermentation from *Fusarium moniliforme*
8.	§172.896	**Dried yeasts**, *Saccharomyces cerevisiae*, *Saccharomyces fragilis*, and dried torula yeast, *Candida utilis*
9.	§172.898	**Bakers yeast glycan** from *Saccharomyces cerevisiae*
10.	§173.110	**Amyloglucosidase** derived from *Rhizopus niveus* for use in degrading gelatinized starch into constituent sugars
11.	§173.120	**Carbohydrase** and cellulase derived from *Aspergillus niger* for use in clam and shrimp processing
12.	§173.130	**Carbohydrase** derived from *Rhizopus oryzae* for use in the production of dextrose from starch
13.	§173.135	**Catalase** derived from *Micrococcus lysodeikticus* for use in the manufacture of cheese
14.	§173.140	**Esterase-lipase** derived from *Mucor miehei* var. Cooney *et* Emerson as a flavor enhancer in cheeses, fats and oils, and milk products
15.	§173.145	***Alpha*-galactosidase** derived from *Morteirella vinaceae* var. *raffinoseutilizer* for use in the production of sucrose from sugar beets
16.	§173.150	**Milk-clotting enzymes**, microbial for use in the production of cheese (Milk-clotting enzymes are derived from *Endothia parasitica*, *Bacillus cereus*, *Mucor pusillus Lindt* and *Mucor miehei* and *Aspergillus oryzae* modified to contain the gene for aspartic proteinase from *Rhizomucor miehei* var *Cooney et Emerson*
17.	§173.160	***Candida guilliermondii*** as the organism for fermentation production of citric acid
18.	§173.165	***Candida lipolytica*** for fermentation production of citric acid.
19.	§173.280	A solvent extraction process for recovery of citric acid from ***Aspergillus niger*** **fermentation liquor**.

Adapted from: http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/MicroorganismsMicrobialDerivedIngredients/default.htm.

must be non-pathogenic and non-toxic in nature, ability to utilize wide range of carbon and nitrogen sources, producing plausible color effect; at the same time show the tolerance with salt concentration, temperature, and pH. The overall process should be simplified to include fewer extraction steps [10].

- Strain development
  Strain development is required to transform the isolated strains that produce pigments in short fermentation time [6••,44]. Research studies demonstrating the application of mutagens like ultraviolet (UV), ethyl methane sulfonate (EMS) and 1-methyl-3-nitro-1-nitrosoguanidine (NTG) enables several fold enhancement of pigments [45,46]. Microwave has also been used to cause mutation in *Serratia marcescens* jxl-1, which enabled high product quality and high yield of the prodigiosin pigment [71••].
- Microbial pigments extracted from agro-industrial waste
  Few research studies have demonstrated the potential of agro-industrial waste from different industries to be used as substrate for large scale production of microbial pigments as described below:
  - Cereal industry — Corn steep liquor acquired from the wet-milling industry has been utilized as a major nitrogen source, however it also contains amino acids, ash and vitamins [72]. This agro waste favored the production of red pigments by *Monascus ruber*. It has also been reported to possess potential for production of fungal pigment from *Penicillium resticulosum* [73]. *Rhodotorula glutinis* uses hydrolyzed waste flour of mung bean as a substrate for carotenoid production [74].
  - Dairy industry — Whey is a prominent waste by-product from the dairy industry; it has a high content of lactose and proteins. Microbes utilizing lactose can be easily cultivated on whey. Various strains of filamentous fungi can utilize whey and soya protein as substrate to produce yellow pigments. *Rhodotorula rubra* MTCC 1446 has been successfully used under submerged fermentation conditions using whey and coconut water to achieve enhanced levels of yellowish-pink pigment [75,76••].
  - Fruit and vegetable industry — A research study has been conducted to assess the feasibility of substrate composed of jackfruit seeds for the production of red pigment by *Monascus purpureus* [77] in solid state fermentation (SSF). Fermented red rice — 'Angkak' has been produced in high yields using durian seeds [78] as substrate by *Monascus* sp. Kinnow peel has shown immense potential for the production of pigments using *M. purpureus* MTCC 369 [79]. Grape waste, apple pomace and tomato waste have also been investigated for optimized processes and designs in order to achieve higher yield of microbial pigments [80,81].
- Fermentation
  Commercial production of microbial pigment is quick and productive through fermentation technology. Also, genetic engineering technology when combined with fermentation results in achieving high yields of pigments [47]. Different types of fermentation techniques are being used depending upon the strain employed and the type of pigment being extracted. However, research studies suggest that it is very crucial to optimize the fermentation processes to get maximum yield; optimization depends on several factors, for example medium composition, operation conditions of pH, temperature, agitation and aeration etc. [6••,48,49]. Currently pigment production is carried out commercially in submerged state fermentation (SmF). However, solid-state fermentation (SSF) systems could be advantageous due to its natural potential [3••,4,50,51].
  Response surface methodology (RSM) technique for screening and optimization of various types of pigments production has gained importance recently [52]. One of the research studies optimized the canthaxanthin production by *Dietzia natronolimnaea* HS-1 using cheese whey as substrate by statistical methods [53]. An effective adsorption method separation of prodigiosin has been demonstrated with very high quantitative recovery (83% higher), which eliminated the one step of separation of microbial-cells and produced the pigment ready for purification [54].
- Genetic engineering
  Wild strains of microbes produce low concentrations of the microbial pigment which usually leads to higher costs of production. It is therefore required to isolate strains capable of producing high yields within shorter fermentation period [71••]. Genetic engineering is the tool that enables to manipulate the microorganism's genetic material by introducing genetic mutations — physical/chemical or by recombinant DNA technology. This technology alters the genetic makeup of a microbe in a way so as to bring about novel functions and increase the yield of pigments. Therefore, genetic engineering has proven to be a highly effective technology to derive valuable pigments in good quantities [41,42]. It helps in developing cost-effective means to produce pigment of interest. Several studies have proven that strains like *Escherichia coli*, *Cornybacterium glutamicum*, *Bacillus subtilis*, and *Pseudomonas putida* can be easily recombined for such processes [56–58].
- Plant tissue culture
  A recent study states that plant tissue culture (PTC) in fermentation can be a robust technique for production of secondary metabolites including pigments. Research has been carried to produce pigments like anthocyanin, betalins, carotenoids, and saffron etc. [40,59].

Current technologies for microbial pigment production

Pigment production using microbes is highly controlled by the composition of the medium, supplemented-stimulators and optimization of culture conditions performed using experimental designs through statistics, aid in developing successful large scale production [71••].

Response surface methodology (RSM), artificial neural networks (ANN) are two of such techniques applied for microbial pigment production. In order to investigate the possible effects of medium components on pigment production a design called Plackett–Burman design was tested at the first stage and significant factors were optimized at the second stage by central composite design, and response of each significant factor was analyzed using regression analysis. This process resulted in designing of process at large-scale [71••,82].

Another effective method of optimizing pigment production is employing immobilized strains; it might enhance cell proliferation thereby increasing their activity. It has been noted if a solid carrier was added to the medium during the fermentative production of prodigiosin, it resulted in rise in yield from 7.05 g/l to 15.6 g/l. It has been reported if microbes in system were subjected to stress conditions (osmotic pressure, elevated temperatures) and were able to adapt through their restricted multiplication, the pigment production could be enhanced [71••,82].

Challenges

Extraction of highly concentrated form of pigments in a purified state remains to be a challenge for the manufacturers [6••]. Microbial pigments are usually extracellular or intracellular; extraction and purification of pigments produced intracellularly adds the stage of cell disruption and increases the downstream processing times. Stability of microbial pigments under environmental stress or when they are exposed to UV leads to degradation of the pigments [60]. Large scale commercial production of pigments with increased yields from microbes within a cost effective manufacturing process is the biggest challenge faced by the industry [6••].

Several difficulties and drawbacks have been reported in pigment extraction and their purification. High temperature, long processes with application of organic solvents leads to degradation and unwanted isomer formation. As an alternative use of enzymes is questionable due to their high costs. Certain microbial pigments are not approved by the regulatory authorities to be used as food colorant like Monascus pigments, which are mixture of azaphilone pigments. These pigments contain mycotoxin citrinin and additional toxic substances hence controversial views for use as colorant in food industry [71••].

Major microbial pigments in food industry

- β-Carotene
  Carotenoids range from yellow to orange-red colored pigments. Microorganisms like *Serratia*, *Micrococcus*, *Mycobacterium*, *Agrobacterium*, *Sulfolobus* and *Streptomyces* [1••,12,61]. It is a recent trend to utilize the agro-industrial byproducts, such as cheese whey, sugarcane molasses, glucose syrup, peat hydrolysate, cellobiose and beet molasses [6••] as nutrients for producing carotenoids. β-Carotene is an antioxidant and has positive effects against few diseases. According to a current research study it is produced mainly using *Blakesleatrispora*, *Mucorcircinelloides* and *Phycomyces blakesleeanus* [3••]. It is used as a food additive in vegetable oils, orange drinks, margarine, various emulsions and microencapsulated beadlets [1••].
- Arprink red
  It is the red colored pigment produced by *Penicillium oxalicum* isolated from soil. The amount of arprink red permitted in various different food products has been recommended by Codex Alimentarius Commission (Rotterdam Meeting, March 11–15, 2002) [3••].
- Riboflavin
  It is a yellow colored pigment, water-soluble vitamin produced by several microbes. It is commercially produced using Ascomycetes *Ashbya gossypii* which is preferred due to its high yield and genetic stability, filamentous fungi *Candida famanta* and bacteria *B. subtilis* [62]. Riboflavin's production through fermentation is of three types: weak over producer, moderate overproducer and strong overproducer. *Eremothicum ashbyii*, *A. gossypii*, *Candida flaleri*, *Sacchaomyces cerevisiae* and *B. subtilis* are amongst the strong producers of riboflavins [3••,63]. Riboflavins are used as food additives in cereals, pastas, sauces, processed cheese, milk products, and energy drinks etc. [12].
- Monascus pigments
  Monascus pigments belong to the family of Monascaceae group of Ascomycetes [65•]. Major strains isolated for application in food industry is of four types: *M. pilosus*, *M. purpureus*, *M. ruberand* and *M. froridanus*. Monascus adds red, orange and yellow color to the food items. Monascus not only adds color to food but also imparts a specific flavor [3••]. They are used as food additives in red wines, tofu, sausages, hams, and meats etc. [64]. Color degradation is a common concern for these pigments; recently a research study investigated the thermal stability of pigments produced by *Monascus ruber* in submerged fermentation and described the behavior of the responses of color degradation using response surface methodology [64].
- Lycopene
  It is a red carotenoid which is an acyclic isomer of beta-carotene. It has been noted that cis-isomer of lycopene is more stable and possesses higher antioxidant property in comparison to trans-lycopenes. The genetic engineering of fungus *Fusarium sporotrichioides* has

been tried to produce the food colorant and antioxidant lycopene using cheap substrate corn fiber [3••].

Antioxidant properties of microbial pigments and their benefits

The unique characteristic of an antioxidant is to trap the free radicals [25]; they quench the photo sensitizer, interact with oxygen singlet and scavenges the peroxy radicals [66]. Most of the chronic diseases like cancer, diabetes, cardiovascular and autoimmune disorders are associated with the presence of free radicals [25]. Microbial pigments are potential antioxidants which when added in food products, exhibited positive effects. Carotenoids, napthaquinone and violacein have demonstrated antioxidant properties due to their biological functions [2]. Similarly, microbial pigment xanthomonadin acts as an antioxidant activity by inhibiting photodynamic lipid peroxidation in liposome. Violacein has exhibited protectin against oxidative damage in gastric ulceration by stimulating mucosal defense mechanism [67]. Such pigments not only act against diseases but are helpful in preventing onset of diseases like atherosclerosis, cataracts, age-related macular degeneration and multiple sclerosis [68].

Pigments found in corn, kale and spinach namely lutein, zeaxanthin and xanthophylls play a crucial role as antioxidants in prevention of age-related macular degeneration (AMRD) which is the main cause of blindness in human retina. Astaxanthin due to its very high antioxidant property has positive effects on disease prevention, boosting immune system, bioactivity against *Helicobacter pylori* and cataract prevention. Carotenoids have been used to prevent cardiovascular diseases (CVD), boosting immune system, sun-burn protection and inhibition of development of few cancers [1••]. A recent research study has concluded that for the first time carotenoid isolated from *M. roseus* and *M. luteus* has demonstrated potential UV-protective, antioxidant and antibacterial activity [61]. Similarly, lycopene aids in oxidation of low density lipoprotein (LDL) cholesterol, prevents arteriosclerosis and coronary diseases. Prodigiosin is an important anticancer microbial pigment [1••].

Novel sources for microbial pigments

Novel strains like *Enterococcus hirae*, *Acinetobacter mufti*, *Pseudomonas aeruginosa* were isolated from soil and cultured on brain heart infusion agar and further investigated *in vitro* for colored pigments and antioxidant activity was tested using DPPH assay which revealed the presence of 54.7% antioxidants; these pigments produced in laboratory have potential to be used as food colorants [22]. A recent research study has revealed the potential in filamentous fungi to produce polyketide-Monascus like pigments and unexplored hydroxyl-anthraquinoid colorant; marine fungi have also been found to be a potential source of new pigments possessing various color types [20•]. Currently filamentous fungi are considered to be important microbial cell factories for producing food grade pigments because of the variety in their pigment colors, chemical structures, and flexibility for large scale production. Similarly, marine fungi have the ability to produce bright colors mainly polyketides [21]. Also, for the first time yeast carotenoid pigment from yeast *Sporobolomyces* has been investigated for better antioxidant and antimicrobial activity and results are promising [25].

Future perspectives and conclusion

Consumers' sensitivity toward application of synthetic additives in food has led to rapid development and large scale production of natural pigments/colorants. Majority of natural food additives are derived from microbes (bacteria, fungi, yeast etc.). Exploitation of microbes for commercial production is the most focus point in development of novel food additives. There is an urgent need for development of novel strains capable of high yield of pigments using a cheap substrate with less downstream processing stages. Certain microbial pigments are extremely sensitive to pH, light, and temperature variations, thereby they exhibit poor stability and their degradation; this aspect of pigments a focus of investigations. Fermentation types combined with genetic engineering techniques and with the study of chemical structures and biosynthetic pathways will play a crucial role to add numerous colors to food in the coming years.

Real time successful commercialization of microbial pigments as food colorants requires investments from key public and private stakeholders. This might overcome the challenges faced in the industry and may lead to development of robust technologies for production, extraction and purification of novel pigments from microbial sources [71••].

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