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Production of Natural Food Colourants Using Food Grade Microbial Pigments - A New Focus in Industrial Microbiology

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Abstract: In today's food industry, the new focus on large-scale microbial production of natural food colourants has emerged among the manufacturers due to the relatively costly production of plant-derived food colourants, and the doubtful safety status of inorganic and synthetic colourants. However, it is still very challenging to change the consumers' reliance on synthetic colourants. In fact, the first European success on the launch of β-carotene from Blakeslea trispora in 1995 has headed more search for new sources of natural food grade microbial pigments. The acceptance and rejection of a microbial food grade pigment by a community of consumers depend on two main contributing factors i.e. the regional and the traditional-based legislations. This paper discusses the classification of natural pigments, the legislation on natural food colourants, the success and new sources of potential pigment-producing microbes, and the advantages of microbial fermentation.

Keywords: Natural food colourants, microbial food grade pigments, food safety, large-scale microbial fermentation

1. Natural Food Colourant: Great Alternative to Synthetic Colourants

Interest in natural food colourants as alternatives to synthetic colourants and other classes of colourants has greatly increased among the modern consumers due to the food safety and health concerns. For years, the safety of inorganic and synthetic colourants has been questioned, resulting in a reduction of the number of permitted colourants. The unmonitored synthetic colourants which some of which were intended for dyeing textiles but not for food had spread through the United States of America (USA) and Europe in a variety of popular food in the past century becoming evidence that the safety of synthetic colourants remains unclear. Additionally, more than 80 synthetic colourants offered by the sellers had never been tested for their toxicity or other adverse effects [1]. Synthetic colourants are likely to be marked as unwelcomed and harmful; some are proven to be responsible for allergenic and intolerance reactions [2]. The prohibition of Red No. 2 and the continued scrutiny of Red No. 40 and Red No. 3 [3] have made red pigments

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*Corresponding author: snajihah91@gmail.com as special interest to the food manufacturing industry, leading the way to search for new sources of red pigments from natural resources. As a result, these limitations of synthetic colourants and the modern consumers' tendencies towards the consumption of natural food colourants have significantly increased the worldwide interest. Producers of alcoholic beverages, soft drinks, confectioneries, and dairy products are among the largest users of natural food colourants, showing the promising and huge market for natural colourants.

Natural food colourants are made from a wide range of renewable sources, and most often, the colourants are extracted from plant materials especially from the division Magnoliophyta, the flowering plants. Saffron, which means yellow in Arabic, is the dried stigma of *Crocus sativus*, and it is the most expensive spice used in food industry especially in Spain [4]. This plant extract is mainly valued as a food additive for flavouring and colouring due to the presence of water-soluble carotenoid, crocin [5] as well as for its therapeutic properties e.g. antitumoural activity [6]. Saffron is usually classified by quality and price based on stigma length, colour, odour, and the presence of floral rest. However, the council of European Union (EU) regards saffron as a spice but not as a colourant. Other sources of natural food grade pigments are algae, microorganisms (e.g. fungi like *Blakeslea trispora* and *Monascus* spp., and the simplest prokaryotes like cyanobacteria *Arthrospira* spp.) and insects (e.g. scale insects like cochineal and lac).

The functions of plant pigments may be classified into five main groups as proposed by [7]: (1) conversion of light energy during photosynthesis with the presence of chromophore (e.g. chlorophylls, phycobilins, and carotenoids), (2) communication medium between plants and animals (e.g. carotenoids and flavonoids), (3) detoxification of reactive oxygen species i.e. antioxidation (e.g. carotenoids), (4) role in response to stress (e.g. flavonoids), and (5) unknown functions of pigments. [8] estimated that approximately three quarter of the plants on earth have not been completely investigated and less than 1% have been studied in detail. This directly shows that there are many unexplored plants that may have potential as new sources of natural food colourants. In fact, the number of permitted synthetic food colourants has reduced resulting from studies started in 1904 and such phenomenon has attracted manufacturers to focus more on production of natural food colourants from plants. However, the production costs for plant-derived food colourants are comparatively expensive and not competitive as compared to synthetic colourants, due to the high dependence on raw materials. Furthermore, every new pigment source requires safety assessment before it can be used as a new food colourant, which is time consuming and costly e.g. a series of assessments through the US (United States) Food and Drug Administration (FDA), Food Standards Australia New Zealand (FSANZ) petitioning for New Zealand and Australia, and the EU approval.

1.1 Classification of Natural Food Colourants

Tetrapyrrols, tetraterpenoids and flavonoids are the three most important classes of natural food colourants [9]. Chlorophylls are the most important member of the tetrapyrrols and they are found in all higher plants. These green colourants are highly susceptible to degradation during processing, resulting in colour changes in food. According to [10], major chemical degradation routes are associated with pheophytinisation, epimerisation, pyrolysis, and also with hydroxylation, oxidation or photo-oxidation with the presence of light; and there is a general agreement that the main cause of green vegetable discolouration during processing is the conversion of chlorophylls to pheophytins by the influence of pH. One of the popular methods to prevent colour degradation of chlorophylls is pH control [11]. Experimentally, at all pH values and at a specific temperature, chlorophyll *a* was found to degrade faster than chlorophyll *b* and the results also proved that chlorophyll *a* was more prone to thermal degradation in acidic conditions [12].

Carotenoids are tetraterpenoids, and they are also as abundant as chlorophylls due to their main role in photosynthesis. Carotenoids representing a group of valuable molecules make up a diverse class of natural pigments with great commercial interest e.g. in pharmaceutical, chemical, food and feed industries. They give the yellow–orange–red colours of many fruits. It is believed that more than 700 different carotenoids are synthesised by plants and microorganisms, for instance the red yeasts from genera *Phaffia*, *Rhodotorula* and *Sporobolomyces* can produce carotenoids of economic value such as β-carotene and astaxanthin [13]; [14].

The third most important class, flavonoids provide the red–purple shade of many fruits, in particular berries. One of the examples of flavonoids is anthocyanins [6]. Approximately 4,000 tonnes of anthocyanins are consumed annually in the United Kingdom (UK), mainly in fruits [8]. Other important classes of natural food colourants that similar to anthocyanins are betalains and anthraquinones. Although betalains and anthraquinones were not widely studied as compared to anthocyanins, they still have much potential as natural food colourants due to having more colouring strength than anthocyanins. The consumption quantities of chlorophyll (tetrapyrrols) and carotenoids (tetraterpenoids), as the major pigments of leafy vegetables, are estimated to exceed the figure shown by the anthocyanins (flavonoids). Needless to say, the intake of natural pigments is an important part of the human diet [15]. Furthermore, [16] have listed common pigments into seven groups with their respective colours as in Table 1.

1.2 Classification of Natural Pigments

The terms “pigment” and “dye” are often misunderstood and misused [17] until recently. By definition, in a given medium, a dye is soluble while a pigment is insoluble [18]. The best example is β-carotene which is pigment in water (because of its insolubility) and dyes in fats, oil and organic solvents due to their hydrophobicity (i.e. solubility). Apart from that, colour hue is dependent on pigment concentration; therefore, yellow β-carotenes will gradually turn orange and even red at increasing concentrations. Besides that, the pigment concentration and the colour parameters of pistachios (*Pistacia vera* and others) have been identified to be influenced by the degree of ripeness and origin, thus represent important elements to differentiate and discriminate samples from different geographic origins [19].

Pigments can be classified into two major groups, hydrophobic and hydrophilic pigments. Hydrophobic pigments primarily consist of carbon and hydrogen meaning that they do not have the properties (i.e. oxygen) to be surrounded by water molecules. Therefore, hydrophobic pigments are non-water soluble. They are quite soluble in other hydrophobic compounds such as fats, oils, and organic solvents. On the contrary, water-soluble pigments or hydrophilic pigments have a considerable amount of oxygen in their chemical structure. Hence, hydrophilic pigments (e.g. anthocyanins) are usually extracted and concentrated using either water or lower alcohols; and organic solvents for hydrophobic or lipophilic pigments (e.g. β-carotene and chlorophyll) yielding an oleoresin rich in pigments and other materials such as triglycerides, sterols, wax, and other lipid-soluble compounds. In the case of hydrophobic pigments, colour formulation is included to make them more stable and more suitable for a variety of food and drinks (e.g. beverages) particularly for extreme food processing conditions [20].

1.3 Legislation on Natural Food Colourants

The rapid emergence of food technologies has enabled the exploration and the development of new natural food colourants from diverse living organisms such as local plants and microorganisms (e.g. bacteria and fungi) that have not yet been scientifically investigated. However, [21] anticipate that it will be impossible to introduce new natural colourants into today’s food industry if the food legislation and consumer attitudes (especially those who are still doubtful or not concerned about natural food colourants) remain unchanged. In other words, three elements must not be ruled out in order to improve the use of natural food colourants in the food manufacturing industry and subsequently to make them more promising: legislation, economy, and consumer awareness and acceptance of the naturally derived colourants. In the food manufacturing industry, the introduction of new natural food grade pigments as colourants is also challenging in ensuring their high stability to processing conditions as compared to the natural pigments that are already available and permitted for industrial applications [22].

Table 2 shows the list of permitted natural and nature-identical colourants in the EU, the USA, Malaysia, New Zealand and Australia. Overall, the legislation put an emphasis on the allowed colourants, sources of colourants, extracting solvents, and the pigment purity. There are two major differences between the EU and the USA legislation: (1) allowed sources of colourants, and (2) allowed food to be coloured, though there are many similarities between the two councils [23]. One of the examples is the use of sodium copper chlorophyllin, by which in the EU the allowed sources are alfalfa, grass, nettle, and edible plant materials, and a long list of food may be coloured; whereas in the USA the allowed source is only alfalfa (*Medicago sativa*) and its use is limited in citrus-based dry beverage mixes (Code of Federal Regulations). In addition, canthaxanthin as a keto carotenoid is generally allowed in the USA but it is only allowed for colouring Saucisses de Strasbourg (a sausage) in the EU. Such differences exist due to the legislation that is (1) region-based (in this case, the regions are EU and USA), and (2) tradition-based usage e.g. in the legal and traditional use of monascus, lac, gardenia, and spirulina in some parts of Asia but prohibited in the EU and USA [13]. For New Zealand and Australia, FSANZ seems following the legislation outlined by the EU based on the same number between the FSANZ code and the E-number. However, FSANZ classifies caramel into Caramel I, Caramel II, Caramel III and Caramel IV. Besides, canthaxanthin is not listed by FSANZ as the permitted colourant, but it is believed that canthaxanthin along with cottonseed flour, fruit juice, and vegetable juice are allowed and do not require FSANZ code following the EU. As mentioned by [24], most systems in Southeast Asia are typically facing the challenges in strengthening the food legislation, food control management, inspection services, laboratory services and information, education, communication, and training, and Table 2 supports the need for better listing and food legislation.

Table 1- Examples and colour characteristics of pigments of biological importance (Adapted from [25] and [26]) Reproduced from [27]

Group	Common Pigments	Predominant Colour
Tetrapyrroles	Chlorophylls	Green
	Billins	
	Cyclic (hemes)	
	Hemoglobin	Red
	Myoglobin	Red
	Linear	
	Phytochrome	Blue-green, yellow-red
Isoprenoid derivatives	Carotenoids	
	Carotenes (e.g. β-carotene, lycopene)	Yellow-red
	Xantophylls (e.g. lutein, zeaxanthin)	Yellow
	Iridoids	
N-Heterocyclic compounds	Purines (e.g. guanine)	Yellow (golden & silvery)
	Pterines	White-yellow
	Flavins (e.g. riboflavine)	Yellow
	Phenazines	Yellow-purple
	Phenoxazines	Yellow-red
	Betalains	Yellow-red
	Eumelanins	Black-brown
	Phaomelanins	Brown
Benzopyran derivatives	Flavonoids (e.g. anthocyanins, flavonols, flavones, anthochlors)	Blue-red, yellow-white, white-cream, yellow
	Tannins	Brown-red
Quinones	Benzoquinones (e.g. plastoquinone)	At high concentrated pink hue
	Naphthoquinone (e.g. vitamin K)	Red-blue-green
	Anthraquinone (e.g. carminic acid)	Red-purple
Melanins	Allomelanins	Yellow-brown
	Eumelanins	Black-brown
	Phaeomelanins	Brown
Metalloproteins	Cu-proteins	Blue-green
	Adenochrome	Purple-red

Table 2 - Permitted natural and nature-identical colourants in the EU (Regulation (EC) No 1333/2008 of the European Parliament and of the Council of 16 December 2008 on food additives), the USA (Code of Federal Regulations), Food Safety and Quality Division (FSQD), Ministry of Health Malaysia (website - http://fsq.moh.gov.my); and Food Standards Australia New Zealand (FSANZ website: http://www.foodstandards.gov.au/). The table was adapted from [28]

No.	EU name	E-number	U.S. name	U.S. CFR number	FSANZ name	Code	FSQD name
1	Curcumin	E 100	Turmeric Turmeric oleoresin	73.600, 73.615	Curcumin or Turmeric	100	Turmeric
2	Riboflavin	E 101	Riboflavin	73.450	Riboflavin or Riboflavin 5'-phosphate sodium	101	+
3	Cochineal, Carminic acid, Carmines	E 120	Cochineal extract, carmine	73.100	Cochineal or carmines or carminic acid	120	Carmine
4	Chlorophylls and chlorophyllins	E 140	Not listed		Chlorophyll	140	Chlorophyll
5	Copper complexes of chlorophylls, chlorophyllins	E 141	Sodium copper chlorophyllin	73.125	Chlorophyll-copper complex; Chlorophyllin copper complex, sodium and potassium salts	141	+
6	Plain caramel Caustic sulphite caramel Ammonia caramel Sulphite ammonia caramel	E 150a E 150b E 150c E 150d	Caramel	73.85	Caramel I, II, III & IV	150a, 150b, 150c & 150d	Caramel
7	Vegetable carbon	E 153	Not listed	Not listed	Carbon blacks or Vegetable carbon	153	+
8	Carotenes	E 160a	β-Carotene	73.95	Carotene	160a	Carotene
9	Annatto, Bixin, Norbixin	E 160b	Annatto extract	73.30	Annatto extracts	160b	Annatto
10	Paprika extract, capsanthin, capsorubin	E 160c	Paprika Paprika oleoresin	73.340, 73.345	Paprika oleoresins	160c	+
11	Lycopene	E 160d	Tomato lycopene extract; tomato lycopene concentrate	73.585	Lycopene	160d	+
12	β-Apo-8'-carotenal (B30)	E 160e	β-Apo-8'-carotenal	73.90	β-apo-8’ Carotenal	160e	β-apo-8’ Carotenal
13	Not listed	Not listed	Not listed	Not listed	β-apo-8’ Carotenoic acid methyl or ethyl ester	160f	Ethyl ester of β-apo-8’ Carotenoic acid
14	Lutein	E 161b	Not listed	Not listed	Lutein	161b	+
15	Canthaxanthin	E 161g	Canthaxanthin	73.75	#	#	Canthaxanthino
16	Beetroot Red,	E 162	Dehydrated beets	73.40	Beet red	162	+

	betanin		(beet powder)				
17	Anthocyanins	E 163	Grape color extract Grape skin extract (enocianina)	73.169, 73.170	Anthocyanins or Grape skin extract or Blackcurrant extract	163	Anthocyanin
18	Not allowed	Not allowed	Toasted partially defatted cooked cottonseed flour	73.140	#	#	+
19	*	*	Fruit juice	73.250	#	#	+
20	*	*	Vegetable juice	73.260	#	#	+
21	*	*	Saffron	73.500	Saffron or crocetin or crocin	164	Saffron

* Allowed as a food ingredient (not an additive) that does not require an E-number.

\# Believed to follow EU, details could not be found in the FSANZ database.

\+ Details could not be found in the FSQD database/from the FSQD officer’s documents

2. Microbial Production of Natural Food Colourant

Microorganisms are becoming a more popular alternative source to produce natural food grade pigments through fermentation. Bacteria and fungi are the most common microbes abundant in the nature. Table 3 lists some pigment-producing microbial sources and the colour shades of their pigments. Natural colourant production by microbial fermentation has a number of advantages: (1) cheaper production, (2) possibly easier extraction, (3) higher yields especially through strain improvement, (4) no lack of raw materials, and (5) no seasonal variations [22]; [21]. Until recently, the use of plant extracts is known to be comparatively expensive and not competitive to synthetic dyes due to their high production cost. Consequently, even though many plants have been used for naturally derived pigment production, synthetic dyes are still considered as the most economical. Such weaknesses could be overcome with the use of microbial pigments which can cut down the high production cost and improve the product quality.

2.1 Regional and Tradition-Based Legislation: A Reality

Obviously, the current legislation on the use of natural colourants as food additives is often based on local and tradition. One obvious example is the disapproval of monascus as a permitted food colourant. Monascus are red and yellow colourants derived from the fermentation of fungus *Monascus purpureus* and *Monascus anka*. Experimentally, *Monascus* pigments have low water solubility, are sensitivite to heat, instable in the pH ranging from 2 to 10, and susceptible to photobleaching. Thus, the patenting has mainly focused on the solubilisation, the stability, and the extraction of pigments. The pigments have been traditionally used as colouring agents for meat, fish, and red rice/red koji or *angkak* for centuries in Asia especially in Japan [29]. Unfortunately, the use of monascus as a food colourant is forbidden in the UK and USA. The concern on the potential secretion of toxic compounds during fermentation process of *Monascus* species is the major reason to the prohibition in the UK and USA. Monascus-fermented rice has been found to contain the mycotoxin citrinin [30]; [31] but citrinin is only produced along with pigments by some and not all strains of *Monascus* sp. [32].The blue shade pigment of *Spirulina* sp. is another natural pigment that is not regarded as a colourant in the EU and the USA. US FDA has classified *Spirulina* sp. as a cyanobacterium, not a vegetable [33]. The blue pigment is used as a food colourant in Japan but limited to non-acidic foodtuffs such as dairy products and chewing gum. The *Spirulina* pigment, phycocyanin, has the highest stability at pH 5-7 [34]; [35]; [36]. Due to the legislation by regional and tradition, cheaper and stable blue synthetic colourant is still conquering the international market.

Table 3 - List of pigment-producing microbial sources and colour shades of their pigments. Reproduced from [22]

No.	Microorganism	Pigment color shade
	Bacteria	
1	*Janthinobacterium lividum*	Bluish purple
2	*Achromobacter* sp.	Creamy
3	*Bacillus* sp.	Brown
4	*Brevibacterium* sp.	Orange, Yellow
5	*Corynebacterium michigannise*	Greyish to creamish
6	*Pseudomonas* sp.	Yellow
7	*Rhodococcus maris*	Bluish red
8	*Streptomyces* sp.	Yellow, red, blue
9	*Serratia* sp.	Red
	Fungi	
10	*Aspergillus* sp.	Orange, red
11	*Blakeslea trispora*	Cream
12	*Monascus purpureus*	Yellow, orange, red
13	*Helminthosporium catenarium*	Red
14	*H. gramineum*	Red
15	*H. cynodontis*	Bronze
16	*H. avenae*	Bronze
17	*Penicillium cyclopium*	Orange
18	*P. nalgeovnsis*	Yellow
	Yeast	
19	*Rhodotorula* sp.	Red
20	*Yarrowia lipolytica*	Brown
21	*Cryptococcus* sp.	Red
22	*Phaffia rhodozyma*	Red
	Algae	
23	*Dunaliella salina*	Red

2.2 Potential Pigment-Producing Microbes: Success and New Sources

The industrial production of natural carotenoids through microbial fermentations is already established and expanding [37]. There are many microbes that have the ability to produce commercial carotenoids such as β-carotene. Fungi are among the microbes that have been screened and used for production of naturally derived food grade pigments. Much of the earlier work on producing colourants from fungi focused on carotenoid pigments. Table 4 lists some fungal food grade pigment producers comprising the potential and commercial species. The list suggests that fungi are among the important sources of carotenoids particularly for natural food colourants.

One of the physiological roles of carotenoids in red yeasts e.g. *Dioszegia* (Tremellales, Heterobasidiomycetes, Fungi) may act as antioxidants in situations of high oxidative stress [38]; [39]. In fact, pigments from fungi were mainly used for species identification and differentiation, and their current values in carotenoid production have made them very important in current natural food colourant industry. For the extraction of carotenoids, since carotenoids are lipophilic, organic solvents such as petroleum ether, chloroform, ethanol, and methanol are used. A number of studies have been done to test the ability of the solvents to isolate natural microbial carotenoids [40]; [41] but the investigation on the carotenoid release by mixtures of solvents had become a low attention. [42] showed that when multiple solvents i.e. dimethyl sulfoxide, petroleum ether and acetone were used together to disrupt the cell wall of the yeast *Rhodotorula glutinis* and thus to release the cellular carotenoid pigments, the mixture demonstrated a synergistic effect on the extent of carotenoid recovery.

The first European success in microbial or fungal production of natural food colourant was in 1995 where a new source of β-carotene from the fungus *Blakeslea trispora* was launched. In 2004, a new natural food colourant called Arpink Red^{TM} (now Natural red^{TM}) was claimed to be produced by a fungal species of *Penicillium oxalicum*. The new red colourant is manufactured by ASCOLOUR BIOTECH in the Czech Republic. The red colourant is claimed as an extracellular metabolite of the anthraquinone class and the pigments are believed to confer anticancer effects when used in food [43]. However, [44] put forward a question on the status of the isolated *P. oxalicum* because none of its characteristics has ever been seen in *Penicillium* species. Therefore, genus and species misidentification might be possible. Beside that, some parties have also raised issues on the possible mycotoxin production in view of the fact that *P. oxalicum* produces a yellow toxic pigment known as secalonic acid D [45] and it is likely to be human pathogen due to the well growth at 37°C [46].

There are many unexplored microbial sources of natural food colourants to date especially from the sea. The functions of marine pigmented heterotrophic bacteria (PHB) pigments are well-known as antioxidants, light protection, and membrane stabilisers [47]; [48]. A large number of marine heterotrophic bacteria (i.e. pigmented and non-pigmented heterotrophic bacteria) are able to synthesise carotenoids, and carotenoid-rich species have been recorded from the coastal and oceanic waters [19]. Thus, the possibility to find new potential naturally derived food grade pigments from microbes are still high without any dependence on the seasons and large spaces, unlike for plant pigments that are seasonal and normally require high availability of raw materials in adequate quantities for industrial extraction. In Malaysia, a Gram-positive bacterium, *Staphylococcus kloosii* was isolated from the respiratory tree of *Holothuria* (*Mertensiothuria*) *leucopilosta* collected from Teluk Nipah, Pangkor Island, Perak and shown to produce orange pigments on tryptone glucose yeast extract agar and in nutrient broth [49]. The findings suggested the presence of pigment–producing microorganisms associated with marine-dwelling sea cucumber. Table 5 presents a list of potential and commercial food grade pigment-producing bacteria. Low production cost is also another factor to be economically feasible, but it would not be a great obstacle in microbial production of microbial food grade pigments due to the emerging technologies of microbial fermentation. As emphasised by [50], the production of plant crops for their pigment content alone does not appear to be economically feasible.

Table 4 - List of potential and commercial food grade pigment-producing fungi

No.	Species	Description
1	*Blakeslea trispora*	This mould is non-pathogenic and non-toxigenic, and it is used for β-carotene production [51].
2	*Monascus purpureus*	This red mould is famous in Asia, for instance in Indonesia, and the pigment has been used for centuries e.g. as colouring agent for red rice/Anka/Angkak [29].
3	*Monascus ruber*	The pigment of this purple mould is also popular as a colouring agent in oriental food. Some applications are in the colouration of processed meats, marine products, surimi, and tomato ketchup [52].
4	*Penicillium oxalicum var. armeniaca* CCM 8242	This fungus can be obtained from soil and its pigment is used as a red colourant. It produces dark red anthraquinone that is stable at pH over 3.5 (without colour loss) and high temperature [51].
5	*Candida guilliermundii*	This yeast is a riboflavin moderate overproducer and its pigment is used as a

		yellow food colourant [51].
6	*Debaryomyces subglobosus*	This yeast is also a riboflavin moderate overproducer and the pigment is used as a yellow food colourant [51].
7	*Eremothecium ashbyii*	This fungus is among the riboflavin strong overproducers and the pigment is extracted for food colourant [51].
8	*Ashbya gossypi*	This fungus is a riboflavin strong overproducer and it is used for yellow food colourant production [44].
9	*Mucor circinelloides*	This fungus produces β-carotene [50]
10	*Phycomyces blakesleeanus*	This fungus is a β-carotene producer [50]
11	*Xanthophyllomyces dendrorhous*	This fungal carotenoid producer was formerly known as *Phaffia rhodozyma*. It produces astaxanthin. One of its pigment applications is as a flesh pigmenter. The pigments are supplemented in the feed of farmed species e.g. salmon and trout since animals cannot synthesise carotenoids [50].
12	*Rhodotorula glutinis*	This red yeast is a carotenoid producer i.e. β-carotene. Feed supplement with *Rhodotorula* cell mass has been found to be safe and nontoxic in animals [53].
13	*Rhodotorula gracilis*	This yeast is a β-carotene producer. The red pigment has been found to be safe and nontoxic in animals [7].
14	*Rhodotorula rubra*	This red yeast is a carotenoid producer i.e. β-carotene. Feed supplement with *Rhodotorula* cell mass has been found to be safe and non-toxic in animals [44]; [58].
15	*Fusarium decemcellulare*	This fungal napthoquinone producer produces soluble extracellular naphthoquinones of the naphthazarin structure (javanicin, anhydrojavanicin, fusarubin, anhydrofusarubin, bostricoidin, and novarubin) or extracellular dimeric naphthoquinone aurofusarin [54].

16	*Fusarium graminearum*	This fungus produces aurofusarin (napthoquinones) [55].
17	*Fusarium bulbigenum*	This fungus produces bikaverin (napthoquinones) [54].

Table 5 - List of potential and commercial food grade pigment-producing bacteria

No.	**Species**	**Description**
1	*Clostridium acetobutylicum*	It is a riboflavin weak overproducer that produces yellow pigment for food colourant [44].
2	*Flavobacterium* sp.	This bacterium produces yellow zeaxanthin pigment [51].
3	*Bradyrhizobium* sp.	This photosynthetic bacterium produces canthaxanthin [51].
4	*Halobacterium* sp.	This extremely halophilic bacterium produces canthaxanthin [55].
5	*Agrobacterium aurantiacum*	An Astaxanthin producer [51].
6	*Paracoccus carotinifaciens*	An Astaxanthin producer [56]
7	*Halobacterium salinarium*	An Astaxanthin producer [55].
8	*Brevibacterium aurantiacum* sp. nov.	This species's previous name was *Brevibacterium linens*. This species was found in the rind of red-smear ripened soft cheeses and can produce isorenieratene and hydroxyl derivatives. Its pigments have therefore been consumed by human beings for a long time [57].
9	*Streptomyces mediolani or Mycobacterium aurum*	This species can produce isorenieratene and hydroxyl derivatives [51].

Conclusions

The growing awareness among the consumers globally towards the harmful effects of synthetic colourants on humans as well as the environment have been increasing the demand for natural colourants in the market. These natural colourants gradually lead the way in the global market due to their non-toxic and non-carcinogenic properties. Although there are some limitations of the natural colourants especially those extracted from plants due to their high production cost, natural colourants from microorganisms can be seen as one of the best alternatives to replace the synthetic colourants considering their low-cost production, easier extraction and high yield production. Apart from that, it was also reported that microbial colourants have great potential from medicinal aspects. However, the production of natural colourants from microorganisms needs to be continuously explored and more scientific studies need to be conducted to assess the potential of microbial pigments as colourants especially in the food industry.

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