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# A review on production of carotenoids from bacteria and it's future aspects

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**Abstract**
Natural carotenoids are secondary metabolites with antioxidant, anti-inflammatory, and cancer-preventative activities. The pharmaceutical, cosmetic, textile, and food industries all have a high need for these molecules, prompting the hunt for alternative natural sources of carotenoids. Carotenoids are light, heat, oxygen, acids, and alkaline bases sensitive. When exposed to light (direct sunlight/UltraViolet), cis–trans photoisomerization occurs, which may result in photodestruction. Biological materials that include carotenoids, as well as their solutions, must be protected from light. Many carotenoids (xanthophylls) are thermolabile, hence they should only be heated when absolutely necessary. The generation of carotenoids from bacteria has sparked a lot of attention in the industrial world in recent years. Some bacteria can manufacture distinctive carotenoids with C30 skeletons in addition to carotenoids with C40 skeletons. This article discusses the isolation and screening of bacteria that produce carotenoids. After primary, secondary, tertiary, and quaternary screening of isolates based on pigmentation, tolerance to pH and NaCl percent (w/v), degree of pigmentation, absorption maxima, free radicle scavenging activities, and it's antimicrobial activity, effective carotenoid producers were identified. In this regard, a wide range of methods for extracting and identifying bacterial carotenoids have been published, and this is the review that summarises most of this knowledge.We present their biosynthetic origin to focus on the strategies used in their extraction and characterisation in order to comprehend the diversity of these carotenoids. For the analysis and identification of bacterial carotenoids, high-performance liquid chromatography-mass spectrometry (HPLC-MS) has received special attention. We'll wrap off this section by discussing the commercial possibilities of bacterial carotenoids. This review is intended to serve as a reference for identifying these metabolites, which are regularly found in new bacterial strains.

**Keywords:** antioxidant, carotenoid, extraction, pigment, screening, xanthophylls

## Introduction
Humans have long preferred natural pigments as a food additive to avoid the negative effects of artificial mineral and metal-based hues. Aniline and hazardous petroleum chemicals were used to make the majority of chemically manufactured colours. Plants create the majority of natural colours, however their availability varies depending on the season and geographical dispersion. Microbial pigment sources are increasingly being emphasised due to their wide range of biodiversity, year-round availability, and high production capability. All photosynthetic species, as well as some photosynthetic and non-photosynthetic bacteria, fungus, yeast, and algae, having carotenoids pigments. Carotenoids are produced by nonphotosynthetic bacteria, and they protect cells from stress by modifying membrane fluidity. Because of their unicellular form, relatively fast growth rate, and ease of handling and processing, bacteria are more suitable for large-scale carotenoid production than algae and fungus [23].
Because carotenoids cannot be produced in the human body, they must be obtained through food and supplementation [39]. Carotenoids (fat-soluble plant pigments that produce much of the colour in nature) are polyisoprenic compounds, meaning they are made up of isoprene units (eight units, forty carbon atoms) [16]. Carotenoid are C40 compounds that can be used as a source of pigment as well as a medicinal molecule that can provide vitamin A, antioxidants, and possibly tumor-inhibiting properties.

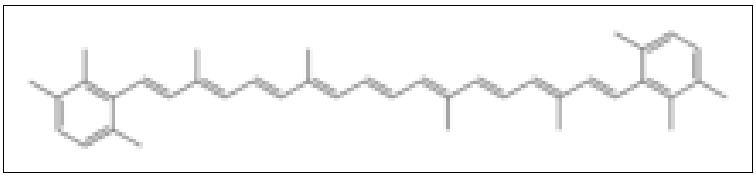

**Fig 1:** General structure of a carotenoid: polyene tail with double bonds, possible terminal rings [16]

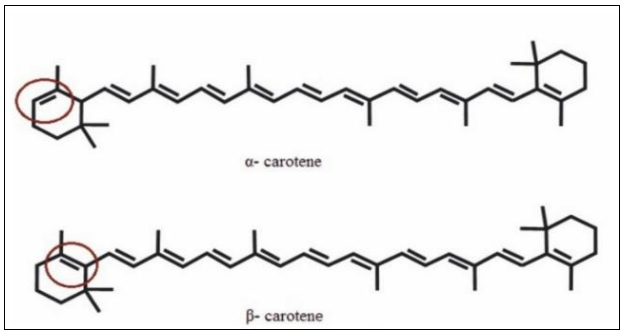


**Fig 2:** Structure of Carotenes. [16]

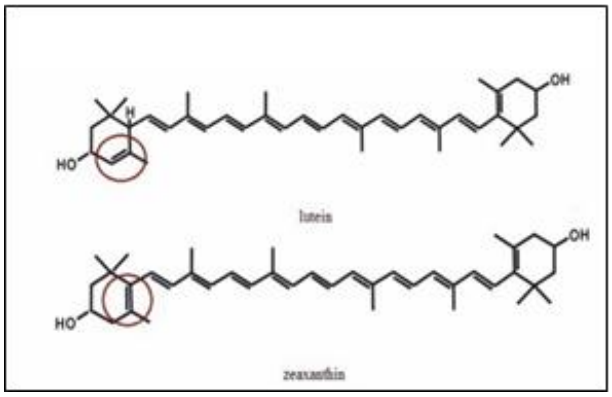


**Fig 3:** Structure of Xanthophylls. [16]

Carotenoids' structure enables biological functions such as photosynthesis, photoprotection, plant colouring, and cell signalling [16]. Isoprenoids are the source of these natural pigments, which are divided into two groups: carotenes (Carotenoids with 40 carbon atoms (formula C40H56) are known as hydrocarbon carotenoids) and oxygenated carotenoids (also known as xanthophylls). Carotenes (e.g. phytoene, lycopene, -carotene) are formed entirely of carbon and hydrogen atoms. Carotenols (e.g., zeaxanthin), carotenals (e.g., -apo-8′-carotenal), carotenones (e.g., cantaxanthin), and carotenoid acids (e.g., 4, 4' Diaponeurosporenoic acid) are all oxygen functional groups found in xanthophyll [21, 47]. Separations of compounds with very similar chemical structures, such as lutein and zeaxanthin or α-carotene and β-carotene, which differ only in the location of one double bond in the cyclohexyl ring, are the most difficult. (See Fig. 2&3) [39].

**Table 1:** Carotenoid's types [24] https://doi.org/10.2147/DMSO.S255783

| Carotenoid types | Chemical structure | Examples |
|---|---|---|
| Carotens | Hydrocarbons | α-carotene, β-carotene, γ-Carotene, δ-Carotene, lycopersene, Phytofluene, Hydrolycopene, Torulene, |
| Xanthophylls | Alcohols | Zeaxanthin, Rhodopin, Alloxanthin, Gazaniaxanthin, Lutein, loroxanthin, lycoxanthin, Saproxanthin |
| | Glycosides | Oscillaxanthin, Phleixanthophyll |
| | Ethers | Rhodovibrin, Spheroidene |
| | Epoxides | DiDinoxanthin, Citroxanthin, Luteoxanthin |
| | Aldehydes | Rhodopinal, Warmington, Torularhodinaldehyde |
| | Ketones | Astaxanthin, Canthaxanthin, Capsanthin, Capsorubin |
| | Esters of alcohols | Fucoxanthin, physalien, Astracein, Siphonein |
| | Apocarotenoids | Crocin, Crocetin, Bixin, Citranaxanthin, Sintaxanthin |

The presence of double bonds in carotenoids' molecules causes cis–trans isomerism, which determines the existence of several geometric isomers. The trans arrangement is seen in the majority of carotenoids. The carotenoids' solubility in lipids is due to the carbon chain's eight isoprene units (also called lipochrome substances). In the blue and ultraviolet regions of the spectrum, all carotenoids absorb light. Carotenoids are hydrophobic compounds that are only weakly soluble in water, if at all. Except in circumstances when they are present in association with proteins in an aqueous medium, they are constrained by hydrophobic parts of the cell and inside the membranes. Polar functional groups change how molecules interact with one another. The isoprene chain in carotenoids has two ionone cycles at the terminations, one of which may have aα–ionone structure and the other of which is normally a β–ionone ring. The carotens that act as provitamin A require the β–ionone ring. The odour of α–ionone in diluted solution is similar to that of violets [16].

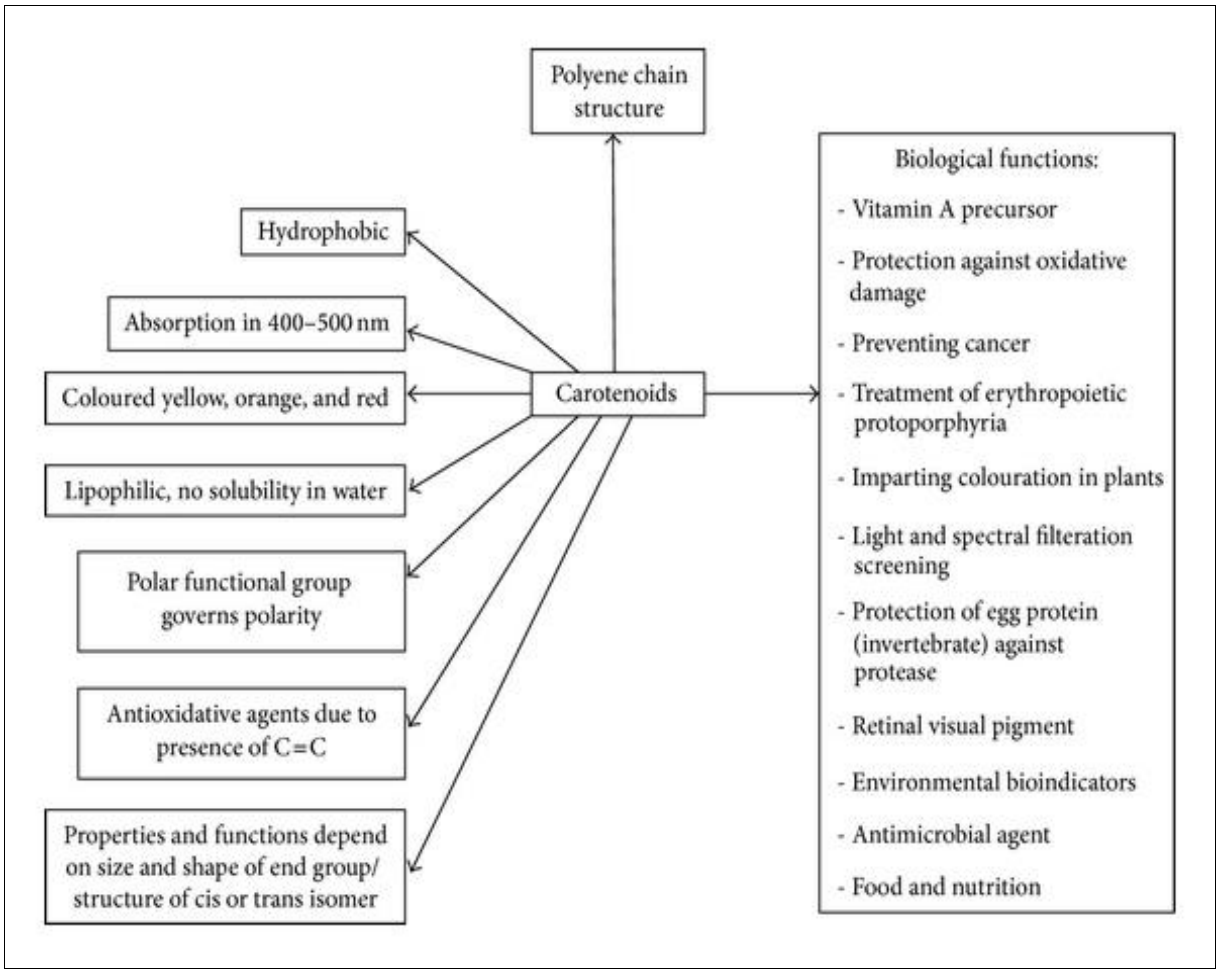


**Fig 4:** Carotenoid's properties and biological functions [38] https://www.hindawi.com/journals/ab/2014/837891/

The insertion of environmentally free radicals and delocalization of charges (positive or negative) along the chains are made possible by the double bonds in these polyenes. Because of their antioxidant properties, carotenoids are extremely sensitive to light, heat, oxygen, acidic and basic environments [14].

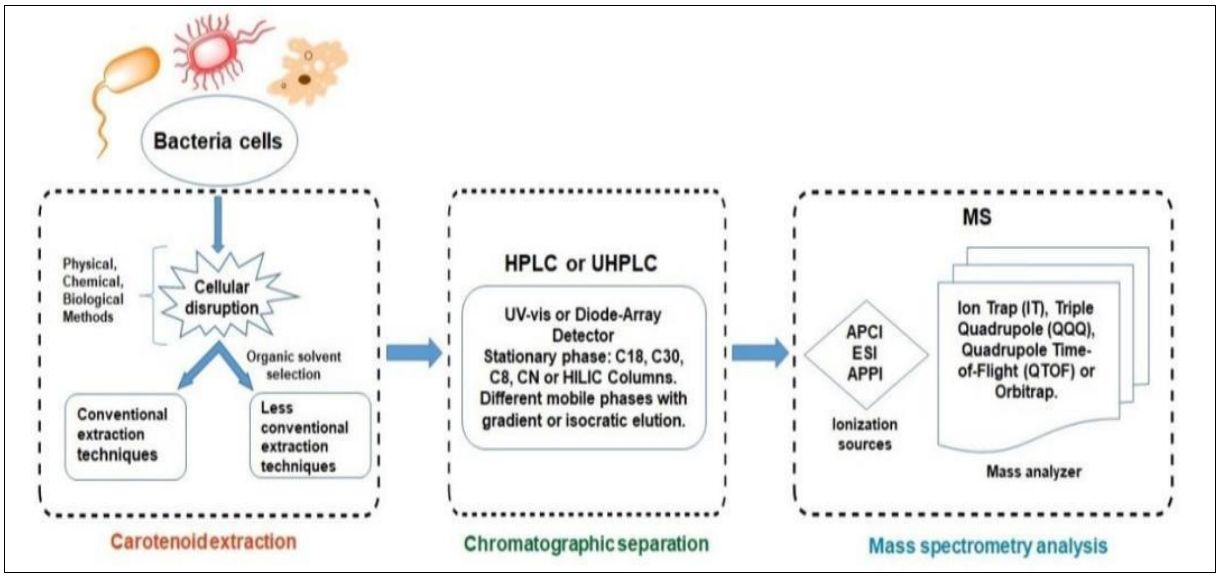


**Fig 5:** Systematic representation of the steps used for the characterization of bacterial carotenoids. https://pubmed.ncbi.nlm.nih.gov/34915787/

**Biosynthesis Pathway of Carotenoids**

Carotenoids are produced as secondary metabolites by plants and bacteria, but they aren't involved in basic survival functions like growth, development, or reproduction. They're linked to light absorption control (photooxidation) and other stress-related activities. Photosynthesis relies on carotenoids to absorb light energy

and protect chlorophyll molecules and other active biosubstances (cytochromes, peroxidases, catalase, flavonoid pigments, vitamin B12, vitamin E, and vitamin K) from autophotolysis. Carotenoids may defend against excessive radiation damage by directly activating the chlorophyll triplet (3Chl) or singlet oxygen (1O2) molecules produced in photodynamic activities. Some bacteria can also synthesise C30 carotenoids, as well as C45, C50, and C60 chain lengths to a lesser extent. The beginning point for the creation of carotenoids derived from bacteria is the C5 isoprenoid isopentenyl pyrophosphate (IPP), which isomerizes into dimethylallyl pyrophosphate (DMAPP) in the mevalonate (MVA) pathway (Figure 2). C10-geranyl diphosphate (GPP) is formed when one IPP molecule and one DMAPP molecule establish a condensation reaction. Depending on the bacterium, additional units of C5 can be added via the 2-C-methyl-D-erythritol-4-phosphate (MEP) pathway [22, 44]. MAV and MEP are both created during bacterial glycolysis. Acetyl CoA makes up the first species, while pyruvate makes up the second. Chain elongation is catalysed by prenyl transferase during a sequence of cyclical events (CrtE). When one or two IPP molecules combine with GPP, C15-farnesyl pyrophosphate (FPP) and C20-geranylgeranyl diphosphate (GGPP) are formed. As a result, carotenoids formed by the C30 and C40 chains go in separate directions. The first group is made by combining two FPP units, whereas the second is made by combining two GGDP units [21, 26]. The C30 carotenoid group produces a colourless carotenoid precursor known as 4, 4'- diaphophytoene, while the C40 group produces 15-cis-phytoene as the initial result. This colourless product is catalysed by the CrtM and CrtB enzymes, respectively. In both pathways, the desaturases CrtN (C30) and CrtI (C30) perform consecutive desaturation activities (C40). Furthermore, Figure 6 shows that the production pathways for C30 and C40 carotenoids have similar first steps. Carotenoids in the C30 group undergo early oxidation, yielding aldehyde, ketone, and/or carboxyl groups, whereas carotenoids in the C40 group undergo later dehydrogenation and cyclization processes. Stereoisomers (cis/trans) and optical isomers (R/S) are produced by various addition, elimination, substitution, and rearrangement reactions [21]. The first coloured carotene is lycopene, which is made up of four successive desaturations in the phytoene molecule. Lycopene cyclization results in the formation of α-carotene and β-carotene. The enzyme lycopene β-cyclase is involved in the production of β-carotene, while a hydroxylation reaction in one of the β-crotene rings produces a β-cryptoxanthin molecule (Figure 6) [48]. In mammals, these three pro-vitamin A carotenoids are required for retinol production.

Two molecules of Vitamin A, a retinoid, are produced from one molecule of β-carotene. Vision, cellular communication, immunological function, and human reproduction all require this vitamin [4, 13]. Because the human body has a limited absorption-conversion capacity for vitamin A, consuming food rings s containing this carotenoid boosts its bioavailability in the digestive system. Xanthophylls, such as lutein, are formed by hydroxylation of α-carotene and have been identified as macular pigments in the human retina [8]. Furthermore, it has been suggested that lutein may assist in the filtering of the light that is responsible for damaging the eyes due to its antioxidant properties [4, 11, 16]. β-carotene and carotene-hydroxylase ketolase catalyse certain zeaxanthin production pathways. Two cetogroups are introduced at positions 4 and 4' of the β-carotene by the β-carotene-ketolase, resulting in cantaxanthine. To make lutein and zeaxanthin, two hydroxyl groups are added to the 3 and 3' of canthaxanthin. The antioxidant activity of zeaxanthin is higher than that of tocopherol, ascorbic acid, β-carotene, and lycopene, making it one of the most beneficial antioxidants. Zeaxanthin is found in lipid globules in the cytoplasm, where it absorbs light in the blue region and oxidises to protect the photosynthetic system. Low density and extremely low density lipoproteins transport nonpolar carotenoids in plasma (β-carotene, α-carotene, lycopene), while high density lipoproteins transport polar carotenoids. Zeaxanthin's mobility guarantees its capacity to penetrate many body structures and mediates the overall favourable effect on a variety of structures and functions.

**Carotenoids producers- Isolation and Screening**

The samples are enriched in nutrient broth and incubated at a preferred temperature for a specific time period. Isolation is accomplished by serially diluting the enriched culture with physiological saline (0.85 percent NaCl) or distilled water and spreading the nutrient agar with 0.1 mL of diluent. Incubated Plates having separated colonies with pigmentation are chosen.

**Primary screening**

The ability to produce yellow, orange, or red pigment, which is likely non-diffusible among other pigment producers and non-pigmented cultures on plates, is used as the major screening criteria for isolates. The isolates with yellow, orange, and red pigmentation were sorted and transferred to nutrient agar slants, keep at 4 degrees Celsius for further research.

**Secondary screening**

Secondary screening is performed on the basis of qualitative tolerance to pH and salt (NaCl percent). All bacterial isolates that have been predominantly screened are streaked on nutrient agar with pH ranging from 5 to 12 and nutrient agar with NaCl (1-6%) separately. At a suitable temperature, the plates are incubated. Tolerant cultures are isolates that grow on nutrient agar with a wide range of pH and are streaked on nutrient agar with increased NaCl, i.e. 1-6 percent. Cultures that show good pigmentation, pH and NaCl tolerance, and vivid pigmentation are screened secondarily and chosen for tertiary screening

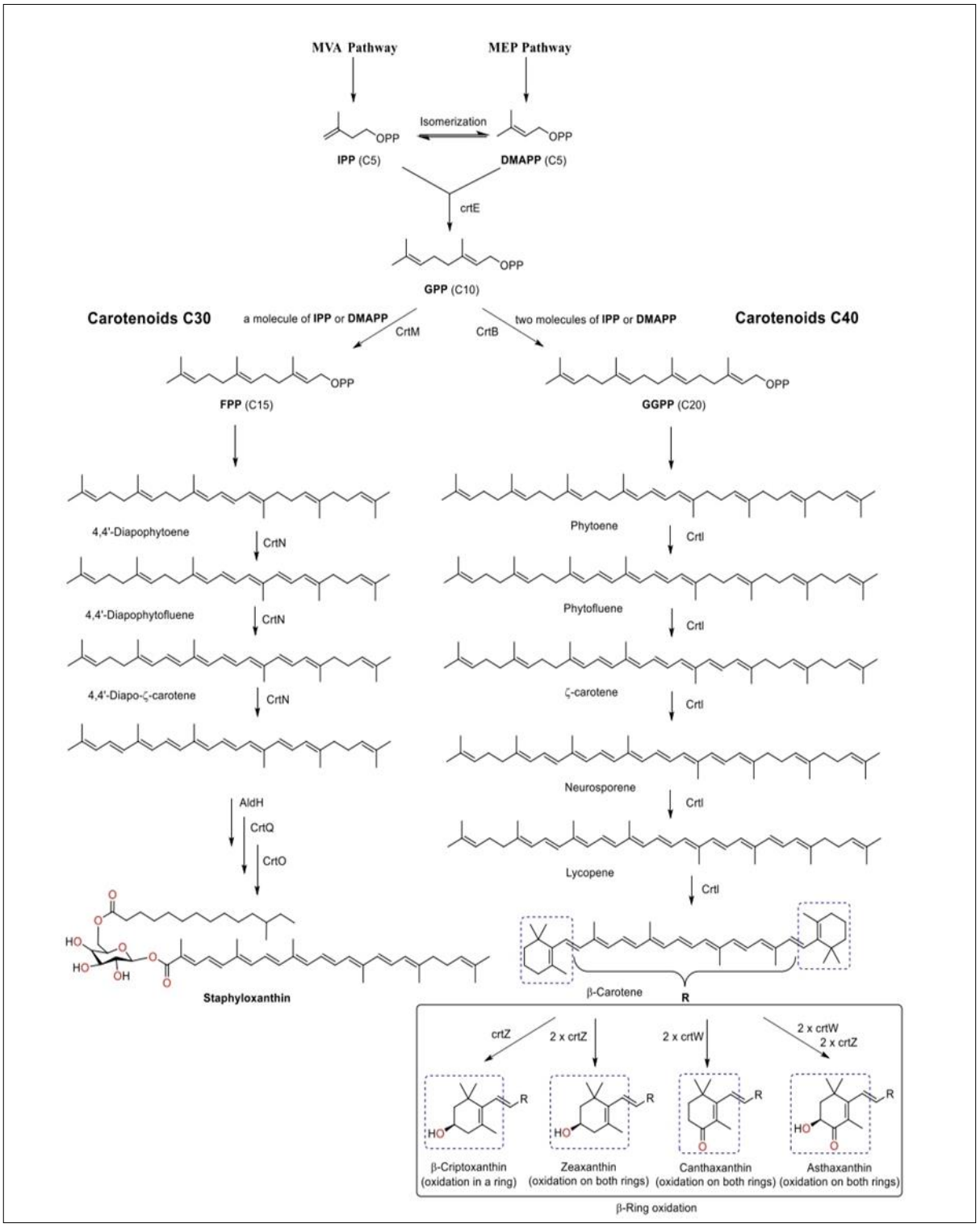


**Fig 6:** Diagrammatic representation of bacterial carotenoids biosynthesis with C30 and C40 carbon units. [Doi:10.1016/j.bbalip.2020.158613.] (48)

**Tertiary screening**

Tertiary screening is based on the isolate's ability to create pigment in both qualitative and quantitative terms. In tertiary screening, actively developing (48 h) cultures of secondarily screened isolates are inoculated individually into nutrient broth and incubated at appropriate temperature for 48 hours under shaking conditions (100 rpm). The culture with robust development and brilliant pigmentation is treated to pigment extraction. To separate cells, the enriched cultures are centrifuged separately at 8000 rpm for 15 min at four degrees celsius based on bacteria. Separated cell pellets are centrifuged twice with sterile distilled water for 10 minutes at 8000 rpm. Separate cell pellets are suspended in 5 mL methanol overnight and then centrifuged at 8000 rpm for 10 minutes at 4 °C [11] During the extraction of carotenoids, biomolecules like proteins, carbohydrates, and fatty acids can cause a slew of issues. As a result, prior to the extraction process, cell disruption is critical. Cell disruption, on the other hand, results in an increase in temperature, which can cause degradation of thermolabile substances

such carotenoids. Cell disruption is achieved through physical, chemical, and/or biological means after pelleting. Spectrophotometric analysis of the supernatants is done separately after centrifugation. The degree of pigmentation is also calculated during tertiary screening. For quaternary screening, isolates with higher levels of pigmentation and distinctive carotenoids absorbance are chosen.

**Quaternary screening**

Quaternary screening is based on antioxidant activity, hence it is carried out by assessing the antioxidant potential of pigment extracts from tertiary screened isolates. The antioxidant potential is determined by assessing free radical scavenging activity using 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) method. DPPH is a stable compound. It is a free radical that is dark violet in colour in solution and is decolorized by powerful reducing agents and antioxidants. The pigment extract's high rate of decolorization suggests that it has stronger antioxidant action. The bacterial isolate extract with the highest free radical scavenging activity (% RSA-Radical Scavenging Activity) is considered a possible antioxidant function, and the isolate as a potential carotenoid generator. The antibacterial activity of the pigment in the solvent is then tested against human infections, as well as its control. The pigment's antimicrobial activity is determined using the well diffusion method. After 24 hours of incubation at 37 °C, pigment's antimicrobial activity against various human infections is demonstrated.

**Extraction Methods**

There is no universally acknowledged method for extracting carotenoids from bacteria because yields vary depending on a variety of parameters (such as carotenoid's polarity, moisture level, and cell wall type). Non-polar solvents, such as hexane, are ideal for extracting carotenes, whereas polar solvents, such as acetone or ethanol, are better for extracting xanthophylls. Furthermore, whether the extraction is accomplished by a liquid-liquid or solid-liquid procedure is determined by the moisture content of the bacterial cells. Another significant thing to consider during the extraction method is the cell wall found in bacteria. Gram-negative bacteria have a thin peptidoglycan layer between their inner and outer membranes, which is adorned with lypopolysacharide (LPS), making them more permeable to organic solvents. Gram-positive bacteria have an inner membrane that is protected by a thick cell wall made mostly of a peptidoglycan network, which makes them more resistant to organic solvents. In both cases, bacterial cell rupture is easier than that of microalgae and yeast, which have more complicated and stiff cell walls. The extraction of these metabolites follows a similar pattern, beginning with cellular destruction or cell-membrane permeabilization, followed by carotenoid solubilization in an organic solvent, and subsequent elimination of components [6].

**Cellular disruption**

During the extraction of carotenoids, biomolecules such as proteins, carbohydrates, and fatty acids can cause a variety of issues. As a result, cell disruption is an important stage in the extraction process. Cell disruption, on the other hand, causes an increase in temperature, which can cause degradation of thermolabile substances such carotenoids. Physical, chemical, and/or biological approaches are used to destroy cells after pelleting. Physical methods include the use of a mortar and pestle, vortex mixing with or without glass beads, orbital shaking, and incubation (which may entail temperature rise). These methods make it easier for organic solvents to enter bacteria, resulting in the solubilization of carotenoids [3]. Biological approaches utilise enzymes to disturb cells, whereas chemical methods use acids, bases, or surfactants. Extraction procedures encompass both traditional and non-conventional processes, which can happen at the same time as cellular disruption. Traditional procedures include atmospheric liquid extraction with maceration or Soxhlet extraction. However, less common methods such as microwaves (microwave-assisted extraction: MAE), ultrasounds (ultrasound-assisted extraction: UAE), supercritical fluid extraction (SFE), pressurised liquid extraction (PLE), and gas expanded liquids (GXLs) can be used to disrupt and extract carotenoids simultaneously.

**Organic solvent selection**

Several solvents, including hexane, acetone, dimethyl sulfoxide (DMSO), dichloromethane (DCM), ethyl acetate (AcOEt), methanol (MeOH), or ethanol (EtOH), can be used to extract bacterial carotenoids during or after cellular disruption, either separately or in combination [6]. As previously stated, the type of carotenoids present in the bacteria will determine the best organic solvent for each extraction (xanthophyll or carotenes). Some bacteria produce solely carotenoids such as -carotene, lycopene, and others. As a result, selecting an extraction solvent may be less difficult. However, bacteria that produce both carotenes and xanthophylls, as well as their esters, make the solvent selection process more difficult. As a result, a wide range of solvent combinations have been employed to extract carotenoids from various types of samples [47], making it difficult to recommend a standardised solvent or solvent mixture for each extraction. Because of their proclivity for forming ester, bacterial carotenoids are notoriously difficult to analyse. As a result, prior to examination, the carotenoid esters must typically be hydrolyzed.

**Saponification**

Carotenes, carotenols, carotenoid esters, carotenoid glycosides, and carotenoids are all found in bacterial carotenoid extracts [47]. Because of the higher hydrophobicity of the molecules, which prevents breakdown by oxidation or isomerization owing to high temperatures and light exposure, carotenoid esters are more stable than

carotenoids. Furthermore, as the degree of esterification of xanthophyll increases, so does the stability of these molecules. Lutein, for example, is less stable than its monoester, which is in turn less stable than its diester. Despite their durability, xanthophyll esters are difficult to identify, and a saponification process is necessary during sample extraction to enable for carotenoids analysis. As a result, saponification is an example of a chemical process for cellular disruption and carotenoids extraction. For example, Bacillus spp. cells were saponified by sonicating a freeze-dried sample in a solution of NaOH (10 percent w/v) for 15 to 20 minutes at room temperature. After that, the NaOH was removed using centrifugation, and the saponified extract's carotenoids were extracted using MeOH: CHCl3 (1:2) and a Tris-buffer saline.

**Extractive procedures used in the past**

Various studies have reported on traditional carotenoids extraction procedures. In the liquid-liquid extraction process, S. aureus was incubated at 40 or 4 °C for 20 to 30 minutes, using EtOH, MeOH, or acetone. [37] A secondary liquid-liquid extraction using an AcOEt:NaCl aqueous solution also allows for the salting-out effect, which cleans the extract of extremely polar chemicals. In both cases, many related STX biosynthesis pathway intermediaries or novel carotenoid compounds in mutant strains were discovered [37]. Recently, a cold extraction approach with MeOH (-20°C) combined with vortex and glass beads to study the Bacillus megaterium carotenoids 4,4′-diapophytoene, 4,4′-diapophytofluene, 4,4′-diaponeurosporene, 4,4′-diapolycopene, 4,4′-diapolycopene, 4,4′-diapolycopene, 4, This approach avoids carotenoids from being thermally degraded throughout the incubation period. Furthermore, 4, 4′- diaponeurosporene is a carotenoid that may be used to treat inflammatory conditions [30]. This carotene was isolated specifically from E. Coli [33]. Extraction followed by shaking at 50 °C allowed the isolation and characterization of astaxanthin, adonixanthin, hydroxy-astaxanthin, and dihydroxy-astaxanthin from marine Gram-negative bacteria Brevundimonas sp. [8, 9]. Astaxanthin is known for its high antioxidant capacity, which is ten times that of beta-carotene and vitamin E. (500 times). This health benefit is commonly used in dermatology [22].

**Analytical Methods**

**Chromatographic separation**

For separation of carotenoids (and certain isomers), HPLC can employ cyano (CN), octylsilane (C8), octadecylsilane (C18), or other more specialised columns as C30 [60]. In the study of bacterial carotenoids, several papers have employed C18 stationary phase columns. The separation of cis/trans isomers of carotenes with or more carbon atoms is difficult with C18 columns due to low resolution. Similar trends are seen using C18 and C30 columns when xanthophylls are examined [6, 44]. The C18 column is more helpful and practical for basic carotenoid analysis than the C30 column because it has superior stability at high pH, is faster, and is less expensive.

Carotenoids were eluted in C18 columns in the order of their polarity, with hydroxycarotenoids being the first to be eluted, followed by carotenes, keto-, and epoxy-carotenoids, which had longer retention durations. Similarly, the retention periods in C30 phases are related to the length of the molecule and the presence/number of polyenes. The C30 stationary phase works well for separating regioisomers from substances like zeaxanthin and lutein, as well as their epoxides.

According to other sources, columns may be used to separate carotenoids in a variety of ways. As a result, C8 columns have a shorter retention period than C18 and C30 columns, depending on the complexity of the carotenoid combination [13]. In general, C8 columns require roughly 20 minutes less than C18 and C30 columns, depending on the complexity of the carotenoid mixture. Carotene isomers (-carotene, -carotene, and lycopene) as well as xanthophyll isomers (canthaxanthin, cryptoxanthin, and -cryptoxanthin) were among the carotenoids identified. In conclusion, the (gold) standard approach for carotenoid separation is HPLC. However, because of the complexity of carotenoid chromatograms owing to the large range of isomers and structurally related molecules, many studies do not provide the quantification of all carotenoids present in a sample. The use of a particular standard is required for carotenoid measurement.

**Mass spectrometry analysis**

The molecular mass and fragmentation patterns recorded in tandem mass spectrometry (MS/MS) mode give structural information about the target chemical [6]. Due to coelution, MS selectivity enables for the identification of chemicals that cannot be separated by HPLC. Ionization procedures including electron ionisation (EI), negative ion chemical ionisation (CI), and rapid atom bombardment (FAB) were used to identify carotenoids. The thermolability of carotenoids is the fundamental problem of EI and CI techniques. For carotenoid analysis, two primary ionisation sources are now used: electrospray ionisation (ESI) and atmospheric pressure chemical ionisation (APCI). ESI may be used to analyse ionic and polar substances such xanthophylls [44].

In APCI, mobile phases may be used without buffers and at high flow rates (more than 800 L/min), but in ESI, effective ionisation requires the use of buffers or organic modifiers and mobile phase flow rates of less than or equal to 500 L/min. The most potent approach for carotenoids analysis is APCI, according to a comparison of the three sources of ionisation at atmospheric pressure (ESI, APCI, and APPI). The effect of four dopants in APPI was investigated in this study, which looked at 11 xanthophylls and four carotenes.

The mass analyzer receives these metabolites after they have been separated and ionised. The ion trap (IT), triple quadrupole (QqQ), quadrupole time of flight (QTOF), and Orbitrap are the most common types of analysers

used to identify carotenoids from bacteria. It enables the structural elucidation of unknown carotenoids using low-resolution tandem MS (MS2, MS3, MS4, etc.). Because of its great sensitivity when operating in Multiple Reaction Monitoring (MRM) mode, QqQ is mostly used for focused and quantitative analysis. The precise mass of both molecule ions and fragments acquired by tandem-MS in high-resolution is used by QTOF and Orbitrap analysers to offer vital structural information. Carotenoid extracts from bacteria frequently include large levels of lipids (mostly triglycerides, TAGs) seen in cell membranes.

**Nuclear magnetic resonance**

NMR is a physical technique for determining the presence of carotenoids. The phenomenon of nuclear magnetic resonance is based on the fact that some atoms' nuclei have the ability to rotate around their own axis. The following sources provide basic information on the chemical structure shown by NMR spectra:

- Signal number, which indicates how many different sorts of protons are present in a carotenoids molecule.
- The type of the protons is determined by the signal's location in the spectrum (alkane, alkene, aromatic, benzilic, hydroxilic, aldehidic etc.)
- Signal intensity, which determines how many protons are used to make each signal.
- Signal breaking in doublets, triplets, and multiplets, which provide information on the number of protons on the carbon atom next to the one carrying the broken signal proton.

The explanation of some structural features, such as those connected to the biopolymer structure, which is the foundation of vital phenomena in plants, became feasible with the use of this technology. Because the energies involved in the commencement of the NMR phenomena are so low, the examined carotenoids are guaranteed to be unaffected by the energy's influence on the carienoidic compounds under research. The value added by dynamic NMR, a method capable of surprising structural aspects characteristic of carotenoid molecules with rigid structures by cooling to low temperatures, would be unimaginable without conformational analysis, which deals with the study of stability and reactivity of conformational isomers. Nuclear magnetic resonance, protonic resonance, or the one involving 13C, 19F, or 31P atoms are used in research in the disciplines of hydrogen bonds, tautomery, proton exchange phenomena, stereochemistry, reaction processes, and quantitative organic chemistry.

**Thin layer chromatography**

The separation and purification of carotenoids is accomplished using TLC. In comparison to column chromatography, this approach purifies to a higher degree. The stationary phase, as well as the mobile phase and plate elution method, are selected according to the carotenoid's nature. There are a few things to keep in mind while doing TLC on carotenoids:

- Quick sample application
- Sample application in poor light
- Chromatogram development right away
- Dark chromatogram development
- Using a nitrogen-rich environment

Because exposing carotenoids to light, especially direct sun light or UV light, causes cis–trans photoisomerization, which can lead to their photodestruction, biological materials containing carotenoids or their solutions must be shielded from the effects of light. Many carotenoids, particularly xanthophylls, are thermolabile, which means that they can only be heated when absolutely essential. Carotenoids must be separated in the dark and at room temperature (up to –20°C) [17]. Warm saponification necessitates the use of solvents with lower boiling temperatures (30–60°C) in order to safeguard the solutions. Many carotenoids can be oxidised in the presence of oxygen (air) or peroxides, since they are oxygen-sensitive in the adsorbed state (in chromatograms, on columns, and thin layers) [36].

Separating carotenoids on a layer of silicagel and developing with petroleum ether (5 percent ethylic ether) or on magnesium oxide and developing with benzene: petroleum ether (9:1, v/v) are the most used TLC methods. For xanthophylls, cromatoplates with zinc carbonate as the stationary system, created with hexane–alcohol, provide excellent resolutions, whilst calcium carbonate, magnesium oxide, and calcium hydroxide are useful in separating both carotenoid hydrocarbons and xanthophylls.

Weak eluents such as ethylic ether and benzene, as well as mixtures of solvents such as acetone–ethylic ether, ethylic alcohol–ethylic ether, ethyl acetate–benzene, and others, are utilised in the TLC separation of carotenoids. Tertiary alcohols are solvents that provide excellent resolution due to their characteristics. Because it contains amounts of chlorhidric acid, which might trigger carotenoid conversions, chloroform is usually avoided. Acetic acid is only utilised when the adsorbent has a high concentration of carotenoids.

**FTIR (Fourier Transform Infrared) Spectrophotometric**

FTIR spectroscopy hasn't been used much to identify the structures of carotenoids. The nature of functional groups contained in a molecule can be determined via FTIR. The FTIR technique is used to get the infrared spectrum of absorption, emission, and photo-conductivity of solids, liquids, and gases. It has a wide range of applications in the elucidation of chemically manufactured or naturally occurring structures. It's a way for

transforming a time domain spectrum to a frequency domain spectrum using mathematics. FTIR can be used to assess the presence of organic and inorganic components in a sample. Based on the infrared absorption frequency range 600-4000 cm-1, the exact chemical groups contained in the sample will be determined utilising spectrum data in automated spectroscopy software. Dehydrated and hydrated with trace amounts of water pigment solutions were prepared in CHCl3 and CCl4. It has been observed that either strong-coupling (e.g. zeaxanthin in hydrated CHCl3) or weak-coupling (e.g. beta-carotene in hydrated CHCl3) aggregated structures form. H2O molecules can be bound to the aggregated structures formed by zeaxanthin in the form of molecular bridges, according to FTIR analysis and molecular modelling, primarily between the terminal hydroxyl groups of adjacent molecules (stabilised by strong hydrogen bonds), but also between the polyene chains (via -type weak hydrogen bonds). [2, 34]

**Applications**

Synthetic carotenoids account for the majority of commercial carotenoids. However, in recent years, customers' interest in goods containing natural carotenoids has grown [18]. Because of its low toxicity, antioxidant activity, stability in acid and neutral pH, high colouring capacity, and ability to combine with other colours, carotenoids are utilised commercially for medicinal, cosmetic, nutraceutical, and food (human and animal) uses, among others. Bacterial carotenoids are important because they have characteristics that can be used in industrial production, such as a large number of colours, no reliance on climatic conditions (seasons), short cultivation times, low extraction complexity, and no toxicity, and they are accepted in a variety of applications.

**Pharmaceutical**

Carotenoids from bacteria, such as esterified astaxanthin, have a better antioxidant potential than manufactured carotenoids (free astaxanthin). Free sarcinaxanthin, mono- and diglucosilated (C50 skeletons), saproxanthin, isorenieratene, and 3, 30-dihydroxyisorenieratene (C40 skeletons) all have 500 times the antioxidant capacity of b-carotene. Adonixanthin is a xanthophyll generated by the bacteria *Paracoccus sp.*, *Erythrobacter sp.*, and *Agrobacterium sp*. that protects the brain against hemorrhagic brain injury due to its stronger antioxidant activity than astaxanthin [32]. Canthaxanthin is employed in the manufacturing of medicinal tablets because of its brilliant red hue [31]. Fucoxanthin coupled with fish oil was shown to significantly reduce weight gain in rats, with data showing that xanthophyll lowers blood glucose and plasma insulin [45]. These characteristics make this class of carotenoids appealing for medicinal uses.

**Cosmetic**

The skin is the human body's biggest organ and the most susceptible to environmental factors such as sun radiation, necessitating the usage of skin photoprotective chemicals on a regular basis. The forehead and palms of the hands contain the highest quantities of carotenoids in human skin. Premature ageing, wrinkles, pigmentation, and dryness are all linked to exposure to UV light. Lycopene has the greatest antioxidant potential for quenching ROS, while b-carotene has shown promising results in UV and infrared light photo-protection in studies. These carotenoid precursors have the potential to replace hydroquinone as a skin depigmenting agent, which has been linked to the development of cancers and inflammation [41]. The dermo-cosmetic sector is interested in bacteria's adaptability in generating new carotenoids as well as classic carotenoids, which may have similar or higher photoprotective, antioxidant, and anticarcinogenic effects. Because of the problem of low stability, the carotenoid potential for use in topical components such as sunscreens remains a barrier to overcome. However, because of its low polarity, it may be easily dissolved in skin emulsions.

**Nutraceutical**

Human health advantages have been linked to eating foods or meals fortified with high concentrations of carotenoids, such as cancer prevention, cardiovascular disease prevention, and eye health. The benefits of drinking b-cryptoxanthin-fortified juice on a regular basis have been linked to bone growth and suppression of bone resorption, avoiding osteoporosis in women. As a result, bacteria such as *Pseudomonas sp*., *Zobellia laminarie 465, or B. linens* [29], might be exploited in the industrial manufacture of this carotenoid, enhancing the nutritional value of other foods and perhaps affecting human health. ROS, on the other hand, are created primarily in the retina of the eye as a result of the interaction of oxygen with photons of light, which is required for human vision. Because of this, macular carotenoids (lutein, zeaxanthin, and meso-zeaxanthin) protect the eye from oxidative damage by absorbing damaging light and quenching (chemical and physical) reactive oxygen species (ROS) [7]. The World Health Organization and the United Nations have set a daily zeaxanthin intake of 0 to 2 milligrammes per kilogramme of body weight as sufficient. Carotenoids may lower human carcinogenesis, according to preclinical, epidemiological, and toxicological investigations. Prostate cancer risk is reduced by 15% when lycopene, beta-carotene, beta-cryptoxanthin, lutein, and zeaxanthin are consumed. According to a meta-analysis of carotenoids, lycopene and b-carotene are linked to a 30% reduction in lung cancer development [1].

**Food**

The food business has concentrated on creating goods with vibrant natural hues. Bacterial carotenoids are used in the food business to provide the distinctive colour of Limburger and Port-du-Salut cheeses [29]. Astaxanthin is

another carotenoid with a wide range of applications. When this carotenoid is produced from *Paracoccus carotinifaciens*, it is employed in salmon and rainbow trout pigmentation; for its antioxidant and photoprotective properties, astaxanthin from *Mycobacterium lacticola* is used in fish feed [40]. Canthaxanthin is also used to colour foods like margarine, butter, and confectionary. The colouring of salmon meat is increased by canthaxanthin from *Haloferax alexandrines*, for example [5]. *Flavobacterium sp*. zeaxanthin is also utilised as a poultry feed ingredient to darken the colour of egg yolks and chicken skin. *Dietzia sp., Bradyrhizobium sp.,* and *Gordonia alkanivorans* are among the bacteria that may produce carotenoids in commercial quantities.

**Textile industry**

Synthetic dyes are widely utilised in the textile sector, with extremely polluting manufacturing methods. Textile colours derived from bacteria may thus be a viable solution to this environmental issue. *Serratia marcescens* and *Janthinobacterium lividum* generate violacein, a pigment that has been demonstrated to be an excellent dye for a variety of materials [19, 35]. The amount of pigment taken from the bacteria and the length of time the cloth was exposed to them will determine the desired colour intensity. A yellow pigment derived from Thermomyces has also been used to effectively colour silk. A red pigment produced by *Talaromyces verruculosus* can be used to colour cotton. Furthermore, *Vibrio spp*. is a Gram-negative bacteria that produces prodigiosin, a brilliant red pigment that has been used to colour wool, nylon, and silk.

**Conclusion**

The growing popularity of natural carotenoids has prompted researchers to look for additional sources of these chemicals. Bacteria stand out as a great alternative because of the wide range of carotenoids that different species can produce. Bacteria can also be thought of as a renewable supply of carotenoids. In this work, the various methods for extracting bacterial carotenoids that are often employed are discussed. Many of these procedures use substantial volumes of organic solvents such as hexane, MeOH, and CHCl3 in numerous phases for extract extraction, concentration, and resuspension. Other microorganisms, such as algae and fungus, have been found to benefit from their use [27, 28, 29]. Furthermore, bacterial carotenoids are frequently discovered at low amounts, making identification difficult. As a result, using sensitive and high-throughput analytical methods for qualitative and quantitative characterization of bacterial carotenoids, such as HPLC-MS, becomes a must. Although C18 and C30 columns are the most often utilised, chromatographic separations have been produced with a variety of stationary phases. Spectral approaches, which have the benefits of wide applicability, high sensitivity, flawless result repeatability, sample integrity preservation, and determination quickness, can be used to identify carotenoids. Visible spectroscopy, IR NMR, and mass spectrometry are some of the spectral approaches used to identify carotenoids. Finally, for years to come, the quest for novel (or modified) microorganisms as natural sources of carotenoids will be a major topic of research.

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**Competing Interests**

The authors declare that there is no conflict of interests.

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