METHOD OF PRODUCING PURIFIED CAROTENOID COMPOUNDS

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See application file for complete search history.

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ABSTRACT
A method of producing a carotenoid in solid form includes culturing a strain of Chlorophyta algae cells in a minimal inorganic medium and separating the algae comprising a solid form of carotenoid. In one embodiment of the invention, the strain of Chlorophyta algae cells includes a strain of Chlamydomonas algae cells.

12 Claims, 3 Drawing Sheets
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METHOD OF PRODUCING PURIFIED CAROTENOID COMPOUNDS

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STATEMENT OF GOVERNMENT INTEREST

Financial Assistance for this project was provided by the U.S. Government through the National Science Foundation under Grant No. DGE-9553456 and through the National Aeronautics and Space Administration (NASA) under Grant No. NAG-W547. The United States Government may own certain rights to this invention.

FIELD OF INVENTION

The invention relates to methods for preparing carotenoids, and in particular, to the production of carotenoids using various strains of algae.

BACKGROUND OF THE INVENTION

It is now generally recognized in medical circles that antioxidants play an important role in nutrition and in the prevention of certain diseases, such as macular degeneration of the eye. Accordingly, it is now routinely recommended that individuals consume a “recommended daily allowance” of antioxidants to maintain good health. Among the important antioxidants are the carotenoids, of which the most commonly known is beta-carotene. Other common carotenoids are the oxygenated form of beta-carotene, known as lutein, and another oxygenated carotenoid found in relatively low amounts in green plants and algae, zeaxanthin.

While the carotenoids are known to have important health effects, these compounds are currently manufactured by expensive processes requiring extraction of the compounds from a natural source, and subsequent purification. As a result, for example, lutein, which is an abundant plant and algal carotenoid, is currently sold for about $50,000 per gram. Zeaxanthin, produced from certain cyanobacteria, has a market price of approximately $100,000 per gram. Zeaxanthin, produced from certain cyanobacteria, has a market price of approximately $100,000 per gram. These prices do not reflect scarcity, but rather the high cost of manufacture. There is a great need for an inexpensive source of carotenoids to promote human health, such as the treatment of hives and other dermatoses, and prevent certain types of eye disorders, such as macular degeneration.

SUMMARY OF THE INVENTION

This summary of invention section is intended to introduce the reader to aspects of the invention and is not a complete description of the invention. Particular aspects of the invention are pointed out in other sections hereinbelow, and the invention is set forth in the appended claims which alone demarcate its scope.

The invention provides a unique method of preparing a carotenoid, zeaxanthin, through use of an alga. In particular, in one embodiment of the invention a Chlorophyta alga is cultured in a medium in the presence of light, and then harvested. The harvested cells are separated, for example, by centrifugation, and contain zeaxanthin and lutein, among other components, in a solid product. The carotenoids are present in a sufficient concentration in the solid for therapeutic or prophylactic administration to humans, or may be further purified to increase carotenoid concentration.
is provided. Stress, such as nutrient deprivation, may also stimulate synthesis and transfer of selected carotenoids to the granule.

**EXAMPLE**

Strain CC-373 (ac-cu-2-21) used in this example was a gift from a stock maintained at the *Chlamydomonas* culture collection at Duke University, Durham, N.C. Cells grown in a minimal inorganic medium at 25° C. in the light for 3 to 4 days (late log to stationary phase) were harvested by centrifugation at 1000 g. The medium was formed of the following: 7.5 mM sodium acetate, 1.0 mM sodium citrate, 3.0 mM K$_2$PO$_4$, 7.0 mM KH$_2$PO$_4$, 7.5 mM NH$_4$Cl, 0.1 mM CaCl$_2$, 1.0 mM MgSO$_4$·0.01 mM FeCl$_3$, supplemented with 1 ml/l trace metals in the concentration of (mg/100 ml) H$_2$BO$_3$, 100; ZnSO$_4$·7H$_2$O, 100; MnSO$_4$·H$_2$O, 40; CoCl$_2$·6H$_2$O, 20; Na$_2$MoO$_4$·2H$_2$O, 20; CuSO$_4$·5H$_2$O. The pelletted cells were suspended in 50 mM phosphate buffer (pH 7.0) or 10 mM Tris-HCl (pH 8.0) containing 1.0% (w/v) Triton X-100, broken by sonication and centrifuged at 1000 g in buffer (50 mM phosphate, pH 7.0) and then twice with buffer. The resulting pellet contains zeaxanthin, lutein, polyphosphate, Ca$^{2+}$, Mg$^{2+}$, several proteins and starch (FIGS. 2-4). Oxygenated carotenoids can be liberated from the pellet fraction by a simple heating treatment.

Analysis of carotenoid composition of *Chlamydomonas reinhardtii* CC-373 demonstrated that the strain was deficient in the epoxidated carotenoids neoxanthin (3S,5R,6R,3'S,5'R)-5,6-epoxy-5,6-dihydroxy-6,5'-tetrahydro-β-carotene-3,3'-diol), violaxanthin ((3S,5R,6S,3'R)-5,6-epoxy-6,5'-tetrahydro-β-carotene-3,3'-diol), and antheraxanthin ((3S,5R,6S,3'R)-5,6-epoxy-5,6'-dihydroxy-β-carotene-3,3'-diol). In whole cells, the amount of zeaxanthin ((3R,3'R)-β,β-carotene-3,3'-diol) was increased to a level greater than that of lutein ((3R,3'R)-β,β-carotene-3,3'-diol). Upon analysis, only a relatively small amount of zeaxanthin was present in the membrane, which is traditionally considered its normal physiological domain. Rather, the bulk of zeaxanthin was recovered in a Triton X-100-insoluble pellet fraction (FIG. 1). The Triton X-100-insoluble fraction pelletted through 1.5 M sucrose, a characteristic of polyphosphate granules that form within cytoplasmic vacuoles. Electron micrographs suggest that the membrane of these vacuoles is derived from the chloroplast envelope. The formation of these dense cytoplasmic vacuoles is not confined to *Chlamydomonas* but is thought to be common through Chlorophyta, i.e. *Dunaliella*, etc. The apparent transfer of zeaxanthin to chloroplast envelope-derived cytosolic vacuoles allows for the simple preparation of a zeaxanthin enriched product without the use of solvents.

The foregoing description provides an enabling disclosure of the invention, which is not limited by the description, but only by the scope of the appended claims. All those other aspects of the invention, and their equivalents, that will become apparent when a person of skill in the art has read the foregoing, are within the scope of the invention and of the claims hereinbelow.

I claim:

1. A method of producing a carotenoid in a detergent-insoluble pellet purified from soluble cellular components comprising:
   a. culturing a strain of *Chlorophyta* algae cells in a minimal inorganic growth medium;
   b. separating said algae cells from said minimal inorganic growth medium to obtain harvested algae;
   c. treating said harvested algae with detergent to obtain a detergent-insoluble pellet fraction comprising a carotenoid; and

2. The method of claim 1, wherein said strain of *Chlorophyta* algae cells comprises a strain of *Chlamydomonas* cells.

3. The method of claim 1, wherein the carotenoid is selected from zeaxanthin and lutein.

4. A detergent-insoluble pellet form of carotenoid, the pellet form obtained from a process comprising:
   a. culturing a strain of *Chlorophyta* algae cells in a minimal inorganic growth medium;
   b. separating said algae cells from said minimal inorganic growth medium to obtain harvested algae;
   c. treating said harvested algae with detergent to obtain a detergent-insoluble pellet fraction comprising a carotenoid wherein liquid chromatography of said pellet fraction produces acetone extracted pigments, as shown in FIG. 1; and
   d. separating said detergent-insoluble pellet fraction from soluble cellular material.

5. The detergent-insoluble pellet form of carotenoid of claim 4, wherein said strain of *Chlorophyta* algae cells comprises a strain of *Chlamydomonas* cells.

6. The detergent-insoluble pellet form of carotenoid of claim 4, wherein the carotenoid is selected from zeaxanthin and lutein.

7. A detergent-insoluble pellet form of carotenoid, the pellet form obtained from a process comprising:
   a. culturing a strain of *Chlorophyta* algae cells in a minimal inorganic growth medium;
   b. separating said algae cells from said minimal inorganic growth medium to obtain harvested algae;
   c. treating said harvested algae with detergent to obtain a detergent-insoluble pellet fraction comprising a carotenoid wherein energy dispersive x-ray analysis of said pellet fraction, having the EDAX analysis as illustrated in FIG. 2, shows phosphate with divalent calcium and magnesium cations as a predominant inorganic component; and
   d. separating said detergent-insoluble pellet fraction from soluble cellular material.

8. The detergent-insoluble pellet form of carotenoid of claim 7, wherein said strain of *Chlorophyta* algae cells comprises a strain of *Chlamydomonas* cells.

9. The detergent-insoluble pellet form of carotenoid of claim 7, wherein the carotenoid is selected from zeaxanthin and lutein.

10. A detergent-insoluble pellet form of carotenoid, the pellet form obtained from a process comprising:
    a. culturing a strain of *Chlorophyta* algae cells in a minimal inorganic growth medium;
    b. separating said algae cells from said minimal inorganic growth medium to obtain harvested algae;
    c. treating said harvested algae with detergent to obtain a detergent-insoluble pellet fraction comprising a carotenoid wherein a sodium dodecyl (lauryl) sulfate—polycrylicamide gel electrophoresis of said pellet fraction indicates a 70 kDa protein as a major protein as illustrated in FIG. 4; and
    d. separating said detergent-insoluble pellet fraction from soluble cellular material.

11. The detergent-insoluble pellet form of carotenoid of claim 10, wherein said strain of *Chlorophyta* algae cells comprises a strain of *Chlamydomonas* cells.

12. The detergent-insoluble pellet form of carotenoid of claim 10, wherein the carotenoid is selected from zeaxanthin and lutein.