METHOD OF PRODUCING PURIFIED CAROTENOID COMPOUNDS

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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
FIG. 2

FIG. 3
METHOD OF PRODUCING PURIFIED CAROTENOID COMPOUNDS

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STATEMENT OF GOVERNMENT INTEREST

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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.
pelleted cells were suspended in 50 mM phosphate buffer
thin, lutein, polyphosphate, Ca", Mg2+, several proteins and
the granule.

Strain CC-373 (ac-ac-c-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 K2PO4, 7.0 mM KH2PO4, 7.5 mM NH4Cl, 0.1 mM
CaCl2, 1.0 mM MgSO4, 0.01 mM FeCl3, supplemented with
1 ml/l trace metals in the concentration of (mg/100 ml)
H3BO3, 100; ZnSO4·7H2O, 100; MnSO4·7H2O, 100; CoC12·6H2O, 40;
CuSO4·5H2O, 20; Na2MoO4·2H2O, 20; CuSO4·5H2O, 6. The
pelleted 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 for 5 min. The pellet was washed two times with 1.0%
Triton X-100 in buffer (50 mM phosphate, pH 7.0) and then
two times with buffer. The resulting pellet contains zeaxan-
thin, lutein, polyphosphate, Ca++, Mg++, 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 defi-
cient in the epoxidated carotenoids neoxanthin (3S,5R,6R,
3'S, 5'R,6'S)-5,6-epoxy-6,7-didehydro-5,6,7,8-tetrahydro-
β-carotene-3,3'-diol); violaxanthin ((3S,5R,6S,3'S,5'R,
6'S)-5,6,5',6'-dideoxy-5,6,5',6'-tetrahydro-β-β-carotene-3,3'-
diol, and antheraxanthin ((3S,5R,6S,3'R)-5,6-epoxy-5,6-
dihydro-β-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,6R)-
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 vacu-
oles is not confined to 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 hereinafter.

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

separating said detergent-insoluble pellet fraction from
soluble cellular material.
2. The method of claim 1, wherein said strain of Chlo-
rophyta 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:
culturing a strain of Chlorophyta algae cells in a minimal
inorganic growth medium;
separating said algae cells from said minimal inorganic
growth medium to obtain harvested algae;
treating said harvested algae with detergent to obtain
a detergent-insoluble pellet fraction comprising a caro-
tenoid wherein liquid chromatography of said pellet
fraction produces acetone extracted pigments, as shown
in FIG. 1; and
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:
culturing a strain of Chlorophyta algae cells in a minimal
inorganic growth medium;
separating said algae cells from said minimal inorganic
growth medium to obtain harvested algae;
treating said harvested algae with detergent to obtain a
detergent-insoluble pellet fraction comprising a caro-
tenoid 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 com-
ponent; and
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:
culturing a strain of Chlorophyta algae cells in a minimal
inorganic growth medium;
separating said algae cells from said minimal inorganic
growth medium to obtain harvested algae;
treating said harvested algae with detergent to obtain a
detergent-insoluble pellet fraction comprising a caro-
tenoid wherein a sodium dodecyl (lauryl) sulfate—
polyacrylamide gel electrophoresis of said pellet fraction
indicates a 70 kDa protein as a major protein as
illustrated in FIG. 4; and
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.