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(54) **MICROORGANISMS AND METHODS FOR PRODUCING CAROTENOIDS AND OTHER COMPOUNDS**

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(73) Assignee: **Wisconsin Alumni Research Foundation**, Madison, WI (US)

(21) Appl. No.: **19/371,081**

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(52) **U.S. Cl.**

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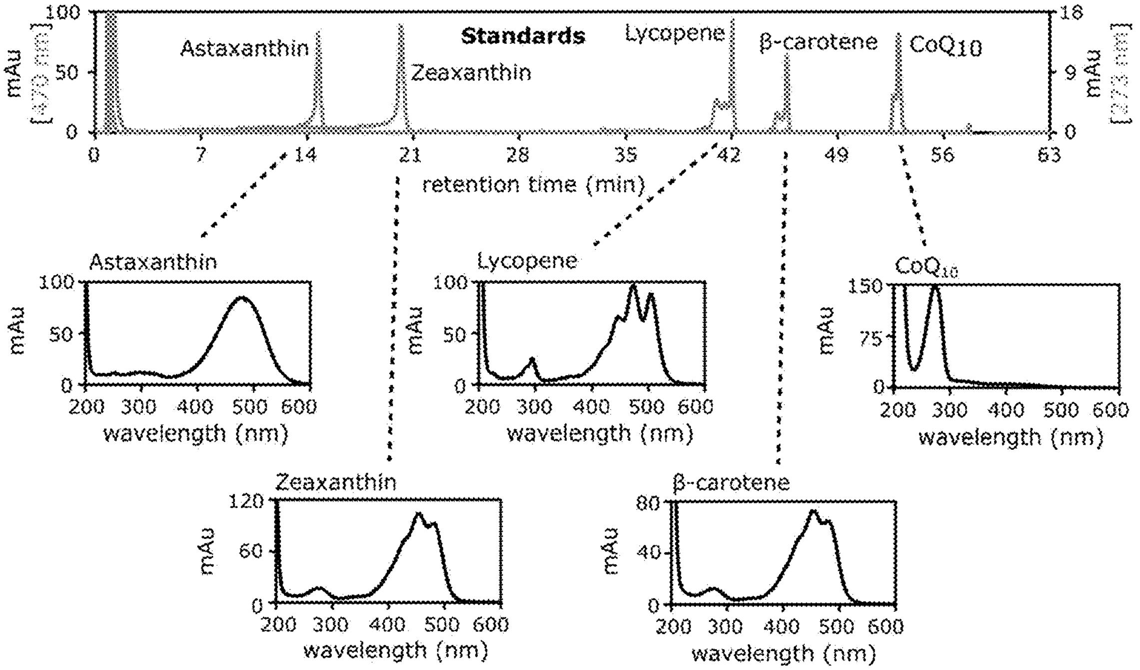
(57) **ABSTRACT**

Recombinant microorganisms configured for enhanced production of carotenoids and other compounds and methods of using the recombinant microorganisms for the production of same. The recombinant microorganism can include a modification that decreases phytoene synthase (CrtB) activity, a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity, a modification that decreases lycopene cyclase (CrtY) activity, a modification that decreases beta-carotene hydroxylase (CrtZ) activity, a modification that decreases 2,2'-beta hydroxylase (CrtG) activity, and/or a modification that increases beta-carotene ketolase (CrtW) activity. The recombinant microorganism can be from the genus *Novosphingobium*, such as *Novosphingobium aromaticivorans*.

Specification includes a Sequence Listing.



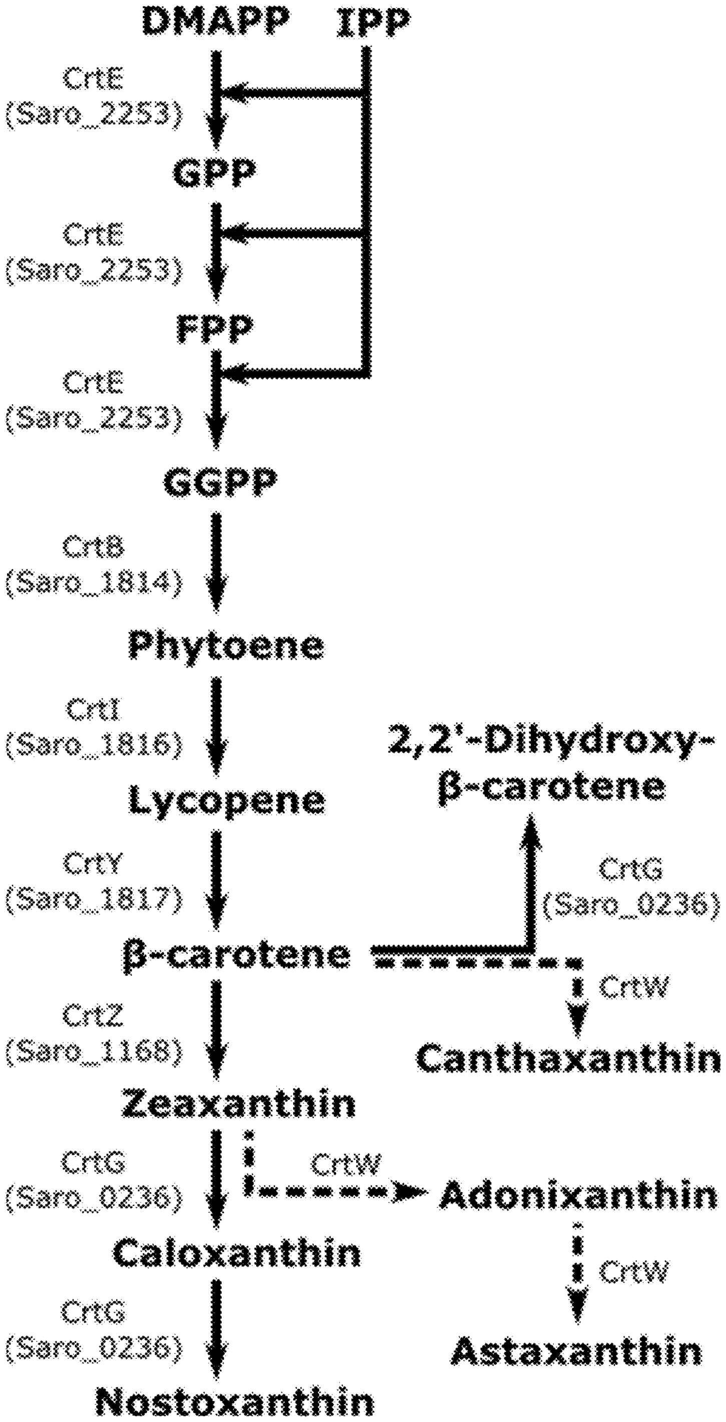

DMAPP
IPP
CrtE
(Saro_2253)
GPP
CrtE
(Saro_2253)
FPP
CrtE
(Saro_2253)
GGPP
CrtB
(Saro_1814)
Phytoene
CrtI
(Saro_1816)
Lycopene
2,2'-Dihydroxy-
β-carotene
CrtG
(Saro_0236)
CrtY
(Saro_1817)
β-carotene
CrtW
CrtZ
(Saro_1168)
Canthaxanthin
Zeaxanthin
CrtG
(Saro_0236)
CrtW
Adonixanthin
Caloxanthin
CrtW
CrtG
(Saro_0236)
Astaxanthin
Nostoxanthin

FIG. 1

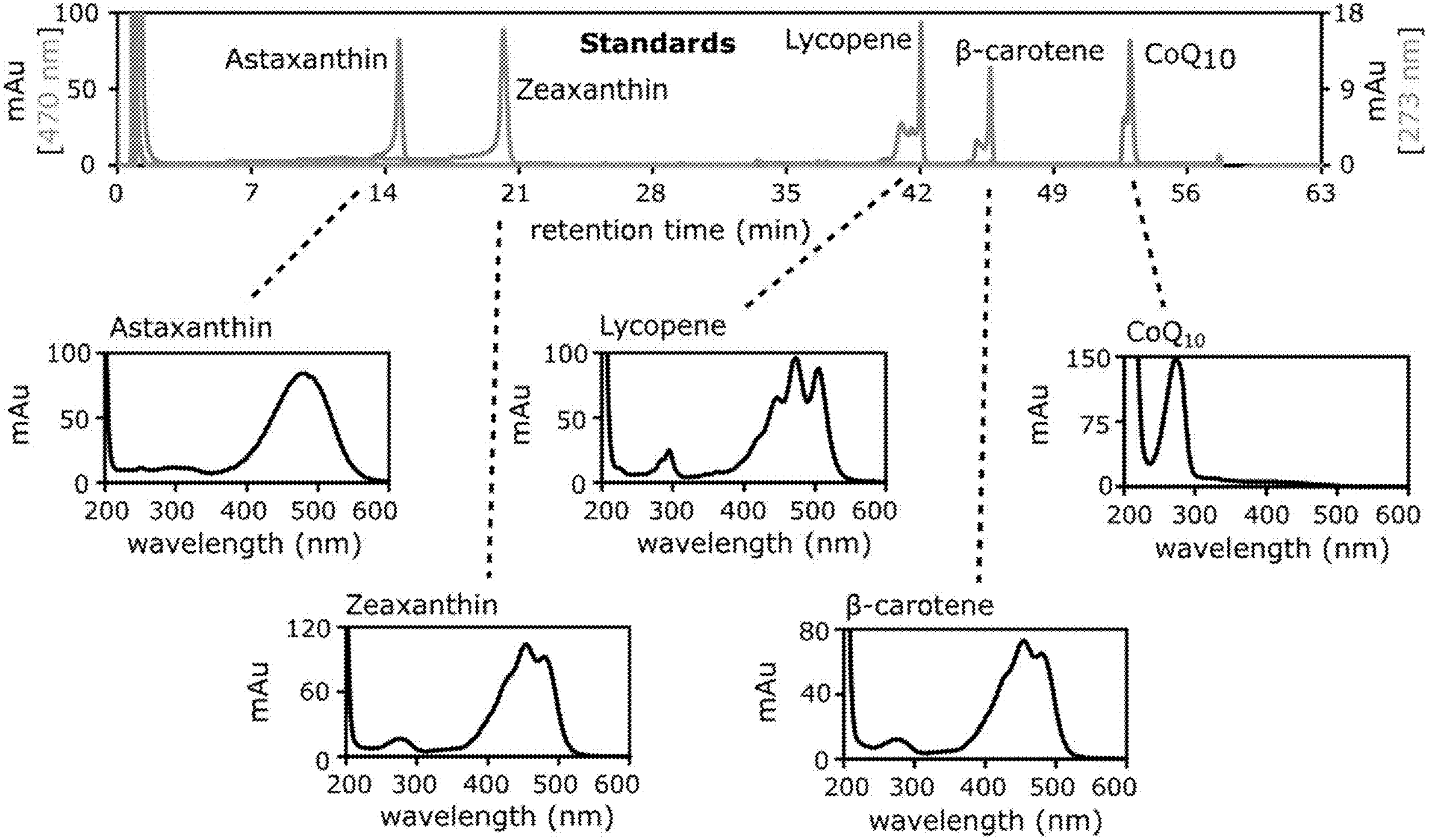

Standards
Astaxanthin
Zeaxanthin
Lycopene
β-carotene
CoQ10
mAu [470 nm]
mAu [273 nm]
100
50
0
18
9
0
0 7 14 21 28 35 42 49 56 63
retention time (min)
Astaxanthin
mAu
100 50 0
200 300 400 500 600
wavelength (nm)
Lycopene
mAu
100 50 0
200 300 400 500 600
wavelength (nm)
CoQ_{10}
mAu
150 75 0
200 300 400 500 600
wavelength (nm)
Zeaxanthin
mAu
120 60 0
200 300 400 500 600
wavelength (nm)
β-carotene
mAu
80 40 0
200 300 400 500 600
wavelength (nm)

FIG. 2

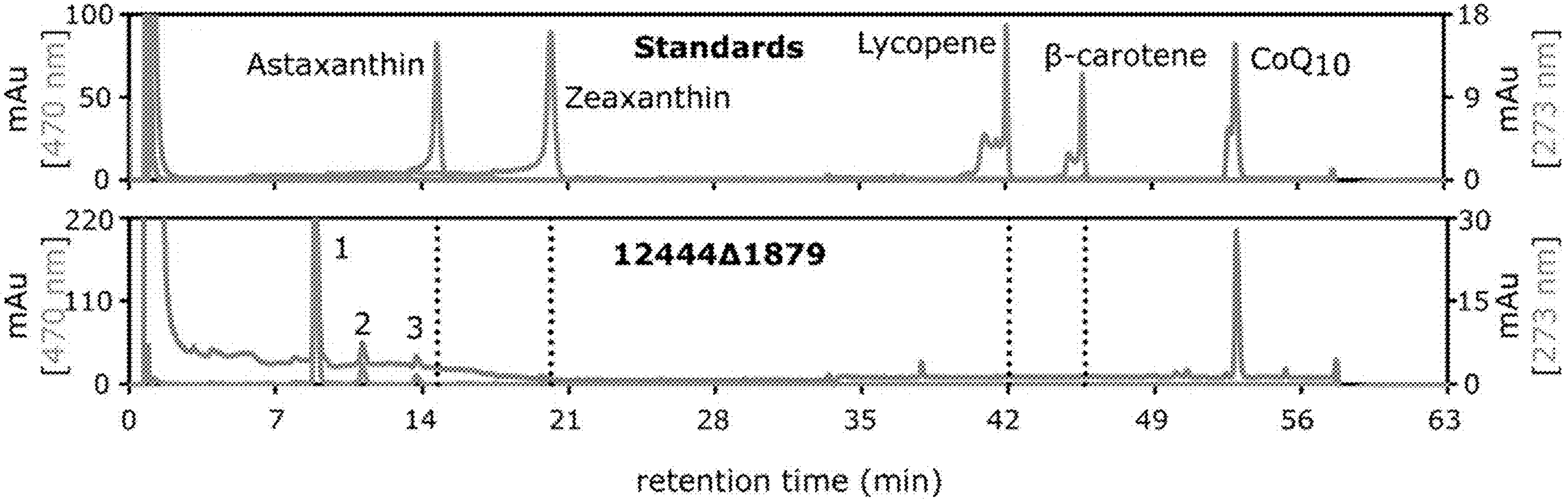

Standards
Astaxanthin
Zeaxanthin
Lycopene
β-carotene
CoQ10
12444Δ1879
1
2
3
mAu [470 nm]
mAu [273 nm]
100
50
0
18
9
0
220
110
0
30
15
0
0
7
14
21
28
35
42
49
56
63
retention time (min)

FIG. 3A

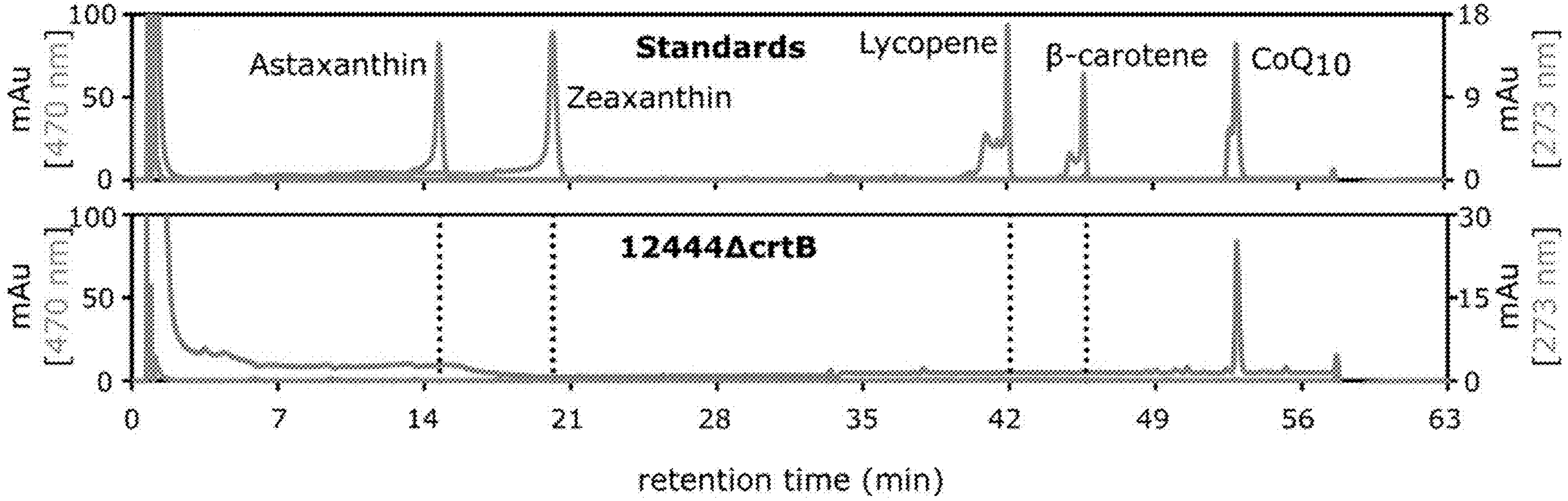

Standards
Astaxanthin
Zeaxanthin
Lycopene
β-carotene
CoQ10
12444ΔcrtB
mAu
[470 nm]
mAu
[273 nm]
100
50
0
18
9
0
100
50
0
30
15
0
0
7
14
21
28
35
42
49
56
63
retention time (min)

FIG. 3B

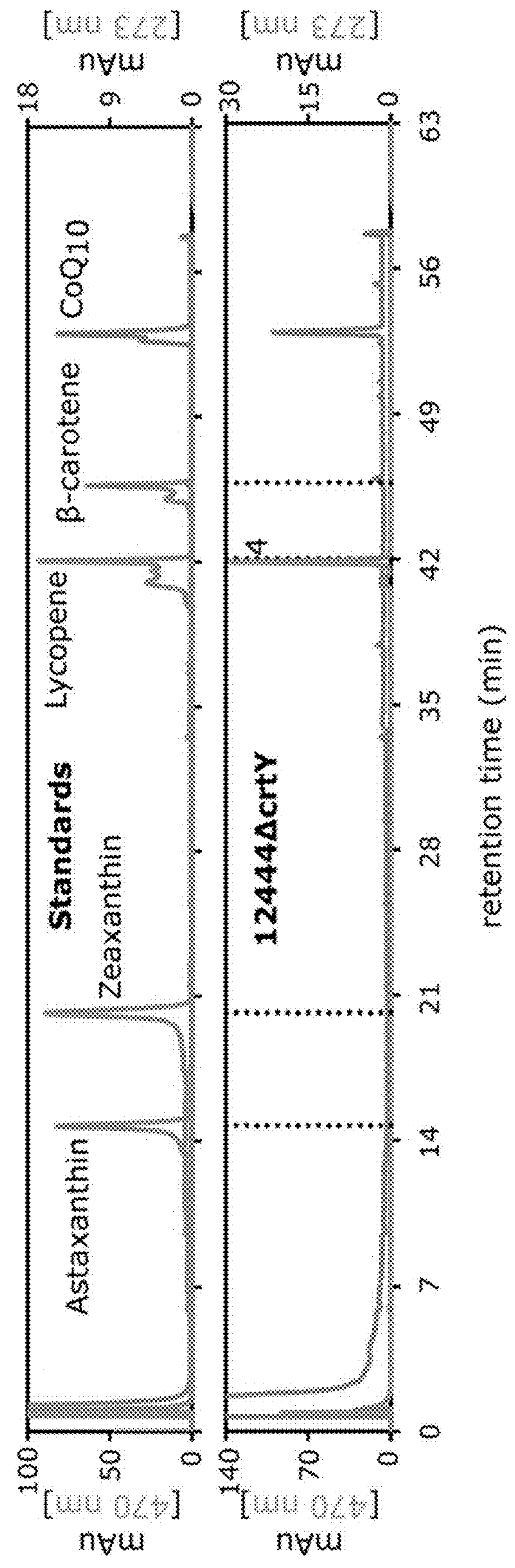

Standards
Astaxanthin
Zeaxanthin
Lycopene
β-carotene
CoQ10
12444ΔcrtY
4
mAu [470 nm]
100
50
0
140
70
0
mAu [273 nm]
18
9
0
30
15
0
0
7
14
21
28
35
42
49
56
63
retention time (min)

FIG. 3C

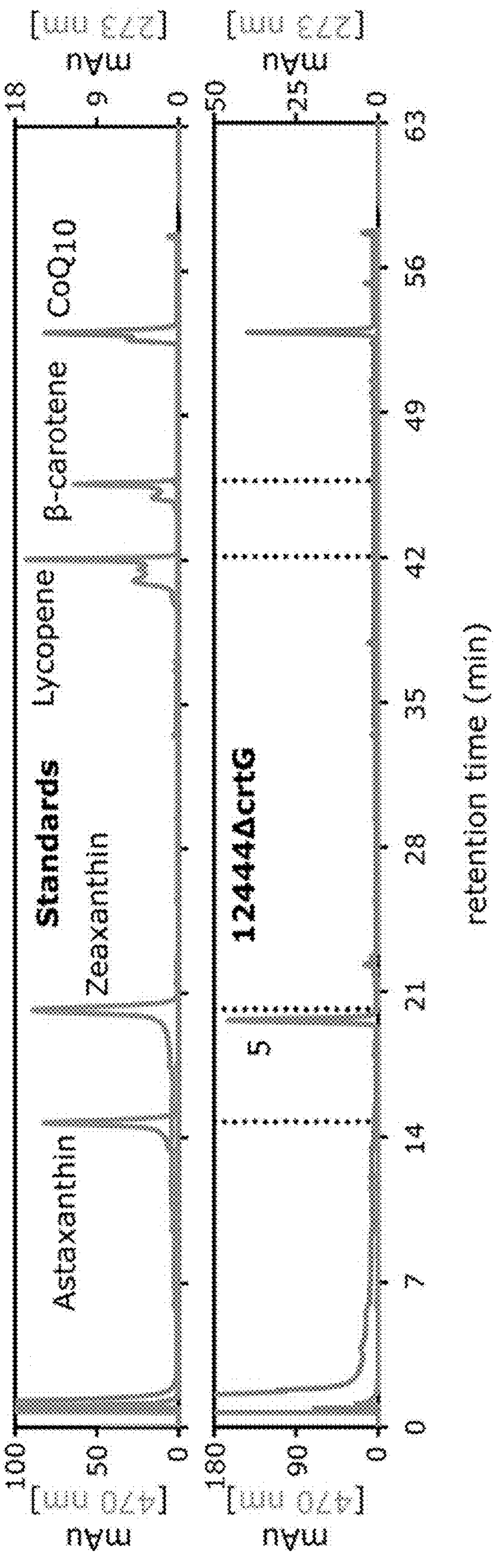

Standards
Astaxanthin
Zeaxanthin
Lycopene
β-carotene
CoQ10
12444ΔcrtG
5
retention time (min)
0
7
14
21
28
35
42
49
56
63
mAu [470 nm]
100
50
0
180
90
0
mAu [273 nm]
18
9
0
50
25
0

FIG. 3D

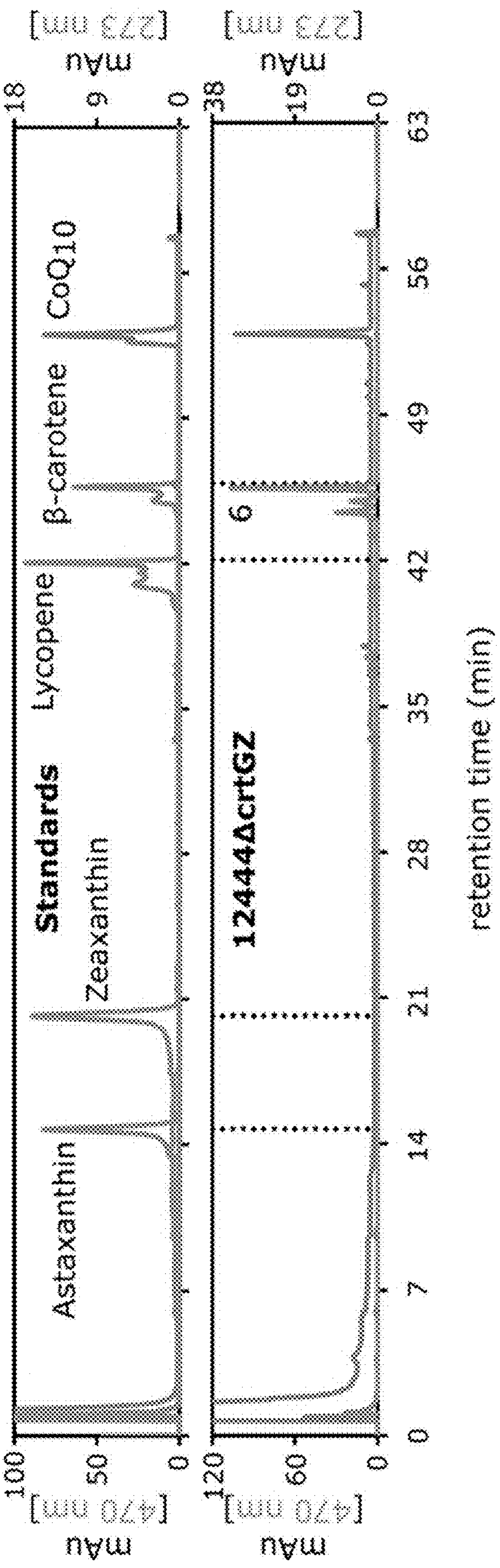

Standards
Astaxanthin
Zeaxanthin
Lycopene
β-carotene
CoQ10
12444ΔcrtGZ
6
mAu [470 nm]
100
50
0
120
60
0
mAu [273 nm]
18
9
0
38
19
0
retention time (min)
0
7
14
21
28
35
42
49
56
63

FIG. 3E

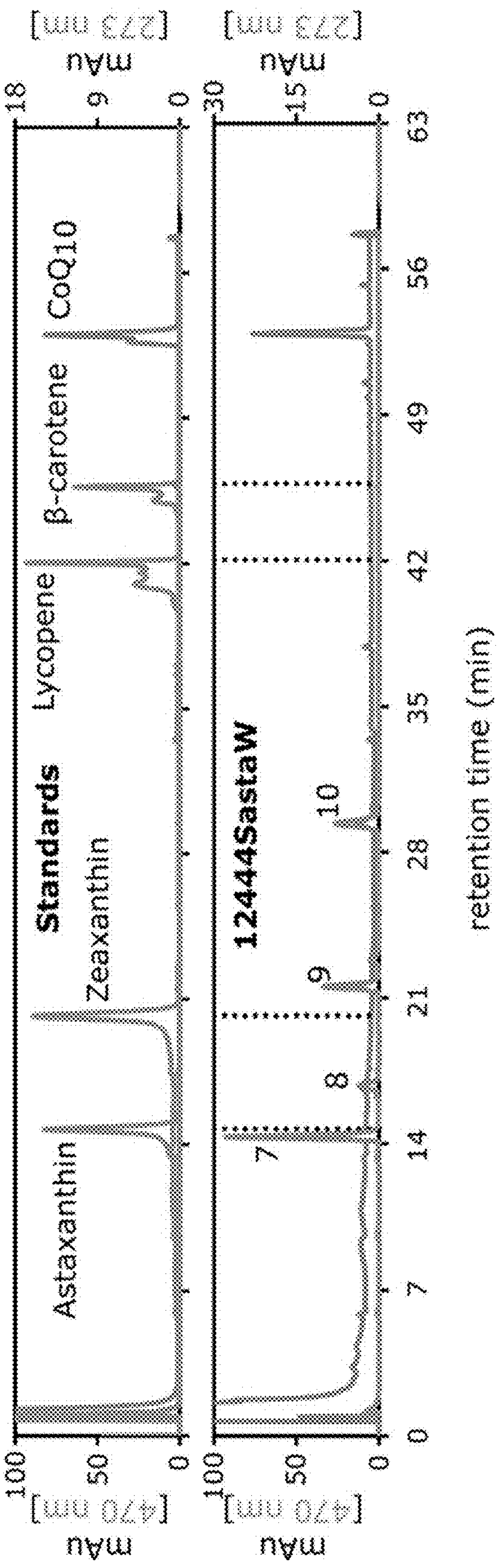

Standards
Astaxanthin
Zeaxanthin
Lycopene
β-carotene
CoQ10
12444SastaW
7
8
9
10
mAu [470 nm]
100
50
0
mAu [273 nm]
18
9
0
30
15
0
retention time (min)
0
7
14
21
28
35
42
49
56
63

FIG. 3F

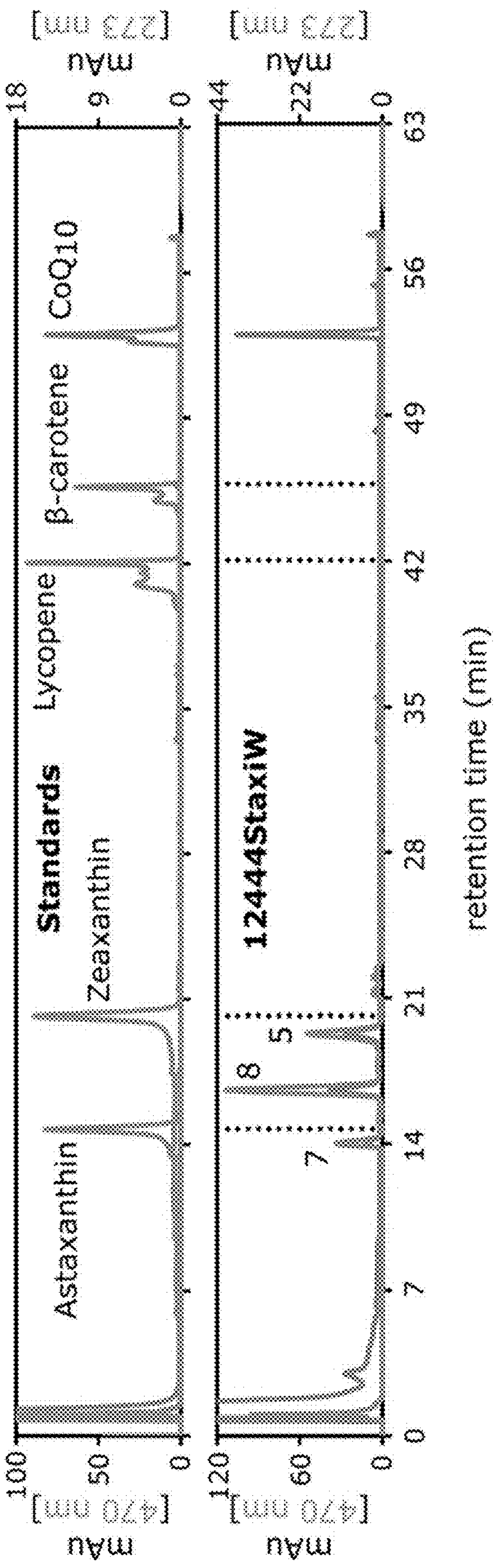

Standards
Astaxanthin
Zeaxanthin
Lycopene
β-carotene
CoQ10
12444StaxiW
7
8
5
mAu [470 nm]
100
50
0
120
60
0
mAu [273 nm]
18
9
0
44
22
0
0
7
14
21
28
35
42
49
56
63
retention time (min)

FIG. 3G

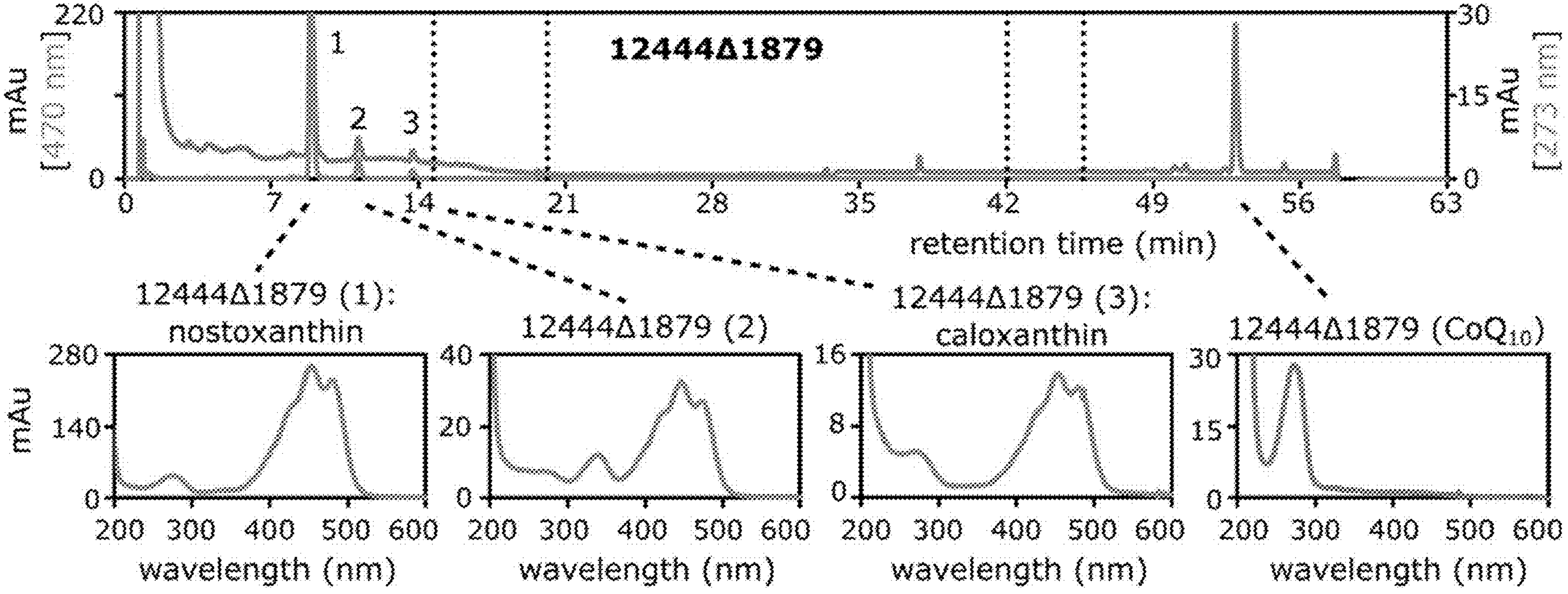

12444Δ1879
mAu [470 nm]
mAu [273 nm]
220
0
30
15
0
1
2
3
0 7 14 21 28 35 42 49 56 63
retention time (min)
12444Δ1879 (1): nostoxanthin
12444Δ1879 (2)
12444Δ1879 (3): caloxanthin
12444Δ1879 (CoQ10)
mAu
280
140
0
40
20
0
16
8
0
30
15
0
200 300 400 500 600
wavelength (nm)

FIG. 4A

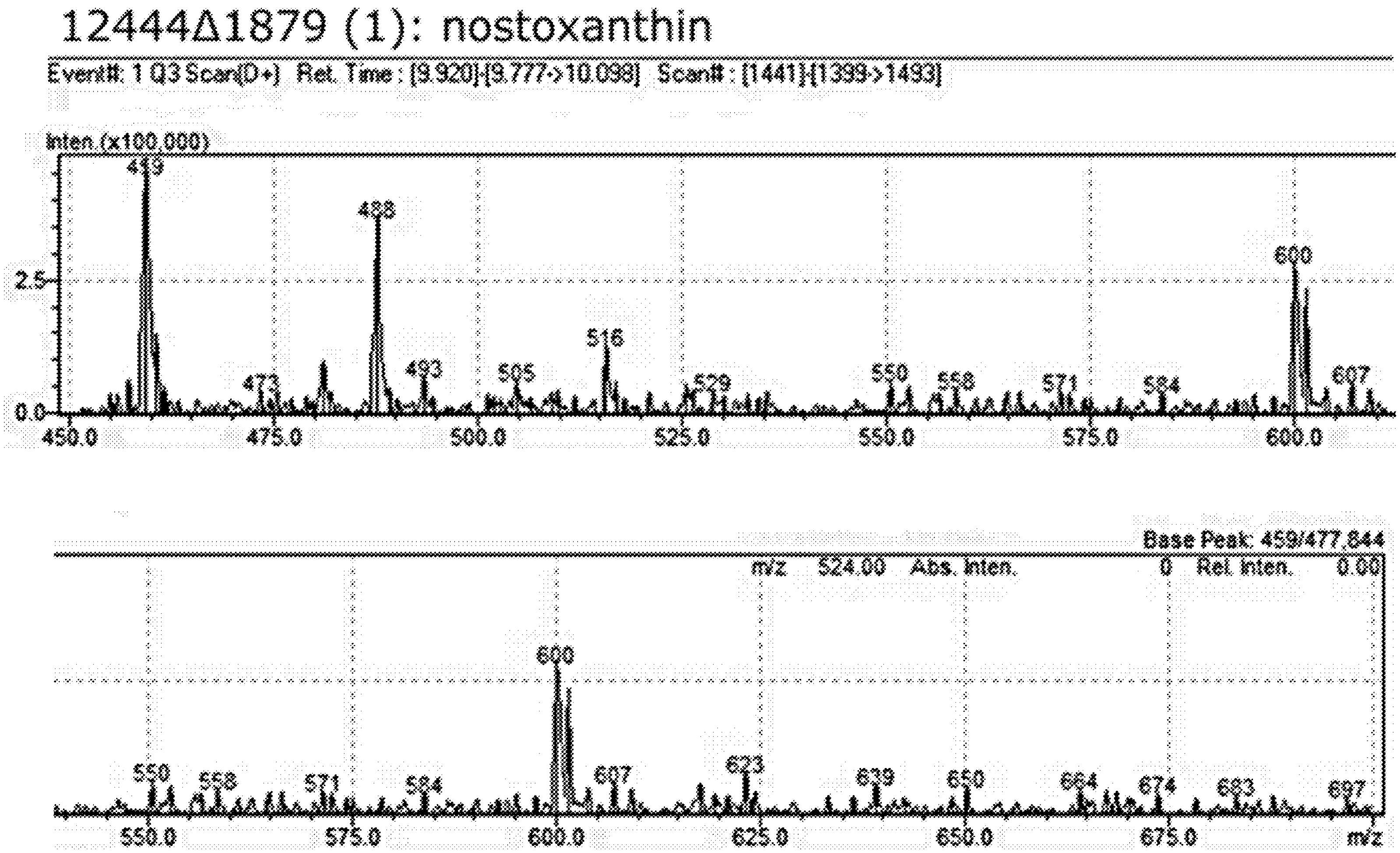

12444Δ1879 (1): nostoxanthin
Event#: 1 Q3 Scan(D+) Ret. Time : [9.920]-[9.777->10.098] Scan# : [1441]-[1399->1493]
Inten (x100,000)
2.5
0.0
459
488
473
493
505
516
529
550
558
571
584
600
607
450.0
475.0
500.0
525.0
550.0
575.0
600.0
Base Peak: 459/477,844
m/z 524.00 Abs. Inten. 0 Rel. Inten. 0.00
600
550
558
571
584
607
623
639
650
664
674
683
697
550.0
575.0
600.0
625.0
650.0
675.0
m/z

FIG. 4B

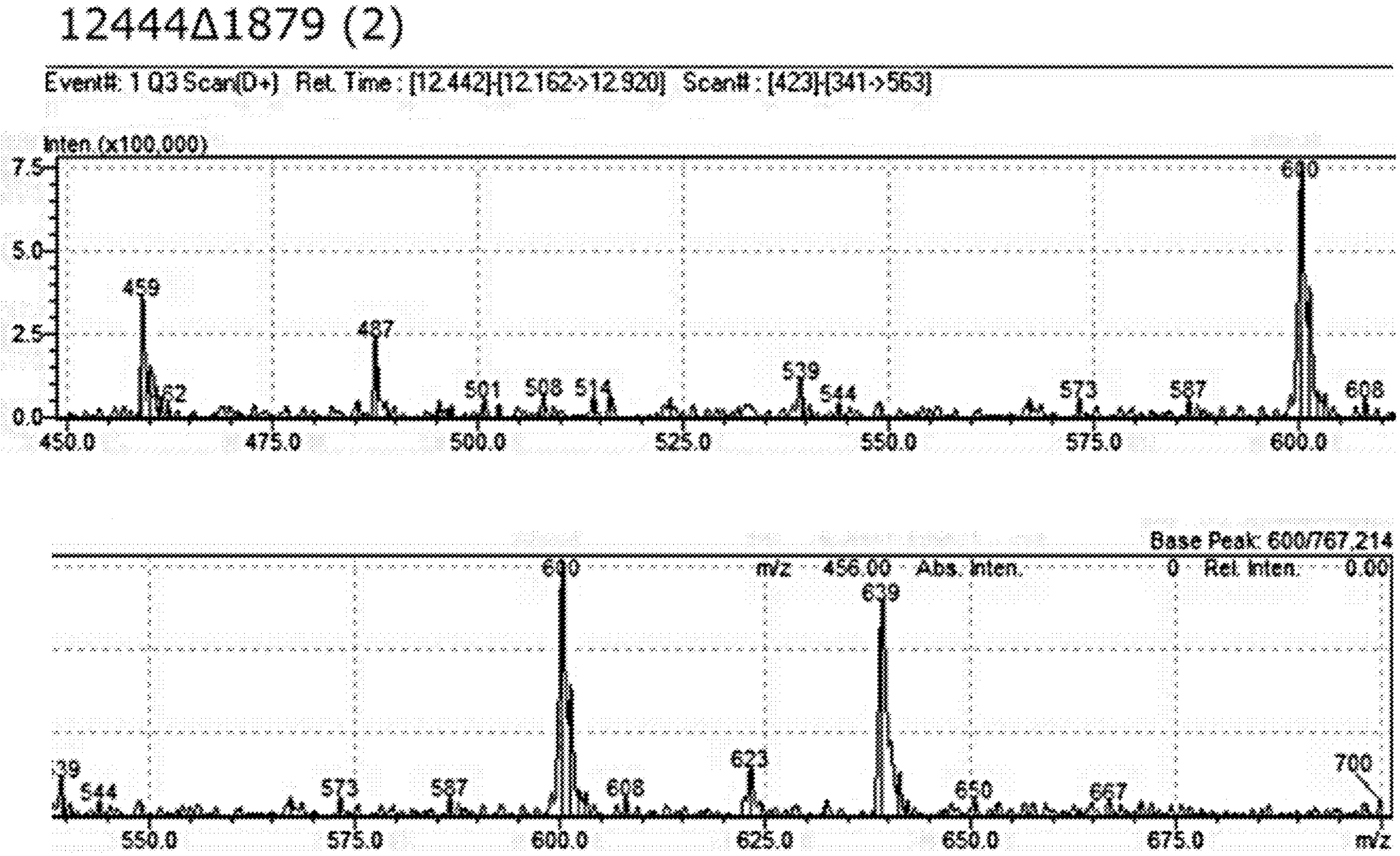

12444Δ1879 (2)
Event#: 1 Q3 Scan(D+) Ret. Time : [12.442]-[12.162->12.920] Scan# : [423]-[341->563]
Inten.(x100,000)
7.5
5.0
2.5
0.0
459
462
487
501
508
514
539
544
573
587
600
608
450.0
475.0
500.0
525.0
550.0
575.0
600.0
Base Peak: 600/767,214
m/z 456.00 Abs. Inten. 0 Rel. Inten. 0.00
600
639
539
544
573
587
608
623
650
667
700
550.0
575.0
600.0
625.0
650.0
675.0
m/z

FIG. 4C

12444Δ1879 (3): caloxanthin

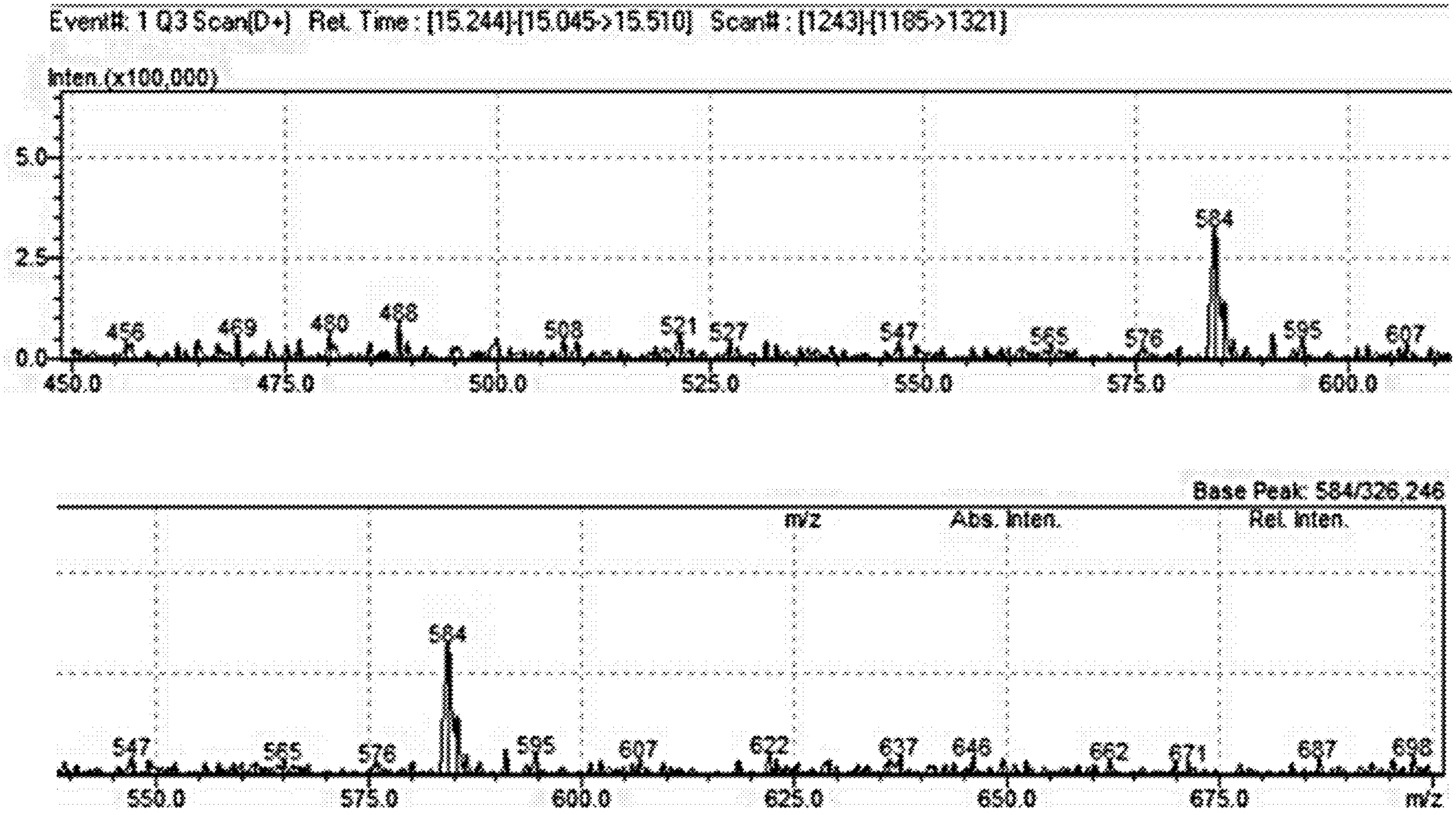


FIG. 4D

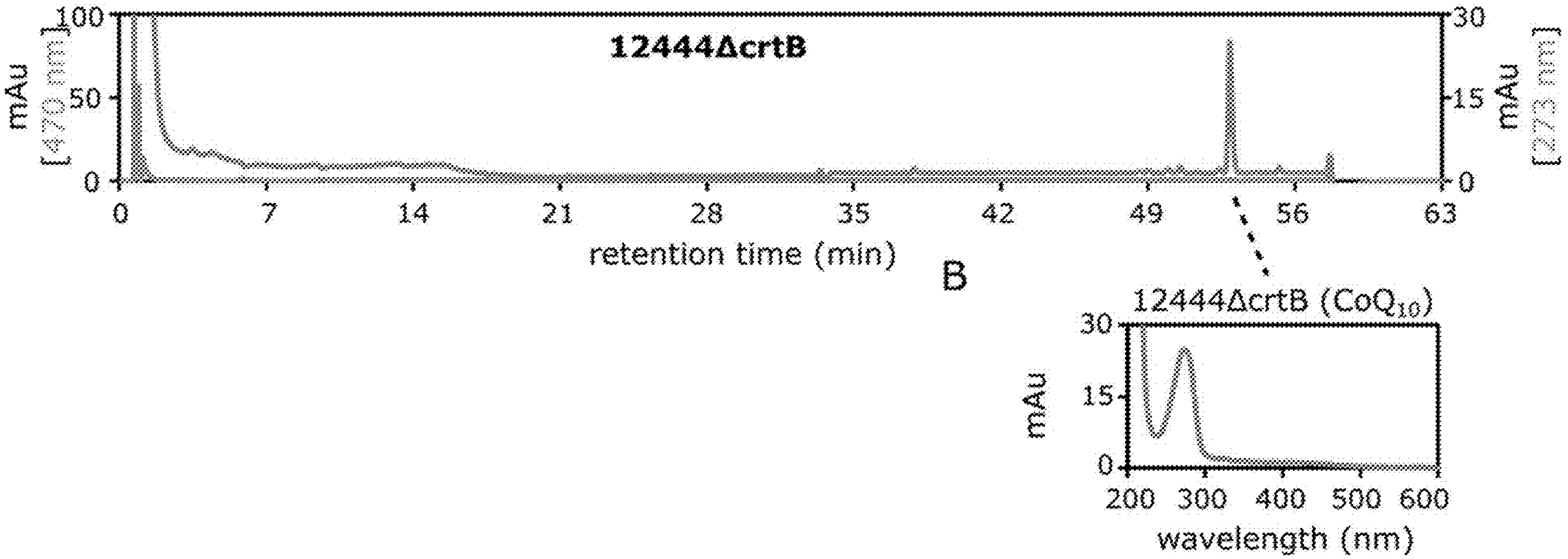

12444ΔcrtB
mAu [470 nm]
100
50
0
mAu [273 nm]
30
15
0
0
7
14
21
28
35
42
49
56
63
retention time (min)
B
12444ΔcrtB (CoQ10)
mAu
30
15
0
200
300
400
500
600
wavelength (nm)

FIG. 5

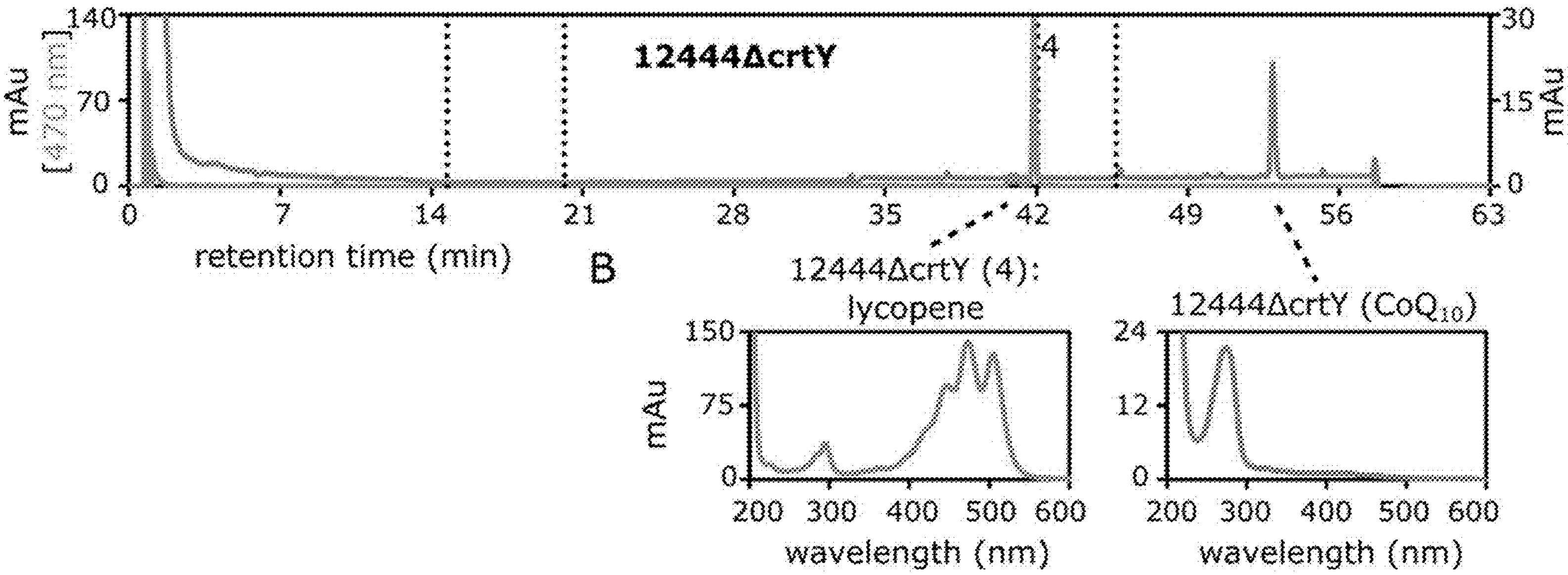

140
70
0
mAu
[470 nm]
12444ΔcrtY
4
30
15
0
mAu
[273 nm]
0
7
14
21
28
35
42
49
56
63
retention time (min)
B
12444ΔcrtY (4):
lycopene
150
75
0
mAu
200 300 400 500 600
wavelength (nm)
12444ΔcrtY (CoQ10)
24
12
0
200 300 400 500 600
wavelength (nm)

FIG. 6

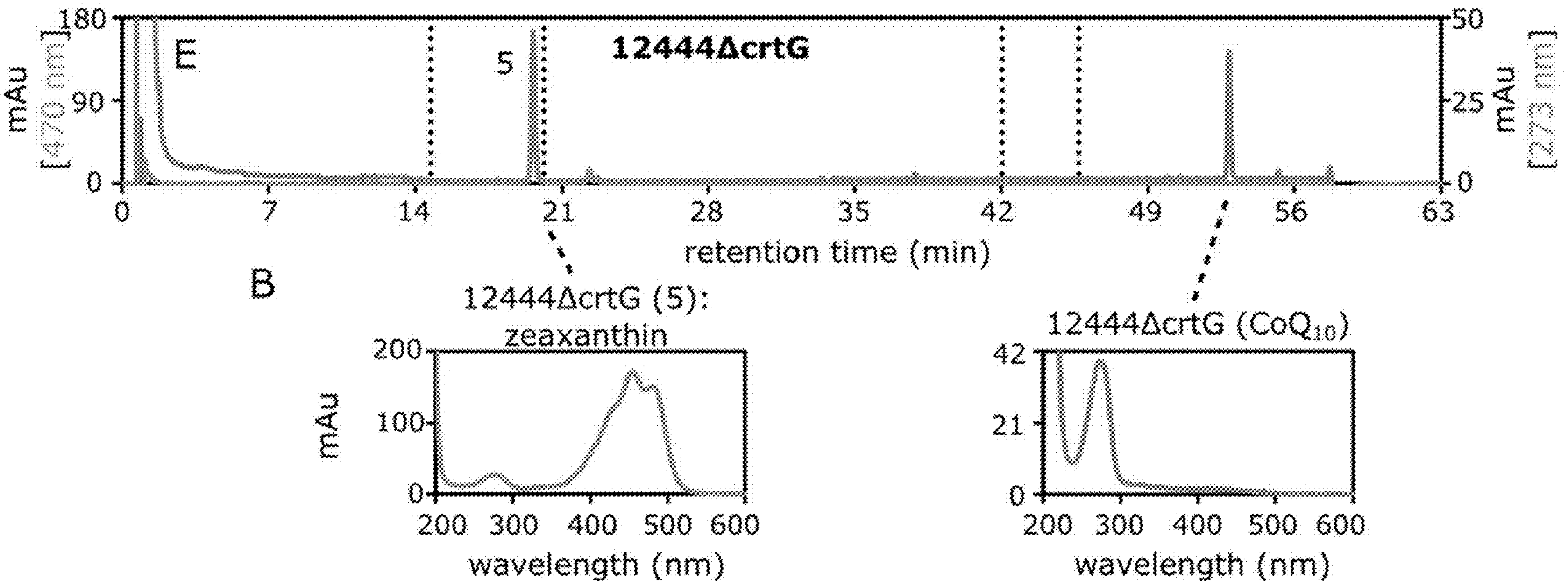

E
5
12444ΔcrtG
mAu [470 nm]
180
90
0
mAu [273 nm]
50
25
0
0
7
14
21
28
35
42
49
56
63
retention time (min)
B
12444ΔcrtG (5): zeaxanthin
200
100
0
mAu
200 300 400 500 600
wavelength (nm)
12444ΔcrtG (CoQ10)
42
21
0
200 300 400 500 600
wavelength (nm)

FIG. 7

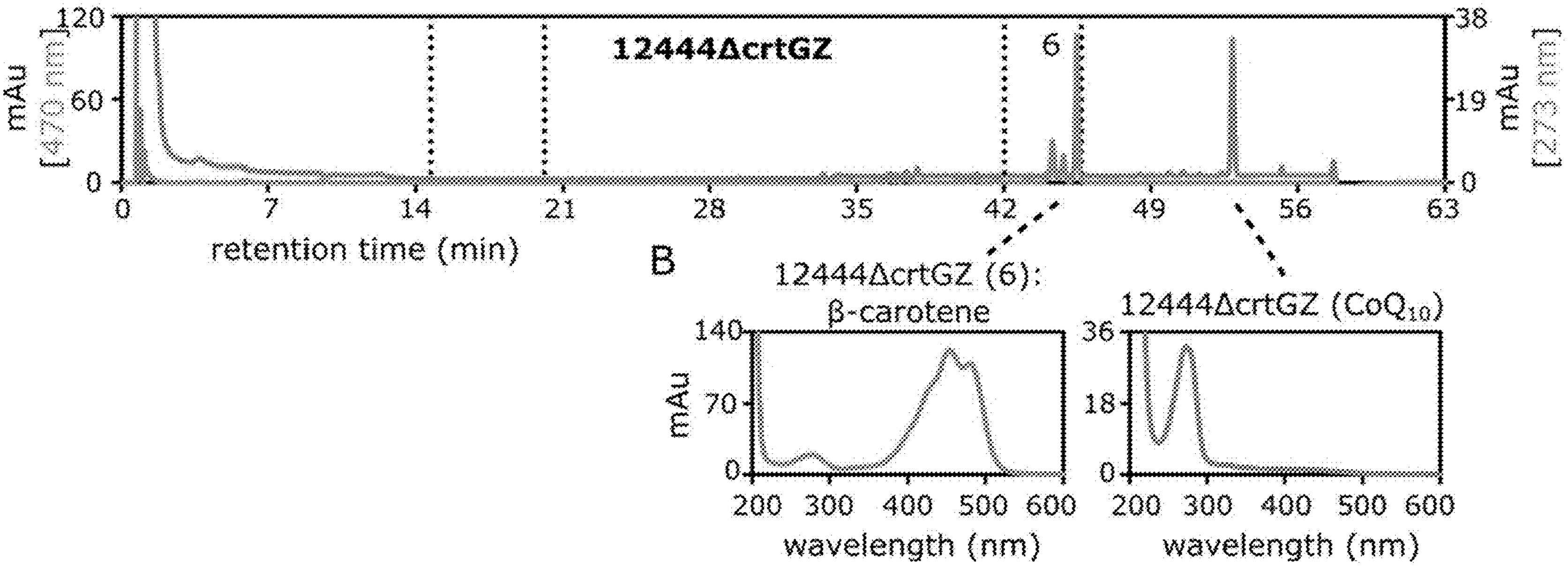

12444ΔcrtGZ
6
mAu [470 nm]
120
60
0
mAu [273 nm]
38
19
0
0
7
14
21
28
35
42
49
56
63
retention time (min)
B
12444ΔcrtGZ (6): β-carotene
mAu
140
70
0
200 300 400 500 600
wavelength (nm)
12444ΔcrtGZ (CoQ10)
36
18
0
200 300 400 500 600
wavelength (nm)

FIG. 8

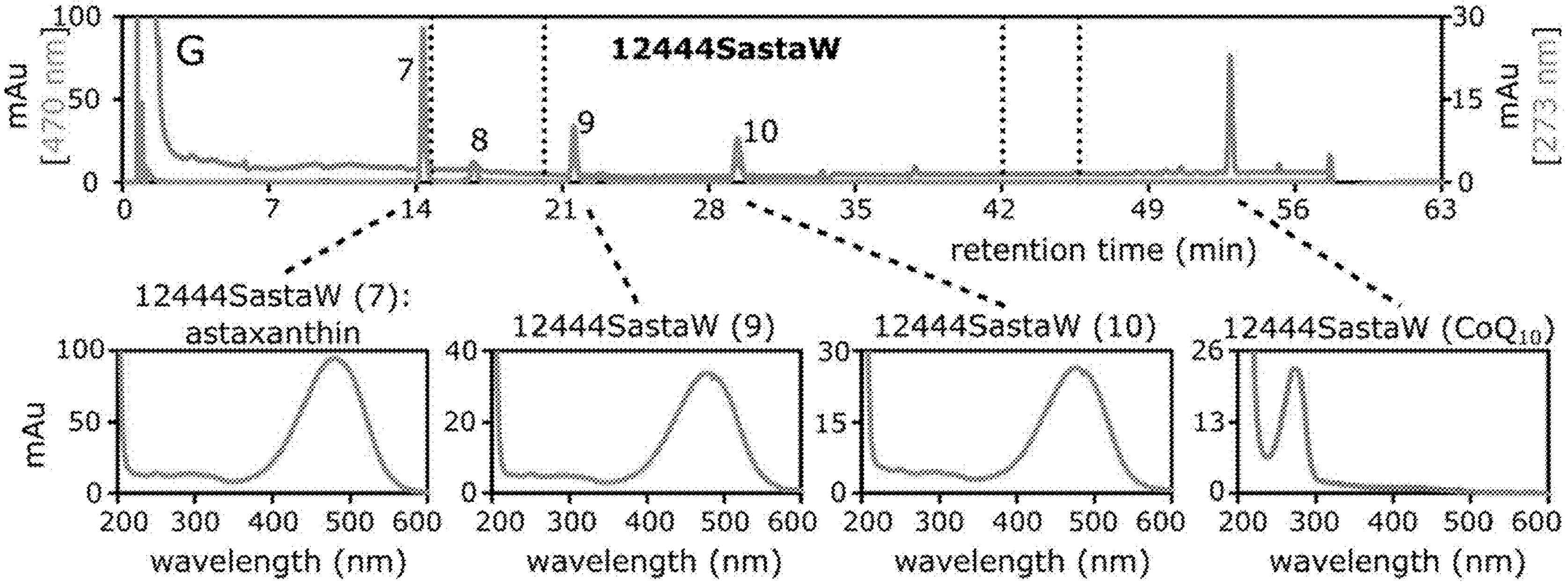

G
12444SastaW
mAu [470 nm]
mAu [273 nm]
100
50
0
30
15
0
7
8
9
10
0
7
14
21
28
35
42
49
56
63
retention time (min)
12444SastaW (7): astaxanthin
12444SastaW (9)
12444SastaW (10)
12444SastaW (CoQ$_{10}$)
mAu
100
50
0
40
20
0
30
15
0
26
13
0
200 300 400 500 600
wavelength (nm)
200 300 400 500 600
wavelength (nm)
200 300 400 500 600
wavelength (nm)
200 300 400 500 600
wavelength (nm)

FIG. 9A

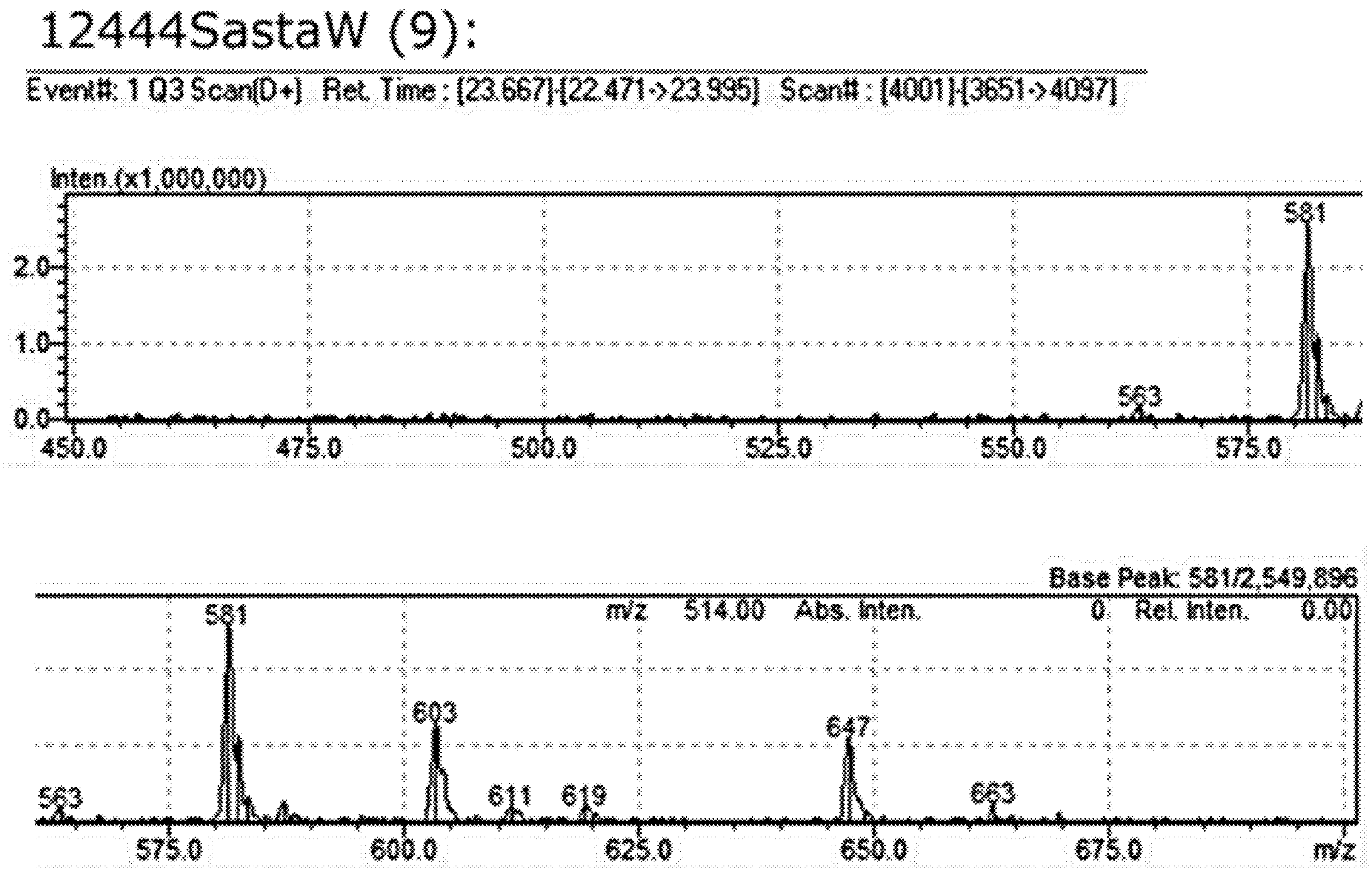

12444SastaW (9):
Event#: 1 Q3 Scan(D+) Ret. Time : [23.667]-[22.471->23.995] Scan# : [4001]-[3651->4097]
Inten.(x1,000,000)
2.0
1.0
0.0
450.0
475.0
500.0
525.0
550.0
575.0
563
581
Base Peak: 581/2,549,896
m/z 514.00 Abs. Inten. 0 Rel. Inten. 0.00
563
581
603
611
619
647
663
575.0
600.0
625.0
650.0
675.0
m/z

FIG. 9B

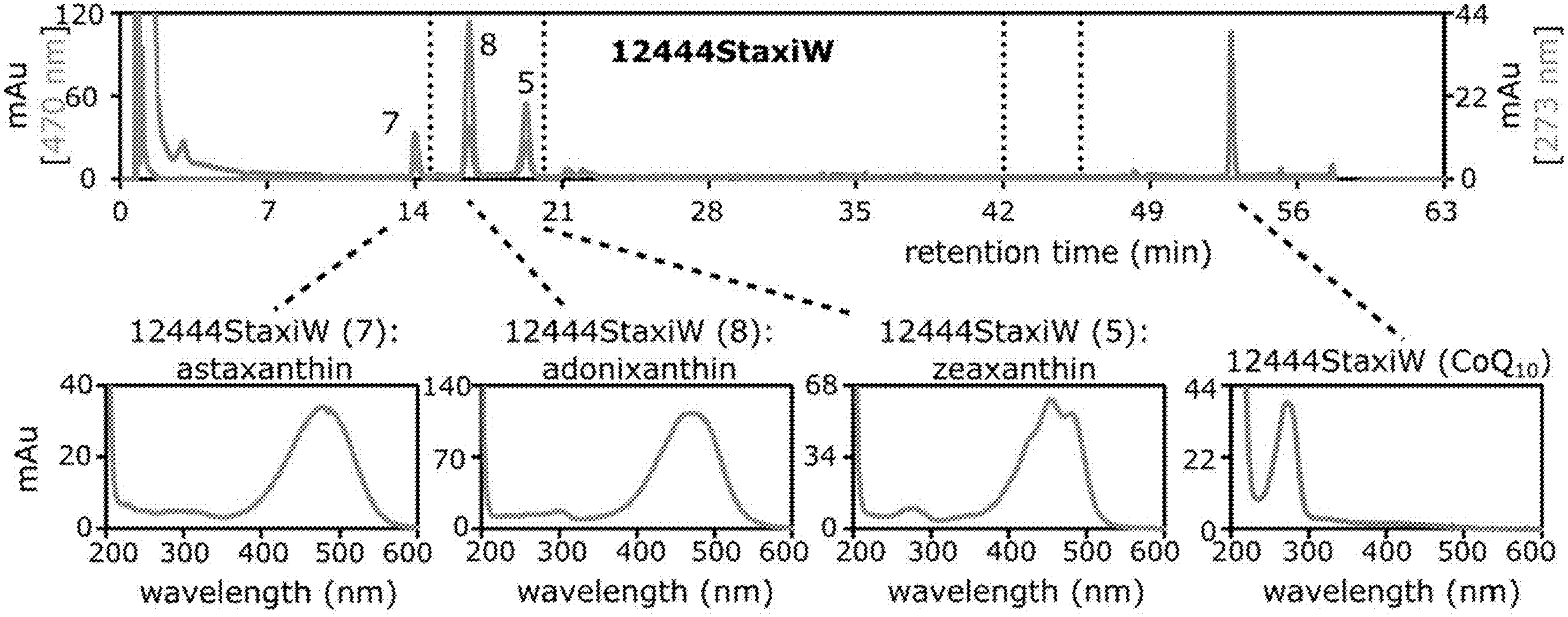

12444StaxiW
mAu [470 nm]
mAu [273 nm]
120
60
0
44
22
0
7
8
5
0
7
14
21
28
35
42
49
56
63
retention time (min)
12444StaxiW (7): astaxanthin
12444StaxiW (8): adonixanthin
12444StaxiW (5): zeaxanthin
12444StaxiW (CoQ10)
mAu
40
20
0
140
70
0
68
34
0
44
22
0
200 300 400 500 600
wavelength (nm)

FIG. 10A

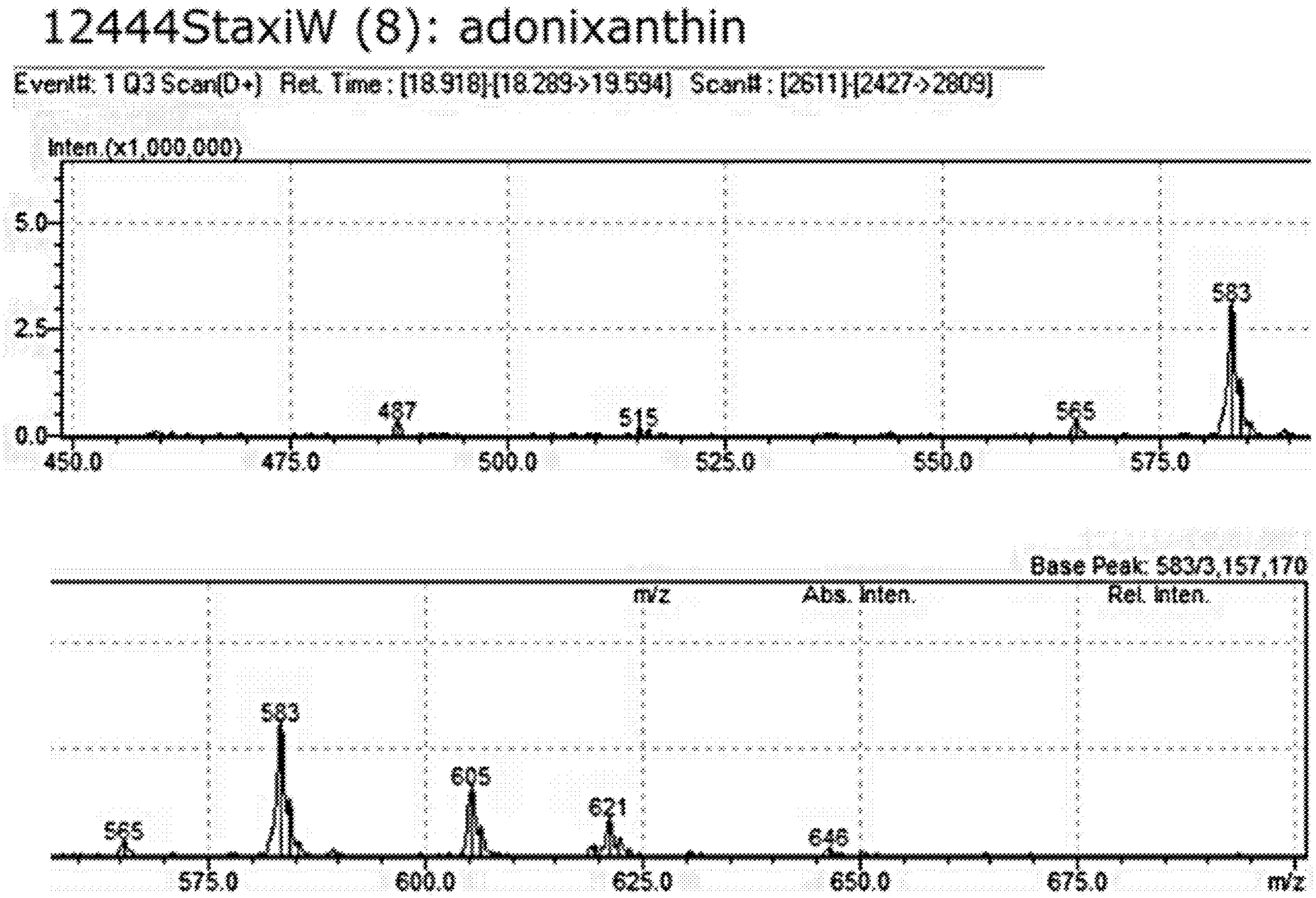

12444StaxiW (8): adonixanthin
Event#: 1 Q3 Scan(D+) Ret. Time : [18.918]-[18.289->19.594] Scan# : [2611]-[2427->2809]
Inten.(x1,000,000)
5.0
2.5
0.0
487
515
565
583
450.0
475.0
500.0
525.0
550.0
575.0
Base Peak: 583/3,157,170
m/z
Abs. Inten.
Rel. Inten.
565
583
605
621
646
575.0
600.0
625.0
650.0
675.0
m/z

FIG. 10B

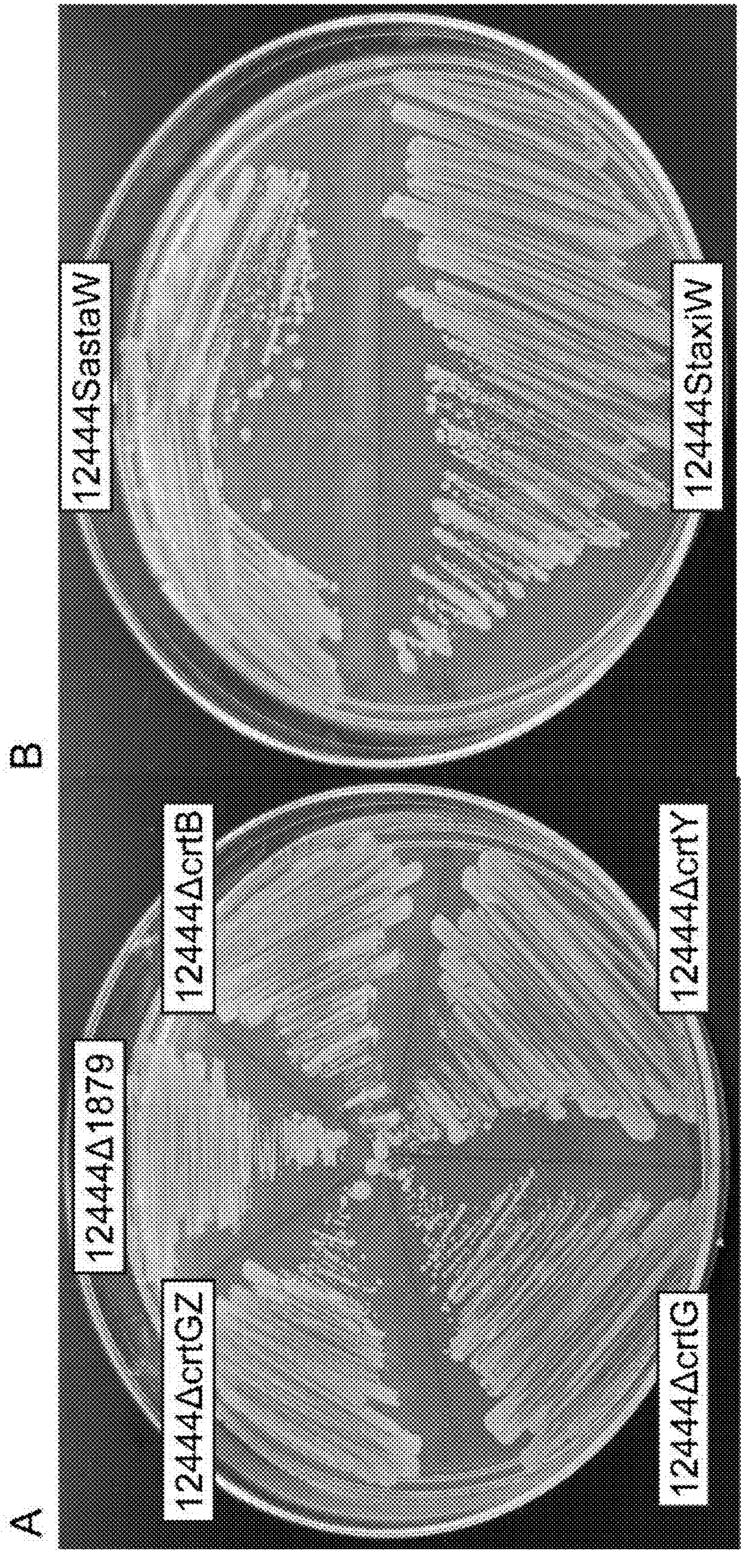

A
12444ΔcrtGZ
12444Δ1879
12444ΔcrtB
12444ΔcrtG
12444ΔcrtY
B
12444SastaW
12444StaxiW

FIG. 11

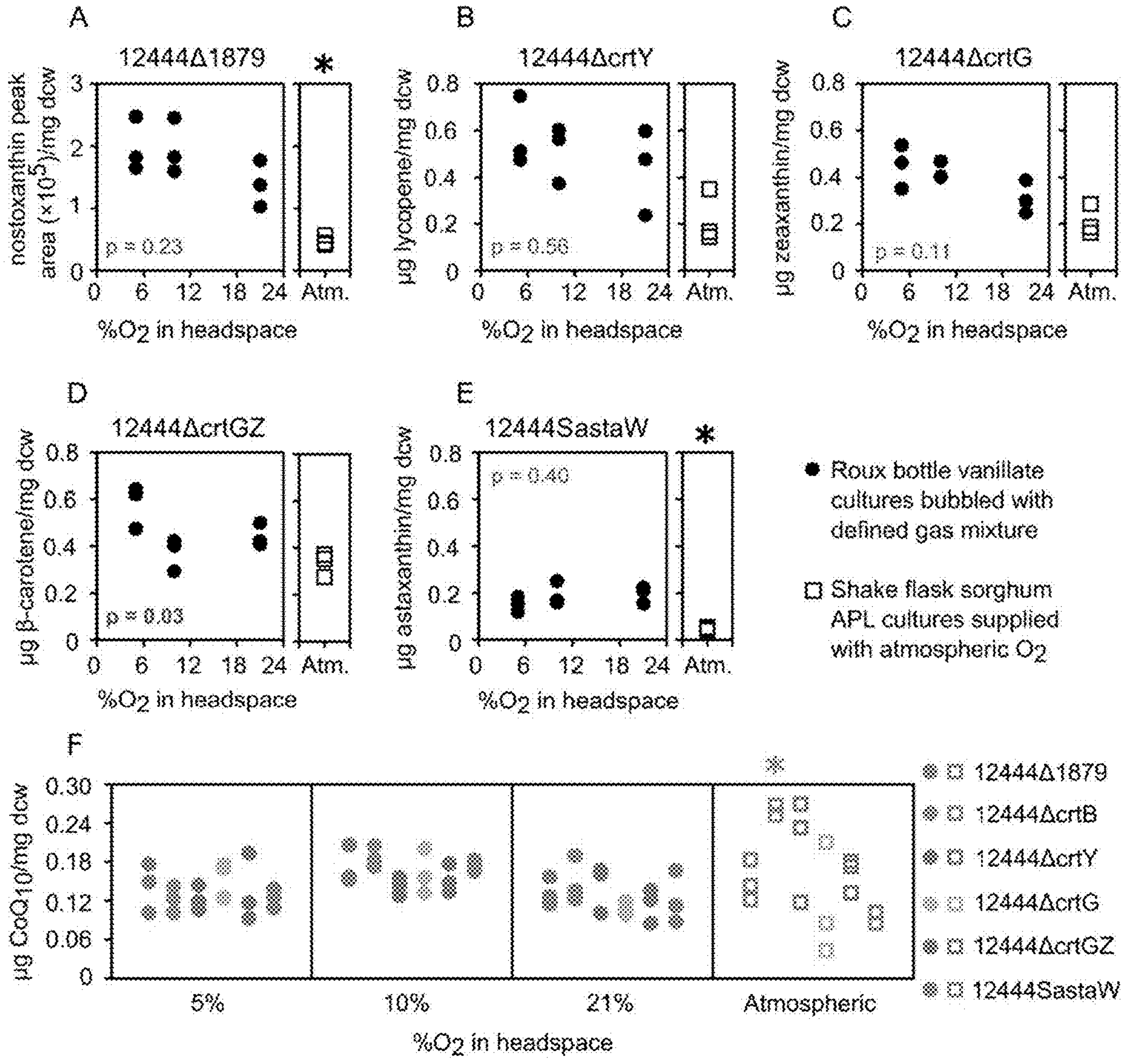

A
12444Δ1879
*
nostoxanthin peak area (×10^5)/mg dcw
0
1
2
3
p = 0.23
0
6
12
18
24
Atm.
%O$_2$ in headspace
B
12444ΔcrtY
µg lycopene/mg dcw
0
0.2
0.4
0.6
0.8
p = 0.56
0
6
12
18
24
Atm.
%O$_2$ in headspace
C
12444ΔcrtG
µg zeaxanthin/mg dcw
0
0.2
0.4
0.6
0.8
p = 0.11
0
6
12
18
24
Atm.
%O$_2$ in headspace
D
12444ΔcrtGZ
µg β-carotene/mg dcw
0
0.2
0.4
0.6
0.8
p = 0.83
0
6
12
18
24
Atm.
%O$_2$ in headspace
E
12444SastaW
*
µg astaxanthin/mg dcw
0
0.2
0.4
0.6
0.8
p = 0.40
0
6
12
18
24
Atm.
%O$_2$ in headspace
● Roux bottle vanillate cultures bubbled with defined gas mixture
☐ Shake flask sorghum APL cultures supplied with atmospheric O$_2$
F
µg CoQ$_{10}$/mg dcw
0
0.06
0.12
0.18
0.24
0.30
*
5%
10%
21%
Atmospheric
%O$_2$ in headspace
● ☐ 12444Δ1879
● ☐ 12444ΔcrtB
● ☐ 12444ΔcrtY
● ☐ 12444ΔcrtG
● ☐ 12444ΔcrtGZ
● ☐ 12444SastaW

FIG. 12

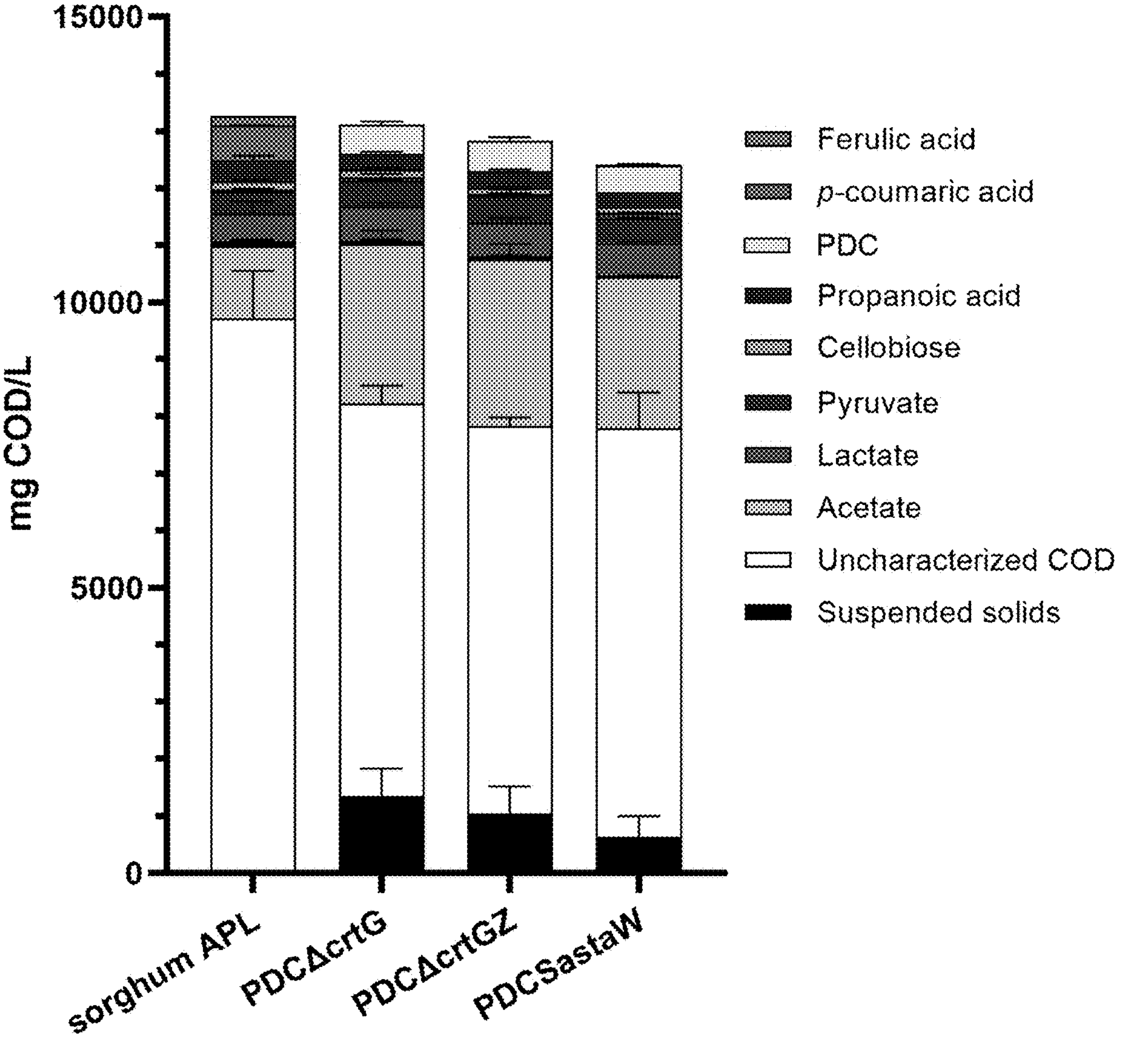

15000
10000
5000
0
mg COD/L
sorghum APL
PDCΔcrtG
PDCΔcrtGZ
PDCSastaW
Ferulic acid
p-coumaric acid
PDC
Propanoic acid
Cellobiose
Pyruvate
Lactate
Acetate
Uncharacterized COD
Suspended solids

FIG. 13

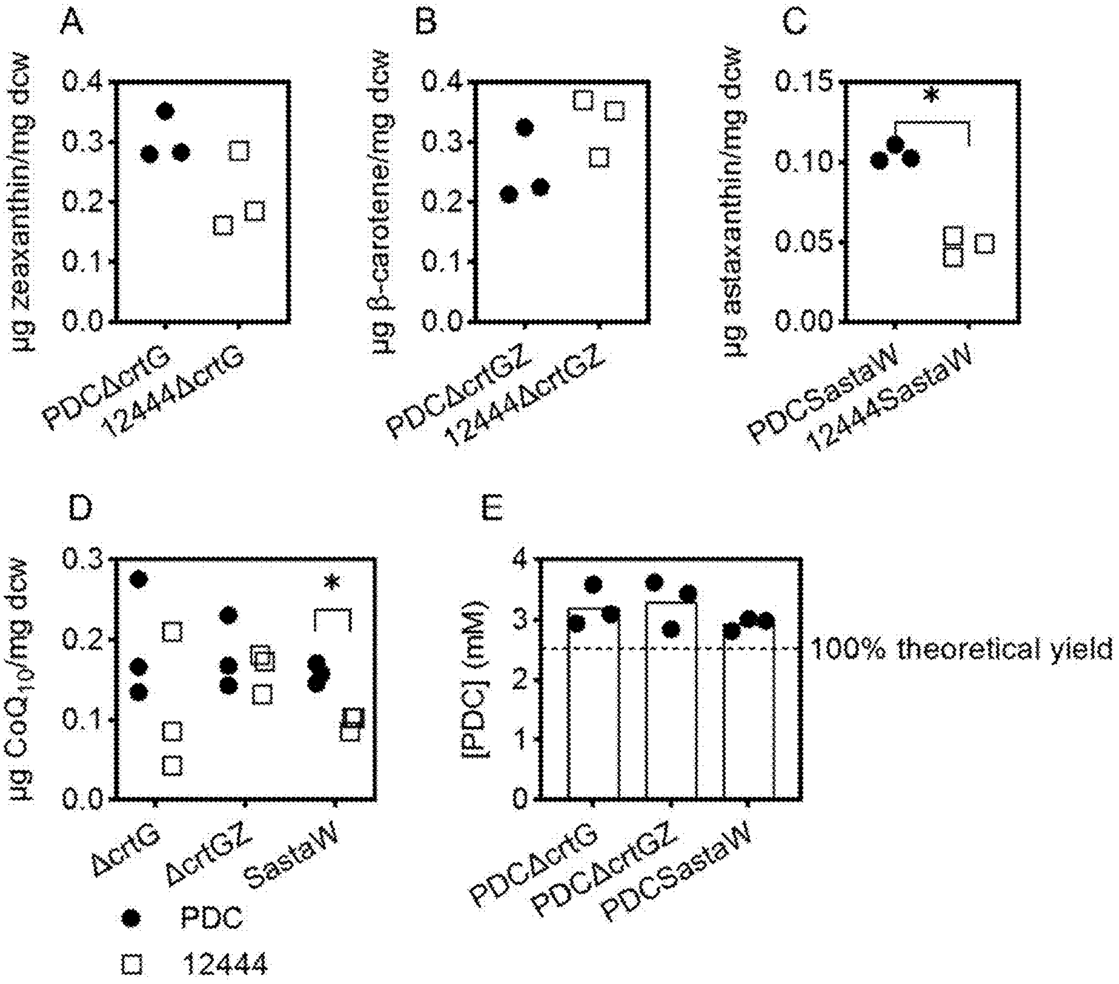

A
µg zeaxanthin/mg dcw
0.4
0.3
0.2
0.1
0.0
PDCΔcrtG
12444ΔcrtG
B
µg β-carotene/mg dcw
0.4
0.3
0.2
0.1
0.0
PDCΔcrtGZ
12444ΔcrtGZ
C
µg astaxanthin/mg dcw
0.15
0.10
0.05
0.00
*
PDCSastaW
12444SastaW
D
µg CoQ10/mg dcw
0.3
0.2
0.1
0.0
*
ΔcrtG
ΔcrtGZ
SastaW
● PDC
□ 12444
E
[PDC] (mM)
4
3
2
1
0
100% theoretical yield
PDCΔcrtG
PDCΔcrtGZ
PDCSastaW

FIG. 14

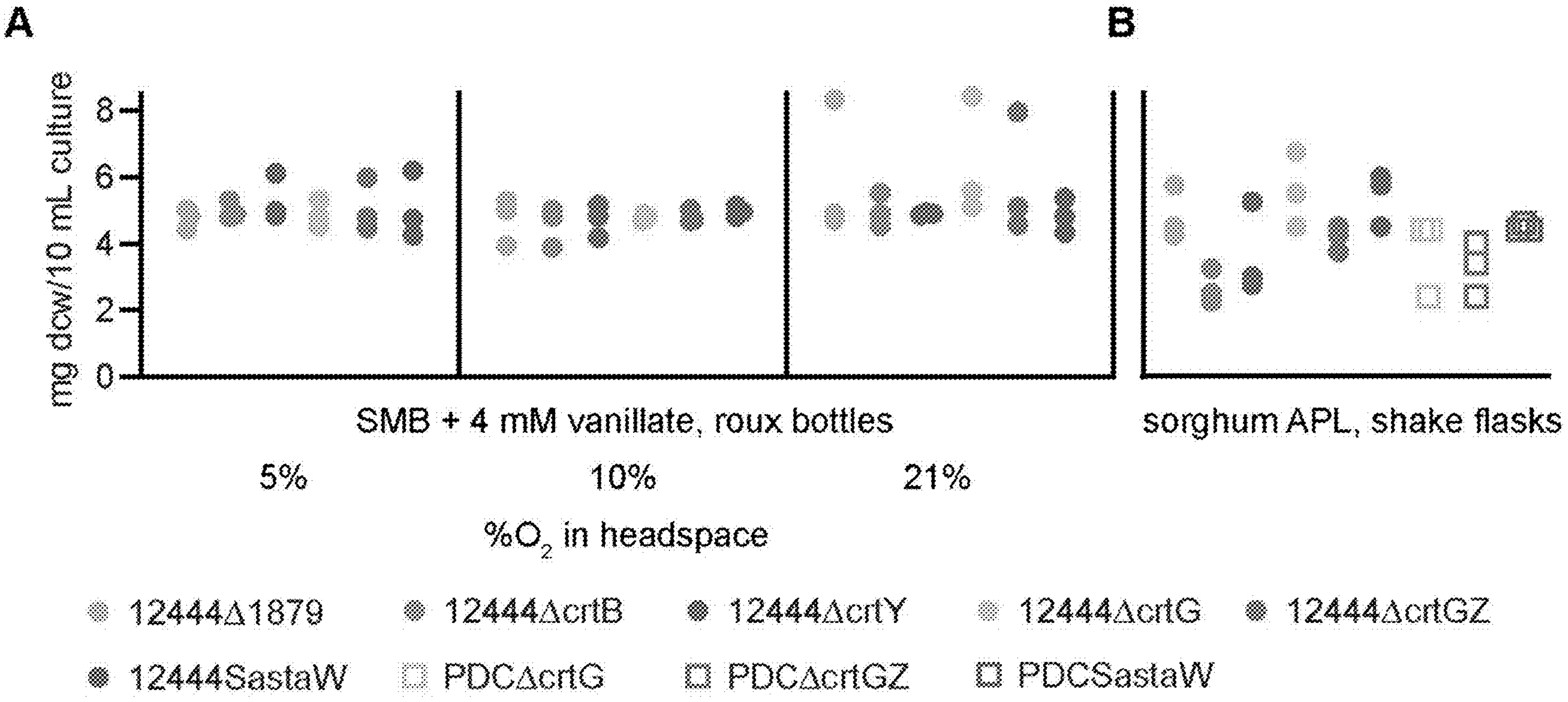

A
B
mg dcw/10 mL culture
0
2
4
6
8
SMB + 4 mM vanillate, roux bottles
5%
10%
21%
%O_2 in headspace
sorghum APL, shake flasks
12444Δ1879
12444ΔcrtB
12444ΔcrtY
12444ΔcrtG
12444ΔcrtGZ
12444SastaW
PDCΔcrtG
PDCΔcrtGZ
PDCSastaW

FIG. 15

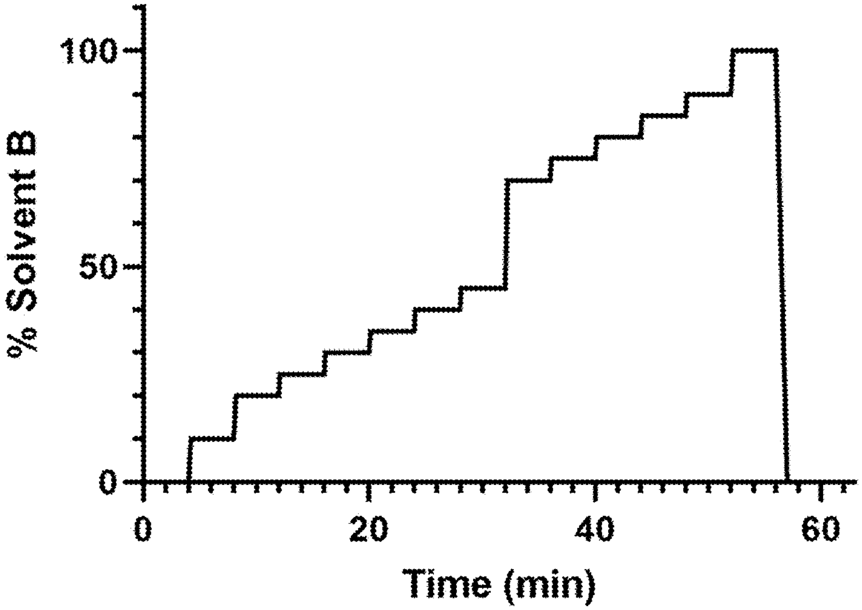

100
50
0
% Solvent B
0
20
40
60
Time (min)

FIG. 16

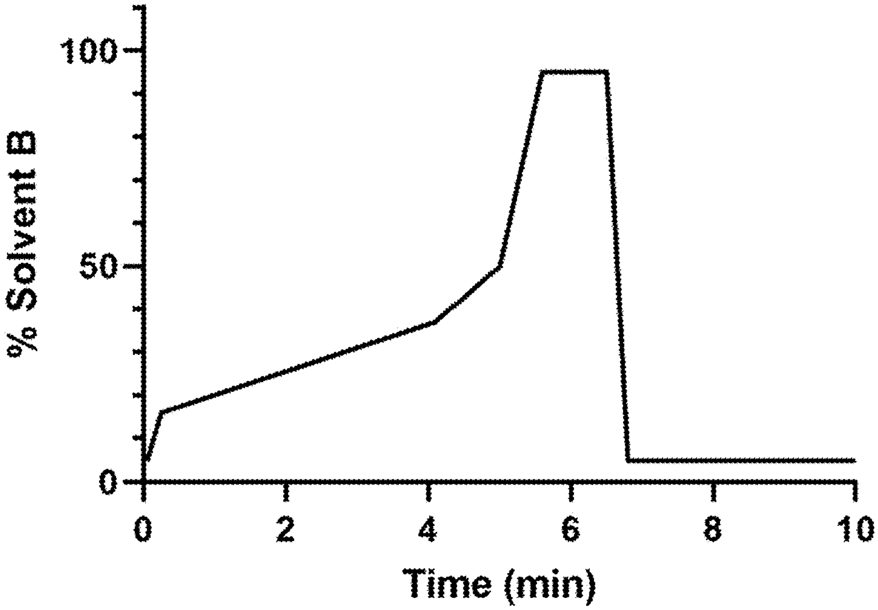

100
50
0
% Solvent B
0
2
4
6
8
10
Time (min)

FIG. 17

MICROORGANISMS AND METHODS FOR PRODUCING CAROTENOIDS AND OTHER COMPOUNDS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] Priority is hereby claimed to U.S. Provisional Application 63/712,785, filed Oct. 28, 2024, which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with government support under DE-SC0018409 awarded by the US Department of Energy. The government has certain rights in the invention.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted in XML format and is hereby incorporated by reference in its entirety. The XML copy, created on Aug. 22, 2025, is named USPTO-250822-09824639-P230405US02-SEQ_LIST.xml and is 56,670 bytes in size.

FIELD OF THE INVENTION

[0004] The invention is directed to recombinant microorganisms configured for enhanced production of carotenoids and other compounds and methods of using the recombinant microorganisms for the production of same.

BACKGROUND

[0005] Carotenoids are lipophilic isoprenoids that are produced by some plants, algae, bacteria, and fungi, and function as membrane-bound light-harvesting pigments and antioxidants (8, 9). Several carotenoids (including astaxanthin, β-carotene, lycopene, and zeaxanthin) are used industrially as animal feed, food coloring, nutritional supplements, cosmetics additives, and pharmaceuticals, with a 2017 global market size of ~$1.5B (10, 11). Most industrial carotenoids are produced synthetically (9, 10, 12), though there are a few biological sources commercially being used, such as the flower *Tagetes erecta* for lutein and the alga *Dunaliella salina* for 3-carotene (10). Thus, there is growing interest in developing new biological sources of carotenoids (9, 10). New sources for the production of carotenoids such as astaxanthin, β-carotene, lycopene, and zeaxanthin are needed.

SUMMARY OF THE INVENTION

[0006] One aspect of the invention is directed to recombinant microorganisms comprising one or more modifications with respect to a corresponding microorganism not comprising the one or more modifications.

[0007] In some versions, the one or more modifications comprise any one or more of: a modification that decreases phytoene synthase (CrtB) activity with respect to the corresponding microorganism; a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity with respect to the corresponding microorganism; a modification that decreases lycopene cyclase (CrtY) activity with respect to the corresponding microorganism; a modification that decreases beta-carotene hydroxylase (CrtZ) activity with respect to the corresponding microorganism; a modification that decreases 2,2'-beta hydroxylase (CrtG) activity with respect to the corresponding microorganism; and a modification that increases beta-carotene ketolase (CrtW) activity with respect to the corresponding microorganism.

[0008] In some versions, the modification that decreases phytoene synthase (CrtB) activity comprises a modification to a gene encoding a phytoene synthase (CrtB) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:2.

[0009] In some versions, the modification that decreases lycopene-forming phytoene (CrtI) desaturase activity comprises a modification to a gene encoding a lycopene-forming phytoene desaturase (CrtI) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:4.

[0010] In some versions, the modification that decreases lycopene cyclase (CrtY) activity comprises a modification to a gene encoding a lycopene cyclase (CrtY) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:6.

[0011] In some versions, the modification that decreases beta-carotene hydroxylase (CrtZ) activity comprises a modification to a gene encoding a beta-carotene hydroxylase (CrtZ) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:8.

[0012] In some versions, the modification that decreases 2,2'-beta hydroxylase (CrtG) activity comprises a modification to a gene encoding a 2,2'-beta hydroxylase (CrtG) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:10.

[0013] In some versions, the modification that increases beta-carotene ketolase (CrtW) activity comprises a recombinant gene encoding a beta-carotene ketolase (CrtW) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:12 or SEQ ID NO:14.

[0014] In some versions, the recombinant microorganism comprises any one or more of: a modification that decreases phytoene synthase (CrtB) activity; a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity; a modification that decreases lycopene cyclase (CrtY) activity; a modification that decreases beta-carotene hydroxylase (CrtZ) activity; and a modification that decreases 2,2'-beta hydroxylase (CrtG) activity. In some versions, the recombinant microorganism comprises any one or more of: a modification that decreases phytoene synthase (CrtB) activity; a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity; and a modification that decreases lycopene cyclase (CrtY) activity. In some versions, the recombinant microorganism comprises a modification that decreases phytoene synthase (CrtB) activity. In some of the foregoing versions, the recombinant microorganism exhibits increased accumulation of coenzyme Q_{10} (CoQ_{10}) with respect to the corresponding microorganism.

[0015] In some versions, the microorganism comprises a modification that decreases lycopene cyclase (CrtY) activity. In some of such versions, the recombinant microorganism exhibits increased accumulation of lycopene with respect to the corresponding microorganism.

[0016] In some versions, recombinant microorganism comprises a modification that decreases 2,2'-beta hydroxylase (CrtG) activity. In some such versions, the recombinant microorganism exhibits increased accumulation of zeaxanthin with respect to the corresponding microorganism.

[0017] In some versions, the recombinant microorganism comprises a modification that decreases 2,2'-beta hydroxylase (CrtG) activity and/or a modification that decreases beta-carotene hydroxylase (CrtZ) activity. In some such versions, the recombinant microorganism exhibits increased accumulation of beta-carotene with respect to the corresponding microorganism.

[0018] In some versions, the recombinant microorganism comprises a modification that decreases 2,2'-beta hydroxylase (CrtG) activity and/or a modification that increases beta-carotene ketolase (CrtW) activity. In some such versions, the recombinant microorganism exhibits increased accumulation of at least one of adonixanthin and astaxanthan with respect to the corresponding microorganism.

[0019] In some versions, the recombinant microorganism further comprises: a modification that decreases 2-pyrone-4,6-dicarboxylic acid (PDC) hydrolase (LigI) activity with respect to the corresponding microorganism; a modification that decreases 4-carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) methyl esterase (DesC) activity with respect to the corresponding microorganism; a modification that decreases 4-carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) cis-trans isomerase (DesD) activity with respect to the corresponding microorganism; and/or a modification that decreases vanillate/3-O-methylgallate O-demethylase (DmtS) activity with respect to the corresponding microorganism. In some such versions, the recombinant microorganism exhibits increased accumulation of 2-pyrone-4,6-dicarboxylic acid (PDC) with respect to the corresponding microorganism.

[0020] In some versions, the modification that decreases PDC hydrolase (LigI) activity comprises a modification to a gene encoding a PDC hydrolase (LigI) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:16.

[0021] In some versions, the modification that decreases CHMOD methyl esterase (DesC) activity a modification to a gene encoding a CHMOD methyl esterase (DesC) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:18.

[0022] In some versions, the modification that decreases CHMOD cis-trans isomerase (DesD) activity comprises a modification to a gene encoding a CHMOD cis-trans isomerase (DesD) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:20.

[0023] In some versions, the modification that decreases vanillate/3-O-methylgallate O-demethylase (DmtS) activity comprises a modification to a gene encoding a vanillate/3-O-methylgallate O-demethylase (DmtS) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:22.

[0024] In some versions, the recombinant microorganism is from the genus *Novosphingobium.*

[0025] In some versions, the recombinant microorganism is *Novosphingobium aromaticivorans.*

[0026] Another aspect of the invention is directed to methods of producing a compound.

[0027] In some versions, the methods comprise culturing a recombinant microorganism of the invention in a medium comprising a plant-derived phenolic.

[0028] In some versions, the compound comprises any one or more of coenzyme Q_{10} (CoQ_{10}), phytoene, lycopene, beta-carotene, zeaxanthin, adonixanthin, astaxanthin, and 2-pyrone-4,6-dicarboxylic acid (PDC).

[0029] In some versions, the medium comprises a plant-derived phenolic selected from the group consisting of a syringyl phenolic, a guaiacyl phenolic, and a p-hydroxyphenyl phenolic.

[0030] In some versions, the medium comprises depolymerized lignin.

[0031] In some versions, the methods comprise further comprising isolating compound from the recombinant microorganism.

[0032] The objects and advantages of the invention will appear more fully from the following detailed description of the preferred embodiment of the invention made in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[0033] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0034] FIG. **1**. Predicted carotenoid biosynthetic pathway based on the annotated genome of *N. aromaticivorans*. Reactions predicted to be present in wild-type *N. aromaticivorans* (13, 23) are shown as full arrows and include the genes predicted to code for the enzymes involved. Reactions predicted to occur in strains in which crtG has been replaced in the genome with a crtW gene are shown as dashed arrows. Abbreviations are: DMAPP=dimethylallyl pyrophosphate, IPP=isopentenyl diphosphate, GPP=geranyl diphosphate, FPP=farnesyl diphosphate, and GGPP=geranylgeranyl pyrophosphate.

[0035] FIG. **2**. HPLC analysis and absorbance spectra of carotenoid and CoQ_{10} standards used in this study. Data are shown in milliabsorbance units (mAu).

[0036] FIGS. **3**A-**3**G. HPLC analyses of acetone:methanol extracts of representative *N. aromaticivorans* cultures. Absorbances are shown for 273 nm (the wavelength of maximum absorbance for CoQ_{10}; blue line) and 470 nm (a wavelength where all carotenoids investigated have some absorbance; orange line). Standard compounds (top panels in FIGS. **3**A-**3**G) analyzed are astaxanthin, zeaxanthin, β-carotene, lycopene, and CoQ_{10}; vertical dotted lines in the bottom panels of FIGS. **3**A-**3**G show the time of maximum absorbance for the carotenoid standards. Strains analyzed are: 12444Δ1879 (FIG. **3**A), 12444ΔcrtB (FIG. **3**B), 12444ΔcrtY (FIG. **3**C), 12444ΔcrtG (FIG. **3**D), 12444ΔcrtGZ (FIG. **3**E), 12444SastaW (FIG. **3**F), and 12444StaxiW (FIG. **3**G). Data are shown as milliabsorbance units (mAu). Major carotenoid peaks are numbered from 1 to 13. Absorbance spectra for all major peaks are shown in FIGS. **2** and **4**A-**10**B.

[0037] FIGS. **4**A-**4**D. Compounds detected in HPLC analyses of *N. aromaticivorans* 12444Δ1879 acetone:methanol extracts. HPLC data reproduced from FIG. **3**A (FIG. **4**A, top panel) is used to organize the absorbance spectra of different compounds (FIG. **4**A, bottom panels), some of which have associated mass spectrometry scans (FIG. **4**B, compound 1 (nostoxanthin); FIG. **4**C, compound 2; FIG. **4**D, compound 3 (caloxanthin)). Dotted lines in the top panel of FIG. **4**A denote retention times of carotenoid standards. The mass spectrometry scans in FIGS. **4**B-**4**D are continued from the top panel to the second panel, with each panel including an overlapping section of the scan.

[0038] FIG. **5**. Compounds detected in HPLC analyses of *N. aromaticivorans* 12444ΔcrtB acetone:methanol extracts. HPLC data reproduced from FIG. **3**B (FIG. **5**, top panel) is used to organize the absorbance spectra of different compounds (FIG. **5**, bottom panel).

[0039] FIG. **6**. Compounds detected in HPLC analyses of *N. aromaticivorans* 12444ΔcrtY acetone:methanol extracts. HPLC data reproduced from FIG. **3**C (FIG. **6**, top panel) is used to organize the absorbance spectra of different compounds (FIG. **6**, bottom panels). Dotted lines in the top panel of FIG. **6** denote retention times of carotenoid standards.

[0040] FIG. **7**. Compounds detected in HPLC analyses of *N. aromaticivorans* 12444ΔcrtG acetone:methanol extracts. HPLC data reproduced from FIG. **3**D (FIG. **7**, top panel) is used to organize the absorbance spectra of different compounds (FIG. **7**, bottom panels). Dotted lines in the top panel of FIG. **7** denote retention times of carotenoid standards.

[0041] FIG. **8**. Compounds detected in HPLC analyses of *N. aromaticivorans* 12444ΔcrtGZ acetone:methanol extracts. HPLC data reproduced from FIG. **3**E (FIG. **8**, top panel) is used to organize the absorbance spectra of different compounds (FIG. **8**, bottom panels). Dotted lines in the top panel of FIG. **8** denote retention times of carotenoid standards.

[0042] FIGS. **9**A and **9**B. Compounds detected in HPLC analyses of *N. aromaticivorans* 12444SastaW acetone:methanol extracts. HPLC data reproduced from FIG. **3**F (FIG. **9**A, top panel) is used to organize the absorbance spectra of different compounds (FIG. **9**A, bottom panels), some of which have associated mass spectrometry scans (FIG. **9**B). Compound 8's absorbance was too low to obtain an accurate absorbance or mass spectrum. Dotted lines in the top panel of FIG. **9**A denote retention times of carotenoid standards. The mass spectrometry scan in FIG. **9**B is continued from the top panel to the second panel, with each panel including an overlapping section of the scan.

[0043] FIGS. **10**A and **10**B. Compounds detected in HPLC analyses of *N. aromaticivorans* 12444StaxiW acetone:methanol extracts. HPLC data reproduced from FIG. **3**G (FIG. **10**A, top panel) is used to organize the absorbance spectra of different compounds (FIG. **10**A, bottom panels), some of which have associated mass spectrometry scans (FIG. **10**B). Dotted lines in the top panel of FIG. **10**A denote retention times of carotenoid standards. The mass spectrometry scan in FIG. **10**B is continued from the top panel to the second panel, with each panel including an overlapping section of the scan.

[0044] FIG. **11**. SMB-Glucose plates of parent and mutant *N. aromaticivorans* strains showing the different colony colors in carotenoid pathway deletion mutants (A, left plate) or in crtW-expressing engineered strains (B, right plate). Some colony colors appear similar despite accumulating different carotenoids (FIGS. **3**A-G) due to the known similarity in the absorption spectra of their carotenoids (FIGS. **2** and **4**A-**10**B).

[0045] FIG. **12**. Carotenoid and CoQ_{10} levels in *N. aromaticivorans* strains. Filled circles show data for roux bottle vanillate-fed cultures bubbled with a gas mixture of defined composition; open squares show data for shake flask *Sorghum* APL cultures supplied with atmospheric O_2. Amounts of select carotenoids, normalized by dry cell weight (dcw), are plotted for *N. aromaticivorans* strains 12444Δ1879 producing nostoxanthin (A), 12444ΔcrtY producing lycopene (B), 12444ΔcrtG producing zeaxanthin (C), 12444ΔcrtGZ producing β-carotene (D), and 12444SastaW producing astaxanthin (E). Data for roux bottle vanillate cultures were plotted against headspace O_2 concentration, and p-values are shown for single-factor ANOVA tests ($\alpha=0.05$) comparing the data at all three O_2 concentrations for each strain (null hypothesis assuming no difference at the three O_2 concentrations). CoQ_{10} levels are also shown for those same strains, plus 12444ΔcrtB, grown in roux bottles with vanillate and in shake flasks with *Sorghum* APL (F). Nostoxanthin accumulation is reported as HPLC peak area due to the lack of a standard. For all other compounds, amounts are reported as mass normalized to dry cell weight. An asterisk (*) denotes data from shake flask *Sorghum* APL cultures that are significantly different from 21% O_2 roux bottle culture data (Student's T-test, $p<0.05$).

[0046] FIG. **13**. Chemical oxygen demand (COD) of *Sorghum* APL before and after incubation with indicated *N. aromaticivorans* strains. All detected compounds in Table 2 are included in the graphs, but many are too low in abundance to be visible in the figure. COD provides an estimate of the available chemical energy in a solution by measuring how much oxygen is required to fully oxidize the organic molecules to CO_2. COD was measured as described herein.

[0047] FIG. **14**. Carotenoid (A-C), CoQ_{10} (D), and PDC (E) levels in *N. aromaticivorans* PDC strains grown in *Sorghum* APL. Filled circles show data for PDC-producing strains, open squares show data for non-PDC producing strains grown in *Sorghum* APL reproduced from FIG. **12**. Amounts of carotenoids and are reported as mass normalized to dry cell weight (dew). PDC levels are reported as concentration measured in the media. An asterisk (*) denotes PDC strain data that are significantly different from the 12444 parent strain data (Student's T-test, $p<0.05$).

[0048] FIG. **15**. Dry cell weights normalized to 10 mL of culture of *N. aromaticivorans* strains grown in SMB+4 mM vanillate at different O_2 concentrations (A) or in *Sorghum* APL in shake flasks (B).

[0049] FIG. **16**. Mobile phase binary gradient for HPLC analysis of compounds in *N. aromaticivorans* acetone:methanol extracts. Solvent A is 70% acetonitrile/30% water and Solvent B is 70% acetonitrile/30% isopropanol. The method proceeds for 63 min.

[0050] FIG. **17**. Mobile phase binary gradient for HPLC analysis of aromatic compounds in *Sorghum* APL. Solvent A is 0.2% formic acid in water and solvent B is methanol. The method ends at 10 min.

DETAILED DESCRIPTION OF THE INVENTION

[0051] One aspect of the invention is directed to recombinant microorganisms. The recombinant microorganisms of the invention can be configured for enhanced production of various carotenoids and/or other compounds. The recombinant microorganisms of the invention comprise one or more modifications that increase the activity of one or more genes or gene products, decrease the activity of one or more genes or gene products, or increase the activity of one or more genes or gene products and decrease the activity of one or more genes or gene products. The recombinant microorganisms with the modifications can exhibit enhanced production of a compound such as coenzyme Q_{10} (CoQ_{10}), phytoene, lycopene, beta-carotene, zeaxanthin, adonixanthin, astaxanthin, and/or 2-pyrone-4,6-dicarboxylic acid (PDC) with respect to corresponding microorganisms not comprising the modifications.

[0052] "Modifications that increase the activity of one or more genes or gene products" refers to any modification to microorganism that increases expression of a gene in producing its gene product or increases the functioning of the gene product. "Increase" in this context encompasses increasing beyond a baseline activity. The baseline activity can be a positive baseline activity or null activity. Exemplary modifications that increase the activity of one or more genes or gene products include genetic modifications. The genetic modifications include genetic modifications to a gene in a manner that increases expression of the gene in producing the gene product. Such modifications include operationally connecting the coding sequence to a stronger promoter or enhancer, etc., and/or introducing additional copies of the gene (whether the native gene or a recombinant version). The genetic modifications also include mutations to a first gene (such as a transcription factor or an inhibitor of a transcription factor) that affects the expression of a second gene. The genetic modifications also include one or more copies of an exogenous or heterologous gene introduced into the microorganism. Other genetic modifications are described herein.

[0053] "Modifications that decrease the activity of one or more genes or gene products" refers to any modification to a microorganism that decreases expression of the gene and thus production of the gene product and/or decreases the functioning of the gene product per se. "Decrease" in this context encompasses reducing below a positive baseline level of expression and/or activity to a lower level of expression and/or activity. The lower level of expression and/or activity can be a lower positive level of expression and/or activity or null expression and/or activity. Decreasing the functioning of a gene product may comprise decreasing the specific activity of a gene product. Exemplary modifications that decrease the activity of one or more genes or gene products include genetic modifications. Exemplary genetic modifications include mutations to a gene that decrease expression of the gene in producing the gene product. Such mutations may include mutations to the coding sequence, the promoter, an enhancer, any other part of the gene, or the entire gene. Other exemplary genetic modifications include mutations to the coding sequence of a gene that decrease the functioning of a gene product expressed from the gene. Exemplary mutations include substitutions, insertions, and deletions, including partial and full deletions of a particular gene. Other exemplary genetic modifications include recombinant nucleotide sequences configured to express antisense RNAs or other molecules that decrease production of a gene product. Other exemplary genetic modifications include mutations to a first gene (such as a transcription factor or an inhibitor of a transcription factor) that affects the expression of a second gene. Other exemplary genetic modifications are described elsewhere herein. Other modifications include epigenetic modifications, such as methylation, etc.

[0054] "Corresponding microorganism" refers to a microorganism of the same species having the same or substantially same genetic and proteomic composition as a recombinant microorganism of the invention, with the exception of genetic and proteomic differences resulting from the modifications specified herein for the recombinant microorganisms of the invention in a given particular embodiment. In some versions, the corresponding microorganism is the native version of the recombinant microorganism of the invention, i.e., the unmodified microorganism as found in nature. The terms "microorganism" and "microbe" are used interchangeably herein.

[0055] In some versions, the recombinant microorganisms comprise one or more modifications with respect to a corresponding microorganism not comprising the one or more modifications. The one or more modifications can comprise, in any combination, a modification that decreases phytoene synthase (CrtB) activity with respect to the corresponding microorganism, a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity with respect to the corresponding microorganism, a modification that decreases lycopene cyclase (CrtY) activity with respect to the corresponding microorganism, a modification that decreases beta-carotene hydroxylase (CrtZ) activity with respect to the corresponding microorganism, a modification that decreases 2,2'-beta hydroxylase (CrtG) activity with respect to the corresponding microorganism, and a modification that increases beta-carotene ketolase (CrtW) activity with respect to the corresponding microorganism.

[0056] Phytoene synthase (CrtB) activity includes activity characterized by EC 2.5.1.32 and comprises the ability to catalyze the conversion of geranylgeranyl pyrophosphate to phytoene. An exemplary phytoene synthase is CrtB (Saro_1814) of *Novosphingobium aromaticivorans*, the nucleic acid coding sequence of which is SEQ ID NO:1 and the protein sequence of which is SEQ ID NO:2. Other exemplary phytoene synthases include proteins with phytoene synthase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:2. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that decreases phytoene synthase activity. A genetic modification that decreases phytoene synthase activity can comprise a genetic modification to a phytoene synthase gene. A genetic modification to a phytoene synthase gene can comprise a substitution or insertion in or a complete or partial deletion of the phytoene synthase gene.

[0057] Lycopene-forming phytoene desaturase (CrtI) activity includes activity characterized by EC 1.3.99.31 and comprises the ability to catalyze the conversion of phytoene to lycopene. An exemplary lycopene-forming phytoene desaturase is CrtI (Saro_1816) of *Novosphingobium aromaticivorans*, the nucleic acid coding sequence of which is SEQ ID NO:3 and the protein sequence of which is SEQ ID NO:4. Other exemplary lycopene-forming phytoene desaturases include proteins with lycopene-forming phytoene desaturase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:4. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that decreases lycopene-forming phytoene desaturase activity. A genetic modification that decreases lycopene-forming phytoene desaturase activity can comprise a genetic modification to a lycopene-forming phytoene desaturase gene. A genetic modification to a lycopene-forming phytoene desaturase gene can comprise a substitution or insertion in or a complete or partial deletion of the lycopene-forming phytoene desaturase gene.

[0058] Lycopene cyclase (CrtY) activity includes activity characterized by EC 5.5.1.19 and comprises the ability to catalyze the conversion of lycopene to beta-carotene. An exemplary lycopene cyclase is CrtY (Saro_1817) of *Novosphingobium aromaticivorans*, the nucleic acid coding sequence of which is SEQ ID NO5; and the protein sequence of which is SEQ ID NO:6. Other exemplary lycopene cyclases include proteins with lycopene cyclase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:6. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that decreases lycopene cyclase activity. A genetic modification that decreases lycopene cyclase activity can comprise a genetic modification to a lycopene cyclase gene. A genetic modification to a lycopene cyclase gene can comprise a substitution or insertion in or a complete or partial deletion of the lycopene cyclase gene.

[0059] Beta-carotene hydroxylase (CrtZ) activity includes activity characterized by EC 1.14.15.24 and comprises the ability to catalyze the conversion of beta-carotene to zeaxanthin, among other activities. An exemplary beta-carotene hydroxylase is CrtZ (Saro_1168) of *Novosphingobium aromaticivorans*, the nucleic acid coding sequence of which is SEQ ID NO:7 and the protein sequence of which is SEQ ID NO:8. Other exemplary beta-carotene hydroxylases include proteins with beta-carotene hydroxylase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:8. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that decreases beta-carotene hydroxylase activity. A genetic modification that decreases beta-carotene hydroxylase activity can comprise a genetic modification to a beta-carotene hydroxylase gene. A genetic modification to a beta-carotene hydroxylase gene can comprise a substitution or insertion in or a complete or partial deletion of the beta-carotene hydroxylase gene.

[0060] 2,2'-Beta hydroxylase (CrtG) activity includes activity characterized by EC 1.14.13.—and comprises the ability to catalyze the conversion of zeaxanthin to caloxanthin, caloxanthin to nostoxanthin, and/or beta-carotene to 2,2'-dihydroxy-beta-carotene. An exemplary 2,2'-beta hydroxylase is CrtG (Saro_0236) of *Novosphingobium aromaticivorans*, the nucleic acid coding sequence of which is SEQ ID NO:9 and the protein sequence of which is SEQ ID NO:10. Other exemplary 2,2'-beta hydroxylases include proteins with 2,2'-beta hydroxylase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:10. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that decreases 2,2'-beta hydroxylase activity. A genetic modification that decreases 2,2'-beta hydroxylase activity can comprise a genetic modification to a 2,2'-beta hydroxylase gene. A genetic modification to a 2,2'-beta hydroxylase gene can comprise a substitution or insertion in or a complete or partial deletion of the 2,2'-beta hydroxylase gene.

[0061] Beta-carotene ketolase activity includes activity characterized by EC 1.14.99.63 and comprises the ability to catalyze the conversion of beta-carotene to canthaxanthin, and/or the conversion of zeaxanthin to adonixanthin, and/or the conversion of adonixanthin to astaxanthin. Beta-carotene ketolase activity is performed by beta-carotene ketolase, sometimes referred to as CrtW. An exemplary beta-carotene ketolase is CrtW of *Sphingomonas taxi*, the nucleic acid coding sequence of which is SEQ ID NO:11 and the protein sequence of which is SEQ ID NO:12. Another exemplary beta-carotene ketolase is CrtW of *Sphingomonas astaxanthinfaciens*, the nucleic acid coding sequence of which is SEQ ID NO:13 and the protein sequence of which is SEQ ID NO: 14. Other exemplary beta-carotene ketolases include proteins with beta-carotene ketolase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NOS:12 or 14. Other beta-carotene ketolases are known in the art. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that increases beta-carotene ketolase activity. A genetic modification that increases beta-carotene ketolase activity can comprise a recombinant beta-carotene ketolase gene. In some versions, the recombinant beta-carotene ketolase gene is an exogenous recombinant beta-carotene ketolase gene newly introduced to the microorganism. In some versions, the recombinant beta-carotene ketolase gene is a modified form of an endogenous beta-carotene ketolase gene already present in the microorganism.

[0062] 2-Pyrone-4,6-dicarboxylic acid (PDC) hydrolase activity (LigI) activity comprises activity characterized by EC 3.1.1.57 and comprises the ability to hydrolyze PDC to produce 4-oxalomesaconate (OMA). An exemplary PDC hydrolase is LigI (Saro_2819) of *Novosphingobium aromaticivorans*, the nucleic acid coding sequence of which is SEQ ID NO15; and the protein sequence of which is SEQ ID NO:16. Other exemplary PDC hydrolases include proteins with PDC hydrolase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO: 16. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that decreases PDC hydrolase activity. A genetic modification that decreases PDC hydrolase activity can comprise a genetic modification to a PDC hydrolase gene. A genetic modification to a PDC hydrolase gene can comprise a substitution or insertion in or a complete or partial deletion of the PDC hydrolase gene.

[0063] 4-Carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) methyl esterase (DesC) activity comprises the ability to demethylate CHMOD to produce OMA. An exemplary CHMOD methyl esterase is desC/DesC (Saro_2864) of *Novosphingobium aromaticivorans*, the nucleic acid coding sequence of which is SEQ ID NO: 17 and the protein sequence of which is SEQ ID NO:18. Other exemplary CHMOD methyl esterases include proteins with CHMOD methyl esterase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:18. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that decreases CHMOD methyl esterase activity. A genetic modification that decreases CHMOD methyl esterase activity can comprise a genetic modification to a CHMOD methyl esterase gene. A genetic modification to a CHMOD methyl esterase gene can comprise a substitution or insertion in or a complete or partial deletion of the CHMOD methyl esterase gene.

[0064] 4-Carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) cis-trans isomerase (DesD) activity comprises the ability to isomerize stereoisomers of CHMOD. An exemplary CHMOD cis-trans isomerase is DesD (Saro_2865) of *Novosphingobium aromaticivorans*, the nucleic acid coding sequence of which is SEQ ID NO: 19 and the protein sequence of which is SEQ ID NO:20. Other exemplary CHMOD cis-trans isomerases include proteins with CHMOD cis-trans isomerase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:20. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that decreases CHMOD cis-trans isomerase activity. A genetic modification that decreases CHMOD cis-trans isomerase activity can comprise a genetic modification to a CHMOD cis-trans isomerase gene. A genetic modification to a CHMOD cis-trans isomerase gene can comprise a substitution or insertion in or a complete or partial deletion of the CHMOD cis-trans isomerase gene.

[0065] Vanillate/3-O-methylgallate O-demethylase (DmtS) activity comprises the ability to 0-demethylate substrates such as vanillate and/or 3-methoxygallic acid. Vanillate/3-O-methylgallate O-demethylases include enzymes having activity characterized under one more of Enzyme Commission (EC) Numbers 2.1.1.341 and 1.14.13.82. An exemplary vanillate/3-O-methylgallate O-demethylase is VanA or DmtS (Saro_2861) of *Novosphingobium aromaticivorans*, the nucleic acid coding sequence of which is SEQ ID NO:21 and the protein sequence of which is SEQ ID NO:22. Other exemplary vanillate/3-O-methylgallate O-demethylases include proteins with phytoene synthase activity having a sequence at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to SEQ ID NO:22. In some versions of the invention, the one or more modifications in the recombinant microorganisms can comprise a genetic modification that decreases vanillate/3-O-methylgallate O-demethylase activity. A genetic modification that decreases vanillate/3-O-methylgallate O-demethylase activity can comprise a genetic modification to a vanillate/3-O-methylgallate O-demethylase gene. A genetic modification to a vanillate/3-O-methylgallate O-demethylase gene can comprise a substitution or insertion in or a complete or partial deletion of the vanillate/3-O-methylgallate O-demethylase gene.

[0066] "Gene" refers to a nucleic acid sequence capable of producing a gene product and may include such genetic elements as a coding sequence together with any other genetic elements required for transcription and/or translation of the coding sequence. Such genetic elements may include a promoter, an enhancer, and/or a ribosome binding site (RBS), among others. In some versions, multiple genes are configured in an operon, in which multiple coding sequences are operationally connected to a single promoter. Each coding sequence and promoter pair in such instances are considered herein to constitute separate genes, despite comprising the same promoter.

[0067] "Gene product" refers to products such as a polypeptide or an mRNA encoded and produced by a particular gene.

[0068] "Operationally connected" refers to a relationship between two genetic elements (e.g., a promoter and coding sequence), in which one of the genetic elements controls or affects the activity of the other genetic element.

[0069] "Endogenous" used in reference to a genetic element means that the genetic element is native to the microorganism in which it is disposed.

[0070] "Exogenous" used in reference to a genetic element means that the genetic element is not native to the microorganism in which it is disposed.

[0071] "Heterologous" used in reference to a genetic element means that the genetic element is derived from a different species than that in which it is disposed or is disposed in relation to another element (genetic element, sequence) in a non-natural arrangement.

[0072] "Recombinant" as used herein with reference to nucleic acid molecules or polypeptides refers to nucleic acid molecules or polypeptides having a non-natural nucleic acid or polypeptide sequence, respectively. Recombinant" as used herein with reference to a gene refers to a gene having a non-natural nucleic acid sequence, is exogenous, is heterologous, or is endogenous to a given microbe but is disposed within the microbe (e.g., within the microbe's genome) at a locus different from the native form of the gene. "Recombinant" as used herein with reference to a cell or microorganism refers to a cell or microorganism that contains a recombinant nucleic acid molecule, polypeptide, or gene.

[0073] "Genetic modification" as used herein refers to any difference in the nucleic acid composition of a cell with respect to a corresponding cell, whether in the cell's native chromosome or in endogenous or exogenous non-chromosomal plasmids harbored within the cell.

[0074] "Overexpress" as used herein means that a particular gene product is produced at a higher level in one cell, such as a recombinant cell, than in a corresponding cell. For example, a microorganism that includes a recombinant nucleic acid configured to overexpress a gene product produces the gene product at a greater amount than a microorganism of the same species that does not include the recombinant nucleic acid.

[0075] A "homologous" gene or protein is a gene or protein inherited in two species from a common ancestor. While homologous genes or proteins can be similar in sequence, similar sequences are not necessarily homologous.

[0076] The terms "identical" or "percent identity", in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described herein (or other algorithms available to persons of skill) or by visual inspection. For sequence comparison and identity determination, one sequence typically acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence based on the designated program parameters. A typical reference sequence of the invention is any nucleic acid or amino acid sequence described herein. Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, Adv. Appl. Math. 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson & Lipman, Proc. Nat'l. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see Current Protocols in Molecular Biology, F. M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2008)). One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity for purposes of defining homologs is the BLAST algorithm, which is described in Altschul et al., *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul et al., supra). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always>0) and N (penalty score for mismatching residues; always<0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=−4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff (1989) Proc. Natl. Acad. Sci. USA 89:10915). In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin & Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001. The above-described techniques are useful in determining sequence identity of sequences described herein.

[0077] In addition to mechanisms described elsewhere herein, genetic modifications for increasing the activity of a gene or protein include but are not limited to placing the coding sequence under the control of a more active promoter, increasing the copy number of genes comprising the coding sequence, introducing a translational enhancer on a gene comprising the coding sequence (see, e.g., Olins et al. *Journal of Biological Chemistry,* 1989, 264(29):16973-16976), and/or modifying factors (e.g., transcription factors or genes therefor) that control expression of a gene comprising the coding sequence. Increasing the copy number of genes comprising a coding sequence can be performed by introducing one or more additional copies of the native gene to the microorganism, introducing one or more heterologous homologs to the microorganism, introducing one or more copies of recombinant versions of the native gene or heterologous homolog to the microorganism, etc. Genes expressing a given coding sequence may be incorporated into the microbial genome or included on an extrachromosomal genetic construct such as a plasmid.

[0078] In addition to mechanisms described elsewhere herein, genetic modifications for decreasing the activity of a gene or protein include but are not limited to substitutions, partial or complete deletions, insertions, or other variations to a coding sequence or a sequence controlling the transcription or translation of a coding sequence, such as placing a coding sequence under the control of a less active promoter, etc. In some versions, the genetic modifications can include the introduction of constructs that express ribozymes or antisense sequences that target the mRNA of the gene of interest. Various other genetic modifications that decrease the activity of a gene or gene product are described elsewhere herein.

[0079] Various methods for introducing genetic modifications are well known in the art and include homologous recombination, among other mechanisms. See, e.g., Green et al., *Molecular Cloning: A laboratory manual,* 4th ed., Cold Spring Harbor Laboratory Press (2012) and Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 3rd ed., Cold Spring Harbor Laboratory Press (2001).

[0080] The recombinant genes of the invention can be codon-optimized for the particular microorganism in which they are introduced. Codon optimization can be performed for any nucleic acid by a number of programs, including "GENEGPS"-brand expression optimization algorithm by DNA 2.0 (Menlo Park, CA), "GENEOPTIMIZER"-brand gene optimization software by Life Technologies (Grand Island, NY), and "OPTIMUMGENE"-brand gene design system by GenScript (Piscataway, NJ). Other codon optimization programs or services are well known and commercially available.

[0081] The recombinant microorganisms of the invention may be prokaryotic or eukaryotic. Suitable prokaryotes include bacteria and archaea. Suitable types of bacteria include α- and γ-proteobacteria, gram-positive bacteria, gram-negative bacteria, ungrouped bacteria, phototrophs, lithotrophs, and organotrophs. Suitable eukaryotes include yeast and other fungi. The microorganism in some versions can be from an order selected from the group consisting of Sphingomonadales and Pseudomonadales. The microorganism in some versions can be from a family selected from the group consisting of Sphingomonadaceae, Pseudomonadaceae, and Enterobacteriaceae. The microorganism in some versions can be from a genus selected from the group consisting of *Sphingomonas, Sphingobium, Sphingosinicella, Sphingopyxis, Novosphingobium, Pseudomonas, Erythrobacter* (e.g., sp. SG61-1L), *Altererythrobacter, Enterobacter*, and *Klebsiella*, among others.

[0082] The microorganism in some versions can be a phenol-degrading microorganism, such as a phenol-degrading bacterium. Phenol-degrading microorganisms, including phenol-degrading bacteria, are well known in the art. See, e.g., Gu et al. 2016 (Gu Q, Wu Q, Zhang J, Guo W, Wu H, Sun M. Community Analysis and Recovery of Phenol-degrading Bacteria from Drinking Water Biofilters. Front Microbiol. 2016 Apr. 12; 7:495), Ramió-Pujol et al. 2013 (Ramió-Pujol S, Baneras L, Artigas J, Romani A M. Changes of the phenol-degrading bacterial community during the decomposition of submersed *Platanus acerifolia* leaves. FEMS Microbiol Lett. 2013 January; 338(2):184-91), Bastos et al. 2000 (Bastos A E, Moon D H, Rossi A, Trevors J T, Tsai S M. Salt-tolerant phenol-degrading microorganisms isolated from Amazonian soil samples. Arch Microbiol. 2000 November; 174(5):346-52), van Schie et al. 1998 (van Schie P M, Young L Y. Isolation and characterization of phenol-degrading denitrifying bacteria. Appl Environ Microbiol. 1998 July; 64(7):2432-8), Paisio et al. 2012 (Paisio C E, Talano M A, González P S, Busto V D, Talou J R, Agostini E. Isolation and characterization of a *Rhodococcus* strain with phenol-degrading ability and its potential use for tannery effluent biotreatment. Environ Sci Pollut Res Int. 2012 September; 19(8):3430-9), Kumari et al. 2013 (Kumari S, Chetty D, Ramdhani N, Bux F. Phenol degrading ability of *Rhodococcus pyrinidivorans* and *Pseudomonas aeruginosa* isolated from activated sludge plants in South Africa. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2013; 48(8):947-53), among others. Examples of phenol-degrading microorganisms include *Pseudomonas putida* (Abu Hamed T., Bayraktar E., Mehmetoğlu Ü., Mehmetoğlu T. (2004). The biodegradation of benzene, toluene and phenol in a two-phase system. Biochem. Eng. J. 19 137-146), *Gliomastix indicus* (Singh R. K., Kumar S., Kumar S., Kumar A. (2008) Biodegradation kinetic studies for the removal of p-cresol from wastewater using *Gliomastix indicus* MTCC 3869. Biochem. Eng. J. 40 293-303), *Sphingomonas chlorophenolica* (Nair C. I., Jayachandran K., Shashidhar S. (2008). Biodegradation of phenol. Afr. J. Biotechnol. 7 4951-4958), *Bacillus brevis* (Arutchelvan V., Kanakasabai V., Elangovan R., Nagarajan S., Muralikrishnan V. (2006). Kinetics of high strength phenol degradation using *Bacillus brevis*. J. Hazardous Materials 129 216-222), and *Cyanobacterium synechococcus* (Song H., Liu Y., Xu W., Zeng G., Aibibu N., Xu L., et al. (2009). Simultaneous Cr (VI) reduction and phenol degradation in pure cultures of *Pseudomonas aeruginosa* CCTCC AB91095. Bioresour. Technol. 100 5079-5084), and *Acinetobacter* sp. (Gu Q, Wu Q, Zhang J, Guo W, Wu H, Sun M. Community Analysis and Recovery of Phenol-degrading Bacteria from Drinking Water Biofilters. Front Microbiol. 2016 Apr. 12; 7:495). Other examples of phenol-degrading microorganisms include *Achromobacter* sp., *Alcaligenes denitripzcans, Arthrobacter* sp., *Arthrobacter sulphureus, Acidovorax delafieldii, Bacillus cereus, Brevibacterium* sp., *Burkholderia* sp., *Burkholderia cepacia, Burkholderia cocovenenans, Burkholderia xenovorans, Chryseobacterium* sp., *Cycloclasticus* sp., *Janibacter* sp., *Marinobacter, Mycobacterium* sp., *Mycobacterium flavescens, Mycobacterium vanbaalenii, Mycobacterium* sp., *Nocardioides aromaticivorans, Pasteurella* sp., *Polaromonas naphthalenivorans, Pseudomonas* sp., *Pseudomonas paucimobilis, Pseudomonas vesicularis, Pseudomonas putida, Pseudomonas fluorescens, Pseudomonas stutzeri, Pseudomonas saccharophilia, Ralstonia* sp., *Rhodococcus* sp., *Rhodococcus erythropolis, Staphylococcus* sp., *Stenotrophomonas maltophilia, Sphingomonas yanoikuyae, Sphingomonas* sp., *Sphingomonas paucimobilis, Sphingomonas wittichii, Terrabacter* sp., and *Xanthamonas* sp. (Seo J-S, Keum Y-S, Li Q X. Bacterial Degradation of Aromatic Compounds. International Journal of Environmental Research and Public Health. 2009; 6(1): 278-309.) Other examples of phenol-degrading microorganism include *Acinetobacter calcoaceticus, Rhodococcus aetherivorans, Rhodococcus ruber* SD3, *Aspergillus oryzae*, and *Aspergillus flavus* (Xu N, Qiu C, Yang Q, Zhang Y, Wang M, Ye C, Guo M. Analysis of Phenol Biodegradation in Antibiotic and Heavy Metal Resistant *Acinetobacter lwoffii* NL 1. Front Microbiol. 2021 Sep. 10; 12:725755), among others.

[0083] An exemplary microorganism from the genus *Novosphingobium* is *Novosphingobium aromaticivorans. Novosphingobium aromaticivorans* DSM12444 can naturally catabolize multiple aromatic compounds containing H, G, and S units via protocatechuic acid.

[0084] The recombinant microorganisms are preferably configured to exhibit enhanced accumulation of various compounds with respect to a corresponding microorganism. "Accumulation" in this context refers to appearance of the compound intracellularly, extracellularly (e.g., secretion), or both. The enhanced accumulation can be exhibited as an increased amount of the compound at steady state or an increased rate of appearance over time. The recombinant microorganisms in such versions may include any one or more of the modifications described herein, in any combination.

[0085] The recombinant microorganisms of the invention preferably exhibit enhanced accumulation of at least one compound with respect to the corresponding microorganism when the recombinant microorganism and the corresponding organism are grown under identical conditions. The accumulation may be enhanced by a factor of at least about 1.1, at least about 1.5, at least about 2, at least about 2.5, at least about 3, at least about 3.5, at least about 4, at least about 4.5, at least about 5, at least about 5.5, at least about 6, or at least about 6.5 and/or up to about 6.5, up to about 7, or more. Such increases may reflect an increase by mass.

[0086] The accumulated compounds of the invention can comprise any one or more of coenzyme Q_{10} (CoQ_{10}), phytoene, lycopene, beta-carotene, zeaxanthin, adonixanthin, astaxanthin, and 2-pyrone-4,6-dicarboxylic acid (PDC).

[0087] Recombinant microorganisms configured for enhanced accumulation of CoQ_{10} can comprise any one or more of a modification that decreases phytoene synthase (CrtB) activity, a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity, a modification that decreases lycopene cyclase (CrtY) activity, a modification that decreases beta-carotene hydroxylase (CrtZ) activity, and/or a modification that decreases 2,2'-beta hydroxylase (CrtG) activity. In some versions, the recombinant microorganisms configured for enhanced accumulation of CoQ_{10} comprises any one or more of a modification that decreases phytoene synthase (CrtB) activity, a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity, and/or a modification that decreases lycopene cyclase (CrtY) activity. In some versions, the recombinant microorganism configured for enhanced accumulation of CoQ_{10} comprises a modification that decreases phytoene synthase (CrtB) activity.

[0088] Recombinant microorganisms configured for enhanced accumulation of lycopene can comprise a modification that decreases lycopene cyclase (CrtY) activity.

[0089] Recombinant microorganisms configured for enhanced accumulation of zeaxanthin can comprise a modification that decreases 2,2'-beta hydroxylase (CrtG) activity.

[0090] Recombinant microorganisms configured for enhanced accumulation of beta-carotene can comprise a modification that decreases 2,2'-beta hydroxylase (CrtG) activity, a modification that decreases beta-carotene hydroxylase (CrtZ) activity, or, preferably, both.

[0091] Recombinant microorganisms configured for enhanced accumulation of adonixanthin and/or astaxanthan can comprise a modification that decreases 2,2'-beta hydroxylase (CrtG) activity, a modification that increases beta-carotene ketolase (CrtW) activity, or, preferably both.

[0092] Recombinant microorganisms configured for enhanced accumulation of PDC can comprise a modification that decreases 2-pyrone-4,6-dicarboxylic acid (PDC) hydrolase (LigI) activity, a modification that decreases 4-carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) methyl esterase (DesC) activity, a modification that decreases 4-carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) cis-trans isomerase (DesD) activity, and/or a modification that decreases vanillate/3-O-methylgallate O-demethylase (DmtS) activity or, preferably, each of the foregoing modifications.

[0093] The recombinant microorganisms of the invention can accordingly be used to produce one or more of the compounds of the invention. Such methods can comprise culturing the recombinant microorganisms of the invention in a manner such that the compound to be produced accumulates intracellularly, extracellularly or both.

[0094] Recombinant microorganisms employed for production of CoQ_{10} can comprise any one or more of a modification that decreases phytoene synthase (CrtB) activity, a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity, a modification that decreases lycopene cyclase (CrtY) activity, a modification that decreases beta-carotene hydroxylase (CrtZ) activity, and/or a modification that decreases 2,2'-beta hydroxylase (CrtG) activity. In some versions, the recombinant microorganisms employed for production of CoQ_{10} comprises any one or more of a modification that decreases phytoene synthase (CrtB) activity, a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity, and/or a modification that decreases lycopene cyclase (CrtY) activity. In some versions, the recombinant microorganism employed for production of CoQ_{10} comprises a modification that decreases phytoene synthase (CrtB) activity.

[0095] Recombinant microorganisms employed for production of lycopene can comprise a modification that decreases lycopene cyclase (CrtY) activity.

[0096] Recombinant microorganisms employed for production of zeaxanthin can comprise a modification that decreases 2,2'-beta hydroxylase (CrtG) activity.

[0097] Recombinant microorganisms employed for production of beta-carotene can comprise a modification that decreases 2,2'-beta hydroxylase (CrtG) activity, a modification that decreases beta-carotene hydroxylase (CrtZ) activity, or, preferably, both.

[0098] Recombinant microorganisms employed for production of adonixanthin and/or astaxanthan can comprise a modification that decreases 2,2'-beta hydroxylase (CrtG) activity, a modification that increases beta-carotene ketolase (CrtW) activity, or, preferably both.

[0099] Recombinant microorganisms employed for production of PDC can comprise a modification that decreases 2-pyrone-4,6-dicarboxylic acid (PDC) hydrolase (LigI) activity, a modification that decreases 4-carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) methyl esterase (DesC) activity, a modification that decreases 4-carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) cis-trans isomerase (DesD) activity, and/or a modification that decreases vanillate/3-O-methylgallate O-demethylase (DmtS) activity or, preferably, each of the foregoing modifications.

[0100] In some versions, the compounds of the invention are produced by culturing a recombinant microorganism of the invention in a medium comprising a plant-derived phenolic. The plant-derived phenolic can comprise any of a number of phenolics obtained from processing plant lignocellulosic biomass. Exemplary plant-derived phenolics comprise syringyl phenolics, guaiacyl phenolics, and p-hydroxyphenyl phenolics. Exemplary syringyl phenolics include syringaldehyde, syringic acid, and S-diketone. Exemplary guaiacyl phenolics include vanillin, vanillic acid, and G-diketone. Exemplary hydroxyphenyl phenolics include p-coumaric acid, p-hydroxybenzaldehyde, and p-hydroxybenzoic acid. Other plant-derived phenolics include methyl guaiacol, propyl guaiacol, dihydroconiferyl alcohol, methyl syringol, p-hydroxy benzoic acid methyl ester, dihydrophydroxy cinnamic acid methyl ester, dihydrosyringol alcohol, and dihydroferulic acid methyl ester, among others.

[0101] The plant-derived phenolic can be derived and/or provided in the form of depolymerized lignin, such as chemically depolymerized lignin. Methods of depolymerizing lignin are well known in the art. See Pandey et al. 2010 (Pandey M P, Kim C S. Lignin Depolymerization and Conversion: A Review of Thermochemical Methods. *Chemical & Engineering Technology,* 2010, Vol. 34, Issue 1, pp. 3-145) and Wang et al. 2013 (Wang H, Tucker M, Ji Y. Recent Development in Chemical Depolymerization of Lignin: A Review. *Journal of Applied Chemistry,* 2013, Volume 2013, Article ID 838645).

[0102] The depolymerized lignin can be derived from pretreated lignocellulosic biomass. Methods of pretreating lignocellulosic biomass are well known in the art. See Kumar et al. 2017 (Kumar A K and Sharma S. Recent Updates on Different Methods of Pretreatment of Lignocellulosic Feedstocks: A Review. *Bioresour. Bioprocess*. (2017) 4:7); Kumar et al. 2009 (Kumar, P.; Barrett, D. M.; Delwiche, M. J.; Stroeve, P., Methods for Pretreatment of lignocellulosic Biomass for Efficient Hydrolysis and Biofuel

Production. Industrial & Engineering Chemistry Research 2009, 48, (8), 3713-3729); Wang et al. 2013 (Wang H, Tucker M, Ji Y. Recent Development in Chemical Depolymerization of Lignin: A Review. (2013) *Journal of Applied Chemistry*. 2013:1-9), and Karlen et al. 2020 (Karlen S D, Fasahati P, Mazaheri M, Serate J, Smith R A, Sirobhushanam S, Chen M, Tymkhin V I, Cass C L, Liu S, Padmakshan D, Xie D, Zhang Y, McGee M A, Russell J D, Coon J J, Kaeppler H F, de Leon N, Maravelias C T, Runge T M, Kaeppler S M, Sedbrook J C, Ralph J. Assessing the viability of recovering hydroxycinnamic acids from lignocellulosic biorefinery alkaline pretreatment waste streams. *ChemSusChem*. 2020 Jan. 26). Examples include chipping, grinding, milling, steam pretreatment, ammonia fiber expansion (AFEX, also referred to as ammonia fiber explosion), ammonia recycle percolation (ARP), CO_2 explosion, steam explosion, ozonolysis, wet oxidation, acid hydrolysis, dilute-acid hydrolysis, alkaline hydrolysis, organosolv, ionic liquids, gamma-valerolactone, and pulsed electrical field treatment, among others.

[0103] The lignocellulosic biomass can be derived from any source, such as corn cobs, corn stover, cotton seed hairs, grasses, hardwood stems, leaves, newspaper, nut shells, paper, softwood stems, *Sorghum*, switchgrass, waste papers from chemical pulps, wheat straw, wood, woody residues, mixed biomass species such as those produced by native prairie, and other sources.

[0104] The medium in some versions can additionally or alternatively comprise a fermentable sugar. Non-limiting examples of suitable fermentable sugars include adonitol, arabinose, arabitol, ascorbic acid, chitin, cellubiose, dulcitol, erythrulose, fructose, fucose, galactose, glucose, gluconate, inositol, lactose, lactulose, lyxose, maltitol, maltose, maltotriose, mannitol, mannose, melezitose, melibiose, palatinose, pentaerythritol, raffinose, rhamnose, ribose, sorbitol, sorbose, starch, sucrose, trehalose, xylitol, xylose, and hydrates thereof, among others.

[0105] In some versions, the fermentable sugar may be replaced by other organic compounds that support growth of the recombinant microorganism. This includes but is not limited to the other organic compounds that are present in the deconstructed biomass fractions from the crops or plant species mentioned above.

[0106] The accumulated compound can be isolated from the recombinant microorganism. An intracellularly accumulated compound can be isolated from the recombinant microorganism by, for example, lysing the recombinant microorganism and purifying the compound from at least some of the lysed components thereof. An extracellularly accumulated compound can be isolated from the recombinant microorganism, for example, by separating the medium in which the recombinant microorganism is cultured from the recombinant microorganism. In some versions, the compound is isolated from the medium.

[0107] A recitation herein of a microorganism "comprising" a mutation in or to a particular gene refers to a gene that would be present were it not for the mutation, e.g., the gene present in a corresponding microorganism. Thus, the recitation of a microorganism "comprising" a mutation in or to a particular gene encompasses a mutated form of the gene present in the microorganism, a partially deleted remnant of the gene present in the microorganism, a complete absence of the gene (e.g., as resulting from a complete deletion of the gene) in the microorganism, or other configurations.

[0108] For generating recombinant microorganisms suitable for producing PDC, the recombinant microorganisms of the invention can include any of the modifications disclosed or claimed in US 2020/0263215 A1, U.S. Pat. No. 11,028,418 B2, US 2021/0261993 A1, and U.S. Pat. No. 11,981,946 B2, which are incorporated herein by reference in their entireties.

[0109] The elements and method steps described herein can be used in any combination whether explicitly described or not.

[0110] All combinations of method steps as used herein can be performed in any order, unless otherwise specified or clearly implied to the contrary by the context in which the referenced combination is made.

[0111] As used herein, the singular forms "a," "an," and "the" include plural referents unless the content clearly dictates otherwise.

[0112] Numerical ranges as used herein are intended to include every number and subset of numbers contained within that range, whether specifically disclosed or not. Further, these numerical ranges should be construed as providing support for a claim directed to any number or subset of numbers in that range. For example, a disclosure of from 1 to 10 should be construed as supporting a range of from 2 to 8, from 3 to 7, from 5 to 6, from 1 to 9, from 3.6 to 4.6, from 3.5 to 9.9, and so forth.

[0113] All patents, patent publications, and peer-reviewed publications (i.e., "references") cited herein are expressly incorporated by reference to the same extent as if each individual reference were specifically and individually indicated as being incorporated by reference. In case of conflict between the present disclosure and the incorporated references, the present disclosure controls.

[0114] It is understood that the invention is not confined to the particular construction and arrangement of parts herein illustrated and described, but embraces such modified forms thereof as come within the scope of the claims.

EXAMPLES

Production of Carotenoids from Aromatics and Pretreated Lignocellulosic Biomass by *Novosphingobium aromaticivorans*

SUMMARY

[0115] Carotenoids are lipophilic compounds found in the membranes of various organisms. Individual carotenoids are also commodity chemicals, produced industrially for use as food additives, nutritional supplements, cosmetics, and pharmaceuticals. The alphaproteobacterium *Novosphingobium aromaticivorans* has previously been established as a potential platform microbe for converting aromatic compounds derived from lignocellulosic plant biomass into valuable extracellular products. Here, we show that *N. aromaticivorans* DSM 12444 cells naturally produce the carotenoid nostoxanthin, and we construct a set of gene deletion mutants that accumulate β-carotene, lycopene, or zeaxanthin, which are predicted intermediates in nostoxanthin biosynthesis as well as commodity chemicals. We also show that a mutant strain heterologously expressing a CrtW protein accumulates the carotenoid astaxanthin. When grown on vanillate as the carbon source, we find that the levels of carotenoids are not significantly affected by O_2 concentration in the tested range of 5% to 21% O_2. We also show that these carotenoids are produced at comparable levels when strains are grown in liquor from alkaline pretreated *Sorghum* biomass (*Sorghum* APL), which contains a mixture of aromatics. Finally, we construct strains that produce zeaxanthin, β-carotene, or astaxanthin concurrently with 2-pyrone-4,6-dicarboxylic acid, a potential building block for biodegradable polymers, when grown in *Sorghum* APL. Combined, our results show that *N. aromaticivorans* can simultaneously produce valuable intracellular and extracellular commodities when grown in the presence of either pure aromatics or pretreated lignocellulosic biomass.

[0116] There is economic and environmental interest in generating commodity chemicals from renewable resources, such as lignocellulosic biomass, that can substitute for chemicals derived from fossil fuels. The bacterium *Novosphingobium aromaticivorans* is a promising microbial platform for producing commodity chemicals from lignocellulosic biomass because it can produce these from compounds in pretreated lignocellulosic biomass, which many industrial microbial catalysts cannot metabolize. Here, we show that *N. aromaticivorans* can be engineered to produce several valuable carotenoids. We also show that engineered *N. aromaticivorans* strains can produce these lipophilic chemicals concurrently with the extracellular commodity chemical 2-pyrone-4,6-dicarboxylic acid when grown in a complex liquor obtained from alkaline pretreated lignocellulosic biomass. Concurrent microbial production of valuable intra- and extracellular products can increase the economic value generated from conversion of lignocellulosic biomass-derived compounds into commodity chemicals and facilitate separation of water- and membrane-soluble products.

INTRODUCTION

[0117] The aromatic polymer lignin is a major component of lignocellulosic plant biomass and is estimated to represent as much as 30% of the organic carbon in the biosphere (1). However, the heterogeneous structure and chemical composition of lignin have limited its economic value to industry. In addition, the mixture of aromatic compounds that results from lignocellulosic biomass deconstruction is often not metabolized by commonly used industrial microbes. We are interested in developing microbial catalysts that can convert heterogeneous mixtures of biomass-derived compounds, including aromatics, into valuable products.

[0118] We and others have been exploring *Novosphingobium aromaticivorans*, an alphaproteobacterium of the Sphingomonadales order, as a platform for producing valuable compounds (2) because it is amenable to genomic modification (3, 4) and can metabolize many components of deconstructed lignocellulosic biomass, including aromatic monomers (2) and some dimers (3, 5). For example, *N. aromaticivorans* DSM 12444 has been engineered to stoichiometrically convert the major aromatic monomers in deconstructed plant biomass into 2-pyrone-4,6-dicarboxylic acid (PDC), a potential polyester precursor (6) that is secreted into the media (2, 7). This study sought to expand the suite of valuable compounds that *N. aromaticivorans* can produce from biomass-derived aromatics.

[0119] The genome sequence of *N. aromaticivorans* DSM 12444 predicts that this bacterium can produce the carotenoid nostoxanthin (13). Amongst the intermediates in the predicted nostoxanthin synthesis pathway of *N. aromaticivorans* are the industrially valuable carotenoids lycopene, β-carotene, and zeaxanthin (FIG. **1**). A recent genome-scale metabolic model of *N. aromaticivorans* suggested that carotenoids could be some of the most profitable products made from plant biomass by *N. aromaticivorans* because of their high economic value and yields (14).

[0120] *N. aromaticivorans* (when it was known as *Sphingomonas aromaticivorans* F199) has also been shown to produce the lipophilic Coenzyme Q_{10} (CoQ_{10}) (15). CoQ_{10} is also the main isoprenoid quinone in humans and is a commodity chemical used in the pharmaceutical and cosmetics industries (16-19). Currently, bacteria that produce CoQ_{10} industrially (16, 20) cannot metabolize the aromatics present in deconstructed plant biomass. Thus, there is potential for *N. aromaticivorans* to also become a source of CoQ_{10} when grown in aromatic-containing solutions derived from plant biomass.

[0121] In this work, we test several predicted reactions in the *N. aromaticivorans* carotenoid biosynthetic pathway (FIG. **1**) by generating defined mutants that accumulate β-carotene, lycopene, or zeaxanthin. Further, we engineer a strain that heterologously expresses a CrtW protein and accumulates the carotenoid astaxanthin. We show that these carotenoids can be produced from vanillate, an aromatic compound commonly present in deconstructed lignocellulosic plant biomass, and from an alkaline pretreatment liquor (APL) made from *Sorghum*. We also engineer a set of strains that produce either zeaxanthin, β-carotene, or astaxanthin concurrently with PDC when fed *Sorghum* APL, showing that *N. aromaticivorans* can be engineered to simultaneously produce extracellular and intracellular products from this renewable carbon source. We discuss how co-production of membrane-bound carotenoids and excreted dicarboxylic acids like PDC could improve the economics of valorizing biomass in a lignocellulosic biorefinery.

Results

Nostoxanthin is the Main Carotenoid Produced by *N. aromaticivorans* DSM 12444

[0122] The *N. aromaticivorans* DSM 12444 genome predicts that this bacterium contains genes that encode previously uncharacterized proteins with 52 to 74% amino acid sequence identity to known enzymes that lead to nostoxanthin production (Table 1).

TABLE 1

Amino acid sequence identity of predicted *N. aromaticivorans* DSM 12444 carotenoid pathway proteins with the most closely related characterized protein identified by PaperBLAST (51).

Protein annotation	Locus tag, accession number	% identity	Most similar protein accession number	Organism encoding most similar protein	Reference for characterization
CrtI	Saro_1816, ABD26256.1	74%	ADO33738.1	*Sphingomonas elodea* ATCC 31461	(23)
CrtY	Saro_1817, ABD26257.1	52%	AEP37353.1	*S. elodea* ATCC 31461	(23)
CrtZ	Saro_1168, ABD25613.1	58%	AIT05942.1	*Sphingomonas taxi* ATCC 55669	(25)
CrtG	Saro_0236, WP_011443898.1	72%	AEP37351.1	*S. elodea* ATCC 31461	(23)

[0123] To test the prediction that *N. aromaticivorans* uses these previously uncharacterized gene products to produce carotenoids, we grew cells in the presence of vanillate and analyzed acetone:methanol (lipophilic) extracts of the cells by LC-MS. Our analysis of these extracts from the parent *N. aromaticivorans* strain (12444Δ1879) was consistent with nostoxanthin being a major carotenoid (compound 1 in FIG. 3A): compound 1 had absorbance maxima at 453 and 480 nm (FIG. **4**A) and an m/z peak of 600 (FIG. **4**B), both characteristic of nostoxanthin (21, 22). Two other compounds in these extracts had slightly longer retention times than compound 1: compound 2 had absorbance maxima at 339, 446, and 474 nm (FIG. **4**A), and m/z peaks of 600 and 639 (FIG. **4**C), and compound 3 had absorbance maxima at 453 and 481 nm (FIG. **4**A), and an m/z peak of 584 (FIG. **4**D). Though we were not able to identify compound 2, the properties of compound 3 are consistent with caloxanthin (22), a predicted precursor to nostoxanthin (FIG. **1**) known to be present in some sphingomonads that produce nostoxanthin (22, 23). However, commercial standards of pure nostoxanthin or caloxanthin were not available to estimate the compounds' abundances or provide further proof of their chemical identity. Another compound detected in the *N. aromaticivorans* lipophilic extracts had the same retention time and absorbance spectrum as a commercial CoQ_{10} standard (FIG. **3**A and FIGS. **2** and **4**A, consistent with previous work showing that CoQ_{10} is the major quinone in this bacterium (15).

Lipophilic Compounds Produced by *N. aromaticivorans* Mutants Containing Deletions of Genes in the Predicted Carotenoid Biosynthetic Pathway

[0124] To further test whether *N. aromaticivorans* uses the predicted carotenoid biosynthesis pathway (FIG. **1**), we generated a set of mutants with in-frame deletions of genes predicted to encode proteins involved in the pathway. We grew these strains with vanillate as sole carbon source and analyzed the compounds present in lipophilic extracts from these mutants, using commercial standards when available to aid in identification and quantification of the lipophilic compounds.

[0125] Deletion of Saro_1814 (encoding a putative CrtB homologue) resulted in a strain (12444ΔcrtB) that formed non-pigmented colonies on solid media (FIG. **11** (A, left panel)), as expected given the predicted role of this gene product in phytoene synthesis (FIG. **1**). We also found that strain 12444ΔcrtB only contained CoQ_{10} as a major lipophilic compound (FIG. **3**B and FIG. **5**). Deletion of Saro_1817 (encoding a putative CrtY homologue) resulted in a strain (12444ΔcrtY) that formed light pink colonies (FIG. **11** (A, left panel)), and contained the predicted pathway intermediate lycopene (compound 4) as well as CoQ_{10} in its lipophilic extract (FIG. **3**C and FIG. **6**). Deletion of Saro_0236 (encoding a putative CrtG homologue) resulted in a strain (12444ΔcrtG) that formed yellow colonies (FIG. **11** (A, left panel)) and contained the predicted pathway intermediate zeaxanthin (compound 5) and CoQ_{10} as the major components of its lipophilic extract (FIG. **3**D and FIG. **7**). Finally, to test if the pathway intermediate β-carotene could be accumulated, we constructed a strain (12444ΔcrtGZ) in which both Saro_0236 and Saro_1168 (encoding a putative CrtZ homologue) were deleted, as deletion of Saro_1168 alone would be expected to also produce 2,2'-dihydroxy-β-carotene (FIG. **1**). Strain 12444ΔcrtGZ formed yellow colonies (FIG. **11** (A, left panel)), and the main components of its lipophilic extract were β-carotene (compound 6) and CoQ_{10} (FIG. **3**E and FIG. **8**).

[0126] In sum, the compounds present in the lipophilic extracts of each of these mutants were consistent with predictions from the annotated carotenoid biosynthetic pathway in the *N. aromaticivorans* genome (FIG. **1**). These experiments illustrate that one or more gene deletions can result in *N. aromaticivorans* strains that produce industrially valuable carotenoids when grown in the presence of vanillate. Furthermore, the lipophilic extracts from all strains tested also contain the electron carrier CoQ_{10}.

Production of Astaxanthin by an Engineered *N. aromaticivorans* Strain

[0127] Astaxanthin is a valuable carotenoid that is not predicted to be produced by *N. aromaticivorans*, since the genome of this organism lacks a crtW gene. While several bacteria, including some other members of the Sphingomonadales order (*Sphingomonas astaxanthinifaciens* (24) and *Sphingomonas taxi* ATCC 55669 (25)), naturally produce astaxanthin (26), none of these are known to metabolize aromatic compounds present in pretreated lignocellulosic biomass. To test if we could engineer *N. aromaticivorans* to produce astaxanthin, we placed a recombinant crtW gene from *S. astaxanthinfaciens* or *S. taxi* separately into the crtG locus of 12444ΔcrtG to generate strains 12444SastaW and 12444StaxiW, respectively. The difference in colony colors of the 12444ΔcrtG, 12444SastaW, and 12444StaxiW strains (FIG. **11**) suggested that the insertion of each of these crtW genes into the *N. aromaticivorans* genome resulted in altered carotenoid profiles.

[0128] To test this hypothesis, we analyzed the lipophilic extracts from vanillate-grown 12444SastaW and 12444StaxiW cells. This analysis showed that 12444SastaW produced astaxanthin (FIG. **3**F; compound 7), as well as small amounts of three other putative carotenoids (compounds 8, 9, 10) and CoQ_{10} (FIG. **3**F and FIG. FIGS. **9**A and **9**B). The lipophilic extract of 12444StaxiW also contained astaxanthin (compound 7) and CoQ_{10} (FIG. **3**G and FIG. **10**A), although its predominant carotenoid (compound 8, which is also a minor component of the 12444SastaW extract) appears to be adonixanthin (FIG. **10**A, based on its measured mass (m/z peak=583; FIG. **10**B). The 12444StaxiW lipophilic extract also contained two additional compounds, one of them identified as zeaxanthin (compound 5; FIG. **3**G and FIG. **10**A). From this, we conclude that introducing the CrtW protein from *S. astaxanthinfaciens* into *N. aromaticivorans* generates a strain that is more effective at accumulating astaxanthin than a strain using the CrtW protein from *S. taxi*. Therefore, in subsequent experiments, we used cells containing the *S. astaxanthinfaciens* crtW gene (strain 12444SastaW) as a chassis for an astaxanthin-producing strain of *N. aromaticivorans.*

Impact of O_2 Tension on Levels of Carotenoids and CoQ_{10} in *N. aromaticivorans*

[0129] The dissolved O_2 concentration of a culture can affect carotenoid levels in various organisms in different ways (27). For example, lower O_2 tensions have been shown to increase CoQ_{10} production in some bacteria (20), while other microbes increase carotenoid production at high O_2 tensions presumably since carotenoids can provide protection against reactive oxygen species (27). Therefore, we tested whether bubbling *N. aromaticivorans* cultures with gas containing 5%, 10%, or 21% O_2 would lead to significant changes in carotenoids and CoQ_{10} levels when using vanillate as a carbon source.

[0130] Carotenoid levels of *N. aromaticivorans* strains grown at different O_2 tensions are shown in FIG. **12** (A-E). Nostoxanthin levels in 12444Δ1879, which are reported as HPLC peak area due to lack of a standard for quantification, showed only modest changes with oxygen (ranging from 1.0 to 2.5×10^5 area units per mg dry cell weight (dcw)). Under the same growth conditions, lycopene levels in 12444ΔcrtY ranged from 0.24 to 0.75 μg/mg dcw, zeaxanthin in 12444ΔcrtG ranged from 0.25 to 0.54 μg/mg dcw, β-carotene in 12444ΔcrtGZ ranged from 0.29 to 0.64 μg/mg dcw, and astaxanthin in 12444SastaW ranged from 0.12 to 0.25 μg/mg dcw. Single-factor ANOVA tests (alpha=0.05) of carotenoid levels suggested that only strain 12444ΔcrtGZ contained significantly different carotenoid levels at the O_2 concentrations tested (FIG. **12** (A-E)). CoQ_{10} levels for each strain at different O_2 tensions (FIG. **12** (F)) ranged from 0.08 to 0.2 μg/mg dcw. ANOVA tests also showed that, for each of the strains, there was no significant difference in CoQ_{10} production at the three O_2 concentrations tested (FIG. **12** (F)). Furthermore, ANOVA tests showed there was no significant difference in CoQ_{10} production between the different strains at any of the individual O_2 concentrations. Therefore, we conclude that O_2 availability does not have a consistent significant impact on *N. aromaticivorans* carotenoid or CoQ_{10} levels over the concentration range tested.

Production of Carotenoids and CoQ_{10} by *N. aromaticivorans* from Alkaline Pretreated *Sorghum* Biomass

[0131] The above results showed that wild type and engineered *N. aromaticivorans* strains accumulate carotenoids and CoQ_{10} when grown on vanillate, an aromatic compound predicted to be found in deconstructed lignocellulosic biomass. However, we also wanted to test whether carotenoids and CoQ_{10} could be generated when cells were grown on the mixture of aromatics directly obtained from plant biomass. We therefore grew several strains in a *Sorghum* alkaline pretreatment liquor (APL) (28) which contains a mixture of aromatic monomers and other organics (FIG. **13**, Table 2). With a few exceptions, the *N. aromaticivorans* strains grown in *Sorghum* APL (in shake flasks with atmospheric O_2 conditions) produced amounts of carotenoids and CoQ_{10} (normalized by dry cell weight; open squares in FIG. **12**) that were within the production ranges of the vanillate-grown cultures grown at different dissolved O_2 tensions (filled circles in FIG. **12**). One exception was strain 12444ΔcrtB, which lacks detectable carotenoids in lipophilic extracts; this strain produced significantly more CoQ_{10} when grown in APL compared to when grown on vanillate (FIG. **12** (F)). In addition, strains 12444Δ1879 and 12444SastaW produced significantly less nostoxanthin and astaxanthin, respectively, when grown in APL compared to when grown on vanillate. Overall, these results show that it is possible to use *N. aromaticivorans* for producing carotenoids from a mixture of aromatic and other organic compounds derived from pretreated lignocellulosic biomass.

TABLE 2

Concentration of aromatic monomers and other organics in sorghum APL used in this study. Values represent mean ± S.D.. Trace levels of indicated aromatic compounds were detected, but concentrations << 0.1 mM prevented quantification. All aromatics in Table 5 were investigated. Other organics were measured as described herein. ND, not detected.

	Concentration (mM)			
Compound	Sorghum APL (n = 2)	PDCΔcrtG (n = 3)	PDCΔcrtGZ (n = 3)	PDCSastaW (n = 3)
Aromatic monomers				
p-coumaric acid	2.01 ± 0.03	ND	ND	ND
Ferulic acid	0.513 ± 0.001	ND	ND	ND
PDC	ND	3.2 ± 0.3	3.3 ± 0.4	2.9 ± 0.1
Acetosyringone	Trace	ND	ND	ND

TABLE 2-continued

Concentration of aromatic monomers and other organics in sorghum APL used in this study. Values represent mean ± S.D.. Trace levels of indicated aromatic compounds were detected, but concentrations << 0.1 mM prevented quantification. All aromatics in Table 5 were investigated. Other organics were measured as described herein. ND, not detected.

	Concentration (mM)			
Compound	Sorghum APL (n = 2)	PDCΔcrtG (n = 3)	PDCΔcrtGZ (n = 3)	PDCSastaW (n = 3)
p-hydroxybenzaldehyde	Trace	ND	ND	ND
p-hydroxybenzoic acid	Trace	ND	ND	ND
Protocatechuic acid	Trace	ND	ND	ND
Vanillin	Trace	ND	ND	ND
Syringic acid	ND	Trace	ND	Trace
Vanillic acid	ND	Trace	ND	Trace
Other organics				
Acetate	19.8 ± 0.9	43 ± 4	45 ± 4	41.5 ± 0.3
Lactate	5 ± 2	6.3 ± 0.7	6.2 ± 0.9	5.8 ± 0.2
Pyruvate	4.8 ± 0.3	5.7 ± 0.3	6.1 ± 0.5	5.4 ± 0.2
Propanoic acid	3.2 ± 0.7	2.5 ± 0.3	2.6 ± 0.2	2.44 ± 0.02
Formate	2.8 ± 0.7	2.0 ± 0.2	0.98 ± 0.08	1.5 ± 0.2
Cellobiose	0.36 ± 0.03	0.32 ± 0.07	0.247 ± 0.005	0.260 ± 0.006
Xylitol	0.25 ± 0.06	0.4 ± 0.3	0.2 ± 0.4	0.54 ± 0.06
Glucose	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	ND
Ethanol	0.03 ± 0.02	0.2 ± 0.1	0.09 ± 0.08	0.08 ± 0.09
Glycerol	0.2 ± 0.2	ND	ND	ND
Succinate	ND	ND	ND	ND
Xylose	ND	ND	ND	ND

Concurrent Production of Carotenoids, CoQ_{10}, and PDC from Alkaline Pretreated *Sorghum* Biomass

[0132] Previous work has shown that engineered *N. aromaticivorans* strains containing defined mutations in aromatic metabolism can convert the three major classes of biomass aromatics (syringyl, guaiacyl, and p-hydroxyphenyl) into PDC and secrete it into the medium (2, 7). We sought to test whether individual *N. aromaticivorans* strains could produce both extracellular PDC and intracellular lipophilic compounds (carotenoids and CoQ_{10}) as valuable products from biomass-derived media. To do this, we generated a set of strains that contained both the mutations needed to accumulate extracellular PDC and those needed to accumulate the carotenoids zeaxanthin, β-carotene, or astaxanthin. We found that these engineered strains produce extracellular PDC, as well as the expected carotenoid and CoQ_{10}, when grown in *Sorghum* APL (FIG. **14**). The levels of individual carotenoid species and CoQ_{10} (normalized by dry cell weight) in the lipophilic cell extracts are comparable to or greater than the amounts produced by the respective non-PDC-producing strains grown in *Sorghum* APL (FIG. **14** (A-D)). In addition, the levels of extracellular PDC are equal to or greater than 100% theoretical yield, based on the measured concentrations of aromatic compounds in APL (FIG. **14** (E), Table 2). This observation is consistent with the stoichiometric conversion of aromatic monomers to PDC reported previously for the PDC-producing strain that was used in these studies (7).

DISCUSSION

[0133] This work sought to expand the types of valuable chemicals that could be produced from pretreated lignocellulosic biomass. We confirmed that *N. aromaticivorans* naturally produces the industrially important isoprenoid CoQ_{10}, and we leveraged its native ability to synthesize carotenoids along with the utility of heterologous expression to engineer mutant strains that accumulate different valuable carotenoids from either a pure aromatic (vanillate) or from *Sorghum* APL, a feedstock derived from lignocellulosic biomass. We also used this new information to engineer a set of *N. aromaticivorans* strains that can concurrently produce CoQ_{10}, a valuable carotenoid, and PDC from *Sorghum* APL.

N. aromaticivorans as a Production Platform for Carotenoids and CoQ_{10}

[0134] Although other microbes can be used as sources of carotenoids and CoQ_{10}, our work is important for several reasons. First, we confirmed predictions from *N. aromaticivorans* genomic and physiological studies that this bacterium contains metabolic pathways that can produce the carotenoid nostoxanthin (13) as well as CoQ_{10} (15). Our work also shows that minimal genomic modifications of *N. aromaticivorans* can lead to strains that accumulate valuable carotenoids such as β-carotene, lycopene, astaxanthin, or zeaxanthin. In addition, we demonstrate that *N. aromaticivorans* cells lacking crtB (Saro_1814) could simplify industrial production of CoQ_{10}, another important commodity chemical used in the pharmaceutical and cosmetics industries, because the crtB mutation prevents the accumulation of other acetone:methanol soluble materials. We also found that changes in O_2 tension did not have a significant impact on *N. aromaticivorans* carotenoid or CoQ_{10} levels, unlike in other microbes where O_2 tensions or the resulting oxidative stress can have a significant impact on accumulation of these products (27). Effects of O_2 tension on carotenoid production can be variable; in some cases lower O_2 tensions lead to higher production due to increased membrane synthesis (29) while in other cases higher O_2 tensions lead to higher production (30) presumably due to the ability of these compounds to quench reactive oxygen species (27). While our data suggests that *N. aromaticivorans* might not regulate carotenoid synthesis in response to changes in O_2 tension, additional studies are needed to test this hypothesis. The ability of *N. aromaticivorans* to synthesize comparable levels of carotenoids and CoQ_{10} at different 02 concentrations could be advantageous in industrial settings, due to the capital and operational costs associated with aeration of large bioreactors.

Microbial Production of Valuable Commodity Chemicals from Pretreated Lignocellulosic Biomass

[0135] As society looks for ways to produce commodity chemicals from abundant renewable resources, pretreated lignocellulosic biomass is an attractive material. *N. aromaticivorans* has natural converging pathways to catabolize major components found in pretreated lignocellulosic biomass, including the most abundant aromatic monomers found in lignin (syringyl, guaiacyl, and p-hydroxyphenyl aromatics) (2), some aromatic dimers (3, 5), and other organic compounds (31). Because carotenoids and CoQ_{10} are produced from central metabolites, *N. aromaticivorans* could thus "funnel" mixtures of compounds found in pretreated lignocellulosic biomass into these commodity chemicals. This is in contrast to existing microbial hosts for producing carotenoids or CoQ_{10} that typically use food-grade sugars as carbon sources (32). To date, relatively few of the microbes being considered for industrially producing carotenoids and CoQ_{10} also have the native ability to metabolize any aromatic compounds (33-37). Thus, *N. aromaticivorans* could be an important microbial catalyst for industrial production of carotenoids and CoQ_{10} from renewable lignocellulosic carbon sources.

[0136] The method used to generate the *Sorghum* APL feedstock is well known to solubilize easily cleavable aromatics from plant cell walls, without the breakdown of the carbohydrate and lignin polymers in the biomass (28). We showed that *N. aromaticivorans* can grow in the presence of *Sorghum* APL alone, unlike previous studies which have supplemented lignocellulosic APL with minerals (38-43) and/or nitrogen/carbon sources (38, 40, 44, 45). We also found that *N. aromaticivorans* produces nearly the same amounts (normalized to dry cell weight) of carotenoids and CoQ_{10} when grown on *Sorghum* APL as when grown in a defined medium containing vanillate (FIG. **12**), in addition to accumulating comparable amounts of cellular material (FIG. **15**). These results suggest that *N. aromaticivorans* could produce these valuable isoprenoids from lignocellulosic substrates without the need to add other nutrients.

Simultaneous Production of Carotenoids, CoQ_{10} and PDC from Pretreated Lignocellulosic Biomass

[0137] Our work illustrates the potential for *N. aromaticivorans* to produce carotenoids and CoQ_{10} as intracellular lipophilic products. We also generated strains that concurrently produce these lipophilic products along with the soluble extracellular product PDC, which has several potential industrial uses (46). In strains that accumulate both intracellular (carotenoids and CoQ_{10}) and extracellular (PDC) products, the cellular and aqueous fractions can be separated and each used as a source of valuable products to increase the economic value derived from pretreated lignocellulosic biomass. Notably, the strains we engineered for simultaneous PDC and carotenoid production accumulate at least as much carotenoid as the non-PDC-producing strains, showing that synthesis of two products does not have a significant negative impact on the overall output. The greater than 100% theoretical yield of PDC observed for these strains (FIG. **14** (E)) likely reflects conversion of aromatic compounds present in the *Sorghum* APL that were not detected by our HPLC-MS/MS analysis, in addition to complete conversion of detected aromatic monomers (Table 2).

[0138] In previous studies with strains that produce PDC from aromatics, a second carbon source (glucose) was added to cells, since the mutations that result in accumulation of PDC block the use of aromatics to support growth (2, 7). The growth of PDC-producing strains in *Sorghum* APL reported here predicts that *N. aromaticivorans* will not need to be supplemented with other nutrients to produce extracellular and intracellular compounds from this and possibly other types of feedstocks derived from plant biomass.

[0139] In sum, this work adds to a growing body of evidence that *N. aromaticivorans* is a promising microbe for converting lignocellulosic biomass into valuable compounds because it is amenable to genomic modifications and can metabolize abundant aromatic components of this biomass. This work establishes *N. aromaticivorans* as a promising host for producing valuable carotenoids and CoQ_{10} from both pretreated lignocellulosic biomass and from purified aromatics. In addition, *N. aromaticivorans* has the ability to produce these intracellular lipophilic compounds concurrently with PDC, which could help to improve the economics of converting plant biomass into industrial commodities. Future work will focus on improving yields of these and other products under industrially relevant conditions.

Materials and Methods

Novosphingobium aromaticivorans Strains

[0140] Details on all strains in this study can be found in Table 3. *N. aromaticivorans* 12444Δ1879 is a derivative of wild-type strain DSM 12444 (also called F199 (31, 47)), in which a putative sacB gene (Saro_1879 or SARO_RS09410) was deleted to create a strain amenable to genomic modifications using a sacB-containing plasmid (3, 48). We used 12444Δ1879 as the parent strain to generate strains 12444ΔcrtB (lacking crtB; Saro_1814 or SARO_RS09080), 12444ΔcrtY (lacking crtY; Saro_1817 or SARO_RS09095), 12444ΔcrtG (lacking crtG; Saro_0236 or SARO_RS01180), 12444ΔcrtGZ (lacking crtG and crtZ; Saro_0236 and Saro_1168 or SARO_RS01180 and SARO_RS05825), 12444StaxiW (replacing Saro_0236 with the gene for the CrtW protein from *S. taxi* ATCC 55669 (NCBI Accession WP_038660513.1)), and 12444SastaW (replacing Saro_0236 with the gene for the CrtW protein from *S. astaxanthinfaciens* (NCBI accession WP_211248127.1)).

TABLE 3

All strains and plasmids used in this study.

Name	Genotype	Description	Reference
E. coli strains			
DH5α	F− Φ80lacZΔM15 Δ(lacZYA-argF) U169 recA1 endA1 hsdR17 (rK−, mk+) phoA supE44 λ-thi− gyrA96 relA1	Used for creating and maintaining plasmids	Bethseda Research Laboratories
S17-1	recA pro hsdR RP4-2-Tc::Mu-Km::Tn7	Used for mobilizing plasmids into *N. aromaticivorans* via conjugation	(50)
N. aromaticivorans strains			
12444Δ1879	DSM 12444 ΔSaro__1879	Parent strain; putative sacB has been deleted to allow genomic modifications using a sacB-containing plasmid	(3)
12444ΔcrtB	DSM 12444 ΔSaro__1879 ΔSaro__1814	Parent with deleted crtB	This work
12444ΔcrtY	DSM 12444 ΔSaro__1879 ΔSaro__1817	Parent with deleted crtY	This work
12444ΔcrtG	DSM 12444 ΔSaro__1879 ΔSaro__0236	Parent with deleted crtG	This work
12444ΔcrtGZ	DSM 12444 ΔSaro__1879 ΔSaro__0236 ΔSaro__1168	Parent with deleted crtG and crtZ	This work
12444StaxiW	DSM 12444 ΔSaro__1879 ΔSaro__0236::crtW from *S. taxi* ATCC 55669	Parent with deleted crtG, with the gene for the CrtW protein from *Sphingomonas taxi* ATCC 55669 (NCBI Accession WP__038660513.1) in the Saro__0236 genomic locus	This work
12444SastaW	DSM 12444 ΔSaro__1879 ΔSaro__0236::crtW from *S. astaxanthinifaciens*	Parent with deleted crtG, with the gene for the CrtW protein from *Sphingomonas astaxanthinifaciens* (NCBI accession WP__211248127.1) in the Saro__0236 genomic locus	This work
12444PDCΔdmtS	DSM 12444 ΔSaro__1879 ΔSaro__2819 ΔSaro__2864-5 ΔSaro__1872	Parent with deleted ligI, desCD, dmtS that accumulates PDC from aromatic monomers	(7)
PDCΔcrtG	DSM 12444 ΔSaro__1879 ΔSaro__2819 ΔSaro__2864-5 ΔSaro__1872 ΔSaro__0236	12444PDCΔdmtS with deleted crtG	This work

TABLE 3-continued

All strains and plasmids used in this study.

Name	Genotype	Description	Reference
PDCΔcrtGZ	DSM 12444 ΔSaro__1879 ΔSaro__2819 ΔSaro__2864-5 ΔSaro__1872 ΔSaro__0236 ΔSaro__1168	12444PDCΔdmtS with deleted crtG and crtZ	This work
PDCSastaW	DSM 12444 ΔSaro__1879 ΔSaro__2819 ΔSaro__2864-5 ΔSaro__1872 ΔSaro__0236::crtW from *S. axtaxanthinifaciens*	12444PDCΔdmtS with deleted crtG, with the gene for the CrtW protein from *S. astaxanthinifaciens* in the Saro__0236 genomic locus	This work
Plasmids			
pK18msB-MCS1		pK18mobsacB lacking the multiple cloning site, with a new XbaI site introduced	(3, 48)
pK18msB/ΔSaro__1814		pK18msB-MCS1 containing genomic regions that naturally flank Saro__1814	This work
pK18msB/ΔSaro__1817		pK18msB-MCS1 containing genomic regions that naturally flank Saro__1817	This work
pK18msB/ΔSaro__0236		pK18msB-MCS1 containing genomic regions that naturally flank Saro__0236	This work
pK18msB/ΔSaro__1168		pK18msB-MCS1 containing genomic regions that naturally flank Saro__1168	This work
pK18msB/ΔSaro__0236::StaxiW		pK18msB/ΔSaro__0236 with the gene for CrtW from *S. taxi* ATCC 55669 between the Saro__0236 flanking regions	This work
pK18msB/ΔSaro__0236::SastaW		pK18msB/ΔSaro__0236 with the gene for CrtW from *S. astaxanthinifaciens* between the Saro__0236 flanking regions	This work

[0141] *N. aromaticivorans* 12444PDCΔdmtS is a derivative of 12444Δ1879 that was genetically modified to accumulate stoichiometric amounts of PDC from syringyl, guaiacyl, and p-hydroxyphenyl aromatic compounds (7). Strain 12444PDCΔdmtS has Saro_2819 (ligI), Saro_2864-5 (desCD), and Saro_1872 (dmtS) deleted from the genome. We used 12444PDCΔdmtS as the parent strain to generate strains PDCΔcrtG (lacking Saro_0236), PDCΔcrtGZ (lacking both Saro_0236 and Saro_1168), and PDCSastaW (replacing Saro_0236 with the gene for the CrtW protein from *S. astaxanthinifaciens*).

[0142] Genes for CrtW proteins were synthesized as gBlocks (Integrated DNA Technologies, Coralville, IA). Plasmids for cloning were constructed with the NEBluilder HiFi DNA Assembly Master Mix (New England Biolabs; Ipswich, MA). Methods for constructing mutants (including PCR primers used (Table 4)) are contained in Supporting Information.

TABLE 4

Primers used to create cloning vectors for genomic modifications of N. aromaticivorans.

Gene deleted/ plasmid linearized	Fragment (relative to gene)	Primers
crtB (Saro_1814)	Upstream	5'-**cgattcattaatgcagctggcacgacag**CAGGACTCTCGATCTACCTGCACCATC-3' (SEQ ID NO: 23) 5'-**CGATAAAGCCCAGCTTG*CTCACAGGTCGTCGGCCTT***CATTGC-3' (SEQ ID NO: 24)
	Downstream	5'-***GAAGGCCGACGACCTGTGAG*CAAGCTGGGCTTTATCG**GCAAAGC-3' (SEQ ID NO: 25) 5'-***gtttctgcggactggctttctagatgttc***CACCATGACGAGGTGGACCAGAATGAAC-3' (SEQ ID NO: 26)
crtY (Saro_1817)	Upstream	5'-**cgattcattaatgcagctggcacgacag**CTTGAAACGGTAGCCGAAGGTGTAAAGGTCG-3' (SEQ ID NO: 27) 5'-***GCAAATGAAAGTGGGTTGG*CGATCCGCTTAGGGACATG**CGGTTG-3' (SEQ ID NO: 28)
	Downstream	5'-**CATGTCCCTAAGCGGATCG*CCAACCCACTTTCATTTGC***AGGAACC-3' (SEQ ID NO: 29) 5'-***gtttctgcggactggctttctagatgttc***GATGGTGCAGGTAGATCGAGAAGTCCTG-3' (SEQ ID NO: 30)
crtG (Saro_0236)	Upstream	5'-**cgattcattaatgcagctggcacgacag**GTCGAACAGTACGTCACCTTCATCAACCAG-3' (SEQ ID NO: 31) 5'-***CGGTATTGCTCGTGATG*CCAACGGCTCCTGC**CTGAACAG-3' (SEQ ID NO: 32)
	Downstream	5'-**GCAGGAGCCGTTGG*CATCACGAGCAATACCG***CTGCAACTATGG-3' (SEQ ID NO: 33) 5'-***gtttctgcggactggctttctagatgttc***CTCGTATCCCACAGCGATATCAGGATGC-3' (SEQ ID NO: 34)
crtZ (Saro_1168)	Upstream	5'-**cgattcattaatgcagctggcacgacag**CACTTCCATCGTCTTCGACTGCTTGAG-3' (SEQ ID NO: 35) 5'-***CCTGCTTCAGCACCGCAG*CGACACTTTCTTACAATTTGC**CCGAAAGTC-3' (SEQ ID NO: 36)
	Downstream	5'-**GGCAAATTGTAAGAAAGTGTCG*CTGCGGTGCTGAAGCAGG***AACTG-3' (SEQ ID NO: 37) 5'-***gtttctgcggactggctttctagatgttc***CTACTGCCGGATTTTCCGGCATGGAAG-3' (SEQ ID NO: 38)
pK18msB-MCS1		5'-CTGTCGTGCCAGCTGCATTAATG-3' (SEQ ID NO: 39) 5'-GAACATCTAGAAAGCCAGTCCGCAGAAAC-3' (SEQ ID NO: 40)
pK18msB/ ΔSaro0236		5'-CCAACGGCTCCTGCCTGAACAG-3' (SEQ ID NO: 41) 5'-CATCACGAGCAATACCGCTGCAACTATGG-3' (SEQ ID NO: 42)

Underlined sequences are extensions to the primers that will not bind to the genomic DNA region being amplified. Lowercase bold and bold italicized sequences are complementary to the end regions of linearized pK18msB-MCS1. The upper case BOLD and BOLD ITALICIZED are complementary to the upper case BOLD and BOLD ITALICIZED sequences of the primer used to create the other flanking region for the same gene.

Bacterial Growth

[0143] *E. coli* strains used for plasmid cloning were grown in lysogeny broth and shaken at ~200 rpm at 30 or 37° C. For routine manipulation, *N. aromaticivorans* cultures were grown in GluSis at 30° C. GluSis is a modification of Sistrom's minimal medium (49) in which the succinate has been replaced by 22.6 mM glucose. The minimal medium used for *N. aromaticivorans* experiments was Standard Mineral Base (SMB) (3) at an initial pH of 7.0. Where needed to select for the presence or absence of plasmids, media were supplemented with 100 μg/mL ampicillin, 50 μg/mL kanamycin, or 10% sucrose (w/v).

Preparation of *Sorghum* APL

[0144] *Sorghum* APL (45) was prepared by mixing samples of milled 2014 GLBRC *Sorghum* (2 g) with a sodium hydroxide solution (1% NaOH in H_2O, 20 mL) in sealed 125 mL Erlenmeyer flasks, before heating for 90 min in an oil bath at 90° C. The flask was then immediately placed in ice for 10 min, after which the biomass and aqueous phases were separated by centrifugation at 4,300×g for 15 min and the supernatant recovered as a source of soluble aromatics. The solid biomass was rinsed three times with ddH_2O (20 mL, 15 mL, and 15 mL), and the washes were recovered through centrifugation. The initial aqueous supernatant and washes were combined and adjusted to pH 7.0 using 1 M HCl. The solution was centrifuged at 20,000×g for 1 h at 4° C. and passed through a 0.2 μm surfactant-free cellulose acetate (SFCA) filter to remove any remaining insoluble material, yielding the alkaline pretreatment liquor (APL) used in further experiments.

Growth of *N. aromaticivorans* in Minimal Medium with Vanillate

[0145] Cultures of each *N. aromaticivorans* strain were initially grown in a 125 mL conical shake flask containing 10 mL SMB supplemented with 4 mM vanillate. Between 3 and 8 mL of this culture was combined with 480 mL of fresh SMB+4 mM vanillate in a glass roux bottle. Roux bottle cultures were attached to a gas mixer using SideTrak 840 mass flow controllers attached to a FloBox 954 (Sierra Instruments; Monterey, CA) in a 30° C. temperature-controlled room. Gas was piped into the bottoms of the cultures and exhausted from the headspace through outlets in stoppers. The gas contained 5, 10, or 21% O_2, 1% CO_2, and N_2 as the remainder. Cell growth was monitored by periodically removing samples for analysis using a Klett-Summerson photoelectric colorimeter with a red filter. Cultures were grown until they reached late exponential growth or early stationary phase. For dry cell weight (dcw) determination, aliquots (~80 mL) were centrifuged in pre-weighed tubes (8,000×g for 15 min), supernatants were removed, cell pellets were air-dried in a fume hood, then the tubes were re-weighed (FIG. **15**). Aliquots (~160 mL) were also harvested (centrifuged at 8,000×g for 15 min) for isolation of lipophilic compounds by extraction with acetone:methanol (see below).

Growth of *N. aromaticivorans* in *Sorghum* APL

[0146] Each *N. aromaticivorans* strain was initially grown in a 125 mL conical shake flask containing 10 mL SMB supplemented with 10 mM glucose. 1 mL aliquots were centrifuged at ~7,000×g for 5 min, the supernatant was removed, and the cell pellet was used to inoculate 18 mL of *Sorghum* APL in a 125 mL conical shake flask. Cultures were shaken at ~200 rpm at 30° C. until they reached early stationary phase. Aliquots of cultures for extraction into acetone:methanol (10 mL) and dcw determination (5 mL) were harvested as described above (see FIG. **15** for dry cell weight measurements).

Preparation of Lipophilic Extracts

[0147] Care was taken to minimize O_2 and light exposure to acetone:methanol extracts, though samples were not handled anaerobically. Cell pellets from were resuspended in water (950 µL for roux bottle samples and 100 µL for pellets from shake flask cultures), then transferred into a 15 mL glass Sorval centrifuge tubes. Extraction solvent (7:2 acetone:methanol solution; 5 mL or 1.5 mL respectively for roux bottle or shake flask samples) was added and the samples were mixed by pipetting. The tube was centrifuged (10,000×g for 20 min), then the supernatant was transferred to a new 15 mL glass tube. The pelleted cells were extracted a second time, after resuspending cells in water (500 µL or 100 µL respectively for roux bottle or shake flask samples) followed by extraction solvent (4.5 mL or 1.5 mL respectively for roux bottle or shake flask samples). After centrifugation, the supernatants from both extractions were combined. The combined supernatants were partially dried under a stream of N_2 (to a final volume of ~1-4 mL) to concentrate materials before analysis by HPLC. The concentration of compounds in lipophilic extracts was calculated after correcting for dry cell weight, any dilution prior to extraction and the final volume of sample after drying under N_2.

HPLC Identification and Quantification of Lipophilic Compounds

[0148] For identification and quantification, the acetone:methanol lipophilic extracts were analyzed via reverse-phase HPLC using a Kinetex 2.6 µm PS C18 100 Å (150×2.1 mm) column (Phenomenex; Torrance, CA) attached to a Shimadzu Nexera XR HPLC system. The mobile phase was a binary gradient (FIG. **16**) of Solvent A (70% acetonitrile/30% water) and Solvent B (70% acetonitrile/30% isopropanol) flowing at 0.45 mL/min. Absorbance was measured between 200 and 600 nm using a Shimadzu SPD-M20 Å photodiode array detector. The following commercial standards were used to identify compounds in the lipophilic extracts: β-carotene (Sigma-Aldrich), lycopene (Pharmaceutical Secondary Standard, Certified Reference Material (7.2%); Supelco), zeaxanthin (United States Pharmacopeia Reference Standard), astaxanthin (Sigma-Aldrich), and Coenzyme Q_{10} (Sigma-Aldrich).

[0149] To identify compounds that were not commercially available for use as standards, the eluent from the HPLC was analyzed via mass spectrometry using a Shimadzu triple quadrupole mass spectrometer LCMS-8045. We used positive mode Q3 scans from 450 m/z to 700 m/z around the retention times of unknown HPLC peaks to obtain mass spectra of compounds eluting at such times (FIGS. **4**B-D, **9**B, and **10**B).

Analysis of Culture Media for PDC and Aromatic Compounds

[0150] Extracellular media samples were prepared by centrifuging 1.5 mL of culture at 20,000×g for 2 minutes before passing the supernatant through a 0.2 µm SFCA membrane filter. The filtered media was analyzed using the Shimadzu Nexera XR HPLC system with the photodiode array detector and LCMS-8045 described above. The mobile phase was a binary gradient (FIG. **17**) of solvent A (0.200 formic acid in water) and solvent B (methanol) flowing at 0.4 mL/min. The stationary phase was a Phenomenex Kinetex F5 column (2.6 cm pore size, 2.1 mm ID, 150 mm length). Aromatic compounds were identified by multiple-reaction-monitoring (MRM) using the transition ions specified in Table 5, which were obtained from analyzing pure standards as previously described (7). Aromatic compounds were quantified by comparing sample absorbance at specific wavelengths and retention times with known standards, as measured by the photodiode array detector (Table 6).

TABLE 5

Multiple reaction module conditions for HPLC-MS/MS identification of aromatic compounds in sorghum APL.

Compound	MW (g/mol)	DUIS mode	Parent m/z	Transition 1 m/z, collision energy (V)	Transition 2 m/z, collision energy (V)	Transition 3 m/z, collision energy (V)
Acetosyringone	196.2	+	197.0	155.1, (−20)	140.1, (−28)	125.1, (−32)
Acetovanillone	166.2	+	167.0	125.1, (−13)	110.0, (−23)	N/A

TABLE 5-continued

Multiple reaction module conditions for HPLC-MS/MS identification of aromatic compounds in sorghum APL.

Compound	MW (g/mol)	DUIS mode	Parent m/z	Transition 1 m/z, collision energy (V)	Transition 2 m/z, collision energy (V)	Transition 3 m/z, collision energy (V)
Catechol	110.1	−	109.2	91.1, (24)	65.1, (24)	41.0, (35)
Ferulic acid	194.2	−	193.0	134.1, (17)	149.1, (16)	178.1, (14)
p-Coumaric acid	164.0	−	163.0	119.1, (16)	93.1, (31)	117.1, (30)
PDC	184.1	−	183.1	139.0, (12)	111, (15)	95.1, (13)
p-OH-Benzaldehyde	122.1	−	121.0	92.1, (25)	93.1, (21)	65.1, (24)
p-OH-benzoic acid	138.1	−	137.0	93.0, (16)	65.1, (33)	75.2, (30)
Protocatechuic acid	154.1	−	153.0	109.1, (17)	91.1, (26)	N/A
Syringaldehyde	182.2	−	181.1	151.1, (26)	166.2, (20)	123.1, (27)
Syringic acid	198.2	−	197.0	182.1, (14)	122.8, (23)	94.9, (33)
Vanillic acid	168.1	−	167.0	152.1, (19)	107.9, (19)	123.0, (14)
Vanillin	152.2	−	151.0	136.0, (17)	92.0, (22)	108.0, (24)

TABLE 6

Photodiode array detection parameters for quantification of aromatic compounds in sorghum APL.

Compound	Peak absorbance wavelength (nm)	Wavelength used for quantification (nm)	Retention time (min)
Ferulic acid	322	325	3.9
p-Coumaric acid	312	334	3.3
PDC	315	325	1.2
Protocatechuic acid	259	258	1.8
Vanillic acid	260	258	2.7

Construction of Plasmids for Generating in-Frame Deletions of Saro_1814, Saro_1817, Saro_0236, or Saro_1168

[0151] Regions of *Novosphingobium aromaticivorans* genomic DNA containing ~1000 bp upstream and downstream of the genes to be deleted were amplified via PCR (see Table 4 for primers). Plasmid pK18msB-MCS1 (a variant of pK18mobsacB (48) in which the multiple cloning site has been removed, and which contains a gene for kanamycin resistance and sacB for sucrose sensitivity) was linearized via PCR as previously described (3). The upstream and downstream flanking regions for each gene were combined with linearized pK18msB-MCS1 using the NEBuilder HiFi Assembly system (New England Biolabs, Ipswich, MA) to produce a plasmid in which the upstream and downstream DNA sequences are adjacent, with no intervening coding region (Table 3). In all cases, a gene's start codon was eliminated; for some genes, a portion of the downstream coding region was retained. The plasmids were transformed into NEB 5-alpha competent *Escherichia coli* cells (New England Biolabs). The transformed *E. coli* cells were cultured in LB media+kanamycin, the plasmids were purified using a Qiagen® Plasmid Maxi Kit (Qiagen, Germany), and DNA sequencing was used to confirm the presence of the desired junction between upstream and downstream fragments.

Plasmids for Recombining a Foreign crtW Gene into the *N. aromaticivorans* Genome

[0152] DNA fragments containing the gene coding for the CrtW protein from either *Sphingomonas taxi* ATCC 55669 (NCBI Accession WP_038660513.1) or *Sphingomonas astaxanthinifaciens* (NCBI accession WP_211248127.1), with sequences complementary to the upstream and downstream regions of Saro_0236 at the crtW fragments' ends, were ordered as gBlocks from Integrated DNA Technologies (Coralville, IA). Genes encoding CrtW from other organisms were constructed to have codon usage frequencies similar to those of other genes in *N. aromaticivorans* (calculated from several genes in the genome), but without making the GC content of individual genes too high for the gBlock synthesis process. Sequences of the fragments were (with sequences complementary to the Saro_0236 flanks in lowercase):

Sphingomonas taxi ATCC 55669 crtW:

(SEQ ID NO: 43)

gtcaaccgcgtcaacctgttcaggcaggagccgttgggcATGAGCCCCGATCGGGGGAATACGCGCCACA

GCCTGCTGCTCGCCGCCGCGATCGGTGCGGCCTGGCTCGCCATCCATATTGGCGGCA

TCTTCTTCTGGCAGTGGCGTGCCGCCACGGTGCCGGTCGCACTTCTGCTGATCGTGGT

GCAGGCGTGGCTGAGCACCGGCCTCTTCATCGTCGCGCACGACTGCATGCACGGATC

GTTCGCACCCGGACGCCGGGCGTGGAACGTCGTCGTCGGCACCCTGTGCCTCGGCGC

CTATGCCGGCCTGTCCTATCGCGCGCTCTACCCGATGCACCACGCGCATCATGCCGC

-continued

GCCCGGCACCGAACACGATCCCGACTTCCATGCCGCCGCGCCTCGCCGCGCGCTTCC

GTGGTTCGTCCATTTCTTCCGCGGGTACTACACCCATGGCCAGATCCTGCGGATCAC

GCTTGCGGCGATCGTCTACATCCTGCTCGGCGCGTCGCTTCTCAACATCGTGCTGTTC

TGGGCGGTGCCGGCGCTGCTCGCGCTTGCGCAATTGTTTCTGTTCGGCACCTATCTGC

CCCACCGTCACGGCGAGACGCCGTTCGCCGACACGCACAACGCGCGCAGCAACTCG

CTGTCGCCGCTCGCCTCGCTGGCGACCTGCTTCCACTTCGGTGCCTATCACCACGAA

CATCACCTCAGCCCGCAGACTCCGTGGTGGCAGCTCCCGCACATCAAGCGCGGCTG

Acatcacgagcaataccgctgcaactatggcctctacttc

Sphingomonas astaxanthinifaciens crtW:

(SEQ ID NO: 44)
gtcaaccgcgtcaacctgttcaggcaggagccgttgggcATGGCAGAACGCCGTCGCCCGGCCTATATGGC

ACCCATGCTCAGTGATGCGCAGCGCCGTCGCCAGGCGATGATCGGCCTTGGCCTTGC

CGCAGCGATCACCGCAGCCTTCGTCGCGCTTCATGTCTGGTCGGTCTTCTTCCTTCCG

CTTGAAGGAGCAGGCTGGTGGCTTGCGCTTCCGATCGTCGCAGTGCAAACCTGGCTT

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CGGCACCCCGTGGTGGCAGCTGCCTCGACGACGGCGAGAGCTTGCACTTCCTGCATG

Acatcacgagcaataccgctgcaactatggcctctacttc

[0153] The plasmid that was used to delete Saro_0236 was linearized using primers 5'-CCAACGGCTCCTGCCTGAACAG-3' (SEQ ID NO:45) and 5'-CATCACGAGCAATACCGCTGCAACTATGG-3' (SEQ ID NO:46). Each of the crtW DNA fragments was separately combined with this linearized plasmid using the NEBuilder HiFi Assembly system (New England Biolabs) to produce plasmids pK18msB/ΔSaro0236::StaxiW and pK18msB/ΔSaro0236::SastaW, each containing crtW in the Saro_0236 genomic locus (with the start and stop codons for crtW located where those codons for Saro_0236 would normally be located). The plasmids were transformed into NEB 5-alpha competent *E. coli* (New England Biolabs), and the plasmids were purified and confirmed as described above.

Modifying the *N. aromaticivorans* Genome

[0154] The gene deletion and crtW-containing plasmids were separately mobilized into *N. aromaticivorans* via conjugation with *E. coli* S17-1. For conjugation, cultures of *E. coli* S17-1 harboring the plasmid (in LB containing kanamycin) and *N. aromaticivorans* (in GluSis) were grown overnight at 30° C. Cultures were diluted and allowed to resume exponential growth before cells were harvested by centrifugation (~7,000×g for 5 min). Each cell pellet was separately washed in LB, then resuspended together into 90 μL LB containing no added antibiotic. Conjugations were allowed to proceed overnight at 30° C. The following day, the cells were harvested via centrifugation, resuspended into GluSis, and shaken at 200 rpm for >1 h at 30° C. Cells from these cultures were plated onto solid GluSis with kanamycin to select for *N. aromaticivorans* cells in which the plasmid had integrated into the genome via homologous recombination (single crossovers). Single crossover strains were confirmed through the inability to immediately grow on GluSis containing 10% sucrose.

[0155] Single crossover strains were cultured in 5 mL of GluSis containing 10% sucrose and shaken at 30° C. until growth commenced (usually several days), which signified loss of the plasmid from the genome via a second round of homologous recombination. These cultures were streaked onto solid GluSis containing 10% sucrose to generate individual colonies that had lost the plasmid, and plasmid loss was confirmed by the inability to grow on GluSis containing kanamycin. The desired genomic modification was confirmed via PCR and sequencing of isolated genomic DNA.

Measuring Chemical Oxygen Demand (COD)

[0156] COD was measured on filtered and unfiltered samples using COD2 mercury-free high range (20-1,500 mg/L) digestion vials following the manufacturer's protocol (2565115, Hach, Loveland, CO, United States). "Suspended solids" COD was calculated by subtracting the filtered sample COD from the unfiltered sample COD. "Uncharacterized" COD refers to the difference between the total filtered COD and the sum of all calculated CODs for every measured aromatic and organic molecule. The COD for each measured molecule was calculated based on the stoichiometry of how many moles of O_2 would be required to fully oxidize the molecule to CO_2 and H_2O.

Analysis of Organics in *Sorghum* APL

[0157] Analysis was performed on an Agilent 1260 Infinity II HPLC equipped with an HPX-87H column at 50° C. and a refractive index detector. The mobile phase was 0.02N sulfuric acid flowing at 0.5 mL/min. Samples were centrifuged to remove cells and particulates, decanted, and diluted 1:9 (v/v) with MilliQ water. The sample injection volume was 50 μL. The analytes quantified were glucose, xylose, pyruvic acid, xylitol, cellobiose, succinic acid, lactic acid, propanoic acid, glycerol, formic acid, acetic acid, and ethanol. Concentrations were calculated from a 9-point calibration curve and are reported of the average of two technical replicates.

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cttcgggccg cgctcgggca ggactcattg atcaccgggg tccgcgcgct cgcgtgcagc  360
gccaccaagg tgacgctttc ggaaggcacc cgcatcgagg cgggcgccgt tatcgacgcg  420
cggggcttgc gcaacctcca gcacctcacc ggcggatggc agaagttcgt cggccgccgg  480
ctccggctcg cgcagccgca cagtctcgac gcgccgatcg tcaaggatgc ttccgtcgaa  540
cagatcgacg gctaccgctt cgtctattgc ctgccctttg ccgccgacga gatcttcgtc  600
gaggacacct attactccga cagccccgcg ctcgacgtgg cggtgctggg cgagaggatc  660
gacgcctatg tcgcggcgcg cgggtggcag gtggccgagg tccttggcga ggagcatggc  720
gtgcttccgg tggtagcggg cggaaacttc gacgccttct ggcggtcgag cggaggcgcg  780
gtcgcccgcg ccggtgttcg cgccggcctg ttccaggcgg tcaccagcta ttcgctgccc  840
gatgccgtgc gctttgccct ggccctcgcc cggcaaccgg acctcggcgg cgaggctctg  900
gccgcgttca gcgaagccta cgcgcgccgg cactggaagc gctcggcgtt ccaccgcgca  960
ctttccgcca tgctgttcgc ggcggcccgg cccgagcatc gctaccgcgt gctcgaacgc  1020
ttctaccgcc tggaccgccg ccttatcgag cgtttctatg ccggtcggac gacccttctc  1080
gacaaggcgc gcattctttc gggcaagccg ccggtaccgg taaccaaggc catcggcgtg  1140
ctcaccggcc ttggcgcgcg ccccaaccca ctttcatttg caggaacccg cgcatga     1197

SEQ ID NO: 6           moltype = AA  length = 398
FEATURE                Location/Qualifiers
source                 1..398
                       mol_type = protein
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 6
MTARRCDIAI LGGGLAGGLV ALALRRARPD LSLLLVEQGE RLGGNHVWSF FGSDVGNEGR  60
ALLDGMFVAA WPEYTVHFPA FRRRLKTSYY SITSRRFDAV LRAALGQDSL ITGVRALACS  120
ATKVTLSEGT RIEAGAVIDA RGLRNLQHLT GGWQKFVGRR LRLAQPHSLD APIVKDASVE  180
QIDGYRFVYC LPFAADEIFV EDTYYSDSPA LDVAVLGERI DAYVAARGWQ VAEVLGEEHG  240
VLPVVAGGNF DAFWRSSGGA VARAGVRAGL FQAVTSYSLP DAVRFALALA RQPDLGGEAL  300
AAFSEAYARR HWKRSAFHRA LSAMLFAAAR PEHRYRVLER FYRLDRRLIE RFYAGRTTLL  360
DKARILSGKP PVPVTKAIGV LTGLGARPNP LSFAGTRA                          398

SEQ ID NO: 7           moltype = DNA  length = 531
FEATURE                Location/Qualifiers
source                 1..531
                       mol_type = genomic DNA
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 7
atgggcgggg ccatgcagac gcttgccgcg atcctcattg tcctgggcac ggtcctcgcg  60
atggaattcg tcgcgtggtc gagccacaag tacatcatgc acggcttcgg ctggggctgg  120
caccgcgacc atcatgagcc gcacgaaggg tttctcgaga agaacgatct ctacgccatt  180
gtcggcgcgg cgctttcgat cctgatgttc gcgctgggca gcccgatgat catgggcgcg  240
gatgcctggt ggccgggcac gtggatcggc ctgggcgtcc tgttctatgg cgtgatctat  300
acgctggtgc atgacgggct ggtccaccag cgctggttcc gatgggtccc gaagcgcggc  360
tatgccaagc ggctggtcca ggcgcacaag cttcaccacg ccacgatcgg caaggaaggc  420
ggcgtcagtt tcggcttcgt cttcgcgcgc gaccctgcgg tgctgaagca ggaactgcgg  480
gcccagcgcg aggcaggcat cgccgttctg cgcgaggcag tggacggcta g           531

SEQ ID NO: 8           moltype = AA  length = 176
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FEATURE                Location/Qualifiers
source                 1..176
                       mol_type = protein
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 8
MGGAMQTLAA ILIVLGTVLA MEFVAWSSHK YIMHGFGWGW HRDHHEPHEG FLEKNDLYAI  60
VGAALSILMF ALGSPMIMGA DAWWPGTWIG LGVLFYGVIY TLVHDGLVHQ RWFRWVPKRG  120
YAKRLVQAHK LHHATIGKEG GVSFGFVFAR DPAVLKQELR AQREAGIAVL REAVDG      176

SEQ ID NO: 9           moltype = DNA  length = 711
FEATURE                Location/Qualifiers
source                 1..711
                       mol_type = genomic DNA
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 9
atgaacggcg tgctggtctc ggcctgggcc atgacggtga tcgtcgccgt ccgctatctt  60
gcgaccagcg gtcttttcgc ctggctgacg gccagggtga ggcccggcca ctatgccggg  120
ctcgaccggc agatccgctc ggagatcggg tggagccttg ccaccgcggc gatctatggc  180
gtgccggcgg gcgtggtcgc ctggggctgg caggagcggg ggtggacgcg gatctacacc  240
gatccgcatg ccttgccgct ctggtggatg ccggtctcgc tcctgctcta cctcttcatc  300
cacgatacct ggttctactg gacccaccgc tggatgcacc ggccggccgt gttccgccgc  360
atccatgcca tccaccacgc cagtcgcccg ccgacggcgt gggccgcaat gaacttccac  420
ccggtcgagg ccgccgtggt ctcgctgctg atccccgcgc tggtcttcgc gatcccgatc  480
cacgtcgcgg ttctggggct ggtgctgctg gtgatgacgg tgatgggcgt gaccaaccac  540
atgggctggg aaatgtttcc acgcgcgctc gttcattccc gggcagggaa ctggctgata  600
accgcaagcc accaccaccg ccatcacgag caataccgct gcaactatgg cctctacttc  660
cggttctggg accggttgtg cggcaccgac aagggattgt ccgccccatg a           711

SEQ ID NO: 10          moltype = AA  length = 236
FEATURE                Location/Qualifiers
source                 1..236
                       mol_type = protein
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 10
MNGVLVSAWA MTVIVAVRYL ATSGLFAWLT ARVRPGHYAG LDRQIRSEIG WSLATAAIYG  60
VPAGVVAWGW QERGWTRIYT DPHALPLWWM PVSLLLYLFI HDTWFYWTHR WMHRPAVFRR  120
IHAIHHASRP PTAWAAMNFH PVEAAVVSLL IPALVFAIPI HVAVLGLVLL VMTVMGVTNH  180
MGWEMFPRAL VHSRAGNWLI TASHHHRHHE QYRCNYGLYF RFWDRLCGTD KGLSAP      236

SEQ ID NO: 11          moltype = DNA  length = 717
FEATURE                Location/Qualifiers
source                 1..717
                       mol_type = genomic DNA
                       organism = Sphingomonas taxi
SEQUENCE: 11
atgagccccg atcgggggaa tacgcgccac agcctgctgc tcgccgccgc gatcggtgcg  60
gcctggctcg ccatccatat tggcggcatc ttcttctggc agtggcgtgc cgccacggtg  120
ccggtcgcac ttctgctgat cgtggtgcag gcgtggctga gcaccggcct cttcatcgtc  180
gcgcacgact gcatgcacgg atcgttcgca cccggacgcc gggcgtggaa cgtcgtcgtc  240
ggcaccctgt gcctcggcgc ctatgccggc ctgtcctatc gcgcgctcta cccgatgcac  300
cacgcgcatc atgccgcgcc cggcaccgaa cacgatcccg acttccatgc cgccgcgcct  360
cgccgcgcgc ttccgtggtt cgtccatttc ttccgcgggt actacaccca tggccagatc  420
ctgcggatca cgcttgcggc gatcgtctac atcctgctcg gcgcgtcgct tctcaacatc  480
gtgctgttct gggcggtgcc ggcgctgctc gcgcttgcgc aattgtttct gttcggcacc  540
tatctgcccc accgtcacgg cgagacgccg ttcgccgaca cgcacaacgc gcgcagcaac  600
tcgctgtcgc cgctcgcctc gctggcgacc tgcttccact tcggtgccta tcaccacgaa  660
catcacctca gcccgcagac tccgtggtgg cagctcccgc acatcaagcg cggctga     717

SEQ ID NO: 12          moltype = AA  length = 238
FEATURE                Location/Qualifiers
source                 1..238
                       mol_type = protein
                       organism = Sphingomonas taxi
SEQUENCE: 12
MSPDRGNTRH SLLLAAAIGA AWLAIHIGGI FFWQWRAATV PVALLLIVVQ AWLSTGLFIV  60
AHDCMHGSFA PGRRAWNVVV GTLCLGAYAG LSYRALYPMH HAHHAAPGTE HDPDFHAAAP  120
RRALPWFVHF FRGYYTHGQI LRITLAAIVY ILLGASLLNI VLFWAVPALL ALAQLFLFGT  180
YLPHRHGETP FADTHNARSN SLSPLASLAT CFHFGAYHHE HHLSPQTPWW QLPHIKRG    238

SEQ ID NO: 13          moltype = DNA  length = 777
FEATURE                Location/Qualifiers
source                 1..777
                       mol_type = genomic DNA
                       organism = Sphingomonas astaxanthinifaciens
SEQUENCE: 13
atggcagaac gccgtcgccc ggcctatatg gcacccatgc tcagtgatgc gcagcgccgt  60
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cgccaggcga tgatcggcct tggccttgcc gcagcgatca ccgcagcctt cgtcgcgctt  120
catgtctggt cggtcttctt ccttccgctt gaaggagcag gctggtggct tgcgcttccg  180
atcgtcgcag tgcaaacctg gcttagcgtc ggtctgttca tcgtcgcgca tgatgcaatg  240
catggcagcc ttgcaccggg ccgccctgcg accaaccttt tctggggacg gcttacgctt  300
ctgctctacg cgggcttctg gttggaccgc ctttcgccca agcatttcga ccaccaccgc  360
catgtcggga ccgagcgcga tcccgatttc tcggtcgatc atccgacccg cttctggccc  420
tggtattatg ccttcatgcg gcgctatttc gggcttcgcg aatatctggt gctgaacgcg  480
ctggtgctgg cctacgtgct ggtgctgaag gcgccgctcg gcaatctgct cctgttctgg  540
gcgctgccct cgatcctgtc ctcgatccag ctcttctatt tcggcaccta ccttccgcac  600
cggcacgagg acgcgccctt cgccgaccag cacaatgccc gcagcaacga ctttccggtc  660
tggctgtcgc tgctgacctg cttccacttc ggctatcacc gcgagcatca cctcagcccc  720
ggcaccccgt ggtggcagct gcctcgacga cggcgagagc ttgcacttcc tgcatga     777

SEQ ID NO: 14          moltype = AA  length = 258
FEATURE                Location/Qualifiers
source                 1..258
                       mol_type = protein
                       organism = Sphingomonas astaxanthinifaciens
SEQUENCE: 14
MAERRRPAYM APMLSDAQRR RQAMIGLGLA AAITAAFVAL HVWSVFFLPL EGAGWWLALP  60
IVAVQTWLSV GLFIVAHDAM HGSLAPGRPA TNLFWGRLTL LLYAGFWLDR LSPKHFDHHR  120
HVGTERDPDF SVDHPTRFWP WYYAFMRRYF GLREYLVLNA LVLAYVLVLK APLGNLLLFW  180
ALPSILSSIQ LFYFGTYLPH RHEDAPFADQ HNARSNDFPV WLSLLTCFHF GYHREHHLSP  240
GTPWWQLPRR RRELALPA                                              258

SEQ ID NO: 15          moltype = DNA  length = 891
FEATURE                Location/Qualifiers
source                 1..891
                       mol_type = genomic DNA
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 15
atgaccgacc agaaccgcat cgtaagctgg cacgccaatc cgtcgaagcc gcgctacacc  60
ccgccgccgg gcgcggtcga cgcgcattgc cacgtcttcg gcccgatggc gcagttcccc  120
ttctcggcca aggccaagta cctgcccgag gacgccggcc ccgacatgct cttcgccctg  180
cgcgaccacc tcggcttcga gcgcaacgtg atcgtccagg caagctgcca cggcaccgac  240
aacgccgcca cgcttgatgc catcgccagg tcgaacggaa aggcgcgcgg cgtcgccgtc  300
gtcgatcccg ccatctcgga gacggacctc caggctctcc acgaaggcgg catccgcggc  360
atccgcttca acttcctcaa gcgcctcgtc gacgatgcgc ccaaggacaa gttcctcgaa  420
gtcgcccggc gcctgcccaa gggttggcac gtcgtgatct atttcgaggc cgacatcctc  480
gaggaactgc gtccgttcat ggatgcgatc cccgtgccgc tggtcatcga ccacatgggc  540
cgccccgatg tccgccaggg ccccgacggc gccgacatga aggccttccg caacttcctg  600
aacagccgcg acgacatctg gttcaaggcc acctgccccg accggctcga cgcgatcaag  660
gaaggcggcg cgggcgatcc gtggaacgcc tttgccgatg ccgtcgcgcc gctcgttgcc  720
gactaccagg accgggtcct gtgggggacc gactggccgc accccaacat ggacaccgag  780
atccccgacg acggccatct cgtcgacatg atcccccgca tcgccccgac cgaggaactg  840
caacgcaagc tcctcgtcga caatccgatg cggctctact gggccgactg a          891

SEQ ID NO: 16          moltype = AA  length = 296
FEATURE                Location/Qualifiers
source                 1..296
                       mol_type = protein
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 16
MTDQNRIVSW HANPSKPRYT PPPGAVDAHC HVFGPMAQFP FSAKAKYLPE DAGPDMLFAL  60
RDHLGFERNV IVQASCHGTD NAATLDAIAR SNGKARGVAV VDPAISETDL QALHEGGIRG  120
IRFNFLKRLV DDAPKDKFLE VARRLPKGWH VVIYFEADIL EELRPFMDAI PVPLVIDHMG  180
RPDVRQGPDG ADMKAFRNFL NSRDDIWFKA TCPDRLDAIK EGGAGDPWNA FADAVAPLVA  240
DYQDRVLWGT DWPHPNMDTE IPDDGHLVDM IPRIAPTEEL QRKLLVDNPM RLYWAD      296

SEQ ID NO: 17          moltype = DNA  length = 738
FEATURE                Location/Qualifiers
source                 1..738
                       mol_type = genomic DNA
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 17
atggccggct tcgtcctgat ccatggttcc tggcatggtg gctggtgctt cgatcccgtc  60
gccgaaatcc tgcgcgcgcg cggccacacg gtcgtcgcgc cgacgctgcc cgggatggga  120
ggcacggcgg aggaaatggc ggcggtcacg ctggatggct ggggcgaatt tgctgcgcag  180
cattgtcgcg acctgaaggc acggggcgtc ggccccgtgg tcctcgccgg ccactcgcgc  240
ggaggccttg tcgtctccac cgcggccgag cgcgaccctt ccgcgatgga cgccatcgtc  300
tacatatgcg cgatgatgct gccctcgggc atgagtcgcg ccgggttcaa ggaactggaa  360
ggcccgaacc ccgctttcga cgcgatcatc tcgaaggttc acggcggcat cgccacggtc  420
atcgacacgc agaatgctgc accggtcttt gcacaaattt cgccgcccga tctggtcgag  480
gcggcaatgg caaggctcgt ggccgaaccc catgctccgc gttcgcagca gatcaaggtc  540
acgccggaac gctggggcag cctcccgcgc acttacgtcg aatgcacgct cgaccgcacc  600
attccgatcg aaagccagcg ccgcatgatc gcgatgtcgc cgggtgcgaa cgtggtgact  660
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ctggaggcgg accacagtcc ctatctgtcg aaaccgcagg aactggccga ggcgctggaa  720
gcggccattc ccgcctga                                                738

SEQ ID NO: 18          moltype = AA  length = 245
FEATURE                Location/Qualifiers
source                 1..245
                       mol_type = protein
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 18
MAGFVLIHGS WHGGWCFDPV AEILRARGHT VVAPTLPGMG GTAEEMAAVT LDGWGEFAAQ  60
HCRDLKARGV GPVVLAGHSR GGLVVSTAAE RDPSAMDAIV YICAMMLPSG MSRAGFKELE  120
GPNPAFDAII SKVHGGIATV IDTQNAAPVF AQISPPDLVE AAMARLVAEP HAPRSQQIKV  180
TPERWGSLPR TYVECTLDRT IPIESQRRMI AMSPGANVVT LEADHSPYLS KPQELAEALE  240
AAIPA                                                              245

SEQ ID NO: 19          moltype = DNA  length = 813
FEATURE                Location/Qualifiers
source                 1..813
                       mol_type = genomic DNA
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 19
atggcggcat gctcggcacc atgcgagtct gaaccgatgg ccttgaaata ctaccacgcc  60
gaaccgctgg ccaactctct caagtcgatg gtcccgctca aggaaaaggg cctcgcctac  120
gagagcatct acgtcgatct gcacaagttc gagcagcatc agccgtggtt caccgcgatc  180
aatcccgaag gccaggtgcc ggtgctcgac catgacggca cgatcatcac gcacacgacg  240
gtgatcaacg aatacctcga ggatgccttc cccgatgccc agcccgccga tgcgcccctg  300
cgtccgcgcg acccggtggg tgcggcgcgc atgcgctact ggaacaagtt catcgacgag  360
cacgtgatga actacgtctc gatgcacgga tggcaccgca tggtcggcgt gatcgcccgc  420
aacatcgcca gcggcgattt cgagaaactg ctcgaaagca ttccgctgcc cgatcagcgc  480
aagaagtggg ctaccgcgcg atcgggcttt tccgaagccg atctcgccaa tgccaccgcc  540
aagatcgaat acgcgctcga caaggtcgag aagcaactcg gcgagacgaa gtggctggcg  600
ggcgacacct acacgcttgc cgacatcaac ttctattcgc actgcggcgc gatggtcgaa  660
cgcatgttcc cggaaatgga agtggcgagg cgcgcgccgc gcctgtgcga atggcgtgat  720
cgcgttgccg cgcggcctgc cgtcgccgaa gcgctgaaaa gcgaagaccg cactgcgccc  780
gggctgcgcg tctggtcggg agaagtgcgc tga                               813

SEQ ID NO: 20          moltype = AA  length = 270
FEATURE                Location/Qualifiers
source                 1..270
                       mol_type = protein
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 20
MAACSAPCES EPMALKYYHA EPLANSLKSM VPLKEKGLAY ESIYVDLHKF EQHQPWFTAI  60
NPEGQVPVLD HDGTIITHTT VINEYLEDAF PDAQPADAPL RPRDPVGAAR MRYWNKFIDE  120
HVMNYVSMHG WHRMVGVIAR NIASGDFEKL LESIPLPDQR KKWATARSGF SEADLANATA  180
KIEYALDKVE KQLGETKWLA GDTYTLADIN FYSHCGAMVE RMFPEMEVAR RAPRLCEWRD  240
RVAARPAVAE ALKSEDRTAP GLRVWSGEVR                                   270

SEQ ID NO: 21          moltype = DNA  length = 1071
FEATURE                Location/Qualifiers
source                 1..1071
                       mol_type = genomic DNA
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 21
atgagcggcg gcgaaccggt tccgaagctg tcggcgaaac ccgccgccac gtatcttcgc  60
aacacctggt acgtggcggg ctgggccagc gatcttgccg gcgagccgca gcagcgcacg  120
ttcctggaag agccggtggc gctcttccgc gacggacacg gtgaggcgaa ggccatcggc  180
gggcgctgcc cgcaccggtt cgcgccgctc ggccatggca gcgtcgtcga cggggcgctg  240
atgtgcccct accacggcct gcgtttcgat ggggatggac gctgcgtcca caacccgcat  300
cccggcggac atcttcccga tgcgcggcag cgggtctatc cgcttgtcga gcggcatgcc  360
ttgctgtgga tatggatggg cgatgcagca aaggctgatc cggcatcgat cccggacttt  420
tcgtggcttt cggaccccag atgggaggcc gtgcgcgggg ccacggtcgc cgagggtcac  480
ttcgagctct acagcgacaa cattctcgac ctcagccacg ccaacttcgt ccacccggcg  540
ctggtcgcca gcgcattcac cgaaggcgag cgcaagttct ggcaggacgg agacaatgtc  600
tttgccgaat acgtgcggct gaacgacgag ctttccgtcg gcatttcggc ggtgatgggg  660
accgaggggc ggccgcagga tttctacggc atggtcaagt ggcatgcgcc ggccgtactc  720
tacttcgatt tccgcgcggg cgagccgggc acgccgcgcg agcaatgcac gctgctgcca  780
tcgctccatg ccttcacgcc ggaaacccct gacacgacgc attacttctg ggcgaccgcg  840
cgcgactaca ggctgggcga cgcggagttc accgccggaa tgcgcgccgc gctcgaattc  900
gcgttcgagc aggaagacat gccgatcatc cgcgacagcc accggctcat gcgcggcgag  960
gacttctggg cgcttcgccc gctgatcctc ggtggcgatg gtggcggggt gcgggcccgg  1020
agaatgctgc aacggctgat cgagcgcgag agacagcagg acgctgcctg a           1071

SEQ ID NO: 22          moltype = AA  length = 356
FEATURE                Location/Qualifiers
source                 1..356
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                       mol_type = protein
                       organism = Novosphingobium aromaticivorans
SEQUENCE: 22
MSGGEPVPKL SAKPAATYLR NTWYVAGWAS DLAGEPQQRT FLEEPVALFR DGHGEAKAIG  60
GRCPHRFAPL GHGSVVDGAL MCPYHGLRFD GDGRCVHNPH PGGHLPDARQ RVYPLVERHA  120
LLWIWMGDAA KADPASIPDF SWLSDPRWEA VRGATVAEGH FELYSDNILD LSHANFVHPA  180
LVASAFTEGE RKFWQDGDNV FAEYVRLNDE LSVGISAVMG TEGRPQDFYG MVKWHAPAVL  240
YFDFRAGEPG TPREQCTLLP SLHAFTPETP DTTHYFWATA RDYRLGDAEF TAGMRAALEF  300
AFEQEDMPII RDSHRLMRGE DFWALRPLIL GGDGGGVRAR RMLQRLIERE RQQDAA      356

SEQ ID NO: 23          moltype = DNA  length = 55
FEATURE                Location/Qualifiers
source                 1..55
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 23
cgattcatta atgcagctgg cacgacagca ggactctcga tctacctgca ccatc       55

SEQ ID NO: 24          moltype = DNA  length = 42
FEATURE                Location/Qualifiers
source                 1..42
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 24
cgataaagcc cagcttgctc acaggtcgtc ggccttcatt gc                     42

SEQ ID NO: 25          moltype = DNA  length = 44
FEATURE                Location/Qualifiers
source                 1..44
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 25
gaaggccgac gacctgtgag caagctgggc tttatcggca aagc                   44

SEQ ID NO: 26          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 26
gtttctgcgg actggctttc tagatgttcc accatgacga ggtggaccag aatgaac     57

SEQ ID NO: 27          moltype = DNA  length = 59
FEATURE                Location/Qualifiers
source                 1..59
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 27
cgattcatta atgcagctgg cacgacagct tgaaacggta gccgaaggtg taaaggtcg   59

SEQ ID NO: 28          moltype = DNA  length = 44
FEATURE                Location/Qualifiers
source                 1..44
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 28
gcaaatgaaa gtgggttggc gatccgctta gggacatgcg gttg                   44

SEQ ID NO: 29          moltype = DNA  length = 45
FEATURE                Location/Qualifiers
source                 1..45
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 29
catgtcccta agcggatcgc caacccactt tcatttgcag gaacc                  45

SEQ ID NO: 30          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 30
gtttctgcgg actggctttc tagatgttcg atggtgcagg tagatcgaga agtcctg     57

SEQ ID NO: 31          moltype = DNA  length = 58
FEATURE                Location/Qualifiers
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source                 1..58
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 31
cgattcatta atgcagctgg cacgacaggt cgaacagtac gtcaccttca tcaaccag    58

SEQ ID NO: 32          moltype = DNA  length = 39
FEATURE                Location/Qualifiers
source                 1..39
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 32
cggtattgct cgtgatgcca acggctcctg cctgaacag                         39

SEQ ID NO: 33          moltype = DNA  length = 43
FEATURE                Location/Qualifiers
source                 1..43
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 33
gcaggagccg ttggcatcac gagcaatacc gctgcaacta tgg                    43

SEQ ID NO: 34          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 34
gtttctgcgg actggctttc tagatgttcc tcgtatccca cagcgatatc aggatgc     57

SEQ ID NO: 35          moltype = DNA  length = 55
FEATURE                Location/Qualifiers
source                 1..55
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 35
cgattcatta atgcagctgg cacgacagca cttccatcgt cttcgactgc ttgag       55

SEQ ID NO: 36          moltype = DNA  length = 48
FEATURE                Location/Qualifiers
source                 1..48
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 36
cctgcttcag caccgcagcg acactttctt acaatttgcc cgaaagtc               48

SEQ ID NO: 37          moltype = DNA  length = 45
FEATURE                Location/Qualifiers
source                 1..45
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 37
ggcaaattgt aagaaagtgt cgctgcggtg ctgaagcagg aactg                  45

SEQ ID NO: 38          moltype = DNA  length = 56
FEATURE                Location/Qualifiers
source                 1..56
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 38
gtttctgcgg actggctttc tagatgttcc tactgccgga ttttccggca tggaag      56

SEQ ID NO: 39          moltype = DNA  length = 23
FEATURE                Location/Qualifiers
source                 1..23
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 39
ctgtcgtgcc agctgcatta atg                                          23

SEQ ID NO: 40          moltype = DNA  length = 29
FEATURE                Location/Qualifiers
source                 1..29
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 40
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gaacatctag aaagccagtc cgcagaaac                                  29

SEQ ID NO: 41          moltype = DNA  length = 22
FEATURE                Location/Qualifiers
source                 1..22
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 41
ccaacggctc ctgcctgaac ag                                         22

SEQ ID NO: 42          moltype = DNA  length = 29
FEATURE                Location/Qualifiers
source                 1..29
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 42
catcacgagc aataccgctg caactatgg                                  29

SEQ ID NO: 43          moltype = DNA  length = 795
FEATURE                Location/Qualifiers
source                 1..795
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 43
gtcaaccgcg tcaacctgtt caggcaggag ccgttgggca tgagccccga tcgggggaat  60
acgcgccaca gcctgctgct cgccgccgcg atcggtgcgg cctggctcgc catccatatt  120
ggcggcatct tcttctggca gtggcgtgcc gccacggtgc cggtcgcact tctgctgatc  180
gtggtgcagg cgtggctgag caccggcctc ttcatcgtcg cgcacgactg catgcacgga  240
tcgttcgcac ccggacgccg ggcgtggaac gtcgtcgtcg gcaccctgtg cctcggcgcc  300
tatgccggcc tgtcctatcg cgcgctctac ccgatgcacc acgcgcatca tgccgcgccc  360
ggcaccgaac acgatcccga cttccatgcc gccgcgcctc gccgcgcgct tccgtggttc  420
gtccatttct tccgcgggta ctacacccat ggccagatcc tgcggatcac gcttgcggcg  480
atcgtctaca tcctgctcgg cgcgtcgctt ctcaacatcg tgctgttctg ggcggtgccg  540
gcgctgctcg cgcttgcgca attgtttctg ttcggcacct atctgcccca ccgtcacggc  600
gagacgccgt tcgccgacac gcacaacgcg cgcagcaact cgctgtcgcc gctcgcctcg  660
ctggcgacct gcttccactt cggtgcctat caccacgaac atcacctcag cccgcagact  720
ccgtggtggc agctcccgca catcaagcgc ggctgacatc acgagcaata ccgctgcaac  780
tatggcctct acttc                                                  795

SEQ ID NO: 44          moltype = DNA  length = 855
FEATURE                Location/Qualifiers
source                 1..855
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 44
gtcaaccgcg tcaacctgtt caggcaggag ccgttgggca tggcagaacg ccgtcgcccg  60
gcctatatgg cacccatgct cagtgatgcg cagcgccgtc gccaggcgat gatcggcctt  120
ggccttgccg cagcgatcac cgcagccttc gtcgcgcttc atgtctggtc ggtcttcttc  180
cttccgcttg aaggagcagg ctggtggctt gcgcttccga tcgtcgcagt gcaaacctgg  240
cttagcgtcg gtctgttcat cgtcgcgcat gatgcaatgc atggcagcct tgcaccgggc  300
cgccctgcga ccaacctttt ctggggacgg cttacgcttc tgctctacgc gggcttctgg  360
ttggaccgcc tttcgcccaa gcatttcgac caccaccgcc atgtcgggac cgagcgcgat  420
cccgatttct cggtcgatca tccgacccgc ttctggccct ggtattatgc cttcatgcgg  480
cgctatttcg ggcttcgcga atatctggtg ctgaacgcgc tggtgctggc ctacgtgctg  540
gtgctgaagg cgccgctcgg caatctgctc ctgttctggg cgctgccctc gatcctgtcc  600
tcgatccagc tcttctattt cggcacctac cttccgcacc ggcacgagga cgcgcccttc  660
gccgaccagc acaatgcccg cagcaacgac tttccggtct ggctgtcgct gctgacctgc  720
ttccacttcg gctatcaccg cgagcatcac ctcagccccg gcaccccgtg gtggcagctg  780
cctcgacgac ggcgagagct tgcacttcct gcatgacatc acgagcaata ccgctgcaac  840
tatggcctct acttc                                                  855

SEQ ID NO: 45          moltype = DNA  length = 22
FEATURE                Location/Qualifiers
source                 1..22
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 45
ccaacggctc ctgcctgaac ag                                         22
```

-continued

```
SEQ ID NO: 46          moltype = DNA  length = 29
FEATURE                Location/Qualifiers
source                 1..29
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 46
catcacgagc aataccgctg caactatgg                                  29
```

1. A recombinant microorganism comprising one or more modifications with respect to a corresponding microorganism not comprising the one or more modifications, wherein the one or more modifications comprise any one or more of:

a modification that decreases phytoene synthase (CrtB) activity with respect to the corresponding microorganism;

a modification that decreases lycopene-forming phytoene desaturase (CrtI) activity with respect to the corresponding microorganism;

a modification that decreases lycopene cyclase (CrtY) activity with respect to the corresponding microorganism;

a modification that decreases beta-carotene hydroxylase (CrtZ) activity with respect to the corresponding microorganism;

a modification that decreases 2,2'-beta hydroxylase (CrtG) activity with respect to the corresponding microorganism; and

a modification that increases beta-carotene ketolase (CrtW) activity with respect to the corresponding microorganism.

2. The recombinant microorganism of claim 1, wherein:

the modification that decreases phytoene synthase phytoene synthase (CrtB) activity comprises a modification to a gene encoding a phytoene synthase phytoene synthase (CrtB) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:2;

the modification that decreases lycopene-forming phytoene (CrtI) desaturase activity comprises a modification to a gene encoding a lycopene-forming phytoene desaturase (CrtI) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:4;

the modification that decreases lycopene cyclase (CrtY) activity comprises a modification to a gene encoding a lycopene cyclase (CrtY) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:6;

the modification that decreases beta-carotene hydroxylase (CrtZ) activity comprises a modification to a gene encoding a beta-carotene hydroxylase (CrtZ) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:8; and/or

the modification that decreases 2,2'-beta hydroxylase (CrtG) activity comprises a modification to a gene encoding a 2,2'-beta hydroxylase (CrtG) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO:10.

3. The recombinant microorganism of claim 1, comprising any one or more of:

the modification that decreases phytoene synthase phytoene synthase (CrtB) activity;

the modification that decreases lycopene-forming phytoene desaturase (CrtI) activity;

the modification that decreases lycopene cyclase (CrtY) activity;

the modification that decreases beta-carotene hydroxylase (CrtZ) activity; and

the modification that decreases 2,2'-beta hydroxylase (CrtG) activity.

4. (canceled)

5. The recombinant microorganism of claim 1, comprising the modification that decreases phytoene synthase phytoene synthase (CrtB) activity with respect to the corresponding microorganism.

6. The recombinant microorganism of claim 5, wherein the recombinant microorganism exhibits increased accumulation of coenzyme Q_{10} (CoQ_{10}) with respect to the corresponding microorganism.

7. The recombinant microorganism of claim 1, comprising the modification that decreases lycopene cyclase (CrtY) activity.

8. The recombinant microorganism of claim 7, wherein the recombinant microorganism exhibits increased accumulation of lycopene with respect to the corresponding microorganism.

9. The recombinant microorganism of claim 1, comprising the modification that decreases 2,2'-beta hydroxylase (CrtG) activity.

10. The recombinant microorganism of claim 9, wherein the recombinant microorganism exhibits increased accumulation of zeaxanthin with respect to the corresponding microorganism.

11. The recombinant microorganism of claim 9, comprising the modification that decreases beta-carotene hydroxylase (CrtZ) activity.

12. The recombinant microorganism of claim 11, wherein the recombinant microorganism exhibits increased accumulation of beta-carotene with respect to the corresponding microorganism.

13. The recombinant microorganism of claim 9, comprising the modification that increases beta-carotene ketolase (CrtW) activity.

14. The recombinant microorganism of claim 13, wherein the modification that increases beta-carotene ketolase (CrtW) activity comprises a recombinant gene encoding a beta-carotene ketolase (CrtW) comprising a sequence having at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% sequence identity to SEQ ID NO: 12 or SEQ ID NO:14.

15. The recombinant microorganism of claim 13, wherein the recombinant microorganism exhibits increased accumulation of at least one of adonixanthin and astaxanthan with respect to the corresponding microorganism.

16. The recombinant microorganism of claim **1**, further comprising:

a modification that decreases 2-pyrone-4,6-dicarboxylic acid (PDC) hydrolase (LigI) activity with respect to the corresponding microorganism;

a modification that decreases 4-carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) methyl esterase (DesC) activity with respect to the corresponding microorganism;

a modification that decreases 4-carboxy-2-hydroxy-6-methoxy-6-oxohexa-2,4-dienoate (CHMOD) cis-trans isomerase (DesD) activity with respect to the corresponding microorganism; and/or

a modification that decreases vanillate/3-O-methylgallate O-demethylase (DmtS) activity with respect to the corresponding microorganism.

17. (canceled)

18. The recombinant microorganism of claim **16**, wherein the recombinant microorganism exhibits increased accumulation of 2-pyrone-4,6-dicarboxylic acid (PDC) with respect to the corresponding microorganism.

19. The recombinant microorganism of claim **1**, wherein the recombinant microorganism is from the genus *Novosphingobium.*

20. The recombinant microorganism of claim **1**, wherein the recombinant microorganism is *Novosphingobium aromaticivorans.*

21. A method for producing a compound comprising culturing the recombinant microorganism of claim **1** in a medium comprising a plant-derived phenolic.

22-25. (canceled)

26. The recombinant microorganism of claim **3**, wherein:

the modification that decreases phytoene synthase phytoene synthase (CrtB) activity comprises a modification to a gene encoding a phytoene synthase phytoene synthase (CrtB) comprising a sequence having at least 95% sequence identity to SEQ ID NO:2;

the modification that decreases lycopene-forming phytoene (CrtI) desaturase activity comprises a modification to a gene encoding a lycopene-forming phytoene desaturase (CrtI) comprising a sequence having at least 95% sequence identity to SEQ ID NO:4;

the modification that decreases lycopene cyclase (CrtY) activity comprises a modification to a gene encoding a lycopene cyclase (CrtY) comprising a sequence having at least 95% sequence identity to SEQ ID NO:6;

the modification that decreases beta-carotene hydroxylase (CrtZ) activity comprises a modification to a gene encoding a beta-carotene hydroxylase (CrtZ) comprising a sequence having at least 95% sequence identity to SEQ ID NO:8; and/or

the modification that decreases 2,2'-beta hydroxylase (CrtG) activity comprises a modification to a gene encoding a 2,2'-beta hydroxylase (CrtG) comprising a sequence having at least 95% sequence identity to SEQ ID NO:10.

* * * * *