

PRODUCTION OF CAROTENOIDS IN OLEAGINOUS YEAST AND FUNGI

Related Applications

[0001] This application claims the benefit of U.S. Provisional Application No. 60/663,621, filed March 18, 2005, the contents of which are hereby incorporated by reference in their entirety.

Background of the Invention

[0002] Carotenoids are organic pigments ranging in color from yellow to red that are naturally produced by certain organisms, including photosynthetic organisms (e.g., plants, algae, cyanobacteria), and some fungi. Carotenoids are responsible for the orange color of carrots, as well as the pink in flamingos and salmon, and the red in lobsters and shrimp. Animals, however, cannot produce carotenoids and must receive them through their diet.

[0003] Carotenoid pigments (e.g., β -carotene and astaxanthin) are used industrially as ingredients for food and feed stocks, both serving a nutritional function and enhancing consumer acceptability. For example, astaxanthin is widely used in salmon aquaculture to provide the orange coloration characteristic of their wild counterparts. Some carotenoids are also precursors of vitamin A. Also, carotenoids have antioxidant properties, and may have various health benefits (see, for example, Jyonouchi et al., *Nutr. Cancer* 16:93, 1991; Giovannucci et al., *J. Natl. Cancer Inst.* 87:1767, 1995; Miki, *Pure Appl. Chem* 63:141, 1991; Chow et al., *Anticancer Res.* 19:1849, 1999; Wang et al., *Antimicrob. Agents Chemother.* 44:2452, 2000). Some carotenoids such as β -carotene, lycopene, and lutein are currently sold as nutritional supplements.

[0004] In general, the biological systems that produce carotenoids are industrially intractable and/or produce the compounds at such low levels that commercial scale isolation is not practicable. Thus, most carotenoids used in industry are produced by chemical synthesis. There is a need for improved biological systems that produce carotenoids. Some efforts have previously been made to genetically engineer certain bacteria or fungi to produce higher levels of carotenoids (see, for example, Misawa et al., *J. Biotechnol.* 59:169, 1998; Visser et al., *FEMS Yeast Research* 4:221, 2003). However, improved systems, allowing higher levels of production and greater ease of isolation, are needed.

Summary of the Invention

[0005] The present invention provides improved systems for the biological production of carotenoids. In one aspect, the invention encompasses the discovery that it is desirable to produce carotenoids in oleaginous organisms. Without wishing to be bound by any particular theory, the present inventors propose that biological systems may be able to

accumulate higher levels of carotenoids if the compounds are sequestered in lipid bodies. Regardless of whether absolute levels are higher, however, carotenoids that are accumulated within lipid bodies in oleaginous organisms are readily isolatable through isolation of the lipid bodies.

[0006] The present invention therefore provides oleaginous fungi (including, for example, yeast or other unicellular fungi) that produce one or more carotenoids. The present invention also provides methods of constructing such yeast and fungi, methods of using such yeast and fungi to produce carotenoids, and methods of preparing carotenoid-containing compositions, such as food or feed additives, or nutritional supplements, using carotenoids produced in such oleaginous yeast or fungi. In particular, the present invention provides systems and methods for generating yeast and fungi containing one or more oleaginic and/or carotenogenic modifications that increase the oleaginic and/or alter their carotenoid-producing capabilities as compared with otherwise identical organisms that lack the modification(s).

[0007] The present invention further encompasses the general recognition that lipid-accumulating systems are useful for the production and/or isolation of lipophilic agents (such as, but not limited to isoprenoids, or isoprenoid-derived compounds). Thus, according to the present invention, it is desirable to engineer organisms to produce such lipophilic agents and/or to accumulate lipid.

[0008] Various other aspects of the present invention will be apparent to those of ordinary skill in the art from the present description, including the appended claims.

Brief Description of the Drawing

[0009] *Figure 1A-1D* depicts certain common carotenoids.

[0010] *Figure 2* depicts how sufficient levels of acetyl-CoA and NADPH may be accumulated in the cytosol of oleaginous organisms to allow for production of significant levels of cytosolic lipids. Enzymes: 1, pyruvate decarboxylase; 2, malate dehydrogenase; 3, malic enzyme; 4, pyruvate dehydrogenase; 5, citrate synthase; 6, ATP-citrate lyase; 7, citrate/malate translocase.

[0011] *Figures 3A and 3B* depict the mevalonate isoprenoid biosynthesis pathway, which typically operates in eukaryotes, including fungi.

[0012] *Figure 4* depicts the mevalonate-independent isoprenoid biosynthesis pathway, also known as the DXP pathway, which typically operates in bacteria and in the plastids of plants.

[0013] *Figure 5* depicts intermediates in the isoprenoid biosynthesis pathway and how they feed into biosynthetic pathways of other biomolecules, including carotenoids as

well as non-carotenoid compounds such as sterols, steroids, and vitamins, such as vitamin E or vitamin K.

[0014] *Figures 6A-6D* illustrate various carotenoid biosynthetic pathways. Figure 6A highlights branches leading to various cyclic and acyclic xanthophylls; Figure 6B shows certain *X. dendrorhous* pathways that generate dicyclic and monocyclic carotenoids, including astaxanthin; Figure 6C shows interconnecting pathways for converting β -carotene into any of a variety of other carotenoids, including astaxanthin; Figure 6D depicts possible routes of synthesis of cyclic carotenoids and common plant and algal xanthophylls from neurosporene.

[0015] *Figures 7A-7C* show an alignment of certain representative fungal HMG-CoA reductase polypeptides. As can be seen, these polypeptides show very high identity across the catalytic region, and also have complex membrane spanning domains. In some embodiments of the invention, these membrane-spanning domains are disrupted or are removed, so that, for example, a hyperactive version of the polypeptide may be produced.

[0016] *Figures 8A-8D* depict schematic representations of plasmids generated and described in detail in the exemplification.

Definitions

[0017] *Carotenogenic modification*: The term "carotenogenic modification", as used herein, refers to a modification of a host organism that adjusts production of one or more carotenoids, as described herein. For example, a carotenogenic modification may increase the production level of one or more carotenoids, and/or may alter relative production levels of different carotenoids. In principle, an inventive carotenogenic modification may be any chemical, physiological, genetic, or other modification that appropriately alters production of one or more carotenoids in a host organism produced by that organism as compared with the level produced in an otherwise identical organism not subject to the same modification. In most embodiments, however, the carotenogenic modification will comprise a genetic modification, typically resulting in increased production of one or more selected carotenoids. In some embodiments, the selected carotenoid is one or more of astaxanthin, β -carotene, canthaxanthin, lutein, lycopene, phytoene, zeaxanthin, and/or modifications of zeaxanthin or astaxanthin (e.g., glucoside, esterified zeaxanthin or astaxanthin). In some embodiments, the selected carotenoid is one or more xanthophylls, and/or a modification thereof (e.g., glucoside, esterified xanthophylls). In certain embodiments, the selected xanthophyll is selected from the group consisting of astaxanthin, lutein, zeaxanthin, lycopene, and modifications thereof. In some embodiments, the selected carotenoid is one or more of astaxanthin, β -carotene, canthaxanthin, lutein, lycopene, and zeaxanthin and/or modifications of zeaxanthin or astaxanthin. In some embodiments, the carotenoid is β -carotene. In some

embodiments, the selected carotenoid is astaxanthin. In some embodiments, the selected carotenoid is other than β -carotene.

[0018] *Carotenogenic polypeptide*: The term “carotenogenic polypeptide”, as used herein, refers to any polypeptide that is involved in the process of producing carotenoids in a cell, and may include polypeptides that are involved in processes other than carotenoid production but whose activities affect the extent or level of production of one or more carotenoids, for example by scavenging a substrate or reactant utilized by a carotenoid polypeptide that is directly involved in carotenoid production. Carotenogenic polypeptides include isoprenoid biosynthesis polypeptides, carotenoid biosynthesis polypeptides, and isoprenoid biosynthesis competitor polypeptides, as those terms are defined herein. The term also encompasses polypeptides that may affect the extent to which carotenoids are accumulated in lipid bodies.

[0019] *Carotenoid*: The term “carotenoid” is understood in the art to refer to a structurally diverse class of pigments derived from isoprenoid pathway intermediates. The commitment step in carotenoid biosynthesis is the formation of phytoene from geranylgeranyl pyrophosphate. Carotenoids can be acyclic or cyclic, and may or may not contain oxygen, so that the term carotenoids include both carotenes and xanthophylls. In general, carotenoids are hydrocarbon compounds having a conjugated polyene carbon skeleton formally derived from the five-carbon compound IPP, including triterpenes (C_{30} diapocarotenoids) and tetraterpenes (C_{40} carotenoids) as well as their oxygenated derivatives and other compounds that are, for example, C_{35} , C_{50} , C_{60} , C_{70} , C_{80} in length or other lengths. Many carotenoids have strong light absorbing properties and may range in length in excess of C_{200} . C_{30} diapocarotenoids typically consist of six isoprenoid units joined in such a manner that the arrangement of isoprenoid units is reversed at the center of the molecule so that the two central methyl groups are in a 1,6-positional relationship and the remaining non-terminal methyl groups are in a 1,5-positional relationship. Such C_{30} carotenoids may be formally derived from the acyclic $C_{30}H_{42}$ structure, having a long central chain of conjugated double bonds, by: (i) hydrogenation (ii) dehydrogenation, (iii) cyclization, (iv) oxidation, (v) esterification/glycosylation, or any combination of these processes. C_{40} carotenoids typically consist of eight isoprenoid units joined in such a manner that the arrangement of isoprenoid units is reversed at the center of the molecule so that the two central methyl groups are in a 1,6-positional relationship and the remaining non-terminal methyl groups are in a 1,5-positional relationship. Such C_{40} carotenoids may be formally derived from the acyclic $C_{40}H_{56}$ structure, having a long central chain of conjugated double bonds, by (i) hydrogenation, (ii) dehydrogenation, (iii) cyclization, (iv) oxidation, (v) esterification/glycosylation, or any combination of these processes. The class of C_{40}

carotenoids also includes certain compounds that arise from rearrangements of the carbon skeleton, or by the (formal) removal of part of this structure. More than 600 different carotenoids have been identified in nature; certain common carotenoids are depicted in Figure 1. Carotenoids include but are not limited to: antheraxanthin, adonirubin, adonixanthin, astaxanthin, canthaxanthin, capsorubin, β -cryptoxanthin, α -carotene, β -carotene, β,ψ -carotene, δ -carotene, ϵ -carotene, echinenone, 3-hydroxyechinenone, 3'-hydroxyechinenone, γ -carotene, ψ -carotene, 4-keto- γ -carotene, ζ -carotene, α -cryptoxanthin, deoxyflexixanthin, diatoxanthin, 7,8-didehydroastaxanthin, didihydrolycopene, fucoxanthin, fucoxanthinol, isorenieratene, β -isorenieratene, lactucaxanthin, lutein, lycopene, myxobactone, neoxanthin, neurosporene, hydroxyneurosporene, peridinin, phytoene, rhodopin, rhodopin glucoside, 4-keto-rubixanthin, siphonaxanthin, spheroidene, spheroidenone, spirilloxanthin, torulene, 4-keto-torulene, 3-hydroxy-4-keto-torulene, uriolide, uriolide acetate, violaxanthin, zeaxanthin- β -diglucoside, zeaxanthin, and C30 carotenoids. Additionally, carotenoid compounds include derivatives of these molecules, which may include hydroxy-, methoxy-, oxo-, epoxy-, carboxy-, or aldehydic functional groups. Further, included carotenoid compounds include ester (e.g., glycoside ester, fatty acid ester) and sulfate derivatives (e.g., esterified xanthophylls).

[0020] *Carotenoid biosynthesis polypeptide:* The term "carotenoid biosynthesis polypeptide" refers to any polypeptide that is involved in the synthesis of one or more carotenoids. To mention but a few, these carotenoid biosynthesis polypeptides include, for example, polypeptides of phytoene synthase, phytoene dehydrogenase (or desaturase), lycopene cyclase, carotenoid ketolase, carotenoid hydroxylase, astaxanthin synthase, carotenoid epsilon hydroxylase, lycopene cyclase (beta and epsilon subunits), carotenoid glucosyltransferase, and acyl CoA:diacylglycerol acyltransferase. Representative examples of carotenoid biosynthesis polypeptide sequences are presented in Tables 17-25.

[0021] *Gene:* The term "gene", as used herein, generally refers to a nucleic acid encoding a polypeptide, optionally including certain regulatory elements that may affect expression of one or more gene products (i.e., RNA or protein).

[0022] *Heterologous:* The term "heterologous", as used herein to refer to genes or polypeptides, refers to a gene or polypeptide that does not naturally occur in the organism in which it is being expressed. It will be understood that, in general, when a heterologous gene or polypeptide is selected for introduction into and/or expression by a host cell, the particular source organism from which the heterologous gene or polypeptide may be selected is not essential to the practice of the present invention. Relevant considerations may include, for example, how closely related the potential source and host organisms are in evolution, or how

related the source organism is with other source organisms from which sequences of other relevant polypeptides have been selected.

[0023] *Host cell:* As used herein, the “host cell” is a yeast or fungal cell that is manipulated according to the present invention to accumulate lipid and/or to express one or more carotenoids as described herein. A “modified host cell”, as that term is used herein, is a host cell that contains at least one oleaginic modification and/or at least one carotenogenic modification according to the present invention.

[0024] *Isolated:* The term “isolated”, as used herein, means that the isolated entity has been separated from at least one component with which it was previously associated. When most other components have been removed, the isolated entity is “purified”. Isolation and/or purification may be performed using any techniques known in the art including, for example, fractionation, extraction, precipitation, or other separation.

[0025] *Isoprenoid biosynthesis competitor polypeptide:* The term “isoprenoid biosynthesis competitor polypeptide”, as used herein, refers to a polypeptide whose expression in a cell reduces the level of geranylgeranyl diphosphate (GGPP) available to enter the carotenoid biosynthesis pathway. For example, isoprenoid biosynthesis competitor polypeptides include enzymes that act on isoprenoid intermediates prior to GGPP, such that less GGPP is generated (see, for example, Figure 5). Squalene synthase is but one isoprenoid biosynthesis competitor polypeptide according to the present invention; representative squalene synthase sequences are presented in Table 16. Prenyldiphosphate synthase enzymes and *para*-hydroxybenzoate (PHB) polyprenyltransferase are yet additional isoprenoid biosynthesis competitor polypeptides according to the present invention; representative prenyldiphosphate synthase enzymes and PHB polyprenyltransferase polypeptides are presented in Table 29 and 30 respectively.

[0026] *Isoprenoid biosynthesis polypeptide:* The term “isoprenoid biosynthesis polypeptide” refers to any polypeptide that is involved in the synthesis of isoprenoids. For example, as discussed herein, acetoacetyl-CoA thiolase, HMG-CoA synthase, HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, IPP isomerase, FPP synthase, and GGPP synthase, are all involved in the mevalonate pathway for isoprenoid biosynthesis. Each of these proteins is also an isoprenoid biosynthesis polypeptide for purposes of the present invention, and sequences of representative examples of these enzymes are provided in Tables 7-15.

[0027] *Isoprenoid pathway:* The “isoprenoid pathway” is understood in the art to refer to a metabolic pathway that either produces or utilizes the five-carbon metabolite isopentenyl pyrophosphate (IPP). As discussed herein, two different pathways can produce the common isoprenoid precursor IPP—the “mevalonate pathway” and the “non-mevalonate

pathway". The term "isoprenoid pathway" is sufficiently general to encompass both of these types of pathway. Biosynthesis of isoprenoids from IPP occurs by polymerization of several five-carbon isoprene subunits. Isoprenoid metabolites derived from IPP are of varying size and chemical structure, including both cyclic and acyclic molecules. Isoprenoid metabolites include, but are not limited to, monoterpenes, sesquiterpenes, diterpenes, sterols, and polyprenols such as carotenoids.

[0028] *Oleaginic modification:* The term "oleaginic modification", as used herein, refers to a modification of a host organism that adjusts the desirable oleaginity of that host organism, as described herein. In some cases, the host organism will already be oleaginous in that it will have the ability to accumulate lipid to at least about 20% of its dry cell weight. It may nonetheless be desirable to apply an oleaginic modification to such an organism, in accordance with the present invention, for example to increase (or, in some cases, possibly to decrease) its total lipid accumulation, or to adjust the types or amounts of one or more particular lipids it accumulates (e.g., to increase relative accumulation of triacylglycerol). In other cases, the host organism may be non-oleaginous (though may contain some enzymatic and regulatory components used in other organisms to accumulate lipid), and may require oleaginic modification in order to become oleaginous in accordance with the present invention. The present invention also contemplates application of oleaginic modification to non-oleaginous host strains such that their oleaginicity is increased even though, even after being modified, they may not be oleaginous as defined herein. In principle, the oleaginic modification may be any chemical, physiological, genetic, or other modification that appropriately alters oleaginity of a host organism as compared with an otherwise identical organism not subjected to the oleaginic modification. In most embodiments, however, the oleaginic modification will comprise a genetic modification, typically resulting in increased production and/or activity of one or more oleaginic polypeptides. In some embodiments, the oleaginic modification comprises at least one chemical, physiological, genetic, or other modification; in other embodiments, the oleaginic modification comprises more than one chemical, physiological, genetic, or other modification. In certain aspects where more than one modification is utilized, such modifications can comprise any combination of chemical, physiological, genetic, or other modification (e.g., one or more genetic modification and chemical or physiological modification).

[0029] *Oleaginic polypeptide:* The term "oleaginic polypeptide", as used herein, refers to any polypeptide that is involved in the process of lipid accumulation in a cell and may include polypeptides that are involved in processes other than lipid biosynthesis but whose activities affect the extent or level of accumulation of one or more lipids, for example by scavenging a substrate or reactant utilized by an oleaginic polypeptide that is directly involved in lipid accumulation. For example, as discussed herein, acetyl-CoA carboxylase,

pyruvate decarboxylase, isocitrate dehydrogenase, ATP-citrate lyase, malic enzyme, and AMP deaminase, among other proteins, are all involved in lipid accumulation in cells. In general, reducing the activity of pyruvate decarboxylase or isocitrate dehydrogenase, and/or increasing the activity of acetyl CoA carboxylase, ATP-citrate lyase, malic enzyme and/or AMP deaminase is expected to promote oleaginity. Each of these proteins is an oleaginic polypeptide for purposes of the present invention, and sequences of representative examples of these enzymes are provided in Tables 1-6.

[0030] *Oleaginous*: The term "oleaginous", refers to the ability of an organism to accumulate lipid to at least about 20% of its dry cell weight. In certain embodiments of the invention, oleaginous yeast or fungi accumulate lipid to at least about 25% of their dry cell weight. In other embodiments, inventive oleaginous yeast or fungi accumulate lipid within the range of about 20-45% of their dry cell weight. In some embodiments, oleaginous organisms may accumulate lipid to as much as about 70% of their dry cell weight. In some embodiments of the invention, oleaginous organisms may accumulate a large fraction of total lipid accumulation in the form of triacylglycerol. In certain embodiments, the majority of the accumulated lipid is in the form of triacylglycerol. Alternatively or additionally, the lipid may accumulate in the form of intracellular lipid bodies, or oil bodies. In certain embodiments, the present invention utilizes yeast or fungi that are naturally oleaginous. In some aspects, naturally oleaginous organisms are manipulated (e.g., genetically, chemically, or otherwise) so as to further increase the level of accumulated lipid in the organism. In other embodiments, yeast or fungi that are not naturally oleaginous are manipulated (e.g., genetically, chemically, or otherwise) to accumulate lipid as described herein. For the purposes of the present invention, *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*) and *Candida utilis* are not naturally oleaginous fungi.

[0031] *Polypeptide*: The term "polypeptide", as used herein, generally has its art-recognized meaning of a polymer of at least three amino acids. However, the term is also used to refer to specific functional classes of polypeptides, such as, for example, oleaginic polypeptides, carotenogenic polypeptides, isoprenoid biosynthesis polypeptides, carotenoid biosynthesis polypeptides, and isoprenoid biosynthesis competitor polypeptides. For each such class, the present specification provides several examples of known sequences of such polypeptides. Those of ordinary skill in the art will appreciate, however, that the term "polypeptide" is intended to be sufficiently general as to encompass not only polypeptides having the complete sequence recited herein (or in a reference or database specifically mentioned herein), but also to encompass polypeptides that represent functional fragments (i.e., fragments retaining at least one activity) of such complete polypeptides. Moreover, those of ordinary skill in the art understand that protein sequences generally tolerate some substitution without destroying activity. Thus, any polypeptide that retains activity and shares

at least about 30-40% overall sequence identity, often greater than about 50%, 60%, 70%, or 80%, and further usually including at least one region of much higher identity, often greater than 90% or even 95%, 96%, 97%, 98%, or 99% in one or more highly conserved regions (e.g., isocitrate dehydrogenase polypeptides often share a conserved AMP-binding motif; HMG-CoA reductase polypeptides typically include a highly conserved catalytic domain (see, for example, Figure 7); acetyl coA carboxylase typically has a carboxyl transferase domain; see, for example, Downing et al., *Chem. Abs.* 93:484, 1980; Gil et al., *Cell* 41:249, 1985; Jitrapakdee et al. *Curr Protein Pept Sci.* 4:217, 2003; U.S. Patent Number 5,349,126, each of which is incorporated herein by reference in its entirety), usually encompassing at least 3-4 and often up to 20 or more amino acids, with another polypeptide of the same class, is encompassed within the relevant term "polypeptide" as used herein.

[0032] *Source organism:* The term "source organism", as used herein, refers to the organism in which a particular polypeptide sequence can be found in nature. Thus, for example, if one or more heterologous polypeptides is/are being expressed in a host organism, the organism in which the polypeptides are expressed in nature (and/or from which their genes were originally cloned) is referred to as the "source organism". Where more than one heterologous polypeptides are being expressed in a host organism, one or more source organism(s) may be utilized for independent selection of each of the heterologous polypeptide(s). It will be appreciated that any and all organisms that naturally contain relevant polypeptide sequences may be used as source organisms in accordance with the present invention. Representative source organisms include, for example, animal, mammalian, insect, plant, fungal, yeast, algal, bacterial, cyanobacterial, archaeobacterial and protozoal source organisms.

Detailed Description of Certain Preferred Embodiments of the Invention

[0033] As noted above, the present invention encompasses the discovery that carotenoids can desirably be produced in oleaginous yeast and fungi. According to the present invention, strains that both (i) accumulate lipid, often in the form of cytoplasmic oil bodies and typically to at least about 20% of their dry cell weight; and (ii) produce carotenoid(s) at a level at least about 1%, and in some embodiments at least about 3-20%, of their dry cell weight, are generated through manipulation of host cells (i.e., strains, including, e.g., naturally-occurring strains, strains which have been previously modified, etc.). These manipulated host cells are then used to produce carotenoids, so that carotenoids that partition into the lipid bodies can readily be isolated.

[0034] In general, it will be desirable to balance oleaginy and carotenoid production in inventive cells such that, as soon as a minimum desirable level of oleaginy is achieved, substantially all further carbon which is capable of being utilized and diverted into

biosynthesis of products is diverted into a carotenoid production pathway. In some embodiments of the invention, this strategy involves engineering cells to be oleaginous; in other embodiments, it involves engineering cells to accumulate a higher level of lipid, particularly cytoplasmic lipid, than they would accumulate in the absence of such engineering even though the engineered cells may not become "oleaginous" as defined herein. In other embodiments, the extent to which an oleaginous host cell accumulates lipid is actually reduced so that remaining carbon can be utilized in carotenoid production.

Host Cells

[0035] Those of ordinary skill in the art will readily appreciate that a variety of yeast and fungal strains exist that are naturally oleaginous or that naturally produce carotenoids. Any of such strains may be utilized as host strains according to the present invention, and may be engineered or otherwise manipulated to generate inventive oleaginous, carotenoid-producing strains. Alternatively, strains that naturally are neither oleaginous nor carotenoid-producing may be employed. Furthermore, even when a particular strain has a natural capacity for oleaginy or for carotenoid production, its natural capabilities may be adjusted as described herein, so as to change the production level of lipid and/or carotenoid. In certain embodiments engineering or manipulation of a strain results in modification of a type of lipid and/or carotenoid which is produced. For example, a strain may be naturally oleaginous and/or carotenogenic, however engineering or modification of the strain may be employed so as to change the type of lipid which is accumulated and or to change the type of carotenoid which is produced.

[0036] When selecting a particular yeast or fungal strain for use in accordance with the present invention, it will generally be desirable to select one whose cultivation characteristics are amenable to commercial scale production. For example, it will generally (though not necessarily always) be desirable to avoid filamentous organisms, or organisms with particularly unusual or stringent requirements for growth conditions. However, where conditions for commercial scale production can be applied which allow for utilization of filamentous organisms, these may be selected as host cells. In some embodiments of the invention, it will be desirable to utilize edible organisms as host cells, as they may optionally be formulated directly into food or feed additives, or into nutritional supplements, as desired. For ease of production, some embodiments of the invention utilize host cells that are genetically tractable, amenable to molecular genetics (e.g., can be efficiently transformed, especially with established or available vectors; optionally can incorporate and/or integrate multiple genes, for example sequentially; and/or have known genetic sequence; etc), devoid of complex growth requirements (e.g., a necessity for light), mesophilic (e.g., prefer growth temperatures with in the range of about 25-32 °C), able to assimilate a variety of carbon and nitrogen sources and/or capable of growing to high cell density. Alternatively or additionally,

various embodiments of the invention utilize host cells that grow as single cells rather than multicellular organisms (e.g., as mycelia).

[0037] In general, when it is desirable to utilize a naturally oleaginous organism in accordance with the present invention, any modifiable and cultivatable oleaginous organism may be employed. In certain embodiments of the invention, yeast or fungi of genera including, but not limited to, *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*, and *Yarrowia* are employed. In certain particular embodiments, organisms of species that include, but are not limited to, *Blakeslea trispora*, *Candida pulcherrima*, *C. revkaufi*, *C. tropicalis*, *Cryptococcus curvatus*, *Cunninghamella echinulata*, *C. elegans*, *C. japonica*, *Lipomyces starkeyi*, *L. lipoferus*, *Mortierella alpina*, *M. isabellina*, *M. ramanniana*, *M. vinacea*, *Mucor circinelloides*, *Phycomyces blakesleanus*, *Pythium irregulare*, *Rhodosporidium toruloides*, *Rhodotorula glutinis*, *R. gracilis*, *R. graminis*, *R. mucilaginosus*, *R. pinicola*, *Trichosporon pullans*, *T. cutaneum*, and *Yarrowia lipolytica* are used.

[0038] Of these naturally oleaginous strains, some also naturally produce carotenoids and some do not. In most cases, only low levels (less than about 0.05% dry cell weight) of carotenoids are produced by naturally-occurring carotenogenic, oleaginous yeast or fungi. Higher levels of β -carotene are sometimes produced, but high levels of other carotenoids are generally not observed.

[0039] In general, any organism that is naturally oleaginous and non-carotenoid-producing (e.g., produce less than about 0.05% dry cell weight, do not produce the carotenoid of interest) may be utilized as a host cell in accordance with the present invention. In some embodiments, the organism is a yeast or fungus from a genus such as, but not limited to, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Pythium*, *Trichosporon*, and *Yarrowia*; in some embodiments, the organism is of a species including, but not limited to, *Mortierella alpina* and *Yarrowia lipolytica*.

[0040] Comparably, the present invention may utilize any naturally oleaginous, carotenoid-producing organism as a host cell. In general, the present invention may be utilized to increase carbon flow into the isoprenoid pathway in naturally carotenoid-producing organisms (particularly for organisms other than *Blakeslea* and *Phycomyces*), and/or to shift production from one carotenoid (e.g., β -carotene) to another (e.g., astaxanthin). Introduction of one or more carotenogenic modifications (e.g., increased expression of one or more endogenous or heterologous carotenogenic polypeptides), in accordance with the present invention, can achieve these goals.

[0041] In certain embodiments of the invention, the utilized oleaginous, carotenoid-producing organism is a yeast or fungus, for example of a genus such as, but not limited to,

Blakeslea, *Mucor*, *Phycomyces*, *Rhodosporidium*, and *Rhodotorula*; in some embodiments, the organism is of a species such as, *Mucor circinelloides* and *Rhodotorula glutinis*.

[0042] When it is desirable to utilize strains that are naturally non-oleaginous as host cells in accordance with the present invention, genera of non-oleaginous yeast or fungi include, but are not limited to, *Aspergillus*, *Botrytis*, *Cercospora*, *Fusarium* (*Gibberella*), *Kluyveromyces*, *Neurospora*, *Penicillium*, *Pichia* (*Hansenula*), *Puccinia*, *Saccharomyces*, *Sclerotium*, *Trichoderma*, and *Xanthophyllomyces* (*Phaffia*); in some embodiments, the organism is of a species including, but not limited to, *Aspergillus nidulans*, *A. niger*, *A. terreus*, *Botrytis cinerea*, *Cercospora nicotianae*, *Fusarium fujikuroi* (*Gibberella zeae*), *Kluyveromyces lactis*, *K. lactis*, *Neurospora crassa*, *Pichia pastoris*, *Puccinia distincta*, *Saccharomyces cerevisiae*, *Sclerotium rolsii*, *Trichoderma reesei*, and *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*).

[0043] It will be appreciated that the term "non-oleaginous", as used herein, encompasses both strains that naturally have some ability to accumulate lipid, especially cytoplasmically, but do not do so to a level sufficient to qualify as "oleaginous" as defined herein, as well as strains that do not naturally have any ability to accumulate extra lipid, e.g., extra-membranous lipid. It will further be appreciated that, in some embodiments of the invention, it will be sufficient to increase the natural level of oleaginity of a particular host cell, even if the modified cell does not qualify as oleaginous as defined herein.

[0044] As with the naturally oleaginous organisms, some of the naturally non-oleaginous fungi naturally produce carotenoids, whereas others do not. Genera of naturally non-oleaginous fungi that do not naturally produce carotenoids (e.g., produce less than about 0.05% dry cell weight, do not produce carotenoid of interest) may desirably be used as host cells in accordance with the present invention include, but are not limited to, *Aspergillus*, *Kluyveromyces*, *Penicillium*, *Saccharomyces*, and *Pichia*; species include, but are not limited to, *Aspergillus niger* and *Saccharomyces cerevisiae*. Genera of naturally non-oleaginous fungi that do naturally produce carotenoids and that may desirably be used as host cells in accordance with the present invention include, but are not limited to, *Botrytis*, *Cercospora*, *Fusarium* (*Gibberella*), *Neurospora*, *Puccinia*, *Sclerotium*, *Trichoderma*, and *Xanthophyllomyces* (*Phaffia*); species include, but are not limited to, *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*).

[0045] As discussed above, any of a variety of organisms may be employed as host cells in accordance with the present invention. In certain embodiments of the invention, host cells will be *Yarrowia lipolytica* cells. Advantages of *Y. lipolytica* include, for example, tractable genetics and molecular biology, availability of genomic sequence (see, for example, Sherman et al. *Nucleic Acids Res.* 32(Database issue):D315-8, 2004), suitability to various

cost-effective growth conditions, and ability to grow to high cell density. In addition, *Y. lipolytica* is naturally oleaginous, such that fewer manipulations may be required to generate an oleaginous, carotenoid-producing *Y. lipolytica* strain than might be required for other organisms. Furthermore, there is already extensive commercial experience with *Y. lipolytica*.

[0046] *Saccharomyces cerevisiae* is also a useful host cell in accordance with the present invention, particularly due to its experimental tractability and the extensive experience that researchers have accumulated with the organism. Although cultivation of *Saccharomyces* under high carbon conditions may result in increased ethanol production, this can generally be managed by process and/or genetic alterations.

[0047] Additional useful hosts include *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*), which is experimentally tractable and naturally carotenogenic. *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*) strains can produce several carotenoids, including astaxanthin.

[0048] *Aspergillus niger* and *Mortierella alpina* accumulate large amounts of citric acid and fatty acid, respectively; *Mortierella alpina* is also oleaginous.

[0049] *Neurospora* or *Gibberella* are also useful. They are not naturally oleaginous and tend to produce very low levels of carotenoids, thus extensive modification may be required in accordance with the present invention. *Neurospora* and *Gibberella* are considered relatively tractable from an experimental standpoint. Both are filamentous fungi, such that production at commercial scales can be a challenge necessary to overcome in utilization of such strains.

[0050] *Mucor circinelloides* is another available useful species. While its molecular genetics are generally less accessible than are those of some other organisms, it naturally produces β -carotene, thus may require less modification than other species available.

[0051] Molecular genetics can be performed in *Blakeslea*, though significant effort may be required. Furthermore, cost-effective fermentation conditions can be challenging, as, for example, it may be required that the two mating types are mixed. Fungi of the genus *Phycomyces* are also possible sources which have the potential to pose fermentation process challenges, and these fungi are also may be less amenable to manipulate than several other potential host organisms.

[0052] Those of ordinary skill in the art will appreciate that the selection of a particular host cell for use in accordance with the present invention will also affect, for example, the selection of expression sequences utilized with any heterologous polypeptide to be introduced into the cell, and will also influence various aspects of culture conditions, etc. Much is known about the different gene regulatory requirements, protein targeting sequence requirements, and cultivation requirements, of different host cells to be utilized in accordance

with the present invention (see, for example, with respect to *Yarrowia*, Barth et al. *FEMS Microbiol Rev.* 19:219, 1997; Madzak et al. *J Biotechnol.* 109:63, 2004; see, for example, with respect to *Xanthophyllomyces*, Verdoes et al. *Appl Environ Microbiol* 69: 3728-38, 2003; Visser et al. *FEMS Yeast Res* 4: 221-31, 2003; Martinez et al. *Antonie Van Leeuwenhoek.* 73(2):147-53, 1998; Kim et al. *Appl Environ Microbiol.* 64(5):1947-9, 1998; Wery et al. *Genet.* 184(1):89-97, 1997; see, for example, with respect to *Saccharomyces*, Guthrie and Fink *Methods in Enzymology* 194:1-933, 1991). In certain aspects, for example, targeting sequences of the host cell (or closely related analogs) may be useful to include for directing heterologous proteins to subcellular localization. Thus, such useful targeting sequences can be added to heterologous sequence for proper intracellular localization of activity. In other aspects (e.g., addition of mitochondrial targeting sequences), heterologous targeting sequences may be eliminated or altered in the selected heterologous sequence (e.g., alteration or removal of source organism plant chloroplast targeting sequences).

Engineering Oleaginy

[0053] All living organisms synthesize lipids for use in their membranes and various other structures. However, most organisms do not accumulate in excess of about 10% of their dry cell weight as total lipid, and most of this lipid generally resides within cellular membranes.

[0054] Significant biochemical work has been done to define the metabolic enzymes necessary to confer oleaginy on microorganisms (primarily for the purpose of engineering single cell oils as commercial sources of arachidonic acid and docosahexaenoic acid; see for example Ratledge *Biochimie* 86:807, 2004, the entire contents of which are incorporated herein by reference). Although this biochemical work is compelling, prior to the present invention, there have been no reports of *de novo* oleaginy being established through genetic engineering with the genes encoding the key metabolic enzymes.

[0055] It should be noted that oleaginous organisms typically only accumulate lipid when grown under conditions of carbon excess and nitrogen or other nutrient limitation. Under these conditions, the organism readily depletes the limiting nutrient but continues to assimilate the carbon source. The "excess" carbon is channeled into lipid biosynthesis so that lipids (usually triacylglycerols) accumulate in the cytosol, typically in the form of bodies.

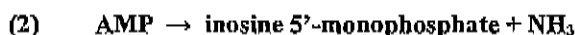
[0056] In general, it is thought that, in order to be oleaginous, an organism must produce both acetyl-CoA and NADPH in the cytosol, which can then be utilized by the fatty acid synthase machinery to generate lipids. In at least some oleaginous organisms, acetyl-CoA is generated in the cytosol through the action of ATP-citrate lyase, which catalyzes the reaction:



[0057] Of course, in order for ATP-citrate lyase to generate appropriate levels of acetyl-CoA in the cytosol, it must first have an available pool of its substrate citric acid. Citric acid is generated in the mitochondria of all eukaryotic cells through the tricarboxylic acid (TCA) cycle, and can be moved into the cytosol (in exchange for malate) by citrate/malate translocase.

[0058] In most oleaginous organisms, and in some non-oleaginous organisms, the enzyme isocitrate dehydrogenase, which operates as part of the TCA cycle in the mitochondria, is strongly AMP-dependent. Thus, when AMP is depleted from the mitochondria, this enzyme is inactivated. When isocitrate dehydrogenase is inactive, isocitrate accumulates in the mitochondria. This accumulated isocitrate is then equilibrated with citric acid, presumably through the action of aconitase. Therefore, under conditions of low AMP, citrate accumulates in the mitochondria. As noted above, mitochondrial citrate is readily transported into the cytosol.

[0059] AMP depletion, which in oleaginous organisms is believed to initiate the cascade leading to accumulation of citrate (and therefore acetyl-CoA) in the cytoplasm, occurs as a result of the nutrient depletion mentioned above. When oleaginous cells are grown in the presence of excess carbon source but under conditions limiting for nitrogen or some other nutrient(s), the activity of AMP deaminase, which catalyzes the reaction:



is strongly induced. The increased activity of this enzyme depletes cellular AMP in both the cytosol and the mitochondria. Depletion of AMP from the mitochondria is thought to inactivate the AMP-dependent isocitrate dehydrogenase, resulting in accumulation of citrate in the mitochondria and, therefore, the cytosol. This series of events is depicted diagrammatically in Figure 2.

[0060] As noted above, oleaginy requires *both* cytosolic acetyl-CoA *and* cytosolic NADPH. It is believed that, in many oleaginous organisms, appropriate levels of cytosolic NADPH are provided through the action of malic enzyme (Enzyme 3 in Figure 2). Some oleaginous organisms (e.g., *Lipomyces* and some *Candida*) do not appear to have malic enzymes, however, so apparently other enzymes can provide comparable activity, although it is expected that a dedicated source of NADPH is probably required for fatty acid synthesis (see, for example, Wynn et al., *Microbiol* 145:1911, 1999; Ratledge *Adv. Appl. Microbiol.* 51:1, 2002, each of which is incorporated herein by reference in its entirety).

[0061] Thus, according to the present invention, the oleaginy of a host organism may be enhanced by modifying the expression or activity of one or more polypeptides involved in generating cytosolic acetyl-CoA and/or NADPH. For example, modification of the expression or activity of one or more of acetyl-CoA carboxylase, pyruvate decarboxylase,

isocitrate dehydrogenase, ATP-citrate lyase, malic enzyme, and AMP-deaminase can enhance oleaginy in accordance with the present invention. Exemplary polypeptides which can be utilized or derived so as to enhance oleaginy in accordance with the present invention include, but are not limited to those acetyl-CoA carboxylase, pyruvate decarboxylase, isocitrate dehydrogenase, ATP-citrate lyase, malic enzyme, and AMP-deaminase polypeptides provided in Table 1, Table 2, Table 3, Table 4, Table 5, and Table 6, respectively.

[0062] In some embodiments of the invention, where an oleaginous host cell is employed, enzymes and regulatory components relevant to oleaginy are already in place but could be modified, if desired, by for example altering expression or activity of one or more oleaginic polypeptides and/or by introducing one or more heterologous oleaginic polypeptides. In those embodiments of the invention where a non-oleaginous host cell is employed, it is generally expected that at least one or more heterologous oleaginic polypeptides will be introduced.

[0063] The present invention contemplates not only introduction of heterologous oleaginous polypeptides, but also adjustment of expression or activity levels of heterologous or endogenous oleaginic polypeptides, including, for example, alteration of constitutive or inducible expression patterns. In some embodiments of the invention, expression patterns are adjusted such that growth in nutrient-limiting conditions is not required to induce oleaginy. For example, genetic modifications comprising alteration and/or addition of regulatory sequences (e.g., promoter elements, terminator elements) may be utilized to confer particular regulation of expression patterns. Such genetic modifications may be utilized in conjunction with endogenous genes (e.g., for regulation of endogenous oleaginic polypeptide(s)); alternatively, such genetic modifications may be included so as to confer regulation of expression of at least one heterologous polypeptide (e.g., oleaginic polypeptide(s)). For example, promoters including, but not limited to Tef1, Gpd1 promoters can be used in conjunction with endogenous genes and/or heterologous genes for modification of expression patterns of endogenous oleaginic polypeptides and/or heterologous oleaginic polypeptides. Similarly, exemplary terminator sequences include, but are not limited to, use of *Y. lipolytica* XPR2 terminator sequences.

[0064] In some embodiments, at least one oleaginic polypeptide is introduced into a host cell. In some embodiments of the invention, a plurality (e.g., two or more) of different oleaginic polypeptides is introduced into the same host cell. In some embodiments, the plurality of oleaginic polypeptides contains polypeptides from the same source organism; in other embodiments, the plurality includes polypeptides independently selected from different source organisms.

[0065] Representative examples of a variety of oleaginic polypeptides that may be introduced into or modified within host cells according to the present invention, include, but are not limited to, those provided in Tables 1-6. As noted above, it is expected that at least some of these polypeptides (e.g., malic enzyme and ATP-citrate lyase) should desirably act in concert, and possibly together with one or more components of fatty acid synthase, such that, in some embodiments of the invention, it will be desirable to utilize two or more oleaginic polypeptides from the same source organism.

[0066] In general, source organisms for oleaginic polypeptides to be used in accordance with the present invention include, but are not limited to, *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodospiridium*, *Rhodotorula*, *Trichosporon*, *Yarrowia*, *Aspergillus*, *Botrytis*, *Cercospora*, *Fusarium* (*Gibberella*), *Kluyveromyces*, *Neurospora*, *Penicillium*, *Pichia* (*Hansenula*), *Puccinia*, *Saccharomyces*, *Sclerotium*, *Trichoderma*, and *Xanthophyllomyces* (*Phaffia*). In some embodiments, the source species for acetyl CoA carboxylase, ATP-citrate lyase, malic enzyme and/or AMP deaminase polypeptides include, but are not limited to, *Aspergillus nidulans*, *Cryptococcus neoformans*, *Fusarium fujikuroi*, *Kluyveromyces lactis*, *Neurospora crassa*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Ustilago maydis*, and *Yarrowia lipolytica*; in some embodiments, source species for pyruvate decarboxylase or isocitrate dehydrogenase polypeptides include, but are not limited to *Neurospora crassa*, *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*), *Aspergillus niger*, *Saccharomyces cerevisiae*, *Mucor circinelloides*, *Rhodotorula glutinis*, *Candida utilis*, *Mortierella alpina* and *Yarrowia lipolytica*.

Engineering Carotenoid Production

[0067] Carotenoids are synthesized from isoprenoid precursors, some of which are also involved in the production of steroids and sterols. The most common isoprenoid biosynthesis pathway, sometimes referred to as the "mevalonate pathway", is generally depicted in Figure 3. As shown, acetyl-CoA is converted, via hydroxymethylglutaryl-CoA (HMG-CoA), into mevalonate. Mevalonate is then phosphorylated and converted into the five-carbon compound isopentenyl pyrophosphate (IPP). Following isomerization of IPP into dimethylallyl pyrophosphate (DMAPP), three sequential condensation reactions with additional molecules of IPP generate the ten-carbon molecule geranyl pyrophosphate (GPP), followed by the fifteen-carbon molecule farnesyl pyrophosphate (FPP), and finally the twenty-carbon compound geranylgeranyl pyrophosphate (GGPP).

[0068] An alternative isoprenoid biosynthesis pathway, that is utilized by some organisms (particularly bacteria) and is sometimes called the "mevalonate-independent pathway", is depicted in Figure 4. This pathway is initiated by the synthesis of 1-deoxy-D-

xyloglucose-5-phosphate (DOXP) from pyruvate and glyceraldehyde-3-phosphate. DOXP is then converted, via a series of reactions shown in Figure 4, into IPP, which isomerizes into DMAPP and is then converted, via GPP and FPP, into GGPP as shown in Figure 3 and discussed above.

[0069] Various proteins involved in isoprenoid biosynthesis have been identified and characterized in a number of organisms. Moreover, various aspects of the isoprenoid biosynthesis pathway are conserved throughout the fungal, bacterial, plant and animal kingdoms. For example, polypeptides corresponding to the acetoacetyl-CoA thiolase, HMG-CoA synthase, HMG-CoA reductase, mevalonate kinase, phosphomevalonate kinase, mevalonate pyrophosphate decarboxylase, IPP isomerase, FPP synthase, and GGPP synthase shown in Figure 3 have been identified in and isolated from a wide variety of organisms and cells. Representative examples of a wide variety of such polypeptides are provided in Tables 7-15. One or more of the polypeptides selected from those provided in any one of Tables 7-15 may be utilized or derived for use in the methods and compositions in accordance with the present invention.

[0070] According to the present invention, carotenoid production in a host organism may be adjusted by modifying the expression or activity of one or more proteins involved in isoprenoid biosynthesis. In some embodiments, such modification involves introduction of one or more heterologous isoprenoid biosynthesis polypeptides into the host cell; alternatively or additionally, modifications may be made to the expression or activity of one or more endogenous or heterologous isoprenoid biosynthesis polypeptides. Given the considerable conservation of components of the isoprenoid biosynthesis polypeptides, it is expected that heterologous isoprenoid biosynthesis polypeptides will often function even in significantly divergent organisms. Furthermore, should it be desirable to introduce more than one heterologous isoprenoid biosynthesis polypeptide, in many cases polypeptides from different source organisms will function together. In some embodiments of the invention, a plurality of different heterologous isoprenoid biosynthesis polypeptides is introduced into the same host cell. In some embodiments, this plurality contains only polypeptides from the same source organism (e.g., two or more sequences of, or sequences derived from, the same source organism); in other embodiments the plurality includes polypeptides independently selected from different source organisms (e.g., two or more sequences of, or sequences derived from, at least two independent source organisms).

[0071] In some embodiments of the present invention that utilize heterologous isoprenoid biosynthesis polypeptides, the source organisms include, but are not limited to, fungi of the genera *Blakeslea*, *Candida*, *Cryptococcus*, *Cunninghamella*, *Lipomyces*, *Mortierella*, *Mucor*, *Phycomyces*, *Pythium*, *Rhodosporidium*, *Rhodotorula*, *Trichosporon*,

Yarrowia, *Aspergillus*, *Botrytis*, *Cercospora*, *Fusarium* (*Gibberella*), *Kluyveromyces*, *Neurospora*, *Penicillium*, *Pichia* (*Hansenula*), *Puccinia*, *Saccharomyces*, *Schizosaccharomyces*, *Sclerotium*, *Trichoderms*, *Ustilago*, and *Xanthophyllomyces* (*Phaffia*). In certain embodiments, the source organisms are of a species including, but not limited to, *Cryptococcus neoformans*, *Fusarium fujikuroi*, *Kluyveromyces lactis*, *Neurospora crassa*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Ustilago maydis*, and *Yarrowia lipolytica*.

[0072] As noted above, the isoprenoid biosynthesis pathway is also involved in the production of non-carotenoid compounds, such as sterols, steroids, and vitamins, such as vitamin E or vitamin K. Proteins that act on isoprenoid biosynthesis pathway intermediates, and divert them into biosynthesis of non-carotenoid compounds are therefore indirect inhibitors of carotenoid biosynthesis (see, for example, Figure 5, which illustrates points at which isoprenoid intermediates are channeled into other biosynthesis pathways). Such proteins are therefore considered isoprenoid biosynthesis competitor polypeptides. Reductions of the level or activity of such isoprenoid biosynthesis competitor polypeptides are expected to increase carotenoid production in host cells according to the present invention.

[0073] In some embodiments of the present invention, production or activity of endogenous isoprenoid biosynthesis competitor polypeptides may be reduced or eliminated in host cells. In some embodiments, this reduction or elimination of the activity of an isoprenoid biosynthesis competitor polypeptide can be achieved by treatment of the host organism with small molecule inhibitors of enzymes of the ergosterol biosynthetic pathway. Enzymes of the ergosterol biosynthetic pathway include, for example, squalene synthase, squalene epoxidase, 2,3-oxidosqualene-lanosterol cyclase, cytochrome P450 lanosterol 14 α -demethylase, C-14 sterol reductase, C-4 sterol methyl oxidase, SAM:C-24 sterol methyltransferase, C-8 sterol isomerase, C-5 sterol desaturase, C-22 sterol desaturase, and C-24 sterol reductase. Each of these enzymes is considered an isoprenoid biosynthesis competitor polypeptide. Regulators of these enzymes may also be considered isoprenoid biosynthesis competitor polypeptides (e.g., the yeast proteins Sut1 (Genbank Accession JC4374 GI:2133159) and Mot3 (Genbank Accession NP_013786 GI:6323715), which may or may not have homologs in other organisms).

[0074] In other embodiments, reduction or elimination of the activity of an isoprenoid biosynthesis competitor polypeptide can be achieved by decreasing activity of the ubiquinone biosynthetic pathway. The commitment step in ubiquinone biosynthesis is the formation of *para*-hydroxybenzoate (PHB) from tyrosine or phenylalanine in mammals or chorismate in bacteria, followed by condensation of PHB and isoprene precursor, resulting in addition of the prenyl group. This reaction is catalyzed by PHB-polyprenyltransferase. The

isoprenoid side chain of ubiquinone is determined by the prenyldiphosphate synthase enzyme. The 3-decaprenyl-4-hydroxybenzoic acid resulting from the condensation of PHB and decaprenyldiphosphate reaction undergoes further modifications, which include hydroxylation, methylation and decarboxylation, in order to form ubiquinone (CoQ10). Thus, inhibition of prenyldiphosphate synthase leading from farnesyldiphosphate to extended isoprenoids, or inhibition of PHB polyprenyltransferase may be useful in increasing the amount of isoprenoid available for carotenoid biosynthesis. (Examples of prenyldiphosphate synthase and PHB-polyprenyltransferase enzymes are depicted in Tables 29 and 30, respectively).

[0075] Known small molecule inhibitors of isoprenoid biosynthesis competitor enzymes include, but are not limited to, zaragasic acid (including analogs thereof such as TAN1607A (Biochem Biophys Res Commun 1996 Feb 15;219(2):515-520)), RPR 107393 (3-hydroxy-3-[4-(quinolin-6-yl)phenyl]-1-azabicyclo[2.2.2]octane dihydrochloride; J Pharmacol Exp Ther. 1997 May;281(2):746-52), ER-28448 (5-{N-[2-butenyl-3-(2-methoxyphenyl)]-N-methylamino}-1,1-pentylidenebis(phosphonic acid) trisodium salt; Journal of Lipid Research, Vol. 41, 1136-1144, July 2000), BMS-188494 (The Journal of Clinical Pharmacology, 1998; 38:1116-1121), TAK-475 (1-[2-[(3R,5S)-1-(3-acetoxy-2,2-dimethylpropyl)-7-chloro-1,2,3,5-tetrahydro-2-oxo-5-(2,3-dimethoxyphenyl)-4,1-benzoxazepine-3-yl] acetyl] piperidin-4-acetic acid; Eur J Pharmacol. 2003 Apr 11;466(1-2):155-61), YM-53601 ((E)-2-[2-fluoro-2-(quinuclidin-3-ylidene) ethoxy]-9H-carbazole monohydrochloride; Br J Pharmacol. 2000 Sep;131(1):63-70), or squalastatin I that inhibit squalene synthase; terbinafine that inhibits squalene epoxidase; various azoles that inhibit cytochrome P450 lanosterol 14 α -demethylase; and fenpropimorph that inhibits the C-14 sterol reductase and the C-8 sterol isomerase. In other embodiments, heterologous isoprenoid biosynthesis competitor polypeptides may be utilized (whether functional or non-functional; in some embodiments, dominant negative mutants are employed).

[0076] One particular isoprenoid biosynthesis competitor polypeptide useful according to the present invention is squalene synthase which has been identified and characterized from a variety of organisms; representative examples of squalene synthase polypeptide sequences are included in Table 16. In some embodiments of the invention that utilize squalene synthase (or modifications of squalene synthase) source organisms include, but are not limited to, *Neurospora crassa*, *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*), *Aspergillus niger*, *Saccharomyces cerevisiae*, *Mucor circinelloides*, *Rhotorula glutinis*, *Candida utilis*, *Mortierella alpina*, and *Yarrowia lipolytica*.

[0077] The carotenoid biosynthesis pathway branches off from the isoprenoid biosynthesis pathway at the point where GGPP is formed. The commitment step in

carotenoid biosynthesis is the formation of phytoene by the head-to-head condensation of two molecules of GGPP, catalyzed by phytoene synthase (often called crtB; see Figure 6). A series of dehydrogenation reactions, each of which increases the number of conjugated double bonds by two, converts phytoene into lycopene via neurosporene. The pathway branches at various points, both before and after lycopene production, so that a wide range of carotenoids can be generated. For example, action of a cyclase enzyme on lycopene generates γ -carotene; action of a desaturase instead produces 3,4-didehydrolycopene. γ -carotene is converted to β -carotene through the action of a cyclase. β -carotene can be processed into any of a number of products (see, for example, Figure 6C), including astaxanthin (via echinone, hydroxyechinone, and phoenicoxanthin).

[0078] According to the present invention, carotenoid production in a host organism may be adjusted by modifying the expression or activity of one or more proteins involved in carotenoid biosynthesis. As indicated, in some embodiments, it will be desirable to utilize as host cells organisms that naturally produce one or more carotenoids. In some such cases, the focus will be on increasing production of a naturally-produced carotenoid, for example by increasing the level and/or activity of one or more proteins involved in the synthesis of that carotenoid and/or by decreasing the level or activity of one or more proteins involved in a competing biosynthetic pathway. Alternatively or additionally, in some embodiments it will be desirable to generate production of one or more carotenoids not naturally produced by the host cell.

[0079] According to some embodiments of the invention, it will be desirable to introduce one or more heterologous carotenogenic polypeptides into a host cell. As will be apparent to those of ordinary skill in the art, any of a variety of heterologous polypeptides may be employed; selection will consider, for instance, the particular carotenoid whose production is to be enhanced. The present invention contemplates not only introduction of heterologous carotenogenic polypeptides, but also adjustment of expression or activity levels of heterologous or endogenous carotenogenic polypeptides, including, for example, alteration of constitutive or inducible expression patterns. In some embodiments of the invention, expression patterns are adjusted such that growth in nutrient-limiting conditions is not required to induce oleaginity. For example, genetic modifications comprising alteration and/or addition of regulatory sequences (e.g., promoter elements, terminator elements) may be utilized to confer particular regulation of expression patterns. Such genetic modifications may be utilized in conjunction with endogenous genes (e.g., for regulation of endogenous carotenogenic); alternatively, such genetic modifications may be included so as to confer regulation of expression of at least one heterologous polypeptide (e.g., carotenogenic polypeptide(s)). For example, promoters including, but not limited to Tef1, Gpd1 promoters

can be used in conjunction with endogenous genes and/or heterologous genes for modification of expression patterns of endogenous carotenogenic polypeptide(s) and/or heterologous carotenogenic polypeptide(s). Similarly, exemplary terminator sequences include, but are not limited to, use of *Y. lipolytica* XPR2 terminator sequences.

[0080] As indicated in Figure 6 and in the literature, proteins involved in carotenoid biosynthesis include, but are not limited to, phytoene synthase, phytoene dehydrogenase, lycopene cyclase, carotenoid ketolase, carotenoid hydroxylase, astaxanthin synthase (a single multifunctional enzyme found in some source organisms that typically has both ketolase and hydroxylase activities), carotenoid epsilon hydroxylase, lycopene cyclase (beta and epsilon subunits), carotenoid glucosyltransferase, and acyl CoA:diacylglycerol acyltransferase. Representative example sequences for these carotenoid biosynthesis polypeptides are provided in Tables 17-25.

[0081] Xanthophylls can be distinguished from other carotenoids by the presence of oxygen containing functional groups on their cyclic end groups. For instance, lutein and zeaxanthin contain a single hydroxyl group on each of their terminal ring structures, while astaxanthin contains both a keto group and a hydroxyl on each terminal ring. This property makes xanthophylls more polar than carotenes such as beta-carotene and lycopene, and thus dramatically reduces their solubility in fats and lipids. Naturally occurring xanthophylls are often found as esters of the terminal hydroxyl groups, both mono- and diesters of fatty acids. They also occur as glucosides in certain species of bacteria. The solubility and dispersibility of xanthophylls can be greatly modified by the addition of ester moieties, and it is known that esterification can also affect the absorbability and/or bioavailability of a given carotenoid. It is an objective of this invention to maximize the amount of a particular xanthophyll accumulating within the intracellular triacylglyceride fraction of oleaginous yeasts, and one mechanism for achieving this goal is to increase the hydrophobic nature of the xanthophyll product that accumulates. One way of achieving this is to engineer the production of fatty-acyl mono- and/or diesters of the target xanthophyll compound.

[0082] A variety of enzymes can function to esterify carotenoids. For example, carotenoid glucosyltransferases have been identified in several bacterial species (see, e.g., Table 24). In addition, acyl CoA:diacylglycerol acyltransferase (DGAT) and acyl CoA:monoacylglycerol acyltransferases (MGAT), which function in the final steps of triacylglycerol biosynthesis, are likely to serve an additional role in the esterification of xanthophylls. Representative DGAT polypeptides are shown in Table 25. Furthermore, other enzymes may specifically modify carotenoids and molecules of similar structure (e.g. sterols) and be available for modification and ester production.

[0083] In some embodiments of the invention, potential source organisms for carotenoid biosynthesis polypeptides include, but are not limited to, genera of naturally oleaginous or non-oleaginous fungi that naturally produce carotenoids. These include, but are not limited to, *Botrytis*, *Cercospora*, *Fusarium* (*Gibberella*), *Mucor*, *Neurospora*, *Phycomyces*, *Puccinia*, *Rhodotorula*, *Sclerotium*, *Trichoderma*, and *Xanthophyllomyces*. Exemplary species include, but are not limited to, *Neurospora crassa*, *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*), *Mucor circinelloides*, and *Rhodotorula glutinis*. Of course, carotenoids are produced by a wide range of diverse organisms such as plants, algae, yeast, fungi, bacteria, cyanobacteria, etc. Any such organisms may be source organisms for carotenoid biosynthesis polypeptides according to the present invention.

[0084] It will be appreciated that the particular carotenogenic modification to be applied to a host cell in accordance with the present invention will be influenced by which carotenoid(s) is desired to be produced. For example, isoprenoid biosynthesis polypeptides are relevant to the production of most carotenoids. Carotenoid biosynthesis polypeptides are also broadly relevant. Ketolase is particularly relevant for production of canthaxanthin, as hydroxylase is for production of lutein and zeaxanthin, among others. Both hydroxylase and ketolase (or astaxanthin synthase) are particularly useful for production of astaxanthin.

Production and Isolation of Carotenoids

[0085] As discussed above, accumulation of lipid bodies in oleaginous organisms is generally induced by growing the relevant organism in the presence of excess carbon source and limiting nitrogen. Specific conditions for inducing such accumulation have previously been established for a number of different oleaginous organisms (see, for example, Wolf (ed.) *Nonconventional yeasts in biotechnology* Vol. 1, Springer-Verlag, Berlin, Germany, pp. 313-338; *Lipids* 18(9):623, 1983; *Indian J. Exp. Biol.* 35(3):313, 1997; *J. Ind. Microbiol. Biotechnol.* 30(1):75, 2003; *Bioresour Technol.* 95(3):287, 2004, each of which is incorporated herein by reference in its entirety).

[0086] In general, it will be desirable to cultivate inventive modified host cells under conditions that allow accumulation of at least about 20% of their dry cell weight as lipid. In other embodiments, the inventive modified host cells are grown under conditions that permit accumulation of at least about 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, or even 80% or more of their dry cell weight as lipid. In certain embodiments, the host cells utilized are cells which are naturally oleaginous, and induced to produce lipid to the desired levels. In other embodiments, the host cells are cells which naturally produce lipid, but have been engineered to increase production of lipid such that desired levels of lipid production and accumulation are achieved.

[0087] In certain embodiments, the host cells of the invention are not naturally oleaginous, but have been engineered to produce lipid such that desired levels of lipid production are obtained. Those of ordinary skill in the art will appreciate that, in general, growth conditions that are effective for inducing lipid accumulation in a source organism, may well also be useful for inducing lipid accumulation in a host cell into which the source organism's oleaginic polypeptides have been introduced. Of course, modifications may be required in light of characteristics of the host cell, which modifications are within the skill of those of ordinary skill in the art.

[0088] It will also be appreciated by those of ordinary skill in the art that it will generally be desirable to ensure that production of the desired carotenoid by the inventive modified host cell occurs at an appropriate time in relation to the induction of oleaginity such that the carotenoid(s) accumulate(s) in the lipid bodies. In some embodiments, it will be desirable to induce production of the carotenoid(s) in a host cell which does not naturally produce the carotenoid(s), such that detectable levels of the carotenoid(s) is/are produced. In certain aspects the host cells which do not naturally produce a certain carotenoid(s) are capable of production of other carotenoid(s) (e.g. certain host cells may, for example, naturally produce β -carotene but may not naturally produce astaxanthin); in other aspects the host cells do not naturally produce any carotenoid(s). In other embodiments, it will be desirable to increase production levels of carotenoid(s) in a host cell which does naturally produce low levels of the carotenoid(s), such that increased detectable levels of the carotenoid(s) are produced. In certain aspects, the host cells which do naturally produce the carotenoid(s) (e.g., β -carotene) also produce additional carotenoid(s) (e.g., astaxanthin, etc.); in still other aspects, the cells which naturally produce the carotenoid(s) (e.g., β -carotene) do not produce additional carotenoid(s).

[0089] In certain embodiments of the invention, it will be desirable to accumulate carotenoids to levels (i.e., considering the total amount of all produced carotenoids together) that are greater than at least about 1% of the dry weight of the cells. In some embodiments, the total carotenoid accumulation in the lipid bodies will be to a level at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 13%, at least about 14%, at least about 15%, at least about 16%, at least about 17%, at least about 18%, at least about 19%, at least about 20% or more of the total dry weight of the cells. In certain embodiments of the invention, it will be desirable to achieve total levels of carotenoid accumulation in the lipid bodies (i.e., considering the total amount of all produced carotenoids together) that are greater than at least about 1% of the dry weight of the cells. In some embodiments, the total carotenoid accumulation in the lipid bodies will

be to a level at least about 2%, at least about 3%, at least about 4%, at least about 5%, at least about 6%, at least about 7%, at least about 8%, at least about 9%, at least about 10%, at least about 11%, at least about 12%, at least about 13%, at least about 14%, at least about 15%, at least about 16%, at least about 17%, at least about 18%, at least about 19%, at least about 20% or more of the total dry weight of the cells.

[0090] Bacterial carotenogenic genes have already been demonstrated to be transferrable to other organisms, and are therefore particularly useful in accordance with the present invention (see, for example, Miura et al., *Appl. Environ. Microbiol.* 64:1226, 1998). In other embodiments, it may be desirable to utilize genes from other source organisms such as plant, alga, or microalgae; these organisms provide a variety of potential sources for ketolase and hydroxylase polypeptides. Still additional useful source organisms include fungal, yeast, insect, protozoal, and mammalian sources of polypeptides.

[0091] In certain embodiments, the *Mucor circinelloides* multi-functional phytoene synthase/lycopene cyclase and the *Neurospora crassa* phytoene dehydrogenase genes can be expressed in *Yarrowia lipolytica*. Subsequent overexpression of the catalytic domain from *N. crassa* hydroxymethylglutaryl-CoA reductase and/or treatment of the modified *Y. lipolytica* strains with the squalene synthase inhibitor zaragozic acid further increases carotenoid production. Finally, *Paracoccus marcusii* genes encoding carotenoid hydroxylase and carotenoid ketolase enzymes are expressed in *Y. lipolytica* β -carotene-producing strains, and this modification results in the accumulation of astaxanthin. Similar approaches to enhance carotenoid production could be employed in other oleaginous or non-oleaginous host organisms can be undertaken, using the same, homologous, or functionally similar carotenogenic polypeptides.

[0092] It should be noted that, for inventive organisms that produce more than one carotenoid, it will sometimes be possible to adjust the relative amounts of individual carotenoids produced by adjusting growth conditions. For example, it has been reported that controlling the concentration of dissolved oxygen in a culture during cultivation can regulate relative production levels of certain carotenoids such as β -carotene, echinenone, β -cryptoxanthin, 3-hydroxyechinenone, asteroideone, canthaxanthin, zeaxanthin, adonirubin, adonixanthin and astaxanthin (see, for example, United States Patent Number 6,825,002 to Tsubokura et al., the entire contents of which are incorporated herein by reference).

[0093] Particularly for embodiments of the present invention directed toward production of astaxanthin, it will often be desirable to utilize one or more genes from a natural astaxanthin-producing organism. Where multiple heterologous polypeptides are to be expressed, it may be desirable to utilize the same source organism for all, or to utilize closely related source organisms.

[0094] One advantage provided by the present invention is that, in addition to allowing the production of high levels of carotenoids, the present invention allows those produced compounds to be readily isolated because they accumulate in the lipid bodies within oleaginous organisms. Methods and systems for isolating lipid bodies have been established for a wide variety of oleaginous organisms (see, for example, United States Patents 5,164,308; 5,374,657; 5,422,247; 5,550,156; 5,583,019; 6,166,231; 6,541,049; 6,727,373; 6,750,048; and 6,812,001, each of which is incorporated herein by reference in its entirety). In brief, cells are typically recovered from culture, often by spray drying, filtering or centrifugation. In some instances, cells are homogenized and then subjected to supercritical liquid extraction or solvent extraction (e.g., with solvents such as chloroform, hexane, methylene chloride, methanol, isopropanol, ethyl acetate, etc.), yielding a crude oil suspension. This oil suspension may optionally be refined as known in the art. Refined oils may be used directly as feed or food additives. Alternatively or additionally, carotenoids can be isolated from the oil using conventional techniques.

[0095] Given the sensitivity of carotenoids generally to oxidation, many embodiments of the invention employ oxidative stabilizers (e.g., tocopherols, vitamin C; ethoxyquin; vitamin E, BHT, BHA, TBHQ, etc. or combinations thereof) during and/or after carotenoid isolation. Alternatively or additionally, microencapsulation, for example with proteins, may be employed to add a physical barrier to oxidation and/or to improve handling (see, for example, U.S. Patent Application 2004/0191365).

Uses

[0096] Carotenoids produced according to the present invention can be utilized in any of a variety of applications, for example exploiting their biological or nutritional properties (e.g., anti-oxidant, anti-proliferative, etc.) and/or their pigment properties. For example, according to the present invention, carotenoids may be used in pharmaceuticals (see, for example, Bertram, *Nutr. Rev.* 57:182, 1999; Singh et al., *Oncology* 12:1643, 1998; Rock, *Pharmacol. Ther.* 75:185, 1997; Edge et al, *J. Photochem Photobiol* 41:189, 1997; U.S. Patent Application 2004/0116514; U.S. Patent Application 2004/0259959), food supplements (see, for example, Koyama et al, *J. Photochem Photobiol* 9:265, 1991; Bauernfeind, *Carotenoids as colorants and vitamin A precursors*, Academic Press, NY, 1981; U.S. Patent Application 2004/0115309; U.S. Patent Application 2004/0234579), electro-optic applications, animal feed additives (see, for example, Krinski, *Pure Appl. Chem.* 66:1003, 1994; Polazza et al., *Meth. Enzymol.* 213:403, 1992), cosmetics (as anti-oxidants and/or as cosmetics, including fragrances; see for example U.S. Patent Application 2004/0127554), etc. Carotenoids produced in accordance with the present invention may also be used as intermediates in the production of other compounds (e.g., steroids, etc.).

[0097] For example, astaxanthin and/or esters thereof may be useful in a variety of pharmaceutical applications and health foods including treatment of inflammatory diseases, asthma, atopic dermatitis, allergies, multiple myeloma, arteriosclerosis, cardiovascular disease, liver disease, cerebrovascular disease, thrombosis, neoangiogenesis-related diseases, including cancer, rheumatism, diabetic retinopathy; macular degeneration and brain disorder, hyperlipidemia, kidney ischemia, diabetes, hypertension, tumor proliferation and metastasis; and metabolic disorders. Additionally, carotenoids and astaxanthin may be useful in the prevention and treatment of fatigue, for improving kidney function in nephropathy from inflammatory diseases, as well as prevention and treatment of other life habit-related diseases. Still further, astaxanthin has been found to play a role as inhibitors of various biological processes, including interleukin inhibitors, phosphodiesterase inhibitors, phospholipase A2 inhibitors, cyclooxygenase-2 inhibitors, matrix metalloproteinase inhibitors, capillary endothelium cell proliferation inhibitors, lipoxigenase inhibitors. See, e.g., Japanese Publication No. 2006022121, published 20060126(JP Appl No. 2005-301156 filed 20051017); Japanese Publication No. 2006016408, published 20060119(JP Appl No. 2005-301155 filed 20051017); Japanese Publication No. 2006016409, published 20060119(JP Appl No. 2005-301157 filed 20051017); Japanese Publication No. 2006016407, published 20060119(JP Appl No. 2005-301153 filed 20051017); Japanese Publication No. 2006008717, published 20060112(JP Appl No. 2005-301151 filed 20051017); Japanese Publication No. 2006008716, published 20060112(JP Appl No. 2005-301150 filed 20051017); Japanese Publication No. 2006008720, published 20060112(JP Appl No. 2005-301158 filed 20051017); Japanese Publication No. 2006008719, published 20060112(JP Appl No. 2005-301154 filed 20051017); Japanese Publication No. 2006008718, published 20060112(JP Appl No. 2005-301152 filed 20051017); Japanese Publication No. 2006008713, published 20060112(JP Appl No. 2005-301147 filed 20051017); Japanese Publication No. 2006008715, published 20060112(JP Appl No. 2005-301149 filed 20051017); Japanese Publication No. 2006008714, published 20060112(JP Appl No. 2005-301148 filed 20051017); and Japanese Publication No. 2006008712, published 20060112 (JP Appl No. 2005-301146 filed 20051017).

[0098] It will be appreciated that, in some embodiments of the invention, carotenoids produced by manipulated host cells as described herein are incorporated into a final product (e.g., food or feed supplement, pharmaceutical, cosmetic, dye-containing item, etc.) in the context of the host cell. For example, host cells may be lyophilized, freeze dried, frozen or otherwise inactivated, and then whole cells may be incorporated into or used as the final product. The host cell may also be processed prior to incorporation in the product to increase bioavailability (e.g., via lysis). Alternatively or additionally, a final product may incorporate only a portion of the host cell (e.g., fractionated by size, solubility), separated from the whole.

For example, in some embodiments of the invention, lipid droplets are isolated from the host cells and are incorporated into or used as the final product. In other embodiments, the carotenoids themselves, or individual carotenoid compounds are isolated and reformulated into the final product.

[0099] As stated above, fatty acid and glucoside esters are the predominant carotenoid esters found in nature, whereas additional esters (e.g. with organic acids or inorganic phosphate) can be synthesized to generate useful product forms. For delivery, carotenoid esters can also be formulated as salts of the ester form (See, e.g., US Patent Publication No. 20050096477).

[00100] The amount of carotenoid incorporated into a given product may vary dramatically depending on the product, and the particular carotenoid(s) involved. Amounts may range, for example, from less than 0.01% by weight of the product, to more than 1%, 10%, 20%, 30% or more; in some cases the carotenoid may comprise 100% of the product.

[00101] In some embodiments of the invention, one or more produced carotenoids is incorporated into a component of food or feed (e.g., a food supplement). Types of food products into which carotenoids can be incorporated according to the present invention are not particularly limited, and include beverages such as teas, juices, and liquors; confections such as jellies and biscuits; fat-containing foods and beverages such as dairy products; processed food products such as rice and soft rice (or porridge); infant formulas; or the like. In some embodiments of this aspect of the invention, it may be useful to incorporate the carotenoids within bodies of edible lipids as it may facilitate incorporation into certain fat-containing food products.

[00102] Examples of feedstuffs into which carotenoids produced in accordance with the present invention may be incorporated include, for instance, pet foods such as cat foods, dog foods and the like, feeds for aquarium fish, cultured fish or crustaceans, etc., feed for farm-raised animals (including livestock and further including fish or crustaceans raised in aquaculture). Food or feed material into which the carotenoid(s) produced in accordance with the present invention is incorporated is preferably palatable to the organism which is the intended recipient. This food or feed material may have any physical properties currently known for a food material (e.g., solid, liquid, soft).

[00103] In some embodiments of the invention, one or more produced carotenoids is incorporated into a cosmetic product. Examples of such cosmetics include, for instance, skin cosmetics (e.g., lotions, emulsions, creams and the like), lipsticks, anti-sunburn cosmetics, makeup cosmetics, fragrances, products for daily use (e.g., toothpastes, mouthwashes, bad breath preventive agents, solid soaps, liquid soaps, shampoos, conditioners), etc.

[00104] In some embodiments, one or more produced carotenoids is incorporated into a pharmaceutical. Examples of such pharmaceuticals include, for instance, various types of

tablets, capsules, drinkable agents, troches, gargles, etc. In some embodiments, the pharmaceutical is suitable for topical application. Dosage forms are not particularly limited, and include capsules, oils, granula, granula subtilae, pulveres, tabellae, pilulae, trochisci, or the like. Oils and oil-filled capsules may provide additional advantages both because of their lack of ingredient decomposition during manufacturing, and because inventive carotenoid-containing lipid droplets may be readily incorporated into oil-based formulations.

[00105] Pharmaceuticals according to the present invention may be prepared according to techniques established in the art including, for example, the common procedure as described in the United States Pharmacopoeia, for example.

[00106] Carotenoids produced according to the present invention may be incorporated into any pigment-containing product including, for example, fabric, paint, etc. They may also be incorporated into a product which is an environmental indicator, or an instrument such as a biosensor for use as a detection agent.

EXEMPLIFICATION:

[00107] Table 26 below describes certain *Yarrowia lipolytica* strains used in the following exemplification:

TABLE 26: *Yarrowia lipolytica* strains.

NRRL Y-1095	Wild type diploid	
ATCC76861	<i>MATB ura2-21 lyc1-5 LYS1-5B</i>	
ATCC76982	<i>MATB ade1 leu2-35 lyc1-5 xpr2</i>	
ATCC201249	<i>MATA ura3-302 leu2-270 lys8-11 PEX17-HA</i>	
MF346	<i>MATA ura2-21</i>	ATCC76861 x ATCC201249
MF350	<i>MATB ura2-21 leu2-35 ade1</i>	ATCC76982 x MF346

[00108] (The genotypes at *LYC1*, *LYS1*, *XPR2*, and *PEX17* were not determined in crosses nor verified for ATCC strains.)

[00109] All basic molecular biology and DNA manipulation procedures described herein are generally performed according to Sambrook *et al.* or Ausubel *et al.* (Sambrook J, Fritsch EF, Maniatis T (eds). 1989. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press: New York; Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds). 1998. *Current Protocols in Molecular Biology*. Wiley: New York).

Example 1: Production of plasmids for carotenoid strain construction.

[00110] Plasmids were generated for construction of carotenoid producing strains. The following subparts describe production of plasmids encoding carotenogenic polypeptides. Plasmids used in these studies and details of their construction are described in Table 27.

Additional plasmid construction details and descriptions of their use are found in the text of the relevant subsection. All PCR amplifications used NRRL Y-1095 genomic DNA as template unless otherwise specified. The *URA5* gene described below is allelic with the *ura2-21* auxotrophy above. The *GPD1* and *TEF1* promoters are from *Y. lipolytica* as is the *XPR2* terminator.

[00111] *GGSI* is the gene encoding the *Y. lipolytica* gene encoding geranylgeranylpyrophosphate synthase. The nucleic acid coding sequence, and encoded Ggs1 protein of pMB4591 and pMB4683 are as follows:

[00112] atggattataacagcgcgggatttcaaggagatatggggcaaggccgcgacaccgcgclgctgggaccgta
caactacctcgcgaacaaccggggccacaacatcagagaacacttgatcgacgcgttcggagcggttatcaagggtgacaagagcg
atctcgagaccatttcgcacatcaccaagattttgcataactcgtcgtctgttgatgacgtggaagacaactcgatctccgacgagg
cctgcggcagccattgtctgtttggagtcctccaaacatcaactccgccaactacatgtactttggtctctgcaggaggtgctcaa
gtctaaagtctatgatgccgtctccatttcaccgaggaaatgatcaacttgcatagaggtcagggatgagatctctacggagagaaca
ctcacttgcctcctcggaagacgagtatctggagatgggtgtgcacaagaccgggtggactgtttcggctggctctgagacttatgtctcgc
gtggcatcgaaacaggaggaccatgaaaagatcaacttgcatacacccttaccgacacactgggagtcatttaccagattctggat
gattacctcaacctgcagtcacgggaattgaccgagaacaagggtattctgcgaagatacagcgaaggaaagtgttcgttcgctgatt
cacagcatacgcaccaaccgggataaccacgagattctcaacattctcaaacagcgaacaagcgcacgtcactcaaaaagtacgcc
gtggactacatgagaacagaacccaagagtttcgactactgcctcaagaggatacaggccatgacactcaaggcaagttcgacattga
tgatctagcagcagctggccacgatgtctccaagctacgagccatttgcattttgtgtccacctctgactgtgaggagagaaaagtact
ttgaggatgcgcagtgga

[00113] mdynsadfkeiwgkaadtallgpynlannrghnrehliaafgavikvdkdletishitkilhnssllvdd
vednsmllrrglpaahclfgvpqtinsanymyfvalevlkksydavsfiteeminlhrqgmdlywretltcpsedylemrv
hktgglfrlrlmlsvaskqedhckinfdlthldtlgviyqildylnlqsteltcnkgfcedisegkfsfplihsrtnpdnheilnilk
qrtsdaslkkyavdymrtetksfdyclkriqamslkassyiddlaaghdvsklraihyfvstsdcecrkyfedaq

TABLE 27: Plasmids

Plasmid	Backbone	Insert	Oligos or source
pMB4529	PCR2.1	3.4 kb <i>ADE1</i> PCR product	MO4475 & MO4476
pMB4534	PCR2.1	2.1 kb <i>LEU2</i> PCR product	MO4477 & MO4478
pMB4535	PCR2.1	1.2 kb <i>URA5</i> PCR product	MO4471 & MO4472
pMB4589	pMB4535 (<i>KpnI</i> + <i>SpeI</i>)	1.2 kb <i>GPD1</i> promoter (<i>KpnI</i> + <i>NotI</i>); 0.14 kb <i>XPR2</i> terminator (<i>NotI</i> + <i>SpeI</i>)	MO4568 & MO4591; MO4566 & MO4593
pMB4590	pMB4535 (<i>KpnI</i> + <i>SpeI</i>)	0.4 kb <i>TEF1</i> promoter (<i>KpnI</i> + <i>NotI</i>); 0.14 kb <i>XPR2</i> terminator (<i>NotI</i> + <i>SpeI</i>)	MO4571 & MO4592; MO4566 & MO4593
pMB4591	pMB4590 (<i>NheI</i> + <i>MluI</i>)	1.0 kb <i>GGSI</i> ORF (<i>XbaI</i> +	MO4534 &

pMB4597	pMB4534 (<i>Acc65I</i> + <i>SpeI</i>)	<i>MluI</i>) <i>GPD1</i> promoter & <i>XPR2</i> terminator (<i>Acc65I</i> + <i>SpeI</i>)	MO4544 From pMB4589
pMB4603	pMB4597 (<i>RsrII</i> + <i>MluI</i>)	Residual backbone & <i>TEF1</i> promoter (<i>RsrII</i> + <i>MluI</i>)	From pMB4590
pMB4616	pMB4529 (<i>RsrII</i> + <i>SpeI</i>)	Residual backbone & <i>GPD1</i> promoter & <i>XPR2</i> terminator (<i>RsrII</i> + <i>SpeI</i>)	From pMB4589
pMB4629	pMB4616 (<i>RsrII</i> + <i>MluI</i>)	Residual backbone & <i>TEF1</i> promoter (<i>RsrII</i> + <i>MluI</i>)	From pMB4590
pMB4631	pMB4603 (<i>KpnI</i> + <i>NheI</i>)	1.2 kb <i>GPD1</i> promoter (<i>KpnI</i> + <i>NheI</i>);	MO4568 & MO4639
pMB4628	pMB4603	<i>Carp</i>	See 1A
pMB4637	pMB4629 (<i>NheI</i> + <i>MluI</i>)	1.5 kb <i>hmg1^{trunc}</i> ORF (<i>XbaI</i> + <i>MluI</i>)	See 1D
pMB4638	pMB4629	<i>carB(i)</i>	See 1B
pMB4660	pMB4638 (+ <i>URA3</i>)	<i>carB(i)</i>	See 1C
pMB4662	pMB4631 (<i>SpeI</i> + <i>XhoI</i>)	1.8 kb <i>URA3</i> fragment (<i>SpeI</i> + <i>BsaI</i>)	MO4684 & MO4685 See 1C
pMB4683	pMB4662 (<i>Acc65I</i> + <i>MluI</i>)	1.4 kb <i>tef1p-GGS1</i> fragment (<i>Acc65I</i> + <i>MluI</i>)	From pMB4591
pMB4692	pMB4662 (<i>Acc65I</i> + <i>MluI</i>)	0.4 kb <i>TEF1</i> promoter (<i>Acc65I</i> + <i>NheI</i>); 0.55 kb <i>crtZ</i> ORF (<i>XbaI</i> + <i>MluI</i>)	See 1E
pMB4698	pMB4629 (<i>NheI</i> + <i>MluI</i>)	0.9 kb <i>crtW</i> ORF (<i>XbaI</i> + <i>MluI</i>)	See 1F
pMB4599	pBluescriptSKII- (<i>EcoRV</i>)	1.9 kb <i>carRP</i> gene	MO4525 & MO4541
pMB4606	pBluescriptSKII- (<i>EcoRV</i>)	1.9 kb <i>carB</i> gene	MO4530 & MO4542
pMB4613	pMB4599 (<i>Acc65I</i> + <i>PvuMI</i>)	<i>carRP(i)</i>	See text
pMB4619	pBluescriptSKII- (<i>BamHI</i> + <i>Acc65I</i>)	<i>carB(i)</i>	See text

[00114] Certain oligonucleotides referred to in Table 27 above are as follows:

MO4471	5'-CTGGGTGACCTGGAAGCCTT
MO4472	5'-AAGATCAATCCGTAGAAGTTCAG
MO4475	5'-AAGCGATTACAATCTTCCTTTGG
MO4476	5'-CCAGTCCATCAACTCAGTCTCA
MO4477	5'-GCATTGCTTATTACGAAGACTAC
MO4478	5'-CCACTGTCCTCCACTACAAACAC
MO4534	5'-CACAAACGCGTTCCTGCGCATCCTCAAAGT
MO4544	5'-CACAATCTAGACACAAATGGATTATAACAGCGCGGAT
MO4566	5'-CACAAACTAGTTTGGCCACCTACAAGCCAGAT
MO4568	5'- CACAAGGTACCAATGTGAAAGTGCGCGTGAT

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tgctggcaattcgaacgagtggaattgctcttcttagcgatacaaccttacaccggcgcaattgcgactagtaglaccacacattgc
agacaggggaaaaaigagaaatctaaataaccatttaa

[00117] mlltymevehlyytlpvlglswlsrpyyatdalkfkflltvaftasawdnyivyhkawsycptcvtavig
yvpleeymffimltltvaftnlvmrwhlshffirpetpvmqsvlrvlpitallitaykawhlavpgkplfygscilwyacpvlall
wfgagcymmrrplavlvsialptlflcwvdvvaigagtwdislatstgkfvvphlpveefmffalintvlvfgtcaidrtmailhlfk
nkspyqrpyqhsksflhqilemtwafclpdqvlhsdtfhdlsvswdilirakasksfytasavfpgdvrgelgvlyafcratddldne
qvpvqrkeqlilthqfvsdlfgqktsaptaidwdfyndqlpascisafksftrhrhveagaikclldgykwlderrrsirdqedlryys
acvassvgemctrilahadkpasrqqtqwiiqraremglvlyqtniardiwdseelgrcylpqdwltekevaliqgglaigeerl
lsishrilyqadelmrvvankgidklpshcggvraacnvayasigtklksykhhypsravhgnskrveiallsvynlytapiatssth
crqgkmmlnti

[00118] Alternatively, pMB4599 was also used as a template for PCR amplification using MO4318, MO4643, MO4644, and MO4639 and

MO4318 5'-GTAAAACGACGGCCAGT

MO4643 5'-CACACGGTCTCATGCCAAGCCTTGTATGCAGTGATTAA

MO4639 5'-CCACTGTGTTTGCTGGCGG

MO4644 5'-CACACGGTCTCTGGCATTTGGCGGTCCCTGGAAA

producing fragments of 0.5 and 0.95 kb, that were subsequently cleaved with *Acc65I* and *BsaI*, and *BsaI* and *PpuMI*, respectively. These fragments were ligated to pMB4599 that had been digested with *Acc65I* and *PpuMI*, yielding pMB4613, harboring intronless *carRP*. The 1.85 kb *XbaI*-*MluI* fragment from pMB4613 can be inserted into *NheI*- and *MluI*-cleaved pMB4603 to yield pCarRPdel.

[00119] 1B: Production of pMB4638 (*tef*1p-*carB ADE1*), encoding phytoene dehydrogenase: Intron-containing *carB* was amplified from *M. circinelloides* (ATCC 90680) genomic DNA using MO4530 and MO4542:

MO4530 5'-CACAAACGCGTTTAAATGACATTAGAGTTATGAAC

MO4542 5'-CACAATCTAGACACAAATGTCCAAGAAACACATTGTC

and the resulting 1.9 kb fragment was phosphorylated with T4 polynucleotide kinase and blunt-end ligated into pBS-SKII- cleaved with *EcoRV*, yielding pMB4606. pMB4606 was then used as a template for PCR amplification using MO4318 and MO4648, and MO4646 and MO4647, and MO4343 and MO4645:

MO4318 5'-GTAAAACGACGGCCAGT

MO4648 5'-CACAAGGTCTCAAGCACGCATCCCGGAACTG

MO4646 5'-CACACGGTCTCAGGCATGTCGCCCTACGATGC

MO4647 5'-CACACGGTCTCATGCTTGACCCACAAAGAATAGG

MO4343 5'-CAGGAAACAGCTATGAC

MO4645 5'-CACACGGTCTCTTGCCCATATACA'TGGTCTGAAACG

producing fragments of 0.4 and 0.85 and 0.7 kb, that were subsequently cleaved with *Acc65I* and *BsaI*, and *BsaI*, and *BsaI* and *BamHI*, respectively. These fragments were ligated to pBS-SKII- that had been cut with *Acc65I* and *BamHI*, yielding pMB4619, harboring intronless *carB*. The 1.75 kb *XbaI*-*MluI* fragment from pMB4619 was inserted into *NheI*- and *MluI*-cleaved pMB4629, yielding pMB4638. The resulting nucleic acid coding sequence and encoded CarB protein of pMB4638 are as follows:

[00120] atgtccaagaaacattgtcattatcgtgctggcgtgggtggcagcggctacagctgctcgtttgcccgcga
aggttcaaggtcactgtggtggagaaaaacgactttggtggcgccgctgctccttgatccatcaccagggccatogctttgatcagg
gcccgtcgtctactgtatgcccaagtaactttgaggacgctttgcccgtatggacgagcgattcaagaccacgtggagctgctgcga
tgcgacaacaactacaaggtgcactttgacgacgggtgagtcgatccagctgtcgtctgacttgacacgcatgaagctgaattggaccg
cgtggaggggcccccttggttttgcccgtatcctggatttcagaaagagacacacatccactacgaaagcggcaccctgattgcgctca
agaagaatttgaatccatctggacacctgattcgcacatagtcgctccagagatctttgccttcacctgtttgcaagatctacgaccg
cgcttccaagtacttcaagaccaagaagatgcgcatggcattcagctttcagaccatgtatatgggcatgtcgcctacagatggcctgc
tgtctacagcctgtttgagtaacacggagttcgctgaaggcaatctggtatcccggtggcggttcaacatgggtggtcagaagctagagg
cgattgcaagcaaaagtacgatgccgagttatctacaatgcgcctgttgcgaagattaacaccgatgatgccaccaacaagtgaca
gggtgaaccttggaaaatggccacatcatcgatccgatcgcggttggtgtaacgcagatctggctatgcttaccacaatctgttgcctcc
ctgcgcatggacgcaaaacacactggcttccaagaaattgacgtctcttccatttcttactgttccatgtccaccaaggtgcctcaat
tgacgtgcacaacatcttttggccgaggttatcaggagagctttgacgaaatctcaaggactttggcctgcctctgaagcctccttc
taogtcaatgtgccctctgcgatccttctgctgctccgacggcaagactctgtcattgtcttgggtccctattggtcatatgaagag
caagacggggcgatgcttccaccgagaactaccggccatggtggacaaggcacgcaagatggtgctggctgtgaltgagcgtcgtct
gggcatgtgaatttcgccgacttgattgagcatgagcaagtaatzatcccgctgtatggcagagcaagttcaatctgtggagaggct
caattctgggtttgtcatgatgtgcttcaggtgctgtggttccgtccagcacaaggattctaccggctgttatgataacctattcttgt
gggtgcaagcacgcattccggaaactggtgttccattgtccttgacggaagcaagctcacctctgaccaagttgtcaagagctttgaa
agacgcccaagccaagaagatcgagatggagaacacgcaagcactttggaggagcctgatgctgaatgcacattccctgtgtggt
tctggttcgcgctgccttttgggtcatgttatgttctttacttctccctcaatccaatggccaaacgcccgcatctttatcaataattgta
cctgaagtattccgcttcataacttaattgcatitaa

[00121] mskkhiviigagvggtataarlareregfkvtvvelndfgggrcslihghqghrfdqgpslylmpkyfedafad
lderiqdhllelrcdnnykvhfdgdesiqldssdlrmkaeldrvcgplgfgfrfldfmkethihyesgtliaalknfsiwdlirikyap
eifrlhlfkgkiydraskyfktdkmmmaftftmymgmsspydapavysllqyfefaeigiwyprggfnnmvvqkdeaiakkydac
fiynapvakintddatqvtgvtlenghiidavvcnadlvayhnlppcrwtqntlaskkltsssisfywsmskvpqldvhn
flaeayqesfdeifkdfglpseasyvynvpsridpsaapdgkdsvivlvpighmksktgdstenypamvdkarkmvlavierl
gmsnfadlieheqvndpavwqskfnlhwrgsilglshdvlqlwfpstkdstgrydnlffvgasthpgtgvpivglagsktsdqvv
ksfgktpkprkiementqapleepdaestfpvfwlraafwvmfmfffyfipqsnqtpasfinllpevfirvhsnvi

[00122] 1C. Production of pMB4660 (*tef1p-carB URA3*) encoding encoding
phytoene dehydrogenase: The 4.3 kb *XhoI*-*NotI* fragment and the 1.8 kb *NotI*-*SpeI* fragment
from pMB4638 were ligated to the 1.9 kb *BsaI*- and *SpeI*-cleaved *URA3* gene generated by

PCR amplification of *Y. lipolytica* genomic DNA using MO4684 and MO4685 to create pMB4660:

MO4684 5'-CATTCACTAGTGGTGTGTTCTGTGGAGCA'ITC

MO4685 5'-CACACGGTCTCATCGAGGTGTAAGTGGTAGTGCAGTG

The resulting nucleic acid coding sequence and encoded CarB(i) protein of pMB4660 are as follows:

[00123] atgtccaaagaaacacattgtcattatcgggtgctggcgtgggtggcacggctacagctgctcgtttggcccgcga
aggcttcaaggtcactgtggtggagaaaaacgacttgggtggcggccgctgctccttgatccatcaccagggccatcgcttgatcagg
gcccgtgctctacctgatcccaagtactttgaggacgcctttgcccgtatcggacgagcgcattcaagaccaccitggagctgtgcga
tgcgacaacaactacaaggtgcactttgacgacgggtgagtcgacgcagctgtgctgacttgacacgcatgaaggctgaattggaccg
cgtggaggggccccctgtgtttggccgattcctggatttcagaaagacacacatccactacgaanaggcaccctgattggcctca
agaagaatttgaatccatcgtggaccgtattcgcacagacgtcctcagagatcttctgctgcacctgtttggcaagatctacgaccg
cgcttccaaagtacttcaagaccaagaagatgcgcagtcgattcacgtttcagaccatgtatatgggcagctgcccctacgatgcgcctgc
tgtctacagcctgttgacgtacaccgagttcgtgaaggcatctggtatcccggtggcggctcaacatgggtggtcagaagctagagg
cgattgcaaaagcaaaagtagcagtcggagtttatctacaatgcgcctgttgcgaagattaacaccgatgatccaccaaaacaagtaca
ggtgttaacottggaaaatggccacalcacgatgcgcgattggtgtgtgaacgcagatctggtctatgcttatcacaatctgttgcctcc
ctgcccgtgacgcaaaacacactggcttccaaagaattgacgtcttctccatttctctactggtccatgtccaccaagggtgctcaat
tggacgtgcacaacatcttttggccgaggcttatcaggagagccttgacgaatcttcaaggacttggcctgccccttgaaagcctcttc
tacgtcaatgtgcctctcgcacgtatccttctgctgctcccgcagcgaaggactctgtcattgtcttggctgcttattggtcatatgaagag
caagacggcgatgcttccaccgagaactaccggccatgtgagacaaggcacgcaagatgggtgctggtgattgagcgtcgtct
gggcatgtgaatttgcgcgacttgattgagcatgagcaagtcaatgatccgctgtatggcagagcaagttcaatctgtgagaggct
caattctgggttgtctcatgatgtgttcagggtgctgtgttccgclccagcacaaaggattctaccggctgttatgataacctattcttgt
gggtgcaagcacgcacccggaactggtgttccattgtccttcaggaagcaagctcaccctgaccgaagtgtcaagagccttggaa
agacgccaagccaagaagatcgagatggagaacacgcaagcacccttggaggagcctgatgctgaatcgacattccctgtgtgtgt
tctggttgcgcgctgcttttgggtcatgtttatgttctttactcttccctcaatccaatggccaaacgcccgcacctttatcaataatttggta
cctgaagtattccgcgttcataactataatgtcatttaa

[00124] mskkhiviigavvggtataarlaregfkvtvvekndfeggrcslihghqhrfdqgpslylmpkyfedafad
lderiqdhllelrcdnnykvhfddgesiqssdltrmkaeldrvegplgfrfldfinkethihyestlialkknfesiwdlirikyap
eifrlhlfkiydraskyftklonrmafftqtmymgmsspydapavysllqytefaegiwyprggfmmvvqkleaiakqkydae
fiynapvakintddatkqvtgvtlenghiidadaavvcnadlvayhnlppcrwtqntlasckltsssisfywsmskvpqldvhn
flaeayqesfdeifkdfglpseasfyvnpvridpsaapdgkdsvivlpighmksktgdastenympamvdkarkmvlavierl
gmsnfadlieheqvndpavwqskfnlwrsgilshdvlqvlwfrpstkdstgrydnlffvgasthpgtgvplvagskltsdqvv
ksfgktpkprkiementqapleepdaestfpvwfwlraafwvnmfmffypqsnqtpasfinllpevfrvhnsvi

[00125] 1D. Production of pMB4637 and pTef-HMG encoding a truncated HMG1.

For production of a truncated variant of the HMG-CoA reductase gene, which also encodes a

77 amino acid leader sequence derived from *S. cerevisiae*, the following oligonucleotides are synthesized:

PRIMER O 5'-TTCTAGACACAAAAATGGCTGCAGACCAATTGGTGA
 PRIMER P 5'-CATTAATTCTTCTAAAGGACGTATTTCTTATC
 PRIMER Q 5'-GTTCTCTGGACGACCTAGAGG
 MO4658 5'-CACACACGCGTACACCTATGACCGTATGCAAAT

Primers O and P are used to amplify a 0.23 kb fragment encoding Met-Ala followed by residues 530 to 604 of the Hmg1 protein of *S. cerevisiae*, using genomic DNA as template. Primers Q and MO4658 are used to amplify a 1.4 kb fragment encoding the C-terminal 448 residues of the Hmg1 protein of *Y. lipolytica*, using genomic DNA as template. These fragments are ligated to the appropriate cloning vector, and the resultant plasmids, designated pOP and pQMO4658, are verified by sequencing. The OP fragment is liberated with *Xba*I and *Ase*I, and the QMO4658 fragment is liberated with *Mae*I and *Mlu*I. These fragments are then ligated to the *ADE1 TEF1p* expression vector pMB4629 cut with *Xba*I and *Mlu*I to produce pTefHMG.

[00126] Alternatively, the native *HMG1* gene from *Y. lipolytica* may be modified without *S. cerevisiae* sequences as described in the table above using primers MO4658 (described above) and MO4657, to create pMB4637:

MO4657 5'-CACACTCTAGACACAAAAATGACCCAGTCTGTGAAGGTGG

The resulting nucleic acid coding sequence and encoded Hmg1^Y protein of pMB4637 are as follows:

[00127] atgacccagctctgtgaagggtgttgagaagcacgttcctatcgtcattgagaagccagcgagaaggaggag
 gacacctcttgaagactccattgagctgactgtcggaaagcagcccaagcccgtagccgagacccgttctctggacgacctagagg
 ctatcatgaaggcaggtaagaccaagcttctgaggaccacgaggtgtcaagctctctctogagggaagcttcttctgtctctga
 gaagcagcttctgtgacaacacccgagctgttggcatccgaatctatcatctccagcagctctataccaagacttagagacctcaa
 agcttcttacctgcactacgactacgacctgttttggagcctgttgcgagaacgttattggttacatgcctctccccgttgggtgtctg
 gcccatgaacattgatggcaagaactaccacattcctatggccaccactgagggtgtcttgttgcctcaaccatgcgaggttgcagg
 ccatcaacgcggtgtggtgttaccactgtgcttactcaggacggtatgacacgaggtcctgtgttcccttccctctcaagcgggc
 ttgagccgctaagatctggttattcogaggagggtcicaagtcctatgcgaagcctcaactccacctctcgattgtctgtctcca
 gtctcttcaactctacccctgtcgttgaacctgtgtttattcattccgaaccaccactgtgtatgcatgggcatgaacatgaictccaagg
 gcgtcgaaactctctgcccgtatgttcaaggagtagcgttccctgatatggacattgtgtctgtctcgggtaactactgcactgacaa
 gaagcccgagcgatcaactggatcgaaggccgaggcaagagtgttggccgaagccaccatccctgctcacattgtcaagtctgtt
 ctcaaaagtgggttgacgtcttgttgagctcaacatcagcaagaatctgatcggtagtgccatggctggctctgtgggaggttcaatg
 cacacgcccgaacctgttgacgccaatctaccttgcactggccaggatcctgctcagaatgtcaggtcttccaactgcattcacgctg
 atgagcaacgtcagcggtaacctgctatctccgtttccatgccttctatcaggtcgggtaccattgttgagggtactatttggagcccc
 agggggctatgtctggagatgttggcgtgcgaggtcctcacatcgagacccccgggtccaacgccaacagcttctcgcacattgc

ttctggagttcttcgagcggagctttcgtctgttctgctcttgcgcggccatcttgcaagtcaratgacccacaacgggtccagg
ctctactccggccaagcagtcctcagggcgaatctgcagcgtctacaaaacgggtogaatattgcatacgggtcatag

[00128] mtqsvlcvvckhvpiviekpsekeedtsedsietvgkqpkpvtctrslddleaimkagtklledhevkv
lslegklplyalekqlgdntavgirrsiisqqsnktletsklpylhydydrvfagaccenvigymplpvgvagpmnidgknyhip
mattegcivastmrgckainagggtvtvtqdgmtgpcvsvfslkragaakiwldsceglksmrkafnstrfarlqslhstlaagl
lfirfrttgdamgmnmmiskgvchslavinvkeygfpdmdivsvsgnyctdkcpaainwiegrgksvvaeatipahivksvlks
evdalvelnisknligsamagsvvgfnahaanlvtailylatqdpqnvessncitlmsnvdgnllisvsmpsievgtigggtilep
qgamlemlgvrghietpganaqqlariasgvlaaelslcsalaaghlvqshmtlnrsqaptpakqsqadlqrlqngsnicirs

[00129] 1E. Production of pMB4692 (*URA3 tef/p-crtZ*) encoding carotene hydroxylase. The following carotene hydroxylase (CrtZ) ORF sequence was synthesized; based on protein sequence of *Novosphingobium aromaticivorans*, using *Y. lipolytica* codon bias:

5'-

ttctagacacaaaaatgggtggagccatgcagaccctcgtctatactcgatcgctcctcggtacagtcctcgtatggagtttctc
gcttggctcttcataagatatacaatgcattgcttcggatgggatggcatagagaccatcagagcccatgagggatttcttc
agaagaatgacttatagccatcggttggcgtcctctctgatactcatgtttgacctggctctcccatgcatggcgctgac
gcctggtggccggaaacctggatcggaactgggtgctctctatgggtgcatctataacctggtgacgaaggctggtgacac
aacgatggtttagatgggtgcctaaacgaggttaagccnaacgactcgtcagggccataagctgcacacgccaacatttgc
aaggaaggaggcgctctcattcggttctgttgcggcgagatcccgcggttctgaagcaggngcttcgagctcaacgagaagc
aggtatcgccctgctcgcagagggctgtgacggctagacgcgt

This sequence was cleaved using *Xba*I and *Mlu*I and ligated, along with an *Acc*65I-*Nhe*I *TEF1* promoter fragment from pMB4629, to pMB4662 cut with *Acc*65I and *Mlu*I to produce pMB4692. The nucleic acid coding sequence is depicted in bold underline above. The resulting encoded crtZ protein of pMB4692 is as follows:

[00130] mggamqtlailivltvlamecfvawsshkyimhgfwwgwhrdhhephgffleknldyaivgaalsil
mfalgspmingadawwpgtwiglgvlfygyiylvhdglvhqrwfrwvprkyakrlvqahklhhatigkeggvsfgfvfar
dpavlkqelraqreagiavlreavdg

[00131] 1F. Production of pMB4698 (*ADE1 tef/p-crtW*), encoding carotene ketolase. The following carotene ketolase (CrtW) ORF sequence was synthesized, based on protein sequence of an environmental sequence isolated from the Sargasso Sea (Genbank accession AACY01034193.1):

5'-

ttctagacacaaaaatgaactcgatctatttcttggccttccacctactggcaccctccagccctctgttcttcttgggtcgcaaacga
attctctctcaagcccgaaaaggctcgtctcctcgctggctcattgttccgcttggtgcttactctcggaattggctttcccttc
ccctccatcaaacgagctggttctcattcggttctctcttctcttagatcttctctgcacacggacttttatcggttgcctatga
cgtatgcacgcttctcttcttctgaccacccctggccttaaccgttggattggacgtgtctgtcttctctcatgtatgctggaactctct

acaaaagatgctgccgaatcacccgtgcacaccaccaagccccgaacagttgaagaccctgactaccaacgatgcactaa
caacaatatctcgaactgtaacgttcactttatgggaattacctcggatggcacaattgcttaattctctcttgcgtttgctcgc
tctcaecttccgtgtttctgaactctctctcaattctctccactgctctttctctctctctctctctctctctctctctctc
tctcgtgggaacctggctgccacccgacgagggcgtactactcgacccggcgttaccactcgcctgaactccacctg
ctctttctctcgtctgtctaccacttgcgttaccacccgtgaacaccatgaatctccctctactccttgggtccaacttccctaaactc
cgagaaggtttctctcatctaaacgcgt

This sequence was cleaved using *Xba*I and *Mlu*I and ligated to pMB4629 cut with *Nhe*I and *Mlu*I to produce pMB4698. The nucleic acid coding sequence is depicted in bold underline above. The resulting encoded crtW protein of pMB4698 is as follows:

[00132] intrsiswpstywhlqpsccsswvanefspqarkglvlaglgsawlltglgfsplphqtswillgclvllrsflh
tgIfvahdamhaslvpdhpglnrwigrvcllmyaglsykrcrcnrhrhqpapetvedpdyqrctnnildwyvhfmgnylgw
qqllnlsclvwaltrfvsdysaqffhllfsvlplivssqflvgtwlprrgatttrpgvttrslnfhpalsfaacyhfigyhrehhespst
pwfqplklregsl

Example 2: Engineering *Yarrowia lipolytica* for increased carotenoid production.

[00133] 2A. Production of *Y. lipolytica* expressing geranylgeranylpyrophosphate synthase and phytoene dehydrogenase: MF350 (*MATB ura2-21 leu2-35 ade1*) was transformed with pMB4591 (*tef1p-GGS1*) that had been cleaved upstream of *URA5* with *Ssp*I; a Ura⁺ transformant carrying the plasmid at the *ura2* locus was identified and named MF364. It was subsequently transformed with pMB4638 (*tef1p-carB*) that had been cleaved at *ADE1* with *Ssp*I and a prototrophic transformant was chosen that harbored the plasmid at the *ade1* locus. This strain was named MF502.

[00134] 2B. Production of *Y. lipolytica* expressing geranylgeranylpyrophosphate synthase, phytoene dehydrogenase and phytoene synthase/lycopene cyclase MF502 was transformed with pMB4628 (*tef1p-carRP*) that had been treated with *Ssp*I. Nine prototrophic colonies were chosen that were uncolored, orange, or very orange on the transformation plate (YNB agar with 1% glucose and 0.1% glutamate [YNBglut]) after two to three days of growth. Two, MF597 and MF600 (the very orange ones), produced greater than 4 mg carotene per g dry cell weight (DCW) after four days of growth in YPD at 30°C. Southern analysis reveals a different single *Kpn*I-*Hind*III band in genomic DNA from MF597 and MF600, neither of which suggested that homologous integration occurred at *leu2-270*.

[00135] 2C. Production of *Y. lipolytica* expressing phytoene synthase/lycopene cyclase and phytoene dehydrogenase: ATCC201249 (*MATA ura3-302 leu2-270 lys8-11*) was transformed with *Ssp*I-cleaved pMB4628. Hundreds of Leu⁺ colonies were pooled, re-grown, and transformed with pMB4660 (*tef1p-carB*) that had been cleaved upstream of *URA3* with *Sal*I. One colony that was noticeably yellow after 5 days at 30°C on YNBglut plus 0.6

mM lysine was selected, named MF447, and found to produce 0.2 mg carotene per gram dry cell weight after 4 days of growth in YPD.

[00136] MF447 was challenged with 1 g/L 5-fluoroorotic acid and Ura⁻ segregants selected. Surprisingly, they were all found to retain the identical yellow appearance of their parent, implying that the loss of a functional *URA3* gene did not coincide with the loss of a functional CarB enzyme. Southern analysis demonstrates that two fragments from a KpnI-HindIII digest of MF447 DNA contain *URA3*p-hybridizing sequences, only one of which also hybridizes to *carB*. The other is absent in MF578, the Ura3⁻ segregant chosen for further manipulation. Plasmid rescue and analysis of the DNA sequence encompassing the carRP intron in strains MF447, MF597 (example 2c), and MF600 (example 2c) revealed that exons 1 and 2 were contiguous and were each separated by an intron sequence that lacked the original internal SspI site (present in pMB4628).

[00137] 2D. Production of *Y. lipolytica* expressing phytoene synthase/lycopene cyclase, phytoene dehydrogenase and geranylgeranylpyrophosphate synthase: MF578 was transformed with pMB4683 (*tef1p-GGS1*) that had been cleaved with *SaII* (upstream of *URA3*) or with *SnaI* (within the *GGS1* ORF). Ura⁺ Leu⁺ colonies in both cases appeared bright orange on YNBglut+Lys and on YPD, and several produced greater than 4 mg carotene per gram of dry cell weight when grown as above. One, MF633, contained a single copy of the plasmid at the *GGS1* locus, as inferred from Southern analysis. The others arose by non-homologous or more complex integrations.

[00138] 2E. Production of *Y. lipolytica* expressing phytoene synthase/lycopene cyclase, phytoene dehydrogenase and geranylgeranylpyrophosphate synthase: MF364 is crossed with MF578, and spores from the resulting diploid are plated on YPD for two to three days at 30°C. Orange Leu⁺ Ade⁻ Ura⁻ colonies are screened for the presence of *tefp-carB*, *tefp-carRP*, and *tefp-GGS1* by PCR, and for high carotenoid (>4 mg/g dry cell weight) production after growth in YPD liquid medium. Colonies meeting these criteria, as well as displaying resistance to 5-fluoroorotic acid, an indication that they harbor the *ura3-302* allele, are chosen for further studies and hereafter referred to as GBRPua strains. Such a strain is selected for further analysis and modification.

Example 3: Extraction of carotenoids from *Yarrowia lipolytica* cells

[00139] Shake-flask testing of generated strains was conducted using YPD medium (1% yeast extract, 2% peptone, 2% glucose). 20 ml cultures in 125 ml flasks were grown at 30°C. *Y. lipolytica* cells were harvested from 72-96 hour cultures, and extractions were performed to determine carotenoid form and quantity. 1.8 ml of culture was placed into an Eppendorf tube. Cells were pelleted and washed twice with 1 ml H₂O. After the second wash, the resuspended cells were transferred to a pre-weighed snap-cap tube with a hole

poked in the top, and the cells were lyophilized overnight. After drying to completion, the tube was weighed in order to calculate dry cell weight. 0.25 ml from the same shake flask culture was placed into a 2 ml screw-cap tube for carotenoid extraction. Cells were pelleted and the supernatant was aspirated. Pelleted cells may be frozen at -80°C and stored. An equal volume of cubic zirconia beads was added to cell pellets, along with 1 ml ice-cold extraction solvent (a 50/50 v/v mix of hexane and ethyl acetate containing 0.01% butylhydroxytoluene (BHT)). The mixture was then agitated (Mini-BeadBeater-8, BioSpec Products, Inc.) at maximum speed for 5 minutes at 4°C. The mixture was then spun at maximum speed for 1 minute, and the supernatant was collected and deposited in a cold 16 ml glass vial. The remaining cell debris was re-extracted at least three times, without the addition of zirconia beads; all supernatants were pooled in the 16 ml glass vial. Following extraction, the glass vial was spun for 5 minutes at 2000 rpm at 4°C in a Sorvall tabletop centrifuge, and the supernatant was transferred to a new cold 16 ml glass vial. A Speed Vac was used to concentrate the supernatant (room temperature in dark), and the samples were stored at -20°C or -80°C until immediately before HPLC analysis. Prior to HPLC analysis, the samples were resuspended in 1 ml ice-cold solvent and then transferred to a cold amber vial. Throughout the protocol, care was taken to avoid contact with oxygen, light, heat, and acids.

Example 4: Quantification of carotenoid production by HPLC

[00140] For carotenoid analysis, samples were resuspended in ice-cold extraction solvent (a 50/50 v/v mix of hexane and ethyl acetate containing 0.01% butylhydroxytoluene (BHT)). An Alliance 2795 HPLC (Waters) equipped with a Waters XBridge C18 column (3.5 μ m, 2.1 x 50 mm) and Thermo Basic 8 guard column (2.1 x 10 mm) was used to resolve carotenoid at 25°C; authentic carotenoid samples were used as standards. The mobile phases and flow rates are shown below (Solvent A= Ethyl Acetate; Solvent B= Water; Solvent C= Methanol; Solvent D= Acetonitrile). The injection volume was 10 μ L. The detector is a Waters 996 photodiode array detector. The retention times for lipophilic molecules include astaxanthin (1.159 min), zcaxanthin (1.335), β -apo-8'-carotenal (2.86 min), ergosterol (3.11 min), lycopene (3.69 min), β -Carotene (4.02 min), and phytoene (4.13 min). Astaxanthin, zcaxanthin, β -apo-8'-carotenal, lycopene and β -Carotene are detected at 475 nm, whereas ergosterol and phytoene were detected at 286 nm.

TABLE 28 Retention Times for Lipophilic Molecules

<i>Time (min)</i>	<i>Flow (mL/min)</i>	<i>% A</i>	<i>% B</i>	<i>% C</i>	<i>% D</i>	<i>Curve</i>
	0.50	0.0	20.0	0.0	80.0	
3.00	1.00	20.0	0.0	0.0	80.0	6

4.50	1.00	80.0	0.0	20.0	0.0	6
5.50	1.00	0.0	0.0	60.0	40.0	6
6.50	1.00	0.0	0.0	80.0	20.0	6
7.50	1.00	0.0	0.0	100.0	0.0	6
8.50	1.00	0.0	0.0	100.0	0.0	6
9.50	1.00	0.0	20.0	0.0	80.0	6
10.50	0.50	0.0	20.0	0.0	80.0	6

Example 5: Expression of a truncated form of HMG-CoA reductase results in increased carotenoid production

[00141] In order to increase carotenoid production, carbon flow through the isoprenoid pathway is enhanced by introducing a truncated variant of the HMG-CoA reductase gene.

[00142] In one approach, a truncated variant of the HMG-CoA reductase gene which also encodes a 77 amino acid leader sequence derived from *S. cerevisiae* Hmg1 is introduced into a GRPBua strain (described in Example 2E above). Plasmid pTefHMG can be cleaved with *Sna*BI, *Bbv*CI, or *Bsu*36I to direct integration at the *ade1* locus, or with *Bam*HI to direct integration at the *HMG1* locus, or with *Eco*RV to promote random integration, in the GRPBua strains, restoring them to adenine prototrophy. Resulting Ade⁺ transformants are screened for increased carotenoid production.

[00143] Alternatively, the native *HMG1* gene from *Y. lipolytica* may be modified without *S. cerevisiae* sequences as described in Example 1D above, to create pMB4637. This plasmid can be digested as described for pTefHMG and transformed into GRPBua strains, and resulting transformants screened as described for increased carotenoid production.

[00144] In still another approach, a truncated variant of the *N. crassa* HMG-CoA reductase gene may be utilized and introduced into *Y. lipolytica* strains. In order to generate a plasmid suitable for expression of the heterologous HMG-CoA reductase, p641P (Yeast 2001; 18 (2001): 97-113) is modified by replacing the *JCL1* promoter with the *GPD* promoter, and by the addition of sequences conferring resistance to phleomycin. *Y. lipolytica* genomic DNA is amplified with two primers.

GPDDist: 5' CACACGGTaccgttaggtgggtgggtg

GPDprox: 5' CACACGGATCCtggttaattcaagaatgaatatagagaagagaag,

and the resulting fragment (0.7 kb) is cleaved with *Bam*HI and *Kpn*I, and ligated to *Bam*HI- and *Kpn*I-cleaved p641P, creating the plasmid "p641Pgpd". The *ble* gene under the control of the *A. nidulans* *GPD* promoter is then excised from pBCphleo (Silar, Fungal Genetics Newsletter 42:73) as a 3.2 kb *Bcl*I-*Bam*HI fragment and inserted into the unique *Bam*HI site

of “p641Pgpd”, in the orientation that preserves the *Bam*HI site proximal to the *GPD* promoter, to create “p641Pgpdble” ,

[00145] *N. crassa* genomic DNA is amplified with two primers:

Neuhmg fwd: 5' CACACGGATCCACATCAACAatggcatctgccaccctccccc

Neuhmg rev: 5' CACACGGATCcaagtgcgtgacgcgggaacttg,

and the resulting fragment is cleaved with *Bam*HI and inserted into *Bam*HI-digested “p641Pgpdble” in the correct orientation. The resulting plasmid, “pZg”, contains sequences encoding a truncated cytosolic catalytic domain of hydroxymethylglutaryl-CoA reductase from *N. crassa* (Genbank accession: XP_324892) under the control of the constitutive *GPD* promoter. This plasmid can be introduced into the *Y. lipolytica* strain created in Example 2E above, and transformants are selected by their resistance to phleomycin (100 µg/ml). Resulting transformants are tested for β-carotene production, as described above.

Example 6: Introduction of heterologous carotene hydroxylase and carotene ketolase genes into *Y. lipolytica* strains producing carotenoid for production of astaxanthin.

[00146] For introduction of carotene hydroxylase and carotene ketolase into carotenoid producing *Y. lipolytica*, pMB4692 and pMB4698, described as in Example 1E and 1F above, can be sequentially introduced into the GRPBua strain (described in Example 2E). For the introduction of pMB4692, the plasmid may be cleaved with *Sa*I or *Bsr*GI to direct integration at the *ura3* locus, or with *Xba*I to promote random integration, selecting for uracil prototrophy. GRPBua *Ura*⁺ transformants harboring pMB4692 are screened for zeaxanthin production in YPD. Zeaxanthin-producing cells are transformed with pMB4698 (which can be cleaved with *Ppu*MI, *Ssp*I or *Bbv*CI to direct integration at the *ade1* locus, or with *Eco*RV to promote random integration) and prototrophic colonies are screened for astaxanthin production.

[00147] Alternatively, the order of plasmid transformation may be reversed wherein pMB4698 is transformed first and transformants are selected for adenine prototrophy. GRPBua *Ade*⁺ transformants harboring pMB4698 are screened for canthaxanthin production. Canthaxanthin-producing GRPBua[pMB4698] cells are transformed with pMB4692 and prototrophic colonies are screened for astaxanthin production.

[00148] In another approach, the carotenoid ketolase and carotenoid hydroxylase genes from *P. marcusii* can be introduced into the strains described in Example 2 above, in order to convert β-carotene into astaxanthin. *P. marcusii* genomic DNA is amplified with two primers.

CrtZfwd: 5' CACACCGTCTCAAatgaccaatttcctgatcgtcgtc

CrtZrev: 5' CACACAGATCtcacgtgcgctcctcgcgcc,

and the resulting fragment is cleaved with *BsmBI*, modified with the Klenow fragment of DNA polymerase, and cleaved with *BglII*. This fragment is inserted into *PmlI*- and *BamHI*-cleaved pINA1269 (J. Mol. Microbiol. Biotechnol. 2 (2000): 207-216), containing the *hp4d* promoter, the *XPR2* terminator, the selectable *LEU2* gene, and sequences necessary for selection and propagation in *E. coli*. The resulting plasmid "pA" contains sequences encoding carotene hydroxylase from *P. marcusii* (*crtZ* gene)(Genbank accession: CAB56060.1) under the control of the *hp4d* promoter.

[00149] "pYEG1TEF" is modified by substituting the *LIP2* terminator for the *XPR2* terminator as follows. pINA1291 is digested with *AvrII*, modified with the Klenow fragment of DNA polymerase, and cleaved with *EcoRI*, and the small *LIP2t* containing fragment is ligated to "pYEG1TEF" that has been digested with *SacII*, modified with T4 DNA polymerase in the presence of dNTP, and cleaved with *EcoRI*. The resulting plasmid is named "pYEG1TEF-LIP2t".

[00150] In order to amplify the carotenoid ketolase gene, *P. marcusii* genomic DNA is amplified with two primers.

CrtWfwd: 5' CACACCCTAGGCCatgagcgccatgccctgc

CrtWrev: 5' CACACAAGCTTtcatgcggtgtcccccttg,

and the resulting fragment is cleaved with *AvrII* and *HindIII*, and inserted into *AvrII*- and *HindIII*-cleaved "pYEG1TEF-LIP2t". The resulting plasmid, "pBt", contains sequences encoding the carotene ketolase (*crtW* gene)(Genbank accession: CAB56059.1) under the control of the constitutive *TEF1* promoter.

[00151] In order to combine the two expression cassettes into a single plasmid, "pBt" is cleaved with *ClaI*, modified with the Klenow fragment of DNA polymerase, and cleaved with *EcoRI*, and the *crtW*-containing fragment is isolated, mixed with the phosphorylated oligonucleotide adaptor pair:

5' AA1TCGCGGCCGCT and

5' AGCGGCCGCG,

cleaved with *NofI*, and ligated to *NofI*-digested "pA". The resulting plasmid, "pABt", contains both the *TEF1p/crtW/LIP2t* cassette and the *hp4d/crtZ/XPR2t* cassette as well as the selectable *LEU2* gene.

[00152] "pABt" can be introduced into the *Y. lipolytica* strain described above in Example 4 (*TEF1p/al-1/XPR2t*; *hp4d/carRP/LIP2t*; *GPDp/HMGR_{mmc}*), and transformants selected for leucine prototrophy.

Example 7: Partial inactivation of *Y. lipolytica* ERG9 gene encoding squalene synthase results in increased carotenoid production:

[00153] 7A. In order to partially inactivate the *ERG9* gene encoding squalene synthase, the neighboring *FOL3* gene is disrupted, resulting in a folinic acid requirement. This strain is then transformed with a mutagenized fragment of DNA partially spanning the two genes, and *Fol*⁺ transformants are screened for decreased squalene synthase activity.

[00154] The following oligonucleotides are synthesized:

PRIMER K 5'-CCTTCTAGTCGTACGTAGTCAGC;

PRIMER L 5'-CCACTGATCTAGAATCTCTTTCTGG

and used to amplify a 2.3 kb fragment from *Y. lipolytica* genomic DNA spanning most of the *FOL3* gene, using *Pfu* polymerase. The resulting fragment is cleaved with *Xba*I and phosphorylated, then ligated into pBluescriptSK⁺ that has been cleaved with *Kpn*I, treated with T4 DNA polymerase (T4pol) in the presence of dNTPs, and subsequently cleaved with *Xba*I. The resultant plasmid, designated pBS-*fol3*, is then cleaved with *Acc65*I and *Eco*RI, treated with T4pol as above, and ligated to the 3.4 kb *Eco*RV-*Spe*I *ADE1* fragment (treated with T4pol) from pMB4529.

[00155] The resulting plasmid, pBS*fol3*Δ*ade*, can be cleaved with *Bst*WI and *Xba*I to liberate a 5.5 kb fragment that is used to transform the GRBPua strains described above to adenine prototrophy. Resulting *Ade*⁺ transformants are screened for a folinic acid requirement, and for homologous integration by PCR analysis.

[00156] Strains that harbor the resultant *fol3*Δ*ADE1* allele can be transformed with a 3.5 kb DNA fragment generated by mutagenic PCR amplification using the primers:

PRIMER M 5'-GGCTCATTGCGCATGCTAACATCG;

PRIMER N 5'-CGACGATGCTATGAGCTTCTAGACG,

and *Y. lipolytica* genomic DNA as template. The resulting fragment containing the N-terminal three-quarters of the *FOL3* ORF and the C-terminal nine-tenths of the *ERG9* ORF is used to transform strains. The resulting *Fol*⁺ *Ade*⁺ transformants are screened for decreased squalene synthase activity by sensitivity to agents such as zaragozic acid, itraconazole, or fluconazole. Additionally, the resulting transformants are screened for increased carotenoid production.

[00157] 7B. Alternatively, the PCR fragment produced in 7A could be cloned and altered in such a way as to remove the 3'-untranslated region of *ERG9* gene. Replacement of the *fol3*Δ*ADE1* disruption by this fragment results in decreased expression of squalene synthase [Schuldiner et al. (2005), Cell 123:507–519][Muhlrad and Parker (1999), RNA 5:1299–1307], which can be confirmed as in 7A. This approach may also be used in a *Fol*⁺ *Ade*⁺ strain, using the *ADE1* marker to disrupt the *ERG9* 3'-UTR.

[00158] 7C. In still another approach, partially defective *ERG9* alleles can be identified in *S. cerevisiae* using plasmid shuffling techniques [Boeke et al. (1987), Methods

Enzymol. 154:164-175], and using drug sensitivities as a phenotype. Defective genes can be transferred to *Y. lipolytica* using standard molecular genetic techniques.

Example 8: Treatment of *Y. lipolytica* strains producing carotenoid with inhibitor of an isoprenoid biosynthesis competitor polypeptide results in increased carotenoid production

[00159] Cultures produced in Example 2 are treated with the squalene synthase inhibitor, zaragozic acid (zaragozic acid at 0.5 μ M) and monitored for β -carotene production, as described above.

Example 9: Constructing an oleaginous strain of *Saccharomyces cerevisiae*

[00160] The genes encoding the two subunits of ATP-citrate lyase from *N. crassa*, the AMP deaminase from *Saccharomyces cerevisiae*, and the cytosolic malic enzyme from *M. circinelloides* are overexpressed in *S. cerevisiae* strains in order to increase the total lipid content. Similar approaches to enhance lipid production could be employed in other host organisms such as *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*), using the same, homologous, or functionally similar oleaginous polypeptides.

[00161] Qiagen RNAEasy kits (Qiagen, Valencia, CA.) are used to prepare messenger RNA from lyophilized biomass prepared from cultures of *N. crassa*. Subsequently, RT-PCR is performed in two reactions containing the mRNA template and either of the following primer pairs.

acl1:

1fwd: 5' CACACGGATCCTATAatgccttcgcaacgacccg

1rev: 5' CACACACTAGttaaatggacctaacaacgaccc

acl2:

2fwd: 5' CACACGGATCCAATATAAatgtctgcgaagagcatcctcg

2rev: 5' CACACGCATGCttaagcttggaactccacgcac

[00162] The resulting fragment from the acl1 reaction is cleaved with *SpeI* and *BamHI*, and that from the acl2 reaction is cleaved with *BamHI* and *SphI*, and both are ligated together into YE24 that has been digested with *NheI* and *SphI*, creating the plasmid "p12". The bi-directional *GALI-10* promoter is amplified from *S. cerevisiae* genomic DNA using the primers.

gal10: 5' CACACGGATCCaatittcaaaaattctacttttttttgatggac

gal1: 5' CACACGGATCCtttttctccttgacgttaaagtataagg,

and the resulting 0.67 kb fragment is cleaved with *BamHI* and ligated in either orientation to *BamHI*-digested "p12" to create "p1gal2" and "p2gal1", containing *GALI-acl1/GALI10-acl2* and *GALI10-acl1/GALI-acl2*, respectively (Genbank accession: *acl1*: CAB91740.2; *acl2*: CAB91741.2).

[00163] In order to amplify the *S. cerevisiae* gene encoding AMP deaminase and a promoter suitable for expressing this gene, *S. cerevisiae* genomic DNA is amplified using two primer pairs in separate reactions:

AMD1 ORF:

AMD1FWD: 5' CACACGAGCTCAAAAatggacaatcaggctacacagag

AMD1rev: 5' CACACCCTAGGtcacttttctcaatggtctcttgaattg

GAL7p:

gal7prox: 5' CACACGAGCTCggaatattcaactgtttttttatcatgttgatg

gal7dist: 5' CACACGGAtcctcttgaaaatatgcacitctatacttttag,

and the resulting fragment from the AMD1 reaction (2.4 kb) is cleaved with *SacI* and *AvrII*, and that from the GAL7 reaction (0.7 kb) is cleaved with *BamHI* and *SphI*, and both are ligated together into YEp13 that has been digested with *NheI* and *BamHI*, creating the plasmid "pAMPD". This plasmid carries the *S. cerevisiae* gene, *AMD1*, encoding AMP deaminase, under the control of the galactose-inducible *GAL7* promoter.

[00164] Messenger RNA is prepared from lyophilized biomass of *M. circinelloides*, as described above, and the mRNA template is used in a RT-PCR reaction with two primers:

MAEfwd: 5' CACACGCTAGCTACAAAatgttgcac(caaacgcata)gcaac

MAErev: 5' CACACGTCGACTtaatgatctcggtatacgagaggaac,

and the resulting fragment is cleaved with *NheI* and *Sall*, and ligated to *XbaI*- and *XhoI*-digested pRS413TEF (Mumberg, D. et al. (1995) *Gene*, 156:119-122), creating the plasmid "pTEFMAE", which contains sequences encoding the cytosolic NADP⁺-dependant malic enzyme from *M. circinelloides* (E.C. 1.1.1.40; *mce* gene; Genbank accession: AY209191) under the control of the constitutive *TEF1* promoter.

[00165] The plasmids "p1gal2", "pAMPD", and "pTEFMAE" are sequentially transformed into a strain of *S. cerevisiae* to restore prototrophy for uracil ("p1gal2"), leucine ("pAMPD"), and histidine ("pTEFMAE") (Guthrie and Fink *Methods in Enzymology* 194:1-933, 1991). The resulting transformants are tested for total lipid content following shake flask testing in either synthetic complete (SC) medium lacking uracil, leucine and histidine, as described in Example 3, or in a 2-step fermentation process. In the 2-step process, 1.5 ml of cells from an overnight 2 ml roll tube culture containing SC medium lacking uracil, leucine and histidine are centrifuged, washed in distilled water, and resuspended in 20 ml of a nitrogen-limiting medium suitable for lipid accumulation (30 g/L glucose, 1.5 g/L yeast extract, 0.5 g/L NH₄Cl, 7 g/L KH₂PO₄, 5 g/L Na₂HPO₄·12H₂O, 1.5 g/L MgSO₄·7H₂O, 0.08 g/L FeCl₃·6H₂O, 0.01 g/L ZnSO₄·7H₂O, 0.1 g/L CaCl₂·2H₂O, 0.1 mg/L MnSO₄·5H₂O, 0.1 mg/L CuSO₄·5H₂O, 0.1 mg/L Co(NO₃)₂·6H₂O; pH 5.5 (J Am Oil Chem Soc 70:891-894 (1993)).

[00166] Intracellular lipid content of the modified and control *S. cerevisiae* strains is analyzed using the fluorescent probe, Nile Red (J Microbiol Meth (2004) 56:331-338). In brief, cells diluted in buffer are stained with Nile Red, excited at 488 nm, and the fluorescent emission spectra in the wavelength region of 400-700 nm are acquired and compared to the corresponding spectra from cells not stained with Nile Red. To confirm results from the rapid estimation method, the total lipid content is determined by gas chromatographic analysis of the total fatty acids directly transmethylesterified from dried cells, as described (Appl Microbiol Biotechnol. 2002 Nov;60(3):275-80). Non-transformed *S. cerevisiae* strains produce 6% and 10% total lipid (dry cell weight basis) after growth in YPD and lipid accumulation medium, respectively. Yeast strains expressing the multiple oleaginic polypeptides produce 17% and 25% total lipid following growth in YPD and lipid accumulation medium, respectively.

Example 10: Introduction of heterologous carotene hydroxylase into *Y. lipolytica* strains producing carotenoid for production of zeaxanthin

[00167] MF578 (*tef-carRP tef-carB*) was transformed with pMB4692 that had been cleaved with *Sall*. Several Ura⁺ colonies inferred to contain *tef-crtZ* by PCR analysis were able to produce zeaxanthin in YPD shake flasks, and in one case, all of the carotene was depleted.

[00168] The following tables are referenced throughout the description:

Table 1. Examples of acetyl-CoA carboxylase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	XP_410263	49097606	41	CAG08536	47226520
2	XP_329580	32418204	42	NP_724636	24586460
3	XP_386756	46124405	43	NP_610342	24586458
4	XP_367702	39972623	44	NP_001084	4501855
5	XP_501721	50548503	45	NP_446374	16758804
6	EAK99708	46440402	46	EAL63219	60465120
7	XP_457211	50413128	47	NP_921034	37533464
8	NP_982612	45184894	48	T07084	7438099
9	XP_449236	50293649	49	AAP78896	32264940
10	NP_593271	19114183	50	AAO62903	29123370
11	NP_014413	6324343	51	BAA07012	1100253
12	XP_455355	50310667	52	AAL02056	15558947
13	T42531	11272737	53	AAG40563	11869927
14	AAA20073	171504	54	D86483	25293894
15	EAL20176	50257469	55	T07920	7438090
16	XP_571316	58268320	56	A57710	2130099
17	XP_402244	49076566	57	AAO62902	29123376
18	S60200	2133343	58	2208491A	1588584
19	BAA24410	2804173	59	T09538	7438102
20	P32874	1708192	60	CAC19875	12057067
21	S55089	7438088	61	AAP78897	32264942
22	NP_990836	45382859	62	T02235	7438095
23	CAE01471	32526576	63	AAG40564	11869928
24	AAR37018	40019048	64	E86483	25293893
25	NP_001...	57164283	65	CAC84161	20975574
26	NP_776649	27806341	66	T07081	7438097
27	CAI25271	56205878	67	CAC19876	12057069
28	XP_109883	51828611			
29	NP_942134	38679971			
30	NP_942131	38679960			
31	NP_942135	38679974			
32	NP_942136	38679977			
33	AAP94122	33112885			
34	NP_071529	11559962			
35	2006242A	740964			
36	AAS13685	42405896			
37	NP_598665	48976025			
38	Q13085	2493311			
39	XP_548250	57091783			
40	XP_314071	58385597			

Table 2. Examples of pyruvate decarboxylase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	IQPBB	7245977	45	P87208	2501326
2	CAA54522	871533	46	EAL18331	50255598
3	IPYDB	515237	47	XP_567475	58260130
4	CAA28380	4109	48	AAM73540	21666011
5	IPVDB	1127233	49	AAM73539	21666009
6	CAA33709	4114	50	XP_502508	50550071
7	AAN77243	25992752	51	CAA93158	1177659
8	NP_013235	6323163	52	XP_412533	49123327
9	Q6FJA3	57012668	53	P51844	1706333
10	S36363	486942	54	XP_455842	50311631
11	Q12629	52788279	55	CAA61155	3688422
12	AAP75898	37359468	56	XP_444902	50284947
13	S70684	2131152	57	CAA47319	4118
14	NP_011601	6321524			
15	AAQ73618	34500072			
16	NP_983270	45185554			
17	AAF78895	8745337			
18	CAB65534	6689662			
19	AAP75899	37359470			
20	NP_982469	45184751			
21	CAA97091	1945321			
22	S50700	1086157			
23	XP_446491	50288125			
24	XP_462338	50427451			
25	AAC03164	17066784			
26	EAK96569	46437219			
27	XP_457131	50412425			
28	AAC03165	2734883			
29	XP_459224	50421349			
30	CAH56494	52673248			
31	XP_502647	50550349			
32	NP_010203	6320123			
33	BAA04886	1786148			
34	XP_449074	50293325			
35	EAL04098	46444826			
36	CAD60727	27803024			
37	T38759	25777585			
38	XP_331173	32421459			
39	NP_594083	19114995			
40	XP_401609	49075036			
41	XP_390010	46136637			
42	XP_409025	49095128			
43	NP_984350	45188127			
44	AAD16178	4323053			

Table 3. Examples of isocitrate dehydrogenase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	O13285	3023996	45	XP_502479	50550013
2	EAK91676	46432179	46	EAK96238	46436883
3	O13285	3023996	47	EAK96305	46436951
4	EAK94305	46434909	48	XP_461797	50426401
5	XP_451683	50303483	49	XP_328403	32415850
6	XP_459772	50422415	50	CAF31997	42820684
7	O13294	27805482	51	XP_389756	46136129
8	XP_460289	50423413	52	XP_363786	39952139
9	XP_390523	46137663	53	AAL73035	18463935
10	XP_367343	39971905	54	XP_405140	49086142
11	XP_323176	32405126	55	NP_595203	19111995
12	XP_445447	50286037	56	NP_014779	6324709
13	AAK76730	15027826	57	XP_447564	50290265
14	NP_010217	6320137	58	NP_985684	45198655
15	NP_984921	45190667	59	XP_566837	58258849
16	AAK76731	15027827	60	XP_454086	50308171
17	P79089	3023999	61	XP_398943	49069308
18	NP_013275	6323203			
19	XP_407136	49091350			
20	NP_982520	45184802			
21	XP_446953	50289047			
22	XP_445184	50285511			
23	XP_455638	50311227			
24	AAA64516	736722			
25	NP_970434	42525054			
26	AAT93173	51013759			
27	XP_569233	58264154			
28	XP_569234	58264156			
29	XP_403726	49080406			
30	XP_503571	50552322			
31	XP_458151	50428131			
32	O13302	13124301			
33	XP_409927	49096934			
34	XP_385909	46122711			
35	XP_365293	39967489			
36	NP_983873	45187650			
37	XP_455266	50310493			
38	NP_594397	19115309			
39	XP_324955	32408949			
40	CAE81942	38636405			
41	NP_014361	6324291			
42	XP_446479	50288101			
43	XP_567378	58259936			
44	XP_398944	49069310			

Table 4. Examples of ATP-citrate lyase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	XP_327071	32413182	45	NP_593246	19114158
2	O93988	30912679	46	XP_398620	49068662
3	XP_370222	39977669	47	XP_567460	58260100
4	XP_406573	49090008	48	AAT94429	51092031
5	XP_504787	50554757	49	NP_725514	24653990
6	Q9P7W3	30912748	50	AAD34754	28372804
7	XP_398620	49068662	51	EAL26601	54637198
8	NP_596202	19112994	52	XP_319323	58392375
9	XP_567460	58260100	53	AAH84776	54311201
10	NP_001008	56118260	54	BAB00624	9229902
11	XP_418154	50760837	55	NP_001008	56118260
12	AAH84253	54038148	56	AAH84253	54038148
13	NP_942127	38569423	57	AAH56378	38614162
14	NP_001087	38569421	58	NP_001087	38569421
15	P53396	20141248	59	NP_942127	38569423
16	AAL34316	17028103	60	P53396	20141248
17	NP_001002	50540366	61	XP_511495	55645405
18	AAH84776	54311201	62	NP_058683	8392839
19	S21173	105392	63	NP_001002	50540366
20	AAT94429	51092031	64	S21173	105392
21	AAD34754	28372804	65	NP_508280	17551266
22	AAH21502	18204829	66	CAE64663	39593194
23	XP_319323	58392375	67	CAE56725	39579419
24	NP_725514	24653990	68	NP_506267	17557344
25	EAL26601	54637198	69	XP_537640	57091075
26	CAE56725	39579419	70	CAF96059	47204551
27	CAE64663	39593194	71	F96633	25404292
28	XP_511495	55645405	72	AAM91141	22136126
29	CAF95829	47210997	73	NP_849634	30681854
30	AAO22565	27754223	74	AAO23582	27764922
31	AAL33788	17065616	75	AAM65078	21593129
32	CAB46077	5304837	76	CAC86996	15919089
33	CAF96044	47204726	77	AAQ75158	34558814
34	AAK13318	13160653	78	AAQ75127	34558782
35	AAQ75159	34558815			
36	AAQ75128	34558783			
37	XP_537640	57091075			
38	XP_327069	32413178			
39	CAB76164	7160184			
40	XP_370223	39977671			
41	XP_386215	46123323			
42	CAA10666	7159697			
43	XP_406572	49090004			
44	XP_503231	50551515			

Table 5. Examples of malic enzyme polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	NP_012896	6322823	45	ZP_001276	53693400
2	XP_448858	50292851	46	YP_044961	50083451
3	XP_454793	50309563	47	YP_128226	54295811
4	NP_986598	45201028	48	NP_719387	24375344
5	XP_460887	50424595	49	XP_572853	58271394
6	EAK97738	46438407	50	NP_252161	15598667
7	XP_504112	50553402	51	ZP_001368	46164263
8	XP_330094	32419237	52	YP_125345	54298976
9	XP_380981	46107844	53	NP_793695	28871076
10	XP_411070	49102552	54	YP_096964	52843165
11	XP_362875	39946676	55	EAH92280	44245125
12	NP_587760	19075260	56	YP_154988	56459707
13	NP_978189	42780942	57	EAI68195	44354928
14	YP_035982	49481098	58	YP_070054	51595863
15	YP_027934	49184682	59	YP_133025	54303032
16	YP_018438	47527089	60	NP_969623	42524243
17	ZP_002365	47565532	61	NP_856009	31793516
18	YP_083209	52143619	62	DECARBOXY (ATING))	
19	XP_571672	58269032	63	NP_935035	37680426
20	NP_391586	16080758	64	YP_050922	50121755
21	YP_092693	52786864	65	E70705	7431223
22	NP_831516	30019885	66	NP_216848	57116971
23	YP_093460	52787631	67	DECARBOXY (ATING))	
24	YP_081030	52082239	68	YP_143786	55980489
25	NP_822689	29828055	69	YP_130202	54309182
26	O34389	33517449	70	NP_415996	16129438
27	EAL19111	50256386	71	NP_819843	29654151
28	NP_825047	29830413	72	NP_753809	26247769
29	ZP_002340	47096498	73	NP_707611	56479957
30	NP_928837	37525493	74	F85728	25283682
31	NP_230833	15641201	75	YP_163690	56552851
32	NP_934257	37679648	76	YP_150562	56413487
33	NP_761613	27366085	77	NP_720610	24378655
34	AC1314	25283688	78	NP_460525	16764910
35	YP_055602	50842375	79	ZP_003193	48865537
36	YP_095310	52841511	80	NP_784797	28377905
37	ZP_002315	47093832	81	T13496	7431227
38	AC1686	25283689	82	AAV65766	55793550
39	YP_126594	54294179	83	A97096	25283683
40	YP_123567	54297198	84	YP_193951	58337366
41	EAJ76260	44510091	85	H97096	25283684
42	YP_114273	53803890	86	ZP_003237	48870993
43	NP_797637	28898032	87	ZP_001460	41689468
44	YP_040250	49483026	88	D86737	25283676

Row	Genbank ACCESSION	Genbank GI
89	ZP 002870	48825851
90	ZP 001439	34762975
91	1922245A	737262
92	YP 169914	56708018
93	YP 055027	50841800
94	ZP 000625	23023297
95	NP 296302	15807565
96	NP 285599	15807938
97	YP 132069	54302076
98	CAA50716	467569
99	ZP 002906	48833596
100	ZP 003155	48861632
101	NP 773109	27381580
102	AAQ95658	37622953
103	CAC19505	56204311
104	AAH80660	51873855
105	P40927	729986
106	AAT02533	46850200
107	BAC37086	26346875
108	T02763	7431235
109	XP 387367	46125627
110	AAC50613	1465733
111	CAA39421	669118
112	CAA39420	669117
113	NP 032641	6678912
114	CAA39419	581228
115	AAB01380	1335389
116	JC4160	1085347
117	E96828	25283677
118	BAD87910	57899974
119	EAI77083	44511304
120	P13697	266504
121	NP 036732	7106353
122	YP 065939	51246055
123	CAC18164	16944467
124	XP 322953	32404680
125	AAK91502	18460985
126	AAQ88396	37147841
127	NP 001003	57525624
128	1GQ2P	21465488
129	AAO26053	28195290
130	AAH84250	54038006
131	XP 362590	39946106
132	AAH03287	13096987
133	Q29558	2497785
134	XP 532217	57094622

Row	Genbank ACCESSION	Genbank GI
135	P28227	126734
136	NP 496968	17537199
137	NP 914533	34906372
138	AAD10504	4096786
139	AAO67523	50897495
140	P43279	1170871
141	AAK83074	15077109
142	AAP33011	30575690
143	AAN86690	27357017
144	P78715	41017288
145	AAP32204	30526303
146	AAV31249	54287505
147	T06402	7431232
148	Q99KE1	55583978
149	XP 399922	49071266
150	P36444	547886
151	AAO30034	28059162
152	AAK83073	15077107
153	NP 002387	4505145
154	AAA33487	168528
155	BAA74735	4239891
156	NP 989634	45383538
157	1GZ3D	31615316
158	AAW56450	57791240
159	AAT02534	46850202
160	S29742	422339
161	1O0SB	34811253
162	P27443	126732
163	T06401	7431231
164	AAL16175	16226466
165	AAF73006	8118507
166	AAK97530	15420975
167	EAI90348	44385841
168	P51615	1708924
169	AAA19575	169327
170	S43718	1084300
171	P34105	1346485
172	AAS38597	42733630
173	BAC54101	27530932
174	AAT02535	46850204
175	CAB66003	6706333
176	AAH84860	54311418
177	CAA39422	669119
178	NP 916713	34910732
179	CAA56354	510876
180	DEFBC	7427668

Row	Genbank ACCESSION	Genbank GI
181	JC5967	7431234
182	NP_197960	15239517
183	NP_651959	21356279
184	CAB64263	6634090
185	BAB20887	54606800
186	EAL27424	54638022
187	NP_006671	5729920
188	AAB08874	1561774
189	1PJLH	33358128
190	1GZ4D	22218682
191	1QR6B	5822327
192	1PJ3D	39654475
193	P22178	126736
194	XP_410305	49097690
195	AAH22472	18490280

Table 6. Examples of AMP deaminase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	AAA34420	171053	45	NP_000471	4502079
2	XP_446684	50288509	46	S68147	2134756
3	NP_983153	45185436	47	AAH56380	38614134
4	XP_453337	50306727	48	O08739	2494043
5	EAL02322	46443037	49	NP_113732	13928736
6	XP_460211	50423261	50	O09178	2494044
7	XP_503822	50552824	51	XP_420973	50747746
8	XP_413009	49131023	52	NP_956142	41054127
9	XP_360256	39941438	53	CAG01709	47222742
10	XP_381547	46108978	54	NP_957187	41053780
11	XP_330167	32419447	55	XP_392957	48104570
12	CAB97316	16945394	56	AAH07183	13938134
13	T50996	11359582	57	CAG05605	47220579
14	NP_595153	19111945	58	NP_620231	20302047
15	EAL22226	50259553	59	XP_540247	57098851
16	XP_402237	49076548	60	CAF99638	47230445
17	CAA62797	995562	61	XP_513671	55587796
18	AAF65407	7638159	62	CAI18828	56203368
19	XP_537039	57088163	63	CAI18829	56203369
20	AAH49119	29145073	64	CAI18830	56203370
21	XP_569691	58265070	65	EAA19931	23484684
22	AAD56303	5922018	66	CAH99706	56500932
23	NP_004028	21264318	67	XP_131103	38076931
24	A44313	345738	68	CAH77387	56523366
25	CAI19307	56206061			
26	AAA62126	644509			
27	CAI19305	56206059			
28	XP_310497	58424203			
29	CAI19306	56206060			
30	AAC50308	608499			
31	CAG06825	47229629			
32	NP_727741	45551453			
33	NP_727739	45551452			
34	NP_727740	24641890			
35	AAN09337	22832227			
36	T01259	7484807			
37	XP_506591	51963676			
38	NP_850294	30687456			
39	CAG07509	47228777			
40	NP_494974	32564190			
41	T15771	7497030			
42	CAE59064	39596837			
43	NP_494973	32564194			
44	BAA06505	1321635			

Table 7. Examples of acetoacetyl-CoA thiolase polypeptides.

Row	Genbank ACCESSION	Genbank GI
1	P10551	135758
2	Q04677	418002
3	Q12598	34925109
4	T10247	7433657
5	T42741	11257345
6	AAL18924	16417944
7	AAM67058	21618008
8	AAO51605	28829030
9	AAU95618	53854350
10	AAU95619	53854352
11	BAA97003	8777413
12	CAE76429	38567134
13	EAK90852	46431255
14	EAL32264	54643520
15	NP_015297	6325229
16	NP_568694	30695411
17	NP_572414	24640423
18	NP_596686	19113478
19	NP_851154	30695409
20	NP_908411	34894172
21	NP_974900	42573608
22	NP_974901	42573610
23	NP_984262	45188039
24	XP_389497	46134945
25	XP_401186	49074048
26	XP_405546	49087148
27	XP_449306	50293789
28	XP_449306	50293789
29	XP_450298	50899020
30	XP_453599	50307241
31	XP_460741	50424309
32	XP_500646	50546253

Table 8. Examples of HMG-CoA synthase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	B55729	1083370	45	XP_456470	50405663
2	P54869	1708235	46	XP_506052	50557288
3	S13887	86312	47	XP_513693	55587844
4	S27197	284048	48	XP_536483	57085299
5	AAA37076	387072	49	XP_569805	58265298
6	AAF89580	9621905	50	XP_571930	58269548
7	AAH00297	33991031			
8	AAH31363	21618633			
9	AAH42929	27552834			
10	AAH79694	50925193			
11	AAH83543	54035469			
12	AAO52569	28830079			
13	AAP35966	30583443			
14	BAB23657	12836439			
15	BAC04559	21754758			
16	BAC05233	21758044			
17	CAA52032	1772495			
18	CAC18553	11602786			
19	CAG33131	48145817			
20	CAH92111	55730782			
21	CAI22408	56205097			
22	EAK97451	46438115			
23	EAL25034	54635631			
24	NP_002121	54020720			
25	NP_013580	6323509			
26	NP_032282	31560689			
27	NP_058964	8393538			
28	NP_593859	19114771			
29	NP_666054	31981842			
30	NP_725570	24654139			
31	NP_775117	27465521			
32	NP_957379	41055180			
33	NP_983739	45187516			
34	NP_990742	45382279			
35	NP_999545	47523816			
36	XP_315872	58387870			
37	XP_323241	32405256			
38	XP_368218	39973655			
39	XP_389442	46134253			
40	XP_397202	48141273			
41	XP_402977	49078452			
42	XP_409060	49095198			
43	XP_446972	50289085			
44	XP_453529	50307101			

Table 9. Examples of HMG-CoA reductase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	A23586	90238	45	XP_503558	50552167
2	O74164	11132850	46	XP_536323	57084803
3	P51639	1708252	47	XP_571450	58268588
4	P54960	1708251			
5	Q12649	18276268			
6	Q29512	2495262			
7	Q9Y7D2	11133211			
8	S30338	422383			
9	S72194	7450066			
10	AAA36989	387052			
11	AAA37077	305355			
12	AAA49740	214237			
13	AAD20975	9817458			
14	AAH74197	49257596			
15	AAL09351	15824453			
16	AAO85434	29468180			
17	AAP72015	32165622			
18	AAR02862	45272118			
19	AAT92819	51013051			
20	BAC20567	23574646			
21	CAA63970	4376229			
22	CAE47850	41581201			
23	CAF92135	47213283			
24	CAH92577	55731745			
25	EAK94577	46435190			
26	EAL20195	50257490			
27	AAF80374	8886086			
28	NP_013555	6323483			
29	NP_013636	6323565			
30	NP_032281	56119096			
31	NP_037266	40538852			
32	NP_588235	19075735			
33	NP_985010	45190756			
34	NP_989816	45383193			
35	NP_999724	47551099			
36	XP_324892	32408825			
37	XP_364130	39955070			
38	XP_389373	46134115			
39	XP_400629	49072680			
40	XP_405730	49087632			
41	XP_407954	49092986			
42	XP_449268	50293713			
43	XP_451740	50303597			
44	XP_458872	50420671			

Table 10. Examples of mevalonate kinase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	XP_386088	46123069	45	Q50559	2497518
2	XP_408006	49093090	46	CAF88123	47200914
3	XP_370449	39978123	47	NP_275189	15678075
4	EAL04797	46445529	48	EAI88745	44383877
5	XP_322935	32404644	49	ZP_002040	46141948
6	NP_001007	55925207	50	XP_543435	57105916
7	XP_460851	50424525	51	EAI38920	44313360
8	XP_567851	58260882	52	NP_148611	14602065
9	XP_567850	58260880	53	EAD08953	43286228
10	AAQ02416	33303805	54	EAD45697	43361720
11	CAA53059	450346	55	YP_134862	55377012
12	AAH16140	16359371	56	NP_720650	24378695
13	AAH05606	13542811	57	NP_614276	20094429
14	XP_403111	49078786	58	E84270	25409931
15	XP_452532	50305147	59	NP_691146	23097680
16	CAG08527	47226511	60	ZP_003233	48870579
17	XP_446138	50287417	61	AAG02440	9937386
18	AAO51522	28828936	62	EAD12278	43292898
19	NP_985191	45190937	63	NP_498328	17555862
20	XP_500956	50546973	64	EAB31483	42928976
21	NP_013935	6323864	65	ZP_003319	50590618
22	AAD45421	5578718	66	NP_814642	29375488
23	NP_920723	37532842	67	AC1434	25514495
24	NP_851084	30690651	68	ZP_003577	53796847
25	AAL18925	16417946	69	EAD82048	43454743
26	NP_788338	28573850	70	CAE73618	39586491
27	AAU20834	51988124	71	YP_012624	46906235
28	AAU87813	52839819	72	NP_988455	45358898
29	AAU20835	51988125	73	ZP_002348	47097293
30	YP_183887	57641409	74	ZP_002862	48824993
31	NP_143478	14591399	75	ZP_002307	47093020
32	BAA24409	2804172	76	NP_597102	19173299
33	NP_126232	14520757	77	CAD24422	20429111
34	XP_522574	55639331	78	NP_785308	28378416
35	NP_071114	11499870	79	EAA39098	29247539
36	XP_423949	50797461	80	NP_819638	29653946
37	NP_633786	21227864	81	EAH49746	44168765
38	ZP_002971	48840229	82	EAH49745	44168764
39	EAH50787	44170778	83	NP_378182	15922513
40	NP_615566	20089491	84	ZP_000459	23002259
41	IVISA	40890012	85	H90181	25393827
42	EAK03559	44549994	86	YP_054120	50405028
43	NP_248080	15669275	87	BAB07790	9695270
44	IKKHA	20150886	88	AAG02435	9937379

Row	Genbank ACCESSION	Genbank GI
89	NP_560495	18313828
90	YP_187834	57866187
91	EAK40782	44602942
92	CAC51370	15212070
93	AAG02424	9937364
94	YP_185521	57651465
95	YP_040044	49482820
96	YP_194037	58337452
97	D86675	25400965
98	NP_763916	27467279
99	CAF89434	47197810
100	EAF38333	43767792
101	EAK46841	44611394
102	H89827	25507776
103	ZP_003149	48861061
104	EAK17824	44570143
105	EAH86276	44235719
106	YP_118418	54024176
107	ZP_003196	48865749
108	AAG02430	9937372
109	NP_269075	15674901
110	NP_802520	28896170
111	AAL97579	19748102
112	ZP_003666	56808907
113	NP_965060	42519130
114	NP_819639	29653947
115	EAD97024	43484567
116	BAD86800	57753870

Table 11. Examples of phosphomevalonate kinase polypeptides.

Row	Genbank ACCESSION	Genbank GI
1	AAA34596	171479
2	XP_452514	50305111
3	NP_985210	45190956
4	XP_446144	50287429
5	XP_462340	50427455
6	EAL04096	46444824
7	EAL03941	46444668
8	XP_503619	50552418
9	XP_389940	46136497
10	XP_329795	32418634
11	XP_369652	39976529
12	XP_406448	49089559
13	NP_593421	19114333
14	XP_568385	58261950
15	EAL17628	50254887
16	AAL18926	16417948
17	BAD43274	51969164
18	BAD44652	51971975
19	XP_398375	49068172
20	BAD44486	51971643
21	F90479	25393214
22	YP_194039	58337454

Table 12. Examples of mevalonate pyrophosphate decarboxylase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	AAT93171	51013755	45	NP_496967	17537203
2	IFI4A	13786942	46	NP_691147	23097681
3	XP_455548	50311049	47	EAL29282	54640164
4	XP_445335	50285813	48	AD1434	25515042
5	XP_456912	50409853	49	ZP_002308	47093021
6	NP_986435	45200865	50	YP_012625	46906236
7	AAF19399	6625790	51	ZP_002348	47097294
8	XP_328845	32416734	52	NP_819637	29653945
9	XP_505041	50555265	53	NP_376888	15921219
10	NP_594027	19114939	54	ZP_003319	50590617
11	XP_364905	39963452	55	NP_585805	19074299
12	XP_390600	46137817	56	YP_187835	57866188
13	XP_408551	49094180	57	CAD24423	20429112
14	AAA34506	7544604	58	AAG02431	9937373
15	EAL18927	50256200	59	NP_763917	27467280
16	XP_568247	58261674	60	AAG02446	9937394
17	XP_402794	49077992	61	ZP_002863	48824994
18	AAH81784	51980639	62	AAG02441	9937387
19	EAL00166	46440864	63	YP_185522	57651466
20	NP_619597	20149736	64	A89828	25505863
21	NP_112324	13592005	65	NP_814641	29375487
22	BAC40852	26354448	66	YP_040045	49482821
23	XP_546783	57087071	67	NP_785307	28378415
24	Q99JF5	23814095	68	ZP_003196	48865750
25	AAH63907	39645379	69	ZP_003233	48870580
26	CAF99534	47230341	70	E86675	25400967
27	AAP35576	30582699	71	EAE31110	43552684
28	AAP36301	30584105	72	BAB07791	9695271
29	AAL18927	16417950	73	CAC51371	15212071
30	AAV32433	54292590	74	ZP_000459	23002258
31	AAP68208	31711704	75	NP_965061	42519131
32	AAM64988	21593039	76	BAD86801	57753871
33	NP_566995	18410026	77	YP_194038	58337453
34	XP_423130	50771155	78	YP_118419	54024177
35	AAM65192	21593243	79	EAK18820	44571499
36	NP_001007	55925435	80	EAI85935	44379784
37	NP_573068	28571205	81	NP_721336	24379381
38	BAD27942	50252009	82	D95044	25388338
39	T47584	11281655	83	AAG02456	9937408
40	XP_307373	31196851	84	C97914	25511486
41	CAE73245	39591192	85	EAK47683	44612560
42	NP_496966	17537201	86	EAB86425	43039778
43	XP_393230	48121058	87	YP_140971	55822530
44	G90479	25393662	88	YP_139081	55820639

Row	Genbank ACCESSION	Genbank GI
89	BAD07376	40882372
90	NP_968512	42523132
91	EAI06705	44265427
92	YP_060018	50914046
93	AAG02451	9937401
94	NP_269076	15674902
95	ZP_003666	56808906
96	NP_688323	22537472
97	NP_735832	25011437
98	EAC40267	43149093
99	AAL97580	19748103
100	EAI76915	44367119
101	EAD35042	43339207
102	YP_073129	51598941
103	EAI90092	44385501
104	BAB07818	9711347
105	EAD72850	43433025
106	NP_212820	15595031
107	YP_124337	54297968
108	YP_096056	52842257
109	EAA39903	29248368
110	EAH06252	44088237
111	YP_127354	54294939
112	EAD45753	43361830
113	NP_802519	28896169

Table 13. Examples of IPP isomerase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	NP_015208	6325140	45	AAL91980	19568939
2	XP_448008	50291151	46	BAB40973	13603406
3	NP_983828	45187605	47	AAF29975	6856554
4	XP_455121	50310203	48	T52027	25493162
5	XP_462358	50427491	49	AAL91979	19568937
6	EAL01685	46442395	50	T46812	11362218
7	XP_504974	50555131	51	T51248	11362217
8	XP_328425	32415894	52	BAB40974	13603408
9	XP_367200	39971619	53	O48965	6225532
10	XP_389898	46136413	54	XP_225509	34877710
11	XP_404716	49085144	55	XP_506401	51963472
12	CAD37150	21627818	56	AAF29978	6856560
13	NP_595164	19111956	57	AAH76541	50369278
14	XP_566641	58258457	58	AAT94033	51038230
15	XP_402453	49077100	59	XP_225502	34876517
16	Q35586	6225528	60	Q39471	6225533
17	AAP36609	30584713	61	AAB67743	1213450
18	AAF37873	7188790	62	NP_197148	22326844
19	NP_445991	16758306	63	BAB09611	9759126
20	O42641	6225529	64	AAD41766	5305669
21	BAA33979	3790386	65	AAB67741	1213442
22	Q13907	6225527	66	XP_395125	48101420
23	AAH22418	48257241	67	AAN28784	23505849
24	AAH19227	48257312	68	AAF36996	7110585
25	AAH57827	35505325	69	BAB16690	15289752
26	NP_004499	40018633	70	AAQ14869	33340598
27	AAH89786	58477715	71	BAC65421	28971819
28	CAH91844	55730243	72	S71369	2129625
29	XP_418561	50732281	73	AAF29979	6856562
30	AAH06999	48257093	74	AAF29980	6856564
31	CAF98782	47225155	75	AAF21674	30267831
32	NP_808875	29366820	76	Q39664	6225534
33	XP_507622	55633353	77	NP_650962	24648688
34	AAH82648	52139082	78	AAM50284	21429130
35	NP_001011	58332496	79	XP_321388	58395620
36	AAF29976	6856556	80	Q9BXS1	20978506
37	AAG10423	9971806	81	T07979	7484383
38	O48964	6225525	82	XP_225508	34876527
39	AAF29973	6856550	83	AAT92102	51011386
40	AAF29977	6856558	84	XP_225507	34876555
41	AAQ84167	35186998	85	XP_344623	34876537
42	AAF29974	6856552	86	S44843	630677
43	Q39472	6225526	87	XP_225498	27687955
44	S49588	1085973	88	AAT08468	47013849

Row	Genbank ACCESSION	Genbank GI
89	EAI79636	44370808
90	CAE75055	39587401
91	EAL04047	46444775
92	XP 225528	34876543
93	XP 544282	57040602
94	XP 225511	27688013
95	P26173	114853
96	EAI04069	44405322
97	EAH27496	44127513
98	AAF91499	9653280
99	AAM48661	21328655
100	EAK17826	44570145
101	EAD59515	43391069
102	YP 128702	54307682
103	EAK66656	44639203
104	YP 118189	54023947
105	T50740	11282665
106	ZP 002077	46193541
107	EAK16470	44568229
108	YP 165403	56695056
109	EAD08775	43285885
110	YP 195623	58616494
111	EAI38918	44313358
112	NP 930583	37527239
113	YP 160254	56478665
114	EAH69842	44206571
115	EAK26254	44582307
116	AAR24381	38569721
117	AAM48607	21328600
118	EAD82049	43454744
119	ZP 001924	45914126
120	YP 056780	50843553
121	YP 050880	50121713
122	EAH29235	43749645
123	NP 630823	21225044
124	Q82MJ7	34582349
125	ZP 003374	52010110
126	AAS75819	45737905
127	Q8KP37	30913023
128	XP 507621	55633351
129	XP 344621	34876521
130	XP 346322	34880719
131	YP 152060	56414985
132	AAT42442	48429280
133	Q9KK75	13878536
134	NP 806649	29143307

Row	Genbank ACCESSION	Genbank GI
135	YP 063124	50955836
136	Q8FND7	46395593
137	CAF20647	41326485
138	Q8NN99	23821718
139	Q7X5H2	46395586
140	NP 336246	15841209
141	Q83MJ9	46395588
142	P60923	46395576
143	Q8FE75	31563050
144	1R67A	38493022
145	Q9KWD1	13878537
146	Q7VEU0	46395585
147	B84333	25410326
148	NP 417365	16130791
149	E85944	25355426
150	1HZTA	15826050
151	1PVFB	50513321
152	EAD63579	43403471
153	1I9AB	13786886
154	YP 012992	46906603
155	ZP 002293	47091503
156	EAI37194	44310821
157	YP 137864	55380014
158	CAD92056	42516867
159	1OW2B	42543244

Table 14. Examples of FPP synthase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	Q92250	2497455	45	AAQ56011	34013692
2	XP_363065	39948036	46	AAQ14872	33340604
3	XP_386960	46124813	47	AAQ14871	33340602
4	Q92235	3122099	48	AAD17204	4324960
5	XP_412149	49116518	49	AAH87886	56789674
6	XP_503599	50552378	50	AAK68152	14573639
7	NP_593299	19114211	51	AAA52423	182399
8	CAD42869	21955860	52	S66470	2129849
9	XP_448787	50292709	53	CAA29064	4725
10	NP_012368	6322294	54	CAI12715	55957735
11	T42081	7433997	55	BAA03523	40788949
12	EAK93751	46434339	56	P14324	1346031
13	XP_451300	50302727	57	S66471	2129850
14	XP_571137	58267962	58	AAA35820	182405
15	XP_460720	50424267	59	CAA59170	1491641
16	NP_984739	45190485	60	BAB16687	15289750
17	BAD15361	46367743	61	CAA72793	1922251
18	S71433	7433991	62	CAH91070	55728661
19	CAA65643	1523990	63	AAK58594	14279425
20	XP_399061	49069544	64	AAB07264	1146159
21	S71432	7433990	65	Q09152	21431776
22	AAH68912	46249832	66	O64905	6016044
23	IFPS	1065289	67	BAB60822	14422406
24	P08836	3915686	68	S52009	1076319
25	AAH83515	53733369	69	NP_917118	34911542
26	IUBX	1942050	70	AAD32648	4894899
27	IUBY	1942051	71	AAA40960	203582
28	AAF37872	7188788	72	AAR27053	38684029
29	NP_803463	29135293	73	AAU43998	52353430
30	AAK63847	14488053	74	AAL82595	18958450
31	AAV58896	55710092	75	NP_917069	34911444
32	T06272	7433988	76	XP_228802	34879769
33	JC4846	2117737	77	BAD81810	56785155
34	P05369	120478	78	AAN62522	24796660
35	O24241	25452945	79	NP_595334	19112126
36	O24242	25452946	80	T52066	25458583
37	AAH59125	37590777	81	AAL49067	17946048
38	AAH48497	28913418	82	CAA08919	3395483
39	AAP74720	32329199	83	XP_547662	57089869
40	CAG11850	47225367	84	EAL26135	54636732
41	AAM51429	21436457	85	BAB60821	14422404
42	AAP74719	32329197	86	AAP74721	32329201
43	AAM08927	20135548	87	XP_496902	51466663
44	XP_537252	57089113	88	XP_474182	50929309

Row	Genbank ACCESSION	Genbank GI
89	CAA87327	1160178
90	BAD20729	47776234
91	BAC53873	30984142
92	BAB69490	15991313
93	NP_974565	42572937
94	CAA08918	5678609
95	AAP86267	32527731
96	AAO17735	30522953
97	AAK71861	14647139
98	AAL73357	18478919
99	AAQ63552	29124957
100	CAI00471	56498227
101	NP_701155	23508486
102	XP_474180	50929305
103	AAL73358	18478922
104	EAH48995	44167328
105	NP_493027	17508563
106	CAE71711	39580204
107	XP_487220	51766977

Table 15. Examples of GGPP synthase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	AAT92871	51013155	45	CAH18006	51469024
2	XP 447025	50289191	46	CAA75568	3549881
3	NP 984623	45190369	47	XP 397455	48143654
4	XP 390273	46137163	48	XP 410947	49101294
5	XP 404791	49085320	49	XP 381914	46109712
6	XP 368486	39974191	50	XP 364478	39959279
7	Q92236	6831550	51	XP 360889	39942704
8	AAO85432	29468176	52	XP 369218	39975655
9	XP 572774	58271236	53	XP 406544	49089926
10	XP 502923	50550901	54	XP 367595	39972409
11	AAK11525	13021716	55	XP 363775	39952117
12	XP 326920	32412880	56	XP 368486	39974191
13	CAF32032	42820719	57	XP 390273	46137163
14	BAD29965	50355599	58	Q92236	6831550
15	XP 384767	46117498	59	AAK11525	13021716
16	BAD29970	50355631	60	CAF32032	42820719
17	CAB89115	7649674	61	XP 404791	49085320
18	CAG09545	47229030	62	AAO85432	29468176
19	CAI13753	55960163	63	BAD29965	50355599
20	AAH69913	47124116	64	BAD29970	50355631
21	AAH67768	45709211	65	BAA90525	6899844
22	XP 455003	50309979	66	AAT65717	49409613
23	P56966	9296978	67	XP 384767	46117498
24	NP 001007	56090562	68	CAB89115	7649674
25	AAT65717	49409613	69	XP 572774	58271236
26	NP 956329	41053321	70	AAK11531	13021724
27	BAA90525	6899844	71	XP 502923	50550901
28	XP 405729	49087630	72	CAI13753	55960163
29	AAK11531	13021724	73	CAG09545	47229030
30	XP 412280	49119197	74	XP 412280	49119197
31	AAC05273	2944400	75	P56966	9296978
32	NP 523958	24660002	76	NP 001007	56090562
33	XP 402074	49076128	77	AAH69913	47124116
34	EAL30191	54641441	78	AAH67768	45709211
35	XP 536340	57084951	79	NP 956329	41053321
36	XP 424685	50811194	80	EAL30191	54641441
37	AAH06798	13905030	81	XP 424685	50811194
38	AAP06018	29841005	82	XP 536340	57084951
39	XP 460338	50423511	83	NP 523958	24660002
40	AAC05595	2957271	84	AAC05273	2944400
41	EAK92197	46432727	85	XP 405729	49087630
42	XP 535573	57108760	86	AAC05595	2957271
43	AAH83212	53734594	87	XP 402074	49076128
44	XP 486466	51827552	88	AAP06018	29841005

Row	Genbank ACCESSION	Genbank GI
89	AAH06798	13905030
90	XP 535573	57108760
91	AAH83212	53734594
92	AAP21085	30097620
93	NP 984623	45190369
94	XP 447025	50289191
95	AAT92871	51013155
96	XP 486466	51827552
97	XP 410947	49101294
98	XP 397455	48143654
99	XP 455003	50309979
100	EAK92197	46432727
101	XP 381914	46109712
102	XP 460338	50423511
103	CAH18006	51469024
104	XP 360889	39942704
105	XP 406544	49089926
106	XP 364478	39959279
107	XP 363775	39952117
108	XP 367595	39972409
109	XP 369218	39975655
110	C39273	483124
111	BAB79600	18143445
112	BAA14124	216682
113	AAN85596	27228290
114	AAA32797	413730
115	Q08291	585326
116	S52584	1073293
117	S53722	1076576
118	AAC44848	1842242
119	BAA19583	1944371
120	S71230	2129674
121	BAA23157	2578822
122	AAC77874	3885426
123	CAB38744	4490594
124	BAA78047	4958920
125	BAA82613	5631295
126	CAB56064	5912297
127	BAA86284	6277254
128	T11021	7447356
129	AAF78199	8650415
130	AAG10424	9971808
131	CAC10561	10637876
132	T50879	11279298
133	BAB01343	11994221
134	Q42698	13431546

Row	Genbank ACCESSION	Genbank GI
135	Q43133	13431547
136	P54976	13878921
137	BAB50600	14023995
138	BAB60678	14325238
139	BAB60820	14422402
140	NP 189589	15228704
141	NP 188651	15231055
142	NP 188069	15231869
143	NP 188073	15231881
144	AAL01997	15553715
145	AAL01998	15553717
146	NP 252732	15599238
147	NP 245470	15602398
148	NP 390308	16079484
149	NP 440010	16329282
150	NP 440010	16329282
151	AAL17614	17352451
152	NP 520343	17546941
153	AAL76349	18645048
154	AAM21638	20386366
155	AAM21639	20386368
156	NP 622916	20807745
157	AAM48650	21328644
158	NP 659794	21492720
159	AAM64496	21592547
160	AAM65107	21593158
161	NP 680811	22297564
162	ZP 000474	23003800
163	ZP 001252	23469933
164	NP 698760	23502633
165	E84566	25313373
166	F85434	25313385
167	AC1245	25313389
168	E83997	25313393
169	G84566	25313395
170	AH2910	25315863
171	D87505	25398795
172	A89932	25505949
173	F97685	25520741
174	AI3285	25527013
175	BAC42571	26450928
176	NP 785195	28378303
177	NP 790546	28867927
178	AAO63392	28950937
179	AAO93113	29893480
180	NP 833891	30022260

Row	Genbank ACCESSION	Genbank GI
181	AAP59037	31621279
182	ZP_001374	32039216
183	NP_864766	32471772
184	NP_875521	33240579
185	NP_881399	33593755
186	NP_884694	33597051
187	NP_888456	33600896
188	NP_893187	33861626
189	NP_894940	33863380
190	NP_896835	33865276
191	NP_896835	33865276
192	AAQ65086	34365549
193	NP_945877	39933601
194	NP_946867	39934591
195	NP_952815	39996864
196	AAR37805	40062934
197	AAR37858	40062988
198	AAR98495	41018904
199	AAR99082	41059107
200	NP_965349	42519419
201	NP_980544	42783297
202	EAA96348	42858148
203	EAB36506	42939031
204	EAB36642	42939300
205	EAC39208	43146996
206	EAD26007	43320598
207	EAE43084	43576643
208	EAE70061	43630884
209	EAF70308	43832107
210	EAG88494	44055952
211	EAH52060	44173220
212	EAH78354	44221788
213	EAH84117	44231960
214	EAI11762	44272832
215	EAI49391	44328289
216	EAI54846	44336042
217	EAI68356	44355138
218	EAI68713	44355672
219	EAI69401	44356609
220	EAI73873	44362658
221	EAI73634	44506168
222	EAI77351	44511694
223	EAK70639	44644254
224	ZP_001751	45523854
225	AAS76253	45752710
226	ZP_001957	45916757

Row	Genbank ACCESSION	Genbank GI
227	IRTRB	46015556
228	ZP_001863	46105954
229	ZP_002002	46107045
230	ZP_001711	46132567
231	ZP_002073	46192680
232	ZP_002074	46192861
233	AAS82860	46241274
234	ZP_002108	46308696
235	YP_010568	46579760
236	BAD18313	47076770
237	ZP_002315	47093750
238	ZP_002335	47095946
239	AAT35222	47531118
240	ZP_002401	47569437
241	ZP_002435	47573473
242	ZP_002626	48728941
243	ZP_002702	48765678
244	ZP_002705	48766028
245	ZP_002732	48768894
246	ZP_002914	48834438
247	ZP_003024	48848203
248	ZP_003129	48858958
249	ZP_003177	48863841
250	ZP_003225	48869790
251	AAT51323	49086036
252	ZP_003301	49236117
253	YP_034222	49476181
254	YP_040995	49483771
255	YP_043579	49486358
256	AAT71982	50253560
257	AAT90315	50952782
258	YP_066435	51246551
259	YP_075673	51892982
260	YP_085511	52141318
261	YP_092166	52786337
262	ZP_001298	53691368
263	YP_105136	53716444
264	YP_111769	53722784
265	ZP_003630	54030933
266	YP_129021	54308001
267	AAV74395	56122554
268	AAV74396	56122556
269	YP_148246	56420928
270	YP_156518	56461237
271	YP_162590	56551751
272	YP_171470	56750769

Row	Genbank ACCESSION	Genbank GI
273	YP_175959	56964228
274	YP_186407	57650478
275	YP_190690	58038726
276	AAW66658	58201026
277	YP_194187	58337602
278	YP_197469	58579257
279	YP_201938	58582922
280	YP_196510	58617311

Table 16. Examples of squalene synthase polypeptides.

Row	Genbank ACCESSION	Genbank GI
1	AAA34597	171481
2	CAA42583	3686
3	Q9HGZ6	51704336
4	BAB12207	9955387
5	XP_453457	50306959
6	Q752X9	51701405
7	O74165	51701378
8	XP_458579	50420093
9	EAK95451	46436082
10	P78589	2499979
11	Q9Y753	51701459
12	XP_407513	49092104
13	XP_364394	39958237
14	Q7S4Z6	51701416
15	CAD60581	27764301
16	XP_389557	46135731
17	NP_595363	19112155
18	B48057	477750
19	NP_034321	34328173
20	CAH92517	55731622
21	AAI00038	6002565
22	P53798	1706773
23	NP_004453	31542632
24	AAP36671	30584837
25	1EZFC	11514497
26	AAH09251	14328083
27	AAH84016	54035372
28	I52090	2136196
29	XP_420043	50745256
30	AAH81810	51858605
31	CAB48363	50841455
32	XP_569783	58265254
33	XP_569782	58265252
34	XP_534557	57105080
35	XP_401989	49075920

Table 17. Examples of phytoene dehydrogenase polypeptides.

Row	Genbank ACCESSION	Row	Genbank ACCESSION
1	1613414B	45	AAP59036
2	1613414F	46	AAP79175
3	1904206A	47	AAQ04224
4	2121278A	48	AAQ04225
5	A86203	49	AAQ65246
6	A96612	50	AAQ65246
7	A99470	51	AAQ88931
8	AAA24820	52	AAR37797
9	AAA34001	53	AAR37802
10	AA150313	54	AAR37850
11	AAA64981	55	AAR37855
12	AAA91161	56	AAR86105
13	AAA99519	57	AAR98491
14	AAC44798	58	AAR98494
15	AAC44850	59	AAR98733
16	AAC48983	60	AAS17750
17	AAF78201	61	AAT01639
18	AAG10426	62	AAT35222
19	AAG14399	63	AAT74579
20	AAG28700	64	AAT74580
21	AAG50743	65	AAT76050
22	AAH85048	66	AAT76434
23	AAK51545	67	AAT90316
24	AAK51557	68	AAU34019
25	AAK64299	69	AAW23161
26	AAL02000	70	AB2035
27	AAL15300	71	AB2064
28	AAL38046	72	AC2446
29	AAL73986	73	AF1557
30	AAL80005	74	AF2029
31	AAL91366	75	AG2103
32	AAM45380	76	AG2509
33	AAM48646	77	AH1199
34	AAM63349	78	AI2185
35	AAM94364	79	AI2273
36	AAN75037	80	B55548
37	AAN85599	81	B84327
38	AAO24235	82	B90061
39	AAO46892	83	BAA14127
40	AAO46894	84	BAA20276
41	AAO53257	85	BAA76534
42	AAO53258	86	BAB10768
43	AAO64750	87	BAB50520
44	AAO93135	88	BAB51896

Row	Genbank ACCESSION
89	BAB68552
90	BAB79603
91	BAB82461
92	BAB82462
93	BAB98016
94	BAC75676
95	BAC77668
96	BAC77671
97	BAD07279
98	BAD07280
99	BAD07287
100	BAD07288
101	CAA52098
102	CAA60479
103	CAA66626
104	CAB38739
105	CAB38743
106	CAB40843
107	CAB56041
108	CAB56062
109	CAB59726
110	CAB65434
111	CAB94794
112	CAC85667
113	CAD19989
114	CAD27442
115	CAD55814
116	CAE00192
117	CAE83576
118	CAF19330
119	CAF21094
120	CAF21337
121	CAH91165
122	E90061
123	EAA90383
124	EAA98598
125	EAB09790
126	EAB14136
127	EAB18725
128	EAB29729
129	EAB30992
130	EAB41377
131	EAB54727
132	EAB76679
133	EAB87028
134	EAB92587

Row	Genbank ACCESSION
135	EAB94459
136	EAB96864
137	EAC01884
138	EAC38895
139	EAC60360
140	EAD05874
141	EAD05999
142	EAD20520
143	EAE06978
144	EAE70773
145	EAF04985
146	EAF51354
147	EAF62819
148	EAF75453
149	EAG09111
150	EAG19412
151	EAG23070
152	EAG25053
153	EAG25054
154	EAG29279
155	EAG39845
156	EAG56100
157	EAG63013
158	EAG68633
159	EAG71574
160	EAG89835
161	EAH04928
162	EAH04936
163	EAH08586
164	EAH22597
165	EAH22853
166	EAH31648
167	EAH55579
168	EAH68071
169	EAH73302
170	EAH79041
171	EAH86965
172	EAH97108
173	EAH99977
174	EAI01660
175	EAI03576
176	EAI06784
177	EAI11087
178	EAI15261
179	EAI15547
180	EAI17521

Row	Genbank ACCESSION
181	EAI21398
182	EAI29728
183	EAI38468
184	EAI43591
185	EAI51589
186	EAI58453
187	EAI72974
188	EAI77885
189	EAI78272
190	EAI80262
191	EAI83937
192	EAI86664
193	EAJ00517
194	EAJ05570
195	EAJ08238
196	EAJ15524
197	EAJ18144
198	EAJ20649
199	EAJ21683
200	EAJ24413
201	EAJ28774
202	EAJ30522
203	EAJ35157
204	EAJ37407
205	EAJ39929
206	EAJ54356
207	EAJ54959
208	EAJ56207
209	EAJ58447
210	EAJ59958
211	EAJ63347
212	EAJ66054
213	EAJ67637
214	EAJ69812
215	EAJ74441
216	EAJ76472
217	EAJ76473
218	EAJ80355
219	EAJ80839
220	EAJ81408
221	EAJ86174
222	EAJ87600
223	EAJ88203
224	EAJ88682
225	EAJ92341
226	EAJ94774

Row	Genbank ACCESSION
227	EAJ97555
228	EAJ97958
229	EAK07654
230	EAK08513
231	EAK08529
232	EAK10609
233	EAK10614
234	EAK12902
235	EAK13034
236	EAK15092
237	EAK22483
238	EAK23222
239	EAK24187
240	EAK24674
241	EAK28785
242	EAK34731
243	EAK34742
244	EAK36883
245	EAK37522
246	EAK42705
247	EAK43213
248	EAK52580
249	EAK53452
250	EAK58759
251	EAK62665
252	EAK63558
253	F84187
254	F90272
255	G87635
256	G90413
257	H83880
258	H84320
259	JC7723
260	NP_060220
261	NP_080435
262	NP_193157
263	NP_214383
264	NP_276913
265	NP_293819
266	NP_294534
267	NP_294585
268	NP_295972
269	NP_338490
270	NP_376437
271	NP_377056
272	NP_388895

Row	Genbank ACCESSION
273	NP 441167
274	NP 441254
275	NP 442491
276	NP 442727
277	NP 562475
278	NP 568712
279	NP 601630
280	NP 601630
281	NP 616426
282	NP 624522
283	NP 626360
284	NP 630834
285	NP 643053
286	NP 647302
287	NP 659552
288	NP 661086
289	NP 661546
290	NP 661701
291	NP 662300
292	NP 681023
293	NP 681127
294	NP 682351
295	NP 693380
296	NP 693382
297	NP 737250
298	NP 763380
299	NP 786524
300	NP 822198
301	NP 822828
302	NP 827278
303	NP 851528
304	NP 857496
305	NP 868798
306	NP 869339
307	NP 870237
308	NP 874530
309	NP 874561
310	NP 874977
311	NP 892236
312	NP 892265
313	NP 892458
314	NP 893232
315	NP 894882
316	NP 895385
317	NP 895793
318	NP 895829

Row	Genbank ACCESSION
319	NP 896854
320	NP 896994
321	NP 898304
322	NP 898346
323	NP 902647
324	NP 923340
325	NP 923639
326	NP 923813
327	NP 925079
328	NP 931515
329	NP 936379
330	NP 940208
331	NP 945754
332	NP 946860
333	NP 946866
334	NP 948851
335	NP 962004
336	NP 968600
337	NP 974222
338	NP 974545
339	O49901
340	P17059
341	P54971
342	P54978
343	P54979
344	P54981
345	P54982
346	P74306
347	Q01671
348	Q02861
349	Q38893
350	Q40406
351	Q9FV46
352	Q9SE20
353	Q9SMJ3
354	Q9ZTN9
355	Q9ZTP4
356	S29314
357	S32171
358	S49624
359	S52586
360	S65060
361	T10701
362	T31463
363	T46822
364	T48646

Row	Genbank ACCESSION
365	T50745
366	T50749
367	T50893
368	T50910
369	T51119
370	T51123
371	XP_324732
372	XP_383241
373	XP_401825
374	XP_470568
375	XP_473486
376	XP_477063
377	XP_525801
378	XP_540198
379	YP_006049
380	YP_013621
381	YP_024310
382	YP_041986
383	YP_041988
384	YP_044561
385	YP_044564
386	YP_062471
387	YP_117947
388	YP_120612
389	YP_135077
390	YP_136483
391	YP_145331
392	YP_145348
393	YP_171014
394	YP_172823
395	YP_173078
396	YP_173207
397	YP_184572
398	YP_187368
399	YP_187371
400	YP_187371
401	YP_187371
402	ZP_000490
403	ZP_000509
404	ZP_000518
405	ZP_000566
406	ZP_000627
407	ZP_000627
408	ZP_001073
409	ZP_001081
410	ZP_001091

Row	Genbank ACCESSION
411	ZP_001116
412	ZP_001117
413	ZP_001119
414	ZP_001124
415	ZP_001510
416	ZP_001591
417	ZP_001593
418	ZP_001602
419	ZP_001614
420	ZP_001645
421	ZP_001650
422	ZP_001722
423	ZP_001746
424	ZP_001752
425	ZP_001770
426	ZP_001777
427	ZP_001787
428	ZP_001837
429	ZP_001867
430	ZP_002073
431	ZP_002077
432	ZP_002339
433	ZP_002680
434	ZP_002705
435	ZP_002771
436	ZP_002892
437	ZP_002916
438	ZP_002963
439	ZP_003022
440	ZP_003036
441	ZP_003107
442	ZP_003202
443	ZP_003258
444	ZP_003268
445	ZP_003269
446	ZP_003276
447	ZP_003283
448	ZP_003557
449	ZP_003559
450	ZP_003565
451	ZP_003577
452	ZP_003593
453	ZP_003595
441	ZP_003685

Table 18. Examples of phytoene synthase and lycopene cyclase polypeptides.

Row	Genbank Accession	Genbank GI	Row	Genbank Accession	Genbank GI
1	1613414C	227040	45	AAP79176	32307542
2	A49558	1076590	46	AAQ91837	37499616
3	AAA19428	506623	47	AAR08445	38037628
4	AAA32836	413732	48	AAR31885	39842609
5	AAA64982	148413	49	AAR37803	40062932
6	AAB87738	29893495	50	AAR37856	40062986
7	AAC44849	1842243	51	AAR86104	40456029
8	AAD38051	13542332	52	AAR87868	40557193
9	AAF78202	8650418	53	AAR98492	41018901
10	AAF82616	9081847	54	AAS02284	41394357
11	AAG10427	9971814	55	AAS17009	42491736
12	AAG28701	11066678	56	AAS18307	42521626
13	AAK07734	18476085	57	AAT28184	47498515
14	AAK07735	18476089	58	AAT35222	47531118
15	AAK15621	13195243	59	AAT38473	47779181
16	AAL02001	15553721	60	AAT46069	48686711
17	AAL76346	18645045	61	AAT74581	50313418
18	AAL82578	21326700	62	AAT90319	50952786
19	AAM45379	21360353	63	AAV74394	56122551
20	AAM48647	21328641	64	AAW23162	56698928
21	AAM62787	21553694	65	AC2035	25366683
22	AAM94363	22296799	66	AC2035	25366683
23	AAN85600	27228294	67	BAB18514	11344507
24	AAO24767	27903500	68	BAB79604	18143449
25	AAO39835	28403302	69	BAD07278	40809739
26	AAO46895	37729028	70	BAD07286	40809755
27	AAO47570	33465823	71	BAD62106	54291340
28	AAO73816	33465821	72	BAD62107	54291341
29	AAP22038	30349414	73	C90061	25506636
30	AAP55451	32350232	74	CAA47625	19347
31	AAP55453	32350236	75	CAA68575	19341
32	AAP55461	32350252	76	CAB07958	1934837
33	AAP55471	32350272	77	CAB38740	4490590
34	AAP55484	32350298	78	CAB51949	5690074
35	AAP55486	32350302	79	CAB56063	5912296
36	AAP56083	32349564	80	CAB86388	7453011
37	AAP56124	32349646	81	CAB93661	8250520
38	AAP56127	32349652	82	CAB94795	8574392
39	AAP56136	32349670	83	CAC19567	11990226
40	AAP56148	32349694	84	CAC27383	12584564
41	AAP56155	32349708	85	CAD19988	18307500
42	AAP56156	32349710	86	CAD29284	57282088
43	AAP56157	32349712	87	CAE76609	38567321
44	AAP56158	32349714	88	E37802	95606

Row	Genbank Accession	Genbank GI
89	E84320	25410251
90	EAA98758	42863045
91	EAB01965	42869439
92	EAB04170	42873822
93	EAB07138	42879858
94	EAB09791	42885235
95	EAB19826	42905452
96	EAB35029	42936011
97	EAB41375	42948740
98	EAB78706	43024004
99	EAB92586	43052355
100	EAC06949	43081493
101	EAC18360	43104624
102	EAC25793	43119723
103	EAC29883	43128092
104	EAC32813	43133973
105	EAC33105	43134560
106	EAC38486	43145552
107	EAC52233	43173313
108	EAC60029	43189028
109	EAC68026	43204953
110	EAC96197	43261031
111	EAD08701	43285745
112	EAD20866	43310220
113	EAD32755	43334458
114	EAD38008	43345761
115	EAD50152	43370658
116	EAD50402	43371147
117	EAD81123	43452903
118	EAD93882	43478303
119	EAE12860	43516265
120	EAE16121	43522884
121	EAE31084	43552634
122	EAE35665	43561764
123	EAE44717	43579862
124	EAE46627	43583580
125	EAE47846	43586023
126	EAE72264	43635190
127	EAE76009	43642573
128	EAE86335	43662748
129	EAE89581	43669163
130	EAF18881	43728007
131	EAF64277	43819669
132	EAF67931	43827263
133	EAF84745	43861327
134	EAF94004	43880040

Row	Genbank Accession	Genbank GI
135	EAG06083	43903395
136	EAG21950	43933540
137	EAG43625	43973477
138	EAG50171	43985555
139	EAG57517	43999205
140	EAG62787	44009110
141	EAG65580	44014171
142	EAG68110	44018715
143	EAG72283	44026322
144	EAG78750	44037938
145	EAG80445	44041116
146	EAG93220	44064453
147	EAH04927	44085694
148	EAH08972	44093217
149	EAH10377	44095788
150	EAH22151	44117864
151	EAH31461	44134654
152	EAH50033	44169323
153	EAH64480	44196848
154	EAH79040	44223009
155	EAH99976	44255671
156	EAI02786	44259828
157	EAI02787	44259829
158	EAI03575	44260943
159	EAI05900	44264266
160	EAI61004	44344824
161	EAI70669	44358327
162	EAI83938	44377067
163	EAJ05110	44406802
164	EAJ05369	44407471
165	EAJ08876	44412338
166	EAJ35156	44449986
167	EAJ38900	44455130
168	EAJ49645	44470504
169	EAJ54357	44477026
170	EAJ60475	44485647
171	EAJ64125	44492007
172	EAJ67499	44497025
173	EAJ76471	44510405
174	EAJ76950	44511114
175	EAJ78637	44513596
176	EAJ78787	44513824
177	EAJ79616	44515082
178	EAJ80356	44516200
179	EAJ81914	44518489
180	EAJ87417	44526623

Row	Genbank Accession	Genbank GI
181	EAK08514	44557109
182	EAK08523	44557119
183	EAK12901	44563097
184	EAK22180	44576315
185	EAK24859	44580262
186	EAK28345	44585276
187	EAK34732	44594324
188	EAK34736	44594329
189	EAK37296	44597942
190	EAK37521	44598256
191	EAK56335	44624430
192	G84363	25410528
193	NP_274195	15677043
194	NP_284085	15794263
195	NP_294586	15805888
196	NP_388961	16078144
197	NP_441168	16330440
198	NP_443763	16519643
199	NP_624523	21218744
200	NP_630832	21225053
201	NP_662273	21674208
202	NP_682350	22299103
203	NP_693381	23099915
204	NP_786525	28379633
205	NP_822199	29827565
206	NP_822829	29828195
207	NP_851527	30795077
208	NP_868799	32475805
209	NP_874560	33239618
210	NP_879992	33592348
211	NP_884101	33596458
212	NP_889809	33602249
213	NP_892264	33860703
214	NP_895828	33864268
215	NP_898345	33866786
216	NP_902648	34498433
217	NP_902649	34498434
218	NP_924690	37521313
219	NP_931516	37528171
220	NP_946861	39934585
221	NP_949079	39936803
222	NP_962005	41409169
223	NP_968601	42523221
224	O07333	3913360
225	P08196	585746
226	P21683	30923192

Row	Genbank Accession	Genbank GI
227	P37269	585009
228	P37271	27735222
229	P37272	585749
230	P53797	1709885
231	P54975	1706137
232	P54977	1706139
233	P65860	54041032
234	Q9SSU8	8928282
235	Q9UUQ6	34922667
236	S22474	7489041
237	S32170	321671
238	S52587	1073300
239	S56668	2129505
240	S68307	2130144
241	T10702	7484346
242	T46594	11291807
243	T50746	11356347
244	T50895	11291816
245	XP_324765	32408567
246	XP_383242	46114448
247	XP_403902	49080862
248	YP_006040	46255128
249	YP_103126	53723680
250	YP_112342	53723357
251	YP_117945	54023703
252	YP_120611	54026369
253	YP_136628	55378778
254	YP_136629	55378779
255	YP_145340	55978284
256	YP_145343	55978287
257	YP_160917	56479328
258	YP_160918	56479329
259	YP_162605	56551766
260	YP_172822	56752121
261	YP_187369	57652299
262	YP_192648	58040684
263	ZP_000044	22956752
264	ZP_001091	53688068
265	ZP_001591	53763709
266	ZP_001657	45514234
267	ZP_001690	46132223
268	ZP_001746	45523280
269	ZP_001837	53771530
270	ZP_001867	45546711
271	ZP_002096	46204978
272	ZP_002248	46324513

Row	Genbank Accession	Genbank GI
273	ZP_002450	47575031
274	ZP_002680	48763469
275	ZP_002710	48766450
276	ZP_002791	48782680
277	ZP_002892	48832182
278	ZP_002916	48834623
279	ZP_003036	48849426
280	ZP_003269	48893702
281	ZP_003351	52007802
282	ZP_003487	53730362
283	ZP_003501	53759405
284	ZP_003591	53798896
285	ZP_003628	54030691

Table 19. Examples of carotenoid ketolase polypeptides.

Row	Accession Number	GI Number	Row	Accession Number	GI Number
1	AAA99932	609575	45	EAI53009	44333409
2	AAB48668	1870215	46	EAI54054	44334878
3	AAC25611	2541936	47	EAI67818	44354362
4	AAF78203	8650419	48	EAI68153	44354875
5	AAH16427	16741158	49	EAI89684	44384943
6	AAN03484	22597194	50	EAJ27674	44439188
7	AAN85497	26541510	51	EAJ45589	44464684
8	AAN86030	33439708	52	EAJ45589	44464684
9	AAO64399	28976134	53	EAJ67118	44496466
10	AAQ23139	33621091	54	EAJ74221	44507022
11	AAT35222	47531118	55	EAJ74653	44507662
12	AAT35555	47558911	56	EAJ88396	44528064
13	AAV41372	55139370	57	EAJ88887	44528792
14	AB2307	25530134	58	EAK06069	44553531
15	AF2204	25533132	59	EAK11467	44561166
16	BAB54999	14028447	60	EAK16824	44568733
17	BAB58879	14270087	61	EAK28828	44585942
18	BAC98366	37360914	62	EAK28828	44585942
19	CAA60478	2654318	63	EAK31112	44589271
20	CAB56059	5912292	64	EAK42591	44605441
21	D87673	25398945	65	NP_045063	11465545
22	EAA79304	42823978	66	NP_081575	27754029
23	EAA80363	42826055	67	NP_338204	15843167
24	EAA81403	42828115	68	NP_440788	16330060
25	EAA84711	42834481	69	NP_441220	16330492
26	EAB82380	43031476	70	NP_682690	22299443
27	EAB86624	43040184	71	NP_770721	27379192
28	EAC05755	43079085	72	NP_848964	30468077
29	EAD12219	43292778	73	NP_857223	31794730
30	EAD71182	43427899	74	NP_881760	33594116
31	EAD94927	43480380	75	NP_882469	33594826
32	EAF11582	43712986	76	NP_886657	33599097
33	EAF98072	43888329	77	NP_895643	33864083
34	EAG19345	43928738	78	NP_896386	33864827
35	EAG38273	43963688	79	NP_897461	33865902
36	EAG79800	44039853	80	NP_924674	37521297
37	EAG96474	44070318	81	NP_927525	37524181
38	EAH00349	44077315	82	NP_947075	39934799
39	EAH36448	44143633	83	P54972	1706150
40	EAH40683	44151265	84	Q39982	2498257
41	EAH53180	44175316	85	Q44261	2498256
42	EAH96648	44250729	86	T31123	11361063
43	EAI05260	44263397	87	XP_330780	32420673
44	EAI17468	44281329	88	XP_368852	39974923

Row	Accession Number	GI Number
89	XP_380194	46102628
90	XP_383758	46115480
91	XP_405100	49086048
92	XP_409222	49095522
93	YP_102417	53725671
94	YP_108945	53719959
95	YP_132414	54302421
96	YP_154670	56459389
97	YP_166682	56696325
98	YP_168846	56698471
99	YP_172377	56751676
100	ZP_001068	23124870
101	ZP_001112	53688676
102	ZP_001607	53764743
103	ZP_001757	46118877
104	ZP_001787	53736018
105	ZP_002218	46321435
106	ZP_002456	47575608
107	ZP_003028	48848557
108	ZP_003107	48856640
109	ZP_003264	48893204
110	ZP_003458	53688805
111	ZP_003513	53763576

Table 20. Examples of carotenoid hydroxylase polypeptides.

Row	Genbank ACCESSION	Genbank GI	Row	Genbank ACCESSION	Genbank GI
1	AAC44852	1842246	44	EAI00766	44256844
2	AAC49443	1575296	45	EAI29017	44298625
3	AAD54243	5852870	46	EAI30844	44443849
4	AAG10430	9971820	47	EAI72524	44504516
5	AAG10793	9988836	48	EAK10611	44559981
6	AAG33636	11245486	49	EAK53455	44620561
7	AAL80006	19071768	50	EAK63955	44635271
8	AAM44971	21280903	51	H90469	25394049
9	AAM51300	21436107	52	NP_745389	26989964
10	AAM77007	21734857	53	NP_922503	37536402
11	AAN85601	27228295	54	P54973	1706152
12	AAO53295	28911949	55	Q44262	2498258
13	AAS48097	44887642	56	S52982	1073291
14	AAS55552	45184599	57	XP_473611	50928167
15	AAS88426	46326968	58	YP_024309	48478603
16	AAT48741	49036137	59	ZP_003055	48851297
17	AAT84408	50844570	60	ZP_003107	48856620
18	AAV85452	56267980			
19	AAV85453	56267982			
20	BAA14129	216687			
21	BAB79605	18143450			
22	BAC77670	31790567			
23	BAD07283	40809749			
24	BAD07291	40809765			
25	CAA70427	2956671			
26	CAA70888	2956717			
27	CAB55625	5870598			
28	CAB55626	5870600			
29	CAB56060	5912293			
30	CAC06712	9968545			
31	CAC95130	33145986			
32	EAB30128	42926157			
33	EAC49462	43167766			
34	EAC86129	43241003			
35	EAD61089	43395962			
36	EAD76156	43443111			
37	EAD88640	43467793			
38	EAE27903	43546376			
39	EAE28203	43546980			
40	EAE78743	43647896			
41	EAH12173	43714211			
42	EAH29370	44130906			
43	EAH44202	44158360			

Table 21. Examples of astaxanthin synthase polypeptides and putative astaxanthin synthase polypeptides.

Row	Genbank ACCESSION	Genbank GI
1	AAM56288	21501451
2	XP_571276	58268240
3	EAL20013	50257304
4	XP_401804	49075484
5	XP_397817	49067054
6	XP_399595	49070612
7	XP_403279	49079218
8	XP_382294	46110473
9	XP_406021	49088382
10	XP_381224	46108332
11	XP_391479	46139577
12	XP_569261	58264210
13	EAL22841	50260180
14	XP_359866	39940658

Table 22. Examples of carotenoid epsilon hydroxylase polypeptides.

ACCESSION	GI	PROTEIN DESCRIPTION
ABB52076	79155148	putative epsilon-ring carotene hydroxylase [<i>Daucus carota</i> subsp. <i>sativus</i>]
BAD94136	62319017	Cytochrom P450 -like protein [<i>Arabidopsis thaliana</i>]
ABD28565	87162770	E-class P450, group I [<i>Medicago truncatula</i>]
AAT28222	47498772	putative 97B2-like cytochrome P450 [<i>Ginkgo biloba</i>]
ABC68396	85001685	cytochrome P450 monooxygenase CYP97A [<i>Glycine max</i>]
ABC59110	84514203	cytochrome P450 monooxygenase CYP97B [<i>Medicago truncatula</i>]
NP_190881	42565881	LUT1 (LUTEIN DEFICIENT 1); oxygen binding [<i>Arabidopsis thaliana</i>]
ABB47954	78708979	cytochrome P450 monooxygenase, putative [<i>Oryza sativa</i> (japonica cultivar-group)]
NP_922604	37536604	putative cytochrome P450 monooxygenase [<i>Oryza sativa</i> (japonica cultivar-group)]

Table 23. Examples of lycopene cyclase polypeptides, beta and epsilon subunits.

ACCESSION	GI	PROTEIN DESCRIPTION
AAK07431	12746307	lycopene epsilon-cyclase [<i>Adonis palaeatina</i>]
ABB52073	79154988	putative lycopene epsilon cyclase [<i>Daucus carota</i> subsp. <i>sativus</i>]
Q38932	27735211	Lycopene epsilon cyclase, chloroplast precursor
AAB53336	1399181	lycopene epsilon cyclase
AAG10428	9971816	epsilon cyclase [<i>Tagetes erecta</i>]
AAK07434	12746313	lycopene epsilon-cyclase [<i>Lactuca sativa</i>]
AAM45382	21360359	epsilon cyclase [<i>Tagetes erecta</i>]
O65837	11132841	Lycopene epsilon cyclase, chloroplast precursor
AAL69394	18419661	lycopene epsilon-cyclase [<i>Spinacia oleracea</i>]
BAE79549	87299433	lycopene epsilon-cyclase [<i>Chrysanthemum x morifolium</i>]
XP_463351	50901836	putative lycopene epsilon-cyclase [<i>Oryza sativa</i>]

		(japonica cultivar-group)]
AAS48096	44887640	epsilon lycopene cyclase [Citrus sinensis]
AAX92679	62638188	lycopene epsilon cyclase [Citrus maxima]
AAL92114	19569601	lycopene epsilon-cyclase [Citrus x paradisi]
AAK07433	12746311	lycopene epsilon-cyclase [Solanum tuberosum]
AAL47019	17864021	lycopene epsilon-cyclase [Citrus sinensis]
AAT46065	48686703	chloroplast lycopene epsilon-cyclase precursor [Chlamydomonas reinhardtii]
BAD07293	40809769	lycopene epsilon-cyclase [Citrus limon]
BAD07285	40809753	lycopene epsilon-cyclase [Citrus sinensis]
BAD07277	40809737	lycopene epsilon-cyclase [Citrus unshiu]
EAJ62839	44489138	unknown [environmental sequence]
BAE43547	73993068	putative lycopene beta cyclase [Taxodium distichum var. distichum]
BAE43550	73993074	putative lycopene beta cyclase [Taxodium distichum var. distichum]
BAE43557	73993088	putative lycopene beta cyclase [Taxodium distichum var. imbricarium]
BAE43558	73993090	putative lycopene beta cyclase [Taxodium distichum var. imbricarium]
BAE43553	73993080	putative lycopene beta cyclase [Taxodium distichum var. imbricarium]
BAE43545	73993064	putative lycopene beta cyclase [Taxodium distichum var. distichum]
BAE43556	73993086	putative lycopene beta cyclase [Taxodium distichum var. imbricarium]
BAE43552	73993078	putative lycopene beta cyclase [Taxodium distichum var. distichum]
BAE43560	73993094	putative lycopene beta cyclase [Taxodium distichum var. imbricarium]
BAE43554	73993082	putative lycopene beta cyclase [Taxodium distichum var. imbricarium]
BAE43551	73993076	putative lycopene beta cyclase [Taxodium distichum var. distichum]
BAE43519	73993012	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43535	73993044	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43541	73993056	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43542	73993058	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43517	73993008	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43534	73993042	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43537	73993048	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43533	73993040	putative lycopene beta cyclase [Cryptomeria japonica]
BAD02774	38603277	putative lycopene beta cyclase [Cryptomeria japonica]
BAD02766	38603261	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43540	73993054	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43514	73993002	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43544	73993062	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43538	73993050	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43528	73993030	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43546	73993066	putative lycopene beta cyclase [Taxodium distichum var. distichum]
BAE43526	73993026	putative lycopene beta cyclase [Cryptomeria japonica]
BAE43543	73993060	putative lycopene beta cyclase [Cryptomeria japonica]

BAD02742	38603213	putative lycopene beta cyclase [<i>Cryptomeria japonica</i>]
BAD02770	38603269	putative lycopene beta cyclase [<i>Cryptomeria japonica</i>]
BAE43522	73993018	putative lycopene beta cyclase [<i>Cryptomeria japonica</i>]
BAE43559	73993092	putative lycopene beta cyclase [<i>Taxodium distichum</i> var. <i>imbricarium</i>]
BAE43527	73993028	putative lycopene beta cyclase [<i>Cryptomeria japonica</i>]
BAE43548	73993070	putative lycopene beta cyclase [<i>Taxodium distichum</i> var. <i>distichum</i>]
AAF44700	14550425	lycopene beta-cyclase [<i>Citrus sinensis</i>]
BAE43555	73993084	putative lycopene beta cyclase [<i>Taxodium distichum</i> var. <i>imbricarium</i>]
BAE43549	73993072	putative lycopene beta cyclase [<i>Taxodium distichum</i> var. <i>distichum</i>]
AAU14144	51922063	lycopene beta-cyclase [<i>Citrus sinensis</i>]
AAN86060	27261727	lycopene cyclase [<i>Citrus unshiu</i>]
AAR89632	40756518	lycopene-beta-cyclase [<i>Citrus maxima</i>]
AAM21152	20530862	lycopene beta-cyclase [<i>Citrus sinensis</i>]
AAD38049	13959731	lycopene cyclase [<i>Citrus x paradisi</i>]
AAU05146	51511939	lycopene beta-cyclase [<i>Citrus sinensis</i>]
AAU05145	51511937	lycopene beta-cyclase [<i>Citrus sinensis</i>]
AAK07430	12746305	lycopene beta-cyclase [<i>Adonis palaestina</i>]
ABB72443	82394885	lycopene beta-cyclase [<i>Citrus sinensis</i>]
BAE79544	87299423	lycopene beta-cyclase [<i>Chrysanthemum x morifolium</i>]
BAE78471	85717882	lycopene beta cyclase [<i>Taraxacum officinale</i>]
Q43415	11133019	Lycopene beta cyclase, chloroplast precursor
AAF23013	6665782	lycopene epsilon-cyclase [<i>Daucus carota</i>]
ABB52071	79154899	putative lycopene beta cyclase [<i>Daucus carota</i> subsp. <i>sativus</i>]
AAW8382	59665024	lycopene beta-cyclase [<i>Lycium barbarum</i>]
AAG10429	9971818	beta cyclase [<i>Tagetes erecta</i>]
AAM45381	21360357	beta cyclase [<i>Tagetes erecta</i>]
AAM14335	20259239	putative lycopene beta cyclase [<i>Arabidopsis thaliana</i>]
AAO18661	27728515	lycopene beta-cyclase [<i>Zea mays</i>]
AAA81880	735882	lycopene cyclase
Q43503	11133022	Lycopene beta cyclase, chloroplast precursor
S66350	2129931	lycopene beta-cyclase (EC 5.5.1.-) - tomato
XP_464409	50905841	putative lycopene beta-cyclase [<i>Oryza sativa</i> (japonica cultivar-group)]
CAD70565	45237491	lycopene cyclase [<i>Bixa orellana</i>]
Q43578	11133025	Lycopene beta cyclase, chloroplast precursor
AAL92175	19569782	beta-lycopene cyclase [<i>Sandersonia aurantiaca</i>]
AAX54906	61742130	putative chloroplast lycopene beta cyclase precursor [<i>Chlamydomonas reinhardtii</i>]
S66349	2129954	lycopene beta-cyclase (EC 5.5.1.-) - common tobacco
AAG21133	10644119	chromoplast-specific lycopene beta-cyclase [<i>Lycopersicon esculentum</i>]
CAB92977	8247354	neoxanthin synthase [<i>Solanum tuberosum</i>]
CAB93342	8249885	neoxanthin synthase [<i>Lycopersicon esculentum</i>]
Q9SEA0	11131528	Capsanthin/capsorubin synthase, chloroplast precursor
Q42435	12643508	Capsanthin/capsorubin synthase, chloroplast precursor
AAO64977	37730608	lycopene beta cyclase [<i>Haematococcus pluvialis</i>]
Q40424	11133011	Lycopene beta cyclase, chloroplast precursor
ABB52072	79154940	putative capsanthin-capsorubin synthase [<i>Daucus carota</i>]

		subsp. sativus]
AAQ02668	33304511	lycopene cyclase [Setaria italica]
CAA54961	840729	putative chromoplastic oxydo-reductase [Capsicum annuum]
EAJ62838	44489136	unknown [environmental sequence]
YP_401079	81300871	Lycopene cyclase, beta and epsilon [Synechococcus elongatus PCC 7942]
YP_172741	56752040	lycopene cyclase [Synechococcus elongatus PCC 6301]
ZP_011...	88808972	lycopene beta cyclase [Synechococcus sp. WH 7805]
EAK50052	44615956	unknown [environmental sequence]
NP_892751	33861190	putative lycopene epsilon cyclase [Prochlorococcus marinus subsp. pastoris str. CCMP1986]
NP_875182	33240240	Lycopene epsilon cyclase [Prochlorococcus marinus subsp. marinus str. CCMP1375]
YP_382237	78213458	Lycopene cyclase, beta and epsilon [Synechococcus sp. CC9605]
YP_397130	78779018	Lycopene cyclase, beta and epsilon [Prochlorococcus marinus str. MIT 9312]
NP_896821	33865262	lycopene beta cyclase [Synechococcus sp. WH 8102]
YP_397570	78779458	Lycopene cyclase, beta and epsilon [Prochlorococcus marinus str. MIT 9312]
ZP_010...	87302144	lycopene cyclase [Synechococcus sp. WH 5701]
EAK17149	44569190	unknown [environmental sequence]
YP_291882	72382527	lycopene cyclase, beta and epsilon [Prochlorococcus marinus str. NATL2A]
NP_875528	33240586	Lycopene beta cyclase related dehydrogenase [Prochlorococcus marinus subsp. marinus str. CCMP1375]
NP_893181	33861620	putative lycopene beta cyclase [Prochlorococcus marinus subsp. pastoris str. CCMP1986]
NP_895600	33864040	putative lycopene epsilon cyclase [Prochlorococcus marinus str. MIT 9313]
EAI47456	44325573	unknown [environmental sequence]
YP_291268	72381913	lycopene cyclase, beta and epsilon [Prochlorococcus marinus str. NATL2A]
ZP_010...	84517806	Lycopene beta cyclase related dehydrogenase [Prochlorococcus marinus str. MIT 9211]
AAF34191	6970079	lycopene epsilon cyclase [Daucus carota]
ZP_010...	84518202	Lycopene epsilon cyclase [Prochlorococcus marinus str. MIT 9211]
YP_376736	78184301	Lycopene cyclase, beta and epsilon [Synechococcus sp. CC9902]
ZP_003...	66796756	Lycopene cyclase, beta and epsilon [Deinococcus geothermalis DSM 11300]
NP_894954	33863394	putative lycopene beta cyclase [Prochlorococcus marinus str. MIT 9313]
AAT76051	50365502	lycopene cyclase [Citrus clementina]
EAK22047	44576122	unknown [environmental sequence]
NP_294525	15805827	lycopene cyclase [Deinococcus radiodurans R1]

Table 24. Examples of carotenoid glucosyltransferase polypeptides.

ACCESSION	GI	PROTEIN DESCRIPTION
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AA121261	148395	CrtX [Pantoea agglomerans]
AAN85597	27228291	Zeaxanthin Glucosyl Transferase [Pantoea stewartii]
BAB79601	18143446	crtX [Pantoea agglomerans pv. millettiae]
AAZ73147	72536082	zeaxanthin glucosyl transferase [Enterobacteriaceae bacterium DC413]
AAZ73128	72536060	zeaxanthin glucosyl transferase [Enterobacteriaceae bacterium DC260]
AAZ73140	72536074	zeaxanthin glucosyl transferase [Enterobacteriaceae bacterium DC416]
Q01330	231911	Zeaxanthin glucosyl transferase
ZP_006...	71674312	UDP-glycosyltransferase, MGT [Trichodesmium erythraeum IMS101]
NP_439972	16329244	zeaxanthin glucosyl transferase [Synechocystis sp. PCC 6803]
EAH29368	44130903	unknown [environmental sequence]
ZP_005...	67926135	zeaxanthin glucosyl transferase, hypothetical protein [Crocospira watsonii WH 8501]
YP_378763	78188425	hypothetical protein Cag_0447 [Chlorobium chlorochromatii CaD3]
ZP_005...	68549418	Glycosyl transferase, group 1 [Pelodictyon phaeoclathratiforme BU-1]
ZP_010...	85713606	glycosyl transferase, group 1 [Nitrobacter sp. Nb-311A]
YP_317171	75674750	glycosyl transferase, group 1 [Nitrobacter winogradskyi Nb-255]
ZP_006...	69929171	Glycosyl transferase, group 1 [Nitrobacter hamburgensis X14]
ZP_009...	84500589	hypothetical protein OB2597_11541 [Oceanicola batsensis HTCC2597]
ZP_009...	83953176	hypothetical protein NAS141_12746 [Sulfitobacter sp. NAS-14.1]
ZP_009...	83942121	hypothetical protein EE36_07793 [Sulfitobacter sp. EE-36]
YP_508020	89052569	glycosyl transferase, group 1 [Jannaschia sp. CCS1]
ZP_010...	85704103	hypothetical protein ROS217_13931 [Roseovarius sp. 217]
ZP_009...	83370850	probable glycosyltransferase [Rhodobacter sphaeroides ATCC 17025]
ZP_006...	69934465	Glycosyl transferase, group 1 [Paracoccus denitrificans PD1222]
ZP_009...	83949880	probable glycosyltransferase [Roseovarius nubinhibens ISM]
YP_376237	78183803	putative glycosyltransferase [Synechococcus sp. CC9902]
YP_376129	78183695	probable glycosyltransferase [Synechococcus sp. CC9902]
YP_374296	78186253	hypothetical protein Plut_0365 [Pelodictyon luteolum DSM 273]
ZP_010...	87301651	Putative glycosyltransferase [Synechococcus sp. WH 5701]
ZP_011...	88809938	Putative glycosyltransferase [Synechococcus sp. WH 7805]

BAE47471	78483937	carotenoid glucosyltransferase [Paracoccus sp. N81106]
ZP_010...	87303273	probable glycosyltransferase [Synechococcus sp. WH 5701]
YP_376127	78183693	probable glycosyltransferase [Synechococcus sp. CC9902]
YP_501334	88196509	hypothetical protein SAOUHSC_02880 [Staphylococcus aureus subsp. aureus NCTC 8325]
YP_187370	57652300	glycosyl transferase, group 2 family protein [Staphylococcus aureus subsp. aureus COL]
CAA66627	1340131u	named protein product [Staphylococcus aureus]
YP_041987	49484763	putative glycosyl transferase [Staphylococcus aureus subsp. aureus MRSA252]
YP_417885	82752144	hypothetical protein SAB2436c [Staphylococcus aureus RF122]
YP_252404	70725490	hypothetical protein SH0489 [Staphylococcus haemolyticus JCSC1435]
NP_693379	23099913	hypothetical protein OB2458 [Oceanobacillus thuyensis HTE831]
ZP_008...	82501285	conserved hypothetical protein [Caldicellulosiruptor saccharolyticus DSM 8903]
ZP_010...	87303565	hypothetical protein WH5701_09900 [Synechococcus sp. WH 5701]

Table 25. Examples of acyl CoA:diacylglycerol acyltransferase (DGAT) polypeptides.

ACCESSION	GI	PROTEIN DESCRIPTION
XP_957022	85082953	hypothetical protein [Neurospora crassa N150]
XP_386864	46124621	hypothetical protein FG06688.1 [Gibberella zeae PH-1]
XP_755172	71000982	diacylglycerol O-acyltransferase DGAT [Aspergillus fumigatus Af293]
XP_663763	67539978	hypothetical protein AN6159.2 [Aspergillus nidulans FGSC A4]
BAE65302	83775179	unnamed protein product [Aspergillus oryzae]
XP_502557	50550169	hypothetical protein [Yarrowia lipolytica]
AAS78662	56199782	diacylglycerol acyltransferase [Glycine max]
ABB84383	82582915	diacylglycerol acyltransferase [Jatropha curcas]
AAV31083	54145459	1,2-diacyl-sn-glycerol:acyl-CoA acyltransferase [Euonymus alatus]
AAG23696	10803053	diacylglycerol acyltransferase [Perilla frutescens]
AAF64065	7576941	putative diacylglycerol acyltransferase [Brassica napus]
AAS01606	41387497	acyl-CoA:diacylglycerol acyltransferase 1 [Olea europaea]
AAT73629	50299542	acyl CoA:diacylglycerol acyltransferase [Glycine max]
AAM03340	67043496	putative diacylglycerol acyltransferase [Tropaeolum majus]
XP_645633	66824557	hypothetical protein DDB0202877 [Dictyostelium discoideum]
AAF19345	6625653	diacylglycerol acylCoA acyltransferase [Nicotiana tabacum]
AAV40785	63376239	diacylglycerol acyltransferase DGAT2 [Brassica juncea]
AAW47581	57231736	diacylglycerol acyltransferase [Oryza sativa (japonica)]

		cultivar-group)]
AAR11479	38146080	diacylglycerol acyltransferase [<i>Ricinus communis</i>]
AAV40784	63376226	diacylglycerol acyltransferase DGAT1 [<i>Brassica juncea</i>]
AAP68322	31711932	At2g19450 [<i>Arabidopsis thaliana</i>]
AAW51456	57545061	diacylglycerol acyltransferase [<i>Lotus corniculatus</i> var. japonicus]
AAD45536	5579408	putative diacylglycerol acyltransferase [<i>Brassica napus</i>]
BAD53762	53791817	putative acyl-CoA:diacylglycerol acyltransferase [<i>Oryza sativa</i> (japonica cultivar-group)]
NP_956024	41054343	hypothetical protein LOC325875 [<i>Danio rerio</i>]
AAL49962	18642598	diacylglycerol acyltransferase 1 [<i>Bos taurus</i>]
XP_930884	89028385	similar to Diacylglycerol O-acyltransferase 1 (Diglyceride acyltransferase) (ACAT-related gene) [<i>Homo sapiens</i>]
NP_777118	27819636	diacylglycerol O-acyltransferase 1 [<i>Bos taurus</i>]
Q9GMF1	18202926	Diacylglycerol O-acyltransferase 1 (Diglyceride acyltransferase)
NP_036211	6912332	diacylglycerol O-acyltransferase 1 [<i>Homo sapiens</i>]
AAH06263	34782946	DGAT1 protein [<i>Homo sapiens</i>]
XP_780515	72006039	similar to Diacylglycerol O-acyltransferase 1 [<i>Strongylocentrotus purpuratus</i>]
AAD40881	5225382	putative diacylglycerol acyltransferase [<i>Brassica napus</i>]
XP_539214	73974769	similar to Diacylglycerol O-acyltransferase 1 (ACAT related gene product 1) isoform 1 [<i>Canis familiaris</i>]
AAZ22403	71063860	diacylglycerol O-acyltransferase 1 [<i>Bubalus bubalis</i>]
NP_999216	47522918	diacylglycerol acyltransferase [<i>Sus scrofa</i>]
NP_001...	50539976	hypothetical protein LOC436731 [<i>Danio rerio</i>]
XP_849176	73974767	similar to Diacylglycerol O-acyltransferase 1 (ACAT related gene product 1) isoform 2 [<i>Canis familiaris</i>]
NP_505828	71997360	H19N07.4 [<i>Caenorhabditis elegans</i>]
AAF82410	9049538	diacylglycerol acyltransferase [<i>Caenorhabditis elegans</i>]
CAE75170	39591950	Hypothetical protein CBG23107 [<i>Caenorhabditis briggsae</i>]
XP_626337	66358318	diacylglycerol acyltransferase 1 [<i>Cryptosporidium parvum</i> Iowa II]
XP_668402	67624239	acyl-CoA:diacylglycerol acyltransferase 1-related enzyme [<i>Cryptosporidium hominis</i> TU502]
AAP94208	33113253	acyl-CoA:diacylglycerol acyltransferase 1-related enzyme [<i>Toxoplasma gondii</i>]
AAP94209	33113255	acyl-CoA:diacylglycerol acyltransferase 1-related enzyme [<i>Toxoplasma gondii</i>]
XP_579557	62652535	PREDICTED: diacylglycerol O-acyltransferase 1 [<i>Rattus norvegicus</i>]
BAC66171	29170489	diacylglycerol acyltransferase [<i>Mus musculus</i>]
Q9ERM3	18202872	Diacylglycerol O-acyltransferase 1 (Diglyceride acyltransferase)
AAL78366	18698659	acyl coenzyme A:diacylglycerol acyltransferase [<i>Drosophila melanogaster</i>]
NP_995724	45552403	CG31991-PD, isoform D [<i>Drosophila melanogaster</i>]
NP_724017	24584734	CG31991-PC, isoform C [<i>Drosophila melanogaster</i>]
XP_858062	73974765	similar to Diacylglycerol O-acyltransferase 1 (ACAT related gene product 1) isoform 3 [<i>Canis familiaris</i>]

XP_728984	82915156	hypothetical protein PY01256 [<i>Plasmodium yoelii</i> yoelii str. 17XNL]
CAG11944	47225461	unnamed protein product [<i>Tetraodon nigroviridis</i>]
BAD27526	50199438	acyl-CoA:diacylglycerol acyltransferase [eukaryotic synthetic construct]
XP_317656	31226099	ENSANGP00000002281 [<i>Anopheles gambiae</i> str. PEST]
AAV59457	55733950	putative diacylglycerol acyltransferase [<i>Oryza sativa</i> (japonica cultivar-group)]
EAL33593	54644853	GA16599-PA [<i>Drosophila pseudoobscura</i>]
XP_678753	68073677	diacylglycerol O-acyltransferase [<i>Plasmodium berghei</i> strain ANKA]
XP_520014	55631434	PREDICTED: similar to Diacylglycerol O-acyltransferase 1 (Diglyceride acyltransferase) [<i>Pan troglodytes</i>]
CAG10815	47219451	unnamed protein product [<i>Tetraodon nigroviridis</i>]
XP_624754	66522700	PREDICTED: similar to ENSANGP00000002281 [<i>Apis mellifera</i>]
CAC69884	15620769	diacylglycerol acyltransferase I [<i>Rattus norvegicus</i>]
XP_686181	68363630	PREDICTED: similar to Diacylglycerol O-acyltransferase 1 (Diglyceride acyltransferase) [<i>Danio rerio</i>]
XP_734008	70921323	diacylglycerol O-acyltransferase [<i>Plasmodium chabaudi</i> chabaudi]
XP_673128	68062248	hypothetical protein PB300300.00.0 [<i>Plasmodium berghei</i> strain ANKA]
AAS72376	45642963	acyl-CoA:cholesterol acyltransferase beta [<i>Toxoplasma gondii</i>]
AAS72375	45642961	acyl-CoA:cholesterol acyltransferase alpha [<i>Toxoplasma gondii</i>]
NP_586145	19074639	STEROL O-ACYLTRANSFERASE [<i>Encephalitozoon cuniculi</i> GB-M1]
XP_640280	66812202	hypothetical protein DDB0205259 [<i>Dictyostelium discoideum</i>]
AAV40783	63376221	diacylglycerol acyltransferase [<i>Brassica juncea</i>]
XP_765774	71032265	diacylglycerol O-acyltransferase [<i>Theileria parva</i> strain Muguga]
Q876L2	34582301	Sterol O-acyltransferase 2 (Sterol-ester synthase 2)
XP_571260	58268208	sterol O-acyltransferase [<i>Cryptococcus neoformans</i> var. <i>neoformans</i> JEC21]
EAL20032	50257323	hypothetical protein CNBF3580 [<i>Cryptococcus neoformans</i> var. <i>neoformans</i> B-3501A]
XP_954478	84999514	acyl transferase [<i>Theileria annulata</i> strain Ankara]
XP_505086	50555355	hypothetical protein [<i>Yarrowia lipolytica</i>]
NP_588558	19076058	hypothetical protein SPCPIE11.05c [<i>Schizosaccharomyces pombe</i> 972h-]
AAC49441	1389739	acyl-CoA:sterol acyltransferase
NP_014416	6324346	Acyl-CoA:sterol acyltransferase, isozyme of Are1p; Are2p [<i>Saccharomyces cerevisiae</i>]
XP_750354	70991010	sterol o-acyltransferase APE2 [<i>Aspergillus fumigatus</i> Af293]
XP_382192	46110268	hypothetical protein FG02016.1 [<i>Gibberella zeae</i> PH-1]
BAE54934	83764790	unnamed protein product [<i>Aspergillus oryzae</i>]

XP_885914	76617939	similar to Sterol O-acyltransferase 2 (Cholesterol acyltransferase 2) (ACAT-2) isoform 2 [Bos taurus]
XP_591251	76617937	similar to Sterol O-acyltransferase 2 (Cholesterol acyltransferase 2) (ACAT-2) isoform 1 [Bos taurus]
BAC00846	21392392	AcylCoA:Cholesterol Acyltransferase 2 [Rattus norvegicus]
NP_649816	28571583	CG8112-PA [Drosophila melanogaster]
NP_666176	22122547	sterol O-acyltransferase 2 [Mus musculus]
O88908	18202245	Sterol O-acyltransferase 2 (Cholesterol acyltransferase 2) (ACAT-2)
XP_761502	71022545	hypothetical protein UM05355.1 [Ustilago maydis 521]
NP_714950	40254723	sterol O-acyltransferase 2 [Rattus norvegicus]
EAQ86094	88178626	hypothetical protein CHGG_07347 [Chaetomium globosum CBS 148.51]
XP_461395	50425599	hypothetical protein DEHA0F25652g [Debaryomyces hansenii CBS767]
XP_661812	67527926	hypothetical protein AN4208.2 [Aspergillus nidulans FGSC A4]
AAH96091	64654094	Sterol O-acyltransferase 2 [Homo sapiens]
O75908	18202149	Sterol O-acyltransferase 2 (Cholesterol acyltransferase 2) (ACAT-2)
AAH96090	64652990	Sterol O-acyltransferase 2 [Homo sapiens]
AAK48829	13898623	acyl coenzyme A: cholesterol acyltransferase-2 [Homo sapiens]
XP_543637	73996435	PREDICTED: similar to sterol O-acyltransferase 2 [Canis familiaris]
O77759	18202176	Sterol O-acyltransferase 2 (Cholesterol acyltransferase 2) (ACAT-2)
AAO32474	28564191	ARE2 [Saccharomyces castellii]
XP_323485	32405744	hypothetical protein [Neurospora crassa]
NP_982606	45184888	AAR065Cp [Eremothecium gossypii]
NP_593708	19114620	hypothetical protein SPAC13G7.06 [Schizosaccharomyces pombe 972h-]
AAO32554	28564940	ARE2 [Saccharomyces kluyveri]
EAL28962	54639560	GA20833-PA [Drosophila pseudoobscura]
XP_449806	50294790	hypothetical protein CAGL0M10571g [Candida glabrata CBS138]
NP_033256	84619697	sterol O-acyltransferase 1 [Mus musculus]
Q61263	18202591	Sterol O-acyltransferase 1 (Cholesterol acyltransferase 1) (ACAT-1)
BAC34925	26342537	unnamed protein product [Mus musculus]
XP_452607	50305295	unnamed protein product [Kluyveromyces lactis]
NP_001...	77735363	hypothetical protein LOC504287 [Bos taurus]
Q60457	18202585	Sterol O-acyltransferase 1 (Cholesterol acyltransferase 1) (ACAT-1)
XP_320321	58393811	ENSANGP00000016512 [Anopheles gambiae str. PEST]
XP_320320	58393809	ENSANGP00000016486 [Anopheles gambiae str. PEST]
O70536	18202126	Sterol O-acyltransferase 1 (Cholesterol acyltransferase 1) (ACAT-1)
XP_714776	68482533	acyl-CoA cholesterol acyltransferase [Candida albicans SC5314]

P84285	56404462	Sterol O-acyltransferase 2 (Sterol-ester synthase) (ASAT)
AAH77916	50416229	Soat1-prov protein [Xenopus laevis]
XP_692855	68364838	PREDICTED: similar to Soat1-prov protein [Danio rerio]
CAI13574	55960156	sterol O-acyltransferase (acyl-Coenzyme A: cholesterol acyltransferase) 1 [Homo sapiens]
AAI56227	18028942	cholesterol acyltransferase 1 [Gorilla gorilla]
AAI56228	18028944	cholesterol acyltransferase 1 [Pongo pygmaeus]
AAC37532	4878022	acyl-coenzyme A: cholesterol acyltransferase [Homo sapiens]
2201440A	1585676	acyl-CoA:cholesterol acyltransferase
Q876L3	34582302	Sterol O-acyltransferase 1 (Sterol-ester synthase 1)
BAE01048	67969393	unnamed protein product [Macaca fascicularis]
XP_514030	55588858	PREDICTED: hypothetical protein XP_514030 [Pan troglodytes]
XP_547445	73961286	similar to Sterol O-acyltransferase 1 (Cholesterol acyltransferase 1) (ACAT-1) [Canis familiaris]
EAQ84619	88177151	hypothetical protein CHGG_08633 [Chaetomium globosum CBS 148.51]
O77761	18202178	Sterol O-acyltransferase 1 (Cholesterol acyltransferase 1) (ACAT-1)
XP_422267	50751122	PREDICTED: similar to Sterol O-acyltransferase 1 (Cholesterol acyltransferase 1) (ACAT-1) [Gallus gallus]
XP_693284	68392980	PREDICTED: similar to Sterol O-acyltransferase 1 (Cholesterol acyltransferase 1) (ACAT-1) [Danio rerio]
AAT92940	51013293	YCR048W [Saccharomyces cerevisiae]
XP_956576	85080625	hypothetical protein [Neurospora crassa N150]
XP_624691	66564061	PREDICTED: similar to ENSANGP00000016486 [Apis mellifera]
CAF96514	47222847	unnamed protein product [Tetraodon nigroviridis]
XP_788209	72085563	PREDICTED: similar to sterol O-acyltransferase 1 [Strongylocentrotus purpuratus]
XP_445307	50285757	unnamed protein product [Candida glabrata]
CAE70002	39596364	Hypothetical protein CBG16409 [Caenorhabditis briggsae]
CAG07990	47225647	unnamed protein product [Tetraodon nigroviridis]
NP_510623	17549960	B0395.2 [Caenorhabditis elegans]
AAI28331	76157393	SJCHGC04421 protein [Schistosoma japonicum]
CAI96158	66347204	Diacylglycerol O-acyltransferase [Bubalus bubalis]
XP_390039	46136695	hypothetical protein FG09863.1 [Gibberella zeae PH-1]
XP_643169	66819019	hypothetical protein DDB0203882 [Dictyostelium discoideum]
AAO53095	28850306	hypothetical protein [Dictyostelium discoideum]
AAB06959	1515472	acyl-CoA:cholesterol acyltransferase [Oryctolagus cuniculus]
NP_945619	39933343	putative alginate o-acetyltransferase AlgI [Rhodopseudomonas palustris CGA009]
ZP_008...	77691302	Membrane bound O-acyl transferase, MBOAT [Rhodopseudomonas palustris BisB5]
XP_465546	50908115	putative wax synthase [Oryza sativa (japonica cultivar-group)]

Table 29: Examples of Prenyldiphosphate synthase polypeptides

29A: Bacteria Proteins that require a mitochondrial targeting sequence		
Accession	GI	Description
ZP_009...	83373595	Trans-hexaprenyltranstransferase [Rhodobacter sphaeroides ATCC 17029]
ZP_009...	83371280	Trans-hexaprenyltranstransferase [Rhodobacter sphaeroides ATCC 17025]
CAD24417	20429105	decaprenyl diphosphate synthase [Paracoccus zeaxanthinifaciens]
ZP_010...	85705714	Geranylgeranyl pyrophosphate synthase/Polyprenyl synthetase [Roseovarius sp. 217]
ZP_010...	84515724	decaprenyl diphosphate synthase [Loktanella vestfoldensis SKA53]
YP_165582	56695234	decaprenyl diphosphate synthase [Silicibacter pomeroyi DSS-3]
ZP_010...	86139019	decaprenyl diphosphate synthase [Roseobacter sp. MED193]
ZP_009...	83941379	decaprenyl diphosphate synthase [Sulfitobacter sp. EE-36]
ZP_009...	83854856	decaprenyl diphosphate synthase [Sulfitobacter sp. NAS-14. 1]
ZP_006...	69299873	Farnesyltranstransferase [Silicibacter sp. TM1040]
ZP_010...	84683979	Geranylgeranyl pyrophosphate synthase/Polyprenyl synthetase [Rhodobacter rubrum HTCC2654]
ZP_009...	84500217	decaprenyl diphosphate synthase [Oceanicola batsensis HTCC2597]
ZP_009...	83952381	decaprenyl diphosphate synthase [Roseovarius nubinhibens ISM]
ZP_006...	69937106	Trans-hexaprenyltranstransferase [Paracoccus denitrificans PD1222]
ZP_005...	68180845	Trans-hexaprenyltranstransferase [Jannaschia sp. CCS1]
ZP_008...	78495595	Polyprenyl synthetase [Rhodopseudomonas palustris BisB18]
AA_Y82368	67866738	decaprenyl diphosphate synthase [Agrobacterium tumefaciens]
NP_353656	15887975	hypothetical protein AGR_C_1125 [Agrobacterium tumefaciens str. C58]
ZP_008...	77688465	Farnesyltranstransferase [Rhodopseudomonas palustris BisB5]
NP_531334	17934544	octaprenyl-diphosphate synthase [Agrobacterium tumefaciens str. C58]
YP_484709	86748213	Farnesyltranstransferase [Rhodopseudomonas palustris HaA2]
AAP56240	37903500	decaprenyl diphosphate synthase [Agrobacterium tumefaciens]
YP_192388	58040424	Decaprenyl diphosphate synthase [Gluconobacter oxydans 621H]
29B: Subunit 1- Proteins that contain mitochondrial targeting sequence		
Accession	GI	Description

T43193	11279237	trans-pentaprenyltransferase homolog - fission yeast (Schizosaccharomyces pombe)
AAD28559	4732024	trans-prenyltransferase [Homo sapiens]
AAI07275	78070698	Trans-prenyltransferase [Mus musculus]
BAE48216	81157931	subunit 1 of decaprenyl diphosphate synthase [Homo sapiens]
AAH49211	29165656	PDSS1 protein [Homo sapiens]
Q33DR2	85700953	Decaprenyl-diphosphate synthase subunit 1 (Solanesyl-diphosphate synthase subunit 1) (Trans-prenyltransferase)
XP_507706	55633583	PREDICTED: similar to TPRT protein [Pan troglodytes]
XP_586717	76632198	PREDICTED: similar to trans-prenyltransferase [Bos taurus]
XP_849908	73948851	PREDICTED: similar to trans-prenyltransferase [Canis familiaris]
29C: Subunit 2- Proteins that contain mitochondrial targeting sequence		
Accession	GI	Description
Q13851	60389474	Decaprenyl-diphosphate synthase subunit 2 (Decaprenyl pyrophosphate synthetase subunit 2)
BAE48218	81157935	subunit 2 of solanesyl diphosphate synthase [Mus musculus]
BAE48217	81157933	subunit 2 of decaprenyl diphosphate synthase [Homo sapiens]

Table 30: Examples of PHB-Polyprenyltransferase polypeptides

GI	PROTEIN DESCRIPTION
51013645	YNR041C [Saccharomyces cerevisiae]
50285815	unnamed protein product [Candida glabrata]
50311051	unnamed protein product [Kluyveromyces lactis]
45200866	AGL231 Wp [Eremothecium gossypii]
50555263	hypothetical protein [Yarrowia lipolytica]
68473193	<i>para</i> -hydroxybenzoate: polyprenyl transferase [Candida albicans SC5314]
50410039	hypothetical protein DEHA0A14212g [Debaryomyces hansenii CBS767]
83769349	unnamed protein product [Aspergillus oryzae]
70994900	<i>para</i> -hydroxybenzoate-polyprenyltransferase precursor [Aspergillus fumigatus Af293]
19114131	hypothetical protein SPAC56F8. 04c [Schizosaccharomyces pombe 972h-]
39973573	hypothetical protein MG01067. 4 [Magnaporthe grisea 70-15]
85078920	protein related to <i>para</i> -hydroxybenzoate polyprenyltransferase precursor [Neurospora crassa N150]
76660839	PREDICTED: similar to <i>para</i> -hydroxybenzoate-polyprenyltransferase, mitochondrial [Bos taurus]
52138578	<i>para</i> -hydroxybenzoate-polyprenyltransferase, mitochondrial [Homo sapiens]
18088424	COQ2 protein [Homo sapiens]
47221448	unnamed protein product [Tetraodon nigroviridis]

58385249	ENSANGP00000012220 [Anopheles gambiae str. PEST]
50746583	PREDICTED: similar to hypothetical protein CL640 [Gallus gallus]
54638587	GA21912-PA [Drosophila pseudoobscura]
21355567	CG9613-PA [Drosophila melanogaster]
71005862	hypothetical protein UM01450. 1 [Ustilago maydis 521]

[00169] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the following claims:

What is claimed is:

1. A genetically engineered *Yarrowia* fungus characterized by:
 - a) the fungus is oleaginous in that it can accumulate lipid to at least 20% of its dry cell weight; and
 - b) the fungus produces at least one carotenoid, and can accumulate the produced carotenoid to at least 1% of its dry cell weight;wherein the fungus comprises at least one modification selected from the group consisting of carotenogenic modifications, oleaginic modifications, and combinations thereof, wherein the term carotenogenic modification comprises a genetic modification resulting in increased production of one or more carotenoids, wherein the term oleaginic modifications comprises a genetic modification resulting in increased production and/or activity of one or more oleaginic polypeptides and wherein the at least one modification alters oleaginic of the fungus, confers to the fungus oleaginity, confers to the fungus the ability to produce the at least one carotenoid to a level at least 1% of its dry cell weight, or confers to the fungus the ability to produce at least one carotenoid which the fungus does not naturally produce.
2. The fungus of claim 1, wherein the at least one oleaginic polypeptide is:
 - (i) selected from the group consisting of acetyl-CoA carboxylase polypeptide, pyruvate decarboxylase polypeptide, isocitrate dehydrogenase polypeptide, ATP-citrate lyase polypeptide, malic enzyme polypeptide, AMP deaminase polypeptide, and combinations thereof; or
 - (ii) at least one polypeptide selected from the group consisting of a polypeptide in any one of Tables 1 through 6.
3. The fungus of claim 1 or 2, wherein the at least one oleaginic modification comprises expression of at least one heterologous oleaginic polypeptide in the fungus.

4. The fungus of any of claim 1 to 3, wherein the at least one oleaginic modification comprises expression of at least one heterologous gene encoding the at least one heterologous oleaginic polypeptide.
5. The fungus of claim 3 or 4, wherein the at least one heterologous oleaginic polypeptide comprises an animal polypeptide, a mammalian polypeptide, an insect polypeptide, a plant polypeptide, a fungal polypeptide, a yeast polypeptide, an algal polypeptide, a bacterial polypeptide, a cyanobacterial polypeptide, an archaeobacterial polypeptide, or a protozoal polypeptide.
6. The fungus of any one of claims 1 to 5 wherein the at least one carotenogenic modification increases or decreases expression of at least one carotenogenic polypeptide wherein the at least one carotenogenic polypeptide is selected from the group consisting of: isoprenoid biosynthesis polypeptides, carotenoid biosynthesis polypeptides, isoprenoid biosynthesis competitor polypeptides, and combinations thereof, and wherein
- (i) the isoprenoid biosynthesis polypeptides are selected from the group consisting of: acetoacetyl-CoA thiolase polypeptide, HMG-CoA synthase polypeptide, HMG-CoA reductase polypeptide, mevalonate kinase polypeptide, phosphomevalonate kinase polypeptide, mevalonate pyrophosphate decarboxylase polypeptide, IPP isomerase polypeptide, FPP synthase polypeptide, and GGPP synthase polypeptide; and/or
 - (ii) the carotenoid biosynthesis polypeptides are selected from the group consisting of: phytoene synthase polypeptide, phytoene dehydrogenase polypeptide, lycopene cyclase polypeptide, carotenoid ketolase polypeptide, carotenoid hydroxylase polypeptide, astaxanthin synthase polypeptide, carotenoid epsilon hydroxylase polypeptide, carotenoid glucosyltransferase polypeptide, lycopene cyclase (beta and epsilon subunits) polypeptides, and acyl CoA:diacylglycerol acyltransferase polypeptide; and/or
 - (iii) the isoprenoid biosynthesis competitor polypeptides are selected from the group consisting of squalene synthase polypeptide, prenyldiphosphate synthase and PHB polyprenyltransferase; and/or

(iv) the carotenogenic polypeptide is selected from the group consisting of any of the polypeptides of any one of Tables 7-25, Table 29, and Table 30, and combinations thereof.

7. The fungus of claim 6, wherein the at least one heterologous carotenogenic polypeptide comprises an animal polypeptide, a mammalian polypeptide, an insect polypeptide, a plant polypeptide, a fungal polypeptide, a yeast polypeptide, an algal polypeptide, a bacterial polypeptide, cyanobacterial polypeptide, an archaeobacterial polypeptide, or a protozoal polypeptide.

8. The fungus of claim 7, wherein the at least one heterologous carotenogenic polypeptide comprises at least two heterologous carotenogenic polypeptides.

9. The fungus of claim 8, wherein the at least two heterologous carotenogenic polypeptides are from a single source organism, or from at least two different source organisms.

10. A fungus according to any of the preceding claims wherein the fungus accumulates the produced at least one carotenoid to a level selected from the group consisting of: above 1%, above 2%, above 3%, above 5%, and above 10% of the fungus' dry cell weight.

11. A fungus according to any of the preceding claims characterized in that the fungus accumulates lipid in the form of cytoplasmic bodies.

12. The fungus of claim 11 wherein the at least one carotenoid accumulates in the cytoplasmic oil bodies.

13. A fungus according to any of the preceding claims, wherein the strain comprises a carotenogenic modification selected from the group consisting of:

a) expression of a polypeptide selected from a group consisting of a truncated endogenous HMG CoA reductase polypeptide that lacks the N-terminal membrane spanning domain, acetoacetyl-CoA thiolase, HMG-CoA synthase, FPP synthase, and GGPP synthase;

b) expression of a heterologous polypeptide selected from the group consisting of phytoene synthase, phytoene desaturase, lycopene cyclase, carotenoid ketolase, carotenoid hydroxylase, and combinations thereof;

c) decreased expression or activity of an endogenous polypeptide selected from the group consisting of squalene synthase polypeptide, prenyldiphosphate synthase polypeptide and PHB polyprenyltransferase polypeptide; and combinations thereof.

14. A fungus according to any one of the preceding claims wherein the strain comprises a carotenogenic modification selected from the group consisting of:

a) expression of a polypeptide selected from a group consisting of a truncated endogenous HMG CoA reductase polypeptide that lacks the N-terminal membrane spanning domain, acetoacetyl-CoA thiolase, HMG-CoA synthase, FPP synthase, and GGPP synthase;

b) expression of a heterologous polypeptide selected from the group consisting of phytoene synthase, phytoene desaturase, lycopene cyclase, carotenoid ketolase, carotenoid hydroxylase, astaxanthin synthase, carotenoid epsilon hydroxylase, lycopene cyclase (beta and epsilon subunits), carotenoid glucosyltransferase, acyl CoA:diacylglycerol acyltransferase, and combinations thereof;

c) decreased expression or activity of an endogenous squalene synthase polypeptide; and combinations thereof.

15. A method of producing a carotenoid, the method comprising steps of:

a) cultivating the fungus of any one of the preceding claims under conditions that allow production of the carotenoid;

b) and isolating the produced carotenoid.

16. The method of claim 15, wherein the step of isolating comprises fractionating the cultivation medium to obtain at least one carotenoid-enriched fraction.
17. The method of claim 15 or 16, wherein the step of cultivating comprises cultivating the fungus under conditions that allow accumulation of the carotenoid in cytoplasmic oil bodies; and the step of isolating comprises isolating oil derived from the cytoplasmic oil bodies.
18. The method of any one of claims 15 to 17, wherein the carotenoid is selected from the group consisting of astaxanthin, β -carotene, canthaxanthin, zeaxanthin, lutein, lycopene, and combinations thereof.
19. The method of any one of claims 15 to 18, wherein the carotenoid comprises astaxanthin.
20. A method of preparing a food or feed additive containing a carotenoid, the method comprising steps of:
- a) cultivating the fungus of any one of claims 1 to 14 under conditions that allow production of the carotenoid;
 - b) isolating the carotenoid; and
 - c) combining the isolated carotenoid with one or more other food or feed additive components.