Office de la Propriété Intellectuelle du Canada
Un organisme d'Industrie Canada

Canadian Intellectual Property Office
An agency of Industry Canada

CA 2722442 A1 2009/11/19

(21) 2 722 442

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2009/05/18
(87) Date publication PCT/PCT Publication Date: 2009/11/19
(85) Entrée phase nationale/National Entry: 2010/10/25
(86) N° demande PCT/PCT Application No.: US 2009/044409
(87) N° publication PCT/PCT Publication No.: 2009/140696
(30) Priorité/Priority: 2008/05/16 (US61/053,955)

(51) Cl.Int./Int.Cl. *C12N 9/02* (2006.01), *C10L 1/02* (2006.01), *C12N 15/53* (2006.01), *C12N 9/88* (2006.01), *C12P 7/04* (2006.01), *C12P 7/24* (2006.01), *C12P 7/64* (2006.01)

(71) Demandeur/Applicant:
LS9, INC., US

(72) Inventeurs/Inventors:
SCHIRMER, ANDREAS, US;
RUDE, MATHEW, US;
BRUBAKER, SHANE, US

(74) Agent: TORYS LLP

(54) Titre : PROCEDES ET COMPOSITIONS POUR PRODUIRE DES HYDROCARBURES
(54) Title: METHODS AND COMPOSITIONS FOR PRODUCING HYDROCARBONS

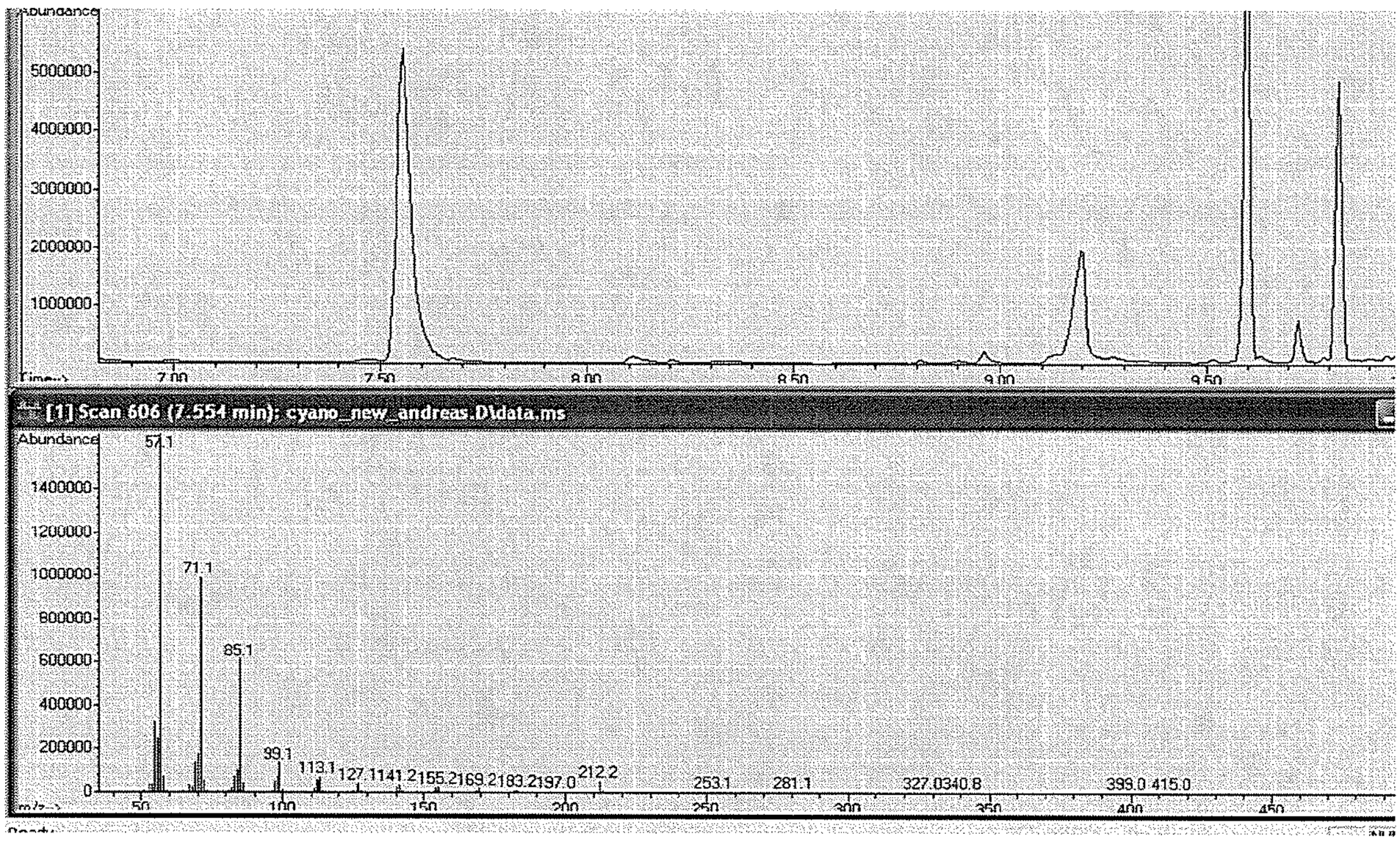

FIG. 1

(57) Abrégé/Abstract:
Compositions and methods for producing hydrocarbons such as aldehydes, alkanes, and alkenes are described herein. Certain hydrocarbons can be used in biofuels.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
19 November 2009 (19.11.2009)

PCT

(10) International Publication Number
WO 2009/140696 A3

(51) International Patent Classification:
C12N 9/02 (2006.01) *C12P 7/64* (2006.01)
C12N 9/88 (2006.01) *C10L 1/02* (2006.01)

(21) International Application Number:
PCT/US2009/044409

(22) International Filing Date:
18 May 2009 (18.05.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/053,955 16 May 2008 (16.05.2008) US

(71) Applicant *(for all designated States except US)*: **LS9, INC.** [US/US]; 100 Kimball Way, South San Francisco, CA 94080 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SCHIRMER, Andreas** [DE/US]; 100 Kimball Way, South San Francisco, CA 94080 (US). **RUDE, Mathew** [US/US]; 100 Kimball Way, South San Francisco, CA 94080 (US). **BRUBAKER, Shane** [US/US]; 100 Kimball Way, South San Francisco, CA 94080 (US).

(74) Agents: **SUPERKO, Colleen** et al.; Wilmer Cutler Pickering Hale and Dorr LLP, 60 State Street, Boston, MA 02109 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
25 March 2010

(54) Title: METHODS AND COMPOSITIONS FOR PRODUCING HYDROCARBONS

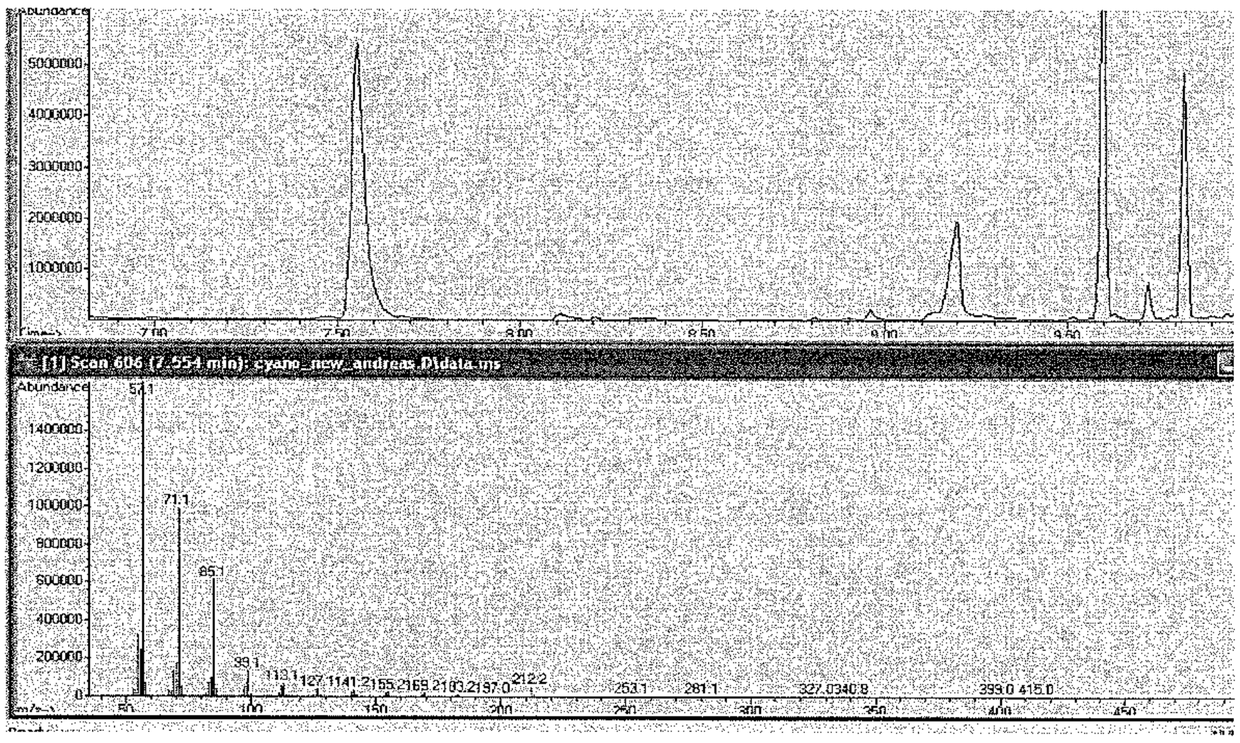

FIG. 1

(57) Abstract: Compositions and methods for producing hydrocarbons such as aldehydes, alkanes, and alkenes are described herein. Certain hydrocarbons can be used in biofuels.

WO 2009/140696 A3

METHODS AND COMPOSITIONS FOR PRODUCING HYDROCARBONS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/053,955, filed May 16, 2008, the contents of which are hereby incorporated in their entirety herein.

BACKGROUND OF THE INVENTION

[0002] Petroleum is a limited, natural resource found in the Earth in liquid, gaseous, or solid forms. Petroleum is primarily composed of hydrocarbons, which are comprised mainly of carbon and hydrogen. It also contains significant amounts of other elements, such as, nitrogen, oxygen, or sulfur, in different forms.

[0003] Petroleum is a valuable resource, but petroleum products are developed at considerable costs, both financial and environmental. First, sources of petroleum must be discovered. Petroleum exploration is an expensive and risky venture. The cost of exploring deep water wells can exceed $100 million. Moreover, there is no guarantee that these wells will contain petroleum. It is estimated that only 40% of drilled wells lead to productive wells generating commercial hydrocarbons. In addition to the economic cost, petroleum exploration carries a high environmental cost. For example, offshore exploration disturbs the surrounding marine environments.

[0004] After a productive well is discovered, the petroleum must be extracted from the Earth at great expense. During primary recovery, the natural pressure underground is sufficient to extract about 20% of the petroleum in the well. As this natural pressure falls, secondary recovery methods are employed, if economical. Generally, secondary recovery involves increasing the well's pressure by, for example, water injection, natural gas injection, or gas lift. Using secondary recovery methods, an additional 5% to 15% of petroleum is recovered. Once secondary recovery methods are exhausted, tertiary recovery methods can be used, if economical. Tertiary methods involve reducing the viscosity of the petroleum to make it easier to extract. Using tertiary recovery methods, an additional 5% to 15% of petroleum is recovered. Hence, even under the best circumstances, only 50% of

the petroleum in a well can be extracted. Petroleum extraction also carries an environmental cost. For example, petroleum extraction can result in large seepages of petroleum rising to the surface. Moreover, offshore drilling involves dredging the seabed which disrupts or destroys the surrounding marine environment.

[0005] Since petroleum deposits are not found uniformly throughout the Earth, petroleum must be transported over great distances from petroleum producing regions to petroleum consuming regions. In addition to the shipping costs, there is also the environmental risk of devastating oil spills.

[0006] In its natural form, crude petroleum extracted from the Earth has few commercial uses. It is a mixture of hydrocarbons (*e.g.*, paraffins (or alkanes), olefins (or alkenes), alkynes, napthenes (or cylcoalkanes), aliphatic compounds, aromatic compounds, *etc.*) of varying length and complexity. In addition, crude petroleum contains other organic compounds (*e.g.*, organic compounds containing nitrogen, oxygen, sulfur, *etc.*) and impurities (*e.g.*, sulfur, salt, acid, metals, *etc.*).

[0007] Hence, crude petroleum must be refined and purified before it can be used commercially. Due to its high energy density and its easy transportability, most petroleum is refined into fuels, such as transportation fuels (*e.g.*, gasoline, diesel, aviation fuel, *etc.*), heating oil, liquefied petroleum gas, *etc*.

[0008] Crude petroleum is also a primary source of raw materials for producing petrochemicals. The two main classes of raw materials derived from petroleum are short chain olefins *(e.g.*, ethylene and propylene) and aromatics (*e.g.*, benzene and xylene isomers). These raw materials are derived from longer chain hydrocarbons in crude petroleum by cracking it at considerable expense using a variety of methods, such as catalytic cracking, steam cracking, or catalytic reforming. These raw materials are used to make petrochemicals, which cannot be directly refined from crude petroleum, such as monomers, solvents, detergents, or adhesives.

[0009] One example of a raw material derived from crude petroleum is ethylene. Ethylene is used to produce petrochemicals such as, polyethylene, ethanol, ethylene oxide, ethylene glycol, polyester, glycol ether, ethoxylate, vinyl acetate, 1,2-dichloroethane, trichloroethylene, tetrachloroethylene, vinyl chloride, and polyvinyl chloride. An additional example of a raw material is propylene, which is used to produce isopropyl alcohol, acrylonitrile, polypropylene, propylene oxide, propylene

glycol, glycol ethers, butylene, isobutylene, 1,3-butadiene, synthetic elastomers, polyolefins, alpha-olefins, fatty alcohols, acrylic acid, acrylic polymers, allyl chloride, epichlorohydrin, and epoxy resins.

[0010] These petrochemicals can then be used to make specialty chemicals, such as plastics, resins, fibers, elastomers, pharmaceuticals, lubricants, or gels. Particular specialty chemicals which can be produced from petrochemical raw materials are: fatty acids, hydrocarbons (*e.g.*, long chain, branched chain, saturated, unsaturated, *etc.*), fatty alcohols, esters, fatty aldehydes, ketones, lubricants, *etc*.

[0011] Specialty chemicals have many commercial uses. Fatty acids are used commercially as surfactants, for example, in detergents and soaps. They can also be used as additives in fuels, lubricating oils, paints, lacquers, candles, salad oil, shortening, cosmetics, and emulsifiers. In addition, fatty acids are used as accelerator activators in rubber products. Fatty acids can also be used as a feedstock to produce methyl esters, amides, amines, acid chlorides, anhydrides, ketene dimers, and peroxy acids and esters.

[0012] Hydrocarbons have many commercial uses. For example, shorter chain alkanes are used as fuels. Methane and ethane are the main constituents of natural gas. Longer chain alkanes (*e.g.*, from five to sixteen carbons) are used as transportation fuels (*e.g.*, gasoline, diesel, or aviation fuel). Alkanes having more than sixteen carbon atoms are important components of fuel oils and lubricating oils. Even longer alkanes, which are solid at room temperature, can be used, for example, as a paraffin wax. Alkanes that contain approximately thirty-five carbons are found in bitumen, which is used for road surfacing. In addition, longer chain alkanes can be cracked to produce commercially useful shorter chain hydrocarbons.

[0013] Like short chain alkanes, short chain alkenes are used in transportation fuels. Longer chain alkenes are used in plastics, lubricants, and synthetic lubricants. In addition, alkenes are used as a feedstock to produce alcohols, esters, plasticizers, surfactants, tertiary amines, enhanced oil recovery agents, fatty acids, thiols, alkenylsuccinic anhydrides, epoxides, chlorinated alkanes, chlorinated alkenes, waxes, fuel additives, and drag flow reducers.

[0014] Fatty alcohols have many commercial uses. The shorter chain fatty alcohols are used in the cosmetic and food industries as emulsifiers, emollients, and

thickeners. Due to their amphiphilic nature, fatty alcohols behave as nonionic surfactants, which are useful as detergents. In addition, fatty alcohols are used in waxes, gums, resins, pharmaceutical salves and lotions, lubricating oil additives, textile antistatic and finishing agents, plasticizers, cosmetics, industrial solvents, and solvents for fats.

[0015] Esters have many commercial uses. For example, biodiesel, an alternative fuel, is comprised of esters (*e.g.*, fatty acid methyl ester, fatty acid ethyl esters, *etc.*). Some low molecular weight esters are volatile with a pleasant odor which makes them useful as fragrances or flavoring agents. In addition, esters are used as solvents for lacquers, paints, and varnishes. Furthermore, some naturally occurring substances, such as waxes, fats, and oils are comprised of esters. Esters are also used as softening agents in resins and plastics, plasticizers, flame retardants, and additives in gasoline and oil. In addition, esters can be used in the manufacture of polymers, films, textiles, dyes, and pharmaceuticals.

[0016] Aldehydes are used to produce many specialty chemicals. For example, aldehydes are used to produce polymers, resins (*e.g.*, Bakelite), dyes, flavorings, plasticizers, perfumes, pharmaceuticals, and other chemicals. Some are used as solvents, preservatives, or disinfectants. Some natural and synthetic compounds, such as vitamins and hormones, are aldehydes. In addition, many sugars contain aldehyde groups.

[0017] Ketones are used commercially as solvents. For example, acetone is frequently used as a solvent, but it is also a raw material for making polymers. Ketones are also used in lacquers, paints, explosives, perfumes, and textile processing. In addition, ketones are used to produce alcohols, alkenes, alkanes, imines, and enamines.

[0018] In addition, crude petroleum is a source of lubricants. Lubricants derived petroleum are typically composed of olefins, particularly polyolefins and alpha-olefins. Lubricants can either be refined from crude petroleum or manufactured using raw materials refined from crude petroleum.

[0019] Obtaining these specialty chemicals from crude petroleum requires a significant financial investment as well as a great deal of energy. It is also an inefficient process because frequently the long chain hydrocarbons in crude

petroleum are cracked to produce smaller monomers. These monomer are then used as the raw material to manufacture the more complex specialty chemicals.

[0020] In addition to the problems with exploring, extracting, transporting, and refining petroleum, petroleum is a limited and dwindling resource. One estimate of world petroleum consumption is 30 billion barrels per year. By some estimates, it is predicted that at current production levels, the world's petroleum reserves could be depleted before the year 2050.

[0021] Finally, the burning of petroleum based fuels releases greenhouse gases (*e.g.*, carbon dioxide) and other forms of air pollution (*e.g.*, carbon monoxide, sulfur dioxide, *etc.*). As the world's demand for fuel increases, the emission of greenhouse gases and other forms of air pollution also increases. The accumulation of greenhouse gases in the atmosphere leads to an increase global warming. Hence, in addition to damaging the environment locally (*e.g.*, oil spills, dredging of marine environments, *etc.*), burning petroleum also damages the environment globally.

[0022] Due to the inherent challenges posed by petroleum, there is a need for a renewable petroleum source which does not need to be explored, extracted, transported over long distances, or substantially refined like petroleum. There is also a need for a renewable petroleum source that can be produced economically without creating the type of environmental damage produced by the petroleum industry and the burning of petroleum based fuels. For similar reasons, there is also a need for a renewable source of chemicals that are typically derived from petroleum.

SUMMARY OF THE INVENTION

[0023] The invention is based, at least in part, on the identification of cyanobacterial genes that encode hydrocarbon biosynthetic polypeptides. Accordingly, in one aspect, the invention features a method of producing an aldehyde, the method comprising producing in a host cell a polypeptide comprising the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, or a variant thereof, and isolating the aldehyde from the host cell.

[0024] In some embodiments, the polypeptide comprises an amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least

about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82.

[0025] In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 with one or more amino acid substitutions, additions, insertions, or deletions. In some embodiments, the polypeptide has reductase activity. In yet other embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, with one or more conservative amino acid substitutions. For example, the polypeptide comprises one or more of the following conservative amino acid substitutions: replacement of an aliphatic amino acid, such as alanine, valine, leucine, and isoleucine, with another aliphatic amino acid; replacement of a serine with a threonine; replacement of a threonine with a serine; replacement of an acidic residue, such as aspartic acid and glutamic acid, with another acidic residue; replacement of a residue bearing an amide group, such as asparagine and glutamine, with another residue bearing an amide group; exchange of a basic residue, such as lysine and arginine, with another basic residue; and replacement of an aromatic residue, such as phenylalanine and tyrosine, with another aromatic residue. In some embodiments, the polypeptide has about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more amino acid substitutions, additions, insertions, or deletions. In some embodiments, the polypeptide has reductase activity.

[0026] In other embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64. In certain embodiments, the polypeptide has reductase activity.

[0027] In another aspect, the invention features a method of producing an aldehyde, the method comprising expressing in a host cell a polynucleotide comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the nucleotide sequence is SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some

embodiments, the method further comprises isolating the aldehyde from the host cell.

[0028] In other embodiments, the nucleotide sequence hybridizes to a complement of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, or to a fragment thereof, for example, under low stringency, medium stringency, high stringency, or very high stringency conditions.

[0029] In other embodiments, the nucleotide sequence encodes a polypeptide comprising: (i) the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82; or (ii) the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 with one or more amino acid substitutions, additions, insertions, or deletions. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 with one or more conservative amino acid substitutions. In some embodiments, the polypeptide has reductase activity.

[0030] In other embodiments, the nucleotide sequence encodes a polypeptide having the same biological activity as a polypeptide comprising the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82. In some embodiments, the nucleotide sequence is SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81 or a fragment thereof. In other embodiments, the nucleotide sequence hybridizes to a complement of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81 or to a fragment thereof, for example, under low stringency, medium stringency, high stringency, or very high stringency conditions. In some embodiments, the biological activity is reductase activity.

[0031] In some embodiments, the method comprises transforming a host cell with a recombinant vector comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the recombinant vector further comprises a promoter operably linked to the nucleotide sequence. In some embodiments, the promoter is a developmentally-regulated, an organelle-specific, a tissue-specific, an inducible, a

constitutive, or a cell-specific promoter. In particular embodiments, the recombinant vector comprises at least one sequence selected from the group consisting of (a) a regulatory sequence operatively coupled to the nucleotide sequence; (b) a selection marker operatively coupled to the nucleotide sequence; (c) a marker sequence operatively coupled to the nucleotide sequence; (d) a purification moiety operatively coupled to the nucleotide sequence; (e) a secretion sequence operatively coupled to the nucleotide sequence; and (f) a targeting sequence operatively coupled to the nucleotide sequence. In certain embodiments, the nucleotide sequence is stably incorporated into the genomic DNA of the host cell, and the expression of the nucleotide sequence is under the control of a regulated promoter region.

[0032] In any of the aspects described herein, the host cell can be selected from the group consisting of a mammalian cell, plant cell, insect cell, yeast cell, fungus cell, filamentous fungi cell, and bacterial cell.

[0033] In some embodiments, the host cell is a Gram-positive bacterial cell. In other embodiments, the host cell is a Gram-negative bacterial cell.

[0034] In some embodiments, the host cell is selected from the genus *Escherichia*, *Bacillus*, *Lactobacillus*, *Rhodococcus*, *Pseudomonas*, *Aspergillus*, *Trichoderma*, *Neurospora*, *Fusarium*, *Humicola*, *Rhizomucor*, *Kluyveromyces*, *Pichia*, *Mucor*, *Myceliophtora*, *Penicillium*, *Phanerochaete*, *Pleurotus*, *Trametes*, *Chrysosporium*, *Saccharomyces*, *Stenotrophamonas*, *Schizosaccharomyces*, *Yarrowia*, or *Streptomyces*.

[0035] In particular embodiments, the host cell is a *Bacillus lentus* cell, a *Bacillus brevis* cell, a *Bacillus stearothermophilus* cell, a *Bacillus licheniformis* cell, a *Bacillus alkalophilus* cell, a *Bacillus coagulans* cell, a *Bacillus circulans* cell, a *Bacillus pumilis* cell, a *Bacillus thuringiensis* cell, a *Bacillus clausii* cell, a *Bacillus megaterium* cell, a *Bacillus subtilis* cell, or a *Bacillus amyloliquefaciens* cell.

[0036] In other embodiments, the host cell is a *Trichoderma koningii* cell, a *Trichoderma viride* cell, a *Trichoderma reesei* cell, a *Trichoderma longibrachiatum* cell, an *Aspergillus awamori* cell, an *Aspergillus fumigates* cell, an *Aspergillus foetidus* cell, an *Aspergillus nidulans* cell, an *Aspergillus niger* cell, an *Aspergillus oryzae* cell, a *Humicola insolens* cell, a *Humicola lanuginose* cell, a *Rhodococcus opacus* cell, a *Rhizomucor miehei* cell, or a *Mucor michei* cell.

[0037] In yet other embodiments, the host cell is a *Streptomyces lividans* cell or a *Streptomyces murinus* cell. In other embodiments, the host cell is an *Actinomycetes* cell.

[0038] In some embodiments, the host cell is a CHO cell, a COS cell, a VERO cell, a BHK cell, a HeLa cell, a Cv1 cell, an MDCK cell, a 293 cell, a 3T3 cell, or a PC12 cell.

[0039] In particular embodiments, the host cell is an *E. coli* cell, such as a strain B, a strain C, a strain K, or a strain W *E. coli* cell.

[0040] In other embodiments, the host cell is a cyanobacterial host cell. In particular embodiments, the cyanobacterial host cell is a cell listed in Table 1.

[0041] In some embodiments, the aldehyde is secreted by the host cell.

[0042] In certain embodiments, the host cell overexpresses a substrate described herein. In some embodiments, the method further includes transforming the host cell with a nucleic acid that encodes an enzyme described herein, and the host cell overexpresses a substrate described herein. In other embodiments, the method further includes culturing the host cell in the presence of at least one substrate described herein. In some embodiments, the substrate is a fatty acid derivative, an acyl-ACP, a fatty acid, an acyl-CoA, a fatty aldehyde, a fatty alcohol, or a fatty ester.

[0043] In some embodiments, the fatty acid derivative substrate is an unsaturated fatty acid derivative substrate, a monounsaturated fatty acid derivative substrate, or a saturated fatty acid derivative substrate. In other embodiments, the fatty acid derivative substrate is a straight chain fatty acid derivative substrate, a branched chain fatty acid derivative substrate, or a fatty acid derivative substrate that includes a cyclic moiety.

[0044] In some embodiments, the fatty acid derivative is a C_3-C_{25} fatty acid derivative. For example, the fatty acid derivative is a C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, or C_{25} fatty acid derivative. In particular embodiments, the fatty acid derivative substrate is tetradecanoyl-ACP, hexadecanoyl-ACP, hexadecenoyl-ACP, or octadecenoyl-ACP.

[0045] In certain embodiments of the aspects described herein, the aldehyde is a C_3-C_{25} aldehyde. For example, the aldehyde is a C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11},

C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, or C_{25} aldehyde. In some embodiments, the aldehyde is tetradecanal, hexadecanal, hexadecenal, octadecanal, octadecenal, methyltetradecanal, methyltetradecenal, methylhexadecanal, methylhexadecenal, methyloctadecanal, or methyloctadecenal.

[0046] In some embodiments, the aldehyde is a straight chain aldehyde, a branched chain aldehyde, or a cyclic aldehyde.

[0047] In some embodiments, the method further includes isolating the aldehyde from the host cell or from the culture medium.

[0048] In another aspect, the invention features a genetically engineered microorganism comprising an exogenous control sequence stably incorporated into the genomic DNA of the microorganism. In one embodiment, the control sequence is integrated upstream of a polynucleotide comprising a nucleotide sequence having at least about 70% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the nucleotide sequence has at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the nucleotide sequence is SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81.

[0049] In some embodiments, the polynucleotide is endogenous to the microorganism. In some embodiments, the microorganism expresses an increased level of an aldehyde relative to a wild-type microorganism. In some embodiments, the microorganism is a cyanobacterium.

[0050] In another aspect, the invention features a method of making an aldehyde, the method comprising culturing a genetically engineered microorganism described herein under conditions suitable for gene expression, and isolating the aldehyde.

[0051] In another aspect, the invention features a method of making an aldehyde, comprising contacting a substrate with (i) a polypeptide having at least 70% identity to the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, or a variant thereof; (ii) a polypeptide encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, or a

variant thereof; or (iii) a polypeptide comprising the amino acid sequence of SEQ ID NO:54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64. In some embodiments, the polypeptide has reductase activity.

[0052] In some embodiments, the polypeptide has at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82. In some embodiments, the polypeptide has the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82.

[0053] In some embodiments, the polypeptide is encoded by a nucleotide sequence having at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the polypeptide is encoded by a nucleotide sequence having SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81.

[0054] In some embodiments, the biological substrate is a fatty acid derivative, an acyl-ACP, a fatty acid, an acyl-CoA, a fatty aldehyde, a fatty alcohol, or a fatty ester.

[0055] In some embodiments, the fatty acid derivative is a C_3-C_{25} fatty acid derivative. For example, the fatty acid derivative is a C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, or C_{25} fatty acid derivative. In particular embodiments, the fatty acid derivative substrate is tetradecanoyl-ACP, hexadecanoyl-ACP, hexadecenoyl-ACP, or octadecenoyl-ACP.

[0056] In certain embodiments, the aldehyde is a C_3-C_{25} aldehyde. For example, the aldehyde is a C_3, C_4, C_5, C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, or C_{25} aldehyde. In some embodiments, the aldehyde is tetradecanal, hexadecanal, hexadecenal, octadecanal, octadecenal, methyltetradecanal, methyltetradecenal, methylhexadecanal, methylhexadecenal, methyloctadecanal, or methyloctadecenal.

[0057] In some embodiments, the aldehyde is a straight chain aldehyde, a branched chain aldehyde, or a cyclic aldehyde.

[0058] In another aspect, the invention features an aldehyde produced by any of the methods or microorganisms described herein. In particular embodiments, the aldehyde has a $\delta^{13}C$ of about -15.4 or greater. For example, the aldehyde has a $\delta^{13}C$ of about -15.4 to about -10.9, for example, about -13.92 to about -13.84. In other embodiments, the aldehyde has an $f_M{}^{14}C$ of at least about 1.003. For example, the aldehyde has an $f_M{}^{14}C$ of at least about 1.01 or at least about 1.5. In some embodiments, the aldehyde has an $f_M{}^{14}C$ of about 1.111 to about 1.124.

[0059] In another aspect, the invention features a method of producing a fatty alcohol, the method comprising producing in a host cell a polypeptide comprising the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, or a variant thereof, and isolating the fatty alcohol from the host cell. In some embodiments, the fatty alcohol is secreted by the cell.

[0060] In some embodiments, the polypeptide comprises an amino acid sequence having at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% identity to SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82.

[0061] In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 with one or more amino acid substitutions, additions, insertions, or deletions. In some embodiments, the polypeptide has reductase activity. In yet other embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, with one or more conservative amino acid substitutions. For example, the polypeptide comprises one or more of the following conservative amino acid substitutions: replacement of an aliphatic amino acid, such as alanine, valine, leucine, and isoleucine, with another aliphatic amino acid; replacement of a serine with a threonine; replacement of a threonine with a serine; replacement of an acidic residue, such as aspartic acid and glutamic acid, with another acidic residue; replacement of a residue bearing an amide group, such as asparagine and glutamine, with another residue bearing an amide group; exchange of a basic residue, such as lysine and arginine, with another basic residue; and replacement of an aromatic residue, such as phenylalanine and tyrosine, with another aromatic residue. In some

embodiments, the polypeptide has about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, or more amino acid substitutions, additions, insertions, or deletions. In some embodiments, the polypeptide has reductase activity.

[0062] In other embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64. In certain embodiments, the polypeptide has reductase activity.

[0063] In another aspect, the invention features a method of producing a fatty alcohol, the method comprising expressing in a host cell a polynucleotide comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the nucleotide sequence is SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the method further comprises isolating the fatty alcohol from the host cell.

[0064] In other embodiments, the nucleotide sequence hybridizes to a complement of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, or to a fragment thereof, for example, under low stringency, medium stringency, high stringency, or very high stringency conditions.

[0065] In other embodiments, the nucleotide sequence encodes a polypeptide comprising: (i) the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82; or (ii) the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 with one or more amino acid substitutions, additions, insertions, or deletions. In some embodiments, the polypeptide comprises the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 with one or more conservative amino acid substitutions. In some embodiments, the polypeptide has reductase activity.

[0066] In other embodiments, the nucleotide sequence encodes a polypeptide having the same biological activity as a polypeptide comprising the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82. In some embodiments, the nucleotide sequence is SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79,

or 81 or a fragment thereof. In other embodiments, the nucleotide sequence hybridizes to a complement of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81 or to a fragment thereof, for example, under low stringency, medium stringency, high stringency, or very high stringency conditions. In some embodiments, the biological activity is reductase activity.

[0067] In some embodiments, the method comprises transforming a host cell with a recombinant vector comprising a nucleotide sequence having at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least about 99% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the recombinant vector further comprises a promoter operably linked to the nucleotide sequence. In some embodiments, the promoter is a developmentally-regulated, an organelle-specific, a tissue-specific, an inducible, a constitutive, or a cell-specific promoter. In particular embodiments, the recombinant vector comprises at least one sequence selected from the group consisting of (a) a regulatory sequence operatively coupled to the nucleotide sequence; (b) a selection marker operatively coupled to the nucleotide sequence; (c) a marker sequence operatively coupled to the nucleotide sequence; (d) a purification moiety operatively coupled to the nucleotide sequence; (e) a secretion sequence operatively coupled to the nucleotide sequence; and (f) a targeting sequence operatively coupled to the nucleotide sequence. In certain embodiments, the nucleotide sequence is stably incorporated into the genomic DNA of the host cell, and the expression of the nucleotide sequence is under the control of a regulated promoter region.

[0068] In some embodiments, the method further includes expressing a gene encoding a recombinant alcohol dehydrogenase in the host cell.

[0069] In any of the aspects of the invention described herein, the methods can produce fatty alcohols comprising a C_6-C_{26} fatty alcohol. In some embodiments, the fatty alcohol comprises a C_6, C_7, C_8, C_9, C_{10}, C_{11}, C_{12}, C_{13}, C_{14}, C_{15}, C_{16}, C_{17}, C_{18}, C_{19}, C_{20}, C_{21}, C_{22}, C_{23}, C_{24}, C_{25}, or a C_{26} fatty alcohol. In particular embodiments, the fatty alcohol is 1-decanol, 1-dodecanol, 1-myristyl alcohol, 1-hexadecanol, octadecenol, tetradecenol, or hexadecenol

[0070] In other embodiments, the fatty alcohol comprises a straight chain fatty alcohol. In other embodiments, the fatty alcohol comprises a branched chain fatty alcohol. In yet other embodiments, the fatty alcohol comprises a cyclic moiety.

[0071] In some embodiments, the fatty alcohol is an unsaturated fatty alcohol. In other embodiments, the fatty alcohol is a monounsaturated fatty alcohol. In yet other embodiments, the fatty alcohol is a saturated fatty alcohol.

[0072] In another aspect, the invention features a fatty alcohol produced by any of the methods or any of the microorganisms described herein, or a surfactant comprising a fatty alcohol produced by any of the methods or any of the microorganisms described herein.

[0073] In some embodiments, the fatty alcohol has a $\delta^{13}C$ of about -15.4 or greater. In certain embodiments, the fatty alcohol has a $\delta^{13}C$ of about -15.4 to about -10.9, or of about -13.92 to about -13.84.

[0074] In some embodiments, the fatty alcohol has an $f_M{}^{14}C$ of at least about 1.003. In certain embodiments, the fatty alcohol has an $f_M{}^{14}C$ of at least about 1.01 or at least about 1.5. In some embodiments, the fatty alcohol has an $f_M{}^{14}C$ of about 1.111 to about 1.124.

[0075] In another aspect, the invention features an isolated nucleic acid consisting of no more than about 500 nucleotides of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the nucleic acid consists of no more than about 300 nucleotides, no more than about 350 nucleotides, no more than about 400 nucleotides, no more than about 450 nucleotides, no more than about 550 nucleotides, no more than about 600 nucleotides, or no more than about 650 nucleotides, of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the nucleic acid encodes a polypeptide having reductase activity.

[0076] In another aspect, the invention features an isolated nucleic acid consisting of no more than about 99%, no more than about 98%, no more than about 97%, no more than about 96%, no more than about 95%, no more than about 94%, no more than about 93%, no more than about 92%, no more than about 91%, no more than about 90%, no more than about 85%, or no more than about 80% of the nucleotides of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81. In some embodiments, the nucleic acid encodes a polypeptide having reductase activity.

[0077] In another aspect, the invention features an isolated polypeptide consisting of no more than about 200, no more than about 175, no more than about 150, or no more than about 100 of the amino acids of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82. In some embodiments, the polypeptide has reductase activity.

[0078] In another aspect, the invention features an isolated polypeptide consisting of no more than about 99%, no more than about 98%, no more than about 97%, no more than about 96%, no more than about 95%, no more than about 94%, no more than about 93%, no more than about 92%, no more than about 91%, no more than about 90%, no more than about 85%, or no more than about 80% of the amino acids of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82. In some embodiments, the polypeptide has reductase activity.

Definitions

[0079] Throughout the specification, a reference may be made using an abbreviated gene name or polypeptide name, but it is understood that such an abbreviated gene or polypeptide name represents the genus of genes or polypeptides. Such gene names include all genes encoding the same polypeptide and homologous polypeptides having the same physiological function. Polypeptide names include all polypeptides that have the same activity (*e.g.*, that catalyze the same fundamental chemical reaction).

[0080] The accession numbers referenced herein are derived from the NCBI database (National Center for Biotechnology Information) maintained by the National Institute of Health, U.S.A. Unless otherwise indicated, the accession numbers are as provided in the database as of April 2009.

[0081] EC numbers are established by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB) (available at http://www.chem.qmul.ac.uk/iubmb/enzyme/). The EC numbers referenced herein are derived from the KEGG Ligand database, maintained by the Kyoto Encyclopedia of Genes and Genomics, sponsored in part by the University of Tokyo. Unless otherwise indicated, the EC numbers are as provided in the database as of March 2008.

[0082] The articles "a" and "an" are used herein to refer to one or to more than one (*i.e.*, to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

[0083] The term "about" is used herein to mean a value ± 20% of a given numerical value. Thus, "about 60%" means a value of between 60 ± (20% of 60) (*i.e.*, between 48 and 70).

[0084] As used herein, the term "aldehyde" means a hydrocarbon having the formula RCHO characterized by an unsaturated carbonyl group (C=O). In a preferred embodiment, the aldehyde is any aldehyde made from a fatty acid or fatty acid derivative. In one embodiment, the R group is at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbons in length..

[0085] As used herein, an "aldehyde biosynthetic gene" or an "aldehyde biosynthetic polynucleotide" is a nucleic acid that encodes an aldehyde biosynthetic polypeptide.

[0086] As used herein, an "aldehyde biosynthetic polypeptide" is a polypeptide that is a part of the biosynthetic pathway of an aldehyde. Such polypeptides can act on a biological substrate to yield an aldehyde. In some instances, the aldehyde biosynthetic polypeptide has reductase activity.

[0087] As used herein, the term "alkane" means a hydrocarbon containing only single carbon-carbon bonds.

[0088] As used herein, an "alkane biosynthetic gene" or an "alkane biosynthetic polynucleotide" is a nucleic acid that encodes an alkane biosynthetic polypeptide.

[0089] As used herein, an "alkane biosynthetic polypeptide" is a polypeptide that is a part of the biosynthetic pathway of an alkane. Such polypeptides can act on a biological substrate to yield an alkane. In some instances, the alkane biosynthetic polypeptide has decarbonylase activity.

[0090] As used herein, an "alkene biosynthetic gene" or an "alkene biosynthetic polynucleotide" is a nucleic acid that encodes an alkene biosynthetic polypeptide.

[0091] As used herein, an "alkene biosynthetic polypeptide" is a polypeptide that is a part of the biosynthetic pathway of an alkene. Such polypeptides can act on a biological substrate to yield an alkene. In some instances, the alkene biosynthetic polypeptide has decarbonylase activity.

[0092] As used herein, the term "attenuate" means to weaken, reduce or diminish. For example, a polypeptide can be attenuated by modifying the polypeptide to reduce its activity (*e.g.*, by modifying a nucleotide sequence that encodes the polypeptide).

[0093] As used herein, the term "biodiesel" means a biofuel that can be a substitute of diesel, which is derived from petroleum. Biodiesel can be used in internal combustion diesel engines in either a pure form, which is referred to as "neat" biodiesel, or as a mixture in any concentration with petroleum-based diesel. Biodiesel can include esters or hydrocarbons, such as aldehydes and alkanes.

[0094] As used therein, the term "biofuel" refers to any fuel derived from biomass. Biofuels can be substituted for petroleum based fuels. For example, biofuels are inclusive of transportation fuels (*e.g.*, gasoline, diesel, jet fuel, *etc.*), heating fuels, and electricity-generating fuels. Biofuels are a renewable energy source.

[0095] As used herein, the term "biomass" refers to a carbon source derived from biological material. Biomass can be converted into a biofuel. One exemplary source of biomass is plant matter. For example, corn, sugar cane, or switchgrass can be used as biomass. Another non-limiting example of biomass is animal matter, for example cow manure. Biomass also includes waste products from industry, agriculture, forestry, and households. Examples of such waste products that can be used as biomass are fermentation waste, straw, lumber, sewage, garbage, and food leftovers. Biomass also includes sources of carbon, such as carbohydrates (*e.g.*, monosaccharides, disaccharides, or polysaccharides).

[0096] As used herein, the phrase "carbon source" refers to a substrate or compound suitable to be used as a source of carbon for prokaryotic or simple eukaryotic cell growth. Carbon sources can be in various forms, including, but not limited to polymers, carbohydrates, acids, alcohols, aldehydes, ketones, amino acids, peptides, and gases (*e.g.*, CO and CO_2). These include, for example, various monosaccharides, such as glucose, fructose, mannose, and galactose; oligosaccharides, such as fructo-oligosaccharide and galacto-oligosaccharide; polysaccharides such as xylose and arabinose; disaccharides, such as sucrose, maltose, and turanose; cellulosic material, such as methyl cellulose and sodium

carboxymethyl cellulose; saturated or unsaturated fatty acid esters, such as succinate, lactate, and acetate; alcohols, such as ethanol or mixtures thereof. The carbon source can also be a product of photosynthesis, including, but not limited to, glucose. A preferred carbon source is biomass. Another preferred carbon source is glucose.

[0097] As used herein, a "cloud point lowering additive" is an additive added to a composition to decrease or lower the cloud point of a solution.

[0098] As used herein, the phrase "cloud point of a fluid" means the temperature at which dissolved solids are no longer completely soluble. Below this temperature, solids begin precipitating as a second phase giving the fluid a cloudy appearance. In the petroleum industry, cloud point refers to the temperature below which a solidified material or other heavy hydrocarbon crystallizes in a crude oil, refined oil, or fuel to form a cloudy appearance. The presence of solidified materials influences the flowing behavior of the fluid, the tendency of the fluid to clog fuel filters, injectors, *etc.*, the accumulation of solidified materials on cold surfaces (*e.g.*, a pipeline or heat exchanger fouling), and the emulsion characteristics of the fluid with water.

[0099] A nucleotide sequence is "complementary" to another nucleotide sequence if each of the bases of the two sequences matches (*i.e.*, is capable of forming Watson Crick base pairs). The term "complementary strand" is used herein interchangeably with the term "complement". The complement of a nucleic acid strand can be the complement of a coding strand or the complement of a non-coding strand.

[0100] As used herein, the term "conditions sufficient to allow expression" means any conditions that allow a host cell to produce a desired product, such as a polypeptide, aldehyde, or alkane described herein. Suitable conditions include, for example, fermentation conditions. Fermentation conditions can comprise many parameters, such as temperature ranges, levels of aeration, and media composition. Each of these conditions, individually and in combination, allows the host cell to grow. Exemplary culture media include broths or gels. Generally, the medium includes a carbon source, such as glucose, fructose, cellulose, or the like, that can be metabolized by a host cell directly. In addition, enzymes can be used in the medium

to facilitate the mobilization (*e.g.*, the depolymerization of starch or cellulose to fermentable sugars) and subsequent metabolism of the carbon source.

[0101] To determine if conditions are sufficient to allow expression, a host cell can be cultured, for example, for about 4, 8, 12, 24, 36, or 48 hours. During and/or after culturing, samples can be obtained and analyzed to determine if the conditions allow expression. For example, the host cells in the sample or the medium in which the host cells were grown can be tested for the presence of a desired product. When testing for the presence of a product, assays, such as, but not limited to, TLC, HPLC, GC/FID, GC/MS, LC/MS, MS, can be used.

[0102] It is understood that the polypeptides described herein may have additional conservative or non-essential amino acid substitutions, which do not have a substantial effect on the polypeptide functions. Whether or not a particular substitution will be tolerated (*i.e.*, will not adversely affect desired biological properties, such as decarboxylase activity) can be determined as described in Bowie *et al.*, *Science* (1990) 247:1306 1310. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine), and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine).

[0103] As used herein, "control element" means a transcriptional control element. Control elements include promoters and enhancers. The term "promoter element," "promoter," or "promoter sequence" refers to a DNA sequence that functions as a switch that activates the expression of a gene. If the gene is activated, it is said to be transcribed or participating in transcription. Transcription involves the synthesis of mRNA from the gene. A promoter, therefore, serves as a transcriptional regulatory element and also provides a site for initiation of

transcription of the gene into mRNA. Control elements interact specifically with cellular proteins involved in transcription (Maniatis *et al.*, *Science* 236:1237, 1987).

[0104] As used herein, the term "ester synthase" means a peptide capable of producing fatty esters. More specifically, an ester synthase is a peptide which converts a thioester to a fatty ester. In a preferred embodiment, the ester synthase converts a thioester (e.g., acyl-CoA) to a fatty ester.

[0105] In an alternate embodiment, an ester synthase uses a thioester and an alcohol as substrates to produce a fatty ester. Ester synthases are capable of using short and long chain thioesters as substrates. In addition, ester synthases are capable of using short and long chain alcohols as substrates.

[0106] Non-limiting examples of ester synthases are wax synthases, wax-ester synthases, acyl CoA:alcohol transacylases, acyltransferases, and fatty acyl-coenzyme A:fatty alcohol acyltransferases. Exemplary ester synthases are classified in enzyme classification number EC 2.3.1.75. Exemplary GenBank Accession Numbers are provided in Figure 40.

[0107] As used herein, the term "fatty acid" means a carboxylic acid having the formula RCOOH. R represents an aliphatic group, preferably an alkyl group. R can comprise between about 4 and about 22 carbon atoms. Fatty acids can be saturated, monounsaturated, or polyunsaturated. In a preferred embodiment, the fatty acid is made from a fatty acid biosynthetic pathway.

[0108] As used herein, the term "fatty acid biosynthetic pathway" means a biosynthetic pathway that produces fatty acids. The fatty acid biosynthetic pathway includes fatty acid enzymes that can be engineered, as described herein, to produce fatty acids, and in some embodiments can be expressed with additional enzymes to produce fatty acids having desired carbon chain characteristics.

[0109] As used herein, the term "fatty acid derivative" means products made in part from the fatty acid biosynthetic pathway of the production host organism. "Fatty acid derivative" also includes products made in part from acyl-ACP or acyl-ACP derivatives. The fatty acid biosynthetic pathway includes fatty acid synthase enzymes which can be engineered as described herein to produce fatty acid derivatives, and in some examples can be expressed with additional enzymes to produce fatty acid derivatives having desired carbon chain characteristics.

Exemplary fatty acid derivatives include for example, fatty acids, acyl-CoA, fatty aldehyde, short and long chain alcohols, hydrocarbons, fatty alcohols, and esters (*e.g.*, waxes, fatty acid esters, or fatty esters).

[0110] As used herein, the term "fatty acid derivative enzymes" means all enzymes that may be expressed or overexpressed in the production of fatty acid derivatives. These enzymes are collectively referred to herein as fatty acid derivative enzymes. These enzymes may be part of the fatty acid biosynthetic pathway. Non-limiting examples of fatty acid derivative enzymes include fatty acid synthases, thioesterases, acyl-CoA synthases, acyl-CoA reductases, alcohol dehydrogenases, alcohol acyltransferases, fatty alcohol-forming acyl-CoA reductase, ester synthases, aldehyde biosynthetic polypeptides, and alkane biosynthetic polypeptides. Fatty acid derivative enzymes convert a substrate into a fatty acid derivative. In some examples, the substrate may be a fatty acid derivative which the fatty acid derivative enzyme converts into a different fatty acid derivative.

[0111] As used herein, the term "fatty alcohol forming peptides" means a peptide capable of catalyzing the conversion of acyl-CoA to fatty alcohol, including fatty alcohol forming acyl-CoA reductase (FAR, EC 1.1.1.*), acyl-CoA reductase (EC 1.2.1.50), or alcohol dehydrogenase (EC 1.1.1.1). Additionally, one of ordinary skill in the art will appreciate that some fatty alcohol forming peptides will catalyze other reactions as well. For example, some acyl-CoA reductase peptides will accept other substrates in addition to fatty acids. Such non-specific peptides are, therefore, also included. Nucleic acid sequences encoding fatty alcohol forming peptides are known in the art, and such peptides are publicly available. Exemplary GenBank Accession Numbers are provided in Figure 40.

[0112] As used herein, "fatty acid enzyme" means any enzyme involved in fatty acid biosynthesis. Fatty acid enzymes can be expressed or overexpressed in host cells to produce fatty acids. Non-limiting examples of fatty acid enzymes include fatty acid synthases and thioesterases.

[0113] As used herein, the term "fatty ester" means an ester. In a preferred embodiment, a fatty ester is any ester made from a fatty acid, for example a fatty acid ester. In one embodiment, a fatty ester contains an A side (*i.e.*, the carbon chain attached to the carboxylate oxygen) and a B side (*i.e.*, the carbon chain comprising

the parent carboxylate). In a preferred embodiment, when the fatty ester is derived from the fatty acid biosynthetic pathway, the A side is contributed by an alcohol, and the B side is contributed by a fatty acid. Any alcohol can be used to form the A side of the fatty esters. For example, the alcohol can be derived from the fatty acid biosynthetic pathway. Alternatively, the alcohol can be produced through non-fatty acid biosynthetic pathways. Moreover, the alcohol can be provided exogenously. For example, the alcohol can be supplied in the fermentation broth in instances where the fatty ester is produced by an organism. Alternatively, a carboxylic acid, such as a fatty acid or acetic acid, can be supplied exogenously in instances where the fatty ester is produced by an organism that can also produce alcohol.

[0114] The carbon chains comprising the A side or B side can be of any length. In one embodiment, the A side of the ester is at least about 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, or 18 carbons in length. The B side of the ester is at least about 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26 carbons in length. The A side and/or the B side can be straight or branched chain. The branched chains may have one or more points of branching. In addition, the branched chains may include cyclic branches. Furthermore, the A side and/or B side can be saturated or unsaturated. If unsaturated, the A side and/or B side can have one or more points of unsaturation.

[0115] In one embodiment, the fatty ester is produced biosynthetically. In this embodiment, first the fatty acid is "activated." Non-limiting examples of "activated" fatty acids are acyl-CoA, acyl-ACP, and acyl phosphate. Acyl-CoA can be a direct product of fatty acid biosynthesis or degradation. In addition, acyl-CoA can be synthesized from a free fatty acid, a CoA, or an adenosine nucleotide triphosphate (ATP). An example of an enzyme which produces acyl-CoA is acyl-CoA synthase

[0116] After the fatty acid is activated, it can be readily transferred to a recipient nucleophile. Exemplary nucleophiles are alcohols, thiols, or phosphates.

[0117] In one embodiment, the fatty ester is a wax. The wax can be derived from a long chain alcohol and a long chain fatty acid. In another embodiment, the fatty ester can be derived from a fatty acyl-thioester and an alcohol. In another embodiment, the fatty ester is a fatty acid thioester, for example fatty acyl Coenzyme A (CoA). In other embodiments, the fatty ester is a fatty acyl

panthothenate, an acyl carrier protein (ACP), or a fatty phosphate ester. Fatty esters have many uses. For example, fatty esters can be used as a biofuel.

[0118] As used herein, "fraction of modern carbon" or "f_M" has the same meaning as defined by National Institute of Standards and Technology (NIST) Standard Reference Materials (SRMs) 4990B and 4990C, known as oxalic acids standards HOxI and HOxII, respectively. The fundamental definition relates to 0.95 times the ^{14}C /^{12}C isotope ratio HOxI (referenced to AD 1950). This is roughly equivalent to decay-corrected pre-Industrial Revolution wood. For the current living biosphere (plant material), f_M is approximately 1.1.

[0119] Calculations of "homology" between two sequences can be performed as follows. The sequences are aligned for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a preferred embodiment, the length of a reference sequence that is aligned for comparison purposes is at least about 30%, preferably at least about 40%, more preferably at least about 50%, even more preferably at least about 60%, and even more preferably at least about 70%, at least about 80%, at least about 90%, or about 100% of the length of the reference sequence. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein, amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps and the length of each gap, which need to be introduced for optimal alignment of the two sequences.

[0120] The comparison of sequences and determination of percent homology between two sequences can be accomplished using a mathematical algorithm. In a preferred embodiment, the percent homology between two amino acid sequences is determined using the Needleman and Wunsch (1970), J. Mol. Biol. 48:444 453, algorithm that has been incorporated into the GAP program in the GCG software

package, using either a Blossum 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6. In yet another preferred embodiment, the percent homology between two nucleotide sequences is determined using the GAP program in the GCG software package, using a NWSgapdna.CMP matrix and a gap weight of 40, 50, 60, 70, or 80 and a length weight of 1, 2, 3, 4, 5, or 6. A particularly preferred set of parameters (and the one that should be used if the practitioner is uncertain about which parameters should be applied to determine if a molecule is within a homology limitation of the claims) are a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

[0121] As used herein, a "host cell" is a cell used to produce a product described herein (*e.g.*, an aldehyde or alkane described herein). A host cell can be modified to express or overexpress selected genes or to have attenuated expression of selected genes. Non-limiting examples of host cells include plant, animal, human, bacteria, yeast, or filamentous fungi cells.

[0122] As used herein, the term "hybridizes under low stringency, medium stringency, high stringency, or very high stringency conditions" describes conditions for hybridization and washing. Guidance for performing hybridization reactions can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y. (1989), 6.3.1 - 6.3.6. Aqueous and nonaqueous methods are described in that reference and either method can be used. Specific hybridization conditions referred to herein are as follows: 1) low stringency hybridization conditions in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by two washes in 0.2X SSC, 0.1% SDS at least at 50°C (the temperature of the washes can be increased to 55°C for low stringency conditions); 2) medium stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 60°C; 3) high stringency hybridization conditions in 6X SSC at about 45°C, followed by one or more washes in 0.2.X SSC, 0.1% SDS at 65°C; and preferably 4) very high stringency hybridization conditions are 0.5M sodium phosphate, 7% SDS at 65°C, followed by one or more washes at 0.2X SSC, 1% SDS at 65°C. Very high stringency conditions (4) are the preferred conditions unless otherwise specified.

[0123] The term "isolated" as used herein with respect to nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively, that are present in the natural source of the nucleic acid. Moreover, an "isolated nucleic acid" includes nucleic acid fragments, such as fragments that are not naturally occurring. The term "isolated" is also used herein to refer to polypeptides, which are isolated from other cellular proteins, and encompasses both purified endogenous polypeptides and recombinant polypeptides. The term "isolated" as used herein also refers to a nucleic acid or polypeptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques. The term "isolated" as used herein also refers to a nucleic acid or polypeptide that is substantially free of chemical precursors or other chemicals when chemically synthesized.

[0124] As used herein, the "level of expression of a gene in a cell" refers to the level of mRNA, pre-mRNA nascent transcript(s), transcript processing intermediates, mature mRNA(s), and/or degradation products encoded by the gene in the cell.

[0125] As used herein, the term "microorganism" means prokaryotic and eukaryotic microbial species from the domains Archaea, Bacteria and Eucarya, the latter including yeast and filamentous fungi, protozoa, algae, or higher Protista. The term "microbial cell", as used herein, means a cell from a microorganism.

[0126] As used herein, the term "nucleic acid" refers to polynucleotides, such as deoxyribonucleic acid (DNA), and, where appropriate, ribonucleic acid (RNA). The term also includes analogs of either RNA or DNA made from nucleotide analogs, and, as applicable to the embodiment being described, single (sense or antisense) and double-stranded polynucleotides, ESTs, chromosomes, cDNAs, mRNAs, and rRNAs.

[0127] As used herein, the term "operably linked" means that a selected nucleotide sequence (*e.g.*, encoding a polypeptide described herein) is in proximity with a promoter to allow the promoter to regulate expression of the selected nucleotide sequence. In addition, the promoter is located upstream of the selected nucleotide sequence in terms of the direction of transcription and translation. By "operably linked" is meant that a nucleotide sequence and a regulatory sequence(s)

are connected in such a way as to permit gene expression when the appropriate molecules (*e.g.*, transcriptional activator proteins) are bound to the regulatory sequence(s).

[0128] The term "or" is used herein to mean, and is used interchangeably with, the term "and/or," unless context clearly indicates otherwise.

[0129] As used herein, "overexpress" means to express or cause to be expressed a nucleic acid, polypeptide, or hydrocarbon in a cell at a greater concentration than is normally expressed in a corresponding wild-type cell. For example, a polypeptide can be "overexpressed" in a recombinant host cell when the polypeptide is present in a greater concentration in the recombinant host cell compared to its concentration in a non-recombinant host cell of the same species.

[0130] As used herein, "partition coefficient" or "P," is defined as the equilibrium concentration of a compound in an organic phase divided by the concentration at equilibrium in an aqueous phase (*e.g.*, fermentation broth). In one embodiment of a bi-phasic system described herein, the organic phase is formed by the aldehyde or alkane during the production process. However, in some examples, an organic phase can be provided, such as by providing a layer of octane, to facilitate product separation. When describing a two phase system, the partition characteristics of a compound can be described as logP. For example, a compound with a logP of 1 would partition 10:1 to the organic phase. A compound with a logP of -1 would partition 1:10 to the organic phase. By choosing an appropriate fermentation broth and organic phase, an aldehyde or alkane with a high logP value can separate into the organic phase even at very low concentrations in the fermentation vessel.

[0131] As used herein, the term "purify," "purified," or "purification" means the removal or isolation of a molecule from its environment by, for example, isolation or separation. "Substantially purified" molecules are at least about 60% free, preferably at least about 75% free, and more preferably at least about 90% free from other components with which they are associated. As used herein, these terms also refer to the removal of contaminants from a sample. For example, the removal of contaminants can result in an increase in the percentage of aldehydes or alkanes in a sample. For example, when aldehydes or alkanes are produced in a host cell, the

aldehydes or alkanes can be purified by the removal of host cell proteins. After purification, the percentage of aldehydes or alkanes in the sample is increased.

[0132] The terms "purify," "purified," and "purification" do not require absolute purity. They are relative terms. Thus, for example, when aldehydes or alkanes are produced in host cells, a purified aldehyde or purified alkane is one that is substantially separated from other cellular components (*e.g.*, nucleic acids, polypeptides, lipids, carbohydrates, or other hydrocarbons). In another example, a purified aldehyde or purified alkane preparation is one in which the aldehyde or alkane is substantially free from contaminants, such as those that might be present following fermentation. In some embodiments, an aldehyde or an alkane is purified when at least about 50% by weight of a sample is composed of the aldehyde or alkane. In other embodiments, an aldehyde or an alkane is purified when at least about 60%, 70%, 80%, 85%, 90%, 92%, 95%, 98%, or 99% or more by weight of a sample is composed of the aldehyde or alkane.

[0133] As used herein, the term "recombinant polypeptide" refers to a polypeptide that is produced by recombinant DNA techniques, wherein generally DNA encoding the expressed polypeptide or RNA is inserted into a suitable expression vector and that is in turn used to transform a host cell to produce the polypeptide or RNA.

[0134] As used herein, the term "substantially identical" (or "substantially homologous") is used to refer to a first amino acid or nucleotide sequence that contains a sufficient number of identical or equivalent (*e.g.*, with a similar side chain, *e.g.*, conserved amino acid substitutions) amino acid residues or nucleotides to a second amino acid or nucleotide sequence such that the first and second amino acid or nucleotide sequences have similar activities.

[0135] As used herein, the term "synthase" means an enzyme which catalyzes a synthesis process. As used herein, the term synthase includes synthases, synthetases, and ligases.

[0136] As used herein, the term "transfection" means the introduction of a nucleic acid (*e.g.*, via an expression vector) into a recipient cell by nucleic acid-mediated gene transfer.

[0137] As used herein, "transformation" refers to a process in which a cell's genotype is changed as a result of the cellular uptake of exogenous nucleic acid. This may result in the transformed cell expressing a recombinant form of an RNA or polypeptide. In the case of antisense expression from the transferred gene, the expression of a naturally-occurring form of the polypeptide is disrupted.

[0138] As used herein, a "transport protein" is a polypeptide that facilitates the movement of one or more compounds in and/or out of a cellular organelle and/or a cell.

[0139] As used herein, a "variant" of polypeptide X refers to a polypeptide having the amino acid sequence of polypeptide X in which one or more amino acid residues is altered. The variant may have conservative changes or nonconservative changes. Guidance in determining which amino acid residues may be substituted, inserted, or deleted without affecting biological activity may be found using computer programs well known in the art, for example, LASERGENE software (DNASTAR).

[0140] The term "variant," when used in the context of a polynucleotide sequence, may encompass a polynucleotide sequence related to that of a gene or the coding sequence thereof. This definition may also include, for example, "allelic," "splice," "species," or "polymorphic" variants. A splice variant may have significant identity to a reference polynucleotide, but will generally have a greater or fewer number of polynucleotides due to alternative splicing of exons during mRNA processing. The corresponding polypeptide may possess additional functional domains or an absence of domains. Species variants are polynucleotide sequences that vary from one species to another. The resulting polypeptides generally will have significant amino acid identity relative to each other. A polymorphic variant is a variation in the polynucleotide sequence of a particular gene between individuals of a given species.

[0141] As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of useful vector is an episome (*i.e.*, a nucleic acid capable of extra-chromosomal replication). Useful vectors are those capable of autonomous replication and/or expression of nucleic acids to which they are linked. Vectors capable of directing

the expression of genes to which they are operatively linked are referred to herein as "expression vectors". In general, expression vectors of utility in recombinant DNA techniques are often in the form of "plasmids," which refer generally to circular double stranded DNA loops that, in their vector form, are not bound to the chromosome. In the present specification, "plasmid" and "vector" are used interchangeably, as the plasmid is the most commonly used form of vector. However, also included are such other forms of expression vectors that serve equivalent functions and that become known in the art subsequently hereto.

[0142] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

[0143] Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0144] FIG. 1A is a GC/MS trace of hydrocarbons produced by *Prochlorococcus marinus* CCMP1986 cells. FIG. 1B is a mass fragmentation pattern of the peak at 7.55 min of FIG. 1A.

[0145] FIG. 2A is a GC/MS trace of hydrocarbons produced by *Nostoc punctiforme* PCC73102 cells. FIG. 2B is a mass fragmentation pattern of the peak at 8.73 min of FIG. 2A.

[0146] FIG. 3A is a GC/MS trace of hydrocarbons produced by *Gloeobaceter violaceus* ATCC29082 cells. FIG. 3B is a mass fragmentation pattern of the peak at 8.72 min of FIG. 3A. **[0147]** FIG. 4A is a GC/MS trace of hydrocarbons produced by *Synechocystic sp.* PCC6803 cells. FIG. 4B is a mass fragmentation pattern of the peak at 7.36 min of FIG. 4A.

[0148] FIG. 5A is a GC/MS trace of hydrocarbons produced by *Synechocystis sp.* PCC6803 wild type cells. FIG. 5B is a GC/MS trace of hydrocarbons produced by *Synechocystis sp.* PCC6803 cells with a deletion of the sll0208 and sll0209 genes.

[0149] FIG. 6A is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 wild type cells. FIG. 6B is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65).

[0150] FIG. 7 is a GC/MS trace of hydrocarbons produced by *E. coli* cells expressing *Cyanothece* sp. ATCC51142 cce_1430 (YP_001802846) (SEQ ID NO:69).

[0151] FIG. 8A is a GC/MS trace of hydrocarbons produced by *E. coli* cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Synechococcus elongatus* PCC7942 YP_400610 (Synpcc7942_1593) (SEQ ID NO:1). FIG. 8B depicts mass fragmentation patterns of the peak at 6.98 min of FIG. 8A and of pentadecane. FIG. 8C depicts mass fragmentation patterns of the peak at 8.12 min of FIG. 8A and of 8-heptadecene.

[0152] FIG. 9 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838) (SEQ ID NO:5).

[0153] FIG. 10 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Synechocystis* sp. PCC6803 sll0208 (NP_442147) (SEQ ID NO:3).

[0154] FIG. 11 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Nostoc* sp. PCC7210 alr5283 (NP_489323) (SEQ ID NO:7).

[0155] FIG. 12 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611

(Synpcc7942_1594) (SEQ ID NO:65) and codon-optimized *Acaryochloris marina* MBIC11017 AM1_4041 (YP_001518340) (SEQ ID NO:46).

[0156] FIG. 13 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and codon-optimized *Thermosynechococcus elongatus* BP-1 tll1313 (NP_682103) (SEQ ID NO:47).

[0157] FIG. 14 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and codon-optimized *Synechococcus sp.* JA-3-3Ab CYA_0415 (YP_473897) (SEQ ID NO:48).

[0158] FIG. 15 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Gloeobacter violaceus* PCC7421 gll3146 (NP_926092) (SEQ ID NO:15).

[0159] FIG. 16 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and codon-optimized *Prochlorococcus marinus* MIT9313 PMT1231 (NP_895059) (SEQ ID NO:49).

[0160] FIG. 17 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Prochlorococcus marinus* CCMP1986 PMM0532 (NP_892650) (SEQ ID NO:19).

[0161] FIG. 18 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and codon-optimized *Prochlorococcus mariunus* NATL2A PMN2A_1863 (YP_293054) (SEQ ID NO:51).

[0162] FIG. 19 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and codon-optimized *Synechococcus* sp. RS9917 RS9917_09941 (ZP_01079772) (SEQ ID NO:52).

[0163] FIG. 20 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611

(Synpcc7942_1594) (SEQ ID NO:65) and codon-optimized *Synechococcus* sp. RS9917 RS9917_12945 (ZP_01080370) (SEQ ID NO:53).

[0164] FIG. 21 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Cyanothece* sp. ATCC51142 cce_0778 (YP_001802195) (SEQ ID NO:27).

[0165] FIG. 22 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Cyanothece* sp. PCC7425 Cyan7425_0398 (YP_002481151) (SEQ ID NO:29).

[0166] FIG. 23 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Cyanothece* sp. PCC7425 Cyan7425_2986 (YP_002483683) (SEQ ID NO:31).

[0167] FIG. 24A is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Prochlorococcus marinus* CCMP1986 PMM0533 (NP_892651) (SEQ ID NO:71). FIG. 24B is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Prochlorococcus marinus* CCMP1986 PMM0533 (NP_892651) (SEQ ID NO:71) and *Prochlorococcus mariunus* CCMP1986 PMM0532 (NP_892650) (SEQ ID NO:19).

[0168] FIG. 25A is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 Δ*fadE lacZ*::P_{trc} '*tesA-fadD* cells. FIG. 25B is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 Δ*fadE lacZ*::P_{trc} '*tesA-fadD* cells expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Acaryochloris marina* MBIC11017 AM1_4041 (YP_001518340) (SEQ ID NO:9).

[0169] FIG. 26A is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 Δ*fadE lacZ*::P_{trc} '*tesA-fadD* cells expressing *Synechocystis* sp. PCC6803 sll0209 (NP_442146) (SEQ ID NO:67). FIG. 26B is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 Δ*fadE lacZ*::P_{trc} '*tesA-fadD* cells expressing *Synechocystis* sp. PCC6803 sll0209 (NP_442146) (SEQ ID NO:67) and *Synechocystis* sp. PCC6803 sll0208 (NP_442147) (SEQ ID NO:3).

[0170] FIG. 27A is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 Δ*fadD lacZ::P*$_{trc}$*-'tesA* cells expressing *M. smegmatis* strain MC2 155 MSMEG_5739 (YP_889972) (SEQ ID NO:85). FIG. 27B is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 Δ*fadD lacZ::P*$_{trc}$*-'tesA* cells expressing *M. smegmatis* strain MC2 155 MSMEG_5739 (YP_889972) (SEQ ID NO:85) and *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838) (SEQ ID NO:5).

[0171] FIG. 28 is a graphic representation of hydrocarbons produced by *E. coli* MG1655 Δ*fadD lacZ::P*$_{trc}$*-'tesA* cells expressing *M. smegmatis* strain MC2 155 MSMEG_5739 (YP_889972) (SEQ ID NO:85) either alone or in combination with *Nostoc* sp. PCC7120 alr5283 (SEQ ID NO:7), *Nostoc punctiforme* PCC73102 Npun02004178 (SEQ ID NO:5), *P. mariunus* CCMP1986 PMM0532 (SEQ ID NO:19), *G. violaceus* PCC7421 gll3146 (SEQ ID NO:15), *Synechococcus* sp. RS9917_09941 (SEQ ID NO:23), *Synechococcus* sp. RS9917_12945 (SEQ ID NO:25), or *A. marina* MBIC11017 AM1_4041 (SEQ ID NO:9).

[0172] FIG. 29A is a representation of the three-dimensional structure of a class I ribonuclease reductase subunit β protein, RNRβ. FIG. 29B is a representation of the three-dimensional structure of *Prochlorococcus marinus* MIT9313 PMT1231 (NP_895059) (SEQ ID NO:17). FIG. 29C is a representation of the three-dimensional structure of the active site of *Prochlorococcus marinus* MIT9313 PMT1231 (NP_895059) (SEQ ID NO:17).

[0173] FIG. 30A is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838) (SEQ ID NO:5). FIG. 30B is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838) Y123F variant. FIG. 30C is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838) Y126F variant.

[0174] FIG. 31 depicts GC/MS traces of hydrocarbons produced *in vitro* using *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838) (SEQ ID NO:6) and octadecanal (A); Npun02004178 (ZP_00108838) (SEQ ID NO:6), octadecanal, spinach ferredoxin reductase, and NADPH (B); octadecanal, spinach ferredoxin,

spinach ferredoxin reductase, and NADPH (C); or Npun02004178 (ZP_00108838) (SEQ ID NO:6), spinach ferredoxin, and spinach ferredoxin (D).

[0175] FIG. 32 depicts GC/MS traces of hydrocarbons produced *in vitro* using *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838) (SEQ ID NO:6), NADPH, octadecanal, and either (A) spinach ferredoxin and spinach ferredoxin reductase; (B) *N. punctiforme* PCC73102 Npun02003626 (ZP_00109192) (SEQ ID NO:88) and *N. punctiforme* PCC73102 Npun02001001 (ZP_00111633) (SEQ ID NO:90); (C) Npun02003626 (ZP_00109192) (SEQ ID NO:88) and *N. punctiforme* PCC73102 Npun02003530 (ZP_00109422) (SEQ ID NO:92); or (D) Npun02003626 (ZP_00109192) (SEQ ID NO:88) and *N. punctiforme* PCC73102 Npun02003123 (ZP_00109501) (SEQ ID NO:94).

[0176] FIG. 33A is a GC/MS trace of hydrocarbons produced *in vitro* using octadecanoyl-CoA, *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:66), NADH, and Mg^{2+}. FIG. 33B is a GC/MS trace of hydrocarbons produced *in vitro* using octadecanoyl-CoA, *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:66), NADPH, and Mg^{2+}. FIG. 33C is a GC/MS trace of hydrocarbons produced *in vitro* using octadecanoyl-CoA, *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:66) and NADPH.

[0177] FIG. 34A is a GC/MS trace of hydrocarbons produced *in vitro* using octadecanoyl-CoA, labeled NADPH, *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:66), and unlabeled NADPH. FIG. 34B is a GC/MS trace of hydrocarbons produced *in vitro* using octadecanoyl-CoA, labeled NADPH, *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:66), and S-(4-^{2}H)NADPH. FIG. 34C is a GC/MS trace of hydrocarbons produced *in vitro* using octadecanoyl-CoA, labeled NADPH, *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:66), and R-(4-^{2}H)NADPH.

[0178] FIG. 35 is a GC/MS trace of hydrocarbons in the cell-free supernatant produced by *E. coli* MG1655 Δ*fadE* cells in Che-9 media expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65).

[0179] FIG. 36 is a GC/MS trace of hydrocarbons in the cell-free supernatant produced by *E. coli* MG1655 Δ*fadE* cells in Che-9 media expressing *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) and *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838) (SEQ ID NO:5).

[0180] FIG. 37 is a GC/MS trace of hydrocarbons produced by *E. coli* MG1655 cells expressing *Nostoc* sp. PCC7120 alr5283 (NP_489323) (SEQ ID NO:7) and *Nostoc* sp. PCC7120 alr5284 (NP_489324) (SEQ ID NO:81).

[0181] FIG. 38 is a list of examples of homologs of *Synechococcus elongatus* PCC7942 YP_400610 (Synpcc7942_1593) (SEQ ID NO:1) from a metagenomic database.

[0182] FIG. 39 is a list of examples of homologs of *Synechococcus elongatus* PCC7942 YP_400611 (Synpcc7942_1594) (SEQ ID NO:65) from a metagenomic database.

[0183] FIG. 40 is a table identifying various genes that can be expressed, overexpressed, or attenuated to increase production of particular substrates.

DETAILED DESCRIPTION

[0184] The invention provides compositions and methods of producing aldehydes, fatty alcohols, and hydrocarbons (such as alkanes, alkenes, and alkynes) from substrates, for example, an acyl-ACP, a fatty acid, an acyl-CoA, a fatty aldehyde, or a fatty alcohol substrate (*e.g.*, as described in PCT/US08/058788, specifically incorporated by reference herein). Such aldehydes, alkanes, and alkenes are useful as biofuels (*e.g.*, substitutes for gasoline, diesel, jet fuel, *etc.*), specialty chemicals (*e.g.*, lubricants, fuel additive, *etc.*), or feedstock for further chemical conversion (*e.g.*, fuels, polymers, plastics, textiles, solvents, adhesives, *etc.*). The invention is based, in part, on the identification of genes that are involved in aldehyde, alkane, and alkene biosynthesis.

[0185] Such alkane and alkene biosynthetic genes include, for example, *Synechococcus elongatus* PCC7942 Synpcc7942_1593 (SEQ ID NO:1), *Synechocystis* sp. PCC6803 sll0208 (SEQ ID NO:3), *Nostoc punctiforme* PCC 73102 Npun02004178 (SEQ ID NO:5), *Nostoc* sp. PCC 7120 alr5283 (SEQ ID NO:7), *Acaryochloris marina* MBIC11017 AM1_4041 (SEQ ID NO:9),

Thermosynechococcus elongatus BP-1 tll1313 (SEQ ID NO:11), *Synechococcus* sp. JA-3-3A CYA_0415 (SEQ ID NO:13), *Gloeobacter violaceus* PCC 7421 gll3146 (SEQ ID NO:15), *Prochlorococcus marinus* MIT9313 PM123 (SEQ ID NO:17), *Prochlorococcus marinus* subsp. pastoris str. CCMP1986 PMM0532 (SEQ ID NO:19), *Prochlorococcus marinus* str. NATL2A PMN2A_1863 (SEQ ID NO:21), *Synechococcus* sp. RS9917 RS9917_09941 (SEQ ID NO:23), *Synechococcus* sp. RS9917 RS9917_12945 (SEQ ID NO:25), *Cyanothece* sp. ATCC51142 cce_0778 (SEQ ID NO:27), *Cyanothece* sp. PCC7245 Cyan7425DRAFT_1220 (SEQ ID NO:29), *Cyanothece* sp. PCC7245 cce_0778 (SEQ ID NO:31), *Anabaena variabilis* ATCC29413 YP_323043 (Ava_2533) (SEQ ID NO:33), and *Synechococcus elongatus* PCC6301 YP_170760 (syc0050_d) (SEQ ID NO:35). Other alkane and alkene biosynthetic genes are listed in Table 1 and Figure 38.

[0186] Aldehyde biosynthetic genes include, for example, *Synechococcus elongatus* PCC7942 Synpcc7942_1594 (SEQ ID NO:65), *Synechocystis* sp. PCC6803 sll0209 (SEQ ID NO:67), *Cyanothece* sp. ATCC51142 cce_1430 (SEQ ID NO:69), *Prochlorococcus marinus* subsp. pastoris str. CCMP1986 PMM0533 (SEQ ID NO:71), *Gloeobacter violaceus* PCC7421 NP_96091 (gll3145) (SEQ ID NO:73), *Nostoc punctiforme* PCC73102 ZP_00108837 (Npun02004176) (SEQ ID NO:75), *Anabaena variabilis* ATCC29413 YP_323044 (Ava_2534) (SEQ ID NO:77), *Synechococcus elongatus* PCC6301 YP_170761 (syc0051_d) (SEQ ID NO:79), and *Nostoc* sp. PCC 7120 alr5284 (SEQ ID NO:81). Other aldehyde biosynthetic genes are listed in Table 1 and Figure 39.

[0187] Using the methods described herein, aldehydes, fatty alcohols, alkanes, and alkenes can be prepared using one or more aldehyde, alkane, and/or alkene biosynthetic genes or polypeptides described herein, or variants thereof, utilizing host cells or cell-free methods.

Table 1: Aldehyde and alkane biosynthetic gene homologs in cyanobacterial genomes

Cyanobacterium	Alkane Biosynth. Gene accession number	% ID	Aldehyde Biosynth. Gene accession number	% ID
Synechococcus elongatus PCC 7942	YP_400610	100	YP_400611	100
Synechococcus elongatus PCC 6301	YP_170760	100	YP_170761	100
Microcoleus chthonoplastes PCC 7420	EDX75019	77	EDX74978	70
Arthrospira maxima CS-328	EDZ94963	78	EDZ94968	68
Lyngbya sp. PCC 8106	ZP_01619575	77	ZP_01619574	69
Nodularia spumigena CCY9414	ZP_01628096	77	ZP_01628095	70
Trichodesmium erythraeum IMS101	YP_721979	76	YP_721978	69
Microcystis aeruginosa NIES-843	YP_001660323	75	YP_001660322	68
Microcystis aeruginosa PCC 7806	CAO90780	74	CAO90781	67
Nostoc sp. PCC 7120	NP_489323	74	NP_489324	72
Nostoc azollae 0708	EEG05692	73	EEG05693	70
Anabaena variabilis ATCC 29413	YP_323043	74	YP_323044	73
Crocosphaera watsonii WH 8501	ZP_00514700	74	ZP_00516920	67
Synechocystis sp. PCC 6803	NP_442147	72	NP_442146	68
Synechococcus sp. PCC 7335	EDX86803	73	EDX87870	67
Cyanothece sp. ATCC 51142	YP_001802195	73	YP_001802846	67
Cyanothece sp. CCY0110	ZP_01728578	72	ZP_01728620	68
Nostoc punctiforme PCC 73102	ZP_00108838	72	ZP_00108837	71
Acaryochloris marina MBIC11017	YP_001518340	71	YP_001518341	66
Cyanothece sp. PCC 7425	YP_002481151	71	YP_002481152	70
Cyanothece sp. PCC 8801	ZP_02941459	70	ZP_02942716	69
Thermosynechococcus elongatus BP-1	NP_682103	70	NP_682102	70
Synechococcus sp. JA-2-3B'a(2-13)	YP_478639	68	YP_478638	63
Synechococcus sp. RCC307	YP_001227842	67	YP_001227841	64
Synechococcus sp. WH 7803	YP_001224377	68	YP_001224378	65
Synechococcus sp. WH 8102	NP_897829	70	NP_897828	65
Synechococcus sp. WH 7805	ZP_01123214	68	ZP_01123215	65
uncultured marine type-A *Synechococcus* GOM 3O12	ABD96376	70	ABD96375	65
Synechococcus sp. JA-3-3Ab	YP_473897	68	YP_473896	62
uncultured marine type-A *Synechococcus* GOM 3O6	ABD96328	70	ABD96327	65
uncultured marine type-A *Synechococcus* GOM 3M9	ABD96275	68	ABD96274	65
Synechococcus sp. CC9311	YP_731193	63	YP_731192	63
uncultured marine type-A *Synechococcus* 5B2	ABB92250	69	ABB92249	64
Synechococcus sp. WH 5701	ZP_01085338	66	ZP_01085337	67
Gloeobacter violaceus PCC 7421	NP_926092	63	NP_926091	67
Synechococcus sp. RS9916	ZP_01472594	69	ZP_01472595	66
Synechococcus sp. RS9917	ZP_01079772	68	ZP_01079773	65
Synechococcus sp. CC9605	YP_381055	66	YP_381056	66
Cyanobium sp. PCC 7001	EDY39806	64	EDY38361	64
Prochlorococcus marinus str. MIT 9303	YP_001016795	63	YP_001016797	66
Prochlorococcus marinus str. MIT9313	NP_895059	63	NP_895058	65
Synechococcus sp. CC9902	YP_377637	66	YP_377636	65
Prochlorococcus marinus str. MIT 9301	YP_001090782	62	YP_001090783	62
Synechococcus sp. BL107	ZP_01469468	65	ZP_01469469	65
Prochlorococcus marinus str. AS9601	YP_001008981	62	YP_001008982	61
Prochlorococcus marinus str. MIT9312	YP_397029	62	YP_397030	61
Prochlorococcus marinus subsp. pastoris str. CCMP1986	NP_892650	60	NP_892651	63
Prochlorococcus marinus str. MIT 9211	YP_001550420	61	YP_001550421	63
Cyanothece sp. PCC 7425	YP_002483683	59	-	
Prochlorococcus marinus str. NATL2A	YP_293054	59	YP_293055	62
Prochlorococcus marinus str. NATL1A	YP_001014415	59	YP_001014416	62
Prochlorococcus marinus subsp. marinus str. CCMP1375	NP_874925	59	NP_874926	64
Prochlorococcus marinus str. MIT 9515_05961	YP_001010912	57	YP_001010913	63
Prochlorococcus marinus str. MIT 9215_06131	YP_001483814	59	YP_001483815	62
Synechococcus sp. RS9917	ZP_01080370	43	-	
uncultured marine type-A *Synechococcus* GOM 5D20			ABD96480	65

Aldehyde, Alkane, and Alkene Biosynthetic Genes and Variants

[0188] The methods and compositions described herein include, for example, alkane or alkene biosynthetic genes having the nucleotide sequence of SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, or 35, as well as polynucleotide variants thereof. In some instances, the alkane or alkene biosynthetic gene encodes one or more of the amino acid motifs described herein. For example, the alkane or alkene biosynthetic gene can encode a polypeptide comprising SEQ ID NO:37, 38, 39, 41, 42, 43, or 44. The alkane or alkene biosynthetic gene can also include a polypeptide comprising SEQ ID NO:40 and also any one of SEQ ID NO:37, 38, or 39.

[0189] The methods and compositions described herein also include, for example, aldehyde biosynthetic genes having the nucleotide sequence of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, as well as polynucleotide variants thereof. In some instances, the aldehyde biosynthetic gene encodes one or more of the amino acid motifs described herein. For example, the aldehyde biosynthetic gene can encode a polypeptide comprising SEQ ID NO:54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64.

[0190] The variants can be naturally occurring or created *in vitro*. In particular, such variants can be created using genetic engineering techniques, such as site directed mutagenesis, random chemical mutagenesis, Exonuclease III deletion procedures, and standard cloning techniques. Alternatively, such variants, fragments, analogs, or derivatives can be created using chemical synthesis or modification procedures.

[0191] Methods of making variants are well known in the art. These include procedures in which nucleic acid sequences obtained from natural isolates are modified to generate nucleic acids that encode polypeptides having characteristics that enhance their value in industrial or laboratory applications. In such procedures, a large number of variant sequences having one or more nucleotide differences with respect to the sequence obtained from the natural isolate are generated and characterized. Typically, these nucleotide differences result in amino acid changes with respect to the polypeptides encoded by the nucleic acids from the natural isolates.

[0192] For example, variants can be created using error prone PCR (see, *e.g.*, Leung *et al.*, *Technique* 1:11-15, 1989; and Caldwell *et al.*, *PCR Methods Applic.* 2:28-33, 1992). In error prone PCR, PCR is performed under conditions where the copying fidelity of the DNA polymerase is low, such that a high rate of point mutations is obtained along the entire length of the PCR product. Briefly, in such procedures, nucleic acids to be mutagenized (*e.g.*, an aldehyde or alkane biosynthetic polynucleotide sequence), are mixed with PCR primers, reaction buffer, $MgCl_2$, $MnCl_2$, Taq polymerase, and an appropriate concentration of dNTPs for achieving a high rate of point mutation along the entire length of the PCR product. For example, the reaction can be performed using 20 fmoles of nucleic acid to be mutagenized (*e.g.*, an aldehyde or alkane biosynthetic polynucleotide sequence), 30 pmole of each PCR primer, a reaction buffer comprising 50 mM KCl, 10 mM Tris HCl (pH 8.3), and 0.01% gelatin, 7 mM $MgCl_2$, 0.5 mM $MnCl_2$, 5 units of Taq polymerase, 0.2 mM dGTP, 0.2 mM dATP, 1 mM dCTP, and 1 mM dTTP. PCR can be performed for 30 cycles of 94°C for 1 min, 45°C for 1 min, and 72°C for 1 min. However, it will be appreciated that these parameters can be varied as appropriate. The mutagenized nucleic acids are then cloned into an appropriate vector and the activities of the polypeptides encoded by the mutagenized nucleic acids are evaluated.

[0193] Variants can also be created using oligonucleotide directed mutagenesis to generate site-specific mutations in any cloned DNA of interest. Oligonucleotide mutagenesis is described in, for example, Reidhaar-Olson *et al.*, *Science* 241:53-57, 1988. Briefly, in such procedures a plurality of double stranded oligonucleotides bearing one or more mutations to be introduced into the cloned DNA are synthesized and inserted into the cloned DNA to be mutagenized (*e.g.*, an aldehyde or alkane biosynthetic polynucleotide sequence). Clones containing the mutagenized DNA are recovered, and the activities of the polypeptides they encode are assessed.

[0194] Another method for generating variants is assembly PCR. Assembly PCR involves the assembly of a PCR product from a mixture of small DNA fragments. A large number of different PCR reactions occur in parallel in the same vial, with the products of one reaction priming the products of another reaction. Assembly PCR is described in, for example, U.S. Pat. No. 5,965,408.

[0195] Still another method of generating variants is sexual PCR mutagenesis. In sexual PCR mutagenesis, forced homologous recombination occurs between DNA molecules of different, but highly related, DNA sequence *in vitro* as a result of random fragmentation of the DNA molecule based on sequence homology. This is followed by fixation of the crossover by primer extension in a PCR reaction. Sexual PCR mutagenesis is described in, for example, Stemmer, *PNAS, USA* 91:10747-10751, 1994.

[0196] Variants can also be created by *in vivo* mutagenesis. In some embodiments, random mutations in a nucleic acid sequence are generated by propagating the sequence in a bacterial strain, such as an *E. coli* strain, which carries mutations in one or more of the DNA repair pathways. Such "mutator" strains have a higher random mutation rate than that of a wild-type strain. Propagating a DNA sequence (*e.g.*, an aldehyde or alkane biosynthetic polynucleotide sequence) in one of these strains will eventually generate random mutations within the DNA. Mutator strains suitable for use for *in vivo* mutagenesis are described in, for example, PCT Publication No. WO 91/16427.

[0197] Variants can also be generated using cassette mutagenesis. In cassette mutagenesis, a small region of a double stranded DNA molecule is replaced with a synthetic oligonucleotide "cassette" that differs from the native sequence. The oligonucleotide often contains a completely and/or partially randomized native sequence.

[0198] Recursive ensemble mutagenesis can also be used to generate variants. Recursive ensemble mutagenesis is an algorithm for protein engineering (*i.e.*, protein mutagenesis) developed to produce diverse populations of phenotypically related mutants whose members differ in amino acid sequence. This method uses a feedback mechanism to control successive rounds of combinatorial cassette mutagenesis. Recursive ensemble mutagenesis is described in, for example, Arkin *et al.*, *PNAS, USA* 89:7811-7815, 1992.

[0199] In some embodiments, variants are created using exponential ensemble mutagenesis. Exponential ensemble mutagenesis is a process for generating combinatorial libraries with a high percentage of unique and functional mutants, wherein small groups of residues are randomized in parallel to identify, at each

altered position, amino acids which lead to functional proteins. Exponential ensemble mutagenesis is described in, for example, Delegrave *et al.*, *Biotech. Res.* 11:1548-1552, 1993. Random and site-directed mutagenesis are described in, for example, Arnold, *Curr. Opin. Biotech.* 4:450-455, 1993.

[0200] In some embodiments, variants are created using shuffling procedures wherein portions of a plurality of nucleic acids that encode distinct polypeptides are fused together to create chimeric nucleic acid sequences that encode chimeric polypeptides as described in, for example, U.S. Pat. Nos. 5,965,408 and 5,939,250.

[0201] Polynucleotide variants also include nucleic acid analogs. Nucleic acid analogs can be modified at the base moiety, sugar moiety, or phosphate backbone to improve, for example, stability, hybridization, or solubility of the nucleic acid. Modifications at the base moiety include deoxyuridine for deoxythymidine and 5-methyl-2'-deoxycytidine or 5-bromo-2'-doxycytidine for deoxycytidine. Modifications of the sugar moiety include modification of the 2' hydroxyl of the ribose sugar to form 2'-O-methyl or 2'-O-allyl sugars. The deoxyribose phosphate backbone can be modified to produce morpholino nucleic acids, in which each base moiety is linked to a six-membered, morpholino ring, or peptide nucleic acids, in which the deoxyphosphate backbone is replaced by a pseudopeptide backbone and the four bases are retained. (See, *e.g.*, Summerton *et al.*, *Antisense Nucleic Acid Drug Dev.* (1997) 7:187-195; and Hyrup *et al.*, *Bioorgan. Med. Chem.* (1996) 4:5-23.) In addition, the deoxyphosphate backbone can be replaced with, for example, a phosphorothioate or phosphorodithioate backbone, a phosphoroamidite, or an alkyl phosphotriester backbone.

[0202] The aldehyde and alkane biosynthetic polypeptides Synpcc7942_1594 (SEQ ID NO:66) and Synpcc7942_1593 (SEQ ID NO:2) have homologs in other cyanobacteria (nonlimiting examples are depicted in Table 1). Thus, any polynucleotide sequence encoding a homolog listed in Table 1, or a variant thereof, can be used as an aldehyde or alkane biosynthetic polynucleotide in the methods described herein. Each cyanobacterium listed in Table 1 has copies of both genes. The level of sequence identity of the gene products ranges from 61% to 73% for Synpcc7942_1594 (SEQ ID NO:66) and from 43% to 78% for Synpcc7942_1593 (SEQ ID NO:2).

[0203] Further homologs of the aldehyde biosynthetic polypeptide Synpcc7942_1594 (SEQ ID NO:66) are listed in Figure 39, and any polynucleotide sequence encoding a homolog listed in Figure 39, or a variant thereof, can be used as an aldehyde biosynthetic polynucleotide in the methods described herein. Further homologs of the alkane biosynthetic polypeptide Synpcc7942_1593 (SEQ ID NO:2) are listed in Figure 38, and any polynucleotide sequence encoding a homolog listed in Figure 38, or a variant thereof, can be used as an alkane biosynthetic polynucleotide in the methods described herein.

[0204] In certain instances, an aldehyde, alkane, and/or alkene biosynthetic gene is codon optimized for expression in a particular host cell. For example, for expression in *E. coli*, one or more codons can be optimized as described in, *e.g.*, Grosjean *et al.*, *Gene* 18:199-209 (1982).

Aldehyde, Alkane, and Alkene Biosynthetic Polypeptides and Variants

[0205] The methods and compositions described herein also include alkane or alkene biosynthetic polypeptides having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36, as well as polypeptide variants thereof. In some instances, an alkane or alkene biosynthetic polypeptide is one that includes one or more of the amino acid motifs described herein. For example, the alkane or alkene biosynthetic polypeptide can include the amino acid sequence of SEQ ID NO:37, 38, 39, 41, 42, 43, or 44. The alkane or alkene biosynthetic polypeptide can also include the amino acid sequence of SEQ ID NO:40 and also any one of SEQ ID NO:37, 38, or 39.

[0206] The methods and compositions described herein also include aldehyde biosynthetic polypeptides having the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, as well as polypeptide variants thereof. In some instances, an aldehyde biosynthetic polypeptide is one that includes one or more of the amino acid motifs described herein. For example, the aldehyde biosynthetic polypeptide can include the amino acid sequence of SEQ ID NO:54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64.

[0207] Aldehyde, alkane, and alkene biosynthetic polypeptide variants can be variants in which one or more amino acid residues are substituted with a conserved

or non-conserved amino acid residue (preferably a conserved amino acid residue). Such substituted amino acid residue may or may not be one encoded by the genetic code.

[0208] Conservative substitutions are those that substitute a given amino acid in a polypeptide by another amino acid of similar characteristics. Typical conservative substitutions are the following replacements: replacement of an aliphatic amino acid, such as alanine, valine, leucine, and isoleucine, with another aliphatic amino acid; replacement of a serine with a threonine or vice versa; replacement of an acidic residue, such as aspartic acid and glutamic acid, with another acidic residue; replacement of a residue bearing an amide group, such as asparagine and glutamine, with another residue bearing an amide group; exchange of a basic residue, such as lysine and arginine, with another basic residue; and replacement of an aromatic residue, such as phenylalanine and tyrosine, with another aromatic residue.

[0209] Other polypeptide variants are those in which one or more amino acid residues include a substituent group. Still other polypeptide variants are those in which the polypeptide is associated with another compound, such as a compound to increase the half-life of the polypeptide (*e.g.*, polyethylene glycol).

[0210] Additional polypeptide variants are those in which additional amino acids are fused to the polypeptide, such as a leader sequence, a secretory sequence, a proprotein sequence, or a sequence which facilitates purification, enrichment, or stabilization of the polypeptide.

[0211] In some instances, an alkane or alkene biosynthetic polypeptide variant retains the same biological function as a polypeptide having the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36 (*e.g.*, retains alkane or alkene biosynthetic activity) and has an amino acid sequence substantially identical thereto.

[0212] In other instances, the alkane or alkene biosynthetic polypeptide variants have at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more than about 95% homology to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36. In another embodiment, the polypeptide variants include a fragment

comprising at least about 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof.

[0213] In some instances, an aldehyde biosynthetic polypeptide variant retains the same biological function as a polypeptide having the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 (*e.g.*, retains aldehyde biosynthetic activity) and has an amino acid sequence substantially identical thereto.

[0214] In yet other instances, the aldehyde biosynthetic polypeptide variants have at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more than about 95% homology to the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82. In another embodiment, the polypeptide variants include a fragment comprising at least about 5, 10, 15, 20, 25, 30, 35, 40, 50, 75, 100, or 150 consecutive amino acids thereof.

[0215] The polypeptide variants or fragments thereof can be obtained by isolating nucleic acids encoding them using techniques described herein or by expressing synthetic nucleic acids encoding them. Alternatively, polypeptide variants or fragments thereof can be obtained through biochemical enrichment or purification procedures. The sequence of polypeptide variants or fragments can be determined by proteolytic digestion, gel electrophoresis, and/or microsequencing. The sequence of the alkane or alkene biosynthetic polypeptide variants or fragments can then be compared to the amino acid sequence of SEQ ID NO:2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, or 36 using any of the programs described herein. The sequence of the aldehyde biosynthetic polypeptide variants or fragments can be compared to the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 using any of the programs described herein.

[0216] The polypeptide variants and fragments thereof can be assayed for aldehyde-, fatty alcohol-, alkane-, and/or alkene-producing activity using routine methods. For example, the polypeptide variants or fragment can be contacted with a substrate (*e.g.*, a fatty acid derivative substrate or other substrate described herein) under conditions that allow the polypeptide variant to function. A decrease in the level of the substrate or an increase in the level of an aldehyde, alkane, or alkene can

be measured to determine aldehyde-, fatty alcohol-, alkane-, or alkene-producing activity, respectively.

Anti-Aldehyde, Anti-Fatty Alcohol, Anti-Alkane, and Anti-Alkene Biosynthetic Polypeptide Antibodies

[0217] The aldehyde, fatty alcohol, alkane, and alkene biosynthetic polypeptides described herein can also be used to produce antibodies directed against aldehyde, fatty alcohol, alkane, and alkene biosynthetic polypeptides. Such antibodies can be used, for example, to detect the expression of an aldehyde, fatty alcohol, alkane, or alkene biosynthetic polypeptide using methods known in the art. The antibody can be, *e.g.*, a polyclonal antibody; a monoclonal antibody or antigen binding fragment thereof; a modified antibody such as a chimeric antibody, reshaped antibody, humanized antibody, or fragment thereof (*e.g.*, Fab', Fab, $F(ab')_2$); or a biosynthetic antibody, *e.g.*, a single chain antibody, single domain antibody (DAB), Fv, single chain Fv (scFv), or the like.

[0218] Methods of making and using polyclonal and monoclonal antibodies are described, *e.g.*, in Harlow *et al.*, Using Antibodies: A Laboratory Manual: Portable Protocol I. Cold Spring Harbor Laboratory (December 1, 1998). Methods for making modified antibodies and antibody fragments (*e.g.*, chimeric antibodies, reshaped antibodies, humanized antibodies, or fragments thereof, *e.g.*, Fab', Fab, $F(ab')_2$ fragments); or biosynthetic antibodies (*e.g.*, single chain antibodies, single domain antibodies (DABs), Fv, single chain Fv (scFv), and the like), are known in the art and can be found, *e.g.*, in Zola, Monoclonal Antibodies: Preparation and Use of Monoclonal Antibodies and Engineered Antibody Derivatives, Springer Verlag (December 15, 2000; 1st edition).

Substrates

[0219] The compositions and methods described herein can be used to produce aldehydes, fatty alcohols, alkanes, and/or alkenes from an appropriate substrate. While not wishing to be bound by a particular theory, it is believed that the alkane or alkene biosynthetic polypeptides described herein produce alkanes or alkenes from substrates *via* a decarbonylation mechanism. In some instances, the substrate is a

fatty acid derivative, *e.g.*, a fatty aldehyde, and an alkane having particular branching patterns and carbon chain length can be produced from a fatty acid derivative, *e.g.*, a fatty aldehyde, having those particular characteristics. In other instances, the substrate is an unsaturated fatty acid derivative, *e.g.*, an unsaturated fatty aldehyde, and an alkene having particular branching patterns and carbon chain length can be produced from an unsaturated fatty acid derivative, *e.g.*, an unsaturated fatty aldehyde, having those particular characteristics.

[0220] While not wishing to be bound by a particular theory, it is believed that the aldehyde biosynthetic polypeptides described herein produce aldehydes from substrates *via* a reduction mechanism. In certain instances, the substrate is an acyl-ACP.

[0221] While not wishing to be bound by a particular theory, it is believed that the fatty alcohols described herein are produced from substrates *via* a reduction mechanism. In certain instances, the substrate is a fatty aldehyde.

[0222] Accordingly, each step within a biosynthetic pathway that leads to the production of these substrates can be modified to produce or overproduce the substrate of interest. For example, known genes involved in the fatty acid biosynthetic pathway, the fatty aldehyde pathway, and the fatty alcohol pathway can be expressed, overexpressed, or attenuated in host cells to produce a desired substrate (see, *e.g.*, PCT/US08/058788, specifically incorporated by reference herein). Exemplary genes are provided in Figure 40.

Synthesis of Substrates

[0223] Fatty acid synthase (FAS) is a group of polypeptides that catalyze the initiation and elongation of acyl chains (Marrakchi *et al., Biochemical Society,* 30:1050-1055, 2002). The acyl carrier protein (ACP) along with the enzymes in the FAS pathway control the length, degree of saturation, and branching of the fatty acid derivatives produced. The fatty acid biosynthetic pathway involves the precursors acetyl-CoA and malonyl-CoA. The steps in this pathway are catalyzed by enzymes of the fatty acid biosynthesis (*fab*) and acetyl-CoA carboxylase (*acc*) gene families (see, *e.g.*, Heath *et al.*, *Prog. Lipid Res.* 40(6):467-97 (2001)).

[0224] Host cells can be engineered to express fatty acid derivative substrates by recombinantly expressing or overexpressing acetyl-CoA and/or malonyl-CoA

synthase genes. For example, to increase acetyl-CoA production, one or more of the following genes can be expressed in a host cell: *pdh, panK, aceEF* (encoding the E1p dehydrogenase component and the E2p dihydrolipoamide acyltransferase component of the pyruvate and 2-oxoglutarate dehydrogenase complexes), *fabH, fabD, fabG, acpP,* and *fabF*. Exemplary GenBank accession numbers for these genes are: *pdh* (BAB34380, AAC73227, AAC73226), *panK* (also known as coaA, AAC76952), *aceEF* (AAC73227, AAC73226), *fabH* (AAC74175), *fabD* (AAC74176), *fabG* (AAC74177), *acpP* (AAC74178), *fabF* (AAC74179). Additionally, the expression levels of *fadE, gpsA, ldhA, pflb, adhE, pta, poxB, ackA,* and/or *ackB* can be attenuated or knocked-out in an engineered host cell by transformation with conditionally replicative or non-replicative plasmids containing null or deletion mutations of the corresponding genes or by substituting promoter or enhancer sequences. Exemplary GenBank accession numbers for these genes are: *fadE* (AAC73325), *gspA* (AAC76632), *ldhA* (AAC74462), *pflb* (AAC73989), *adhE (*AAC74323*), pta* (AAC75357), *poxB* (AAC73958), *ackA* (AAC75356), and *ackB* (BAB81430). The resulting host cells will have increased acetyl-CoA production levels when grown in an appropriate environment.

[0225] Malonyl-CoA overexpression can be effected by introducing *accABCD* (*e.g.*, accession number AAC73296, EC 6.4.1.2) into a host cell. Fatty acids can be further overexpressed in host cells by introducing into the host cell a DNA sequence encoding a lipase (*e.g.*, accession numbers CAA89087, CAA98876).

[0226] In addition, inhibiting PlsB can lead to an increase in the levels of long chain acyl-ACP, which will inhibit early steps in the pathway (*e.g.*, accABCD, fabH, and fabI). The *plsB* (*e.g.*, accession number AAC77011) D311E mutation can be used to increase the amount of available acyl-CoA.

[0227] In addition, a host cell can be engineered to overexpress a *sfa* gene (suppressor of fabA, *e.g.*, accession number AAN79592) to increase production of monounsaturated fatty acids (Rock *et al.*, *J. Bacteriology* 178:5382-5387, 1996).

[0228] In some instances, host cells can be engineered to express, overexpress, or attenuate expression of a thioesterase to increase fatty acid substrate production. The chain length of a fatty acid substrate is controlled by thioesterase. In some instances, a *tes* or *fat* gene can be overexpressed. In other instances, C_{10} fatty acids

can be produced by attenuating thioesterase C_{18} (*e.g.*, accession numbers AAC73596 and P0ADA1), which uses $C_{18:1}$-ACP, and expressing thioesterase C_{10} (*e.g.*, accession number Q39513), which uses C_{10}-ACP. This results in a relatively homogeneous population of fatty acids that have a carbon chain length of 10. In yet other instances, C_{14} fatty acids can be produced by attenuating endogenous thioesterases that produce non-C_{14} fatty acids and expressing the thioesterases, that use C_{14}-ACP (for example, accession number Q39473). In some situations, C_{12} fatty acids can be produced by expressing thioesterases that use C_{12}-ACP (for example, accession number Q41635) and attenuating thioesterases that produce non-C_{12} fatty acids. Acetyl-CoA, malonyl-CoA, and fatty acid overproduction can be verified using methods known in the art, for example, by using radioactive precursors, HPLC, and GC-MS subsequent to cell lysis. Non-limiting examples of thioesterases that can be used in the methods described herein are listed in Table 2.

Table 2: Thioesterases

Accession Number	Source Organism	Gene	Preferential product prod
AAC73596	*E. coli*	*tesA* without leader sequence	$C_{18:1}$
AAC73555	*E. coli*	*tesB*	
Q41635, AAA34215	*Umbellularia california*	*fatB*	$C_{12:0}$
Q39513; AAC49269	*Cuphea hookeriana*	*fatB2*	$C_{8:0}$ - $C_{10:0}$
AAC49269; AAC72881	*Cuphea hookeriana*	*fatB3*	$C_{14:0}$ - $C_{16:0}$
Q39473, AAC49151	*Cinnamonum camphorum*	*fatB*	$C_{14:0}$
CAA85388	*Arabidopsis thaliana*	*fatB* [M141T]*	$C_{16:1}$
NP 189147; NP 193041	*Arabidopsis thaliana*	*fatA*	$C_{18:1}$
CAC39106	*Bradyrhiizobium japonicum*	*fatA*	$C_{18:1}$
AAC72883	*Cuphea hookeriana*	*fatA*	$C_{18:1}$
AAL79361	*Helianthus annus*	*fatA1*	

* Mayer *et al.*, *BMC Plant Biology* 7:1-11, 2007

Formation of Branched Aldehydes, Fatty Alcohols, Alkanes, and Alkenes

[0229] Aldehydes, fatty alcohols, alkanes, an alkenes can be produced that contain branch points by using branched fatty acid derivatives as substrates. For example, although *E. coli* naturally produces straight chain fatty acid derivatives (sFAs), *E. coli* can be engineered to produce branched chain fatty acid derivatives (brFAs) by introducing and expressing or overexpressing genes that provide branched precursors in the *E. coli* (*e.g.*, *bkd*, *ilv*, *icm*, and *fab* gene families).

Additionally, a host cell can be engineered to express or overexpress genes encoding proteins for the elongation of brFAs (*e.g.*, ACP, FabF, *etc.*) and/or to delete or attenuate the corresponding host cell genes that normally lead to sFAs.

[0230] The first step in forming brFAs is the production of the corresponding α-keto acids by a branched-chain amino acid aminotransferase. Host cells may endogenously include genes encoding such enzymes or such genes can be recombinantly introduced. *E. coli*, for example, endogenously expresses such an enzyme, IlvE (EC 2.6.1.42; GenBank accession YP_026247). In some host cells, a heterologous branched-chain amino acid aminotransferase may not be expressed. However, *E. coli* IlvE or any other branched-chain amino acid aminotransferase (*e.g.*, IlvE from *Lactococcus lactis* (GenBank accession AAF34406), IlvE from *Pseudomonas putida* (GenBank accession NP_745648), or IlvE from *Streptomyces coelicolor* (GenBank accession NP_629657)), if not endogenous, can be introduced and recombinantly expressed.

[0231] The second step is the oxidative decarboxylation of the α-ketoacids to the corresponding branched-chain acyl-CoA. This reaction can be catalyzed by a branched-chain α-keto acid dehydrogenase complex (*bkd*; EC 1.2.4.4.) (Denoya *et al.*, *J. Bacteriol.* 177:3504, 1995), which consists of E1α/β (decarboxylase), E2 (dihydrolipoyl transacylase), and E3 (dihydrolipoyl dehydrogenase) subunits. These branched-chain α-keto acid dehydrogenase complexes are similar to pyruvate and α-ketoglutarate dehydrogenase complexes. Any microorganism that possesses brFAs and/or grows on branched-chain amino acids can be used as a source to isolate *bkd* genes for expression in host cells, for example, *E. coli*. Furthermore, *E. coli* has the E3 component as part of its pyruvate dehydrogenase complex (*lpd*, EC 1.8.1.4, GenBank accession NP_414658). Thus, it can be sufficient to express only the *E1 α/β* and *E2 bkd* genes. Table 3 lists non-limiting examples of *bkd* genes from several microorganisms that can be recombinantly introduced and expressed in a host cell to provide branched-chain acyl-CoA precursors.

Table 3: *Bkd* genes from selected microorganisms

Organism	Gene	GenBank Accession #
Streptomyces coelicolor	*bkdA1* (E1α) *bkdB1* (E1β) *bkdC1* (E2)	NP_628006 NP_628005 NP_638004
Streptomyces coelicolor	*bkdA2* (E1α) *bkdB2* (E1β) *bkdC2* (E2)	NP_733618 NP_628019 NP_628018
Streptomyces avermitilis	*bkdA* (E1a) *bkdB* (E1b) *bkdC* (E2)	BAC72074 BAC72075 BAC72076
Streptomyces avermitilis	*bkdF* (E1α) *bkdG* (E1β) *bkdH* (E2)	BAC72088 BAC72089 BAC72090
Bacillus subtilis	*bkdAA* (E1α) *bkdAB* (E1β) *bkdB* (E2)	NP_390288 NP_390288 NP_390288
Pseudomonas putida	*bkdA1* (E1α) *bkdA2* (E1β) *bkdC* (E2)	AAA65614 AAA65615 AAA65617

[0232] In another example, isobutyryl-CoA can be made in a host cell, for example in *E. coli*, through the coexpression of a crotonyl-CoA reductase (Ccr, EC 1.6.5.5, 1.1.1.1) and isobutyryl-CoA mutase (large subunit IcmA, EC 5.4.99.2; small subunit IcmB, EC 5.4.99.2) (Han and Reynolds, *J. Bacteriol.* 179:5157, 1997). Crotonyl-CoA is an intermediate in fatty acid biosynthesis in *E. coli* and other microorganisms. Non-limiting examples of *ccr* and *icm* genes from selected microorganisms are listed in Table 4.

Table 4: *Ccr* and *icm* genes from selected microorganisms

Organism	Gene	GenBank Accession #
Streptomyces coelicolor	*Ccr*	NP_630556
	icmA	NP_629554
	icmB	NP_630904
Streptomyces cinnamonensis	*ccr*	AAD53915
	icmA	AAC08713
	icmB	AJ246005

[0233] In addition to expression of the *bkd* genes, the initiation of brFA biosynthesis utilizes β-ketoacyl-acyl-carrier-protein synthase III (FabH, EC 2.3.1.41) with specificity for branched chain acyl-CoAs (Li *et al.*, *J. Bacteriol.* 187:3795-3799, 2005). Non-limiting examples of such FabH enzymes are listed in Table 5. *fabH* genes that are involved in fatty acid biosynthesis of any brFA-containing microorganism can be expressed in a host cell. The Bkd and FabH enzymes from host cells that do not naturally make brFA may not support brFA production. Therefore, *bkd* and *fabH* can be expressed recombinantly. Vectors containing the *bkd* and *fabH* genes can be inserted into such a host cell. Similarly, the endogenous level of Bkd and FabH production may not be sufficient to produce brFA. In this case, they can be overexpressed. Additionally, other components of the fatty acid biosynthesis pathway can be expressed or overexpressed, such as acyl carrier proteins (ACPs) and β-ketoacyl-acyl-carrier-protein synthase II (*fabF*, EC 2.3.1.41) (non-limiting examples of candidates are listed in Table 5). In addition to expressing these genes, some genes in the endogenous fatty acid biosynthesis pathway can be attenuated in the host cell (*e.g.*, the *E. coli* genes *fabH* (GenBank accession # NP_415609) and/or *fabF* (GenBank accession # NP_415613)).

Table 5: *FabH, ACP and fabF* genes from selected microorganisms with brFAs

Organism	Gene	GenBank Accession #
Streptomyces coelicolor	*fabH1*	NP_626634
	ACP	NP_626635
	fabF	NP_626636
Streptomyces avermitilis	*fabH3*	NP_823466
	fabC3 (ACP)	NP_823467
	fabF	NP_823468
Bacillus subtilis	*fabH_A*	NP_389015
	fabH_B	NP_388898
	ACP	NP_389474
	fabF	NP_389016
Stenotrophomonas maltophilia	SmalDRAFT_0818 (*FabH)*	ZP_01643059
	SmalDRAFT_0821 (*ACP)*	ZP_01643063
	SmalDRAFT_0822 (*FabF)*	ZP_01643064
Legionella pneumophila	*FabH*	YP_123672
	ACP	YP_123675
	fabF	YP_123676

Formation of Cyclic Aldehydes, Fatty Alcohols, Alkanes, and Alkenes

[0234] Cyclic aldehydes, fatty alcohols, alkanes, and alkenes can be produced by using cyclic fatty acid derivatives as substrates. To produce cyclic fatty acid derivative substrates, genes that provide cyclic precursors (*e.g.*, the *ans*, *chc*, and *plm* gene families) can be introduced into the host cell and expressed to allow initiation of fatty acid biosynthesis from cyclic precursors. For example, to convert a host cell, such as *E. coli*, into one capable of synthesizing ω-cyclic fatty acid derivatives (cyFA), a gene that provides the cyclic precursor cyclohexylcarbonyl-CoA (CHC-CoA) (Cropp *et al.*, *Nature Biotech.* 18:980-983, 2000) can be introduced and expressed in the host cell. Non-limiting examples of genes that provide CHC-CoA in *E. coli* include: *ansJ*, *ansK*, *ansL*, *chcA,* and *ansM* from the ansatrienin gene cluster of *Streptomyces collinus* (Chen *et al., Eur. J. Biochem.* 261: 98-107, 1999) or *plmJ*, *plmK*, *plmL*, *chcA,* and *plmM* from the phoslactomycin B gene cluster of *Streptomyces* sp. HK803 (Palaniappan *et al.*, *J. Biol. Chem.* 278:35552-35557, 2003) together with the *chcB* gene (Patton *et al.*, *Biochem.* 39:7595-7604, 2000) from *S. collinus*, *S. avermitilis,* or *S. coelicolor* (*see* Table 6). The genes listed in Table 5 can then be expressed to allow initiation and elongation of ω-cyclic fatty acids. Alternatively, the homologous genes can be isolated from microorganisms that make cyFA and expressed in a host cell (*e.g.*, *E. coli*).

Table 6: Genes for the synthesis of CHC-CoA

Organism	Gene	GenBank Accession #
Streptomyces collinus	*ansJK*	U72144*
	ansL	
	chcA	
	ansM	
	chcB	AF268489
Streptomyces sp. HK803	*pmlJK*	AAQ84158
	pmlL	AAQ84159
	chcA	AAQ84160
	pmlM	AAQ84161
Streptomyces coelicolor	*chcB/caiD*	NP_629292
Streptomyces avermitilis	*chcB/caiD*	NP_629292

*Only *chcA* is annotated in GenBank entry U72144, *ansJKLM* are according to Chen *et al.* (*Eur. J. Biochem.* 261:98-107, 1999).

[0235] The genes listed in Table 5 (*fabH, ACP,* and *fabF*) allow initiation and elongation of ω-cyclic fatty acid derivatives because they have broad substrate specificity. If the coexpression of any of these genes with the genes listed in Table 6 does not yield cyFA, then *fabH, ACP,* and/or *fabF* homologs from microorganisms that make cyFAs (*e.g.*, those listed in Table 7) can be isolated (*e.g.*, by using degenerate PCR primers or heterologous DNA sequence probes) and coexpressed.

Table 7: Non-limiting examples of microorganisms that contain ω-cyclic fatty acids

Organism	Reference
Curtobacterium pusillum	ATCC19096
Alicyclobacillus acidoterrestris	ATCC49025
Alicyclobacillus acidocaldarius	ATCC27009
Alicyclobacillus cycloheptanicus *	Moore, *J. Org. Chem.* 62:pp. 2173, 1997

*Uses cycloheptylcarbonyl-CoA and not cyclohexylcarbonyl-CoA as precursor for cyFA biosynthesis.

Aldehyde, Fatty Alcohol, and Alkene Saturation Levels

[0236] The degree of saturation in fatty acid derivatives can be controlled by regulating the degree of saturation of fatty acid derivative intermediates. The *sfa*, *gns*, and *fab* families of genes can be expressed or overexpressed to control the saturation of fatty acids. Figure 40 lists non-limiting examples of genes in these gene families that may be used in the methods and host cells described herein.

[0237] Host cells can be engineered to produce unsaturated fatty acids by engineering the host cell to overexpress *fabB* or by growing the host cell at low temperatures (*e.g.*, less than 37 °C). FabB has preference to cis-δ3decenoyl-ACP and results in unsaturated fatty acid production in *E. coli*. Overexpression of *fabB* results in the production of a significant percentage of unsaturated fatty acids (de Mendoza *et al.*, *J. Biol. Chem.* 258:2098-2101, 1983). The gene *fabB* may be inserted into and expressed in host cells not naturally having the gene. These unsaturated fatty acid derivatives can then be used as intermediates in host cells that are engineered to produce fatty acid derivatives, such as fatty aldehydes, fatty alcohols, or alkenes.

[0238] In other instances, a repressor of fatty acid biosynthesis, for example, *fabR* (GenBank accession NP_418398), can be deleted, which will also result in increased unsaturated fatty acid production in *E. coli* (Zhang *et al.*, *J. Biol. Chem.* 277:15558, 2002). Similar deletions may be made in other host cells. A further increase in unsaturated fatty acid derivatives may be achieved, for example, by overexpressing *fabM* (trans-2, cis-3-decenoyl-ACP isomerase, GenBank accession DAA05501) and controlled expression of *fabK* (trans-2-enoyl-ACP reductase II,

GenBank accession NP_357969) from *Streptococcus pneumoniae* (Marrakchi *et al.*, *J. Biol. Chem.* 277: 44809, 2002), while deleting *E. coli fabI* (trans-2-enoyl-ACP reductase, GenBank accession NP_415804). In some examples, the endogenous *fabF* gene can be attenuated, thus increasing the percentage of palmitoleate (C16:1) produced.

Other Substrates

[0239] Other substrates that can be used to produce aldehydes, fatty alcohols, alkanes, and alkenes in the methods described herein are acyl-ACP, acyl-CoA, a fatty aldehyde, or a fatty alcohol, which are described in, for example, PCT/US08/058788. Exemplary genes that can be altered to express or overexpress these substrates in host cells are listed in Figure 40. Other exemplary genes are described in PCT/US08/058788.

Genetic Engineering of Host Cells to Produce Aldehydes, Fatty Alcohols, Alkanes, and Alkenes

[0240] Various host cells can be used to produce aldehydes, fatty alcohols, alkanes, and/or alkenes, as described herein. A host cell can be any prokaryotic or eukaryotic cell. For example, a polypeptide described herein can be expressed in bacterial cells (such as *E. coli*), insect cells, yeast or mammalian cells (such as Chinese hamster ovary cells (CHO) cells, COS cells, VERO cells, BHK cells, HeLa cells, Cv1 cells, MDCK cells, 293 cells, 3T3 cells, or PC12 cells). Other exemplary host cells include cells from the members of the genus *Escherichia*, *Bacillus*, *Lactobacillus*, *Rhodococcus*, *Pseudomonas*, *Aspergillus*, *Trichoderma*, *Neurospora*, *Fusarium*, *Humicola*, *Rhizomucor*, *Kluyveromyces*, *Pichia*, *Mucor*, *Myceliophtora*, *Penicillium*, *Phanerochaete*, *Pleurotus*, *Trametes*, *Chrysosporium*, *Saccharomyces*, *Schizosaccharomyces*, *Yarrowia*, or *Streptomyces*. Yet other exemplary host cells can be a *Bacillus lentus* cell, a *Bacillus brevis* cell, a *Bacillus stearothermophilus* cell, a *Bacillus licheniformis* cell, a *Bacillus alkalophilus* cell, a *Bacillus coagulans* cell, a *Bacillus circulans* cell, a *Bacillus pumilis* cell, a *Bacillus thuringiensis* cell, a *Bacillus clausii* cell, a *Bacillus megaterium* cell, a *Bacillus subtilis* cell, a *Bacillus amyloliquefaciens* cell, a *Trichoderma koningii* cell, a *Trichoderma viride* cell, a

Trichoderma reesei cell, a *Trichoderma longibrachiatum* cell, an *Aspergillus awamori* cell, an *Aspergillus fumigates* cell, an *Aspergillus foetidus* cell, an *Aspergillus nidulans* cell, an *Aspergillus niger* cell, an *Aspergillus oryzae* cell, a *Humicola insolens* cell, a *Humicola lanuginose* cell, a *Rhizomucor miehei* cell, a *Mucor michei* cell, a *Streptomyces lividans* cell, a *Streptomyces murinus* cell, or an *Actinomycetes* cell.

[0241] Other nonlimiting examples of host cells are those listed in Table 1.

[0242] In a preferred embodiment, the host cell is an *E. coli* cell. In a more preferred embodiment, the host cell is from *E. coli* strains B, C, K, or W. Other suitable host cells are known to those skilled in the art.

[0243] Various methods well known in the art can be used to genetically engineer host cells to produce aldehydes, fatty alcohols, alkanes and/or alkenes. The methods include the use of vectors, preferably expression vectors, containing a nucleic acid encoding an aldehyde, fatty alcohol, alkane, and/or alkene biosynthetic polypeptide described herein, or a polypeptide variant or fragment thereof. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell and are thereby replicated along with the host genome. Moreover, certain vectors, such as expression vectors, are capable of directing the expression of genes to which they are operatively linked. In general, expression vectors used in recombinant DNA techniques are often in the form of plasmids. However, other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses, and adeno-associated viruses), can also be used.

[0244] The recombinant expression vectors described herein include a nucleic acid described herein in a form suitable for expression of the nucleic acid in a host

cell. The recombinant expression vectors can include one or more control sequences, selected on the basis of the host cell to be used for expression. The control sequence is operably linked to the nucleic acid sequence to be expressed. Such control sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Control sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc*. The expression vectors described herein can be introduced into host cells to produce polypeptides, including fusion polypeptides, encoded by the nucleic acids as described herein.

[0245] Recombinant expression vectors can be designed for expression of an aldehyde, fatty alcohol, alkane, and/or alkene biosynthetic polypeptide or variant in prokaryotic or eukaryotic cells (*e.g.*, bacterial cells, such as *E. coli*, insect cells (using baculovirus expression vectors), yeast cells, or mammalian cells). Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, for example, by using T7 promoter regulatory sequences and T7 polymerase.

[0246] Expression of polypeptides in prokaryotes, for example, *E. coli*, is most often carried out with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide. Such fusion vectors typically serve three purposes: (1) to increase expression of the recombinant polypeptide; (2) to increase the solubility of the recombinant polypeptide; and (3) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide. This enables separation of the recombinant polypeptide from the fusion moiety after purification of the fusion

polypeptide. Examples of such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin, and enterokinase. Exemplary fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith *et al.*, *Gene* (1988) 67:31-40), pMAL (New England Biolabs, Beverly, Mass.), and pRITS (Pharmacia, Piscataway, N.J.), which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the target recombinant polypeptide.

[0247] Examples of inducible, non-fusion *E. coli* expression vectors include pTrc (Amann *et al.*, *Gene* (1988) 69:301-315) and pET 11d (Studier *et al.*, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a coexpressed viral RNA polymerase (T7 gn1). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

[0248] One strategy to maximize recombinant polypeptide expression is to express the polypeptide in a host cell with an impaired capacity to proteolytically cleave the recombinant polypeptide (see Gottesman, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, Calif. (1990) 119-128). Another strategy is to alter the nucleic acid sequence to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the host cell (Wada *et al.*, *Nucleic Acids Res.* (1992) 20:2111-2118). Such alteration of nucleic acid sequences can be carried out by standard DNA synthesis techniques.

[0249] In another embodiment, the host cell is a yeast cell. In this embodiment, the expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari *et al.*, *EMBO J.* (1987) 6:229-234), pMFa (Kurjan *et al.*, *Cell* (1982) 30:933-943), pJRY88 (Schultz *et al.*, *Gene* (1987) 54:113-123), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.).

[0250] Alternatively, a polypeptide described herein can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of proteins in cultured insect cells (*e.g.*, Sf 9 cells) include, for example, the pAc series (Smith *et al.*, *Mol. Cell Biol.* (1983) 3:2156-2165) and the pVL series (Lucklow *et al.*, *Virology* (1989) 170:31-39).

[0251] In yet another embodiment, the nucleic acids described herein can be expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, *Nature* (1987) 329:840) and pMT2PC (Kaufman *et al.*, *EMBO J.* (1987) 6:187-195). When used in mammalian cells, the expression vector's control functions can be provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40. Other suitable expression systems for both prokaryotic and eukaryotic cells are described in chapters 16 and 17 of Sambrook *et al.*, eds., Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

[0252] Vectors can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, or electroporation. Suitable methods for transforming or transfecting host cells can be found in, for example, Sambrook *et al.* (supra).

[0253] For stable transformation of bacterial cells, it is known that, depending upon the expression vector and transformation technique used, only a small fraction of cells will take-up and replicate the expression vector. In order to identify and select these transformants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) can be introduced into the host cells along with the gene of interest. Selectable markers include those that confer resistance to drugs, such as ampacillin, kanamycin, chloramphenicol, or tetracycline. Nucleic acids encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a polypeptide described herein or can be introduced on a separate vector. Cells stably

transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

[0254] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) can be introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin, and methotrexate. Nucleic acids encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a polypeptide described herein or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

[0255] In certain methods, an aldehyde biosynthetic polypeptide and an alkane or alkene biosynthetic polypeptide are co-expressed in a single host cell. In alternate methods, an aldehyde biosynthetic polypeptide and an alcohol dehydrogenase polypeptide are co-expressed in a single host cell.

Transport Proteins

[0256] Transport proteins can export polypeptides and hydrocarbons (*e.g.*, aldehydes, alkanes, and/or alkenes) out of a host cell. Many transport and efflux proteins serve to excrete a wide variety of compounds and can be naturally modified to be selective for particular types of hydrocarbons.

[0257] Non-limiting examples of suitable transport proteins are ATP-Binding Cassette (ABC) transport proteins, efflux proteins, and fatty acid transporter proteins (FATP). Additional non-limiting examples of suitable transport proteins include the ABC transport proteins from organisms such as *Caenorhabditis elegans*, *Arabidopsis thalania*, *Alkaligenes eutrophus*, and *Rhodococcus erythropolis*. Exemplary ABC transport proteins that can be used are listed in Figure 40 (*e.g.*, CER5, AtMRP5, AmiS2, and AtPGP1). Host cells can also be chosen for their endogenous ability to secrete hydrocarbons. The efficiency of hydrocarbon

production and secretion into the host cell environment (*e.g.*, culture medium, fermentation broth) can be expressed as a ratio of intracellular product to extracellular product. In some examples, the ratio can be about 5:1, 4:1, 3:1, 2:1, 1:1, 1:2, 1:3, 1:4, or 1:5.

Fermentation

[0258] The production and isolation of aldehydes, fatty alcohols, alkanes and/or alkenes can be enhanced by employing beneficial fermentation techniques. One method for maximizing production while reducing costs is increasing the percentage of the carbon source that is converted to hydrocarbon products.

[0259] During normal cellular lifecycles, carbon is used in cellular functions, such as producing lipids, saccharides, proteins, organic acids, and nucleic acids. Reducing the amount of carbon necessary for growth-related activities can increase the efficiency of carbon source conversion to product. This can be achieved by, for example, first growing host cells to a desired density (for example, a density achieved at the peak of the log phase of growth). At such a point, replication checkpoint genes can be harnessed to stop the growth of cells. Specifically, quorum sensing mechanisms (reviewed in Camilli *et al.*, *Science* 311:1113, 2006; Venturi *FEMS Microbio. Rev.* 30:274-291, 2006; and Reading *et al.*, *FEMS Microbiol. Lett.* 254:1-11, 2006) can be used to activate checkpoint genes, such as *p53*, *p21*, or other checkpoint genes.

[0260] Genes that can be activated to stop cell replication and growth in *E. coli* include *umuDC* genes. The overexpression of *umuDC* genes stops the progression from stationary phase to exponential growth (Murli *et al., J. of Bact.* 182:1127, 2000). UmuC is a DNA polymerase that can carry out translesion synthesis over non-coding lesions – the mechanistic basis of most UV and chemical mutagenesis. The *umuDC* gene products are involved in the process of translesion synthesis and also serve as a DNA sequence damage checkpoint. The *umuDC* gene products include UmuC, UmuD, umuD', $UmuD'_2C$, $UmuD'_2$, and $UmuD_2$. Simultaneously, product-producing genes can be activated, thus minimizing the need for replication and maintenance pathways to be used while an aldehyde, alkane and/or alkene is being made. Host cells can also be engineered to express *umuC* and *umuD* from *E.*

coli in pBAD24 under the *prpBCDE* promoter system through *de novo* synthesis of this gene with the appropriate end-product production genes.

[0261] The percentage of input carbons converted to aldehydes, fatty alcohols, alkanes and/or alkenes can be a cost driver. The more efficient the process is (*i.e.*, the higher the percentage of input carbons converted to aldehydes, fatty alcohols, alkanes and/or alkenes), the less expensive the process will be. For oxygen-containing carbon sources (*e.g.*, glucose and other carbohydrate based sources), the oxygen must be released in the form of carbon dioxide. For every 2 oxygen atoms released, a carbon atom is also released leading to a maximal theoretical metabolic efficiency of approximately 34% (w/w) (for fatty acid derived products). This figure, however, changes for other hydrocarbon products and carbon sources. Typical efficiencies in the literature are approximately less than 5%. Host cells engineered to produce aldehydes, alkanes and/or alkenes can have greater than about 1, 3, 5, 10, 15, 20, 25, and 30% efficiency. In one example, host cells can exhibit an efficiency of about 10% to about 25%. In other examples, such host cells can exhibit an efficiency of about 25% to about 30%. In other examples, host cells can exhibit greater than 30% efficiency.

[0262] The host cell can be additionally engineered to express recombinant cellulosomes, such as those described in PCT application number PCT/US2007/003736. These cellulosomes can allow the host cell to use cellulosic material as a carbon source. For example, the host cell can be additionally engineered to express invertases (EC 3.2.1.26) so that sucrose can be used as a carbon source. Similarly, the host cell can be engineered using the teachings described in U.S. Patent Nos. 5,000,000; 5,028,539; 5,424,202; 5,482,846; and 5,602,030; so that the host cell can assimilate carbon efficiently and use cellulosic materials as carbon sources.

[0263] In one example, the fermentation chamber can enclose a fermentation that is undergoing a continuous reduction. In this instance, a stable reductive environment can be created. The electron balance can be maintained by the release of carbon dioxide (in gaseous form). Efforts to augment the NAD/H and NADP/H balance can also facilitate in stabilizing the electron balance. The availability of intracellular NADPH can also be enhanced by engineering the host cell to express an

NADH:NADPH transhydrogenase. The expression of one or more NADH:NADPH transhydrogenases converts the NADH produced in glycolysis to NADPH, which can enhance the production of aldehydes, alkanes and/or alkenes.

[0264] For small scale production, the engineered host cells can be grown in batches of, for example, around 100 mL, 500 mL, 1 L, 2 L, 5 L, or 10 L; fermented; and induced to express desired aldehydes, fatty alcohols, alkanes and/or alkenes based on the specific genes encoded in the appropriate plasmids. For example, *E. coli* BL21(DE3) cells harboring pBAD24 (with ampicillin resistance and the aldehyde, fatty alcohol, alkane, or alkene synthesis pathway) as well as pUMVC1 (with kanamycin resistance and the acetyl CoA/malonyl CoA overexpression system) can be incubated overnight in 2 L flasks at 37°C shaken at > 200 rpm in 500 mL LB medium supplemented with 75 μg/mL ampicillin and 50 μg/mL kanamycin until cultures reach an OD_{600} of > 0.8. Upon achieving an OD_{600} of > 0.8, the cells can be supplemented with 25 mM sodium proprionate (pH 8.0) to activate the engineered gene systems for production and to stop cellular proliferation by activating UmuC and UmuD proteins. Induction can be performed for 6 hrs at 30°C. After incubation, the media can be examined for aldehydes, fatty alcohols, alkanes and/or alkenes using GC-MS.

[0265] For large scale production, the engineered host cells can be grown in batches of 10 L, 100 L, 1000 L ,or larger; fermented; and induced to express desired aldehydes, fatty alcohols, alkanes and/or alkenes based on the specific genes encoded in the appropriate plasmids. For example, *E. coli* BL21(DE3) cells harboring pBAD24 (with ampicillin resistance and the aldehyde and/or alkane synthesis pathway) as well as pUMVC1 (with kanamycin resistance and the acetyl-CoA/malonyl-CoA overexpression system) can be incubated from a 500 mL seed culture for 10 L fermentations (5 L for 100 L fermentations, *etc.*) in LB media (glycerol free) with 50 μg/mL kanamycin and 75 μg/mL ampicillin at 37°C, and shaken at > 200 rpm until cultures reach an OD_{600} of > 0.8 (typically 16 hrs). Media can be continuously supplemented to maintain 25 mM sodium proprionate (pH 8.0) to activate the engineered gene systems for production and to stop cellular proliferation by activating umuC and umuD proteins. Media can be continuously supplemented with glucose to maintain a concentration 25 g/100 mL.

[0266] After the first hour of induction, aliquots of no more than 10% of the total cell volume can be removed each hour and allowed to sit without agitation to allow the aldehydes, alkanes and/or alkenes to rise to the surface and undergo a spontaneous phase separation. The aldehyde, fatty alcohols, alkane and/or alkene component can then be collected, and the aqueous phase returned to the reaction chamber. The reaction chamber can be operated continuously. When the OD_{600} drops below 0.6, the cells can be replaced with a new batch grown from a seed culture.

Producing Aldehydes, Fatty Alcohols, Alkanes and Alkenes using Cell-free Methods

[0267] In some methods described herein, an aldehyde, fatty alcohols, alkane and/or alkene can be produced using a purified polypeptide described herein and a substrate described herein. For example, a host cell can be engineered to express aldehyde, fatty alcohols, alkane and/or alkene biosynthetic polypeptide or variant as described herein. The host cell can be cultured under conditions suitable to allow expression of the polypeptide. Cell free extracts can then be generated using known methods. For example, the host cells can be lysed using detergents or by sonication. The expressed polypeptides can be purified using known methods. After obtaining the cell free extracts, substrates described herein can be added to the cell free extracts and maintained under conditions to allow conversion of the substrates to aldehydes, fatty alcohols, alkanes and/or alkenes. The aldehydes, fatty alcohols, alkanes and/or alkenes can then be separated and purified using known techniques.

Post-Production Processing

[0268] The aldehydes, fatty alcohols, alkanes and/or alkenes produced during fermentation can be separated from the fermentation media. Any known technique for separating aldehydes, fatty alcohols, alkanes and/or alkenes from aqueous media can be used. One exemplary separation process is a two phase (bi-phasic) separation process. This process involves fermenting the genetically engineered host cells under conditions sufficient to produce an aldehyde, fatty alcohols, alkane and/or alkene, allowing the aldehyde, fatty alcohols, alkane and/or alkene to collect in an

organic phase, and separating the organic phase from the aqueous fermentation broth. This method can be practiced in both a batch and continuous fermentation setting.

[0269] Bi-phasic separation uses the relative immiscibility of aldehydes, fatty alcohols, alkanes and/or alkenes to facilitate separation. Immiscible refers to the relative inability of a compound to dissolve in water and is defined by the compound's partition coefficient. One of ordinary skill in the art will appreciate that by choosing a fermentation broth and organic phase, such that the aldehyde, alkane and/or alkene being produced has a high logP value, the aldehyde, alkane and/or alkene can separate into the organic phase, even at very low concentrations, in the fermentation vessel.

[0270] The aldehydes, fatty alcohols, alkanes and/or alkenes produced by the methods described herein can be relatively immiscible in the fermentation broth, as well as in the cytoplasm. Therefore, the aldehyde, fatty alcohols, alkane and/or alkene can collect in an organic phase either intracellularly or extracellularly. The collection of the products in the organic phase can lessen the impact of the aldehyde, fatty alcohols, alkane and/or alkene on cellular function and can allow the host cell to produce more product.

[0271] The methods described herein can result in the production of homogeneous compounds wherein at least about 60%, 70%, 80%, 90%, or 95% of the aldehydes, fatty alcohols, alkanes and/or alkenes produced will have carbon chain lengths that vary by less than about 6 carbons, less than about 4 carbons, or less than about 2 carbons. These compounds can also be produced with a relatively uniform degree of saturation. These compounds can be used directly as fuels, fuel additives, specialty chemicals, starting materials for production of other chemical compounds (*e.g.*, polymers, surfactants, plastics, textiles, solvents, adhesives, *etc.*), or personal care product additives. These compounds can also be used as feedstock for subsequent reactions, for example, hydrogenation, catalytic cracking (*via* hydrogenation, pyrolisis, or both), to make other products.

[0272] In some embodiments, the aldehydes, fatty alcohols, alkanes and/or alkenes produced using methods described herein can contain between about 50% and about 90% carbon; or between about 5% and about 25% hydrogen. In other

embodiments, the aldehydes, fatty alcohols, alkanes and/or alkenes produced using methods described herein can contain between about 65% and about 85% carbon; or between about 10% and about 15% hydrogen.

Fuel Compositions and Specialty Chemical Compositions

[0273] The aldehydes, fatty alcohols, alkanes and/or alkenes described herein can be used as or converted into a fuel or as a specialty chemical. One of ordinary skill in the art will appreciate that, depending upon the intended purpose of the fuel or specialty chemical, different aldehydes, fatty alcohols, alkanes and/or alkenes can be produced and used. For example, a branched aldehyde, fatty alcohol, alkane and/or alkene may be desirable for automobile fuel that is intended to be used in cold climates. In addition, when the aldehydes, fatty alcohols, alkanes and/or alkenes described herein are used as a feedstock for fuel or specialty chemical production, one of ordinary skill in the art will appreciate that the characteristics of the aldehyde, fatty alcohol, alkane and/or alkene feedstock will affect the characteristics of the fuel or specialty chemical produced. Hence, the characteristics of the fuel or specialty chemical product can be selected for by producing particular aldehydes, fatty alcohols, alkanes and/or alkenes for use as a feedstock.

[0274] Using the methods described herein, biofuels having desired fuel qualities can be produced from aldehydes, fatty alcohols, alkanes and/or alkenes. Biologically produced aldehydes, fatty alcohols, alkanes and/or alkenes represent a new source of biofuels, which can be used as jet fuel, diesel, or gasoline. Some biofuels made using aldehydes, fatty alcohols, alkanes and/or alkenes have not been produced from renewable sources and are new compositions of matter. These new fuels or specialty chemicals can be distinguished from fuels or specialty chemicals derived from petrochemical carbon on the basis of dual carbon-isotopic fingerprinting. Additionally, the specific source of biosourced carbon (*e.g.*, glucose vs. glycerol) can be determined by dual carbon-isotopic fingerprinting (*see*, *e.g.*, U.S. Patent No. 7,169,588, in particular col. 4, line 31, to col. 6, line 8).

[0275] The aldehydes, fatty alcohols, alkanes and/or alkenes and the associated biofuels, specialty chemicals, and mixtures can be distinguished from their petrochemical derived counterparts on the basis of ^{14}C (f_M) and dual carbon-isotopic

fingerprinting. In some examples, the aldehyde, fatty alcohol, alkane and/or alkene in the biofuel composition can have a fraction of modern carbon (f_M ^{14}C) of, for example, at least about 1.003, 1.010, or 1.5.

[0276] In some examples, a biofuel composition can be made that includes aldehydes, fatty alcohols, alkanes and/or alkenes having $\delta^{13}C$ of from about -15.4 to about -10.9, where the aldehydes, fatty alcohols, alkanes and/or alkenes account for at least about 85% of biosourced material (*i.e.*, derived from a renewable resource, such as biomass, cellulosic materials, and sugars) in the composition.

[0277] The ability to distinguish these biologically derived products is beneficial in tracking these materials in commerce. For example, fuels or specialty chemicals comprising both biologically derived and petroleum-based carbon isotope profiles can be distinguished from fuels and specialty chemicals made only of petroleum-based materials. Thus, the aldehydes, fatty alcohols, alkanes and/or alkenes described herein can be followed in commerce or identified in commerce as a biofuel on the basis of their unique profile. In addition, other competing materials can be identified as being biologically derived or derived from a petrochemical source.

[0278] Fuel additives are used to enhance the performance of a fuel or engine. For example, fuel additives can be used to alter the freezing/gelling point, cloud point, lubricity, viscosity, oxidative stability, ignition quality, octane level, and/or flash point. In the United States, all fuel additives must be registered with Environmental Protection Agency. The names of fuel additives and the companies that sell the fuel additives are publicly available by contacting the EPA or by viewing the agency's website. One of ordinary skill in the art will appreciate that the aldehyde- and/or alkane-based biofuels described herein can be mixed with one or more fuel additives to impart a desired quality.

[0279] The aldehyde, fatty alcohols, alkane and/or alkene-based biofuels described herein can be mixed with other fuels, such as various alcohols, such as ethanol and butanol, and petroleum-derived products, such as gasoline, diesel, or jet fuel.

[0280] In some examples, the mixture can include at least about 10%, 15%, 20%, 30%, 40%, 50%, or 60% by weight of the aldehyde, fatty alcohols, alkane, or

alkene. In other examples, a biofuel composition can be made that includes at least about 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90% or 95% of an aldehyde, fatty alcohols, alkane, or alkene that includes a carbon chain that is 8, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or 22 carbons in length. Such biofuel compositions can additionally include at least one additive selected from a cloud point lowering additive that can lower the cloud point to less than about 5°C, or 0°C; a surfactant; a microemulsion; at least about 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, or 95% diesel fuel from triglycerides; petroleum-derived gasoline; or diesel fuel from petroleum.

EXAMPLES

[0281] The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

Example 1. Detection and verification of alkane biosynthesis in selected cyanobacteria

[0282] Seven cyanobacteria, whose complete genome sequences are publicly available, were selected for verification and/or detection of alkane biosynthesis: *Synechococcus elongatus* PCC7942, *Synechococcus elongatus* PCC6301, *Anabaena variabilis* ATCC29413, *Synechocystis sp.* PCC6803, *Nostoc punctiforme* PCC73102, *Gloeobacter violaceus* ATCC 29082, and *Prochlorococcus marinus* CCMP1986. Only the first three cyanobacterial strains from this list had previously been reported to contain alkanes (Han *et al.*, *J. Am. Chem. Soc.* 91:5156-5159 (1969); Fehler *et al.*, *Biochem.* 9:418-422 (1970)). The strains were grown photoautotrophically in shake flasks in 100 mL of the appropriate media (listed in Table 8) for 3-7 days at 30°C at a light intensity of approximately 3,500 lux. Cells were extracted for alkane detection as follows: cells from 1 mL culture volume were centrifuged for 1 min at 13,000 rpm, the cell pellets were resuspended in methanol, vortexed for 1 min and then sonicated for 30 min. After centrifugation for 3 min at 13,000 rpm, the supernatants were transferred to fresh vials and analyzed by GC-MS. The samples were analyzed on either 30 m DP-5 capillary column (0.25 mm internal diameter) or a 30 m high temperature DP-5 capillary column (0.25mm internal diameter) using the following method.

[0283] After a 1 μL splitless injection (inlet temperature held at 300°C) onto the GC/MS column, the oven was held at 100°C for 3 mins. The temperature was ramped up to 320°C at a rate of 20°C/min. The oven was held at 320°C for an additional 5 min. The flow rate of the carrier gas helium was 1.3 mL/min. The MS quadrapole scanned from 50 to 550 m/z. Retention times and fragmentation patterns of product peaks were compared with authentic references to confirm peak identity.

[0284] Out of the seven strains, six produced mainly heptadecane and one produced pentadecane (*P. marinus* CCMP1986); one of these strains produced methyl-heptadecane in addition to heptadecane (*A. variabilis* ATCC29413) (see Table 8). Therefore, alkane biosynthesis in three previously reported cyanobacteria was verified, and alkane biosynthesis was detected in four cyanobacteria that were not previously known to produce alkanes: *P. marinus* CCMP1986 (see Figure 1), *N. punctiforme* PCC73102 (see Figure 2), *G. violaceus* ATCC 29082 (see Figure 3) and *Synechocystis sp.* PCC6803 (see Figure 4).

[0285] Figure 1A depicts the GC/MS trace of *Prochlorococcus marinus* CCMP1986 cells extracted with methanol. The peak at 7.55 min had the same retention time as pentadecane (Sigma). In Figure 1B, the mass fragmentation pattern of the pentadecane peak is shown. The 212 peak corresponds to the molecular weight of pentadecane.

[0286] Figure 2A depicts the GC/MS trace of *Nostoc punctiforme* PCC73102 cells extracted with methanol. The peak at 8.73 min has the same retention time as heptadecane (Sigma). In Figure 2B, the mass fragmentation pattern of the heptadecane peak is shown. The 240 peak corresponds to the molecular weight of heptadecane.

[0287] Figure 3A depicts the GC/MS trace of *Gloeobaceter violaceus* ATCC29082 cells extracted with methanol. The peak at 8.72 min has the same retention time as heptadecane (Sigma). In Figure 3B, the mass fragmentation pattern of the heptadecane peak is shown. The 240 peak corresponds to the molecular weight of heptadecane.

[0288] Figure 4A depicts the GC/MS trace of *Synechocystic sp.* PCC6803 cells extracted with methanol. The peak at 7.36 min has the same retention time as heptadecane (Sigma). In Figure 4B, the mass fragmentation pattern of the

heptadecane peak is shown. The 240 peak corresponds to the molecular weight of heptadecane.

Table 8: Hydrocarbons detected in selected cyanobacteria

Cyanobacterium	ATCC#	Genome	Medium	Alkanes	
				reported	verified [2]
Synechococcus elongatus PCC7942	27144	2.7 Mb	BG-11	C17:0	**C17:0**, C15:0
Synechococcus elongatus PCC6301	33912	2.7 Mb	BG-11	C17:0	**C17:0**, C15:0
Anabaena variabilis	29413	6.4 Mb	BG-11	C17:0, 7- or 8-Me-C17:0	**C17:0**, Me-C17:0
Synechocystis sp. PCC6803	27184	3.5 Mb	BG-11	-	**C17:0**, C15:0
Prochlorococcus marinus CCMP1986 [1]	-	1.7 Mb	-	-	**C15:0**
Nostoc punctiforme PCC73102	29133	9.0 Mb	ATCC819	-	**C17:0**
Gloeobacter violaceus	29082	4.6 Mb	BG11	-	**C17:0**
[1] cells for extraction were a gift from Jacob Waldbauer (MIT)					
[2] major hydrocarbon is in bold					

[0289] Genomic analysis yielded two genes that were present in the alkane-producing strains. The *Synechococcus elongatus* PCC7942 homologs of these genes are depicted in Table 9 and are Synpcc7942_1593 (SEQ ID NO:1) and Synpcc7942_1594 (SEQ ID NO:65).

Table 9: Alkane-producing cyanobacterial genes

Gene Object ID	Locus Tag	Genbank accession	Gene Name	Length	COG	Pfam	InterPro	Notes
637800026	Synpcc7942_1593	YP_400610	hypothetical protein	231 aa	-	pfam02915	IPR009078 IPR003251	ferritin/ribonucleotide reductase-like rubreryhtrin
637800027	Synpcc7942_1594	YP_400611	hypothetical protein	341 aa	COG5322	pfam00106	IPR000408 IPR016040 IPR002198	predicted dehydrogenase NAD(P)-binding short chain dehydrogenase

Example 2. Deletion of the sll0208 and sll0209 genes in *Synechocystis* sp. PCC6803 leads to loss of Alkane Biosynthesis

[0290] The genes encoding the putative decarbonylase (sll0208; NP_442147) (SEQ ID NO:3) and aldehyde-generating enzyme (sll0209; NP_442146) (SEQ ID NO:67) of *Synechocystis* sp. PCC6803 were deleted as follows. Approximately 1 kb of upstream and downstream flanking DNA were amplified using primer sll0208/9-KO1 (CGCGGATCCCTTGATTCTACTGCGGCGAGT) with primer sll0208/9-KO2
(CACGCACCTAGGTTCACACTCCCATGGTATAACAGGGGCGTTGGACTCC TGTG) and primer sll0208/9-KO3
(GTTATACCATGGGAGTGTGAACCTAGGTGCGTGGCCGACAGGATAGGG-CGTGT) with primer sll0208/9-KO4
(CGCGGATCCAACGCATCCTCACTAGTCGGG), respectively. The PCR products were used in a cross-over PCR with primers sll0208/9-KO1 and sll0208/9-KO4 to amplify the approximately 2 kb sll0208/sll0209 deletion cassette, which was cloned into the *Bam*HI site of the cloning vector pUC19. A kanamycin resistance cassette (aph, KanR) was then amplified from plasmid pRL27 (Larsen *et al.*, *Arch. Microbiol.* 178:193 (2002)) using primers Kan-aph-F
(CATGCCATGGAAAGCCACGTTGTGTCTCAAAATCTCTG) and Kan-aph-R (CTAGTCTAGAGCGCTGAGGTCTGCCTCGTGAA), which was then cut with *Nco*I and *Xba*I and cloned into the *Nco*I and *Avr*II sites of the sll0208/sll0209 deletion cassette, creating a sll0208/sll0209-deletion KanR-insertion cassette in pUC19. The cassette-containing vector, which does not replicate in cyanobacteria, was transformed into *Synechocystis* sp. PCC6803 (Zang et al., 2007, J. Microbiol.,

vol. 45, pp. 241) and transformants (*e.g.*, chromosomal integrants by double-homologous recombination) were selected on BG-11 agar plates containing 100 μg/mL Kanamycin in a light-equipped incubator at 30 °C. Kanamycin resistant colonies were restreaked once and then subjected to genotypic analysis using PCR with diagnostic primers.

[0291] Confirmed deletion-insertion mutants were cultivated in 12 mL of BG11 medium with 50 μg/mL Kanamycin for 4 days at 30°C in a light-equipped shaker-incubator. 1 mL of broth was then centrifuged (1 min at 13,000 g) and the cell pellets were extracted with 0.1 mL methanol. After extraction, the samples were again centrifuged and the supernatants were subjected to GC-MS analysis as described in Example 1.

[0292] As shown in Fig. 5, the *Synechocystis* sp. PCC6803 strains in which the sll0208 and sll0209 genes were deleted lost their ability to produce heptadecene and octadecenal. This result demonstrates that the sll0208 and sll0209 genes in *Synechocystis* sp. PCC6803 and the orthologous genes in other cyanobacteria (see Table 1) are responsible for alkane and fatty aldehyde biosynthesis in these organisms.

Example 3. Production of Fatty Aldehydes and Fatty Alcohols in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594

[0293] The genomic DNA encoding *Synechococcus elongatus* PCC7942 orf1594 (YP_400611; putative aldehyde-generating enzyme) (SEQ ID NO:65) was amplified and cloned into the *Nco*I and *Eco*RI sites of vector OP-80 (pCL1920 derivative) under the control of the P_{trc} promoter. The resulting construct ("OP80-PCC7942_1594") was transformed into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media with 1% (w/v) glucose as carbon source and supplemented with 100 μg/mL spectinomycin. When the culture reached OD_{600} of 0.8-1.0, it was induced with 1mM IPTG and cells were grown for an additional 18-20 h at 37°C. Cells from 0.5 mL of culture were extracted with 0.5 mL of ethyl acetate. After sonication for 60 min, the sample was centrifuged at 15,000 rpm for 5 min. The solvent layer was analyzed by GC-MS as described in Example 1.

[0294] As shown in Fig. 6, *E. coli* cells transformed with the *Synechococcus elongatus* PCC7942 orf1594 -bearing vector produced the following fatty aldehydes and fatty alcohols: hexadecanal, octadecenal, tetradecenol, hexadecenol, hexadecanol and octadecenol. This result indicates that PCC7942 orf1594 (i) generates aldehydes *in-vivo* as possible substrates for decarbonylation and (ii) may reduce acyl-ACPs as substrates, which are the most abundant form of activated fatty acids in wild type *E. coli* cells. Therefore, the enzyme was named Acyl-ACP reductase. *In-vivo*, the fatty aldehydes apparently are further reduced to the corresponding fatty alcohols by an endogenous *E. coli* aldehyde reductase activity.

Example 4. Production of Fatty Aldehydes and Fatty Alcohols in *E. coli* through Heterologous Expression of *Cyanothece* sp. ATCC51142 cce_1430

[0295] The genomic DNA encoding *Cyanothece* sp. ATCC51142 cce_1430 (YP_001802846; putative aldehyde-generating enzyme) (SEQ ID NO:69) was amplified and cloned into the *Nco*I and *Eco*RI sites of vector OP-80 (pCL1920 derivative) under the control of the P_{trc} promoter. The resulting construct was transformed into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media with 1% (w/v) glucose as carbon source and supplemented with 100 μg/mL spectinomycin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0296] As shown in Fig. 7, *E. coli* cells transformed with the *Cyanothece* sp. ATCC51142 cce_1430 -bearing vector produced the following fatty aldehydes and fatty alcohols: hexadecanal, octadecenal, tetradecenol, hexadecenol, hexadecanol and octadecenol. This result indicates that ATCC51142 cce_1430 (i) generates aldehydes *in-vivo* as possible substrates for decarbonylation and (ii) may reduce acyl-ACPs as substrates, which are the most abundant form of activated fatty acids in wild type *E. coli* cells. Therefore, this enzyme is also an Acyl-ACP reductase.

Example 5. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Synechococcus elongatus* PCC7942 orf1593

[0297] The genomic DNA encoding *Synechococcus elongatus* PCC7942 orf1593 (YP_400610; putative decarbonylase) (SEQ ID NO:1) was amplified and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 1.

[0298] As shown in Fig. 8, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *S. elongatus* PCC7942_1593-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that PCC7942_1593 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 6. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Nostoc punctiforme* PCC73102 Npun02004178

[0299] The genomic DNA encoding *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838; putative decarbonylase) (SEQ ID NO:5) was amplified and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 1.

[0300] As shown in Fig. 9, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *N. punctiforme* PCC73102 Npun02004178-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also tridecane, pentadecene, pentadecane and heptadecene. This result indicates that Npun02004178 in *E. coli* converts tetradecanal, hexadecenal, hexadecanal and

octadecenal to tridecane, pentadecene, pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 7. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Synechocystis* sp. PCC6803 sll0208

[0301] The genomic DNA encoding *Synechocystis* sp. PCC6803 sll0208 (NP_442147; putative decarbonylase) (SEQ ID NO:3) was amplified and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 1.

[0302] As shown in Fig. 10, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *Synechocystis* sp. PCC6803 sll0208-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that Npun02004178 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 8. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Nostoc* sp. PCC7210 alr5283

[0303] The genomic DNA encoding *Nostoc* sp. PCC7210 alr5283 (NP_489323; putative decarbonylase) (SEQ ID NO:7) was amplified and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 1.

[0304] As shown in Fig. 11, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *Nostoc* sp. PCC7210 alr5283-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that alr5283 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 9. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Acaryochloris marina* MBIC11017 AM1_4041

[0305] The genomic DNA encoding *Acaryochloris marina* MBIC11017 AM1_4041 (YP_001518340; putative decarbonylase) (SEQ ID NO:9) was codon optimized for expression in *E. coli* (SEQ ID NO:46), synthesized, and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0306] As shown in Fig. 12, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *A.marina* MBIC11017 AM1_4041-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also tridecane, pentadecene, pentadecane and heptadecene. This result indicates that AM1_4041 in *E. coli* converts tetradecanal, hexadecenal, hexadecanal and octadecenal to tridecane, pentadecene, pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 10. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Thermosynechococcus elongatus* BP-1 tll1313

[0307] The genomic DNA encoding *Thermosynechococcus elongatus* BP-1 tll1313 (NP_682103; putative decarbonylase) (SEQ ID NO:11) was codon

optimized for expression in *E. coli* (SEQ ID NO:47), synthesized, and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0308] As shown in Fig. 13, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *T. elongatus* BP-1 tll1313-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that tll1313 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 11. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Synechococcus sp.* JA-3-3Ab CYA_0415

[0309] The genomic DNA encoding *Synechococcus sp.* JA-3-3Ab CYA_0415 (YP_473897; putative decarbonylase) (SEQ ID NO:13) was codon optimized for expression in *E. coli* (SEQ ID NO:48), synthesized, and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0310] As shown in Fig. 14, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *Synechococcus sp.* JA-3-3Ab CYA_0415-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that Npun02004178 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 12. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Gloeobacter violaceus* PCC7421 gll3146

[0311] The genomic DNA encoding *Gloeobacter violaceus* PCC7421 gll3146 (NP_926092; putative decarbonylase) (SEQ ID NO:15) was amplified and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 1.

[0312] As shown in Fig. 15, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *G. violaceus* PCC7421 gll3146-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that gll3146 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 13. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Prochlorococcus marinus* MIT9313 PMT1231

[0313] The genomic DNA encoding *Prochlorococcus marinus* MIT9313 PMT1231 (NP_895059; putative decarbonylase) (SEQ ID NO:17) was codon optimized for expression in *E. coli* (SEQ ID NO:49), synthesized, and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0314] As shown in Fig. 16, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *P.marinus* MIT9313 PMT1231-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that PMT1231 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 14. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Prochlorococcus marinus* CCMP1986 PMM0532

[0315] The genomic DNA encoding *Prochlorococcus marinus* CCMP1986 PMM0532 (NP_892650; putative decarbonylase) (SEQ ID NO:19) was amplified and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 1.

[0316] As shown in Fig. 17, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *P.marinus* CCMP1986 PMM0532-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that PMM0532 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 15. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Prochlorococcus mariunus* NATL2A PMN2A_1863

[0317] The genomic DNA encoding *Prochlorococcus mariunus* NATL2A PMN2A_1863 (YP_293054; putative decarbonylase) (SEQ ID NO:21) was codon optimized for expression in *E. coli* (SEQ ID NO:51), synthesized, and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of

the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0318] As shown in Fig. 18, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *P.mariunus* NATL2A PMN2A_1863-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that PMN2A_1863 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 16. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Synechococcus* sp. RS9917 RS9917_09941

[0319] The genomic DNA encoding *Synechococcus* sp. RS9917 RS9917_09941 (ZP_01079772; putative decarbonylase) (SEQ ID NO:23) was codon optimized for expression in *E. coli* (SEQ ID NO:52), synthesized, and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0320] As shown in Fig. 19, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *Synechococcus* sp. RS9917 RS9917_09941-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that RS9917_09941 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 17. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Synechococcus* sp. RS9917 RS9917_12945

[0321] The genomic DNA encoding *Synechococcus* sp. RS9917 RS9917_12945 (ZP_01080370; putative decarbonylase) (SEQ ID NO:25) was codon optimized for expression in *E. coli* (SEQ ID NO:53), synthesized, and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0322] As shown in Fig. 20, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *Synechococcus* sp. RS9917 RS9917_12945-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also pentadecane and heptadecene. This result indicates that RS9917_12945 in *E. coli* converts hexadecanal and octadecenal to pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 18. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Cyanothece* sp. ATCC51142 cce_0778

[0323] The genomic DNA encoding *Cyanothece* sp. ATCC51142 cce_0778 (YP_001802195; putative decarbonylase) (SEQ ID NO:27) was synthesized and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0324] As shown in Fig. 21, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *Cyanothece* sp. ATCC51142 cce_0778 -bearing vectors

produced the same fatty aldehydes and fatty alcohols as in Example 3, but also tridecane, pentadecene, pentadecane and heptadecene. This result indicates that ATCC51142 cce_0778 in *E. coli* converts tetradecanal, hexadecenal, hexadecanal and octadecenal to tridecane, pentadecene, pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 19. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Cyanothece* sp. PCC7425 Cyan7425_0398

[0325] The genomic DNA encoding *Cyanothece* sp. PCC7425 Cyan7425_0398 (YP_002481151; putative decarbonylase) (SEQ ID NO:29) was synthesized and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0326] As shown in Fig. 22, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *Cyanothece* sp. PCC7425 Cyan7425_0398 -bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also tridecane, pentadecene, pentadecane and heptadecene. This result indicates that Cyan7425_0398in *E. coli* converts tetradecanal, hexadecenal, hexadecanal and octadecenal to tridecane, pentadecene, pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 20. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Cyanothece* sp. PCC7425 Cyan7425_2986

[0327] The genomic DNA encoding *Cyanothece* sp. PCC7425 Cyan7425_2986 (YP_002483683; putative decarbonylase) (SEQ ID NO:31) was synthesized and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-

PCC7942_1594 into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0328] As shown in Fig. 23, *E. coli* cells cotransformed with the *S. elongatus* PCC7942_1594 and *Cyanothece* sp. PCC7425 Cyan7425_2986-bearing vectors produced the same fatty aldehydes and fatty alcohols as in Example 3, but also tridecane, pentadecene, pentadecane and heptadecene. This result indicates that Cyan7425_2986 in *E. coli* converts tetradecanal, hexadecenal, hexadecanal and octadecenal to tridecane, pentadecene, pentadecane and heptadecene, respectively, and therefore is an active fatty aldehyde decarbonylase.

Example 21. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Prochlorococcus marinus* CCMP1986 PMM0533 and *Prochlorococcus mariunus* CCMP1986 PMM0532

[0329] The genomic DNA encoding *P.mariunus* CCMP1986 PMM0533 (NP_892651; putative aldehyde-generating enzyme) (SEQ ID NO:71) and *Prochlorococcus mariunus* CCMP1986 PMM0532 (NP_892650; putative decarbonylase) (SEQ ID NO:19) were amplified and cloned into the *Nco*I and *Eco*RI sites of vector OP-80 and the *Nde*I and *Xho*I sites of vector OP-183, respectively. The resulting constructs were separately transformed and cotransformed into *E. coli* MG1655 and the cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0330] As shown in Fig. 24A, *E. coli* cells transformed with only the *P.mariunus* CCMP1986 PMM0533-bearing vector did not produce any fatty aldehydes or fatty alcohols. However, *E. coli* cells cotransformed with PMM0533 and PMM0532-bearing vectors produced hexadecanol, pentadecane and heptadecene (Fig. 24B). This result indicates that PMM0533 only provides fatty aldehyde substrates for the decarbonylation reaction when it interacts with a decarbonylase, such as PMM0532.

Example 22. Production of Alkanes and Alkenes in a Fatty Acyl-CoA-producing *E. coli* strain through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Acaryochloris marina* MBIC11017 AM1_4041

[0331] The genomic DNA encoding *Acaryochloris marina* MBIC11017 AM1_4041 (YP_001518340; putative fatty aldehyde decarbonylase) (SEQ ID NO:9) was synthesized and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed with OP80-PCC7942_1594 into *E. coli* MG1655 Δ*fadE lacZ*::P_{trc} '*tesA-fadD*. This strain expresses a cytoplasmic version of the *E. coli* thioesterase, 'TesA, and the *E. coli* acyl-CoA synthetase, FadD, under the control of the P_{trc} promoter, and therefore produces fatty acyl-CoAs. The cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 1.

[0332] As shown in Fig. 25, these *E. coli* cells cotransformed with *S. elongatus* PCC7942_1594 and *A. marina* MBIC11017 AM1_4041 also produced alkanes and fatty alcohols. This result indicates that *S. elongatus* PCC7942_1594 is able to use acyl-CoA as a substrate to produce hexadecenal, hexadecanal and octadecenal, which is then converted into pentadecene, pentadecane and heptadecene, respectively, by *A. marina* MBIC11017 AM1_4041.

Example 23. Production of Alkanes and Alkenes in a Fatty Acyl-CoA-producing *E. coli* Strain through Heterologous Expression of *Synechocystis* sp. PCC6803 sll0209 and *Synechocystis* sp. PCC6803 sll0208

[0333] The genomic DNA encoding *Synechocystis* sp. PCC6803 sll0208 (NP_442147; putative fatty aldehyde decarbonylase) (SEQ ID NO:3) was synthesized and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The genomic DNA encoding *Synechocystis* sp. PCC6803 sll0209 (NP_442146; acyl-ACP reductase) (SEQ ID NO:67) was synthesized and cloned into the *Nco*I and *EcoR*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting constructs were cotransformed with into *E. coli* MG1655 Δ*fadE lacZ*::P_{trc} '*tesA-fadD*. This

strain expresses a cytoplasmic version of the *E. coli* thioesterase, 'TesA, and the *E. coli* acyl-CoA synthetase, FadD, under the control of the P_{trc} promoter, and therefore produces fatty acyl-CoAs. The cells were grown at 37°C in M9 minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 26.

[0334] As shown in Fig. 26, these *E. coli* cells transformed with *Synechocystis* sp. PCC6803 sll0209 did not produce any fatty aldehydes or fatty alcohols. However, when cotransformed with *Synechocystis* sp. PCC6803 sll0208 and sll0209, they produced alkanes, fatty aldehydes and fatty alcohols. This result indicates that *Synechocystis* sp. PCC6803 sll0209 is able to use acyl-CoA as a substrate to produce fatty aldehydes such as tetradecanal, hexadecanal and octadecenal, but only when coexpressed with a fatty aldehyde decarbonylase. The fatty aldehydes apparently are further reduced to the corresponding fatty alcohols, tetradecanol, hexadecanol and octadecenol, by an endogenous *E. coli* aldehyde reductase activity. In this experiment, octadecenal was converted into heptadecene by *Synechocystis* sp. PCC6803 sll0208.

Example 24. Production of Alkanes and Alkenes in a Fatty Aldehyde-producing *E. coli* Strain through Heterologous Expression of *Nostoc punctiforme* PCC73102 Npun02004178 and Several of its Homologs

[0335] The genomic DNA encoding *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838; putative fatty aldehyde decarbonylase) (SEQ ID NO:5) was amplified and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The genomic DNA encoding *Mycobacterium smegmatis* strain MC2 155 orf MSMEG_5739 (YP_889972, putative carboxylic acid reductase) (SEQ ID NO:85) was amplified and cloned into the *Nco*I and *EcoR*I sites of vector OP-180 (pCL1920 derivative) under the control of the P_{trc} promoter. The two resulting constructs were cotransformed into *E. coli* MG1655 Δ*fadD lacZ::*P_{trc}*-'tesA*. In this strain, fatty aldehydes were provided by MSMEG_5739, which reduces free fatty acids (formed by the action of 'TesA) to fatty aldehydes. The cells were grown at 37°C in M9

minimal media supplemented with 100 μg/mL spectinomycin and 100 μg/mL carbenicillin. The cells were cultured and extracted as in Example 3 and analyzed by GC-MS as described in Example 1.

[0336] As shown in Fig. 27, these *E. coli* cells cotransformed with the *N. punctiforme* PCC73102 Npun02004178 and *M. smegmatis* strain MC2 155 MSMEG_5739-bearing vectors produced tridecane, pentadecene and pentadecane. This result indicates that Npun02004178 in *E. coli* converts tetradecanal, hexadecenal and hexadecanal provided by the carboxylic acid reductase MSMEG_5739 to tridecane, pentadecene and pentadecane. As shown in Fig. 28, in the same experimental set-up, the following fatty aldehyde decarbonylases also converted fatty aldehydes provided by MSMEG_5739 to the corresponding alkanes when expressed in *E. coli* MG1655 Δ*fadD lacZ::*P_{trc}*-'tesA*: *Nostoc* sp. PCC7210 alr5283 (SEQ ID NO:7), *P. mariunus* CCMP1986 PMM0532 (SEQ ID NO:19), *G. violaceus* PCC7421 gll3146 (SEQ ID NO:15), *Synechococcus* sp. RS9917_09941 (SEQ ID NO:23), *Synechococcus* sp. RS9917_12945 (SEQ ID NO:25), and *A. marina* MBIC11017 AM1_4041 (SEQ ID NO:9).

Example 25: Cyanobacterial Fatty Aldehyde Decarbonylases belong to the class of Non-heme Diiron Proteins. Site-directed mutagenesis of conserved histidines to phenylalanines in *Nostoc punctiforme* PCC73102 Npun02004178 does not abolish its catalytic function

[0337] As discussed in Example 13, the hypothetical protein PMT1231 from *Prochlorococcus marinus* MIT9313 (SEQ ID NO:18) is an active fatty aldehyde decarbonylase. Based on the three-dimensional structure of PMT1231, which is available at 1.8 Å resolution (pdb2OC5A) (see Fig. 29B), cyanobacterial fatty aldehyde decarbonylases have structural similarity with non-heme diiron proteins, in particular with class I ribonuclease reductase subunit β proteins, RNRβ (Stubbe and Riggs-Gelasco, TIBS 1998, vol. 23., pp. 438) (see Fig. 29A). Class Ia and Ib RNRβ contains a diferric tyrosyl radical that mediates the catalytic activity of RNRα (reduction of ribonucleotides to deoxyribonucleotides). In *E. coli* RNRβ, this tyrosine is in position 122 and is in close proximity to one of the active site's iron molecules. Structural alignment showed that PMT1231 contained a phenylalanine

in the same position as RNRb tyr122, suggesting a different catalytic mechanism for cyanobacterial fatty aldehyde decarbonylases. However, an aligment of all decarbonylases showed that two tyrosine residues were completely conserved in all sequences, tyr135 and tyr138 with respect to PMT1231, with tyr135 being in close proximity (5.5 Å) to one of the active site iron molecules (see Fig. 29C). To examine whether either of the two conserved tyrosine residues is involved in the catalytic mechanism of cyanobacterial fatty aldehyde decarbonylases, these residues were replaced with phenylalanine in Npun02004178 (tyr 123 and tyr126) as follows.

[0338] The genomic DNA encoding *S. elongatus* PCC7942 ORF1594 (SEQ ID NO:65) was cloned into the NcoI and EcoRI sites of vector OP-80 (pCL1920 derivative) under the control of the P_{trc} promoter. The genomic DNA encoding *N. punctiforme* PCC73102 Npun02004178 (SEQ ID NO:5) was also cloned into the NdeI and XhoI sites of vector OP-183 (pACYC177 derivative) under the control of the P_{trc} promoter. The latter construct was used as a template to introduce a mutation at positions 123 and 126 of the decarbonylase protein, changing the tyrosines to phenylalanines using the primers
gttttgcgatcgcagcatttaacatttacatccccgttgccgacg and
gttttgcgatcgcagcatataacattttcatccccgttgccgacg, respectively. The resulting constructs were then transformed into *E. coli* MG1655. The cells were grown at 37°C in M9 minimal media supplemented with 1% glucose (w/v), and 100 μg/mL carbenicillin and spectinomycin. The cells were cultured and extracted as in Example 3.

[0339] As shown in Fig. 30, the two Npun02004178 Tyr to Phe protein variants were active and produced alkanes when coexpressed with *S. elongatus* PCC7942 ORF1594. This result indicates that in contrast to class Ia and Ib RNRβ proteins, the catalytic mechanism of fatty aldehyde decarbonylases does not involve a tyrosyl radical.

Example 26: Biochemical characterization of *Nostoc punctiforme* PCC73102 Npun02004178

[0340] The genomic DNA encoding *N. punctiforme* PCC73102 Npun02004178 (SEQ ID NO:5) was cloned into the *Nde*I and *Xho*I sites of vector pET-15b under the control of the T7 promoter. The resulting Npun02004178 protein contained an

N-terminal His-tag. An *E. coli* BL21 strain (DE3) (Invitrogen) was transformed with the plasmid by routine chemical transformation techniques. Protein expression was carried out by first inoculating a colony of the *E. coli* strain in 5 mL of LB media supplemented with 100 mg/L of carbenicillin and shaken overnight at 37 °C to produce a starter culture. This starter cultures was used to inoculate 0.5 L of LB media supplemented with 100 mg/L of carbenecillin. The culture was shaken at 37 °C until an OD_{600} value of 0.8 was reached, and then IPTG was added to a final concentration of 1 mM. The culture was then shaken at 37 °C for approximately 3 additional h. The culture was then centrifuged at 3,700 rpm for 20 min at 4 °C. The pellet was then resuspended in 10 mL of buffer containing 100 mM sodium phosphate buffer at pH 7.2 supplemented with Bacterial ProteaseArrest (GBiosciences). The cells were then sonicated at 12 W on ice for 9 s with 1.5 s of sonication followed by 1.5 s of rest. This procedure was repeated 5 times with one min intervals between each sonication cycle. The cell free extract was centrifuged at 10,000 rpm for 30 min at 4 °C. 5 mL of Ni-NTA (Qiagen) was added to the supernatant and the mixture was gently stirred at 4 °C. The slurry was passed over a column removing the resin from the lysate. The resin was then washed with 30 mL of buffer containing 100 mM sodium phosphate buffer at pH 7.2 plus 30 mM imidazole. Finally, the protein was eluted with 10 mL of 100 mM sodium phosphate buffer at pH 7.2 plus 250 mM imidazole. The protein solution was dialyzed with 200 volumes of 100 mM sodium phosphate buffer at pH 7.2 with 20% glycerol. Protein concentration was determined using the Bradford assay (Biorad).
5.6 mg/mL of Npun02004178 protein was obtained.

[0341] To synthesize octadecanal for the decarbonylase reaction, 500 mg of octadecanol (Sigma) was dissolved in 25 mL of dichloromethane. Next, 200 mg of pyridinium chlorochromate (TCI America) was added to the solution and stirred overnight. The reaction mixture was dried under vacuum to remove the dichloromethane. The remaining products were resuspended in hexane and filtered through Whatman filter paper. The filtrate was then dried under vacuum and resuspended in 5 mL of hexane and purified by silica flash chromatography. The mixture was loaded onto the gravity fed column in hexane and then washed with two column volumes of hexane. The octadecanal was then eluted with an 8:1 mixture of

hexane and ethyl acetate. Fractions containing octadecanal were pooled and analyzed using the GC/MS methods described below. The final product was 95% pure as determined by this method.

[0342] To test Npun02004178 protein for decarbonylation activity, the following enzyme assays were set-up. 200 μL reactions were set up in 100 mM sodium phosphate buffer at pH 7.2 with the following components at their respective final concentrations: 30 μM of purified Npun02004178 protein, 200 μM octadecanal, 0.11 μg/mL spinach ferredoxin (Sigma), 0.05 units/mL spinach ferredoxin reductase (Sigma), and 1 mM NADPH (Sigma). Negative controls included the above reaction without Npun02004178, the above reaction without octadecanal, and the above reaction without spinach ferredoxin, ferredoxin reductase and NADPH. Each reaction was incubated at 37°C for 2 h before being extracted with 100 μL ethyl acetate. Samples were analyzed by GC/MS using the following parameters: run time: 13.13 min; column: HP-5-MS Part No. 19091S-433E (length of 30 meters; I.D.: 0.25 mm narrowbore; film: 0.25ìM); inject: 1 ìl Agilent 6850 inlet; inlet: 300 C splitless; carrier gas: helium; flow: 1.3 mL/min; oven temp: 75 °C hold 5 min, 320 at 40 °C/min, 320 hold 2 min; det: Agilent 5975B VL MSD; det. temp: 330 °C; scan: 50-550 M/Z. Heptadecane from Sigma was used as an authentic reference for determining compound retention time and fragmentation pattern.

[0343] As shown in Fig. 31, *in-vitro* conversion of octadecanal to heptadecane was observed in the presence of Npun02004178. The enzymatic decarbonylation of octadecanal by Npun02004178 was dependent on the addition of spinach ferredoxin reducatase, ferredoxin and NADPH.

[0344] Next, it was determined whether cyanobaterial ferredoxins and ferredoxin reductases can replace the spinach proteins in the *in-vitro* fatty aldehyde decarbonylase assay. The following four genes were cloned separately into the *Nde*I and *Xho*I sites of pET-15b: *N. punctiforme* PCC73102 Npun02003626 (ZP_00109192, ferredoxin oxidoreductase petH without the n-terminal allophycocyanin linker domain) (SEQ ID NO:87), *N. punctiforme* PCC73102 Npun02001001 (ZP_00111633, ferredoxin 1) (SEQ ID NO:89), *N. punctiforme* PCC73102 Npun02003530 (ZP_00109422, ferredoxin 2) (SEQ ID NO:91) and *N. punctiforme* PCC73102 Npun02003123 (ZP_00109501, ferredoxin 3) (SEQ ID

NO:93). The four proteins were expressed and purified as described above. 1 mg/mL of each ferredoxin and 4 mg/mL of the ferredoxin oxidoreductase was obtained. The three cyanobacterial ferredoxins were tested with the cyanobacterial ferredoxin oxidoreductase using the enzymatic set-up described earlier with the following changes. The final concentration of the ferredoxin reductase was 60 μg/mL and the ferredoxins were at 50 μg/mL. The extracted enzymatic reactions were by GC/MS using the following parameters: run time: 6.33 min; column: J&W 122-5711 DB-5ht (length of 15 meters; I.D.: 0.25 mm narrowbore; film: 0.10μM); inject: 1 μL Agilent 6850 inlet; inlet: 300 °C splitless; carrier gas: helium; flow: 1.3 mL/min; oven temp: 100 °C hold 0.5 min, 260 at 30 °C/min, 260 hold 0.5 min; det: Agilent 5975B VL MSD; det. temp: 230 °C; scan: 50-550 M/Z.

[0345] As shown in Fig. 32, Npun02004178-dependent *in-vitro* conversion of octadecanal to heptadecane was observed in the presence of NADPH and the cyanobacterial ferredoxin oxidoreductase and any of the three cyanobacterial ferredoxins.

Example 27. Biochemical characterization of *Synechococcus elongatus* PCC7942 orf1594

[0346] The genomic DNA encoding *S. elongatus* PCC7492 orf1594 (SEQ ID NO:65) was cloned into the *Nco*I and *Xho*I sites of vector pET-28b under the control of the T7 promoter. The resulting PCC7942_orf1594 protein contained a C-terminal His-tag. An *E. coli* BL21 strain (DE3) (Invitrogen) was transformed with the plasmid and PCC7942_orf1594 protein was expressed and purified as described in Example 22. The protein solution was stored in the following buffer: 50 mM sodium phosphate, pH 7.5, 100 mM NaCl, 1 mM THP, 10% glycerol. Protein concentration was determined using the Bradford assay (Biorad). 2 mg/mL of PCC7942_orf1594 protein was obtained.

[0347] To test PCC7942_orf1594 protein for acyl-ACP or acyl-CoA reductase activity, the following enzyme assays were set-up. 100 μL reactions were set-up in 50 mM Tris-HCl buffer at pH 7.5 with the following components at their respective final concentrations: 10 μM of purified PCC7942_orf1594 protein, 0.01-1 mM acyl-CoA or acyl-ACP, 2 mM $MgCl_2$, 0.2-2 mM NADPH. The reactions were incubated

for 1 h at 37°C and where stopped by adding 100 μL ethyl acetate (containing 5 mg/l 1-octadecene as internal standard). Samples were vortexed for 15 min and centrifuged at max speed for 3 min for phase separation. 80 μL of the top layer were transferred into GC glass vials and analyzed by GC/MS as described in Example 26. The amount of aldehyde formed was calculated based on the internal standard.

[0348] As shown in Fig. 33, PCC7942_orf1594 was able to reduce octadecanoyl-CoA to octadecanal. Reductase activity required divalent cations such as Mg^{2+}, Mn^{2+} or Fe^{2+} and NADPH as electron donor. NADH did not support reductase activity. PCC7942_orf1594 was also able to reduce octadecenoyl-CoA and octadecenoyl-ACP to octadecenal. The K_m values for the reduction of octadecanoyl-CoA, octadecenoyl-CoA and octadecenoyl-ACP in the presence of 2 mM NADPH were determined as 45 ± 20 μM, 82 ± 22 μM and 7.8 ± 2 μM, respectively. These results demonstrate that PCC7942_orf1594, *in vitro*, reduces both acyl-CoAs and acyl-ACPs and that the enzyme apparently has a higher affinity for acyl-ACPs as compared to acyl-CoAs. The K_m value for NADPH in the presence of 0.5 mM octadecanoyl-CoA for PCC7942_orf1594 was determined as 400 ± 80 μM.

[0349] Next, the stereospecific hydride transfer from NADPH to a fatty aldehyde catalyzed by PCC7942_orf1594 was examined. Deutero-NADPH was prepared according to the following protocol. 5 mg of $NADP^+$ and 3.6 mg of D-glucose-1-d was added to 2.5 mL of 50 mM sodium phosphate buffer (pH 7.0). Enzymatic production of labeled NADPH was initiated by the addition of 5 units of glucose dehydrogenase from either *Bacillus megaterium* (USB Corporation) for the production of R-(4-^{2}H)NADPH or *Thermoplasma acidophilum* (Sigma) for the production of S-(4-^{2}H)NADPH. The reaction was incubated for 15 min at 37°C, centrifuge-filtered using a 10 KDa MWCO Amicon Ultra centrifuge filter (Millipore), flash frozen on dry ice, and stored at -80°C.

[0350] The *in vitro* assay reaction contained 50 mM Tris-HCl (pH 7.5), 10 μM of purified PCC7942_orf1594 protein, 1 mM octadecanoyl-CoA, 2 mM $MgCl_2$, and 50 μL deutero-NADPH (prepared as described above) in a total volume of 100 μL. After a 1 h incubation, the product of the enzymatic reaction was extracted and analyzed as described above. The resulting fatty aldehyde detected by GC/MS was

octadecanal (see Fig. 34). Because hydride transfer from NADPH is stereospecific, both *R*-(4-^{2}H)NADPH and *S*-(4-^{2}H)NADPH were synthesized. Octadecanal with a plus one unit mass was observed using only the *S*-(4-^{2}H)NADPH. The fact that the fatty aldehyde was labeled indicates that the deuterated hydrogen has been transferred from the labeled NADPH to the labeled fatty aldehyde. This demonstrates that NADPH is used in this enzymatic reaction and that the hydride transfer catalyzed by PCC7942_orf1594 is stereospecific.

Example 28. Intracellular and Extracellular Production of Fatty Aldehydes and Fatty Alcohols in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594

[0351] The genomic DNA encoding *Synechococcus elongatus* PCC7942 orf1594 (YP_400611; acyl-ACP reductase) (SEQ ID NO:65) was amplified and cloned into the *Nco*I and *Eco*RI sites of vector OP-80 (pCL1920 derivative) under the control of the P_{trc} promoter. The resulting construct was cotransformed into *E. coli* MG1655 Δ*fadE* and the cells were grown at 37°C in 15 mL Che-9 minimal media with 3% (w/v) glucose as carbon source and supplemented with 100 μg/mL spectinomycin and carbenicillin, respectively. When the culture reached OD_{600} of 0.8-1.0, it was induced with 1mM IPTG and cells were grown for an additional 24-48 h at 37°C. Che-9 minimal medium is defined as: 6g/L Na_2HPO_4, 3 g/L KH_2PO_4, 0.5 g/L NaCl, 2 g/L NH_4Cl, 0.25 g/L $MgSO_4$ x 7 H_2O, 11 mg/L $CaCl_2$, 27 mg/L Fe_3Cl x 6 H_2O, 2 mg/L ZnCl x 4 H_2O, 2 mg/L Na_2MoO_4 x 2 H_2O, 1.9 mg/L $CuSO_4$ x 5 H_2O, 0.5 mg/L H_3BO_3, 1 mg/L thiamine, 200 mM Bis-Tris (pH 7.25) and 0.1% (v/v) Triton-X100. When the culture reached OD_{600} of 1.0-1.2, it was induced with 1 mM IPTG and cells were allowed to grow for an additional 40 hrs at 37°C. Cells from 0.5 mL of culture were extracted with 0.5 mL of ethyl acetate for total hydrocarbon production as described in Example 26. Additionally, cells and supernatant were separated by centrifugation (4,000 g at RT for 10 min) and extracted separately.

[0352] The culture produced 620 mg/L fatty aldehydes (tetradecanal, heptadecenal, heptadecanal and octadecenal) and 1670 mg/L fatty alcohols (dodecanol, tetradecenol, tetradecanol, heptadecenol, heptadecanol and

octadecenol). Fig 35 shows the chromatogram of the extracted supernatant. It was determined that 73 % of the fatty aldehydes and fatty alcohols were in the cell-free supernatant.

Example 29. Intracellular and Extracellular Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Synechococcus elongatus* PCC7942 orf1594 and *Nostoc punctiforme* PCC73102 Npun02004178

[0353] The genomic DNA encoding *Synechococcus elongatus* PCC7942 orf1594 (YP_400611; acyl-ACP reductase) (SEQ ID NO:65) was amplified and cloned into the *Nco*I and *Eco*RI sites of vector OP-80 (pCL1920 derivative) under the control of the P_{trc} promoter. The genomic DNA encoding *Nostoc punctiforme* PCC73102 Npun02004178 (ZP_00108838; fatty aldehyde decarbonylase) (SEQ ID NO:5) was amplified and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting constructs were cotransformed into *E. coli* MG1655 Δ*fadE* and the cells were grown at 37°C in 15 mL Che9 minimal media with 3% (w/v) glucose as carbon source and supplemented with 100 μg/mL spectinomycin and carbenicillin, respectively. The cells were grown, separated from the broth, extracted, and analyzed as described in Example 28.

[0354] The culture produced 323 mg/L alkanes and alkenes (tridecane, pentadecene, pentadecane and heptadecene), 367 mg/L fatty aldehydes (tetradecanal, heptadecenal, heptadecanal and octadecenal) and 819 mg/L fatty alcohols (tetradecanol, heptadecenol, heptadecanol and octadecenol). Fig. 36 shows the chromatogram of the extracted supernatant. It was determined that 86% of the alkanes, alkenes, fatty aldehydes and fatty alcohols were in the cell-free supernatant.

Example 30. Production of Alkanes and Alkenes in *E. coli* through Heterologous Expression of *Nostoc* sp. PCC7210 alr5284 and *Nostoc* sp. PCC7210 alr5283

[0355] The genomic DNA encoding *Nostoc* sp. PCC7210 alr5284 (NP_489324; putative aldehyde-generating enzyme) (SEQ ID NO:81) was amplified and cloned into the *Nco*I and *Eco*RI sites of vector OP-80 (pCL1920 derivative) under the control of the P_{trc} promoter. The genomic DNA encoding *Nostoc* sp. PCC7210

alr5283 (NP_489323; putative decarbonylase) (SEQ ID NO:7) was amplified and cloned into the *Nde*I and *Xho*I sites of vector OP-183 (pACYC derivative) under the control of the P_{trc} promoter. The resulting constructs were cotransformed into *E. coli* MG1655 and the cells were grown at 37°C in 15 mL Che9 minimal media with 3% (w/v) glucose as carbon source and supplemented with 100 μg/mL spectinomycin and carbenicillin, respectively (as described in Example 28). Cells from 0.5 mL of culture were extracted and analyzed as described in Example 3 and analyzed by GC-MS as described in Example 26.

[0356] As shown in Fig. 37, *E. coli* cells cotransformed with the *Nostoc* sp. PCC7210 alr5284 and *Nostoc* sp. PCC7210 alr5283-bearing vectors produced tridecane, pentadecene, pentadecane, tetradecanol and hexadecanol. This result indicates that coexpression of *Nostoc* sp. PCC7210 alr5284 and alr5283 is sufficient for *E. coli* to produce fatty alcohols, alkanes and alkenes.

OTHER EMBODIMENTS

[0357] It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

CLAIMS:

1. A method of producing an aldehyde, the method comprising producing in a host cell a polypeptide comprising the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, or a variant thereof, and isolating the aldehyde from the host cell.

2. A method of producing an aldehyde, the method comprising producing in a host cell a polypeptide comprising an amino acid sequence having at least about 70% identity to SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, and isolating the aldehyde from the host cell.

3. A method of producing an aldehyde, the method comprising producing in a host cell a polypeptide comprising the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 with one or more amino acid substitutions, additions, insertions, or deletions, wherein the polypeptide has reductase activity.

4. The method of claim 3, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82 with one or more conservative amino acid substitutions.

5. A method of producing an aldehyde, the method comprising expressing in a host cell a polynucleotide comprising a nucleotide sequence having at least about 70% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, and isolating the aldehyde from the host cell.

6. A method of producing an aldehyde comprising expressing in a host cell a polynucleotide that hybridizes to a complement of a nucleotide sequence of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, or to a fragment thereof, wherein the polynucleotide encodes a polypeptide having the same biological activity as a polypeptide comprising ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82.

7. The method of any one of claims 1-6, wherein the polypeptide or the polynucleotide is from a cyanobacterium.

8. A method of producing an aldehyde, the method comprising transforming a host cell with a recombinant vector comprising a nucleotide sequence having at least about 70% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, and isolating the aldehyde from the host cell.

9. The method of claim 8, wherein the recombinant vector further comprises a promoter operably linked to the nucleotide sequence.

10. The method of any one of claims 1-9, wherein the host cell is selected from the group consisting of a mammalian cell, plant cell, insect cell, yeast cell, fungus cell, filamentous fungi cell, and bacterial cell.

11. The method of claim 10, wherein the host cell is an *E. coli* cell.

12. The method of claim 11, wherein the *E. coli* cell is a strain B, a strain C, a strain K, or a strain W *E. coli* cell.

13. The method of claim 8, wherein the host cell produces a polypeptide encoded by the nucleotide sequence of the recombinant vector.

14. The method of claim 10, wherein the aldehyde is secreted by the host cell.

15. The method of claim 14, wherein the aldehyde comprises a C_{13}-C_{21} aldehyde.

16. The method of claim 14, wherein the aldehyde is selected from the group consisting of tetradecanal, hexadecanal, hexadecenal, octadecanal, octadecenal, methyltetradecanal, methyltetradecenal, methylhexadecanal, methylhexadecenal, methyloctadecanal, and methyloctadecenal.

17. The method of any one of claims 1-16, further comprising culturing the host cell in the presence of at least one biological substrate for the polypeptide or for a polypeptide encoded by the nucleotide sequence.

18. The method of claim 17, wherein the substrate is a fatty acid derivative.

19. The method of claim 18, wherein the fatty acid derivative is a C_{14}-C_{22} fatty acid derivative.

20. The method of claim 17, wherein the fatty acid derivative is selected from the group consisting of tetradecanoyl-ACP, hexadecanoyl-ACP, hexadecenoyl-ACP, octadecenoyl-ACP, and their derivatives.

21. A genetically engineered microorganism comprising an exogenous control sequence stably incorporated into the genomic DNA of the microorganism upstream of a polynucleotide comprising a nucleotide sequence having at least about

70% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, wherein the microorganism produces an increased level of an aldehyde relative to a wild-type microorganism.

22. The microorganism of claim 21, wherein the microorganism is a cyanobacterium.

23. A method of producing an aldehyde, the method comprising culturing the microorganism of claim 21 under conditions suitable for gene expression.

24. An aldehyde produced by any one of the methods of claims 1-20 and 23.

25. The aldehyde of claim 24, wherein the aldehyde has a $\delta^{13}C$ of about -15.4 or greater.

26. The aldehyde of claim 24, wherein the aldehyde has a $f_M{}^{14}C$ of at least about 1.003.

27. A method of making an aldehyde, comprising contacting a substrate with (i) a polypeptide comprising the amino acid sequence of ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, or a variant thereof, or (ii) a polypeptide encoded by a nucleotide sequence having at least 70% identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, or a variant thereof.

28. The method of claim 27, wherein the aldehyde comprises a C_{13}-C_{21} aldehyde.

29. The method of claim 27, wherein the aldehyde is selected from the group consisting of tetradecanal, hexadecanal, hexadecenal, octadecanal, octadecenal, methyltetradecanal, methyltetradecenal, methylhexadecanal, methylhexadecenal, methyloctadecanal, and methyloctadecenal.

30. The method of claim 29, wherein the substrate is selected from the group consisting of tetradecanoyl-ACP, hexadecanoyl-ACP, hexadecenoyl-ACP, octadecenoyl-ACP, and their derivatives.

31. A method of producing an aldehyde, the method comprising producing in a host cell a polypeptide comprising the amino acid sequence of SEQ ID NO:54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64, wherein the polypeptide has reductase activity.

32. The method of claim 31, wherein the polypeptide is from a cyanobacterium.

33. The method of claim 31 or 32, wherein the host cell is selected from the group consisting of a mammalian cell, plant cell, insect cell, yeast cell, fungus cell, filamentous fungi cell, and bacterial cell.

34. The method of claim 33, wherein the host cell is an *E. coli* cell.

35. The method of claim 31, wherein the aldehyde is secreted by the host cell.

36. The method of claim 31, wherein the aldehyde comprises a C_{13}-C_{21} aldehyde.

37. The method of claim 36, wherein the aldehyde is selected from the group consisting of tetradecanal, hexadecanal, hexadecenal, octadecanal, octadecenal, methyltetradecanal, methyltetradecenal, methylhexadecanal, methylhexadecenal, methyloctadecanal, and methyloctadecenal.

38. The method of any one of claims 31-37, further comprising culturing the host cell in the presence of at least one biological substrate for the polypeptide.

39. The method of claim 38, wherein the substrate is a fatty acid derivative.

40. The method of claim 39, wherein the fatty acid derivative is selected from the group consisting of tetradecanoyl-ACP, hexadecanoyl-ACP, hexadecenoyl-ACP, octadecenoyl-ACP, and their derivatives.

41. A method of making an aldehyde, comprising contacting a substrate with a polypeptide comprising the amino acid sequence of SEQ ID NO:54, 55, 56, 57, 58, 59, 60, 61, 62, 63, or 64, wherein the polypeptide has reductase activity.

42. The method of claim 41, wherein the aldehyde comprises a C_{13}-C_{21} aldehyde.

43. The method of claim 42, wherein the aldehyde is selected from the group consisting of tetradecanal, hexadecanal, hexadecenal, octadecanal, octadecenal, methyltetradecanal, methyltetradecenal, methylhexadecanal, methylhexadecenal, methyloctadecanal, and methyloctadecenal.

44. The method of claim 41, wherein the substrate is a fatty acid derivative.

45. The method of claim 44, wherein the substrate is selected from the group consisting of tetradecanoyl-ACP, hexadecanoyl-ACP, hexadecenoyl-ACP, octadecenoyl-ACP, and their derivatives.

46. A method of producing a fatty alcohol, the method comprising producing in a host cell a polypeptide comprising the amino acid sequence of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, or a variant thereof, and isolating the fatty alcohol from the host cell.

47. A method of producing a fatty alcohol, the method comprising producing in a host cell a polypeptide comprising an amino acid sequence having at least about 70% identity to SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82, and isolating the fatty alcohol from the host cell.

48. A method of producing a fatty alcohol, the method comprising expressing in a host cell a polynucleotide comprising a nucleotide sequence having at least about 70% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, and isolating the fatty alcohol from the host cell.

49. A method of producing a fatty alcohol, the method comprising transforming a host cell with a recombinant vector comprising a nucleotide sequence having at least about 70% sequence identity to SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81, and isolating the fatty alcohol from the host cell.

50. The method of any one of claims 46-49, further comprising expressing a gene encoding a recombinant alcohol dehydrogenase in the host cell.

51. The method of claim 50, wherein the fatty alcohol is secreted by the host cell.

52. The method of claim 50, wherein the fatty alcohol comprises a C_6-C_{26} fatty alcohol.

53. The method of claim 50, wherein the fatty alcohol is 1-decanol, 1-dodecanol, 1-myristyl alcohol, 1-hexadecanol, octadecenol, tetradecenol, or hexadecenol.

54. The method of claim 50, wherein the fatty alcohol comprises a straight chain fatty alcohol.

55. The method of claim 50, wherein the fatty alcohol comprises a branched chain fatty alcohol.

56. The method of claim 50, wherein the fatty alcohol comprises a cyclic moiety.

57. The method of claim 50, wherein the fatty alcohol is an unsaturated fatty alcohol.

58. The method of claim 50, wherein the fatty alcohol is a monounsaturated fatty alcohol.

59. The method of claim 50, wherein the fatty alcohol is a saturated fatty alcohol.

60. An isolated nucleic acid consisting of no more than 500 nucleotides of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81.

61. An isolated nucleic acid consisting of no more than 90% of the nucleotides of SEQ ID NO:65, 67, 69, 71, 73, 75, 77, 79, or 81.

62. The nucleic acid of claims 60 or 61, wherein the nucleic acid encodes a polypeptide having reductase activity.

63. An isolated polypeptide consisting of no more than 200 amino acids of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82.

64. An isolated polypeptide consisting of no more than 90% of the amino acids of SEQ ID NO:66, 68, 70, 72, 74, 76, 78, 80, or 82.

65. The isolated polypeptide of claim 63 or 64, wherein the polypeptide has reductase activity.

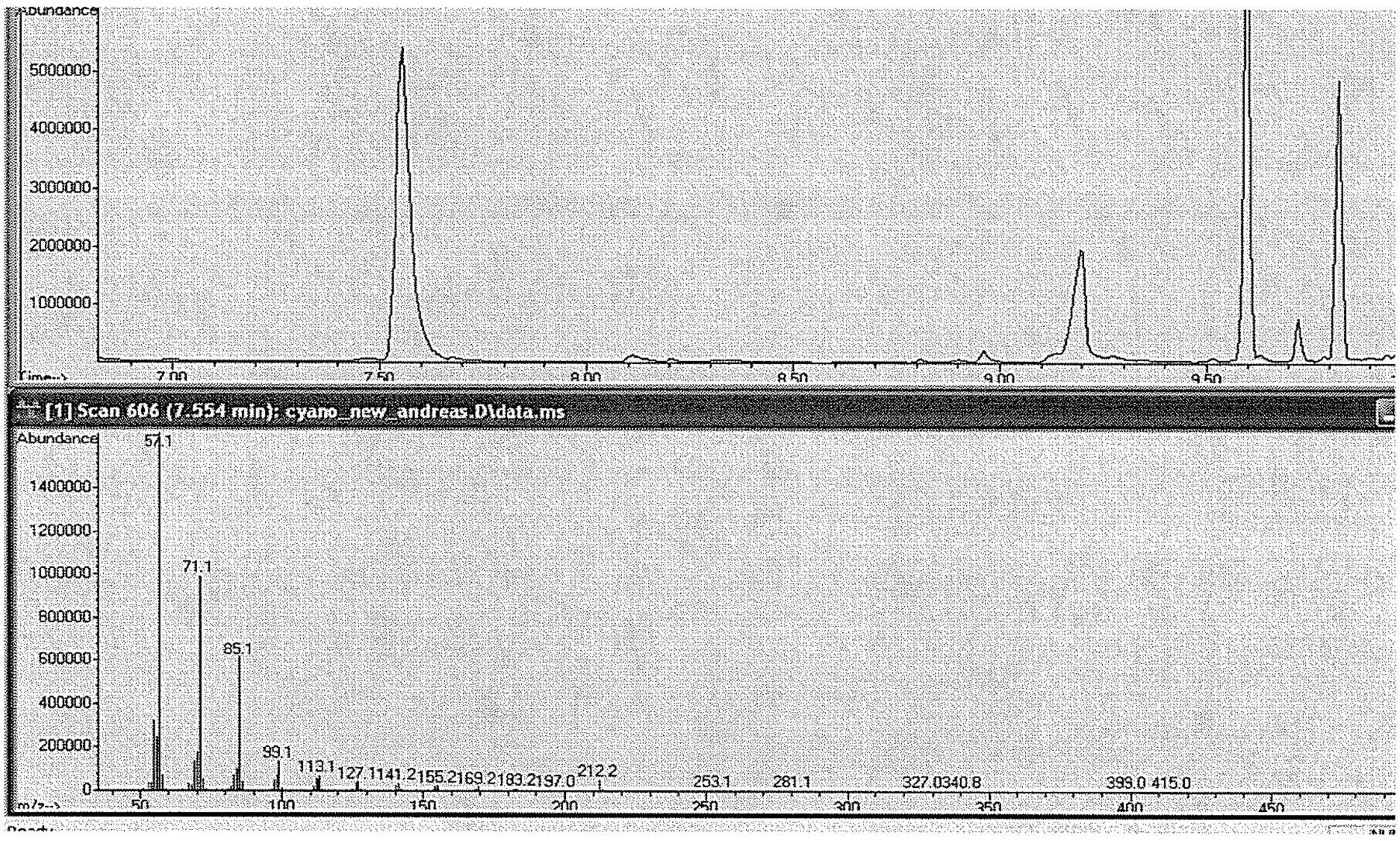
Abundance
5000000
4000000
3000000
2000000
1000000
7.00
7.50
8.00
8.50
9.00
9.50
[1] Scan 606 (7.554 min): cyano_new_andreas.D\data.ms
Abundance
1400000
1200000
1000000
800000
600000
400000
200000
0
57.1
71.1
85.1
99.1
113.1
127.1
141.2
155.2
169.2
183.2
197.0
212.2
253.1
281.1
327.0
340.8
393.0
415.0
m/z
50
100
150
200
250
300
350
400
450

FIG. 1

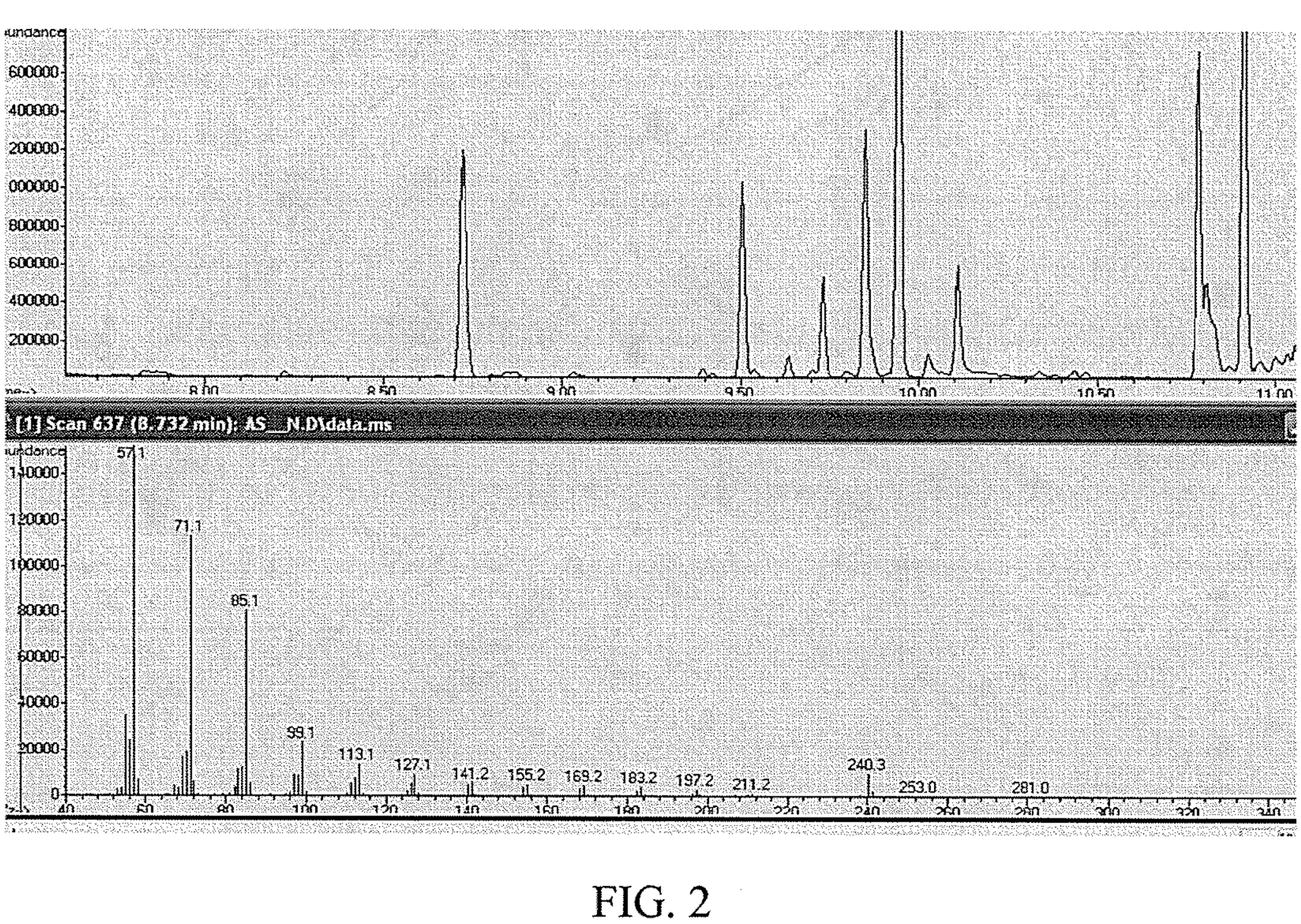
600000
400000
200000
000000
800000
600000
400000
200000
8.00
8.50
9.00
9.50
10.00
10.50
11.00
[1] Scan 637 (8.732 min): AS__N.D\data.ms
57.1
71.1
85.1
99.1
113.1
127.1
141.2
155.2
169.2
183.2
197.2
211.2
240.3
253.0
281.0
20000
0
40
50
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340

FIG. 2

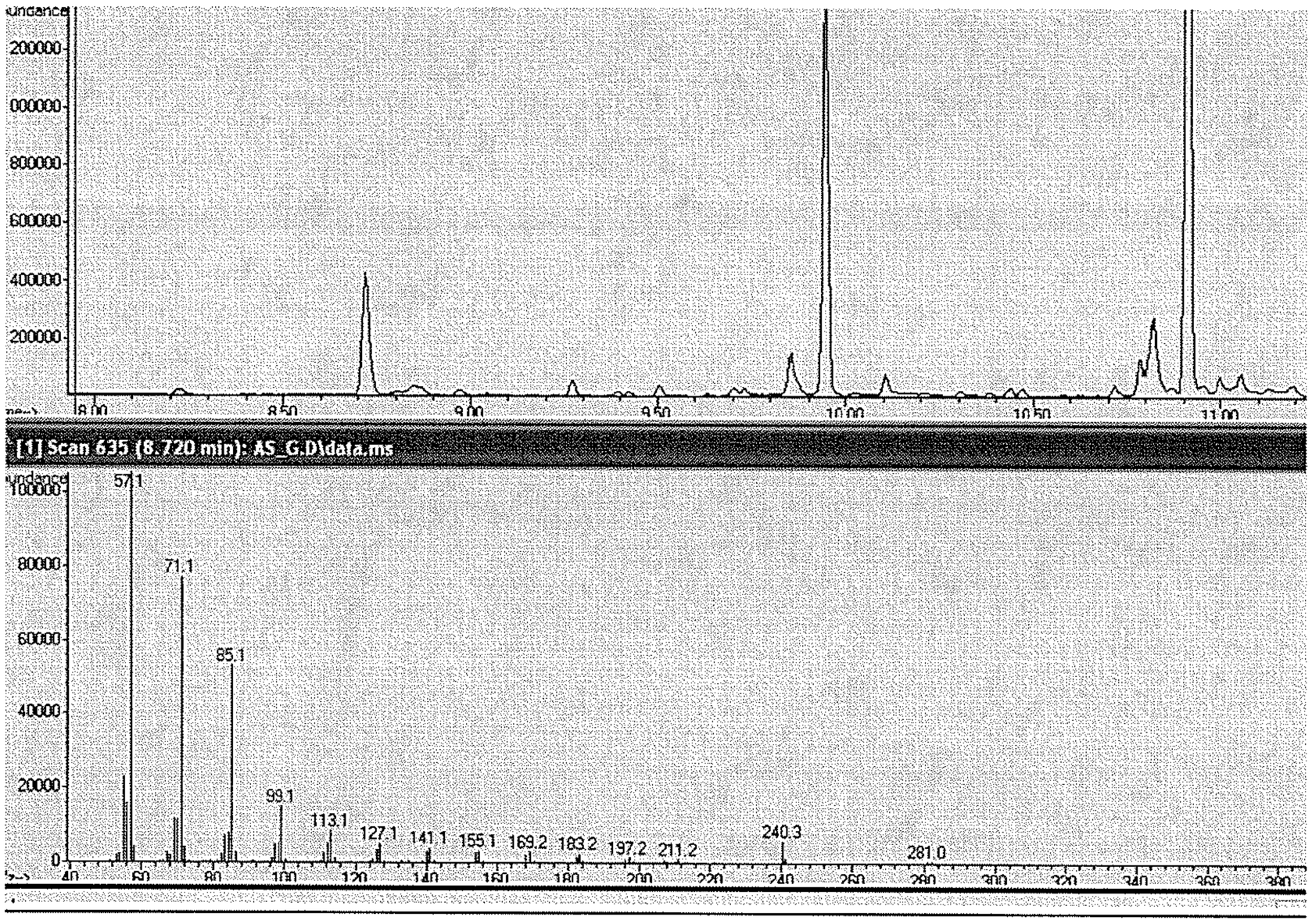
Abundance
200000
000000
800000
600000
400000
200000
8.00
8.50
9.00
9.50
10.00
10.50
11.00
[1] Scan 635 (8.720 min): AS_G.D\data.ms
Abundance
100000
80000
60000
40000
20000
0
57.1
71.1
85.1
99.1
113.1
127.1
141.1
155.1
169.2
183.2
197.2
211.2
240.3
281.0
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380

FIG. 3

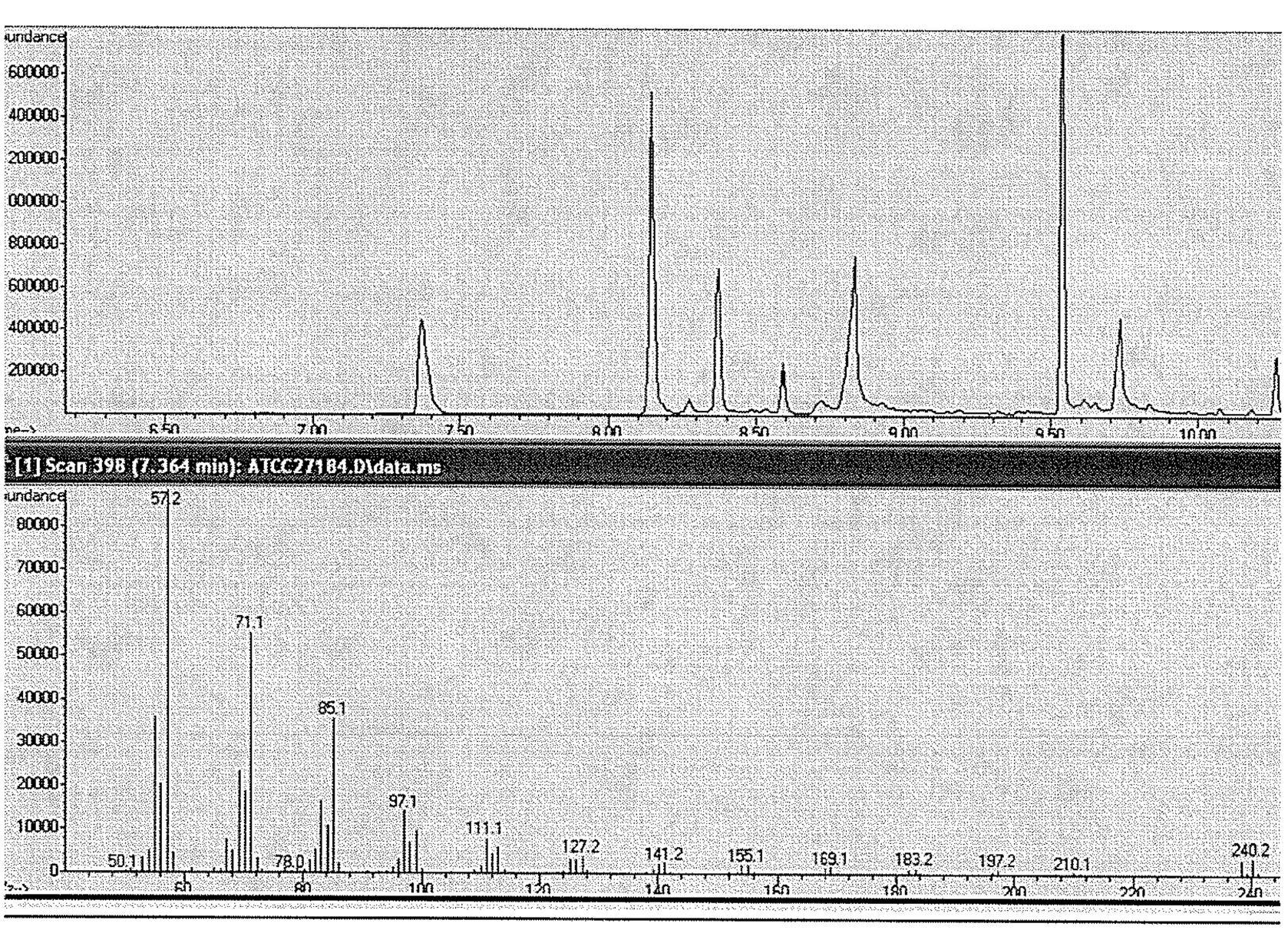
Abundance
600000
400000
200000
000000
800000
600000
400000
200000
6.50
7.00
7.50
8.00
8.50
9.00
9.50
10.00
[1] Scan 398 (7.364 min): ATCC27184.D\data.ms
Abundance
80000
70000
60000
50000
40000
30000
20000
10000
0
57.2
71.1
85.1
97.1
111.1
127.2
141.2
155.1
169.1
183.2
197.2
210.1
240.2
50.1
78.0
60
80
100
120
140
160
180
200
220
240

FIG. 4

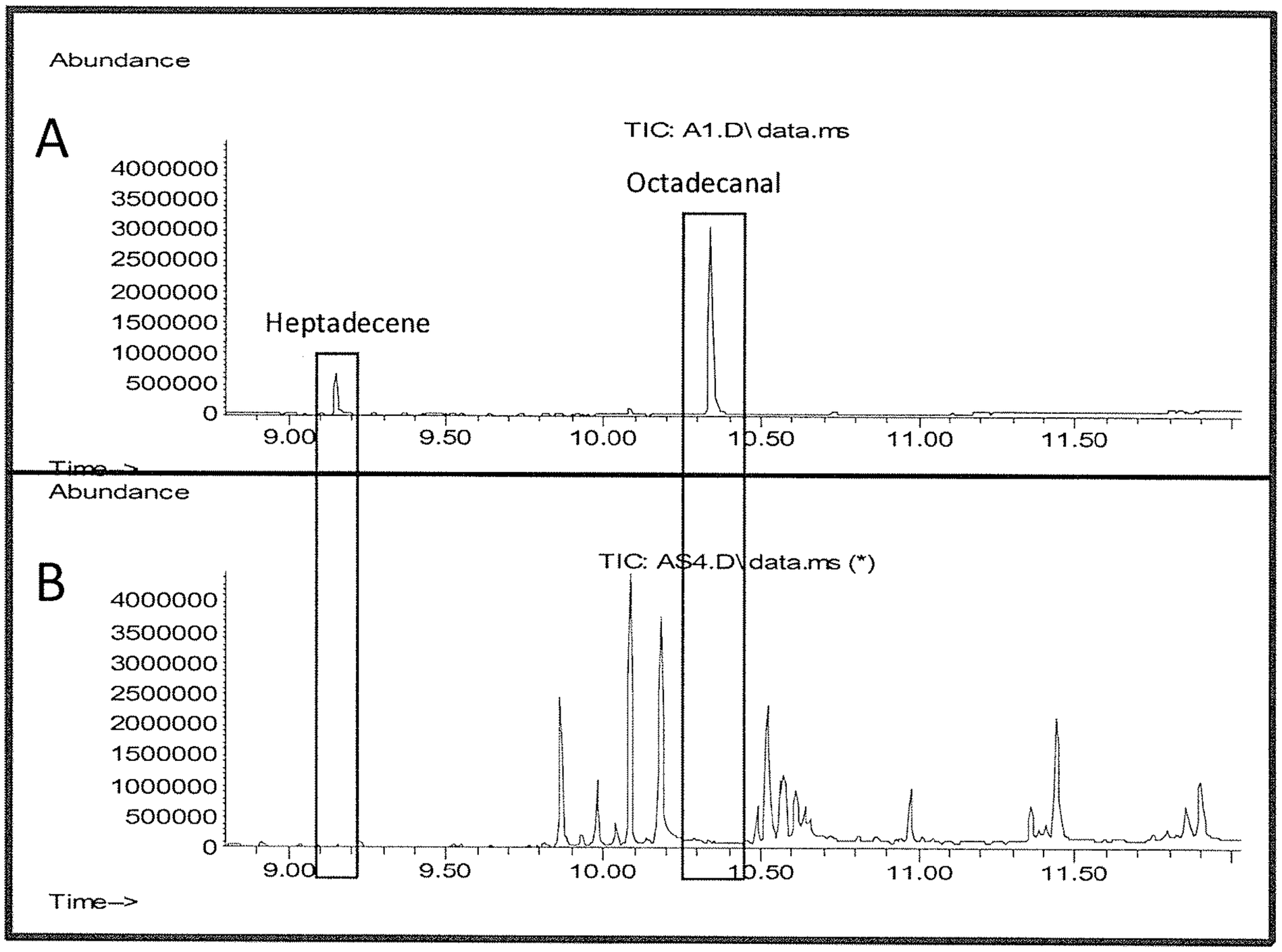
Abundance
A
TIC: A1.D\ data.ms
Octadecanal
Heptadecene
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
9.00
9.50
10.00
10.50
11.00
11.50
Time -->
Abundance
B
TIC: AS4.D\ data.ms (*)
Time-->

FIG. 5

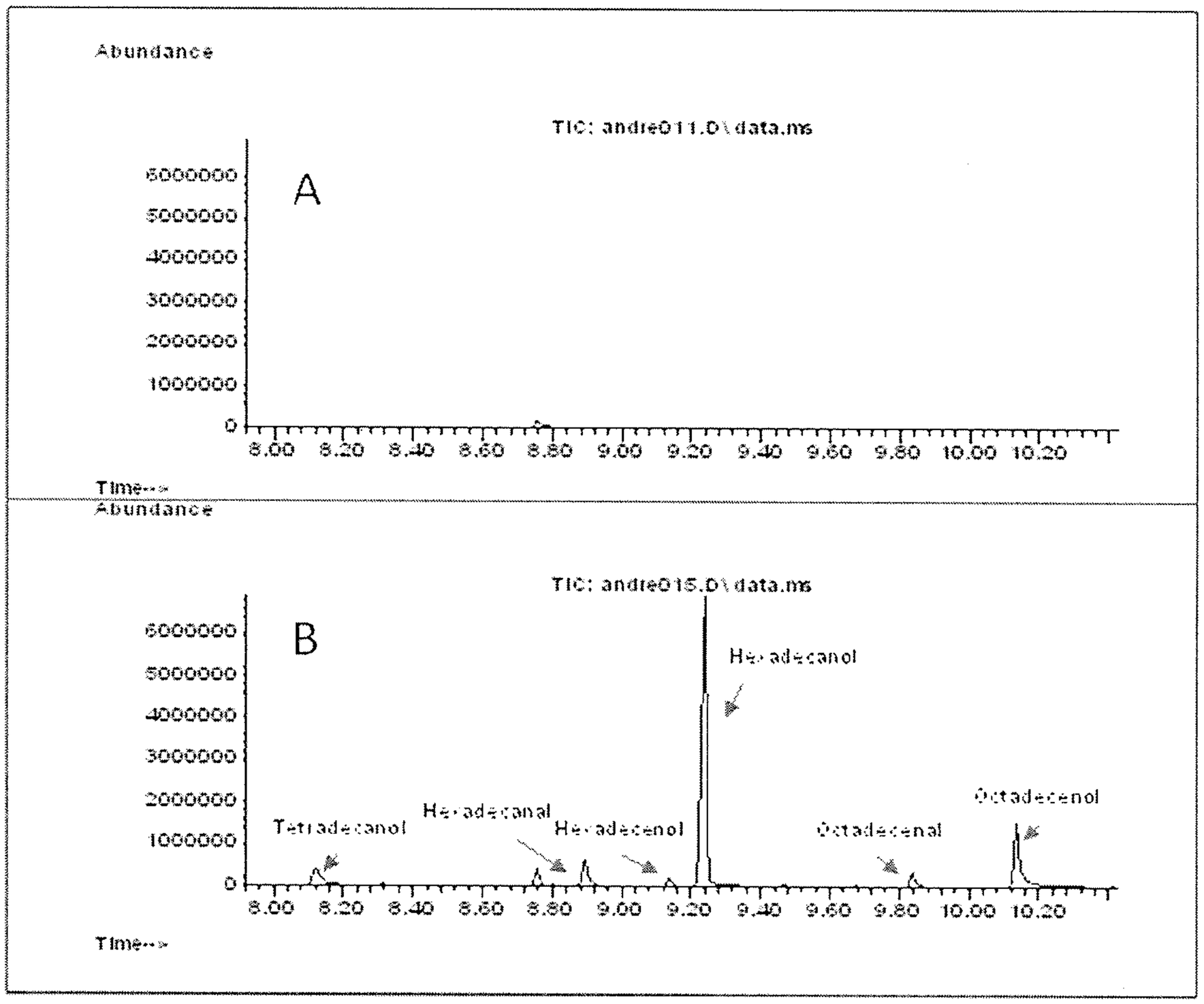
Abundance
TIC: andre011.D\data.ms
A
6000000
5000000
4000000
3000000
2000000
1000000
0
8.00 8.20 8.40 8.60 8.80 9.00 9.20 9.40 9.60 9.80 10.00 10.20
Time-->
Abundance
TIC: andre015.D\data.ms
B
Hexadecanol
Octadecenol
Hexadecanal
Tetradecanol
Hexadecenol
Octadecenal
6000000
5000000
4000000
3000000
2000000
1000000
0
8.00 8.20 8.40 8.60 8.80 9.00 9.20 9.40 9.60 9.80 10.00 10.20
Time-->

FIG. 6

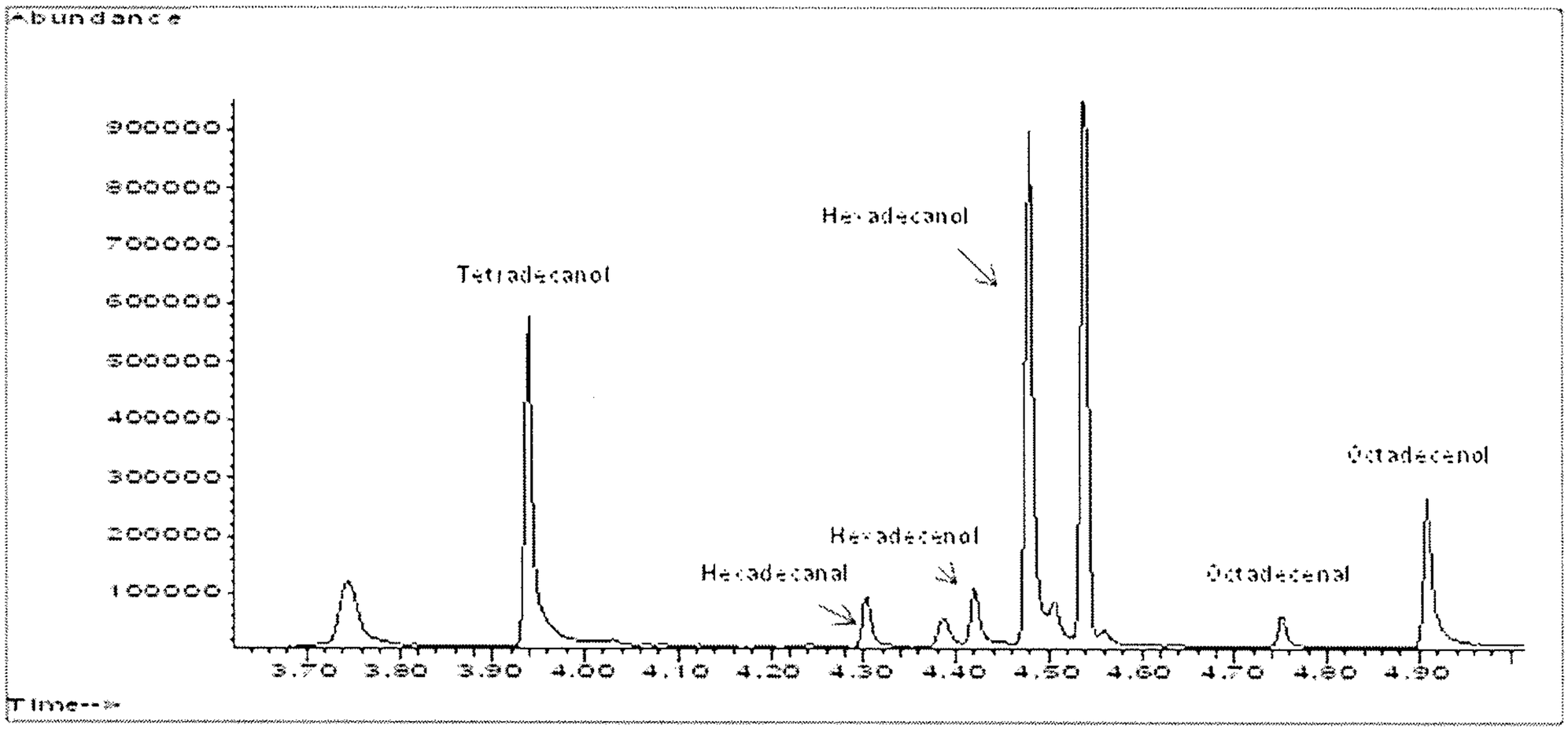
Abundance
900000
800000
700000
600000
500000
400000
300000
200000
100000
Tetradecanol
Hexadecanol
Hexadecanal
Hexadecenol
Octadecenal
Octadecenol
3.70 3.80 3.90 4.00 4.10 4.20 4.30 4.40 4.50 4.60 4.70 4.80 4.90
Time-->

FIG. 7

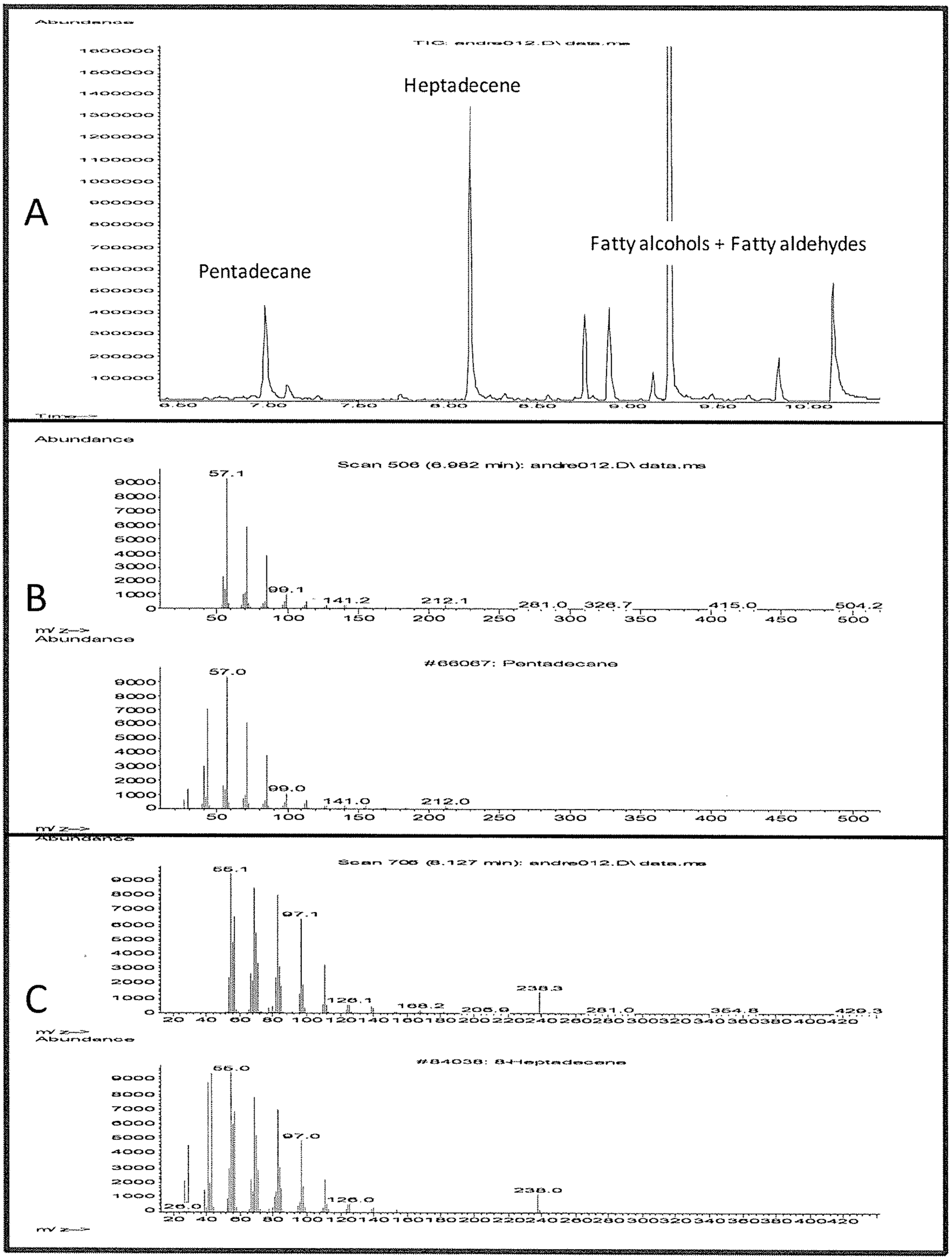
A
Heptadecene
Fatty alcohols + Fatty aldehydes
Pentadecane
B
Scan 506 (6.982 min): andre012.D\ data.ms
#86087: Pentadecane
C
Scan 708 (8.127 min): andre012.D\ data.ms
#84038: 8-Heptadecene

FIG. 8

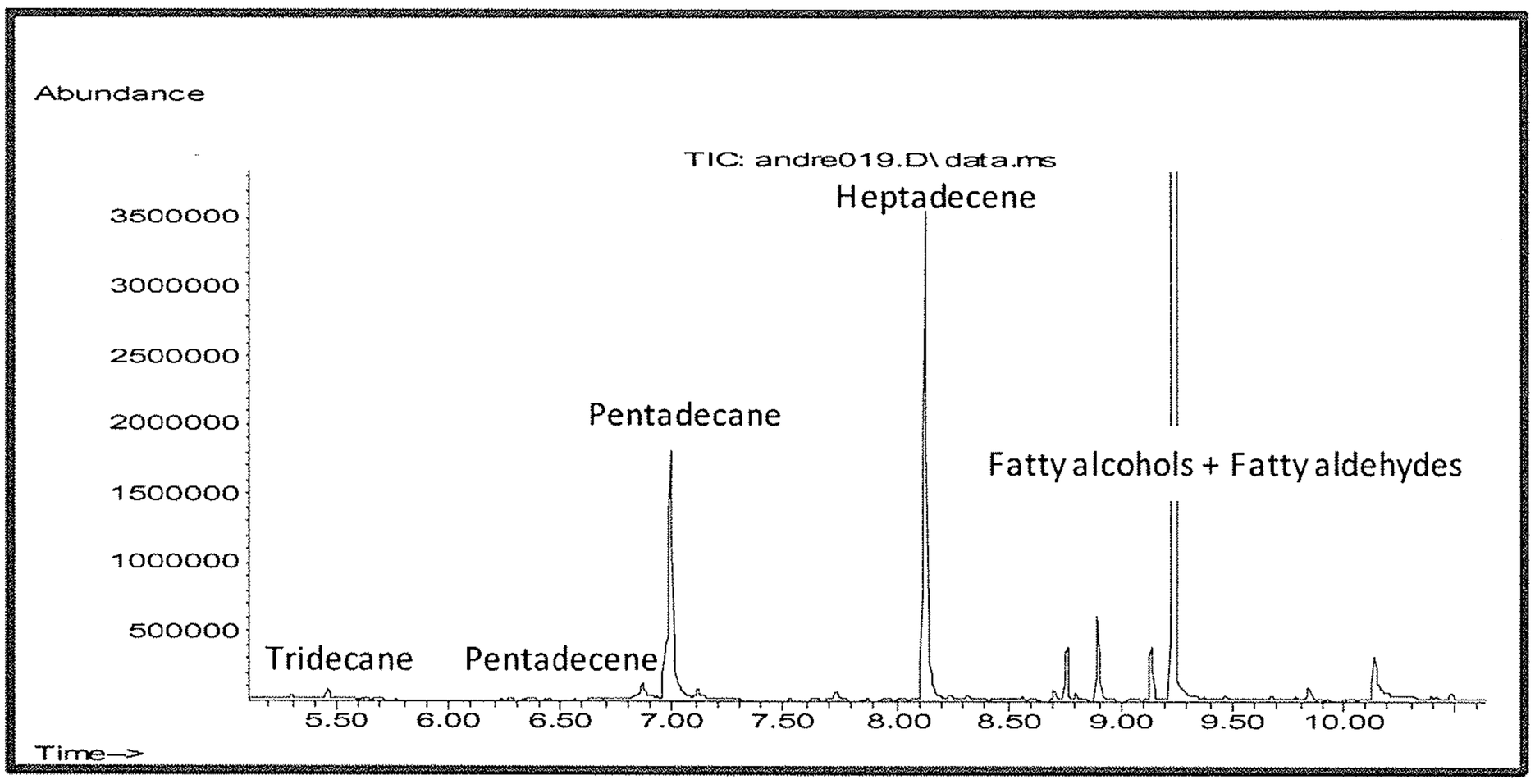
Abundance
TIC: andre019.D\ data.ms
3500000
3000000
2500000
2000000
1500000
1000000
500000
Heptadecene
Pentadecane
Fatty alcohols + Fatty aldehydes
Tridecane
Pentadecene
5.50
6.00
6.50
7.00
7.50
8.00
8.50
9.00
9.50
10.00
Time-->

FIG. 9

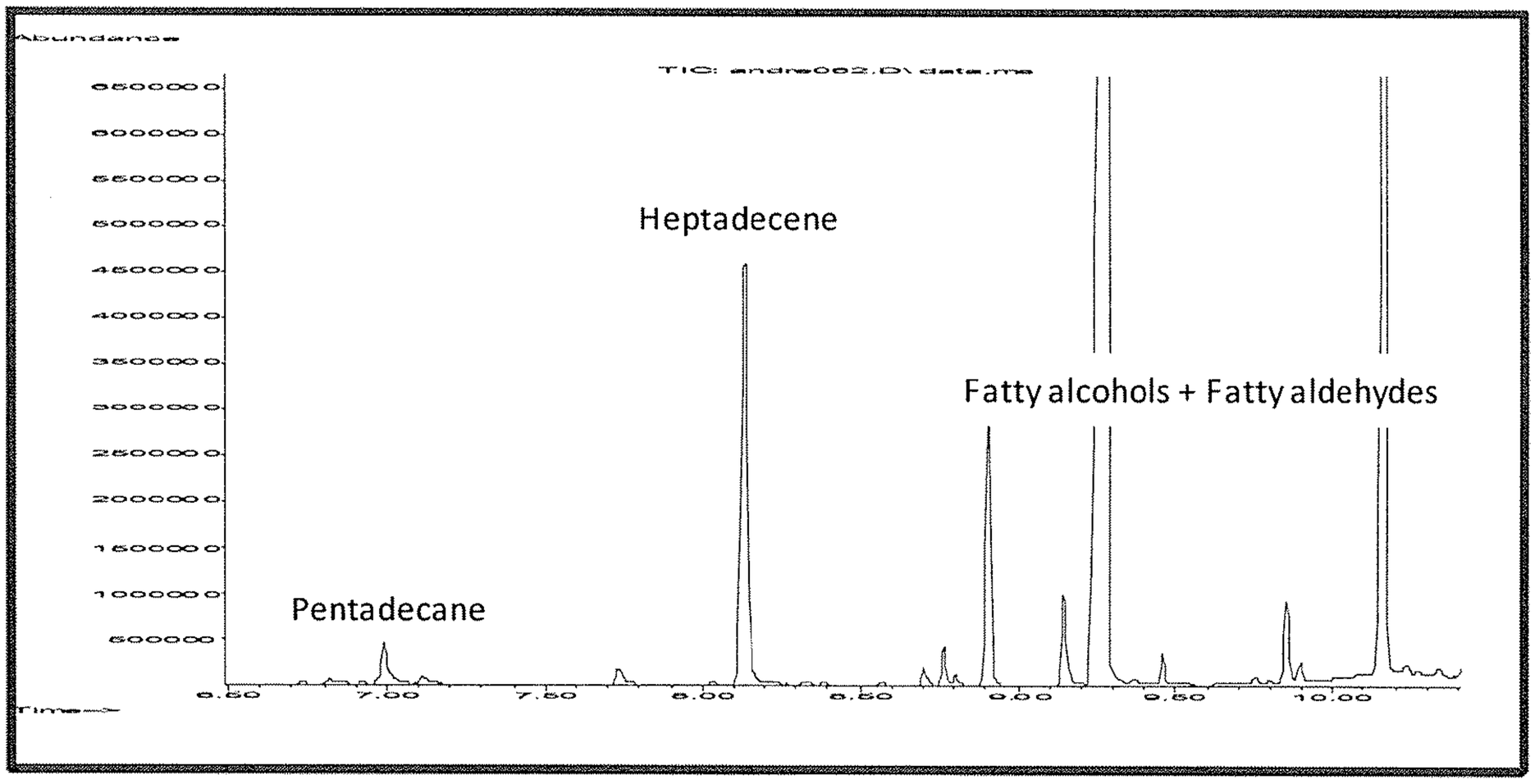
Heptadecene
Fatty alcohols + Fatty aldehydes
Pentadecane

FIG. 10

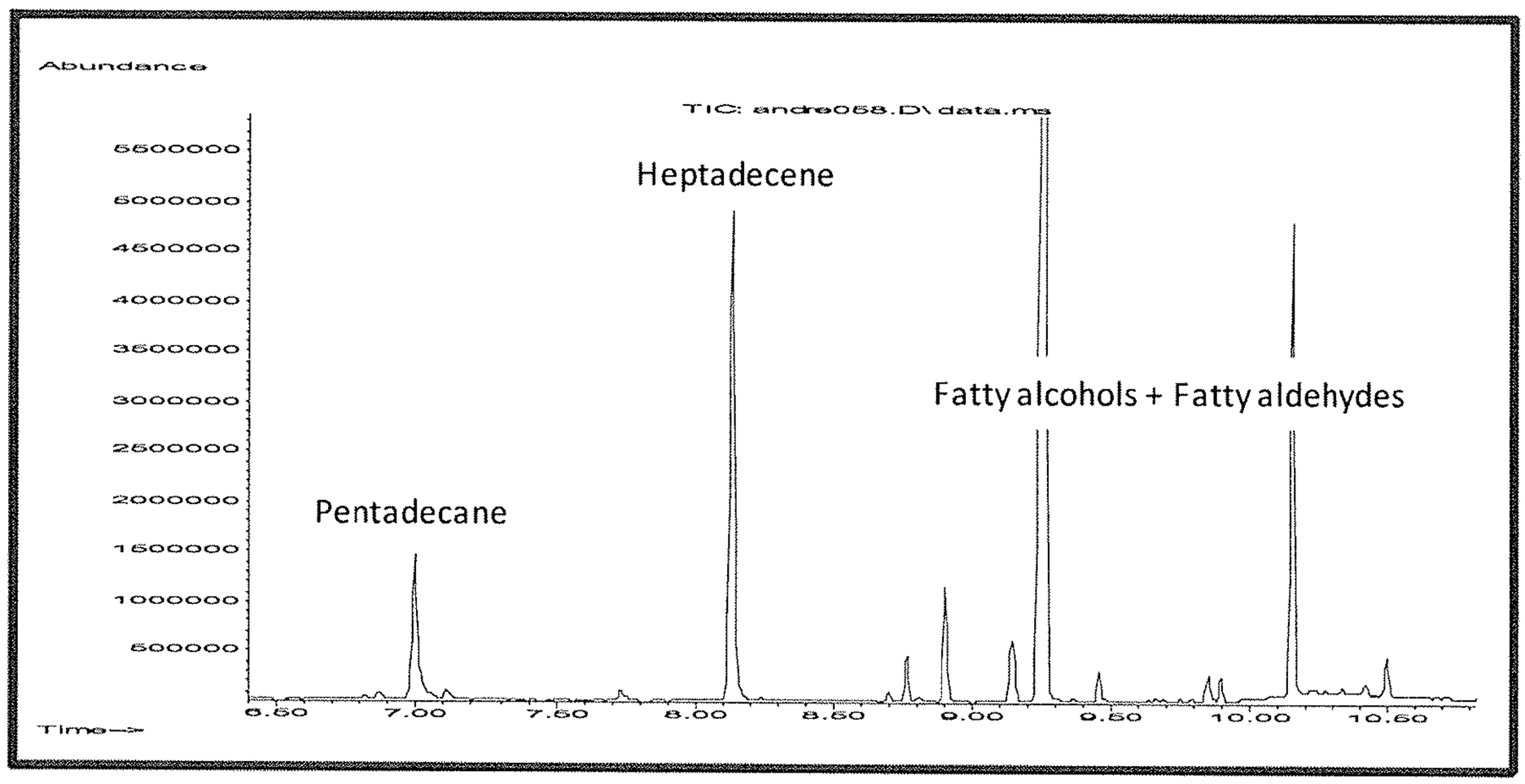
Abundance
TIC: andre068.D\ data.ms
Heptadecene
Fatty alcohols + Fatty aldehydes
Pentadecane
5600000
5000000
4600000
4000000
3600000
3000000
2500000
2000000
1600000
1000000
500000
6.50
7.00
7.50
8.00
8.50
9.00
9.50
10.00
10.50
Time-->

FIG. 11

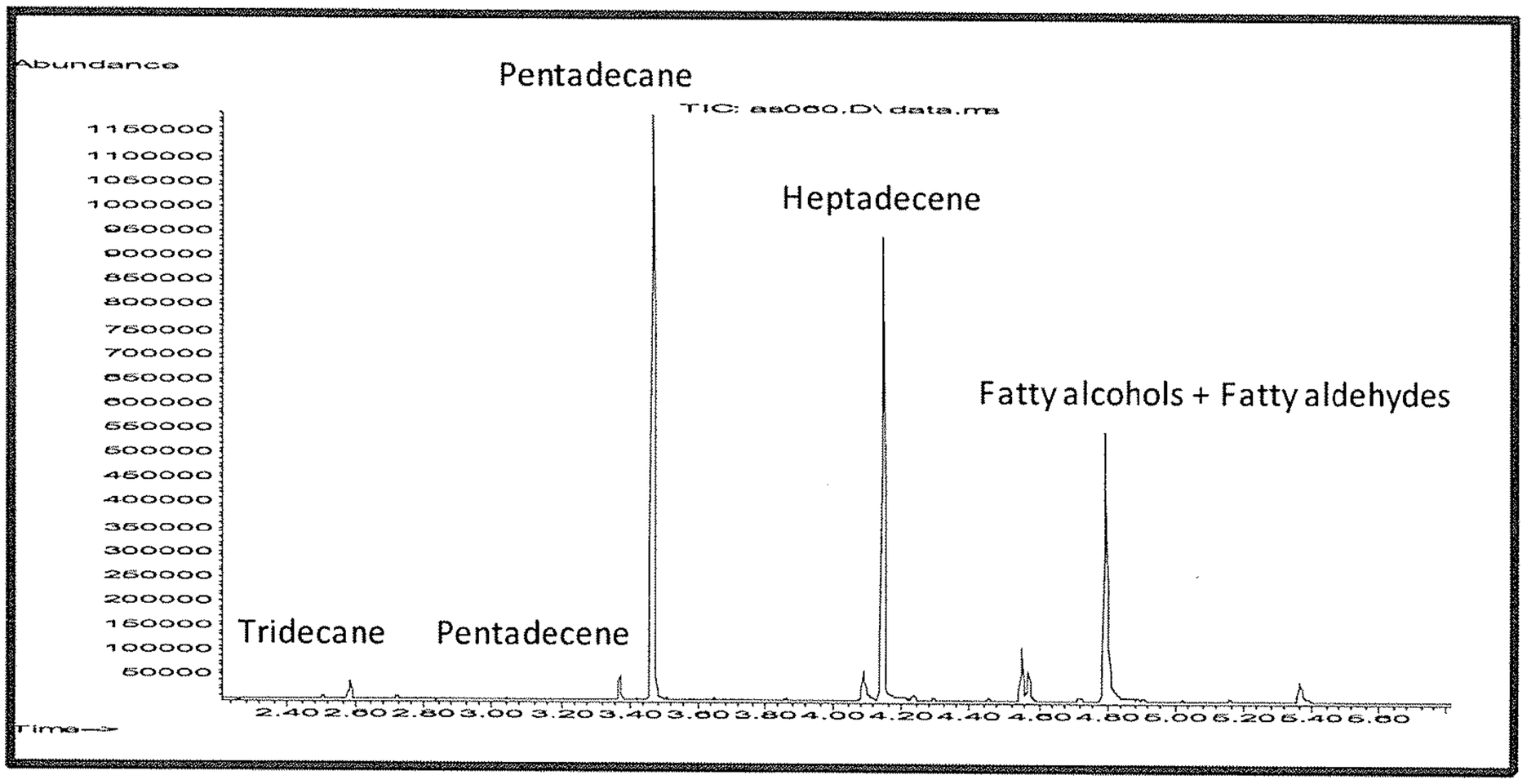
Abundance
Pentadecane
TIC: as060.D\ data.ms
Heptadecene
Fatty alcohols + Fatty aldehydes
Tridecane
Pentadecene
1160000
1100000
1060000
1000000
960000
900000
860000
800000
750000
700000
650000
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
2.40 2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20 5.40 5.60
Time-->

FIG. 12

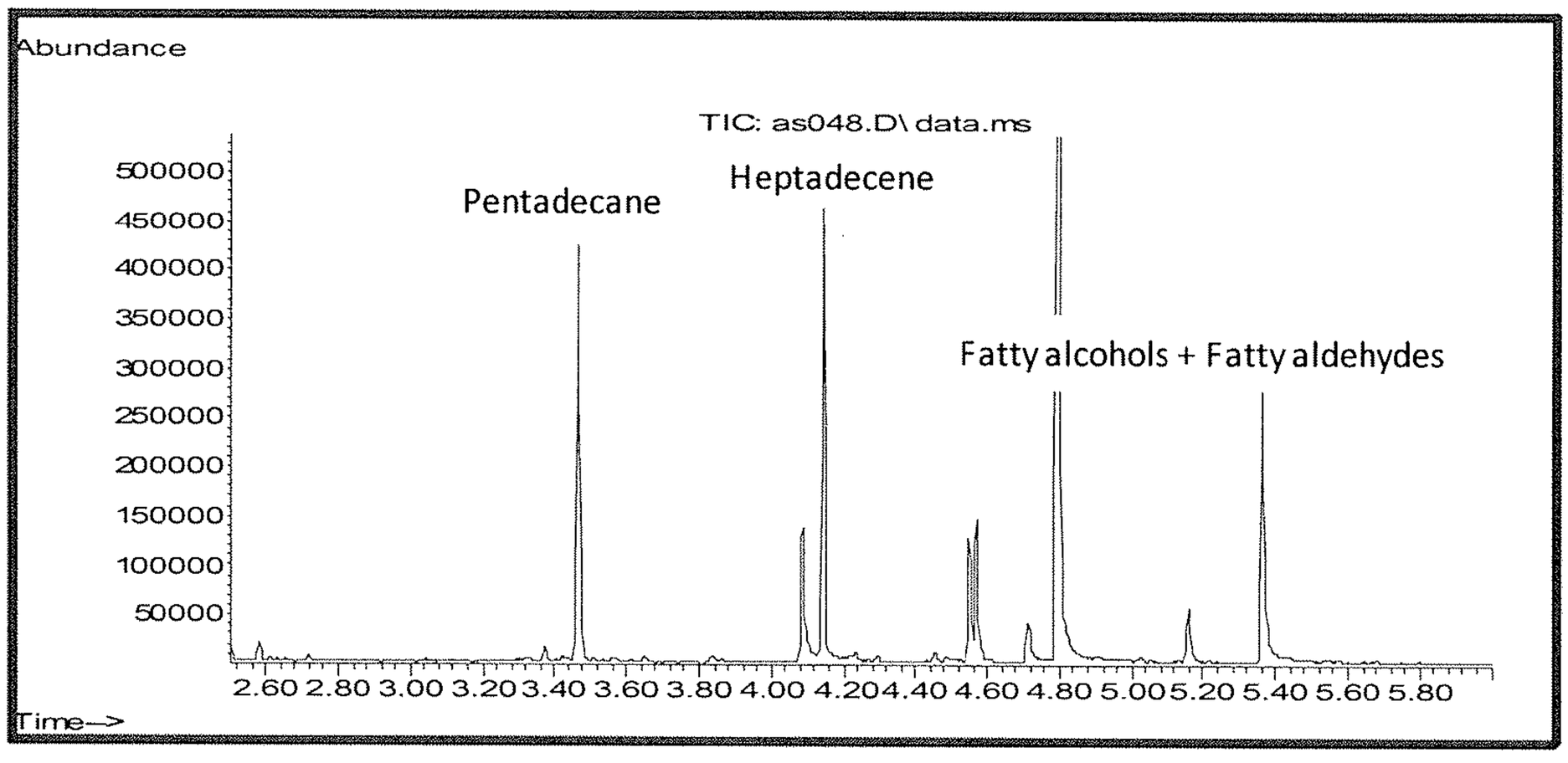
Abundance
TIC: as048.D\ data.ms
Pentadecane
Heptadecene
Fatty alcohols + Fatty aldehydes
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20 5.40 5.60 5.80
Time-->

FIG. 13

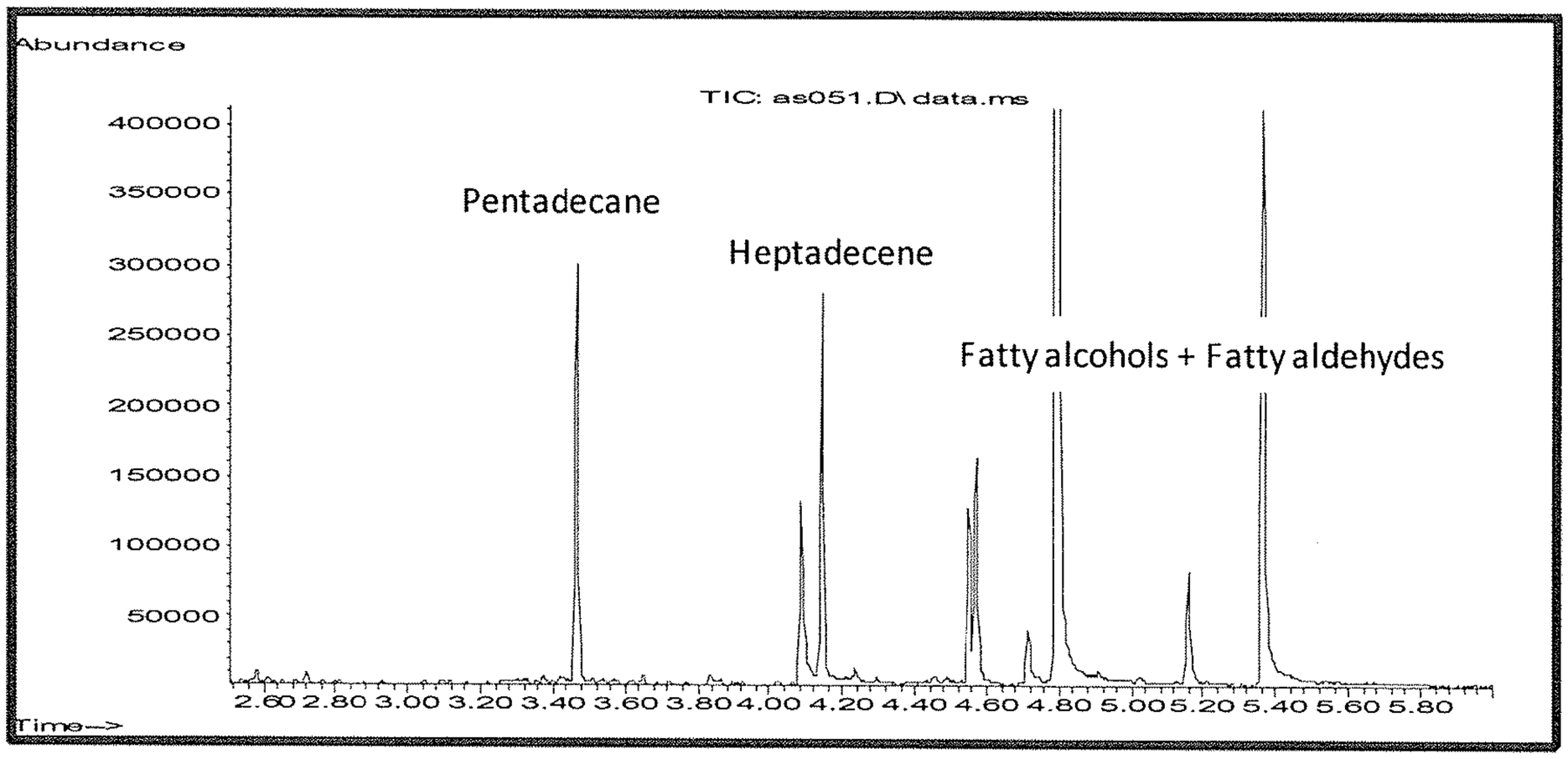
Abundance
TIC: as051.D\ data.ms
Pentadecane
Heptadecene
Fatty alcohols + Fatty aldehydes
400000
350000
300000
250000
200000
150000
100000
50000
2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20 5.40 5.60 5.80
Time-->

FIG. 14

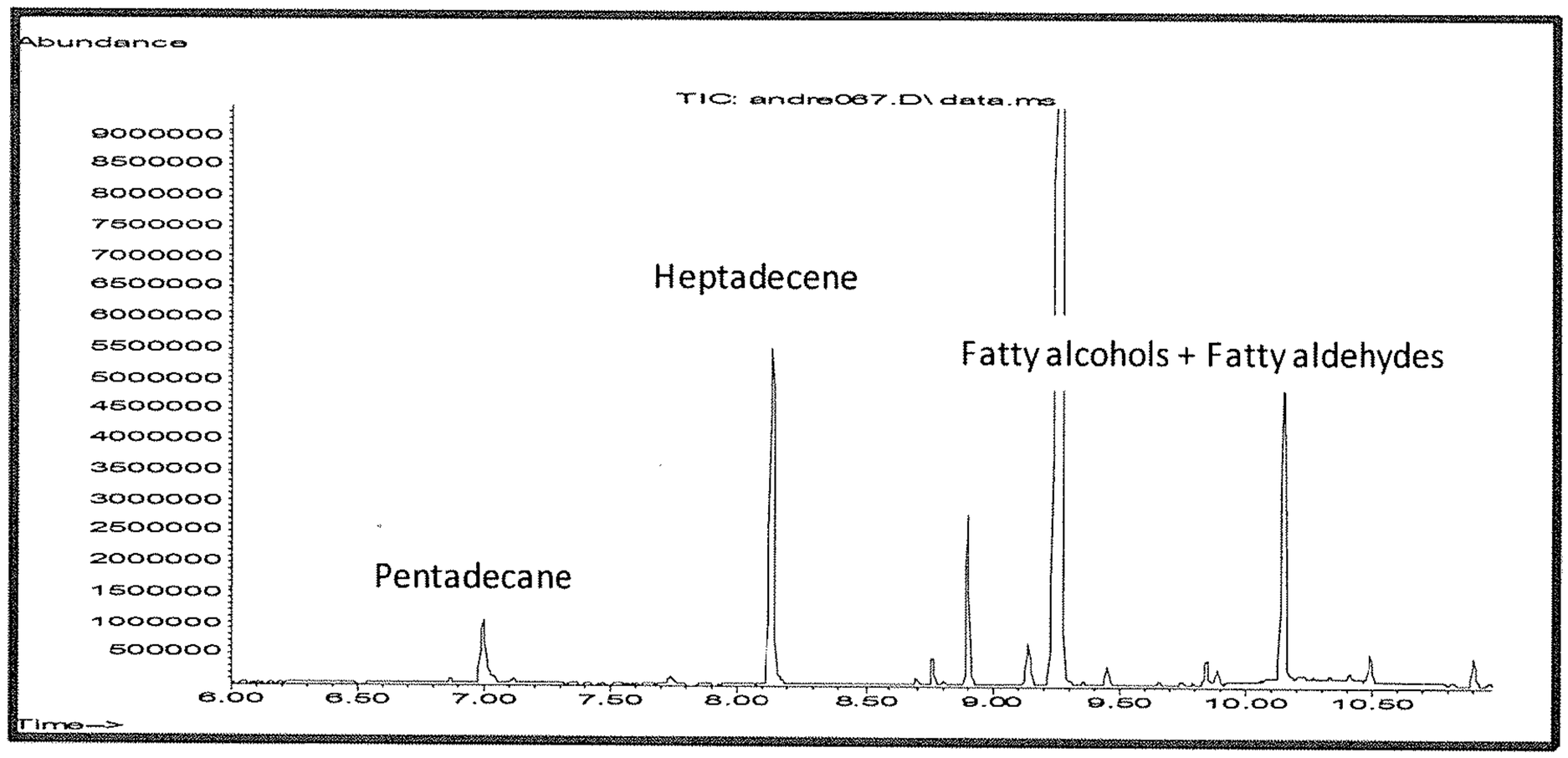
Abundance
TIC: andre067.D\ data.ms
9000000
8500000
8000000
7500000
7000000
6500000
6000000
5500000
5000000
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
Heptadecene
Fatty alcohols + Fatty aldehydes
Pentadecane
6.00
6.50
7.00
7.50
8.00
8.50
9.00
9.50
10.00
10.50
Time-->

FIG. 15

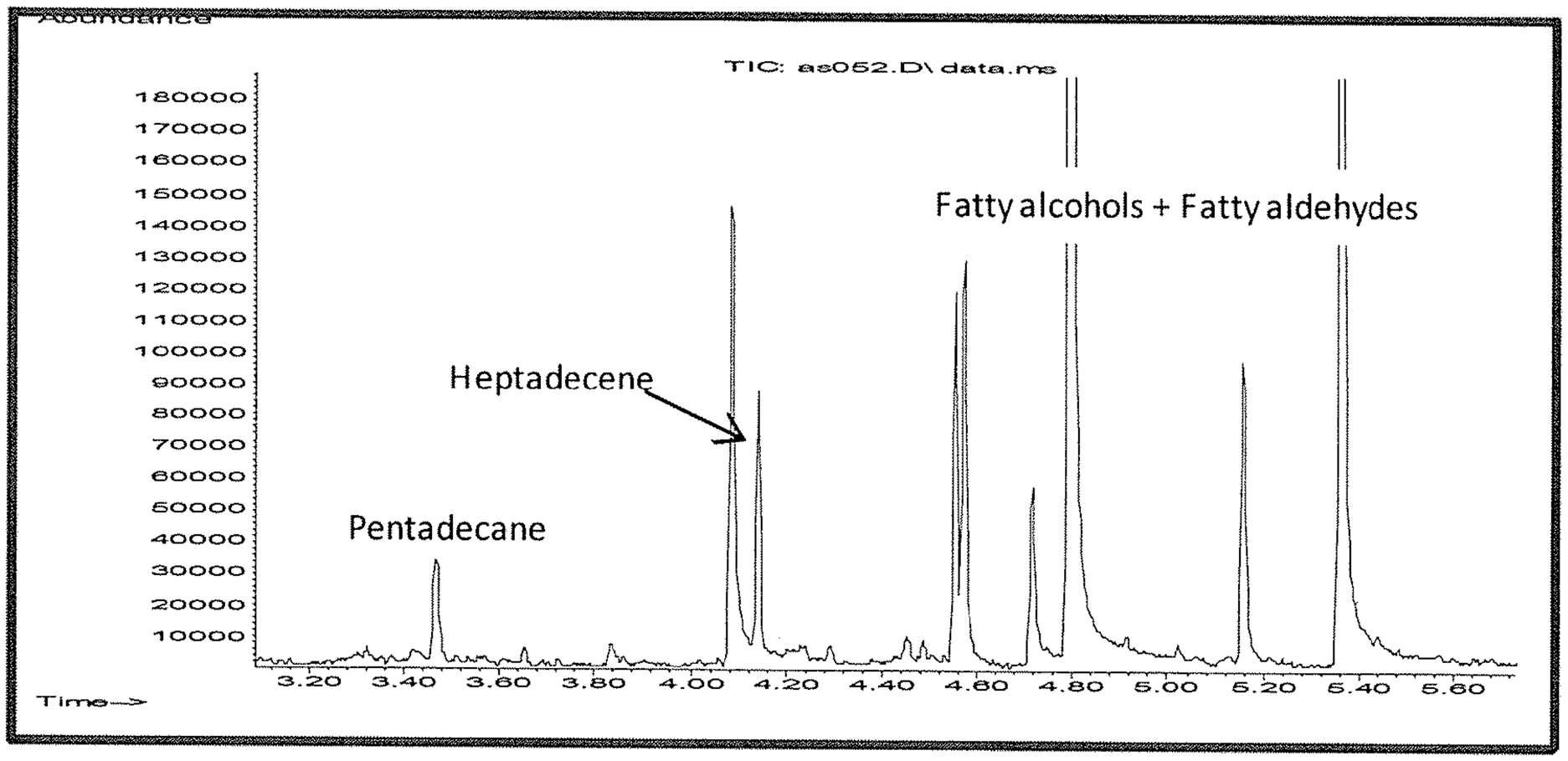
Abundance
TIC: as052.D\ data.ms
180000
170000
160000
150000
140000
130000
120000
110000
100000
90000
80000
70000
60000
50000
40000
30000
20000
10000
Fatty alcohols + Fatty aldehydes
Heptadecene
Pentadecane
3.20
3.40
3.60
3.80
4.00
4.20
4.40
4.60
4.80
5.00
5.20
5.40
5.60
Time-->

FIG. 16

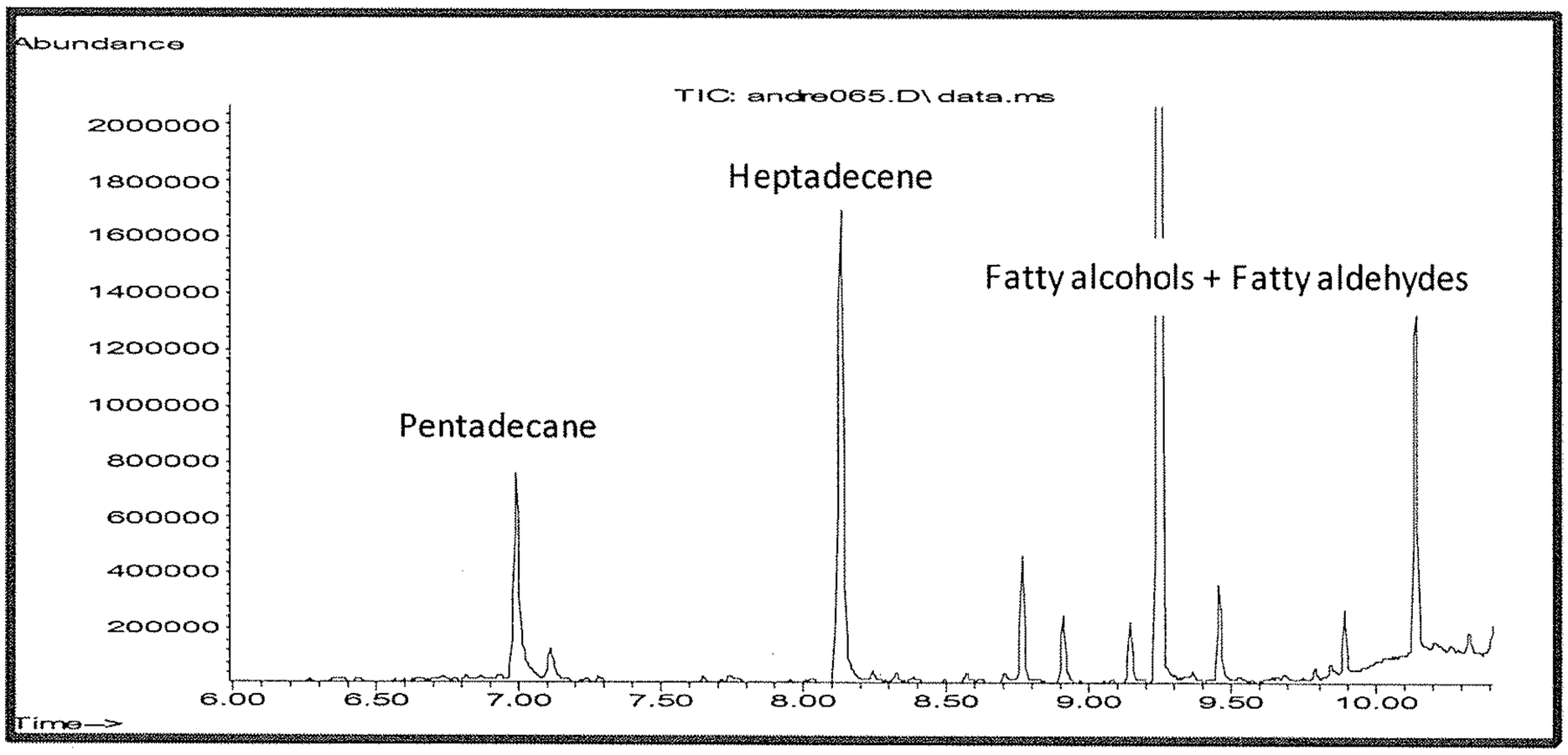
Abundance
TIC: andre065.D\ data.ms
Heptadecene
Fatty alcohols + Fatty aldehydes
Pentadecane
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
6.00
6.50
7.00
7.50
8.00
8.50
9.00
9.50
10.00
Time-->

FIG. 17

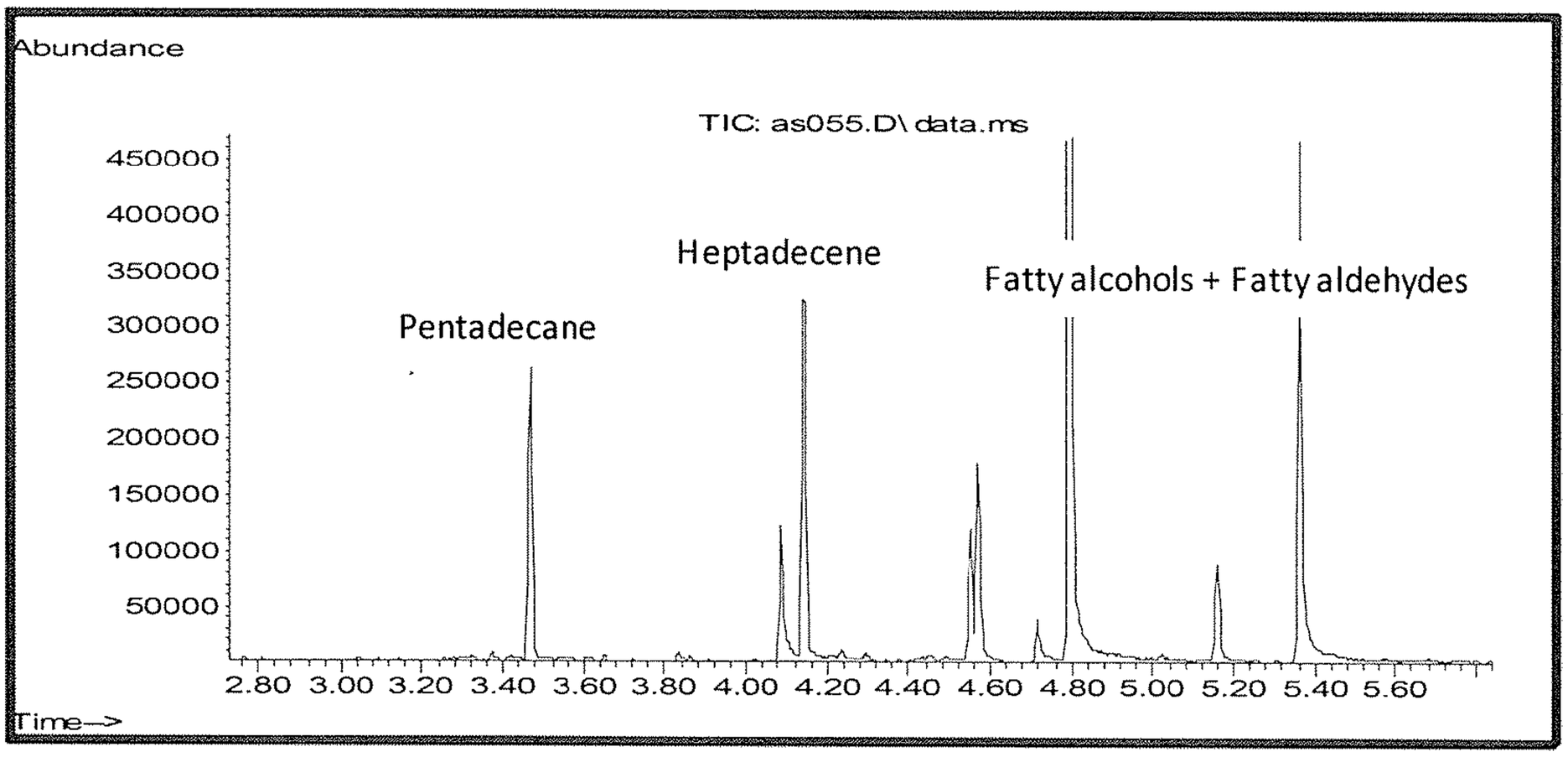
Abundance
TIC: as055.D\ data.ms
Pentadecane
Heptadecene
Fatty alcohols + Fatty aldehydes
450000
400000
350000
300000
250000
200000
150000
100000
50000
2.80
3.00
3.20
3.40
3.60
3.80
4.00
4.20
4.40
4.60
4.80
5.00
5.20
5.40
5.60
Time-->

FIG. 18

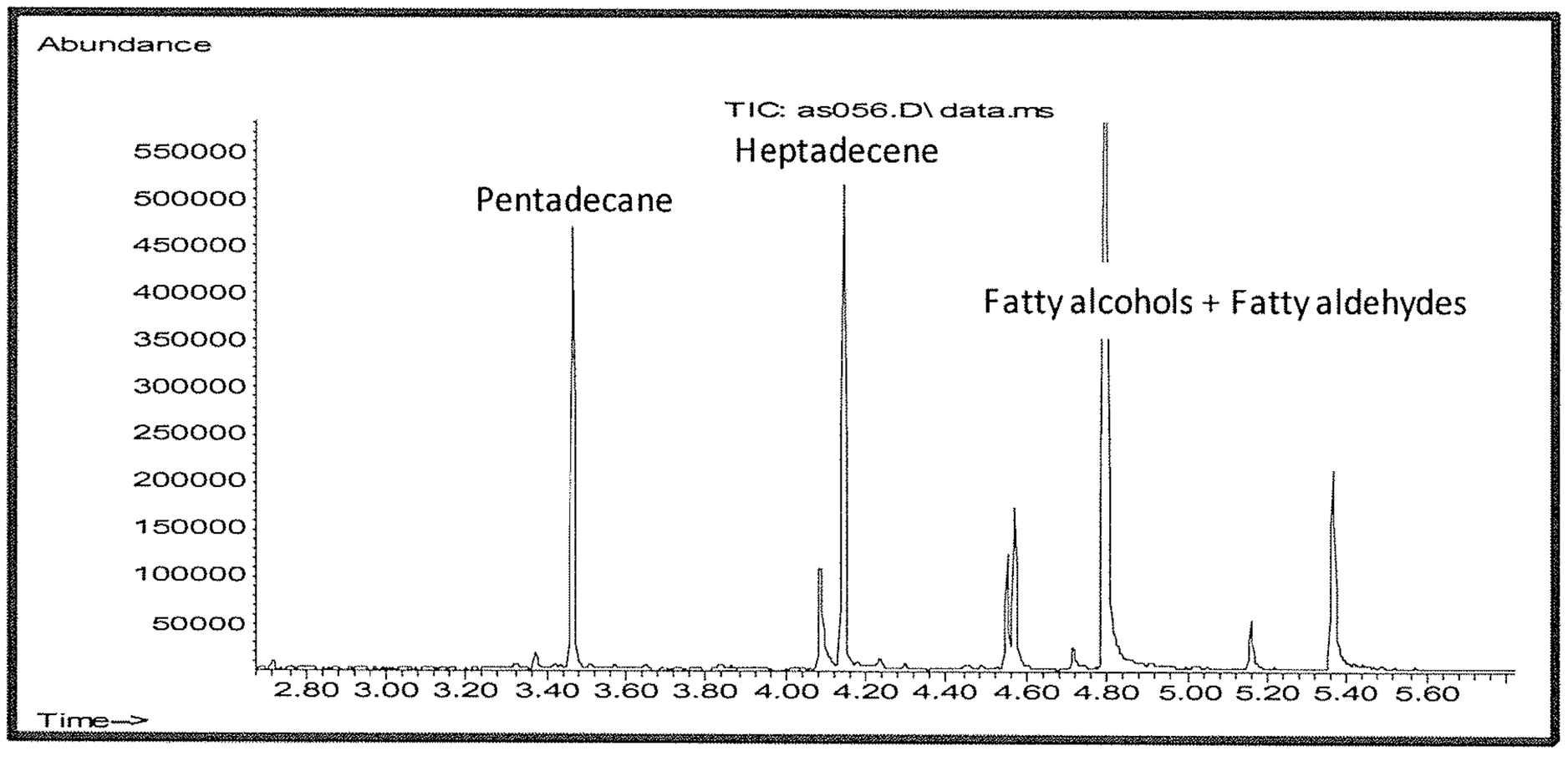
Abundance
TIC: as056.D\ data.ms
Heptadecene
Pentadecane
Fatty alcohols + Fatty aldehydes
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20 5.40 5.60
Time-->

FIG. 19

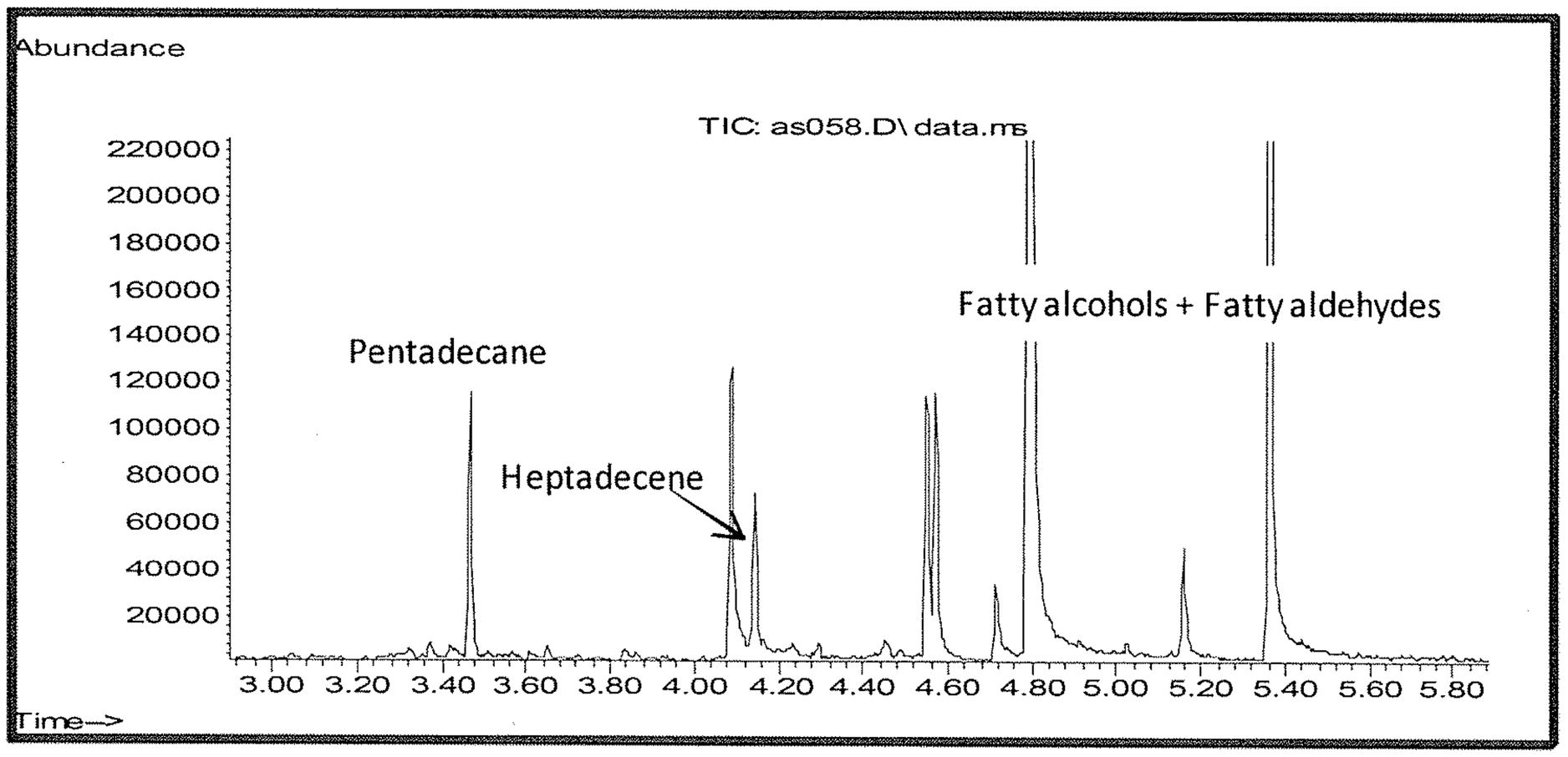
Abundance
TIC: as058.D\ data.ms
Fatty alcohols + Fatty aldehydes
Pentadecane
Heptadecene
220000
200000
180000
160000
140000
120000
100000
80000
60000
40000
20000
3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20 5.40 5.60 5.80
Time-->

FIG. 20

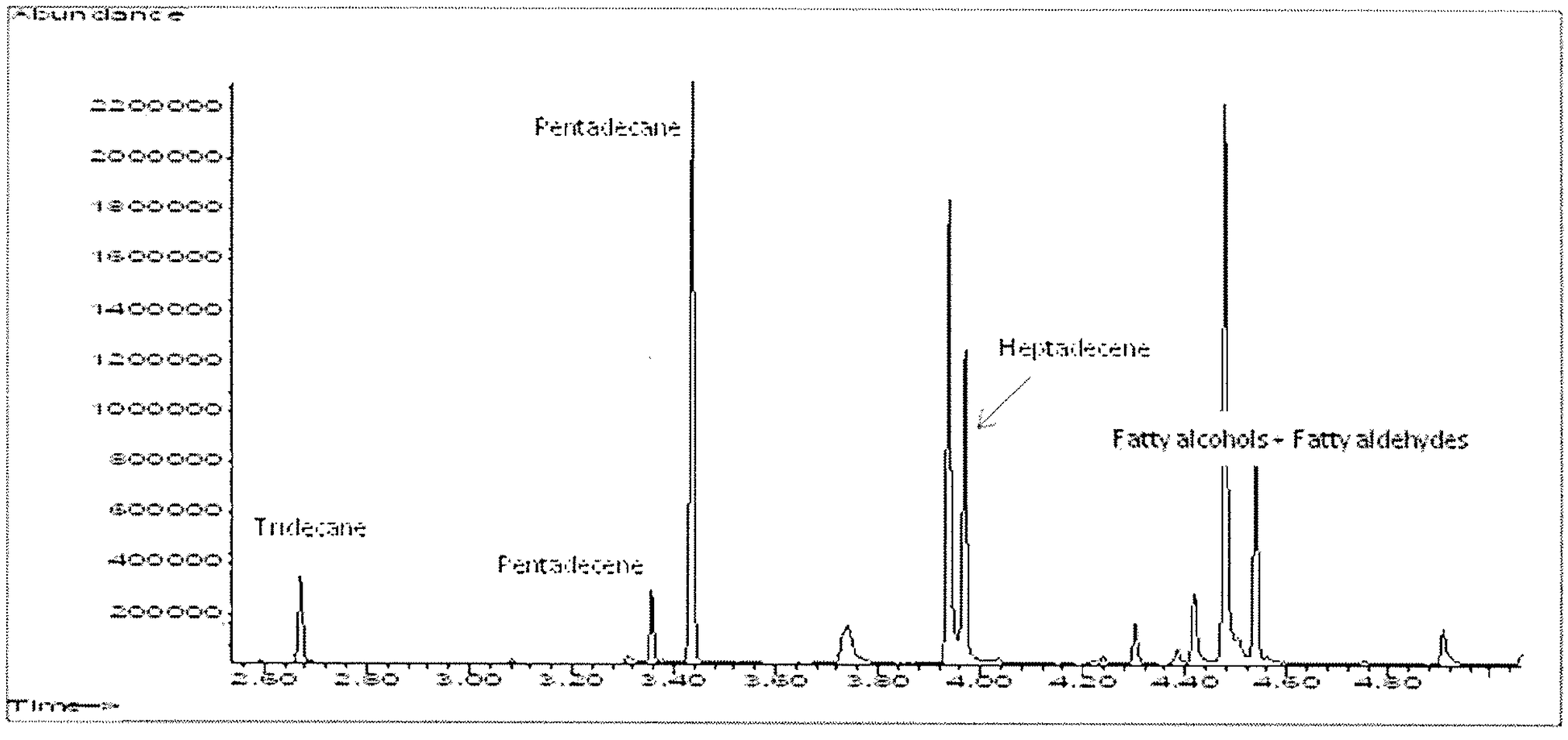
Abundance
2200000
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
Tridecane
Pentadecane
Pentadecane
Heptadecene
Fatty alcohols + Fatty aldehydes
2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80
Time

FIG. 21

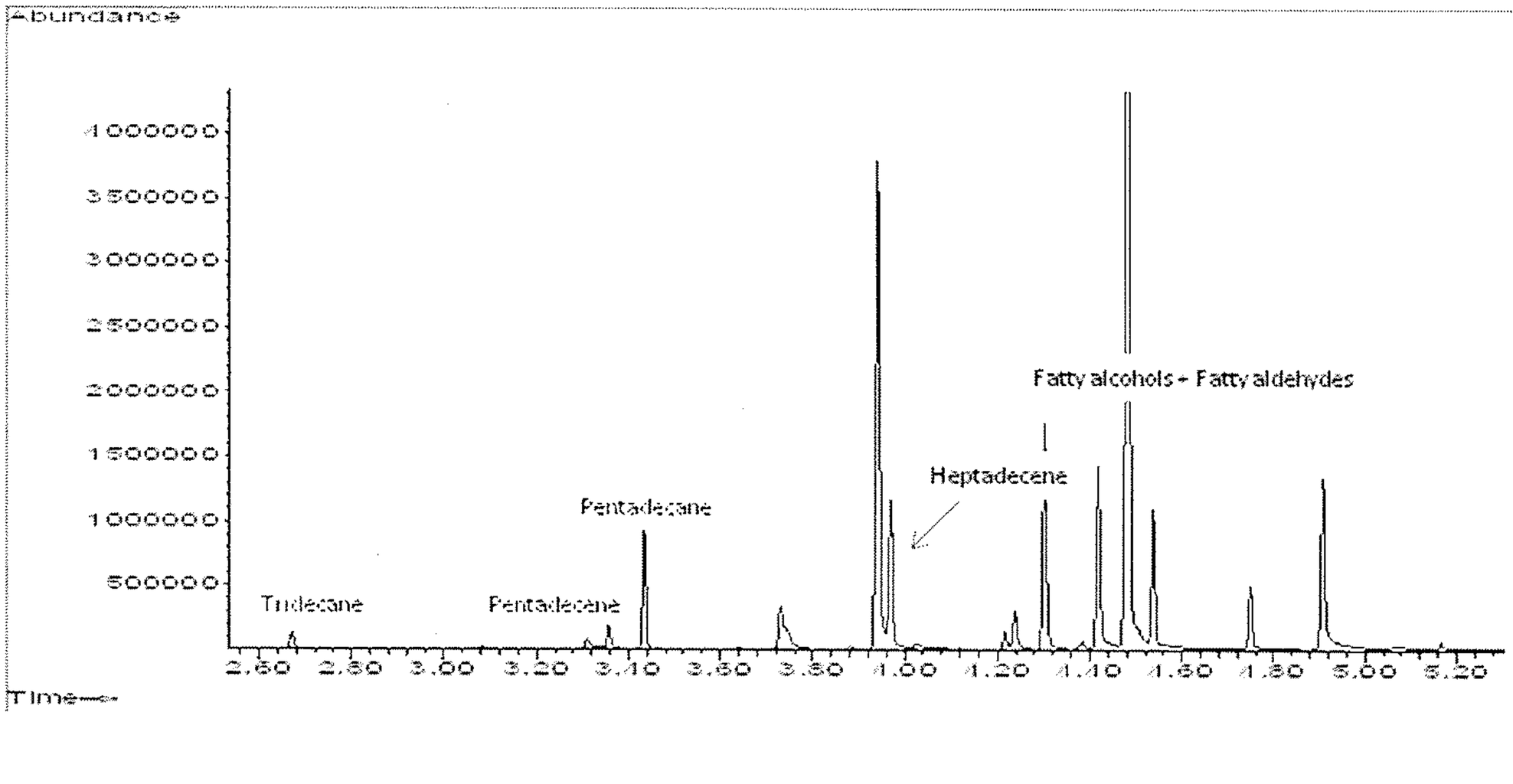
Abundance
1000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
Tridecane
Pentadecane
Pentadecane
Heptadecene
Fatty alcohols + Fatty aldehydes
2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20
Time

FIG. 22

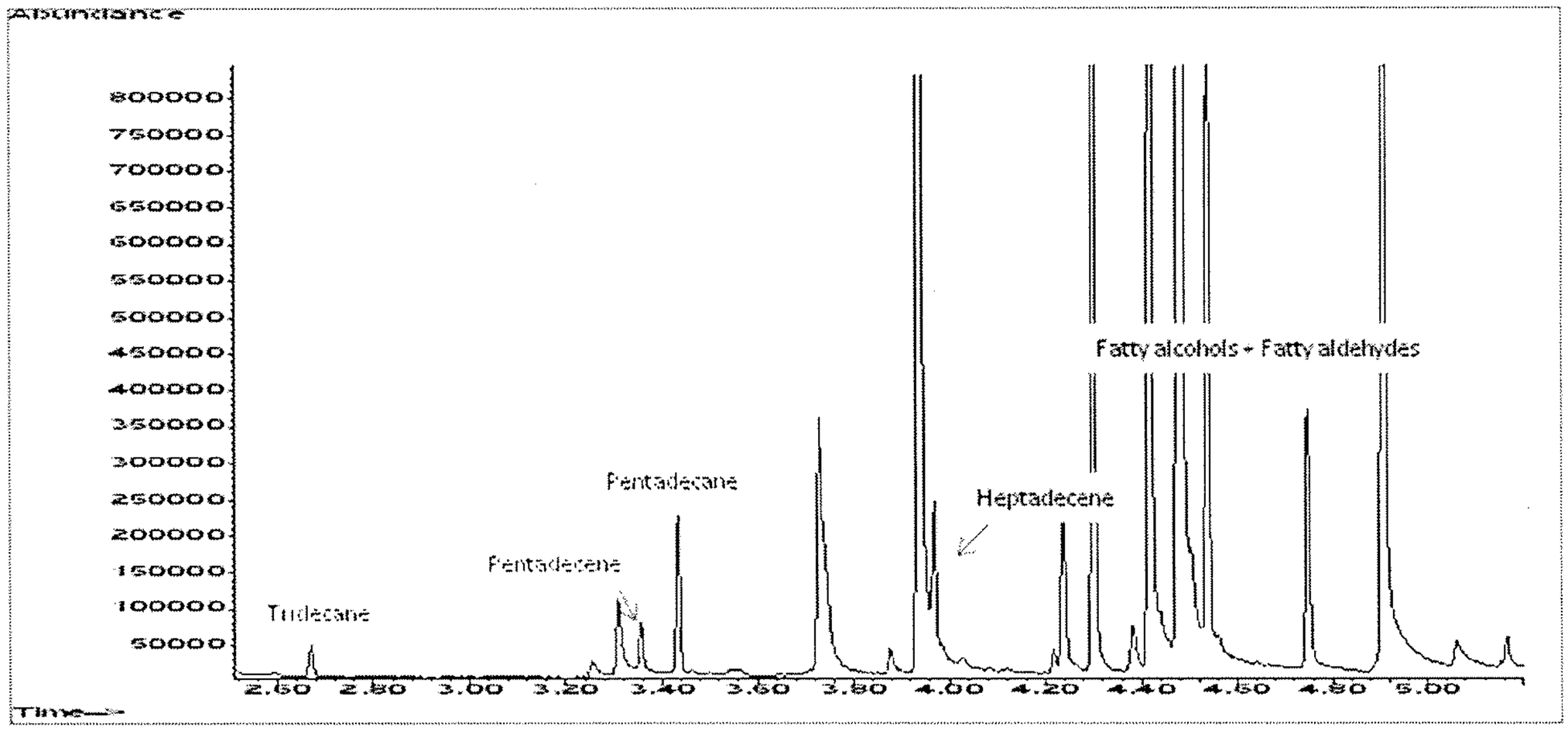
Abundance
800000
750000
700000
650000
600000
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
Tridecane
Pentadecene
Pentadecane
Heptadecene
Fatty alcohols + Fatty aldehydes
2.60 2.80 3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.50 4.80 5.00
Time

FIG. 23

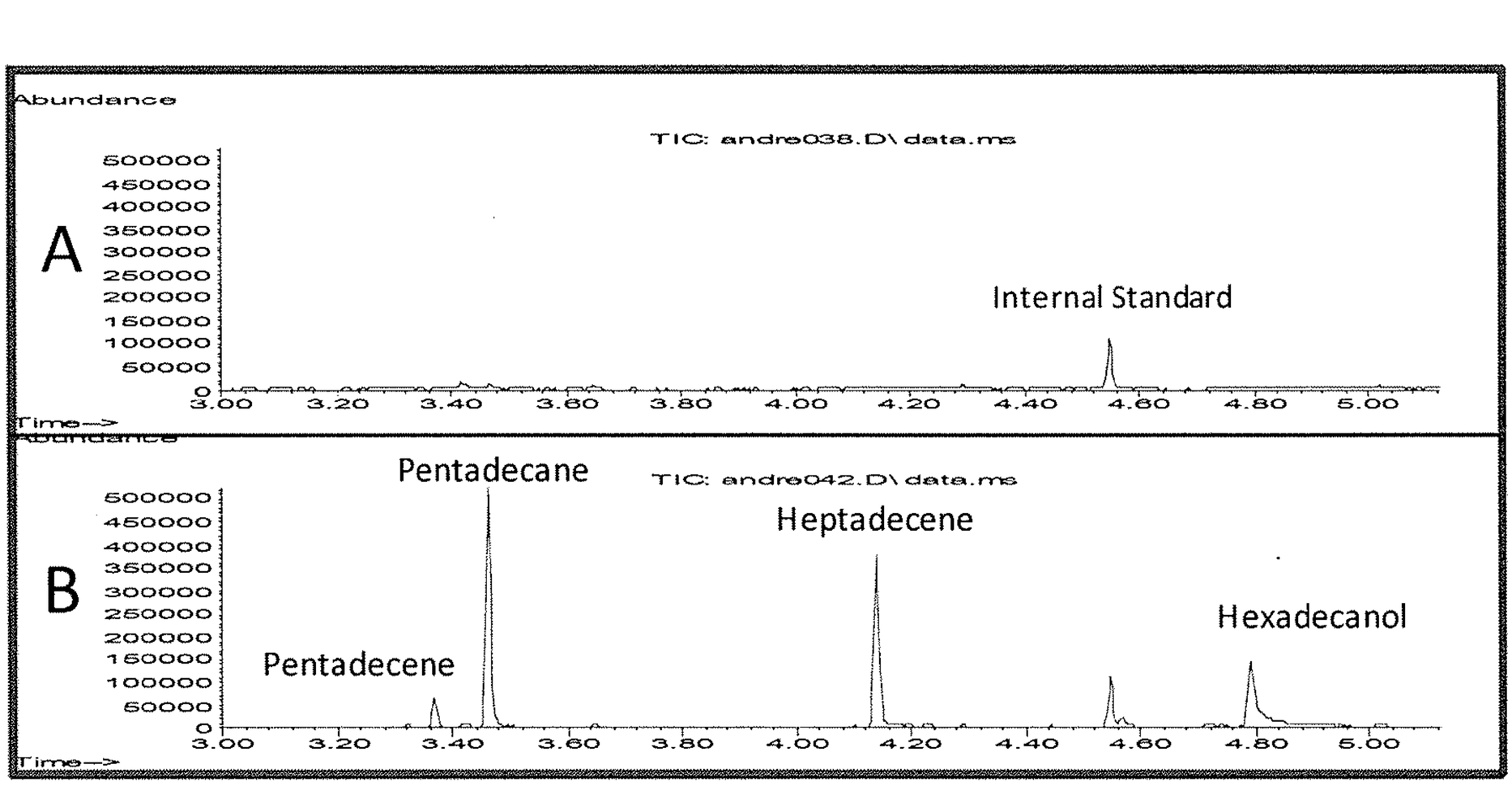
Abundance
TIC: andre038.D\data.ms
A
Internal Standard
Time
Abundance
TIC: andre042.D\data.ms
B
Pentadecane
Heptadecene
Hexadecanol
Pentadecene
3.00 3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00
Time

FIG. 24

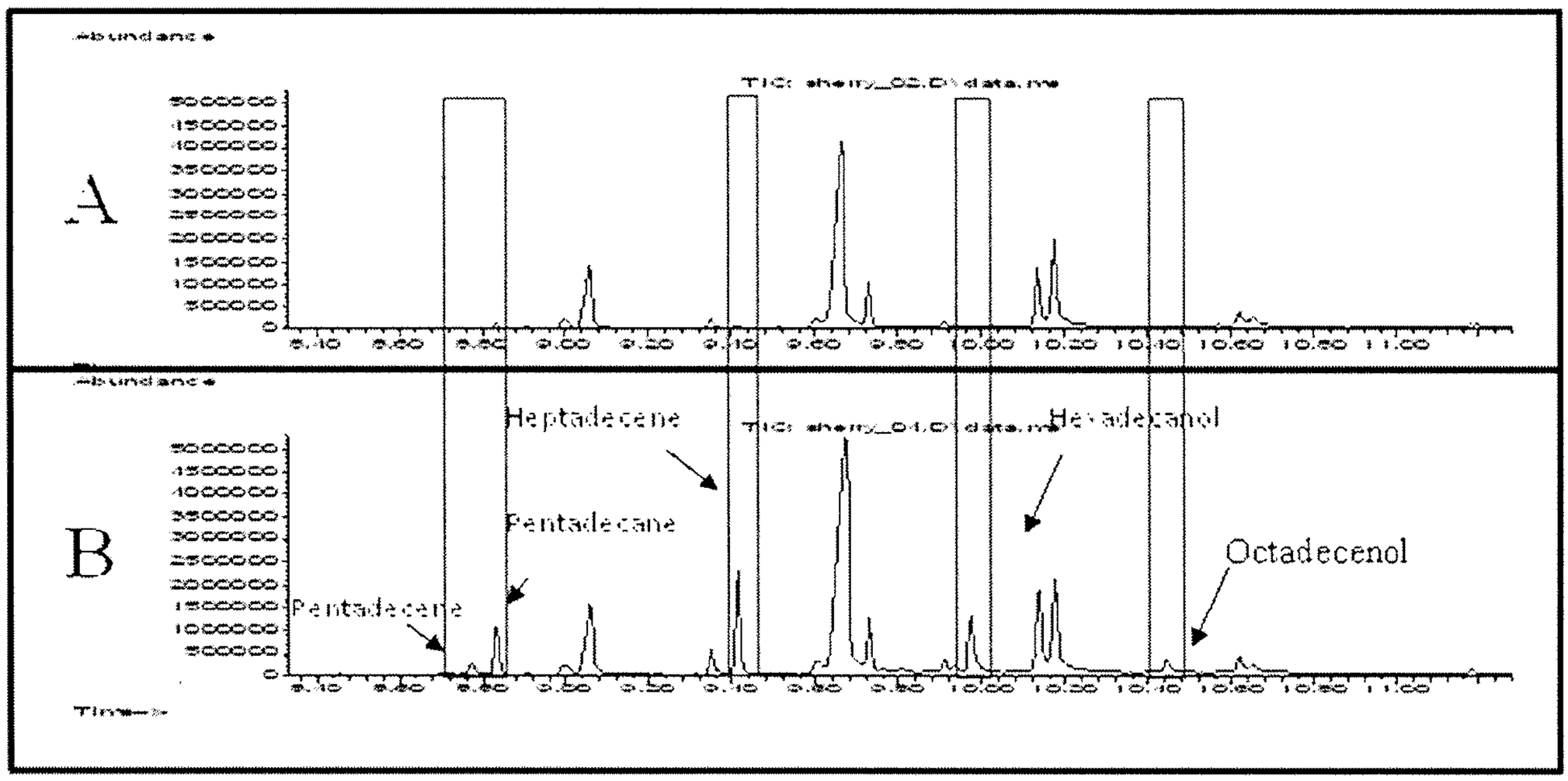
A
B
Heptadecene
Pentadecane
Pentadecene
Hexadecanol
Octadecenol

FIG. 25

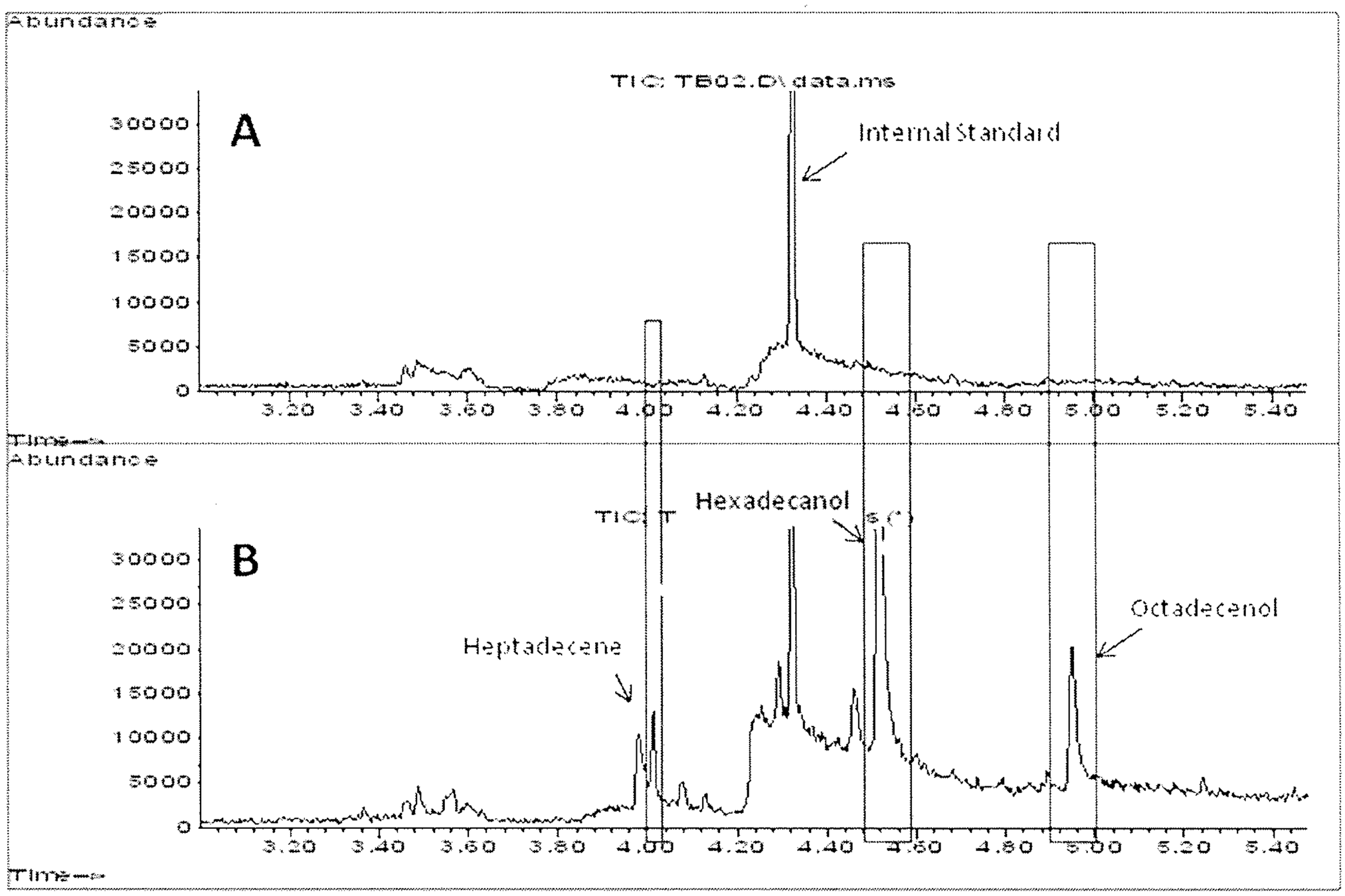
Abundance
TIC: T502.D\data.ms
A
Internal Standard
30000
25000
20000
15000
10000
5000
0
3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20 5.40
Time
Abundance
TIC: T
B
Hexadecanol
Heptadecene
Octadecenol
30000
25000
20000
15000
10000
5000
0
3.20 3.40 3.60 3.80 4.00 4.20 4.40 4.60 4.80 5.00 5.20 5.40
Time

FIG. 26

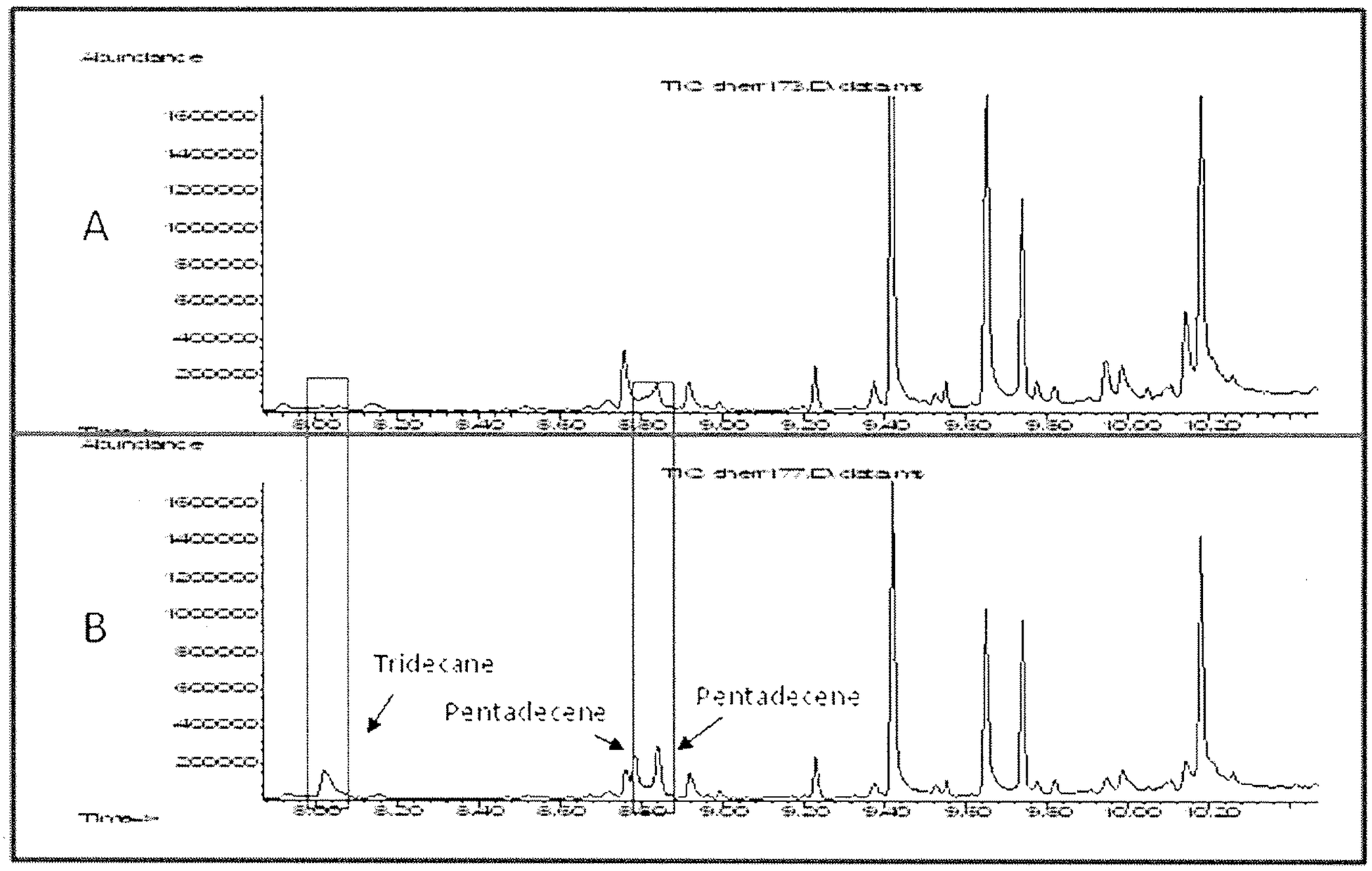

FIG. 27

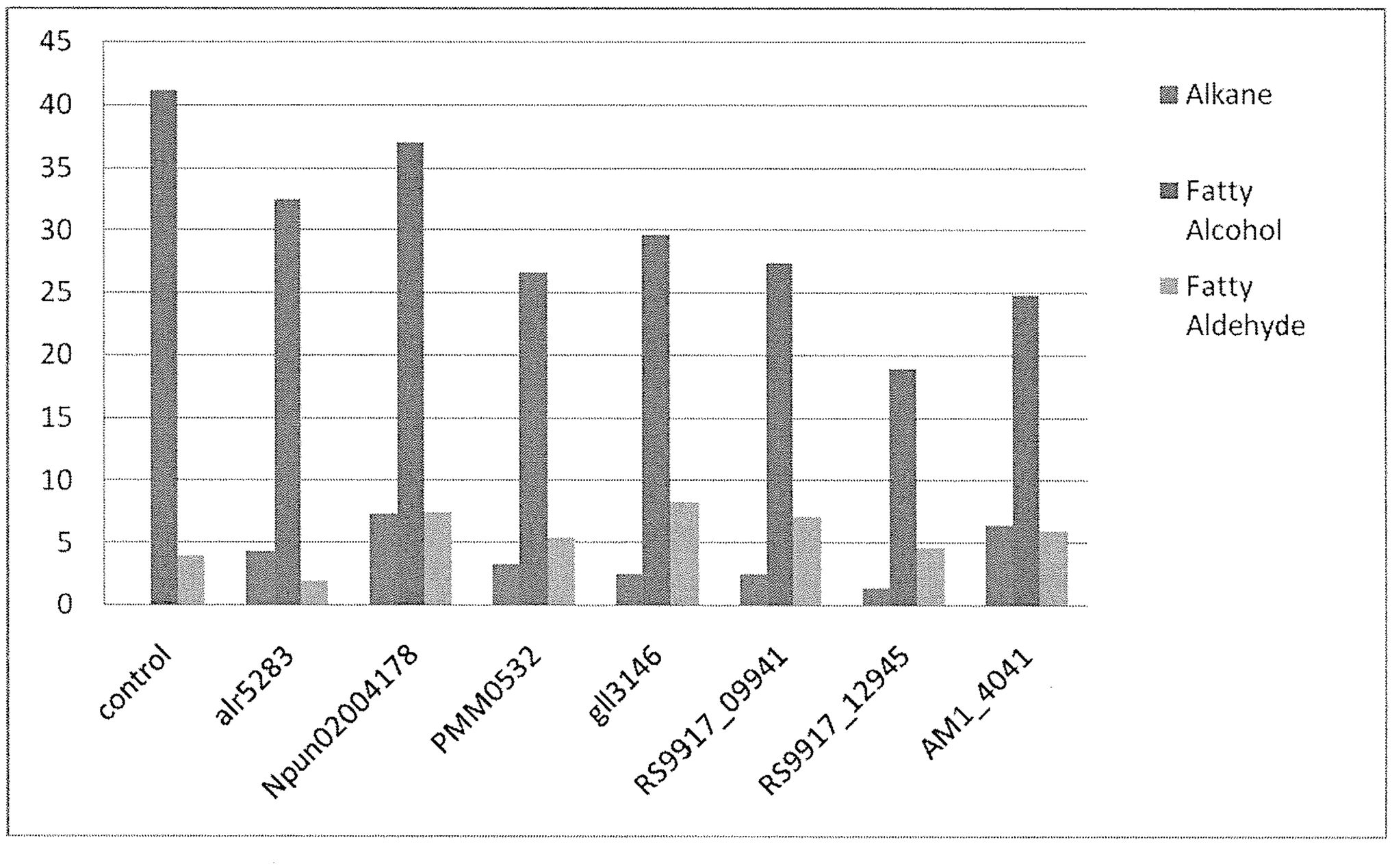
45
40
35
30
25
20
15
10
5
0
control
alr5283
Npun02004178
PMM0532
gll3146
RS9917_09941
RS9917_12945
AM1_4041
Alkane
Fatty Alcohol
Fatty Aldehyde

FIG. 28

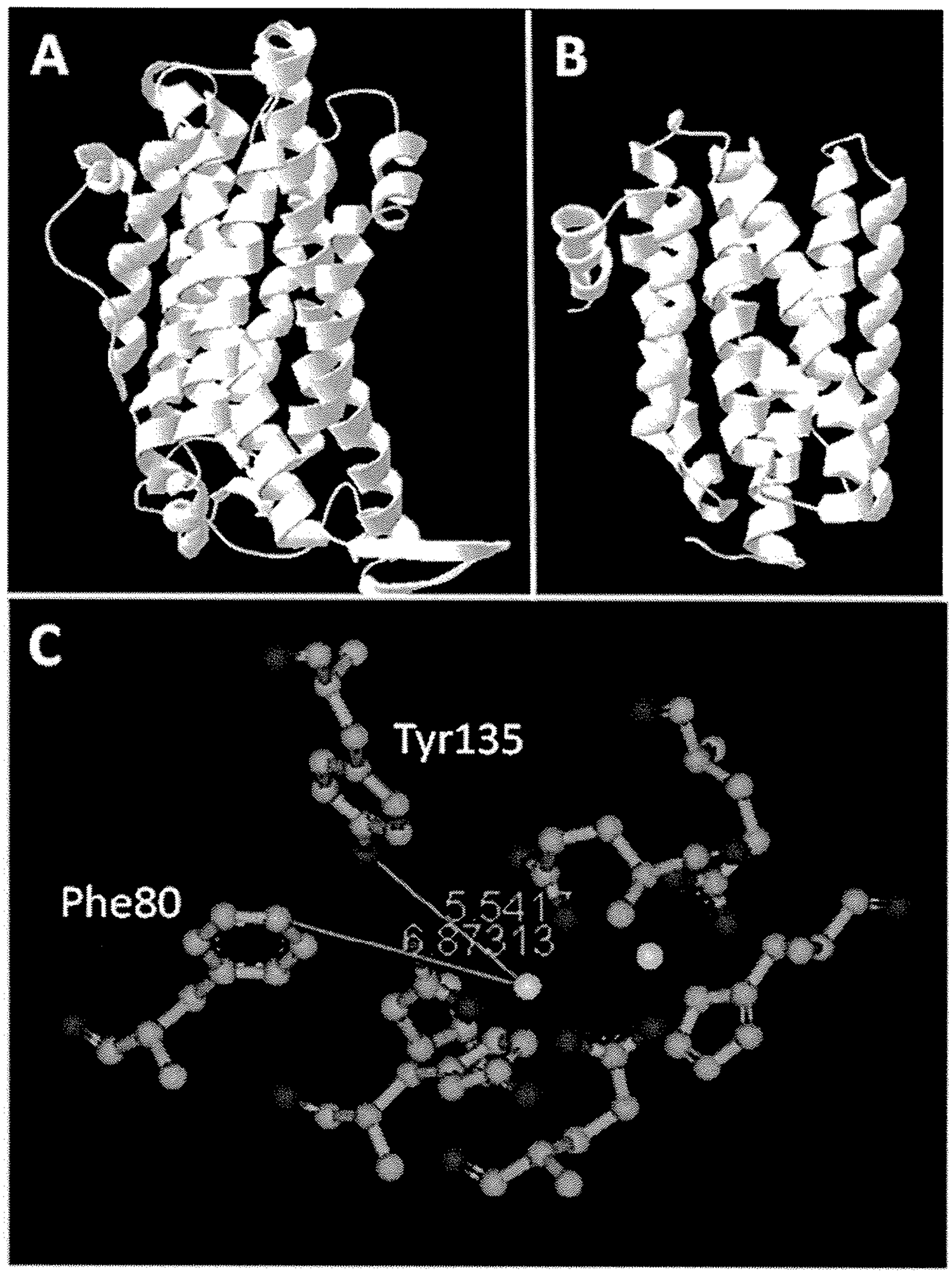

FIG. 29

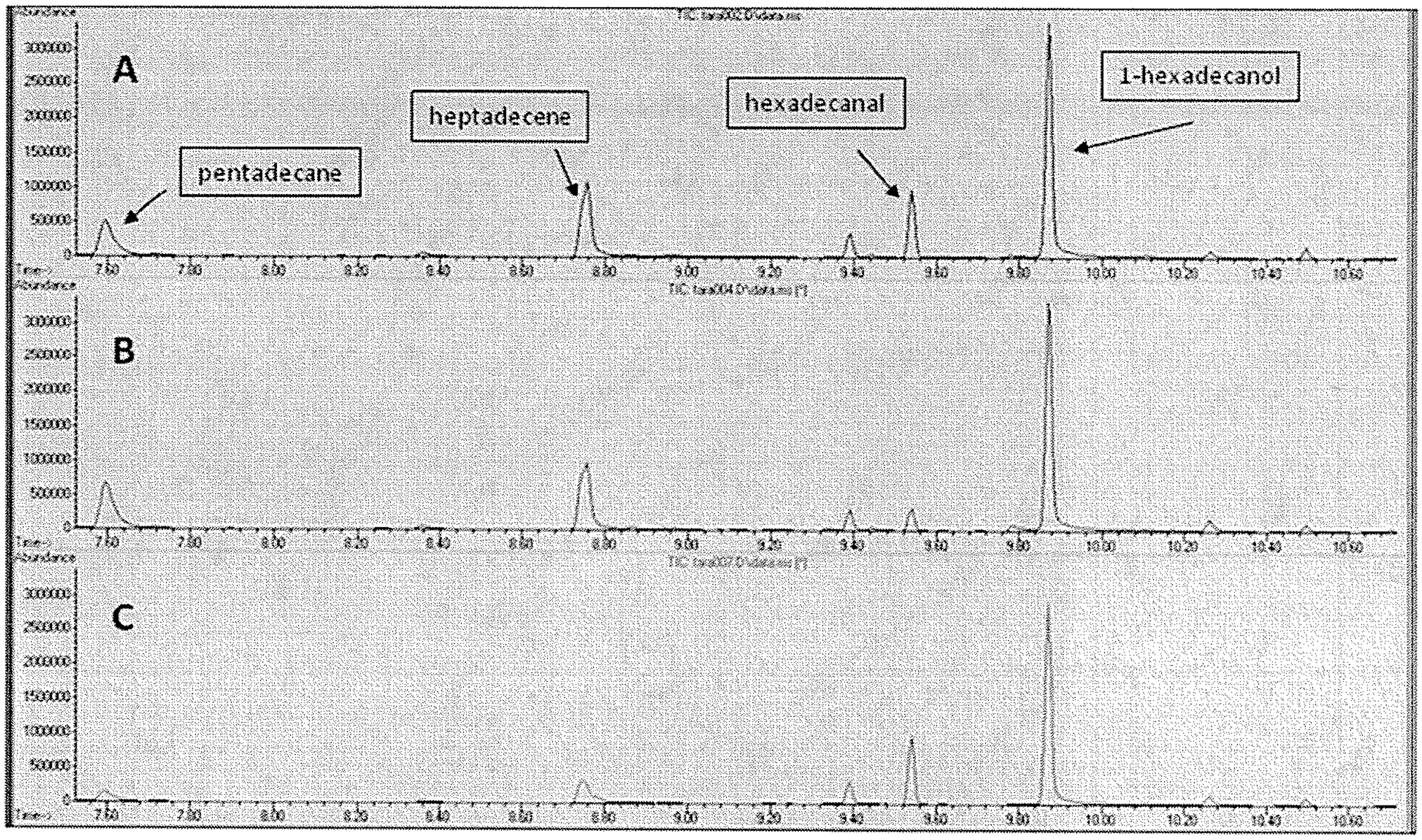

FIG. 30

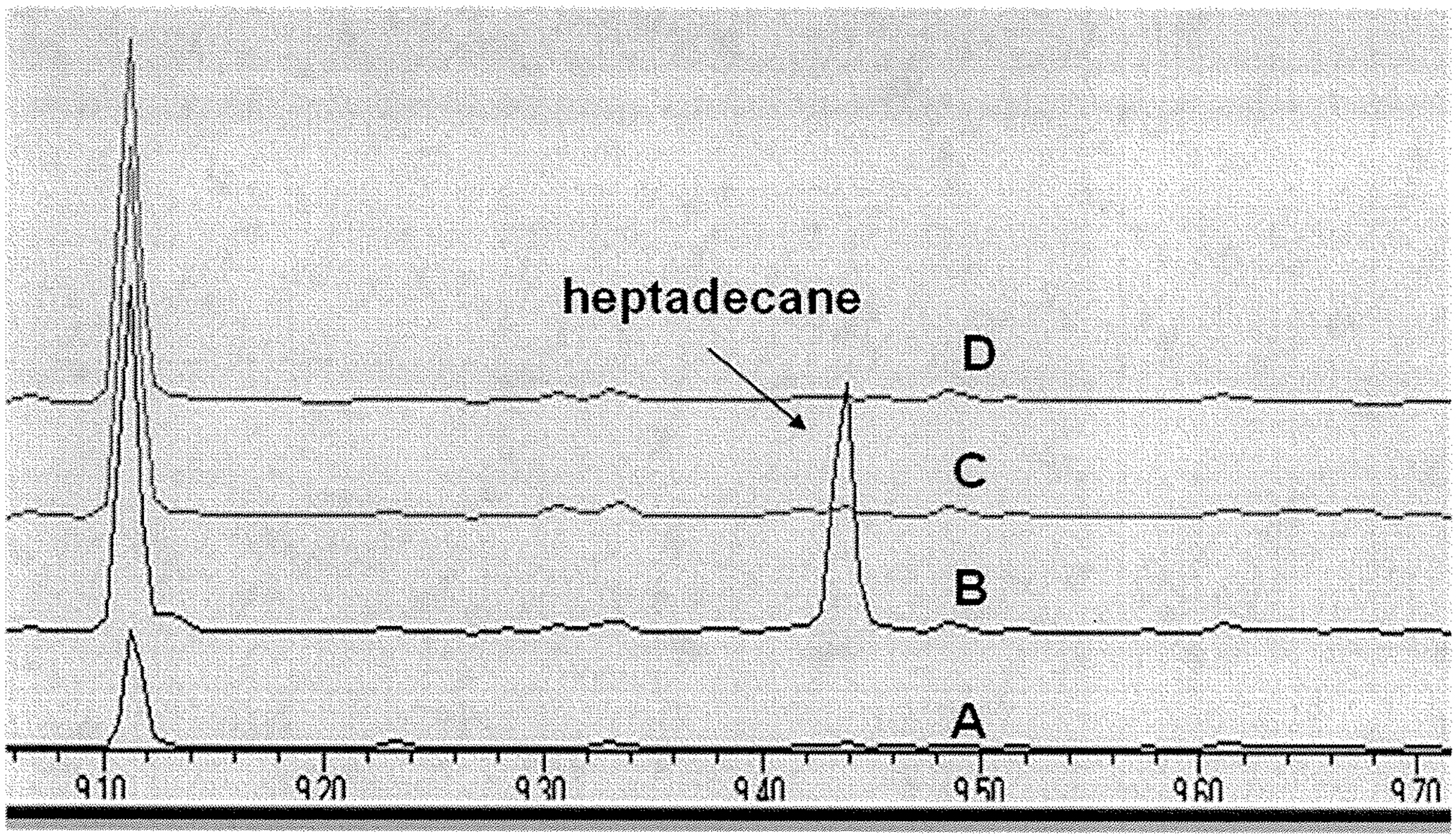

FIG. 31

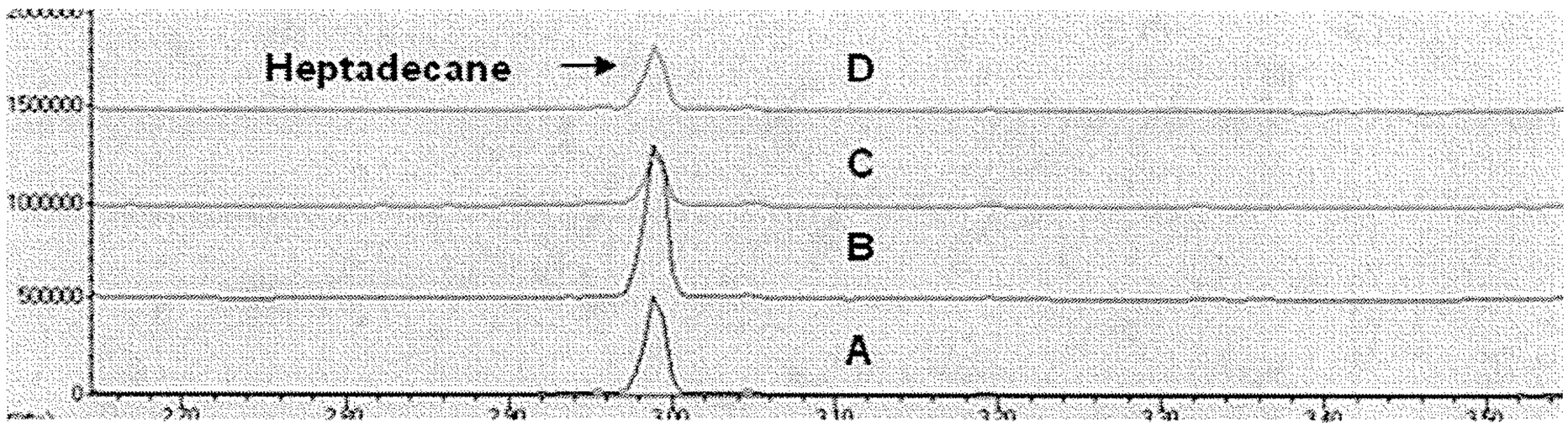
Heptadecane
D
C
B
A
1500000
1000000
500000
0

FIG. 32

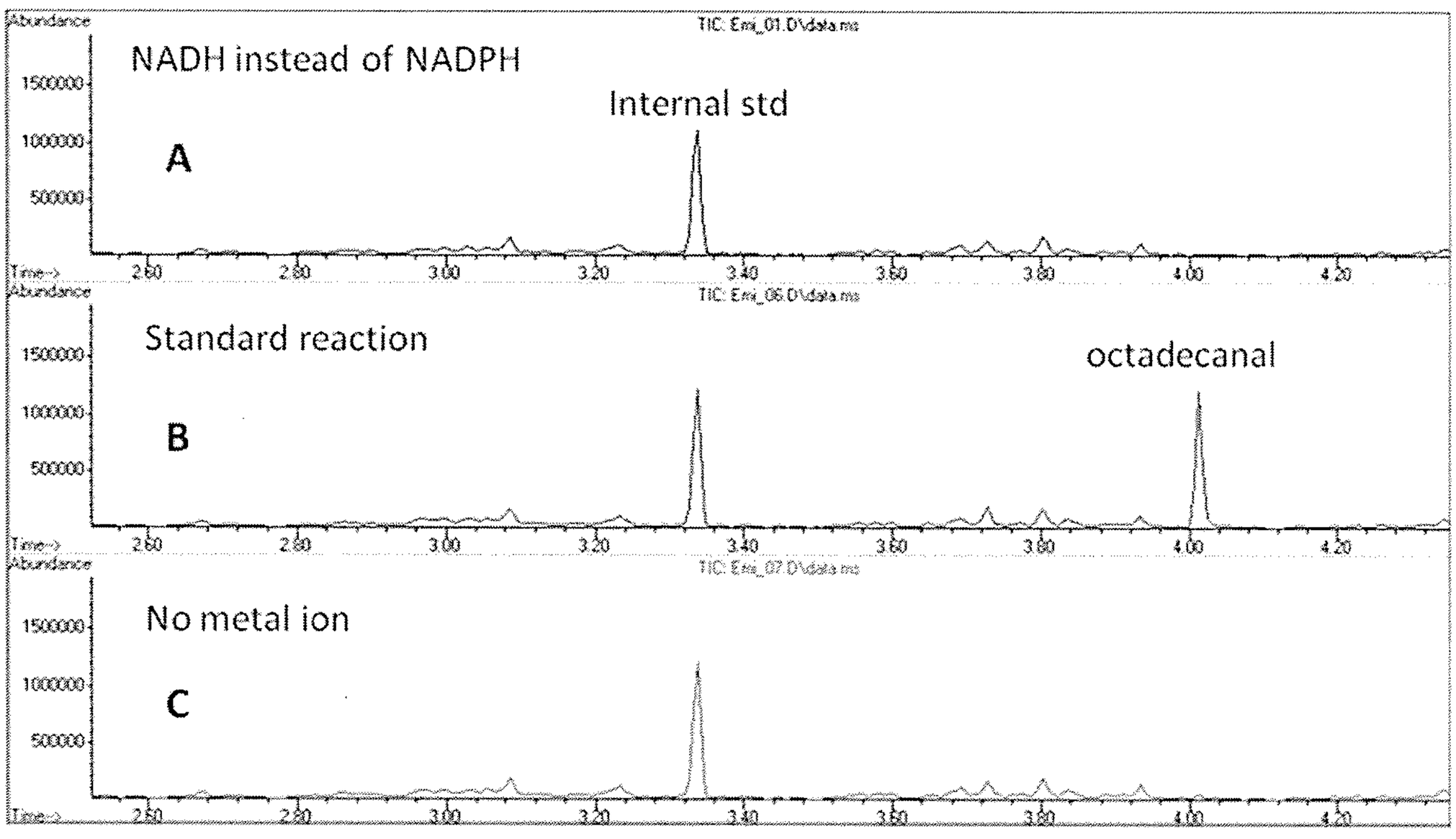
Abundance
TIC: Emj_01.D\data.ms
NADH instead of NADPH
Internal std
A
1500000
1000000
500000
Time->
2.60
2.80
3.00
3.20
3.40
3.60
3.80
4.00
4.20
Abundance
TIC: Emj_06.D\data.ms
Standard reaction
octadecanal
B
1500000
1000000
500000
Time->
Abundance
TIC: Emj_07.D\data.ms
No metal ion
C
1500000
1000000
500000
Time->
2.60
2.80
3.00
3.20
3.40
3.60
3.80
4.00
4.20

FIG. 33

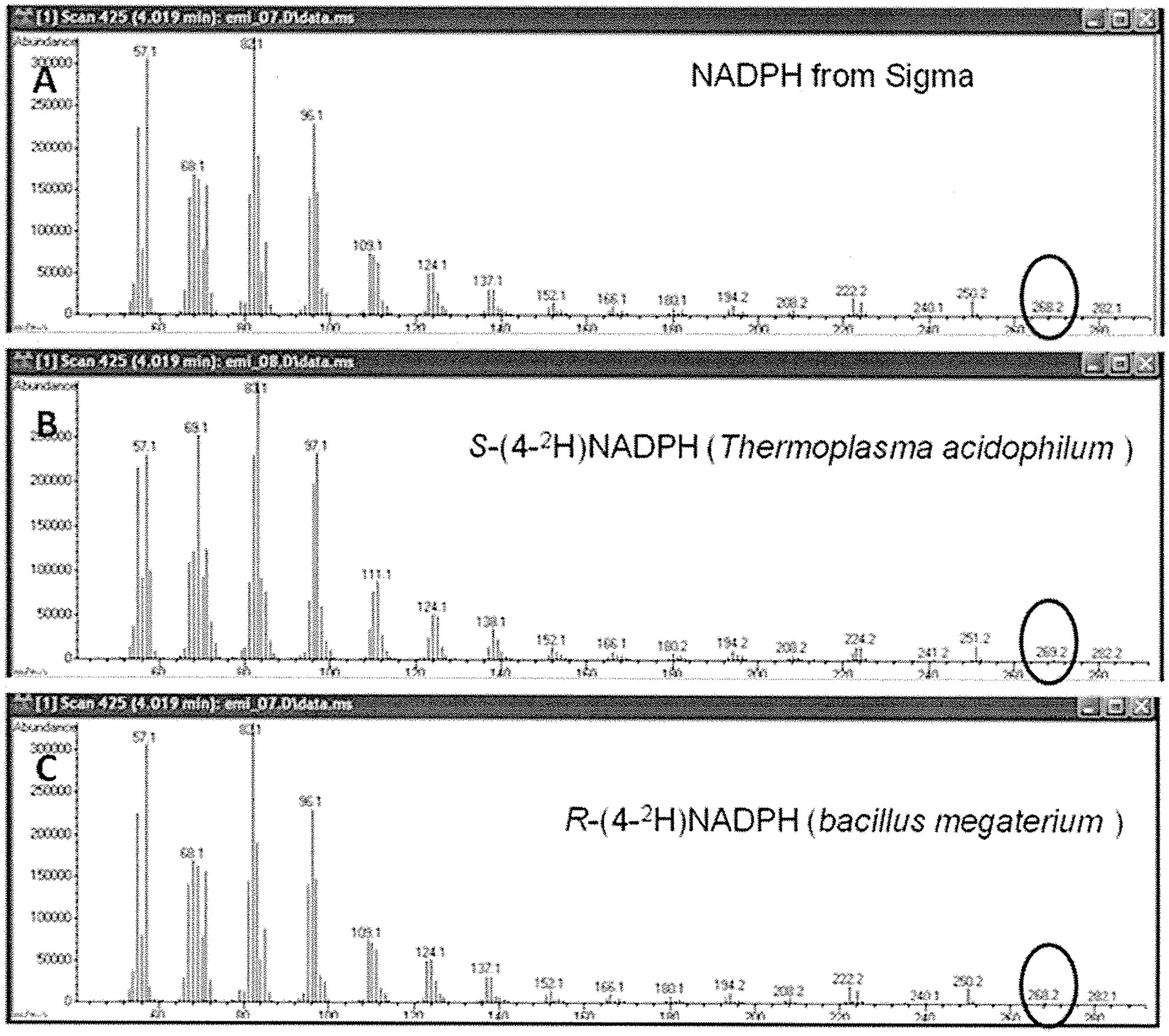
A
NADPH from Sigma
B
S-(4-2H)NADPH (Thermoplasma acidophilum)
C
R-(4-2H)NADPH (bacillus megaterium)

FIG. 34

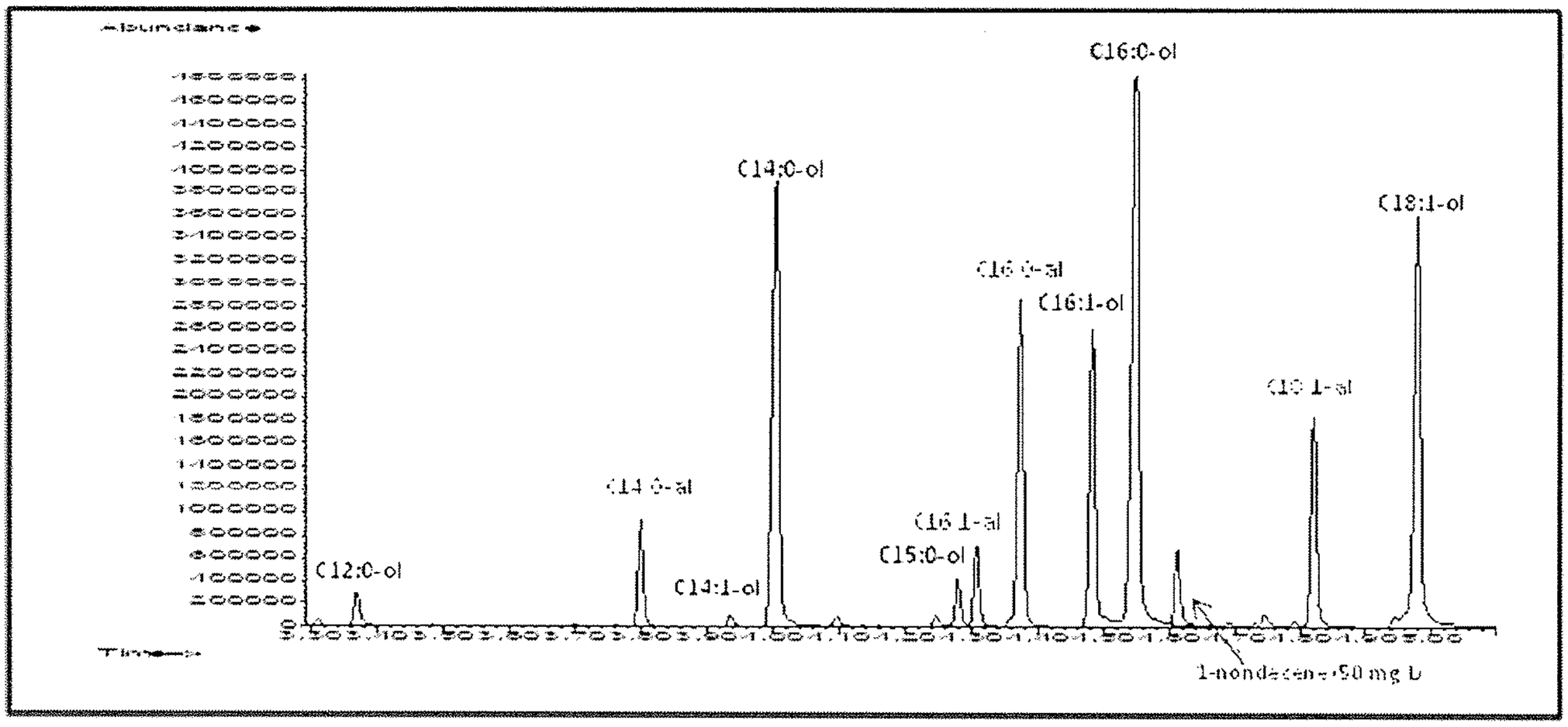
Abundance
C16:0-ol
C14:0-ol
C18:1-ol
C16:0-al
C16:1-ol
C18:1-al
C14:0-al
C16:1-al
C15:0-ol
C12:0-ol
C14:1-ol
Time
1-nondecene-50 mg/L

FIG. 35

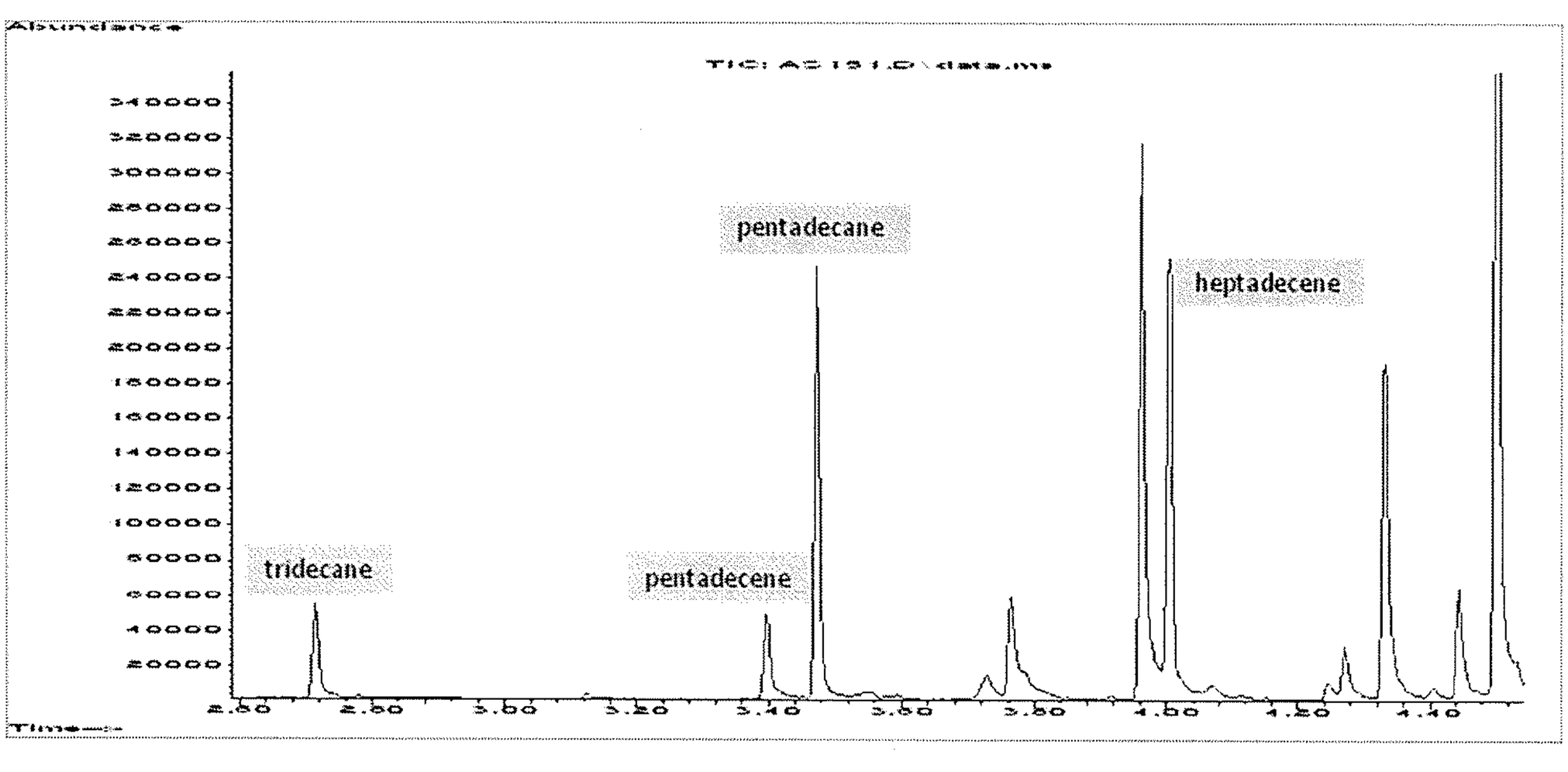
Abundance
TIC: A2151.D\data.ms
pentadecane
heptadecene
tridecane
pentadecene
Time

FIG. 36

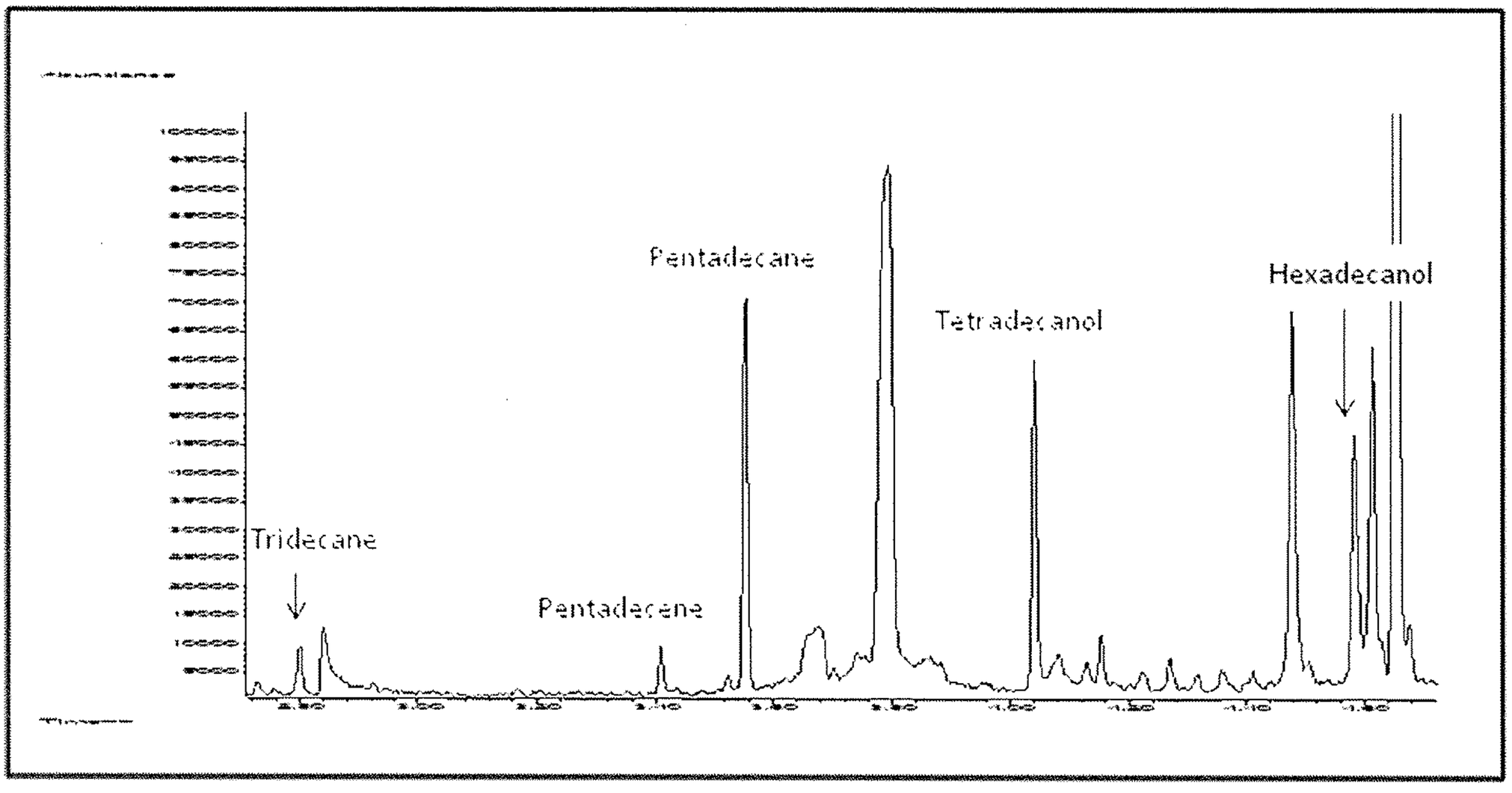
Tridecane
Pentadecane
Pentadecane
Tetradecanol
Hexadecanol

FIG. 37

FIG. 38

Accession Numbers as of April 10, 2009

Accession Number	% Identity	% Similarity	Alignment Length
gi|135970898|gb|EBL05614.1|	59.5	74.3	237
gi|134964254|gb|EBE59803.1|	57.6	74.2	229
gi|142528845|gb|ECY73505.1|	60.4	77.9	222
gi|135713677|gb|EBJ38387.1|	61.1	78.7	221
gi|141225813|gb|ECQ49060.1|	59.7	77.8	221
gi|144115151|gb|EDI97334.1|	67.7	80.9	220
gi|142133005|gb|ECV83152.1|	67.3	80.9	220
gi|137965371|gb|EBX01252.1|	67.3	80.9	220
gi|134786157|gb|EBD42319.1|	67.3	80.9	220
gi|136216894|gb|EBM66672.1|	62.3	77.7	220
gi|143271262|gb|EDE04654.1|	63.0	78.5	219
gi|135973786|gb|EBL07573.1|	63.0	78.1	219
gi|140222739|gb|ECK35865.1|	63.0	77.6	219
gi|139710482|gb|ECG93903.1|	62.6	78.1	219
gi|140109767|gb|ECJ60294.1|	62.6	78.5	219
gi|137939755|gb|EBW86789.1|	62.6	78.1	219
gi|140086977|gb|ECJ44922.1|	62.6	78.1	219
gi|143729007|gb|EDG48416.1|	62.6	78.1	219
gi|143217179|gb|EDD66368.1|	62.6	78.1	219
gi|138728422|gb|ECB60007.1|	62.6	78.1	219
gi|143540790|gb|EDF53461.1|	62.6	78.1	219
gi|143580323|gb|EDF73830.1|	62.6	78.1	219
gi|137317024|gb|EBT41871.1|	62.6	78.1	219
gi|143567212|gb|EDF67415.1|	62.6	78.1	219
gi|140726723|gb|ECN09681.1|	62.6	78.1	219
gi|136249402|gb|EBM88688.1|	62.6	78.1	219
gi|141156650|gb|ECQ02018.1|	62.6	78.1	219
gi|143550472|gb|EDF58473.1|	62.6	78.1	219
gi|139581046|gb|ECG04925.1|	62.6	78.1	219
gi|141904835|gb|ECU06854.1|	62.6	78.1	219
gi|143596624|gb|EDF78559.1|	62.1	78.1	219
gi|142808717|gb|EDA73967.1|	62.1	78.1	219
gi|137639134|gb|EBV19468.1|	62.1	78.1	219
gi|140970945|gb|ECO75236.1|	62.1	77.6	219

gi|137724335|gb|EBV66164.1|	62.1	78.1	219
gi|143095952|gb|EDC78454.1|	66.1	80.3	218
gi|135919851|gb|EBK71172.1|	66.1	80.3	218
gi|143083445|gb|EDC69308.1|	66.1	80.3	218
gi|137949431|gb|EBW92260.1|	63.1	77.6	214
gi|136336883|gb|EBN48108.1|	62.2	78.0	214
gi|136008042|gb|EBL28916.1|	62.2	78.0	214
gi|134796061|gb|EBD49256.1|	60.8	76.6	214
gi|143142117|gb|EDD12240.1|	60.8	76.6	214
gi|141842906|gb|ECT63492.1|	60.3	77.6	214
gi|137436109|gb|EBU09046.1|	60.3	77.1	214
gi|142796298|gb|EDA64685.1|	71.0	83.3	210
gi|138143480|gb|EBX98705.1|	70.5	83.3	210
gi|136004890|gb|EBL27104.1|	68.6	82.4	210
gi|142206956|gb|ECW39307.1|	67.1	82.4	210
gi|135813588|gb|EBK00762.1|	62.4	78.6	210
gi|137008728|gb|EBR69250.1|	62.7	79.0	209
gi|141443295|gb|ECR86509.1|	63.0	78.9	208
gi|135999440|gb|EBL23890.1|	63.1	79.1	206
gi|136001501|gb|EBL25083.1|	62.6	77.7	206
gi|137008499|gb|EBR69124.1|	62.3	77.5	204
gi|135813080|gb|EBK00444.1|	60.8	77.5	204
gi|139947991|gb|ECI56786.1|	63.6	79.8	198
gi|136255251|gb|EBM92608.1|	61.1	77.3	198
gi|141717528|gb|ECS91623.1|	58.6	76.8	198
gi|141153056|gb|ECP99448.1|	59.3	75.3	194
gi|140654921|gb|ECM59517.1|	67.9	81.9	193
gi|140993407|gb|ECO91072.1|	63.9	79.6	191
gi|143171676|gb|EDD33295.1|	63.9	79.6	191
gi|143659341|gb|EDG12240.1|	59.5	75.8	190
gi|138539256|gb|ECA29247.1|	68.1	81.9	188
gi|141964470|gb|ECU48335.1|	64.5	80.3	183
gi|139227663|gb|ECE28885.1|	63.9	79.2	183
gi|135926500|gb|EBK75672.1|	58.2	76.4	182
gi|140708786|gb|ECM97174.1|	68.4	81.4	177
gi|139523141|gb|ECF65392.1|	62.6	79.3	174
gi|137874181|gb|EBW49523.1|	61.5	76.4	174
gi|143221750|gb|EDD69688.1|	60.9	75.9	174
gi|140086962|gb|ECJ44914.1|	63.4	80.2	172
gi|142781070|gb|EDA53384.1|	61.8	77.1	170
gi|139775004|gb|ECH37282.1|	61.0	76.3	169

gi|136260534|gb|EBM96169.1|	61.0	75.7	169
gi|137441185|gb|EBU11854.1|	58.1	75.5	167
gi|136330550|gb|EBN43814.1|	65.1	80.7	166
gi|139650149|gb|ECG51660.1|	67.3	80.6	165
gi|143638003|gb|EDF99869.1|	63.6	80.0	165
gi|137949739|gb|EBW92432.1|	61.7	75.9	162
gi|143382653|gb|EDE68551.1|	63.9	81.0	158
gi|138989189|gb|ECC70595.1|	63.9	80.4	158
gi|138408887|gb|EBZ46853.1|	64.1	78.2	156
gi|137230040|gb|EBS93199.1|	61.3	76.1	155
gi|141605381|gb|ECS53894.1|	65.6	81.8	154
gi|137858747|gb|EBW40699.1|	64.9	81.8	154
gi|140209383|gb|ECK27191.1|	66.0	79.1	153
gi|142753984|gb|EDA33411.1|	60.7	75.3	150
gi|137242084|gb|EBS99775.1|	64.9	82.4	148
gi|136229422|gb|EBM75188.1|	64.6	82.3	147
gi|140311369|gb|ECK89744.1|	73.3	85.6	146
gi|140866197|gb|ECO03647.1|	65.1	82.2	146
gi|139229558|gb|ECE29833.1|	61.4	76.6	145
gi|141659030|gb|ECS68172.1|	61.4	76.6	145
gi|139580852|gb|ECG04786.1|	65.3	81.9	144
gi|138338712|gb|EBZ05758.1|	65.3	81.9	144
gi|136204827|gb|EBM58548.1|	61.3	76.8	142
gi|139095530|gb|ECD38154.1|	64.0	81.3	139
gi|136351648|gb|EBN58190.1|	60.1	76.1	138
gi|138155154|gb|EBY06350.1|	67.9	80.3	137
gi|137644530|gb|EBV22059.1|	63.1	80.8	130
gi|143775710|gb|EDG72409.1|	61.5	79.2	130
gi|143500330|gb|EDF32920.1|	63.1	77.9	122
gi|139709584|gb|ECG93249.1|	71.1	85.1	121
gi|142537519|gb|ECY79816.1|	62.8	81.0	121
gi|137944410|gb|EBW89433.1|	64.2	78.3	120
gi|137387955|gb|EBT81682.1|	63.9	78.2	119
gi|139955976|gb|ECI62054.1|	71.2	84.8	118
gi|137251843|gb|EBT05348.1|	62.1	77.6	116
gi|138442523|gb|EBZ70326.1|	62.1	76.7	116
gi|141590592|gb|ECS49420.1|	60.7	78.6	112
gi|143187997|gb|EDD45026.1|	60.7	77.7	112
gi|143655969|gb|EDG10472.1|	61.5	78.0	109
gi|139459255|gb|ECF24788.1|	62.9	79.1	105
gi|141976584|gb|ECU56751.1|	62.9	79.1	105

gi|139233104|gb|ECE31190.1|	69.6	83.3	102
gi|139233107|gb|ECE31193.1|	70.3	82.2	101
gi|138582711|gb|ECA59616.1|	61.4	77.2	101
gi|138442855|gb|EBZ70514.1|	61.4	75.3	101
gi|137662676|gb|EBV31757.1|	60.4	76.2	101
gi|139846062|gb|ECH87248.1|	62.6	74.8	99
gi|136935327|gb|EBR27657.1|	60.6	74.5	94
gi|137466252|gb|EBU25703.1|	67.0	80.2	91
gi|137820604|gb|EBW18665.1|	63.7	80.2	91
gi|138539295|gb|ECA29276.1|	62.6	79.1	91
gi|136239262|gb|EBM81844.1|	64.7	81.2	85
gi|136294902|gb|EBN19768.1|	64.7	81.2	85
gi|137413136|gb|EBT96003.1|	61.2	76.5	85
gi|137641041|gb|EBV20354.1|	60.0	72.9	85
gi|142508710|gb|ECY58869.1|	64.3	82.1	84
gi|140096399|gb|ECJ51008.1|	62.2	81.7	82
gi|137938664|gb|EBW86178.1|	64.0	81.3	75
gi|137275448|gb|EBT18729.1|	58.1	70.3	74
gi|141955842|gb|ECU42610.1|	63.0	80.8	73
gi|139221707|gb|ECE24659.1|	66.2	83.1	71
gi|142508709|gb|ECY58868.1|	52.9	68.6	70
gi|137523719|gb|EBU55323.1|	65.2	82.6	69
gi|140781524|gb|ECN46583.1|	65.6	82.8	64
gi|137627577|gb|EBV13553.1|	60.0	73.3	60
gi|141951833|gb|ECU39722.1|	59.7	73.7	57
gi|137232510|gb|EBS94613.1|	59.7	73.7	57

Cut-off used: >50% Identity to and >25% length of synpcc7942_1593

FIG. 39

Accession Numbers as of April 10, 2009

Accession Number	% Identity	% Similarity	Alignment Length
gi|143288250|gb|EDE13503.1|	71.3	80.5	87
gi|142342310|gb|ECX39602.1|	71.3	80.5	87
gi|137949588|gb|EBW92346.1|	70.7	85.9	92
gi|139984340|gb|ECI81897.1|	70.6	78.8	85
gi|140249046|gb|ECK54318.1|	69.7	82.0	267
gi|142111437|gb|ECV67406.1|	69.2	81.3	182
gi|142994709|gb|EDC04737.1|	68.8	81.2	138
gi|143066602|gb|EDC56955.1|	67.7	81.5	248
gi|138840827|gb|ECC11022.1|	67.1	78.8	146
gi|137829071|gb|EBW23606.1|	66.7	77.1	96
gi|142133008|gb|ECV83155.1|	66.5	79.5	337
gi|143095956|gb|EDC78458.1|	66.4	79.1	339
gi|144115152|gb|EDI97335.1|	66.4	79.4	339
gi|140732156|gb|ECN13587.1|	66.1	79.5	254
gi|136241230|gb|EBM83170.1|	66.0	78.6	103
gi|140001769|gb|ECI93451.1|	65.9	76.9	91
gi|139305662|gb|ECE48752.1|	65.9	80.0	205
gi|137634503|gb|EBV17219.1|	65.8	80.7	114
gi|138584841|gb|ECA61142.1|	65.5	81.9	116

gi|135919849|gb|EBK71170.1|	65.4	76.6	107
gi|141153057|gb|ECP99449.1|	65.4	79.6	280
gi|141976585|gb|ECU56752.1|	65.3	77.6	98
gi|142206955|gb|ECW39306.1|	65.3	78.5	340
gi|141804802|gb|ECT36785.1|	65.3	76.8	95
gi|138931154|gb|ECC47219.1|	65.1	76.7	86
gi|138408888|gb|EBZ46854.1|	65.1	78.0	255
gi|134743188|gb|EBD14908.1|	64.8	78.8	307
gi|138168794|gb|EBY16028.1|	64.8	77.6	304
gi|135749749|gb|EBJ60721.1|	64.8	79.1	105
gi|138338711|gb|EBZ05757.1|	64.6	80.0	175
gi|142827948|gb|EDA88477.1|	64.5	77.7	121
gi|138361576|gb|EBZ15968.1|	64.4	78.7	267
gi|140517919|gb|ECM08416.1|	64.4	77.4	115
gi|135813081|gb|EBK00445.1|	64.3	79.7	143
gi|137627576|gb|EBV13552.1|	64.3	80.1	171
gi|141161845|gb|ECQ05757.1|	64.2	77.1	109
gi|140992134|gb|ECO90156.1|	64.2	76.8	95
gi|137796334|gb|EBW04596.1|	64.2	78.9	279
gi|137619413|gb|EBV08950.1|	64.1	77.6	223
gi|140517917|gb|ECM08414.1|	64.1	79.6	181
gi|137232509|gb|EBS94612.1|	64.1	79.0	181
gi|135811491|gb|EBJ99446.1|	64.0	77.7	314

gi|141167040|gb|ECQ09480.1|	64.0	78.8	250
gi|143441820|gb|EDE97777.1|	64.0	78.2	261
gi|140311368|gb|ECK89743.1|	64.0	79.3	261
gi|140970943|gb|ECO75234.1|	64.0	76.6	111
gi|136817739|gb|EBQ60666.1|	63.8	78.5	340
gi|141717529|gb|ECS91624.1|	63.7	78.8	146
gi|137632337|gb|EBV16047.1|	63.7	77.7	256
gi|137662677|gb|EBV31758.1|	63.6	79.7	143
gi|140091056|gb|ECJ47190.1|	63.6	78.2	280
gi|143217178|gb|EDD66367.1|	63.5	78.8	137
gi|139984339|gb|ECI81896.1|	63.5	76.3	156
gi|139382506|gb|ECE73591.1|	63.4	78.9	194
gi|140096397|gb|ECJ51006.1|	63.4	79.7	153
gi|134606350|gb|EBC34611.1|	63.4	77.9	131
gi|140705175|gb|ECM95033.1|	63.4	77.0	191
gi|139846064|gb|ECH87250.1|	63.1	76.0	179
gi|137953535|gb|EBW94572.1|	63.1	78.0	241
gi|143738737|gb|EDG53066.1|	63.1	76.6	111
gi|141951832|gb|ECU39721.1|	63.1	76.6	111
gi|143271261|gb|EDE04653.1|	63.1	76.6	111
gi|139846065|gb|ECH87251.1|	63.0	78.8	146
gi|137251844|gb|EBT05349.1|	63.0	78.8	146
gi|136249401|gb|EBM88687.1|	63.0	78.8	146

gi|134628580|gb|EBC48074.1|	63.0	77.0	100
gi|136312048|gb|EBN31461.1|	62.9	76.2	143
gi|143221751|gb|EDD69689.1|	62.9	78.9	194
gi|141955844|gb|ECU42612.1|	62.9	75.7	140
gi|143395654|gb|EDE73119.1|	62.8	77.5	218
gi|142781071|gb|EDA53385.1|	62.8	79.3	164
gi|136303394|gb|EBN25555.1|	62.7	77.7	319
gi|143557688|gb|EDF62238.1|	62.6	77.9	131
gi|136008043|gb|EBL28917.1|	62.6	77.6	294
gi|143596625|gb|EDF78560.1|	62.6	77.0	318
gi|137641042|gb|EBV20355.1|	62.6	79.1	163
gi|136231267|gb|EBM76426.1|	62.5	76.7	339
gi|143175604|gb|EDD36054.1|	62.5	76.7	339
gi|142508708|gb|ECY58867.1|	62.5	76.7	339
gi|135926501|gb|EBK75673.1|	62.5	76.6	320
gi|141955884|gb|ECU42641.1|	62.4	77.8	189
gi|142821119|gb|EDA83282.1|	62.4	78.7	202
gi|134609411|gb|EBC36492.1|	62.4	75.8	194
gi|142885864|gb|EDB27722.1|	62.3	75.4	207
gi|136204828|gb|EBM58549.1|	62.3	77.0	318
gi|143580324|gb|EDF73831.1|	62.2	76.7	339
gi|143766375|gb|EDG67769.1|	62.2	77.0	339
gi|143500332|gb|EDF32922.1|	62.2	76.4	339

gi|139233105|gb|ECE31191.1|	62.1	76.5	132
gi|143738779|gb|EDG53089.1|	62.1	76.8	314
gi|134964255|gb|EBE59804.1|	62.1	77.1	153
gi|140863545|gb|ECO01751.1|	62.1	77.0	269
gi|137944409|gb|EBW89432.1|	62.0	78.5	158
gi|143411619|gb|EDE81261.1|	62.0	76.7	339
gi|142753988|gb|EDA33415.1|	62.0	77.0	339
gi|139580853|gb|ECG04787.1|	61.9	75.7	202
gi|141227933|gb|ECQ50606.1|	61.9	75.1	173
gi|143659340|gb|EDG12239.1|	61.8	78.3	157
gi|136935328|gb|EBR27658.1|	61.8	77.7	157
gi|137275449|gb|EBT18730.1|	61.8	77.5	204
gi|138585243|gb|ECA61437.1|	61.8	76.5	136
gi|139195947|gb|ECE06889.1|	61.7	76.2	269
gi|139424973|gb|ECF02640.1|	61.7	76.3	274
gi|141380828|gb|ECR42772.1|	61.7	75.8	227
gi|136351647|gb|EBN58189.1|	61.7	77.0	339
gi|136304410|gb|EBN26254.1|	61.6	75.7	185
gi|139948037|gb|ECI56814.1|	61.6	76.8	224
gi|135970899|gb|EBL05615.1|	61.6	76.8	211
gi|138627165|gb|ECA90647.1|	61.5	75.5	143
gi|137395720|gb|EBT86160.1|	61.5	74.8	143
gi|140086960|gb|ECJ44912.1|	61.5	77.1	249

gi|141024916|gb|ECP11582.1|	61.4	76.7	223
gi|139095531|gb|ECD38155.1|	61.4	76.7	210
gi|141659029|gb|ECS68171.1|	61.4	77.2	127
gi|139969430|gb|ECI71470.1|	61.4	76.7	215
gi|136986729|gb|EBR56775.1|	61.4	75.7	202
gi|143634197|gb|EDF97600.1|	61.3	76.7	313
gi|135973785|gb|EBL07572.1|	61.3	76.3	279
gi|143200944|gb|EDD54508.1|	61.3	74.6	173
gi|137787263|gb|EBV99371.1|	61.2	76.3	232
gi|139204136|gb|ECE12313.1|	61.2	76.7	219
gi|136001500|gb|EBL25082.1|	61.2	76.3	219
gi|141874476|gb|ECT85572.1|	61.2	76.4	237
gi|137905325|gb|EBW67375.1|	61.1	74.9	175
gi|140089341|gb|ECJ46519.1|	61.1	75.4	203
gi|140855194|gb|ECN95754.1|	61.1	75.9	203
gi|134965622|gb|EBE60718.1|	61.1	75.8	339
gi|141527125|gb|ECS15588.1|	61.1	76.0	208
gi|136218988|gb|EBM68086.1|	61.0	73.4	154
gi|142364499|gb|ECX54765.1|	60.9	76.1	330
gi|141603393|gb|ECS53340.1|	60.8	74.8	143
gi|136216893|gb|EBM66671.1|	60.8	76.3	245
gi|143743653|gb|EDG56305.1|	60.8	73.7	148
gi|140222741|gb|ECK35867.1|	60.7	73.0	163

gi|142389887|gb|ECX71634.1|	60.6	74.8	198
gi|139315697|gb|ECE51398.1|	60.6	76.4	241
gi|136255250|gb|EBM92607.1|	60.5	76.0	291
gi|137387954|gb|EBT81681.1|	60.5	76.6	124
gi|139229559|gb|ECE29834.1|	60.4	75.3	202
gi|140866196|gb|ECO03646.1|	60.1	74.6	303
gi|139229561|gb|ECE29836.1|	60.0	74.0	100
gi|140957440|gb|ECO66006.1|	59.9	75.9	274
gi|143567213|gb|EDF67416.1|	59.8	74.8	286
gi|139955973|gb|ECI62051.1|	59.8	72.2	97
gi|140726724|gb|ECN09682.1|	59.7	73.4	154
gi|139775003|gb|ECH37281.1|	59.6	73.7	99
gi|137949740|gb|EBW92433.1|	58.7	71.7	92
gi|139650150|gb|ECG51661.1|	57.7	73.2	97
gi|142528844|gb|ECY73504.1|	56.7	70.0	90

Cut-off used: >50% Identity to and >25% length of synpcc7942_1594

Fig. 40

Accession Numbers are from NCBI, GenBank, Release 159.0 as of April 15, 2007
EC Numbers are from KEGG, Release 42.0 as of April 2007 (plus daily updates up to March, 2008)

CATEGORY	GENE	NAME	ACCESSION	EC NUMBER	MODIFICATION	USE	ORGANISM
1. Fatty Acid Production Increase / Product Production Increase							
increase acyl-CoA							
reduce catabolism of derivatives and intermediates							
reduce feedback inhibition							
attenuate other pathways that consume fatty acids							
						increase	
	accA	Acetyl-CoA carboxylase, subunit A (carboxyltransferase alpha)	AAC73296, NP_414727	6.4.1.2	Over-express	Malonyl-CoA production	*Escherichia coli, Lactococci*
	accB	Acetyl-CoA carboxylase, subunit B (BCCP: biotin carboxyl carrier protein)	NP_417721	6.4.1.2	Over-express	increase Malonyl-CoA production	*Escherichia coli, Lactococci*
	accC	Acetyl-CoA carboxylase, subunit C (biotin carboxylase)	NP_417722	6.4.1.2, 6.3.4.14	Over-express	increase Malonyl-CoA production	*Escherichia coli, Lactococci*
	accD	Acetyl-CoA carboxylase, subunit D (carboxyltransferase beta)	NP_416819	6.4.1.2	Over-express	increase Malonyl-CoA production	*Escherichia coli, Lactococci*
	aceE	pyruvate dehydrogenase, subunit E1	NP_414656, AAC73226	1.2.4.1	Over-express	increase Acetyl-CoA production	*Escherichia coli*

	aceF	pyruvate dehydrogenase, subunit E2	NP_414657	2.3.1.12	Over-express	increase Acetyl-CoA production	*Escherichia coli*
	ackA	acetate kinase	AAC75356, NP_416799	2.7.2.1	Delete or reduce	increase Acetyl-CoA production	*Escherichia coli*
	ackB	acetate kinase AckB	BAB81430	2.7.2.1	Delete or reduce	increase Acetyl-CoA production	*Escherichia coli*
	acpP	acyl carrier protein	AAC74178	NONE	Over-express	increase Acetyl-CoA production	*Escherichia coli*
	fadD	acyl-CoA synthase	AP_002424	2.3.1.86, 6.2.1.3	Over-express	increase Fatty acid production	*Escherichia coli W3110*
	adhE	alcohol dehydrogenase	CAA47743	1.1.1.1, 1.2.1.10	Delete or reduce	increase Acetyl-CoA production	*Escherichia coli W3111*
	cer1	Aldehyde decarbonylase	BAA11024	4.1.99.5	Over-express	increase Acetyl-CoA production	*Arabidopsis thaliana*
	fabA	beta-hydroxydecanoyl thioester dehydrase	NP_415474	4.2.1.60	express	fatty acyl-CoA production	*E. coli K12*
	fabD	[acyl-carrier-protein] S-malonyltransferase	AAC74176	2.3.1.39	Over-express	increase Acetyl-CoA production	*E. coli K12*
	fabF	3-oxoacyl-[acyl-carrier-protein] synthase II	AAC74179	2.3.1.179	Delete or OverExpress	increase Acetyl-CoA production	*E. coli K12*

	fabG	3-oxoacyl-[acyl-carrier protein] reductase	AAC74177	1.1.1.100	Over-express	increase Acetyl-CoA production	*E. coli K12*
	fabH	3-oxoacyl-[acyl-carrier-protein] synthase III	AAC74175	2.3.1.180	Over-express	increase Acetyl-CoA production	*E. coli K12, lactococci*
	fabI	enoyl-[acyl-carrier-protein] reductase, NADH-dependent	NP_415804	1.3.1.9	express	fatty acyl-CoA production	*E. coli K12, lactococci*
	fabR	Transcriptional Repressor	NP_418398	NONE	Delete or reduce	modulate unsaturated fatty acid production	*E. coli K12*
	fabZ	(3R)-hydroxymyristol acyl carrier protein dehydratase	NP_414722	4.2.1.-			*E. coli K12*
	fadE	acyl-CoA dehydrogenase	AAC73325	1.3.99.3, 1.3.99.-	Delete or reduce	increase Acetyl-CoA production	
	acr1	Fatty Acyl-CoA reductase	YP_047869, AAC45217	1.2.1.42	Over-express	for fatty alcohol production	*Acinetobacter sp., i.e. calcoaceticus*
	GST, gshB	Glutathione synthase	P04425	6.3.2.3	Delete or reduce	increase Acyl-CoA	*E. coli K12*
	gpsA	biosynthetic sn-glycerol 3-phosphate dehydrogenase	AAC76632, NP_418065	EC: 1.1.1.94	Delete or reduce	increase Acetyl-CoA production	*E. coli K12*
	ldhA	lactate dehydrogenase	AAC74462, NP_415898	EC: 1.1.1.27, 1.1.1.28	Delete or reduce	increase Acetyl-CoA production	*E. coli K12*
	Lipase	Triglyceride Lipase	CAA89087, CAA98876	3.1.1.3	express	increase Fatty acid production	*Saccharomyces cerevisiae*
		Malonyl-CoA decarboxylase	AAA26500	4.1.1.9, 4.1.1.41	Over-express		*Saccharopolyspora erythraea*
	panD	aspartate 1-decarboxylase	BAB96708	4.1.1.11	Over-express	increase Acyl-CoA	*Escherichia coli W3110*

	panK a.k.a. coaA	pantothenate kinase	AAC76952	2.7.1.33	Over-express	increase Acetyl-CoA production	*E.coli*
	panK a.k.a. coaA, R106K	pantothenate kinase	AAC76952	2.7.1.33	Express, Over-express, R106K mutation	increase Acetyl-CoA production	*E. coli*
	pdh	Pyruvate dehydrogenase	BAB34380, AAC73226, NP_415392	1.2.4.1	Over-express	increase Acetyl-CoA production	
	pflB	formate acetyltransferase (pyruvate formate lyase)	AAC73989, P09373	EC: 2.3.1.54	Delete or reduce	increase Acetyl-CoA production	
	plsB	acyltransferase	AAC77011	2.3.1.15	D311E mutation	reduce limits on Acyl-CoA pool	*E. coli K12*
	poxB	pyruvate oxidase	AAC73958, NP_415392	1.2.2.2	Delete or reduce	increase Acetyl-CoA production	
	pta	phosphotransacetylase	AAC75357, NP_416800	2.3.1.8	Delete or reduce	increase Acetyl-CoA production	
	udhA	pyridine nucleotide transhydrogenase	CAA46822	1.6.1.1	Over-express	conversion NADH to NADPH or vice versa	
	fadB	fused 3-hydroxybutyryl-CoA epimerase/delta(3)-cis-delta(2)-trans-enoyl-CoA isomerase/enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase	AP_003956	4.2.1.17, 5.1.2.3, 5.3.3.8, 1.1.1.35	Delete or reduce	Block fatty acid degradation	*E. coli*

	fadJ	3-hydroxyacyl-CoA dehydrogenase; K01692 enoyl-CoA hydratase; K01782 3-hydroxybutyryl-CoA epimerase	AAC75401	1.1.1.35, 4.2.1.17, 5.1.2.3	Delete or reduce	Block fatty acid degradation	*E. coli*
	fadA	3-ketoacyl-CoA thiolase	BAE77458	2.3.1.16	Delete or reduce	Block fatty acid degradation	*E. coli*
	fadI	beta-ketoacyl-CoA thiolase	AAC75402	2.3.1.16	Delete or reduce	Block fatty acid degradation	*E. coli*
	YdiO	acyl-coA dehydrogenase	YP_852786	1.3.99.-	Delete or reduce	Block fatty acid degradation	*E. coli*
2. Structure Control							
2A. Chain Length Control							
2	tesA	thioesterase	P0ADA1	3.1.2.-, 3.1.1.5	Delete and/or express	C18 Chain Length	
	tesA without leader sequence	thioesterase	AAC73596, NP_415027	3.1.2.-, 3.1.1.5	express or overexpress	C18:1	*E.coli*
	tesA without leader sequence:L109P	thioesterase	P0ADA1	3.1.2.-, 3.1.1.5	Express and/or overexpress mutation L109P	<C18 Chain Length	*E. coli*
	fatB1 (umbellularia)	thioesterase	Q41635	3.1.2.14	express or overexpress	C12:0	*Umbellularia californica*
	fatB2 (umbellularia)DELETE	thioesterase	AAC49269	3.1.2.14	express or overexpress	C8:0 - C10:0	*Cuphea hookeriana*

	umbellu ria)						
	fatB3	thioesterase	AAC72881	3.1.2.14	express or overexpress	C14:0 - C16:0	*Cuphea hookeriana*
	fatB (cinnam onum)	thioesterase	Q39473	3.1.2.14	express or overexpress	C14:0	*Cinnamomum camphora*
	fatB[M 141T]*	thioesterase	CAA85388	3.1.2.14	express or overexpress	C16:1	*Arabidopsis thaliana*
	fatA1 (Heliant hus)	thioesterase	AAL79361	3.1.2.14	express or overexpress	*C18:1*	*Helianthus annuus*
	atfata (ARAB IDOPSI S FATA ACYL-ACP THIOE STERA SE)	thioesterase	NP_189147, NP_193041	3.1.2.14	express or overexpress	C18:1	*Arabidopsis thaliana*
	fatA	thioesterase	CAC39106	3.1.2.14	express or overexpress	C18:1	*Brassica juncea*
	fatA (cuphea)	thioesterase	AAC72883	3.1.2.14	express or overexpress	C18:1	*Cuphea hookeriana*
2B. Branching Control							
	attenuat e FabH						

	express FabH from S. glaucescens or S. coelicolor and knock out endogenouse FabH					increase branched chain fatty acid derivatives	
	express FabH from B. subtilis and knock out endogenouse FabH						
	bdk - E3 - dihydroplipoyl dehyrodgenase subunit			EC 1.2.4.4			
	bkd - E1-alpha/beta subunit	decarboxylase subunits of branched-chain a-ketoacid dehydrogenase complex		EC 1.2.4.4			

	bkd - E2 - dihydrolipoyl transacylase subunit			EC 1.2.4.4			
	bkdA1	branched-chain a-ketoacid dehydrogenase a-subunit (E1a)	NP_628006	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces coelicolor*
	bkdB1	branched-chain a-ketoacid dehydrogenase a-subunit (E1b)	NP_628005	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces coelicolor*
	bkdC1	dihydrolipoyl transacetylase (E2)	NP_628004	EC 2.3.1.168	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces coelicolor*
	bkdA2	branched-chain a-ketoacid dehydrogenase a-subunit (E1a)	NP_733618	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces coelicolor*
	bkdB2	branched-chain a-ketoacid dehydrogenase b-subunit (E1b)	NP_628019	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces coelicolor*
	bkdC2	dihydrolipoyl transacetylase (E2)	NP_628018	EC 2.3.1.168	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces coelicolor*

	bkdA	branched-chain a-ketoacid dehydrogenase a-subunit (E1a)	BAC72074	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces avermitilis*
	bkdB	branched-chain a-ketoacid dehydrogenase b-subunit (E1b)	BAC72075	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces avermitilis*
	bkdC	dihydrolipoyl transacetylase (E2)	BAC72076	EC 2.3.1.168	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces avermitilis*
	bkdF	branched-chain a-ketoacid dehydrogenase a-subunit (E1a)	BAC72088	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces avermitilis*
	bkdG	branched-chain a-ketoacid dehydrogenase b-subunit (E1b)	BAC72089	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces avermitilis*
	bkdH	dihydrolipoyl transacetylase (E2)	BAC72090	EC 2.3.1.168	express or Over-Express	make branched-chain acyl-CoA precursors	*Streptomyces avermitilis*
	bkdAA	branched-chain a-ketoacid dehydrogenase a-subunit (E1a)	NP_390285	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Bacillus subtilis*
	bkdAB	branched-chain a-ketoacid dehydrogenase b-subunit (E1b)	NP_390284	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-	*Bacillus subtilis*

						CoA precursors	
	bkdB	dihydrolipoyl transacetylase (E2)	NP_390283	EC 2.3.1.168	express or Over-Express	make branched-chain acyl-CoA precursors	*Bacillus subtilis*
	bkdA1	branched-chain a-ketoacid dehydrogenase a-subunit (E1a)	AAA65614	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Pseudomonas putida*
	bkdA2	branched-chain a-ketoacid dehydrogenase b-subunit (E1b)	AAA65615	EC 1.2.4.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Pseudomonas putida*
	bkdC	dihydrolipoyl transacetylase (E2)	AAA65617	EC 2.3.1.168	express or Over-Express	make branched-chain acyl-CoA precursors	*Pseudomonas putida*
	lpd	dihydrolipoamide dehydrogenase (E3)	NP_414658	1.8.1.4	express or Over-Express	make branched-chain acyl-CoA precursors	*Escherichia coli*
	IlvE	branched-chain amino acid aminotransferase	YP_026247	2.6.1.42	express or Over-Express	make branched a-ketoacids	*Escherichia coli*
	IlvE	branched-chain amino acid aminotransferase	AAF34406	2.6.1.42	express or Over-Express	make branched a-ketoacids	*Lactococcus lactis*
	IlvE	branched-chain amino acid aminotransferase	NP_745648	2.6.1.42	express or Over-Express	make branched a-ketoacids	*Pseudomonas putida*

	IlvE	branched-chain amino acid aminotransferase	NP_629657	2.6.1.42	express or Over-Express	make branched a-ketoacids	*Streptomyces coelicolor*
	ccr	crotonyl-CoA reductase	NP_630556	1.6.5.5,1.1.1.1	express or Over-Express	Converting crotonyl-CoA to butyryl-CoA	*Streptomyces coelicolor*
	ccr	crotonyl-CoA reductase	AAD53915	1.6.5.5,1.1.1.1	express or Over-Express	Converting crotonyl-CoA to butyryl-CoA	*Streptomyces cinnamonensis*
	IcmA, isobutyryl-CoA mutase	isobutyryl-CoA mutase, subunit A	NP_629554	5.4.99.2	express or Over-Express	converting butyryl-CoA to isobutyryl-CoA	*Streptomyces coelicolor*
	IcmA, isobutyryl-CoA mutase	isobutyryl-CoA mutase, subunit A	AAC08713	5.4.99.2	express or Over-Express	converting butyryl-CoA to isobutyryl-CoA	*Streptomyces cinnamonensis*
	IcmB, isobutyryl-CoA mutase	isobutyryl-CoA mutase, subunit B	NP_630904	5.4.99.2	express or Over-Express	converting butyryl-CoA to isobutyryl-CoA	*Streptomyces coelicolor*
	IcmB, isobutyryl-CoA mutase	isobutyryl-CoA mutase, subunit B	CAB59633	5.4.99.2	express or Over-Express	converting butyryl-CoA to isobutyryl-CoA	*Streptomyces cinnamonensis*

	FabH, ACPs and fabF genes with specificity for branched chain acyl-CoAs						
	IlvE	branched-chain amino acid aminotransferase	CAC12788	EC2.6.1.42	over express	branched chain amino acid amino transferase	Staphylococcus carnosus
	FabH1	beta-ketoacyl-ACP synthase III	NP_626634	2.3.1.180	express or Over-Express	initiation of branched-chain fatty acid biosynthesis	Streptomyces coelicolor
	ACP	acyl-carrier protein	NP_626635	NONE	express or Over-Express	initiation and elongation of branched-chain fatty acid biosynthesis	*Streptomyces coelicolor*
	FabF	beta-ketoacyl-ACP synthase II	NP_626636	2.3.1.179	express or Over-Express	elongation of branched-chain fatty acid biosynthesis	*Streptomyces coelicolor*

	FabH3	beta-ketoacyl-ACP synthase III	NP_823466	2.3.1.180	express or Over-Express	initiation of branched-chain fatty acid biosynthesis	*Streptomyces avermitilis*
	FabC3 (ACP)	acyl-carrier protein	NP_823467	NONE	express or Over-Express	initiation and elongation of branched-chain fatty acid biosynthesis	*Streptomyces avermitilis*
	FabF	beta-ketoacyl-ACP synthase II	NP_823468	2.3.1.179	express or Over-Express	elongation of branched-chain fatty acid biosynthesis	*Streptomyces avermitilis*
	FabH_A	beta-ketoacyl-ACP synthase III	NP_389015	2.3.1.180	express or Over-Express	initiation of branched-chain fatty acid biosynthesis	*Bacillus subtillis*
	FabH_B	beta-ketoacyl-ACP synthase III	NP_388898	2.3.1.180	express or Over-Express	initiation of branched-chain fatty acid biosynthesis	*Bacillus subtillis*

	ACP	acyl-carrier protein	NP_389474	NONE	express or Over-Express	initiation and elongation of branched-chain fatty acid biosynthesis	*Bacillus subtillis*
	FabF	beta-ketoacyl-ACP synthase II	NP_389016	2.3.1.179	express or Over-Express	elongation of branched-chain fatty acid biosynthesis	*Bacillus subtillis*
	SmalD RAFT_ 0818	beta-ketoacyl-ACP synthase III	ZP_01643059	2.3.1.180	express or Over-Express	initiation of branched-chain fatty acid biosynthesis	*Stenotrophomonas maltophilia*
	SmalD RAFT_ 0821	acyl-carrier protein	ZP_01643063	NONE	express or Over-Express	initiation and elongation of branched-chain fatty acid biosynthesis	*Stenotrophomonas maltophilia*
	SmalD RAFT_ 0822	beta-ketoacyl-ACP synthase II	ZP_01643064	2.3.1.179	express or Over-Express	elongation of branched-chain fatty acid biosynthesis	*Stenotrophomonas maltophilia*

	FabH	beta-ketoacyl-ACP synthase III	YP_123672	2.3.1.180	express or Over-Express	initiation of branched-chain fatty acid biosynthesis	*Legionella pneumophila*
	ACP	acyl-carrier protein	YP_123675	NONE	express or Over-Express	initiation and elongation of branched-chain fatty acid biosynthesis	*Legionella pneumophila*
	FabF	beta-ketoacyl-ACP synthase II	YP_123676	2.3.1.179	express or Over-Express	elongation of branched-chain fatty acid biosynthesis	*Legionella pneumophila*
	FabH	beta-ketoacyl-ACP synthase III	NP_415609	2.3.1.180	delete or reduce	initiation of branched-chain fatty acid biosynthesis	*Escherichia coli*
	FabF	beta-ketoacyl-ACP synthase II	NP_415613	2.3.1.179	delete or reduce	elongation of branched-chain fatty acid biosynthesis	*Escherichia coli*
To Produce Cyclic Fatty Acids							

	AnsJ	dehydratase (putative)	not available	not available	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces collinus*
	AnsK	CoA ligase (putative)	not available	not available	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces collinus*
	AnsL	dehydrogenase (putative)	not available	not available	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces collinus*
	ChcA	enoyl-CoA reductase	U72144	EC 1.3.1.34	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces collinus*
	AnsM	oxidorecutase (putative)	not available	not available	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces collinus*
	PlmJ	dehydratase (putative)	AAQ84158	not available	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces sp. HK803*
	PlmK	CoA ligase (putative)	AAQ84158	not available	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces sp. HK803*
	PlmL	dehydrogenase (putative)	AAQ84159	not available	express or Over-Express	cyclohexylcarbonyl-CoA	*Streptomyces sp. HK803*

						boiosynthesis	
	ChcA	enoyl-CoA reductase	AAQ84160	EC 1.3.1.34	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces sp. HK803*
	PlmM	oxidorecutase (putative)	AAQ84161	not available	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces sp. HK803*
	ChcB	enoyl-CoA isomerase	AF268489	not available	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces collinus*
	ChcB/CaiD	enoyl-CoA isomerase	NP_629292	4.2.1.-	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces coelicolor*
	ChcB/CaiD	enoyl-CoA isomerase	NP_824296	4.2.1.-	express or Over-Express	cyclohexylcarbonyl-CoA boiosynthesis	*Streptomyces avermitilis*
2C. Saturation Level Control							
	Sfa	Suppressor of FabA	AAN79592, AAC44390	NONE	Over-express	increase monounsaturated fatty acids	*E.coli*
	also *see* FabA in sec. 1				express	produce unsaturated fatty acids	

	GnsA	suppressors of the secG null mutation	ABD18647.1	NONE	Over-express	increase unsaturated fatty acid esters	*E.coli*
	GnsB	suppressors of the secG null mutation	AAC74076.1	NONE	Over-express	increase unsaturated fatty acid esters	*E.coli*
	also *see* section 2A - items with :0 are unsaturated (no double bonds) and with :1 are saturated (1 double bond)						
	fabB	3-oxoacyl-[acyl-carrier-protein] synthase I	BAA16180	EC:2.3.1.41	overexpress	modulate unsaturated fatty acid production	*Escherichia coli*
	fabK	trans-2-enoyl-ACP reductase II	AAF98273	1.3.1.9	express	modulate unsaturated fatty acid production	*Streptococcus pneumoniae*
	fabL	enoyl-(acyl carrier protein) reductase	AAU39821	1.3.1.9	express	modulate unsaturated fatty acid production	*Bacillus licheniformis DSM 13*

	fabM	trans-2, cis-3-decenoyl-ACP isomerase	DAA05501	4.2.1.17	Over-express	modulate unsaturated fatty acid production	*Streptococcus mutans*
3. Final Product Output							
3A. Wax Output							
	AT3G51970	long-chain-alcohol O-fatty-acyltransferase	NP_190765	2.3.1.26	express	wax production	*Arabidopsis thaliana*
		thioesterase (*see* chain length control section)			express	increase fatty acid production	
		fatty alcohol forming acyl-CoA reductase		1.1.1.*	express	convert acyl-coa to fatty alcohol	
	acr1	acyl-CoA reductase (ACR1)	YP_047869	1.2.1.42	express	convert acyl-coa to fatty alcohol	*Acinetobacter sp. ADP1*
	yqhD	alcohol dehydrogenase	AP_003562	1.1.-.-	express	increase	*E. coli W3110*
	ELO1	Fatty acid elongase	BAD98251	2.3.1.-	express	produce very long chain length fatty acids	*Pichia angusta*
	plsC	acyltransferase	AAA16514	2.3.1.51	express		*Saccharomyces cerevisiae*
	DAGAT/DGAT	diacylglycerol acyltransferase	AAF19262	2.3.1.20	express	wax production	*Arabidopsis thaliana*
	hWS	acyl-CoA wax alcohol acyltransferase	AAX48018	2.3.1.20	express	wax production	*Homo sapiens*

	aft1	bifunctional wax ester synthase/acyl-CoA:diacylglycerol acyltransferase	AAO17391	2.3.1.20, 2.3.1.75	express	wax production	*Acinetobacter sp. ADP1*
	mWS	wax ester synthase (simmondsia)	AAD38041	2.3.1.-, 2.3.1.75	express	wax production	*Simmondsia chinensis*
3B. Fatty Alcohol Output							
		various thioesterases (refer to Sec. 2A)			express	produce	
	acr1	acyl-CoA reductase	YP_047869	1.2.1.42	express	produce	*Acinetobacter sp. ADP1*
	yqhD	alcohol dehydrogenase	AP_003562	1.1.-.-	express	produce	*Escherichia coli W3110*
	BmFAR	FAR (fatty alcohol forming acyl-CoA reductase)	BAC79425	1.1.1.*	express	reduce fatty acyl-CoA to fatty alcohol	*Bombyx mori*
	Akr1a4	Mammalian microsomal aldehyde reductase	NP_067448	1.1.1.2	express	produce	*Mus musculus*
	GTNG_1865	Long-chain aldehyde dehydrogenase	YP_001125970	1.2.1.3	express	produce	*Geobacillus thermodenitrificans NG80-2*
	FadD	acyl-CoA synthase	NP_416319	EC 6.2.1.3	express	produce more	*E. Coli K12*
To make Butanol							
	atoB	acetyl-CoA acetyltransferase	YP_049388	2.3.1.9	express	produce	*Erwinia carotovora*
	hbd	Beta-hydroxybutyryl-CoA dehydrogenase	BAD51424	1.1.1.157	express	produce	*Butyrivibrio fibrisolvens*
	CPE0095	crotonase	BAB79801	4.2.1.55	express	produce	*Clostridium perfringens*
	bcd	butyryl-CoA dehydryogenase	AAM14583	1.3.99.2	express	produce	*Clostridium beijerinckii*

	ALDH	coenzyme A-acylating aldehyde dehydrogenase	AAT66436	1.2.1.3	express	produce	*Clostridium beijerinckii*
	AdhE	aldehyde-alcohol dehydrogenase	AAN80172	1.1.1.1 1.2.1.10	express	produce	*Escherichia coli CFT073*
3C. Fatty Acid Ester Output							
	thioesterase	*see* chain length control section			express	produce	
	acr1	acyl-CoA reductase	YP_047869	1.2.1.42	express	produce	*Acinetobacter sp. ADP1*
	yqhD	alcohol dehydrogenase	AP_003562	1.1.-.-	express	produce	*E. Coli K12*
	AAT	alcohol O-acetyltransferase	AAG13130	2.3.1.84	express	produce	*Fragaria x ananassa*
4. Export							
	Wax ester exporter (FATP family, Fatty Acid (long chain) Transport Protein)		NP_524723	NONE	express	export wax	*Drosophila melanogaster*
	ABC transport protein	putative alkane transporter	AAN73268	NONE	express	export products	*Rhodococcus erythropolis*
	CER5	wax transporter	At1g51500, AY734542, At3g21090, At1g51460	NONE	express	export products	*Arabidopsis thaliana*

	AtMRP 5	Arabidopsis thaliana multidrug resistance-associated	NP_171908	NONE	express	export products	*Arabidopsis thaliana*
	AmiS2	ABC transporter AmiS2	JC5491	NONE	express	export products	*Rhodococcus sp.*
	AtPGP1	ARABIDOPSIS THALIANA P GLYCOPROTEIN1	NP_181228	NONE	express	export products	*Arabidopsis thaliana*
	AcrA	putative multidrug-efflux transport protein acrA	CAF23274	NONE	express	export products	*Candidatus Protochlamydia amoebophila UWE25*
	AcrB	probable multidrug-efflux transport protein, acrB	CAF23275	NONE	express	export products	*Candidatus Protochlamydia amoebophila UWE25*
	TolC	Outer membrane protein [Cell envelope biogenesis,	ABD59001	NONE	express	export products	*Francisella tularensis subsp. novicida*
	AcrE	transmembrane protein affects septum formation and cell membrane permeability	YP_312213	NONE	express	export products	*Shigella sonnei Ss046*
	AcrF	Acriflavine resistance protein F	P24181	NONE	express	export products	*Escherichia coli*
	tll1618	multidrug efflux transporter	NP_682408.1	NONE	express	export products	*Thermosynechococcus elongatus BP-1]*
	tll1619	multidrug efflux transporter	NP_682409.1	NONE	express	export products	*Thermosynechococcus elongatus BP-1]*
	tll0139	multidrug efflux transporter	NP_680930.1	NONE	express	export products	*Thermosynechococcus elongatus BP-1]*
5. Fermentation							

	replication checkpoint genes					increase output efficiency	
	umuD	DNA polymerase V, subunit	YP_310132	3.4.21.-	Over-express	increase output efficiency	*Shigella sonnei Ss046*
	umuC	DNA polymerase V, subunit	ABC42261	2.7.7.7	Over-express	increase output efficiency	*Escherichia coli*
	NADH: NADPH transhydrogenase (alpha and beta subunits) (pntA, pntB)		P07001, P0AB70	1.6.1.2	express	increase output efficiency	*Shigella flexneri*

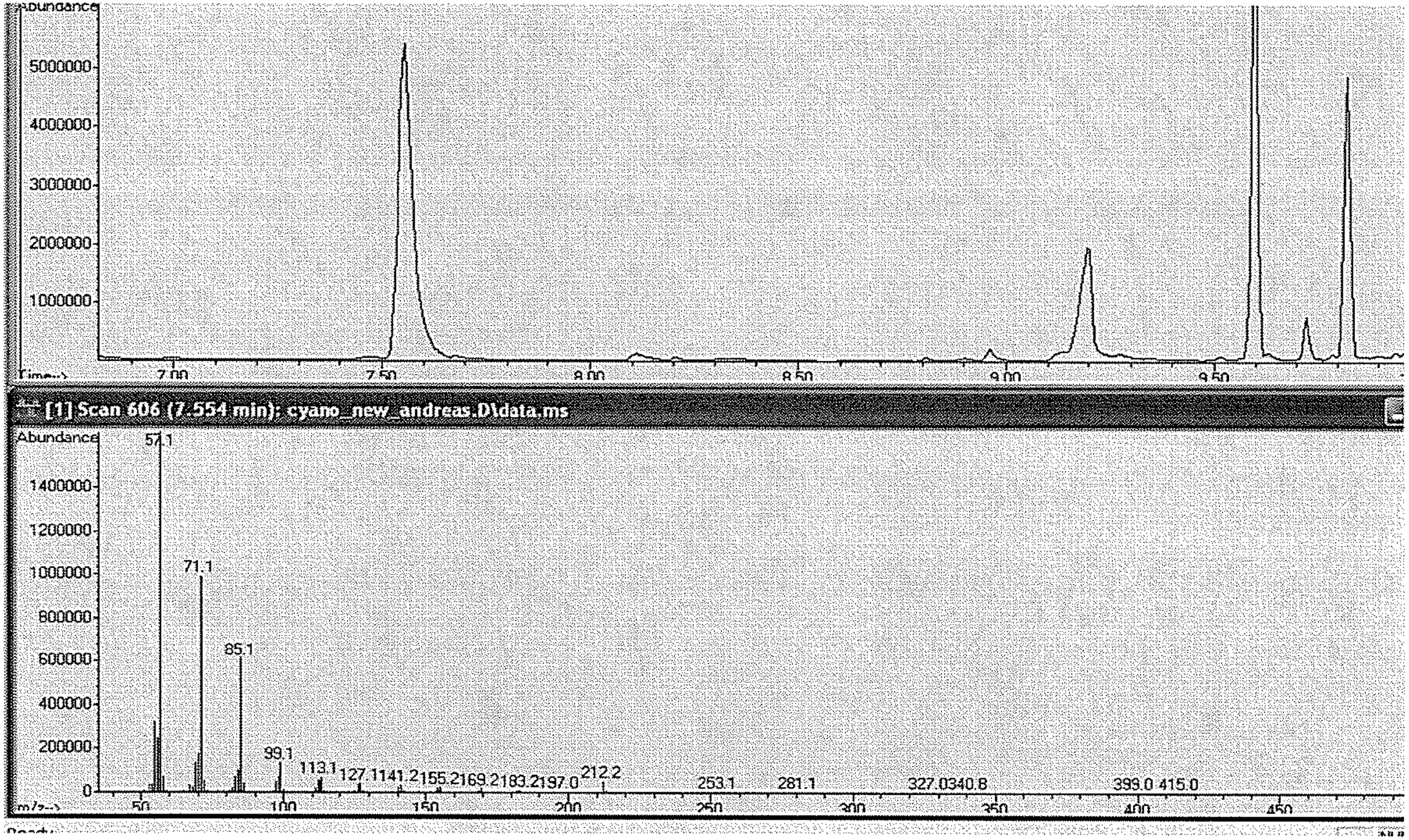
Abundance
5000000
4000000
3000000
2000000
1000000
7.00
7.50
8.00
8.50
9.00
9.50
[1] Scan 606 (7.554 min): cyano_new_andreas.D\data.ms
Abundance
57.1
1400000
1200000
1000000
800000
600000
400000
200000
0
71.1
85.1
99.1
113.1
127.1
141.2
155.2
169.2
183.2
197.0
212.2
253.1
281.1
327.0
340.8
398.0
415.0
50
100
150
200
250
300
350
400
450

FIG. 1