Technical

Biochemical Modification of Fats by Microorganisms: A Preliminary Survey

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Over 100 different strains of bacteria, actinomycetes, fungi and yeasts were incubated at 28 C for five days in the presence of soybean oil. Some soybean oil was consumed by many microorganisms, and some was also hydrolyzed to free fatty acids. *Aspergillus oryzae,* two different strains of *Amylomyces rouxii* and *Rhizopus oligosporus* hydrolyzed the oil completely (95%). The fatty acids from *Aspergillus flavus* fermentation contained less linolenic acid than the original soybean oil. Lipase was found intra- and extracellularly when microorganisms were grown in the presence of soybean oil.

Many industrially important fatty chemicals are manufactured from fats and oils by chemical modifications that often require high temperatures, pressures and metallic catalysts. Some of these products could be produced by enzymes or whole microbial cells with greater rapidity and better specificity under milder conditions.

Some reactions of industrial significance that have been accomplished by enzymes or whole microbial cells include oxidation (1), hydrolysis (2,3), esterification (4), interesterification (5,6), transesterification (7) and epoxidation (8). A recent report (9) indicates that lipase is being used in Japan for the commercial production of fatty acids from triglycerides. To our knowledge, this is the only example of industrial application of an enzyme for the production of fatty chemicals. If enzymatic reactions are to compete with other chemical processes, considerable basic research into the mechanisms and kinetics of these reactions needs to be carried out.

At the Northern Regional Research Center, we have instituted a survey of microorganisms capable of growing in the presence of soybean oil. It is hoped that this program will ultimately lead to microorganisms that, in the process of using soybean oil, alter a portion of it to produce useful derivatives such as hydroxy acids. The findings of this preliminary survey are included in this report.

MATERIALS AND METHODS

Cultivation of microorganisms. All microorganisms used in this study were obtained from the ARS Culture Collection, Northern Regional Research Center. The synthetic liquid medium used for growing microorganisms contained 0.2 g aspargine, 0.1 g K_2HPO_4, 0.05 g $MgSO_4$, 0.2 g glucose, 0.5 mg thiamine hydrochloride, 0.145 mg $Fe(NO_3)_3 \cdot 9H_2O$, 0.088 mg $ZnSO_4 \cdot 7H_2O$ and 0.031 mg $MnSO_4 \cdot 4H_2O$/100 ml water. About 120 ml of medium was sterilized in a 300-ml flask by autoclaving at 121 C. Refined, bleached soybean oil (5 ml) (16:0=10.4%; 18:0=3.8%; 18:1=22.0%; 18:2=55.6%; 18:3=8.2%) obtained from Central Soya Co. Inc. was added. A loopful of microorganisms grown on yeast-malt agar slants was transferred to the sterile medium, and then the flask and its contents were shaken (200 rpm) on a rotary shaker at 28 C for five days. With selected microorganisms, 750 ml medium containing 32 ml soybean oil was used for more detailed studies.

Extraction of lipids. During preliminary screening, the cells and the cultured medium were extracted three times with hexane. The combined hexane extract was dried over anhydrous magnesium sulfate, and then the solvent was removed from the combined extracts on a rotary evaporator. For larger fermentations, the cells were separated from the medium by filtration. Subsequently, both the cells and supernatant were extracted separately with hexane. The combined hexane extract was treated as above. The intracellular lipids were extracted by soaking the cells in chloroform:methanol (2:1, v/v). After removing the solvent on a rotary evaporator, the residue was extracted with hexane to separate the lipids from non-lipid material. Chloroform:methanol was also used to extract the intracellular lipids of microorganisms grown in oil-free medium, because hexane did not extract them. This method completely extracted the lipids because the extracted cells after saponification and acidification yielded only traces of fatty acids.

Analytical methods. Lipid extracts were monitored by thin layer chromatography (TLC) (10). Triglycerides were converted to methyl esters according to the method of Christopherson and Glass (11). This method was modified by replacing petroleum ether with diethyl ether. Fatty acids were esterified with diazomethane (12). Fatty acid composition was determined by gas liquid chromatography (GC) with 1/8-in. stainless steel columns packed with 15% EGSS-X on 100-120 mesh Gas-Chrom P (Applied Science Laboratories, Inc., State College, Pennsylvania). Capillary GC was performed on a Hewlett-Packard 5890 instrument equipped with flame ionization detectors and a 30-m 0.32 mm i.d. fused silica column coated with SP-2330 (Supelco Inc., Bellefonte, Pennsylvania). The peak areas were integrated with the aid of a ModComp Computer. Separation of fatty acids and triglycerides from fermentation broth extracts was achieved by preparative TLC. Free fatty acids (FFA) calculated as oleic acid in lipid mixtures were estimated by the official American Oil Chemists' Society method Ca 5a-40 (13).

RESULTS

At the start of the survey, microorganisms were selected for evaluation because of their known ability to grow in hydrocarbon or fat (14). Later, several microorganisms were chosen at random to include many genera. Following five days of incubation at 28 C, the cell-containing medium was extracted three times with hexane. The combined hexane extract was dried over anhydrous magnesium sulfate, and then the solvent was evaporated. The lipid residue was weighed. TLC analysis indicated that in addition to triglycerides, some samples consisted of

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[1]Deceased.

TABLE 1

Screening of Microorganisms Grown in Soybean Oil

NRRL No.	Genus and species	% Added oil extracted	% FFA in extract
Bacteria			
B-3712[a]	*Bacillus megaterium*	62	0.1
B-172	*Bacillus macerans*	88	0.1
NRS-604	*Bacillus brevis*	92	0.2
NRS-609	*Bacillus stearothermophilus*	88	0.1
NRS-744	*Bacillus subtilis*	100	1.4
NRS-1105	*Bacillus polymyxa*	94	0.2
B-3711	*Bacillus cereus*	94	0.2
B-10	*Pseudomonas fluorescens*	69	0.1
B-3429	*Pseudomonas oleovorans*	100	0.1
B-1855	*Pseudomonas aureofaciens*	89	3.4
B-562	*Enterobacter aerogenes*	—	0.1
B-3157	*Arthrobacter simplex*	100	0.2
B-2979	*Arthrobacter globiformis*	97	0.2
B-14092	*Arthrobacter terregens*	91	0.2
B-36	*Agrobacterium tumefaciens*	100	0.1
B-609	*Mycobacterium phlei*	90	0.1
B-633	*Streptococcus lactis*	99	0.2
B-634	*Streptococcus cremoris*	96	0.2
B-4377	*Staphylococcus epidermidis*	99	0.2
B-4369	*Chromobacterium violaceum*	80	63.5
B-2270	*Azotobacter vinelandii*	94	0.2
B-1459	*Xanthomonas campestris*	100	0.2
B-137	*Erwinia aroideae*	99	0.2
B-4291	*Salmonella arizonae*	96	0.2
B-54	*Flavobacterium devorans*	91	0.2
B-3522	*Klebsiella pneumoniae*	100	0.2
B-1036	*Acetobacter aceti*	100	0.2
B-14023	*Zymomonas mobilis*	96	0.2
B-402	*Cellulomonas fimi*	99	0.2
Actinomyces			
B-3287	*Nocardia aurantia*	70	0.4
5767	*Nocardia cholesterolicum*	88	0.0
B-3906	*Nocardia corallina*	77	0.1
B-5476	*Nocardia corallina*	61	0.0
B-1532	*Nocardia erythropolis*	86	0.2
B-5477	*Nocardia minima*	18	0.0
B-3068	*Nocardia* sp.	88	0.0
5635	*Nocardia* sp.	82	0.1
B-12178	*Actinoplanes armeniaca*	93	0.1
5325	*Actinoplanes caeruleus*	91	0.1
B-3342	*Actinoplanes missouriensis*	94	0.1
B-16090	*Actinoplanes rectilineatus*	93	0.1
2209	*Streptomyces aureofaciens*	95	0.3
2338	*Streptomyces erythraeus*	97	0.3
2331	*Streptomyces halstedii*	100	1.1
B-1679	*Streptomyces viridifaciens*	98	0.3
2234	*Streptomyces rimosus*	85	2.3
B-2208	*Streptomyces albus*	94	0.3
Fungi-Ascomycetes			
468	*Aspergillus oryzae*	42	94.2
1957	*Aspergillus flavus*	0	—
3228	*Aspergillus niger*	49	57.1
405	*Aspergillus ochraceus*	44	78.6
3248	*Aspergillus candidus*	84	0.4
67	*Aspergillus carbonarius*	42	62.6
78	*Aspergillus chevalieri*	88	0.8
163	*Aspergillus fumigatus*	2	—
255	*Aspergillus terreus*	8	12.7
187	*Aspergillus nidulans*	58	35.7
573	*Aspergillus versicolor*	84	0.1
849	*Penicillium roqueforti*	48	37.8
1640	*Penicillium thomii*	85	0.2
790	*Penicillium oxalicum*	17	62.7
811	*Penicillium chrysogenum*	9	71.8
1916	*Penicillium frequentans*	88	0.2
1843	*Penicillium citrinum*	1	—
821	*Penicillium notatum*	88	0.5
2039	*Penicillium raistrickii*	85	0.5
895	*Penicillium lilacinum*	88	1.9
1002	*Penicillium claviforme*	96	0.5
5883	*Fusarium graminearum*	89	11.6
2374	*Fusarium moniliforme*	32	30.8
1871	*Fusarium oxysporum*	44	60.9
3078	*Fusarium solani*	39	69.0
5232	*Helminthosporium oryzae*	62	0.4
1647	*Sclerotinia convoluta*	91	0.5
Fungi-Basidiomycetes			
2366	*Pleurotus ostreatus*	93	0.2
Fungi-Phycomycetes			
A-26225	*Mucor circinelloides*	85	61.8
1894	*Mucor rouxii*	77	34.5
3469	*Mucor pusillus*	60	81.1
5281	*Mucor racemosus*	5	67.2
5926	*Absidia coerulea*	51	66.0
2700	*Gilbertella persicaria*	33	61.7
5191	*Amylomyces rouxii*	92	3.7
2928	*Amylomyces rouxii*	84	80.8
3139	*Amylomyces rouxii*	54	100.4
3160	*Amylomyces rouxii*	33	94.5
2710	*Rhizopus oligosporus*	96	42.1
2549	*Rhizopus oligosporus*	84	95.4
2904	*Rhizopus chinensis*	79	56.9
3133	*Rhizopus oryzae*	67	76.0
5192	*Rhizopus chlamydosporus*	87	0.1
1309	*Absidia ramosa*	72	51.6
2453[a]	*Phycomyces nitens*	86	74.0
1554[a]	*Phycomyces blakesleeana*	85	1.0
1399	*Helicostylum piriforme*	98	0.1
1369	*Cunninghamella blakesleeana*	34	47.7
2941	*Mortierella parvispora*	83	0.1
Fungi-Imperfectii			
6437	*Cladosporium resinae*	99	0.6
2178	*Curvularia lunata*	85	0.3
3108	*Beauveria bassina*	63	5.9
2325	*Alternaria solani*	62	7.4
Yeasts			
Y-552	*Geotricum candidum*	54	93.4
Y-1095	*Candida lipolytica*	32	45.9
Y-1091	*Rhodotorula glutinis*	94	0.1
Y-2022	*Debaryomyces polymorphus*	94	0.1
Y-2075	*Pichia gulliermondii*	91	0.1
Y-11528	*Pichia pinus*	90	0.1
Y-900	*Candida utilis*	94	0.1
Y-1603	*Pichia pastoris*	97	0.1

[a]Medium also contained 2 g yeast extract.

fatty acids and traces of di- and monoglycerides. No hydroxy fatty acids were detected by TLC. FFA contents were determined, and the results are shown in Table 1. Bacteria and actinomycetes in general did not increase the FFA of added soybean oil. *Chromobacterium violaceum* was the only species tested to show high (63.5%) FFA. The next highest was shown in the *Pseudomonas aureofaciens* fermentation, which resulted in only 3.4% of the soybean oil as FFA. Even though no increase in FFA was found in soybean oil after fermentation, some of the added soybean oil was metabolized by a few bacteria and actinomyces. It would appear, therefore, that as the oil is hydrolyzed, the FFA are quickly adsorbed and metabolized.

Extensive hydrolysis of soybean oil was most prevalent with fungi. Essentially complete hydrolysis (95%) was evident with *Aspergillus oryzae, Rhizopus oligosporus* and *Amylomyces rouxii.* Four different strains of the latter were tested. Only two strains hydrolyzed soybean oil completely, and one showed 80% hydrolysis while the fourth (NRRL 5191) had only 3.7% FFA in soybean oil after fermentation. With *Aspergillus flavus* no fat could be extracted, and no visible fat was found in the medium. Later results (Table 4) showed that soybean oil probably was adsorbed on the extracellular membrane and can only be extracted by soaking the cells in hexane. Only two strains among the yeasts hydrolyzed soybean oil. Lipids extracted from the medium after incubation with *Geotrichum candidum* and *Candida lipolytica* were 93% and 46% FFA.

To study the intracellular lipids, selected microorganisms were grown four days at 28 C in 750 ml medium to which 32 ml soybean oil was added. Extracellular lipids were extracted with hexane, and intracellular lipids were extracted with a chloroform:methanol (2:1) mixture. The results are shown in Table 2. FFA were absent in both intra- (chloroform-methanol extract) and extracellular (hexane extract) lipid fractions of *Nocardia minima* (NRRL B-5477). The fatty acid compositions of the triglyceride fractions are typical of soybean oil which contained 10.3% 16:0, 3.8% 18:0, 21.8% 18:1, 55.6% 18:2 and 8.5% 18:3. Only 66% of the added soybean oil was extracted; the remainder presumably was assimilated. Even though FFA were absent, the liquid medium showed lipase activity. After dialysis and freeze-drying of the cell-free broth, 0.2 g of solid was obtained. The solid material was stirred with 2 g of soybean oil and 5 ml of 0.1M NaH_2PO_4 (pH 5.8) for 48 hr at 25 C. The oil extracted from this reaction mixture contained 35% FFA, whereas in a control experiment the FFA remained unaltered at 0.1%. Apparently, FFA formed during fermentation of *Nocardia minima* were quickly adsorbed and metabolized. All other microorganisms showed products of hydrolysis, with *Amylomyces rouxii* (NRRL 3160) exhibiting the highest content of FFA. The compositions of the fatty acid fractions were different from those of the triglycerides. Fatty acid fractions contained more saturated fatty acids, while the triglyceride fractions were richer in polyunsaturates. The fatty acid composition of the intracellular lipids reflects that of soybean oil. However, when these organisms were grown in a high-glucose (3%) lipid-free medium, saturated long chain fatty acids up to 26 carbon atoms were more abundant with correspondingly lower amounts of unsaturated fatty acids, as shown in Table 3. Interestingly, two of the three organisms had a high content (10 to 12%) of γ-linolenic acid. Palmitoleic acid was found when lipid-free medium was used, whereas inclusion of soybean oil in the medium suppressed formation of this acid.

Intra- as well as extracellular lipase activity was observed in many microorganisms grown in soybean oil. For example, *Candida lipolytica* (Y-1095) culture broth was extracted with hexane and then dialyzed and lyophilized yielding 0.2 g solid. Half of this material was stirred with 5 ml of 0.1M NaH_2PO_4 (pH 5.8) and 2 g soybean oil for 48 hr at 25 C. The oil extracted from this mixture contained 24% FFA. The cells were extracted with hexane followed by chloroform:methanol. The lipid-free cells and glass beads suspended in phosphate buffer were ruptured in a cell homogenizer (Bead-Beater, Biospec Products, Bartlesville, Oklahoma) until microscopic examination in-

TABLE 2

Fatty Acid Composition of FFA and TG Fractions from Microorganisms Grown on Soybean Oil

Organism NRRL No.	Hexane extract g	$CHCl_3$-MeOH extract g	FFA %	Fatty acid composition									
				Fatty acid fraction					Triglyceride fraction				
				16:0	18:0	18:1	18:2	18:3	16:0	18:0	18:1	18:2	18:3
B-5477	19.8		0.1						9.9	3.5	21.9	56.2	8.5
		6.0	0.1						10.8	3.7	22.9	54.4	8.2
Y-1095	20.0		50.7	12.4	5.3	23.4	51.4	7.2	8.3	3.7	23.5	57.6	6.4
		1.6	47.2	12.5	4.1	21.5	54.4	7.2	7.0	2.2	21.1	60.0	9.3
1369	17.8		41.2	17.5	7.2	23.7	45.8	5.8	5.4	2.0	24.2	60.6	7.5
		4.9	29.2	10.8	4.3	26.3	53.9	4.7	10.4[a]	3.7	19.3	55.6	8.3
3160	23.5		88.4	11.6	4.1	22.0	54.7	7.6	4.0[b]	1.4	24.2	58.8	8.1
		1.1	83.0	7.1	2.2	25.2	58.3	7.2	6.1[c]	1.2	16.8	62.5	12.4

[a]Also 2.7% unknown.

[b]Also 3.5% 14:0.

[c]Also 1.0% unknown.

sured complete cell rupture. The ruptured cells were filtered through cheesecloth, and the filtrate was dialyzed and lyophilized to yield 1.18 g. A portion of this material (0.2 g) treated as above gave oil containing 25% FFA.

To study the change in fatty acid composition during growth, *Aspergillus flavus* was grown on a medium containing soybean oil, for two, three, four and five days, and the lipid contents and fatty acid compositions were followed. The data are shown in Table 4. The FFA increased in the hexane extracts while the $CHCl_3$-MeOH extracts contained mostly triglycerides. The linolenate contents of the fatty acid fractions from the medium (hexane extracts) continuously decreased from 7.3% at two days to 3.4% at five days growth. The linolenate content in the corresponding triglyceride fractions increased concomitantly. Apparently some kind of selective metabolism of linolenate is taking place. The intracellular fatty acid fractions ($CHCl_3$:MeOH extract) were similar in composition to the extracellular (hexane extract) fatty acids fractions. This is not surprising because the liberated free fatty acids are adsorbed by the cellular membrane without discrimination. However, the triglycerides inside the cells have high linolenate (12–15%) and linoleate (65–67%) contents. Presumably the fatty acids are selectively esterified by the enzymes inside the cells. Capillary GC and infrared spectroscopy of selected samples revealed no *trans*- or conjugated isomers. The fatty acids of soybean oil were not altered by the microorganisms.

From the dry weight of the lipid-free cells, it appears that there was a pause in growth at day 3. Similar observations were made by Ellis et al. (15) on the growth of *Amylomyces* strains. The free fatty acids in the medium increased to 96% at day 3 and then decreased to 83% at day 4. This decrease in FFA can be explained if cell lysis was assumed to be occurring. If some cells lysed and the triglycerides from inside the cells spilled into the medium, then the free fatty acid content decreased. The fatty acid composition of the triglyceride fractions can also be explained if lysis is assumed to be occurring. By day 3, most of the soybean oil had been hydrolyzed, and at day 4 and later, the triglycerides in the medium came from lysis of the cells. Therefore, the fatty acid composition of the triglycerides from inside the cells and from the medium were similar; whereas at days 2 and 3 when cell lysis was at a minimum, the fatty acid composition of the intracellular triglycerides was different from that of the medium.

The *A. flavus* cells grown on soybean oil contained more than 50% lipid as measured by the amount of lipid extracted from the cells. In a control experiment, 27.5 g of soybean oil was extracted from the culture medium, and the amount of oil extracted from *A. flavus* during five-day growth was much less. Therefore, some oil was assimilated by *A. flavus*. When the fatty acid composition of the total lipid (hexane extract + lipid extract) was calculated, the linolenate content dropped from 7.5% on day 2 to 6.0% on day 5, whereas the other fatty acids remained essentially the same as in soybean oil. This indicated that *A. flavus* selectively catabolized linolenic acid. It might be argued that the decrease in linolenic acid during fermentation was the result of oxidation rather than selective assimilation. Two control experiments with either soybean oil or soybean oil fatty acids did not change the linolenate content after five days of shaking at 28 C.

TABLE 3

Fatty Acid Composition of Lipids from Microorganisms Grown on High Glucose Lipid-Free Medium

Fatty acid	NRRL No. 3610	Y-1095	1369
14:0	0.9	0.2	0.7
16:0	22.4	10.4	14.8
16:1	5.8	9.9	0.9
18:0	4.2	3.0	9.5
18:1	23.7	45.9	44.0
18:2	23.9	29.7	12.7
18:3[a]	11.7	0.0	9.8
20:0	0.6	0.9	1.5
22:0	0.0	0.0	1.3
24:0	4.1	0.0	3.9
26:0	2.7	0.0	0.8

[a]γ-Linolenic acid.

TABLE 4

Yield of Oil and Cells and Fatty Acid Composition of FFA and TG Fractions During Growth of *Aspergillus flavus*

No. of days (wt. lipid-free dry cells)	Hexane extract g	$CHCl_3$-MeOH extract g	FFA %	Fatty acid composition of: Fatty acids 16:0	18:0	18:1	18:2	18:3	Triglycerides 16:0	18:0	18:1	18:2	18:3
2 (2.42)	23.67		58.7	12.0	4.2	22.9	53.6	7.3	9.9[a]	3.7	22.4	55.8	7.6
		1.0	14.6	12.5[a]	3.8	20.3	55.6	7.1	10.0	2.1	10.5	64.6	12.6
3 (3.22)	19.64		96.2	10.9	4.9	26.3	52.9	5.0	5.1[a]	1.8	20.7	60.8	8.3
		4.38	5.0	11.1[a]	6.0	28.8	47.4	4.7	8.8	2.3	8.0	65.9	15.1
4 (3.08)	16.47		82.8	11.1	5.0	26.4	53.2	4.3	8.8[a]	2.7	9.7	65.8	12.2
		3.53	7.8	11.7[a]	3.3	19.3	60.1	4.9	9.2	2.4	9.1	66.6	12.7
5 (4.00)	17.40		85.0	12.0	6.0	29.2	49.4	3.4	9.5[a]	2.6	10.9	65.0	10.6
		4.72	4.3	11.0[a]	4.9	27.4	50.5	4.2	9.1	2.3	9.9	66.6	12.1

[a]Contains 14:0.

DISCUSSION

In our survey for modification of vegetable oils, we had hoped to find evidence for reactions such as hydroxylation or hydrogenation. If these reactions occurred, they were so small as to be obscured by the predominant reaction, hydrolysis.

Aspergillus flavus hydrolyzed soybean oil completely (96%) in three days of fermentation, and the fatty acids obtained contained less linolenic acid (5% vs 8.5%) than originally contained in soybean oil. It thus appears that this organism has the potential of modifying the fatty acid composition. If the fatty acids containing lower linolenic acid were re-esterified, the resulting triglycerides should be more stable than the original soybean oil (16).

Amylomyces rouxii (NRRL 3160) and *Cunninghamella blakesleeana* (NRRL 1369) when grown on fat-free medium produced lipids containing greater amounts of saturated fatty acids than those grown on soybean oil. However, the rather high amounts of γ-linolenic acid present in these triglycerides make them, potentially, a rich source of γ-linolenic acid, a precursor of biologically active prostaglandins.

The diversity of types of microorganisms surveyed is quite broad. In the beginning of the survey, microorganisms from bacteria, yeasts, actinomycetes and filamentous fungi were selected that were known to be associated with lipid materials. *Cladosporium resinae,* which often is found growing in jet fuel, neither assimilated nor produced any change in soybean oil. Following failure to obtain interesting modified oils other than hydrolysis products, we looked at other microorganisms in the groups above but not known to have an association in nature with fats. These included several genera not examined before, such as the genus *Streptomyces.* The question now can be asked, why did the survey fail to identify metabolic products while other surveys have turned up organisms that modified molecules at high levels? It may be that microorganisms that have not had contact with certain compounds do not have the enzyme systems to break down the material completely to CO_2 and water or assimilate the substrate directly. Compounds not commonly found in nature often are only slightly modified by the microorganism. For example, Detroy and Hesseltine (17) found that *Dactylium dendroides* (NRRL 2575) could convert aflatoxin B_1 into aflatoxinol by a reduction of cyclopentenone, thereby reducing biological activity. The transformation of steroids by certain fungi to give high yields of useful products is well documented.

When one examines the compounds that were transformed, such as aflatoxin, it is apparent that these materials are not commonly found in nature. Aflatoxin occurs only in a few ecological situations, and microorganisms would not come into contact with the molecule, or at most only a few times in thousands of generations. In contrast, vegetable oils and other triglycerides have been around since the beginning of life and occur widely. Thus, practically all microorganisms, which have had continual contact with glycerides, have evolved an efficient system of using vegetable oils for energy and growth. Therefore, they are less likely to make intermediate metabolites that remain stable and accumulate. Two approaches to getting around this dilemma are obvious. One would be to examine autotrophs which may not have evolved an efficient system to break down lipids. A second would involve the development of mutants that lack enzyme activity so that the breakdown of the vegetable oil is effectively blocked at the desired step. Another approach would be to isolate known enzyme systems to execute reactions in vitro.

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