REVIEW

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Overproduction of Microbial Lipids and Lipases

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ABSTRACT. The nomenclature of simple and complex lipids, and their analysis are introduced and the overproduction of lipids and lipases are discussed.

CONTENTS

1 Introduction

Lipids have been known and used for a long time – not only as food for man and animals but also in the industry. It is thus thought important to introduce here the chemistry and biotechnology of lipids and of their characteristic constituents, fatty acids.

2 Nomenclature

In general, the nomenclature of lipids consists of two basic parts, *viz.* the nomenclature of fatty acids and complex lipids. At the onset of research in organic chemistry and biochemistry, newly discovered organic compounds were given trivial names, as is shown in Table I, only the most common names of selected fatty acids being given. As both the systematic (formed for acids derived from hydrocarbons by the suffix -oic) and trivial names are somewhat cumbersome, the following type of abbreviations are used. The figure preceding the colon gives the number of carbons in the molecule, the figure following the colon denotes the number of double bonds. In case that the chain is branched, the abbreviation *br* is put in front of the first figure with the understanding that the numbering starts from carboxyl number one. This nomenclature is used only when the main chain is substituted with methyl group(s) which is, as will be shown further, the most common case from the biogenetic point of view. A small index at *br*, *e.g.* br_{10}-19:0, specifies tuberculostearic acid, *i.e.* 10-methyloctadecanoic acid. The abbreviations *i* and *ai* are used for the two most common cases, viz *iso* and *anteiso* branching, which is branching at the last but not one and last but two carbon, respectively (these are biosynthetic precursors of valine, leucine and isoleucine). The prefix *c* means cyclopropane ring, as a rule with the number of link of a methylene group (see Table I).

The position of double bonds, assuming the *cis* (*i.e. Z*) geometric configuration, can be described in two ways. The first gives the position of all double bonds, starting from the carboxyl

(number 1), the other from the end methyl carbon (numbering n; so called "ω"-nomenclature). The second, however, can be used only at the allylic-type systems of double bonds (cf. Fig. 1). Examples of this nomenclature are in Table I.

Table I. Examples of some of the more common fatty acids

Systematic name	Common name	Short notation	Systematic name	Common name	Short notation
Saturated			*Unsaturated*		
			Monoenoic		
Dodecanoic	lauric	12:0	5-Tetradecenoic	physeretic	5-14:1
Tetradecanoic	myristic	14:0	9-Tetradecenoic	myristoleic	9-14:1
Pentadecanoic		15:0	9-Hexadecenoic	palmitoleic	9-16:1
Hexadecanoic	palmitic	16:0	9-Octadecenoic	oleic	9-18:1
Heptadecanoic	margaric	17:0	11-Octadecenoic	*cis*-vaccenic	11-18:1
Octadecanoic	stearic	18:0			
Eicosanoic	arachidic	20:0	*Polyenoic*		
Docosanoic	behenic	22:0	9,12-Octadeca-dienoic	linoleic	9,12-18:2 or 18:2$n-6$
Tetracosanoic	lignoceric	24:0	9,12,15-Octadeca-trienoic	α-linolenic	9,12,15-18:3 or 18:3$n-3$
Hexacosanoic	cerotic	26:0	6,9,12-Octadeca-trienoic	γ-linolenic	6,9,12-18:3 or 18:3$n-6$
Octacosanoic	montanic	28:0	5,8,11,14-Eicosa-tetraenoic	arachidonic	5,8,11,14-20:4 or 20:4$n-6$
Triacontanoic	melissic	30:0			
Dotriacontanoic	lacceroic	32:0			
12-Methyltri-decanoic	isomyristic	*i*-14:0			
12-Methyltetra-decanoic	sarcinic	*ai*-15:0			
14-Methylpenta-decanoic	isopalmitic	*i*-16:0			
cis-9,10-Methylene-octadecanoic	dihydrosterculic	*c*-9,10-19:0			
cis-11,12-Methylene-octadecanoic	lactobacilic	*c*-11,12-19:0			

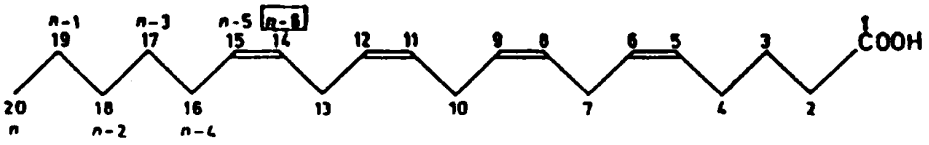

Fig. 1. Structure of arachidonic acid; *systematic name*: all-*cis*-5,8,11,14-eicosatetraenoic acid *short notation*: 5,8,11,14-20:4 or 20:4$n-6$

Hence it is recommended, after studying Figs. 2–7, to consult the original papers or special books, *e.g.* Kates (1988).

3 Isolation and identification

In general, identification of lipids has become possible only in the late fifties, marked by the experiments of James and Martin with the gas chromatography of fatty acid methyl esters (FAME). Lipids are, as a rule, mixtures of homologs. Hence the usual separation methods of organic chemistry, *e.g.* crystallization and distillation, fail to provide the desired results. The development of thin-layer chromatography (TLC), together with gas chromatography, contributed to the separation of intact lipids. With the development of other instrumental techniques and their establishment in biochemical laboratories at the beginning of the seventies, such as linking a gas chromatograph to a mass spectrometer, GC–MS, and use of nuclear magnetic resonance permitted unprecedented discoveries in

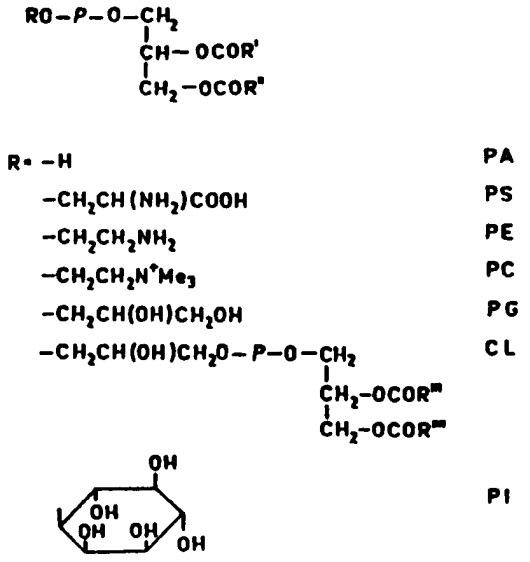

Fig. 2. Some structures of common phospholipids and their abbreviations;

PA phosphatidic acid
PS phosphatidylserine
PE phosphatidylethanolamine
PC phosphatidylcholine
PG phosphatidylglycerol
CL diphosphatidylglycerol (cardiolipin)
PI phosphatidylinositol
R', R'', R''', R'''' - alkyls, usually C_{12} to C_{24}.

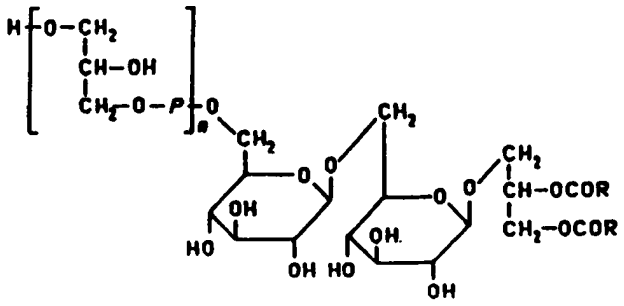

Fig. 5. Structure of lipoteichoic acid from Gram-positive bacteria.

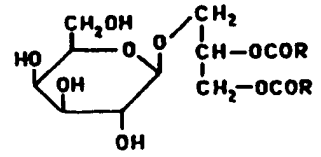

Fig. 3. Structure of monogalactosyl-diacylglycerols (MGDG).

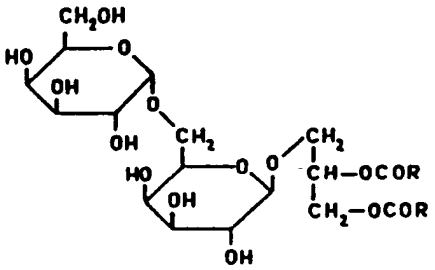

Fig. 4. Structure of digalactosyl-diacylglycerols (DGDG).

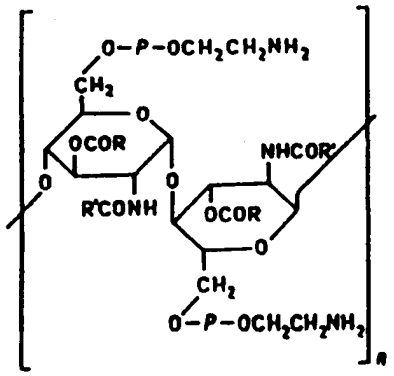

Fig. 6. Structure of lipopolysaccharide from Gram-negative bacteria.

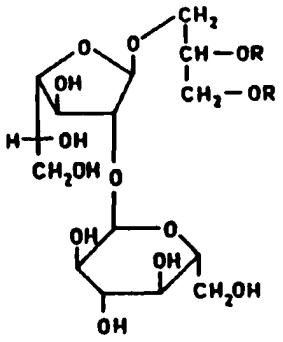

Fig. 7. Structure of diphytanylglycerol from methanogenic bacteria; R = $C_{20}H_{41}$ (phytanyl); saccharides are α-D-glucopyranose and β-D-galactofuranose.

lipidology to be made. The most up-to-date technique is the combination of high-performance liquid chromatography (HPLC) and mass spectrometry (LC-MS). Individual compounds can be separated in an HPLC column and the eluate is directly transferred to the MS (*e.g.*, by the "thermospray" or "moving belt" method). MS makes use of the so-called "soft" ionization technique, *viz.* chemical ionization (both positive and negative), field desorption or fast atom bombardment. This leads to excellent separation and spectra of individual compounds of the sample. Moreover, wide-spread computerization simplifies and

speeds up the identification of the compounds by comparing the detected spectra with libraries comprising tens of thousands of spectra of known compounds. The same applies to GC-MS making use of capillary columns with phases stable at temperatures above 350 °C and performing at rates as high as 5000 plates per m. Using a 60-m column with a suitable phase, one can separate almost any mixture into individual components.

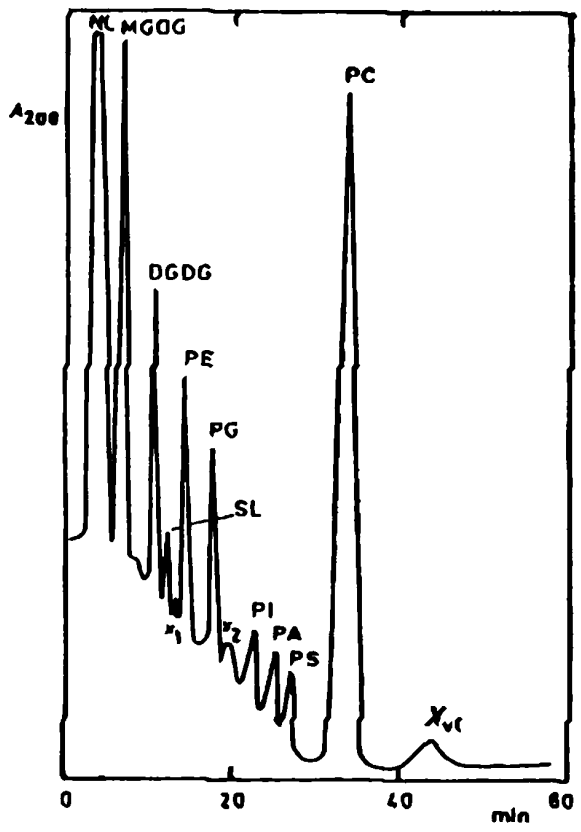

Fig. 8. Separation of total phospholipids into single classes by HPLC; NL – neutral lipids (triglycerides, waxes, *etc.*); x_1 and x_2 – unidentified lipids; SL – sulfolipids.

The isolation of lipids consists of several steps. *First, one has to obtain total* lipids, if possible free of nonlipidic compounds (proteins, saccharides, pigments, *etc.*). Extraction with chloroform–methanol is most frequently used for this purpose (although other mixtures, such as toluene–ethanol, have been also applied). Disintegrated cells are supplemented with such an amount of chloroform–methanol (usually in the ratio 2:1 till 1:2) that two layers are not formed. After a thorough shaking, chloroform and water are added whereupon lipids accumulate in the lower chloroform layer. Contamination by nonlipid compounds can be eliminated by careful washing or elution through a Sephadex or DEAE-cellulose column. The total mixture can be further separated by chromatography on silica gel, yielding three basic groups of compounds, viz. nonpolar lipids (*e.g.*, triacylglycerols), phospholipids and glycolipids. These individual *groups can be again separated* both preparatively and/or analytically by TLC (one- and two-dimensional) or HPLC. After obtaining the individual classes of lipids, one can continue to separate them into individual molecular species, making use of reverse-phase chromatography (RP-HPLC). Figs 8 and 9 show the separation of lipids from algae into individual classes and further the separation of phosphatidylcholines into individual molecular species. For illustration, the result of GC–MS analysis of trimethylsilylated phosphatidylcholine after hydrolysis with phospholipase C is shown in Fig. 10.

It is rather simple to obtain the composition of total fatty acids or fatty acids in the individual fractions. Total lipids can be modified to methyl esters by acidic or basic transesterification (protic or Lewis acid as HCl, H_2SO_4, BF_3, BCl_3, *etc.*, and sodium methanolate and sodium hydroxide, respectively). Lipids can also be subjected to basic hydrolysis; the nonsaponifiable fraction (sterols, hydrocarbons) can be removed by extraction with diethyl ether or hexane, and fatty acids after acidification by extraction with chloroform or diethyl ether. Conversion to methyl esters can be again achieved by acidic esterification in methanol or by reaction with diazomethane. The obtained derivatives can be subjected to chromatography on a polar or nonpolar, usually capillary column, depending on the sample type. Identification by GC is possible only according to the retention time or a derived value, such as the equivalent chain length.

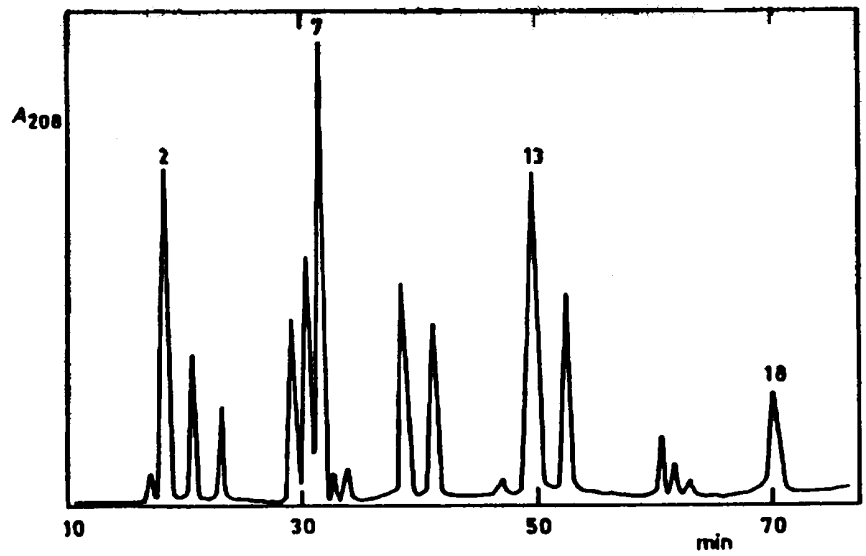

Fig. 9. Separation of phosphatidylcholine into single molecular species by reversed-phase HPLC; peak numbers - molecular species of PC with acyls: 2 - 18:3 + 18:3; 7 - 16:0 + 16:1; 13 - 16:0 + 18:1; 18 - 18:0 + 18:1.

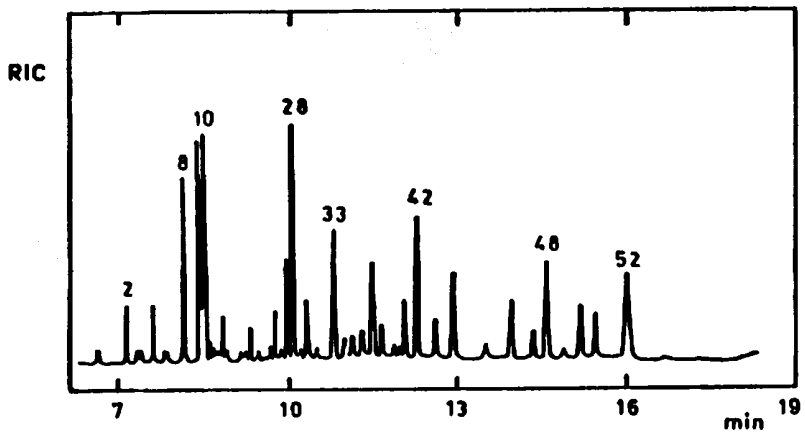

Fig. 10. Gas chromatography-mass spectrometry of diacylglycerols obtained from phosphatidylcholine; peak numbers - molecular species of PC with acyls: 2 - 14:0 + 16:0; 8 - 16:0 + 16:0; 10 - 16:0 + 18:1; 28 - 16:0 + 18:1; 33 - 16:0 + 18:2; 42 - 18:1 + 18:1; 48 - 18:1 + 18:3; 52 - 18:3 + 18:3.

Using GC-MS, it is possible to determine the position of double bonds and of branching after conversion of the compounds to nitrogenous derivatives, such as pyrrolidides, picolinyl esters, *etc.* Mass spectra make it possible to determine the position of double bonds by cleaving off the CH_2 groups, as well as and branching. GC-MS was successfully applied to the identification of intact lipids, *e.g.* tri- and diacylglycerides, wax esters, esters of sterols, *etc.* However, the use of GC-MS is limited to a few specialized laboratories where the expensive equipment and theoretical and practical background in the field are available. The same holds for LC-MS.

Even if the above physico-chemical methods are available, it is recommended to use lipid standards. In general, while the more common fatty acids can be purchased either pure, or in mixtures the rarer ones may cause difficulties. The basic classes of lipids, phospho- and glycolipids, both synthetic and in natural mixtures, can also be obtained. A disadvantages may be in the high cost and low stability of the compounds even when stored sealed at -18 °C and under nitrogen (*e.g.*, phosphatidylserine). Further information can be found in Christie (1982, 1987) and Kates (1988) and in the original articles cited there.

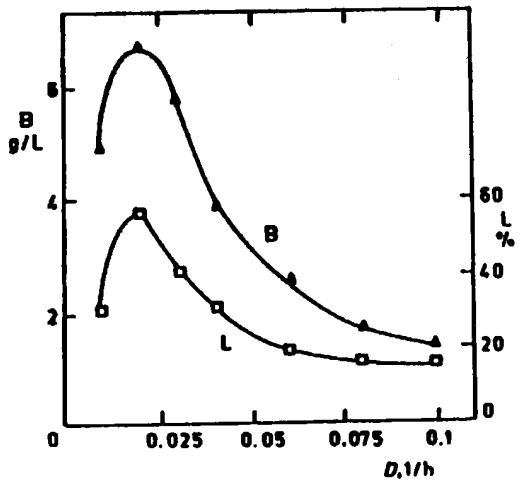

Fig. 12. Accumulation of lipids by yeasts growing in continuous culture (dilution rate *D*); B - biomass, L - lipids.

Under optimum conditions, a nearly 22-% conversion of glucose and 31-% conversion of ethanol to lipids can be achieved. An important factor is the amount of acetyl-CoA formed. One mole of ethanol is converted into 1 mol of acetyl-CoA, while 1 mol of glucose yields 2 mol of acetyl-CoA. One has to take into consideration that the molar mass of ethanol is 46 g/mol while that of glucose is 180 g/mol. The calculation gives a different pattern according to which ethanol is almost nearly a twice more efficient substrate than glucose. It is clear that a part of the substrate is used for other reactions in the cell and cannot be used for the formation of lipids. Another advantage of ethanol as substrate is that its oxidation yields high amounts of NADH (NADPH) which is further used for the synthesis of fatty acids (reduction of double bonds). An explanation why the excess oxygen prevents the utilization of saccharides (glucose) is the catabolic inhibition of phosphofructokinase (a key enzyme in glucose degradation catalyzing the conversion of fructose 6-phosphate into fructose 1,6-bisphosphate.

Table II. Examples of oleaginous microorganisms

Microorganisms	Lipids % (*W*/*W*)	Microorganisms	Lipids % (*W*/*W*)
Bacterium		*Molds*	
Arthrobacter sp.	78	*Aspergillus nidulans*	51
		A. terreus	57
Algae		*Cunninghamella elegans*	56
Monalanthus salina	70	*Fusarium bulbigenum*	50
Neochloris oleoabundans	54	*Mortierella isabelina*	86
Ochromonas danica	71	*M. vinacea*	66
		Mucor circinelloides	65
Yeasts		*M. ramannianus*	56
Candida curvata	58	*Penicillium lilacinum*	56
Cryptococcus albidus	63	*P. spinulosum*	64
C. terricolus	65	*Ustilago zeae*	59
Endomycopsis vernalis	65		
Geotrichum candidum	50		
Lipomyces lipofer	64		
L. starkeyi	63		
Rhodosporidium toruloides	66		
Rhodotorula glutinis	71		
Trichosporon pullulans	65		

The oil-forming organisms which accumulate mostly triacylglycerols are shown in Table II. Only those organisms are listed here which contain more than 50 % lipids (related to dry mass). It follows from Table III, which gives the contents of individual lipid classes, that triacylglycerols are the main part of the microbial lipids. They occur usually as oil droplets in vacuoles. The representation of individual fatty acids is given in Table IV.

Table III. Lipid analysis (%) of some oleaginous microorganisms[a,b]

Organism	HC	SE	TG	DG	MG	FFA	GL	PL
Bacterium								
Arthrobacter sp.	0	2.2	91	*tr*	0	0	0	6.7
Algae								
Neochloris oleabundans	0.7	1.5	81	0.8	0.1	1.2	0	9.3
Yeasts								
Cryptococcus terricolus	0	0.4	92	2.5	0.3	3.2	0	1.8
Endomycopsis vernalis	0	10	84	1	0	1	0	4
Lipomyces lipofer	0	*tr*	72	0	0	8	4	14
L. starkeyi	0	*tr*	79	0	0	5	5	15
Rhodotorula glutinis	0	3	84	0	0	10	0	2
Moulds								
Mortierella isabelina	0	0.4	82	6.6	0.4	3.9	0	7
Penicillium lilacinum	0	0.2	85	3.4	2.8	2.0	0	6.4
Ustilago zeae	0	21	16	*tr*	0	50	0	12

[a]HC = hydrocarbons, SE = steroid esters, TG = tri-, DG = di-, MG = monoacylglycerols, respectively, FFA = free fatty acids, GL = glycolipids, PL = phospholipids.
[b]*tr* = trace.

In general, algae are photoautotrophic microorganisms synthesizing high amounts of oligoenoic fatty acids, mainly C_{16} and C_{18}. In contrast, yeast cells do not synthesize oligoenoic acids, but mostly palmitic and oleic acids, whose contents reach 70–80 %. The sum of stearic and linoleic acid does not exceed 15 %; traces of linolenic and palmitoleic acids were also detected. Lower fatty acids (less than C_{16}) usually occur only in traces. Higher amounts of linoleic and linolenic acids were described in moulds. Moulds of the genus *Mortierella* exhibit high levels of C_{20} oligoenoic acids (as much as 34 % of arachidonic acid and, under different cultivation conditions, as much as 21 % of

Table IV. Composition of fatty acids (%) of various oleaginous microorganisms

Microorganisms	Fatty acid							
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	others[a]
Bacterium								
Arthrobacter sp.	3	43	5	15	24	0	0	*br ev* 10
Algae								
Neochloris oleoabundans	2	15	4	11	36	7	0	*br ev un* 25
Yeasts								
Endomycopsis vernalis	0	15	0	2	57	24	2	
Lipomyces lipofer	0	37	4	7	48	3	0	*hi* 1
L. starkeyi	0	32	2	7	53	6	0	
Rhodotorula glutinis	1	13	0	7	53	20	5	12:0 1
Molds								
Penicillium lilacinum	0	28	0	13	37	20	0	*ev* 2

[a]*br* – branched, *ev* – even, *un* – unsaturated, *hi* – higher.

γ-dihomolinolenic acid). These organisms are a source of γ-linolenic acid which is important from the dietetic point of view (Yamada *et al.* 1988). Unusual fatty acids, including hydroxy acids, are minor, except for the genus *Claviceps* which contains high amounts of ricinoleic acid (Křen *et al.* 1987).

The basic factors bearing on the representation of fatty acids are pH, temperature, the amount of dissolved oxygen and the growth (carbon) substrate (Table V). When yeast cells are cultivated in a mixture of alkanes, the length of the fatty acid chain depends on the number of carbon atoms in the alkanes. The intracellular contents of fatty acids are rather stable if fatty acids or their esters serve as the carbon source (Table VI) but occasionally, desaturation of fatty acids (conversion of stearic to oleic acid) occurs. When a common vegetable oil is used as carbon source the content of cell lipids resembles that of the vegetable oil. However, the content of polyenoic fatty acids drops (from 54 % on linseed oil to 29 % on linolenic acid in *Candida lipolytica*), which is, from the dietetic point of view, undesirable.

Table V. Fatty acid composition (%) of the yeast *Candida curvata* grown on different carbon a sources

Carbon source	Lipids %(W/W)		Lipid yield g per 100 g		Major fatty acids							
	B	C	B	C	B				C			
					16:0	18:0	18:1	18:2	16:0	18:0	18:1	18:2
Glucose	33	29	11	13	33	12	43	7	36	14	40	6
Sucrose	37	28	15	15	32	11	42	7	37	10	44	6
Lactose	39	31	17	19	33	11	49	6	28	13	47	11
Xylose	49	37	17	18	41	14	43	4	30	15	45	4
Ethanol	30	35	10	13	27	13	49	9	26	12	51	10

[a]B – batch culture, C – continuous culture.

Table VI. Conversion methyl and ethyl esters of palmitic and stearic acids into triacylglycerols by the genus *Candida*

Fatty acid	Substrate added Lipids, %	*C. guilliermondii* 16:0 + 18:0 (Me) 34.6	*Candida sp.* 16:0 (Et) 51.3	*Candida sp.* 18:0 (Et) 44.8
16:0		26.0	60.8	13.4
16:1		4.3	10.9	0.8
18:0		26.2	5.2	37.8
18:1		38.8	17.4	40.7
18:2		4.6	2.2	5.2

Microbial biomass can serve as a source of other compounds whose biosynthesis starts from acetyl-CoA, above all carotenoids and sterols. Although sterols were found also in cellular membranes of prokaryotes, their low contents in cyanobacteria prevents their practical use. Hence eukaryotic microorganisms, such as algae, yeasts and moulds, are economically much more interesting.

The most abundant microbial sterol is ergosterol occurring in almost all the mentioned organisms. Ergosterol is always accompanied by other related sterols, *e.g.* by chondrillasterol and poriferasterol in algae. The most frequently used industrial microorganism is the yeast *Saccharomyces cerevisiae*, containing ergosterol as the prevailing sterol plus as many as ten minor sterols. The typical range of total sterols is 0.1 – 4 % of the dry mass, depending on strain.

A high sterol content was found in moulds, ergosterol being in most cases the dominant sterol. Usually, the remnants of mycelium after extraction of antibiotics (*e.g.* in the case of the genus *Penicillium*) are used as sterol source. As much as 5 % ergosterol, related to the dry mass, was reported for *Aspergillus fumigatus*. Ergosterol is used for the production of vitamin D_2 which is not very active (only

10 % of activity for broilers compared with that of vitamin D_3 which is prepared from 7-dehydrocholesterol). Hence experiments were done to prepare *S. cerevisiae* strains accumulating 7-dehydrocholesterol.

Carotenoids occur in almost all microbial cells. Although all algae contain carotenoids as components of their photosynthetic system in chloroplasts, their content is low. On the other hand, it was calculated that brown algae alone produce globally as much as 12 Tg (*i.e.* 1.2×10^7 tons) carotenoids per year. However, the maximum yield of total carotenoids never exceeds 1 % of total biomass. β-Carotene is the third most abundant compound. Typically, the content of carotenoids is not higher than a few mg per g dry mass. In the blue-green alga *Spirulina platensis*, an important food source for the inhabitants at the Chad Lake in Africa, growing in brackish water, the content of β-carotene reaches 0.4 % dry mass. Similarly, *Dunaliella salina* produces as much as 400 mg carotene per m^2 per day. β-Carotene is especially important as the source of vitamin A. It is present in fungi as a dominant carotenoid. The highest producers of β-carotene are the species *Blakeslea trispora* and *Phycomyces blakesleeanus*, whose mutants produce as much as 2 % of their dry mass as β-carotene. The influence of cultivation conditions on carotenoid production can be summarized as follows. At low temperatures (about 10 °C) β-carotene is formed preferentially while above 25 °C the share of β-carotene drops and rather its oxidation products are formed (torulen, torularhodin). The optimum pH of production is 6.5. Overproduction of carotenes is enhanced by light even in heterotrophic organisms. Nitrogen-containing compounds can block the biosynthesis of carotenoids at different stages, *e.g.* imidazole leads to the biosynthesis of lycopene (a colorless precursor), diphenylamine suppresses the production of torulene and promotes the formation of β-carotene.

The alga *Botryococcus braunii* is known to produce a mixture of polyisoprenoid hydrocarbons, the dominant one being botryococcene. The hydrocarbon content may reach 85 % of total biomass, and these can be subjected to thermic processing, leading to paraffin and other oil fractions that are commercially interesting.

The prohibition of whale killing during the last decade forced the pharmaceutical industry to look for a compensation of cetaceum, *i.e.* hexadecyl palmitate. The subtropical plant *Simmondsia californica* was found to contain wax esters (jojoba oil) in its seeds instead of triacylglycerols but, disappointingly, the plant is not easy to grow. A significant amount of wax esters is produced by the bacterium *Acinetobacter* growing on alkanes. When cultivated on hexadecane, it produces hexadecyl palmitate as the main compound and also esters of the same length but with one or two double bonds which makes these compounds liquid at room temperature. Similarly the photosynthetic *Euglena gracilis* produces wax esters upon autotrophic cultivation. C_{20} to C_{22} hydrocarbons, whose isolation is expensive, have to be used to reach the same chain lengths as those in the jojoba oil.

As already stated, the number of unsaturated bonds decreases with growing temperature. The yields of wax esters are about 10 mg/L which is by two orders of magnitude lower than would be economically interesting. Wax esters from mycolic bacteria, such as from species of the genus *Corynebacterium*, have only been investigated from the point of view of basic research.

Some microbial glycolipids have been studied, such as the sophorose glycolipid from *Torulopsis bombicola* (putatively produced in Japanese pilot plants) or dimycolyl esters from the genus *Mycobacterium*, that might be applied as biological antifoam agents and detergents. Similarly, the active proteolipid surfactin from *Bacillus subtilis* decreases water surface tension from 27 to 5 mN/m.

Another interesting bacterial product is poly-3-hydroxybutyrate. This compound is synthesized *e.g.* by *Alcaligenes eutrophus* and has excellent emulgation properties. Moreover, it is easily thermodegradable and hence is not ecologically objectionable, in contrast to polyethylene, etc. Under suitable cultivation conditions (exhaustion of nitrogen from the cultivation medium but not a complete consumption of all utilizable carbon), it can reach as much as 80 % of bacterial mass. The degree of polymerization can be as high as 10^4. The critical factor in the production of poly-3-hydroxybutyrate is its cultivation, including the proper choice of substrate. Microorganisms are also used for the isolation of ubiquinones (substitute for vitamin K), phospholipids (substances with emulgation properties) and unusual fatty acids (*see above*).

However, none of the above microbial processes and products, with the exception of the production of ergosterol, is not presently profitable. It does not exclude the possibility that these processes

will be applicable with the change of economical conditions (as was said about Germany during the World War II). This change will be feasible also with the growing ecologization of industry.

5 Overproduction of lipases

From the point of view of practical application, lipases can be ranked into four groups.

The first group consists of esterases catalyzing, *e.g.*, the esterification of higher fatty acids by higher alcohols (*e.g.* the formation of hexadecyl palmitate (cetylpalmitate) from palmitic acid and hexadecanol (cetylalcohol)). Such a reaction can occur only in a water-free environment. An example is the above esterification catalyzed by *Rhizopus arrhizus* cells.

The second group comprises industrial lipases. These enzymes (EC 3.1.1.3) hydrolyze triacylglycerols by way of di- and monoacylglycerols to free fatty acids.

The hydrolytic properties of microbial lipases are routinely applied in food industry. The so-called "*in situ*" formation of lipases by microorganisms is especially important for dairy industry. An example is the production of Limburg cheese whose ripening is markedly influenced by lipases from the yeasts *Candida mycoderma* and *Debaryomyces kloeckeri*. The typical flavor of the Roquefort-type cheese depends on the lipolytical properties of *Penicillium roquefortii*. "In situ" action of lipases is also applied in the meat industry. The presence of lipolytic organisms of the genera *Micrococcus* and *Lactobacillus* is necessary for reaching the characteristic taste properties of some types of smoked meat. The lipase-producing microorganisms *Candida lipolytica* and *Geotrichum candidum* are used in fish processing to eliminate excess fat.

Besides the "*in situ*" production, purified preparations of microbial lipases are also used in food industry. Taste and aromatic properties of milk, butter, cheeses and yoghurts can be improved by the addition of a lipase isolated from *Rhizopus delemar*.

Enzymic hydrolysis of fats is applied also in other branches of industry. Lipases can be applied in fur-processing for careful removal of fat remnants from furs. Lipases in a mixture with other enzymes can be used in the purification of waste water. *Candida lipolytica*, *C. cylindracea* and *Pseudomonas stutzeri* lipases are used as components of detergents and washing and cleaning preparations.

The increasing production of fats is in correlation with the effort to make use of lipases for their processing. Although the conventional chemical method of fat degradation at high temperature and pressure is still economically more profitable, more interest is now being shown for the preparation of fatty acids and glycerol from triacylglycerols present in fats by hydrolysis with microbial lipases.

All the above possibilities of lipase utilization are based on their hydrolytic properties. However, if the water content is substantially reduced in the reaction system, lipases catalyze also specific "non-hydrolytic" reactions whose occurrence would not be possible in the aqueous environment. A nonaqueous environment changes the substrate, as well as the enzyme and markedly increases its thermostability. In the environment of organic solvents, lipases catalyze interesterifications, transesterifications, synthesis of new esters and also modifications of saccharides, peptides or the formation of optically active compounds. The specificity of lipases makes it possible to obtain compounds difficult to prepare by conventional chemical methods. These compounds can be used, *e.g.*, in pharmaceutical, food, and agrochemical industry.

An example of utilization of lipase-catalyzed ester synthesis is the synthesis of esters with characteristic taste and flavor ("flavor" esters). Immobilized *Candida cylindracea* lipase catalyzes the formation of ethyl butyrate from butyric acid and ethanol in an *n*-heptane environment.

Lipase-catalyzed interesterification can be applied to the modification of fats and oils and thus to achieving the required composition of triacylglycerols mixtures. An example is the substitution of cacao butter that is an important raw material for the production of candies or production of triacylglycerols with concentrated oligounsaturated fatty acids.

The acylation of saccharides with fatty acids from vegetable oil catalyzed by lipases in a nonaqueous environment can be important for pharmaceutical and food industry as these enzymic reactions lead to the formation of surface-active compounds of biological origin.

The ability of lipases to synthesize peptides is used for the preparation of peptides containing unusual amino acids. Lipases can accept D-amino acids as substrate, these being constituents of

molecules of some antibiotics and neuroactive peptides. An example is the lipase-catalyzed formation of N-phenylacetyl-L-cysteinyl-D-valine, the precursor of penicillin G.

Optically active compounds formed by the action of some lipases can be used also for the production of various pharmaceuticals and pheromones.

The third group comprises phospholipases (A–D) cleaving ester bonds or, in the case of phospholipases C and D, the polar part of the molecule. The typical source of phospholipase A_2 is snake venom, although it was detected also in microorganisms (*Leptospira pomona*, *Bacillus cereus*, *Escherichia coli*). Phospholipase B_1 is typical of microorganisms; it was found in *E. coli* and its molar mass is about 60 kg/mol, although the molar mass of 29 kg/mol of this enzyme has also been reported. Phospholipase B_1 with molar mass of 26 kg/mol was isolated from *Bacillus megaterium*. The most important microbial phospholipase is phospholipase C which was prepared from *Clostridium perfringens* and *Bacillus cereus*. This enzyme has been reported from over 100 species of microorganisms including bacteria, moulds and yeasts. Data on the properties of phospholipases depend on the isolation methods and are rather varied, especially in terms of substrate specificity. An activity resembling that of phospholipase D was reported in *Corynebacterium pseudotuberculosis* and in many other microorganisms. In conclusion, industrial production and application of these enzymes have not started yet, although *e.g.* phospholipase C is being produced for experimental reasons.

The last group comprises enzymes with lipids as substrates. These are, above all, desaturases (catalyzing the formation of double bonds in carbon chains), hydrogenases, and various oxidases and peroxidases.

Although most of the enzymes can be purchased from companies as more or less pure preparations, their utilization in industry is virtually zero and has not left research laboratories where they serve as excellent tools for studying lipids.

6 Chemotaxonomy based on lipids

It is difficult to coordinate classical taxonomy with a chemosystematic application of data on fatty acids and lipids in general (Wassef 1977). It is attempted here, with some simplification as the relatively abundant exceptions are not generally included.

6.1 Myxomycota, Chytridiomycetes *and* Oomycetes

The finding that most genera produce α-linolenic and γ-linolenic acids, in contrast to other fungi, was striking. The presence of higher polyenoic acids (20:2, 20:3, 20:4 and 22:3) that are linked to the biosynthesis of γ-linolenic acid is not surprising. Besides these acids, the usual fatty acids have been detected in these fungi.

6.2 Ascomycetes *and* Deuteromycetes

Members of this group which includes also yeasts usually contain 16:0, 18:0 and 18:1 fatty acids. Polyenoic fatty acids are minor, with the exception of some *Saccharomyces* species which contain as much as 50 % palmitoleic acid. The representation of fatty acids differs significantly in the mycelium and in spores.

Ricinoleic acid, reported from green plants, was found in the genus *Claviceps* but its biosynthesis in *Claviceps* and in plant seeds differ.

6.3 Basidiomycetes

This group is very variable, its members produce both the usual C_{16} and C_{18} acids and linoleic acid. In the spores, acids with over 18 carbon atoms may be present.

Simple and complex lipids, such as triglycerides, glyco- and phospholipids, were detected. In yeasts, glycosphingolipids have been found that were reported to be the usual component of vertebrate

nervous tissue. The cultivation conditions have a great impact on the contents of lipids and fatty acids in these organisms.

Table VII. Chemosystematical classification of fungi by means fatty acids

Taxonomical groups		Type of fatty acids[a]
Myxomycota	$n-6$;	20:2, 20:3, 20:4
Oomycota	$n-3$;	20:5, 22:6
	$n-6$;	18:3, 20:0, 20:3, 20:4, 22:0, 22:3
Chytridomycota	$n-3$;	18:3, 18:4, 20:5
	$n-6$;	18:3, 20:4
Eumycota		
Zygomycetes		
Entomophthoraceae		14:0, 16:1, 18:1, 18:2, ω-18:3
Other orders		16:0, 18:2, α-18:3, not longer than C_{18}
Ascomycetes (including *Saccharomycetaceae*) and *Deuteromycetes*		no oligoenoic fatty acids 16:1, 18:2
Claviceps		hydroxy- and epoxy fatty acids, *e.g.* ricinoleic acid
Basidiomycetes		heterogenous contents, enormous variability, no ω-18:3

[a]Some characteristic examples. Without exception are present: 16:0, 18:1 and 18:2.

Bacterial fatty acids differ from those of higher organisms to a greater extent. Most bacteria contain $C_{10}-C_{20}$ fatty acids; those with $C_{14}-C_{19}$ predominate and can be divided into five groups.

First, there are saturated acids with a direct chain, second, those with a branched chain (the most frequent ones being *iso*- and *anteiso*-acids), third, monoenoic acids with, preferentially, *cis* isomerism at the double bond, fourth, acids with a cyclopropane ring (in *Mycobacteria* also with several rings), and finally, hydroxy acids with the hydroxy group in position 2 or 3 (*i.e.* α or β). Rarely, oligoenoic acids were reproted, above all in photosynthetizing prokaryotes (blue-green algae).

Gram-negative bacteria (with *E. coli* as a typical example) possess high contents of the usual fatty acids and also acids with an odd number of carbon atoms and a cyclopropane ring.

In contrast, Gram-positive bacteria contain high amounts of acids with an odd carbon number and mostly with a branched chain, *i.e. iso*- and *anteiso*-acids. A typical representative of this group is *Bacillus subtilis*. All the studied bacteria, and all organisms in general, contain palmitic acid.

Archaebacteria do not contain esterically linked fatty acids but derivatives of phytol and glycerol linked by ether bonds.

Fatty acids with less than 10 and more than 20 carbon atoms are rare in bacteria but very long fatty acids have been reported recently from a number of organisms (Řezanka 1989). Mycobacteria and related organisms form a very interesting group featuring the presence of mycolic and similar acids, *i.e.* acids with a carbon side chain in position 2 and a hydroxy group in position 3.

The great variability in fatty acids makes these compounds excellent chemotaxonomical markers. Processing of chemotaxonomic data is now aided by computer software and various statistical programs including the cluster analysis are routinely used.

7 Conclusion

Two directions can be foreseen for the future of microbial lipids. First, it is the production of oils enriched with essential oligoenoic fatty acids. Second, it might be the production of important lipolytic enzymes, especially by means of immobilized cells, *e.g.* production of triacylglycerols with oligounsaturated fatty acids.

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