BIOTRANSFORMATION OF OILS AND FATS: A REVIEW

YEONG SHOOT KIAN, SALMIAH AHMAD, OOI TIAN LYE and CHEAH SUAN CHOO*

Biotechnology is not something new. Since prehistoric times, humans have exploited microorganisms for their own use. By trial and error, they have developed the production of alcoholic beverages and food without knowing that microbes were the responsible agent. With the discovery of the existence of microorganisms, and the subsequent development of culture methods, came the birth of modern biological technology or in short, biotechnology (Steele et al., *1991).*

The term biotechnology has a very broad meaning. It could mean genetic manipulation of mammalian, plant or microbial cells to the use of microorganisms to aid a process. One of the aspects of biotechnology is biotransformation. Biotransformation could be defined as the use of biocatalyst to convert a raw material into a value-added product. The choice of biocatalyst is between isolated enzymes or microbial whole cells. In cases where the biotransformation is a one-step reaction and non-cofactor requiring enzyme is available, then an isolated enzyme often immobilized onto a support is generally the most efficient biocatalyst. An excellent example is the use of lipases for ester synthesis (Eigtved et al., *1988; Lazar* et al., *1986 & Staal, 1991). In other instances where the biotransformation is a*

* Palm Oil Research Institute of Malaysia
P. O. Box 10620, 50720 Kuala Lumpur.

relatively complex multistep reaction, especially when these enzymatic steps require cofactors, then the only possible approach is to employ a living biocatalyst, usually genetically modified microbial cells (Casey and Macrae, 1992).

One of the main advantages of biotransformations is their well-known enantioselectivity. Although chemical reactions with optically active catalysts or auxiliaries could be used successfully, enzymatic reactions are often more economical (Kieslich, 1992).

Cheap raw materials such as glucose, complex carbohydrates like starch, molasses or even waste waters are among the favourite substrates for biotransformation (Buhler and Wandrey, 1992). However a number of interesting novel value-added products can also be derived from oils and fats. These products may find new industrial applications.

PRODUCTION OF OLEOCHEMICALS

Oleochemicals are chemicals that can be derived from renewable resources such as vegetable oils and animal tallow. The oleochemical industry is centred around the chemical modification of natural triglycerides from plants or animals to give products having a wide range of applications. The majority of these chemical modifications are performed at the carboxylic group (approx. 96%) with others at the mid-chain unsaturation centres of the fatty acid moieties (Casey and Macrae, 1992; Wandrey, 1990 & Zoebelein, 1992). These reactions could be catalyzed by a chemical catalyst or biocatalyst.

Enzymatic reactions at the carboxylic end have been extensively studied by a number of researchers. Examples of reactions at the carboxylic end of free fatty acids are esterification, amination and reduction while in the case of oils and fats, hydrolysis and interesterification. These reactions are usually catalyzed by immobilized enzymes.

Reactions at the hydrocarbon chain are usually more difficult to effect and require co-factors. Therefore microbial whole cells are usually employed to bring about transformations in the hydrocarbon chain. In this paper we would like to focus on those types of reactions that use oils and fats or their derivatives as raw materials. *Figure 1* shows the possible types of reactions that could be catalyzed by a biocatalyst (Werdelmann and Schmid, 1982).

DESATURATION OF FATTY ACIDS

Desaturation of a saturated fatty acid or its derivatives may mean an addition of an important functionality to the oils especially palm and coconut oils which contain quite a high percentage of saturated fatty acids. Palm oil consists of 50% of saturated fatty acids in its fatty acid composition while coconut oil contains 90%. Desaturation *via* chemical method is often difficult and non-stereospecific. Therefore desaturation through biotechnological method seemed to be the next possible alternative. A number of microorganisms that demonstrated desaturation activities is discussed in this paper.

Yeast has been reported to be able to desaturate saturated fatty acids. An oleaginous yeast *Rhodotorula glutinis* IIP-30 isolated from hydrocarbon-contaminated soil was grown on coconut and groundnut oils. It was found to be able to increase the unsaturated fatty acid composition in coconut oil from 9.5% to 68.2%. However, the fatty acid compositions of groundnut oil was not altered

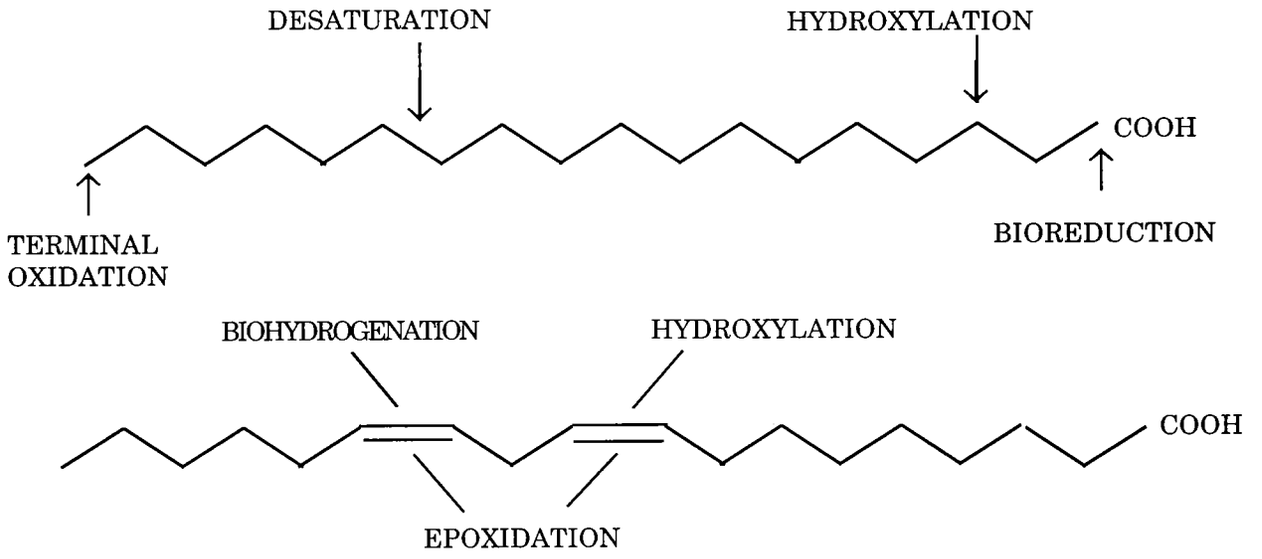


Figure 1. Biotransformations of fatty acids. (Note that the reaction types are not all limited to the positions indicated.)

(Von Johnson *et al.*, 1992).

Another yeast, *Candida lipolytica* YB 423-12 was grown on rapeseed oil, soapstock and palm stearin. The microorganism greatly absorbed the fatty acids and modified their compositions. In all the cases, the unsaturated fatty acid content of the cell oils was greater than that of the substrates. The increases were respectively 5% for rapeseed oil, 9.4% for soapstock and 22.8% for palm oil stearin (Montet *et al.*, 1985).

Another group of unsaturated acid-producing microorganism is bacteria. *Rhodococcus* sp. KSM-B-3M was isolated by a Japanese group. The microorganism was cultured in medium containing a saturated fatty acid or its derivative. *Table 1* shows the results of some of the production of unsaturated fatty esters (Kimura *et al.*, 1991).

Rhodococcus sp. KSM-B-MT66, a mutant strain obtained by treatment of *Rhodococcus*

TABLE 1. PRODUCTION OF UNSATURATED FATTY ACID OR DERIVATIVES BY *RHODOCOCCUS SP.*

Substrate[a]	Product	Amount (g/L)
Sodium palmitate	cis-Hexadecenoic acid	0.03
Methyl palmitate	Methyl cis-hexadecenoate	0.07
Propyl palmitate	Propyl cis-hexadecenoate	2.10
Isopropyl palmitate	Isopropyl-cis-hexadecenoate	8.10

[a] Amount of substrate was 1 ml/L of culture broth.

sp. KSM-B-3, was able to *cis*-desaturate a variety of long chain alkanes, haloalkanes and acyl fatty acids at the central position of the chain. Hexadecane was desaturated to *cis*-7-hexadecene, chloroalkanes were converted to *n*-chloro-*cis*-9-alkenes, acyl hexadecanoates were converted to acyl-*cis*-6-hexadecenoates (Takeuchi *et al.*, 1990).

Gamma-linolenic acid (GLA; C18:3γ) is an unsaturated fatty acid of the ω-6 series. It is used for cosmetic and pharmaceutical applications. GLA can be derived from plant sources. Some of the GLA-producing plants are evening primrose (8% GLA), blackcurrant (17% GLA), gooseberry (11% GLA) and borage (23% GLA). GLA can also be derived from unicellular microorganisms. For example, the production of GLA by *Mucor javanicus* has been commercialized. The fungal oil contained 11% of GLA (Ratledge, 1987). Another *Mucor* strain, *Mucor circillenoides* CBS 172-27 was able to accumulate GLA at a higher percentage, 17.4% GLA in its mycelium when cultured in a linoleic acid-rich medium like sunflower oil that contained 65.5% of C18:2 (Aggelis *et al.*, 1992).

Polyunsaturated fatty acids (PUFAs) such as arachidonic acid (ARA) and eicosapentaenoic acid (EPA) are used in the human body for the biosynthesis of eicosanoid hormones *viz* protaglandins, thromboxanes and leukotrienes. To date the major source of PUFAs has been fish oil (Radwan, 1991).

Within the past decade, much work has been done to look into the possibilities of producing PUFAs from microbial sources. *Mortierella* sp. was reported to be one of the potent producers of PUFAs. *Mortierella alpina* 1S-4 grown in the presence of linolenic acid-rich medium like linseed oil has been found to be able to accumulate EPA (5,8,11,14,17-*cis*-eicosapentaenoic acid; C20:5ω3) up to 1.88 mg/ml of culture broth or 66.6 mg/g of dry mycelia (Shimizu *et al.*, 1989 & 1989a). Mutants of *Mortierella alpina* 1S-4, which was obtained by treating the wild type with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine were found to be desaturase defective at certain positions and gave different types of PUFAs. Mut44 was found to have low Δ-5 desaturase activities. It accumulated a high level of dihomo-γ-linolenic acid (DGLA, 8,11,14-*cis*-eicosatrienoic acid; C20:3ω6) at 28.6% w/w in its mycelia but a low level of arachidonic acid (5,8,11,14-*cis*-eicosatetraenoic acid; C20:4ω6) (10.6%) compared to wild type, which had levels of 6.3% and 47%, respectively. Mut48, on the other hand was found to be Δ-12 desaturase defective. Therefore the accumulation of fatty acid in the ω-6 series was not detected. Instead some of the ω-9 series fatty acids such as Mead acid (5,8,11-*cis*-eicosatrienoic acid; C20:3ω9), 6,9-*cis*-octadecadienoic acid (C18:2ω9) and 8,11-*cis*-eicosadienoic acid (C20:2ω9) were detected in its mycelia (Jareonkitmongkol *et al.*, 1992a). The accumulation of Mead acid by this particular fungal source is exceptionally high. Under optimum conditions, the fungus produced *ca.* 0.8 g of MEAD acid per litre of culture broth which is equivalent to 15% w/w of the total mycelial fatty acids. An addition of 2% (v/v) of vegetable oils like coconut, olive and camellia seemed to increase the production of Mead acid to about three times of that without the oil supplement (Jareonkitmongkol *et al.*, 1992).

KETONES

Some microorganisms have shown the ability to convert triglycerides or their fatty acids into ketones. These microorganisms were found to be able to accumulate 2-ketones (also known as methyl alkyl ketones), which have one carbon atom less than their substrates in the culture medium. So far the accumulation of 2-ketones having more than 15 carbon atoms has not been detected. 2-Alcohols and hydroxy ketones were sometimes detected as minor by-products.

Table 2 shows the accumulation of 2-ketones by various microorganisms (Kranz *et al.*, 1992; Yagi *et al.*, 1989; 1991 & 1991a).

TABLE 2 ACCUMULATION OF 2-KETONES BY VARIOUS MICROORGANISMS

Microorganism	Substrate	Product	Yield[d]	Reference
Trichoderma sp. SM-30	Tricaproin[a]	2-Pentanone	60	Yagi *et al.*, 1989
	Tricaprylin[a]	2-Heptanone	100	
	Tricaprin[a]	2-Nonanone	200	
	Palm kernel oil[a]	2-Heptanone	4	
		2-Nonanone	5	
		2-Undecanone	29	
		2-Tridecanone	1	
Penicillium decumbens IFO 7091	Palm kernel oil[b]	2-Heptanone	225	Yagi *et al.*, 1990
		2-Nonanone	248	
		2-Undercanone	1556	
		2-Tridecanone	25	
Aureobasidium sp. SM-25	Tricaproin[a]	2-Pentanone	160	Yagi *et al.*, 1991
	Tricaprylin[a]	2-Heptanone	2.3	
	Tricaprin[a]	2-Nonanone	0.9	
	Palm kernel oil[a]	2-Heptanone	2.4	
		2-Nonanone	2.2	
		2-Undecanone	6.8	
Fusarium avenaceum f. sp. *fabae* IFO 7158	Tricaproin[a]	2-Pentanone	10	Yagi *et al.*, 1991[a]
	Tricaprylin[a]	2-Heptanone	102	
	Tricaprin[a]	2-Nonanone	106	
Monascus pupureus DSM 1379	Caproic acid[c]	2-Pentanone	100	Kranz *et al.*, 1992
	Caprylic acid[c]	2-Heptanone	80	
	Capric acid[c]	2-Nonanone	47	
	Lauric acid[c]	2-Undecanone	5	

[a]Carbon source 1g/100ml.
[b]Carbon source 10g/100ml.
[c]Substrate concentration 1.5 mM.
[d]Yield in mg/100ml except for *M. purpureus*, where the formation of methyl ketone in percent relative to the formation of 2-pentanone which is regarded as 100%.

HYDROXY FATTY ACIDS

Chemical treatment of oleic acid with sulphuric acid and subsequent hydrolysis leads to mixtures of 9- and 10-hydroxystearic acids. Such hydroxystearic acids and the corresponding keto-fatty acids are useful as lubricants; as surfactants; as plasticizer; as components in detergent; in coating and paint industries and in the synthesis of resins (El-Sharkawy *et al.*, 1992). Presently the only commercial source of hydroxy fatty acids is ricinoleic acid from castor oil.

Microbiological conversion of oleic acid to hydroxy fatty acid by the Pseudomonads was reported in the 60s (Schroepfer, 1965 & 1966; Wallen *et al.*, 1962). The conversion of oleic acid to 10-hydroxystearic acid (10-HSA) was observed by Wallen *et al.* to reach a 14% yield (Wallen *et al.*, 1962). 10-HSA produced by *Pseudomonas* sp. was found to be stereospecific, the hydroxy group having the *D*-configuration (Schroepfer, 1965 & 1966). *Corynebacterium* sp. S-401, which was reported to be able to catalyze the hydration of squalene, also converted oleic acid to 10-ketostearic acid[1] (10-KSA) and 10-HSA with 22.4 and 9.1% yields, respectively (Seo *et al.*, 1981). These findings indicate the possibility of producing hydroxy fatty acid, which fetches a premium price compared to oleic acid, *via* microbiological processes. Oleic acid is abundant in most vegetable oils such as olive, rape seed and palm oils.

In 1986, a patent was granted to Litchfield and Pierce for the discovery of the conversion of unsaturated fatty acids by *Rhodococcus rhodochrous* ATCC 12674 to hydroxy- and keto-fatty acids. *R. rhodochrous* is also known as *Norcadia aurantia*. Two types of unsaturated fatty acids were used as substrates. They were oleic and linoleic acids. When oleic acid was used as substrate, 55.1% of 10-HSA was produced and when linoleic acid was used as substrate, 22.2% of 10-hydroxy-12-octadecenoic acid was produced. Together with the hydroxy fatty acids, keto-acids were also produced. They were 10-KSA from oleic acid and 10-keto-12-octadecenoic acids from linolenic acid (Litchfield and Pierce, 1986).

Another microbial isolates, *Norcadia cholesterolicum* NRRL 5767 was reported to be able to produce similar types of hydroxy fatty acids when cultured on unsaturated fatty acids. Resting cell suspensions of *N. cholesterolicum* converted oleic acid to 10-HSA, linoleic acid to 10-hydroxy-12-octadecenoic acid and linolenic acid to 10-hydroxy-12,15-octadecadienoic acid. Under optimum culture conditions the conversions were 75-80%. Minor amounts of 10-KSA were formed as a by-product (Koritala *et al.*, 1989 & 1992). Conversion of oleic acid to 10-HSA was also observed in yeasts. Resting cells of *Saccharomyces cerevisiae* converted oleic acid into 10-HSA in 45% yield (El-Sharkawy *et al.*, 1992).

In contrast, a growing culture of *Staphylococcus* sp. seemed to produce 10-KSA as the major product from oleic acid. The yield of 10-KSA achieved 90% while 10-HSA was only 5% of the 10-KSA produced (Lancer, 1993). Similarly *Flavobacterium* sp. strain DS5 (NRRL B-14859) also produced 10-KSA (85%) as the major product from oleic acid and 10-HSA as minor product (10% of 10-KSA)(Hou, 1994).

The conversion of oleic acid to 10-KSA is likely to be *via* the 10-HSA (Hou, 1994). *Figure 2* is a schematic diagram of the proposed pathway. Initial hydration of the olefinic bond of the oleic acid would afford 10-HSA, and an enzymatic dehydrogenation by alcohol dehydrogenase would afford 10-KSA. The nature of oleic acid metabolites formed during the microbial transformation reaction was highly dependent upon biotransformation conditions. When the concentration of oxygen in the culture is reduced, the formation of 10-HSA would be higher (El-Sharkawy *et al.*, 1992). Conversely, when the contribution of oxygen in the culture is increased, the formation of 10-KSA would be higher.

In the case of *Micrococcus luteus* BL0-3, a new product, 4-ketolauric acid was observed in addition to 10-HSA and 10-KSA. 4-Ketolauric was presumably produced after three cycles of β-oxidation of oleic acid or its

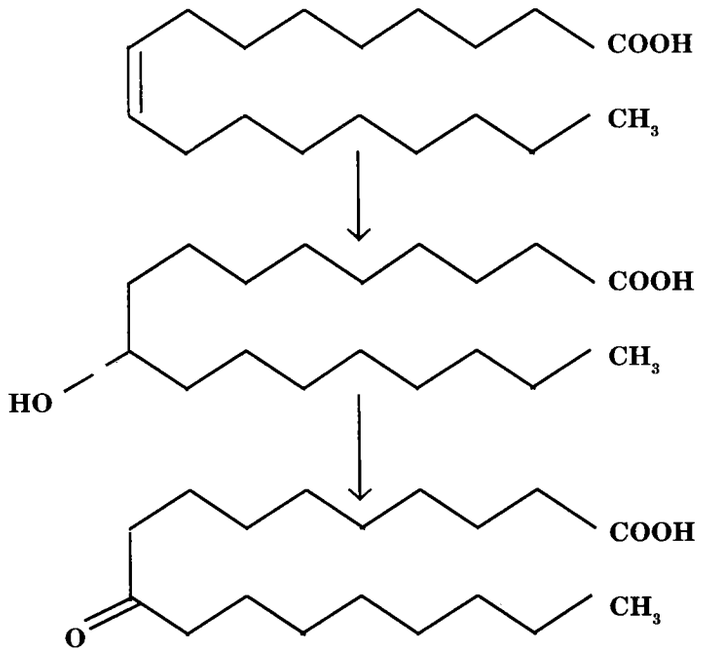


Figure 2. Proposed pathway of microbial tranformation of oleic acid to 10-HSA and 10-KSA.

derivatives in the *M. luteus* cells (Esaki *et al.*, 1994). In the transformation of oleic acid and its esters by *Sarcina lutea*, 1,7-heptanedicarboxylic acid was also detected. 1,7-heptanedicarboxylic acid was probably produced from oleic acid by a fission at the double bond with subsequent oxidation of an aldehyde or other derivatives. The detailed transformation pathway is being investigated (Blank *et al.*, 1991).

The conversions of unsaturated fatty acids into dihydroxy fatty acids were also observed in other microorganisms. *Pseudomonas* sp. 42A2, when cultivated in a mineral salt medium with olive oil as a sole carbon source; produced a new surface active compound which was later identified to be dihydroxyoctadecenoic acid (Mercade *et al.*, 1988). The bioconversion of another *Pseudomonas* sp. afforded 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD) from oleic acid. The maximal yield of DOD was 72% (Hou *et al.*, 1991; Hou and Bagby, 1991). The bacterium was later identified as *P. aeruginosa* and has been deposited in the ARS Culture Collection as *P. aeruginosa* NRRL B-18602 (Hou *et al.*, 1993)

ω-HYDROXY FATTY ACIDS

Cell-free preparations of *Bacillus megaterium* in the presence of NADPH and oxygen, catalyzed the hydroxylation of saturated long-chain fatty acids (Miura and Fulco, 1974), fatty amides and fatty alcohols to a mixture of ω-2, ω-3 and ω-4 monohydroxy[2] isomers.

Several monounsaturated fatty acids were also tested as substrates. They were *cis*-9-hexadecenoate, *cis*-9-octadecenoate and *cis*-12-octadecenoate (Miura and Fulco, 1975). The distribution of the three monohydroxy isomeric products varied from one substrate to another but generally hydroxylation at the ω-3 position

was favoured (Miura and Fulco, 1974). For example, when palmitic acid was used as a substrate, the major products were 15- (30%; ω-2), 14- (50%; ω-3) and 13- (20%; ω-4) hydroxypalmitates.

When palmitoleic acid was used as substrate, two other products; 9,10-epoxypalmitate and 9,10-dihydroxypalmitate were also identified as reaction products in addition to the expected isomeric mixture of monohydroxypalmitates (Buchanan and Fulco, 1978). It was later found that epoxidation and hydroxylation were catalyzed by the same cytochrome P-450 monooxygenase complex (Ruettiger and Fulco, 1981).

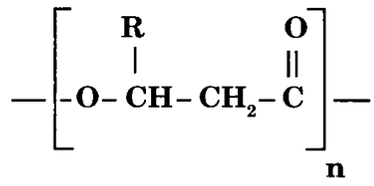
Another *Bacillus* strain, *Bacillus pumilus*, also showed hydroxylation capability. Two of the *B. pumilus strains*, BD-174 and BD-226 hydroxylated oleic acid to produce 15-, 16- and 17-hydroxy-9-octadecenoic acids (Lancer *et al.*, 1992).

MICROBIAL POLYMERS

Many bacteria are able to accumulate intracellular reserve materials varying from internal *n*-alkane pools to polyphosphate. Most common is poly-3-hydroxybutyric acid or poly-3-hydroxybutanoate (PHB). A large amount of PHB is accumulated when cells are grown under nitrogen limiting conditions and in the presence of excess carbon source (Huisman *et al.*, 1991; Lageveen *et al.*, 1988). The accumulation of PHB may amount to 80% of the cell dry weight (Lageveen *et al.*, 1988). PHB possesses the properties of a biodegradable thermoplastic. The first industrial production of PHB (poly-3-hydroxybutanoate) and PHA (poly-3-hydroxyalkanoate) did not happen until 1982 when ICI marketed them under the trade name Biopol (Anderson and Dawes, 1990).

PHB can be used to manufacture bottles and films like conventional oil-based thermoplastic. Besides applications as packaging material, it can also be used as surgical sutures, as a biodegradable matrix for drug release or for the manufacture of devices for bone replacement (Valentin *et al.*, 1992).

Most of the known polyhydroxyalkanoates are polymers of 3-hydroxyacids having the following general formula (*Figure 3*).



R = H
= alkyl or alkene group
= branched alkyl or alkene group
n = 4 000 to 10 000

Figure 3. General formula of poly-3-hydroxyalkanoates or poly-3 hydroxyalkenoates.

The bulk of the published research on PHAs and PHB has been concentrated on two bacteria, *Alcaligenes eutrophus* and *Pseudomonas oleovorans*. Other bacteria that also produce PHAs are *Rhodospirillum rubum*, *Halobacterium mediterranei*, *Rhodococcus* sp., *Corybacterium* sp., *Nocardia* sp. *etc.* The accumulation of polyesters could be in the form of homopolymer or co-polymer (Anderson and Dawes, 1990).

Two types of polyesters that are commonly synthesized are short-chain-length PHAs (SCL-PHAs) and medium-chain-length PHAs (MCL-PHAs). SCL-PHAs are made up of monomer units of 3 to 5 carbon atoms while MCL PHAs are made up of monomer units of 6-14 carbon atoms. *Alcaligenes eutrophus*, *Rhodospirillum rubum* and *Pseudomonas pseudoflava* are known to accumulate polyesters composed of SCL monomer units only while *Pseudomonas putida* and other fluorescent *Pseudomonas* strains principally MCL PHAs (Choi and Yoon, 1994). For example, *Alcaligenes* sp. when grown on a wide range of substrates such as *n*-alkanoic acids from C_2 to C_{22}, plant oils and animal fats accumulated only 3-HB and 3-HV (3-hydroxyvalerate) (Haywood *et al.*, 1989). On the other hand, fluorescent Pseudomonads such as *Pseudomonas aeruginosa*, *P. putida* and *P. fluorescens* accumulated only MCL-PHAs with 6-12 carbon atoms when grown on *n*-alkanoic acid (C_4-C_{18}) and unsaturated fatty acids (Huisman *et al.*, 1989).

CONCLUSIONS

Many microorganisms have shown their abilities to produce novel fatty acids that may find new industrial applications or higher value-added fatty acids. Eventhough some of these microorganisms gave very promising yields of such fatty acids and the production of such microbial oils has reached pilot scale, the successful commercialization of these processes would need further development work. The future prospects for microbial oils might lie in three possible areas: (1) as substitutes for the existing high value plant oils, (2) as novel materials that are not available from other sources, and (3) as a saleable end-product from waste processing (Ratledge and Boulton, 1985). The success would also depend very much on the collaboration between microbiologists, chemists, genetic engineers and biochemical engineers. First the microbiologists would screen for possible new strains. Then the chemists would help to identify potentially useful material. Once the strain and the product have been identified, the genetic engineers would improve on the strain to increase the yield of the desired product. Lastly the biochemical engineer would design the bioreactor to give the maximal yield.

NOTES

[1]According to IUPAC nomenclature, it is also known as 10-oxo-stearic acid.

[2]In the original publication, the author defined the terminal methyl carbon as ω-carbon and the methylene carbon adjacent to the methyl group as ω-1 and the subsequent carbons as ω-2 and ω-3. However, we would like to follow Christie (1982) and name terminal methylene carbon as ω-1 and the subsequent carbons as ω-2 and ω-3 and so on.

ABBREVIATIONS:

GLA = *gamma* linolenic acid

PUFA = polyunsaturated fatty acid

ARA = arachidonic acid

EPA = eicosapentaenoic acid

HSA = hydroxystearic acid

KSA = ketostearic acid

PHB = polyhydroxybutanoate

PHA = polyhydroxyalkanoate

SCL = short chain length

MCL = medium chain length

HB = hydroxybutanoate

HV = hydroxyvalerate

REFERENCES

AGGELIS, G; KOMAITIS, M E; DIMITROULIAS, G; PINA, M and GRAILLE, J (1992). Possibilité de production d'acide gamma linolénique par culture de *Mucor circinelloïdes* CBS 172-27 sur quelques huiles végétales. *Oléagineux 47*: 49-53.

ANDERSON, A J and DAWES, E A (1990). Occurrence, metabolism, metabolic role and industrial uses of bacterial polyhydroxyalkanoates. *Microbiol. Rev. 54*: 450-472.

BLANK, W; TAKAYANAGI, H; KIDO, T; MEUSSDOERFFER, F; ESAKI, N and SODA, K (1991). Transformation of oleic acid and its esters by *Sarcina lutea*. *Agric. Biol. Chem. 55*: 2651-2652.

BUCHANAN, J F and FULCO A J (1978). Formation of 9,10-epoxypalmitate and 9,10-dihydroxypalmitate from palmitoleic acid by a soluble system from *Bacillus megaterium*. *Biochem. Biophys. Res. Comm. 85*: 1254-1260.

BUHLER, M and WANDREY, C (1992). Oleochemicals by biochemical reactions?, *Fat Sci. Technol. 94*: 82-94.

CASEY, J and MACRAE, A (1992). Biotechnology and the oleochemical industry. *Inform 3*: 203-207.

CHOI, M H and YOON, S C (1994). Polyester biosynthesis characteristics of *Pseudomonas citronellolis* grown on various carbon sources, including 3-methyl-branched substrates. *Appl. Environ. Microbiol. 60*: 3245-3254.

CHRISTIE, W W (1982). The structure, chemistry and occurrence of lipids in Lipid Analysis, 2nd ed., Pergamon Press, GB, p. 3.

EIGTVED, P; HANSEN, T T and MILLER C A (1988). Ester synthesis with immobilized lipases. In: TH Applewhite (ed). *Proceedings of World Conference on Biotechnology for the Fats and Oils Industry,* edited by T H Applewhite, Illinois, pp. 134-137.

EL-SHARKAWY, S H; YANG, W; DOSTAL, L and ROSAZZA, P N (1992). Microbial oxidation of oleic acid. *Appl. Environ. Microbiol. 58*: 2116-2122

ESAKI, N; ITO, S; BLANK, W and SODA, K (1994). Biotransformation of oleic acid by *Micrococcus luteus* cells. *Biosci. Biotech. Biochem. 58*: 319-321.

HAYWOOD, G W; ANDERSON, A J and DAWES, E A (1989). A survey of the accumulation of novel polyhydroxyalkanoates by bacteria. *Biotechnol. Lett. 11*: 471-476.

HOU, C T (1994). Production of 10-ketostearic acid from oleic acid by *Flavobacterium* sp. strain DS5 (NRRL B-14859). *Appl. Environ. Microbiol. 60*: 3760-3763.

HOU, C T; BAGBY, M O; PLATTNER, R D and KORITALA, S (1991). A novel compound, 7,10-dihydroxy-8-(*E*)-octadecenoic acid from oleic acid by bioconversion. *J. Am. Oil Chem. Soc. 68*: 99-101.

HOU, C T and BAGBY, M O (1991). Microbial production of a novel compound, 7,10-dihydroxy-8-octadecenoic acid from oleic acid. *PCT/US91/01806.*

HOU, C T; NAKAMURA, L K; WEISLEDER, D; PETERSON R E and BAGBY, M O (1993). Identification of NRRL strain B-18602 (PR3) as *Pseudomonas aeruginosa* and effect of phenazine 1-carboxylic acid formation on 7,10-dihydroxy-8(*E*)-octadecenoic acid accumulation. *W. J. Microbiol. Biotechnol. 9*: 570-573.

HUISMAN, G W; De LEEUW, O; EGGINK, G and WITHOLT, B (1989). Synthesis of poly-3-hydroxyalkanoates is a common feature of fluorescent Pseudomonads. *Appl. Environ. Microbiol. 55*: 1949-1954.

HUISMAN, G W; WONINK, E; MEIMA, R; KAZEMIER, B; TERPSTRA, P and WITHOLT, B (1991). Metabolism of poly(3-hydroxyalkanoates)(PHAs) by *Pseudomonas oleovorans*. *J. Biol. Chem. 266*: 2191-2198.

JAREONKITMONGKOL, S; KAWASHIMA, H; SHIMIZU, S and YAMADA, H (1992). Production of 5,8,11-*cis*-eicosatrienoic acid by a Δ-12-desaturase defective mutant of *Mortierella alpina* 1S-4. *J. Am. Oil Chem. Soc. 69*: 939-944.

JAREONKITMONGKOL, S; SHIMIZU, S and YAMADA, H (1992a). Fatty acid desaturation-defective mutants of an arachidonic-acid-producing fungus, *Mortierella alpina* 1S-4. *J. Gen. Microbiol. 138*: 997-1002.

KIESLICH, K (1992). Aspects for the future of biotransformations. *W. J. Microbiol. and Biotechnol. 8 suppl. 1*: 67-71.

KIMURA, Y; ADACHI, S and ARA, K (1991). Microorganism belonging to genus *Rhodococcus* and a process for producing alkene derivative and unsaturated fatty acid. *US Patent No. 5,059,532.*

KORITALA, S; HOSIE, L; HOU, C T, HESSELTINE, C W and BAGBY, M O (1989). Microbial conversion of oleic acid to 10-hydroxystearic acid. *Appl. Microbiol. Biotechnol. 32*: 299-304.

KORITALA, S and BAGBY, M O (1992). Microbial conversion of linoleic and linolenic acids to unsaturated hydroxy fatty acids. *J. Am. Oil Chem. Soc. 69*: 575-578.

KRANZ, C; PANITZ, C and KUNZ, B (1992). Biotransformation of free fatty acids in mixtures to methyl ketones by *Monascus purpureus. Appl. Microbiol. Biotechnol. 36*: 436-439.

LAGEVEEN, R G; HUISMAN, G W; PREUSTING, H; KETELAAR, P; EGGINK, G and WITHOLT, B (1988). Formation of polyesters by *Pseudomonas oleovorans:* Effect on substrates on formation and composition of poly-(*R*)-3-hydroxyalkanoates. *Appl. Environ. Microbiol. 54*: 2924-2932.

LANCER, A C; PLATTNER, R D and BAGBY, M O (1992). Production of 15-, 16- and 17-hydroxy-9-octadecenoic acids by bioconversion of oleic acid with *Bacillus pumilus. J. Am. Oil Chem. Soc. 69*: 363-366.

LANCER, A C (1993). Conversion of oleic acid to 10-ketostearic acid by a *Staphylococcus* species. *J. Am. Oil Chem. Soc. 70*: 543-545.

LAZAR, G; WEISS, A and SCHMID, R D (1986). Synthesis of esters by lipases. *In: A R Baldwin (ed). Proceedings of World Conference on Emerging Technologies in the Fats and Oils Industry,* edited by A. R. Baldwin, AOCS, USA, pp. 346-354.

LITCHFIELD, J H and PIERCE, G E (1986). Microbiological synthesis of hydroxy-fatty acids and keto-acids. *US Patent 4,582,804.*

MERCADE, E; ROBERT, M; ESPUNY, M J; BOSCH, M P; MANRESA, M A; PARRA, J L and GUINEA, J (1988). New surfactant isolated from Pseudomonas 42A2. *J. Am. Oil Chem. Soc. 65*: 1915-1916.

MIURA, Y and FULCO, A J (1975). ω-1, ω-2 and ω-3 Hydroxylation of long-chain fatty acids, amides and alcohols by a soluble enzyme system from *Bacillus megaterium. Biochim. Biophys. Acta 388*: 305-317.

MIURA, Y and FULCO, A J (1974). (ω-2) Hydroxylation of fatty acids by a soluble system from *Bacillus megaterium. J. Biol. Chem. 249*: 1880-1888.

MONTET, D; RATOMAHENINA, R and GALZY, P (1985). A study of the influence of the growth media on the fatty acid composition in *Candida lipolytica* diddens and lodder. *Biotechnol. Lett. 7*: 733-736.

RADWAN, S S (1991). Sources of C_{20}-polyunsaturated fatty acids for biotechnological use. *Appl. Microbiol. Biotechnol. 35*: 421-430.

RATLEDGE, C (1987). Lipid biotechnology: A wonderland for the microbial physiologist. *J. Am. Oil Chem. Soc. 64*: 1647-1656.

RATLEDGE, C and BOULTON, C A (1985). *In: Moo-Young M (Ed-in-chief).* Comprehensive Biotechnology, Vol. 3, Chap. 48: Fats and oils, Ed-in-chief Moo-Young, M, Pergamon Press, GB, pp. 983-1003.

RUETTINGER, R T and FULCO A J (1981). Epoxidation of unsaturated fatty acids by a soluble cytochrome P-450-dependent system from *Bacillus megaterium. J. Biol. Chem. 256*: 5728-5734.

SCHROEPFER, G J Jr. (1965). Enzymatic stereospecificity in the conversion of oleic acid to 10-hydroxystearic acid. *J. Am. Oil Chem Soc. 87*: 1411-1412.

SCHROEPFER, G J Jr. (1966). Stereospecific conversion of oleic acid to 10-hydroxystearic acid. *J. Biol. Chem. 241*: 5441-5447.

SEO, C W; YAMADA, Y; TAKADA, N and OKADA, H (1981). Hydration of squalene and oleic acid by *Corynebacterium* sp. S-401. *Agric. Biol. Chem. 45*: 2025-2030.

SHIMIZU, S; KAWASHIMA, H; AKIMOTO, K; SHINMEN, Y and YAMADA, H (1989). Microbial conversion of an oil containing α-linolenic acid to an oil containing eicosapentaenoic acid. *J. Am. Oil Chem. Soc. 66*: 342-347.

SHIMIZU, S; KAWASHIMA, H; AKIMOTO, K; SHINMEN, Y and YAMADA H (1989a). Conversion of linseed oil to an eicosapentaenoic acid containing oil by *Mortierella alpina* 1S-4 at low temperature. *Appl. Microbiol. Biotechnol. 32*: 1-4.

STAAL, L OH (1991). To esters *via* biotechnology.*In: T H Appwhite (ed). Proceedings of World Conference on Oleochemical into the 21st Century*, edited by T H Applewhite, Illinois, pp 279-283.

STEELE, D B and STOWERS, M D (1991). Techniques for selection of industrially important microorganisms. *Annu. Rev. Microbiol. 45*: 89-106.

TAKEUCHI, K; KOIKE, K and ITO, S (1990). Production of *cis*-unsaturated hydrocarbons by a strain of *Rhodococcus* in repeated batch culture with a phase-inversion, hollow-fiber system. *J. Biotechnol. 14*: 179-186.

VALENTIN, H E; SCHONEBAUM, A and STEINBUCHEL, A (1992). Identification of 4-hydroxyvaleric acid as a constituent of biosynthetic polyhydroxyalkanoic acids from bacteria. *Appl. Microbiol. Biotechnol. 36*: 507-514.

VON JOHNSON; SINGH, M and YADAV, N K (1992). Transformation of vegetable oils by an oleaginous yeast: *Rhodotorula glutinis* IIP-30. *Biotechnol. Lett. 14*: 801-804.

WALLEN, L L; BENEDICT, R G and JACKSON, R W (1962). The microbiological production of 10-hydroxystearic acid from oleic acid. *Arch. Biochem. Biophy. 99*: 249-253.

WANDREY, C (1990). Biochemical engineering for oleochemicals. *In: T H Appwhite (ed). Proceedings of World Conference on Oleochemicals into the 21st Century.* edited by T H Applewhite, AOCS, Illinois, pp. 194-210.

WERDELMANN, B W and SCHMID, R D (1982). The biotechnology of fats a challenge and an opportunity. *Fette Seifen Anstrichnittel 84*: 436-443.

YAGI, T; HATANO, A; KAWAGUCHI, M; HATANO, T; FUKUI, F and FUKUI, S (1990). Methylalkylketone fermentation from palm-kernel oil by *Penicillium decumbens* IFO 7091. *J. Ferment. Bioeng. 70*: 100-103.

YAGI, T; KAWAGUCHI, M; HATANO, T; FUKUI, F and FUKUI, S (1989). Production of ketoalkanes from fatty acid esters by a fungus, *Trichoderma. J. Ferment. Bioeng. 68*: 188-192.

YAGI, T; KAWAGUCHI, M; HATANO, T; HATANO, A; NAKANISHI, T; FUKUI, F and FUKUI, S (1991). Formation of *n*-alkane-2-ones and *n*-alkane-2-ols from triglycerides by a black yeast, *Aureobasidium. J. Ferment. Bioeng. 71*: 93-99.

YAGI, T; HATANO, A; HATANO, T; FUKUI, F and FUKUI, S (1991a). Formation of monohydroxy-*n*-nonane-2-ones from tricaprin by *Fusarium avenaceum* f. sp. *fabae* IFO 7158. *J. Ferment. Bioeng. 71*: 176-180.

ZOEBELEIN, H (1992). Renewable resources for the chemical industry. *Inform 3*: 721-725.