FUNGAL LIPID METABOLISM

KÜFLERDE YAĞ METABOLİZMASI

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ABSTRACT: Present biological research has concentrated on the commercial exploitation of microorganisms. Among the diverse range of microbial products with potential economic value, lipids and lipid related compounds are attracting considerable attention. The focus should rather be on the more expensive lipid products with medical applications, including certain polyunsaturated fatty acids as well as hydroxy polyunsaturated fatty acids, prostaglandins, thromboxanes and leukotrienes.

Key words: *Fungi, lipid, polyunsaturated fatty acids*

ÖZET: Günümüz biyolojik araştırmaları, mikroorganizmaların ticari amaçlarla kullanılabilmesi üzerinde yoğunlaşmıştır. Potansiyel anlamda ekonomik değeri olan birçok mikrobiyel ürün arasında, lipidler ve lipid benzeri bileşikler önemli bir yer tutmaktadır. Yapılan çalışmalar daha çok tıbbi uygulaması olan pahalı lipid bileşikleri üzerinde olmalıdır ki, bunlar bazı doymamış yağ asitlerinin yanı sıra hidroksi doymamış yağ asitleri, prostaglandinler, tromboksanlar ve lökotrienleri içermektedir.

Anahtar Kelimeler: *Küf, yağ, doymamış yağ asitleri*

DEFINITION AND CLASSIFICATION OF LIPIDS

Biological materials have been classified as lipids, proteins, carbohydrates and minerals. One of the main characteritics that distinguishes lipids from other natural pdoducts is their solubility in organic solvents such as ether, chloroform, benzene, etc. and their insolubility in water. There is no universally-accepted definition of "lipid" as the term covers an extremely diverse range of molecular species which makes it impossible to provide a single chemical definition (RATLEDGE & WILKINSON, 1988; CHRISTIE, 1989).

The most common definition, however, is "Lipids are fatty acids and their derivatives, and substances related biosynthetically or functionally to these compounds". The fatty acids are compounds synthesised in nature via condensation of malonyl-coenzyme. A units by a fatty acid synthetase complex (MCMURRY, 1984; CHRISTIE, 1989).

Lipids may be relatively simple molecules, as for example the fatty acids themselves, or more complex and contain phospho or sulpho groups, amino acids, peptides and derivatives, sugars and even oligosaccharides. The diversity of lipids signifies a diversity of function. Lipids can act as storage materials in animals, plant and microbial cells and are also responsible for the structure of cell membranes (RATLEDGE & WILKINSON, 1988).

The sutructure of more common lipids can be divided loosely into two categories: (i) those sutructures based on long chain fatty acids or their immediate derivatives such as alkanes (and some alkenes) and fatty alcohols, and (ii) sutructures derived from isoprene unit and which are loosely known as terpenoid lipids. The former lipids encompass the natural fats and oils found in plants and animals as well as phoshpholipids and glycolipids which are also of ubiquitous distribution. The terpenoid lipids range from the simplest monoterpenes found fragrant oils, often confusingly known as essential oils, to the more complex steroid and carotenoid molecules (RATLEDGE & WILKINSON, 1988).

Approximately 80 % of the world's oil and fat need is derived from agricultural products, and the remainder coming from animal and marine sources. It is essential to find new sources for oil and protein supplement with concern to the nutritional problems accompanying the rapid growth of world population (RATLEDGE, 1979; HOLDSWORTH, 1987).

Recent developments in technology have demonstrated the possiblitiy of cultivanting microorganisms on extremely large scales for the production of single cell protein. This led to the realisation that microorganisms

can compete on equal terms with cheap plant products provided that the scale of operation is sufficient. Therefore, there seems little reason why a process for the production of microbial oils and fats should not compete with conventional sources in an attempt to produce a better more cost effective commodity (RATLEDGE, 1982; HOLDSWORTH et al., 1988).

Micro-organisms display a whole panoply of lipids. The lipid within a cell may vary not only in amount but in type according to how the micro-organism is grown, or what stage during growth the micro-organism is taken for analysis (WEETE, 1980). Nevertheless, sometimes, they are thought to be more limited in the range and type of lipid which they possess in comparison with animal or plant cells; this is totally incorrect. For example, although, as far as we know, micro-organisms do not contain prostaglandins or the related leukotrienes and thromboxanes, certain ones do contain the precursors of these molecules-such as highly polyunsaturated fatty acids, arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid (HAMBERG, 1986; RATTRAY, 1988; LOSEL, 1988). However, COTTRELL (1989), KOCK et al. (1991) and SMIT (1993) have reported the evidence of prostaglandins in yeasts.

OLEAGINICITY AND BIOCHEMISTRY OF LIPID ACCUMULATION

Organisms, principally eukaryotes, which can accumulate 20% or more of their biomass as lipid have been known for many years. Such organisms were termed as "oleaginous" in keeping with oil-bearing plants that are similarly named (RATLEDGE, 1982).

Biochemical studies on the mechanism of lipid accumulation suggested a biochemical definition for oleaginicity based on the possession of one of the key enzymes for lipogenesis, namely ATP: citrate lyase (ACL) (BOTHAM & RATLEDGE, 1979; BOULTON & RATLEDGE, 1981a, b, 1984a,b). Another key enzyme involved in lipid accumulation is malic enzyme. Whilst ATP: citrate lyase provides the acetyl building units for fatty acid biosynthesis, malic enzyme generates the NADPH by which the acetyl units can be reduced and used as the backbone of the fatty acids (KENDRICK, 1991; RATLEDGE, 1992). Eukaryotic organisms without ATP: citrate lyase appear unable to achieve the same degree of lipid accumulation as those which possess it (BOULTON & RATLEDGE, 1983a,b; RATLEDGE, 1989).

The amount of lipid which an oleaginous micro-organism can accumulate is determined by the culture conditions. Lipid accumulates in oleaginous micro-organisms when there is an excess of carbon available to the cells during a period when another nutrient which is required for cell proliferation is exhausted from the medium. Usully, this limiting nutrient is nitrogen, but it can be phosphate, potassium, magnesium, sulphate or even iron (RATLEDGE, 1982).

After the nitrogen becomes exhausted the cells continue to assimilate the carbon source. During this transition from primary to secondary metabolsim, the intracellular concentrations of various key intermediates change. Of principal significance is the rapid decrease in AMP concentration immediately following nitrogen exhaustion (EVANS & RATLEDGE, 1983a,b). This is due to activation of AMP deaminase (EVANS et al., 1983) which deaminates AMP to yield IMP and NH_3.

The immediate metabolic consequence of the drop in AMP concentration is a decrease in NAD^+ - isocitrate dehydrogenase activity. The activity of this enzyme in oleaginous cells, unlike non-oleaginous organisms) is dependent completely upon the presence of AMP (Figure 1) (RATLEDGE, 1989). As a consequence, isocitrate can no longer be effectively metabolised trough the citric acid cycle, causing both isocitrate and citric acid to accumulate. The equilibrium of isocitrate and citrate via acotinase favours citrate. Thus, it is citrate which accumulates in the early period following the onset of nitrogen exhaustion from the medium (EVANS & RATLEDGE, 1983 b).

As citric acid accumulates, it is transported across the mitochondrial membrane in exchange for malate (EVANS et al., 1983). Malate serves both as the counter-ion to pyruvate uptake into the mitochondrion and citrate transport out of the mitochondrion. It is also converted back into pyruvate via malic enzyme, an enzyme which produces NADPH to be used subsequently in the biosynthesis of fatty acids.

The key to oleaginicity resides not only in the ability of oleaginous organism to accumulate citrate but also subsequently to deal with citrate. Therefore, the principal key to oleaginicity is the possession of ATP: citrate lyase (RATLEDGE, 1982).

Early studies of lipid accumulation were carried out either in batch culture or in two-stage continuous culture. It was generally believed that lipid concentrations equal to those attained in batch culture could not occur in a single stage chemostat. Although the advantages of continuous culture techniques for studying lipid accumulation have been described, the commercial use of continuous culture is much less evident due to the high capital costs. Therefore, most lipid accumulation studies are conducted with batch fermentations.

The usual course of lipid accumulation during batch fermentations can be thought of as a two-stage process: (a) cell proliferation and balanced growth culminating with exhaustion of another nutrient from the medium, and (b) a lipid accumulation phase (RATLEDGE, 1992).

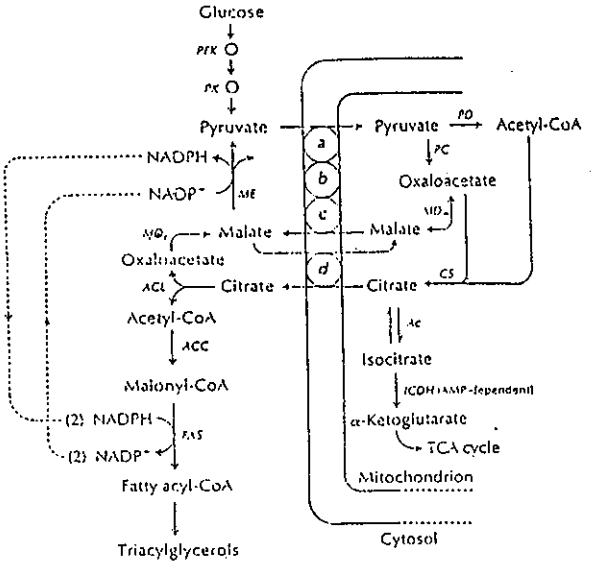

Figure 1. The biochemical pathway for triacylglycerol biosynthesis in oleaginous yeatsts (RATLEDGE, 1989)

The course of lipid accumulation in moulds may be similar to that described for yeasts. However, it may be just a coincidence and may due to the relatively poor conditions which fungi are grown. These conditions lead to arithmetic growth rates rather than any extended exponential period of growth (RATLEDGE, 1989). Lipid accumulation is a growth-linked process. If the growth rate were to be increased then the rate of lipid accumulation would also increase. The sutudies on microbial lipid production have shown that the optimum growth conditions are not necessarily best for maximum lipid production. The rate of lipid synthesis relative to the rate of synthesis of other cellular products determines whether lipid accumulates (WEETE, 1980).

FUNGAL LIPIDS

The greater cell-size and complexity of fungi is accompanied by a corresponding diversity of lipid components (RATLEDGE, 1987). In fungi, lipids occur not only as major constituents of membrane systems, but also as cell wall components, as storage material in abundant and readily observed lipid bodies and, in some cases, as extracellular products (BRENNAN & LÖSEL, 1978). The amounts and types of lipid at individual fungal sites vary not only from one organism to another but also with age, stages of development, nutrition and environmental conditions (WEETE, 1980; LÖSEL, 1988, 1989). The lipid content of fungal species can be manipulated by varying culture conditions, therefore, the records of total lipid content are of limited value unless the parameters of growth are specified.

LIPID COMPOSITION OF FUNGAL LIPIDS

More information is available on lipid composition than on any other aspect of fungal lipids, largely because of the ease of preparation and analysis of total lipid samples. The lipid fractions from a variety of moulds, showed wide range of values for the contents of both polar and neutral lipids (WEETE, 1980). Triacylglycerols represent the major lipid component. These are generally considered as storage lipids that may be used for energy and carbon skeletons during growth and development. The proportion of triacylglycerols change with cultural conditions, such as in *Mortierella isabellina* the proportion of triacylglycerols was consistently less when the growth temperature was increased (LÖSEL, 1988).

The other major lipid components of oleaginous moulds are no different from the nonolegainous species. Numerous sterols are observed in fungi (BRENNAN & LÖSEL, 1978; WEETE, 1980). Squalene and other hydro-

carbons do occur frequently (FARAG, *et al.*, 1981). There is evidence that sterols exhibit a condensing or liquefying effect on acyl lipids depending on the physical state of the lipid. They may regulate permeability by affecting internal viscosity and molecular motion of lipids in the membrane (CONTTRELL, 1989). They may also serve as precursors of steroid hormones involved in the sexual reproduction of some fungi.

Polar lipids of moulds are mainly the phospholipids, but also include glycolipids. Phospholipids are important structural components of biological membranes. They have been impliceted in the active transport of ions across memranes and are also essential for the activity of some membrane-bound enzymes (LOSEL, 1988, 1989).

The relative proportions of the all the lipid components may vary according to the stage of fungal development, age and conditions under which fungus is cultured (WEETE, 1980).

The advantages of using moulds rather than yeasts to accumulate lipid must be seen either in the ability of moulds to handle and upgrade a greater range of waste materials than yeasts or in their ability to produce a wider diversity of fatty acids. The fatty acids of moulds have been well described in numerous reviews (SHAW, 1965, 1966; WEETE, 1980, LÖSEL, 1988).

Fatty acids are of the widest distribution in all living cells which are aliphatic monocarboxylic acids that occur in nature a homologous series ranging in chain length from C_{10} to C_{36} (RATLEDGE & WILKINSON, 1988).

They are rarely found naturally in the free from, but are linked to a variety of molecules of which glycerol is the most common. They may be fully saturated and may be linear, branched, or contain alicyclic rings. Unsaturated fatty acids as frequently as the saturated ones: they may contain several double bonds, though 1 or 2 is the most usual. The position of the double bond is dictated by the route of biosynthesis. Dienoic fatty acids and others containing additional double bonds (polyunsaturated acids) are normally found as major components only in eukaryotic organisms, though many cyanobacteria also contain them. Besides being saturated or unsaturated, fatty acids may contain other functional groups in addition to the carboxyl group. This may, for example, be hydroxyl, oxo, epoxy, or even a second carboxyl group (RATLEDGE & WILKINSON, 1988).

Fungi show a greater diversity of fatty acids than yeasts which is a simple reflection of the far greater number of moulds. But the majority of species contain, in order of abundance, oleic acid ($C_{18:1}$) palmitic acid ($C_{16:0}$) and linoleic acid ($C_{18:2}$) as the major acids, with stearic acid ($C_{18:0}$), linolenic acid ($C_{18:3}$) and palmitoleic acid ($C_{16:1}$) as the minor ones (SHAW 1966).

Besides the occurence of high amounts of the polyunsaturated fatty acids, $C_{18:2}$ and $C_{18:3}$, *Mucorales* uniquely contain γ-linolenic acid ($C_{18:3}$, n-6) rather than α-linolenic acid (SHAW, 1965, 1966). Highly polyunsaturated fatty acid such as arachidonic acid ($C_{20:4}$), eicosapentaenoic acid ($C_{20:5}$) and docosahexanoic acid ($C_{22:6}$) occur in the same species (TOTANI and OBA, 1987).

CONCLUSION

Although the lipid metabolism concerned with physiology or biiochemistry, taxonomy, spoilage problems and drugs, antibiotic-, or food-related industries have generated an extensive literature, it is still a relatively unexplored field. Deriving oils from micro-organisms will never replace the bulk plant oils, but production of polyunsaturated fatty acids for nutritional, and perhaps medical purposes using mould technology can be a challenge, and also feasible in terms of using various and cheap carbon sources. Therefore, investigation of fungal lipid metabolism can develop the biochemical understanding of lipids to obtain essential fatty acids, such as long-chain PUFAs, hydroxy PUFAs, and eicosanoids that are important in the function of cardiovascular, nervous and immune system of the body. Fungi has a panoply of lipid components that can easily be manipulated by varying culture conditions in the way of production of speciality fats and oils.

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