Biotechnology of Single Cell Oils

A. Makri, S. Bellou, S. Fakas and G. Aggelis

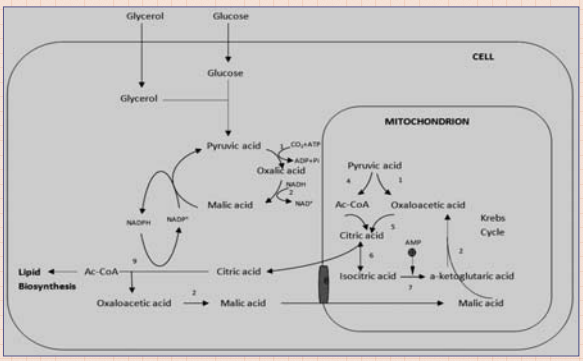
Laboratory of Microbiology, Division of Genetics, Cell and Development Biology, Department of Biology, University of Patras, 26504, Patras-Greece

Introduction

Microbial lipids, known as Singe Cell Oils (SCO), are in the forefront of biotechnological products for many years. However, before SCO to be considered as commercially viable products, they have to compete with the abundance of oils available from agricultural sources (Ratdlege, 1993). In fact, SCO production cost is much higher than the respective plant oils, so only SCO which commanded a high price could be economically produced. The high-priced SCO encompass oils containing high amounts of polyunsaturated fatty acids (PUFAs) such as arachidonic and docosahexanoic acids with high nutrient and dietary importance and gamma linolenic acid (GLA) with unique anticancer properties (Kenny *et al*., 2000; Das and Undurti, 2004). Current research in SCO production is focused on the use of selected industrial and agro-industrial by-products as substrates in order to eliminate the production cost. E.g. tomato waste is produced in huge amounts in all Mediterranean countries where this waste is uncontrollably disposed to landfills near to the processing units, causing environmental pollution. An alternative way for the valorization of tomato waste is the use of tomato waste hydrolysate (TWH) as a growth inducer of oleaginous fungi (Fakas *et al*., 2008a). Raw glycerol is another substrate of particular interest since it is produced in vast quantities in the bio-diesel production units. The use of raw glycerol as the sole carbon substrate for SCO production is an alternative way to recycle this by-product and simultaneously decrease the production cost of both, SCO and bio-diesel, processes (Papanikolaou *et al*., 2008).

Biochemistry of lipid accumulation

Although glucose is the most common substrate for SCO production, other carbon sources, such as glycerol, could be assimilated and converted into microbial lipids. Oleaginous microorganisms accumulate lipid in nitrogen limited conditions and when an suitable carbon substrate is found in the growth environment in excess. Lipid accumulation is triggered by the exhaustion of nitrogen, in the growth medium. In nitrogen limited conditions the AMP concentration in the cell is diminished and the activity of isocitrate dehydrogenase within the mitochondrion slows or even stops. Although TCA is disrupted, assimilation of the carbon substrate remains high leading to the accumulation of citric acid which is finally excreted into the cytosol. There, citric acid is cleaved by the ATP:citrate lyase (enzyme that is present only in oleaginous species) and acts as acetyl-CoA donor in the biosynthetic pathway of storage triacylglycerols (Scheme 1 – Ratledge and Wynn, 2002, adapted).



Scheme 1. Lipid biosynthesis in oleaginous microorganisms (Ratledge and Wynn 2002, adapted). **Enzymes:** 1: Pyruvate carboxylase, 2: Malic dehydrogenase, 3: Malic enzyme, 4: Pyruvate dehydrogenase, 5: Citrate synthase, 6: Aconitase, 7: Isocitrate dehydrogenase, 8: citrate/malate translocase, 9: ATP citrate lyase.

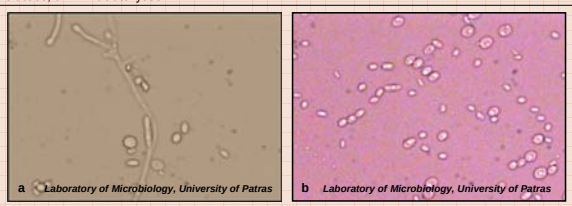


Figure 1: The morphology of the unconventional yeast *Yarrowia lipolytica* grown on glycerol depends on the growth phase: yeast-like and short mycelial cells (a) were predominant during exponential growth phase while yeast cells were appeared in the stationary growth phase (b).

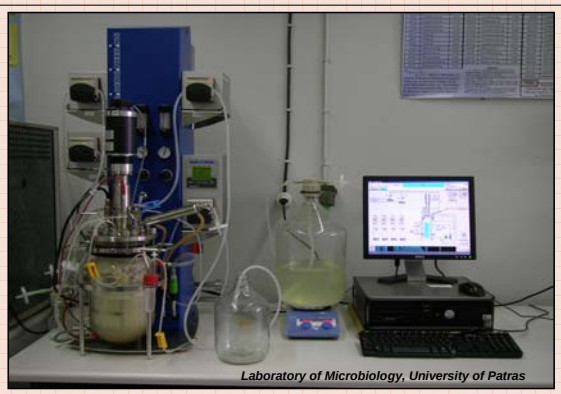


Figure 2: Continuous culture of *Yarrowia lipolytica* on raw glycerol in a laboratory scale bioreactor

SCO production from raw glycerol and other agro-industrial by-products

The oleaginous mould *Cunninghamella echinulata* was cultivated on various agro-industrial residues, tested as substrates for the production of GLA-containing SCO (Table 1, Fakas *et al*., 2008b). In these substrates microbial growth and lipid accumulation depend on the availability of the ammonium nitrogen liberated from protein structures. Lipid accumulation was marginally achieved on corn steep, whey concentrate and yeast extract, where the oil yield was almost 20% in biomass (at total nitrogen concentration 0.4 g/l), while growth on corn gluten yielded only 11% of oil in biomass. On the contrary, oil yield on Tomato waste hydrolysate reached around 40% at the highest nitrogen concentration utilized. It is concluded that, besides carbon, the nitrogen source plays an important role in lipid synthesis.

Lipid contents in *C. echinulata* cultivated on C-limited TWH media were 5, 7.4, and 8.2% of mycelial dry weight at, mid exponential (ME), late exponential (LE), and stationary (S) growth phases respectively. Each lipid fraction contributes in different percentage to total lipids within each growth phase (Table 2). The proportion of neutral lipids (N) increased sharply during exponential phase, and a further increase was observed at S phase, while the percentage of glycolypids and sphingolipids (G+S) fraction was high at the beginning of growth (ME phase) but declined thereafter. Finally, P concentration demonstrated a pronounced decrease with time. N consisted predominantly of TAG (accounting for 75–92% w/w of total N) and to a lesser degree of diacylglycerol (DAG) (7–8.7% w/w), while monoacylglycerol (MAG) (0.5–3% w/w) was only present in small amounts. G+S fraction comprised of three major components and four minor, while all of them were present throughout growth. Monoglycosyldiacylglycerol (MGDG) was the main glycolipid (accounting for almost 50% w/w of the total G+S), followed by diglycosyldiacylglycerol (DGDG) (14–33% w/w). The third more abundant compound was an unidentified glycolipid (G), which was more polar than DGDG. The major P class was phosphatidylcholine (PC) (accounting for 40.2–50.2% w/w of total P), followed by phosphatidylethanolamine (PE) (22.4–25.1% w/w), phosphatidylinositol (PI) (17.4–20% w/w), and phosphatidylserine (PS) (6.7–10% w/w), while diphosphatidylglycerol (DPG) was a minor component (1.2–7.8% w/w). The concentrations of all P classes in the total lipid decreased with time, except for PS that maintained a constant concentration during LE and S phases. Upon exiting the exponential phase, PC increased its proportion in P fraction at the expense of the other classes, mainly DPG. During S phase, there was a drop in PC concentration accompanied by an increase in both PI and PS concentrations (Fakas *et al*., 2006).

Other microbial species, such as *Yarrowia lipolytica* (Figure 1), can efficiently convert raw glycerol into various biotechnological products. According to Papanikolaou and Aggelis, (2002), this yeast cultivated on raw glycerol in a continuous system (Figure 2) accumulated 43% (w/w/) of lipid while the produced biomass was 8.1 g/l. Also, Papanikolaou et al., (2008), reported that *Mortierella isabellina*, when grown on raw glycerol, produced 8.5 g/l biomass that contained 51.7% (w/w) lipid.

Table 1
Lipid and GLA production during growth of *Cunninghamella echinulata* on various concentrations of organic nitrogen sources (Fakas et al., 2008b)

Nitrogen source	Total nitrogen (g/l)	Biomass (g/l)	Oil in biomass (%)	GLA in oil (%)	GLA production (mg/l)
Corn gluten[a]	0.4	3.7	11.9	3	10
	0.7	10.3	11.2	2	18
	1	9.8	13.3	2.6	27
Corn steep[a]	0.4	8.9	19.8	12.6	189
	0.7	18.5	14	12.9	274
	1	16.7	7.6	14	137
Whey concentrate[a]	0.4	8.2	19.3	14.4	194
	0.7	12.7	15.8	13.8	228
	1	17.1	14.3	14.1	282
Yeast extract[a]	0.4	13	16.6	12.1	216
	0.7	17.7	16.3	13.6	326
	1	9.5	12	12.8	116
Tomato waste hydrolysate[b]	0.2	19.5	18.4	18.3	662
	1.2	18.9	17.4	17.5	574
	1.5	17.6	39.6	11.5	802

[a] Strain CCF-103.
[b] Strain ATHUM 4411.

Table 2 Lipid composition during growth *of C. echinulata* on tomato waste hydrolysate (Fakas *et al*., 2006)

Growth phases	Neutral lipidsa [% (w/w) of total lipids]				Glycolipids plus sphingolipids [% (w/w) of total lipids]			Phospholipids [% (w/w) of total lipids]						
	N	TAG	DAG	MAG	G+S	MGDG	DGDG	G	P	PC	PE	PI	PS	DPG
Mid exponential	45	37.6	4.6	1.6	41	21.3	13.5	1.2	14	5.6	3.5	2.7	1.1	1.1
Late exponential	67	48.2	10.6	1.4	24	11.5	6.2	1.0	9	4.5	2.0	1.6	0.6	0.3
Stationary	74	65.3	5.2	0.4	20	9.1	2.8	0.4	6	2.8	1.4	1.2	0.6	0.1

Neutral, glycolipid plus sphingolipid, and phospholipid fractions in total lipids and absolute changes in individual lipid classes.
N neutral lipids, TAG triacylglycerol, DAG diacylglycerol, MAG monoacylglycerol, SE steryl esters, G+S glycolipids+sphingolipids, MGDG monoglycosyldiacylglycerol, DGDG diglycosyldiacylglycerol, G unidentified glycolipid, P phospholipids, PC phosphatidylcholine, PE phosphatidylethanolamine, PI phosphatidylinositol, PS phosphatidylserine, DPG diphosphatidylglycerol
aMinor neutral lipids were free fatty acids, wax esters, and hydrocarbons.

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