ΓΕΩΠΟΝΙΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ
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Microbial oil production by newly isolated yeast strains and novel industrial applications based on waste and by-product valorisation

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Microbial oil

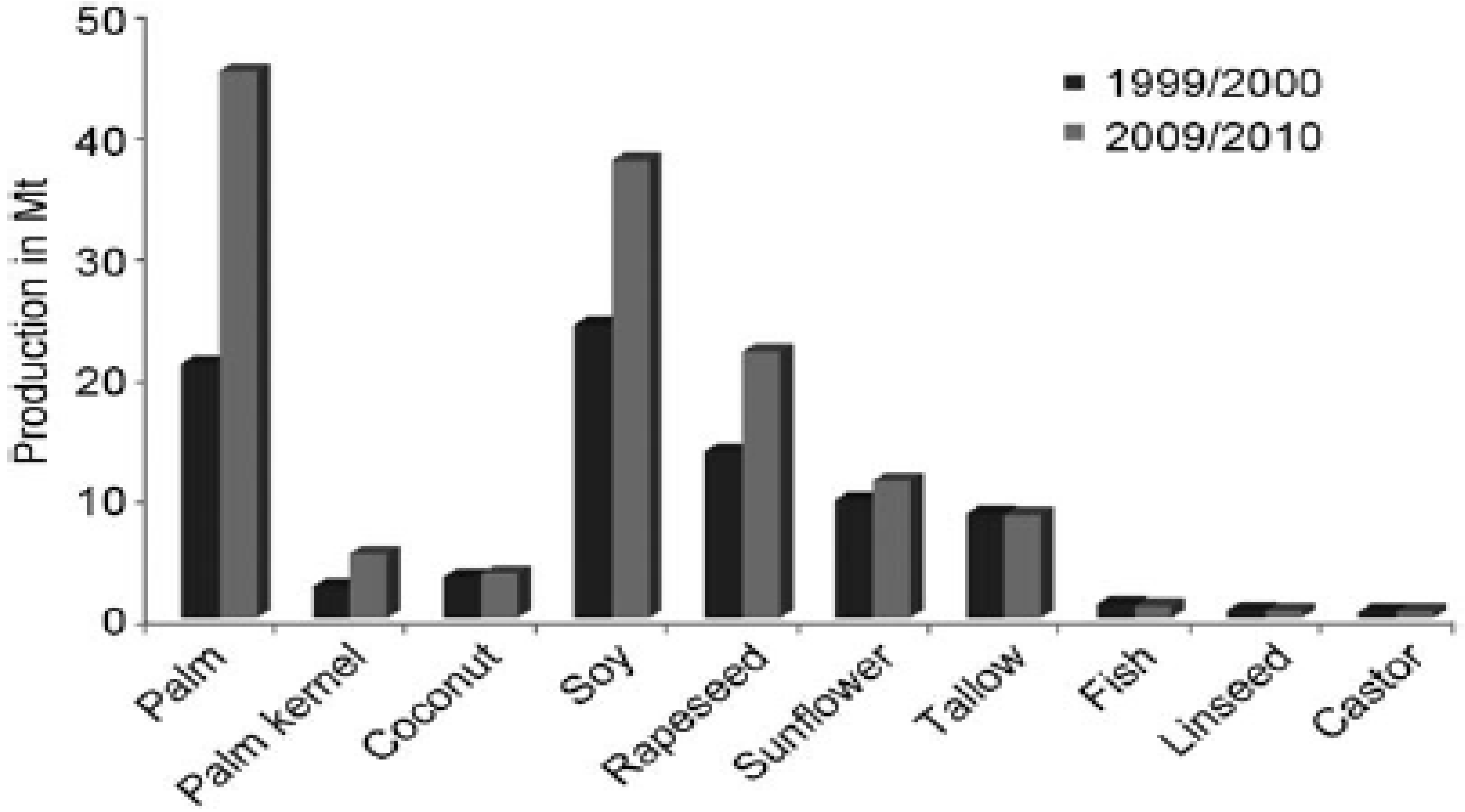
- ✓ Microbial oils or single cell oils are lipids that are produced by different microorganisms
- ✓ Microorganisms that can accumulate lipids to more than 20% of their dry weight are considered oleaginous.
- ✓ Microbial oil production could be achieved via fermentation using renewable resources (agro-industrial wastes and residues)

Applications of microbial oil

Conventional used of microbial oil include:

- ✓ Biodiesel production
- ✓ Medical and dietary uses when MO contain polyunsaturated fatty acids such as γ-linolenic acid and arachidonic acids
- ✓ Cocoa butter substitutes
- ✓ Substitutes for natural oils and fats as feedstock for chemical production eg. biolubricants, surfactants, wax esters

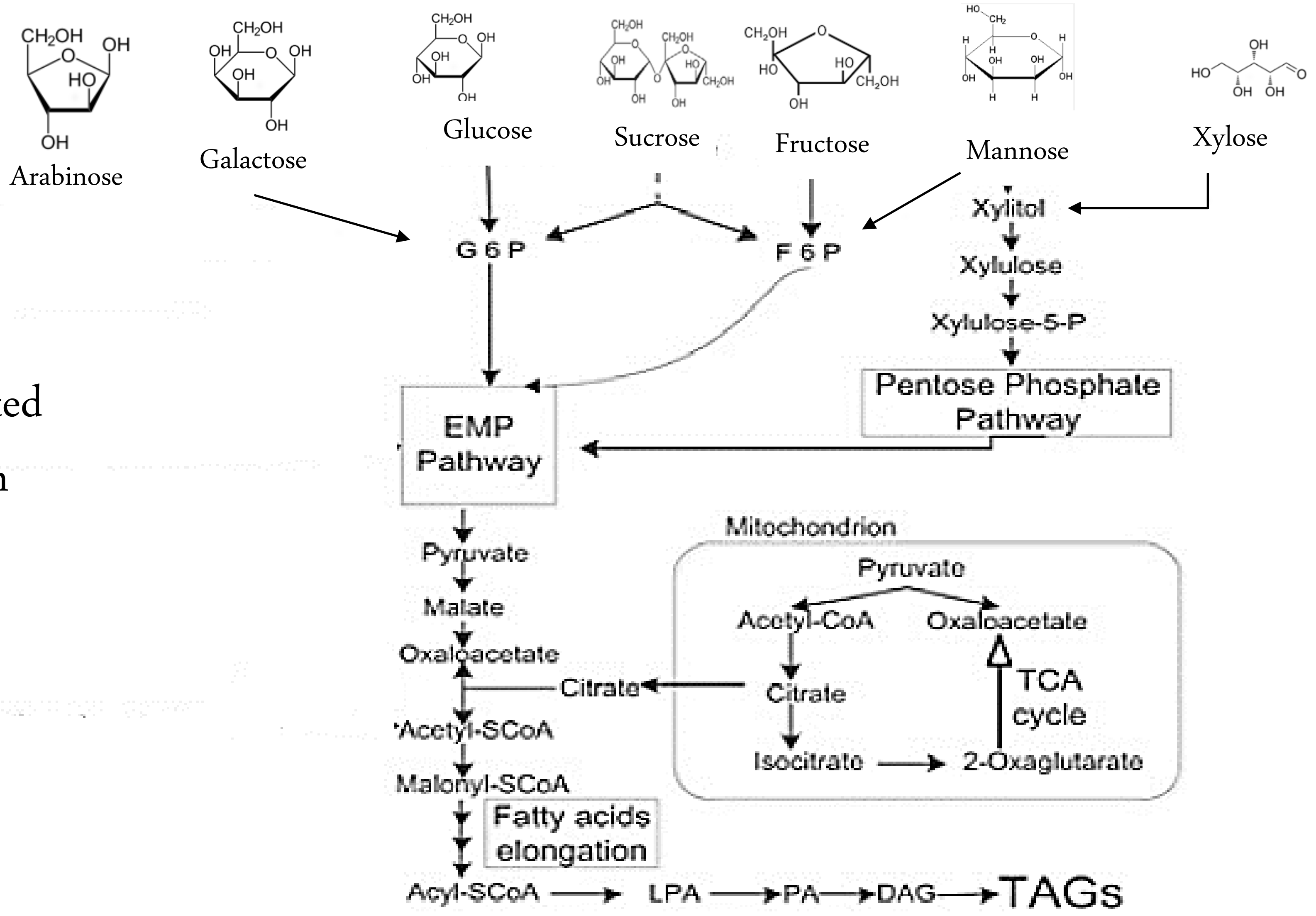
Production of oils fats for oleochemical industry



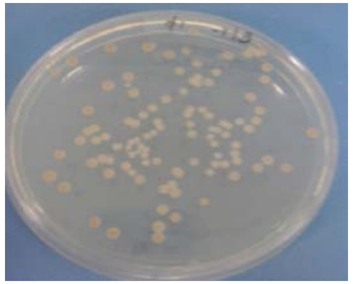
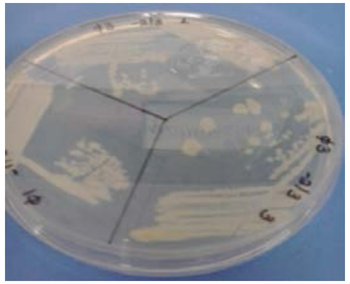
Alternative sources for oleochemical production are still needed

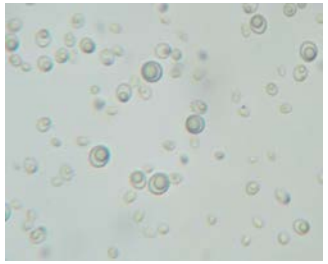
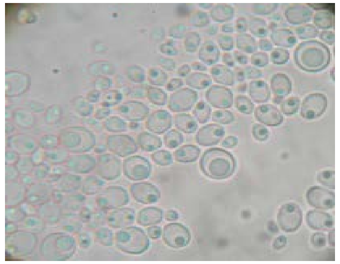
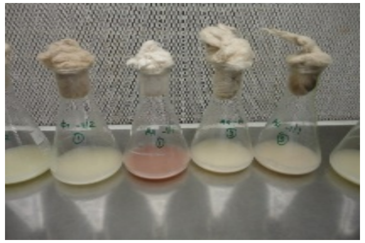
Metabolic pathway

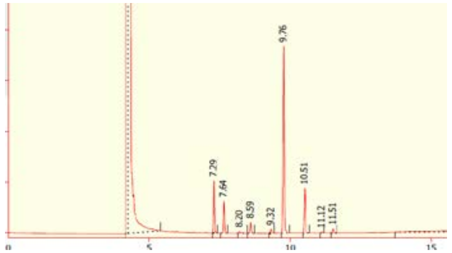
Microbial oil production occurred under nutrient limited conditions e.g. when nitrogen is depleted



Isolation and screening of oleaginous yeasts



Isolation of yeasts

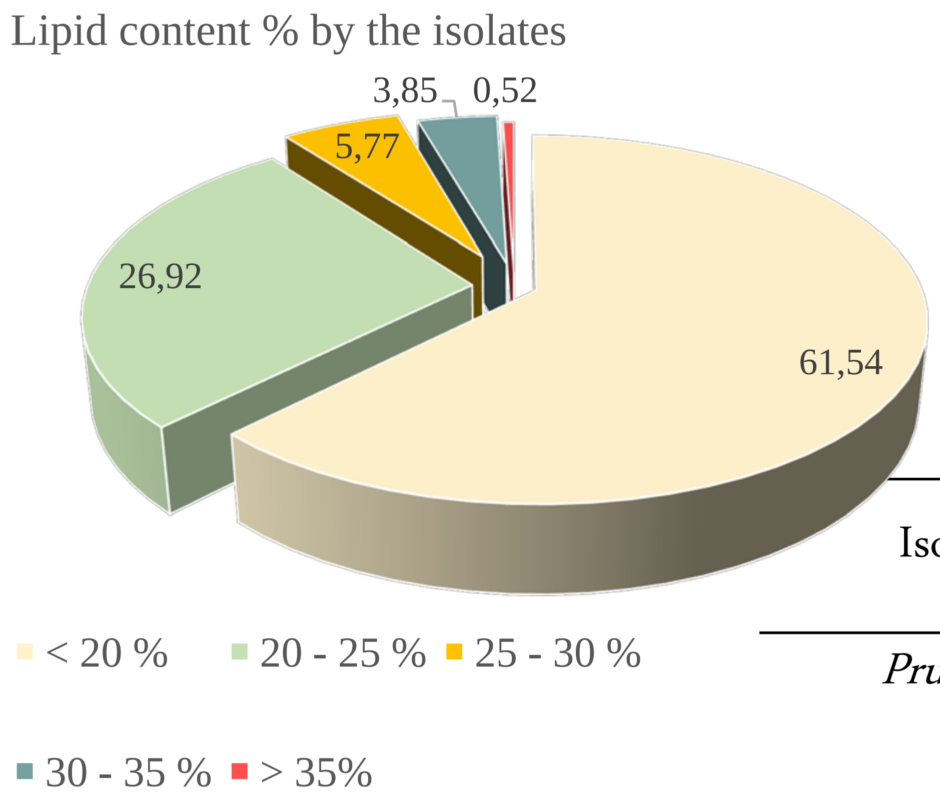
Screening of oleaginous yeasts using sucrose as carbon source.

Screening of isolates in various carbon sources

Extraction of oil

Fatty acid analysis

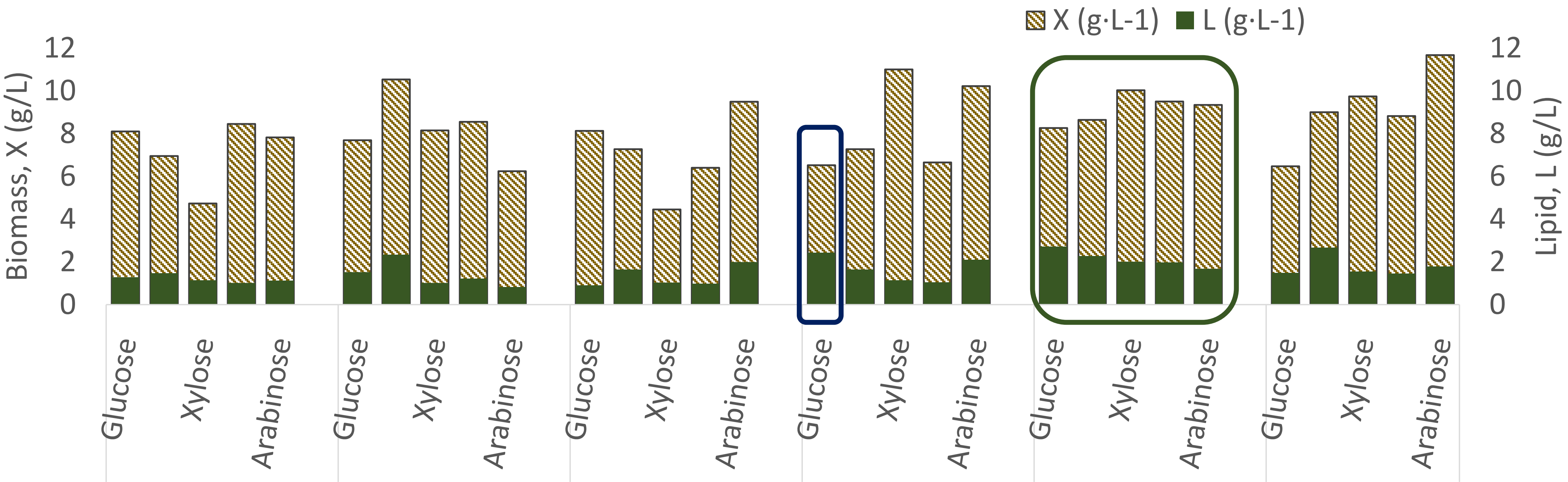
Isolation and Screening on sucrose



- 88 yeast strains were isolated
- The total lipid content of 21 strains varied from 21-48 %
- 6 isolates which showed higher lipid content were selected for screening process.

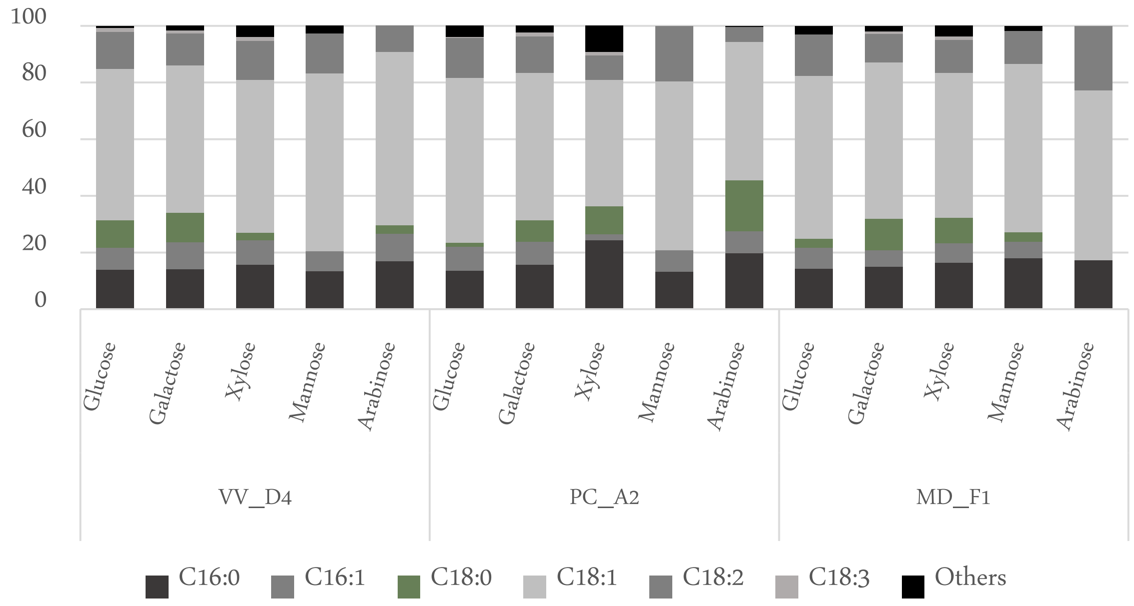
Isolation source	Code name	Time (h)	X ($g{\cdot}L^{-1}$)	L ($g{\cdot}L^{-1}$)	$Y_{L/X}$ (% wt/wt)
Prunus domestica	PD_D2	48	8.0	2.8	34.7
	PD_F1	48	7.3	2.0	26.5
Vitis vinifera	VV_D4	48	8.0	2.7	34.4
Pyrus communis	PC_A2	24	5.6	2.7	48.2
Pyrus pyrifolia	PP_D3	48	8.4	2.1	24.6
Malus domestica	MD_F1	48	8.0	2.5	31.3

Screening of selected yeast strains on different carbon sources



- ❑ PP_D3 strain achieved 18,86 – 32,93% lipid content in all carbon sources
- ❑ Galactose → lipid content higher than 20% w/w for all strains
- ❑ Glucose → PD_F1 (37%)

Fatty acid composition

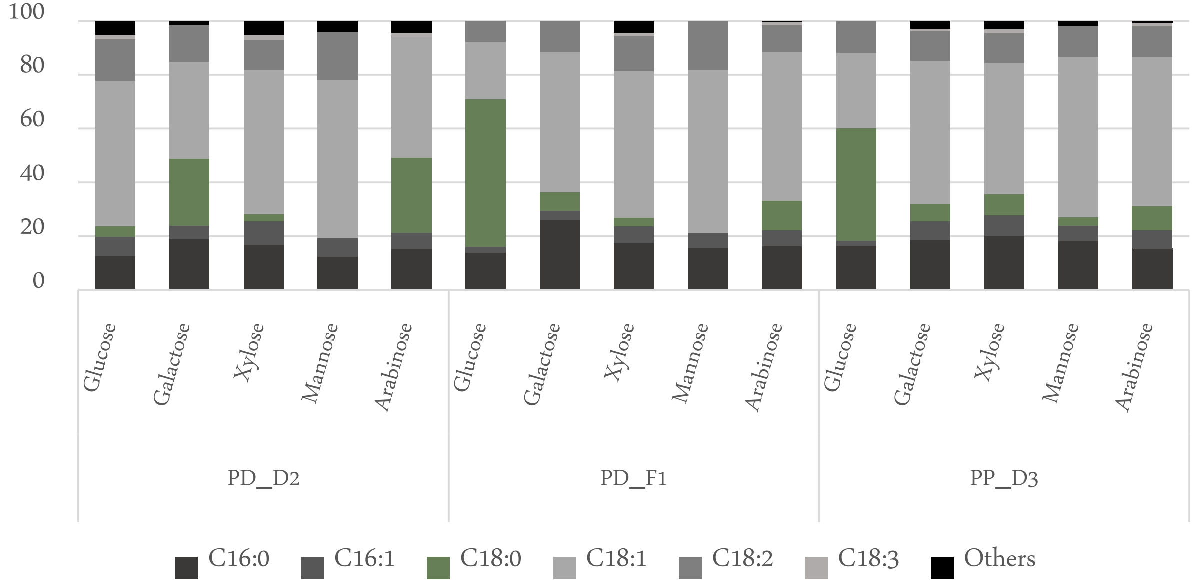


Main fatty acids palmitic and oleic acid

palm oil

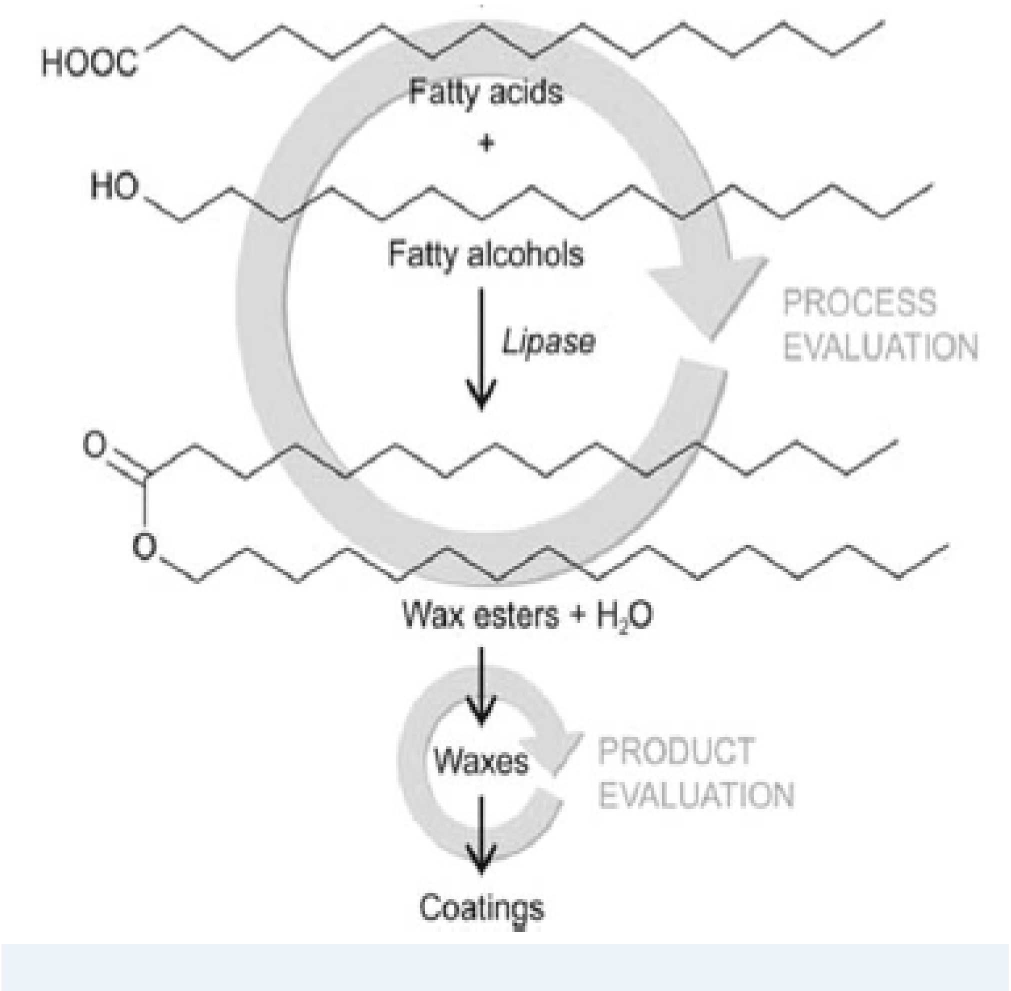
Different fatty acid profiles

- High saturated content
- High oleic acid content



Wax esters production from microbial oil

- Esters of long-chain carboxylic acids and long-chain alcohols
- Chemical or enzymatic methods
- Wax esters with more than 26 carbon atoms can be used as ingredients in coatings.



Petersson et al, 2005

Synthesis of Wax esters

Optimization of the reaction conditions:

- Fatty alcohols: **oleyl alcohol**
  cetyl alcohol
- Immobilized lipases: **Novozyme 435**
  Lipozyme

Parameters tested:

✓ *temperature*

✓ *oil to alcohol molar ratio*

✓ *amount of enzyme*

Identification of reaction mixture components by TLC

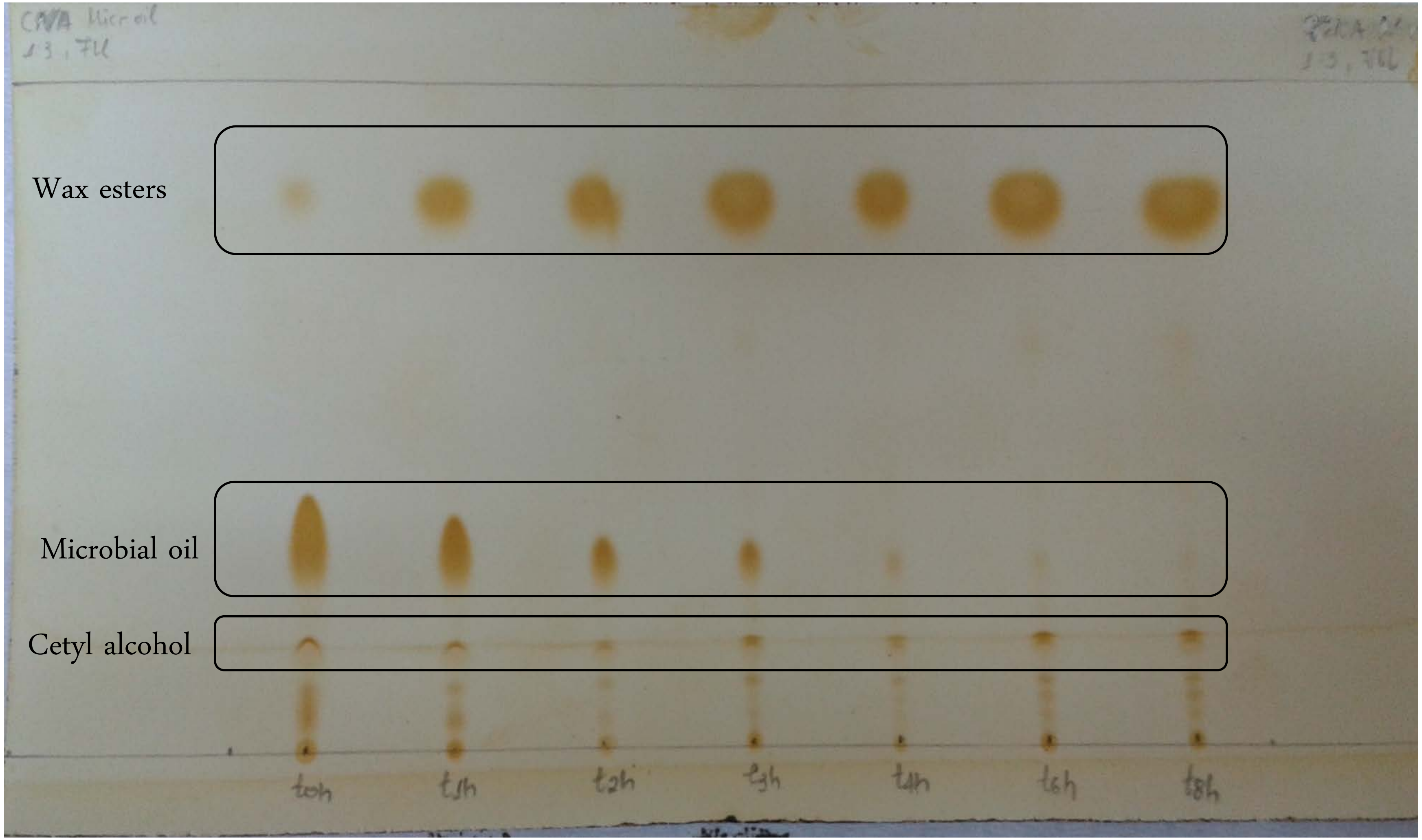
Quantification of wax esters by GC

Fatty acid	Palm oil %	Microbial oil %
Lauric (C12:0)	1.8	-
Myristic (C14:0)	1.7	0.42
Palmitic (C16:0)	48.1	26
Palmitelaidic (C16:1)	0.2	0.9
Stearic (C18:0)	5.6	10.7
Oleic (C18:1)	33.5	52.1
Linoleic (C18:2)	6.7	7.3
α-Linoleic acid(C18:3)	-	-
Arachidic acid (C20:0)	-	0.4
Arachidonic (C20:4)	0.3	-
Behenic acid (C22:0)	-	0.3

Reaction progress of microbial oil transesterification

Reaction conditions:

- 70 °C
- Novozyme amount 7Unit
- Microbial oil to cetyl alcohol molar ratio 1:3



The highest wax ester conversion yield (83 %) achieved at 6-8 hours of the reaction

Conclusions

- 6 newly isolated yeast strains could be evaluated for microbial oil production on various carbon sources
- The fatty acid composition is significantly affected by the cultivation conditions and type of carbon sources
- Different fatty acid profiles have various potential applications
- Microbial oil can be effectively used for wax esters production in a solvent free system

Thank you for your attention

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