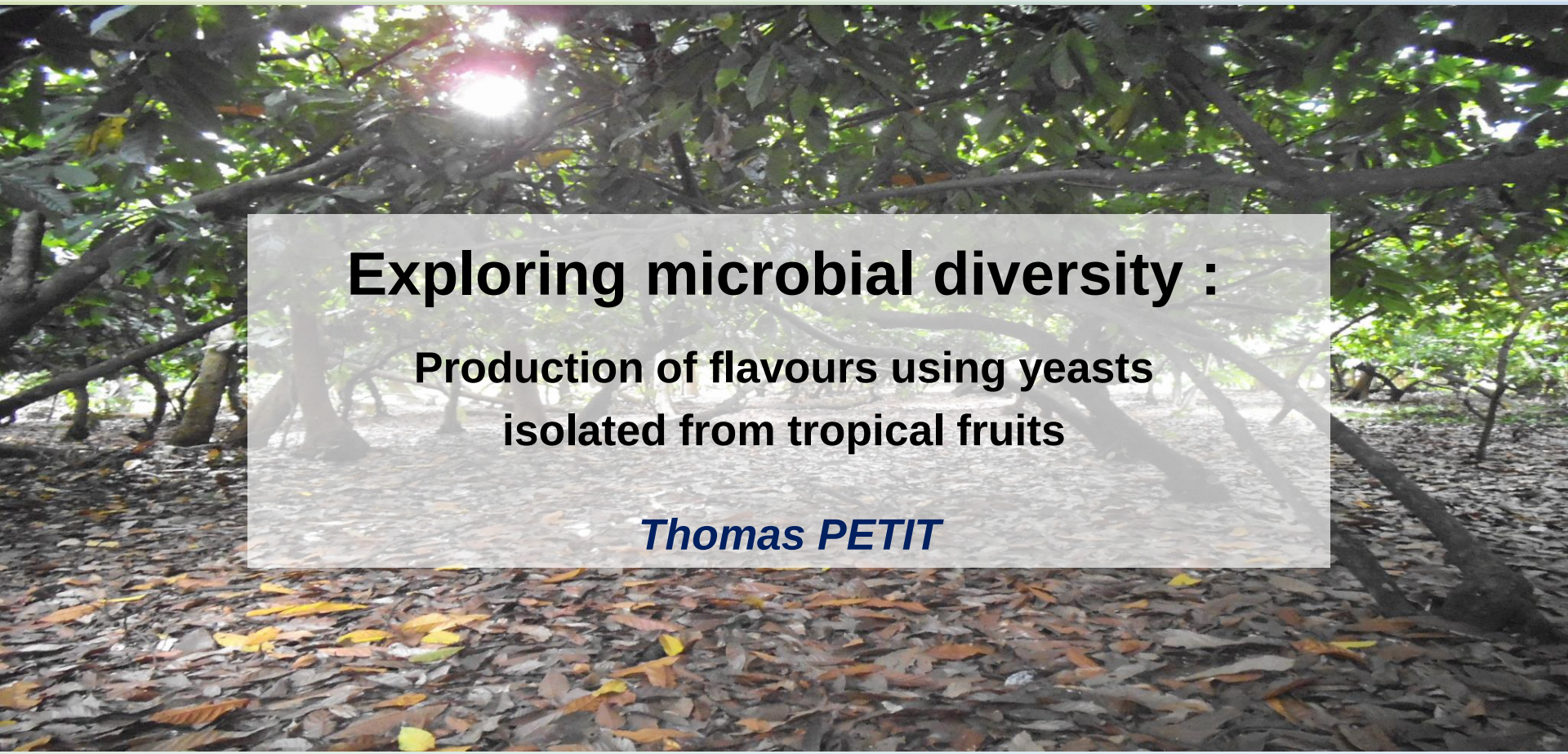


Exploring microbial diversity :

Production of flavours using yeasts isolated from tropical fruits

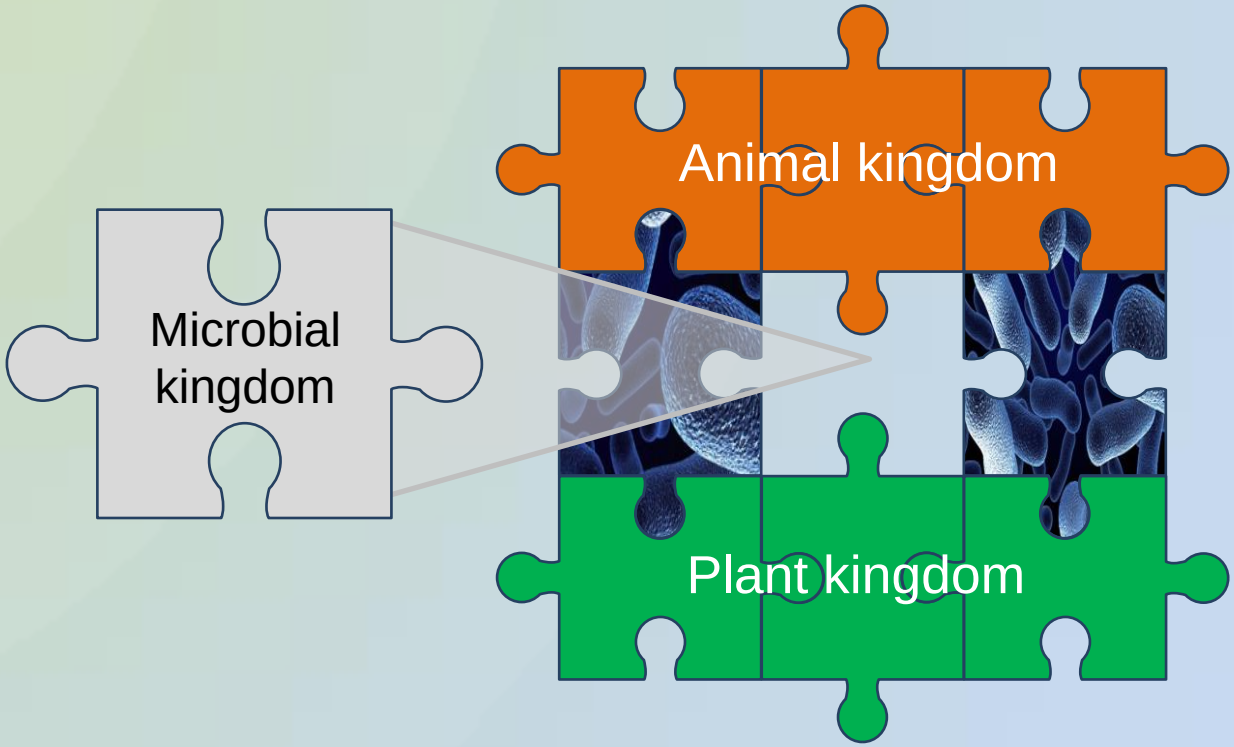
Thomas PETIT

Colloque "Connaissance et Conservation des milieux naturels" – 15/09/2015

Interest in studying microbial diversity?

- What can we expect from microbial diversity?
- Potential benefit in Indian Ocean area?

Hot spots of biodiversity



92% of the microbial kingdom remains to be discovered

- Hanson et coll. (2009) ; Fernández et coll. (2012) -

Potential benefit for industry ?

F&F market size: $23.9 billion (2013)

Flavour production by yeast

- ✓ Flavours (lactones, esters, alcohols, …) are described as the combined perception of taste, smell and mouth feel
- ✓ Flavours are important ingredients for food industries

LESAFFRE SAFISIS

Nestlé

LALLEMAND

DSM

- ✓ Increased consumer demand towards ‘natural’ ingredients
- ✓ Yeasts are suitable microorganisms for production of VOCs (GRAS)

Step 1. Screening for yeast …

Passiflora sp. 1

Physalis peruviana

Hylocereus polyrhizus (Dragon fruit)

Theobroma cacao (during fermentation)

Diospyros kaki 1

Passiflora sp. 2

307 isolated strains from fruits and fermented foods

Diospyros kaki 2

Anana comosus

Persea Americana

Malus sp.

Eugenia jambos

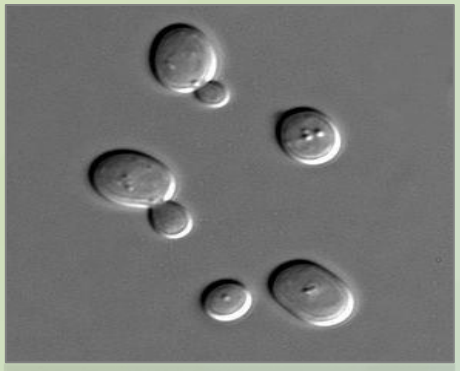
Pyrus sp.

Prunus persica

Step 2. Strain identification…

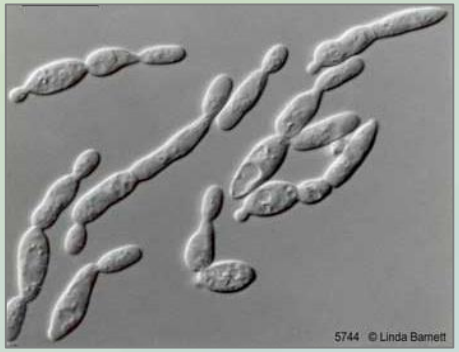
Identified species

(> 99% homology with known sequences)



Saccharomyces cerevisiae

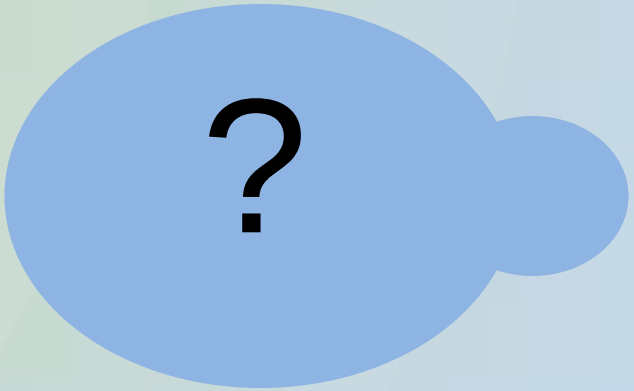
EB27 – 99,5%



Sporidiobolus pararoseus

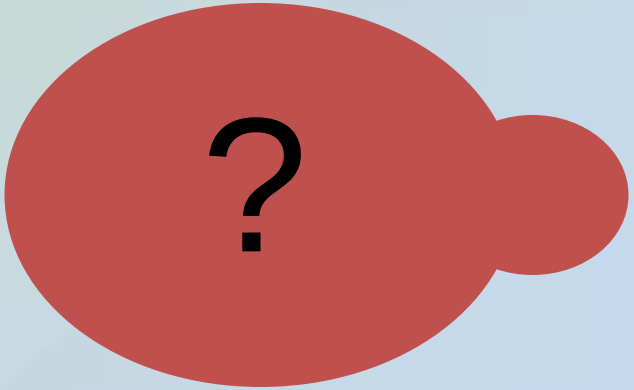
NRKAP3 – 100%

New yeast species



Candida sp. ?

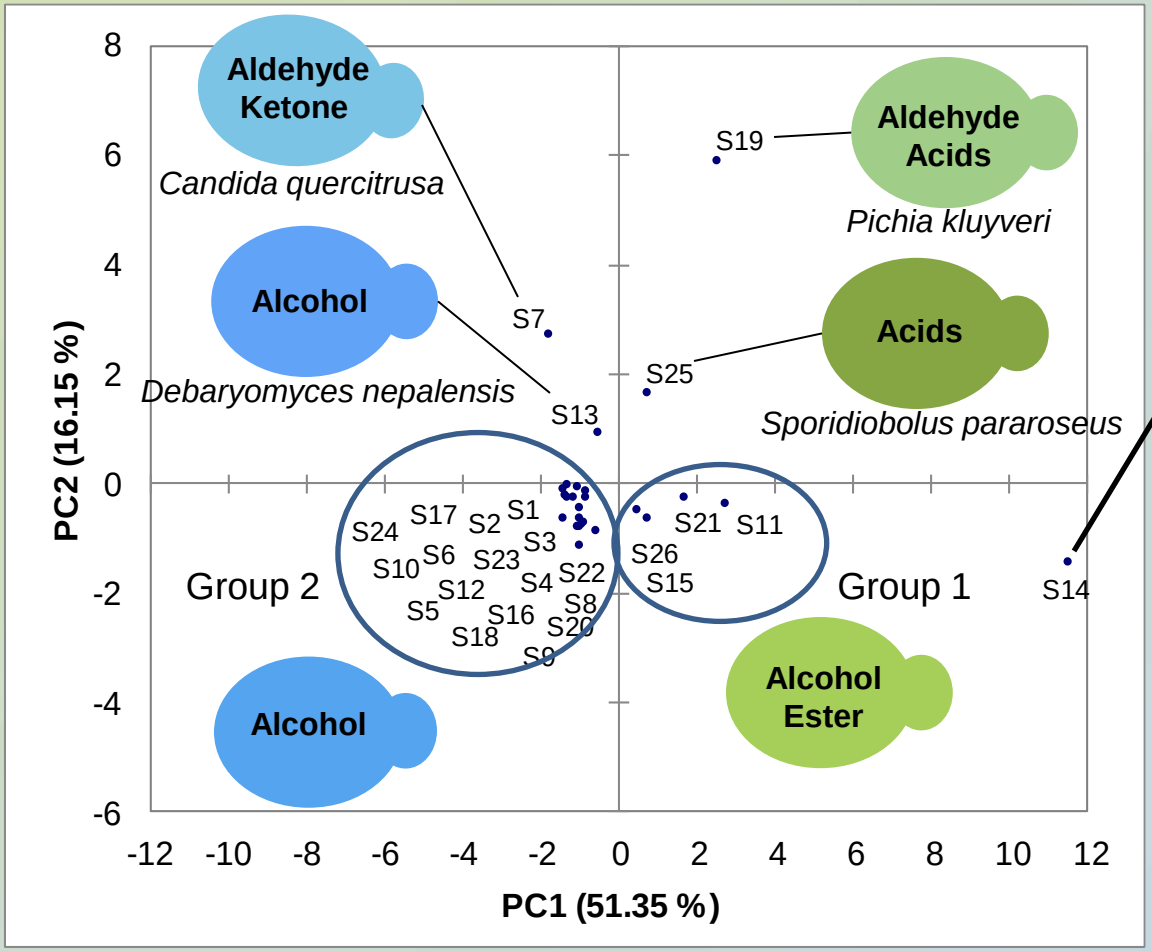
EB23 97,4%



Rhodotorula sp. ?

EGPOC17 – 97,1%

Step 3. Determination of volatile compounds



Principal Component Analysis (PCA) was used to compare the performance of the strains according to their flavor profile (analyzed by HS–SPME–GC/MS)

Geotrichum fragrans

32 VOCs
28 Esters
(6 unsaturated esters)
4 alcohols

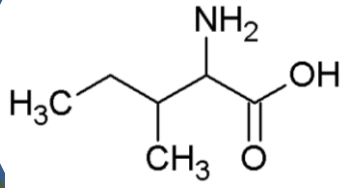
Ethyl tiglate

Grondin et al. (2014). Yeast 32, 57-66
Grondin et al. (2015). IJFM 203,101-108

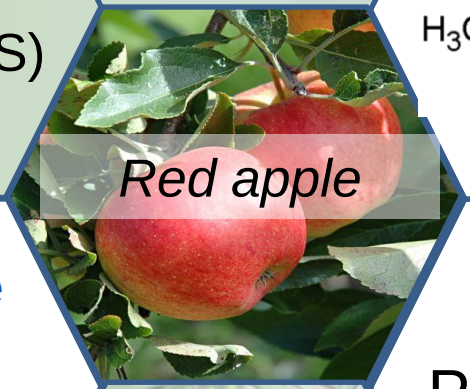
Ethyl-tiglate production by *S. suaveolens*

Natural flavour (FEMA GRAS)

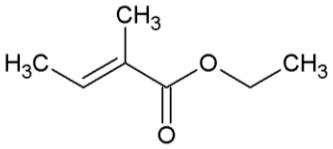
Isoleucine



Perfums
Foods

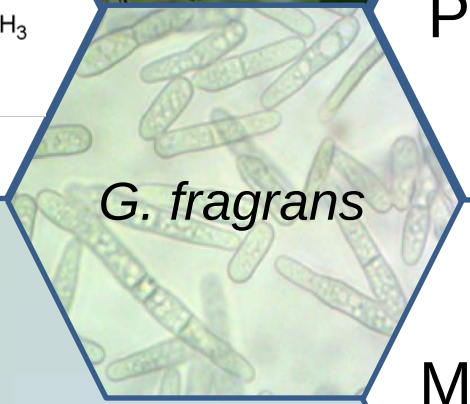


Ethyl tiglate



Precursors?

Mainly produced by chemical synthesis



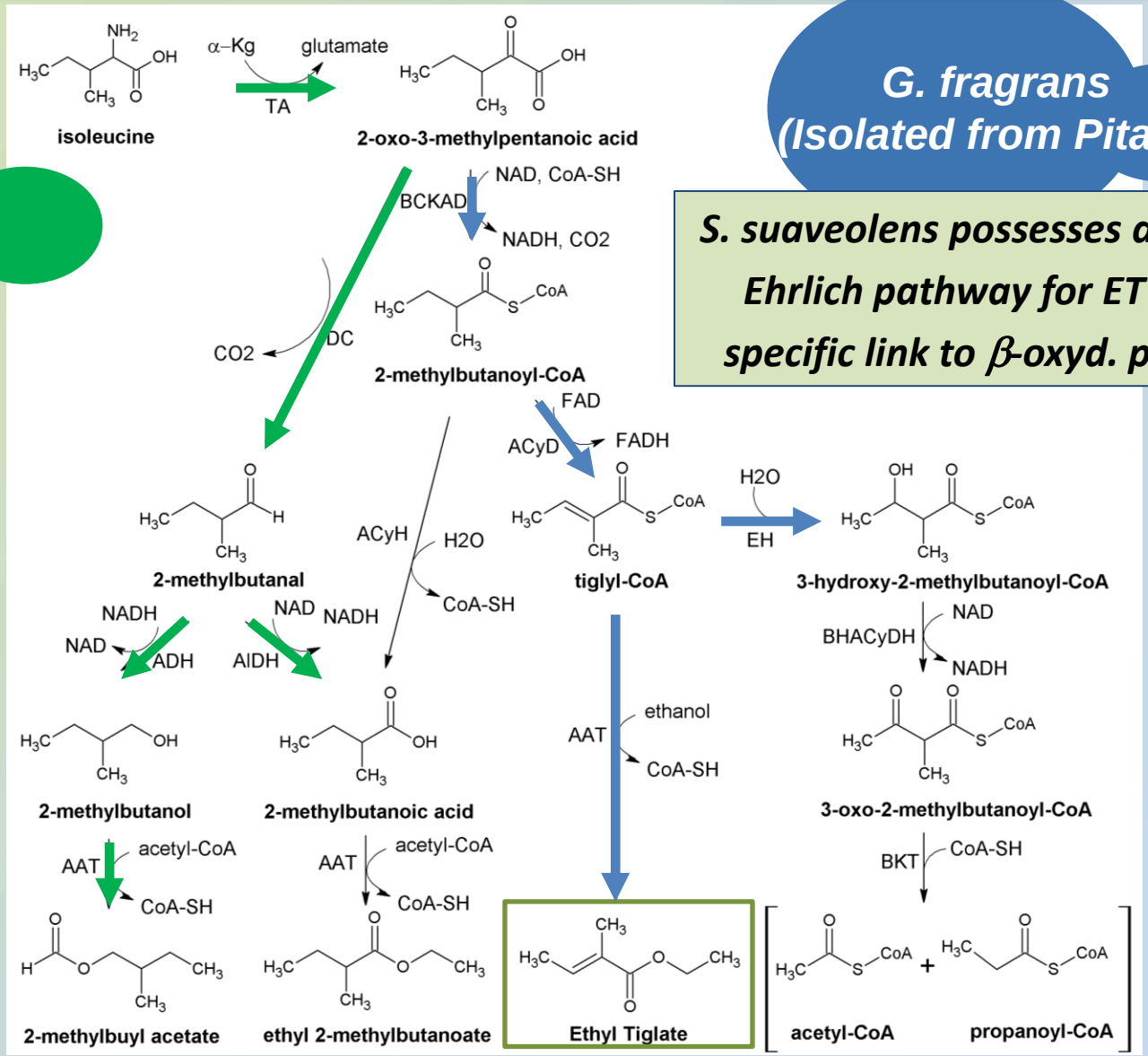
Métabolism?

Ethyl-tiglate production by *S. suaveolens*

***S. cerevisiae* (Control strain)**

***G. fragrans* (Isolated from Pitaya)**

S. suaveolens possesses a modified Ehrlich pathway for ET (with a specific link to β-oxyd. pathway)

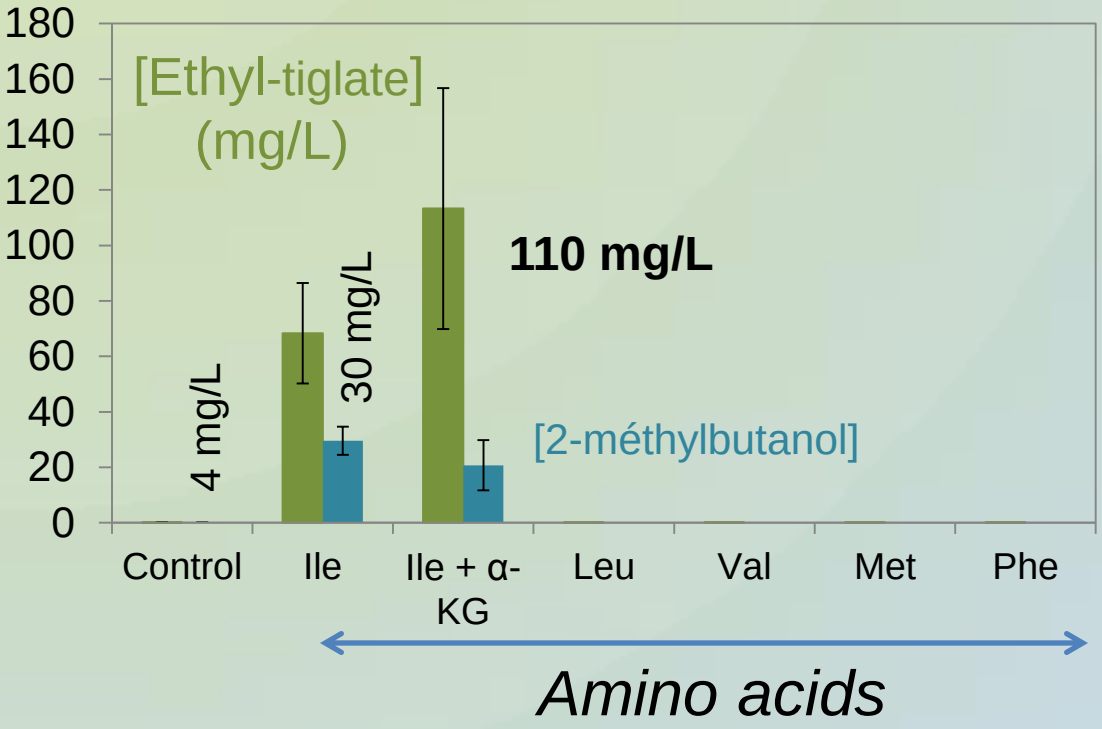


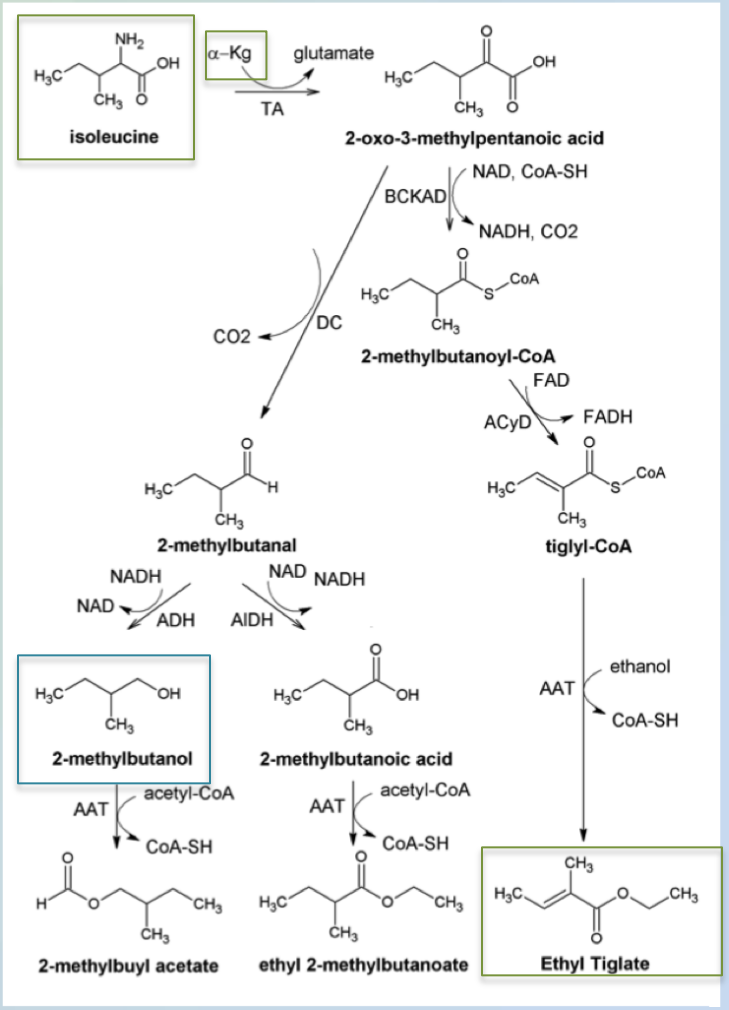
Ehrlich pathway

Ethyl tiglate and β-ox pathways

Ethyl-tiglate production by *S. suaveolens*

Ehrlich pathway





Ethyl tiglate production could be significantly improved using precusors like iseuleucine and αKG

Concluding remarks…

- **Functionnal screening of microbial diversity** is a powerfull tool to discover new biocatalysts to produce natural chemicals for industry that can compete with synthetic chemistry and biology (GMO)

- **European « Cost Bioflavour » project** was a good starting point to explore microbial diversity in Indian Ocean area

- The work could be generalized to explore the microbial diversity from other ecological niches and different industrial applications (flavours, pigments, industrial enzymes, biofuels, food processing…)...

Follow-up discussion on next Thursday (14h FST/LCSNSA)…

THAT'S ALL FOR NOW FOLKS !

Microbial community of fermented food (Cocoa)

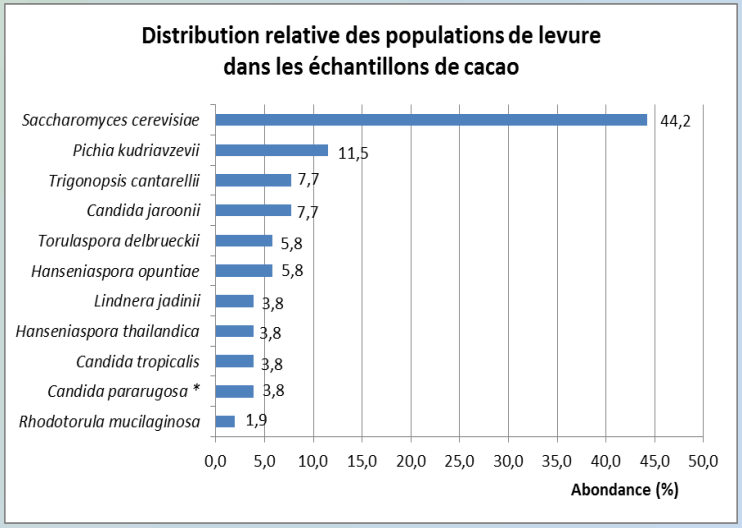
Role of microorganisms in cocoa processing (*var. Criollo from Malagasy*) and links between microflora and product quality?

Maturation of Cocoa pod

Fève fraîche avec mucilage

Fève sèche fermentée

3 to 5 days fermentation

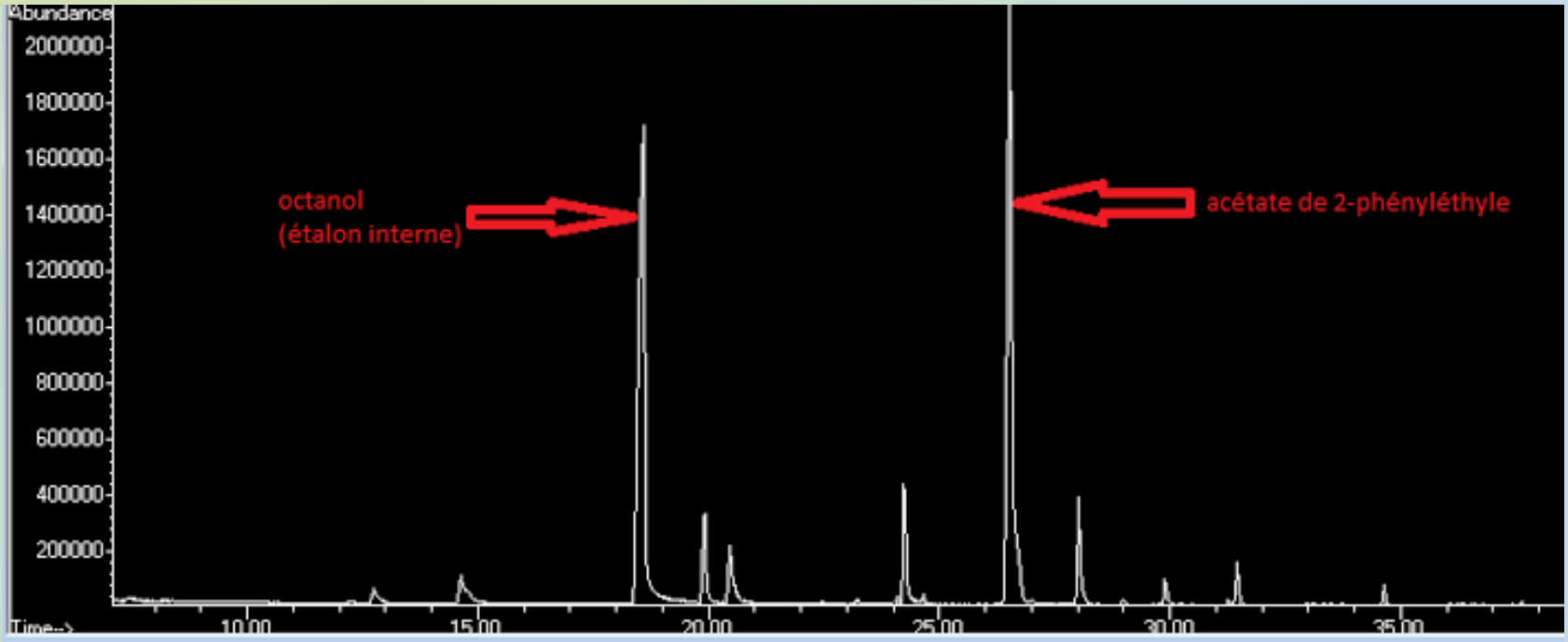


Distribution of native flora

- More than 500 isolated strains (206 yeasts, 265 LAB, 98 AAB)
- Atypical *S. cerevisiae* strains predominate during fermentation (44%)
- Lots of putative new strains isolated (Seq. identity D1/D2 < 97%)

Microbial community of fermented food (Cocoa)

- Strong production of natural 2-phenylethyl acetate 2PEAc (> 4 g/L !!!)



- 2-PEAc is a highly valued natural volatile ester with a rose/honey-like odour and is widely used to add flavour to soaps, foods and drinks
- 2-PEAc production is generally around 1 g/L (GMO)
- Best reported resistance of cells to 2-PEAc is 2,5 g/L
- Room for implementation in industry as new biocatalyst? Patenting?