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Yeasts: Mini-factories Producing Tailored Lipids For Green Chemistry. When Infrared Light Reveals Cell Metabolism

Marine Froissard

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Yeasts: Mini-factories Producing Tailored Lipids For Green Chemistry. When Infrared Light Reveals Cell Metabolism.



LIPIDS ARE STORED IN LIPID DROPLETS (LDs)

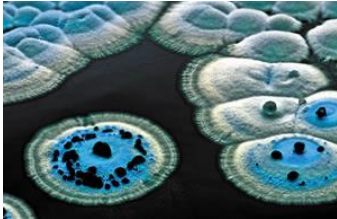
Mammals
Homo sapiens



From Asterix official website

Christelle CEBO
INRA GABI

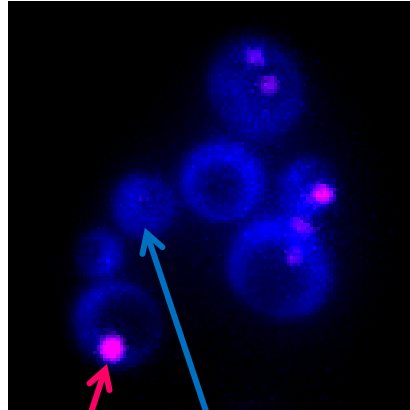
Bacteria
Streptomyces sp.



From Thompson et al. Genome Biology 2002

Marie-Joëlle VIROLLE
CNRS IGM

Unicellular eukaryotes
Saccharomyces cerevisiae



LD Tryptophan

Frédéric JAMME
Matthieu REFREGIERS



Plants
Brassica napus



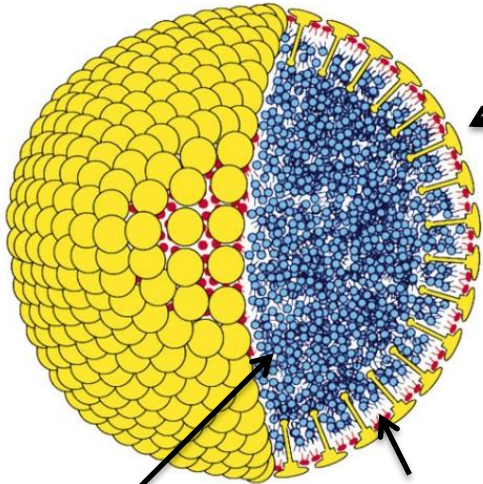
Jean WEBER / © INRA

LIPIDS ARE STORED IN LIPID DROPLETS (LD)

Plants

Arabidopsis thaliana / *Brassica napus*

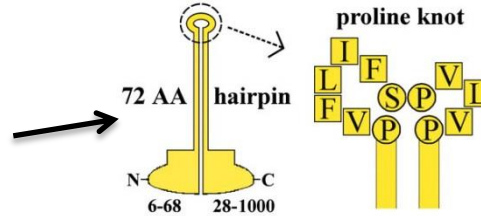
seed lipid droplet = oleosome



Lipid core =
triacylglycerols
and sterol esters

phospholipid
monolayer

oleosin
caleosin



From Hsieh et al. (2004) Plant Physiol

Predicted structure = tri-block organization

- ➔ variable N- and C- termini
- ➔ hydrophobic central domain

WHY STUDYING LDs?

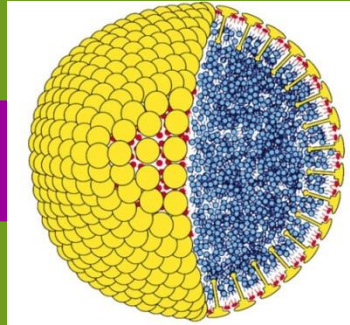
Understanding the dynamics

➔ **Oils** for food, biofuel and green chemistry are extracted from LDs

BUT

➔ LDs have a crucial role in **diseases with increasing prevalence** (obesity, diabetes).

filling



mobilization

stabilization



WHY STUDYING LDs AND ASSOCIATED PROTEINS?

Valorization of LDs and associated proteins

➔ **food processing industry, cosmetic and health** : LDs are natural emulsions and **nanovectors**, and oleosins can form **suprastructures**

BUT

➔ **Oleosins** (from peanut and hazelnut) are **allergens**



Biotechnol. Prog. 2005, 21, 1297–1301

1297

Elevating Bioavailability of Cyclosporine A via Encapsulation in Artificial Oil Bodies Stabilized by Caleosin

Miles C. M. Chen,[†] Jui-Ling Wang,[‡] and Jason T. C. Tzen^{*,†}

Graduate institute of Biotechnology, National Chung-Hsing University, Taichung, Taiwan, and Department of Physiology, National Yang-Ming University, Medical College, Taipei, Taiwan



Self-assembly of tunable protein suprastructures from recombinant oleosin

Kevin B. Vargo^a, Ranganath Parthasarathy^a, and Daniel A. Hammer^{a,b,1}

^aChemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA 19104; and ^bBioengineering, University of Pennsylvania, Philadelphia, PA 19104

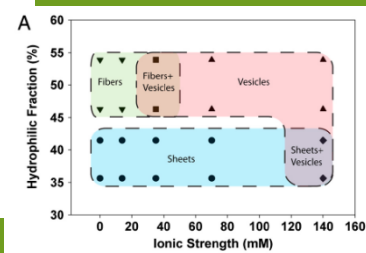
Edited by* David A. Tirrell, California Institute of Technology, Pasadena, CA, and approved June 5, 2012 (received for review April 3, 2012)

Using recombinant amphiphilic proteins to self-assemble suprastructures would allow precise control over surfactant chemistry and the facile incorporation of biological functionality. We used cryo-TEM to confirm self-assembled structures from recombinantly produced mutants of the naturally occurring sunflower protein, oleosin. We studied the phase behavior of protein self-assembly

although the direct visualization of a bilayer membrane or vesicular encapsulation has not been explicitly shown (12).

While a number of naturally occurring proteins, such as hydrophobins (13), oleosins (14), latherin (15), and ranaspumin (16), are known to stabilize interfaces, only oleosins are structurally reminiscent of a chain surfactant. Oleosins are a family of plant

PNAS



.05

WHY STUDYING LDs AND ASSOCIATED PROTEINS?

Valorization of LDs and associated proteins

- ➔ **food processing industry, cosmetic and health** : LDs are natural emulsions and **nanovectors**, and oleosins can form **suprastructures**



T. Chardot talk Structural studies of proteins inserted in a half membrane using SOLEIL synchrotron Light



Self-assembly of tunable protein suprastructures from recombinant oleosin

Kevin B. Vargo^a, Ranganath Parthasarathy^a, and Daniel A. Hammer^{a,b,1}

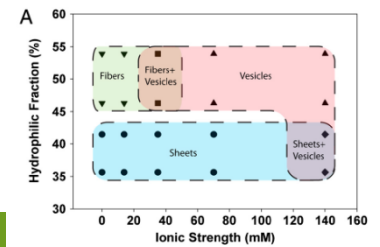
^aChemical and Biomolecular Engineering, University of Pennsylvania, Philadelphia, PA 19104; and ^bBioengineering, University of Pennsylvania, Philadelphia, PA 19104

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WHY STUDYING LDs AND ASSOCIATED PROTEINS?

Valorization

→ for
are n
can f

→ Ole

US 2012/0301932 A1

DU PONT DE NEMOURS AND CO

Nov. 29, 2012

EXPRESSION OF CALEOSIN IN RECOMBINANT OLEAGINOUS MICROORGANISMS TO INCREASE OIL CONTENT THEREIN

[0001] This application claims the benefit of U.S. Provisional Application No. 61/490,337, filed May 26, 2011, which is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0002] This invention is in the field of biotechnology. More specifically, this invention pertains to recombinant oleaginous microorganisms that are capable of producing more oil due to the expression of a caleosin polypeptide.

BACKGROUND OF THE INVENTION

[0003] Microorganisms such as filamentous fungi, yeast and algae produce a variety of lipids, including fatty acids,

However, there is still a need for recombinant oleaginous microorganisms having increased oil content relative to the oil of currently known strains.

[0006] U.S. Pat. No. 7,256,014 discloses that the expression of at least one plant oleosin gene in a microbial cell engineered to produce a hydrophobic/lipophilic compound, such as a carotenoid, significantly increases the overall titer of the compound.

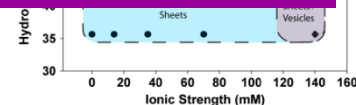
[0007] Froissard et al. (*FEMS Yeast Res.* 9:428-438, 2009) disclose that the non-oleaginous yeast, *Saccharomyces cerevisiae*, transformed with a heterologous gene encoding a caleosin polypeptide (*Arabidopsis thaliana* caleosin 1, AtClol), exhibited an increase in the number and size of lipid bodies and accumulated more fatty acids than the parent strain.

[0008] However, there are no reports of recombinant oleaginous microorganisms transformed with a gene encoding a caleosin polypeptide to increase the oil content of such recombinant microbial cells.



and the facile incorporation of biological functionality. We used cryo-TEM to confirm self-assembled structures from recombinantly produced mutants of the naturally occurring sunflower protein, oleosin. We studied the phase behavior of protein self-assembly

While a number of naturally occurring proteins, such as hydrophobins (13), oleosins (14), latherin (15), and ranaspumin (16), are known to stabilize interfaces, only oleosins are structurally reminiscent of a chain surfactant. Oleosins are a family of plant



1297

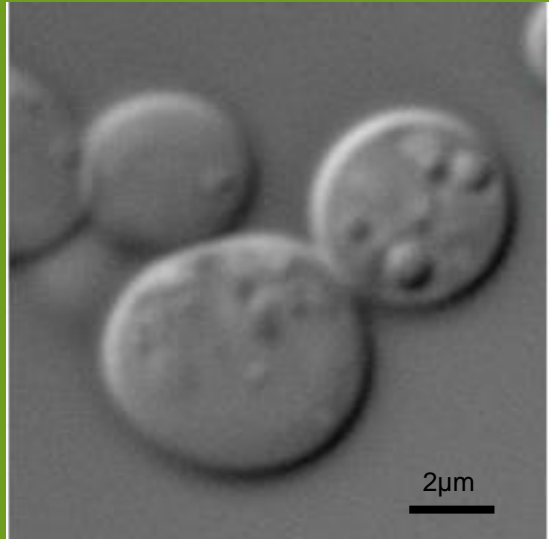
ulation in

Department of

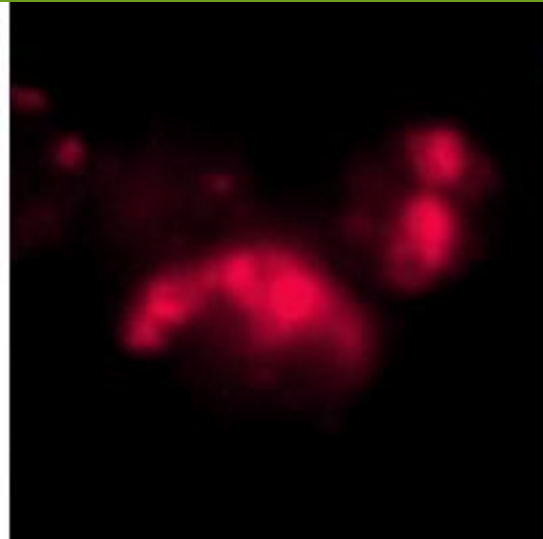
.07

HETEROLOGOUS EXPRESSION OF OLEOSINS IN YEAST

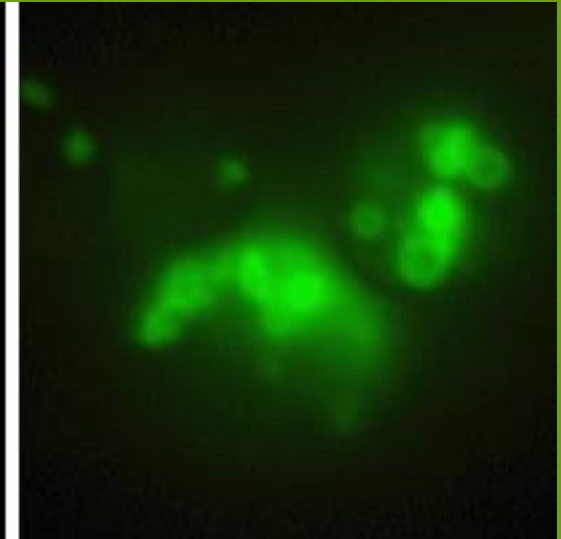
Ole1 and Clo1 oleosins are targeted to LDs in yeast



Nomarski



Erg6p-RFP



AtClo1-GFP

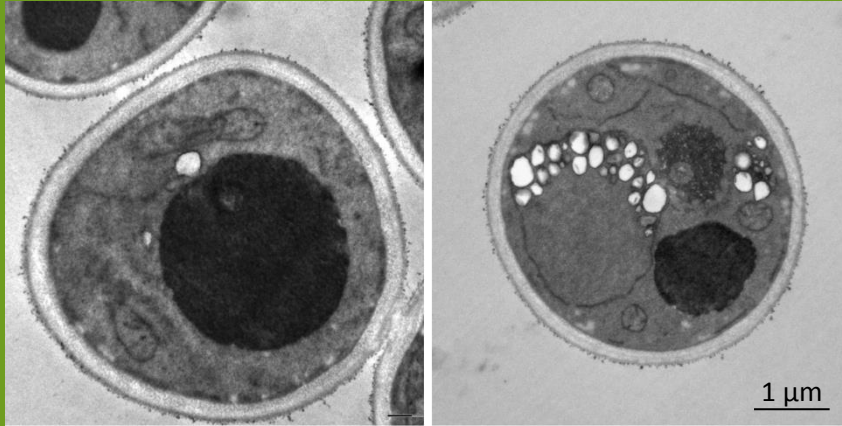
Froissard *et al.* 2009. FEMS Yeast Research

Boulard and Froissard. 2012. Techniques de l'Ingénieur. re209

.08

HETEROLOGOUS EXPRESSION OF OLEOSINS IN YEAST

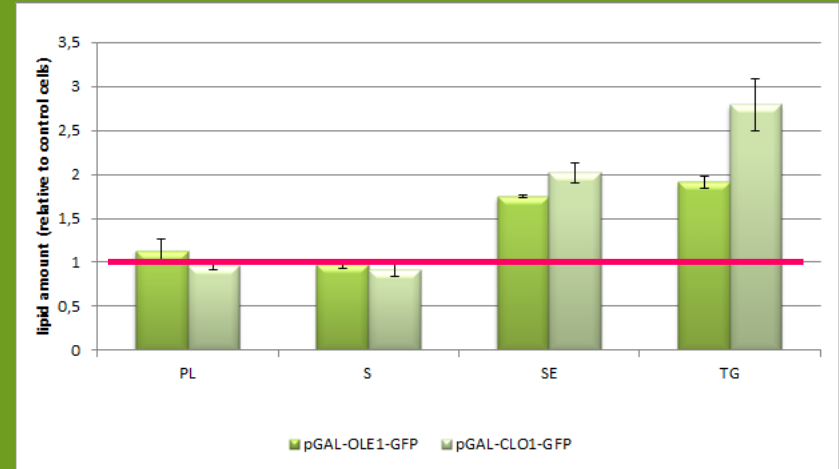
Oleosins induce LD proliferation



Control

+ AtOle1-GFP

oleosins induce triacylglycerols (TG) and steryl esters (SE) accumulation

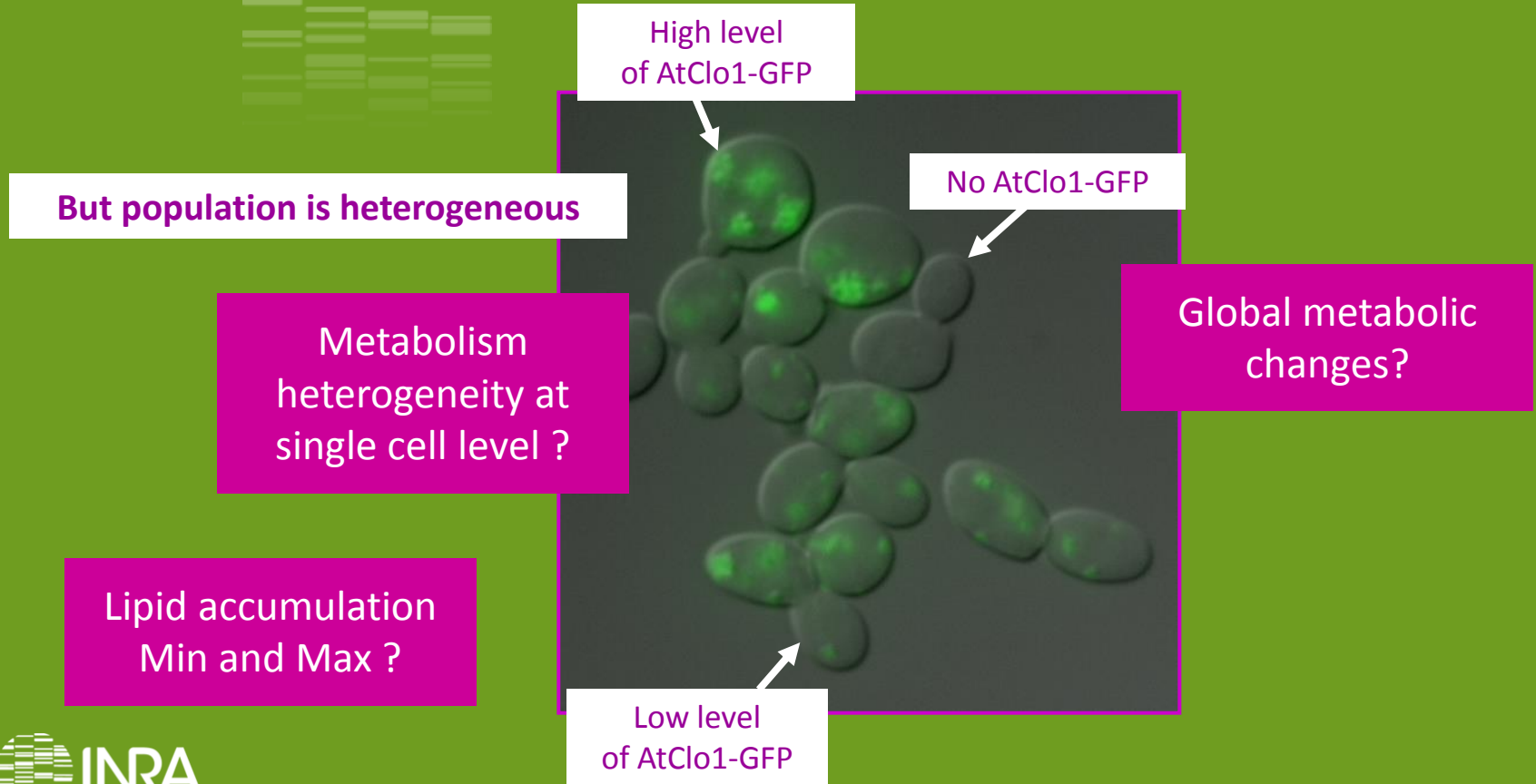


Christine LONGIN
Sophie CHAT

Froissard *et al.* 2009. FEMS Yeast Research
Boulard and Froissard. 2012. Techniques de l'Ingénieur. re209

.09

METABOLISM EXPLORATION USING SYNCHROTRON FTIR



METABOLISM EXPLORATION USING SYNCHROTRON FTIR



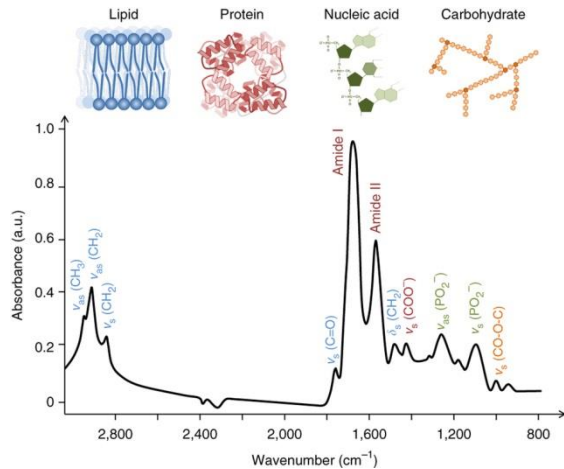
= Spectroscopie et Microscopie Infrarouge avec Synchrotron



FTIR = Vibrational spectroscopy

Spectral fingerprints of biological macromolecules

➔ Cell metabolism overview



From Baker et al. Nature Protocols. 2014

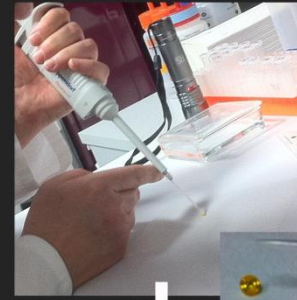
Brightness of Synchrotron Light

+ ZnSe hemisphere

high spectral and spatial resolution

+

Deposit of 2 μL cell suspension
on ZnSe hemisphere
(50 μm diameter window)

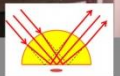


low vacuum drying

Hemisphere installation under the microscope
and FT-IR spectra acquisition



- 4 x 4 μm spatial resolution
- single cell spectra acquisition



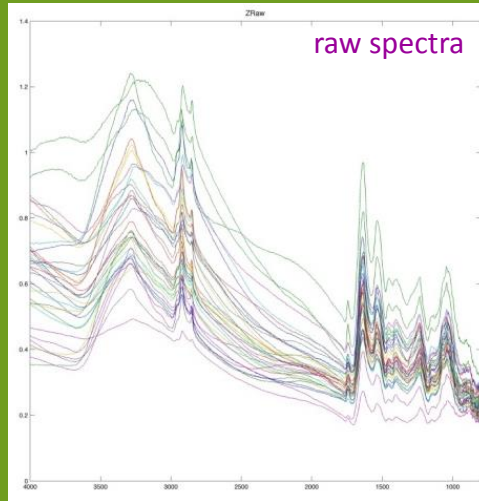
METABOLISM EXPLORATION USING SYNCHROTRON FTIR



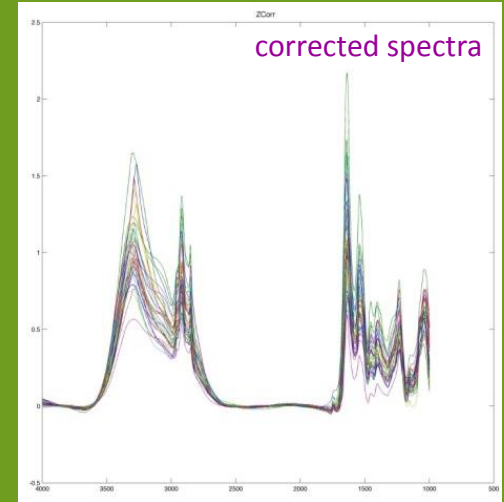
= Spectroscopie et Microscopie Infrarouge avec Synchrotron



Acquisition of 50 spectra



Matlab
Pretreatment
RMieS-EMSC
correction



Spectra baseline deformation due to light scattering

- Round shape of cells
- Small size of cells
- Wavelength range of mid-infrared



Frédéric JAMME

METABOLISM EXPLORATION USING SYNCHROTRON FTIR

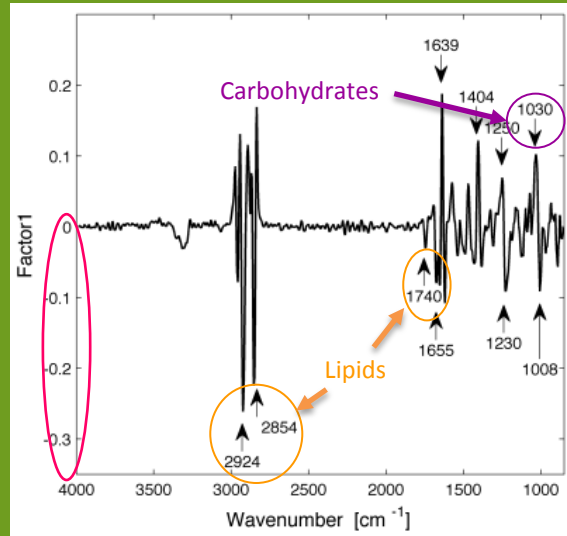
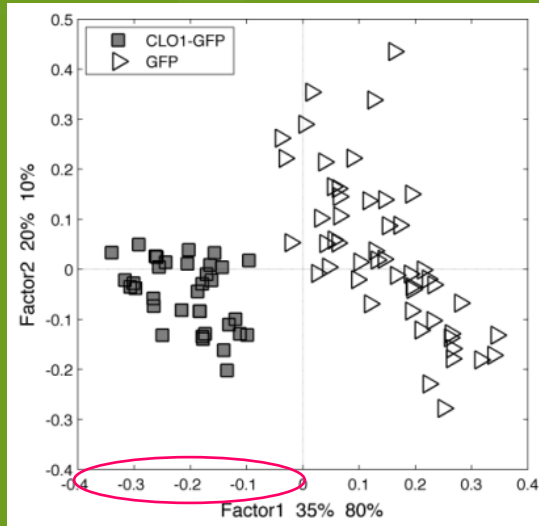


= Spectroscopie et Microscopie Infrarouge avec Synchrotron



Frédéric JAMME

Principal Component
Analysis (PCA)

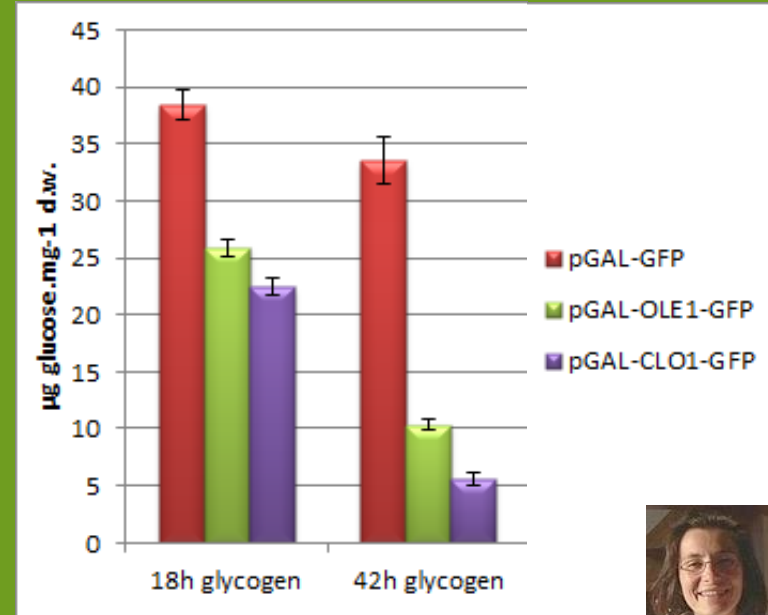
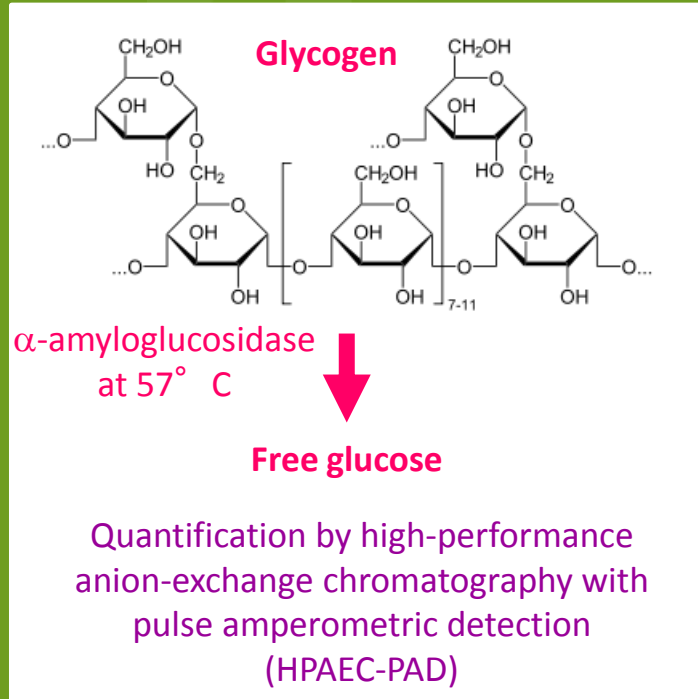


Population separation among Factor 1

More lipids and less carbohydrates in AtCLO1-GFP expressing cells

METABOLISM EXPLORATION USING SYNCHROTRON FTIR

Metabolic modifications were confirmed using biochemical analysis



Valérie MECHIN
INRA IJPB

.014

Jamme *et al.* 2013. PLOS ONE.

ADDITIONAL INFORMATION

SOLEIL communication



RECHERCHER SUR LE SITE OK PLAN DU SITE | ACCÈS | ANNUAIRE | CONTACTS | PARTENAIRES | LIENS

RECHERCHE INSTRUMENTATION
INDUSTRIE et VALORISATION LA SOCIÉTÉ SOLEIL
SOURCES et ACCÉLÉRATEURS

DISCO
Huiles végétales et dérivés. Etude structurale d'une oléosine, protéine au centre d'enjeux énergétiques et médicaux



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RECHERCHE INSTRUMENTATION
INDUSTRIE et VALORISATION LA SOCIÉTÉ SOLEIL
SOURCES et ACCÉLÉRATEURS

SMIS
Chimie verte : la lumière infrarouge dévoile le métabolisme cellulaire des levures, mini-usines de production de lipides à façon

Dans le contexte actuel d'épuisement des ressources des huiles issues de la biomasse et la chimie verte ne remplacent un jour les produits d'origine fossile. L'équipe DISCO (Dynamique et Structure des Complexes) travaille à identifier des facteurs influant sur le métabolisme des microorganismes, mais aussi à favoriser le développement d'alternatives durables. La technique de dichroïsme circulaire permet d'étudier les conditions physiologiques, des données sur la structure des huiles chez les plantes oléagineuses.

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RECHERCHE INSTRUMENTATION
INDUSTRIE et VALORISATION LA SOCIÉTÉ SOLEIL
SOURCES et ACCÉLÉRATEURS

SMIS - Chimie verte

L'utilisation combinée du faisceau synchrotron de la ligne SMIS et d'hémisphère en ZnSe a permis aux chercheurs de l'INRA d'obtenir des spectres infrarouge sur des levures uniques. L'analyse comparative de spectres infrarouge de levures riches en lipides en huile a révélé de fortes modifications du métabolisme entre souches ayant une capacité variable de stockage de lipides. Ce travail permet de comprendre les mécanismes cellulaires mis en jeu lors du stockage de l'huile, afin de développer des levures performantes pour des applications en chimie verte.



Mise en évidence des modifications métaboliques induites par l'accumulation de lipides dans la levure *S. cerevisiae* par sFTIR sur cellules uniques

quiers
ammi³ J.D. Vindigni¹
Institut Jean-Pierre Bourgin, INRA AgroParisFrance
INRA, Nantes, France
SOLEIL, Gif-sur-Yvette, France
Institut Jean-Pierre Bourgin, Versailles
versailles.inra.fr

Dans le contexte actuel d'épuisement des ressources fossiles, d'augmentation du pétrole et de protection de l'environnement, la valorisation énergétique des huiles issues de la biomasse et la chimie verte prennent de l'importance. En effet, ces huiles et leurs dérivés biodégradables sentent un intérêt grandissant en remplacement de produits d'origine fossile. Deux sources sont privilégiées, celle des huiles végétales déjà bien implantées, celle des huiles de microorganismes (algues) et celle des huiles de déchets. C'est dans ce cadre et actuellement en plein essor. C'est dans ce cadre et actuellement nos recherches visant à identifier des facteurs influant sur la qualité et la quantité de lipides

INRA en lumière, 5 ans de partenariat avec SOLEIL

INRA communication



LES CENTRES INRA
VERSAILLES-GRIGNON

ACCUEIL ACTUALITÉS LA CENTRE ET LES RECHERCHES OUTILS ET RESSOURCES PARTENAIRES et VALORISATION ÉVÉNEMENTS CONTACT

Centre Inra - Versailles-Grignon - Toutes les actualités - 201512 Production Lipides levures

1, 2, 3 Soleil ou la merveilleuse histoire d'un système innovant de production de lipides d'intérêt chez la levure

L'expression d'une protéine de plante chez une levure induit une suraccumulation de lipides. Dans le cadre d'un partenariat avec le Synchrotron Soleil, les chercheurs de l'Inra Versailles-Grignon ont exploré plus avant les mécanismes dans la perspective d'utiliser ces microorganismes pour produire des lipides d'intérêt dans les secteurs de l'énergie, la nutrition, la santé ou encore la chimie du carbone renouvelable.

LIRE AUSSI
Des levures qui carburent
Synchrotron Soleil

MÉDIATHÈQUE
Les médias les plus récents :
Accéder à la médiathèque

THANKS TO



Institut Jean-Pierre Bourgin

Dynamique et Structure des Corps Lipidiques (Actual)

Thierry CHARDOT, INRA (Head)

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Pascale JOLIVET, INRA

Sabine D'ANDREA, AgroParisTech

Yann GOHON, AgroParisTech

Isabelle BOUCHEZ, INRA

Carine DERUYFFELAERE, INRA

Franjo JAGIC, INRA

Michel CANONGE, AgroParisTech

Roselyne TACHE, INRA

Bernard CINTRAT, AgroParisTech

Zita PURKRTOVA CDD CAER DGA

Jean-David VINDIGNI (PhD, past)

Différentiation et Polarité Cellulaire (Future)

Jean-Denis FAURE, AgroParisTech (Head)

SOLEIL

DISCO

Matthieu REFREGIERS (Beamline leader)

Frédéric JAMME

Alexandre GIULIANI

Frank WIEN

Valérie ROUAM

Bertrand CINQUIN



SMIS

Paul DUMAS (Beamline leader)

Frédéric JAMME



and SOLEIL staff



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