Targeted genome engineering, a promising solution for redesigning the metabolic potential of microalgae

Fayza Daboussi, Head of the Synthetic Biology in Microalgae Team
Microalgae, photosynthetic cell biofactories

Photosynthesis

- CO₂ as a substrate for the synthesis of biomolecules
- Short doubling time (few hours)

Combine properties of plant and microorganism

Nucleus

- Organic compounds
- Nutrients

Calvin cycle

- ATP
- NADPH
- O₂

Biomass

- Carbohydrates
- Lipids
- PUFA

Carotenogenesis

- Astaxanthin
- β-Carotene

Isoprenoids

- β-Carotene
- Astaxanthin

Microalgae
Microalgae, rough diamonds of biotechnology

A huge reservoir of marketable products

- **Nutrition**: $4 Billion
  - 2 Years
- **Aquaculture**: $700 Million
  - 2 Years
- **Nutraceutics**:
  - 10 Years
  - 20 Years
  - Energy
- **Green chemistry**: Mass Market
  - Low value
  - 20 Years

Barriers

Pulz and Gross, 2004
Hurdles and challenges for microalgae biotechnological field

Barriers at each steps of the value chain

- Economic
  - Valorization
    - Market visibility
    - Legislation
  - Extraction
    - Co-products
    - Secretion
  - Harvesting
    - Dewatering
    - Flocculation/Flotation
  - Cultivation
    - Water management
    - Crop protection
  - Strains
    - Species selection
    - Creation of new tools

Research axes to overcome biological bottlenecks

- Biological
  - Exploring the biodiversity (Oceanographic campaigns)
  - Exploiting the biodiversity (Starvation, light, salinity)
  - Engineering strain to improve their performances

Technological
Objective: Create platform organism for algal synthetic biology

Synthetic Biology:
- Re-design existing biosynthetic pathways
- Design and construct novel functions and systems

- Develop genome engineering methodologies
- Redesign the metabolic potential of microalgae
- Increase Knowledge on cellular metabolism
Genome engineering methodologies available

**Forward genetic**
- Random mutagenesis
  - UV radiation
  - or EMS
- Insertionnal mutagenesis
  - Antibiotic resistance marker
- Direct Screening
- Oil production
- Light
- Lipid modification
- Fast growing
- Chemical
- Temperature fluctuation

**Reverse genetic**
- Heterologous expression
- Metabolic engineering
- Gene silencing
- Homologous Recombination
- Overexpression
- Transgenics

D'après Vuttipongchaikij, Thai J Genet (2012)
Genome engineering methodologies available

**Forward genetic**
- Random mutagenesis
  - UV radiation
  - UV radiation or EMS
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  - Antibiotic resistance marker

**Reverse genetic**
- Heterologous expression
- Metabolic engineering
- Gene silencing
- Homologous Recombination

**Major advances**
- Trophic conversion / Resistance to oxydative damages / Modification of the quality/quantity of lipids

**Limitations**
- Uncontrol genome modification
- Switch-off often incomplete
  - HR occurred at low frequency
  - Deletion of specific target genes difficult
Targeted genome engineering using engineered nucleases

In microalgae, homologous recombination frequency $<10^{-6}$

Frequency induced using engineered nucleases

Gene insertion

Gene Inactivation

Smith et al., Nucleic acid research 2006
Moscou and Bogdanove, Science 2010
Christian et al., Genetics 2011
Targeted genome engineering using engineered nucleases

In microalgae, homologous recombination frequency <10^-6

Frequency induced using engineered nucleases

Major Breakthrough for reverse genetic
A revolution for metabolic engineers

Gene insertion
Overexpress enzymes of a limiting step
Introduce new metabolic pathways

Gene Inactivation
Delete competing pathways

Smith et al., Nucleic acid research 2006
Moscou and Bogdanove., Science 2010
Christian et al., Genetics 2011

Engineered Nuclease
Chromosome
Cut

Paste
Loss of a few base pairs
Several classes of molecular scissors

- Meganucleases (2000)
- TALE Nucleases (2011)
- CRISPR/Cas9 system (2012)

*Puchta and Fauser, Plant J, 2013*
**Transcription Activator Like effectors**

In an infected plant cell, *Xanthomonas* uses TAL effectors to bind DNA. TALEs bind DNA and activate disease-promoting genes. This leads to disease (S). Simultaneously, resistance genes are activated (R), leading to resistance. Adapted from Bogdanove, 2010.
**Transcription Activator Like effectors Nucleases**

**TALE structure**

- Nterm
- DNA binding domain
- Cterm

**Natural code**

```
CTAGAGTCGCGTGT
```

*Moscov & Bogdanove, Science 2009*

**First generation of TALEN**

```
TCTGGAGCTGACAGTG
```

*Dna sequence to edit*

```
ATACGCATGACAATG
```

*Christian et al, Genetics 2010*
**Functioning of the type II CRISPR-Cas systems in bacteria**

**Defense mechanism against phage**

**Memorisation step:**
- Viral DNA inserted into the bacterial genome

**«immune response» step:**
1) Transcription of viral fragment + recognition RNA #1 (crRNA)
2) Assembly with recognition RNA # 2 (TracrRNA) and Cas9
3) Maturation
4) Recognition of phage DNA and cleavage by Cas9
An engineered Cas9 system

Genome-editing platforms

Mahlouz et al 2011

Mahlouz et al 2014
Targeted genome engineering in various application fields
Phaeodactylum tricornutum, an attractive organism

Microalgae, a huge diversity (100,000 to 1 million species) **BUT** less than 15 species suited as a biotechnological crop

- 30% lipids per dry weight
- 50% lipids per dry weight in nitrogen starvation
- Fatty-acid profile with 30% of C20:5 and 26% of C16:1
- Ability to be cultivated in mixotrophy
- Growth in the absence of silica
- Availability of the genomic sequence (2.10^7 pb)
- Provision of genetic tools

Domergue et al. Plant Physiology 2003
Proof of concept in genetic and metabolic engineering

- Demonstration of both targeted gene insertion and targeted gene inactivation using engineered meganucleases

- Creation of a strain with a high increase in triacylglycerol content
Open the way

Biochemical Pathways
Establish *P. tricornutum* as an industrial biotechnology host

Rewriting the lipid metabolic network
Multi-gene modifications

Building new metabolic pathways
Fine-tune gene expression

Increase fatty acids or TAG production
Modify the quality of fatty acids

Biodiesel/ Biokerosen $<$ C16:0

Omega3: C20:5, C22:4

Radakovits, 2010

Catabolism
Develop new strategies to succeed

New challenges

1. Improve transformation efficacy
2. Achieve high genome modification frequencies
3. Evaluate and control the safety of genome engineering
4. Develop an efficient gene expression toolbox

Proof of concept of metabolic engineering
Opening towards vast fields of investigation

- Overcome sensitivity to stress
- Improve photosynthetic activity
- Tolerate or defend against invasive species
- Optimize cultivation and harvesting processes
- Produce new compounds
- Increase compounds productivity

Genome engineering solutions
Thank you for your attention