award

ENI AWARD 2014

CANDIDATURE PROPOSAL

1. TITLE OF THE RESEARCH

Genetic Engineering of Cyanobacteria for Free Fatty Acid Production

2. PRIZE SECTION Please indicate the Prize section for which you propose your candidature by crossing the box

only one section can be chosen

3. SUBMISSION TO THE ENI AWARD Please indicate if the same research is being submitted for the first time or not

First time submission	x
Re-submission	☐

3.1. <u>IN CASE OF RESUBMISSION:</u> BRIEF EXPLANATION OF THE MOTIVES OF RESUBMITTING THE RESEARCH RESULTS ALREADY PRESENTED IN PAST EDITIONS.

Please specify in which edition of the ENI AWARD it had been submitted and justify the motives of the representation in this edition by highlighting the new developments of the research – **<u>1000 characters max incl. Spaces</u>**

4. MAIN AUTHOR(S) ACCOUNTABLE FOR THE RESEARCH RESULTS

In case of two main authors copy and paste the section below

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5. SECONDARY AUTHOR(S) OF THE RESEARCH RESULTS (IF ANY)

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6. DESCRIPTION OF THE RESEARCH RESULTS

Please note that the maximum number of **characters** allowed is **4500**, spaces included

Fuels derived from microalgae provide a renewable source of hydrocarbon-based energy that is not only compatible with the existing energy infrastructure, but will also help to reduce greenhouse gas emissions. While conventional algal biofuel systems produce natural lipids (fuel precursors) from eukaryotic microalgae, cyanobacteria offer the potential to directly produce drop-in replacement fuels and to tailor-design fuel properties via control of carbon chain length and degree of saturation through genetic engineering. Additionally, cyanobacteria are capable of excreting biodiesel precursors such as free fatty acids (FFAs). Biofuel precursor excretion enables the application of continuous production systems rather than the traditional batch production of algal biofuels. Moreover, this excretion mechanism allows for fuel separation without harvesting the microalgal biomass, reducing the amount of nutreints required for microalgal growth as well as the time required for growth. Research in my laboratory focuses on developing genetically engineered cyanobacteria for production of hydrocarbon-based fuels and fuel precursors. Over the past 4 years, we have demonstrated that cyanobacteria can be engineered to directly convert carbon dioxide into FFAs, precursors for the prodution of fatty acid methyl esters (biodiesel). In addition to this proof-of-principle demonstration, our work investigated new gene sources for improved FFA production. Two essential steps in FFA production include FFA cleavage from the acyl-acyl carrier protein (acyl-ACP), catalyzed by a thioesterase, and carboxylation of acetyl-CoA by acetyl-CoA carboxylase. The chloroplast associated acyl-ACP thioesterase and acetyl-CoA carboxylase genes were cloned from a green alga, *Chlamydomonas reinhardtii*, for FFA production in cyanobacteria. The algal acyl-ACP thioesterase was active in the cyanobacterial host and showed activity similar to a thioesterase from *Escherichia coli*. Expression of the acetyl-CoA carboxylase did not show increased FFA production, but this may be due to the negative effects of FFA production. Our research shows that FFA production is harmful to the cyanobacterial host, leading to reduced cell growth, decreased photosynthetic yields, reactive oxygen species generation, and significant changes in cell pigment concentration and localization. Analysis of cellular gene expression changes using RNA-seq confirmed that a severe stress response and other metabolic changes accompany FFA production in cyanobacteria. These physiological, genetic, and metabolic responses will ultimately limit cyanobacterial FFA production, and the reduced fitness of the host cell may lead to additional problems at large-scale production, including increased susceptiblity for contamination and instability of continuous processes. To address this problem, my work identified several genetic targets which led to improved cyanobactieral tolerance to FFA production. These targets include proteins that degrade reactive oxygen species, membrane transport proteins, and hypothetical proteins. Manipulation of these targets led to improved cell growth, photosynthetic yields, and FFA production in the cyanobacterial host. Through this research, we have demonstrated FFA production directly from carbon dioxide, identified new gene sources for FFA production, and improved FFA tolerance, an important obstacle in advancing cyanobacterial FFA production.

7. PLEASE LIST FROM ONE (minimum**) TO FIVE (**maximum**) PUBLICATIONS CONTAINING THE RESULTS OF THE RESEARCH PRESENTED WITH THIS CANDIDATURE AND AUTHORED BY THE MAIN SCIENTIST (**see point n° 4**).**
THE AFORESAID PUBLICATIONS HAVE TO BE SENT IN ELECTRONIC COPY AS MENTIONED IN THE OFFICIAL ANNOUNCEMENT:

Please insert Authors, Title and References for each publication

1. Ruffing AM. RNA-seq analysis and targeted mutagenesis for improved free fatty acid production in an engineered cyanobacterium. *Biotechnology for Biofuels.* 2013. 6:113.
2. Ruffing AM. Borrowing genes from *Chlamydomonas reinhardtii* for free fatty acid production in engineered cyanobacteria. *Journal of Applied Phycology.* 2013. 25(5): 1495-1507.

3. Ruffing AM, Jones HDT. Physiological effects of free fatty acid production in genetically engineered *Synechococcus elongates* PCC 7942. *Biotechnology and Bioengineering.* 2012. 100(9): 2190-2199. (Cover, Spotlight)

N.B.:

a. Candidatures must be compulsorily presented in English.

b. The Candidature, CV and list of publications must be sent to the Scientific Secretariat **before 22 NOVEMBER 2013 no later than 5.00 pm CET (Central European Time),** as requested in the Official Announcement.

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