Genetic Engineering of algae for higher biofuel value and biosafety issues

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Algae Production share by Country, World Markets: 2020

- Australia/New Zealand: 14%
- China: 21%
- Argentina: 6%
- Brazil: 2%
- EU: 10%
- USA: 47%

(from PIKE Research, LLC)
Less dependence on fossil Biodiesel

Advantage

CO₂ EMISSIONS OUTPUT BASED ON 100% BIODIESEL BURNT

Pitfalls

High production cost due to:
- Low lipid
- Low biomass
- Harvesting

Algal biofuel production is neither environmentally nor commercially sustainable

Source: National Research Energy Laboratory (US) and Massachusetts Institute of Technology
Diglyceride acyltransferase (DGAT) catalyzes the formation of triglycerides from diacylglycerol and Acyl-CoA. (Final and only committed step in TGs biosynthesis)
Impact of GM alga on yield (neutral lipid)

Altered lipid composition and enhanced lipid production in green microalga by introduction of brassica diacylglycerol acyltransferase 2

Irshad Ahmad¹, Anil K. Sharma², Henry Danielli³ and Shashi Kumar¹.*

<table>
<thead>
<tr>
<th>Algal cells</th>
<th>Dry Biomass (mg)</th>
<th>Total Lipid (mg)</th>
<th>Total Lipid (%)</th>
<th>Total FAME (mg)</th>
<th>Neutral Lipid (mg)</th>
<th>Neutral Lipid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>12.33±2.08</td>
<td>12.33</td>
<td>1.42±0.18</td>
<td>2.98±0.37</td>
<td>2.98</td>
</tr>
<tr>
<td>Transformed</td>
<td>100</td>
<td>18.76±1.96</td>
<td>18.76</td>
<td>3.68±0.16</td>
<td>7.69±0.34</td>
<td>7.69</td>
</tr>
</tbody>
</table>
Carbon Concentration Mechanism (CCM): Photosynthetic organisms develop CCM by inducing a set of genes (i.e. low carbon inducible transporter proteins, LCIA- LCIB). The algae concentrate dissolved inorganic carbon (DIC/Ci/HCO$_3^-$) and fix the CO$_2$ with the help of RuBisCo enzyme (in pyrenoid).
<table>
<thead>
<tr>
<th>Gene</th>
<th>Location</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCIA</td>
<td>CP Envelope</td>
<td>Transporter for bicarbonate (HCO₃⁻) from cytosol to stroma</td>
</tr>
<tr>
<td>LCIB</td>
<td>CP Stroma</td>
<td>Recycle unused CO₂ from photosynthetic process</td>
</tr>
</tbody>
</table>
Fig. Molecular analysis of wild type and transgenic *P. kessleri*-I. (a) LCIA–LCIB expression cassette in pCCMAB. (b) Transformed cell lines (T5, T8, T10, T12 and T14) were selected on hygromycin. (c) Transformed lines analysed by PCR primers, using LCIA and LCIB gene to produce amplicons ~726bp and ~565bp respectively. (d) RT-PCR of transforemd lines using LCIA-LCIB transgenes. (f) LCIB protein detection in transformed lines.
Transgene-proteins expression

Localization of LCIB in pyrenoid by antibody
TEM of transformed *P. kessleri*-l

Transmission Electron microscopy of wild type (A) and transgenics (B, T12; C, T14). S: starch, P: pyrenoid. Bars= 5µm.
Nile red stained & confocal images of *P. kessleri-I* (1) Differential interference contrast (DIC), (2) Fluorescences from lipid droplets, (3) Overlay image of fluorescences and DIC.
## Scale up study on 5\textsuperscript{th} and 10\textsuperscript{th} day

<table>
<thead>
<tr>
<th>Strain</th>
<th>Fresh Wt Biomass (g)</th>
<th>Dry Wt Biomass (g)</th>
<th>DW biomass productivity (mg/L/Day)</th>
<th>Lipid yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>55.0</td>
<td>7.0</td>
<td>21.65 ± 2.98</td>
<td>0.86</td>
</tr>
<tr>
<td>T12</td>
<td>75.0</td>
<td>10.0</td>
<td>39.37 ± 2.57</td>
<td>1.43</td>
</tr>
<tr>
<td>T14</td>
<td>80.0</td>
<td>12.0</td>
<td>83.25 ± 4.99</td>
<td>1.96</td>
</tr>
</tbody>
</table>

20-L growth of WT and Transgenic on 5\textsuperscript{th} day (left) and 10\textsuperscript{th} day (right)
Algae Biofuel

Developed CCM/GM marine alga Pk-I for commercial use

Neutral lipid ↑ via Bn-DGAT2 expression

Genetic Transformation Tools

Direct FAME from wet biomass

Biomass & lipid ↑ using endophytic fungi and easy harvesting.

Biomass + Bioremediation of wastewater

Efficient PBRs run on solar energy

Sustainable algae biofuels

Robust strain; High biomass-lipid; 0 cost medium for growth; Harvesting; FAME
• Green algae and cyanobacteria are photosynthetic organisms, but it may be ambiguous for algal species to be regulated under existing biotechnology regulations related to the Plant Protection Act.

• Due to the lack of experience with engineered algae, USA regulatory agencies (i.e. FDA, USDA, EPA) had difficulty in determining, how to evaluate the application from Mera Inc. to grow GM *Chlamydomonas* in Hawaii for pharmaceutical production in 2005.
Hypothetical risks from GM Algae?

• **Escape to environment:** Several biosafety and environmental risks are associated with GM organisms related to mass cultivation in open ponds.

• **HGT:** Horizontal gene transfer in cyanobacteria can cause transgene transfer to wild type relatives or other organisms.

• **Sexual reproduction:** Sexual reproduction in green algae with their wild type counterparts can easily transfer transgenes to wild relatives and possibility of forming a new combinations.
Mitigation of GM algae risks

**GM Cyanophytes:** Higher receptiveness of foreign gene and associated with risk of horizontal gene transfer

- **Growth in photobioreactors:** Closed systems *i.e.* **photobioreactors can be placed outdoors** to use the natural sunlight or in greenhouses, to reduce the risk of spillage of GM algae into the environment.

- **Dominance of GM alga** can be decreased by coupling the gene of interest with antisense or RNAi to prevent the expression of genes that increase the fitness of GM alga in the natural ecosystem.

- **Linking growth** of GM strain to specific nutrient
Mitigation of GM algae risks

GM Chlorophytes: Prefer to reproduce asexually (transgene containment). Drastic conditions (lack of nutrients or moisture) trigger the haploid daughter cells to undergo sexual reproduction by forming gametes of plus and minus strains.

• **Closed systems** (PBRs) may reduce the risk of spillage of GM algae

• **Reduce the ability of genetically transformed alga to compete with the wildtype.** The dominance of GM alga can be decreased by coupling the gene of interest with antisense or RNAi approach.

• **Chloroplast transformation:** maternal inheritance of chloroplast genes occurs in isogamous green alga.

• The surrounding extra water source or **buffer zone** may be erected around the open pond systems to prevent weather extremes and accidental spillage
Procedures for using genetically modified microalgal culture developed in India for industrial applications

| Review Committee on Genetic Manipulations (RCGM), DBT | Approves GMO research & biosafety studies; authorizes import-export of DNA vectors & GM germplasm material for research and academic purposes. |
| Genetic Engineering Appraisal Committee (GEAC) | **Recommends approvals of field trials**, import-export & commercial cultivation of GMOs; monitors trait efficacy, sustainability & environmental biosafety of GMOs after release; implements environmental rules and regulations in consonance with state and district level coordination committees. |
| Institutional Biosafety Committee (IBSC) | Examines and **approves the institutional R&D on GM** and biosafety issues of GMOs developed by the institute/organization. |
| Indian Council of Agricultural Research (ICAR) under Ministry of Agriculture (Govt. of India) | **Evaluates GM crops for trait efficacy and agronomic performance** for at least two seasons through the All India Coordinated Research Project (AICRP) trials of the Indian Council for Agricultural Research (ICAR) |
| Biotechnology Regulatory Authority of India (BRAI) (Proposed) | Autonomous and statutory agency to regulate the GM research, transport, import, production and use of organisms and products of modern biotechnology, which is designed to be a single-window mechanism for approval of all GMOs. |
Genetically transformed alga are more fit than wildtype strain and could take over the wildtype population.

- GM is less fit than wild type due to transgene load and requirement of higher nutrition to produce the desirable product.
- GM/Alga usually may not survive long if transferred to any new habitat.

GMs could harm the ecosystem.

- The genetic modification of alga is aimed for modifying either photosynthesis, biomass or lipid biosynthesis, not to generate harmful strains with respect to human health or for perturbing the surrounding natural ecosystem.
In the final year of CAB-Comm funding, we also completed the world’s first EPA approved outdoor field tests of GMO algae, produced the world’s first algae based surfboard, and working with commercial partners Heliae and Triton Health and Nutrition, completed development of strains for commercial production of protein co-products.
Potentials and limitations of using the genetically engineered microorganism

- No robust strain of algae yet discovered from the nature that could be commercially viable
- Cost is higher than fossil...
- Toxic element released from industries can be remediate using the genetically modified algal species

*Success of GM crops like cotton, soybean, corn etc*
*Higher yields in fermentation from genetically modified microbes rationalized the use of GM approach*

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Algal biofuel production is neither environmentally nor commercially sustainable

Kevin Flynn, Chair Professor, Swansea University
The Conversation 8 August 2017
There is no scientific evidence to prove that GM crops would harm soil, human health and environment,” a written reply in the Rajya Sabha

Acknowledgments

Research Group
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A Centre of excellence for research, training and technology transfer to industry in the field of biotechnology to promote sustainable global development

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