Genetically Engineered Algae for Biofuels: A Key Role for Ecologists

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Genetically engineered (GE) microalgae are nearing commercial release for biofuels production without sufficient public information or ecological studies to investigate their possible risks. Blue-green algae (cyanobacteria) and eukaryotic green algae are likely to disperse widely from open ponds and, on a smaller scale with lower probability, from enclosed photobioreactors. With powerful molecular techniques, thousands of algal strains have been screened, hybridized, and redesigned to grow quickly and tolerate extreme conditions. Some biologists do not expect GE microalgae to survive in the wild. However, thorough ecological and evolutionary assessments are needed to test this assumption and, if the algae do survive, to confirm that their persistence is highly unlikely to cause environmental harm. Cyanobacteria are especially difficult to evaluate because of the chance of horizontal gene transfer with unrelated microbes. Before novel GE algae enter the environment, key biosafety and environmental risk issues should be formally addressed by teams of experts that include ecologists.

Keywords: transgenic organisms, biofuel, algae, risk assessment, synthetic biology

Research on obtaining biofuels from algae is heating up, as are efforts to design algae that produce high-value pharmaceuticals, plastics, and food or feed additives (e.g., Rosenberg et al. 2008, Pollack 2010, Rasala et al. 2010, Sayre 2010). Microalgae, the main focus of our attention in the present article, include prokaryotic cyanobacteria and eukaryotic algae such as *Chlamydomonas* and *Nannochloropsis*. Bioengineers forecast that microalgae will be redesigned to produce biofuels using insights from synthetic biology, an advanced method of creating genetically engineered organisms (GEOs; e.g., Dana et al. 2012). High-throughput methods for DNA sequencing, metagenomics, hybridization, accelerated evolution, and genetic engineering have become increasingly efficient and affordable, and thousands of algal strains have been collected worldwide for raw genetic materials. For example, Rismani-Yazdi and colleagues (2011) recently identified pathways and genes of importance related to biofuel production in *Dunaliella tertiolecta*, a nonmodel marine flagellate. In addition, DNA constructs that confer faster growth rates and increased nitrogen-use efficiency in microalgae have been the subject of several patents (e.g., Unkefer et al. 2005). With the support of large public and private investments, patents and publications on GE algae are proliferating rapidly.

Despite these investments, growing algae for biofuels in open ponds or in photobioreactors may be too expensive to be practical, even with major economic subsidies (e.g., USDOE 2010a, Hall and Benemann 2011). It is widely felt that further innovation is needed, including the mass cultivation of microalgae that are genetically engineered (GE; e.g., Gressel 2008, Rosenberg et al. 2008, Flynn et al. 2010, Robertson et al. 2011). Although novel traits can be obtained by nontransgenic methods and therefore merit evaluation, the use of recombinant DNA offers the greatest freedom to improve on the performance of wild strains. Indeed, the Monsanto company is collaborating with Sapphire Energy to discover new genes that confer rapid growth and other beneficial traits in order to accelerate Sapphire's road to commercializing algae, with possible spin-off applications in crop plants (*http://monsanto.mediaroom.com/index.php?s=43&item=934*). Another company, Joule Unlimited, has patented GE cyanobacteria with a host of foreign microbial genes that enhance ethanol production (*www.jouleunlimited.com*). Pilot GE algal biofuel projects are now underway, with ambitious plans to scale up in the United States and abroad (e.g., Rosenberg et al. 2008).

Before novel GEOs are introduced into the environment, however, key biosafety issues should be addressed in order to evaluate potential levels of exposure and unintended sources of harm (e.g., Snow et al. 2005, Wolt et al. 2010). The possibility that novel and very hardy types of GE microalgae could be cultured near natural surface waters raises questions similar to those that come up repeatedly with GE crops and farm-raised GE fish, as well as with non-GEOs that have the potential to become invasive. For example, how frequently would GE algae escape from cultivation and processing facilities? This could occur through aerosolization (Genitsaris et al. 2011, Sharma and Singh 2011), wildlife vectors (Kristiansen 1996), turbulent weather that damages or destroys these facilities, accidents, human error, or other

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events. How far would GE algae disperse (Kristiansen 1996, Marshall and Chalmers 1997), and how long would they survive (Ehresmann and Hatch 1975)? Could transgenes designed to enhance the growth and fitness of released GE algae subsequently spread across metapopulations, species, habitats, and regions, and, if so, at what scales and over what time frames?

In a hypothetical worst-case scenario, escaped GE algae might persist and produce toxins or might become so abundant that they create harmful algal blooms. If it is possible for free-living GE algae to become more invasive, more toxic, or more tolerant of extreme abiotic conditions than their wild counterparts are, this would be cause for concern. Most important, we need to know whether there are plausible scenarios under which GE "superalgae" or other organisms that acquire particular genes from them could proliferate to levels that harm human or environmental health. In many cases, the persistence of free-living GE algae is not expected to have unwanted consequences, but the scientific basis for reaching this conclusion should be compelling and unambiguously clear.

So far, too little attention has been paid to these ecological questions, and relevant funding opportunities from agencies such as the US Department of Energy, the Department of Agriculture (USDA), or the US Environmental Protection Agency have been limited. We suggest that efforts to create GE microalgae should be accompanied by the cogeneration of a strong foundation of conference proceedings and peer-reviewed articles that address their possible risks or lack thereof. As with other GEOs, research to support environmental risk assessment of novel microalgae should involve scientists with broad expertise and minimal conflicts of interest. Below, we describe a few initial information gaps that merit careful consideration.

Survival and persistence

The ability of GE algae (or their DNA) to survive outside of open ponds or enclosed bioreactors is crucial baseline information for risk assessment. Proponents of GE algae often suggest that lab-created strains should not be able to survive in the wild, especially if they are domesticated and bred to produce large volumes of industrial by-products (e.g., Gressel 2008, Maron 2010, Pollack 2010). This scenario would obviate many environmental concerns, but ecological research is needed to determine how well seemingly weak GE microalgae would fare under a wide range of natural biotic and abiotic conditions. We are concerned here about the potential that some GE algae or their evolving progeny might have that would allow them to thrive and persist in natural environments if an unintended release were to occur. Certain engineered traits, such as tolerance of harsh conditions or enhanced growth, may facilitate the survival and growth of GE algae in unmanaged ecosystems.

Unfortunately, much more is known about the baseline performance of microalgae under controlled laboratory conditions than in complex, uncontrolled natural habitats. To begin to fill this gap, new GE lab strains that are intended for large-scale cultivation should be examined in comparison with their natural, non-GE counterparts in contained mesocosm experiments and modeling studies. A recent simulation analysis by Flynn and colleagues (2010) concluded that, depending on the specific nature of genetic modifications, escaping populations of GE algae may be quickly outcompeted by natural forms. However, these authors also noted the possibility that introduced changes in algal metabolic processes may adversely affect the food value of such organisms for zooplankton, which often provide top-down regulation of algal populations. Flynn and colleagues (2010) stressed that, in some cases, unintended effects of genetic engineering might inadvertently produce new strains of harmful algae that could become persistent.

Clearly, it is important that both the emerging algal biofuel industry and the scientific community make every effort to evaluate and minimize the potential risk of undesirable outcomes. As a precaution, J. Craig Venter (Synthetic Genomics) and other researchers have stressed that GE algae should be equipped with transgenic "suicide genes" or other characteristics that would make it impossible for feral strains to survive following an environmental release (e.g., Davison 2005, Pollack 2010, Venter 2010). If such precautions are taken in lieu of thorough environmental assessments, more information should be required to ensure their long-term success and to prevent GE algae from evolving to silence or overcome biological traits that are designed to kill them.

Potential for gene flow and rapid evolution

Several features that make microalgae attractive for gene discovery and metabolic engineering also complicate efforts to assess gene flow and evolutionary consequences over the long term. The short life span and high photosynthetic rates of microalgae lead to high productivity, which may facilitate rapid population growth and evolutionary adaptation through natural or artificial selection. Cyanobacteria are especially efficient at photosynthesis, and some are easy to transform (e.g., Robertson et al. 2011), but they are challenging to monitor, because horizontal gene transfer (HGT) can occur between different cyanobacterial taxa (Herrero and Flores 2008), as well as between cyanobacteria and eukaryotic algae (Waller et al. 2006). In addition, some cyanobacteria can release and take up "naked" DNA from their surroundings (Thomas and Nielson 2005) and can both import and export their DNA through viral vectors (Lindell et al. 2004). Significant HGT can also take place in eukaryotic algae through viral transmission (e.g., Monier et al. 2009). Moreover, successful HGT events among cyanobacteria appear to be most frequent among genes involved in photosynthesis or respiration and specific signature genes of unknown function; this trend is consistent with the hypothesis that HGT often involves genes directly affecting the competition and adaptation of similar cyanobacterial species in neighboring niches (Yerrapragada et al. 2009).

Over evolutionary time, gene transfers often facilitate the acquisition of functions in prokaryotes by eukaryotic organisms, allowing them to colonize new environments; transfers between eukaryotes also occur, mainly into larger organisms that ingest eukaryotic cells and also between plant lineages (Andersson 2005). Remarkably, even HGT from algae to animals has been observed: Rumpho and colleagues (2008) reported the transfer of the algal nuclear gene *psbO* to the photosynthetic sea slug *Elysia chlorotica*. Therefore, a key question for risk assessment is how pervasive contemporary HGT involving microalgae might be in the context of specific transgenic traits, realistic selection pressures, and relevant environmental conditions.

Confidential business information

Unfortunately, the proprietary nature of much research on GE algae makes it difficult to know which species and novel traits are in the research and development pipeline. Relevant details are just beginning to emerge from patent applications, funding agencies, and published interviews with chief executive officers (CEOs). For example, Algenol Biofuels is developing intergeneric, genetically engineered strains of blue-green algae known as "hybrid algae" that have been engineered to overexpress two fermentation enzymes, pyruvate dehydrogenase (PDC) and alcohol dehydrogenase (ADH; USDOE 2010b); these "enhanced" or "hybrid" algae also have high salt and heat tolerance (Schwartz 2011). In a US patent, Algenol's CEO, Paul Woods, described temperature-inducible transgenes coding for PDC and ADH from the anaerobic bacterium *Zymomonas mobilis*, which were inserted into a lab strain of the cyanobacterium *Synechococcus* along with a gene for ampicillin resistance as a selectable marker (Woods et al. 2001). It is possible that constructs similar to those in the patent are being developed by Algenol for commercial use, but such details are not available to the public.

For GE algae, federal reviews of commercial activities or environmental releases of GE algae will be posted at *http://epa.gov/biotech_rule/pubs/submiss.htm*. This much more limited access to information about GE algae, which can be developed and evaluated indoors, is in contrast with GE for agricultural crops, for which biological information is provided on a USDA Web site and elsewhere during outdoor field trials and petitions for nonregulated status (*www.aphis.usda.gov/biotechnology/status.shtml*, *www.nbiap.vt.edu/data.aspx*), albeit with frequent omissions due to confidential business information. Access to such public information makes it possible for outside parties to begin to evaluate possible risks, rule out unlikely scenarios, and carry out baseline research on relevant ecological questions.

Conclusions

As GE algal strains are developed for large-scale facilities, we suggest that ecologists and other researchers will need access to basic information, access to relevant strains, independent authority to conduct research, and funding to build a rigorous scientific foundation that can support objective and quantitative risk assessment. Dana and colleagues (2012) provided recommendations for comprehensive environmental assessments of synthetic organisms, including GE algae, that evaluate new technologies at multiple stages, but their call for action is just a starting point. Given historical lessons from the sometimes-devastating effects of invasive species (e.g., Pimentel et al. 2000) and from societal responses to GE foods (e.g., Somerville 2000), we are also concerned that the general public might develop and express significant fears about potential future problems with GE-based algal biofuel production, whether or not these fears are justified. Therefore, we suggest that transparent and formal risk assessments of GE algae are urgently needed to guide and inform the development of sustainable algal biofuel production. Ecologists and evolutionary biologists who study algae in natural environments have key expertise that should not go untapped in developing GE algae that are both safe for the environment and regarded as safe by society.

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