Opinion

Cultivated microalgae spills: hard to predict/easier to mitigate risks

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Cultivating algae on a large scale will inevitably lead to spills into natural ecosystems. Most risk analyses have dealt only with transgenic algae, without considering the risks of cultivating the corresponding non-transgenic wild type species. This is despite the long-studied 'paradox of the plankton', which describes the unsuitability of laboratory experimentation or modeling to predict the outcome of introducing non-native algae into a new ecosystem. Risk analyses of transgenic strains of native algae can be based on whether they are more fit or less fit than their wild type, but these are not possible with non-native species. Risks from spills can be minimized by mutagenically or transgenically deleting genes that are unnecessary in culture but obligatory in nature.

Raison d'être for large-scale algal cultivation

The massive cultivation of microalgae for animal feed (especially as a replacement for fishmeal) and eventually even as a replacement of soybeans for biofuels [1], as well as for specialty products [2], has been contemplated for a long time, with the first commercial productions now coming on line. When such production is performed on wastelands, using seawater mixed with fertilizer as a culture medium together with industrial carbon dioxide, the yields can be over an order of magnitude greater than crops [3]. This is without competing for agricultural land and fresh water, while utilizing fertilizer more efficiently than agriculture. Such cultivation is clearly environmentally positive, if it can be done efficiently, with low energy input, and could vastly contribute to global food and fuel security. The average power plant provides enough carbon dioxide to support intensive alga culture on 5000–10 000 hectares, with over $>10^{13}$ algal cells per hectare. What will be the effect on nearby bodies of water if a storm, earthquake, tsunami, or human ineptitude causes the release of such massive amounts of ultra-dense algae?

Many of the algae are not native to the geographic areas where they are being cultivated. They are then being selected, mutated, and in some cases genetically engineered to select for an array of desired traits. The risks from spills of genetically engineered algae have been discussed in four recent articles [4–7], but only the one we authored [5] discusses the possible risks of wild type algae spills. We argued that the complexity of such risk assessment may be greater than previously assumed. The authors dealing solely with transgenic algae [4,6,7] call for a considerable investment in risk assessment at the laboratory scale prior to their release. Non-native and native species modified by non-recombinant techniques are exempt from their risk assessment, but any assessment should logically compare transgenics to their parent strains as experimental controls. There is extensive ecological literature about the inability of laboratory experiments or modeling to predict what will happen when non-native algae are released into natural ecosystems, as exemplified by 'the paradox of the plankton' (Box 1 and Figure 1) [8–11]. We discuss the implications of this paradox below, along with the potential risks from different types of strains of non-transgenic and domesticated transgenic algae, along with suggestions for transgenic and non-transgenic ways to mitigate the risks to a most unlikely level.

The risks from non-native algae – the paradox of the plankton

Spills of non-native algae in aquatic (or terrestrial) ecosystems may have massive ecological repercussions, regardless of whether the algae are genetically modified. The actual environmental risk from a given domesticated alga (whether native, introduced, transgenic, or domesticated by mutagenic strain selection) is a compounded function of the effects that mutagenesis or the addition of transgenes may have on the competitive ability (positive or negative fitness) of the alga to compete in the local natural ecosystem. The question then is whether it is possible to predict the influence of the introduction of transgenic or wild type species of algae that are not indigenous to the natural ecosystem.

Interspecific plankton population dynamics are complex and can appear counter-intuitive, as reflected in the concept of 'the paradox of the plankton' (Box 1). In a seemingly homogeneous environment, hundreds of plankton species can coexist despite the presence of limiting factors, such as

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Box 1. Paradox of the plankton

The term 'the paradox of the plankton' was coined by Hutchinson [10], who asked the question 'how it is possible for a number of species to coexist in a relatively isotropic or unstructured environment all competing for the same sort of materials?' Such a situation is expected to reach a final equilibrium as a population consisting of a single or only a few species, not more than the number of limiting factors, as is borne out in laboratory experiments [11]. However, in the environment we see hundreds of plankton species coexisting, even where competition is heavy. The causes for this apparent paradox have been studied extensively, and although various mechanisms have been proposed, it remains basically unsolved [11]. Recent studies bear out the characteristic chaotic behavior of plankton communities [22], which may be seen as complex adaptive systems [23]. It may be that 'when species are limited by multiple factors, the coexistence of a large number of species is the most probable outcome and that habitat heterogeneity interacts with network structure to favor diversity' [24]. The emerging picture is that of the existence of a large number of niches that continuously come and go locally, in which a large number of planktonic organisms can thrive in coexistence next to each other. The complexity of the situation appears to be such that the actual phytoplankton diversity observed in nature is systematically underestimated based on experimental results as well as those derived from modeling [25].

unavailability of nutrients. However, based on laboratory experiments, one would expect that the number of coexisting species cannot be greater than the number of limiting factors, and this is what constitutes the apparent paradox. The expectation, however, presumes that resources are homogenously mixed in the environment, which may be true in the laboratory, but false in a natural ecosystem. A sufficient lack of homogeneity in the natural environment, which also changes over time, may lead to much more variation in selective forces, allowing the coexistence of a much larger number of species than predicted from laboratory experiments.

A definitive risk assessment would thus require in-depth analysis of the fitness of an invading organism, the fitness of indigenous algae, and the intricacies of the ecological niches in the nearby natural environment. Consequently, it will be very difficult, or even impossible, to make firm predictions about the risks of non-native algae based on algal fitness characteristics determined in laboratory experiments or in modeling studies. It is thus difficult if not impossible, based on laboratory experimentation, to have a meaningful discussion about regulatory decisions and policies on the risks emanating from environmental release of introduced, non-native microalgae, whether wild type, mutated, or transgenic. The regulatory discussion would be facilitated if the strain in question were severely debilitated in its environmental functionality, either by mutation or by transgenic means. We argue that this debilitation should be accomplished by introducing mitigating traits into cultivated algae that produce a phenotype that leads to an extremely severe reduction in possibilities for long-term survival in nature, but will be neutral or may even be beneficial under the special conditions of culture.

The risks from transgenic algae

Most of the algae being cultivated have no known sexual cycle and thus have a low genetic glass ceiling, that is, they are quite limited in the genomic diversity that may be required for domestication [12]. They need traits that they do not possess to become domesticated, and a feasible way to obtain those traits is by bringing them from other species. Two general types of traits are being considered: so called 'platform traits', which increase the reliability and robustness of the cultures, and 'value-added traits', which encode products not synthesized by the wild type (Table 1 and Table S1 in the supplementary material online). The platform traits include plant protection traits that limit contamination by other organisms, as well as those that allow dense growth without dying when reaching a high population density, increased abiotic stress tolerance, especially to heat, and those that increase photosynthetic capacity. The value-added traits include traits that optimize the organism for production of biofuels, animal feeds, feedstocks for industrial processes and pharmaceuticals, industrial and feed enzymes, as well as components of cosmetics. The specific genes under consideration have been widely reviewed (Table S1 in the supplementary material online).

Although we cannot provide a definitive risk analysis for all the transgenes being discussed here, we can make some preliminary generalizations. Most of the transgenes being used reduce the fitness of the recipient alga below the fitness of its respective wild type. In such cases, the risk analysis needs to be based solely on the environmental risks involved in cultivating the wild type. One cannot generalize within a group of transgenes whether specific transgenes will be environmentally riskier than the wild type. For example, contamination by alien algae from the environment is a major problem, and several transgenes may be candidates to overcome this problem. One could engineer protection against such contamination by using transgenes encoding toxins that can kill the alien algae, which would give the transgenic algae a competitive advantage in natural ecosystems. One could also protect against algal contamination by conferring herbicide resistance on the cultivated algae, which would have little environmental impact because the herbicides would be diluted to ineffective levels in the case of a spill. Genes conferring resistance to bacteria, fungi, and protozoa would clearly provide transgenic algae with a fitness advantage in nature. Genes conferring traits such as heat tolerance would clearly also broaden the ecosystems where a transgenic species of algae could grow. Adding the ability to utilize near-infrared light in photosynthesis would increase growth rate and confer a competitive advantage in any ecosystem.

Some of the value-added traits might actually lower fitness, for example, those that render the algae recognizably more nutritious to other organisms. Overexpression of other traits might render algae less fit than their respective wild types. Genes conferring added mineral nutritional value to transgenic algae used in animal feed could increase algal fitness above that of their corresponding wild type, by making them better competitors for essential minerals such as iron and zinc. Therapeutic additives to feeds can be risk-neutral if they do not affect organisms in the natural ecosystem, but if they do affect native species, environmental balances can be disrupted.

An alga transformed with a gene conferring a high environmental risk need not require that such an organism