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Review article

Large scale cultivation of genetically modified microalgae: A new era for environmental risk assessment

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ABSTRACT

The genetic modification of microalgal strains for enhanced or modified metabolic activity shows great promise for biotechnological exploitation. However, of key concern for many is the safety of genetic modification technology and genetically modified organisms with regard to both the environment and human health, and how these concerns are met will play a key role in ensuring how successful commercialisation of genetically modified (GM) algae is achieved. Commercialisation opportunities for GM microalgae will inevitably require translation from laboratory to industrial settings, on scales beyond those typically associated with the current biotechnology sector. Here we provide an overview of the current situation with regards to genetic modification techniques and legislation, and the implications of large-scale cultivation with regards to developing a safe and effective risk assessment system for contained and uncontained activities. We discuss the rationale and options for modification and the implications for risks associated with scale up to human health and the environment, current grey areas in political/technical legislation, the use of contained/uncontained production systems, deliberate release and monitoring strategies. We conclude that while existing procedures are not entirely sufficient for accurate and exhaustive risk assessment, there exists a substantial knowledge base and expertise within the existing aquaculture, fermentation and (algal) biotechnology industries that can be combined and applied to ensure safe use in the future.

1. Introduction

Microalgae represent a highly diverse assemblage of photosynthetic microorganisms found over a wide range of environmental habitats, from fresh water through to hyper saline, and spanning a wide range of both temperature and pH tolerances [1,2]. Containing both eukaryotic and prokaryotic (cyanobacteria) members, the general term 'microalgae' is used here to encapsulate this broad grouping of photosynthetic microorganisms with their diverse metabolic potential and function.

Production of microalgal biomass does not require high quality land resources, as is the case of plant crops, and in comparison to large scale fermentation vessel grown yeast or bacteria, these photosynthetic microorganisms have low input requirements (light and micronutrients) whilst producing large amounts of biomass over short periods of time [3]. Microalgal culturing has a significant requirement for water resources which are often scarce. However many species can be grown in saline or brackish waters, reducing impact on increasingly valuable fresh water supplies, or on nutrient rich waste waters that are not suitable for agriculture or human consumption [4]. Combining photosynthetic/heterotrophic growth with waste water treatment/remediation and/or CO_2 capture could not only reduce production costs but has the potential to offer "added value services" to the process of algal biomass generation.

Commercial viability of algal derived products will most likely be achieved by combining commercialisation of high-value, low-volume products such as β-carotene, docosahexaenoic acid and eicosahexaenoic acid with the production of low-value, high-volume products like feeds, fertilisers and biofuels [5].

1.1. GM microalgae and current legislation

Many algal species have become successfully established as suitable for mass culture [6,7], predominantly aquaculture related, but including production for food and feeds, waste water treatment, fertiliser, biofuels, fine chemicals, and pharmaceuticals [8,9]. The advent of the genomic era has heralded a new dawn in microalgal exploitation potential by allowing the combination and selection of key physiological characteristics with modified metabolic activities, enhancing

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production of native compounds relative to wild type strains or introducing genes for the production of additional non-native compounds or added functionality.

Microalgae have been commercially cultured for well over 40 years and the systems currently utilised at scale tend to be unsophisticated shallow open ponds with no artificial mixing or, alternatively, paddle wheel mixed raceway ponds, both of which can cover hundreds of hectares in size [10]. Commercialisation of genetically modified (GM) microalgae for industrial purposes will inevitably require the culturing of GM microalgae at this kind of large-scale, but this will require more stringent risk assessment and environmental management strategies than those utilised for the unmodified wild type algae currently being grown. Much can be learnt from existing 'large-scale' enclosed culture practices exploiting GM bacterial and yeast strains which are typically grown in fermenter-style reactors. Even at smaller scales (e.g. for the production of the highest value products), the utilisation of 'closed' photobioreactor (PBR) systems still requires the effective exposure of the algae to light, the agitation of liquid media to enhance nutrient mixing, and for the removal of toxic oxygen build up; creating multiple opportunities for environmental exposure and, therefore, potentially a significant barrier to commercialisation when these organisms are genetically modified.

The industrial biotechnology sector has so far been slow to respond to GM algae with most projects never leaving the research laboratory setting. Only a few collaborative ventures such as a recent project carried out by Plymouth Marine Laboratory and Rothamsted Research utilising a genetically modified *P. tricornutum* strain expressing heterologous Δ5-elongase for the accumulation of high value omega 3 long chain fatty acids [11], and a commercial venture between Sapphire Energy and UC San Diego ever reach pilot scale. This is in part due to a fundamental lack of information and assessment tools available to researchers, industrial developers or regulators on the risks associated with the large scale propagation of GM microalgae, as well as a lack of suitable facilities to undertake essential pilot scale trials. Yet, even these relatively small trials (< 2000 l) have highlighted the pressing need for the development of tools and mechanisms to aid the technical aspects of GM microalgal cultivation, containment and risk assessment, and crucially to consider the legislative and political aspects of such activities.

To begin with, it is important to define exactly what is meant by the term 'Genetic Modification'. The term *genetically modified organism* (GMO) is used to refer to any microorganism, plant, or animal in which genetic engineering techniques have been used to introduce, remove, or modify specific parts of its genome. It should be noted however that techniques that replicate naturally occurring phenomenon such as random mutagenesis are not generally considered to result in GMOs under European guidelines and are therefore not subject to GM control measures or legislation [12]. Indeed, it is worthy of note that more than 2500 plant varieties in 175 plant species, both crop and decorative, have been created by random mutagenesis and released without fanfare into the environment over the past 75 years [13].

There are many strategies for enhancing algal phenotypes, including random mutagenesis, traditional recombinant nucleic acid technologies, and genome editing tools including transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and RNA-guided engineered nucleases (RGENs) derived from the bacterial clustered regularly interspaced short palindromic repeat (CRISPR)–Cas9 system [14].

Whether any of these new technologies produce a 'GMO' depends largely on the country involved: e.g. in European countries the definition of GMO is mostly associated with the synthetic introduction of genetic material into an organism to create a novel organism via the use of recombinant nucleic acid technologies, though there are ongoing debates about the definition of what constitutes a GMO and the genetic technologies involved. It is unclear how existing legislations around the world will address the new developments and capabilities around genome editing techniques such as CRISPR/Cas9. Direct delivery of guide RNA alongside purified Cas 9 protein into microalgal cells, as opposed to plasmid-mediated delivery for example, is likely to bypass the GMO legislation in the USA, since the genome editing complex is degraded in the recipient cell leaving no trace of foreign DNA [15]. Indeed, it is worthy of note that the US Department of Agriculture (USDA) has decided that it will not regulate a mushroom which has been genetically modified using the CRISPR/Cas9 gene editing tool [16], thus setting a precedent of CRISPR/Cas9 derived plants being considered non-GMO in the USA. Whether this technique will fall under GMO legislation in the European Union will depend on the interpretation of the 2001 Directive on the Deliberate Release of GM Organisms into the Environment [12] which stipulates that techniques of genetic modification include "recombinant nucleic acid techniques involving the formation of new combinations of genetic material by the insertion of nucleic acid molecules produced by whatever means outside an organism into any virus, bacterial plasmid or other vector system and their incorporation into a host organism in which they do not naturally occur but which they are capable of continued propagation". This legislation was formulated before the advent of gene editing techniques such as the CRISPR/Cas9 technology and whether this technique is considered "targeted mutagenesis" (not GM) or the formation of new genetic material (GM) is likely to create significant debate in the future as more R & D projects are commercialised that incorporate this versatile and powerful technology. This failure of regulation to keep up to date with the GM technology advances has created an element of unease; while the European Commission debates this conundrum and repeatedly delays the decision, the legal limbo of gene editing is having a big impact on research [17] which will inevitably impact any commercialisation of genetically edited microalgae.

Currently, within Europe there is legislation covering aspects of GMOs from deliberate release [12], environmental protection and remedying of environmental damage [18], GMOs in food and feed [19], and labelling [20], to list but a few. However, within the scope of these directives each member state is able to take further measures of regulation, management and control of GMOs. Other countries around the world follow their own sets of legislative rules. Despite the potential for wide disparity globally, fortunately most legislation is built on the requirements of the Cartagena Protocol on Biosafety to the Convention on Biological Diversity [21] which provides international guidelines on the regulation and management of living modified organisms (LMOs).

1.2. Public concern

A major factor holding back industry uptake of GMOs is public concern resulting from intensive campaigns by both media and NGOs. Sensationalised press coverage and lack of appropriate communication from the scientific community to the general public has left many fearful and suspicious of GM technologies and, as a result, resistant to buying products containing them. Several reports commissioned by the UK Government and Research Councils have indicated that communication between those involved in science and the general public must be improved and that engagement at an early stage is important for improving understanding [22]. It was also found that through free-flowing dialog, many issues surrounding the use of industrial biotechnology could be addressed and no longer present significant concerns to the general public [23]. Of key concern for many is the safety of GM technology and GMOs with regard to both the environment and human health, and how these concerns are met will play a key role in ensuring how successful commercialisation of GM algae is achieved. Thus, it is important that the potential of microalgae to contribute to future energy and food security, as well as human and environmental health, is not undermined before the platforms can become established. In a new era of increasingly ready access to genetically modified microalgae, there is a crucial requirement for an environmental risk assessment (ERA) system which can uphold and withstand the rigours of