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Research review paper

# Improving microalgae for biotechnology — From genetics to synthetic biology

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ABSTRACT

Microalgae have traditionally been used in many biotechnological applications, where each new application required a different species or strain expressing the required properties; the challenge therefore is to isolate or develop, characterize and optimize species or strains that can express more than one specific property. In agriculture, breeding of natural variants has been successfully used for centuries to improve production traits in many existing plant and animal species. With the discovery of the concepts of classical genetics, these new ideas have been extensively used in selective breeding. However, many biotechnologically relevant algae do not possess the sexual characteristics required for traditional breeding/crossing, although they can be modified by chemical and physical mutagens. The resulting mutants are not considered as genetically modified organisms (GMOs) and their cultivation is therefore not limited by legislation. On the other hand, mutants prepared by random or specific insertion of foreign DNA are considered to be GMOs. This review will compare the effects of two genetic approaches on model algal species and will summarize their advantages in basic research. Furthermore, we will discuss the potential of mutagenesis to improve microalgae as a biotechnological resource, to accelerate the process from specific strain isolation to growth optimization, and discuss the production of new products. Finally, we will explore the potential of algae in synthetic biology.



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## 1. Introduction

In recent years, algae have attracted wide interest as potential tools to produce different compounds, including specialty chemicals, pharmaceuticals, food supplements and biofuels. Research in algal physiology (including algal biotechnology) dates back to the 1950s, when their potential was first noted and exploited (Aach, 1952; Geoghegan, 1951; Milner, 1951; Spoehr and Milner, 1949), for review see (Borowitzka,

*Abbreviations:* CRISPR, clustered regularly interspaced short palindromic repeats; DSB, double stranded break; EMS, ethyl methane sulfonate; EPA, eicosapentaenoic acid; GMO, genetically modified organism; MNNG, methylnitronitrosoguanidine; TAG, triacylglycerol; TALEs, transcription activator-like effectors; T-DNA, transfer DNA; ZFN, zinc-finger nucleases

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2013). Over the past 50 years we have learnt a considerable amount about algal physiology, growth and regulation, optimization of growth conditions and improvements in energy balance for algal production. However, to this day our knowledge is mostly limited to wild type strains of different origins. Traditionally, different strains or species of algae are used for different purposes, thus specific rapidly growing strains are used for biomass production, while others are used for the production of specific compounds such as astaxanthin, eicosapentaenoic acid (EPA) and recently, oils (Pulz and Gross, 2004; Trentacoste et al., 2013). This illustrates an inherent limitation of wild type strains, where ideally, the strain should combine high growth rate and maximum product formation with ease and low cost of harvest. Some strains fulfill one or two of these criteria, but it is not feasible to achieve optimal production through simple strain selection. Naturally occurring strains did not evolve under conditions required for the production of many biotechnologically and economically important products. Therefore, selective breeding technologies akin to those successfully applied in the development of cereal cultivars of wheat and corn must be used (Georgianna and Mayfield, 2012). The time course of these processes can be further accelerated with basic knowledge accumulated through higher plant breeding, as well as the availability of emerging methods and technologies. Therefore, in order to fully exploit the potential of algae, it is crucial to invest in basic research on algal physiology and reactions to changing environments. This knowledge, combined with genetic approaches, will enable the exploitation of novel algal strains having optimized growth and production characteristics.

## 2. Mutagenesis

Genetic material of all living organisms is transferred from parents to progeny, thus forming the basis of hereditary traits. Genes, the simplest units of heredity, are transferred to the next generation following a set of basic genetic rules. Since their discovery in the mid-19th century by Gregor Mendel, these fundamental laws have been exploited extensively by breeders in order to produce and combine desired traits in the progeny, and by researchers to study mechanisms of heredity, as well as other aspects of biology. Naturally occurring mutants arise by interactions between environmental effectors such as UV irradiation, or metabolically produced reactive oxygen species, and the genetic material. Such mutations, and the resulting mutants, are a major source of genetic variability with potential for evolution (Barton, 2010; Eyre-Walker and Keightley, 2007). However, the natural processes are too slow for immediate applications in breeding or research. Mutation frequency can be increased by several orders of magnitude using different mutagens (see below), leading to the production of mutant populations. To maximize these, thousands to tens of thousands of independent mutants must be generated in order to cover the entire genome. The mutant population can then be screened for the desired phenotype. While the generation of mutant populations can be a simple task, especially in the case of chemical mutagenesis, the real strength of mutational screening lies in the selection of mutants with desired phenotypes. This is also one of the major bottlenecks of any mutagenesis screen and is discussed in more detail below. The required phenotypes can be complex, including increased cell size, improved growth, resistance to different compounds or improved productivity of a specific compound, all of which will require specific (and different) screening protocols. Both breeders and researchers can search for similar phenotypes, but with different motivations. Breeders are concerned with the organism itself, in order to produce a specific progeny. In contrast, researchers are more interested in mutational mechanisms and their intracellular connections. This difference may seem trivial, but it has far-reaching consequences. The first step in characterizing a mutational mechanism is to identify the mutated gene(s). This is traditionally done by crossing mutants to other strains, and requires the existence of sexual reproduction in the specific species. While generally obvious in higher plants, this prerequisite is sometimes complicated to fulfill in algae, where the conditions needed for sexual reproduction are sometimes not known and the strains are usually maintained asexually. In basic research, this obstacle can be overcome by sequencing the mutant genome using next-generation sequencing and comparing this to the parental strain (Dutcher et al., 2011). Importantly, prior knowledge, or the existence of sexual reproduction, is not required for obtaining and propagating mutant algal strains. Mutant strains can be selected based on phenotype and reproduced asexually, giving rise to a culture expressing that phenotype. Because most algae are haploid, even recessive mutants can be propagated in this way. Traditionally, only strains with desired phenotype/s are selected from the mutant population. However, it is also possible to save and characterize mutants of all genes in the collection, irrespective of phenotype, with the benefits described below.

### 2.1. Chemical and physical mutagens

Chemical and physical mutagens are among the most widely used, both in basic and applied science, particularly because most of them are easy to apply at different doses and their mutagenic potentials are well characterized (Table 1). Although so far there have only been a few reports describing mutagenesis of wild type algal cells in order to improve their biotechnological properties, all major mutagens were used to this end and proved their usefulness. The most widely used chemical mutagens are alkylating agents such as ethyl methane sulfonate (EMS) and methylnitronitrosoguanidine (MNNG). They were also the first ones used in mutagenic screenings to increase EPA production in *Nannochloropsis oculata* (Chaturvedi and Fujita, 2006) and to enhance growth properties of *Chlorella* (Ong et al., 2010). Typical physical mutagens include different types of irradiation such as UV, gamma or heavy ion beams. The mode of action and mutagenic potential of each type of radiation on cells depends on the energy while the frequency of their use depends on ease of application. Mutagenesis by UV is very simple, since it requires neither specialized equipment nor chemicals and can be very easily performed, essentially by exposing cells to germicidal UV lamps in a sterile hood. Given its simplicity and potential, this method has been used both in basic research to prepare algal strains with specific features (Neupert et al., 2009), and in applied science to produce strains with increased production of oil (de Jaeger et al., 2014; Vigeolas et al., 2012). Gamma and particularly heavy ion beam irradiation requires specific equipment so they are not so widely used. Nevertheless, the applicability of gamma irradiation to mutagenesis was demonstrated by improved productivity of astaxanthin (Najafi et al., 2011). Clearly all mutagens have proven their merits in production of mutants with desired phenotypes. However, as we mention above and discuss in more detail below the main limitation of any mutagenesis screen is the screening procedure itself.

#### 2.1.1. Conditional mutations

Essential genes are less amenable to a classical genetic approach since an inactivating mutation cannot be recovered due to its lethality. One way to circumvent this is through point mutations that could affect only the activity or behavior of a gene product without its inactivation,

**Table 1**
Different mutagens, their mode of action and mutations caused.

| Mutagen | Mode of action | Most common mutation caused |
|---|---|---|
| EMS, MNNG | Alkylation of DNA base, particularly guanine | Point mutations |
| UV irradiation | Photochemical reaction leading to cyclobutane ring | Point mutations, deletions |
| Gamma irradiation | Ionization leading to double stranded break | Deletions |
| Heavy ion beams | Ionization leading to double stranded break | Chromosome breaks and exchanges |
| T-DNA, antibiotics resistance gene | DNA fragment insertion | Insertions, deletions |



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