GENETIC ENGINEERING OF ALGAL SPECIES

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Contents

Summary

Genetic engineering of algae is not common due to problems related to the design of vectors (i.e. plasmids or viruses) that can be successfully incorporated into the algae, accepted by the cell and expressed in a satisfying way.

Most studies have therefore been made on the "green yeast" *Clamydomonas reinhartii* and some cyanobacterial species. However, in this review we are presenting examples of studies performed on a broad collection of algal species ranging from cyanobacteria to macroalgae like *Laminaria*.

We have included different kinds of applications, within physiology, biochemistry, molecular biology, phylogeny, industry and environmental science. This ongoing and forthcoming research will undoubtedly increase our knowledge and usage of these important and fascinating primary-producing organisms.

1. Introduction

1.1. What are Algae?

Algae are a heterogeneous group of organisms. They are aquatic or live in damp habitats on land. Some are prokaryotic but most are eukaryotic. Cell size can vary from 1 µm up to tenths of meters and the complexity from a rather simple spherical cell to a highly differentiated plant (Figure 1 and 2).

They reproduce sexually, with complex lifecycles, or asexually. Some can produce resting stages called cysts that can survive in sediments for at least 10 to 50 years. The only feature that the algae seem to have in common is their ability to use light to fix carbon from CO_2 and to produce oxygen in the process.

However, even this autotrophic mode is not true for all algae. Some have a strict heterotrophic mode of life, while others can switch between obtaining carbon from fixation or by eating other organisms or organic particles.

All algae are not related evolutionary, i.e. they do not share a common ancestor, but seem to have evolved on several separate occasions. Indeed, the only really common feature that algae seem to share is the inclination to occupy damp places.

The definition “algae” are thus more of a traditional and practical naming and should not be considered as a group of organisms of common ancestry.

Figure 1. Ice-floe holding millions of marine diatoms, Weddel Sea, Antarctica. (Photo: Anna Godhe, Marine Botany, Göteborg University, Sweden.).

Figure 2. Marine kelp, Cape of Good Hope, South Africa. (Photo: Anna Godhe, Marine Botany, Göteborg University, Sweden).

1.2. What is Genetic Engineering?

The words “genetic engineering” are also hard to delimit [see also *Methods in Genetical Engineering*; *Genetics and Society*]. We have chosen to interpret it generously here, so that all kinds of genetic work performed on algae will be considered.

Strict genetic engineering studies, i.e. the insertion of another organism's gene into the genome of an alga, are scarce, and nearly all work in this field has been performed on very few organisms like the “green yeast” *Chlamydomonas reinhardtii* and some species of cyanobacteria.

Thus, in this summary we have included representative molecular studies on genetical diversity, phylogeny and taxonomy as well as physiological mechanisms and applied genetic engineering.

1.3. The Importance of Algae

The use of algae in biotechnological research and industry is significant. Algae play roles as biocatalysts for the production of food, chemicals and fuels and they are becoming important in the development of solar energy technology, biodegradation and bioremediation. In addition, some species of algae are eaten directly by humans.

The red macroalgae *Porphyra* sp. is a common ingredient in East Asian cuisine. The markets for other algae, like the microalgae *Spirulina* sp., *Chlorella* sp. and *Dunaliella* sp., are expanding as a food supplement in western world health stores.

For instance, *Spirulina* (a cyanobacteria) has a protein content above 70 percent, which also makes it attractive as fodder in the aquaculture industry. Many of these algal species are retailed because of their antioxidant properties.

Figure 3. Red tide caused by dinoflagellates. Skagerrak, NE Atlantic.
(Photo: Anna Godhe, Marine Botany, Göteborg University, Sweden)

Algae are also sometimes causing severe problems (Figure3). The expanding international aquaculture industries often encounter severe problems due to harmful algae.

Some algal species carry spines that can physically damage fish gills. Other algae produce toxins, which accumulate in filter feeders, like commercially important oysters and mussels. Oysters and mussels are usually not affected, but human consumers might experience different diseases.

2. Classification of Algae

Before one considers bioengineering of algae, it is necessary to define the taxonomic position of these organisms. This is not an easy task since algae are an extremely cohesive group of organisms and clearly not relatives in the evolutionary (phylogenetic) sense like animals are.

Alga phylogeny can most clearly be visualized as a tree (Figure 4). Many different characters can be evaluated to construct such trees and the most widely used feature today is DNA sequence data (see below; section 5).

Such data has provided evidence for the existence of ten major phyla of algae. These are the *Glaucophyta, Euglenophyta, Cryptophyta, Haptophyta, Dinophyta, Heterocontophyta* (including diatoms, brown algae), *Rhodophyta* (red algae), *Chlorophyta* (green algae) and the prokaryotic *Cyanophyta* (cyanobacteria) and *Prochlorophyta*.

When more molecular data becomes available, it is highly likely that this division might change. Two other groups of organisms, the apicomplexans and chlorarachniophytes, which contain plastid genomes (the genome of chloroplasts), may in the future be identified as algae. Some groups of algae are closely related to non-photosynthetic organisms (protozoans).

One striking example is the relationship between *Trypanosoma*, the cause of sleeping sickness and Chagas disease, and the chlorophyll containing hay infusion organism *Euglena*.

Another is the relationship between ciliates (such as *Paramecium*), the apicomplexans (like the malaria parasite *Plasmodium*) and dinoflagellates (like toxic *Alexandrium*). How is this possible?

The answer is endosymbiosis, where one or a few endosymbiotic organisms have been incorporated in a host cell, and the movement of genes from one organism to another.

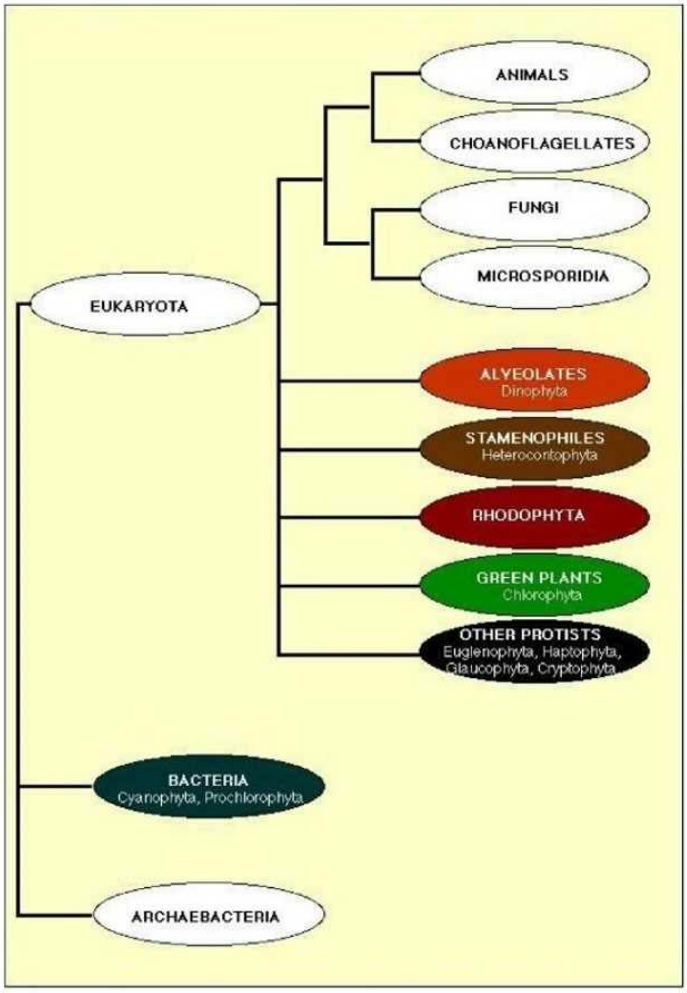

Figure 4. Phylogenetic tree based on ribosomal RNA sequences. Major groups which includes algae are indicated with color (adapted from D.J. Patterson & M.L.Sogin, Tree of Life at http://phylogeny.arizona.edu/tree/phylogeny.html)

3. Principles of Microalga Culture

To be able to isolate algae from its natural environment one has to mimic both its chemical and physical habitat. The basic problem in establishing algal cultures is the design of the media (see also *Algal cell culture*). Natural water is very dilute but at the same instance a very complex media. This is why purified offshore water (or artificial seawater) is used as the basis for marine alga culturing media. To this, precisely defined quantities of major nutrients (i.e. nitrogen, phosphorus, silica), minor nutrients (i.e. copper, zinc, cobalt, manganese, molybdenum, iron, selenium) and vitamins (i.e. B12, thiamin and biotin) are added.

Light intensity, light quality and day length are parameters that can have profound effects on algal growth. In general, cultured algae are adapted to rather low light intensities and the temperature range is quite broad.

Beside the physiological parameters, many algae also need specific biological

parameters to be able to grow. These parameters are often completely unknown, which creates problems.

Many marine algal species can only be cultivated for some generations in natural, untreated seawater. The exclusion of some of the accompanying species is usually possible (i.e. predators), but often the algae of interest cease to grow after exclusion of all accompanying species (i.e. small flagellates and bacteria).

If this is due to the algal species need for prey organisms or if they live in a mutualistic kind of mood, still remains to be solved. Mixed cultures create problems when studying algae.

It should therefore be pointed out that, at the moment, there are very limited numbers of algal species that are used in biogenetic engineering studies, since these studies nearly always require one to grow the algal species without other organisms present in the culture (i.e. axenic cultures).

It should also be mentioned here that several species of macroalgae are commercially and scientifically cultivated (see further the section on Genetic engineering of algae: examples of environmental and industrial applications).

4. Gene Technology

4.1. Polymerase Chain Reaction

The capacity to amplify specific regions of DNA, by using the polymerase chain reaction (PCR), has in many ways revolutionized the molecular biology discipline (see also *Physical methods of analysis; Methods in genetic engineering*). In PCR reactions specific DNA fragments are synthesized *in vitro*. The product obtained contains millions of copies of the fragment and can therefore easily be identified and isolated from the rest of the DNA genome. The PCR technology is nowadays used as a routine tool in most molecular studies, including genetic engineering. An important property of the PCR is the capacity to amplify a target sequence from a crude DNA template. This has become very helpful in many applications within the algal field of research. PCR on crude template preparations are very useful in phylogenetic and taxonomic studies on species that can not be obtained in pure culture. The PCR technology has also become irreplaceable within ecological and physiological research.

4.2. Cloning

One of the major problems when applying genetic engineering on new kinds of organisms is the problem to design specific vectors that can both be transformed into the cells, accepted by the cell and expressed in an adequate way. The ability to introduce and achieve desired levels of expression of foreign genes have been made possible by:

a) Technical development for the incorporation of DNA into algal cells. Techniques used in transformation of algal cells include injection of DNA through fine glass needles (microinjection), bombardment of cells with DNA coated gold particles, and

virus infection. Other methods used to make the cells prepared for uptake of DNA fragments or plasmids are the use of electrical charge to temporarily open pores in the cell membrane (electroporation) or agitation of algal protoplasts, i.e. algae without cell walls, with glass beads.

b) Development of promoter systems so that the introduced DNA can be expressed by the algal cells in a satisfying way. Homologous promoters are usually preferred since heterologous promoters (those from other organisms) sometimes do not drive the expression of the transformed genes in an efficient way.

c) Selection of reporter genes, which identify the cell that has been successfully transformed. In bacteria, genes conferring antibiotic resistance are the most widely used reporter genes. Usually antibiotic resistance genes are not used as reporter genes in algae due to their often- natural resistance to antibiotic compounds. Reporter genes that have been used include the gene that encodes the enzyme arylsulfatase. This enzyme in normally expressed under sulfur starvation and it causes the algal cells to produce an easily detectable coloured substance. Pesticide resistance is popular as selective markers in plant genetic engineering, and can probably also be used as such in similar studies on algae

4.3. Hybridization

Artificial construction of a double-stranded nucleic acid by complementary base paring of two single stranded nucleic acids (RNA or DNA) is called hybridization (see also *Genetics and Molecular Biology*). This technique has become a powerful tool in genetic research. It also permits the detection of smaller stretches of nucleic acid that are complementary to a known sequence. Such a single-stranded molecule of known sequence is called a probe. A probe labeled with some kind of detection molecule (radioactive, fluorescent or color) can be used to locate a sequence complementary to the probe within a mixture of nucleic acids of unknown composition and origin. Hybridization can be performed both on isolated DNA bound on a matrix support (filter, beads, plastic wells), in solution or directly on preserved whole cells or tissue. Within algal research, whole cell hybridization has been used to distinguish between closely related strains or for the enumeration of a single species within a large assembly of species (i.e. natural water samples).

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