

Ethical values regarding the use of CRISPR/Cas9 in *Chlamydomonas reinhardtii*

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The field of synthetic biology has increased explosively during the last decade, but there is a great importance to evaluate and chart eventual side effects. With the help from the office at the Swedish Gene Technology Advisory Board, the Technical Research Institute and the Work Environmental Authority we have been able to analyze our project design from many perspectives. Together with the Gene Technology Advisory Board we evaluated our project from three different points of view where we focus on the environmental, the economical and the health related values. Additionally, we got input from the Technical Research Institute and the Work Environmental Authority which helped us to complement the evaluation of the production line as well as environmental and health related risks.

Conclusion

It is important that possibilities, regarding the work with CRISPR/Cas9 in *C. reinhardtii*, are evaluated against any potential risks. The future prospect is to grow the algae in an isolated production area in open ponds, which enables the algae to absorb CO₂ from the environment. There are no major risks with the open pond cultivation while the production occurs in an isolated area, and if the algae would be released in to nature they would be self-extinct eventually due to their light induced system (LIP promoter). There are no comprehensive health risks for individuals neither before nor after the optimizing of the algae, and other than special waste sorting no further actions need to be taken. Our project is a valuable asset in a global perspective. The project has a great economical value since it enables a green fuel production that is economically sustainable. Most importantly this type of biofuel production can be installed on almost any type of areas. This could find a purpose for unused areas in developing countries, giving them an opportunity for these types of industries thereby resulting in an economic strength.

References

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Values



Environmental

Applying the CRISPR/Cas9 system with the inducible LIP promoter on *Chlamydomonas reinhardtii* has many advantages. The system entails that through high intensity light, the gene regulation can be controlled. Unlike many other methods there is no need of using harmful chemicals or other expensive technics to get the system going¹. The entire procedure can be executed in an environmentally friendly way and additionally contribute to a cleansing effect of carbon dioxide from the atmosphere.

From a safety perspective the algae are planned to be cultured in an isolated area. This means that the outside ecosystem would not be involved in this process, thereby maintained in its original condition. Instead, atmospheric carbon dioxide would be removed at same time as sunlight is absorbed, resulting in a decreased greenhouse effect.



Economical

Some of today's possible problems regarding biofuel production from algae include insufficient amount of biomass recovery for commercial viability, as well as the need for highly advanced bioreactors/photo-bioreactors. Additionally, there is a need to develop technologies for harvesting, oil extraction etc. at low a cost. New technologies such as genetic engineering is being explored to enhance the production of biofuels from algae and make the process more cost-effective².

Since algae are easy to grow, production in large-scale is possible, for instance open pond cultivation. This way the process can use carbon dioxide from coal-fired industries, thus leading to a greater reduction and control over nearby emissions². The fatty acids extracted from the algae for biofuels are not all that can be used for a specific purpose. Some examples are protein residues used as enrichment in food for both humans and animals³, glycerin products for food as well as drug additives⁴ and carbohydrates for ethanol production and other algae parts which can be used for bio gas production⁵.

Algae can be grown in large ponds, as mentioned earlier but also in places such as pools. This quality can provide purpose to locations that are not suitable for other agricultural productions. This could be an important way of using unoccupied areas in for example development countries with extreme climate and weather conditions⁶. The efficiency of the fatty acid production is profitable, depending on which specie the oil content can be up to 80 %². The application of these methods can result in new job opportunities and new collaborations between countries.



Health

Before practical studies of our project applications for working with biological agents were made according to *the Swedish Work Environment Authority*, where our project was confirmed to be at a high level of protection for both the environment and individual's health⁷.

The algae *C. reinhardtii* are eukaryotic single cell chlorophytes⁸, they are not harmful neither before nor after the modification. The modification itself affects the starch gene and makes the algae toxic in no way.

Waste products and other materiel used in the practical studies of the algae are taken care of as recommended in the application forms mentioned earlier⁷.