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Letter to the Editor

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Methodological Analysis of Non-Model Microalgae Genetic Engineering Systems

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Letter to the Editor

Despite algae's enormous potential to contribute to the future bio economy, there are practical and theoretical constraints to how well organically derived species can operate in an outdoor setting. The use of biotechnology to modify and design algal metabolism with the goal of boosting biomass productivity, channeling carbon toward a more favorable composition, insect resistance or creating novel compounds can help overcome some of the most significant commercialization challenges. There are several rational genetic engineering efforts published in the literature with varying degrees of success, but they frequently lack a full explanation of the possible underlying causes of failed attempts. We examine the published methods and approaches used in recent non-model algal genetic engineering breakthroughs. We are particularly interested in identifying difficulties related to genetic engineering that have been documented in recent literature based on native algal defense and resistance systems. The purpose of this research is to shed light on common mechanisms and suggest ways that could aid in the development of successful genetic engineering strategies in non-model algae.

Non-model algae lag significantly behind in terms of efficient and successful genetic engineering, despite the fact that the model alga *Chlamydomonas reinhardtii* has been studied for decades. Non-model algae are being adapted to employ the tools and methodologies established and demonstrated in *C. reinhardtii*. Several beneficial characteristics helped *C. reinhardtii* establish itself as the model alga early on. Unicellular morphology, growth in simple media, autotrophic, heterotrophic and mix trophic growth ability and sexual mating to allow for more complex genetics are among these features. This species was one of the first algae to have its nuclear, mitochondrial and chloroplast genome sequenced to aid in tool development. With the development of these advanced tools, interest shifted to genetic modifications useful on an industrial scale for the production of biomass or products of interest. However, in *C. reinhardtii*, transgene expression efficiency and stability were initially low and appeared to be linked to gene silencing. With the advancement of these powerful techniques, the focus switched to genetic alterations that could be used on a large scale to produce biomass or other products of interest. Transgene expression efficiency and stability in *C. reinhardtii*, on the other hand, were initially low and thought to be associated to gene silencing *C. reinhardtii* mutants were screened for reporter gene expression in order to better understand low transgenic expression levels. UVM-4 was one of the strains that had higher levels of nuclear transgene expression and is currently frequently used. Other achievements included increasing production or introducing new items. Culture yields in outdoor ponds, for example, have been increased by reducing antenna size inserting additional bicarbonate transporters improving central carbon metabolism modifying energy carrier pools or adding nitrogen fixation capabilities. The intricate and durable cell wall is perhaps the most significant hurdle for microalgae genetic engineering and the transformation methods that drive the process. Exogenous DNA must be introduced into cells and must pass through the cell wall barrier in order for transgenes to be expressed.

This stage is species-dependent and is likely to pose a considerable obstacle to researchers in developing a viable genetic engineering strategy. Algae cell walls are made up of a complex heteropolymer of carbohydrates, hydrocarbons, proteins, and other components that make them extremely resistant to penetration. With the advancement of these powerful techniques, the focus switched to genetic alterations that could be used on a large scale to produce biomass or other products of interest. Transgene expression efficiency and stability in *C. reinhardtii*, on the other hand, were initially low and thought to be associated to gene silencing *C. reinhardtii* mutants were screened for reporter gene expression in order to better understand low transgenic expression levels UVM-4 was found to have higher amounts of nuclear transgene expression and is now often used the mutation(s) responsible for greater transgenic expression in the UVM-4 strain are unknown, but have been connected to epigenetic gene silencing machinery other achievements included increasing production or introducing new items. Culture yields in outdoor ponds, for example, have been increased by reducing antenna size adding additional bicarbonate transporters, improving central carbon metabolism, modifying energy carrier pools or adding nitrogen fixation capabilities. There has also been mention of the generation of bio products such as terrene antigens and vaccines and immunotoxins.

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