Algal Productivity Enhancements by Rapid Screening and Selection of Improved Biomass and Lipid Producing Phototrophs (APEX)

Increasing the economic feasibility and scalability of algal biofuels has been the ultimate goal of the Bioenergy Technologies Office Advanced Algal System Program. While impressive strides towards improved algal biomass outputs has been made, productivities from these photoautotrophs require further improvements in terms of biomass yields and composition (e.g. higher oil content) for the economically successful production of algal biofuels. We propose to implement a strain improvement strategy on two strains already proven to be suitable for outdoor production that will deliver a feedstock production platform that has the potential to produce >23 g/m^2/day of algal biomass on an annualized average basis with a targeted lipid content >31% and an overall improvement in cultivation robustness by 50% in outdoor ponds. Improved strains will be tested in a large-scale outdoor algal production system capable of mass culturing and multi-acre production of algae biomass. To achieve these goals, we will use novel and powerful mutagenesis techniques, e.g. atmospheric and room temperature plasma mutagenesis, coupled with turbidostat selection and fluorescence-assisted cell sorting (FACS) to isolate higher oil accumulating strains that still retain high biomass productivities. This will be augmented by molecular breeding to ensure the stability of the high-oil producing phenotypes. Random mutagenesis will also be complemented by targeted genome engineering that will explore the disruption of storage carbohydrate synthesis using genetic engineering as a way to improve carbon partitioning to lipid biosynthesis. Promising mutants will be assessed under relevant culturing conditions using unique state-of-the-art outdoor "simulators" that mimic the outdoor active radiation (PAR) outputs, and temperature swings. Additional random mutagenesis, turbidostat selection and FACS will be applied, as needed, to these strains to improve lipid content, biomass productivities and tolerances to oxidative stress. Finally, bioprospecting efforts and selective pressure culturing will be used to identify rapidly growing cells that prosper in high-bicarbonate, high-pH media. The isolates will be screened for the most oleaginous strains, which will be evaluated for outdoor deployment. Environmental samples will also be investigated for strains able to genetically cross with our oil accumulating mutants to improve trait stability and robustness in outdoor production ponds. Our extensive bioinformatics and functional genomics capabilities will be leveraged to perform detailed comparative transcriptomics and metabolomics (i.e., lipidomics) between laboratory and pond conditions to correlate algal gene expression patterns to biomass productivities and composition as a function of biotic and abiotic conditions, including cultivation regimes, medium composition and pond microbiome. Overall, consolidation of robust genetic/genome engineering and breeding approaches coupled with robust culturing platforms capable of mimicking outdoor cultivation conditions that promote simultaneous selection of the most productive and highest lipid-accumulating strains in an iterative indoor-outdoor fashion provides a powerful framework for accelerated algal strain discovery and improvement. Importantly, as the individual steps have been demonstrated, we have a high level of confidence that the implementation of the proposed unified and integrated process is relatively low risk, and will be successful in reaching the targeted cultivation improvements of 23 g/m^2/day on an annualized average basis and improve lipid content to >31%, thereby yielding ≥80 GGE per ton of biomass.

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