Summary/Abstract for Public Release

Name of Applicant	Ginkgo Bioworks, Inc. (Ginkgo)
Principal Investigator	Dr. Nikos Reppas
Project Title	Integrated pest management-inspired approach to algal crop protection based on laboratory evolution and engineered antimicrobial peptides
Major Participants	Pacific Northwest National Laboratories (PNNL) Global Algae Innovations (GAI) Colorado School of Mines (Mines)

Summary

Objectives: The project aims to achieve significant reductions in the number of pest-induced pond growth slowdown events, crashes, and periods of decreased productivity in commercial algal ponds with a holistic approach that combines treatment by targeted antimicrobial peptides (AMPs) with evolution of algal systems for tolerance to the AMP's effects. There are three primary objectives which we will address in the context of this larger goal: (1) identify the major pests and pathogens (along with any other relevant biotic/abiotic factors) responsible for loss of productivity in *Nitzschia* crops, (2) establish a library of AMPs with demonstrated and specific activity against the most critical *Nitzschia* pests and evaluate performance under simulated real-world conditions at industrially-relevant scales, and (3) evolve *Nitzschia* cultures to be tolerant to the direct and indirect effects of AMP treatment while maintaining high productivity/good performance.

Methods: We will leverage commercially proven genomic/bioengineering tools and high-throughput (HT) testing workflows to achieve our project objectives. Metagenomics/transcriptomics analyses will be conducted on healthy and unhealthy *Nitzschia* ponds to identify key algal pests that negatively impact productivity. Process-relevant (model) algae-pest systems will be developed to enable rapid screening, testing and evaluation of conditions impacting biomass yields and for assessing AMP activity. Database mining (including proprietary assets) and protein engineering combined with HT screening will be used to identify AMPs with activity against target pests. In addition, automated adaptive laboratory evolution (ALE) will be used to evolve *Nitzschia* and its protective phycosphere community toward improved resistance to those AMPs found to have activity against pests of interest.

Impact. This proposed multi-pronged pest control strategy, which combines innovative metagenomics and transcriptomics for pest identification, production and HT screening of a large set of novel AMPs, and innovative adaptive laboratory evolution, have the potential to accelerate the process of translating the results of laboratory research to larger-scale outdoor systems as a first critical step on the road to industrial decarbonization. The proposed AMP-and-ALE approach to crop protection will: (i) identify algal pests via metagenomics and transcriptomics of relevant environmental samples, provide rich new datasets, and enable a holistic ecosystem characterization for various pond health statuses, (ii) inform the design and HT screening of novel antimicrobial peptides with optimized pest specificity, and (iii) evolve and selecting commercially relevant algal strains and its beneficial phycosphere with improved resistance to AMPs via ALE.