Enabling Experimental Evolution: Multi-parameter Sensor System Integration into a Culture/Stressor Biofluidics System

Chinmayee Govinda Raj[1], Aryamitra Bake[1], Ali-Imran Tayeb[1], Carrie Ludman[1], Purva Joshi[2], Jonathan Wang[3], Diana Gentry[4]
[1]San Jose State Univ., [2]Sacred Heart Cathedral – NASA Ames VIP Program, [3]Millennium Engineering & Integration Co., [4]NASA Ames Research Center

Experimental evolution (EE) exposes microbial communities to ecological stressors, simulating dynamics up to near-extinction events. Combined with comparative sequencing and other molecular tools, such data can inform the genetic and other biological mechanisms underlying extremophile adaptation, and other observed effects. Automating this type of experiment using biofluidics can mitigate many traditional obstacles, including delays in assay results and environment adjustment and the need for many replicates.

A first-generation device for automating EE procedures, the Automated Adaptive Directed Evolution Chamber (AADEC), was developed at NASA Ames. UV-C radiation was the stressor, an LED-photodiode array measured optical density, magnetic agitation and peristaltic pump systems ensured nutrient availability, and Arduino microcontrollers provided control. *Escherichia coli* in LB kanamycin media was used for testing and performance verification. A manual laboratory procedure with timed exposure to UV-C was performed to typify tolerance acquisition. Approximately a 10^6 factor increase in survival ratio was recorded over multiple iterations.

Currently, a second-generation device is being developed integrating more real-time sensors: redox potential (ORP), indicating available/consumed metabolic energy; dissolved oxygen (DO), indicating aerobic/anaerobic growth; pH, indicating metabolic products; and electrical conductivity (EC), another indicator of metabolic products. The EC sensor system was constructed and calibrated in-house and matched commercial sensors in the required range. A Raspberry Pi computer automated the electrical system, allowing real-time data acquisition. The fluidics card was made of CNC-milled polycarbonate for biocompatibility.

Each sensor parameter can also be used as a selection pressure alone or in combination with others to create extreme microbial environments. As a proof of concept, this work demonstrated sensor operation in one pair of growth-sensor chambers. It can be expanded to a multi-chamber system to enable inter-culture comparisons and multi-population studies. The prior Arduino system will be ported to the RPi system. Future stressors to be added include thermal, reactive oxygen species, and varying nutrient availability.