Employing Automated Experimental Evolution to Understand Survival Strategies of Lab-Grown Extremophiles

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Introduction: Experimental evolution (EE) exposes microbes to intentional stressors to improve resistance through artificial mutation. The resulting changes to metabolic pathways, protein structure, and genetic sequences, along with traditional genetic engineering tools, to can help understand the mechanisms of improved tolerance. Multiple iterations of a predetermined UV-C exposure led to approximately 10^6 factor increase in *Escherichia coli* survival ratio.[1] However, manual EE experimental procedures proved to be tedious, time consuming, highly prone to errors, and challenging to reproduce. An automated experimental set-up – the Automated Adaptive Directed Evolution Chamber (AADEC) -- with minimal scope for human interference was developed at NASA Ames.[2] *E. coli* in LB kanamycin media was used for performance verification. The system was equipped with an optical system including two 260 nm UV-C LEDs, an array of 600 nm LEDs, and their corresponding photodiodes. Media temperature measurement was done using RTD sensors placed in contact with the cell growth chamber. The 600 nm optics measured optical density and hence overall microbe population health. The choice of stressors was between UV-C radiation and temperature, or both. A miniature magnetic agitation and peristaltic pump systems confirmed continuous nutrient availability and media mixing. Arduino microcontrollers provided system control.

Current Device Description: A second-generation device integrating more real-time biochemical sensors has been developed recently. Added sensors include pH for indicating metabolic products, oxidation-reduction potential (ORP) for indicating available/consumed metabolic energy, dissolved oxygen (DO) for indicating aerobic/anaerobic growth cycles, and electrical conductivity (EC) as an additional indicator of metabolic products. The EC sensor system, Microbial Electrical Conductivity (MECON) was developed end-to-end in-house to suit the needs of the growth chamber dimensions. MECON was optimized to have performance comparable to commercial microsensors in the range of interest. A multilayered fluidic growth chamber was redesigned to have dedicated growth/exposure and sensor chambers to effectively accommodate the sensor microprobes. Rapid prototyping techniques such as laser cutting and CNC milling were employed for machining. The entire sensor system was automated using a Raspberry Pi computer allowing continuous, real-time data acquisition. The fluidics card also has slots for solenoids for magnetic agitation, flow valves compatible with previous generation peristaltic pump, and growth/exposure chamber windows fitted with quartz disks allowing excellent UV-C transmission.

As a proof-of-concept, we have demonstrated multi-parameter sensor system operation in one pair of growth-sensor chambers. *E. coli* electrochemical parameters were recorded over 24 hr growth cycle. Pronounced electrochemical changes can be observed during the switch from aerobic to anaerobic growth at ~6 hr because of the media's dissolved oxygen depletion (Figure 1). Multiple chamber experiments can be easily done to assist in inter-culture comparisons and multi-population studies.[3]

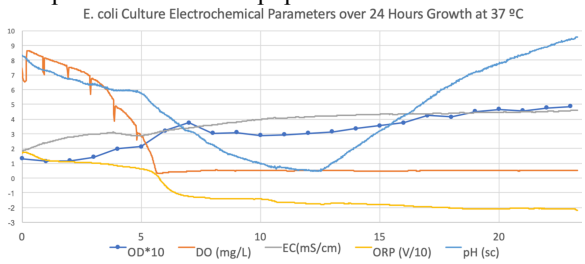

Figure 1: 24 hr E. coli growth biochemical data.

Conclusion and Future Scope: With four additional sensors, the system is biochemically more informative in real-time. More importantly, each sensor parameter can be used as a selection pressure, individually or in combination with others, to artificially create and control inhospitable environments analogous to extremophile habitats for microbial growth in the lab. Potential stressors to be added in the future include thermal, reactive oxygen species, metal-ion concentrations, and varying nutrient availability.

References:

1. Blaich et. al. (2016) Instrumentation for examining microbial response to changes in environmental pressures. Abstract #178038.
2. J.Wang. (2017) Developing and Parallel Array Growth System for Automated Directed Evolution Experiments. Master's thesis. San José State University.
3. Govinda Raj et al. (2018) Enabling Experimental Evolution: Multi-parameter Sensor System Integration into a Culture/Stressor Bio-fluidics System. American Geophysical Union. Abstract #467264.