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Title: An Autonomous System for Experimental Evolution of Microbial Cultures: Test Results using Ultraviolet-C Radiation and Escherichia coli.

Abstract:
At its core, the field of microbial experimental evolution seeks to elucidate the natural laws governing the history of microbial life by understanding its underlying driving mechanisms. However, observing evolution in nature is complex, as environmental conditions are difficult to control. Laboratory-based experiments for observing population evolution provide more control, but manually culturing and studying multiple generations of microorganisms can be time consuming, labor intensive, and prone to inconsistency.

We have constructed a prototype, closed system device that automates the process of directed evolution experiments in microorganisms. It is compatible with any liquid microbial culture, including polycultures and field samples, provides flow control and adjustable agitation, continuously monitors optical density (OD), and can dynamically control environmental pressures such as ultraviolet-C (UV-C) radiation and temperature. Here, the results of the prototype are compared to iterative exposure and survival assays conducted using a traditional hood, UV-C lamp, and shutter system.

The shutter system acts as a manually-controlled counterpart system. It allows the user to precisely control the UV-C exposure time within an enclosed sterile hood in which open liquid cultures or plated samples can be placed. The shutter system consists of two motor-controlled opaque acrylic blades beneath a pair of UV-C bulbs. The motors open the blades in response to a button push and close after a period of time determined by a user-adjustable potentiometer. The system's 'open' position exposes microorganisms to UV-C radiation at a flux determined by user-swappable aperture plates, and the 'closed' position blocks UV-C light to a limit of 1-6 μW/cm^2 (reducible to zero through additional shielding).

The shutter system was used to assay several previously generated experimental samples of Escherichia coli for UV-C tolerance. Each stock was created after one of fifteen iterations of UV-C radiation exposure. Cultures were regrown in 5 mL liquid LB Broth to an OD600 of 0.8, washed twice with 0.9% saline, serially diluted over eight orders of magnitude, and plated in 10 μL aliquots onto LB agar plates. One of two duplicate plates was then exposed to UV-C radiation at a dose of 15 J/m^2 using the shutter system; the other was placed in the same hood, but not exposed. The survival ratio, which compares the mean number of colonies in an iteration that survive UV-C exposure to the control group that was never exposed, was then calculated to determine the UV-C tolerance of each lineage after each of the iterations.

Preliminary results from 6 iterations show that, as expected, resistance to UV-C radiation increases as the iteration number increases. The ideal region for exposure within the UV-C hood was found to yield a consistent UV-C intensity of .29-.31 mW/cm^2 . The exposure time of the shutter was accordingly adjusted to 5 seconds to achieve the specified exposure dose of 15 J/m^2 . Experiments for the remaining iterations, including comparing "by hand" survival assays to assays completed using the shutters, are underway to determine the impact of the shutter system and the effectiveness of the system.

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