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A method to analyze, sort, and retain viability of obligate anaerobic microorganisms from complex microbial communities

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ABSTRACT

A high speed flow cytometric cell sorter was modified to maintain a controlled anaerobic environment. This technology enabled coupling of the precise high-throughput analytical and cell separation capabilities of flow cytometry to the assessment of cell viability of evolved lineages of obligate anaerobic organisms from cocultures.

Mapping functional interactions across microorganisms is essential to fully characterize microbial community structure and ecology, with important implications in human medicine, resource management and extraction, and environmental science. Anaerobic microorganisms are critical components of many important microbiomes, such as in anaerobic digesters (Fernández et al., 1999), anaerobic hydrocarbon deposits (Aitken et al., 2004), and anoxic waters and sediments (Musat et al., 2008). Notwithstanding major advances in laboratory microbiology techniques to study the physiology, systems biology, and microbial interactions of anaerobic microorganisms (Walker et al., 2009, 2012; Hillesland and Stahl, 2010; Hillesland et al., 2014; Yoon et al., 2011, 2013; Stolyar et al., 2007), a major challenge in characterizing anaerobic communities emerges from the technical difficulty of performing high throughput single cell analysis of live cells in complete absence of oxygen.

Flow cytometry is a useful technique for high-throughput analysis and isolation of live single cells for cultivation or experimentation (Chisholm et al., 1992; Davey and Kell, 1996; Wallner et al., 1997; Czechowska et al., 2008). Recently, flow cytometry has facilitated single-cell analyses of genotypic and phenotypic variation that are particularly useful for understanding microbial population community structure and diversity. However, most applications have been limited to aerobic microbial systems because the sort stream and cell deposition areas of flow cytometers are open to the oxygenated atmosphere. Here, we present a new method that enables the separation of target cells from complex samples and preserves the viability of anaerobic cells for downstream experimentation by flow cytometric analysis, cell sorting, and single cell growth under anoxic conditions.

A BD Influx cell sorter (BD Biosciences) was equipped for anaerobic work by eliminating oxygen from the sort stream and cell deposition areas (Fig. 1). An anodized aluminum column was placed around the sort stream, seated in a groove on the cytometer frame, and sealed to the nozzle by a vinyl gasket, which spanned the gap between the aluminum column and upper aspect of the nozzle, creating a sealed interrogation chamber (Fig. 1B). Windows of 12.5 mm diameter MgF_2-coated glass (Edmund Optics, Inc., Barrington, NJ) were installed in the interrogation chamber at 180 and 90° angles to the incident laser line to allow forward scattered light (FSC), side scattered light (SSC), and fluorescence to reach the detectors. The internal seams of the cytometer frame were sealed with acrylic latex caulk (DAP Products Inc., Baltimore, MD). A glove box (12 × 14 × 9 in.) made of anodized

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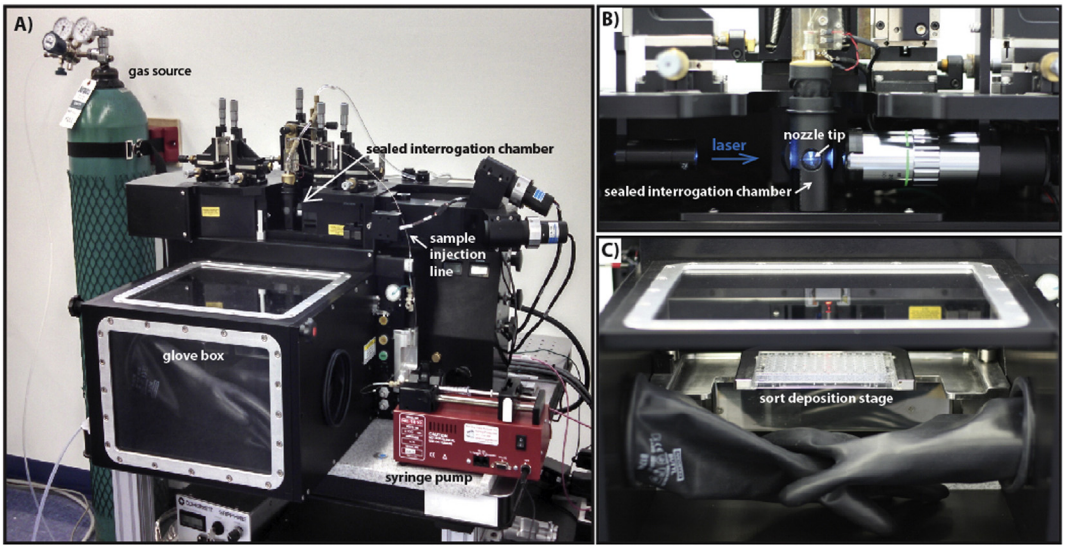

Fig. 1. A BD Influx cell sorter (BD Biosciences) (A) was equipped for anaerobic work by eliminating oxygen from the sort stream (B) and cell deposition area (C).

aluminum with clear polycarbonate thermoplastic windows and 4-inch diameter holes for placement of 16-mil chemical-resistant butyl gloves (Honeywell, Morris Township, New Jersey) was secured to the sort deposition chamber with aluminum bolts, in place of the standard deposition area door, forming a sealed sort chamber and workspace (Fig. 1 C). A port in the glove box was plumbed to a regulated (5–10 psi) tank of high purity nitrogen gas to create a positive-pressure oxygen-free environment during sorting. Oxygen concentration was measured with an Oxygen Pen (Sper Scientific, Scottsdale, AZ) placed inside the sealed sort chamber and was maintained at less than 0.1% during sorting.

Culture samples were loaded into the cytometer by a New Era Pump Systems (Farmingdale, NY) syringe pump. Each sample was run for 5 min at 20 μL per minute before sorting to ensure removal of residual oxygen from the sample line. Cells were interrogated with a 200 mW 488-nm laser (Coherent, Santa Clara, CA) and SSC and FSC were collected for each cell, with FSC as the data collection trigger. Sort gates

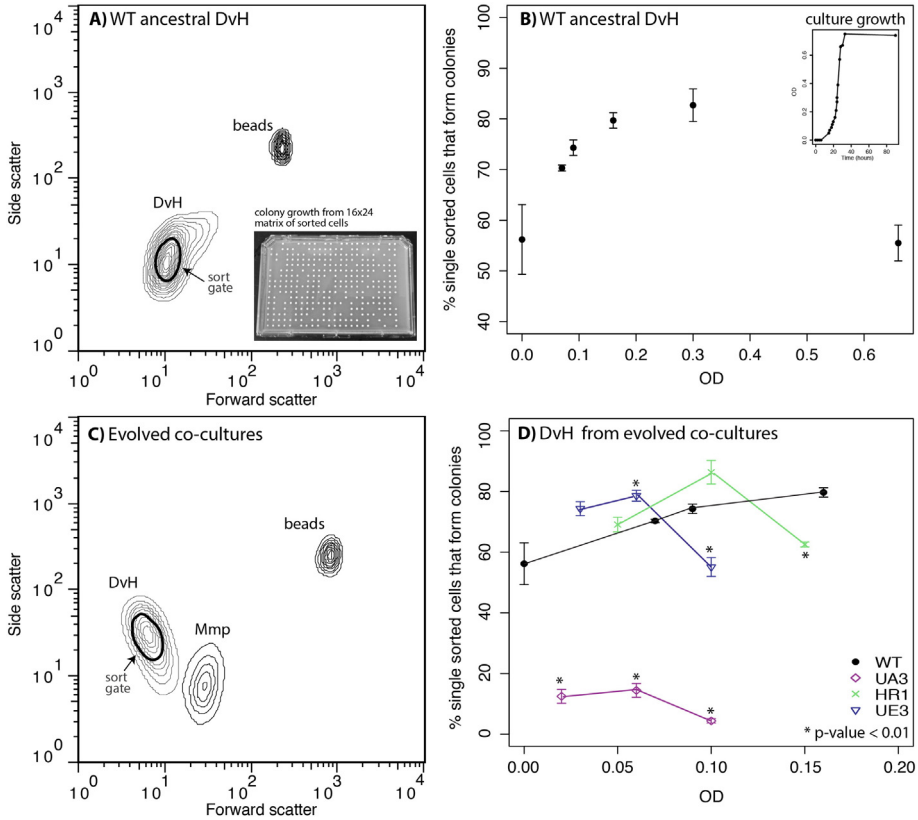

Fig. 2. Comparison of DvH single cell recovery rates from an ancestral monoculture (A, B) and three 1000-generation evolved co-culture lineages with *Methanococcus maripaludis* (UA3, HR1, and UE3) (C, D) in log phase and early stationary phase growth (WT only). Note that the characteristics of the DvH populations varied between wild type (A) and coculture (C) relative to standard beads (1 μm diameter). Inset in (A) displays a typical matrix of colonies growing for the 16 × 24 sort matrix. In (D), p-values are calculated to compare each evolved line to the WT at similar OD. Inset in (B) displays growth data from the DvH WT culture from which single cells were sampled to test single cell growth efficiency plotted in (B).