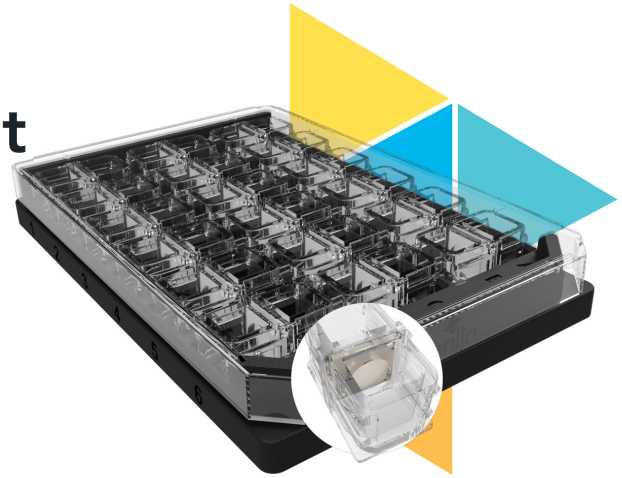
unlocking contact-independent microbial interactions

co-culture



introduction

Microbial co-culture is a technique widely used to study interaction dynamics within populations consisting of multiple microbial strains. While characterizing interactions between microbial populations is essential to understanding different microbial communities, from gut flora to coral reefs, limited tools exist to study individual populations or strains growing within a community. Cerillo's vertical membrane co-culture plate enables quantification of diffusion-mediated, contact-independent interactions between distinct bacterial populations in co-culture. The plate reader-compatible format enables real-time optical density measurements and facilitates the independent characterization and analysis of the co-cultured microbial populations.

co-cultured microbial populations

Cerillo's co-culture devices were used to characterize interactions between Escherichia coli populations followed by multi-species co-cultures with two bacterial species commonly found in the cystic fibrotic lung, Pseudomonas aeruginosa and Burkholderia cenocepacia. Microbes were grown in Lysogeny Broth (LB), LB media diluted with 1x Dulbecco's Phosphate Buffered Saline (DPBS), or DPBS. LB, a nutritionally rich medium for bacterial culture, is widely used for its high bacterial yield and convenience [1]. DPBS creates an environment suitable to microbes but devoid of nutrients, requiring nutrients to diffuse across the membrane to support microbial growth.

Each well of the co-culture plate was loaded with 2 mL of media. Where appropriate, wells were inoculated at a calculated OD600 of 0.0005 with the bacterial strain specified. The co-culture plate was then placed into a Tecan Infinite M200 Pro plate reader, incubated at 37˚C, shaken linearly with 3 mm of travel at 7.5 hertz (450 RPM), and OD measurements were recorded at 600 nm every 5 minutes. All of the experiments were conducted in triplicate with biological replicates.

The impact that diffusion of metabolites across the membrane might have on growth characteristics was studied first. E. coli was grown in two conditions, 'pre-mixed' and 'gradient', at four concentrations of LB (Fig 1) and the growth measured using OD600. The 'pre-mixed' condition was inoculated on one side of the membrane with equal concentrations of LB (diluted to 50% with DPBS) on either side. The 'gradient' condition was also inoculated on one side of the membrane, but started with DPBS in the inoculated reservoir and all of the LB, undiluted, on the opposite side. The total quantity of LB provided between each condition was held constant.

For growth to occur on the DPBS-inoculated side, substrates from LB must diffuse across the membrane. Minimal differences between the paired 'pre-mixed' and 'gradient' conditions were observed at each of the four concentrations tested indicating that the essential metabolites in LB are able to diffuse across the membrane at a sufficiently rapid rate to allow E. coli to grow similarly to the control case for different concentration gradients and population densities.

Co-culture between competing cultures was then characterized by culturing in one co-culture duet E. coli in isolation on one side with LB in the adjacent reservoir and, in another duet, two E. coli populations separated by the membrane, thus competing for nutrients (Fig 2). Both of these conditions were assessed at 50% LB (diluted with1x DPBS) and100% LB.

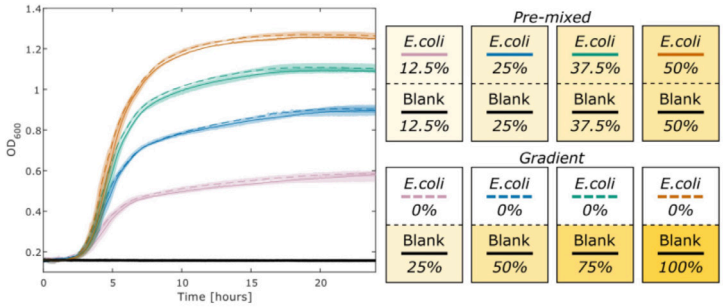


Fig1. Substrate diffusion across co-culture duet porous membrane supports microbial population growth comparable to that of microbes grown in pre-mixed media with equivalent nutritional carrying capacity.

The condition in which E. coli is isolated on just one side of the co-culture chamber acts as a reference point compared to the case in which two E. coli populations are competing for the same metabolites. For the condition in which E. coli is competing and cultured with 100% LB, the growth characteristics are similar to those observed when E. coli is isolated and cultured in 50% LB. This result indicates that there is a comparable amount of biomass produced on either side of the membrane in the 100% LB competing case to the amount of biomass produced in the 50% LB isolated case. The slight differences between these curves demonstrate fascinating growth dynamics, captured by utilizing co-culture duets.

Multi-species co-culture was then studied for P. aeruginosa and B. cenocepacia by, for each species, culturing in one co-culture duet one microbial species in isolation on one side with LB in the adjacent reservoir and, in another duet, two reservoirs with the same microbial populations separated by the membrane, and, in another duet, P. aeruginosa grown in one reservoir and B. cenocepacia grown across the membrane, in the adjacent reservoir (Fig 3).

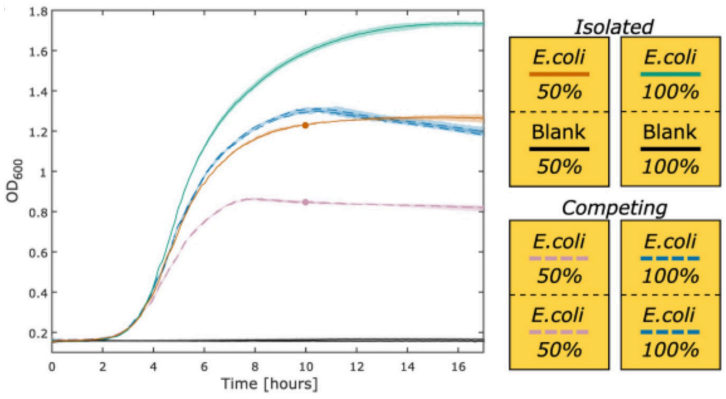


Fig 2. Growth curve for competitive microbial growth deviates from population growth with comparable resources but without competition.

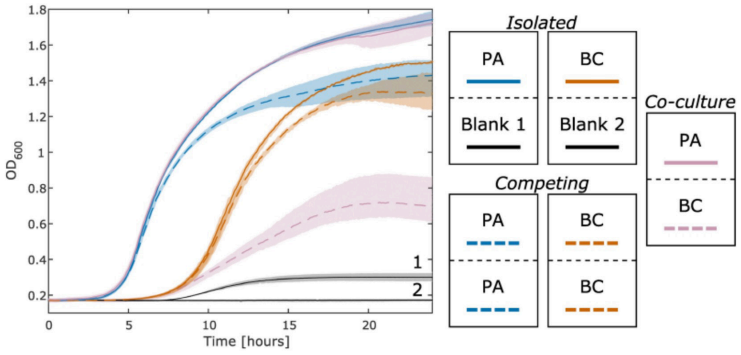


Fig 3. Co-culture duets enable quantitative analysis of growth curves of P. aeruginosa (PA) and B. cenocepacia (BC) in co-culture

summary

Cerillo co-culture duets enable distinct microbial populations to be grown together, physically separated by a vertical membrane, allowing measurement of contact-independent interactions. This culture plate allows for high-throughput and high-resolution phenotypic assessment of microbial interactions. Compatibility with the Cerillo Stratus and other commercially available plate readers allows for the rapid generation of optical density growth curves.

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