Live-Fish-Facs-Droplet Cultivation: A Strategy to Explore Recalcitrant Acidobacteria

Nita, Iuliana; Buijs, Yannick; Bentzon-Tilia, Mikkel

Publication date:
2023

Document Version
Publisher's PDF, also known as Version of record

[P159] LIVE-FISH-FACS-DROPLET CULTIVATION: A STRATEGY TO EXPLORE RECALCITRANT ACIDOBACTERIA

Iuliana Nita[1], Yannick Buijs, Mikkel Bentzon-Tilia

[1]*Technical University of Denmark, Department of Biotechnology and Biomedicine, Kgs. Lyngby, Denmark*

Antibiotics from few culturable microorganisms have prevented millions of deaths over the past many decades. While initially effective, their efficacy has dramatically decreased due to the emergence and spread of antibiotic resistance. As antibiotic resistant microorganisms are predicted to cause 10 million deaths by 2050, we are in dire need of novel antimicrobial molecules. The potential of natural microbiomes to produce diverse secondary metabolites (SMs) with therapeutic properties is an attractive solution to mitigate this crisis, and the abundant acidobacterial phylum has proven to harbor a vast repertoire of biosynthetic gene clusters encoding potentially bioactive SMs. However, most clades of the Acidobacteria are recalcitrant to cultivation, which impedes our ability to harness the potential of these SMs. To circumvent this limitation, we present a strategy aiming at coupling Fluorescence in situ Hybridization within living bacteria (live-FISH) with Fluorescence-Activated Cell Sorting (FACS) and droplet cultivation to selectively isolate these bacteria in order to gain access to previously uncultured and biosynthetically talented acidobacterial strains. Employing DNA probes hybridizing to the 16S rRNA of Acidobacteria subdivision 1 in a conventional FISH experiment allowed us to identify members of this group, including the uncultivable Angelobacter clade, from soil samples. Following this, we utilized Acidobacterium capsulatum as a model organism to demonstrate the feasibility of a live-FISH methodology targeting Acidobacteria. Despite a severe reduction in cell viability, we were able to improve cell survival through a modified hybridization approach. Separate from the Live-FISH procedure, we encapsulated A. capsulatum cells in minuscule droplets, and after three days of incubation, we observed proliferation, demonstrating the capacity of A. capsulatum to thrive in 200 pL-droplets. Further refinement of the live-FISH method and its integration with droplet encapsulation and cultivation will lead to targeted isolation of previously uncultured acidobacterial strains, allowing us to tap into the biosynthetic repertoire of this promising phylum.