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Microtiter Dish Biofilm Formation Assay

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ABSTRACT

Biofilms are communities of microbes attached to surfaces, which can be found in medical, industrial and natural settings. In fact, life in a biofilm probably represents the predominate mode of growth for microbes in most environments. Mature biofilms have a few distinct characteristics. Biofilm microbes are typically surrounded by an extracellular matrix that provides structure and protection to the community. Microbes growing in a biofilm also have a characteristic architecture generally comprised of macrocolonies (containing thousands of cells) surrounded by fluid-filled channels. Biofilm-grown microbes are also notorious for their resistance to a range of antimicrobial agents including clinically relevant antibiotics. The microtiter dish assay is an important tool for the study of the early stages in biofilm formation, and has been applied primarily for the study of bacterial biofilms, although this assay has also been used to study fungal biofilm formation. Because this assay uses static, batch-growth conditions, it does not allow for the formation of the mature biofilms typically associated with flow cell systems. However, the assay has been effective at identifying many factors required for initiation of biofilm formation (i.e, flagella, pili, adhesins, enzymes involved in cyclic-di-GMP binding and metabolism) and well as genes involved in extracellular polysaccharide production. Furthermore, published work indicates that biofilms grown in microtiter dishes do develop some properties of mature biofilms, such a antibiotic tolerance and resistance to immune system effectors. This simple microtiter dish assay allows for the formation of a biofilm on the wall and/or bottom of a microtiter dish. The high throughput nature of the assay makes it useful for genetic screens, as well as testing biofilm formation by multiple strains under various growth conditions. Variants of this assay have been used to assess early biofilm formation for a wide variety of microbes, including but not limited to, pseudomonads, Vibrio cholerae, Escherichia coli, staphylocci, enterococci, mycobacteria and fungi. In the protocol described here, we will focus on the use of this assay to study biofilm formation by the model organism Pseudomonas aeruginosa. In this assay, the extent of biofilm formation is measured using the dye crystal violet (CV). However, a number of other colorimetric and metabolic stains have been reported for the quantification of biofilm formation using the microtiter plate assay. The ease, low cost and flexibility of the microtiter plate assay has made it a critical tool for the study of biofilms.

ATTACHMENTS

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EXTERNAL LINK

https://dx.doi.org/10.3791%2F2437O'Toole GA. Microtiter Dish Biofilm Formation Assay. JoVE (Journal of Visualized Experiments) [Internet]. 2011 Jan 30 [cited 2019 Mar 19];(47):e2437. Available from: https://www.jove.com/video/2437/microtiter-dish-biofilm-formation-assay

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Growing a Biofilm

1 Grow a culture of the wild-type *Pseudomonas aeruginosa* or mutant strain over night in a rich medium (i.e. LB)

2 Dilute the over night culture 1:100 into fresh medium for biofilm assays. A standard biofilm assay medium for *P. aeruginosa* is M63 minimal medium supplemented with magnesium sulfate, glucose and casamino acids (see Table). As an alternative biofilm-promoting medium that stimulates less planktonic growth and a more robust biofilm, the glucose and casamino acids can be replaced with arginine as the sole carbon and energy source.

3 Add 100 µL of the dilution per well in a 96 well dish. For quantitative assays, we typically use 4-8 replicate wells for each treatment.

4 Incubate the microtiter plate for 4-24 hrs at 37°C.

Staining the Biofilm

5 After incubation, dump out cells by turning the plate over and shaking out the liquid.

6 Gently submerge the plate in a small tub of water (i.e., use the bottoms of pipette tip boxes for P1000 pipetmen as the tub). Shake out water. Repeat this process a second time. This step helps remove unattached cells and media components that can be stained in the next step, and significantly lowers background staining.

7 Add 125 µL of a 0.1% solution of crystal violet in water to each well of the microtiter plate. Wear gloves and a lab coat while making the solution. Use caution when weighing out the CV as the powder is hydroscopic and readily stains clothing, skin, etc.

8 Incubate the microtiter plate at room temperature for 10-15 min.

9 Rinse the plate 3-4 times with water by submerging in a tub of water as outlined above, shake out and blot vigorously on a stack of paper towels to rid the plate of all excess cells and dye.

10 Turn the microtiter plate upside down and dry for a few hours or overnight.

11 For qualitative assays, the wells can be photographed when dry.

Quantifying the Biofilm

12 Add 125 µL of 30% acetic acid in water to each well of the microtiter plate to solubilize the CV.

13 Incubate the microtiter plate at room temperature for 10-15 min.

14 Transfer 125 µL of the solubilized CV to a new flat bottomed microtiter dish.

15 Quantify absorbance in a plate reader at 550 nm using 30% acetic acid in water as the blank.