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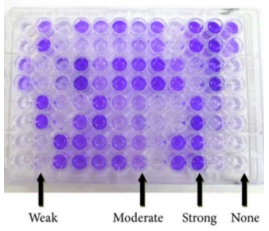
High-throughput assay for quantifying bacterial biofilm formation



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Protocol status: Working
We use this protocol and it's working

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Abstract

Biofilms are assemblages of single-celled organisms attached to a surface and encased in a sticky extracellular polymeric substance that the cells produce. Difficult to eradicate, they can have profound effects on human health and industrial processes. Studying biofilm formation and testing potential biofilm treatments or mitigation strategies requires a robust protocol for quantifying bacterial biofilms with high-throughput and reproducibility. We have developed such a protocol using readily-available staining materials and a plate reader.

Materials

96-well plates
Incubator
Multichannel pipette (P200)
Crystal violet (0.04%)
Ethanol (95%)
Plate reader

Set-up biofilms

1 Dilute overnight bacterial culture to an optical density (OD600) of 0.01 with fresh media.

2 For the blank samples, pipette 100 uL of sterile media into at least 8 wells of a 96 well plate. These wells have media but no cells and no treatment. Purpose: account for the effect of media on optical density.

3 For the blank+treatment samples, pipette 100 uL of sterile media + treatment into at least 8 wells of the 96 well plate. Do this for each treatment. These wells have media + treatment but no cells. Purpose: account for the effect of treatment on optical density.

4 For the positive control samples, pipette 100 uL of diluted bacterial culture into at least 8 wells of a 96 well plate. These wells have cells but no treatment. Purpose: assess maximum biofilm formation.

5 For the experimental samples, pipette 100 uL of diluted bacterial culture plus 1-10 uL of treatment into at least 8 wells. Purpose: assess effect of treatment on biofilm formation.

6 Incubate 96 well plate with lid for 24 hours without shaking at optimal temperature for bacterial growth. To reduce evaporation in the wells, keep the lidded plate inside a bag along with a moist paper towel.

1d

Remove planktonic cells

7 After 24 hours incubation, measure OD600 of the wells with a plate reader. This will be a measure of overall growth, not biofilm formation. Check for contamination in the blank wells.

8 The biofilms should be adhered to the bottom and sides of each well. To remove media and planktonic cells, gently pour off supernatant into sink with running water or container with bleach-water.

9 Wash the biofilms - with a multichannel pipette, gently transfer 200 uL sterile water into each well. Swirl without disturbing biofilms.

10 Pour off the sterile water by inverting the plate and shaking it gently over the drain/container. You want to remove the planktonic cells without disturbing the biofilms.

11 Repeat step 9: Gently transfer another 200 uL sterile water into each well. Swirl without disturbing biofilms.

12 Repeat step 10: Pour off the sterile water by inverting the plate and shaking it gently over the drain/container. You want to remove the planktonic cells without disturbing the biofilms.

Stain biofilms

5m

13 Using a multichannel pipette, add 150 uL 0.04% crystal violet per well. Make sure to coat all of the cells that are stuck in the biofilm. Incubate for 5 minutes.

5m

14 Pour off the crystal violet by inverting the plate and shaking gently over the drain/container with water running.

15 To wash away excess crystal violet, gently transfer 200 uL sterile water into each well. Swirl without disturbing biofilms.

16 Pour off the sterile water by inverting the plate and shaking it gently over the drain/container. Do not disturb the biofilms.

17 Repeat step 15: Gently transfer another 200 uL sterile water into each well. Swirl without disturbing biofilms.

18 Repeat step 16: Pour off the sterile water by inverting the plate and shaking it gently over the drain/container. Do not disturb the biofilms.

Solubilize the cells

19 Using a multichannel pipette, add 100 uL of 95% ethanol to each sample well. Make sure to coat all cells in the biofilm. Pipette up and down to release the cells from the biofilm and mix with the ethanol. DO NOT POUR OFF THE ETHANOL.

Quantify biofilms

20 Remove any water from the bottom of the 96 well plate.

21 Use a plate reader to measure the OD492 of each well. The amount of biofilm in each well is proportional to the amount of crystal violet released from the ethanol-solubilized biofilm cells.

22 Determine if there are outliers in each set. A common rule for outliers is any data point that is 1.5x the IQR above the upper quartile or below the lower quartile of a set of data.

23 Calculate average optical density of each set of samples.

24 Subtract appropriate blank averages from the positive control samples and experimental samples.