Crystal Violet Staining & Quantification

Purpose

Crystal violet is used in the microscopic examination of cells to highlight and give contrast to specific features of the cell, e.g. nuclei and cytoplasm.

Materials

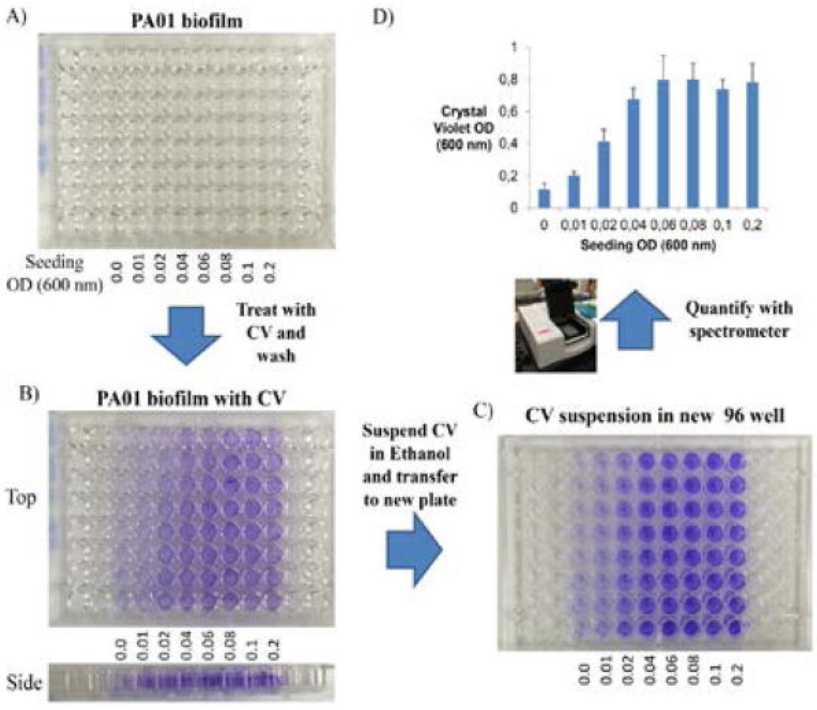
- 100 mL M9 media (CONCENTRATION at 1000x) and glutamine (CONCENTRATION at 100x), filtered through a 0.2µm vacuum filtration unit once at 100mL)
- 51 mL LB media (no antibiotics present)
- agar plates containing streaked bacteria of desired strains
- 5 50mL conical tubes
- 11 15mL conical tubes
- 4 U-bottom 96 well tissue culture plates
- 2 flat bottom 96 well tissue culture plates
- 0.1% crystal violet (0.2g crystal violet in 200mL water)
- 33.6 mL 30% acetic acid

Procedure

Note: steps 4-9 should be done within the sterile field of an ethanol-cleaned lab bench under a Bunsen burner to minimize contamination.

1. Add 10 mL LB into conical tubes tubes
2. Pick and place one colony from each plate in each 50 mL tube.
3. Tighten and tape caps and set to incubate in shaker overnight at 37°C and 175 RPM
4. Dilute all cultures except in 15mL conical tubes
5. Check OD600 of each culture, veru=ifying that it is 0.1
6. Pipet 100µL of the OD600= 0.1 solutions into each of the first 6 wells of the first row of each of the four plates. Repeat with the next four strains and the next four rows.
7. Pipet 100µL of M9 into the first 6 wells of the sixth row of each of the four plates.

8. Set two of the plates in the incubator at 37°C. Leave the other two on the lab bench.
9. After 48 hours, take one plate from the incubator and one plate from the bench.
10. Rinse each plate two to three times with distilled water.
11. Pipet 125µL of 0.1% crystal violet into each well previously containing liquid (rows A-F, columns 1-6 of both plates).
12. After 10 minutes, rinse both plates with distilled water three to four times.
13. Pipet 200µL of 30% acetic acid into each well previously containing liquid (rows A-F, columns 1-6 of both plates).
14. Pipet up and down four times then transfer 100mL of each well from the room temperature plate into the corresponding well in a flat bottom 96 well plate. Repeat for the 37°C plate, but transfer into the empty wells (A7 - F12) on the right side of the same flat bottom 96 well plate.
15. Pipet 100µL of 30% acetic acid into each well of row G of the flat bottom 96 well plate.
16. Process OD595 in an absorbance plate reader.
17. After 24 hours, repeat steps 12 - 18 with the remaining plates.



Safety Precautions

Crystal violet can lead to permanent pigmentation of the skin when in contact with granulation tissue and should not be used on lesions of the face. May produce allergic contact dermatitis or necrosis in the intertriginous areas. Has seen reports on animal carcinogenicity when used as bacterial and fungi treatment. Is a toxic substance and should be handled with care. Is unsafe in animal feed and has shown dose-related carcinogenic properties on different organs.

Reference

Fünfhaus, A., Göbel, J., Ebeling, J. *et al.* Swarming motility and biofilm formation of *Paenibacillus larvae*, the etiological agent of American Foulbrood of honey bees (*Apis mellifera*). *Sci Rep* 8, 8840 (2018). https://doi.org/10.1038/s41598-018-27193-8

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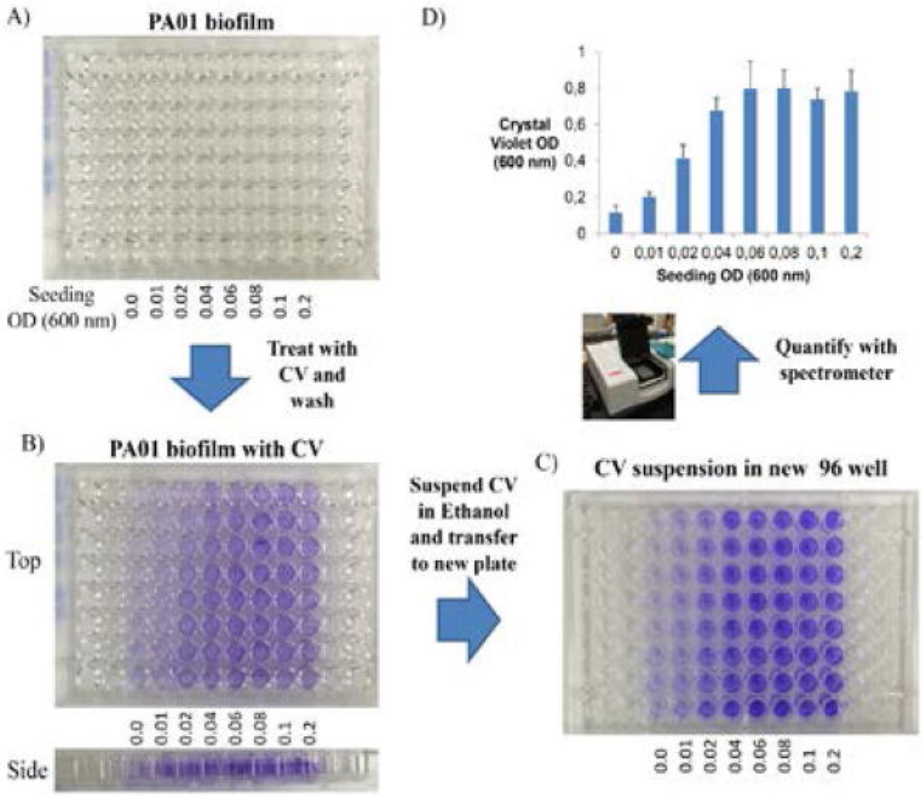
https://www.nature.com/articles/s41598-018-27193-8

a. NOTE: Need generate standard curve!
b. 1% solution CV in deionized water, incubate room temp (5-30min)
c. wash growth media + planktonic cells w DI water
d. Decoloring solution (V greater or equal original culture media) + incubate 10-30min
    i. Solution 90-95% ethanol, but other solution can use
e. CV infused decoloring solution to 96 well plate→ assess absorbance 530-600nm

2.
3.
4. Place the cells to be examined on the ice and wash them with cold PBS.
5. Fix the cells with 100% ice-cold methanol for about 10 minutes.
6. Then aspirate methanol from plates. After this, the cells can be stained with crystal violet or stored for future use. For storage, the cells should be covered with 50% glycerol in PBS, wrapped in a plastic wrap or parafilm and refrigerated.
7. To stain the cells, move them to room temperature and add 0.5% crystal violet solution in 25% methanol. Then incubate the cells for about 10 minutes and remove the crystal violet stain by washing in water until the dye comes off. After washing, allow the cells to dry at room temperatures.
8.



9.