Biofilms, Let's Stick Together

Bianca Cerda , Daniela Gonzalez , Gabriela Verde, Karina Lizama

Motivation

Biofilms have been the cause of many health issues across the world while showing dominant resistance against various antibiotics.

Background

Biofilms are a surface- connected bacterial community which play a crucial role in various environments.(Lene et.al, 2011). Biofilms consist of protein , DNA , polysaccharides, and polymer matrix. They are known to cause persistent infections due to their tolerance against antibiotics creating a global health issue (Niels et.al,2010).

Methods

Growing bacteria

- Bacteria 19A in LB broth was incubated at 29 °C in 3 separate petri dishes with inserted cover slip.

Fixing

- 4% of formaldehyde was added throughout every hour; 3 hour period- 3 dishes.

Staining

- 500 microliters of SYBR green was added and left for 20 minutes.

Affinity labeling application

- Ulex lectin (UEA-1)
- Goat antibody biotin
- Rabbit anti-goat biotin
- Coverslip was removed and placed on glass slide with moveol.
- Slides were observed under the fluorescent microscope.
  - SYBR green- nucleic acids shown through fluorescence.
  - Orange/Red = extracellular matrix shown through binding.

Results

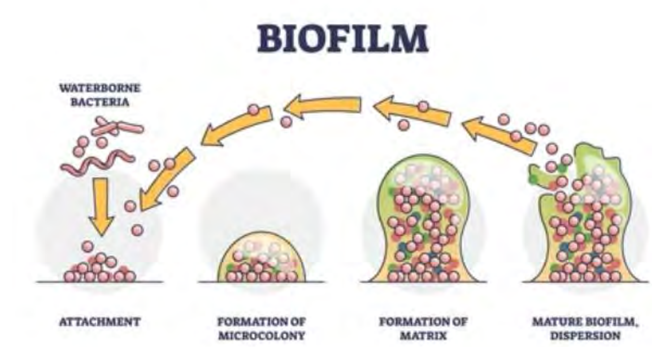

Figure 1: A visual diagram that shows the processes of biofilm formation.

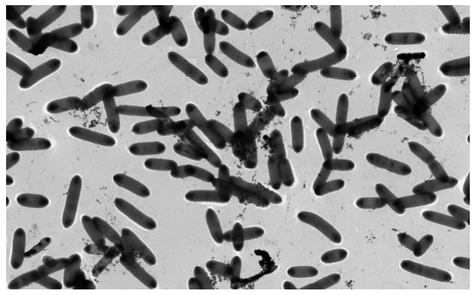

Fig 4: An Image of bacteria 19A taken with electron microscope (TEM). The bacteria found was mostly a mixture of bacillus, diplobacilli, as well as a few palisades.

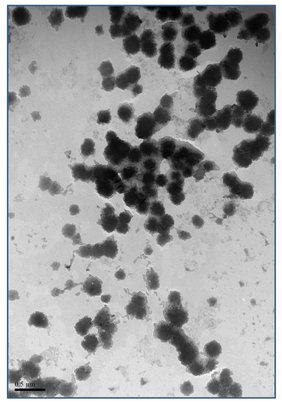

Fig 3: This Image shows bacteria 19A in biofilm formation taken with electron microscope. In order to capture the image negative staining was used;Which allowed the bacteria to be seen more clearly.

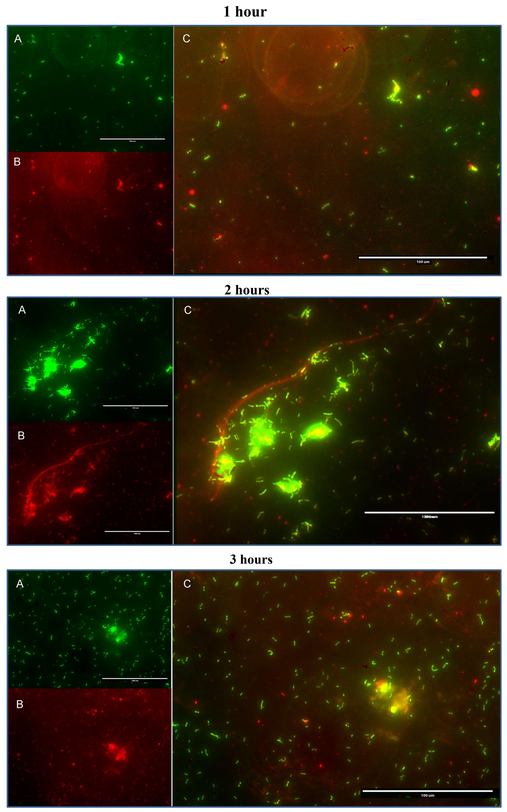

Figure 2: Had a time frame of 3 hours; Fixed every hour with 4 % formaldehyde at a 3 hour period.- Bacteria stained with SYBR green and affinity labeling, observed with fluorescent microscope at 40x magnification.
A: SYBR green used to stain nucleic acids.
B: Affinity labeling used to label extracellular matrix.
C: Both staining and labeling overlapping.

Discussion

As shown in figure head 2; During the time between 1 to 3 hours different amounts of biofilms were found on our coverslips,which were fixed every hour. And while we were able to partly answer our original question by finding out what could stimulate biofilm formation, we changed from using a crystal violet assay to a lectin assay for bacteria staining. The reason being because the crystal violet would penetrate the biofilms too much making it very difficult to study our chosen bacteria under the microscope.While the group did expect to find biofilms in our coverslides we were surprised to find extracellular bacteria along with the biofilm; This being because the lectin that we used, a ulex lectin, binds to specific proteins or enzymes in the bacteria. So by having the lectin bind to it we know it has this specific protein or enzyme but we haven't able to identify it yet, with more time we would have like to identify the unknown extracellular bacteria.

Conclusions

Biofilm were discovered in our coverslips, some that were separated were in the shape of bacillus with very few cocci. Along with the biofilms, some extracellular matrix, that has yet to be identified, was also present. The staining of the bacteria was accomplished by using a SYBR green stain, ulex lectin, and affinity labeling to label the bacteria on our coverslips. We concluded that the bacteria did stick down to a surface (the cover slip) creating a biofilm colony.

Future Work

If we had extra time we would have liked to continue researching:

- At what concentration does the lectin become a stimulus/inhibitor?
- To discover if the extracellular that was found on our biofilm coverslips is truly extracellular or something else?

References

Yan J, Bassler BL. Surviving as a Community: Antibiotic Tolerance and Persistence in Bacterial Biofilms. Cell Host Microbe. 2019 Jul 10;26(1):15-21. doi: 10.1016/j.chom.2019.06.002. PMID: 31295420; PMCID: PMC6629468.

Vestby LK, Grønseth T, Simm R, Nesse LL. Bacterial Biofilm and its Role in the Pathogenesis of Disease. Antibiotics (Basel). 2020 Feb 3;9(2):59. doi: 10.3390/antibiotics9020059. PMID: 32028684; PMCID: PMC7167820.

Acknowledgements

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For more information, contact me@my.email.com

Alternate Text

Daniela Gonzalez, Gabriela Verde, Karina Lizama, Bianca Cerda

Oak Crest

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