# Detection of Catecholamines Produced in Planktonic *P. aeruginosa* and *S. aureus* Treated with Adult Bovine Serum

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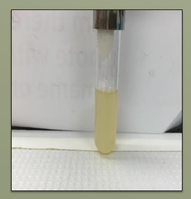
## Introduction

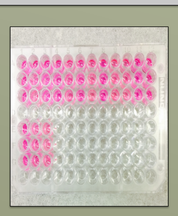
Bacterial biofilms play a critical role in inducing and sustaining chronic wounds that are serious health threats. Bacterial biofilms can also be found on medical prosthetics and implants that sustain infections in patients and cause life threatening situations. Bacteria self-produce these sticky extracellular substances termed a biofilm which help them to adhere to each other forming a community of microorganisms. One of the major issues is that biofilms have antimicrobial characteristics and provide protection from the immune system; biofilms are found in over 80% of human bacterial infections. Formation of a bacterial biofilm occurs when an individual (planktonic) bacterial cell attaches to a surface such as collagen exposed in a wound. The planktonic bacterial cell then converts into a biofilm phenotype which allows it to grow and divide on the surface thereby forming layers of microcolonies. After maturation, which is characterized by the production of an extracellular matrix, cells detach from the biofilm and disperse to re-enter the planktonic mode and repeat the biofilm cycle. Under conditions of stress, namely injury or disease, the human body releases adrenaline-like hormones called catecholamines such as epinephrine (adrenaline) and norepinephrine (noradrenaline). Many studies have indicated a close relationship between the presence of catecholamine hormones in a human host and the growth, formation, and virulence of bacterial biofilms. Furthermore, studies from Dr. Isseroff's dermatology lab at UC Davis confirm that the presence of these catecholamines at dermal wound sites impair the healing process by generating a cellular response through activation of beta-adrenergic receptors. However, few species of bacterial biofilms have been shown to produce catecholamines independently, and none have been shown to produce epinephrine. We examined two species of bacteria commonly found in chronic wounds, *Pseudomonas aeruginosa* (Gram negative) and *Staphylococcus aureus* (Gram positive), to determine whether they can produce catecholamines in eukaryotic cell growth conditions. We examined the supernatants of the media after the bacteria were cultured with 0% and 10% concentrations of Adult Bovine Serum (ABS) and then detected for the presence of catecholamines by High Pressure Liquid Chromatography Electrochemical Detection (HPLC-ED).

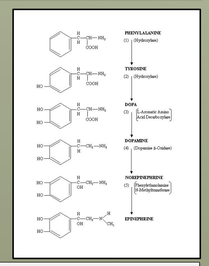
## Materials/Methods

### Planktonic Bacterial Culture Preparation

Overnight cultures are prepared using aseptic technique as follows. Briefly, colonies of *Staphylococcus aureus* and *Pseudomonas aeruginosa* are inoculated in Tryptic Soy Broth or Luria-Bertani media, respectively. Cultures are grown with shaking aeration at 37 degrees Celsius. The next day, the cultures are spun down to pellet and supernatant is removed. The pelleted bacterial cells are resuspended in Dulbecco's Modified Eagle Medium (DMEM) and normalized to cell number as determined by optical density. These cultures were passaged into 30 mL DMEM and allowed to grow for 12 hours with samples being monitored for turbidity at 0, 4, 8, and 12 hours. Approximately 1 mL samples were aliquoted from 30 mL cultures intended for 12 hour growth curves at 0, 4, 8, and 12 hours. The samples were then acidified with 110 microliters of 0.2M perchloric acid to prevent oxidation of catecholamines prior to HPLC analysis. Samples were stored at -80 degrees Celsius until processing.








### HPLC Analysis of Catecholamines

The catecholamines within the acidified media from the above planktonic growth assays were extracted using MonoSpin® PBA solid phase extraction spin columns. An internal standard (3, 4-dihdroxybenzylamine; DHBA) was added to each sample (2.5ng) to determine the efficiency of extractions. Retained catecholamines were eluted in 200uL of 2% acetic acid, and 70uL was injected into the HPLC for electrochemical detection analysis (performed in duplicate). For HPLC, a Synergi™ 4um fusion-reverse phase column (250 x 4.6 mm) maintained at 28°C was used for separation of compounds. Catecholamine detection was performed using a LC-4C amperometric detector using an oxidizing potential of 700mV at a flow rate of 1mL/min.

## Results

| | Average Yield | Accuracy |
|---|---|---|
| Norepinephrine | 77.4 ±3.7% | 94.9% |
| Epinephrine | 76.4 ±1.4% | 93.2% |
| Dopamine | 75.4 ±1.6% | 92.0% |

Figure 1: Percent yield of PBA extractions and accuracy of HPLC-ED catecholamine analyses. Known amounts of standards were extracted using PBA monospin columns and then quantified by HPLC-ED. After correcting for the percent yield of catecholamines using the internal standard DHBA, the accuracy was determined by comparing the PBA extracted values to the known amounts. The experiment was repeated 5 times to generate averages.

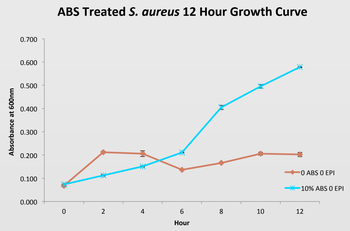



Figure 2: DMEM containing 10% ABS enhances *S. aureus* growth rate.

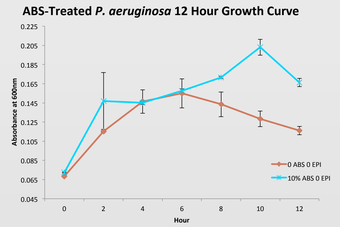



Figure 3: DMEM containing 10% ABS promotes *P. aeruginosa* growth rate.

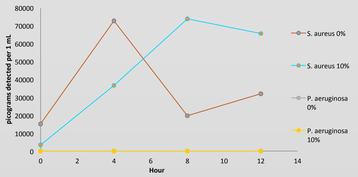



Figure 4: *S. aureus* produced considerable amounts of norepinephrine during both 12 hour growth curves. 10% ABS treatment resulted in higher production of norepinephrine in *S. aureus*. *P. aeruginosa* did not produce norepinephrine (nor any other catecholamines) in either 0% or 10% growth curve.

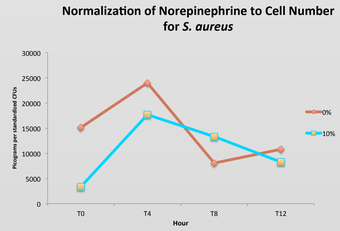



Figure 5: Norepinephrine production in *S. aureus* normalized to cell number as determined by determining the colony forming units (CFU).

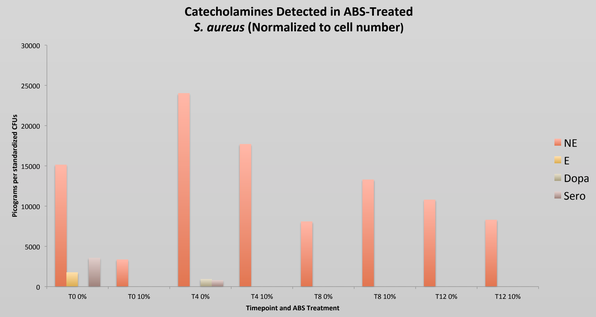



Figure 6: Twelve hour culture of *S. aureus* with or without ABS demonstrated the production of catecholamines, predominantly norepinephrine. Interesting the amount of norepinephrine decreased over time. Also, it is notable that epinephrine was found at the initial time point (T0).

## Results Continued

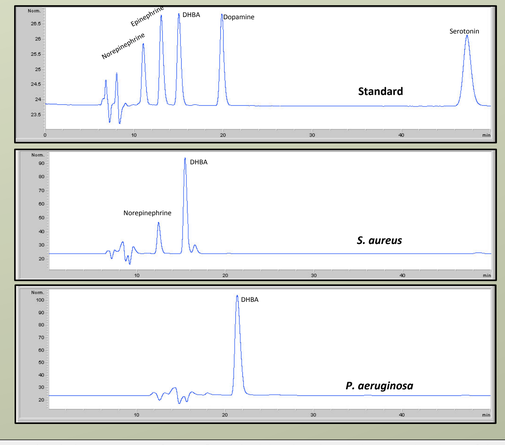



Figure 7: (**Top**) Chromatogram of a $1x10^{-5}$ standard detailing the relative retention times of the catecholamines observed. (**Middle**) Chromatogram of *S. aureus* at the 4 hour time point and 0% ABS conditions indicating the presence of catecholamines. (**Bottom**) Chromatogram of *P. aeruginosa* at the 4 hour time point and 0% ABS conditions indicating an absence of any detectable catecholamines.

## Conclusions and Future Work

- Growth of both *S. aureus* and *P. aeruginosa* was found to be higher with 10% ABS treatment.
- HPLC analysis of planktonic *S. aureus* indicates the production of epinephrine and serotonin at the initial time points, which could be a carry over effect from the overnight cultures.
- HPLC analysis of planktonic *P. aeruginosa* indicated no production of any catecholamine products at any time point throughout the growth curve.
- *S. aureus* was found to produce considerable amounts of norepinephrine during both 0% and 10% growth curves. Higher production of norepinephrine was indicated under 10% ABS treatment.
- Future efforts will be focused on:
  - → Repeating these experiments to provide biological replicates
  - → Detecting catecholamine production in *S. aureus* and *P. aeruginosa* growth in bacteriological media.
  - → Catecholamine analysis of biofilm samples from *S. aureus* and *P. aeruginosa.*

## Acknowledgments

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This material is based upon work supported by the Howard Hughes Medical Institute, National Science Foundation, and S.D. Bechtel, Jr. Foundation. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do not necessarily reflect the views of the funders.The STAR program is administered by the Cal Poly Center for Excellence in STEM Education (CESAME) on behalf of the California State University.