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

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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 at 37 degrees Celsius. The next day, the cultures are spun down to pellet and supernatant is removed. The pellet 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 centrifuged and 100 microliters of 0.2M phosphoric 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 conditioned media from the above planktonic growth assays were extracted using Monospin® PBA solid phase extraction spin columns. An internal standard [3, 4-Dihydroxybenzylamine; DHBA] was added to each sample (2.5μg) to determine the efficiency of extractions. Retained catecholamines were eluted in 200μL of 0.2% acetic acid, and 70μL was injected into the HPLC for electrophysiological detection (performed in duplicate). For HPLC, a Synergi® 4μm Ammonium phosphate column (250 x 4.6mm) maintained at 25°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

Figure 1: Chromatogram of a standard containing dopamine, norepinephrine, and epinephrine.

Table 1: Results of catecholamine detection in S. aureus and P. aeruginosa.

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 norepinephrine 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

References:
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