Methods: Quantitative Techniques

OEST 740
050608
Conventional Techniques

- **Dry weight**
- **Tetrazolium Reduction Assay**
  - Tetrazolium salts – colorimetric determinates measured spectrophotometrically
  - Reduced by mitochondrial dehydrogenases
    - MTT – violet – DMSO
    - XTT – brown – easier – water soluble product
- **Biofilm formation**

Hawser & Douglas 1994
Conventional Techniques

- Radiolabelling
  - Incorporation of $[^3H]$ Leucine
  - Precipitated by TCA and radioactivity measured
  - Used to assess the effects of antibiotics, biofilm formation
  - *E. coli*, *Candida spp.*

Dix et al. 1988

Hawser & Douglas 1994
Conventional Techniques

- Imaging
  - (ex. Confocal Laser Scanning Microscopy)
    - Thickness, cell density, composition

- Cell counts
  - Direct viable cell counts (DVC)
    - To assess disinfection efficiency
  - Plate counts
    - Estimate survival populations after disinfection
MBEC Assay

- Relies on CFU’s as a proxy for the presence of a microbial biofilm
- Assumes bacteria are only present if they formed a biofilm
- Pro: high-throughput, commercially available
- Con: does not delineate other effects
MBEC Assay

- Primarily utilized to analyze the effects of chemical treatment on biofilm formation.

Harrison et al. 2005
MBEC Assay

Harrison et al. 2005
MBEC Assay

- Relies on the recovery of attached cells from substratum exposed to chemical treatment.

Harrison et al. 2005
MBEC Assay

- Provides 3 comparison measurements in each experiment: MIC, MBC and MBEC

Harrison et al. 2005
MBEC Assay

- Growth of bacterial biofilms in MBEC assay have been found to be very reproducible

Harrison et al. 2005
Electrochemical Impedance Spectroscopy

- Bacterial adhesion and biofilm proliferation
- Utilizes a one-compartment, 3-electrode cell
  - Working electrodes
    - Grids - colonized
    - Coupons – non-colonized
- Can account for non-specific adsorption of substances

Dheilly et al. 2008
Electrochemical Impedancemetry

- Impedance could be correlated to biofilm growth time and confirmed by CLSM

Dheilly et al. 2008
Electrochemical Impedancemetry

- Can provide real-time monitoring of both biofilm formation and growth.
  - *P. aeruginosa*

Dheilly et al. 2008
Crystal violet

- Applied to pre-formed biofilms to assess biofilm formation.
- Dissolved with ethanol
- Measured spectrophotometrically

O’Toole
Crystal violet

- Biofilm formation of avirulent and WT strain of *B. pertussis*.

Setta et al. 2007
Fourier Transform Infrared Spectroscopy

Setta et al. 2007
Fourier Transform Infrared Spectroscopy

Setta et al. 2007
Alamar Blue

- Redox indicator
- Antibiotic susceptibility testing
- Simple and amendable to high-throughput technologies.

Pettit et al. 2005
**Alamar Blue**

- Method verified using XTT reduction and CFU/ml

Pettit et al. 2005
Summary

- The importance of biofilm formation and the roles it plays in biocorrosion and antibiotic resistance has lead to the advancement of several quantitative methods.
  - detect the formation of biofilms
  - susceptibility to different chemical agents.

- Despite advances conventional biofilm techniques remain invaluable to the verification of new methods.