CYTOCHROME *BD* IS REQUIRED FOR UROPATHOGENIC *ESCHERICHIA COLI* PATHOGENESIS AND BIOFILM DEVELOPMENT

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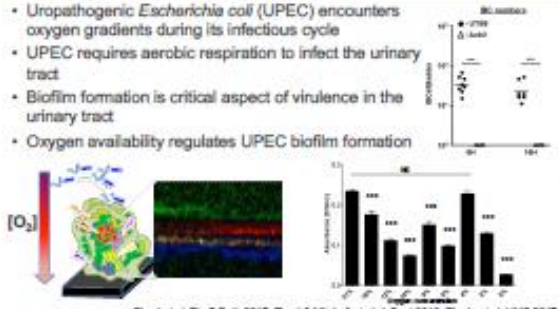
Biofilm formation is a common bacterial survival strategy

- Bacteria form multicellular communities known as biofilms in the natural environment and during infection
- Bacteria in biofilms are highly resistant to antibiotics and immunity
- Biofilms are a major contributor to chronic bacterial infection and treatment failure

Oxygen is a regulator of UPEC biofilms

- Uropathogenic *Escherichia coli* (UPEC) encounters oxygen gradients during its infectious cycle
- UPEC requires aerobic respiration to infect the urinary tract
- Biofilm formation is critical aspect of virulence in the urinary tract
- Oxygen availability regulates UPEC biofilm formation



Floyd et al. PLoS Path 2015; Floyd & Mitchell et al. J. Bact 2018; Eberly et al. UMS 2017

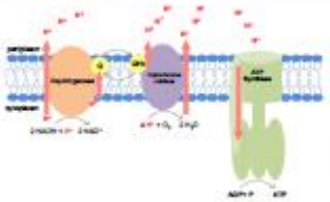
Quinol oxidases mediate aerobic respiration

Heme copper oxidase

- Cytochrome *bo* (*cyoABCD*)
- Atmospheric (21%) oxygen

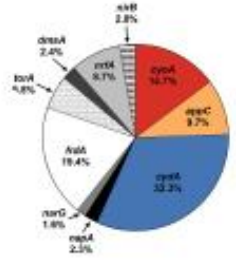
***bd*-type oxidases**

- Cytochromes *bd* (*cydABX*) and bd_2 (*appBC*)
- Low (2 – 15%) oxygen



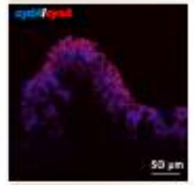
Adapted from Eberly et al. UMS 2017

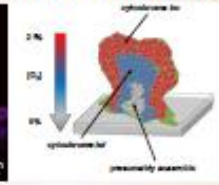
Cytochrome *bd* is the most abundant respiratory transcript in UPEC biofilms



- UPEC exhibits respiratory heterogeneity in biofilms
- The majority of transcript corresponds to aerobic respiratory operons
- *cydABX* (cytochrome *bd*) is the most abundant respiratory operon in biofilms

Quinol oxidases are expressed in spatially distinct subpopulations



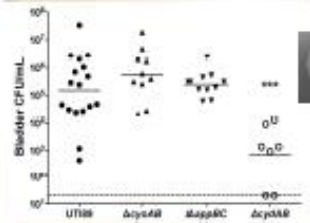


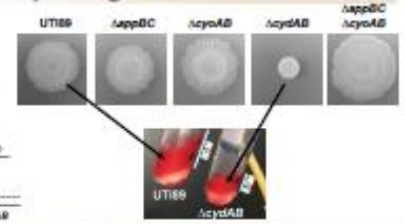
- Cytochrome *bo* and *bd* are inversely organized along the oxygen gradient
- UPEC organizes into differentially respiring subpopulations in biofilms

Hypothesis

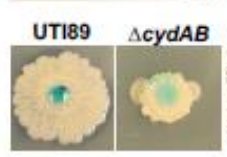
Differentially respiring subpopulations regulate biofilm development and stress tolerance

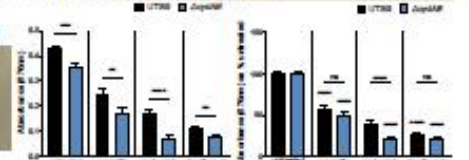
Loss of cytochrome *bd* disrupts biofilm formation and UTI pathogenesis

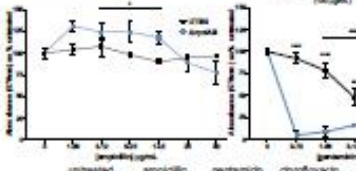


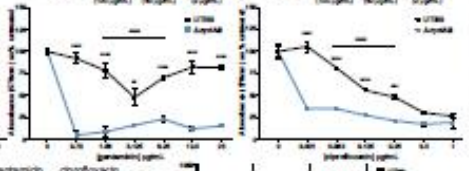


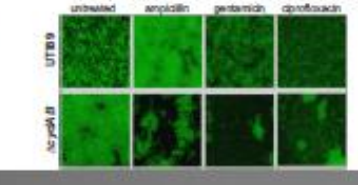
Loss of cytochrome *bd* increases biofilm sensitivity to antibiotics

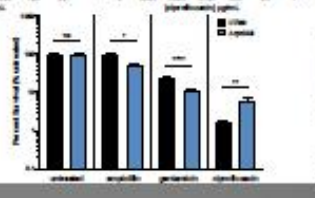




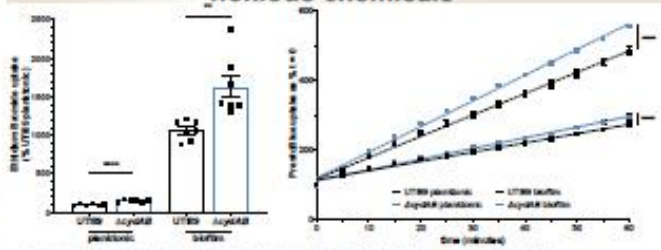






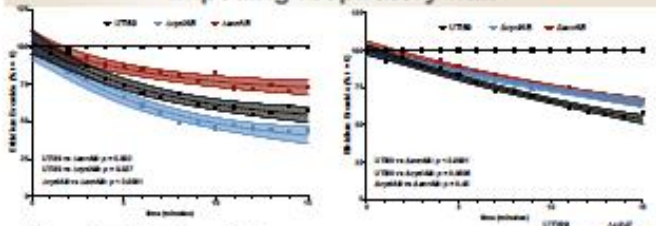


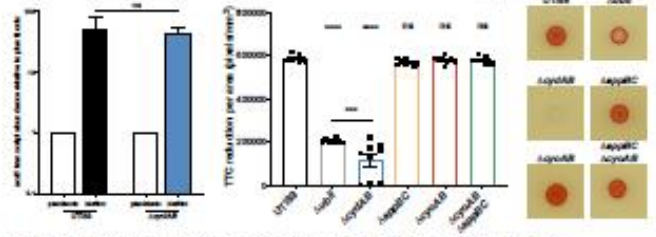
ΔcydAB biofilms cells have increased influx of noxious chemicals



- Loss of cytochrome *bd* increases expression of outer membrane porins
- Δ*cydAB* biofilm cells have increased influx of noxious chemicals

Loss of cytochrome *bd* decreases efflux by impeding respiratory flux





- Δ*cydAB* cells have a biofilm-specific decrease in efflux of noxious chemicals
- Δ*cydAB* reduces efflux pump activity without affecting expression

Conclusions

- Quinol oxidases are spatially organized along biofilm oxygen gradients
- Cytochrome *bd* is central regulator of biofilm development
- Loss of cytochrome *bd* increases biofilm sensitivity to antibiotics by influencing the accumulation of antibiotics and other noxious chemicals
- Inhibition of cytochrome *bd* is a potential therapeutic strategy

Future Directions

- Identify mechanisms by which cytochrome *bd* promotes host colonization
- Define mechanisms by which cytochrome *bd* influences ECM production
- Investigate inhibition of cytochrome *bd* as a potential anti-biofilm therapeutic approach

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