Physiological adaptation in biofilms –

The Achilles’ heel of attached cells?

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... but even AB for which MIC predicts clinical success fail to eradicate the biofilm...
Resistance contributes to failure of therapy

Enterococcus faecium as example

Arrias & Murray, 2012
Tolerance contributes to failure of therapy
Persistence contributes to failure of therapy

Fauvert, ..., Michiels, 2011
**MIC**: minimum inhibitory concentration

**MDK$_{99}$**: minimum duration for killing for 99% of cells in the population

Brauner, ..., Balaban, 2016
Therapy failure linked to physiological adaptations in sessile cells

- Metabolic changes
- Response to oxidative stress

(Expression of sRNA in response to growth arrest/starvation)

(SOS response)

(Stringent response)
Bactericidal antibiotics induce ROS

Gram-positives
Gram-negatives
Fungi
Mitochondria

Van Acker & Coenye, 2017
Burkholderia cenocepacia biofilms contain persister cells that are impossible to eradicate.

Van Acker, ..., Coenye, 2013

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Antibiotic treatment induces ROS in biofilms

Tobramycin (4 x MIC) - DCFHDA staining + flow cytometry

Untreated

Treated

21 %*

72 %*

*n = 7, p<0.05

Van Acker, ..., Coenye, 2013
Antibiotic treatment induces ROS in biofilms

Ciprofloxacin (4 x MIC)
Luminescence from oxyR::lux promotor fusion

Van Acker, ..., Coenye, 2016
Gene expression in treated cells

Glyoxylate shunt

Surviving cells

TCA cycle

Van Acker, ..., Coenye, 2013
Downregulation of metabolism

NADH ↓
Glyoxylate shunt ↑

Reduced AB uptake
AB targets unavailable
SURVIVAL

ROS ↓

Metabolic active cells

TCA cycle ↑
NADH ↑

ROS ↑
AB targets available
Increased AB uptake
CELL DEATH

Resistance development
Can we use this information to develop novel approaches for treatment?

• Does antibiotic treatment result in more killing if we shut down defense mechanisms against ROS?
Inhibition of *B. cenocepacia* SOD by DETC increases TOB-mediated killing

Van Acker, ..., Coenye, 2013
Knocking out *B. cenocepacia* catalases increases TOB-mediated killing

Van Acker, ..., Coenye, 2013
Antioxidants decrease sensitivity of *B. cenocepacia* towards TOB

% survival compared to TOB alone

- TOB
- TOB+Glutathione
- TOB+Mannitol
- TOB+Cysteine

Van Acker, ..., Coenye, 2016
Can we use this information to develop novel approaches for treatment?

• Does antibiotic treatment result in more killing if we shut down defense mechanisms against ROS?

• Can we increase antibiotic-mediated killing if we shut down the glyoxylate shunt?
Inhibiting the glyoxylate shunt in *B. cenocepacia* increases TOB-mediated killing

Van Acker, ..., Coenye, 2013
Can we use this information to develop novel approaches for treatment?

• Does antibiotic treatment result in more killing if we shut down defense mechanisms against ROS?

• Can we increase antibiotic-mediated killing if we shut down the glyoxylate shunt?

• Can we force cells to become metabolically more active and by doing so increase the effect of treatment with antibiotics?
Metabolite-enabled eradication of bacterial persisters by aminoglycosides

Kyle R. Allison¹, Mark P. Brynildsen¹† & James J. Collins¹,2,3

Escherichia coli & Staphylococcus aureus, 2011
Exogenous Alanine and/or Glucose plus Kanamycin Kills Antibiotic-Resistant Bacteria

Bo Peng,1,2,3 Yu-bin Su,1,3 Hui Li,1,3 Yi Han,1 Chang Guo,1 Yao-mei Tian,1 and Xuan-xian Peng1,*
Antibiotic efficacy is linked to bacterial cellular respiration

Michael A. Lobritz, Peter Belenky, Caroline B. M. Porter, Arnaud Gutierrez, Jason H. Yang, Eric G. Schwarz, Daniel J. Dwyer, Ahmad S. Khalil, and James J. Collins

Escherichia coli, 2015
To conclude ...

- Physiological adaptation plays an important role in reduced susceptibility of sessile cells to antibiotics.
- These physiological adaptations take many forms ...
- ... and are often linked.
To conclude ...

• Physiological adaptation plays an important role in reduced susceptibility of sessile cells to antibiotics

• These physiological adaptations take many forms ...

• ... and are often linked

• Fundamental insights into these adaptations could open up novel approaches to combat biofilm-related infections
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