Precision medicine in microbiology: personalizing antimicrobial and anti-inflammatory treatments in people with cystic fibrosis

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Prof. Paul Cos
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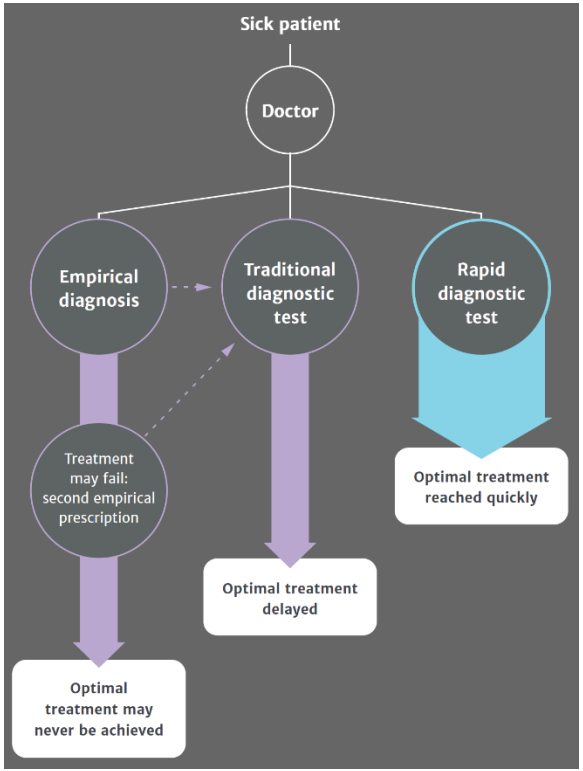
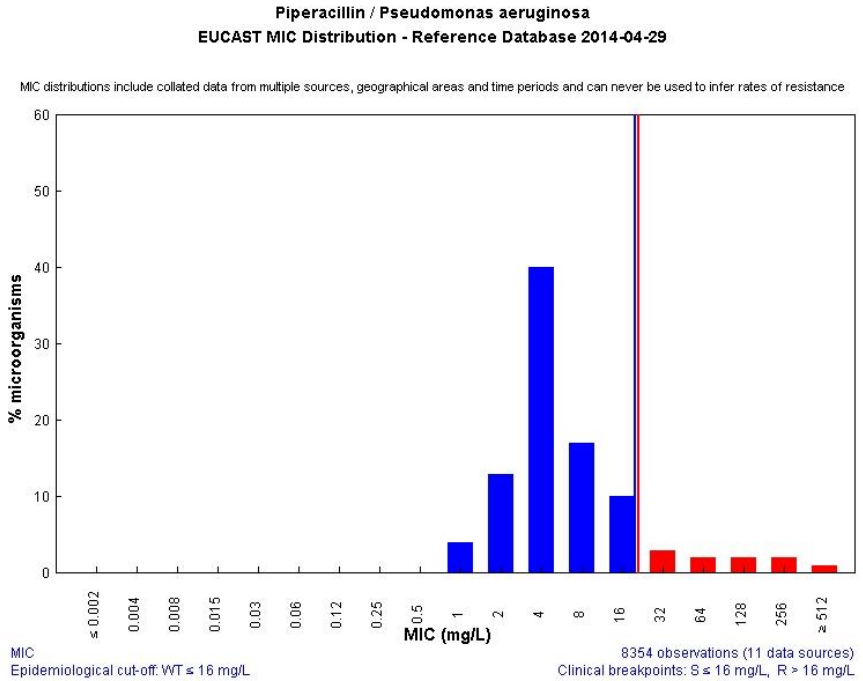
Precision medicine in microbiology

- Treatment of infectious diseases has been benefiting from ‘precision medicine’ for a long time as treatment is often based on susceptibility of infecting micro-organism



O’Neill *et al*., 2016

Review on Antimicrobial Resistance
Tackling drug-resistant infections globally



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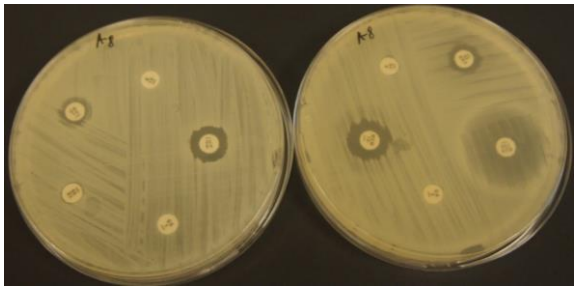

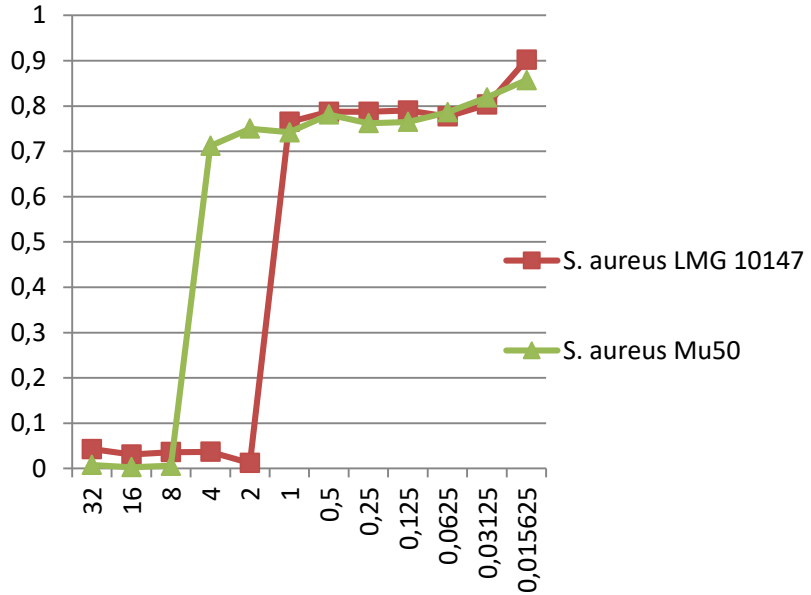

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- Let’s take a closer look at these issues

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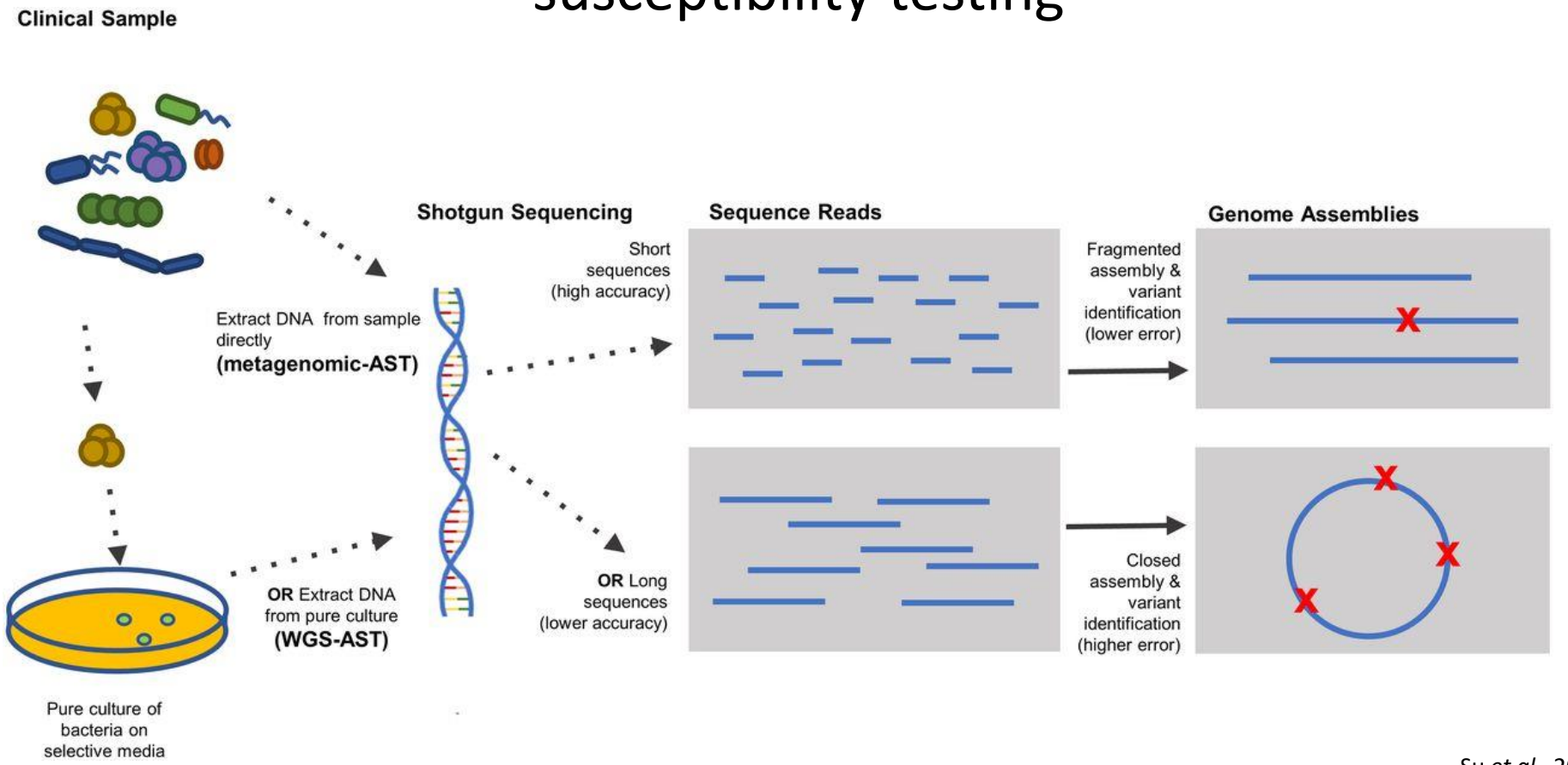
Speed - whole genome sequencing for antimicrobial susceptibility testing



Su *et al.*, 2019

Predicting antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneumoniae* isolates using whole genomic sequence data

N. Stoesser[1,2*], E. M. Batty[3], D. W. Eyre[1], M. Morgan[2], D. H. Wyllie[4], C. Del Ojo Elias[1], J. R. Johnson[5], A. S. Walker[1], T. E. A. Peto[1] and D. W. Crook[1,2]

Prediction of *Staphylococcus aureus* Antimicrobial Resistance by Whole-Genome Sequencing

N. C. Gordon,[a] J. R. Price,[b] K. Cole,[b] R. Everitt,[c] M. Morgan,[d] J. Finney,[a] A. M. Kearns,[e] B. Pichon,[e] B. Young,[a] D. J. Wilson,[a] M. J. Llewelyn,[b] J. Paul,[f] T. E. A. Peto,[a] D. W. Crook,[a] A. S. Walker,[a] T. Golubchik[a]

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The CRyPTIC Consortium and the 100,000 Genomes Project

Rapid antibiotic-resistance predictions from genome sequence data for *Staphylococcus aureus* and *Mycobacterium tuberculosis*

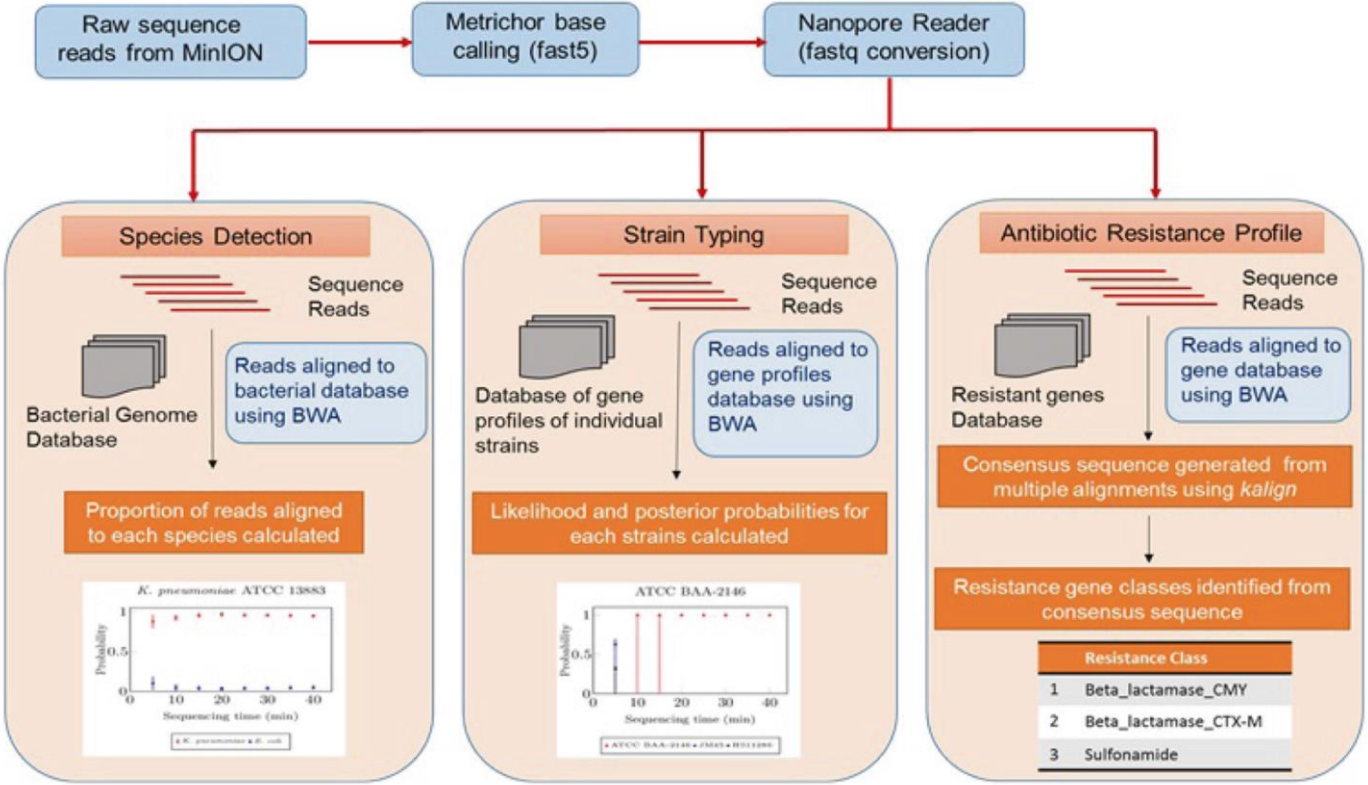
Phelim Bradley[1], N. Claire Gordon[2], Timothy M. Walker[2], Laura Dunn[2], Simon Heys[1], Bill Huang[1], Sarah Earle[2], Louise J. Pankhurst[2], Luke Anson[2], Mariateresa de Cesare[1], Paolo Piazza[1], Antonina A. Votintseva[2], Tanya Golubchik[2], Daniel J. Wilson[1,2], David H. Wyllie[2], Roland Diel[3], Stefan Niemann[4,5], Silke Feuerriegel[4,5], Thomas A. Kohl[4], Nazir Ismail[6,7], Shaheed V. Omar[6], E. Grace Smith[8], David Buck[1], Gil McVean[1], A. Sarah Walker[2,9], Tim E.A. Peto[2,9], Derrick W. Crook[2,9,10] & Zamin Iqbal[1]

Evaluation of Machine Learning and Rules-Based Approaches for Predicting Antimicrobial Resistance Profiles in Gram-negative Bacilli from Whole Genome Sequence Data

Mitchell W. Pesesky[1†], Tahir Hussain[1,2†], Meghan Wallace[3], Sanket Patel[3], Saadia Andleeb[2], Carey-Ann D. Burnham[3,4] and Gautam Dantas[1,3,5,6*]*

Streaming algorithms for identification of pathogens and antibiotic resistance potential from real-time MinION™ sequencing

Minh Duc Cao[1†], Devika Ganesamoorthy[1†], Alysha G. Elliott[1], Huihui Zhang[1], Matthew A. Cooper[1] and Lachlan J.M. Coin[1,2*]



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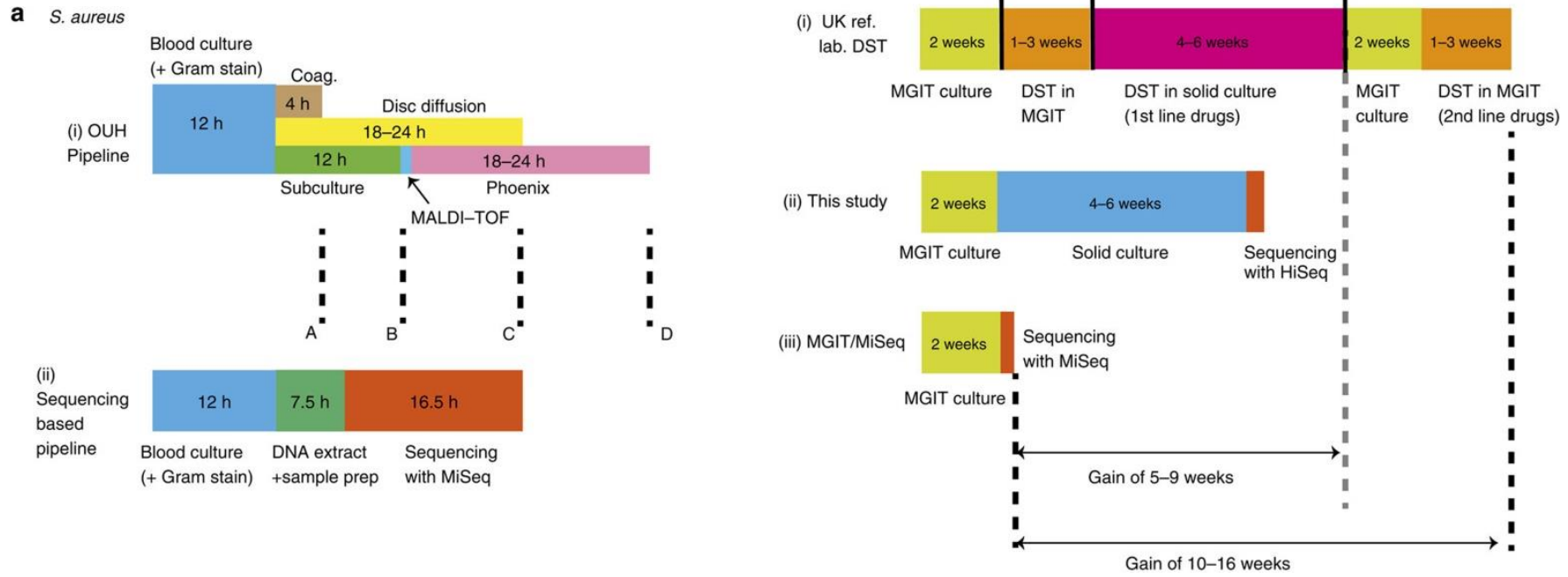

Speed - whole genome sequencing for antimicrobial susceptibility testing

- Potential is enormous
- Downside: focus on *known* resistance mechanisms
- Downside: prediction of gene expression in *in vivo* situation based on whole-genome sequencing is very difficult at best
- This means that acurately predicting a bacterial phenotype will not always be possible

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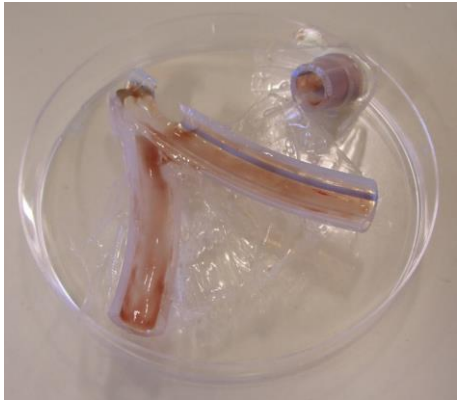
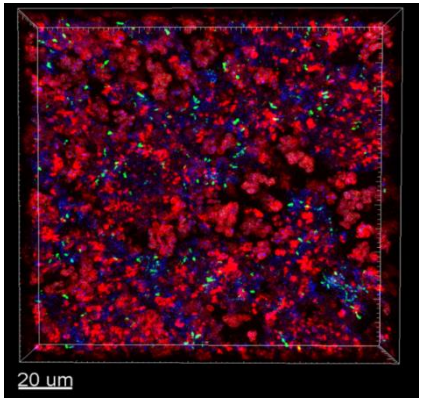
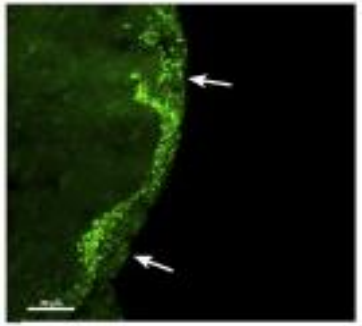
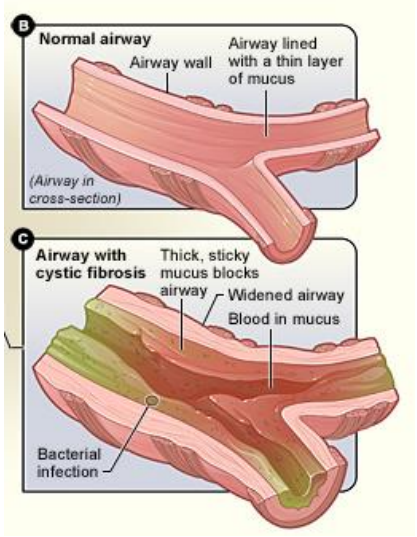
Biofilm phenotype is not taken into account!

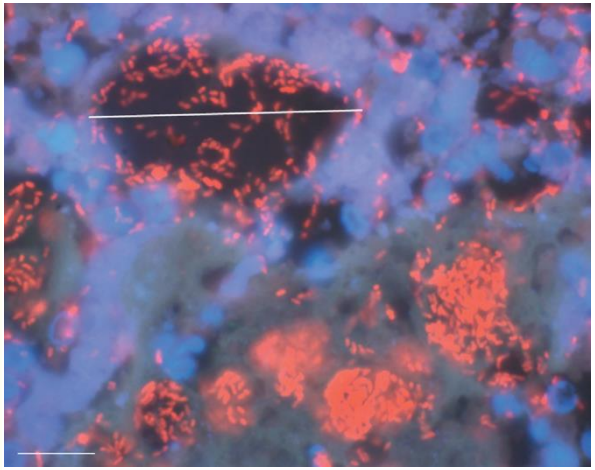


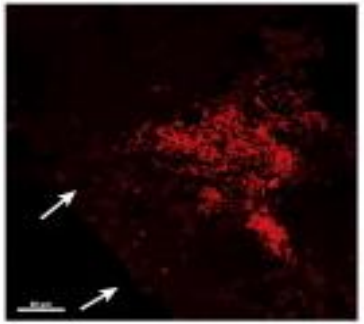




S. aureus

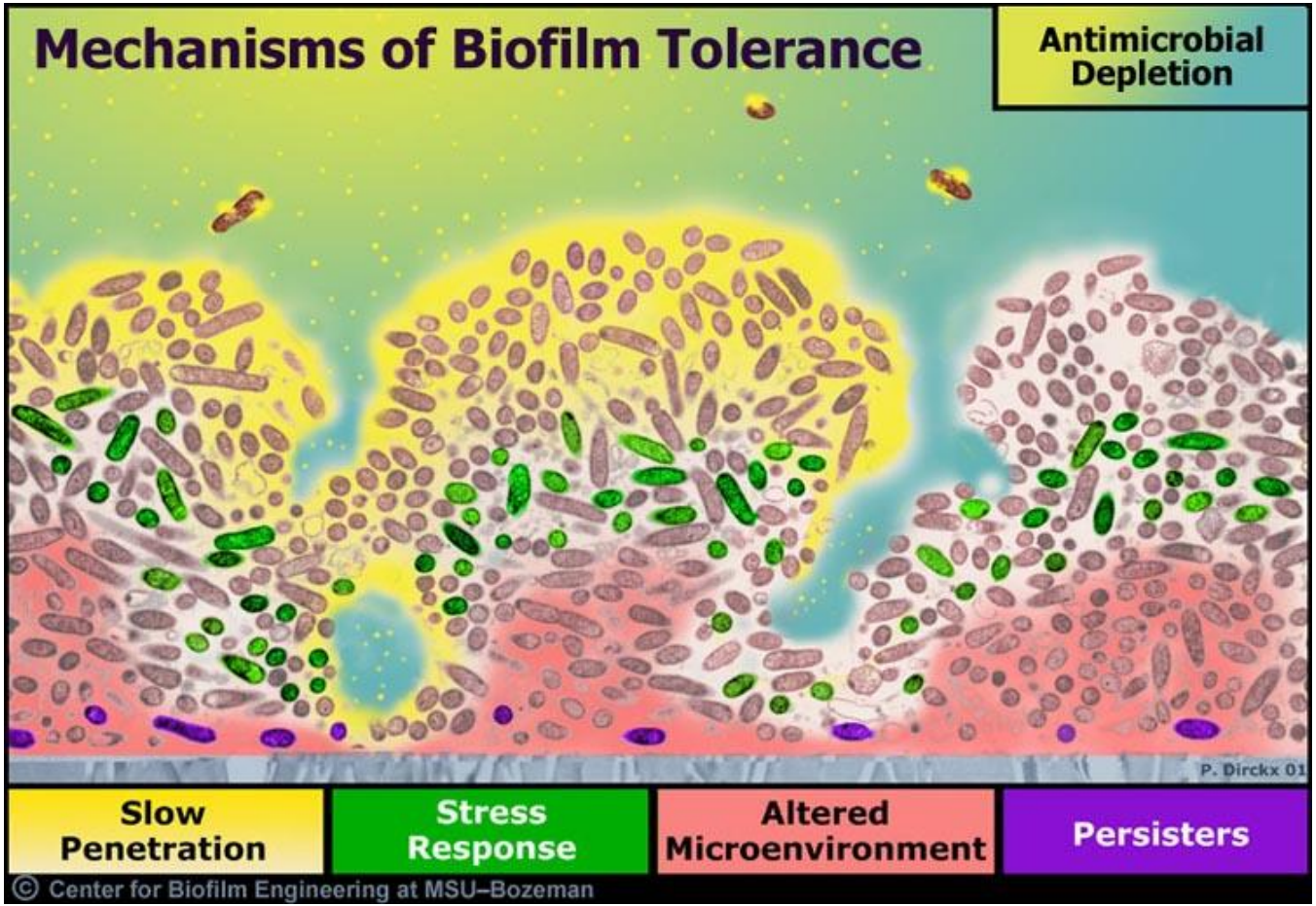




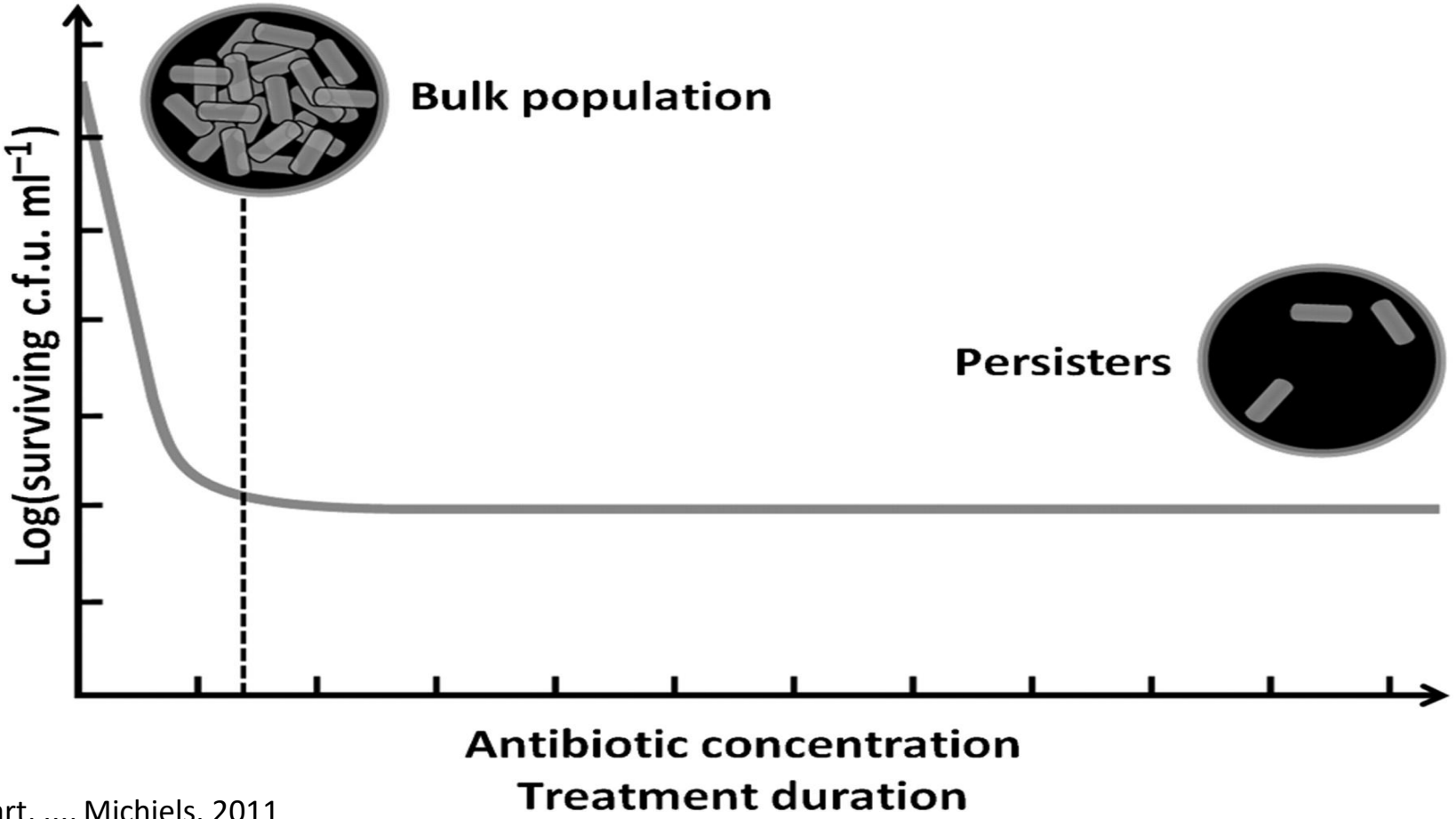


P. aeruginosa

Biofilm tolerance contributes to therapy failure



Persistence contributes to failure of therapy



Fauvart, ..., Michiels, 2011

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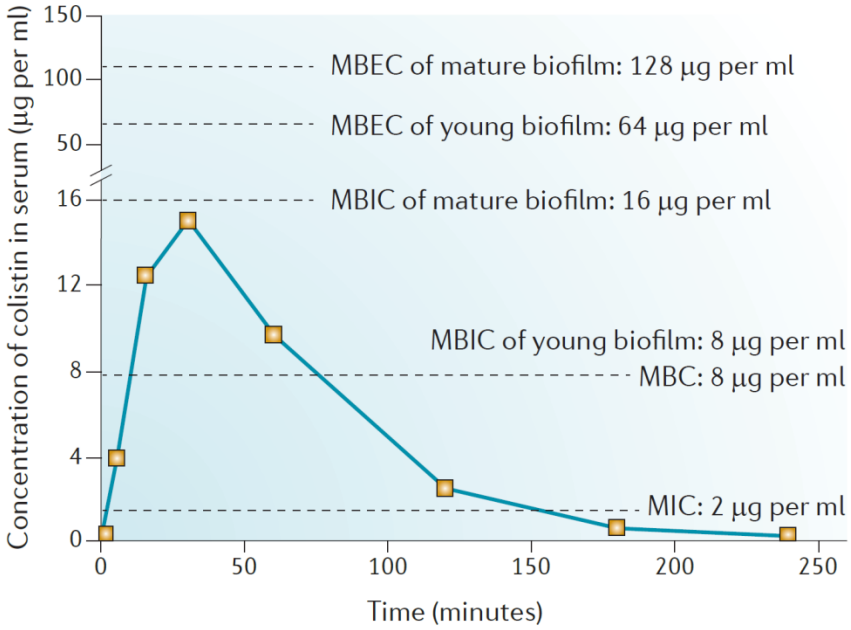
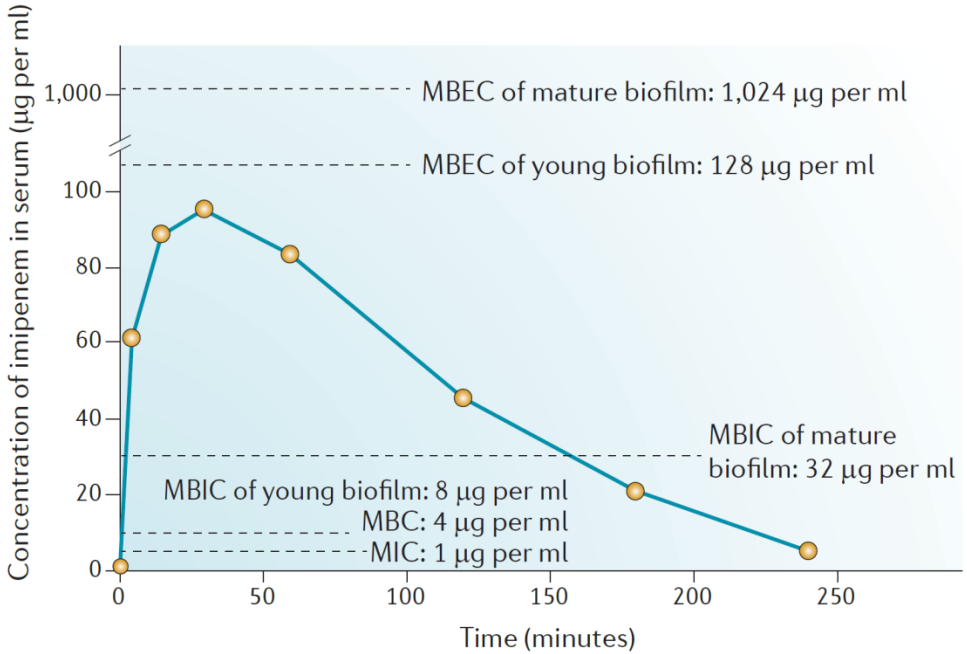
What is the effect *in vivo*?

In vivo PK/PD in a mouse model of biofilm lung infections





Bjarnsholt et al. Nat Rev Drug Discov 2013 12:791-808

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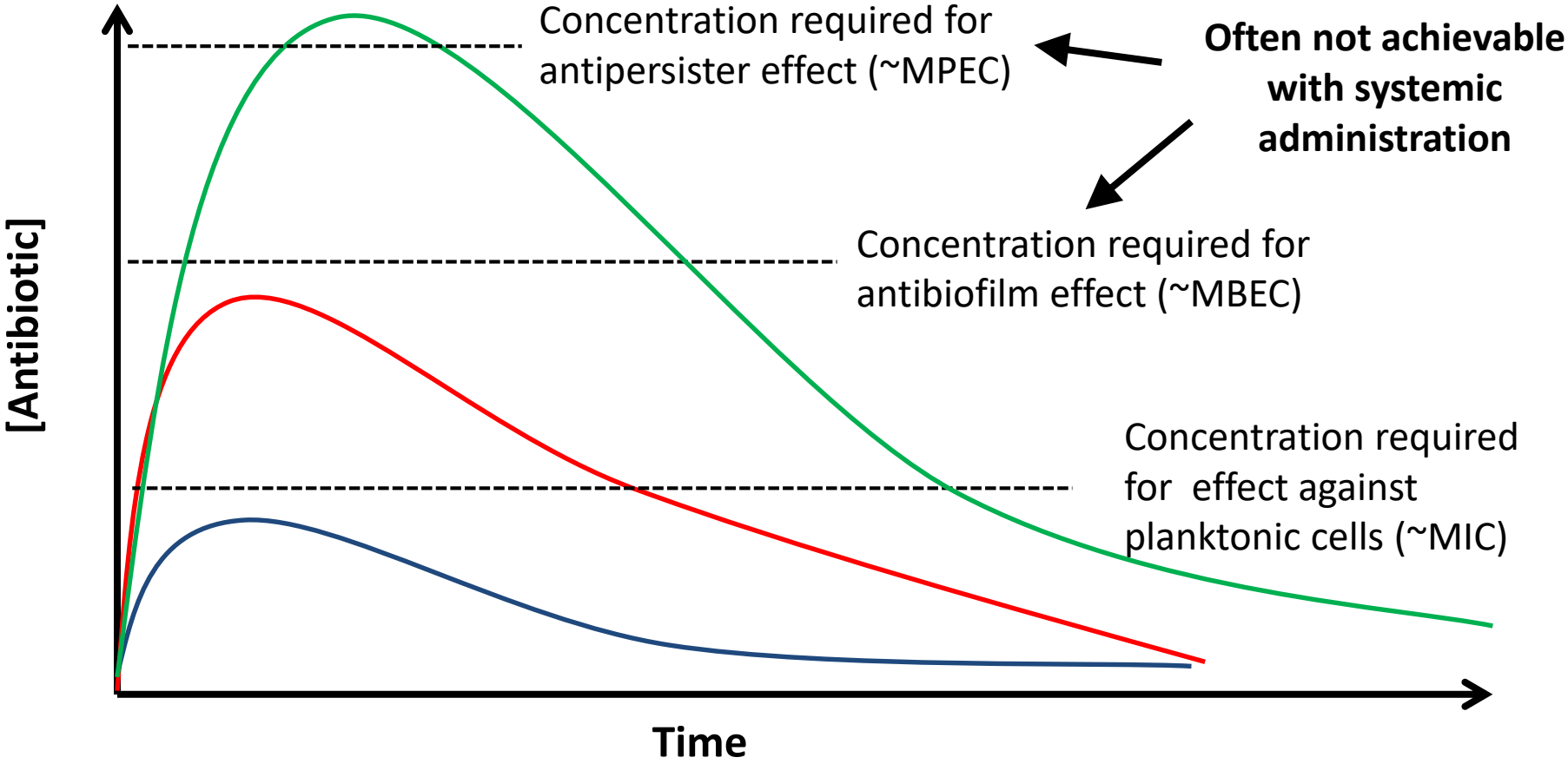

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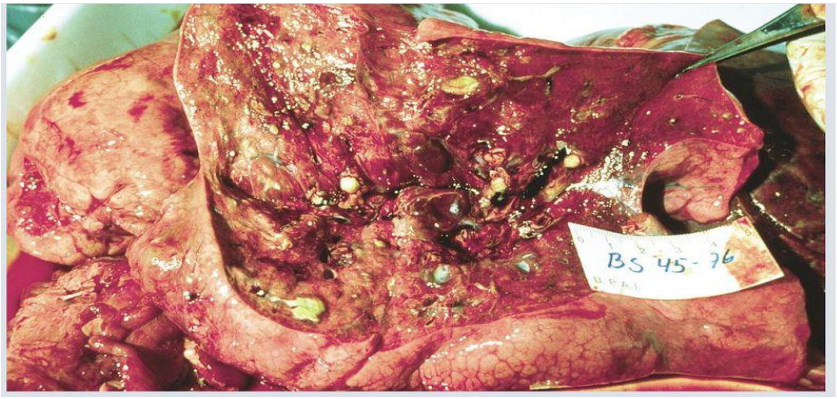
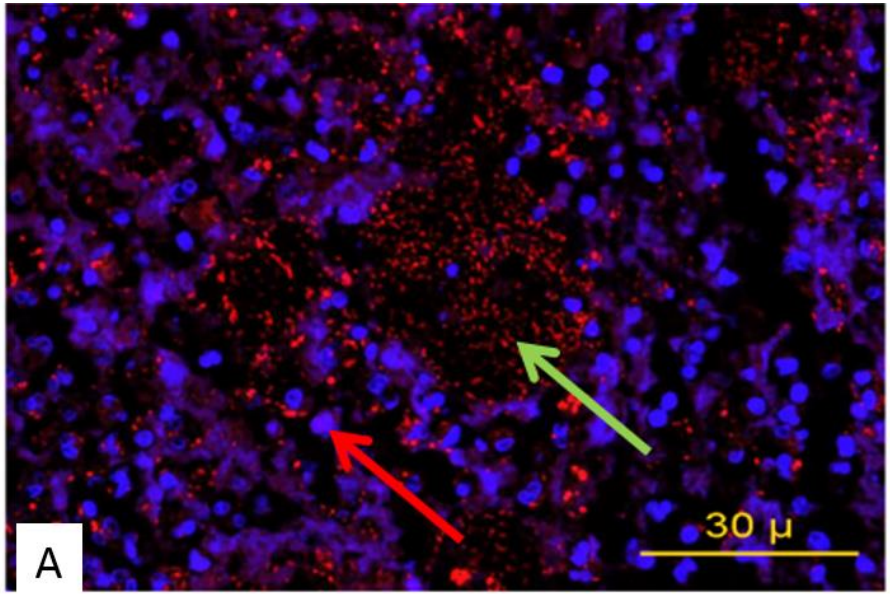
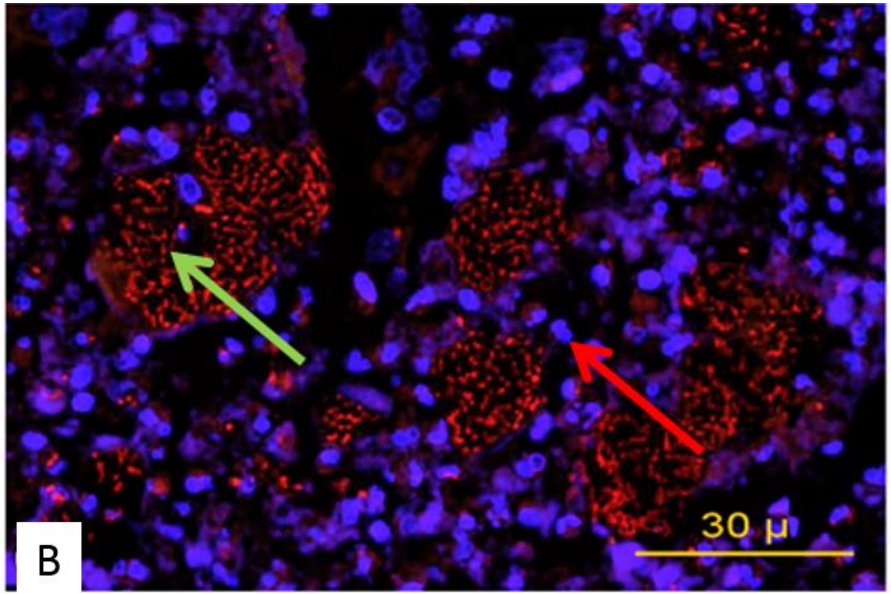
- **Now what about the host? Does the host influence the outcome of an antimicrobial treatment?**



Chronic lung infections by *P. aeruginosa* are caused by biofilms in the lung mucosa

(Thomas Bjarnsholt, Peter O. Jensen, Niels Hoiby)





3-D lung cell cultures

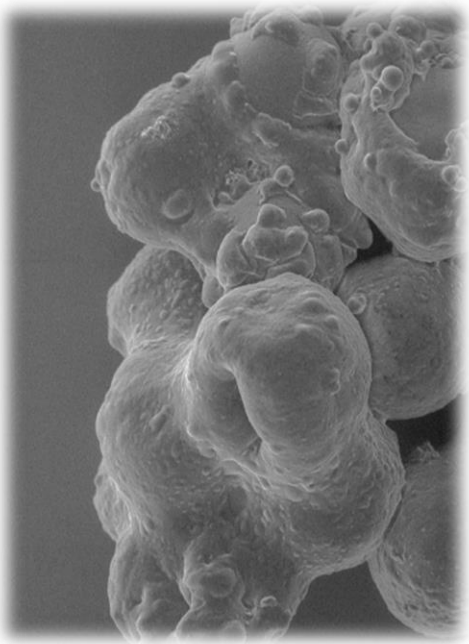
mimic key features of the *in vivo* tissue

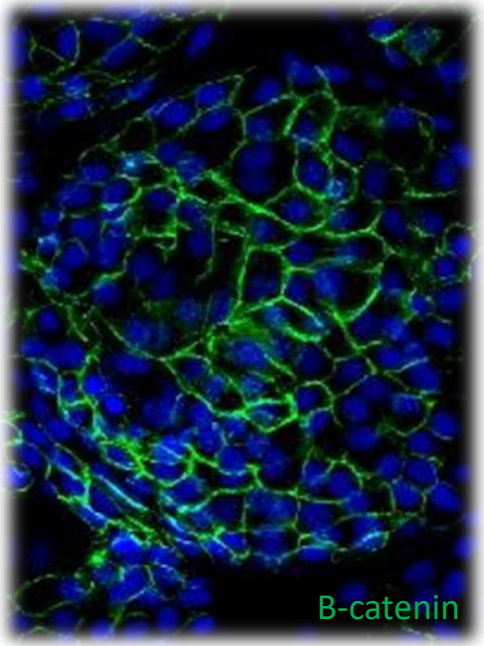
Architecture

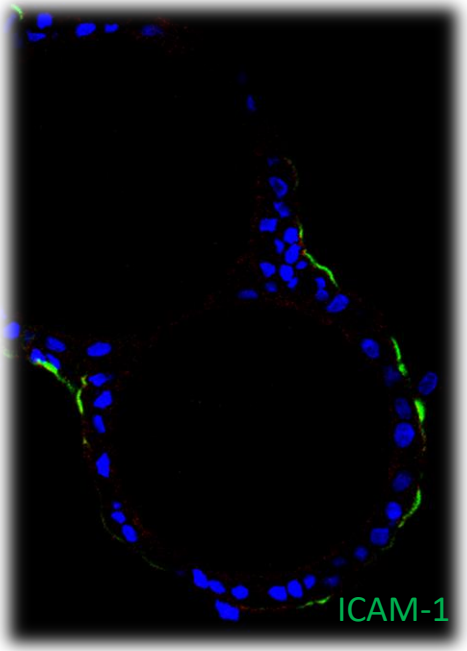
Barrier function

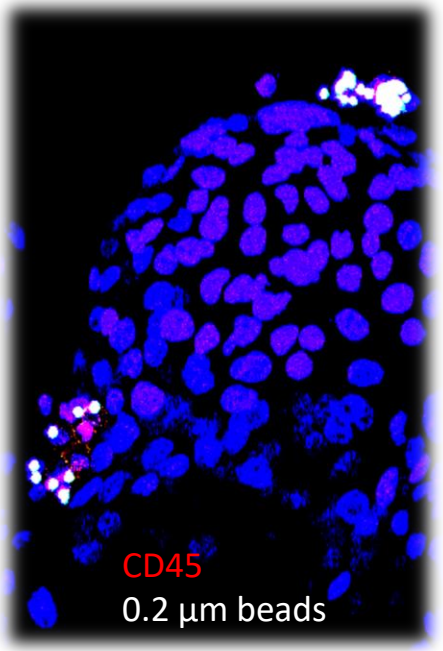
Polarity

Multicellular complexity





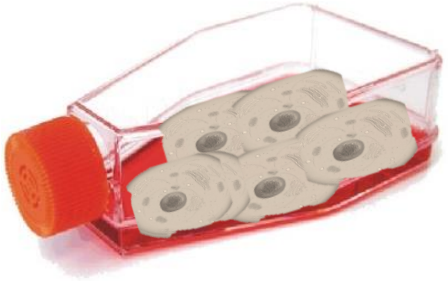




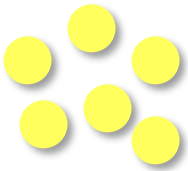
Carterson et al, 2005. Infect Immun; Barrila et al. 2010, Nat Rev Microbiol; Crabbé et al, 2011. Cell Microbiol; Crabbé et al. 2014. Pathog Dis

3-D lung cell cultures

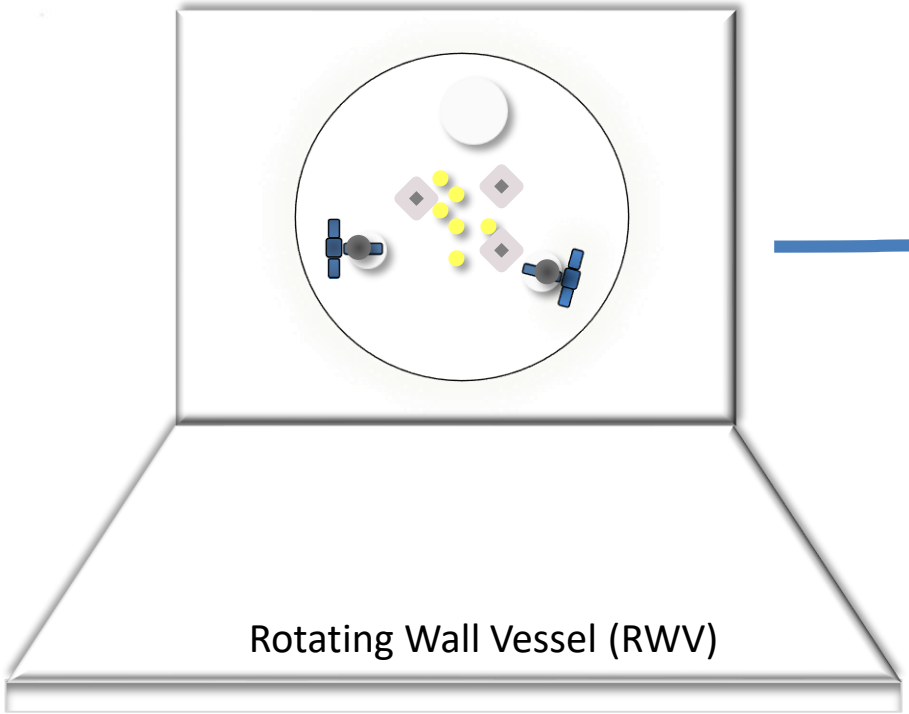
using the rotating wall vessel (RWV) technology

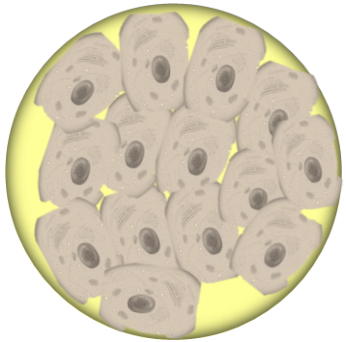


Monolayer
(A549 lung epithelial cells)



Microcarrier beads
(Collagen I – coated)
(175 µm)





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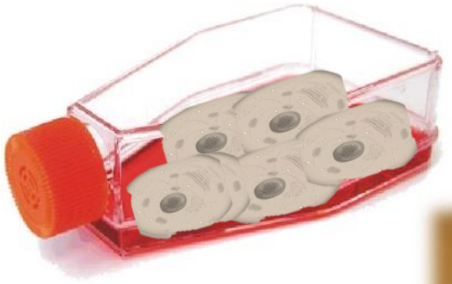

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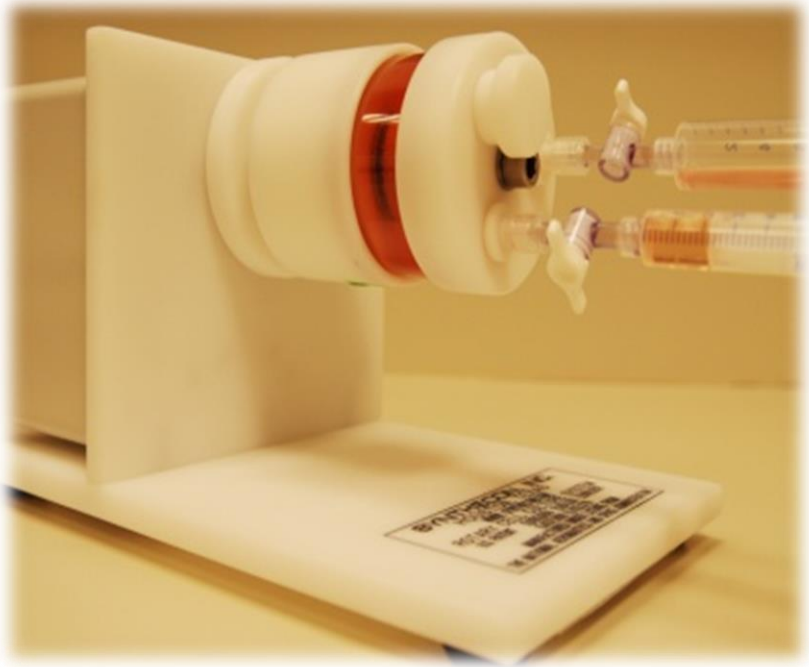
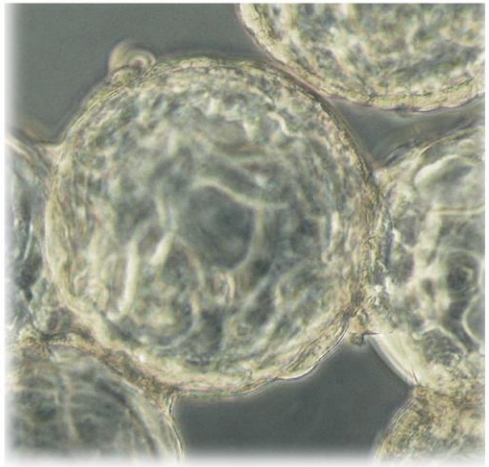
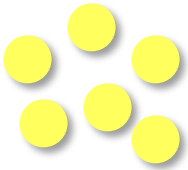




Cells grown on microcarrier beads

(3-D aggregates)



Microcarrier beads

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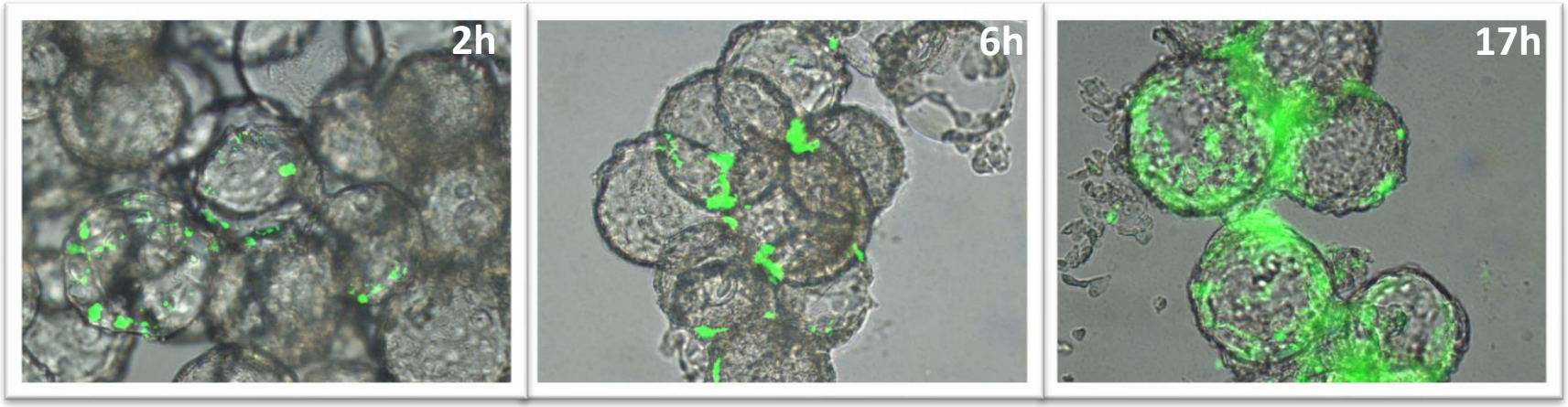
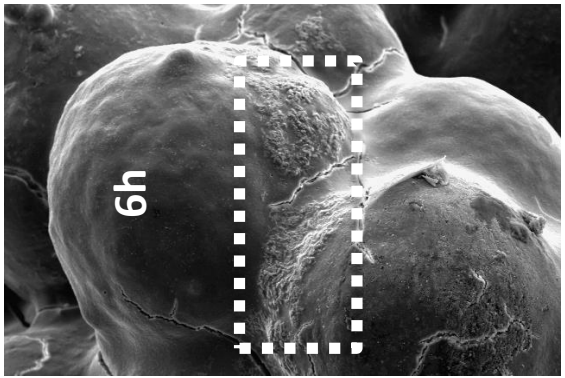
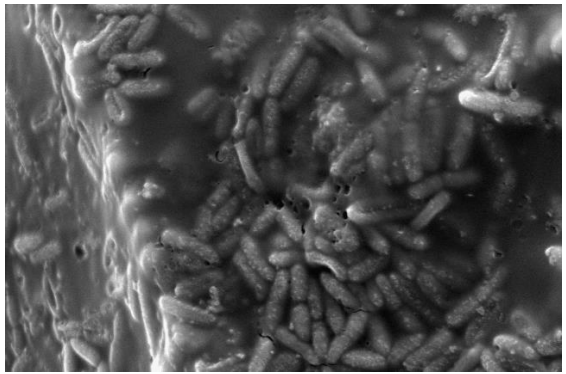
Biofilm-like structures on 3-D lung cells

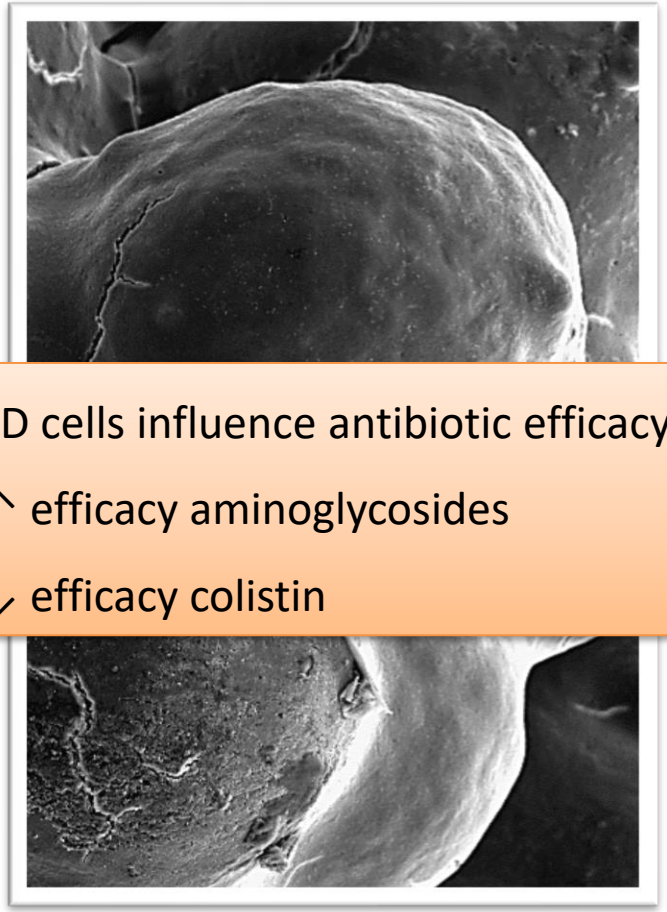






Crabbé et al. 2017 Sci Rep

Does the host influence bacterial tolerance?

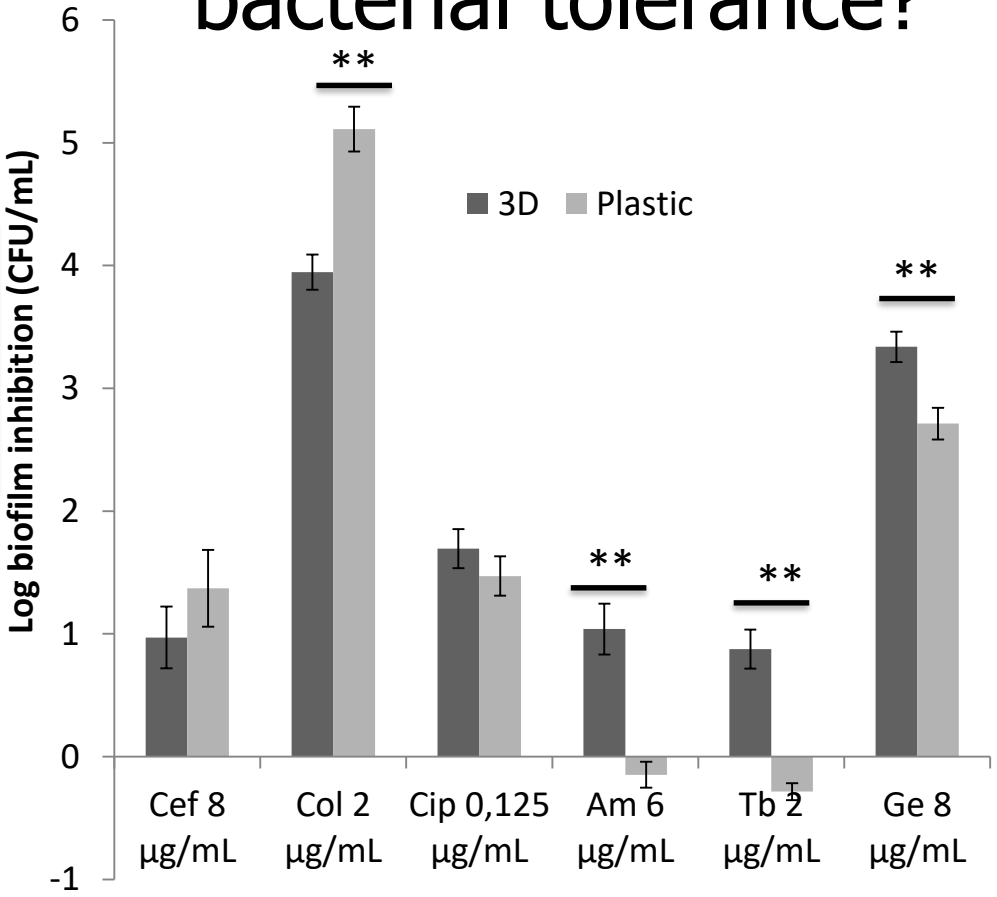


3D cells influence antibiotic efficacy:

↑ efficacy aminoglycosides

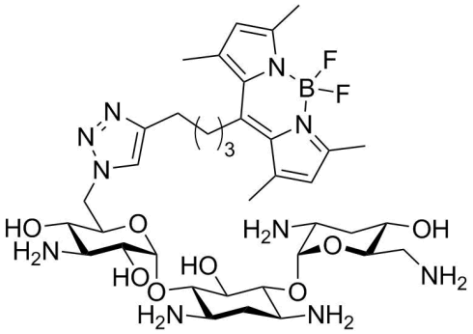
↓ efficacy colistin

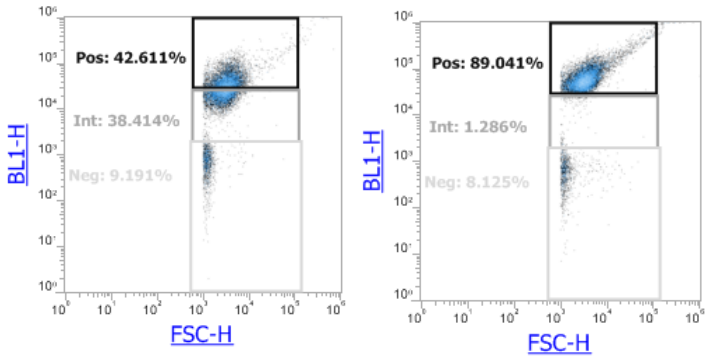
P. aeruginosa biofilms on 3D-A549 cells

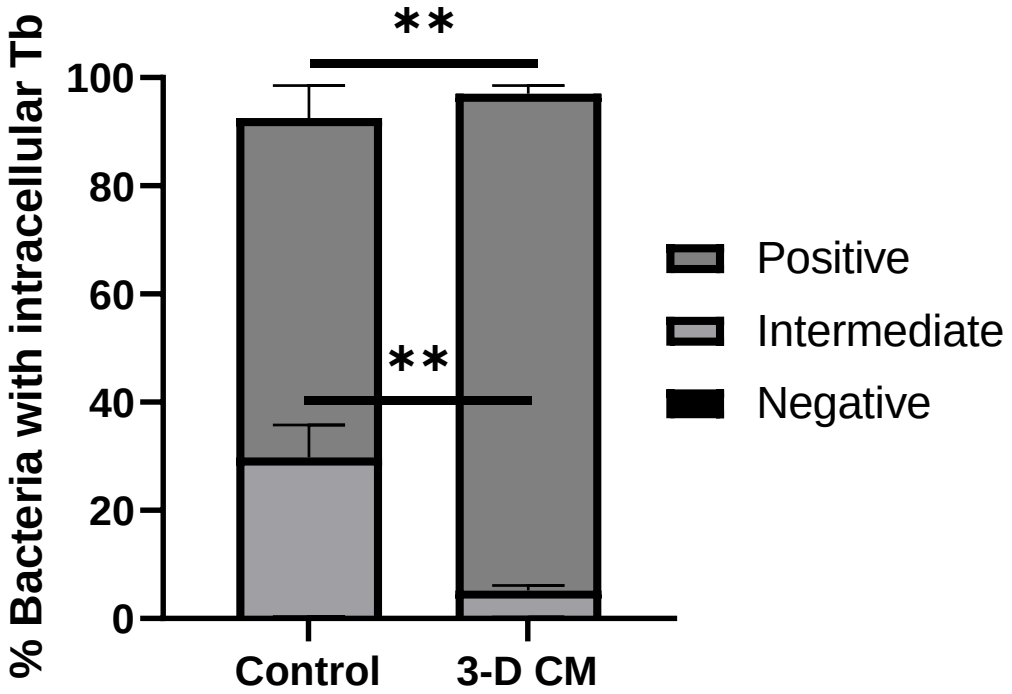


Crabbé et al. 2017. Sci Rep.

What causes this potentiating effect?







- Potentiating effect of 3-D CM is due to higher intracellular Tb levels and depends on PMF

Crabbé et al, PLoS Path, 2019

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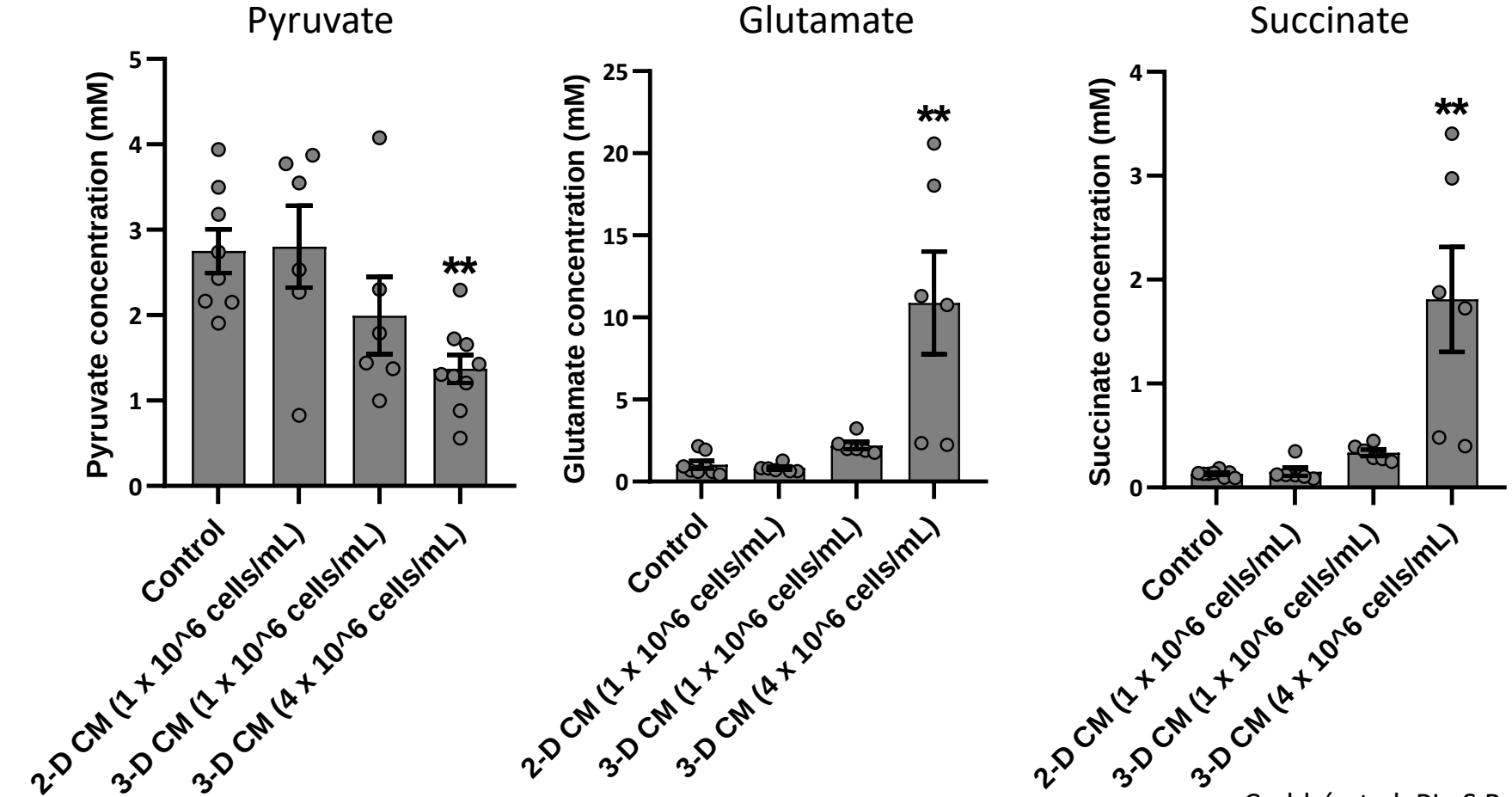

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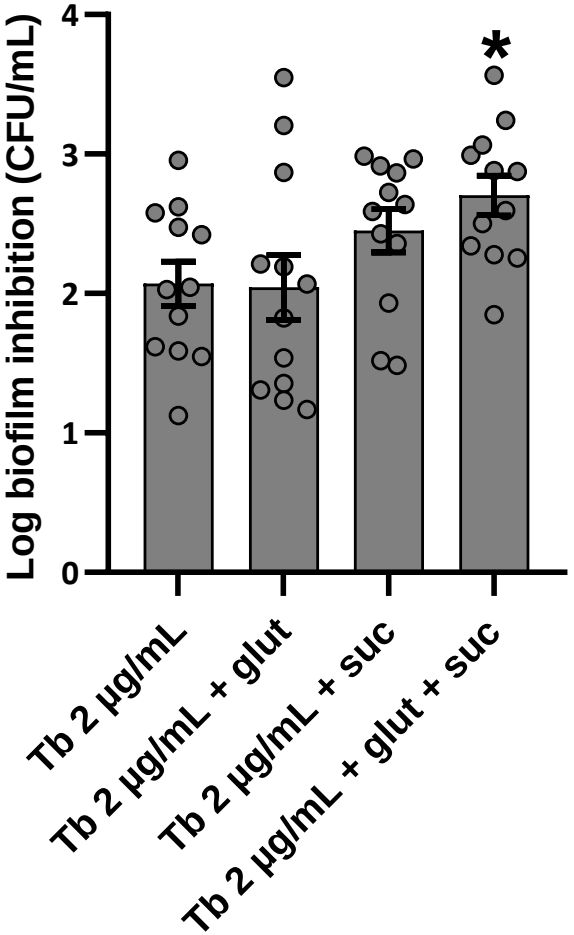
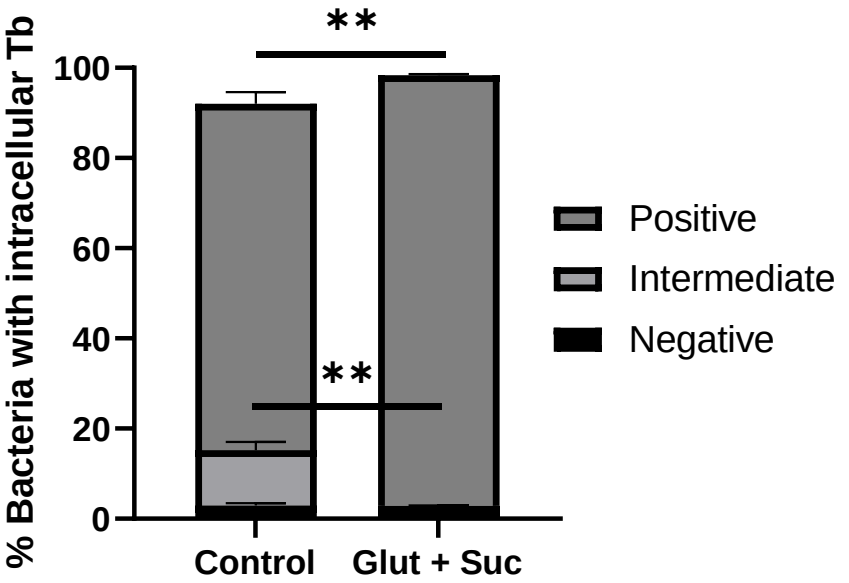
What causes this potentiating effect?



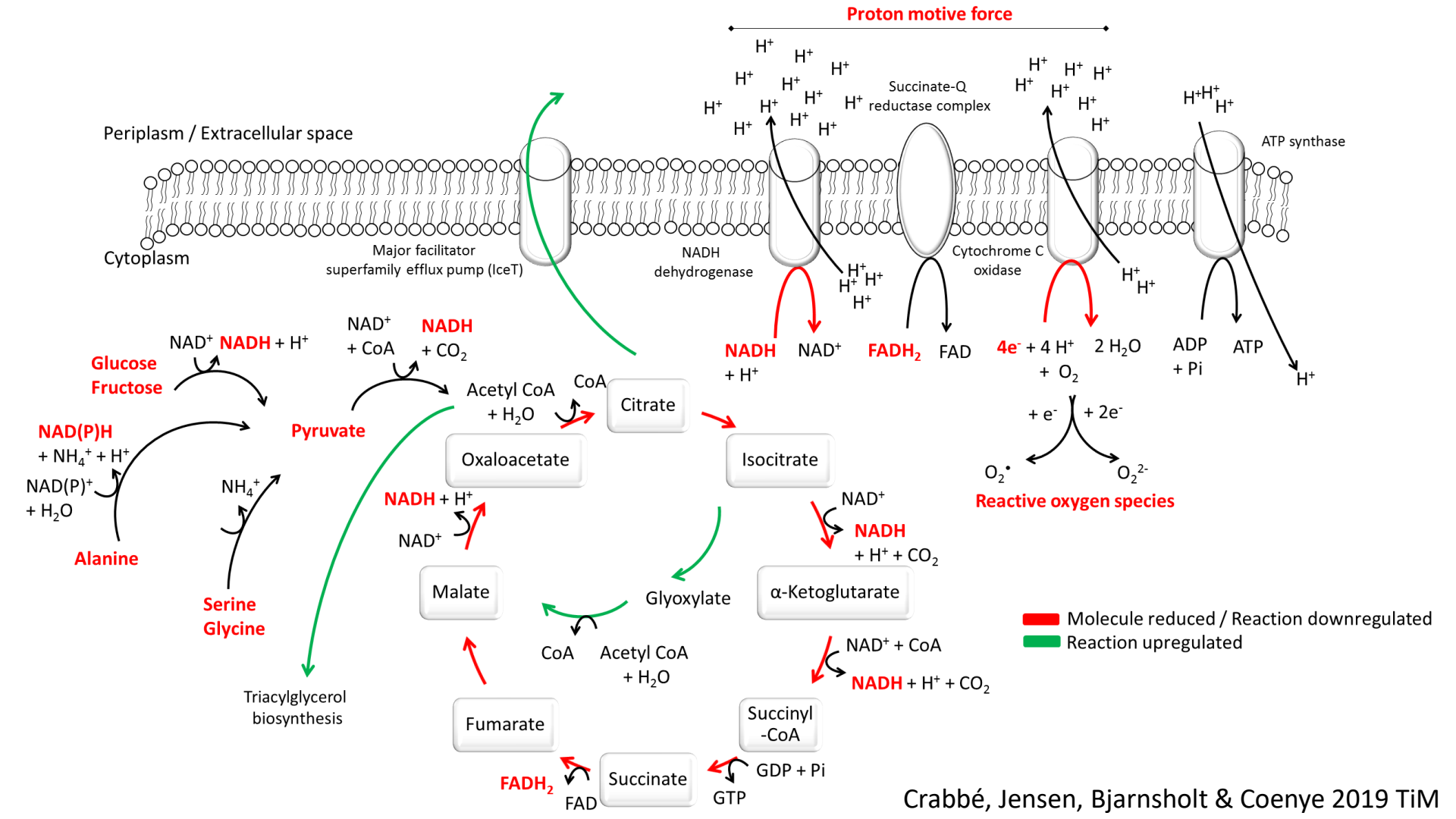


- 3-D-CM contains higher levels of glutamate and succinate
- Glutamate and succinate increase Tb uptake and potentiate Tb activity

Crabbé et al, PLoS Path, 2019

Wrap-up - what about the host?

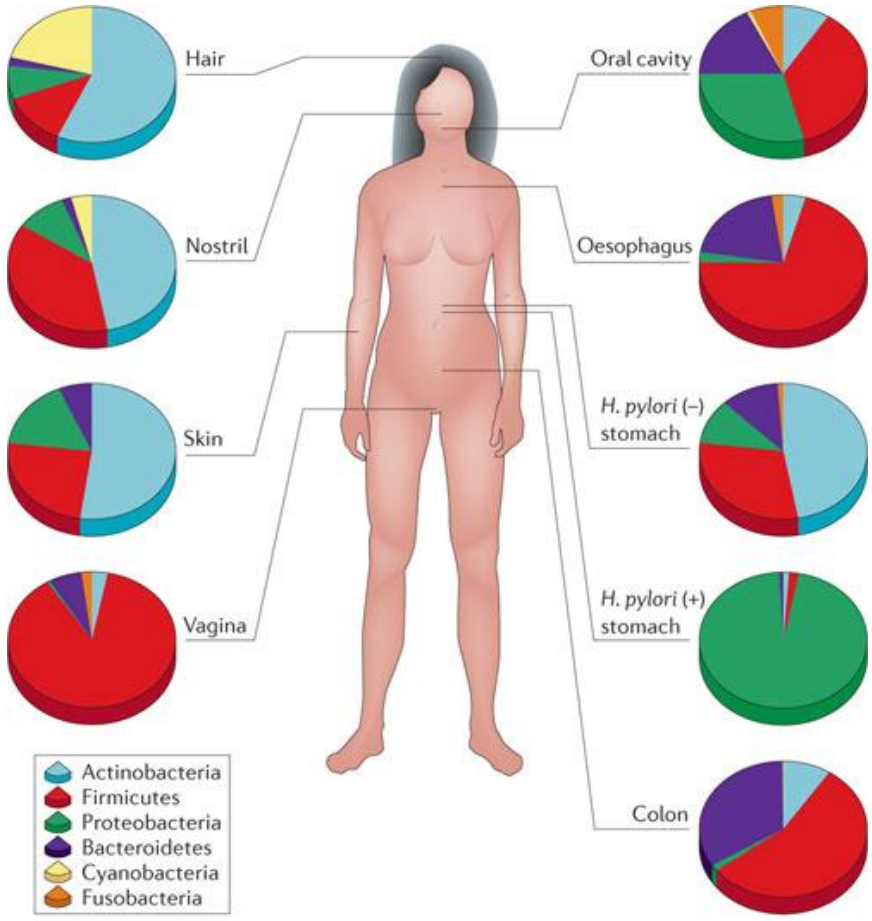
- Also the host produces (secreted) compounds that influence biofilm susceptibility
- This interaction between pathogen and host likely can have a significant impact on the efficacy of antimicrobial therapy
- Clinical implications unclear
- What about patient-to-patient variation and its impact?
- We need to expand our model and way of thinking



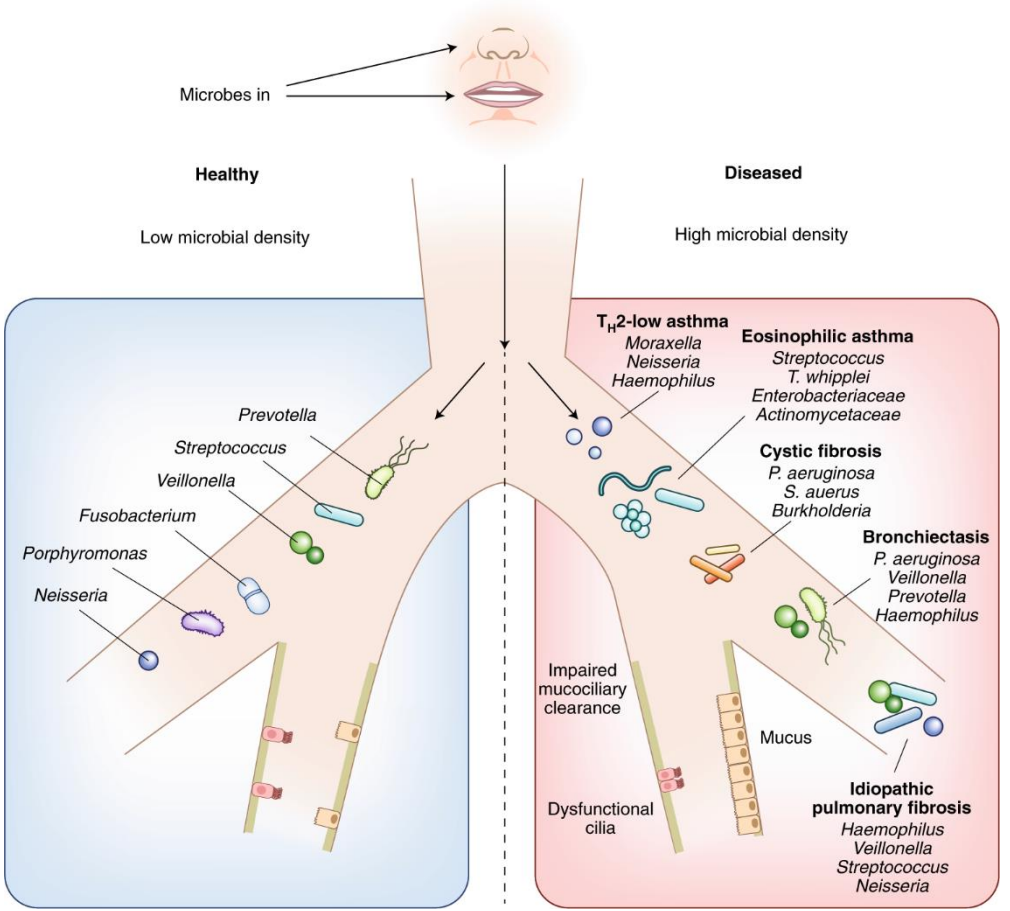
Crabbé, Jensen, Bjarnsholt & Coenye 2019 TiM

Wrap-up - what about the host?

- Also the host produces (secreted) compounds that influence biofilm susceptibility
- This form of ‘communication’ likely can have a significant impact on the efficacy of antimicrobial therapy
- Clinical implications unclear
- What about patient-to-patient variation and its impact?
- We need to expand our model and way of thinking
- **What about influence of bacteria on the host? Role of commensals and the microbiome**

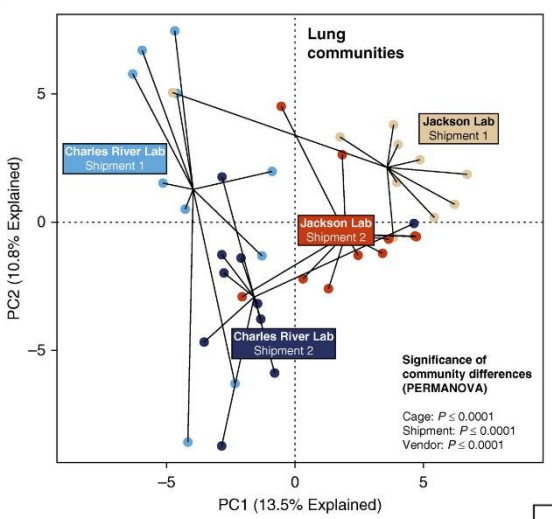


Nature Reviews | Genetics



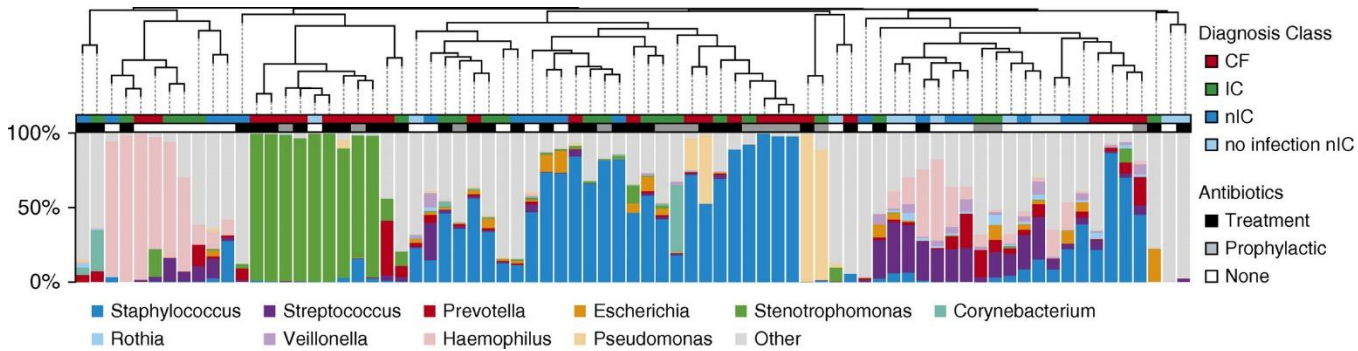
The Lung Microbiota of Healthy Mice Are Highly Variable, Cluster by Environment, and Reflect Variation in Baseline Lung Innate Immunity

Robert P. Dickson[1,2], John R. Erb-Downward[1], Nicole R. Falkowski[1], Ellen M. Hunter[1], Shanna L. Ashley[1], and Gary B. Huffnagle[1,3,4,5]



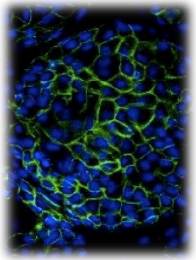
Culture-Independent Analysis of Pediatric Bronchoalveolar Lavage Specimens

Philip Zachariah[1,2*], Chanelle Ryan[1,3*‡], Sruti Nadimpalli[1§], Gina Coscia[1‖], Michelle Kolb[1‡], Hannah Smith[1], Marc Foca[1], Lisa Saiman[1,2], and Paul J. Planet[1,2,3,4,5‡¶]

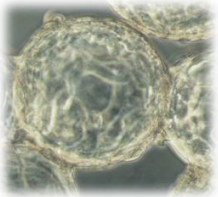


Do commensal bacteria mediate the inflammatory response of the host to pathogens?

An *in vivo*-like 3D-model of lung epithelial cells (A549) is used



ZO-1
DAPI



[1]Barrila *et al*, 2010, Nat Rev Microbiol 8(11)

Lung epithelial cells are infected with various CF lung microbiome members

Pseudomonas aeruginosa PAO1

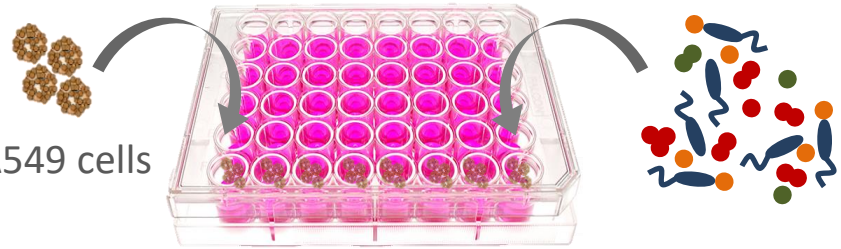
Staphylococcus aureus SP123

Streptococcus anginosus LMG 14696

Achromobacter xylosoxidans LMG 26680

Gemella haemolysans LMG 18984

Rothia mucilaginosa DSM 20746

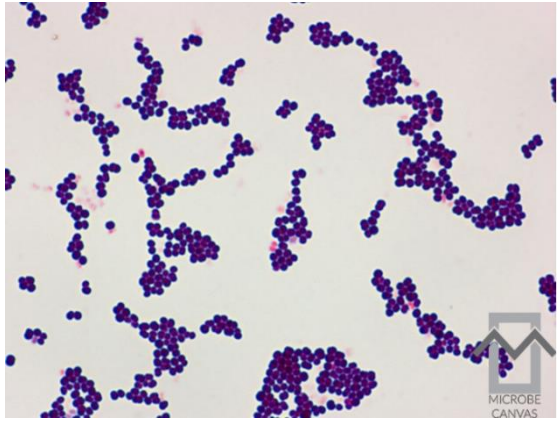


3-D A549 cells

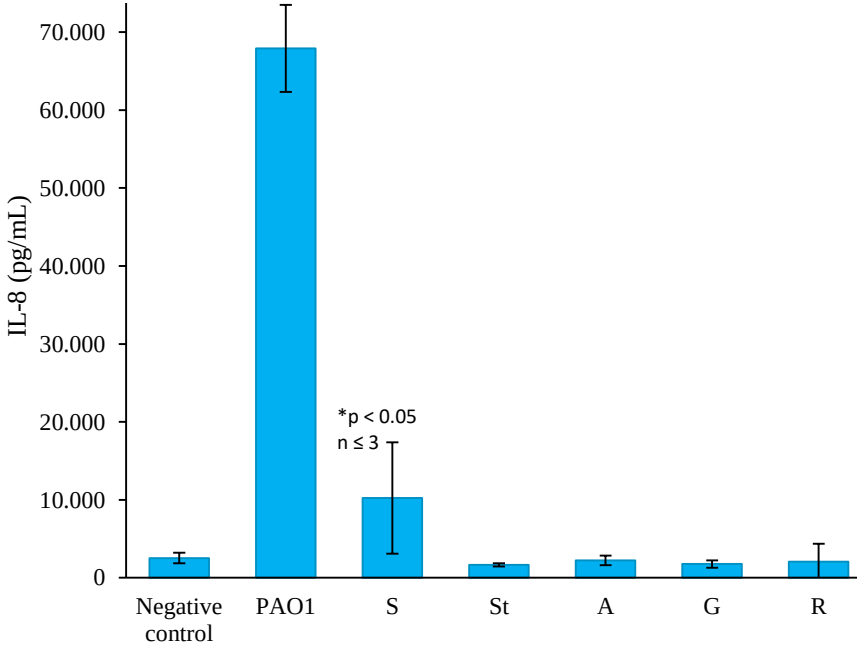
Rothia mucilaginosa

- Gram positive coccus
- Facultative anaerobic (aerotolerant)
- Usually encapsulated with a small amount of polysaccharide
- Normal flora of the oral cavity and upper respiratory tract
- Forms biofilms

Classification	
Kingdom	Bacteria
Phylum	Actinobacteria
Class	Actinobacteria
Order	Actinomycetales
Family	Micrococcaceae
Genus	*Rothia*
Species	*R. mucilaginosa*



Screening of effect of various bacteria on IL-8 response induced by *P. aeruginosa* PAO1



Negative control = Uninfected 3-D A549 cells

PAO1 = *Pseudomonas aeruginosa* PAO1

S = *Staphylococcus aureus* SP123

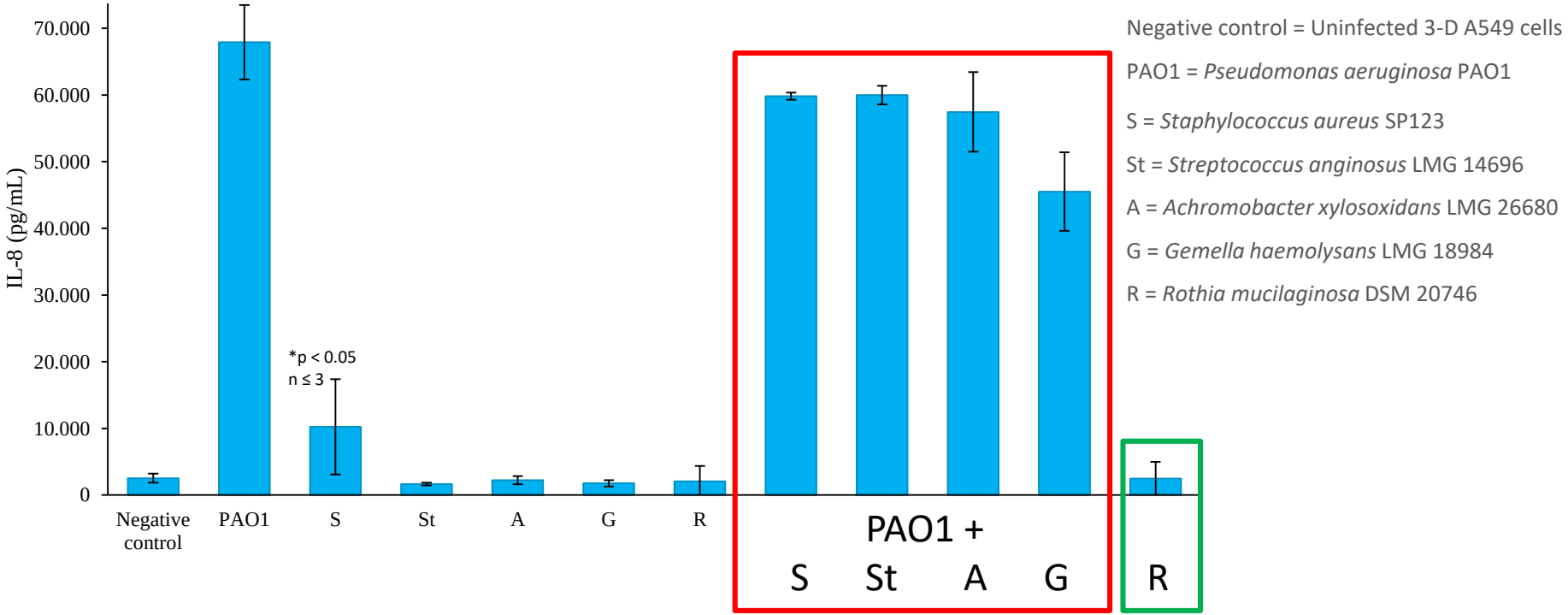
St = *Streptococcus anginosus* LMG 14696

A = *Achromobacter xylosoxidans* LMG 26680

G = *Gemella haemolysans* LMG 18984

R = *Rothia mucilaginosa* DSM 20746

Screening of effect of various bacteria on IL-8 response induced by *P. aeruginosa* PAO1



- *R. mucilaginosa* lowers the PAO1-induced IL-8 response in 3-D A549 cells

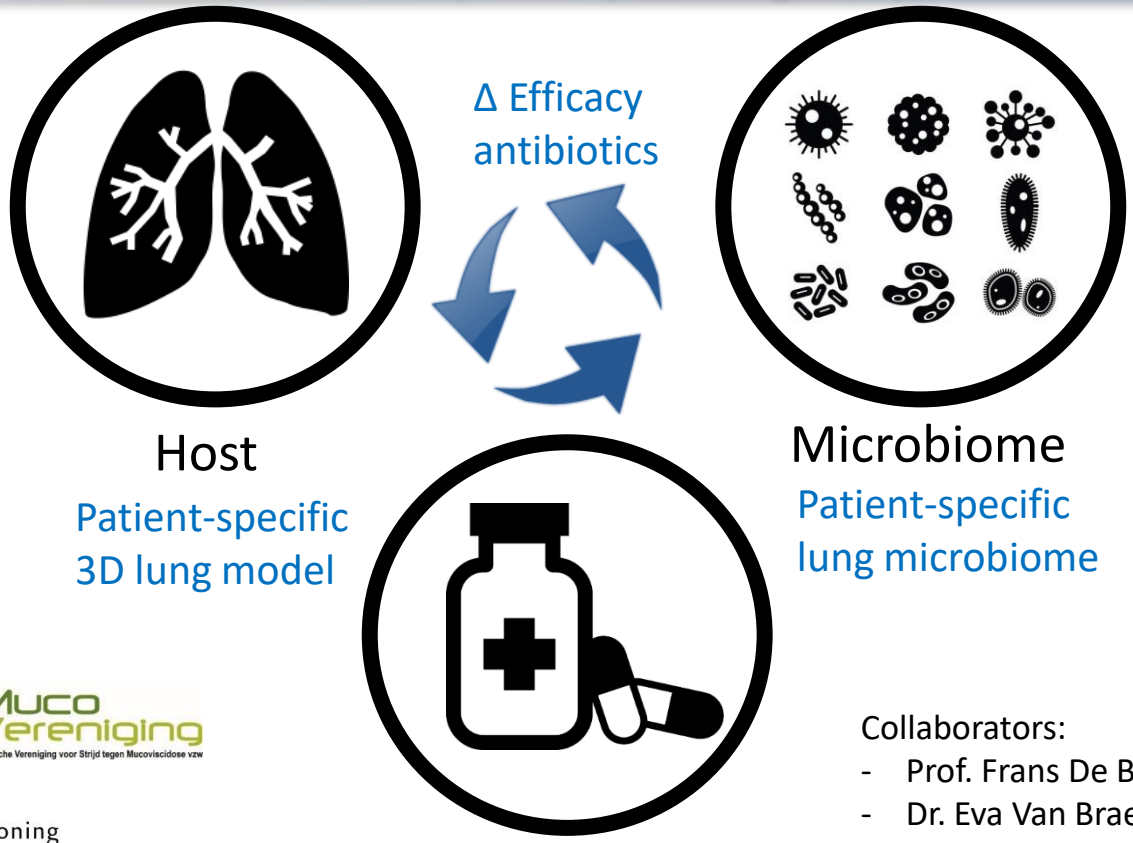
Effect of *R. mucilaginosa* on IL-8 response

All data point to involvement of (inhibition of) the NF-κB pathway

Wrap-up – does the lung microbiome influence the host?

- Yes!
- *R. mucilaginosa* lowers the pathogen-induced IL-8 response in lung epithelial cells
- xxxx

Ongoing work: developing a personalized approach for antimicrobial susceptibility testing in cystic fibrosis



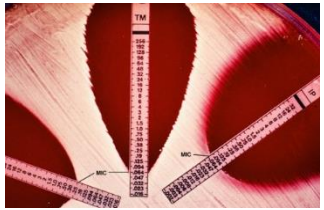
Muco Vereniging
Belgische Vereniging voor Strijd tegen Mucoviscidose vzw

Koning Boudewijnstichting
Samen werken aan een betere samenleving

Collaborators:
- Prof. Frans De Baets
- Dr. Eva Van Braeckel
- Prof. Anne Malfroot (UZB)
- Dr. Elke De Wachter (UZB)

Ongoing work: developing a personalized approach for antimicrobial susceptibility testing in cystic fibrosis

Conventional susceptibility assay



Minimal inhibitory concentration (MIC)

- Not personalized
- Poor predictor for *in vivo* susceptibility
- No guarantee for clinical success

Approach: Mimic each patient in the lab

- Genetic background (CFTR mutation)

Develop 3D patient-specific models derived from nasal epithelial cells

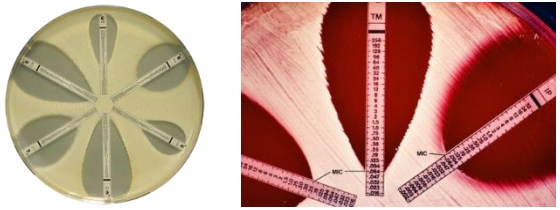
- Lung microbiome

Use patient-specific microbiome + *P. aeruginosa* derived from sputum

Approach: Mimic each patient in the lab

Personalized antibiotic susceptibility profile

Conventional susceptibility assay



Minimal inhibitory concentration (MIC)

- ✗ Not personalized
- ✗ Poor predictor for *in vivo* susceptibility
- ✗ No guarantee for clinical success

Personalized susceptibility assays

Biofilm eradicating concentration (BEC)

- ✓ Personalized
- ✓ Better predictor for *in vivo* susceptibility
- ✓ Better prediction of clinical success (?)

Precision medicine in microbiology: personalizing antimicrobial and anti-inflammatory treatments in people with cystic fibrosis

Tom Coenye

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FACULTY OF PHARMACEUTICAL SCIENCES

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