Transcriptional Regulation of Antibiotic Resistance Mechanisms in *Pseudomonas aeruginosa* Biofilms

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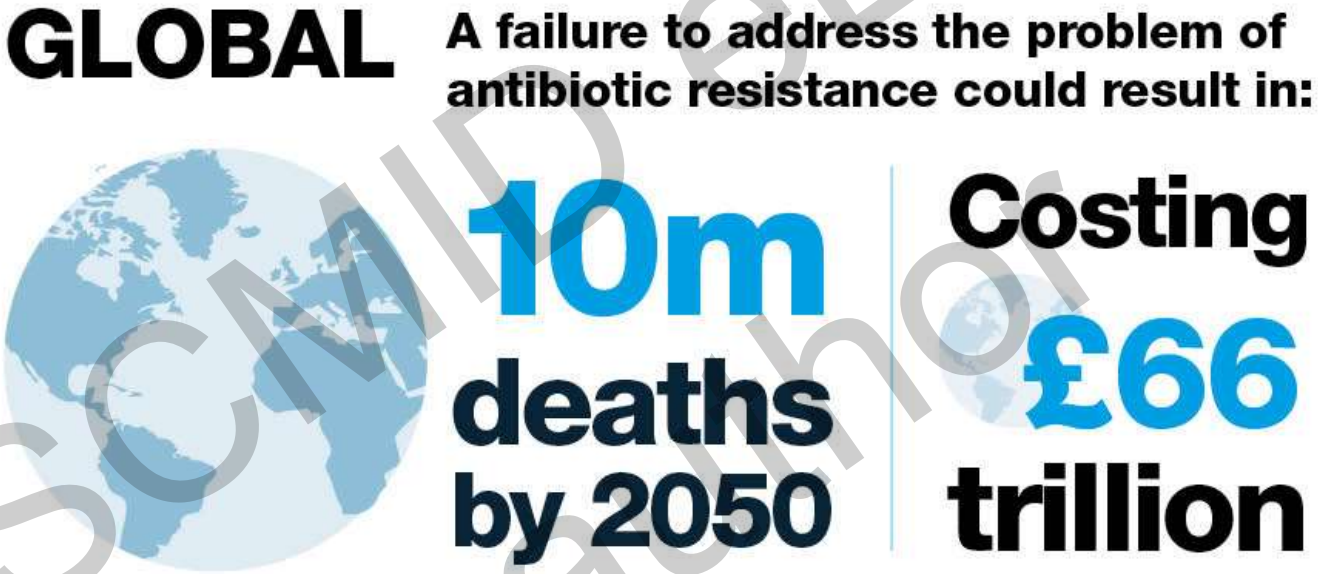
Antibiotic resistance is a problem



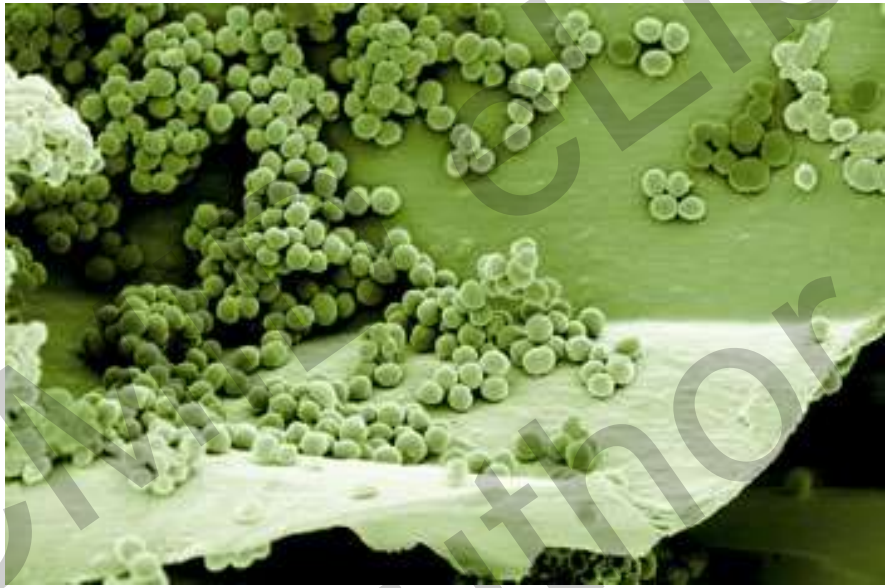
https://www.gov.uk/government/publications/health-matters-antimicrobial-resistance/health-matters-antimicrobial-resistance

Antibiotic resistance research in the Mah lab

- Biofilm antibiotic resistance genes*
- Planktonic resistance genes identified through studying transcriptional regulators involved in antibiotic resistance

What is a biofilm?

- Defined as a community of microbes* attached to a surface (biotic or abiotic), encased in a matrix



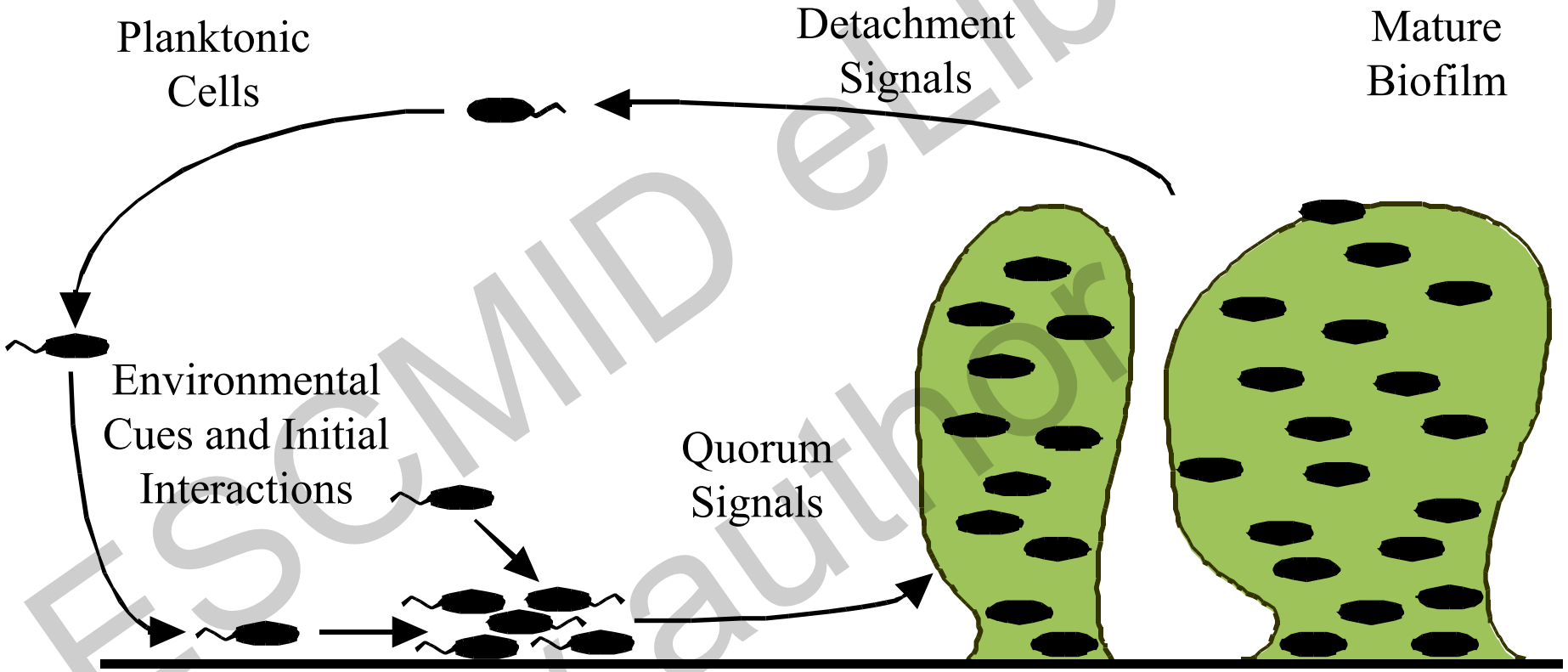
Staphylococcus aureus biofilm on vascular prosthesis

*** -most biofilms are multispecies**

-most bacteria can form a biofilm under certain conditions

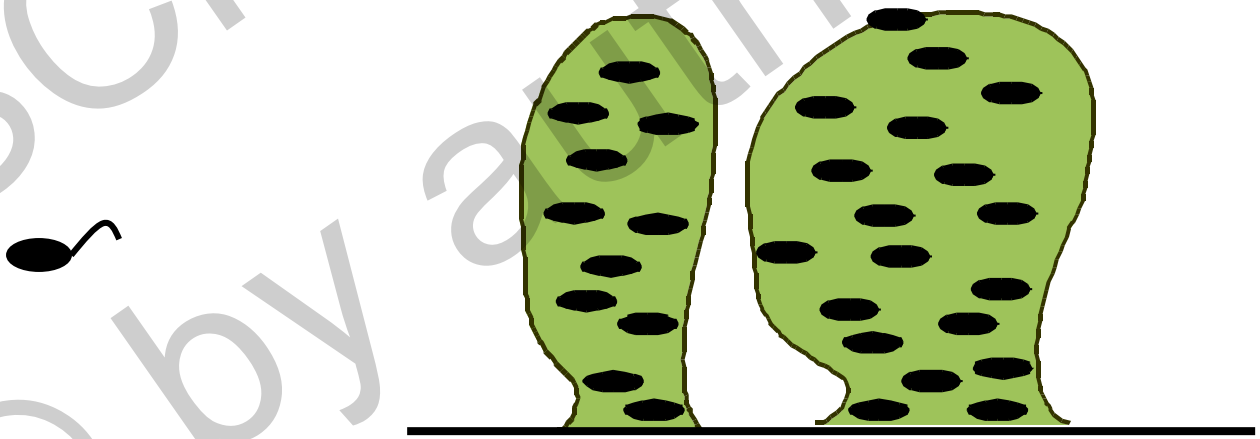
http://www2.cnrs.fr/sites/en/image/2002n01268h_hd.jpg

A model of biofilm development



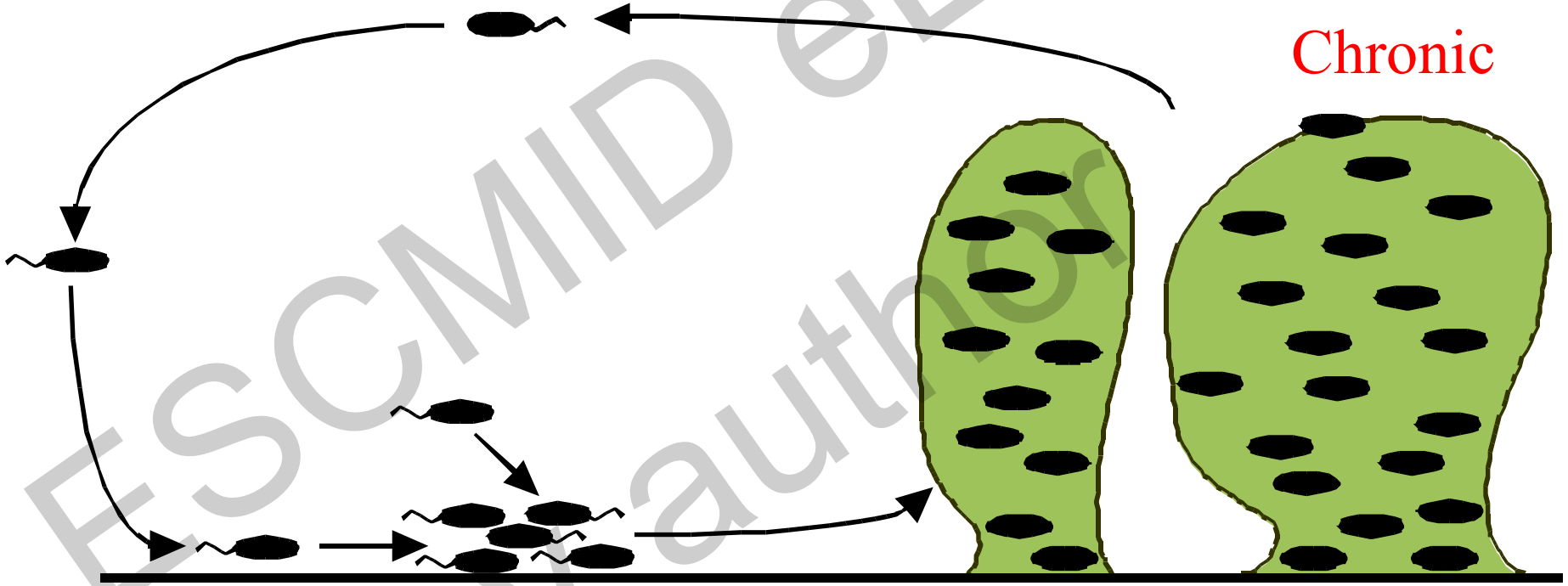
What are the properties of a mature biofilm?

- Surrounded by matrix composed of sugars, proteins and extracellular DNA (eDNA)
- Gene expression differences
  - virulence factors (eg Type III secretion system and effectors) not expressed
- Resistant to antibiotics



Acute vs. chronic infections

Planktonic cells express virulence factors and cause symptoms of acute infection



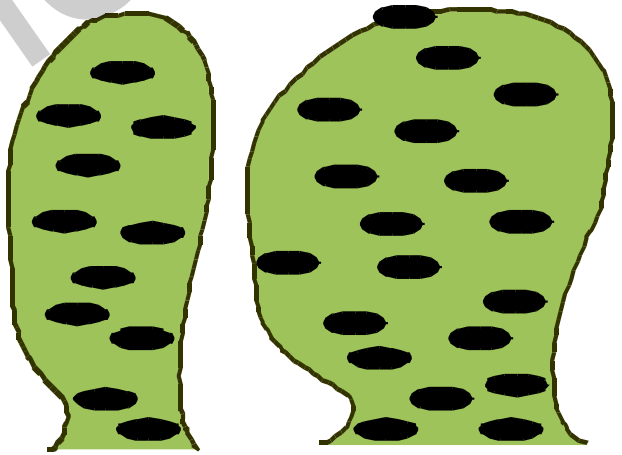
Antibiotics will kill the planktonic cells but not the biofilm cells

Examples of planktonic antibiotic resistance mechanisms

- Intrinsic (part of genome)
  - Low outer membrane permeability
  - Action of efflux pumps
- Acquired (through mutation)
  - Upregulation of drug efflux pumps
  - Alteration of antibiotic target

Biofilm antibiotic resistance mechanisms

- Multiple mechanisms
- Not based on mutations (intrinsic)
- Matrix (eDNA)
- Biofilm-specific gene expression



Is there a genetic basis for *Pseudomonas aeruginosa* biofilm antibiotic resistance?

George O' Toole, Dartmouth Medical School, Hanover, NH

The screen

- Screened a *Pseudomonas aeruginosa* transposon-insertion mutant library for mutants that were more sensitive to tobramycin (Tb) than the wild type strain

Mutants identified in the screen

- Isolated 6 mutants

- Properties of these mutants
  - Grow as well as the wild type
  - Have same planktonic antibiotic resistance profile as the wild type
  - Make a biofilm by microtiter plate assay
  - More sensitive to antibiotics only when growing in a biofilm

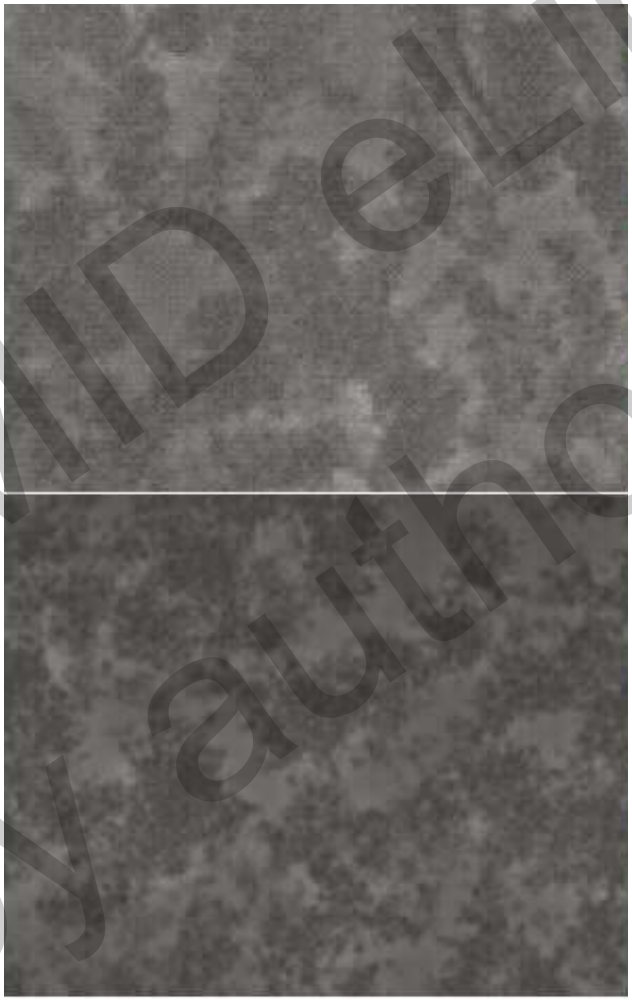
Genetic loci identified in the screen

PA number/gene	Annotation	Biofilm sensitivity to Tb compared to wild type
Δ*ndvB*	Glucosyltransferase	3-fold
Δ*tssC1*	Type VI secretion protein	3-fold
ΔPA0757	2-component sensor	3-fold
ΔPA1874-77	Novel efflux pump	3-fold
ΔPA2070	Hypothetical membrane protein	2-fold
ΔPA5033	Hypothetical protein	3-fold

Mah *et al* 2003 Nature 426
Zhang and Mah 2008 *J. Bact* 190
Zhang *et al* 2011 *J. Bact* 193
Beaudoin *et al* 2012 *J. Bact* 194
Zhang *et al* 2013 PLoS ONE 8
Taylor *et al* 2019 mSphere 4

Δ*ndvB* forms a biofilm as well as wild-type

Wild-type



Δ*ndvB*

Top down view,
24 hour old biofilm
grown in flowing system

Mah *et al* 2003 Nature 426

Δ*ndvB* is more sensitive to Tb compared to wild-type

MBC (mg/ml Tb)

Strain	Planktonic	Biofilm	Fold Change
wt	0.008	0.40	50x
Δ*ndvB*	0.008	0.025	3x

MBC- minimal bactericidal concentration

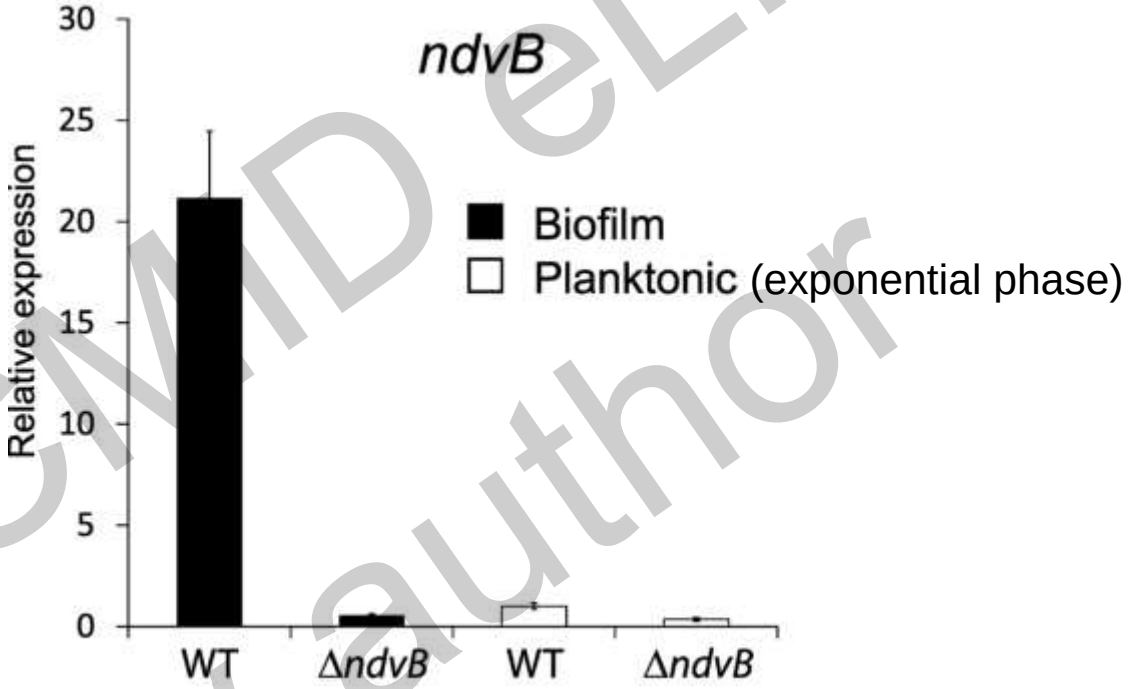
Δ*ndvB* biofilms are sensitive to two different classes of antibiotic

MBC (mg/ml Tb)

Strain	Planktonic	Biofilm	Fold Change
wt	0.008	0.40	50x
Δ*ndvB*	0.008	0.025	3x

Similar results with gentamycin (aminoglycoside) and ciprofloxacin (fluoroquinolone)

Biofilm-specific expression of *ndvB*



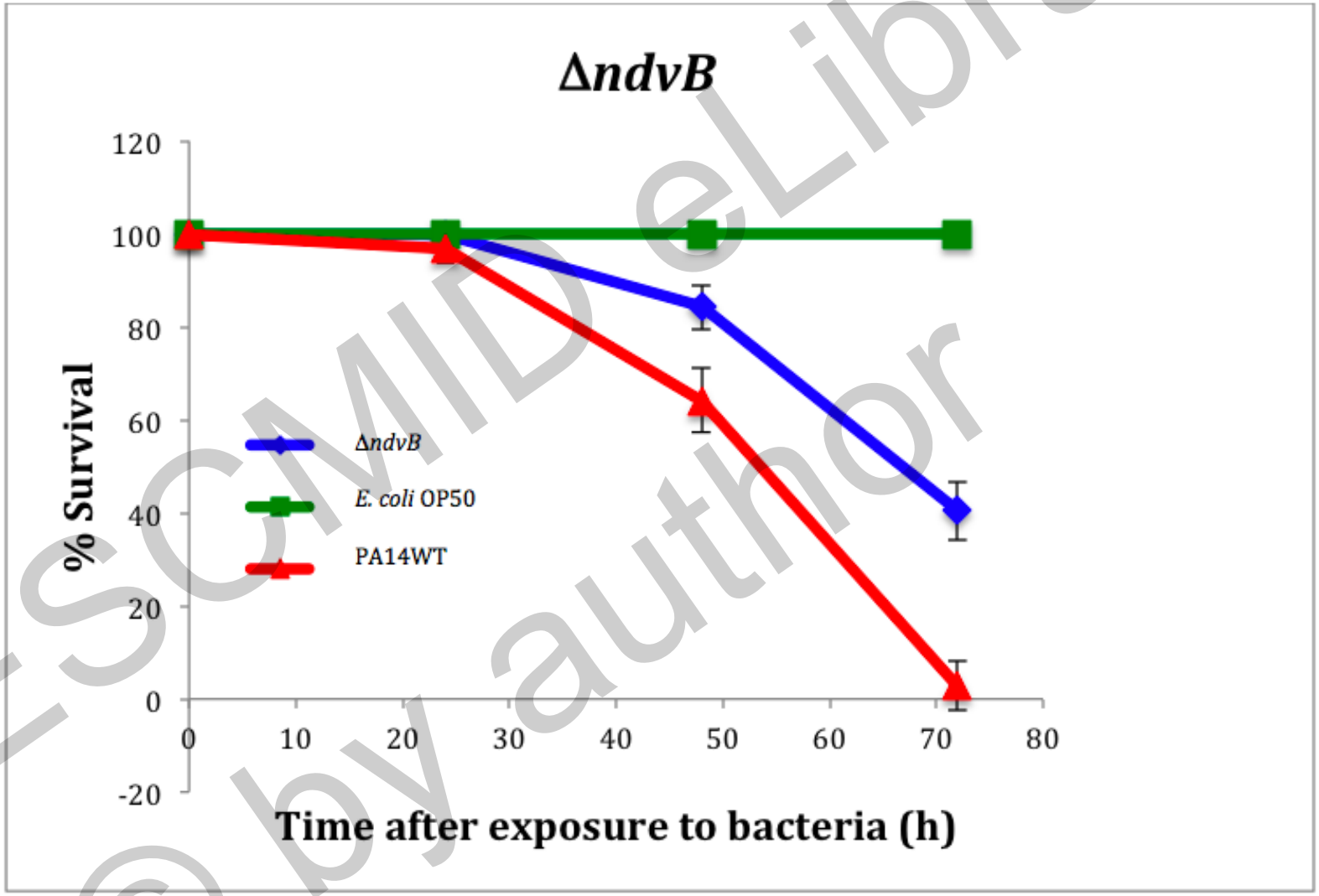
Beaudoin *et al* 2012 J Bacteriol 194

Caenorhabditis elegans model for microbial pathogenesis

- *C. elegans* feeds normally feeds on *E. coli*
- *P. aeruginosa* kills worms in 3 days; based on biofilm formation and persistence in worm

http://fangyenlab.seas.upenn.edu/images.html

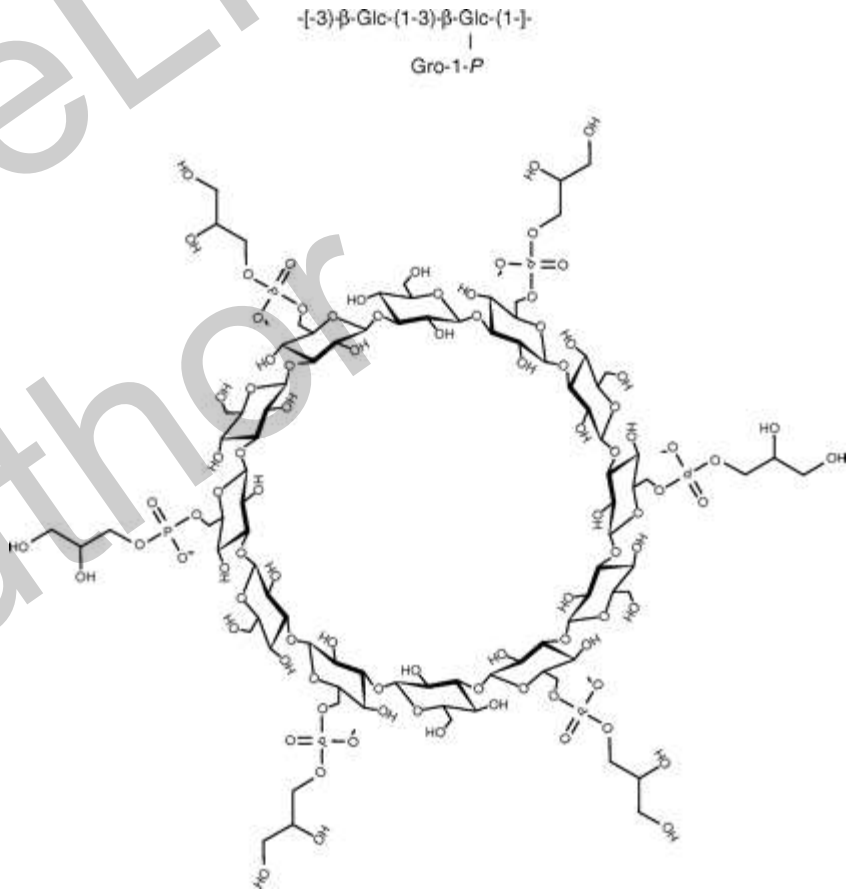
ndvB contributes to persistence *in vivo*



Zhang *et al* 2013 PLoS ONE 8

What is *ndvB*?

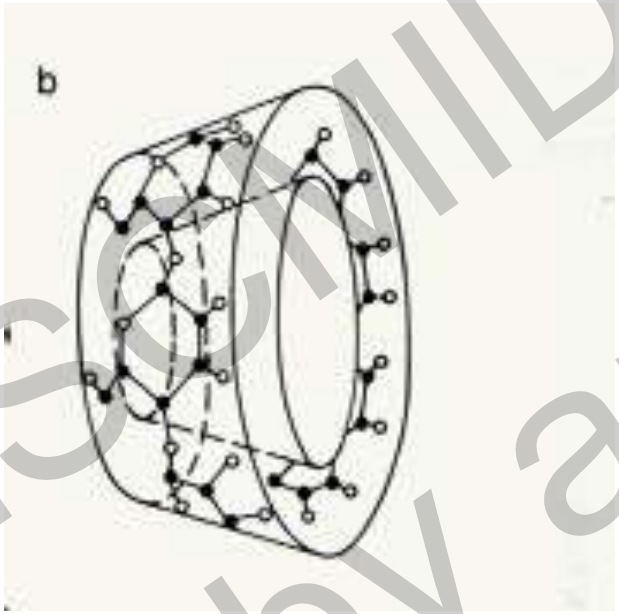
- Encodes a glucosyltransferase required for the formation of cyclic periplasmic glucans



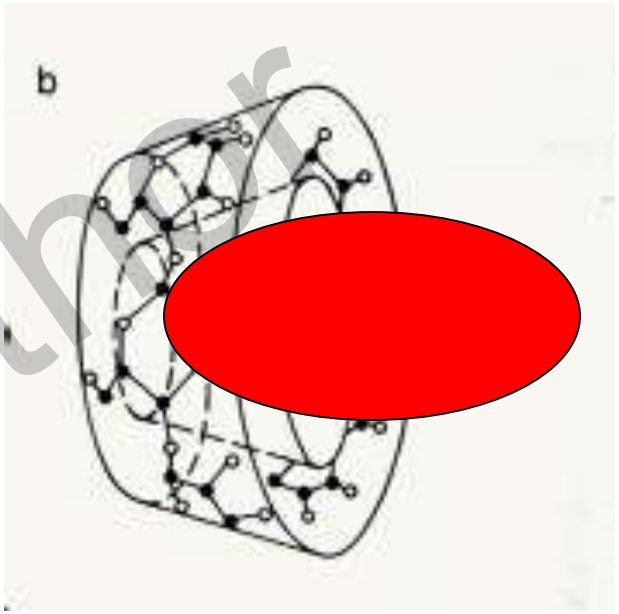
Sadovskaya *et al* 2010 Glycobiology **20**

What is the role of glucans in biofilm antibiotic resistance?

- Sequestration of antibiotics away from their cellular targets



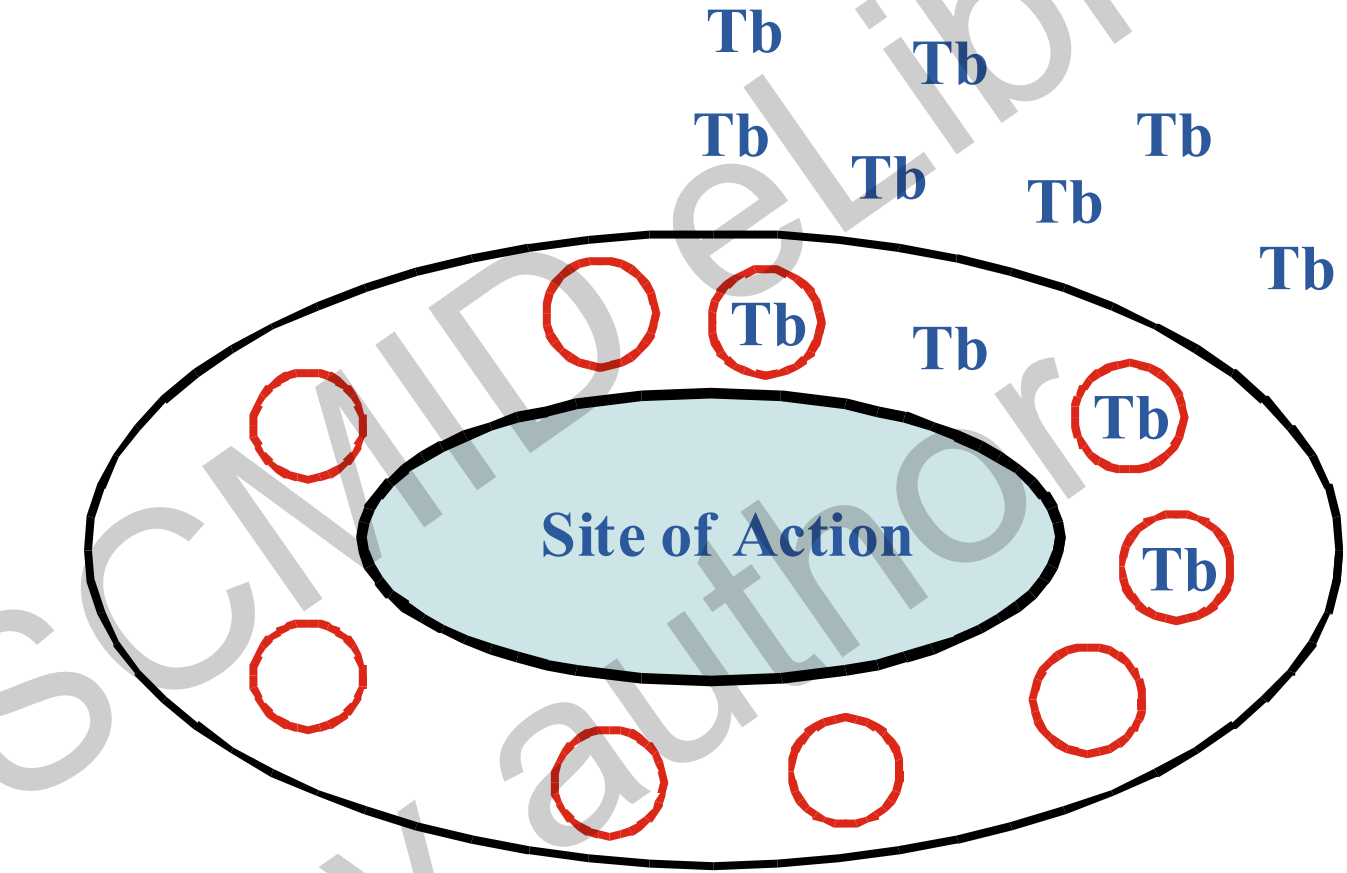
3D structure of cyclodextrin



Guest molecule interaction

Loftsson and Brewster 1996 J. Pharm. Sci. **85**

Model for *ndvB*-driven biofilm antibiotic resistance



Mah *et al* 2003 Nature 426

Biofilm antibiotic resistance genes are important for biofilm stress response

PA number	Annotation	Biofilm sensitivity to Tb compared to wild type
Δ*ndvB*	Glucosyltransferase	3-fold
Δ*tssC1*	Type VI secretion protein	3-fold
ΔPA0757	Probable 2-component sensor	3-fold
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Biofilm-specific expression of 6 genes important for biofilm resistance to antibiotics

- *ndvB*, PA0084, PA0756, PA1875-77, PA2070 and PA5033 are more highly expressed in biofilms compared to exponential phase planktonic cells (qPCR by Li Zhang)

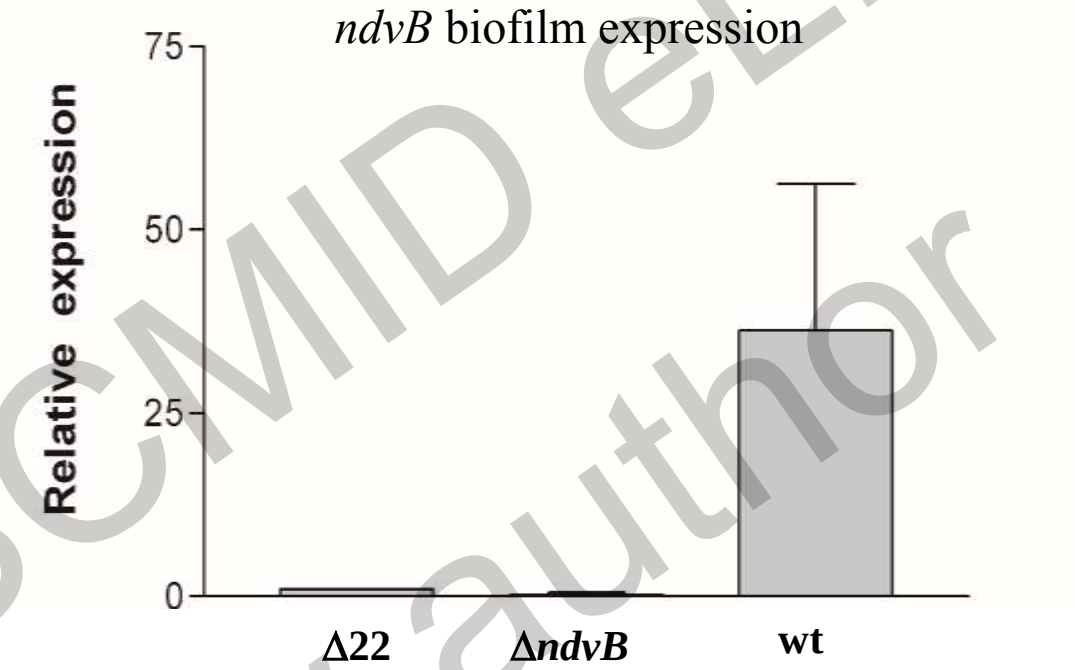
- Is there a DNA element that drives biofilm-specific expression?

22-bp motif present in promoters of 6 biofilm antibiotic resistance genes

ndvB, *tssC1*, PA1874-1877, PA0757, PA2070 and PA5033

Multiple EM for Motif Elicitation (MEME): Bailey *et al* 2006 Nucleic Acids Res **34**

The 22-bp motif in the *ndvB* promoter is important for biofilm-specific expression



L. Zhang, unpublished data

What binds to the 22-bp motif?

Approach to identify the 22-bp motif binding protein

- Constructed sub-library of 550 transposon-insertion mutants* predicted to be transcriptional regulators
- Screened for mutants that were more sensitive to 100 ug/mL Tb when growing as biofilms

*Liberati *et al* 2006 PNAS 103

Prediction

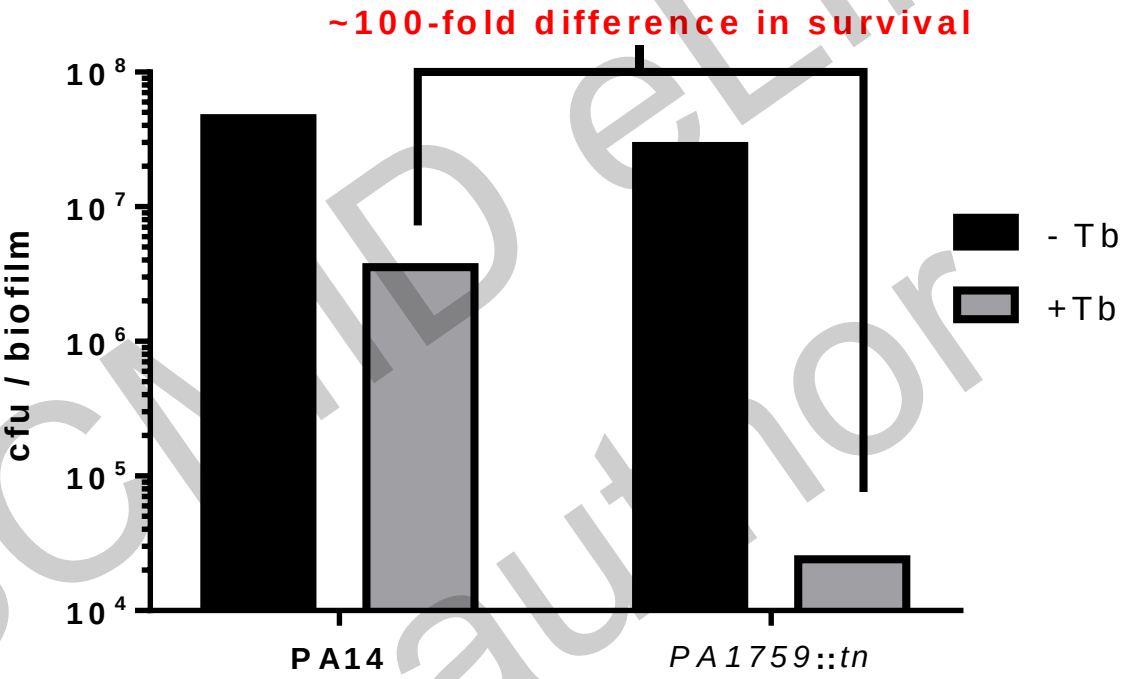
- 22 bp-motif binding protein tn-mutant should be far more sensitive to antibiotics compared to individual deletion mutants of biofilm antibiotic resistance genes (eg Δ*ndvB)*

Success!

- 13 transcriptional regulators were identified as being involved in biofilm antibiotic resistance
- PA1759 (probable transcriptional regulator) had the strongest phenotype

C. Hall, unpublished data

PA1759 tn-mutant biofilm is 100-fold more sensitive to 100 µg/mL Tb compared to wt



after exposure to Tb, surviving cells were enumerated by colony counting

C. Hall, unpublished data

Future work

- Is PA1759 the 22-bp motif binding protein?
  - Construct deletion mutant
    - Antibiotic resistance assays
    - qPCR (using primers for *ndvB, tssC1* ect)
  - Express protein and perform gel shifts with 22-bp motif-containing promoters
- What is the PA1759 regulon?
  - RNAseq comparing wt and ΔPA1759 biofilms

Summary

- 6 (unrelated) genetic loci contribute to biofilm antibiotic resistance
  - Transcriptional regulator may control expression of 6 different mechanisms of biofilm resistance
- Several transcriptional regulators are important for antibiotic resistance in biofilms
  - PA1759 may be the 22-bp motif binding protein that controls the expression of 6 biofilm antibiotic resistance mechanisms

Future directions

- The ultimate goal of this work is to identify compounds that prevent expression of high-level antibiotic resistance in biofilms by targeting transcriptional regulators of biofilm antibiotic resistance

Acknowledgements

Clayton Hall*
Mel Novakovic
Patrick Taylor
Caetanie Tchagang
Li Zhang*

Financial Support
NSERC
Cystic Fibrosis Canada