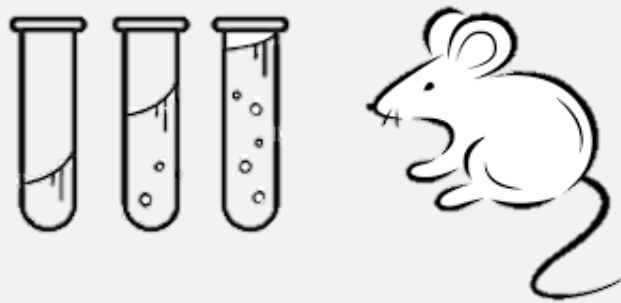


*Transcriptomic data to support in vitro semi-mechanistic PK/PD modelling of Polymyxin B against *Acinetobacter baumannii**

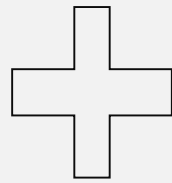
Mathilde Lacroix, Andrej Trauner, Claudia Zampaloni, Caterina Bissantz, Hamasseh Shirvani, Sandrine Marchand, William Couet, Alexia Chauzy

Institut Roche
Inserm U1070 – University of Poitiers

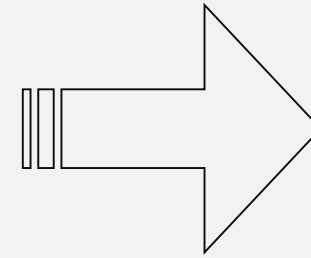
PK/PD modelling



In vitro & in vivo studies

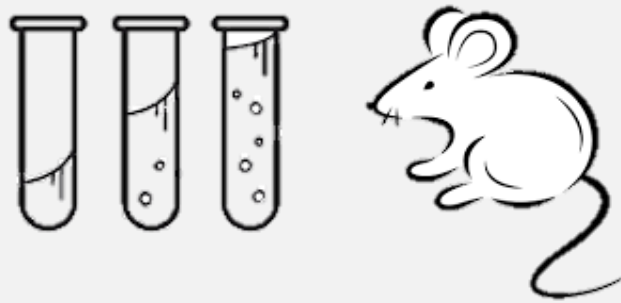


PK/PD modelling

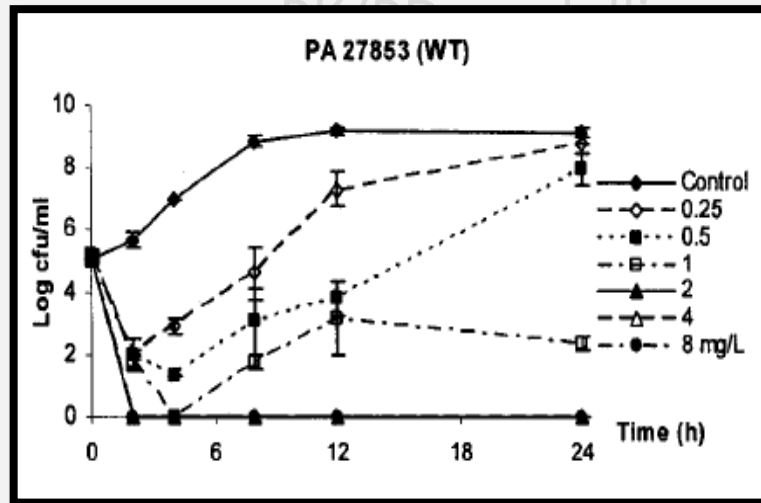


Prediction of human dosing regimens

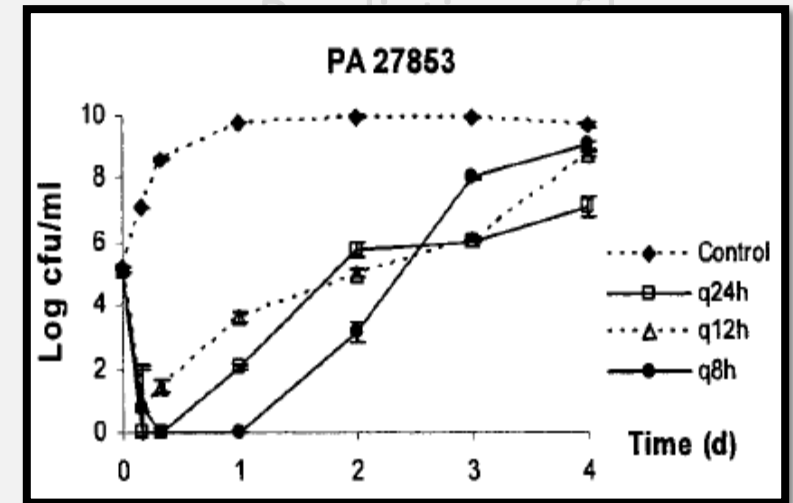
PK/PD modelling



In vitro & in vivo studies



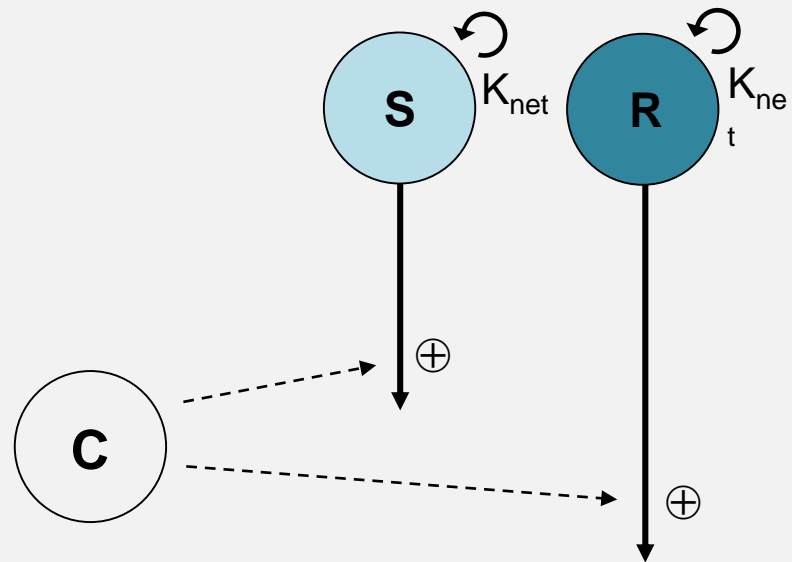
Time-kill



Hollow fiber

PK/PD model types

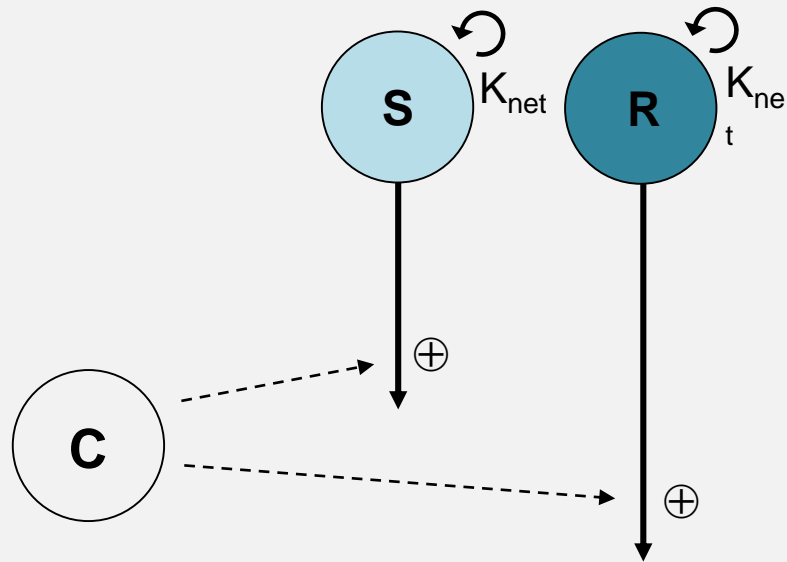
Hetero-resistance



Mouton *et al.*, 1997 Antimicrobial Agents Chemotherapy
Meagher *et al.*, 2004 Antimicrobial Agents Chemotherapy

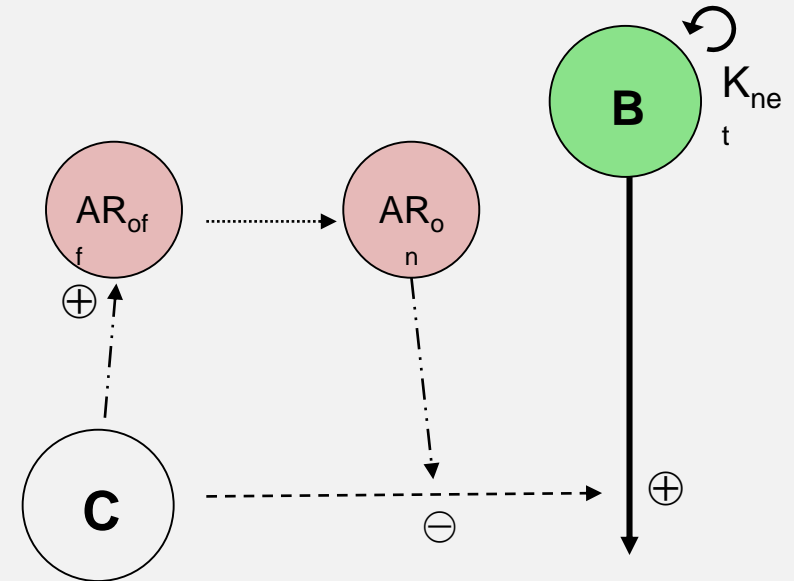
PK/PD model types

Hetero-resistance



Mouton *et al.*, 1997 Antimicrobial Agents Chemotherapy
 Meagher *et al.*, 2004 Antimicrobial Agents Chemotherapy

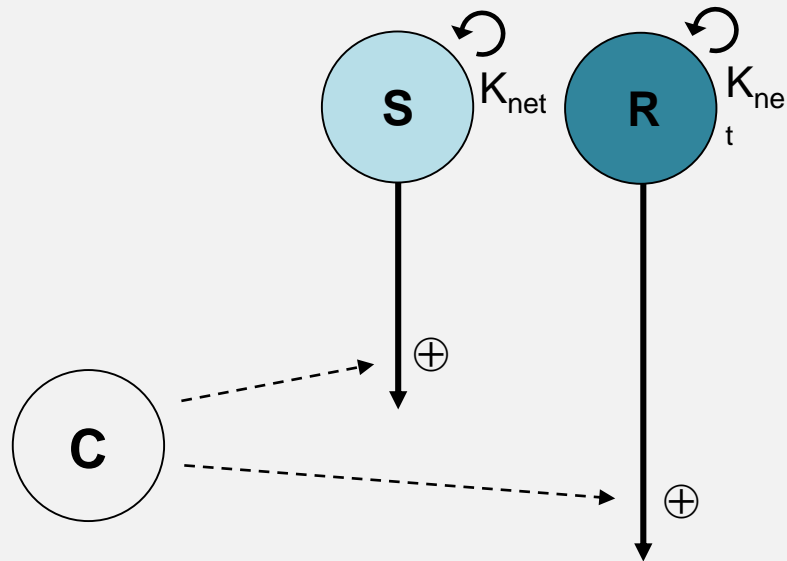
Adaptation



Mouton *et al.*, 1997 Antimicrobial Agents Chemotherapy
 Tam *et al.*, 2005 Journal of Antimicrobial Chemotherapy

PK/PD model types

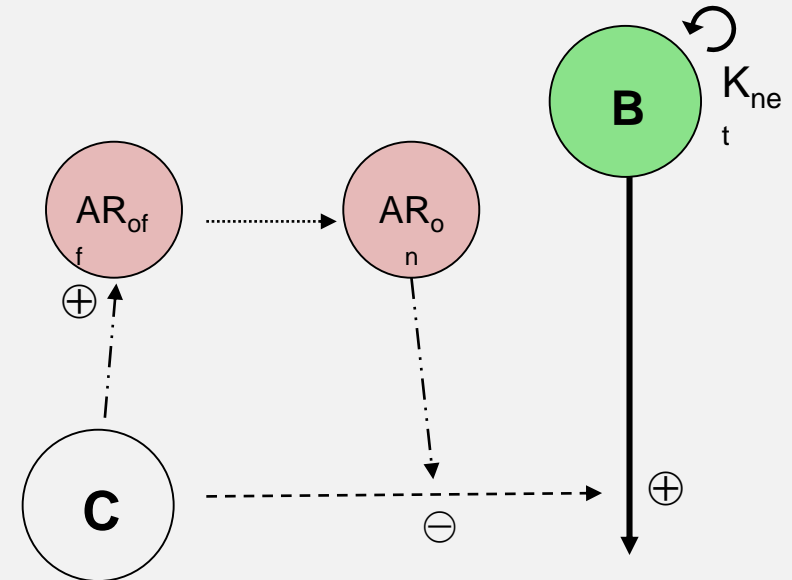
Hetero-resistance



Mouton *et al.*, 1997 Antimicrobial Agents Chemotherapy
 Meagher *et al.*, 2004 Antimicrobial Agents Chemotherapy

- ⇒ Population analysis profiles (PAPs)
- ⇒ Sequencing

Adaptation



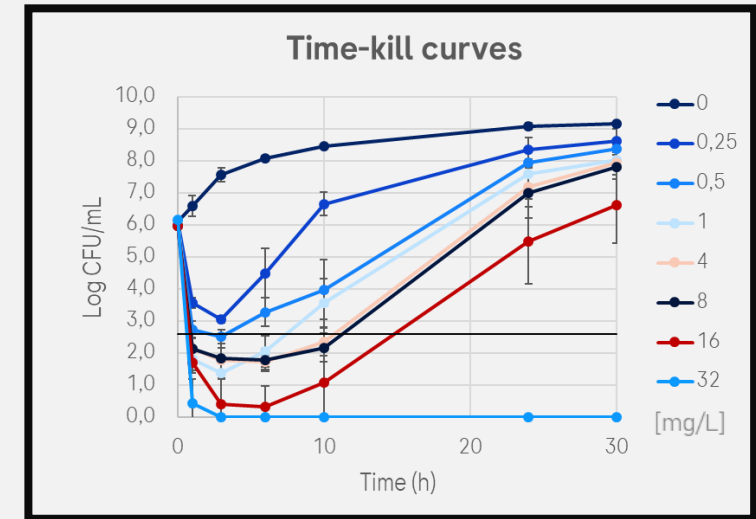
Mouton *et al.*, 1997 Antimicrobial Agents Chemotherapy
 Tam *et al.*, 2005 Journal of Antimicrobial Chemotherapy

- ⇒ RT-qPCR
- ⇒ Transcriptomics

Bacteria and Compound

→ *Acinetobacter baumannii*: ESKAPE pathogen

→ Polymyxin B : last resort antibiotic for MDR Gram- bacteria



Molecular Omics

ROYAL SOCIETY OF CHEMISTRY

RESEARCH ARTICLE

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Cite this: *Mol. Omics*, 2020, 16, 327

Pan-transcriptomic analysis identified common differentially expressed genes of *Acinetobacter baumannii* in response to polymyxin treatments†

Mengyao Li,^a Su Mon Aye,^a Maizbha Uddin Ahmed,^b Mei-Ling Han,^a Chen Li,^{b,c} Jiangning Song,^{b,c,d} John D. Boyce,^a David R. Powell,^e Mohammad A. K. Azad,^a Tony Velkov,^f Yan Zhu,^{g,*a} and Jian Li^{*,a}

MICROBIAL GENOMICS

RESEARCH ARTICLE

Boinett et al., *Microbial Genomics* 2019;5
DOI 10.1099/mgen.0.000246

MICROBIOLOGY SOCIETY

OPEN DATA OPEN MICROBIOLOGY

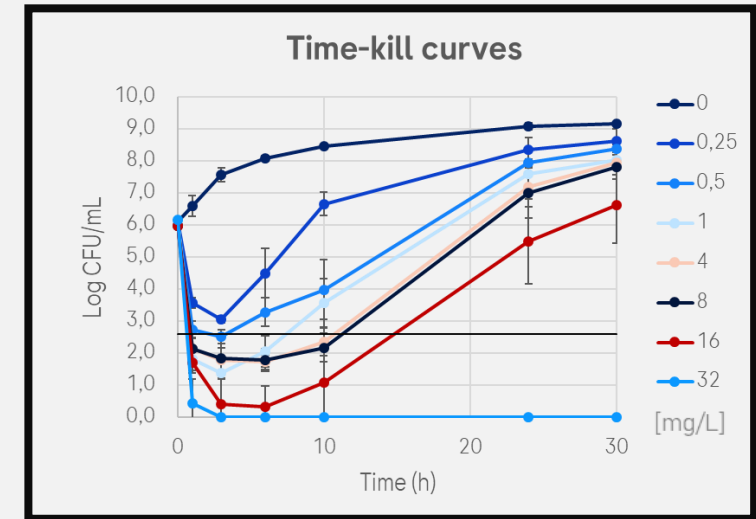
Clinical and laboratory-induced colistin-resistance mechanisms in *Acinetobacter baumannii*

Christine J. Boinett,^{1,2,3} Amy K. Cain,^{1,4} Jane Hawkey,^{5,6,7} Nhu Tran Do Hoang,² Nhu Nguyen Thi Khanh,⁸ Duy Pham Thanh,² Janina Dordel,^{1,9} James I. Campbell,^{2,3} Nguyen Phu Huong Lan,^{2,10} Matthew Mayho,¹ Gemma C. Langridge,^{1,11} James Hadfield,¹ Nguyen Van Vinh Chau,¹⁰ Guy E. Thwaites,^{2,3} Julian Parkhill,¹ Nicholas R. Thomson,^{1,12} Kathryn E. Holt^{5,6} and Stephen Baker^{2,3,13,*}

Bacteria and Compound

→ *Acinetobacter baumannii*: ESKAPE pathogen

→ Polymyxin B : last resort antibiotic for MDR Gram- bacteria



Molecular Omics

ROYAL SOCIETY OF CHEMISTRY

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Objective: Integrate transcriptomics data into *in vitro* PK/PD models

Strains

- ❖ Two clinical MDR *Acinetobacter baumannii* isolates obtained before and after colistin treatment ¹
 - **AB121** *pmbS* (MIC = 0.5 mg/L)
 - **AB122** *pmbR* (MIC = 64 mg/L)

¹ Jaidane *et al.*, 2018 International Journal of Antimicrobial Agents

Strains

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- ❖ AB122 carries a 10 AA insertion into *pmrB*, absent in AB121

	1	10	20	30	40	50																																												
AB121	V	H	S	L	K	K	R	L	I	W	G	T	S	I	-	-	-	-	-	-	F	S	V	I	L	G	C	I	L	I	F	S	A	Y	K	V	A	L	Q	E	V	D	E	I	L	D				
AB122	V	H	S	L	K	K	R	L	I	W	G	T	S	I	F	S	V	I	L	G	C	I	L	I	F	S	V	I	L	G	C	I	L	I	F	S	A	Y	K	V	A	L	Q	E	V	D	E	I	L	D

¹ Jaidane *et al.*, 2018 International Journal of Antimicrobial Agents

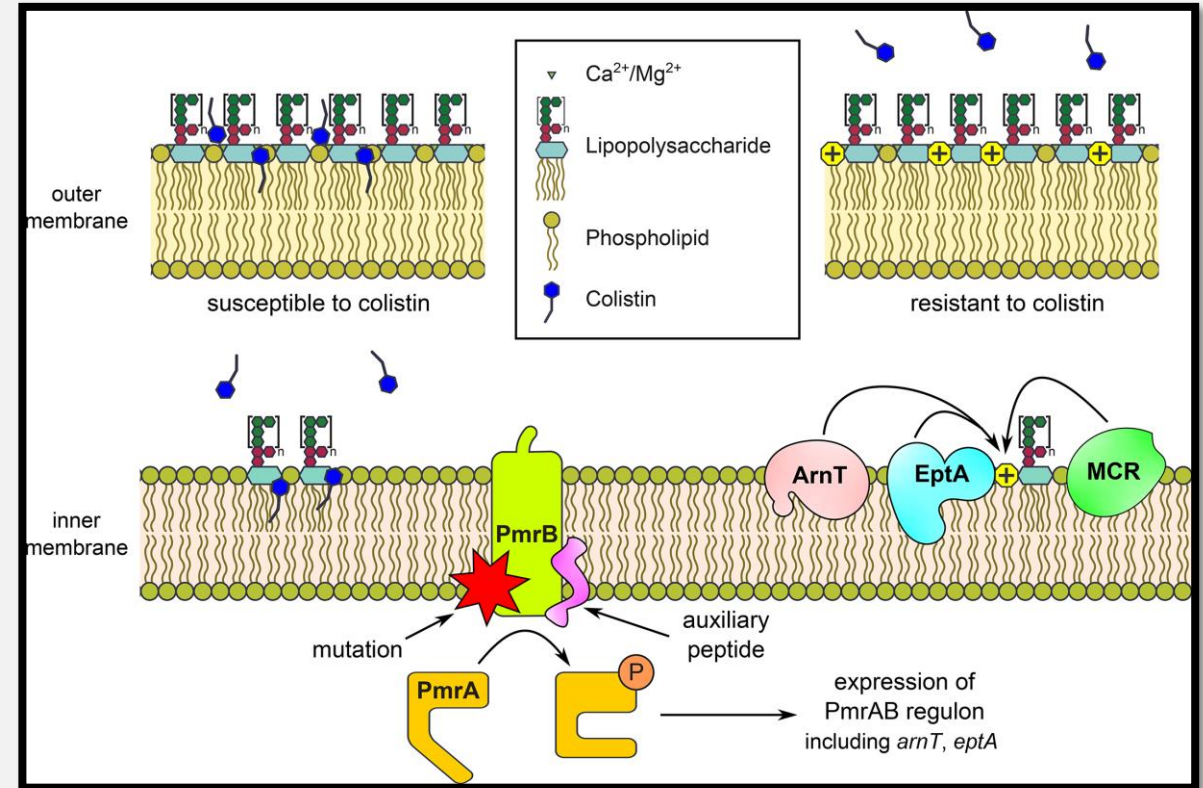
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AB121	V	H	S	L	K	K	R	L	I	W	G	T	S							F	S	V	I	L	G	C	I	L	I	F	S	A	Y	K	V	A	L	Q	E	V	D	E	I	L	D
AB122	V	H	S	L	K	K	R	L	I	W	G	T	S							F	S	V	I	L	G	C	I	L	I	F	S	A	Y	K	V	A	L	Q	E	V	D	E	I	L	D



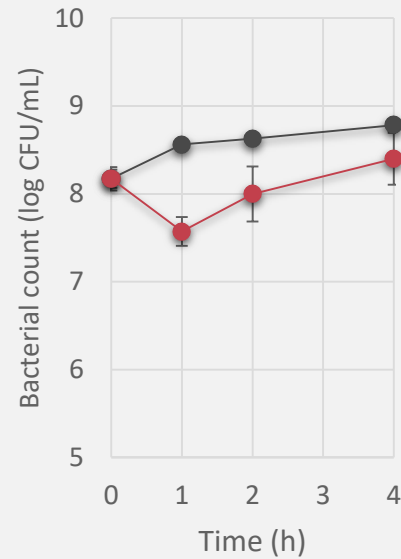
¹ Jaidane *et al.*, 2018 International Journal of Antimicrobial Agents

² Janssen *et al.*, 2021 PLoS Genetics

Bacterial samples preparation

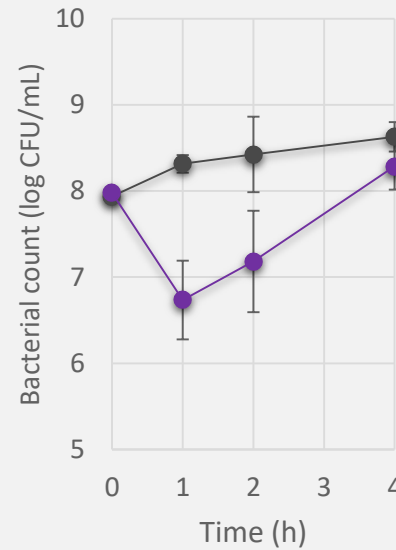
Time-kill curves for transcriptomics

AB121 *pmbS*



● Control
● PMB - 1mg/L

AB122 *pmbR*



● Control
● PMB - 32mg/L

→ Transcriptomics study for both strains on T0, T1h, T2h and T4h samples

WGCNA

Weighted Gene Correlation Networks for Analysis ^{1,2}

- **Dataset preparation** (with DESeq2 ³)

Normalization and thresholding by variance (>90% quantile to reduce noise) - *from 3799 to 363 genes* -

¹ Zhang *et al.*, 2005 Statistical applications in genetics and molecular biology

² Langfelder *et al.*, 2008 BMC bioinformatics

³ Love *et al.*, 2014 Genome Biology

WGCNA

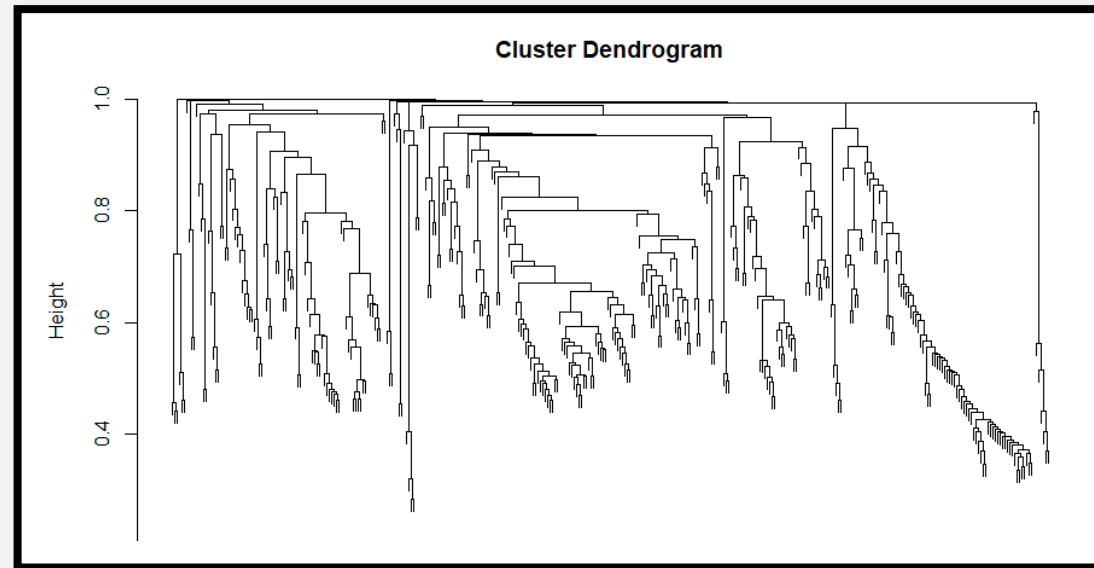
Weighted Gene Correlation Networks for Analysis ^{1,2}

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- **Construction of a gene co-expression network**

Use of interaction patterns among genes



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WGCNA

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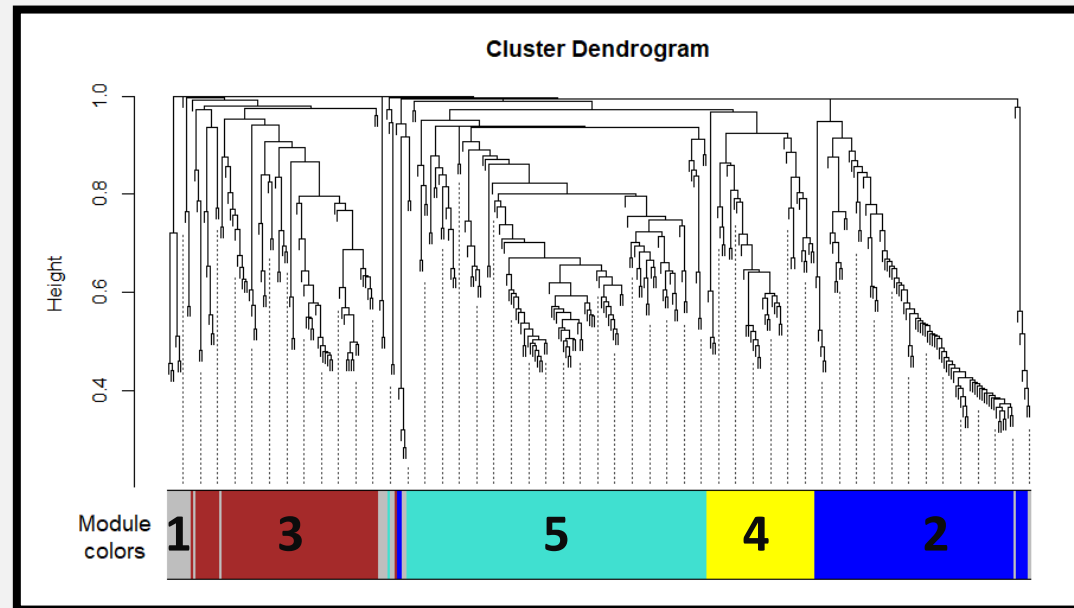
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Use of interaction patterns among genes

- **Identification of modules**



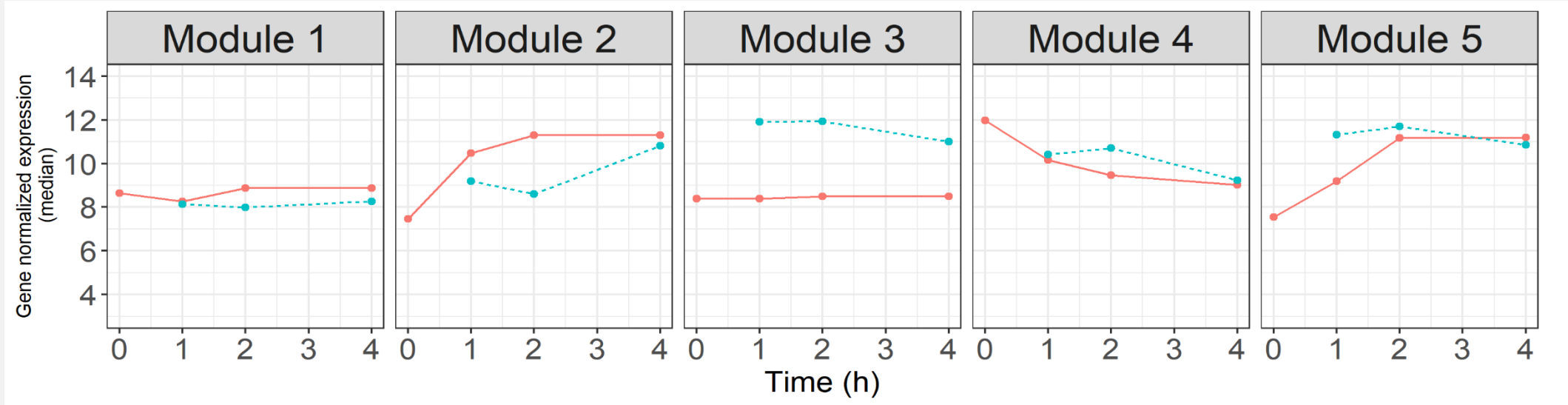
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AB121 *pmbS*

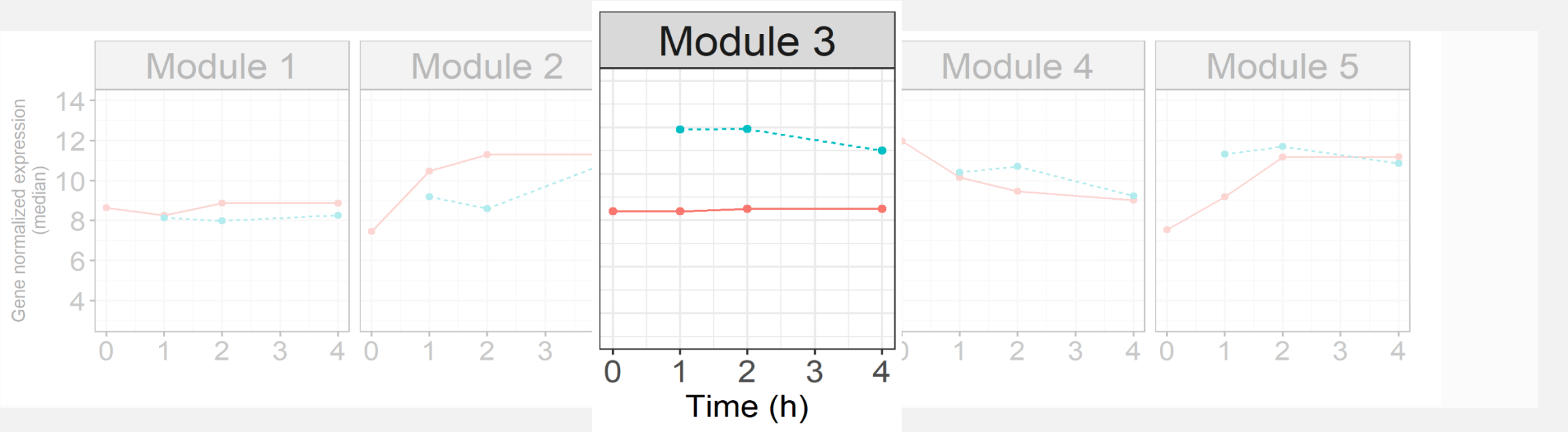
Modules expression profiles



—●— Control
- -●- - with PMB

AB121 *pmbS*

Modules expression profiles



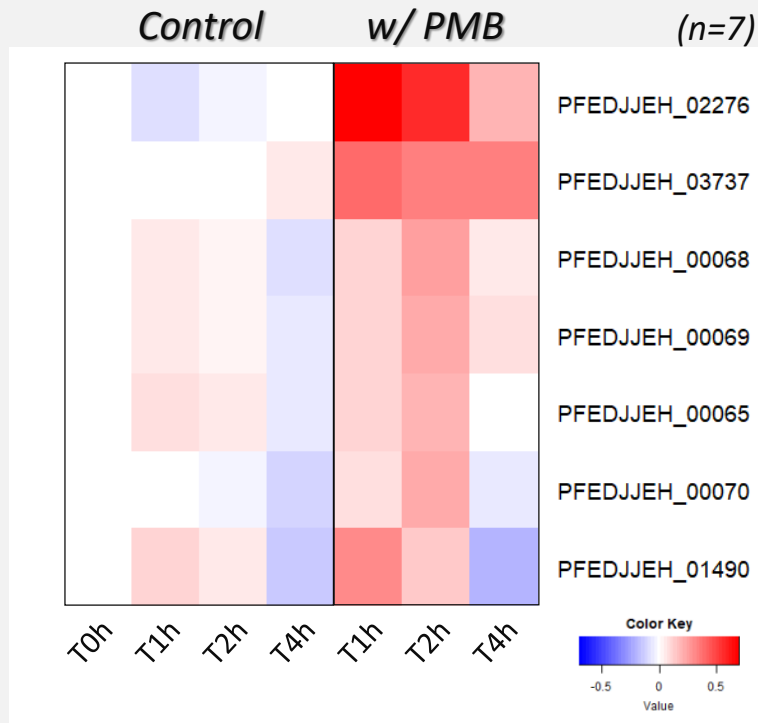
—●— Control
- - -●- - with PMB

AB121 *pmbS*

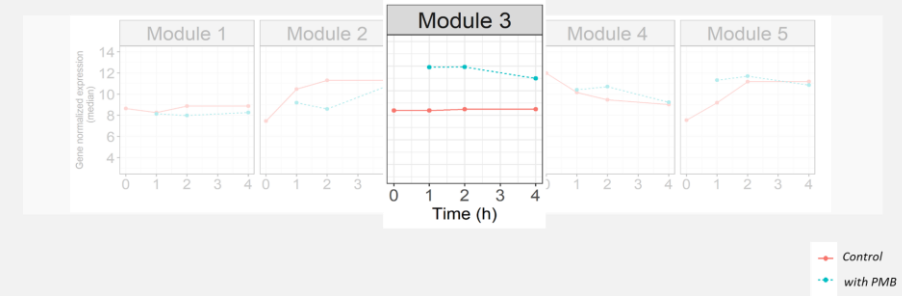
→ 91 genes allocated to this module

Gene expressions per pathway

Amino-acids metabolism



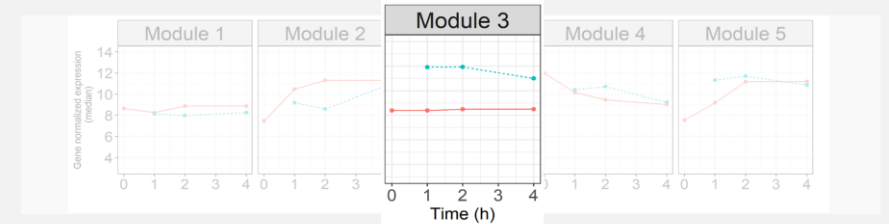
- 35 genes associated with general metabolisms
- 21 hypothetical proteins



AB121 *pmbS*

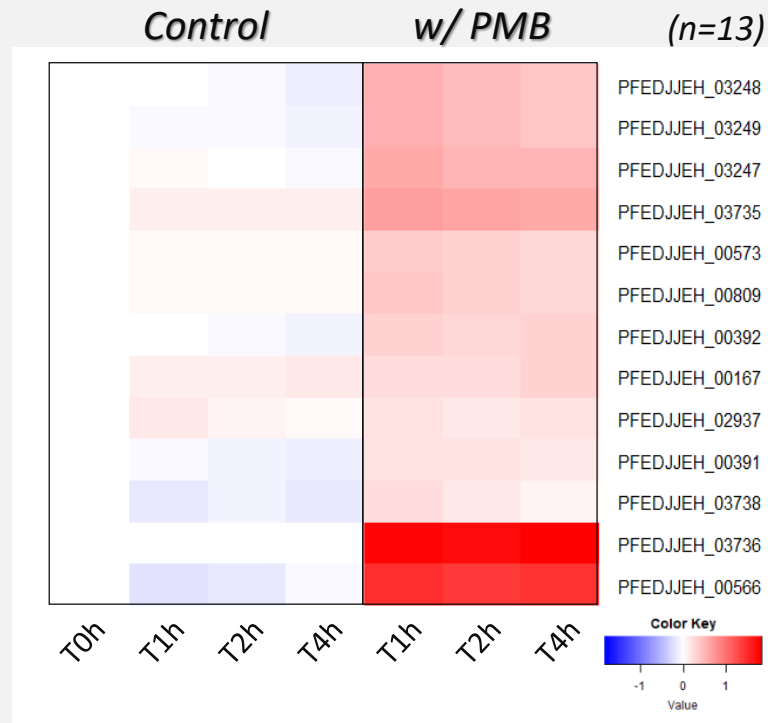
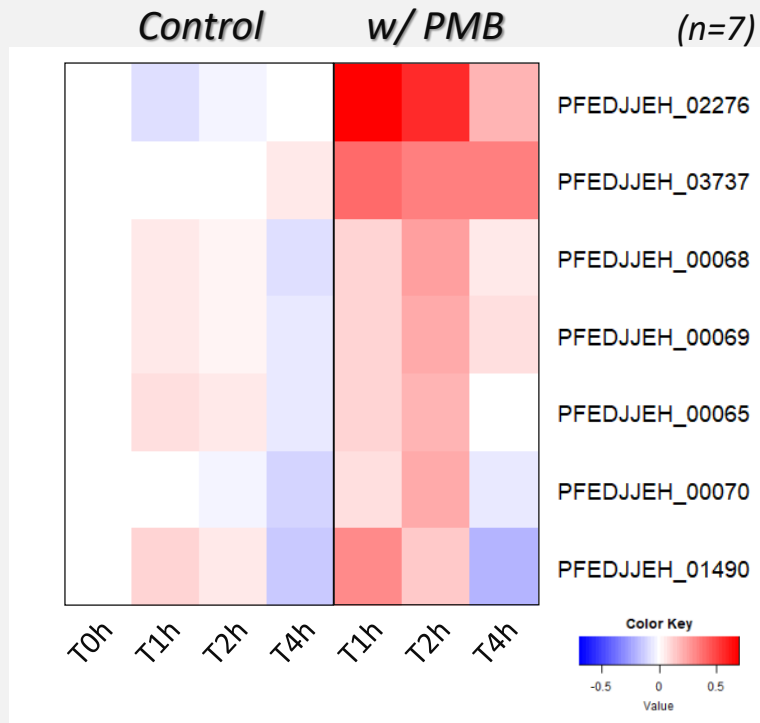
→ 91 genes allocated to this module

Gene expressions per pathway



Amino-acids metabolism

Efflux systems

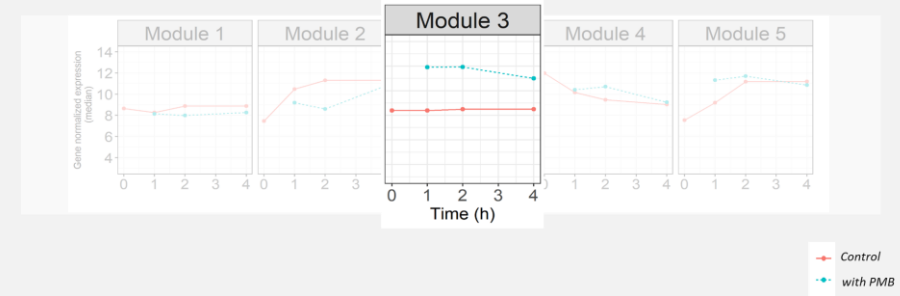


- 35 genes associated with general metabolisms
- 21 hypothetical proteins

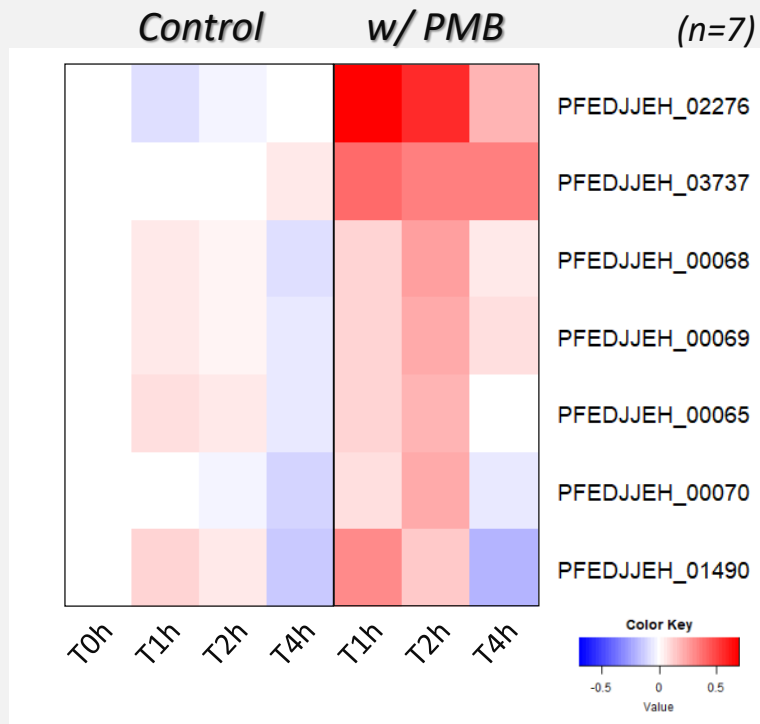
AB121 *pmbS*

→ 91 genes allocated to this module

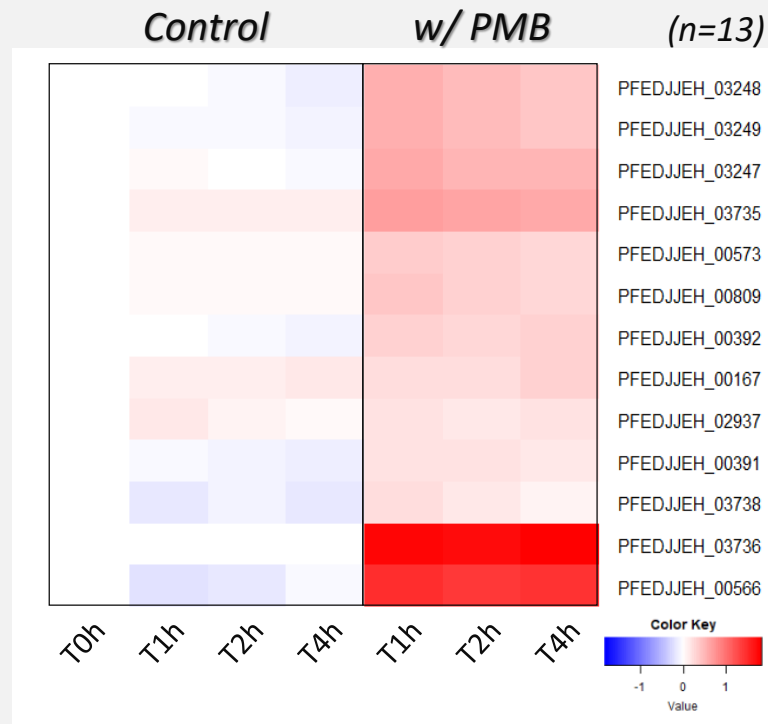
Gene expressions per pathway



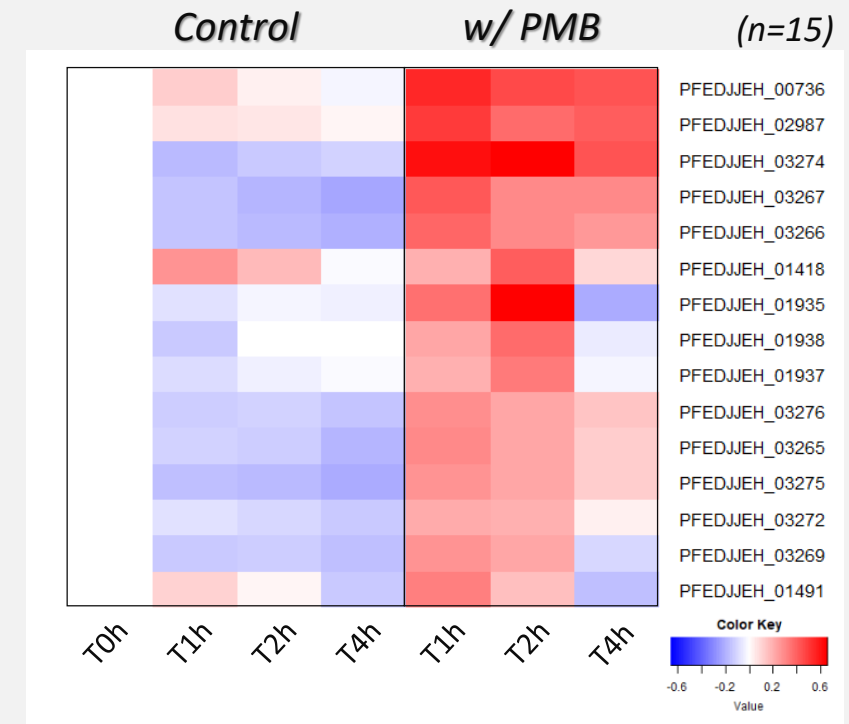
Amino-acids metabolism



Efflux systems



Membrane integrity

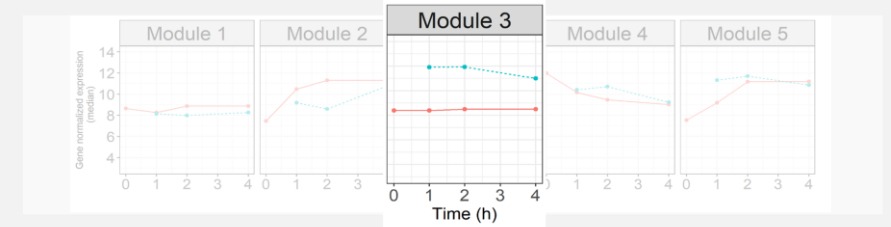


- 35 genes associated with general metabolisms
- 21 hypothetical proteins

AB121 *pmbS*

→ 91 genes allocated to this module

Gene expressions per pathway



Amino-acids metabolism

Efflux systems

→ Compensation of PMB membrane disruption activity by a over-production of membrane components ?

Confirming literature results

Henry et al., 2012 *Antimicrobial Agents and Chemotherapy*

Park et al., 2015 *Clinical Microbiology and Infection*

Henry et al., 2015 *Journal of Antimicrobial Chemotherapy*

Cheah et al., 2016 *Scientific Reports*

Hua et al., 2017 *Front. Cell. Infect. Microbiol.*

Boll et al., 2020 *PNAS*

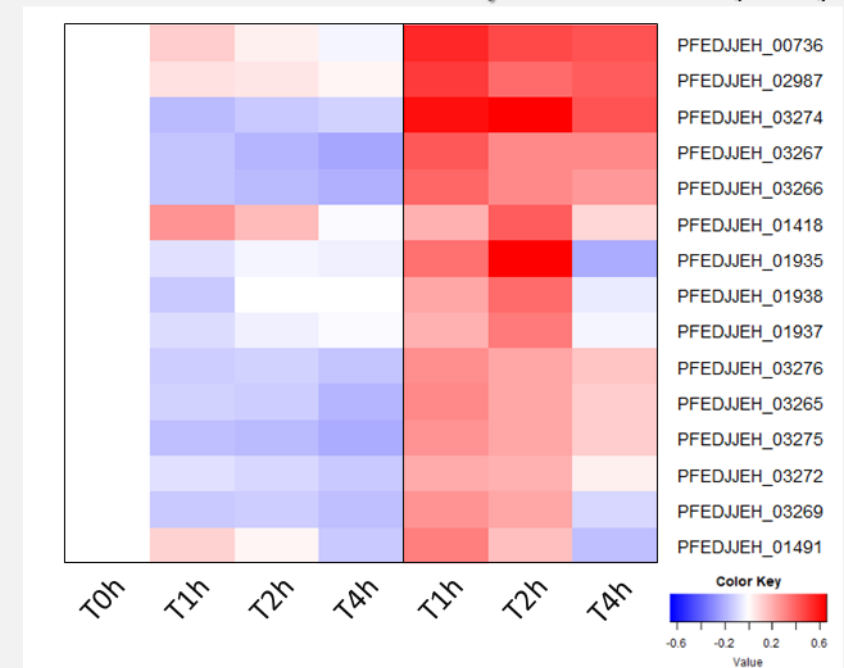
Chamoun et al., 2021 *Int. Journal of Molecular Science*

Membrane integrity

Control

w/ PMB

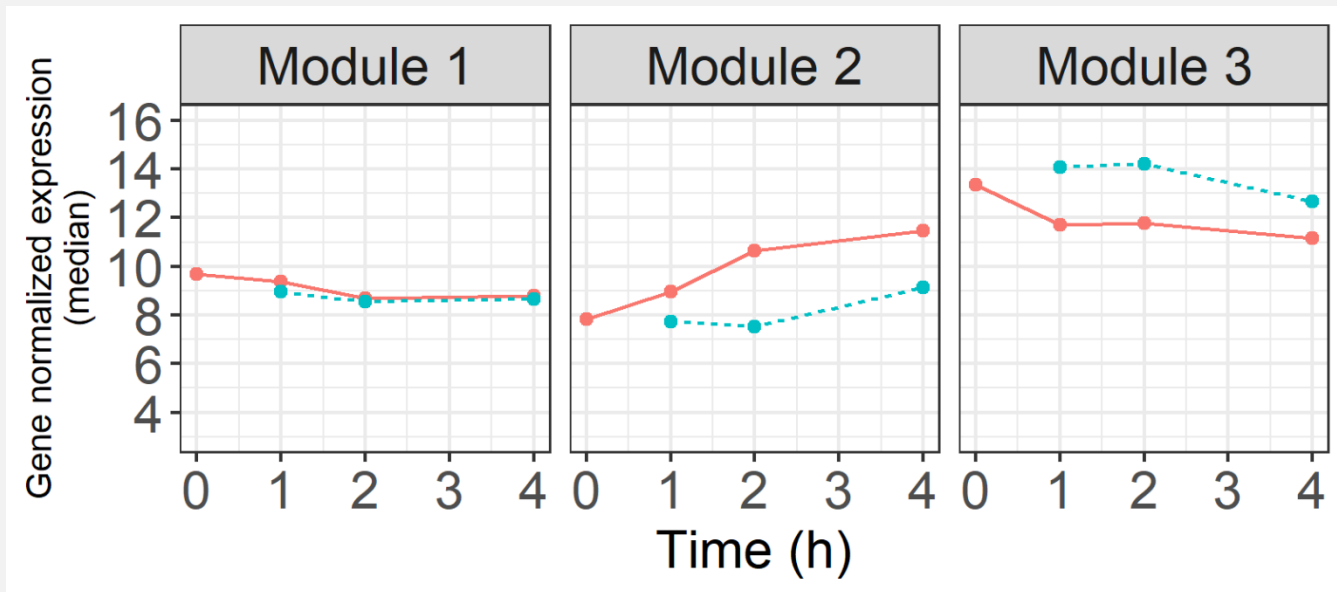
(n=15)



- 35 genes associated with general metabolisms
- 21 hypothetical proteins

AB122 *pmbR*

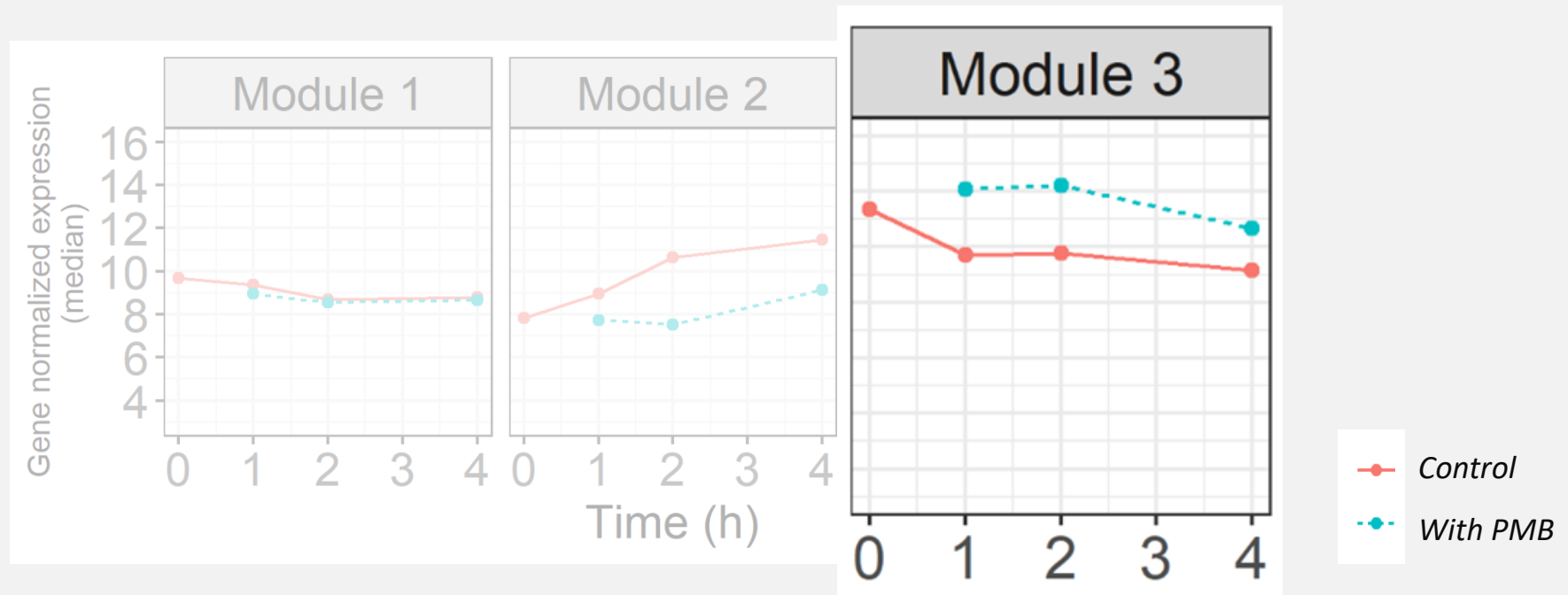
Modules expression profiles



—●— Control
- -●- - With PMB

AB122 *pmbR*

Modules expression profiles

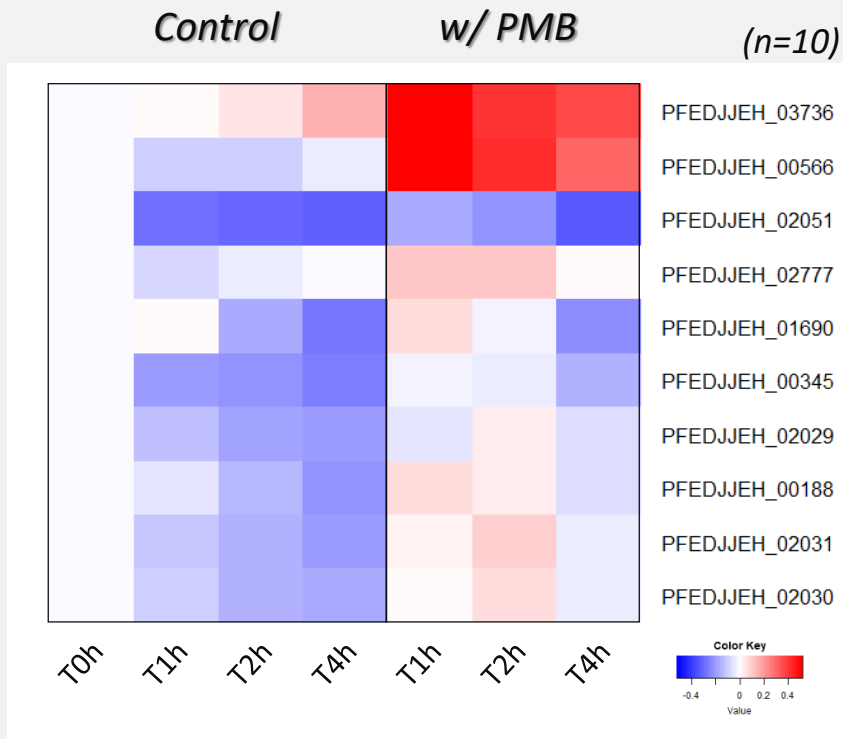


AB122 *pmbR*

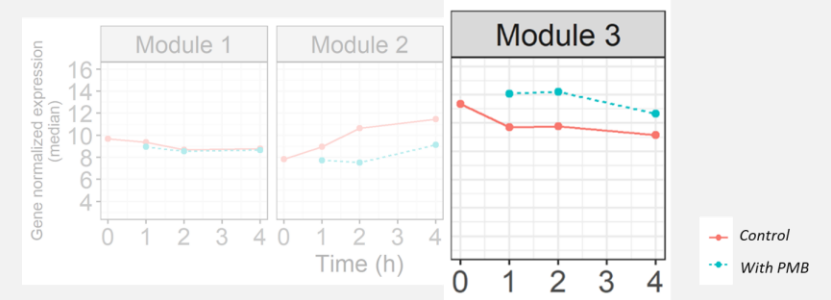
→ 105 genes allocated to this module

Gene expressions per pathway

Efflux systems



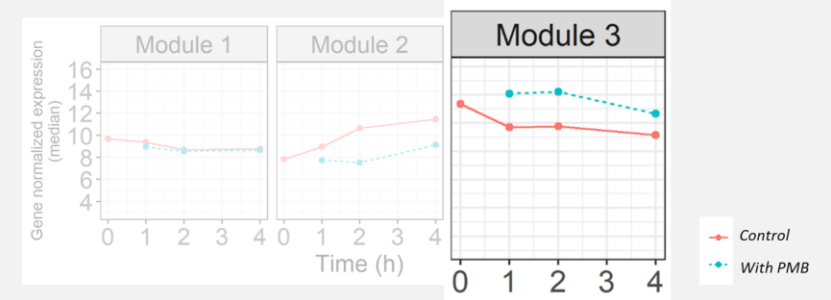
- 21 genes associated with general metabolisms
- 11 hypothetical proteins



AB122 *pmbR*

→ 105 genes allocated to this module

Gene expressions per pathway

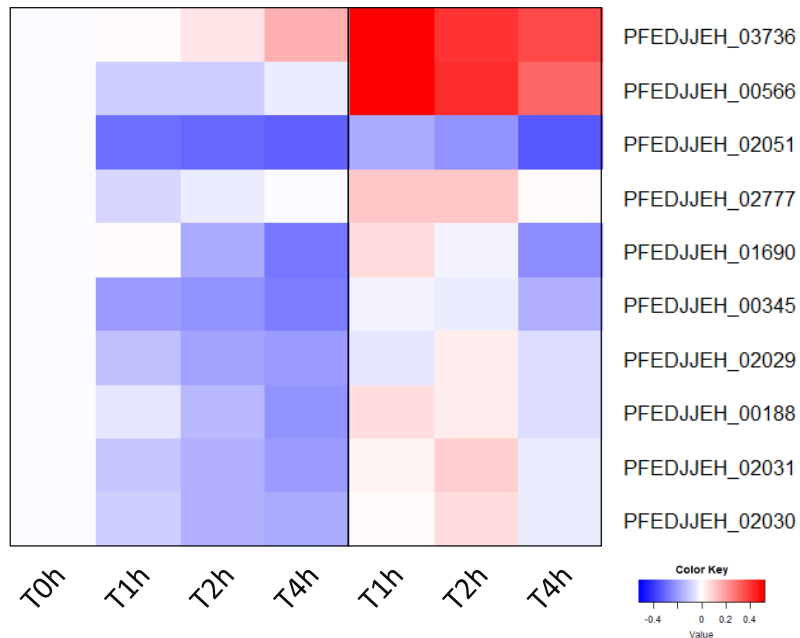


Efflux systems

Control

w/ PMB

(n=10)

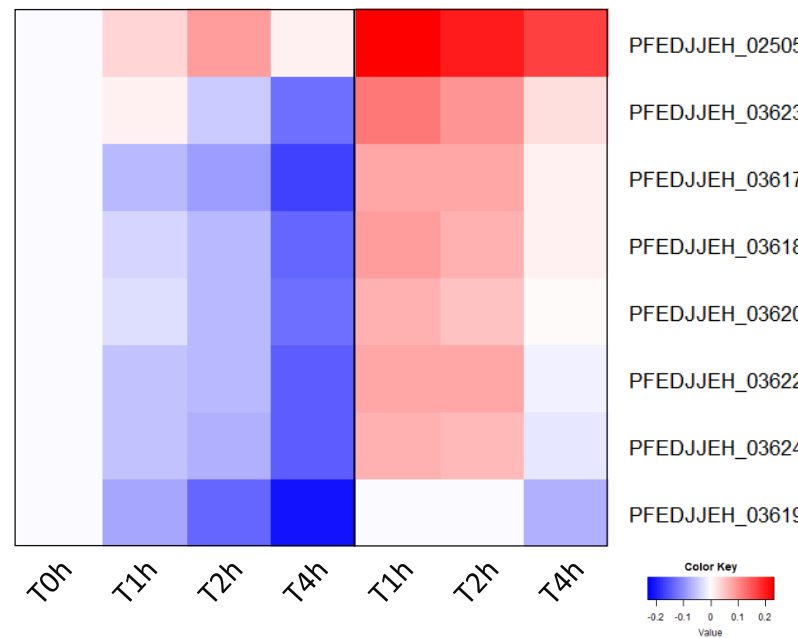


Oxidative phosphorylation

Control

w/ PMB

(n=8)

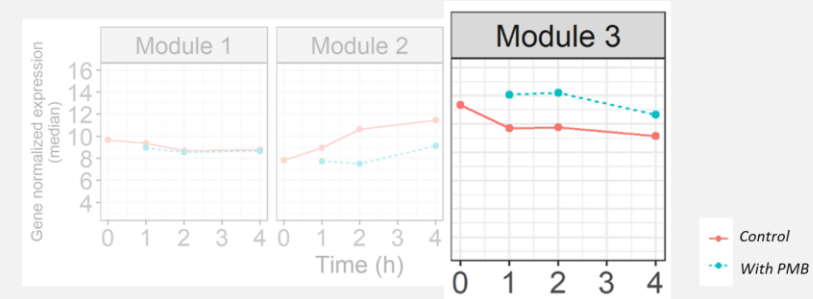


- 21 genes associated with general metabolisms
- 11 hypothetical proteins

AB122 *pmbR*

→ 105 genes allocated to this module

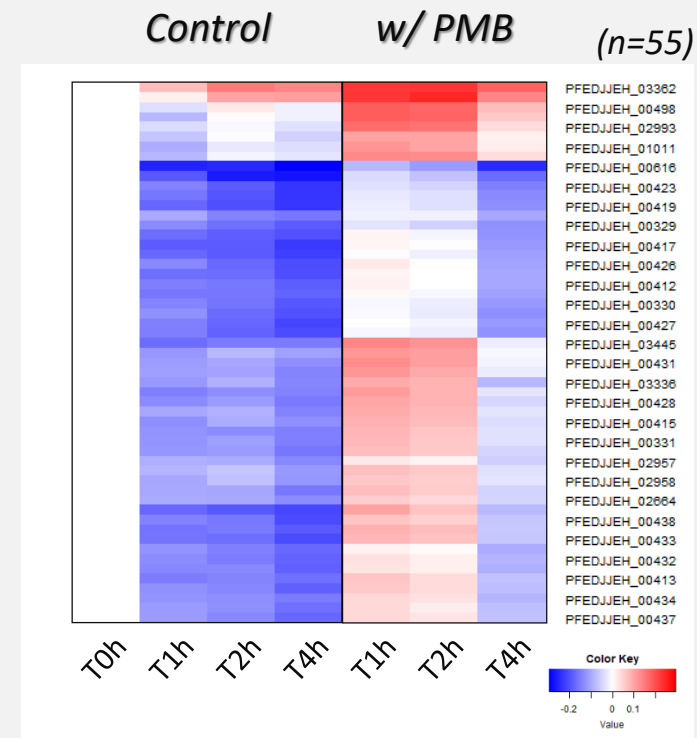
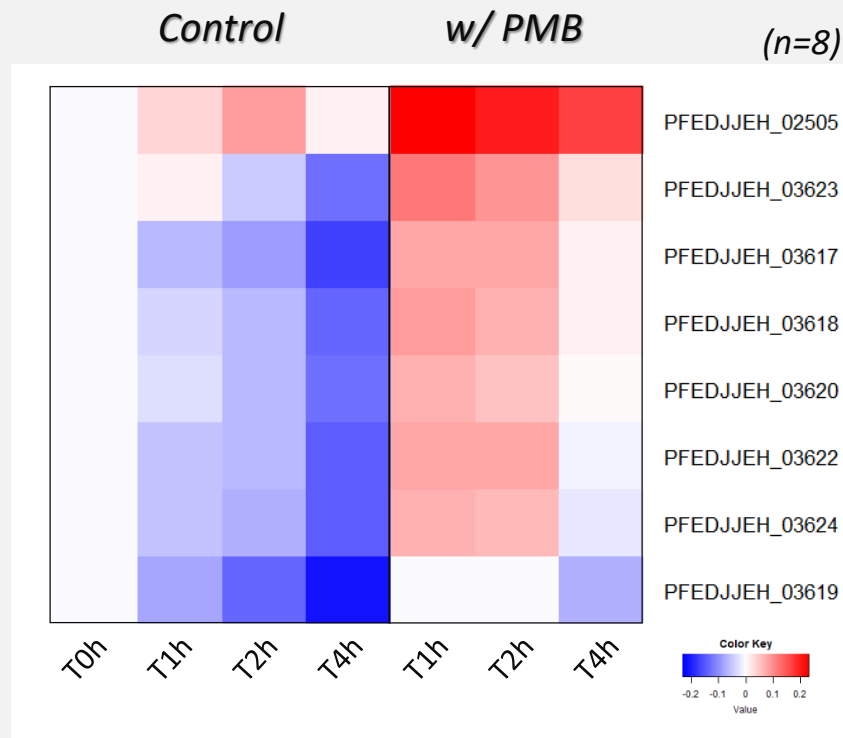
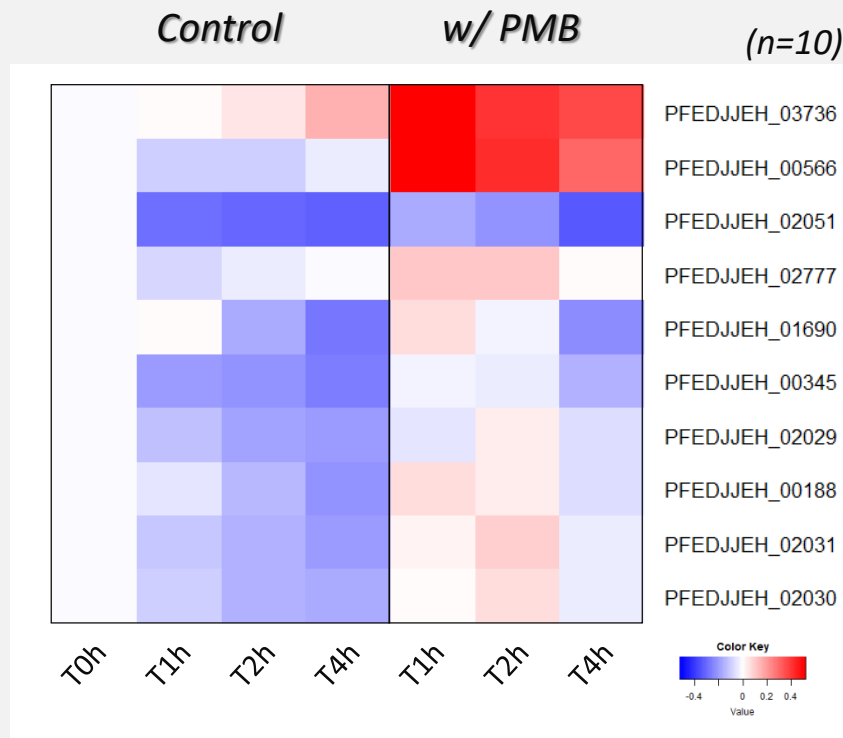
Gene expressions per pathway



Efflux systems

Oxidative phosphorylation

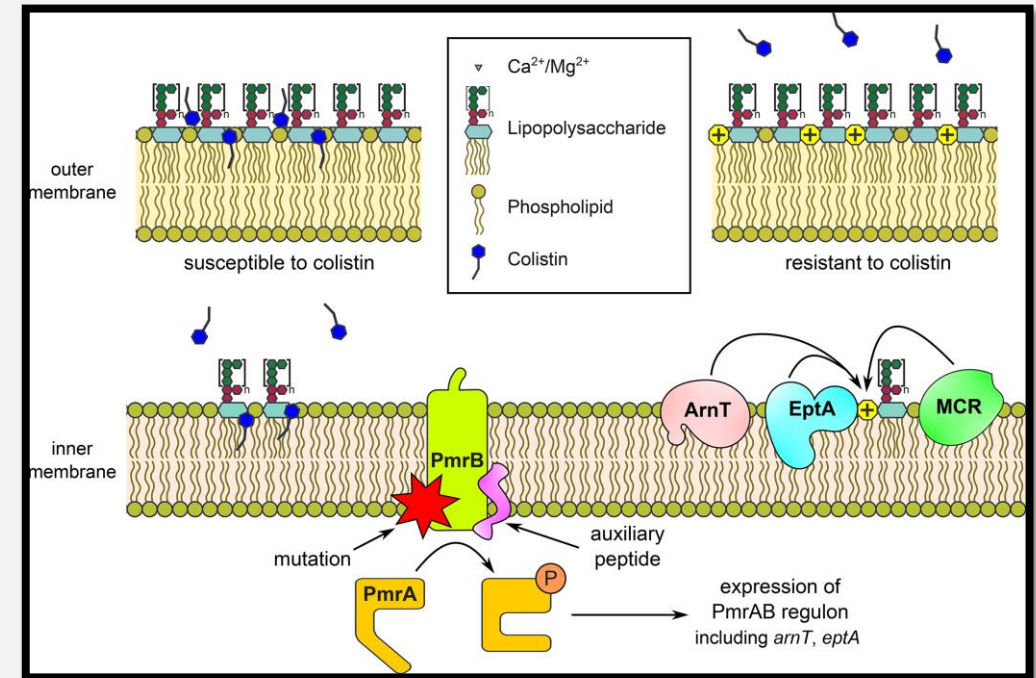
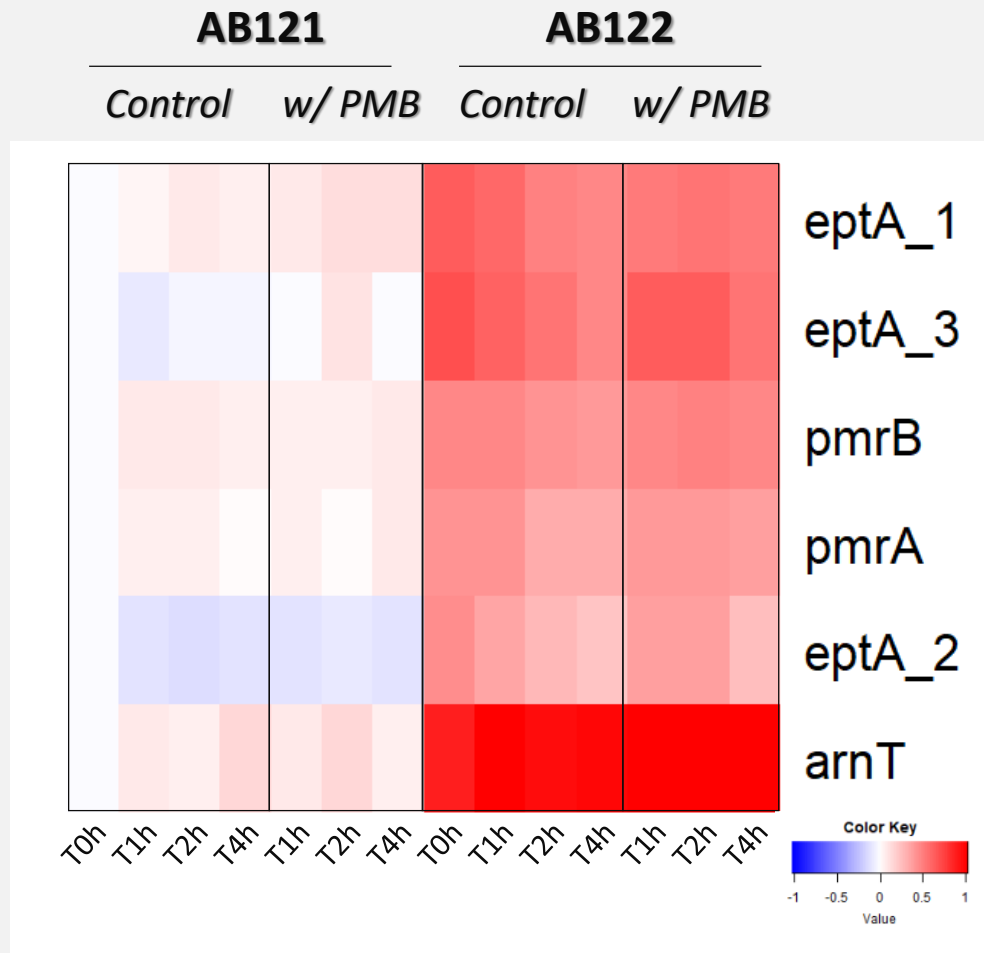
Protein synthesis



- 21 genes associated with general metabolisms
- 11 hypothetical proteins

AB122 *pmbR*

Resistance genes



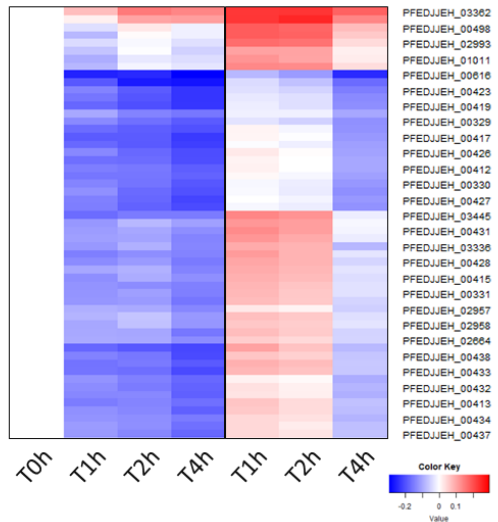
No expression change of genes involved in resistance with PMB addition

AB122 *pmbR*

Potential resistance mechanism

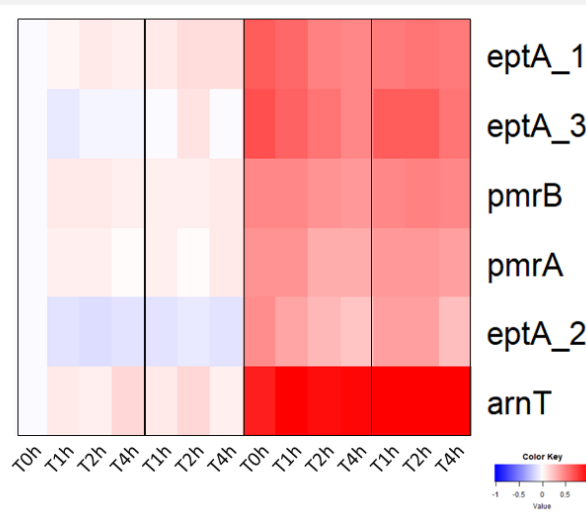
Protein synthesis

Control w/ PMB (n=55)

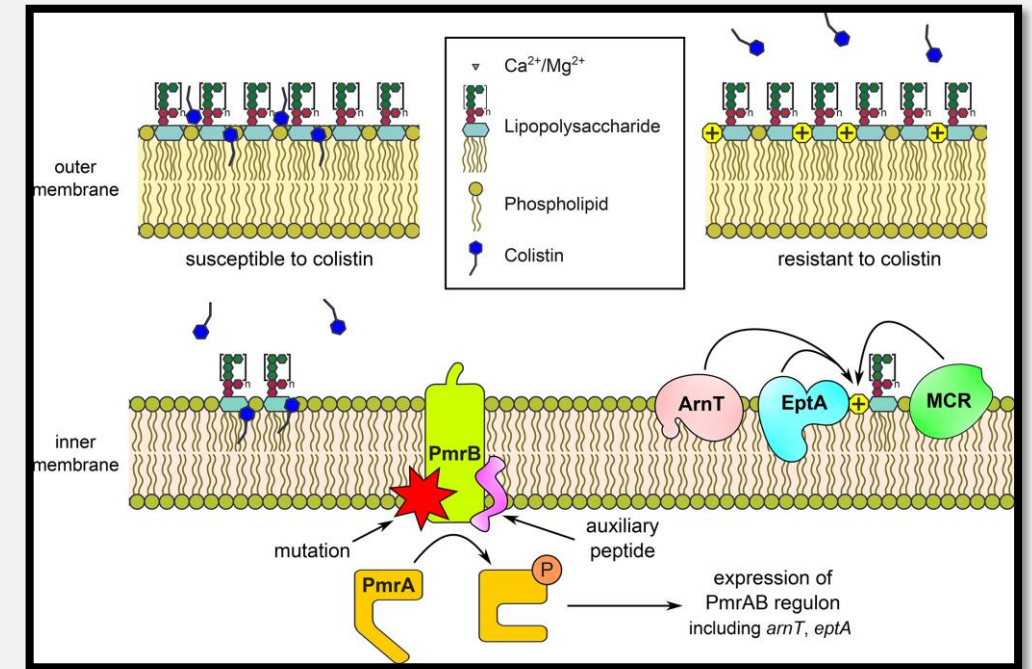


Increase of protein synthesis induced by PMB

AB121 AB122
Control w/ PMB Control w/ PMB



No expression change with PMB addition of genes involved in resistance

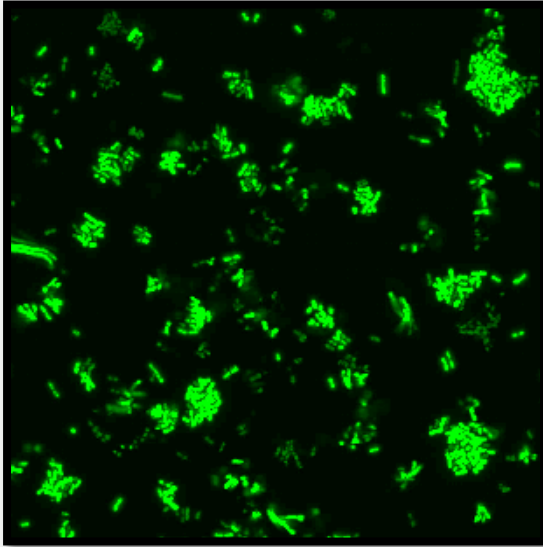


Synthesis increase of *eptA* and *arnT* to change lipid A charge and prevent PMB binding ?

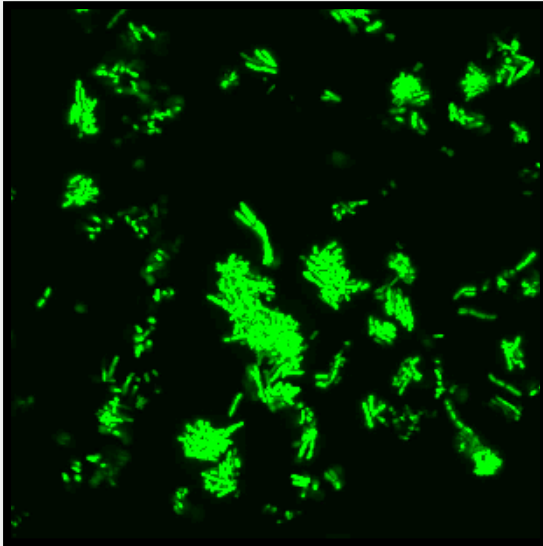
Study of PMB fixation to bacterial membrane by confocal microscopy

AB121 & AB122 *Confocal microscopy*

All bacteria



AB121 pmbS



AB122 pmbR

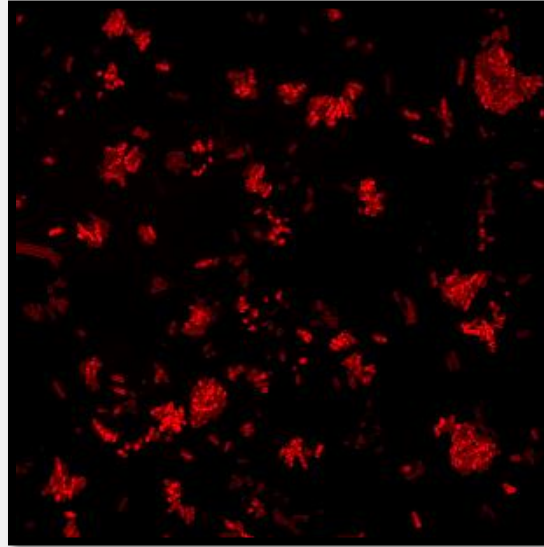
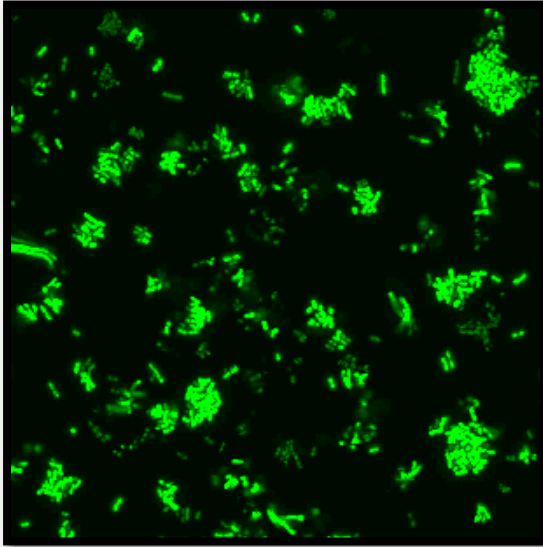
Green -> Syto 9
PMB_R -> PMB-Rhodamine

AB121 & AB122 *Confocal microscopy*

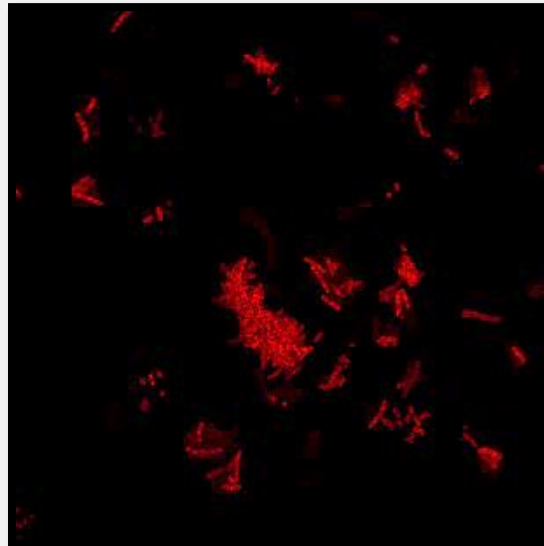
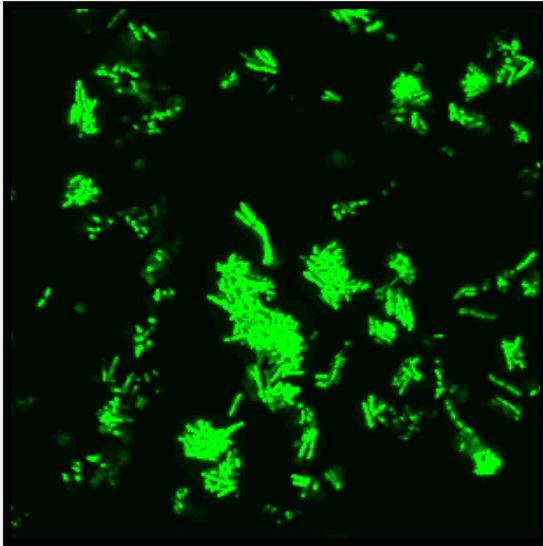
All bacteria

PMB_R-fixing bacteria

AB121 pmbS



AB122 pmbR



AB121 & AB122 *Confocal microscopy*

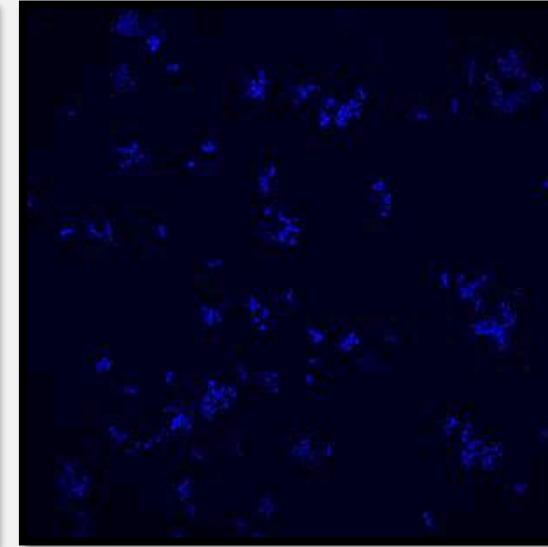
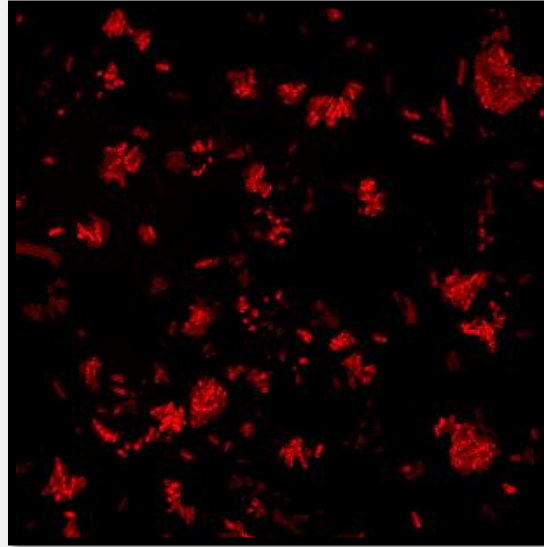
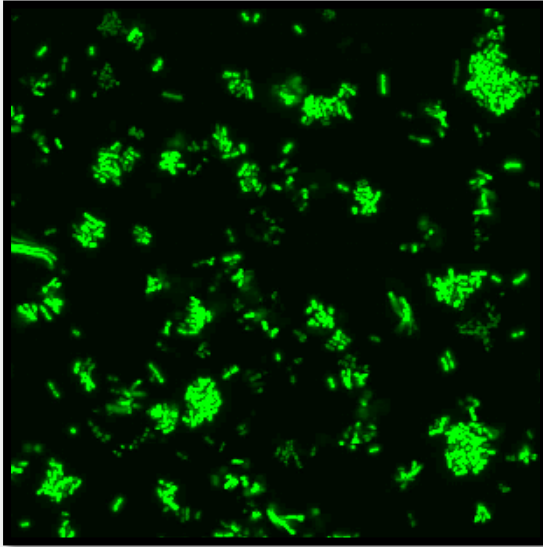
Green -> Syto 9
PMB_R -> PMB-Rhodamine
Blue -> Sytox Blue

All bacteria

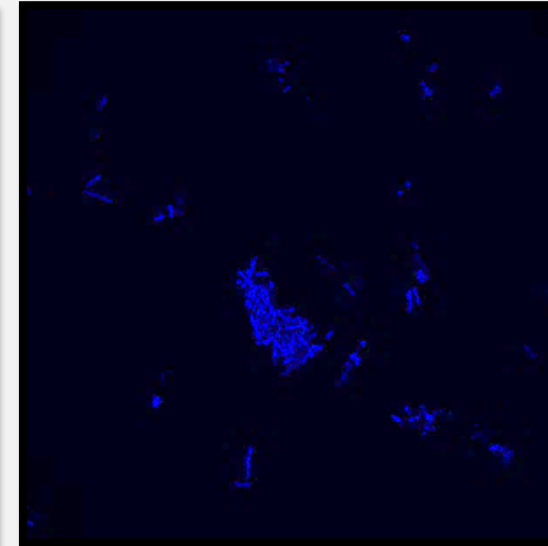
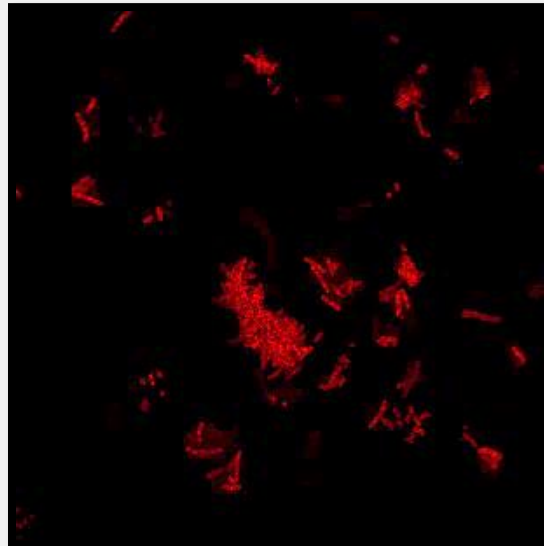
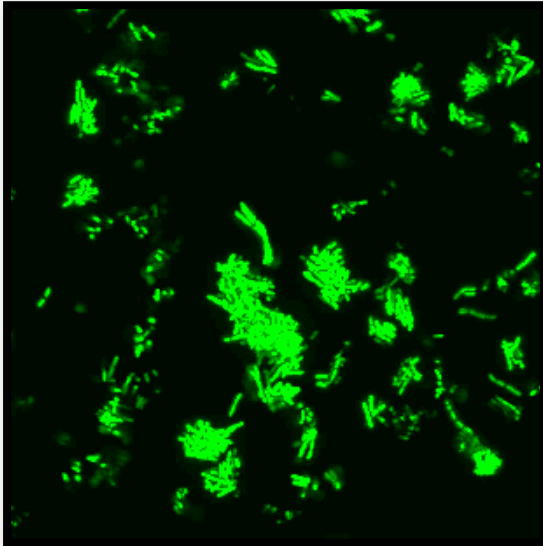
PMB_R-fixing bacteria

Dead bacteria

AB121 pmbS



AB122 pmbR



AB121 & AB122 *Confocal microscopy*

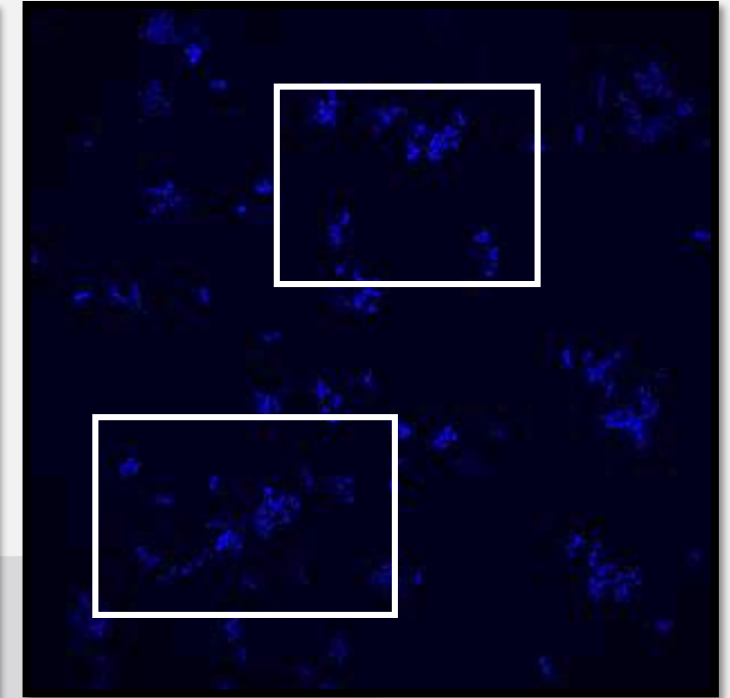
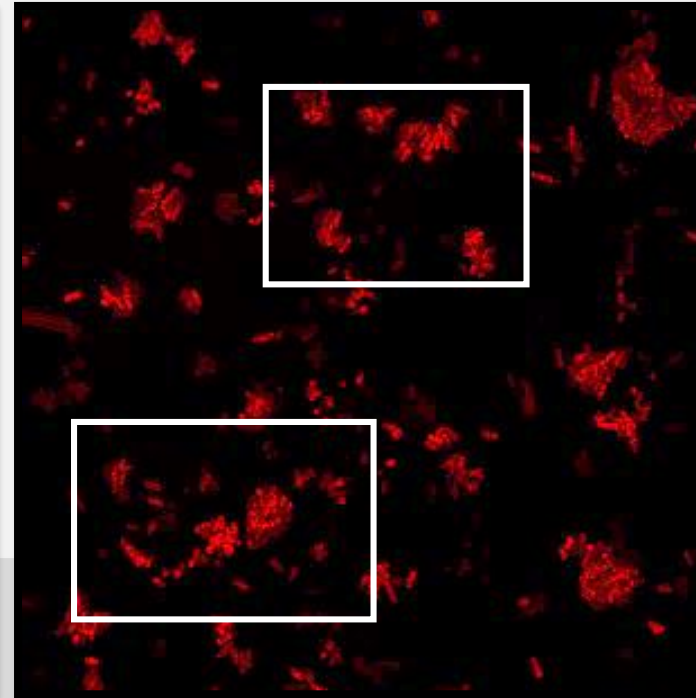
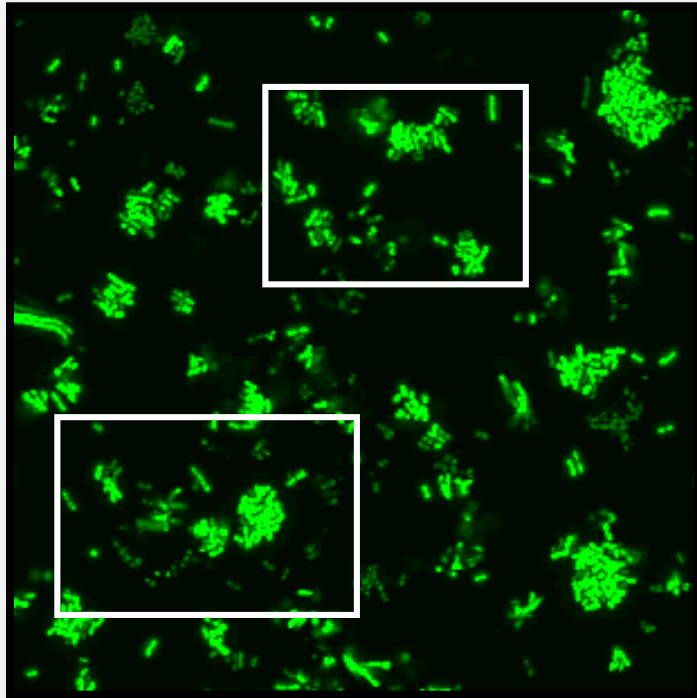
Green -> Syto 9
PMB_R -> PMB-Rhodamine
Blue -> Sytox Blue

All bacteria

PMB_R-fixing bacteria

Dead bacteria

*AB121
pmbS*



AB122 pmbR

→ For AB121: all bacteria are labelled with PMB_R

Green -> Syto 9
PMB_R -> PMB-Rhodamine
Blue -> Sytox Blue

AB121 & AB122 *Confocal microscopy*

All bacteria

Polymyxin B - Rhodamine

Dead bacteria

→ For AB122: presence of bacteria subpopulations not fixing PMB on their membranes

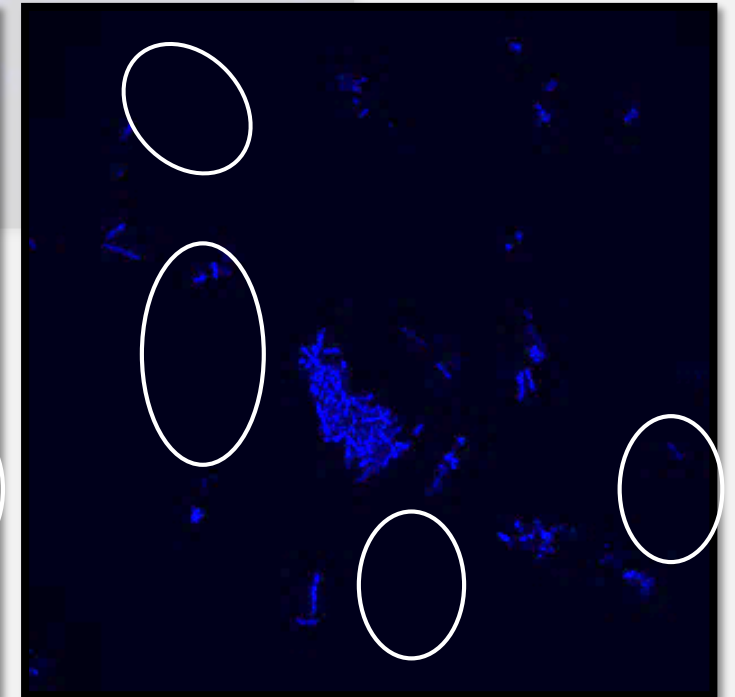
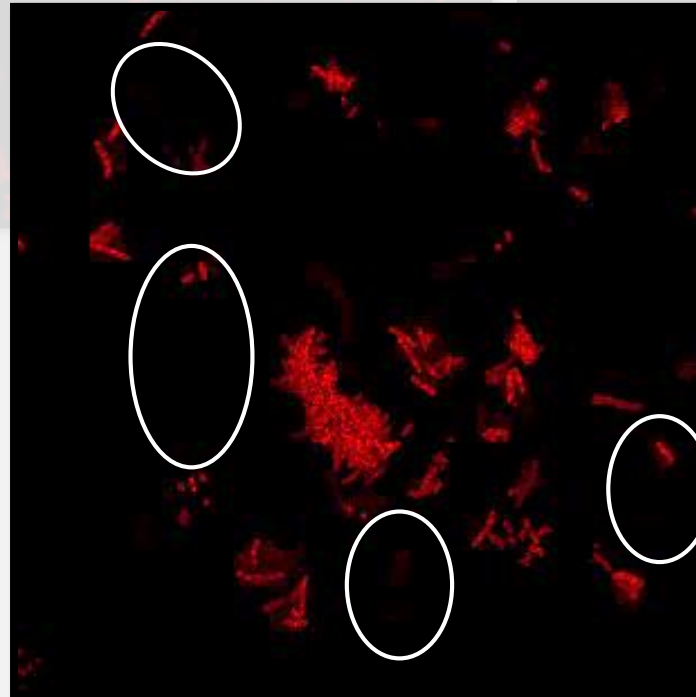
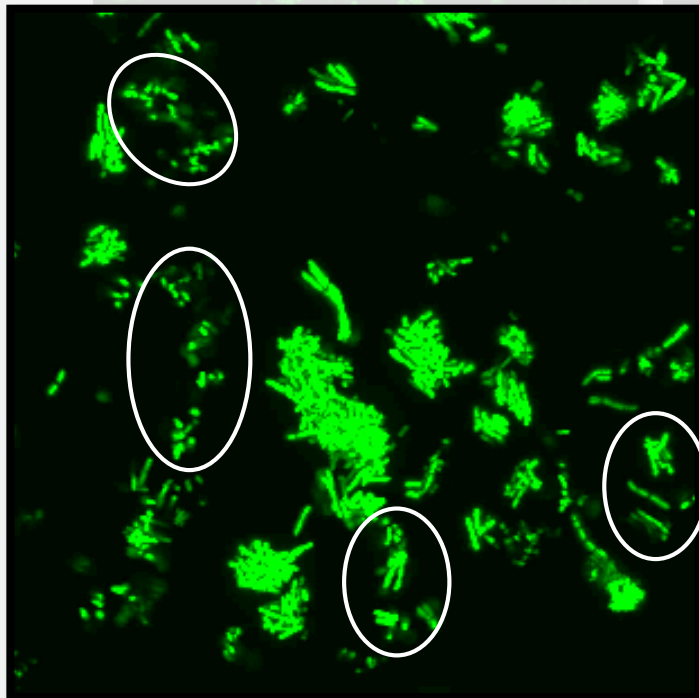
AB121 pmbS

All bacteria

PMB_R-fixing bacteria

Dead bacteria

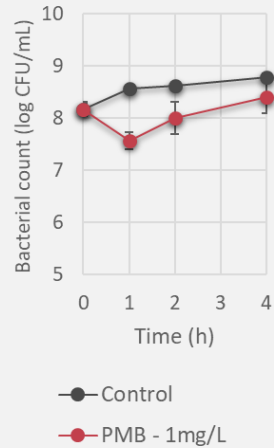
AB122 pmbR



Conclusion – Resistance mechanisms

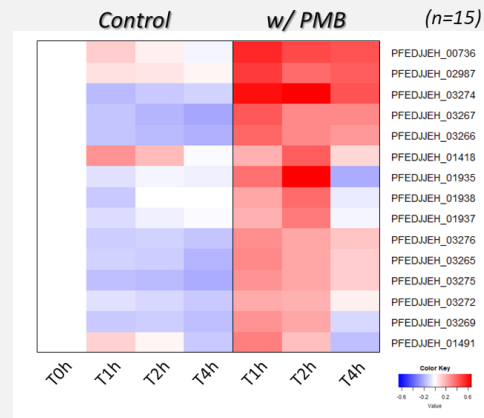
Time-kill curves

AB121 *pmbS*



Transcriptomic and confocal microscopy results

Membrane remodeling



Potential resistance mechanism

Over-production of membrane components to prevent PMB membrane disruption ?

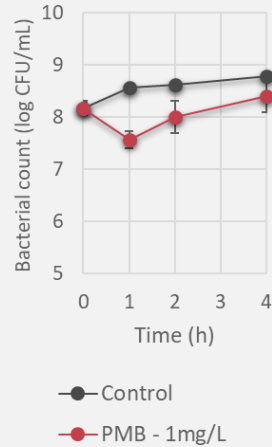


Adaptation ?

Conclusion – Resistance mechanisms

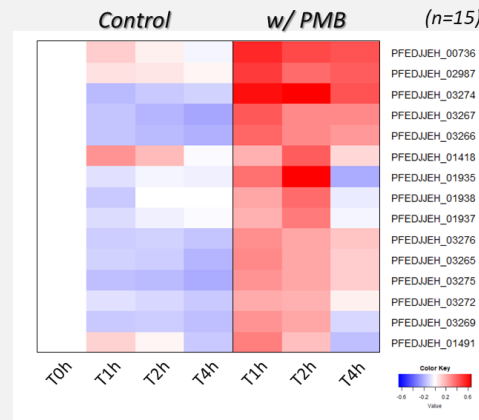
Time-kill curves

AB121 *pmbS*



Transcriptomic and confocal microscopy results

Membrane remodeling



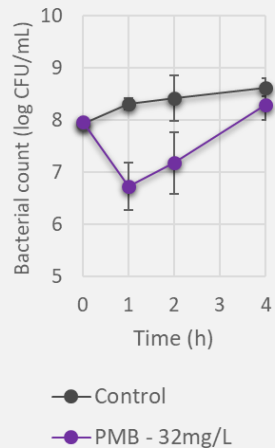
Potential resistance mechanism

Over-production of membrane components to prevent PMB membrane disruption ?

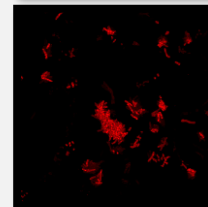
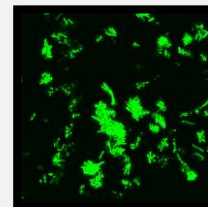
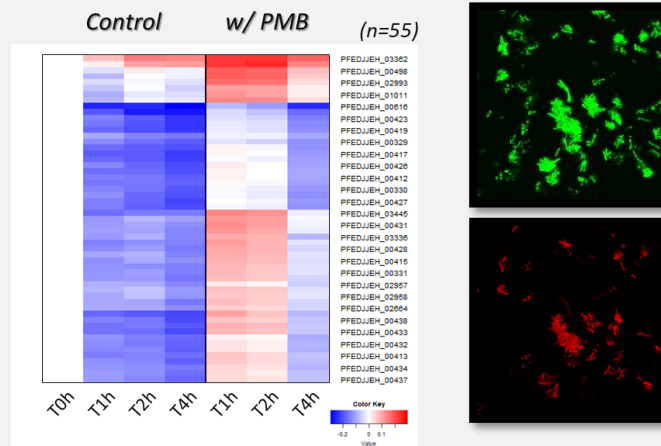


Adaptation ?

AB122 *pmbR*



Protein synthesis



Synthesis increase of *eptA* to change lipid A charge and prevent PMB binding ?



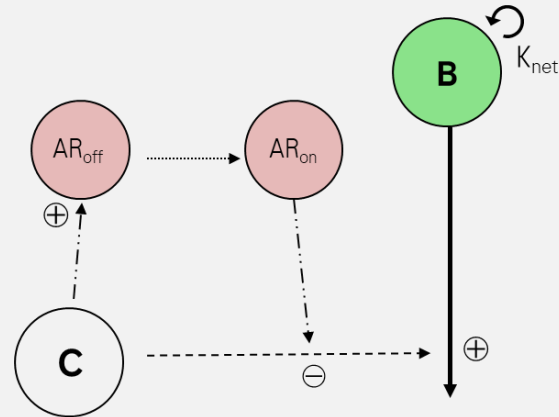
Hetero-resistance + Adaptation ?

Conclusion – Modelling

AB121
pmbS



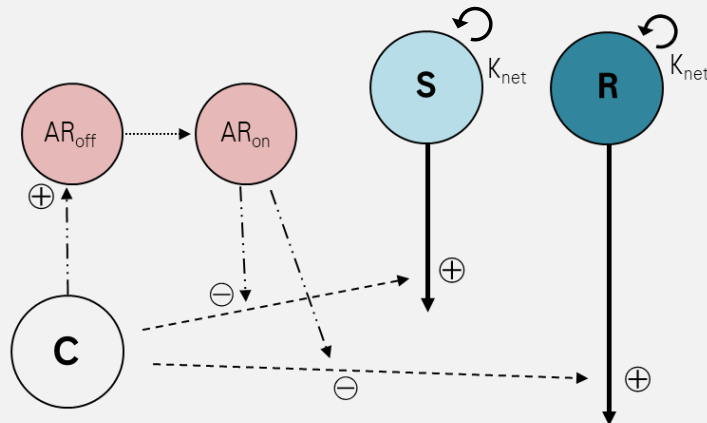
Adaptation



AB122
pmbR



Hetero-resistance + Adaptation



• PK/PD model choice

- ✓ Transcriptomics may orient the selection of model structure
- ✓ Results need to be expanded (Proteomics)

Thanks for your attention

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