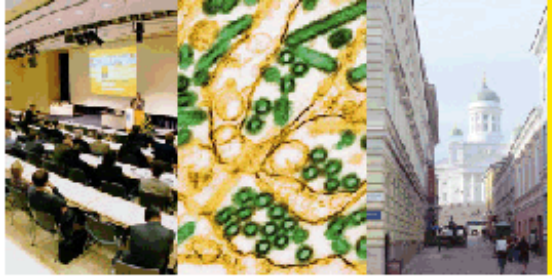

Inducibility of PatA/PatB efflux pumps by fluoroquinolones (FQ) in *Streptococcus pneumoniae*

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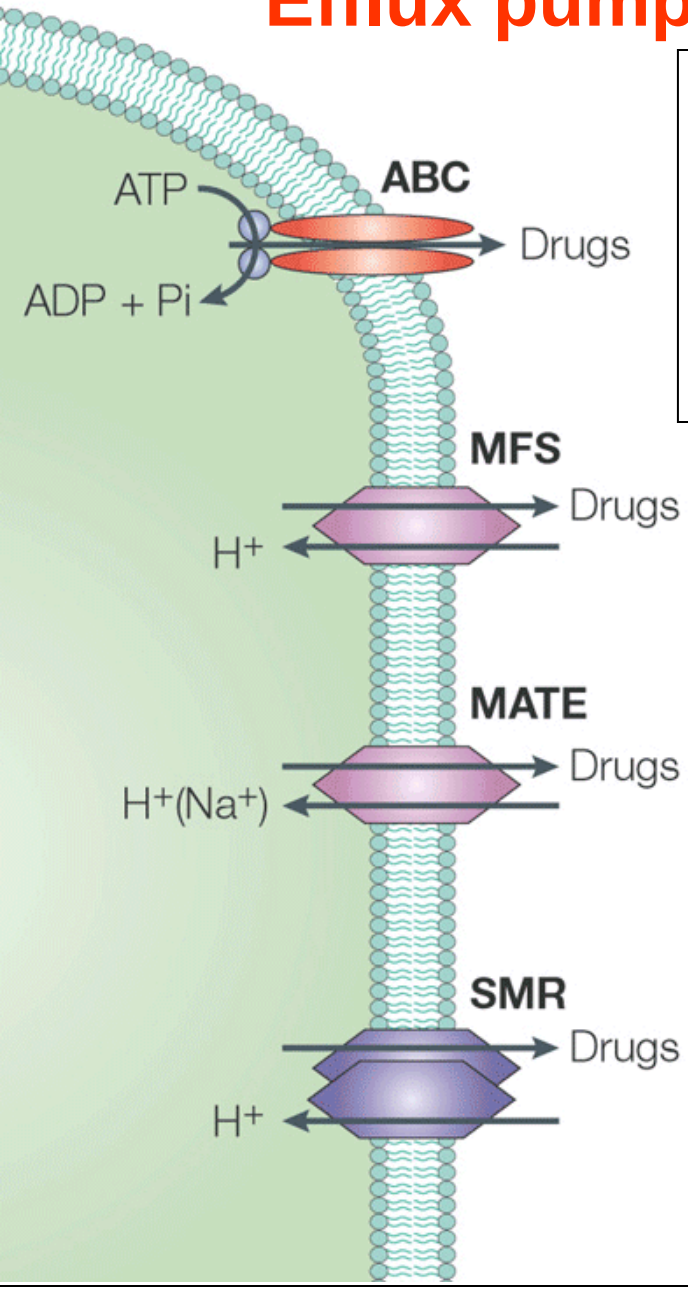
Efflux pumps in Gram-positive bacteria



Primary transporters
« **A**TP-**B**inding **C**assette »

PatA/PatB
Marrer et al, AAC 2006; 50:685-93

Secondary transporters
(Proton motive force)

PmrA
Gill et al, AAC 1999; 43:187-9

Terry et al., Nature Reviews Microbiology 2005; 3: 566-572

Aim of the study

- To examine whether the expression of FQ efflux pumps is inducible upon exposure to sub-MIC concentrations of FQ
  - using strains with variable basal level of expression of these transporters
  - comparing FQ that are substrates or not of efflux transporters

Strains used in this study

ATCC 49619

reference strain,
fully susceptible

SP334

SP335

resistant mutants
selected in vitro
by 13 days exposure
to CIP sub-MIC
concentrations
of ATCC49619
or of a clinical isolate

Avrain et al. JAC 2007; 60, 965-72

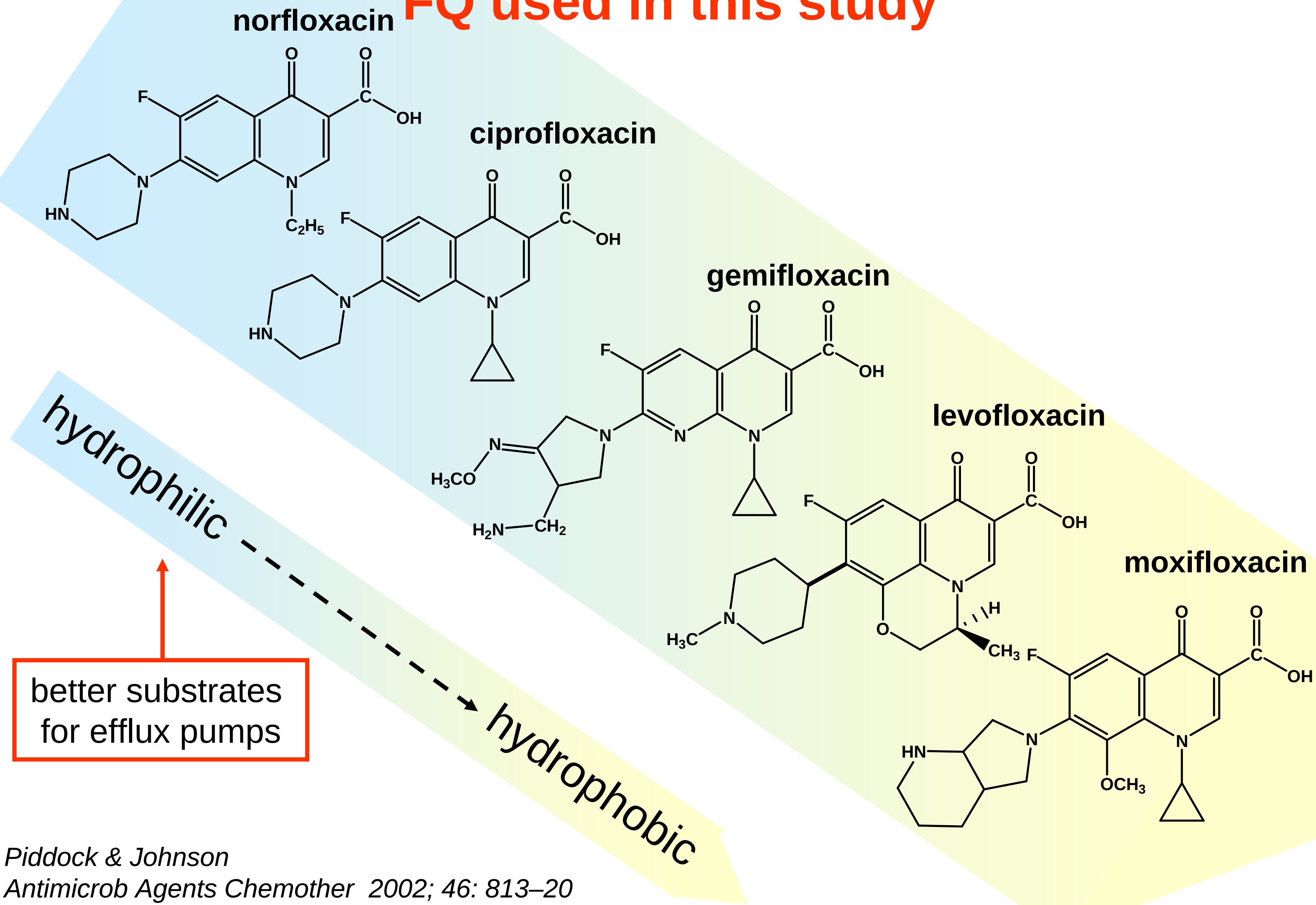
SP295

SP13

2 clinical isolates
with low and high
levels of FQ resistance

Helsinki railway station

FQ used in this study



Piddock & Johnson
Antimicrob Agents Chemother 2002; 46: 813–20

MIC of fluoroquinones

- or + Reserpine as efflux pump inhibitor

FQ / strains	NOR		CIP		LVX		MXF		GMF	
	-R	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**
49619	4		1		1		0.25		0.125	
SP334	**32**		**4**		2		0.5		0.25	
SP335	**64**		**32**		**4**		0.5		**0.5**	
SP295	**16**		2		1		0.125		0.063	
SP13	**64**		**16**		2		0.25		0.25	

- NOR and CIP show elevated MICs in the 4 resistant strains
- LVX MIC is close to the EUCAST Bkpt (± 1 dil) in all strains
- MXF and GMF consistantly show low MICs

MIC of fluoroquinones

- or + Reserpine as efflux pump inhibitor

FQ / strains	NOR		CIP		LVX		MXF		GMF	
	-R	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**
49619	4	2	1	0.5	1	0.5	0.25	0.25	0.125	0.125
SP334	**32**	4	**4**	1	2	1	0.5	0.5	0.25	0.125
SP335	**64**	**8**	**32**	**2**	**4**	**2**	0.5	0.25	**0.5**	0.125
SP295	**16**	2	2	0.5	1	1	0.125	0.125	0.063	0.032
SP13	**64**	**16**	**16**	**2**	2	1	0.25	0.25	0.25	0.125

- reserpine reverses resistance but only partially in 2 strains
- MFX not affected; LVX and GMF poorly affected

- efflux contributes to resistance in the 4 strains
- other mechanisms also present in 2 strains

Target mutations ?

FQ / strains	NOR		CIP		LVX		MXF		GMF	
	-R	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**	**-R**	**+R**
49619	4	2	1	0.5	1	0.5	0.25	0.25	0.125	0.125
SP334	**32**	4	**4**	1	2	1	0.5	0.5	0.25	0.125
SP335	**64**	**8**	**32**	**2**	**4**		**ParE (Ile460Val)**		**0.5**	0.125
SP295	**16**	2	2	0.5	1	1	0.125	0.125	0.063	0.032
SP13	**64**	**16**	**16**		**ParE (Ile460Val); ParC (Ser79Phe;Lys137Asn)**					

- reserpine reverses resistance but only partially in 2 strains

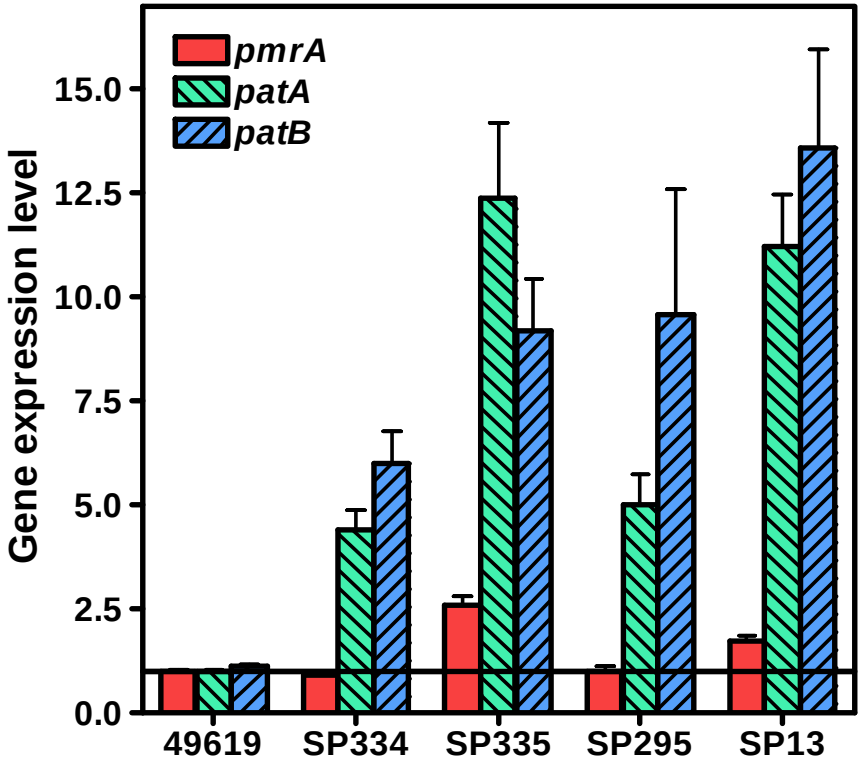
- efflux contributes to resistance in the 4 strains
- target mutations evidenced in 2 strains

Expression of *pmrA*, *patA*, *patB* by Real-Time PCR

- bacteria grown overnight on MH agar plates
- resuspended in THY broth (OD 0.2-0.4)
  - or + fluoroquinolone at ½ MIC for up to 4 h
- RNA extraction and reverse transcription *(Avrain et al. JAC 2007; 60, 965-72)*
- real-time PCR (SYBRGreen Supermix), with *rpoD* and *proC* as housekeeping genes

Basal expression level

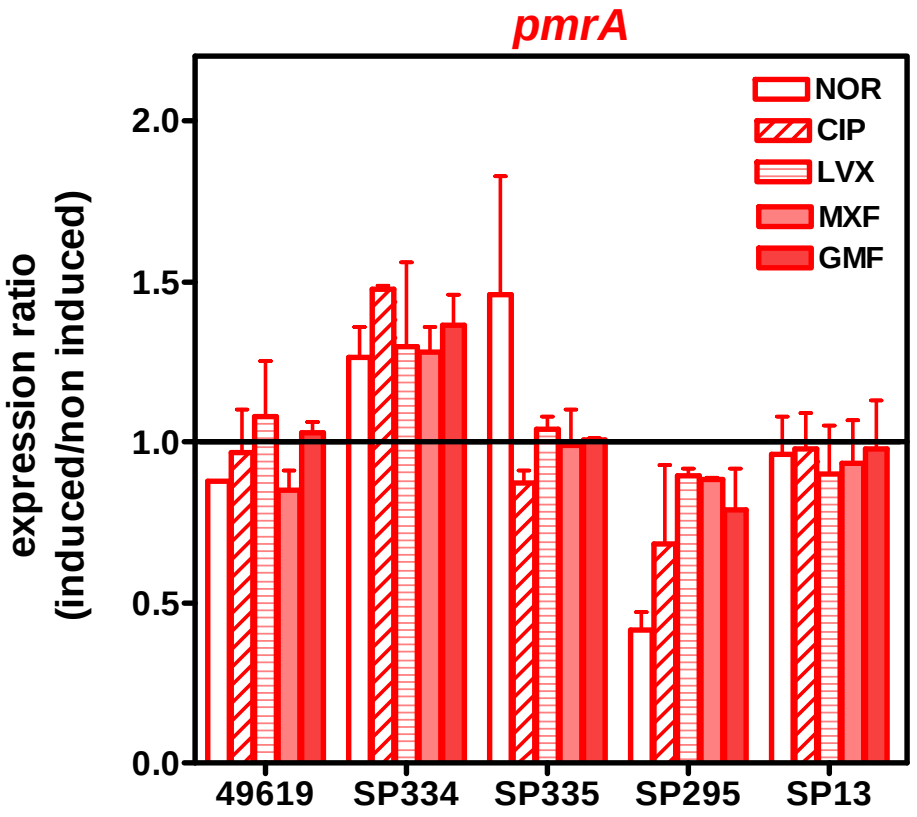
Culture at OD ~ 0.5-0.6



- all strains overexpress *patA*/*patB* to variable level
- SP335 and SP13 show a low level of *pmrA* overexpression

Induced expression level

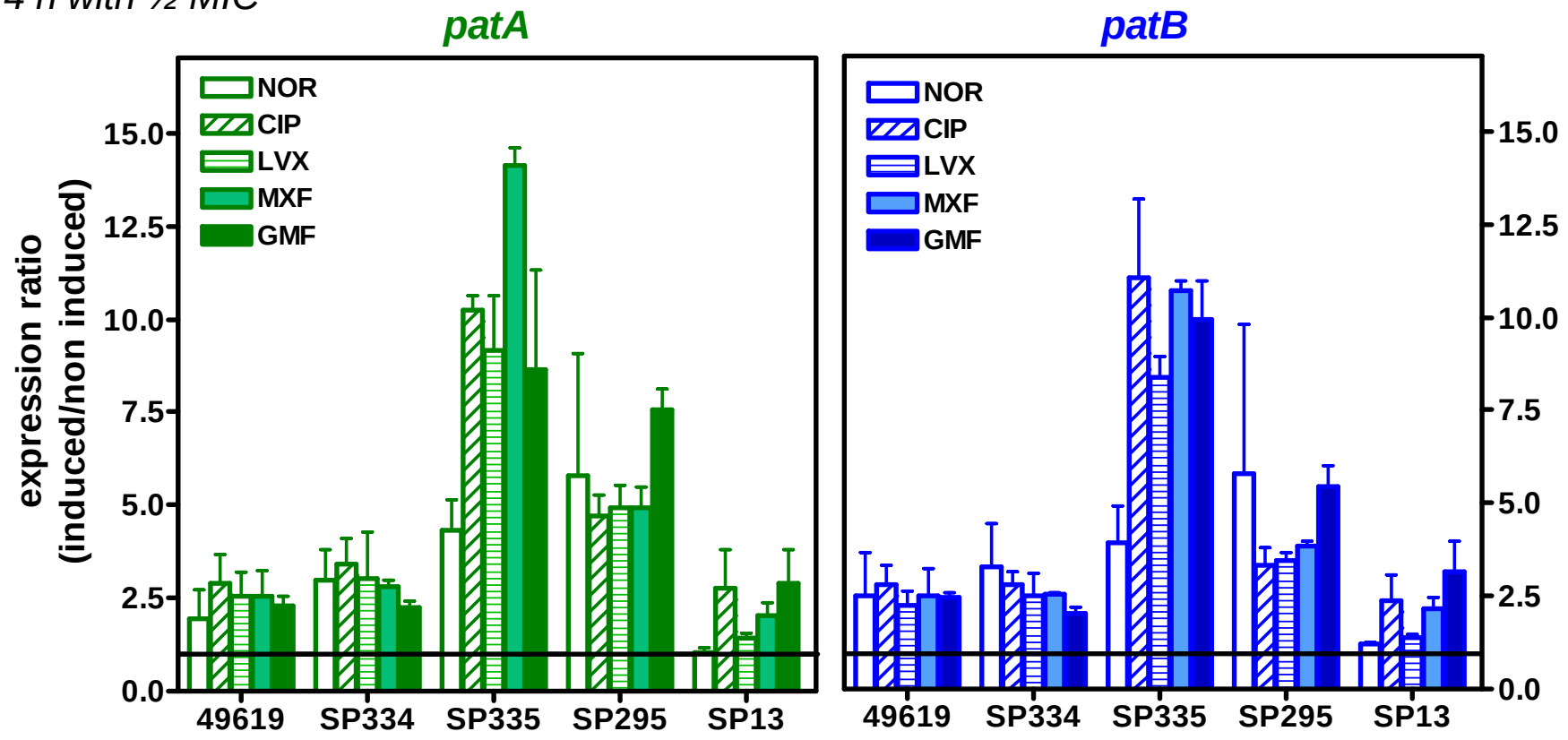
4 h with ½ MIC



- no induction of *pmrA* wathever the FQ used as inducer

Induced expression level

4 h with ½ MIC



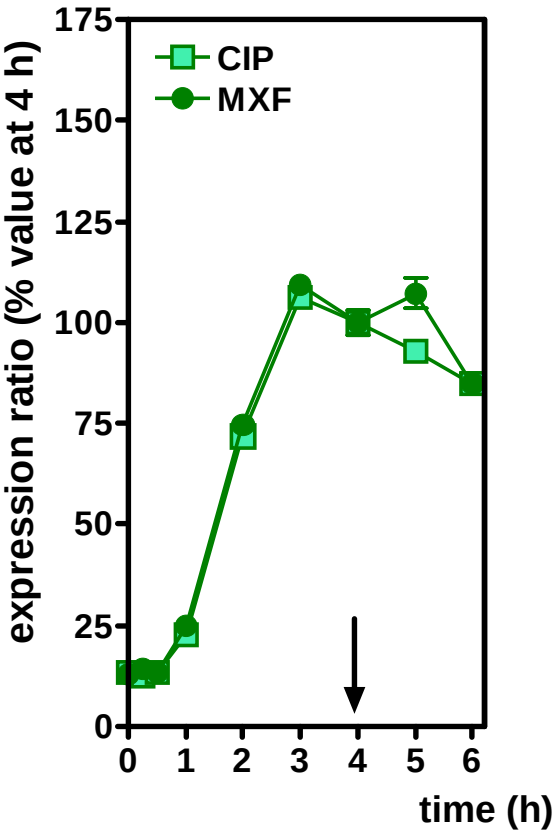
- induction of *patA*/*patB*
  - in all strains but to highly variable levels
  - by all FQ, whether substrates or not

Kinetics of induction & reversibility

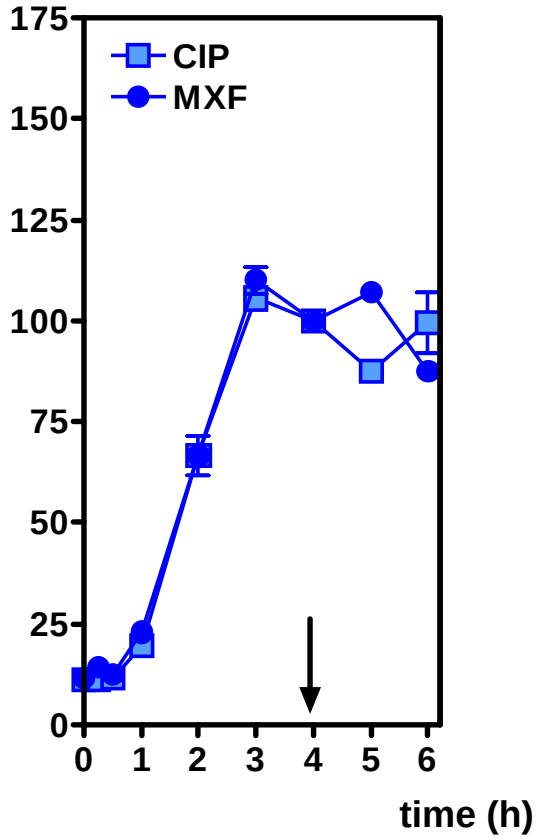
Up to 6 h with ½ MIC

ATCC49619 (low basal level)

patA



patB

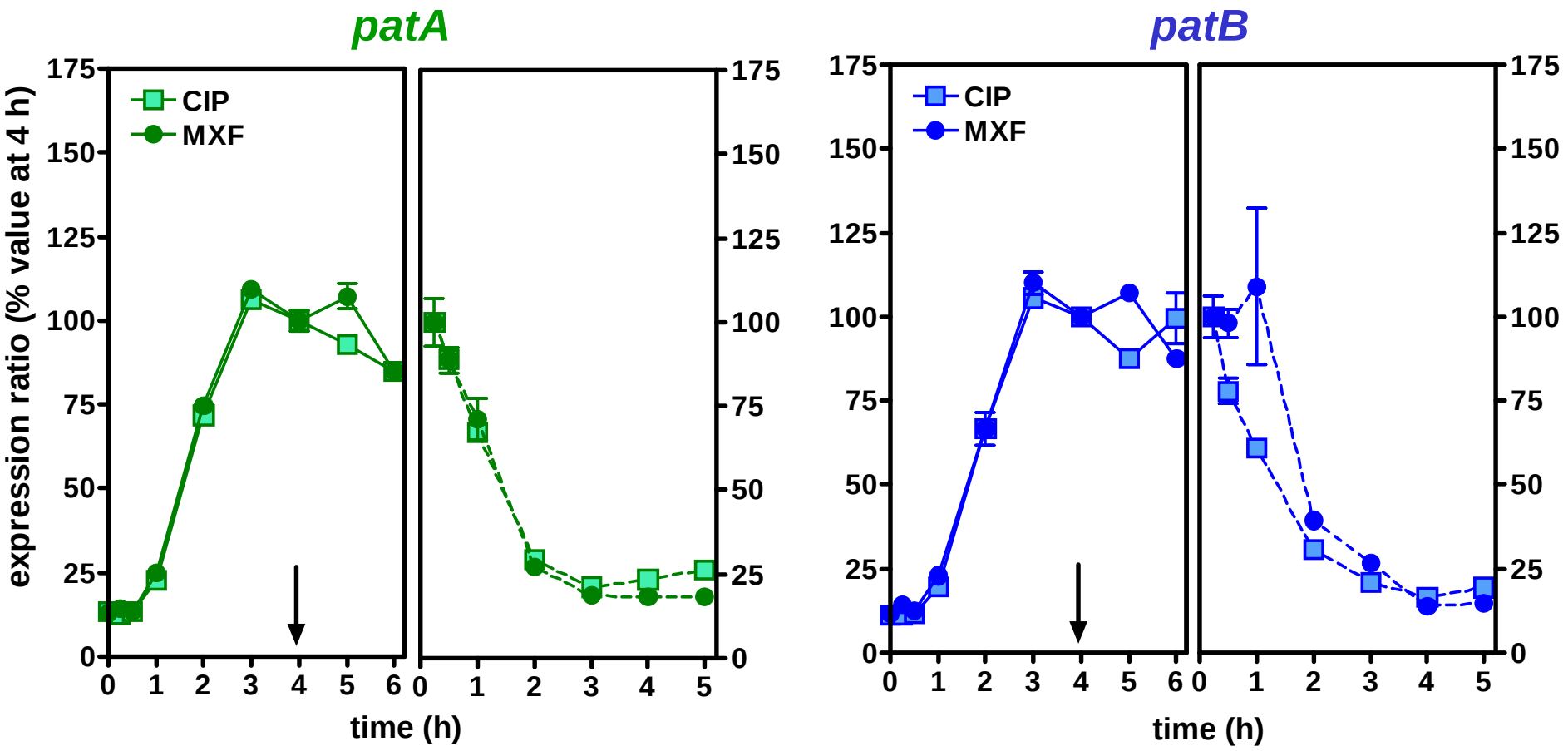


- lag phase ~ 30 min
- max at 4 h

Kinetics of induction & reversibility

4 h with ½ MIC; up to 5 h without FQ

ATCC49619 (low basal level)

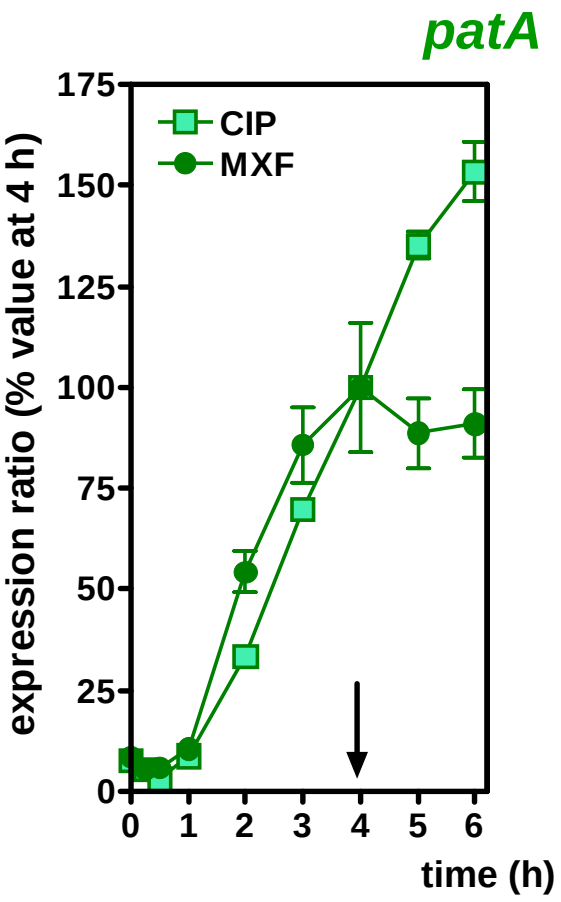


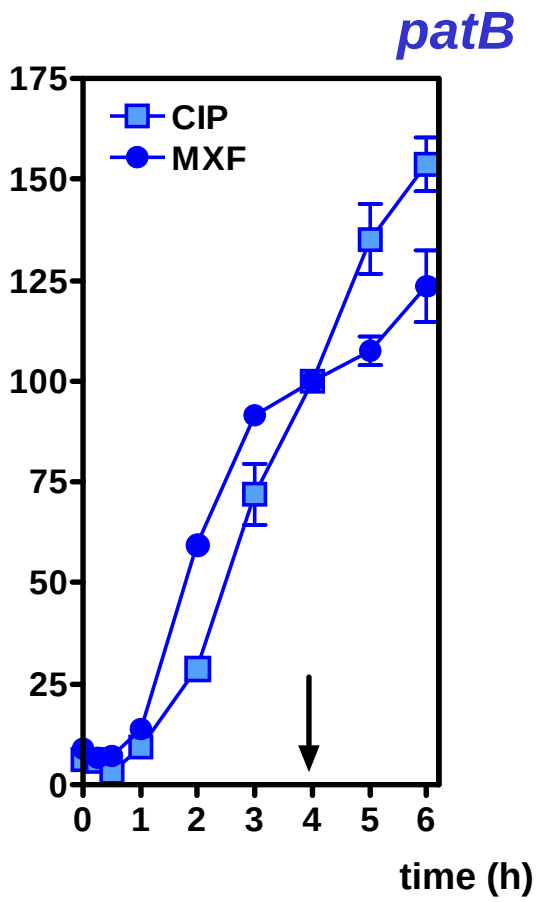
- fully reversible in 4 h

Kinetics of induction & reversibility

Up to 6 h with ½ MIC

SP335 (high basal level, CIP-selected)



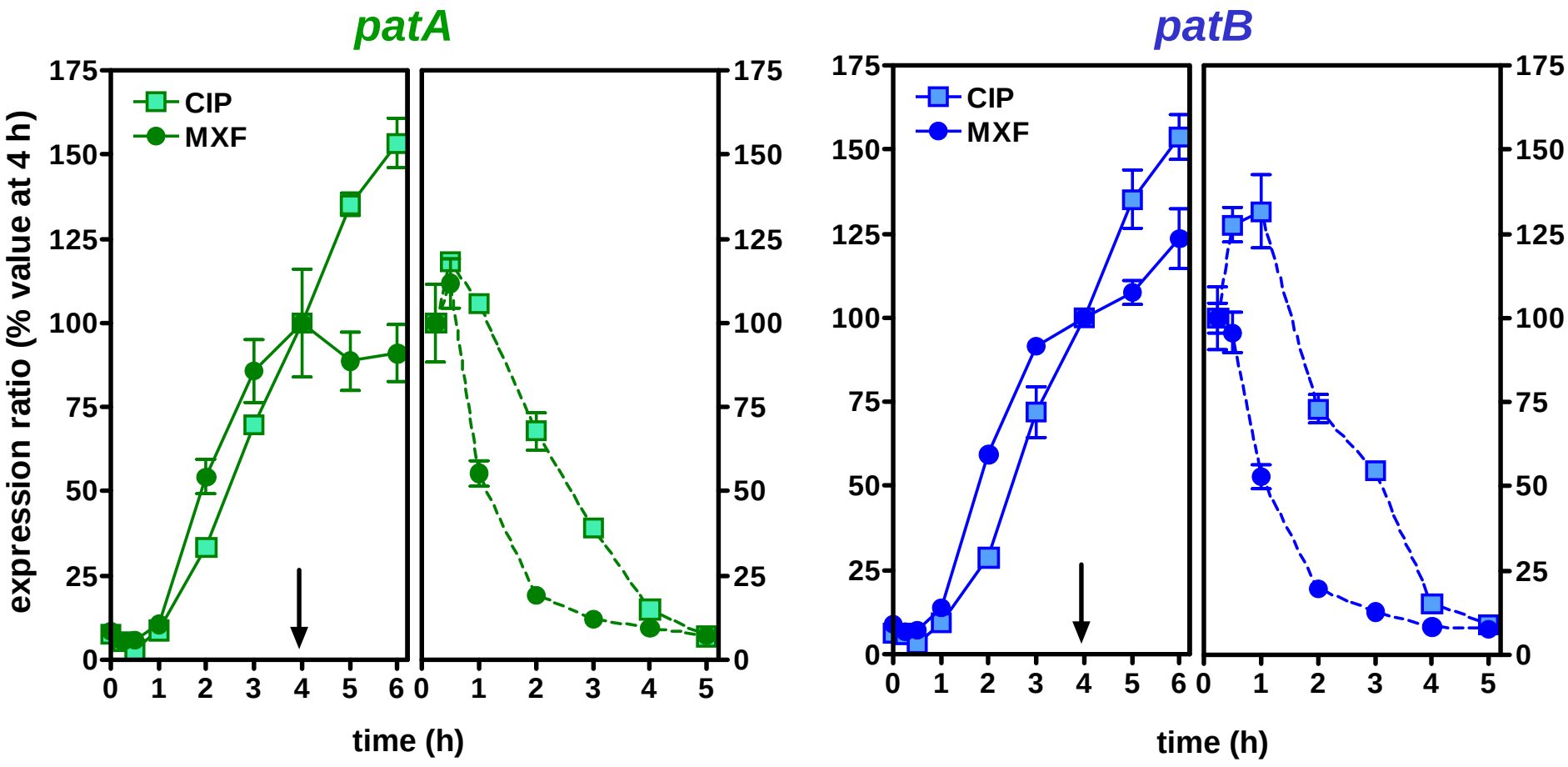


- lag phase ~ 60 min

Kinetics of induction & reversibility

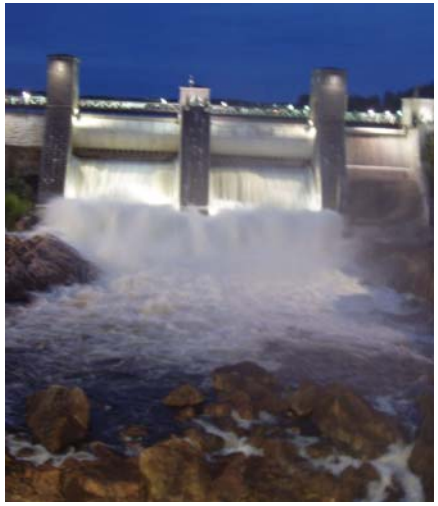
4 h with ½ MIC; up to 5 h without FQ

SP335 (high basal level; CIP selected)



- fully reversible in 4 h

Conclusions



Imatra rapids, Finland

Efflux and resistance

- NOR and CIP highly affected by efflux
  (+ Reserpine ↘ MIC ≥ 2 dilutions)
- LVX and GMF: modestly affected by efflux
  (+ Reserpine ↘ MIC 1 dilution)
- MXF not affected

Conclusions

Induction of efflux mechanisms

- PmrA is not inducible by FQ
- PatA/PatB are induced
  - by all tested FQ (substrates or not)
  - to levels depending
    - ++ on the strain and of its basal expression level
    - - - on the inducer
  - on a quick and fully reversible manner

exposure to sub-MIC concentrations of FQ may trigger induction of PatA/PatB, causing loss of susceptibility