Biofilm og antibiotikaresistens

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Biofilm arbejdsgruppen under DSKM
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- Innate
  - Diffusion
  - Limited growth
    - Oxygen limitation, metabolism
    - Stationary phase and stress response
  - Persisters
  - Biofilm-specific

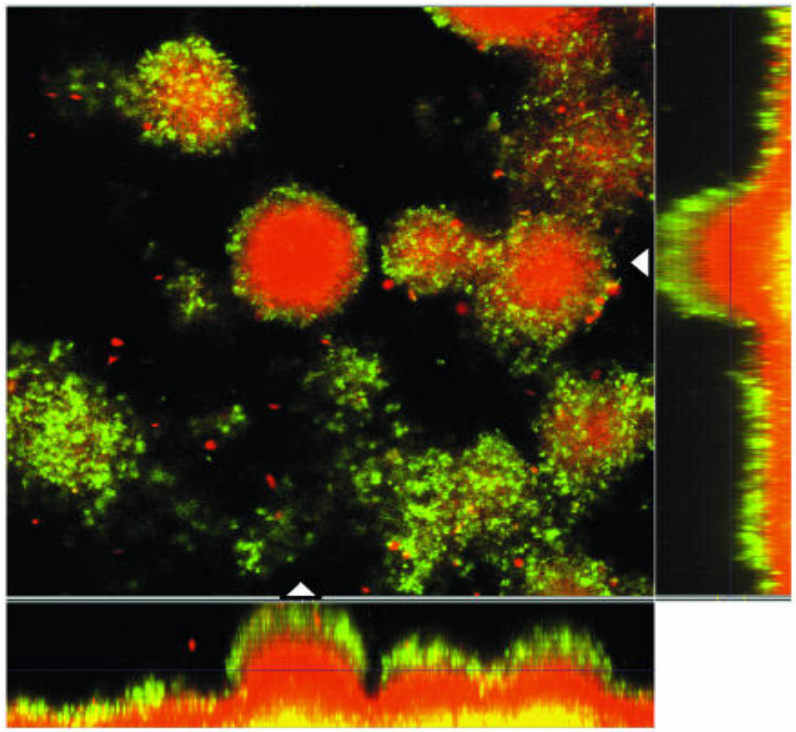
- Antibiotic-induced (planktonically-related)
  - beta-lactamase
  - Efflux pumps

Genetic variation in biofilms (hypermutability and increased gene transfer)

(Anderson and O´Toole, Current Topics in Microbiology and Immunology, 2008)

Antibiotic induced-beta-lactamase

Expression of AmpC β-lactamase in PAO1 biofilm (weak inducer 125 X MIC)



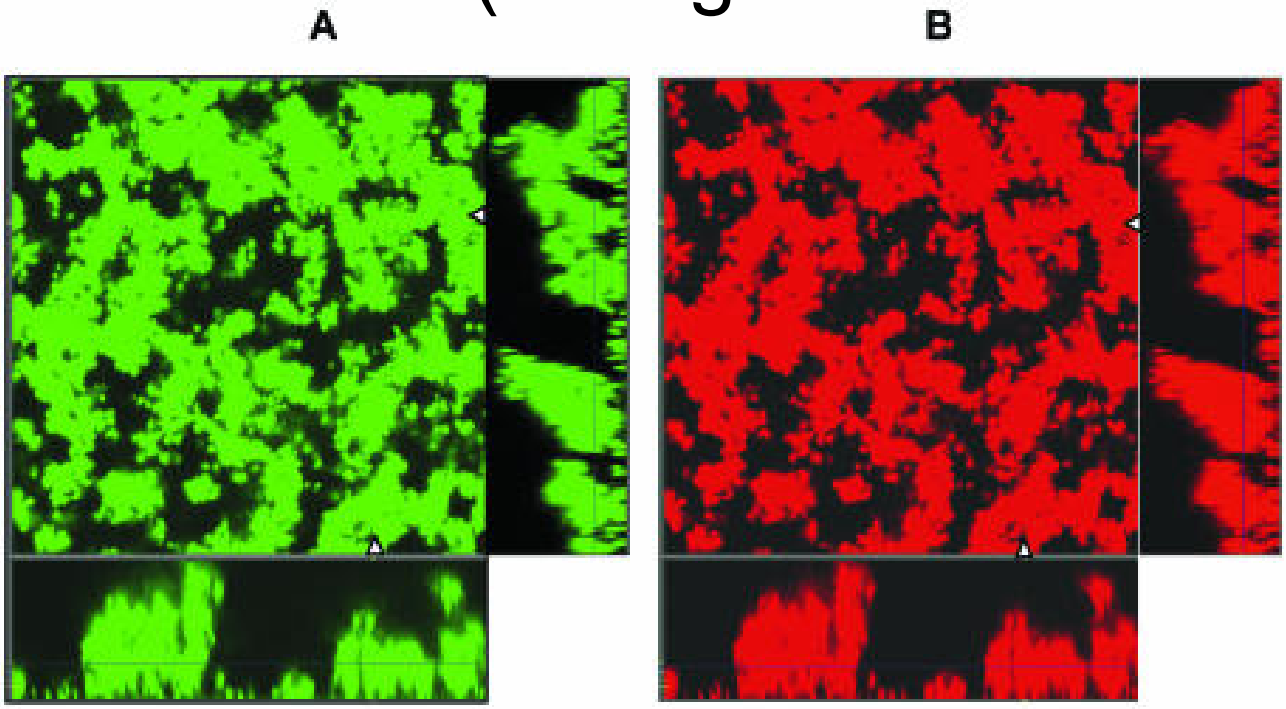
Cells without induction of beta-lactamase

Cells with induction of beta-lactamase

PAO1 with P*ampC-gfp* (ASV): 6 days old biofilm exposed to 100 µg/ml ceftazidime for 4 h. Detection level of the monitor: 10 µg/ml ceftazidime

(Bagge, Antimicrob Agents Chemother. 2004;48: 1168–74)

Expression of AmpC β-lactamase in PAO1 biofilm (strong inducer 2.5 X MIC)



Cells with induction of beta-lactamase

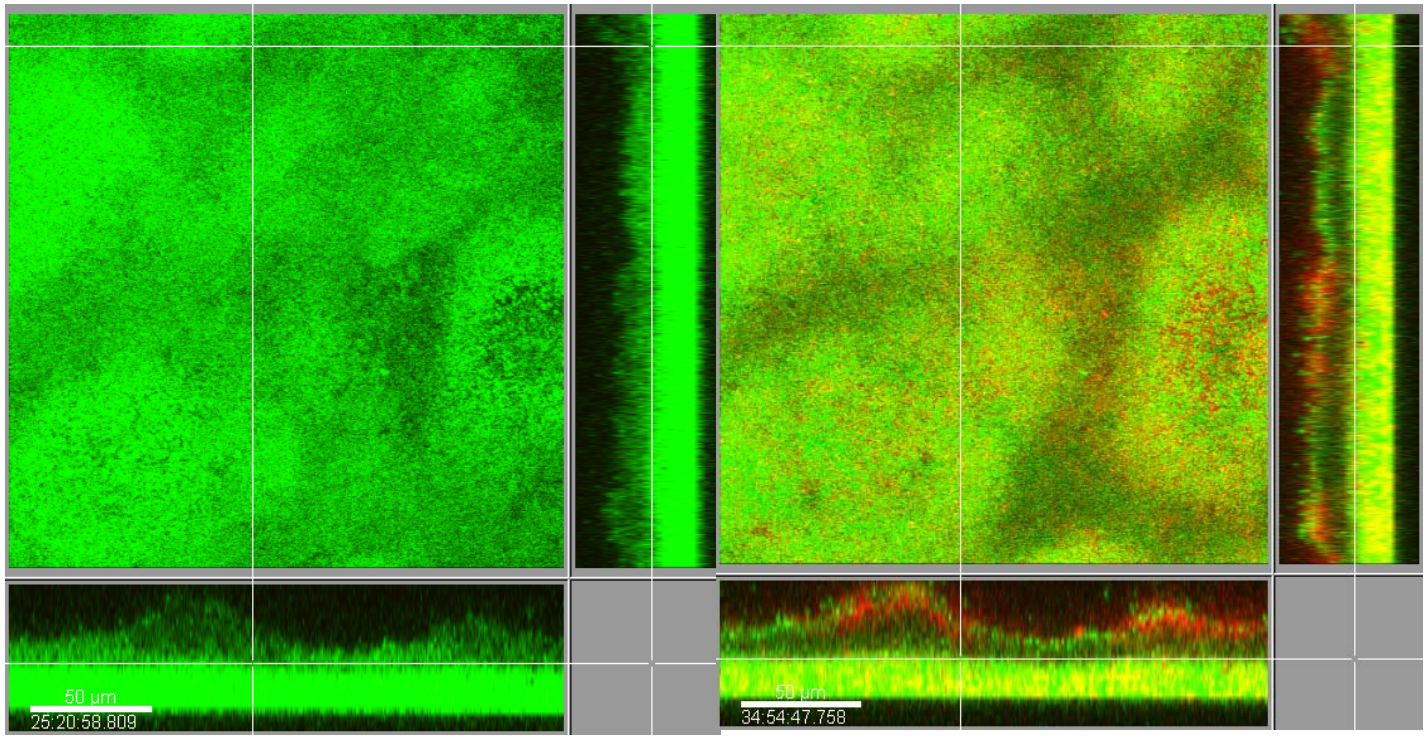
Cells without induction of beta-lactamase

PAO1 with P*ampC-gfp* (ASV): 6 days old biofilm exposed to 10 µg/ml imipenem (2.5x MIC) for 4 hours (A) and uninduced biofilm (B). (Detection level of the monitor ≥0.1 µg/ml imipenem)

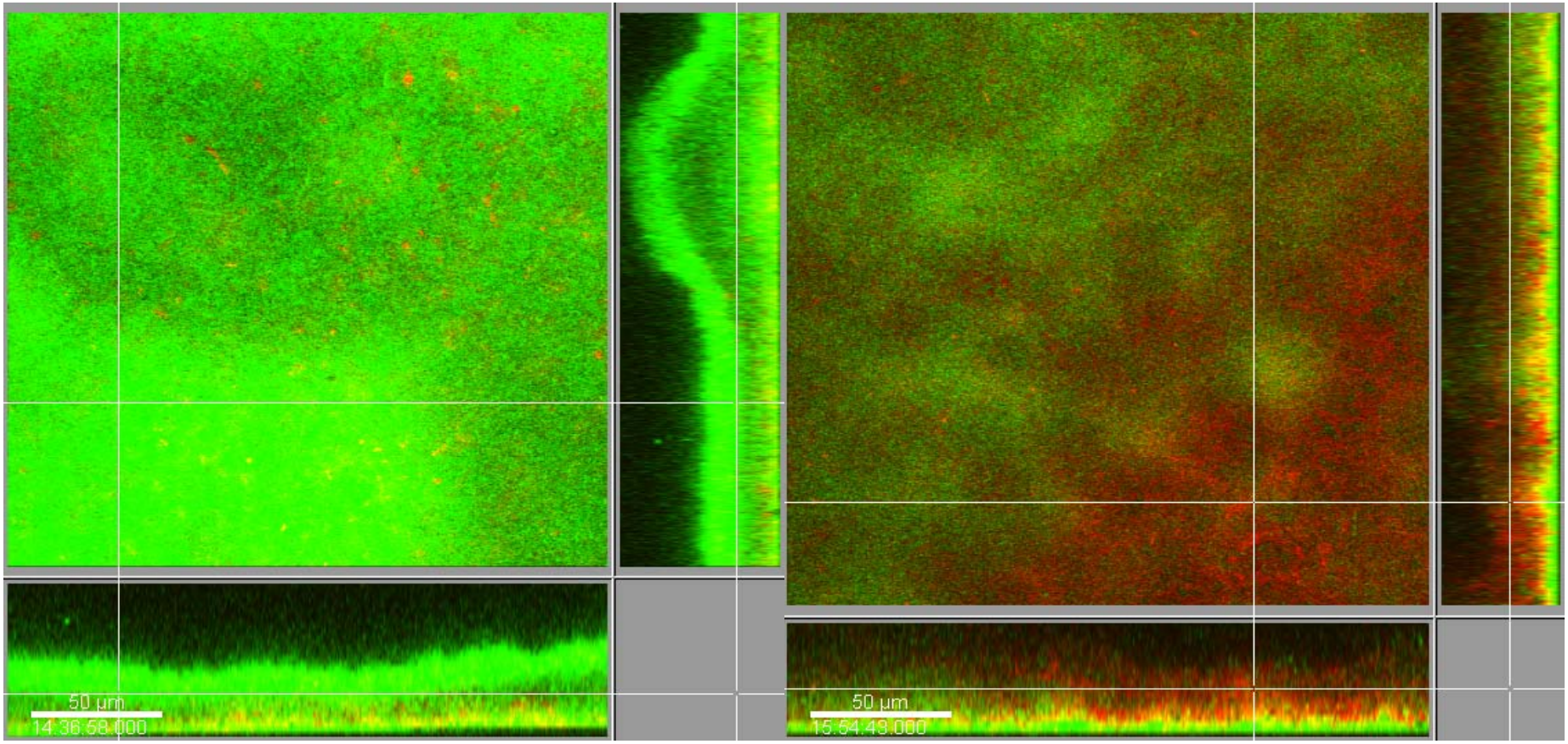
(Bagge, Antimicrob Agents Chemother. 2004;48: 1168–74)

Retarded difussion decreases the concentration of antibiotic entering the biofilm helping beta-lactamase to destroy the antibiotic.

- P.a. *ampD*$^{-}$ gfp 7 days old biofilm treated with 10 X MIC ceftazidime and a beta-lactamase inhibitor (aztreonam)



CF P.a. *ampD*- gfp 7 days old biofilm treated with 10 X MIC meropenem



P. aeruginosa live cells *P. aeruginosa* dead cells

(Ciofu, O, Bjarnsholt, T. 2007)

Genetic variation: hypermutability
Mutation frequencies in biofilm

	Mutation freq. (rifampicin)	8oxodG/10^6dG
PAO1 planktonic	$1xE^{-8}$	22.26
PAO1 biofilm	$2.5xE^{-7}$	57.75

(Mandsberg,L. 2004)

Increased mutability of *P. aeruginosa* in biofilms

- Biofilm cultures of *P. aeruginosa* displayed up to a 105-fold increase in mutability compared with planktonic cultures.

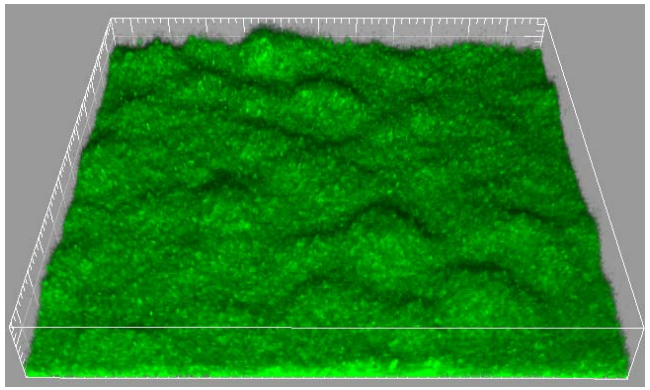
- Down-regulation of antioxidant enzymes in *P. aeruginosa* biofilms may enhance the rate of mutagenic events due to the accumulation of oxidative DNA damage

(Driffield, K. et al 2008 J. Antimicrobial Chemotherapy)

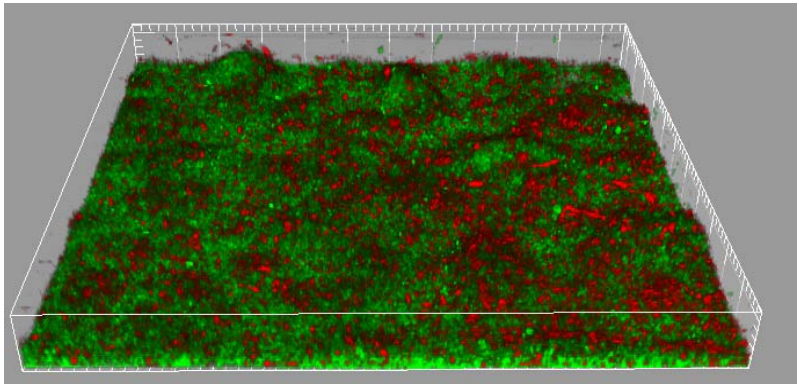
Hypermutable PAO1 mutant

Mutation frequency Rifampicin **$1{,}8 \times 10^{-8}$**

PAO1 day 4 biofilm

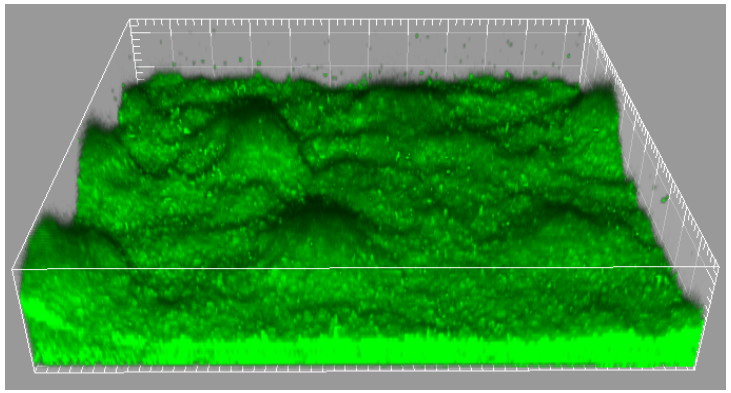


treated with piperacillin/tazobactam 20X MIC



5×10^{-7}

PAO1 *mutT*$^{-}$ day 4 biofilm



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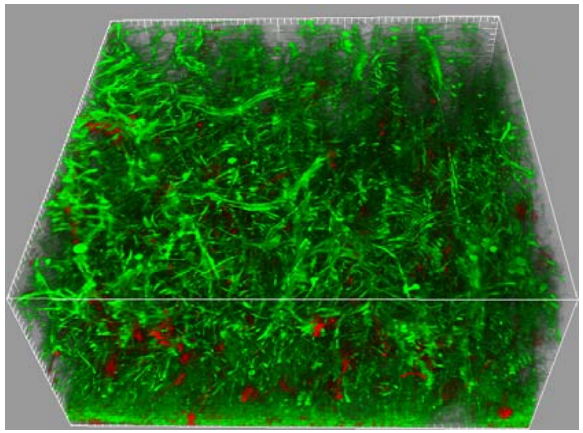

(Lotte Mandsberg, 2007)