

Biofilm architecture of Pseudomonas aeruginosa: a relevant key to improve the infectious diseases treatments

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Outline

- Problematics
- Biofilm: complex ecosystem
- Strategy
- Microscopy
- Prospects



RESISTANCE :

- Increasing threat for human and animal health, food safety and agriculture
- > Failure in infection prevention and treatment \rightarrow 25 000 deaths/year.
- Without effective antibiotics, some medical cares will be more unsafe

The WHO list :

> Priority 1: CRITICAL

Acinetobacter baumannii

Pseudomonas aeruginosa

Enterobacteriaceae

Priority 2: HIGH
 Enterococcus faecium
 Staphylococcus aureus
 Helicobacter pylori
 Campylobacter spp.
 Salmonellae
 Neisseria gonorrhoeae



Pseudomonas aeruginosa

- Gram negative and non-fermenting bacterium
- Resistant to a large range of antimicrobial agents
 - Amoxicilline
 - Cefotaxime
 - Kanamycine
 - Tigecycline



Pseudomonas aeruginosa

- Opportunistic and ubiquitous pathogen
- Major cause of nosocomial infections

Virulence factors : Cellular factors

Virulence factors : secreted factors

- Community of microorganisms fixed to a substrate
- Microorganisms live in an extracellular matrix
 - Proteins
 - Nucleic acids
 - Lipids
 - Exopolysaccharides (50-90% of the total organic matter)
- Physical barrier against host immune defenses



Scanning electron micrographs ΔPA3476Δrhl strain. Colonies were grown for 18H at 37°C on Biofilm broth medium.





Bacterial communication

- = Quorum sensing (QS)
- = Global regulatory mechanism
- Depends on the bacterial density
- Bacteria population is organised
- Works with autoinducer signals, acyl homoserin lactones (AHL) and quinolones

























Biofilm & Resistance

Physical barrier against host immune defenses and antibiotic attacks Extrapolymeric substances slow down the antibiotic penetration into the biofilm and/or inhibit the antibiotic effects

Several phenotypes into the community

Sub-population of persistant cells

The bacteria closeness into the biofilm make easier the horizontal genes transfer



Strategy

QS

Biofilm

Disruption of the biofilm architecture to improve the antibiotic penetration

Resistance

Strategy



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An Active Fraction from DalbergiaTrichocarpa Baker Disrupts the Formation and Maintenance of Biofilms in Pseudomonas Aeruginosa PAO1

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Activity assays of *Dalbergia trichocarpa* extracts from roots, barks and leaves. PAO1 WT, 18h, 37°C . Naringenin (Nar 4mM) was used as positive control and Naringine (Nin 4mM) as negative control.







P. aeruginosa strains visualized by electronic scanning and confocal laser scanning microscopy.

 \checkmark (A) PAO1 wild type,

 \checkmark (B) Δ wspF-cdrA-GFP,

✓ (C) Δrsm Z/Y

Images after 18 hours at 37°C on biofilm broth medium. Confocal laser microscopy: Zeiss LSM 710; staining with a LIVE/DEAD[™] BacLight[™] kit. 313µm X 313µm.









Colonies were grown for 18 hours at 37°C on Biofilm broth medium. The samples were dried with methanol bath and were coated with an alloy of gold and platinum. SEM: Jeol JSM-6100.

- \checkmark (A) PAO1 wild type,
- ✓ (B) ∆wspF-cdrA-GFP,
- ✓ (C) $\Delta rsm Z/Y$







A. Schematic representation of the CDC bioreactor.The biofilm grow on the intern face of the coupon schematised in the red square. B. A coupon carry the PAO1 biofilm. The biofilm has been grown in the CDC Bioreactor with TSB (100mg/L) medium during 24H and stained with a crystal violet solution (0,1%).



P. aeruginosa strains visualized by 3D Digital microscopy.

Images after 48 hours at 37°C on biofilm broth medium. The culture were stained with crystal violet for initial tests. Digital microscopy Hirox KH-8700.





P. aeruginosa strains visualized by 3D Digital microscopy.

Images after 36 hours at 37°C on biofilm broth medium. No pre-treatment.

Digital microscopy Hirox KH-8700.





















The measurement of the volume



The profile with the measurement of the extrema







Improve the HIROX technics: normalize the observations Improve the observations: correlate it with other analyses Multiply the observations: mutant strains, biofilm treated





Acknowledgments









