



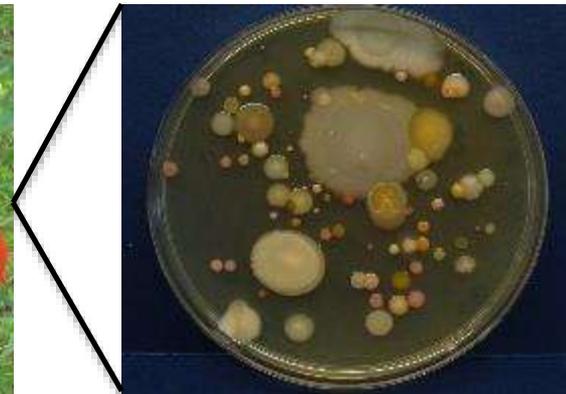
Lidija Senerovic

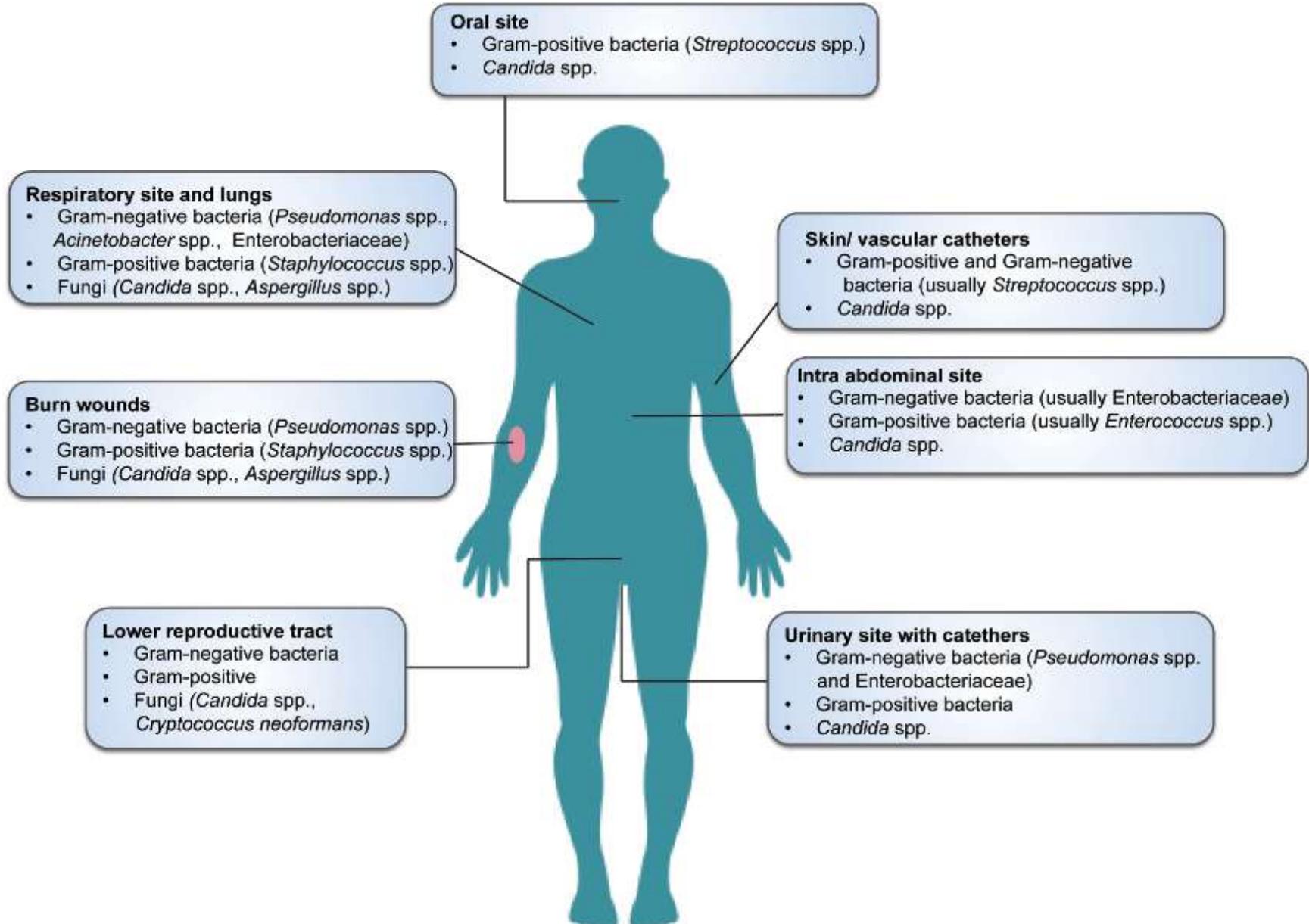
Novel therapeutic strategies for mixed infections: Controlling bacterial-fungal interaction, signaling and *quorum sensing*

Lidija Senerovic

IMGGE, UB

Instead of leading solitary lives, microorganisms occupy the same niches





TISSUE

Periodontitis



Otitis media



Common mixed species infections



Chronic wound

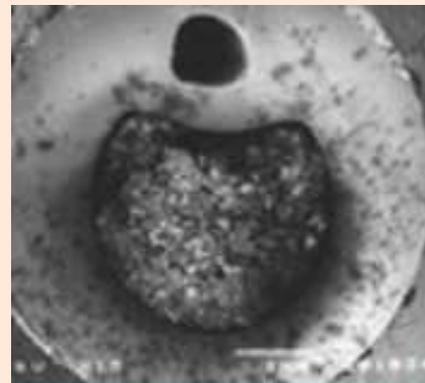


MEDICAL DEVICES

Prosthetic joint



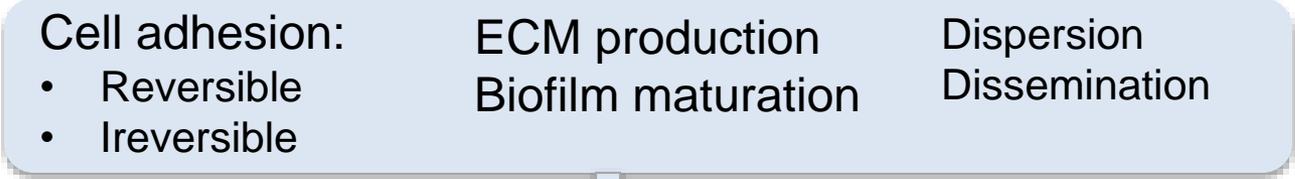
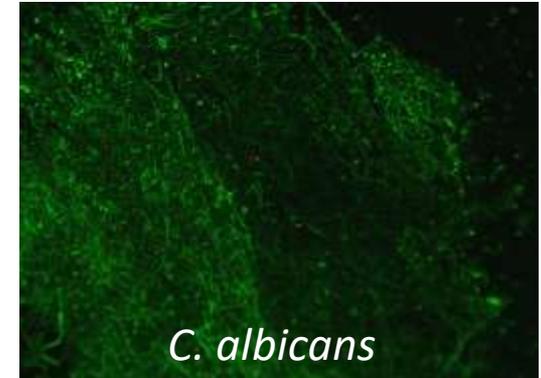
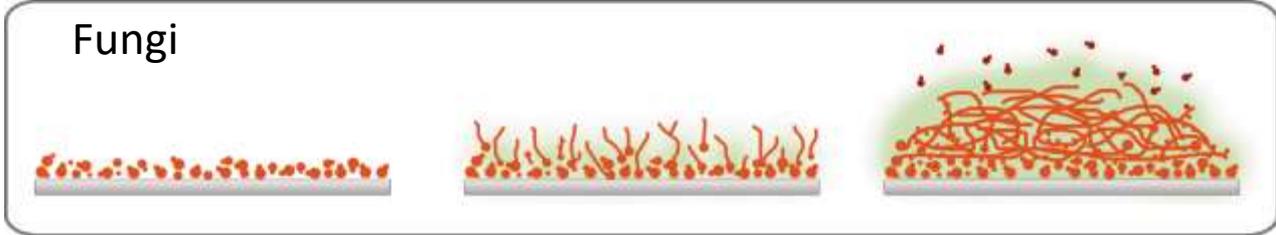
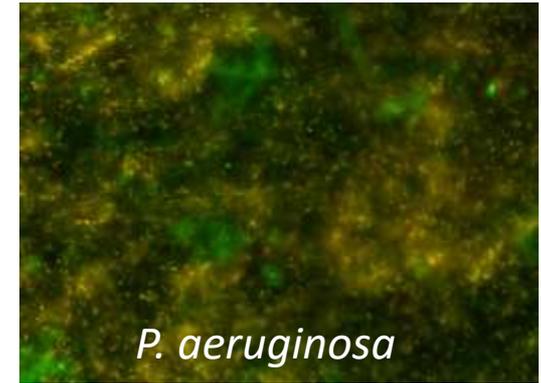
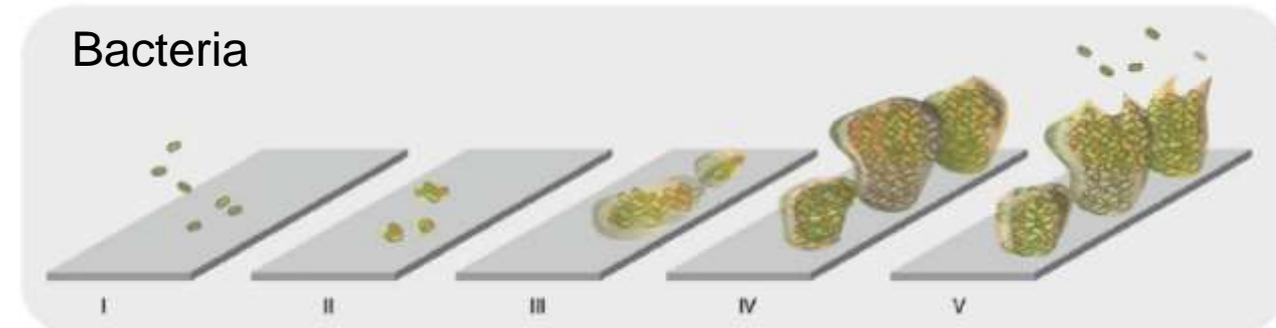
Central venous catheter



20% of very low weight infants develop mixed species infections

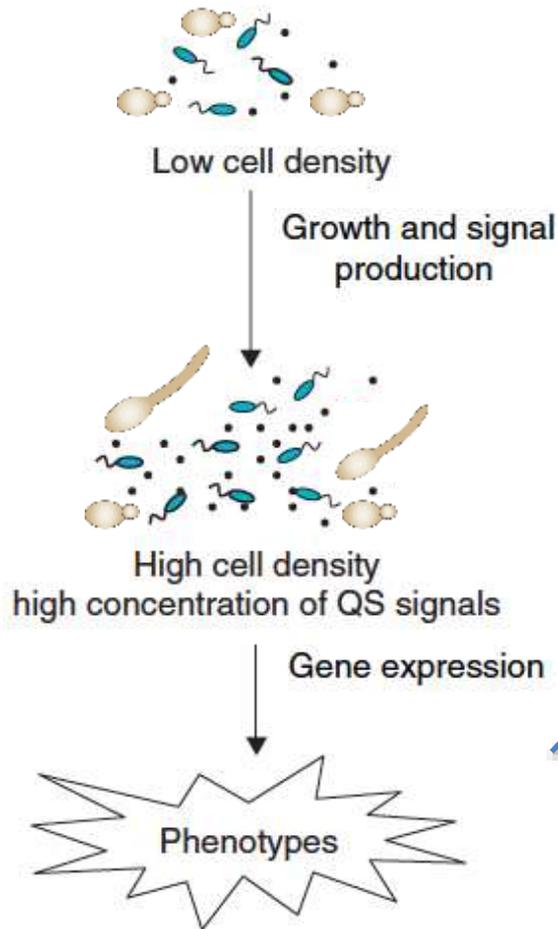
Biofilms

Layers of microorganisms embedded in a self produced extracellular matrix



Quorum sensing-regulated processes

Quorum sensing - cell–cell communication process that involves the production, detection, and response to extracellular signaling molecules called autoinducers



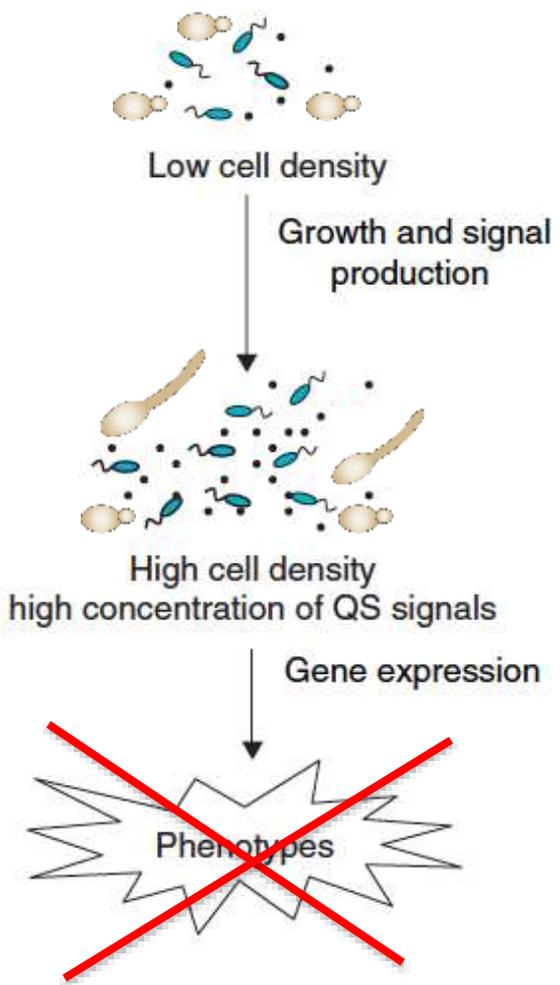
Bacteria virulence phenotypes:

- Virulence factor production (pyocyanin, elastase, proteases)
- Swarming and motility
- Antibiotic resistance
- **Biofilm formation**

Fungal phenotypes:

- Growth
- Morphogenesis
- Phylamentation
- **Biofilm formation**

Quorum sensing inhibition



QS inhibition:

- ❖ Repression of signal generation
- ❖ Degradation of QS signals
- ❖ Blockage of signal-receptor binding

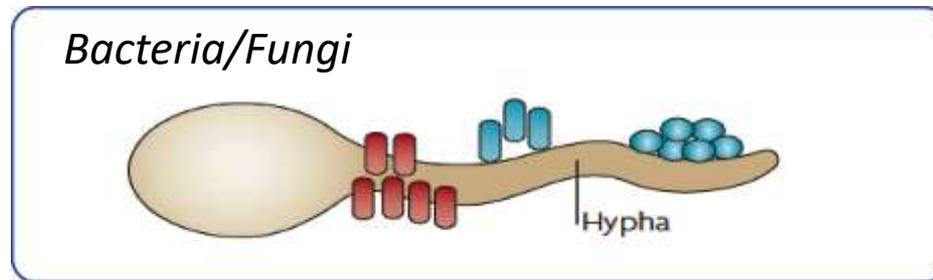
QS in bacteria

- Gram negative: AHLs (*P. aeruginosa* uses C4-AHL, 3oxo-C12-AHL, PQS)
- Gram positive: peptides

QS in fungi

- Alcohols: farnesol, tyrosol, alcohols of aromatic amino acids in *Candida albicans*
- Peptides: quorum sensing like-peptide 1 in *Cryptococcus neoformans*
- Oxilipins in *Aspergillus nidulans*

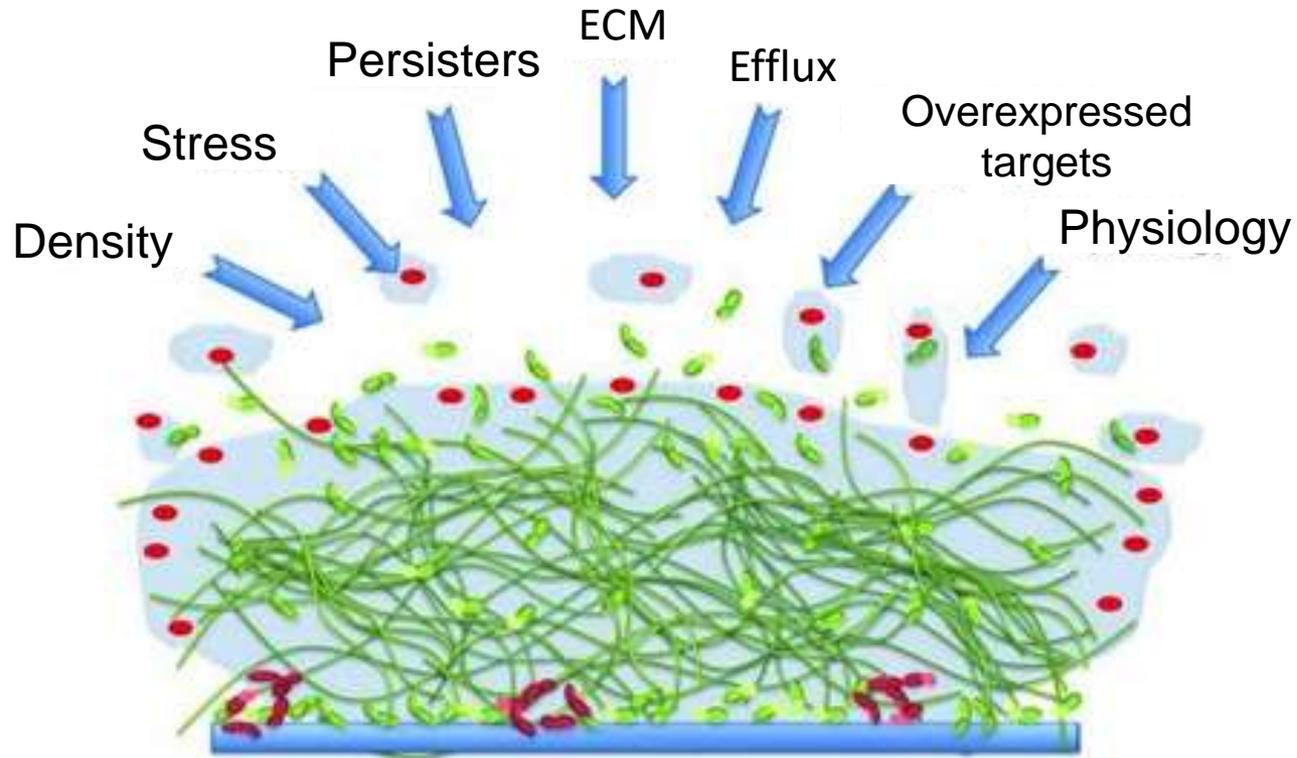
Mixed species polymicrobial biofilms



Interspecies interactions:

- Synergism (*C. albicans* and *Staphylococcus aureus*; *C. albicans* and *Escherichia coli*)
 - Antagonism (*C. albicans* and *P. aeruginosa*)
1. phenazines and phospholipase C kill *Candida* filaments
 2. 3-oxo-C12-AHL inhibits filamentation
 3. Farnesol and other secreted factors: inhibit QS in *P. aeruginosa*, inhibit swarming and stimulate biofilm formation, stimulate phenazines increasing virulence

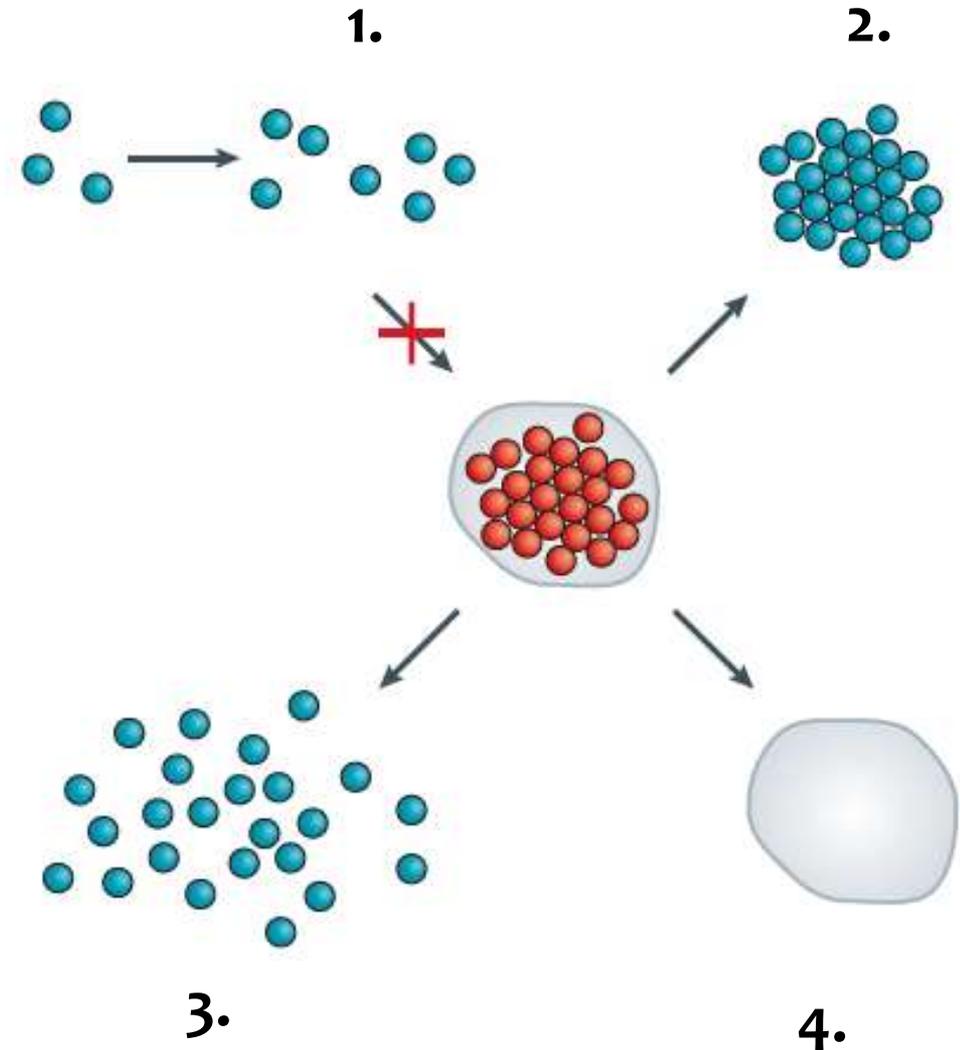
Biofilm infections are harder to treat because of increased resistance to antimicrobial therapy



All of these resistance mechanisms can be reverted by biofilms dispersal strategies

Biofilm treatment strategies

1. Prevention:
 - antibiotic/antifungal prophylaxis
 - targeting of surface molecules
 - targeting c-diGMP signalling
2. Weakening:
 - inhibition of efflux pumps
 - degradation of extracellular matrix
 - targeting QS
3. Disruption:
 - mechanical
 - enzymatic
 - QS inhibition
4. Killing:
 - one or more antimicrobials
 - targeting of the cellular membrane



In the search for novel antibiofilm agents

1. Bacteria from different niches:

- soil (mountains, caves, soda lakes)
- ethno-medicinal plants
- strains associated with animal species



2. Plant extracts:

- Organic solvent extracts
- Essential oils
- Supercritical CO₂ extraction



Citrus essential oils

Pompia (*Citrus x monstrosa*)



Grapefruit (*Citrus x paradisi*)

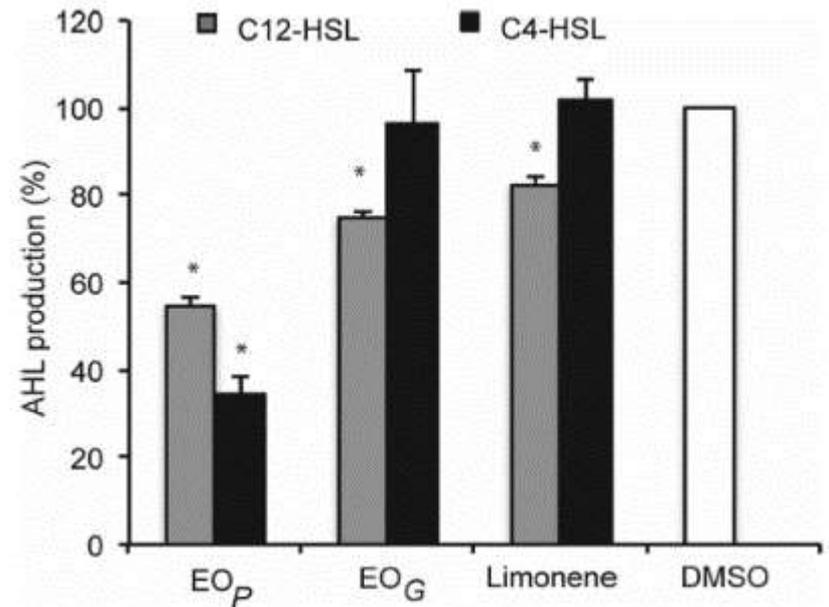


Pathogen	EO _P	MIC (mg L ⁻¹) ^a	
		EO _G	Limonene
<i>Pseudomonas aeruginosa</i>	>500	>500	>500
<i>Candida albicans</i>	50	50	100
<i>Candida parapsilosis</i>	100	50	100
<i>Aspergillus fumigatus</i>	100	100	>250
<i>Aspergillus terreus</i>	250	250	>250
<i>Scedosporium apiospermum</i>	100	100	>250

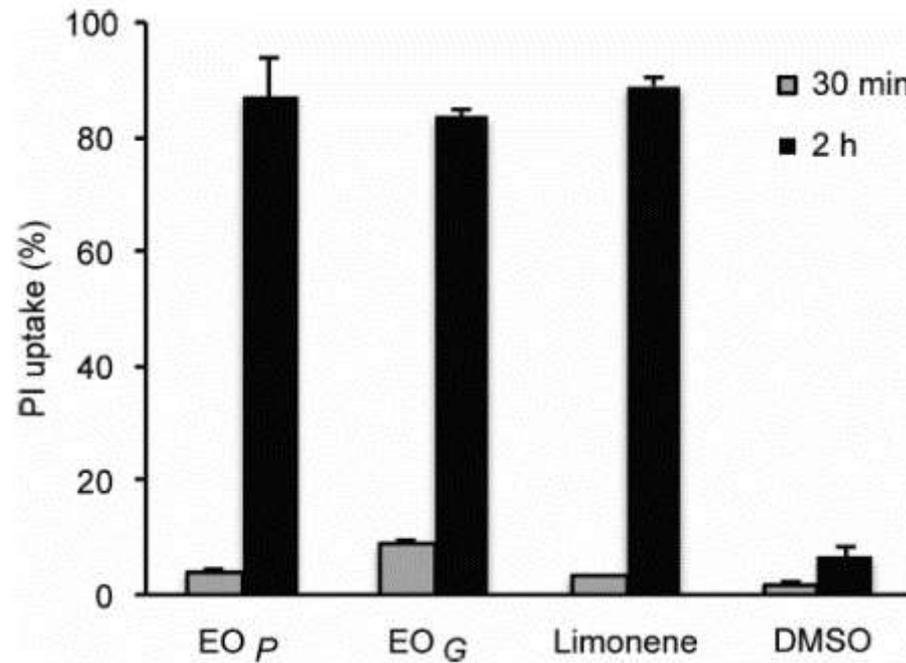
QS inhibition in *Pseudomonas aeruginosa*



Chromobacterium violaceum CV026

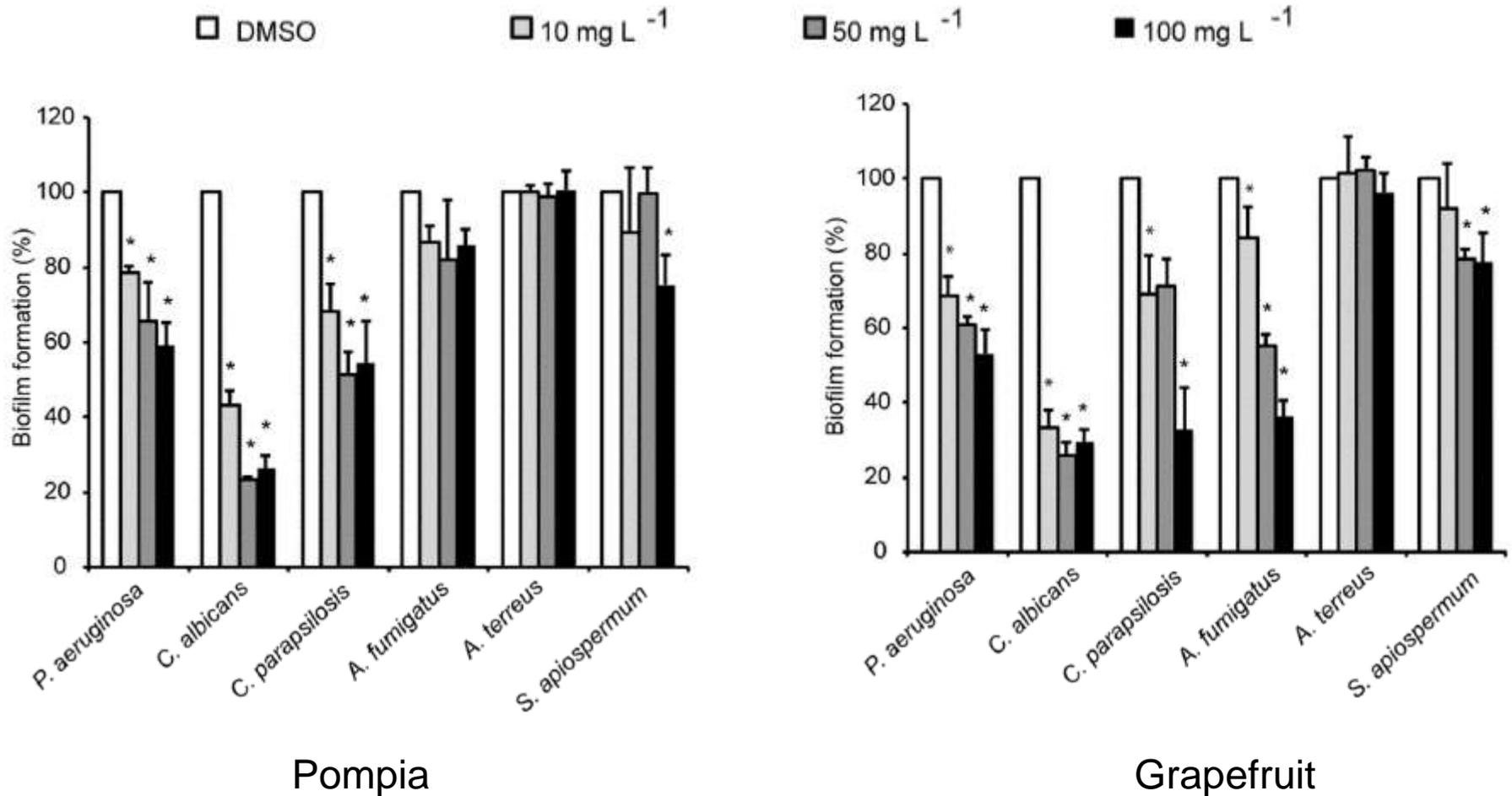


Membrane damage assay

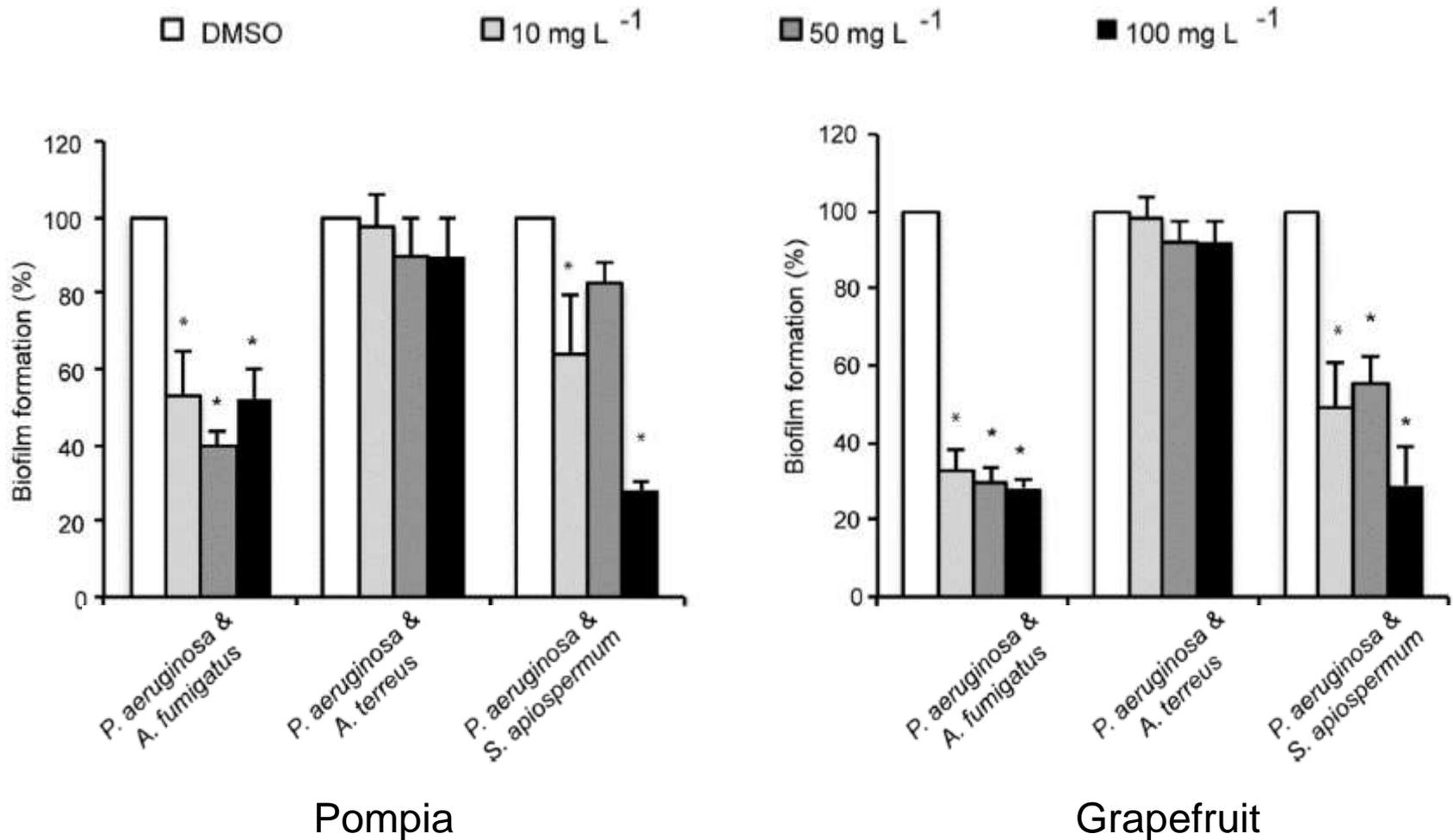


Candida albicans

Inhibition of monomicrobial biofilms

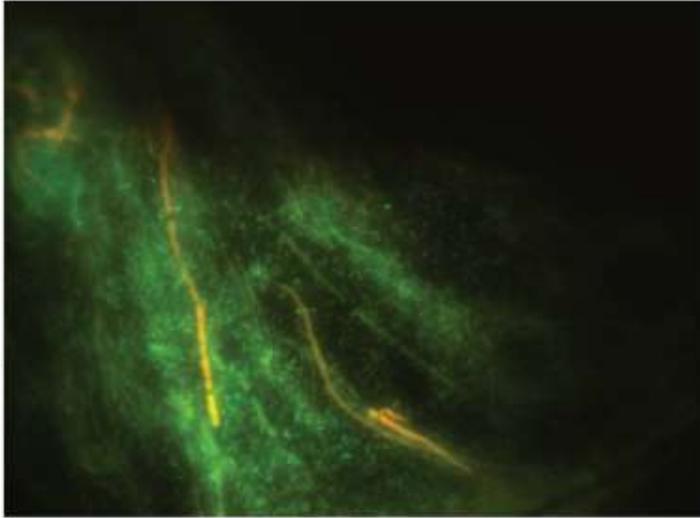


Inhibition of mixed species biofilms

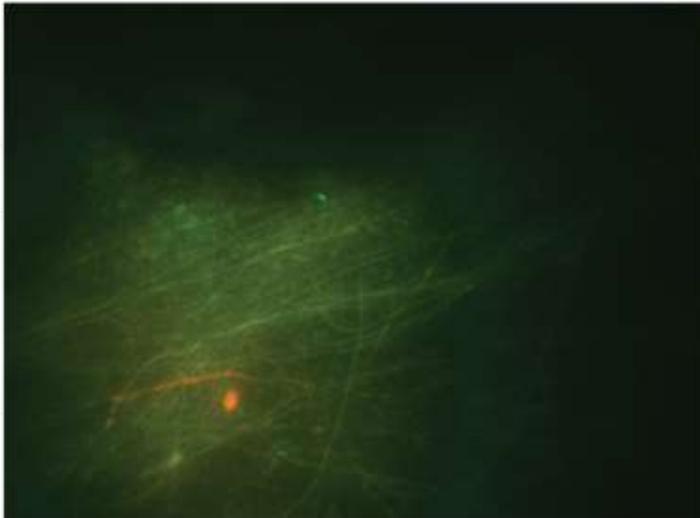


Biofilms+DMSO

P. aeruginosa/A. fumigatus



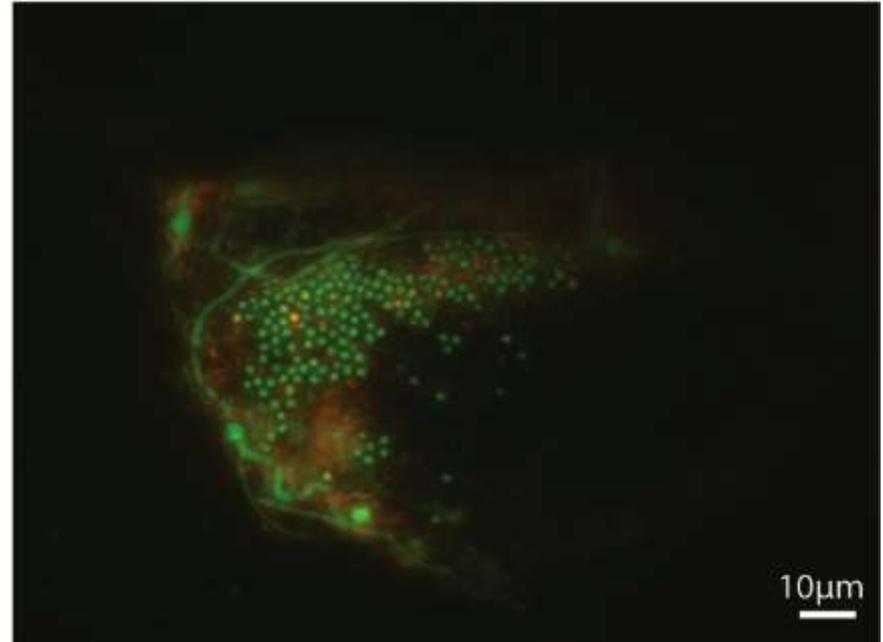
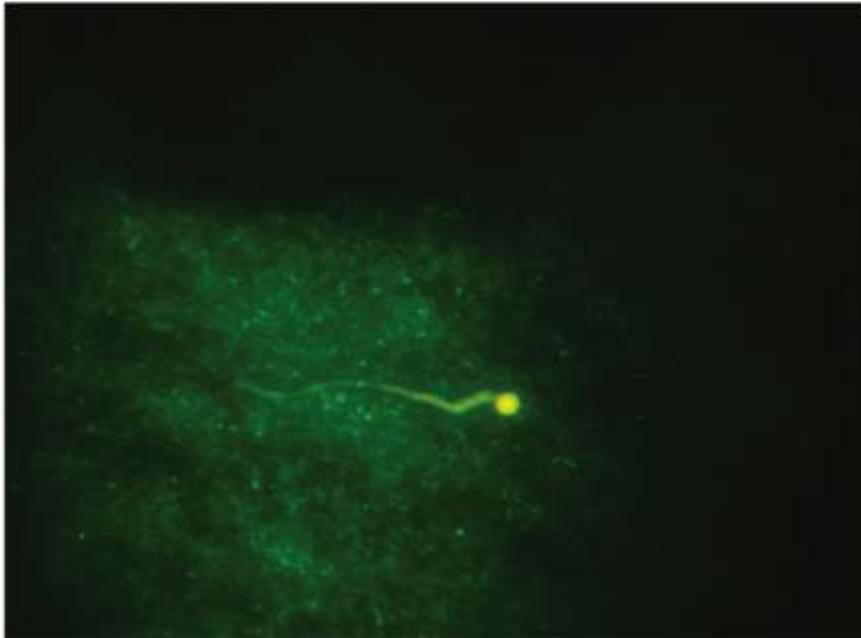
P. aeruginosa/S. apiospermum



Biofilms+DMSO

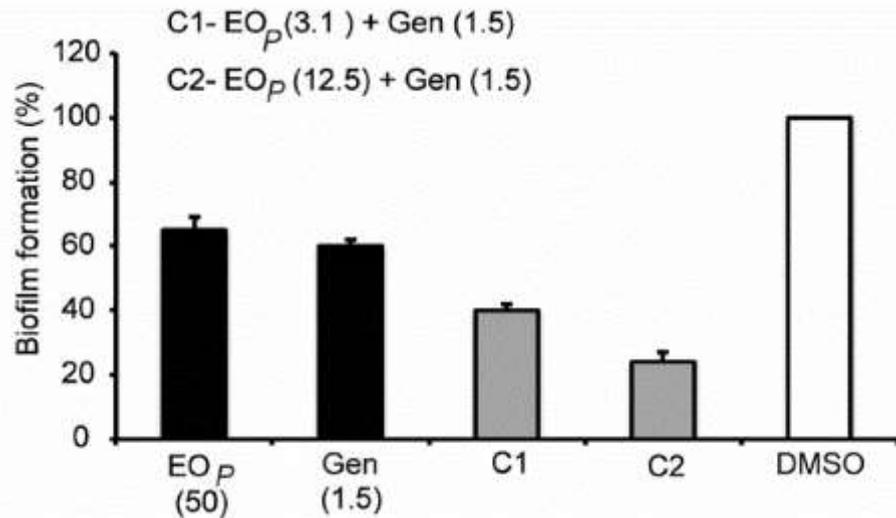
Biofilms+EOp

P. aeruginosa/A. terreus

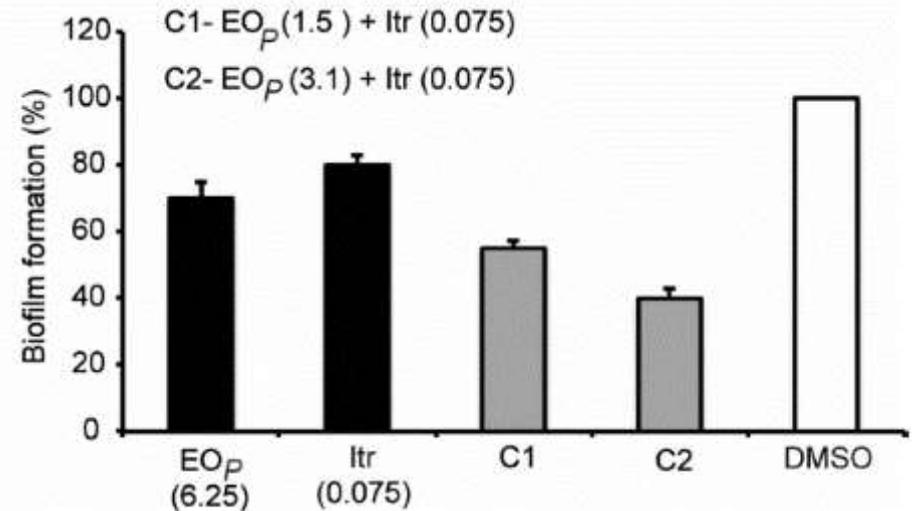


Synergistic activity with clinically used antimicrobials

P. aeruginosa



C. albicans



Conclusions

- Combination of anti-QS and membrane targeting compounds effectively inhibit formation of mixed biofilms
- Citrus EOs could be used in prophylaxis of mixed species infections
- Citrus EOs could be used to potentiate activity of clinical drugs during the treatment of mixed species infections

Acknowledgements

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Thank you!