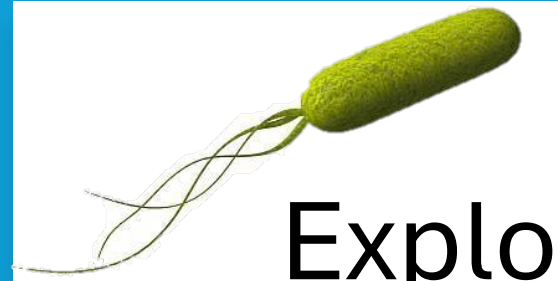
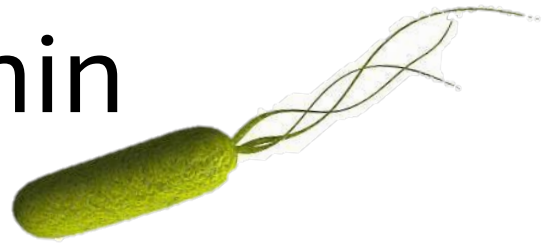


Exploring the biological activities of carotenoids on preformed *Pseudomonas aeruginosa* biofilms: focus on β-carotene and astaxanthin

Ing.Carette Julie



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Belgian Pharmacognosy Day 2024

UMONS RESEARCH INSTITUTE FOR HEALTH SCIENCES AND TECHNOLOGY

Université de Mons

Outline

- Antimicrobial resistance
- *Pseudomonas aeruginosa*
  - Biofilm & virulence factors
- Carotenoids
  - Structure-activity
  - Carotenoid known for its anti-biofilm activity
- Astaxanthin
- β-carotene
- Prospects

Antimicrobials

- Most effective drug treatments
  - Life expectancy has increased by 23 years since the first antibiotic in 1924
- Flemming discovered Penicillin
- 150 new antibiotics have been developed

→Widespread overuse leads to worldwide spreading of resistance

Antimicrobial resistance: numbers talk

- Among top 10 global health threats
- Murray et al. estimated that 4,95 million deaths/year were associated with bacterial antibiotic resistance
- The World Bank estimates that up to 3.8% of the global gross domestic product could be lost due to antimicrobial resistance by 2050
- Antimicrobial involvement in agriculture and food systems have an impact on driving antimicrobial resistance

Antimicrobial resistance: Silent Pandemic

- Urgent action
- If not : antimicrobial resistance become the world’s primary cause of death = 10 million deaths/year by 2050
- Not only a global public health but also socioeconomic problem
- Modern medicine depends on effective antimicrobials
- WHO published a list of antibiotic-resistant "priority pathogens"

Priority pathogens

WHO priority pathogens list for R&D of new antibiotics

Priority 1: CRITICAL

- *Acinetobacter baumannii*, carbapenem-resistant
- *Pseudomonas aeruginosa*, carbapenem-resistant
- *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing

Priority 2: HIGH

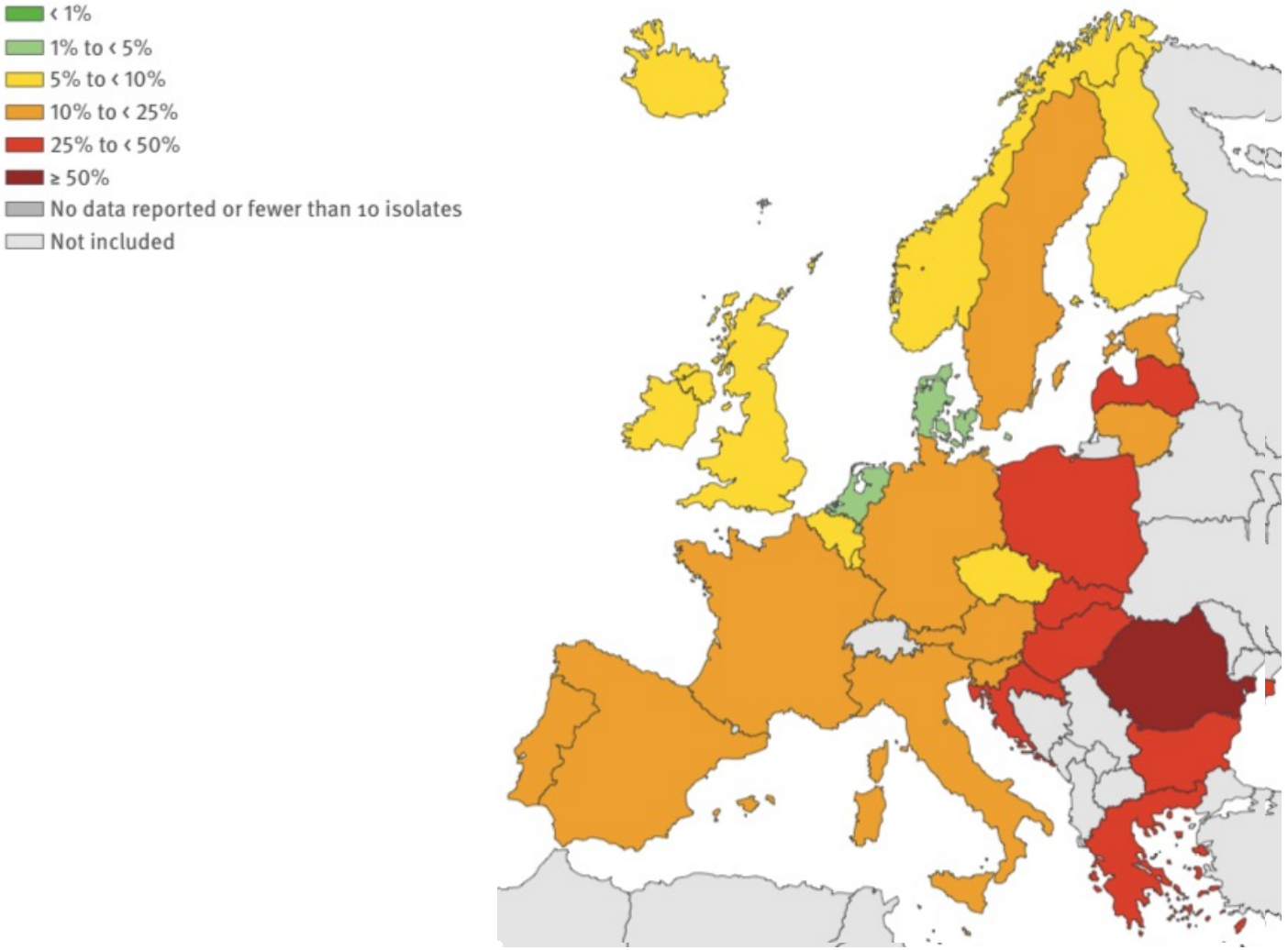
- *Enterococcus faecium*, vancomycin-resistant
- *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant
- *Helicobacter pylori*, clarithromycin-resistant
- *Campylobacter* spp., fluoroquinolone-resistant
- *Salmonellae*, fluoroquinolone-resistant
- *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant

Priority 3: MEDIUM

- *Streptococcus pneumoniae*, penicillin-non-susceptible
- *Haemophilus influenzae*, ampicillin-resistant
- *Shigella* spp., fluoroquinolone-resistant

Pseudomonas aeruginosa

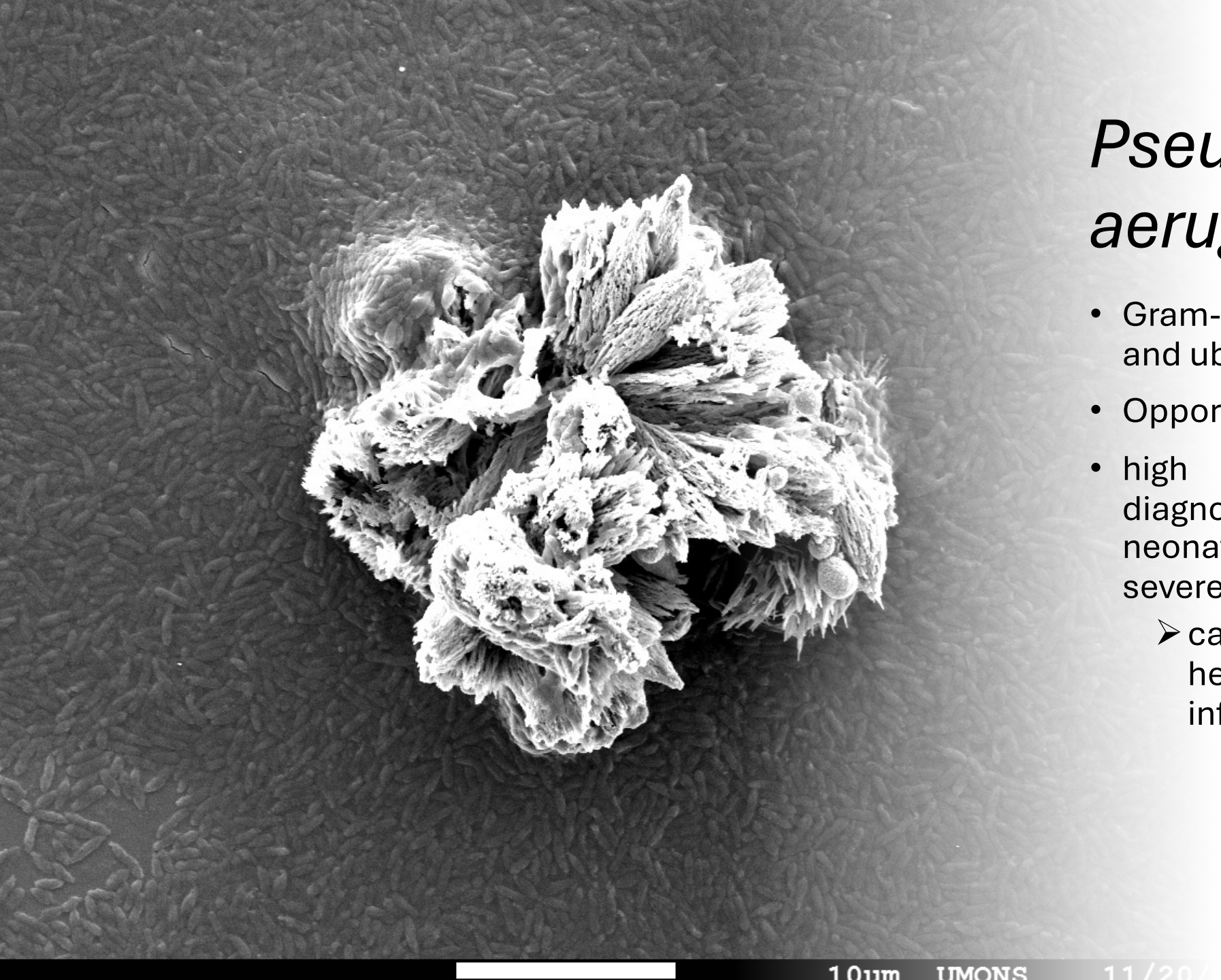
- Worrying situation in Europe



World Health Organization

Fluoroquinolones

Piperacillin-tazobactam

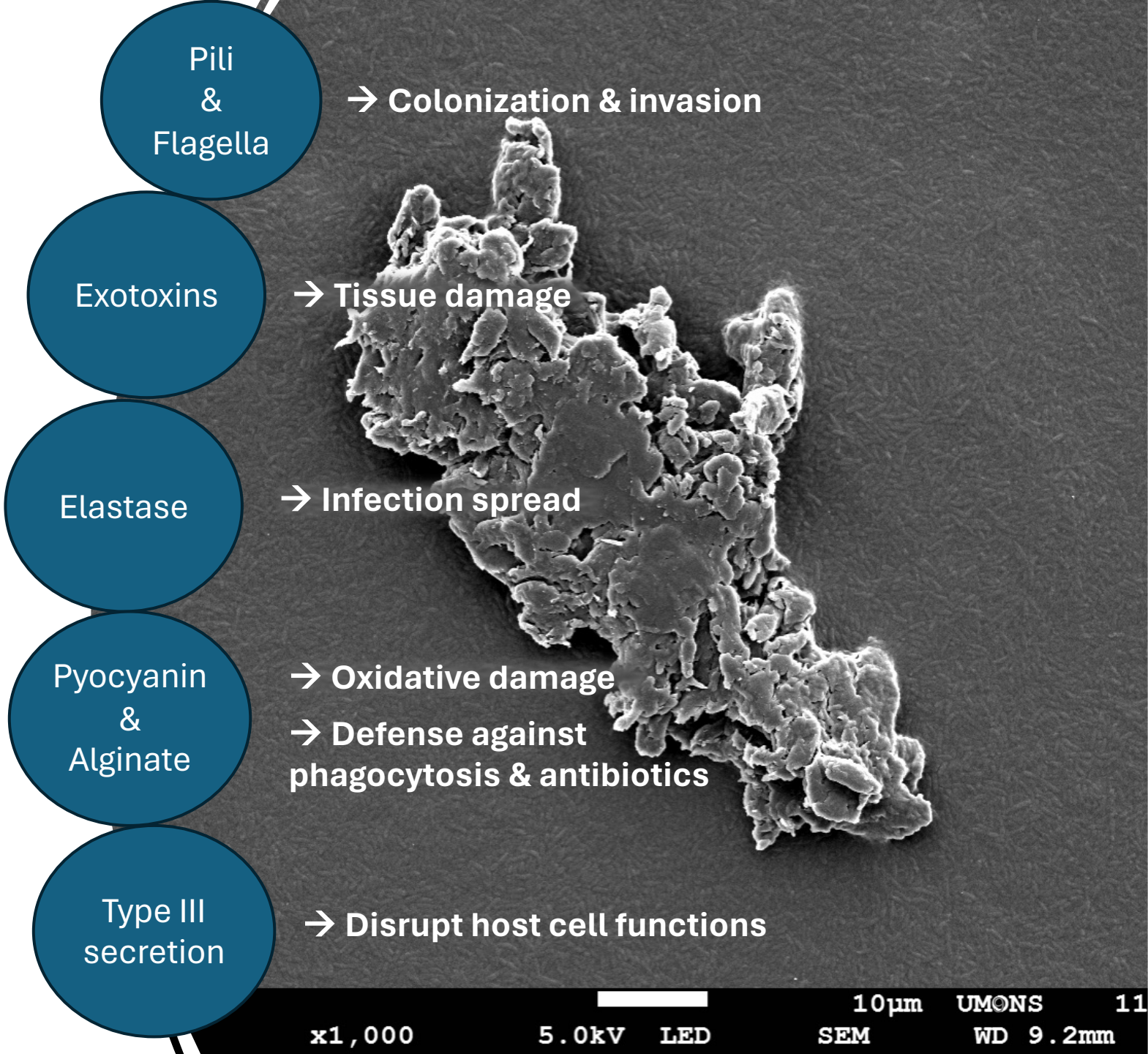


Pseudomonas aeruginosa

- Gram-negative aerobic rod-shaped and ubiquitous bacterium
- Opportunistic!
- high mortality rate in patients diagnosed with cystic fibrosis, neonatal infections, cancer, and severe burns = Life-threatening
  - causing more than 50% of healthcare-acquired infections

Pseudomonas aeruginosa

- Ability to form biofilm = most important virulence determinants
  - Leading to chronic infections
  - Increasing resistance to antibiotics



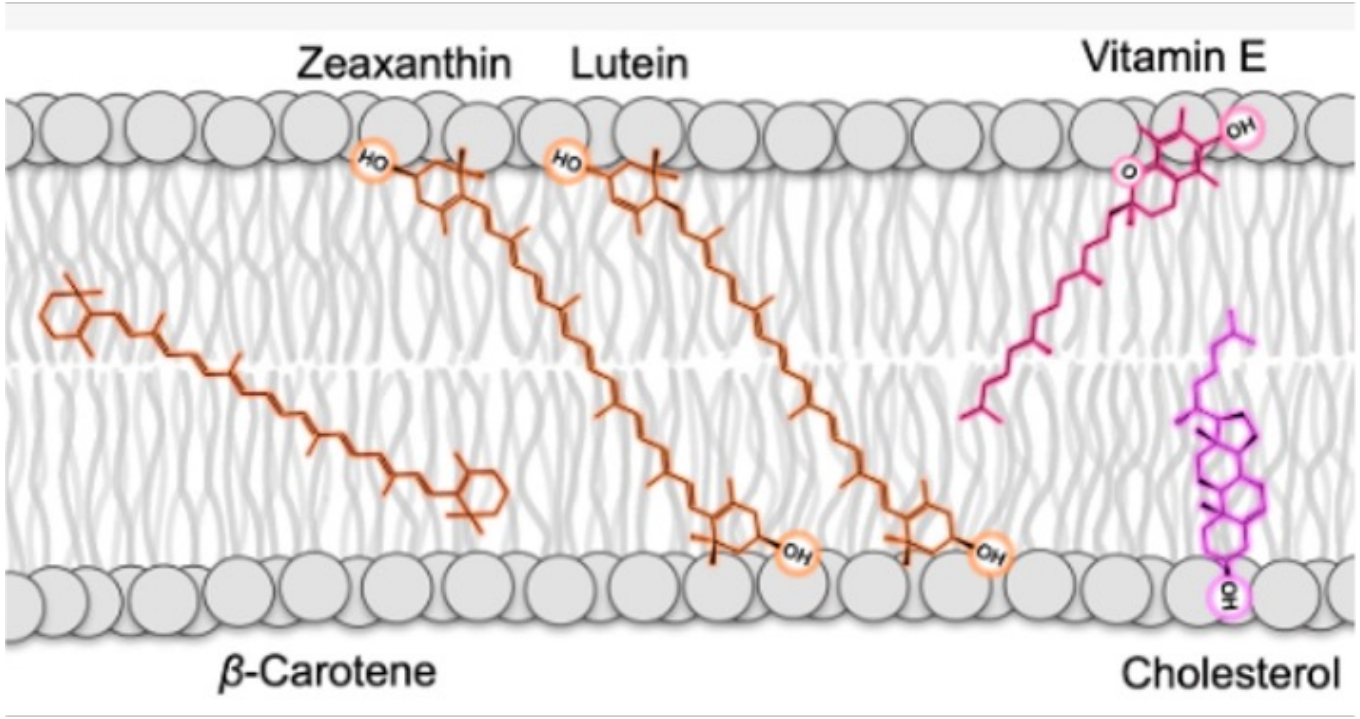
Carotenoids

Antioxidant and Pro-oxidant Effects

Cell Communication Disruption

Bacterial Membrane Disorganization

Impact on Biofilm Matrix

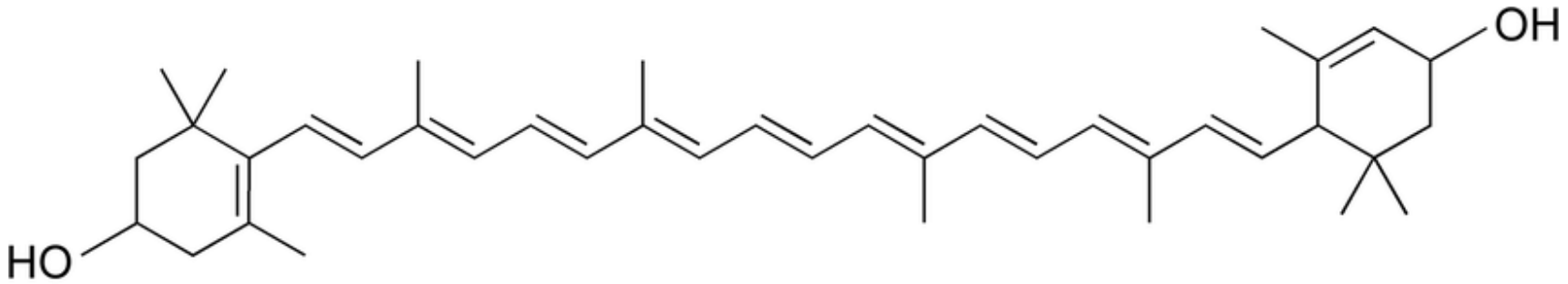


➔ Structure-activity relationship ?

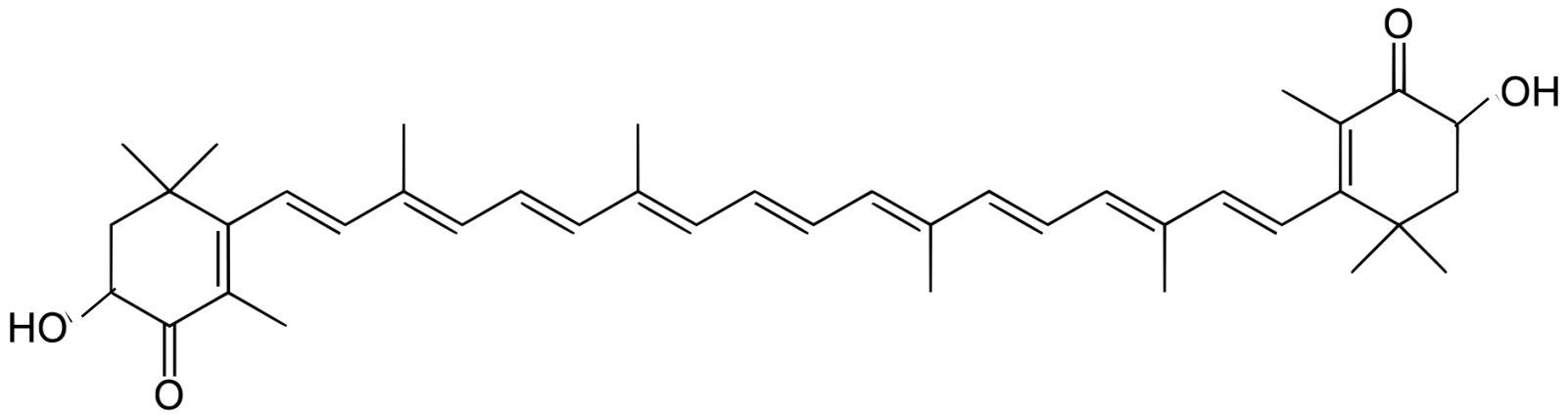
Carotenoids

Zeaxanthin	acts as a quorum sensing inhibitor, reducing biofilm formation and virulence factor expressions in *Pseudomonas aeruginosa*
Lutein	disrupts biofilm formation in *Pseudomonas aeruginosa* and enhances the bactericidal effects of tobramycin.
Carotenoid pigment from *Rhodotorula glutinis*	disrupts biofilm formation in food spoilage bacteria by suppressing quorum-sensing genes.
Carotenoids from *Fenneropenaeus indicus, Penaeus semisulcatus*	Bioactive carotenoids extracted from the head and carapace of three shrimp species demonstrated significant antibacterial and biofilm inhibition properties, particularly against Gram-positive bacteria.
Yellow carotenoid pigment extracted from *Kocuria sp.* GMA	Effective antimicrobial and antibiofilm properties against various pathogens.
Pigments from *Rhodococcus sp. SC1*	These pigments, primarily carotenoids, demonstrated antibacterial and antibiofilm properties, showing potential for medical applications due to ability to inhibit biofilm formation and bacterial growth.
.....	

Astaxanthin ?



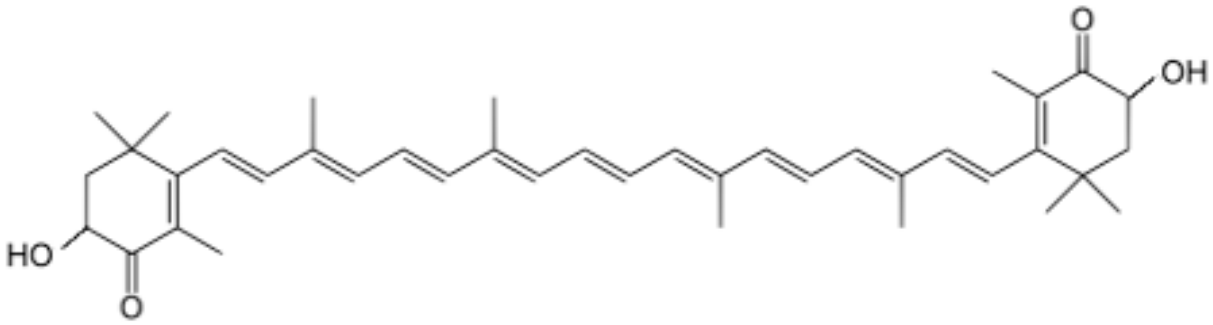
Lutein



Astaxanthin

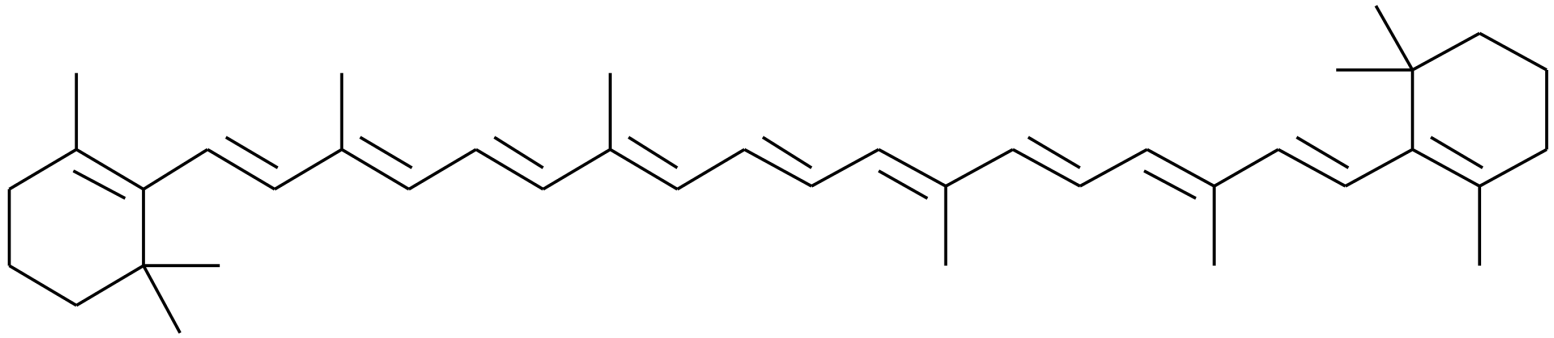
Astaxanthin ?

Effective against *Aeromonas hydrophila* and *Aeromonas sobria* strains,
In toothpaste to inhibit the growth of cariogenic bacteria, reducing tooth decay and enhancing oral health.
Demonstrated antibacterial effects against common bacterial strains including *Escherichia coli*.
Role in combating various diseases which may include mechanisms useful in anti-biofilm activity.
Showing significant antibacterial activity, which implies potential in anti-biofilm applications.
Explores astaxanthin's cytotoxic and antibacterial properties from endophytic bacteria, hinting at its anti-biofilm capabilities.
….

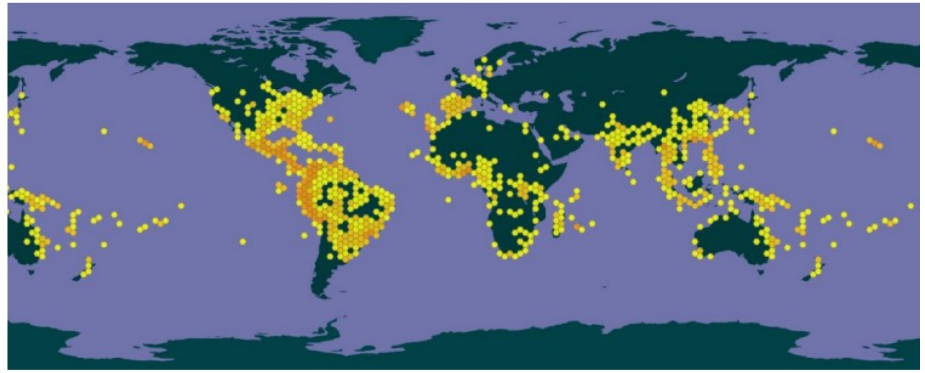


β-carotene ?

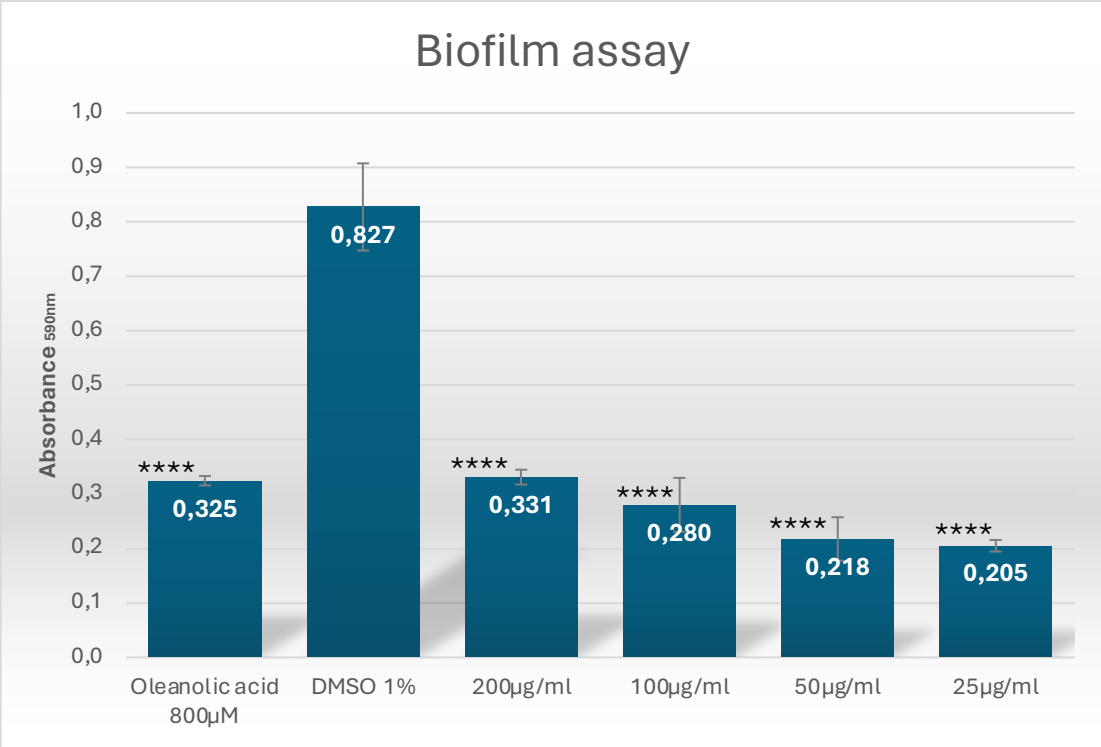
Plant extracts from several ethnomedicinal plants containing β-carotene show a biofilm activity against *P.aeruginosa*
The extract of *Prangos ferulacea*, which contains beta-carotene, significantly reduces biofilm formation in *Listeria monocytogenes* by altering the expression of key virulence and stress response genes.
The extract of *Lagenaria siceraria*, which contains beta-carotene, demonstrates significant antibiofilm activity, particularly effective against *Streptococcus pneumoniae* and *Staphylococcus aureus*.
The study on *Lactuca serriola* extract, which contains beta-carotene, demonstrated significant antibacterial and anti-biofilm effects against clinically isolated strains of *Porphyromonas gingivalis* and *Prevotella intermedia*.



Ipomoea batatas extracts

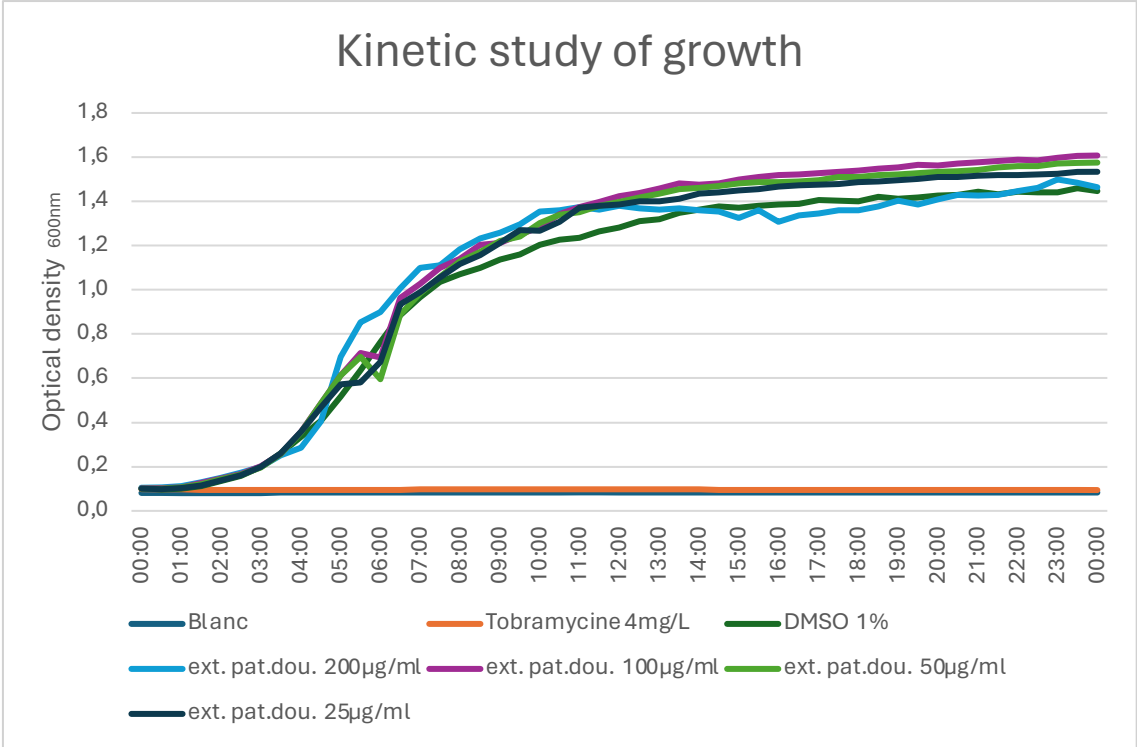


- **β-carotene** : About 90% or more of the total carotenoids
- **α-carotene** : About 5% or less of the total carotenoids
- **Lutein** : Less than 5% of the total carotenoids
- **Zeaxanthin** : Less than 1% of the total carotenoids



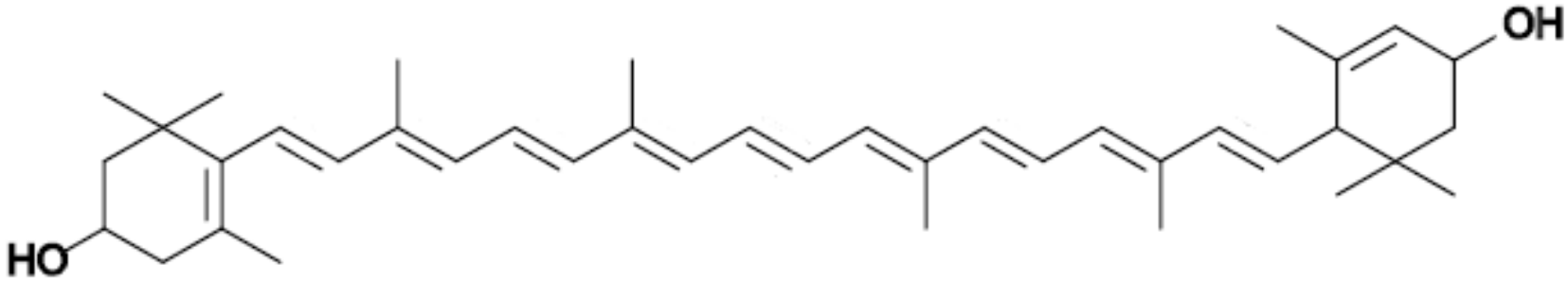
By crystal violet staining

**** p < 0,0001 (n=3; biological replicates)

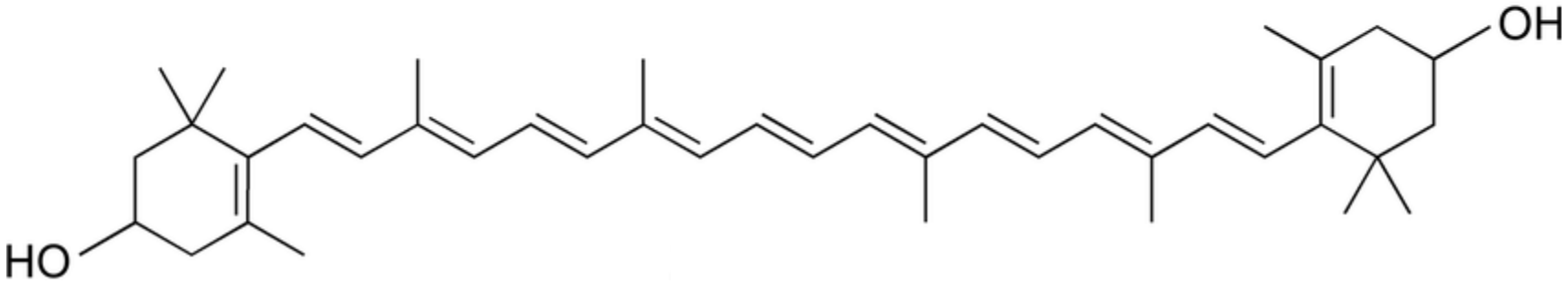


(n=6; average from 6 biological replicates)

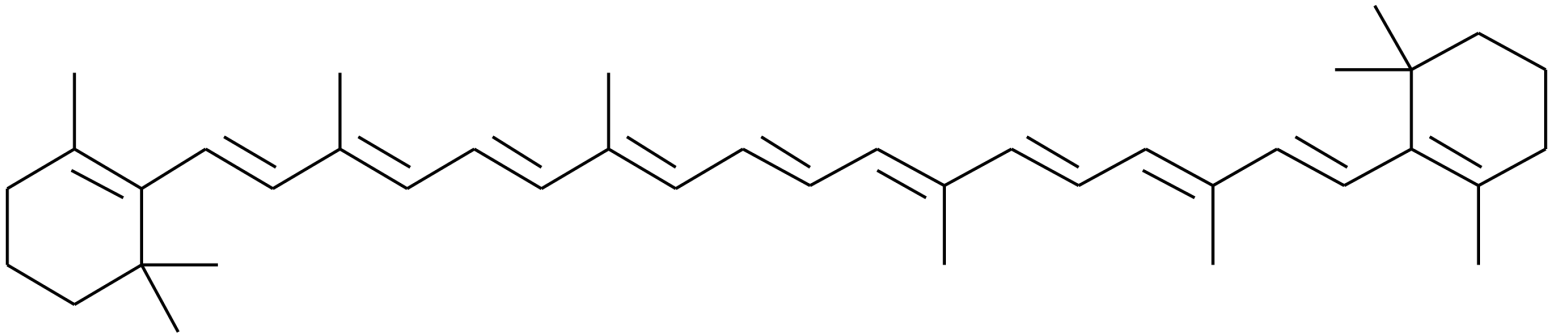
β-carotene ?



Lutein

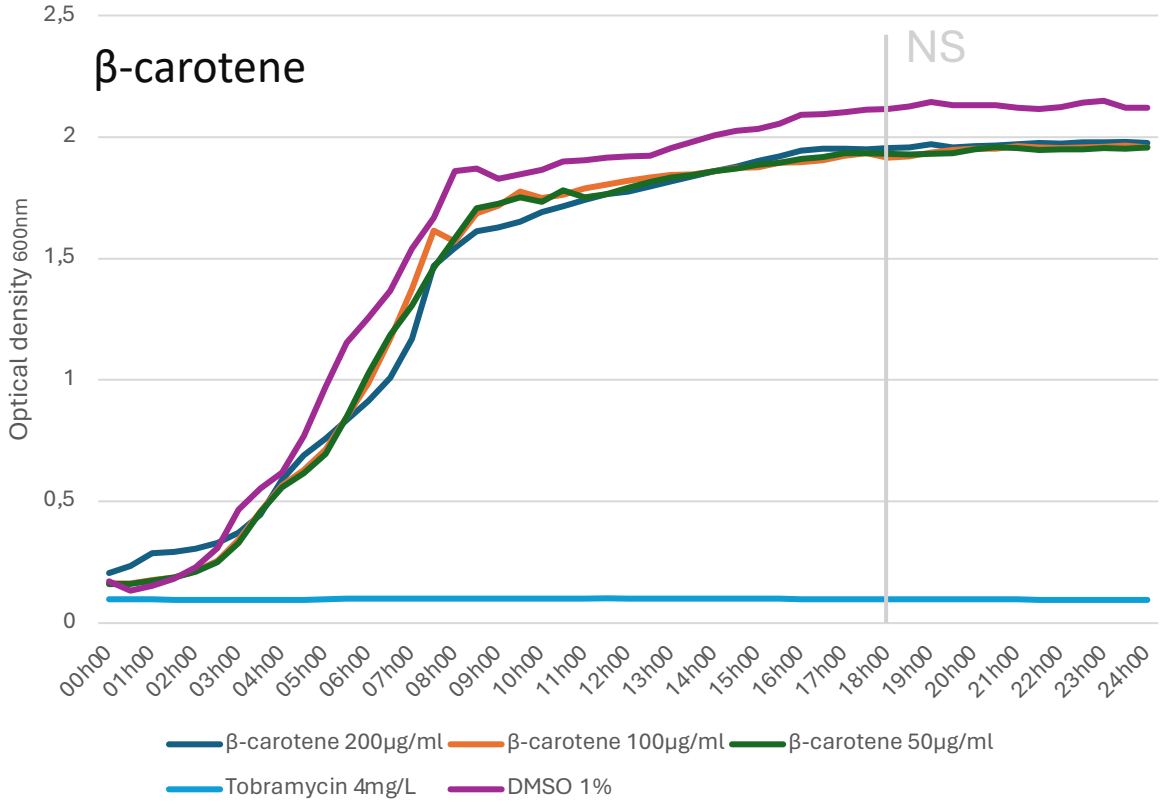


Zeaxanthin

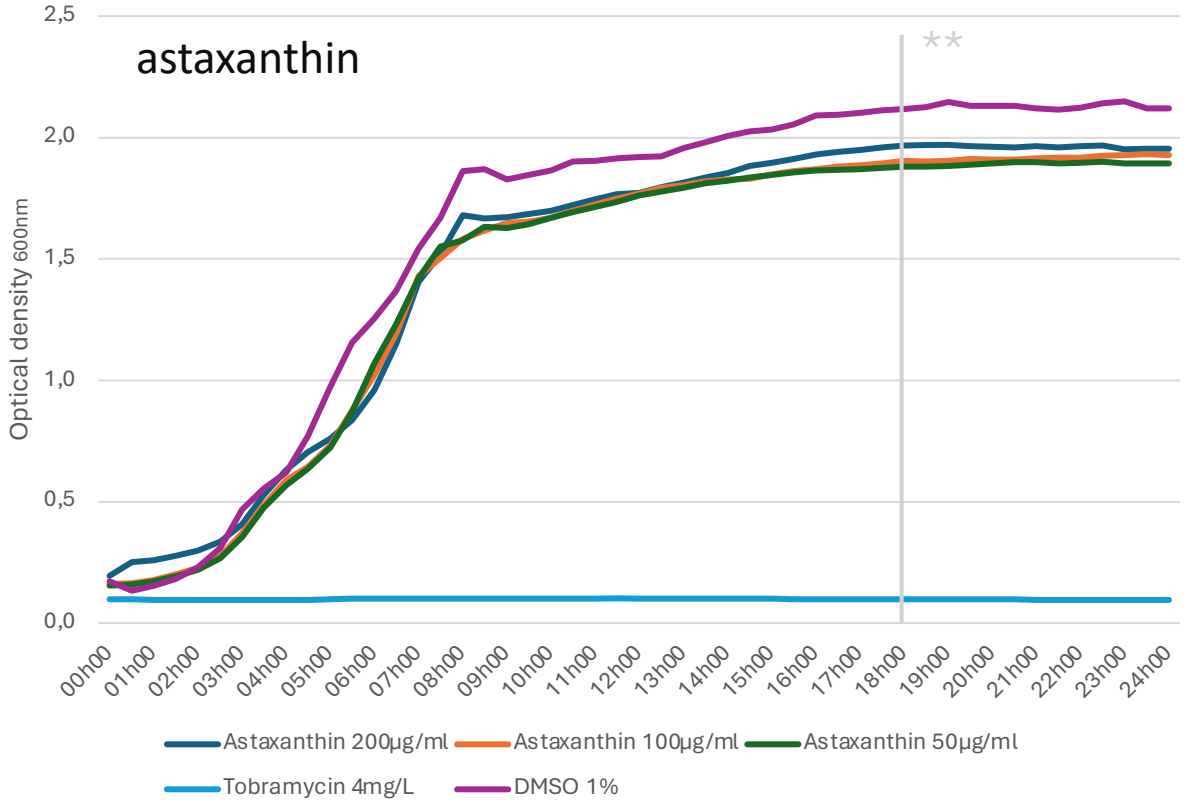


β-carotene

Kinetic analysis of *Pseudomonas aeruginosa* growth in presence of β-carotene or astaxanthin

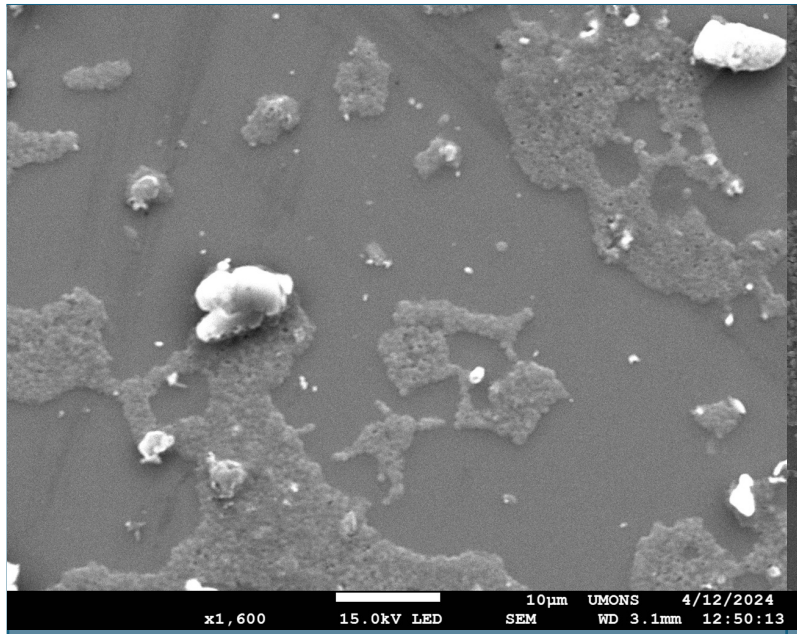


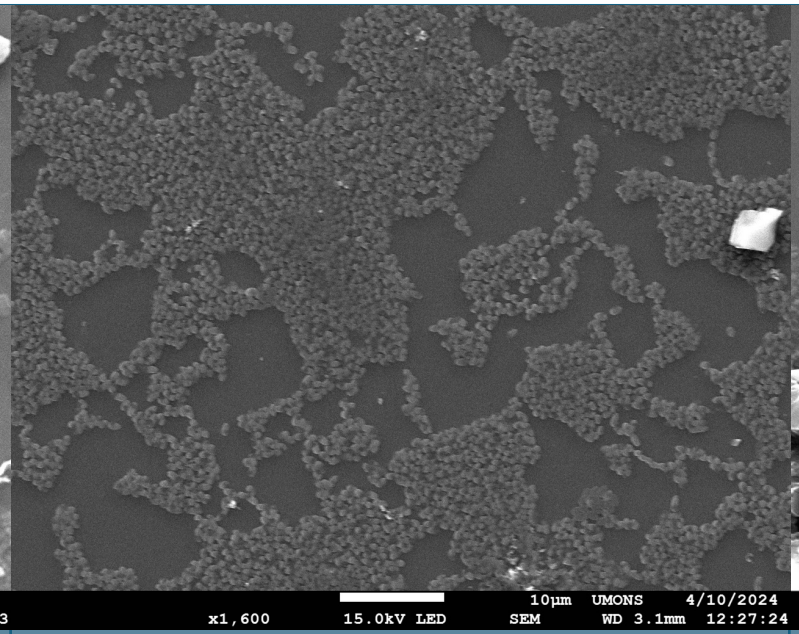
*** p < 0,05 (n=6; average from 6 biological replicates)

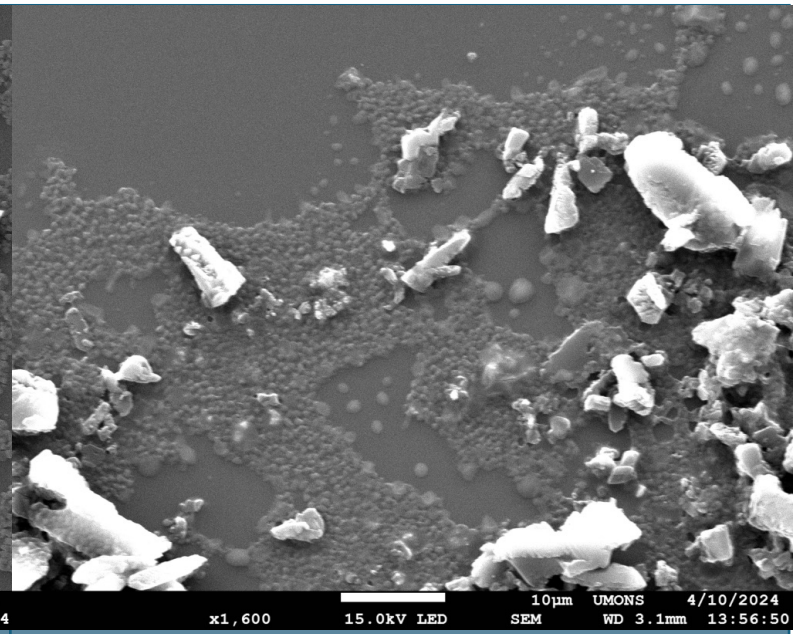


*** p < 0,05 (n=6; average from 6 biological replicates)

Scanning electron microscopy of *Pseudomonas aeruginosa* biofilm with Astaxanthin

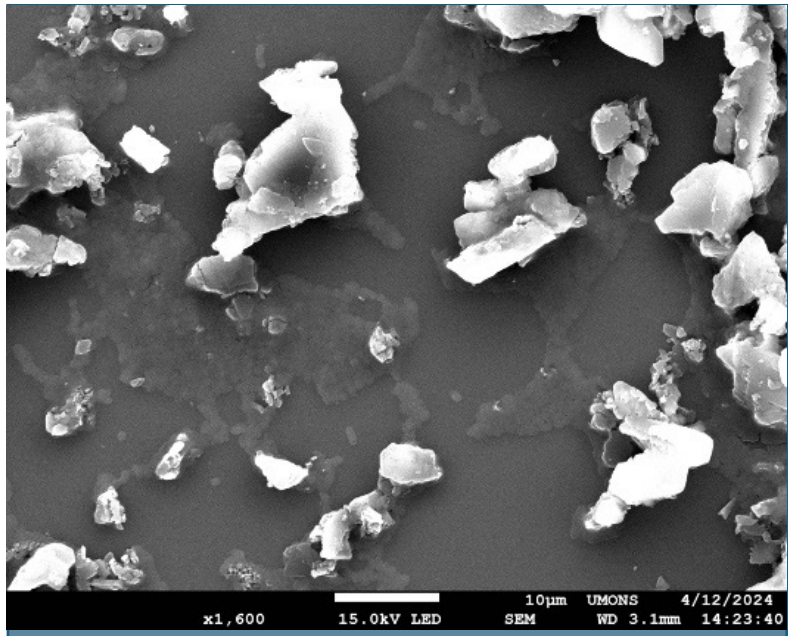






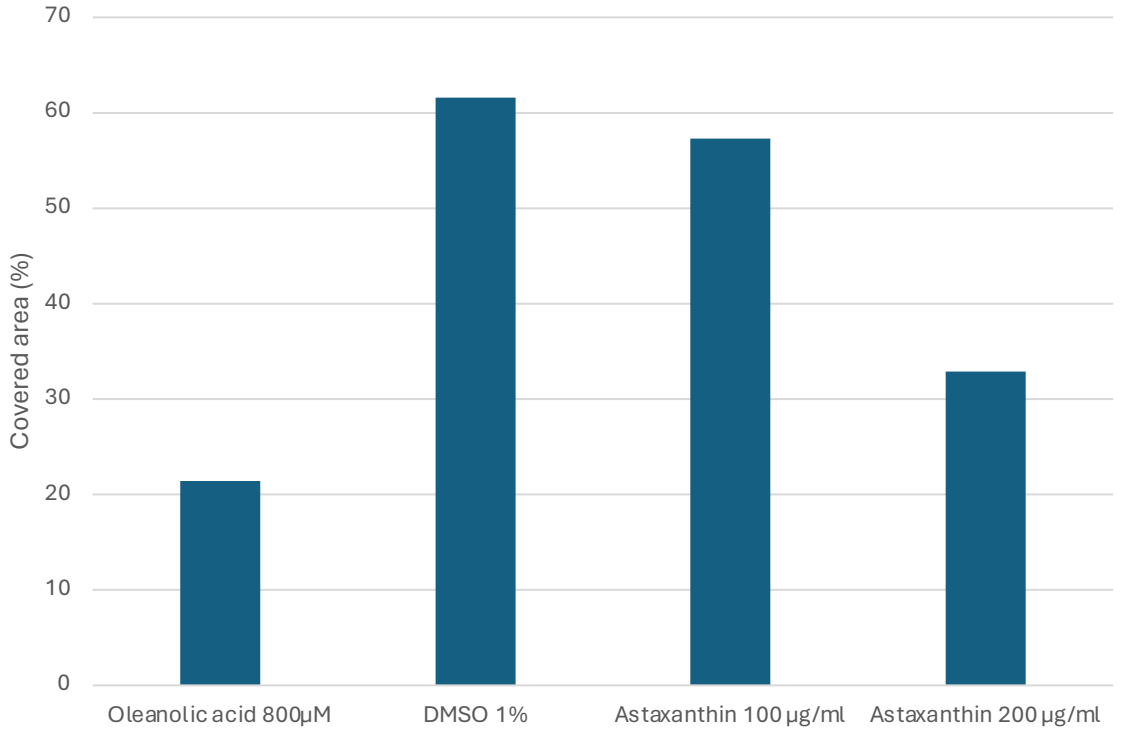
Oleanolic acid 800µM	DMSO 1%	Astaxanthin 100 µg/ml
21,4% of covered area Biofilm inhibiteur- positive control	61,6 % of covered area Negative control	57,3 % of covered area

Scanning electron microscopy of *Pseudomonas aeruginosa* biofilm with Astaxanthin



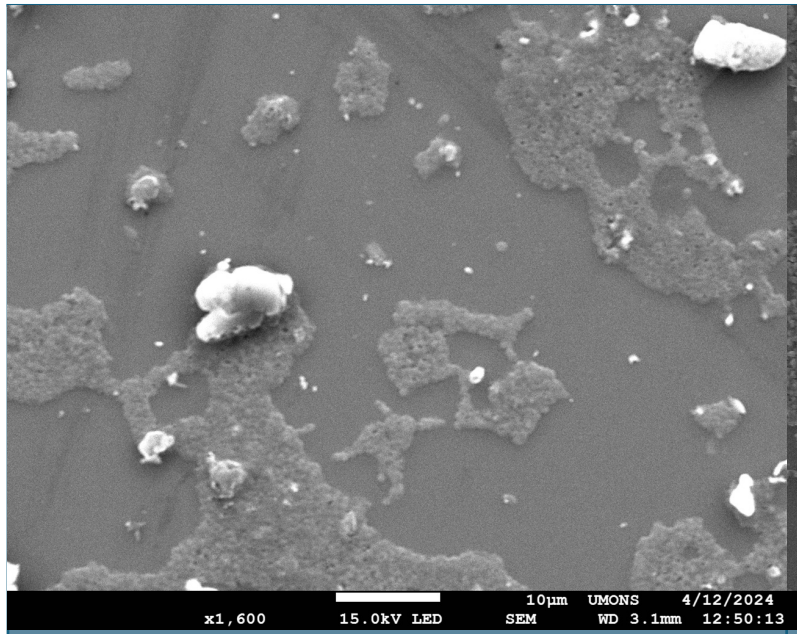
Astaxanthin 200 µg/ml

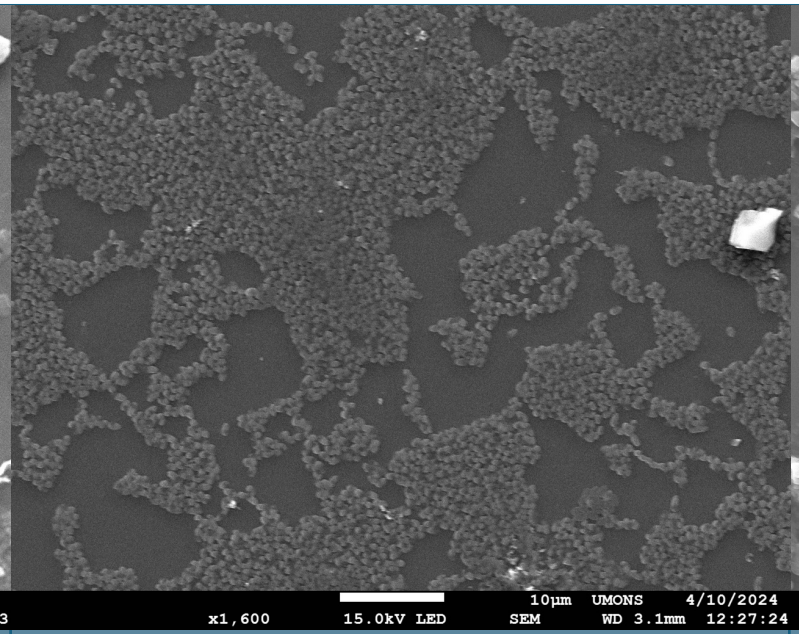
32,9 % of covered area

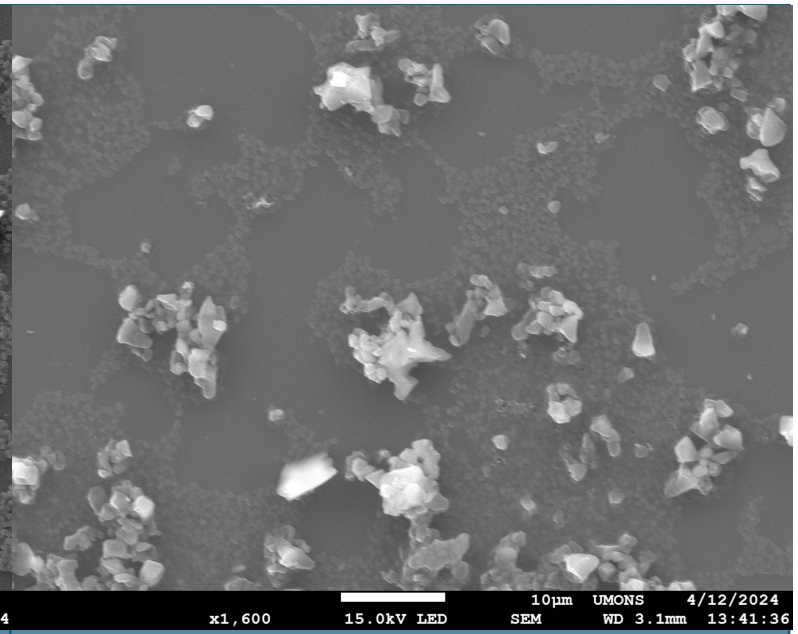


(n=3; biological replicates)

Scanning electron microscopy of *Pseudomonas aeruginosa* biofilm with β-carotene

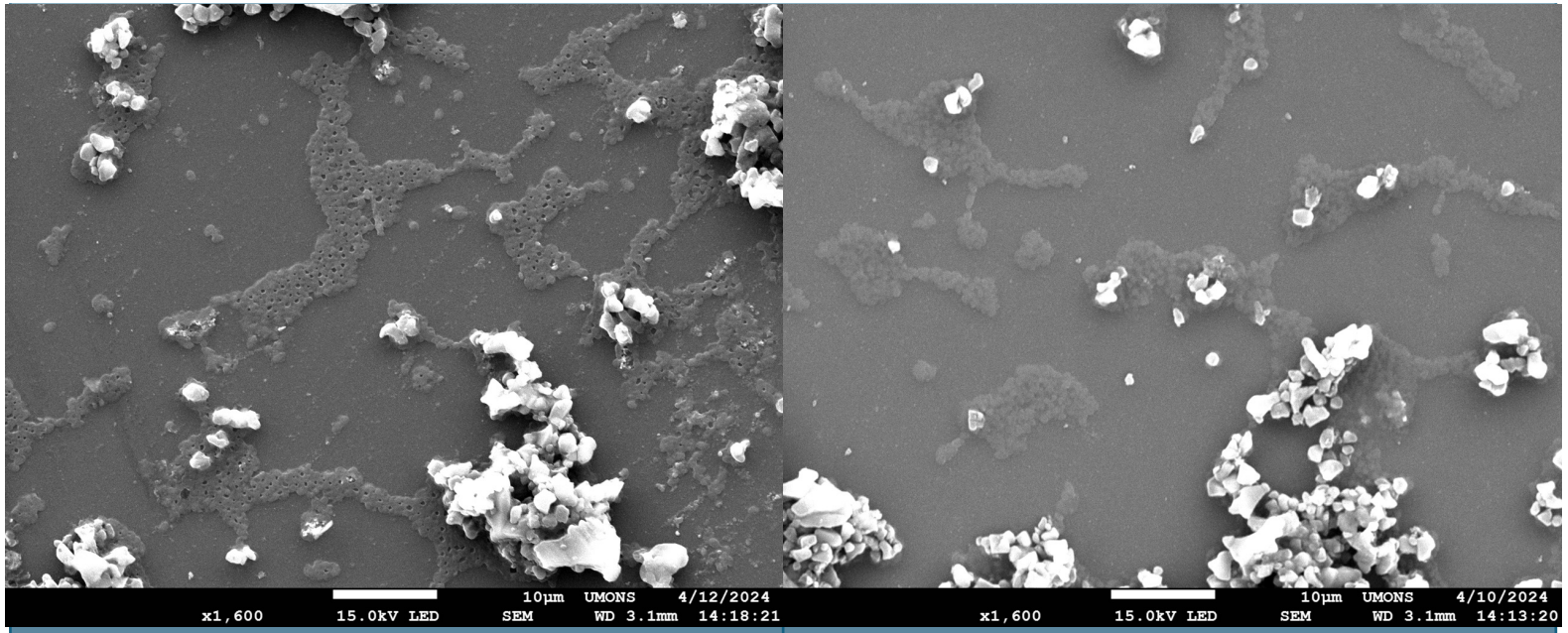




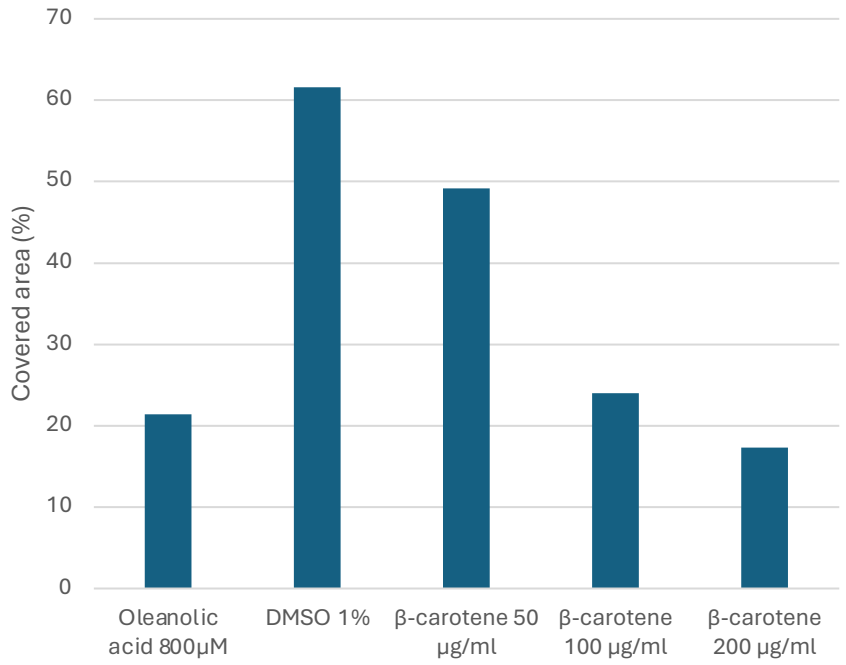


Oleanolic acid 800µM	DMSO 1%	β-carotene 50 µg/ml
21,4% of covered area Biofilm inhibiteur- positive control	61,6 % of covered area Negative control	49,2% of covered area

Scanning electron microscopy of *Pseudomonas aeruginosa* biofilm with β-carotene



β-carotene 100 µg/ml	β-carotene 200 µg/ml
24% of covered area	17,3% of covered area



(n=3; biological replicates)

Prospects

- Confirm the biofilm results for *Ipomoea batatas* extract, astaxanthin & β-carotene
- Perform motility assays & pyocyanin assays
- Perform biofilm assay on preformed biofilm & bioreactor biofilm
- HPTLC and HPLC profiling & measurement of primary carotenoids for *Ipomoea batatas* extract