Tracking bacilli swimmers in microalgae biofilms

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Introduction

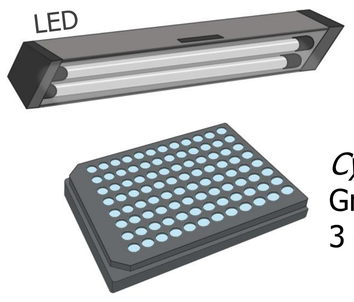
Some *Bacillus* biofilms have been shown to spontaneously contain a subpopulation of motile bacteria capable of navigating through the matrix in three dimensions (Houry *et al.*, 2012). These swimming bacteria vascularize the biofilms and facilitate the penetration of specific substances to the deeper layers. Microalgae are rich in molecules of interest, and are at the heart of the challenges of the 21st century. Their biofilm culture is attracting growing interest due to their high productivity potential. In this innovative study, the aim is to characterize *Bacillus* swimming in a *Cylindrotheca closterium* biofilm using swimming bacilli to further increase biomass productivity.

The swimmers criss-cross a biofilm of bacteria, but are they capable of vascularizing a biofilm of microalgae, given that these microorganisms are at least three times larger?

Using confocal microscopy (CLSM), an experimental method was developed to study the swimming of these bacilli. Although the microalgae biofilm becomes thicker with increasing matrix production (Fanesi *et al.*, 2019), *Bacillus* swimming is still observable. Precise characterization of the swimmers requires further optimization and trajectory analyses.

Materials and Methods

Culture of microalgae biofilms

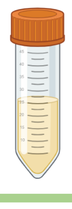


Cylindrotheca closterium
Growth medium : f/2
3 days adhesion

Inoculation
50 µL.puits^{-1}
Each day, inoculated wells are sacrificed

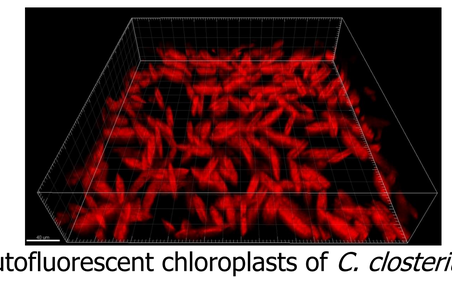
Culture of swimming bacteria:

Bacillus thuringiensis Bt407-GFP
Growth medium : TSB
DO_{600nm} = 0,4

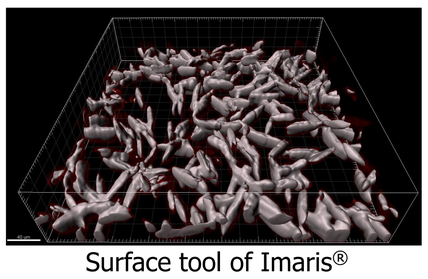


CLSM Acquisitions 30 minutes after inoculation:

Biofilm biovolume extraction

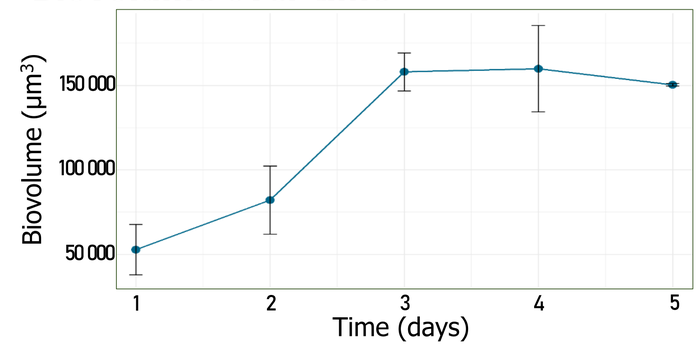


Autofluorescent chloroplasts of *C. closterium*

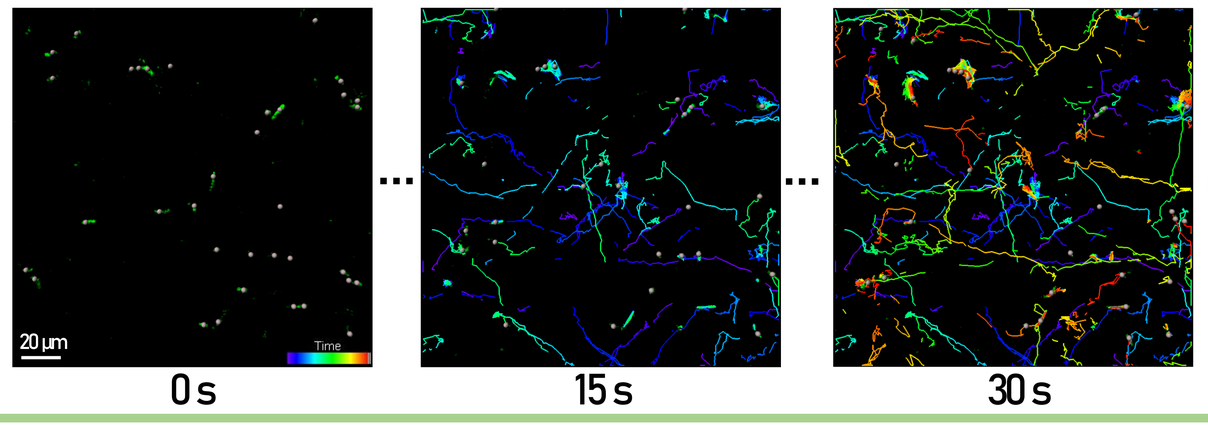


Surface tool of Imaris®

Biovolume over time



Bacteria tracking at the bottom of the wells

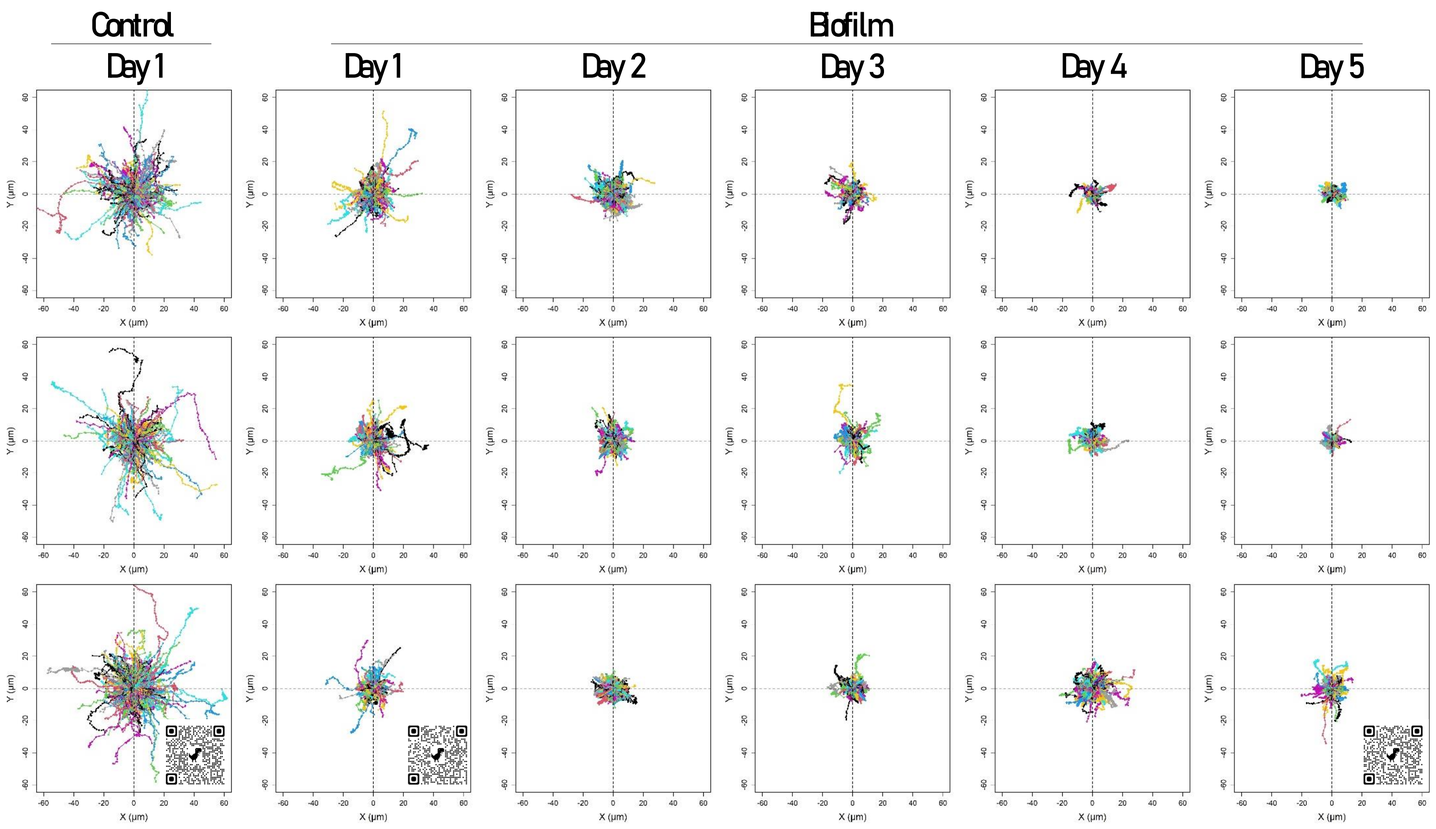


Motility caracterisation:

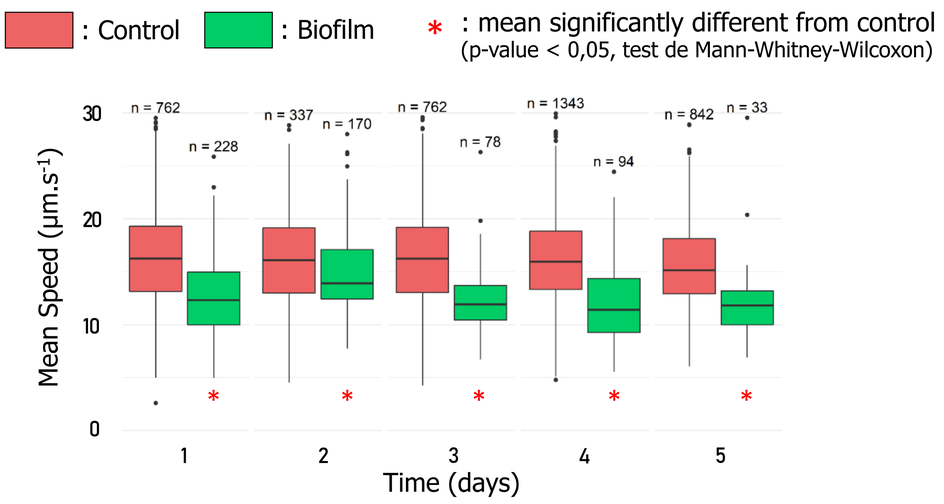
- Position
- Swimming parameters (speed, length, duration…)
- Mean square displacement
- Straightness index

Results

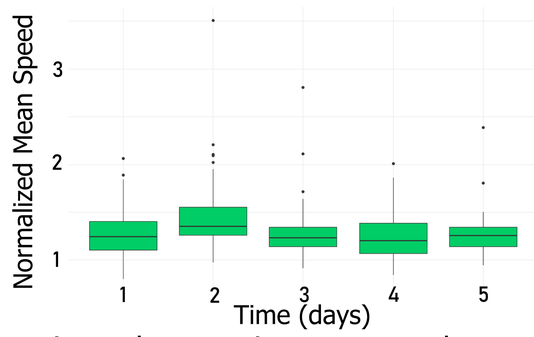
Normalized trajectories of swimming bacteria over time



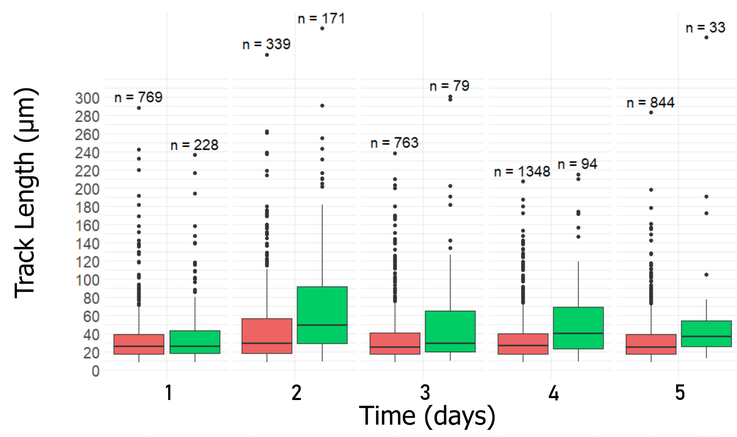
Swimming parameters over time



Control mean speed (16 µm.s^{-1}) > Biofilm mean speed (13 µm.s^{-1})
There is a decrease in the number of bacteria over time.



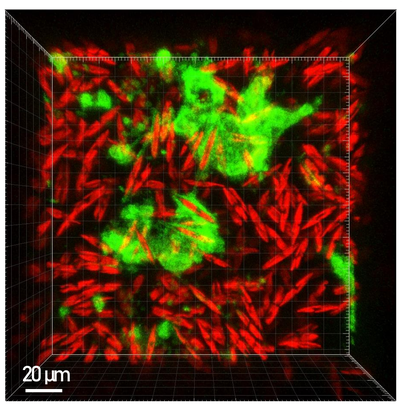
There is no decrease in mean speed over time.



Track Length in control < Track Length in biofilm

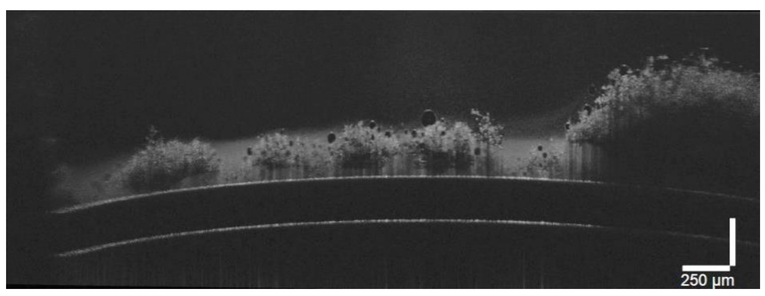
Heterogeneity of microalgal biofilms

Autofluorescent clusters (560 nm):



C. closterium (> 5 days)

Mesoscopic structure by Optical Coherence Tomography (B-scan):



C. closterium (> 3 weeks)

Conclusion

This project, at the convergence of the fields of biofilms, microalgae and bacterial motility, has demonstrated the remarkable ability of bacilli to move within the matrix of a microalgal biofilm. However, the swimmers used have a reduced average speed of movement, more confined trajectories and a reduced number of swimmers observed at the bottom of the well. This is consistent with the increased biovolume of the *C. closterium* biofilm and its substantial thickening.

This groundbreaking study highlights the heterogeneity of this phenomenon in a complex biofilm. The impact of biofilm vascularization by bacteria swimming through the matrix has not yet been assessed. Exploring these vascularization phenomena could encourage the entry of nutrients or the release of molecules such as O_2 trapped in biofilms, and thus promote their growth.