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OMICS International is a pioneer and leading science event organizer, which publishes around 500 open access journals and conducts over 500 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

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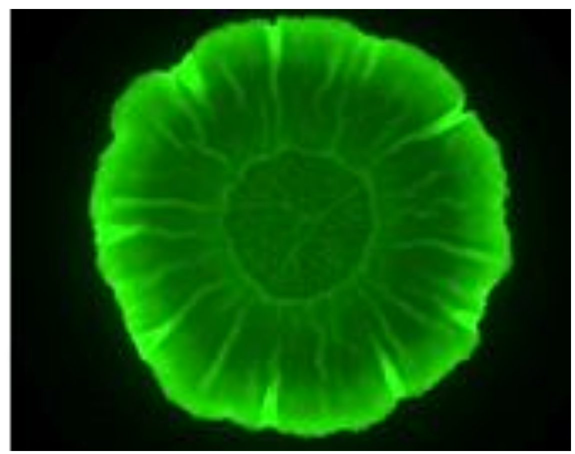
מכון ויצמן למדע
WEIZMANN INSTITUTE OF SCIENCE

Novel forces shaping Beneficial Bacterial Biofilms

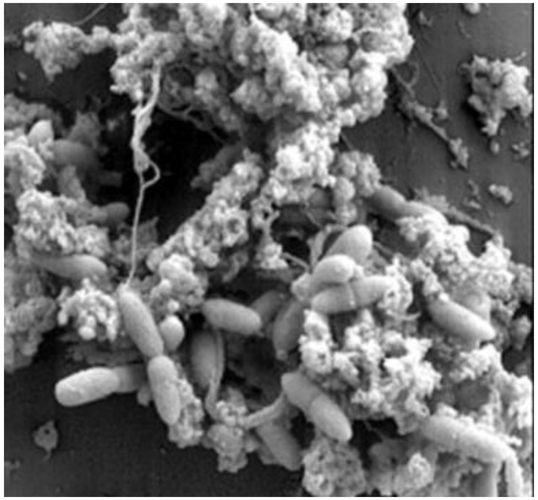


Dept. of Mol. Genetics
Weizmann institute of Science

Biofilms are multicellular communities of bacteria

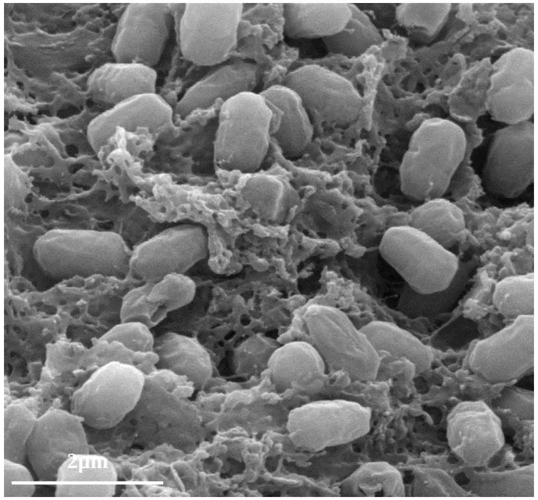
Pseudomonas aeruginosa

Dietrich *et al.*, 2013



Sriramulu *et al.*, 2005

Bacillus subtilis



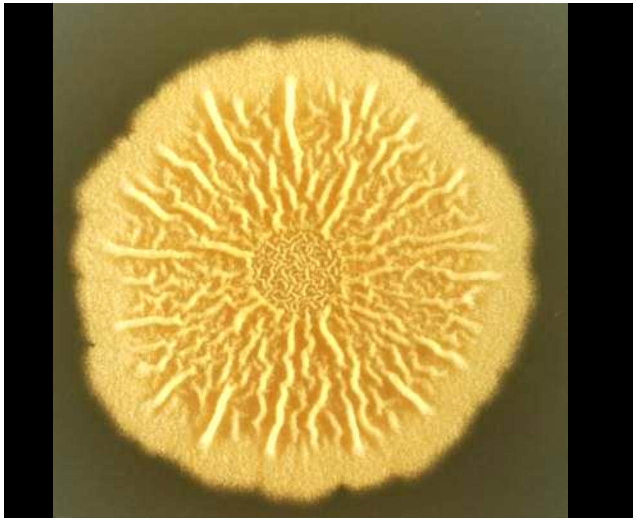
Bacteria in the biofilm state:

- Adhere stronger to the host
- Are more resistant to: antibiotics, sterilizing agents, and the immune system

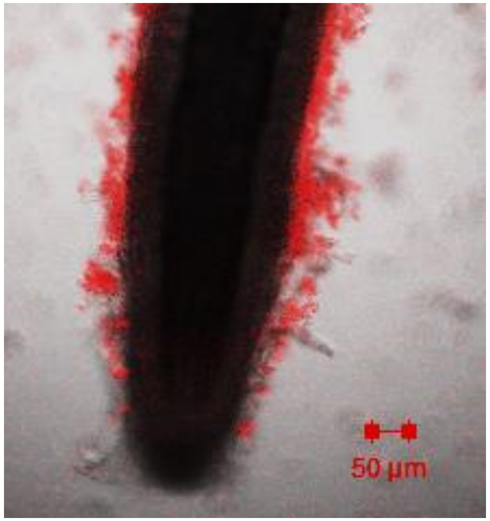
-Biofilm formation Plays an important role in promoting beneficial interactions, and in pathogenicity

Bacillus subtilis is a Gram-positive beneficial bacterium forms biofilms in soils and on plant roots

Biofilm formation in the lab



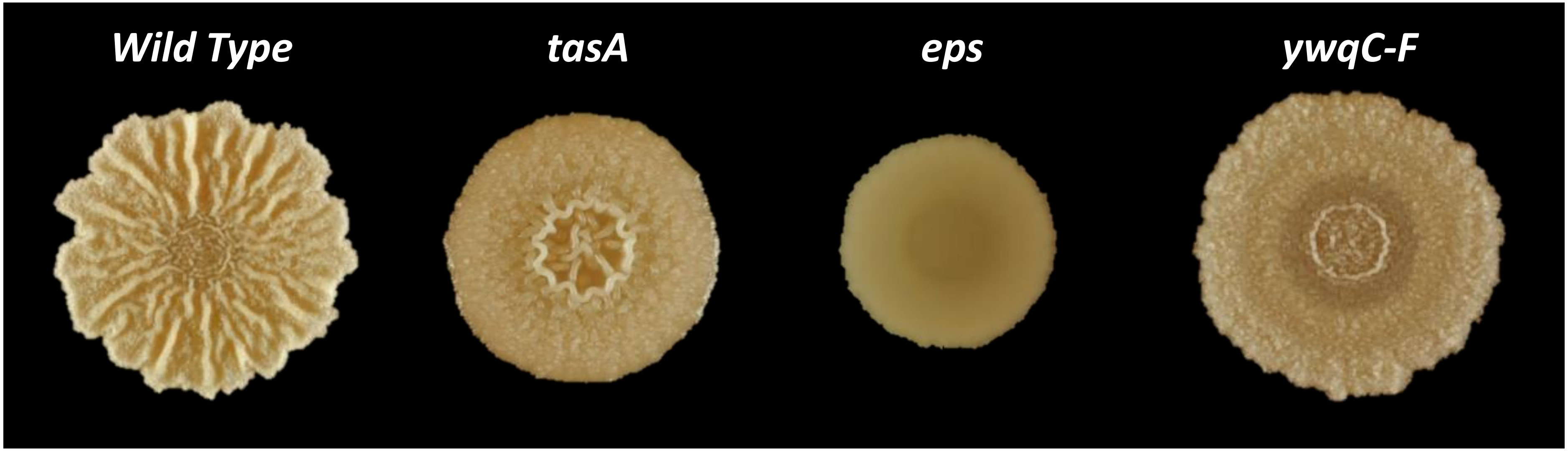
Biofilm formation on the root



Yaara Oppenheimer-Shaanan

What genetic and biochemical factors are essential for biofilm formation?

So far, biofilm morphology was considered a direct output of organic matter secretion, a protein and carbohydrate-rich polymeric extracellular matrix (ECM)



tasA; amyloid-like fibers
epsA-O; exopolysaccharides
ywqC-F; acidic exopolysaccharides

What genetic and biochemical factors are essential for biofilm formation?

Gram-Positive

Bacillus subtilis

Mycobacterium smegmatis

Gram-Negative

Pseudomonas chlororaphis

Salmonella enterica

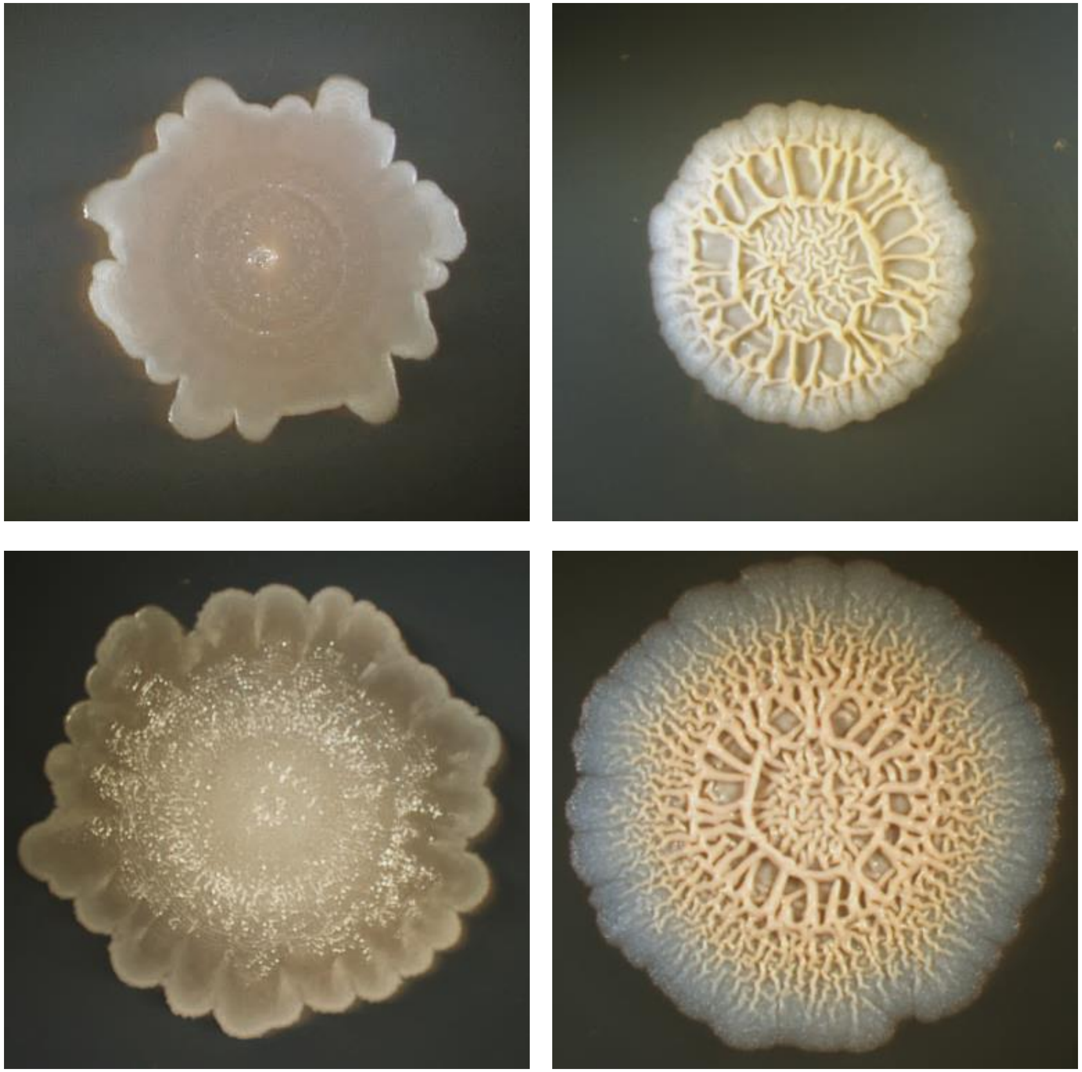
- No similarity in ECM genes and in extracellular matrix components.
- In many cases organic ECM is highly produced but biofilm architecture is defective.
- Oxygen depravation triggers morphogenesis (Kolodkin-Gal et al., Gene Dev 2013, Mcloon and Kolodkin-Gal et al., JBact, 2012).

Biofilm formation is dependent on calcium

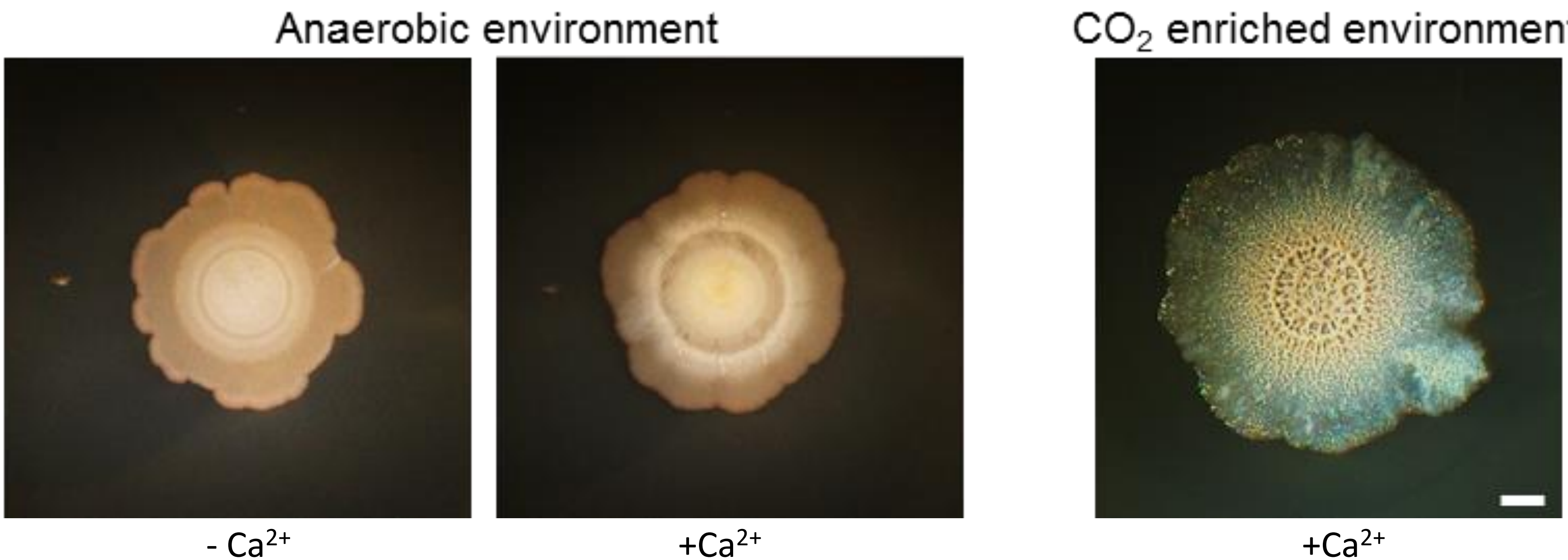
- Ca^{2+} +Ca^{2+}

Day3

Day7



Biofilm formation is dependent on carbon dioxide



Carbonate precipitation $Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$

Calcium carbonate is a mineral produced in access of calcium and carbon dioxide, and participates in shaping multicellular organisms.

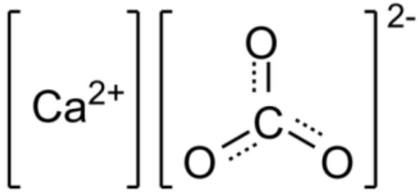
Calcium carbonate minerals are used to build shells and skeletons of higher organism

Starfish (calcite)

Corals (argonite)

Biomineralization; The process through which organisms are involved in mineral formation

Do bacterial colonies form minerals similar to skeleton formation?

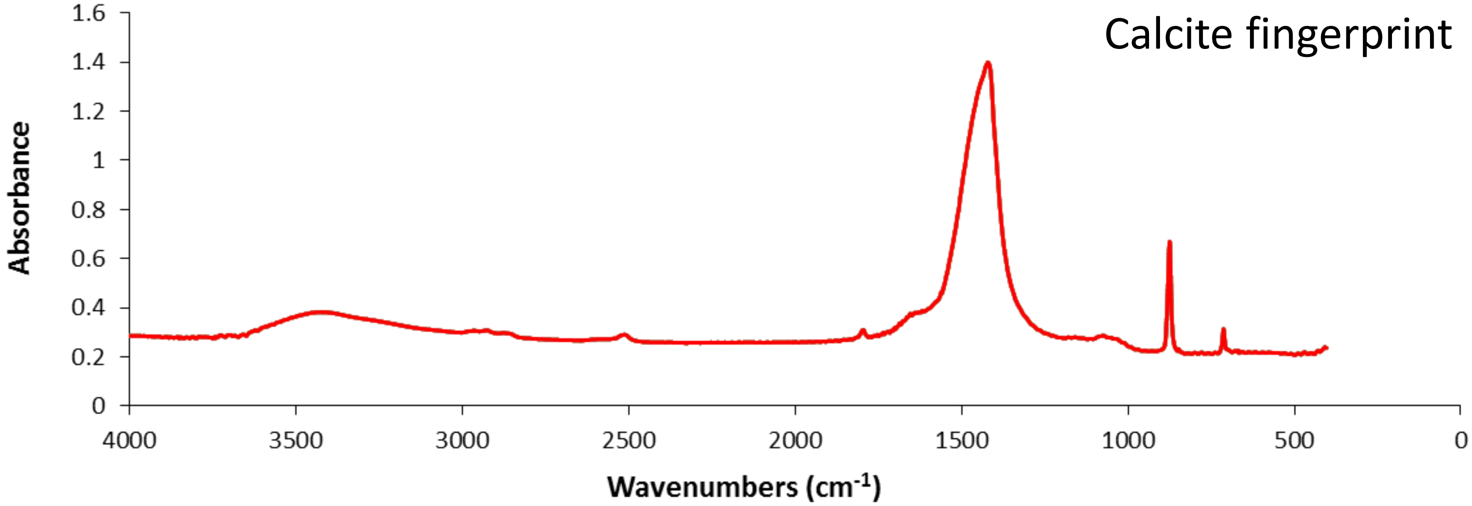


Calcite

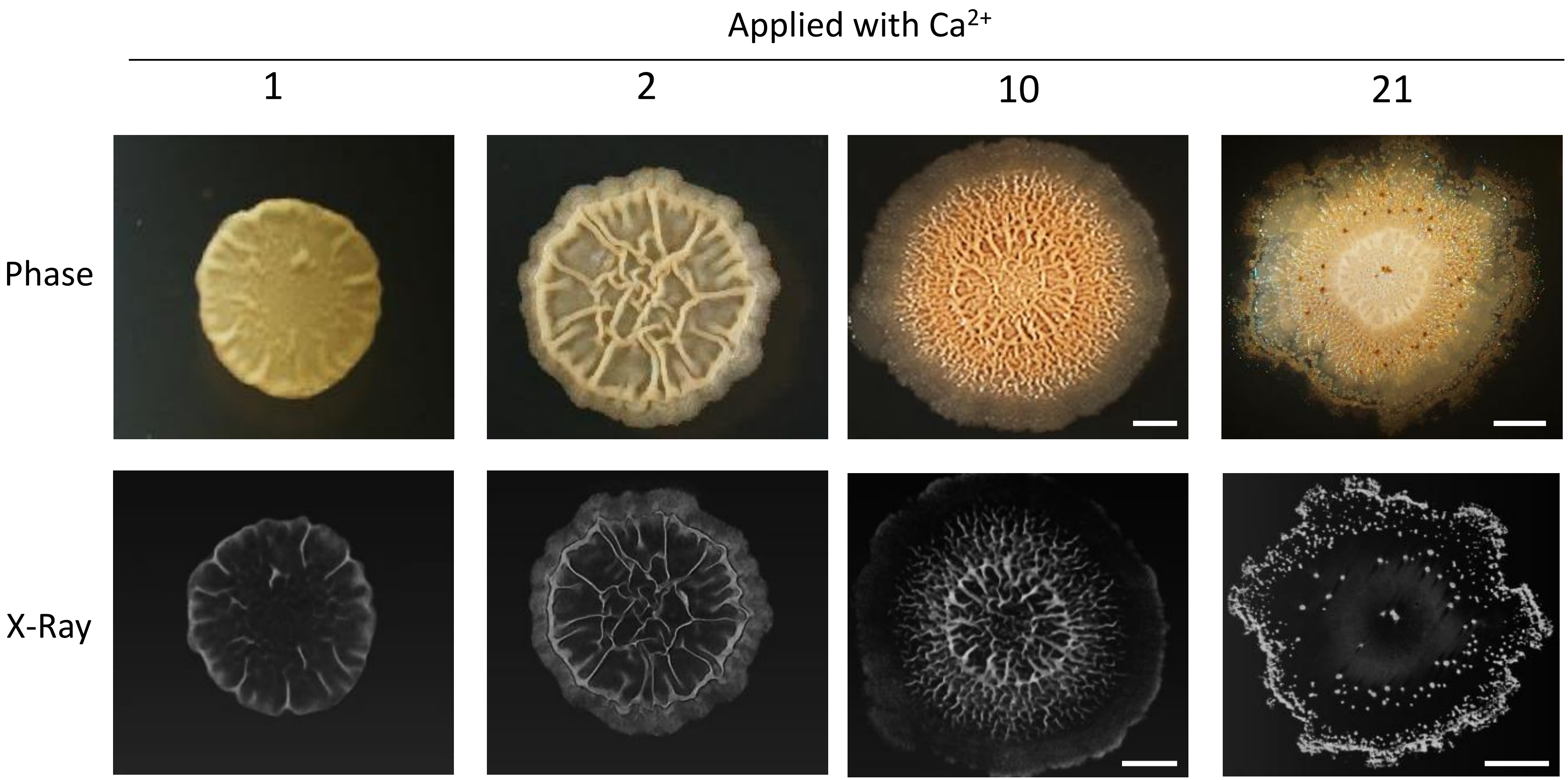
Aragonite

Vaterite

FTIR



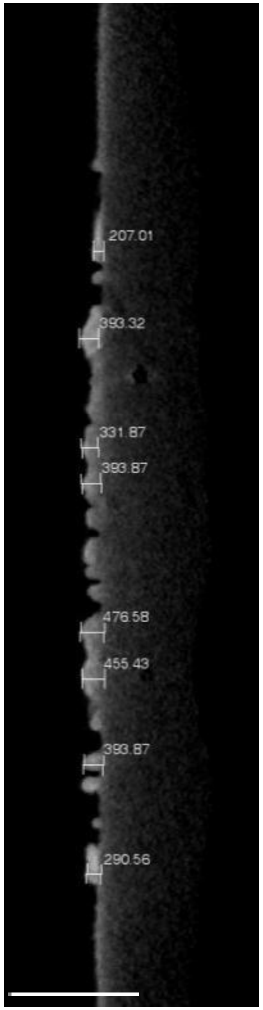
Are mineral scaffolds involved in morphogenesis of bacterial biofilms?

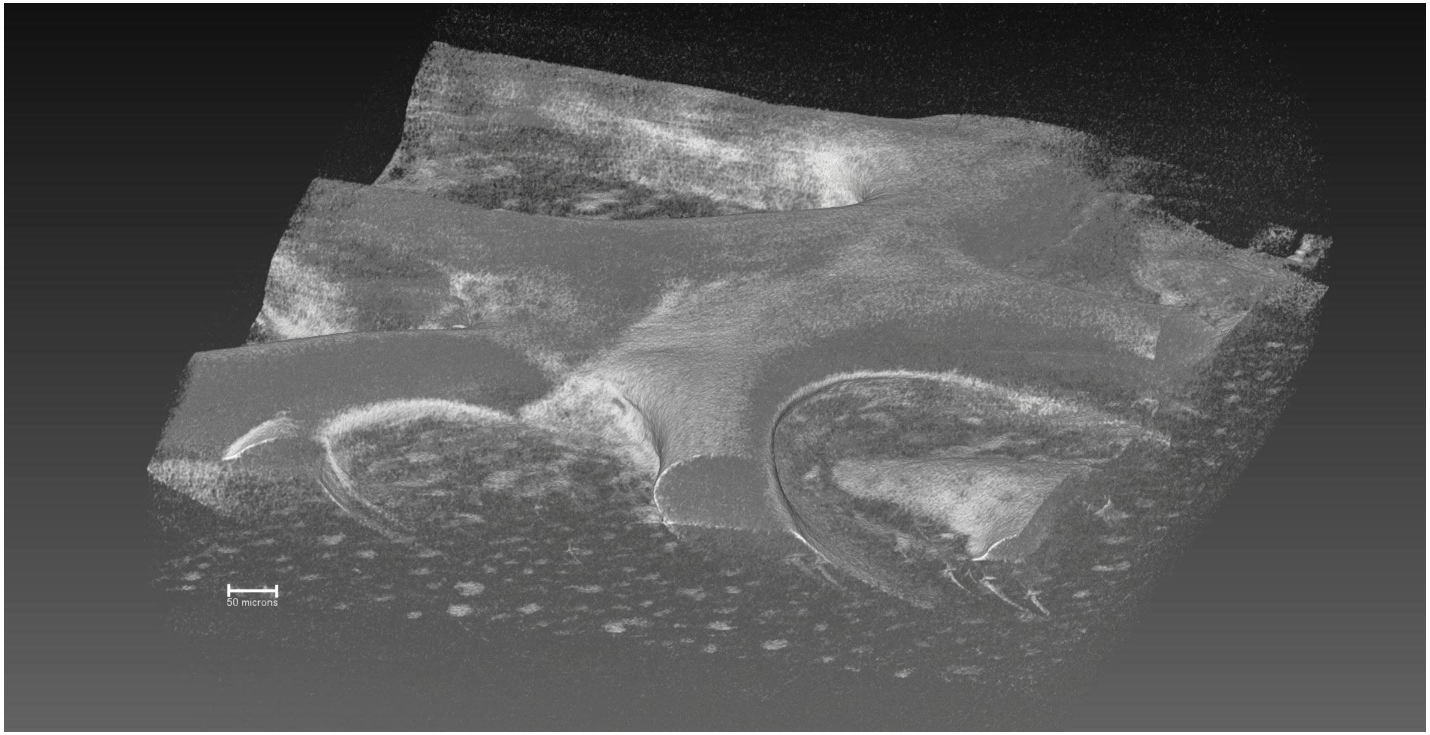


Minerals provide mechanical support throughout the wrinkles

Mineral thickness

Wild type





Do mutants defective in biomineralization have a developmental defect?

Urease

Urea

1 $CO(NH_2)_2 + H_2O \rightarrow CO_2 + 2NH_3$

Ammonia

2 $2NH_3 + CO_2 + H_2O \rightarrow 2NH_4^+ + CO_3^{2-}$ ↑ pH

Spontaneous

3 $HCO_3^- \leftrightarrow CO_3^{2-} + H^+$

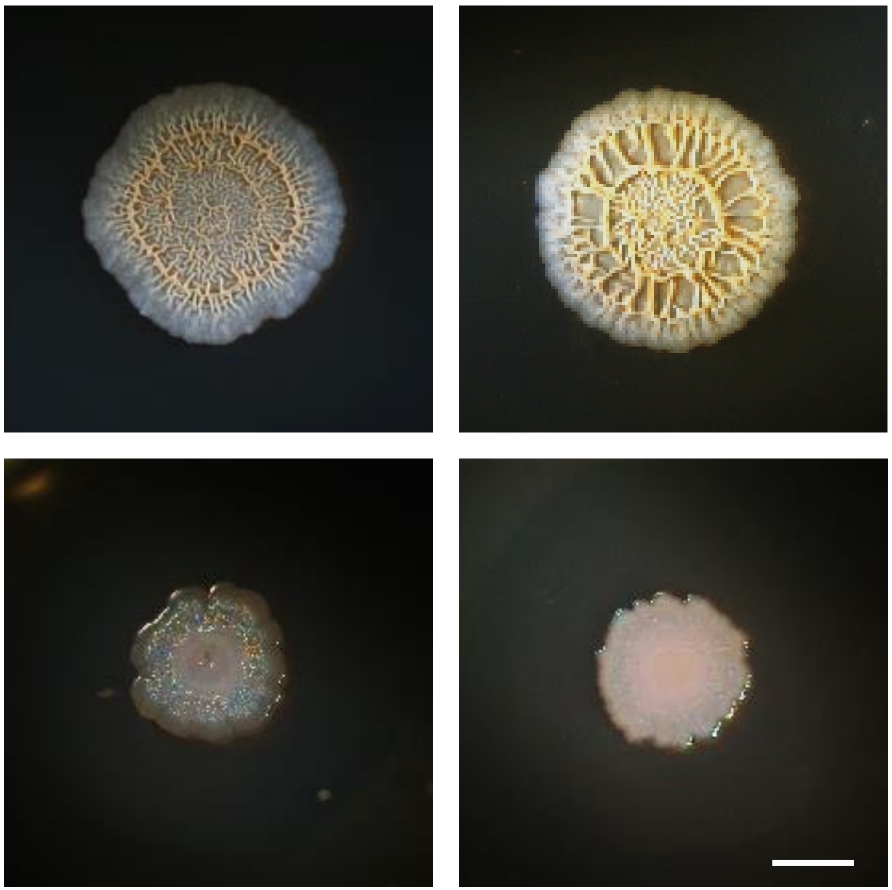
4 $Ca^{2+} + CO_3^{2-} \leftrightarrow CaCO_3$

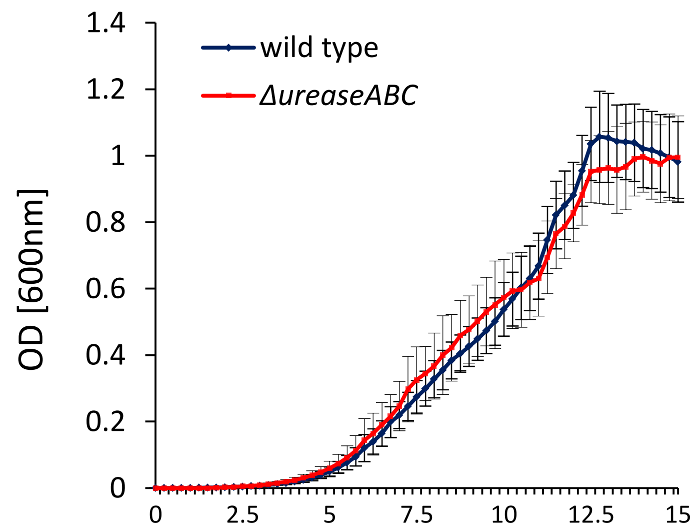
Do mutants defective in biomineralization have a developmental defect?

pH 5.5 pH 7.0

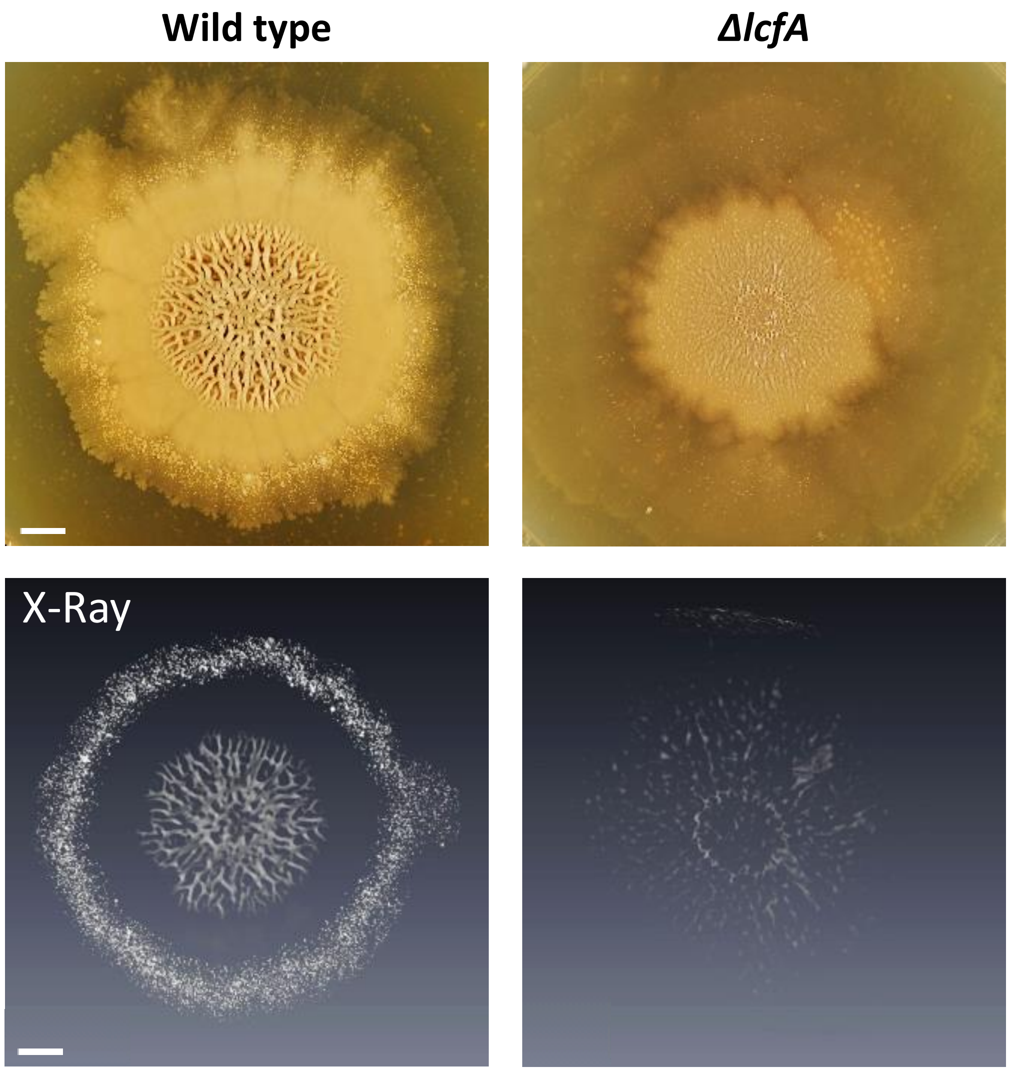
Wild type

ΔureA-C

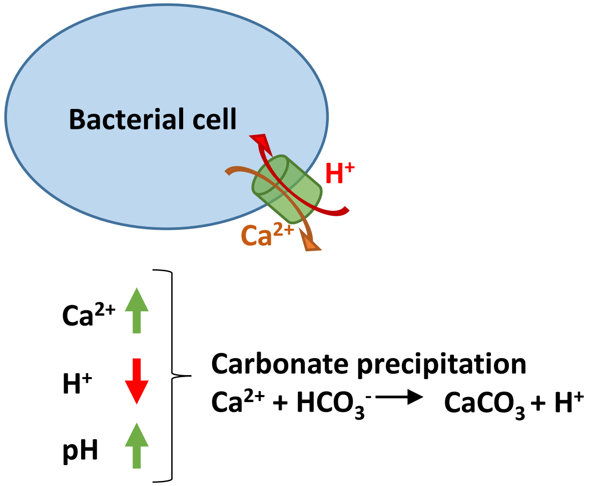




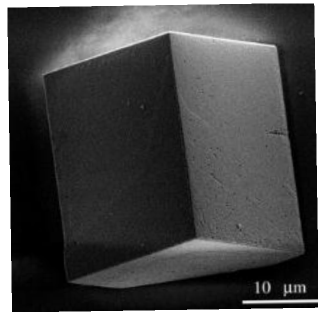
Mutants defective in MVPs synthesis have a biomineralization and a morphology defect

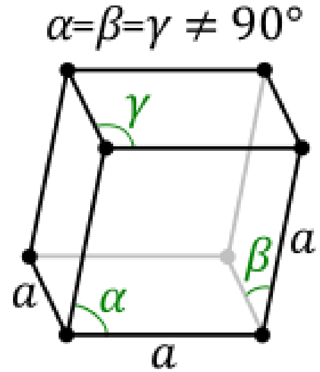


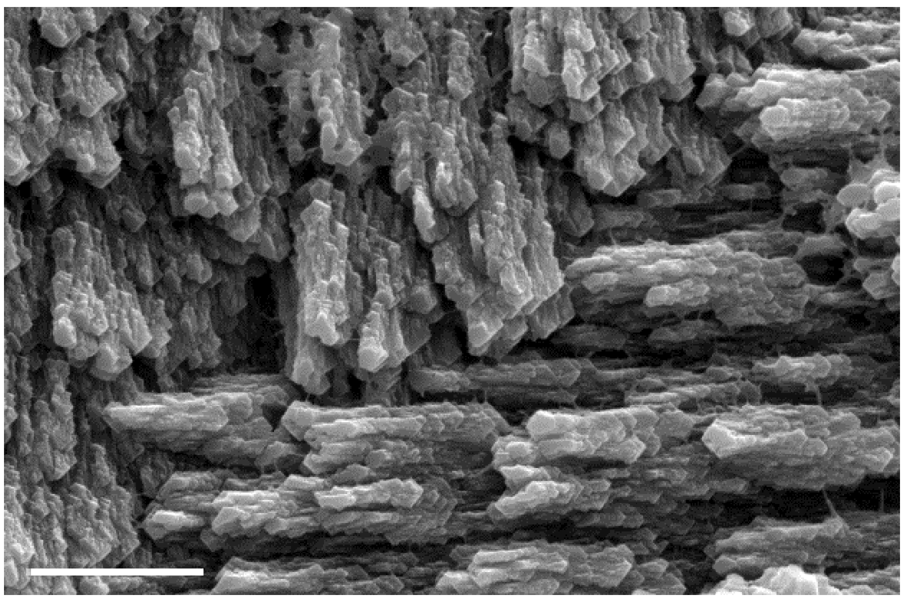
lcfA serves to compartmentalize the membrane



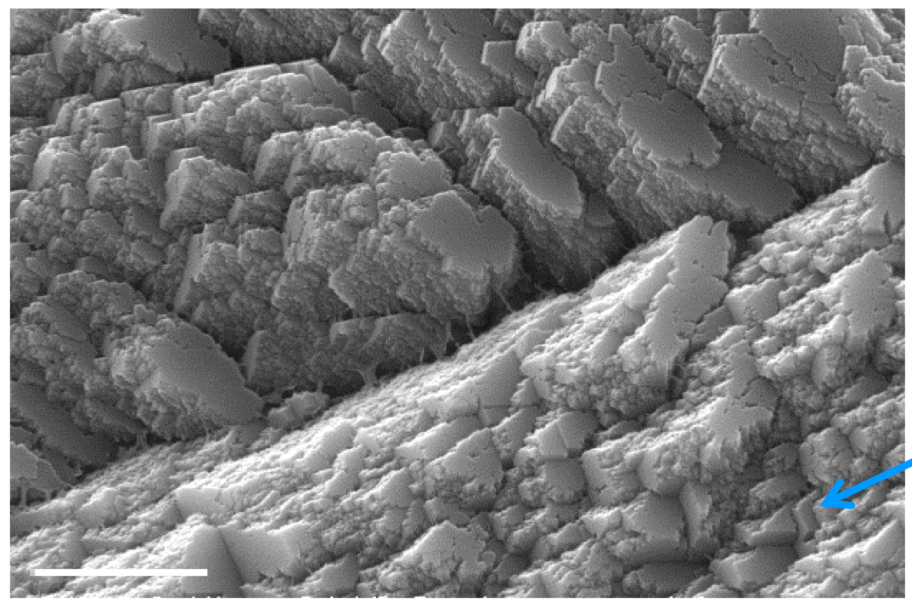
Calcite morphology is determined via interaction with charges EPS





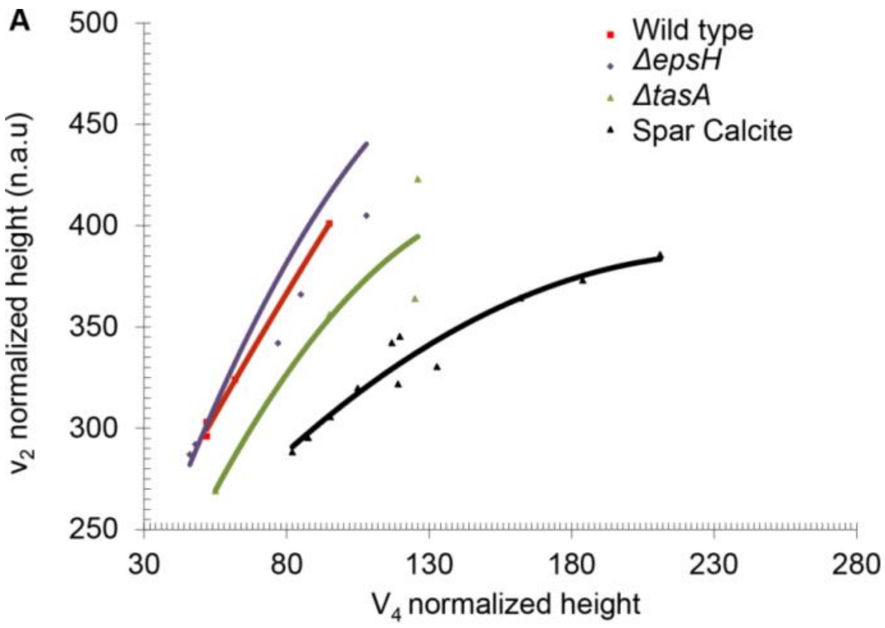


Wild-Type



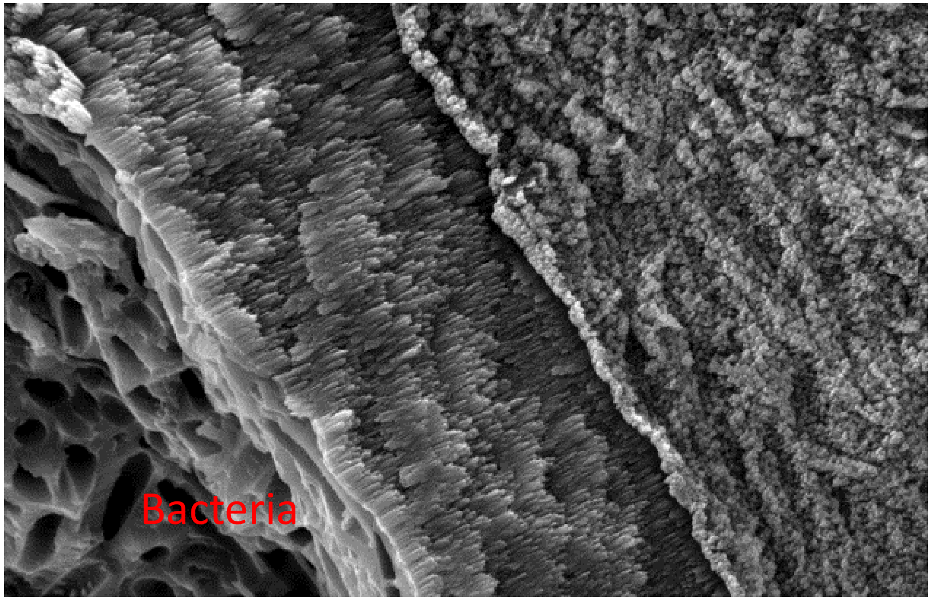
ΔywqC-F

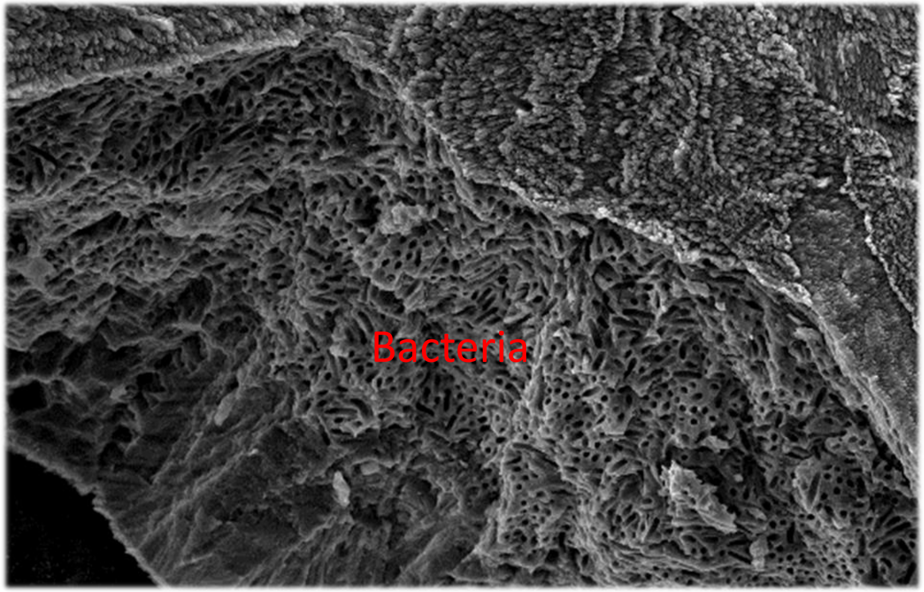
ywqC-F **is a novel operon responsible for producing negatively charged exopolysaccharides that have a significant influence on the calcite morphology**



Calcite morphology is determined via interaction with charges EPS

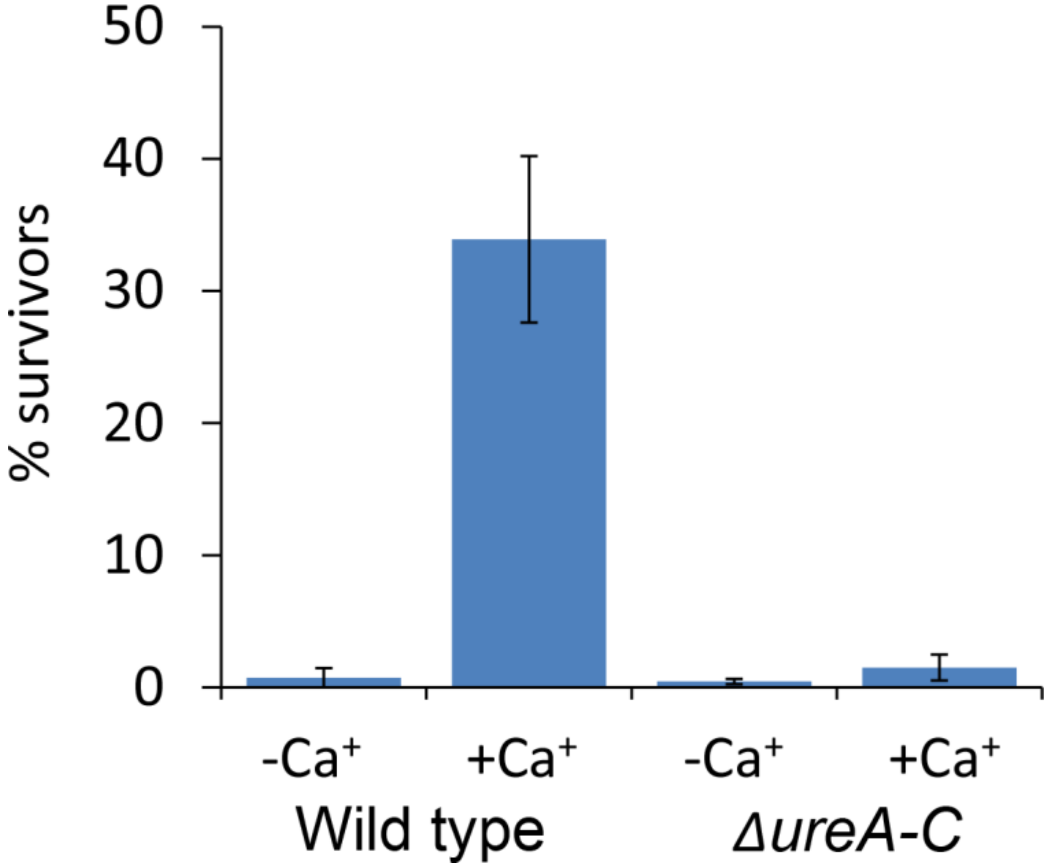
Calcite crystals provide phenotypic Antibiotic resistance?



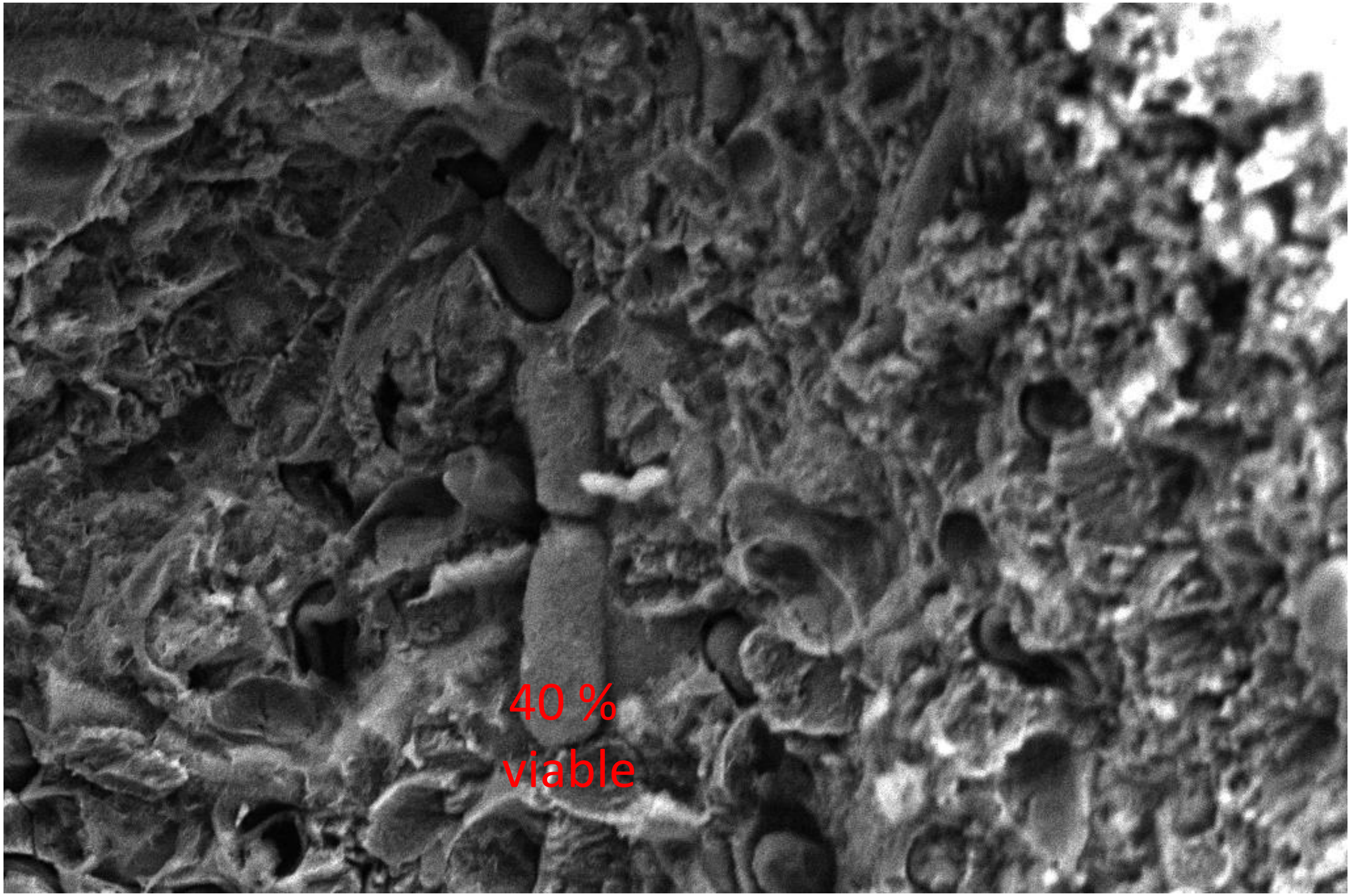


Water Diffusion coefficient:
Polysaccharides film (cellulose)- 4-14 $\times 10^{-8}$ cm^2/sec
Calcium carbonate- 20 $\times 10^{-6}$ cm^2/sec (May-Crespo *et al.*, 2010)
Calcite crystal- up to 5 $\times 10^{-2}$ cm^2/sec (*Alkattan et al.*, 1998)

Mineral accumulation provides phenotypic resistance to 70% Ethanol

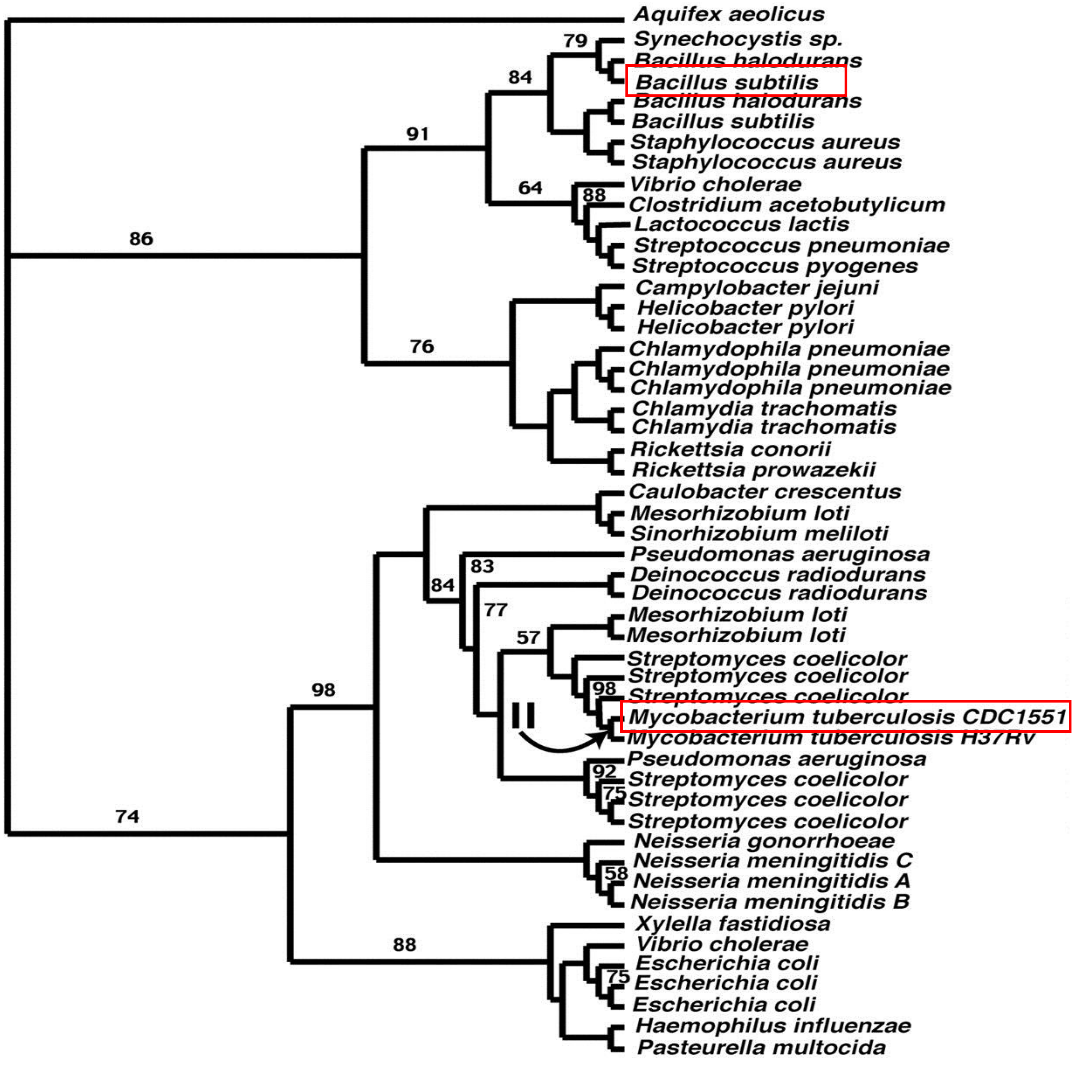


Living bacteria reside within mineral scaffolds



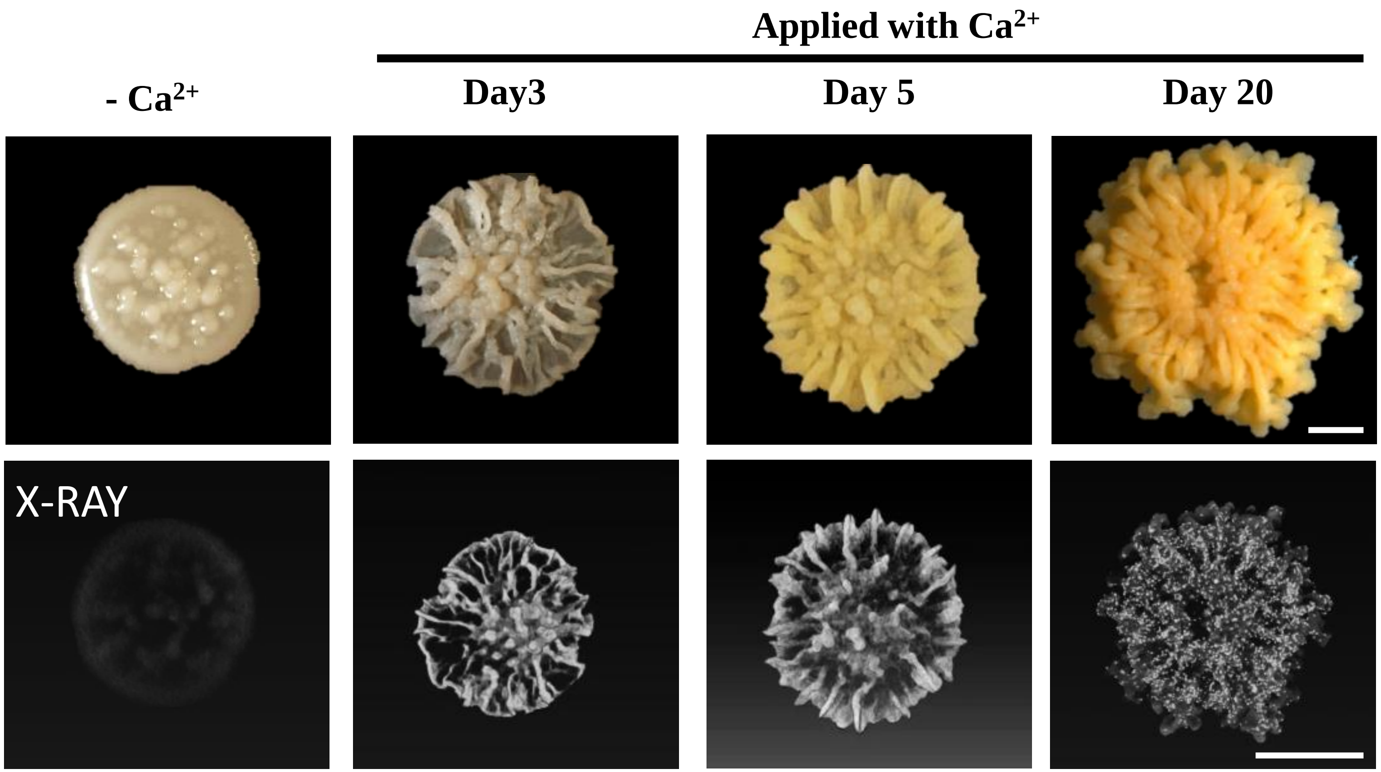
Can mineral precipitation promote morphogenesis in other bacterial species?

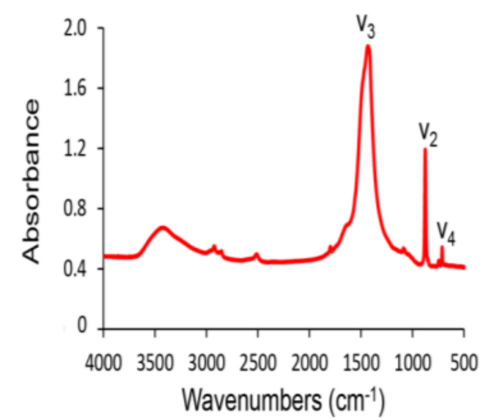
Phylogenetic tree



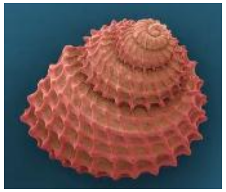
Kinsella R J et al. PNAS 2003

Calcite scaffolds promote morphogenesis in *Mycobacterium smegmatis*





Summary



- Rigidity and structuring of bacterial biofilms requires biologically controlled biomineralization
- ECM is essential, but Calcite scaffolds are also essential for bacterial development.
- The organic and non-organic scaffolds strongly shape each other
- **How are bacteria survive within minerals?**
- **Bacterial "Osteoblasts"?**

Oppenheimer-Shaanan & Sibony-Nevo et al, Under review

Current Lab members

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Kamin program for R & D
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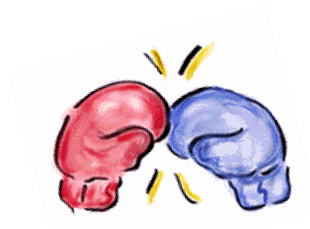
Prof. Stephen Weiner, Prof. Lia Addadi, Prof. Michael Meijler, Prof. Rotem Sorek, Prof. Avraham Shenzer, Prof. Ian Baltimore (Max Plank), Dr. Shmuel Rubinstein (Harvard)

How is flexibility achieved?

B. subtilis

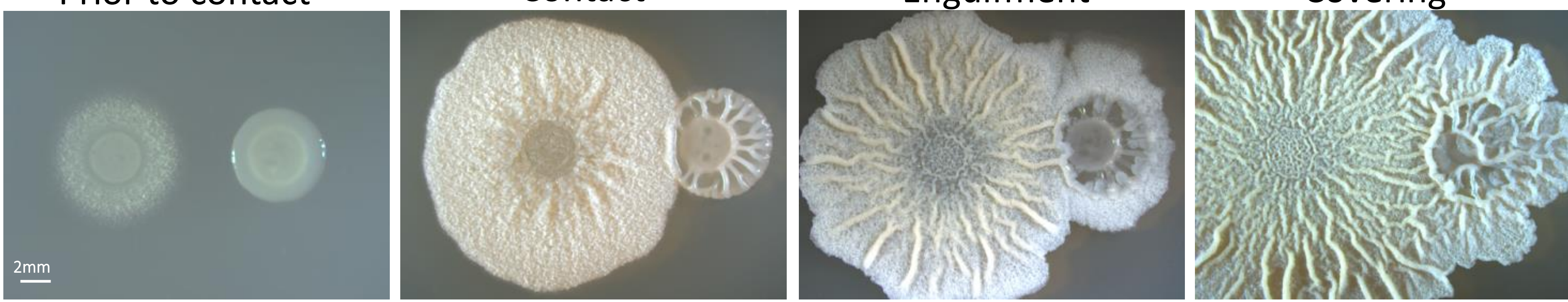
B. simplex



What happens when two complex biofilms meet?

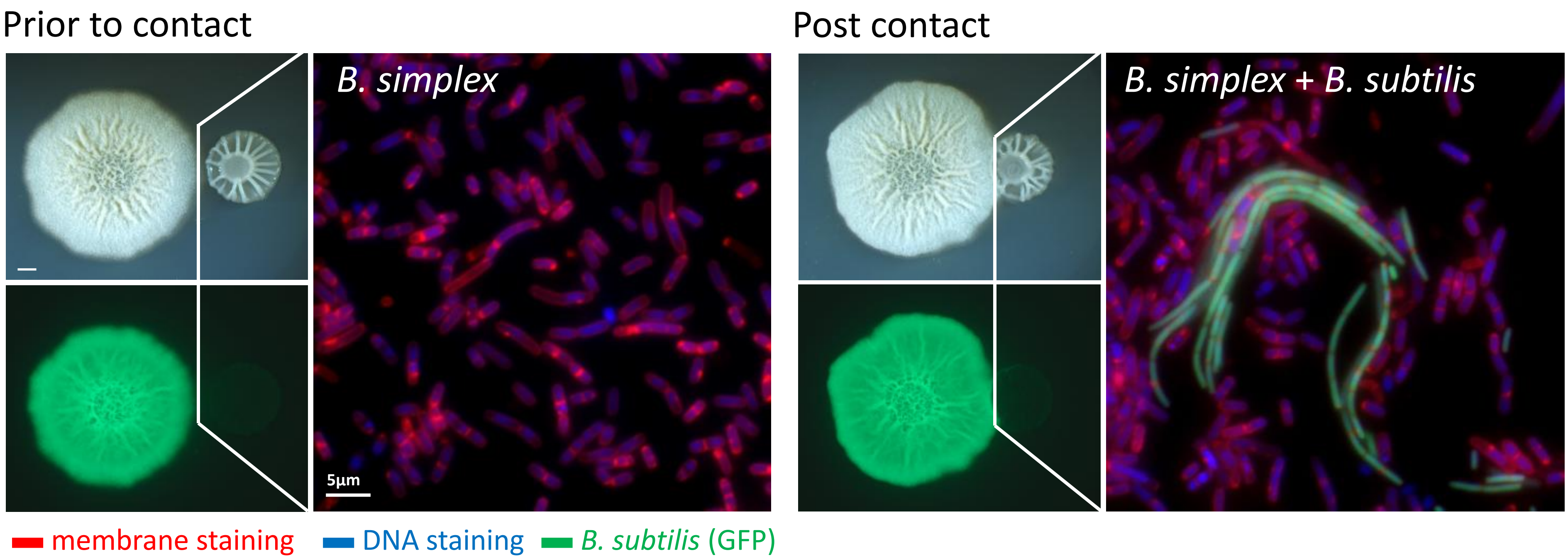
The interaction between *B. subtilis* and *B. simplex* occurs in coordinated stages

Prior to contact | Contact | Engulfment | Covering

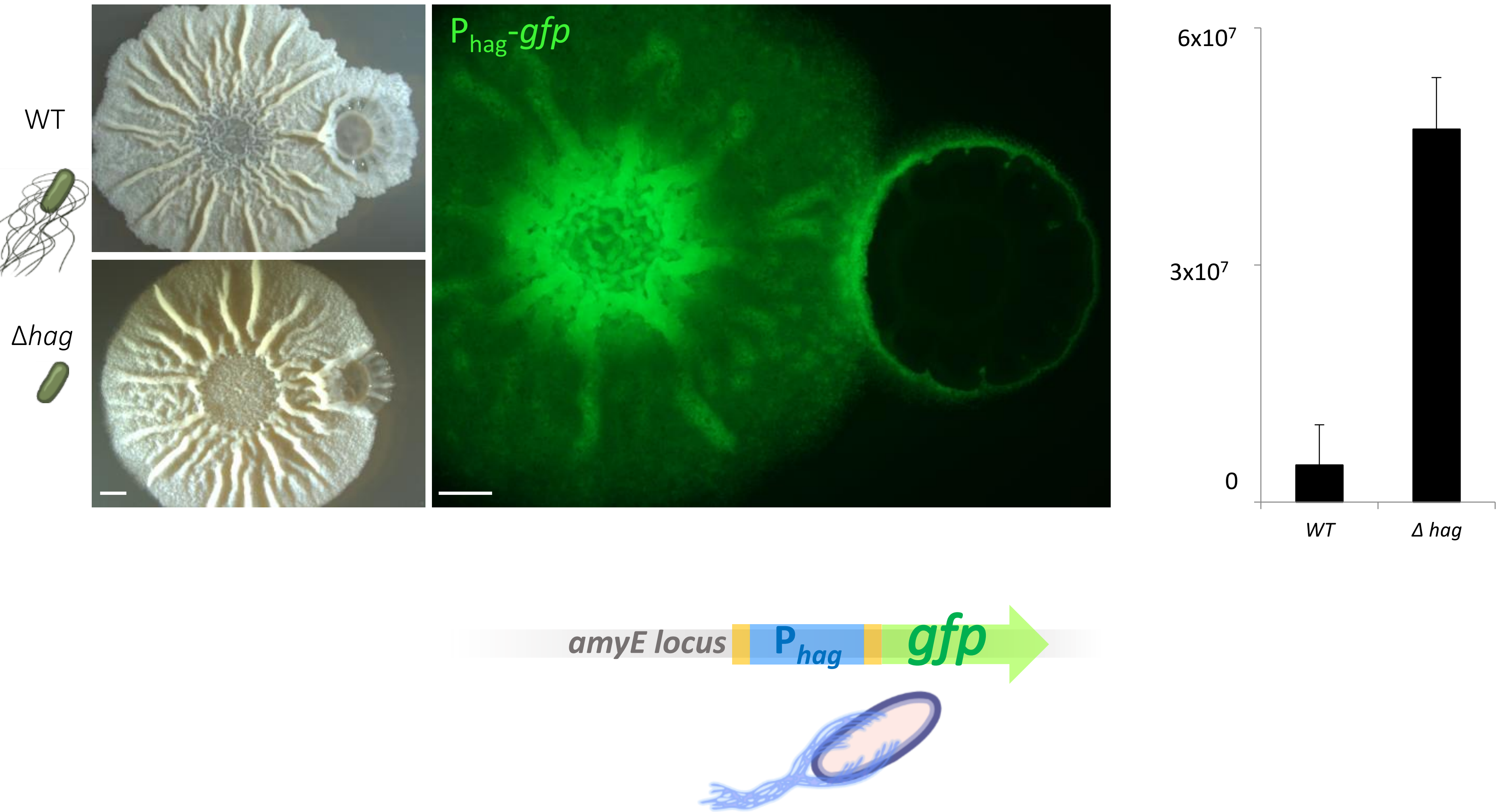


24h | 48h | 72h | 96h

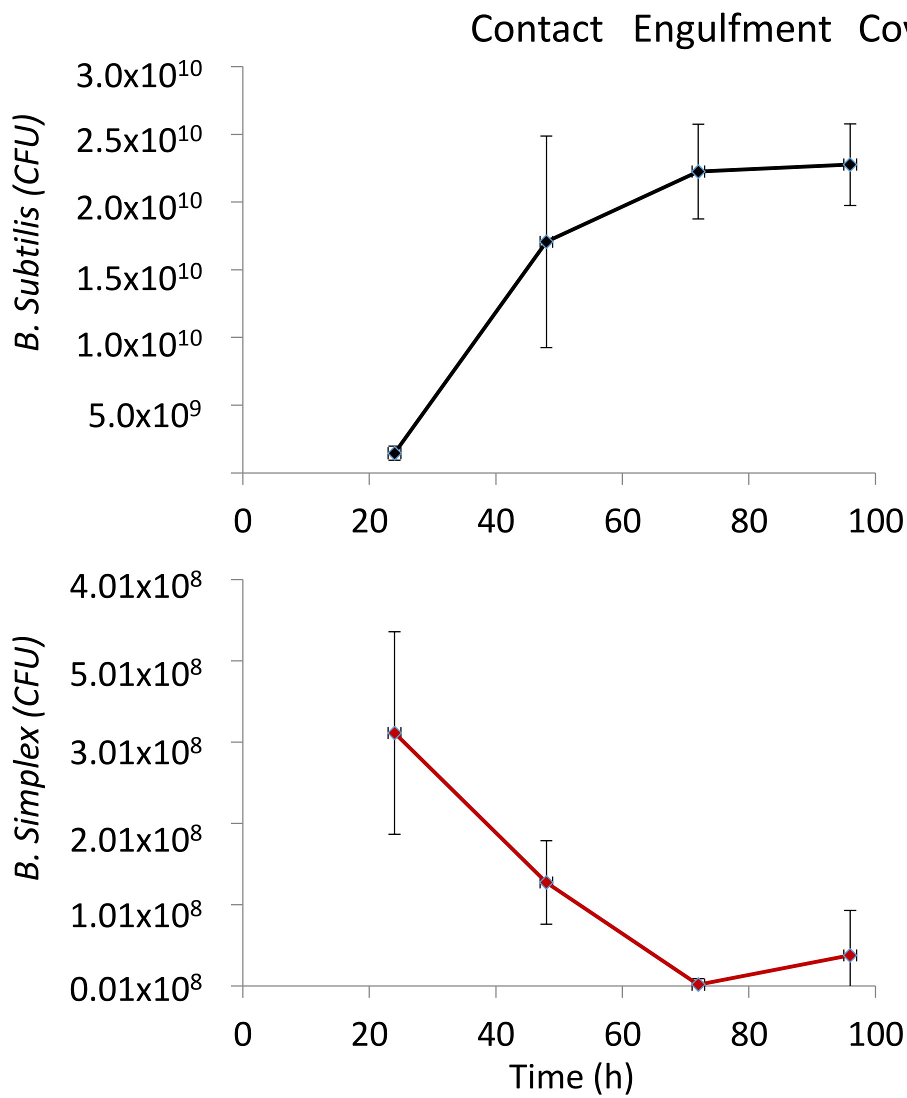
Stage 1: Contact between the two biofilms leads to the invasion of *B. subtilis* into *B. simplex* colony

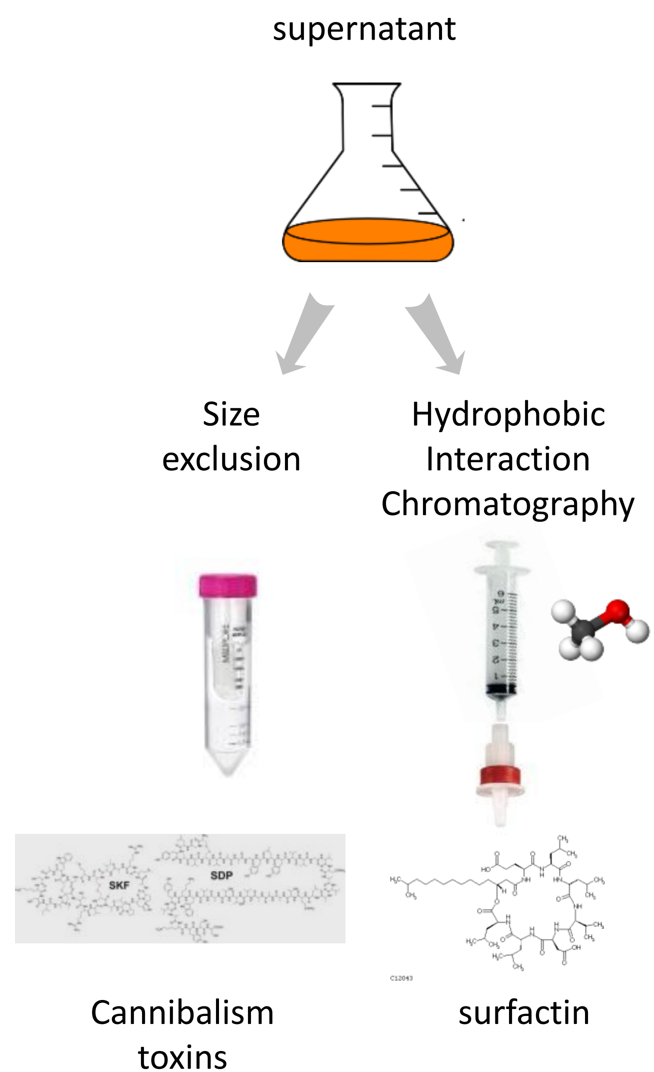


Stage 2: *B. subtilis* engulfs the biofilm of *B. simplex* by recruiting flagellated motility



Stage 3: *B. simplex* population is eradicated by secretion of small diffusible molecules by *B. subtilis*

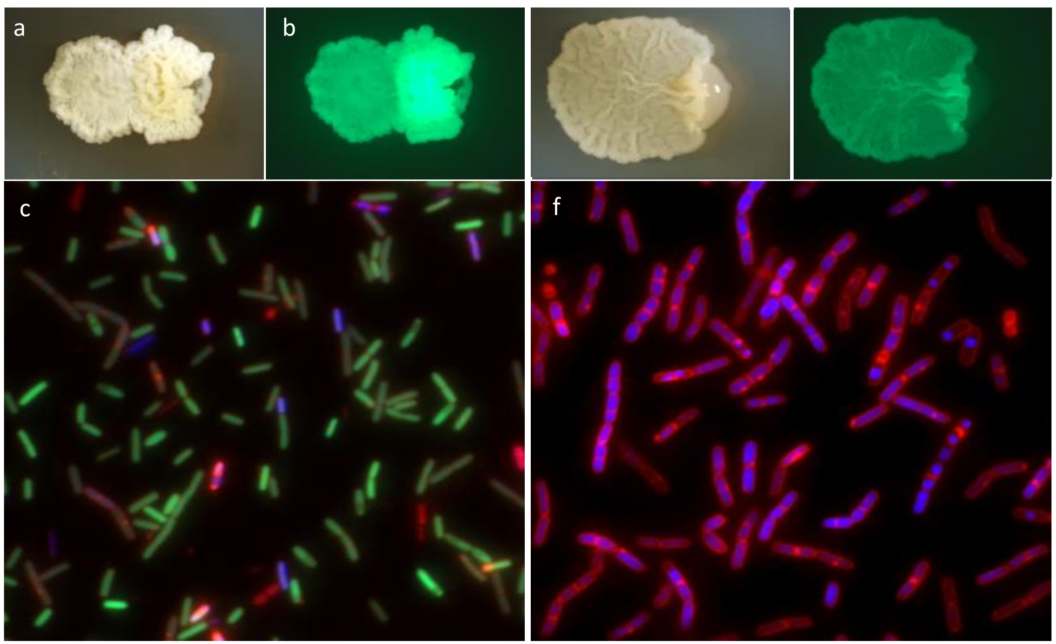


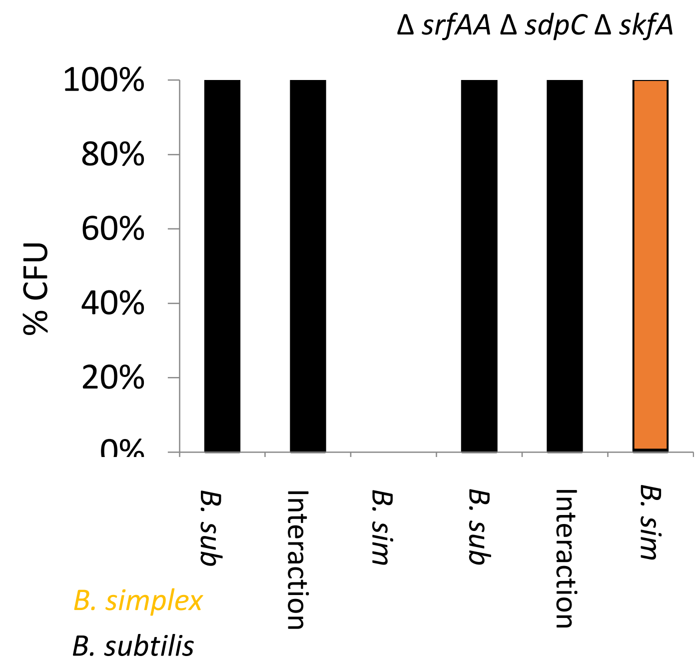


Stage 3: *B. simplex* population is eradicated by secretion of Surfactin and "cannibalism" toxins by *B. subtilis*

WT

Δ Sdp Δ skf Δ srfAA

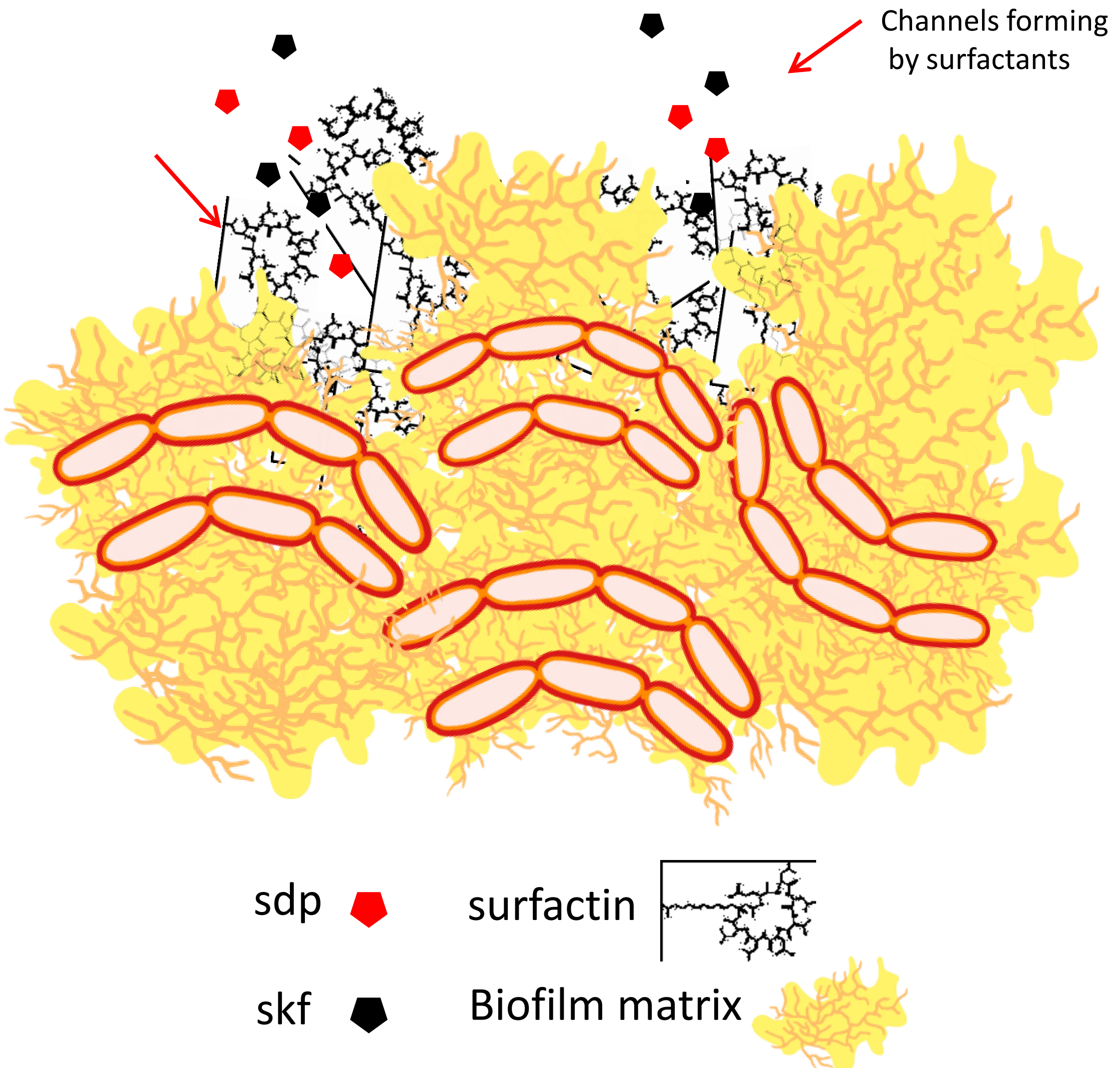




Novel roles for molecules previously considered solely as biofilm regulators

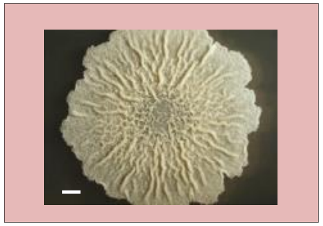
surfactin

SdpC & SkfA
Cannibalism
toxins

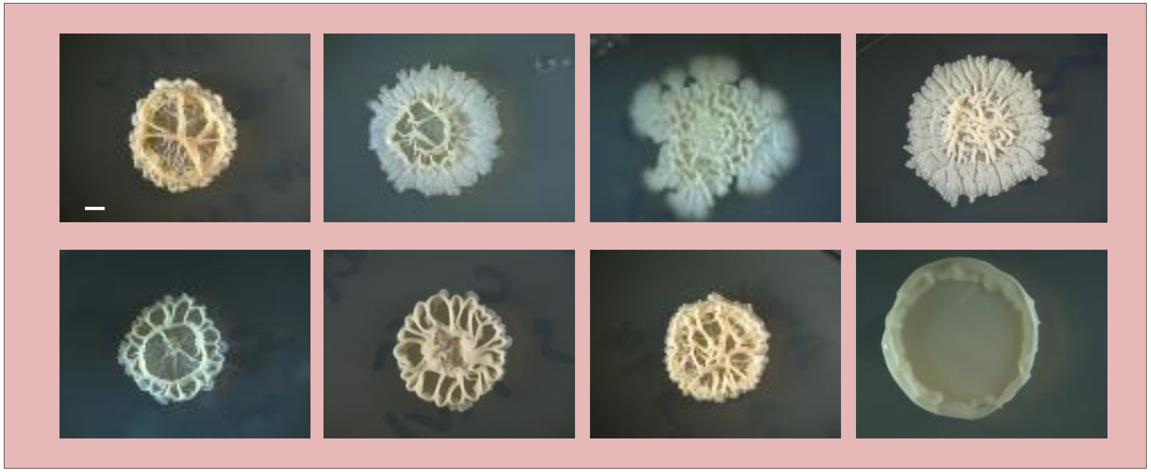


Stage 4: *B. subtilis* population changes genetically in the course of the interspecies interaction

WT



Interaction "mutants"



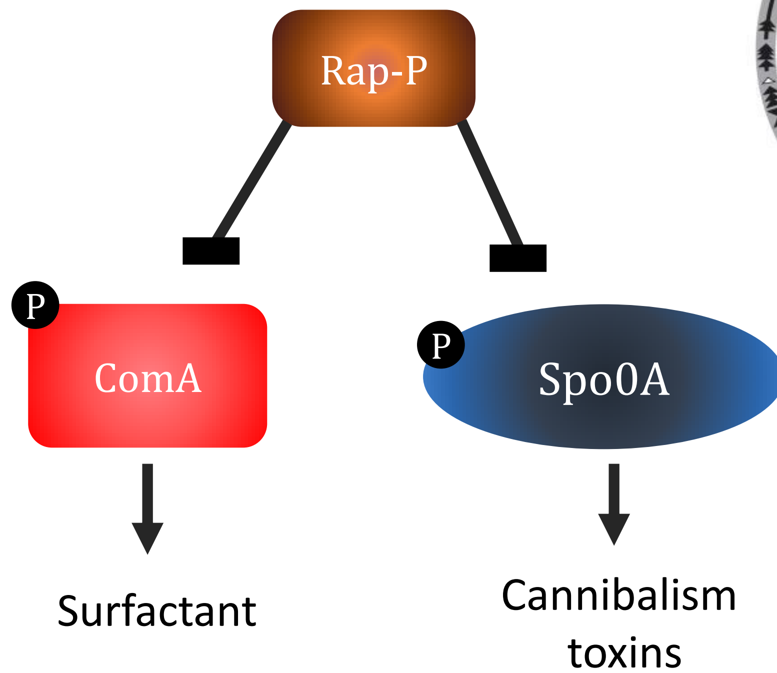
Whole genome sequencing of the strains derived from the interspecies interaction had no chromosomal mutations

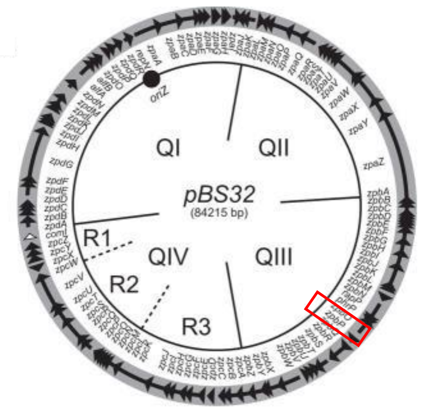
Loss of *B. subtilis* natural plasmid leads to the hyperrugose biofilm phenotype reminiscent of interaction-derived strains

WT (3610)

Plasmid cured

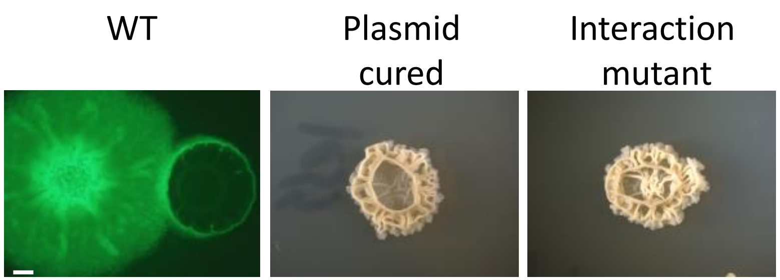
Interaction derived strain

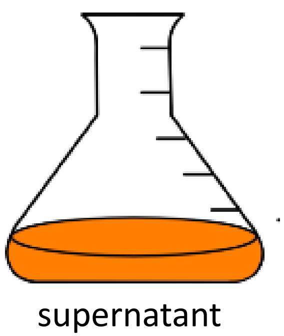


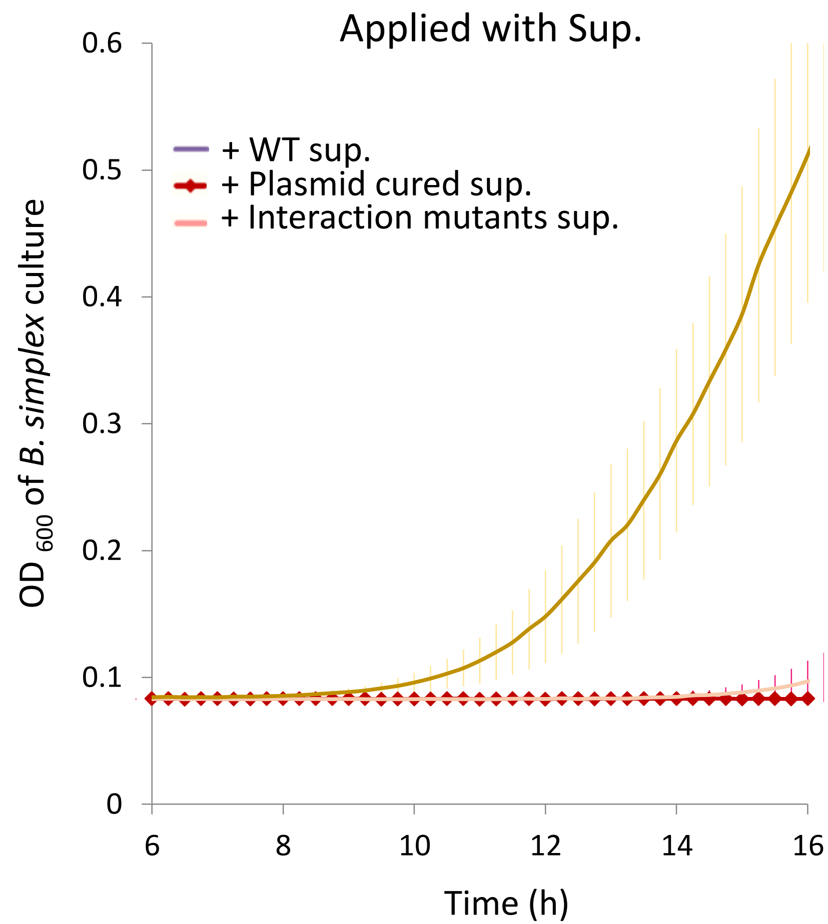


Neiditch MB *et al.*, 2013

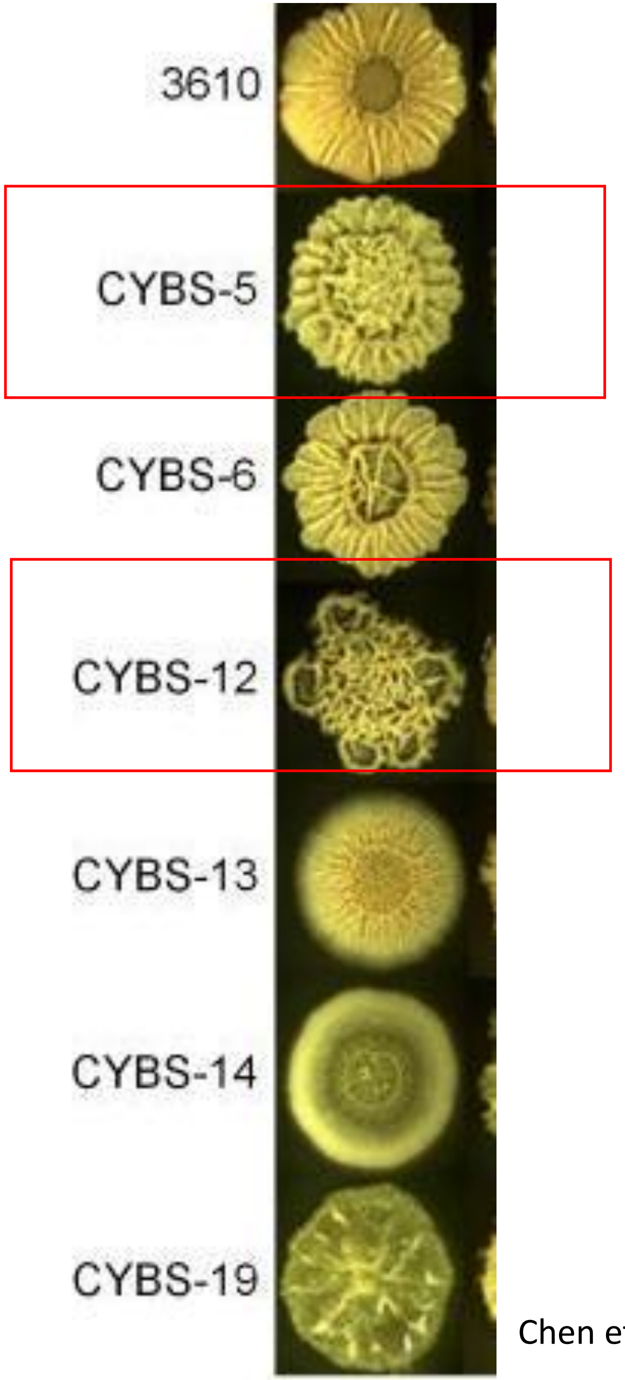
Stage 5: The loss of *B. subtilis* natural plasmid increases the virulence factors that allow the elimination of *B. simplex* biofilms







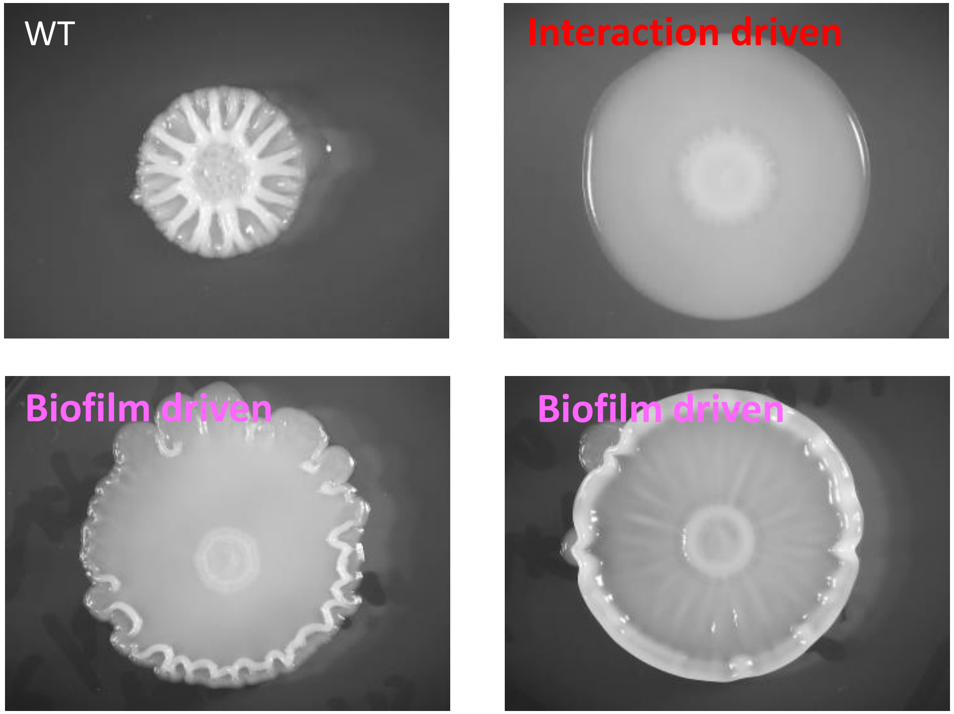
The loss of *B. subtilis* natural plasmid may be common among root-associated *B. subtilis*



We Suspect plasmid loss occurs for Isolates with Enhanced biocontrol properties

Chen et al., 2011

B. simplex population changes genetically in the course of the interspecies interaction



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B. simplex - mutation analysis

Mutant	Protein	Affect	Change
Mut 1	Spo0A	Nonsynonymous	G643A
Mut 2	Spo0A	Frameshift deletion	534_535del
Mut 3	Spo0A	Nonsynonymous	T193G
Mut 4	Tyrosine - protein kinase EpsD	Frameshift deletion	556_559del
Mut 5	UDP - glucose dehydrogenase	Frameshift deletion	918delA

Strategies to enable flexibility of *Bacillus subtilis* biofilms to improve competition during interspecies interactions

- Reservoir of motile cells is actively maintained to enable spreading to new niches from the rigid structures

- Bet-hedging strategy of plasmid loss enables rising of violent isolates during interspecies interactions

- The changes in the single cell morphology and in the toxin secretion are reversible

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