Bofilm: the essence of a symbiotic system!

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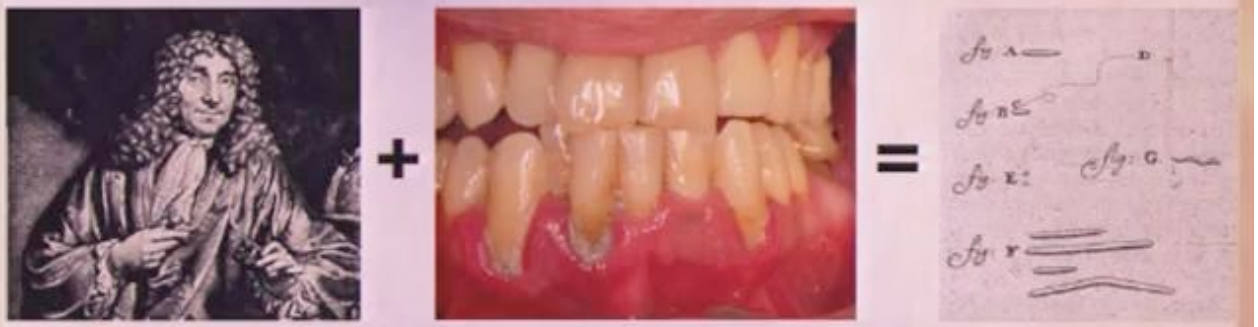
Caravagio 1571-1610

Bofilm: the essence of a symbiotic system!

Early observations: body teeming with microbial life



"...animalcules were in such enormous numbers, that all the water...seemed to be alive." — van Leeuwenhoek (1683)

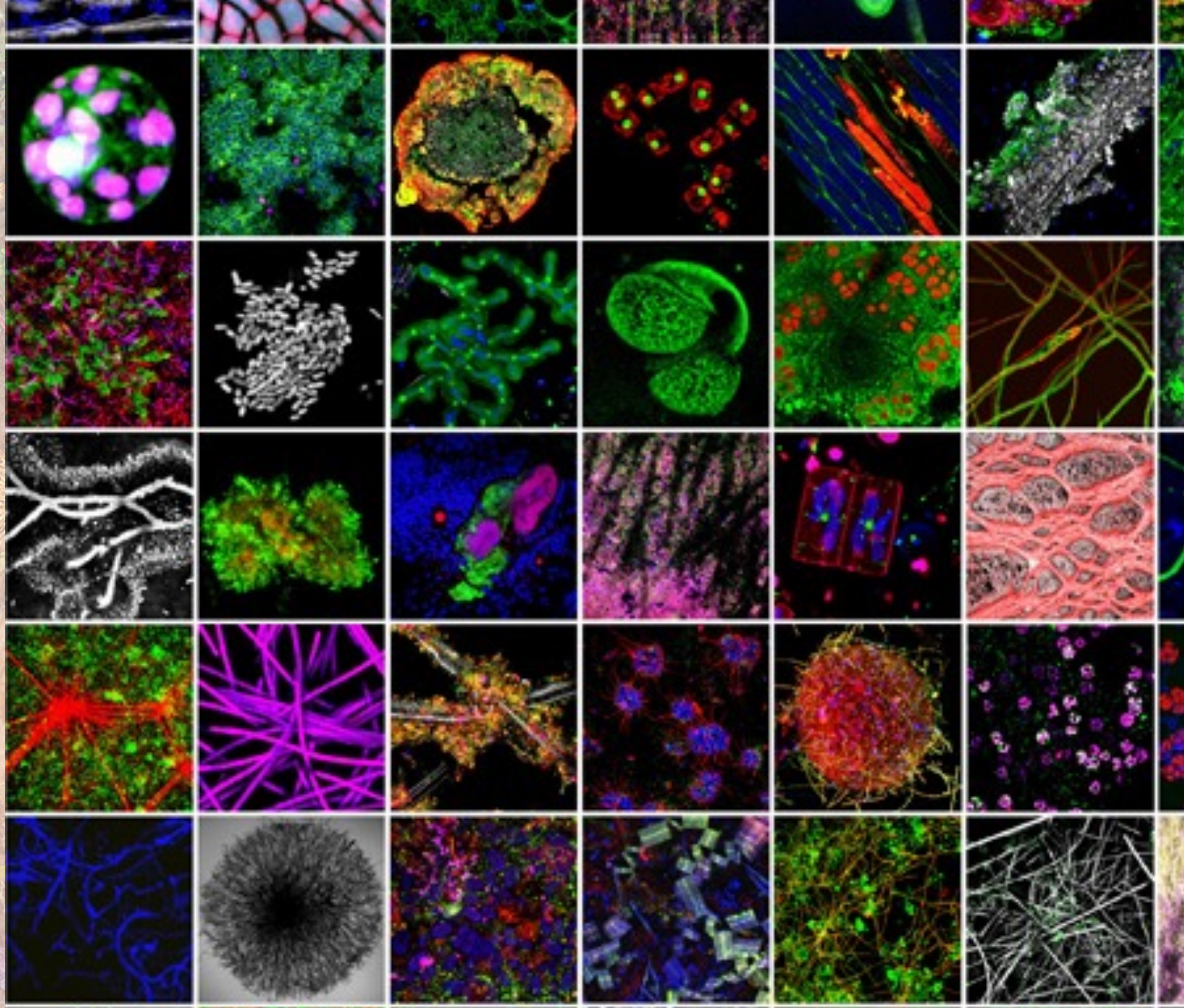
Definition of Biofilm

- Biofilms are communities of microorganisms in a matrix that joins them together and to living or inert substrates

- Biofilms are surface-attached communities of bacteria, encased in an extracellular matrix of secreted proteins, carbohydrates, and/or DNA, that assume phenotypes distinct from those of planktonic cells

- ……..

Biofilms are everywhere



Biofilms are everywhere

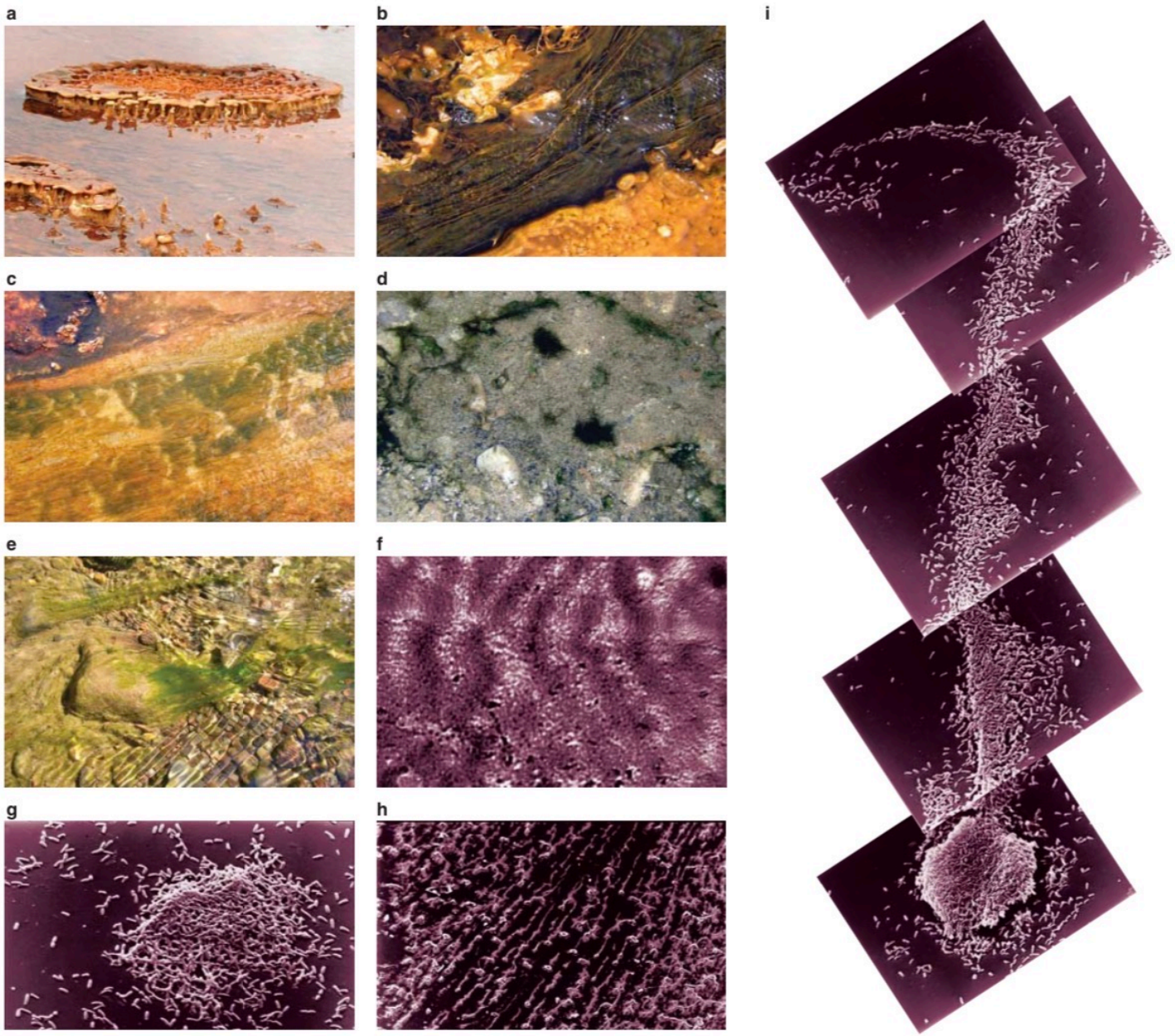


Figure 2 | **Structural similarity of biofilms growing in hydrothermal hot springs, freshwater rivers and laboratory flow cells.** Similar structures are seen in biofilms growing in hydrothermal hot springs (**a**–**c**), biofilms growing in freshwater rivers (**d**,**e**) and laboratory flow cells (**f**–**h**). Biofilms growing in quiescent or low-shear environments tend to form circular structures, such as 'mushrooms' or mounds (**a**,**d**,**g**). Biofilm streamers (**b**,**e**,**h**,**i**) and ripple structures (**c**,**f**) form in faster, high-shear flows. Biofilms are from the Biscuit Basin thermal area, Yellowstone National Park, USA (**a**–**c**), Gardener River, Yellowstone National Park, USA (**d**), Hyalite Creek, Bozeman, Montana, USA (**e**), mixed species biofilm grown at a flow velocity of 1 m s^{-1} (REF. 14) and a *Pseudomonas aeruginosa* PAN067 biofilm grown in a flow cell with a flow of 0.03 m s^{-1} (**g**) or 1 m s^{-1} (**h**, **i**) (REF. 43). Panel **c** is modified with permission from REF. 53 © (2004) ASM Press.

Biofilms are everywhere

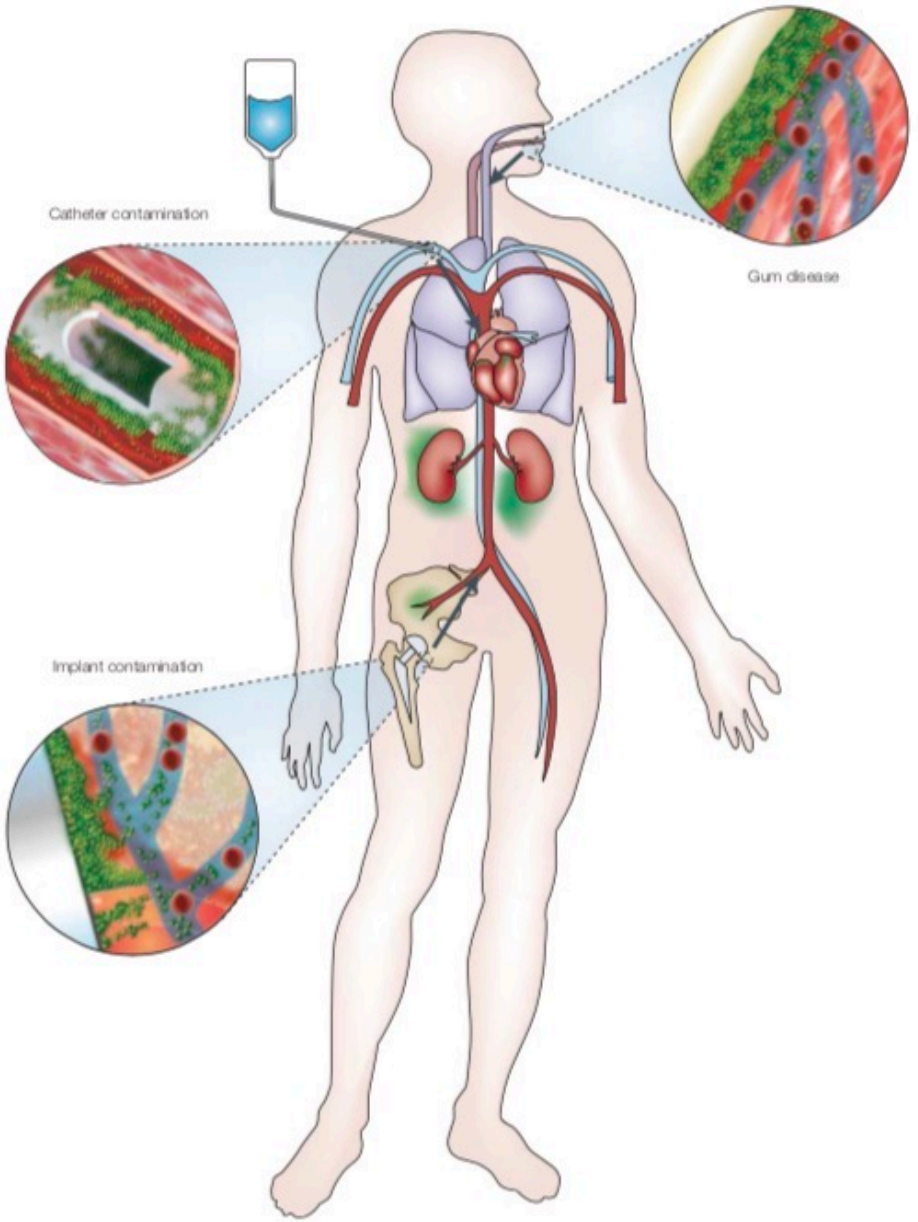
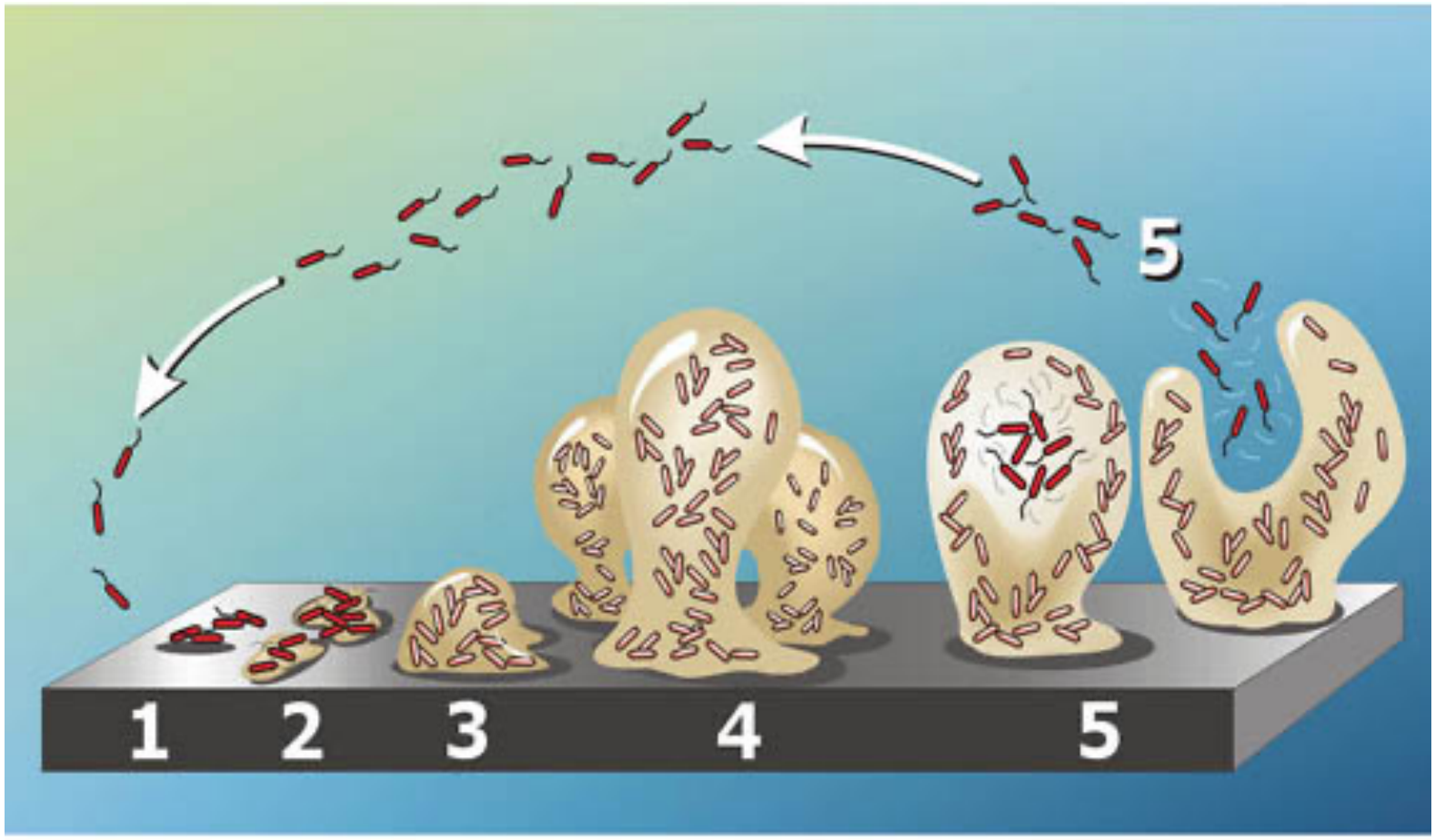
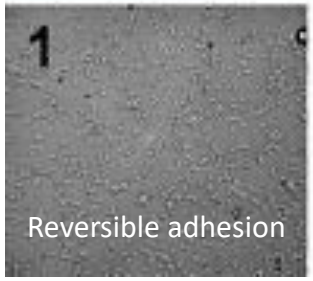
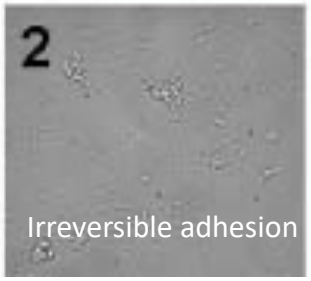


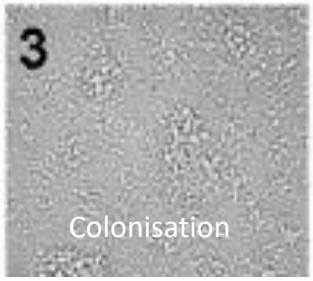
Figure 4 | **Schematic showing three examples of possible points of entry into the body for infectious biofilms; catheter, hip replacement, and periodontal disease.** Arrows show how the biofilm (green) might be disseminated around the body, either by single cells or clumps of protected emboli, using the example of native or artificial heart valve infective endocarditis as a common central location for embolization. Sporadic detachment could lead to cycles of bacteraemia. Image courtesy of P. Dirckx, Center for Biofilm Engineering.

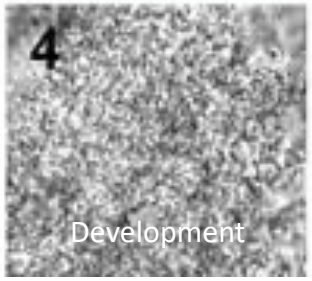
How do biofilms form?

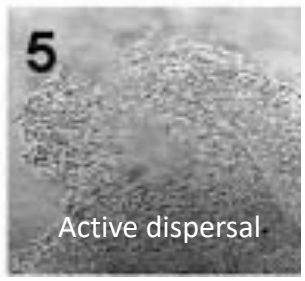




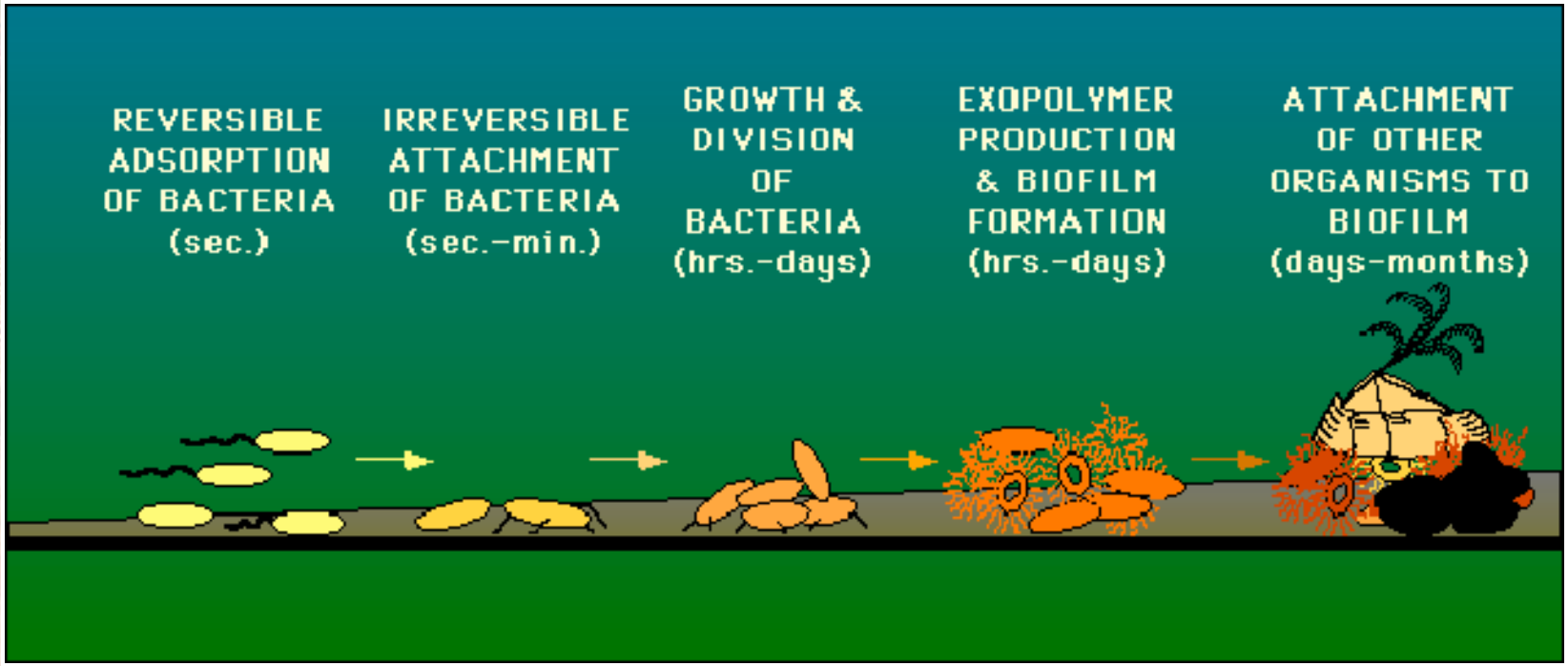








How do biofilms form?



Biofilms are everywhere

How do biofilms form?

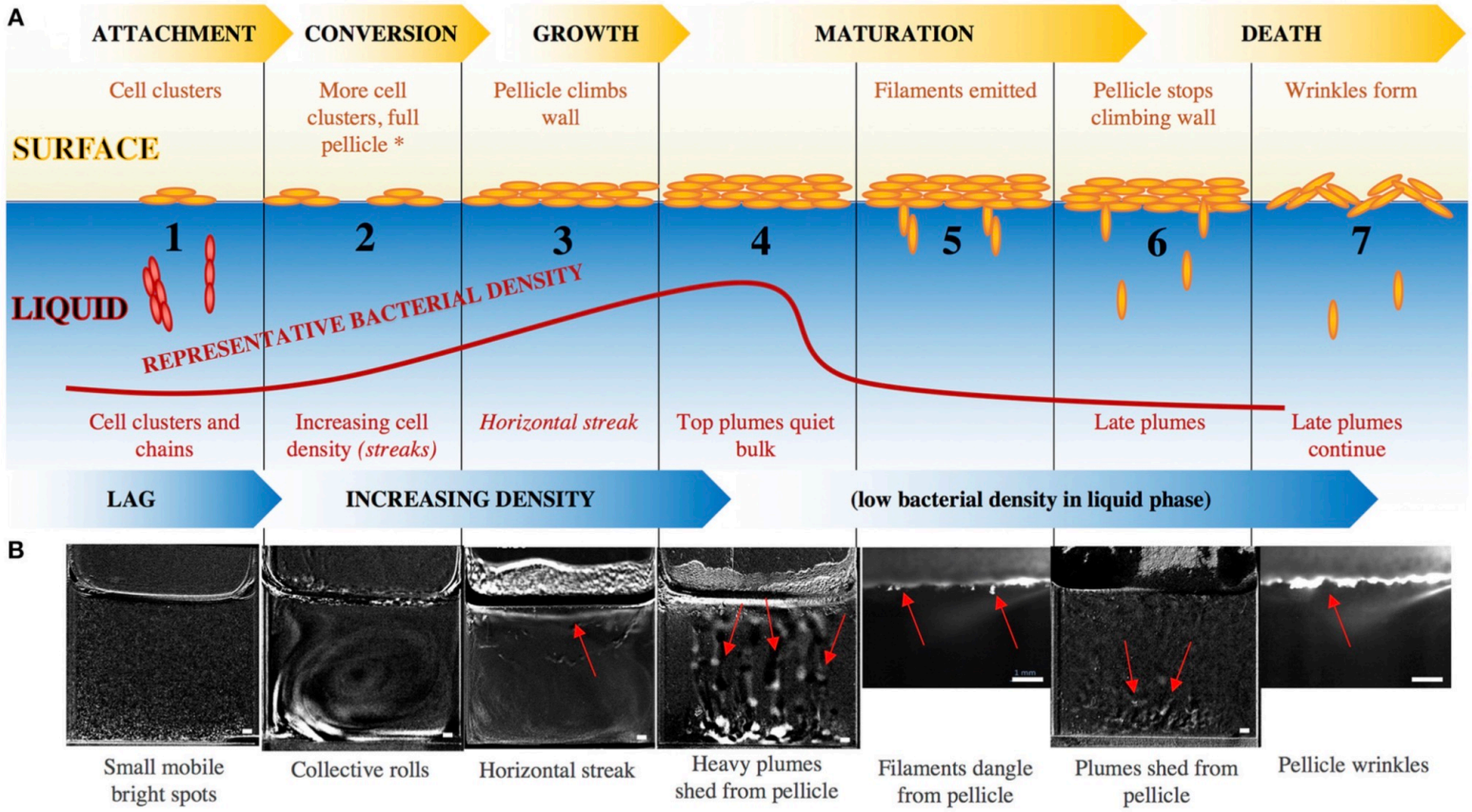


FIGURE 2 | (A) Surface pellicle formation progresses through the standard steps of attachment, conversion, growth, maturation, and dispersal, while the bacterial density in the liquid bulk increases until the formation of a mature surface pellicle, after which the density of bacteria in the liquid drops. The red curve indicates representative OD of the bacteria in the liquid, and captioned are the steps. Beyond stage 2, the motility/chemotaxis mutants maintain the same surface events as the wild-type, but have altered liquid bulk events, indicated by the italics. The asterisk at stage 2 signals the point beyond which EPS mutants do not progress. **(B)** Snapshots of the series of events in the development of a wild-type pellicle. Smaller images (stages 5, 7) are a zoomed-in view using the laser sheet, and larger images (stages 1–4, 6) are views of the entire well with the previous time frame subtracted from the current time frame to emphasize features. Red arrows point to the relevant features. Scale bar is 1 mm. For larger images, see **Figure S5**.

How do biofilms form?

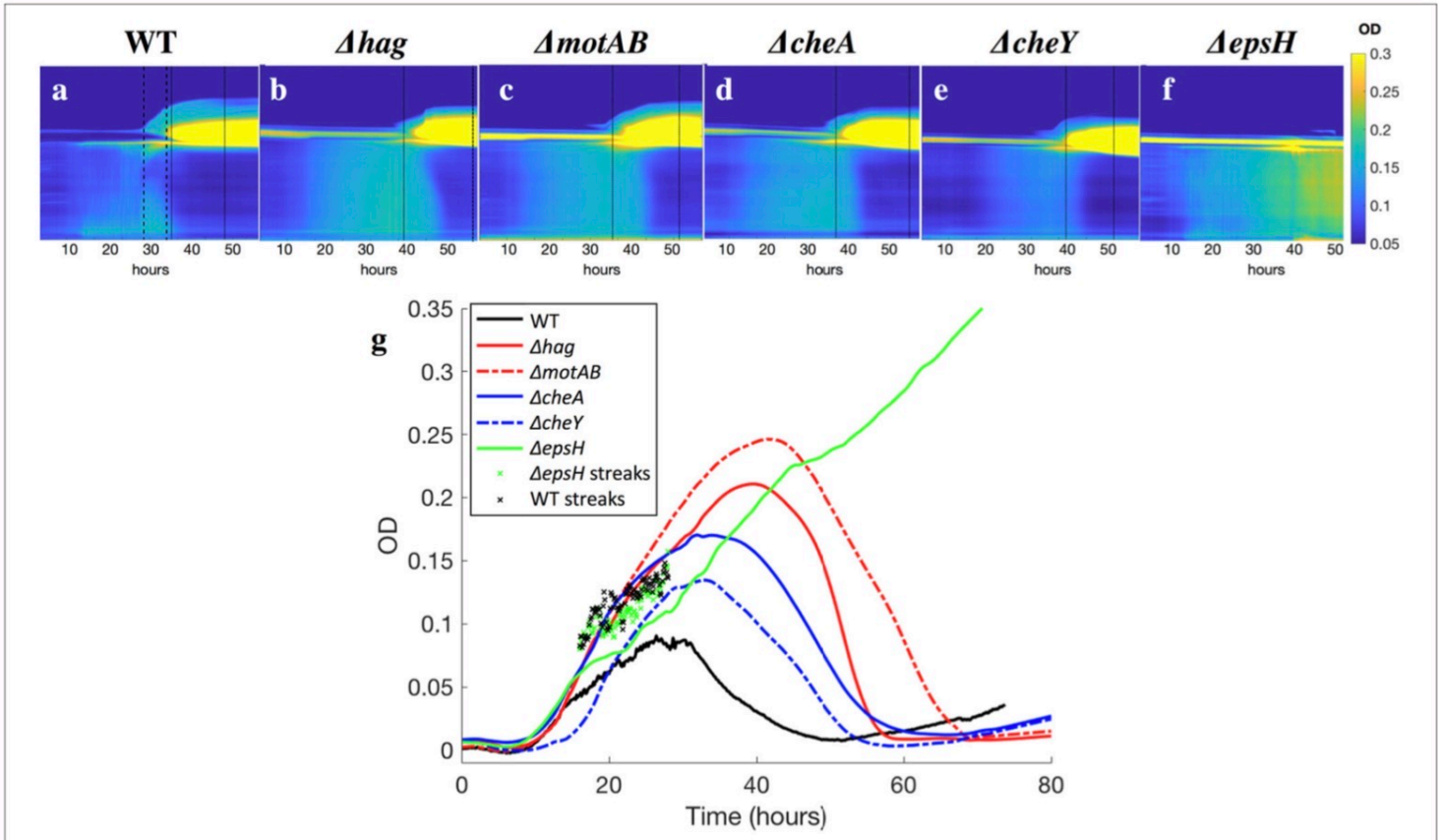
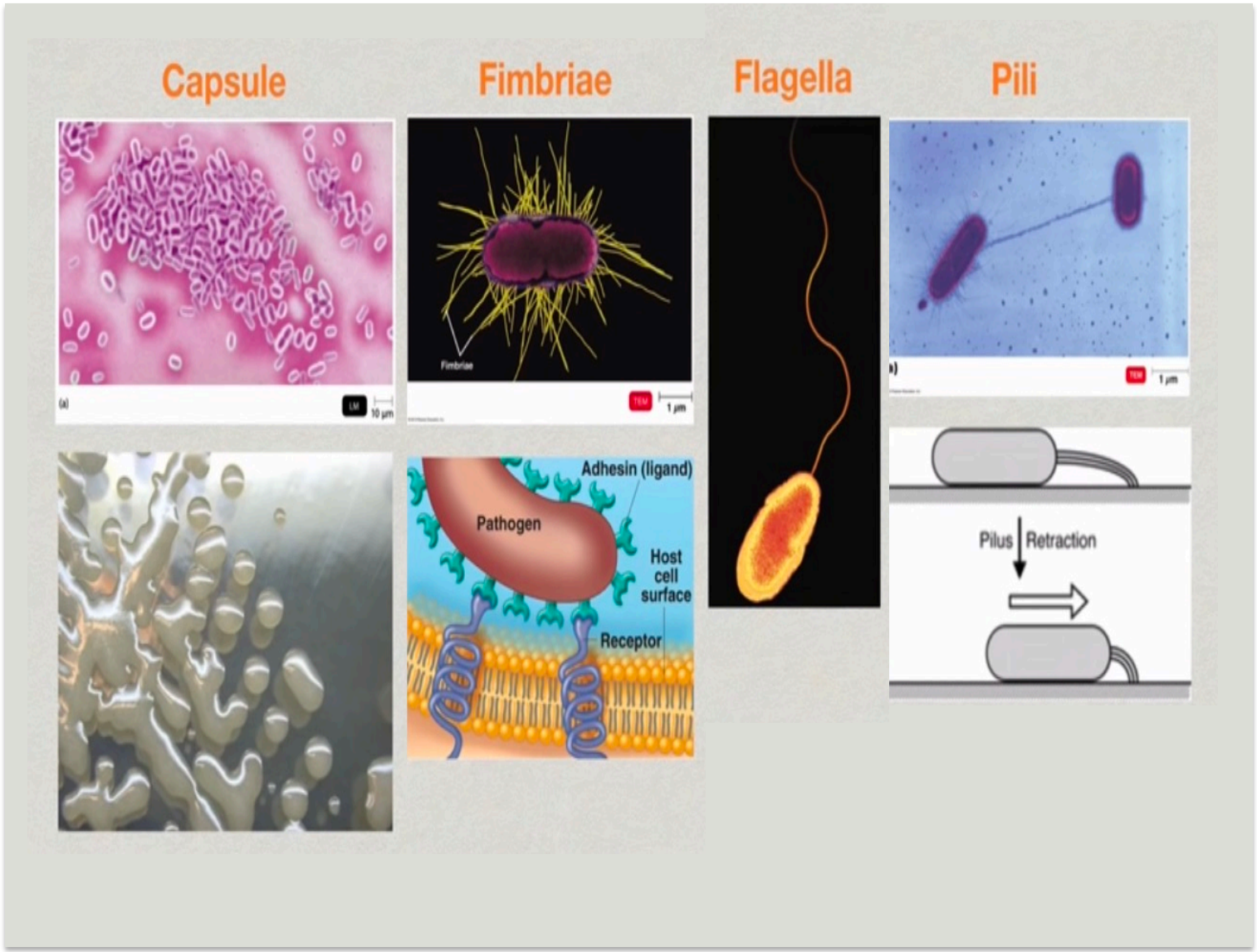
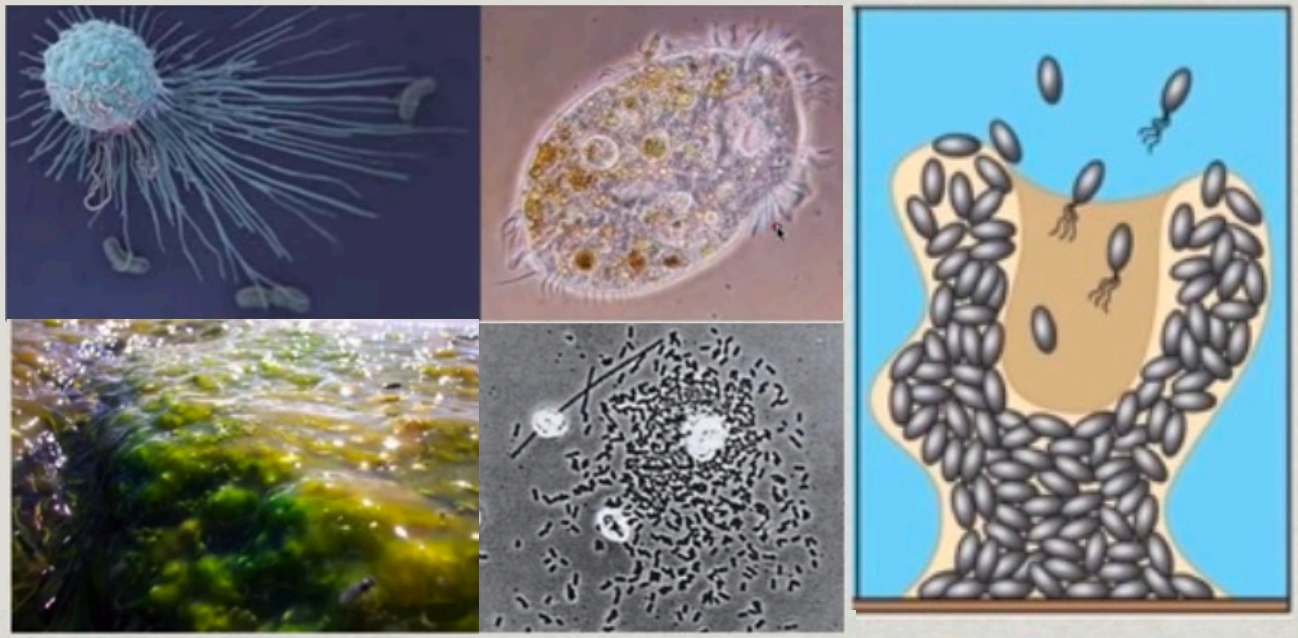


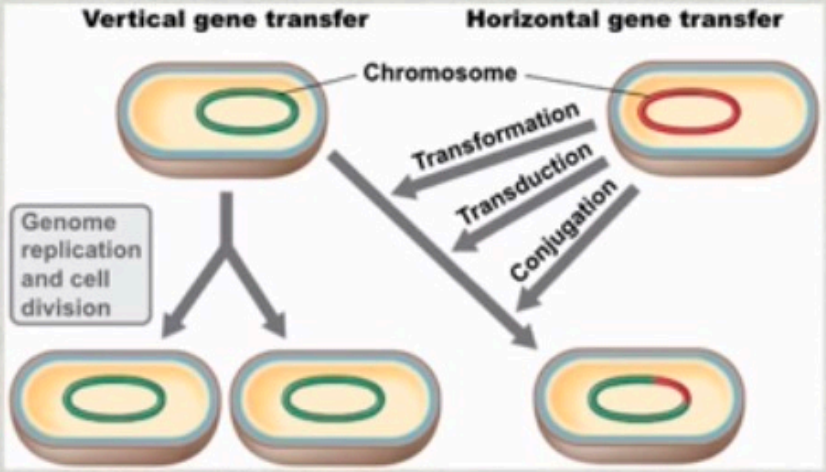
FIGURE 5 | (a–f) Kymographs of the OD of the liquid underneath developing pellicles, for all strains. Time is plotted on the x-axis, and the y-axis is the horizontally-averaged density of vertical position in the cuvette. The bright yellow section is the surface pellicle climbing the wall. Dotted lines indicate when top plumes start and end (only in the wild-type), and dashed lines indicate when late plumes begin and end. Top plumes correlate with a drop in the bacterial density in the liquid of the wild-type, while late plumes correlate with a drop in the bacterial density of the Δ*hag*, Δ*motAB*, Δ*cheA*, and Δ*cheY* mutants. In Δ*epsH*, the bulk is never quieted, and additionally plumes do not appear. Late plumes also correspond to the pellicle no longer climbing the wall in the wild-type. **(g)** Average ODs in the liquid for all strains. Across all strains, the bacterial density rises and then drops, with the exception of the Δ*epsH* mutant, who does not form a pellicle and whose density also does not drop. Wild-type bacteria density is suppressed fastest at approximately 30 h when the average cuvette OD reaches ~ 0.08, followed by chemotaxis mutants at 35 h (OD ~ 0.15) and motility mutants at 40 h (OD ~ 0.22). In 'x's are the local OD's within the collective streaks of the WT and Δ*epsH* strains, which are higher locally than in the rest of the cuvette.

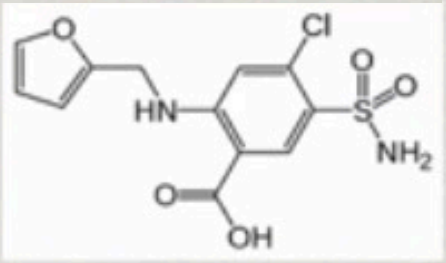
Linking bacterial structures to biofilm



Why to form biofilms?

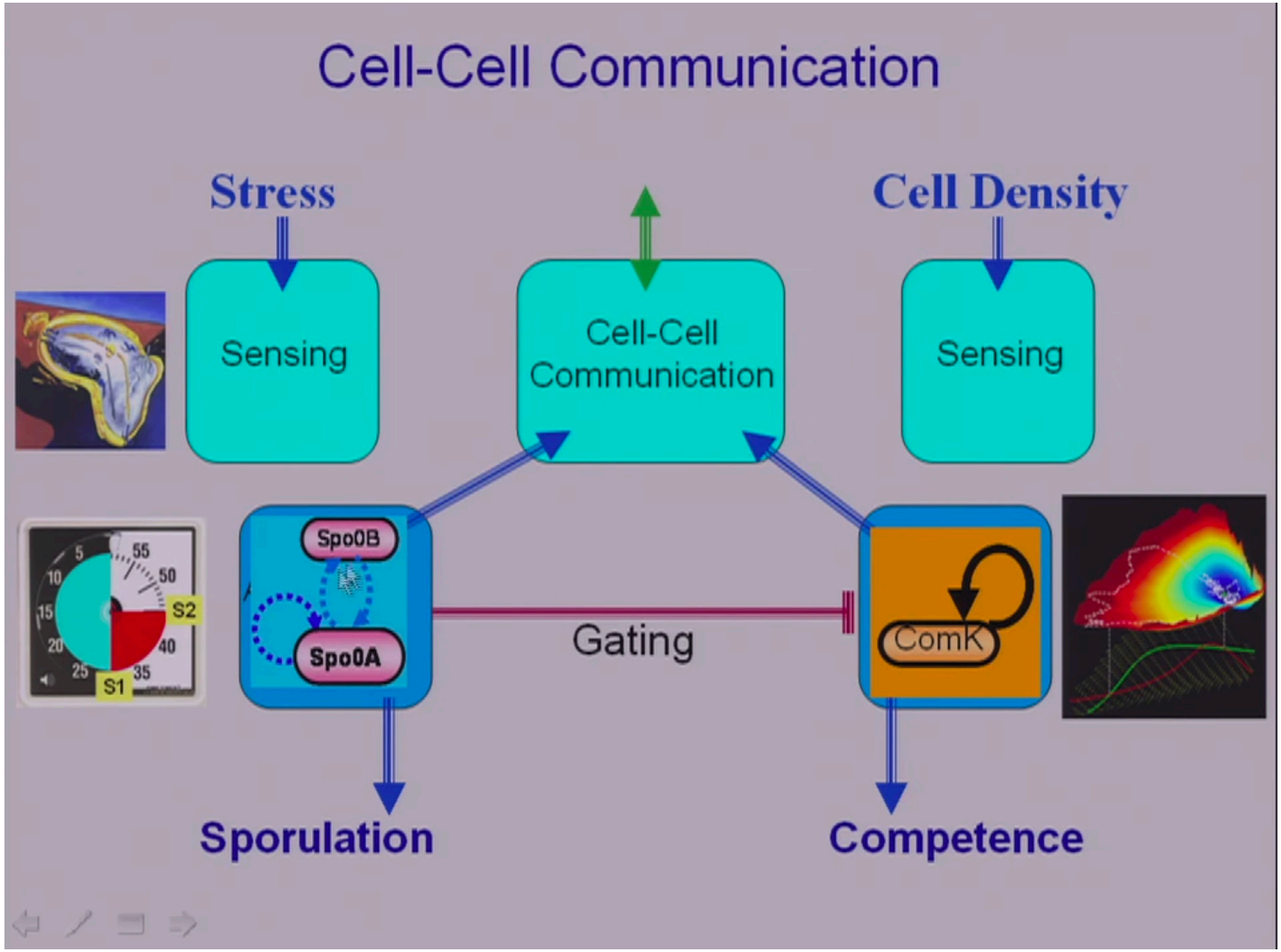




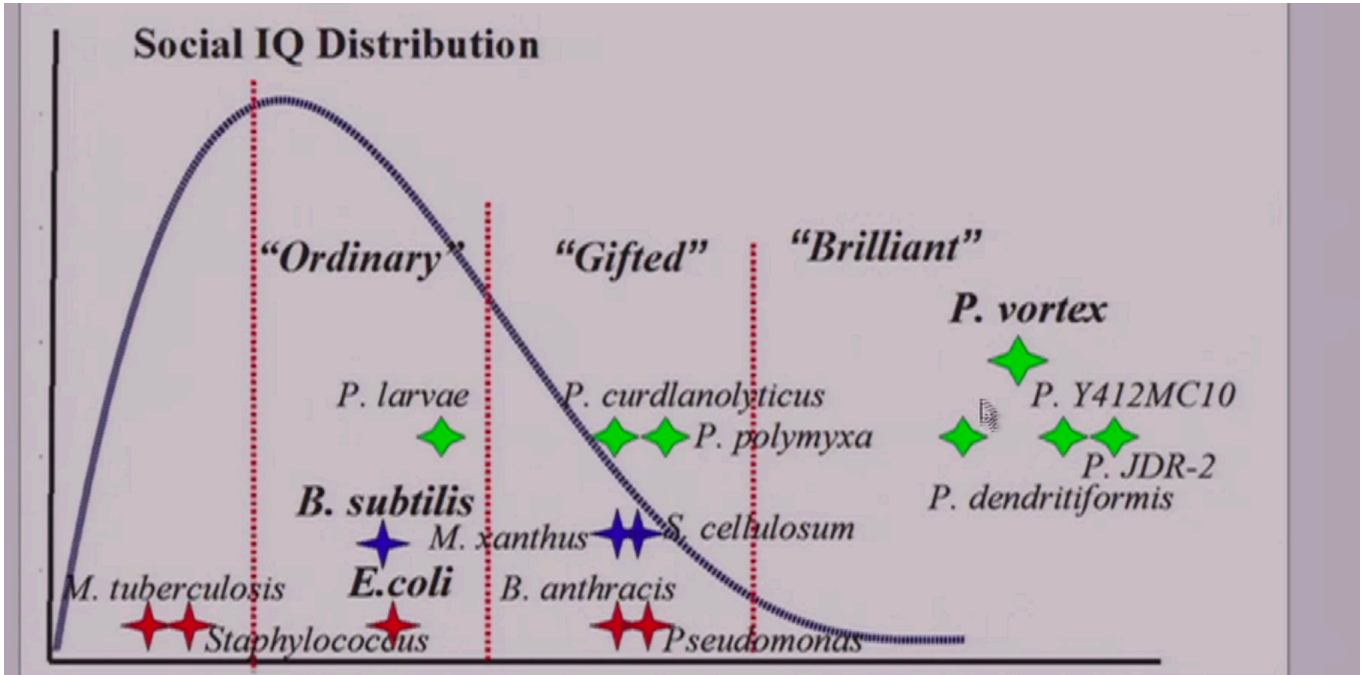


Resitance to protozoa
Nutrient acquisition
Increased tolerance to toxics
Genetic eschange

Biofilms and cell defence

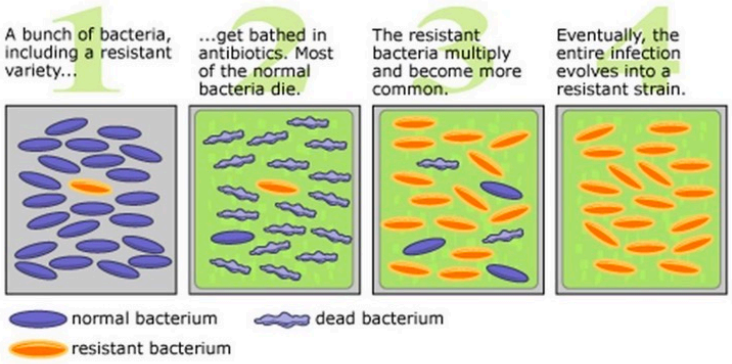


Biofilms and cell defence



The number of genes to communicate and process environmental information (two-component and transcription-factor genes), to make decisions and to synthesize offensive (toxic) and defensive (neutralizing) agents

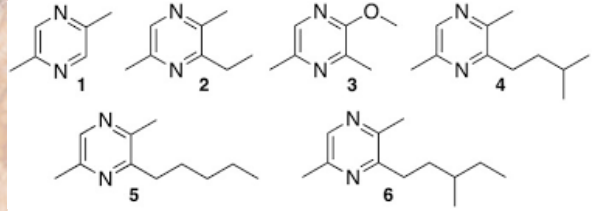
With Sirota et al BMC Genomics 2010



Biofilms and cell defence

Antibiotic resistance

Examples of pheromones produced by bacteria and ants



Competent but complex communication: The phenomena of pheromone-responsive plasmids

Amy J. Sterling, William J. Snelling, Patrick J. Naughton, Nigel G. Ternan, James S. G. Dooley

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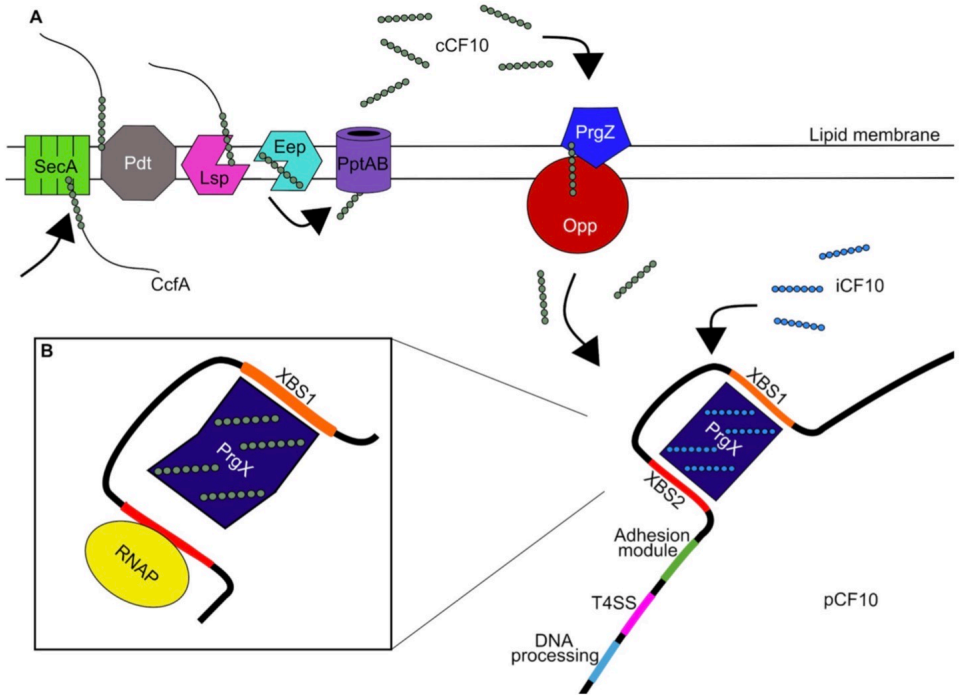
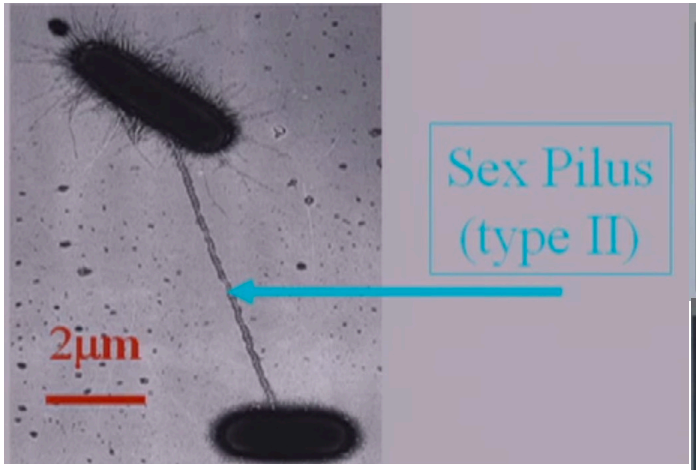


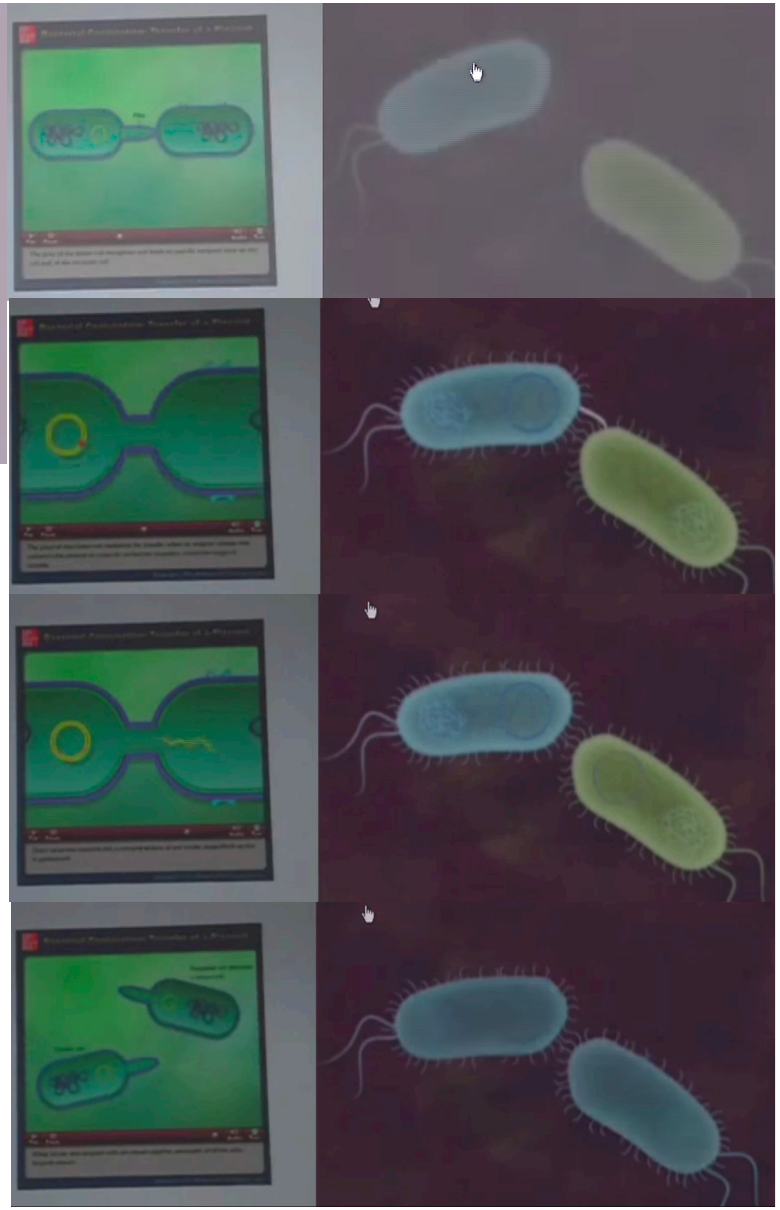
Fig 1. Model for induction of conjugation genes in pCF10. (A) Chromosomally encoded CcfA lipoprotein (grey) is exported to the extracellular region, where it is attached to the lipid membrane by Pdt. Subsequently, Lsp II cleaves the precursor on its cysteine residue before mature cCF10 is cut from the lipoprotein by Eep. Hydrophobic cCF10 is actively transported out of the cell through PptAB. Exogenous cCF10 (grey) is recognised by pCF10-encoded and externally presented PrgZ and is then passed to Opp for active uptake. In cCF10 absence, the PrgX tetramer is bound in a 1:1 ratio with iCF10 peptides (blue) and maintains tight binding to the pCF10 DNA through binding sites XBS1 and XBS2, thereby sterically inhibiting the binding of RNAP. (B) Within the induced state, the PrgX/cCF10 complex replaces the PrgX/iCF10 complex on the pCF10 DNA. The PrgX/cCF10 complex fails to maintain tight binding to XBS2, allowing RNA polymerase to access and then transcribe the downstream conjugation inducing genes. Eep, enhanced expression of pheromone; Opp, oligopeptide permease; Pdt, prolipoprotein diacylglyceryl transferase; Prg, pheromone-responsive gene; RNAP, RNA polymerase; T4SS, type 4 secretion system; XBS, PrgX binding site.

https://doi.org/10.1371/journal.ppat.1008310.g001

Biofilms and cell defence

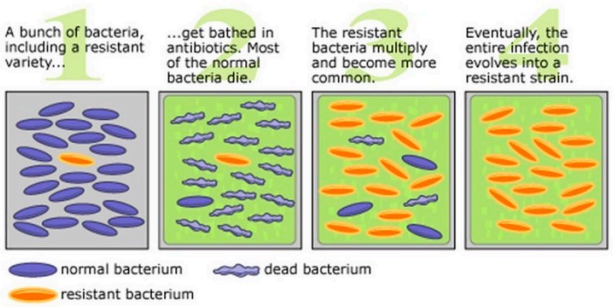
Antibiotic resistance



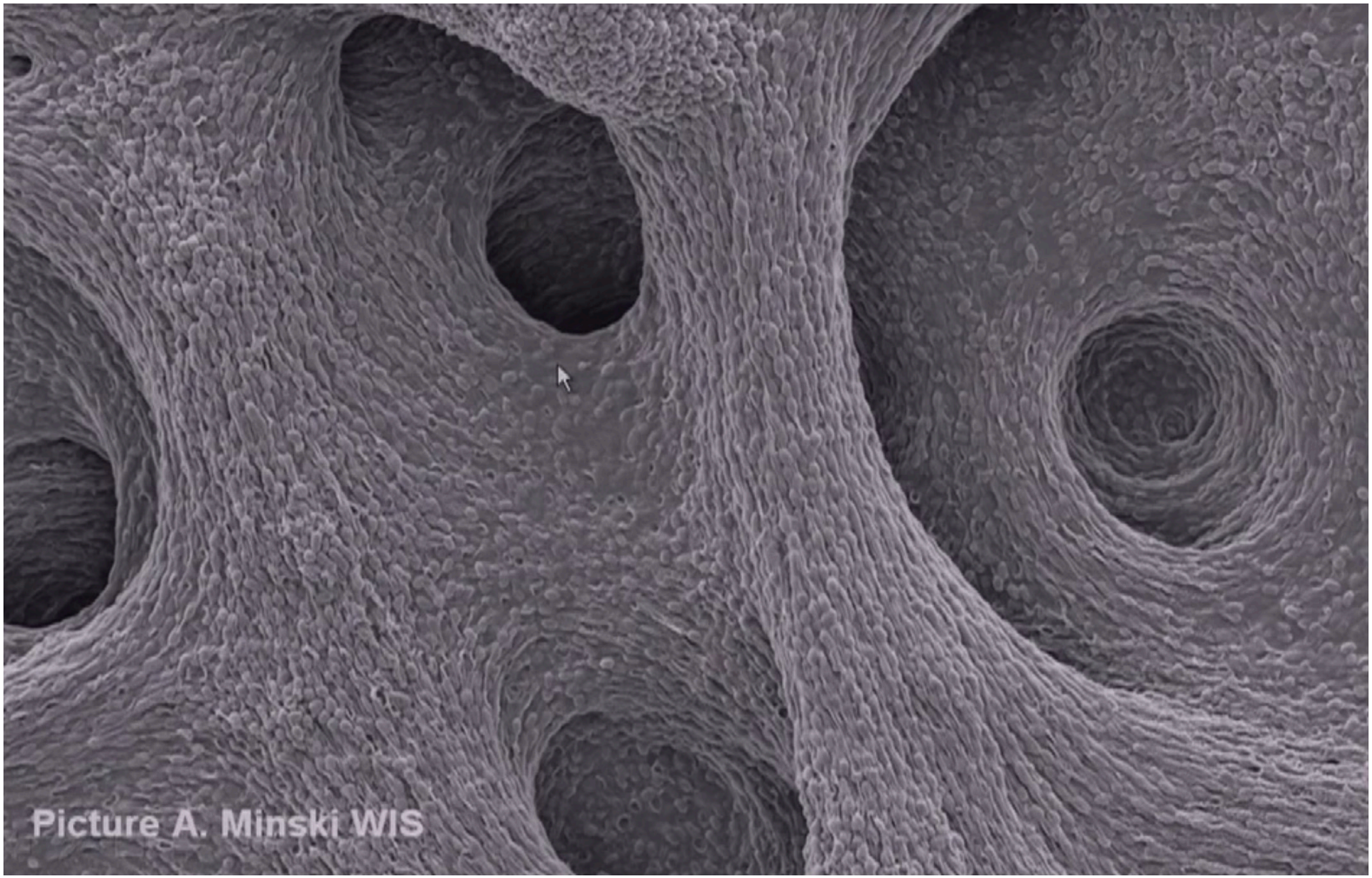


Pheromone production in 15 min

Plasmid exchange in 15-25 min



Biofilms have a structure



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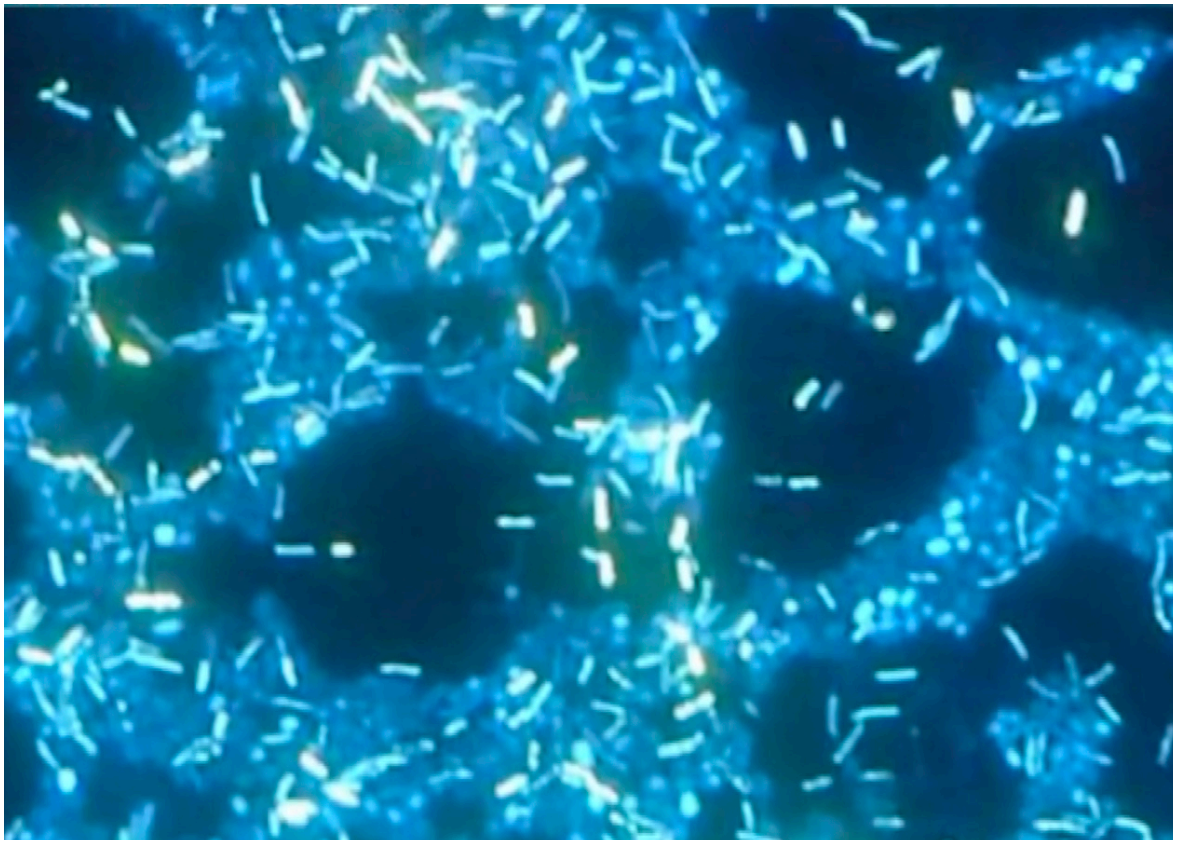

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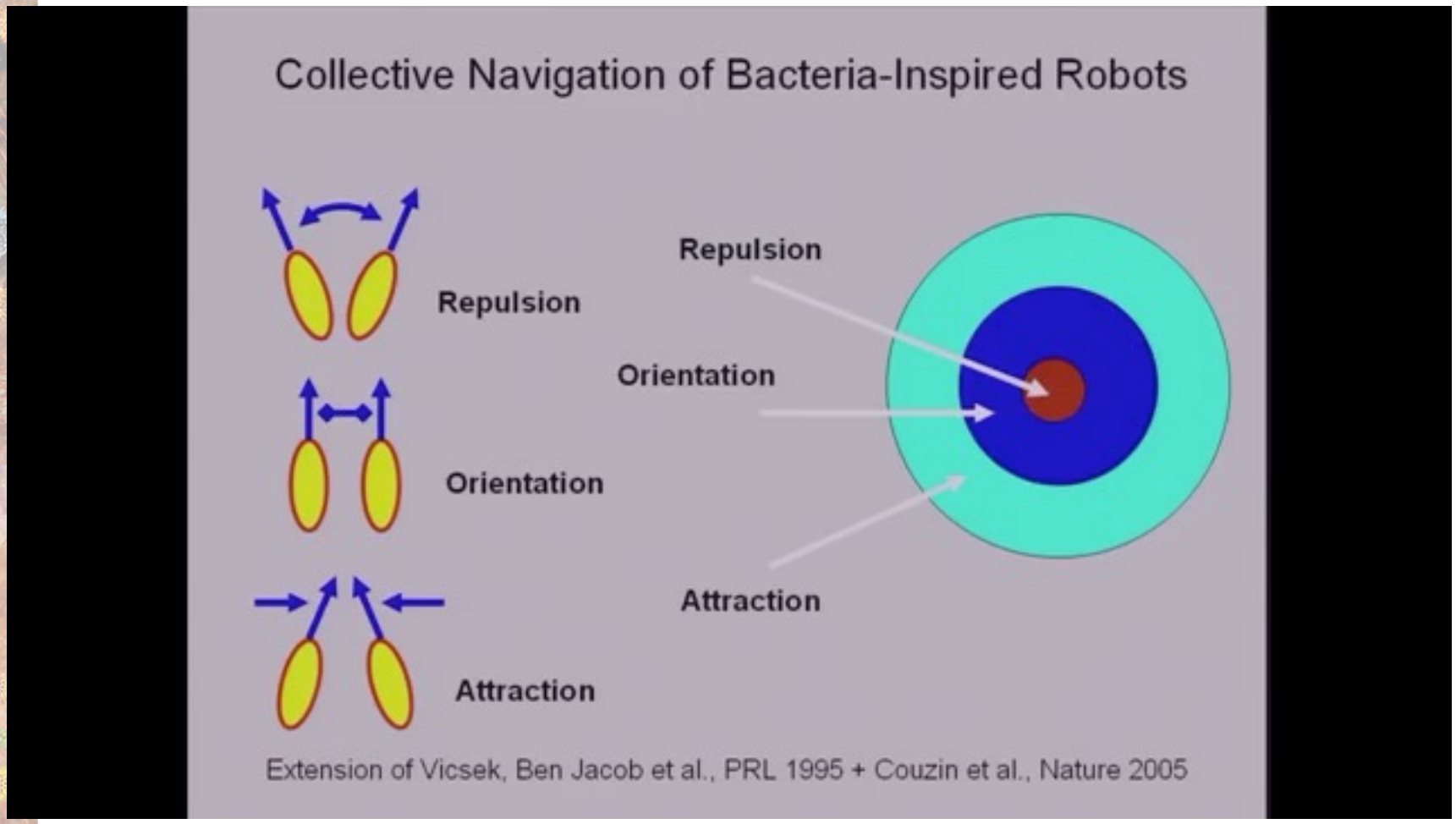
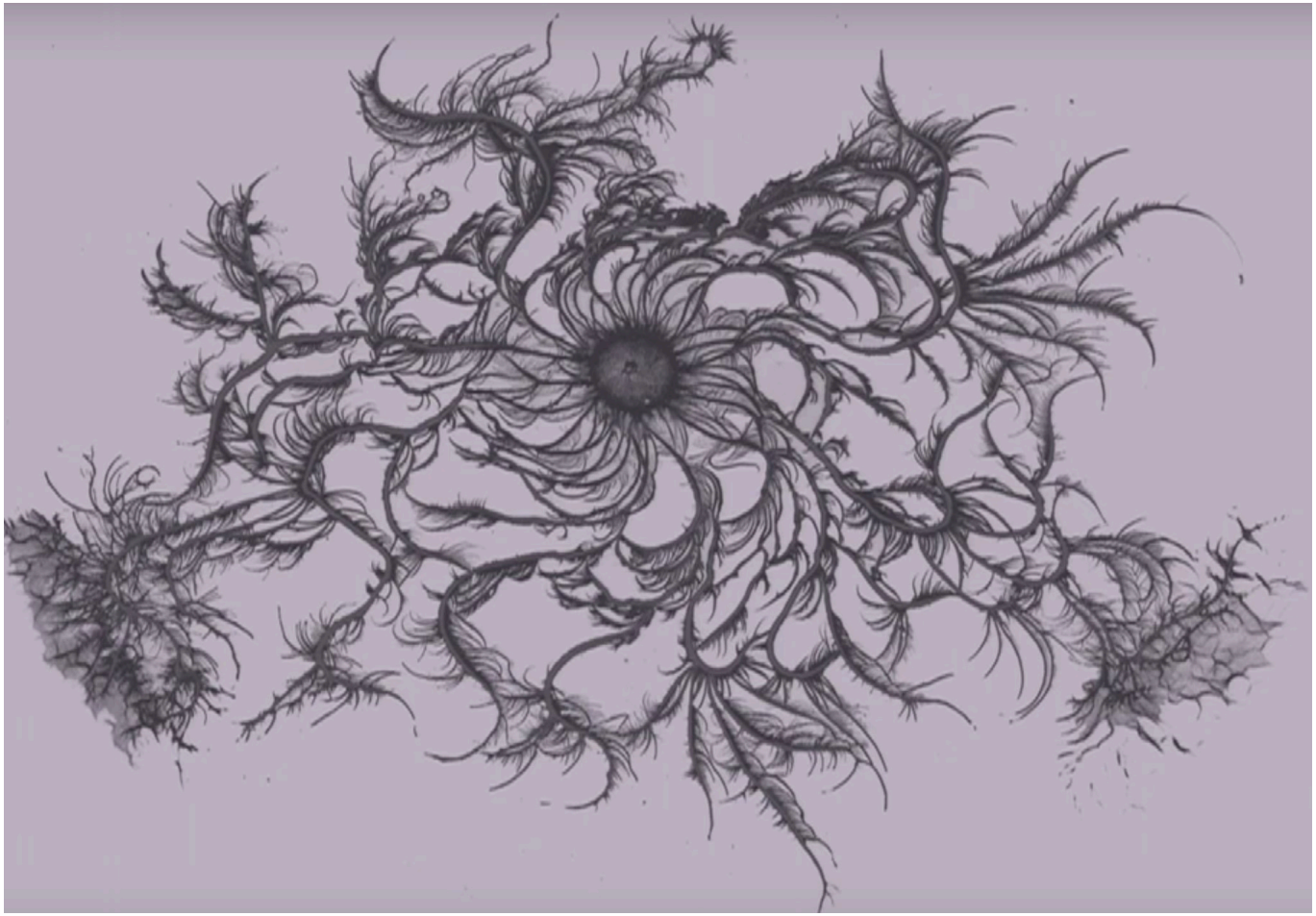


Biofilms have a structure

Biofilms have a structure



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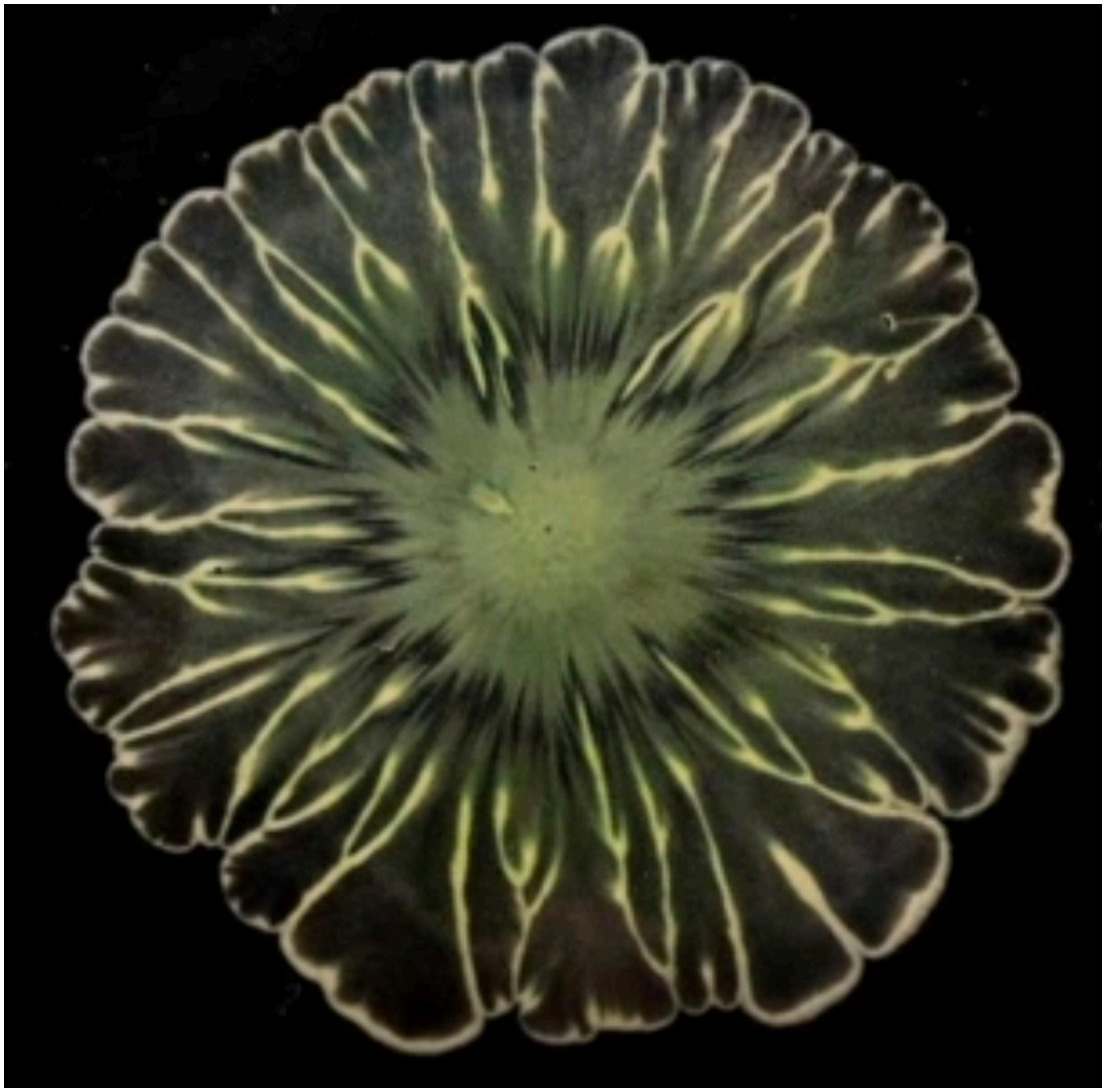

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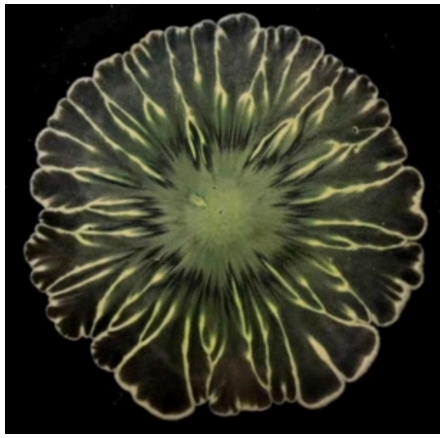
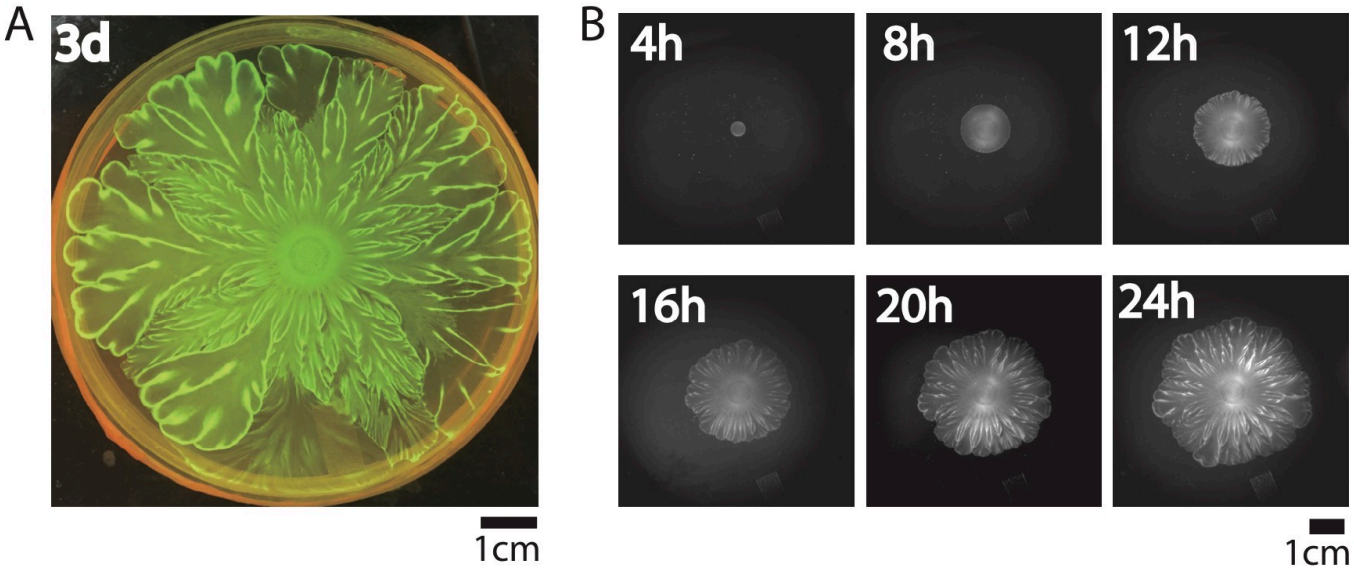


The spatial structure of biofilms can determine growth and survival of different species, but the mechanisms driving formation of this structure are not fully understood.

non-motile bacteria “hitchhike” on the motile bacteria as the latter migrate outward. The non-motile bacteria accumulate at the boundary of the colony and trigger an instability that leaves behind striking flower-like patterns.

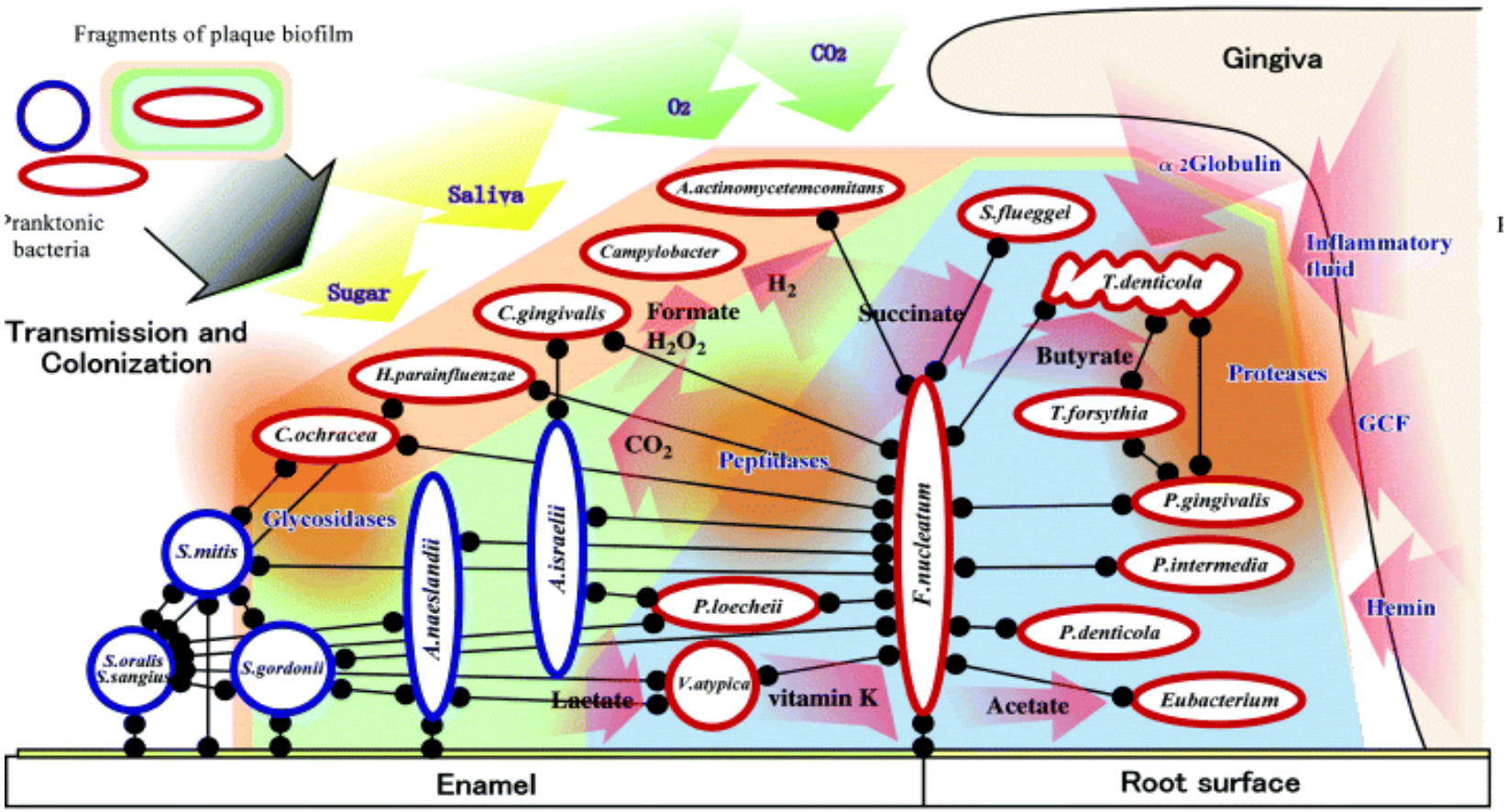
Acinetobacter baylyi, a gram-negative bacterium that easily moves on soft surfaces using twitching motility , and an ***Escherichia coli*** strain that is almost non-motile on soft agar.

Biofilms have a structure



Biofilms have a structure

Biofilms are everywhere



Early colonizer ⇨ Secondary colonizer ⇨ Late colonizer : Microbial succession

(Gram ⊕ cocci) (Gram ⊕ ⊖ rod) (Gram ⊖ rod/ motile) (Dominant microorganisms)

Aerobic ⇨ Capnophilic / Facultative anaerobic ⇨ Anaerobic : Environment modification

Saliva (saccharolytic) ⇨ GCF (proteolytic) : Nutrition supply

Biofilms are everywhere

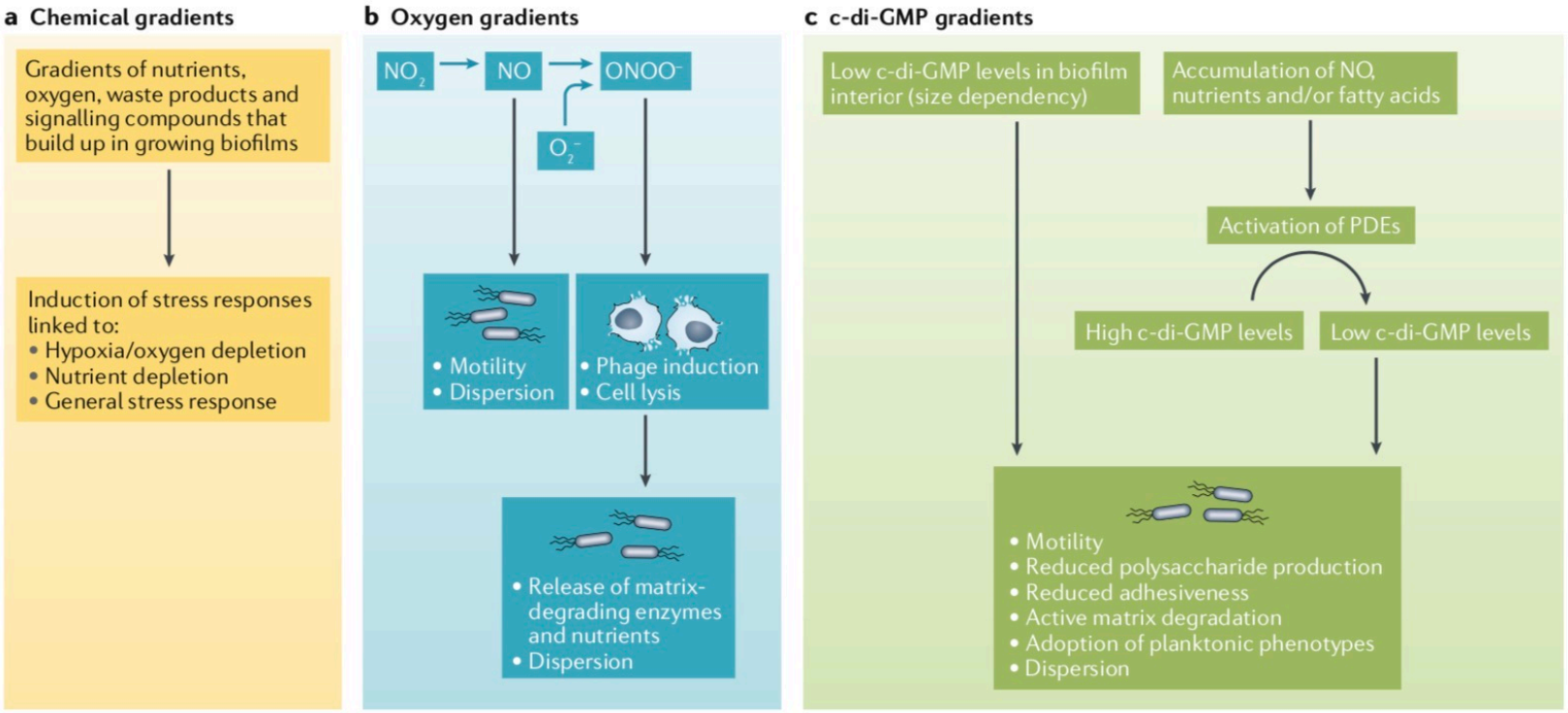


Fig. 2 | **Environmental conditions initiating dispersion.** Each part indicates various chemical gradients that are likely to be present in biofilms; low concentrations of those chemicals have been linked to dispersion. Moreover, phenotypic changes such as motility or induction of events that are triggered in response to the limited availability of compounds are shown. **a** | Bacteria residing at different locations within the biofilm structure experience concentration gradients of nutrient resources, oxygen, waste products and extracellular signalling molecules. Biofilm cells respond to these gradients by inducing various stress responses, such as the RpoS-dependent general stress response, as well as increased expression of genes involved in the response to oxygen limitation and nutrient deprivation. **b** | Under limiting oxygen conditions, such as found in the interior of the biofilm, anaerobic denitrification leads to the generation of nitric oxide (NO). Exposure to NO is linked to the reduction in cellular bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) levels and thus increased motility and dispersion. Furthermore, NO can react with superoxide to generate the cell-toxic radical $ONOO^-$. $ONOO^-$ causes cellular damage, bacteriophage induction and cell lysis. Cell lysis results in the release of degradative enzymes that contribute to the enzyme-mediated breakdown of the biofilm matrix and/or loosening of the biofilm matrix. In addition, cell lysis generates nutrients for growth. **c** | The levels of c-di-GMP differ throughout the biofilm, with the lowest levels being detected in the biofilm interior. Low c-di-GMP levels contribute to phenotypes generally associated with the planktonic mode of growth, including increased motility, increased drug susceptibility but reduced adhesiveness and matrix production. Bacteria residing at different locations within the biofilm structure experience the accumulation of native dispersion cues, including NO, *cis*-2-decenoic acid and nutrients. Sensing of dispersion cues activates phosphodiesterases (PDEs) capable of degrading c-di-GMP, ultimately resulting in an overall reduction in the levels of c-di-GMP. Phenotypes associated with low c-di-GMP levels include increased motility, reduced adhesiveness, reduced matrix production and dispersion.

Biofilms are everywhere

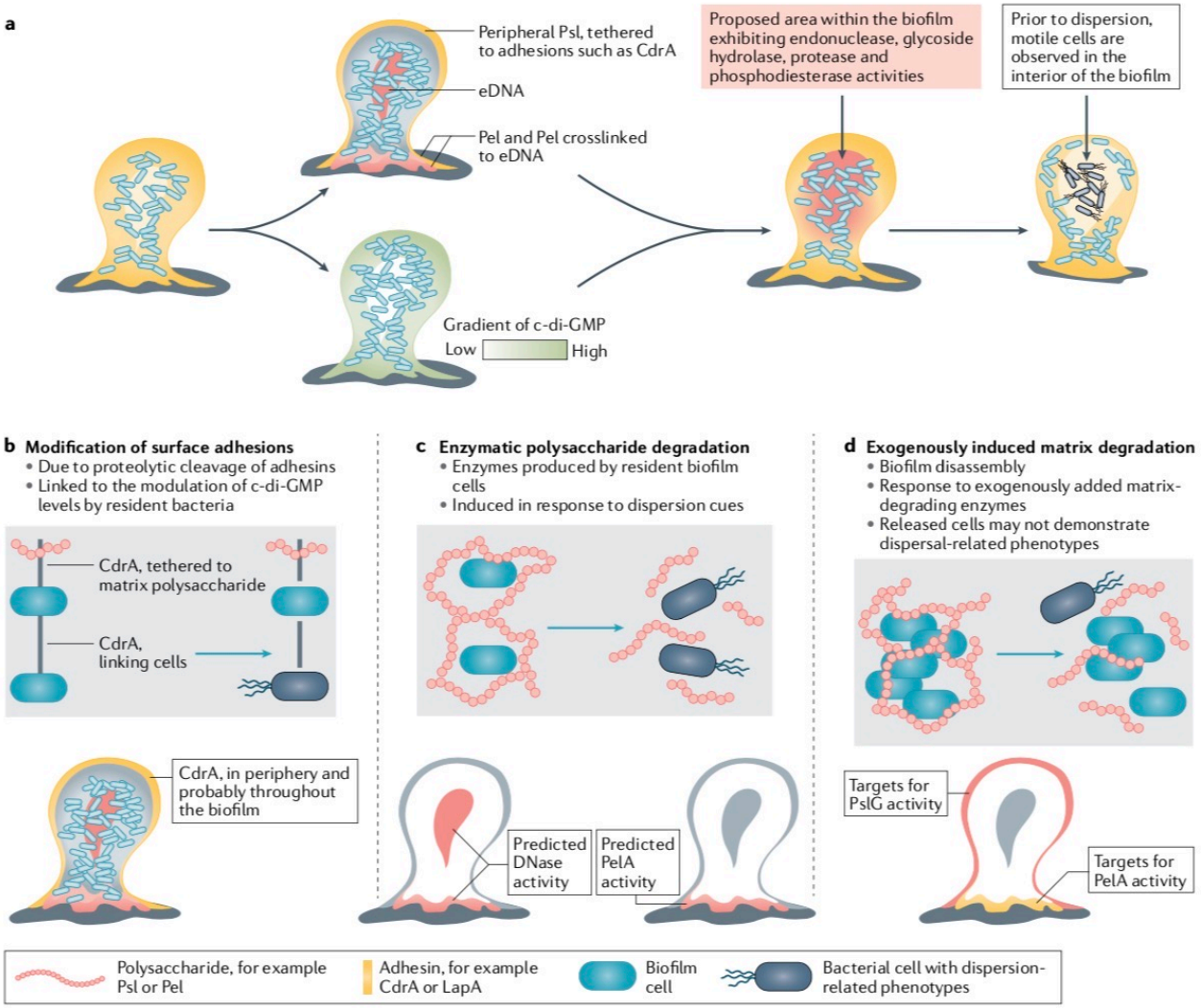


Fig. 4 | **Mechanisms resulting in biofilm dispersal. a** | Model of spatial localization of biofilm matrix components and bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) and proposed biofilm areas with active matrix- and c-di-GMP-degrading activities that lead to biofilm dispersion. In *Pseudomonas aeruginosa* PAO1, the matrix polysaccharide Psl is localized at the periphery and the base of biofilms. Psl interacts with the adhesin protein CdrA and forms a protective but non-rigid structure in between cells and around the biofilm structure. The polysaccharide Pel is primarily located at the base of the biofilm and crosslinked to extracellular DNA (eDNA). However, eDNA is not limited to the biofilm base but has also been detected in the biofilm interior. Mature biofilms are characterized by low c-di-GMP levels in the interior of the biofilm, whereas c-di-GMP levels in immature or less structured biofilms are more uniform. Considering the link between dispersion, low c-di-GMP levels and matrix degradation, the biofilm periphery and biofilm interior are to the locations within the biofilm most likely to experience increased matrix- and c-di-GMP-degrading activities. Moreover, these locations coincide with observed dispersion events such as void formation and erosion of the biofilm structure. **b** | Surface adhesins such as CdrA or LapA are cleaved to untether the polysaccharide matrix and break cell–cell interactions. **c** | Matrix components are enzymatically degraded (by intrinsic DNases or the hydrolase PelA) in response to native or environmental dispersion cues (nitric oxide, nutrients or *cis*-2-decenoic acid). **d** | Biofilm disassembly can be induced by exogenously added matrix-degrading enzymes. As enzymes are exogenously added, matrix degradation is expected to primarily target the periphery of the biofilm structure (BOX 1).

Biofilms as biofactories

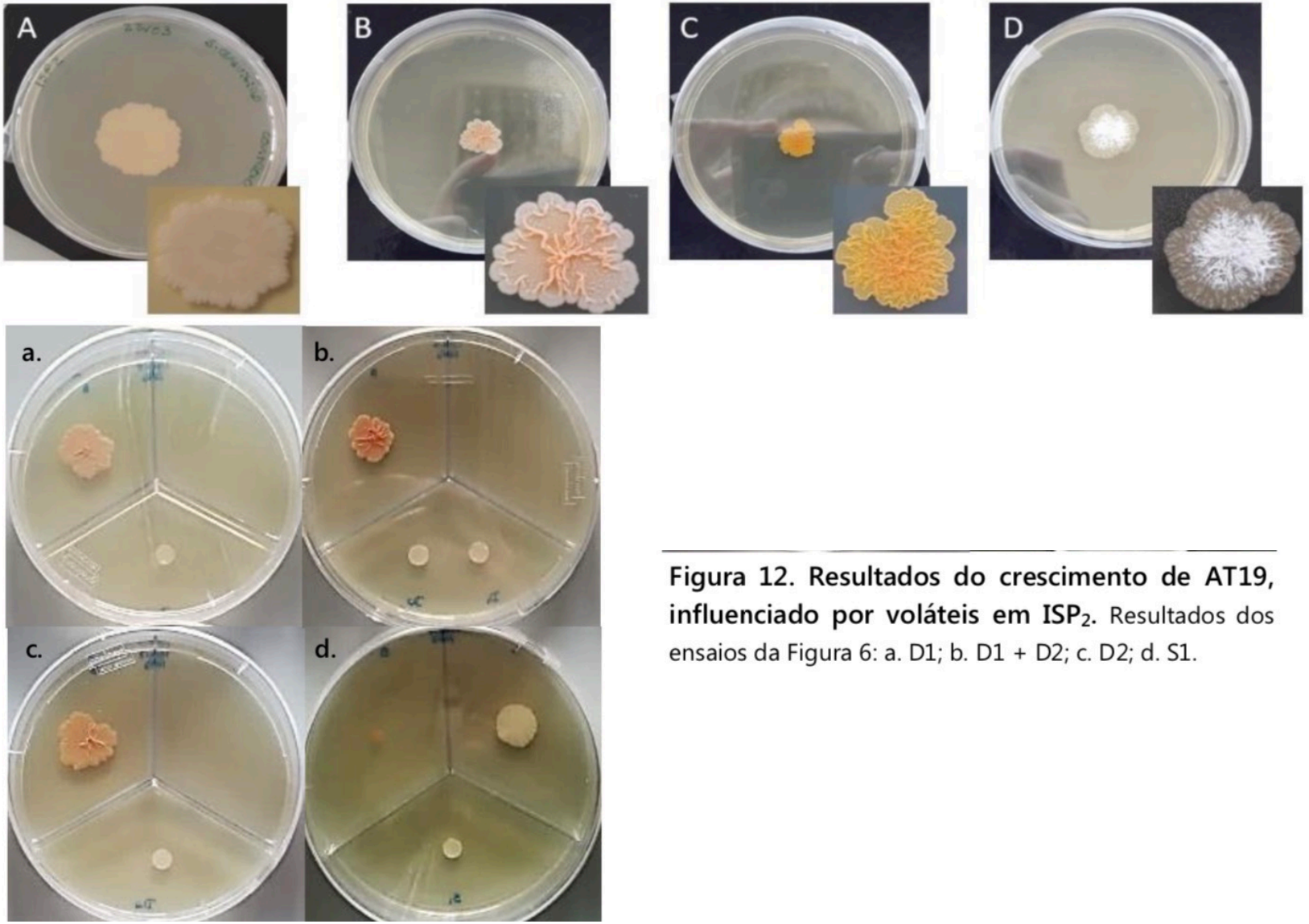
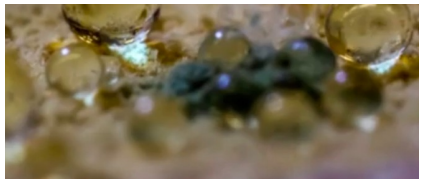


Figura 12. Resultados do crescimento de AT19, influenciado por voláteis em ISP_2. Resultados dos ensaios da Figura 6: a. D1; b. D1 + D2; c. D2; d. S1.

Biofilms as biofactories



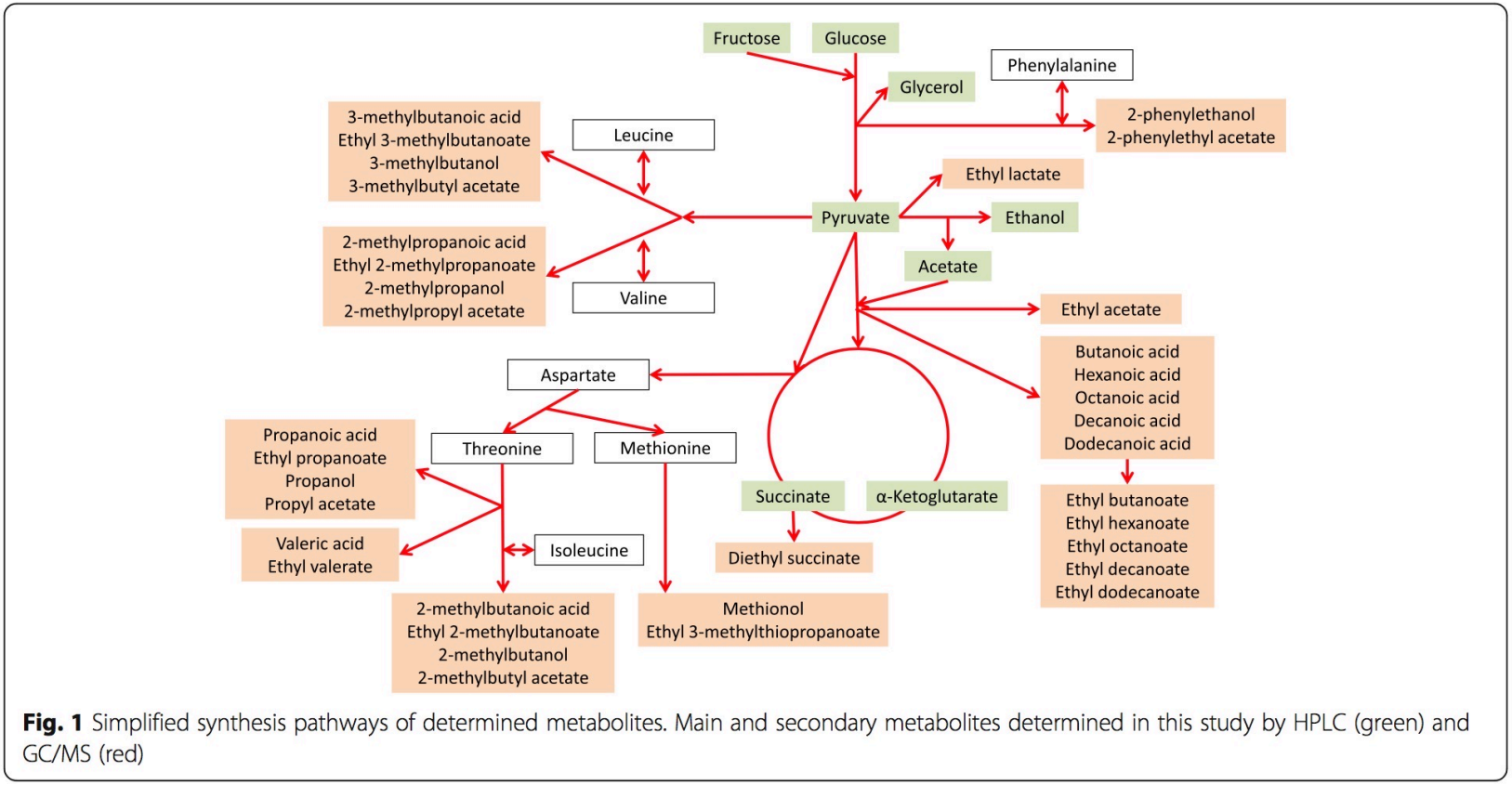
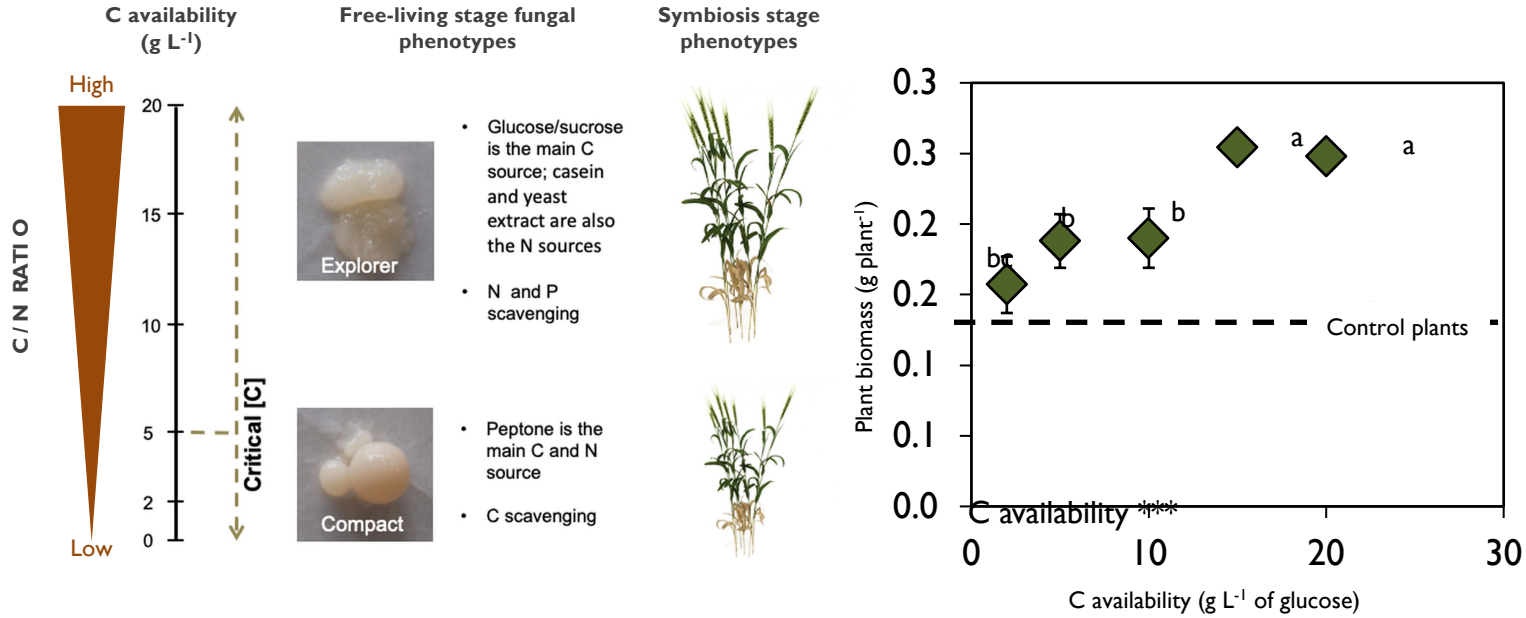


Fig. 1 Simplified synthesis pathways of determined metabolites. Main and secondary metabolites determined in this study by HPLC (green) and GC/MS (red)

Biofilms as biofactories

Enzymes



Ensaio de vaso com trigo (*Triticum aestivum*) inoculado ou não com *Serendipita indica* (n=12)

Dias, under review. Front Microbiol.

Biofilms as biofactories

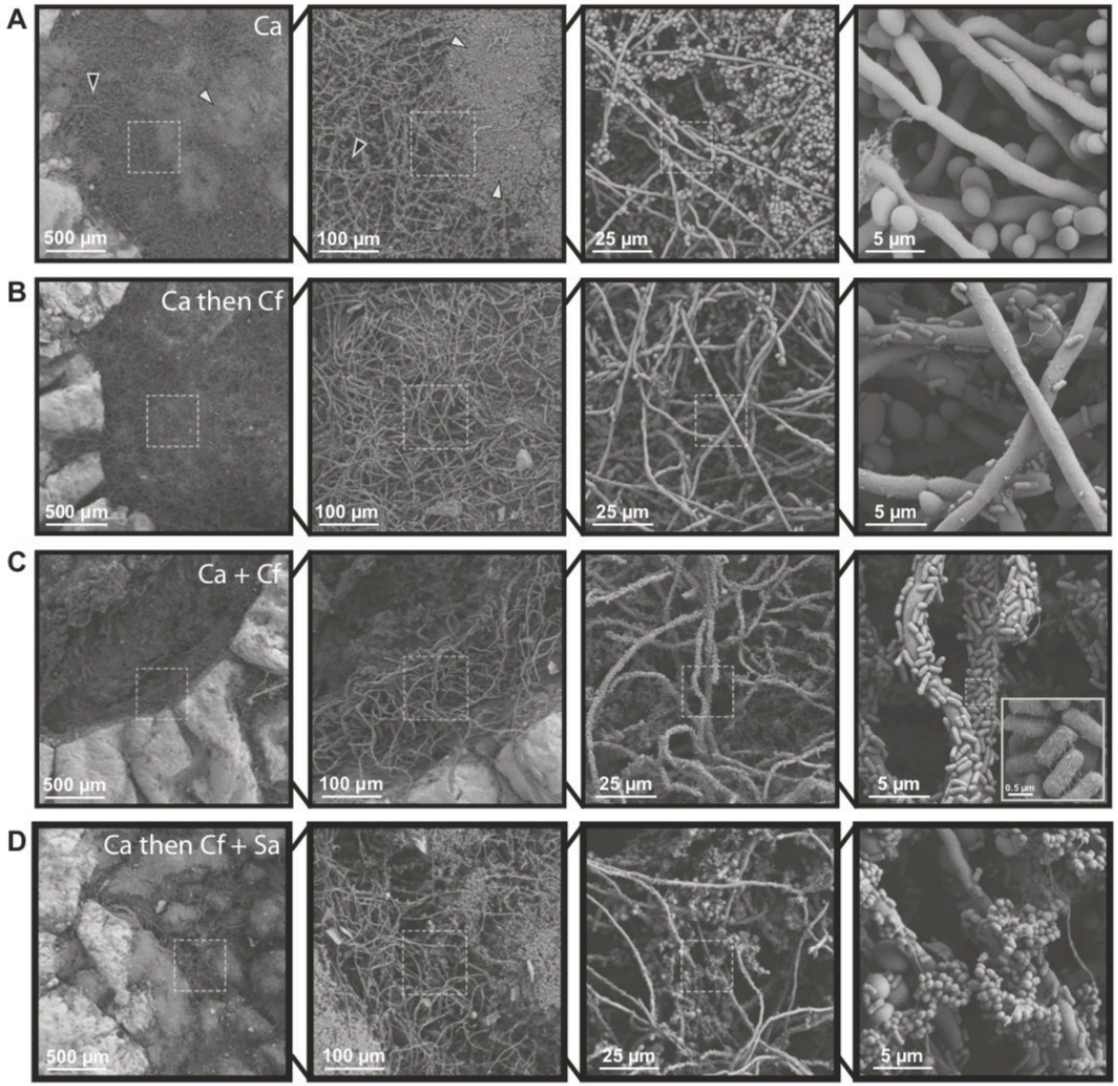


Fig. 1 Fungal-bacterial interactions and morphological heterogeneity within wound environments. Scanning electron micrographs of ex vivo wounds at four different magnifications (×100, ×500, ×2000, ×10000). Fungal-bacterial biofilms were grown using both staggered and simultaneous inoculation models in a subset of combinations to illustrate effects of priority and interbacterial competition. Microbes were growth for up to 48 h before SEM processing in 6 mm excisional wounds on 12 mm punch biopsies of human skin suspended in a DMEM-agarose gel at 37 °C, 5% CO_2. **A** *C. albicans* mono-infection. White arrowheads point to examples of yeast aggregates while black arrowheads point to hyphal networks. **B** *C. albicans* as early colonizer and *C. freundii* as late colonizer. **C** *C. albicans* and *C. freundii* simultaneously coinoculated. For imaging of putative pili on *C. freundii*, magnification was increased to ×20,000 as needed. **D** *C. albicans* as early colonizer and *C. freundii* + *S. aureus* as late colonizers. Dashed outlines represent region magnified.

Biofilms are everywhere

Biofilms have a structure

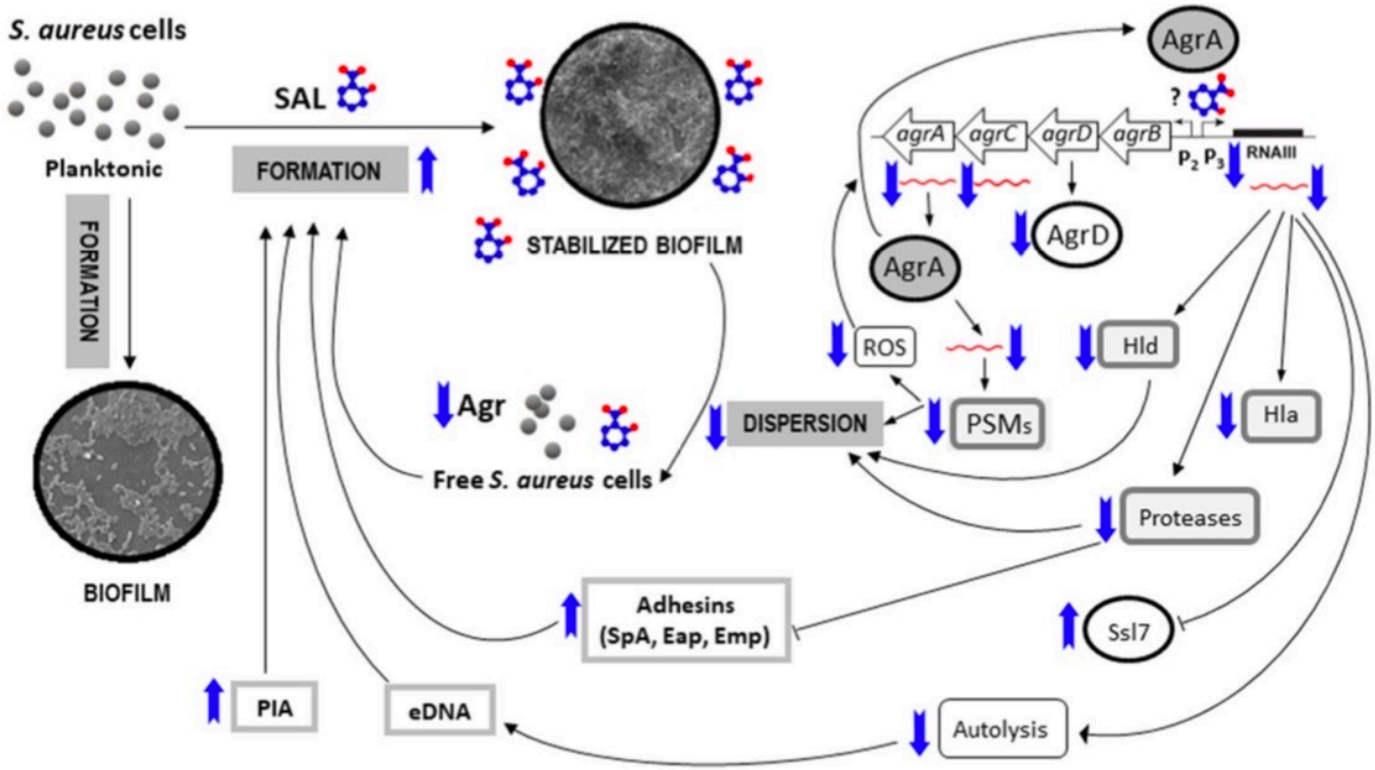


Figure 7. Schematic representation of SAL effects on stabilization of biofilm formed by *S. aureus*. Summary of changes determined in mRNA or protein expression, biochemical and phenotypic levels. Black lines with arrowheads endings denote stimulation and flat endings inhibition. Up and down blue arrows represent an increased or diminished change induced by SAL, respectively.

[agrA - Accessory gene regulator protein A - Staphylococcus ...]
Required for high-level post-exponential phase expression of a series of secreted proteins
PSM – Phenol soluble modulins
Hia – Adhesine gene.

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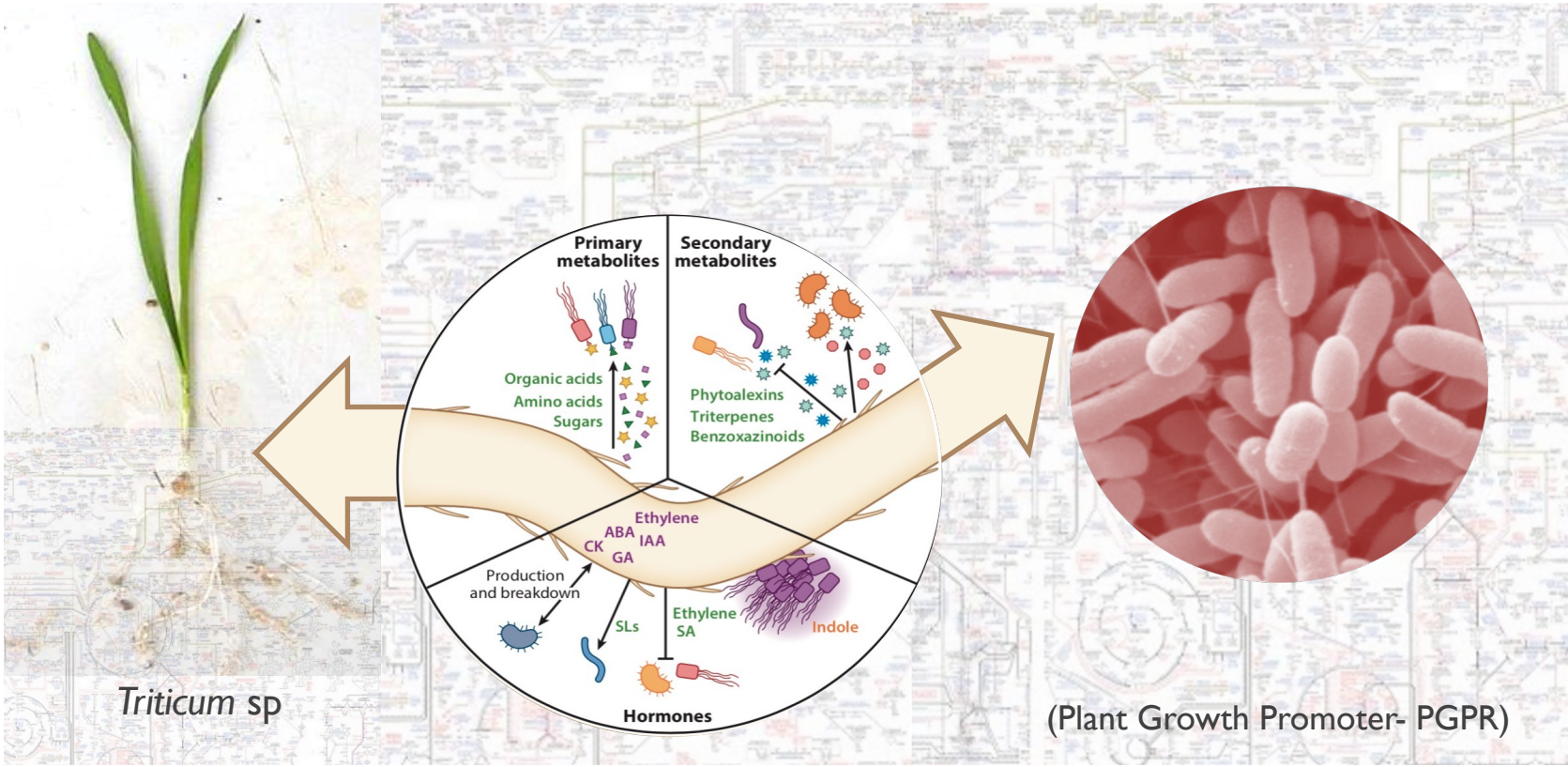

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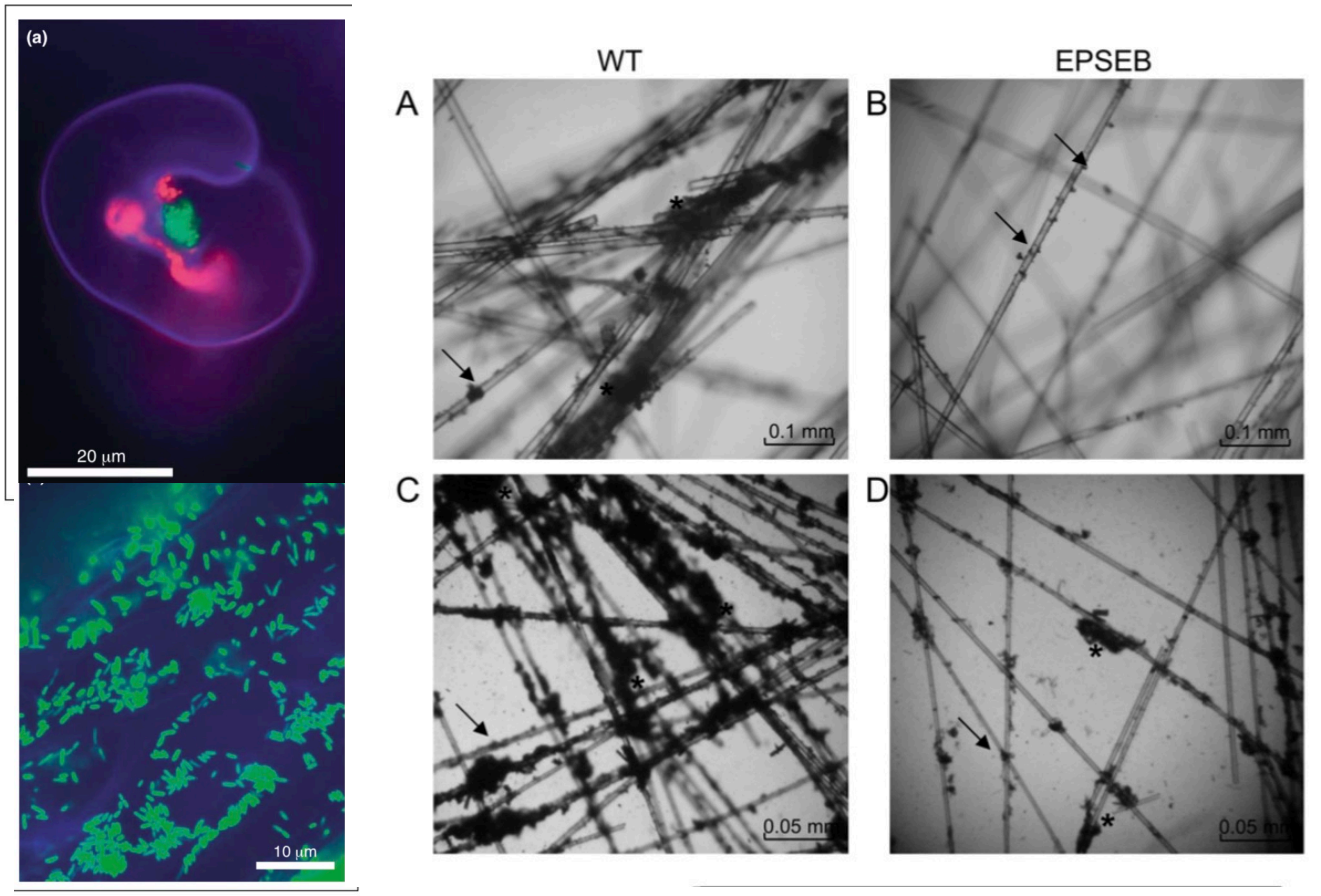

Figure 2. ***H. seropedicae*** **biofilm formation on glass fiber.** Light microscopy was performed with *H. seropedicae* SmR1 and EPSEB (*epsB* mutant) grown in the presence of glass fiber for 12 hours, without (A,B) and with (C,D) addition of purified wild-type EPS (100 µg.mL^{-1}). Arrows indicate attached bacteria. Asterisks indicate mature biofilm colonies. For biofilm expression analyses (E), *H. seropedicae* MHS-01 cells were grown for 12 h in the presence or absence of glass fiber, the free living bacteria were directly used and biofilm bacteria were recovered from glass fiber by vortex. β-galactosidase activity was determined, standardized by total protein concentration, and expressed as nmol ONP.(min.mg protein) $^{-1}$± standard deviation. Different letters indicate significant differences ($p<0.01$, Duncan multiple range test) in *epsG* expression between the tested conditions.
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