Bacterial Biofilms

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Synonyms

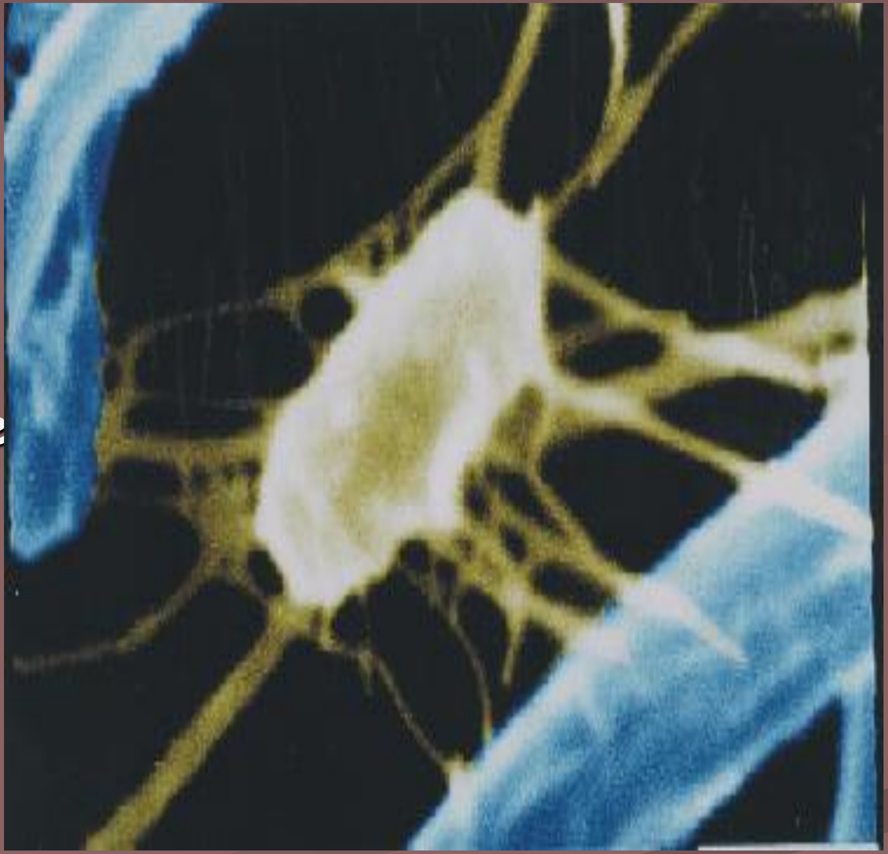
- Biofilm formation
- Adhesion factor
- Slime factor

WHAT IS BIOFILM?

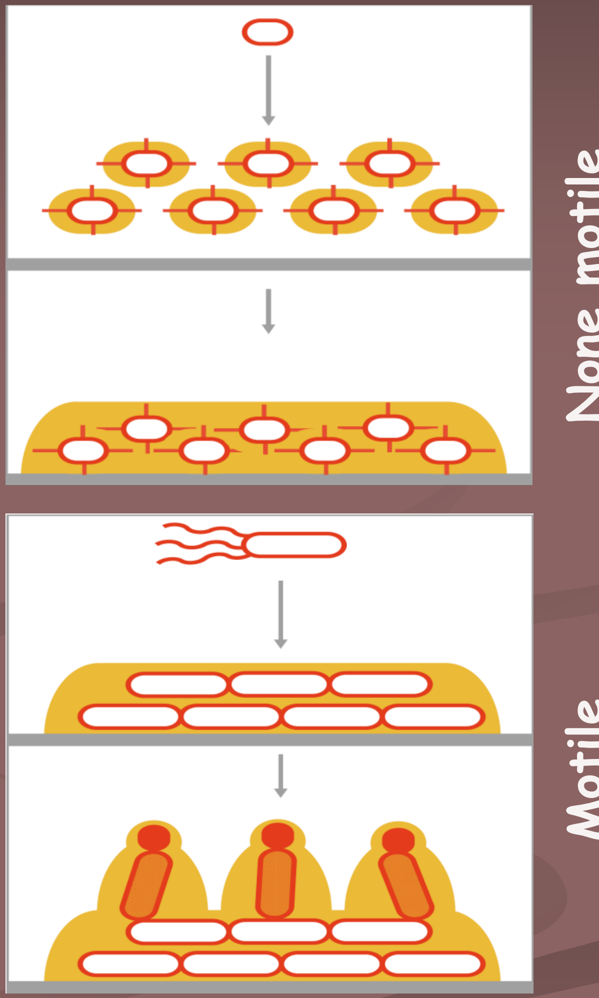
- Biofilms are surface-attached bacteria in an extracellular matrix of secreted
- **carbohydrates (extrapolysaccharides), proteins,** and/or **DNA,** forming **planktonic** free floating bacterial cells **(Bjarnshol et al 2017).**
- Bacterial pathogens live in biofilms **adhere** to **biotic or abiotic** surfaces forming **biomass. (Miguel et al 2017).**
- Biofilm is a complex community of **single** or different types (**polymicrobial**) of micro-organisms **attached** to a surface and **stick** to each other **(Hannan et al 2018).**

Matrix

- Components :
- Extracellular Polysaccharides
- Proteins
- Some micro chemical structures
- Dead cells have also been identifie biofilms



The most species produce biofilm



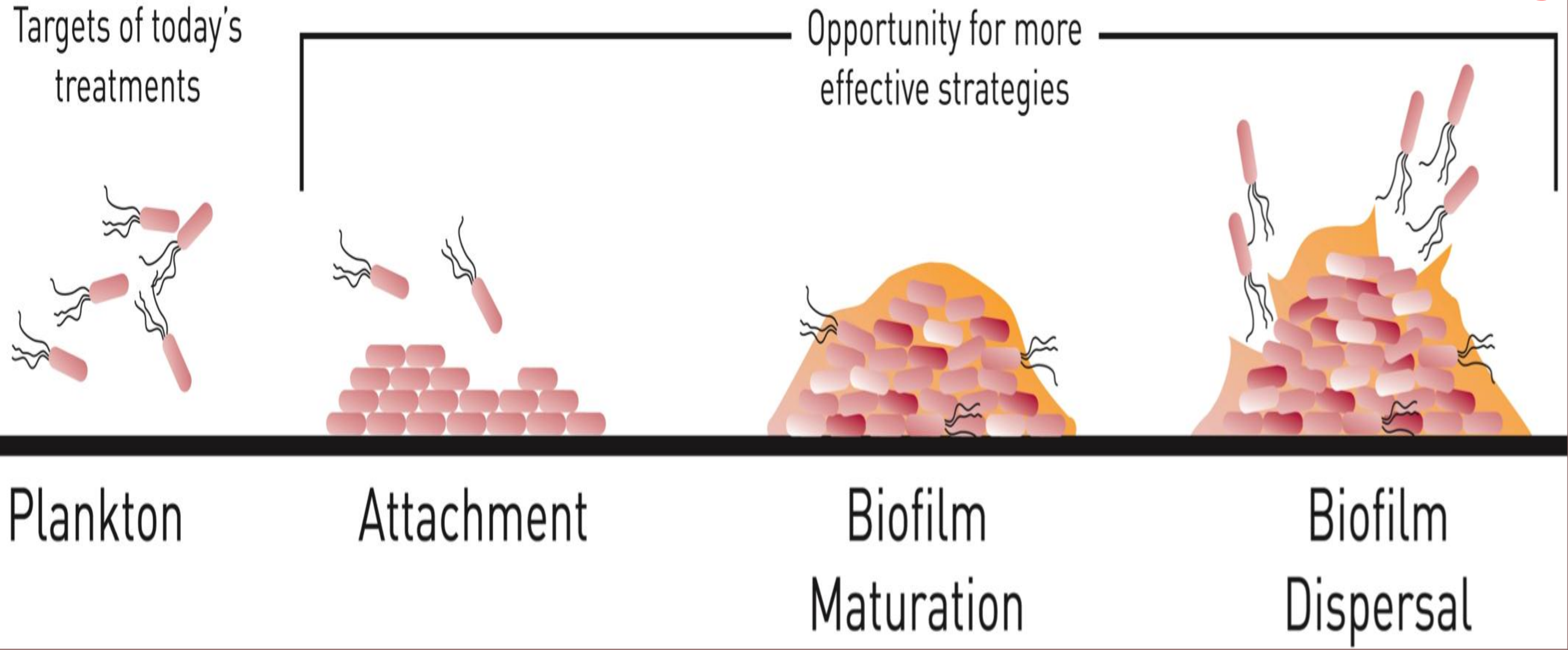
- *Strept mutans.*
- *St. aureus* esp.
- MRSA.
- *Ps. aurogenosa.*
- *St. epidermedis.*
- *E. coli.*
- *Salmonella sp.*
- *Enterococci.*

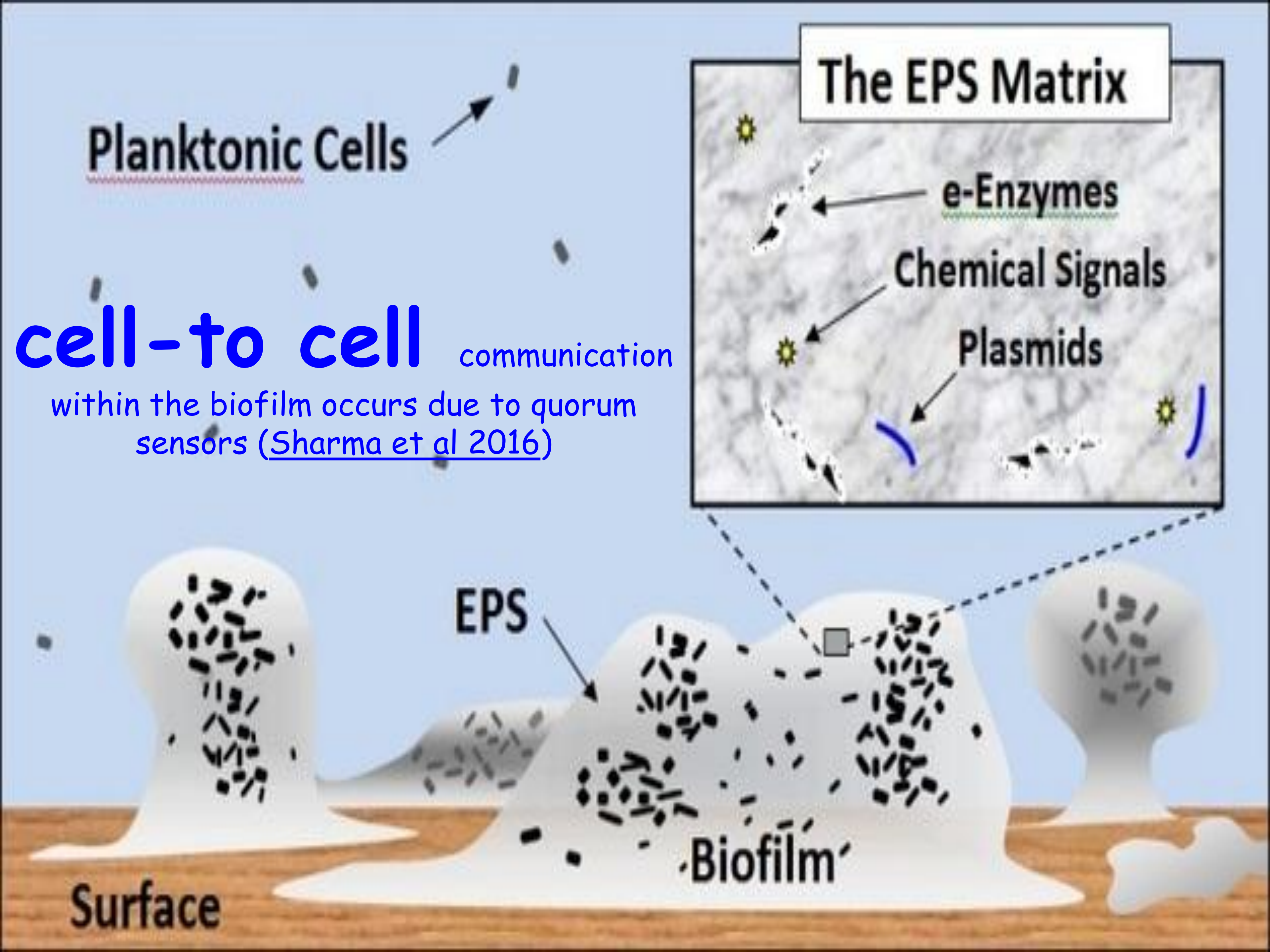
Biofilms – Now a Universal Feature for most bacterial sp.

How biofilm is developed ?

Planktonic

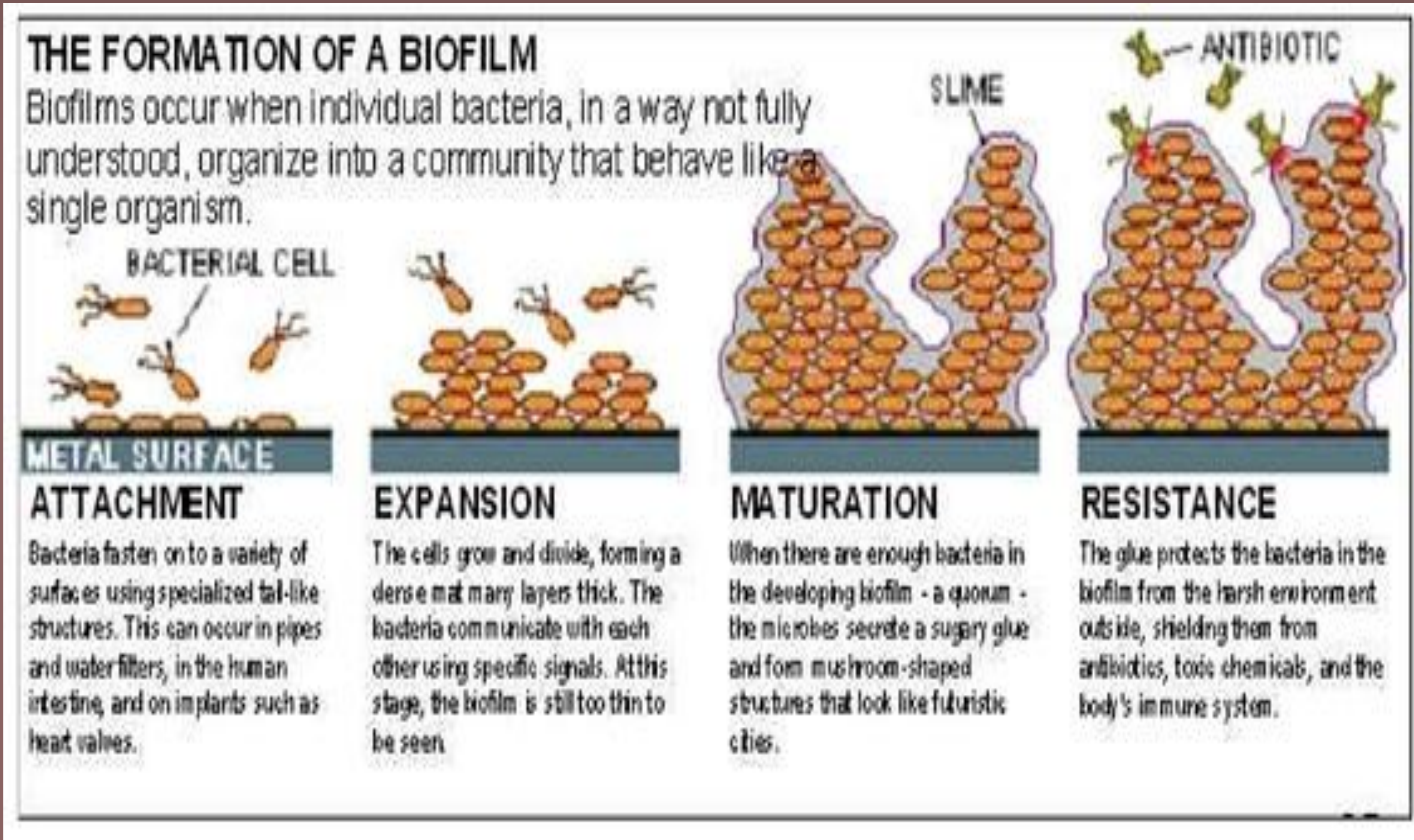
Quorum sensing



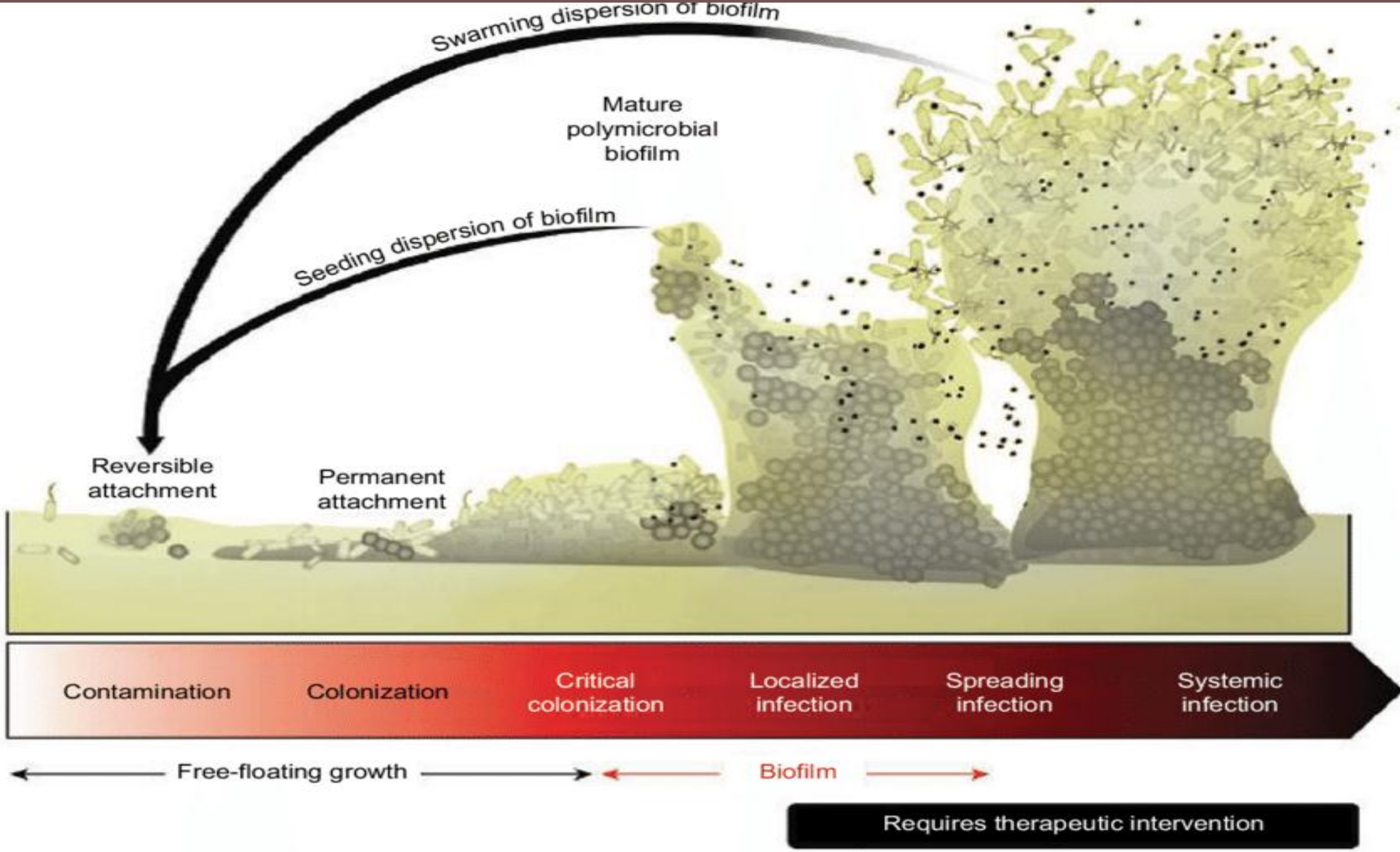


cell-to cell communication within the biofilm occurs due to quorum sensors (Sharma et al 2016)

Antibiotic can't penetrate mature biofilm



Polymicrobial biofilm community



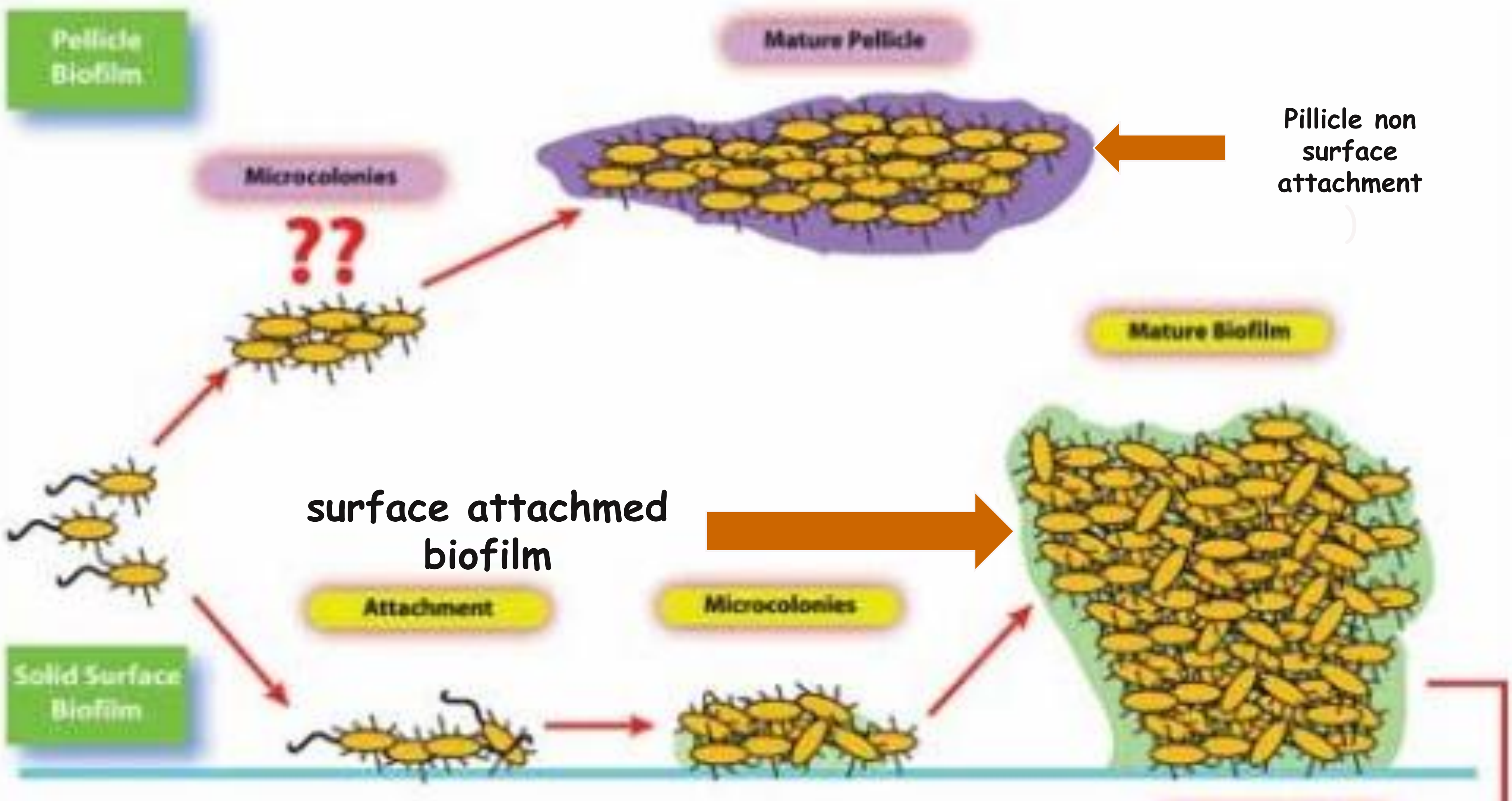
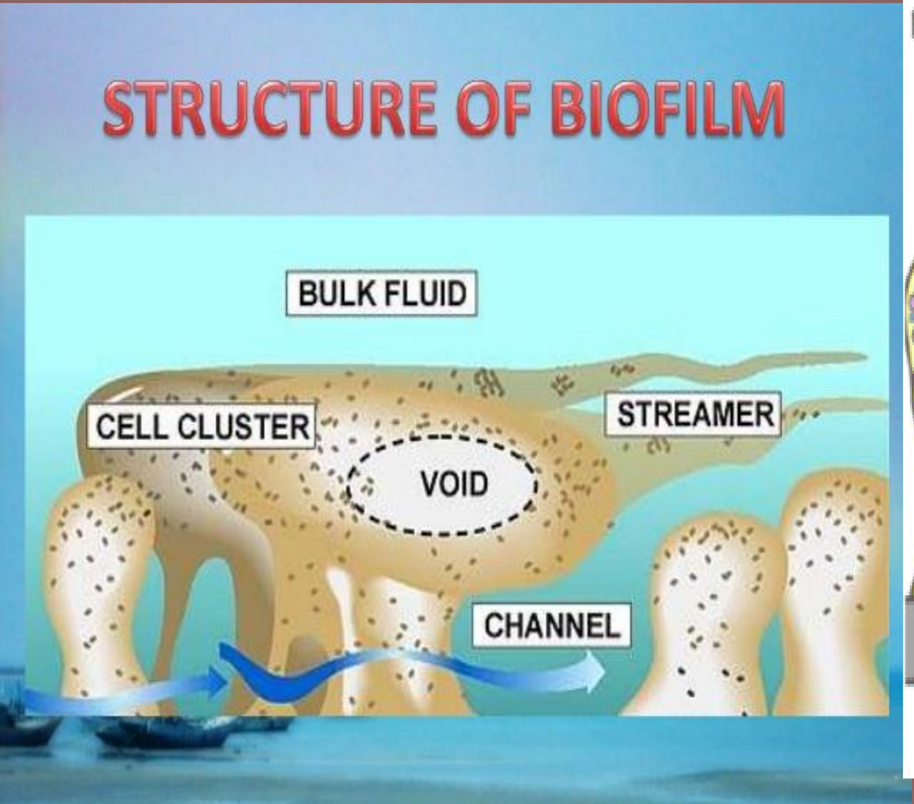


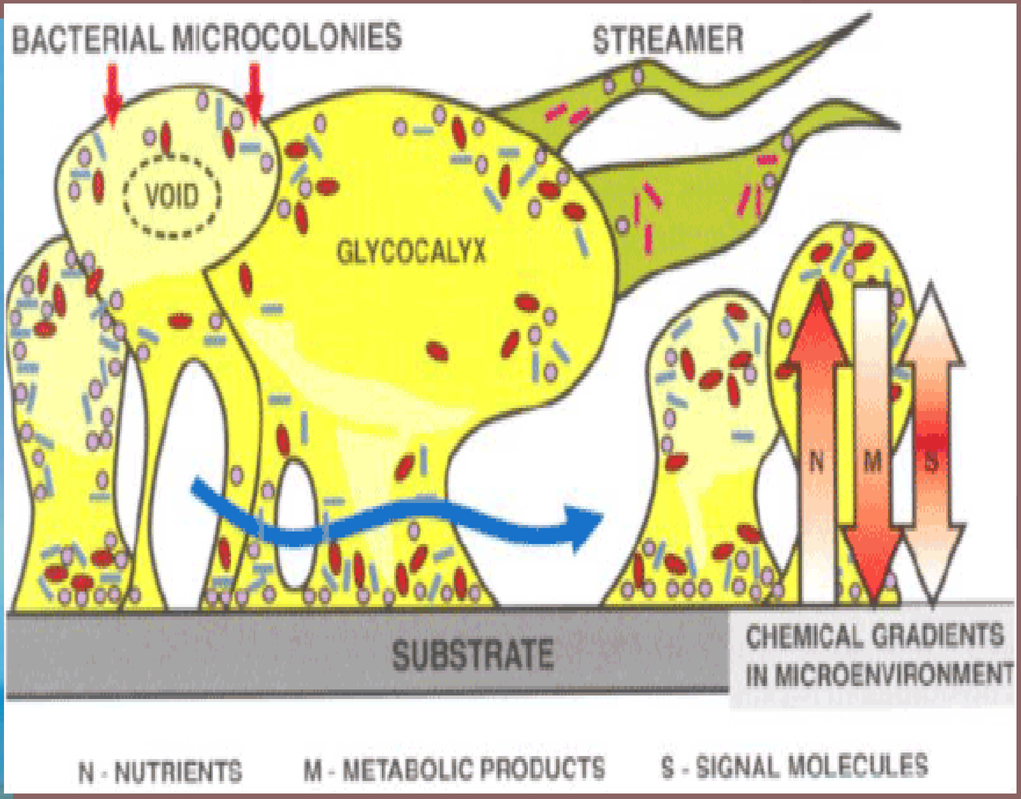
Figure 1
Biofilm Formation Model
Schematic diagrams depict bacterial biofilm formation. Both solid surface and pellicle biofilm contain exopolymeric matrices, although they may differ in composition. It is not clear if microcolony formation precedes mature pellicle biofilm formation.

Does the biofilm attach in a fluid stream ?

YES

Biofilms seems
to form columns and mushroom like projections
that are separated by water filled channels





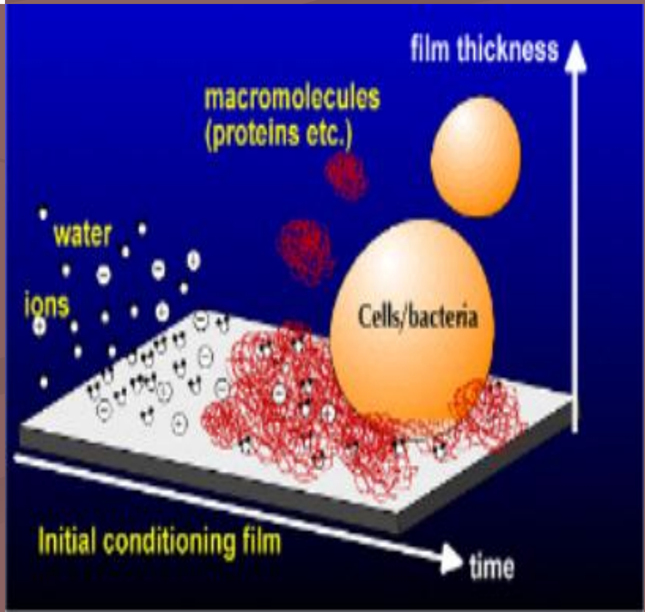
Does biofilm attach to

SURFACES ?

It develops on either :

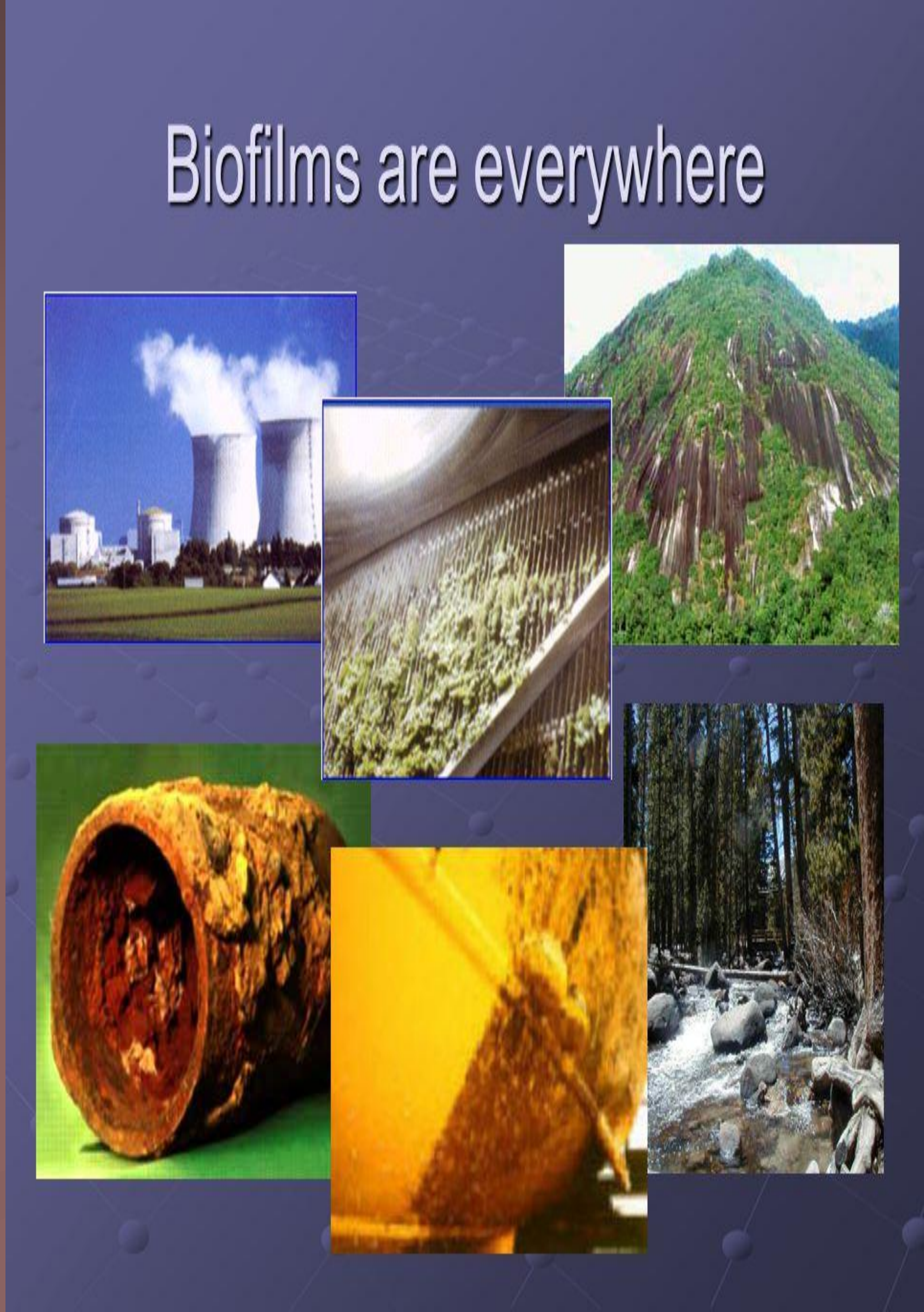
WET or DRY

BIOTIC or ABIOTIC



Solid food preparation surfaces


BIO FILM lab
an engineering and biology inquiry lab
www.STEMmom.org


Biofilms are everywhere

Genotypically

Biofilm **synthesis** is mainly encoded by:

- Intra cellular adhesion (ica) gene
- Biofilm associating protein (bap) gene
- Assisting gene regulator (agr) gene

(Tan et al 2018)

- Biofilm is influenced mostly by means of *ica* operons (*icaA, icaB, icaC and icaD*) genes.
- The most prodominant genes are *icaA* & *icaD* (Torlak et al 2017).
- Matrix is mainly exopolysaccharide
- Its composition varies between species strain and controlled by the bacterial growth conditions and the age of the biofilm (Godefroid et al 2010)

Biofilm-associated genes detected in CNS

- Accumelation Associated Protein (*aap)*
- Extracellular Matrix Binding Protein (*embP*), *fbe*, *atlE* and *eno* (Srednik et al 2017)
- -------------------

Brucella,
Bartonella
Herpes virus
Cytomegalo virus, **secrete exopolysaccharide under the overexpression of a gene called (*AiiD*)**

Aquired Immunity against Infectious Diseases

(Muraill et al 2016).

Amyloid production (curli fibers)

- Certain species such as *E. coli, Salmonella spp., Citrobacter spp., and T.B.* secrete cellulose and produce amyloid fibers (curli) which can provide structural, adhesive and protective properties to a biofilm by means of :

Bacterial cellulose synthesis gene (bcs)

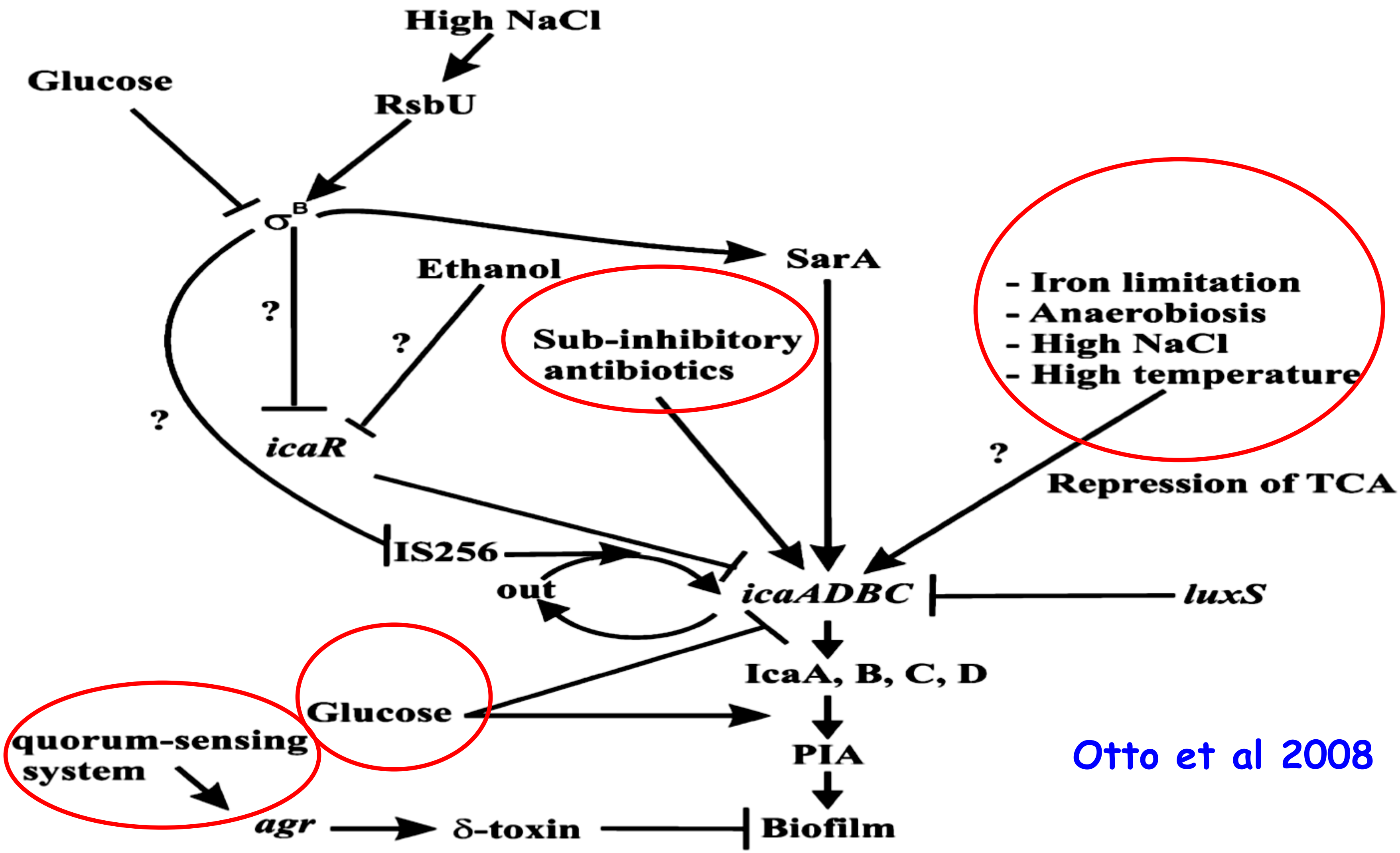
- In *E. coli*, curli fibers are required for the arckiticture of extraoplysaccharide matrix (Kikuchi et al 2005)

Bacterial cellulose is an organic compound with the formula $(C_6H_{10}O_5)_n$ produced by certain types of bacteria. characterized by high purity, strength, moldability

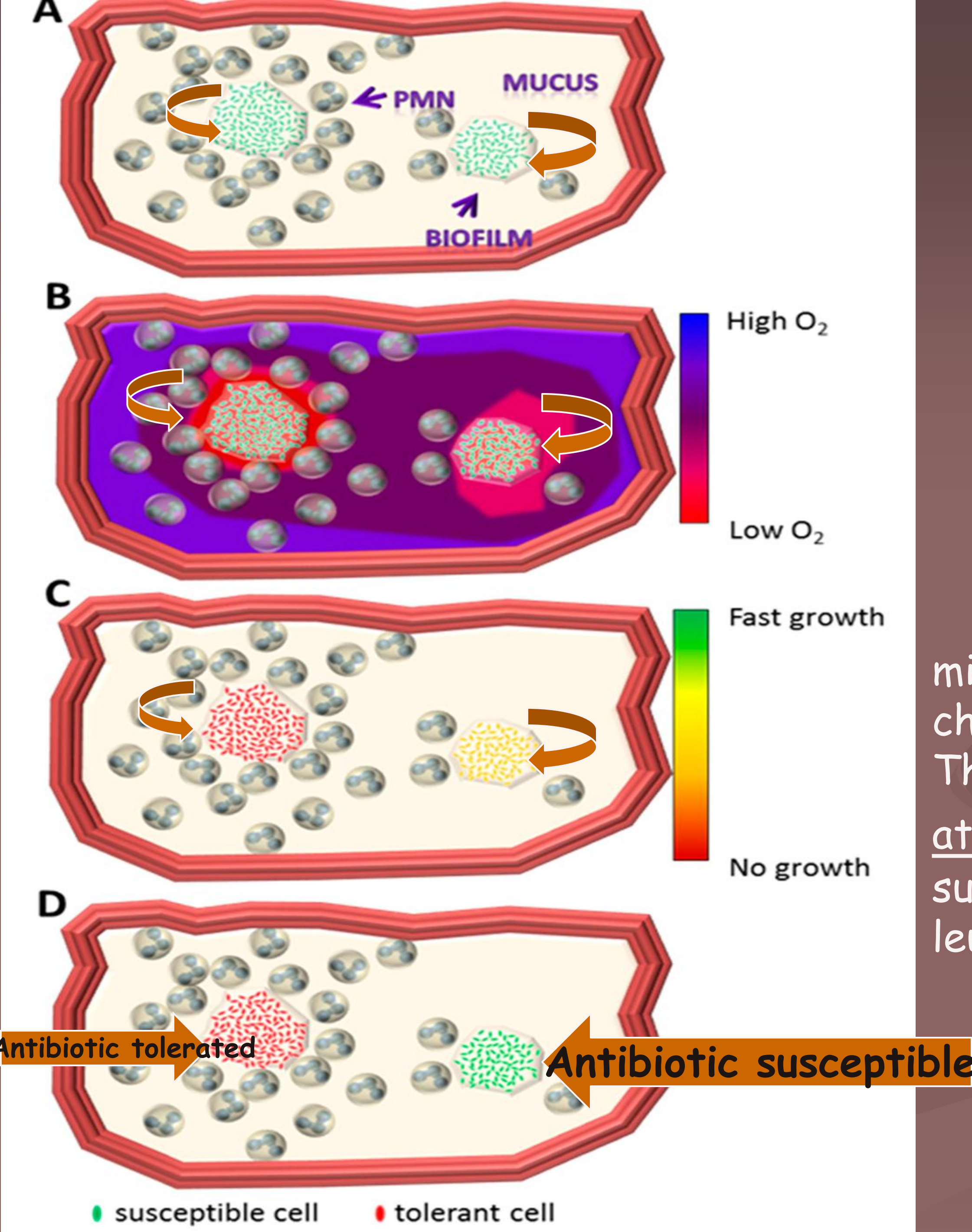
E. Coli biofilm genes

- csgD : Increases curli fimbriae production
- bcsA : cellulose synthesis
- pgaC : helps in biofilm adhesion
- fimB : protein regulates fimbriae production
- Hha : haemolysis expression and decreases biofilm
  - (Sharma et al 2016)

Factors affectting *ica* operon



Otto et al 2008



Oxygen microrequirenments and antibiotic tolerence

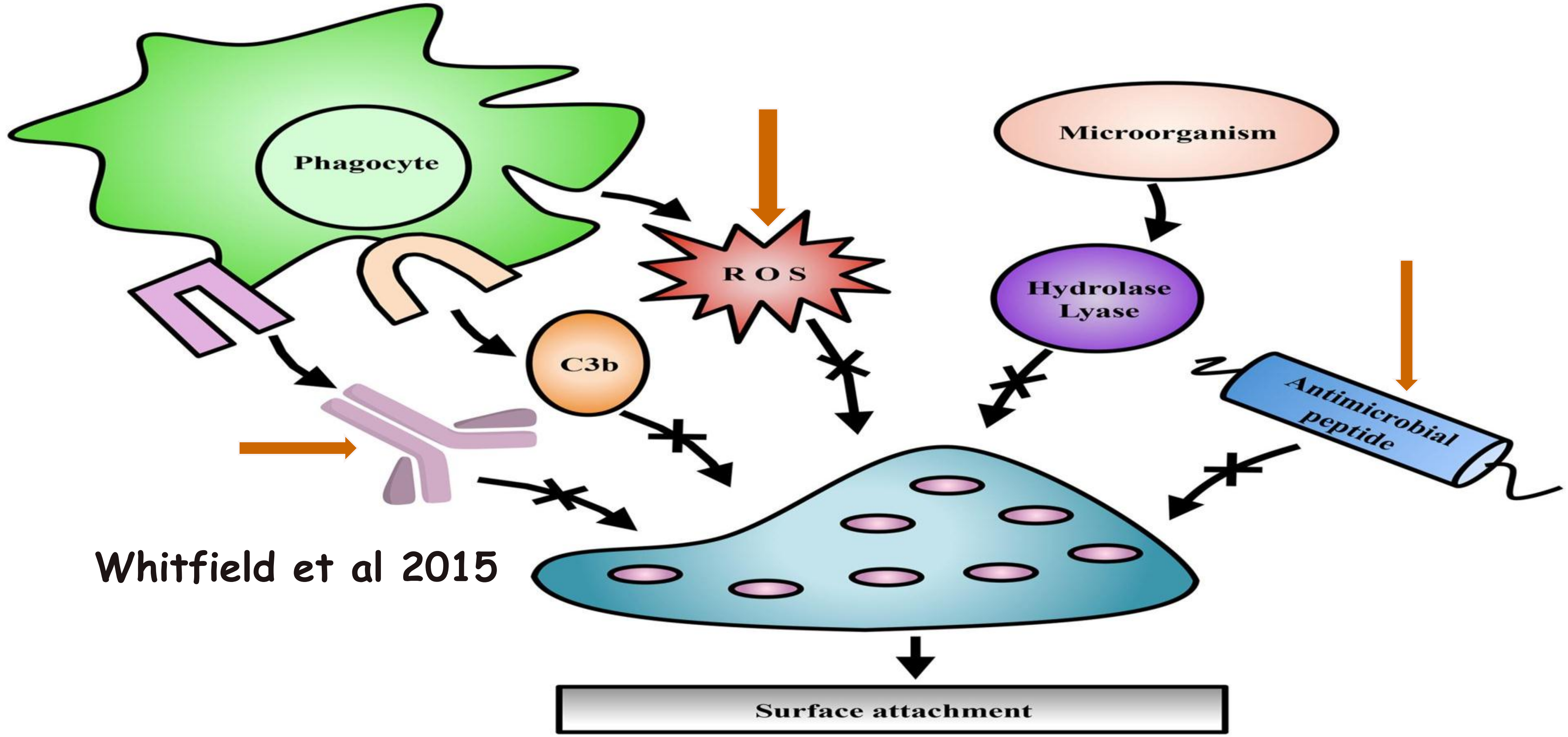
microenvironment of biofilms in chronic infections ..
The bronchial lumen with two non-attached (pillcle) biofilms surrounded by polymorph nuclear leukocyte (PMN) infiltrated mucus

Sønderholmet et al 2017

Virulence of biofilm

- MRSA biofilms are 1000- fold more resistant to antibiotics *in vitro*. (Gowrishankar et al 2016) but mechanism of these drug tolerances observed in biofilms is still unclear (Jimi et al 2017).
- Bacteria in the protected biofilm environment have 10 to 1000 fold more antibiotic resistance compared to planktonic bacteria (Piotrowski et al 2017).

- Bacteria living in biofilms are better able to survive more by slowing their growth, reducing metabolism resulting in
- Increased resistance and reduced penetration of antimicrobial into the biofilm structure (Notcovich et al 2018)

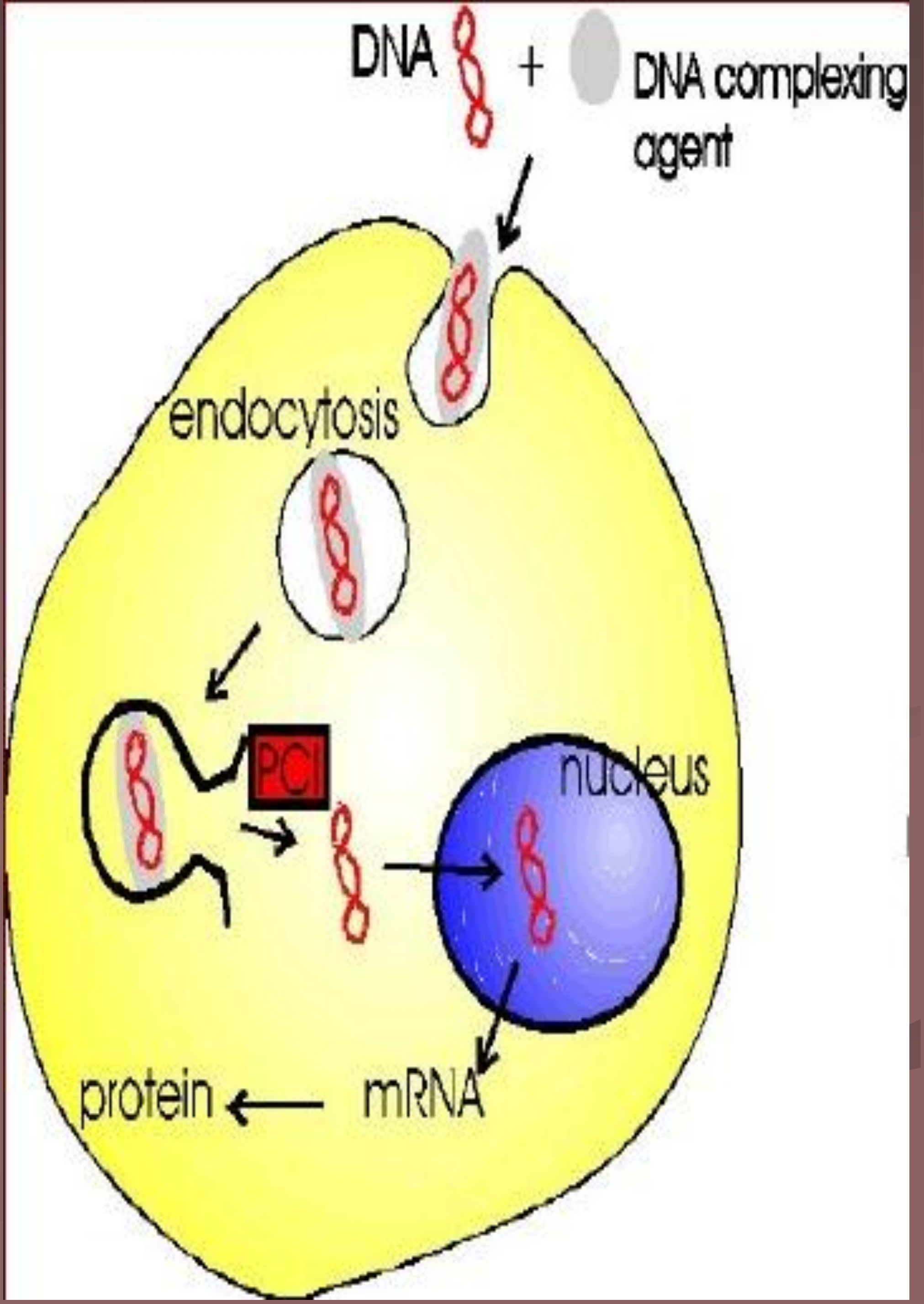


Whitfield et al 2015

- *in vivo,* biofilms reduce the effectiveness of the host innate immune system by reducing phagocytosis during infection (Asli et al 2017).

Biofilm help in Gene transfer between / among bacteria which can convert the avirulent commensals

to highly virulent pathogens



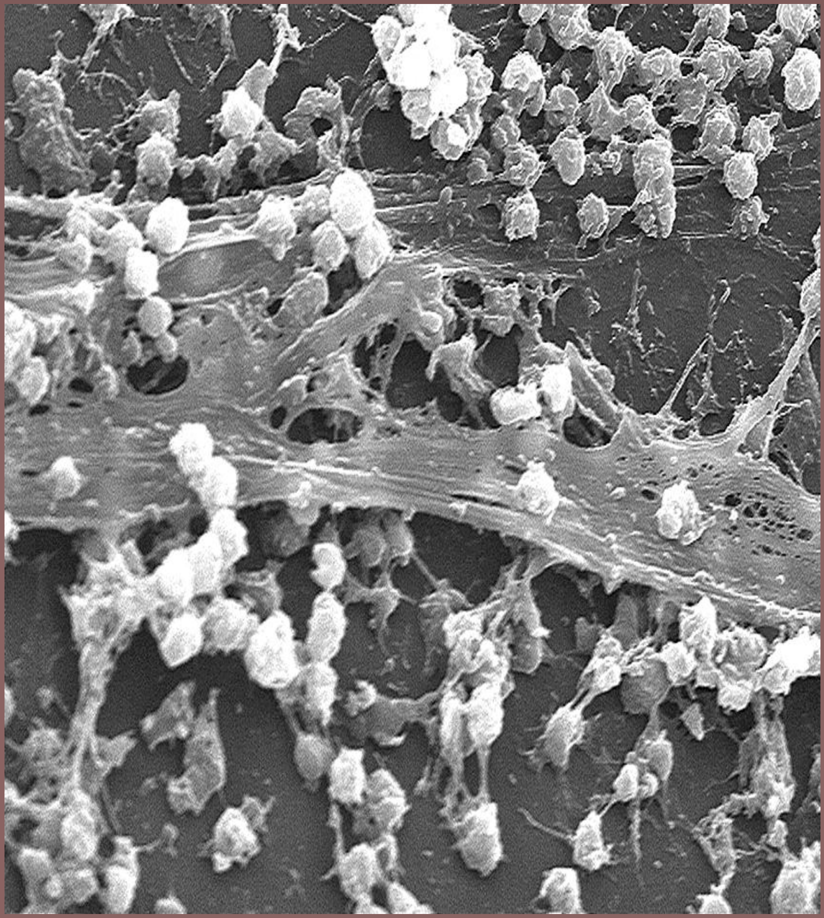
Even some biocides !

- **Sodium hypochlorite** (oxidizing biocide) as one of the most effective antibacterial agents — requires a **600 fold** - increase in concentration to kill **biofilm cells** of *S. aureus* compared with **planktonic cells** of the same species (Luppens et al 2002).
- **Goldshield (GS5)** is a novel organosilane biocide marketed as a single application product **with no effect** on either biofilm **formation** or **development** (Murray et al 2017).

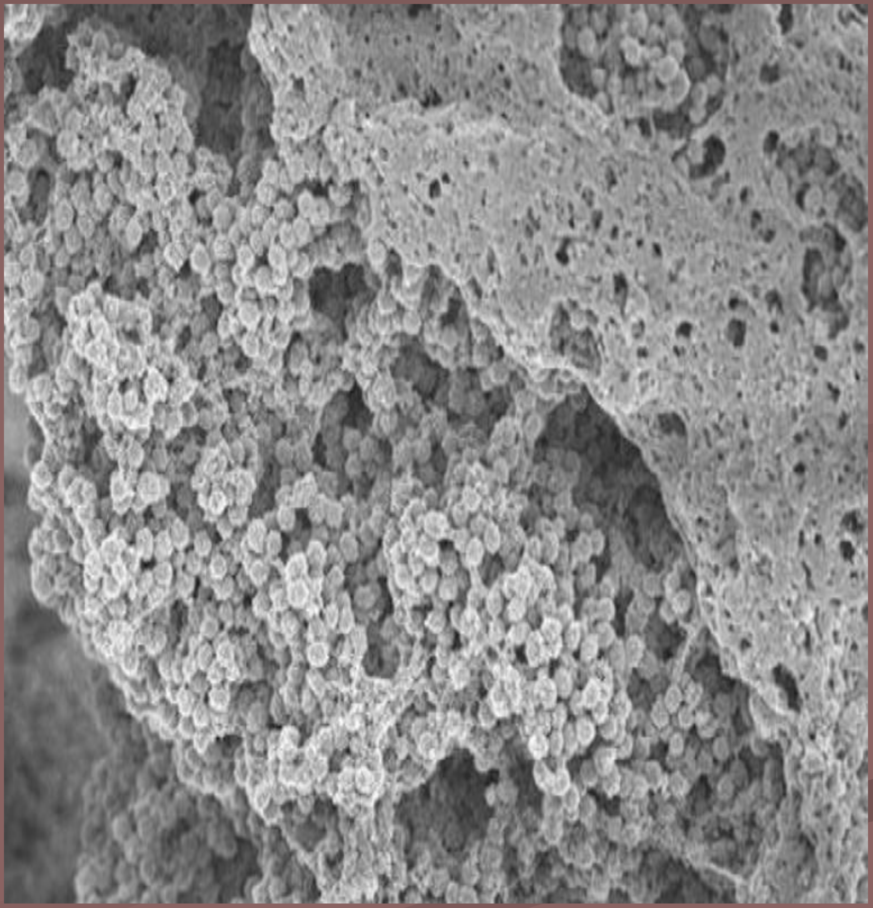
Polymicrobial biomass

- Biofilms act as **reservoirs** of different pathogenic microorganisms favouring the **dissemination** of infection to other body sites (Zago et al 2015) even in drinking water pipes (Luo et al 2017)
- **Synergistic, mutualistic, and antagonistic interactions** that occur between microorganisms contribute to the development of polymicrobial biofilm communities [Kuramitsu et al 2007].

SEM of MRSA biofilm
indwelling catheter

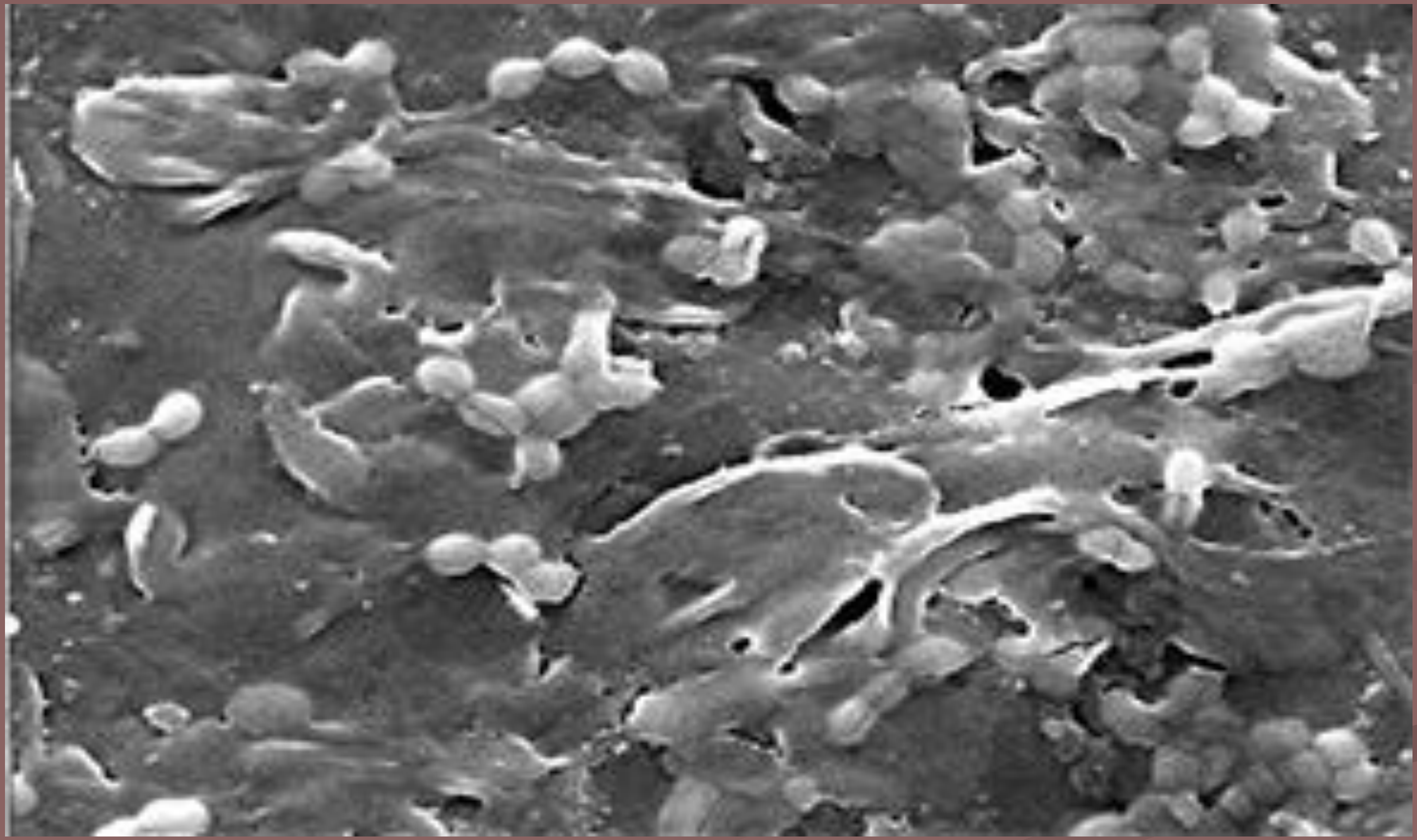


Biofilm early developed



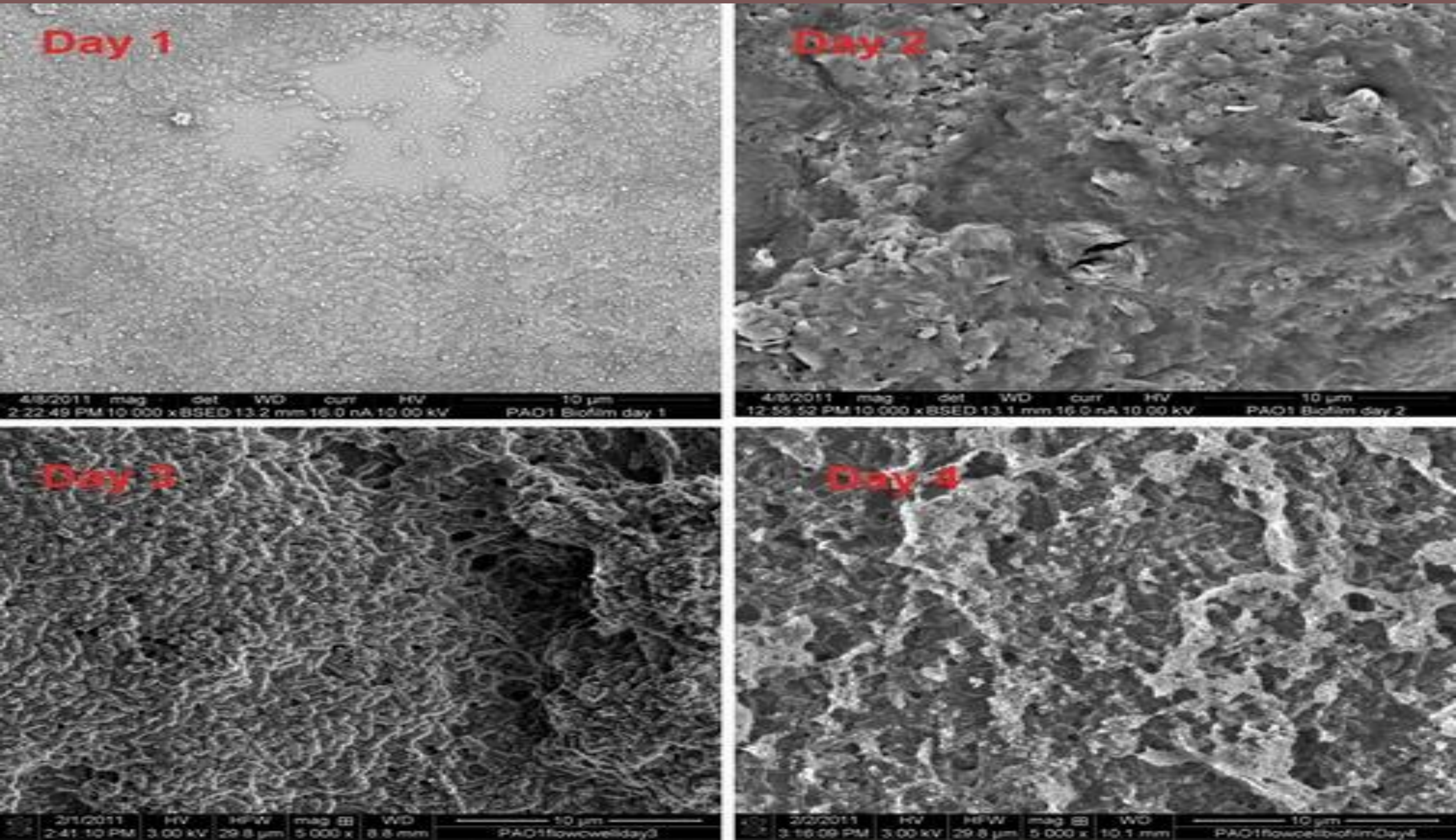
Biofilm 4 days old

SEM of MRSA
biofilm on plastic surface of food equipment

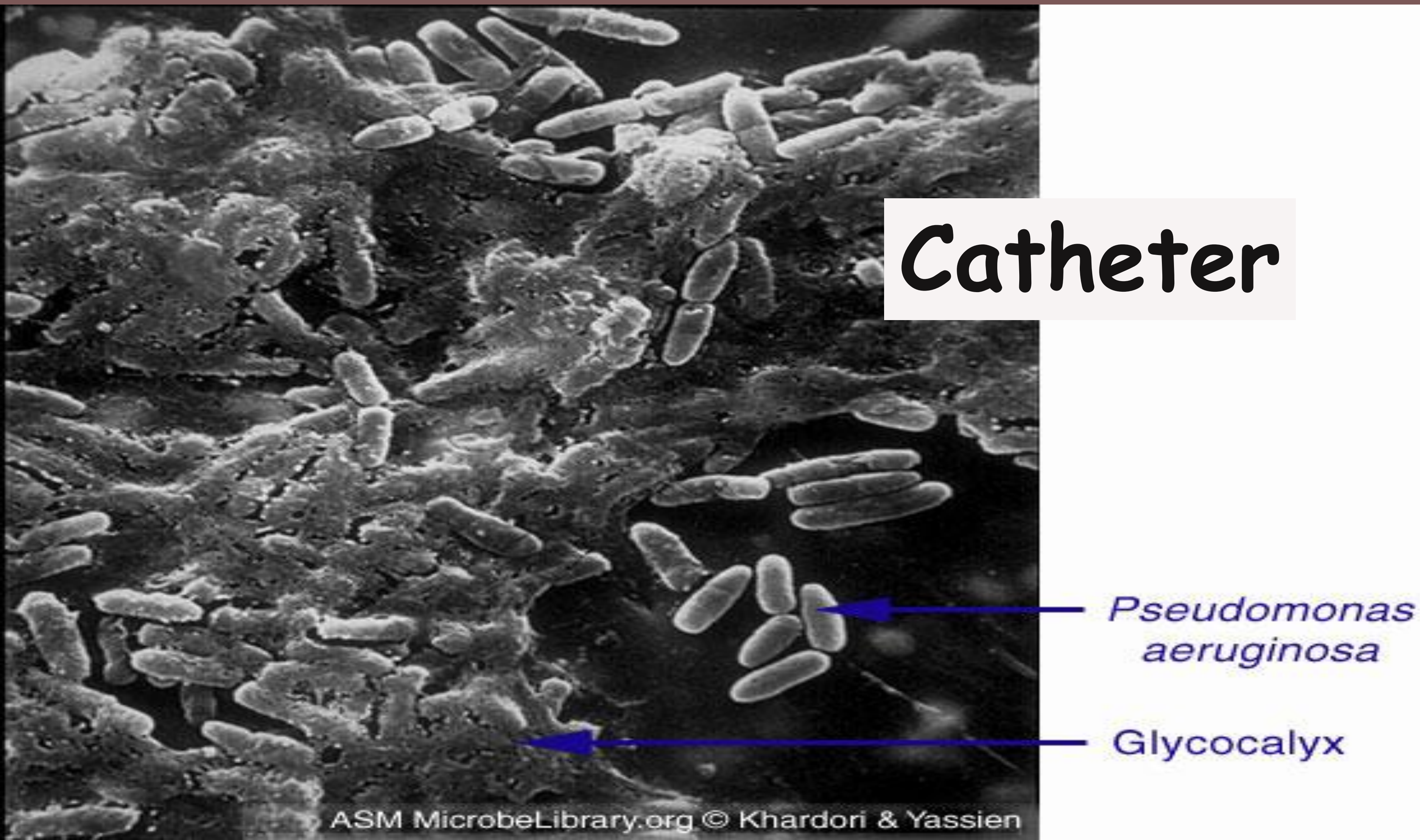


SEM of *Ps. aurogenosa* biofilm

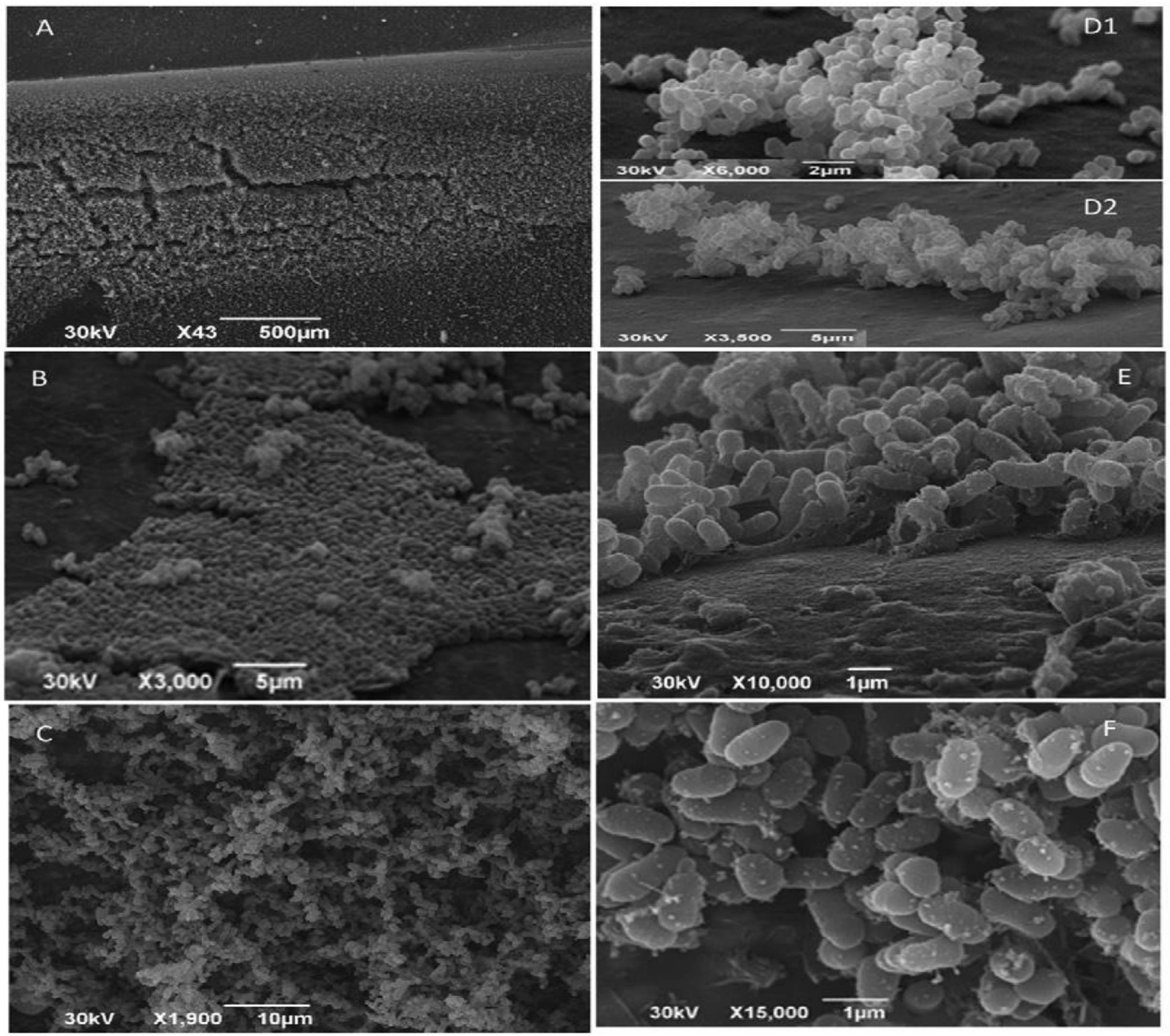
along 4 days



Ps. aurogenosa from renal catheters

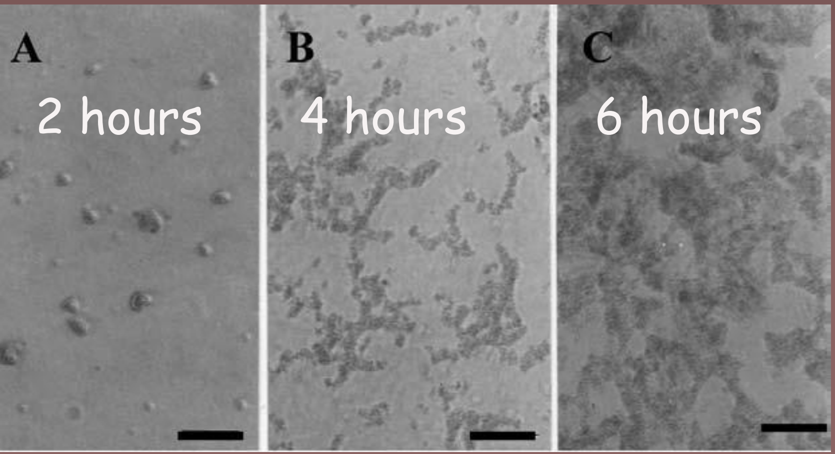


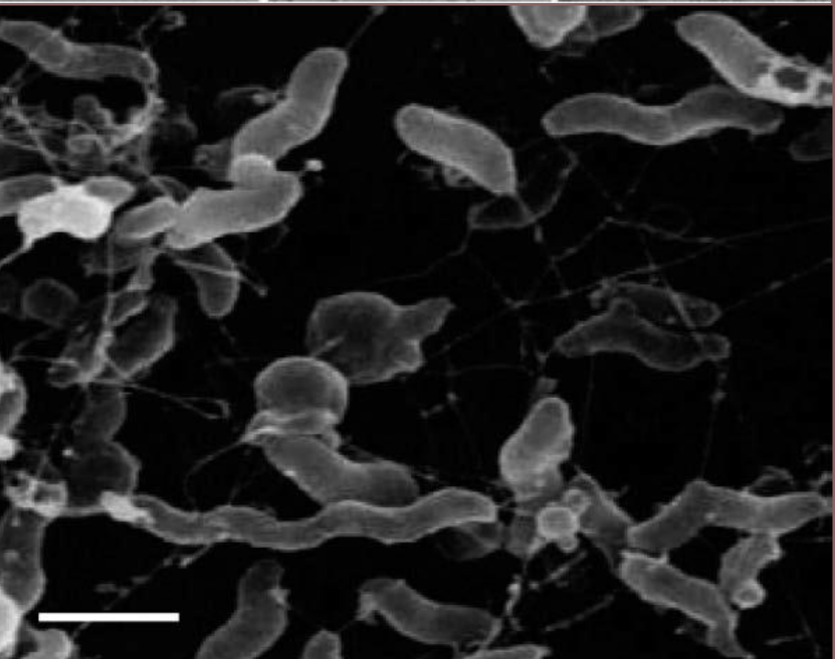
Corynebacterium striatum from renal catheter (Souza et al 2015)



Campylobacter jejuni

(MOE et al 2009)

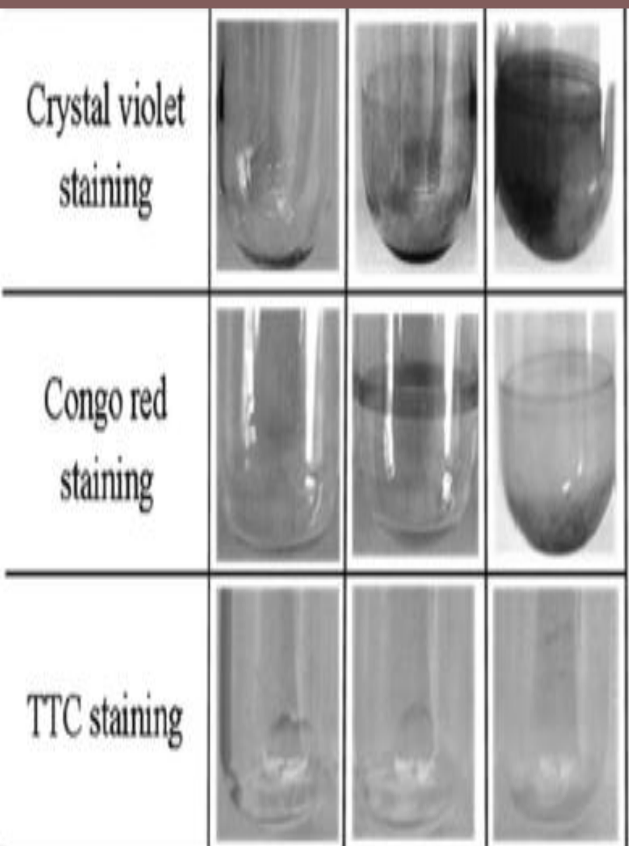




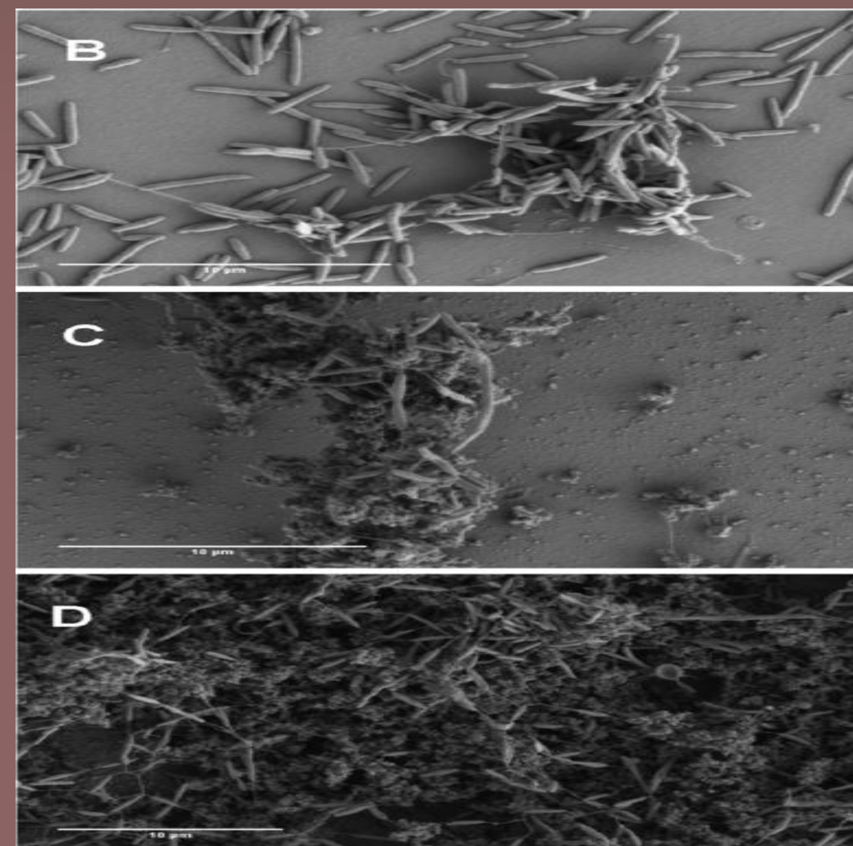
Time course of biofilm formation by *C. jejuni*

Scanning electron microscopy observation of the biofilm formed on a glass coverslip. Flagella act as bridges forming net-like connections between the organisms.
Bar =1 μm.

Chicken meat juice enhance *Campylobacter jujeni* biofilm (Brown et al 2014)



Juice 0% 5% 100%



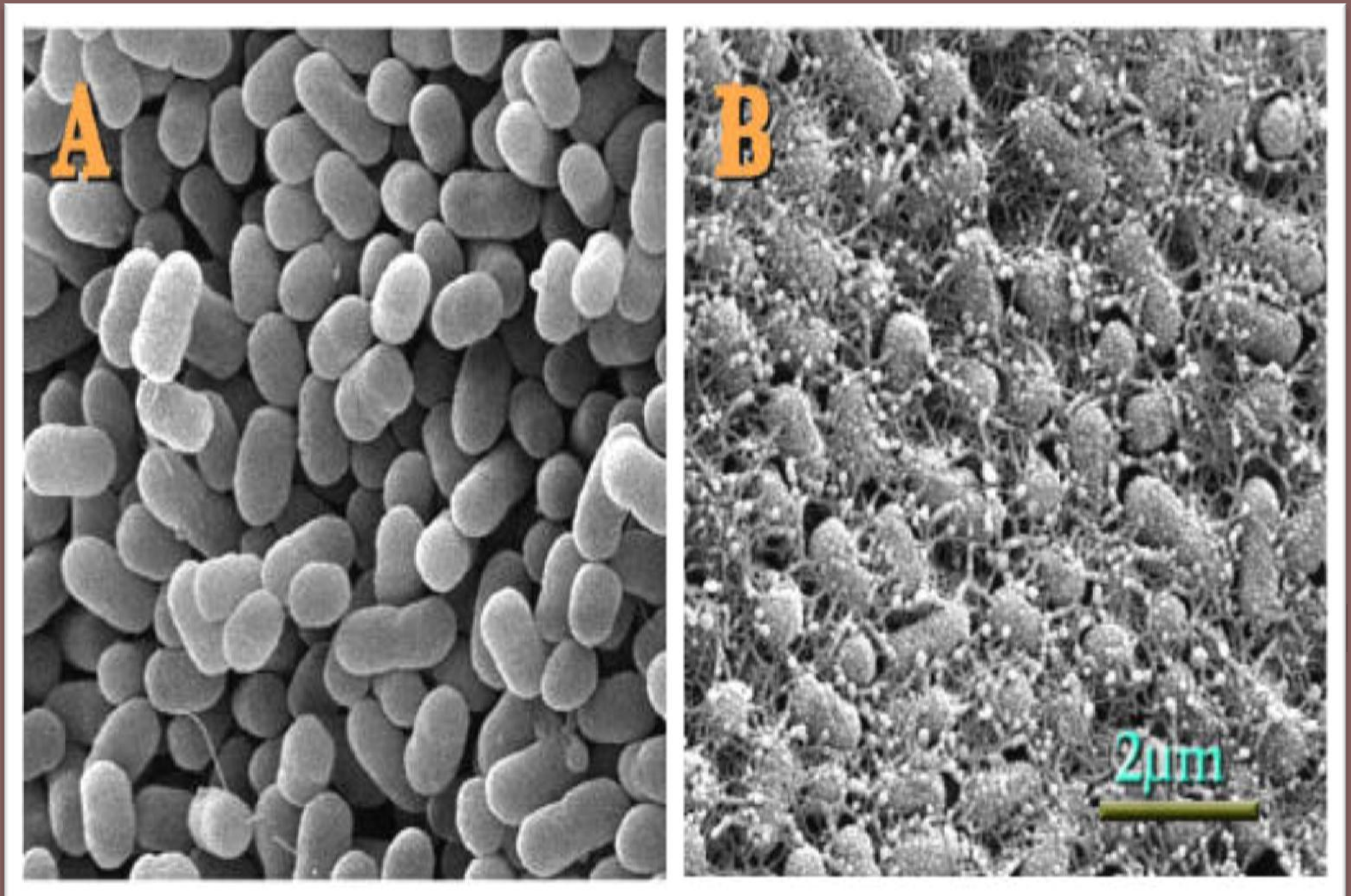
Pure brucella broth

brucella broth with 5% Juice

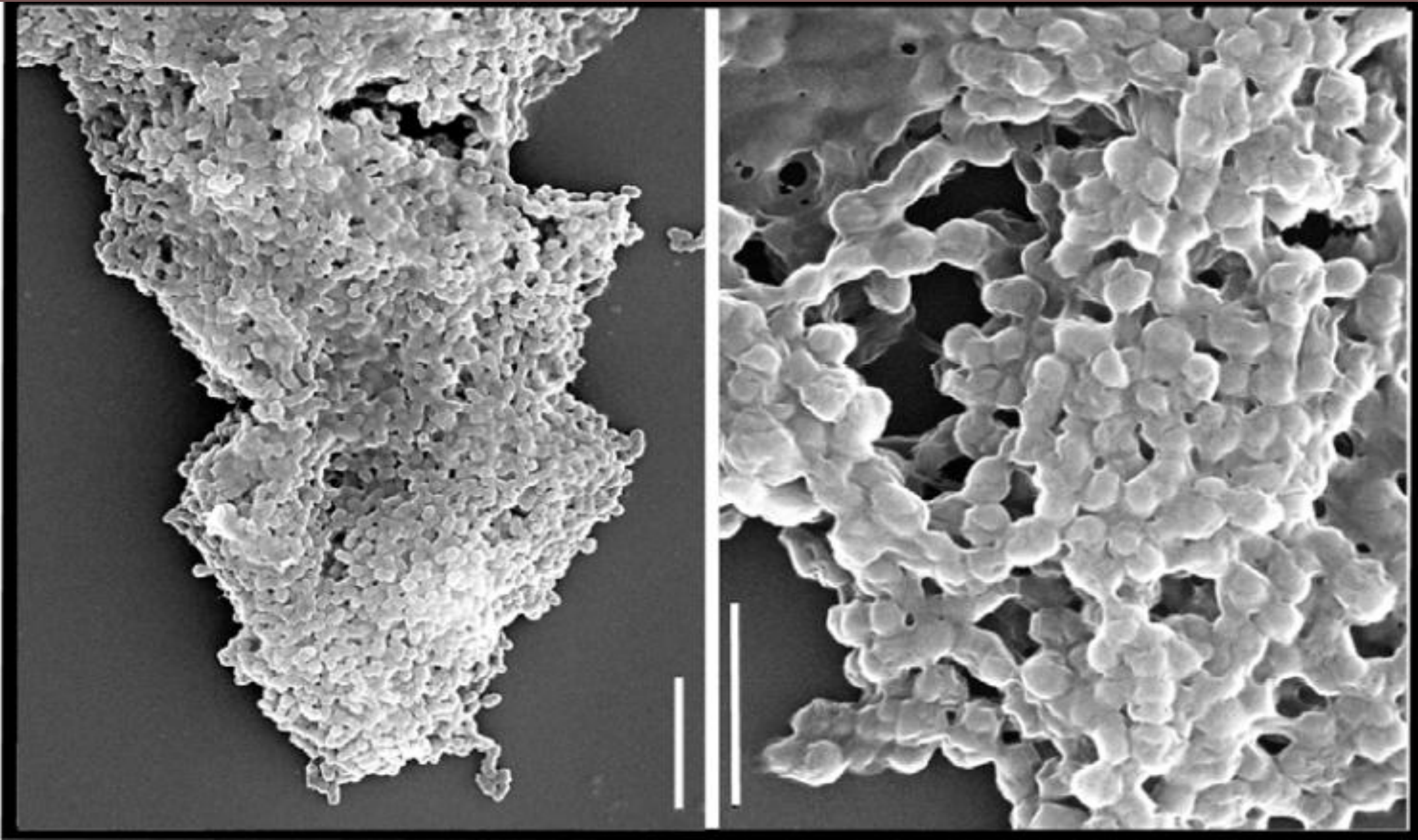
Juice 100%

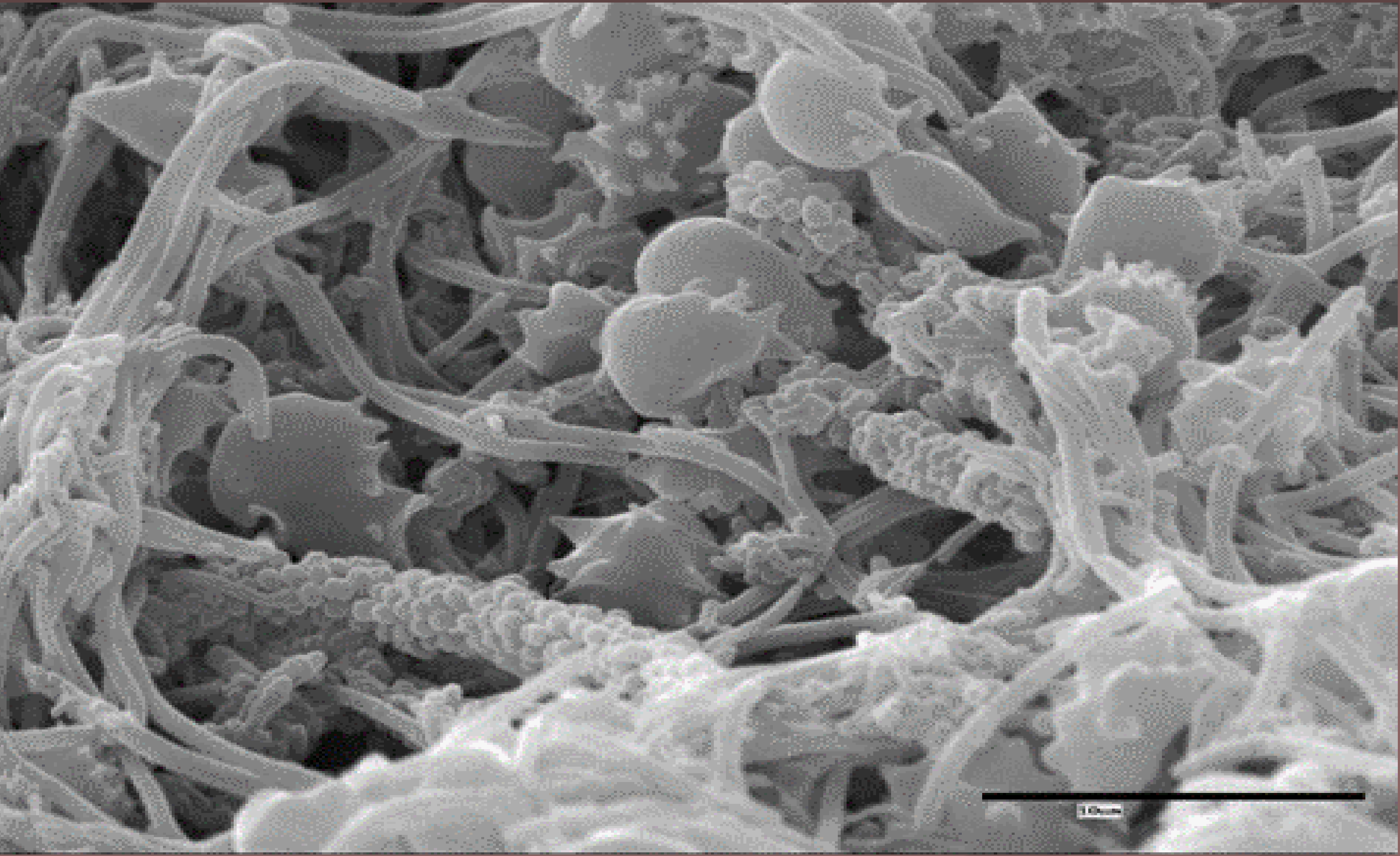
E.coli O 157 biofilm

- A -ve
- B +ve

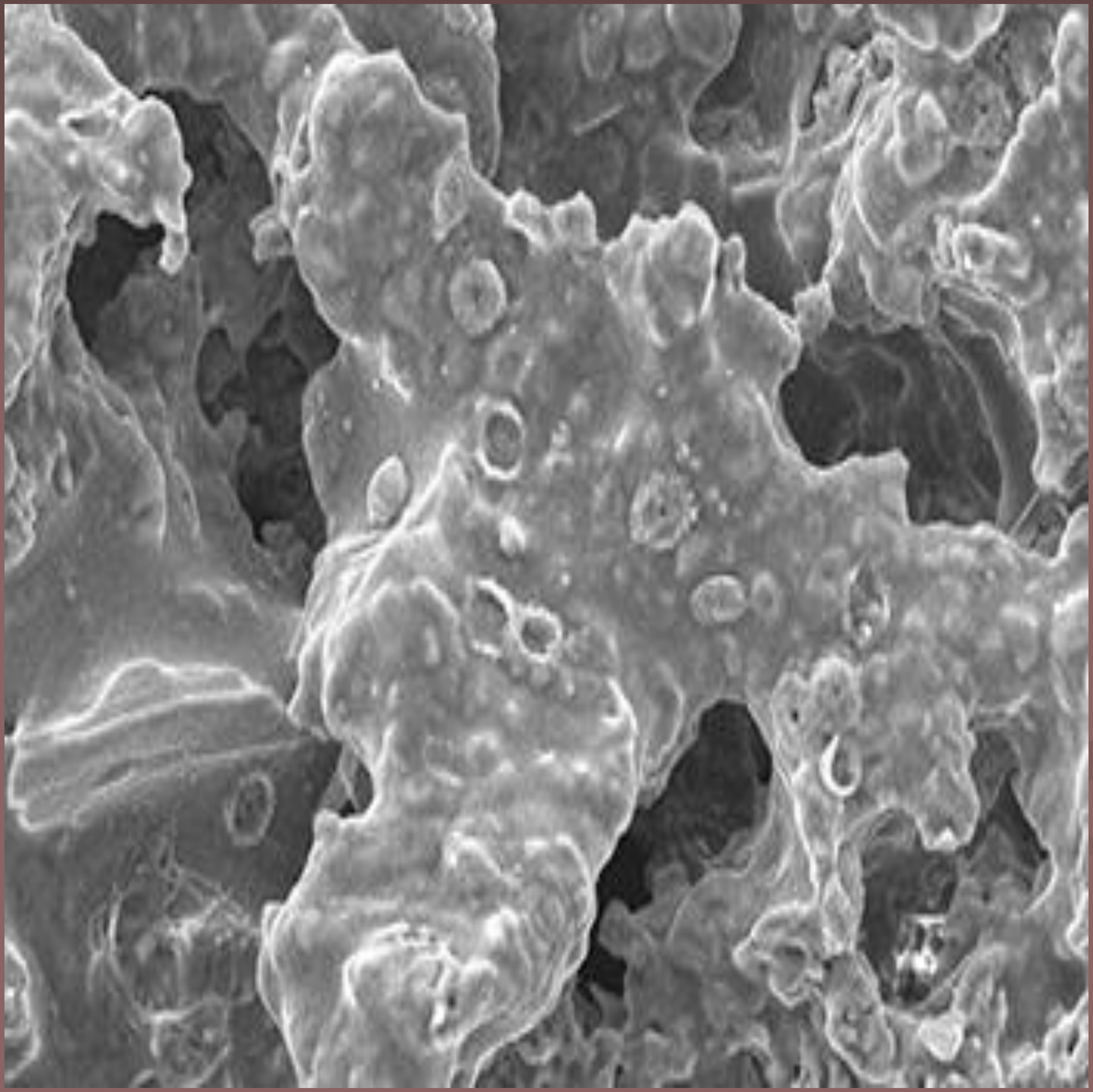


SEM of *Brucella abortus 2308* biofilm
(Almiron et al 2013)





Dental polymicrobial biomass



Biomass of chronic wounds (*S. aureus & Ps. aur*) are very difficult to be **treated** (Pereira et al 2017)

Severe polymicrobial community biofilm formation

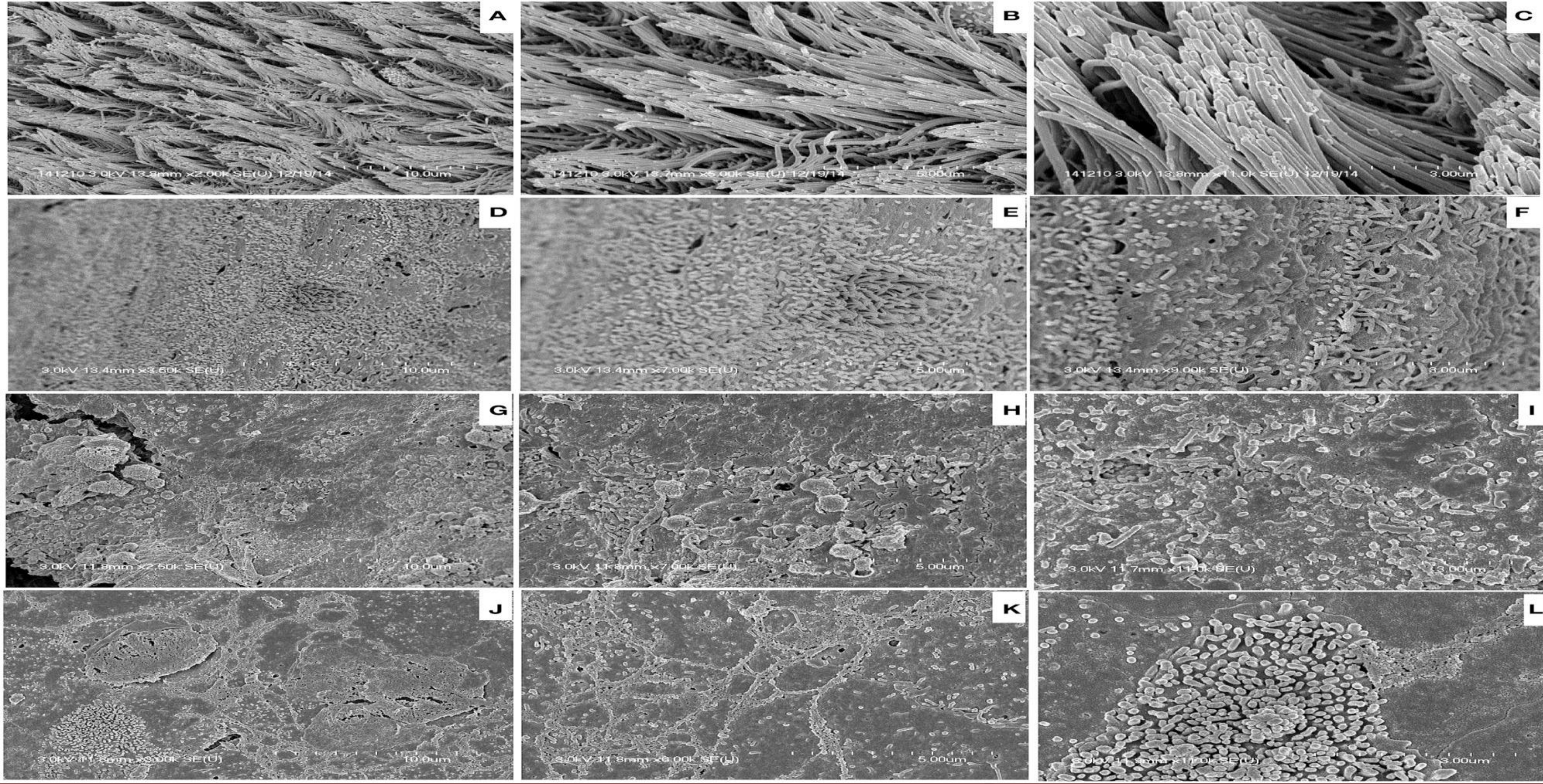
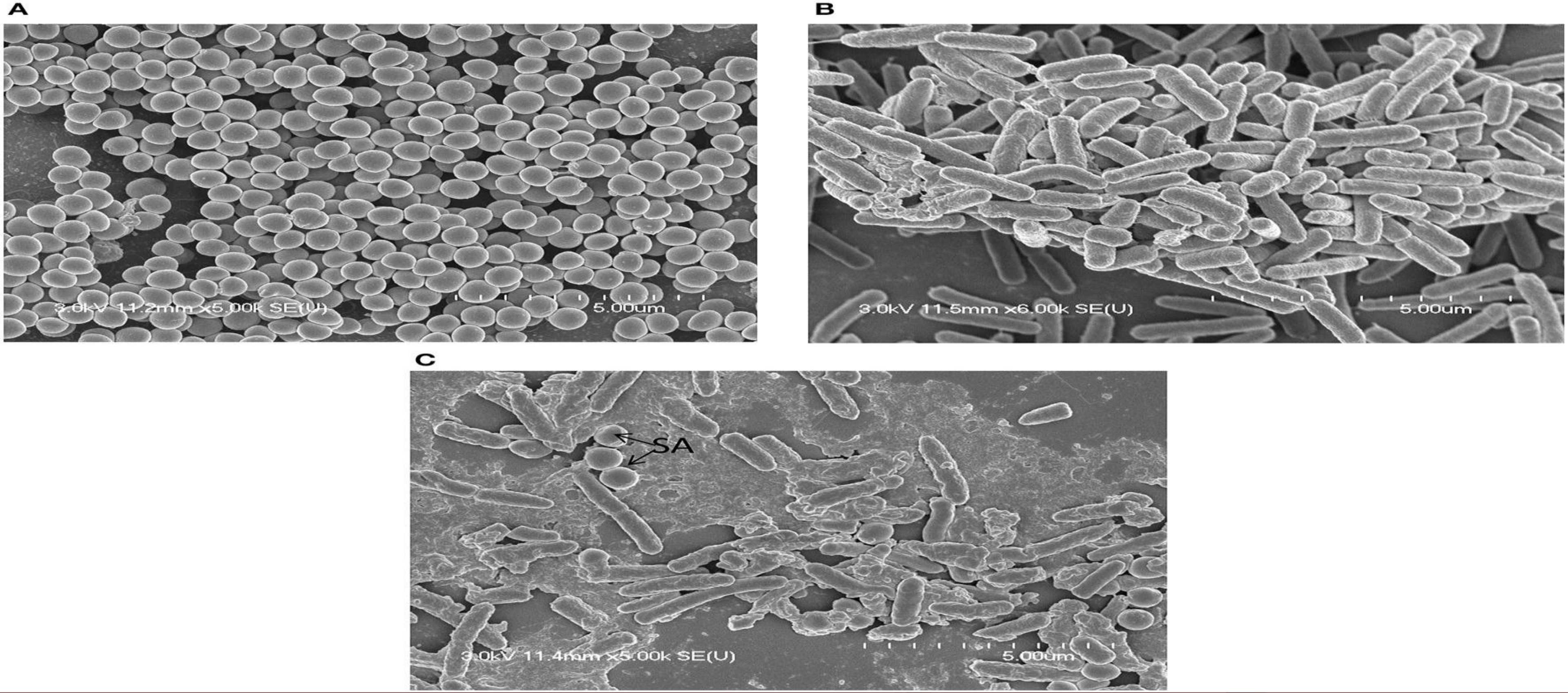


FIGURE :Scanning electron microscope (SEM) images of bullae inoculated with MRSA) and *Ps. aeruginosa* (Animal inoculation)

- **(A–C) Representative SEM images of normal control.**
- **(D–F) Representative SEM images of rat bullae inoculated with MRSA only.**
- **(G–I) Representative SEM images of rat bullae inoculated with *Ps.* only.**
- **(J–L) Representative SEM images of rat bullae inoculated with a mixed culture.** **(Yadav et 2017)**



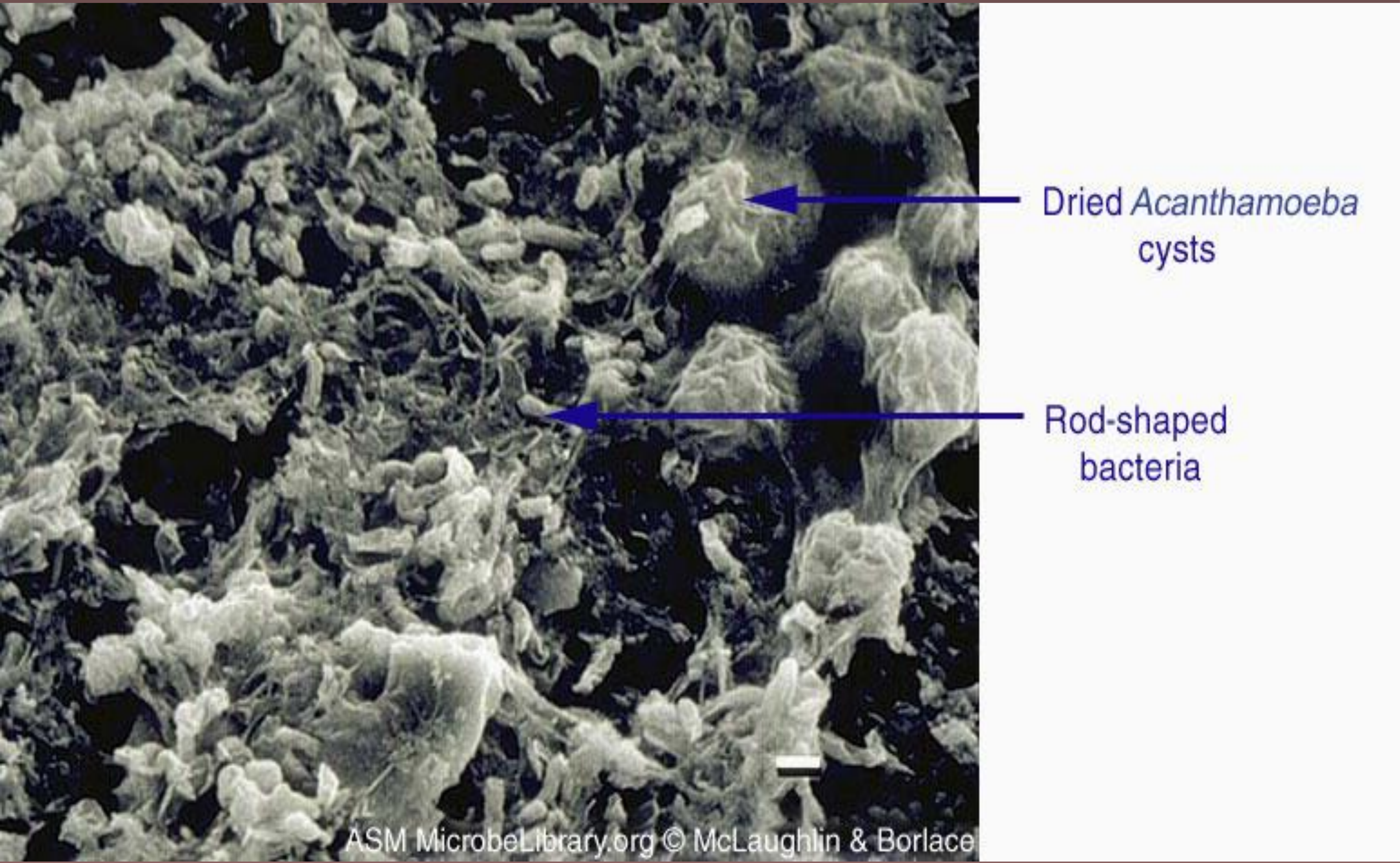
- FIGURE : Scanning electron microscope (SEM) images of *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* (Ps. a) isolated from **chronic wound**
- (A) Representative SEM image of MRSA (single species biofilms).
- (B) Representative SEM images of Ps. a (single species biofilms).
- (C) Representative SEM images of (multi-species biofilms of MRSA and Ps. a)

BIOFILMS AND CONTACT LENSES

Common in 20 - 80 %

- Bacterial biofilm formation on contact lenses and contact lens storage cases may be a risk factor in contact lens-associated corneal infections. Studies have shown that contamination of lens cases by bacteria, fungi, and amoebae is common with 20% to 80% of lens wearers having a contaminated lens case.

Contact lens

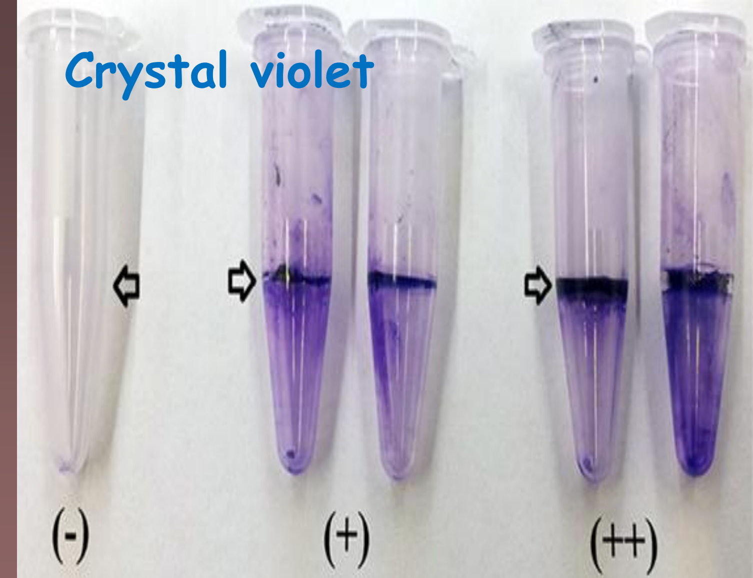


Diagnosis

How to be detected phenotypically ?

- A - Qualitatively:
  - Crystal violet ependorf tube test
  - Safranin tube test
  - Inoculation onto
  - Congo Red Agar.
  - Modified Congo Red Agar
- B – Quantitatively:
  - Microtitration assay by crystal violet stain of serial dilution of 96 well plate and read the result by ELISA (El-Banna et al 2016).

Qualitative



Quantitative

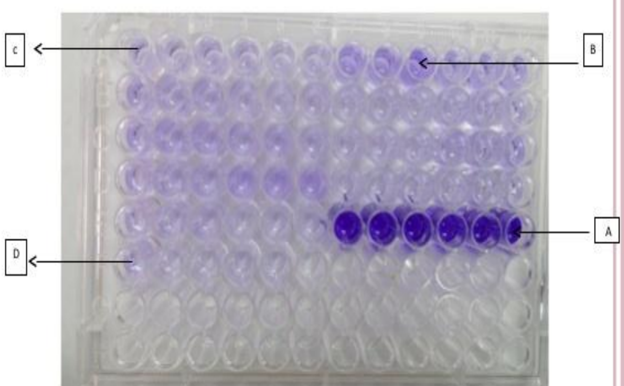


Fig.6 Detection of biofilm formation by microtiter plate method ,A:strong,B: weak C: non biofilm former D:negative control.

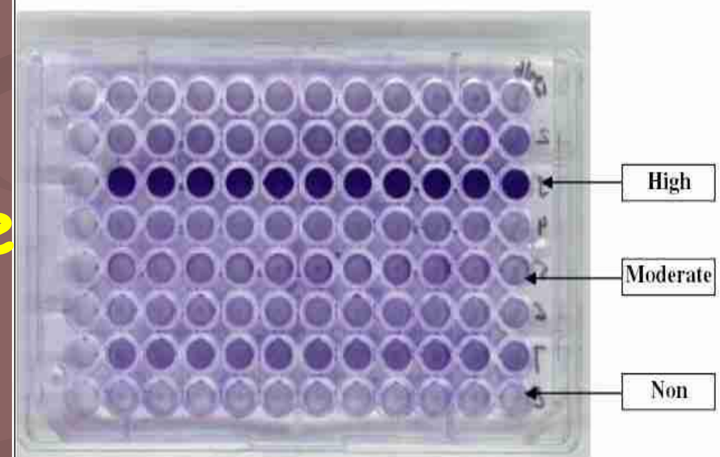
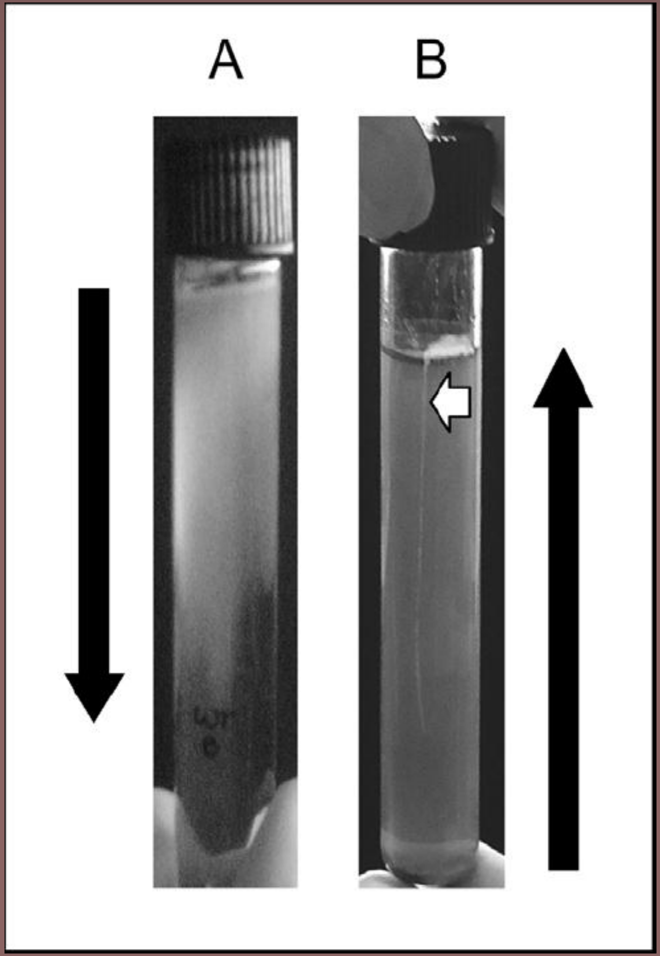


Figure 1: Screening of biofilm producers by TCP method: high, moderate and non slime producers differentiated with crystal violet staining in 96 well tissue culture plate.

Biofilm development in *brucella mellitensis*



- *B. abortus* growth under microaerobic conditions. **10-day old cultures grown** in semi-solid (**A**) or liquid (**B**) media at 37°C.
- Bacterial aggregates are visualized at the **top** of the liquid medium at small arrow.

Bacterial mass at the **bottom** of the tube, large arrows shows growth direction (**Almiron et al 2013**) .

Semi-solid **Liquid**

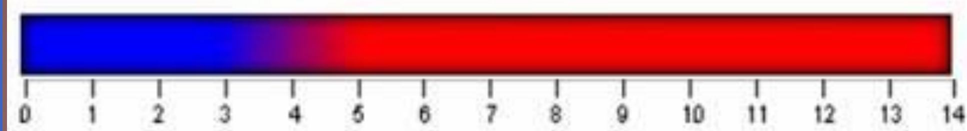
Brucella biofilm detection

Microaerobic Anaerobic

- *B. abortus* biofilm development on polystyrene flasks.
- **Crystal-violet** stained flasks from cultures grown under anaerobic (right) or microaerobic (left) conditions at 37°C during 18 days after saturation (**Almiron et al 2013**).

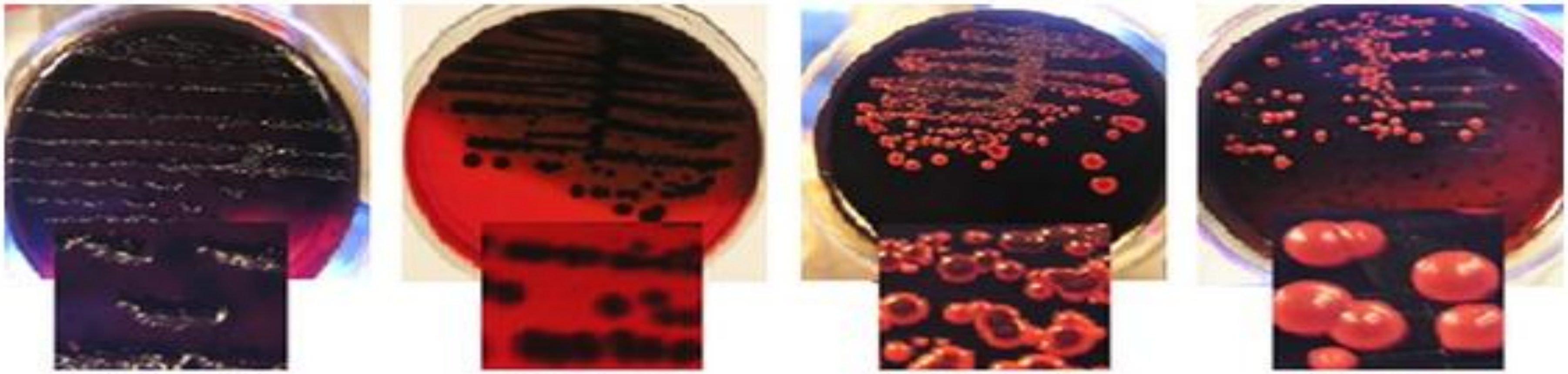
BIOFILM on Congo Red Agar

Congo red



Approximate pH range for color change: 3.0-5.0
Color of acid form: blue
Color of base form: red

S. aureus
on congo red agar (Mariana et al 2009)



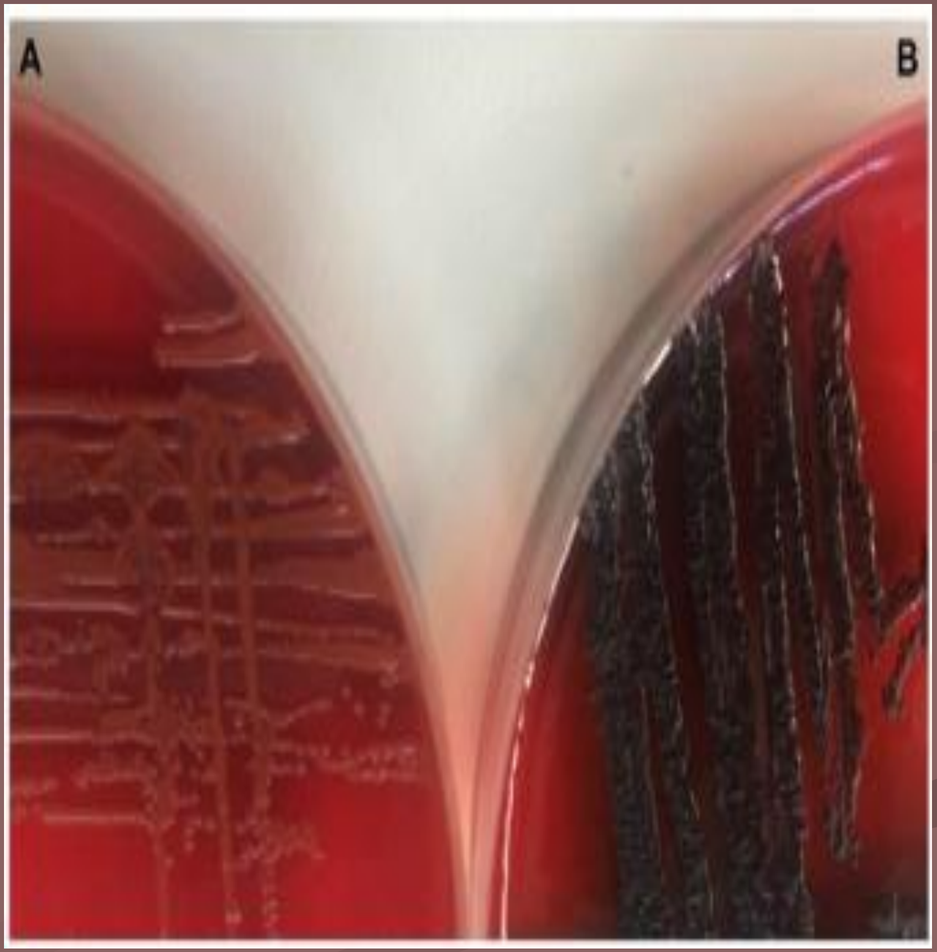
Very black colonies | Black colonies | Weak black colonies | Red colonies

B

100% black colour

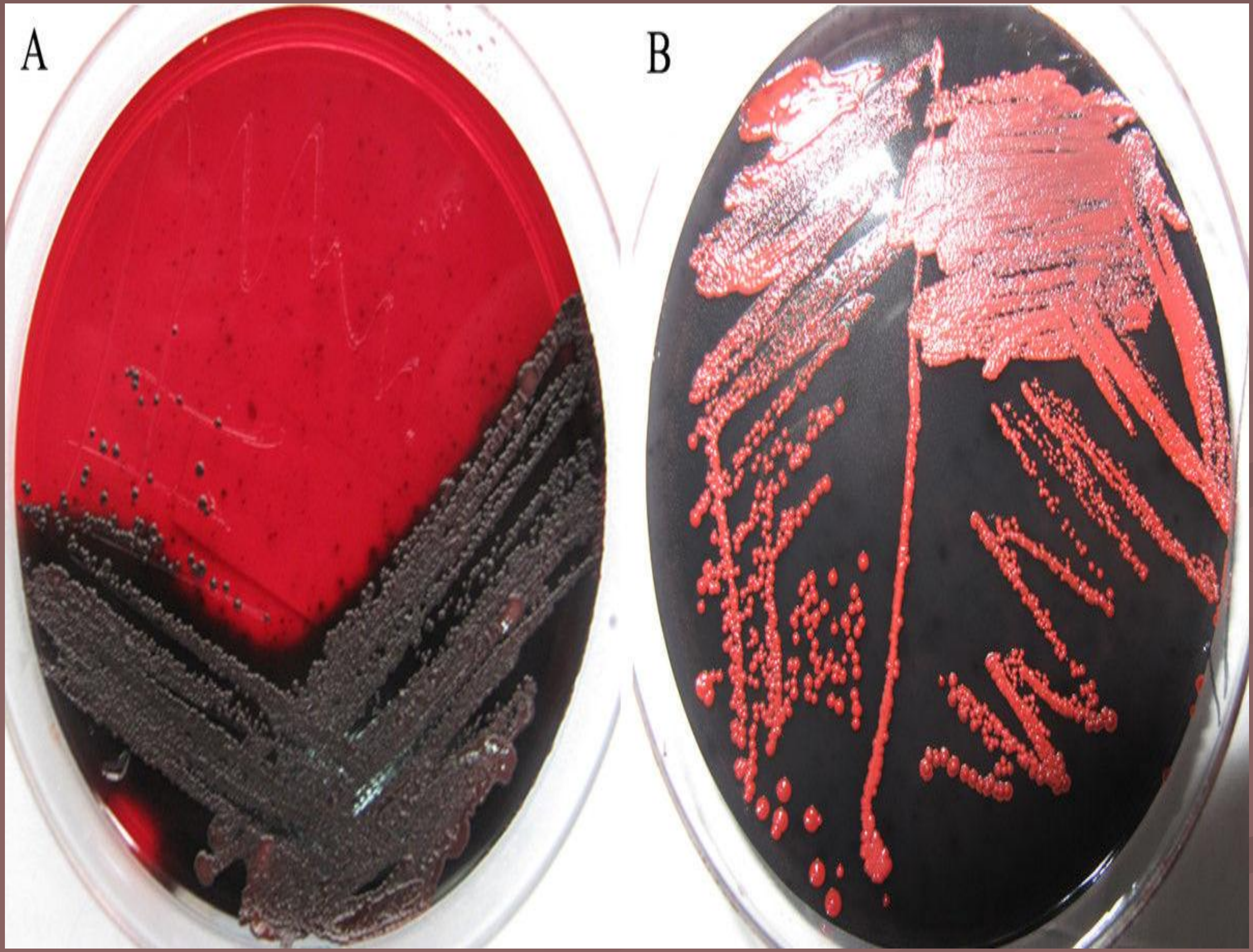
After 48hr at 37◦C ➔ After 2 days at room tem. ➔ After 4 days at room tem.

MRSA



S. epidermedis

NEGATIVE

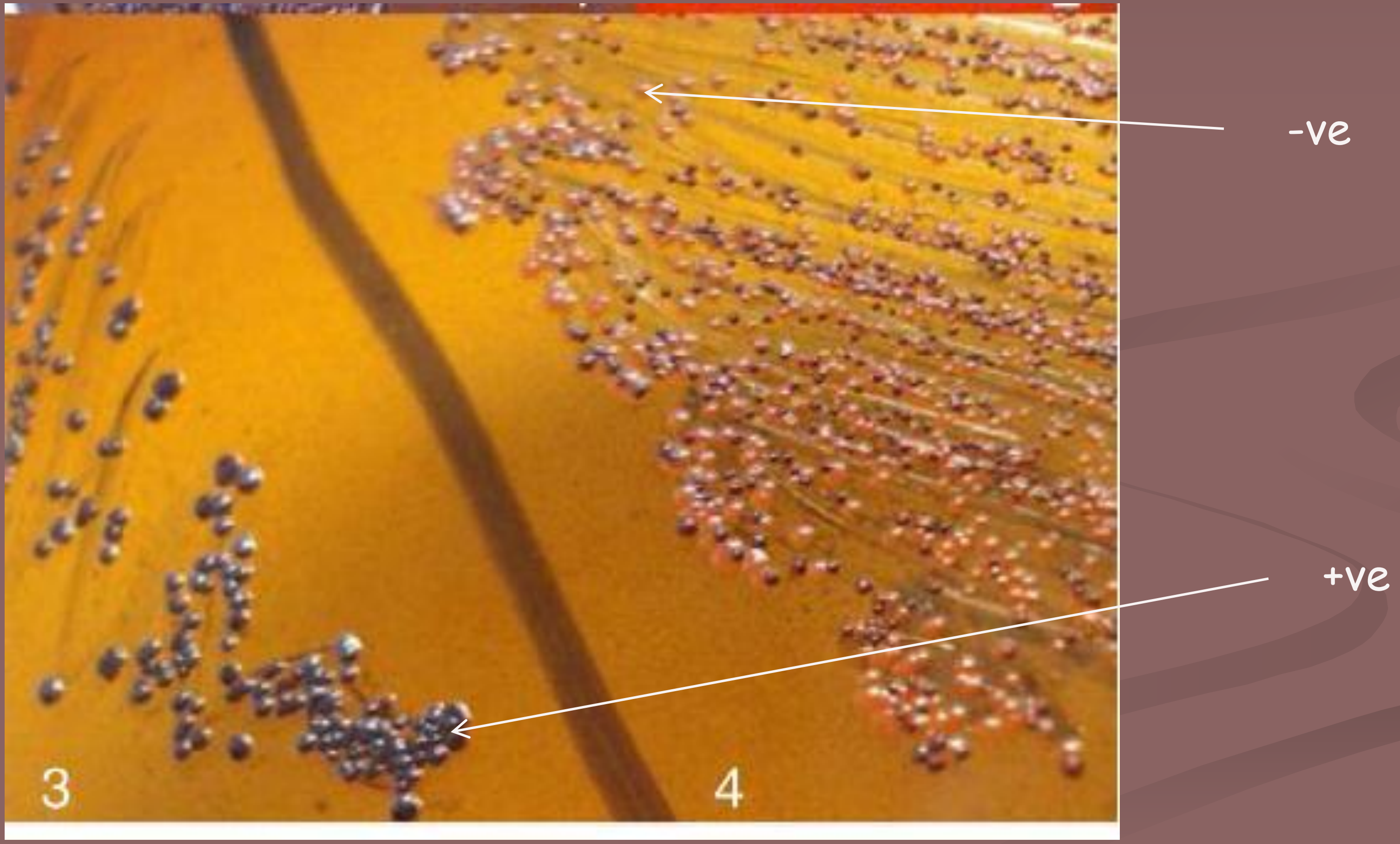


POSITIVE

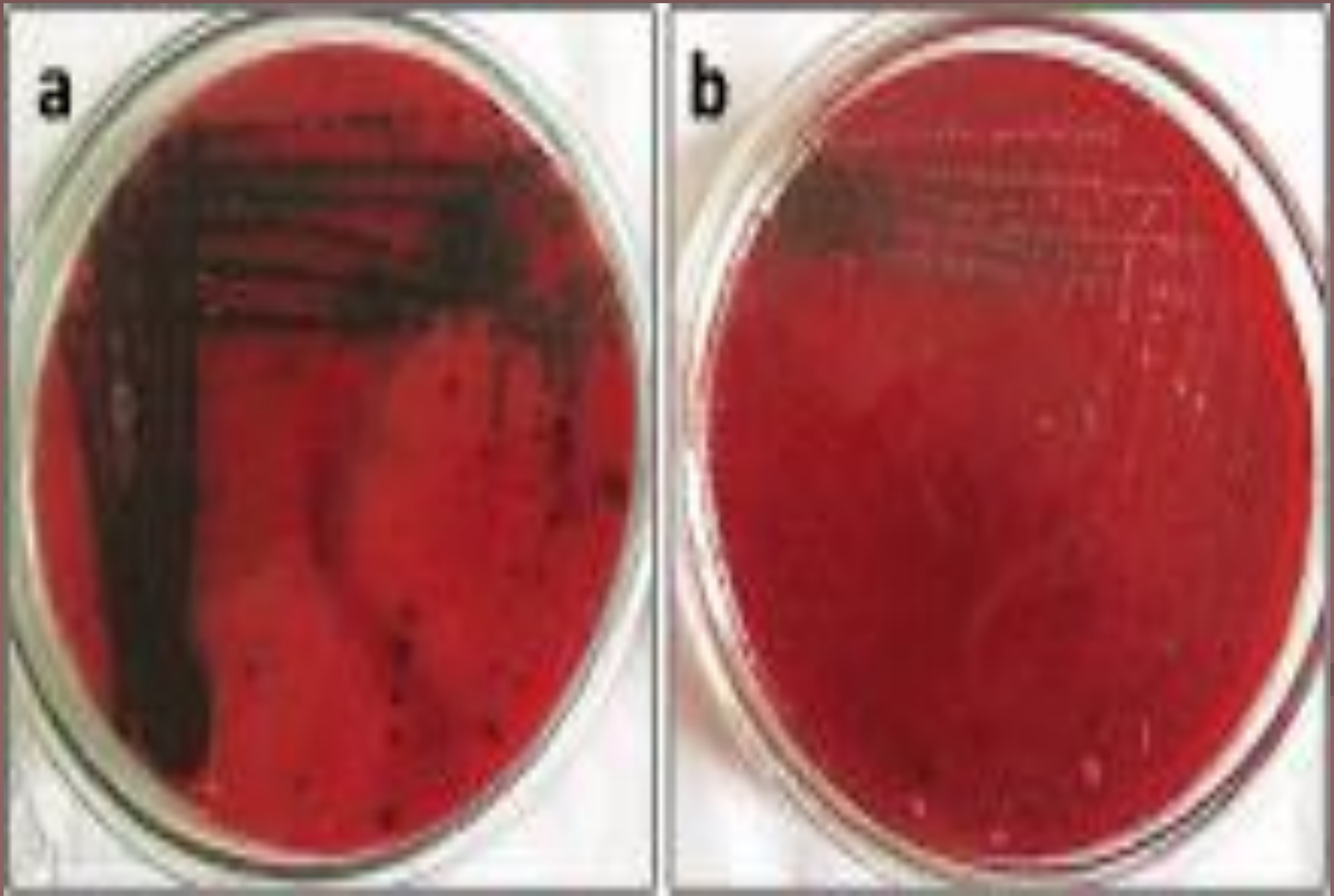
S. epidermedis

on congo red magnesium agar

(Kaiser et al 2013)

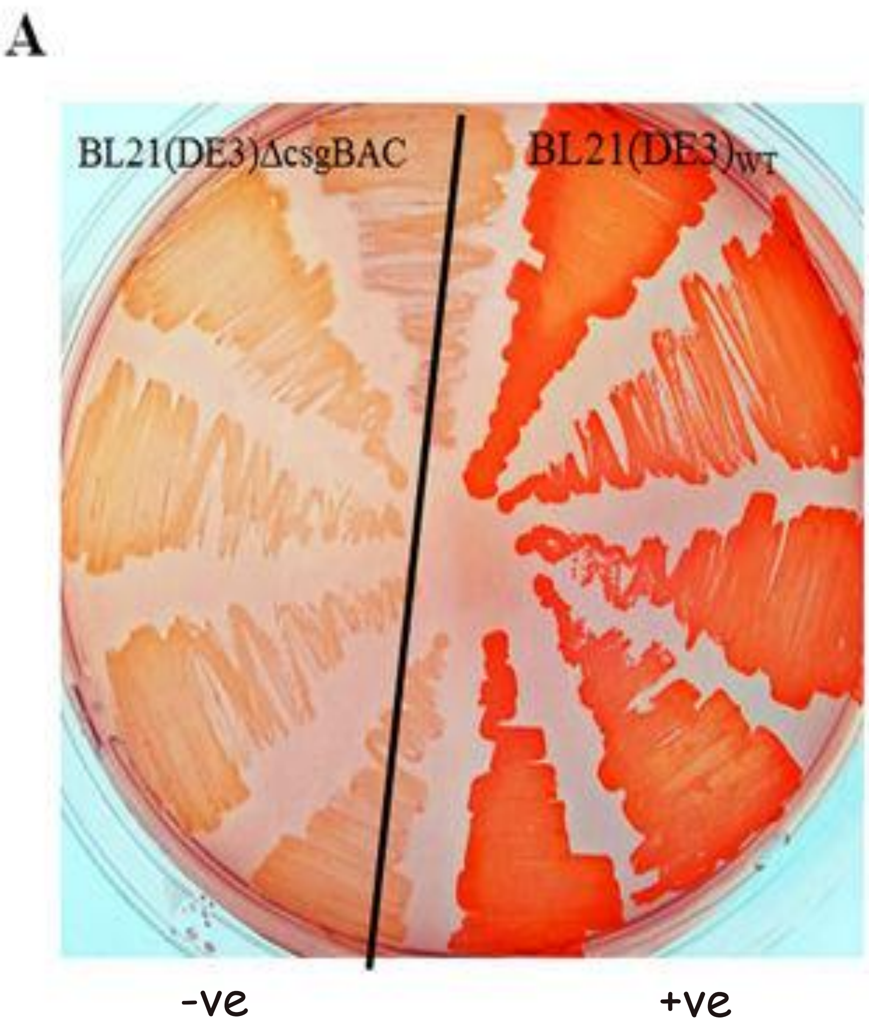


Ps. aurogenosa
on congo red agar

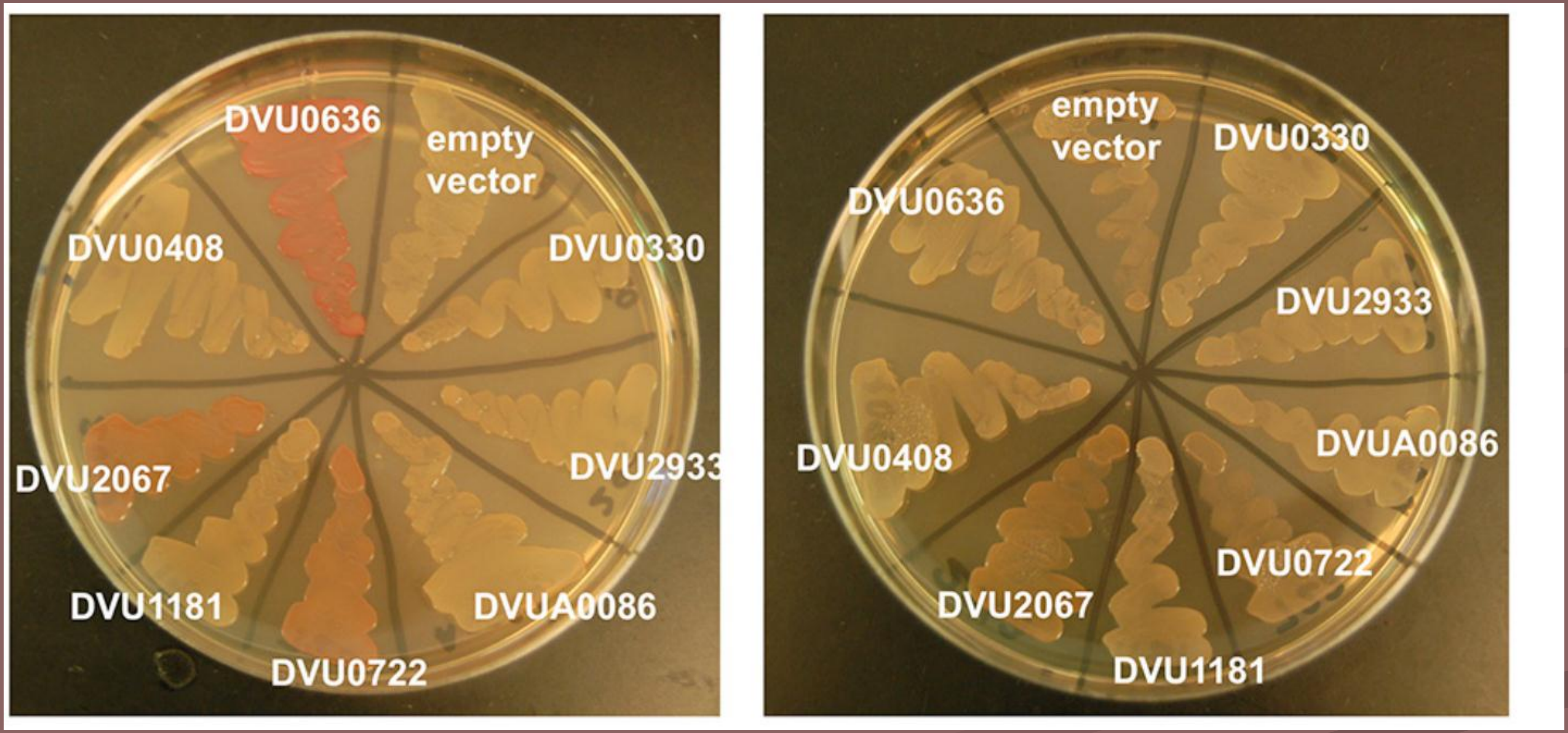


E. Coli on congo red agar

(Aljanaby and Alfaham 2017)

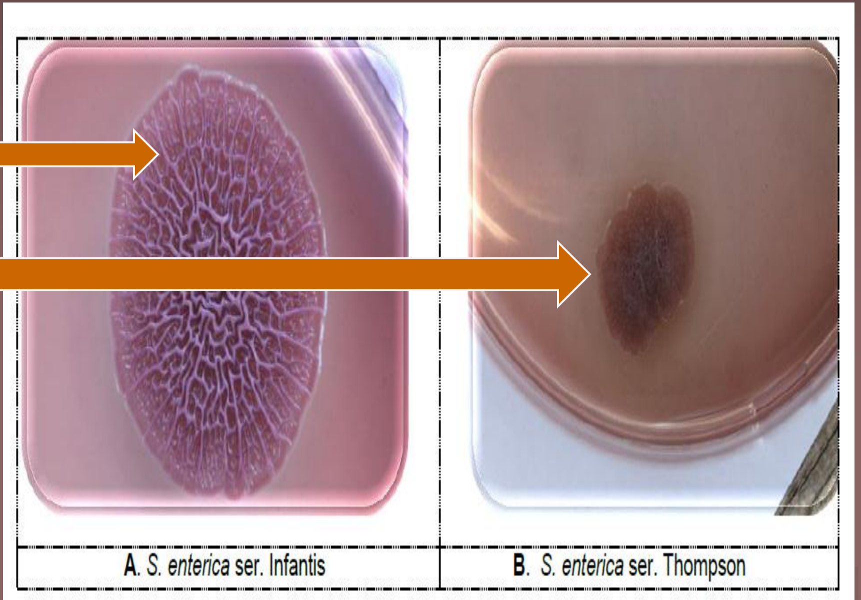


E. Coli on Congo red agar
(Rajeev et al 2014)

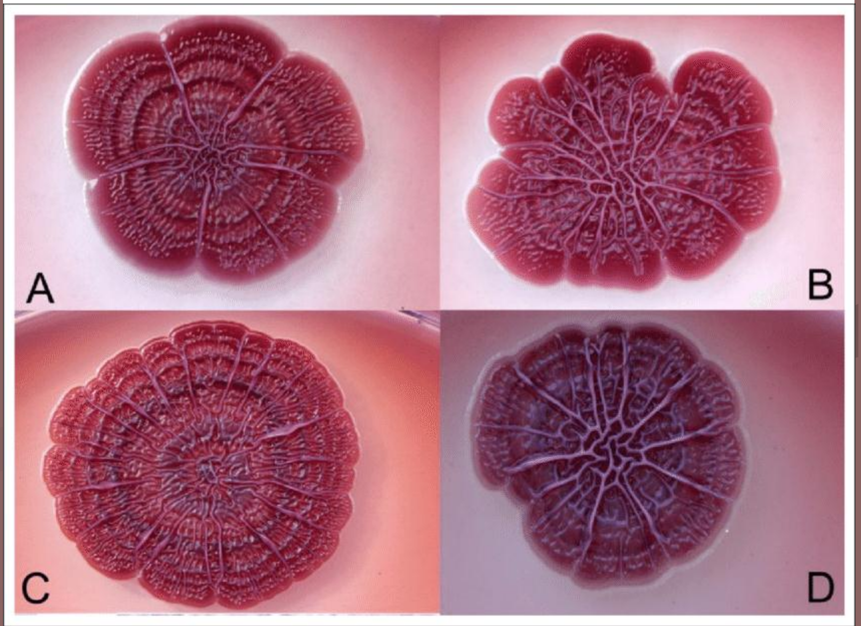


Salmonella spp. Congo Red binding assay

- Strains producing cellulose appear pink dry and rough (pdar)
- Strains producing curli fimbriae results in brown dry and rough (bdar) Bokranz et al., 2005



Different serovars of *Salmonella* isolated from feed, on Congo red agar at 20 °C, resulted in forming of the characteristic red dry and rough (rdar) morphotypes (Steenackers et al 2012)

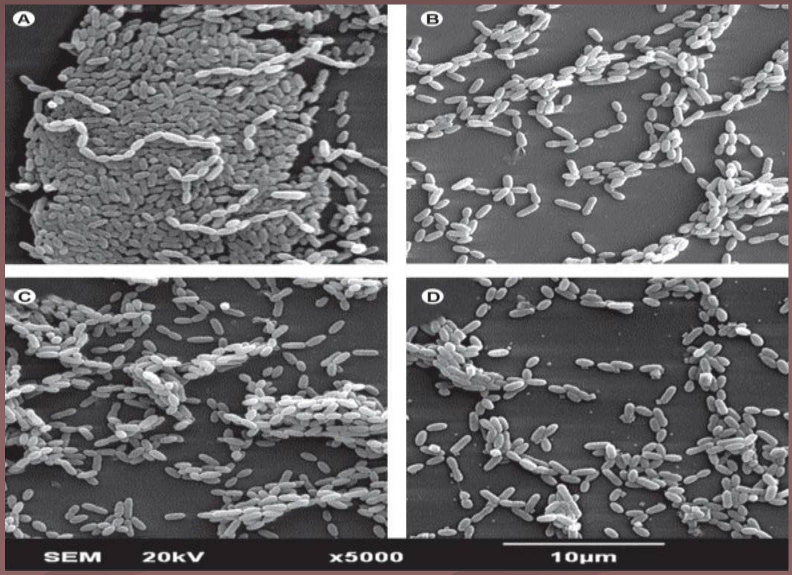



THE BEST
ANTI-BIOFILM
TREATMENTS
depositphotos

PHYSICALLY

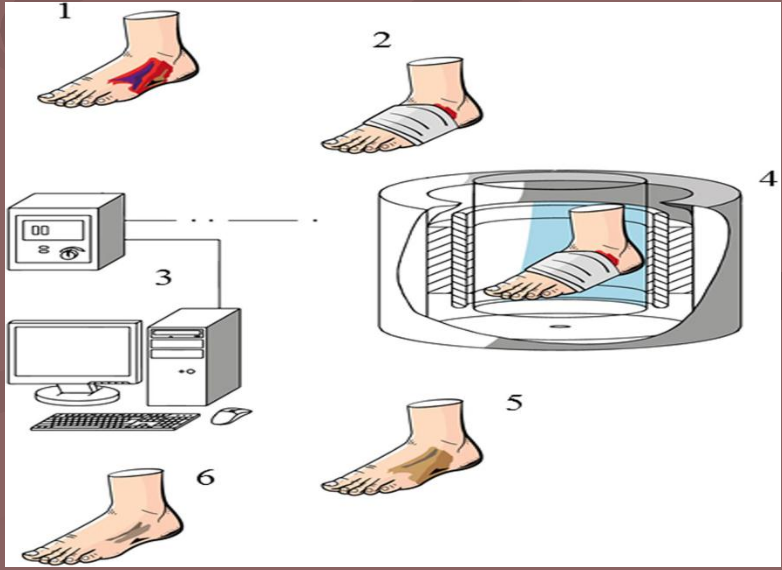
A - Laser therapy of oral *St. mutans* biofilm

SEM micrograph showing the morphology and structure of SSB of *S. mutans* (Zancope et al 2016).
A = Control
B-D = Biofilm after irradiation



B - Rotating magnetic fields in

- **Biotic surface** : wound pathogens *S. aureus* and *Ps. Aurogenosa* (Junka et al 2018)
- **Abiotic surface** drinking water pipes poly biomass (Han et al 2018)



Honey has antibiofilm activity

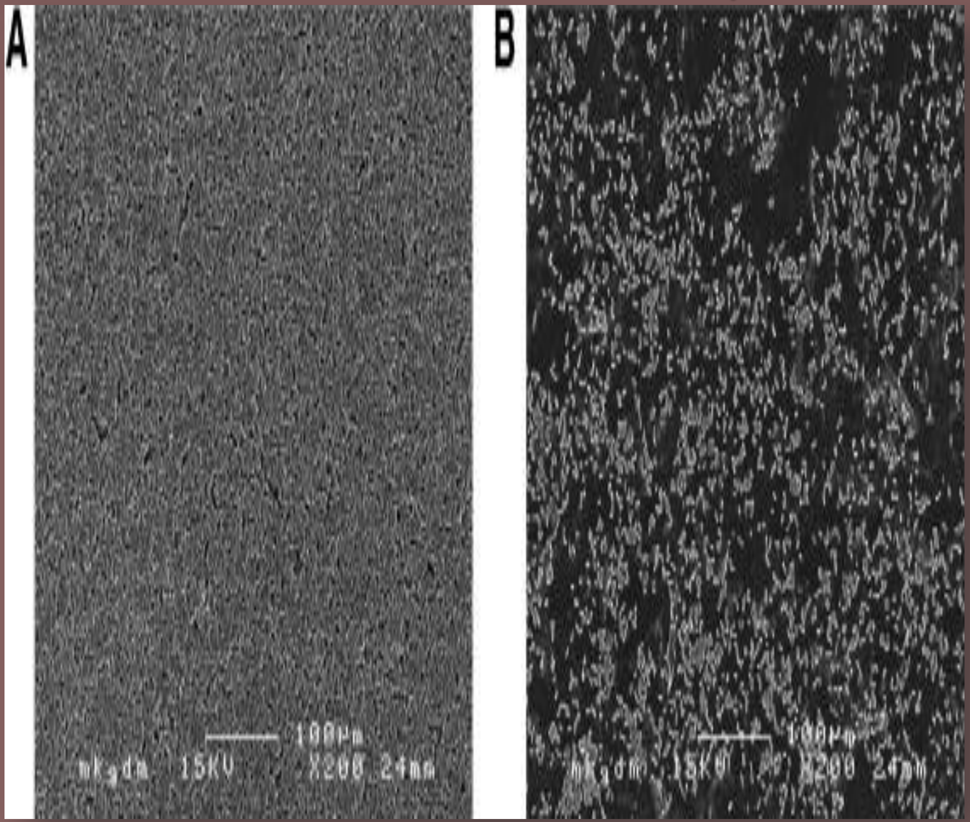
- New Zealand honey against ***S. aureus*** & ***Ps. aurogenosa*** (Alandejani et al 2009)
- New Zealand honey against ***S. typhi*** biofilm developed on cholesterol gallstone (Hannan et al 2018).
- Against ***S. aureus*** biofilm (Lu et al 2017 & Zamora et al 2017)
- Against ***Aspirgillus*** biofilm(Gatherton 2017)
- Against ***Proteus*** (Majtan et al 2014)

Synergy of honey with:

- Vancomycin against *S. aureus* (Campeau & Patel 2014)
- Rifampcin against *S. aureus* (Lui et al 2018)
- Phage against *E. coli* biofilm *in vitro* (Oliveira et al 2017 & Emineke et al 2017)
- كركم Curcumin against *P. aurogenosa* (Jadaunet al 2015)

- **Hyperoside** is a type of modified flavonoid (Sun et al 2017) inhibits biofilm formation of *Ps. aeruginosa*

- Antibiofilm activity of **carboxymethyl chitosan**



A : control B : after treatment

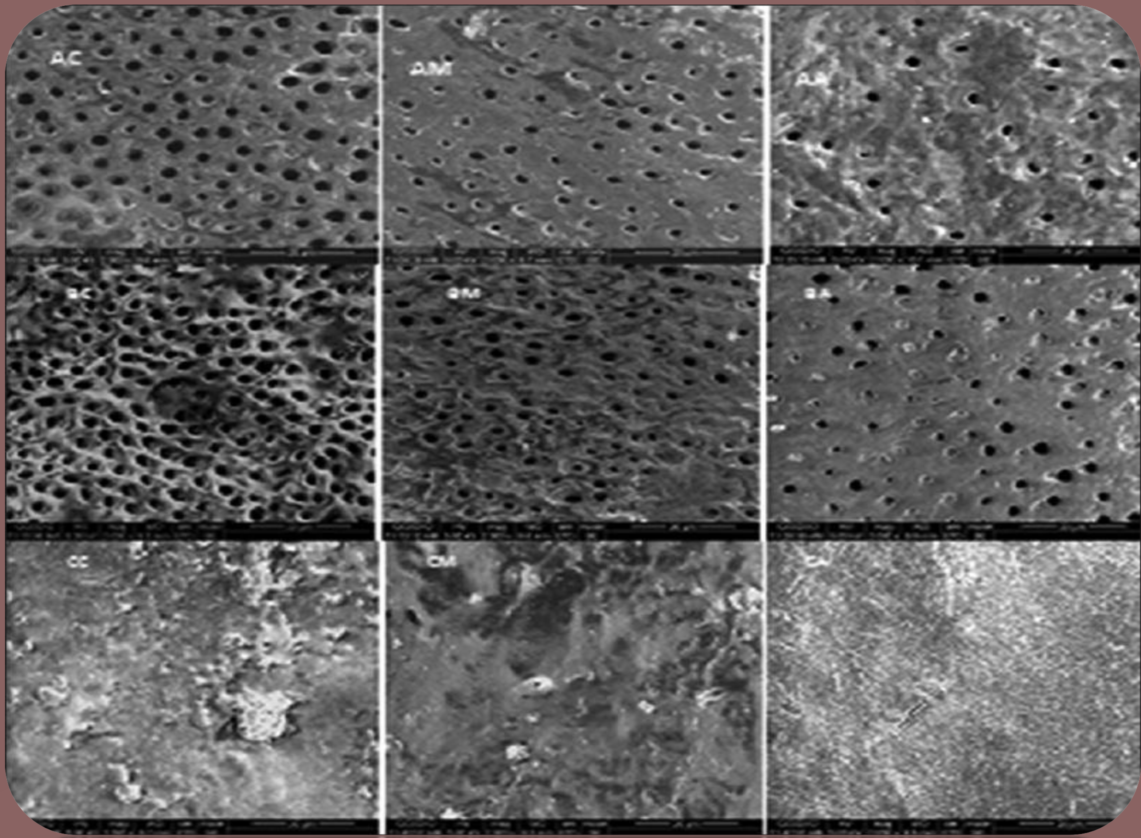
Chitosan-EDTA 1:1 Tooth treatment (Geethapriya et al 2016).

Chitosan-copper nanoparticle Tooth treatment (Covarrubias et al 2018).

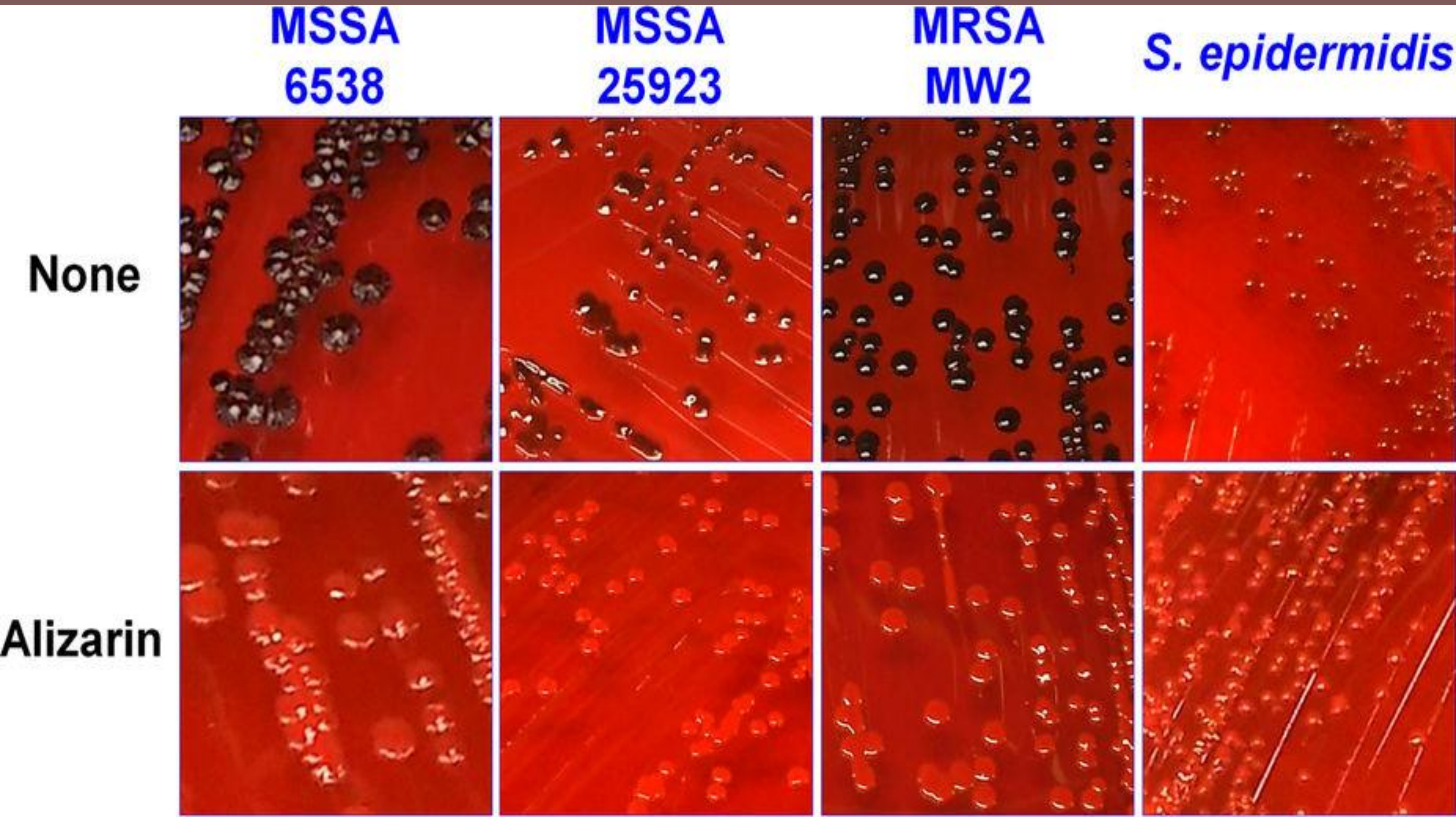
Apical tooth treated

Middle tooth treated

Control



Alezarin is a natural compound derived from the roots of the madder genus. It has **antibiofilm a ctivity** (Manoharan et al 2017)



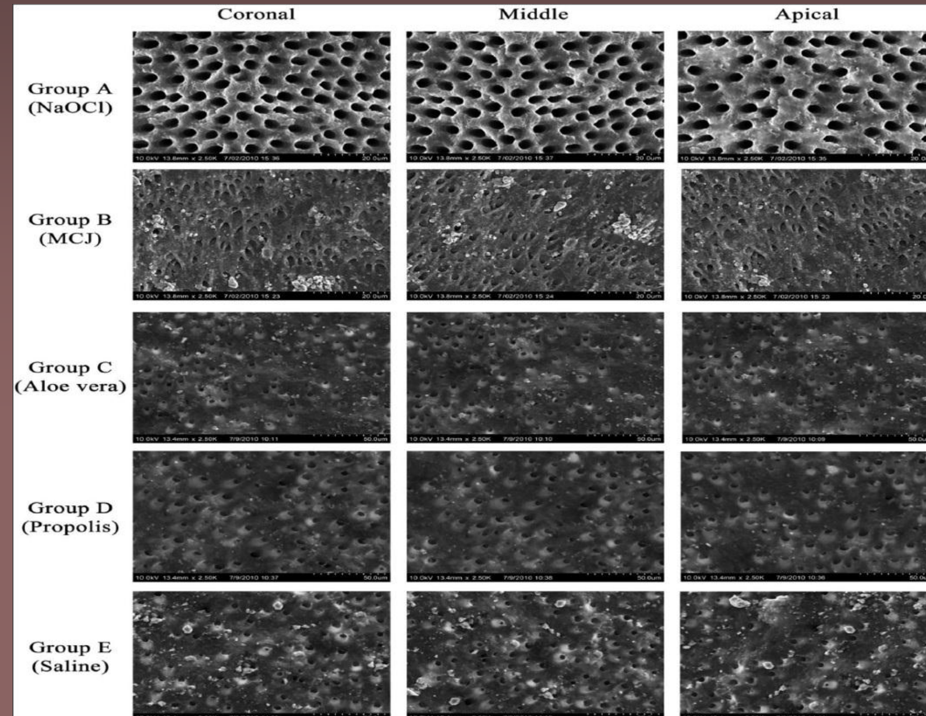
Natural Anti- dental biofilm

At the three levels evaluated, 1% NaOCl with PUI was the most effective in removing completely biofilm compared to the natural irrigants

Propolis,

Aloe Vera

Morinda citrifolia juice.

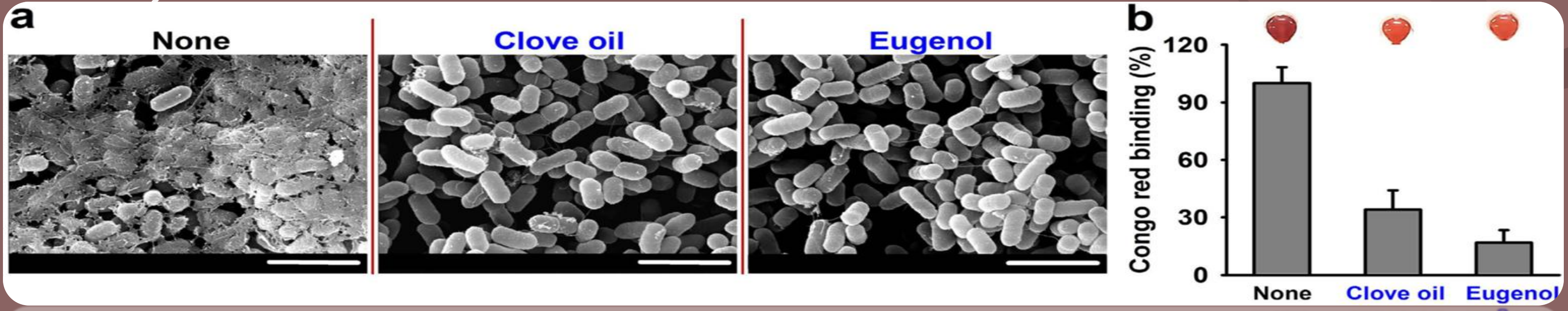


- Tunisian propolis has antibiofilm activity against 33 oral pathogens (Kouidhi et al 2010)
- Italian propolis and bud poplar resins extract have antibiofilm action against Ps aurogenosa (De Marco et al 2017)

Propolis throat spray

Essential oils of medicinal plants

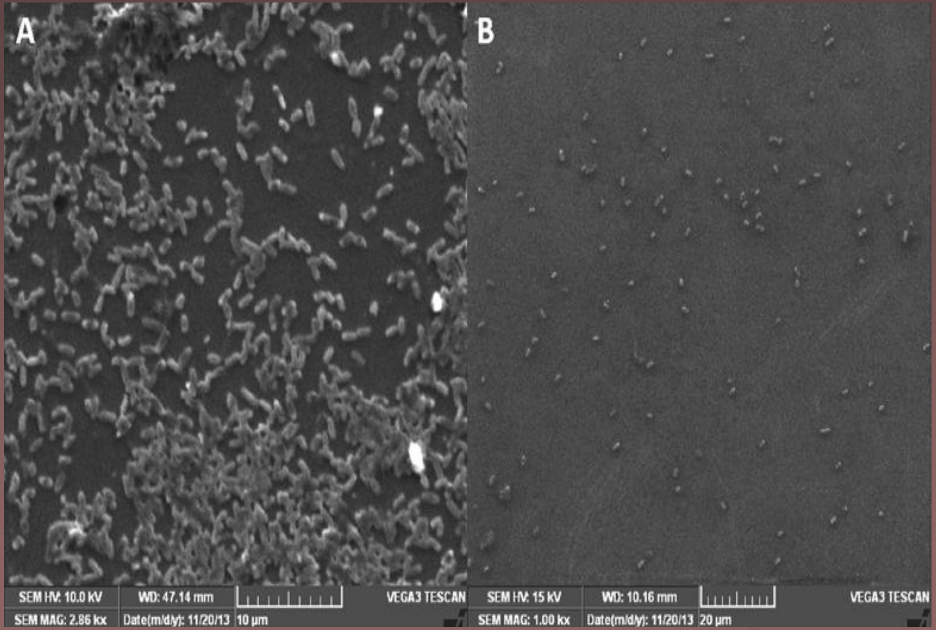
- Kumari et al 2017 determined essential oils Oregano oil (Carvacrol), Cinnamon oil زيت قرفة(Cinnamaldehyde), Lemongrass oil (Citral), Clove oil (Eugenol), Peppermint oil (Menthol) and Thyme oil (thymol) against *Fungal* biofilm.
- Citrus bergamia, قرفةCinnamomum zeylanicum, Leptospermum scoparium and Thymus vulgaris زعتر against *S. aureus* of bovine origin (Pedonese et al 2017)
- قرنفل Clove essential oil & eugenol against *E. coli O15H7* (Kim et al 2016).



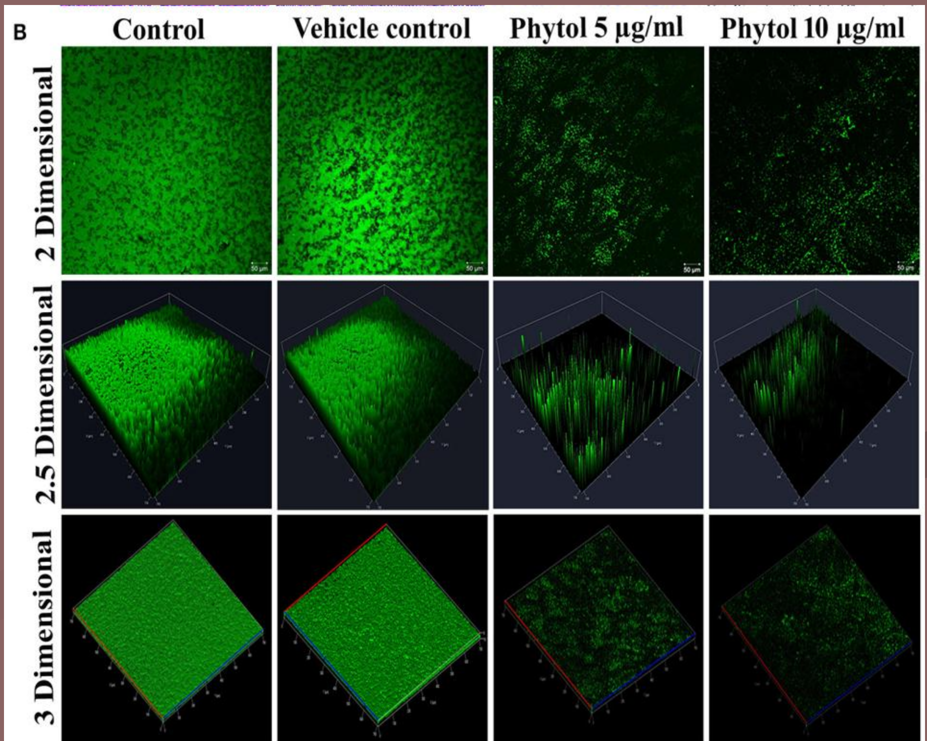
- Artini et al 2018 examined 89 medicinal plant samples against *Ps. aurogenosa* concluded many of them biofilm inhibitors.

- عرقسوس licorice extract has antibiofilm action against *S. aureus* (Rohinishree and Negi 2016).
- زنجبيل Ginger extract **inhibits biofilm formation** by *Ps. aeruginosa PA14* (Kim & Park 2013).
- كركم curcumin **reduces biofilm formation by** *S. aureus and Escherichia coli* (Moshe et al 2011).
- Ascorbic acid **antibofilm produced by** *MRSA* (Mirani et al 2018)

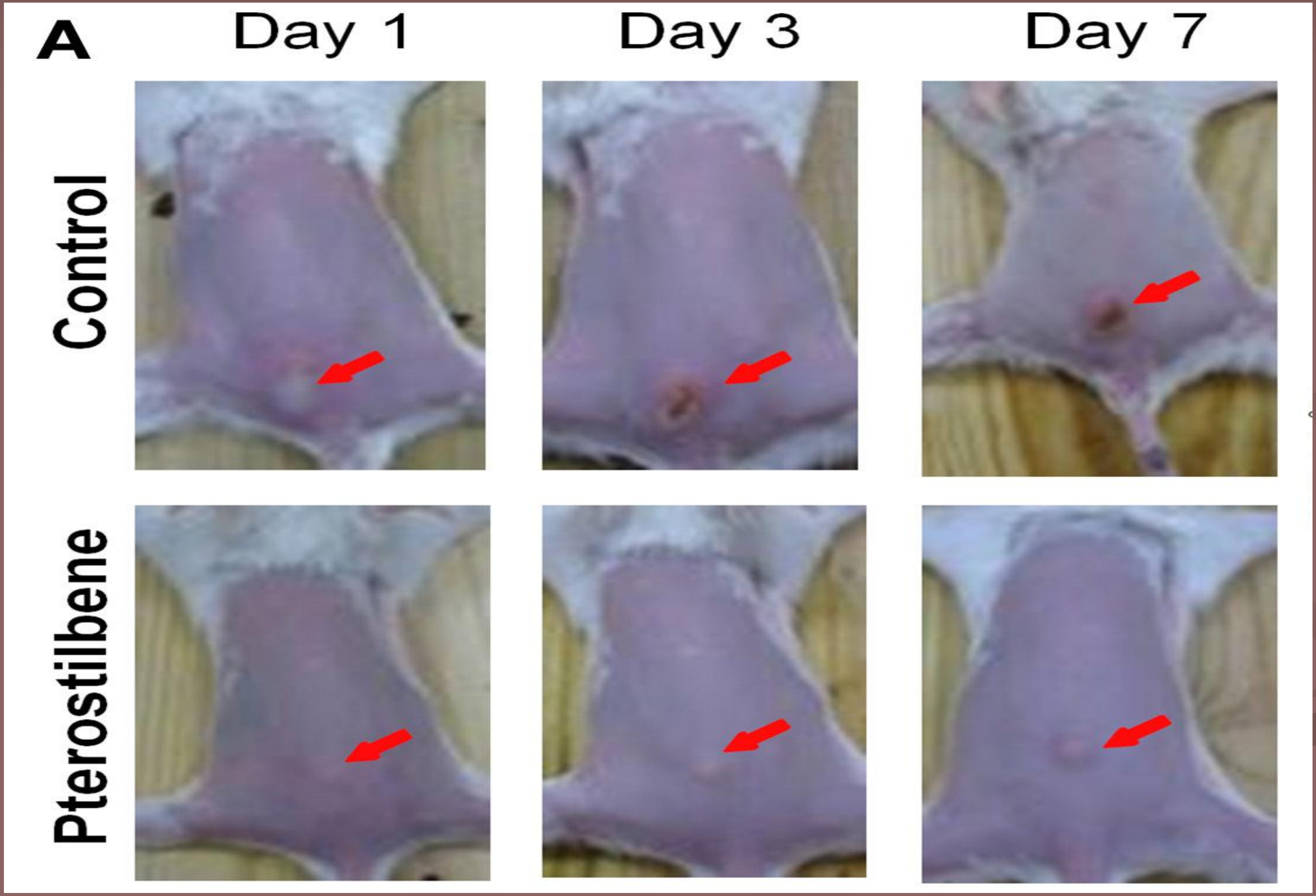
- Among different medicinal plants, *T. Bellerica* extract reduces biofilm formation in *Ps. aeruginosa* (Ganesh et al 2018)



- Phytol extract exhibit antibiofilm and (Srinivasan et al 2017) concluded its efficacy ***in vivo*** against *Serratia marcescens* (induced pyelonephritis in rats).



MRSA biofilm eradication by *In vivo* topical application of pterostilbene Which is methoxylate of natural some plants (Grapes) Yang et al 2017

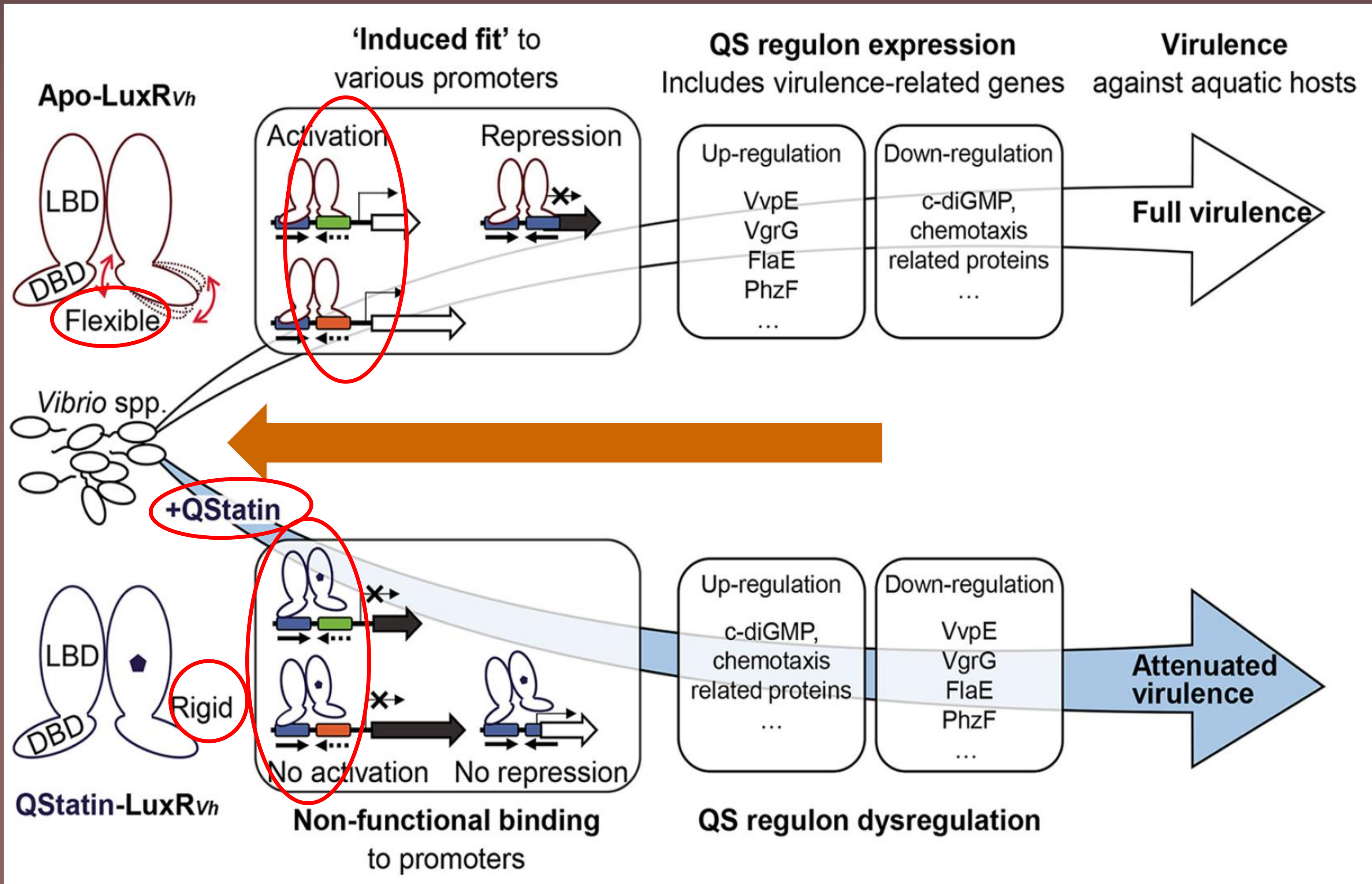


Biocidin is a blend of herb extracts

Nano therapy

- Silica dioxide is a Surface-modified nanoparticles as anti-biofilm for dental polymers (Zaltsman et al 2017)
- Cationic ultrashort antimicrobial peptides as nanoparticles (Almaaytah et al 2018)
- Nanostructure of thiourea derevatives against fungal biofilm (Limban et al 2018)
- Lauric acid which is one of medium chain fatty acid (MCFAs) has antimicrobial activity due to reactive oxygen species (ROS) . It inhibits *Cl difficile* (Yang et al 2018) and MRSA (Yang et al 2017) biofilm in vitro.

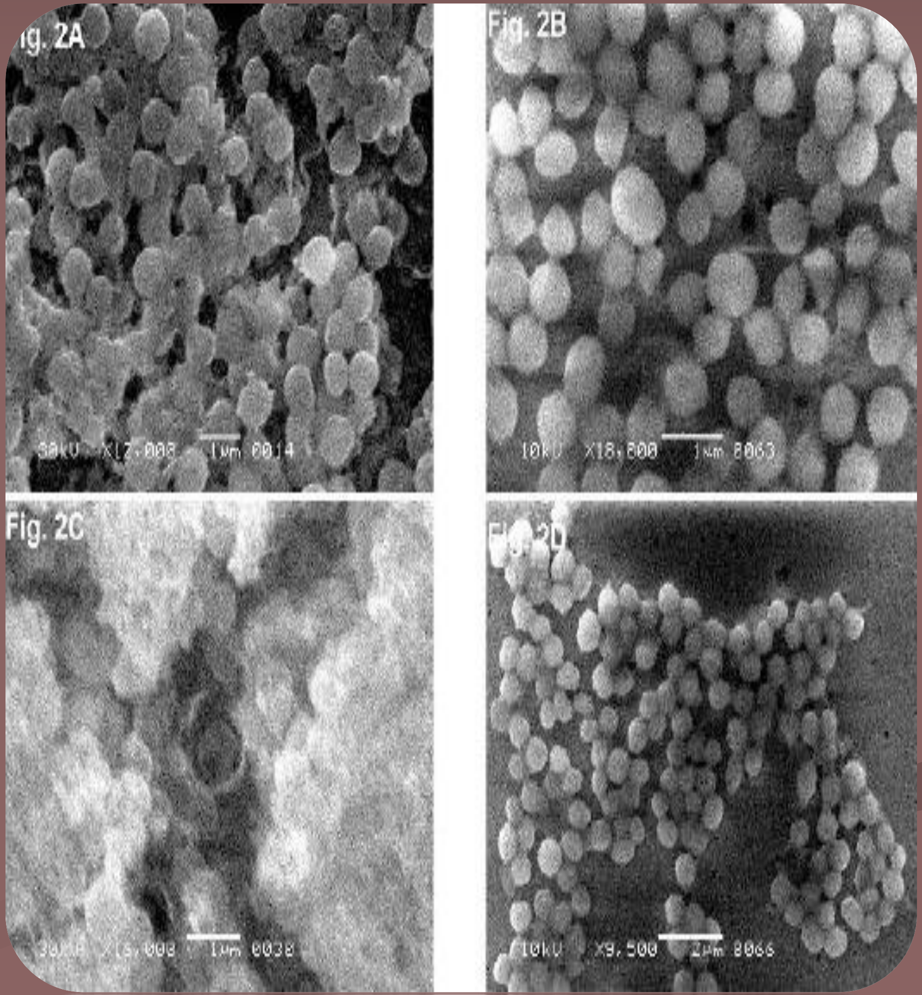
Q statin nanoparticles against *Vibrio spp.*
(Kim et al 2018)



Ascorbic acid antibofilm produced by MRSA
Mirani et al 2018

(A) after exposure to sub MIC of oxacillin produced EPS and adapted biofilm mode of growth.
(B) Cells devoid of EPS in the presence of 1 mg/ml ascorbic acid.
(C) Multicellular aggregates of biofilms embedded in EPS after long exposure to sub MIC of oxacillin
(D) Control growth on tryptic soy agar plate after antibiofilm action

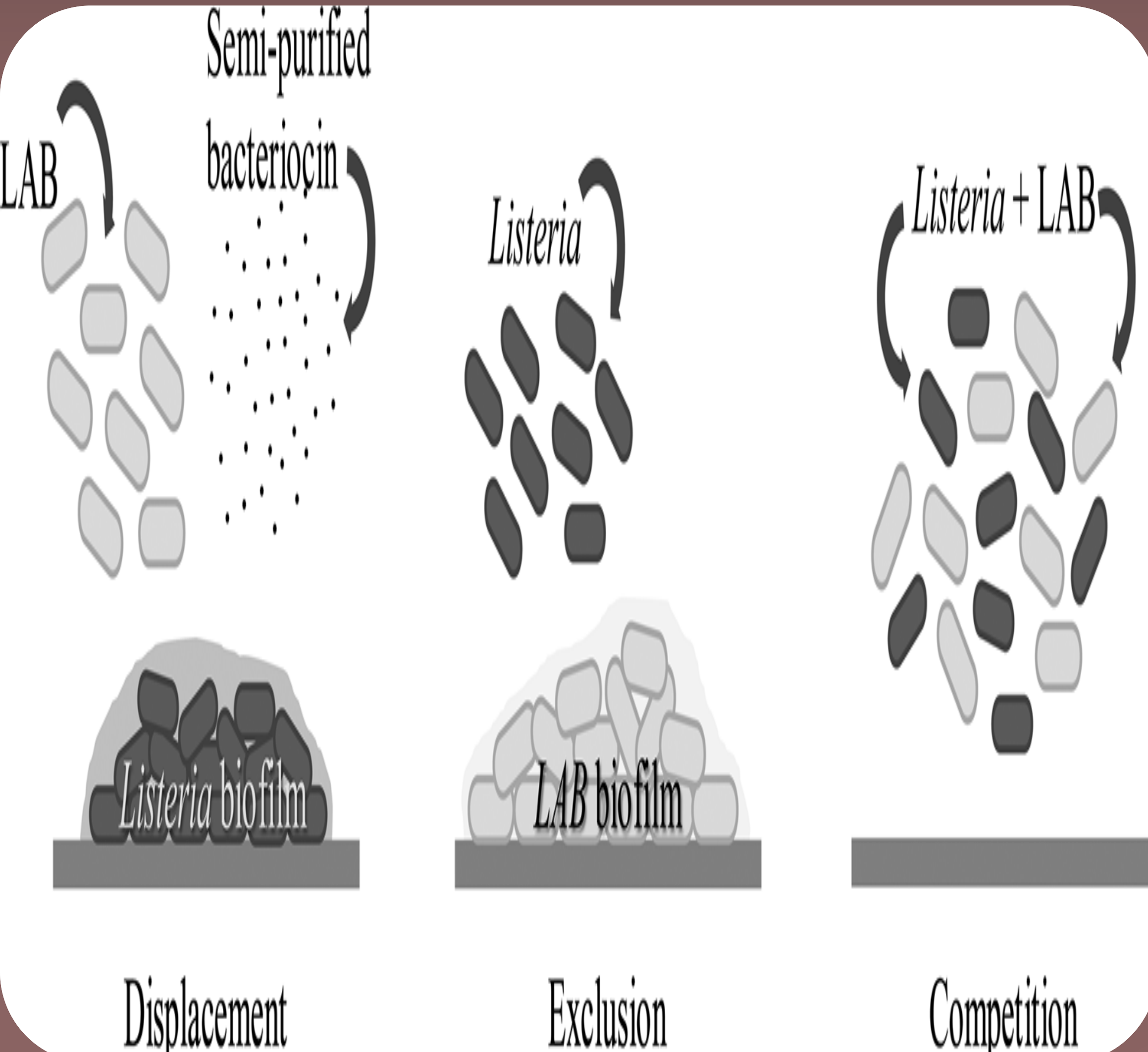
A B



C D

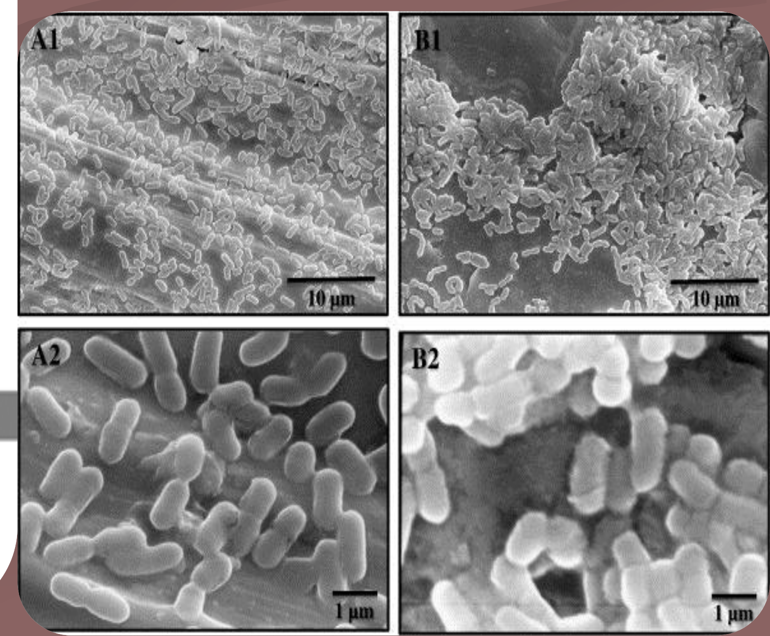
- **Probiotic *E. coli*** strains in inhibits biofilm formation of pathogenic *E. coli* via via extracellular activity of **DegP** gene.
- Probiotic *E. coli* Nissle 1917 (EcN) is capable to outcompete the biofilm formation of pathogens (*E. coli* O157H7, *Ps. aeruginosa, S. aureus* and *S. epidermidis* (Fang et al 2018)
- *Strept. sanguinis and Strept. Gordonii* are able to produce significant amounts of H_2O_2 to declare oral biofilm (Redanz et al 2018)

Lactobacillus sakei has antibiofilm activity



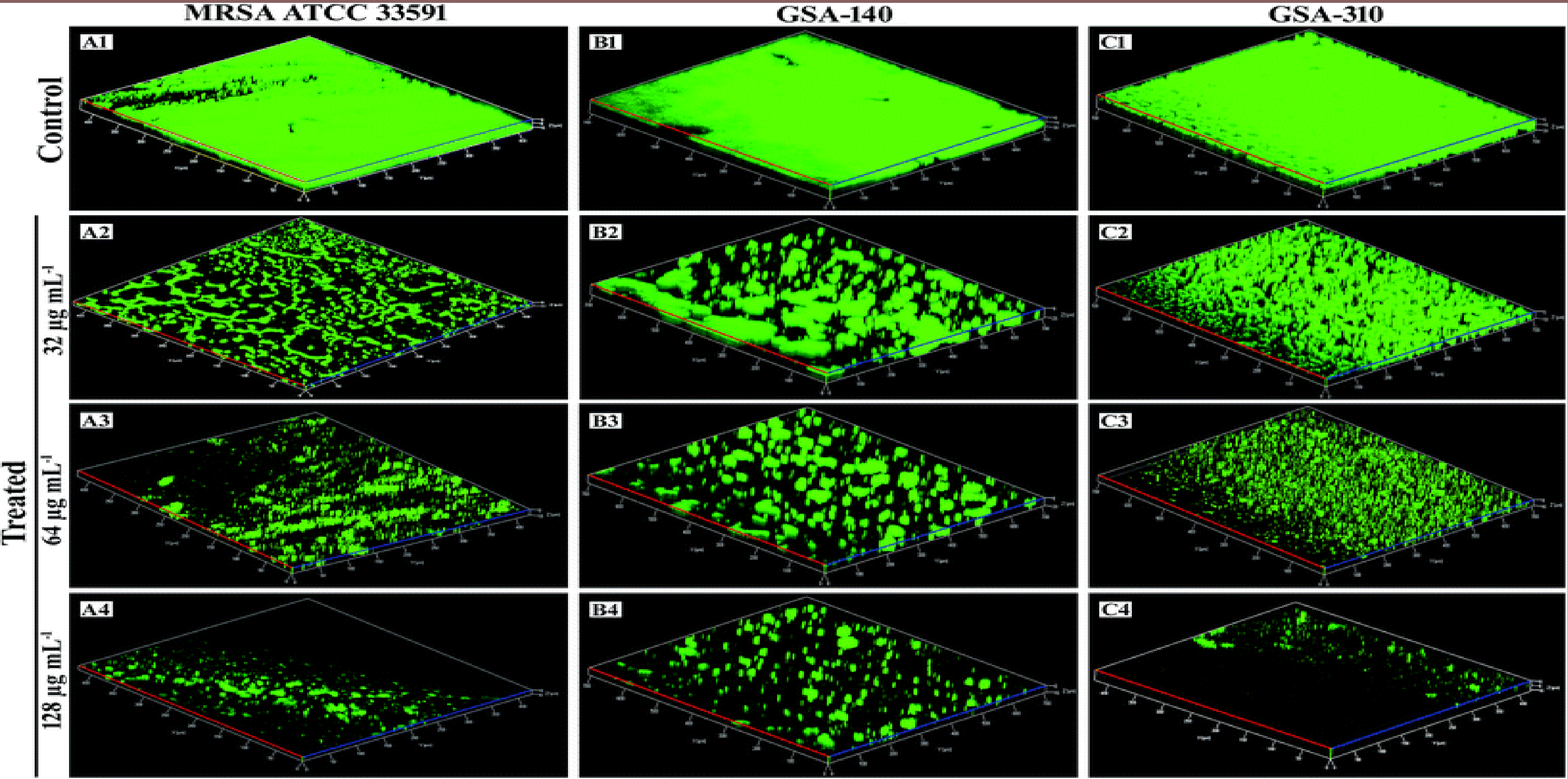
Control of *Lesteria monocytogen* biofilm by means of *Lactobacillus sakei* bacteriocin

(Ibarreche et al 2016)



- 4 different Lactobacillus species have antibiofilm activities against *S. aureus & E. coli* (Cui et al 2017)

  - *Bacillus myloliquefaciens* against a reference MRSA and two clinical strains (Gowrishankar et al 2015)

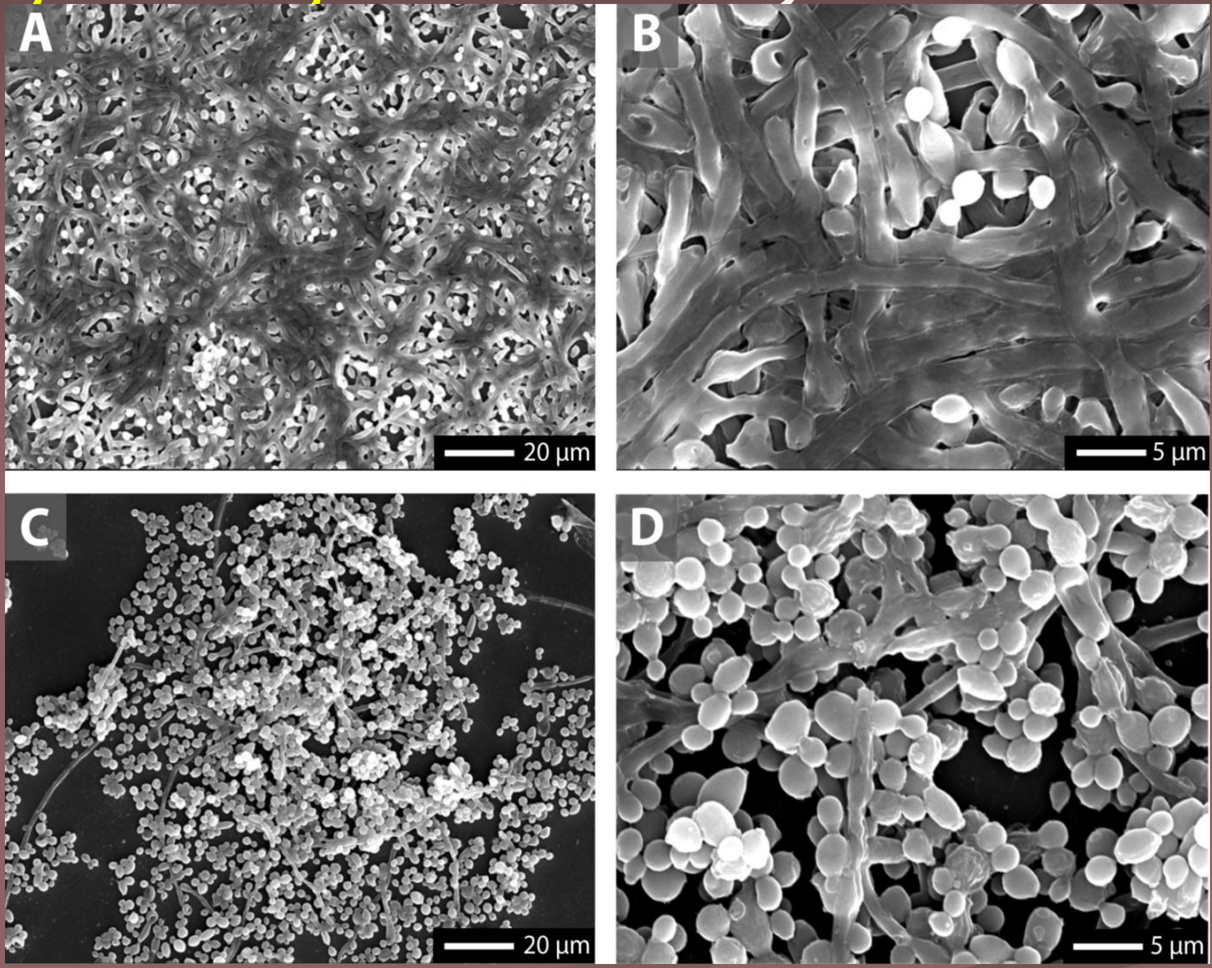


Aspirin antibiofilm activity
(Madariaga-Venegas et al 2017)

- Nitric oxide-releasing aspirin against *Candida albicans* biofilm

A & B --- before

C & D --- after



- Nitric Oxide-Based therapeutic Agent against *S. aureus* Biofilms (Jardeleza et al 2014)

شكراً لكم علي حسن
استماعكم