Botanicals, Biofilms, and Chronic Infections

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Editor, *Medical Herbalism* Journal

http://naimh.com/northwest/seminar

Introduction to Microbiome and Biofilms

The New Microbiology

Terms

- **Microbe.** A microscopic living organism. Many be single or multi-celled. Includes bacteria, archaea, most protozoans, yeasts, and (depending on who you ask) viruses.
- **Microbiota** - the microorganisms of a particular site or anatomical zone
- **Microbiome** – the combined genetic material of the microorganisms of a particular site
- **Bacteriome** and **Archaeome** – Bacteria and Archaea
- **Virome** – The viruses in the human, or in one region of the organism
- **Fungome** – Yeasts and other fungi

New concepts in microbiology

- Old Model. Freely mobile microbial invaders infect a sterile body. They can be cultured on lab plates. Antibiotics can kill and remove them.
- New methods of detection have increased not only the number of microorganisms we can recognize but have completely overthrown the previous model of infection.


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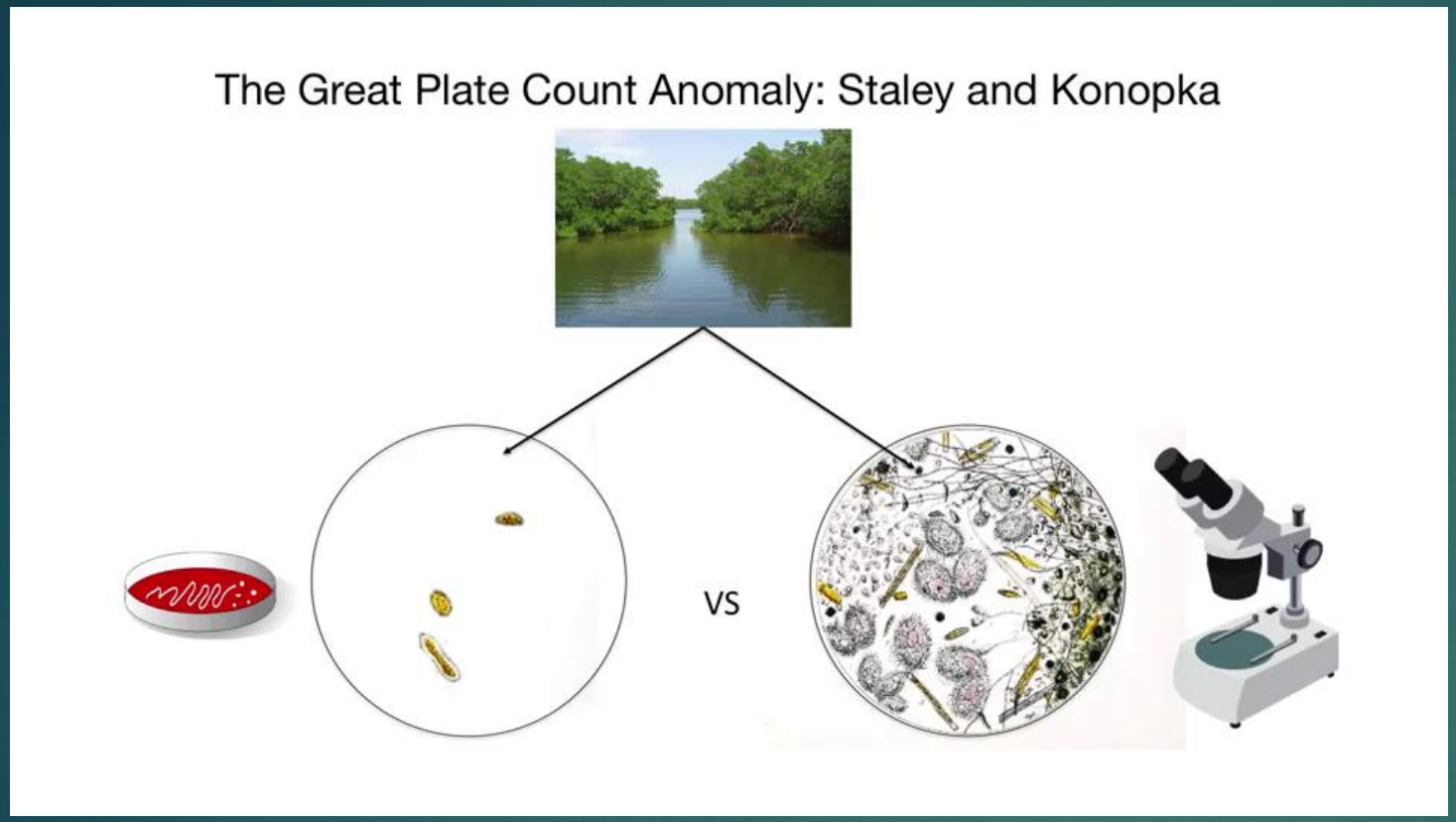
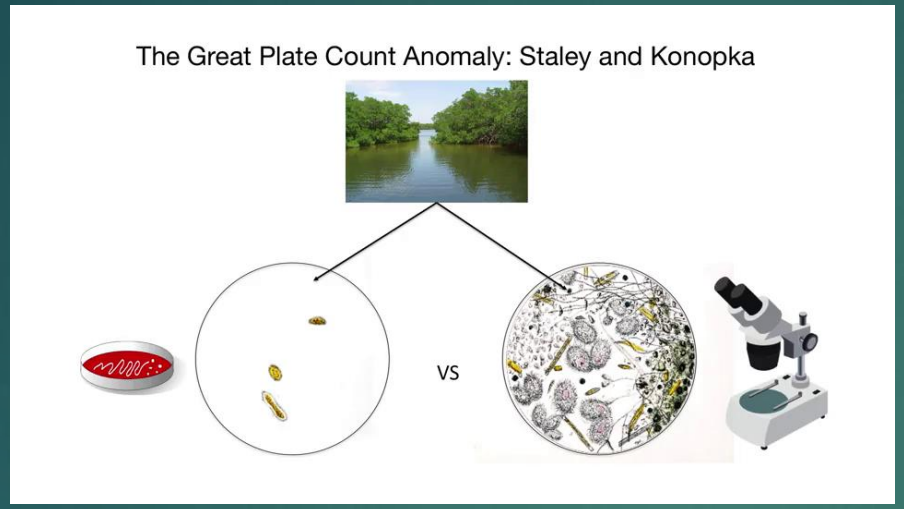
Photo credit: National Cancer Institute, NIH

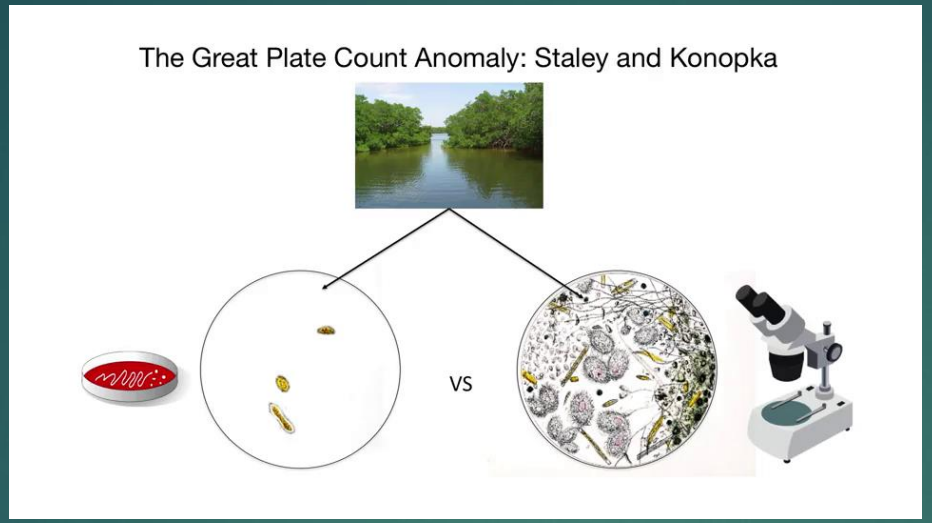
New methods of detection have increased not only the number of microorganisms we can recognize but have completely overthrown the previous model of infection.



- Typically of bacteria observed in lake water in a microscope, only 0.1 to 1% can be cultured in media.



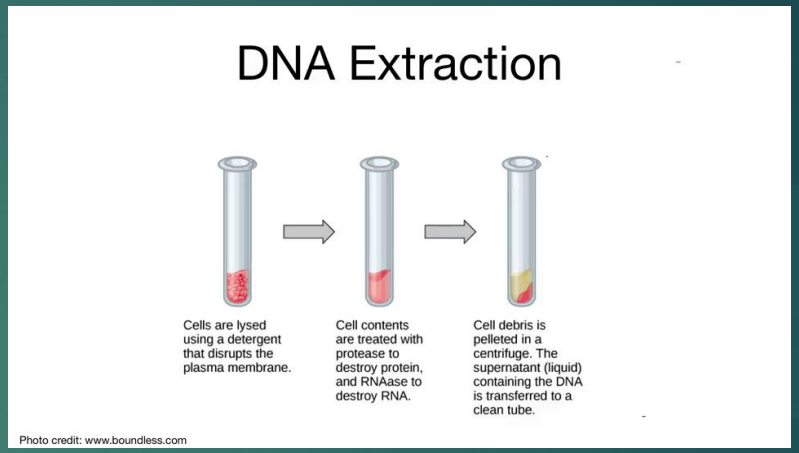
The great majority of microbes in the human gut cannot be cultured, including some of the dominant species.

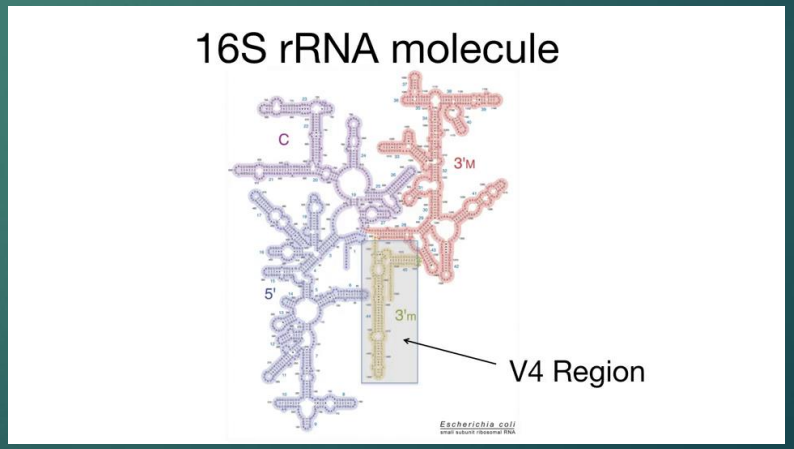


We know a lot about *Escherichia coli* because it is easy to culture but It is a very minor part of the gut population, less than 0.1%.

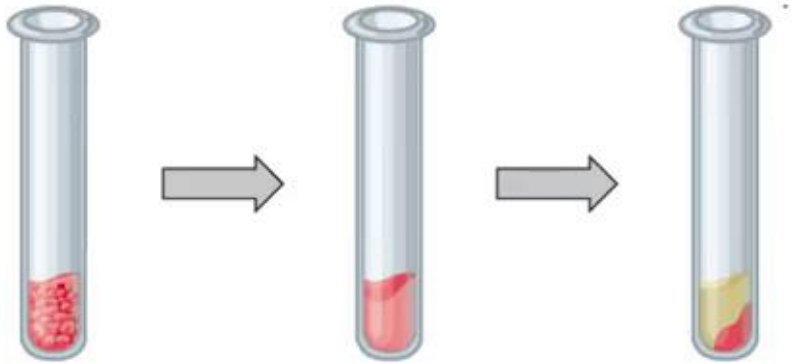
DNA sequencing to identify bacteria

- DNA Extraction
- Polymerase Chain Reaction
- Sequencing





DNA Extraction



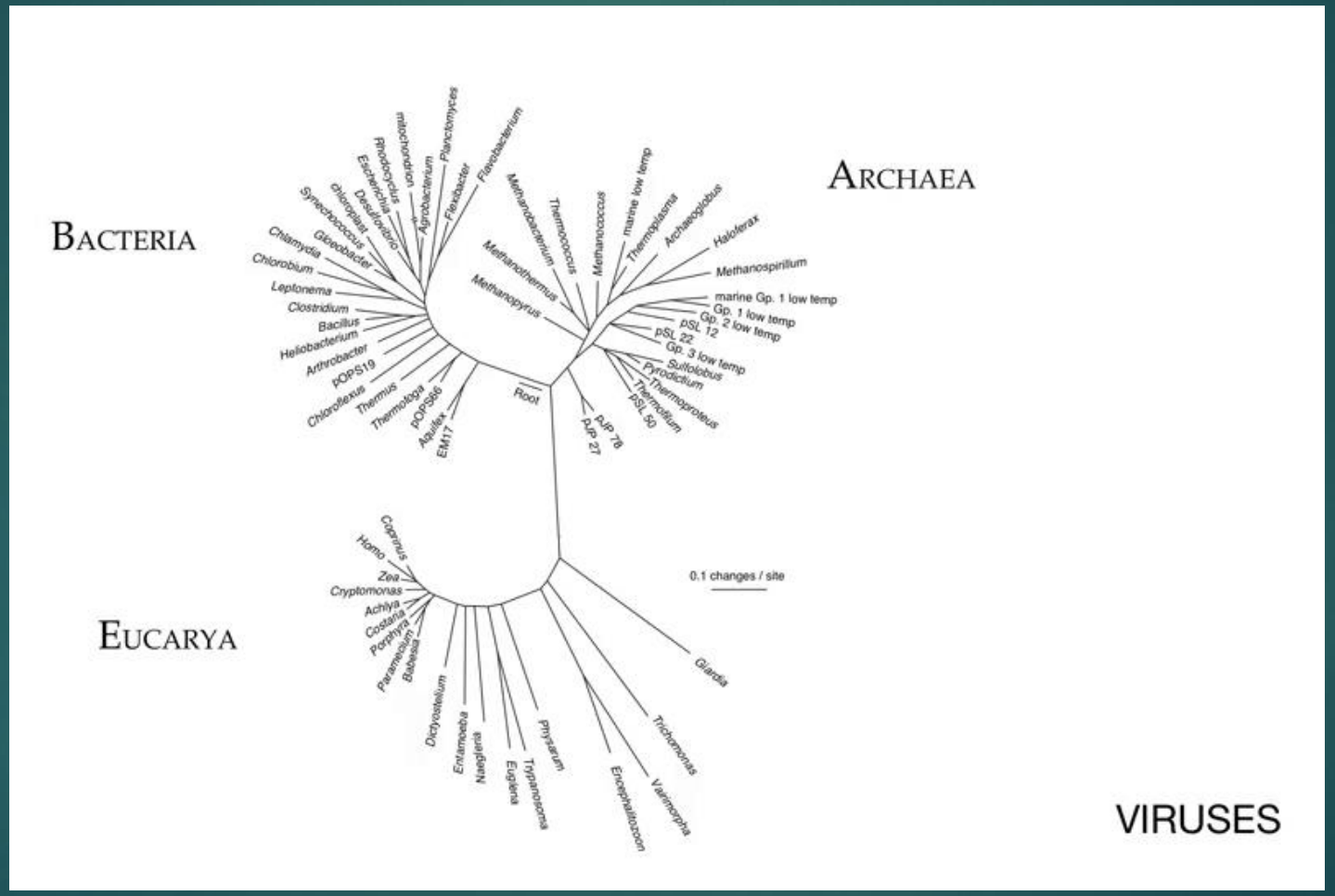
Cells are lysed using a detergent that disrupts the plasma membrane.

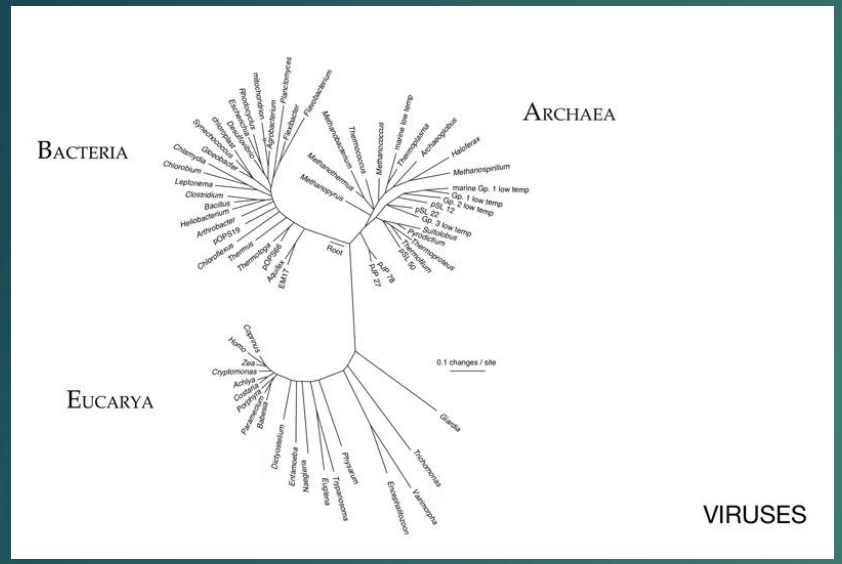
Cell contents are treated with protease to destroy protein, and RNAase to destroy RNA.

Cell debris is pelleted in a centrifuge. The supernatant (liquid) containing the DNA is transferred to a clean tube.

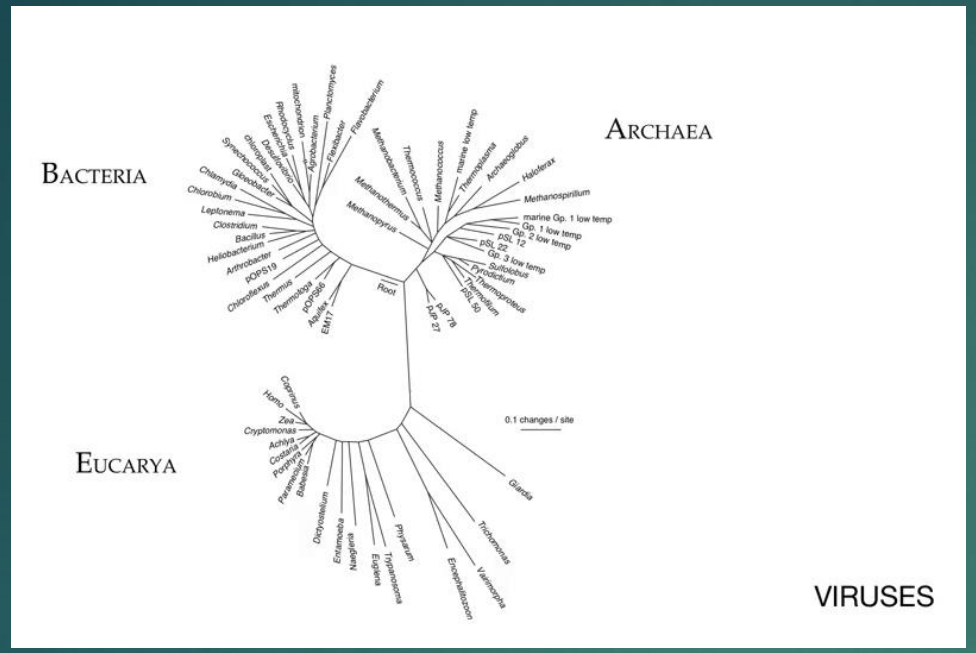
Photo credit: www.boundless.com

Phylogenetic tree

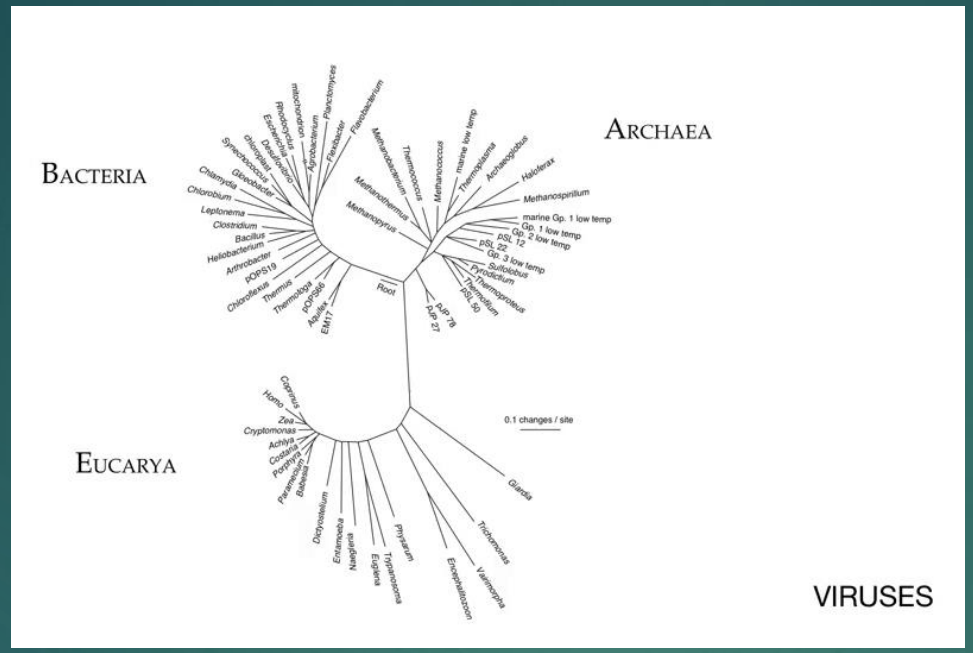




Archaea distinguished from Bacteria by DNA sequencing. Their metabolic pathways are different, and they can live in environments of extreme heat, cold, or other adverse sitatuions. Some Archaea are normal residents of the human microbiome.



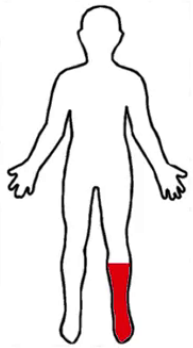
From 1980 to 2012, the number of known Bacterial families has grown from 12 to more than 100. The great majority have never been cultured or identified in a microscope, and their existence could not be detected through traditional methods



In July 2016, scientists reported identifying a set of 355 genes from the last universal common ancestor (LUCA) of all life, including microorganisms, living on Earth

The Human Microbiome

How human are we?



10 trillion human cells
vs.
100 trillion microbial cells!

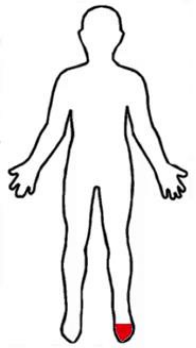
10%????

How human are we?

There are 20,000 human genes…

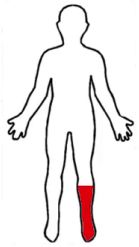
…and between 2-20 million microbial genes!

0.1 - 1%????



- About ten times as many viruses as bacteria exist in and on the human body and its biome. Most viruses are bacteriophages.

How human are we?



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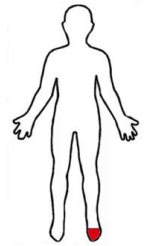


A fungome also exists in normal health, in and on most of the body.
The interdependent community of bacteria, virus, and fungi has been called the "Third Arm of the Immune System" due to its barrier function, resistance to invasion, and secretion of antimicrobial compounds.
It also fulfills essential nutritional and metabolic functions.

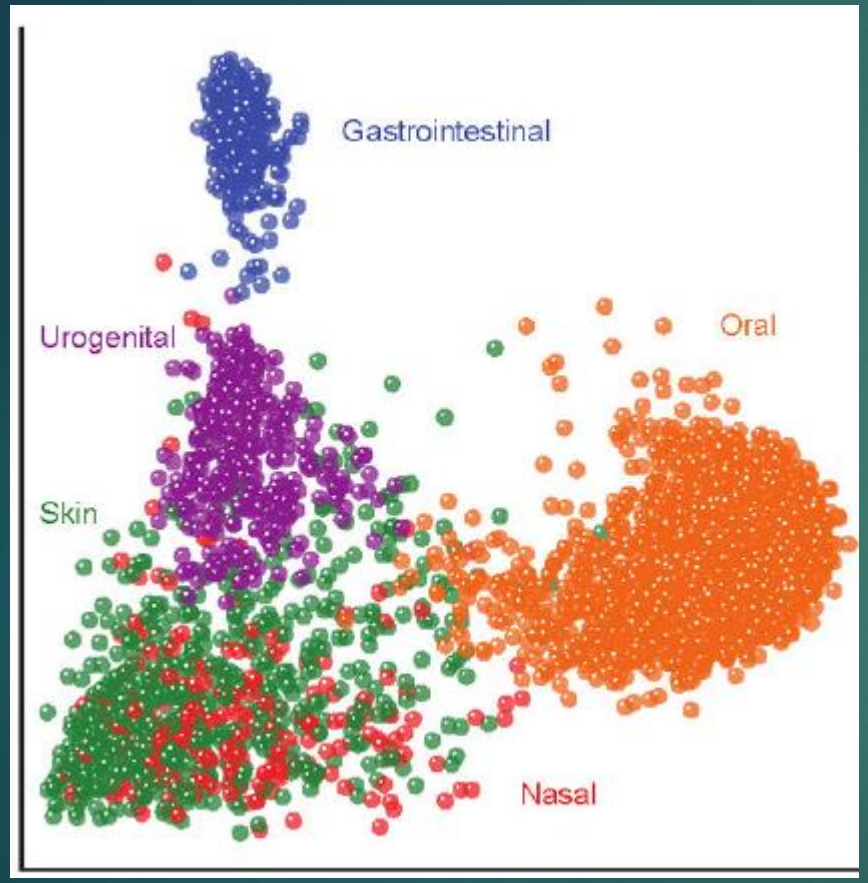
Some surprising results

- An estimated 1,000 different microbial species inhabit the human gut. No more 400 of these have been identified through genetic studies, and only a small minority have been cultured.

- More than 30 bacteria species have been found in the normal flora of the bladder which had never been detected on urine culture or microscopy.

- A microbiome has been discovered in the normal placenta. It is composed of bacteria similar to the oral microbiome with no similarity to the vaginal microbiome

Human microbiome



- " . . . healthy individuals differ remarkably in the microbes that occupy habitats such as the gut, skin, and vagina."
- " . . . diversity and abundance of each habitat's signature microbes vary widely among healthy subjects."
- Strong niche specialization occurs both within and among individuals

Human Microbiome Project Consortium. Structure, function and diversity of the healthy human microbiome. Nature. 2012 Jun 13;486(7402):207-14.

Likewise, each body site contains a distinct microbiome

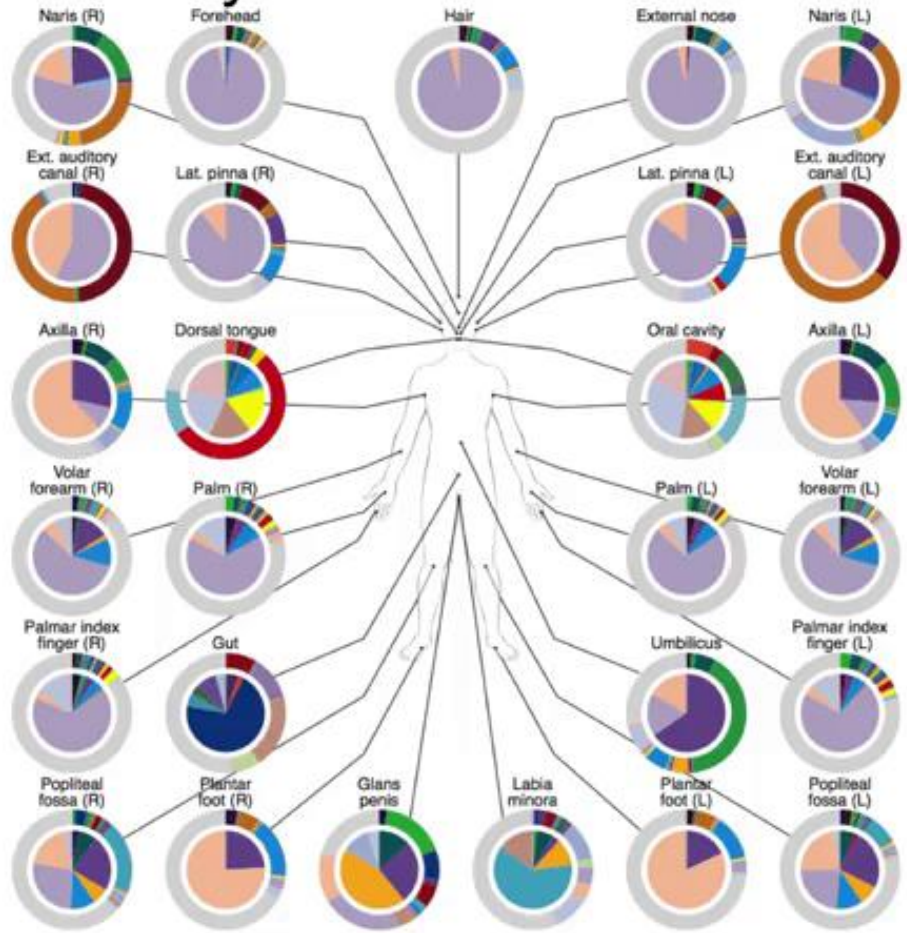
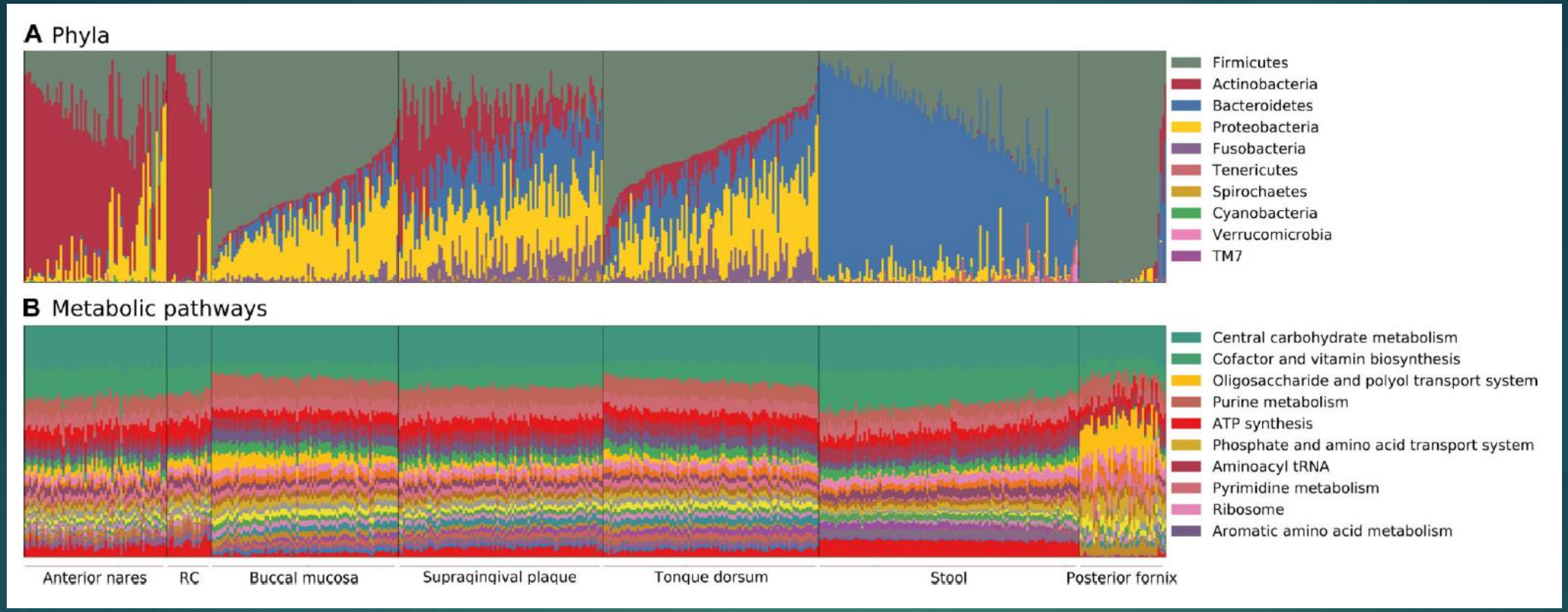


Figure adapted from Costello et. al (2009) *Supplementary materials*



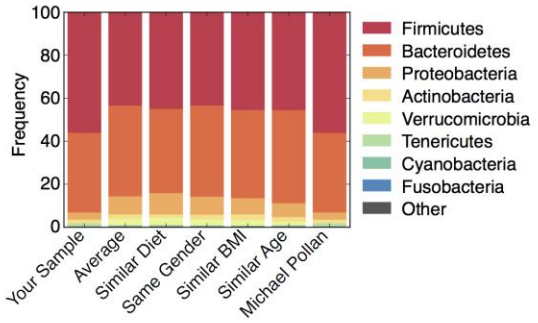
Top series shows dominant bacterial types in each of the 7 regions

Bottom series shows functional bacterial metabolic pathways in each region.

YOUR AMERICAN GUT SAMPLE

MICHAEL POLLAN

What's in your American Gut sample?



Your most abundant microbes:

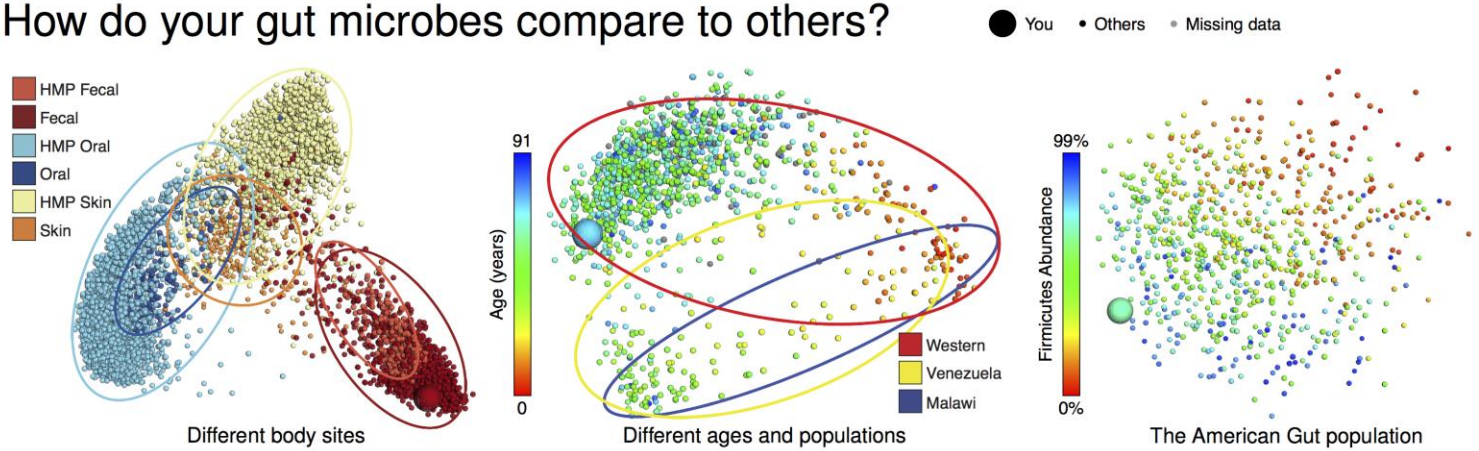
Taxonomy	Sample
Genus *Prevotella*	24.9%
Family Ruminococcaceae	13.4%
Family Lachnospiraceae	10.1%
Genus *Bacteroides*	10.0%

Your most enriched microbes:

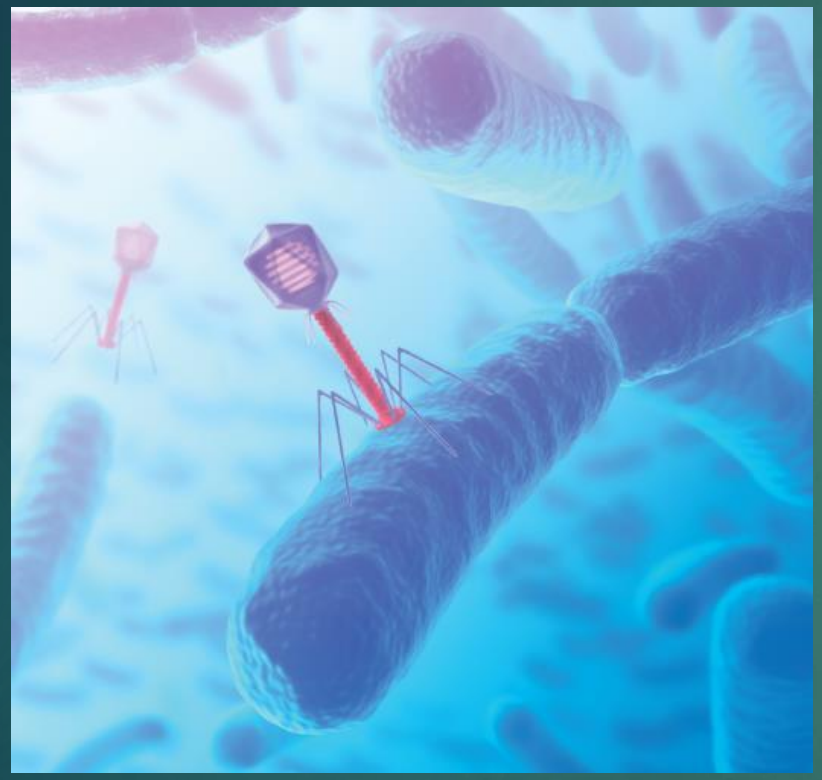
Taxonomy	Sample	Population	Fold
Genus *Clostridium*	2.5%	0.3%	7x
Genus *Finegoldia*	0.7%	0.0%	17x
Genus *Prevotella*	24.9%	2.6%	9x
Genus *Collinsella*	0.9%	0.1%	8x

This sample included the follow rare taxa: Genus *Varibaculum*, Genus *Neisseria*, Genus *Campylobacter*, Order ML615J-28

How do your gut microbes compare to others?



Human gut virome



- Humans are colonized by immense populations of viruses, which metagenomic analysis shows are mostly unique to each individual.
- "This study did not yield any clear examples of known DNA viruses infecting animal cells."
- Most of the human virome are bacteriophages, and may serve as important controls on the bacterial population

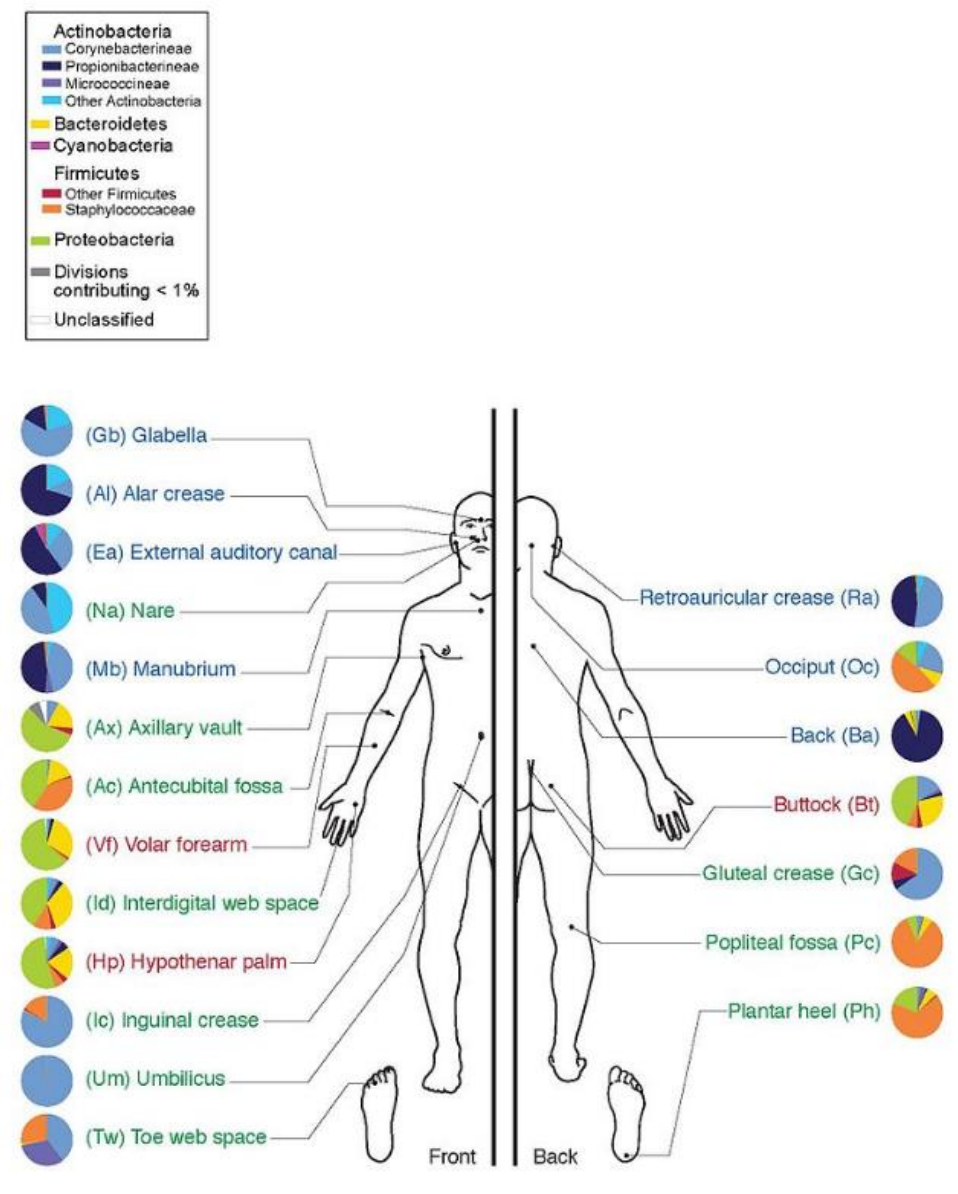
Minot S, Bryson A, Chehoud C, Wu GD, Lewis JD, Bushman FD. Rapid evolution of the human gut virome. Proc Natl Acad Sci U S A. 2013 Jul 23;110(30):12450-5.

Minot S, Sinha R, Chen J, Li H, Keilbaugh SA, Wu GD, Lewis JD, Bushman FD. The human gut virome: inter-individual variation and dynamic response to diet. Genome Res. 2011 Oct;21(10):1616-25.

Research on the human virome: where are we and what is next

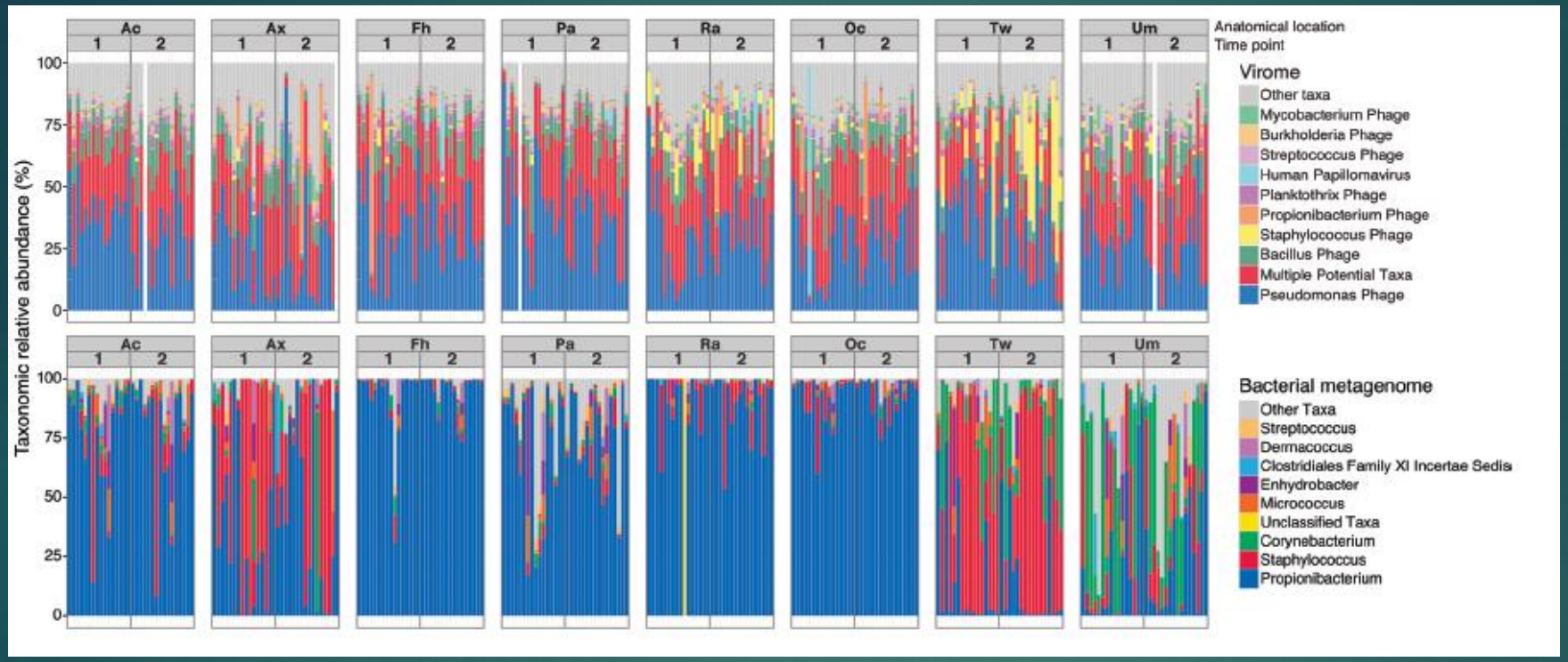
Zou S, Caler L, Colombini-Hatch S, Glynn S, Srinivas P. Research on the human virome: where are we and what is next. Microbiome. 2016 Jun 24;4(1):32.

Skin Microbiome



- The skin microbiome varies widely between different areas.
- The microbiomes of different areas are more similar between individuals than with other areas in the same individual.

Skin Virome and Bacteriome



Hannigan GD, Meisel JS, Tyldsley AS, Zheng Q, Hodkinson BP, SanMiguel AJ, Minot S, Bushman FD, Grice EA. The human skin double-stranded DNA virome: topographical and temporal diversity, genetic enrichment, and dynamic associations with the host microbiome. MBio. 2015 Oct 20;6(5):e01578-15.

Urinary Microbiome

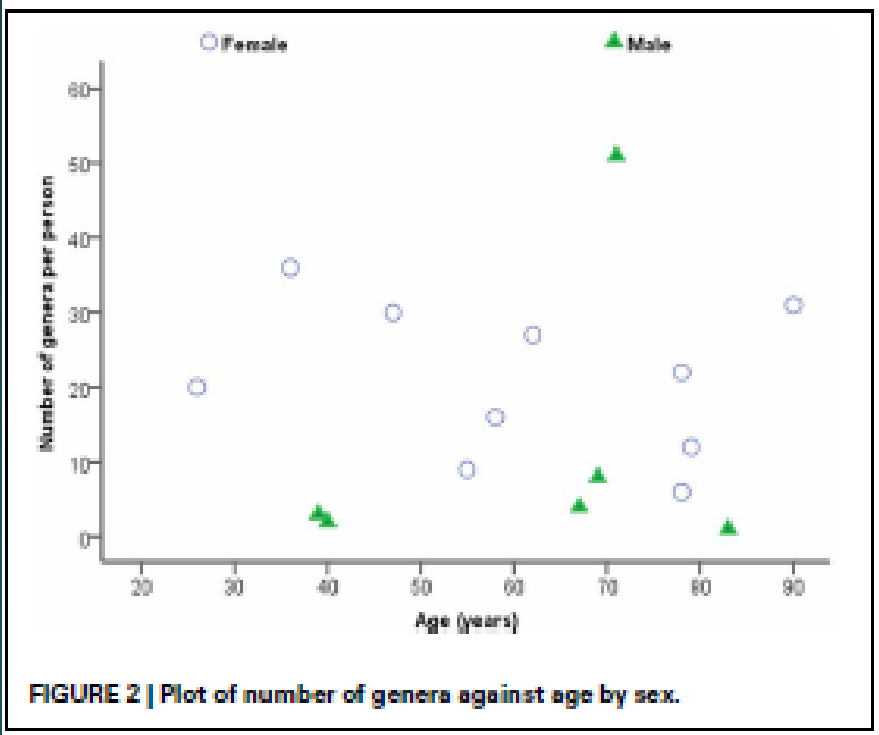


FIGURE 2 | Plot of number of genera against age by sex.

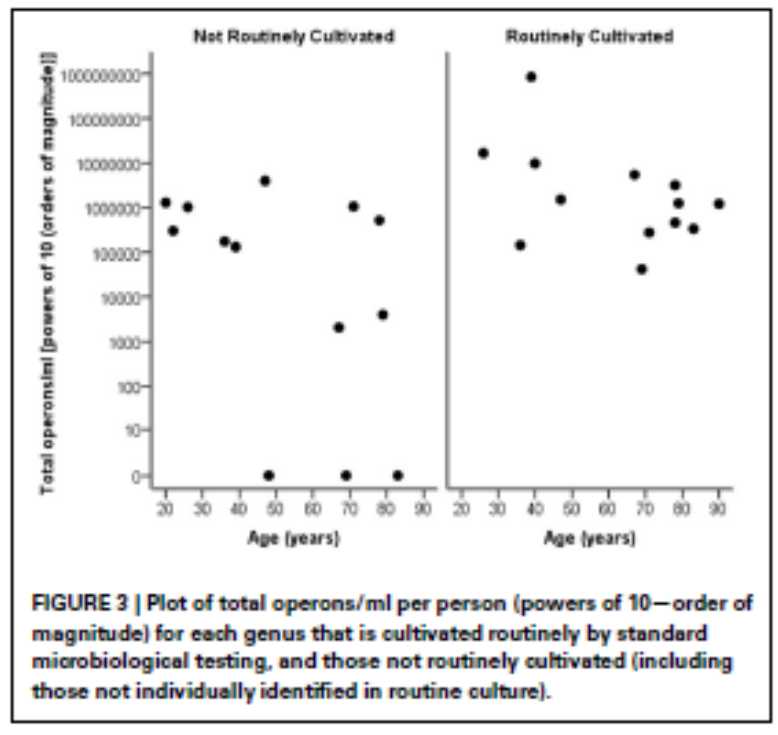
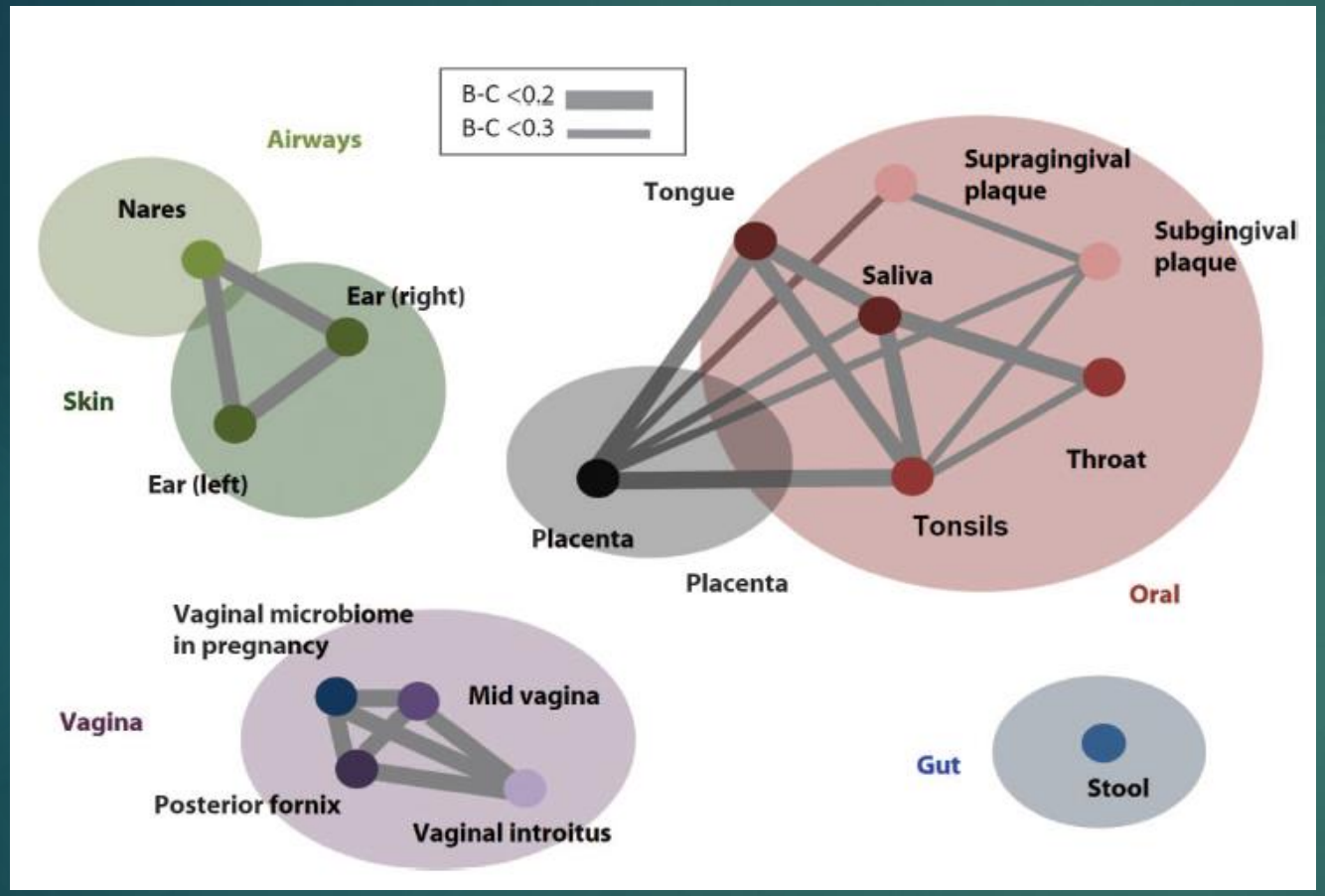


FIGURE 3 | Plot of total operons/ml per person (powers of 10—order of magnitude) for each genus that is cultivated routinely by standard microbiological testing, and those not routinely cultivated (including those not individually identified in routine culture).

- Analysis by by genetics rather than by culturing nearly doubles the known genera inhabiting the healthy male or female bladder.
- Female have much high numbers of bacterial genera.

Placental microbiome



- Taxonomic comparisons between regions of the body
- The thicker the connecting line, the greater the similarity of the taxonomic profile.
- Study has been criticized for possible contamination of samples with oral bacteria
- Implications for the theory of the sterile body

Aagaard K, Ma J, Antony KM, Ganu R, Petrosino J, Versalovic J. The placenta harbors a unique microbiome. Sci Transl Med. 2014 May 21;6(237):237ra65.

How much do we know?

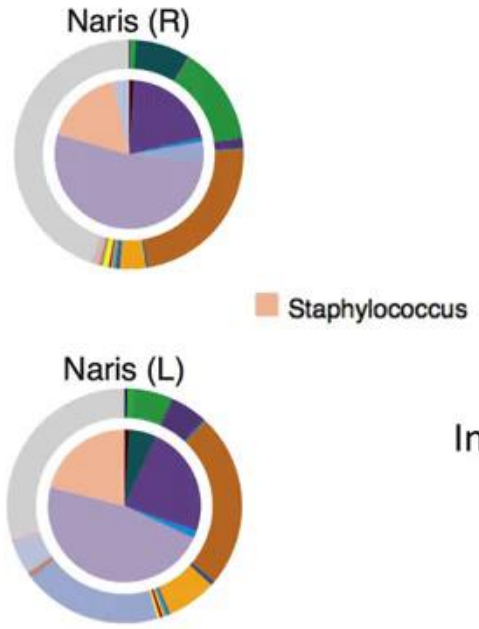
- "The field is still young, and we have only a limited knowledge of the confounding factors, the natural variation in the healthy population (and also variation over time), the impact of low-abundance species that are difficult to detect . . . ."
- We indeed cannot be confident about the significance of the many observed differences between healthy and diseased individuals.
- The enthusiasm generated by early discoveries of correlations between elements of the microbiome and disease must be tempered by the sobering fact that correlation does not equate with causality, and the potential confounding factors across populations are many.
- See monthly ***Microbiome*** and ***Biofilms and Microbiomes*** open access journals

Blaser M, Bork P, Fraser C, Knight R, Wang J. The microbiome explored: recent insights and future challenges. Nat Rev Microbiol. 2013 Mar;11(3):213-7.

Questions about infection in the era of the microbiome

- Could host conditions and microbial ecology and community structure be more important than the nature of the infectious agent?.
- Does a microbial-killing strategy resolve the infection?
- Can a microbial-killing strategy further damage microbial ecology?
- What factors can restore a damaged ecology?

Respiratory Microbiome - Nose



Figures adapted from Costello et. al (2009) *Supplementary materials*

Despite the prescence of *Staphylococcus aureus*, infections don't occur

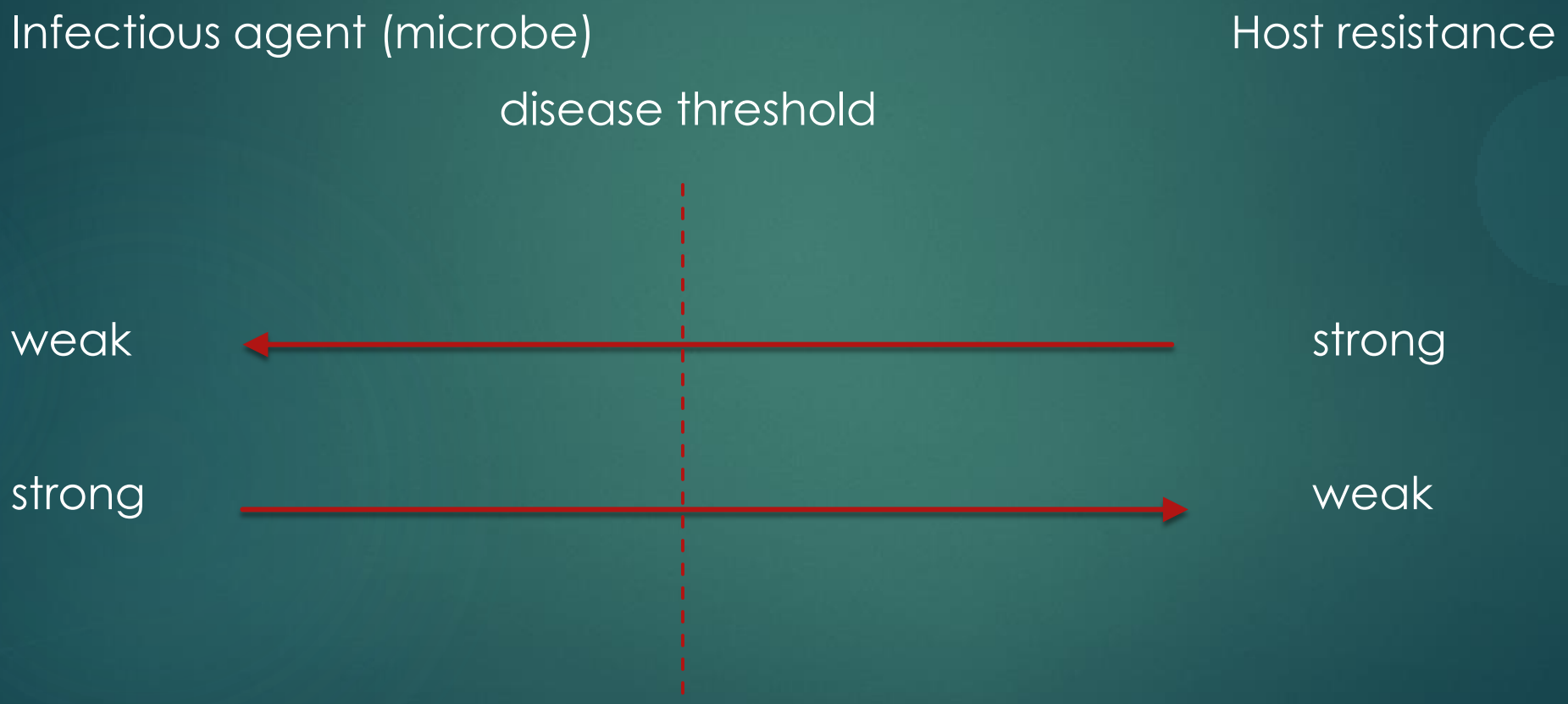
Innocuous bacteria keep infectious bacteria at bay

In many conditions, establishment of colonies of pathogenic bacteria more likely to be due to ***disruption of the biofilms*** of the normal microbiome than to simple invasion and infection.

We should use great caution in viewing biofilms as “the enemy”
In the same manner that we have viewed microbes as “the enemy.”

Infection

Old model of infection



Therapeutic choices: Kill the organism or strengthen the host

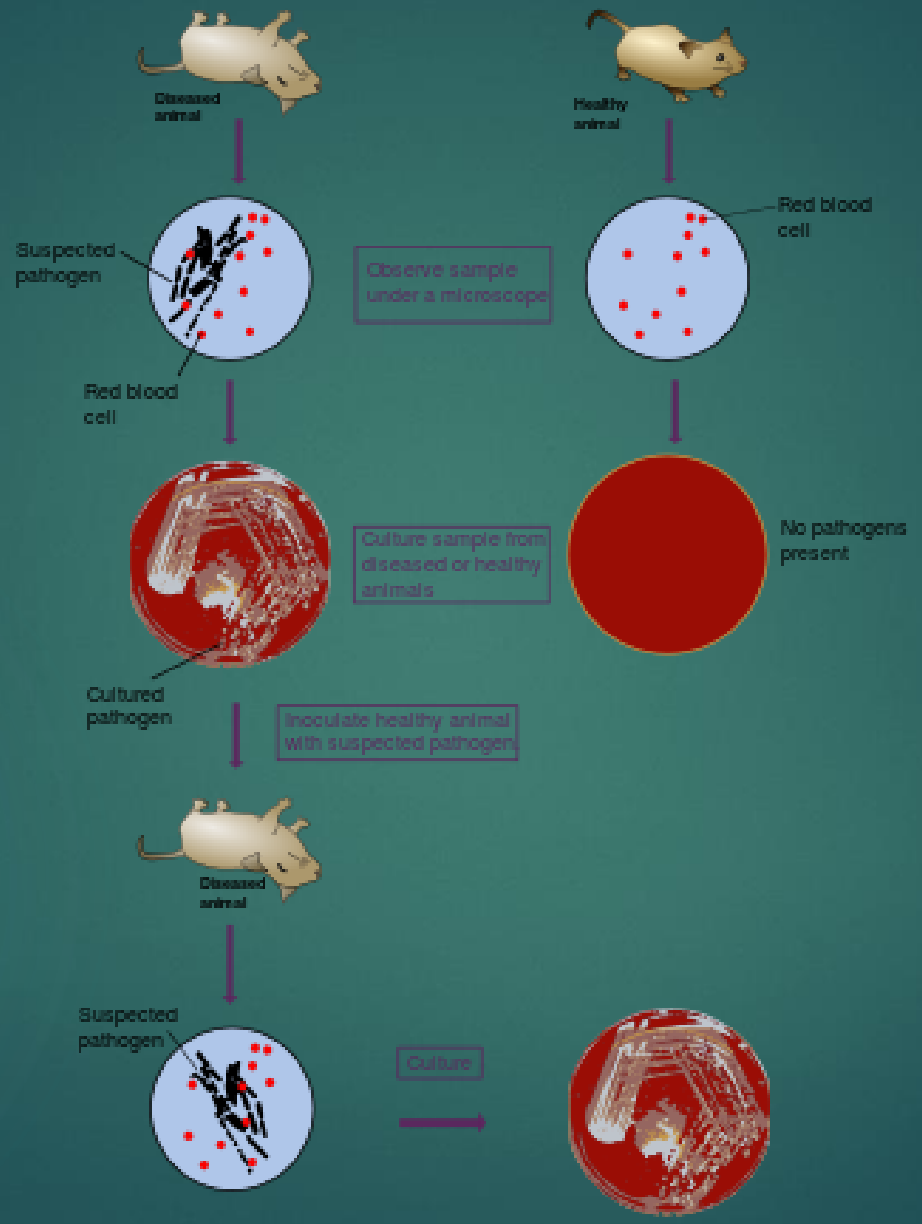
Koch's Postulates:

① The microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms.

② The microorganism must be isolated from a diseased organism and grown in pure culture.

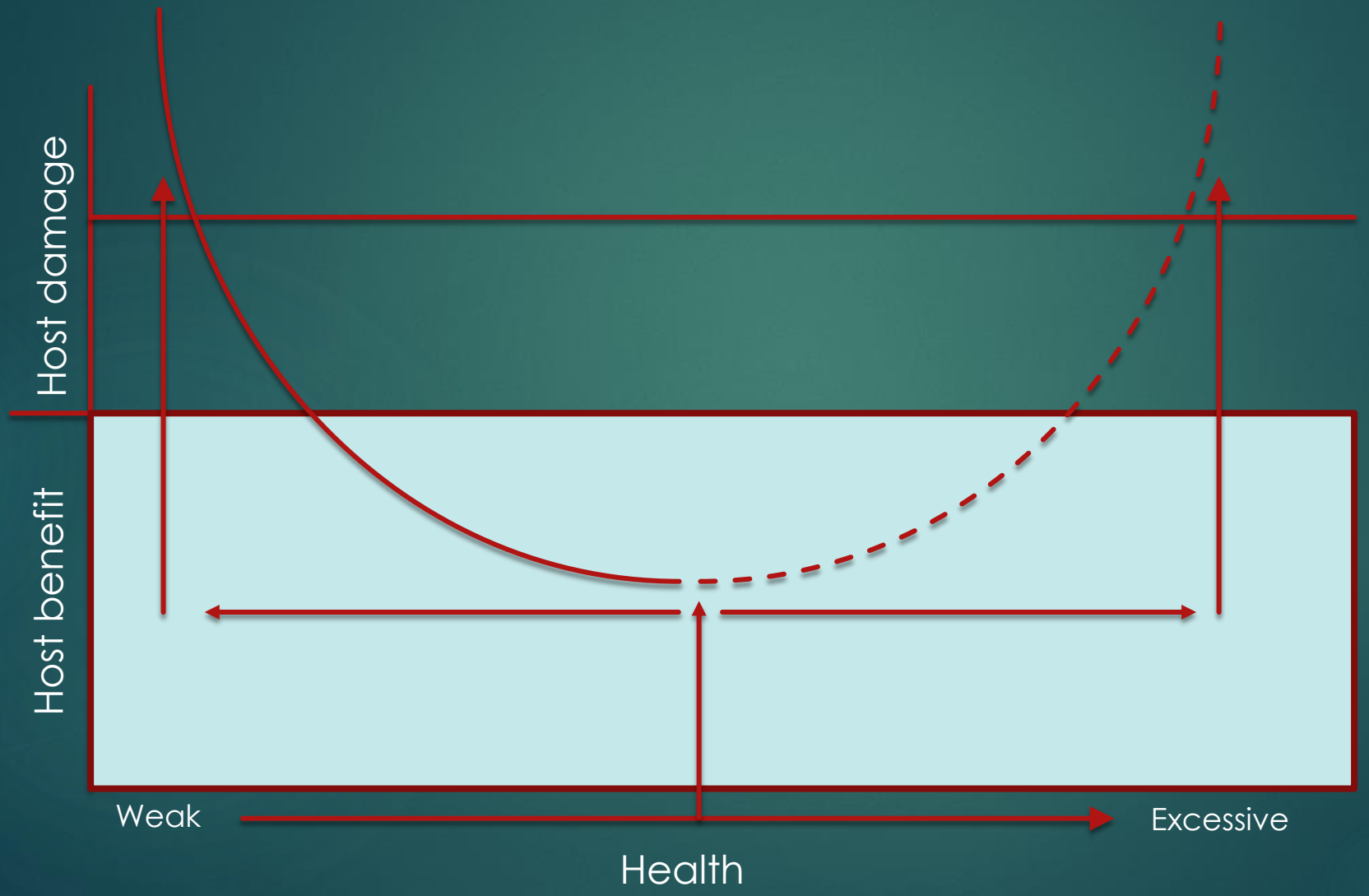
③ The cultured microorganism should cause disease when introduced into a healthy organism.

④ The microorganism must be reisolated from the inoculated, diseased experimental host and identified as being identical to the original specific causative agent.



1. Koch himself abandoned the first point in the face of evidence for asymptomatic carriers of potentially pathogenic organisms
2. The vast majority of microorganisms known to inhabit the human body cannot be cultured in any known medium
3. Many potentially pathogenic organisms can be injected into animals or humans without causing any symptoms at all.

Damage-response model of infection

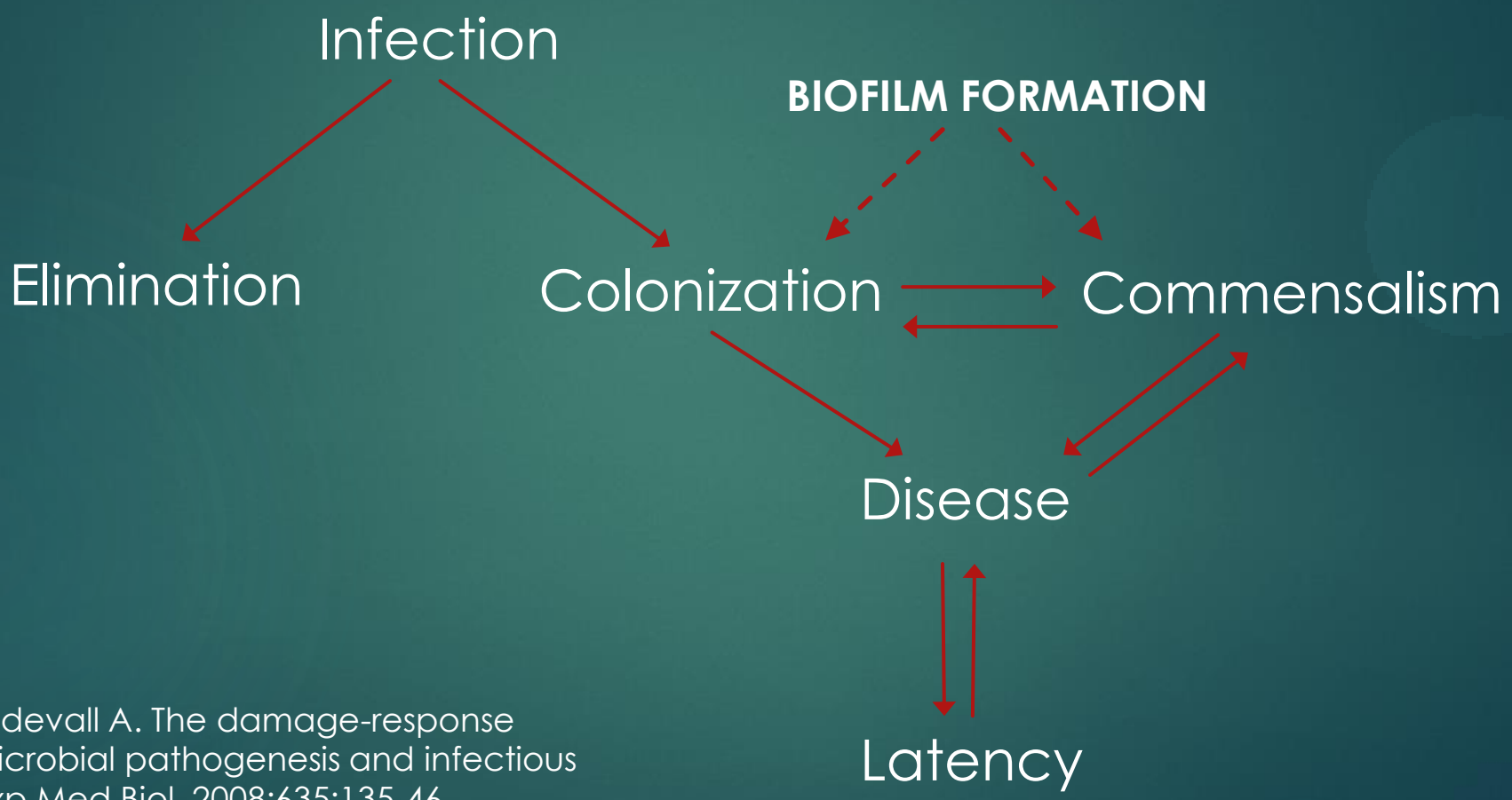


Pirofski LA, Casadevall A. The damage-response framework of microbial pathogenesis and infectious diseases. Adv Exp Med Biol. 2008;635:135-46.

Examples of host damage

- Non-healing wounds and ulcers. Immune response damages tissues.
- Tuberculosis. Damage to lung by immune response.
- Chronic viral hepatitis. Damage to liver by immune response.
- HIV infection triggering autoimmune response.
- Chronic Lyme infection. Damage to connective tissues by response.
- Possible chronic-infection triggered autoimmunity
- Permanent presence of high volumes of antigenic food substances produce systemic inflammation.

Possible outcomes of chronic infection



Pirofski LA, Casadevall A. The damage-response framework of microbial pathogenesis and infectious diseases. Adv Exp Med Biol. 2008;635:135-46.

Old Model

- Single infectious agents responsible for single disease
- Allopathic strategy: Kill the organism with antimicrobials.
- Natural strategies: Enhance host resistance through lifestyle and/or antimicrobial strategy

New Model

- Bacteria, viruses, and fungi exist throughout the organism in complex structured communities.
- They are essential to life and primarily beneficial.
- ***Avoid disrupting essential or beneficial commensal structures.***
- Natural therapeutics to restore disrupted communities or their structures
- Acute systemic anti-microbial strategy for serious infection only.
- Anti-microbial and anti-biofilm strategies for local chronic infection when topical application is possible.
- Internal biofilms: a Great Unknown

Avoid disrupting essential or beneficial commensal structures.

Natural therapeutics to restore disrupted communities or their structures

Acute systemic anti-microbial strategy for serious infection only.

Anti-microbial and anti-biofilm strategies for local chronic infection when topical application is possible.

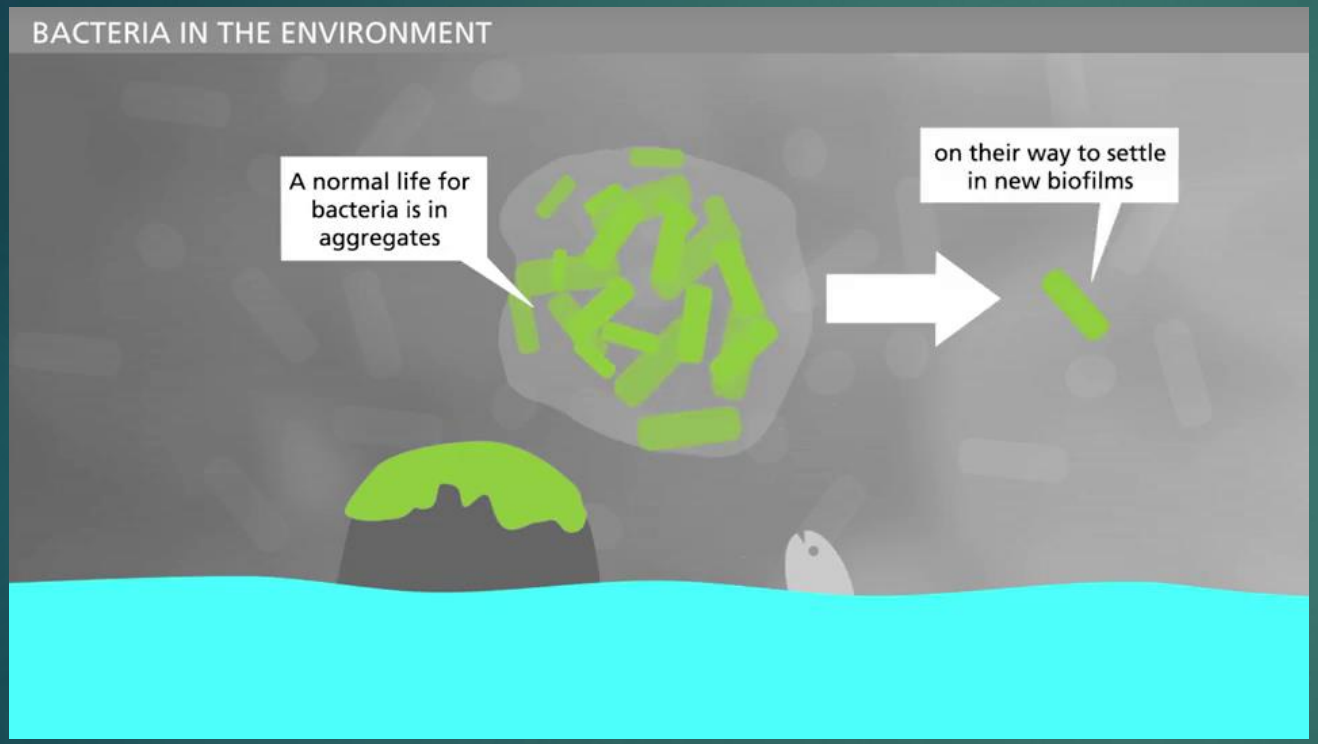
Internal biofilms: a Great Unknown

Biofilms

Definitions

- **Planktonic** form. Bacterial for as a single organism
- **Biofilm** form. Bacteria in complex matrix of single or multiple species which may include yeasts and fungi.
- **MIC.** Minimum inhibitory concentration. The minimum amount of an antimicrobial substance to stop growth of an organism in culture.
- **SIC**. Sub Inhibitory Concentration. Amounts of antimicrobial substances below the level necessary to stop growth of an organism in culture.

Bacteria live in a biofilm state



Planktonic form.
Free moving

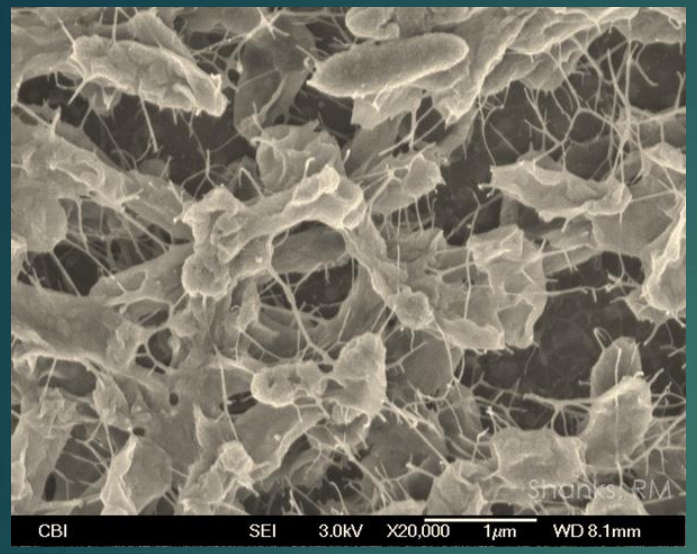
Biofilm form.
Non-mobile, linked in a matrix

The biofilm for of bacteria is resistant to both antibiotic therapy and the immune system

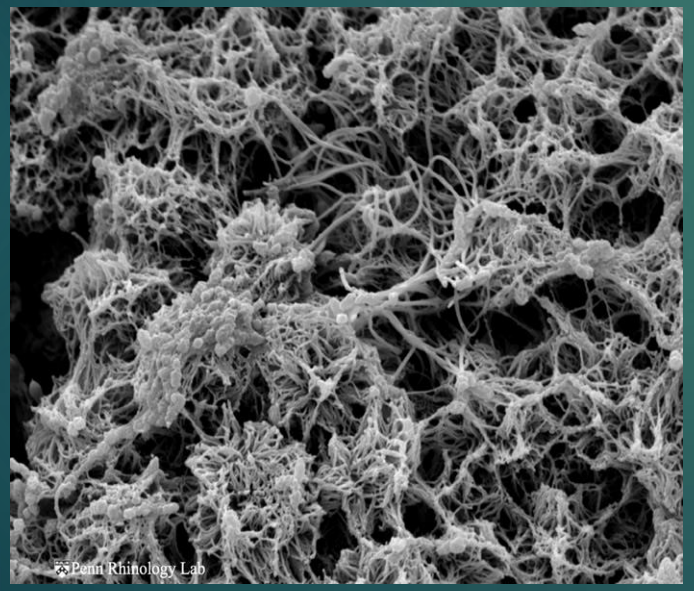
Most bacteria in an on the human body exist in biofilm form. Most are ***beneficial*** commensal bacteria and provide barrier, immune, and metabolic functions

Biofilm matrix

- Under specific conditions, bacteria secrete matrix substances.
- These are typically polysaccharides, genetic material, or proteins
- The bacteria may also secrete gel substances into the matrix.
- The biofilm structure may take on the qualities of a complex organism, with specialization of function at the various levels and among the various component organisms of the biofilm.
- The structural substances create a mechanical barrier protecting the internal organisms from the immune system, or from antimicrobial substances.
- The structure may also produce a metabolic gradient, with different metabolism of the superficial or deep organisms.



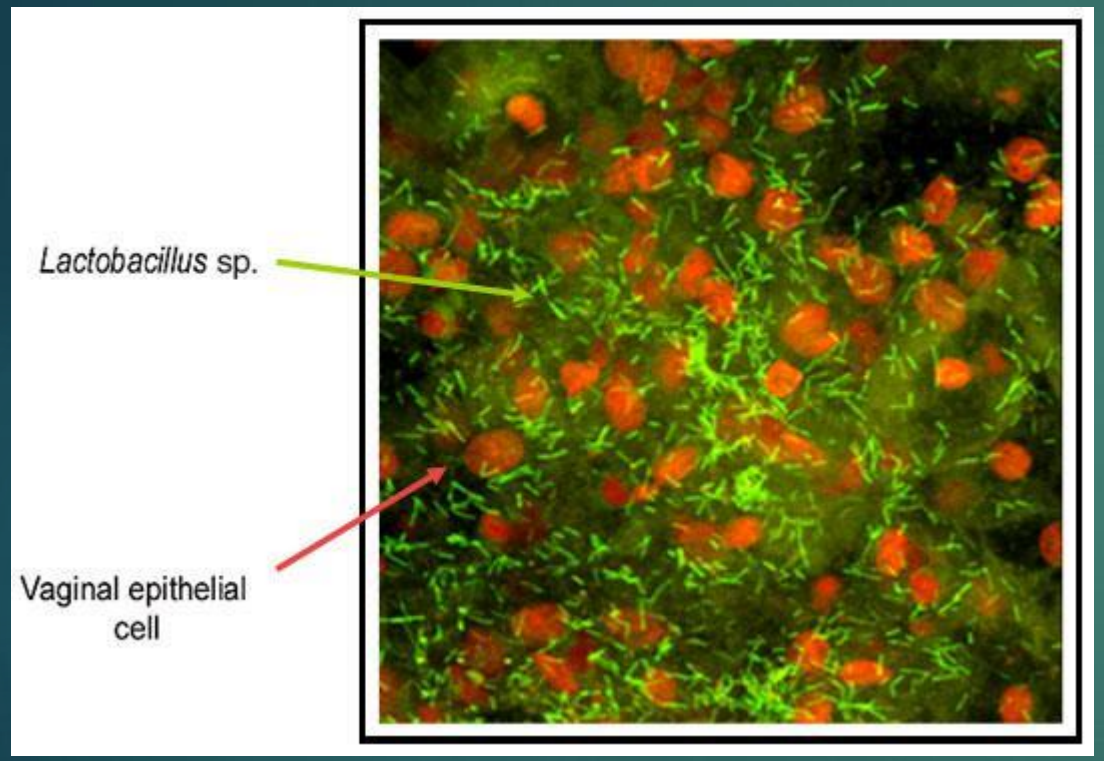
MRSA

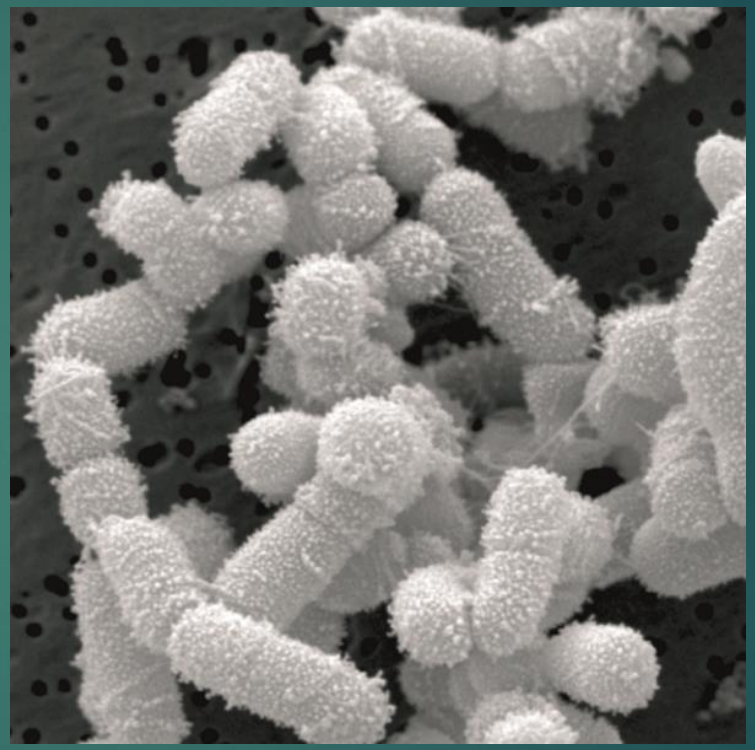


Pseudomonas

Biofilms are part of normal microbiome defense of the body but pathological biofilms are nearly universally present in:

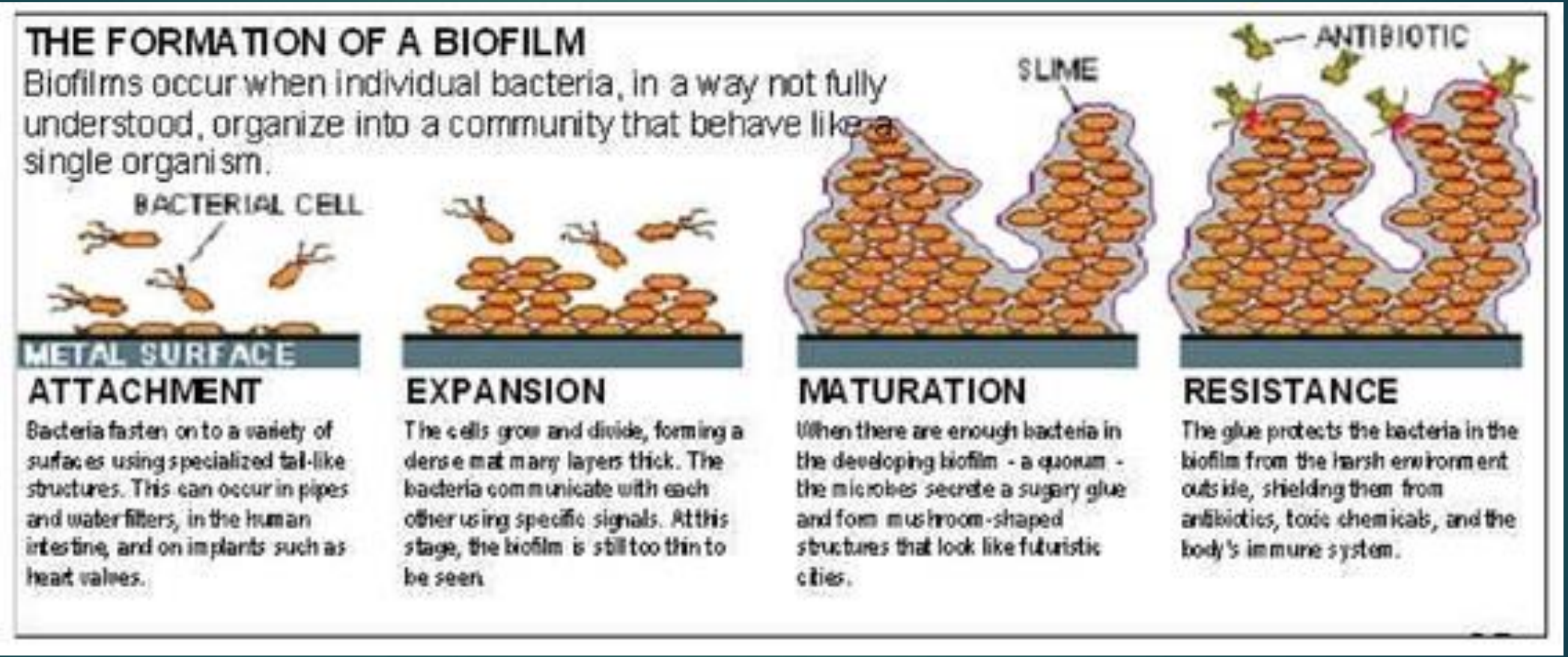
- Oral plaque, periodontal disease, abscess
- MRSA infections on skin
- Other skin infections
- Chronic wounds and ulcers
- Chronic sinus infection
- Upper GI disturbances
- Vaginal infection
- Bladder infection





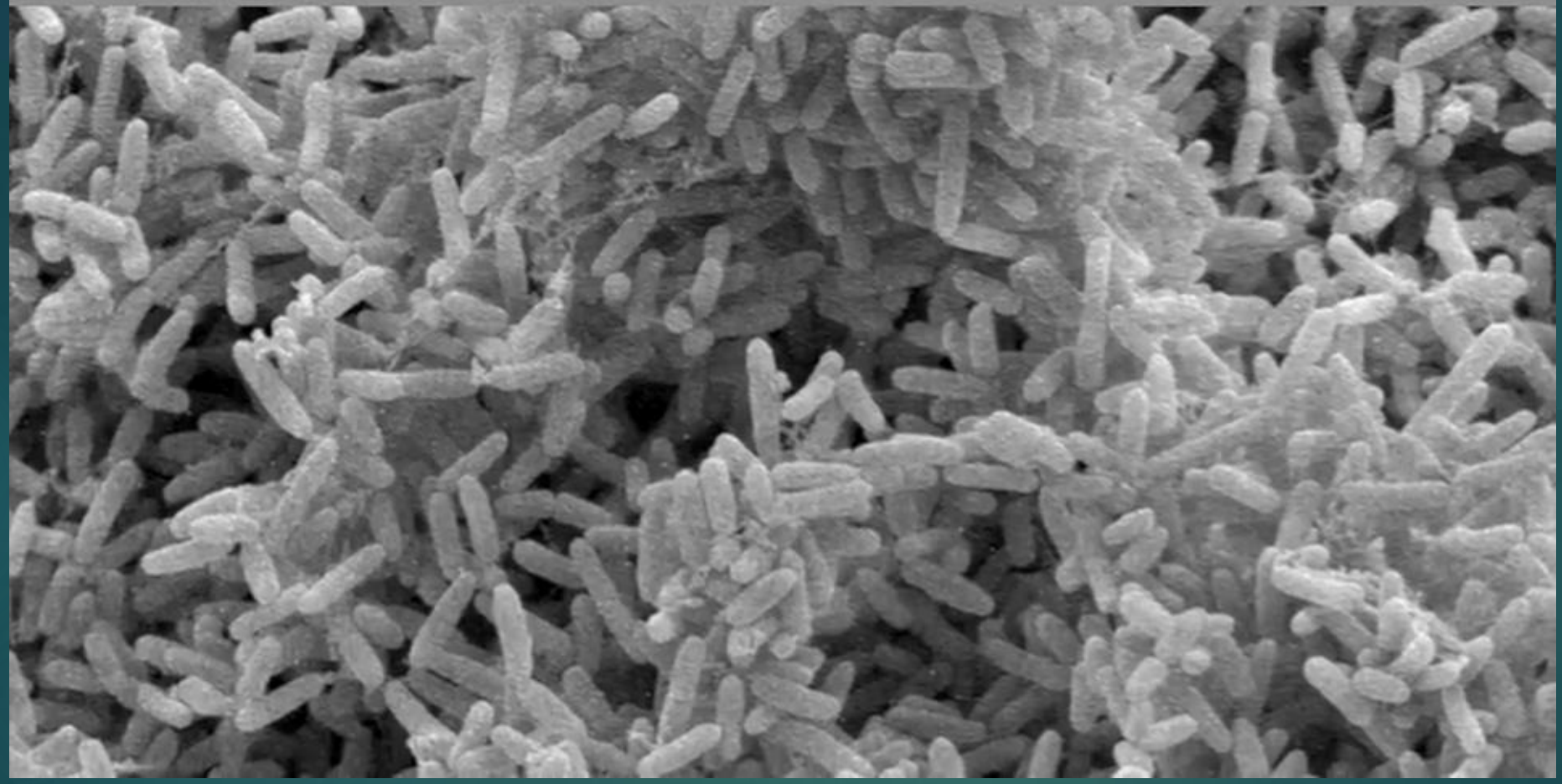
Lactobacillus

Biofilms


THE FORMATION OF A BIOFILM
Biofilms occur when individual bacteria, in a way not fully understood, organize into a community that behave like a single organism.
BACTERIAL CELL
METAL SURFACE
SLIME
ANTIBIOTIC
ATTACHMENT
Bacteria fasten on to a variety of surfaces using specialized tail-like structures. This can occur in pipes and water filters, in the human intestine, and on implants such as heart valves.
EXPANSION
The cells grow and divide, forming a dense mat many layers thick. The bacteria communicate with each other using specific signals. At this stage, the biofilm is still too thin to be seen.
MATURATION
When there are enough bacteria in the developing biofilm - a quorum - the microbes secrete a sugary glue and form mushroom-shaped structures that look like futuristic cities.
RESISTANCE
The glue protects the bacteria in the biofilm from the harsh environment outside, shielding them from antibiotics, toxic chemicals, and the body's immune system.

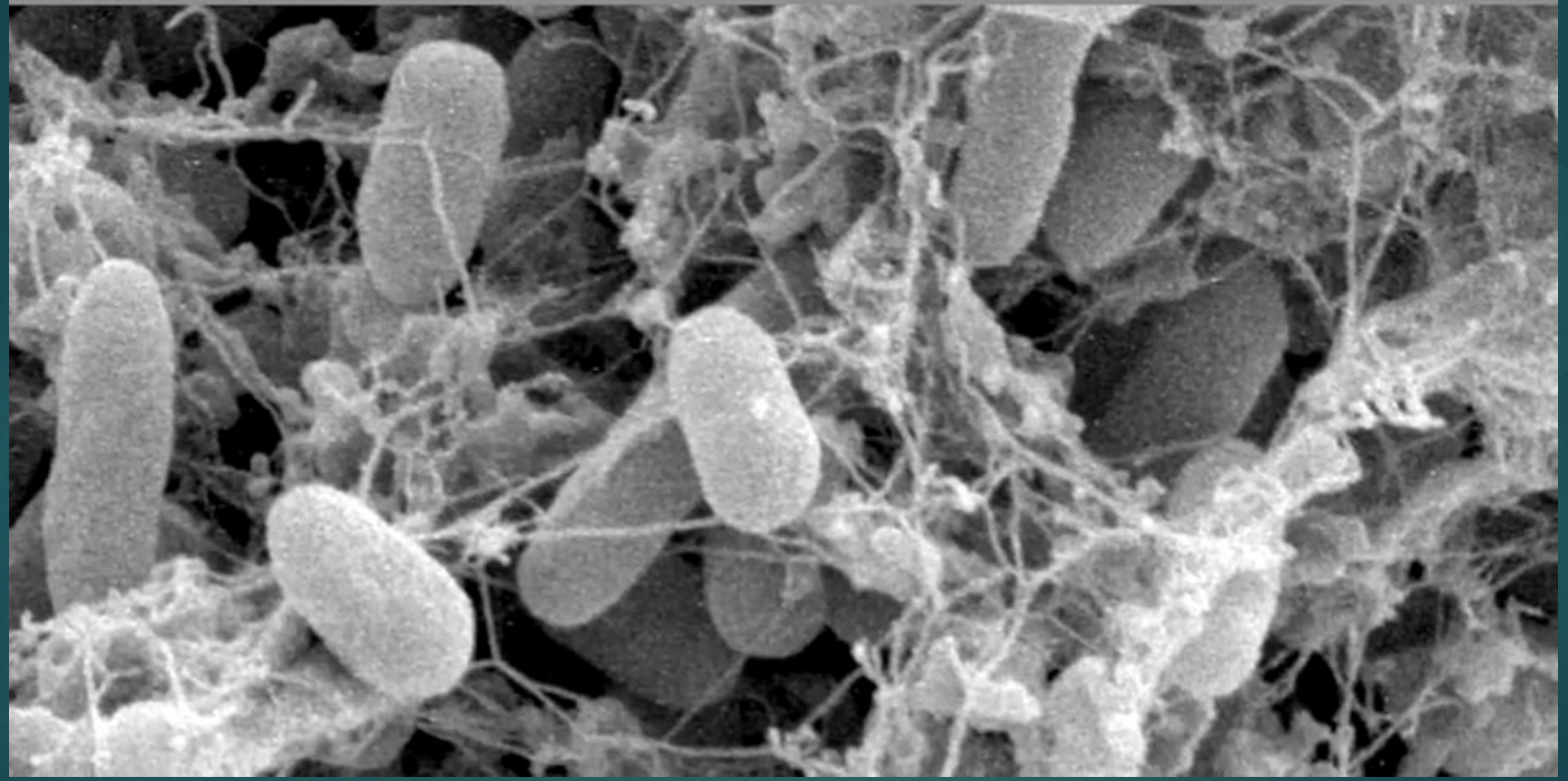
Bjarnsholt & Quortrup

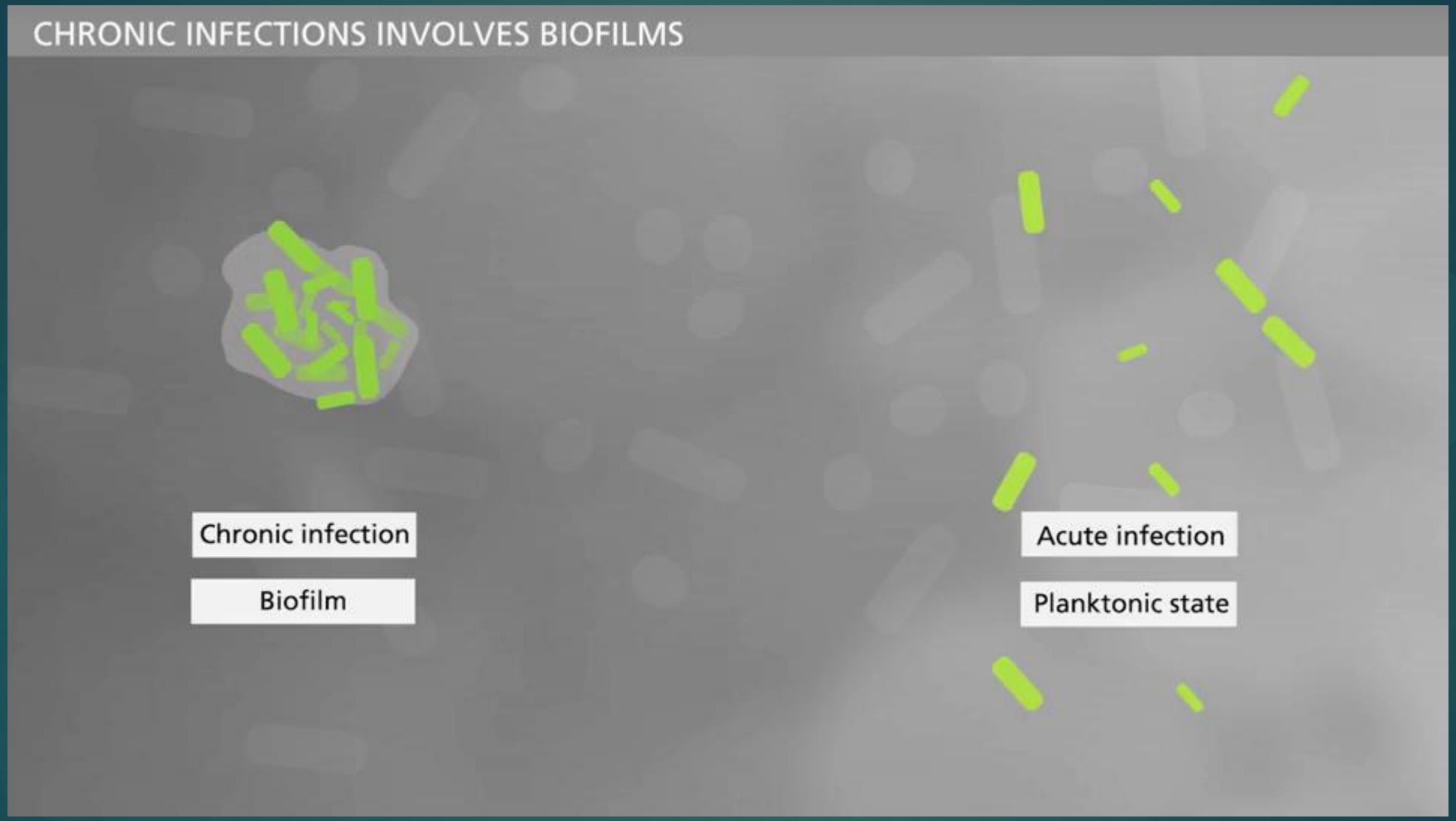
BIOFILM EMBEDDED IN A MATRIX



Bjarnsholt & Quortrup

BIOFILM EMBEDDED IN A MATRIX




CHRONIC INFECTIONS INVOLVES BIOFILMS
Chronic infection
Biofilm
Acute infection
Planktonic state

CHRONIC INFECTIONS INVOLVES BIOFILMS

Chronic infection

Biofilm

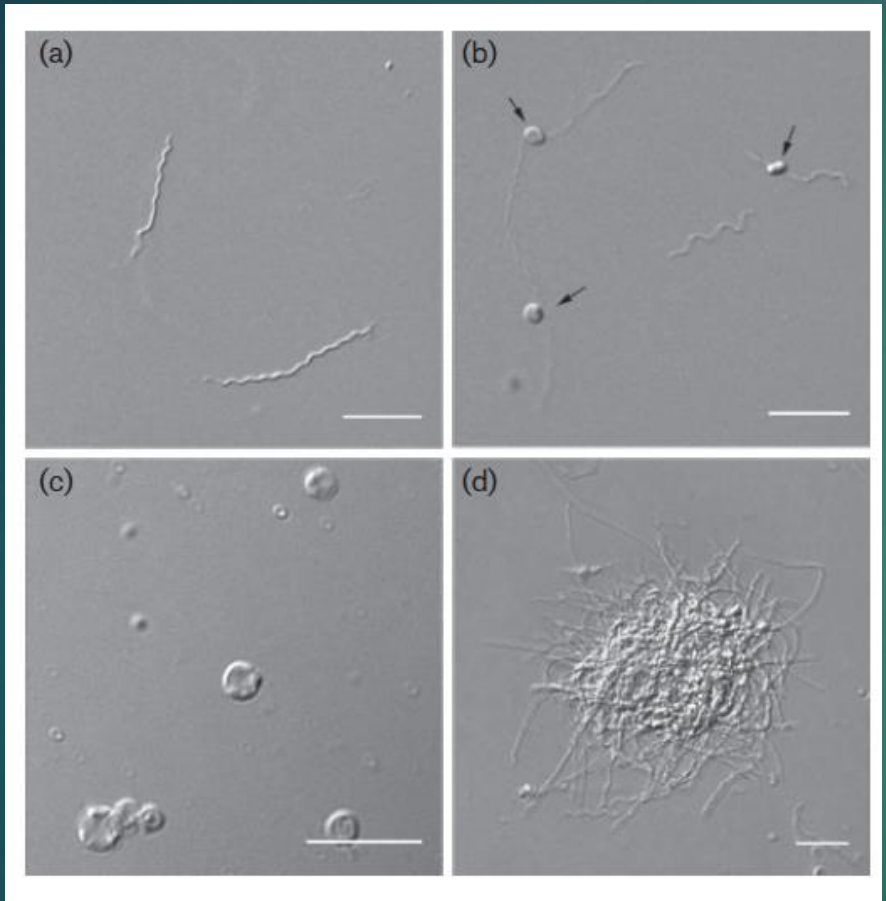
Protected from both the immune defence and antibiotics

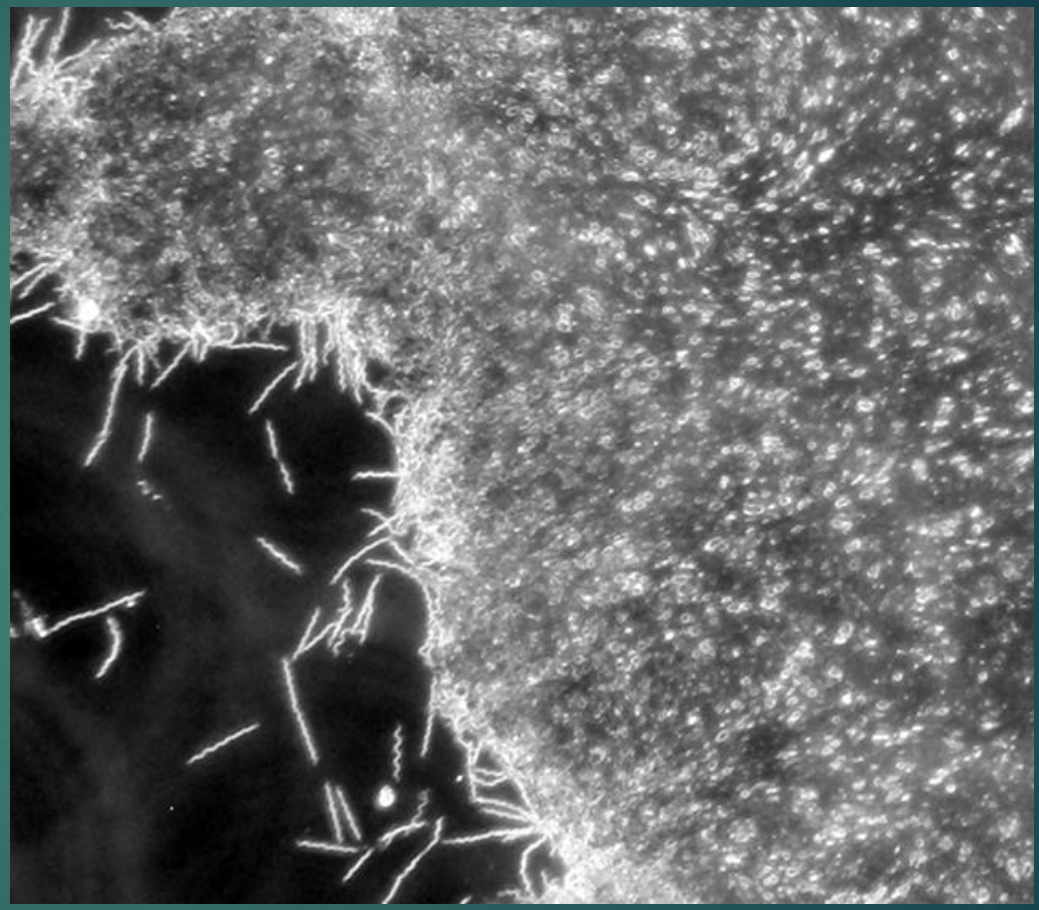
Acute infection

Planktonic state

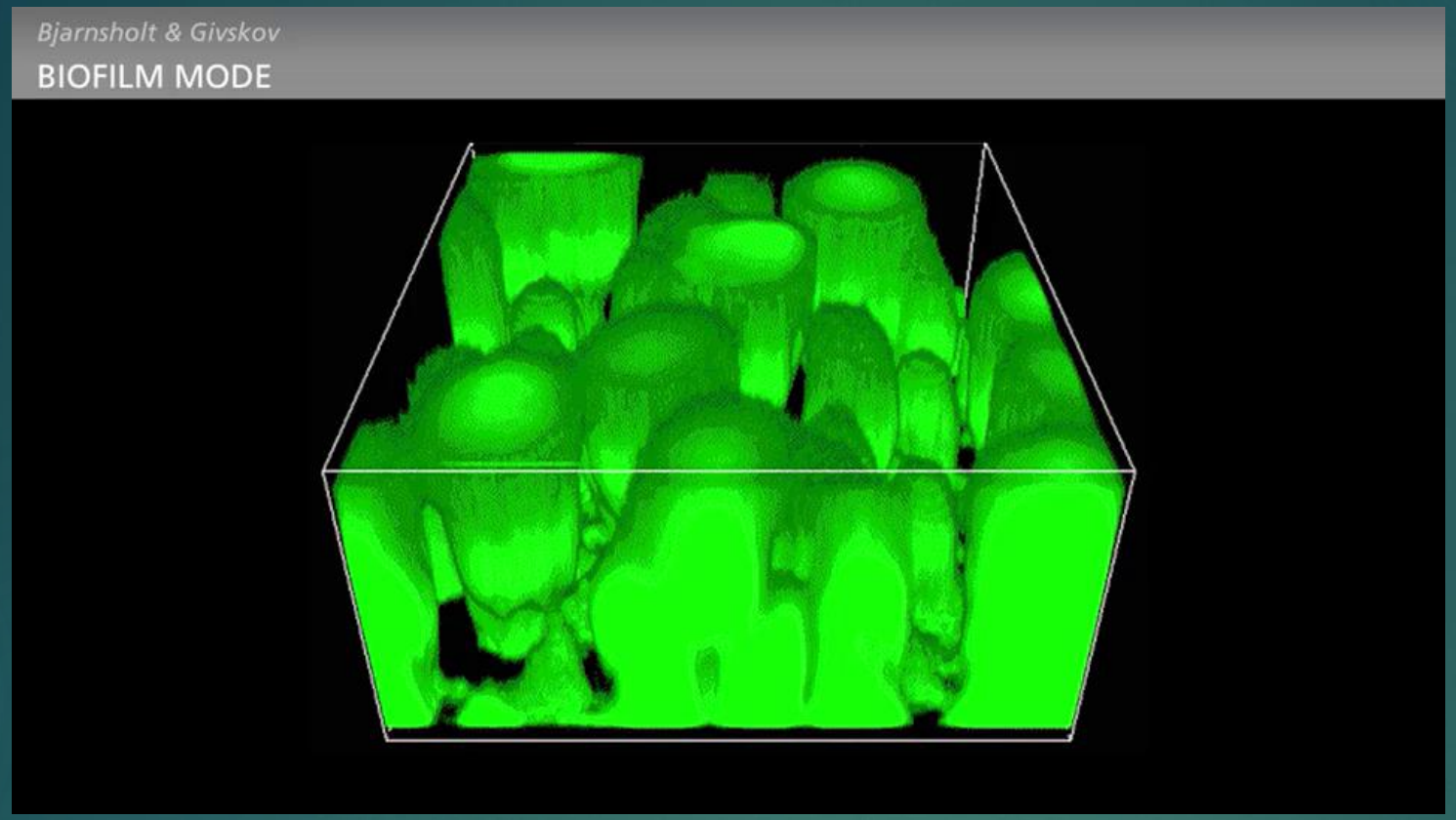
Can be eradicated by the immune defence and by antibiotics

Borrelia biofilm on slide





- Biofilms are the normal life state for bacteria and some fungi
- The gradient of metabolism from aerobic at the surface to anaerobic at the core allows resistance to substances which might attack the metabolism.
- In some species, an attached biofilm layer provides nutrients to a superficial layer, which may the secrete antibiotics, reproduce, etc.
- Once aggregated, bacteria in biofilms can dramatically change their functions and secretions.
- Commensal biofilms in the gut, on the skin, and elsewhere may be essential or beneficial for the life of the organism.

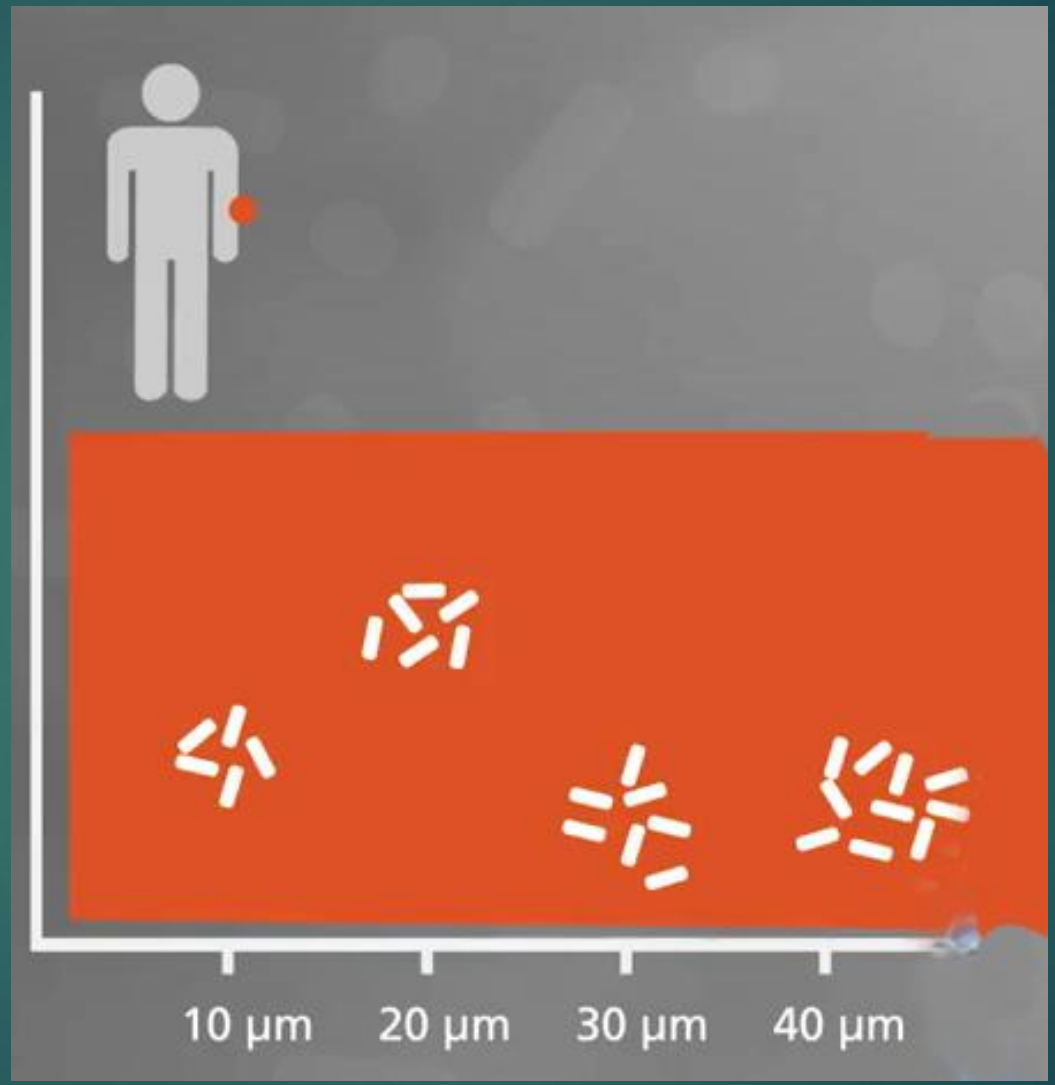


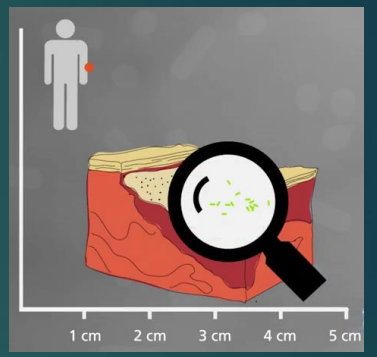
The biofilm “mushrooms” in the picture are about actual size, this only occurs in lab conditions

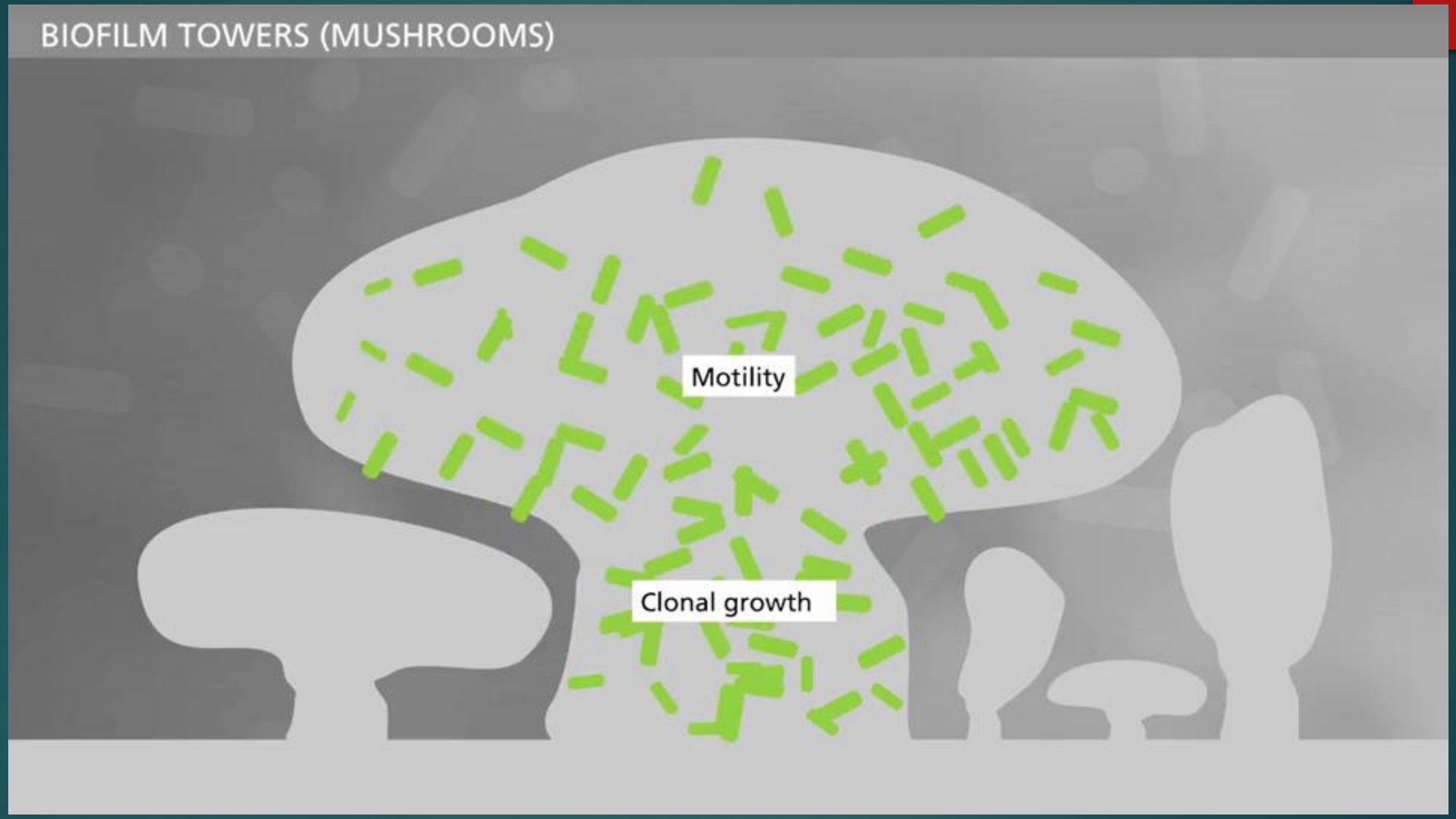
Biofilms have not been studied in the living organism.

Biofilms in infected wounds are typically in the range of 5 to 10 micrometers, or 1/100 of a millimeter. Requires about 100x magnification to be visible.

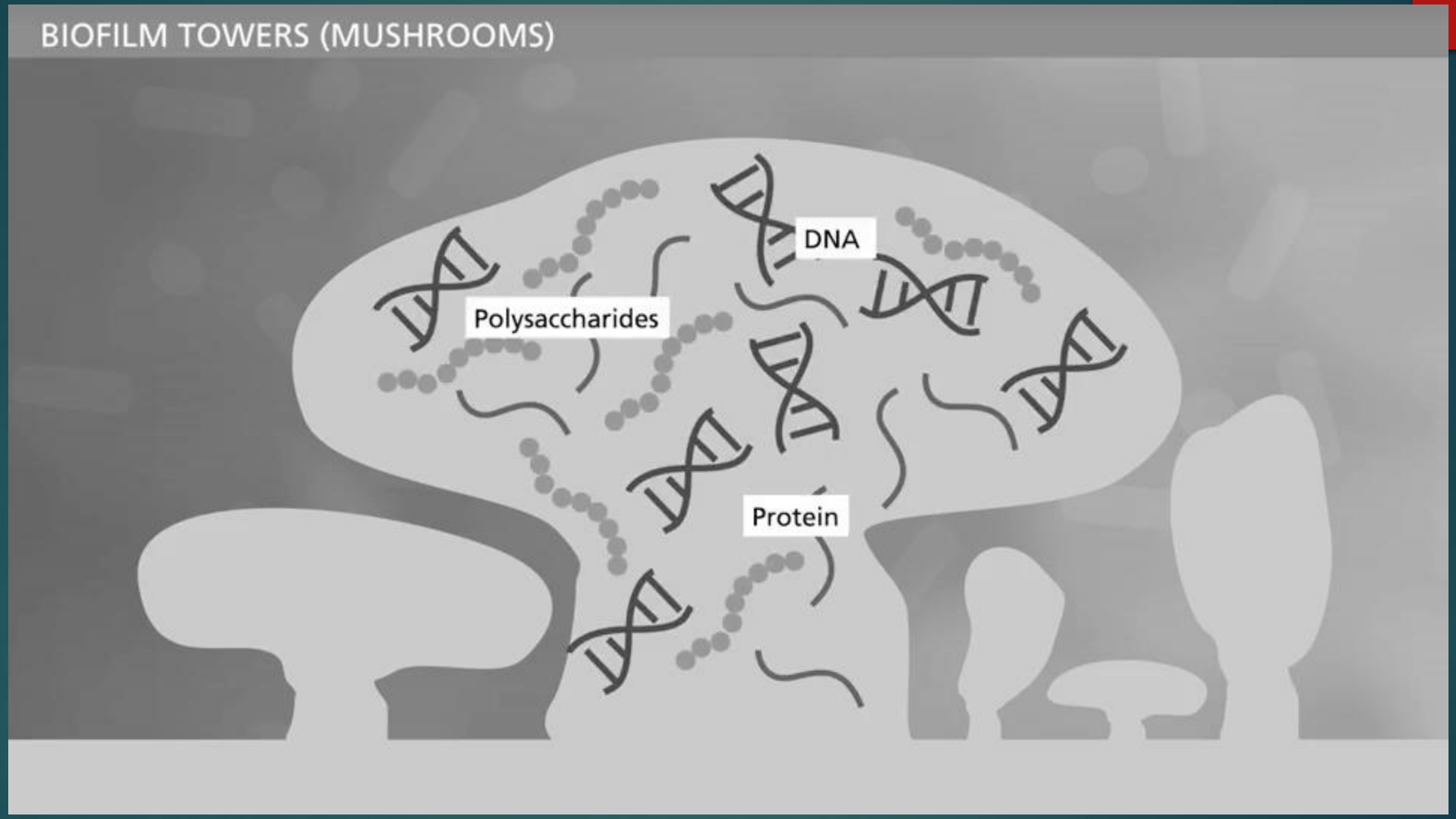
One sample of *Borrelia* biofilm in tissue samples required 400X magnification.

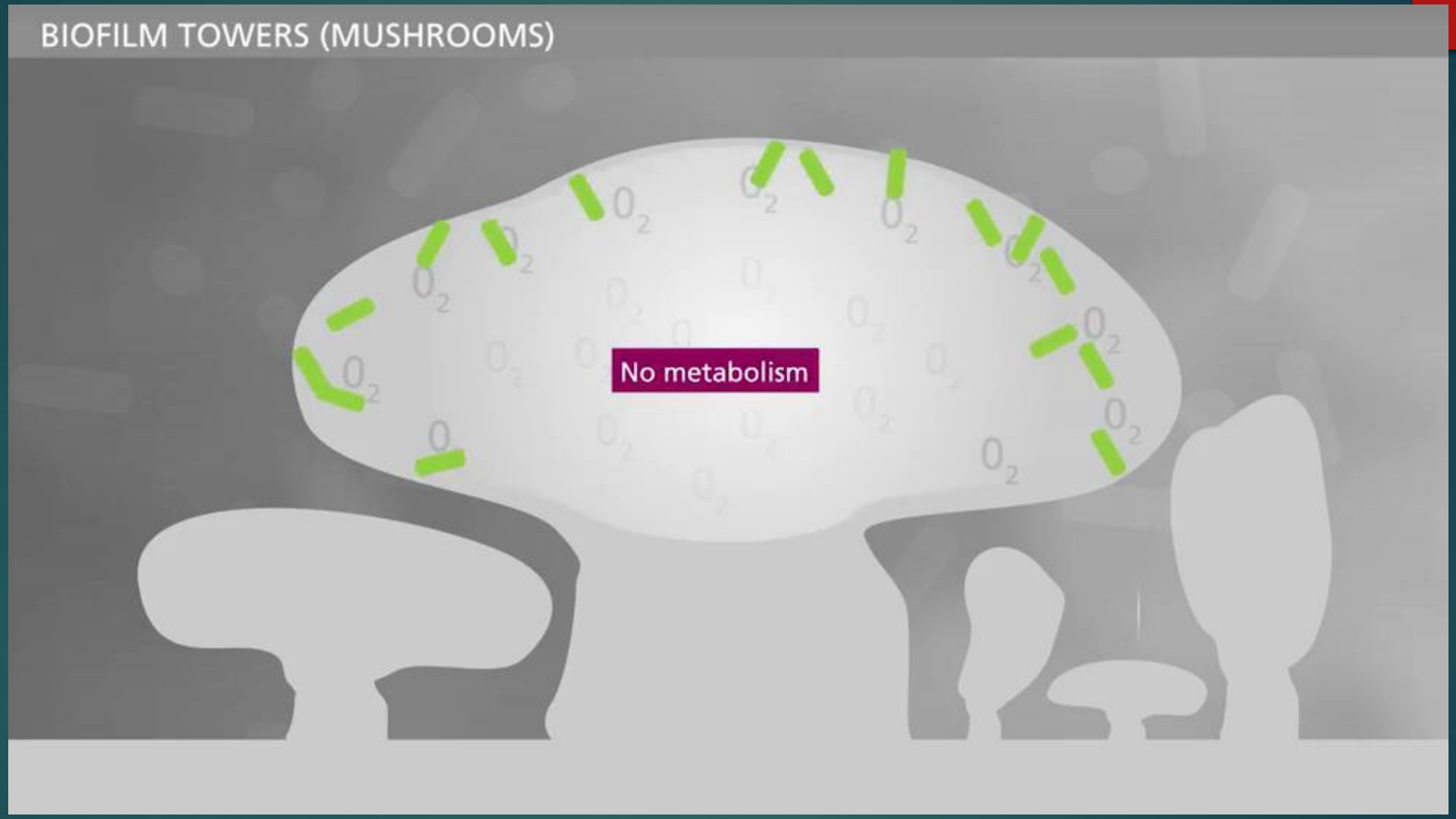


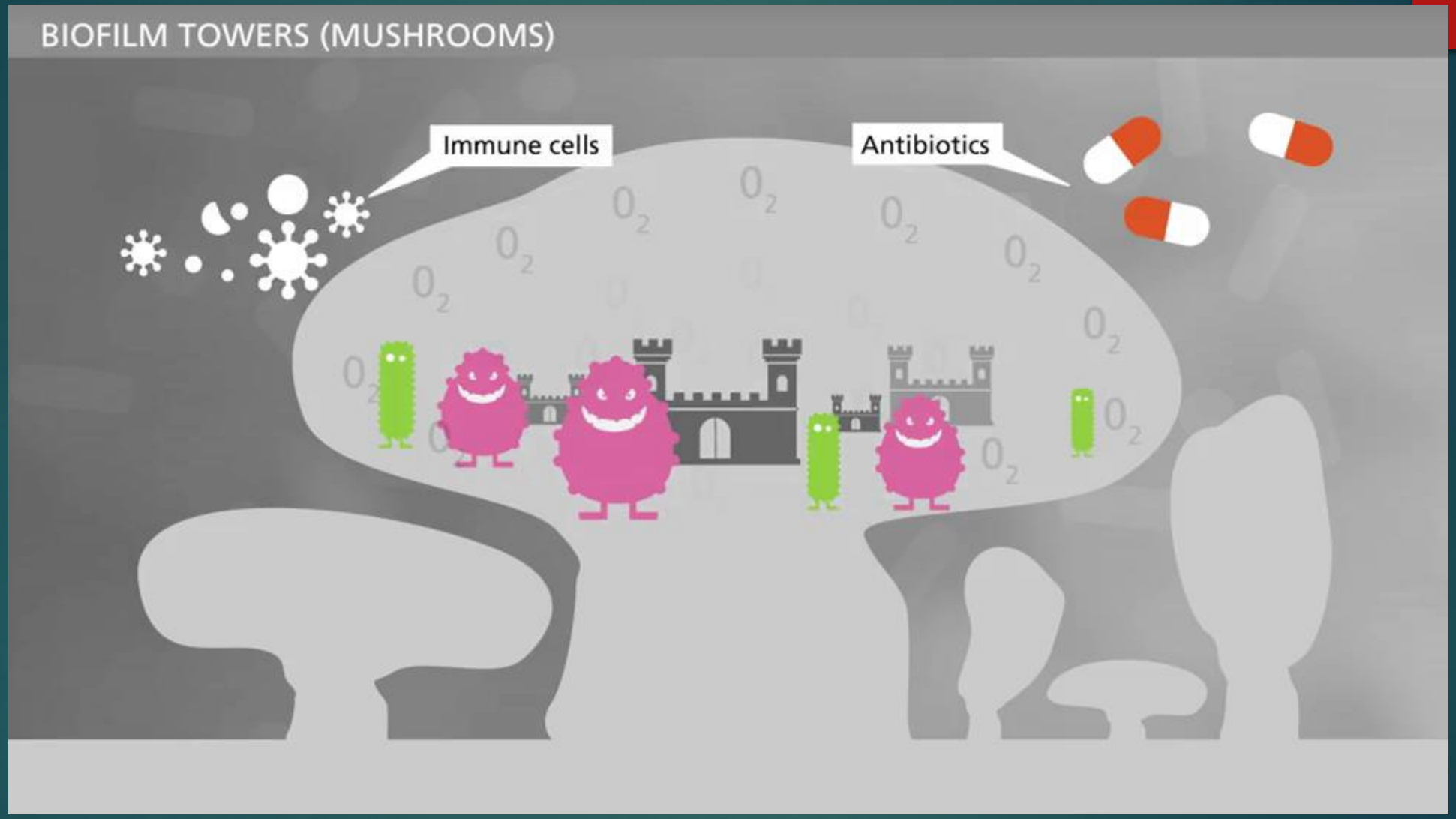


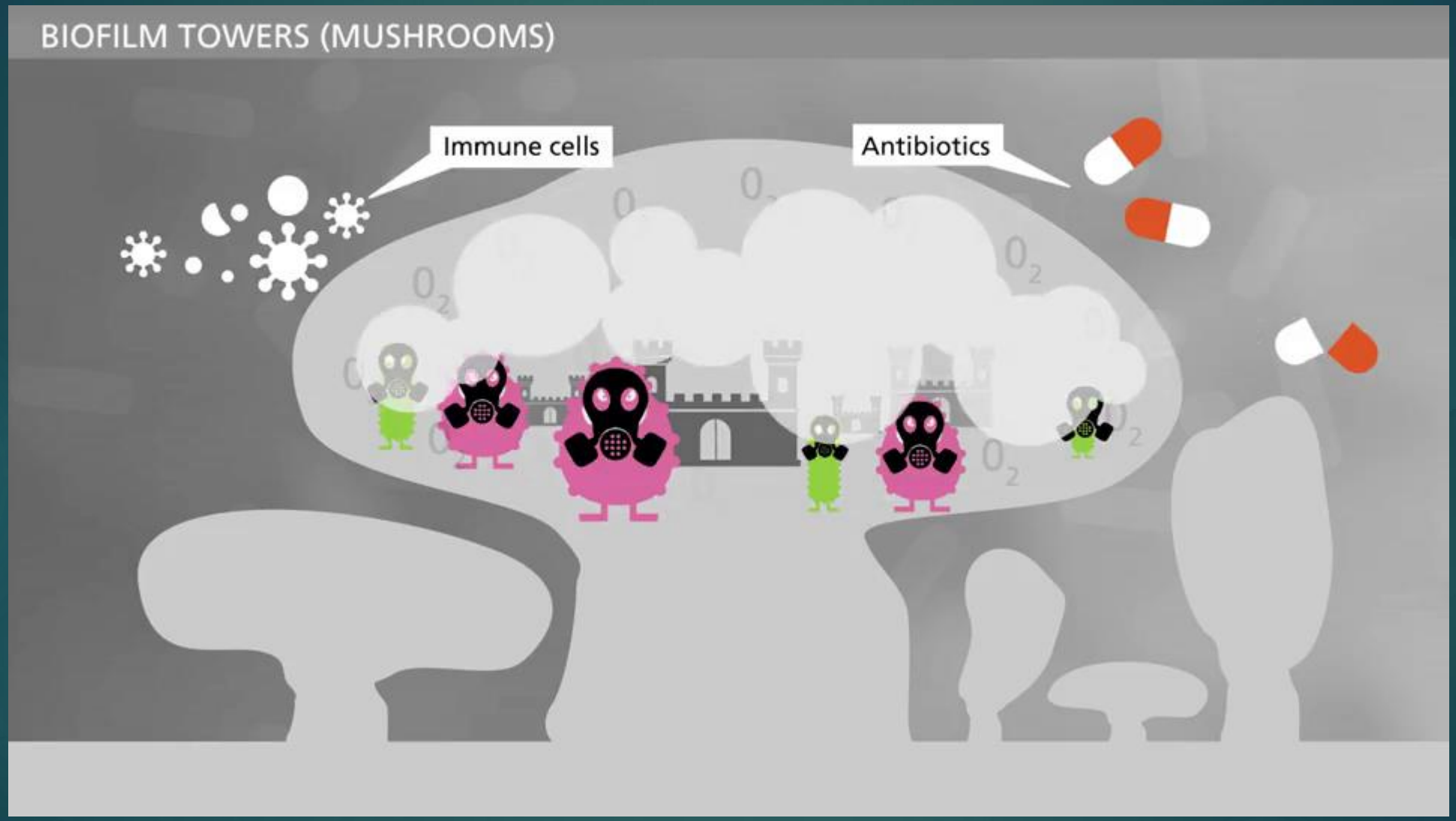


Pattern of growth in the laboratory dish

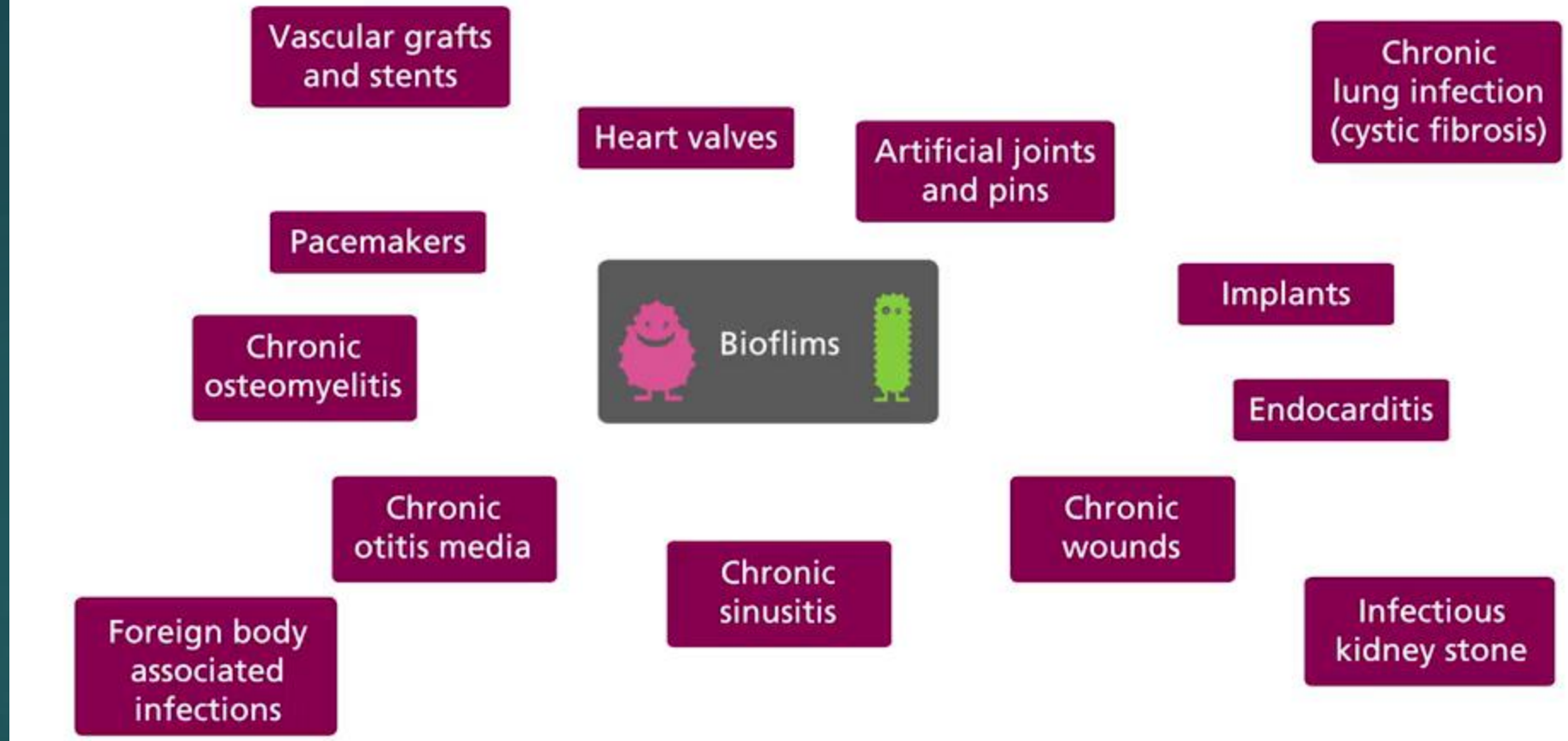

BIOFILM TOWERS (MUSHROOMS)
DNA
Polysaccharides
Protein


BIOFILM TOWERS (MUSHROOMS)
No metabolism


BIOFILM TOWERS (MUSHROOMS)
Immune cells
Antibiotics

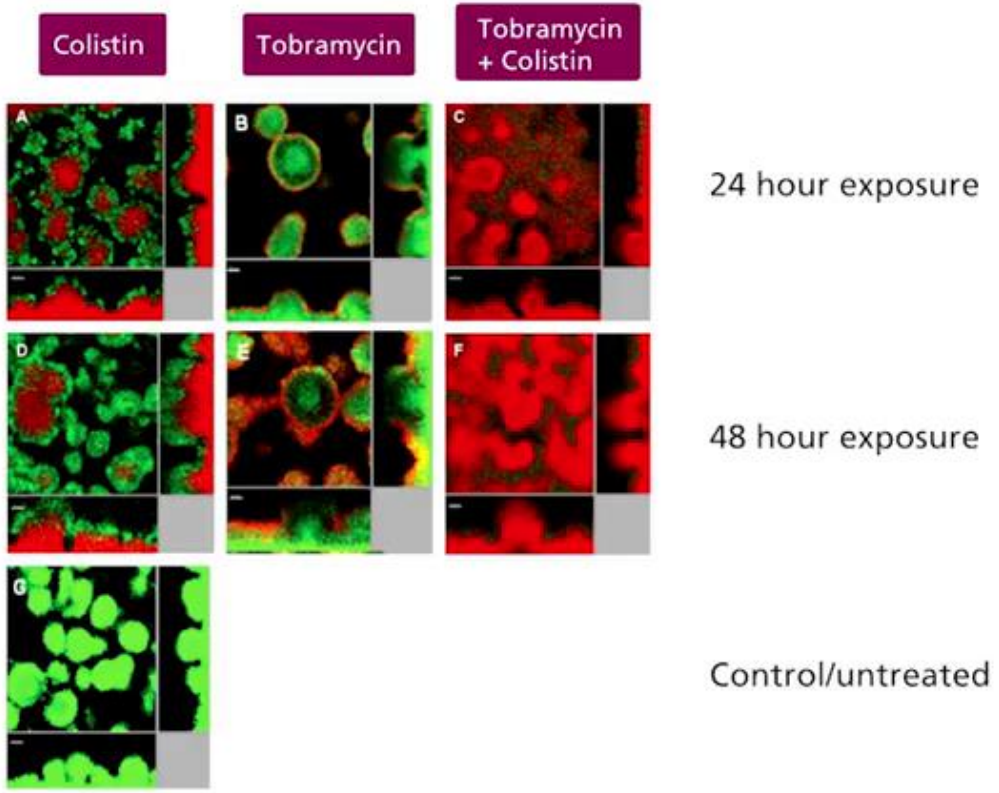

BIOFILM TOWERS (MUSHROOMS)
Immune cells
Antibiotics

BIOFILMS HAVE BEEN FOUND



Herrmann, G. et al., J Infect Dis. 2010 Nov 15;202(10):1585-92

COLISTIN COMBINED WITH TOBRAMYCIN



*nephrotoxic last resort antibiotic used in Cystic Fibrosis infections

Killed cells in the biofilm are red. Colistin* kills the anaerobes at the center of the biofilm, but leaves the metabolically active aerobes at the surface intact, and the biofilm is completely restored. Tobramycin kills the aerobes, but leaves the anaerobes intake. The combination can kill the biofilm.

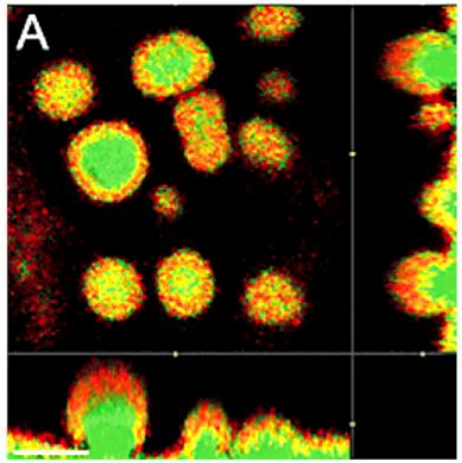
Pamp SJ et al. Mol Microbiol. 2008 Apr;68(1):223-40

CIPROFLOXACIN KILLS THE METABOLICALLY ACTIVE SUBPOPULATION

Red = Dead cells

Green = alive cells

+ Ciprofloxacin

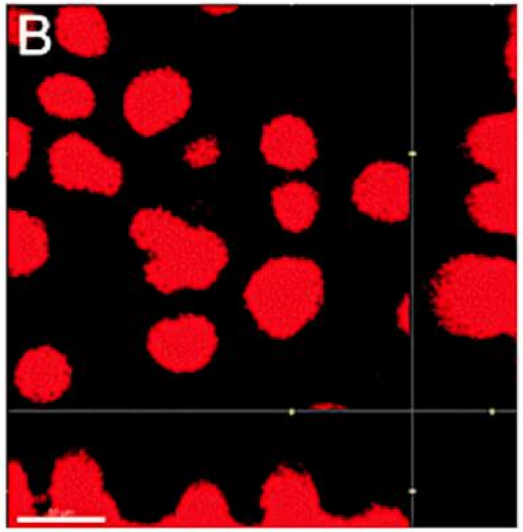


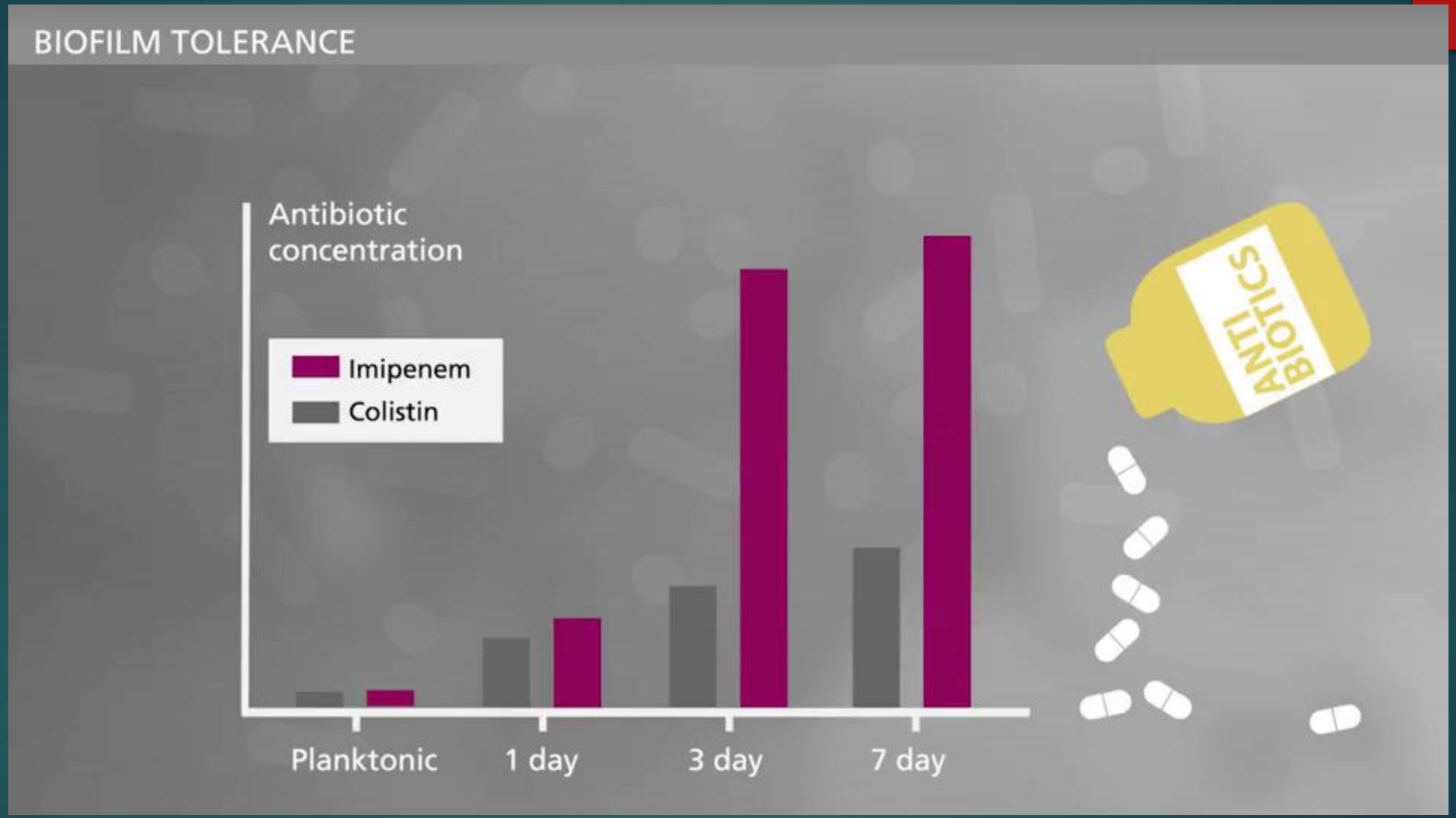
Pamp SJ et al. Mol Microbiol. 2008 Apr;68(1):223-40

CIPROFLOXACIN KILLS THE METABOLICALLY ACTIVE SUBPOPULATION

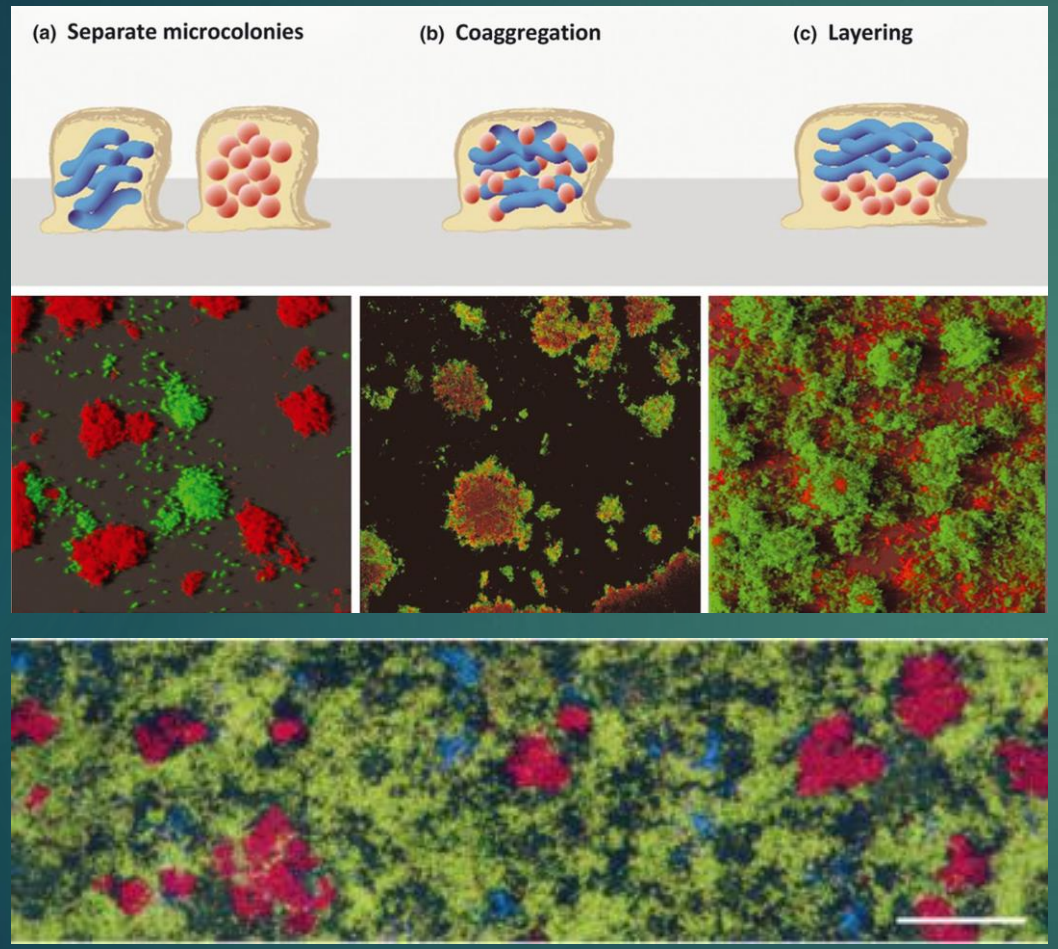
+ Ciprofloxacin

+ Colistin




BIOFILM TOLERANCE
Antibiotic concentration
Imipenem
Colistin
Planktonic
1 day
3 day
7 day
ANTI BIOTICS

Multispecies biofilms

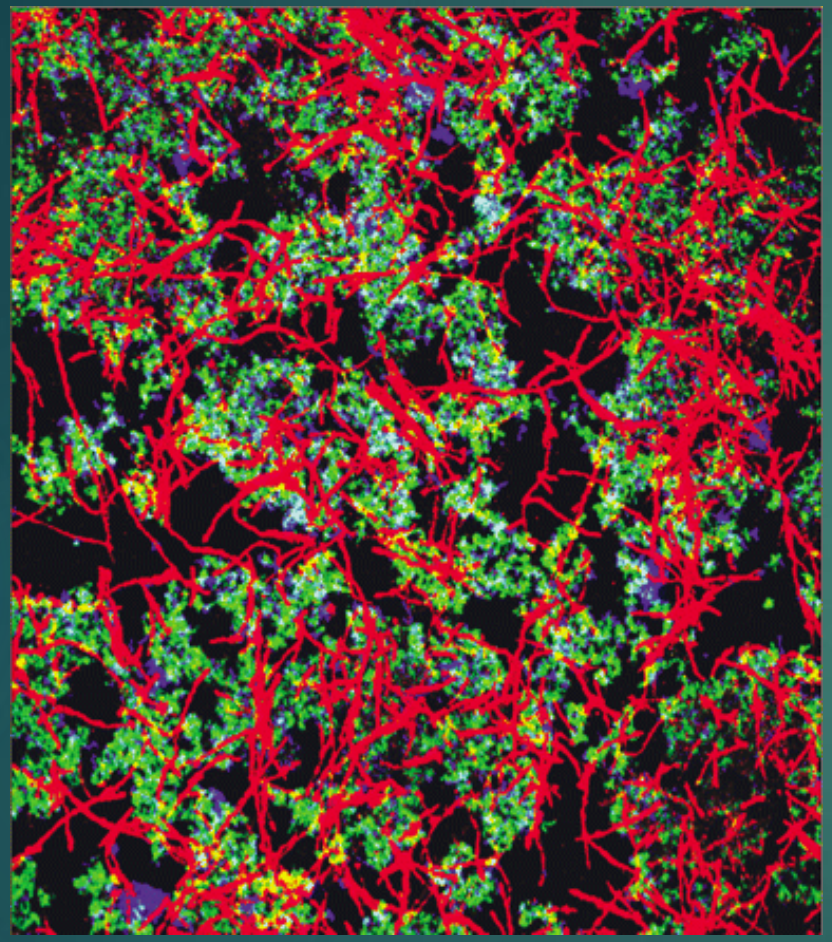


Microorganisms typically form multispecies biofilms which may also include fungi.

Below: Oral plaque is a multispecies biofilm with constantly changing and evolving components

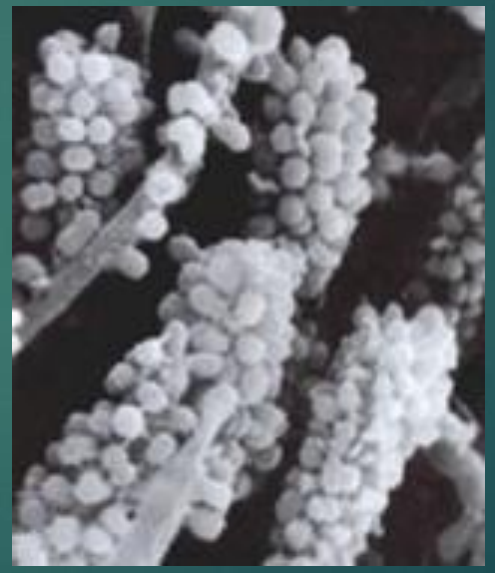
Tolerance genes are most easily spread in multi-species biofilms. Multispecies biofilms evolve in their composition and their resistance with each dose of antibiotics

Burmølle M, Webb JS, Rao D, Hansen LH, Sørensen SJ, Kjelleberg S. Enhanced biofilm formation and increased resistance to antimicrobial agents and bacterial invasion are caused by synergistic interactions in multispecies biofilms. Appl Environ Microbiol. 2006 Jun;72(6):3916-23.



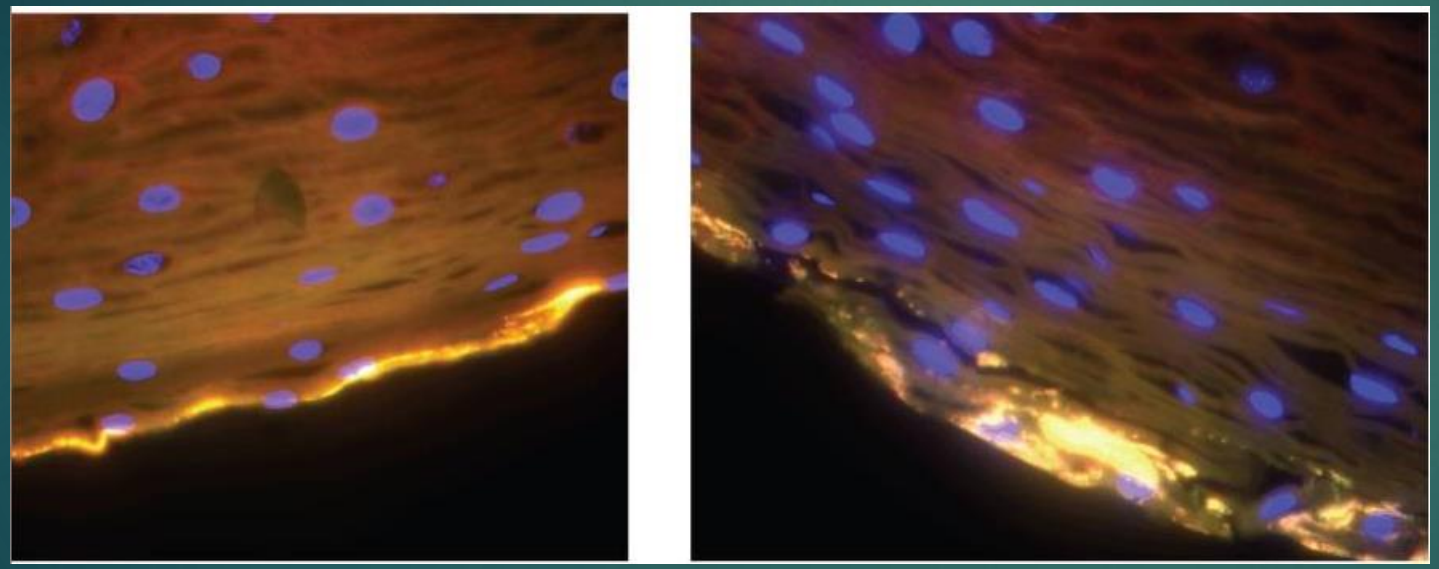
Right: A 3 species biofilm grown in saliva

Below: a “corncob” biofilm with cocci attached to bacilli



Bacterial vaginosis multispecies biofilm

"Currently, it is consensus that BV involves the presence of a dense, structured and polymicrobial biofilm, primarily constituted by *G. vaginalis* clusters, strongly adhered to the vaginal epithelium"



Red, yellow, and green hues show different species.
Blue circles are nuclear DNA from the host cells

Machado D, Castro J, Palmeira-de-Oliveira A, Martinez-de-Oliveira J, Cerca N. Bacterial Vaginosis Biofilms: Challenges to Current Therapies and Emerging Solutions. Front Microbiol. 2016 Jan 20;6:1528.

Berberine and companion alkaloids

MAY ACT AGAINST BIOFILMS BY ATTACKING BOTH AEROBES AND ANAEROBES

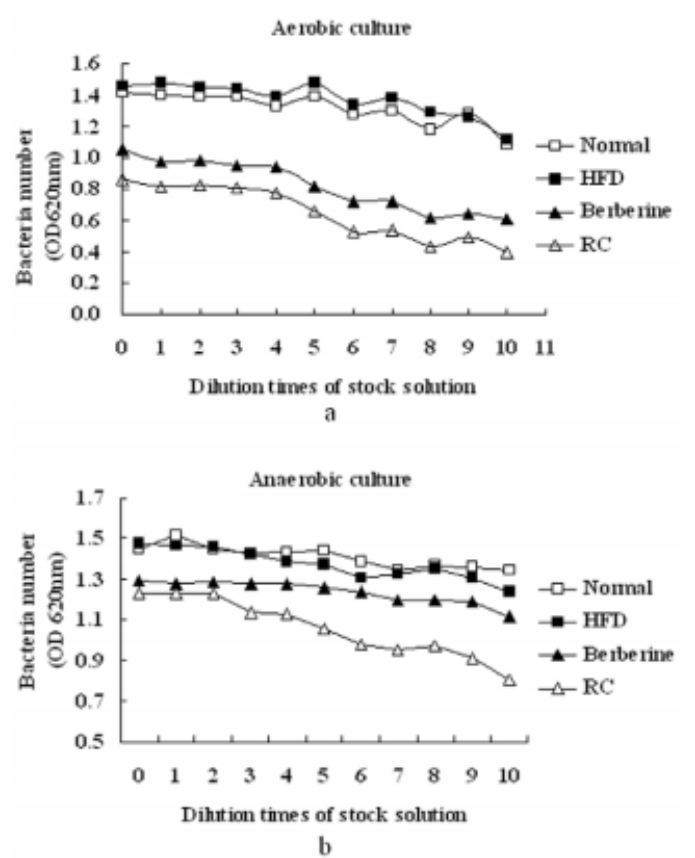


Figure 3. Effects of RC and berberine on growth of fecal bacteria ex vivo under (a) aerobic and (b) anaerobic conditions. "Normal", normal chow diet-fed mice; "HFD", high-fat diet-fed control mice; "Berberine", Berberine-treated HFD mice; "RC", Rhizoma Coptidis-treated HFD mice (n = 6).
doi:10.1371/journal.pone.0024520.g003

In this *ex vivo* trial both *Coptis* root and it constituent *berberine* significantly inhibit the growth of gut bacteria under both aerobic and anaerobic conditions. In *in vitro* trials, both RC and berberine significantly inhibit the growth of *Firmicutes* under anaerobic conditions.

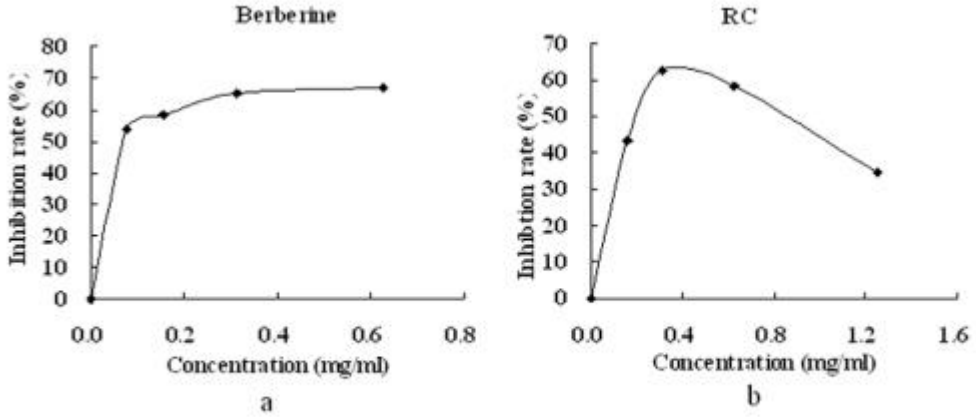


Figure 4. Effects of (a) berberine and (b) RC on the growth of *Lactobacillus* sp. under anaerobic conditions in vitro.
doi:10.1371/journal.pone.0024520.g004

Xie W, Gu D, Li J, Cui K, Zhang Y. Effects and action mechanisms of berberine and Rhizoma coptidis on gut microbes and obesity in high-fat diet-fed C57BL/6J mice. PLoS One. 2011;6(9):e24520.

The Odwalla Juice E-coli epidemic

- Odwalla juice marketing unpasteurized juices during the 1990s.
- In 1996, a batch of their apple juice because infected with pathogenic E-coli bacteria. The apple juice is a component in most of their juices.
- An epidemic followed across the American West, with cases reported in Washington State, Colorado, and California. One child died in Colorado, and 13 more were hospitalized with kidney damage.
- A number of individuals in Boulder, CO became sick. None were ever recorded in the official statistics of the epidemic.
- A tincture formula of equal parts of *Hydrastis, Mahonia, Berberis* v., and *Coptis chinensis* proved rapidly effective against a case with fever and bloody diarrhea (blood resolved after two moderate doses)

Alkaloids in some berberine-containing plants.

Most of these alkaloids have anti-microbial or other pharmacological effects in scientific trials

Alkaloid	***Hydrastis***	***Mahonia***	***Berberis***	***Coptis***
Berberine	x	x	x	x
Berbamine		x	x	
Berberastine	x			x
Berberubine			x	x
Canadine	x			
Chondocurine			x	
Columbamine			x	x
Coptisine			x	x
Epiberberine				x
Hydrastine	x			
Hydrastinine	x			
Jatrorrhizine		x	x	x
Oxicanthine			x	
Oxyacanthine		x	x	
Palmatine		x	x	x
Tetrahydroberberastine	x			

Berberine compound formula

Potential synergistic alkaloids from *Hydrastis, Mahonia, Berberis,* and *Coptis* combination

New alkaloids with each addition are marked ***bold italic*.**

The possible synergistic auxiliary compounds in each plant may also be present.

Hydrastis	***H + M***	***H + M + B***	***H + M + B + C***
Berberine	Berberine	Berberine	Berberine
Berberastine	***Berbamine***	Berbamine	Berbamine
Canadine	***Berberastine***	Berberastine	Berberastine
Hydrastine	Canadine	***Berberubine***	Berberubine
Hydrastinine	Hydrastine	Canadine	Canadine
OH-4-berberastine	Hydrastinine	***Chondocurine***	Chondocurine
	Jatrorrhizine	***Columbamine***	Columbamine
	Oxyacanthine	Hydrastine	***Coptisine***
	Palmatine	Hydrastinine	***Epiberberine***
	OH-4-berberastine	Jatrorrhizine	Hydrastine
		Oxicanthine	Hydrastinine
		Oxyacanthine	Jatrorrhizine
		Palmatine	Oxicanthine
		OH-4-berberastine	Oxyacanthine
			Palmatine
			OH-4-berberastine

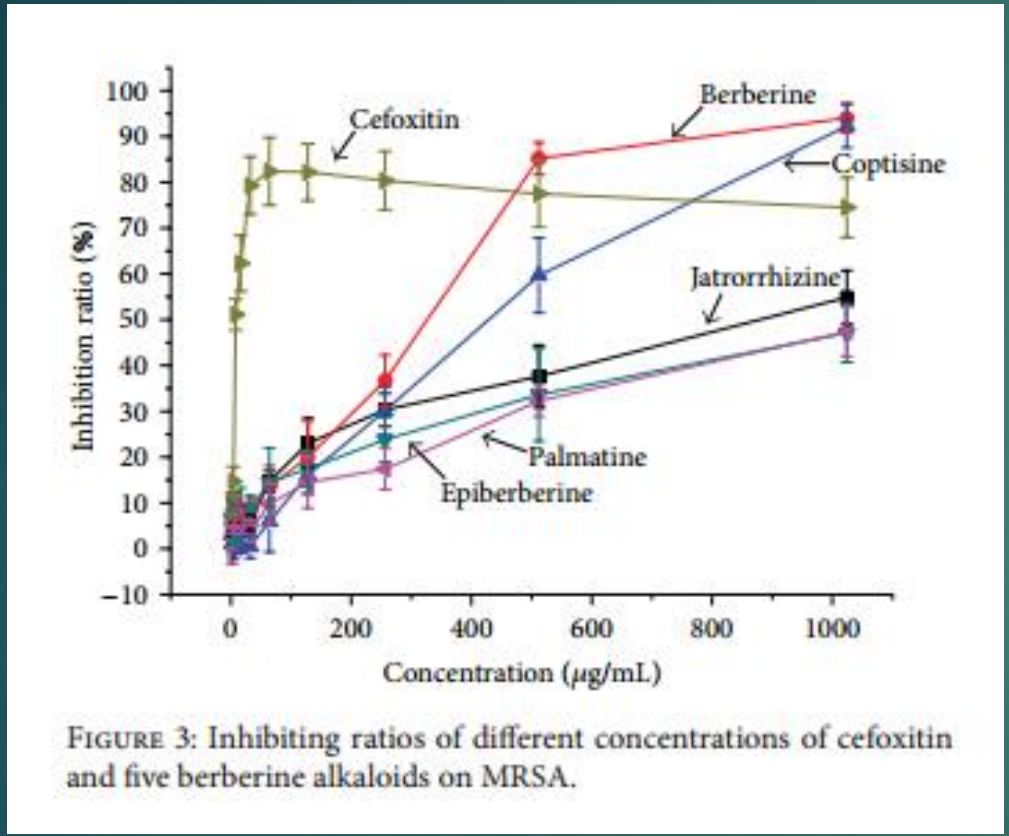


FIGURE 3: Inhibiting ratios of different concentrations of cefoxitin and five berberine alkaloids on MRSA.

Berberine and its related alkaloids common in berberine-containing plants each inhibit bacteria individually

Luo J, Yan D, Yang M, Dong X, Xiao X. Multicomponent therapeutics of berberine alkaloids. *Evid Based Complement Alternat Med*. 2013;2013:545898. doi: 10.1155/2013/545898. Epub 2013 Mar 24.

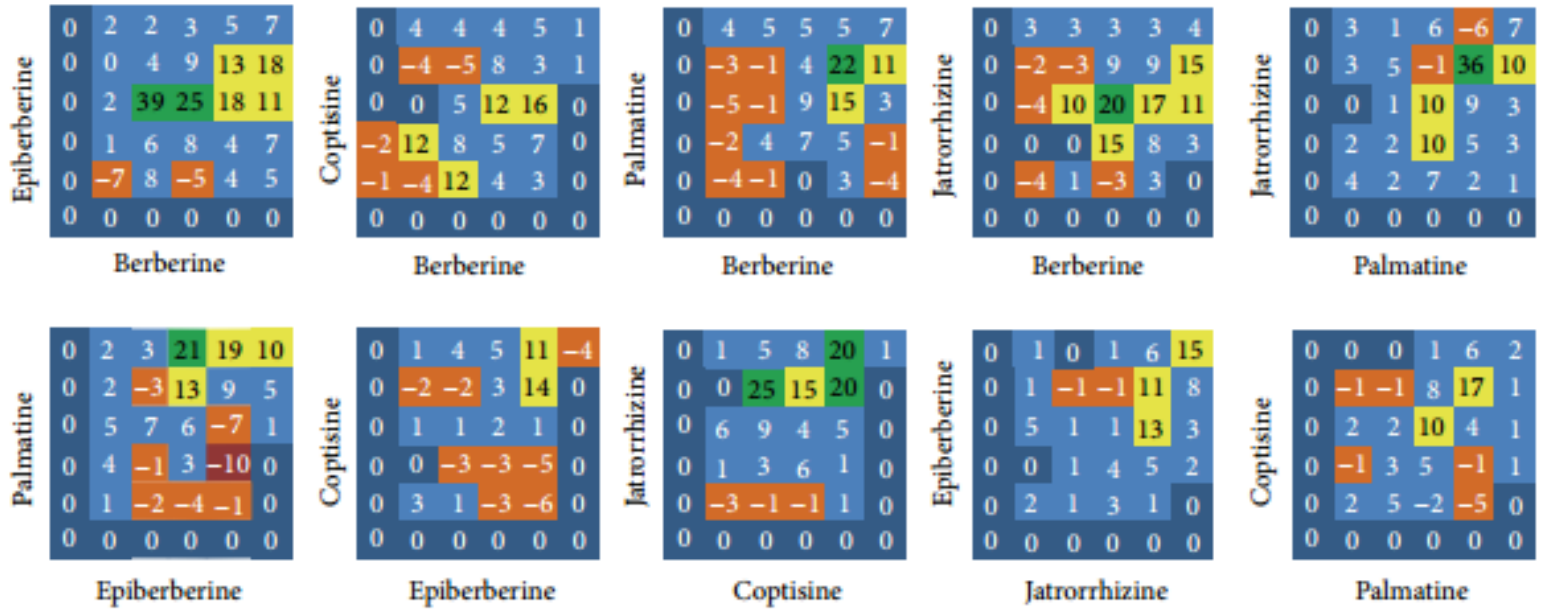


FIGURE 5: The excess over the highest single agent model, including 10 combinations of the 5 berberine alkaloids. Purple squares indicate strong antagonistic effect, orange squares indicate slight antagonistic effect, dark blue squares indicate additive effect, light blue squares indicate slight synergistic effect, brilliant yellow squares indicate strong slight synergistic effect, and green squares indicate strong synergistic effect.

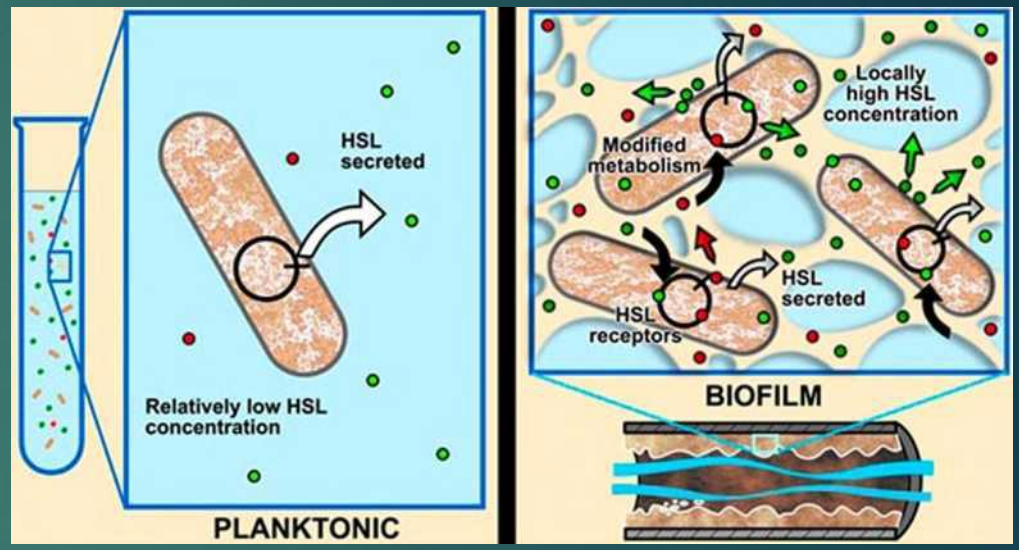
Pairs of alkaloids usually show synergistic effects against bacteria.

- The effects of internal use of berberine-containing plants on diarrhea/dysentery is not likely due to antimicrobial effects.
- Has effects throughout the gut, reducing watery secretions, secretions, bile flow, peristalsis, and also astringency.
- **Topical treatments** of biofilm-related infections with a combination of berberine-containing herbs may be more effective than a single herb.

Microbial defenses

This Biofilm will now come to order
"Quorum sensing" by bacteria

- Planktonic bacteria secrete signaling molecules.
- As the population grows, the concentration of signaling molecules rises, and bind to surface receptors on the bacteria.
- This triggers bacterial DNA activation
- Increased production of the triggering molecule
- Expression of matrix materials to form a biofilm
- Production of antibiotics to protect the colony from other bacteria, fungi, etc.
- Production of adhesion molecules
- Production of proteases and other substances enabling invasion of tissues.



PUBMED search tip:
(biofilm* OR quorum)

Some plants with anti-biofilm/quorum properties

- The discovery of the quorum-sensing property essential to formation and functioning of a biofilm has led to a research quest for plant constituents with anti-quorum or anti-biofilm properties.

Science + tradition

- *Allium*
- *Hydrastis (leaf)*
- *Commiphora myrrha*
- *Boswellia*
- *Achillea*
- *Aloe*
- *Hypericum*
- *Althaea*
- *Arctostaphylos*
- *Acalypha*
- *Quercus* and **tannins**

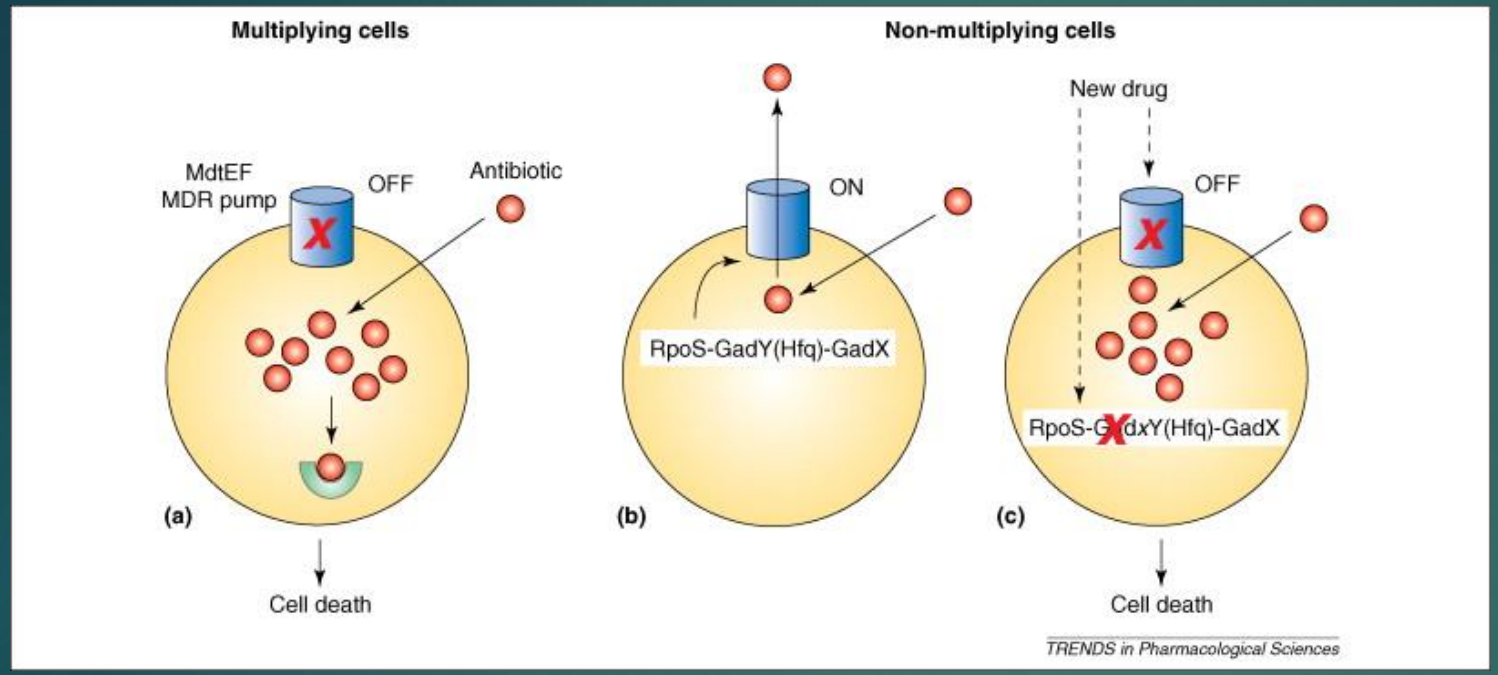
Traditional use

- *Anemopsis*
- *Larrea*
- *Baptisia*
- *Thuja*
- *Bursera*

Multiple Drug Resistant Efflux Pumps (MDR)

- Bacteria contain transporters in their membranes which actively pump harmful substances back out of the cell.
- The process is non-specific, evicting a wide variety of substances. It can result in complete inactivation of anti-biotic substances.
- MDR activity is responsible for bacterial resistance to both plant and pharmaceutical antibiotics.
- A bacterial population will evolve to contain robust MDR pump activity in response to plant or pharmaceutical antibiotics.
- Bacteria of unrelated species can acquire the MDR pump resistance genes from each other in a multispecies biofilm
- The pharmaceutical quest for MDR pump inhibitors (MDRi) has led to a flurry of research into plant compounds in the last few years.

Efflux pumps



- Efflux pumps allow microorganisms to expel many kinds of substances harmful to them.
- Genes coding for more efficient efflux pumps are part of bacterial resistance.
- Efflux pump inhibition is a potential target for antimicrobial therapy with plants or drugs.

Reviews of efflux pump structures, functions, and roles in bacteria

Zhou G, Shi QS, Huang XM, Xie XB. The Three Bacterial Lines of Defense against Antimicrobial Agents. Int J Mol Sci. 2015 Sep 9;16(9):21711-33. doi: 10.3390/ijms160921711. Review. PubMed PMID: 26370986; PubMed Central PMCID: PMC4613276.

… and structures and function common to prokaryocytes and eukaroycytes

Ughachukwu P, Unekwe P. Efflux pump-mediated resistance in chemotherapy. Ann Med Health Sci Res. 2012 Jul;2(2):191-8. doi: 10.4103/2141-9248.105671. PubMed PMID: 23439914; PubMed Central PMCID: PMC3573517.

MDR pump inhibitors in plants

- Most *isolated* plant antimicrobial substances are not effective against gram negative bacteria, due to membrane functions and MDR pumps, but the whole plants themselves may be very effective due to synergistic constituents, including MDR inhibitors.
- Addition of MDRi constituents can multiply effectiveness dramatically 100-1000x.
- Many whole plants contain MDR pump inhibitors.
- Likewise, plant materia rich in MDR pump inhibitors may be added in formula to topical preparations or other herbs.

Some plants containing MDRi

- *Hydrastis* (leaf)
- Some *Berberis* species (leaf)
- *Allium sativum*
- *Allium spp.*
- *Calendula*
- *Plantago*
- *Echinacea*
- *Artemisia spp.*
- *Hypericum*
- *Althaea*
- *Achillea*
- *Commiphora*
- *Boswellia*
- *Baptisia*
- *Arctostaphylos*
- *Arbutus*

Some widely dispersed MDRi constituents

Luteolin	**Apigenin**	**Kaempferol**	**Myricetin**
Artemisia	*Artemisia*	*Allium*	*Arctostaphylos spp.*
Echinacea	*Echinacea*	*Echinacea*	*Arbutus spp.*
Plantago	*Plantago*	*Althaea*	*Other Ericacaea*
Baptisia		*Berberis v*	
		Calendula	

Plants and biofilms

Plants can do through multiple constituents what no drug can do. These functions are essential to plant survival

- Attack microbial cell wall
- Attack microbial metabolism
- Disrupt bacterial resistance functions (MDR pumps for instance)
- Disrupt quorum sensing
- Disrupt quorum-dependent functions
- ***In humans,*** they may also stimulate local host resistance or circulation

Plant defenses against biofilms are proof of the concept of constituent-synergy in herbal medicine

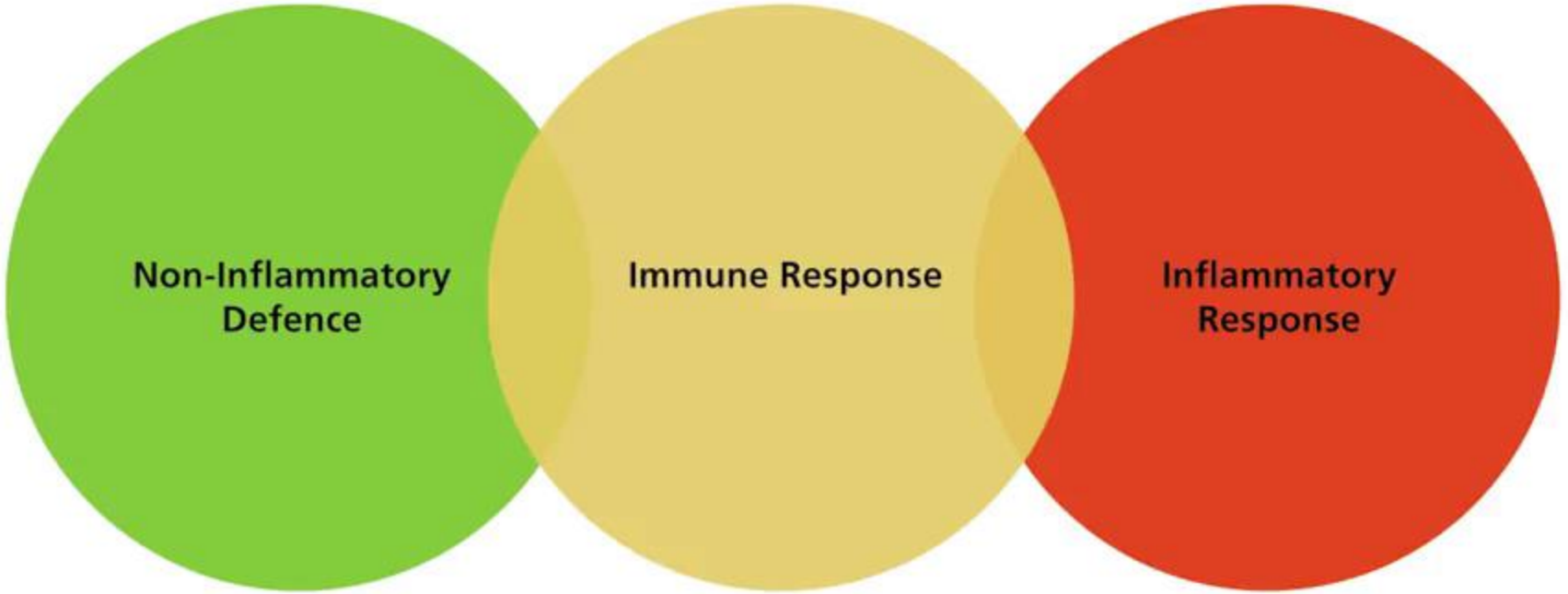
Hydrastis leaf

- Contains all the *Hydrastis* alkaloids but in lower concentration than the root.
- Contains at least 2 MDRi which ***effectively double the potency of berberine***
- ***Also*** contains anti-quorum ***and*** anti-biofilm properties unrelated to its alkaloids or the MDRi
- Sustainably grown *Hydrastis* leaf may be added in formula to almost any topical antimicrobial to improve results

Cech NB, Junio HA, Ackermann LW, Kavanaugh JS, Horswill AR. Quorum quenching and antimicrobial activity of goldenseal (Hydrastis canadensis) against methicillin-resistant Staphylococcus aureus (MRSA). Planta Med. 2012 Sep;78(14):1556-61.

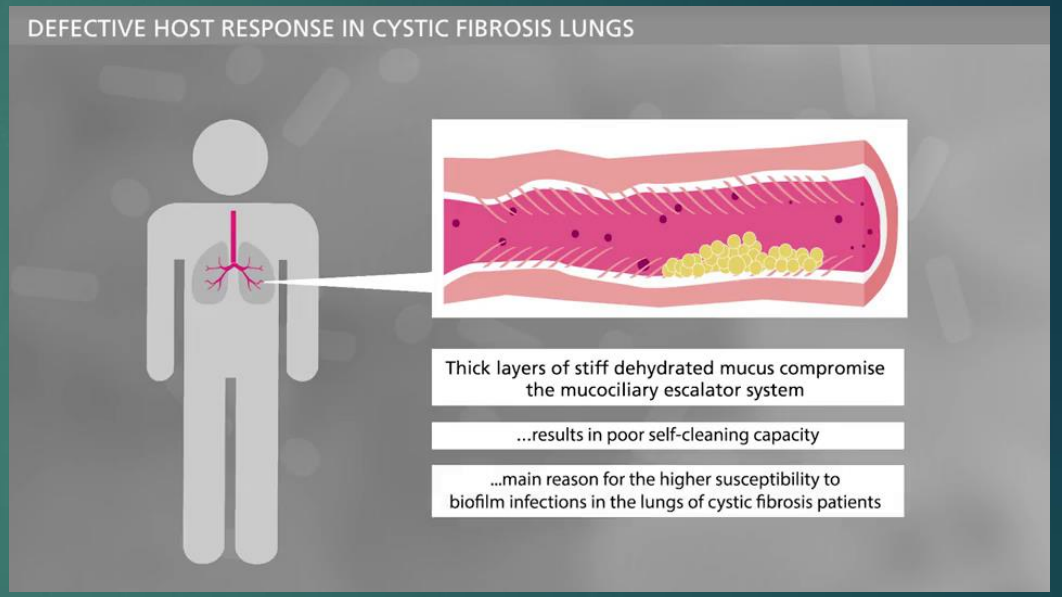
Host defense against biofilms

HOST RESPONSE TO INFECTION



Non-inflammatory defense

- Mechanisms which "self-clean" the body, especially in the skin, respiratory, and urinary tracts.
- Action of cilia
- Urinary flow
- Eustachian tube function
- Circulation to skin.

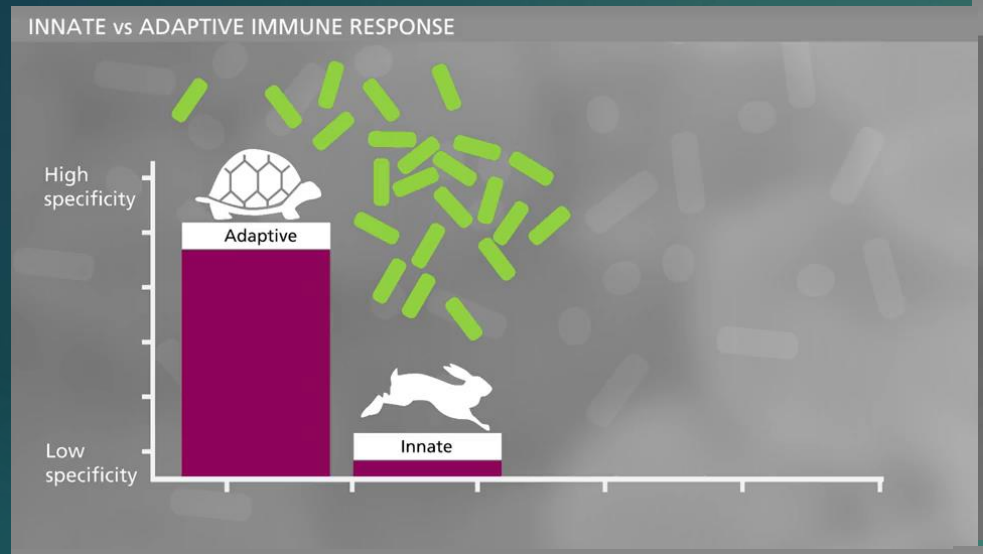


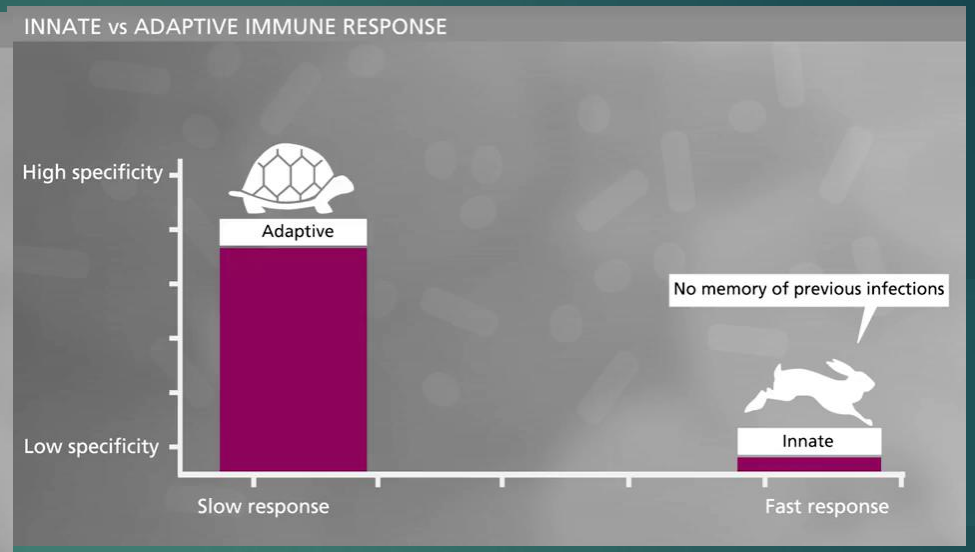
Small intestine

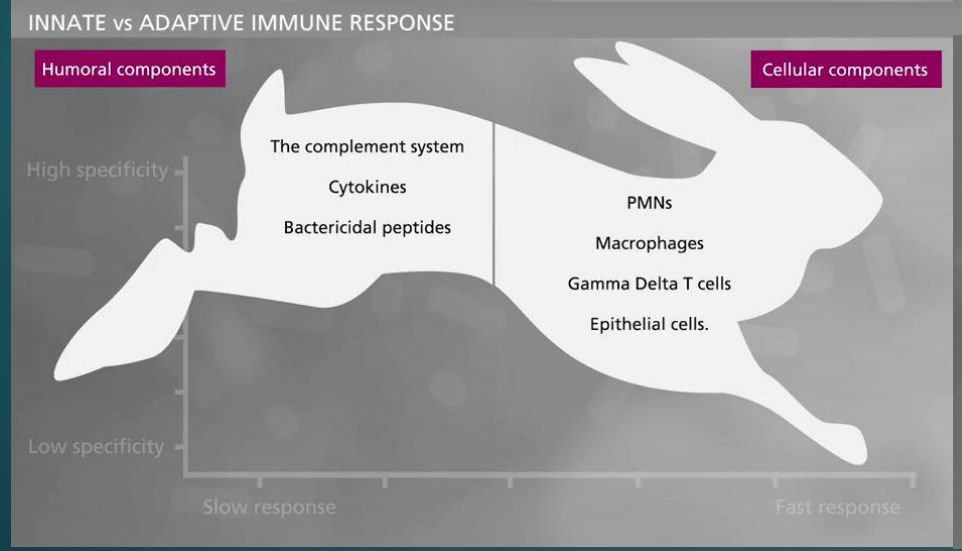
Bacteria count and biofilm formation is kept low by non-immune factors

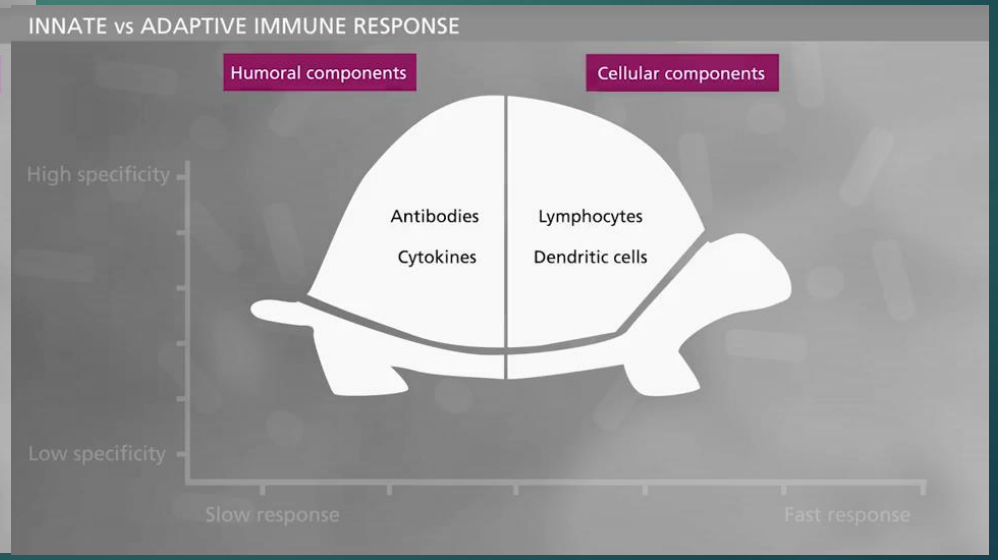
- Gastric acidity that kills ingested organisms
- Antimicrobial peptides secreted by epithelial cells
- Propulsive intestinal motility producing a fast transit time of the small intestine (about 2 hours).
- Long-chain fatty acids in conjugated bile.
- Preview: overgrowth of bacteria and/or biofilms in the upper GI may be due to loss of these factors in a hypochlorhydria/amotility syndrome

Immune responses









Polymorphal nuclear leukocytes

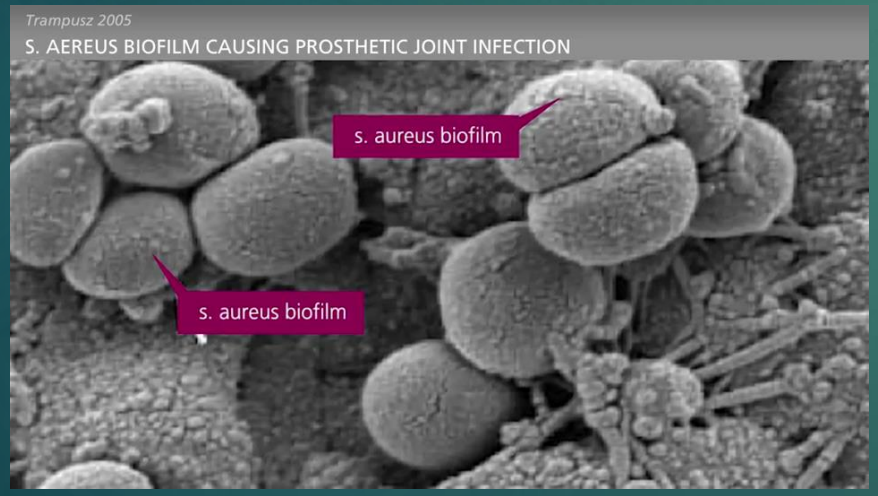
In the host response to chronic biofilms, the cellular components normally present only during the acute phase of the innate immune system are chronically activated, especially ***Polymorphal nuclear leukocytes (PMN).*** This ***chronic activation of an acute response*** can result in tissue inflammation and damage.

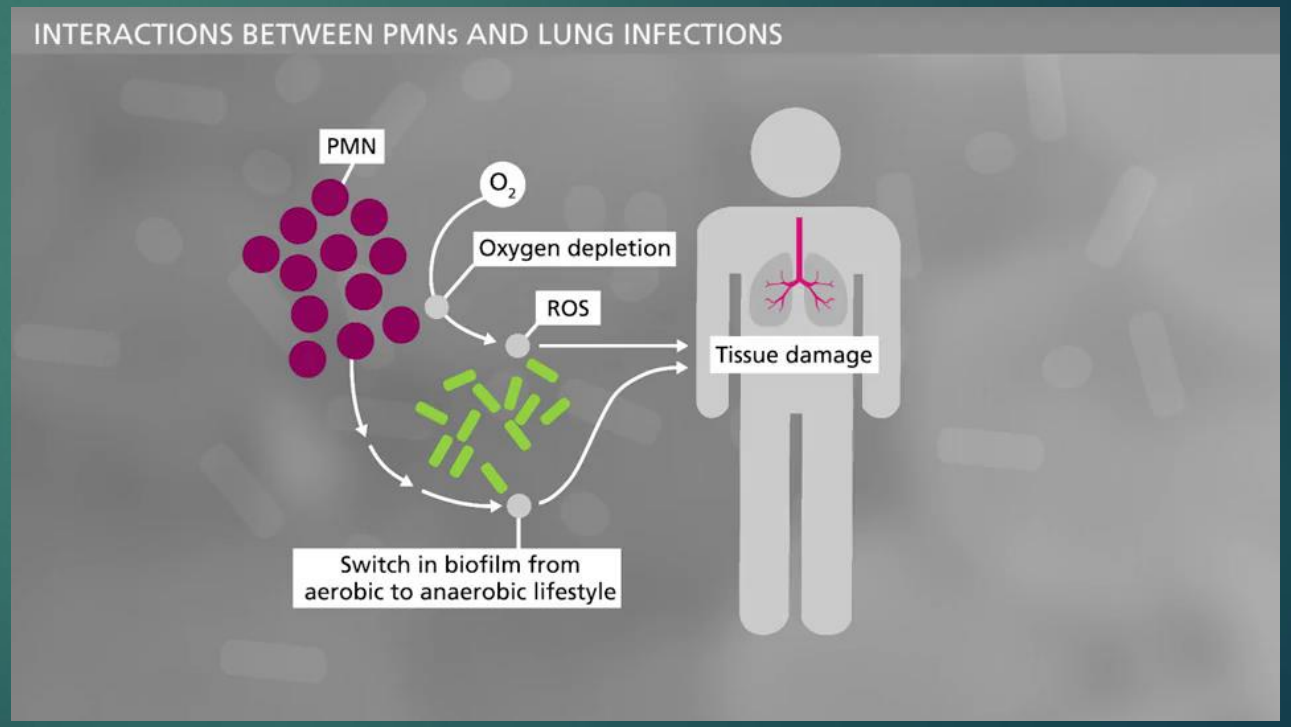
PMN: Neutrophils, Eosinophils, Basophils, Mast Cells

Polymorphal nuclear leukocytes (PMN)

The biofilm protects bacteria from otherwise bactericidal PMNs.

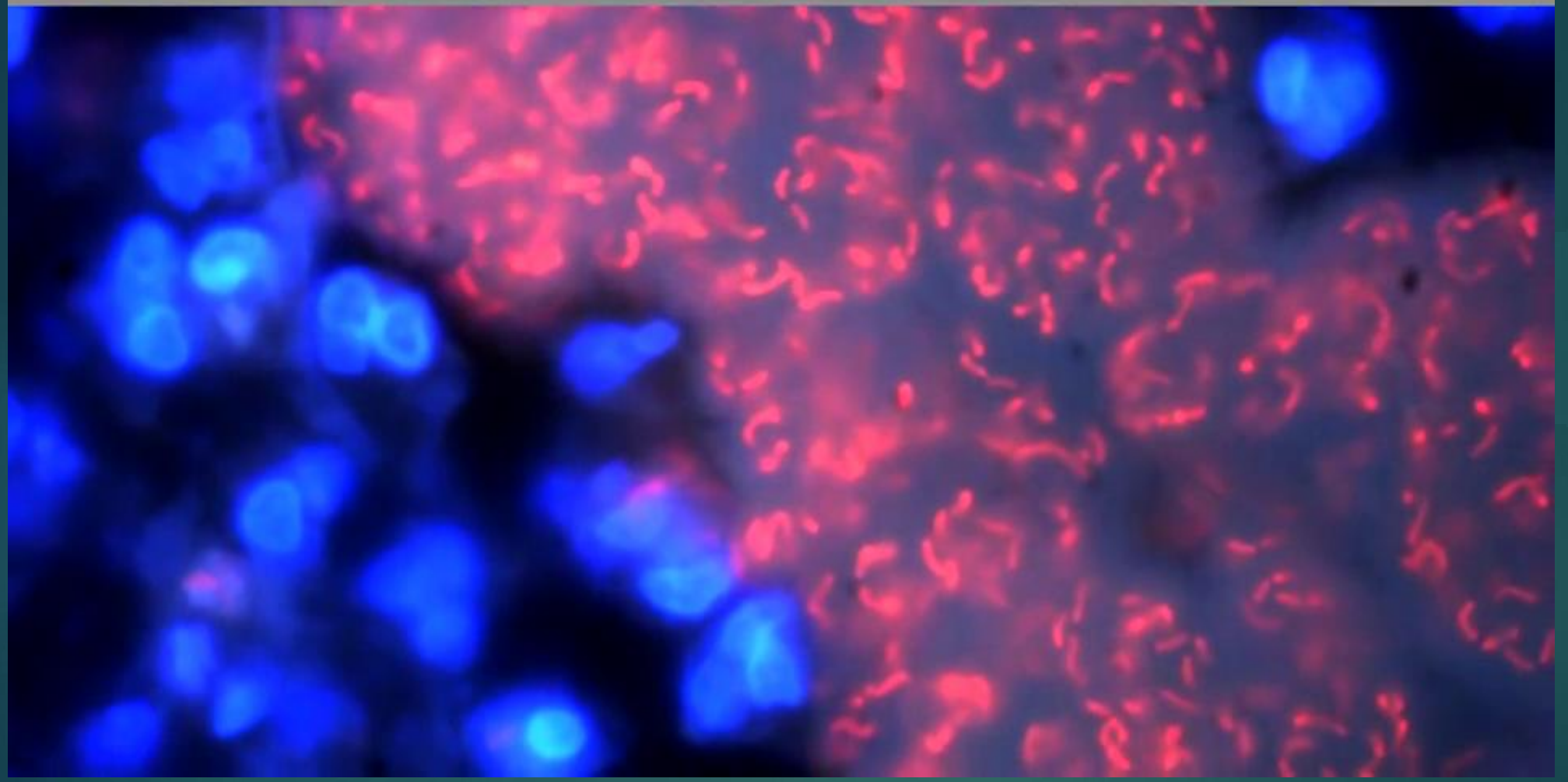
Oxidative bursts from the PMN ***damage the tissues*** around the biofilm and produce inflammation.





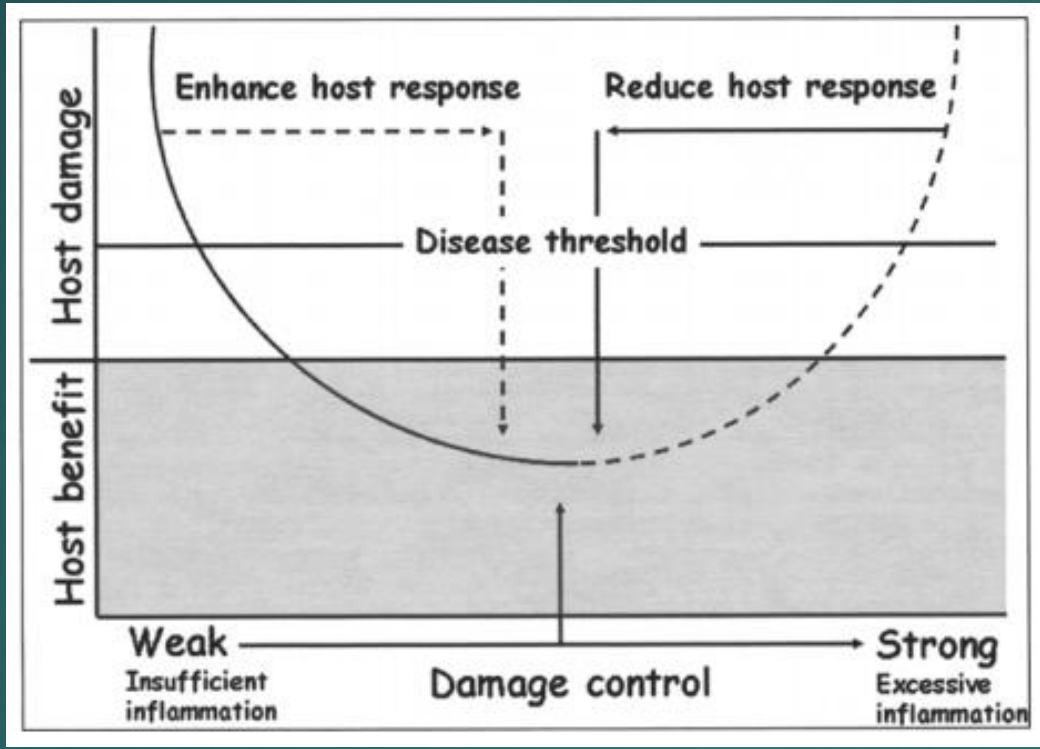
Bjarnsholt et al., Pediatr Pulmonol 2009

ENDOBRONCHIAL MICROGRAPH FROM INFECTED CF LUNG



PMN stained in blue surround the biofilm. Their oxidative bursts can damage tissues.

Damage-response model of infection



Pirofski LA, Casadevall A. The damage-response framework of microbial pathogenesis and infectious diseases. Adv Exp Med Biol. 2008;635:135-46.

The “Biofilm Complex”

- Planktonic microorganisms.
- A biofilm matrix with evolved or enhanced quorum-dependent functions
- Active resistance to antimicrobial substances through efflux pumps.
- A continuous and ongoing evolution of resistance to host and antimicrobials through robust exchange of resistance genes
- Damage to the tissues through invasion, toxins, or other quorum-dependent activities
- A strong but ineffective active immune response which may further damage the tissues through non-resolving inflammation

A Plant Constituent Synergy model of therapeutics for the chronic biofilm complex

Damage-response therapeutics

A synergy model for multi-constituent topical applications

Antimicrobial Effects

Direct antimicrobial effects
MDR pump inhibition
Anti-quorum effects

Enhance immunity

Increase local circulation
Enhance local immunity
Support systemic immunity

Reduce damage

Modify local inflammation
Repair local tissue damage

These properties are all possessed by some single plants, and with some simple plant combinations. Formulas combining "superstar" plants in each category may be extremely potent for topical use.

The constituent synergy model requires that all constituents come into contact with the biofilm simultaneously in a manner similar to plant defense.

May be applied in different topical contexts: skin, ear, mouth, throat, sinus, stomach, (duodenum?), vagina.

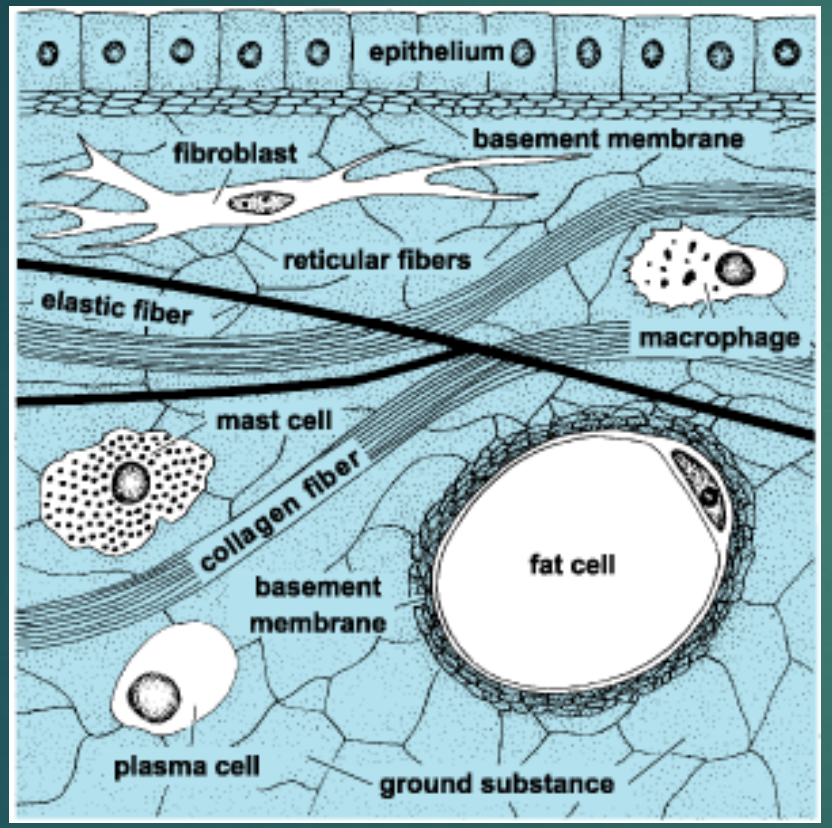
Application below the duodenum is questionable due to digestion/degradation of constituents.

Application for possible internal biofilms is unlikely, due to pass through gut and liver and unlikely parallel pharmacokinetics of all the constituents at the same time.

Topical applications

- The constituent synergy model requires that all constituents come into contact with the biofilm simultaneously in a manner similar to plant defense.
- May be applied in different topical contexts: skin, ear, mouth, throat, sinus, stomach, (duodenum?), vagina.
- Plants may be combined for multiple effects
- Plants may be delivered in media with anti-biofilm effects
- Application below the duodenum is questionable due to digestion/degradation of constituents.
- Application for possible internal biofilms is unlikely, due to pass through gut and liver and unlikely parallel pharmacokinetics of all the constituents at the same time.

Immunity and healing in the dermis



- Circulation can increase or decrease from external or internal (or herbal) stimuli
- The immune-cell-rich dermis is semi-independent of the larger immune system, and can be regulated or stimulated by local factors, including herbal applications.
- Collagen and elastin forming fibrocytes circulate in the system in the same manner as white blood cells, and can migrate into an injured or inflamed dermis to produce healing and **scarring.**

Potential synergistic actions against the biofilm complex

	Anti-inflammatory	Vulnerary	Antiseptic	Anti-biofilm	MRDi	Local Immunity
Calendula	x	x	x		x	x
Plantago	x	x	x	x	x	x
Hypericum	x	x	x	x	x	x
Echinacea	x	x	x		x	x
Althaea	x	x	x	x	x	x

Infused oils: Olive oil also has wound healing and anti-inflammatory effects
Echinacea wash from decoction of 1 ounce per liter for 40 minutes.
Echinacea wash from tincture 1 part Echinacea to 3-6 parts water.

Some cautions

- Caution in applying herbs with strong wound healing effects to suppurating wounds. Potential to "seal in" a biofilm and produce septicemia.
- Case: A man applied comfrey poultices to an extensive burn on his hand. This resulted in severe infection of the hands, swollen nodes in the armpit, and fever from septicemia.
- Caution in applying topical herbs in salve form (with wax) to an infection, even if the herbs may be antimicrobial. May create anaerobic environment.
- Case: A young man with fungal infection in pubic hair region shaved the hair and applied a salve. The bacteria flourished in the anaerobic environment and entered the body through the micro-tears. Result: nearly a week in the hospital on IV antibiotics. Systemic infection with both staph and strep.

Herbs with synergistic effects against biofilms

	Anti septic	Immune	Anti Biofilm	MRDi
Larrea	x	x	trad	
Thuja	x	x	trad	
Anemopsis	x		trad	
Baptisia	x	x	trad	x
Hypericum	x	x	science	x
Althaea	x	x	science	x

	Antiseptic	Immune	Biofilm	MRDi
Aloe	x		science	(-)
Commiphora	x	x	science	x
Boswellia	x	x	science	x
Allium	x	x	science	x
Hydrastis	x		science	x
Achillea	x		science	x

Stimulate local circulation

	Stimulant	Antiseptic	Immunity	Biofilm	MDRi
Thuja	x	x	x	trad	
Anemopsis	x	x		trad	
Myrica	x	x	x	trad	
Baptisia	x	x	x	trad	x
Commiphora	x	x	x	yes	x
Achillea	x	x		yes	x
Capsicum	x	x			
Arnica	x	x	x	yes	

Some historical combinations

Garden variety infused topical oil

	cool	Anti-inflammatory	Vulnerary	Antiseptic	Anti biofilm	MRDi	Local Immunity
Calendula	x	x	x	x		x	x
Plantago	x	x	x	x	x	x	x
Hypericum	x	x	x	x	x	x	x

Samuel Thomson's Number Six

	Stimulant	Anti inflammatory	Antiseptic	Immunity	Biofilm	MDRi	Vulnerary
Commiphora	x	X	x	x	x	x	
Capsicum	xxx		x				
Echinacea		X	x	x		x	x

- "Rheumatic drops" taken internally, topical antiseptic, throat spray
- Externally: "The most powerful antiseptic known, and is on that account highly serviceable in all putrid affections whatever"
- Used as surgical disinfectant with simultaneous internal immune stimulation by the later Physiomedicalists (post germ-theory)
- RS Clymer later recommended substitution of Echinacea for Capsicum in the formula. Can use all three in suitable proportions

A classical pair

	Stimulant	Anti-inflammatory	Antiseptic	Immune	Biofilm	MDRi
Hydrastis			x		x	x
Myrrh	x	x	x	x	x	x

Traditionally used for oral infections and non-healing wounds

Hydrastis and Myrrh

- Topical wash for infection
- Antibacterial, antiviral, antifungal
- Spray for sore throat
- Gum disease
- Topical for gastric mucosa
- Powerful systemic effects (mucous membrane tonic, general alterative and tonic, antimicrobial through separate mechanisms, in low dose is balanced warm, cold, moist and dry.)

Sinusitis spray

- Get a 2 ounce sinus spray bottle
- Add 1 teaspoon of glycerine. Not more.
- Add 15 drops each of *Hydrastis* and *Myrrh**. Not more.
- Fill to 2 oz with water.
- Spray into sinuses up to 4 times per day.
- Frequently will clear chronic sinusitis within 4 days.

*Original recipe called for 30 drops of *Anemopsis*

Possible combinations

		Stimulant	Anti inflammatory	Antiseptic	Immune	biofilm	MDRi
Bursera	warm	x	x	x	x	trad	?
Larrea	cool		x	x	x	trad	?
Anemopsis	warm	x	x	x	trad	trad	?

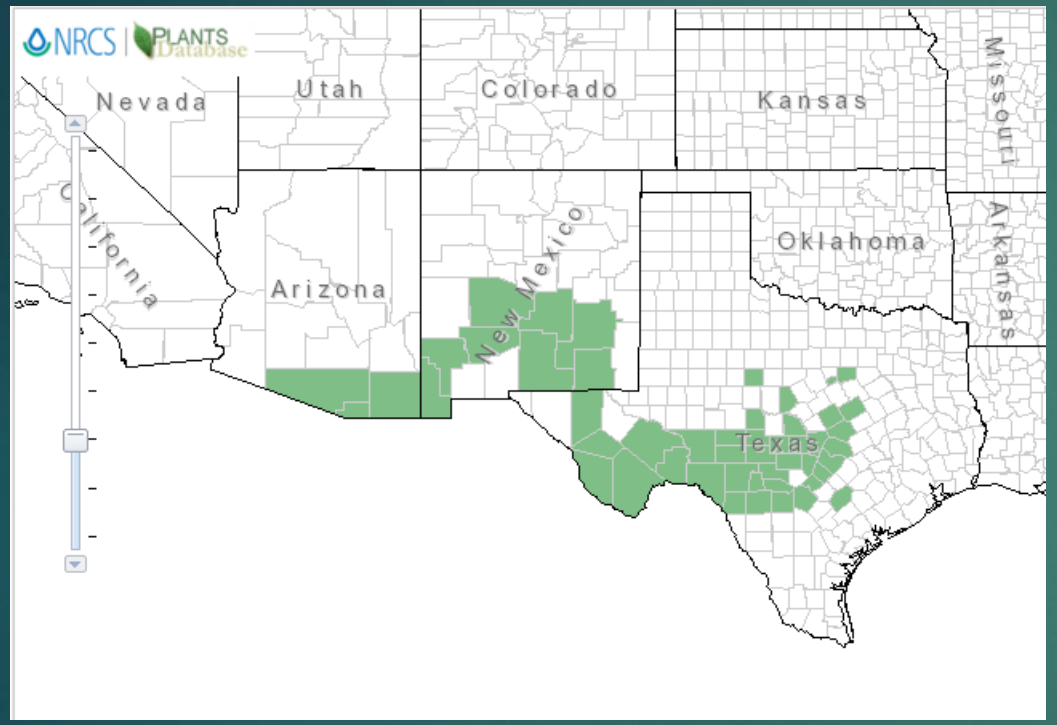
Esberitox

		Stimulant	Anti inflammatory	Vulnerary	Antiseptic	Immune	Biofilm	MDRi
Echinacea	cool		x	x	x	x	x	x
Baptisia	cold	x			x	x	trad	x
Thuja	warm	x	x		x	x	trad	

- Developed in Europe for internal use as an immune stimulant.
- A very potent topical treatment. Prepare as decoction.
- Note traditional use of Baptisia was primarily external application of the tea

Acalypha spp. Yerba del Cancer.

A universal folk remedy for wounds in Mexico



Acalypha phleoides (syn: *lindheimeri)*

A. californica

Michael Moore: "For chronic infections when nothing else has worked. "

Acalypha and Arctostaphylos

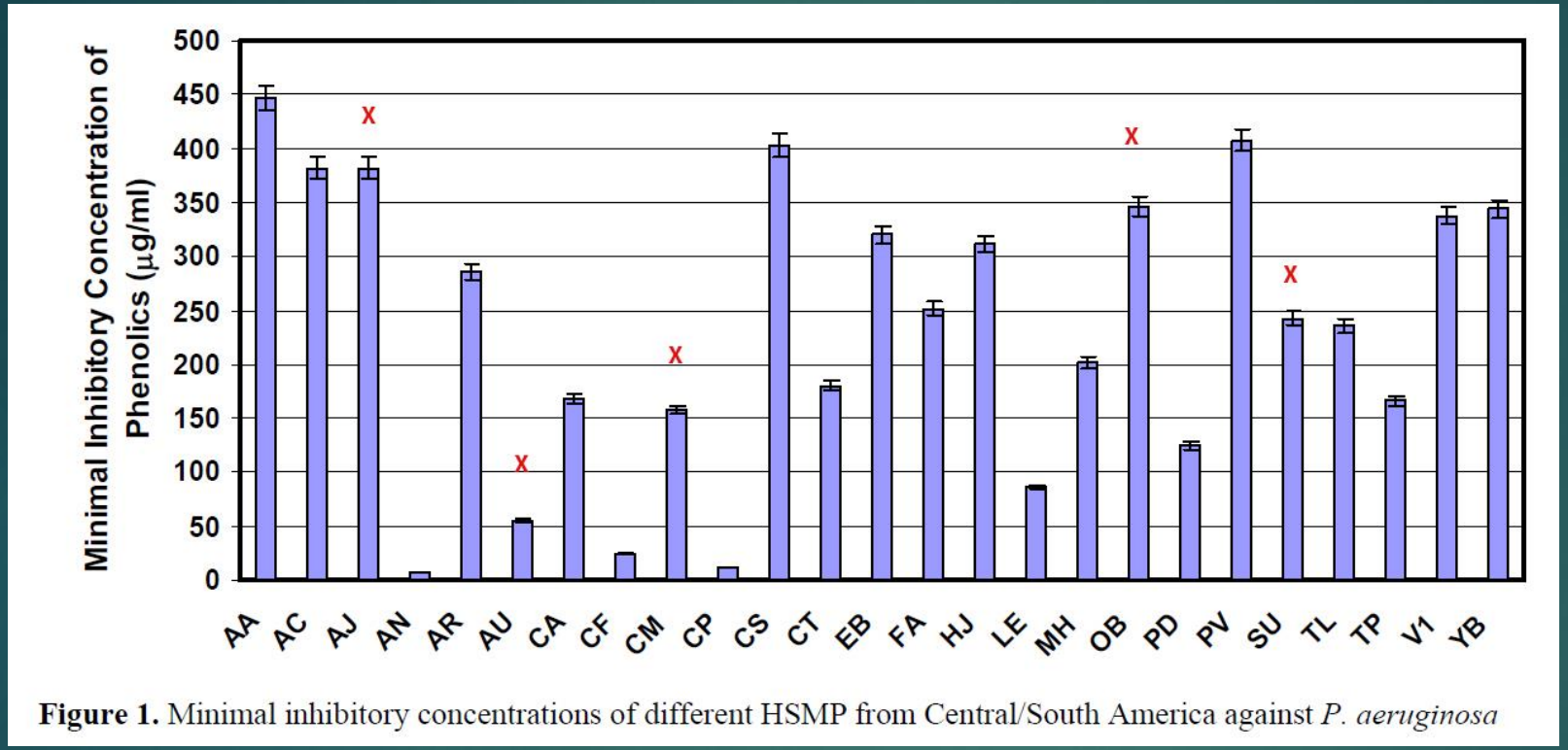


Figure 1. Minimal inhibitory concentrations of different HSMP from Central/South America against *P. aeruginosa*

Acalypha (AJ) is a relatively ***poor antimicrobial***.
Arctostaphylos u. (AU) is ***very strong***

Huerta V, Mihalik K, Crixell SH, and Vattem, DA*
Herbs, Spices and Medicinal Plants Used In Hispanic Traditional Medicine Can Decrease Quorum Sensing Dependent Virulence in *Pseudomonas aeruginosa*
International Journal of Applied Research in Natural Products
Vol. 1(2), pp. 9-15, June/July 2008

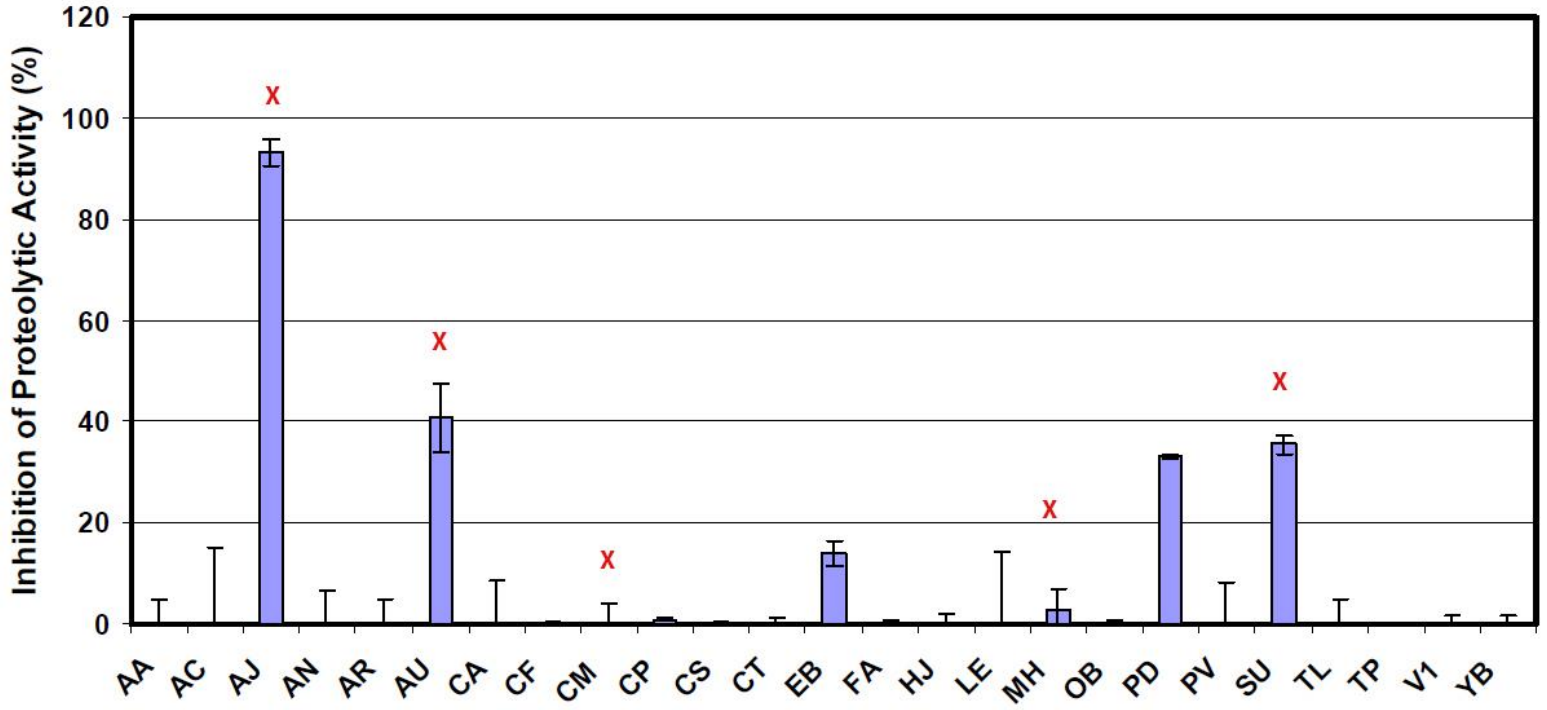


Figure 3. Effect of sub-lethal concentrations of HSMP from Central/South America on quorum sensing dependent total proteolytic enzyme acticity in *P. aeruginosa*.

Of 25 Mexican plants tested, Acalypha and Uva ursi were #1 and #2 in one measurement of anti-quorum activity Most had no activity

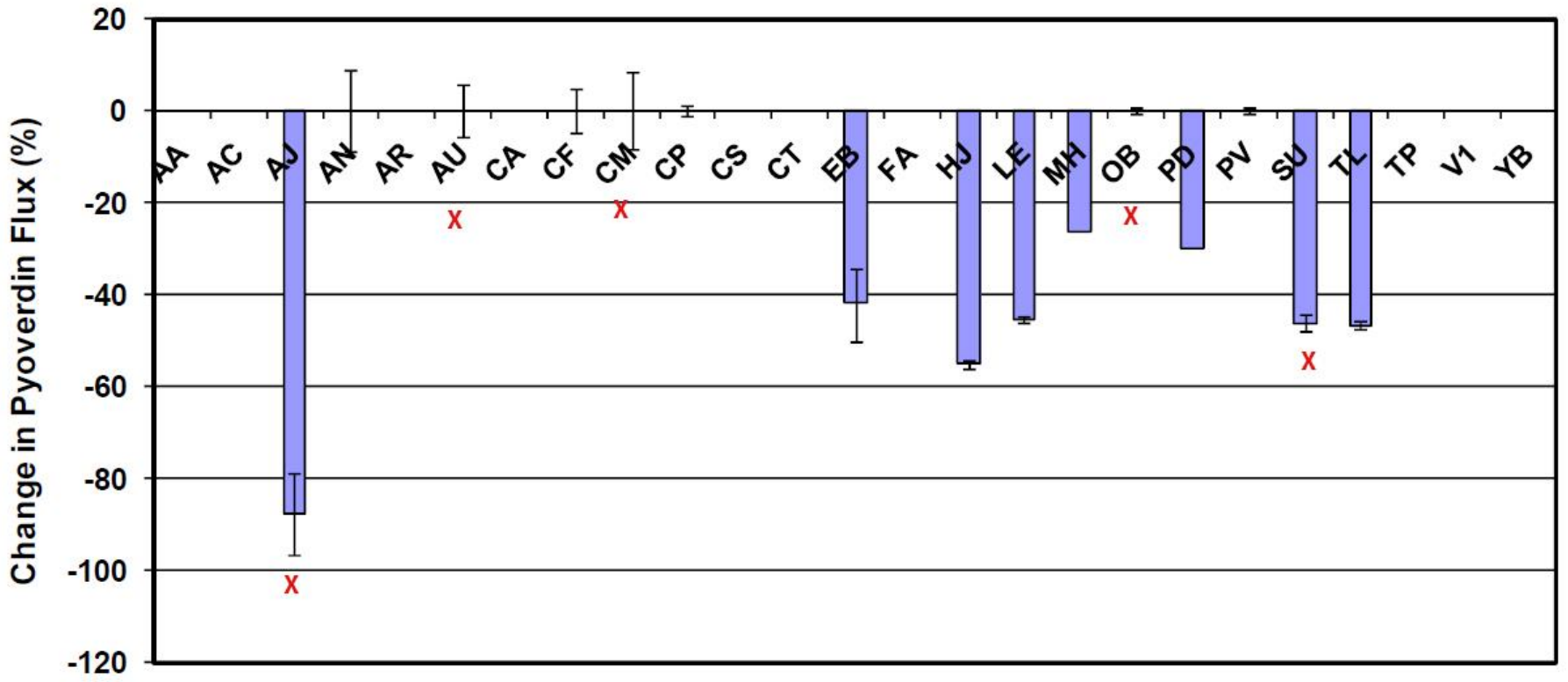


Figure 5. Effect of sub-lethal concentrations of HSMP from Central/South America on formation of pyoverdin pigment formation in *P. aeruginosa*.

In another measure of quorum sensing activity, Acalypha was #1 of the 25.

Anti-microbial and anti-biofilm effects of Uva Ursi

Table II. Anti-microbial and anti-QS activity of the screened aqueous and ethanolic plant extracts against wild-type C. violaceum *ATCC 31532 and reporter* C. violaceum *NCTC 13274 strain*

Plant species	Inhibition zone area (mm^2) against wild-type *C. violaceum* ATCC 31532 strain				Inhibition zone areas (mm^2) against reporter *C. violaceum* ACTC 13274 strain[a]			
	Growth inhibition		Pigment inhibition		Growth inhibition		Pigment inhibition	
	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
Arctostaphylos uva-ursi	235	207	126	189	207	235	153	126
Betula verrucosa	0	19	89	105	0	9	113	105
Calendula officinalis	0	0	0	19	0	0	0	31
Chelidonium majus	9	19	0	0	9	14	0	0
Comarum palustre	0	0	0	19	0	0	0	75
Eucalyptus viminalis	19	31	75	127	19	31	276	204
Inula helenium	0	0	0	35	0	0	0	85
Juniperus communis	0	0	31	31	0	0	0	19
Ledum palustre	0	31	0	19	0	19	19	25
Quercus robur	0	0	75	94	0	0	94	94
Rosa majalis	0	0	0	9	0	0	0	14
Salvia officinalis	0	31	0	0	0	25	0	0
Vaccinium vitis-idaea	31	44	0	69	31	59	83	54

[a] Supplemented with C_6-AHL (5×10^{-7} mol L^{-1}).

Achillea millefolium, Bidens tripartita, Matricaria chamomilla, Plantago major, Taraxacum fficinale, Tussilago farfara and *Viola tricolor* did not show any activity.

Tolmacheva AA, Rogozhin EA, Deryabin DG. Antibacterial and quorum sensing regulatory activities of some traditional Eastern-European medicinal plants. Acta Pharm. 2014 Jun;64(2):173-86.

Quercus species
Constituent synergy for anti-quorum properties

- Dried then rehydrated *Quercus* bark
- The whole plant had mild anti-microbial but very strong anti-quorum sensing activity.
- Ten constituents tested individually
- Two of ten showed anti-microbial and anti-quorum activity
- Five more showed anti-quorum activity without anti-microbial activity
- Only a recombination of all constituents together showed activity equal to the whole plant.

Deryabin DG, Tolmacheva AA. Antibacterial and Anti-Quorum Sensing Molecular Composition Derived from Quercus cortex (Oak bark) Extract. Molecules. 2015 Sep 17;20(9):17093-108.

Allium sativum

- Raw fresh cut garlic contains high amounts of allicin, which has broad spectrum antimicrobial and anti-biofilm effects
- Allicin breaks down rapidly once garlic is cut or crushed. Breakdown products have anti-biofilm and antimicrobial effects.
- The constituent *ajoene*, which is abundant in oil-infused garlic preparations, has a potent anti-biofilm effect.
- Half life of allicin is about 18 hours.
- Some of these non-allicin constituents may be delivered to a biofilm systemically after oral ingestion.
- ***Fresh garlic can produce second and third degree in burns***.

Allium sativum applications

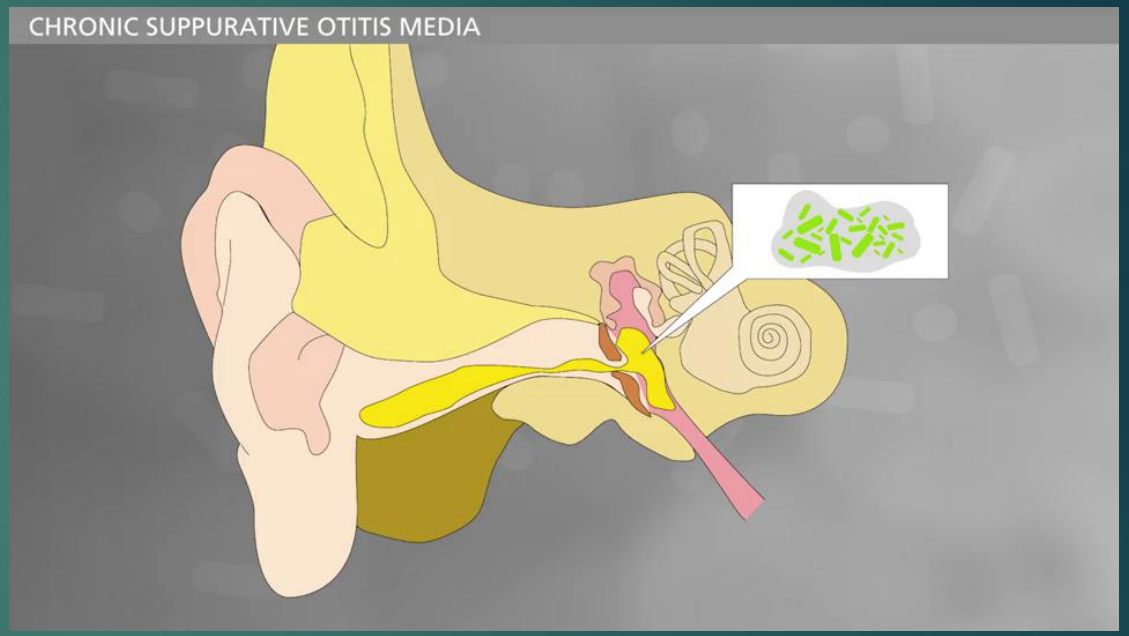
- Two cloves (not whole bulbs) in liter of water, blended and strained through cheesecloth.
- Poultice
- Foot bath or handbath.
- Mouthwash for thrush
- Douche
- Infused oil to ear

Galen's treatment for arterial wounds in gladiators

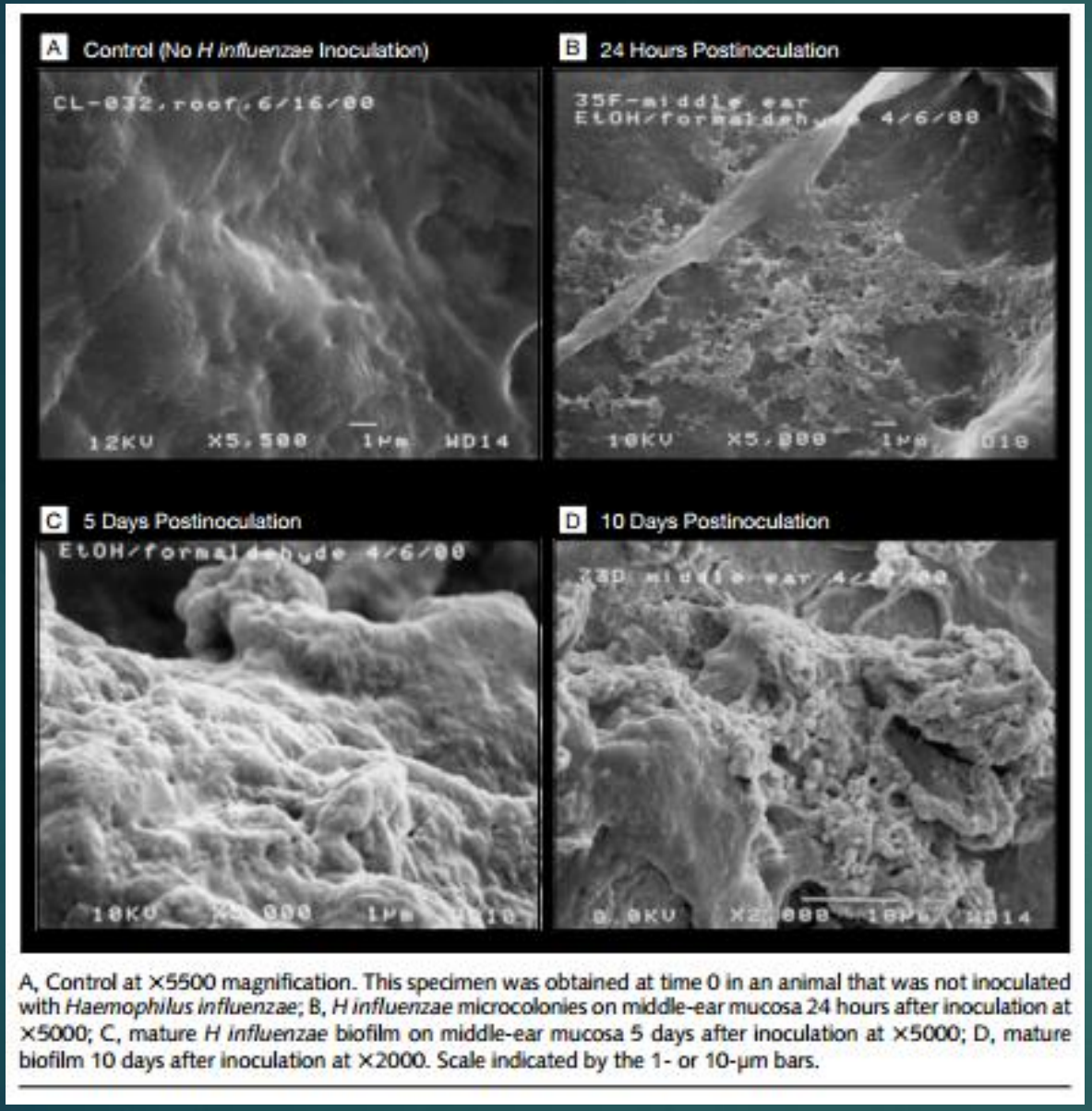
- Crush and soak garlic in wine overnight. Retains a reduced level of allicin, and adds the allicin breakdown products.
- Make a wet poultice of flour with this and pack into the wound to stop bleeding.
- Cover with a cloth, and keep moist with the garlic/wine tincture.
- Stops bleeding with the flour matrix, and prevents infection and formation of biofilm with the garlic/wine mixture.

Chronic otitis media

- Normal oral flora to form chronic biofilms in the middle ear.
- Biofilms readily detected in the exudate after eardrum rupture.
- Traditional treatment with warm infused oil of garlic.
- Antimicrobial/anti-biofilm constituents including oil-soluble *ajoene*.
- Eardrum is permeable to medications and plant constituents.
- Administration in outer ear results in expectoration in the sinuses with garlic flavor to the discharge.



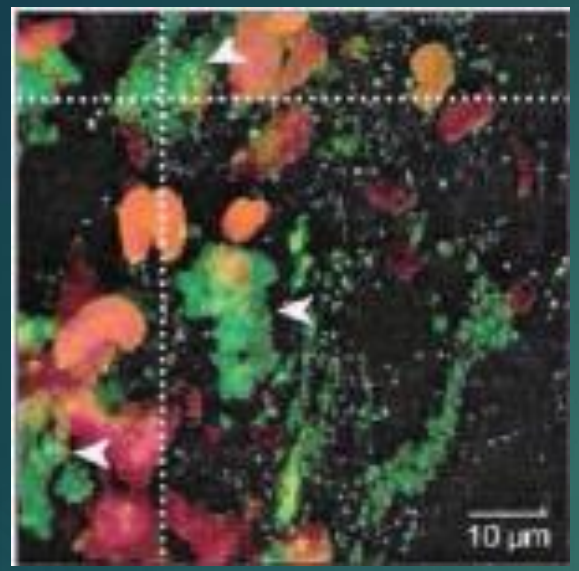
May add the astringent *Verbascum* and antimicrobial *Phytolacca* to the formula.

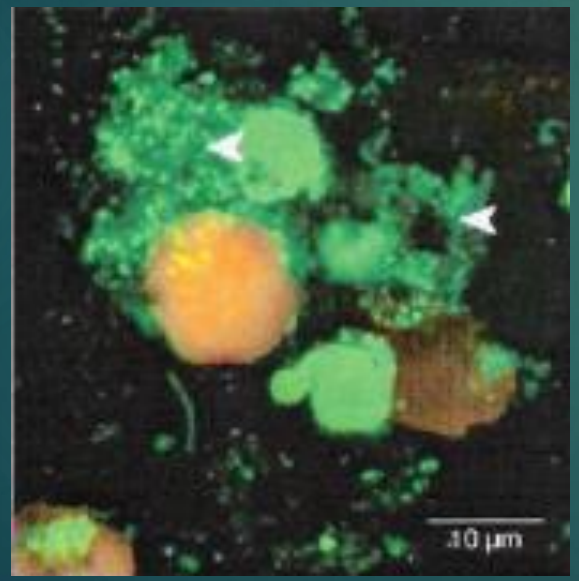


A, Control at ×5500 magnification. This specimen was obtained at time 0 in an animal that was not inoculated with *Haemophilus influenzae*; B, *H influenzae* microcolonies on middle-ear mucosa 24 hours after inoculation at ×5000; C, mature *H influenzae* biofilm on middle-ear mucosa 5 days after inoculation at ×5000; D, mature biofilm 10 days after inoculation at ×2000. Scale indicated by the 1- or 10-μm bars.

H. influenzae biofilm in middle ear biopsy In animal models on days 0, 1, 5, and 10.

Ehrlich GD, Veeh R, Wang X, Costerton JW, Hayes JD, Hu FZ, Daigle BJ, Ehrlich MD, Post JC. Mucosal biofilm formation on middle-ear mucosa in the chinchilla model of otitis media. JAMA. 2002 Apr 3;287(13):1710-5.



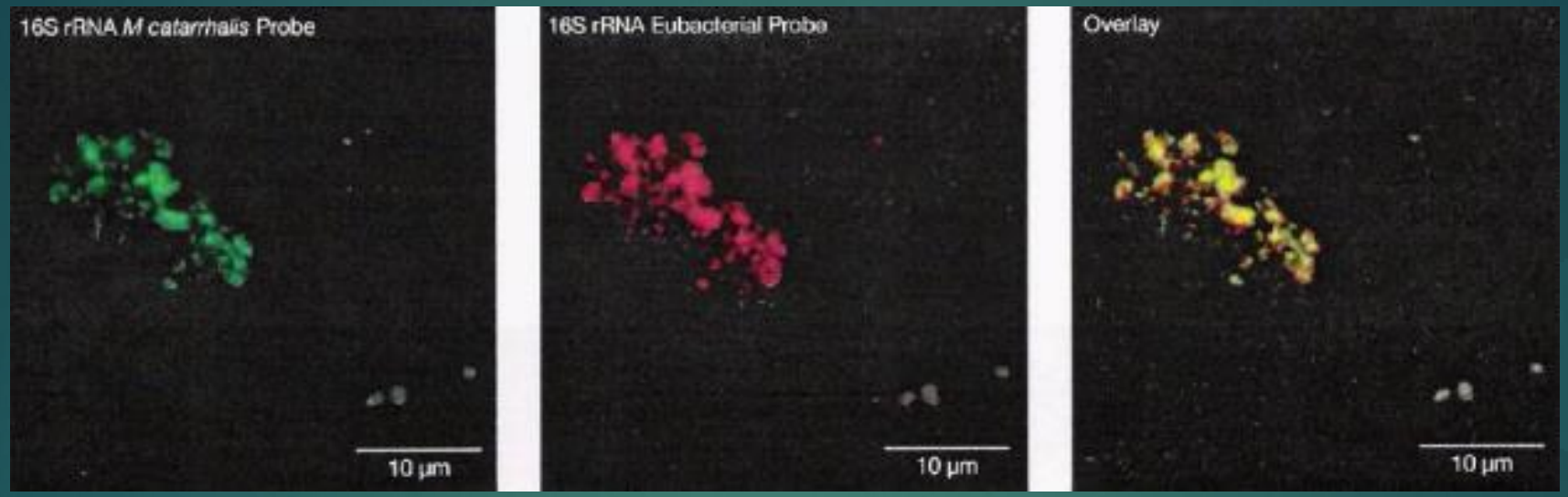


Human middle ear biopsy.
White arrows show biofilms
Green *H. influenza* biofilms from middle ear biopsy.
In both pictures the host cells are yellow, orange, or red

Hall-Stoodley L, Hu FZ, Gieseke A, Nistico L, Nguyen D, Hayes J, Forbes M, Greenberg DP, Dice B, Burrows A, Wackym PA, Stoodley P, Post JC, Ehrlich GD, Kerschner JE. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA. 2006 Jul 12;296(2):202-11.

Multispecies biofilm in middle ear, *H influenza* (L) *S pneumoniae* (M), overlay (R)

Hall-Stoodley L, Hu FZ, Gieseke A, Nistico L, Nguyen D, Hayes J, Forbes M, Greenberg DP, Dice B, Burrows A, Wackym PA, Stoodley P, Post JC, Ehrlich GD, Kerschner JE. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA. 2006 Jul 12;296(2):202-11.



Imaging probes for *Moraxella catarrhalis,* eubacteria, and their overlay. A biopsy from the middle ear of a child with otitis media with effusion

Hall-Stoodley L, Hu FZ, Gieseke A, Nistico L, Nguyen D, Hayes J, Forbes M, Greenberg DP, Dice B, Burrows A, Wackym PA, Stoodley P, Post JC, Ehrlich GD, Kerschner JE. Direct detection of bacterial biofilms on the middle-ear mucosa of children with chronic otitis media. JAMA. 2006 Jul 12;296(2):202-11.

Otitis externa

- Chronis otitis externa had the presence of biofilms in 23/25 patients.
- Results from the standard treatment with antibiotics and steroids was compared with "chemical peeling" of the biofilm and superficial epithelium of the ear canal.
- Result were similar for the first 3 months.
- At 6 months, 89% of the "peeling group" were symptom free, vs 64% of the antibiotic group
- At 9 months, the corresponding rates were 80% and 60%

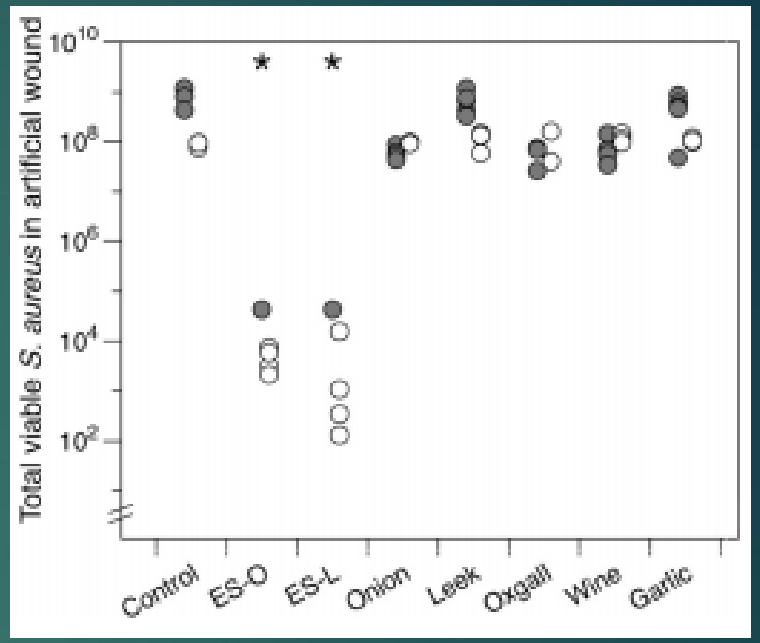
Fusconi M, Petrozza V, Taddei AR, Vinciguerra V, De Virgilio A, Chiarini F, Cirenza M, Gallinelli C, Conte M, de Vincentiis M. Is biofilm the cause of chronic otitis externa? Laryngoscope. 2011 Dec;121(12):2626-33.

Ancient formula from *Balds Leechbook**

- The recipe instructs the reader to crush garlic and a second *Allium* species (whose translation into modern English is ambiguous), combine these with wine and oxgall (bovine bile), and leave the mixture to stand in a brass or bronze vessel for 9 days and nights
- The researchers made two versions of the formula, exactly as described, with *Allium cepa* (onion) in one and *Allium ampeloprasum* (leek) in the other.
- These were tested against *Staphylococcus aureus* in both planktonic and established biofilm form in synthetic wound fluid

* Cockayne O. 1864 –1866. Leechdoms, wortcunning and starcraft: being a collection of documents, for the most part never before printed, illustrating the history of science before the Norman conquest. Rolls series 35th, 3 Vols. Longman, Green, Longman, Roberts, and Green, London, United Kingdom

- Both formulas were 100% bactericidal against planktonic bacteria.
- Both also significantly reduced the biofilm (see chart)
- *None of the elements individually had any effect on the biofilm*.
- The combination of wine, garlic, and leek demonstrated the full effect of the formula
- If onion was used instead of leek, then the bile was necessary also for the full effect of the formula (bile salts = strong antimicrobial)
- Brass had no effect, but because it is sterile, it was probably valuable in medieval times
- Take-home: ***Addition of leek or possibly onion to a topical antimicrobial garlic preparation has strong synergistic antibiofilm activity.***



Harrison F, Roberts AE, Gabrilska R, Rumbaugh KP, Lee C, Diggle SP. A 1,000-Year-Old Antimicrobial Remedy with Antistaphylococcal Activity. MBio. 2015 Aug 11;6(4):e01129.

Antimicrobial effects on biofilms

- Antimicrobials whether pharmaceutical drugs or isolated plant consitutuents, can kill or inhibit some planktonic forms of bacteria.
- Some drugs (macrolides?) or antimicrobial constituents may inhibit quorum-sensing, but generally will not eradicate an established biofilm.
- Antibiotics used on biofilms ***promote resistance genes*** which are then acquired throughout the biofilm.
- Some antibiotics and some plant consituents, when given in sub-inhibitory doses (SIC) ***trigger quorum sensing*** and biofilm formation.
- SIC doses may also promote "Darwinian" evolution of more robust metabolism or other properties of the microorganisms.

Antimicrobial and biofilm effects of herbs used in traditional Chinese medicine

- "Of the twenty-two components of tea decoctions commonly used to treat infections, only Scutellaria, Taraxacum, Tussilago and Glycyrrhiza exhibited antimicrobial activity. The activity, when present, was organism-specific, i.e., anti-Staphylococcus aureus, including anti-MRSA activity under aerobic and/or anaerobic conditions. However, with the exception of Scutellaria, ***sub-inhibitory concentrations of the herbs exhibited a pattern of inducing enhanced production of biofilm***."

Lau D, Plotkin BJ. Antimicrobial and biofilm effects of herbs used in traditional Chinese medicine. Nat Prod Commun. 2013 Nov;8(11):1617-20.

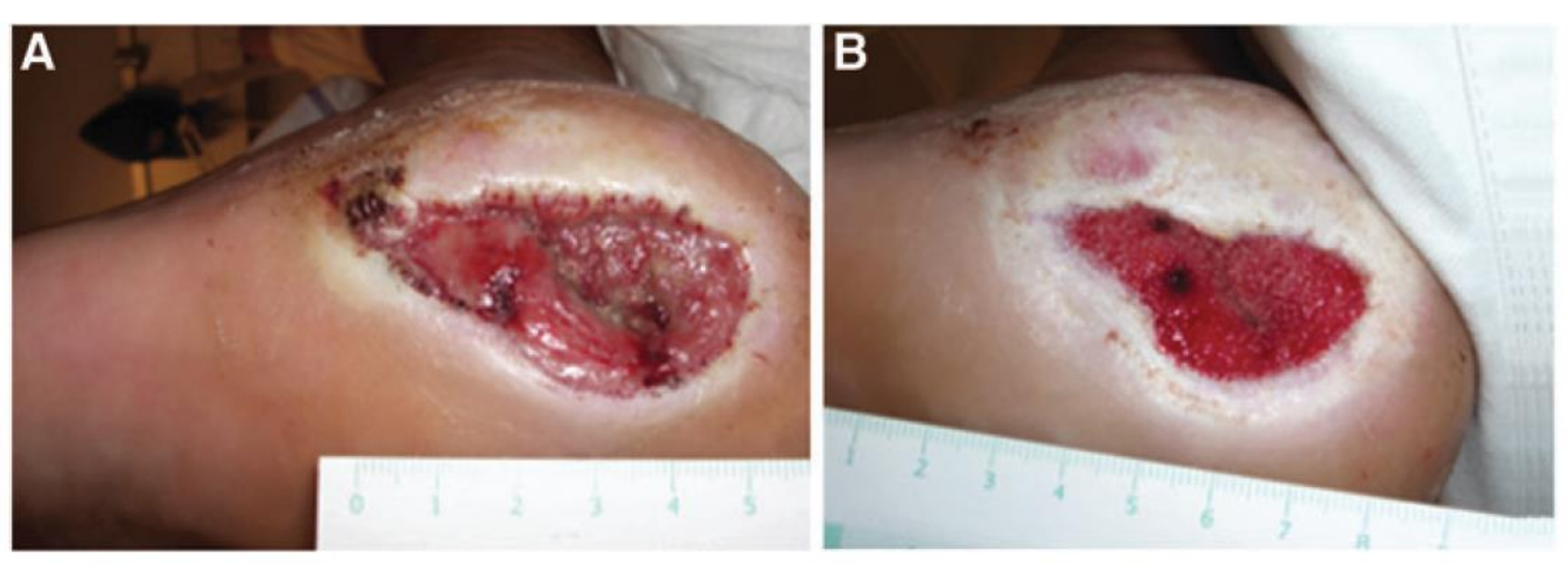
Antimicrobial effect on the microbiome

- Systemic antibiotics can promote the development of resistance in every compartment of the microbiome.
- Systemic antibiotics can force the metabolic evolution of the microbiome to produce more energy (SFCA)
  - Relationship to the fattening of farm animals with SIC of antibiotics
  - Relationship to obesity in U.S. population.
- Systemic antibiotics can reduce the diversity in every compartment of the microbiome.

Media

Vinegar and biofilms

- Acetic acid has an anti-microbial effect against established biofilms both in-vitro and in open wounds.
- It is effective for 100% eradication of established *P. aeruginosa* and *S. aureus* at a concentration of 1% acetic acid.
- The anti-biofilm effect is not due to pH value of the bacteria, because HCl at the same pH has no effect.
- The effect is due to the ***acetic acid molecule*** itself.
- Application six times a day for twenty minutes on non-healing diabetic ulcers. (See following slides)



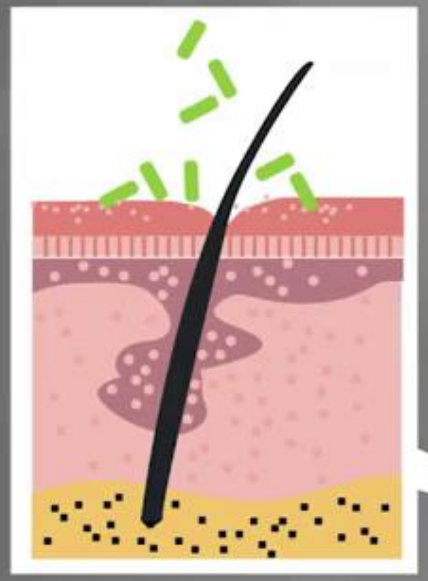
Day 0 vs Day 11 of antibiotic resistant diabetic foot ulcer treated with vinegar. Note complete lack of suppuration.

Days 0, 3, and 6 of vinegar treated antibiotic resistant diabetic foot ulcers. Note disappearance of suppuration and appearance of circulation by day 3.

Treatment of a year-long antibiotic resistance diabetic foot ulcer with vinegar. Days 0 and 6. See method of application in middle slide.

Stages of chronic ulcers

MICROORGANIMS

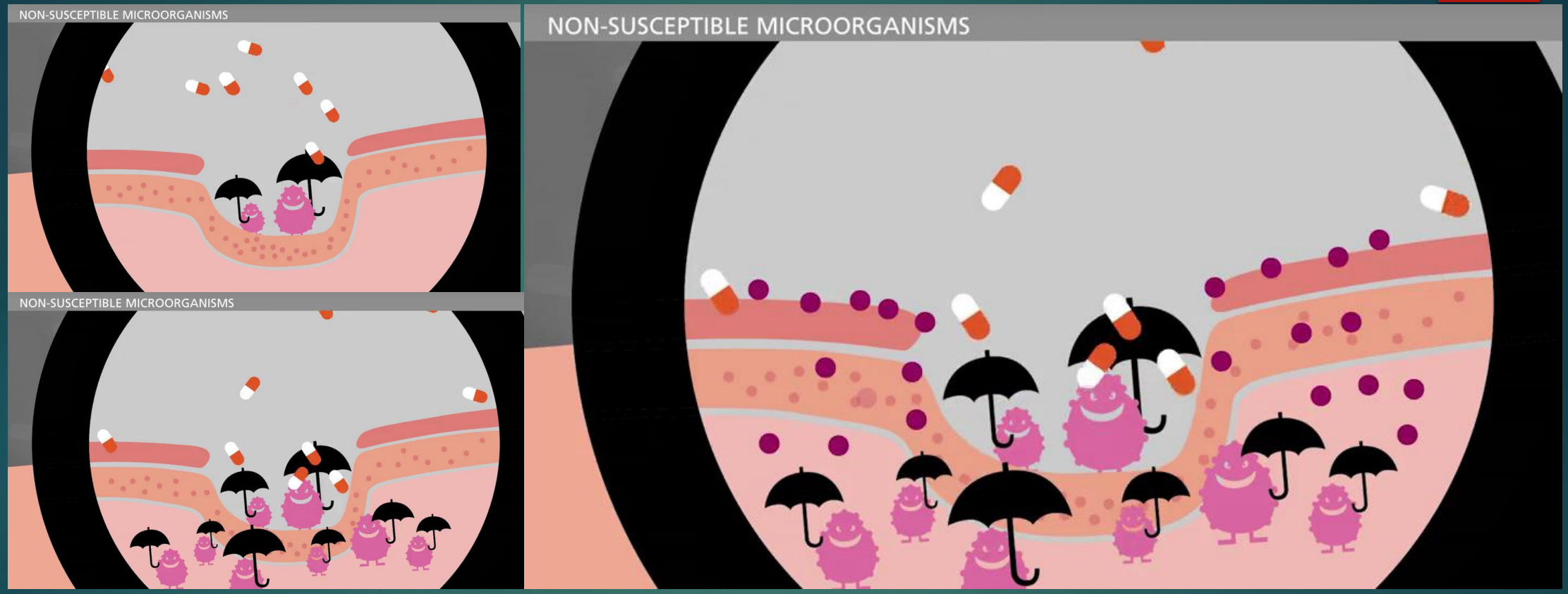


When a wound occurs it is contaminated with microorganisms

...normally cleared by the innate and adaptive immune defense system

- if virulent microorganisms
- if skin with edema
- if diabetic foot ulcers

Bacteria cannot be erradicated



Antibiotic treatment results in resistance, evolution of the biofilm, and ultimately to co-infection by additional species and yeasts (purple circles) in multispecies biofilms

Honey

- Honey in a dilution of ½ was tested against planktonic and biofilm forms of antibiotic resistant *P. aeruginosa and S. aureus*
- Tested honey was Manuka honey, which may contain antimicrobial volatile substances. Some Canadian honey samples were ineffective.
- The honey completely eradicated planktonic forms and reduced biofilm forms of both bacteria by 63-91%
- Manuka honey contains essential oil of *Melaleuca alternifolia.*
- *Other essential oils could be used to make a medicated honey.*

Alandejani T, Marsan J, Ferris W, Slinger R, Chan F. Effectiveness of honey on Staphylococcus aureus and Pseudomonas aeruginosa biofilms. Otolaryngol Head Neck Surg. 2009 Jul;141(1):114-8.

Oral biofilms

- A healthy microbiome may exist in the biofilm on the teeth.
- Sugars drive evolution of the biofilm on the teeth toward acid producing bacteria and caries.
- Poor hygiene results in evolution of the a multispecies biofilm of anaerobes which can live under the gum line. Subsequent inflammation is destructive to the tissues.
- An entirely new biofilm of anaerobes evolves in a tooth abscess.
- Anaerobes in severely infected gum pockets or abscesses may spread through virulent planktonic bacteria to other areas of the body, to medical implants, kidney stones, atherosclerotic plaque, etc.

Treatments for oral infection

- Combinations of *Hydrastis* and Myrrh, applied generously, diligently, and persistently have saved teeth that were due to be pulled because of severe gum disease. Consider Hydrastis leaf.
- May also work with powdered Myrrh and sea salt.
- Will not work without first mechanical cleaning of the teeth.
- Abscesses or infected root canals cannot be addressed with herbs.
- Strong *Echinacea angustifolia* teas internally, and also held as a mouth wash, have effectively prevented or treated oral infections following gum surgery when antibiotics were refused.
- Also effective internally in a case study of facial cellulitis following root canal, when antibiotics were refused.

Tooth powder

For treatment or maintenance after cleaning

	Parts	Stimulant	Anti-inflammatory	Antiseptic	Immune	Biofilm	MDRi
Quercus alba	4		x	x		x	
Myrrh	4	x	x	x	x	x	x
Myrica	2	x		x		x	
Hydrastis	1			x		x	x
Cinnamomum cassia	1	x	x	x			
Eugenia	1	x		x			

This is a formula from Candis Cantin Kiriagis

Bacterial vaginosis

- The normal biome of the vagina is dominated by one of several vagina-specific *Lactobacillus* species.
- BV is characterized by strongly tissue-adherent multi species biofilms constructed on a dominant *Gardnerella* matrix.
- Antibiotics are ineffective because of the biofilm, and because restoration of the ***vaginal-specific Lactobacillus*** is necessary.
- The general pattern of therapy is:
  - Keep the environment acidic with vinegar and/or boric acid
  - Apply probiotics of vaginal-specific *Lactobacillus.*
  - Apply topical therapeutics with antimicrobial and anti-biofilm effects.

Some traditional treatments

- Vinegar douches. May have anti-biofilm effects independent of pH effects.
- Boric acid capsules. BID. Boron may have anti-biofilm effects independent of pH.
- Boric acid mixed with powder of *Hydrastis, Mahonia*, or *Berberis*. Might be enhanced by the use of leaf of *Hydrastis* or *Mahonia*.
- Douche of *Hydrastis* tea. Consider adding the leaf, with the entire Berberine compound formula.
- Douche of *Allium sativum*. Strain the blended preparation through cheesecloth (*allicin* from cut garlic can cause burns)

Vinegar and vaginal biofilms

- Acetic acid has an anti-microbial effect against established biofilms both in-vitro and in open wounds.
- It is effective at 100% eradication of established *P. aeruginosa* and *S. aureus* at a concentration of 1% acetic acid.
- The anti-biofilm effect is not due to pH value of the bacteria, because HCl at the same pH has no effect.
- The effect is due to the acetic acid molecule itself.
- Application six times a day for twenty minutes on non-healing diabetic ulcers.

Boric acid and biofilm formation

Bacteria	Biofilm production					
	In the absence of either of the B compounds		In the presence of H_3BO_3		In the presence of $Na_2B_8O_{13}$-$4H_2O$	
	OD	Bf	OD	Bf	OD	Bf
S. aureus ATCC 25923	0.055 ± 0.002	+	0.02 ± 0.003	–	0.013 ± 0.002	–
V. anguillarum No: 218	0.078 ± 0.001	++	0.031 ± 0.003	–	0.019 ± 0.001	–
A. hydrophila ATCC 19570	0.08 ± 0.06	++	0.03 ± 0.002	–	0.02 ± 0.001	–
A. hydrophila No: 219	0.079 ± 0005	++	0.025 ± 0.001	–	0.021 ± 0.001	–
Y. ruckerii No: 217	0.076 ± 0.011	++	0.03 ± 0.001	–	0.025 ± 0.002	–
P. aeruginosa ATCC 27853	0.216 ± 0.014	+++	0.04 ± 0.002	+	0.035 + 0.001	–
P. aeruginosa No: 266	0.09 ± 0.07	++	0.06 ± 0.001	+	0.011 ± 0.001	–
L. garviea M28-K280	0.180 ± 0.012	+++	0.014 ± 0.002	–	0.04 ± 0.001	+
B. melitensis Rev-1	0.05 ± 0.003	+	0.02 ± 0.002	–	0.018 ± 0.001	–
B. abortus No: 31	0.075 ± 0.004	++	0.024 ± 0.002	–	0.022 ± 0.001	–

Bf biofilm formation

Beneficial effects in BV may be due to the effect of the Boron molecule on biofilm formation rather than to the acidity.

Sayin Z, Ucan US, Sakmanoglu A. Antibacterial and Antibiofilm Effects of Boron on Different Bacteria. Biol Trace Elem Res. 2016 Feb 11.

Topical wound oil

- *Arctostaphylos*
  - *Powerful anti-microbial, anti-biofilm, efflux pump inhibitors*
- *Acalypha*
  - *Powerful anti-biofilm, anti-inflammatory*
- *Mahonia*
  - *Powerful antimicrobial via berberine and companion alkaloids; efflux pump inhibitors*
- *Hydrastis* leaf
  - Antimicrobial via berberine and companion alkaloids; anti-biofilm; efflux pump inhibitors * 2
- *Commiphora*
  - *Antimicribial, anti-biofilm; efflux pump inhibitors, anti-inflammatory, circulatory stimulant, immune*
- *Larrea*
  - Antimicrobial, anti-biofilm, anti-inflammatory, immune

Infused in warm olive oil.

Unilateral otitis externa of 6 days duration, so severe that the ear canal was swollen shut and the drum not visible. A recurrence of a similar infection 4 months prior. Unresponsive to a garlic oil preparation.

Inflammation reduced after one dose, canal was open by half way through day 3 of treatment 4x per day.

Otitis externa

- Chronis otitis externa had the presence of biofilms in 23/25 patients.
- Results from the standard treatment with antibiotics and steroids was compared with "chemical peeling" of the biofilm and superficial epithelium of the ear canal.
- Result were similar for the first 3 months.
- At 6 months, 89% of the "peeling group" were symptom free, vs 64% of the antibiotic group
- At 9 months, the corresponding rates were 80% and 60%

Fusconi M, Petrozza V, Taddei AR, Vinciguerra V, De Virgilio A, Chiarini F, Cirenza M, Gallinelli C, Conte M, de Vincentiis M. Is biofilm the cause of chronic otitis externa? Laryngoscope. 2011 Dec;121(12):2626-33.

Paronychia

Stock photos showing paronychia and its resolution through discharge. The topical wound oil on previous slide resolved a paronychia going from the side past the middle of the nail. Consolidation first to the bottom corner of the nail, then clearing by half way through day-3, without any need for drainage.

Topical wound treatment

- *Scutellaria baicalensis*
- *Mahonia* or *Berberis* leaf
- Another berberine containing herb
- *Echinacea*
- *Larrea*

The combined formula has multiple antimicrobial Antibiofilm, efflux pump inhibiting, anti-inflammatory Local immune stimulating properties.

Formula from herbalist Sam Coffman of The Human Path school

Topical wound treatment

- Usnea
- Betula
- Calendula
- Artemisia

Each plant has anti-microbial and anti-biofilm properties

Wash for sores, has successfully prevented amputations. Formula from herbalist/wildcrafter Patti Leahy used as standard wash for non-healing wounds and ulcers at Occupy Medical clinic in Eugene, Oregon

Biofilms in the gut

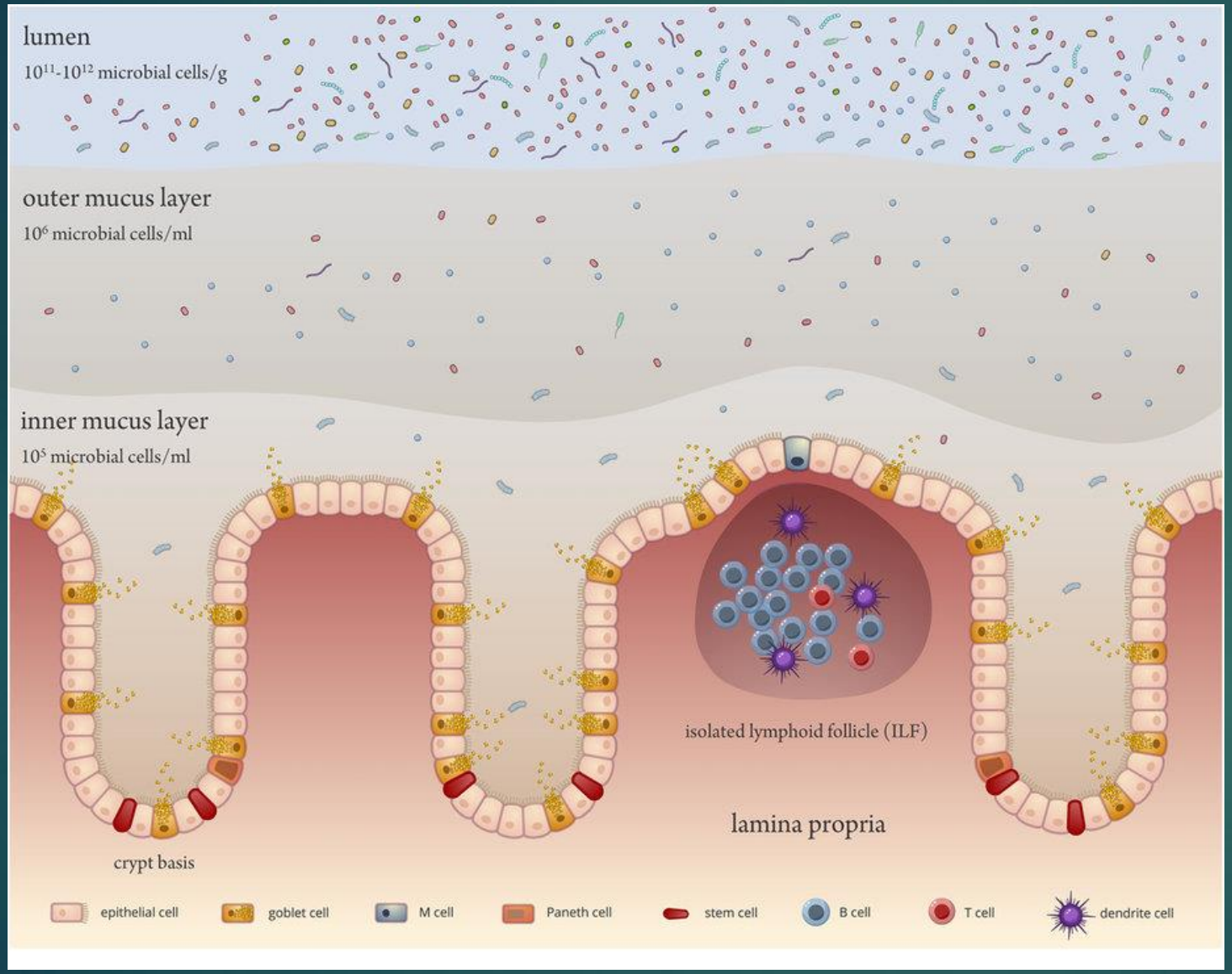
GASTROINTESTINAL BIOFILM STRUCTURES OF COMMENSAL MICROORGANISMS ARE ESSENTIAL TO NORMAL IMMUNOLOGICAL, METABOLIC, AND NEUROLOGICAL HEALTH

Biofilms in the gut

- Biofilms may form in the luminal food contents of the digestive tract.
- They also form within the mucin layer of the gut wall throughout the tract.
- The composition of the luminal and mucosal biofilms is different.
- The functions of the mucosal biofilms may be different from the identical organisms in the lumen due to quorum-sensing activation.
- Details of the composition of the mucin-layer biofilm in health and disease are scanty, and in some cases controversial.

Macfarlane S, Bahrami B, Macfarlane GT. Mucosal biofilm communities in the human intestinal tract. Adv Appl Microbiol. 2011;75:111-43.

de Bos WM. Microbial biofilms and the human intestinal microbiome. *npj Biofilms and Microbiomes* Article number: 15005 (2015)



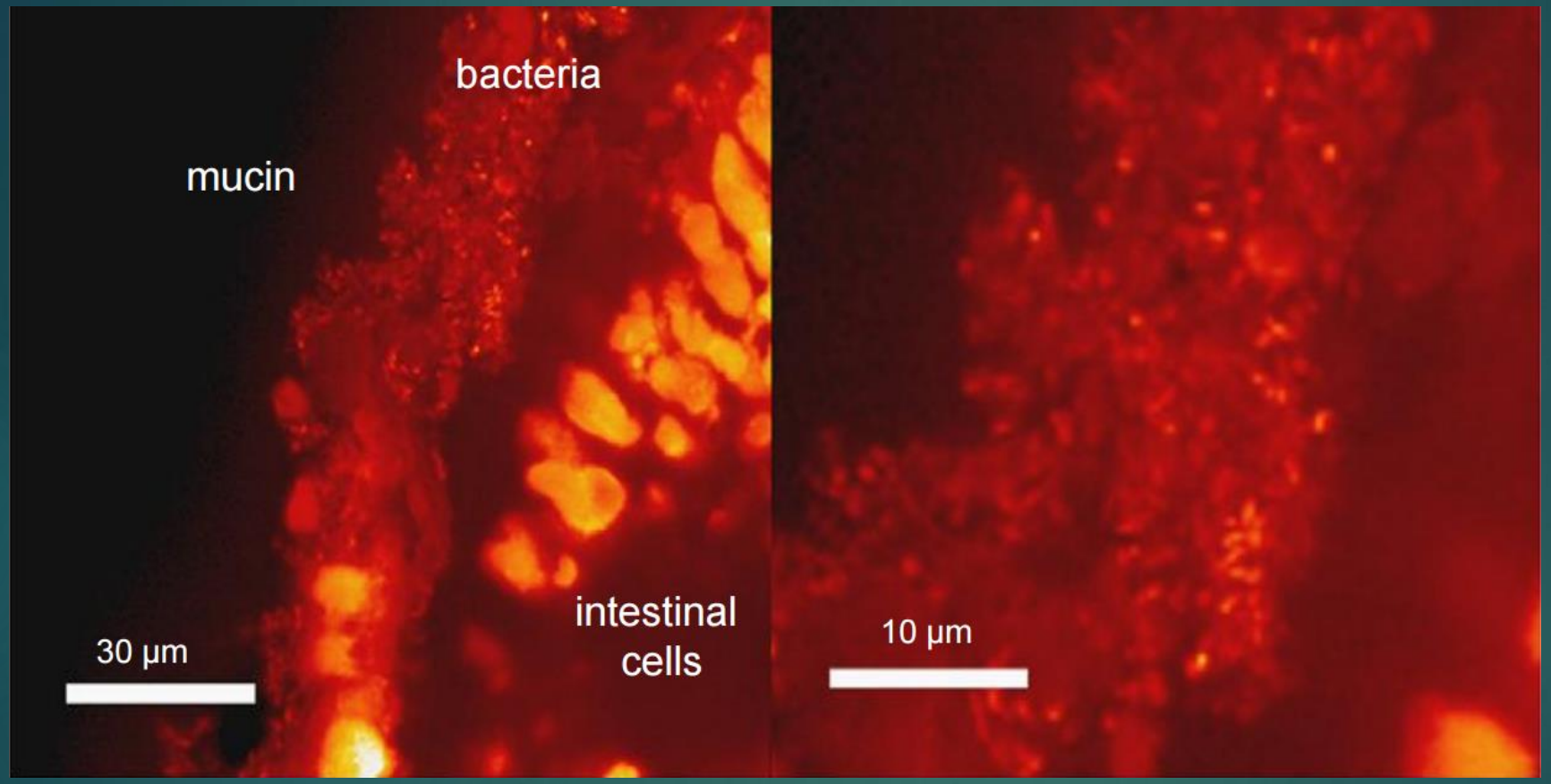
100,000,000,000/g

1,000,000 /mL

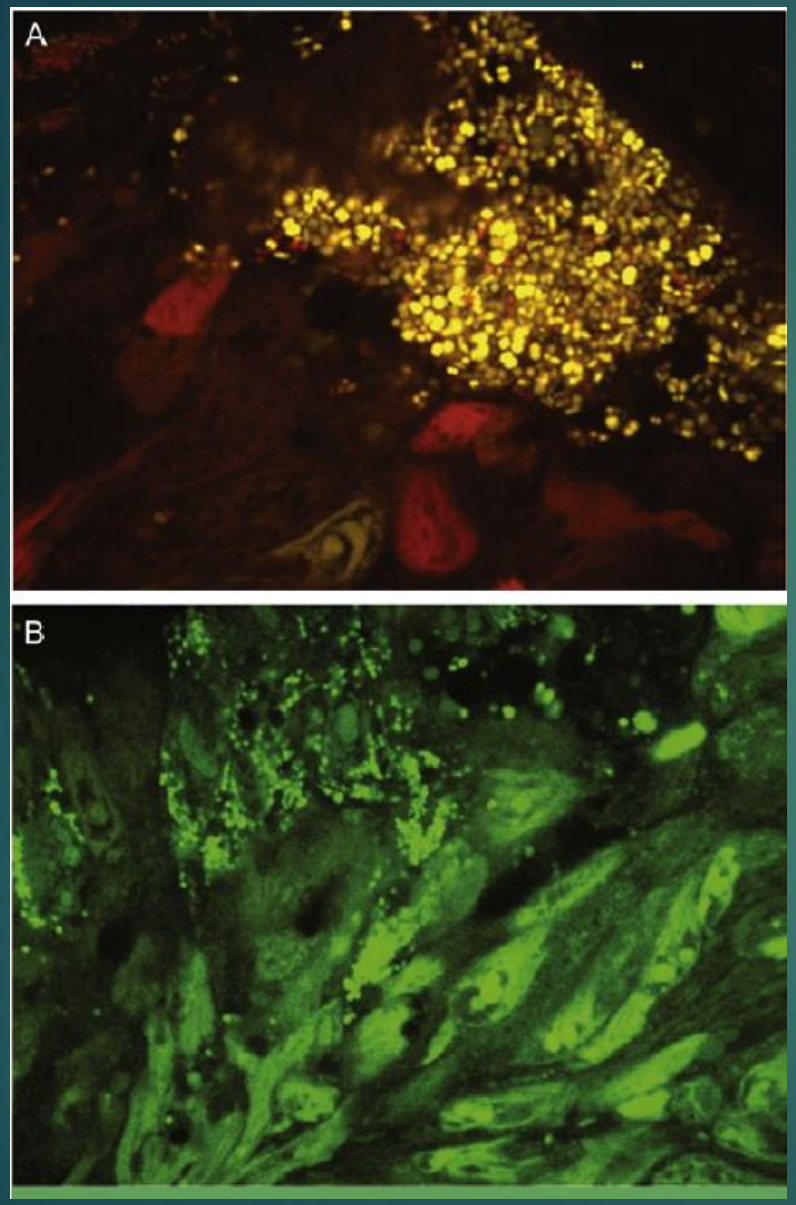
100,000/mL

About 5% of bacteria in feces is in biofilm form. Most bacteria in the mucus layer is in biofilms

De Weirdt R, Van de Wiele T. Micromanagement in the gut: microenvironmental factors govern colon mucosal biofilm structure and functionality. Biofilms and Microbiomes (2015) 1, 15026

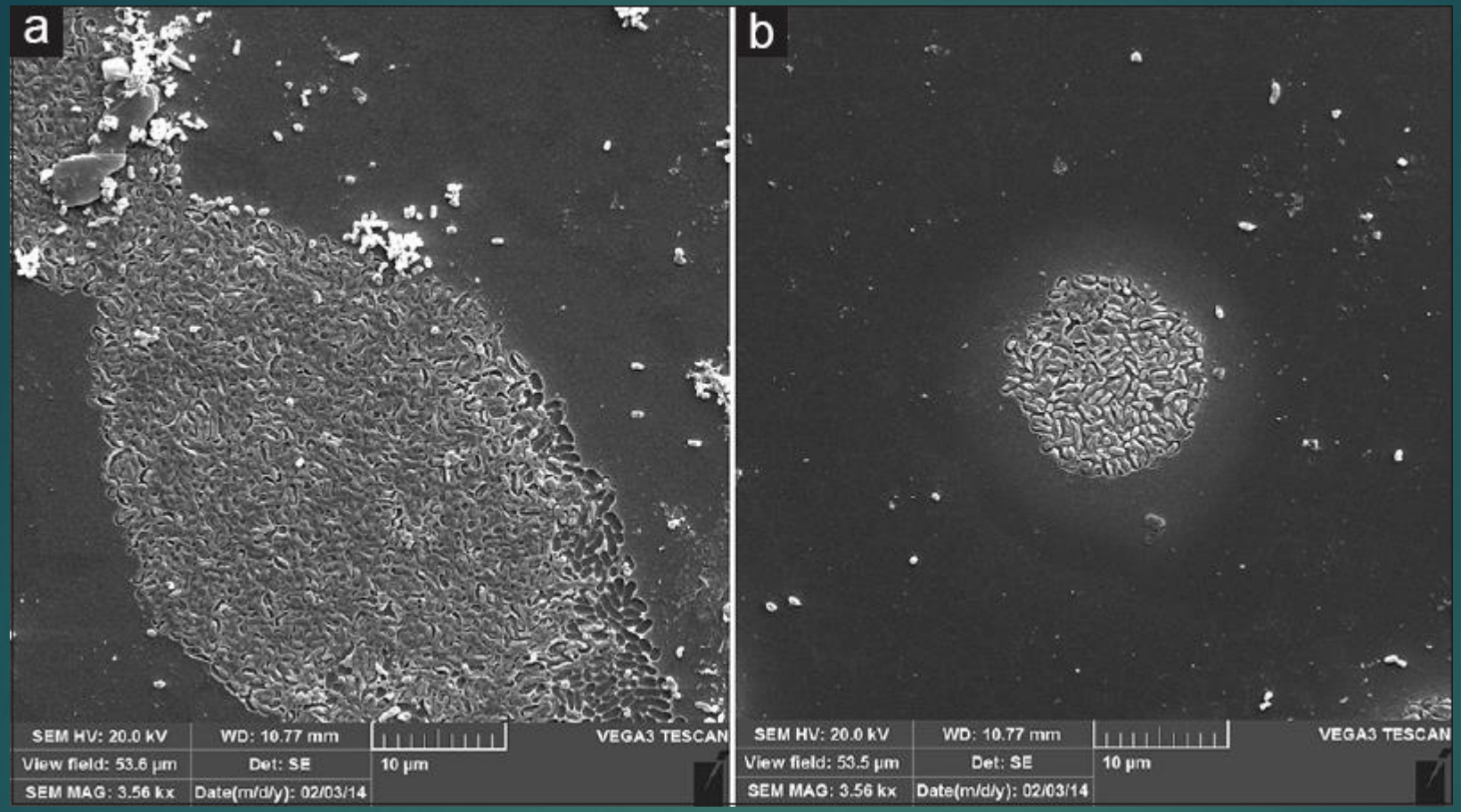


Bacterial biofilm in gut mucosal layer. Site not specified.

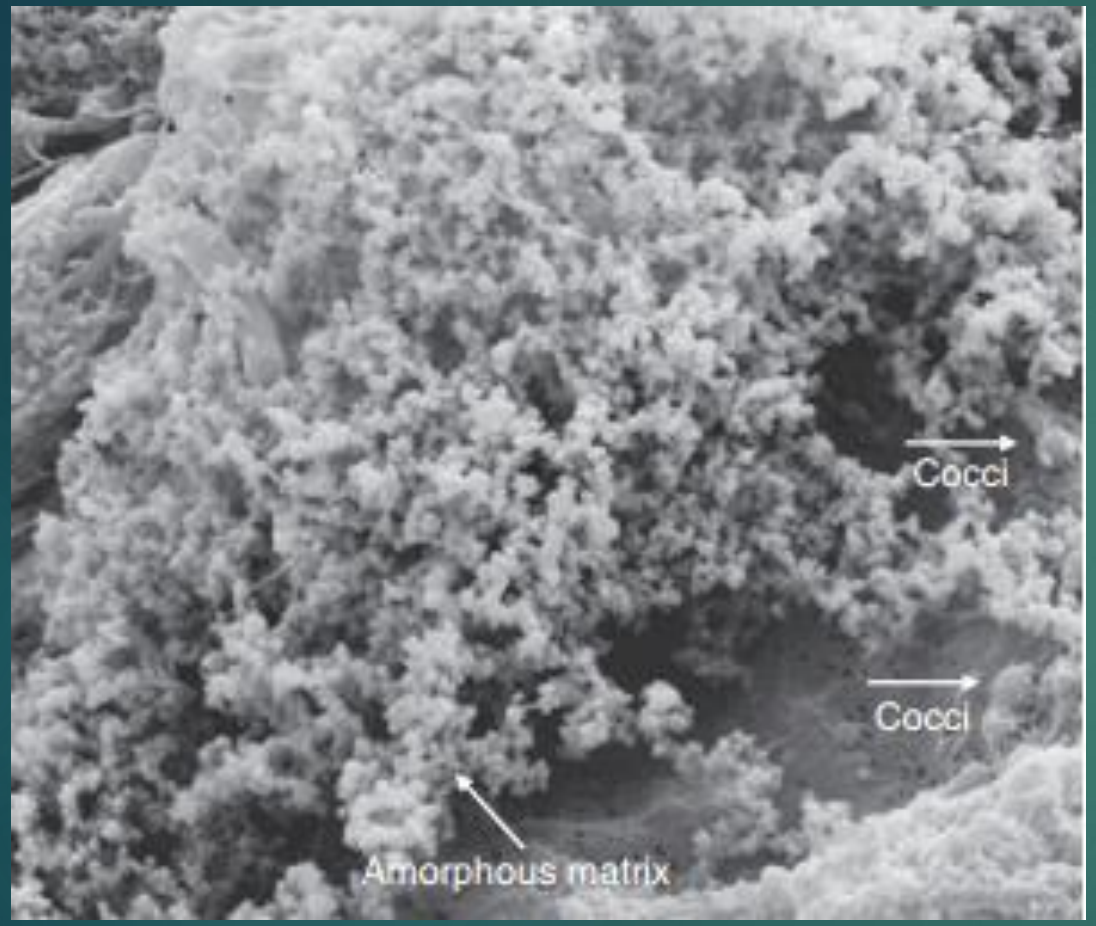


Stained multispecies biofilms in the esophageal mucus of patient with Barrett's esophagus (A) and normal (B)

Macfarlane S, Bahrami B, Macfarlane GT. Mucosal biofilm communities in the human intestinal tract. Adv Appl Microbiol. 2011;75:111-43



Scanning electron micrographs of *Helicobacter pylori* biofilms in the lab dish. *H pylori* settles into the mucous layer of the stomach wall where the pH remains neutral.

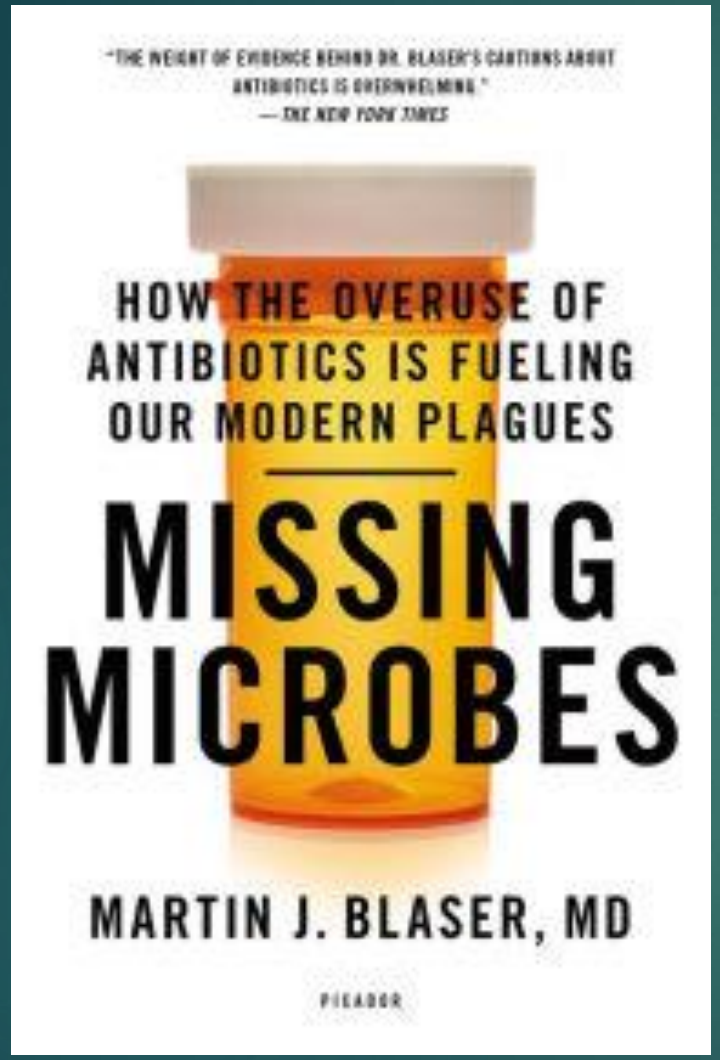


Dense extracellular matrix in gastric mucosal biopsy patient positive for *H. pylori* infection on breath analysis.

Cammarota G, Sanguinetti M, Gallo A, Posteraro B. Review article: biofilm formation by Helicobacter pylori as a target for eradication of resistant infection. Aliment Pharmacol Ther. 2012 Aug;36(3):222-30.

Helicobacter pylori

- *H. pylori* is a normal component of the gastric microbiome, present in the majority of the world's population, and in up to 90% of the population of third world countries.
- Sometimes the dominant species, and commensal, and produces no symptoms in 20% or fewer individuals in first-world studies.
- In one sample, 10/23 healthy individuals with no gastric symptoms tested positive for *H pylori*.
- Normally grows in a mucosal biofilm at neutral pH, in multispecies biofilm
- Pathology may be due to disruption or loss of the mucous layer.



- Blaser is one of the original researchers that discovered *H. pylori*.
- Eradication of *H. pylori* can assist in the healing of ulcers and chronic gastritis, but at a cost to the microbiome and the immune system.
- Patients without *H pylori*, either naturally or after eradication have a 2-8x incidence of GERD.
- Likewise patients without H pylori have a 30-40% increased incidence of asthma. Allergies, as measured on skin prick tests, are also increased.

Roberts formula for ulcers

- Roberts formula for ulcers was developed mid 20th century, long before the possible infectious basis of gastric ulcers was known, and before the discovery of H pylori.
- Most of the herbs are those that would traditionally be used on topical ulcers or poorly healing wounds.
- Most of the herbs have antiseptic and/or anti-biofilm properties.
- Later in the 20th century, J. Bastyr added *Baptisia* and pancreatic enzymes to the formula.

Roberts Formula for Ulcers

		Antiseptic	Anti biofilm	MRDi	Local Immunity	Anti-inflammatory	Vulnerary
Althaea	cool	x	x	x	x	x	x
Geranium maculatum	cool	X*	x	x			
Hydrastis (leaf)	cold	X*	(x)	(x)			
Echinacea	cool	x		x	x	x	x
Phytolacca	cold	x			x		
(Baptisia)	cold	x	x	x	x		

*Specific strong activity against *H. pylori* in vitro

Duodenal ulcer

Exemplar drawn from of many similar cases at NCNM clinic during the 1980s before the discovery of *H. pylori,* using capsules of Bastyr's modified Roberts Formula

- **Patient:** 26 year-old high school teacher.
- **History:** Duodenal ulcer diagnosed by MD.
- **Botanical treatment**:
- 8 parts Marshmallow *(Althea off.)*
- 4 parts Wild Indigo *(Baptisia tinctoria)*
- 8 parts *Echinacea angustifolia*
- 8 parts Spotted Cranebill *(Geranium mac.)*
- 8 parts Golden Seal *(Hydrastis canadensis)*
- 8 parts Poke *(Phytolacca americana)*
- 8 parts Slippery Elm *(Ulmus fulva)*
- 2 parts pancreatin
- 1 part niacinamide
- 2 parts duodenal substance
- One quart of cabbage juice per day.
- **Other treatments:**
- Eliminate alcohol and coffee. Stress reduction techniques.
- **Follow up:** two weeks
- Patient is cutting back on the herbal formula and cabbage juice, and all symptoms are relieved.

N-acetyl cysteine and UGI biofilms

- N-acetyl cysteine has been used in clinical trials for *Helicobacter pylori* eradication.
- Pretreatment with N-acetyl-cysteine at 10 mg.
- All patients receive standard antibiotic therapy.
- Antibiotics more effective after pretreatment

Cammarota G, Sanguinetti M, Gallo A, Posteraro B. Review article: biofilm formation by Helicobacter pylori as a target for eradication of resistant infection. Aliment Pharmacol Ther. 2012 Aug;36(3):222-30.

Lactobacillus in stomach and duodenum

- Patients with bacterial overgrowth in stomach and duodenum (SIBO) after long-term PPI use.
- 10 billion mixed *Lactobacillus* organisms
- 30 mg of N-acetyl-cysteine (anti-biofilm)
- 2.34 g potato maltodextrin (prebiotic)
- QD with main meal for 10 days.

Del Piano M, Anderloni A, Balzarini M, Ballarè M, Carmagnola S, Montino F, Orsello M, Pagliarulo M, Tari R, Soattini L, Sforza F, Mogna L, Mogna G. The innovative potential of Lactobacillus rhamnosus LR06, Lactobacillus pentosus LPS01, Lactobacillus plantarum LP01, and Lactobacillus delbrueckii Subsp. delbrueckii LDD01 to restore the "gastric barrier effect" in patients chronically treated with PPI: a pilot study. J Clin Gastroenterol. 2012 Oct;46 Suppl:S18-26.

- Strong bacterial overgrowth in the stomach and duodenum of people treated with PPIs compared with subjects with a normal intragastric acidity.
- Overgrowth was stronger after long-term treatment with a PPI
- "Marked antagonistic activity" of *Lactobacillus* towards 5 strains of *E. coli*
- Significantly reduced bacterial overgrowth.
- *Lactobacilli* dominated bacterial counts (gastric/duodenal) post-treatment.
- Demonstrated ability to colonize/recolonize the stomach and duodenum.
- A significant decrease in fecal enterococci, total coliforms, E. coli, molds, and yeasts in subjects treated was recorded at the end probiotic supplementation compared with baseline.

TABLE 3. Quantification of Total Bacterial Cells and Total Lactobacillus (Mean ± SEM, log10 CFU/mL of Gastric Juice or Gram of Duodenal Brushing) at d_0 (all Groups) and d_{10} (Group B): Comparison Between Time 0 (d_0) and d_{10} in Group B

	Group B		
Time	log CFU/mL or log CFU/g	% of Total *Lactobacillus*	*P*†
d_0			
Gastric juice			
Total bacteria	8.60 ± 0.17		*
Total Lactobacillus	7.15 ± 0.25	3.51	*
Duodenal brushing			
Total bacteria	8.32 ± 0.33		*
Total Lactobacillus	6.76 ± 0.33	2.74	*
d_{10}			
Gastric juice			
Total bacteria	7.71 ± 0.27		0.0023
Total Lactobacillus	7.70 ± 0.27	98.03	0.0742
Duodenal brushing			
Total bacteria	7.47 ± 0.32		0.0256
Total Lactobacillus	7.44 ± 0.32	93.50	0.0355

*Comparison reference time (d_0).
†Comparison between baseline (d_0) and d_{10}.

	Day 0	Day 10
Gastric juice		
Total bacteria	600,000,000	71,000,000
Lactobacillus	11,500,000	70,000,000

Duodenal brushing		
Total bacteria	320,000,000	47,000,000
Lactobacillus	7,600,000	44,000,000

TABLE 8. Quantification of Specific Microbial Groups in Fecal Samples at d_0 (all Groups) and d_{10} (Group B): Comparison Between Baseline (d_0) and d_{10} in Group B

Time	Group B $\log_{10}$ CFU/g	*P*†
d_0		
Enterococcus spp.	7.80 ± 0.25	*
Total coliforms	9.55 ± 0.16	*
Escherichia coli	9.44 ± 0.18	*
Yeasts	5.95 ± 0.14	*
Molds	5.64 ± 0.14	*
d_{10}		
Enterococcus spp.	6.99 ± 0.23	0.0155
Total coliforms	8.01 ± 0.24	0.0064
Escherichia coli	7.97 ± 0.23	0.0105
Yeasts	3.56 ± 0.18	0.0066
Molds	4.30 ± 0.15	0.0053

Results are expressed as $\log_{10}$ CFU/g of feces (mean ± SEM).
*Comparison reference time (d_0).
†Comparison between baseline (d_0) and d_{10} in group B.

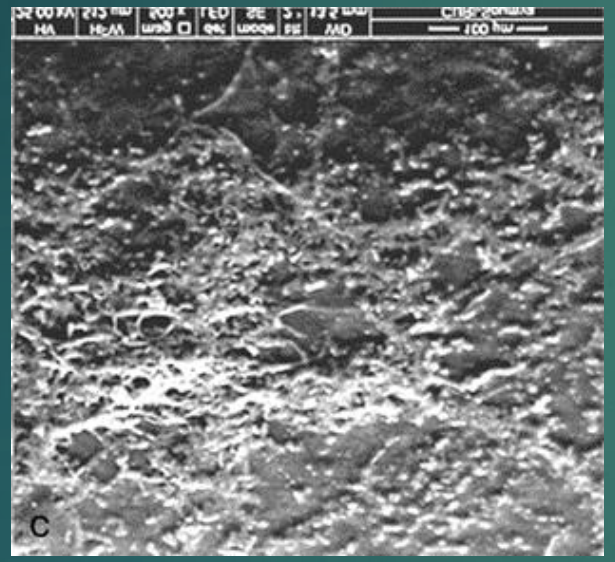
Stool bacterial counts of the measured species declined on the order of 10-100 times.

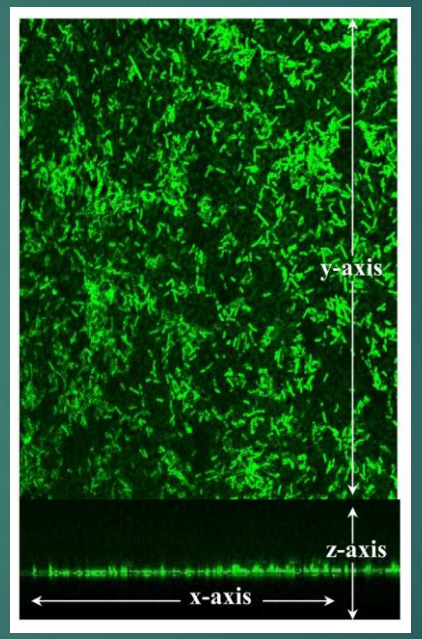
N-acetylcysteine and biofilms clinical trials review

Promising results when NAC can directly contact the biofilm

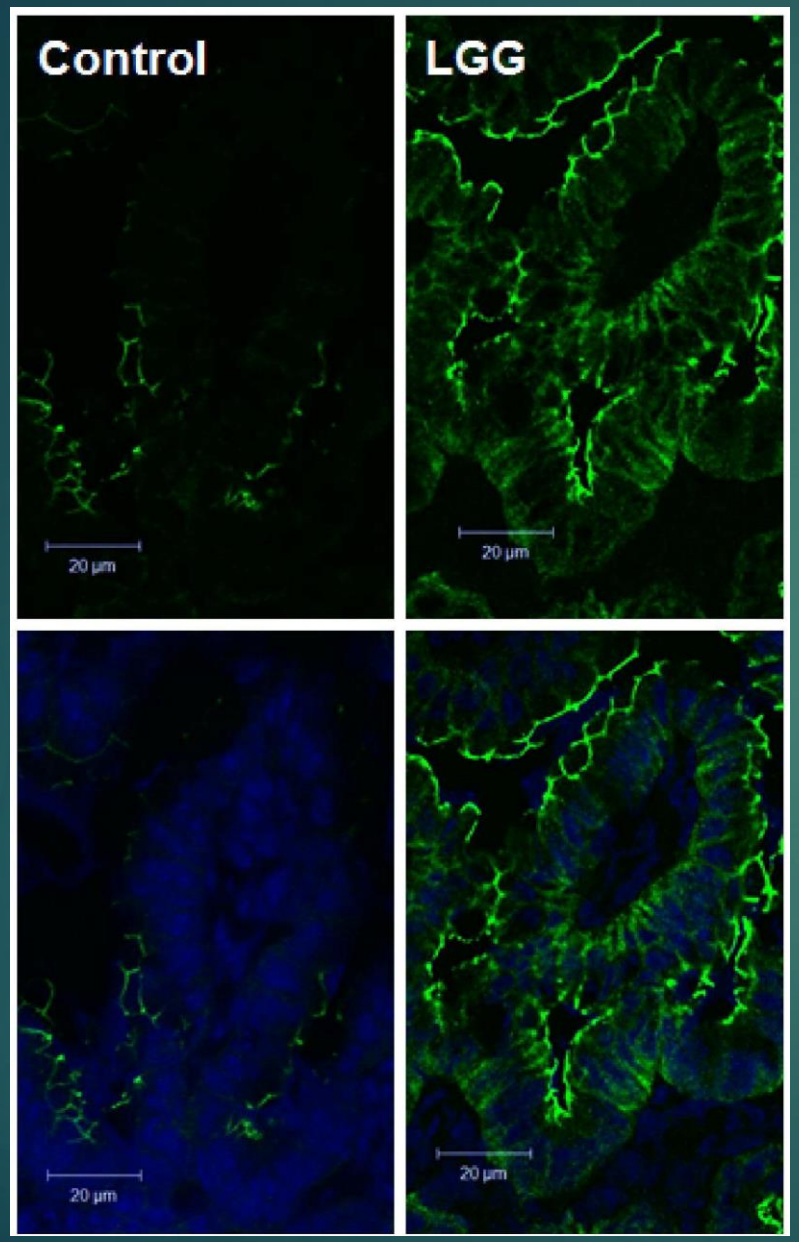
Dinicola S, De Grazia S, Carlomagno G, Pintucci JP. N-acetylcysteine as powerful molecule to destroy bacterial biofilms. A systematic review. Eur Rev Med Pharmacol Sci. 2014 Oct;18(19):2942-8. Review.

Lactobacillus and *Bifidobacterium* form complex biofilms within the mucosal layer of the intestinal tract





Lactobacillus biofilms on surface of an olive (left) and in lab dish (right).



Images of intestinal *Lactobacillus* biofilms in humans are difficult to find. More common are images in mice and birds.

In this scan of a mouse intestine, before and after treatment with a Lactobacillus species (LGG), the effects of the LGG mucosal colonization on the expression of junction protein claudin-3 is shown with the luminescent dye.

Patel RM, Myers LS, Kurundkar AR, Maheshwari A, Nusrat A, Lin PW. Probiotic bacteria induce maturation of intestinal claudin 3 expression and barrier function. Am J Pathol. 2012 Feb;180(2):626-35.

Multispecies commensal biofilms in distal ileum

- Terminal ileum and proximal colon characterized by multi-species heterogenous mucosal biofilms Of SCFA producing bacteria.
- Acetate production is highest in these adjacent areas.
- SFCA production and its benefits may be dependent on mucosal bound commensal biofilms.
- SCFA provide essential calories (10% of total mitochondrial fuel)
- SCFA promote tight junction in the gut, and surface immunity.
- SCFA have systemic metabolic effects, promote insulin sensitivity.

Macfarlane, S., Macfarlane, G. T., Composition and metabolic activities of bacterial biofilms colonizing food residues in the human gut. *Appl. Environ. Microbiol.* 2006, *72*, 6204–6211.

Macfarlane S, Bahrami B, Macfarlane GT. Mucosal biofilm communities in the human intestinal tract. Adv Appl Microbiol. 2011;75:111-43.

Lactobacillus in distal small intestine

Table 1
Rod-shaped bacteria tightly associated with ileal epithelial cells.

Biopsy n°	Strain name	Species[a]	Gene bank accession number
1	SF1031	*Lactobacillus mucosae*	FN400925
1	SF1036	*Bifidobacterium breve*	FN400926
2	SF1087	*Lactobacillus mucosae*	FN400927
2	SF1091	*Lactobacillus mucosae*	FN400928
2	SF1108	*Lactobacillus mucosae*	FN400929
2	SF1109	*Lactobacillus gasseri*	FN400930
3	SF1111	*Lactobacillus mucosae*	FN400931
4	SF1146	*Lactobacillus mucosae*	FN400932
5	SF1183	*Lactobacillus gasseri*	FN400933
6	SF1232	*Lactobacillus mucosae*	FN400934
7	SF1233	*Lactobacillus mucosae*	FN400935

[a] Assessed on the basis of the nucleotide sequence of the gene coding for 16S RNA and of biochemical (API) tests.

On recovery, most species

- Produced antimicrobial substances
- Formed biofilms
- Degraded mucin
- Survived simulated gastric environment

Fakhry S, Manzo N, D'Apuzzo E, Pietrini L, Sorrentini I, Ricca E, De Felice M, Baccigalupi L. Characterization of intestinal bacteria tightly bound to the human ileal epithelium. Res Microbiol. 2009 Dec;160(10):817-23.

Clinical questions

- If biofilms are part of the normal and even essential functioning of the microbiome, should we use caution before we attempt to eradicate them in the gut?
- Is it clinically possible to deliver biofilm disrupting agents to the middle or lower digestive tract?
- If we attempt to treat internal biofilms with agents which disrupt biofilms in the lab dish, but with unknown pharmacokinetics, can we damage healthy structures in the gut?

Small Intestine Bacterial Overgrowth (SIBO)

- SIBO biofilms documented secondary to PPI or achlorhydria in the duodenum.
- A form of SIBO specific to the distal ileum, and characterized by diarrhea and weight loss, has been recognized since the 1950s. This area is especially rich in mucosal biofilms.
- Biofilms form readily on enteric feeding tubes.
- Poor correlations between jejunal aspiration, mucosal biopsy, and hydrogen/methane breath tests may be due to the confounding effects of biofilm.

Chandra S, Dutta U, Noor MT, Taneja N, Kochhar R, Sharma M, Singh K. Endoscopic jejunal biopsy culture: a simple and effective method to study jejunal microflora. Indian J Gastroenterol. 2010 Nov;29(6):226-30.

Multiple factors contribute to the normally low bacterial content of the human small intestine

- Gastric acidity that kills ingested organisms
- Immune components (IgA) secreted in bile and by the intestinal epithelium
- Antimicrobial peptides secreted by epithelial cells
- Propulsive intestinal motility producing a fast transit time of the small intestine.
- Long-chain fatty acids in conjugated bile

Internal biofilms

Internal biofilms

- These usually require surgical/mechanical assistance to remove.
- High doses of single antibiotics are usually ineffective
- High doses antibiotic combinations *may* be effective.
- Medical devices and implants
- Tissue fillers
- Osteomyelitis
- Endocarditis
- Infectious kidney stone
- Tooth abscess
- Chronic tissue infection (Borrelia, other?)

“Biofilm busters”

- Many products now purporting to break up biofilms within the gut or within the body are now available.
- Play into the paradigm of “biofilm as enemy.”
- The basis for their use is in-vitro effects against biofilm or matrix material.
- The positive assertions for their clinical worth have greatly exceeded any evidence of their value.
- Almost nothing known about the pharmacokinetics of anti-biofilm constituents.
- Because biofilms can’t be studied within the tissues, effects of constituents or drugs on the biofilms can’t be directly determined.

Plant activity (topical)

- Antimicrobial
- MRDi
- Anti quorum
- Anti biofilm

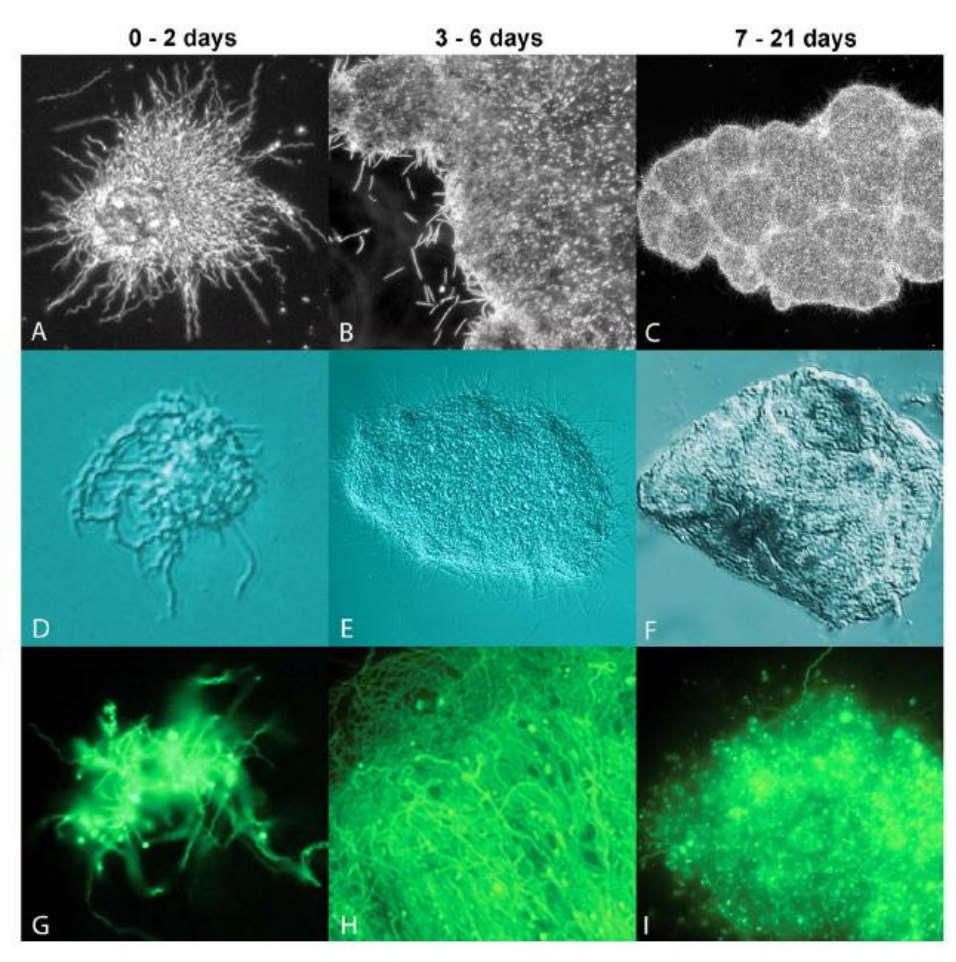
- Properties are due to different constituents which come in direct contact with microorganism/biofilm
- These constituents, taken internally, may have radically different pharmacokinetics.
- Gastric environment, small intestine, effect of microbiome, first pass through intestine, route of excretion, elevation of serum level to effective dose.
- Pharmacokinetics not identified for more than a handful of plant constituents.

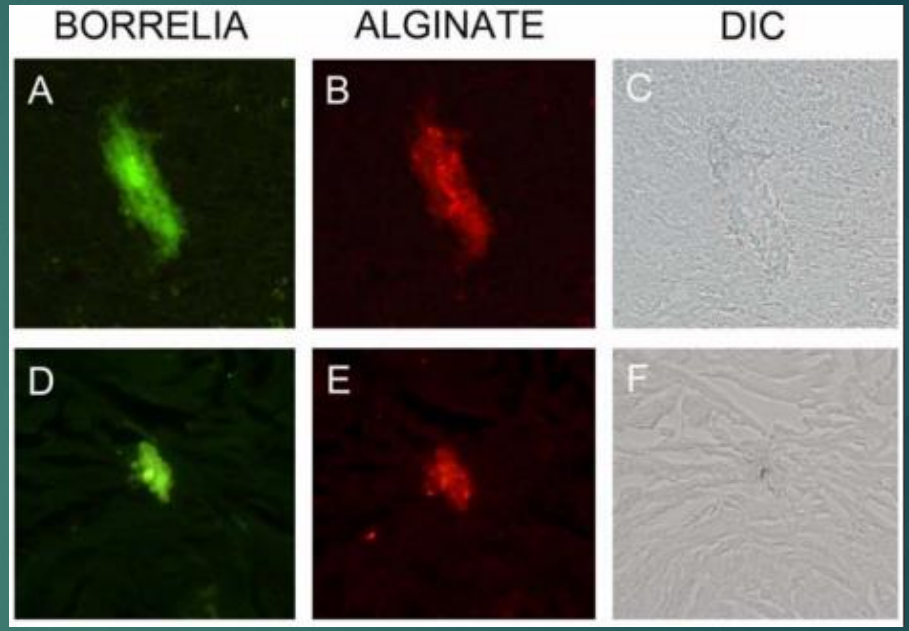
Many enzymes can disrupt existing biofilms in the lab dish.
Can these survive the gut and liver when taken orally to produce
An effective serum dose for internal biofilms?

Some substances with anti-biofilm effects in the lab

- Lactoferrin/apolactoferrin
- N-acetyl-cysteine
- Lumbrokinase and nattokinase
- Various formulas of enzymes
  - InterFase Plus
  - Biofilm Defense
- Of these, evidence for effectiveness is based on in-vitro effects, or effects in the upper GI.
- Because of difficulty in evaluating internal biofilms, ***we may need to rely on empirical results.***

Borrelia biofilms *in vitro* and *in vivo*

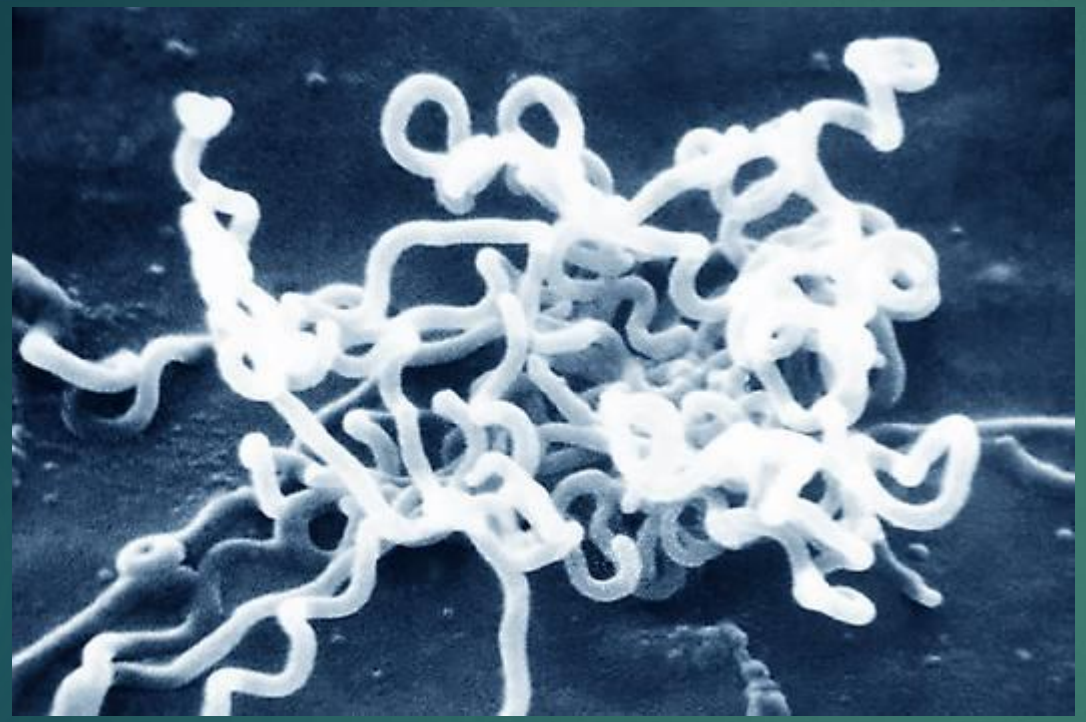




Borrelia biofilms *in vitro* are large enough to be seen with the naked eye. In human tissue, the samples above were visible only at 400x magnification.

Sapi E, Balasubramanian K, Poruri A, Maghsoudlou JS, Socarras KM, Timmaraju AV, Filush KR, Gupta K, Shaikh S, Theophilus PA, Luecke DF, MacDonald A, Zelger B. Evidence of In Vivo Existence of Borrelia Biofilm in Borrelial Lymphocytomas. *Eur J Microbiol Immunol (Bp).* 2016 Feb 9;6(1):9-24.

Free full text at PubMed



Treponema palladium biofilm *in vitro*

Subspecies are responsible for syphilis and yaws

Smilax glabra

- History of use in China against chronic spirochetal disease.
- *Treponema pallidum* subspecies -- syphilis, yaws
- Reportedly will, in formula, effect complete cure of chronic syphilis in some cases, even third stage.
- These likely include internal biofilms, though these have not been imaged.
- Contemporary reports effective to reduce symptoms of Lyme
- Contemporary report of rapid relief of recurrent symptoms of neurological Lyme. Specifically *S glabra* and not other Smilax species tested according to one practitioner

Classical Chinese formula for spirochete disease

- *Smilax glabra*
- *Scutellaria* radix
- *Coptis chinensis*
- *Taraxacum* herba
- *Lonicera* flos
- *Polygonum cuspidatum* rhizome
- *Glycyrrhiza uralensis*

Most of these have not been tested in lab trials for anti-biofilm activity.
Such activity can be implied in the formula because of clinical effects.

Garlic vs Flagyl for Bacterial Vaginosis

- 500 mg powder of *Allium sativum*
- 250 mg Metronidazole
- Two tablets with meals orally each 12 hrs.
- Successful oral application with reduction of the biofilm implies that the anti-microbial and possibly the anti-biofilm constituents are delivered systemically to the vaginal mucosa

Mohammadzadeh F, Dolatian M, Jorjani M, Alavi Majd H, Borumandnia N. Comparing the therapeutic effects of garlic tablet and oral metronidazole on bacterial vaginosis: a randomized controlled clinical trial. Iran Red Crescent Med J. 2014 Jul;16(7):e19118.

Table 4. Comparison of Laboratory Improvement in Women With Bacterial Vaginosis [a,b]

Group	Garlic	Metronidazole	Total
Lab Improvement	41 (68.3)	33 (55)	74 (61.7)
Lack of Lab Improvement	19 (31.7)	27 (45)	46 (38.3)
Total	60 (100)	60 (100)	120 (100)

[a] Data are presented as No. (%).
[b] Chi square = 2.256 and P > 0.05.

Table 5. Comparison of Treatment Success in Women With Bacterial Vaginosis [a,b]

Group	Garlic	Metronidazole	Total
Successful Treatment	38 (63.3)	29 (48.3)	67 (55.8)
Failure in Treatment	22 (35.7)	31 (51.7)	53 (44.2)
Total	60 (100)	60 (100)	120 (100)

[a] Data are presented as No. (%).
[b] Chi square = 2.737 and P > 0.05.

Table 6. Comparison of Medication Side Effects in Women With Bacterial Vaginosis [a,b]

Group	Garlic	Metronidazole	Total
With Side Effect	9 (15)	20 (33.3)	29 (24.2)
Without Side Effect	51 (85)	40 (66.7)	91 (75.8)
Total	60 (100)	60 (100)	120 (100)

[a] Data are presented as No. (%).
[b] Chi square = 5.502 and P > 0.032.

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http://naimh.com/northwest/seminar