

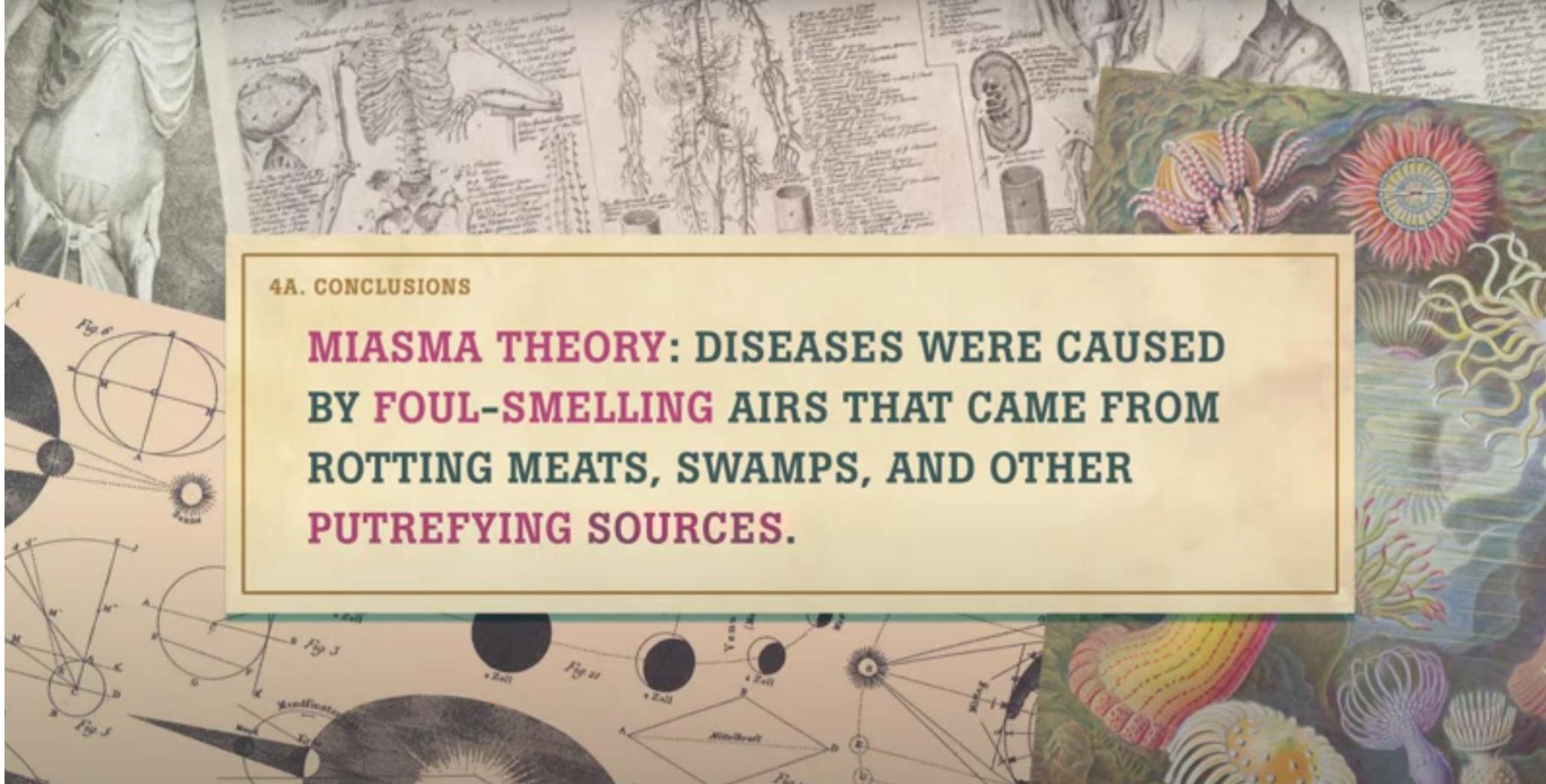
# MICROBIOLOGY



Dr. Manu Smriti Singh  
Department of Biotechnology  
Bennett University

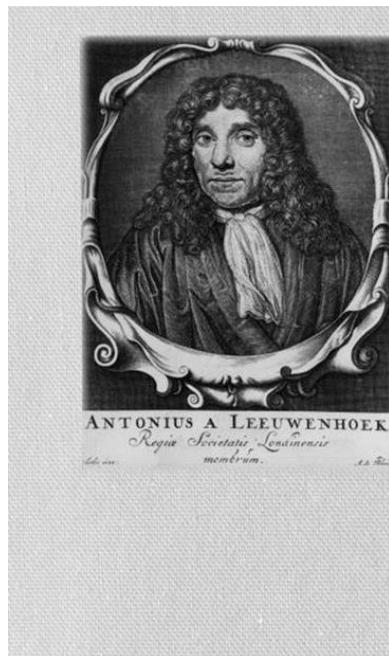
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# WHERE DID DISEASE COME FROM?



# ANTON VON LEEUWENHOEK

- Probably the first person to observe living cells with a simple microscope, amateur scientist, ground his own lenses and described what we know today as bacteria – rod shaped , spiral shaped , etc. “animalcules”



- October 24, 1632 – August 26, 1723
  - Dutch tradesman and scientist
  - Invented the first practical microscopes
  - “the Father of Microbiology”
  - Discovered many microorganisms



protozoa  
"animalcules".

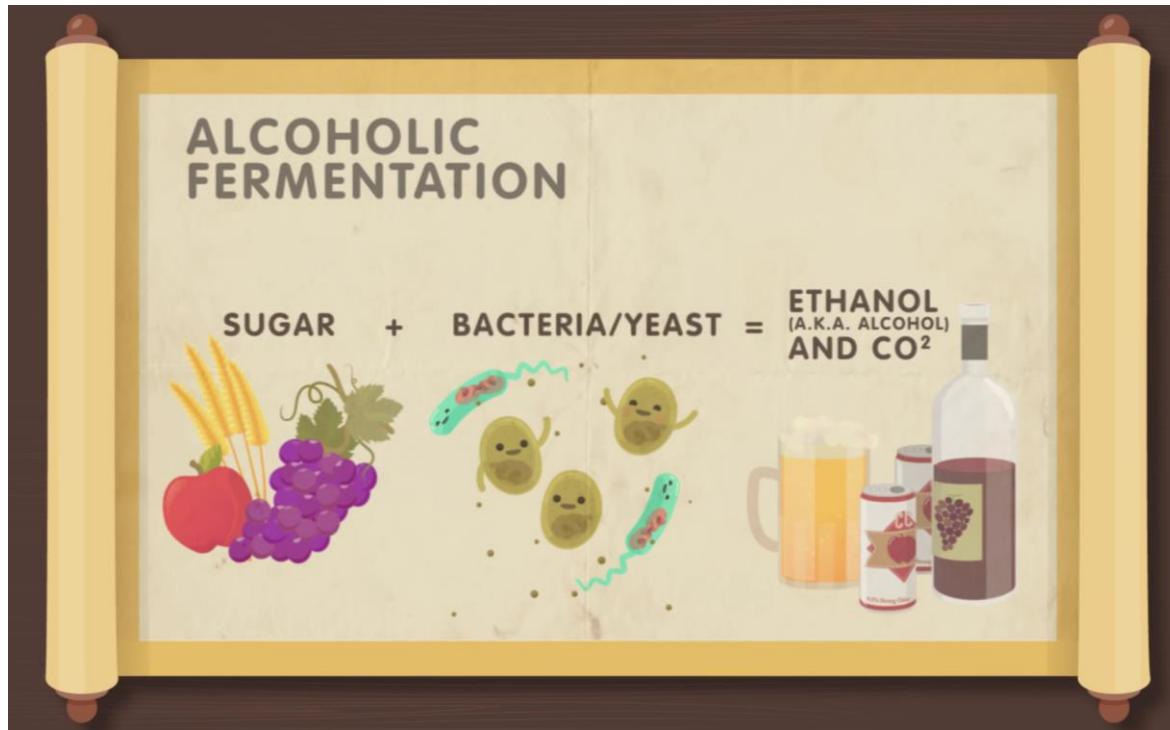


## bacteria



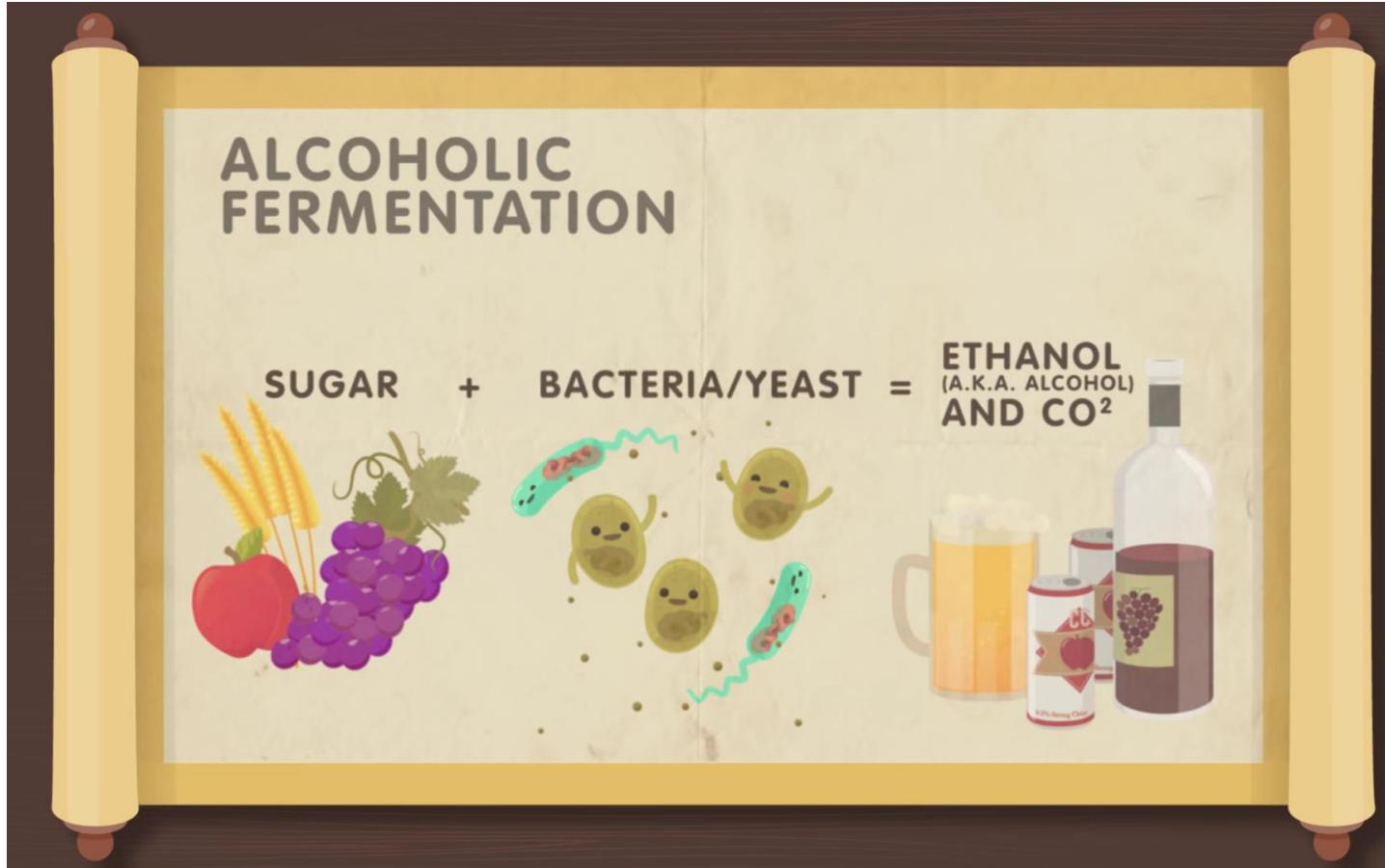
sperm

# LOUIS PASTEUR



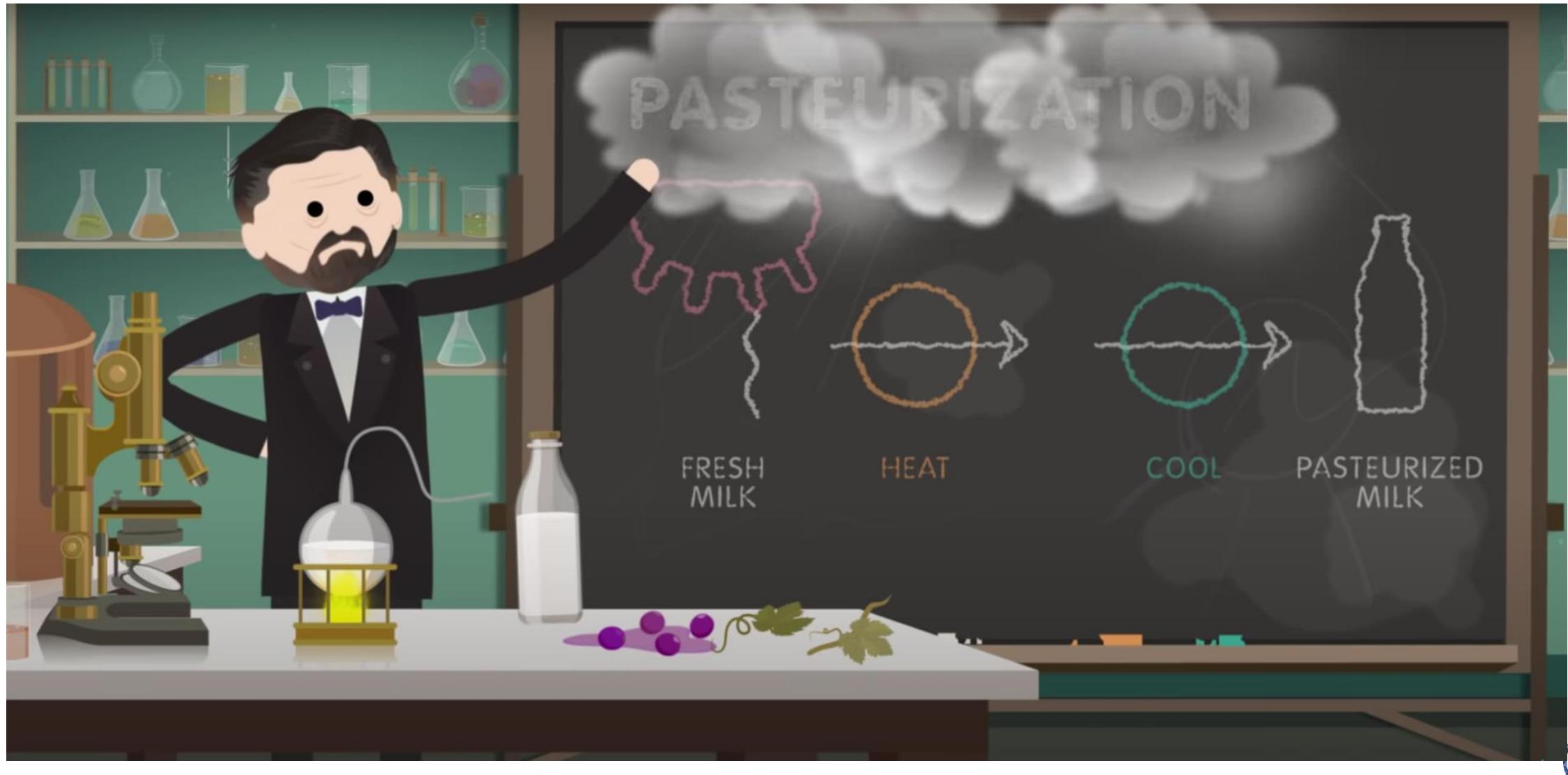
- Local tradesman asked Pasteur to help find out why some of the casks of fine vinegar made from beet juice were spoiling.
- Pasteur examined the good vinegar and the spoiled vinegar under the microscope.
- Yeast that caused the beet juice to ferment was a living organism.
- Casks producing good vinegar → healthy yeast; while those producing the spoiled product → microscopic rods that harmed the yeast.

# LOUIS PASTEUR



Heating fermented products!

# PASTEURIZATION



# IGNAZ SEMMELWEIS



- Physician and scientist
- Hand washing reduced mortality to below 1%

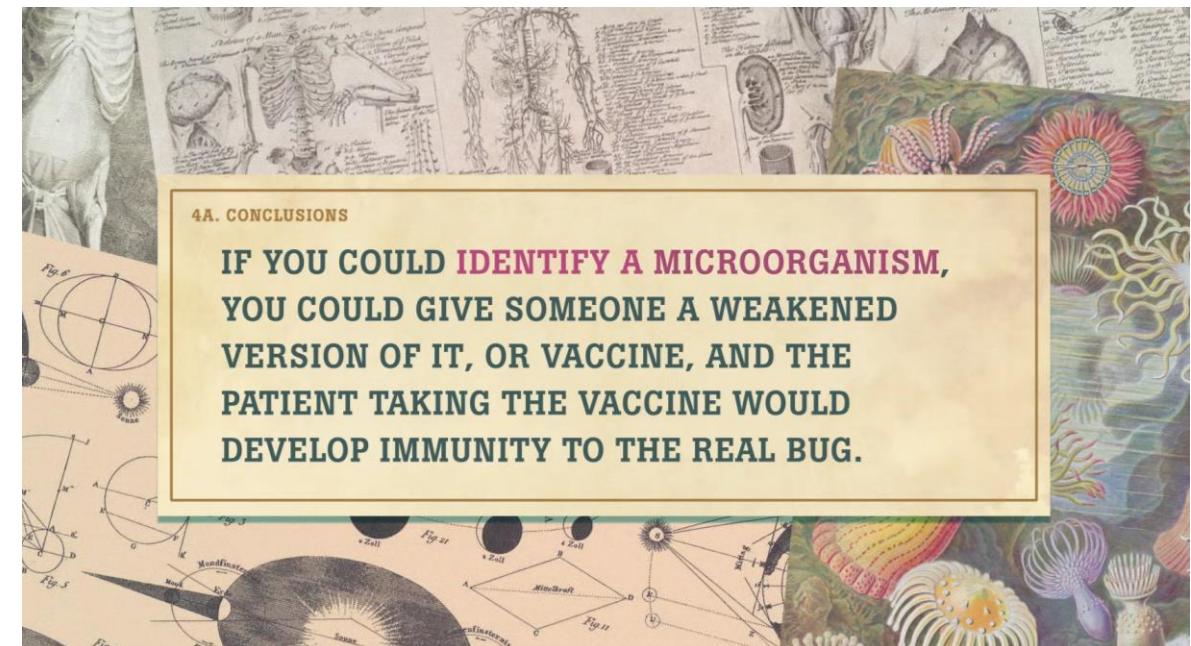
# ANTISEPTIC SURGERY



- Joseph Lister- British Surgeon
- Infection in wounds
- Post-operative infections
- Sterilization of surgical instrumentation
- Read work of Pasteur on food spoilage
- Collaborations with Pasteur
- Sprayed surgical incisions and dressings with carbolic acid solution
- Pioneer of antiseptic surgery

# LOUIS PASTEUR

- In 1885, Pasteur tested his new rabies vaccine on man for the first time.
- Joseph Meister, a young boy was bitten by a rabid dog.
- His mother urged Pasteur to treat with new method.
- Pasteur gave Meister the rabies vaccine and saved his life.

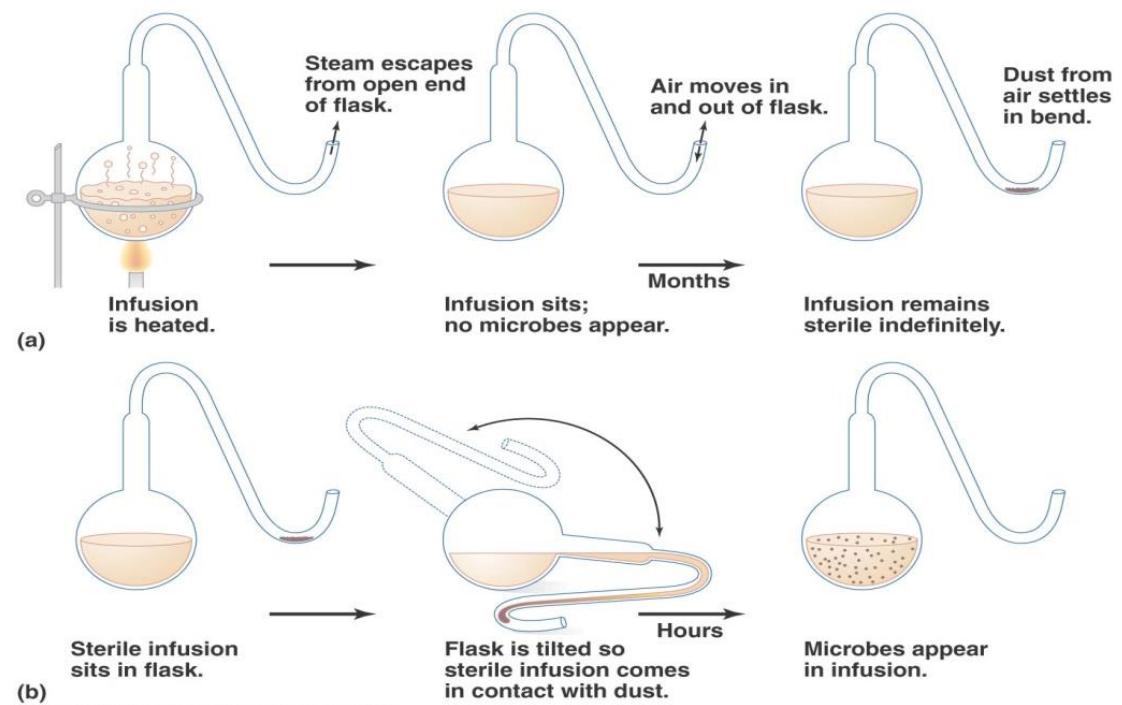




# GERM THEORY OF DISEASE

## Pasteur's Experiments

- When the “swan-necked flasks” remained upright, no microbial growth appeared
- When the flask was tilted, dust from the bend in the neck seeped back into the flask and made the infusion cloudy with microbes within a day



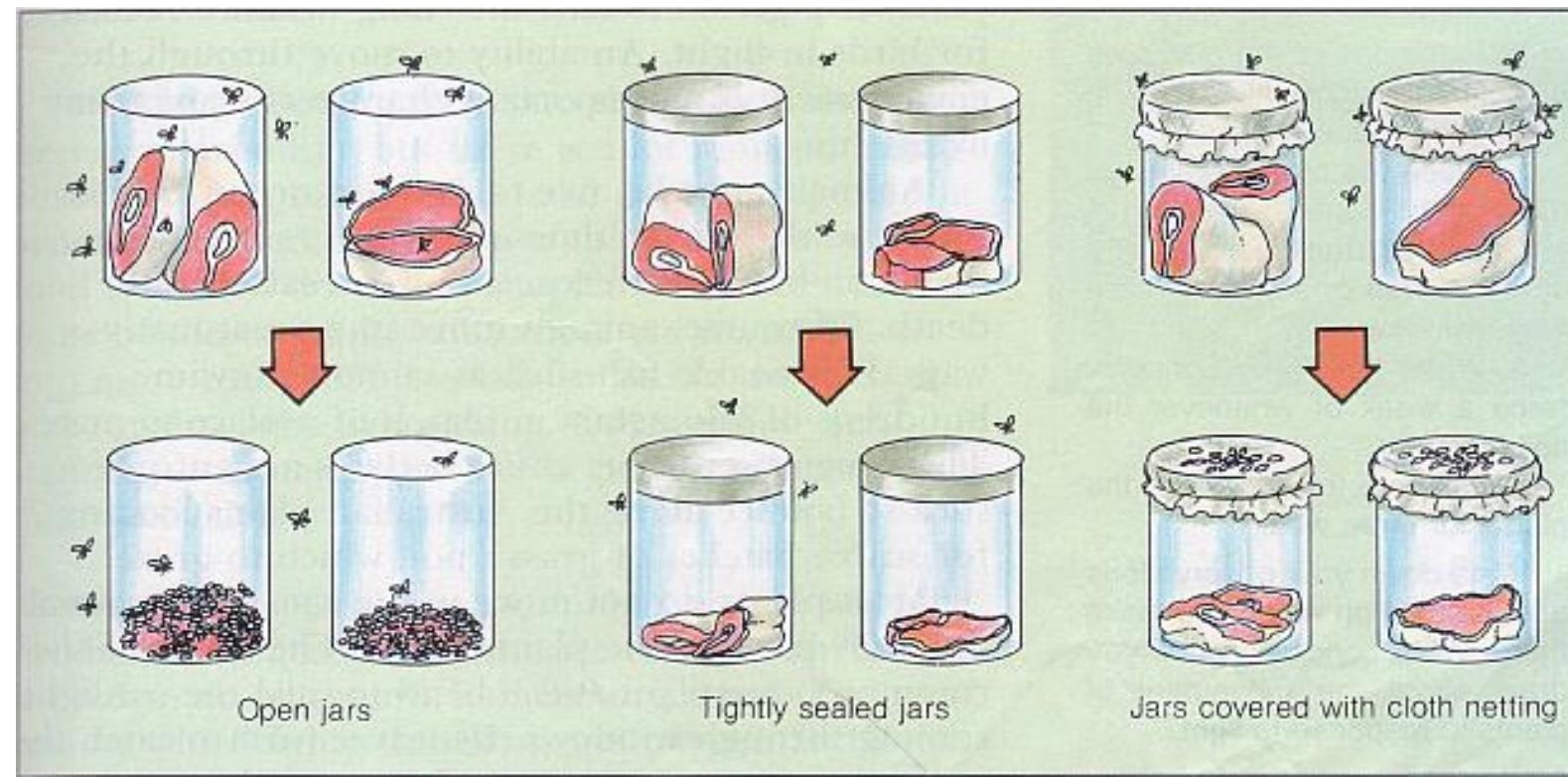
## Miasma Theory

- Robert Seymour's depiction of spread of cholera through poisonous air
- Or the Theory of Spontaneous Generation
- Proven wrong through experiments:
  - Pasteur's pasteurization
  - Francesco Redi
  - Koch's Postulates

# FRANCESCO REDI

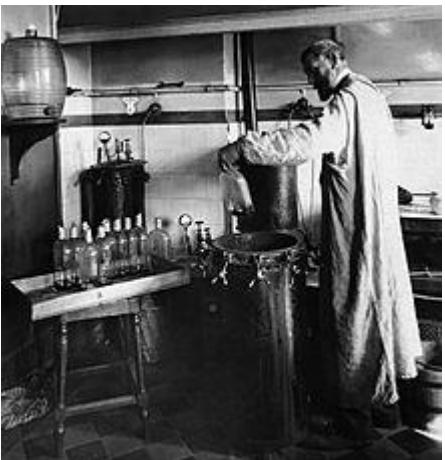
Opposed the prevailing theory of Spontaneous Generation.

Redi used covered jars to show that maggots on meat came from flies –strong evidence against spontaneous generation

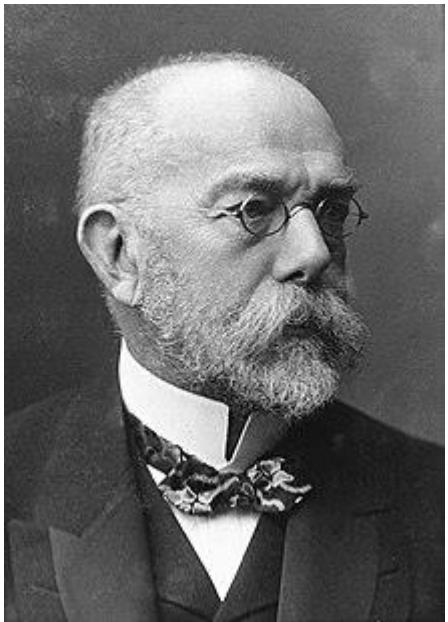


# LOUIS PASTEUR

- Beet juice vinegar spoilage
- Pasteurization- Wine/ Milk
- Silk Industry
- Disproved theory of spontaneous generation
- Vaccine- Chicken cholera/ Rabies



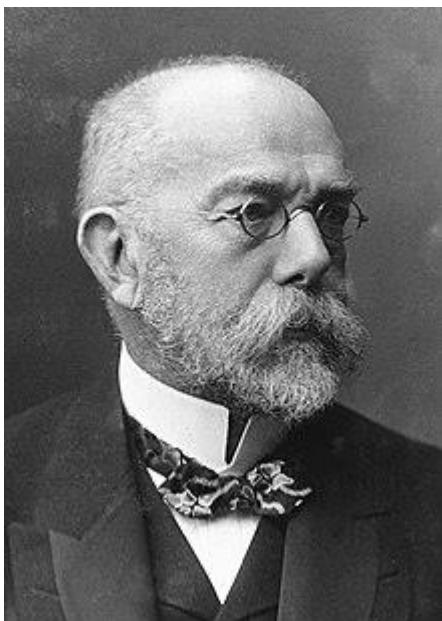
# KOCH'S POSTULATES



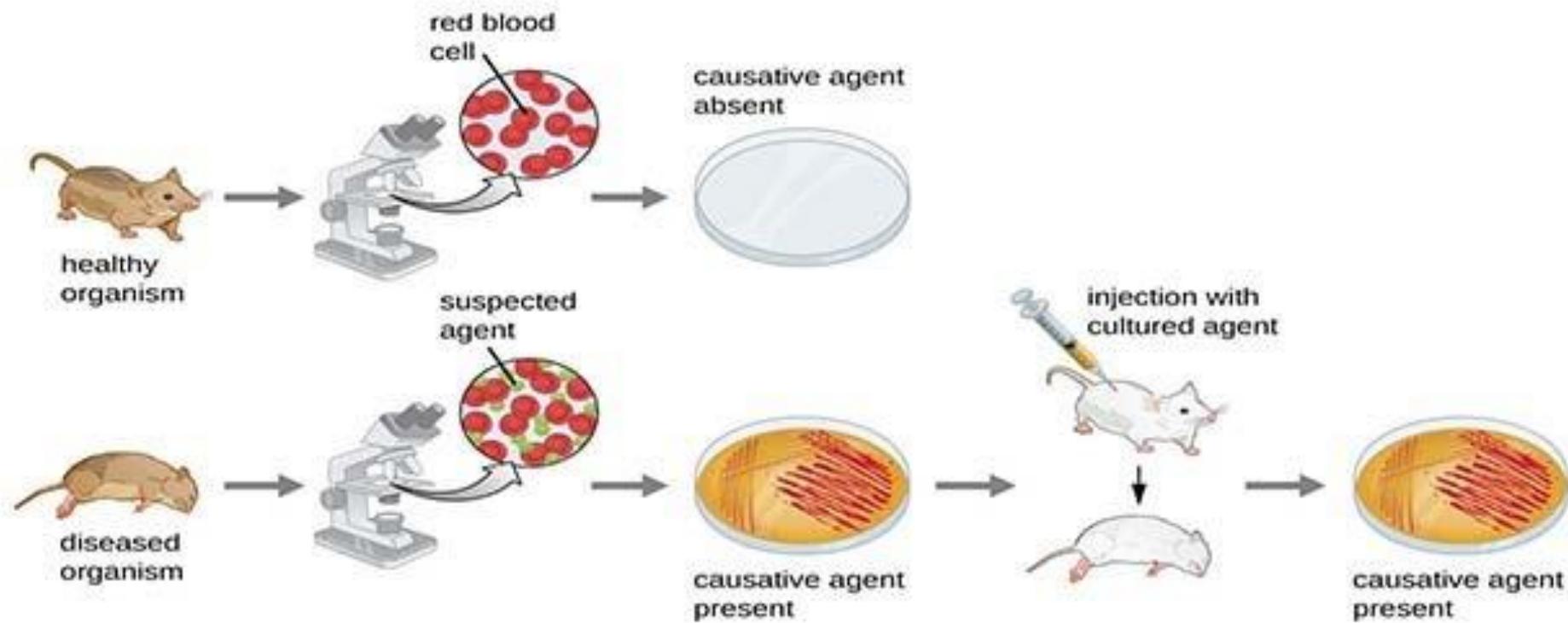
- He discovered the cause of anthrax (*Bacillus anthracis*) –
- from blood of dead cattle
- cultured bacteria in pure culture
- injected bacteria in live cattle and they died,
- then again cultured the bacteria in pure culture.
- This led to the establishment of a procedure for determining microbial cause of disease

Robert Koch, 1876

# KOCH'S POSTULATES



Robert Koch, 1876



1 The suspected causative agent must be absent from all healthy organisms but present in all diseased organisms.

2 The causative agent must be isolated from the diseased organism and grown in pure culture.

3 The cultured agent must cause the same disease when inoculated into a healthy, susceptible organism.

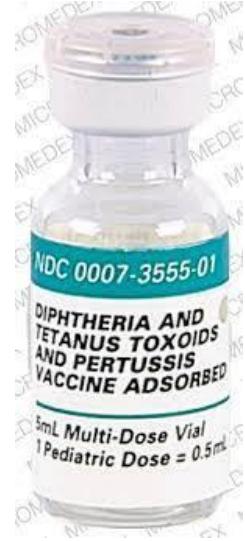
4 The same causative agent must then be reisolated from the inoculated, diseased organism.

# ROBERT KOCH

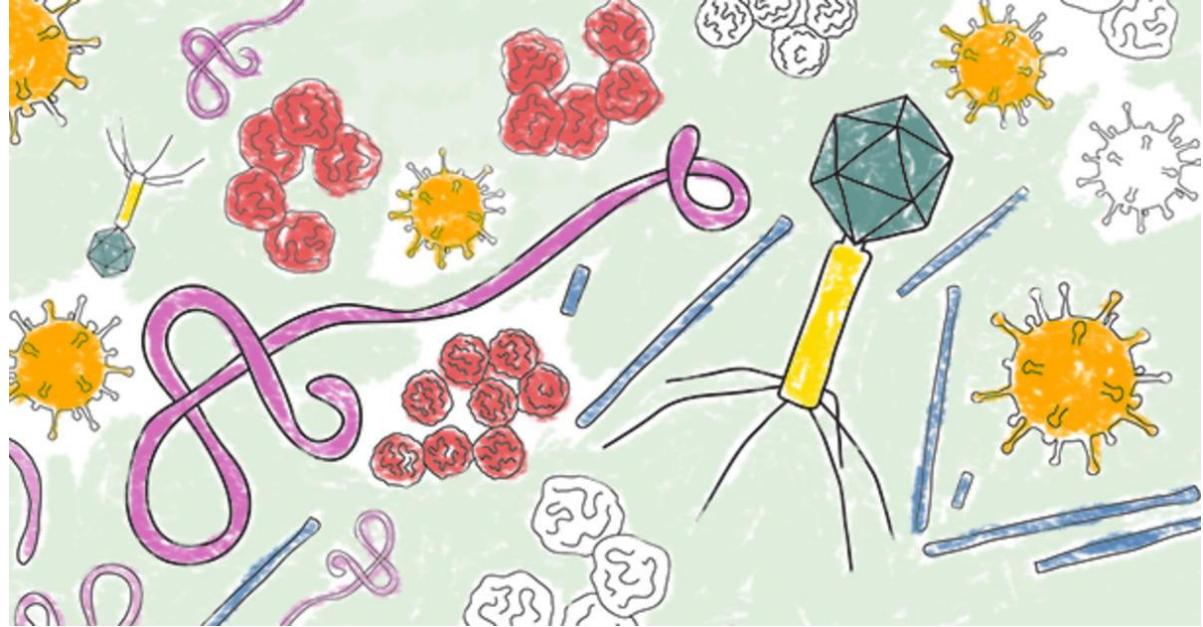
- Anthrax
- Cholera
- Typhoid
- Pneumonia
- Tetanus
- Plague
- Leprosy
- Tuberculosis



Name of the bacterial disease	Causative pathogen	Shape	General Symptoms
Cholera	<i>Vibrio cholerae</i>	Comma-shaped (Vibrio)	Severe diarrhea, irritation of skin around anus, very watery stool; vomiting and muscular cramps, dehydration of the body etc.
E. coli Infection	<i>Escherichia coli</i>	Rod-shaped (Bacillus)	Abdominal cramping, sudden severe watery diarrhea that may change to bloody stools, gas, loss of appetite/nausea, vomiting (uncommon), fatigue, fever etc.
Dysentery	<i>Shigella dysenteriae</i>	Rod-shaped (Bacillus)	Watery diarrhea initially that may evolve to contain mucus and blood. Loss of appetite, straining during bowel movements, with accompanying rectal pain.
Typhoid fever	<i>Salmonella typhi</i>	Rod-shaped (Bacillus)	Characterized by sustained fever up to 40 °C (104 °F), headache and lethargy. It is followed by enlargement of spleen, pain in stomach and sometime rose coloured rashes on body.
Tuberculosis	<i>Mycobacterium tuberculosis</i>	Rod-shaped (Bacillus)	Symptoms may include chest pain and a prolonged cough producing sputum, fever, chills, night sweats, loss of appetite, weight loss, and fatigue.



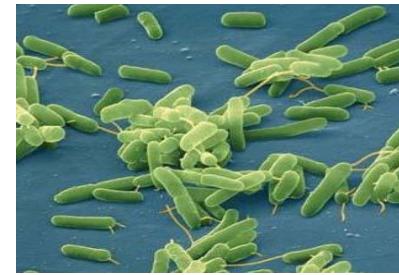
Name of the bacterial disease	Causative pathogen	Shape	General Symptoms
Pertussis (Kali Khansi)	<i>Bordetella pertussis</i>	Intermediate spherical and rod shaped bacteria (Coccobacillus)	Early symptoms include cough, sneezing & runny nose. Late symptoms include ever hacking cough followed by high intake of breath. Gasping
Gonorrhea	<i>Neisseria gonorrhoeae</i>	Group of two cocci (Diplococcus)	Burning and pain during urinating. Leads to sterility
Tetanus	<i>Clostridium tetani</i>	Rod-shaped (Bacillus)	Painful stiffness of the neck (lockjaw) and difficulty in swallowing.
Diphtheria	<i>Corynebacterium diphtheriae</i>	Rod-shaped (Bacillus)	Upper respiratory tract illness having sore throat, an adherent layer on the tonsils, nasal cavity, pharynx. Toxins produce high fever, damage the nervous system and heart
Meningitis (Bacterial)	<i>Neisseria meningitidis</i>	Group of two cocci (Diplococcus)	Headache and neck stiffness associated with fever, confusion or altered consciousness, vomiting, and an inability to tolerate light or loud noises.
Impetigo (Bacterial skin infection)	<i>Staphylococcus aureus</i>	Clusters of cocci (Staphylococcus)	Fluid-filled blisters, itchy rash, skin lesions, swollen lymph nodes etc.
	<i>Streptococcus pyogenes</i>	Chain of cocci (Streptococcus)	



# MICROBIOLOGY

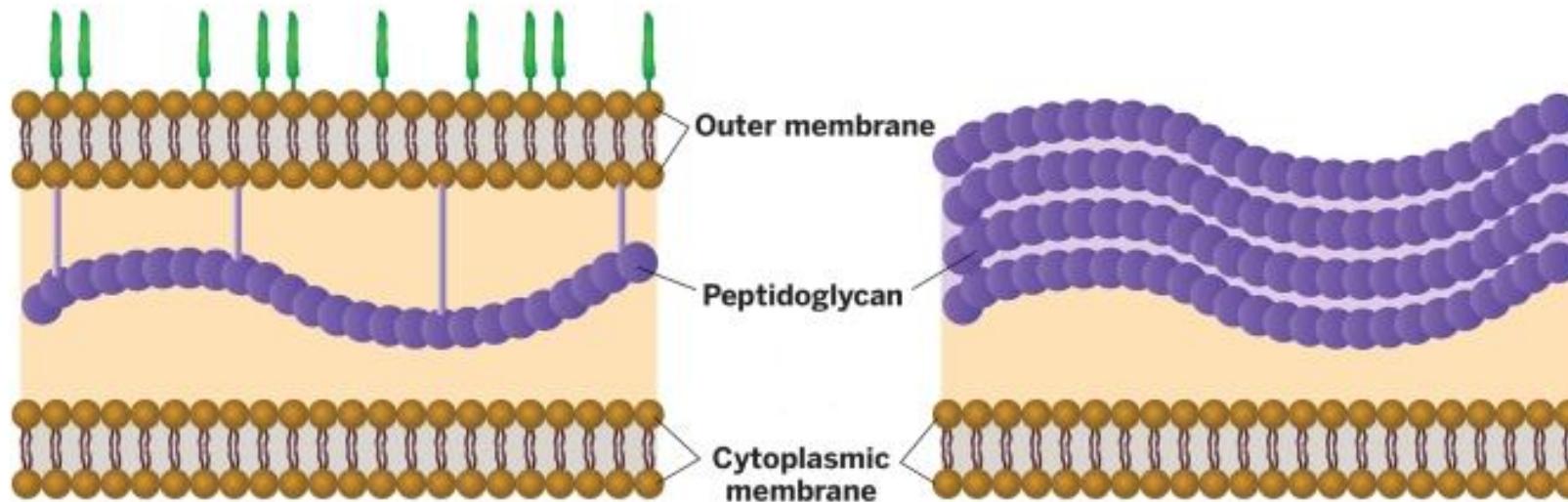
Study of microscopic organisms:

- Archaebacteria
- Bacteria
- Protozoa
- Virus
- Prions

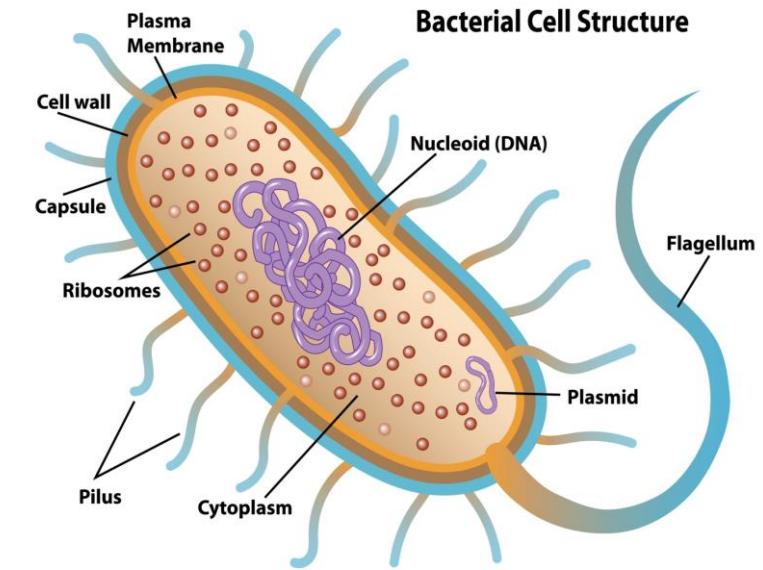


# BACTERIA: MEMBRANE

## GRAM-NEGATIVE

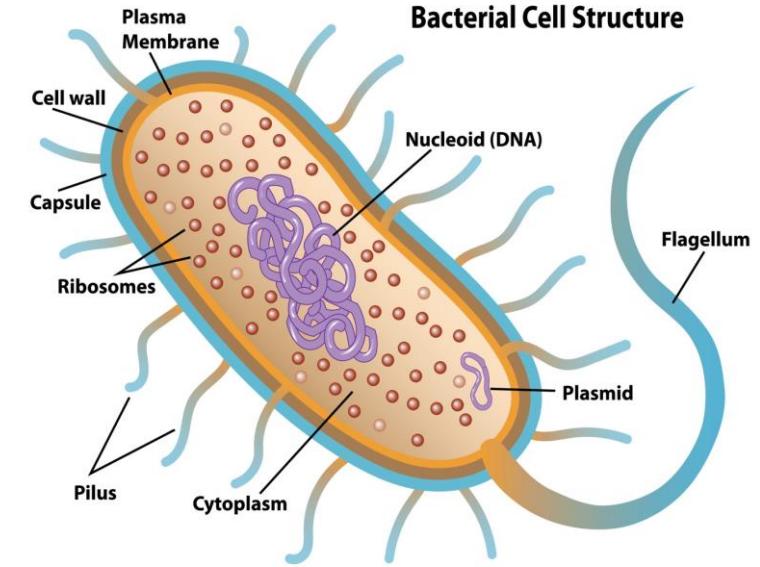


## GRAM-POSITIVE

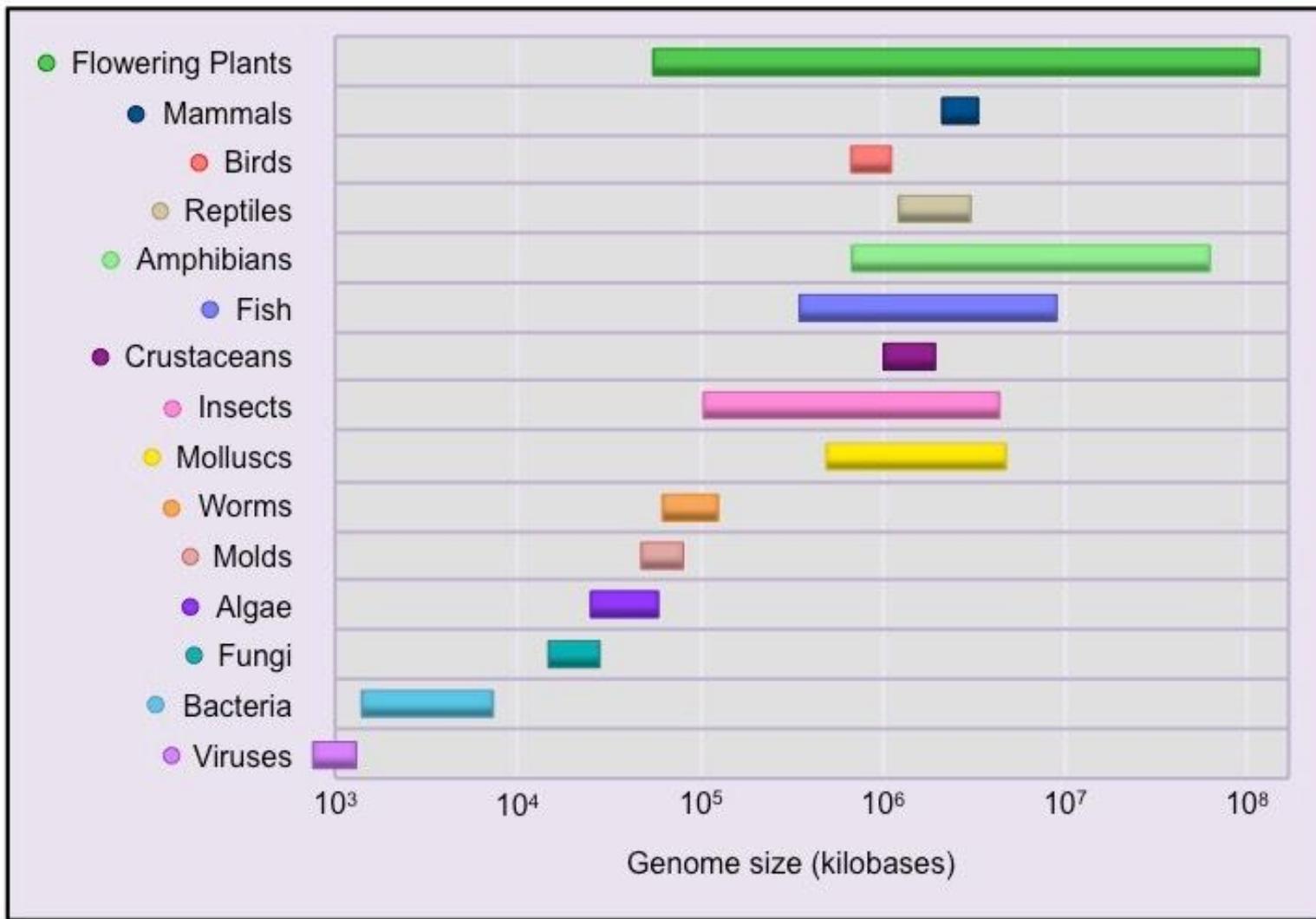


# BACTERIA: PLASMID

- Autonomous DNA molecule (extrachromosomal)
- It is a replicon (self-replicating)
- 5kbp-2mbp
- Mostly circular, double-stranded
- Copy number- 1-100 or more
- Provides special characteristics to the host bacteria:
  1. Antibiotic resistance (*E. coli, Staphylococcus sp.*)
  2. Killer properties by producing toxin (*Bacillus thuringiensis*)
  3. Degradative- Oil spills (*Pseudomonas sp.*)
  4. Virulence- Pathogenicity (ability to infect)
  5. Tumor formation in plants (*Agrobacter tumefaciens*)



# BACTERIA: GENOME SIZE COMPARISON



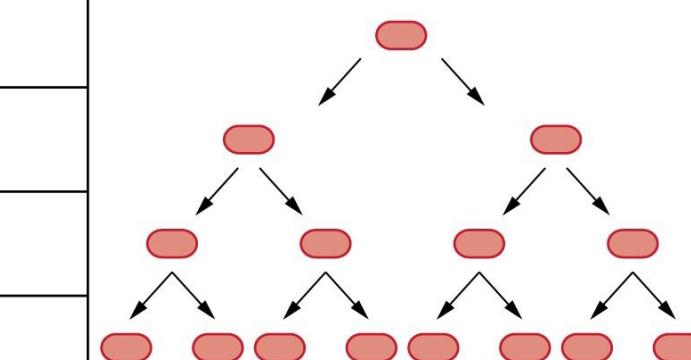
# CALCULATION OF GENERATION TIME

Generation time =  $t$  (time in minutes)/  $n$  (no. of generations)

Calculation of final density of bacterial culture:

$$N_t = N_0 \times 2^n$$

- ( $N_t$ ) number of cells in population
- ( $N_0$ ) original number of cells in the population
- ( $n$ ) number of divisions
- Example
  - $N_0 = 10$  cells in original population
  - $n = 12$ 
    - 4 hours assuming 20 minute generation time
  - $N_t = 10 \times 2^{12}$
  - $N_t = 10 \times 4,096$
  - $N_t = 40,960$

Number of generations ( $n$ )	Number of cells	Each division adds two new cells
0	1	
1	2	
2	4	
3	8	

# CALCULATION OF NO. OF GENERATION

$$N = \frac{\log N_t - \log N_0}{\log 2}$$

N = Number of generations in a given amount of time

$\log N_0$  = number of cells at a early time point in the exponential phase

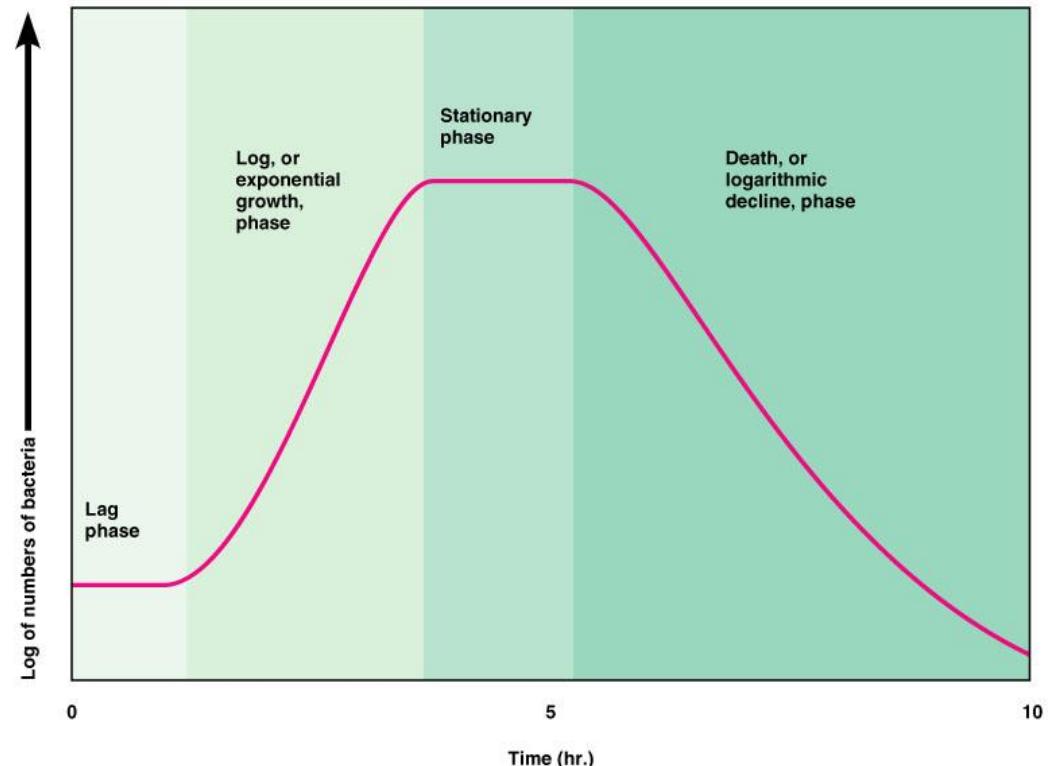
$\log N_t$  = number of cells at a later time point in the exponential phase

t = time in minutes between  $N_0$  and  $N_t$

Example: What is generation time for bacterial population starting at 100 cells and growing logarithmically for 5 hours, resulting in  $1.7 \times 10^6$  cells?

$$n = \frac{\log (1.7 \times 10^6) - \log 100}{\log 2} = 14 \text{ gen in 5 hrs}$$

$$g = 5 \text{ hrs} / 14 \text{ gen} = 0.357 \text{ hrs} = 21 \text{ min}$$



**Not all are disease causing, many are necessary for human life too!**

Product or Process	Contribution of Microorganism
<b>Foods and Beverages</b>	
Cheese	Flavoring and ripening produced by bacteria and fungi; flavors dependent on the source of milk and the type of microorganism
Alcoholic beverages	Alcohol produced by bacteria or yeast by fermentation of sugars in fruit juice or grain
Soy sauce	Produced by fungal fermentation of soybeans
Vinegar	Produced by bacterial fermentation of sugar
Yogurt	Produced by bacteria growing in skim milk
Sour cream	Produced by bacteria growing in cream
Artificial sweetener	Amino acids synthesized by bacteria from sugar
Bread	Rising of dough produced by action of yeast; sourdough results from bacteria-produced acids

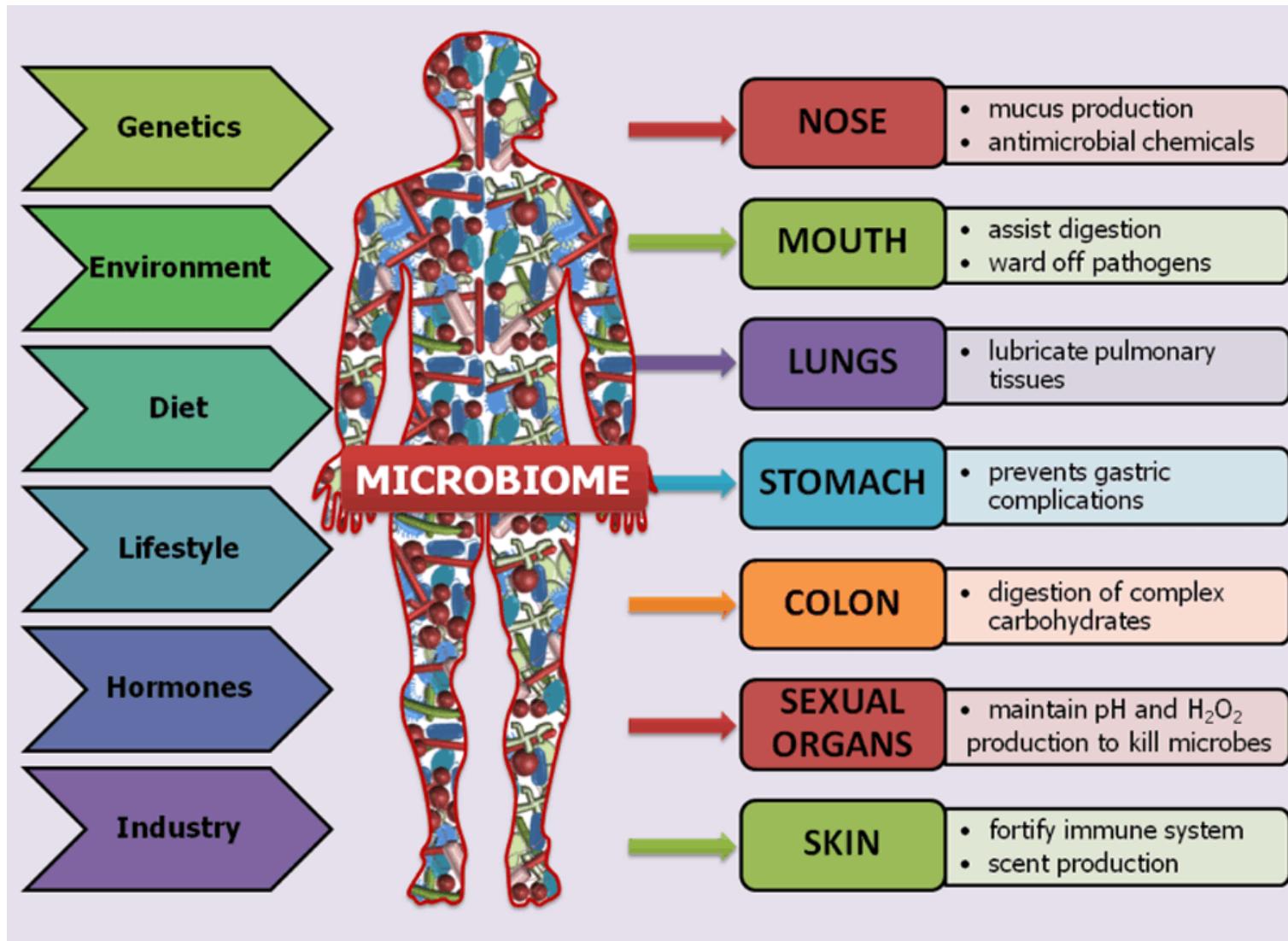


Product or Process	Contribution of Microorganism
<b>Other Products</b>	
Antibiotics	Produced by bacteria and fungi
Human growth hormone, human insulin	Produced by genetically engineered bacteria
Laundry enzymes	Isolated from bacteria
Vitamins	Isolated from bacteria
Diatomaceous earth (used in polishes and buffering compounds)	Composed of cell walls of microscopic algae
Pest control chemicals	Insect pests killed or inhibited by bacterial pathogens

# ECONOMICALLY IMPORTANT BACTERIA

- Role in soil fertility (Nitrogen fixing bacteria- Nitrosomonas)
- Produce antibiotics- Streptomycin/ Tetracycline/ Erythromycin
- Production of butanol (Biofuel) and Methane (Biogas)
- Leather industry (tannery)
- Retting of fibres (jute, cotton, flax, hemp)
- Biotech industry for production of proteins- insulin

# HUMAN MICROBIOME



# HUMAN MICROBIOME

- On an average we have ~40 trillion bacterial cells
- Total cells in our body ~30 trillion
- Foetus born → coated with mother's microbes
- Present in nose, mouth, gut, skin.
- Help digest food/ boost immunity/ synthesize Vitamin B12
- Antibiotics destroy the healthy bacteria
- Takes long time to replenish
- Probiotics enrich gut microbiota

## The Importance of the **MICROBIOME** by the Numbers



**90%**

Up to 90% of all disease can be traced in some way back to the gut and health of the microbiome



**>10,000**

Number of different microbe species researchers have identified living in the human body

**100**

**100 to 1**

The genes in our microbiome outnumber the genes in our genome by about 100 to 1

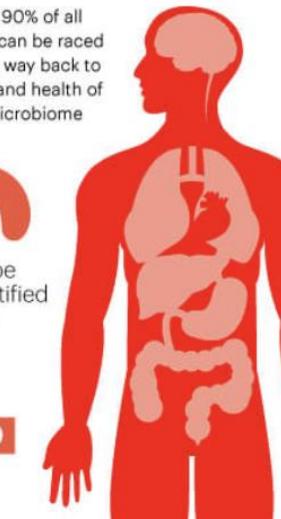
**3.3 million**

Number of non-redundant genes in the human gut microbiome



**99.9%**

Percentage individual humans are identical to one another in terms of host genome



**10-100 trillion**

Number of symbiotic microbial cells harbored by each person, primarily bacteria in the gut, that make up the human microbiota

**10X**

There are 10 times as many outside organisms as there are human cells in the human body



**22,000**

Approximate number genes in the human gene catalog

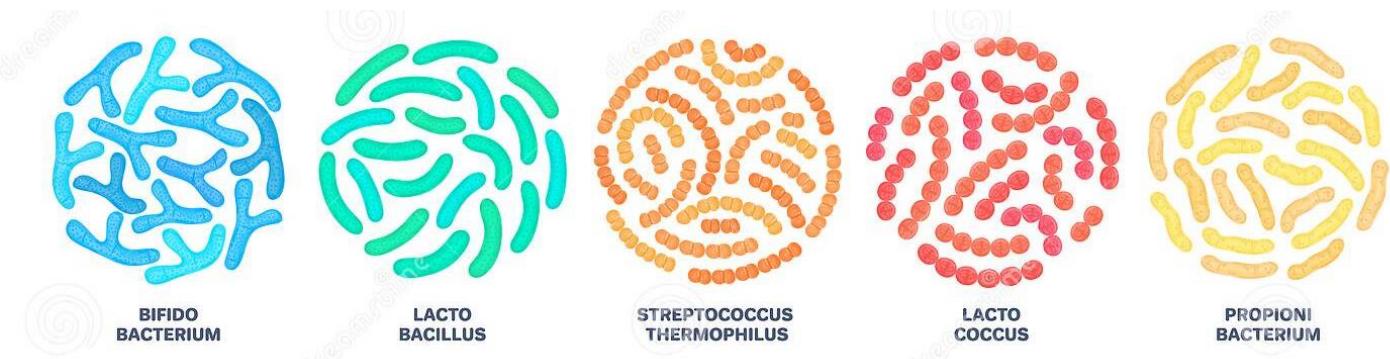


**80%-90%**

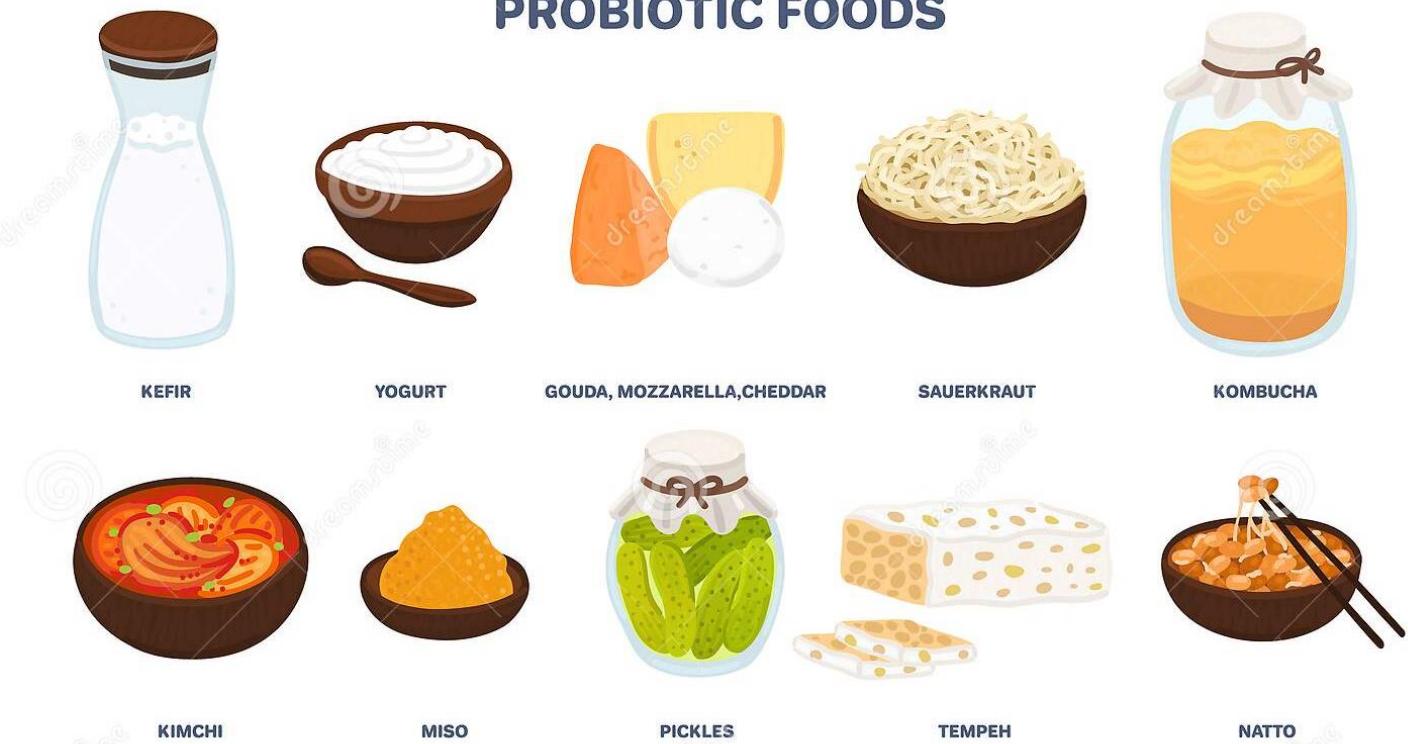
Percentage individual humans are different from one another in terms of the microbiome

# PROBIOTICS

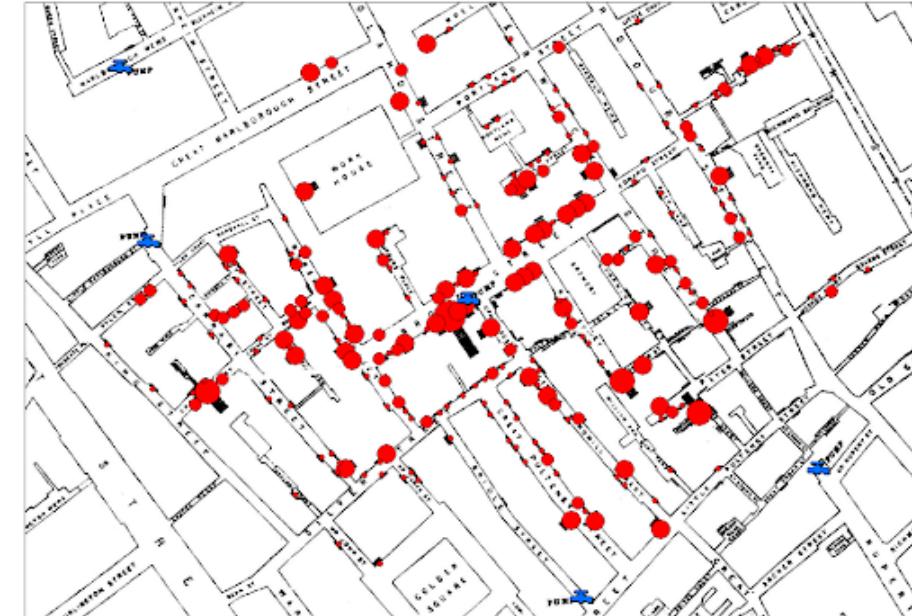
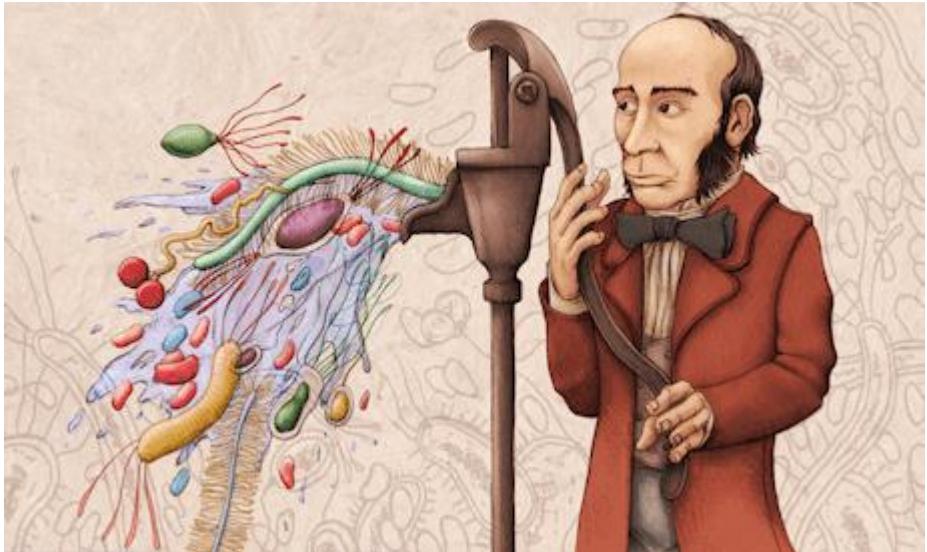
- Keep digestive tract healthy
- Boost immune system
- Help lower blood pressure
- Improve cholesterol level
- Aid weight loss
- Reduce belly fat
- Mental well-being



## PROBIOTIC FOODS



# MAPPING LONDON EPIDEMIC



- Cholera Epidemic, 1854

John Snow- Anesthesiologist

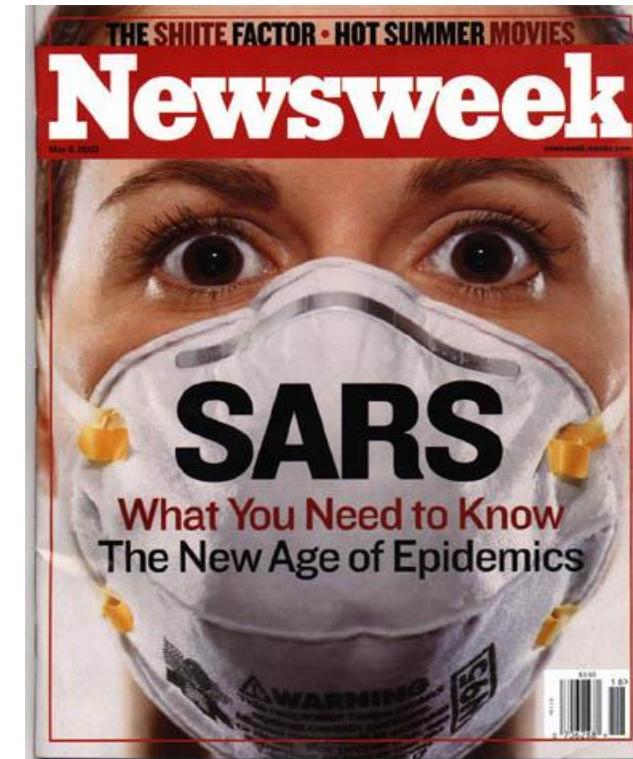
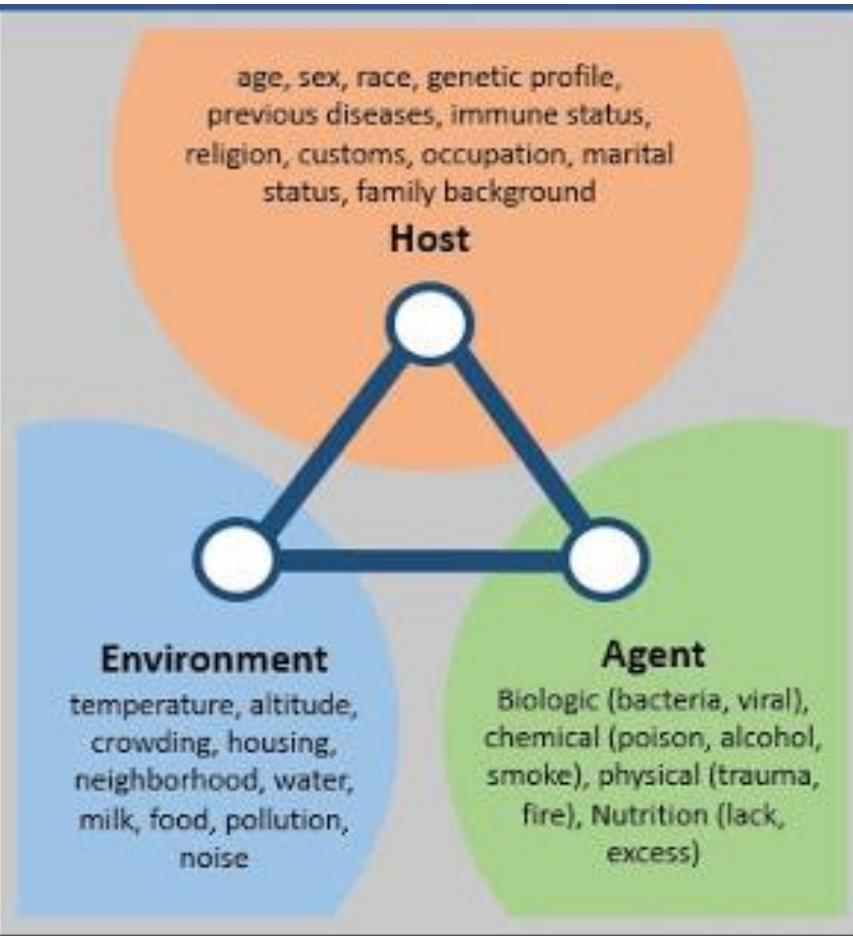
- Proposed that cholera was attributable to a self-replicating agent which was excreted in the cholera evacuations and inadvertently ingested through medium of water.
- Water pumps are zones of cholera outbreak

# EPIDEMIOLOGY



- “Epidemiology is the study of patterns of disease and injury in human populations and the application of this study to the control of health problems. With its focus on disease causation and prevention, this field is a fundamental science of both preventive medicine and public health.”<sup>1</sup>

- Basic model to study health problems
- 3 factors
  - Host
  - Environment
  - Agent
- Disease is produced by exposure of a susceptible **host** to an noxious **agent** in the presence of **environmental factors** that aid or hinder agents of disease



# Epidemics vs. Pandemics



## Epidemic

- Event in which a disease is actively spreading
- Often used to describe problem that has grown out of control



## Pandemic

- Relates to geographic spread
- Describes disease that affects a whole country or the entire world

# EPIDEMIOLOGY



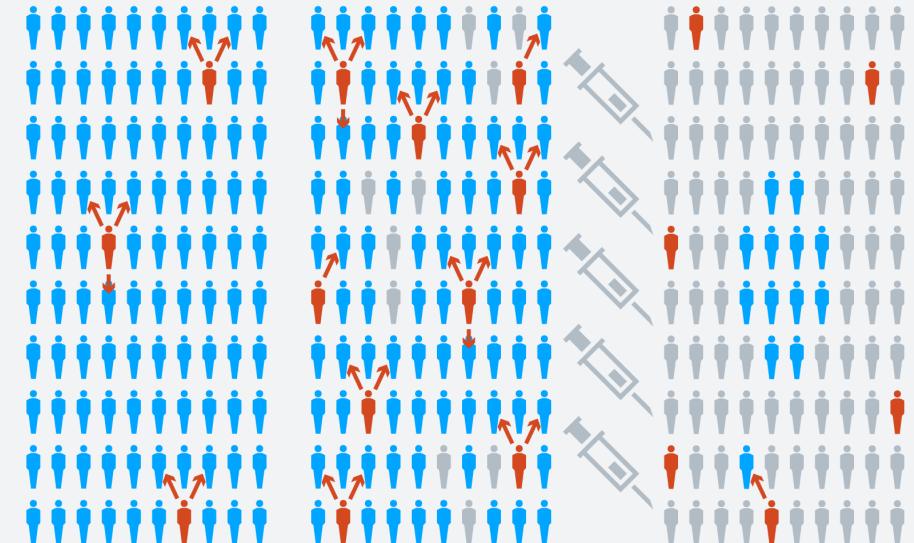
Flatten the curve- Steps taken to manage the disease (Quarantine/ more testing/ healthcare management/ masking)

Herd Immunity- when 60-70% of the population is immunized against a disease

## How does herd immunity work?

- Healthy (not immune)
- Ill (infectious)
- Immune (either after vaccination or recovery from an infection)

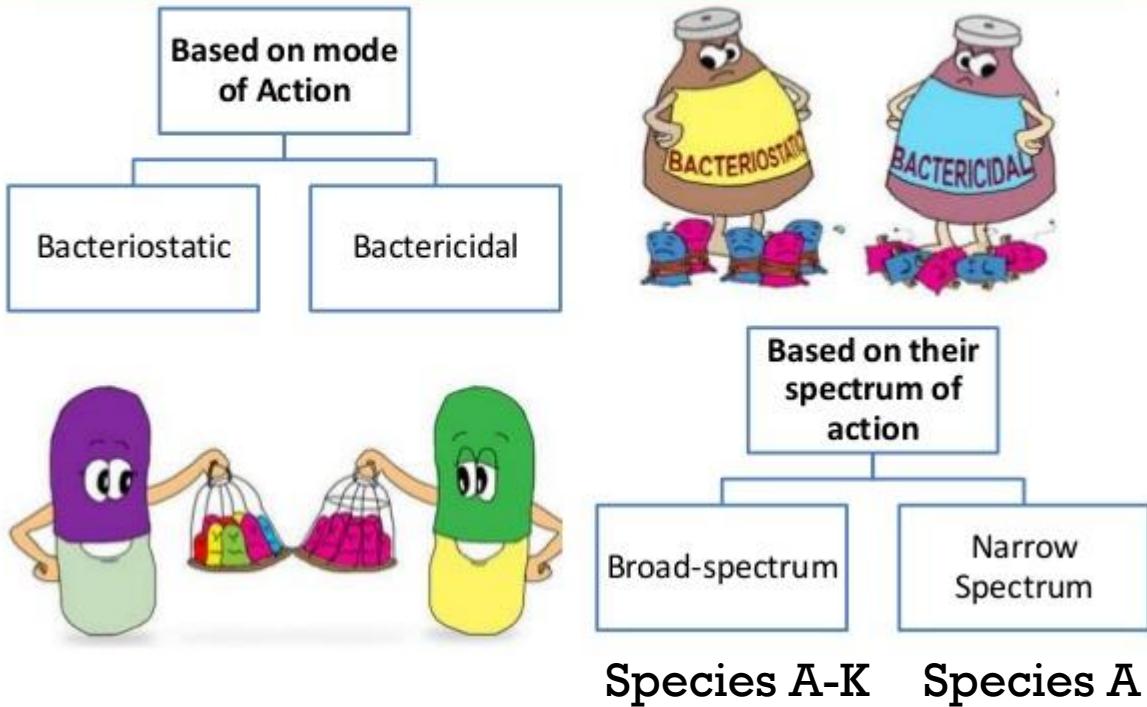
### Patterns of virus spread



### Herd immunity

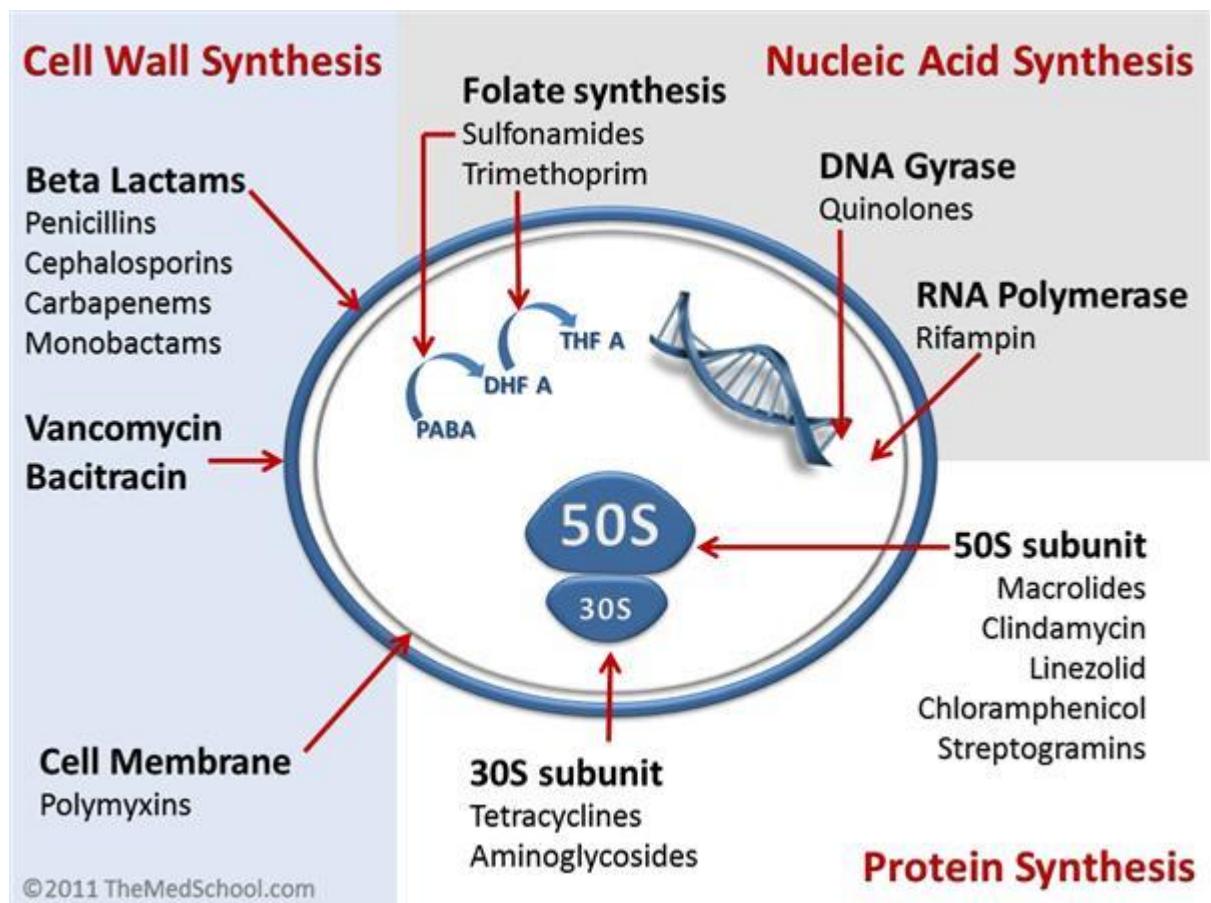
The spread of viruses is limited. People without immunity are better protected.

# Classification of Antibiotics

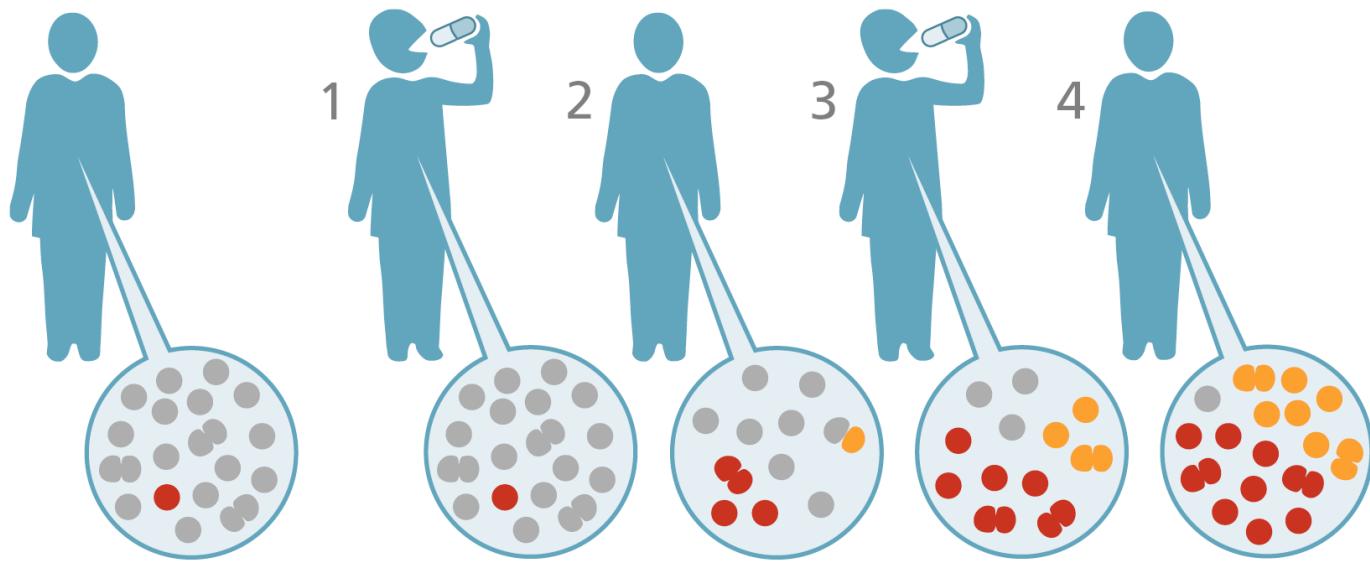


# ANTIBIOTICS

## How antibiotics kill bacteria: mode of action

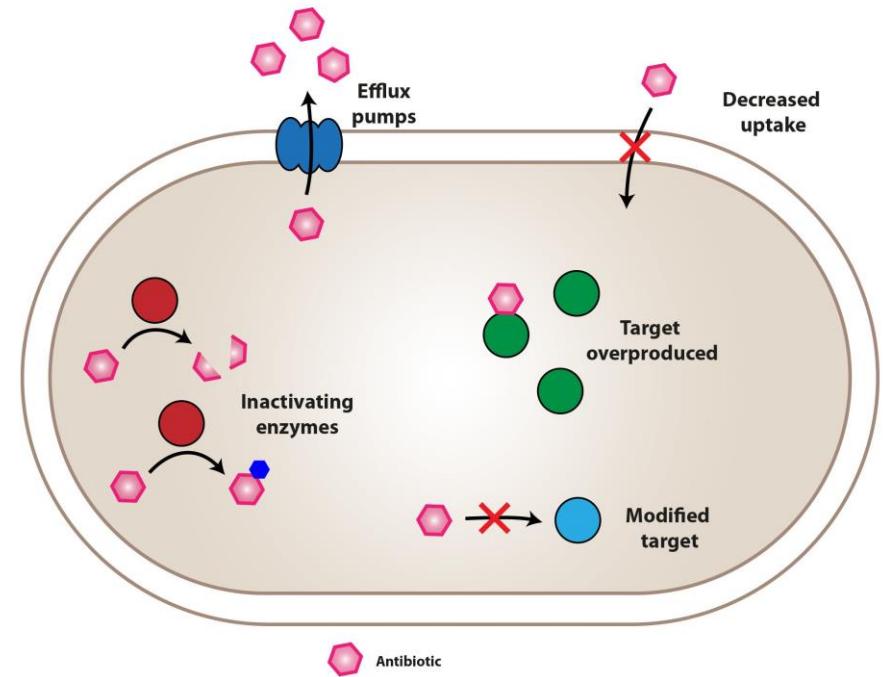


# How does antibiotic resistance occur?



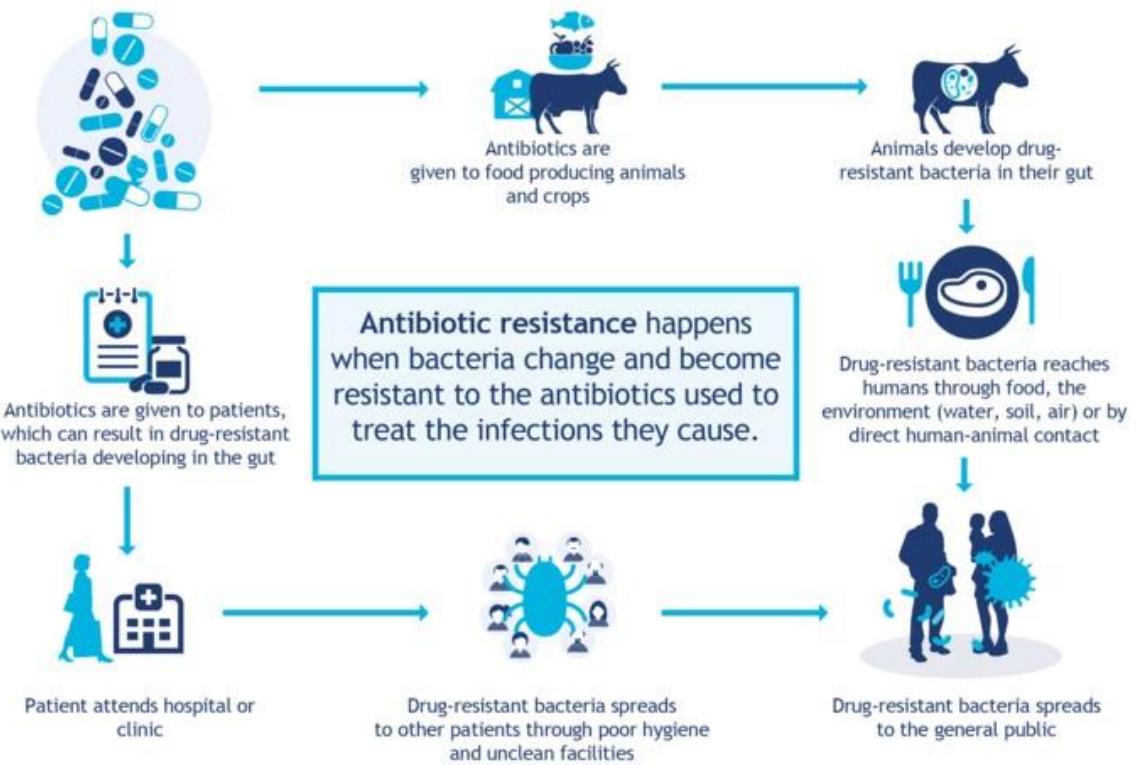
- bacterium sensitive to the antibiotic drug
- antibiotic-resistant bacterium present before initiation of treatment
- antibiotic-resistant bacterium appearing during treatment (by mutation)

## Mechanisms developed by bacteria against the effect of antibiotic



# ANTIBIOTIC RESISTANCE

## HOW IT SPREADS



[www.who.int/drugresistance](http://www.who.int/drugresistance)

#AntibioticResistance



### What you can do to prevent Antibiotic Resistance?

- Avoid broad spectrum antibiotics
- Take full prescription
- Prevent infections by maintaining hygiene (washing hands frequently, avoiding contact with sick people etc.)

# ANTIBIOTIC RESISTANCE WHAT YOU CAN DO



Antibiotic resistance happens when bacteria change and become resistant to the antibiotics used to treat the infections they cause.



- 1 Only use antibiotics when prescribed by a certified health professional
- 2 Always take the full prescription, even if you feel better
- 3 Never use left over antibiotics
- 4 Never share antibiotics with others
- 5 Prevent infections by regularly washing your hands, avoiding contact with sick people and keeping your vaccinations up to date

[www.who.int/drugresistance](http://www.who.int/drugresistance)

#AntibioticResistance

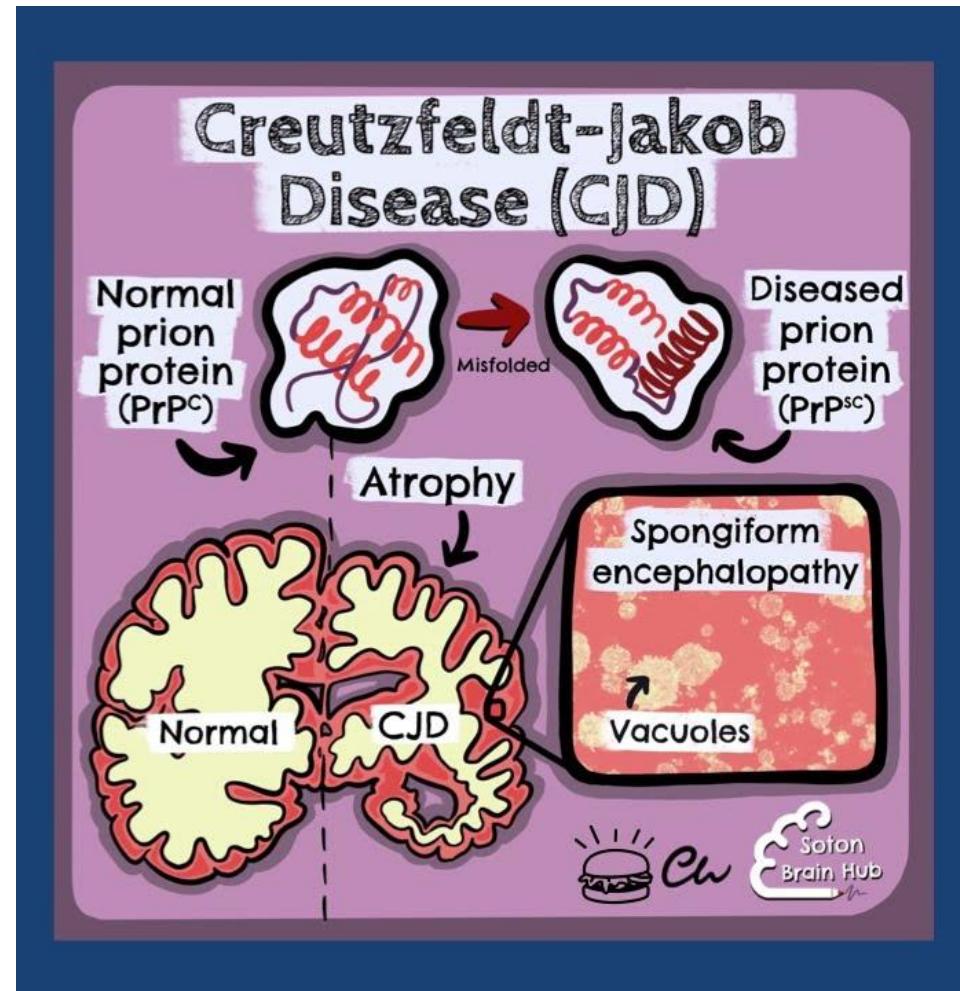


Microbiology- Study of microscopic organisms:

- Archaebacteria
- Bacteria
- Protozoa
- Virus
- Prions

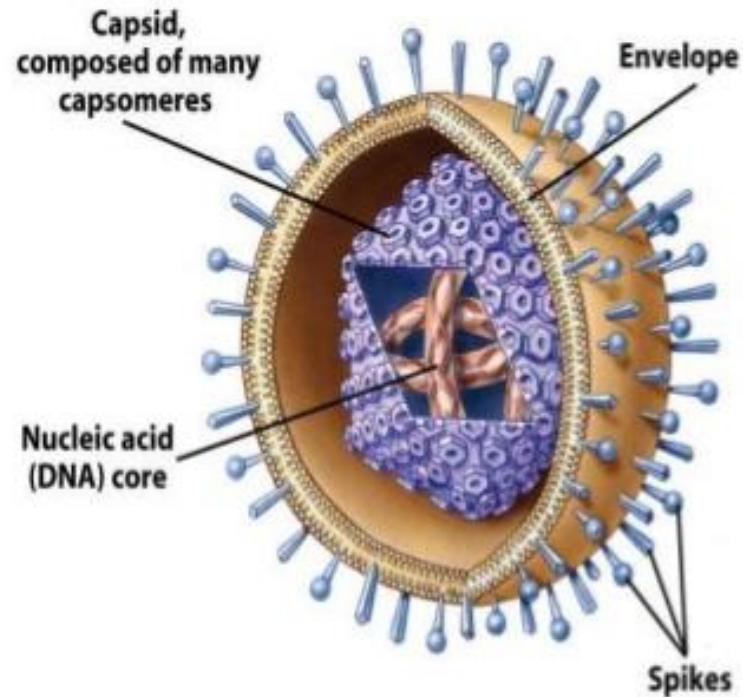
- Transmissible spongiform encephalopathy (TSE) are a group of neurodegenerative disorders characterized by accumulation of prion in central and peripheral nervous system in both animals and humans.
- Can be sporadic/ infective

# PRIONS

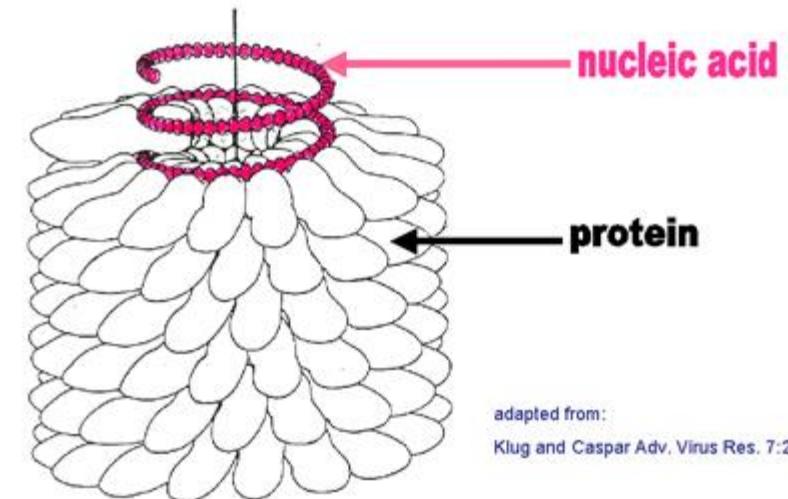


# VIRUS: STRUCTURE

- Protective Shell – Capsid
  - Made of many identical protein subunits
  - Protect the genetic material
  - May be involved in cell entry
  - Symmetrically organized
  - 50% of weight
  - Enveloped or non-enveloped
- Envelope: A lipid-containing membrane that surrounds some virus particles.  
✓ located outside the capsid)  
✓ It is acquired during viral maturation

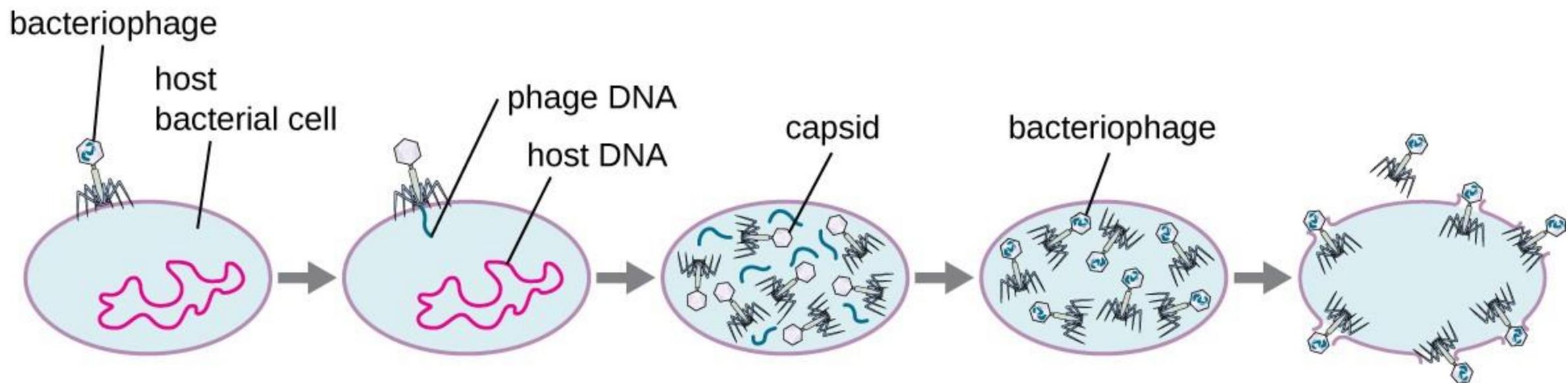


## TOBACCO MOSAIC VIRUS



adapted from:  
Klug and Caspar Adv. Virus Res. 7:225

# VIRUS- LYTIC CYCLE



**1 Attachment**  
The phage attaches to the surface of the host.

Attach using spike proteins

**2 Penetration**  
The viral DNA enters the host cell.

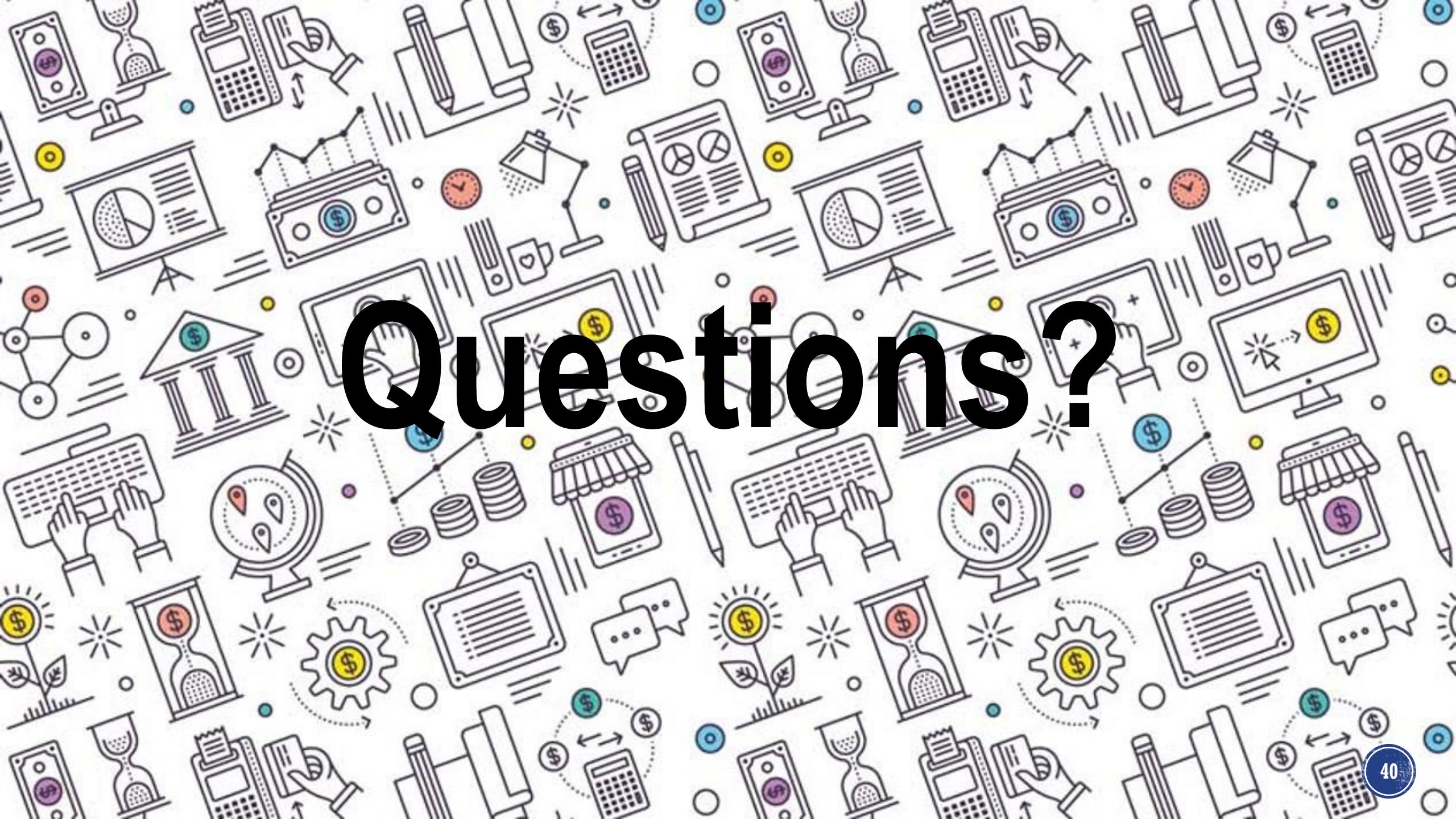
The cell lyses, releasing the newly made phages.

**3 Biosynthesis**  
Phage DNA replicates and phage proteins are made.

Acquire envelope during Lysis

**4 Maturation**  
New phage particles are assembled.

**5 Lysis**  
The cell lyses, releasing the newly made phages.



# Questions?