Microbiology

Lecture 6
Microbial Nutrition, Growth and Control (II)

Outline

- **Microbial growth in natural environments**
- **Measurement of microbial population size**
- **Principles of microbial control**
- **Physical, chemical and biological control**
- **Antibiotics**
- **Bacterial resistance to antibiotics***

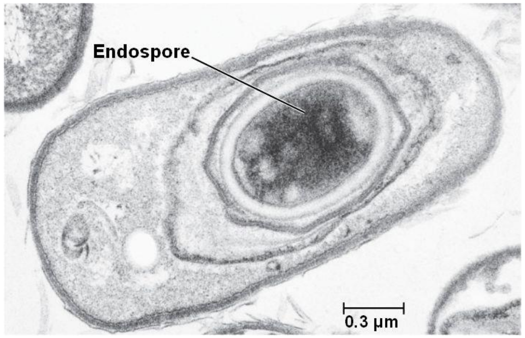
Microbial Growth in Natural Environments

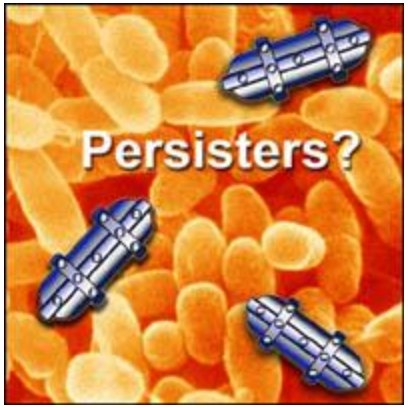
(**Different from lab cultivation**)

Oligotrophy (寡营养) in natural environments

To survive starvation conditions:

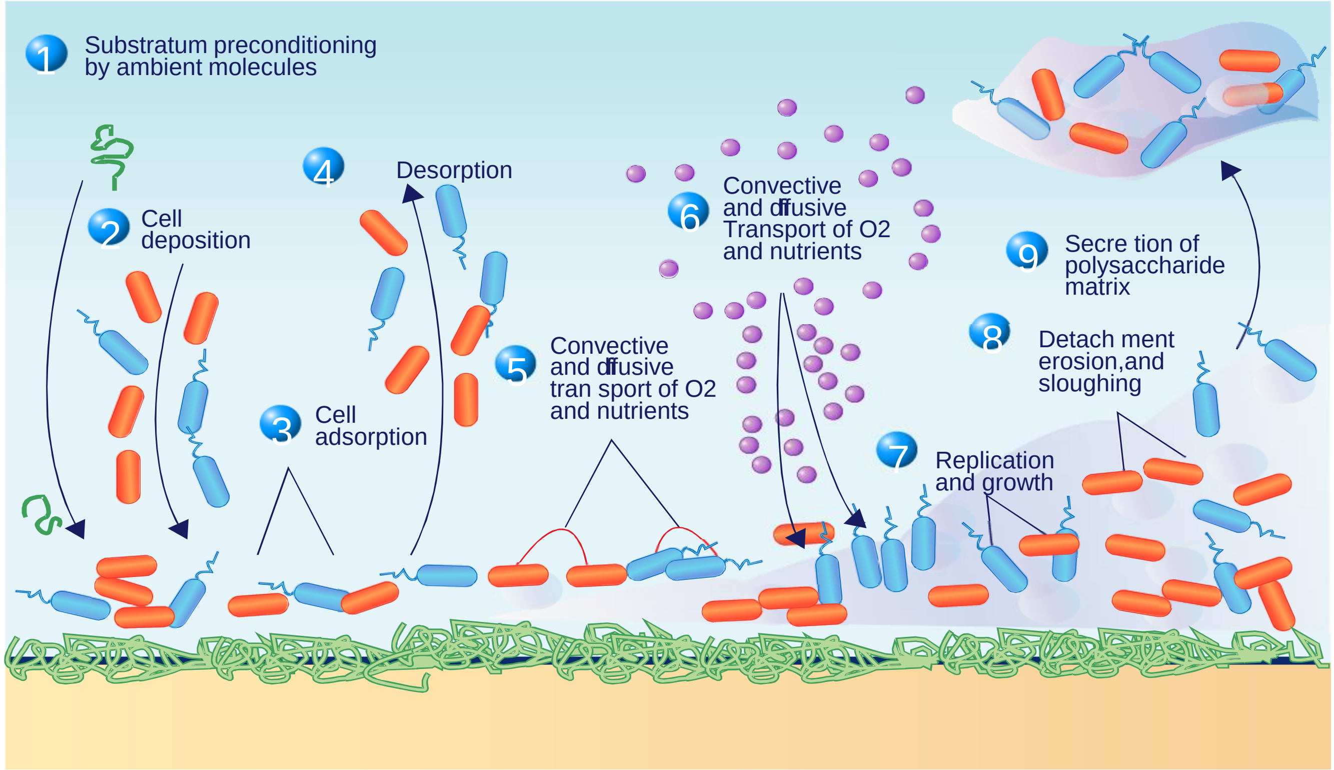
1. Form endospores (spore vs. endospore)
2. Form persisters
3. Function of RpoS (Transcription in bacteria)
4. Function of starvation proteins
    a. increase peptidoglycan cross-linking and cell wall strength
    b. protects DNA
    c. prevent protein denaturation and renature damaged proteins





Growth in biofilm(生物膜)

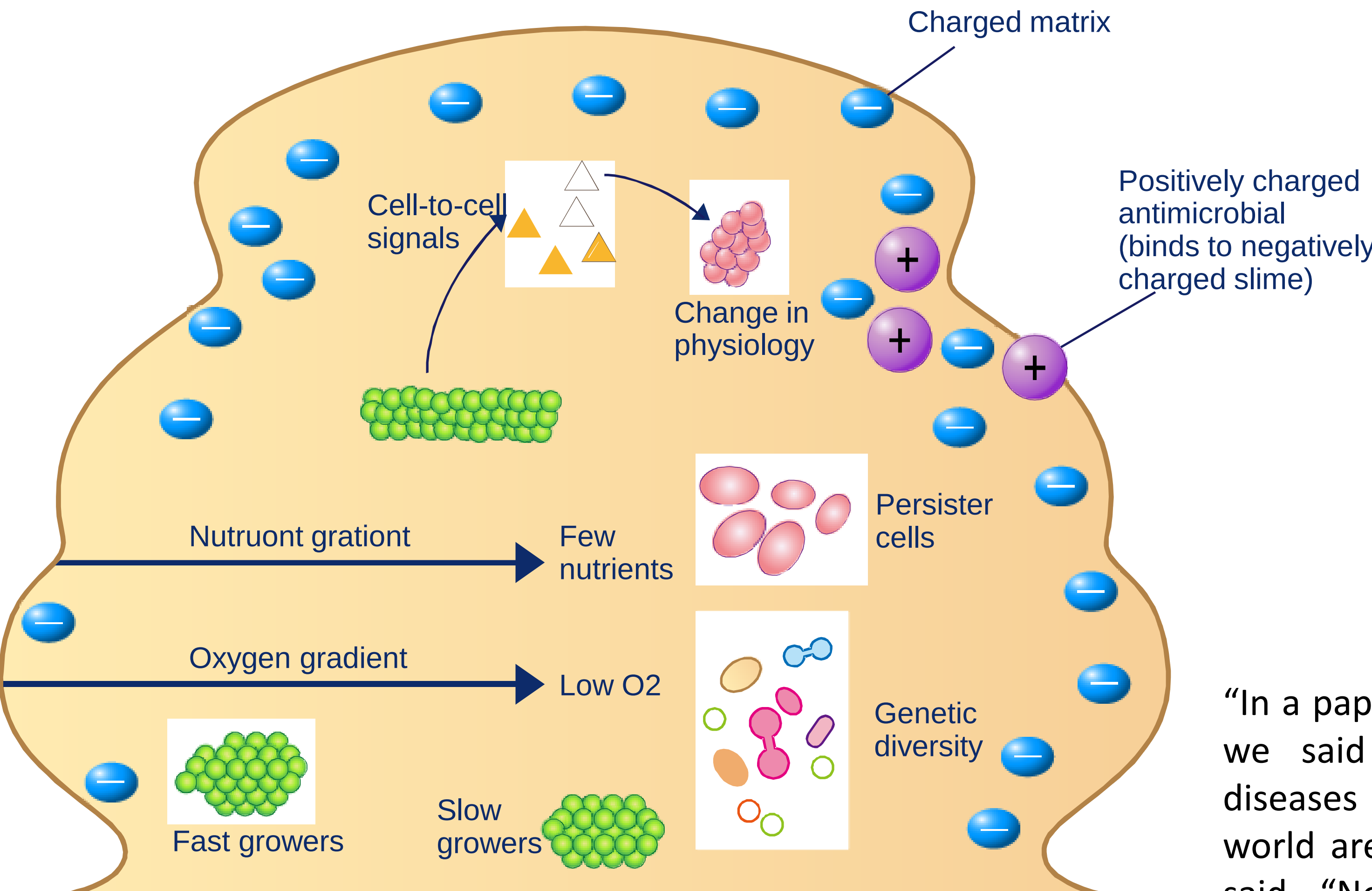
Although ecologists observed as early as the 1940s that more microbes in aquatic environments were found attached to surfaces than were free-floating, only relatively recently has this fact gained the attention of microbiologists.



Biofilm vs. membranes

"The attached microbes are members of complex, slime-encased communities called biofilms."

Heterogeneity in biofilm



Prof. Costerton

"In a paper in Science in 1999, we said 65 percent of all diseases in the developed world are biofilms," Costerton said. "Now the NIH says **80** percent.

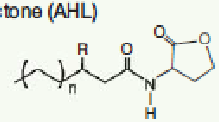
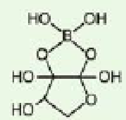
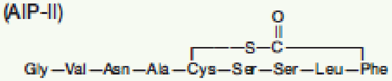
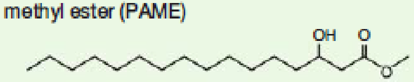
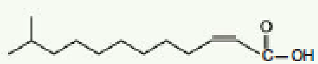
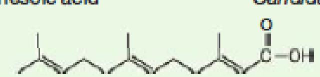
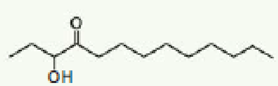
Cell-cell communication within microbial populations

Quorum sensing (群感效应)：Bacterial cells use **molecular signals** to communicate with each other in a **density-dependent manner**.

AHL(N-酰基高丝氨酸内酯): **G-/AI-1**

Furanosylborate (呋喃酰硼酸): **AI-2**

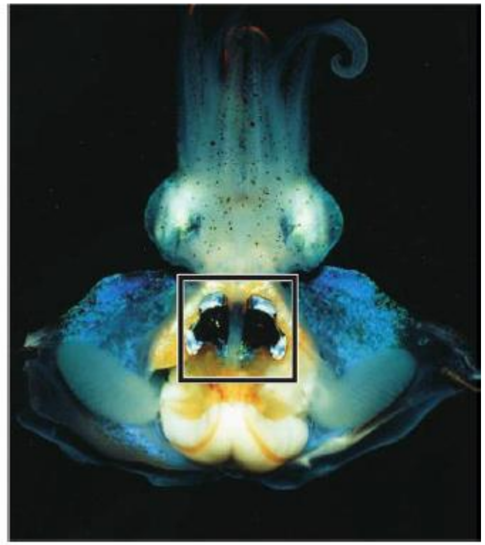
Peptide(寡肽): **G+**

Signal and Structure	Representative Organism	Function Regulated
N-acylhomoserine lactone (AHL) 	*Vibrio fischeri* *Agrobacterium tumefaciens* *Erwinia carotovora* *Pseudomonas aeruginosa* *Burkholderia cepacia*	Bioluminescence Plasmid transfer Virulence and antibiotic production Virulence and biofilm formation Virulence
Furanosylborate (AI-2) 	*Vibrio harveyi*[a]	Bioluminescence
Cyclic thiolactone (AIP-II) 	*Staphylococcus aureus*	Virulence
Hydroxy-palmitic acid methyl ester (PAME) 	*Ralstonia solanacearum*	Virulence
Methyl dodecenoic acid 	*Xanthomonas campestris*	Virulence
Farnesoic acid 	*Candida albicans*	Dimorphic transition and virulence
3-hydroxytridecan-4-one 	*Vibrio cholerae*	Virulence

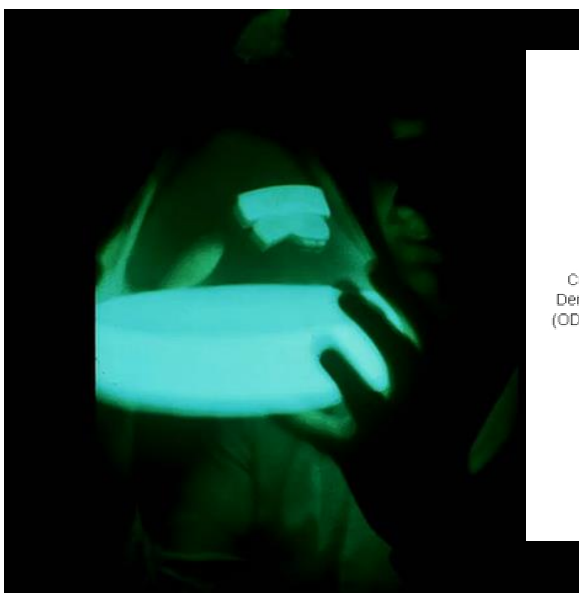
Quorum sensing often relates with bacteria in biofilm. Why?

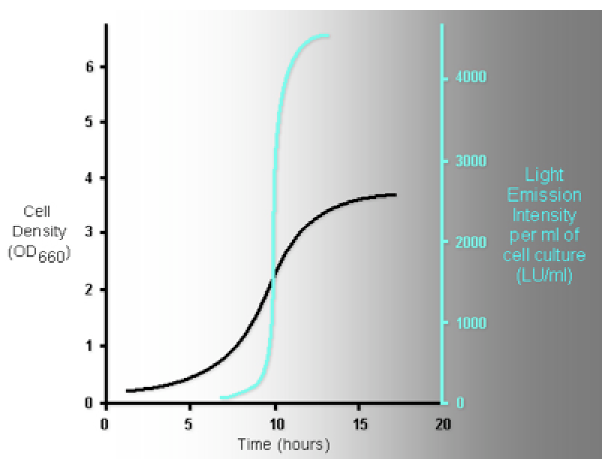
A story from squid (see“视频资源”)

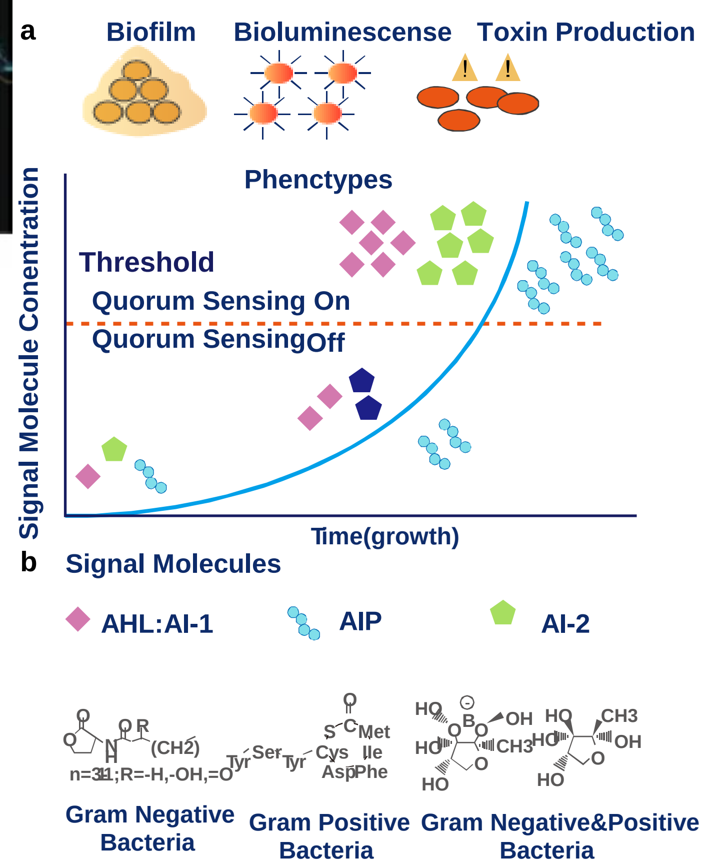
(a) *E. scolopes*, the bobtail squid



(b) Light organ







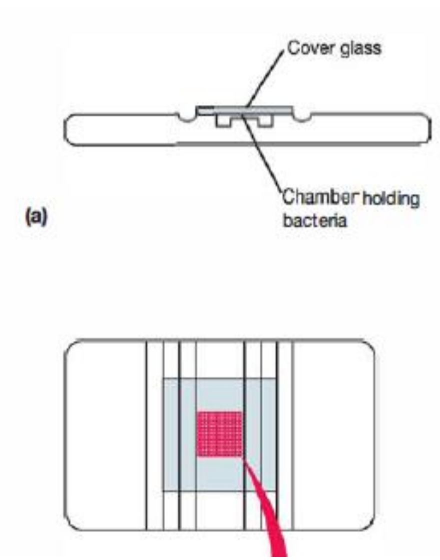
Measurement of Microbial Population Size

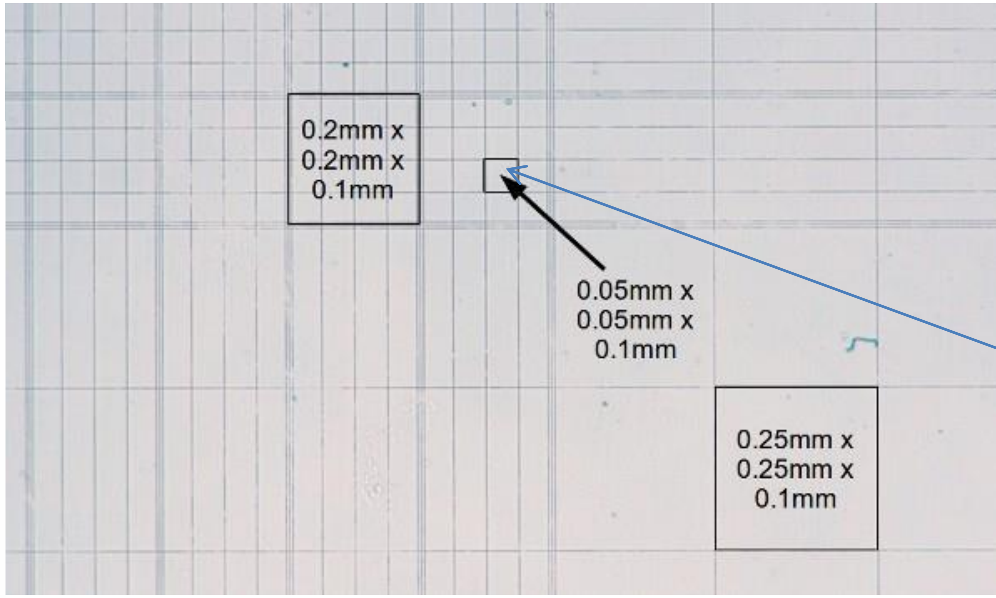
- **Direct measurement of cell numbers**

- ***Viable*** **counting methods**

- **Measurement of cell mass**

Using counting chambers (计数板)



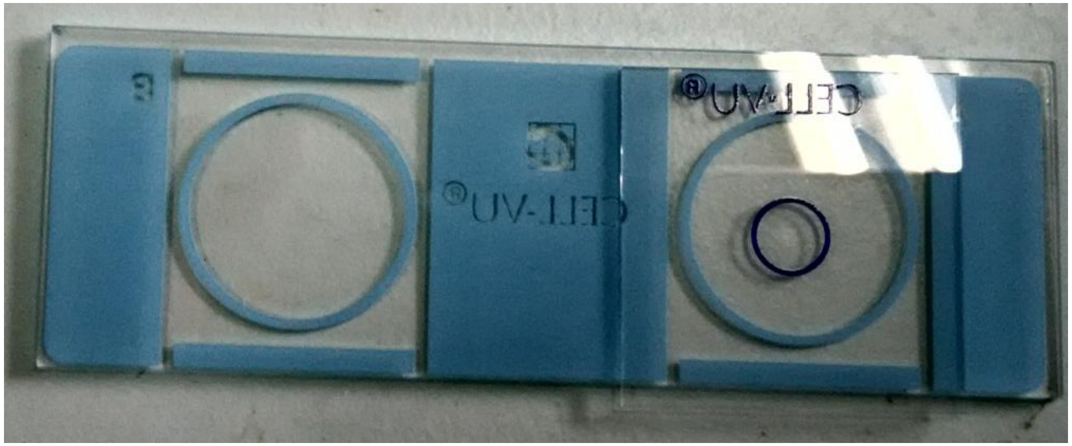


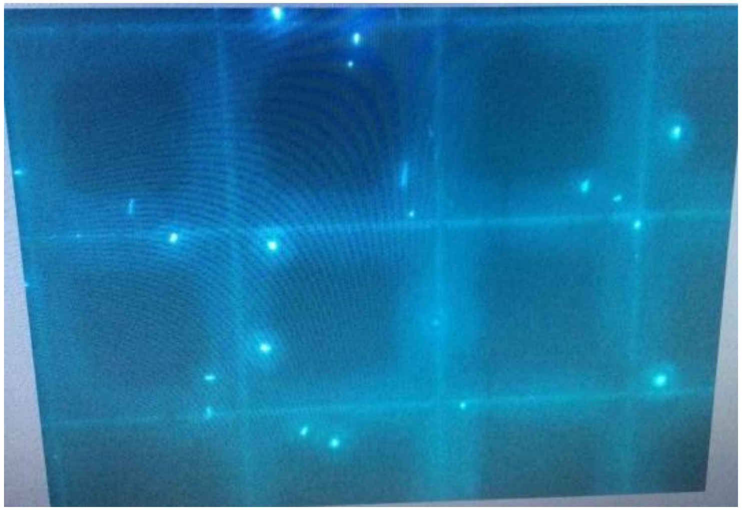
Volume= 2.5×10^{-4} ul
=2.5×10^{-7} mL

1 cell in each of the area= cell density?

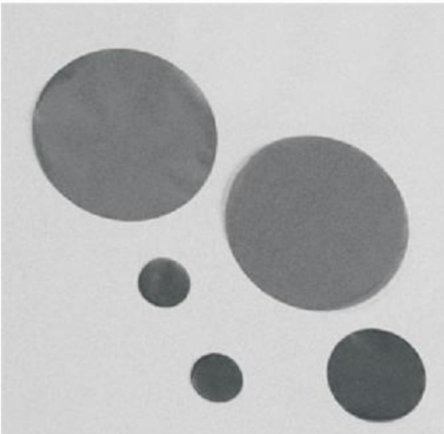
Petroff-Hausser counting chamber

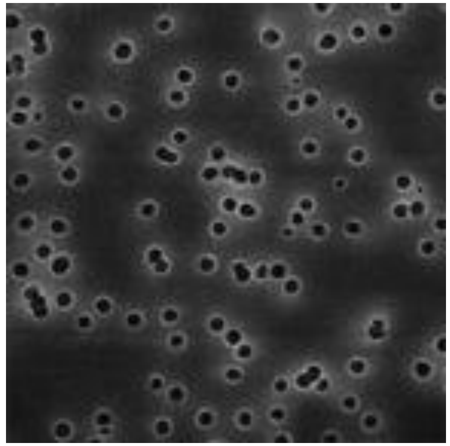
- ◆**easy, inexpensive, and quick**
- ◆**count all cells**
- ◆**cannot distinguish living from dead cells**
- ◆**can only determine relatively high concentration of cells**



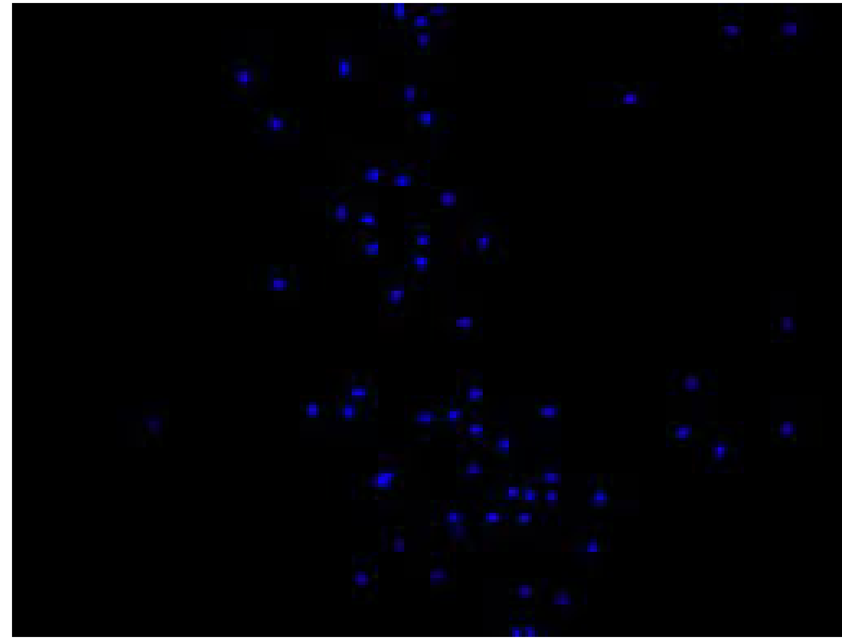


Use thinner chamber (10-20 µm) and fluorescence (more specific and clearer)





Black polycarbonate
membrane (poresize: **0.2μm**)



DAPI stained microbial cells

Cell density (no. of cells mL^{-1}) =

1cm^2(10^8 μm^2) **100×100=10^{4}μm^2** **100 cells**

$$\frac{\text{(Total filter area/Area of a field)} \times \text{average cell number per field}}{\text{volume of filtered water}}$$

10 mL

10^5 mL^{-1}

Filtration and fluorescent microscope

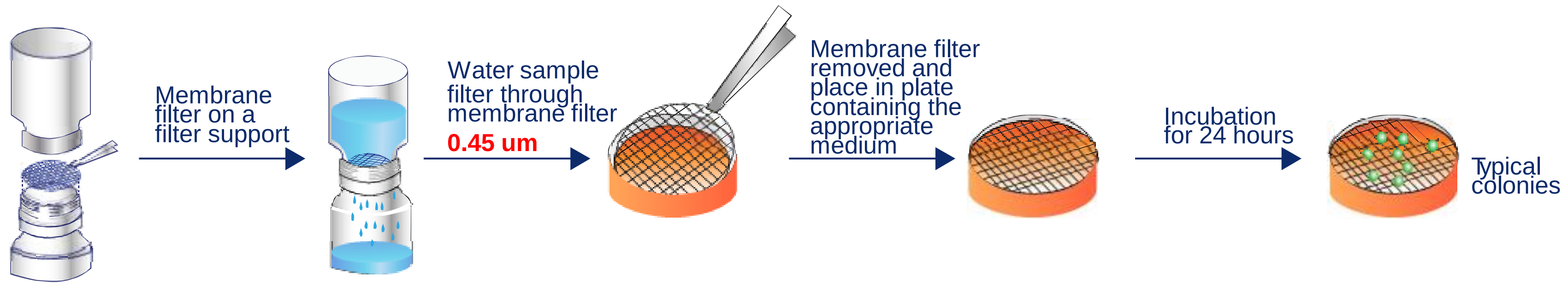
Viable Counting Methods

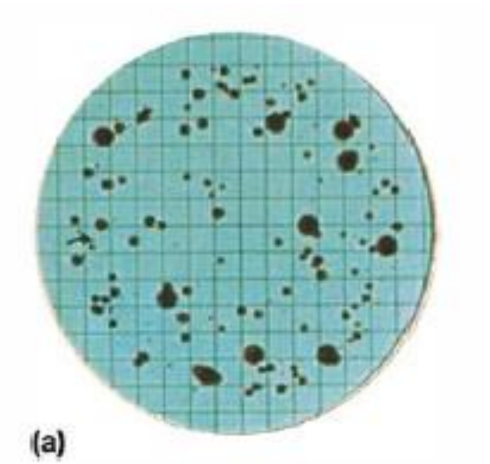
Live

- Actively metabolizing cell
- Cell with reduced metabolic activity
- Some metabolic activity and plasma membrane intact, but RNA content is reduced
- Plasma membrane intact, but no detectable metabolic activity
- Extensive damage to plasma membrane
- Cellular DNA degraded
- Cell fragmented

Dead

Viable cell ≈ CFU





Pour plate 倾注平板 and spread plate 涂平板 are also work.

Typically 30-300 CFUs are preferred.

- **Labor-consuming (need cultivation equipment) and time-costing**
- **Only count cultivable cells**

  uncultivable microbes （about 90-99.9% for nature environments）
- **Can obtain the isolates**
- **Good detective limitation (think about filtration of 100 mL water)**

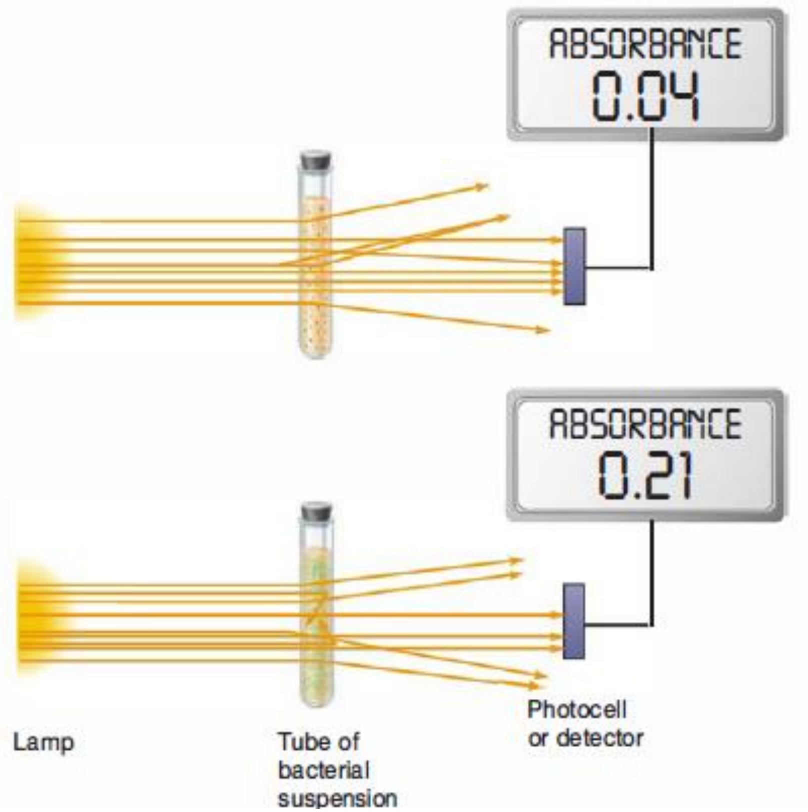
How to quantify CFU in soil sample?

Measurement of cell mass

Weighing (balance, 天平)

measuring the turbidity

(spectrometer, 分光光度计OD 600)



- **Convenient**
- **can only determine relatively high concentration of cells**
- **Not distinguish live/dead cells**
- **Not comparable among different microorganisms**

OD600=1, for

***Escherichia coli*, around 1$\times 10^9$ cell per ml**

***Saccharomyces cerevisiae* (酿酒酵母), around 3$\times 10^7$ cell per ml**

How to measure the growth of *Mycoplasma* (支原体) cells in broth medium by weighing?

1. Sample preparation?
2. The precision of balance?
3. How to calculate the growth rate?

Principles of Microbial Control

Terminology

- **Sterilization (灭菌)**

All living cells, spores, and acellular entities are either destroyed or removed from an object or habitat.

- **Disinfection (消毒)**

Killing, inhibition, or removal of microorganisms that may cause disease.

- **Antisepsis (组织防腐)**

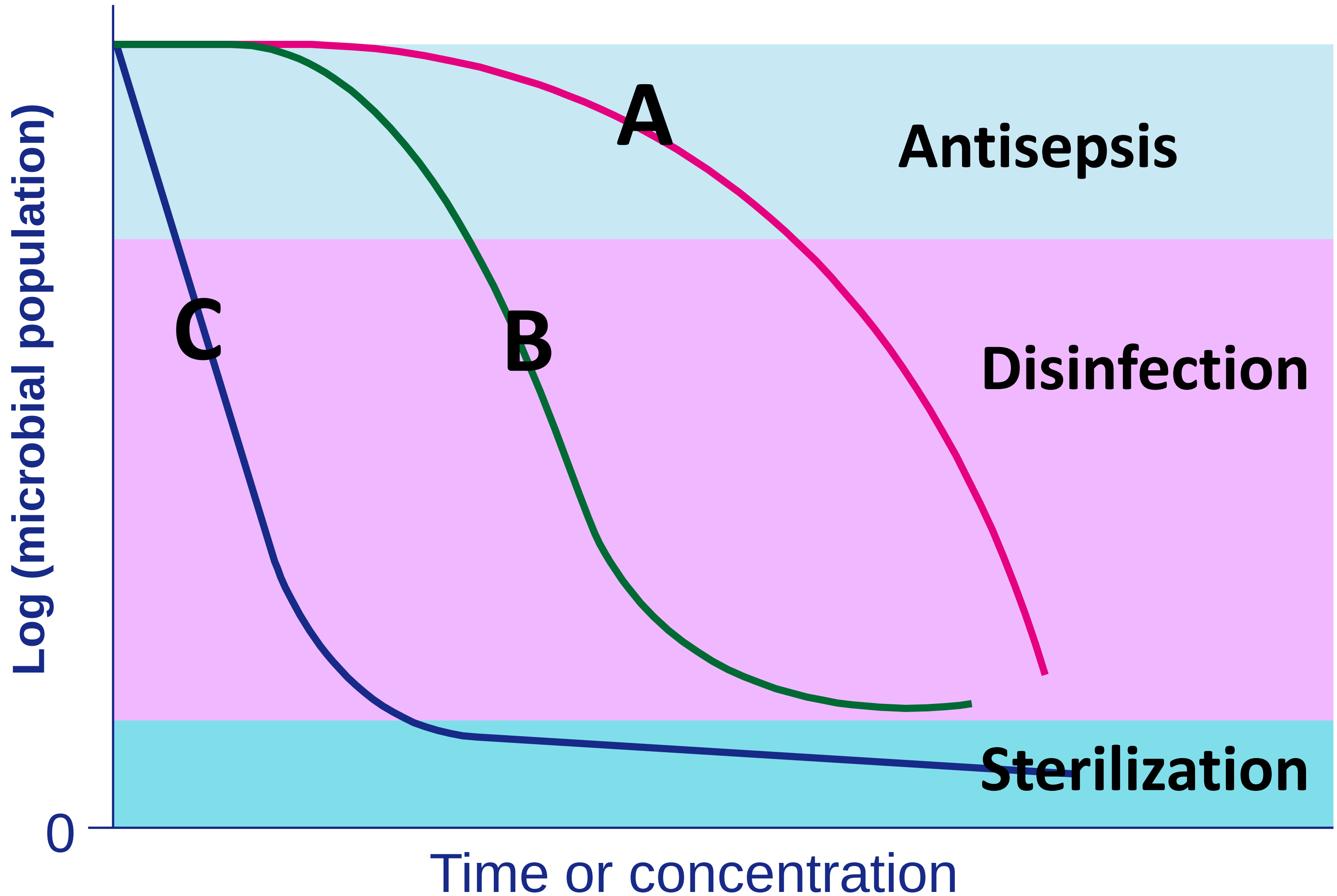
Chemical agents applied to tissue to prevent infection by killing or inhibiting pathogen growth.

- **Chemotherapy (化疗)**

Use of chemical agents to kill or inhibit the growth of microorganisms within host tissue

Cases: 1. boiling water; 2. use mercurochrome (红药水) on wound

Three examples of microbial control agents



Terminology

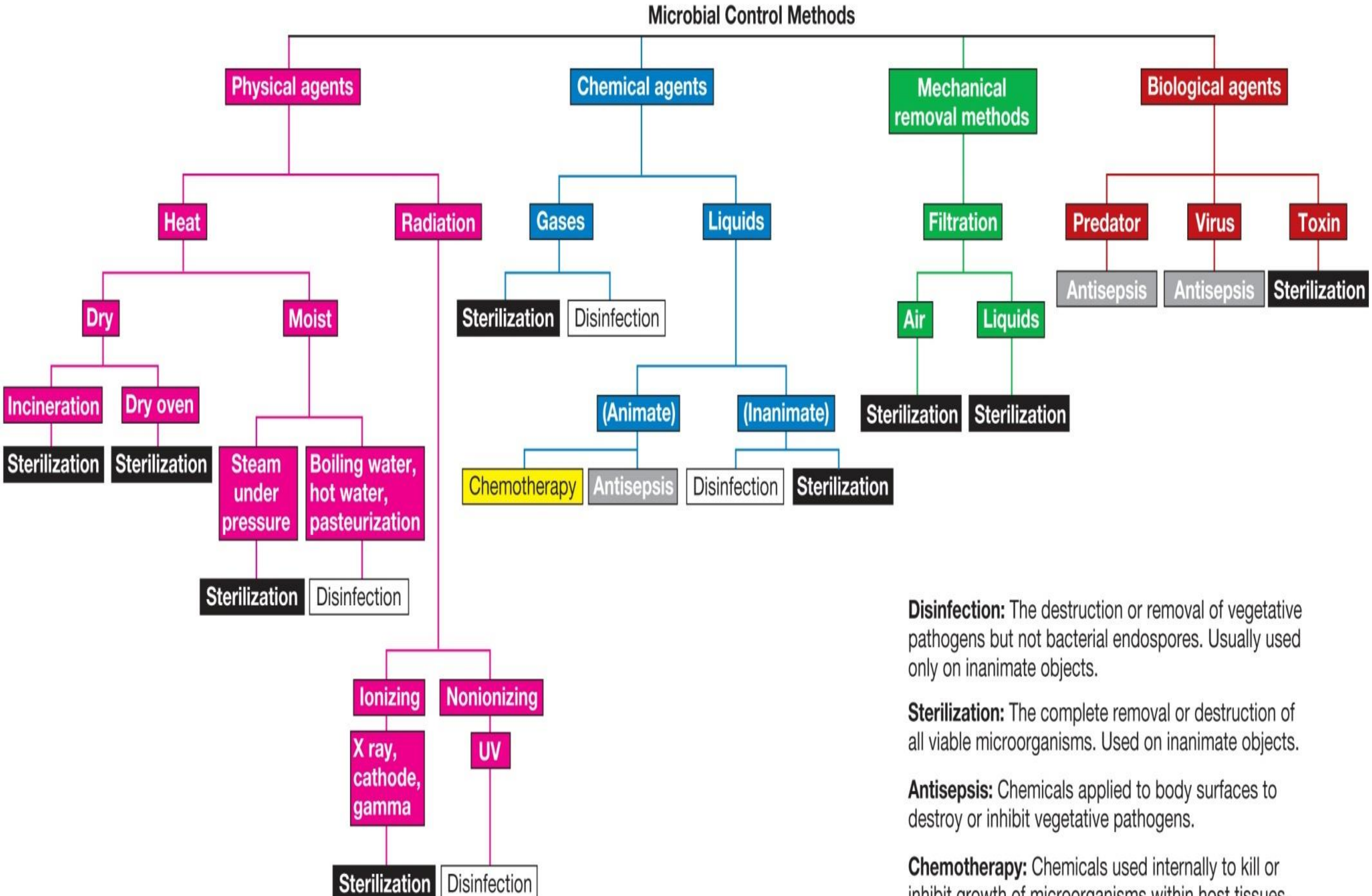
- **suffix –cide (杀菌物, live/dead)**

Bactericide (杀细菌剂)
Fungicide (杀真菌剂)
Viricide (杀病毒剂)

- **-static (抑菌物, growth/no growth)**

Bacteriostatic (抑细菌剂)
Fungistatic (抑真菌剂)

The same agent can be -cide or -static under higher and lower concentration, respectively.



Disinfection: The destruction or removal of vegetative pathogens but not bacterial endospores. Usually used only on inanimate objects.

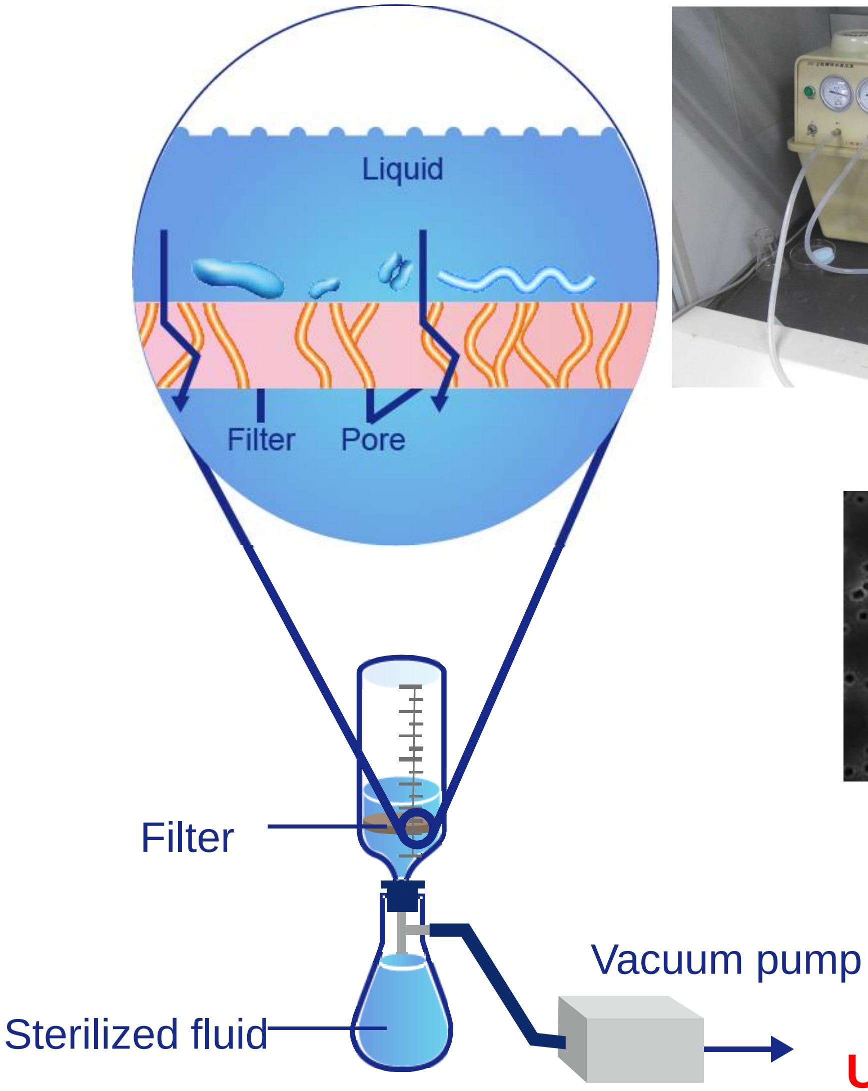
Sterilization: The complete removal or destruction of all viable microorganisms. Used on inanimate objects.

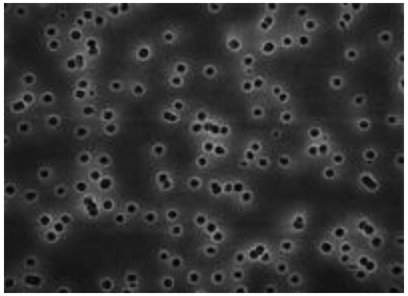
Antisepsis: Chemicals applied to body surfaces to destroy or inhibit vegetative pathogens.

Chemotherapy: Chemicals used internally to kill or inhibit growth of microorganisms within host tissues.

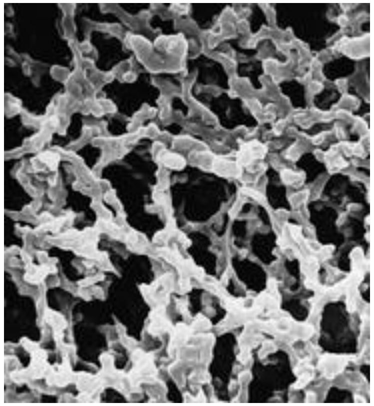
Physical, Chemical and Biological Control

Mechanical Removal Methods





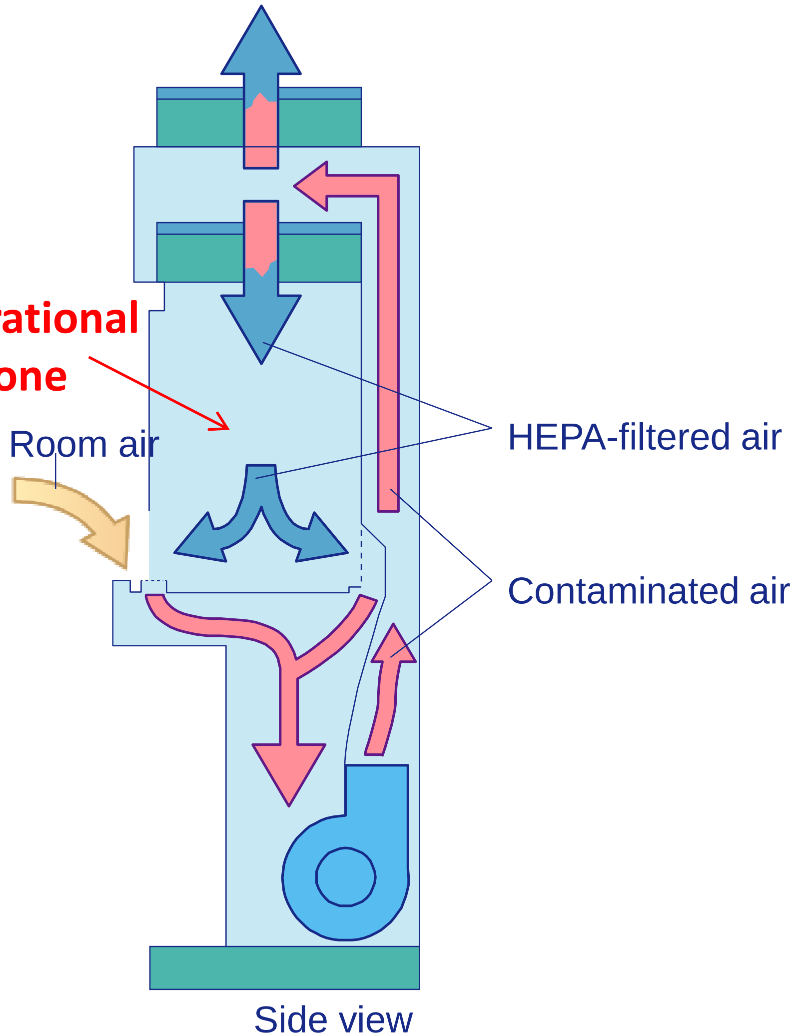
Isopore membrane



Mixed cellulose membrane

Usually use 0.2 µm poresize membranes

Air filtration in lab (biosafety cabinet)



Biosafety cabinet
生物安全柜

Physical Control Methods (Heat)

Usually 160-170 degree C and 2-4 h in oven (烘箱)

Dry heat

Autoclave
灭菌锅
121 ^{o}C and 0.1Mpa

Heating and boiling

↓

Excluding air by generating steam

↓

T at 121 ^{o}C and the pressure at 0.1 Mpa

↓

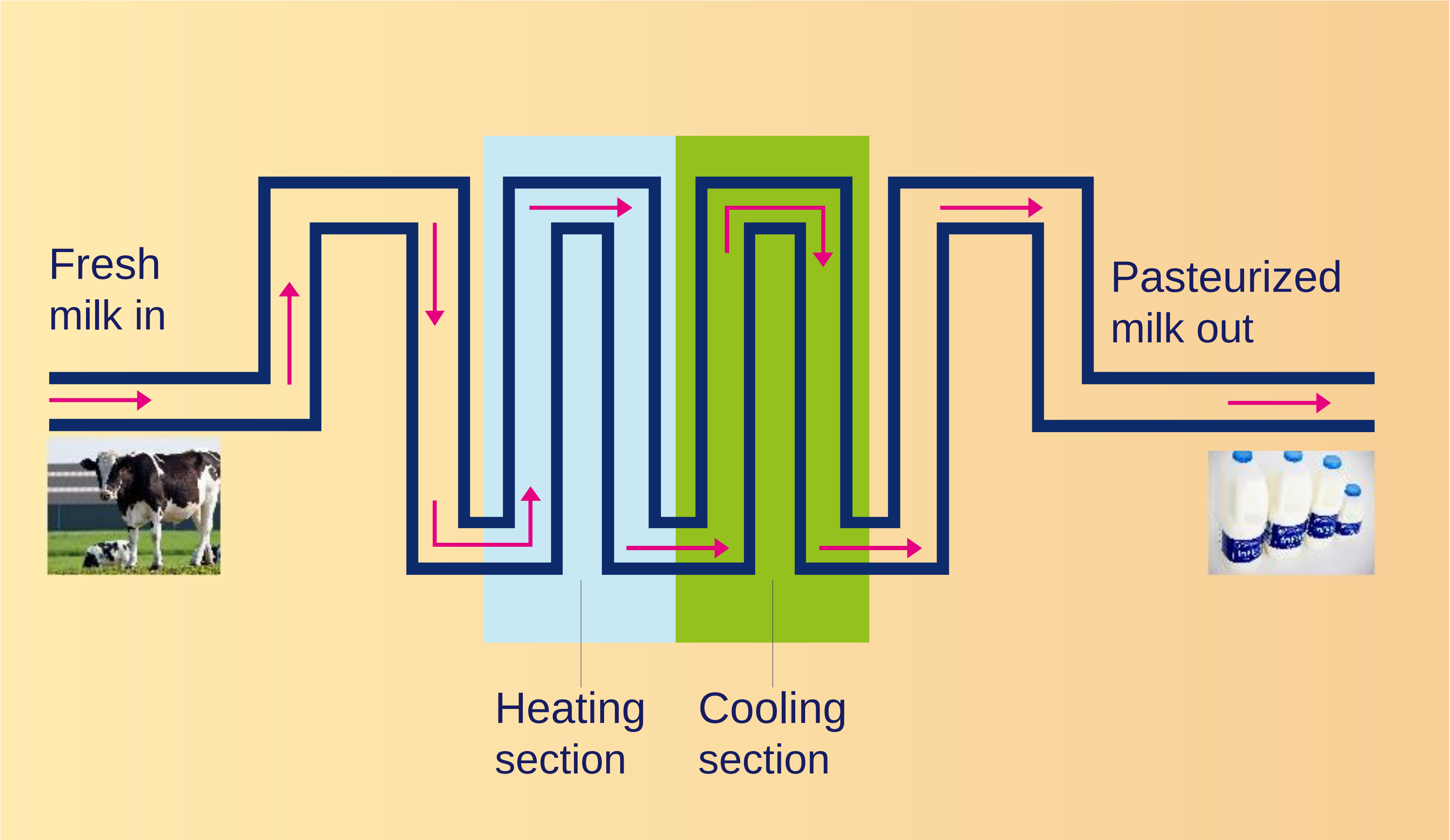
Keep 15-30 min and excluding steam

↓

Cooling down

Moist heat

Pasteurization (70-85^{o}C, 15 s)



Radiation

- **Ultraviolet (UV)**

  260 nm

  DNA damage

  poor penetration

- **Ionizing radiation (致电离辐射)**

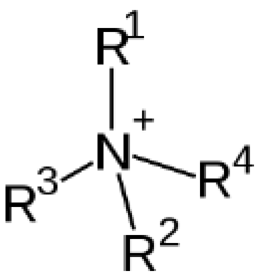
  gamma radiation and electron beams

  dislodge electrons from atoms or molecules

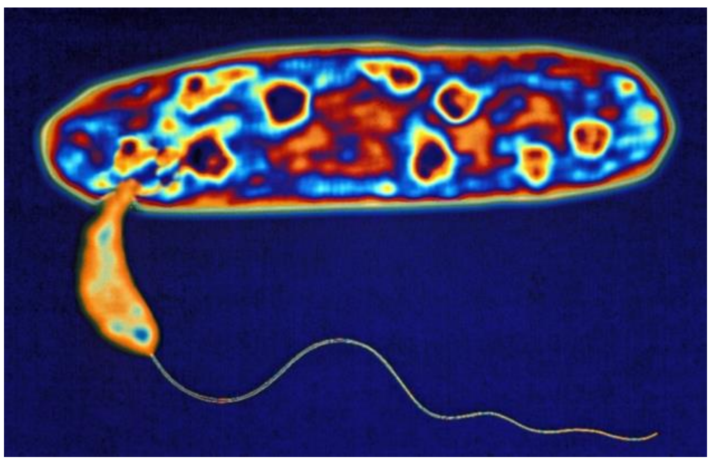
  suitable for antibiotics, hormones, and plastic disposable

Chemical Control Agents

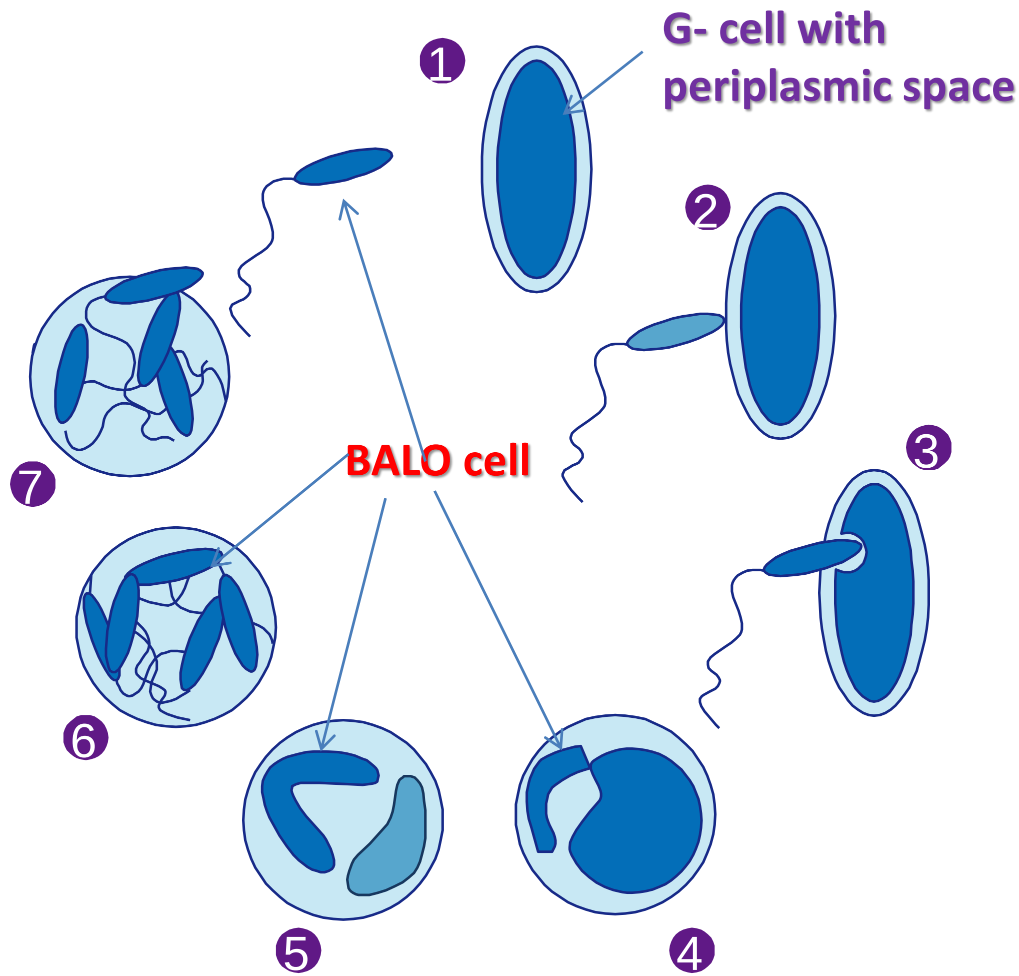
Chemical	Commonly used concentration	Activity level
Ethylene oxide (环氧乙烷，气态)	450-500 mg/L	High
Formaldehyde (甲醛)	6-8%	High to intermediate
Hydrogen peroxide (过氧化氢)	6-30%	High to intermediate
Alcohols (酒精类)	around 75%	Intermediate
Chlorine (氯，主要是次氯酸)	500-5,000 mg/L	Intermediate
Quaternary ammonium (季铵盐类)	0.1-0.2%	Low



Biological Control of Microorganisms



Reardon, 2015



Bdellovibrio (蛭弧菌) And Like Organisms, BALOs

Use phage to control bacteria

Steffanie Strathdee and her husband

UC San Diego's Global Health Institute

超级细菌—鲍曼不动杆菌

Acinetobacter baumannii

ESK**A**PE (六大超级细菌)

噬菌体疗法

Evaluation of antimicrobial agent effectiveness

- Population size
  Larger size requires a longer time to die
- Population composition
  Susceptibility of different cells
- Concentration or intensity of an antimicrobial agent
  Mostly positive but not always
- Contact time
- Temperature
- Local environment (pH/free-living or biofilm)

Antibiotics

Antimicrobials & Antibiotics

- In general, any chemical, physical, or biological product that controls microorganisms is referred to as an **antimicrobial agent**.

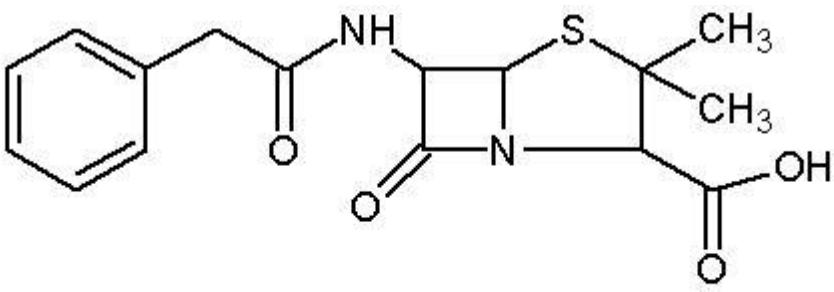
- **Antibiotic**: a microbial product or its derivative that kills susceptible microorganisms or inhibits their growth.

- **Antibiotics** are chemotherapeutic agents (化疗药物).

Some typical antibiotics

- Fleming accidentally rediscovered **penicillin** (青霉素) from mold ***Penicillium notatum*** (青霉菌) in 1928

- Streptomycin (链霉素), chloramphenicol (氯霉素), neomycin (新霉素), oxytetracycline (土霉素), and tetracycline (四环素) were discovered from ***Streptomyces*** (链霉菌属), an ***Actinobacteria*** (放线菌).



General Characteristics of Antimicrobial Drugs

Therapeutic index (治疗指数)

$$\frac{\textbf{Toxic dose(毒性剂量)}}{\textbf{Therapeutic dose (治疗剂量)}}$$

Larger is better!

Types of antibiotics:

Naturally produced
Synthetic
Semisynthetic

Narrow-spectrum drugs
Broad-spectrum drugs

Cidal
Static

Determining the Level of Antimicrobial Activity

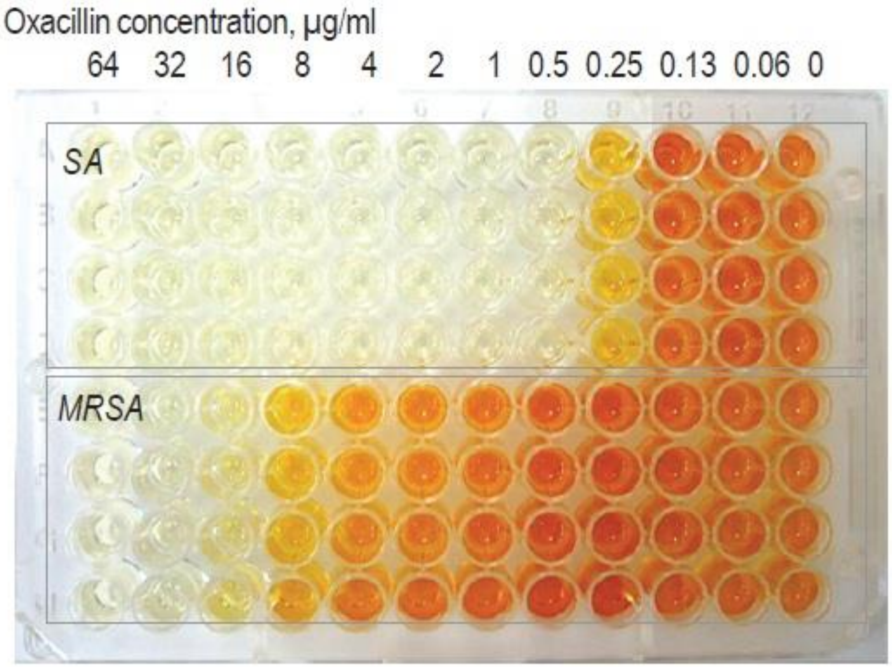
- Minimal inhibitory concentration (MIC)

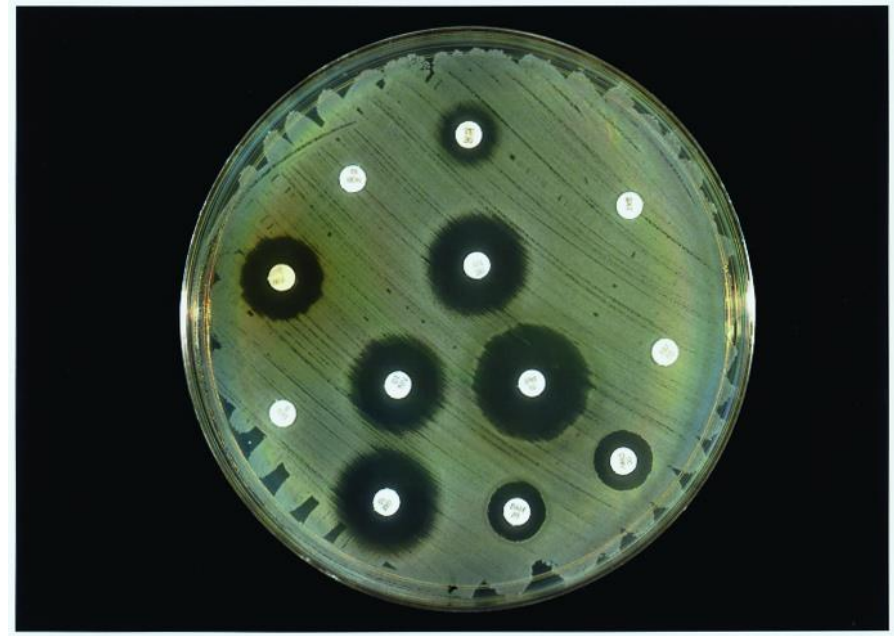
- Minimal lethal concentration (MLC)

- Methods to determine MIC

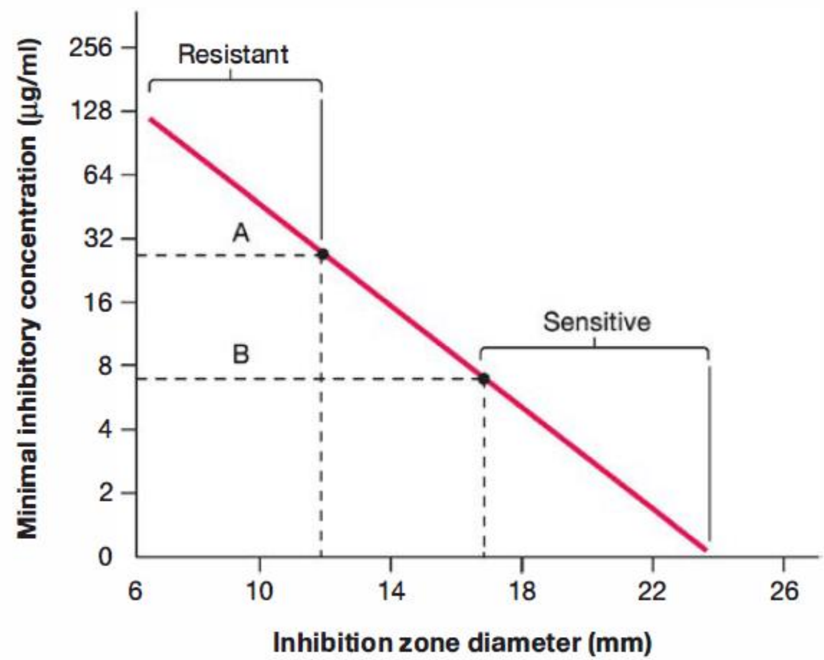
  Dilution Susceptibility Tests (稀释法药敏试验)

  Disk Diffusion Tests (纸片扩散实验)





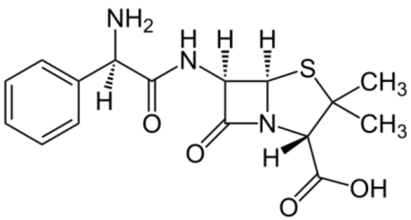
Kirby-Bauer method

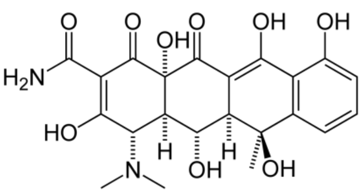


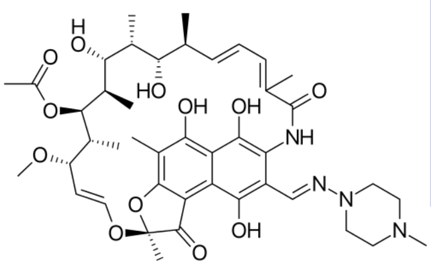
Dilution susceptibility tests on 金黄色葡萄球菌

Think about how to determine MLC?

Examples of antibiotics





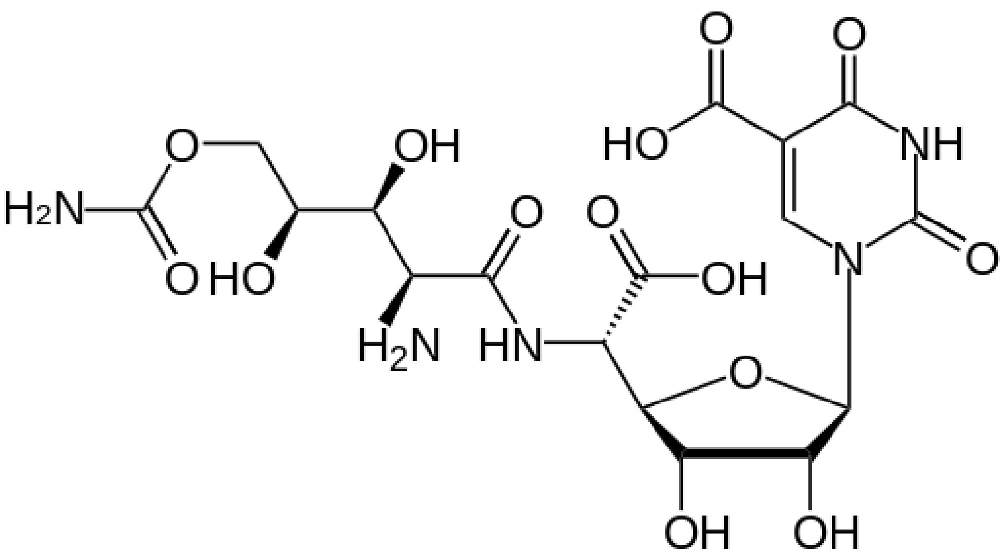


Antibiotics	Primary Effects	Spectrum	Mechanism
Ampicillin (氨苄青霉素)	Cidal	Broad (G+ and some G-)	**Cell wall synthesis inhibition** (transpeptidation enzyme)
Oxytetracycline (土霉素)	Static	Broad	Bind to small ribosomal subunit protein S30 and **inhibit protein synthesis**
Rifampin (利福平)	Cidal	Broad (*Mycobacterium et al.*)	**Inhibits** bacterial DNA-dependent **RNA polymerase**

Why antibiotics do not affect host's cellular functions?

Antifungal Drugs

Treatment of fungal infections are usually much more difficult than the bacterial ones. ***Why?***



Polyoxyin (多氧菌素) D targeting on chitin synthase in fungi

Factors Influencing Antimicrobial Drug Effectiveness

- First, drug must be able to reach the site of infection.

  unstable in stomach acid

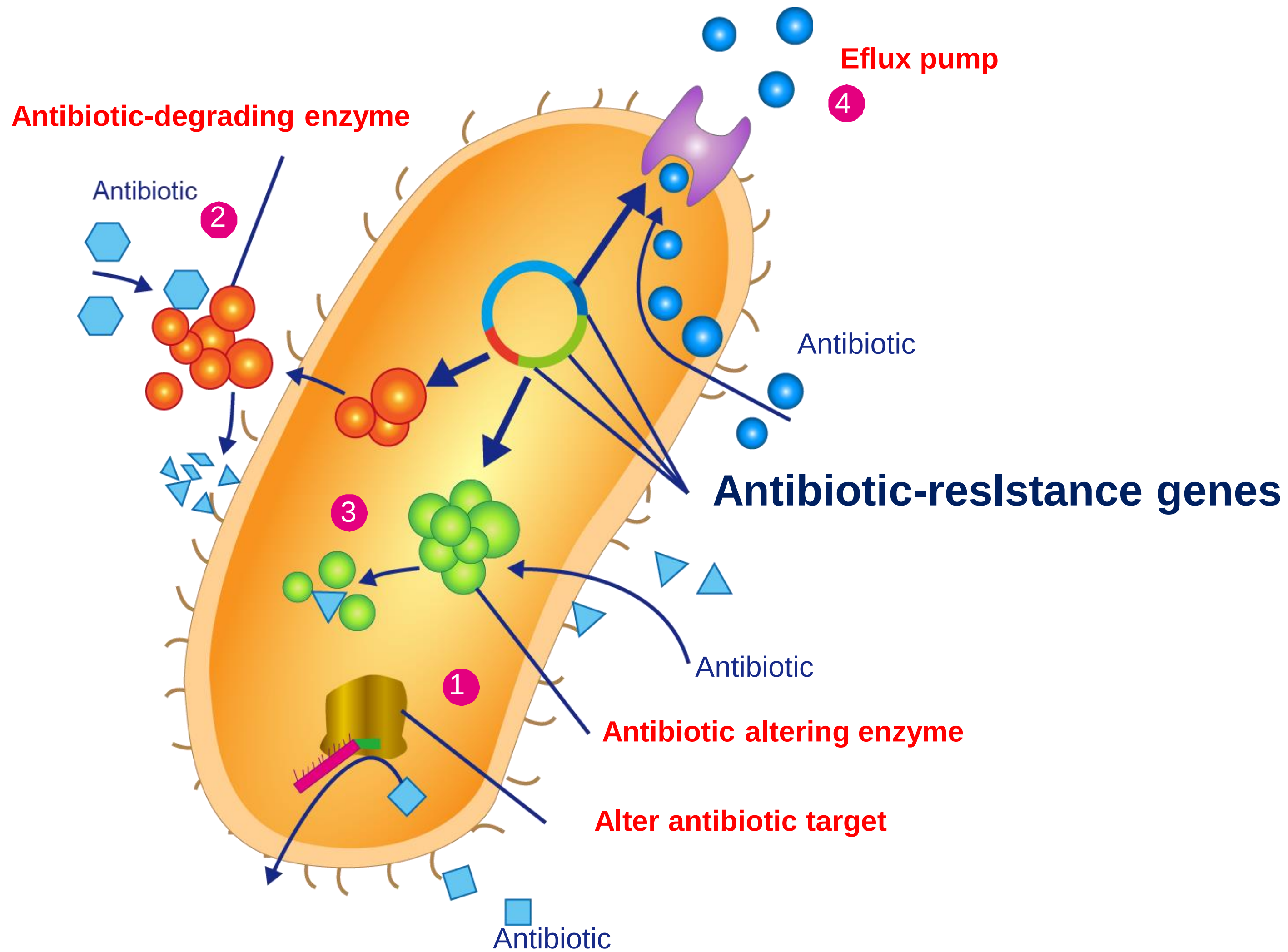
  not well absorbed from the intestinal tract

- Second, the pathogen must be susceptible to the drug.

- Third, dose must high enough at the site of infection.

- **Drug resistance in bacterial pathogens**

Mechanisms of Drug Resistance



超级细菌”ESKAPE”

***E**nterococcus faecium* (屎肠球菌)
***S**taphylococcus aureus* (金黄色葡萄球菌)
***K**lebsiella pneumoniae* (肺炎克雷伯氏菌)
***A**cinetobacter baumannii* (鲍氏不动杆菌)
***P**seudomonas aeroginosa* (铜绿假单胞菌)
***E**nterobacter* spp. (肠杆菌)

Discussion

1. Consider cell-cell communication: bacteria that "subvert“ and "cheat" have been described. Describe a situation in which it would be advantageous for one species to subvert another, that is, degrade an intercellular signal made by another species. Also, describe a scenario whereby bacterial cheaters-defined as bacteria that do not make a molecular signal but profit by the uptake and processing of signal made by another microbe-might have a growth advantage
2. Which physical or chemical agent would be the best choice for sterilizing the following items: glass pipettes, tryptic soy broth tubes, nutrient agar, antibiotic solution, interior of a biological safety cabinet, wrapped package of plastic Petri plates? Explain your choices.

3. How would you explain to a patient that a virus can be used to eliminate a bone infection caused by bacteria that do not respond to antibiotics?

4. Suppose hospital custodians have been assigned the task of cleaning all showerheads in patient rooms to prevent the spread of infectious disease. What two factors would have the greatest impact on the effectiveness of the disinfectant the custodians use? Explain what that impact would be.

5. What advantage might soil bacteria and fungi gain from the synthesis of antibiotics?

6. You are a pediatrician treating a child with an upper respiratory infection that is clearly caused by a virus. The child's mother insists that you prescribe antibiotics-she's not leaving without them! How do you convince the child's mother that antibiotics will do more harm than good?