厦门大学
生命科学学院
SCHOOL OF LIFE SCIENCES XIAMEN UNIVERSITY

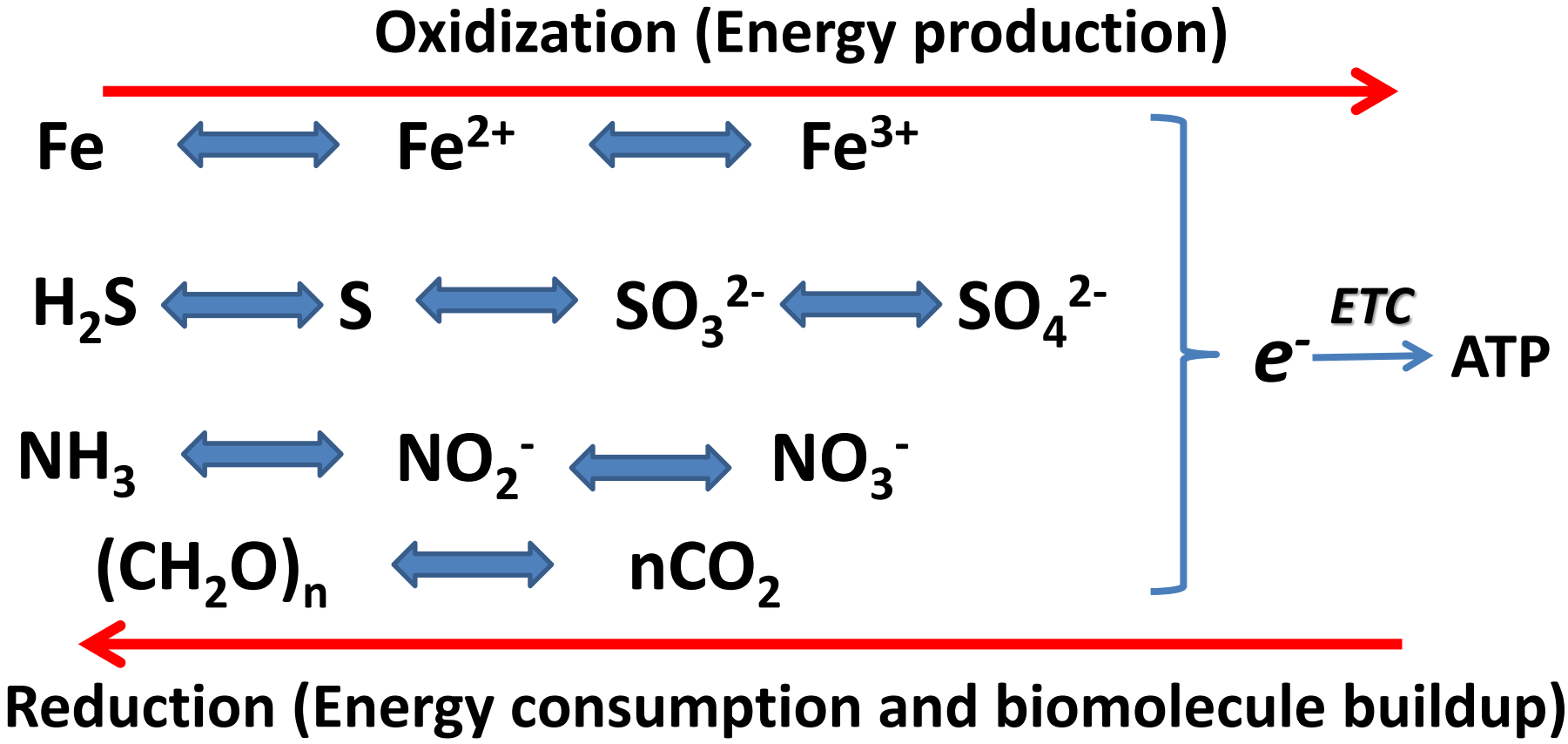
MICROBIOLOGY

Lecture 5
Microbial Nutrition, Growth and Control (I)

Outline

- **Types of nutrients***
- **Nutritional types (see 11.1)***
- **Reproductive strategies**
- **Bacterial cell cycle**
- **Influences of environmental factors on growth**
- **Laboratory culture of cellular microbes**
- **Growth curve**

Nutrition vs. Nutrient
Reduction vs. Oxidization

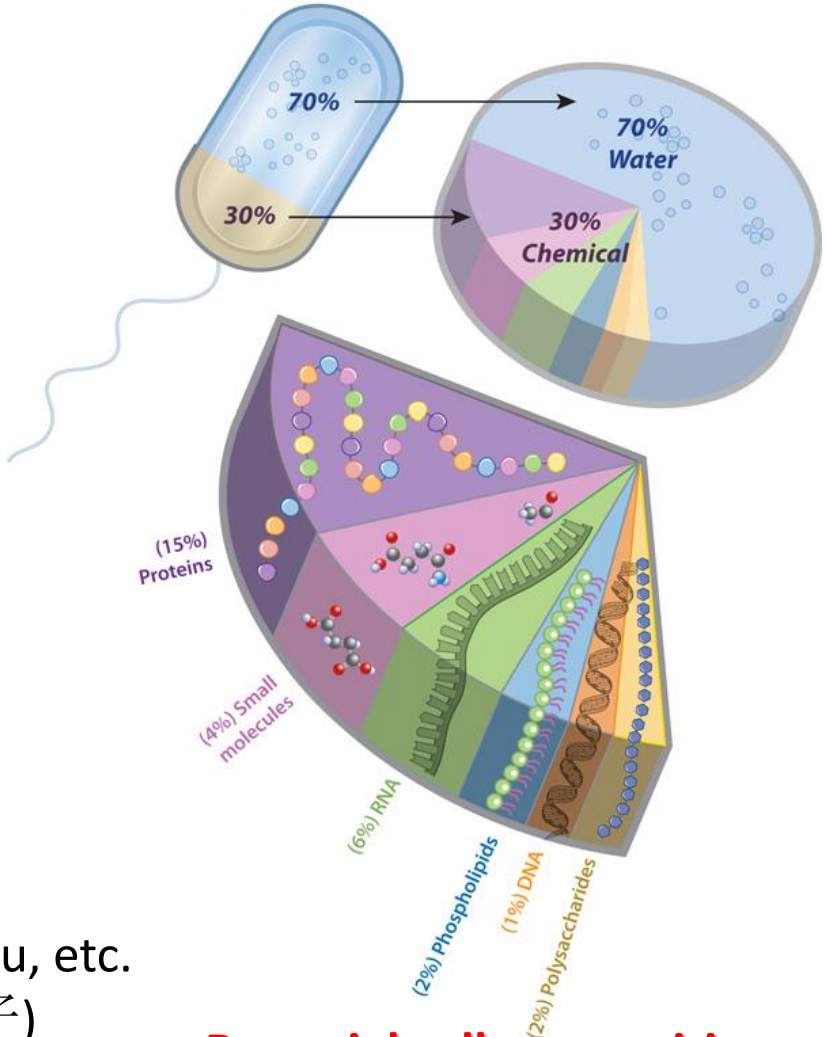


Elements required for growth

Macroelements

Molecule	Elements
Proteins	C, H, O, N, S
Lipids	C, H, O, P
Carbohydrate	C, H, O
Nucleic acid	C, H, O, N, P

Metal elements: K, Ca, Mg, and Fe



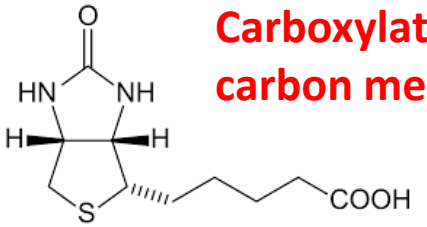
Bacterial cell composition

Microelements (trace elements): Mn, Co, Mo, Cu, etc.

- serve as cofactors of enzymes (酶的辅助因子)
- required in trace amounts
- some unique substances may be required (Si for diatom)

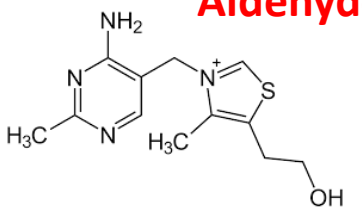
Growth factors

- Organic compounds
- Essential cell components (or their precursors) that the cell cannot synthesize
- Must be supplied by environment if cell is to survive and reproduce
  - **amino acids**
  - **purines and pyrimidines**
  - **vitamins**
    - **function as co-enzyme(辅酶)**



Carboxylation, one-carbon metabolism

Biotin (生物素)



Aldehyde transfer

Thiamine (硫胺素，B1)

Microbial production of growth factors

- Microorganisms can synthesize many growth factors
- Large-scale industrial production of growth factors, such as vitamins

Nutritionally deficient mutant= auxotroph

In genetics, a strain is said to be auxotrophic if it carries a mutation that renders it unable to synthesize an essential compound.

A mutation converting a prototroph （原营养型） into an auxotroph （营养缺陷型）

谷氨酰胺to终止密码子

甘氨酸to天冬氨酸

Two leucine auxotroph mutants of yeast

Lin et al., 2012

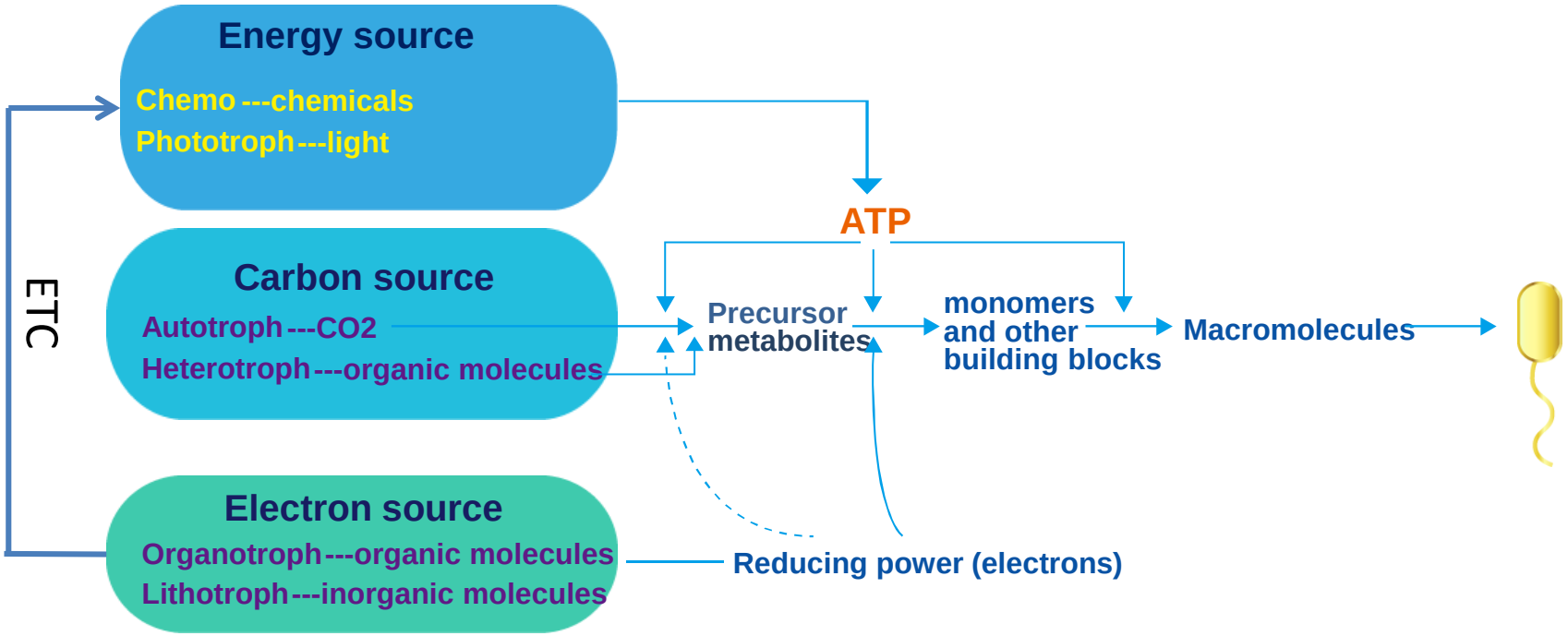
How to screen the auxotroph?

Replica plate (影印平板) for auxotroph screening

	Prototroph	Auxotroph
Complete medium (all nutrients)	+	+
Minimal medium (lack of certain growth factor)	+	-

Nutritional Types

Nutritional types are determined by energy source, carbon source and electron source.



Diversity of microbial nutritional types

Nutritional Type	Carbon Source	Energy Source	Electron Source	Type organisms
Photo-litho-autotroph (光能无机自养型) (similar with plants)	CO_2	Light	Inorganics	*Cyanobacteria* Green/purple sulfur bacteria
Chemo-organo-heterotroph (化能有机异养型) (similar with animals)	Organics	Organics	Organics	Most pathogens Most colonies on agar plates
Photo-organo-heterotroph (光能有机异养型)	Organics	Light	Organics	Purple non-sulfur bacteria
Chemo-litho-autotroph (化能无机自养型)	CO_2	Inorganics	Inorganics	Some nitrogen cycling microbes Sulfur oxidizing bacteria Iron oxidizing bacteria
Chemo-litho-heterotroph （化能无机异养型）	Organics	Inorganics	Inorganics	Some sulfur oxidizing bacteria (*Beggiatoa* sp.)

Photo-litho-autotroph

Microcystis aeruginosa
铜绿微囊藻

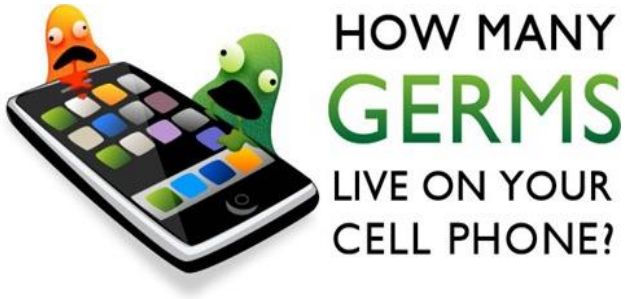
***Cyanobacteria* blooming (水华)**

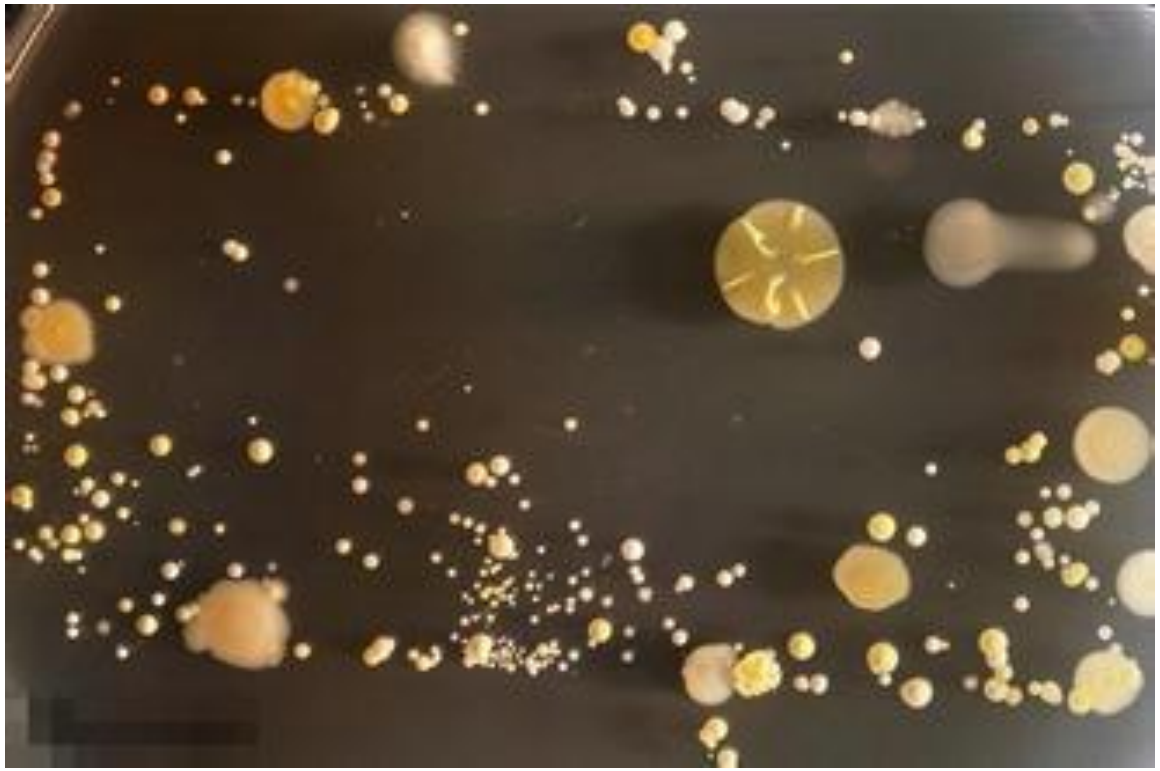
Electron source: H_2O

Purple sulfur bacteria

Electron source: H_2S

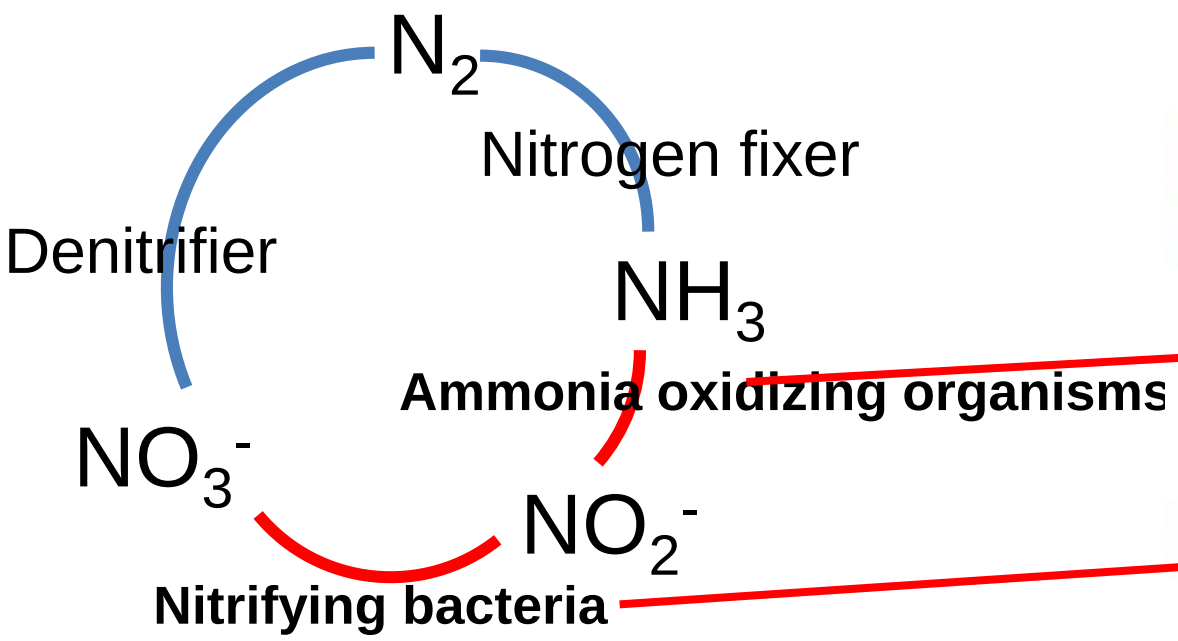
Chemo-organo-heterotroph





Bacteria on the cellphone screen cultivated with nutrient agar

Chemo-litho-autotroph



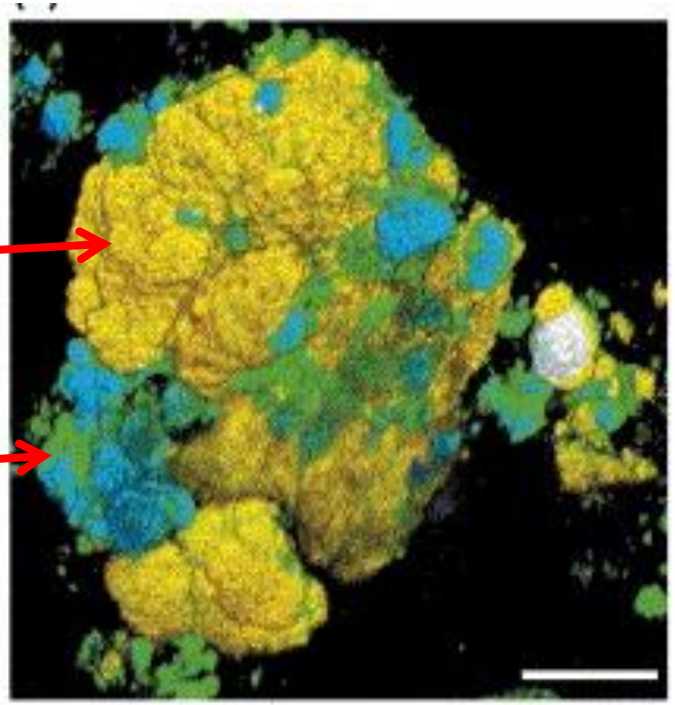
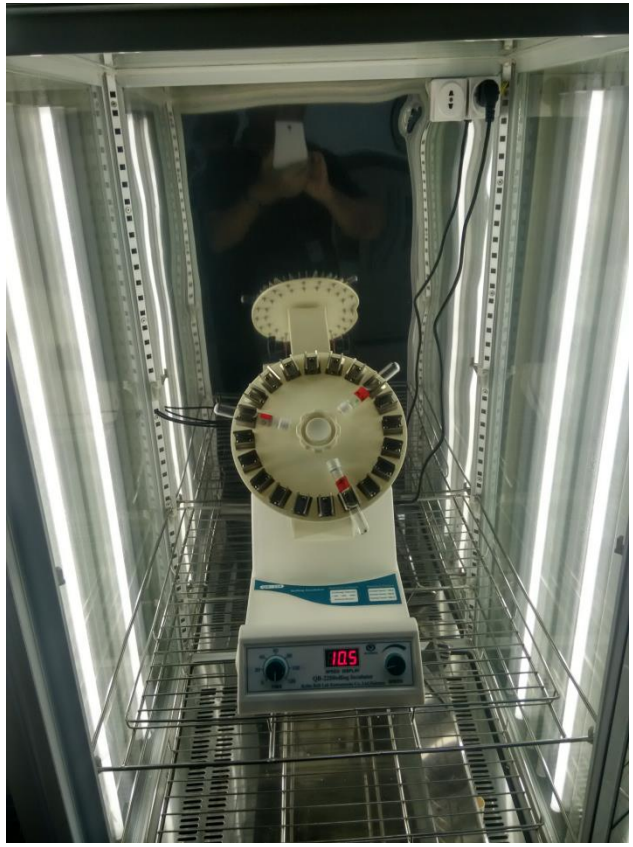
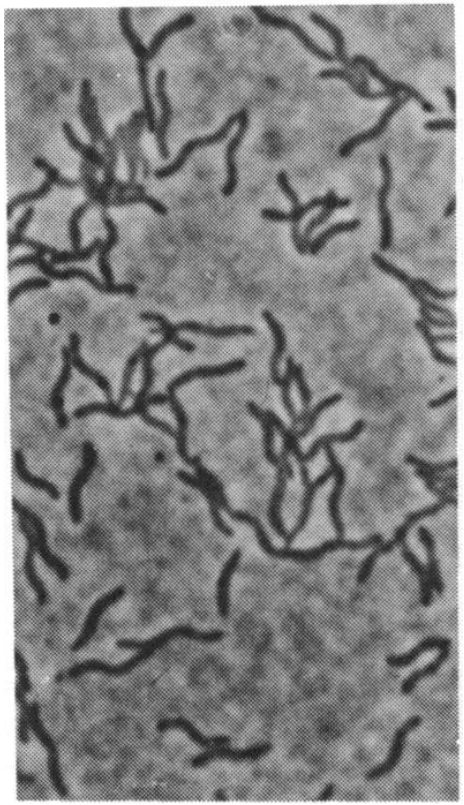


Photo-organo-heterotroph





Yeast extract	0.30	g
Na_2-succinate	1.00	g
(NH_4)-acetate	0.50	g
Fe(III) citrate solution (0.1% in H_2O)	5.00	ml
KH_2PO_4	0.50	g
$MgSO_4$ x 7 H_2O	0.40	g
NaCl	0.40	g
NH_4Cl	0.40	g
$CaCl_2$ x 2 H_2O	0.05	g
Vitamin B_{12} solution (10 mg in 100 ml H_2O)	0.40	ml
Trace element solution SL-6 (see below)	1.00	ml
L-Cysteiniumchloride	0.30	g
Resazurin(0,1%)	0.50	ml
Distilled water	1000.00	ml

Adjust pH to 6.8.
Boil the medium for a few minute. Bubble the medium with nitrogen gas and fill 15 ml tubes with a rubber septum under a stream of nitrogen gas. Autoclave at 15 min. Sterile syringes are used to inoculate and remove samples.
Incubate in the light using a tungsten lamp.

Trace element solution SL-6:

$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.30	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.02	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

Rhodocyclus tenuis DSM109

Mixotroph: organism with different nutritional types

Obligate photoorganoheterotroph

Photolithautotroph and facultative chemoorganoheterotroph

Summary

- The growth of microbial cells needs macronutrients, micronutrients and growth factors

- There are five microbial nutritional types classified by the energy source, carbon source and electronic source

Bacterial growth

Reproductive Strategies

- Many eukaryotic microbes exhibit both asexual reproduction（无性繁殖）, involving mitosis（有丝分裂）, and sexual reproduction, involving meiosis （减数分裂）to produce gametes or gamete-like cells.
- Unlike eukaryotes, bacterial and archaeal cells are haploid（单倍体）. Most reproduce by ***binary fission***（二分裂）.

Molecules double in amounts for **binary fission**

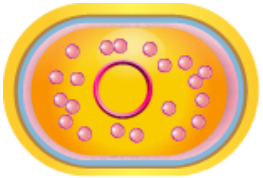
- Protein
- RNA and ***DNA***
- Lipids for membrane
- Cell wall components
- Small organic and inorganic molecules

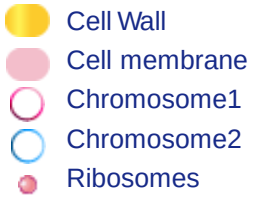
Each **daughter cell** have ½ old cell material and ½ new cell material.

All biosynthetic events must **coordinate** carefully.

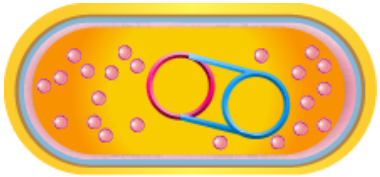
Binary fission

A new cell

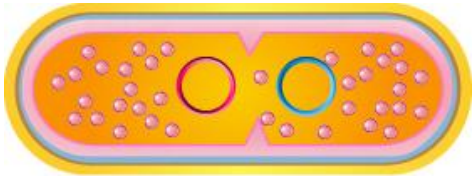




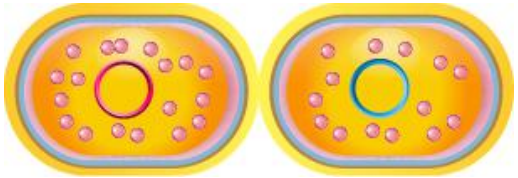
DNA replication



Septation starts



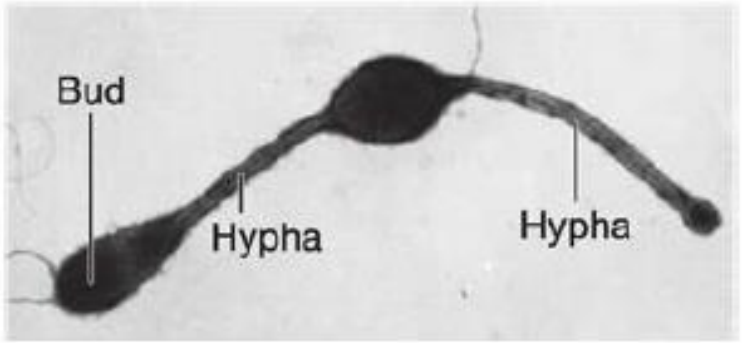
Septum (横隔) formation



Two daughter cells

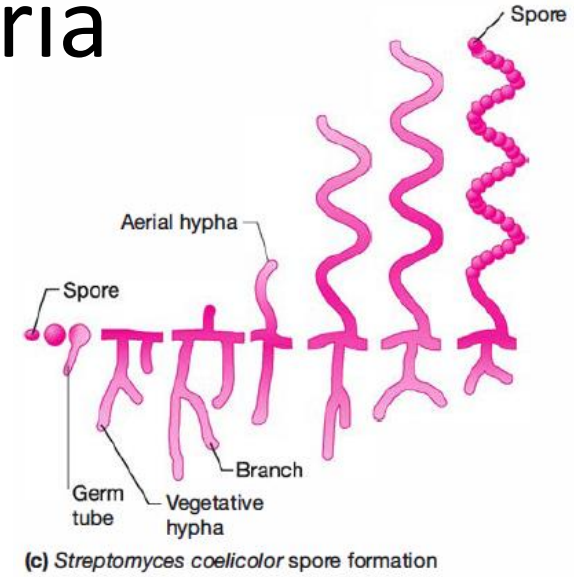
Binary fission

Other reproductive strategies in bacteria

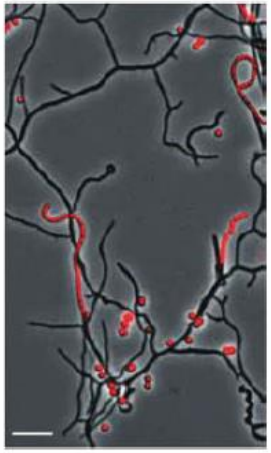


(a) *Hyphomonas* mother cell and bud

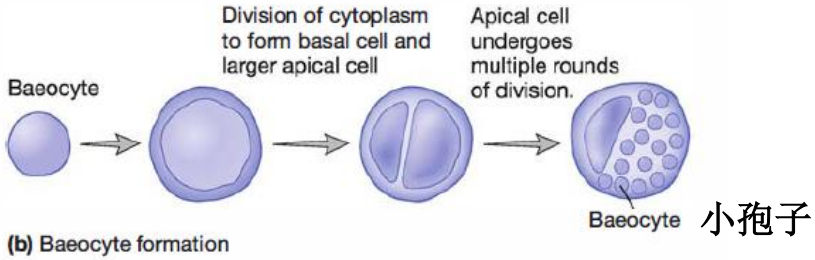
Budding (出芽生殖)



(c) *Streptomyces coelicolor* spore formation

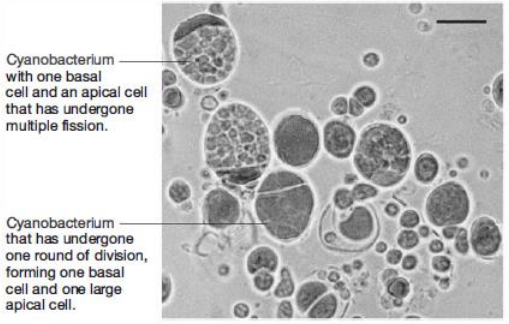


Spore of actinobacteria (放线菌)



(b) Baeocyte formation 小孢子

Multiple fission (复分裂) in Cyanobacteria (蓝细菌)



The ***cell cycle*** is the complete sequence of events extending from the formation of a new cell through the next division.

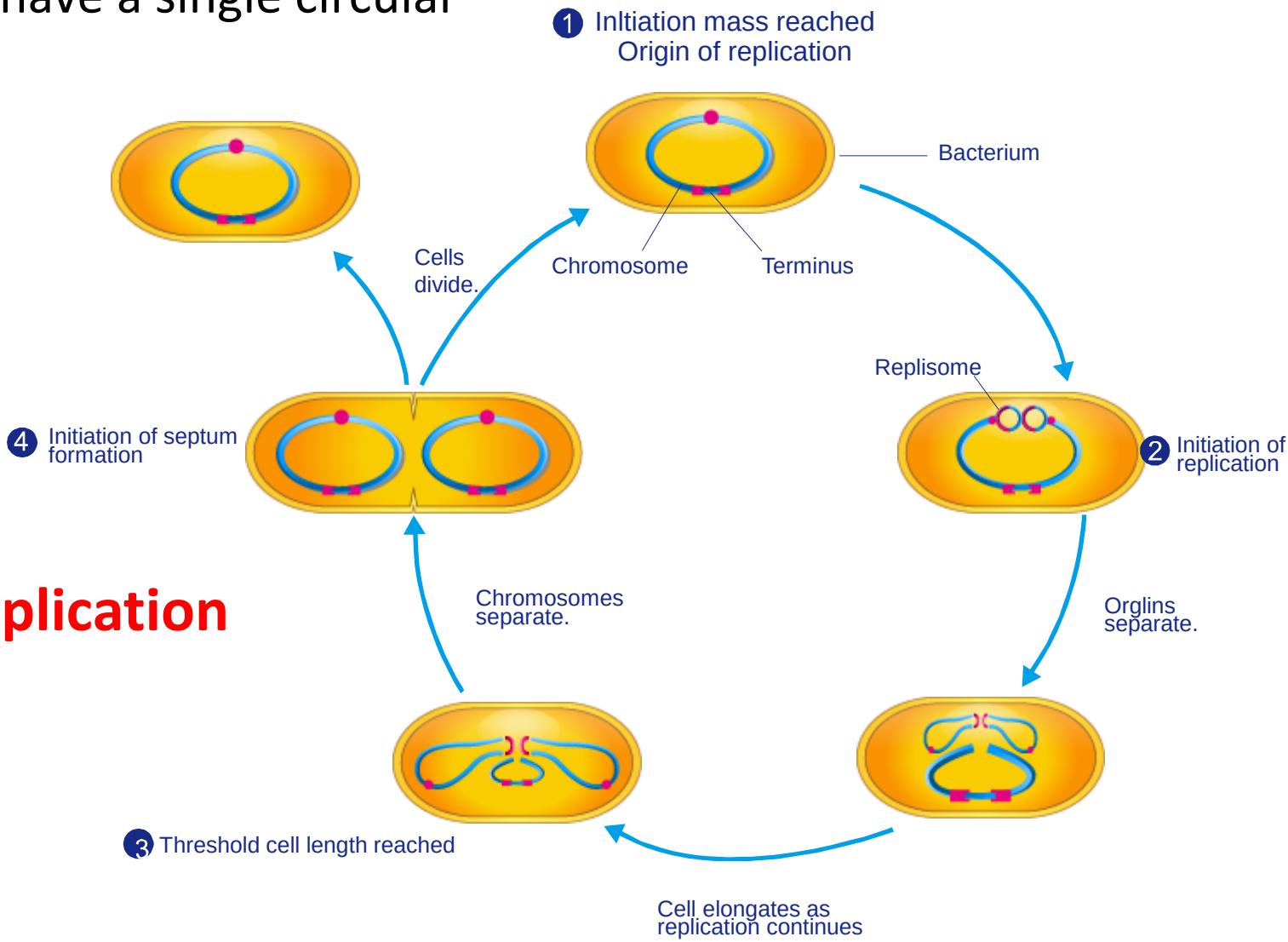
Two events during the bacterial cell cycle:

1. **Replication and partitioning of DNA** into the progeny cells

2. **Cytokinesis**（胞质分裂）
   ----formation of the **septum** (横隔) and progeny cells

Replication and partitioning of DNA

Most bacteria have a single circular chromosome.

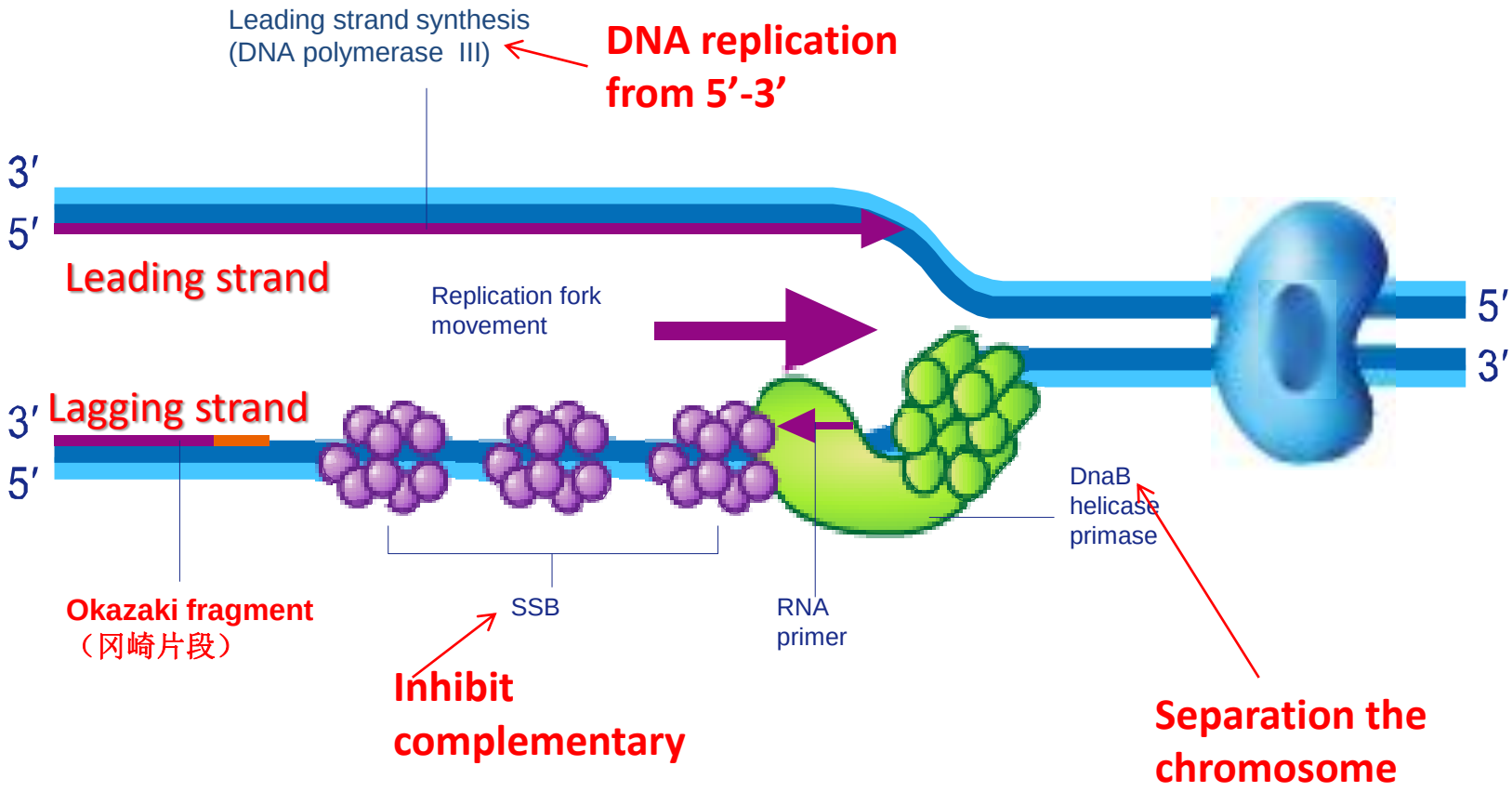


Origin of replication

Terminus

Replisome

Replisome (DNA replication fork)



DNA partitioning?

Cytokinesis

- **Septation (=cytokinesis)** is the process of forming a cross wall between two daughter cells

- Septation is divided into 4 steps:

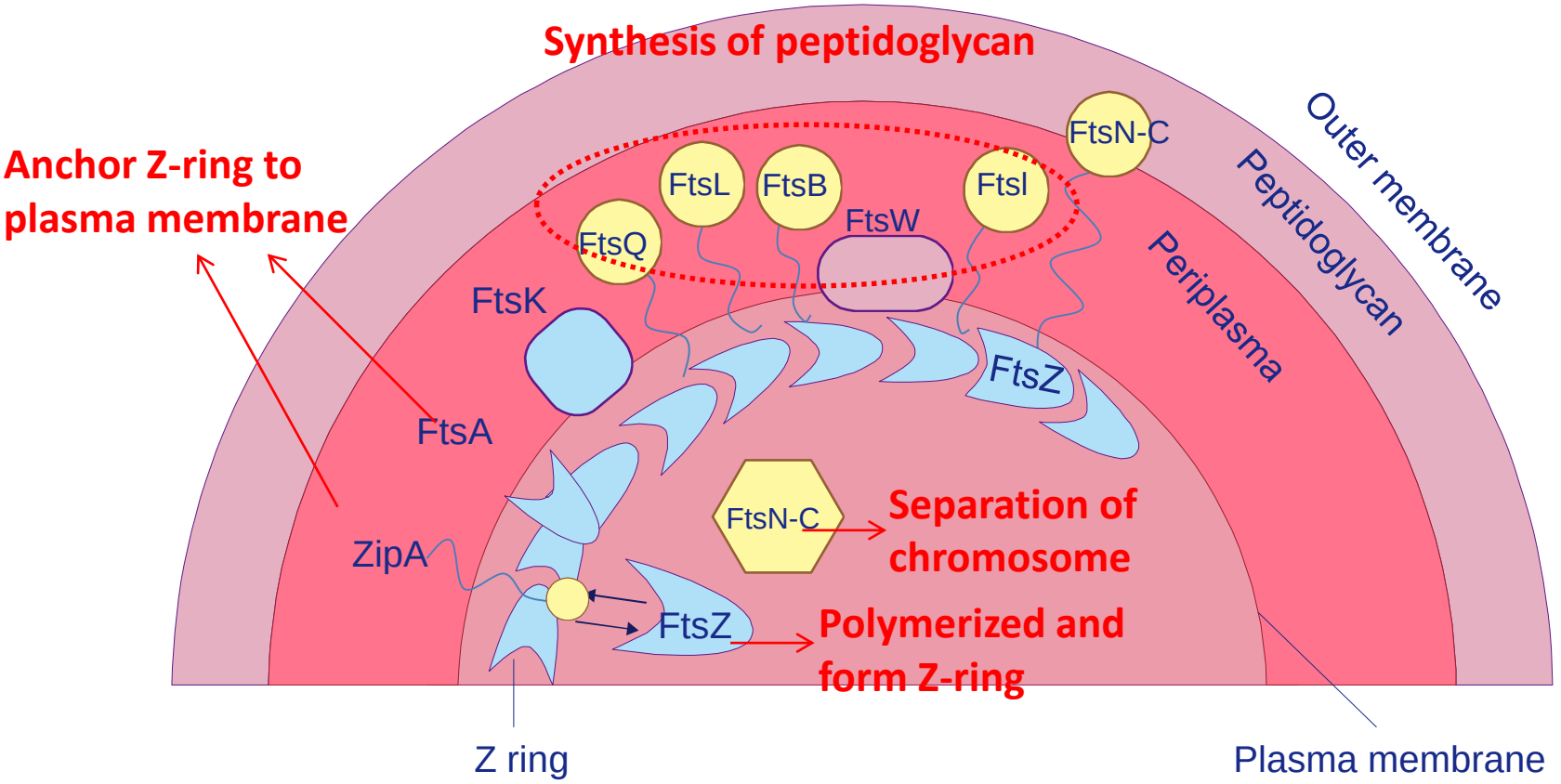
(1)selection of the site where the septum will be formed;

(2)assembly of the Z ring, which is composed of the cytoskeletal protein FtsZ;

(3)assembly of the cell wall-synthesizing;

(4)constriction of the cell and septum formation.

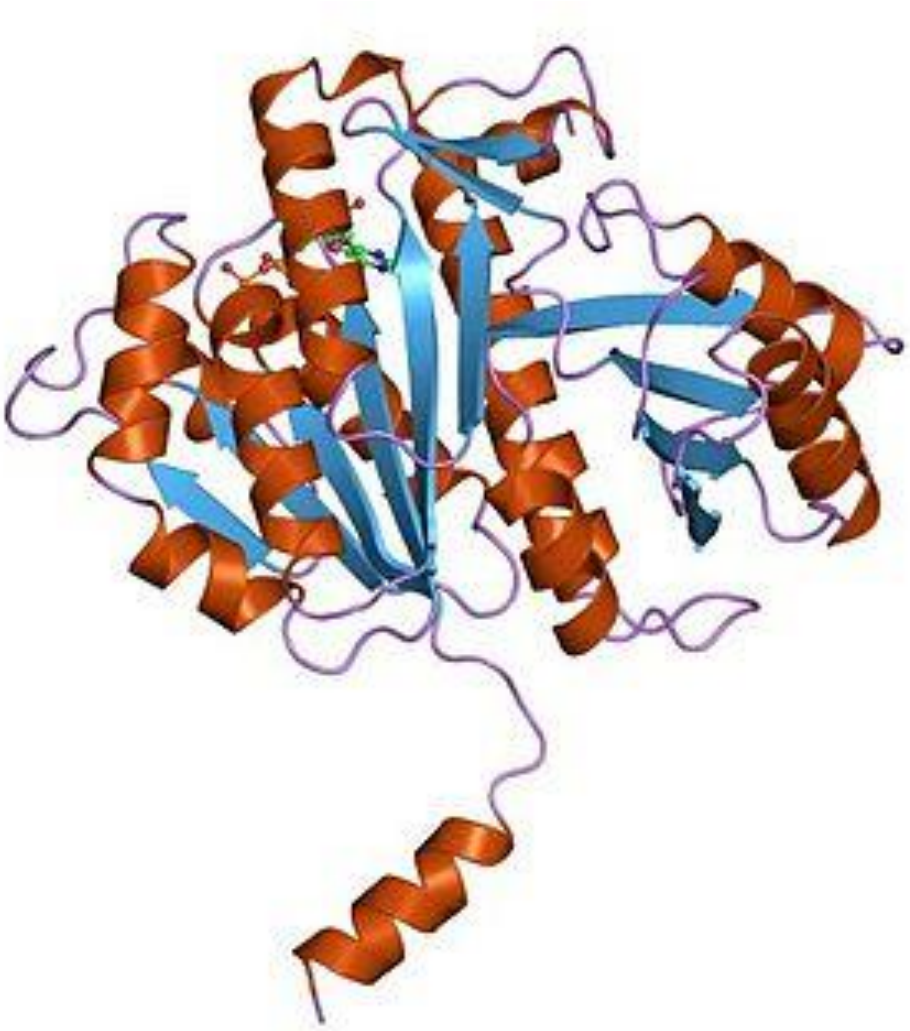
The Z-ring and divisome



The *E.coli* divisome

Figure 7.5 The Cell Division Apparatus in E.coli. The cell division apparatuts is composed of numerous proteins.The first step in divisome formation is the polymerization of FtsZ to form the Z ring.FtsA and ZipA proteins anchor the Z ring to the plasma a membrane,and then other proteins in the divisome assemble along the Z ring.

Filamenting temperature-sensitive mutant Z



FtsZ is a prokaryotic homologue to the eukaryotic protein tubulin (微管蛋白)

Also found in dividing chloroplasts and some mitochondria

But why Z-ring forms only in the middle of the cell?

The formation of Z-ring limited in the center of cell by **oscillation** of MinCDE proteins

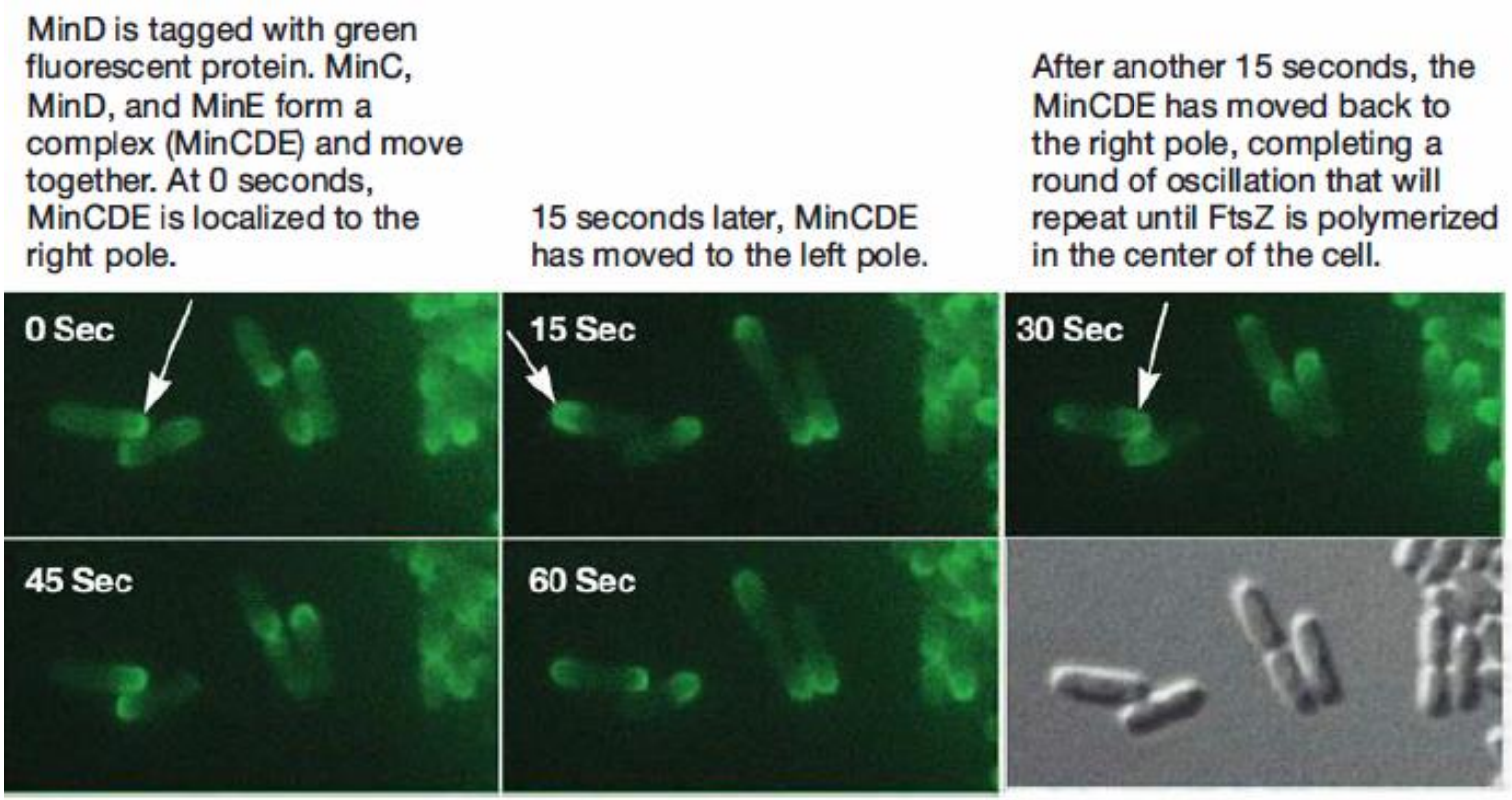


Figure 7.4 MinCDE Proteins and Establishment of the Site of Septum Formation. A GFP-MinD fusion protein is shown oscillating from one end of an *E. coli* cell to the other. MinC blocks septum formation. It oscillates with MinD, and since concentrations of MinC are highest at the poles, septum formation is forced to occur at the center of the cell.

Peptidoglycan synthesis (略)

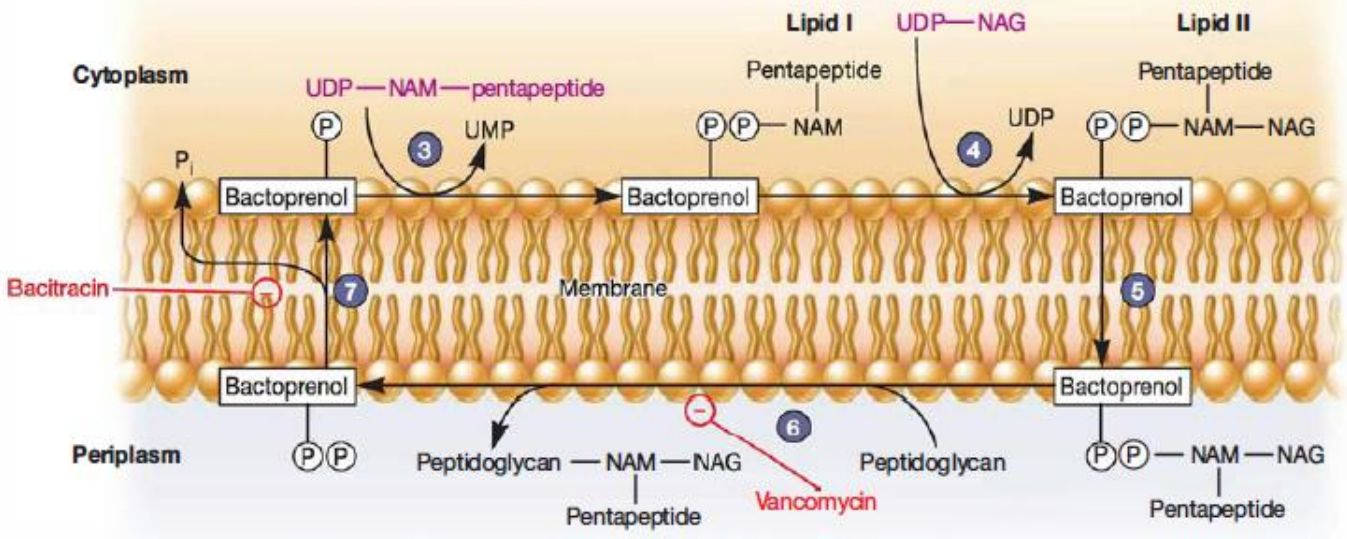
1. UDP derivatives of NAM and NAG are synthesized (figure 12.9).

2. Sequential addition of amino acids to UDP-NAM to form the NAM-pentapeptide (figure 12.9b).

3. NAM-pentapeptide is transferred to bactoprenol phosphate. They are joined by a pyrophosphate bond.

4. UDP transfers NAG to the bactoprenol-NAM-pentapeptide. If a pentaglycine interbridge is required, it is created using special glycyl-tRNA molecules but not ribosomes. Interbridge formation occurs in the membrane.

5. The bactoprenol carrier transports the completed NAG-NAM-pentapeptide repeat unit across the membrane.



6. The NAG-NAM-pentapeptide is attached to the growing end of a peptidoglycan chain, increasing the chain's length by one repeat unit.

7. The bactoprenol carrier moves back across the membrane. As it does, it loses one phosphate, becoming bactoprenol phosphate. It is now ready to begin a new cycle.

8. Peptide cross-links between peptidoglycan chains are formed by transpeptidation (figure 12.12).

Figure 12.10 Peptidoglycan Synthesis. NAM is *N*-acetylmuramic acid and NAG is *N*-acetylglucosamine. The pentapeptide contains L-lysine in *Staphylococcus aureus* peptidoglycan and diaminopimelic acid (DAP) in *E. coli*. Inhibition by bacitracin and vancomycin also is shown.

Cell wall synthesis during growth of cocci and rod bacteria

AI. Spherical cells build new peptidoglycan only at midcell, where the septum will form during division. This leads to daughter cells that have one old and one new cell wall hemisphere.

BI. During growth, prior to division, new cell wall is made along the side of the cell but not at the poles. This placement is thought to be determined by the position of MreB homologues.

BII. As division begins, FtsZ polymerization forms a Z ring and new cell wall growth is confined to the midcell.

BIII. Rod-shaped daughter cells are formed with one new pole and one old pole.

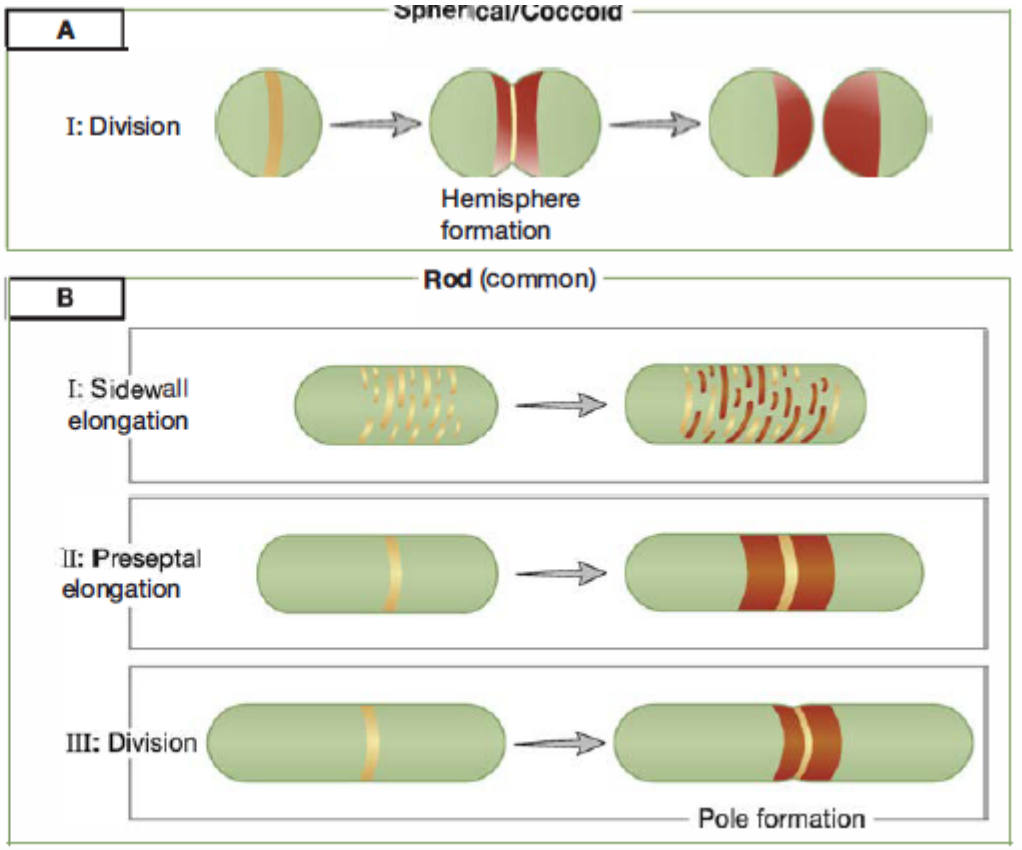


Figure 7.7 Cell Wall Biosynthesis and Determination of Cell Shape in Spherical and Rod-Shaped Cells.

Determination of cell shape

- Microbial cell shape is faithfully heritable.

- Microbial cell shape is not unchangeable. Some microorganisms show distinct cell shape under different physiological conditions.

- Cell wall play a key role for determining the cell shape.

Summary

- Most prokaryotic microorganisms reproduce by ***binary fission***
- Bacterial cell cycle has two related pathways: ***replication and partitioning of DNA*** and ***Cytokinesis.***
- Key function of Z-ring (divisome) during cell division

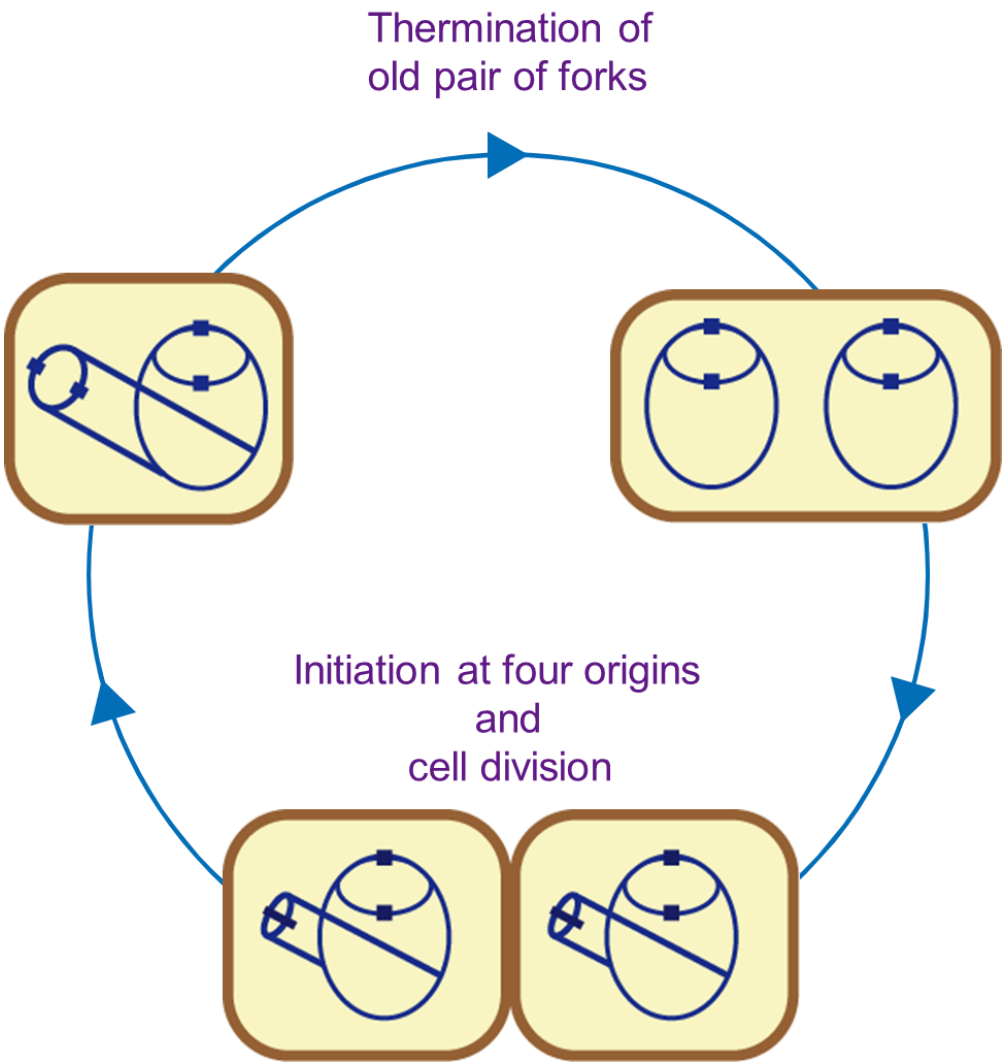
*Molecules in each *E. coli* cell

macromolecule	percentage of total dry weight	weight per cell (fg)	characteristic molecular weight (Da)	number of molecules per cell
protein	55	165	3×10^4	3,000,000
RNA	20	60		
23 S rRNA		32	1×10^6	20,000
16 S rRNA		16	5×10^5	20,000
5 S rRNA		1	4×10^4	20,000
transfer		9	2×10^4	200,000
messenger		2	1×10^6	1,400
DNA	3	9	3×10^9	2
lipid	9	27	800	20,000,000
lipopolysaccharide	3	9	8000	1,000,000
peptidoglycan	3	9	$(1000)_n$	1
glycogen	3	9	1×10^6	4,000
metabolites and cofactors pool	3	9		
inorganic ions	1	3		
total dry weight	100	300		
water (70% of cell)		700		
total cell weight		1000		

composition rules of thumb

- carbon atoms ~10^{10}
- 1 molecule per cell gives ~1 nM conc.
- ATP required to build and maintain cell over a cell cycle ~10^{10}
- glucose molecules needed per cell cycle ~3×10^9 (2/3 of carbons used for biomass and 1/3 used for ATP)

How does an E. coli cell divided each 20 min although its DNA replication will be finished in 40 min?



Influences of environmental factors on growth

Environmental factors

Salinity

Osmotic pressure

pH

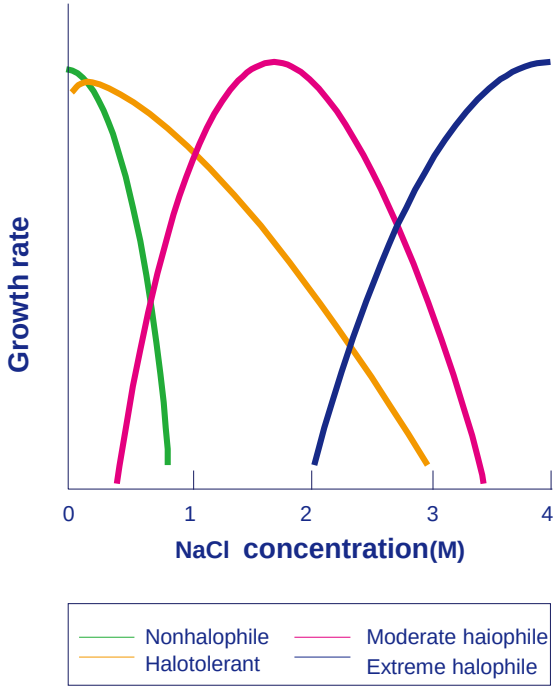
Temperature

Oxygen

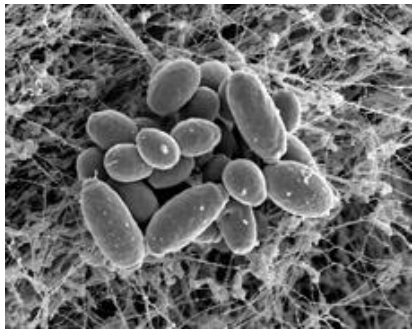
Pressure

Osmotic pressure or salinity

Nonhalophile	**Cannot sustain high salinity**
Halotolerant	**Grow optimally in low salinity but sustain high salinity**
Moderate halophile	**Require high levels of salt, usually over 0.2 M**
Extreme halophile	**Require high or nearly saturated salinity, usually over 2 M**



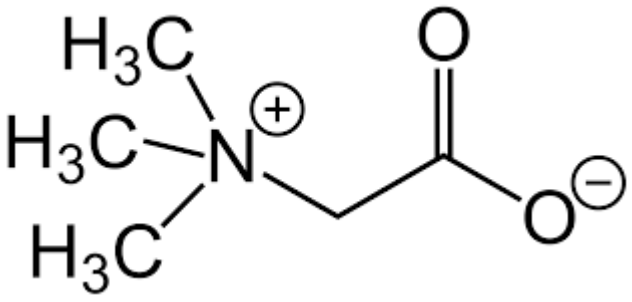
Nonhalophile: Freshwater species
Halotolerant: *Staphylococcus* spp. (葡萄球菌)
Moderate halophile: Seawater species
Extreme halophile: Brackish water (nearly saturated)



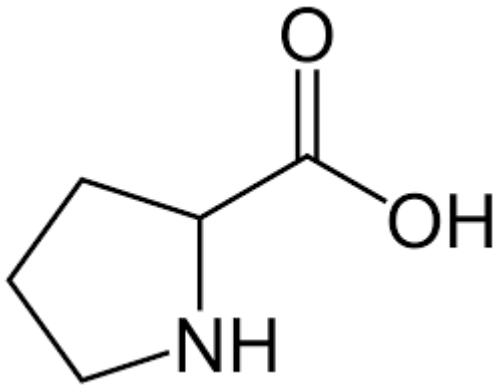
Natronobacterium gregoryi

Compatible solutes补偿溶质

- Balance the high osmotic pressure outside cell
- Potassium chloride
- Betaine (甜菜碱), and amino acids such as proline (脯氨酸) and glutamic acid (谷氨酸)



Betaine



Proline

Water activity*

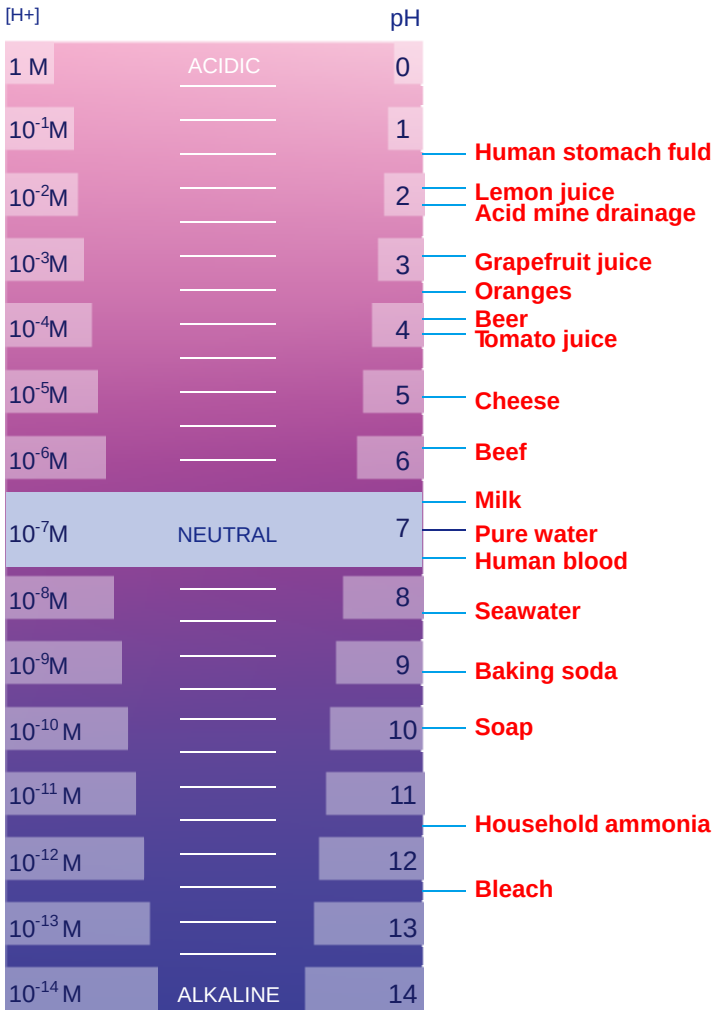
- Water activity or $\mathbf{a_w}$ is the partial vapor pressure of water in a substance divided by the standard state partial vapor pressure of water.
- Higher $\mathbf{a_w}$ substances tend to support more microorganisms. Bacteria usually require at least 0.91, and fungi at least 0.7.

Substance	a_w	Source
Distilled **Water**	1.00	[5]
Tap **water**	0.99	[*citation needed*]
Raw meats	0.99	[5]
Milk	0.97	[*citation needed*]
Juice	0.97	[*citation needed*]
Salami	0.87	[5]
Shelf-stable cooked bacon	< 0.85	[6]
Saturated NaCl solution	0.75	[*citation needed*]
Point at which cereal loses crunch	0.65	[*citation needed*]
Dried fruit	0.60	[5]
Typical indoor air	0.5 - 0.7	[*citation needed*]
Honey	0.5 - 0.7	[*citation needed*]

Microorganism Inhibited	a_w
Clostridium botulinum A, B	0.97
Clostridium botulinum E	0.97
Pseudomonas fluorescens	0.97
Clostridium perfringens	0.95
Escherichia coli	0.95
Salmonella	0.93
Vibrio cholerae	0.95
Bacillus cereus	0.93
Listeria monocytogenes	0.92, (0.90 in 30% glycerol)
Bacillus subtilis	0.91
Staphylococcus aureus	0.86
Most molds	0.80
No microbial proliferation	0.50

pH

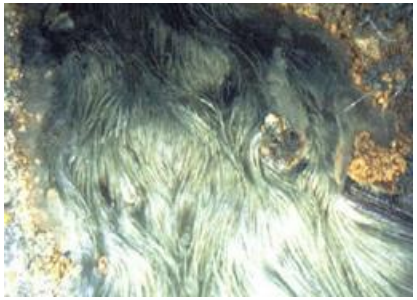
Acidophile	**Growth optimum between pH 0-5.5**
Neutrophile	**Growth optimum between pH 5.5-8.0**
Alkaliphile	**Growth optimum between pH 8.0-11.5**



pH opyima of some microbes

Ferroplasma spp.(A)

Dunaliella acidophila(E)
Cyanidium caldarium(E)
Thiabacillus thiooxdans(B)
Suifolabus acido caldarius(A)



Physanum polyciphalum(E)

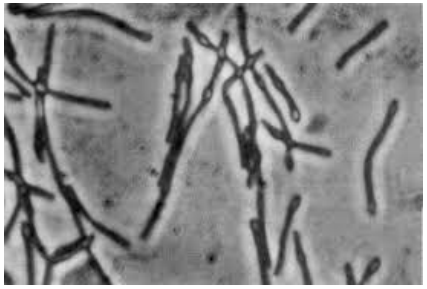
Lactobacillus acidophilus(B)
E.coli,Pseudomonas aeruginosa(B)

Staphylacoccus aureus(B)

Nitrosomonas spp.(B)

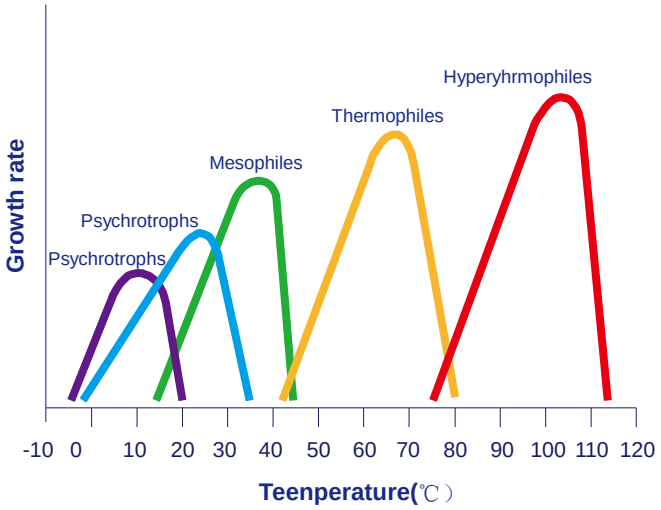
Microcystis aeruginosa(B)

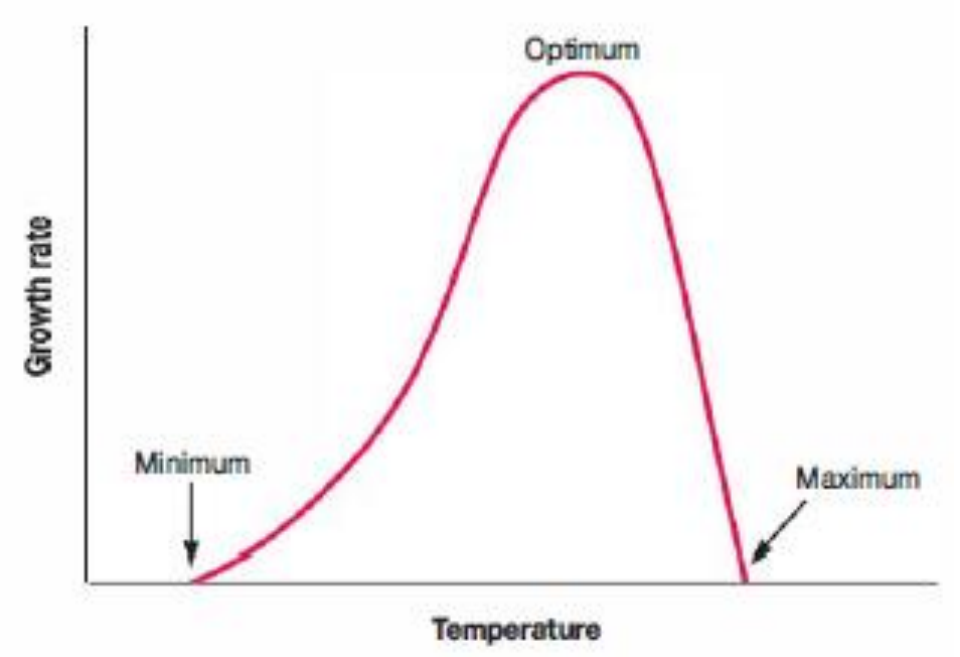
Bacillus alcalaphilus(B)



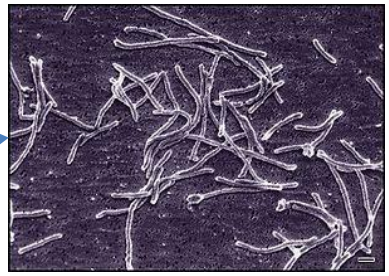
Temperate

Psychrophile(嗜冷)	Growth at 0°C and has an optimal growth temperature of 15°C or lower
Psychrotroph(耐冷)	Can grow at 0-7°C; has an optimum between 20-30°C and a maximum around 35°C
Mesophile(嗜温)	Has growth optimum between 20-45°C
Thermophile(嗜热)	Can grow at 55°C or higher; optimum often between 55 and 60°C
Hyperthermophile (超嗜热)	Has an optimum between 85 and about 113°C

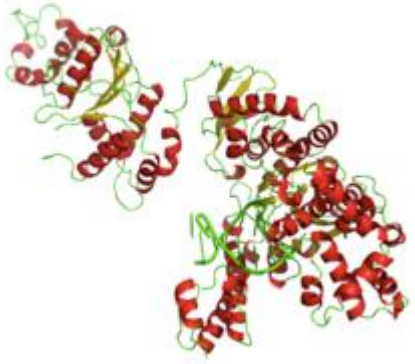




Microorganism	Minimum	Optimum	Maximum
Nonphotosynthetic Bacteria and Archaea			
Bacillus psychrophilus	–10	23–24	28–30
Pseudomonas fluorescens	4	25–30	40
Enterococcus faecalis	0	37	44
Escherichia coli	10	37	45
Neisseria gonorrhoeae	30	35–36	38
Thermoplasma acidophilum	45	59	62
Thermus aquaticus	40	70–72	79
Pyrococcus abyssi	67	96	102
Pyrodictium occultum	82	105	110
Pyrolobus fumarii	90	106	113
Photosynthetic Bacteria			
Anabaena variabilis	ND[1]	35	ND
Synechococcus eximius	70	79	84
Protists			
Chlamydomonas nivalis	–36	0	4
Amoeba proteus	4–6	22	35
Skeletonema costatum	6	16–26	>28
Trichomonas vaginalis	25	32–39	42
Tetrahymena pyriformis	6–7	20–25	33
Cyclidium citrullus	18	43	47
Fungi			
Candida scotti	0	4–15	15
Saccharomyces cerevisiae	1–3	28	40
Mucor pusillus	21–23	45–50	50–58

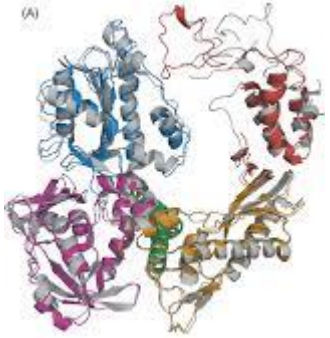


Thermus aquaticus
栖热水生菌



Taq DNA polymerase

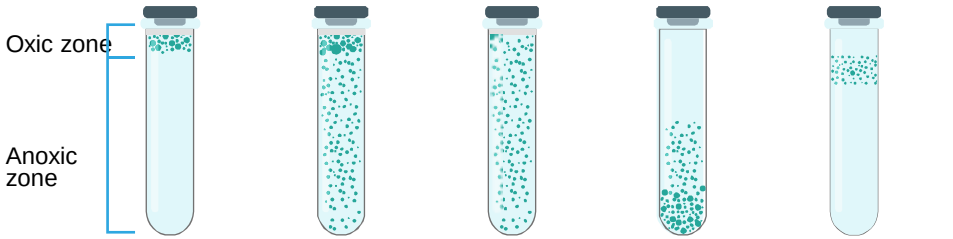
Pyrococcus furiosus



Pfu DNA polymerase

Oxygen concentration

Obligate aerobe 严格好氧菌	**Complete dependent on atmospheric O_2 for growth**
Facultative anaerobe 兼性厌氧菌	**Does not require O_2 for growth but grow better in its prescence**
Aerotolerant anaerobe 耐氧厌氧菌	**Grow equally well in presence or absence of O_2**
Obligate anaerobe 严格厌氧菌	**Does not tolerate O_2 and die in its presence**
Microaerophile 微需氧细菌	**Require O_2 levels between 2-10% for growth and is damaged by atmospheric O_2 level**



Enzyme content

Obligate aerobe	Facultative anaerobe	Aerotderant anaerobe	Strict anaerobe	Microaerophle
+SOD +Catalase	+SOD +Catalase	+SOD -Catalase	-SOD -Catalase	+SOD +/- Catalase (low levels)

Questions for thinking:

Why there are anaerobic environments? Give some examples of anaerobic environments.

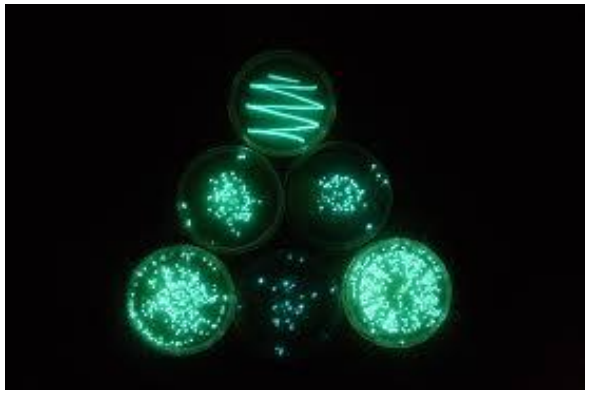
Pressure

Piezophile or barophile	**Growth more rapid at high hydrostatic pressures**

Seawater: 10m = 1 atm

Crust(地壳): 3-4m= 1 atm

Barotolerant (耐压) vs. **Barophilic (嗜压)**



Photobacterium profundum

grow under 0.1 MPa to 70 MPa

- Increase the amount of unsaturated fatty acids in their membrane lipids as pressure increases
- Shorten the length of their fatty acids

Laboratory Culture of Cellular Microbes

Culture Media

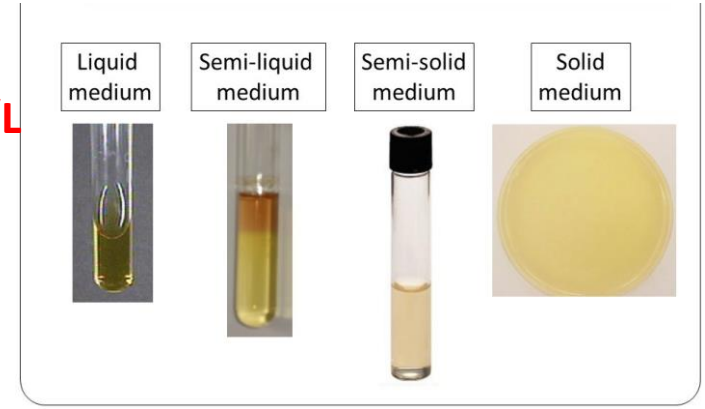
Basis for classification	Types
Chemical composition	Defined (synthetic), complex
Physical nature	Liquid, semisolid, soild
Function	Supportive基本, enriched丰富, selective选择, differential鉴别

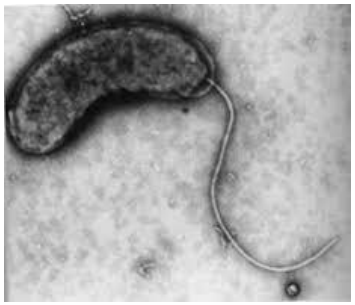
M9 medium for E. coli

Formula for 1X M9 (per liter):
0.3% (22mM) Potassium Phosphate (KH_2PO_4) (3.0g/L)
0.6% (22mM) Sodium Phosphate (Na_2HPO_4) (6.0g/L)
0.5% (85mM) Sodium chloride (NaCl) (5.0g/L)
0.1% Ammonium Chloride (NH_4Cl)(1.0g/L)
2mM Magnesium Sulfate ($MgSO_4$)
0.1mM Calcium Chloride ($CaCl_2$) (15.0mg/L) (optional)
0.4% Glucose (or Glycerol) (4.0g/L) (optional)

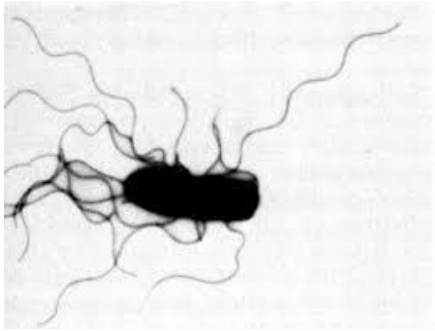
LB meidum

Yeast extract酵母粉 5.0g/L
Tryptone蛋白胨 10.0g/L
NaCl 10.0g/L

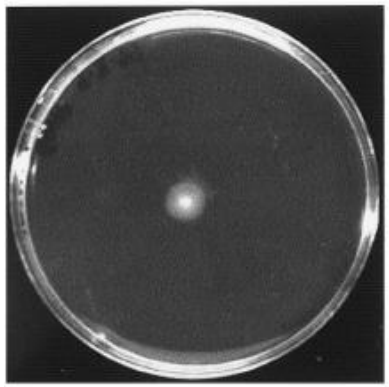


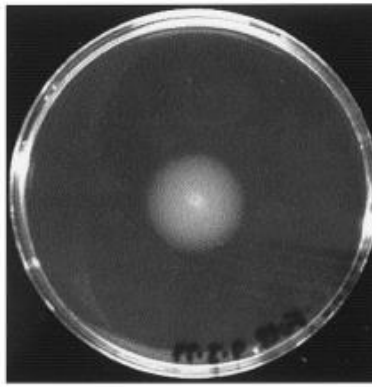


Polar flagellum
端生鞭毛



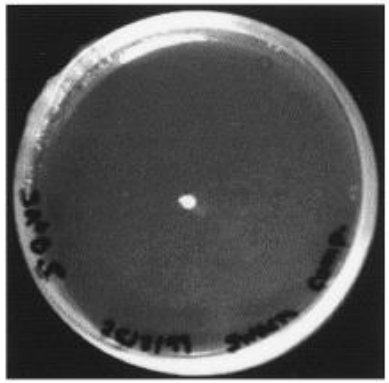
Peritrichous flagellum
周生鞭毛

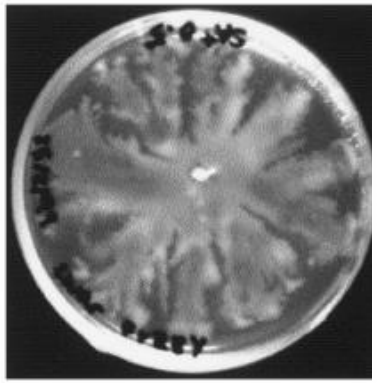




Agar
0.3%

Swimming





Agar
0.5%

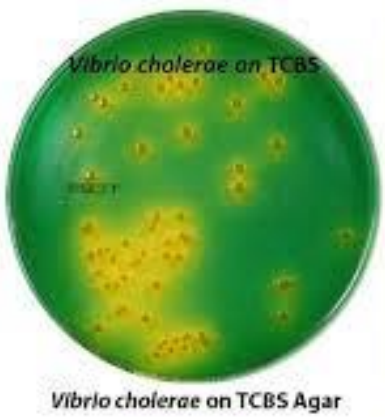
Swarming

Sánchez-Contreras et al., 2002

Functional types of media

- **Supportive media**: general purpose and sustain the growth of many microorganisms
- **Enriched media**: other nutrients may be added to supportive media to encourage the growth of fastidious microbes.
- **Selective media**: allow the growth of particular microorganisms, while inhibiting the growth of others.
- **Differential media**: distinguish among different groups of microbes and even permit tentative identification of microorganisms based on their biological characteristics.

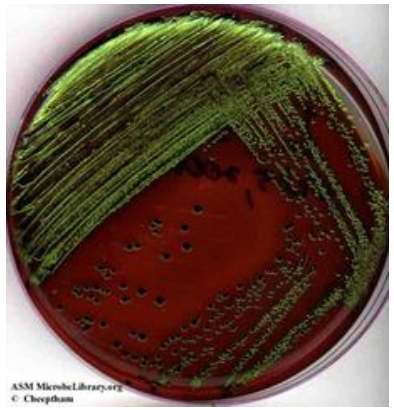
TCBS Medium



Vibrio cholerae on TCBS Agar

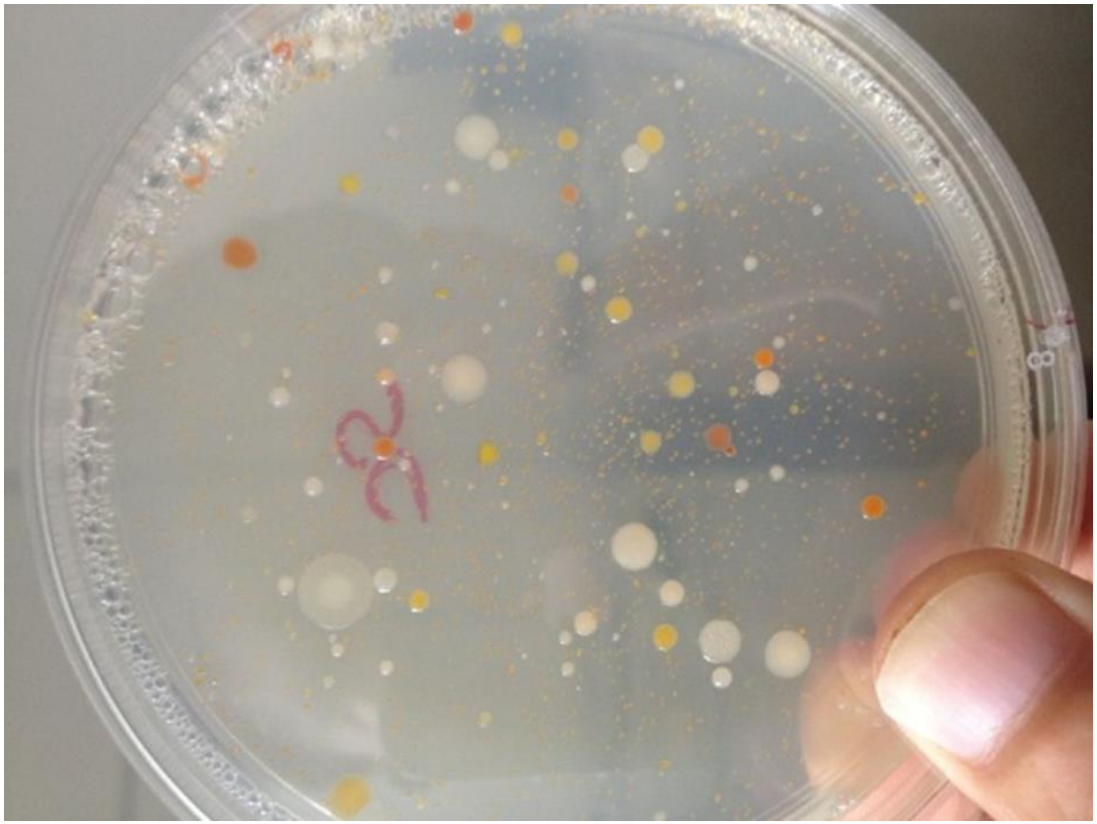

Vibrio parahaemolyticus on TCBS Agar

EMB medium
伊红美蓝培养基



Culture methods

Culture in **solid** and liquid medium



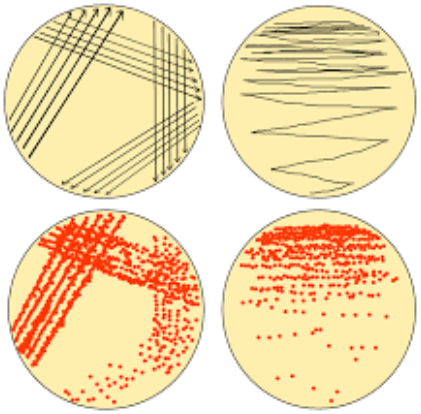
Used in both cell counting and isolation

CFU (colony forming unit)

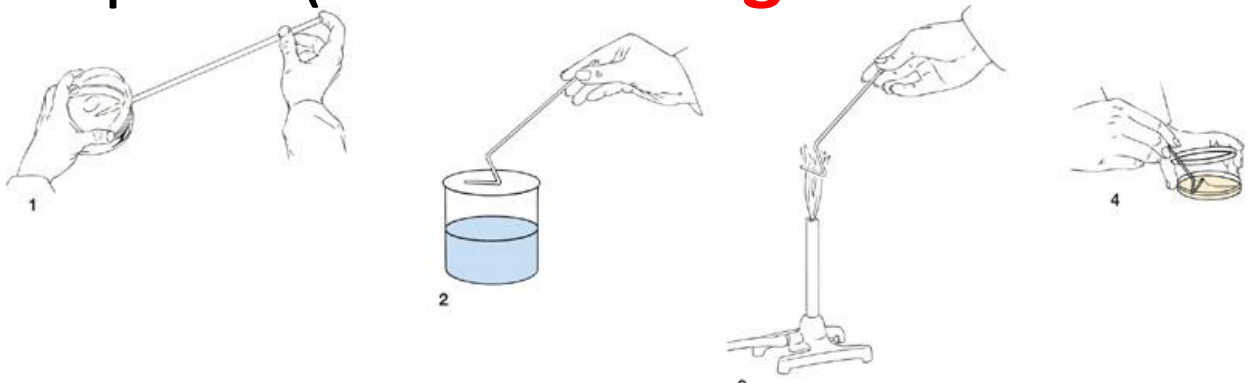
colony from a single viable (可育的) and cultivable cell

Inoculation on agar plate

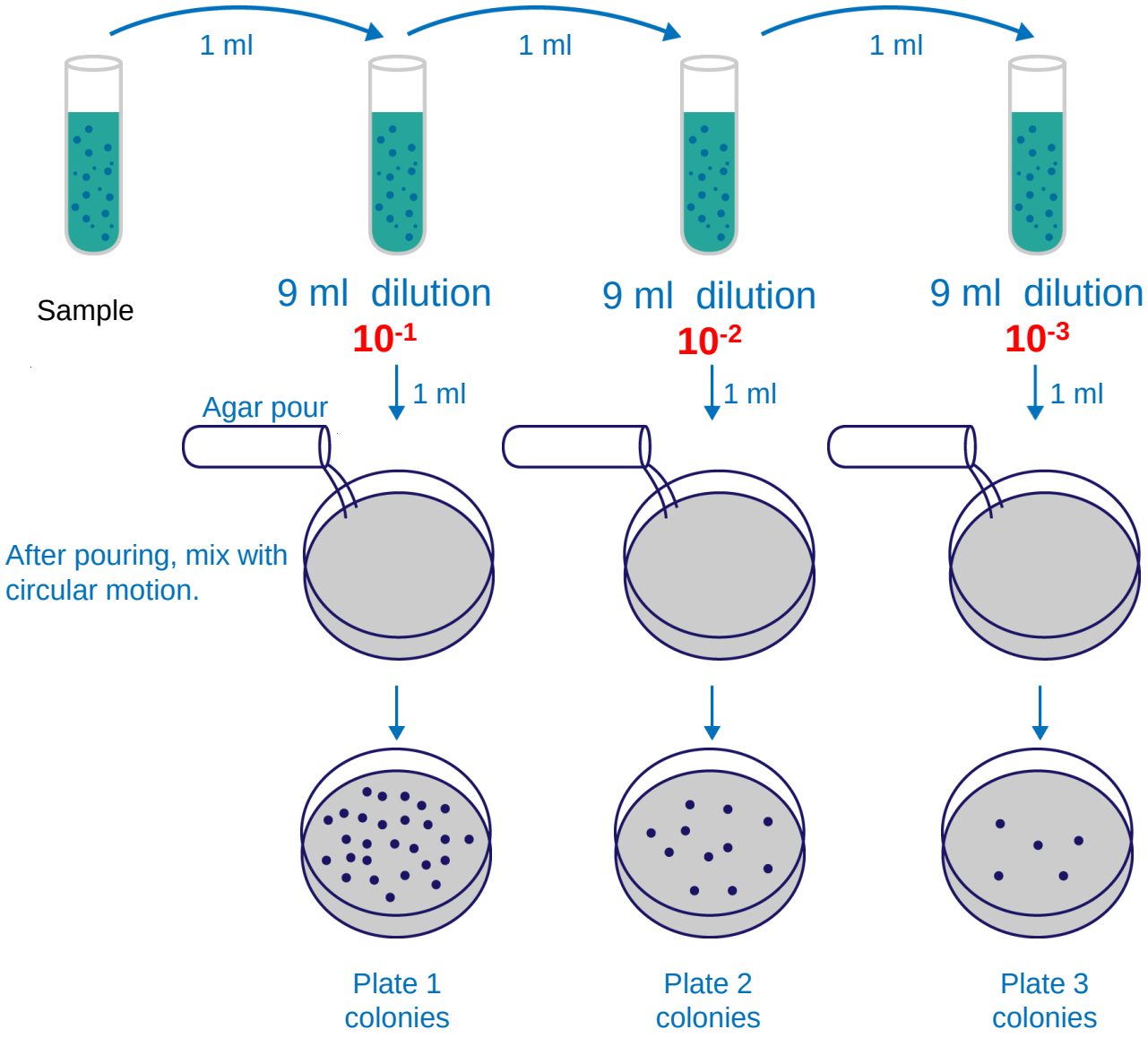
- Streak plate (**Isolation**)



- Spread plate (**Cell counting and isolation**)

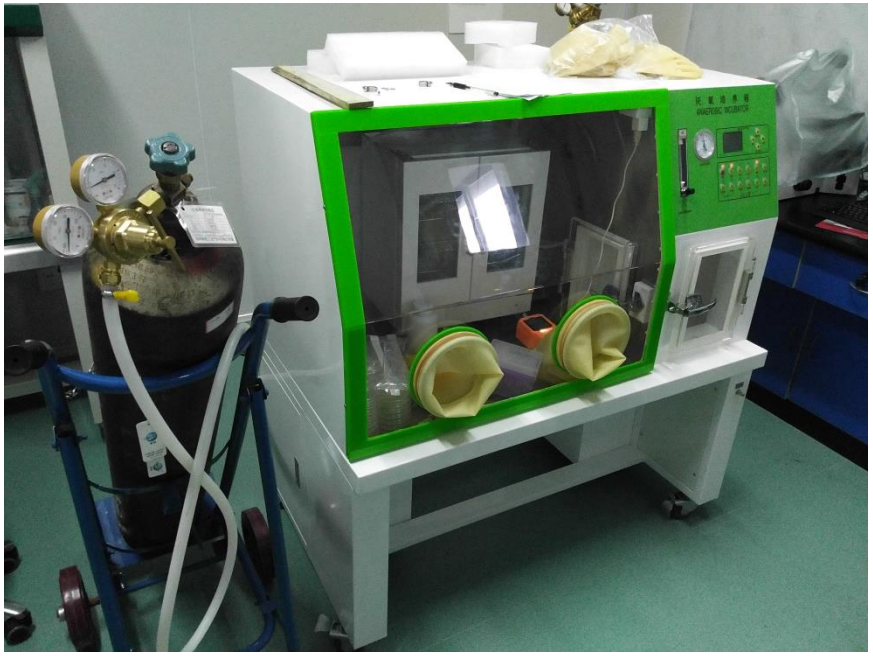


- Pour plate (**Cell counting and isolation**)



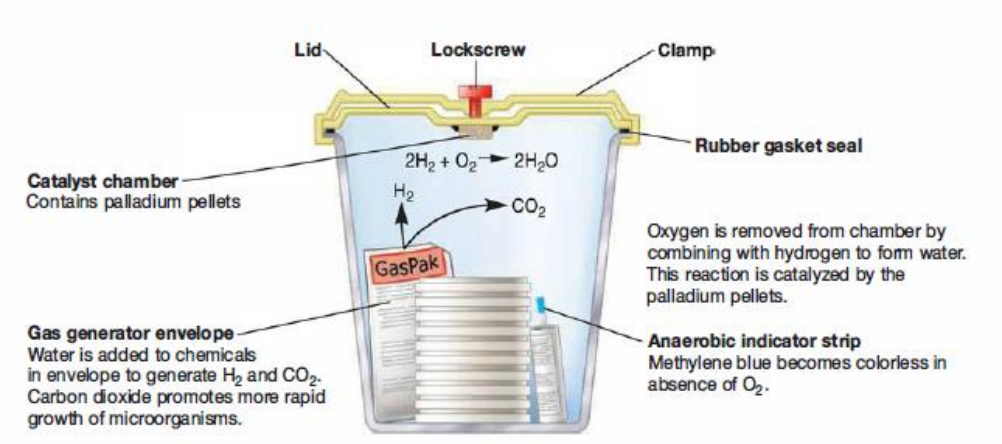
Any difference between the pour plate and spread plate?

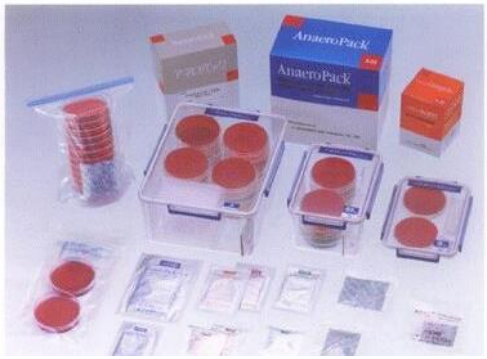
Cultivation of anaerobes (厌氧微生物)



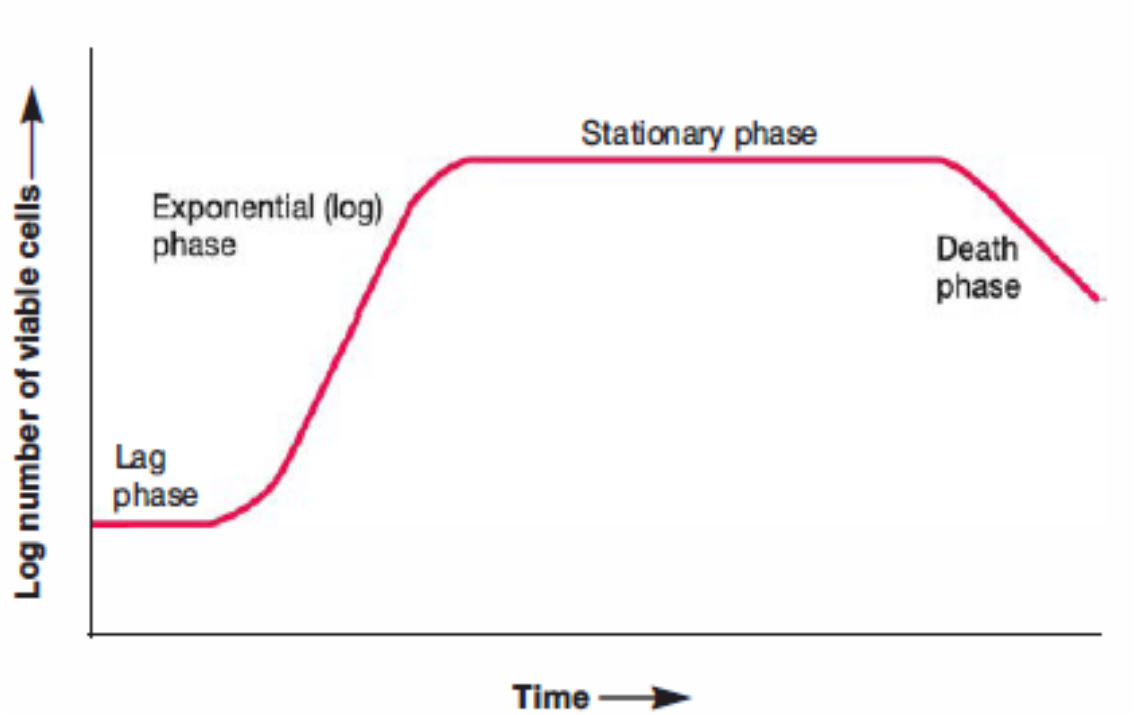
***Glovebox*手套箱**

Need H_2 and palladium catalyst (钯触媒) to keep oxygen-free

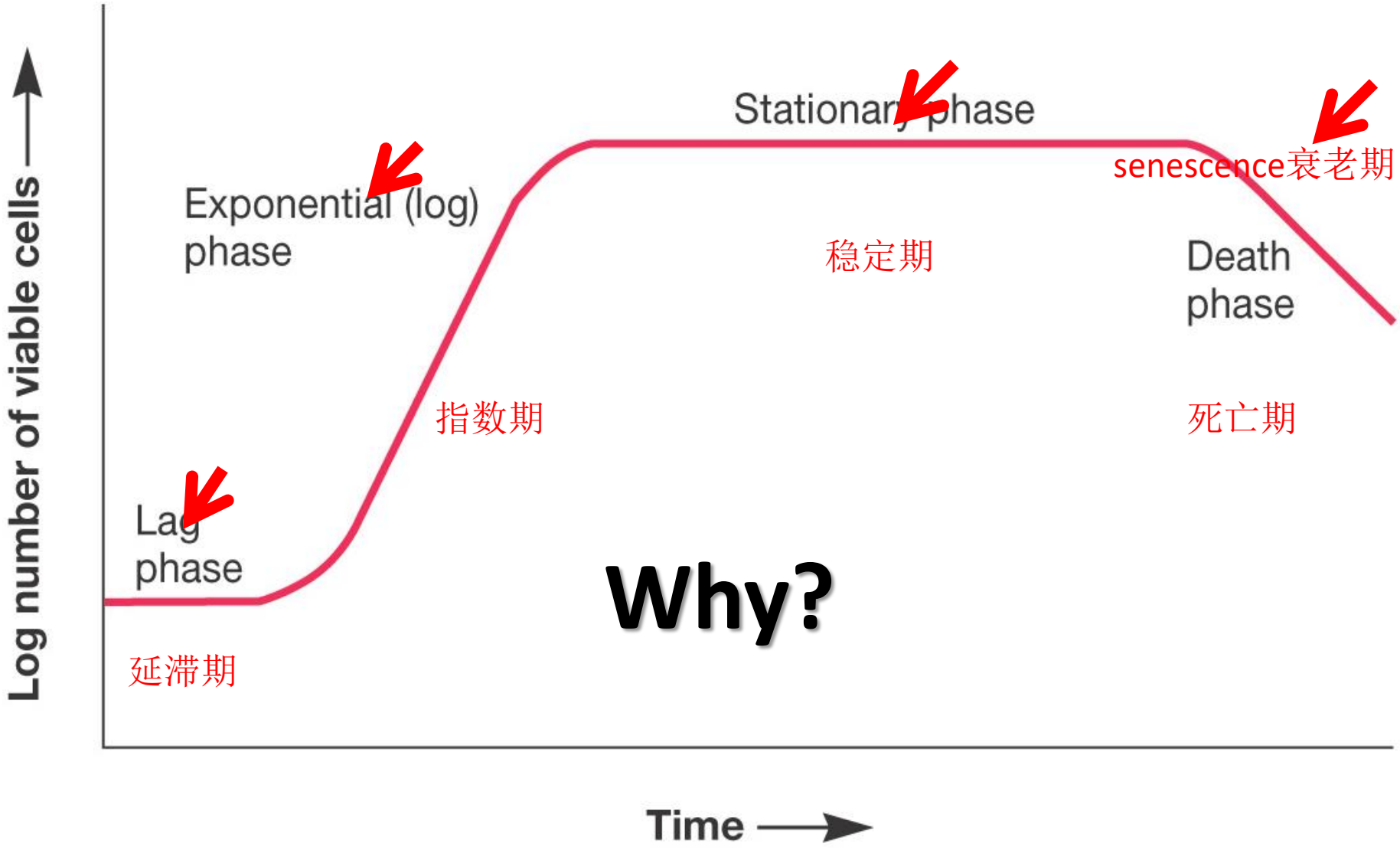




Growth Curve



1. Refer to growth in the size of a population;
2. Grow in liquid medium;
3. Grow in **batch culture (a closed system);**
4. Although it is “life in the lab”, it is important for understanding the growth in natural environments.

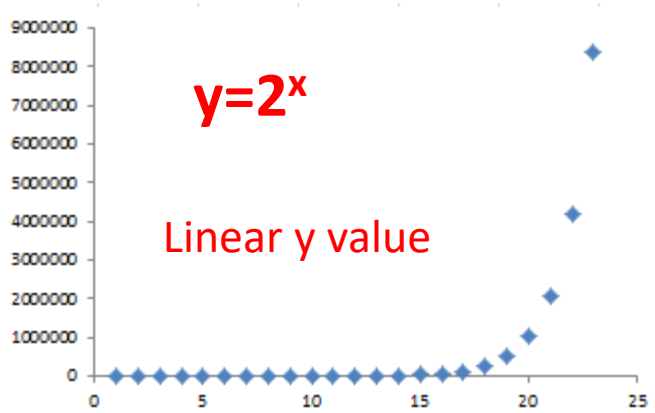

Log number of viable cells
Exponential (log) phase
Stationary phase
senescence衰老期
稳定期
Death phase
指数期
死亡期
Lag phase
Why?
延滞期
Time

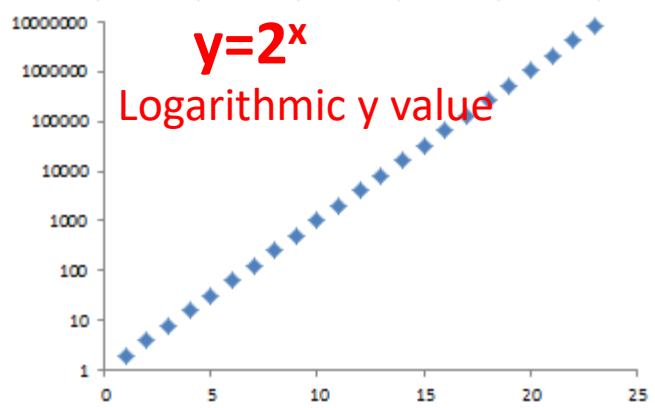
Lag phase

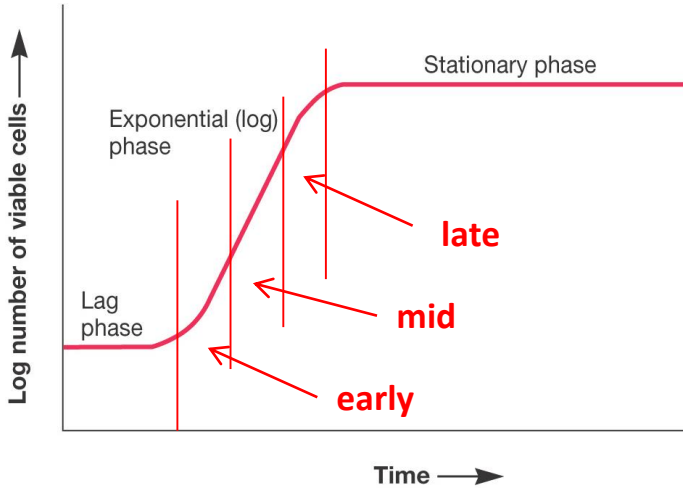
- **Cell synthesizing new components**
- **Adapt to new medium or other conditions**
- **Varies in length (in some cases can be very short or even absent)**

Exponential phase

- also called log phase
- rate of growth and division is **constant**
- population is most **uniform** in terms of chemical and physiological properties during this phase
- usually used in biochemical and physiological study

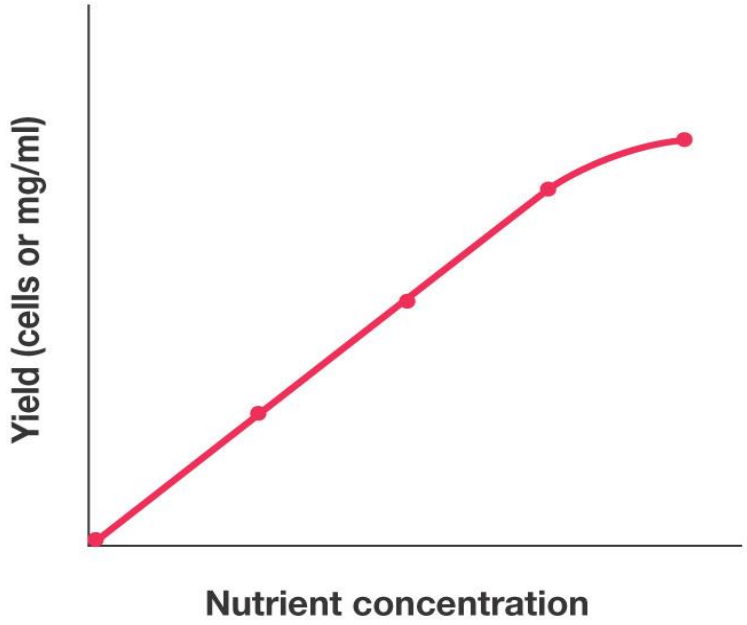


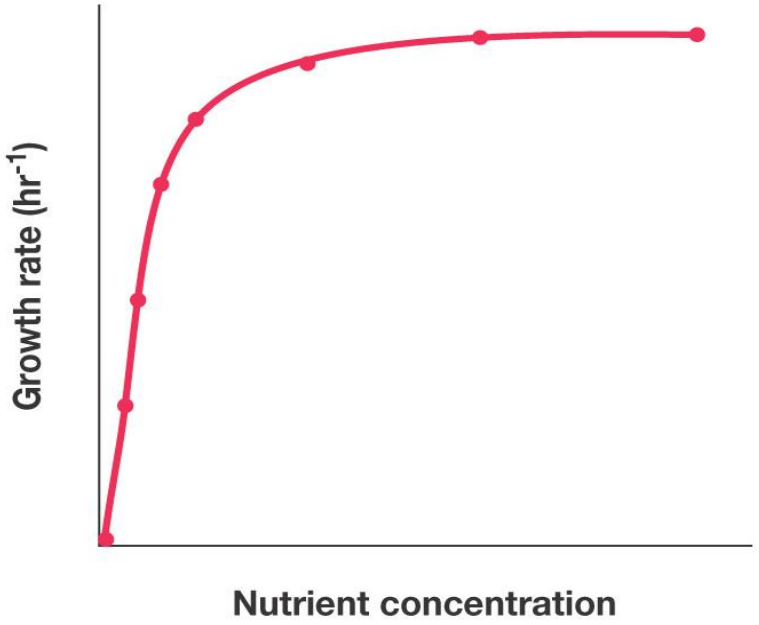




Growth rate in the exponential phase is determined by

- Microbial characteristics
- The nature of the medium
- The environmental conditions



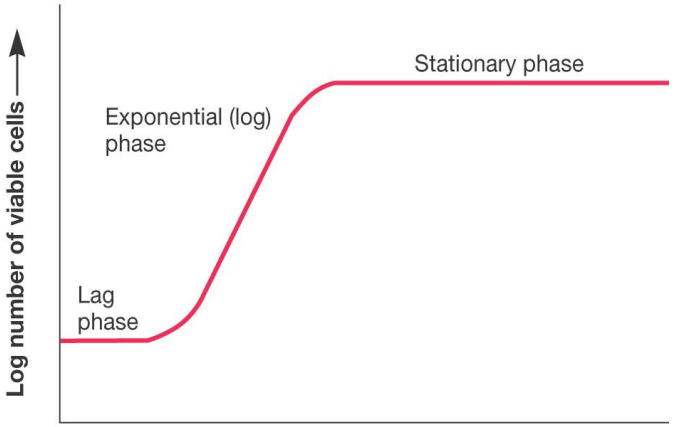


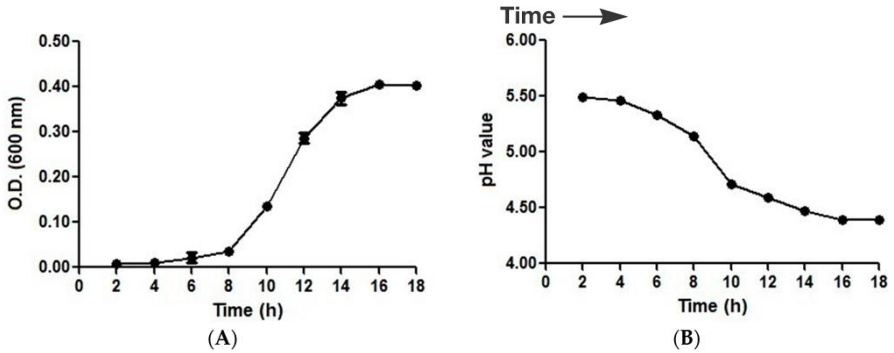
Stationary phase

Closed system population growth eventually ceases, total number of viable cells remains constant (**active cells stop reproducing** or **reproductive rate is balanced by death rate**)

stress

- Essential nutrient limitation
- Limited oxygen availability
- Toxic waste accumulation





Senescence (细胞衰老) and death phase

Two alternative hypotheses:

1. Cells are Viable But Not Culturable (VBNC)

Cells are alive, but dormant, capable of new growth when conditions are right

2. Programmed cell death (PCD)

Fraction of the population genetically programmed to die (commit suicide, altruists, 利他主义者)

Mathematics of Growth

Given N_0(cell number or density at beginning), N_t (cell number or density at time point t)

and t (time point)

Growth rate= $[\log_2(N_t/N_0)]/t$ (***why $\log_2$?***)

Generation time (代时) =$t/[\log_2(N_t/N_0)]$

How to determine N_0 and N_t?

Discussion

1. If you wished to obtain a pure culture of bacteria that could degrade benzene and use it as a carbon and energy source, how would you proceed?
2. Design an experiment to screen an auxotroph of *E. coli* that cannot synthesize proline. How can you verify it at gene-level?
3. Suppose you discovered a new bacterial strain from the permafrost of Alaska. You are able to culture the bacterium in a define medium, but it grows very slowly. How could you optimize its growth? Explain your choices.
4. Give one bacterial example of chemolithoautotroph and describe the relationships between its distribution and function
5. Introduce the event https://en.wikipedia.org/wiki/GFAJ-1 to classmates. You may give your personal comments on the false discovery.
6. What are peptones, yeast extract, beef extract, thioglycollate and agar? Why are they used in media?