＜＜亦敵亦友：探索微生物的世界＞＞

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TOPICS

- Introduction
- Microbial utilization & Biotechnology
- Pathogenic microorganisms
- The genes we share with yeast
- Genes to genomics, proteomics & biological networks

微生物之簡介

微生物之發現與微生物學之發展

什麼是微生物 (microorganisms / microbes) ？

細胞結構：原核與真核細胞

微生物在分類學上之地位

Microbiology

mikros = 微小 small

bios = 生命 life

logos = 科學

古代: 醬油、酒、麵包等釀造發酵食品

雷文霍克 (Leeuwenhock), 十七世紀：使用自製的簡單顯微镜觀察酵母菌、紅血球、原生動物以及細菌

自然發生說 (spontaneous generation):
認為生命乃是自然發生的

巴斯德 (Louis Pasteur, 1822-1895)

(1) 推翻了自然發生說, 而提倡了生物發生說 (biogenesis), 確認了「生命源自於生命的觀念」

(2) 確認了酒精發酵與酵母的關係、殺菌問題等，並說明了抗體（antibodies）在寄主內產生

(3) "微生物之父"

Pasteur Institute Study of biology, micro-organisms, diseases & vaccines

http://en.wikipedia.org/wiki/Pasteur_Institute

什麼是微生物 (microorganisms / microbes) ?

- 肉眼所無法見到之生物體

- 五大類微生物:

細菌 (Bacteria)　　原生生物 (Protoza)

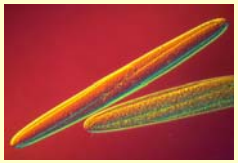
藻類 (Algae)　　病毒（Virus）

真菌 (Fungi): 酵母菌 (Yeast) & 黴菌 (Molds)

Some "monster" bacteria:

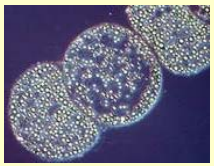
Epulopisium fishelson (1991): 刺尾魚(surgeonfish)之內臟，紅海 / 澳大利亞, ~ 600 micrometers

Acanthurus lineatus, the striped surgeonfish



http://www.micro.cornell.edu/faculty/Angert/faculty.EAngert.html

Thiomargarita namibiensis (1999): 納米比亞之海岸, 100-750 micrometer



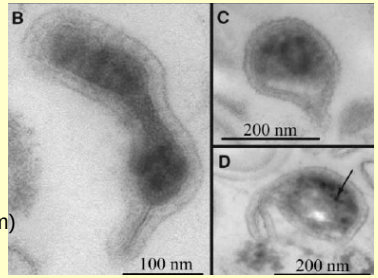
http://www.bact.wisc.edu/Microtextbook/index.php?module=Book&func=displayfigure&book_id=4&fig_number=34&chap_number=2

How small an organism can be?

December 2006
University of California, Berkeley

Three new archaea

Diameter: 200 nm (= 1/50,000 cm)
~ 1/5 of common bacteria



4,000,000 of them = 1 period (句點)

How big is a…. ? http://www.cellsalive.com/howbig.htm

細胞結構: 原核與真核細胞

- 細胞: 生命之最基本單位
- 根據它們之細胞結構處組成, 所有生活細胞可被分成兩類:

原核（Prokaryotic）& 真核（Eukaryotic）細胞

Prokaryotic cells	Eukaryotic cells
Bacteria	Animals Plants Fungi Protoza

Selected characteristics of prokaryotic & eukaryotic cells

	Prokaryote	Eukaryote
Cell size	0.5-2 μm	2-200 μm
Nuclear body	No nuclear membrane; no mitosis	True nucleus; nuclear membrane; mitosis
DNA	Single molecule; not in chromosomes	Several chromosomes
Organelles	None	Mitochondria, chloroplasts, vacuoles, others
Cell wall	Relatively thin; usually peptidoglycan	Thick or absent; chemically different

(Brock & Madigan, 1991)

微生物之分類: The three domain system

- 1977年前，原核生物被認為是最原始之生物，隱喻此微生物為複雜生物之祖先。但是Carl Woese等人發現此兩類生物非相互演化而成，而是源自共同祖先，以完全不同的途徑各自進化而成。
- 此觀念是源自核醣體核醣核酸(ribosomal ribonucleic acid；rRNA)之研究
- 將生物分為三域（domains):

Archaebacteria（太古生物）: methanogens, extreme halophiles, hyperthermophiles

Eubacteria（真細菌）: Gram (+) & Gram (-) bacteria

Eukaryotes（真核生物）: algae, protozoa, fungi, plants, animals

微生物之特性、細胞結構、營養需求與細胞生長

細菌之特性

- 原核細胞
- 單細胞生物
- 一般比真核細胞較小
- 雖然很小、但是結構及生理能仍很複雜
- 大多數細菌具有下列其中一種形狀：

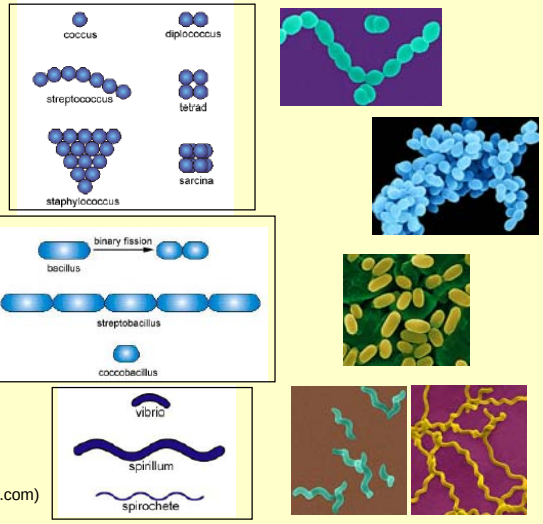
  球狀 （Coccus）
  桿狀 （Rod or bacillus）
  螺旋狀 （Spiral）

Coccus

Rod / Bacillus

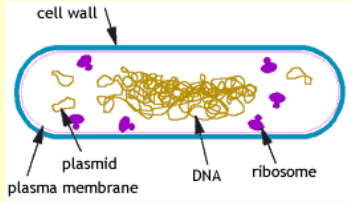
Spiral

(http://denniskunkel.com)



細菌之外觀結構

- 細胞壁（cell wall）、細胞膜（cell membrane）與外膜（outer membrane)
- 流體之細胞質（cytoplasm），其中含有一個 nuclear region (nucleoid) & 核醣體（ribosomes）等

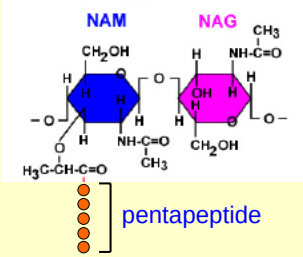


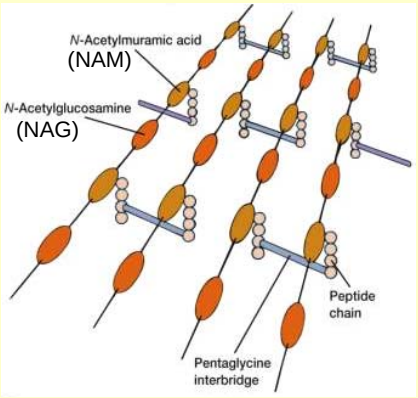
- 其他外在之結構（例如：鞭毛及纖毛）

細菌之細胞壁

- 由 **Peptidoglycan 肽聚糖 (或稱胞壁質murein) 多元體 (polymer)** 所組成
- **Peptidoglycan多元體:** 由成鏈相同之peptidoglycan單元體組成
- **Peptidoglycan單元體 (monomer):**

N-acetyl glucosamine (NAG), N-acetyl muramic acid (NAM) and pentapeptide





Peptidoglycan (murein)

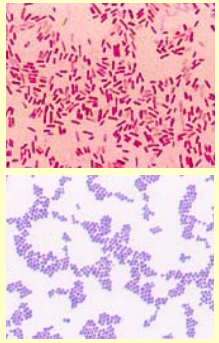
(Prescott et al., Microbiology, 2005)

格蘭陽性與格蘭陰性細菌

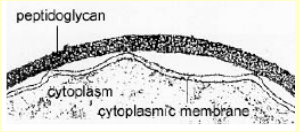
＜格蘭染色法＞

- Staining w/ crystal violet
- Treat with iodine
- Rinsed with alcohol
- Counterstain w/ safranine

Gram (-) : Pink to Red
Gram (+): Dark purple

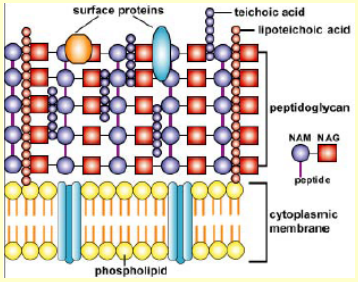


格蘭陽性細菌之細胞壁



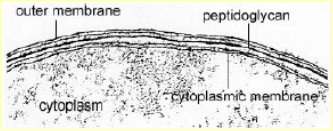
Peptidoglycan (60-80%)
Teichoic acids
Lipoteichoic acids
Proteins

- **Peptidoglycan 防止細胞因滲透壓造成溶菌（lysis)**
- **Techoic acids 可能幫助增強細胞壁之強度**
- **蛋白質之功能:**
  - **酵素**
  - **adhesins**
  - **invasins**
  - **抗拒吞噬細胞之攻擊**



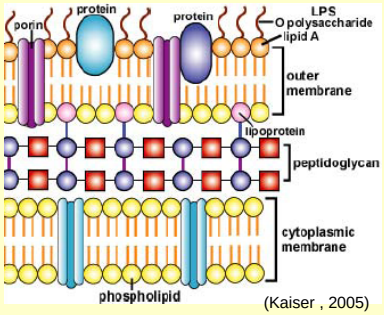
(Kaiser, 2005)

格蘭陰性細菌之細胞壁



Peptidoglycan (10-20%)
Outer membrane (phospholipids, Lipoproteins, proteins & lipopolysaccharides, LPS)

- **Peptidoglycan 防止細胞因滲透壓造成溶菌（lysis)**
- **LPS是內毒素，可促進免疫之反應**
- **蛋白質之功能:**
  - 酵素
  - adhesins
  - invasins
  - 抗拒吞噬細胞之攻擊



(Kaiser , 2005)

格蘭陰性細菌, 例如:

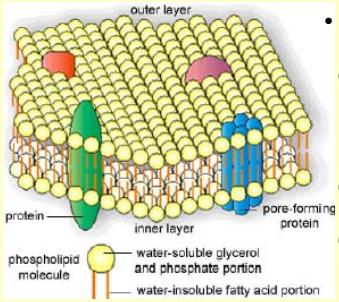
Escherichia coli（大腸桿菌）
Salmonella typhosa（傷寒菌）
Pseudomomas aeruginosa（綠膿桿菌）
Vibrio comma（霍亂菌）

格蘭陽性細菌, 例如:

Staphylococcus aureus（金黃葡萄球菌）
Bacillus subtilis（枯草桿菌）
Clostridium tetani（破傷風菌）

細菌之細胞膜

- 主要之化學組成份: 磷脂質(phospholipid) & 蛋白質（proteins）



(Kaiser, 2005)

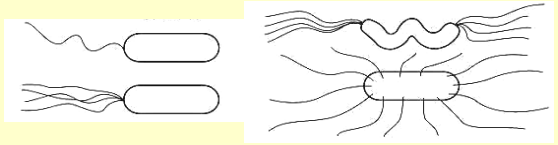
- **主要之功能:**

(1)選擇性之通透膜：決定物質之進出（例如：營養素、氣體）

(2)廢物之排出、胞子之形成

(3)抗生素（例如：**polymyxins**）作用之目標

細菌之鞭毛 (flagella)

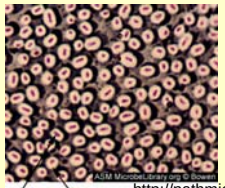


- 分為週鞭毛、端鞭毛、多鞭毛、單鞭毛等
- 細菌的運動器官，使細菌能在液體環境中泳動
- In general, counterclockwise rotation causes forward motion, clockwise rotation causing a tumble

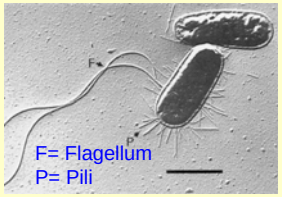
細菌之莢膜 (Capsule) & 鐵毛 (Pili)

Capsule

- Resistance to phagocytosis
- Adherence to surfaces



http://pathmicro.med.sc.edu/fox/protype.htm



zbio.net/pictures/e_coli_pili.jpg

Pili

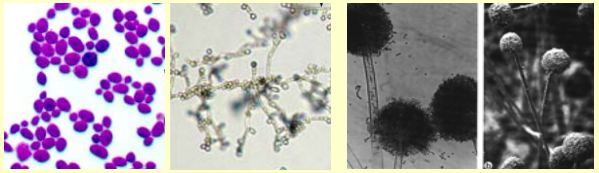
- Attachment to surface
- Bacterial mating

在細菌細胞質之內（within the cytoplasm)

- 細胞質：大多數細菌代謝反應發生之場所
- Cytosol：細胞質之液體成份
- Nucleoid: chromosome (染色體) 之所在，不具有核膜
- Plasmids（質體): small, closed circular DNA
- Ribosomes（核醣體）: 蛋白質之合成

真菌之特性

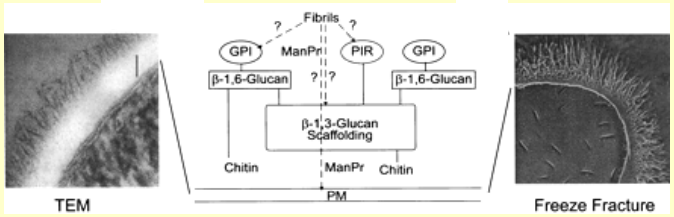
- 包含酵母菌（Yeast）與黴菌（Molds）
- 真核細胞: 有核膜及核仁，二或二條以上之染色體
- 有一個較堅固之細胞壁



Yeasts　　Molds

真菌之細胞結構

- 細胞膜：phospholipid, proteins & glycoproteins
- 細胞壁： chitin and/or cellulose, proteins
  glucans (carbohydrate chains): glucose, mannose & N-acetyl-D-glucosamone



部份之功能:防止因滲透壓造成溶菌（lysis), 與寄主之接觸, drug targets

Membrane-bound organelles:

細胞進行代謝、提供能量、傳遞化學物質之場所

The nucleus: nuclear membrane, multiple chromosomes within

The endoplasmic reticulum (內質網): protein & lipid synthesis, transport molecules within the cells, molecules storage

The Golgi complex (高基氏體): sort proteins & lipids received from the ER, sort/package molecules into vesicle for transport to other part of the cell or secretion

Mitochondria (粒線體) and others

微生物之營養需求與細胞生長

微生物營養及環境之需求

- 對物理因素之需求

Temperature Oxygen pH Osmosis

- 對營養成份及環境之需求

Energy source: phototrophs & chemotrophs

Carbon source: autotrophs (CO_2) & heterotrophs (organic comp'd)

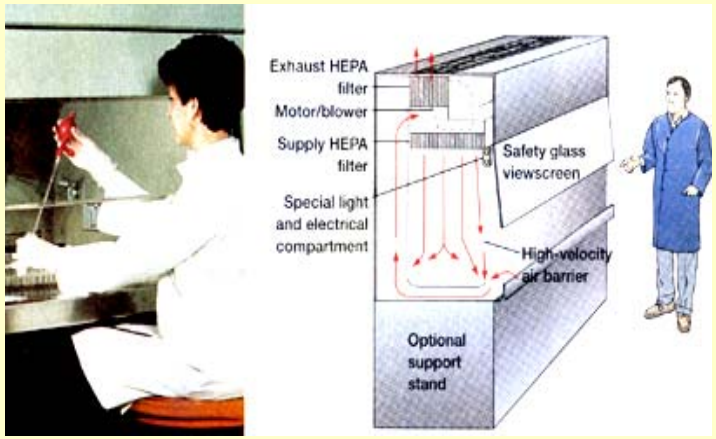
Nitrogen source: nitrogen, nitrates, ammonia or organic N comp'd

Minerals: S, P, K/Mg/Ca, Fe, trace elements

微生物培養基及其他器具之滅菌操作：高壓釜（autoclave）

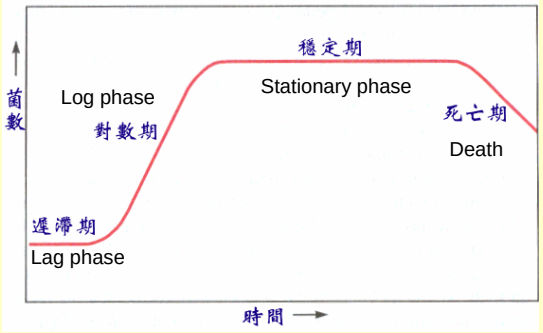
121°C, 15 pounds/inch^2
15 分鐘

無菌操作台,去除 99.97 % > 0.3 μm 之物質



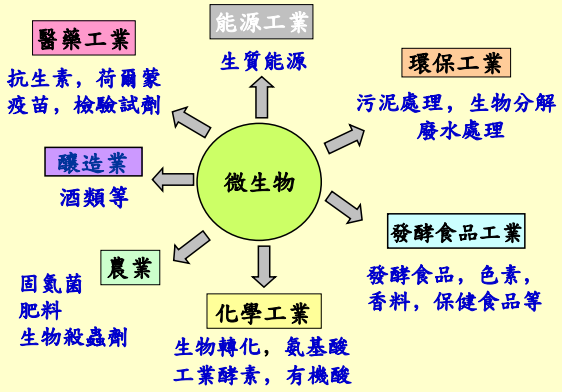
微生物之細胞生長

生長曲線:當一菌落被移到一新培養基時， 菌落生長(菌數變化)之情形



微生物之利用與生物技術

微生物之利用



抗生素 (antibiotics) 之發現：penicillin 之故事

”由生物，尤其是微生物所生產之物質，阻止其他微生物與其他生活細胞機能、生長之物質”

- **Alexander Fleming (1929):** 發現在洋菜培養基上之葡萄球菌，在偶然進入之青黴菌(*Penicillium notatum*)菌落周圍，葡萄球菌呈透明狀。發現青黴菌可分泌抑制葡萄球菌生長之物質，即penicillin 。

- **Florey & Chain (1940):** 將 penicillin成功的純化與結晶化

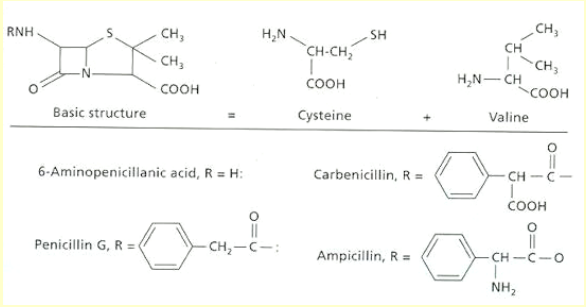
Penicillic 之故事（續）

- Penicillin prevents the cross-linking of small peptide chains in **peptidoglycan**
- Pre-existing cells are unaffected, but all newly produced cells grow abnormally, unable to maintain their wall rigidity, and they are susceptible to osmotic lysis.

Bacillus cereus

抗生素之發展與近況（current status)

- 抗生素之量產：1941年之後，研究大量生產之方法，促成抗生素工業之起始
- 抗生素之改良 (例：菌種及化學結構改良)與新抗生素之開發

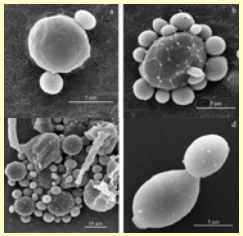


Some clinically important antibiotics

Antibiotic	Producer organism	Activity	Site or mode of action
Penicillin	*Penicillium chrysogenum*	Gram-positive bacteria	Wall synthesis
Cephalosporin	*Cephalosporium acremonium*	Broad spectrum	Wall synthesis
Griseofulvin	*Penicillium griseofulvum*	Dermatophytic fungi	Microtubules
Bacitracin	*Bacillus subtilis*	Gram-positive bacteria	Wall synthesis
Polymyxin B	*Bacillus polymyxa*	Gram-negative bacteria	Cell membrane
Amphotericin B	*Streptomyces nodosus*	Fungi	Cell membrane
Erythromycin	*Streptomyces erythreus*	Gram-positive bacteria	Protein synthesis
Neomycin	*Streptomyces fradiae*	Broad spectrum	Protein synthesis
Streptomycin	*Streptomyces griseus*	Gram-negative bacteria	Protein synthesis
Tetracycline	*Streptomyces rimosus*	Broad spectrum	Protein synthesis
Vancomycin	*Streptomyces orientalis*	Gram-positive bacteria	Protein synthesis
Gentamicin	*Micromonospora purpurea*	Broad spectrum	Protein synthesis
Rifamycin	*Streptomyces mediterranei*	Tuberculosis	Protein synthesis

微生物之色素: 紅色酵母菌之故事

- 菌種: *Xanthophyllomyces dendrorhous*
- Astaxanthin-producing yeast
- Astaxanthin (蝦青素/蝦紅素):
  為脂溶性分子屬於類葫羅蔔素的一種



- Astaxanthin:
  (1) 對健康的可能益處 (2) 天然色素

http://www.microbelibrary.org/ASMOnly/details.asp?id=1971&Lang=

致病性微生物

Before birth: microorganisms-free

Normal microbiota/normal flora (正常微生物叢):
microorganisms that establish permanent colonies inside or on the body without causing disease

Brain, blood and muscles are normally microorganisms-free

Skin and mucous membranes are readily colonized

Some benefits of the normal microbiota:

提供維生素等營養物供人類吸收與利用

幫助人類抵抗病原菌的侵襲及健全人類的免疫力

健康成人身體上的 normal microbiota

皮膚：少數Gram(+)菌，包括幾種的葡萄球菌及厭氧性棒桿菌等

呼吸道：葡萄球菌、鏈球菌、類白喉桿菌及Gram(-)菌等

口腔: 複雜的生物環境

胃(分泌鹽酸，消化蛋白質)：乳桿菌
十二指腸及空腸：鏈球菌、乳桿菌。

大腸: 兼氣性好氧菌(如大腸桿菌、糞鏈球菌)、厭氧菌(如梭狀芽胞桿菌等)

生殖泌尿道：因解剖與生理上的差異，normal microbiota 有異
男性：厭氧性的Gram(-)桿菌和球菌
女性：厭氧性的Gram(-)桿菌和球菌外，乳桿菌、酵母菌、鏈球菌和大腸桿菌等

(http://microbiology.scu.edu.tw/lifescience/)

Pathogens（致病菌）: disease-causing microorganisms

(1) invade the human body or produce toxins
(2) overcome the immune defenses & cause disease

Opportunistic pathogens（伺機性病原菌）：一些平時為normal microbiota 組成之微生物或是外來的微生物。在宿主健康時，因normal microbiota的存在被控制在一定的繁殖數量與宿主共存，或被抑制生長而無法留存於宿主體內；當宿主的免疫力減弱或正常菌叢遭受破壞時，它們便能乘機大量繁殖並造成宿主生病。

http://microbiology.scu.edu.tw/lifescience/

科克假說 (Koch postulates)

特異微生物引起特殊疾病時之必備標準：

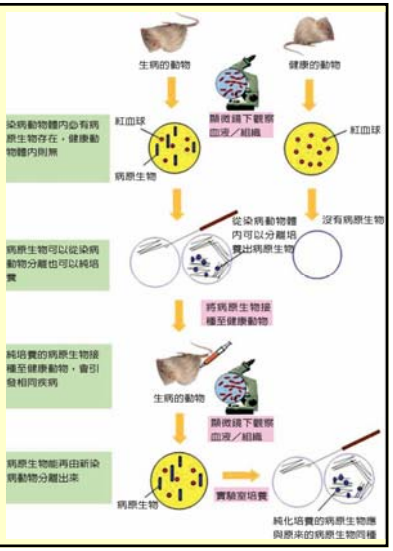
(1)某一特異微生物常與某一特定疾病有關

(2) 此微生物可於染病動物中分離出，且可以純培養

(3) 該微生物之純培養注入健康動物時，將引起致病

(4) 自實驗感染之動物，可分離出該注入之微生物

http://www.nsc.gov.tw/_newfiles/popular_science.asp?add_year=2006&popsc_aid=146



致病菌與疾病

疾病名稱	致病菌	一般感染人類方式
炭疽熱	*Bacillus anthracis*	直接接觸，孢子吸入或攝食
梨漿蟲病	*Babesia bovis, B.divergens, B. microti, B. equi*	傳染性壁蝨的咬傷
布魯士氏菌病(波形熱)	*Brucella melitensis, B. abortus, B. suis*	牛奶，直接或非直接接觸
彎曲桿菌病	*Campylobacter fetus, C. jejuni*	污染的水或食物
貓抓病	*Bartonella henselae*	貓或狗的抓傷
科羅拉多壁蝨熱	*Coltivirus*	壁蝨咬傷
牛痘病	Cowpox virus	皮膚擦傷
隱鞭孢子蟲症	*Cryptosporidium* spp.	與傳染性的小牛接觸
腦炎(加州型)	Arbovius	蚊子叮咬
腦炎(St. Louis)	Arbovius	蚊子叮咬
腦脊髓癌(Eastern equine)	Arbovius	蚊子叮咬
腦脊髓癌(Venezuelan equine)	Arbovius	蚊子叮咬
腦脊髓癌(Western equine)	Arbovius	蚊子叮咬
梨型鞭毛蟲病	*Giardia lamblia*	污水
鼻疽	*Pseudomonas mallei*	皮膚接觸，空氣吸入

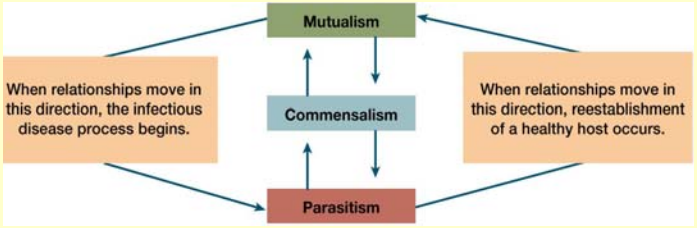
http://microbiology.scu.edu.tw/lifescience/

B型皰疹病毒性腦炎	*Herpesvirus simiae*	猴子咬傷，接觸來自猴子的物質
鉤端螺旋體病	*Leptospira interogens*	與污水、尿、感染的組織直接接觸
李斯特氏菌病	*Listera monocytogenes*	食物性感染
萊姆關節炎	*Borrelia burgdorferi*	感染的壁蝨咬傷
淋巴細胞性脈絡叢腦膜炎	Arobovirus	污塵的吸入、吃污染的食物
地中海熱	*Rickettsia conorii*	壁蝨咬傷
類鼻疽	*Pseudomonas pseudomallei*	節肢動物、水、食物
瘟疫	*Yersinia pestis*	跳蚤咬傷
鸚鵡熱	*Chlamydia psittaci*	直接接觸、呼吸道吸入
Q熱	*Coxiella burnetii*	吸入感染的泥土或塵埃
狂犬病	Rabies virus	患狂犬病動物的咬傷
回歸熱	*Barrelia* spp.	壁蝨或蝨的咬傷
立克次氏菌痘	*Rickettsia akari*	小蟲咬傷
落磯山脈斑點熱	*Rickettsia rickettsii*	壁蝨咬傷
沙門桿菌病	*Salmonella* spp.	直接接觸、食物
叢林型斑疹傷寒	*Rickettsia tsutsugamushi*	小蟲咬傷
結核病	*Mycobacterium bovis*	牛奶、直接接觸
兔熱病	*Francisella tularensis*	直接接觸感染的動物屍體、壁蝨咬傷、蒼蠅叮咬
斑疹傷寒熱	*Rickettsia mooseri*	蚤的咬傷
水泡性口腔炎	Virus（Rhabdovirus group）	直接接觸
黃熱病	Yellow fever virus	蚊子

(http://science.scu.edu.tw/micro/1024/micro_encyc/index.htm)

Symbiosis

（互利共生）



（寄生狀態）

微生物疾病之發生

影響因子： 環境因素　微生物致病力　寄主之抵抗力與敏感性

微生物之致病因子：

病原菌之附著力(adhension)：莢膜、表面多醣物、附著性表面蛋白及菌絲等

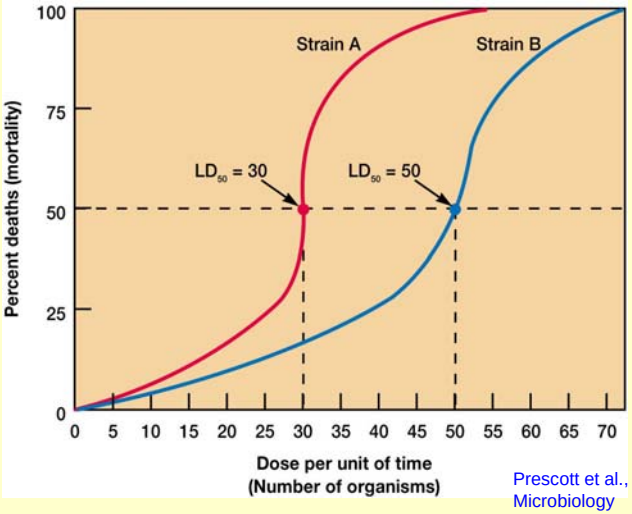
穿透(entry)：產生破壞組織或細胞的物質，如:蛋白酶、脂酶等

侵入(invasion)與增殖(multiplication)

毒素：外毒素—包括神經毒素、細胞毒素、腸毒素
內毒素—如Gram(-)細菌外膜之LPS

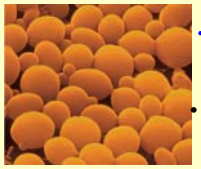
How do we measure microbial virulence ?

- **Lethal dose 50 (LD_{50})**
  number of pathogens that will kill 50% of an experimental group of hosts
- **Infectious dose 50 (ID_{50})**
  number of pathogens that will infect 50% of an experimental group of hosts



Prescott et al., (2007) Microbiology

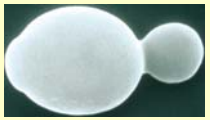
微生物在生物醫學上之應用:
使用麵包酵母菌為模式生物去研究人類疾病



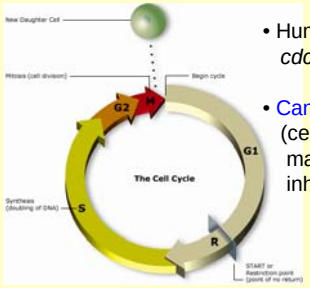
- **麵包酵母菌 (*Saccharomyces cerevisiae*)**: a one-cell (or unicellular) type of fungi
- They multiply as single cells that divide by budding (as shown in the figure)

- Yeast as a model organism (**模式生物**):
  - it is easy & cheap to replicate
  - the average cell cycle for yeast is ~90 min (~24 h for human cells)
- Genome sequencing of *S. cerevisiae*
  - Just over 12 million base pairs, containing ~6,000 genes
  - About 20% of human disease genes have counterparts in yeast

Similarities yield clues to human disease: cancer as an example



- As long as it has enough food, the yeast just eats & reproduces every ~90 min (cell-division cycle)
- Only abnormal yeast cells stop growing while their food supply is still plentiful (e.g. cell-division cycle or *cdc* mutants)



- Human equivalents of the yeast *cdc* genes are identified
- Cancer results from cell division gone wild (cell growth & multiply uncontrollably), may be due to genetic mutations that are inherited & caused by environmental factors

微生物研究之前瞻

微生物基因體、蛋白質體與功能分析

Microbial genome projects（微生物基因體計劃）

- 1995: completion of the first two microbial genomes (*Haemophilus influenzae, Mycoplasma genitalium*)
- Development of faster, more accurate sequencing methods
- Current status of microbial genomics [NCBI January 7, 2009]

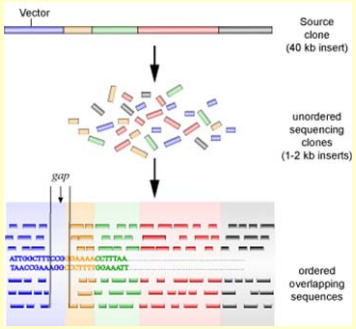
  813 microbial genome completed (54 archaeal, 749 bacterial, 10 fungal)

  Many (> 1,000 ?) microbial genome sequencing is in progress

Overview of a microbial genome project

- DNA sequencing
- Genome assembly
- Gene annotation
- Databases

DNA sequencing: the shotgun method

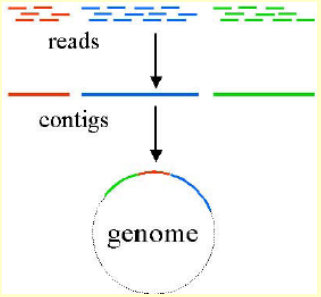


(http://www.bioteach.ubc.ca/Bioinformatics/GenomeProjects/)

- Generate library representing whole genome
- Pick random clones for sequencing
- Assemble from overlap of clone sequences
- Rapid & simple

Genome Assembly

- Sequencing reading from clones are overlapped – using sequence identities - to obtain large segments "Contigs"
- Resulting contigs are combined to assembly the whole genome
- By overlapping individual readings, a genome may be covered several times



(Bini, 2005)

Gene annotation

- Gene finding: determine putative open-reading-frames (ORFs)
- Translate ORFs
- Searching against databases of sequences

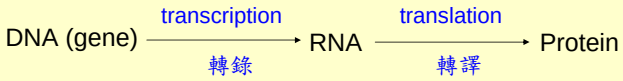
  homolog (hit a protein of known function)
  conserved hypothetical protein (hit a protein of unknown function)
  hypothetical proteins (if no match)

- Assignments for molecular functions

  Functional assignments (Pfam, etc)
  Structural assignments (NCBI, PDB)

Microbial Genome Databases

- The National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/genomes/lproks.cgi)
- The Institute for Genomic Research (TIGR) (http://www.tigr.org/db.shtml)
- The DOE (Department of Energy) Joint Genome Institute (DGI) (http://genome.jgi-psf.org/mic_home.html)
- Individual genome sequencing projects
  (e.g.) *Saccharomyces* Genome Database (SGD)



Applications of genome sequences

- Characterization of metabolic & regulatory pathways
- Identification of targets for antimicrobial agents
- Study of antibiotic resistance
- Identification of virulence factors for microbial pathogens
- Comparative genomics & evolution studies & human diseases
- Global regulation of gene expression

Some tools for global analysis of gene functions

- DNA microarrays (基因晶片)

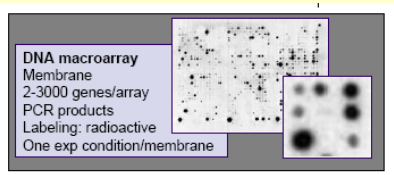
  Monitor the expression of thousands of gene all at once

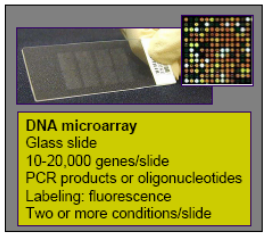
- 2-D PAGE (二維式蛋白電泳分析) & mass spectrometry (質譜分析)

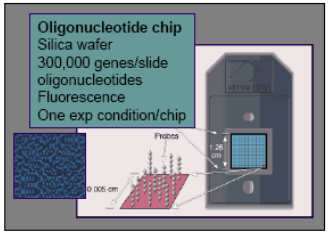
  Analysis of protein expression "Proteome" (蛋白組)
  ----→其分析研究即稱為蛋白組分析或蛋白質體(Proteomics)

DNA microarrays

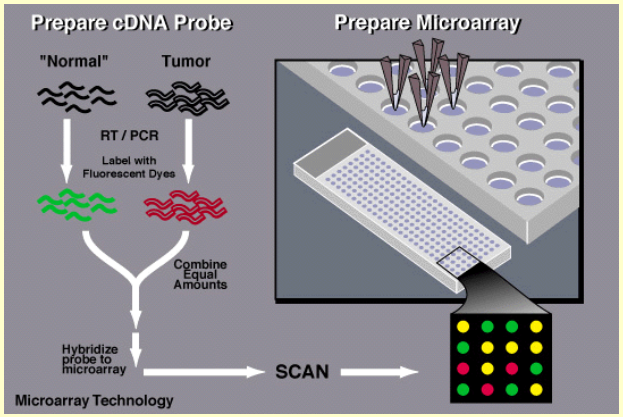
Solid supports:
Membrane
Glass slide
Plastic chip







(Bini, 2005)

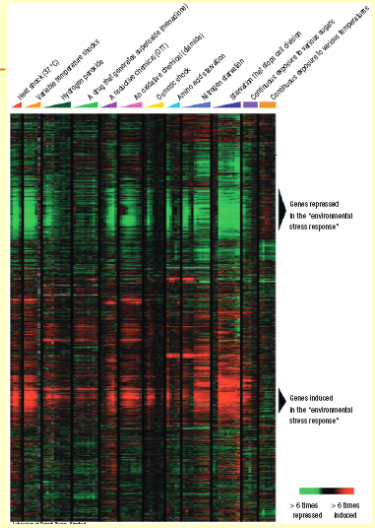


(http://www.accessexcellence.org/RC/VL/GG/microArray.html)

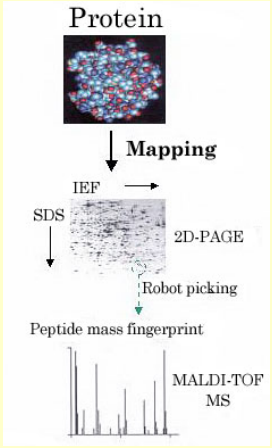
Clustering of gene expression

Comparing gene expression:

- Among different time points
- In response to different environmental conditions
- From different microbial strains (virulent strain *vs*. avirulent strain)
- Between normal cell *vs*. tumor cell
- Others



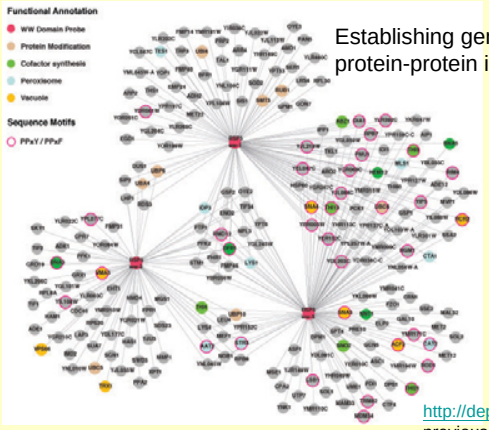
Proteome analysis



You can also compare proteome from different samples (e.g. virulent vs. avirulent strains)

Protein identification

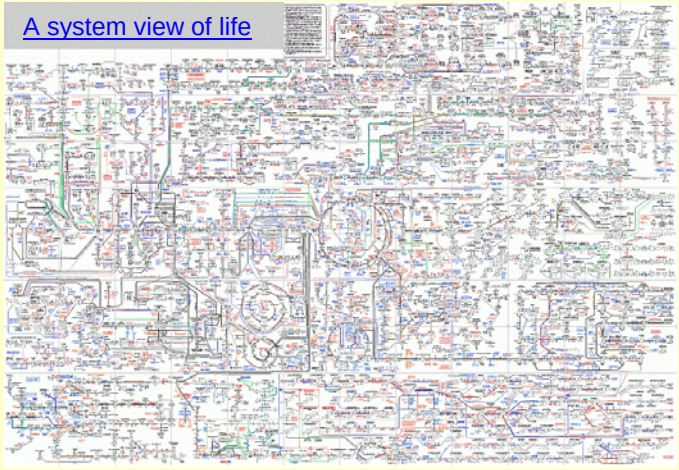
Biological networks & Systems Biology



Establishing gene regulatory & protein-protein interaction networks

http://depts.washington.edu/sfields/previous_projects/index.html

A system view of life



http://www.bio-pro.de/en/life/magazin/04653/index.html