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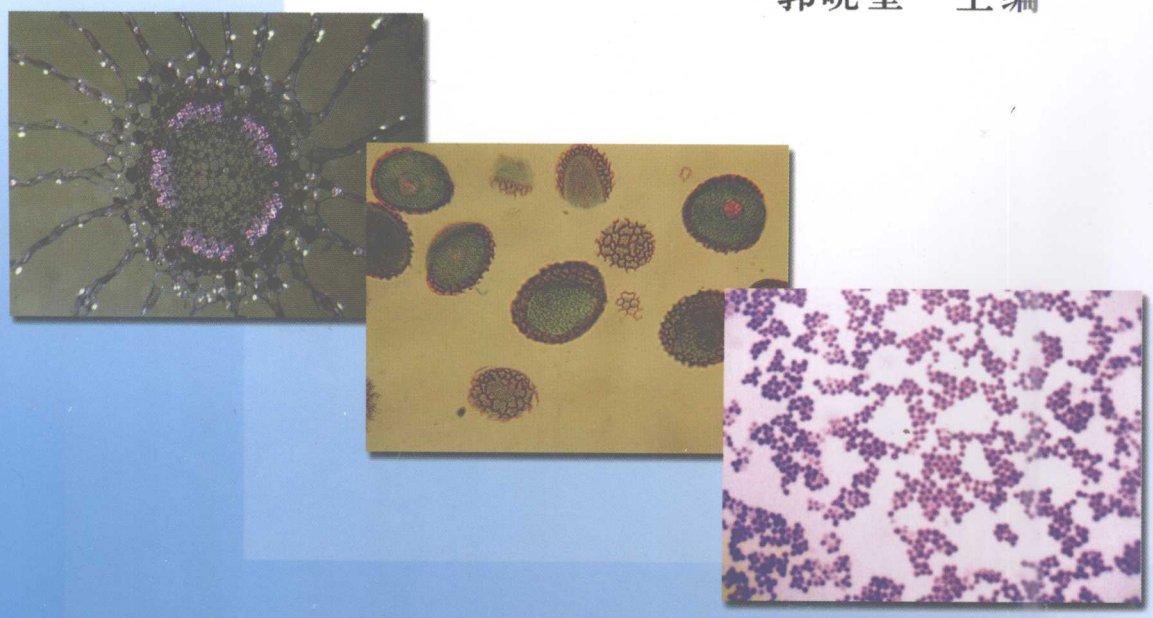
REVIEW OF MEDICAL MICROBIOLOGY AND PARASITOLOGY

病原生物学纲要

第2版

郭晓奎 主编



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病原生物学纲要

双语版　第2版

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内 容 简 介

本书是普通高等教育“十一五”国家级规划教材《病原生物学》的姊妹教材。根据教育部指示精神，为配合国内高等医药院校开展双语教学而编写。全书以英语为主体，重点叙述病原生物学基本理论和主要知识点，对主要专业词汇进行了中文注释；分医学微生物学和医学寄生虫学两部分，包括医学细菌学、医学真菌学、医学病毒学、医学蠕虫学、原虫学和医学节肢动物学。

本书适用于高等医药院校的本科生，特别是长学制学生，也可供从事病原生物学的教师、临床执业医师以及从事预防医学工作的专业人员参考。

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Preface for Sencond Edition

The goals of *Review of Medical Microbiology and Parasitology* is to provide a brief, accurate, and up-to-date presentation of the medically important aspects of Microbiology and Parasitology. It covers both the basic and clinical aspects of bacteriology, virology, mycology and parasitology.

It is hoped that *Review of Medical Microbiology and Parasitology* will be a useful learning tool and functional reference, not only for undergraduate students taking the course of medical science, medical technology, dentistry, but also for other students taking more advanced programs in the biological science, and biotechnology.

As with any book, numerous people have been involved and have helped in its production, and I would like to take this opportunity to acknowledge them. I am particularly grateful to professor Guo-Ping Zhao for review and helpful suggestions. We wish to acknowledge our students who provide the stimulation for continuation of this work.

If you have any comments and suggestions for this book or the next edition, please send them to the department of medical microbiology and parasitology, Shanghai Jiao Tong University School of Medicine, Room 511, Building 5, 280 South Chongqing Rd, Shanghai, China 200025, or via E-mail to microbiology@ sjtu. edu. cn.

Xiao-Kui Guo, Ph. D.

2008. 1. 4

第1版前言

为配合双语教学，我室教师以极大的热情编写了这本《病原生物学纲要（双语版）》。期望本教材在双语教学中将有助于学生学习和老师教课。

本教材以英文为主体，语言全部来自原版书籍，对英语专业单词或短语以及语言比较复杂、专业内容深奥的长句、段落进行了中文翻译，形成了形式比较特别的英汉双语教材。中文加入的原则是：①如英文句法简单明确，仅有个别生词或短语，即将相应的中文直接插在其后。②对语言比较复杂、生词较多的长句、段落，则在其后全部予以翻译。为打印方便起见，均未使用括号。

本教材参照全国规划教材和医师资格考试大纲以及本校教学大纲中要求掌握和熟悉的内容编写，包含了医学微生物学和医学寄生虫学的基本理论和主要知识，适用于五年制本科及七年制学生病原生物学的教学。

本教材寄生虫学图谱来自《人体寄生虫学》（第六版）；微生物学彩图来自《医学微生物学与寄生虫学》；在本教材的编写过程中，寄生虫学老师在相关编写内容选择上给予了极大支持；两位学术秘书的积极配合及插图的制备、扫描，为本书的增色和完成起了很大作用，在此一并感谢！

由于我们的学术水平、英语水平、翻译水平和编写能力有限，错误、遗漏等不当之处敬请批评指正。

刘晶星

于上海第二医科大学

2004年10月12日

Contents

Chapter 1 INTRODUCTION TO MEDICAL MICROBIOLOGY 医学微生物学绪论

MICROORGANISMS AND MICROBIOLOGY(微生物与微生物学)

Microbiology is the scientific study of small living organisms. These organisms are called microorganisms because they require magnification to be seen. Some microbial species can have devastating effects on human beings by causing **infectious diseases**(感染性疾病). A great success of the science of microbiology has been the control of fatal infectious diseases in developed countries. However, these diseases are still important causes of death in less developed parts of the world. Despite these threats by some species, most microorganisms are beneficial. The proper function of the **biosphere**(生物圈) and soil depends upon their activities. Of more direct impact upon humans is the industrial production of antibiotics, food products, organic chemicals, and biomass. Microbes are also important in agriculture and food spoilage.

In the microbial world (ref. table 1-1), there are two structural types of cells: **prokaryotic cells**(原核细胞) are relatively simple in structure; **eukaryotic cells**(真核细胞) are more complex, in that they contain **organelles**(细胞器) that are compartments for special metabolic functions. These organelles include a true nucleus, the mitochondrion and the chloroplast. In addition, microbiologists also deal with **viruses**(病毒), which are non-cellular entities that use the metabolic machinery of host cells to replicate themselves. The dichotomy in the structural types of cells does not accurately represent the evolutionary relationships among organisms. Analysis of the nucleotide sequences of ribosomal RNA has indicated that there are two groups of prokaryotes: the **archaea**(古菌) and the **bacteria**(细菌). These groups are no more closely related to each other than they are to the **eukarya**(真核).

Table 1-1 Microbial world

Domins	Types of cells	Microorganisms	Pathogenesis of human
	Non-cellular	Prions#	+
		Viroids	
		Virus#	+
Bacteria	Prokaryotic	Bacteria#	+
Archae		Archaebacteria	
Eukarya	Eukaryotic	Fungi#	+
		Slime molds	
		Algae	
		Protozoa *	+
		Helminth *	+

#: medical microorganisms * : see medical parasite

MAJOR CATEGORIES OF MEDICAL MICROORGANISMS

Medical microorganisms are usually divided into four groups, including prion, viruses, bacteria, fungi.

Prion(朊粒)

A number of remarkable discoveries in the past 3 decades have led to the molecular and genetic characterization of the transmissible agent causing **scrapie**(羊搔痒病), a degenerative central nervous system disease of sheep. Studies have identified a scrapie-specific protein in preparations from scrapie-infected brains of sheep which is capable of reproducing the symptoms of scrapie in uninfected sheep. Attempts to identify additional components, such as nucleic acid, have been unsuccessful. To distinguish this agent from viruses and viroids, the term **prion** was introduced to emphasize its proteinaceous and infectious nature.

Viruses

Viruses lack many of the attributes of cells, especially the ability to replicate. Only when it infects a

cell does a virus acquire the key attribute of a living system: reproduction. Viruses are known to infect all cells, including microbial cells. Host-virus interactions tend to be highly specific, and the biologic range of viruses mirrors the diversity of potential host cells. Further diversity of viruses is exhibited by their broad array of strategies for replication and survival.

Bacteria

Bacteria are the smallest living cells. They have a cytoplasmic membrane surround by a cell wall; a unique interwoven polymer called **peptidoglycan** (肽聚糖) makes the wall rigid. They have no nucleus, but all the chemical elements of nucleic acid and protein synthesis are present. They divide by binary fission and can be grown in artificial culture.

Fungi

The **fungi** (真菌) are nonphotosynthetic protists growing as a mass of branching, interlacing filaments ("hyphae") known as a mycelium (菌丝体). Although the hyphae exhibit cross-walls, the cross-walls are perforated and allow free passage of nuclei and cytoplasm. The entire organism is thus a coenocyte (多核细胞) (a multinucleated mass of continuous cytoplasm) confined within a series of branching tubes. These tubes, made of polysaccharides such as chitin, are homologous with cell walls. The mycelial forms are called **molds** (霉菌); a few types, **yeasts** (酵母), do not form a mycelium but are easily recognized as fungi by the nature of their sexual reproductive processes and by the presence of transitional forms. The fungi probably represent an evolutionary offshoot of the protozoa.

HISTORY OF MICROBIOLOGY

The science of microbiology was born with the discovery of the simple microscope by **Anton van Leeuwenhoek** around 1683 and his reporting of small forms of life that could not be seen with the naked eye, but most developments in microbiology have occurred in the past 100 years. **Louis Pasteur** and **Robert Koch** were two leaders in developing the discipline. Among many other accomplishments, Pasteur disproved the theory of **spontaneous generation** (自然发生说) conclusively. This was important to demonstrate that experiments with microbes were reproducible, and not the consequence of new life arising. Koch developed rigid criteria for proving a specific disease was caused by a specific bacterium (**Koch's postulates**) (郭霍法则). Obtaining pure cultures of microbes was essential for fulfilling Koch's criteria and the use of pure cultures remains an essential tool in the study of infectious disease today.

MEDICAL MICROBIOLOGY

Even though the science of microbiology has developed only in the last 100 years, it has been a very important science for two reasons: (1) microorganisms have been excellent research tools for understanding the molecular biology of cells, and (2) many problems important to human society are consequences of microbial activity.

Owing to practical consideration, microbiology has been divided usually according to its application as general microbiology, agricultural microbiology, industrial microbiology and veterinary microbiology etc. The medical microbiology is one of the essential basic sciences. It is the study of biological characteristics of microorganisms and their relationships with human hosts. The aim in studying is to learn the basic core of the fundamental knowledge of medical microbiology as well as to understand for the diagnosis, prophylaxis and treatment of microbiological infections and infectious disease, and thus to control these and to raise the health standard of general population.

(郭晓奎)

Chapter 2 MORPHOLOGY AND STRUCTURE OF BACTERIA 细菌的形态与结构

Typical bacteria are unicellular prokaryotic organisms that have no membrane-bound cytoplasmic organelles except ribosomes (核糖体), no membrane-enclosed nucleus and have normal peptidoglycan.

BACTERIAL SIZE, SHAPE AND GROUPINGS

Unit for Measurement

Bacterial cells are most conveniently measured in microns (μm, 微米). They range in size from large cells such as *Bacillus anthracis* (1.0—1.3) μm × (3—10) μm to very small cells such as *Pasteurella tularensis* 0.2μm × (0.2—0.7) μm.

Shape and Groupings

Bacteria have characteristic shapes. The common microscopic morphologies are cocci; rods; long, filamentous branched cells; and comma-shaped and spiral cells. The arrangement of cells is also typical of various species or groups of bacteria.

A. Coccus

The **coccus** (球菌) is spherical bacterium. If the cells remain attached following division, certain groupings result. Depending on the plane of division and the number of division through which the cells remain attached, the following groupings may occur: chain (streptococcus [链球菌]), pairs (diplococcus), grape-like irregular cluster (staphylococcus [葡萄球菌]), cubical bundles (tetrad [四联球菌] and sarcina [八叠球菌]).

B. Bacillus

The **bacillus** (杆菌) is rod-shaped bacterium. A median size of bacillus is about 2—3μm long and 0.5—1.0μm wide. In addition, there are streptobacillus (链杆菌), coccobacillus (球杆菌), fusiform bacillus (梭形杆菌), corynebacterium (棒状杆菌) and mycobacterium (分枝杆菌).

C. Spiral bacterium (螺形菌)

1. Vibrio (弧菌) The bacterium may be comma-shaped (逗点状).

2. Spirillum (螺菌) The bacterium may be spiral-shaped (螺旋状).

Bacterial Structure

The essential structures include cell wall, cytoplasmic membranes, cytosol and nucleoid. In some species of bacteria, other particular structures may be present: capsule, flagella, pili and spore.

A. Cell wall (细胞壁)

The **Gram stain** (革兰染色) broadly differentiates bacteria into **Gram-positive** (革兰阳性) and **Gram-negative** (革兰阴性) groups. Gram-positive and Gram-negative organisms differ drastically in their cell wall structures. Most Gram-positive bacteria have a relatively thick cell wall which is composed largely of peptidoglycan and other polymers such as the teichoic acids. In contrast, the peptidoglycan layer in Gram-negative bacteria is thin, and outside the peptidoglycan there is an outer membrane structure.

1. Common chemical composition peptidoglycan.

Unique features of almost all prokaryotic cells are cell wall peptidoglycan and the specific enzymes involved in its biosynthesis. These enzymes are target sites for inhibition of peptidoglycan synthesis by specific antibiotics.

(1) Peptidoglycan: The primary chemical structures of peptidoglycans of both Gram-positive and Gram-negative bacteria consist of:

—polysaccharide backbones (聚糖骨架), composed of alternating *N*-acetylglucosamine (*N*-乙酰葡萄糖胺) and *N*-acetylmuramic acid (*N*-乙酰胞壁酸) connected by β-1,4 glycosidic bone (β-1,4 糖苷键).

—tetrapeptide side chains (四肽侧链), attached to the *N*-acetylmuramic acid.

—pentapeptide cross-bridges (五肽交联桥) (e.g. a glycine$_5$ peptide) that exist only in Gram-positive bacteria.

The backbone is the same in all bacterial species. The tetrapeptide side chains and the peptide cross-bridges vary from species to species and the peptide cross-bridges exist only in gram-positive bacteria (Fig. 2-1, Fig. 2-2).

The peptidoglycan in Gram-positive bacteria is often cross-linked in three dimensions, providing a very strong, rigid cell wall.

(2) Enzymes and antibiotics that attack the peptidoglycan (破坏肽聚糖的酶和抗生素)

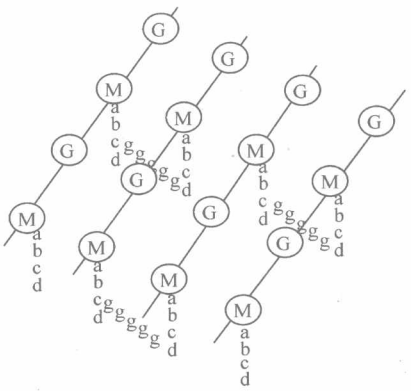

Fig. 2-1 Schematic drawing of the polysaccharide backbones composition of *S. aureus* (Gram-positive bacteria)

G: *N*-acetylglucosamine; M: *N*-acetylmuramic acid; g: glycine; a、b、c、d: amino acid

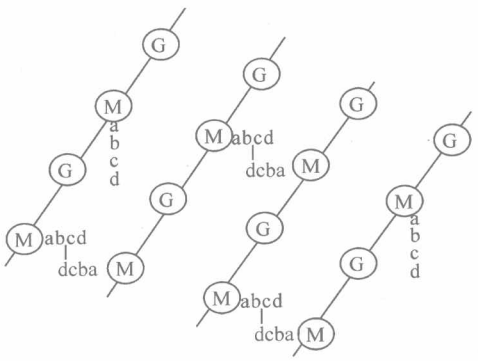

Fig. 2-2 Schematic drawing of the polysaccharide backbones composition of *E. coli* (Gram-negative bacteria)

G: *N*-acetylglucosamine; M: *N*-acetylmuramic acid; a、b、c、d: amino acid

1) Lysozyme (溶菌酶): The β-1,4 glycosidic bone of polysaccharide backbone can be hydrolyzed by the lysozyme.

2) Penicillin (青霉素) and related beta-lactam antibiotics (β-内酰胺抗生素): The cross-linking reaction in the synthesis of peptidoglycan is catalyzyed by transpeptidases (转肽酶) that are targets for penicillin and related beta-lactam antibiotics. These enzymes are called penicillin-binding proteins (PBP) (青霉素结合蛋白). Therefore, penicillin and related beta-lactam antibiotics can inhibit the synthesis of peptidoglycan.

In Gram-positive bacteria, there are as many as 40 sheets of peptidoglycan, comprising up to 50% of the cell wall material. In Gram-negative bacteria, there appears to be only one or two sheets, comprising 5% —10% of the wall material.

2. Special components

(1) Gram-positive cell wall: Most Gram-positive cell wall contain considerable amount of teichoic acid (磷壁酸). There are two types of teichoic acids: wall teichoic acid (壁磷壁酸), covalently linked to muramic acid residues of the peptidoglycan; and membrane teichoic acid (膜磷壁酸) (lipoteichoic acid [脂磷壁酸]) covalently linked to membrane glycolipid (Fig. 2-3). The teichoic acid molecules extend through the cell wall and constitute major surface antigens.

(2) Gram-negative cell wall: Outer membrane is external to the peptidoglycan layer, it contains three components: lipid bilayer, lipoprotein and lipopolysaccharide.

1) **Lipid bilayer** (脂质双层): The major proteins of the outer membrane are named outer membrane protein (OMP 外膜蛋白). Among these, the

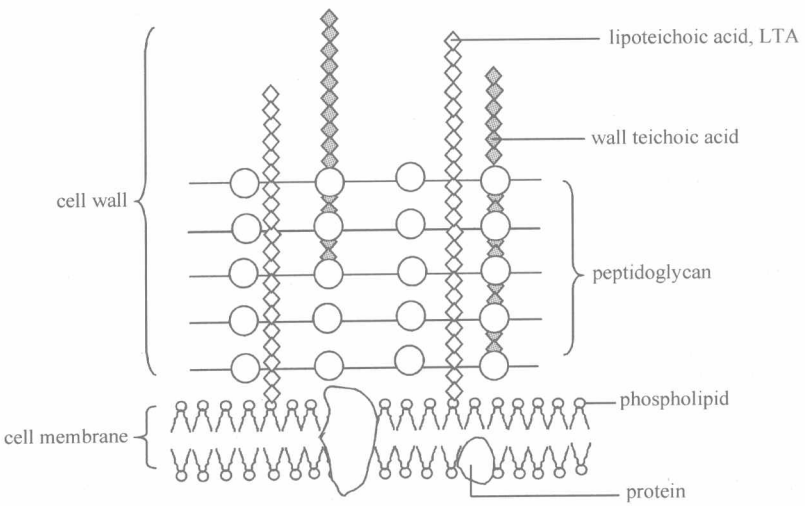

Fig. 2-3 A model of the cell wall structure of Gram negative bacteria

porins（孔蛋白）form the special channels that permit the passive diffusion of low-molecular-weight hydrophilic compounds like sugars, amino acids and certain ions.

2）**Lipoprotein**（脂蛋白）: Its function is to anchor（锚定）outer membrane to the peptidoglycan layer.

3）**Lipopolysaccharide, LPS**（脂多糖）: The lipopolysaccharides form part of the outer leaflet of the outer membrane structure. It consists of lipid A（脂质 A）, to which is attached a core polysaccharide（核心多糖）and a specific polysaccharide（特异性多糖）, terminal repeat units（末端重复单位）.

LPS, which is extremely toxic to animals, has been called the endotoxin（内毒素）of Gram-negative bacteria. When LPS is split into lipid A and polysaccharide, all the toxicity is associated with the lipid A. The polysaccharide, on the other hand, represents a major surface antigen of the bacteria cell the so-called O antigen（O 抗原）. Antigenic specificity is conferred by the terminal repeat units.

The area between the external surface of the cytoplasmic membrane and the internal surface of the outer membrane is referred to as the periplasmic space（周浆间隙）. This space is actually a compartment containing a variety of hydrolytic enzyme, which are important to the cell for breakdown of large macromolecules for metabolism.

3. Functions of the cell wall

Functions of the cell wall include:

—maintain bacterial shape, because the pepditoglycan provides rigidity（坚韧性）;

—give osmotic protection the internal osmotic pressure（内部渗透压）of most bacteria ranges from 5 atm（大气压）to 25 atm;

—play an essential role in cell division（分裂）;

—be the sites of major antigenic determinants（抗原决定簇）of the cell surface;

—be the permeablity barrier;

—facilitate the attachment to host cells.

4. L forms of bacteria（细菌 L 型） In osmotically protective media, removal of the bacterial wall with lysozyme or penicillin liberate protoplasts（原生质体）from Gram-positive cells and spheroplasts（原生质球）from Gram-negative cells. If such wall-defective cells are able to grow and divide, they are called L forms.

L forms are difficult to cultivate. They require a special media. Some L form can revert to（回复）the normal bacillary form. L form in the host may produce chronic infection（慢性感染）that are relatively resistant to antibiotic treatment.

B. The cell membrane

The cell membrane is responsible for many of the functions that include electron transport（电子传递）and energy production. In addition, the membrane contains transport proteins（转运蛋白）that allow the uptake（吸收）of metabolites and release of other substances, ion pumps（离子泵）to maintain a membrane potential（膜电势）, and enzymes.

The membrane of prokaryotes are distinguished from those of eukaryotic cells by the absence of sterols（固醇）.

Convoluted invaginations of the cell membrane form specialized structures called mesosome（中介体）, which function in the formation of cross-wall during cell division.

C. Cytoplasm（细胞质）

The cytoplasm of the bacterial cell contains some important granules. They are the ribosomes, plasmids（质粒）and metachromatic granules（异染颗粒）.

Ribosomes have a sedimentation coefficient of 70S and composed of 30S and 50S subunits. This is unlike the eukaryotic 80 S（40 S + 60 S）ribosome. They are the sites of action of many antibiotics that inhibit protein biosynthesis.

Plasmids are small, circular, extrachromosomal, double-stranded DNA molecules. They are capable of self-replication and contain genes that confer some properties, such as antibiotic resistance and virulence factors. Plasmids are not essential for cellular survival.

Metachromatic granules contain RNA and polyphosphates（多聚磷酸盐）. They are deeply stained with aniline dyes（苯胺染料）. They are characteristic features of the corynebacteria（棒状杆菌）.

D. Nuclear material（核质）

Bacterial nucleus are absence of a nuclear membrane（核膜）. The nuclear region is filled with DNA that consist of single continuous circular molecule with a molecular weight（分子量）of approximately 3×10^9. Bacterial nucleus are a single, haploid（单倍体）chromosome. Approximately 1mm long in the unfold state.

E. Capsule（荚膜）

Many bacteria synthesize large amounts of extracellular polymer when growing in their natural environments. When the polymer forms a condensed, well-defined layer closely surrounding the cell, it is called the capsule. With one known exception（the polypeptide capsule）, the polymer is polysaccharide.

The capsules are hard to stain directly, and usually capsules are demonstrated by the negative staining（负染）procedure.

The capsule is a major virulence factor（毒力因子）.

—protect the encapsulated cells from phagocytosis（吞噬）.

—act as a barrier to antimicrobial substances in the blood, body fluids.

—promote adherence to other bacteria or to host tissue surfaces or to the surfaces of certain medical

equipments（医疗器械）.

F. *Flagella*（鞭毛）

Bacterial **flagella** are thread-like appendages（附属结构）, and are constructed of a class of proteins called flagellins. The number and distribution of flagella on the bacterial surface are characteristic for a given species and hence are useful in identifying and classifying bacteria There are four flagella types（Fig. 1-7）:

Monotrichous（单毛菌）—single polar flagellum.

Lophotrichous（丛毛菌）—multiple polar flagella.

Amphitrichous（双毛菌）—two polar flagella.

Peritrichous（周毛菌）—flagella distributed over the entire cell.

They are the organs of locomotion（运动）and provide motility（动力）for bacteria, allowing the cell to swim（chemotaxis 趋化性）toward food and away from poisons.

They are highly antigenic（H antigens）.

G. *Pili*(*Fimbriae*)（菌毛）

Pili are hairlike structures on the outside of bacteria. They are shorter and finer than flagella.

There are two classes of pili, ordinary pili and sex pili.

1. Ordinary pili（普通菌毛） Generally, several hundred pili are arranged over the entire surface of the bacterial cell. They promote adherence of pathogenic bacteria to host cells and are important virulence factors.

2. Sex pili（性菌毛） They promote the transfer of the genetic material（遗传物质的转移）between bacteria in bacterial conjugation（接合）.

H. *Spores*（芽孢）

Under conditions of nutritional depletion, some Gram-positive bacteria can convert from a vegetative（繁殖状态）state to a dormant state（休眠状态）, and form a single internal spore, endospore（内芽孢）. The spore looks bright, often ovoid in the microscope.

The spore contains a complete nucleus, all of the components of the protein-synthesizing apparatus and an energy-generating system.

Each vegetative cell forms only one spore. When nutritional conditions become favorable, each spore germinate（出芽）to produce a single vegetative cell（繁殖体）.

The location of the spore within a cell is a characteristic of the bacteria and assist in identification of the bacterium.

The spore is a dehydrated, multishelled structure that is highly resistant to environment factors, such as desiccation（干燥）, heat, radiation and chemical agents. They can exist for many years as viable spores.

When the spores coming from pathogenic bacteria are introduced into organism from contaminated wounds, the spores germinate to produce vegetative cells that can elicit the disease in the host.

Spores are difficult to decontaminate with standard disinfectants（消毒方法）. A temperature of 121℃ for 15 minutes is utilized to kill spores, sterilization with autoclaves（高压蒸汽灭菌器）or pressure cookers（高压锅）are used for this purpose.

THE GRAM STAIN

The Gram-staining procedure（步骤）begins with the application of a basic dye（碱性染料）, crystal violet（甲紫）. A solution of iodine（碘液）is then applied. All bacteria are stained blue at this point in the procedure. The cells are then treated with alcohol. Gram-positive cells retain the crystal violet-iodine complex, remaining blue. Gram-negative cells are completely decolorized（脱色）by alcohol. As a last step, a counterstain（复染）（such as the dilute carbol fuchsine［稀释苯酚复红］）is applied so that the decolorized Gram-negative cells will taken a red color, The Gram-positive cells now appear purple.

The basic differences in surface structures of Gram-positive and Gram-negative bacteria explain the results of Gram staining. Both Gram-positive and Gram-negative bacteria take up the same amounts of crystal violet（CV）and iodine（I）. The CV-I complex, however, is trapped inside the Gram-positive cell by the dehydration and reduced porosity of the thick cell wall as a result of the differential washing step with 95 percent ethanol or other solvent mixture. In contrast, the thin peptidoglycan layer and probable discontinuities at the membrane adhesion sites do not impede solvent extraction of the CV-I complex from the Gram-negative cell.

Gram stain allows clinician to distinguish the gram positive bacteria and the Gram negative bacteria to initiate antibiotherapy（抗生素治疗）.

（潘　卫）

Chapter 3 BACTERIAL PHYSIOLOGY
细菌的生理

BACTERIAL NUTRITION AND GROWTH

Classification Based on Their Source of Carbon

A. Autotrophs (自养菌)

Autotrophs require only carbon dioxide as a carbon source. An autotroph can synthesize organic molecules from inorganic nutrients. Thereinto, phototrophs (光能自养菌) use radiant energy (light) as their primary energy source, and chemotrophs (化能自养菌) use the oxidation and reduction of chemical compounds as their primary energy source.

B. Heterotrophs (异养菌)

Heterotrophs require organic forms of carbon. A Heterotroph cannot synthesize organic molecules from inorganic nutrients. Thereinto, saprophytes (腐生菌) live on dead organic matter, while parasites (寄生菌) get their nutrients from a living host.

Nutrient Requirements of Bacterial Growth

1. Water.

2. Carbon source Carbon is the structural backbone of the organic compounds that make up a living cell, and is the main energy source of bacteria.

3. Nitrogen source Nitrogen is needed for the synthesis of such molecules as amino acids, DNA, RNA and ATP. Depending on the organism, nitrogen, nitrates, ammonia, or organic nitrogen compounds may be used as a nitrogen source.

4. Minerals Minerals include sulfur needed to synthesize sulfur-containing amino acids and certain vitamins; phosphorus needed to synthesize phospholipids, DNA, RNA, and ATP; potassium, magnesium, iron, and calcium required for certain enzymes; and some trace elements function as cofactors in enzyme reactions.

5. Growth factors (生长因子) Growth factors are organic compounds such as amino acids, purines, pyrimidines, and vitamins that a cell must have for growth but cannot synthesize itself.

Environmental Factors Affecting Growth of Bacteria

1. Nutrients.

2. Temperature Bacteria may grow at a variety of temperatures from close to freezing to near to the boiling point of water. Psychrophilic forms (嗜冷菌) grow best at low temperatures (15—20℃); mesophilic forms (嗜温菌) grow best at 30—37℃; and most thermophilic forms (嗜热菌) grow best at 50—60℃. Mesophiles include all human pathogens and opportunists.

Bacteria can synthesize temporarily a set of "heat-shock proteins (热休克蛋白)" when exposed to a sudden rise in temperature above the growth optimum. These proteins appear to be unusually heat-resistant and to stabilize the heat-sensitive proteins of the cell.

3. Hydrogenion concentration (pH) Microorganisms can be placed in one of the following groups based on their optimum pH requirements: Neutrophiles (嗜中性菌) grow best at a pH range of 5 to 8. Acidophiles (嗜酸性菌) grow best at a pH below 5.5. Alkaliphiles (嗜碱性菌) grow best at a pH above 8.5.

4. Oxygen requirements Microorganisms show a great deal of variation in their requirements for gaseous oxygen. Most can be placed in one of the following groups:

(1) Obligate aerobes (专性需氧菌): They are organisms that grow only in the presence of oxygen. They obtain their energy through aerobic respiration (需氧呼吸).

(2) Microaerophiles (微需氧菌): They are organisms that require a low concentration of oxygen (2% to 10%) for growth, but higher concentrations are inhibitory. They obtain their energy through aerobic respiration.

(3) Facultative anaerobes (兼性厌氧菌): They are organisms that grow with or without oxygen, but generally better with oxygen. They obtain their energy through aerobic respiration if oxygen is present, but use fermentation (发酵) or anaerobic respiration (厌氧呼吸) if it is absent. Most bacteria are facultative anaerobes.

(4) Obligate anaerobes (专性厌氧菌): They are organisms that grow only in the absence of oxygen and, in fact, are often inhibited or killed by the presence of oxygen. They obtain their energy through anaerobic respiration or fermentation. They are killed by oxygen because they lack certain enzymes (catalase [触酶], peroxidase [过氧化物酶], superoxide dismutase [SOD, 超氧化物歧化酶]) that detoxify both hydrogen peroxide and oxygen free radicals (superoxide) produced as side-products during metabolism in the presence of oxygen.

5. Osmotic pressure Most bacteria require an isotonic environment or a hypotonic environment for optimum growth. Organisms requiring high salt concentrations are called halophilic (嗜盐菌); those requiring high osmotic pressures are called osmophilic (嗜高渗菌).

Bacterial Growth

A. Bacterial division and generation time

Bacteria multiply by binary fission (二分裂), a process by which one bacterium splits into two. Therefore, bacteria increase their numbers by geometric progression.

Generation time (代时) is the time it takes for a population of bacteria to double in number. For many common bacteria, the generation time is quite short, 20—60 minutes under optimum conditions. Bacteria can astronomically increase their number in a short period of time due to their short generation time. For most common pathogens in the body, the generation time is probably closer to 5—10 hours.

B. Population dynamics (群体动力学) —the growth curve (生长曲线)

If a liquid medium is inoculated with microbial cells taken from a culture that has previously been grown to saturation and the number of viable cells per milliliter determined periodically and plotted, a curve frequently illustrated in a plot of logarithmic number of bacteria versus time is obtained.

The growth curve consists of four phases (Fig. 3-1):

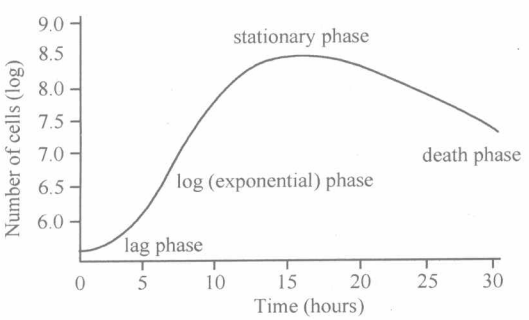

Fig. 3-1 Growth curve of *E. coli*

1. The lag phase (迟缓期, A) The lag phase represents a period during which the cells depleted of metabolites and enzymes as the result of the unfavourable conditions that existed at the end of their previous culture history, adapt to their new environment. Enzymes and intermediates are formed and accumulate until they are present in concentrations that permit growth to resume.

2. The exponential phase (指数期, C) Cell biomass is synthesized at a constant rate and increases in an exponential manner. This continues until one of two things happens: either one or more nutrients in the medium become exhausted, or toxic metabolic products accumulate and inhibit growth. Cells in this stage are generally more susceptible to antibiotics.

3. The maximum stationary phase (稳定期, E) Eventually, the exhaustion of nutrients or the accumulation of toxic products causes growth to cease completely. In most cases, however, there is a slow loss of cells through death, which is just balanced by the formation of new cells through growth and division. When this occurs, the total cell count slowly increases although the viable count stays constant.

4. The death or decline phase (衰亡期, F) Cells may die due to toxic products.

MICROBIAL METABOLISM

Bacterial metabolism is the sum of anabolic processes (synthesis of cellular constituents, requiring energy, [合成代谢]) and catabolic processes (breakdown of cellular constituents with concomitant release of waste products and energy-rich compounds, [分解代谢]).

Cellular Respiration and Exergonic Pathway

Cellular respiration is the process cells use to convert the energy in the chemical bonds of nutrients to ATP energy. Depending on the organism, cellular respiration can be aerobic, anaerobic, or both. Aerobic respiration is an exergonic pathway that requires molecular oxygen (O_2). Anaerobic exergonic pathways do not require oxygen and include anaerobic respiration and fermentation.

A. Aerobic respiration

It is the aerobic catabolism of nutrients to carbon dioxide, water, and energy, and involves an electron transport system in which molecular oxygen is the final electron acceptor. This type of ATP production is seen in aerobes and facultative anaerobes. The overall reaction is:

$C_6H_{12}O_6 + 6O_2$ yields $6CO_2 + 6H_2O$ + energy (as 38 ATP)

B. Anaerobic respiration

In this respiration, an inorganic molecule other than oxygen (e. g., NO_3^-, SO_4^{-2}, CO_2) is the final

electron acceptor.

C. Fermentation

It is an anaerobic breakdown of carbohydrates in which an organic molecule is the final electron acceptor. It does not involve an electron transport system. It involves glycolysis (糖酵解), and is found in anaerobic and facultative anaerobic bacteria. The overall reaction is:

Glucose (6C) + 2 NAD^+ + 2 ADP + 2 inorganic phosphates (P_i)

Yields 2 pyruvate (3C) + 2 NADH + 2 H^+ + 2 net ATP

Medical Important Metabolic Products

A. Catabolic products and identification of bacteria through biochemical testing

When identifying a suspected organism, you inoculate a series of differential media (鉴别培养基). After incubation, you then observe each medium to see if specific end products of metabolism are present. This can be done by adding indicators to the medium that react specifically with the end product being tested, giving some form of visible reaction such as a color change. The results of these tests on the suspected microorganism are then compared to known results for that organism to confirm its identification.

1. Carbohydrate fermentation test(糖发酵试验)

This test is used to determine the ability of an organism to ferment various simple carbohydrates (sugars). The fermentation characteristics are used in identification of the bacteria, particularly the Gram negative enteric (gut) bacteria. When carbohydrates are fermented as a result of bacterial enzymes, the following fermentation end products may be produced: ① acid end products, or ② acid and gas end products.

2. Methyl red (MR) test (甲基红试验) This test is used to identify bacteria that produce stable acid end products by means of fermentation of glucose. The combination medium used for this test, MR/VP broth, includes peptone, glucose, and a phosphate buffer. Some bacteria perform a fermentation of glucose and produce large amounts of stable acids. The pH indicator, methyl red, is added to a 48 hour culture. If the pH is less than 4.4, the indicator will turn red. A red color is read as positive, a yellow color (pH greater than 6.0) is negative.

3. Voges-proskauer (VP) test This test is used to identify organisms able to produce acetoin (乙酰甲基甲醇) from the degradation of glucose during a 2,3-butanediol (丁二醇) fermentation. Both the MR and VP tests are especially useful in differentiating members of the enterobacteriaceae (肠杆菌科). The Voges-Proskauer test uses the same MR/VP broth. Some fermentative organisms do not produce enough stable acids to lower the pH of the medium. For these organisms, the chief end products of glucose metabolism are acetoin and 2,3-butanediol. After 48 hours of incubation, Barritt's Reagent A (alpha-napthol [α-萘酚]) and Barritt's Reagent B (potassium hydroxide [氢氧化钾]) are added to the sample. After gently shaking the tube for aeration, formation of a red color will indicate a positive reaction. No color change or a copper color is negative results.

4. Citrate utilization test (枸橼酸盐利用试验)

This test is designed to determine the ability of an organism, using the enzyme citrase, to use citrate as its sole carbon source. This is part of the IMViC test (Indole, Methyl red, Voges-Proskauer, and Citrate) used for differentiating the Enterobacteriaceae. Simmon's citrate agar contains sodium citrate as the sole carbon source and the ammonium ion as the sole nitrogen source. The pH indicator, bromthymol blue (溴麝香草酚蓝), will turn from green at neutral pH (6.9) to blue when a pH higher than 7.6 is reached (basic or alkaline). If the citrate is utilized, the resulting growth will produce alkaline products (pH > 7.6), changing the color of the medium from green to blue.

5. Indole test (吲哚试验) Some bacteria use the enzyme tryptophanase (色氨酸酶) to convert the amino acid tryptophan into molecules of indole, pyruvic acid and ammonia. Indole production is a key test for the identification of *Escherichia coli*. By adding Kovac's reagent to the medium after incubation we can determine if indole was produced. Kovac's reagent will react with the indole and turn red.

6. Hydrogen sulfide (H_2S) production Some bacteria are capable of breaking down sulfur containing amino acids (cystine, methionine) or reducing inorganic sulfur-containing compounds (such as sulfite[亚硫酸盐], sulfate[硫酸盐], or thiosulfate[硫代硫酸盐]) to produce hydrogen sulfide (H_2S). If the sulfur is reduced and hydrogen sulfide is produced, it will combine with the iron salt to form a visible black ferric sulfide (Fe S) in the tube.

7. Urease test (尿素酶试验) This test is used to differentiate organisms based on their ability to hydrolyze urea with the enzyme urease, particularly useful in distinguishing the genus *Proteus* (变形杆菌属) from other enteric bacteria. Urea test media (either broth or slant) contains urea and the pH indicator phenol red. This indicator turns to a dark pink (magenta) color when the pH is greater than 8.4. When urea is hydrolyzed, ammonia will be produced, raising the pH of the medium above 8.4. A positive reaction is dark pink, and a negative reaction will be either yellow or orange color.

B. Synthetic Products

1. Pyrogen (热原质/致热原) This is a fever-producing substance synthesized by bacteria. In fact, it is the lipopolysaccharide of Gram-negative bacteria. For the injectable medicament, it is especially important to avoid the contamination of pyrogen in the course of pharmic production.

2. Toxins and invasive enzyme

(1) **Exotoxin** (外毒素): proteins produced inside Gram-positive bacteria cells and secreted into the environment. These toxins are some of the strongest poisons known to man and cause violent reactions in host organisms.

(2) **Endotoxin** (内毒素): made up of lipids and carbohydrates associated with the outer membrane of gram-negative bacteria. These toxins usually produce fever, weakness, and capillary damage.

(3) **Invasive Enzyme** (侵袭性酶): These are a group of tissue-degrading enzymes that facilitate bacterial dissemination in the body, e. g., coagulase (凝固酶) secreted by staphylococci (葡萄球菌); hyaluronidase (透明质酸酶) secreted by streptococci (链球菌).

3. Pigments (色素) Some bacteria may produce different pigments, helping differentiate bacteria.

4. Antibiotics (抗生素) A metabolic product produced by one microorganism that inhibits or kills other microorganisms.

5. Bactericin (细菌素) Many Gram-negative bacteria produce bacteriocins. These bactericidal substances are produced by certain strains of bacteria active against some other strains of the same or closely related species. Their production is controlled by plasmids. Bacteriocin-producing strains are resistant to their own bacteriocin; thus bacteriocins can be used for "typing" of bacteria.

6. Vitamins (维生素).

ARTIFICIAL CULTIVATION OF BACTERIA

Microorganisms exist in nature as mixed populations. However, to study microorganisms in the laboratory we must have them in the form of a pure culture (纯培养), that is, one in which all organisms are descendants of the same organism. Two major steps are involved in obtaining pure cultures from a mixed population: First, the mixture must be diluted until the various individual microorganisms become separated far enough apart on an agar surface that after incubation they form visible colonies isolated from the colonies of other microorganisms. This plate is called an isolation plate. Then, an isolated colony can be aseptically "picked off" the isolation plate and transferred to new sterile medium. After incubation, all organisms in the new culture will be descendants of the same organism, that is, a pure culture.

The Medium

A suitable growth medium must contain all the nutrients required by the organism to be cultivated, and such factors as pH, temperature and aeration must be carefully controlled.

A. Classification according to basic ingredients

1. Minimal essential growth medium It contains only the primary precursor compounds essential for growth and demands that a bacterium synthesize most of the organic compounds required for its growth.

2. Enrichment medium (加富/营养培养基) It contains additives that enhance the growth of certain organisms. This is useful when the organism you wish to culture is present in relatively small numbers compared to the other organisms growing in the mixture.

3. Selective medium (选择培养基) It has agents added which will inhibit the growth of one group of organisms while permitting the growth of another. For example, Columbia CNA agar has the antibiotics colistin (多黏菌素) and nalidixic acid (萘啶酸) added which inhibit the growth of Gram-negative bacteria but not the growth of Gram-positives. It is, therefore, said to be selective for Gram-positive organisms, and would be useful in separating a mixture of Gram-positive and Gram-negative bacteria.

4. Differential medium (鉴别培养基) It contains additives that cause an observable color change in the medium when a particular chemical reaction occurs. They are useful in differentiating bacteria according to some biochemical characteristic.

B. Classification according to physical condition

1. Liquid medium.

2. Solid medium A liquid medium can be gelled by adding argar of 1.5% —2.5%. Agar, a polysaccharide extract of a marine alga, is uniquely suitable for microbial cultivation because it is resistant to microbial action and because it dissolves at 100℃ but does not gel until cooled below 45℃.

3. Semisolid medium It contains 0.3% —0.5% of agar.

Phenomena of Bacterial Growth

A. In liquid medium

There are 3 phenomena of growth: surface growth pellicle, uniformly turbid and sediment in bottom. If the medium is unstirred, strict aerobes tend to grow on the surface, micro-aerophiles just under the sur-

face and anaerobes in the body of the medium away from the surface. Growth of anaerobes is often improved by addition of a reducing agent to mop up any free oxygen.

B. On plate

If few enough cells are placed in or on a gelled medium, each cell will grow into an isolated colony. The colony has a high probability of being derived from a single cell, and consists of the descendants of the same organism.

Purposes of Bacterial Artificial Cultivation

From the clinical perspective, cultivation is used for detection and identification, and for the assessment of antibiotic effects, while scientific and industrial objectives are often served by cultivation in bulk to obtain sufficient biomass for detailed biochemical analysis and to produce the desirable products of the brewing and biotechnology industries.

BACTERIAL CLASSIFICATION

Principle of Bacterial classification

A. Phenotypic classification

The microscopic and macroscopic morphologies of bacteria were the first characteristics used to identify bacteria and form the cornerstones for most identification algorithms used today. For example, bacteria can be classified by their ability to retain the purple crystal violet-iodine complex in Gram stain (Gram-positive or Gram-negative) and by the shape of the individual organisms (cocci, bacilli, curved, or spiral). The macroscopic appearance of colonies of bacteria can also be used to identify bacteria (e. g., haemolytic properties on agar containing blood, pigmentation of the colonies, size and shape of the colonies).

The most common methods that are still used to identify bacteria consist of measuring the presence or absence of specific biochemical markers. These techniques are referred to as biotyping (生物型分型). Many bacteria possess antigens that are unique, and antibodies used to detect these antigens are powerful tools for their identification (serotyping [血清型分型]). Other examples of phenotypic methods used to classify bacteria include analysis of antibiogram (抗菌谱) patterns (patterns of susceptibility to different antibiotics) and phage typing (噬菌体型分型) (susceptibility to bacteriophages).

B. Analytic Classification

Analysis of the analytic characteristics of bacteria has also been used to classify bacteria at the genus (属), species (种), or subspecies (亚种) level. It includes: the chromatographic pattern analysis of cell wall mycolic acids (分枝菌酸), analysis of the lipids in the entire cell, and analyses of the whole cell proteins and cellular enzymes.

C. Genotypic Classification

The most precise methods for classifying bacteria are by analysis of their genetic material, including analysing the ratio of guanine to cytosine, DNA hybridisation, nucleic acid sequence analysis, and various other methods primarily to classify organisms at the subspecies level for epidemiologic investigations such as plasmid analysis, ribotyping (核糖分型), and analysis of chromosomal DNA fragments.

Formal Ranks Used in the Taxonomy of Bacteria (Table 3-1)

Table 3-1 Taxonomic ranks

Formal Rank	Example
Kingdom (界)	Prokaryotae (原核生物)
Division (门)	Gracilicutes (薄壁细菌)
Class (纲)	Scotobacteria (暗细菌)
Order (目)	Eubacteriales (真细菌)
Family (科)	Enterobacteriaceae (肠杆菌科)
Genus (属)	*Escherichia* (埃希菌属)
Species (种)	*Escherichia Coli* (大肠埃希菌)

For practical purpose, only the rank of the family, genus, and species are commonly used.

A bacterial species is defined as a distinct group of organisms that have certain distinguishing features and generally bear a close resemblance to one another in the more essential features of organization.

The species is designated by a Latin binominal (拉丁双名法), the first term of which is the *genus*, e. g., *Staphylococcus* (genus) *aureus* (species).

Bergey's Manual of Systematic Bacteriology

First published in 1923, the Bergey's Manual of Systematic Bacteriology is an effort to classify known bacteria and to make this information accessible in the form of a key (keys organize bacterial traits in a manner that permits efficient identification of organisms).

(袁建平　郭晓奎)

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