THE BACTERIAL CELL

BACTERIAL STRUCTURES

Despite their lack of complexity compared to eukaryotes, a number of eubacterial structures may be defined. Not all bacteria possess all of these components.

Plasmids

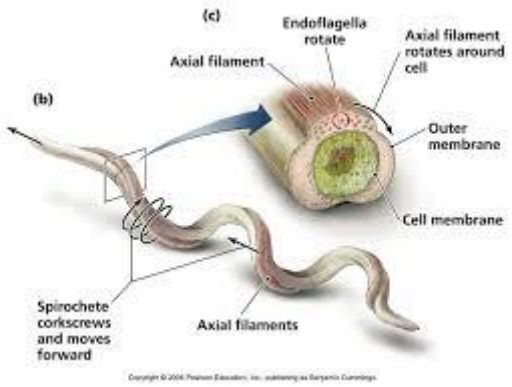
These are extra-chromosomal DNA, usually present in multiple copies, that often code for pathogenesis factors and antibiotic resistance factors. Some forms are also involved in bacterial replication.

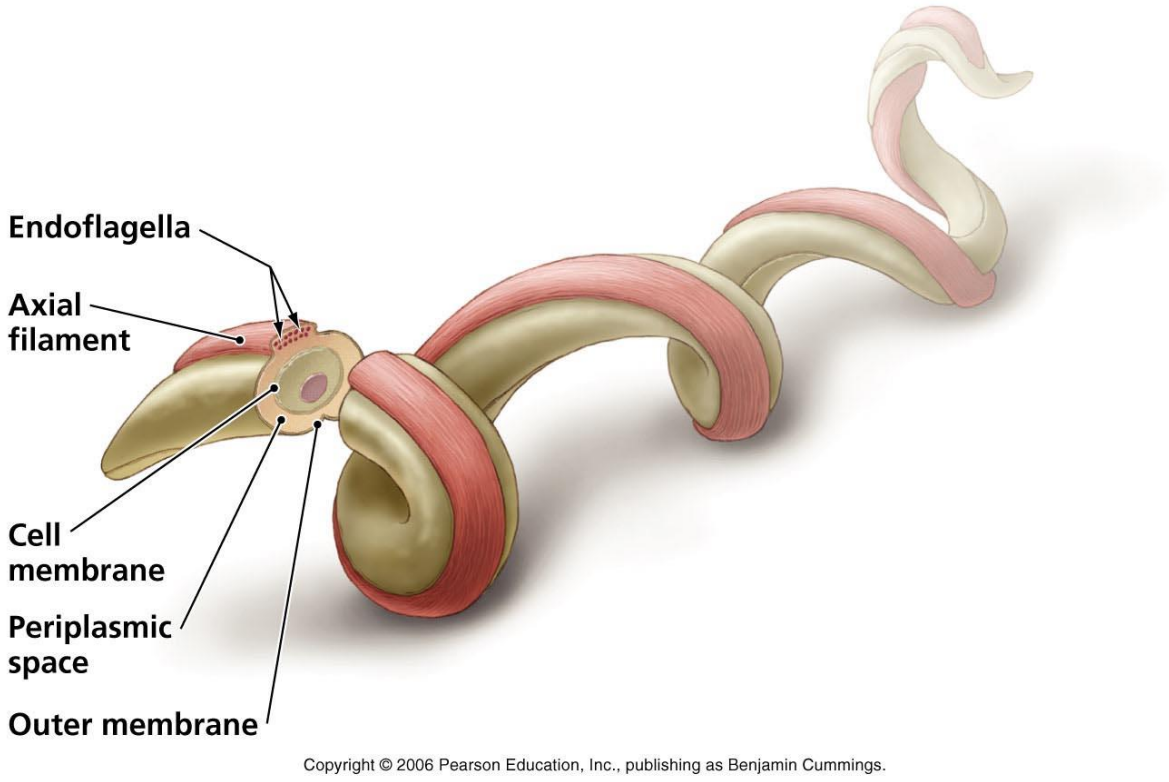
The cell envelope

Bacteria can be divided into two groups on the basis of staining with the Gram stain; Gram positive bacteria remain stained by crystal violet on washing, Gram negative do not. All bacteria have a cell membrane where oxidative phosphorylation occurs (since there are no mitochondria).

Flagella

Some bacterial species are mobile and possess locomotory organelles - flagella. Axial filaments in spirochetes have a similar function to flagella.





Pili (synonym: fimbriae)

The types of pili (or whether they are produced at all) varies both among and between species.

Capsules and slime layers

These are structures surrounding the outside of the cell envelope. When more defined, they are referred to as a capsule when less defined as a slime layer or glycocalyx. They usually consist of polysaccharide; however, in certain bacilli they are composed of a polypeptide (polyglutamic acid).

Endospores (spores)

These are a dormant formساكن طور of a bacterial cell produced by certain bacteria when starved; the actively growing form of the cell is

referred to as vegetative. Spores are commonly found in the genera *Bacillus* and *Clostridium*.

NUTRITION, GROWTH AND ENERGY METABOLISM

Bacterial requirements for growth include sources of energy, "organic" carbon (e.g., sugars and fatty acids) and metal ions (e.g., iron). Optimal temperature, pH and the need (or lack of need for oxygen) are important.

Oxygen Requirements

Obligate aerobes must grow in the presence of oxygen; they cannot carry out fermentation. Obligate anaerobes do not carry out oxidative phosphorylation. Furthermore, they are killed by oxygen; they lack certain enzymes such as catalase [which breaks down hydrogen peroxide, H_2O_2, to water and oxygen], peroxidase [by which NADH + H_2O_2 are converted to NAD and O_2] and superoxide dismutase [by which superoxide, $O_2^{\cdot}$, is converted to H_2O_2]. These enzymes detoxify peroxide and oxygen free radicals produced during metabolism in the presence of oxygen.

Nutrient Requirements

These include sources of organic carbon, nitrogen, phosphorus, sulfur and metal ions including iron.

Temperature

Bacteria may grow at a variety of temperatures from close to freezing to near to the boiling point of water.

pH

Many bacteria grow best at neutral pH.

METABOLISM OF SUGARS (as an example of metabolic pathways)

Glycolysis (Embden, Meyerhof and Parnas [EMP] Pathway)

This is the most common pathway in bacteria for sugar catabolism (It is also found in most animal and plant cells).

Anaerobic Respiration :Anaerobic respiration includes glycolysis and fermentation.

Aerobic Respiration: Aerobic Respiration involves glycolysis and the tricarboxylic acid cycle (Krebs cycle).

CULTURE AND IDENTIFICATION OF INFECTIOUS AGENTS

Bacterial identification in the diagnostic laboratory versus taxonomy

Isolation and identification of bacteria from patients aids treatment since infectious diseases caused by different bacteria have a variety of clinical courses and consequences. Susceptibility testing of isolates (i.e., establishing the minimal inhibitory concentration or MIC) can help in selection of antibiotics for therapy. Recognizing that certain species (or strains) are being isolated atypically may suggest that a disease outbreak has occurred e.g., from contaminated hospital supplies or poor aseptic technique on the part of hospital personnel.

When patients are suspected of having a bacterial infection, it is usual to isolate visible colonies of the organism in pure culture (on agar plates), and then speciate the organism. The identification is based on taxonomic principles applied to the clinical microbiological situation. In the diagnostic laboratory, many samples must be characterized each day and results obtained as quickly as possible. Tests must be easily learned, low in cost and rapidly performed. These classical methods for speciation of bacteria are based on morphological and metabolic characteristics. The diagnostic tests have been selected on the basis that empirically they provide discriminating information. There are numerous different tests for each of the many target pathogens.

Additionally, molecular biology techniques (for characterization of specific genes or gene segments) are now commonplace in the clinical laboratory.

Modern taxonomic approaches often employ technically more complex methodology and are concerned with profiling the structural composition of bacteria. This often involves "molecular biology" or "analytical chemistry" –based approaches. It is now recognized that many of the classical schemes for differentiation of bacteria provide little insight into their genetic relationships and in some instances are scientifically incorrect. New information has resulted in renaming of certain bacterial species and in some instances has required totally reorganizing relationships within and between many bacterial families.

Taxonomic terms (classification)

Family: a group of related genera.

Genus: a group of related species.

Species: a group of related strains.

Type: sets of strain within a species (e.g. biotypes, serotypes).

Strain: one line or a single isolate of a particular species.

The most commonly used term is the species name (e.g. *Streptococcus pyogenes* – abbreviation *S.pyogenes*). There are always two parts to the species name, one defining the genus in this case "*Streptococcus*" and the other the species (in this case "*pyogenes*"). The genus name is always capitalized but the species name is not. Both species and genus are underlined or in italics.

Steps in diagnostic isolation and identification of bacteria

Step 1. Samples of body fluids (e.g. blood, urine, cerebrospinal fluid) are streaked on culture plates and isolated colonies of bacteria (which are visible to the naked eye) appear after incubation for one to several days (Figure 1). Each colony consists of millions of bacterial cells. Observation of these colonies for size, texture, color, and (if grown on blood agar) hemolysis reactions, is highly important as a first step in bacterial identification. Whether the organism requires oxygen for growth is another important differentiating characteristic.

Step 2. Colonies are Gram stained and individual bacterial cells observed under the microscope.

Step 3. The bacteria are speciated using these isolated colonies. This often requires an additional 24 hours of growth.

Under the microscope, the appearance of bacteria is observed. Questions to be asked include:

- Are they Gram positive or negative?
- What is the morphology (rod, coccus, spiral, pleomorphic [variable form] etc)?
- Do cells occur singly or in chains, pairs etc?
- How large are the cells?

Besides the Gram stain, there are other less commonly employed stains available (e.g. for spores and capsules).

Another similar colony from the primary isolation plate is then examined for biochemical properties; for example, will the bacteria ferment a sugar such as lactose? In some instances, the bacteria are identified (e.g. by

aggregation) with commercially available antibodies recognizing defined surface antigens. Other commercial molecular tests are now widely used.

Approaches to rapid diagnosis without prior culture

Certain human pathogens (including the causative agents of tuberculosis, Lyme disease and syphilis) either cannot be isolated in the laboratory or grow extremely poorly. Successful isolation can be slow and in some instances impossible. Direct detection of bacteria without culture is possible in some cases.

A simple approach to rapid diagnosis (as an example of antigen detection) is used in many doctor's offices for the group A streptococcus. The patient's throat is swabbed and streptococcal antigen extracted directly from the swab (without prior bacteriological culture). The bacterial antigen is detected by aggregation (agglutination) of antibody coated latex beads.

Bacterial DNA sequences can be amplified directly from human body fluids (the polymerase chain reaction, PCR). In this fashion large amounts of specific genes or portions of genes can be generated and readily detected. For example, great success has been achieved in rapid diagnosis of tuberculosis.

Finally, direct microscopic observation of certain clinical samples for the presence of bacteria can be helpful (e.g. detection of *M. tuberculosis* in sputum).

Serologic identification of an antibody response (in patient's serum) to the infecting agent can only be successful several weeks after an infection has occurred.